ESTABLISHING A FORMULATION DESIGN SPACE FOR A GENERIC CLOBETASOL 17-PROPIONATE CREAM USING THE PRINCIPLES OF QUALITY BY DESIGN

By

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ABSTRACT

The pharmaceutical industry is global, is highly regulated and is able to achieve reasonable product quality but at high cost with maximum effort. Numerous challenges face the pharmaceutical industry and include a shrinking research pipeline, less innovation, outsourcing, investments, increasing research and development costs, long approval times, growth of the generic industry, failure to understand or analyze manufacturing failure and wastage as high at fifty percent for some pharmaceutical products. An efficient and flexible pharmaceutical sector should be able to consistently produce high quality pharmaceutical products at a reduced cost with minimal waste. As a result, Food and Drug Administration (FDA) and other agencies such as the International Conference on Harmonization (ICH) have embraced a "Quality by Design" (QbD) paradigm and this has become the "desired state" so as to shift manufacturing from being empirical to a science, engineering, and risk based approach.

QbD is a systematic approach for the development of high quality pharmaceutical dosage forms that begins with predefined objectives based on the premise that quality must be built into and not tested into a product. QbD together with the establishment of a design space for dosage forms is a fairly new concept and there is limited published data on QbD concepts that report the entire process of identifying Critical Quality Attributes (CQA), design of a formulation and manufacturing process to meet product CQA, understanding the impact of material attributes and process parameters on product CQA, identification and controlling sources of variability in materials and processes that affect the CQA of a product and finally establishing, evaluating and testing a design space using both *in vitro* and *in vivo* approaches to assure that a product of consistent quality can always be produced.

The objective of these studies was to implement a QbD approach to establish a design space for the development and manufacture of a safe, effective, stable generic formulation containing 0.05% w/w clobetasol 17-propionate (CP) that had similar *in vitro* and *in vivo* characteristics to an innovator product, Dermovate® (Sekpharma® Pty Ltd, Sandton, Gauteng, RSA). Such a product would pose a minimal risk of failure when treating severe skin disorders such as seborrhoeic dermatitis, extreme photodermatitis and/or severe psoriasis in HIV/AIDS patients in Southern Africa.

QbD requires a scientific approach to identify desired dosage form and performance attributes through establishing a Target Product Profile (TPP) and an initial list of CQA was produced. A risk assessment was undertaken to identify possible formulation variables and unit operations that were most likely to have an impact on the CQA of the product and/or manufacturing process. This data was used to focus development activities on potential high risk areas. An Ishikawa diagram was used to conduct an exhaustive analysis and included all factors that may impact product quality. Risk analysis started with an assessment of the physico-chemical characteristics of CP and led to the identification of a viable formulation and manufacturing approach for a generic product. It was observed that the impact of excipient attributes such as those of glyceryl monostearate, cetostearyl alcohol, Gelot® 64, propylene glycol and manufacturing parameters such as homogenization speed, homogenization time, anchor speed, mixing time, heating temperature, batch size and cooling time could have an impact on the resultant quality of the manufatured cream formulations.

Three screening designs *viz.*, Taguchi (TD), Plackett-Burman (PBD) and 2-Level Fractional Factorial (2-LFFD) designs were used to identify significant factors due to the large number of excipient and process parameters that could impact the resultant quality of the CP formulations. These screening designs are commonly used by the pharmaceutical industry and were evaluated to compare their effectiveness for formulation and process optimization for cream manufacture. Twelve batches of cream were manufactured using the TD and PBD approaches whereas sixteen were required for the 2-LFFD approach. All product formulations were manufactured using a Wintech® cream/ointment plant (Wintech® Pharmachem Equipment PVT, Ltd, Mumbai, India) and were assessed in terms of their viscosity, spreadability, pH, content uniformity, extrudability, electrical conductivity and *in vitro* release characteristics using a Franz diffusion cell apparatus over 2, 4, 8, 12, 24 and 72 hours. Spreadability, electrical conductivity and cumulative % CP released over 2, 12, 48 and 72 hours were found to be the significant formulation responses. TD identified ten significant responses and was considered to be the least selective statistical model when compared to the PBD and 2-LFFD approaches. The PBD and 2-LFFD models also generated low coefficients of variation and produced more accurate models than the TD. The graphical plots confirmed that formulation parameters had the most significant effect on the quality of the creams when compared to process related parameters.

A Central Composite Design (CCD) in conjunction with Response Surface Methodology (RSM) approach was used to further investigate formulation variables for design optimization. The % v/v propylene glycol, % w/w Gelot® 64, % w/w cetostearyl alcohol and % w/w glyceryl monostearate were investigated. Thirty batches (6 kg per batch) were manufactured and analyzed in terms of their viscosity, spreadability, pH, content uniformity, extrudability, electrical conductivity and *in vitro* release characteristics using a Franz diffusion cell apparatus over 2, 4, 8, 12, 24 and 72 hours. Model fitting using Design-Expert® software revealed that a correlation between the formulation variables and the cream responses was most suitably described by quadratic polynomial relationships. The % w/w cetostearyl alcohol had the most significant effect on the quality of the cream formulations whereas the % w/w propylene glycol had the least significant effect on the measured responses. The % w/w Gelot® 64 and glyceryl monostearate also have a significant effect on the quality of the cream formulations, albeit not as pronounced. Qualitative interpretation and statistical analysis of the *in vitro* release data from the formulations using f_1, f_2 and S_d factors revealed that the optimized formulation consisted of approximately 46.0% v/v propylene glycol, 8.6% w/w cetostearyl alcohol, 10.5% w/w glyceryl monostearate and 3.8% w/w Gelot® 64 and released CP at a similar rate and extent to Dermovate®. A diffusion-controlled mechanism appeared to be the dominant factor controlling CP release from this formulation.

The RSM concept was also used to establish a design space for the CP cream formulations and the *in vitro* release profile of Dermovate® was used to establish formulation constraints and the levels of key excipients that if used would result in the manufacture of a cream formulation that was similar to Dermovate® in terms of all quality attributes. The lower and upper limits of propylene glycol, Gelot® 64, cetostearyl alcohol and glyceryl monostearate content were established as 44.0% v/v and 46.4% v/v, 2.9% w/w and 3.8% w/w, 7.5% w/w and 10.0% w/w and 10.9% w/w and 12.8% w/w, respectively. The formulations manufactured with the excipients levels set at either the extreme lower or upper limit produced *in vitro* release profiles that were similar to the reference product and were bioequivalent to Dermovate®. The cream formulations were placed on two stability conditions at 40°C, 75% RH and 25°C, 60% RH and were found to be stable for eight weeks.

The human skin blanching study using both visual and chromameter data was performed to confirm the bioequivalence of test formulations established through use of the design network and all formulations were bioequivalent to Dermovate® following calculation of a 90% confidence interval using Locke’s method. A design space for the manufacture of a CP cream in which the levels of key exicpients known to affect product quality has been developed and was shown to be practical in an *in vivo* study.

This thesis is dedicated to the following:

To Dr Sandile Khamanga
(My Mentor)

&

To the Fauzee & Sohawon Families.

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STUDY OBJECTIVES

Dermatological diseases have not been recognized as a public health problem in developing countries, despite a recent report by the World Health Organization that estimates that 21 – 87% of the general population in developing countries presents with skin disease [1,2]. The HIV and AIDS epidemic in Africa adds to the burden of dermatological conditions [1]. Consequently there is a demand for high-potency topical corticosteroid dosage forms such as clobetasol 17-proprionate (CP) creams [3]. For decades, the pharmaceutical industry has faced challenges such as a failure to analyze manufacturing failures and product wastage as high at fifty percent for some products [4,5]. FDA and other organizations such as ICH have introduced the concept of "Quality by Design" that involves the establishment of a design space to reduce the risks associated with the formulation and manufacture of pharmaceutical products [6]. This approach would facilitate the manufacture of high quality topical corticosteroid products that are safe, effective, stable and that have a minimal risk of failure when treating severe skin disorders such as seborrhoeic dermatitis, extreme photodermatitis and/or severe psoriasis in HIV/AIDS patients in Southern Africa.

The objectives of this study were therefore:

i) To identify desired dosage form and performance attributes through establishing a Target Product Profile taking into consideration the intended use and route of administration.

ii) To develop an initial list of Critical Quality Attributes.

iii) To develop, validate and optimize a Reversed-Phase High Performance Liquid Chromatographic (RP-HPLC) method using Central Composite Design (CCD) for the quantitation of CP in semi-solid dosage forms.

iv) To conduct process validation of a Wintech® cream/ointment mixer to provide evidence that the processes operated within specified design parameters are capable of repeatedly and reliably producing a finished product of the desired quality.

v) To conduct a risk assessment analysis for the development of a high quality 0.05% CP cream by assessing the physico-chemical characteristics of the excipients, evaluating the potential high risk areas of the formulation and establishing an Ishikawa diagram to evaluate factors affecting product quality.

vi) To compare three screening design techniques commonly used in the pharmaceutical industry for formulation and manufacture of pilot scale cream formulations with the view to defining a design space for this product.

vii) To investigate the effect of formulation variables on the optimization of CP cream formulations and to develop an optimized cream formulation with an *in vitro* release profile similar to that of a reference formulation *viz.*, Dermovate® cream.

viii) To establish a design space for a bioequivalent and stable generic CP cream formulation.

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CHAPTER ONE

PHARMACEUTICAL DEVELOPMENT AND BIOEQUIVALENCE OF TOPICAL FORMULATIONS

1.1. INTRODUCTION

1.1.1. Global Challenges for the Pharmaceutical Industry

Over the past 10 years, the pharmaceutical industry has been a powerful and dynamic industry despite the economic crisis in industries such as the automotive, aviation, telecommunications and computer electronics sectors. The downturn was due to the financial crisis of 2008 and the subsequent economic recession and economists predict that the pharmaceutical industry sector growth will begin to slow in the coming years, despite their recent strong performances [8,9]. Globally the pharmaceutical industry is being pressured by two major factors *viz.*, pricing and regulatory and the sector is confronting a variety of internal and external challenges [10,11]. The future profitability of the innovator product industry has been threatened by several factors including a shrinking research pipeline, lack of sustainability of innovation, outsourcing, investments, increasing Research and Development (R&D) costs, long approval times, growth of the generic industry, price control, parallel importation and ever-increasing counterfeiting [12]. The confluence of these factors has reduced the profit margins for the industry and has contributed to an increasingly challenging global environment. Other factors that impact the industry include reduced supply of late-stage molecules to replace drugs for which the patent is due to expire and the need to stay ahead of more diverse competitors [12-14]. Drug molecules that are due to come off patent protection have been estimated to be worth $ 30 billion worldwide and as businesses shrink, expenses are reduced to maintain high levels of profitability [15-17]. Pharmaceutical companies are constantly being challenged to accelerate the process of development to bring to markets products that are effective and differentiated from competitor products. While trying to address these challenges, manufacturers must continuously manage the difficult balance between the market-driven “need for speed” and the absolute need for scientific rigor and safety in product development [18-21].

Due to shrinking revenues and new drug approvals declining, companies are being pushed to tighten their R&D budgets [22,23]. The reduced R&D spending, in conjunction with severe budgetary constraints such as those imposed by government-run healthcare systems, health insurance and the government debt crisis present an ideal opportunity for generic companies to expand and build their capability to capture large sectors of the global market [24]. Price cuts have encouraged the use of generics and the generic industry has seen excellent growth. To cut healthcare costs, governments are encouraging the use of generic products to sustain the health of the global population [25-30]. The impact of the economic downturn on various sectors of the pharmaceutical industries is depicted in Figure 1.1.

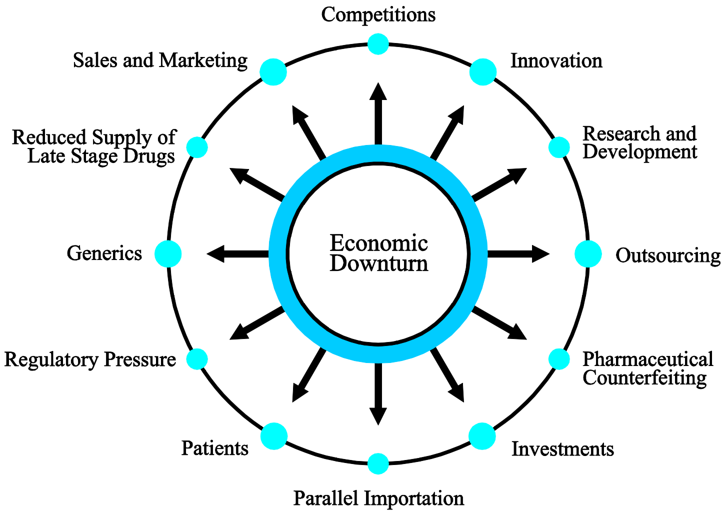


Figure 1.1 The impact of the economic downturn on various sectors of the pharmaceutical industry adapted and redrawn from [15]

In the array of an increasingly aging population and an increasing prevalence of chronic diseases such as Human Immunodeficiency Virus (HIV)/Acquired Immune Deficiency Syndrome (AIDS), cancer, Alzheimer's and Parkinson's disease, medical innovation has the potential to improve quality of life, life expectancy, reduce disability, and improve worker productivity, all of which are vital to increase the prosperity of a nation [9]. Furthermore, innovation and investment in R&D has led to many important benefits for society such as scientific, medical, and technological progress, the improvement of human health, well-being and economic development [31,32]. Pharmaceutical companies have commenced investigation in emerging fields such as research-intensive and product-incentive pharmaceutical sectors that will define economic success worldwide in the 21st century [9,33,34]. The Food and Drug Administration (FDA) of the United States Department of Health, in conjunction with the International Conference on Harmonization (ICH), have created guidelines such as ICH Q8 (R1 and R2), Q9 and Q10, that provide direction for the pharmaceutical industry to facilitate prolongation of the lifecycle of a product by emphasizing the application of pharmaceutical and manufacturing sciences to form the basis for a flexible regulatory approach [35-38]. The guidelines recognize the need for a pharmaceutical product (innovator or generic) to be of high quality for its intended purpose and include tests for characteristics such as identity, strength, and purity. Pharmaceutical quality must represent a low risk of failure in achieving desired clinical outcomes [35,36].

1.1.2. Infectious and Related Diseases and Access to Medicines in Southern Africa

Developing nations such as the Republic of South Africa (RSA) continue to struggle with the burden of HIV/AIDS, malaria, tuberculosis, diarrhea, pneumonia, measles and other infectious diseases [12,39]. Sixty per cent of all deaths in developing nations are due to infectious diseases, as compared to only 10% in developed nations. In the developing world, diarrhea is the cause of death of two million children, measles 700000, malaria 2.0 million lives and HIV/AIDS claims about 2.3 million lives on an annual basis. In sub-Saharan Africa, 25.4 million people are infected with HIV/AIDS and there are 3.1 million new cases reported annually. In total 7.4% of the adult population in sub-Saharan Africa is infected with the virus [12,40]. In some countries, the infection rate among adults is ≥ 20%. Infectious diseases including HIV/AIDS, Hepatitis B and C have an economic and social impact and therefore the diseases restrict productivity, erode economic growth, discourage foreign investment, disrupt families, undermine education, resulting in spending valuable resources on healthcare [12,41-43]. The impact of malaria alone reduces the Gross Domestic Product (GDP) of Africa by $ 100 billion a year [17]. In sub-Saharan Africa 12.3 million children have been orphaned by HIV/AIDS. Many developing nations have little hope of breaking this cycle of poverty and disease. HIV/AIDS for example, represents a major success story in terms of R&D, with over 20 new medicines having been produced in as many years, generating sales in excess of $ 5.3 billion. There are still gaps in AIDS-related R&D, especially for patients in poor countries. For example HIV/AIDS-related topical disorders such as extreme dermatitis, photodermatitis and severe eczema are seldom diagnosed or treated regularly [12,17,44].

Many essential drugs are simply too expensive for use by patients in developing nations [45-48]. In 2003, the per capita income in Ethiopia, Congo, Sierra Leone, Malawi, Niger, Rwanda and Uganda was $ 90, $ 100, $ 150, $ 170, $ 200, $ 220, and $ 240 respectively. Despite drug discounts and foreign aid programs, the cost of ART for treating HIV/AIDS ranges between $ 300 and $ 1200 for patients in the developing world [12]. Access to affordable medications especially generic medications therefore can and does play an important role in assisting developing nations to deal with infectious and related diseases. However, increased availability and access to pharmaceuticals for populations in the developing countries is a key challenge [44,49-51]. This challenge entails reducing the gap between population needs and medicines supply, requiring reconciliation of needs, supply and demand at affordable prices. It is therefore imperative that the market trends adapt, encourage and are directed in a more socially responsible manner [12].

1.1.3. Generic Pharmaceutical Development in Southern Africa

Over the last three decades, bioavailability, bioequivalence and the selection of pharmaceutical products have emerged as critical issues in the pharmaceutical and medical fields due to an enormous increase in manufacture of generic products [52-56]. Approximately 50% of all prescriptions are for molecules that can be substituted with a generic product. Over 80% of the approximately 10000 prescription drugs available in 1990 were available from more than one source [54,57]. With the increasing availability and use of generic pharmaceutical products, healthcare professionals are confronted with multisource products from a variety of manufacturers, from which they must select those that are therapeutically equivalent. The phenomenal growth of the generic pharmaceutical industry and the abundance of multisource products have prompted questions amongst healthcare professionals and scientists regarding therapeutic equivalency of these products, particularly those in certain critical therapeutic categories, such as anticonvulsants and cardiovascular therapies [53,54].

The South African healthcare system consists of a large public sector and a small, yet fast-growing private sector. The system covers basic primary healthcare that is offered free to patients by the government and highly specialized and technologically advanced healthcare services in the private sector [58]. Although the government contributes approximately 40% of all healthcare expenditure, the public sector is under pressure to deliver healthcare services to 80% of the population. The South African public sector expenditure on pharmaceuticals rose by 58% per annum between 1995 and 1999, since the majority of the population relies on the public sector for access to appropriate healthcare [59]. In 2000, approximately R 8.25 billion was spent on pharmaceuticals in RSA, with the government spending only 24% of that total, resulting in an average expense of R 59.36 per person in the public sector, as opposed to R 800.29 per person in the private sector [60]. Increased substitution of generic for original drug products has long been advocated as a means of decreasing consumer expenditure on prescription medicines. The majority of consumers realize major cost-savings when using generic products [59,61,62].

Bioavailability and bioequivalence of pharmaceutical products therefore play a critical role in pharmaceutical formulation development, regulatory review, approval and in the clinical use of pharmaceutical products [26,61,63,64]. The regulatory requirements for bioavailability and bioequivalence were established approximately 40 years ago in response to numerous reports of therapeutic failures that were linked to formulation effects on product performance. Formulation factors affecting bioavailability and bioequivalence are those characteristics that affect the dissolution of a drug or release from the dosage form and excipients that may affect stability (at the site of administration), absorption and/or metabolic processes [65-69]. The Office of Generic Drugs (OGD) has therefore developed a critical understanding and has published reviews for Chemistry, Manufacturing and Control (CMC) for the evaluation of Abbreviated New Drug Applications (ANDA) that are focused on Critical Quality Attributes (CQA) of pharmaceutical products [25,64,70-72]. An ANDA is required to contain data that show a drug product to be pharmaceutically equivalent and bioequivalent to the Reference Listed Drug (RLD). The main goal of CMC and ANDA documentation is to ensure that the generic product is appropriately designed *viz.,* a product that is pharmaceutically equivalent to the RLD and that sponsors have methods and controls in place for the manufacture, processing and packaging of an Active Pharmaceutical Ingredient (API) that are adequate for assuring and preserving the identity, strength, quality and purity of the proposed product [73].

The OGD has ensured that the quality, safety and efficacy of generic products are based on these two important requirements *viz.,* pharmaceutical equivalence and bioequivalence to the RLD [74-76]. The term “Pharmaceutical Equivalence” has been used to ensure that quality generic drug products provide the same therapeutic benefit to a patient as the RLD. Over the years, the design complexity of drug products has increased tremendously. As a result, approaches to ensure therapeutic equivalence of generic and other products has had to evolve to provide an assurance of quality of these products [77,78]. Unlike the case for new drug products, the risk of total failure of generic products is fairly low, since the safety and efficacy of the API has already been established. However cost efficiency is also important because of much lower profit margins and competition with other generic manufacturers. Time efficiencies are also important for both generic and new drug product manufacturers and unanswered scientific questions that may retard the progress of products through a development process should be avoided [79,80]. Another aspect of efficient drug product development is the use of current scientific understanding to limit unnecessary human testing of drugs. The critical pathway of generic pharmaceutical formulation development is summarized in Figure 1.2 [79].

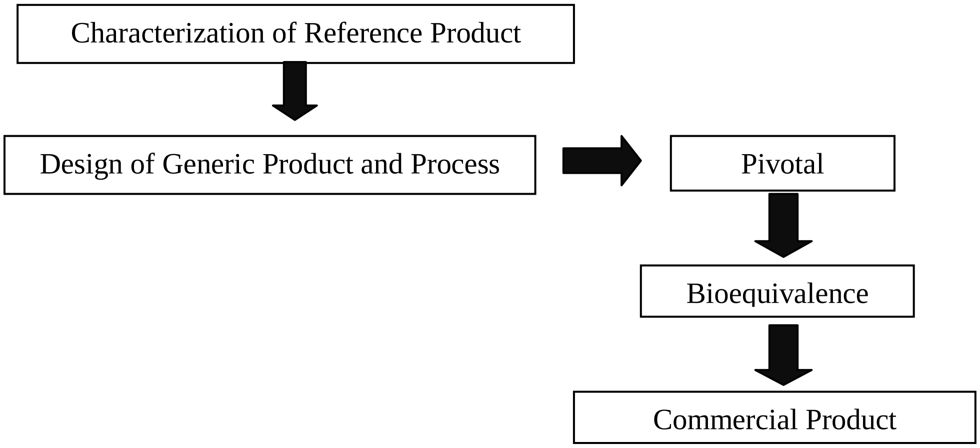


Figure 1.2 Steps involved in the process and development of a potential generic product [79]

A generic drug in the same dosage form and administered via the same route, should also be identical in strength or concentration [26,27,65,81,82]. The FDA classifies products as therapeutically equivalent **i)** if they are proved to be safe and effective, **ii)** if they are pharmaceutically equivalent in that they contain identical amounts of the same API in the same dosage form and route of administration and meet the compendial or other applicable standards of strength, quality, purity and identity, **iii)** if they are bioequivalent in that they do not present a known or potential bioequivalence problem and they meet an acceptable *in vitro* standard and **iv)** if they are adequately labelled and are manufactured in compliance with current Good Manufacturing Practice (cGMP) regulations [65,83,84]. However, pharmaceutically equivalent drugs may differ in shape, score line, release mechanism, packaging, excipients including colors, flavors, and preservatives, expiration time and labeling within certain limits. In general most generic products are an example of pharmaceutically equivalent products [26,76,85]. Once an ANDA sponsor has provided sufficient data to demonstrate that a generic drug product is pharmaceutically equivalent and bioequivalent to a RLD, FDA deems these two pharmaceutical products to be therapeutic equivalent and assigns the generic product a therapeutic equivalence code for example AB in the Approved Drug Products with Therapeutic Equivalence Evaluations or "Orange Book" [74,86]. Therapeutically equivalent drug products are expected to have the same clinical efficacy and safety profiles when administered to patients under the conditions specified in the labeling, and may be substituted for each other without any adjustment in dose or additional monitoring. A generic drug product is therefore described as a pharmaceutical product that is therapeutically equivalent to an innovator or first version of the drug product approved by FDA [53,87,88].

1.2. QUALITY BY DESIGN FOR BIOEQUIVALENCE

1.2.1. Quality by Design

Quality by Design (QbD) is a concept first outlined by well-known quality expert, Joseph M. Juran in various publications, the most notable of which was on Quality by Design in the 1970s [89]. Juran believed that quality could be planned and that most quality crises are related to the way in which quality was planned in the initial stages of manufacture of a product. The principles of QbD have been used to advance product and process quality in every industry, particularly in the automotive industry [90]. A notable example is provided by the success of the Japanese car industry and how the top three spots in car reliability surveys are consistently held by Japanese companies [91]. QbD has also been implemented in fields such as biochemical and biotechnology studies [92,93] and electronic engineering [94,95], but it has most recently been adopted by FDA as a vehicle for the transformation of drug discovery, development and commercial manufacture [96-99]. QbD has therefore become an important paradigm for the pharmaceutical industry since introduction by the FDA. The overarching goal of QbD is to embed quality into pharmaceutical products to ultimately protect patient safety [100]. Many other regulatory agencies around the world have also adopted similar approaches. The ICH defines QbD as a systematic approach that may be used for the development of pharmaceutical products. It commences with predefined dosage form performance objectives and uses science and risk management approaches to gain product and process understanding and ultimately process control, as shown in Figure 1.3. The ICH Q10 guideline [38] describes a holistic and integrated quality management system applicable to QbD environment [100-103].

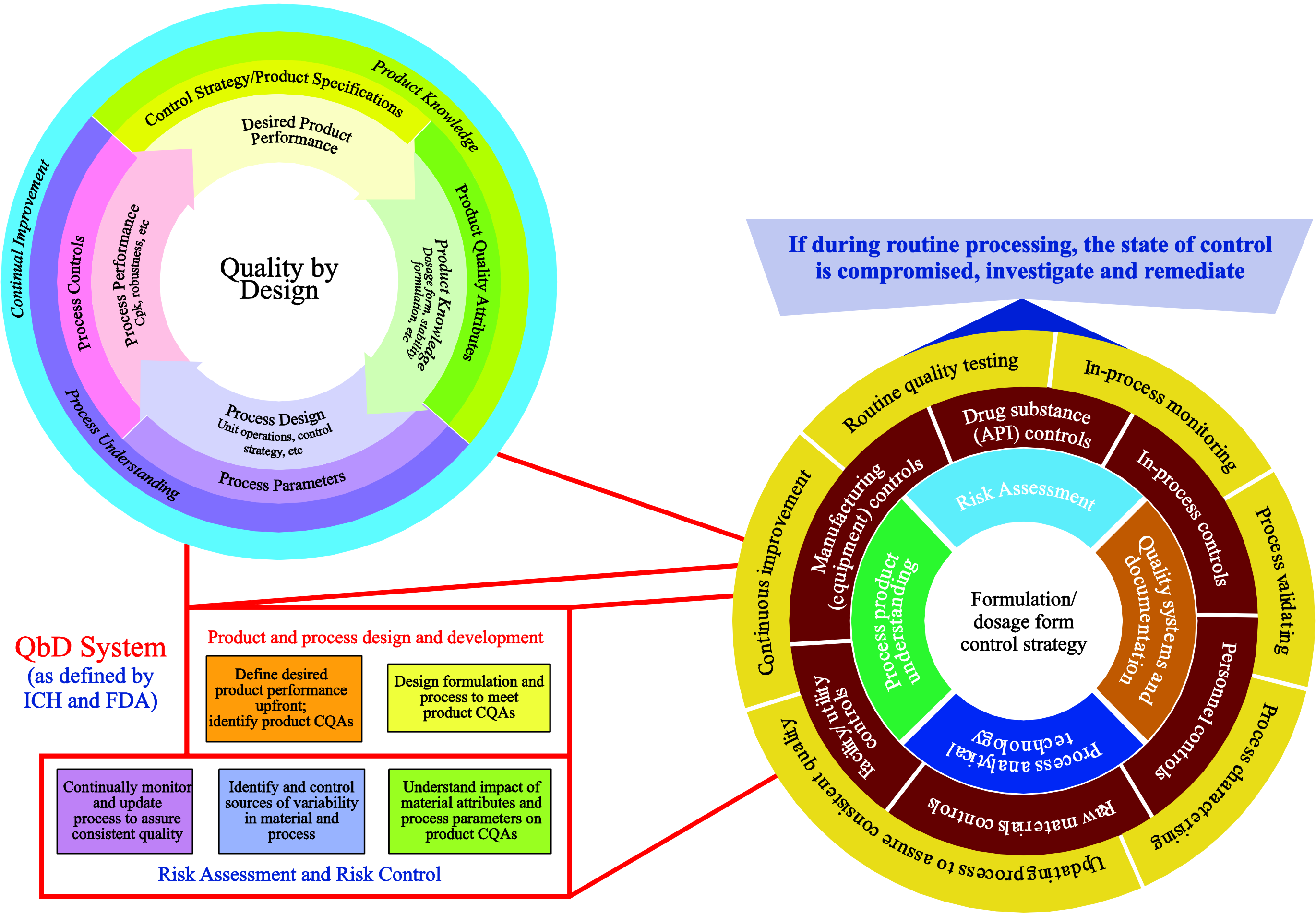


Figure 1.3 QbD adapted and redrawn from [6,104]

1.2.2. Historical Aspects

Over the past few decades, the industrial landscape has undergone significant changes that have been dominated by the shift from a supplier to a customer-dominated market. Following the Second World War, the overall product demand exceeded the general capacity of the pharmaceutical industry [105]. Hence, the quality of a product was mainly defined by the view of the producer as to what constitutes quality, compared to the partial analytical understanding of quality. This situation has changed dramatically and the industry faces a market that is often characterized by intense “cut-throat” competition in many sectors, increasing product complexity and diversity and by growing customer awareness of the requirements and need for high product quality and functionality. In addition, more stringent legislative and regulatory measures imposed by society comprise an increasing powerful driving force towards comprehensive public health and environmental compatibility of industrial processes [105]. In contrast to the earlier years of industrialization, production enterprises nowadays can no longer stratify their market position by increasing the mass-per-hour throughput of specific products. The sustainable success of an industrial company depends more critically than ever on the cost-effective realization of customer-tailored products of high quality that are required to meet the rapidly changing demands of the end user [106]. This approach reflects the overall trend towards an increased focus on custom-made quality, increasingly sophisticated and holistic quality management systems that have been developed over the years, as summarized in Figure 1.4.

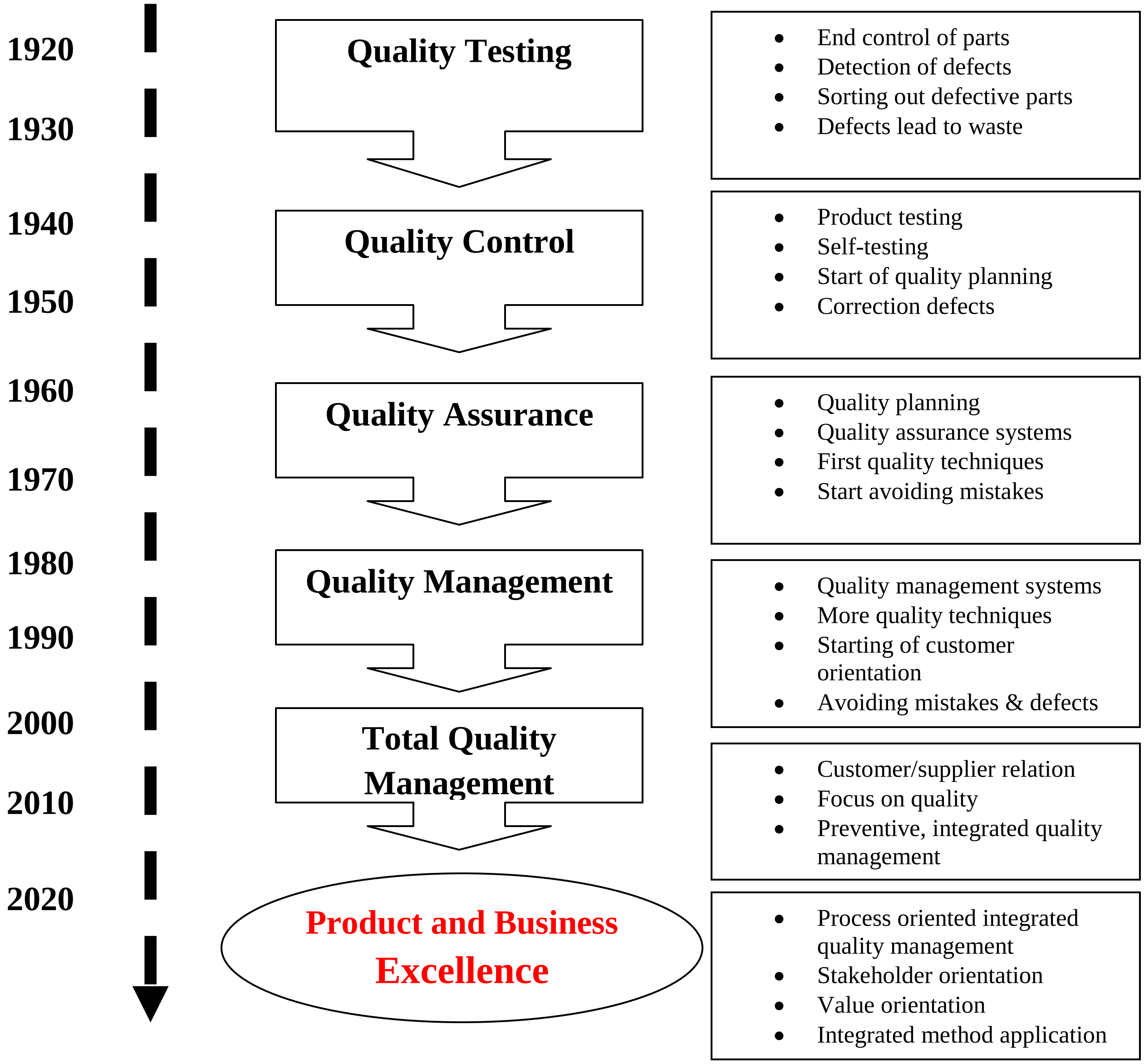


Figure 1.4 Historical development of important quality concepts and their major elements, adapted and redrawn from [106]

Quality management systems range from simple inspection of finished parts for defects and elimination of inferior units, to the implementation of increasingly complex quality systems to avoid the production of any defective parts during manufacture, and finally to the modern view of process oriented, integrated and comprehensive total quality management systems. It is in this context that the modern concepts of Process Analytical Technology (PAT) and QbD have to be viewed [38,106].

1.2.3. Quality by Design in the Pharmaceutical Industry

In the year 2000, a suboptimal state of drug manufacture and an outmoded review process at FDA had undesirable consequences for drug regulation. As far as the pharmaceutical industry was concerned, product quality was adequate and there was hesitation in the implementation of new technologies since the perception of the regulator to innovation was unknown [107]. Although the amount of product waste as a result of mistakes in the manufacturing was generally high, many companies placed little emphasis on the manufacturing process and associated challenges. In some cases, waste was reported to be as much as 50% of the product manufactured and much of the information developed and shared with FDA, was derived empirically [5]. There appeared to be an inability to predict the effects of scale-up on final product quality as well as an inability to analyze or understand the root causes of manufacturing failure. Furthermore, the industry had become global and regional differences in product regulation lengthened preparation and approval times and created additional paperwork to meet regulatory requirements, which in turn led to a higher cost of drug product manufacture [4,107].

At the same time, FDA recognized that additional controls were needed for the drug manufacturing process because of concerns over the state of manufacturing. FDA oversight of firms increased and at one point it seemed there was a tendency to require supplemental changes to the application for every change or modification of a manufacturing process. In 2007, FDA received approximately 5000 supplements for NDA, ANDA and Biological License Applications (BLA). The data required original applications and supplements have focused mainly on chemistry, without consideration of other important aspects of the manufacturing process such as engineering. Furthermore, data have usually not included information about the product development process [107]. Apart from the requirement for supplements to applications, in the 1990s, several other changes to the regulatory process were implemented by FDA to address concerns in respect of the manufacturing process. Although these changes gave FDA greater assurance in respect of product quality, it resulted in additional requirements for the industry and thereby increasing product review times [107,108]. Numerous prescriptive guidance documents were produced by FDA to ensure that the industry understood the process changes and was able to submit adequate data for evaluation that consequently reduced the flexibility of addressing the science of products and associated manufacturing processes. As FDA took on more responsibility for product quality, the workload increased further and applications contained greater amounts of information, some of which were not relevant to the science behind the product development and manufacturing processes. Regulatory processes became more onerous and less flexible and despite specific guidance documents it became increasingly difficult to ensure consistency in regulatory decision-making [107].

In August 2002, FDA announced a new initiative to implement changes through cGMP for the 21st century. This has the primary goal of placing responsibility for product quality on the industry and modernizing the regulation of pharmaceutical manufacturing and product quality processes [6,109,110]. As part of this initiative, the CMC and pharmaceutical aspects were evaluated with the objectives of **i)** encouraging the early adoption of new and technological advances, **ii)** facilitating industry application of modern quality management techniques to all aspects of production and quality assurance, **iii)** encouraging implementation of risk-based approaches that focus both industry and FDA attention on critical areas, **iv)** ensuring that regulatory review, compliance and inspection policies are based on state of the art pharmaceutical science and **v)** enhancing the consistency and coordination of FDA drug quality regulatory programs by further integrating enhanced quality systems approaches into the business of FDA processes and regulatory policies concerning review and plant inspection activities [6,111].

In the early stages of this initiative, several multidisciplinary working groups were formed to complete an assessment of existing cGMP programs and practices in addition to evaluation of the available new tools for enhancing manufacturing science [6,109]. This enabled the creation of a new framework for regulatory oversight of manufacturing quality and is based on quality systems and risk management approaches. Implementation of the envisioned new framework requires a highly educated, well-trained and integrated team of individuals throughout FDA who use risk- and science-based approaches for regulatory decision making throughout the life-cycle of a product [38,112]. A number of specific accomplishments have resulted from the cGMP initiatives. One of these accomplishments is science-based regulation of product quality. The key objective of this initiative is to use a scientific framework to find ways of mitigating risk, while facilitating continuous improvement and innovation in pharmaceutical manufacturing [6]. This new system encourages the implementation of new technologies *viz.*, PAT that facilitates continuous manufacturing improvement via implementation of an effective quality system. In addition to the new concepts considered by FDA in these cGMP initiatives, two important guidance documents were published as part of the ICH guidelines *viz.*, Q8 Pharmaceutical Development [36] and Q9 Quality Risk Management [37]. The former describes the expectation for the drug product pharmaceutical development section of the Common Technical Document (CTD) whereas the latter presents some approaches to producing quality pharmaceutical products using current scientific and risk-based approaches. The guideline, Q10 Quality Systems Approach to Pharmaceutical cGMP Regulations, provides a model for an effective quality management system for the pharmaceutical and biotechnology industries [38].

1.2.4. QbD Implementation in the Generic Pharmaceutical Industry

A few years after FDA introduced the critical path initiative that focused on the challenges involved in the development of new drugs, although some areas of generic development equally applied the concept to the original initiative, it was recognized that there were scientific challenges unique to the development of generic drugs [29,79,113-115]. In May 2007, FDA released the document “Critical Path Opportunities for Generic Drugs” [29] to bring these challenges to the attention of interested parties and identify opportunities for the development of collaborative solutions. The need to address generic drug development was motivated by the large and growing use of generic drugs since they represented 66.4% of the total prescription doses sold in 2004, compared to 61% in 2001 [79]. Efforts to encourage efficient pharmaceutical manufacturing processes and QbD will have maximum impact only when they are applied to generic drug product development and manufacture. While the primary focus of the critical path initiative for generic drugs was on generic drug development for the USA, the scientific challenges and their resolution may have a significant impact on ensuring product quality of generic medicines available in other markets [79].

The OGD website summarizes an analysis of Information Management System (IMS) sales data (wholesale prices) for single-ingredient brand name and generic drug products sold in the USA from 1999 through 2004. This analysis indicates that the appearance of a second generic product reduces the average generic price to nearly half that of the brand name price [79]. As additional generic manufacturers market products, prices continue to fall and for products that attract a large number of generic manufacturers, the average generic price falls approximately to 20% of the price of a branded product. These results suggest value saving for the consumer by access to multiple generic drug products that compete on the market. However, there are still products and product categories for which there is little or no generic competition, despite expiration of all patent and exclusivity protection for the reference product [79,116,117]. Based on this analysis of generic drug development, there are several areas of opportunity where scientific progress could accelerate the development and approval of generic products and expand the range of products for which generic versions are available, while maintaining high standards of quality, safety and efficacy [29,79]. FDA has identified four areas of opportunity for which collaborative activities may advance public health through more efficient development of high quality generic products. These include **i)** improving the science underlying QbD for the development and manufacture of generic drug products, **ii)** improving the efficiency of current methods for the assessment of bioequivalence of systemically-acting drugs, including products that use complex and novel drug delivery technologies, **iii)** developing methods for the assessment of bioequivalence of locally acting drugs such as topical and inhalation products and **iv)** developing methods for the characterization of complex drug substances and products [29,79,118].

Using a QbD paradigm allows for the identification of characteristics that are critical to quality from a safety and efficacy perspective, translating them into the attributes that a drug product should possess, and establishing how the critical process parameters can be varied to consistently produce a drug product with the desired quality and other attributes [36,71,107,119,120]. This is achieved by establishing a relationship between formulations and manufacturing process variables, including drug substance and excipient attributes, process parameters and product quality and identifying the sources of product variability. Testing is usually undertaken to confirm the ultimate quality of the product [66]. Consumers will receive a high quality product, while manufacturers may have the ability to improve their process through appropriate manufacturing changes. Although FDA is encouraging the implementation of QbD for all pharmaceutical products, there are unique issues in the application of QbD to the development and manufacture of generic products. To use QbD to develop a product that is bioequivalent to a reference product, a generic sponsor must have an understanding of what attributes of the formulation and manufacturing process have the potential to change the bioavailability of a particular API, as summarized in Figure 1.5 [64,79,121].

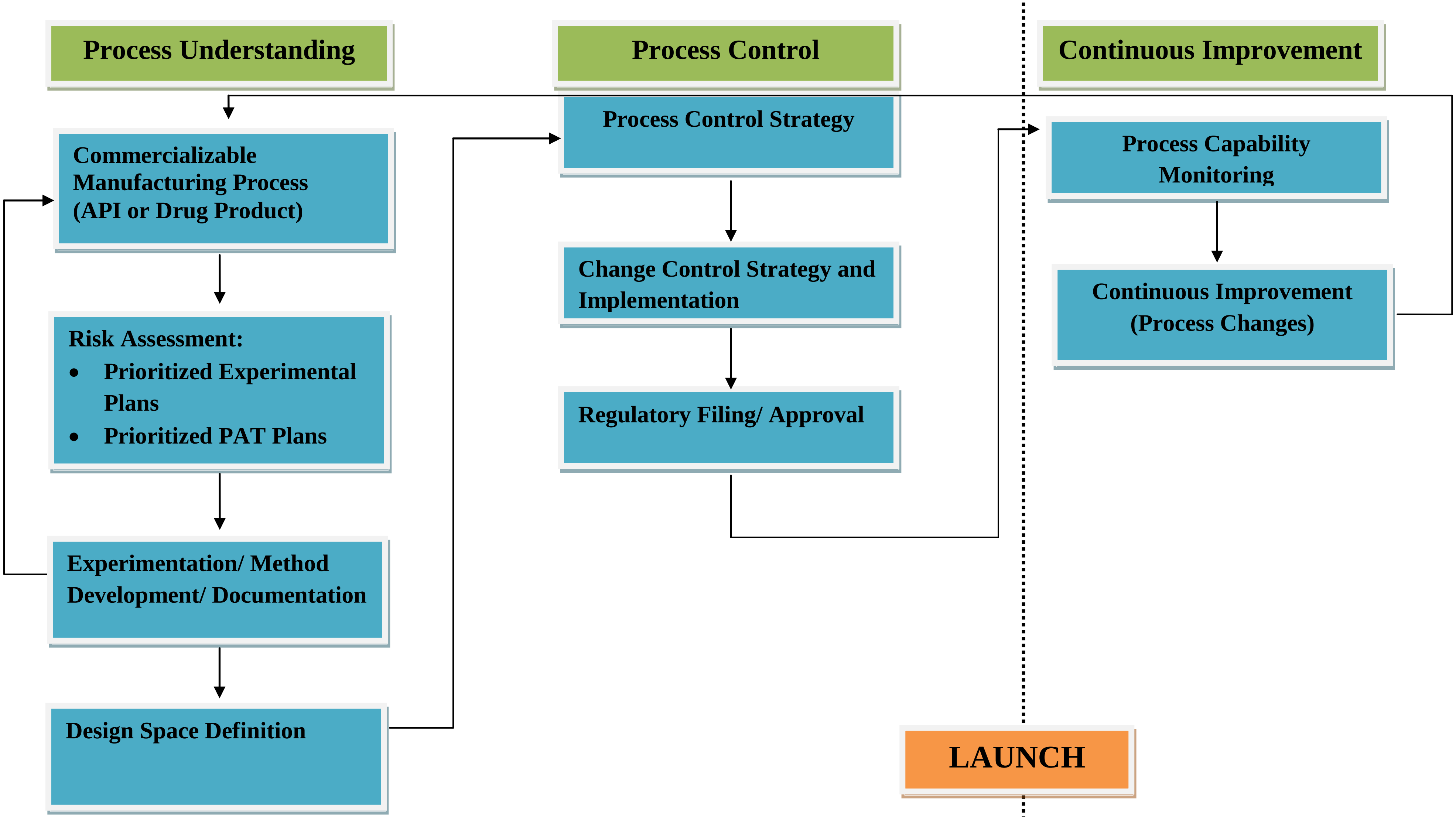


Figure 1.5 QbD enhancing knowledge of process understanding, process control and continuous improvement in a product lifecycle, redrawn and adapted from [122]

Current formulation development strategies for generic drug products are mainly based on trial and error, in-house databases and/or the experience of the formulation scientist. Within a QbD process, modeling and simulation using improved absorption models could help a generic sponsor to identify the formulation and manufacturing variables and to determine what drug release profile is required to ensure a product that exhibits bioequivalence to the reference product [29,118]. Consequently the challenge for a generic developer becomes the selection of excipients and the design of a formulation and release mechanism that will provide the intended *in vivo* release profile. A mechanistic understanding of how the physical properties of an API and the excipients may affect drug product performance will enable rational selection of excipients and reduce the number of experimental formulations that need to be manufactured [71,123]. As it is not feasible to test every trial formulation in an *in vivo* study, the development of better *in vitro*/*in vivo* Correlations (IVIVC) will provide better feedback within an iterative design process. When an *in vitro* dissolution or release test can be related to the *in vivo* dissolution of a product, a generic sponsor would have an efficient tool to evaluate different formulations and select an optimal formulation for use in a pivotal bioequivalence study. However, the correlation of dissolution testing with *in vivo* performance varies from product to product. FDA data generated from dissolution testing and pharmacokinetic studies may help external collaborators develop and test models capable of predicting the relationship between dissolution and bioavailability/bioequivalence [36,72,79,124].

1.2.5. Traditional versus QbD Approaches to Pharmaceutical Development

In pharmaceutical development for API and dosage forms, the traditional approach to product development usually involves limited formulation batches as a univariate approach is being used and therefore limited scale-up investigations were conducted. Following formulation, test batches are manufactured and product performance is assessed in terms of quality by undertaking quality testing and inspection and consequently the process is validated accordingly. The traditional techniques usually use in-process monitoring and the monitoring usually applies a combination of mathematical and knowledge-based models. In-process testing has played a significant role in monitoring and control of pharmaceutical processes. If in any in-process test, a failing result is observed or a product fails to meet predefined limits, the batch is scrapped and the root cause of the failure is identified and with luck, remedied. If necessary the process is modified and updated so that the in-process or end-process testing results will once again meet predefined limits [64].

In contrast, the QbD approach is more proactive and involves experiments using a multivariate approach with subsequent optimization of a formulation using several statistical approaches. During the design phase, process steps for which failure may result in an inability to meet quality targets are initially identified. As a first step toward QbD, process monitoring is established to provide an advance indication of potential failure. The complete implementation of QbD requires intimate process control of critical steps so as to ensure that product quality is maintained. QbD enables enhanced process understanding, and is a systematic and scientific approach to product development in order to implement better controls. The end goal is a more robust manufacturing process than those that typically result from traditional approaches to drug product development [100,106]. The differences between traditional and QbD approaches for the pharmaceutical development sector are summarized in Table 1.1.

Table 1.1 Comparison of traditional and QbD approaches to pharmaceutical development, adapted from [125]

	Traditional Approach	**QbD Approach**
Pharmaceutical Development	Univariate approach.	Multivariate approach.
Quality	Quality assured by testing and inspection; Post-manufacture sampling and quality testing. Process Validation.	Quality built into product and process by design, based on scientific understanding and management of variability. Process control focused on critical quality attributes. Continuous Quality Verification.
Analysis	Data-intensive submission.	Knowledge-rich submission- showing product knowledge and process understanding.
Systems	Systems designed to inhibit changes & minimise business risks. Discourage improvement & innovation.	Changes managed within company's quality system. Real-time batch release feasible. Higher reliance / trust / understanding on systems.
Strategy	Discourage changes.	Flexible process within design space, allowing continuous improvement.
Management	Focus on reproducibility- often avoiding or ignoring variation.	Focus on robustness- understanding and controlling variation.
Regulatory	Compliance focus. Changes require prior approval.	Regulatory scrutiny adjusted to level of Process Understanding. Continuous improvement allowed within Design Space.

1.2.6. Traditional versus QbD Considerations for Topical Formulation Development

Pharmaceutical development using the principles of QbD emphasizes a systematic approach, commencing with predefined objectives and application of scientific understanding and risk management approaches. Two key concepts have been introduced to further aid the implementation and understanding of QbD. The first concept is that of the “Design Space” defined in the ICH Q8 guideline [36] document as an established multidimensional combination and interaction of material attributes and/or process parameters that are demonstrated to provide an assurance of product quality. Understanding the design space for a pharmaceutical process generally involves the identification of critical attributes for the input materials, the process, and the final product as shown in Figure 1.6. A modified definition of the design space has been proposed for analytical methods, where the design space includes any combination of input variables to a method that has been demonstrated to provide an assurance of the quality of the data produced by that method. Another important concept of QbD is that of the development of a “control strategy”.

The purpose of the control strategy is to ensure the final quality of the product. This is obtained from an understanding of the process established after modeling the design space. The analytical adaptation of the control strategy has been proposed from an analytical perspective and is defined as the control of input factors of a method that ensures that the method meets system suitability criteria and wider performance-related goals [100].

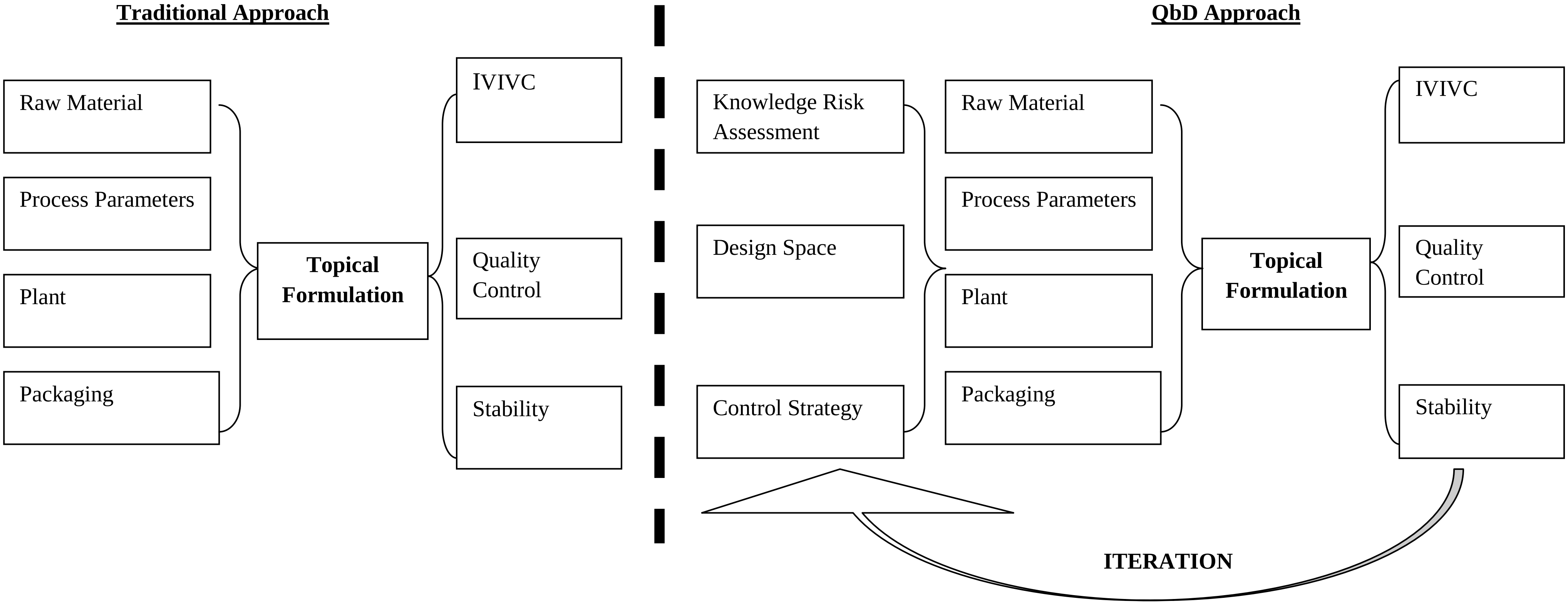


Figure 1.6 Traditional and QbD considerations for topical formulation development

1.3. QbD IMPLEMENTATION IN TOPICAL FORMULATION DEVELOPMENT

1.3.1. Target Product Profile (TPP)

FDA [126] defines the TPP as the statement of the overall intent of the drug development program and it provides information about the product at a particular time in the development process. Usually the TPP is organized according to the key sections in drug labeling and it links drug development activities to specific concepts intended for inclusion in the labeling. ICH Q8 [36] states that pharmaceutical development should include "...identification of those attributes that are critical to the quality of the drug product, taking into consideration intended usage and route of administration" and requires the consideration of the intended use and route of administration through the TPP [127]. The TPP is a patient- and labeling-centered concept that is the "user interface" of the drug product. Consequently a generic version and reference product would be expected to have the same TPP. However a generic product may use different formulation and/or design to implement the TPP. The characteristics and performance tests for a drug product would depend on a particular implementation and may differ between a generic and reference product. For a new drug, changes to the TPP may require new or additional safety or efficacy data to be submitted, but changes to the product characteristics or performance that are a result of reformulation may not [127].

In this study, the design, development, manufacture and production of a safe, effective, stable generic formulation of a 0.05% w/w clobetasol 17-propionate (CP) cream was undertaken in order to produce a product that poses a minimal risk of failure for the treatment of severe skin disorders such as seborrhoeic dermatitis, extreme photodermatitis and/or severe psoriasis in HIV/AIDS patients in RSA. The target pharmaceutical profile for CP is a safe efficacious convenient dosage form, preferably a cream that will enhance the percutaneous absorption of CP into the skin. The manufacturing process for the cream formulation had to be robust and reproducible in order to produce a product that meets the appropriate product CQA such as identity, assay, appearance, chemical and microbiological purity, stability, *in vitro* and *in vivo* analysis in addition content uniformity. The drug product had to be packaged into a container and closure system that would provide adequate protection from moisture vapor, through distribution and use in addition to ensuring patient convenience. However these are aspects of the TPP that constrain or determine the actions of formulation and process development scientists including the common aspects of drug product quality that are implicitly in the TPP. For example, if a label states that a tablet contains 100 mg of an API, this is a claim relating to assay and content uniformity. It is the role of the pharmaceutical scientist to translate a qualitative TPP into what is defined as the "Quality Target Product Profile" (QTPP) for further use in a QbD process [127].

1.3.2. Quality Target Product Profile (QTTP)

The ICH Q8 (R2) guideline [36] defines the QTPP as the basis of design for the development of a product and it is a quantitative surrogate for aspects of clinical safety and efficacy that can be used to design and optimize the formulation and manufacturing process. The International Society of Pharmaceutical Engineers (ISPE) Product Quality Lifecycle Implementation (PQLI) guideline calls this the "Pharmaceutical Target Product Profile" (PTPP) [71]. It should include quantitative targets for impurities, release profiles (dissolution) and other product specific performance requirements including stability. Product-specific examples include for example re-suspendability for an oral suspension, adhesion for a transdermal system, or viscosity for a topical cream formulation. For multisource or generic products this would include bioequivalence to a RLD as an integral part of a QTPP [127]. The QTPP is not a specification as it includes bioequivalence and/or stability testing that are not performed for batch to batch release and the QTPP should also only include patient relevant product performance characteristics. The physicochemical and biological properties of a drug molecule are crucial for ultimate product quality and dosage form performance and must therefore be addressed during the development process [36,127]. An example of the QTPP for a generic CP, 0.05% w/w cream is shown in Table 1.2.

Table 1.2 QTTP for a 0.05% w/w CP generic cream

QTPP Element	Target	Criticality
Dosage form	Cream	Ensure correct release of API at the right time, efficacy and ease of administration
Route of administration	Topical	Patient compliance to therapy
Dosage strength	0.05% w/w	Efficacy
Appearance	Cream conforming to smoothness	Patient acceptability and compliance
Proposed indications	Severe photodermatitis, extreme eczema and psoriasis for HIV/AIDS patients	Ensure therapeutic efficacy
Drug product quality attributes	Physical attributes: Rheological behavior Identification Assay Homogeneity and tube uniformity Preservative content	Pharmaceutical equivalence requirement: Meeting the same compendial or other applicable (quality) standards i.e., identity, assay, purity, and quality
Container closure system	Identical primary packaging to RLD and for patient acceptability	Identical primary packaging to original formulation and for patient acceptability

In Chapter 5, *vide infra*, several aspects of the physicochemical and biological properties of CP that may affect the manufacturability and assessment of a generic 0.05% w/w CP cream formulation are discussed further. In addition, since the 0.05% w/w CP creams were formulated and manufactured, a physical evaluation, *in vitro* release and stability studies on a commercially available innovator cream, Dermovate® (Sekpharma® (PTY) Ltd, Cape Town, RSA) were performed in detail. These data were used to set the bioequivalence criteria for the test formulation(s) that had similar QTPPs to Dermovate® cream in order to produce a safe, effective and high quality generic product.

1.3.3. Identification of Critical Quality Attributes (CQA)

The ICH Q8 (R1) guideline [35] defines CQA as physical, chemical, biological and/or microbiological properties or characteristics of a product that should be within an appropriate limit, range or distribution to ensure that a product of the desired quality is achieved. The CQA are generally associated with an API, excipients, intermediates and drug product in addition to describing elements of the QTTP. For example dissolution and mechanistic factors such as particle size and hardness that may impact product performance. Thus CQA are used to describe aspects of product performance and are determinants of product performance. The CQA of drug products include properties that impart the desired quality, safety and efficacy. The CQA of topical dosage forms are typically those aspects that affect product purity, potency, stability and API release.

The selection of CQA from all potential quality attributes is based on the potential impact of that attribute on patient safety and product efficacy. The product specifications and regulatory guidance for topical formulation development were a suitable starting point for the determination of a list of potential CQA for this project. For a topical formulation, a formulation scientist should specifically consider essential CQA for example assay, content uniformity, impurities, pH, water content, microbial limits and antimicrobial preservative content. In addition, attributes such as viscosity, spreadability, tube (content) uniformity and particle size may be considered. However, the primary indicator of generic product performance in comparison to an innovator product is the *in vitro* assessment of API release, as summarized in Table 1.3. In Chapter 5, *vide infra*, most of the CQA of the generic CP creams formulated and manufactured in this study were compared to the data derived from Dermovate® cream. The *in vitro* release profiles of the test formulations were evaluated over a period of 72 hours and were compared to the *in vitro* release profile of CP from the commercially available innovator formulation. This profile was used as the reference formulation profile for all optimization procedures conducted in all formulation development studies.

Table 1.3 CQA for a 0.05% w/w CP Cream

CQA	Target	Criticality
Identification	Positive for API	Ensure clinical effectiveness
Assay	97% – 102%	Ensure clinical effectiveness
Impurities	Any individual unknown: NMT 1.0% Total impurities: NMT 2.5%	Ensure safety
Homogeneity and Tube Uniformity	Top, middle and bottom of three containers, nine assay values should be within 90.0% to 110.0% label claim and RSD is not more than 5%	Ensure clinical effectiveness
Physical Attributes Rheological Behavior	Match RLD	Needed for clinical effectiveness and patient acceptability
***In Vitro* Release Test**	Match RLD	*In vitro* surrogate used to guide bioequivalence
pH	4.5 - 7.0	Ensure skin non-irritancy
Microbial Limits	Meet USP 34/NF 29 [128]	Ensure safety

1.3.4. Linking Material Attributes and Process Parameters to CQA - Risk Assessment

Risk assessment is a valuable science-based process in quality risk management and can aid in the identification of material attributes and process parameters that have an effect on product CQA. While risk assessment is typically performed in the early stages of pharmaceutical development, it can be helpful to repeat risk assessment analyses as more information and knowledge about the product or materials become available [35]. Risk assessment tools can also be used to identify and rank parameters such as operational, equipment or input material characteristics that have the potential to impact product quality-based on prior knowledge and/or experimental data. The initial list of potential parameters may be quite extensive, but is likely to be reduced as process understanding increases. The list can be refined further through experimentation to determine the significance of individual variables and potential interactions on product quality. Following identification of the significant parameters, they can be further studied through a combination of design of experiments, mathematical modeling, or studies that lead to a mechanistic understanding in order to achieve a higher level of process understanding [35]. The principles of risk assessment and management are described in Chapter 5. These principles were used to evaluate the potential impact of formulation and manufacturing aspects on the CQA of these formulations.

1.3.5. Formulation Design Space

From an early draft of the ICH Q8 guideline, “Design Space” was defined as the established range of process parameters that have been demonstrated to provide an assurance of quality. The change emphasizes the multidimensional interactions of input variables and closely binds the establishment of a design space to the conduct of Design of Experiments (DOE) studies, including interactions among input variables. A design space may be constructed for a single unit operation, multiple unit operations or for the entire manufacturing process [120,129]. Submission of a design space to FDA is a pathway to deriving an ability to operate within that design space without further regulatory approval. A design space is a way to represent all aspects of process understanding that have been established. The benefits of establishing a design space are clear, however one challenge to the effective development and use of a design space is the cost of establishing that space. In a typical design space approach, a sponsor must identify unclassified parameters and then perform a DOE on some of these unclassified parameters whilst fixing other of those parameters. Thus the end point is a regulatory scenario in which there is flexibility or space for selected parameters but not others. This operating parameter-based design space is limited to the equipment used to develop the design space and may well change during scale-up or equipment-change procedures.

Understanding the formulation design space in terms of material attributes allows for scale-up and equipment changes to be linked to the data generated through previous experimentation. The scalability of the design space can be evaluated during the transfer from a laboratory perspective to exhibit batch manufacture. In these studies, DOE was used as a tool during formulation development to screen for excipients and process parameters that would provide optimum formulation performance and stability with adequate robustness in order to characterize the design space for the formulation. Different statistical approaches *viz.*, Taguchi, Plackett-Burman Design, 2-Level Factorial and Central Composite Design were used to determine the impact of selected formulation excipients and/or process variables on the CQA of CP cream formulations and to establish whether interactions between the input variables existed. These studies are described in detail in Chapters 6 and 7, *vide infra*. Thus the formulation design space can be developed to define the limits for excipients composition and other critical characteristics established during formulation development that would ensure and maintain product quality, stability and performance.

1.3.6. Control Strategy

The ICH Q8 (R1) guideline [35] states that a “Control Strategy” is designed to consistently ensure product quality on an ongoing basis. The elements of a control strategy describe and justify how in-process testing and control of input materials *viz.*, API and excipients, container closure system, intermediates and end products may contribute to the quality of the final product. These controls are based on product, formulation and process understanding and include, at the very minimum, control of critical parameters and attributes. Critical sources of variability that can lead to formulation and product failures must be identified, appropriately understood, and managed or controlled. Understanding the source(s) of variability and their impact on processes, intermediate products and finished product quality can minimize or eliminate the need for some end product testing [127]. The quality of raw materials, including API and excipients, is usually monitored by testing. If these materials meet specifications or other standards such as those listed in the United States Pharmacopeia (USP) for drug substance or excipients, they can be used for manufacture of pharmaceutical products [128]. As the drug substance specification alone may not be sufficient to ensure adequate quality, the manufacturing process should also be tightly controlled [127]. The finished drug products are tested for quality by establishing whether they meet specifications. In addition, manufacturers are usually expected to conduct extensive in-process testing such as assay, content uniformity and stability studies for topical formulations, as shown in Figure 1.7.

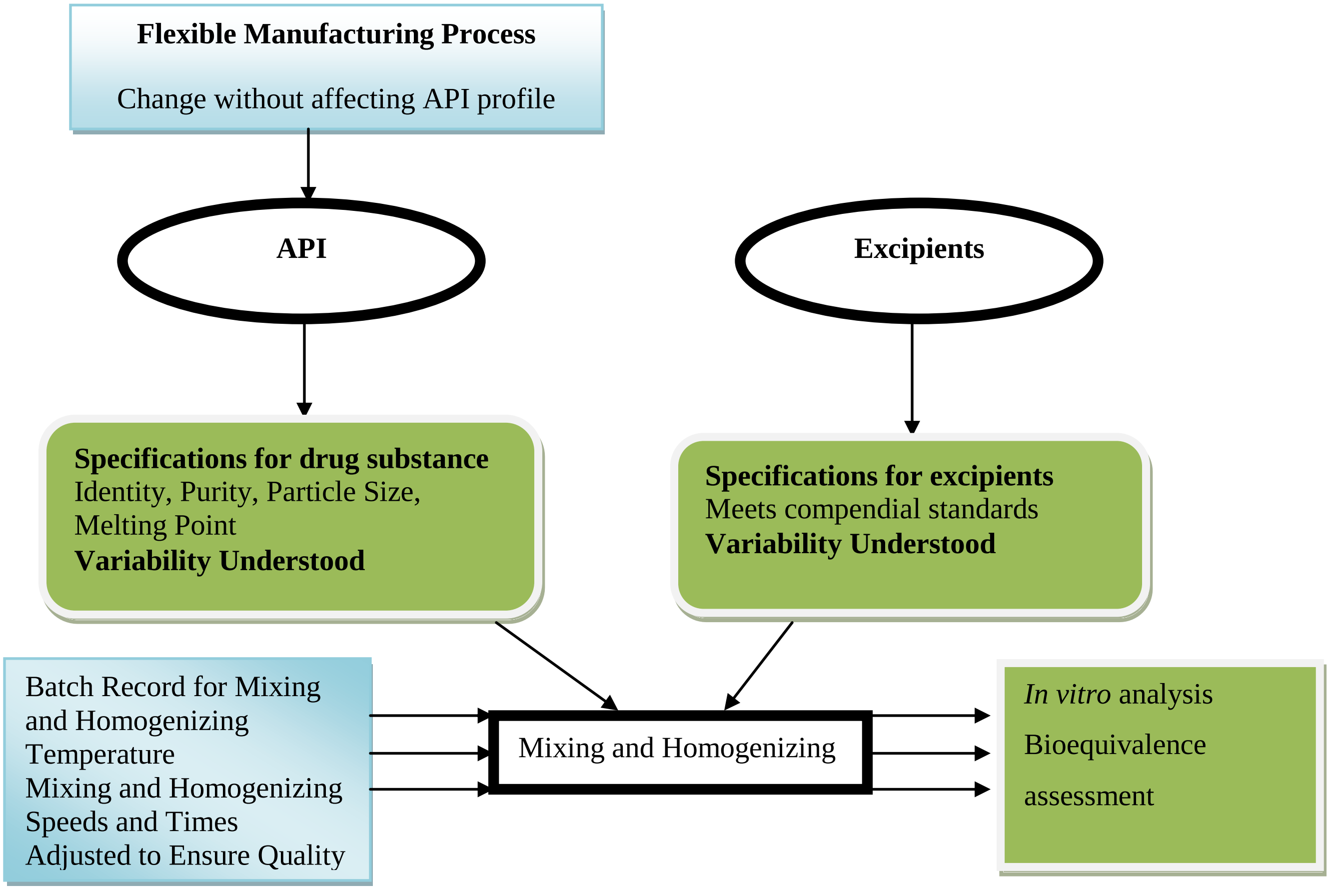


Figure 1.7 Example of a control strategy for a QbD process during development of topical formulations

Pharmaceutical quality can be assured only by understanding and controlling formulation composition and manufacturing process variables to establish the quality of the finished product. Consequently end product testing is used to only confirm the quality of a finished product [127].

1.3.7. Product Lifecycle Management

Throughout the lifecycle of a product, companies have the opportunity to evaluate innovative approaches to improve product quality and to facilitate continued process and/or product improvement [36]. The ICH Q10 guideline [38] is a model for a pharmaceutical quality system that can be implemented throughout the stages of the lifecycle of a product. It describes at least four essential elements that must be reviewed and acted on so as to promote a lifecycle approach to product quality. These elements include implementation of **i)** a process performance and product quality monitoring system, **ii)** Correction Action and Prevention Action (CAPA) system, **iii)** change management system and **iv)** a management review of process performance and product quality [38].

Following the launch of a new drug product, efforts should be made to extend and modify the lifecycle of the product. These may include the use of a continuing process performance and product quality monitoring system in addition to assessing combination therapy, therapeutic indications, target patient populations, dosing regimens and modified formulation approaches or technologies [130-132]. Pharmaceutical companies have recognized that the development of new drug delivery technologies is a powerful and strategic marketing tool to differentiate products and extend the lifecycle, thereby overcoming some market place challenges. Consequently they are pursuing stronger alliances with drug delivery technology companies, including acquisition, to enable them to develop superior products and remain competitive. The application of drug delivery is therefore a valuable, cost-effective lifecycle management resource. By introducing drugs with new and innovative therapeutic benefits, drug delivery systems can be used to extend the lifecycle of a profitable product, thereby creating competitive and financial advantages for a company whilst providing patients with improved medications and therapeutic options [133,134]. Dosage forms can also be improved through the appropriate selection of excipients. To develop a Cremophor EL-free paclitaxel formulation, a full factorial combination of many compounds at three different concentrations was screened using an automated liquid dispenser. It revealed that of the 9880 combinations that were initially tested, only 19 were identified as hit combinations. These studies demonstrate the power of using high-throughput combinatorial approaches for the development of alternative formulations and suggest that this approach can improve the speed and efficiency of drug product and formulation design [135].

1.4. CONCLUSIONS

Currently the demand for access to generic drugs is increasing on a daily basis as through the use of generic products, consumers save between $ 8 and $ 10 billion annually. The FDA has a backlog of more than 800 applications to bring new generic products to market. This is reported to be an all-time high [72].

In an attempt to allow more generic products onto the market in short time, whilst assuring the quality of generics throughout the product lifecycle, it has become evident that the implementation of a concept such as QbD in conjunction with PAT may be a suitable approach for generic drug product development. The QbD concept ensures better design of products with fewer problems in manufacturing, thereby reducing the number of manufacturing supplements required for post-marketing changes, reduces product approval time, reduces the total cost of a product, decreases the rate of failure of batches, decreases end product testing costs and provides a potentially better overall business model. The implementation of QbD will be valuable for the industry and regulatory authorities to achieve the ultimate goal of the modernization of product quality. In this study, a QbD approach was implemented for the design, development and manufacture of safe, effective, stable generic topical formulations containing 0.05% w/w CP. Such formulations would pose a minimal risk of failure for the treatment of severe skin disorders such as seborrhoeic dermatitis, extreme photodermatitis and/or severe psoriasis in HIV/AIDS patients in Southern Africa. Ultimately the establishment of a design space for the successful development of a CP cream formulation was developed.

CHAPTER TWO

A REVIEW OF TOPICAL CORTICOSTEROIDS (TC)

2.1. INTRODUCTION

2.1.1. General Review of Corticosteroids

Corticosteroids are hormones derived from cholesterol [136,137]. They are involved in a wide range of physiological processes including stress, regulation of inflammation, carbohydrate metabolism, protein catabolism, blood electrolyte levels and behavior [138]. Corticosteroids are used to treat neurodegenerative disorders on the assumption that suppression of post-injury inflammation responses at the site of injury will benefit tissue survival [138]. Corticosteroids have dominated the class of anti-inflammatory agents for the past 50 years and are the most commonly used anti-inflammatory drugs [139,140]. Due to the remarkable anti-inflammatory and immuno-regulatory effects of corticosteroids, they have been used as the initial step in the management of different disease and sometimes are the only drugs of choice in daily medical practice [137]. All therapeutic corticosteroids have a 21-carbon skeleton similar to hydrocortisone (cortisol) depicted in Figure 2.1 [141,142].

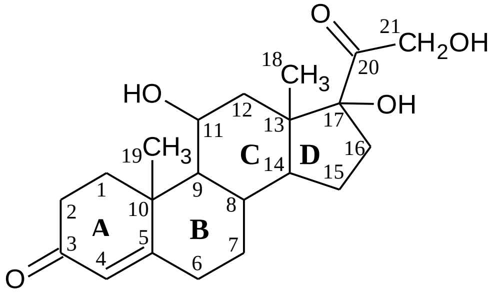


Figure 2.1 Structure of the steroid cortisol, showing the 21-carbon skeleton [141,142]

Modification of the skeleton can alter the degree of anti-inflammatory, metabolic effects, the duration of activity and protein-binding affinity of the resultant compound. This has led to the synthesis of a number of corticosteroids with increased anti-inflammatory potency and reduced mineralocorticoid effects. Therapeutic corticosteroids are typically classified on their relative glucocorticoid and mineralocorticoid potency as well as the duration of biologic effects. In general, those compounds with the most potent glucocorticoid activity are also potent suppressors of the Hypothalamic-Pituitary-Adrenal Axis (HPAA), which can result in clinically significant adverse effects [143,144].

2.2. CLASSIFICATION

2.2.1. Drug Class Review

Not long after Sulzberger and Witten [140] demonstrated the effectiveness of hydrocortisone in the 1950s, new and more effective fluorinated hydrocortisone analogues were made available in the 1960s. In the 1970s and 1980s, super-potent steroids were developed. This meant that there was a wide range of compounds of varying potency available for clinical use. In 1984, Cornell and Stoughton [145] proposed a potency rating of topically applied corticosteroids based primarily on the vasoconstrictor assay or skin-blanching effect of the corticosteroid molecules [146-148]. In 1989, an original classification of corticosteroids was proposed by Coopman *et al.* [149] that placed the molecules into four groups *viz.*, A, B, C and D, according to their molecular/chemical structure. Allergic reactions to one member of a class typically indicate an intolerance to all members of that class. This was known as the "Coopman classification" [137,150,151] and is summarized in Table 2.1.

Table 2.1 Classification of corticosteroids according to Coopman *et al.* [149]

Group	Characteristic of Group	Typical Members
A	No substitution on the D-ring except a short-chain ester or a thioester on C21	Cloprednol Fluorometholone Fluprednisolone acetate Hydrocortisone Hydrocortisone acetate Medrysone Methylprednisolone acetate Methylprednisolone hemisuccinate Prednisolone Prednisolone sodium metasulphobenzoate Prednisolone succinate Prednisolone pivalate Prednisolone caproate Prednisone Tixocortol pivalate
B	C16, C17 *cis* Ketal or diol structure (Possibly a side chain on C21)	Amcinonide Budesonide Desonide Fluchloronide Flumoxonide Flunisolide Fluocinolone acetonide Fluocinonide Halcinonide Triamcinolone Triamcinolone diacetate Triamcinolone hexacetonide Triamcinolone benetonide Triamcinolone acetonide

C	C16 methyl substitution on the D-ring Halogen substitution No side chain on C17 Possibly a side chain on C21	Betamethasone Betamethasone sodium phosphate Desoxymethasone Dexamethasone Dexamethasone acetate Dexamethasone sodium phosphate Diflucortolone valerate Flumethasone pivalate Fluocortin butyl Fluocortolone Fluocortolone caprylate Fluocortolone pivalate Fluprednidene acetate Halometasone Meprednisone
D1 **Halogenated (Less Labile)**	Methyl substitution on C16 Halogen substitution Side chain ester on C17 Possibly a side chain on C21	Alclometasone dipropionate Beclomethasone dipropionate Betamethasone dipropionate Betamethasone 17-valerate Clobetasol 17-propionate Clobetasone butyrate Diflorasone diacetate Fluticasone propionate Mometasone fuorate
D2 **Labile Prodrug Esters**	No methyl substitution on C16 No halogen substitution Side chain ester on C17 Possibly a side chain on C21	Difluprednate A Hydrocortisone 17-butyrate Budesonide (S isomer) Hydrocortisone aceponate Methylprednisolone aceponate Prednicarbate

In 1995 Lepoittevin *et al.* [152] performed conformational analysis of observed cross-reactions, which supported this classification and the central role of constituents of the D-ring, as shown in Figure 2.2.

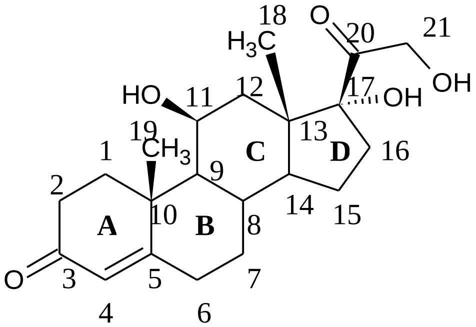
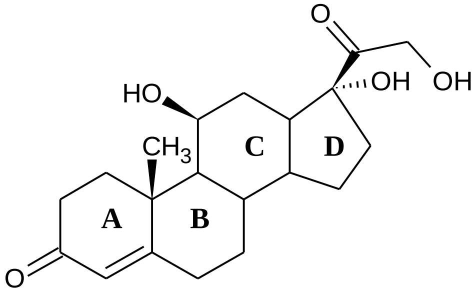
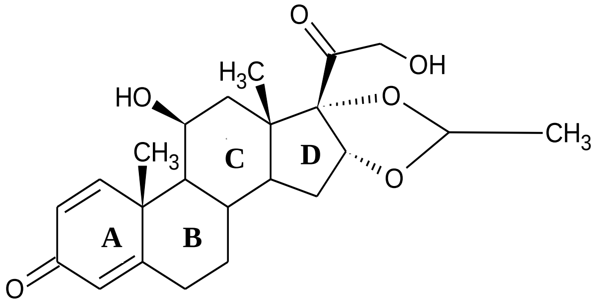
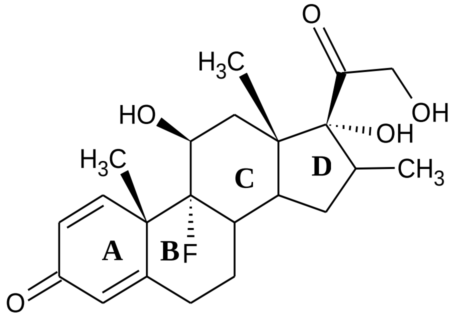


Figure 2.2 Chemical structure of hydrocortisone showing the conventional numbering of carbon atoms [151]

Further clinical evidence led Matura and Goossens [153,154] to divide Group D into two subgroups *viz.*, stable D1 and labile D2 esters, as listed in Table 2.1. Beside the recognition site of the D-ring influenced by C16, C17 substitution, Wilkinson *et al.* [155] considered the A-ring to be a secondary immune recognition site and halogenation of the corticosteroid structure at positions C6 and/or C9 was of utmost importance in respect of the cross-sensitivity patterns observed with corticosteroids. On the basis of patch test results and molecular modeling of corticosteroids, this classification was further simplified into three groups *viz.*, Group 1, non-methylated and most often non-halogenated molecules (Group A, Group D2 and budesonide), which produce most of the allergic reactions; Group 2, halogenated molecules with a C16/C17 *cis* ketal/diol structure (acetonide Group B) and Group 3, halogenated and C16-methylated molecules (Group C and Group D1) only rarely exhibiting an allergic response. A simplified classification is shown in Table 2.2. More recently the United States Pharmacopeia (USP) has created a classification of potency ranking for these molecules into low, medium, high and very high based, on the consensus of the USP Dermatology Advisory Panel [140,156].

Table 2.2 Simplified corticosteroid classification system [154]

Group	**1**	**2**	**3**
Characteristics	No C16-methyl substitution No halogenation	C16/C17 cis ketal diol structure Halogenation	C16-methyl substitution Halogenation
Indicative Structure			
Typical members	Budesonide	Amcinonide	Alclomethasone dipropionate
	Cloprednol	Budesonide (R-isomer)	Beclomethasone dipropionate
	Cortisone acetate	Desonide	Betamethasone
	Dichlorisone acetate	Fluchloronide	Betamethasone 17-valerate
	Difluprednate	Flumoxonide	Betamethasone dipropionate
	Fludrocortisone acetate	Flunisolide	Betamethasone sodium phosphate
	Fluorometholone	Fluocinolone acetonide	Clobetasol propionate
	Fluprednisolone acetate	Fluocinonide	Clobetasone butyrate
	Hydrocortisone	Halcinonide	Cortivazol
	Hydrocortisone aceponate	Triamcinolone acetonide	Desoxymethasone
	Hydrocortisone acetate	Triamcinolone benetonide	Dexamethasone
	Hydrocortisone-17-butyrate	Triamcinolone diacetate	Dexamethasone acetate
	Hydrocortisone-21-butyrate	Triamcinolone hexacetonide	Dexamethasone sodium phosphate
	Hydrocortisone hemisuccinate		Diflucortolone valerate
	Isofluprednone acetate		Diflorasone diacetate
	Mazipredone		Flumethasone pivalate
	Medrysone		Fluocortin butyl
	Methylprednisolone aceponate		Fluocortolone
	Methylprednisolone acetate		Fluocortolone caprylate

Methylprednisolone hemisuccinate Prednicarbate Prednisolone Prednisolone caproate Prednisolone pivalate Prednisolone sodium metasulphobenzoate Prednisolone succinate Prednisone Tixocortol pivalate	Fluocortolone pivalate Fluprednidene acetate Halomethasone Meprednisone Fluticasone propionate Mometasone furoate

2.2.2. Routes of Administration

Corticosteroids are used to treat inflammation of the body [157,158] and are often used as part of the treatment for a number of diseases such as severe allergy or skin conditions, breathing problems, certain cancers, blood disorders, ophthalmological conditions, arthritis, digestive system issues and hormone replacement [159-164]. Corticosteroids have been made available as **i)** oral dosage forms such as tablets including disintegrating technologies, capsules including delayed and extended release technologies, syrups, suspensions, elixirs and solutions, **ii)** systemic dosage forms such as small or large volume parenterals, **iii)** topical steroids including creams, ointments, gels and pastes to treat conditions of the skin, eyes and mucous membranes and **iv)** inhalable formulations such as aerosol or nebulizer products to treat the sinuses, nasal mucosae and lungs [159,165-167].

2.3. TOPICAL CORTICOSTEROIDS (TC)

2.3.1. Overview of TC

Humans have used salves, lotions and/or potions to treat their skin since ancient times and the concept of delivering drugs through the skin is a practice which dates as far back as the 16th century [168]. In the early 1950s Topical Corticosteroids (TC) were introduced into clinical practice and the introduction of topical hydrocortisone represented an advance over previously available therapies. However it was the introduction of the first halogenated glucocorticoid, triamcinolone acetonide, which started a revolution, culminating in the appearance of the highly potent agents available today. Enthusiasm for use of these highly effective agents was at its peak during the 1960s and 1970s, and inevitably the potent corticosteroids were often used inappropriately and indiscriminately. Adverse effects became apparent and the subsequent backlash of opinion against TC has created confusion and prejudice against all steroid-containing preparations. In its extreme form this is known as “steroid phobia” and is still of considerable concern today [169]. TC have made a dramatic contribution to the treatment of dermatologic conditions due to a wide range of non-infectious diseases. Since their introduction almost 50 years ago, TC have been used to treat inflammatory skin diseases due to their anti-inflammatory, immuno-suppressive and anti-proliferative properties [150,170]. The renewed interest in the development of topical formulations intended for local and/or regional activity stems from the potential use of topical formulations to deliver molecules intended for systemic activity. TC are among the most commonly prescribed medications in an ambulatory care setting [170,171] and are the cornerstones of therapy for a wide variety of conditions such as atopic dermatitis, contact dermatitis, psoriasis and seborrheic dermatitis [172].

Several corticosteroid molecules are available in topical formulations, either alone or in combination with other compounds such as antibiotics *viz.*, Betnovate N® (betamethasone and neomycin) and Fucidin H® (hydrocortisone acetate and fusidic acid) or antifungal agents *viz.*, Daktacort® (hydrocortisone and miconazole) and Travocort® (diflucortolone valerate and isoconazole nitrate). TC can induce a great variety of well-known adverse events, many of which are associated with prolonged use or local immuno-suppression. Contact allergy to TC is another possible untoward side effect, which was regarded as a paradoxical phenomenon. Since the first descriptions of contact sensitization to TC in 1959 [1, 2] other reports have been published, but the real extent of the problem was recognized only in the late 1980s [3]. Hypersensitivity to TC is of dermatological and allergological concern, despite the fact that it is thought to affect only a minority of patients with skin disease(s) treated with these compounds. However, in view of the wide use of TC, the incidence of TC sensitization is likely to be underestimated, when considering the difficulty in diagnosing these complaints owing to the peculiar characteristics of steroid allergens and the possible inadequacy of current diagnostic methods [4, 5].

2.3.2. TC Dosage Forms available in RSA

Topical corticosteroids are readily available with and/or without prescription in many countries [173]. Commercially available TC intended for local, regional or systemic activity registered in the Republic of South Africa (RSA) are listed in Table 2.3 [173].

Table 2.3 Commercially available preparations of TC in RSA [173]

Trade Name	Active Ingredient	Manufacturer	Strength	Dosage Form
Biocort®	Hydrocortisone acetate	Thebe Pharma®	1.0%	Cream
Dilucort®	Hydrocortisone acetate	Aspen Pharmacare®	0.5%	Cream, Ointment
Mylocort®	Hydrocortisone acetate	Aspen Pharmacare®	1.0%	Cream, Ointment
Procutan®	Hydrocortisone	Schering-Plough®	1.0%	Cream
Skincalm®	Hydrocortisone	Aspen Pharmacare®	0.5%	Cream
Stopitch®	Hydrocortisone acetate	Restan®	1.0%	Cream
Betnovate half-strength®	Betamethasone valerate	Sekpharma®	0.05%	Cream
Eumovate®	Clobetasone butyrate	Sekpharma®	0.05%	Cream
Beclate®	Beclometasone dipropionate	Cipla Medpro®	0.025%	Cream
Betnovate®	Betamethasone valerate	Sekpharma®	0.1%	Cream, Ointment, Lotion
Diprosone®	Betamethasone dipropionate	Schering-Plough®	0.05%	Cream, Ointment
Adco-Betamethasone®	Betamethasone valerate	Adco Generics®	0.1%	Cream
Lenovate®	Betamethasone valerate	Aspen Pharmacare®	0.1%	Cream, Ointment
Persivate®	Betamethasone valerate	Aspen Pharmacare®	0.1%	Cream, Ointment
Repivate®	Betamethasone valerate	Cipla Medpro®	0.1%	Cream
Nerisone®	Diflucortolone valerate	Bayer Schering®	0.1%	Cream, Fatty ointment
Synalar®	Fluocinolone acetonide	Glenmark®	0.025%	Cream, Ointment, Gel
Cortoderm®	Fluocinolone acetonide	Aspen Pharmacare®	0.025%	Cream, Ointment
Cutivate®	Fluticasone propionate	GlaxoSmithKline®	0.05%,	Cream (0.05%), Ointment

			0.005%	(0.005%)
Locoid®	Hydrocortisone butyrate	Astellas®	0.1%	Cream, Ointment, Lotion, Topical emulsion
Advantan®	Methylprednisolone aceponate	Bayer Schering®	0.1%	Cream, Ointment, Fatty ointment, Milk
Elocon®	Mometasone furoate	Schering-Plough®	0.1%	Cream, Ointment, Lotion
Aspen Mometasone®	Mometasone furoate	Aspen Pharmacare®	0.1%	Cream
Dermovate®	Clobetasol propionate	Sekpharma®	0.05%	Cream, Ointment
Dovate®	Clobetasol propionate	Aspen Pharmacare ®	0.05%	Cream, Ointment
Xenovate®	Clobetasol propionate	Aspen Pharmacare ®	0.05%	Cream, Ointment
Nerisone Forte®	Diflucortolone valerate	Bayer Schering®	0.3%	Ointment

2.3.3. Classification of TC

The preferred approach to determining topical steroid potency is through vasoconstrictor or skin blanching assays. This assay classifies steroids based on the extent to which the agent causes cutaneous vasoconstriction, or a “blanching effect” in healthy persons [145,146,148,174,175]. This is a useful but imperfect method for predicting the clinical effectiveness of steroids. The anti-inflammatory potency of some steroids may vary between patients, depending on the frequency of administration, duration of treatment and site of application. A ranking system that compares clinical outcomes or an effectiveness-to-safety ratio may be of greater benefit, but does not currently exist [176].

TC have been ranked into four groups in terms of potency. Hydrocortisone and clobetasol are examples of low- and ultra-high-potency TC [177,178]. TC are the weakest of the steroids and Class IV compounds are the most potent as listed in the South African Medicines Formulary [173,174,179]. The efficacy is therefore better with the Class IV ultra-high-potency preparations which should only be used for limited periods of time (2-3 weeks). Low-potency TC are known to be the safest agents for long-term use. More potent agents are required to treat severe diseases and areas of the body where the skin is thicker such as the palms of the hands and soles of the feet. High- and ultra-high-potency TC should not be used to treat facial conditions or those of the groin, axilla, or under occlusion, except in rare situations and only for a short duration. These groups may vary depending on formulation and concentration and should be considered approximate [176,178]. In general, ointments are more potent than creams or lotions. Potency is also increased when TC are used under occlusive dressings or in the intertriginous areas [176]. A list of TC and available preparations listed by group, formulation and generic availability in RSA is given in Table 2.4 [173]. Brand name products tend to be more expensive and may impact patient adherence or compliance. The cost of therapy should be considered when selecting TC agents and physicians should be aware that generic products cost less and can reduce the cost of therapy for patients [176,180].

Table 2.4 Potency ratings of TC [173]

Potency (Group)	**Active Ingredient**	**Brand**	**Generic**	**Strength**	**Dosage Forms**
Ultra-High IV	Clobetasol 17-propionate	Dermovate®	Dovate® Xenovate®	0.05%	Cream, Ointment
	Diflucortolone valerate	Nerisone Forte®	-	0.3%	Ointment
High III	Mometasone furoate	Elocon®	Aspen Momentasone®	0.1%	Cream, Ointment, Lotion
	Methylprednisolone aceponate	Advantan®	-	0.1%	Cream, Ointment, Milk
	Fluocinolone acetonide	Synalar®	Cortoderm®	0.025%	Cream, Ointment, Gel
	Diflucortolone valerate	Nerisone®	-	0.1%	Cream, Fatty ointment
	Betamethasone valerate	Betnovate®	Adco-betamethasone® Lenovate® Persivate® Repivate®	0.1%	Cream, Ointment, Lotion
Medium II	Clobetasone butyrate	Eumovate®	-	0.05%	Cream, Ointment
	Betamethasone valerate	Betnovate® half-strength	-	0.05%	Cream
Low/Weak I	Hydrocortisone acetate	Biocort®	Mylocort® Stopitch®	1%	Cream

2.3.4. Clinical Indications

An accurate diagnosis is essential when selecting a specific TC to treat a diagnosed condition. A skin scraping and potassium hydroxide test can clarify whether a corticosteroid or an antifungal is an appropriate choice, since concomitant use of steroids can exacerbate fungal infections [181]. TC are primarily effective in treating conditions characterized by hyperproliferation, inflammation and immunologic involvement. They are also widely used in the treatment of vesiculo-erosive diseases of the oral mucosa to reduce pain and inflammation. They can therefore provide symptomatic relief for burning and pruritic lesions [174,176].

Many skin conditions are treated with topical steroids, but evidence of effectiveness has been established for only a small number of conditions [182]. For example, ultra-high and high-potency TC alone or in combination with other topical therapy are the mainstay of treatment for psoriasis [183]. The high-potency TC are also reserved for treating alopecia areata, resistant atopic dermatitis, discoid lupus, hyperkeratotic eczema, lichen planus, lichen sclerosus of the skin, lichen simplex chronicus, nummular eczema, severe poison ivy, psoriasis and severe hand eczema [176]. Medium-potency TC may be used to treat severe anal inflammation, asteatotic eczema, atopic dermatitis, lichen sclerosus of the vulva, nummular eczema, scabies following treatment with a scabicide, seborrheic dermatitis, severe dermatitis, severe intertrigo for short-term treatment only and stasis dermatitis [176,184]. Low-potency TC may be effective in dermatitis of the diaper area, eyelids or face, intertrigo and perianal inflammation. These agents are the safest for long-term use, for application over large surface areas, for use on the face or areas of the body with thinner skin and for use in paediatric patients [176]. TC are the first-line agents for the treatment of mild-to-moderate psoriasis as well as a number of other inflammatory skin conditions [185]. Psoriasis is relatively common in HIV-infected populations in Africa and may present with typical sharply demarcated, round, thick, scaly papules and plaques favoring the extensor extremities as shown in Figure 2.3 [3,186].

Figure 2.3 Psoriasis (A). Typical sharply demarcated erythematous plaques with "silvery" scale. **Inverse psoriasis (B).** Erythema and scale of the skin folds [3], Permission for including pictures was obtained from authors on 20th October 2013

Most HIV-associated psoriasis will improve when patients are placed on antiretroviral therapy. Additional treatment is usually limited to use of TC and short-contact anthralin therapy [3]. TC modulate intracellular signaling in cells of the immune system and by limiting vascular permeability that allows for propagation of the immune-mediated inflammatory response. More specifically, TC result in a decrease in the local production of cytokines and the presence of vasodilatory substances in skin affected by psoriasis. Corticosteroids are thus effective in the treatment of localized psoriasis since they suppress the local immune response, reduce local skin inflammation and impede or prevent hyperproliferation of skin cells [183,187]. Atopic dermatitis also known as atopic eczema is a chronic pruritic skin condition which can lead to significant morbidity. Questioning patients about the presence and frequency of symptoms allows physicians an opportunity to grade the severity of the disease and the response to treatment [188]. Therapeutic management consists of relieving symptoms and lengthening the time between reoccurrence of the condition. Regular, liberal use of emollients is recommended and the primary pharmacologic treatment is achieved through the use of TC. Twice-daily or more frequent application has not been shown to be more effective than once-daily application and a maintenance regimen of TC may reduce relapse rates in patients who have moderate to severe recurrent atopic dermatitis [182]. TC are also effective for treating vitiligo involving a limited area of skin, lichen sclerosus, bullous pemphigoid, and pemphigus foliaceus [176,189,190].

HIV is the virus that causes AIDS. The virus weakens the immune system and HIV positive patients have AIDS when they develop full blown infection or cancer [191]. Cutaneous manifestations of HIV may result from HIV infection itself or from opportunistic disorders secondary to the decline in the immune-competence of patients infected with the disease [192]. Cutaneous disorders may be the initial signs of HIV-related immuno-suppression. Recognizing HIV-related skin changes may lead to the early diagnosis of HIV infections, allowing early initiation of appropriate antiretroviral therapy. In fact certain skin diseases may be the initial sign that a person is infected with HIV [186,192,193]. Dermatologic diseases are common in HIV-infected individuals and clinicians caring for patients with HIV infection or AIDS in Africa are routinely confronted with skin disorders in their patients. Scarce access to dermatologic specialty care and limited educational resources describing unique clinical characteristics of HIV-related skin diseases can make diagnosing and treating skin diseases a challenge in Africa [192,193]. Examples of HIV-related skin diseases with divergent epidemiologic characteristics include Popular Pruritic Eruption (PPE) associated with HIV, Kaposi Sarcoma (KS), cryptococcosis, and extreme photodermatitis [3,186].

Counter intuitively, dermatitis caused by exposure to the sun is more frequently observed in persons with darker skin types and is very common in HIV-infected persons and can sometimes be quite difficult to differentiate from seborrhea [186]. Photodermatitis presents as an itchy, scaly rash affecting the sun-exposed regions of the skin *viz.*, the face, neck, “v” of the chest, dorsal arms and sometimes the lower legs, the dorsal surface of the feet and generally spares skin that is protected from the sun anatomically such as areas under the chin, or through use of protective clothing as shown in Figure 2.4 [3,192,193].

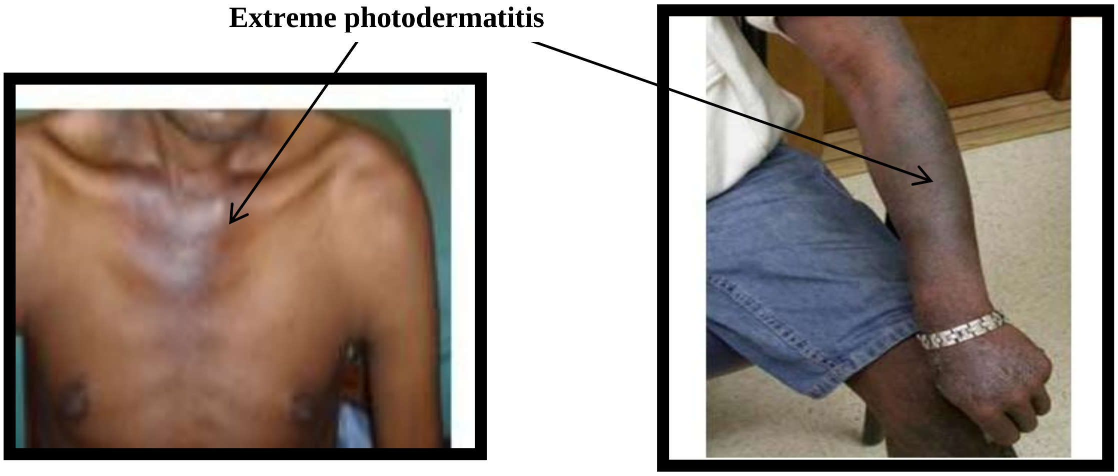


Figure 2.4 Photodermatitis, itching, scaling, and hyperpigmentation involving sun-exposed skin [3,186], Permission for including pictures was obtained from authors on 20th October 2013

The distribution of skin eruptions is often clinically apparent when the shirt is removed. HIV infection itself is photosensitizing and many HIV-infected patients are on therapy that includes photosensitizing drugs such as the sulfonamides. Treatment of these conditions includes immune restoration, use of sun protective clothing such as hats and long-sleeved shirts and potent topical steroids. Many of these patients earn a living working outdoors and sunscreens are not widely available, making avoidance of exposure to the sun extremely difficult [3,186,192]. Furthermore, mutually common conditions such as severe eczema, seborrheic dermatitis and extreme psoriasis have also been shown to be HIV-related skin disorders**.** Advanced HIV disease causes dry skin which can lead to eczema that is always pruritic and may be acute and weeping or chronic dry and scaly, as shown in Figure 2.5 [3,193].

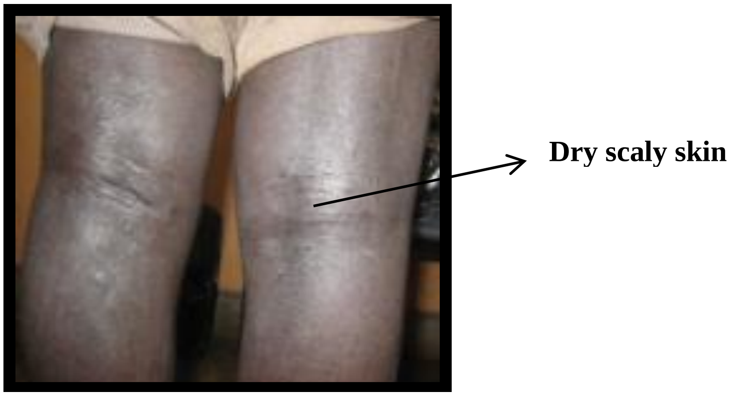


Figure 2.5 Chronic dry, scaly pruritic skin affecting the popliteal fossae [3], Permission for including pictures was obtained from authors on 20th October 2013

Xerosis or dry skin is often present and in adults typically infected areas include the eyelids, neck, flanks, hands, antecubital and popliteal fossae, and lower legs. Moist areas of the skin, such as the axillary regions are typically spared. Treatment focuses on use of TC and emollients and avoidance of desiccating agents such as soaps. Emollients such as petrolatum should be applied immediately after bathing, while the skin is still moist [3,186].

2.3.5. Vehicles for Topical Steroids

A vehicle for topical steroid refers specifically to the type of base in which the medication is included. The most common vehicles used for these agents include creams and ointments, however TC can also be included in gel, lotion, solution, foam or aerosol delivery technologies. The potency of corticosteroids may vary based on the vehicle in which they are formulated, and certain vehicles should be used on specific parts of the body [190]. Ointments provide greater lubrication and occlusion than other vehicles such as creams and lotions and are therefore the most useful for treating dry or thick, hyperkeratotic lesions [176,185]. Their occlusive nature also improves steroid absorption, however they should not be used on hirsute areas as they may cause maceration and folliculitis if used on intertriginous areas such as the groin, gluteal cleft and axilla. Their greasy nature may result in poor patient satisfaction and adherence [176]. Creams on the other hand are mixtures or emulsions of oil suspended in water (O/W) or water suspended in oil (W/O). They have excellent lubricating qualities, and their ability to vanish into the skin makes them cosmetically appealing formulations [194]. Cream formulations of TC are generally less potent than ointments, and they often contain preservatives which can cause irritation, stinging and allergic reactions [195]. Acute exudative inflammation responds well to creams because of their dehydrating effects. Creams are also useful in treating conditions of the intertriginous areas where ointments may not be used, however they do not produce the occlusive effects that ointments achieve [176].

Lotions and gels are the least greasy and occlusive of all TC vehicles. Lotions contain alcohol which has a dehydrating effect on oozing lesions and they are useful for hirsute areas because they penetrate onto the skin and leave little residue. Gels have a jelly-like consistency and are beneficial for exudative inflammation associated with poison ivy. Gels dry rapidly and can be applied to the scalp or other hairy areas as they do not cause matting [174,176]. Foams, mousses and shampoos are also effective vehicles for delivering corticosteroids to the scalp and are easily applied and readily spread, particularly in hairy areas, however foams are usually more expensive formulations [174,176,196].

2.3.6. Frequency of Administration and Duration of Treatment

Once-or twice-daily application for most TC preparations is recommended and the frequency of dosing is dependent on the severity of the skin condition being treated. Frequent administration does not appear to produce better therapeutic outcomes. The optimal dosing schedule can be determined by trial and error and titrating the patient to a minimum frequency of application that provides relief of the symptoms and resolves the condition [176,190,197]. Chronic application of TC can induce tolerance and tachyphylaxis. Ultra-high-potency TC should not be used for more than three weeks continuously. If a longer duration of therapy is required, the TC should be gradually tapered to avoid rebound symptoms and treatment should be resumed after a steroid-free period of at least one week [174,198]. An intermittent schedule can be repeated on a chronic basis or until the condition is resolved. Side effects are rare when low- to high-potency TC are used for periods of three months or less except when treating intertriginous areas, the face and neck under occlusion conditions [174,176]. The amount of TC formulation the patient should apply to a particular area can be determined by using the fingertip unit method. A fingertip unit is defined as the amount of TC that can be squeezed from a standard tube along the fingertip of an adult, assuming that the tube has a standard 5 mm nozzle [176,199]. A fingertip is defined as the length from the very end of the finger to the first crease in the finger as shown in Figure 2.6.

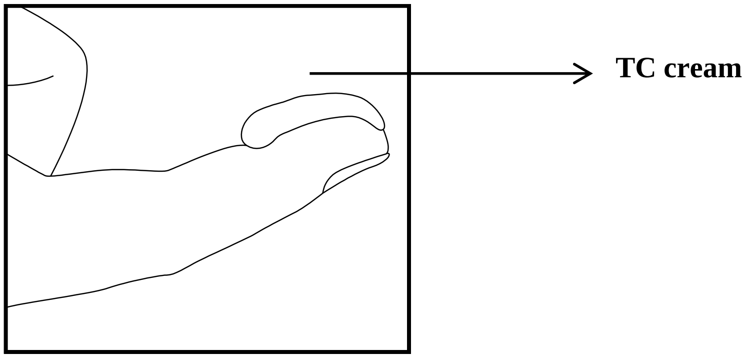


Figure 2.6 Topical corticosteroid cream from the tip of the finger to the first crease in the finger, adapted and redrawn from [199]

Two fingertip units are approximately equivalent to 1 g of TC formulation. One hand-size area *viz.*, the area of one side of the hand of skin requires 0.5 fingertip units or 0.25 g of corticosteroid for effective treatment. The amount dispensed and applied should be considered carefully since application of too little steroid can lead to a poor response and excess can increase the incidence of side effects [200].

2.3.7. Pharmacokinetics

The extent of percutaneous absorption of TC is determined by many factors including the composition and type of vehicle used, the integrity of the epidermal barrier and the use of occlusive dressings. TC can be absorbed through healthy intact skin. Inflammation and/or other disease processes of the skin can increases percutaneous absorption [201] and the use of occlusive dressings substantially increase the percutaneous absorption of TC. Therefore occlusive dressings may be valuable from a therapeutic perspective when treating resistant dermatoses [202]. Following absorption through the skin, TC are handled through pharmacokinetic and metabolic pathways similar to those involved with systemically administered corticosteroids. Corticosteroids modify the functions of epidermal, dermal and leukocyte cells that participate in the precipitation of proliferative and inflammatory skin diseases. Following passage through a cell membrane, corticosteroids react with receptor proteins in the cytoplasm of cells to form a steroid-receptor complex. This complex relocates into the nucleus of the cell, where it binds to DNA. The result of the binding process changes the transcription of messenger RNA (mRNA) and since mRNA acts as a template for protein synthesis, corticosteroids can either stimulate or inhibit the synthesis of specific proteins. Corticosteroids are known to stimulate the production of a glycoprotein called lipocortin. The formation of lipocortin results in the inhibition of phospholipase A2 activity, which releases arachidonic acid, the precursor of prostanoids and leukotrienes, from phospholipids. In contrast, corticosteroids inhibit mRNA responsible for interleukin-1 formation. This activity on arachidonic acid metabolism and interleukin-1 formation results in anti-inflammatory, immuno-suppressive and anti-mitogenic effects. Although the theory relating to activity on protein synthesis may not explain all effects of corticosteroids, these examples illustrate that a specific action at the molecular level can explain some of the characteristic and typical pharmacological effects of topically applied corticosteroids [203,204]. Corticosteroids are primarily metabolized in the liver and are then excreted via the kidneys. Some of the TC and their metabolites are also excreted in the bile [205].

2.3.8. Safety and Side Effects

When TC are used in a controlled situation, they can provide dramatic relief for distressing conditions such as eczema, psoriasis and seborrhoeic dermatitis. Fluorinated TC are associated with a variety of side effects and the fluorinated steroids are generally rated amongst the most potent steroids. They include betamethasone and clobetasol. Except for severe, acute dermatoses, a non-fluorinated steroid such as hydrocortisone is the preferred therapeutic agent because of the relative lack of side effects and lower cost [206]. As with all medications, the prolonged use of TC may cause local and systemic side effects. To reduce potential risk, the least potent TC should be used for the shortest possible duration, whilst maintaining effectiveness. The most common local side effect of TC use is skin atrophy and all TC can induce atrophy. Highly potent steroids, occlusion, thin skin and patient age increase the risk of atrophy. The face, backs of the hands and intertriginous areas are particularly susceptible to atrophy. Resolution often occurs following discontinuation of therapy, but may take months. Concurrent use with 0.1% w/w topical tretinoin such as Retin-A® may reduce the incidence of atrophy derived from chronic use of TC formulations. Other local side effects of TC include permanent dermal atrophy, telangiectasia and striae [176,206].

TC use can also induce rosacea which may include the eruption of erythema and formulation of papules and/or pustules. Steroid-induced rosacea occurs when a facial rash is treated with low-potency TC that produces resolution of the lesions. If the symptoms recur and steroid potency is gradually increased, the rosacea may become refractory to further treatment, making it necessary to discontinue treatment with steroids [207,208]. This may induce severe rebound erythema and pustule outbreak, which may be treated with a 10-day course of tetracycline (250 mg q.i.d.) or erythromycin (250 mg q.i.d.). For severe rebound symptoms, slow tapering of low-potency TC and use of cool, wet compresses applied on the affected area may also help. The normal presentation of superficial infections can be altered when TC are inappropriately used to treat bacterial and/or fungal infections of the skin. Steroids interfere with the natural course of inflammation, potentially allowing infections to spread more rapidly. The application of high-potency TC can induce deep-tissue tinea infections known as Majocchi granuloma [176,209]. This tinea folliculitis requires oral antifungal therapy. Combinations of antifungal agents and corticosteroids such as Daktacort® (miconazole nitrate and hydrocortisone) should be avoided to reduce the risk of severe, persistent or recurrent tinea infections [176,181].

Topical applications of corticosteroids can also result in hypopigmentation. This is more apparent with darker skin tones, but can occur with all skin types. Repigmentation often occurs after discontinuing steroid use [210]. Steroids can induce contact dermatitis in a small number of patients but many cases result from the presence of preservatives, lanolin or other components of topical vehicles. Non-fluorinated steroids such as hydrocortisone or budesonide are more likely to cause contact dermatitis than the fluorinated compounds [151,176,211]. Systemic side effects of TC occur less frequently than local side effects and will usually occur only if the patient does not apply the TC appropriately. Systemic side effects occur since the steroids contained in the formulation are absorbed into the circulatory system and affect other regions of the body, such as the adrenal gland. The systemic side effects of TC include growth suppression in children, hyperglycaemia, hypothalamic-pituitary-adrenal suppression glaucoma, septic necrosis of the femoral head, hypertension and Cushing's syndrome. Cushing's syndrome is a condition caused by excessive use of steroids [212,213] and the symptoms include weight gain, headache and back pain. The concept of an 'ante-drug' promises new developments in TC therapy. An 'ante-drug' such as difluprednate has potent efficacy but fewer side effects than those observed with conventional therapy, since it acts at the site of application prior to metabolism to less active or inactive compounds before reaching the systemic circulation. It is likely that future corticosteroids will have the attributes of an ante-drug, thereby reducing the risk of systemic effects [214]. Topical TC can induce birth defects in animals when used in large quantities, under occlusive conditions, or for long duration. They have not been shown to be teratogenic, and are classified by FDA as pregnancy category C compounds. It is unclear whether topical steroids are excreted in breast milk but as a precaution the application of topical steroids to the breasts should be performed immediately following nursing to allow as much time as possible for removal from the site of application prior to the next feeding [176,215].

2.3.9. Precautions

2.3.9.1. Porphyria

Drugs are a common factor precipitating an acute attack of porphyria which is potentially fatal. It is essential to identify those drugs that may precipitate an acute crisis and avoid prescribing them to porphyric patients except where no safer alternative for therapy exists and the benefits outweigh the risks of use. Most topical TC are safe for use, but caution should be exercised when there is risk of systemic absorption of the TC. For example with dermatological preparations applied to large areas of denuded skin or under occlusive dressings or with vaginal administration of steroid containing products [173].

2.3.9.2. Geriatrics

Topical steroids are indicated for the treatment of several skin disorders affecting the elderly, including forms of dermatitis and psoriasis. TC should generally be used moderately for geriatric patients due to the fact that there is high risk of local and systemic side effects. Treatment of this patient population should reflect the evidence of decreased hepatic, renal, cardiac function and of the presence of concomitant disease or drug therapies. Proper selection of a TC is based on several factors, including the disorder and anatomical site to be treated, the potency, dosage form, application technique of the steroid, potential side effects and/or the presence of coexisting factors such as infection and pre-existing atrophic conditions [173,216].

2.3.9.3. Paediatric Patients

Children especially neonates are more susceptible to the side effects of TC, however they can be used safely in this group. Paediatric patients can safely be prescribed mild to moderate TC and these should not cause local or systemic side effects. Potent and very potent TC are not usually recommended to treat this population group and particularly should not be used in very young children, since they carry a greater risk of side effects in this population sub group than in adults. Ultra-high potency steroids should not be used in infants under one year of age and should be avoided or used for as short a time as possible to control rebound conditions in older children [173].

Exceptions can be made if a patient presents with severe symptoms and it is felt that the benefit of treatment outweighs the risks of side effects. For example potent TC are sometimes recommended for treating cases of severe atopic eczema, usually under the supervision of a dermatologist. Children with chronic severe eczema should have their growth monitored as in these cases steroids may be absorbed in sufficient quantities and may begin to affect the normal growth in these patients [173,206]. Dressings are not suitable for application to the skin of children who have been treated with TC since increased absorption of the steroid may occur, thereby increasing the risk of side effects. It is important to be aware that diapers act as an occlusive dressing and so should not be used if steroid formulations are to be applied to a baby to treat skin conditions [176,189].

2.3.9.4. Pregnancy

TC should generally not be used during pregnancy unless considered essential. Mild, moderate and potent TC are however considered safe to use during pregnancy. Ultra-high and high-potency TC use during pregnancy is not recommended. Studies in animals have shown that corticosteroids applied to the skin of pregnant animals can cause abnormalities in the development of the foetus and manifest as cleft palate or retarded growth of the foetus in the womb. Studies in animals do not necessarily relate to effects in humans, but there may be a small risk of such effects if sufficient steroid is absorbed through the skin into the systemic circulation of pregnant women. For this reason, topical steroid use during pregnancy should be discussed with a physician and if required its use should minimize absorption through the skin and the weakest possible strength is preferred. Application to large areas of skin, underneath airtight dressings, or over prolonged periods of time should be avoided [173].

2.3.9.5. Breastfeeding

Mild, moderate and potent TC are considered safe to use when breastfeeding. To minimize the possibility of steroid absorption through the skin and passing through the breast milk, application to large areas of skin, underneath airtight dressings over prolonged periods of time should be avoided. If TC application to the breasts is necessary, the area should be washed prior to feeding. It is uncertain whether very potent TC are safe to use while breastfeeding and their use is not recommended in breastfeeding mothers [173].

2.4. CONCLUSIONS

Corticosteroids are a group of natural and synthetic analogues of hormones secreted by the hypothalamic-anterior pituitary-adrenocortical axis, more commonly referred to as the pituitary gland. They are often used as part of the treatment for a number of different diseases, such as severe allergies or skin conditions, breathing, certain cancers, blood disorders, conditions of the eye, arthritis, digestive tract issues and in hormone replacement therapy. Prolonged use of systemic corticosteroids may lead to serious side effects such as glaucoma, cataracts, hypertension, cardiac disease, diabetes mellitus and obesity. For these reasons the dose and duration of use should be minimized whenever possible. There is overwhelming evidence in more than 100 placebo-controlled studies that topical therapy is highly effective and safe for the treatment of allergic rhinitis and other allergic conditions. Although reports of antiretroviral controlled drug-comparative studies are limited, they have indicated that topical treatment is at least as effective as low-dose treatment with 7.5 mg prednisolone administered systemically. The advantage of using topical instead of systemic administration is relatively obvious for corticosteroids and TC are among the most commonly prescribed medications in an ambulatory care setting. They are the cornerstones of therapy for a wide variety of dermatoses including atopic dermatitis, contact dermatitis, psoriasis and seborrhoeic dermatitis. In this study, clobetasol 17-propionate, one of the ultra-high-potency TC was used in the formulation of high quality topical creams to treat HIV-related skin disorders in Southern African communities.

CHAPTER THREE

THE DEVELOPMENT AND OPTIMIZATION OF A STABILITY-INDICATING RP-HPLC METHOD FOR THE QUANTITATION OF CLOBETASOL 17-PROPIONATE (CP) IN CREAMS

3.1. INTRODUCTION

3.1.1. Chromatography

High Performance Liquid Chromatography (HPLC) is a popular method for the quantitative analysis of clobetasol 17-propionate (CP) in pharmaceutical dosage forms such as creams [61,217-219], ointments [198,218,220], emollients [6-8], gels, foams, sprays [221,222], lotions [223], shampoos [217], more recently in loaded-poly(D,L-lactic-co-glycolic acid) microspheres [224] and solid lipid nanoparticles [225,226]. HPLC is also a commonly used method for the qualitative analysis of CP in short and long term stability studies [61,227,228]. HPLC is recommended as the method of choice in official compendia including the British Pharmacopoeia (BP) [229,230] and the United States Pharmacopoeia-National Formularies (USP-NF) [231,232], indicating that the technique is suitable for the analysis of CP during formulation development studies.

The objective of these studies was to develop and optimize a Reversed-Phase High Performance Liquid Chromatography (RP-HPLC) method for the quantitation of CP in topical dosage forms and for the assessment of the rate and extent of CP release from topical formulations. A multifactor optimization technique was successfully applied to the development and optimization of a RP-HPLC method. This method was used to analyze CP and the optimal conditions of HPLC separation were established with the aid of Response Surface Methodology (RSM). The method validation of the RP-HPLC technique for the analysis of CP in topical dosage forms is reported in Chapter 4, *vide infra.*

3.1.2. High Performance Liquid Chromatography (HPLC)

Liquid Chromatography (LC) is a method of chromatographic separation based on the difference in distribution of an analyte between two immiscible phases, in which the mobile phase is a liquid that percolates through a stationary phase, usually contained in a column [61,233-235]. LC has developed into the most widely practiced analytical method today. While there are alternatives including spectroscopic and other techniques, for the analysis of small molecules and materials, LC plays an increasingly important role in unraveling the secrets of the modern life sciences. Furthermore LC is the only method that offers equal performance in analytical as well as in preparative and process applications [236]. Thin layer and paper chromatography are older examples of LC and modern techniques including High Speed LC, High Efficiency LC, High Pressure LC (HPLC), Ultra Performance Liquid Chromatography (UPLC) have been used to describe LC [237-241]. High performance liquid chromatography is now the generally accepted terminology [242,243]. Applications of LC appeared more rapidly between 1960 and 1970 when HPLC was developed as an analytical tool in addition to Gas Chromatography (GC). The acronym HPLC originally indicated the fact that high pressure was used to generate the flow required for liquid chromatography in packed columns [244]. In the beginning, pumps had a pressure capability of only 500 psi and this was called high pressure liquid chromatography. The early 1970s saw a tremendous leap in technology and these new HPLC instruments could develop up to 6000 psi and incorporated improved injectors, detectors and columns. HPLC really began to take hold in the mid-to-late-1970s. With continued advances in performance, the acronym HPLC remained the same, but the name was changed to high performance liquid chromatography [244]. In 2012 the fifth decade of HPLC commenced and between 1970 and 2012, it became one of the most applied analytical techniques in the pharmaceutical, food, nutraceutical, cosmetic, environmental, forensic, industrial and chemical industries, leading to validated results in therapeutics and diagnostics [236,245-247].

Two principle types of HPLC *viz.*, Normal-Phase HPLC (NP-HPLC) and Reversed-Phase HPLC (RP-HPLC) can be differentiated by the nature of the stationary phases. The phases are used to effect a separation in addition to the corresponding mobile phase composition and the nature of the interaction of functional groups present in solute molecules with these phases [227,248,249]. In a highly non-polar environment, hydrophilic molecules tend to associate with each other as do water drops on an oily surface. The hydrophilic molecules in a mobile phase will tend to adsorb to the surface of the inside and outside of a particle if that surface is also hydrophilic. Increasing the polarity of a mobile phase subsequently decreases the adsorption and ultimately causes the sample molecules to exit the column. This approach is called NP-HPLC [248]. In contrast, RP-HPLC results from the adsorption of hydrophobic molecules onto a hydrophobic solid support bathed in a polar mobile phase. Decreasing the mobile phase polarity by adding organic solvent reduces the hydrophobic interactions between the solute and the solid support resulting in desorption. The more hydrophobic the molecule, the more time it will spend on the solid support and the higher the concentration of organic solvent required to promote desorption [248,249]. RP-HPLC is the most popular separation technique on an analytical scale [250] because **i)** it applies to a very wide range of molecules including charged and polar molecules, **ii)** it allows precise control of variables such as organic solvent type and concentration, pH and temperature, **iii)** RP-HPLC columns are efficient and stable and **iv)** it is a robust technique [251-253].

3.1.3. Experimental Design for RP-HPLC

Chromatographic method development can be a time-consuming subjective process. As companies accelerate drug development programs and candidate compounds move through this process, fast and robust HPLC method development has become increasingly important [254,255]. Most method development is done using a manual, one-factor-at-a-time process where the approach is to vary one parameter at a time and examine the effect on performance [255]. This procedure is continued until no further improvement is observed, at which time another parameter is selected to evaluate [255]. These separations are often sub-optimal in terms of resolution, tailing, retention time and often lack robustness [256]. The use of statistical tools has been an effective strategy for the development and optimization of chromatographic systems [103,257,258]. Response Surface Methodology (RSM) has proven to be useful as chromatography exhibits multiple responses that need to be optimized simultaneously. These responses include retention factors of chromatographic peaks, tailing and resolution factors [259,260]. RSM is a collection of mathematical and statistical techniques useful for modeling and analysis of response(s) of interest that are influenced by a number of variables. The primary objective of this approach is to optimize the response(s) of interest [258,261-263]. RSM has become an important statistical tool for the design, formulation, development and analysis of scientific investigation and product manufacture. It is also an efficient approach for the improvement of existing studies and products. The most common applications of RSM are in industrial, biological, clinical, social, food, physical and engineering sciences [261,264].

A RSM approach is critical in studying factors that are important in RP-HPLC method development. The successful analysis by RP-HPLC of drugs in complex pharmaceutical formulations relies upon the optimization of chromatographic conditions, sample preparation and post-column detection. The optimization of RP-HPLC methods defines the simultaneous influence of some important conditions, such as column type, mobile phase composition, pH of mobile phase, column temperature and flow rate, on the separation and analysis [265-267]. In the context of this study, a RP-HPLC method was developed for the optimal quantitation of CP in semi-solid dosage forms. Selectivity factor values defined the optimal conditions, which were confirmed by analyzing mathematical models. With the aid of the RSM, it was possible to anticipate precisely to a certain degree, the selection of optimal experimental conditions.

3.1.4. Application of Central Composite Design (CCD) in RP-HPLC

In RSM, Central Composite Design (CCD) is the most popular approach for fitting data to a second order model [268-270]. The CCD approach combines two-level full or fractional factorial designs with additional axial or star points with at least one point at the center of the experimental region being investigated. It allows the determination of both linear and quadratic models [271,272]. Box and Hunter in 1957 discussed the choice of α extensively for CCD when the experimental runs are completely randomized. They derived the conditions required to make a design "rotatable" [268,273], a design that ensures that the quality of the prediction is a function of the distance, ρ, from the design center. The CCD approach is known to be a better alternative to the full factorial three-level design since it demands a smaller number of experiments be undertaken while providing comparable results [274]. In general, a CCD for *k* factors, coded as ($x_1, . . ., x_k$), consists of three parts *viz.*, **i)** a factorial (or cubic) design, containing a total of n fact points with coordinates $x_i = -1$ or $x_i = +1$, for $i = 1, . . ., k$, **ii)** an axial (or star) part, formed by $n_{ax} = 2k$ points with all their coordinates null except for one that is set equal to a certain value α (or −α) and **iii)** a total of n_c runs performed at the center point, where, of course, $x_1 = . . . x_k = 0$ [274]. To build a CCD, each of these three parts needs to be specified and it is essential to decide on how many cubic points are to be used, where they will be located, what the value of α will be and how many replicate runs should be conducted at the center point. Two examples of designs are presented in Figure 3.1and Table 3.1[274].

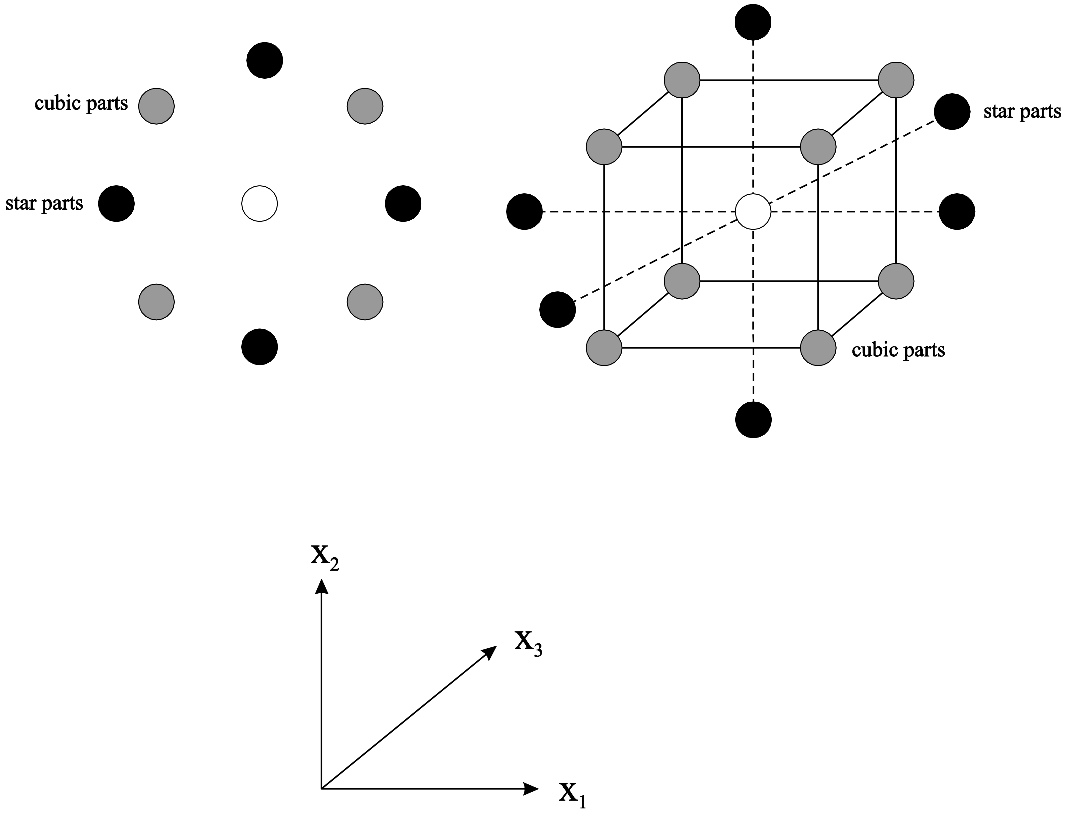


Figure 3.1 Central composite designs for two and three factors [274]

Table 3.1 Coded factor levels for CCD for two- and three-factor systems

Two-factor		Three-factor		
x_1	x_2	x_1	x_2	x_3
-1	-1	-1	-1	-1
1	-1	1	-1	-1
-1	1	-1	1	-1
1	1	1	1	-1
0	0	-1	-1	1
0	0	1	-1	1
0	0	1	1	1
-1.414	0	0	1	1
1.414	0	0	0	0
0	-1.414	0	0	0
0	-1.414	0	0	0
		0	0	0
		-1.683	0	0
		1.683	0	0
		0	-1.683	0
		0	-1.683	0
		0	0	-1.683
		0	0	1.683

The Table shows a two and three factor design. The first four runs of the two factor (2^2 factorial design) make up the cubic part of the CCD and the star design makes the last four (with α = √2) with three replicate runs at the center point. The three-factor case has $2^3 = 8$ runs with four center points and six axial points with α = 1.683. The cubic points in Table 3.1and Figure 3.1 are the same as those of full factorial designs although this is not strictly necessary. Depending on the number of factors, it might be inadvisable to include these points, because it would require too many experiments to be conducted. The total number of distinct levels in CCD is $n_{fact} + 2k + 1$. Therefore there are $2^k + 2k + C_0$ total points where C_0 is the number of center points. The complete quadratic model for k factors contains $(k + 1)(k + 2)/2$ parameters and is depicted in Equation 3.0.

$$y = \beta_0 + \sum_i \beta_i \, x_i + \sum_i \beta_i \, x_i^2 + \sum_{i<j} \sum_j \beta_{ij} \, x_i x_j + \varepsilon \qquad \textbf{\textit{Equation 3.0}}$$

Consequently with two factors, this model has six parameters and the two-factor design in Table 3.1. has nine different combinations of levels. All the model parameters could be estimated using only two cubic points, corresponding to one of the two 2^{2-1} fractions. Selecting a fractional design, instead of a complete factorial, to define the cubic points becomes more attractive as the number of factors increases. The value of α usually ranges from 1 to $\sqrt{k}$ and when $\alpha = \sqrt{k}$, the cubic and axial points are located on the (hyper) surface of a (hyper) sphere and the design is spherical. This is the case of the two-factor design depicted

Table 3.1. At the other extreme, when $\alpha = 1$, the axial points are located at the centers of the sides of the square portion of the two-factor design and at the center of the faces of the cuboidal part of the three-factor design. This type of design is advantageous when the experimental space is square or cubical, which occurs in a natural way when the factors are varied independently of one another. It also has the advantage of requiring only three factor levels, which can be of consequence if one of the factors is qualitative [274,275].

CCD is one of the chemometric techniques used for quantification and optimization of chromatographic systems [274,276-279]. It has been used for the determination of the critical conditions for experimental factors during optimization of extraction steps [280-283], derivatization reactions [283,284], separation steps, quantification processes [285-287] and for robustness studies [267]. These methods were used for the determination of organic and inorganic species in samples such as water, drugs, biological matrices, wines, human plasma and others [167,233,283,288-291]. These applications using several chromatographic techniques for quantification are summarized in Table 3.2.

Table 3.2 Applications of the CCD for the optimization of chromatographic methods

Analytes	**Samples**	**Optimization**	**Chromatographic Technique**	**Reference**
Fatty acids and essential oils	*Borago officinalis L.* flower	Extraction step	GC-MS	[280]
Baicalin and baicalein	*Scutellaria baicelensis*	Extraction step	HPLC	[282]
Human antibodies	Pure proteins systems and then an artificial mixture of proteins containing 1 mg/mL human immunoglobulin G (IgG), 10 mg/mL serum albumin and 2 mg/mL myoglobin.	Extraction step	SEC	[290]
Essential oils	*Myrtus communis L.* Leaves	Extraction step	GC-MS and GC-FID	[292]
Oleuropein	Olive's processing wastewater and olive leaves extracts	Extraction step	HPLC	[293]
Fatty acids	Quince (*Cydonia oblonga Miller*) seeds	Extraction step	GC-MS	[294]
BHA, BHT	Vegetable oil	Extraction step	HPLC	[295]
Volatile compounds	Roasted coffee beans	Extraction step	GC, HS-SPME	[296]
Selected pharmaceuticals and endocrine-disrupting substances	Water	Derivatization step	GC-MS	[283]
Formaldehyde	Supercritical medium	Derivatization step	UV-Vis	[297]
1-nor-Δ(9)-tetrahydrocannabinol-9-carboxylic acid (THCCOOH)	Urine	Derivatization step	GC-MS/MS	[284]
Ten primary amines	Sewage sludge	Derivatization step	GC-IT-MS-MS	[298]
Methyl mercury	Biological samples	Derivatization step	GC-MAE	[289]
Seven antihistamines (chlorpheniramine, cyproheptadine, diphenhydramine, doxylamine, methapyrilene, terfenadine, and triprolidine)	Drugs	Separation step	NACE-EC/ECL .	[299]
Neuroprotective peptides	Peptides	Separation step	Ion pair RP-HPLC	[300]
Atropine	Plant extract	Separation step	CZE	[301]

Active ingredients	*Scutellaria baicelensis*	Separation step	HPLC	[282]
5P12-Rantes	*Pichia pastoris*	Separation step	HPLC	[302]
Azo dye	Waste water	Separation step	UV-Vis	[288]
Volatile phenols	Wines	Separation step	GC	[291]
Organic acid in tobacco	Tobacco	Separation step	AMMS-ICE II	[303]
Triazolopyrimidine sulfoanilide herbicides	Soy milk	Separation step	CE-MS	[304]
Citalopram, fluoxetine, paroxetine and their metabolites	Plasma and whole blood	Separation step	HPLC	[305]
Withaferin A	*Withania somnifera dunal*	Quantification step	MAE	[306]
Chloropicrin	Soil	Quantification step	GC-MS	[307]
Amphetamines	Chiral mixtures	Robustness study	CE	[308]
Carboxylic acids	Industrial reaction mixtures	Robustness study	RP-HPLC	[309]

GC-MS: Gas Chromatography-Mass Spectrometry; HPLC: High Performance Liquid Chromatography; SEC: Size Exclusion Chromatography; GC-FID: Gas Chromatography-Flame Ionization Detector; HS-SPME: Headspace-Solid Phase Microextraction; UV-VIS: UltraViolet-Visible Spectroscopy; GC-IT-MS-MS: Gas Chromatography-Ion Trap Tandem-Mass Spectrometry; GC-MAE: Gas Chromatography Microwave-Assisted Extraction; NACE-EC/ECL: Non-Aqueous Capillary Electrophoresis-ElectroChemical and ElectroChemiLuminescence; RP-HPLC: Reverse-Phase High Performance Liquid Chromatography; CZE: Capillary Zone Electrophoresis; AMMS-ICE II: Anion Micro Membrane Suppressor-Ion Chromatography Exclusion; CE-MS: Capillary Electrophoresis-Mass Spectrometry; MAE: Microwave-Assisted Extraction; CE: Capillary Electrophoresis.

Published data reveal that a CCD approach was primarily used for the determination of the critical conditions of experimental factors during the optimization of separation steps in HPLC [248,282,300,302,305]. HPLC is a chromatographic technique of choice, with many applications in pharmaceutical processes in addition to research and development [267,310]. However it has proved to be a practical analytical tool that has been applied in the cosmetic [311,312], biotechnological [290,313,314], biomedical [315,316], environmental [317-319], food [280,320,321] and biochemical [322,323] industries. In the pharmaceutical industry, HPLC is more widely used than other analytical techniques since sample preparation is rapid and records of qualitative and quantitative data, for samples of widely varying polarity, can be generated in a single analytical run [310]. HPLC has therefore become a powerful technique in natural product analysis as well as drug analysis because of high efficiency, short analysis time, low sample and solvent consumption [324,325].

3.2. LITERATURE REVIEW OF CP

A summary of the chromatographic conditions that have been used for the successful separation of CP from a variety of matrices is given in Table 3.3. The analysis of CP in dosage forms *viz.,* creams, cosmetics, emulgel and nanocapsule suspensions and in human and rat plasma, has been primarily performed on reverse-phased silica-based C_{18} stationary phases [326-330] and is the stationary phase of choice for use in 2013 [331], 2012 [332], 2011 BP [333], 2011 [128] and 2010 USP-NF [334]. However silica-based columns of different diameters and lengths with different particle sizes have been used. Musmade *et al.* [330] and Fontana *et al.* [326] used silica-based C_{18} packed columns of internal diameters 4.6 mm and 250 mm in length to determine CP in rat plasma and in topical nanocapsule suspensions whereas Badilli *et al.* [224] selected a silica-based C_{18} packed column of internal diameter 3.9 mm and length of 150 mm to determine CP in a microparticulate based topical delivery system. As the length of an HPLC column increases, the mechanical separation power increases. This approach may result in longer analytical run times and greater solvent consumption [238]. Organic solvents such as acetonitrile (ACN) and methanol (MeOH) are commonly used in analytical methods reported for the analysis of CP in dosage forms [224,326-328,330,335,336]. MeOH was preferred due to a worldwide shortage and high cost of ACN [218,337,338]. MeOH is also used as an organic modifier in official compendia such as the 2013 [331], 2012 BP [332] and the 2011 [128] and 2010 USP-NF [334]. Although CP is a non-ionized molecule, the use of a buffer, phosphate buffer have also been reported [330]. HPLC with UV detection is the preferred method for monitoring CP in sample matrices, is well-defined [61] and is reported in the USP-NF [231,232]. A flow rate of 1.0 mL/min was commonly used with detection at 240 nm for most analyses [224,328-330,339].

Table 3.3 Published HPLC analytical methods for the determination of CP from 2010 - 2013

Year	Column	Mobile Phase	Flow Rate	Detection	Retention Time	Reference
2013	Nova-Pak® C_{18}, 3.9 × 150 mm, 4 µm	68% Methanol: 32% water	0.9 mL/min	UV 239 nm	8.0 minutes	[327]
2012	Zorbax SB® C_{18}, 4.0 × 250 mm, 5 µm	80% Methanol: 20% Phosphate buffer pH 2.5	1.0 mL/min	UV 240 nm	5.0 minutes	[339]
2011	Nova-Pak® C_{18}, 3.9 × 150 mm, 4 µm	74% Methanol: 26% water	1.0 mL/min	UV 240 nm	3.0 minutes	[224]
2011	ORBAX® Eclipse XDB-C_{18}, 4.6 x 150 mm, 5 µm	0.1% Phosphoric acid in water and Acetonitrile	1.0 mL/min	UV 240 nm	15.5 minutes	[329]
2010	Phenomenex® Luna C_{18}, 3.0 × 150 mm	45 % Acetonitrile: 55% water	1.0 mL/min	UV 240 nm	Not specified	[225]
2010	Variance® C_{18}, 4.6 × 250 mm, 5 µm	65% Acetonitrile: 35% Phosphate buffer pH 7.0, 25.0 mM containing 0.2% v/v triethylamine	1.0 mL/min	UV 240 nm	Not specified	[330]
2010	RP18 Gemini® 4.6 × 250 mm, 5 µm	80% Methanol: 20% water	1.0 mL/min	UV 241 nm	6.75 minutes	[326]
2010	YMC-Pack ODS-A C_{18}	70% Methanol: 30% 0.02 mol/L Potassium dihydrogen phosphate	1.0 mL/min	UV 240 nm	Not specified	[328]
2010	Phenomenex® Luna C_{18}, 2.0 × 150 mm, 5 µm	46% Acetonitrile: 54% water	0.5 mL/min	UV 238 nm	8 minutes	[336]
2010	Polaris® 3 C_{18}-A, 2.0 × 50 mm, 3 µm	70% Methanol: 30% water containing 1% formic acid	0.5 mL/min	LC-MS	Selected reaction monitoring (SRM) transitions were 489.0 → 415.0 (−10 V; 110 V; 0.4 s)	[335]

3.3. EXPERIMENTAL

3.3.1. Reagents and Materials

Micronized CP USP standard and internal standard, Betamethasone 17-valerate (BV) were purchased from Symbiotec$^{®}$ Pharmalab P. Ltd (Indore, India). CP was used for the manufacture of pilot scale cream formulations. Dermovate$^{®}$ 0.05% w/w cream (Batch number 305628, Sekpharma$^{®}$ Pty Ltd, Sandton, Gauteng, RSA), the innovator product commercially available on the South African Market, was purchased from a local pharmacy. HPLC grade MeOH-215 far UV Romil-SpS$^{®}$ Super Purity Solvent was purchased from Romil$^{®}$ Ltd (Waterbeach, Cambridge, UK). HPLC grade water was prepared by reverse osmosis, using a Milli-RO$^{®}$ 15 water purification system (Millipore$^{®}$, Bedford, MA, USA), consisting of a Super-C carbon cartridge, two Ion-X$^{®}$ ion-exchange cartridges and an Organex-Q$^{®}$ cartridge. The water was filtered through a 0.22 μm Millipak$^{®}$ stack filter (Millipore$^{®}$, Bedford, MA, USA) prior to use. All reagents were used without further preparation and were at least of analytical reagent grade.

3.3.2. Instrumentation and Analytical Conditions

Preliminary HPLC method development was performed on a modular HPLC system consisting of a Model P100 dual piston pump (Thermo Separation$^{®}$ Products, San Jose, CA, USA) and a Model AS100 auto sampler (Thermo Separation$^{®}$ Products, San Jose, CA, USA) equipped with a Model 7010 Rheodyne$^{®}$ injector (Rheodyne$^{®}$, Reno, NV, USA) fitted with a 20 µL fixed volume loop. A Linear Model 6200-9060 UV/VIS-500 detector (Linear Instrument Co., CA, USA) and a Spectra Physics SP$^{®}$ 4600 integrator (Thermo Separation$^{®}$ Products, San Jose, CA, USA) were used to capture the data. Optimization studies using CCD were performed on an HPLC system consisting of a Model 2695 Alliance Separations$^{®}$ module and a Model 2487 Waters$^{®}$ Dual λ Absorbance detector coupled to Empower data acquisition software (Waters$^{®}$, Milford, MA, USA). Chromatographic separation was achieved using a Nova Pak$^{®}$ C_{18} (5 μm, 150 mm × 3.9 mm i.d.) column (Waters$^{®}$ Corporation, Milford, MA, USA) protected by a guard column. The column was maintained at specific temperature of 24ºC and the separation was achieved under isocratic conditions with UV detection of 238 nm. The volume of injection was 20 µL and a flow rate of 1.0 mL/min was used for the separation.

3.3.3. Software

Experimental design, data analysis and desirability function calculations were performed using Design-Expert$^{®}$ software (version 8.02, Stat-Ease Inc., Minneapolis, USA). The balance of the calculations for the analysis was performed with Microsoft$^{®}$ Excel 2007 (Microsoft$^{®}$, USA).

3.3.4. Preparation of Stock Solutions

A standard stock solution (100 µg/mL) of CP was freshly prepared by accurately weighing approximately 10 mg of CP into a 100 mL A-grade volumetric flask and making up to volume with MeOH. The solution was then sonicated using a Branson® B12 sonicator (Branson® Inc., Shelton, Conn, USA) for two minutes to ensure complete dissolution of CP. After sonication, the 100 mL A-grade volumetric flask was covered with foil since CP degrades in the presence of light [61,327]. The stock solution was serially diluted with MeOH to obtain CP concentrations of 0.25, 0.5, 1.0, 3.0, 6.0, 9.0, 12.0 and 15.0 µg/mL respectively. A stock solution of BV was prepared by accurately weighing 5 mg BV on a Mettler® Model AE 163 analytical balance (Mettler® Inc., Zurich, Switzerland) and dissolving BV in 20 mL MeOH in a 20 mL A-grade volumetric flask. An 800 µL aliquot volume of this solution was further diluted to 20 mL in an A-grade volumetric flask to produce a solution of approximately 10 µg/mL.

3.3.5. Preparation of Mobile Phase

Approximate volumes of MeOH and water were measured separately, using 1000 mL and 500 mL A-grade measuring cylinders and were then mixed in a 1000 mL Schott® Duran bottle (Schott® Duran GmbH, Wertheim, Germany). The mobile phase was filtered and degassed under an Eyela® Aspirator A-2S vacuum pump (Rikakikai® Co. Ltd, Tokyo, Japan) using a Millipore® HVLP 0.45 µm filter membrane (Millipore®, Bedford, MA, USA).

3.4. METHOD DEVELOPMENT

3.4.1. Column Selection

Silica gel and monolithic octadecyl silane (ODS) silica gel are two of the most common stationary phase packing materials used for NP-HPLC and RP-HPLC applications [340,341]. Numerous normal- and reversed-phase packings are available, however most of which are based on chemical modification of the silica gel surface. In recent years stationary phases with organic polymeric backbones have also become available. For a neutral compound such as CP, the balance between the polarity of the mobile phase and lipophilicity of the molecule will determine the retention time on the HPLC column. The pH of the mobile phase will theoretically have no effect on retention times of neutral molecules [342,343]. Dimethyl octadecylsilyl amorphous silica has been used for the analysis of CP in dosage forms as summarized in Table 3.1. Since CP is a non-ionized molecule and pH manipulation is not required to effect a separation, a reversed-phase silica-based column was chosen. Specifically a Nova Pak® C_{18}, 150 mm x 3.9 mm i.d. with a 4 µm particle size (Waters® Corporation, Milford, MA, USA) was used in these studies.

3.4.2. Method of Detection

Variable wavelength UV detectors offer the best option to monitor an eluent emerging from an analytical column for a large range of solutes. Typically the UV detector has a narrow flow cell of approximately 1 mm in diameter, with a 10 mm path length and a resultant internal volume of approximately 8 µL. The linear range of UV detectors is normally between 0.0001 and 2 Absorbance Units (AU) and samples must be sufficiently diluted to fall within this range to avoid deviation from the Beer-Lambert Law [342]. UV detectors have mostly been applied to the detection of CP in multiple dosage forms as well as in plasma, as summarized in Table 3.2.

3.4.3. Wavelength Detection

The majority of analyses of CP have used wavelengths in the UV range between 238 nm and 241 nm. Prior to HPLC method development, a solution of CP in MeOH:Water (70:30% v/v) was scanned over a wavelength of 210 - 300 nm using a dual beam UV-VIS Model GBC 916 spectrophotometer (GBC Scientific Equipment Pty Ltd, Melbourne, Victoria, Australia). This experiment was done to determine the wavelength at maximum absorption and was observed at a wavelength of 238 nm (Figure 3.2). This wavelength was selected for use during HPLC method development and for *in vitro* analyses of the CP cream formulations manufactured in these studies.

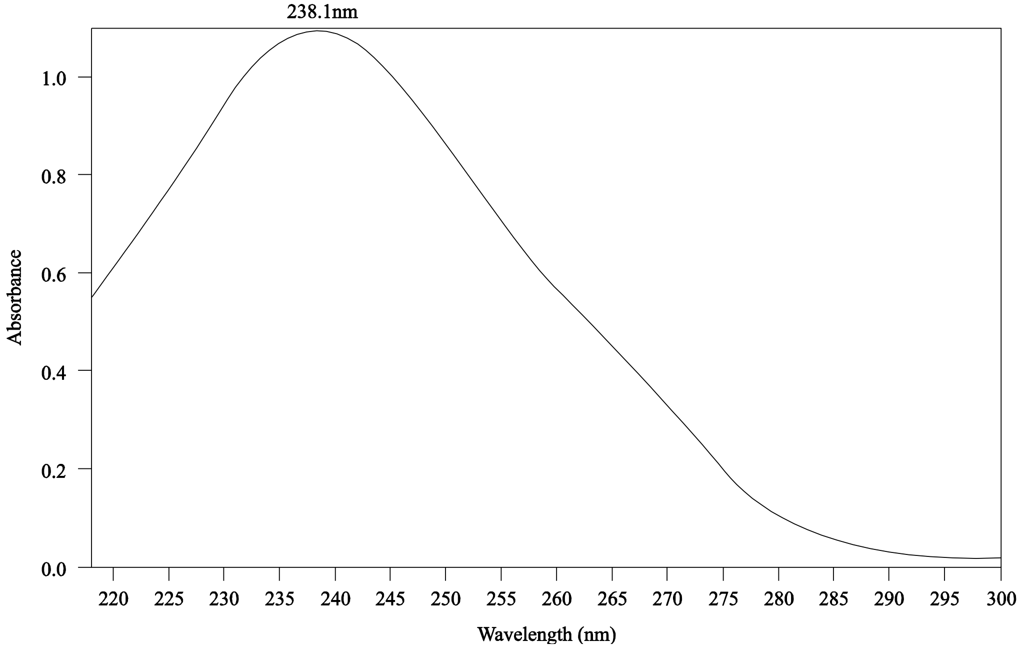
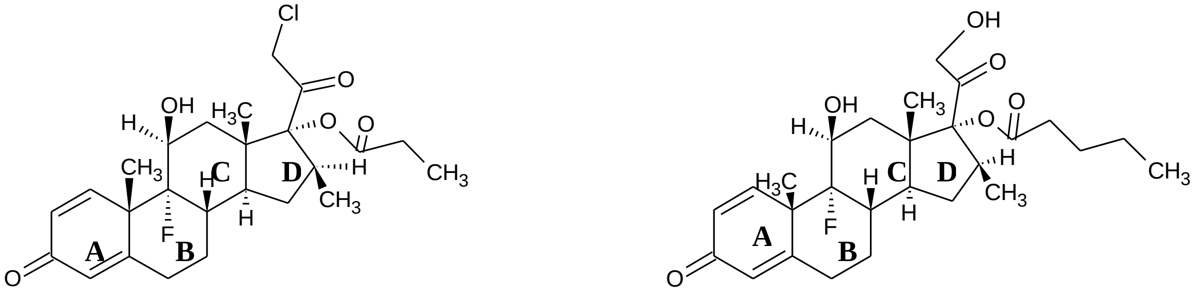


Figure 3.2 Ultraviolet absorption spectrum of CP in MeOH and water (70:30% v/v)

3.4.4. Internal Standard Selection

If an HPLC system is not accurate or precise, the use of an internal standard can improve accuracy by correcting for variable injection volumes of a test solution [344,345]. A solution containing a fixed amount of internal standard is added to a sample in a precisely measured volume. Any subsequent loss of the analyte is accounted for, since loss of the analyte will be mirrored by loss of the internal standard. A substance may be used as an internal standard if it is stable and elutes as close as possible but adequately resolved from the analyte of interest and any possible excipients that may be present in the sample matrix being analyzed [342,346].

Betamethasone 17-valerate [227,347], mometasone furoate [336], clobetasol 17-butyrate [218,335] and betamethasone dipropionate [329] have been used as internal standards for the chromatographic analyses developed for the quantitation of CP in dosage forms. Prior internal standard selection studies revealed that BV was well resolved from CP, had sharp symmetrical peaks and exhibited no peak tailing at a retention time appropriate for the analysis of CP [61]. Betamethasone 17-valerate (BV, [8S, 9R, 10S, 11S, 13S, 14S, 16S, 17R)-9-fluoro-11-hydroxy-17-(2-hydroxyacetyl)-10, 13, 16-trimethyl-3-oxo-6, 7, 8, 11, 12, 14, 15, 16-octahydrocyclopenta[a]phenanthren-17-yl] pentanoate) [348] is a medium to high potency corticosteroid [349,350] that has similar structural and chemical properties to CP as shown in Figure 3.3.



Clobetasol 17-propionate **Betamethasone 17-valerate**

Figure 3.3 Chemical structures of CP and BV, with molecular weights of 466.98 g/mol and 476.58 g/mol respectively

Both molecules possess a 21-carbon steroid structure, characterized by the presence of four fused rings (Figure 3.3.). The steroidal structure consists of three 6-membered rings with one 5-membered ring that is a hydrogenated cyclopentanoperhydrophenanthrene ring system. CP and BV contain a fluorine (-F) atom attached on ring B at position C-9α, contributing to the lipophilicity of those molecules. However CP has a chlorine (-Cl) atom whereas BV contains a hydroxyl (-OH) group on the 5-membered ring D at position C-21, with two extra carbon chains on ring D at position C-17. This difference on the ring D possibly makes BV more lipophilic than CP. Billich *et al.* [347] revealed that BV is slightly more lipophilic than

CP, with BV having a log D_{oct} value of 4.74 ± 0.02 and CP of 4.34 ± 0.02. The retention characteristics of CP and BV in RP-HPLC are therefore likely to be similar and BV was selected as an internal standard for the analytical method developed for the analysis of CP in dosage forms.

3.4.5. Mobile Phase Composition

The initial mobile phase selected for evaluation was based on the published methods and is summarized in Table 3.3. The majority of the recently published data indicate that MeOH was used as the organic solvent for the analysis of CP in dosage forms as well as in plasma [224,326,328,335,339]. A mobile phase of 70% v/v MeOH and 30% water was used at the start of the study. A typical chromatogram of CP and BV developed using these conditions, is depicted in Figure 3.4.

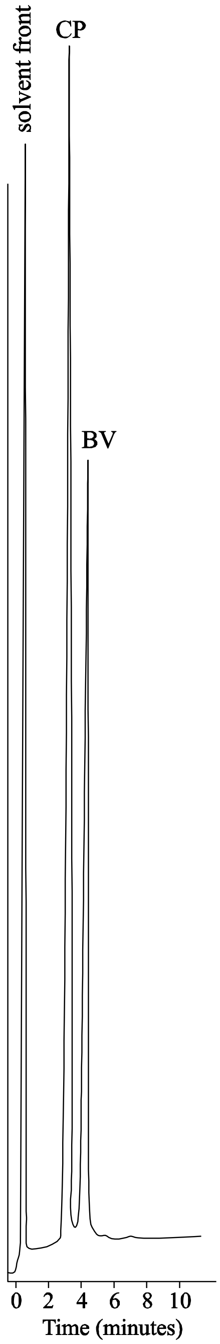


Figure 3.4 Typical chromatogram of CP and BV with a mobile phase of MeOH and water (70:30% v/v)

The efficiency of each of the separations was assessed by considering the resolution factor R_s, peak asymmetry factor A_s and Peak Tailing Factor (PTF) calculated using Equations 3.1, 3.2 and 3.3 respectively. Figure 3.5 depicts a schematic representation for the determination of peak asymmetry and peak tailing factors.

$$R_S = \frac{(Rt_1 - Rt_2)}{\frac{1}{2}(t_{w1} - t_{w2})}$$ ***Equation 3.1***

Where
R_s=Resolution factor
Rt_1=Retention time for first eluting peak
Rt_2=Retention time for second eluting peak
t_{w1}=Width of first eluting peak at the base
t_{w2}=Width of second eluting peak at the base

$$A_s = \frac{B}{A}$$ ***Equation 3.2***

$$PTF = \frac{(A + B)}{2A}$$ ***Equation 3.3***

Where
A_s=Peak asymmetry factor
PTF=Peak tailing factor
B=Distance between the middle point and the right side of the peak
A=Distance between the middle point and the left side of the peak

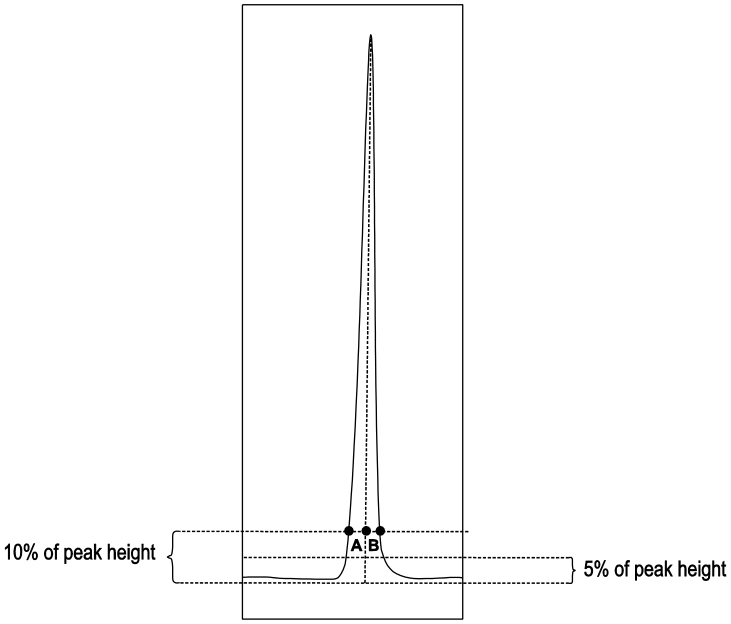


Figure 3.5 Calculation of peak asymmetry factor and peak tailing [351]

The results of the analyses of CP and BV are summarized in Table 3.4.

Table 3.4 Chromatographic parameter analysis of CP and BV using a mobile phase of MeOH and water (70:30% v/v)

Chromatographic Parameter	Results
Resolution Factor, R_s	0.8
Peak Asymmetry Factor of CP Peak, A_s	1.13
Peak Tailing Factor of CP, PTF	1.09

The Resolution Factor (R_s) is a useful indicator of the degree and quality of a separation between components of a mixture. The R_s is normally a measure of the extent of separation between two or more peaks of interest [352]. A R_s of > 2.0 normally indicates that an appropriate separation has been achieved between peaks and that desirable resolution has been achieved [353]. A mobile phase of MeOH and water (70:30% v/v) with a flow rate of 1.0 mL/min resulted in a R_s value of 0.8, indicating that CP and BV were not well resolved. This separation was therefore not acceptable for HPLC analysis of CP. The Peak Asymmetry Factor (A_s), also known as the Peak Tailing Factor (PTF), is an important parameter for assessing band shape. The A_s is usually measured at 10% of the full peak height, whereas PTF is calculated at 5% of the full peak height [351]. An A_s of 1.13 and a PTF of 1.09 was established for CP, indicating that the peak was symmetrical and no tailing was evident. These values confirm that a Nova-Pak® C_{18}, 150 mm x 3.9 mm i.d., 4 µm column is satisfactory for this purpose. The FDA Guidance [353] recommends that PTF should be less than or equal to 2.0 for a column to be considered appropriate for a separation.

MeOH has the ability to form hydrogen bonds with water and this might affect the efficiency of a separation [104]. Different concentrations of an organic modifier in a mobile phase may also change the shape of eluting peaks [267,354]. It was therefore necessary to investigate the effect of different concentrations of MeOH on the retention times and R_s factors of CP and BV respectively. The effect of changing mobile phase composition on the retention times and R_s factors are shown in Figures 3.6 and 3.7 respectively. The actual values of the retention times and R_s factors of CP and BV are summarized in Table 3.5.

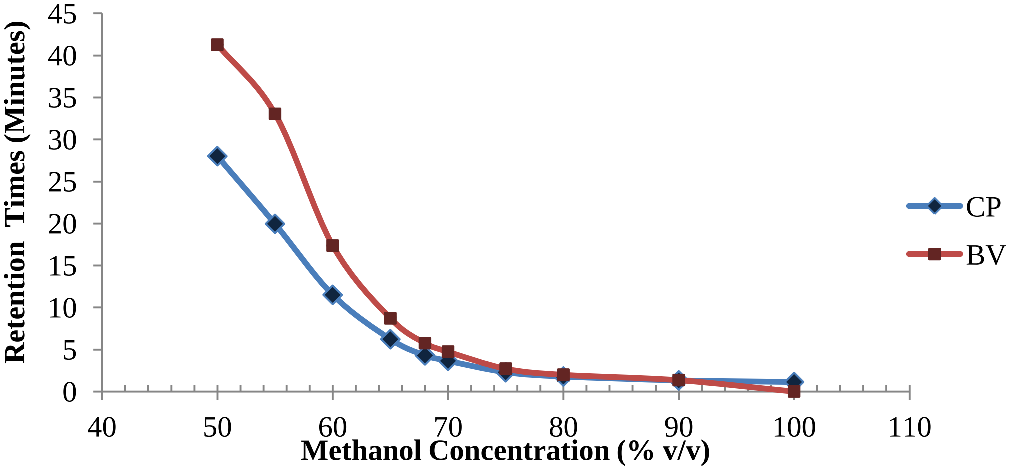


Figure 3.6 Effect of MeOH concentration on the retention time of CP and BV

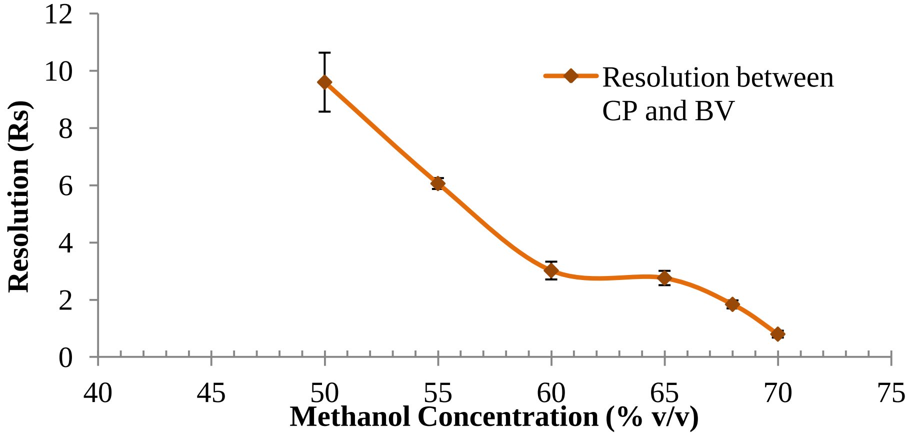


Figure 3.7 Effect of MeOH concentration on the resolution factor of CP and BV

Table 3.5 A summary of the effect of percent MeOH content on retention times and resolution factors of CP and BV

MeOH %v/v	**Retention Time (Minutes)**		**Comments**	**Resolution Factor (R_s)**
	CP	**BV**		
50	28	41.27	Highly separated peaks	9.6±1.03
55	19.94	33.03	Highly separated peaks	6.06±0.19
60	11.49	17.35	Highly separated peaks	3.02±0.31
65	6.21	8.7	Well separated peaks	2.76±0.25
68	4.29	5.75	Well separated peaks	2.54±0.14
70	3.63	4.73	Well separated peaks	0.8±0.01
75	2.28	2.71	Overlapping peaks	-
80	1.77	1.99	Overlapping peaks	-
90	1.31	1.35	Overlapping peaks	-
100	1.12	1.00	-	-

The data given in Figure 3.6 and Table 3.5 reveal that an increase in organic modifier content results in a drastic decrease in the retention time of CP and BV. As the percent volume of MeOH increases from 50% v/v to 100% v/v, the solubility of the drug in MeOH increases and therefore enhances the rapid elution of CP from silica-based columns. When the content of MeOH is increased from 50% v/v to 60% v/v, the retention time of CP and BV decreases by almost 50% from 28 minutes and 41 minutes to 11 minutes and 17 minutes respectively. However when a 50% v/v MeOH concentration was increased to 55% v/v MeOH, a significant decrease in retention times of CP and BV, from 28 minutes and 41 minutes to 19 minutes and 33 minutes was observed. In general even a slight increase in the MeOH content of the mobile phase mixture increases the dispersive character of the mobile phase, resulting in the molecule eluting rapidly. At a MeOH content between 70% v/v and 100% v/v, the retention time of CP and BV was reduced to approximately one minute and eluted close to the solvent front. At high concentrations of MeOH, the mixture consists largely of MeOH and water associated with MeOH and therefore CP and BV elute too rapidly from the silica-based analytical columns to produce an adequate separation.

The data in Figure 3.7 reveal that an increase in organic modifier content results in a decrease in the R_s factors for CP and BV. At MeOH concentration between 50% v/v and 60% v/v, CP and BV were highly resolved from each other, with high R_s and long run times. This might be due to the fact that these molecules have different lipophilic characteristics, BV is more lipophilic than CP and is retained on the silica-based stationary phase and elutes slowly. At MeOH concentrations between 65% v/v and 68% v/v, the peaks of CP and BV were well separated, with an R_s value of approximately 2.5. At those concentrations, the run time was approximately 10 minutes, which is appropriate for an HPLC method. As the MeOH content increases further to 80% v/v, CP and BV elute very rapidly from the column with very short retention times and both peaks overlap, resulting in no resolution (Table 3.5).

3.4.6. Flow Rate Selection

The flow rate is the volume of mobile phase passing through a column in unit time. Flow rate is usually measured at the outlet of the column at ambient pressure (Pa) and temperature (Ta, in K) [355]. Reported analyses of CP indicate that a flow rate of 1.0 mL/min was used in most HPLC methods, as seen from the data listed in Table 3.3. However it was important to investigate the effect of different flow rates on the retention times and R_s factors for CP and BV with a mobile phase composition of MeOH and water (65:35% v/v). The effect of different flow rates on the factors affecting the retention time and resolution of CP and BV are depicted in Figures 3.8 and 3.9. The actual values of retention times and R_s factors of CP and BV due to the effect of different flow rates are summarized in Table 3.6.

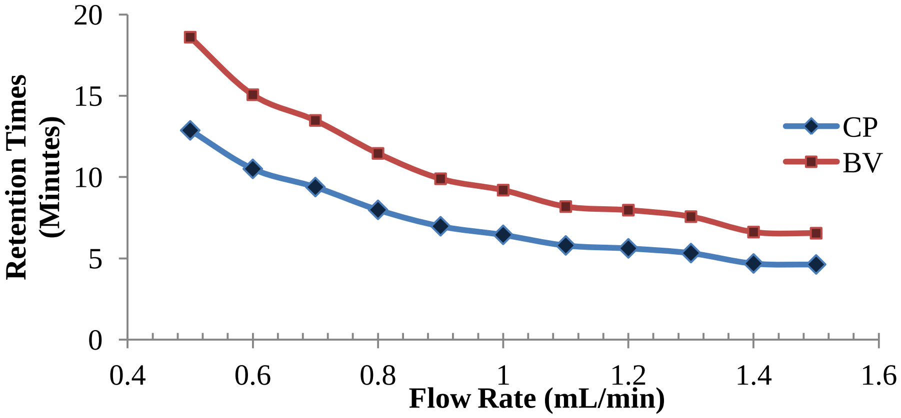


Figure 3.8 Effect of flow rate on the retention time of CP and BV

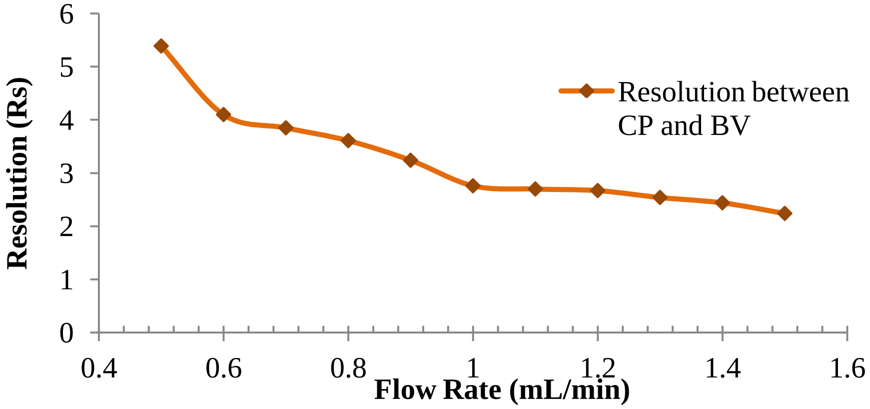


Figure 3.9 Effect of flow rate on the resolution factor of CP and BV

Table 3.6 A summary of the effect of flow rates on the retention times and resolution factors of CP and BV

Flow Rate mL/min	**Retention Time (Minutes)**		**Comments**	**Resolution Factor (R_s)**
	CP	**BV**		
0.5	12.87	18.6	Highly separated peaks	9.6±1.03
0.6	10.5	15.06	Highly separated peaks	6.06±0.19
0.7	9.38	13.48	Highly separated peaks	3.02±0.31
0.8	7.98	11.45	Well separated peaks	2.76±0.25
0.9	6.97	9.89	Well separated peaks	2.54±0.14
1.0	6.44	9.19	Well separated peaks	2.15±0.12
1.1	5.79	8.18	Well separated peaks	2.70±0.08
1.2	5.61	7.96	Separated	2.67±0.02
1.3	5.32	7.56	Separated	2.54±0.03
1.4	4.68	6.61	Separated	2.44±0.04
1.5	4.62	6.54	Separated	2.24±0.05

The data shown in Figure 3.8 indicate that an increase in flow rate from 0.5 mL/min to 1.5 mL/min decreases the retention time of CP from 12.47 minutes to 4.62 minutes and that of BV from 18.60 minutes to 6.54 minutes. It can be noted that the retention times of CP were lower with increasing flow rates than were those of BV. An increase in flow rate from 0.5 mL/min to 1.0 mL/min significantly reduces the retention time of CP and BV 2 fold, as indicated in Figure 3.8 and Table 3.6. However, an increase in flow rate from 1.0 mL/min to 1.5 mL/min slightly decreases the retention times of CP from 6.44 minutes to 4.62 minutes and the R_s factor is also affected by the different flow rates. As the flow rate increases from 0.5 mL/min to 1.5 mL/min, the R_s for CP and BV decreased significantly from 9.6 to 2.24. A low flow rate results in highly separated peaks due to the small volume of mobile phase passing through the column per minute compared to that observed for higher flow rates. The use of high flow rates reveals separation between CP and BV with minimal resolution. At a flow rate of 1.0 mL/min, CP and BV have retention times of 6.44 minutes and 9.19 minutes and were well resolved with a R_s of 2.15. This flow rate was chosen for further RP-HPLC development and validation.

3.5. OPTIMIZATION DESIGN AND ANALYSIS USING CCD

3.5.1. Background

Retention time of a compound in HPLC is a function of numerous parameters. A systematic approach is required to obtain a suitable separation of peaks that are evenly distributed throughout a chromatogram so as to prevent peak overlap [356]. Therefore the successful analysis of drugs in complex pharmaceutical formulations by RP-HPLC relies on the optimization of chromatographic conditions, sample preparation and post–column detection [265,356,357]. The traditional method development or the optimization approach was based on a one-factor-at-a-time design, in which the effect of a single factor on response(s) was monitored whilst all other factors were held constant. Consequently there was no evaluation of the interaction effect of factors on responses. This has prompted scientists to develop other experimental designs for identifying optimum conditions [356,358]. Statistical experimental designs are efficient and have been used for decades as an optimization strategy [359]. The Design Of Experiment (DOE) is a method which enables scientists to simultaneously evaluate the effects and interactions of a large number of factors with a limited number of runs. In this respect, Factorial Design (FD), Fractional Factorial Designs (FFD) and Central Composite Design (CCD) using RSM are important tools for determining the optimal conditions of a process or separation. The major benefit of applying a FFD or CCD in comparison with FD is that a reduced number of experiments needs to be carried out and uses the best experimental points to obtain maximum information about a process [356,357]. Optimization enables the selection of optimal experimental conditions and provides maximum relevant information by analyzing experimental data. The optimization of this RP-HPLC method was performed using RSM for defining the optimal chromatographic conditions of the separation. The impetus for this study was therefore to optimize a RP-HPLC method for the simultaneous determination of CP and BV by investigating the effects of different factors on chromatographic responses with the aid of a CCD.

3.5.2. HPLC Variables Investigated

Mellor [360] explained that the factors affecting retention time, the resolution factor and peak shape are flow rate, column length, particle size of packing, polarity, amount of bonded phase, polarity of mobile phase, column temperature and data handling systems (indirectly). Excellent theoretical analyses may be found in books on chromatography, most notably that by Kirkland and Synder [238]. The objective of the present study was to assess in a simple and practical way some of the factors affect retention and resolution efficiency. Organic modifier content (X_1), flow rate (X_2) and column temperature (X_3) were therefore selected according to CCD principles as three main factors to monitor in this study. The levels of the independent variables were set at -α^a, -1, 0, 1 and +α^a. The experimental factors and levels of coding are summarized in Table 3.7.

Table 3.7 Actual and coded values of the HPLC variables monitored using CCD

Variable	**Symbol**	**Real Value of Coded Levels**				
		-α^a	**-1**	**0**	**+1**	**+α^a**
Methanol Concentration (% v/v)	**X_1**	48.2	55	65	75	81.8
Flow Rate (mL/min)	**X_2**	0.5	0.8	1.0	1.2	1.5
Column Temperature (°C)	**X_3**	22.0	24.0	28.0	32.0	35.0

α^a= 1.68 (star or axial point in the case of three independent variables)

These levels were selected according to preliminary work undertaken in § 3.4 for the selection of mobile phase composition and flow rate. In other words the studies covered the entire region and include the space where optimal levels are likely to exist. The independent variables were coded to the (-1, +1) interval where the low and high levels are coded as -1 and +1 respectively. The axial points are located at (±α, 0, 0), (0, 0, ±α) and (0, 0, ±α), where α is the distance of the axial point from the center point and this makes the design rotatable. In these studies, the α value was fixed at 1.68 (rotatable). The experimental sequence was randomized to minimize the effects of uncontrolled factors. Variables that were not evaluated in these studies were considered as held-constant factors.

3.5.3. HPLC Responses

The retention time of CP (Y_1) and BV (Y_2), peak asymmetry factor of the CP Peak (Y_3), resolution factor between CP and BV peaks (Y_4) and run time (Y_5) were selected as the HPLC responses to be monitored.

3.5.4. Experimental Design

A 2^3 full factorial CCD was used for evaluation of three independent variables consisting of eight factorial points, six axial points and six replicates at the center points. This resulted in the need to conduct 20 experiments as calculated using Equation 3.4.

$$\begin{aligned} N &= 2^n + 2n + nc \\ &= 2^3 + 2 \times 3 + 6 = 20 \end{aligned} \qquad \textit{Equation 3.4}$$

Where

N=Total number of experiments required
n=Number of factors

A CCD was generated to evaluate the Response Surface Models for retention time, A_s, R_s factors and run time of CP and BV using different HPLC variables to study effect of experimental factors on the responses of interest. The CCD data are summarized in Table 3.8.

Table 3.8 Experimental design for the HPLC method optimization for the analysis of CP

Run	**Variables**						**Responses**				
	Methanol Concentration (X_1)		**Flow Rate (X_2)**		**Column Temperature (X_3)**		**RT (CP)* (Y_1)**	**RT (BV)* (Y_2)**	**As (CP)* (Y_3)**	**Rs* (Y_4)**	**Run Time (Y_5)**
	Level	**% v/v**	**Level**	**mL/min**	**Level**	**°C**	**Minutes**	**Minutes**	**-**	**-**	**Minutes**
1	-1	55	+1	1.2	+1	32.0	14.40	23.00	1.25	4.67	25
2	0	65	0	1.0	0	28.0	5.42	7.45	1.05	2.00	10
3	0	65	0	1.0	-1.68	22.0	5.890	8.25	1.10	2.02	10
4	0	65	-1.68	0.5	0	28.0	10.93	15.21	1.17	2.40	20
5	+1	75	+1	1.2	+1	32.0	1.88	2.20	0.93	0	5
6	-1	55	-1	0.8	+1	32.0	21.77	35.08	1.22	5.50	40
7	0	65	+1.68	1.5	0	28.0	3.91	5.44	1.00	2.00	8
8	0	65	0	1.0	0	28.0	5.89	8.22	1.10	2.00	10
9	0	65	0	1.0	0	28.0	5.90	8.22	1.05	2.20	10
10	0	65	0	1.0	0	28.0	6.04	8.46	1.10	2.10	10
11	0	65	0	1.0	0	28.0	6.03	8.44	1.05	2.00	10
12	-1.68	48.2	0	1.0	0	28.0	50.0	62.25	1.32	10.10	65
13	-1	55	+1	1.2	-1	24.0	17.41	28.74	1.23	4.50	30
14	0	65	0	1.0	+1.68	35.0	5.33	7.27	1.15	2.12	10
15	+1.68	81.8	0	1.0	0	28.0	1.67	1.83	1.00	0	5
16	+1	75	-1	0.8	-1	24.0	3.05	3.67	0.90	0	5
17	+1	75	+1	1.2	-1	24.0	2.02	2.44	0.91	0	5
18	0	65	0	1.0	0	28.0	6.00	8.41	1.00	2.00	10
19	+1	75	-1	0.8	+1	32.0	2.86	3.31	0.94	0	5
20	-1	55	-1	0.8	-1	24.0	26.63	37.45	1.20	5.10	40

***RT (CP)= Retention time of CP, RT (BV)= Retention time of BV, A_s (CP)= Peak asymmetry factor of CP peak, R_s= Resolution factor for CP and BV peaks**

To define the relationship between the responses and the input variables, a quadratic regression model can be applied on the basis of Multiple Linear Regression (MLR) [361]. The selected model used 10 coefficients including a constant term, β_0, three main effects, three quadratic terms and three interaction terms, as indicated in Equation 3.5 [361-363].

$$Y = \beta_0 + \sum_{i=1}^{n} \beta_i x_i + \sum_{i=j=1}^{n} \beta_{ij} x_i x_j$$

Equation 3.5

Where

Y=Predicted response of the process
β_0=Constant coefficient
β_i=Linear coefficients
β_{ij}=Interaction coefficients
x_i, x_j=Coded levels of the factors *viz.*, independent or control variables

Based on the CCD method, each response was used to develop an empirical model that correlated the responses to the three HPLC variables using a second-degree polynomial equation as shown in Equation 3.6.

$$Y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{33} x_3^2$$

Equation 3.6

Where

Y=Response to be modeled
β=Regression coefficient
x_1, x_2, x_3=Factors X_1, X_2, X_3

3.5.5. Model Fitting and Statistical Analysis

Statistical analysis of the HPLC data was performed by fitting the data to models using Design-Expert® software (version 8.02, Stat-Ease Inc., Minneapolis, USA). The significance of the parameters can be assessed by using classical statistical tools such as Analysis of Variance (ANOVA). ANOVA showed that the correlation between HPLC variables and their HPLC responses was most suitably described by a quadratic polynomial model. A quadratic polynomial equation was therefore generated for each HPLC response from the CCD.

3.5.6. Quadratic Polynomial Equations, Analysis of Variance and Regression Coefficients

To have a better grasp of the significance of HPLC factor influence on response, it is useful to observe the variables on comparable scales. Experimental data are easily coded. The coefficients that are obtained are approximately on the same scale and significance could be determined by examining the magnitude of the reported coefficients. Provided that the data are coded correctly, a larger value means greater significance [364]. The results of the coefficient data are presented in Equations 3.7 - 3.11 for retention time of CP and BV, A_s factor of CP, R_s for CP and BV and for run time.

Retention time of CP (Y_1) = $6.03 - 11.11X_1 - 2.23X_2 - 0.67X_3 + 6.58X_1^2 + 0.066X_2^2 - 0.57X_3^2 + 1.82X_1X_2 + 0.94X_1X_3 - 0.24X_2X_3$ ***Equation 3.7***

Retention time of BV (Y_2) = $8.37 - 15.69X_1 - 2.90X_2 - 0.76X_3 + 8.32X_1^2 + 0.64X_2^2 - 0.26X_3^2 + 2.31X_1X_2 + 0.94X_1X_3 - 0.41X_2X_3$ ***Equation 3.8***

Peak asymmetry factor of CP peak (Y_3) = $1.06 - 0.13X_1 - 0.017X_2 + 0.022X_3 + 0.027X_1^2 + 4.803E\text{-}004X_2^2 + 2.248E\text{-}003X_3^2 - 7.5E\text{-}003X_1X_2 + 2.5E\text{-}003X_1X_3 - 2.5E\text{-}003X_2X_3$ ***Equation 3.9***

Resolution between CP and BV peaks (Y_4) = $2.07 - 2.69X_1 - 0.15X_2 + 0.054X_3 + 0.91X_1^2 - 0.093X_2^2 - 0.14X_3^2 + 0.18X_1X_2 - 0.071X_1X_3 - 0.029X_2X_3$ ***Equation 3.10***

Run time (Y_5) = $10.03 - 15.81X_1 - 3.31X_2 - 0.37X_3 + 8.65X_1^2 + 1.23X_2^2 - 0.19X_3^2 + 3.13X_1X_2 + 0.63X_1X_3 - 0.62X_2X_3$ ***Equation 3.11***

The polynomial equations represent the quantitative effects of HPLC variables X_1, X_2, and X_3 and their interactions on the five responses monitored *viz.*, Y_1, Y_2, Y_3, Y_4 and Y_5. The values of the coefficients X_1, X_2, and X_3 are related to the effects of these variables on the five responses. Coefficients with more than one factor term and those with higher order terms represent interaction terms and a quadratic relationship, respectively. A positive value (+) represents an effect that favors the optimization while a negative value (-) indicates an antagonistic effect. The values of X_1, X_2, and X_3 were substituted in the equations to obtain theoretical values for the HPLC responses. The quality of the quadratic response surface model developed was also based on R^2 and Standard Deviation (SD) values. The closer the value for R^2 is to unity and the smaller the SD, the more accurate the response that can be predicted by the model. If a R^2 value is > 0.9, there is a good correlation between the experimental and predicted HPLC responses [365]. A summary of the R^2 values for these studies is listed in Table 3.9.

Table 3.9 Model summary statistics of the appropriate HPLC quadratic response surface models

	Response Factor		**Quadratic Response Surface Model**					
	SD*	**F-value**	**Prob > F**	**R^2***	**Adj R^2***	**Pred R^2***	**Adeq Prec***	**C.V (%)***
Y_1	3.47	22.56	<0.05	0.9531	0.9108	0.6434	17.63	34.08
Y_2	2.32	93.99	<0.05	0.9883	0.9778	0.9108	35.19	16.20
Y_3	0.061	7.50	<0.05	0.8710	0.7548	0.1738	10.22	5.61
Y_4	0.50	50.49	<0.05	0.9785	0.9591	0.8389	25.98	19.61
Y_5	2.17	111.58	<0.05	0.9901	0.9813	0.9241	37.64	13.06

***SD= Standard Deviation, R^2= Regression Coefficient, Adj R^2= Adjusted R^2, Pred R^2= Predicted R^2, Adeq Prec= Adequate Precision, C.V (%)= Coefficient of Variation**

The R^2 values indicate that 95.31%, 98.55%, 87.10%, 97.85% and 99.01% of the total variation in Y_1, Y_2, Y_3, Y_4 and Y_5 can be attributed to the HPLC variables respectively. The R^2 values of 0.9531, 0.9883, 0.9785 and 0.9901 for Equations 3.7, 3.8, 3.10 and 3.11 were deemed appropriate to validate the fit due to all R^2 values being > 0.9. Their predicted and observed values were found to be in good agreement except for retention time of CP (Y_1). Moreover Equation 3.11 with an R^2 value of 0.9901 was considered to be in closest agreement between the experimental and predicted run times for this model. However Equation 3.9, with an R^2 of 0.8710 was deemed inappropriate to validate the fit which led to large variation in A_s factor of CP predicted when using this model. In the present study, the adjusted R^2 were well within acceptable limits of $R^2 \geq 0.8044$, except for Equation 3.9. The adjusted R^2 for Equations 3.7, 3.8, 3.10 and 3.11 revealed that the experimental data are well fitted to the second-order polynomial equations. In addition to these models, the predicted R^2 values for retention time of BV (Y_2), R_s factor between CP and BV (Y_4) and run time (Y_5) are in reasonably close agreement with the adjusted R^2 values, indicating the reliability of these models. However the predicted R^2 values for the retention time of CP (Y_1) and A_s factor of CP peak (Y_3) were not in close agreement to the adjusted R^2 values, showing the poor reliability of those quadratic models (Table 3.9).

Furthermore the higher values of > 4 for adequate precision indicate an adequate signal has been observed [365]. All responses showed adequate signals since their values are > 4. A relatively low value for the coefficient of variation (C.V) indicates the precision and reliability of the experiments performed and the A_s factor of CP peak had a low coefficient of variation, showing that the model was reproducible with high precision. ANOVA analysis of the quadratic regression models was used to estimate the quality of the quadratic response surface models and to validate the models [366]. These models were tested at a 95% confidence interval. ANOVA analysis for the response surface quadratic models for Y_1, Y_2, Y_3, Y_4 and Y_5 of the HPLC method optimization are summarized in Table 3.9. F-values of 22.56, 93.99, 7.50, 50.49 and 111.58 for CP and BV retention time, A_s factor for CP, R_s factor between CP and BV and run time were obtained, implying that all quadratic models are significant. The largest F-value of 111.58 was observed for run time, indicating that this model was highly significant, followed by BV retention time with a F-value of 93.99. The P-value is a tool that can be used to check the significance of each coefficient, which also indicates the strength of interaction between each independent variable. The smaller the P-value, the more evidence there is to support rejecting the null hypothesis [367]. Therefore a value of Prob > F < 0.05 confirms that the five quadratic models were significant.

3.5.7. Contour Plots and Response Surface Plots

Contour (2-Dimensional) and response surface (3-Dimensional) plots were constructed according to the model equations. Contour and response surface plots between factors can be generated using Design-Expert® software. The impact of independent factors *viz.*, MeOH concentration, flow rate and column temperature at different levels on the five HPLC responses is shown on 2-D and 3-D plots.

3.5.7.1. Retention Time of CP and BV

The largest F-values of 140.09 and 624.92 were observed for MeOH concentration (X_1), indicating that this factor had the most significant effect on CP and BV retention times in comparison to other HPLC variables. Flow rate (X_2) is another variable that was seen to have an effect on the retention times of both analytes since the F-values were 5.63 and 21.30 respectively. However it was observed that flow rate has more effect on the retention times of BV than of CP. The increase in effect on the retention time of BV might be due to the presence of two extra carbon chains on ring D at position 17, leading to a high lipophilicity of the molecule.

From the data presented, it is clear that even small structural differences can express themselves as a change in chromatographic behavior [368]. Minor structural modifications often can have unpredictable effects on the retention times of corticosteroids, especially if the modification causes a change in the conformation of the molecule which results in changes in its overall lipophilicity [368].

The quadratic effects of MeOH concentration (X_1^2) on CP and BV retention times were also found to be significant (P-value < 0.0001). The effects of column temperature (X_3) on the retention times of those molecules was quite similar, with F-values of 0.51 and 1.46 respectively, indicating that these parameters had the least effect on the retention time of these molecules. Furthermore, it was observed that all three HPLC variables studied have an antagonistic effect on the retention time of CP and BV. The contour and 3-D response surface plots that were constructed to show the interaction effects of HPLC variables on the retention times of CP (Y_1) are depicted in Figures 3.10., 3.11., 3.12. and 3.13. The contour plots (Figures 3.10 and 3.11) reveal that flow rate has a minimal effect on the retention times of CP and BV, whereas MeOH concentration has the most significant effect on this HPLC parameter. An increase in MeOH concentration from 55% v/v to 75% v/v decreased the retention time of CP from 26.63 minutes to 1.88 minutes (Figure 3.10) and decreased the retention time of BV from 37. 45 minutes to 2.20 minutes (Figure 3.11).

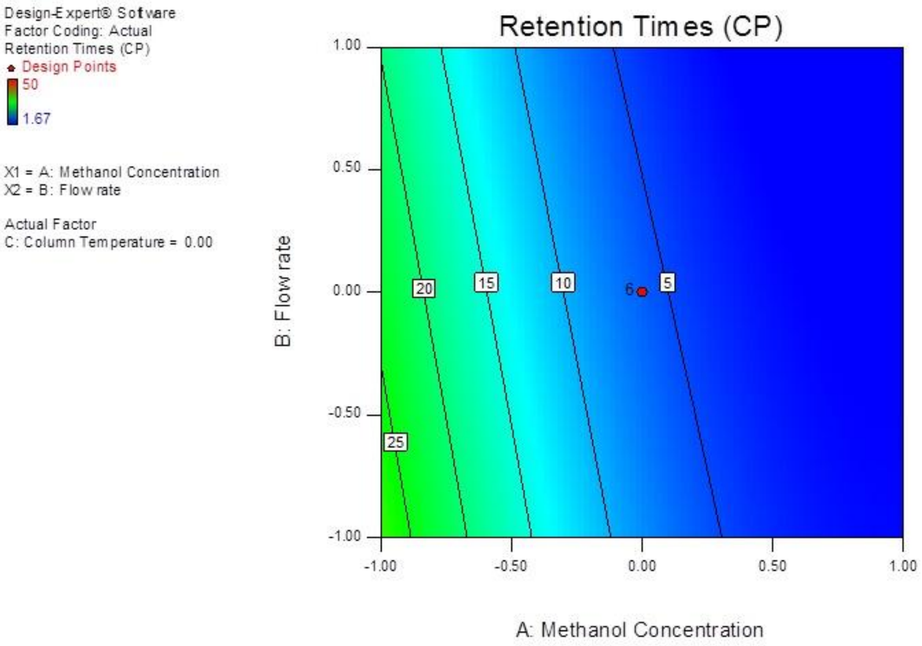


Figure 3.10 Contour plot showing the effect of MeOH concentration and flow rate on the retention time of CP

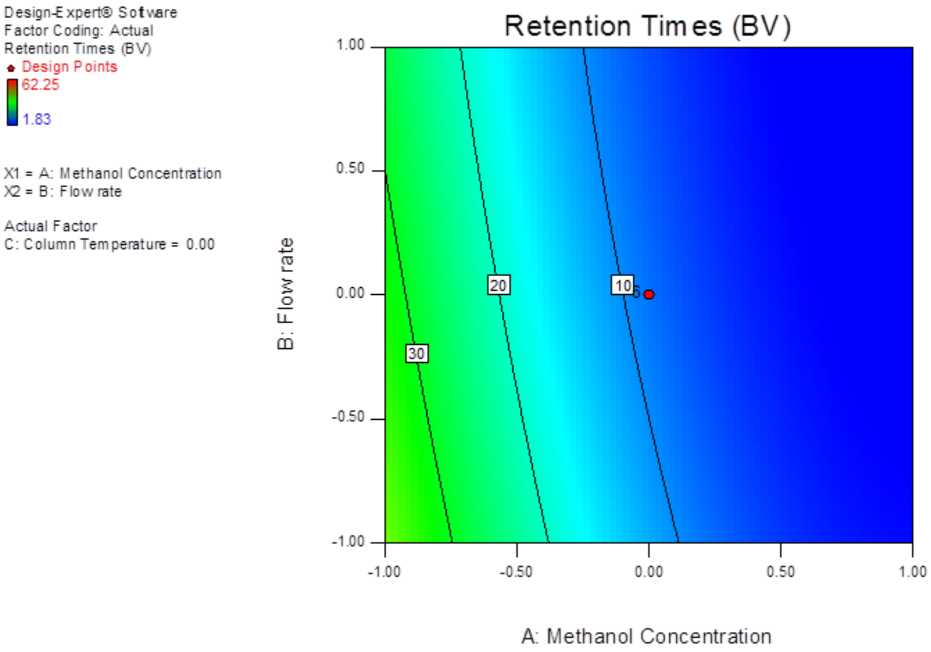


Figure 3.11 Contour plot showing the effect of MeOH concentration and flow rate on the retention time of BV

RP-HPLC uses a non-polar stationary phase and an aqueous moderately polar mobile phase to effect a separation. It is common for the silica-based stationary phase to have surfaces modified with RMe_2SiCl, where R is a straight chain alkyl group such as $C_{18}H_{37}$ or C_8H_{17} [369,370]. With such stationary phases, the retention times are longer for molecules of low polarity whereas polar molecules elute more readily from these systems. An investigator can increase the retention time of a compound by adding additional water to the mobile phase. This alters the affinity of a hydrophobic analyte for a hydrophobic stationary phase to be stronger relative to the more hydrophilic mobile phase. Similarly, an investigator can decrease the retention time of an analyte by adding organic solvent to the mobile phase [370,371]. The 3-D response surface plot of CP retention time (Figure 3.12) indicates that an increase in MeOH concentration has a major and significant effect on the HPLC response, with resultant retention times ranging between 26 minutes and 1 minute, while an increase in flow rate resulted in a slight decrease in CP retention time from 17 minutes to 10 minutes. On the other hand the 3-D response surface plot of BV retention time (Figure 3.13) shows a similar effect to that observed for CP, with slightly longer retention times. A decrease in the retention time for BV from 37 minutes to 15 minutes was observed with increasing flow rate and an increase in MeOH concentration resulted in a decrease in the retention time for BV. A greater effect was observed for flow rate for BV than that observed in CP.

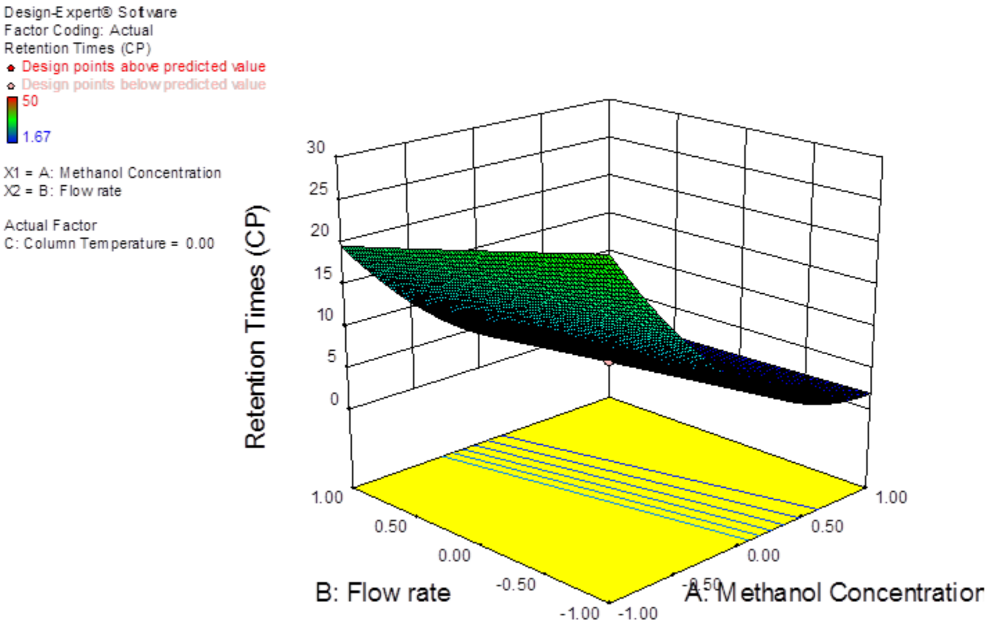


Figure 3.12 3-D response surface plot showing the effect of MeOH concentration and flow rate on the retention time of CP

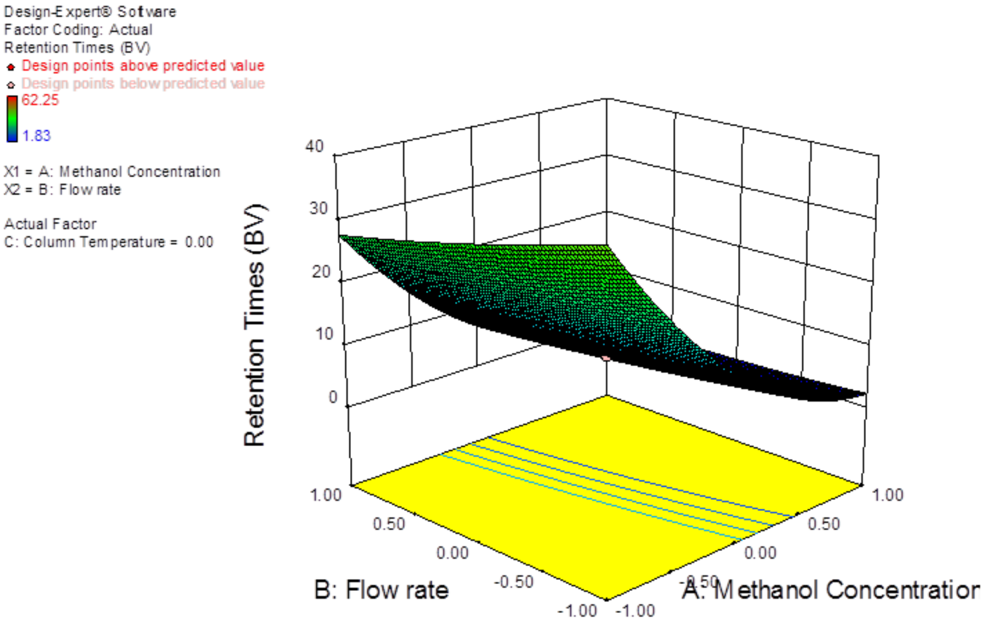


Figure 3.13 3-D response surface plot showing the effect of MeOH concentration and flow rate on the retention time of BV

3.5.7.2. Peak Asymmetry of CP (A_s)

The largest F-value of 61.64 was observed for MeOH concentration (X_1), indicating that this factor has the most significant effect on A_s factor of CP in comparison to other HPLC variables investigated. The flow rate (X_2) and column temperature (X_3) were other variables evaluated that were shown to have little or no effect on the A_s factor for CP since the resultant F-values were 1.02 and 1.82 respectively. However it was observed that column temperature had slightly more of an effect on A_s factor than did the flow rate of the separation. Furthermore, it was observed that two HPLC variables *viz.*, MeOH concentration and flow rate had an antagonistic effect on the A_s factor for CP while column temperature has a synergistic effect on that factor. The contour and 3-D response surface plots that were constructed to show the interaction effects of HPLC variables on the A_s factor for CP (Y_3) are depicted in Figures 3.14 and 3.15. The contour plot (Figure 3.14) shows that MeOH concentration had the most significant effect on this HPLC parameter. An increase in MeOH concentration from 55% v/v to 75% v/v decreased the A_s factor from 1.32 to 0.9.

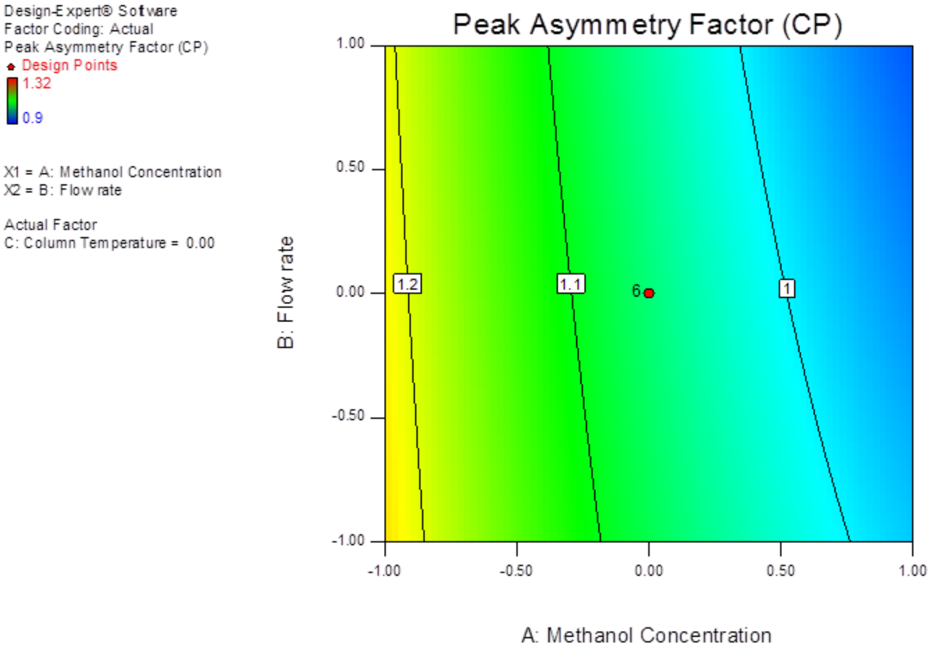


Figure 3.14 Contour plot showing the effects of MeOH concentration and flow rate on CP A_s factor

In general, most chromatographic peaks are not symmetrical, so it is necessary to introduce an asymmetry factor, which characterizes the peak shape [372]. Symmetrical peaks should have a A_s factor close to 1.0 (range: 0.95 – 1.1) and columns producing values of > 1.2 should preferably not be used for a separation [238]. The A_s factor is a measure of peak tailing and values > 1.2 indicate that the peaks are not symmetrical and some peak tailing may be observed [373]. A low MeOH concentration resulted in asymmetrical CP peaks with A_s factors > 1.2. The peak tailing is more probably caused by the high degree of hydration of silica in the column due to high water content, thereby causing peak tailing [374]. The flow rate does not significantly affect the asymmetry of peaks in reversed-phase HPLC, since desorption from hydrophobic surfaces is not affected by flow rate [375] as desorption is the result of reaching a critical concentration of organic modifier. The flow rate therefore plays a minor role as depicted in Figure 3.15.

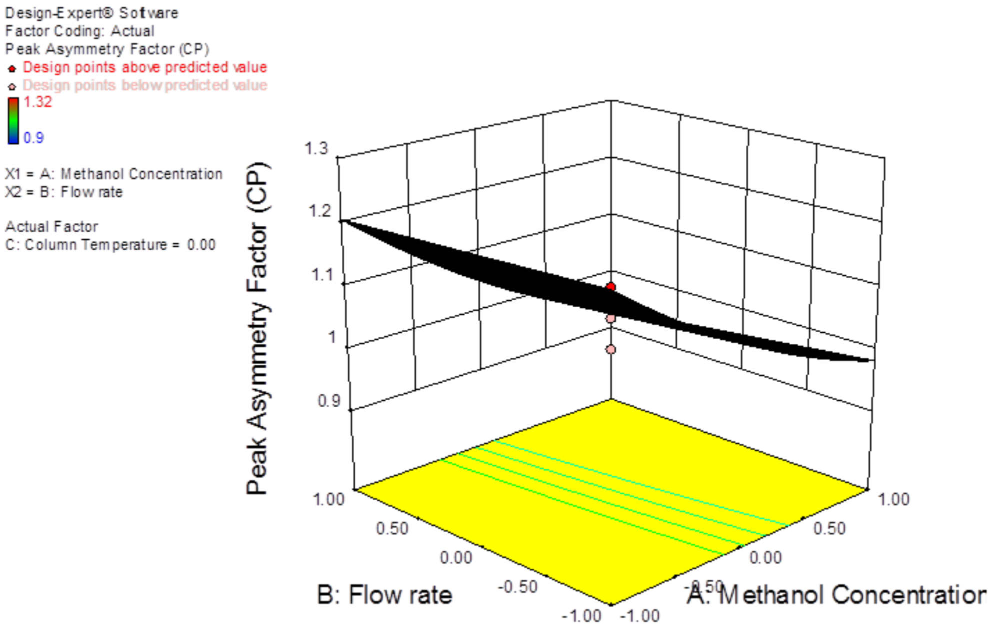


Figure 3.15 3-D response surface plot showing the effect of MeOH concentration and flow rate on CP A_s factor

3.5.7.3. Resolution Factor for CP and BV (**R_s**)

The largest F-value of 399.14 was observed for MeOH concentration (X_1), indicating that this parameter had a significant effect on the R_s between CP and BV. The effects of flow rate (X_2) and column temperature (X_3) were quite similar, with F-values of 1.31 and 0.36 respectively. These parameters had therefore the lowest impact on the R_s factor. Two HPLC variables *viz.*, MeOH concentration and flow rate had an antagonistic effect on the R_s factor, while synergistic effects were contributed through column temperature. The quadratic effects of MeOH concentration (X_1^2) on the R_s factor between CP and BV peaks were also significant (P-value < 0.0001). The contour and 3-D response surface plots that were constructed to show the interaction effects of these HPLC variables on the R_s factor for CP and BV (Y4) are depicted in Figures 3.16 and 3.17. The contour plot (Figure 3.16) reveals that MeOH concentration has the most significant effect on this HPLC parameter. An increase in MeOH concentration from 55% v/v to 75% v/v decreases R_s factor from 5 to 0. Flow rate was found to have no effect on the R_s factor.

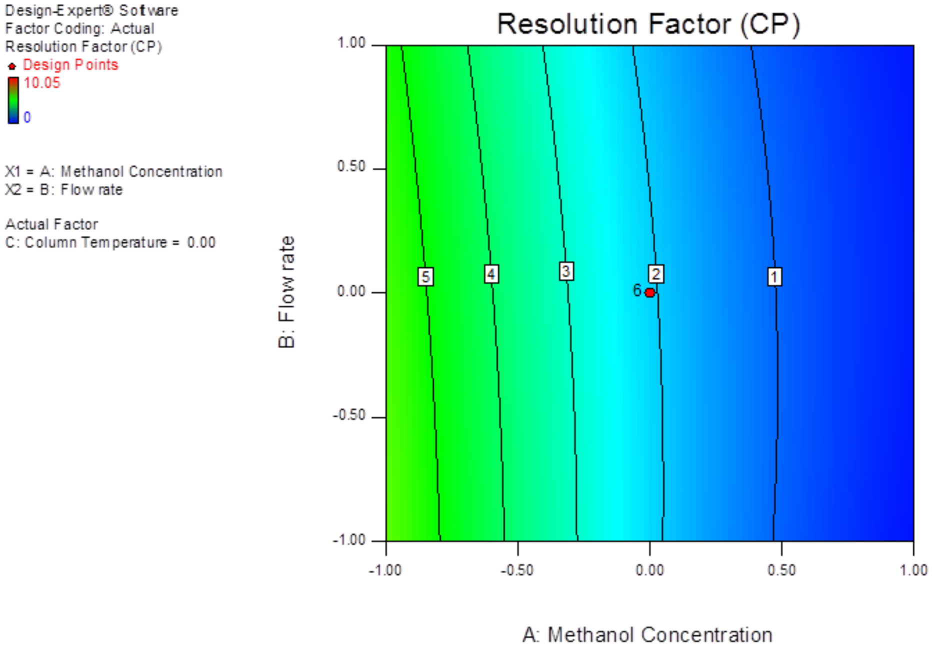


Figure 3.16 Contour plot showing the effect of MeOH concentration and flow rate on the R_s factor for CP and BV

In RP-HPLC, the addition of a polar organic modifier allows sufficient leeway for the solutes to be adequately separated [376]. A very high MeOH concentration of 75% v/v however resulted in no resolution for CP and BV, thus poor separation of these peaks was observed. Conversely low concentrations of MeOH resulted in a high degree of resolution with a factor of approximately 5 for these well separated peaks. The highly resolved peaks exhibited very long run times of approximately 40 minutes. The 3-D response surface plot for the R_s factor for CP and BV (Figure 3.17) reveals that an increase in MeOH concentration was found to have the most significant effect on this HPLC response with R_s factor values ranging between 5 and 0, while an increase in flow rate resulted in only a slight decrease in the R_s factor from 2.1 to 1.7.

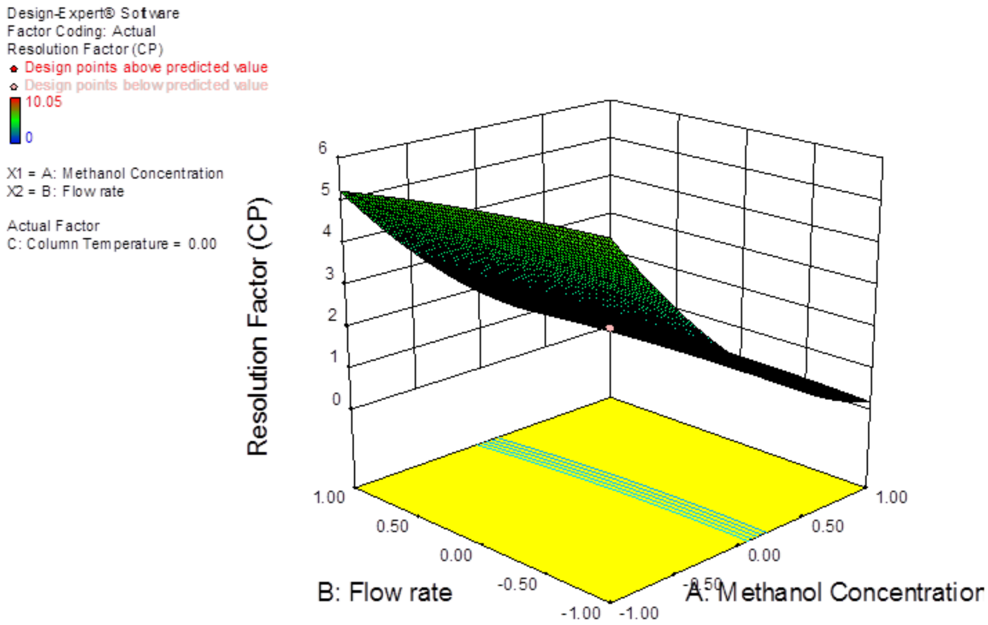


Figure 3.17 3-D response surface plot showing the effect of MeOH concentration and flow rate on resolution factor for CP and BV

3.5.7.4. HPLC Run Time

The largest F-value of 772.06 was observed for MeOH concentration (X_1), indicating that this parameter had the most significant effect on HPLC run time when compared to the other analytical variables investigated. The flow rate (X_2) also had a significant effect on the run time, since the F- and P-values were 31.62 and < 0.05 respectively. The column temperature (X_3) had the lowest impact on run time since the F-value for the parameter was 0.39. Furthermore, it was observed that all three independent variables had an antagonistic effect on the run time. The quadratic and interaction contributions of X_1^2, X_2^2, X_1X_2 and X_1X_3 showed some positive synergistic effects on the run time. The contour and 3-D response surface plots that were constructed to show the interaction effects of the HPLC variables on HPLC run time (Y_5) are depicted in Figures 3.18 and 3.19. The contour plot reveals that the flow rate has a slight effect on run time while MeOH concentration has the most significant effect on this HPLC parameter. An increase in MeOH concentration decreases the run time from approximately 40 minutes to 5 minutes, and an increase in flow rate decreases the HPLC run time from approximately 40 minutes to 30 minutes (Figure 3.18).

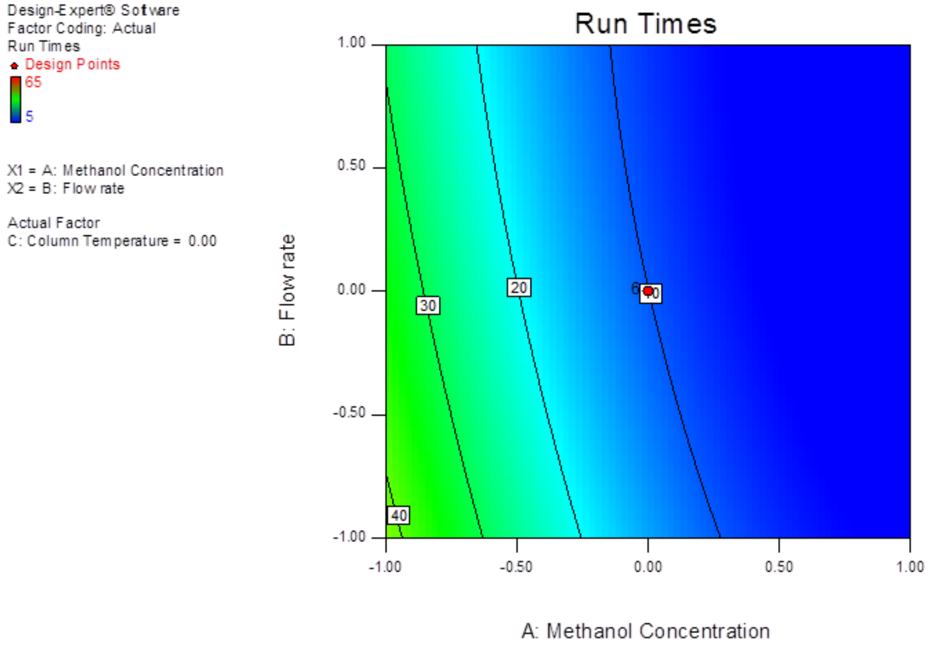


Figure 3.18 Contour plot showing the effect of MeOH concentration and flow rate on the HPLC run time

The HPLC run time is the time taken to complete a chromatographic cycle [377]. Long HPLC run times, such as 40 minutes required to resolve peaks can be tedious, time consuming and expensive. It is advisable to shorten run times and increase selectivity of methods as far as possible [378,379]. Shorter diffusion distances are required and this can be achieved by decreasing the particle size of the silica in particulate columns. However, as the particle size decreases, the backpressure of that column increases proportionally [380]. Pressure is inversely proportional to the square of the particle size. When the particle size is halved, the pressure increases by a factor of four. As the particle sizes get smaller, the interstitial voids get smaller and it therefore becomes increasingly difficult to move the compounds through these smaller spaces [381]. The backpressures in a system present a significant limitation to chromatographers and modern HPLC systems are generally designed to withstand backpressures of up to 6000 pounds per square inch (340 bar). This limit is reached rapidly when trying to decrease HPLC run times by adjusting parameters such as flow rate [380]. The flow rate through a column must be slow enough to allow for diffusion of the analyte(s) of interest into and out of the pores in the column [380]. The 3-D response surface plot for HPLC run time (Figure 3.19.) indicates that an increase in MeOH concentration was found to have the most significant effect on this HPLC response, with run time values ranging between 5 minutes and 30 minutes, while an increase in flow rate resulted in only a slight decrease in the run time.

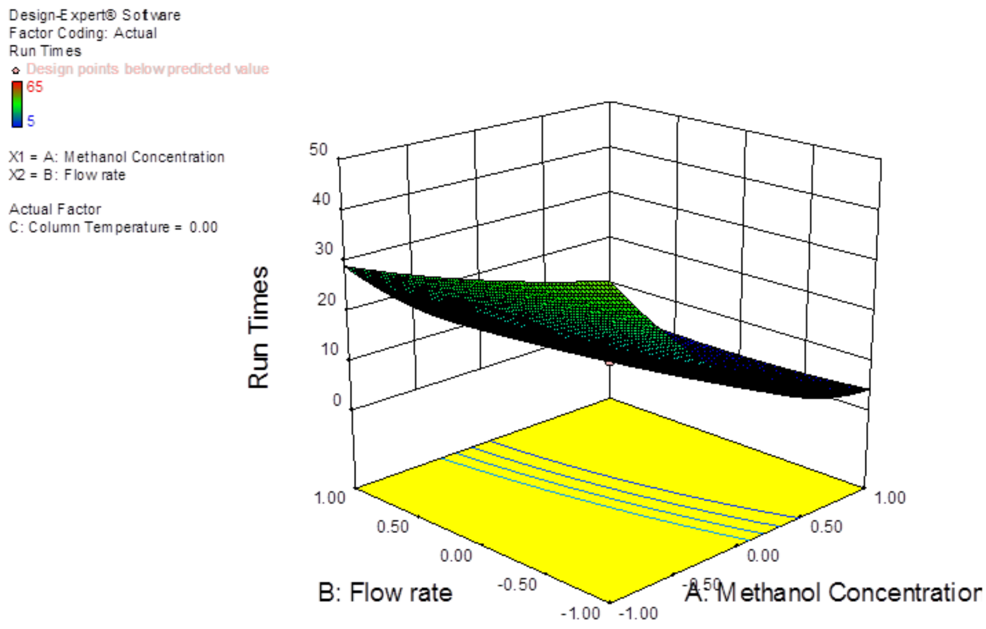


Figure 3.19 3-D response surface plot showing the effect of MeOH concentration and flow rate on the HPLC run time

3.6. OPTIMAL CHROMATOGRAPHIC CONDITIONS

Optimization means improvement of or the best utilization of a specific set of conditions. Thus, one of the aims of an analytical chemist is the successful optimization of a chromatographic separation. To optimize a chromatographic separation, it is necessary to optimize the conditions for the RP-HPLC method. It is clear that different HPLC responses can be manipulated by modification of variables such as MeOH concentration, flow rate and column temperature. The optimal conditions for the HPLC analysis of CP and BV were predicted using the optimization function of the Design-Expert® software. Analysis of contour and 3-D response surface plots showed that the optimal composition of the mobile phase should be established according to adequate separation of CP and BV. The best separation of these two peaks was achieved with the optimal mobile phase MeOH–water composition of 65:35% v/v. With a flow rate of 1.0 mL/min and MeOH–water mobile phase, an optimal separation could be achieved. The predicted values for the HPLC variables *viz.*, MeOH concentration (X_1), flow rate (X_2) and column temperature (X_3) in addition to the respective HPLC responses obtained from numerical optimization are summarized in Table 3.10.

Table 3.10 Predicted values for HPLC variables and their respective responses

HPLC Variables			HPLC Responses					
X_1 (%)	X_2 (mL/min)	X_3 (ºC)	Y_1 (mins)	Y_2 (mins)	Y_3	Y_4	Y_5 (mins)	Desirability
65	1.0	25	6.00	8.60	1.05	2.00	10.00	0.967

The optimal conditions were found in the desirability zone as the desirability value of the model was 0.967. Therefore the optimum HPLC conditions were achieved by using a MeOH concentration of 65% v/v, a flow rate of 1.0 mL/min and a column temperature of 25ºC. The optimal HPLC method revealed a retention time for CP of 6.00 minutes, 8.00 minutes for BV, A_s for CP of 1.05, a R_s factor for CP and BV of 2.00 and an HPLC run time of 10 minutes (Table 3.11).

Table 3.11 Predicted and experimental responses at the optimized conditions

Predicted Responses					Experimental Responses					% Prediction Error				
Y_1	Y_2	Y_3	Y_4	Y_5	Y_1	Y_2	Y_3	Y_4	Y_5	Y_1	Y_2	Y_3	Y_4	Y_5
6.00	8.60	1.05	2.00	10.00	6.17	8.66	1.1	2.00	10.00	2.83	0.69	4.76	0	0

The optimum HPLC variables were found to be near the high concentration of MeOH, flow rate and column temperature. The low magnitude of difference reported as a percentage prediction error indicates the robustness of mathematical modeling and the high prognostic ability of RSM, suggesting that RSM is an efficient tool for this optimization [365]. A typical chromatogram of the separation of CP and BV using the optimized conditions is shown in Figure 3.20.

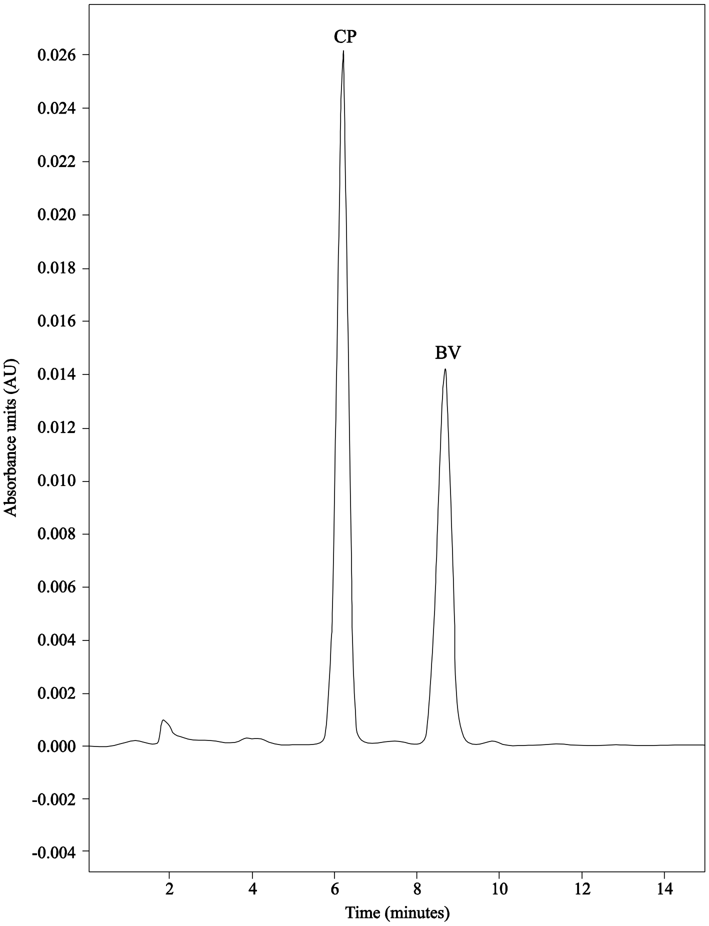


Figure 3.20 Typical chromatogram of the separation of CP (6.0 minutes) and BV (8.0 minutes)

3.7. CONCLUSIONS

HPLC is a liquid chromatographic technique in which a sample is forced through a column under high pressure [244]. In NP-HPLC, the mobile phase is non-polar and the stationary phase is polar. In RP-HPLC, the polarities of the stationary and mobile phases are reversed, allowing only hydrophobic interactions of the stationary phase with the analyte(s) of interest. Polar analyte(s) elute rapidly followed by elution of the non-polar compounds. The benefit of RP-HPLC, which is a more commonly used technique than NP-HPLC, is that small changes in mobile phase composition, flow rate and temperature, can profoundly affect a separation, making it a sensitive and flexible analytical technique [382,383]. RP-HPLC is a sensitive, selective, highly efficient chromatographic technique that is a rapid, repeatable and reliable method for identification and purification purposes [238]. The application of RSM in combination with CCD to optimize the performance of a HPLC method to quantitate CP in cream formulations has been discussed. CCD was used to design an experimental program for modeling the effects of mobile phase composition, flow rate and column temperature on retention times of two analytes, A_s of CP, R_s factor for CP and BV and HPLC run time. Twenty experiments including six center points were conducted. A model-fitting technique using Design-Expert® software (version 8.02, Stat-Ease Inc., Minneapolis, USA) revealed that a correlation existed between the HPLC variables and the responses were most suitably described with quadratic polynomial models. Statistical analysis was undertaken using ANOVA, evaluation of regression coefficient and graphically using contour and 3-dimensional response surface plots.

The retention time of CP, BV, R_s factor between CP and BV and run time generated $R^2 > 0.90$, indicating a good agreement between the experimental and predicted values for these HPLC responses. However the A_s of CP peak generated R^2 values < 0.90, suggesting that the fit to this model was poor as there is a large variations in the experimental data compared to the predicted values using this model. The A_s of CP was found to have the lowest coefficient of variation with a reliable quadratic response surface model. The SD for each quadratic model showed that the predicted value for A_s of CP peak was more accurate and closer to its actual value to the other responses. ANOVA of the quadratic regression models at a 95% level of significance showed that MeOH content and flow rate had a significant effect on all HPLC responses, although MeOH content had the most significant effect. Column temperature had almost no effect on the HPLC responses for CP and BV. The contour plots confirmed that MeOH concentration has the most significant effect on the quality of the HPLC responses. An increase in MeOH concentration resulted in a decrease in retention times of CP and BV, A_s factor of CP, R_s factor for CP and BV and run time. The contour plots showed that flow rate had no effect on the responses, however the 3-D response surface plots showed that flow rate had a slight effect on most responses. An increase in flow rate caused a slight decrease in the HPLC responses. This study demonstrates that CCD can be applied to modeling and optimizing HPLC responses. CCD is an economic method that can be used to generate a maximum amount of data in a short period of time with a limited number of experiments in the laboratory [384].

The experimental responses were close to the predicted values for the optimized HPLC method and the optimum HPLC conditions were found to be a MeOH content of 65% v/v, a flow rate of 1.0 mL/min and a column temperature of 25ºC. The optimum separation conditions yielded a retention time of 6.00 minutes for CP, a retention time of 8.00 minutes for BV, an A_s factor for CP of 1.05, a R_s factor of 2.00 and a 10 minute run time. The optimized method was validated as reported in Chapter 4, *vide infra* to determine whether the method was suitable for application in routine analysis, *in vitro* analysis and content uniformity testing of CP in topical dosage forms. The optimization of a chromatographic method of analysis was performed relatively quickly with a small number of experimental runs, thus minimizing cost and use of materials. The use of experimental design has thus allowed the extraction of maximum chromatographic information within a limited time and will facilitate the validation of the analytical method.

CHAPTER FOUR

VALIDATION OF A STABILITY-INDICATING RP-HPLC METHOD FOR THE QUANTITATION OF CLOBETASOL 17-PROPIONATE (CP) IN CREAMS

4.1. INTRODUCTION

4.1.1. Chromatographic Method Validation

The objective of any analytical measurement is to obtain consistent, reliable and accurate data. The validation of an analytical method is the process by which a method is tested for reliability, accuracy and precision to establish whether it is appropriate for its intended purpose [353,385]. The results from chromatographic method validation can be used to judge the quality, reliability and consistency of analytical data, which is an integral part of good analytical practice [386]. Validation of analytical methods is also required by most regulatory bodies and other quality standard authorities that may have an impact on laboratory practices [385]. Analytical methods need to be validated, verified or revalidated prior to use in routine testing. The validation of analytical methods is also required when transferred to another laboratory, when conditions or method parameters for which the method has been validated change, an instrument with different characteristics is included or when samples in a different matrix are to be tested and the change is outside the original scope of the method [385-387]. Method validation has received considerable attention in the literature from a number of industrial committees and regulatory agencies [353,385,388].

Several guidelines for the development and validation of analytical methods have been produced and recommended by bodies such as FDA [353], ICH [385], International Union of Pure and Applied Chemistry (IUPAC) [389], EURACHEM [390] and the Association of Official Analytical Chemists (AOAC) [391]. The FDA cGMP mandate in Section 211.165 (e) of the Code of Federal Regulations (CFR) stipulates that methods must be validated in respect of accuracy, sensitivity and specificity, and that the reproducibility of the test methods used must be established and documented. Such validation and documentation may be accomplished in accordance with Section 211.194 (a) CFR [353]. These requirements include a statement for each method used in testing a sample that indicates it meets the proper standards of accuracy and reliability, as applied to the analysis of products. The International Organization for Standardization (ISO) and the International Electrotechnical Commission (IEC 17025) have produced guidelines that include a chapter on the validation of methods, with a list of nine validation parameters [386]. The ICH has developed a consensus text on the validation of analytical procedures, and this document includes definitions for eight validation characteristics. ICH have also developed a guidance document with detailed methodology in respect of validation [385]. The AOAC and other scientific organizations have method applications that are validated through multi-laboratory studies [391].

A method should be validated when it is necessary to verify that the performance parameters are adequate when the method is used to meet a particular analytical challenge. Method validation is required when a new method has been developed, a revised method or established method has been adapted to solve a new problem and a review of quality control data indicates that an established method is changing over time. It is also required when an established method is used in a different laboratory and when different analysts use the method. Method validation is also critically used when different equipment is used and in case where it is necessary to demonstrate the equivalence between a new method and a standard analytical approach. Specific areas of analytical practices, in clinical chemistry have specified the validation requirements relevant to certain techniques [387] thereby ensuring that a particular validation terminology together with the statistics used to interpret the data generated is conducted in a manner that is consistent with the requirements relevant to that sector. Official recognition of a method may require characterization of that method using collaborative studies [387,391]. A RP-HPLC method using UV detection for the analysis of CP in cream formulations was validated in accordance with ICH guidelines [385].

4.2. VALIDATION

4.2.1. Calibration, Linearity and Range

Calibration of instruments is the most important step in bioactive compound analysis [324,387]. Good precision and accuracy can be obtained only when an appropriate calibration procedure has been adopted and implemented. For spectrophotometric methods, the concentration of a sample cannot be measured directly and is usually determined using a physical measurement quantity such as the absorbance of an analyte in a solution. This is undertaken to establish an unambiguous empirical or theoretical relationship between the concentration of an analyte in solution and the physical measurement [392]. The calibration of the function $y = g(x)$ can be achieved by fitting the experimental data to an adequate mathematical model. The most common calibration function is usually linear in nature, passes through the origin and is applicable over a dynamic and wide range. For the majority of analytical techniques, the calibration equation as shown in Equation 4.0 is used. For HPLC, deviations from an ideal calibration line can and often do occur [387].

$$y = c + mx \qquad \textit{Equation 4.0}$$

Where

y=Peak area
m=Slope
x=Concentration
c=Intercept

The ICH guidelines [385] recommend that a minimum of five concentration levels of API in combination with an internal standard be used to establish linearity. The calibration standard solutions prepared as described in § 3.3.4, Chapter 3 were injected in ascending order of concentration *viz.*, 0.25, 0.50, 1, 3, 6, 9, 12 and 15 µg/mL of CP in replicates of five (n=5). The Peak Height Ratio (PHR) of CP to BV were determined and a calibration curve of PHR versus concentration was plotted and is shown in Figure 4.1.

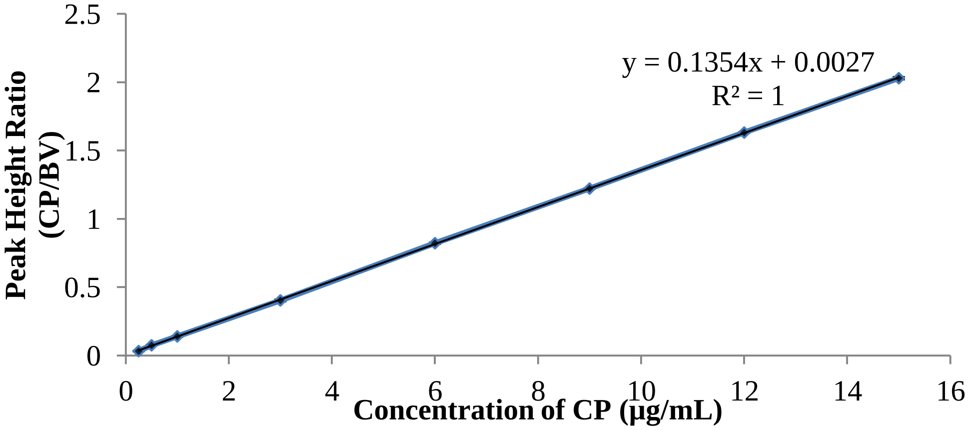


Figure 4.1 Typical calibration curve for CP over the concentration range 0.25 - 15 µg/mL

The equation for the best-fit line was y = 0.135x + 0.002, with a correlation coefficient $R^2 = 1.000$. To establish linearity between a response and sample concentration, the R^2 value should be ≥ 0.999. Furthermore the y-intercept should be < 2% of the response or near zero to establish the accuracy and linearity of a method [393,394]. Since the value of $R^2 = 1.000$ and the y-intercept was 0.002, these values satisfied the specific criteria for linearity and a direct response-concentration relationship was found and the calibration curve was therefore considered linear.

4.2.2. Precision

The precision of an analytical method is the degree of agreement among individual test results when a procedure is applied repeatedly to multiple aliquots of a uniform sample [395,396]. Precision is usually method- and concentration-specific. The two most common precision measures used are “repeatability” and “reproducibility”. They represent the two extreme measures of precision that can be established [387]. According to the ICH guidelines [385], precision tests should be performed at three different levels *viz.*, repeatability (intra-day precision), intermediate precision (inter-day precision) and reproducibility (between laboratory precision). Precision at only two levels was established for this method *viz.*, repeatability and intermediate precision and the tolerance for percent Relative Standard Deviation (% RSD) was set at ± 5% for these studies. The third level of precision was not established as the method that was developed and validated was to be used by the same analyst in the same laboratory using the same equipment.

4.2.2.1. Repeatability (Intra-Day Precision)

Repeatability refers to the precision of measurements undertaken under the same operating conditions by a single analyst using the same equipment on the same day [397]. Repeatability was determined following multiple injections (n=5) of freshly prepared CP solutions at low, medium and high concentrations located within the calibration range on Day 1. A summary of the intra-day precision for the low, medium and high concentrations studied is listed in Table 4.1.

Table 4.1 Intra-day precision (n=5)

Theoretical Concentration (µg/mL)	**PHR (CP/BV) n=5**	**Standard Deviation**	**% RSD**
0.25	0.0318	0.0005	1.57
0.5	0.0743	0.0005	0.67
1.0	0.1393	0.0008	0.57
3.0	0.4027	0.0007	0.17
6.0	0.8214	0.0004	0.04
9.0	1.2215	0.0021	0.17
12.0	1.6315	0.0028	0.17
15.0	2.0290	0.0009	0.04
	Concentration (µg/mL) (Low)	**Concentration (µg/mL) (Medium)**	**Concentration (µg/mL) (High)**
1	0.371	7.561	13.091
2	0.373	7.532	13.092
3	0.372	7.542	12.993
Average	0.372	7.544	13.059
Standard Deviation	0.0010	0.0147	0.0568
%RSD	0.27	0.19	0.44

For an assay method, the precision criterion for instrument precision or % RSD can be ≤ 1%. For intra-day precision, the % RSD can be ≤ 2%, whereas for impurity assays, the intra-assay precision can be ≤ 10% [398]. These results reveal that the method is precise and the % RSD values ranged between 0.04% and 1.57% respectively for the samples studied.

4.2.2.2. Intermediate (Inter-Day Precision)

Intermediate precision refers to the precision of a method with reference to variability within a laboratory such as running analyses on different days and/or through the use of different equipment or analyses undertaken by different analysts [385,397]. The ICH guidelines [385] indicate the extent to which intermediate precision should be established and is dependent on the circumstances under which an analytical procedure is intended to be used. To establish intermediate precision of the HPLC method, samples of the same concentration at low, medium and high levels were freshly prepared and analyzed on two additional, consecutive days *viz.*, Day 2 and 3. The PHR of CP:BV was calculated and the % RSD reported. A summary of the data generated on three consecutive days of analysis is shown in Table 4.2.

Table 4.2 Inter-day precision (n=5)

DAY 1 **Sample Number**	**Concentration µg/mL (Low)**	**Concentration µg/mL (Medium)**	**Concentration µg/mL (High)**
1	0.378	7.512	13.101
2	0.371	7.523	13.131
3	0.376	7.480	13.123
Average	0.375	7.505	13.118
Standard Deviation	0.0036	0.0208	0.1164
%RSD	0.96	0.28	0.12
95% C.I	0.000131	0.000754	0.000553
DAY 2			
1	0.379	7.541	13.081
2	0.370	7.582	13.070
3	0.378	7.531	12.990
Average	0.375	7.551	13.047
Standard Deviation	0.0049	0.0264	0.0493
%RSD	1.31	0.35	0.38
95% C.I	0.000179	0.000958	0.001786
DAY 3			
1	0.371	7.531	13.100
2	0.376	7.500	13.100
3	0.379	7.511	13.121
Average	0.375	7.514	13.107
Standard Deviation	0.0040	0.0153	0.0114
%RSD	1.07	0.20	0.08
95% C.I	0.000146	0.000553	0.000418

The intermediate precision results were found to be adequate, with all % RSD values < 2% for the three concentrations tested. This indicates that there are no significant differences between the mean values for the PHR that were generated on different days on which the analyses were conducted. It can therefore be confirmed that the analytical method has the ability to generate precise results on a day-to-day basis.

4.2.2.3. Reproducibility

Reproducibility is defined as the precision of a method when analyses are conducted in different laboratories or using different analysts or equipment. It is a parameter that is determined in collaborative studies or method transfer experiments [385]. Reproducibility was not assessed for the duration of these studies since all analyses were performed by the same analyst, using the same equipment in the same laboratory.

4.2.3. Accuracy and Bias

The accuracy of an analytical method is a measure of the closeness of an individual test result to a true value and is thus a measure of exactness [397,399,400]. Accuracy serves as a tool to evaluate whether measured values are exactly the same as or as close as possible to a theoretical value. The FDA [353] recommends that accuracy studies for drug substances and products be performed at 80%, 100% and 120% of a target concentration, as stated in the guidelines for submitting samples and analytical data for analytical method validation. However for assay, accuracy may be measured as the percent recovery of a known amount of analyte that is added to a sample or the difference between a mean and true value calculated with the aid of confidence intervals [353].

The ICH guidelines [385] state that the establishment of accuracy requires a minimum of nine determinations over a minimum of three concentration levels covering the specific range of the analytical procedure. The accuracy of the method was established by analyzing samples at low, medium and high concentrations within the calibration range and assessing the percent recovery, Relative Error (R.E), % bias and % RSD for each level investigated. A tolerance of 2% was set for the value for % RSD, as this complies with the limits set by a number of pharmaceutical industries [353]. A % bias of ≤ 5% was set in our laboratory as the test limit since the % bias is the extent of deviation of a sample result from the true value for that sample. A low % bias may result in a high recovery and therefore a more accurate analytical procedure is available for use. The PHR of CP:BV were calculated and accuracy was reported as the percent recovered in the samples tested using a freshly constructed calibration curve. The accuracy results for low, medium and high concentrations are summarized in Table 4.3.

Table 4.3 Accuracy data

	Theoretical Concentration (µg/mL)	Actual Concentration (µg/mL)	Standard Deviation	%RSD	R.E	% Recovery	%Bias
Low	0.37	0.373	0.0052	1.39	0.008	100.8	-0.81
	0.37	0.371	0.0049	1.32	0.000	100.0	0.00
	0.37	0.376	0.0059	1.57	0.000	100.0	0.00
Medium	7.50	7.471	0.0430	0.57	0.004	99.6	0.33
	7.50	7.540	0.0390	0.52	0.005	100.5	-0.58
	7.50	7.461	0.0489	0.65	0.005	99.5	0.52
High	13.10	13.161	0.0401	0.30	-0.004	100.5	-0.57
	13.10	13.097	0.0509	0.38	0.007	99.9	0.11
	13.10	13.091	0.0590	0.45	0.007	99.9	0.11

The % recovery for all samples ranged between 99.5% and 100.8% and the % bias values were between -0.81% and 0.52%, indicating that the analytical method is accurate. The % RSD were well below the 5% threshold set, thereby confirming the accuracy of this method.

4.2.4. Limits of Quantitation (LOQ) and Detection (LOD)

Recent publications have discussed the determination of the Limits of Quantitation (LOQ) and Detection (LOD) for HPLC methods [395,401-403]. Shrivastava and Gupta [402] described four techniques that may be used to establish the LOQ and LOD of an analytical method, that include the use of **i)** visual definition, **ii)** the signal-to-noise ratio, **iii)** a plot of standard deviation versus concentration and **iv)** the lowest concentration for which the % RSD ≤ 5% [402]. In general, the LOQ is the lowest concentration of an analyte in a sample that can be determined with acceptable precision and accuracy under the stated conditions of the test procedure. The LOD is taken as the lowest concentration of an analyte in a sample that can be detected, but not necessarily quantified, under the stated conditions of the test method [385,401]. In this study, The LOQ and LOD were established by injecting progressively decreasing samples in the concentration range of 0 - 0.7 µg/mL of CP solutions and constructing a calibration curve in this region. The LOQ and LOD were established using Equations 4.1 and 4.2 respectively.

$$\text{LOD} = \frac{10\sigma}{S} \qquad \textit{Equation 4.1}$$

$$\text{LOQ} = \frac{3.3\sigma}{S} \qquad \textit{Equation 4.2}$$

Where

σ=Standard deviation of the response
S=Slope of calibration curve

The slope was determined from the calibration curve of CP in the region of the LOQ and LOD, and the standard deviation was calculated from the residual standard deviation of the y-intercept of the regression lines plotted in the LOQ and LOD range. Samples were tested in replicate (n=5) and the % RSD was calculated from the variation in PHR for CP and BV. A calibration curve in the range of 0 - 0.7 µg/mL was generated for the analysis of CP and is depicted in Figure 4.2.

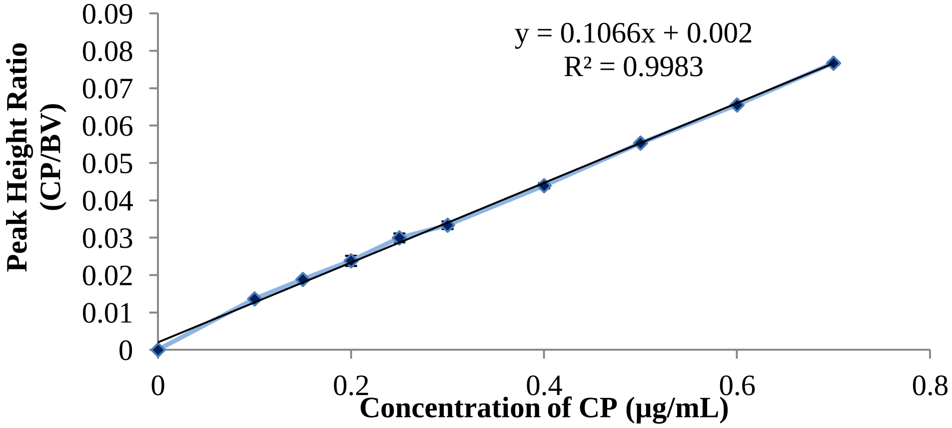


Figure 4.2 Typical calibration curve for CP in the concentration range 0 - 0.7 µg/mL

The LOQ was found to be 0.25 µg/mL with an associated % RSD of 4.94% and the LOD was found to be 0.1 µg/mL.

4.2.5. Robustness

The ICH guidelines [385] define the robustness of an analytical method as the ability of a procedure to remain unaffected by small but deliberate changes in the method. A design of experiment (DOE) approach was used to test the robustness of the RP-HPLC analytical method. Three factors were considered important and are described in § 3.5.2, Chapter 3. The experiments were performed in a randomized manner to minimize the effects of uncontrolled factors that may contribute bias to the final method response. ANOVA data revealed that the retention time of CP, Y_1 is not robust for factors such as MeOH concentration, X_1 and flow rate, X_2. Therefore precautionary measures need to be taken into account for this analytical procedure as small changes in MeOH content may result in a large variation in the retention time for CP and BV.

4.2.6. Assay

The analytical procedure that was developed and validated in these studies was used to assay commercially available Dermovate® 0.05% w/w cream.

4.2.6.1. Extraction Procedure for CP from Creams

Aliquots (1310 ± 5 mg) of Dermovate® cream (0.05% w/w CP, Lot 305628, Sekpharma® (PTY) Ltd, RSA) were accurately weighed and dispersed in 50 mL of MeOH containing BV. Samples were processed either by ultrasonication using a Branson® B12 ultrasonicator (Branson® Inc., Shelton, Conn, USA) wit or without heating at 65°C or magnetic stirring at 1000 rpm with and without heating for 5, 15, 25, 35, 45 and 60 minutes. Six samples were used for each extraction experiment. The solutions were then placed in a Model CF3555 KIC® freezer (KIC® Manufacturing, Stanger, RSA) (-20°C) for 20 minutes, after which the sample was centrifuged at 3000 rpm using a Model 5415 Eppendorf® centrifuge (Geratebau Netheler+ Hinz GmbH 2000, Hamburg 63, West Germany) for 15 minutes. This extraction procedure ensured complete precipitation of the lipophilic components of the cream while the API remained soluble in MeOH [404]. Five mL aliquots of the resulting suspensions were filtered through a 0.45 μm hydrophilic PVDF syringe filter (Millipore® Co., Bedford, MA, USA) and injected in triplicate onto the chromatographic system (6 samples x 3 injections at each time interval for all protocols). A schematic representation of the extraction procedure is shown in Figure 4.3.

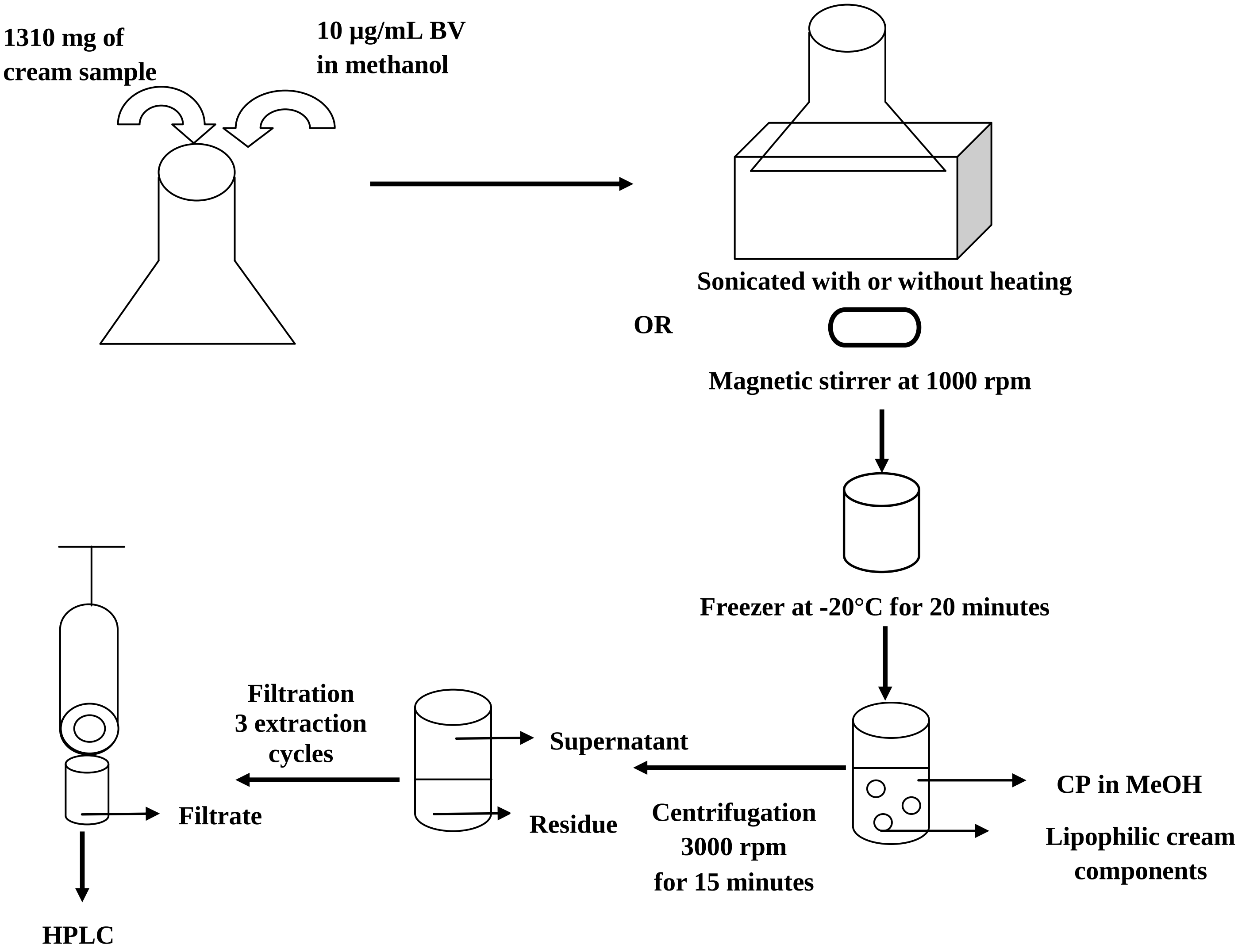


Figure 4.3 Schematic diagram of the extraction procedure for analysis of cream formulations

The results for ultrasonic extraction with and without heating and magnetic stirring extraction processes are listed in Table 4.4 and graphical comparison are shown in Figure 4.4.

Table 4.4 Efficiency of the extraction

Extraction Time (minutes)	**Extraction Efficiency (EE) (%)**											
	Ultrasonic			**Ultrasonic with Heating**			**Magnetic Stirrer**			**Magnetic Stirrer with Heating**		
	EE	**SD**	**%RSD**	**EE**	**SD**	**%RSD**	**EE**	**SD**	**%RSD**	**EE**	**SD**	**%RSD**
5	99.3	0.01	0.01	96.3	0.01	0.01	97.3	0.02	0.02	96.0	0.01	0.01
15	99.5	0.01	0.01	96.6	0.01	0.01	98.6	0.08	0.08	97.4	0.03	0.03
25	99.6	0.02	0.02	97.9	0.03	0.03	98.3	0.09	0.09	97.7	0.04	0.04
35	100.4	0.02	0.02	99.2	0.01	0.01	98.4	0.10	0.10	97.9	0.01	0.01
45	101.1	0.01	0.01	99.9	0.01	0.01	99.2	0.14	0.14	97.8	0.09	0.09
60	101.4	0.01	0.01	99.9	0.02	0.02	99.2	0.13	0.13	97.5	0.09	0.09

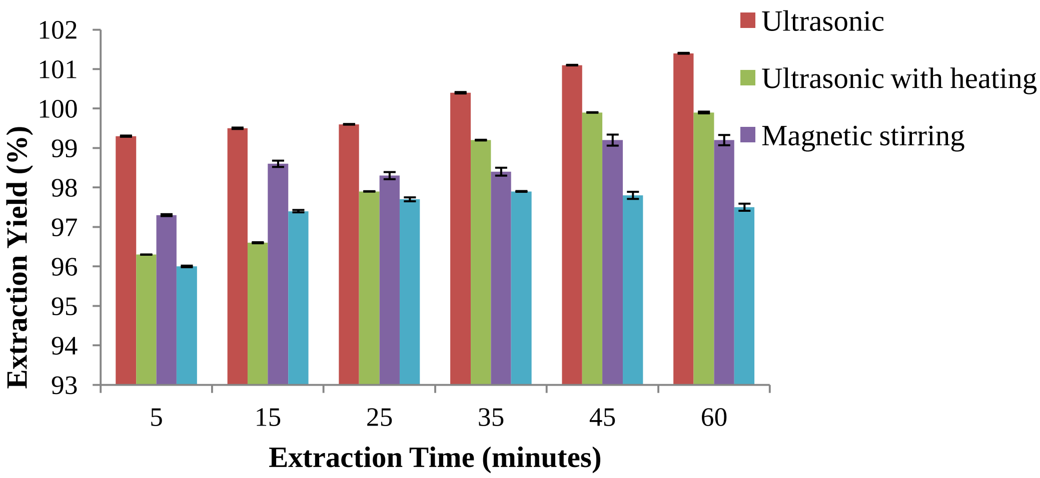


Figure 4.4 Graphical comparison of CP extraction

The experimental results clearly reveal that CP can be quantitatively and reproducibly extracted within 35 minutes by use of a Branson® B12 ultrasonicator bath (Branson® Ltd, Shelton, Conn, USA). The use of a magnetic stirrer required 45 minutes for adequate extraction with associated poor reproducibility. The combination of ultrasonication with simultaneous heating and magnetic stirrer with heating resulted in poor extraction yields. Thirty five minutes of extraction with ultrasound was selected and used for all further studies.

4.2.6.2. Optimization of Ultrasonic Extraction

4.2.6.2.1. Effect of MeOH Concentration

The effect of the different concentrations of MeOH used in the extraction medium was studied over the range of 40% v/v to 100% v/v. The extraction efficiency of different MeOH content used for the analysis of Dermovate® cream is summarized in Table 4.5.

Table 4.5 Efficiency of extraction with solvents of different MeOH content

MeOH Content (% v/v)	Extraction Efficiency (%)	SD	%RSD
40	117.5	0.03	0.03
50	107.7	0.02	0.02
70	96.3	0.02	0.02
90	98.6	0.01	0.01
100	99.3	0.01	0.01

100% v/v MeOH was established to have the highest extraction efficiency with a 99.3% recovery and was used for all extraction studies.

4.2.6.2.2. Effect of Number of Extraction Cycles

The effect of the number of extraction cycles that were used to extract CP from the cream was also investigated *viz.*, extraction cycle 1, extraction cycle 2 and extraction cycle 3. The samples were re-extracted and filtered through a 0.45 μm hydrophilic PVDF syringe filter (Millipore® Co., Bedford, MA, USA) for each extraction cycle and injected in triplicate onto the chromatographic system. The results of the effect of number of extraction cycles on extraction efficiency using different MeOH content are tabulated in Table 4.6 and the results are shown graphically in Figure 4.5.

Table 4.6 Efficiency of extraction with additional extraction cycles with different MeOH content

MeOH Content (% v/v)	Extraction Efficiency (EE) (%)								
	Cycle 1			Cycle 2			Cycle 3		
	EE	SD	%RSD	EE	SD	%RSD	EE	SD	%RSD
40	117.5	0.21	0.18	111.7	0.07	0.06	123.1	0.15	0.12
50	107.7	0.07	0.06	102.9	0.08	0.07	101.0	0.09	0.09
70	96.3	0.01	0.01	98.3	0.87	0.88	99.0	0.27	0.27
90	98.6	0.04	0.04	99.9	1.60	1.60	99.1	1.60	1.60
100	99.3	0.05	0.05	99.9	0.01	0.01	100.8	0.01	0.01

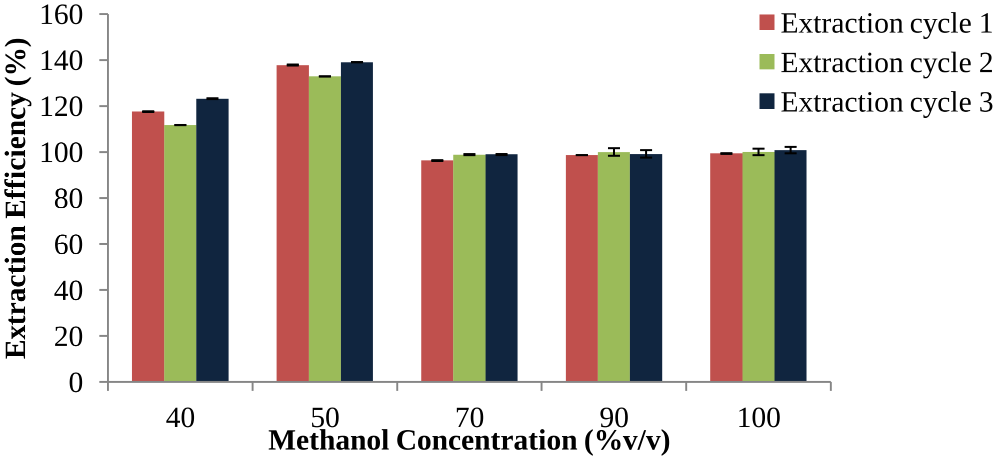


Figure 4.5 Comparison of extraction cycles using different MeOH content

The use of additional extraction cycles ensured quantitative extraction of the analyte. Additional cycles proved to recover less CP. An optimal extraction efficiency of 100% was observed at a MeOH concentration of 100% v/v with 3 extraction cycles. These conditions were selected for further experiments.

4.2.6.2.3. Effect of Sample Size

The effect of the amount of CP cream to be extracted was also studied in the range of 3% w/w to 13.10% w/w in which the impact of the amount of CP cream on the extraction are tabulated in Table 4.7.

Table 4.7 Efficiency of extraction using different amounts of sample

Amount of CP Cream (mg)	Extraction Efficiency (%)	SD	%RSD
1310	99.8	0.29	0.29
1200	99.0	1.64	1.66
900	98.9	1.59	1.60
600	97.7	1.73	1.77
300	97.5	2.54	2.61

The use of 300 mg of CP cream had a relatively poor extraction recovery of 97.5%. An increase in the amount of CP cream used resulted in an increase in the efficiency of extraction from 97.5% to 99.8%. Poor reproducibility was also observed when attempts were made to extract small amounts of cream. An optimal extraction recovery of 99.8% was observed when 1310 mg of CP cream was tested with an associated SD of 0.29. Consequently this amount of cream was used for all assay studies.

4.2.6.3. Validation of Ultrasonic Extraction Procedure

The extraction of 1310 mg of CP cream using ultrasound for 35 minutes using 100% MeOH as the extraction medium for three extraction cycles was validated by assessing intra- and inter-day precision.

4.2.6.3.1. Intra- and Inter-Day Precision

Three extractions were performed at one hour interval on a single day (within-day precision), while two extractions were performed on three consecutive days (day-to-day precision). All samples from day to day experiments were filtered through a 0.45 µm hydrophilic PVDF syringe filter (Millipore® Co., Bedford, MA, USA) prior to refrigeration in light-protected flasks and were analyzed together in order to avoid additional day-to-day variations that might be introduced by chromatographic analysis. A summary of the within-day and day-to-day precision of the extraction experiments is given in Tables 4.8 and 4.9.

Table 4.8 Intra-day precision of extraction

Intra-Day Precision	**Actual Concentration (µg/mL) (n=3)**	**Extraction Yield (%)**
1	13.071	99.80
2	13.072	99.80
3	13.069	99.70
Average	13.070	99.80
Standard Deviation	0.088	0.072
%RSD	0.67	0.07
95% C.I	0.003214	0.002611

Table 4.9 Inter-day precision of extraction

Day 1	**Actual Concentration (µg/mL) (n=3)**	**Extraction Yield (%)**
1	12.971	99.06
2	13.052	99.63
3	13.091	99.99
Average	13.038	99.56
Standard Deviation	0.061	0.467
%RSD	0.46	0.47
95% C.I	0.002218	0.016930
Day 2		
1	12.961	98.90
2	13.200	100.80
3	13.091	99.80
Average	13.084	99.80
Standard Deviation	0.121	0.931
%RSD	0.93	0.93
95% C.I	0.004415	0.033739
Day 3		
1	12.960	99.00
2	13.291	101.5
3	12.950	98.80
Average	13.067	99.70
Standard Deviation	0.192	1.466
%RSD	1.47	1.47
95% C.I	0.006954	0.053087

The results of extraction precision studies were satisfactory for all cases, with a mean extraction yield of 99.7% and inter-day % RSD of 0.47 - 1.47%.

4.2.7. Assay of Commercially Available Generic CP Creams

Commercially available generic CP cream formulations were purchased from a local pharmacy and were assayed. These analyses established the applicability of this method for the analysis of CP dosage forms. The generic products tested were Dovate® (Aspen Pharmacare®, Port-Elizabeth, RSA) and Xenovate® (Aspen Pharmacare®, Port-Elizabeth, RSA). Approximately 1310 mg of cream was accurately weighed and transferred to a 100 mL Schott® Duran round-neck Erlenmeyer flask (Schott® Duran GmbH, 51 Hattenbergstrasse, Germany). A stock solution of BV in MeOH was prepared as described in § 3.3.4, Chapter 3 to produce a solution of final concentration 10 µg/mL. A 50 mL aliquot of the BV solution was added to the stoppered flask. The flask was sonicated for 35 minutes using a Branson® B12 ultrasonicator (Branson® Ltd, Shelton, Conn, USA). The suspension was then placed in a Model CF3555 K.I.C® freezer (K.I.C® Manufacturing, Stanger, RSA) for 20 minutes, after which the sample was centrifuged at 3000 rpm using a Model 5415 Eppendorf® centrifuge (Geratebau Netheler+ Hinz GmbH 2000, Hamburg 63, West Germany) for 15 minutes. A 4.0 mL aliquot of the supernatant was filtered in three cycles through a 0.45 µm HVLP Millipore® filter membrane (Millipore® Co., Bedford, MA, USA) and an aliquot of the filtered sample was injected onto the HPLC system in replicate (n=5). A calibration curve was constructed on the same day that the samples were prepared for analysis using the same extraction procedure and the concentration of CP was interpolated from the resultant curve.

The % recovery following extraction was found to be 99.78% and 98.98% for Dovate® and Xenovate® cream formulations. No interfering peaks were observed in the resultant chromatograms developed for the analysis of Dovate® and Xenovate® indicating that there was no interference from excipients used in the manufacture of these cream formulations. Chlorocresol (CH), the preservative used in these formulations had a retention time of 2.4 minutes. Typical chromatograms following the analysis of Dovate® and Xenovate® creams are shown in Figure 4.6.

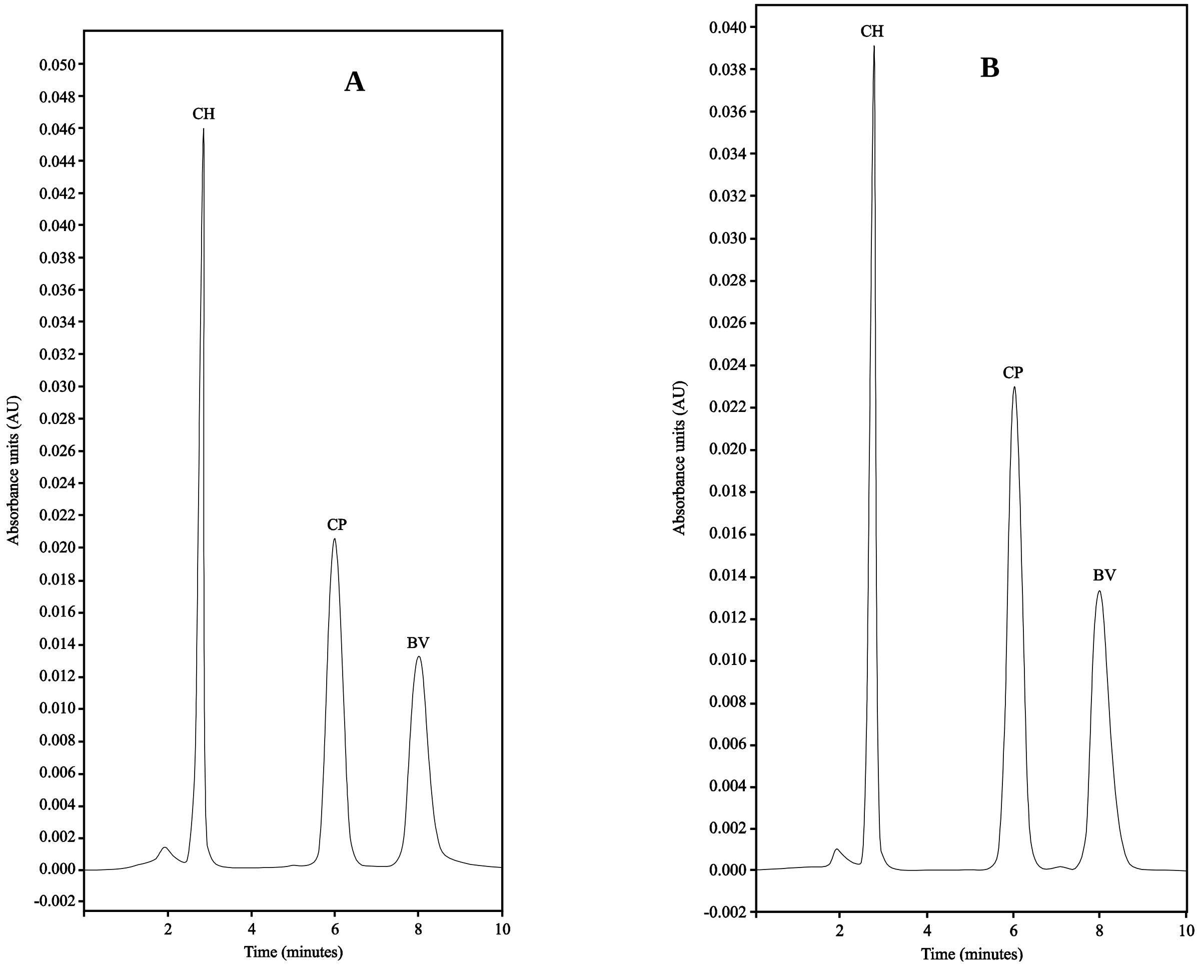


Figure 4.6 Typical chromatogram of CH and CP from Dovate® (A) and Xenovate® (B)

4.2.8. Stability of CP in MeOH

It is important to determine the stability of an analyte for a specified period, in the solvents that will be used for analysis. An analyte is considered stable under specific conditions for specific periods, following comparison of the responses of stored samples to those of freshly prepared standard samples [267]. The stability of CP in acetonitrile (ACN) has been investigated and CP was found to be stable at + 4°C in ACN for 14 days [227]. There are however no precise data available for the stability of CP in MeOH. Therefore a stability study of CP in MeOH was undertaken for a period of 14 days. The aim of the stability study was to detect any potential degradation of CP during sample collection, processing and storage prior to and during analysis. The stability study was conducted at room temperature (+ 22°C) and at + 4°C (Fridge). Stock solutions of CP were prepared as described in § 3.3.4, Chapter 3 and stored under specified temperature conditions, protected from light using aluminium foil.

Aliquots (2.4 mL and 0.6 mL) of samples were withdrawn from the CP stock solution and were added to a 20 mL A-grade volumetric flask and made to volume with MeOH, to produce solutions with concentrations of 12 μg/mL and 3 μg/mL. Samples were withdrawn and analyzed following storage for 1, 2, 3, 7 and 14 days. Aliquots of each sample were mixed with freshly prepared internal standard solution (10 μg/mL) and analyzed by HPLC. The statistical method proposed by Timm *et al.* [405], for the investigation of the stability of drugs in biological samples, was adopted to determine whether the resultant differences in concentrations constituted a relevant and/or significant change. The proposed statistical method takes into account the quality of the experimental procedure or in other words the precision of the method and the number of replicate samples analyzed [405]. The statistical interpretation of the stability data is based on the construction of a 90% confidence interval for the percentage change (Δ) in concentration between the stored and freshly prepared samples. The true change in response on storage is enclosed by Lower (LL) and Upper Limits (UL) of the confidence interval, with a probability of 90% certainty. In other words, there exists a probability of 5% that the true Δ is lower than the LL and a probability of 5% that the true Δ is higher than the UL [405]. Timm *et al.* [405] proposed that a change may be relevant only if both the upper and lower limits of the confidence interval are either < -10% or > 10%. The possible results that could be obtained when this method is used to analyze stability of stored samples are shown in Figure 4.7. The bars above the axis represent the 90% confidence intervals for the % Δ between stored and freshly prepared samples.

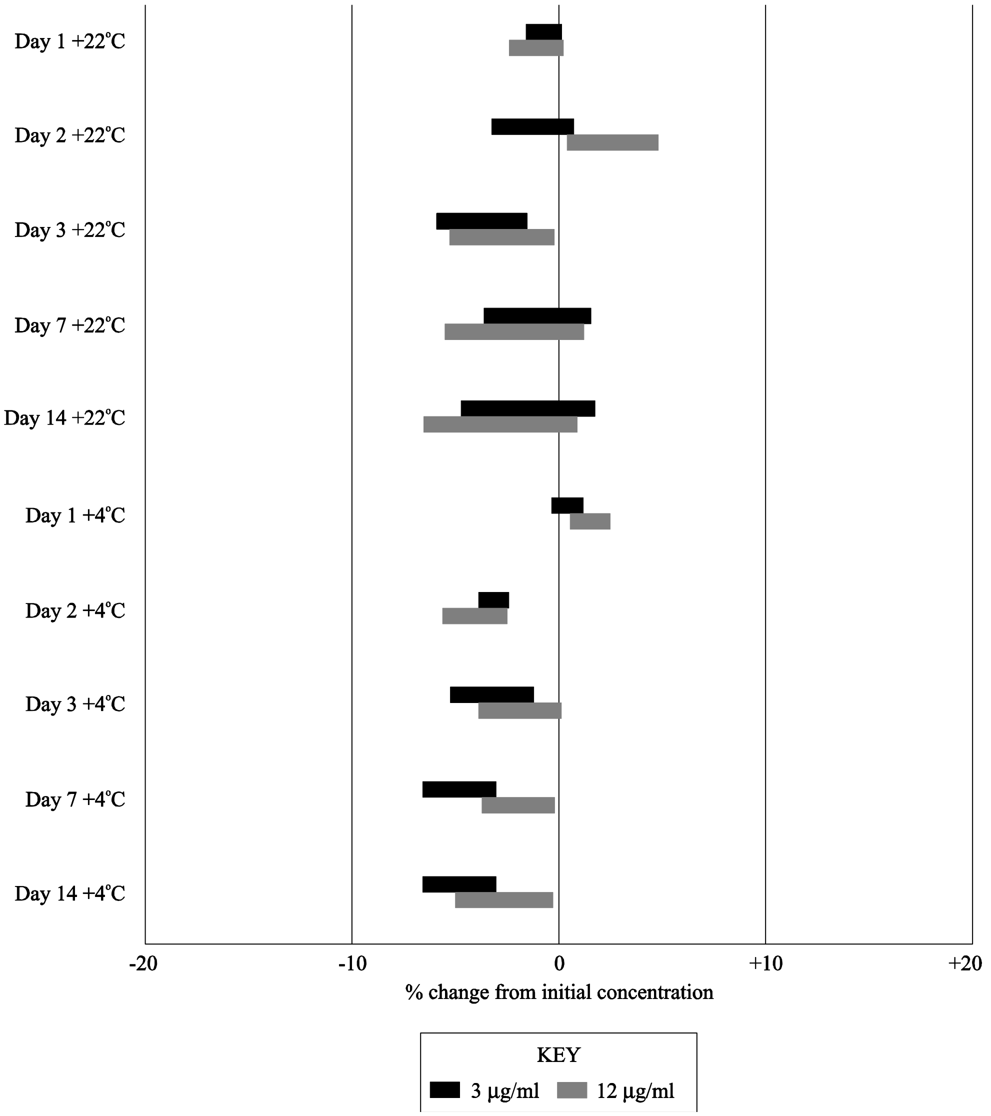


Figure 4.7 Stability of CP in MeOH at two different concentrations, stored at + 4ºC and + 22ºC for 1, 2, 3, 7 and 14 days

The graphical presentation in Figure 4.7 reveals that no significant decrease of concentration was detected when CP was stored in MeOH for 14 days at the temperatures specified. A slight increase in response was observed for both concentrations *viz.*, 3 µg/mL and 12 µg/mL from Day 1 to Day 14, however these results indicate that the change of responses for CP at both the lower (3 µg/mL) and upper (12 µg/mL) concentrations prepared in MeOH and stored at + 22°C and + 4°C for 14 consecutive days were not significant or relevant. CP was therefore considered stable when stored in MeOH at + 4°C and + 22°C for 14 days.

4.3. CONCLUSIONS

A RP-HPLC method for the *in vitro* analysis and quantitation of CP in pilot scale cream formulations has been successfully validated and was applied to the assessment of commercially available CP cream formulations. This method was found to be linear in the range of 0.25 - 15 µg/mL and was found to have a coefficient of determination of 1.000. Statistical analysis confirmed that the method was also precise and reproducible with adequate inter- or intra-day precision. The % RSD values for the intra-day and inter-day precision were found to be < 2%, therefore there was no significant difference between CP:BV PHR for low, medium and high concentration samples analyzed over three consecutive days. The RP-HPLC method was also found to be accurate, with the % bias ranging between -2.79% and 0.58%, and the chromatographic run time of 10 minutes permits the analysis of a large number of samples over a short period of time. This method can therefore be recommended for use in routine quality control testing of CP topical formulations. CP was found to be stable in MeOH at 4ºC and 22ºC for 14 days, permitting the storage of samples prior to analysis. The application of a simple, accurate, precise, stability indicating analytical method is vital for the success of formulation development studies using the principles of QbD whilst establishing a design space for the product under development. The RP-HPLC method developed in this study will be used to assist in making formulation decisions for the establishment of a design space for generic CP cream formulations.

CHAPTER FIVE

MANUFACTURING DESIGN, RISK AND QUALITATIVE ANALYSIS

5.1. INTRODUCTION

5.1.1. Quality Risk Management (QRM)

Risk is defined as “the combination of the probability of occurrence of harm and the severity of that harm” [406]. Risk management principles are successfully used in many areas of business and government including finance, insurance, occupational safety, public health and pharmaco-vigilance, in addition to the agencies that regulate these industries. Quality Risk Management (QRM) has become a mandatory regulatory requirement for health care organizations that are active in the sectors of medical device or pharmaceutical manufacture. However in the pharmaceutical industry, the use of QRM is limited and does not represent the full potential and contribution that risk management has to offer in this sector [37,406]. Although there are several stakeholders, including patients, medical practitioners, government and industry, the protection of the patient by managing the risk to quality should be considered of major importance as shown in Figure 5.1 [406].

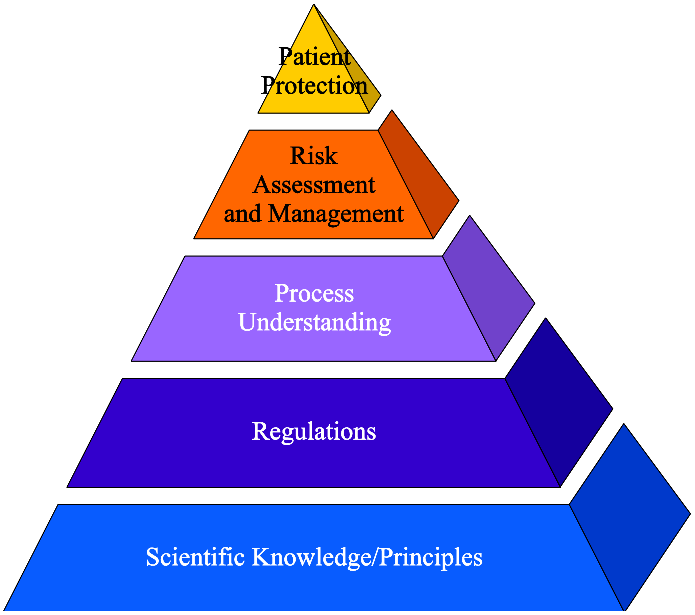


Figure 5.1 Quality Risk Evaluation Pyramid, adapted and redrawn [407]

The ICH Q9 guideline [37] concerning QRM in the pharmaceutical field of API and medicinal product manufacture was initially adopted by the European Union (EU) and PIC/S in Annex 20 of the EU and PIC/S Guide to Good Manufacturing Practice (GMP). The guideline was gradually applied by drug manufacturers, in particular in respect of Sections 1.5 and 1.6 of Part I of the aforementioned document [408]. These sections define “Hazard Analysis and Critical Control Points as a systematic, proactive and preventive tool for assuring product quality, reliability, and safety. It is a structured approach that applies technical and scientific principles to analyze, evaluate, prevent and control the risk or adverse consequence(s) of hazard(s) due to the design, development, production and use of products". QRM is defined as "a systematic process for the assessment, control, communication and review of the risks to the quality of medicinal products across the entire lifecycle of the product". It can be applied proactively and/or retrospectively [37,408]. In most countries, compliance with cGMP, drug regulatory activities and inspections provide a high level of assurance that the risks in manufacture are largely under control. However in countries where control is not as rigorous, patients may be put at risk through the production of medicines of inadequate quality. The assessment of individual risks related to specific products and/or starting materials and the recognition of potential hazards at specific stages of production or distribution should permit regulatory authorities to improve the control of medicines by increasing the effectiveness of their activities within the limits of available resources [409].

The manufacturing and use of a medicinal product, including components necessarily entail some degree of risk. The risk to quality is just one component of the overall risk for that product [406,410]. It is important to understand that product quality should be maintained throughout the lifecycle of the product and attributes that are important to quality should remain consistent to the products originally used in clinical studies [37,411,412]. An effective QRM approach can further ensure a high quality medicinal product that is accessible to the patient by providing a proactive means to identify and control potential quality issues during development and manufacture [37,409]. In addition the use of QRM can improve decision making if and when a quality problem arises. Effective QRM can facilitate better and more informed decisions, can provide regulators with greater assurance that a company has the ability to deal with potential risks and can effect the extent and level of direct regulatory oversight in a beneficial manner [406,413]. The appropriate use of QRM can facilitate but does not hinder the obligation of the industry to comply with regulatory requirements and does not replace appropriate communication between the industry and regulators. The QRM principles are linked to the concepts of QbD in Chapter 1, *vide infra*. Several aspects of the composition of creams and associated manufacturing processes are thought to affect the quality of topical products and a risk assessment exercise was used to identify areas of concern. Risk identification and analysis in accordance with ICH Q9 was used to establish which formulation variables and unit operations were likely to have the greatest potential impact on product quality.

5.1.2. Principles of QRM

The ICH Q9 guideline [37] mentions that there are two primary principles of QRM *viz.,* **i)** evaluation of the risk to quality should be based on scientific knowledge and ultimately link to the protection of the patient and **ii)** level of effort, formality and documentation of the QRM process should be commensurate with the level of risk. However WHO have added two extra principles to the concepts described in ICH Q9 in their guideline [409] as illustrated in Figure 5.2 (reproduced from ICH Q9) *viz.,* **iii)** QRM should be dynamic, iterative and responsive to change and **iv)** the capability for continual improvement and enhancement should be embedded in the QRM process.

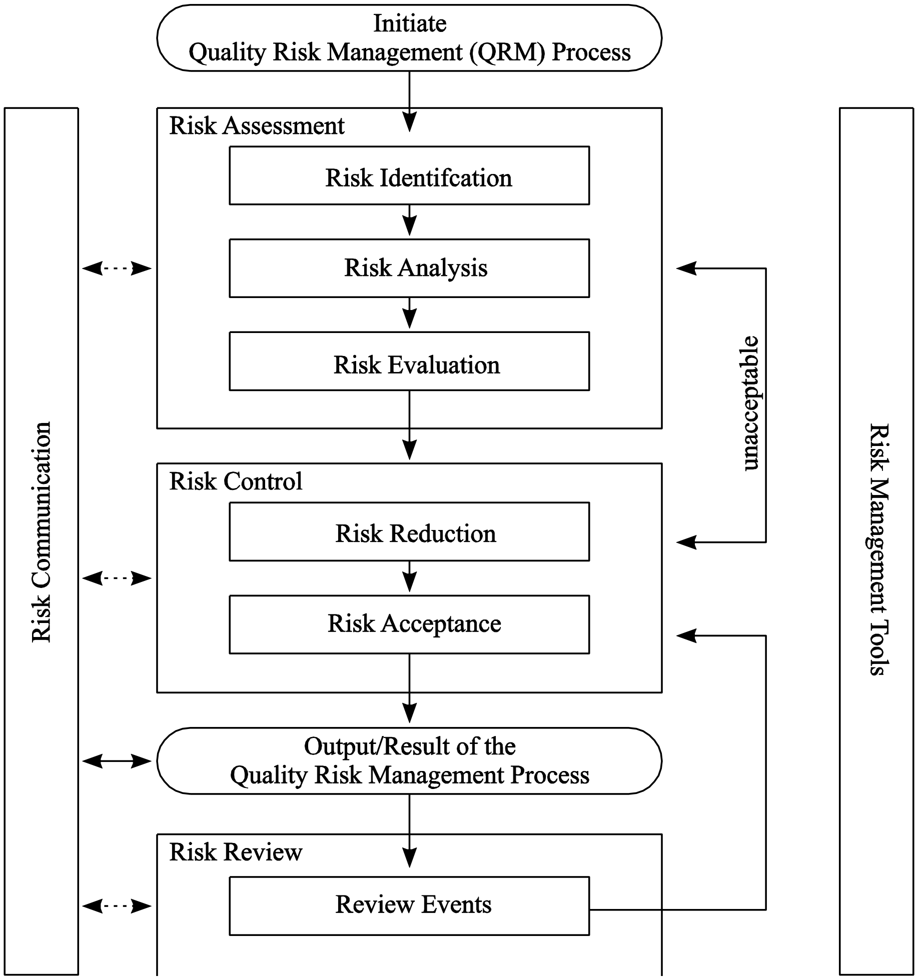


Figure 5.2 Overview of a typical QRM process, redrawn from [37]

The emphasis on each component of the framework might differ from case to case but a robust process must incorporate consideration of all elements at a level of detail commensurate with the specific risk associated with that product. Decision nodes are not shown in Figure 5.2, since decisions can occur at any point in the process and a decision might be taken to return to a previous step and seek further information to adjust the risk models or even to terminate the risk management process based on information that supports such a decision. The term "unacceptable" in the flowchart refers to statutory, legislative or regulatory requirements for risk management and is indicative of when the risk assessment process needs to be revisited [37,409].

In the early stages of formulation development, the main purpose of the RM process is to manage risks and knowledge gaps associated with formulation development of a Finished Pharmaceutical Product (FPP) according to a Pharmaceutical Target Product Profile (PTPP) [409]. In recognizing risks and knowledge gaps, the QRM process plays a significant role in enabling the prioritization and mitigation of risks proactively rather than retrospectively. As the development of the FPP progresses, in addition to supporting development, the secondary purpose of QRM is to determine and manage potential risks to bioavailability, safety, efficacy and product quality with respect to processing parameters and product attributes. QRM in pharmaceutical development should differentiate Quality Process Parameters (QPP) and Quality Attributes (QA) from Quality Critical Process Parameters (QCPP) and Critical Quality Attributes (CQA), each aspect that contributes to defining and refining the control strategy [409]. The long process of pharmaceutical product development is complex and therefore requires continual evaluation and exchange of data, decisions and updates both internally within an organization and externally with stakeholders. An important aspect of pharmaceutical product development and QRM is the maintenance of an effective and secure knowledge management and documentation system that must facilitate transparent communication and the identification of key issues to stakeholders whilst preserving data in a well-structured archive [38,409,414].

5.1.3. Initiating a QRM Process

QRM should include systematic processes designed to coordinate, facilitate and improve science-based decision making with respect to risk. Some possible steps that are used to initiate and plan a QRM process include **i)** defining the problem and/or risk question, including making pertinent assumptions that identify the potential for risk, **ii)** assembling background information and/or data on the potential hazard, harm or human health impact relevant to risk assessment, **iii)** identification of a leader and necessary resources and **iv)** specifying timelines, deliverables and appropriate levels of decision-making for the risk management process [37].

5.1.4. Risk Analysis

Risk analysis is a product of hazard and consequence analysis, and the output is a mathematical expression of risk given by a general term "risk estimation". Risk estimation can be expressed in a number of ways, for example the cost of saving a life, probability of life loss or injury, cost of damage, or the extent of environmental impact [415].

5.1.5. Risk Assessment

Risk assessment consists of hazard identification and risk analysis is associated with exposure to hazards [37,409]. Quality Risk Assessment (QRA) begins with a well-defined problem description or risk question. The QRM team should list all hazards that may be reasonably expected to occur at each step of a process such as production, testing and/or distribution of a product to the point of use [37]. A hazard analysis should be conducted to identify details for the QRM plan in which hazards are of such a nature that their elimination or reduction to acceptable levels is essential. A thorough risk analysis is required to ensure an effective control point and two-stage risk analysis is the recommended approach [409]. During the initial stages, the QRM team should review all materials, activities, equipment, storage, distribution and intended use of a product. A list of potential hazards including biological, chemical and physical that may be introduced, increased or controlled in each step should be made and the subsequent risk analysis should address the basic questions. Three fundamental questions that assist and can help to clearly define the risk(s) for risk assessment purposes include **i)** what is the nature of possible hazards?, **ii)** what is the probability of their occurrence and how easy is it to detect them? and **iii)** what are the consequences i.e. the severity of these hazards? [37].

The output of risk assessment is either a quantitative estimate of risk or a qualitative description of a range of risk(s). When risk is expressed quantitatively, a numerical probability is used. Alternatively risk can be expressed using qualitative descriptors such as high, medium or low which should be defined in detail [416]. Sometimes a "risk score" is used to further define descriptors in risk ranking. In quantitative risk assessment, a risk estimate provides the likelihood of a specific consequence, given a set of risk-generating circumstances [407]. Thus, quantitative risk estimation is useful for one particular consequence at a time. Some appropriate QRM tools use a relative risk measure to combine multiple levels of severity and probability into an overall estimate of relative risk [37,409].

5.1.5.1. Risk Assessment Tools

An early step in the execution of risk analysis is to determine the appropriate risk assessment tool or methodology to be used [37]. There is generally no single best approach for the assessment process and the selection of the appropriate risk methodology should be based on the depth of analysis required, complexity of the subject risk of concern and familiarity with the assessment tool to be used [417]. Based on industry examples, risk ranking and filtering that is sometimes referred to as a risk matrix or flowchart construction were popular tools used for basic risk assessment activities. Failure Mode Effect Analysis (FMEA) appeared to be the most frequently used methodology for more advanced risk analysis efforts. Some examples have demonstrated the power of combining tools to assist with more complex analyses [418]. For example Fault Tree Analysis (FTA) or a Cause and Effect or Fish-bone/Ishikawa diagram, can be used initially to scope and evaluate the fault modes of a particular problem and then be used to supply a Hazard Analysis and Critical Control Point (HACCP) or similar document to evaluate overall system control and effectiveness [406-409]. The development of pilot scale CP cream formulations and associated manufacturing processes used prior knowledge from previous product development projects as a starting point for the risk analysis [61]. A risk analysis, in accordance with ICH Q9, was used to establish which variables and unit operations were likely to have the greatest impact on product quality. The initial risk assessment is shown in Table 5.1.

Table 5.1 Risk assessment to identify variables that have the potential to impact product quality

	Variables and Unit Operations Attributes							
Drug Product CQA	**Formulation Composition**	**Homogenization Speed**	**Homogenization Time**	**Anchor Speed**	**Mixing Time**	**Heating Temperature**	**Cooling Time**	**Batch Size**
Appearance	High	Low	Low	Low	Low	Low	Low	Low
Identity	Low	Low	Low	Low	Low	Low	Low	Low
Assay	Low	High	Low	High	High	Low	Low	High
Impurities	High	Low	Low	Low	Low	Low	Low	Low
Content Uniformity	High	High	High	High	High	Low	Low	High
***In vitro* analysis**	High	High	High	High	High	High	High	low

Through prior knowledge and experience [61,419], the cells highlighted in green were concluded to present a low risk to the product CQA. The cells highlighted in red represent potential risk to the product and form areas for further study during product development efforts. A Cause and Effect diagram was selected as an additional tool for conducting risk assessment. This tool allows for an exhaustive analysis to be conducted and includes analysis of all factors that may affect a process. It is an easy technique to use and is commonly used to gather and organize data, structure risk management processes, facilitate decision making. It is also used to identify the links and interrelationships between factors, as shown in Figure 5.3 [420].

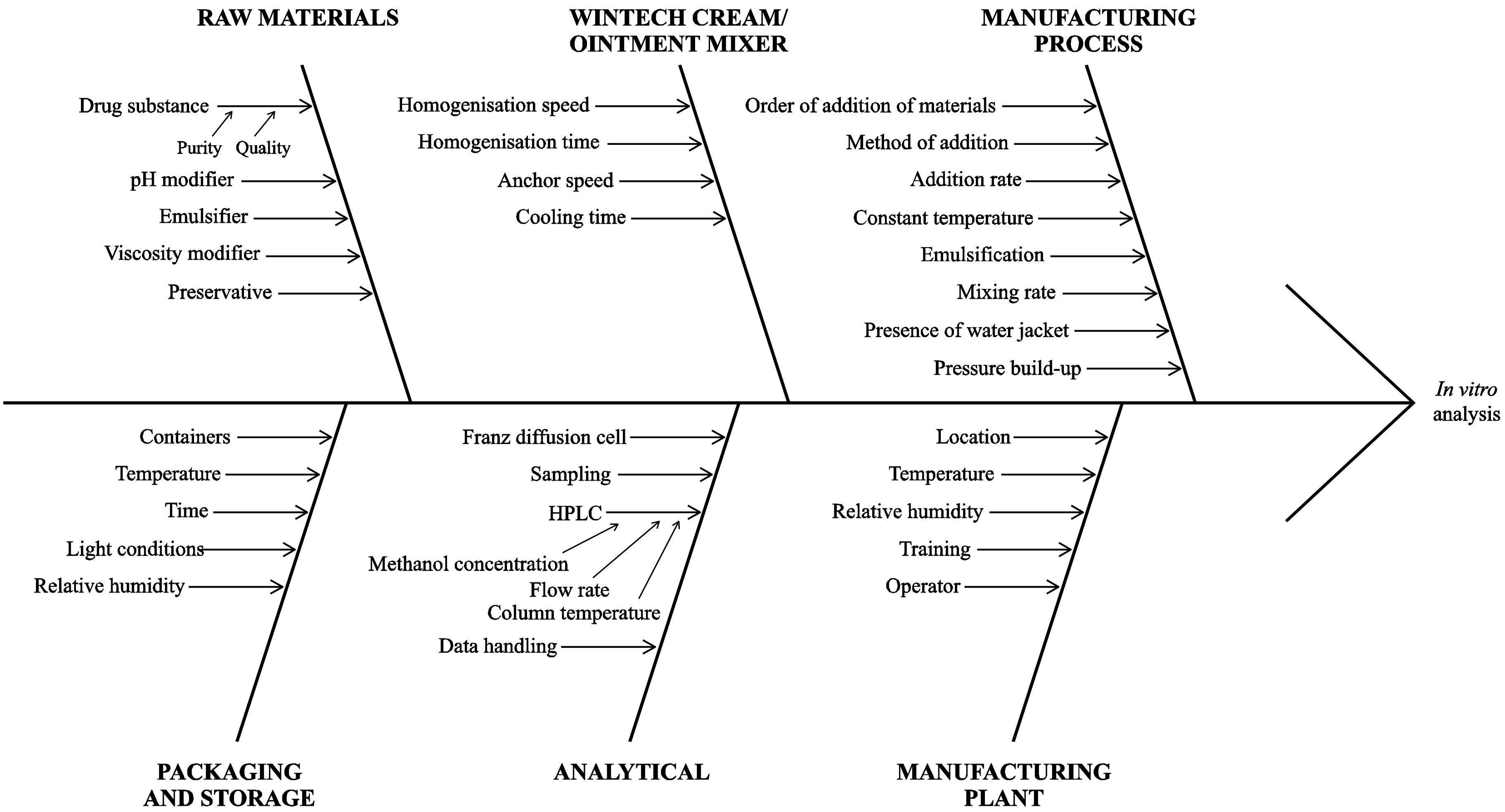


Figure 5.3 Ishikawa diagram of the sources of risk for the manufacture of a 0.05% w/w CP cream formulation

The Ishikawa diagram shows the main sources of risk that may impact the *in vitro* analysis of an API from a topical formulation and includes raw material, manufacturing plant, manufacturing processes, cream/ointment mixer, packaging and storage considerations in addition to analytical methods and data that are generated.

5.2. SELECTION OF THE COMPONENTS OF THE DRUG PRODUCT

5.2.1. Active Pharmaceutical Ingredient (API)

The TPP for CP cream formulation was identified, following an investigation and selection of the highly potent corticosteroid, CP. CP is a neutral molecule, does not possess any ionizable functional groups and therefore has no dissociation constant [205]. Formulation and analytical issues normally associated with pH change are therefore unlikely to affect the product. CP has an octanol/water partition coefficient, Log $P_{o/w}$ = 3.5. The partition coefficient of CP suggests that CP preferentially partitions into lipophilic media during *in vitro* testing and *in vivo* studies [421]. This highly potent corticosteroid was therefore selected for further pharmaceutical development. An evaluation of the attributes of the API that represents a risk with respect to final drug product quality is summarized in Table 5.2.

Table 5.2 Potential impact of attributes of CP on product attributes

	API Attributes					
Drug Product CQA	**Particle Size**	**Moisture**	**Stability**	**Solvent Content**	**Purity**	**Solubility**
Appearance	Low	Low	Low	Low	Low	Low
Identity	Low	Low	Low	Low	Low	Low
Assay	Low	Low	Low	Low	High	Low
Impurities	Low	High	High	High	High	Low
Content Uniformity	Low	Low	Low	Low	Low	Low
***In vitro* analysis**	High	Low	Low	Low	Low	High

Those attributes of CP considered to have the potential to impact product quality are highlighted in red. A certificate of analysis for micronized CP USP powder was provided by Symbiotech® Pharmalab Ltd (Appendix I). Analysis included an assessment of total impurities that were 0.78% and complied with USP specifications [128]. However to confirm the purity of CP, the melting point was determined using a Model SMP30 Stuart® melting point apparatus (Bibby® Scientific Limited, Staffordshire ST15 0SA, UK). Each sample was filled to a depth of at least one mm in a melting tube. The melting point tube was then placed in the melting point apparatus to determine the melting point of the material. CP samples were analyzed in triplicate (n=3) and the melting point range of CP was found to be between 195.5ºC and 196.9ºC which comply with the USP specifications [128], thereby confirming that CP is relatively free of impurities.

Nuclear Magnetic Resonance (NMR) studies were also conducted to assess the purity of three different batches of CP. One-dimensional NMR data were acquired using a Bruker Avance® 600 MHz spectrometer (Bruker Avance®, Rheinstetten, Germany) in the ^{2}H lock mode. Chemical shifts were analyzed using MestRe Nova® software and were recorded in parts per million (ppm) in reference to residual deuterated $CDCl_3$ δ_H 7.26, δ_C 77.0. All coupling constants were reported in Hz. The CP sample was transferred to 178 mm glass ultra-precision ASTM Type 1 Class A borosilicate thin-walled NMR tubes (Norell® Inc. Mays Landing, NJ). These tubes are specifically designed to be used for high resolution NMR and are recommended for chemical structure determination, low and high temperature applications and low temperature sample storage [258]. The ^{1}H NMR spectrum of CP is depicted in Figure 5.4.

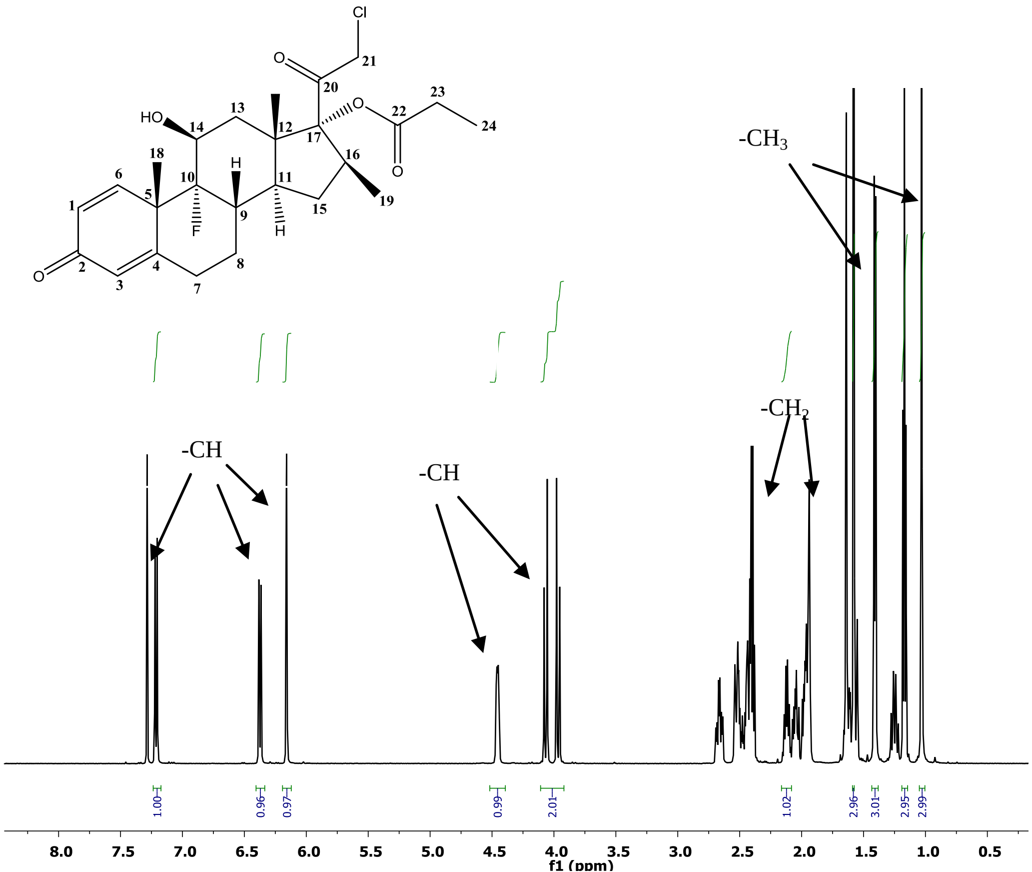


Figure 5.4 ^{1}H NMR (600 MHz; $CDCl_3$) spectrum of CP

For steroid compounds, limited number of resonance signals is required for identification purposes. The presence of three deshielded olefinic protons at δ 7.21 (d, J=10.1 Hz, IH), 6.37 (d, J=10.1 Hz, 1H) and 6.16 (s, 1H) confirmed that the dienone system of ring **A** was present and on the basis of chemical shifts and spin-spin coupling constants these signals were assigned to the C-1, C-6 and C-3 protons respectively. Signals for rings **B**, **C** and **D** match those reported previously [422,423]. Characteristic signals for CP are observed on C-17, a propionate functionality corresponding to resonance at δ 2.41 (q, J=7.6Hz, 2H) and δ 1.17 (t, J=7.5Hz, 3H) for the C-23 and C-24 components respectively. Several unassigned protons also match, but are not necessary for identification purposes. The presence of three carbonyl functional groups can be observed on the ^{13}C NMR spectrum shown in Figure 5.5.

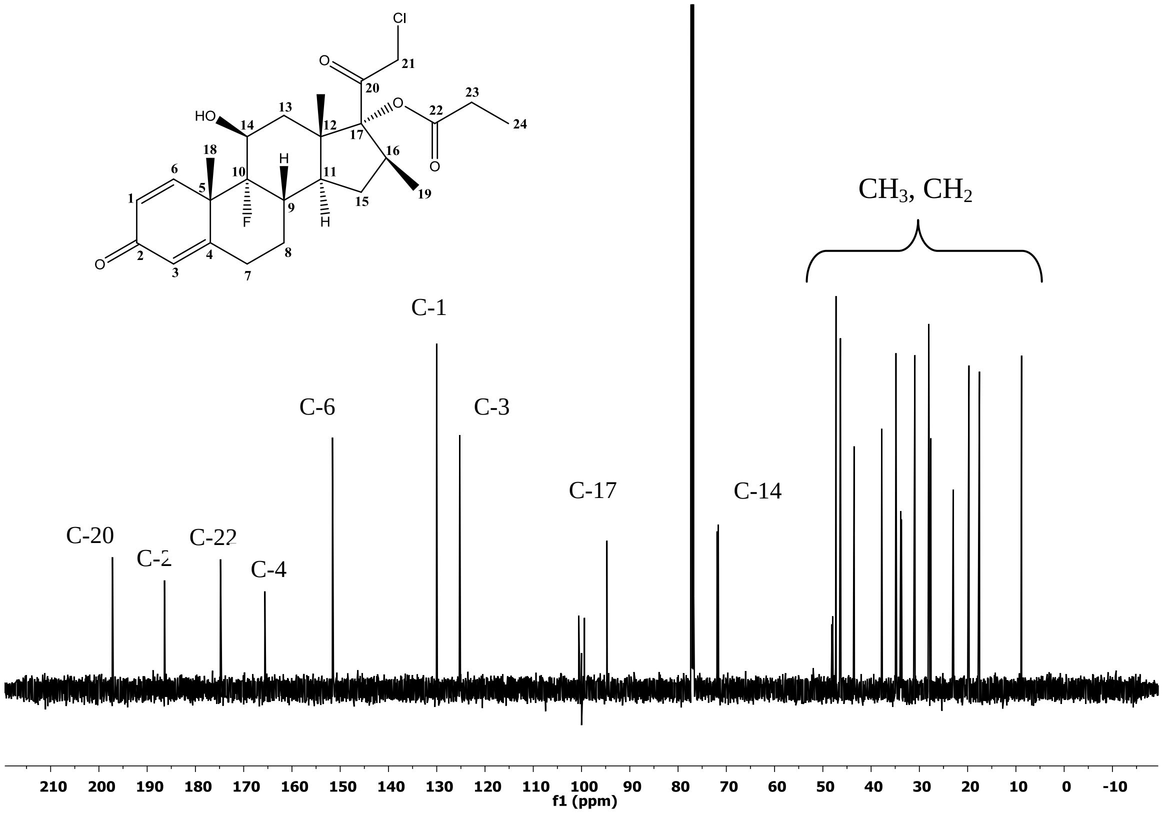


Figure 5.5 ^{13}C NMR spectrum (150 MHz; $CDCl_3$) of CP

The ^{13}C NMR signals at 197.11, 185.94 and 174.13 indicated the presence of three carbonyl (C=O) functional groups corresponding to C-20, C-2 and C-22, respectively. Other assigned carbon signals are consistent with that for the skeleton backbone of corticosteroids. Consistency between batches of CP acquired was also assessed using ^{1}H and ^{13}C NMR spectroscopy (Figures 5.6 and 5.7). No differences in the batches were observed and all signals in the spectra corresponded accurately for each batch tested.

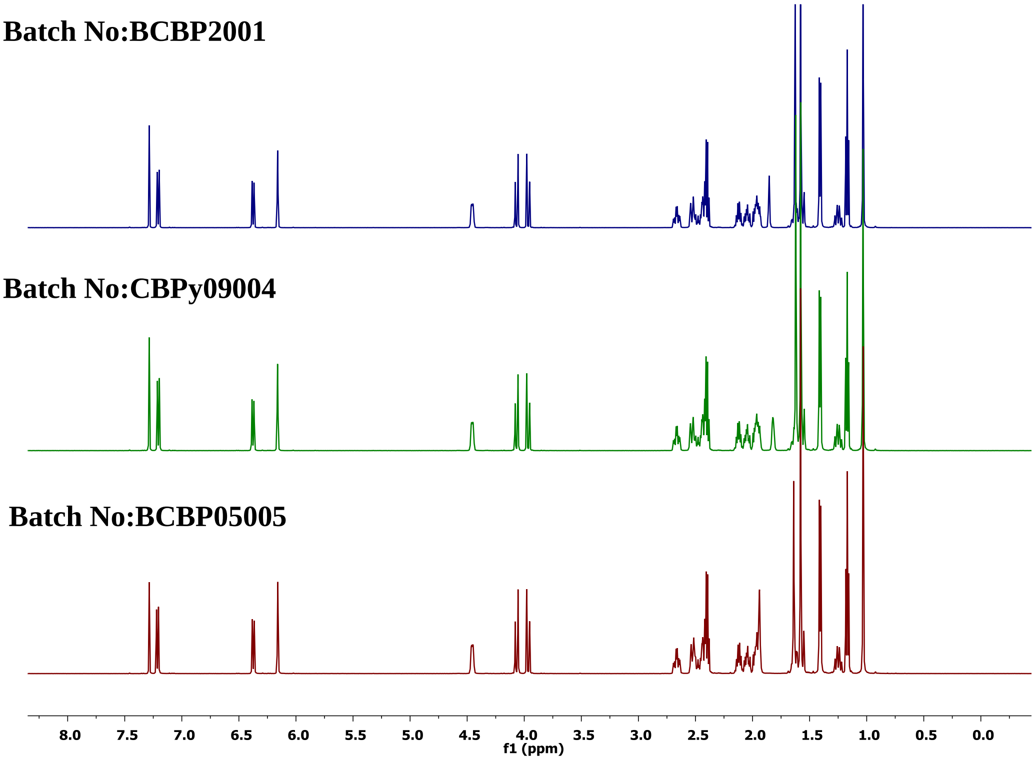


Figure 5.6 Stacked ^{1}H NMR spectra of three different batches of CP

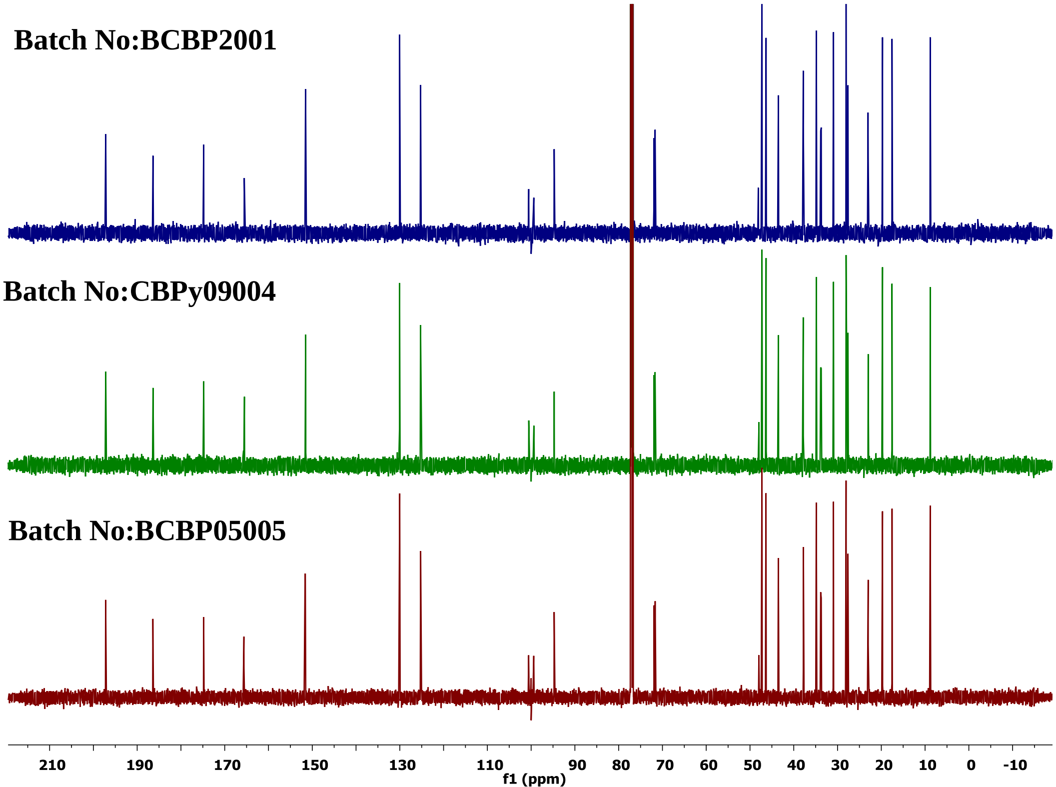


Figure 5.7 Stacked ^{13}C NMR spectra of three different batches of CP

5.2.2. Excipients

To meet the TPP, excipients with appropriate functionality were assessed based on prior scientific knowledge [419]. Compatibility studies were previously performed using infrared (IR) spectrophotometric and thermogravimetric analysis (TGA) [61]. IR spectrophotometric analysis reveals that there were no possible drug-excipient interactions between CP and excipients, since no major chemical bond shifts were evident in the IR spectra. The results generated during preformulation studies facilitated the selection of appropriate excipients for the development and optimization of a high quality CP topical cream formulation [61]. The excipients selected for use included propylene glycol, sodium citrate, citric acid, glyceryl monostearate, cetostearyl alcohol, white beeswax, Gelot® 64 (a mixture of glyceryl stearate and poly ethylene glycol-75 stearate), chlorocresol and distilled water. Previous studies performed during the formulation and manufacture of a laboratory scale CP cream formulation [61] and the % composition for the optimized CP cream that exhibited an *in vitro* release profile similar to Dermovate® cream are listed in Table 5.3.

Table 5.3 Excipients used to manufacture 500 g of a CP cream formulation

Excipient	Abbreviation	% Composition	Batch Quantity (g)	Function
Propylene glycol	PG	46.00	230.00	Cosolvent and penetration enhancer
Sodium citrate	SC	0.05	0.250	pH adjusting agent
Citric acid	CA	0.05	0.250	Buffering agent
Glyceryl monostearate	GMS	11.00	55.00	Emollient, stabilizing and emulsifying agents
Cetostearyl alcohol	CETO-A	8.40	42.00	Stiffening agent, viscosity modifier
Gelot® 64	G-64	3.00	15.00	o/w emulsifier
White beeswax	WB	1.15	5.75	Emulsion stabilizer
Chlorocresol	CH	0.025	0.125	Preservative
Distilled water	DW	30.225	151.13	Vehicle

Based on scientific and prior knowledge of the excipients used in CP cream formulations, a risk assessment was conducted to establish the potential impact of these excipients on final product quality. Excipients can be used to produce a formulation that mimics the dissolution profile of the innovator product and therefore the levels and quality of raw materials used in a formulation must be carefully controlled [63]. An evaluation of excipient attributes that present a risk with respect to final product quality is summarized in Table 5.4.

Table 5.4 Potential impact of excipients on product attributes

	Formulation Attributes							
Drug Product CQA	**Propylene Glycol**	**Sodium Citrate**	**Anhydrous Citric Acid**	**Glyceryl Monostearate**	**Cetostearyl Alcohol**	**Gelot® 64**	**White Beeswax**	**Chlorocresol**
Appearance	High	Low	Low	High	High	High	Low	Low
Identity	Low	Low	Low	Low	Low	Low	Low	Low
Assay	Low	Low	Low	Low	Low	Low	Low	Low
Impurities	High	High	High	High	High	High	High	High
Content Uniformity	Low	Low	Low	High	High	High	Low	Low
***In vitro* analysis**	High	Low	Low	High	High	High	Low	Low

The excipients identified as high risk were investigated throughout the formulation development and manufacturing process and the results are presented in § 6.2, Chapter 6. The excipients used in the formulation of CP creams are conventionally used and the quantities of excipients were generally within the standard range of use. The certificates of analysis for these excipients were provided by the supplier companies that included Croda® (SA) Limited (Johannesburg, RSA), Aspen Pharmacare® (Port Elizabeth, RSA), Medicolab CC® (Johannesburg, RSA) and Comhan Trading® Co Ltd (Johannesburg, RSA) and are listed in Appendix I. The important CQA were established to be propylene glycol, glyceryl monostearate, cetostearyl alcohol and Gelot® 64 content and included an assessment of appearance, impurities, content uniformity and *in vitro* analysis. Although a certificate of analysis was provided for each excipient, quality control tests were performed for all excipients to confirm the quality of the raw materials prior to use in these studies. The samples were analyzed in accordance with the 2012 BP [332] and 2011 USP/NF [128]. The results of quality control tests revealed that most of the excipients met the specifications in the BP [331], the USP/NF [128] and conformed to the data reported in the certificates of analysis. These quality control documents are included in Appendix I. The excipients were confirmed to be of suitable quality for the manufacture of a high quality topical corticosteroid cream formulation.

5.3. RISK CONTROL

Risk management and analysis are important to ensure that control of risk variables during formulation development and manufacturing are adequately monitored and controlled [36]. This is performed to ensure product quality and to minimize the risk of product variability. The same batch of each excipient, verified in terms of batch size was used to manufacture the cream formulations. Propylene glycol was provided from Aspen Pharmacare® (batch number X112573) and was used for all manufacturing processes. By using this approach, the potential impact of different batches of raw material as a risk factor was eliminated.

Similarly the same manufacturing plant or cream/ointment mixer was used for all processing to eliminate the potential risk effect of using different pieces of manufacturing equipment. It was also crucial that the manufacturing conditions such as surrounding temperature and water flowing through the heating jacket were recorded and monitored to assess the potential risk of these external factors on pilot scale cream formulation manufacture. Distilled water from the same supply was used during all manufacturing to minimize this as a source of variability. The batches of cream were manufactured in compliance with GMP and existing Standard Operating Procedures (SOP) at the Faculty of Pharmacy production plant. Process validation of the cream mixer was performed prior to the manufacture of all cream formulations. The analytical method used to assess the CP pilot scale cream formulation was validated and is reported in Chapter 4 *vide infra* to ensure that the method was accurate, reliable, precise and would not produce false product outcomes. Other instruments such as the analytical balances, viscometer, pH meter, electrical conductivity meter and tube filling machine underwent regular maintenance calibration and testing procedures prior to and during production to ensure that they were in appropriate operating condition.

5.4. PROCESS VALIDATION OF THE CP CREAM FORMULATIONS

The ICH and FDA guidelines [36,424] define process validation as a means of ensuring and providing documentary evidence that processes operated within their specified design parameters are capable of repeatedly and reliably producing a finished product of the required quality. Therefore the purpose of process validation is intended to establish that a proposed manufacturing process is suitable and consistently yields a product of the desired quality thereby ensuring that the process is suitable and under control [425]. The critical parameters considered during process validation for the manufacture of pilot scale cream formulation were mixing, homogenization and cooling.

5.4.1. Mixing

Mixing involves blending the API and other excipients with an anchor and blades. Following mixing, the CP content of the cream was assessed so as to establish whether the mixing process was efficient and the process was validated in this way. The mixing speed and time are critical variables that may affect the quality of a mixing process and therefore content uniformity. While mixing speed is kept constant, the mixing time can be studied so as to validate the mixing process. Three batches *viz.*, CP-PV1, CP-PV2 and CP-PV3 were used as validation batches for the mixing process. Each side sampled (n=3) from top (CP-PV1T, CP-PV2T, CP-PV3T), middle (CP-PV1M, CP-PV2M, CP-PV3M) and bottom (CP-PV1B, CP-PV2B, CP-PV3B) layers of CP cream formulations for QC analysis of assay, as shown in Figure 5.8 and Table 5.5.

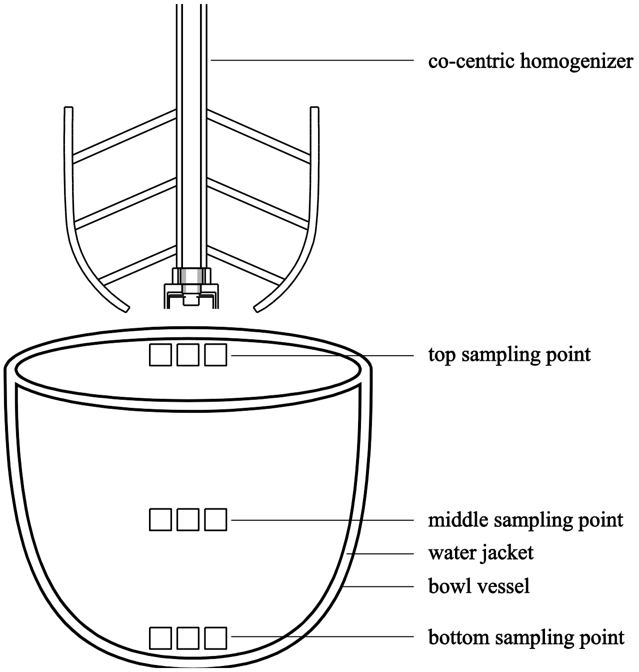


Figure 5.8 Top, middle and bottom sampling points on the mixing bowl

Table 5.5 CP content following 60 minutes of mixing

Assay Results	CP-PV1	CP-PV2	CP-PV3
Top (%)	99.98	100.02	100.23
	100.01	99.86	99.01
	100.01	99.17	99.98
Mean	100.00	99.68	99.74
SD	0.02	0.45	0.64
%RSD	0.02	0.45	0.65
Middle (%)	99.78	100.00	99.98
	100.02	100.23	100.01
	99.89	100.18	99.99
Mean	99.90	100.14	99.99
SD	0.12	0.12	0.02
%RSD	0.12	0.12	0.02
Bottom (%)	99.19	99.13	100.79
	99.89	100.03	100.56
	99.50	100.49	100.50
Mean	99.52	99.88	100.62
SD	0.35	0.70	0.15
%RSD	0.35	0.70	0.15

The % CP content of the three batches of cream were found to be well within the acceptance criteria, with no CP content < 90% or > 115% of the label claim for these creams [128,331].

5.4.2. Homogenization

The homogenization process involves high shear agitation of the cream to ensure that the composition is uniformly distributed and the cream homogeneous. The % CP content of the cream formulation was tested to validate the homogenization process in a similar manner to the process used for the mixing operation as homogenization speed and time are critical variables that may affect content uniformity. Samples of cream were collected following homogenization at different speeds *viz.*, at a maximum rpm of 2500, a minimum rpm of 500 and at a value of 1200 rpm, considered the optimal speed for the process at start, middle and end of the homogenization process. Content uniformity and physical parameters such as viscosity, spreadability and pH were monitored and three batches of cream *viz.*, CP-PV1, CP-PV2 and CP-PV3 were manufactured for the validation process. The results of the effect of homogenization at low, medium and high homogenization speeds on content are summarized in Table 5.6.

Table 5.6 Validation of homogenization

Parameter	Speed	CP-PV1T	CP-PV1M	CP-PV1B	CP-PV2T	CP-PV2M	CP-PV2B	CP-PV3T	CP-PV3M	CP-PV3B	Mean	SD	%RSD
% CP Content (%)	**Min**	99.90	100.01	100.45	99.43	99.98	100.34	100.45	99.35	99.99	99.99	0.39	0.40
	Max	100.04	100.34	100.04	99.09	100.04	100.5	99.90	100.04	100.04	100.00	0.39	0.39
	Opt	99.94	99.90	99.90	100.03	100.45	99.09	99.90	101.45	99.09	99.97	0.71	0.71
Viscosity (cP)	**Min**	5900	5803	5920	5590	5610	5902	5590	5903	5777.25	5777	143.86	2.49
	Max	60820	59400	59510	61700	58200	60600	62100	59200	60750	60253	1264.89	2.58
	Opt	30405	29506	30450	31304	29045	30600	30965	31405	30900	30508	787.99	2.58
Spreadability (cm^2)	**Min**	20.03	21.45	20.89	19.99	20.21	21.45	21.45	20.98	19.99	20.72	0.66	3.19
	Max	35.45	34.43	35.56	34.99	35	35.99	35.89	36	35.9	35.45	0.55	1.56
	Opt	27.88	28.01	28.88	27.99	28.01	28.9	28.92	29.39	28.09	28.45	0.56	1.98
pH	**Min**	6.51	6.54	6.45	6.66	6.57	6.21	6.35	6.45	6.24	6.44	0.15	2.33
	Max	6.43	6.46	6.55	6.45	6.23	6.34	6.24	6.45	6.23	6.37	0.12	1.86
	Opt	6.57	6.56	6.42	6.34	6.45	6.67	6.34	6.24	6.35	6.43	0.14	2.15

The homogenization step for all three batches was validated for high, low and optimum rpm. A maximum speed of 2500 rpm resulted in creams of high viscosity with an average value of 60820 ± 1264 cP and a spreadability of 34.43 ± 0.55 cm^2, whereas use of the minimum speed resulted in creams of low viscosity with an average value of 5777 ± 143 cP and a spreadability of 20.72 ± 0.66 cm^2. The % CP content and pH were found to be constant for all three batches manufactured at the different homogenization speeds. The physical parameters for the creams were found to be accurate, with % RSD < 5% and the homogenization process was therefore considered appropriate and validated.

5.4.3. Cooling

Cooling the creams for 60 and 120 minutes at set cooling rates at the end of the manufacturing process may result in the presence of congealed material at specific sites of the mixing bowl. These sites are termed "cold spots" and the presence of congealed material is an important factor potentially affecting the content uniformity of a cream. If the cooling time is long, there may be a tendency to facilitate the formation of congealed materials at the "cold spots" which may be a result of a lack of accessibility of those locations to the anchor blades or the homogenizer. Samples of CP cream were therefore collected at different time intervals to investigate the effect of cooling times on the presence of congealed material in the mixing bowl and the samples were tested for content uniformity. Three batches CP-PV1, CP-PV2 and CP-PV3 were manufactured during the validation process. The creams were sampled from top (CP-PV1T, CP-PV2T, CP-PV3T), middle (CP-PV1M, CP-PV2M, CP-PV3M) and bottom (CP-PV1B, CP-PV2B, CP-PV3B) of the mixing bowl and the results are summarized in Table 5.7.

Table 5.7 Impact of cooling time

Assay	Presence of Congealed Material	CP-PV1	CP-PV2	CP-PV3
Top (%) 60 mins	No	99.99	101.34	100.57
120 mins	No	100.10	100.86	99.15
	Mean	100.0	101.10	99.86
	SD	0.14	0.34	1.00
	%RSD	0.14	0.33	1.00
Middle (%) 60 mins	No	99.99	100.19	99.67
120 mins	No	100.06	100.23	100.01
	Mean	100.03	100.21	99.84
	SD	0.05	0.03	0.24
	%RSD	0.05	0.03	0.25
Bottom (%) 60 mins	No	99.99	99.33	100.8
120 mins	No	100.89	100.78	100.46
	Mean	100.44	100.06	100.63
	SD	0.64	1.03	0.24
	%RSD	0.63	1.02	0.24

There was no apparent formation of congealed material at any of the locations sampled from the mixing bowl for the 60 and 120 minutes cooling periods. The % CP content for all the batches was well within the acceptance criteria following cooling and therefore cooling did not appear to impact content uniformity of CP.

5.5. CONCLUSIONS

Quality risk management promotes a scientific and practical approach to decision-making and provides a well-documented, transparent and reproducible method for accomplishing the necessary steps of the QRM process based on current approaches and knowledge about assessing the probability, severity and sometimes detectability of a hazard and/or risk [37]. Traditionally the risks to quality have been assessed and managed in many informal ways whether they are empirical or internal procedures based on compilation of observations, trends and other information. Such approaches continue to provide useful information that might support topics such as the handling of complaints, quality defects, deviations and allocation of resources. The WHO [409], FDA [6] and the ICH [37] have recently established QRM guidelines and the pharmaceutical industry and regulators can now assess and manage risk using recognized risk management tools and/or internal procedures such as SOP. In these studies, a scientific approach was used to identify desired dosage form and performance attributes through establishing a TPP. From the TPP, an initial list of CQA was developed and risk assessment was undertaken to identify possible formulation variables and unit operations that were most likely to have an impact on the CQA of the product and/or process. These data were then used to focus development activities on potential high risk areas. An Ishikawa diagram was used as an additional tool for conducting risk assessment. This approach allowed an exhaustive analysis to be conducted and included all factors that may have an impact on product quality [409].

Risk analysis was undertaken commencing with an assessment of the physico-chemical characteristics of CP, leading to identification of a viable formulation and manufacturing approach. Formulation development involved the use of information obtained from previous work and structured experimentation to investigate the relationship between formulation component levels, CP attributes and drug product quality attributes. Using prior knowledge, models, extrapolation and risk assessment processes, the material attributes and process parameters which could impact final product quality, were identified. The impact of excipient attributes such as glyceryl monostearate, cetostearyl alcohol, Gelot® 64, propylene glycol and manufacturing parameters such as homogenization speed, homogenization time, anchor speed, mixing time, heating temperature, batch size and cooling time on *in vitro* release of CP from cream formulations were further studied using three screening design approaches, the results of which are discussed in detail in Chapter 6. The critical parameters considered during process validation for the pilot scale manufacture of CP were established and validated according to the ICH guidelines [385,424]. It can therefore be concluded that the use of risk assessment tools, preliminary formulation studies and knowledge obtained from previous work are essential in designing and conducting statistical experiments for the establishment of a desired design space for the manufacture of CP cream formulations to ensure that a design space for a product that is bioequivalent to a commercially available dosage form could be established.

CHAPTER SIX

THE EVALUATION OF FORMULATION VARIABLES AND PROCESS PARAMETERS FOR THE DEVELOPMENT OF PILOT SCALE CP FORMULATIONS USING THREE SCREENING DESIGNS

6.1. INTRODUCTION

6.1.1. Statistical Screening Designs

Design of Experiments (DOE) was invented by the statistician and geneticist Sir Ronald A. Fisher at Rothamsted Experimental Station situated 25 miles north of London during the 1920s and 1930s [426,427]. The proposed methodology for designing experiments was described in his books “The Arrangement of Field Experiments” in 1926 and “The Design of Experiments” in 1935 [428]. In 1926, Fisher argued that “complex designs” such as factorial designs were more efficient in research than studying only one factor at a time. Much of his pioneering work dealt with agricultural applications of statistical methods. At Rothamsted Fisher designed plant-breeding experiments that provided greater information with less investment of time, effort, and money [427,429]. In his first book on DOE, Fisher explained how valid conclusions could be drawn efficiently from experiments with natural fluctuations such as temperature, soil conditions and rain fall in the presence of “nuisance variables”. The known “nuisance variables” usually cause systematic bias in groups of results such as batch-to-batch variation. The unknown “nuisance variables” usually cause random variability in the results and are called inherent variability or noise [426]. Another important concept suggested by Fisher is Analysis of Variance or ANOVA and he suggested that the outcomes of factorial experiments could be analyzed using ANOVA. This approach enabled researchers to answer several questions at the same time and the experiments were conducted as a set of partitioned sub-experiments that differ from each other in one or more of the factors or treatments applied in them [430,431].

George Box, a statistician and engineer at the British chemical firm Imperial Chemical Industries applied a statistical technique initially used in agriculture to boost the efficiency of industrial manufacturing. He became a leading developer of experimental design procedures for optimizing chemical processes [432]. In the 1940s, some efficient statistical designs for estimating several main effects were found independently and in near succession by Raj Chandra Bose and K. Kishen at the Indian Statistical Institute. Their work remained unknown until Plackett-Burman designs were published in *Biometrika* in 1946 [433]. At about the same time, Raj Chandra Rao introduced the concept of orthogonal arrays as an experimental design approach. This concept played a critical role in the development of the Taguchi methods which were established during his visit to the Indian Statistical Institute in the early 1950s [434].

Taguchi’s methods were successfully adopted and applied by the Japanese and Indian industries and subsequently were also embraced by US industry, albeit with some reservation. Total Quality Management (TQM) and Continuous Quality Improvement (CQI) are management techniques that have been spawned from this statistical quality revolution or statistical quality control and DOE [435].

Around the mid-1990s, “Six Sigma” became popular as a new approach of representing CQI. The Six Sigma approach to quality is said to have begun with a reliability engineer at Motorola in 1985. Six Sigma took off as a significant quality movement when Jack Welch, CEO of General Electric used this approach that facilitates decision making based on statistics, quality and feedback loops and has subsequently been adopted by many large manufacturing companies. Six Sigma first appeared in healthcare in the year 2000 and growth in its use was slow but steady [436]. However by 2002 a number of healthcare institutions such as Charleston Area Medical Center in Charleston, West Virginia had adopted Six Sigma as their major quality program. At about the same time, the Valley Baptist Health System in Southern Texas began to develop a Six Sigma culture that resulted in over one hundred projects being completed in areas such as admissions, laboratories, the emergency department and operating rooms [437].

Pharmaceutical scientists encounter complex technical challenges during pharmaceutical development and therefore it is critical to use an effective optimization methodology to address these challenges. Statistical modeling and DOE have been applied to formulation development and are useful in process optimization and validation in the pharmaceutical arena [438-441]. Recent manuscripts have explained the uses of factorial design and multivariate methods have been used to improve API solubility, to test robustness of an analytical method and aid in the selection of solvents during analytical method development [439,442,443]. The Taguchi, Plackett-Burman and 2-Level Fractional Factorial DOE are well known in the pharmaceutical industry for their application for formulation development [441,444,445]. The main objective of this work was to compare the three screening design techniques, commonly applied in the pharmaceutical industry, to the formulation and manufacture of pilot scale cream formulations for comparative purposes with a view to defining a design space for a bioequivalent product.

6.1.2. Taguchi Design of Experiments

Following World War II many Japanese manufacturers were struggling to cope with limited resources and due to the approach proposed by Taguchi, manufacturers were able to survive and eventually flourish [446,447]. In Japan, Taguchi revolutionized manufacturing processes through cost savings and understood that all manufacturing processes are affected by external influences such as noise due to variability. However Taguchi realized that the methods of identifying sources of noise have the greatest effect on product variability. His ideas have been adopted by successful manufacturers around the globe since this approach results in creating advanced production processes at much lower cost [446]. Taguchi Design of Experiments are statistical methods that can be used to improve the quality of manufactured goods and has been applied to the engineering [434,448,449], biotechnology [450,451], marketing [452], advertising [446,453] and formulation [441,444,445] sectors. Interestingly the Taguchi approach in the field of quality engineering was not made public in the Western Hemisphere until the early 1980s [448]. A number of professional statisticians have welcomed the goals and improvements brought about by the Taguchi methods, particularly in respect of development of designs for studying variability, however the inefficiency of some of the Taguchi proposals has been criticized [446,454]. The elementary work undertaken by Taguchi includes three principle contributions to statistics *viz.*, **i)** a specific loss function, **ii)** the philosophy of off-line quality control and **iii)** innovations in the design of experiments [455].

Since the core parameter of a Taguchi design is based on experimental methods, he decided to make T-DOE user-friendly [454]. Taguchi simplified the use of DOE by incorporating a standard set of experimental design matrices *viz.*, orthogonal arrays, a graphical aid to assign the factors in an experimental matrix such as linear graphs, clear guidelines for the interpretation of results including cookbook, special data transformation such as Signal/Noise Ratios to achieve reduced and a formal study of uncontrollable factors using a robust design technique [454]. A proper orthogonal array can be selected after identifying the number of parameters and the number of levels to be evaluated. Using an array selector table, the name of the respective array can be found by looking at the column and row, corresponding to the number of parameters and number of levels to be evaluated as shown in Table 6.1 [456]. Taguchi also simplified Tolerance Analysis through the use of DOE [454]. The main objective in Taguchi approach is to design robust systems that become reliable when used under uncontrollable conditions. The method aims to adjust design parameters, known as control factors, to their optimal levels such that the system response is always robust. A robust system is insensitive to noise factors, which are difficult, if not impossible to control [446]. Some informative studies have used the Taguchi parameter design method for the purpose of optimizing turning parameters [457-459]. These studies made use of various workpiece materials and controlled parameters to optimize surface roughness, dimensional accuracy, or tool wear. Each evaluation used different combinations and levels of cutting speed, feed rate, depth of cut, cutting time, workpiece length, cutting tool material, cutting tool geometry, coolant and other machine parameters. These studies revealed that clear and useful correlations existed between their control and response parameters, indicating that several different parameters can be included in this type of study and an exclusive combination of parameters can be tailored for a specific situation [446,459].

Table 6.1 An array selector table for Taguchi Design of Experiments [446,456]

Number of Levels	Number of Parameters (P)																													
	2	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**31**
2	L4	L4	L8	L8	L8	L8	L12	L12	L12	L12	L16	L16	L16	L16	L32	L32	L32	L32	L32	L32	L32	L32	L32	L32	L32	L32	L32	L32	L32	L32
3	L9	L9	L9	L18	L18	L18	L18	L27	L27	L27	L27	L27	L36	L36	L36	L36	L36	L36	L36	L36	L36	L36								
4	L16	L16	L16	L16	L32	L32	L32	L32	L32																					
5	L25	L25	L25	L25	L25	L25	L50	L50	L50	L50	L50																			

In the array selector table links are provided to many of the predefined arrays and the arrays are created using an algorithm developed by Taguchi, thereby allowing each variable and setting to be tested equally in a specific set of experiments. For example, if there are three parameters to be investigated at two levels, the proper array to use is L_4 [446,456].The Taguchi design can also be viewed as being a conventional design for the inner array factors with the addition of a “small” outer array fractional designs at each corner of the “inner array” box, as shown in Figure 6.1 [7].

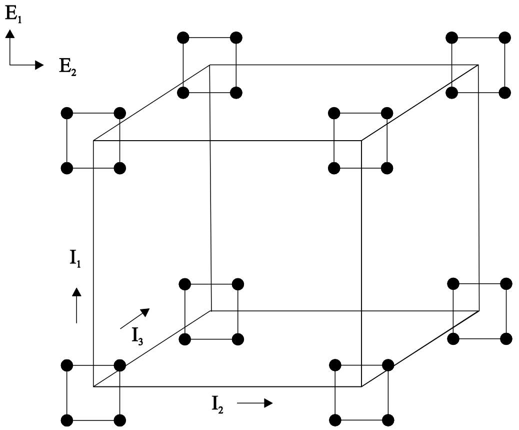


Figure 6.1 Inner $2^3$and outer 2^3arrays for robust design (“I” is the inner array and “E” is the outer array) [7]

6.1.3. Plackett-Burman Experimental Design

In 1946 Plackett and Burman developed an experimental design titled “Plackett-Burman Design” while working in the British Ministry of Supply and published a manuscript titled “The design of optimal multifactorial experiments” in *Biometrika* [460]. The goal of Plackett and Burman was to identify experimental designs for investigating the dependence of some measured quantity on a number of independent factors at different levels (*L*) in order to minimize the variance of the estimates of the dependent factors with a limited number of experiments [461]. Interactions between the factors were considered negligible. The solution to the problem was to find an experimental design where each combination of levels for any pair of factors occurred with the same frequency throughout all experimental runs, as described in Table 6.2. A complete factorial design would satisfy this criterion, but their initiative was to establish designs requiring fewer experiments than would be necessary if a full factorial approach was used [460]. In 1933 Plackett and Burman used a method described by Raymond Paley for the case of two levels ($L = 2$) to generate orthogonal matrices with elements that were either 1 or -1. Paley’s method could also be used to identify such matrices of size *N* for most *N* equal to a multiple of 4. This method worked for all values of *N* up to 100, except for a value of $N = 92$. However, in cases where *N* is a power of 2, the resulting design is identical to a Fractional Factorial design. Therefore the Plackett–Burman experimental designs are mostly used when *N* is a multiple of 4 but not a power of 2 i.e. $N = 12, 20, 24, 28$ etc. [462]. In cases of more than two levels ($L > 2$), Plackett and Burman revealed designs that had previously been reported by Bose and Kishen [433].

Table 6.2 Plackett-Burman design for 12 runs and 11 two-level factors [463]

Run	**X_1**	**X_2**	**X_3**	**X_4**	**X_5**	**X_6**	**X_7**	**X_8**	**X_9**	**X_{10}**	**X_{11}**
1	+	+	+	+	+	+	+	+	+	+	+
2	-	+	-	+	+	+	-	-	-	+	-
3	-	-	+	-	+	+	+	-	-	-	+
4	+	-	-	+	-	+	+	+	-	-	-
5	-	+	-	-	+	-	+	+	+	-	-
6	-	-	+	-	-	+	-	+	+	+	-
7	-	-	-	+	-	-	+	-	+	+	+
8	+	-	-	-	+	-	-	+	-	+	+
9	+	+	-	-	-	+	-	-	+	-	+
10	+	+	+	-	-	-	+	-	-	+	-
11	-	+	+	+	-	-	-	+	-	-	+
12	+	-	+	+	+	-	-	-	+	-	-

For any two X_i, each combination (--, -+, +-, ++) appears three *viz,* the same number of times

In Plackett-Burman designs, the main effects have a complex confounding relationship with two-factor interactions. These designs should therefore be used to study main effects when it can be assumed that two-way interactions are negligible [464]. In practical use, Plackett-Burman designs are often used to screen or identify important factors that influence process output measures or product quality. These designs are useful for fitting first-order models that can detect linear effects and provide information on the existence of second-order effects (curvature) when the design includes experiments undertaken at the center points of the design [465].

6.1.4. 2-Level Fractional Factorial Design

Factorial experimental designs were used in the 19th century by Lawes and Gilbert at the Rothamsted Experimental Station [466]. A full factorial design usually consists of two or more factors, each with discrete possible values or "levels" for which the experimental units take on all possible combinations of levels of factors. For instance, with five factors, two at two levels and three at three levels (denoted $2^2 3^3)^1$, 108 combinations are possible [467]. In such a design, the investigator studies the effect of each factor on a response variable as well as interactions between factors that have an impact on that response variable [468]. In fact all main effects, all two-way interactions and all higher-order interactions can be estimated and uncorrelated in a full factorial design. A full factorial design is therefore known to be balanced and orthogonal in shape [469]. However the main concern with a full factorial design is that, for most practical situations is a costly and tedious process if subjects have to rate all possible combinations. For this reason researchers often use fractional factorial designs, which require fewer experiments to be conducted than undertake full factorial experimental designs [469,470].

Fractional factorial designs are experimental designs consisting of a carefully selected fraction of the experimental runs that make up a full factorial design [471,472]. Fractional designs are normally expressed using the notation l^{k-p}, where l is the number of levels of each of the factors investigated, k is the number of factors to be investigated, and p describes the size of the fraction of the full factorial that was used. For example, a 2^{11-7} design is an eleven factor factorial design and therefore this experimental design requires 16 runs. In practice, one rarely encounters $l > 2$ levels in fractional factorial designs and the levels of a factor are commonly coded as +1 for the highest level, and −1 for the lowest level to be investigated [473,474].

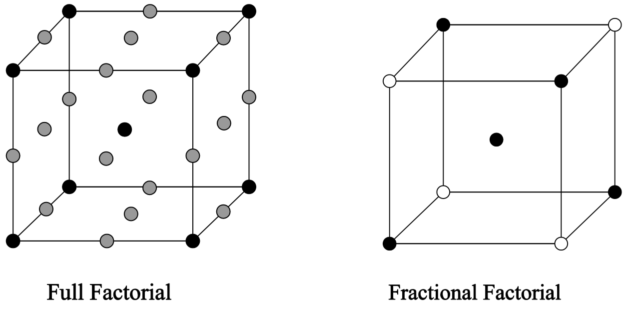


Figure 6.2 Full factorial and fractional factorial designs [475]

Another important property of fractional factorial designs is the resolution or ability of the technique to separate main effects and low-order interactions from each other [426,471]. The most important fractional factorial designs are those of resolution III, IV and V. In general, designs of resolution III and sometimes IV, seek to screen out a few main yet important effects from the many less important effects whereas designs of resolution V, and higher are used for focusing on more than just main effects in an experimental situation [471]. Therefore resolutions below III are not useful and resolutions above V tend to waste resources due to the need for expanded experimentation in most cases. The basic purpose of a fractional factorial design is to investigate cause-and-effect relationships of significance in a given experimental setting as economically as possible [476,477].

6.2. MATERIALS AND METHODS

6.2.1. Materials

Clobetasol 17-propionate (CP) was purchased from Symbiotec® Pharmalab P. Ltd (Indore, India). Propylene glycol, sodium citrate, citric acid, chlorocresol and white beeswax were donated by Aspen Pharmacare® (Port Elizabeth, RSA). Glyceryl monostearate and cetostearyl alcohol were purchased from Croda® Chemicals (SA) Limited (Johannesburg, RSA) whilst Gelot® 64 was purchased from Gattefossé® (SAS, Saint-Priest Cedex, France). All chemicals and reagents used were at least of analytical grade and were used without further modification.

6.2.2. Manufacturing Procedure for Pilot Scale CP Formulations

Batches of CP cream (3/6 Kg) were manufactured using a Model WLP/13/2006-2007 Wintech® cream/ointment mixer (Wintech® Pharmachem Equipment PVT. Ltd, Mumbai, India). Prior to manufacture, the 25 L mixing bowl was thoroughly cleaned with Liquid K® detergent (Ecolab®, Eden Glen, RSA) and distilled water. After draining the water, the mixing bowl was allowed to dry and equilibrate to room temperature (± 22°C).The temperatures of the cream manufacturing facility and the Wintech® cream/ointment mixer were recorded during use and are reported in the batch production records (Appendix II). The manufacture of cream formulations was performed using current Good Manufacturing Practice principles. The water jacket surrounding the mixing bowl was filled with distilled water for heating and cooling purposes. Appropriate quantities of CP, citric acid, sodium citrate, propylene glycol, chlorocresol, Gelot® 64, cetostearyl alcohol, white beeswax, glyceryl monostearate and distilled water were weighed on a Model PM460 Mettler® balance (Mettler® Instruments, Zurich, Switzerland) and introduced to the mixing bowl through the charging port. The mixture of excipients was stirred at a set anchor speed for a specified mixing period at the desired heating temperature and following mixing, the blend was homogenized at a specific homogenization speed for a specific time as defined by the particular experimental design and run in use at that time. Homogenizing time is the time taken for the cream to be homogenized using a co-centric homogenizer whereas mixing time is the time the cream is being mixed by the anchor blades prior to the homogenizing time. The melt was then cooled with continual stirring at a set anchor speed. Cooling was achieved by allowing distilled water (± 20°C) to pass through the heating jacket at a set cooling rate. The temperature of the distilled water was recorded on the batch manufacturing record. Slow continuous agitation of the anchor was permitted until the temperature on the operating panel touch screen displayed between 30°C and 35°C. Rotation of anchor was then stopped and the pressure build-up in the mixing bowl was released by activating the pressure vent. The final CP cream was stored in opaque containers until all quality control tests had been completed, after which it was packed into 50 g aluminium collapsible cream tubes. A schematic representation of the Wintech® cream/ointment mixer is shown in Figure 6.3.

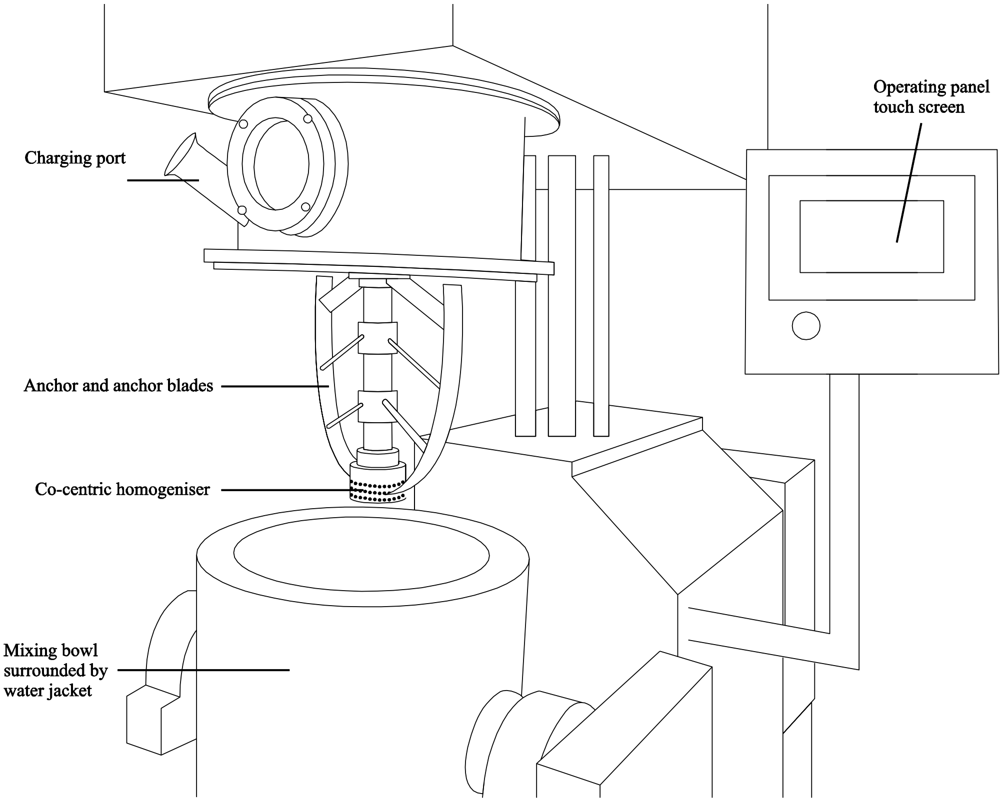


Figure 6.3 A schematic diagram of the Wintech® cream/ointment mixer

6.2.3. Quality Control Parameters

Quality control assessment of the CP cream formulations included tests for viscosity, spreadability, pH, % CP content, tube extrudability, electrical conductivity and *in vitro* release using a Franz diffusion cell system.

6.2.3.1. Rheology

The viscosity of the manufactured topical creams was determined using a Model-RVDI+ Brookfield® Viscometer fitted with a helipath stand (Brookfield® ENG Labs Inc., Stoughton, USA) at 10 rpm fitted with a T-F spindle (code 96). The T-F spindle was selected for use to maintain a torque of between 10% and 90% of the measurement range of the viscometer. Viscosity measurements were performed at room temperature (± 22°C) 60 seconds after the commencement of rotation of the spindle. Viscosity readings were taken 24 hours following the manufacture of each batch. Three consecutive readings (n=3) were recorded and the average viscosity calculated for each batch.

6.2.3.2. Spreadability

Spreadability is the term used to indicate the extent to which a cream may spread when applied to the skin or affected part(s) of the body. The therapeutic potency of a formulation also depends on the ability to be spread that product over the diseased area [478,479]. The parallel-plate method is widely used for measuring the spreadability of semi-solid preparations. It is a relatively inexpensive, rapid and simple method [480]. Approximately three grams of each formulation was sandwiched between two sheets of glass, with dimensions of 15 cm x 15 cm. One 100 gram mass piece was placed in each corner on the upper sheet of glass to place uniform pressure on the formulation. The weights were left in place for three minutes and the radius of the formulation was measured as shown in Figure 6.4 [61].

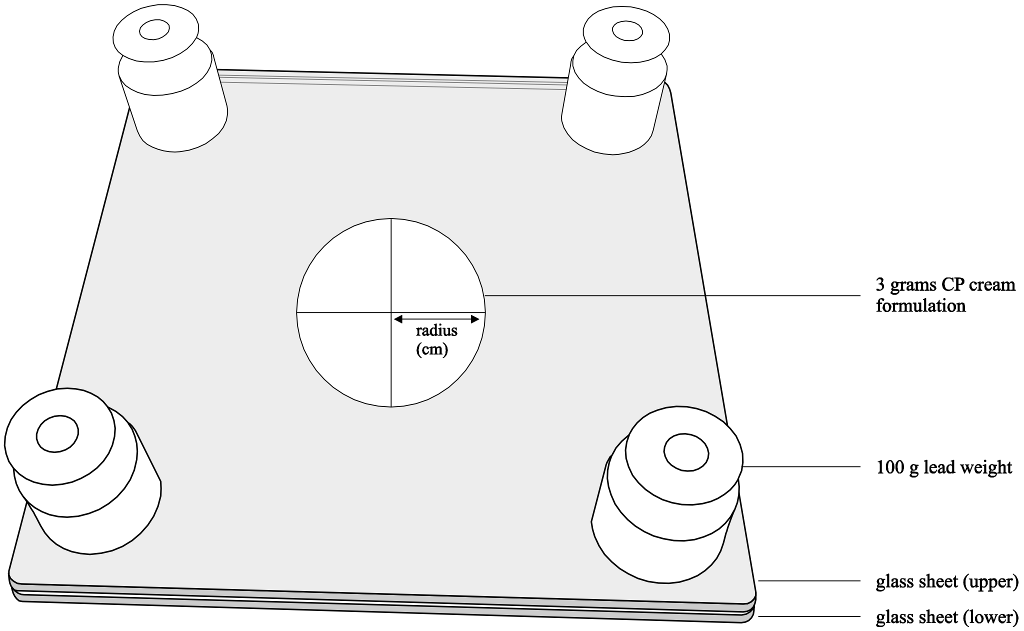


Figure 6.4 Schematic representation of the spreadability test apparatus

Spreadability measurements were performed in triplicate (n=3) for each manufactured batch and the average spreadability calculated and recorded. This approach is not precise or sensitive and data generated must be manually interpreted. However, the results obtained from spreadability testing providessome information in respect of the spreadibility of the cream [61]. Following the measurement of the radius of cream, the area covered by the cream formulation was calculated using Equation 6.0. Spreadability is calculated by using the formula:

$$A = \Pi r^2 \qquad \textbf{\textit{Equation 6.0}}$$

Where

A=Area of the formulation spread on the surface of the glass sheet (cm^2)
Π=Constant (3.141)
r=Radius of the formulation spread on the surface of the glass sheet (cm) [61]

6.2.3.3. pH Determination

The pH of the creams was measured at room temperature (±22°C) using a Model GLP 21 Crison® pH meter (Crison® Instruments, Barcelona, Spain) by inserting the electrode to a depth of 0.5 cm into a beaker containing the cream. The pH was measured in triplicate (n=3) and the average of the three readings was calculated and recorded.

6.2.3.4. CP Content

Approximately 1310 mg of cream was accurately weighed and transferred to a 100 mL Schott® Duran round neck Erlenmeyer flask (Schott® Duran GmbH, 51 Hattenbergstrasse, Germany). A stock solution of BV in MeOH was prepared as described in § 3.3.4, Chapter 3, to produce a solution of final concentration 10 µg/mL. A 50 mL aliquot of the BV solution was added to the stoppered flask. The flask was sonicated for 35 minutes using a Branson® B12 ultrasonicator (Branson® Ltd, Shelton, Conn, USA). The suspension was then placed in a Model CF3555 K.I.C® freezer (K.I.C® Manufacturing, Stanger, RSA) for 20 minutes, after which the sample was centrifuged at 3000 rpm using a Model 5415 Eppendorf® centrifuge (Geratebau Netheler+ Hinz GmbH 2000, Hamburg 63, West Germany) for 15 minutes. An aliquot of the filtered sample was injected in triplicate (n=3) onto the chromatographic system and the CP content was calculated for the linear regression equation obtained from the calibration data.

6.2.3.5. Tube Extrudability

Tube extrudability testing is a useful empirical test to measure the force required to extrude the contents of a tube [480,481]. Since the packaging of creams may have a considerable and significant impact on the delivery of the desired quantity of cream from a container, the extrusion of cream from a collapsible tube is an important criterion to be assessed during quality testing [481].

The formulations were filled into standard capped collapsible aluminium tubes and sealed using a manual Shreeji® collapsible tube filling and crimping machine (Shreeji® Pharmaceutical Scientific and Laboratory Instruments, Mumbai, India). The filled tube was initially weighed using a Model PM460 Mettler® top loading balance (Mettler® Instruments, Zurich, Switzerland) and the weight recorded. The tube was then placed between two sheets of glass and clamped into place. A one kilogram mass piece was placed on top of the glass sheet and the cap removed from the tube. The amount of cream extruded and collected in 10 seconds was weighed and recorded. A schematic illustration of the apparatus is shown in Figure 6.5.

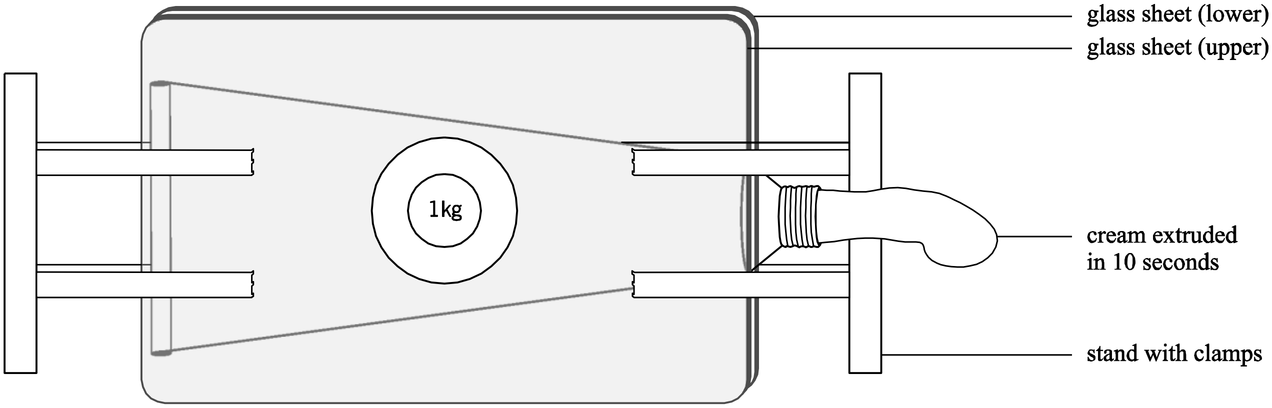


Figure 6.5 Schematic representation of the apparatus used to evaluate tube extrudability

The measurements of extrudability for each batch were undertaken in triplicate (n=3) and the average extrudability value recorded. The larger the amount of cream extruded from a tube, the better the extrudability of that formulation. The extrudability was calculated by using Equation 6.1.

$$E = \frac{W}{A}$$ ***Equation 6.1***

Where

E=Extrudability (g/cm^2)
M=Applied weight to extrude cream from tube (grams)
A=Area (cm^2)

6.2.3.6. Electrical Conductivity

The electrical conductivity of manufactured creams was measured at room temperature (± 22°C) using a digital Model AG Mettler Toledo® Five Easy FE 30 electrical conductivity meter (Mettler Toledo® Instruments, Schwerzenbach, Switzerland), equipped with an inbuilt magnetic stirrer. All formulations were tested in triplicate (n=3) and the average conductivity was recorded.

6.2.3.7. In Vitro Diffusion Studies

In vitro CP release studies were performed using a Franz Diffusion cell system (Crown Glass Company Inc., Branchburg, NJ, USA), a diagramof which is depicted in Figure 6.6.

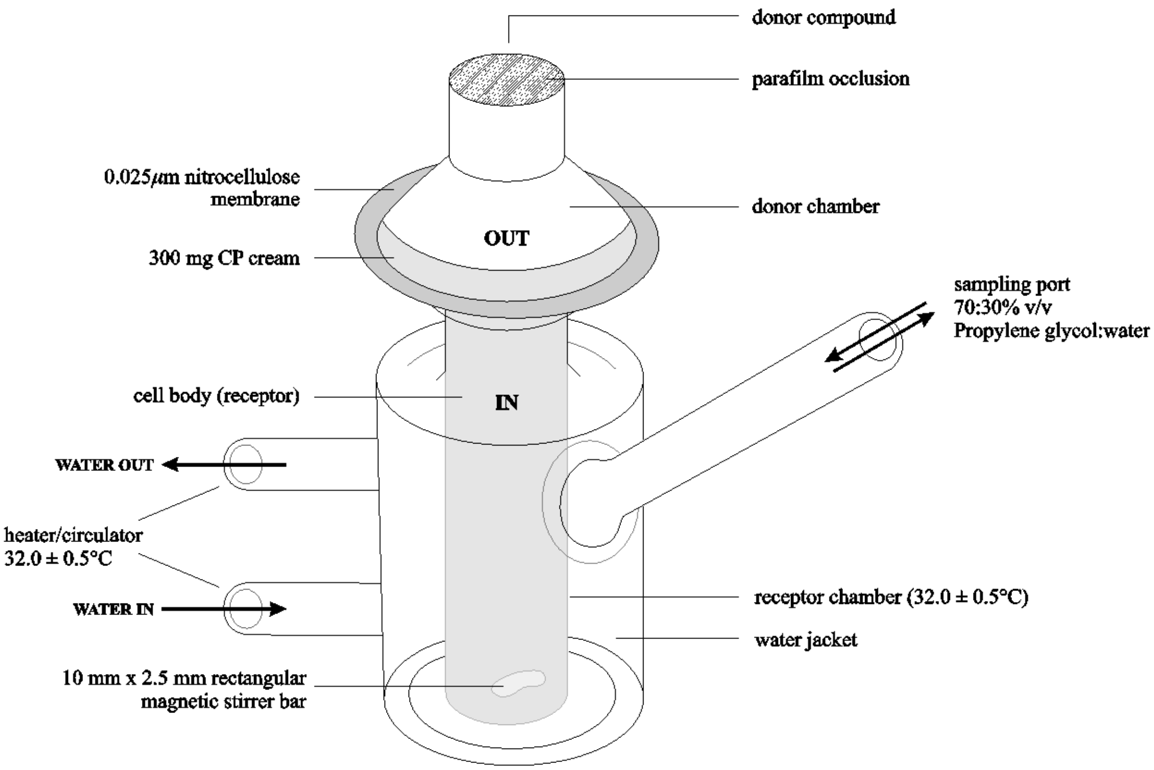


Figure 6.6 Schematic representation of a Franz Diffusion cell

A 300 mg aliquot of the formulation to be tested was applied to a 0.025 μm nitrocellulose MF-Millipore® membrane (Millipore® Co., Bedford, MA, USA) using a glass rod. The receptor medium placed in the receptor chamber was 12.6 mL of propylene glycol and water in a ratio of 70:30% v/v. The receptor medium was maintained at 32 ± 0.5°C using a Model 109046022 Grant® circulating water bath (Grant Instruments® Ltd., Cambridge, UK). The nitrocellulose membrane was soaked in the receptor fluid for two hours prior to use to allow the membrane to equilibrate to the composition of the receptor medium. This assembly was placed on a magnetic stirrer and the receptor solution was continuously stirred at 10 rpm with a 10 mm x 2.5 mm magnetic stirrer bar (Merck Chemicals® Ltd, Darmstadt, Germany). The donor compartment was covered with Parafilm® (Bemis® Flexible Packaging, Neenah, Winconsin) to prevent the cream dehydrating. The receptor fluid samples were withdrawn through the sample port at 0, 2, 4, 8, 12, 24, 48 and 72 hours following commencement of the study and the receptor compartment was filled with fresh medium after each sample removal. Each cream that was manufactured was tested in triplicate (n=3) and the samples were analyzed using HPLC with UV detection at 238 nm (§ 3.3, Chapter 3). The cumulative % CP released was determined and *in vitro* CP release data were plotted for comparative purposes.

6.3. EXPERIMENTAL DESIGN

6.3.1. Statistical Screening Design

Three screening design approaches were evaluated in these studies and included the Taguchi, Plackett-Burman and a 2-Level Fractional Factorial design. The designs were generated and analyzed using Design-Expert® software (version 8.02, Stat-Ease Inc., Minneapolis, USA). The independent variables evaluated included formulation and process parameters and included % v/v propylene glycol, % w/w cetostearyl alcohol, % w/w glyceryl monostearate, % w/w Gelot® 64, mixing time, anchor speed, homogenization time and speed, heating temperature, batch size and cooling time. The composition of input variables at low (-1) and high (+1) levels is listed in Table 6.3.

Table 6.3 Actual and coded values for the variables used during screening design evaluation

Variable		**Levels**	
	Symbol	**Low (-1)**	**High (+1)**
% v/v Propylene glycol (%)	x_1	41	51
% w/w Gelot® 64 (%)	x_2	1	5
% w/w Cetostearyl alcohol (%)	x_3	3	13
% w/w Glyceryl monostearate (%)	x_4	6	16
Homogenization speed (rpm)	x_5	1000	2000
Homogenization time (mins)	x_6	60	120
Anchor speed (rpm)	x_7	30	40
Mixing time (mins)	x_8	60	120
Heating temperature (°C)	x_9	65	75
Cooling time (mins)	x_{10}	60	120
Batch size (Kg)	x_{11}	3	6

These levels were selected according to preliminary studies that were undertaken to cover the range of settings within which the cream/ointment plant could be operated [61]. Viscosity (y_1), spreadability (y_2), pH (y_3), % CP content (y_4), tube extrudability (y_5), electrical conductivity (y_6) and *in vitro* CP released at 2 (y_7), 4 (y_8), 8 (y_9), 12 (y_{10}), 24 (y_{11}), 48 (y_{12}) and 72 (y_{13}) hours were the responses monitored. The formulation response values for batches TD-CP-01 – TD-CP-12, PBD-CP-01 – PBD-CP-12 and 2-LFFD-CP-01- 2-LFFD-CP-16 are included in Appendix III, together with the batch summary records.

6.3.1.1. Taguchi Design (TD)

A standard orthogonal array L_{12} was used to examine this two-factor system. The letter L and the subscript 12 denote Latin square and the number of the experimental runs, respectively. The experimental run involves a corresponding combination of levels to which the factors are evaluated in an experiment set [445]. The eleven variables studied were investigated for screening purposes using TD and twelve pilot scale cream formulations were manufactured according to an orthogonal L_{12} array as listed in Tables 6.4 and 6.5.

Table 6.4 TD used to evaluate the impact of formulation and Wintech® mixer variables on the response of interest for pilot scale cream formulations

Run	**Variables**											**Responses**					
	PG* ***(Tx_1)***	**G-64*** ***(Tx_2)***	**Ceto-A*** ***(Tx_3)***	**GMS*** ***(Tx_4)***	**HS*** ***(Tx_5)***	**HT*** ***(Tx_6)***	**AS*** ***(Tx_7)***	**MT*** ***(Tx_8)***	**Heat-T*** ***(Tx_9)***	**CT*** ***(Tx_{10})***	**BS*** ***(Tx_{11})***	**VSC*** ***(Ty_1)***	**SPD*** ***(Ty_2)***	**pH** ***(Ty_3)***	**% DC*** ***(Ty_4)***	**EXD*** ***(Ty_5)***	**EC*** ***(Ty_6)***
	%	**%**	**%**	**%**	**rpm**	**min**	**rpm**	**min**	**°C**	**min**	**Kg**	**cP**	**cm^2**	**-**	**%**	**g/cm^2**	**µS/cm**
1	51	5	3	16	1000	120	20	60	65	120	6	37000	27.04	6.54	100.10	352.40	490.30
2	41	1	3	6	1000	120	40	120	75	120	6	6500	47.82	5.74	99.74	72.02	899.70
3	41	1	3	6	1000	60	20	60	65	60	3	11900	37.75	6.14	99.71	98.59	719.70
4	41	5	13	16	1000	120	40	60	75	60	3	53467	20.16	6.10	100.60	884.80	408.70
5	41	5	13	6	2000	120	20	120	65	120	3	35700	27.37	5.33	100.51	321.31	150.10
6	51	1	3	16	2000	120	20	120	75	60	3	19400	32.92	6.40	100.72	203.12	916.70
7	51	1	13	16	1000	60	40	120	65	120	3	72733	15.61	6.25	100.51	1052.00	367.00
8	51	5	13	6	1000	60	20	120	75	60	6	66700	17.17	5.74	100.20	898.21	114.70
9	51	5	3	6	2000	60	40	60	75	120	3	45800	24.28	5.91	99.08	393.10	164.80
10	41	5	3	16	2000	60	40	120	65	60	6	84267	12.67	6.03	99.84	1084.00	392.00
11	51	1	13	6	2000	120	40	60	65	60	6	32466	27.82	5.82	100.45	302.71	189.50
12	41	1	13	16	2000	60	20	60	75	120	6	46100	24.04	5.89	100.51	450.00	202.30

***Responses: PG= % v/v Propylene glycol; G-64= % w/w Gelot® 64; Ceto-A= % w/w Cetostearyl alcohol; GMS= % w/w Glyceryl monostearate; HS= Homogenization speed; HT= Homogenization time; AS= Anchor speed; MT= Mixing time; Heat-T= Heating temperature; CT= Cooling time; BS= Batch size; VSC= Viscosity; SPD= Spreadability; % DC= % Drug content; EXD= Extrudability; EC= Electrical conductivity**

Table 6.5 TD used to evaluate the impact of formulation and Wintech® mixer variables on the *in vitro* release of CP from pilot scale cream formulations

Run	Variables											Responses						
	PG* (Tx_1)	**G-64*** (Tx_2)	**Ceto-A*** (Tx_3)	**GMS*** (Tx_4)	**HS*** (Tx_5)	**HT*** (Tx_6)	**AS*** (Tx_7)	**MT*** (Tx_8)	**Heat-T*** (Tx_9)	**CT*** (Tx_{10})	**BS*** (Tx_{11})	**2 hr*** (Ty_7)	**4 hr*** (Ty_8)	**8 hr*** (Ty_9)	**12 hr*** (Ty_{10})	**24hr*** (Ty_{11})	**48hr*** (Ty_{12})	**72 hr*** (Ty_{13})
	%	**%**	**%**	**%**	**rpm**	**min**	**rpm**	**min**	**°C**	**min**	**Kg**	**%**	**%**	**%**	**%**	**%**	**%**	**%**
1	51	5	3	16	1000	120	20	60	65	120	6	5.32	9.06	20.58	27.06	36.04	48.89	55.26
2	41	1	3	6	1000	120	40	120	75	120	6	8.29	13.48	21.21	28.51	42.11	49.92	66.14
3	41	1	3	6	1000	60	20	60	65	60	3	6.75	11.38	20.82	27.64	41.69	47.47	61.94
4	41	5	13	16	1000	120	40	60	75	60	3	1.17	4.25	9.21	12.86	17.99	34.61	40.48
5	41	5	13	6	2000	120	20	120	65	120	3	5.44	8.27	23.23	28.58	36.11	51.02	55.35
6	51	1	3	16	2000	120	20	120	75	60	3	6.69	10.56	22.91	26.39	38.44	40.47	57.47
7	51	1	13	16	1000	60	40	120	65	120	3	1.21	2.81	5.90	8.02	16.21	23.60	31.00
8	51	5	13	6	1000	60	20	120	75	60	6	1.29	2.84	6.62	8.86	19.65	32.41	34.12
9	51	5	3	6	2000	60	40	60	75	120	3	4.41	6.58	13.87	24.99	33.71	44.65	49.06
10	41	5	3	16	2000	60	40	120	65	60	6	1.33	2.72	4.77	7.81	13.95	23.10	26.97
11	51	1	13	6	2000	120	40	60	65	60	6	4.32	9.41	22.30	29.87	35.60	49.95	54.58
12	41	1	13	16	2000	60	20	60	75	120	6	5.26	5.54	14.05	24.81	32.72	42.02	49.94

***Responses: PG= % v/v Propylene glycol; G-64= % w/w Gelot® 64; Ceto-A= % w/w Cetostearyl alcohol; GMS= % w/w Glyceryl monostearate; HS= Homogenization speed; HT= Homogenization time; AS= Anchor speed; MT= Mixing time; Heat-T= Heating temperature; CT= Cooling time; BS= Batch size; 2hr= Cumulative % drug released per unit area over a 2 hour period; 4 hr= Cumulative % drug released per unit area over a 4 hour period; 8 hr= Cumulative % drug released per unit area over a 8 hour period; 12 hr= Cumulative % drug released per unit area over a 12 hour period; 24 hr= Cumulative % drug released per unit area over a 24 hour period; 48 hr= Cumulative % drug released per unit area over a 48 hour period; 72hr= Cumulative % drug released per unit area over a 72 hour period**

6.3.1.2. Plackett-Burman Design (PBD)

Plackett-Burman designs are screening approaches used to identify main factors from a large number of potential contributing elements for a set of responses under evaluation. PBD examines up to N-1 factors in N experiments, where N is a multiple of four *viz.*, 12, 16, 20, 24, etc. Consequently these designs are useful tools for preliminary studies where the aim is to identify variables that can be fixed or eliminated prior to further investigation [482,483]. The PBD used in this study correlated the dependent and independent variables using the polynomial model, shown in Equation 6.2.

$$Y = A_0 + A_1X_1 + A_2X_2 + A_3X_3 + A_4X_4 + \cdots A_nX_n$$

Equation 6.2

Where

Y=Response,
A_0=Constant,
A_1 to A_n=Coefficients of the response values [482]

The design analyzes input data and presents a rank order of the variables with the magnitude of effect, and designates a sign for each of the effects [462]. An 11-factor-12 run PBD was investigated as shown in Tables 6.6 and 6.7.

Table 6.6 PBD used to evaluate the impact of formulation and Wintech® mixer variables on the response of interest for pilot scale cream formulations

Run	**Variables**											**Responses**					
	PG* ***(Px_1)***	**G-64*** ***(Px_2)***	**Ceto-A*** ***(Px_3)***	**GMS*** ***(Px_4)***	**HS*** ***(Px_5)***	**HT*** ***(Px_6)***	**AS*** ***(Px_7)***	**MT*** ***(Px_8)***	**Heat-T*** ***(Px_9)***	**CT*** ***(Px_{10})***	**BS*** ***(Px_{11})***	**VSC*** ***(Py_1)***	**SPD*** ***(Py_2)***	**pH** ***(Py_3)***	**% DC*** ***(Py_4)***	**EXD*** ***(Py_5)***	**EC*** ***(Py_6)***
	%	**%**	**%**	**%**	**rpm**	**min**	**rpm**	**min**	**°C**	**min**	**Kg**	**cP**	**cm^2**	**-**	**%**	**g/cm^2**	**μS/cm**
1	51	1	3	6	2000	60	40	60	65	120	6	5633	58.10	6.65	100.38	57.66	599.66
2	51	1	13	16	1000	120	40	120	65	60	3	51166	18.17	6.67	100.52	116.60	327.66
3	51	5	13	6	1000	60	40	60	75	120	3	58133	21.77	6.55	101.46	141.51	132.83
4	41	1	3	16	1000	120	40	60	75	120	6	16466	26.11	6.33	99.14	288.93	826.00
5	41	5	13	16	1000	60	20	120	65	120	6	81466	12.51	6.97	100.25	497.42	312.00
6	41	5	13	6	2000	120	40	60	65	60	6	95933	17.17	6.36	100.76	221.64	124.50
7	51	1	13	16	2000	60	20	60	75	60	6	62066	19.88	6.08	99.08	263.33	328.77
8	51	5	3	16	2000	120	20	60	65	120	3	43866	16.37	6.46	99.19	318.71	705.66
9	41	1	13	6	2000	120	20	120	75	120	3	38300	26.11	6.68	100.98	392.97	265.66
10	41	1	3	6	1000	60	20	60	65	60	3	5566	43.40	6.71	100.20	50.56	703.66
11	41	5	3	16	2000	60	40	120	75	60	3	48933	14.33	6.36	100.72	203.64	212.76
12	51	5	3	6	1000	120	20	120	75	60	6	23800	28.97	6.83	100.47	80.03	375.00

***Responses: PG= % v/v Propylene glycol; G-64= % w/w Gelot® 64; Ceto-A= % w/w Cetostearyl alcohol; GMS= % w/w Glyceryl monostearate; HS= Homogenization speed; HT= Homogenization time; AS= Anchor speed; MT= Mixing time; Heat-T= Heating temperature; CT= Cooling time; BS= Batch size; VSC= Viscosity; SPD= Spreadability; % DC= % Drug content; EXD= Extrudability; EC= Electrical conductivity**

Table 6.7 PBD used to evaluate the impact of formulation and Wintech® mixer variables on the *in vitro* release of CP from pilot scale cream formulations

Run	Variables											Responses						
	PG* (Px_1)	**G-64*** (Px_2)	**Ceto-A*** (Px_3)	**GMS*** (Px_4)	**HS*** (Px_5)	**HT*** (Px_6)	**AS*** (Px_7)	**MT*** (Px_8)	**Heat-T*** (Px_9)	**CT*** (Px_{10})	**BS*** (Px_{11})	**2 hr*** (Py_7)	**4 hr*** (Py_8)	**8 hr*** (Py_9)	**12 hr*** (Py_{10})	**24 hr*** (Py_{11})	**48 hr*** (Py_{12})	**72 hr*** (Py_{13})
	%	**%**	**%**	**%**	**rpm**	**min**	**rpm**	**min**	**°C**	**min**	**Kg**	**%**	**%**	**%**	**%**	**%**	**%**	**%**
1	51	1	3	6	2000	60	40	60	65	120	6	1.71	5.08	10.49	17.07	31.97	49.27	62.91
2	51	1	13	16	1000	120	40	120	65	60	3	1.21	2.92	5.44	7.80	16.45	25.73	34.93
3	51	5	13	6	1000	60	40	60	75	120	3	1.32	3.24	6.23	8.99	14.70	22.49	28.42
4	41	1	3	16	1000	120	40	60	75	120	6	1.12	3.22	9.54	15.11	33.05	40.00	43.63
5	41	5	13	16	1000	60	20	120	65	120	6	1.07	3.05	6.85	12.74	22.19	32.55	39.81
6	41	5	13	6	2000	120	40	60	65	60	6	1.12	2.68	6.32	12.17	18.77	33.21	48.49
7	51	1	13	16	2000	60	20	60	75	60	6	1.09	2.89	5.91	8.26	14.73	24.74	33.47
8	51	5	3	16	2000	120	20	60	65	120	3	1.66	4.79	10.68	11.44	22.85	37.70	46.40
9	41	1	13	6	2000	120	20	120	75	120	3	1.09	3.19	6.80	10.90	19.60	28.07	35.15
10	41	1	3	6	1000	60	20	60	65	60	3	1.39	4.86	11.15	15.77	29.32	44.51	55.89
11	41	5	3	16	2000	60	40	120	75	60	3	1.20	3.93	9.24	13.44	25.24	43.43	55.09
12	51	5	3	6	1000	120	20	120	75	60	6	1.23	4.12	11.51	16.61	32.21	52.01	64.93

***Responses: PG= % v/v Propylene glycol; G-64= % w/w Gelot® 64; Ceto-A= % w/w Cetostearyl alcohol; GMS= % w/w Glyceryl monostearate; HS= Homogenization speed; HT= Homogenization time; AS= Anchor speed; MT= Mixing time; Heat-T= Heating temperature; CT= Cooling time; BS= Batch size; 2hr= Cumulative % drug released per unit area over a 2 hour period; 4 hr= Cumulative % drug released per unit area over a 4 hour period; 8 hr= Cumulative % drug released per unit area over a 8 hour period; 12 hr= Cumulative % drug released per unit area over a 12 hour period; 24 hr= Cumulative % drug released per unit area over a 24 hour period; 48 hr= Cumulative % drug released per unit area over a 48 hour period; 72hr= Cumulative % drug released per unit area over a 72 hour period**

6.3.1.3. 2-Level Fractional Factorial Design (2-LFFD)

A fractional factorial experiment is generated from a full factorial experiment by selecting an alias structure [471]. In this work, 11 influencing factors, at two levels, were investigated using a Fractional Factorial experimental design. A 2^{11-7} design, an eleven factor fractional factorial design requiring 16 runs with resolution III was conducted in this study as summarized in Tables 6.8 and 6.9.

Table 6.8 2-LFFD used to evaluate the impact of formulation and Wintech® mixer variables on the responses of interest for pilot scale cream formulations

Run	**Variables**											**Responses**					
	PG* ***(Fx_1)***	**G-64*** ***(Fx_2)***	**Ceto-A*** ***(Fx_3)***	**GMS*** ***(Fx_4)***	**HS*** ***(Fx_5)***	**HT*** ***(Fx_6)***	**AS*** ***(Fx_7)***	**MT*** ***(Fx_8)***	**Heat-T*** ***(Fx_9)***	**CT*** ***(Fx_{10})***	**BS*** ***(Fx_{11})***	**VSC*** ***(Fy_1)***	**SPD*** ***(Fy_2)***	**pH** ***(Fy_3)***	**% DC*** ***(Fy_4)***	**EXD*** ***(Fy_5)***	**EC*** ***(Fy_6)***
	%	**%**	**%**	**%**	**rpm**	**min**	**rpm**	**min**	**°C**	**min**	**Kg**	**cP**	**cm^2**	**-**	**%**	**g/cm^2**	**µS/cm**
1	51	1	3	16	2000	120	20	60	75	60	3	24780	21.51	6.24	99.84	72.02	913.66
2	41	5	3	16	2000	60	40	60	75	60	6	18806	14.30	6.25	100.50	244.17	978.66
3	51	1	13	6	1000	120	20	120	75	60	6	16106	26.73	6.06	100.47	203.20	504.33
4	41	1	3	6	1000	60	20	60	75	120	6	7773	33.18	5.08	99.84	67.40	203.66
5	41	5	13	16	1000	120	20	60	65	60	3	23913	9.26	5.44	100.25	337.25	98.33
6	41	1	13	16	2000	60	20	120	75	120	3	34900	7.71	6.26	100.43	352.35	283.66
7	51	1	3	6	2000	60	40	120	65	60	3	6000	24.04	5.68	100.47	63.32	529.00
8	41	5	3	6	2000	120	20	120	65	60	6	24710	16.62	5.60	100.38	313.53	518.33
9	41	1	3	16	1000	120	40	120	65	120	6	8826	28.29	6.23	100.52	72.37	1578.00
10	51	5	13	6	2000	60	20	60	65	120	6	24266	12.99	5.53	101.46	313.56	148.33
11	51	5	3	16	1000	60	20	120	65	120	3	18540	15.68	6.27	99.14	180.49	687.66
12	41	1	13	6	2000	120	40	60	65	120	3	12266	29.24	5.57	100.50	98.58	308.33
13	41	5	13	6	1000	60	40	120	75	60	3	28000	16.37	5.67	100.15	323.61	129.00
14	51	1	13	16	1000	60	40	60	65	60	6	23040	20.93	6.06	99.71	314.52	309.00
15	51	5	13	16	2000	120	40	120	75	120	6	35023	7.22	5.27	99.71	352.37	128.00
16	51	5	3	6	1000	120	40	60	75	120	3	11000	28.00	6.04	100.55	98.58	392.66

***Responses: PG= % v/v Propylene glycol; G-64= % w/w Gelot® 64; Ceto-A= % w/w Cetostearyl alcohol; GMS= % w/w Glyceryl monostearate; HS= Homogenization speed; HT= Homogenization time; AS= Anchor speed; MT= Mixing time; Heat-T= Heating temperature; CT= Cooling time; BS= Batch size; VSC= Viscosity; SPD= Spreadability; % DC= % Drug content; EXD= Extrudability; EC= Electrical conductivity**

Table 6.9 2-LFFD used to evaluate the impact of formulation and Wintech® mixer variables on the *in vitro* release of CP from pilot scale cream formulations

Run	Variables											Responses						
	PG* (*Fx*$_1$)	G-64* (*Fx*$_2$)	Ceto-A* (*Fx*$_3$)	GMS* (*Fx*$_4$)	HS* (*Fx*$_5$)	HT* (*Fx*$_6$)	AS* (*Fx*$_7$)	MT* (*Fx*$_8$)	Heat-T* (*Fx*$_9$)	CT* (*Fx*$_{10}$)	BS* (*Fx*$_{11}$)	2 hr* (*Fy*$_7$)	4 hr* (*Fy*$_8$)	8 hr* (*Fy*$_9$)	12 hr* (*Fy*$_{10}$)	24 hr* (*Fy*$_{11}$)	48 hr* (*Fy*$_{12}$)	72 hr* (*Fy*$_{13}$)
	%	%	%	%	rpm	min	rpm	min	°C	min	Kg	%	%	%	%	%	%	%
1	51	1	3	16	2000	120	20	60	75	60	3	4.98	8.89	24.55	32.91	40.89	47.63	56.52
2	41	5	3	16	2000	60	40	60	75	60	6	2.01	5.74	11.80	18.36	26.20	29.68	42.16
3	51	1	13	6	1000	120	20	120	75	60	6	4.05	6.68	13.27	20.27	28.58	32.65	44.44
4	41	1	3	6	1000	60	20	60	75	120	6	4.98	10.89	15.55	23.91	31.89	35.62	47.52
5	41	5	13	16	1000	120	20	60	65	60	3	3.91	6.64	15.10	19.85	26.44	35.86	40.54
6	41	1	13	16	2000	60	20	120	75	120	3	1.01	3.74	10.80	13.36	22.20	25.68	37.16
7	51	1	3	6	2000	60	40	120	65	60	3	6.09	9.89	15.55	20.91	30.89	36.63	48.52
8	41	5	3	6	2000	120	20	120	65	60	6	3.01	6.04	15.00	18.85	26.04	40.04	48.52
9	41	1	3	16	1000	120	40	120	65	120	6	4.99	8.19	14.05	21.91	30.59	40.04	46.02
10	51	5	13	6	2000	60	20	60	65	120	6	2.99	6.94	15.12	19.23	26.54	40.84	46.89
11	51	5	3	16	1000	60	20	120	65	120	3	2.21	5.94	12.18	18.96	26.29	40.83	42.96
12	41	1	13	6	2000	120	40	60	65	120	3	4.95	8.68	16.27	21.27	28.58	42.94	45.05
13	41	5	13	6	1000	60	40	120	75	60	3	2.59	6.04	14.12	19.05	26.04	40.18	45.35
14	51	1	13	16	1000	60	40	60	65	60	6	2.99	7.05	15.92	19.95	26.94	40.18	40.94
15	51	5	13	16	2000	120	40	120	75	120	6	1.99	3.06	10.04	13.96	22.48	37.60	39.60
16	51	5	3	6	1000	120	40	60	75	120	3	4.95	8.68	15.27	20.27	30.58	45.43	48.43

***Responses: PG= % v/v Propylene glycol; G-64= % w/w Gelot® 64; Ceto-A= % w/w Cetostearyl alcohol; GMS= % w/w Glyceryl monostearate; HS= Homogenization speed; HT= Homogenization time; AS= Anchor speed; MT= Mixing time; Heat-T= Heating temperature; CT= Cooling time; BS= Batch size; 2hr= Cumulative % drug released per unit area over a 2 hour period; 4 hr= Cumulative % drug released per unit area over a 4 hour period; 8 hr= Cumulative % drug released per unit area over a 8 hour period; 12 hr= Cumulative % drug released per unit area over a 12 hour period; 24 hr= Cumulative % drug released per unit area over a 24 hour period; 48 hr= Cumulative % drug released per unit area over a 48 hour period; 72hr= Cumulative % drug released per unit area over a 72 hour period**

6.4. MODEL FITTING AND STATISTICAL ANALYSIS

6.4.1. Screening Design Analysis

The statistical package Design-Expert® (version 8.02, Stat-Ease Inc., Minneapolis, USA) was used to generate a mathematical relationship between the independent variables and observed responses. Fitting of the data to the models and subsequent ANOVA revealed a correlation between the formulation and process variables with batch responses that were best described by linear and two factorial models. Therefore first order equations were generated for each response from TD and PBD designs whereas two factorial equations were generated from the 2-LFFD. ANOVA analysis, evaluation of the P-value and the Regression Coefficient (R^2) were used to analyze the data and Pareto and Main Effect plots were used to visualize the data.

6.4.1.1. Statistical Design Equations and their Regression Coefficients

Analysis of data generated following TD suggests that the viscosity of the cream ranged between 6500 cP and 84267 cP, spreadability between 12.67 cm^2 and 47.82 cm^2, pH between 5.33 and 6.54, % CP content between 99.08% and 100.72%, extrudability between 77.02 g/cm^2 and 1084 g/cm^2 and electrical conductivity between 114.70 µS/cm and 916.70 µS/cm. The percent CP released from the formulations for TD ranged between 1.17% and 8.29%, 2.72% and 13.48%, 4.77% and 23.23%, 7.31% and 28.58%, 13.96% and 42.11%, 23.10% and 51.02% and 26.97% and 66.14% for samples collected at 2, 4, 8, 12, 24, 48 and 72 hours respectively. The factors that had maximum influence on the response can be deduced from the magnitude of the coefficient for each term generated following data analysis [484] and the first order equations derived from analysis of the effect of input factors on significant responses are listed in terms of coded factors in Equations 6.3 – 6.12.

Spreadability (Ty_2) = $+27.68 - 2.08Tx_1 - 4.77Tx_2 - 4.19Tx_3 - 4.15Tx_4 - 1.37Tx_5 + 4.30Tx_6 - 1.49Tx_7 - 0.63Tx_8 + 1.51Tx_9 + 1.47Tx_{10}$ ***Equation 6.3***

pH (Ty_3) = $+5.99 + 0.12Tx_1 - 0.049Tx_2 - 0.14Tx_3 + 0.21Tx_4 - 0.094Tx_5 - 0.016Tx_7 - 0.076Tx_8 - 0.028Tx_9 - 0.048Tx_{10} - 0.031Tx_{11}$ ***Equation 6.4***

% CP Content (Ty_4) = $+100.14 - 0.11Tx_2 + 0.30Tx_3 + 0.20Tx_4 + 0.026Tx_5 + 0.19Tx_6 - 0.12Tx_7 + 0.092Tx_8 - 0.038Tx_9 - 0.079Tx_{10} - 0.028Tx_{11}$ ***Equation 6.5***

Electrical Conductivity (Ty_6) = $+417.95 - 44.12Tx_1 - 131.18Tx_2 - 179.24Tx_3 + 44.88Tx_4 - 82.05Tx_5 + 91.21Tx_6 + 35.91Tx_7 + 55.41Tx_8 - 38.91Tx_9 - 36.54Tx_{10}$ ***Equation 6.6***

Cumulative % CP Released over 2 Hours (Ty_7) = $+4.29 - 0.42Tx_1 - 1.31Tx_2 - 1.18Tx_3 - 0.79Tx_4 + 0.28Tx_5 + 0.91Tx_6 - 0.84Tx_7 - 0.25Tx_8 + 0.23Tx_9 + 0.70Tx_{10}$ ***Equation 6.7***

Cumulative % CP Released over 4 Hours (Ty_8) = $+7.24 - 0.37Tx_1 - 1.62Tx_2 - 1.72Tx_3 - 1.42Tx_4 - 0.062Tx_5 + 1.93Tx_6 - 0.70Tx_7 - 0.46Tx_8 + 0.38Tx_9 - 0.067Tx_{10}$ ***Equation 6.8***

Cumulative % CP Released over 8 Hours (Ty_9) = $+15.46 - 2.41Tx_2 - 1.90Tx_3 - 2.55Tx_4 + 1.40Tx_5 + 4.45Tx_6 - 2.58Tx_7 - 1.35Tx_8 - 0.81Tx_9 + 1.02Tx_{10} - 0.53Tx_{11}$ ***Equation 6.9***

Cumulative % CP Released over 12 Hours (Ty_{10}) = $+21.28 - 0.42Tx_1 - 2.92Tx_2 - 2.45Tx_3 - 3.46Tx_4 + 2.46Tx_5 + 4.26Tx_6 - 2.61Tx_7 - 3.25Tx_8 - 0.21Tx_9 + 2.38Tx_{10}$ ***Equation 6.10***

Cumulative % CP Released over 48 Hours (Ty_{12}) = $+40.68 - 0.68Tx_1 - 1.56Tx_2 - 1.74Tx_3 - 5.23Tx_4 + 1.19Tx_5 + 5.13Tx_6 - 3.04Tx_7 - 3.92Tx_8 + 2.67Tx_{10} + 0.37Tx_{11}$ ***Equation 6.11***

Cumulative % CP Released over 72 Hours (Ty_{13}) = $+48.53 - 1.61Tx_1 - 4.99Tx_2 - 4.28Tx_3 - 5.01Tx_4 + 6.35Tx_5 - 3.82Tx_6 - 3.35Tx_7 + 1.01Tx_8 + 2.60Tx_{10} - 0.69Tx_{11}$ ***Equation 6.12***

Analysis of data generated following PBD reveals that the viscosity values were between 5566 cP and 95933 cP, spreadability values between 12.51 cm^2 and 58.10 cm^2, pH values between 6.08 and 6.97, % CP content values between 99.08% and 101.46%, extrudability values between 50.56 g/cm^2 and 497.42 g/cm^2 and electrical conductivity values between 132.83 µS/cm and 826.00 µS/cm. The cumulative percent CP release ranged between 1.07% and 1.71%, 2.68% and 5.08%, 5.44% and 11.51%, 7.80% and 17.07%, 14.70% and 33.05%, 22.49% and 49.27% and 28.42% and 64.93% for samples collected at 2, 4, 8, 12, 24, 48 and 72 hours respectively. The first order equations derived from analysis for the factors for the significant responses are given below in terms of coded factors in Equations 6.13 – 6.20.

Spreadability (Py_2) = $+25.24 + 1.96Px_1 - 6.72Px_2 - 5.97Px_3 - 7.34Px_4 - 3.11Px_6 + 0.69Px_7 + 1.12Px_8 - 2.38Px_9 + 1.61Px_{10} + 1.88Px_{11}$ ***Equation 6.13***

Electrical Conductivity (Py_6) = $+390.73 - 16.70Px_1 - 80.27Px_2 - 179.73Px_3 + 23.84Px_4 - 55.46Px_5 + 46.69Px_6 - 20.16Px_7 - 41.94Px_8 - 71.46Px_9 + 82.91Px_{10}$ ***Equation 6.14***

Cumulative % CP Released over 2 Hours (Py_7) = $+1.27 + 0.10Px_1 - 0.12Px_3 - 0.042Px_4 + 0.044Px_5 - 0.029Px_6 + 0.012Px_7 - 0.016Px_8 - 0.093Px_9 + 0.061Px_{10} - 0.044Px_{11}$ ***Equation 6.15***

Cumulative % CP Released over 8 Hours (Py_9) = $+8.35 + 0.13Px_2 - 2.09Px_3 - 0.40Px_4 - 0.11Px_5 + 0.035Px_6 - 0.47Px_7 + 0.042Px_8 - 0.14Px_9 + 0.085Px_{10} + 0.090Px_{11}$ ***Equation 6.16***

Cumulative % CP Released over 12 Hours (Py_{10}) = $+12.53 - 0.83Px_1 - 2.38Px_3 - 1.06Px_4 - 0.31Px_5 - 0.91Px_6 - 0.095Px_7 + 0.57Px_8 - 0.31Px_9 + 0.18Px_{10} + 1.13Px_{11}$ ***Equation 6.17***

Cumulative % CP Released over 24 Hours (Py_{11}) = $+23.42 - 1.27Px_1 - 0.76Px_2 - 5.68Px_3 - 1.00Px_4 - 1.23Px_5 + 0.40Px_6 + 1.19Px_7 - 0.17Px_8 + 0.64Px_9 + 2.06Px_{10}$ ***Equation 6.18***

Cumulative % CP Released over 48 Hours (Py_{12}) = $+36.14 - 0.82Px_1 + 0.76Px_2 - 8.34Px_3 - 2.12Px_4 - 0.072Px_5 - 0.45Px_7 + 2.37Px_8 - 1.02Px_9 - 1.13Px_{10} + 2.49Px_{11}$ ***Equation 6.19***

Cumulative % CP Released over 72 Hours (Py_{13}) = $+45.76 - 0.58Px_1 + 1.43Px_2 - 9.05Px_3 - 3.54Px_4 + 1.16Px_5 - 0.18Px_7 + 3.04Px_8 - 2.31Px_9 - 3.04Px_{10} + 3.11Px_{11}$ ***Equation 6.20***

Analysis of data generated following 2-LFFD shows that the viscosity values were between 6000 cP and 35023 cP, spreadability values between 7.22 cm^2 and 33.18 cm^2, pH values between 5.08 and 6.27, % CP content values between 99.71% and 101.46%, extrudability values between 63.32 g/cm^2 and 352.37 g/cm^2 and electrical conductivity values between 128.00 µS/cm and 1578.00 µS/cm. The cumulative percent CP released ranged between 1.01% and 6.07%, 3.06% and 10.89%, 10.44% and 16.27%, 13.36% and 23.91%, 22.20% and 31.89%, 29.68% and 45.43% and 37.16% and 48.52% for samples collected at 2, 4, 8, 12, 24, 48 and 72 hours respectively. The 2-LFFD equations derived from analysis for the factors on the significant responses are given below in terms of coded factors in Equations 6.21 – 6.28.

Spreadability *(Fy_2)* = $+19.51 + 0.14Fx_1 - 4.45Fx_2 - 3.20Fx_3 - 3.89Fx_4 - 2.80Fx_5 + 1.35Fx_6 + 1.55Fx_7 - 1.67Fx_8 + 0.78Fx_9 + 0.53Fx_{11} + 0.59Fx_1Fx_4 - 0.40Fx_1Fx_5 - 1.13Fx_1Fx_7 + 0.45Fx_1Fx_8$ ***Equation 6.21***

Extrudability *(Fy_5)* = $+212.96 - 13.20Fx_1 + 57.49Fx_2 + 73.97Fx_3 + 27.73Fx_4 + 13.28Fx_5 - 19.47Fx_6 + 17.02Fx_7 + 19.70Fx_8 - 21.00Fx_9 + 22.18Fx_{11} + 2.36Fx_1Fx_4 - 12.72Fx_1Fx_5 - 24.46Fx_1Fx_7 - 19.61Fx_1Fx_8$ ***Equation 6.22***

Electrical Conductivity *(Fy_6)* = $+481.92 - 30.33Fx_1 - 96.79Fx_2 - 243.29Fx_3 + 140.21Fx_4 + 73.29Fx_6 + 62.17Fx_7 + 62.83Fx_8 - 40.21Fx_9 - 15.62Fx_{10} + 64.12Fx_{11} - 82.21Fx_1Fx_4 - 15.92Fx_1Fx_5 - 174.08Fx_1Fx_7 - 52.17Fx_1Fx_8$ ***Equation 6.23***

Cumulative % CP Released over 2 Hours *(Fy_7)* = $+3.61 + 0.18Fx_1 - 0.65Fx_2 - 0.55Fx_3 - 0.60Fx_4 - 0.23Fx_5 + 0.50Fx_6 + 0.21Fx_7 - 0.36Fx_8 - 0.29Fx_9 - 0.23Fx_{11} - 0.14Fx_1Fx_4 + 0.46Fx_1Fx_5 + 0.17Fx_1Fx_7$ ***Equation 6.24***

Cumulative % CP Released over 4 Hours *(Fy_8)* = $+7.07 + 0.073Fx_1 - 0.93Fx_2 - 0.96Fx_3 - 0.91Fx_4 - 0.45Fx_5 + 0.039Fx_6 + 0.098Fx_7 - 0.87Fx_8 - 0.35Fx_9 - 0.053Fx_{10} - 0.24Fx_{11} + 0.50Fx_1Fx_5 - 0.069Fx_1Fx_7 + 0.12Fx_1Fx_8$ ***Equation 6.25***

Cumulative % CP Released over 12 Hours *(Fy_{10})* = $+19.56 - 1.00Fx_2 - 1.20Fx_3 - 0.91Fx_4 - 0.96Fx_5 + 0.35Fx_6 - 0.10Fx_7 - 1.16Fx_8 - 0.55Fx_9 - 0.45Fx_{10} - 8.75\text{E-}003Fx_{11} + 0.29Fx_1Fx_4 + 0.65Fx_1Fx_5 - 0.68Fx_1Fx_7 + 0.12Fx_1Fx_8$ ***Equation 6.26***

Cumulative % CP Released over 48 Hours *(Fy_{12})* = $+38.70 + 0.67Fx_2 + 2.53Fx_3 - 0.34Fx_4 - 3.02Fx_5 - 0.87Fx_6 + 1.13Fx_7 + 1.10Fx_8 - 0.52Fx_9 - 2.67Fx_{10} + 0.57Fx_{11} + 2.20Fx_1Fx_4 + 0.46Fx_1Fx_5 - 0.51Fx_1Fx_7 - 2.23Fx_1Fx_8$ ***Equation 6.27***

Cumulative % CP Released over 72 Hours *(Fy_{13})* = $+42.87 + 0.54Fx_1 - 1.65Fx_2 - 2.03Fx_3 - 1.13Fx_4 - 0.63Fx_5 + 0.34Fx_6 + 0.37Fx_7 - 0.75Fx_8 - 0.24Fx_9 - 0.43Fx_{11} - 0.27Fx_1Fx_4 + 0.60Fx_1Fx_5 - 0.65Fx_1Fx_7 + 0.73Fx_1Fx_8$ ***Equation 6.28***

The magnitude and direction of the factor coefficients in the derived equations explain the nature of the effect of input factors on the responses. It can be observed from the first equation for TD data that the input factors with a greater magnitude have a profound effect on the spreadability of the CP cream formulations. The response "spreadability" increases when the factor Tx_6 with a positive coefficient is increased. A decrease in response occurs when factors ($Tx_1 - Tx_5$, Tx_7 and Tx_8) with negative coefficients are increased. Factor Tx_9 has the smallest positive influence on spreadability. Except for factors Tx_2, Tx_3, Tx_4, all others have very small negative coefficients and thus influence the response minimally.

Furthermore it was observed that TD having ten significant responses was found to be a less selective statistical model in comparison to PBD and 2-LFFD, which has only eight significant responses. The Standard Deviation (SD) and R^2 values obtained for the linear and two factorial models indicate the quality of these models. A summary of the R^2 values for all the three screening statistical designs is listed in Table 6.10. The SD for the responses (y_1 - y_{13}) reveals that the predicted values for pH (y_3) are the most accurate and are closer to its actual values than any of the other responses observed for all three screening designs. Furthermore the results of model summary statistics show that most formulation responses result in R^2values > 0.9, for all models, which are considered appropriate to validate the fit of the predicted models. However, the R^2 values = 1.000 observed for PBD were considered high, indicating a good correlation between the experimental and predicted responses for most models and that there is no significant lack of fit for all response variables investigated. Of the three screening designs, PBD was found to be the most accurate design with eleven response models having R^2 values close to unity whereas TD was considered the least accurate approach, with only eight response models showing a high degree of correlation.

In addition to the adequacy of the models tested, the predicted R^2 values must be in reasonably close agreement with adjusted R^2 values to establish the reliability of models [61]. Of the 13 responses, spreadability (y_2), electrical conductivity (y_6), cumulative % CP released over 2 hours (y_7), 4 hours (y_8) and 48 hours (y_{12}) were the only responses that showed good reliability for all models with adjusted and predicted R^2 values that were in close agreement (Table 6.10). The Coefficient of Variation (C.V) indicates the precision and reliability of the experiments performed [365]. Therefore relatively low values for C.V indicate better precision and reliability and pH, % CP content, cumulative % CP released over 2 hours and 48 hours had the lowest C.V, showing that precise models had been developed for the three screening designs. Furthermore it was observed that PBD and 2-LFFD generated low C.V for most responses, indicating that they are good reliable designs.

6.4.1.2. ANOVA

The significance of the linear and 2 factorial models was tested at a 0.05 level of significance using ANOVA for each of the screening designs. For all designs, spreadability, electrical conductivity and cumulative % CP released over 72 hours were found to be the most significant responses and therefore these data were thoroughly investigated. A summary of ANOVA analysis for these models for the most significant responses is summarized in Tables 6.11 - 6.14.

Table 6.10 Model summary statistics for the three screening designs examined

	Taguchi						**Plackett-Burman**						**2-Level Fractional Factorial**					
	SD*	**F-value**	**R^{2*}**	**Adj R^{2*}**	**Pred R^{2*}**	**C.V (%)***	**SD***	**F-value**	**R^{2*}**	**Adj R^{2*}**	**Pred R^{2*}**	**C.V (%)***	**SD***	**F-value**	**R^{2*}**	**Adj R^{2*}**	**Pred R^{2*}**	**C.V (%)***
y_1	4455.00	31.80	0.9969	0.9655	0.5486	10.44	2232.42	177.47	0.9994	0.9938	0.9189	5.04	810.00	142.77	0.9995	0.9925	0.8720	4.29
y_2	0.44	27.68	0.9998	0.9980	0.9738	1.60	0.30	2112.46	1.0000	0.9995	0.9932	1.20	0.51	273.94	0.9997	0.9961	0.9333	2.63
y_3	0.01	1573.62	0.9983	0.9747	0.5677	0.14	0.03	76.46	0.9999	0.9993	0.9908	0.08	0.03	194.00	0.9996	0.9945	0.9058	0.52
y_4	0.01	3308.56	0.9983	0.9815	0.7574	0.01	0.10	59.26	1.0000	0.9997	0.9956	0.10	0.08	42.23	0.9983	0.9747	0.5677	0.08
y_5	59.44	42.06	0.9976	0.9739	0.6585	11.67	18.37	63.61	0.9984	0.9827	0.7740	8.37	5.02	591.63	0.9999	0.9982	0.9691	2.36
y_6	0.03	557.07	0.9998	0.9980	0.9742	0.55	2.24	14089	1.0000	0.9999	0.9990	0.57	23.67	302.66	0.9998	0.9965	0.9396	4.91
y_7	0.04	4171.34	1.0000	0.9997	0.9965	0.94	0.01	6269.00	1.0000	0.9998	0.9977	0.23	0.28	31.63	0.9952	0.9637	0.6902	0.28
y_8	0.12	1108.87	0.9999	0.9990	0.9870	1.59	0.10	79.07	0.9987	0.9861	0.8181	2.76	0.22	9169.12	1.0000	0.9999	0.9980	0.32
y_9	0.32	562.90	0.9998	0.9980	0.9744	2.07	0.10	534.45	0.9998	0.9979	0.9731	1.25	0.25	98.13	0.9993	0.9891	0.8138	1.41
y_{10}	0.45	437.75	0.9998	0.9975	0.9671	51.28	0.14	585.30	0.9998	0.9981	0.9754	1.11	1.39	13043.51	1.0000	0.9999	0.9986	0.13
y_{11}	1.18	84.86	0.9988	0.9871	0.8305	24.97	0.21	1202.25	0.9999	0.9991	0.9880	0.89	0.38	61.74	0.9988	0.9827	0.7042	1.39
y_{12}	0.01	5.354E+005	1.0000	1.0000	1.0000	0.04	0.08	17712.00	1.0000	0.9999	0.9992	0.22	0.34	361.73	0.9998	0.9970	0.9495	0.88
y_{13}	1.28	106.61	0.9991	0.9897	0.8651	2.64	0.59	446.88	0.9998	0.9975	0.9678	1.30	0.19	356.02	0.9998	0.9970	0.9486	0.44

***SD= Standard Deviation, R^2= Regression Coefficient, Adj R^2= Adjusted R^2, Pred R^2= Predicted R^2, Adeq Prec= Adequate Precision, C.V (%)= Coefficient of Variation**

6.4.1.2.1. Spreadability

ANOVA analysis for the response models for spreadability of formulations resulted in F-values of 549.57, 2112.46 and 273.94 for TD, PBD and 2-LFFD, implying that the models are significant for the screening designs. P-values < 0.05 indicate that model terms are significant and Table 6.9 shows the significant model terms to the response, spreadability for the designs.

Table 6.11 ANOVA test results for spreadability

Taguchi						Plackett-Burman					
Source	**SS***	**DF***	**MS***	**F-value**	**Prob>F**	**Source**	**SS***	**DF***	**MS***	**F-value**	**Prob>F**
Model	1072.07	10	107.21	549.57	0.0332[a]	**Model**	1940.14	10	194.01	2112.46	0.0169[a]
Tx_1	51.96	1	51.96	266.35	0.0390[a]	**Px_1**	46.21	1	46.21	503.17	0.0284[a]
Tx_2	273.32	1	273.32	1401.11	0.0170[a]	**Px_2**	542.07	1	542.07	5902.17	0.0083[a]
Tx_3	210.92	1	210.92	1081.25	0.0194[a]	**Px_3**	427.83	1	427.83	4658.33	0.0093[a]
Tx_4	206.42	1	206.42	1058.16	0.0196[a]	**Px_4**	645.85	1	645.85	7032.16	0.0076[a]
Tx_5	22.55	1	22.55	115.60	0.0590[b]	**Px_6**	116.13	1	116.13	1264.42	0.0179[a]
Tx_6	221.97	1	221.97	1137.85	0.0189[a]	**Px_7**	5.72	1	5.72	62.30	0.0802[b]
Tx_7	26.79	1	26.79	137.33	0.0542[b]	**Px_8**	15.03	1	15.03	163.60	0.0497[a]
Tx_8	4.73	1	4.73	24.22	0.1276[b]	**Px_9**	67.75	1	67.75	737.65	0.0234[a]
Tx_9	27.39	1	27.39	140.41	0.0536[b]	**Px_{10}**	31.08	1	31.08	338.43	0.0346[a]
Tx_{10}	26.02	1	26.02	133.38	0.0550[b]	**Px_{11}**	42.47	1	42.47	462.41	0.0296[a]
Residual	0.20	1	0.20			**Residual**	0.092	1	0.092		
Cor Total	1072.26	11				**Cor Total**	1940.23	11			

2-Level Factorial					
Source	**SS***	**DF***	**MS***	**F-value**	**Prob>F**
Model	1007.00	14	71.93	273.94	0.0473[a]
Fx_1	0.29	1	0.29	1.12	0.4824[b]
Fx_2	317.00	1	317.00	1207.29	0.0183[a]
Fx_3	163.34	1	163.34	622.07	0.0255[a]
Fx_4	242.13	1	242.13	922.16	0.0210[a]
Fx_5	125.80	1	125.80	479.10	0.0291[a]
Fx_6	29.18	1	29.18	111.13	0.0602[b]
Fx_7	38.36	1	38.36	146.07	0.0526[b]
Fx_8	44.82	1	44.82	170.71	0.0486[a]
Fx_9	9.77	1	9.77	37.23	0.1034[b]
Fx_{11}	4.50	1	4.50	17.13	0.1509[b]
$Fx_1 x_4$	5.61	1	5.61	21.35	0.1357[b]
$Fx_1 x_5$	2.54	1	2.54	9.66	0.1982[b]
$Fx_1 x_7$	20.45	1	20.45	77.88	0.0718[b]
$Fx_1 x_8$	3.21	1	3.21	12.24	0.1773[b]
Residual	0.26	1	0.26		
Cor Total	1007.26	15			

***SS= Sum of Squares, DF= Degree of Freedom, MS= Mean Square, [a]= Significant at "Prob > F" less than 0.05, [b]= Insignificant at "Prob > F" more than 0.05**

Large F-values (Tx_2, Px_2, Fx_2) of 1401.11, 5902.17 and 1207.29 were observed for % w/w Gelot® 64 for all three designs tested, indicating that this formulation parameter had the most significant effect on the spreadability of the formulations compared to other formulation variables evaluated. The % w/w cetostearyl alcohol (x_3) and glyceryl monostearate (x_4) also had significant yet secondary effects on the formulations, since the F- and P-values were high and < 0.05, respectively (Table 6.11). Mixing time (x_8) had the least effect on spreadability, since the F-values for these parameters were low, with values of 24.2, 163.6 and 170.7 for each of the designs. Furthermore, the Main Effect plot (Figure 6.7) shows that the formulation variables had an antagonistic effect on the spreadability of the creams and that heating temperature, cooling time and batch size had some positive synergistic effects on spreadability. The Main Effect and Pareto plots that were constructed to provide a graphical illustration of the effect of each variable on the spreadability (y_2) of the cream formulations are shown in Figures 6.7 - 6.9.

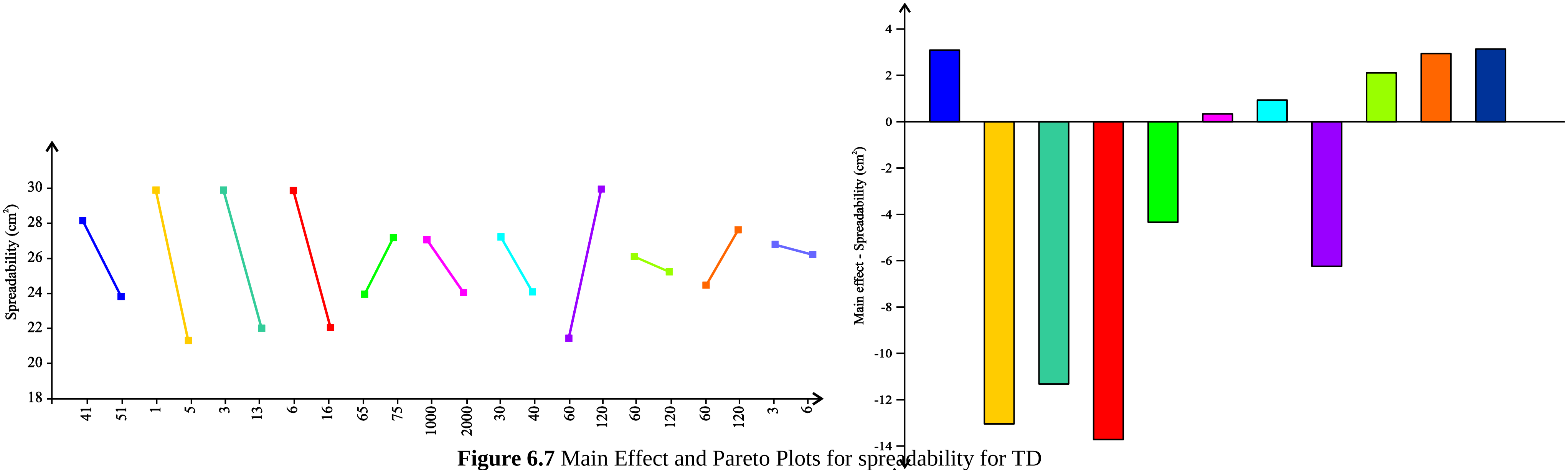


Figure 6.7 Main Effect and Pareto Plots for spreadability for TD

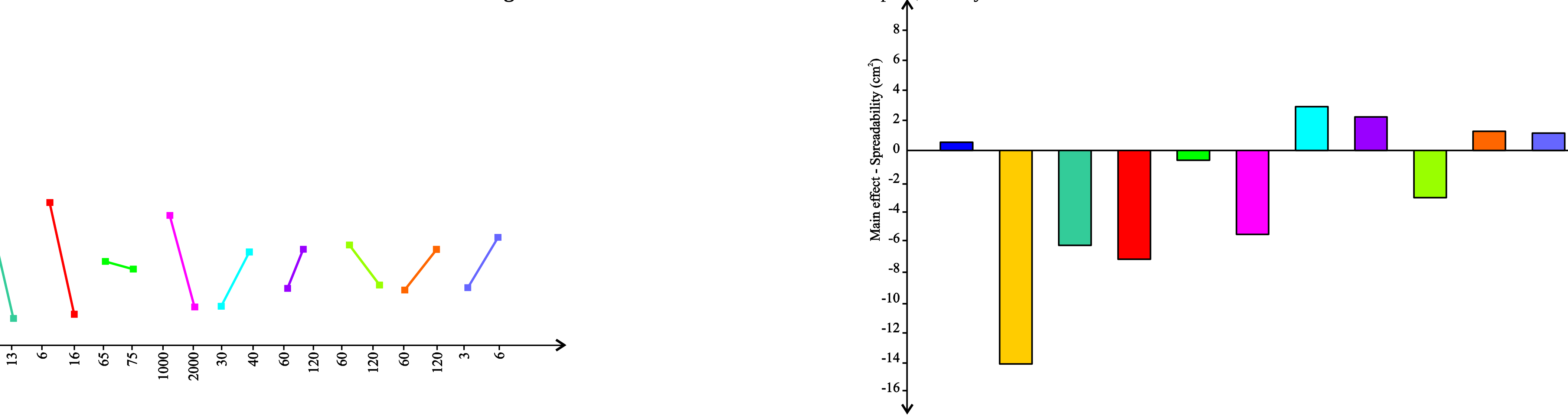


Figure 6.8. Main Effect and Pareto Plots for spreadability for PBD

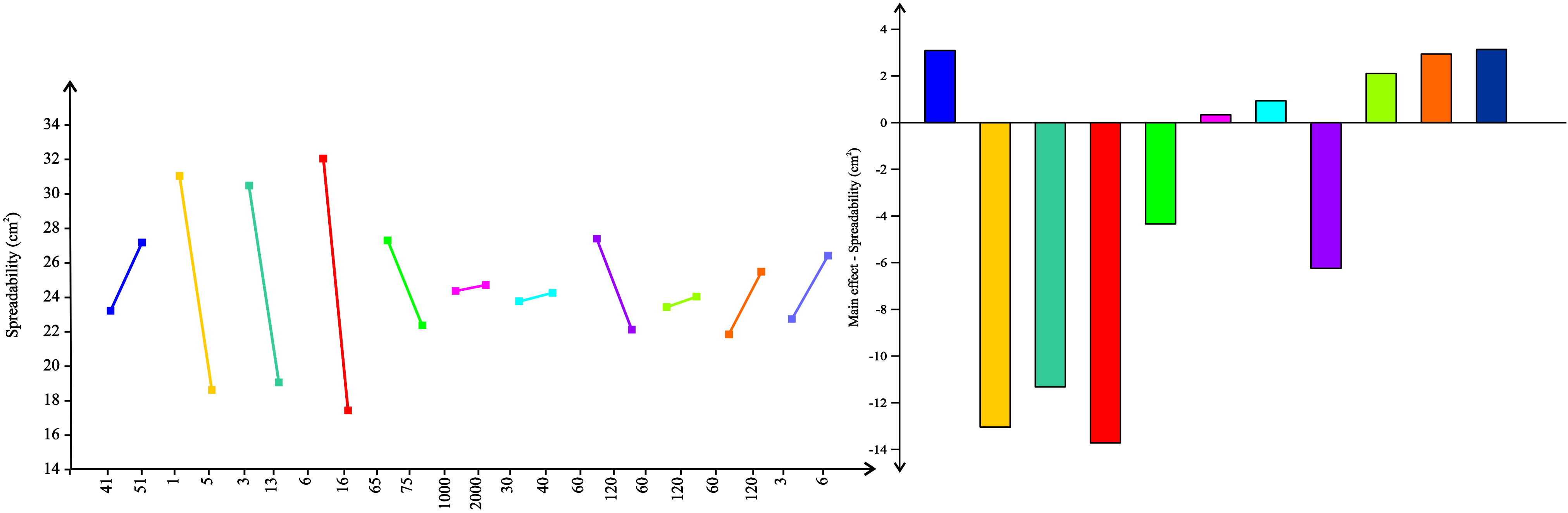


Figure 6.9 Main Effect and Pareto Plots for spreadability for 2-LFFD

Key

🟦 % v/v Propylene glycol
🟨 % w/w Gelot®64
🟩 % w/w cetostearyl alcohol
🟥 % w/w Glyceryl monostearate
🟩 Heating temperature (°C)
🟪 Homogenization speed (rpm)
🟦 Anchor speed (rpm)
🟪 Homogenization time (minutes)
🟩 Mixing time (minutes)
🟧 Cooling time (minutes)
🟦 Batch size (kg)

An increase in the o/w emulsifier content, Gelot® 64, from 1% w/w to 5% w/w resulted in a significant decrease in spreadability from approximately 30 cm^2 to 14 cm^2. This effect may be explained by an increase in the number of emulsified particles in the three-dimensional network of the semi-solid. These particles are packed closer together and therefore are unable to flow readily due to the strong attraction and resistive forced between the particles of the emulsion. There may also be an increase in the particle-particle interactions due to the high particle population per unit weight of the semi-solid, which restricts particle movement and results in a reduction in spreadability [485,486]. An increase in the viscosity-modifying agents and stabilizers such as cetostearyl alcohol and glyceryl monostearate also results in a decrease in spreadability of the formulations as the thickeners increase the viscosity of semi-solids with a simultaneous decrease of shear stressin the formulation [487].

6.4.1.2.2. Electrical Conductivity

ANOVA analysis for the response models for electrical conductivity of the formulations resulted in F-values of 557.07, 14089.54 and 302.66 for TD, PBD and 2-LFFD, implying that the models are significant. The significant model terms for the electrical conductivity for the TD, PBD and 2-LFFD Designs are summarized in Table 6.12. Large F-values (Tx_3, Px_3, Fx_3) of 2256.66, 77147.65 and 1690.80 were observed for % w/w cetostearyl alcohol for the three designs, indicating that this formulation parameter had a significant effect on electrical conductivity of the creams compared to the other formulation variables evaluated. The % w/w Gelot® 64 (x_2) and glyceryl monostearate (x_4) also had significant yet secondary effects on the creams, since the F- and P-values were high and < 0.05 (Table 6.12). The % v/v propylene glycol (x_1), anchor speed (x_7) and heating temperature (x_{10}) had the least impact on electrical conductivity, since the F-values for these parameters were low.

Table 6.12 ANOVA results for electrical conductivity

Taguchi						Plackett-Burman					
Source	**SS***	**DF***	**MS***	**F-value**	**Prob>F**	**Source**	**SS***	**DF***	**MS***	**F-value**	**Prob>F**
Model	5.63	10	0.56	557.07	0.0330^a	**Model**	7.079E+005	10	70794.35	14089.54	0.0066^a
Tx_1	0.23	1	0.23	229.23	0.0420^a	**Px_1**	3347.74	1	3347.74	666.27	0.0247^a
Tx_2	1.09	1	1.09	1075.71	0.0194^a	**Px_2**	77318.47	1	77318.47	15387.98	0.0051^a
Tx_3	2.28	1	2.28	2256.66	0.0134^a	**Px_3**	3.876E+05	1	3.876E+05	77147.65	0.0023^a
Tx_4	0.62	1	0.62	609.47	0.0258^a	**Px_4**	6821.18	1	6821.18	1357.56	0.0173^a
Tx_5	0.64	1	0.64	630.92	0.0253^a	**Px_5**	36914.73	1	36914.73	7346.80	0.0074^a
Tx_6	0.51	1	0.51	501.86	0.0284^a	**Px_6**	26154.96	1	26154.96	5205.38	0.0088^a
Tx_7	0.012	1	0.012	11.79	0.1804^b	**Px_7**	4876.37	1	4876.37	970.50	0.0204^a
Tx_8	0.054	1	0.054	53.85	0.0862^b	**Px_8**	21103.79	1	21103.79	4200.09	0.0098^a
Tx_9	0.090	1	0.090	89.09	0.0672^b	**Px_9**	61284.81	1	61284.81	12196.95	0.0058^a
Tx_{10}	0.11	1	0.11	112.06	0.0600^b	**Px_{10}**	82485.22	1	82485.22	16416.27	0.0050^a
Residual	1.010 E-003	1	1.010 E-003			**Residual**	5.02	1	5.02		
Cor Total	5.63	11				**Cor Total**	7.079E+005	11			

2-Level Factorial					
Source	**SS***	**DF***	**MS***	**F-value**	**Prob>F**
Model	2.373E+006	14	1.695E+005	302.66	0.0450^a
Fx_1	14721.78	1	14721.78	26.28	0.1226^b
Fx_2	1.499E+005	1	1.499E+005	267.62	0.0389^a
Fx_3	9.471E+005	1	9.471E+005	1690.83	0.0255^a
Fx_4	242.13	1	242.13	922.16	0.0210^a
Fx_5	125.80	1	125.80	479.10	0.0291^a
Fx_6	29.18	1	29.18	111.13	0.0602^b
Fx_7	38.36	1	38.36	146.07	0.0526^b
Fx_8	44.82	1	44.82	170.71	0.0486^a
Fx_9	9.77	1	9.77	37.23	0.1034^b
Fx_{11}	4.50	1	4.50	17.13	0.1509^b
$Fx_1\ x_4$	5.61	1	5.61	21.35	0.1357^b
$Fx_1\ x_5$	2.54	1	2.54	9.66	0.1982^b
$Fx_1\ x_7$	20.45	1	20.45	77.88	0.0718^b
$Fx_1\ x_8$	3.21	1	3.21	12.24	0.1773^b
Residual	0.26	1	0.26		
Cor Total	1007.26	15			

***SS= Sum of Squares, DF= Degree of Freedom, MS= Mean Square, ^{a}= Significant at “Prob > F” less than 0.05, ^{b}= Insignificant at “Prob > F” more than 0.05**

Furthermore, the Main Effect plot shows that the formulation variables except glyceryl monostearate had an antagonistic effect on the electrical conductivity of the creams and homogenization time, mixing time and batch size had some positive synergistic effect on this response. The Main Effect and Pareto plots that were constructed to provide a graphical illustration of the effect of each variable on electrical conductivity (y_6) of the creams are shown in Figures 6.10 - 6.12.

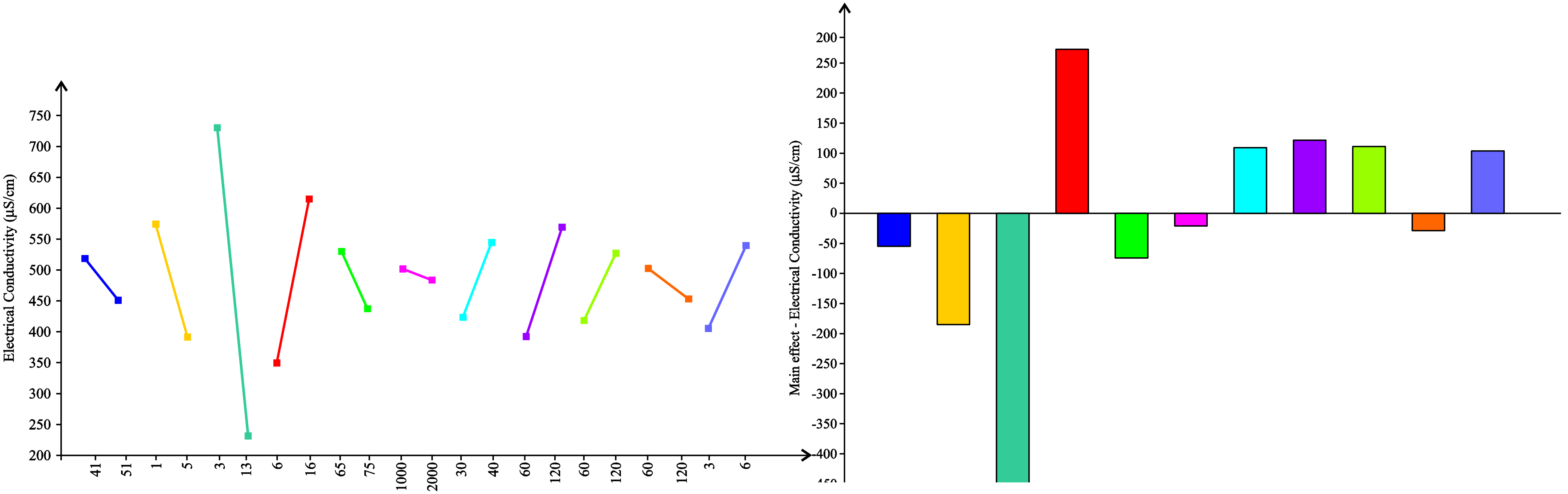


Figure 6.10 Main Effect and Pareto Plots for electrical conductivity fo

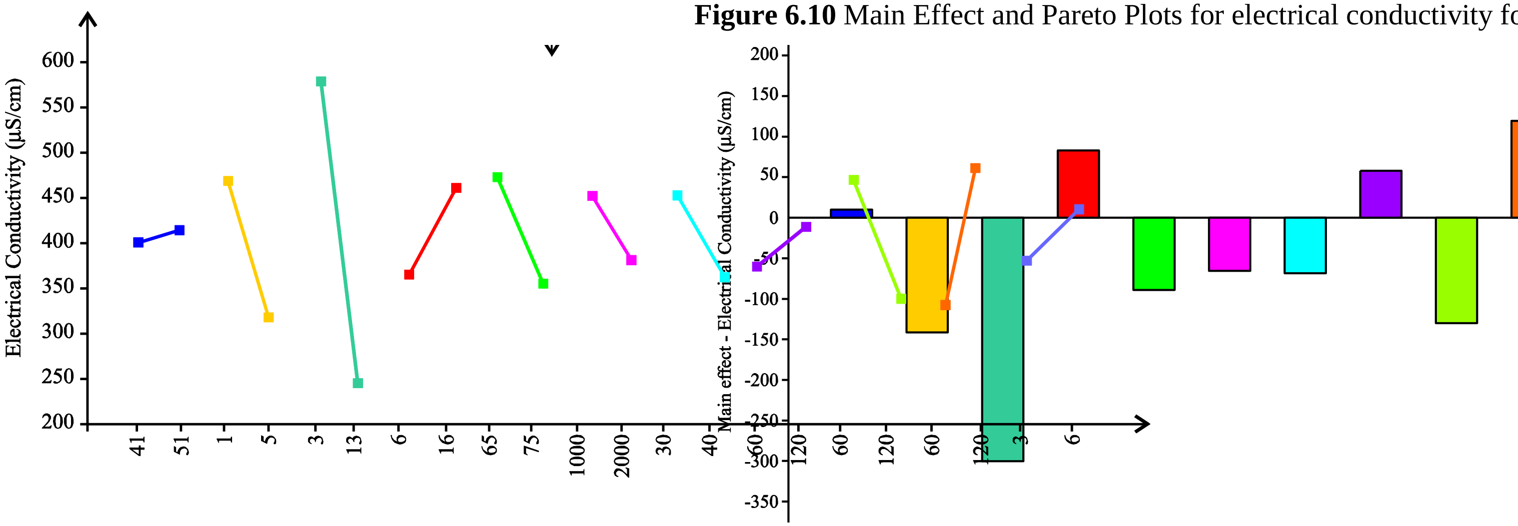


Figure 6.11 Main Effect and Pareto Plots for electrical conductivity for PBD

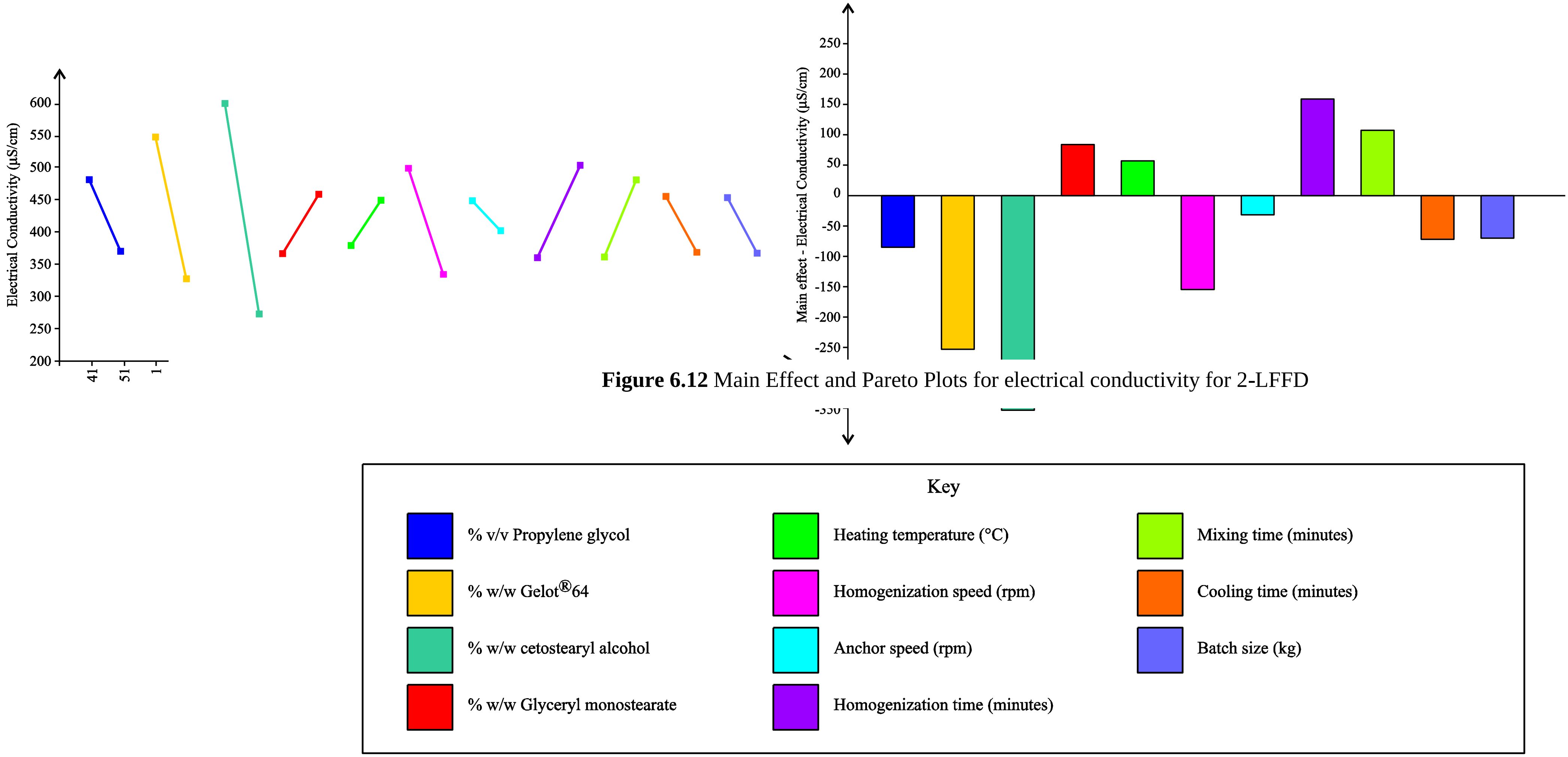


Figure 6.12 Main Effect and Pareto Plots for electrical conductivity for 2-LFFD

An increase in cetostearyl alcohol content from 3% w/w to 13% w/w resulted in a significant decrease in the electrical conductivity of the creams, from 700 µS/cm to 250 µS/cm. Electrical conductimetry is usually used to determine the nature and stability of an emulsion over a period of time [488,489]. Electrical conductimetry is also known to be sensitive to small changes in the microstructure of an emulsion, especially when instability such as creaming, sedimentation or phase inversion are observed [490]. The creams that contained greater amounts cetostearyl alcohol exhibited poor electrical conductivity, which may be due to the fact it is a mixture of fatty alcohols, consisting predominantly of cetyl and stearyl alcohols which increases the oily nature of the cream base.

6.4.1.2.3. Cumulative % CP Released over 72 Hours

ANOVA analysis for the response models for cumulative % CP released from the creams over 72 hours resulted in F-values of 5.354E+005, 446.88 and 356.02 for the TD, PBD and 2-LFFD respectively, implying that the models were significant for the three screening designs. P-values < 0.05 indicate that the model terms are significant and the significant model terms for the response for the TD, PBD and 2-LFFD for this parameter are summarized in Table 6.13.

Large F-values (Tx_3, $Px_{3,}$ Fx_3) of 134.47, 2778.21 and 1819.70 were also observed also for the % w/w cetostearyl alcohol for the three designs. This parameter had the most significant effect on cumulative % CP released from the cream formulations. The % w/w Gelot® 64 (x_2) and glyceryl monostearate (x_4) had secondary yet significant effects on the *in vitro* release of CP from the creams since the F- and P-values were high and < 0.05 respectively (Table 6.13). Homogenization speed (x_5), anchor speed (x_7) and heating temperature (x_{10}) had the least effect on the % CP released over 72 hours with low F-values. The Main Effect and Pareto plots show that most formulation variables had an antagonistic effect on the % CP released. These plots that were constructed to provide a graphical illustration of the effect of each variable on the % CP released from the creams are shown in Figures 6.13 - 6.15.

Table 6.13 ANOVA test results for cumulative % CP released over 72 hours

Taguchi					
Source	**SS***	**DF***	**MS***	**F-value**	**Prob>F**
Model	1115.36	10	111.54	5.354E+005	0.0011^a
Tx_1	31.14	1	31.14	19.04	0.1434^b
Tx_2	298.30	1	298.30	182.40	0.0471^a
Tx_3	219.91	1	219.91	134.47	0.0548^b
Tx_4	300.70	1	300.70	183.87	0.0469^a
Tx_6	484.51	1	484.51	296.26	0.0369^a
Tx_7	175.19	1	175.19	107.12	0.0613^b
Tx_8	134.74	1	134.74	82.39	0.0699^b
Tx_9	12.22	1	12.22	7.47	0.2233^b
Tx_{10}	81.07	1	81.07	49.57	0.0898^b
Tx_{11}	5.73	1	5.73	3.50	0.3124^b
Residual	1.64	1	1.64		
Cor Total	1745.13	11			

Plackett-Burman					
Source	**SS***	**DF***	**MS***	**F-value**	**Prob>F**
Model	1580.31	10	158.03	446.88	0.0368^a
Px_1	4.08	1	4.08	11.55	0.1822^b
Px_2	24.54	1	24.54	69.39	0.0761^b
Px_3	982.47	1	982.47	2778.21	0.0121^a
Px_4	150.24	1	150.24	424.84	0.0309^a
Px_5	16.10	1	16.10	45.53	0.0937^b
Px_7	0.40	1	0.40	1.12	0.4820^b
Px_8	111.14	1	111.14	314.29	0.0359^a
Px_9	64.13	1	64.13	181.33	0.0472^a
Px_{10}	110.90	1	110.90	313.60	0.0359^a
Px_{11}	116.31	1	116.31	328.91	0.0351^a
Residual	0.35	1	0.35		
Cor Total	1580.66	11			

2-Level Factorial					
Source	**SS***	**DF***	**MS***	**F-value**	**Prob>F**
Model	179.93	14	12.85	356.02	0.0415^a
Fx_1	4.60	1	4.60	127.45	0.0562^b
Fx_2	43.63	1	43.63	1208.48	0.0149^a
Fx_3	65.69	1	65.69	1819.70	0.0149^a
Fx_4	20.52	1	20.52	568.45	0.0267^a
Fx_5	6.43	1	6.43	178.01	0.0476^a
Fx_6	1.80	1	1.80	49.74	0.0897^b
Fx_7	2.16	1	2.16	59.86	0.0818^b
Fx_8	9.12	1	9.12	252.64	0.0400^a
Fx_9	0.95	1	0.95	26.33	0.1225^b
Fx_{11}	2.89	1	2.89	80.06	0.0709^b
$Fx_1\ x_4$	1.16	1	1.16	32.01	0.1114^b
$Fx_1\ x_5$	5.71	1	5.71	158.23	0.0505^b
$Fx_1\ x_7$	6.79	1	6.79	187.98	0.0464^b
$Fx_1\ x_8$	8.50	1	8.50	235.38	0.0414^b
Residual	0.036	1	0.036		
Cor Total	179.97	15			

***SS= Sum of Squares, DF= Degree of Freedom, MS= Mean Square, ^{a}= Significant at "Prob > F" less than 0.05, ^{b}= Insignificant at "Prob > F" more than 0.05**

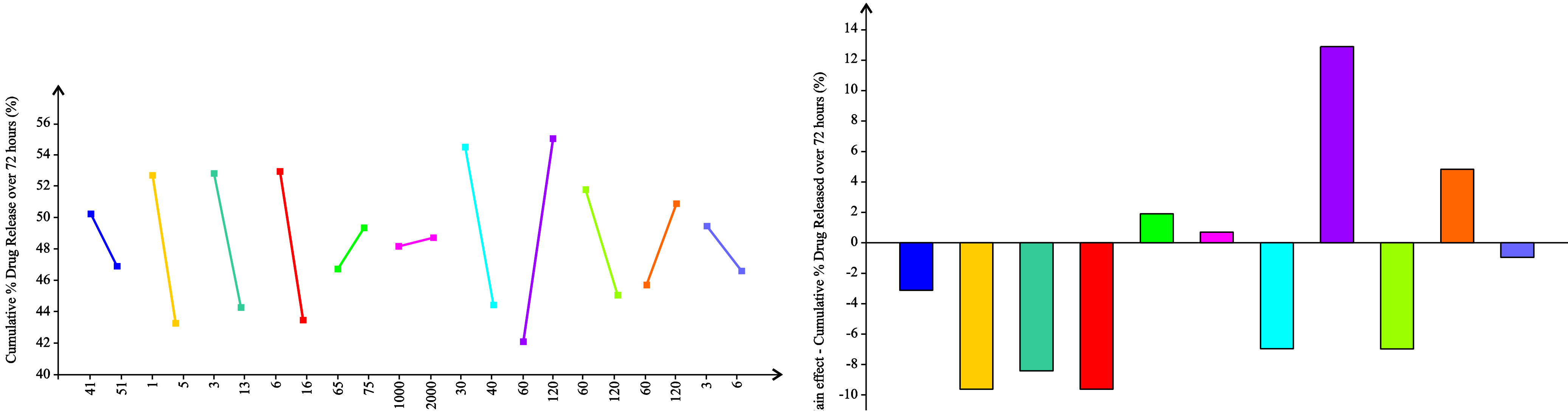


Figure 6.13 Main Effect and Pareto Plots for cumulative % CP released over 72 hours for TD

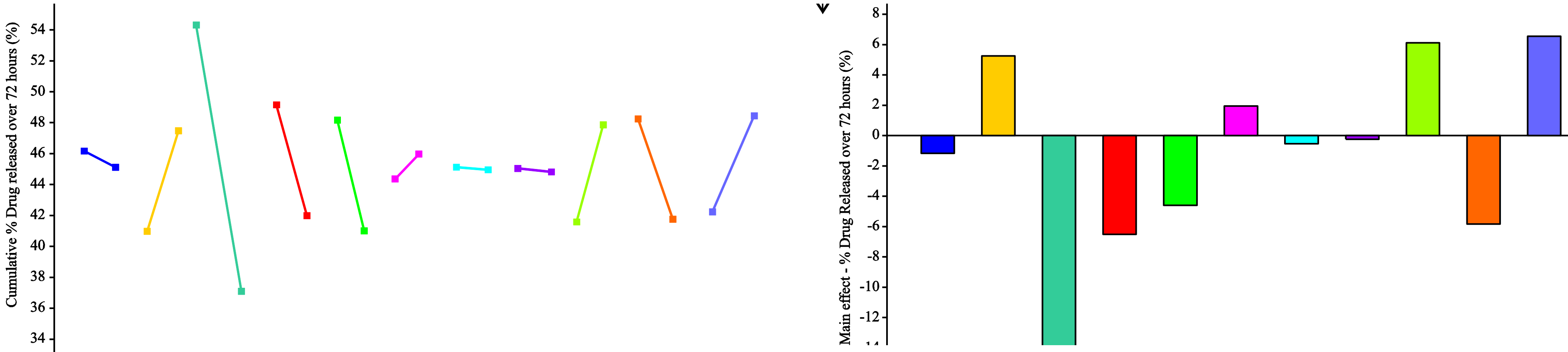


Figure 6.14. Main Effect and Pareto Plots for cumulati

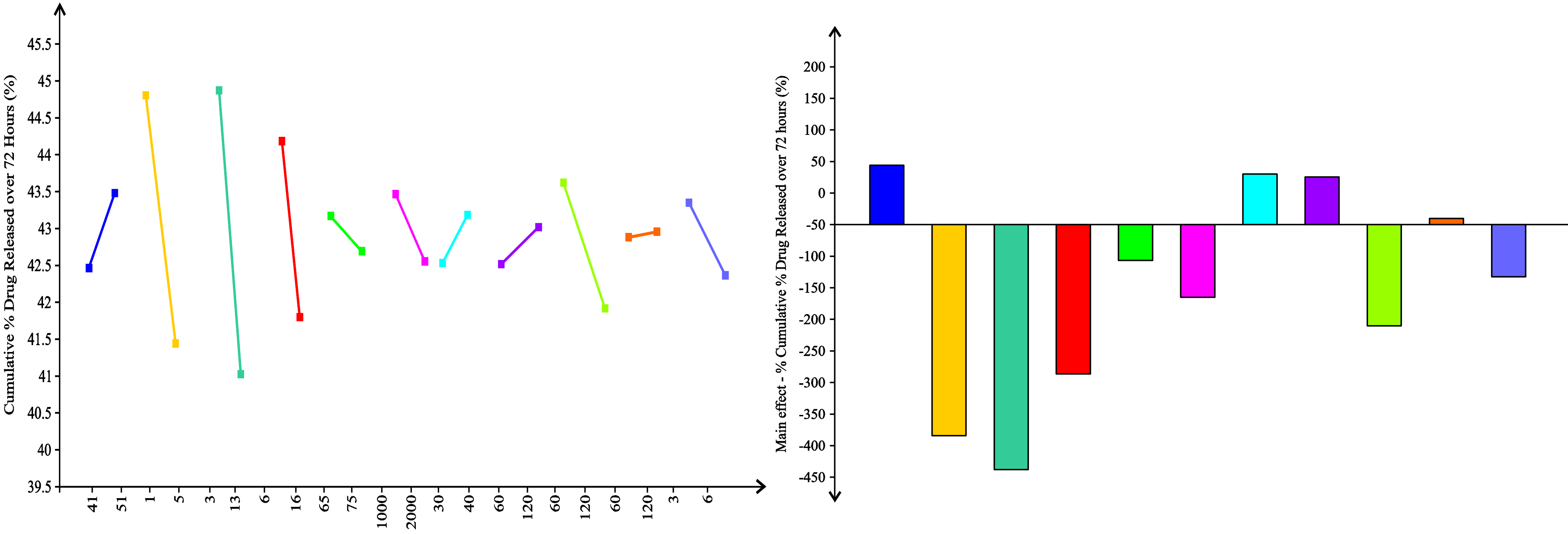


Figure 6.15 Main Effect and Pareto Plots for cumulative % CP released over 72 hours for 2-LFFD

Key

🟦 % v/v Propylene glycol

🟨 % w/w Gelot$^{®}$64

🟩 % w/w cetostearyl alcohol

🟥 % w/w Glyceryl monostearate

🟩 Heating temperature (°C)

🟪 Homogenization speed (rpm)

🟦 Anchor speed (rpm)

🟪 Homogenization time (minutes)

🟩 Mixing time (minutes)

🟧 Cooling time (minutes)

🟦 Batch size (kg)

An increase in cetostearyl alcohol content from 3% w/w to 13% w/w resulted in a significant decrease in the % CP released from the creams over 72 hours from 54% to 32%. Solid aliphatic fatty alcohols such as cetostearyl alcohol are generally used in oil-in-water emulsions to form a visco-elastic continuous phase in combination with the aqueous emulsifier solution that imparts semi-solid properties to the emulsion [491]. Increasing the concentration of fatty alcohol materials in a formulation significantly decreases the burst effect of API from semi-solid bases due to the high viscosity of these formulations. Therefore this results in a decreased affinity of the base for water and subsequently reducing drug diffusion in the base and consequently release from the base [492].

6.5. CONCLUSIONS

Screening designs are economical experimental plans intended to identify significant factors for a specific formulation or process parameter from an extensive list of potential variables [493,494]. Therefore a screening design approach was used to evaluate the significance of eleven formulation parameters and mixer variables on specific and critical formulation responses. Since there are many potential fractional screening designs, three screening design techniques commonly used in the pharmaceutical industry were evaluated to compare their effectiveness for formulation and process optimization for cream manufacture. The Taguchi, Plackett-Burman and 2-Level Fractional Factorial DOE were selected as the screening designs to generate data from a minimum number of runs. Compared to a full factorial design, the TD and PBD consisted of twelve runs while the 2-LFFD required a minimum of sixteen runs. The eleven variables investigated were % v/v propylene glycol, % w/w cetostearyl alcohol, % w/w glyceryl monostearate, % w/w Gelot® 64, mixing time, anchor speed, homogenization time and speed, heating temperature, batch size and cooling time. The viscosity, spreadability, pH, % CP content, tube extrudability, electrical conductivity and *in vitro* CP released over 2, 4, 8, 12, 24, 48 and 72 hours were the thirteen response variables monitored. The maximum (+1) and minimum (-1) levels were selected according to preliminary studies that were performed in addition, covering the range of settings within which the cream/ointment plant could be operated.

A model-fitting technique using Design-Expert® software (version 8.02, Stat-Ease Inc., Minneapolis, USA) showed that a correlation between the formulation and process variables and the cream responses was most suitably described by use of linear and two factorial models. Linear and two factorial equations were generated for each response from the screening designs and statistical analysis was undertaken using ANOVA, evaluation of the regression coefficient and by evaluation of Main Effect and Pareto plots. Of the 13 responses, spreadability, electrical conductivity and cumulative % CP release over 2, 12, 48 and 72 hours were found to be significant, with P-values < 0.05. The formulation parameters, spreadability, electrical conductivity, cumulative % CP released over 2, 4 and 48 hours were the only responses that showed good reliability for all models as their adjusted and predicted R^2 values were in close agreement. The formulation responses, pH, % CP content, cumulative % CP released over 2 hours and 48 hours had the lowest coefficients of variation, thereby indicating precise models had been developed for the three screening designs. The predicted values for pH were shown to be the most accurate and closer to its actual values as compared to the other responses monitored for the three screening designs. Each statistical design generated its own significant responses with TD identifying ten significant responses and found to be a less selective statistical model than the other two designs which have only eight significant responses. Furthermore, it was observed that the PBD and 2-LFFD generated low coefficients of variation for most of the responses, indicating that these approaches may generate more accurate models than a TD.

The Main Effect and Pareto plots confirmed that formulation parameters had the most significant effect on the creams rather than process-related parameters. Among all mixer variables evaluated, the homogenization and mixing time had some significant effects on the responses monitored. Cetostearyl alcohol was the formulation variable that had the most significant effect on the quality of formulations. An increase in cetostearyl alcohol content resulted in an increase in viscosity, a decrease in spreadability and a decrease in the cumulative % CP released over time. Gelot® 64 and glyceryl monostearate had secondary yet significant effects on formulation responses and propylene glycol showed a slight effect in all the three designs. It was therefore deemed necessary to further investigate the formulation variables for design optimization using Response Surface Methodology (RSM).

CHAPTER SEVEN

THE OPTIMIZATION OF CLOBETASOL 17-PROPIONATE (CP) CREAM FORMULATIONS USING RESPONSE SURFACE METHODOLOGY

7.1. INTRODUCTION

7.1.1. Formulation Optimization

Formulation optimization is the science and art of allocating available resources to establish the best possible solution to facilitate the production of high quality pharmaceutical products [495]. Since the 1970s, mathematical and statistical models, aided by computer optimization techniques such as Response Surface Methodology (RSM) have been used for the optimization of formulations and manufacturing processes [104,258,367,496]. This powerful approach has been successfully applied to the development, design and optimization of topical drug delivery systems such as self nano-emulsifying systems, creams, gels and pastes [66,442,497,498]. In the development of topical formulations, the design of an optimal formulation composition with the appropriate penetration and/or absorption characteristics, is essential [499]. RSM describes the behavior of a set of data with the primary objective of simultaneously optimizing the levels of all variables under consideration to produce a product with the best possible performance characteristics following fitting of experimental data to polynomial equation(s) [498].

The methodology of RSM for the optimization of topical formulations encompasses the use of various types of experimental design, generation of polynomial equations and mapping of responses over the experimental domain to assist in determining an optimum formulation, in addition to quantifying the influence of formulation variables on drug permeation [499,500]. RSM reduces the number of experimental runs necessary to establish a mathematical trend in the experimental design, thereby permitting the determination of optimum levels of experimental factors required to achieve a specific and defined response(s). This technique is fairly rapid and leads to a reduction in the number of experimental runs by optimization of a formulation during drug product development and may lead to a further and significant reduction in production cost [499,501]. The ICH Q8 (R2) guideline [36] recommends the application of mathematical and statistical models such as RSM to ensure the development of a high quality pharmaceutical product and to establish an appropriate design space for these products [36]. This study aimed to investigate the effect of formulation variables on the performance characteristics of CP cream formulations for the purpose of optimizing the composition with a minimum number of experimental runs. This target was to achieve an optimized cream formulation with *an in vitro* release profile similar to that of a reference formulation *viz.,* Dermovate® cream.

7.1.2. Response Surface Methodology (RSM)

RSM is a combination of mathematical and statistical techniques that explores the relationship(s) between several exploratory variables and one or more responses at the same time [502]. RSM can be applied when a response or set of responses are influenced by one or several factors [503]. The main effect of a factor is defined as the variation in response caused by a change in the level of the factor under consideration, when all others are kept constant. There is an interaction between variables when the effect of one factor depends on the behavior of another. Following identification of the significant factors affecting a response, it is necessary to explore the relationship between that factor and the dependent response(s) within an experimental region and not only at the borders of that region [504].

RSM is a useful tool for conducting experiments in a rational and systematic manner, thereby providing precise information with fewer experiments. Furthermore RSM has the potential to increase the understanding of the principles of pharmaceutical sciences with respect to formulation composition and is useful for predicting dosage form performance from mathematical models that have been or are to be developed [104,357]. RSM has the ability to identify significant input variables for determining CQA that are essential for the production of a high quality pharmaceutical product. This statistical approach can be used to detect possible interactions and synergies between input variables that can be critical for formulation optimization [261,505]. RSM requires the use of an experimental technique *viz*, a reduced experimental design called a Central Composite Design (CCD). The CCD is formulated on the basis of factorial designs with added star points, a central point and three types of structures that can be used, as shown in Figure 7.1 [505].

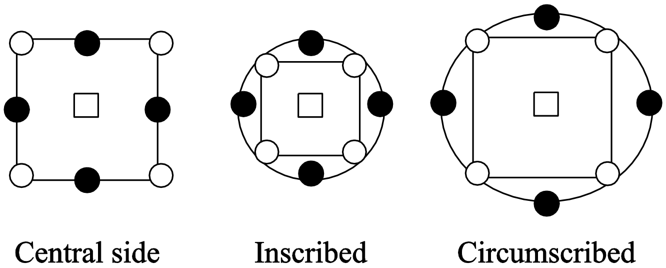


Figure 7.1 Structures of CCD: central side (CCF), inscribed (CCI) and circumscribed (CCC), adapted and redrawn from [505]

7.2. MATERIALS AND METHODS

7.2.1. Materials

Dermovate® 0.05% w/w cream (Sekpharma® Pty Ltd, Sandton, Gauteng, RSA) was purchased from a local pharmacy and stored at 22°C. All other chemicals/reagents used are similar to those reported in §

6.2.1, Chapter 6. All chemicals were at least of analytical grade and were used without further modification.

7.2.2. Equipment

All excipients were weighed using a Model PM4600 Mettler® top-loading analytical balance (Zurich, Switzerland). The cream formulations were manufactured using a Wintech® cream/ointment plant (Wintech® Pharmachem Equipment PVT Ltd, Mumbai, India).

7.2.3. Method of Manufacture

The manufacturing procedure used for the production of all creams (30 experiments) is described in § 6.2.2, Chapter 6. Thirty batches of cream of 6 Kg each were manufactured using an anchor speed of 40 rpm for 120 minutes, a homogenization speed of 2000 rpm for 120 minutes and a cooling time of 120 minutes. The heating temperature used to melt the waxy excipients was set at 75°C. The formulation composition was different for each of the cream formulations manufactured and these are summarized in Appendix IV.

7.2.4. Topical Dosage Form Analysis

Following manufacture, all creams were evaluated in terms of a number of formulation responses including viscosity, spreadability, pH, % CP content, extrudability, electrical conductivity and *in vitro* testing to establish the performance characteristics of the test formulations. A description of the analytical procedures used to assess the creams is reported in § 6.2.3, Chapter 6.

7.2.5. Experimental Design

The formulation variables *viz.*, % v/v propylene glycol (A_1), % w/w Gelot® 64 (A_2), % w/w cetostearyl alcohol (A_3) and % w/w glyceryl monostearate (A_4) were the main factors considered to have the potential to effect on the performance of the creams. Therefore a CCD approach was used to systematically investigate the influence of four critical formulation variables on viscosity (B_1), spreadability (B_2), pH (B_3), % CP content (B_4), extrudability (B_5), electrical conductivity (B_6), and % CP released over 2 (B_7), 4 (B_8), 8 (B_9), 12 (B_{10}), 24 (B_{11}), 48 (B_{12}) and 72 hours (B_{13}). The formulation variables were studied at three levels *viz.*, low (-1), medium (0) and high (+1) and the design data are listed in Table 7.1.

Table 7.1 Actual and coded values for the CCD used to assess formulation variables

Input Factor	**Symbol**	**Real values of coded levels**				
		-α^a	**-1**	**0**	**+1**	**+α^a**
% v/v Propylene glycol	**A_1**	36	41	46	51	56
% w/w Gelot® 64	**A_2**	0	1	3	5	7
% w/w Cetostearyl alcohol	**A_3**	0	3	8	13	18
% w/w Glyceryl monostearate	**A_4**	1	6	11	16	21
Output Factor		**Constraints**				
Viscosity	**B_1**	**~45000 cP**				
Spreadability	**B_2**	**~ 24 cm^2**				
pH	**B_3**	**4 – 7**				
Extrudability	**B_5**	**~77 g/cm^2**				
% Drug Content	**B_4**	**100%**				
Electrical Conductivity	**B_6**	**~200 µS/cm**				
% Drug Released at 2 Hours	**B_7**	**$3 \leq B_7 \leq 6$**				
% Drug Released at 4 Hours	**B_8**	**$8 \leq B_8 \leq 12$**				
% Drug Released at 8 Hours	**B_9**	**$14 \leq B_9 \leq 16$**				
% Drug Released at 12 Hours	**B_{10}**	**$22 \leq B_{10} \leq 24$**				
% Drug Released at 24 Hours	**B_{11}**	**$32 \leq B_{11} \leq 36$**				
% Drug Released at 48 Hours	**B_{12}**	**$42 \leq B_{12} \leq 44$**				
% Drug Released at 72 Hours	**B_{13}**	**$48 \leq B_{13} \leq 52$**				

[a] α= 2.0 (star or axial point in the case of four independent variables)

The range for each factor was selected on the basis of preliminary experimental results generating from testing of laboratory scale and scale-up manufacture of CP cream formulations [61]. The constraints for the formulation responses were established following these preliminary studies and analysis of the *in vitro* release profile generated for the reference product, Dermovate® cream using the *in vitro* test conditions described in § 6.2.2.7, Chapter 6. The formulation response values for batches CCD-CP-01 - CCD-CP-30 are included in Appendix V, together with the batch summary records for these batches. A summary of the thirty experimental runs and associated levels for the coded factors are listed in Tables 7.2 and 7.3.

Table 7.2 CCD Factorial design used to evaluate formulation variable impact on the CP cream responses

Run	**Variables**								**Responses**					
	% v/v Propylene glycol (A_1)		**% Gelot® 64 (A_2)**		**% w/w Cetostearyl alcohol (A_3)**		**% w/w Glyceryl monostearate (A_4)**		**VSC* (B_1)**	**SPD* (B_2)**	**pH (B_3)**	**% DC* (B_4)**	**EXD* (B_5)**	**EC* (B_6)**
	Level	**%**	**Level**	**%**	**Level**	**%**	**Level**	**%**	**cP**	**cm^2**	**-**	**%**	**g/cm^2**	**µS/cm**
1	0	46	0	3	0	8	0	11	44066	24.34	6.15	100.8	72.02	200.02
2	0	46	0	3	+2	18	0	11	89633	12.15	6.62	99.72	244.17	101.50
3	+1	51	-1	1	+1	13	-1	6	46333	22.07	6.63	102.33	77.45	191.33
4	0	46	0	3	0	8	0	11	45500	25.68	6.03	100.64	76.51	198.30
5	0	46	0	3	0	8	0	11	45433	24.65	6.14	103.33	76.31	197.90
6	+1	51	+1	5	-1	3	+1	16	55700	13.89	6.35	98.76	107.69	391.56
7	0	46	+2	7	0	8	0	11	87766	13.44	6.03	102.54	204.96	229.93
8	0	46	-2	0	0	8	0	11	44500	17.14	6.22	100.18	79.44	198.06
9	-2	36	0	3	0	8	0	11	68600	12.57	6.20	101.18	115.47	285.36
10	-1	41	-1	1	+1	13	+1	16	84900	16.46	6.26	103.47	313.56	315.33
11	-1	41	-1	1	-1	3	+1	16	46466	18.87	6.27	104.33	80.22	1066.33
12	+2	56	0	3	0	8	0	11	35766	16.13	6.41	102.56	70.34	288.66
13	-1	41	+1	5	-1	3	+1	16	82300	12.38	6.83	100.48	323.61	898.33
14	0	46	0	3	0	8	-2	1	41133	21.91	6.40	100.75	83.67	177.63
15	-1	41	+1	5	-1	3	-1	6	54800	18.35	6.98	100.36	102.01	311.20
16	0	46	0	3	0	8	0	11	44400	24.90	6.00	100.41	77.80	182.00
17	0	46	0	3	0	8	0	11	44666	24.38	6.00	101.92	75.40	198.66
18	+1	51	+1	5	+1	13	-1	6	71766	14.44	6.11	101.66	179.23	272.33
19	0	46	0	3	-2	0	0	11	71433	16.15	6.05	103.79	176.27	188.83
20	+1	46	-1	1	+1	13	+1	16	92900	13.67	6.35	101.76	462.59	98.36
21	-1	41	-1	1	+1	13	-1	6	72000	22.38	6.84	100.55	191.50	209.66
22	-1	41	+1	5	+1	13	-1	6	90600	9.99	6.70	98.65	445.63	143.80
23	+1	51	-1	1	-1	3	+1	16	35066	19.63	6.35	102.53	49.13	963.00
24	0	46	0	3	0	8	+2	21	89500	11.80	6.68	98.76	180.57	112.03
25	-1	41	+1	5	+1	13	+1	16	88633	9.86	6.37	99.77	195.32	31.13
26	0	46	0	3	0	8	0	11	44800	24.22	6.07	100.10	76.48	198.63
27	+1	51	-1	1	-1	3	-1	6	16600	27.68	6.21	99.78	54.42	433.00
28	-1	41	-1	1	-1	3	-1	6	27433	22.62	6.03	101.73	65.35	734.00
29	+1	51	+1	5	+1	13	+1	16	88367	12.35	6.09	104.15	194.29	252.66
30	+1	51	+1	5	-1	3	-1	6	50800	16.14	6.13	100.55	165.56	307.33

VSC*= Viscosity, SPD*= Spreadability, % DC*= % Drug content, EXD*= Extrudability, EC*= Electrical conductivity

Table 7.3 CCD Factorial design used to evaluate formulation variable impact on CP release from experimental cream formulations

Run	Variables								Responses						
	% v/v Propylene glycol (A_1)		**% Gelot® 64 (A_2)**		**% w/w Cetostearyl alcohol (A_3)**		**% w/w Glyceryl monostearate (A_4)**		**2HR* (B_7)**	**4HR* (B_8)**	**8HR* (B_9)**	**12HR* (B_{10})**	**24HR* (B_{11})**	**48HR* (B_{12})**	**72HR* (B_{13})**
	Level	**%**	**Level**	**%**	**Level**	**%**	**Level**	**%**	**%**	**%**	**%**	**%**	**%**	**%**	**%**
1	0	46	0	3	0	8	0	11	4.83	9.87	15.44	22.85	34.97	44.75	50.96
2	0	46	0	3	+2	18	0	11	1.61	3.70	6.42	9.58	14.43	26.46	34.28
3	+1	51	-1	1	+1	13	-1	6	5.64	10.25	16.35	23.80	31.84	47.96	50.68
4	0	46	0	3	0	8	0	11	4.81	9.84	15.36	22.84	35.44	44.90	48.89
5	0	46	0	3	0	8	0	11	4.72	9.75	15.84	22.43	35.14	44.56	50.00
6	+1	51	+1	5	-1	3	+1	16	2.97	3.86	8.86	14.31	23.00	36.67	49.44
7	0	46	+2	7	0	8	0	11	1.12	4.90	7.12	10.21	14.81	24.13	29.10
8	0	46	-2	0	0	8	0	11	5.45	10.38	16.38	25.51	37.18	45.90	55.47
9	-2	36	0	3	0	8	0	11	1.34	2.16	10.11	13.53	22.78	39.11	45.75
10	-1	41	-1	1	+1	13	+1	16	1.64	3.23	7.34	9.80	14.84	27.96	35.68
11	-1	41	-1	1	-1	3	+1	16	5.45	10.38	16.38	23.51	33.18	45.90	50.47
12	+2	56	0	3	0	8	0	11	7.16	13.02	19.54	25.31	39.90	50.35	65.92
13	-1	41	+1	5	-1	3	+1	16	1.33	5.00	8.17	12.11	17.68	23.84	36.38
14	0	46	0	3	0	8	-2	1	6.30	11.05	17.44	22.48	38.75	47.76	61.85
15	-1	41	+1	5	-1	3	-1	6	1.31	3.98	6.40	10.21	22.57	35.68	52.64
16	0	46	0	3	0	8	0	11	4.81	9.83	15.55	23.87	34.75	46.57	51.95
17	0	46	0	3	0	8	0	11	4.86	9.84	15.15	22.54	35.01	45.68	50.67
18	+1	51	+1	5	+1	13	-1	6	1.33	2.81	7.58	14.91	22.54	42.53	61.06
19	0	46	0	3	-2	0	0	11	5.12	10.90	16.12	24.21	36.81	48.13	52.10
20	+1	46	-1	1	+1	13	+1	16	1.12	2.90	5.12	7.21	10.81	16.13	22.10
21	-1	41	-1	1	+1	13	-1	6	1.09	2.43	6.64	14.17	23.29	41.25	59.54
22	-1	41	+1	5	+1	13	-1	6	1.13	2.80	5.34	7.56	11.81	17.39	21.18
23	+1	51	-1	1	-1	3	+1	16	8.48	15.62	17.37	25.54	39.79	50.64	65.06
24	0	46	0	3	0	8	+2	21	1.61	3.70	6.42	9.58	13.43	25.46	34.28
25	-1	41	+1	5	+1	13	+1	16	1.59	3.69	6.56	9.60	15.44	26.54	35.53
26	0	46	0	3	0	8	0	11	4.34	9.23	15.43	22.42	35.43	44.32	50.74
27	+1	51	-1	1	-1	3	-1	6	9.80	20.19	32.15	40.47	52.17	62.59	70.97
28	-1	41	-1	1	-1	3	-1	6	5.49	12.74	19.10	28.64	42.39	58.32	65.11
29	+1	51	+1	5	+1	13	+1	16	1.47	3.73	6.38	10.53	16.56	27.96	35.55
30	+1	51	+1	5	-1	3	-1	6	1.31	3.99	6.41	12.21	25.54	37.64	54.61

***2hr, 4hr, 8hr, 12 hr, 24hr, 48hr and 72 hr*= Cumulative % CP released per unit area over a 2, 4, 8, 12, 24, 48 and 72 hour period**

7.3. MODEL FITTING AND STATISTICAL ANALYSIS

7.3.1. Response Surface Analysis

The statistical tool, Design-Expert® software (version 8.02, Stat-Ease Inc., Minneapolis, USA) was used to assess the response models and to depict the relationship(s) between input variables and formulation responses. Polynomial models including interaction and quadratic terms were generated for all response variables using a Multiple Linear Regression Analysis (MLRA) approach [506]. The significant terms (P-value < 0.05) were selected from the final equations generated. The validity of each polynomial was established on the basis of ANOVA and R^2 values generated by the software. Normal plots of residuals, two dimensional (2-D) contour plots and three-dimensional (3-D) response surface plots were constructed based on the polynomial functions for the models and they were used to establish any interactive effects for each of the factors on the responses that were monitored [506].

7.3.1.1. Statistical Design Equations and Regression Coefficients

The experimental results concerning the variables tested on each formulation response *viz.*, viscosity, spreadability, pH, % CP content, extrudability, electrical conductivity and % CP released are summarized in Tables 7.1 and 7.2. The viscosity of the CP formulations ranged between 16600 cP and 89600 cP, spreadability between 9.86 cm^2 and 27.68 cm^2, pH between 6.00 and 6.84, % CP content between 98.76% and 104.15%, tube extrudability between 49.13 g/cm^2 and 462.59 g/cm^2 and electrical conductivity between 98.36 µS/cm and 1066.33 µS/cm. The percent CP released from the topical formulations ranged between 1.12% and 9.80% after 2 hours, 2.43% and 20.19% after 4 hours, 5.12% and 32.15% after 8 hours, 7.21% and 40.47% after 12 hours, 10.81% and 52.17% after 24 hours, 16.13% and 62.59% after 48 hours and 21.18% and 70.97% after the 72 hour samples. A quadratic polynomial model was selected for each formulation response monitored, using statistical software. The final empirical models in terms of coded factors for viscosity (B_1), spreadability (B_2), pH (B_3), % CP content (B_4), extrudabiliy (B_5), electrical conductivity (B_6), and cumulative % CP released at 2,4, 8, 12, 24, 48 and 72 hours ($B_{7\text{-}13}$) are shown in Equations 7.1 - 7.13.

Viscosity (B_1) $= +53644.4 - 6469.44A_1 + 10325.00A_2 + 12613.89A_3 + 10030.56A_4 - 612.50A_1A_2 + 1004.17A_1A_3 + 1816.67A_1A_4 - 4675.00A_2A_3 - 3120.83A_2A_4 + 262.50A_3A_4 - 906.94A_1^2 + 2580.56A_2^2 + 6180.56A_3^2 + 2376.39A_4^2$ ***Equation 7.1***

Spreadability (B_2) $= +16.37 + 0.67A_1 - 2.64A_2 - 1.51A_3 - 2.37A_4 + 0.22A_1A_2 - 0.079A_1A_3 - 0.31A_1A_4 + 6.135E\text{-}003A_2A_3 + 0.98A_2A_4 + 0.22A_3A_4 - 0.21A_1^2 + 0.026A_2^2 - 0.26A_3^2 + 0.42A_4^2$ ***Equation 7.2***

pH (B_3) $= +6.47 - 0.068A_1 + 9.861E\text{-}003A_2 + 0.056A_3 - 7.917E\text{-}003A_4 - 0.15A_1A_2 + 4.792E\text{-}003A_1A_3 + 0.056A_1A_4 - 0.14A_2A_3 + 0.013A_2A_4 - 0.10A_3A_4 - 0.026A_1^2 - 0.072A_2^2 - 0.019A_3^2 + 0.032A_4^2$ ***Equation 7.3***

% Drug Content (B_4) = $+101.05 + 0.21A_1 - 0.31A_2 - 0.18A_3 + 0.24A_4 + 0.24A_1A_2 + 0.60A_1A_3 + 0.80A_1A_4 - 0.24A_2A_3 + 0.27A_2A_4 - 0.36A_3A_4 + 0.23A_1^2 + 0.099A_2^2 + 0.20A_3^2 - 0.30A_4^2$ ***Equation 7.4***

Extrudability (B_5) = $+98.76 - 21.55A_1 + 27.92A_2 + 51.97A_3 + 26.63A_4 + 25.80A_1A_2 - 2.38A_1A_3 + 14.30A_1A_4 - 30.02A_2A_3 - 36.77A_2A_4 + 6.16A_3A_4 + 5.81A_1^2 + 18.13A_2^2 + 35.14A_3^2 + 15.61A_4^2$ ***Equation 7.5***

Electrical Conductivity (B_6) = $+274.25 - 33.07A_1 - 55.79A_2 - 156.87A_3 + 53.45A_4 + 29.94A_1A_2 + 64.36A_1A_3 - 25.68A_1A_4 + 73.32A_2A_3 - 21.00A_2A_4 - 103.33A_3A_4 + 39.18A_1^2 + 20.93A_2^2 + 3.72A_3^2 + 3.64A_4^2$ ***Equation 7.6***

Cumulative % Drug Released over 2 Hours (B_7) = $+2.84 + 1.03A_1 - 1.46A_2 - 0.84A_3 - 0.52A_4 - 0.60A_1A_2 - 0.30A_1A_3 - 0.31A_1A_4 + 1.15A_2A_3 + 0.48A_2A_4 - 0.23A_3A_4 + 0.35A_1^2 + 0.11A_2^2 - 0.37A_3^2 + 0.28A_4^2$ ***Equation 7.7***

Cumulative % Drug Released over 4 Hours (B_8) = $+5.45 + 1.70A_1 - 2.45A_2 - 1.93A_3 - 1.06A_4 - 1.33A_1A_2 - 0.25A_1A_3 - 0.72A_1A_4 + 2.27A_2A_3 + 1.01A_2A_4 + 0.083A_3A_4 + 0.54A_1^2 + 0.55A_2^2 - 0.29A_3^2 + 0.48A_4^2$ ***Equation 7.8***

Cumulative % Drug Released over 8 Hours (B_9) = $+11.13 + 1.80A_1 - 3.47A_2 - 2.29A_3 - 1.91A_4 - 1.17A_1A_2 - 0.32A_1A_3 - 1.61A_1A_4 + 2.85A_2A_3 + 2.02A_2A_4 + 0.17A_3A_4 + 0.87A_1^2 + 0.10A_2^2 - 1.14A_3^2 + 0.15A_4^2$ ***Equation 7.9***

Cumulative % Drug Released over 12 Hours (B_{10}) = $+17.16 + 2.37A_1 - 4.68A_2 - 2.95A_3 - 2.72A_4 - 0.53A_1A_2 - 0.17A_1A_3 - 1.77A_1A_4 + 3.56A_2A_3 + 2.67A_2A_4 - 0.45A_3A_4 + 0.69A_1^2 + 0.30A_2^2 - 1.69A_3^2 - 0.16A_4^2$ ***Equation 7.10***

Cumulative % Drug Released over 24 Hours (B_{11}) = $+26.79 + 3.14A_1 - 5.16A_2 - 4.75A_3 - 4.65A_4 - 0.049A_1A_2 - 0.52A_1A_3 - 1.44A_1A_4 + 4.02A_2A_3 + 2.58A_2A_4 - 0.17A_3A_4 + 1.36A_1^2 - 0.85A_2^2 - 2.57A_3^2 + 0.047A_4^2$ ***Equation 7.11***

Cumulative % Drug Released over 48 Hours (B_{12}) = $+42.22 + 2.82A_1 - 6.08A_2 - 4.29A_3 - 5.51A_4 + 2.34A_1A_2 - 0.15A_1A_3 - 1.93A_1A_4 + 4.05A_2A_3 + 3.20A_2A_4 - 0.83A_3A_4 + 0.92A_1^2 - 1.50A_2^2 - 3.68A_3^2 - 1.11A_4^2$ ***Equation 7.12***

Cumulative % Drug Released over 72 Hours (B_{13}) = $+52.54 + 3.05A_1 - 5.25A_2 - 4.96A_3 - 6.70A_4 + 3.56A_1A_2 - 1.13A_1A_3 - 1.55A_1A_4 + 2.74A_2A_3 + 2.52A_2A_4 - 1.35A_3A_4 + 2.37A_1^2 - 2.26A_2^2 - 4.54A_3^2 - 0.82A_4^2$ ***Equation 7.13***

The positive sign for the responses for A_2, A_3 and A_4 revealed that a synergistic effect was observed for these input factors on the viscosity of the cream formulations whereas the negative sign for the responses for A_2, A_3 and A_4 indicates an antagonistic effect of these parameters on the spreadability of the cream. The regression coefficient and associated standard deviation for each parameter were also assessed to establish the accuracy of the responses predicted following modeling of the data. The calculation of SD for "viscosity" and "pH" was obtained using the Design-Expert® software. The viscosity (B_1) was shown to be the most significant formulation response following the manufacture of CP formulations with an F-value of 7.09. The spreadability (B_2) was not as significant (F-value = 4.36) whereas CP content and pH were found to be the least significant formulation responses as the F-values for these responses were 0.37 and 1.43 and were deemed to be low. The viscosity response was found to have the highest standard deviation with value of 10847 whereas pH had the lowest standard deviation value of 0.25. The two formulation responses, pH and % CP content were shown to have low coefficient of variation values of 2.46 and 3.49, indicating that precise and reliable quadratic polynomial models had been developed for these parameters.

7.3.1.2. Viscosity

The largest F-value of 32.45 was observed for cetostearyl alcohol (A_3), indicating that this factor has the most significant effect on the viscosity of the cream formulations compared to other formulation variables. Gelot® 64 (A_2) and glyceryl monostearate (A_3) were other variables that were seen to have some effect on the viscosity of the cream formulations, with F-values of 21.74 and 20.52. The effect of propylene glycol on the viscosity was not as pronounced, with a F-value of 8.54, clearly indicating that this parameter had the least effect on the viscosity of the creams. The contour and 3-D response surface plots constructed to show the interaction effects of formulation variables on cream viscosity (B_1) are shown in Figures 7.2 and 7.3. The contour plot suggests that Gelot® 64 and cetostearyl alcohol were found to have a significant effect on this formulation response, as depicted in Figure 7.2.

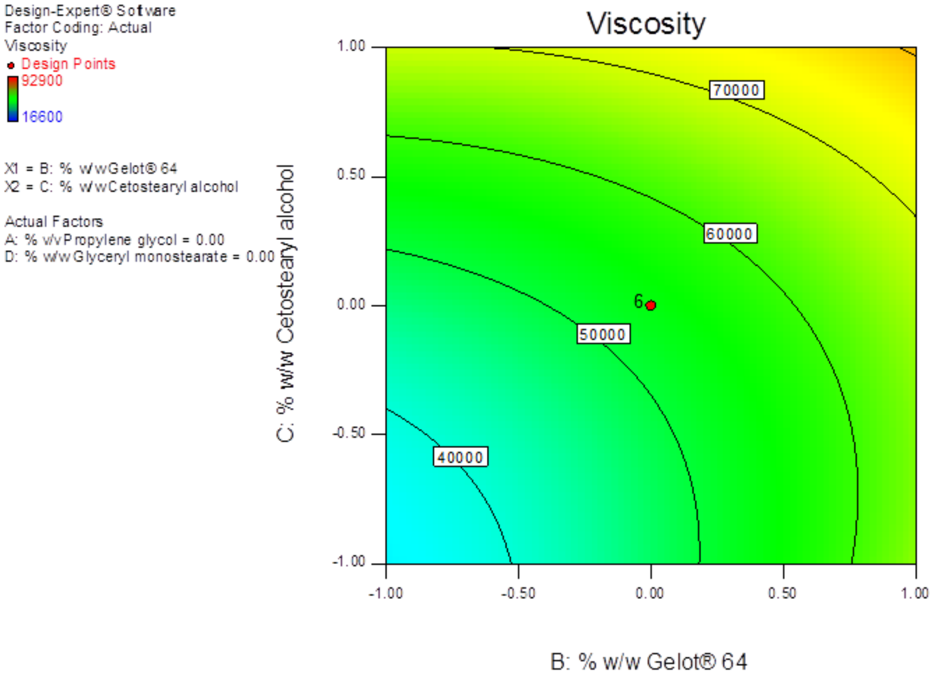


Figure 7.2 Contour plot showing the effect of % w/w cetostearyl alcohol and Gelot® 64 on cream viscosity

An increase in the cetostearyl alcohol content from 3% w/w to 13% w/w resulted in a significant increase in the viscosity of the cream from approximately 41000 cP to 71000 cP. Furthermore an increase Gelot® 64 content from 1% w/w to 5% w/w also resulted in an increase in the viscosity of the cream formulations from approximately 44000 cP to 87000 cP, indicating that cetostearyl alcohol and Gelot® 64 may have the greatest impact on the quality of this semi-solid product. Cetostearyl alcohol is a mixture of solid aliphatic alcohols and plays a dual role in cream dosage forms as it is a stiffening agent and an emulsion stabilizer [507]. This dual action could be the reason for the impact on viscosity of the creams. The viscosity-enhancing properties usually reduce coalescence of the dispersed phase of a topical formulation, thereby improving the physical stability of creams [195].

The addition of o/w emulsifiers often results in the production of a soft formulation that leads to a mixed crystal bilayer network of high viscosity [508]. Furthermore the formation of a separate crystalline lipophilic network in formulations containing high concentrations of an emulsifier(s) usually leads to an increase in viscosity and therefore a greater physico-chemical stability of the cream [485]. However high concentration of emulsifier usually results in an increase in crystallization on storage that would produce optical inhomogenity and a lack of softness which is essential for a suitable cosmetic appearance [485]. The maximum viscosity value was observed when the Gelot® 64 and cetostearyl alcohol content were at their highest levels as can be seen in Figure 7.3.

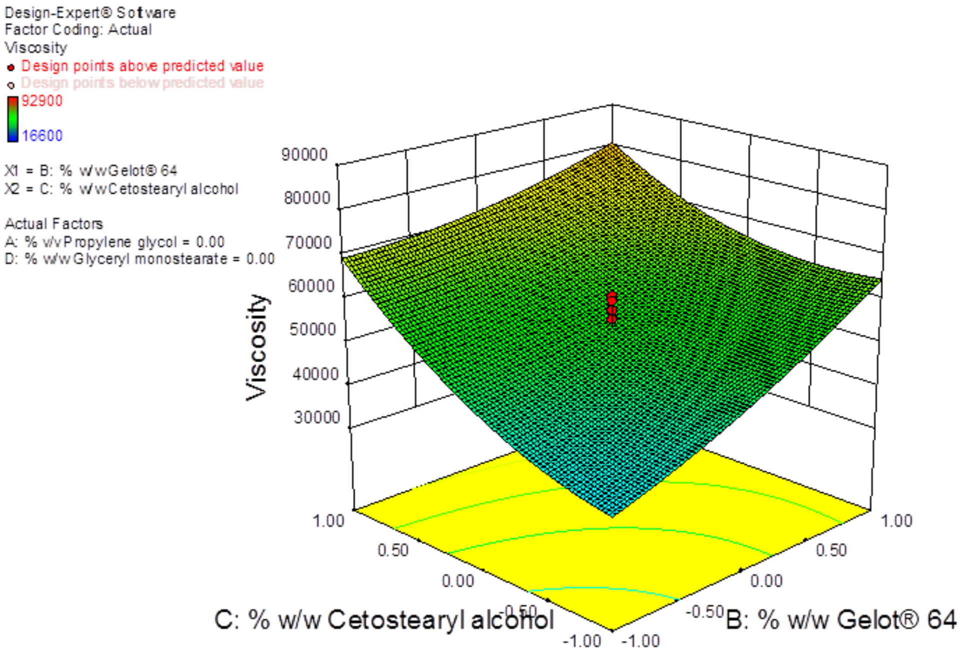


Figure 7.3 3-D response surface plot showing the effect of % w/w cetostearyl alcohol and Gelot® 64 on cream viscosity

7.3.1.3. Spreadability

The largest F-value of 25.88 was observed for cetostearyl alcohol (A_3) indicating that this parameter had the most significant effect on spreadability of the creams, compared to the other formulation variables evaluated. Glyceryl monostearate (A_4) had some secondary effect on the cream formulations with F- and P-values of 8.50 and < 0.05, respectively. The % v/v propylene glycol (A_1) and % w/w Gelot® 64 (A_2) had the least effect on this formulation response, since the F-values for these parameters were 4.36 and 1.67, respectively. The contour plot in Figure 7.4 suggests that both cetostearyl alcohol and glyceryl monostearate had a significant effect on this response when the composition of the formulation included increased amounts of these excipients.

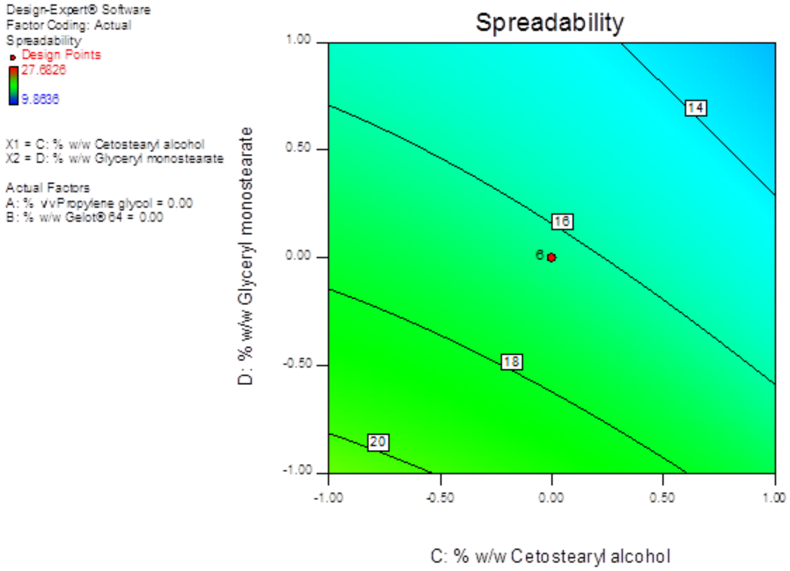


Figure 7.4 Contour plot showing the effect of % w/w cetostearyl alcohol and Gelot® 64 on cream spreadability

An increase in cetostearyl alcohol content resulted in a decrease in the spreadability of the cream formulations with the value for this parameter reducing from approximately 16.0 cm^2 to 12.0 cm^2. An increase in the glyceryl monostearate content also resulted in a decrease in spreadability from approximately 21.1 cm^2 to 11.8 cm^2. Highly viscous formulations are characterized by a high surface tension between particles, exhibiting a decrease in spreadability which would result in less absorption of the base or API into the skin [509]. The 3-D response surface plot (Figure 7.5) reveals that the overall combined effect of cetostearyl alcohol and glyceryl monostearate resulted in a significant decrease in the spreadability of the formulations. The minimum spreadability was observed when the cetostearyl alcohol and glyceryl monostearate content were at the highest levels.

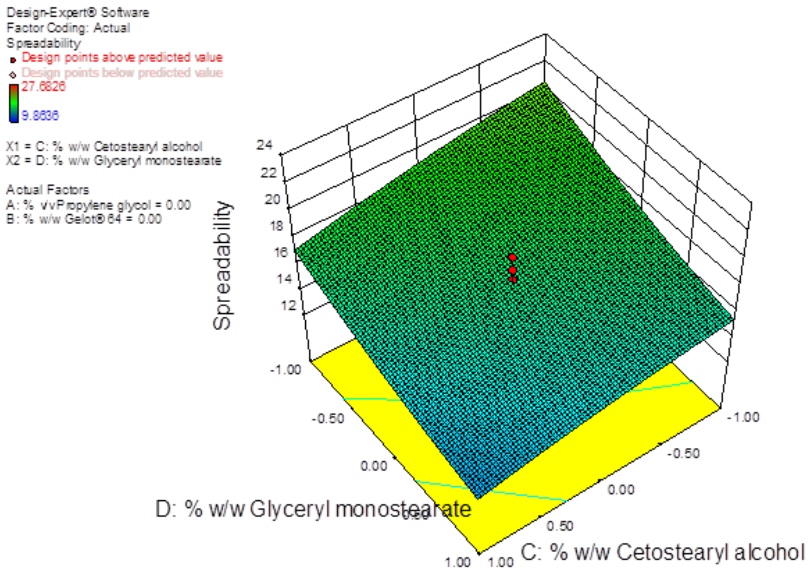


Figure 7.5 3-D response surface plot showing the effect of % w/w cetostearyl alcohol and Gelot® 64 on cream spreadability

7.3.1.4. pH

The largest F-values of 1.73 and 1.19 were observed for propylene glycol (A_1) and cetostearyl alcohol (A_3) content respectively, indicating that these excipients had the most significant effect on the pH of the cream formulations, compared to the other formulation variables evaluated. Gelot® 64 (A_2) and glyceryl monostearate (A_4) had the least effect on pH with resultant F-values of 0.037 and 0.024 respectively. However all cream batches exhibited a pH range between 6.00 and 6.98, irrespective of increasing or decreasing the amount of these excipients in the formulation. From a physiological perspective this result suggests that it is unlikely that any skin irritation will occur following topical application of these cream formulations.

7.3.1.5. CP Content

All formulation variables for this parameter *viz.*, A_1, A_2, A_3 and A_4 resulted in an F-value < 5.0, indicating that there was no significant effect on the CP content of these creams. The emulsifiers Gelot® 64 and cetostearyl alcohol were found to have the most significant effects on the majority of the responses monitored. However these formulation variables had no effect on the % CP content of the cream batches. An increase in Gelot® 64 and cetostearyl alcohol resulted in uniform CP content for all cream batches that ranged between 98% and 104% of label claim with no extreme minimum or maximum values. It is therefore clear that variation of the formulation composition had no impact on the uniform distribution of CP for all 30 batches that were manufactured.

7.3.1.6. Extrudability

The largest F-value of 7.37 was observed for cetostearyl alcohol (A_3) content, indicating that this parameter had the most significant effect on extrudability of the cream from packaging tubes, compared to the other formulation variables investigated. An increase in cetostearyl alcohol content resulted in a highly viscous formulation that in turn affects the extrudability of the creams. The impact of other formulation variables on extrudability *viz.*, A_1, A_2 and A_4 produced F-values of < 5.0, indicating that they did not have a significant effect on the cream extrudability.

7.3.1.7. Electrical Conductivity

The impact of cetostearyl alcohol content (A_3) resulted in a F-value of 12.16 which was the most significant effect observed when monitoring electrical conductivity responses. Propylene glycol (A_1), glyceryl monostearate (A_4) and Gelot® 64 (A_2) had the least effect on the electrical conductivity properties of the creams, with F-values < 5.0. An increase in cetostearyl alcohol composition from 3% to 13% resulted in a significant decrease in the electrical conductivity from approximately 800 µS/cm to 200 µS/cm. This is due to the increase in lipophilicity of the formulations as the cetostearyl alcohol content is increased.

7.3.1.8. In Vitro Release Results for Extreme Formulation Compositions

The *in vitro* release profiles (n=3) generated for CP cream formulations manufactured according to a CCD approach are depicted in Figures 7.6 - 7.9. The formulations have been classified according to similarity of composition for simplicity of comparison and to gain insight into formulation factors that may impact the release of CP from the cream formulations. The notation used to depict the formulation composition uses ratios of % v/v propylene glycol: % w/w Gelot® 64: % w/w cetostearyl alcohol: % w/w glyceryl monostearate and these ratios have been used in the following discussions. The effect of the % content at the extreme composition of propylene glycol in cream formulations in comparison to a formulation composition representing the center points of the CCD *viz.,* 46:3:8:11 is depicted in Figure 7.6.

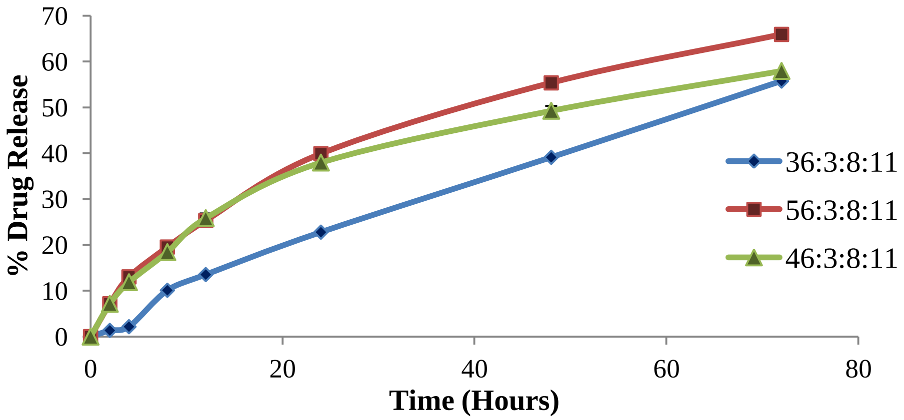


Figure 7.6 *In vitro* release profiles for cream formulations containing propylene glycol at the lower and upper limits of composition in comparison to a center composition

The resultant *in vitro* release profiles reveal that low levels of propylene glycol *viz.*, 36% v/v in a cream formulation, resulted in a decrease in the overall rate and extent of CP release. However after 72 hours the cumulative % CP released was found to be approximately 48% and was similar to that observed for the center formulation. A high propylene glycol content of 56% v/v revealed an overall increase in % CP released from the base. From 2 hours to 24 hours, the cumulative % CP released was similar to that observed for center formulation, but as the time increased, a distinct increase in the amount of CP released at 72 hours was observed. No linear relationship was observed between the amount of propylene glycol and the *in vitro* release rate of CP, which may be attributed to the dual action of propylene glycol as co-solvent to dissolve CP in the medium and as a penetration enhancer in formulations. Propylene glycol is a polyvalent alcohol and is able to penetrate the skin and transport lipophilic substances such as CP via a solvent drag system [510]. Low PG content may decrease the solubility of CP and affect the thermodynamic activity of the system, resulting in a reduction in CP release. The effect of Gelot® 64 in CP cream formulations at the minimum and maximum levels are depicted in Figure 7.7.

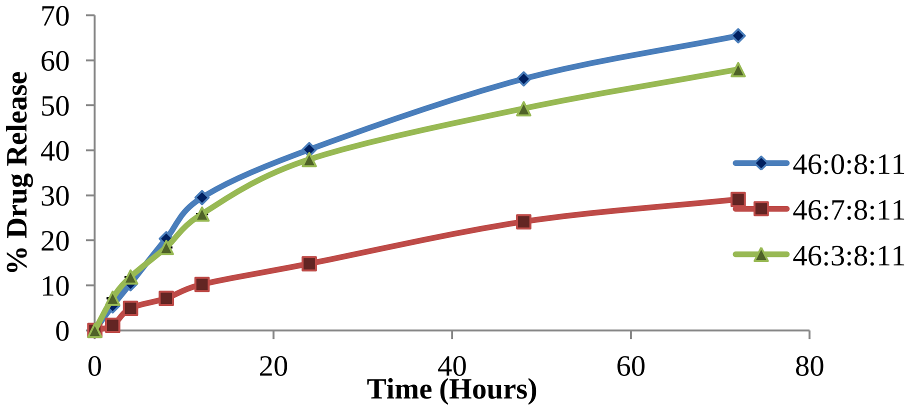


Figure 7.7 *In vitro* release profiles for cream formulations that contain Gelot® 64 at the lower and upper limits of composition in comparison to a center composition

It is evident that the exclusion of Gelot® 64 that is an o/w emulsifier, resulted in an overall increase in the rate of CP release from the creams, whilst an increase in the emulsifier content resulted in a significant decrease in the rate and extent of CP release from the test formulations. However the exclusion of Gelot® 64 in topical formulations can also result in product instability [227] and high levels of Gelot® 64 usually result in highly viscous formulations due to the fact that Gelot® 64 consists of a mixture of glyceryl stearate and polyethylene glycol (PEG)-75 stearate. This mixture of emulsifiers creates a rigid microstructure in viscous formulations and the surface tension between the base and the receptor medium decreases, thereby preventing the CP diffusion from the cream base. The impact of % w/w cetostearyl alcohol on CP release when included at lower and upper extreme of composition is shown in Figure 7.8.

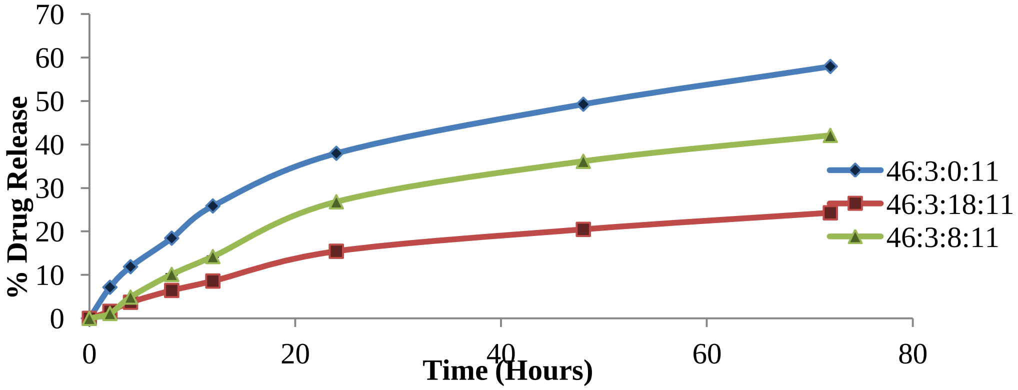


Figure 7.8 *In vitro* release profiles for cream formulations that contain cetostearyl alcohol at the lower and upper limits of composition in comparison to a center composition

It is clear from Figure 7.8. that using a high concentration of cetostearyl alcohol in the formulations resulted in an overall and significant decrease in the rate of CP release from the cream bases when comparing formulations 46:3:0:11 and 46:3:18:11. At 72 hours, the cream formulation with 0% w/w, 8% w/w and 18% w/w cetostearyl alcohol had cumulative % CP released of approximately 60%, 40% and 20% respectively. The incorporation of high levels of cetostearyl alcohol in topical formulations increases the visco-elastic structure of the semi-solid base, thereby contributing to a low flux and overall decrease in CP release. The *in vitro* release profile assessing the impact of glyceryl monostearate on CP release from cream formulations manufactured with high and low levels of the compound are depicted in Figure 7.9.

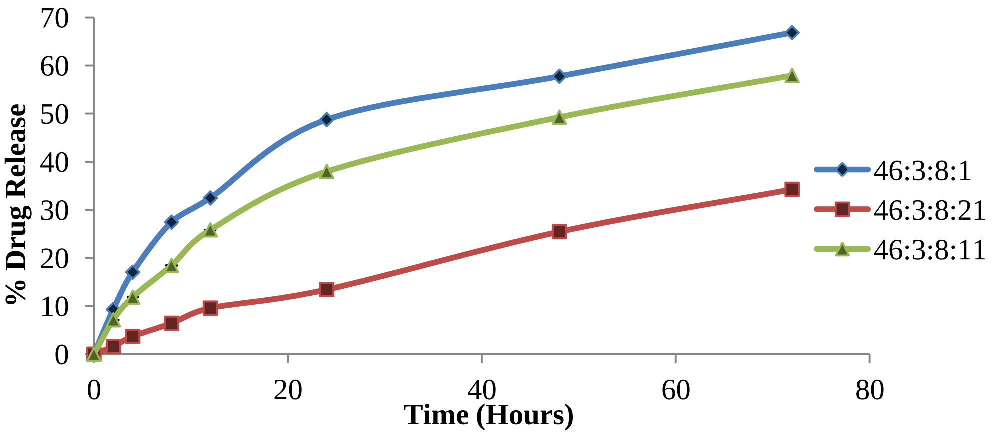


Figure 7.9 *In vitro* release profiles for cream formulations that contain glyceryl monostearate at the lower and upper limits of composition in comparison to a center composition

Glyceryl monostearate is an excellent, commonly used excipient that is included over a wide range of concentration, and can affect the consistency and quality of finished cream products [507,511,512]. Glyceryl monostearate is a mixture of monoglycerides of stearic and palmitic acids and includes variable quantities of di- and triglycerides. It usually contains not less than 90% total fatty acid glycerides, of which at least 35% are monoglycerides [511]. A high concentration of glyceryl monostearate in the formulations resulted in an overall decrease in the release rate of CP from the base, whilst a very low % content of glyceryl monostearate resulted in an increase in release of CP from topical formulations. The decrease in CP release rate might be explained by the fact that the high content of glyceryl monostearate renders the microstructure of the base lipophilic and consequently the structure is less wettable when in contact with the receptor medium. As a result receptor medium penetration rates into the bulk cream structure are decreased, resulting in a decrease in CP release [512].

7.4. FORMULATION OPTIMIZATION

Design-Expert® software (version 8.02, Stat-Ease Inc., Minneapolis, USA) was used to optimize the generic CP cream formulation composition that produced similar responses to that of the reference product, Dermovate® cream. A Normal Boundary Intersection method was used to solve the multi-objective problem of *in vitro* release of CP by using the constraints listed in Table 7.1 as the limits for the optimization process. Objective slice plots were used to study the relationship between input variables and *in vitro* release of CP at different sample times by using a curve showing the change in each response as the level of the input factor is changed [104]. The vertical orange line illustrates the amount of excipient required in a formulation to produce a cream with the desired release characteristics for CP and is depicted in Figure 7.10.

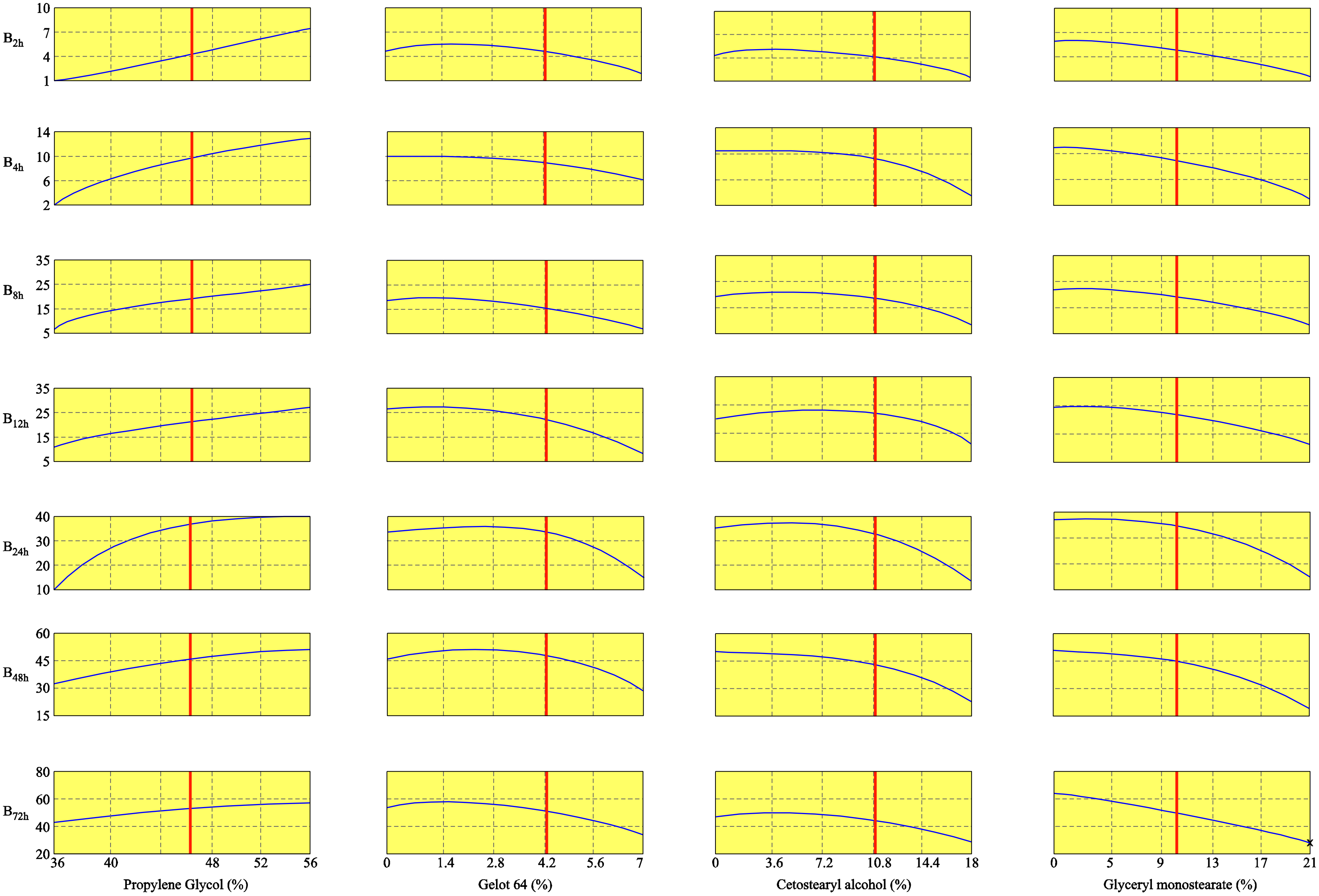


Figure 7.10 Objective slice graph for optimization of the generic CP cream formulation

The predicted values for the formulation variables *viz.*, % v/v propylene glycol (A_1), % w/w Gelot® 64 (A_2), % w/w cetostearyl alcohol (A_3) and % w/w glyceryl monostearate (A_4) in addition to the respective formulation responses observed by numerical optimization, are summarized in Table 7.4.

Table 7.4 Optimized CP formulation

Formulation	**% w/w**	**Predicted Drug Release Profile**	
Propylene glycol	46.1	$\mathbf{B_{2hr}}$	4.8%
Gelot® 64	3.8	$\mathbf{B_{4hr}}$	9.2%
Cetostearyl alcohol	8.6	$\mathbf{B_{8hr}}$	15.2%
Glyceryl monostearate	10.5	$\mathbf{B_{12hr}}$	22.9%
Clobetasol 17-propionate	0.05	$\mathbf{B_{24hr}}$	35.0%
Citric acid	0.05	$\mathbf{B_{48hr}}$	44.9%
Sodium Citrate	0.05	$\mathbf{B_{72hr}}$	50.5%
White beeswax	1.15		
Chlorocresol	0.075		
Distilled water	30.225		

Cream Parameters	
Viscosity	44633 cP
Spreadability	24.91 cm^2
pH	6.02
% drug content	101.23%
Extrudability	76.98 g/cm^2
Electrical Conductivity	198.23µS/cm

The *in vitro* release profiles generated following testing of the optimized generic CP cream formulation compared to the reference and predicted formulations are shown in Figure 7.11. It is clearly evident that there is similarity between the optimized and reference CP formulations.

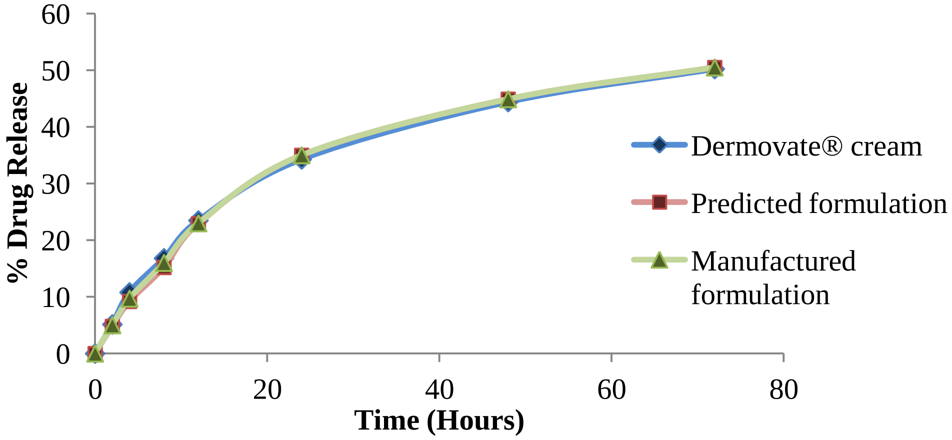


Figure 7.11 *In vitro* release profile of the optimized CP cream compared to the predicted and reference products

The predictability relationship between the results obtained for the optimized formulation that was manufactured versus the predicted formulation from the numerical optimization procedure is shown in Figure 7.12.

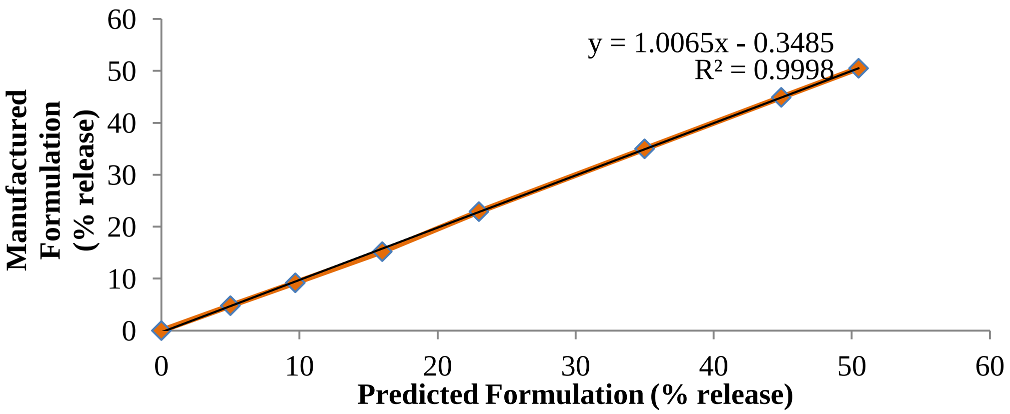


Figure 7.12 Percent CP release from the manufactured formulation versus the predicted data

The plot clearly indicates that the relationship between the manufactured CP cream formulation and predicted CP formulation is linear, with an R^2 value of 0.9998, showing the excellent predictability of RSM for optimization of this formulation.

7.5. STATISTICAL COMPARISON AND MATHEMATICAL MODELLING OF *IN VITRO* CP RELEASE PROFILES

7.5.1. Model-Independent Methods

In recent guidance documents, FDA [29,513] have emphasized the importance of comparison of in *vitro* release profiles and statistical data when testing of diverse formulations. The FDA guidance [513] recommends the use of model-independent methods to calculate the difference (f_1) and similarity (f_2) factors for comparing *in vitro* release profiles. The main advantage of using f_1 and f_2 approach is that it provides a simple statistical way of comparing *in vitro* release data. The use of f_1 and f_2 provides a single value from a number of data points to establish the closeness between two resultant *in vitro* release profiles [514]. The calculation of f_1 determines the percent difference between two curves at each time point and is a measurement of the relative error between *in vitro* release profiles of the test and the reference product at all-time points, by using Equation 7.14.

$$f_1 = \left\{\frac{\sum_{i=1}^{p}|R-T|}{[\sum_{i=1}^{p}R]}\right\} x\ 100$$

Equation 7.14

Where

f_1=Difference factor
R=*In vitro* measurement for the reference formulation at P time points
T=*In vitro* measurement for the test formulation at P time points
P=Number of time points

The calculation of f_2 is a measure of the similarity in percent drug released between *in vitro* release profiles of a test and reference product at all-time points and determines the logarithmic reciprocal square root transformation of the sum of the squared error using Equation 7.15.

$$\mathrm{f}_2 = 50 \log \left\{ [1 + \left(\frac{1}{p}\right) \sum_{i=1}^{p} (R - T)^2]^{\frac{1}{2}} \ x \ 100 \right\} \qquad \textbf{\textit{Equation 7.15}}$$

Where

f_2=Similarity factor
R=*In vitro* measurement for the reference formulation at P time points
T=*In vitro* measurement for the test formulation at P time points
P=Number of time points

A newer model-independent mathematical approach, Gohel similarity factor can be used for the comparison of *in vitro* profiles of a test and reference product. The Gohel similarity factor, S_d can be calculated using Equation 7.16.

$$S_d = \frac{\sum_{t=1}^{n-1} |\log (\frac{AUC_{Rt}}{AUC_{Tt}})|}{n-1} \qquad \textbf{\textit{Equation 7.16}}$$

Where

n=Number of data points collected during an *in vitro* release test
AUC_{Rt}=Area under the curve of the reference product at time t
AUC_{Rt}=Area under the curve of the test product at time t [515]

A value between 0 and 15 for f_1 and between 50 and 100 for f_2 is indicative of the sameness or equivalence between two *in vitro* release profiles evaluated. The $f_1$value is zero when the *in vitro* release profiles of the test and reference product are identical, and this value increases proportionally as *in vitro* profiles become dissimilar, whilst an f_2 value close to 100 indicates that the *in vitro* profiles of a test and reference product are identical [514]. Although the *in vitro* release profiles are usually compared when the number of *in vitro* units used is ≥ 12, the difference and similarity factors are useful to compare the *in vitro* release profiles generated for CP from cream formulations of different formulation composition. It is a limitation to use only n=3 when calculating f_2 value since 12 units are usually required, but the *in vitro* release tests were shown to be predictive in previous work [61]. Two *in vitro* release profiles are considered similar when S_d is zero or close to 0. The S_d factor was calculated for the comparison of the cream formulation to Dermovate® and was compared to the f_2 factor to determine whether relationship between these two parameters could be established to determine the similarity in the *in vitro* release profiles [515,516]. For assessing the discriminatory behaviour of the *in vitro* release test, Dermovate® cream was used as the reference product and the results of these analyses are summarized in Table 7.5.

Table 7.5 f_1 and f_2 values for CP formulations and Dermovate® cream

Comparison*	f_1	f_2	S_d
01	1.5	99.2	0.0001
02	31.7	60.7	0.0185
03	0.9	99.7	0.0001
04	2.7	97.5	0.0001
05	0.5	99.9	0.0001
06	1.6	99.1	0.0001
07	42.1	54.7	0.0807
08	10.4	82.7	0.0100
09	8.9	85.3	0.0089
10	29.0	62.6	0.0130
11	0.9	99.9	0.0001
12	31.2	61.0	0.0165
13	27.6	63.7	0.0120
14	23.1	67.3	0.0112
15	4.8	93.4	0.0001
16	3.4	96.2	0.0001
17	3.4	96.2	0.0001
18	21.6	68.8	0.0115
19	3.7	95.6	0.0001
20	56.0	48.6	0.1320
21	0.8	99.6	0.0001
22	57.8	47.9	0.1220
23	29.5	62.2	0.0168
24	31.7	60.7	0.0108
25	29.3	62.4	0.0165
26	0.5	99.9	0.0001
27	41.3	55.1	0.0150
28	29.6	62.2	0.0108
29	29.2	62.4	0.0108
30	8.7	85.7	0.0090
Predicted formulation	0.5	99.9	0.0001
Optimized formulation	0.5	99.9	0.0001

***Comparison= Dermovate® cream versus CP cream formulation (-01 to -30)**

All batches except 02, 07, 08, 10, 12, 13, 14, 18, 20, 22, 23, 24, 25, 27, 28 and 29 were found to be similar to the *in vitro* release profile of Dermovate® with f_1 values < 15 for these comparisons. The other CP cream batches had f_1 values > 15.0 demonstrating their difference for the *in vitro* release profiles of the reference product. Batches 5, 26, the predicted and optimized formulations produced data, for which the test and reference products were similar, with an f_1 value of 0.5. Batch 7 showed the greatest discrimination between the *in vitro* release profiles in comparison to Dermovate® cream with an f_1 value of 42.1. This batch contained the highest level of % w/w Gelot® 64 which significantly reduced the *in vitro* release of CP from the cream formulation. These cream formulations were manufactured using different amounts of propylene glycol, Gelot® 64, cetostearyl alcohol and glycerol monostearate and resulted in the production of formulations with different physical properties and *in vitro* release profiles.

The similarity factor for comparison of the formulations indicates that all batches except batches 20 and 22 showed values $f_2 > 50$ showed similarity to the *in vitro* release profile of the reference product. Batches 1, 3, 5, 6, 11, 21, 26, the predicted and optimized formulations had f_2 values very close to 100, indicating that the *in vitro* profiles of the test and reference products were almost identical. It can be clearly seen from the data listed in Table 7.3 that there seems to be a relationship between the f_2 values and the similarity factor, S_d. Batches that showed values of f_2 greater than 50 resulted in S_d values of ≤ 0.02. Batches 05, 26, the predicted and optimized formulations had f_2, f_1 and S_d values of 99.9, 0.5 and 0.0001 and had *in vitro* release profiles similar to that observed for Dermovate®.

7.5.2. Mathematical Modeling of Drug Release

Mathematical modeling of drug release data derived from cream formulations generates important information on the transport mechanism of an API from the product [514,517]. Mathematical modeling can also be used to predict and/or design an *in vitro* release profile for a topical system based on the release kinetics of the API that are desired and this approach was used in these studies. The results of modeling provide an understanding of which formulation parameters can alter the *in vitro* release of an API from a dosage form, thereby providing a means of optimizing the formulation development process [518]. Commonly used model-dependent approaches included fitting data to zero order, first order, Higuchi and the Korsmeyer-Peppas models shown in Table 7.6 [514,518].

Table 7.6 Mathematical models used to investigate the kinetics of drug release in this study

Mathematical Model	**Formula**
Zero Order	$Q_t = Q_0 + K_0 t$
First Order	$Ln\ Q_t = Ln\ Q_0 + K_1 t$
Higuchi	$Q_t = Q_0 + K_H t^{1/2}$
Korsmeyer-Peppas	$Q_t = K_{KP} t^n$

Q_t =Amount of drug remaining in the delivery system, Q_0 =Initial amount of drug in the delivery system, $K_{0,\ 1,\ H,\ Kp}$= Zero, first, Higuchi and Korsmeyer-Peppas release constants, t= time, n= Release exponent and indicates the overall mechanism of drug release [518]

CP release data from manufactured and Dermovate® creams were fitted to the zero, first order, Higuchi and Korsmeyer-Peppas models to establish the mechanism of CP release from these formulations and the results are summarized in Table 7.7. CP release from most batches was best fitted to the Higuchi model as most of the R^2 values for this modeling were 0.99. This indicates that diffusion was the predominant factor controlling the release of CP from these formulations. The reference product, Dermovate® followed a Higuchi release model. The Korsemeyer-Peppas model may also be considered appropriate to describe the mechanism of release of CP from the cream formulations as the R^2 values for the model fit range between 0.91 and 0.98 and the values of the release exponent, n, confirm that diffusion is the mechanism of release for CP from these cream formulations.

Table 7.7 Summary of CP release kinetics for CCD formulations

Batch	**Zero Order**		**First Order**		**Higuchi**		**Korsmeyer-Peppas**		**Best Fit Model**
	K_0	**R^2**	**K_1**	**R^2**	**K_H**	**R^2**	**n**	**R^2**	
01	0.8395	0.9081	0.0174	0.7061	7.4500	0.9830	0.7046	0.9759	Higuchi
02	0.5208	0.9899	0.0219	0.7722	4.4323	0.9854	0.8523	0.9893	Korsmeyer-Peppas
03	0.8734	0.9504	0.0169	0.7496	7.6239	0.9953	0.6671	0.9866	Higuchi
04	0.8466	0.9058	0.0175	0.7062	7.5167	0.9813	0.7089	0.9759	Higuchi
05	0.8381	0.9063	0.0715	0.7005	7.4445	0.9827	0.7103	0.9749	Higuchi
06	0.7374	0.9751	0.0219	0.7849	6.3462	0.9925	0.8444	0.9810	Higuchi
07	0.4629	0.9634	0.0212	0.6333	4.0108	0.9938	0.8808	0.9175	Higuchi
08	0.8527	0.8828	0.0168	0.6943	7.6261	0.9705	0.6852	0.9717	Korsmeyer-Peppas
09	0.8123	0.9759	0.0276	0.6897	6.9857	0.9919	1.1159	0.9526	Higuchi
10	0.5520	0.9881	0.0225	0.7652	4.6967	0.9829	0.8801	0.9875	Korsmeyer-Peppas
11	0.8381	0.9307	0.0167	0.7261	7.3852	0.9932	0.6676	0.9821	Higuchi
12	0.9054	0.9140	0.0155	0.7359	8.0197	0.9855	0.6166	0.9848	Higuchi
13	0.4573	0.9069	0.0202	0.6017	4.0657	0.9850	0.8602	0.9181	Higuchi
14	0.8891	0.9107	0.0165	0.7460	7.8641	0.9792	0.6519	0.9867	Korsmeyer-Peppas
15	0.7546	0.9814	0.0361	0.7588	6.4395	0.9822	1.0238	0.9840	Korsmeyer-Peppas
16	0.8662	0.9125	0.0177	0.6777	7.6891	0.9882	0.7274	0.9657	Higuchi
17	0.8542	0.7964	0.0132	0.6652	7.8245	0.9182	0.5466	0.9561	Korsmeyer-Peppas
18	0.8877	0.9859	0.0280	0.7348	7.5574	0.9821	1.1132	0.9802	Higuchi
19	0.9019	0.9179	0.0174	0.7118	7.9758	0.9866	0.7032	0.9756	Higuchi
20	0.3015	0.9522	0.0200	0.6916	2.7132	0.9989	0.8131	0.9670	Higuchi
21	0.8774	0.9836	0.0295	0.7274	7.4922	0.9857	1.1783	0.9794	Higuchi
22	0.3406	0.9521	0.0208	0.6961	2.9757	0.9989	0.8453	0.9717	Higuchi
23	0.8884	0.9243	0.0144	0.7763	7.7998	0.9796	0.5573	0.9737	Higuchi
24	0.4947	0.9862	0.0214	0.7669	4.2079	0.9807	0.8337	0.9854	Korsmeyer-Peppas
25	0.5270	0.9875	0.0221	0.7625	4.5064	0.9925	0.8654	0.9896	Higuchi
26	0.8448	0.8988	0.0181	0.6899	7.5195	0.9788	0.7379	0.9714	Higuchi
27	1.0279	0.8210	0.0134	0.6133	9.3879	0.9411	0.5705	0.9344	Higuchi
28	1.0963	0.9315	0.0180	0.7039	9.6534	0.9925	0.7291	0.9713	Higuchi
29	0.5616	0.9828	0.0229	0.7450	4.8158	0.9933	0.9067	0.9843	Higuchi
30	0.8050	0.9648	0.0226	0.7366	6.9304	0.9826	1.0574	0.9794	Higuchi
Optimized	0.8505	0.9085	0.0178	0.7114	7.5454	0.9827	0.7173	0.9787	Higuchi
Dermovate®	0.8096	0.9061	0.0165	0.6923	7.1991	0.9847	0.6722	0.9704	Higuchi

7.6. CONCLUSIONS

An experimental design such as CCD is useful for the development of topical formulations. This approach allows the successful development of topical dosage forms with a minimum number of experimental runs compared to a traditional formulation development approach. The objectives of this study were to evaluate the effects of certain excipients on the formulation in addition to optimizing a generic CP cream formulation. The use of the CCD allowed for the establishment of high and low limits for composition to expand the experimental domain and to get a better understanding of the product design. Different formulations with varying composition of % v/v propylene glycol, % w/w Gelot® 64, % w/w cetostearyl alcohol and % w/w glyceryl monostearate were manufactured to establish the impact of each excipient on the viscosity, spreadability, pH, electrical conductivity, extrudability, % CP content and *in vitro* release characteristics of the creams. Model fitting using Design-Expert® software (version 8.02, Stat-Ease Inc., Minneapolis, USA) revealed a correlation between formulation variables and cream responses was best described using quadratic polynomial models. Response surface and contour plots were used to examine the interaction effects of the excipients in a graphical manner. Viscosity, spreadability, CP released at 12, 24, 48 and 72 hours proved to be the most significant responses with P-values < 0.05.

The 2-D and 3-D plots confirmed that cetostearyl alcohol was the formulation variable with the most significant effect on the ultimate quality of the cream formulations. An increase in cetostearyl alcohol content increased the viscosity, with a simultaneous decrease in spreadability and cumulative % CP released per unit area. The other formulation variable that seems to have a similar effect was glyceryl monostearate. These two formulation variables have a dual action as they act as emulsifiers and viscosity modifying agents. Propylene glycol was found to have the least effect on formulation responses. The pH of the creams was between 4 and 7 and it is therefore unlikely that any skin irritation would occur during and following application of these formulations. The content uniformity of the thirty batches of product ranged between 98% and 104% and therefore met compendial limits for this parameter [128]. Further statistical analysis of the *in vitro* release data for CP was undertaken to facilitate the selection of an optimized CP cream formulation. Qualitative interpretation and statistical data analysis using the difference, f_1 and similarity, f_2 factors were used to compare the release rate profiles of CP cream formulations to the reference product. The use of model-independent methods to characterize the *in vitro* release profiles of CP from the formulations revealed that batches 5, 26, the predicted and optimized CP formulations released CP at a similar rate and extent to Dermovate®. These formulations consist of approximately 46.0% v/v propylene glycol, 8.6% w/w cetostearyl alcohol, 10.5% w/w glyceryl monostearate and 3.8% w/w Gelot® 64. These batches had an f_1 value of 0.5 and an f_2 value of 99.9, revealing their similarities to Dermovate®.

Mathematical modeling was used to establish which dosage form had a release mechanism for CP that mimicked that of the reference product. The optimized formulation followed Higuchi-type release kinetics with a R^2 value of > 0.99 similar to that of Dermovate® and therefore a diffusion-controlled mechanism appeared to be the dominant factor controlling CP release for this formulation. The use of RSM has been recommended for the establishment of a design space within which product quality can be assured [104]. Therefore the outputs of RSM that were generated were further interrogated to facilitate the establishment of a design space that could be used to ensure that CP cream formulations satisfy product quality criteria specifically in terms of *in vitro* release. The quality standards specified and this approach is further described in Chapter 8 of this thesis.

CHAPTER EIGHT

DEVELOPMENT AND ESTABLISHMENT OF A DESIGN SPACE

8.1. INTRODUCTION

8.1.1. Design Space

The risk assessment and process development experiments lead to an understanding of the effect of process parameters and material attributes on critical quality attributes of a product and facilitate identification of key variables and ranges within which a product of consistent quality can be achieved. The process parameters and material attributes can be selected for inclusion in establishing the design space for the product and process [519]. The design space concept is one of the fundamental paradigms of a QbD framework and is based on the description of which is expected to be one of the results of pharmaceutical development investigations according to a QbD approach [115,519]. The design space concept introduces a revolutionary approach for pharmaceutical development and in the relationship between pharmaceutical companies and regulatory authorities. When a design space is established for a manufacturing process, working within that design space is not considered change. However movements out of the predefined design space are considered to be a change and would normally initiate a regulatory post-approval change process [119]. The design space is therefore considered a final achievement of process understanding in the development of a new product and/or process. However it is likely that the design space would not be constant and it may require updating over the lifecycle of the product as additional knowledge is about the product or process is generated [411,519].

The ICH Q8 (R2) guideline [36] specifies that a design space can also be developed for formulations only, in terms of composition rather than components. For a manufacturing process, independent design spaces for one or more unit operations or a single design space that spans multiple unit operations can be established. A design space can be developed on any scale, however an applicant should justify the importance of the design space that is developed at a small or pilot scale to the proposed production scale manufacturing process and discuss the potential risks that may be evident on scaled-up operations. If the applicant proposes the design space to be applicable to multiple operational scales, the design space should be described in terms of relevant scale-independent parameters [102]. The ICH guidelines provide general indications as to how to describe and establish a design space for different situations. The design space is multivariate *in* nature and is suited for exploring the effects of the attributes of a single material or process parameters in addition to interactions between variables and potential combined effects. Hence multivariate experiments that can highlight possible parameter interactions should be performed to guide identification of such events. Nevertheless, a design space can be described in terms of ranges of material attributes and/or process parameters and in terms of more complex mathematical relationships, time dependent functions, or as a combination of variables such as components of a multivariate model [102,115,519].

Regardless of how a design space is developed, it is expected that operation within that space will result in a product meeting the defined quality criteria. However the term "modeling" in QbD documents is not clearly defined and indeed is often used loosely in discussions of QbD. A number of modeling approaches have been proposed for developing design spaces for pharmaceutical manufacturing and can be divided into three categories *viz.*, **i)** data driven models based solely on extensive statistically-designed experiments, **ii)** semi-empirical approaches using dimensional analysis to develop operating regime maps and scaling rules in which case, the role of experiments is to confirm the design space used to produce a product of acceptable quality and **iii)** first-principle models with which the design of the process at all scales is driven by the model. A limited number of experiments are required to validate the model and perhaps fit a small number of model parameters [520]. The purpose of this work is to establish a design space for a stable high quality bioequivalent CP generic cream formulation that can be used to treat severe eczema and extreme photodermatitis in patients in Southern African infected HIV/AIDS communities. The *in vitro* release performance Dermovate® cream (Sekpharma® Pty Ltd, Gauteng, RSA) was used to define appropriate dosage form performance and to establish the CQA for the generic product.

8.1.2. Design Space Determination

Within the pharmaceutical industry, a conceptual illustration of various spaces *viz.*, knowledge, design and control spaces is used to describe the design space [521,522]. The knowledge space is the space where all information about the impact of formulation and process variables on CQA that are measured, is provided. The design space represents the space within which the quality variables are acceptable, that is the space where product quality satisfies the requirements for safety and efficacy for all combinations in that space. The control space is known as some space, lying within the design space within which companies operate. Control spaces are defined in the CQA space as ± 3σ limits about the target, where the variance σ^2 is based on the common cause variability of a manufacturing process [521]. Clearly in the space for product quality or CQA, it is desirable for the control space to be much smaller than the design space and centered as far as possible from the edges of the design space in all directions. This enables minimization of the probability of any lot of finished product having a combination of properties that lies outside the multivariate specification region as shown in Figure 8.1.

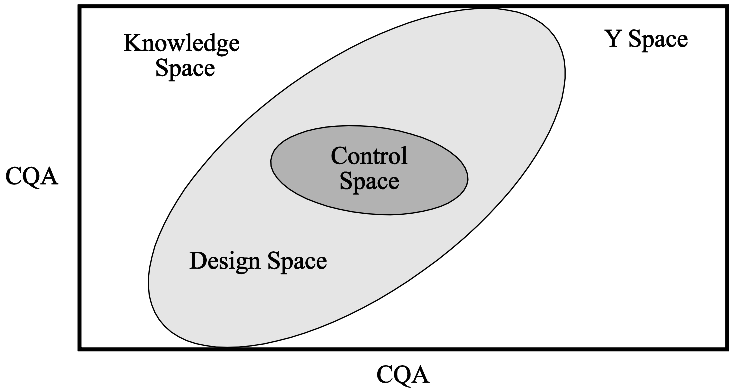


Figure 8.1 Design and control spaces in the space of the CQA, adapted and redrawn from [521]

8.1.3. Presentation of Design Space

The ICH Q8 (R2) guideline [36] proposes the use of interaction, contour and response surface plots to present a design space. The design space can be established through proper characterization techniques and is often an extrapolation from the response surface. Two-dimensional contour and three-dimensional response surface plots are typically used in the visualization of a design space. Visualization, documentation and communication of the design space help to assure that the product and/or process set points are well defined and are within safe and robust operating regions. The design space should include both material and process parameter relationships to permit acceptance of the CQA [522].

A combination of multivariate permutations of interactions and response surface plots may need to be used to understand a specific design space completely. For instance, a combination of interaction and contour plots generated from a Box-Behnken experimental design were used to highlight the relationship between formulation variables and responses for the optimization of stomach specific delivery of rifampicin [418]. Contour plots were also used to determine the design space for drug encapsulation efficiency since this is one of the most important as well as the most difficult properties to predict and control for liposomes containing hydrophilic APIs [523]. Response surface and contour plots were used to identify the effect of solvent type and atomization parameters on quality attributes of spray dried polymer-based solid-dispersions and these plots proved to be useful tools to visualize the effects in detail [524]. Box-Behnken and CCD experimental designs are sometimes inappropriate for Artificial Neural Network (ANN) modeling of highly curved responses and extensive internal mapping of the design space can be undertaken using a pseudo-random design in order to generate predictive ANN models and to map the interior of a design space [525].

8.2. METHODS

8.2.1. Response Surface Methodology

Design-Expert® software (version 8.02, Stat-Ease Inc., Minneapolis, USA) was used for the formulation optimization of CP cream, as described in Chapter 7 *vide infra*. The *in vitro* release profile of Dermovate® (Sekpharma® Pty Ltd, Gauteng, RSA) was used to establish the constraints and to determine the level of each excipient that would result in the manufacture of a cream formulation that was similar to Dermovate® in terms of formulation responses. The impact of the levels of % v/v propylene glycol, % w/w Gelot® 64, % w/w cetostearyl alcohol and % w/w glyceryl monostearate on the extent of CP released from the cream formulations at different time intervals was determined from the resultant constraint plots.

8.2.2. Manufacture of Design Space Cream Formulations

The manufacturing procedure used to produce the cream was generated from the design space network is described in § 6.2.2, Chapter 6. The cream formulations were manufactured depending on the excipient limits that may show bioequivalence to Dermovate®. Only one excipient content changed in a formulation while the other excipient contents remained the same (Appendix VI).

8.2.3. *In Vitro* Analysis of Design Space Formulations

Following manufacture, the creams manufactured from the design space network were evaluated in terms of their *in vitro* release to determine the performance of formulations. The analyses of the cream formulations were performed as described in § 6.2.3, Chapter 6. The formulation response values for batches DS-CP-01 – DS-CP-08 are included in Appendix VII, together with the batch summary records for these batches.

8.2.4. Stability Studies on Design Space Formulations

Stability studies on the formulations obtained from the design space network were conducted using two Model KBF-240 Binder® climatic chambers (Binder GmbH® Ltd, Munchen, Germany) at two different settings *viz.*, 40 ± 2°C, 75 ± 5% RH and 25 ± 2°C, 60 ± 5% RH. A week prior to use, the stability chambers were allowed to equilibrate to the temperature and relative humidity conditions set for these studies. Five aluminium collapsible cream tubes containing 50 g of cream were placed in each equilibrated chamber. At 1, 2, 4 and 8 weeks, a 50 g sample was removed from each chamber and immediately analyzed in terms of the following test parameters including viscosity, spreadability, pH, extrudability, electrical conductivity, % CP content and % CP released from these topical formulations. The confidence intervals were calculated for the *in vitro* release over 72 hours used as described by Timm *et al*. [405]. The percentage difference in this response between the initial sample at time 0 week and stored samples *viz.,* at times 1, 2, 4 and 8 weeks were calculated and used to construct a 90% C.I. The data are reported as confidence intervals depicting the percentage change (Δ) from the initial reading for the CP *in vitro* release rates from the formulations (n=3).

8.3. ESTABLISHMENT OF A DESIGN SPACE

8.3.1. Design Space for CP Cream Formulations

Constraints plots were used to determine the regions of the experimental domain that corresponded to the limits of the *in vitro* release profile of the reference product. The blue curves in the constraint plots indicate the change in the extent of CP release at each stage of the *in vitro* test and the red curves represent the constraints within which the quality of the product will be maintained. The impact of each of the formulation variables that were investigated and described in Chapter 7 on the different stages of *in vitro* release test are shown in Figures 8.2 - 8.7 respectively.

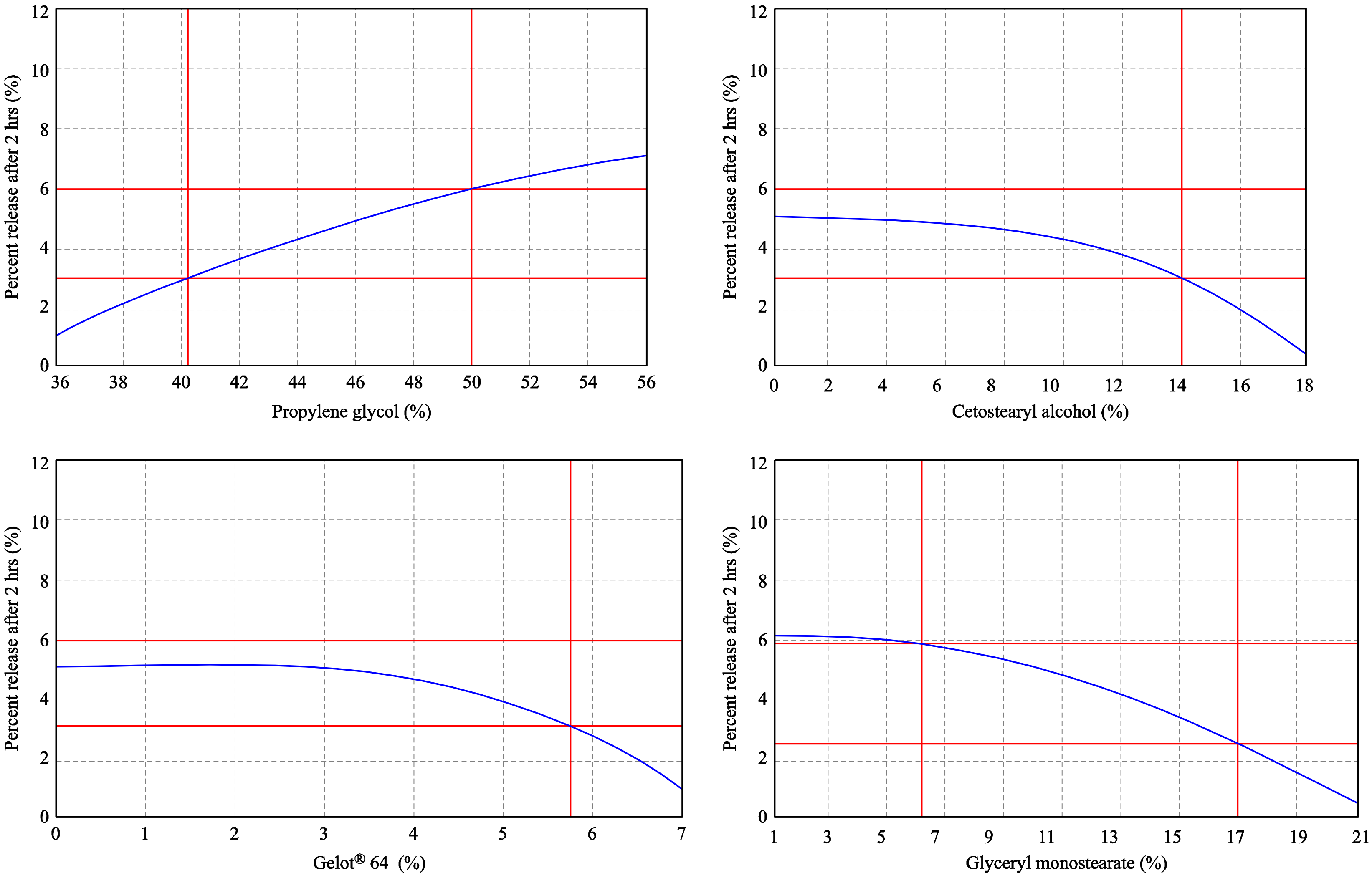


Figure 8.2 Constraint plots for percent CP released after 2 hours from cream formulations

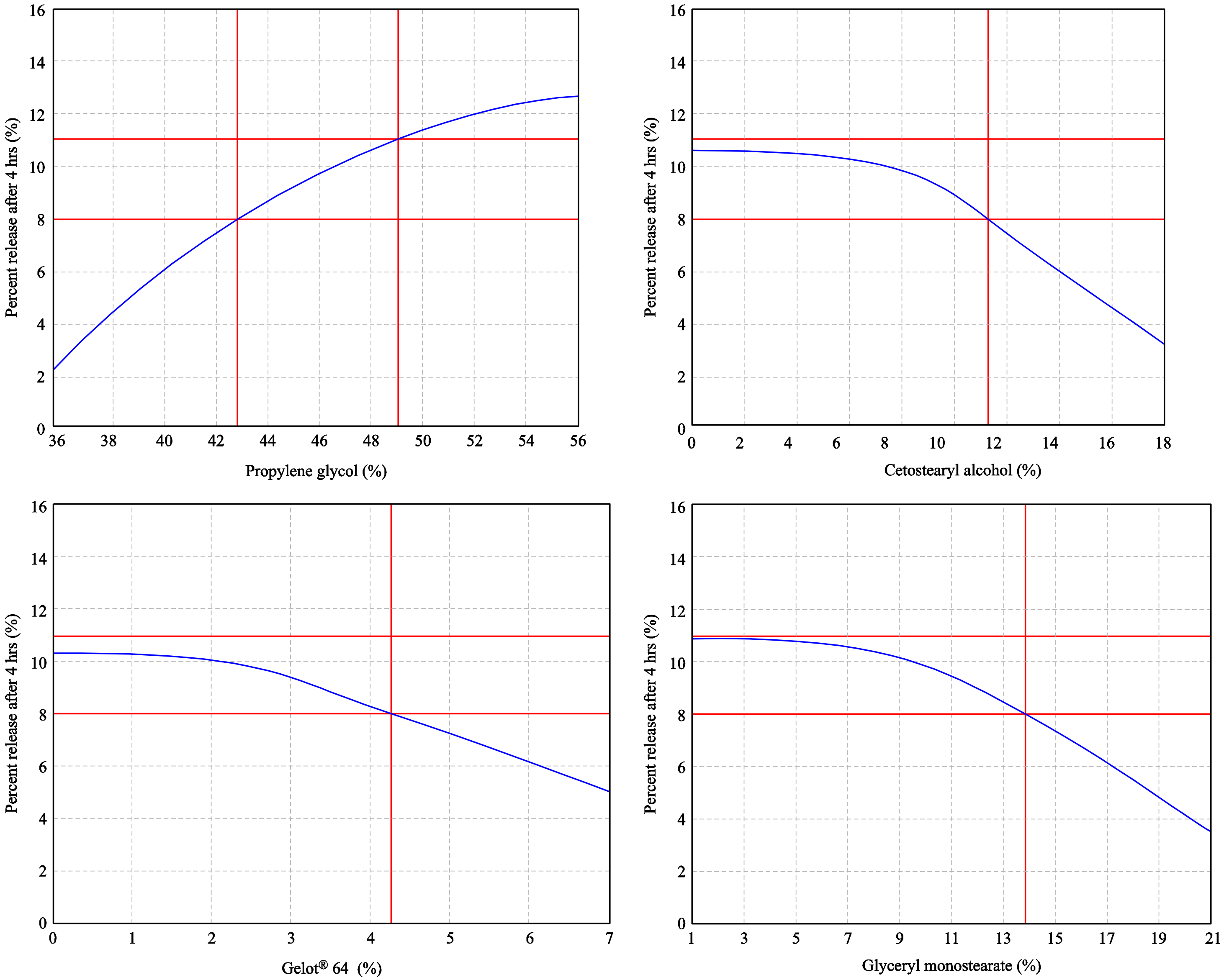


Figure 8.3 Constraint plots for percent CP released after 4 hours from cream formulations

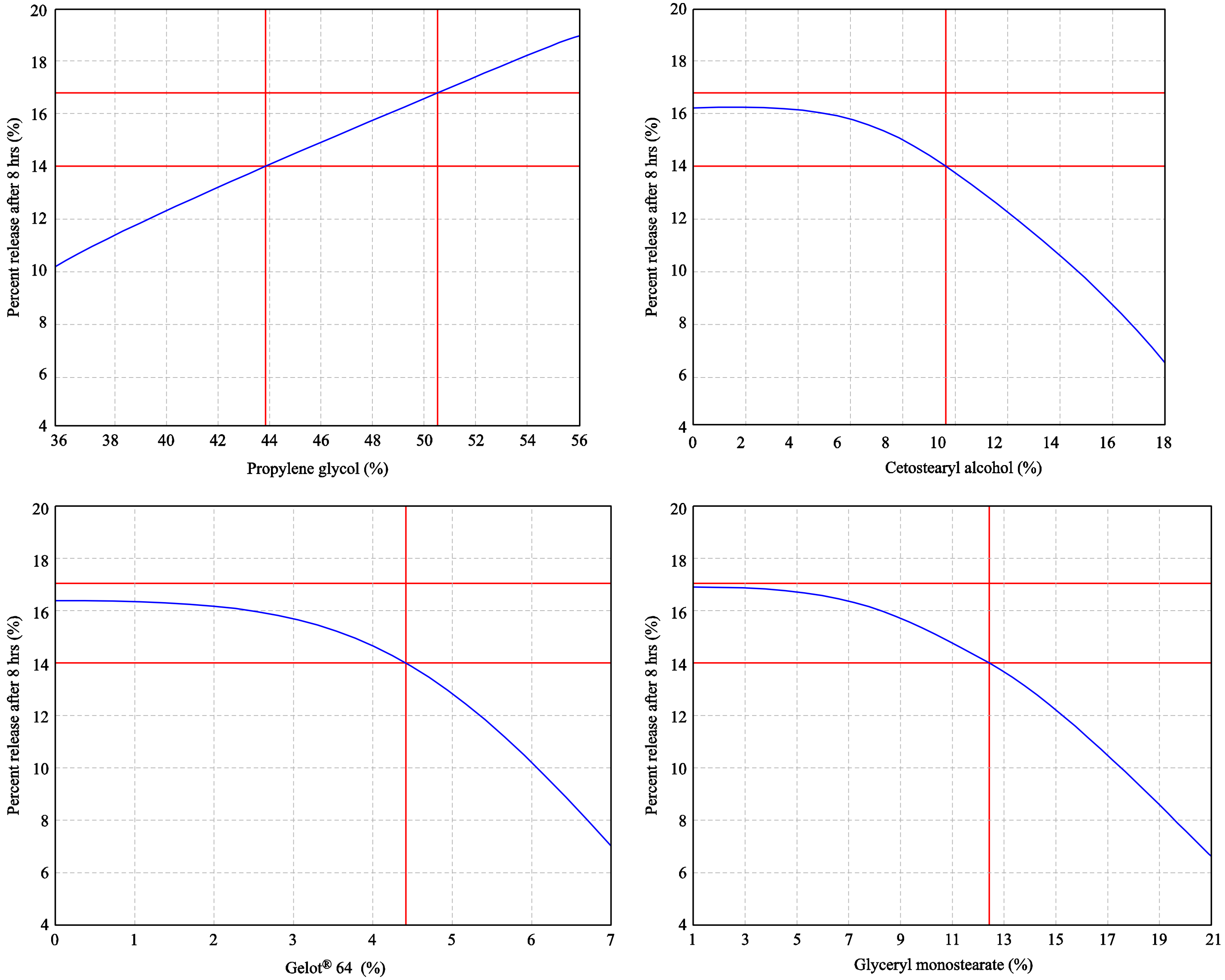


Figure 8.4 Constraint plots for percent CP released after 8 hours from cream formulations

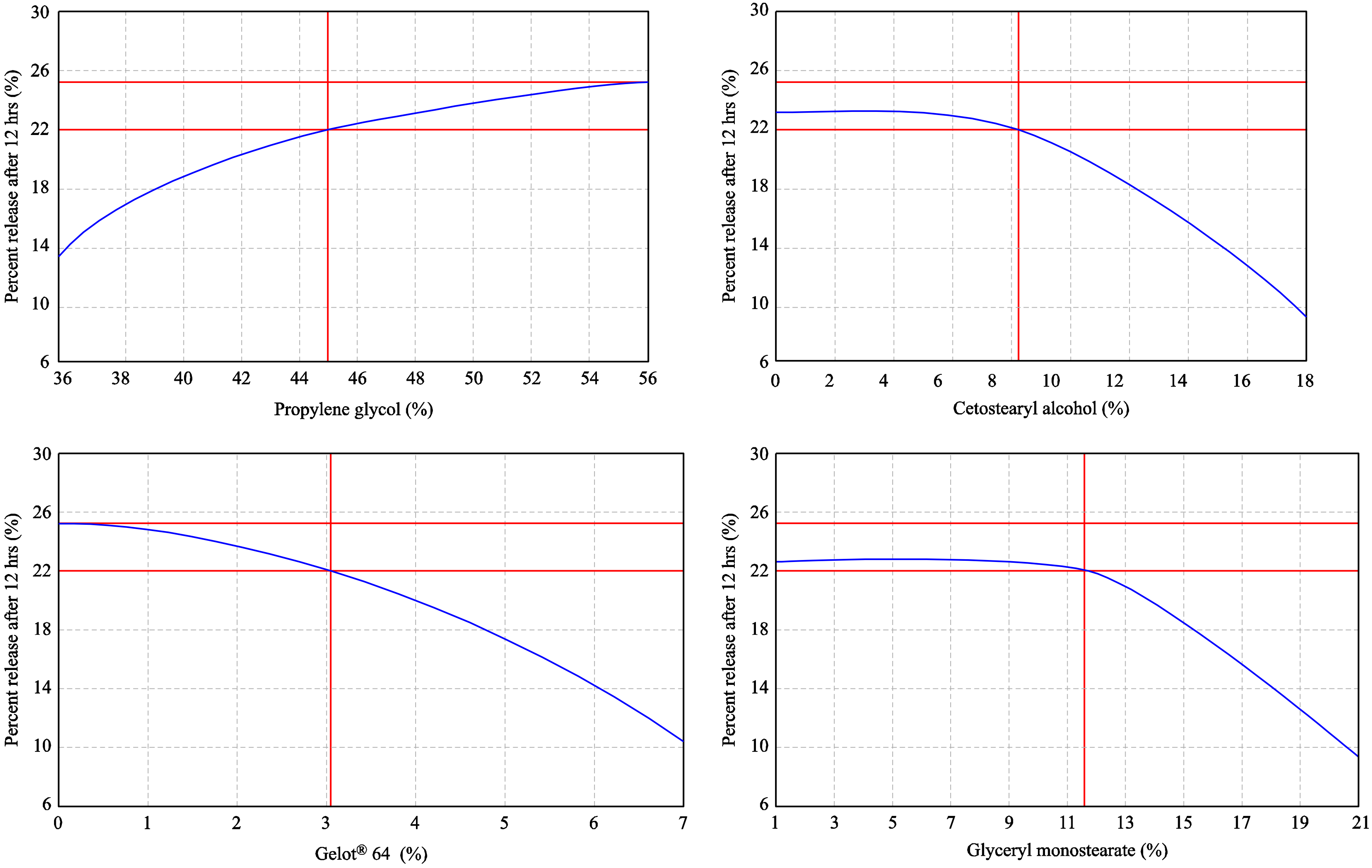


Figure 8.5 Constraint plots for percent CP released after 12 hours from cream formulations

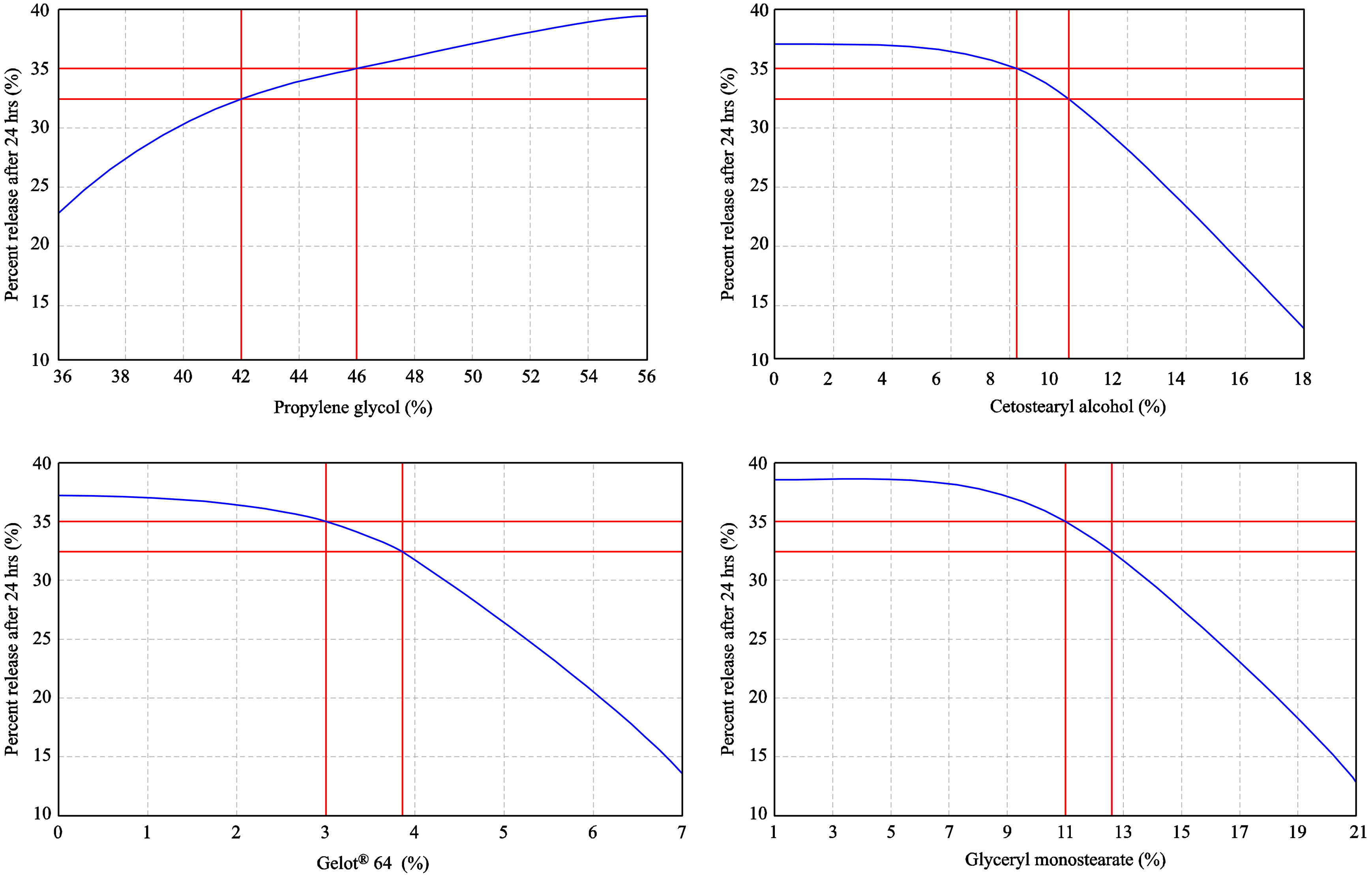


Figure 8.6 Constraint plots for percent CP released after 24 hours from cream formulations

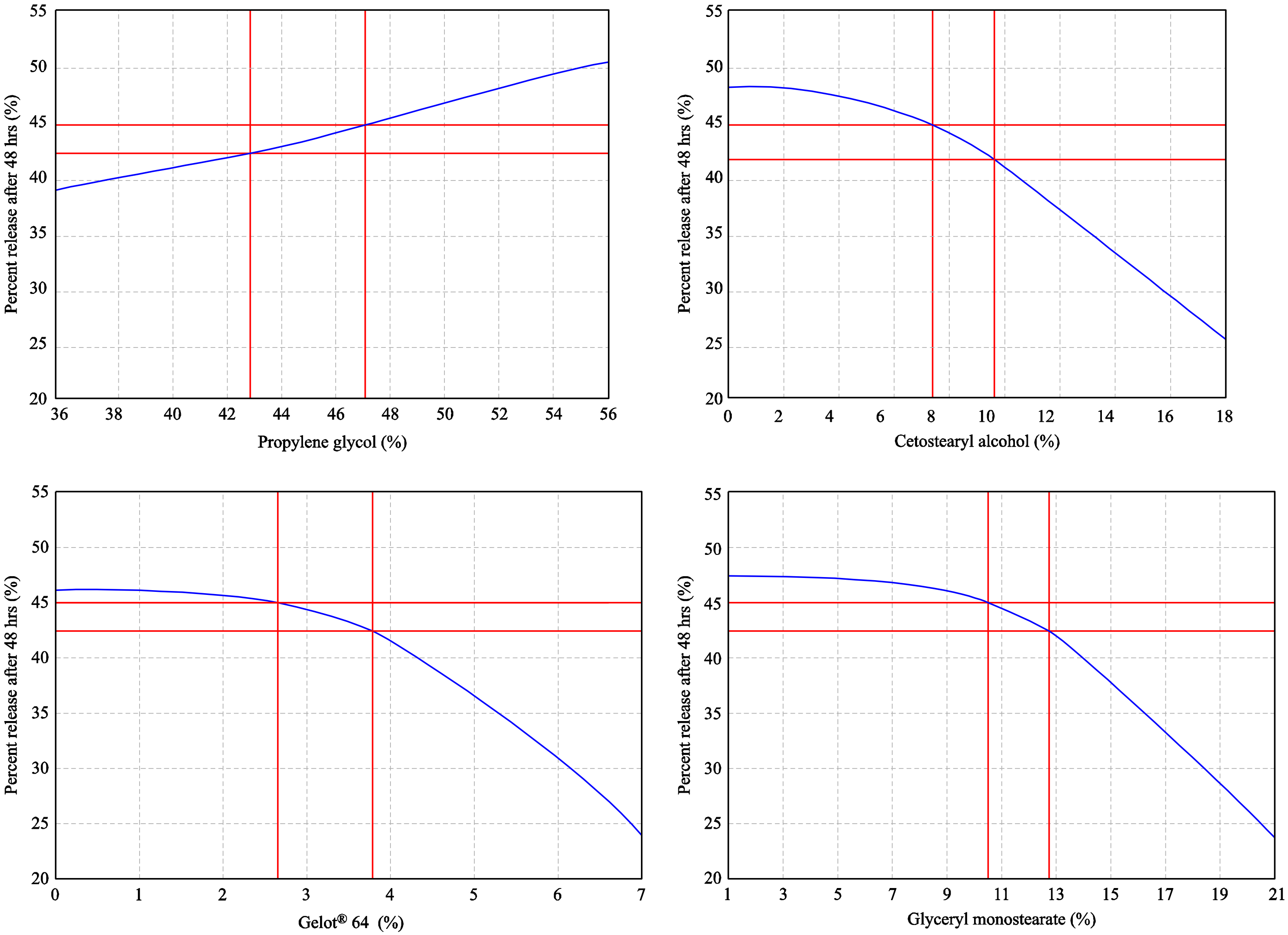


Figure 8.7 Constraint plots for percent CP released after 48 hours from cream formulations

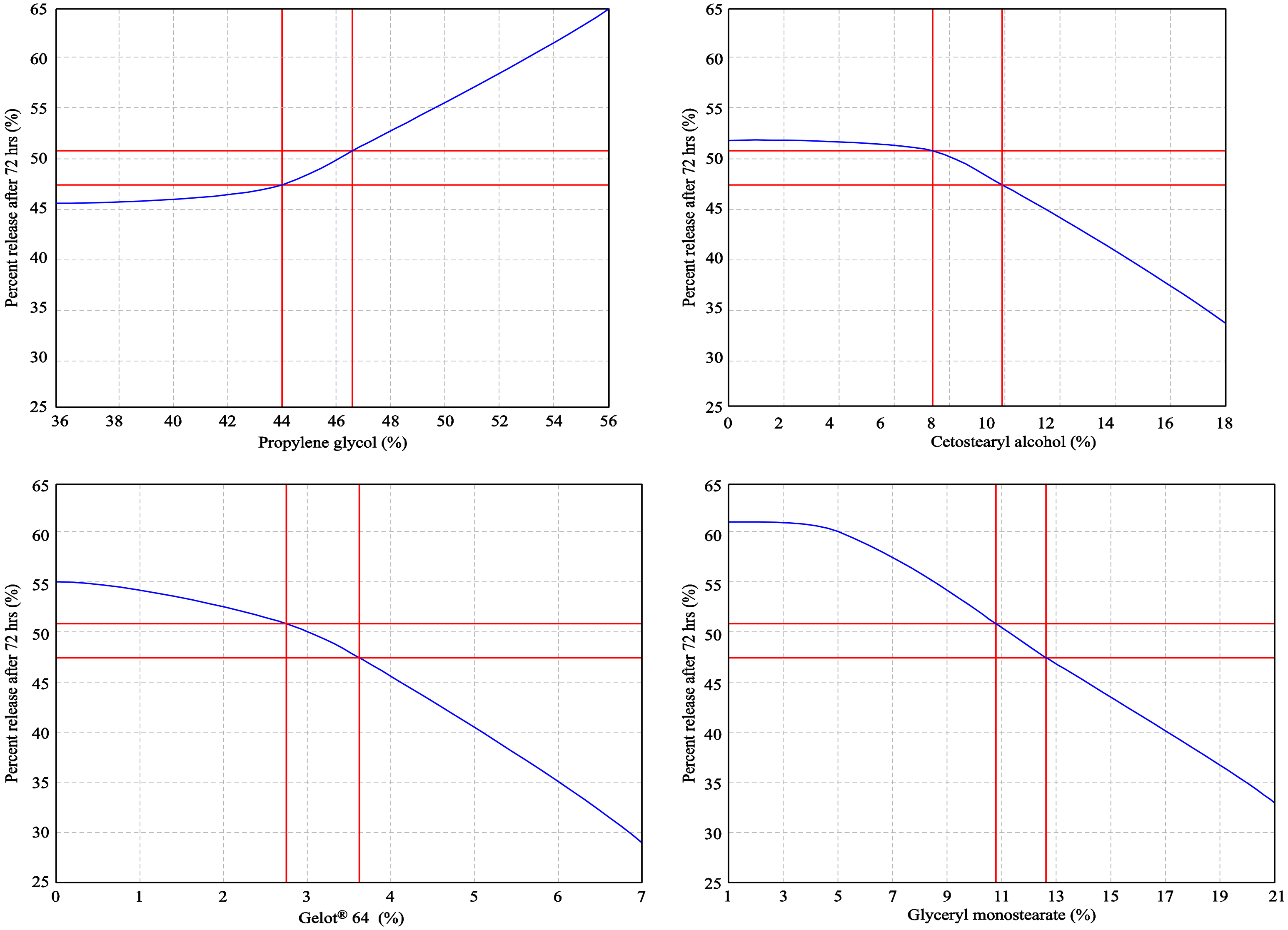


Figure 8.8 Constraint plots for percent CP released after 72 hours from cream formulations

The design space limit values were obtained from analyzing each individual constraint plot at different *in vitro* release times. A lower and upper limit was attained from each plot and a summary of the formulation constraints for the optimized CP cream formulation that was developed following assessment of Figures 8.2 - 8.7 are listed in Table 8.1.

Table 8.1 Established Design Space Limits Following RSM Evaluation

	Propylene Glycol (% v/v)		**Gelot®64 (% w/w)**		**Cetostearyl Alcohol (% w/w)**		**Glyceryl Monostearate (% w/w)**	
	Lower Limit	**Upper Limit**	**Lower Limit**	**Upper Limit**	**Lower Limit**	**Upper Limit**	**Lower Limit**	**Upper Limit**
$\mathbf{B_{2hr}}$	40.5	50.0	0	5.9	0	14.0	6.0	17.0
$\mathbf{B_{4hr}}$	43.0	49.0	0	4.2	0	11.4	0	13.8
$\mathbf{B_{8hr}}$	44.0	50.3	0	4.5	0	9.4	3.5	12.5
$\mathbf{B_{12hr}}$	36.0	45.8	0	3.2	0	8.4	0	11.4
$\mathbf{B_{24hr}}$	42.0	46.0	3.0	3.8	8.0	10.0	11.0	12.8
$\mathbf{B_{48hr}}$	43.0	46.8	2.7	3.8	7.5	9.8	10.8	12.8
$\mathbf{B_{72hr}}$	44.0	46.4	2.9	3.8	7.5	10.0	10.9	12.8

From Table 8.1, it was apparent that during the early stages of *in vitro* testing, the projected lower limit levels of Gelot® 64, cetostearyl alcohol and glyceryl monostearate are extremely low and in some cases even zero. This might be due to a lag phase prior to the release of CP. The lag time therefore corresponds to a delay in the movement of CP in the cream and subsequent release from the dosage form. Consequently diffusion across the synthetic membrane is not the rate-limiting step in release characterization. It was observed that at the cumulative CP released after 24, 48 and 72 hours showed limits that were similar and the formulation limits can only be maintained within certain ranges of excipient composition in the formulation design. It was evident that a specific excipient has a narrow limit range for instance; Gelot® 64 had a narrow range of between 2.9% w/w and 3.8% w/w whereas that for cetostearyl alcohol ranged between 7.5% w/w and 10.0% w/w. These limits that have been identified can therefore be considered to be the limits of the design space for formulation composition for these materials in the composition of the cream formulation that was manufactured in this study. The 72 hour design space limit was taken into further consideration by manufacturing eight cream formulations of different formulation compositions at the lower and upper limits in order to assess bioequivalence of these dosage forms. The composition of the eight design space formulations selected is summarized in Table 8.2.

Table 8.2 Percentage composition (% w/w) of design space cream formulations manufactured and tested

No.	Excipients	Dermovate®	01	02	03	04	05	06	07	08
1	CP	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
2	Propylene glycol	47.50	44.00	46.40	46.10	46.10	46.10	46.10	46.10	46.10
3	Sodium citrate	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
4	Citric acid	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
5	Arlacel® 165	1.50	-	-	-	-	-	-	-	-
6	Gelot® 64	-	3.80	3.80	2.90	3.80	3.80	3.80	3.80	3.80
7	Glyceryl monostearate	11.00	10.50	10.50	10.50	10.50	10.50	10.50	10.90	12.80
8	Cetostearyl alcohol	8.40	8.60	8.60	8.60	8.60	7.50	10.00	8.60	8.60
9	White beeswax	1.15	1.15	1.15	1.15	1.15	1.15	1.15	1.15	1.15
10	Chlorocresol	0.075	0.075	0.075	0.075	0.075	0.075	0.075	0.075	0.075
11	Distilled water	30.225	30.225	30.225	30.225	30.225	30.225	30.225	30.225	30.225

8.3.2. *In Vitro* Release and Assessment of Design Space CP Creams

In vitro analysis was performed on the eight design space formulations using Franz diffusion cells in § 6.2.2 of Chapter 6. All *in vitro* analysis was performed in triplicate (n=3) and the *in vitro* release profiles of the design space cream formulations and that of Dermovate® are shown in Figures 8.9 – 8.12.

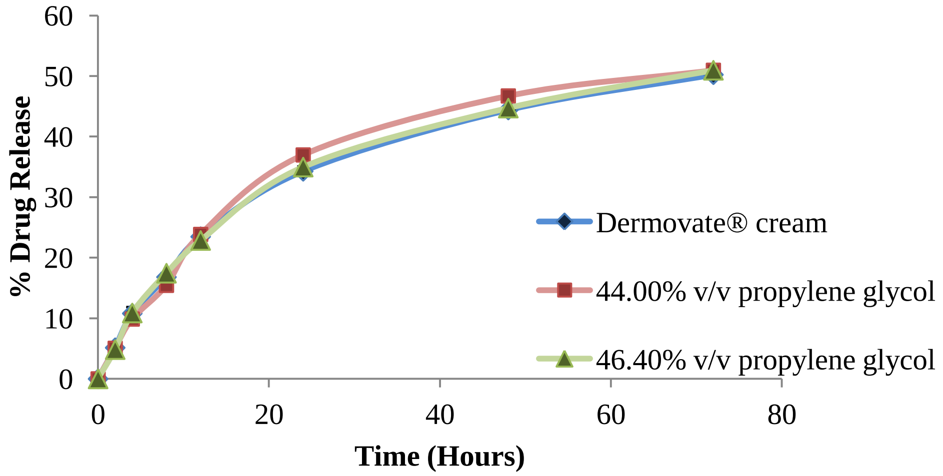


Figure 8.9 *In vitro* release profiles for the design space formulations containing acceptable lower and upper limits of propylene glycol

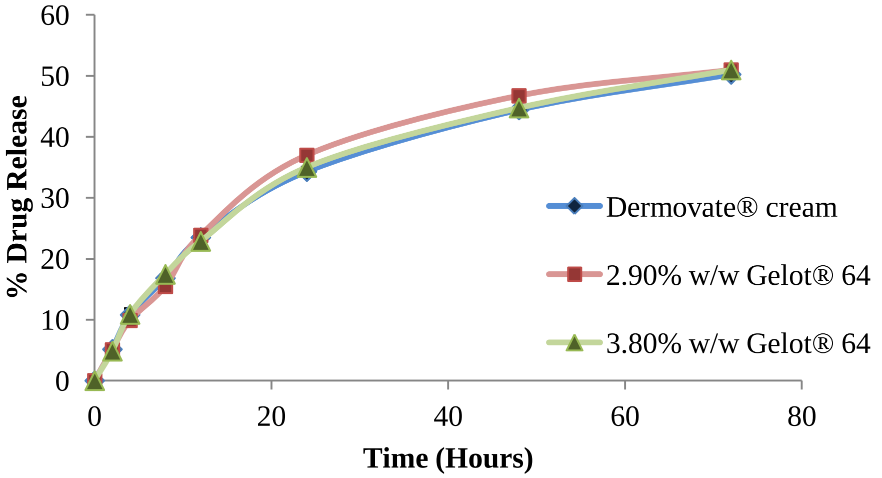


Figure 8.10 *In vitro* release profiles for the design space formulations containing acceptable lower and upper limits of Gelot® 64

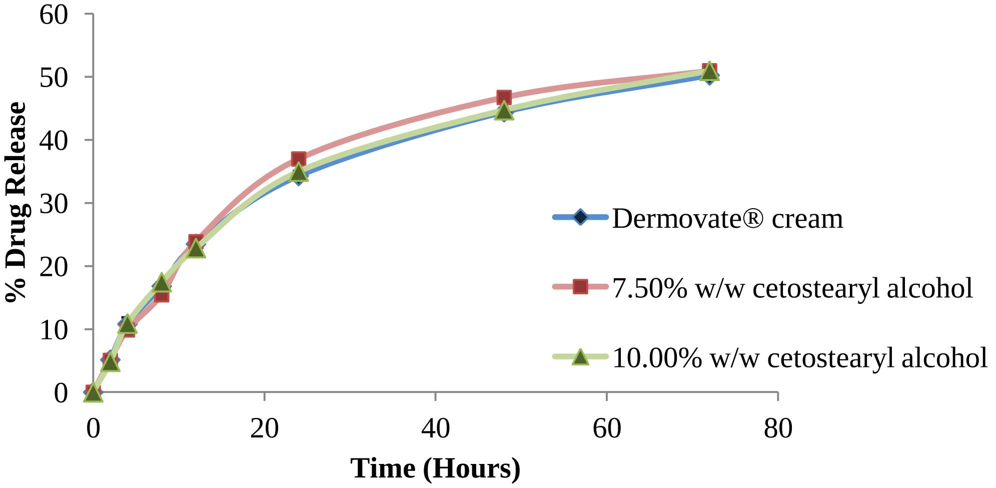


Figure 8.11 *In vitro* release profiles for the design space formulations containing acceptable lower and upper limits of cetostearyl alcohol

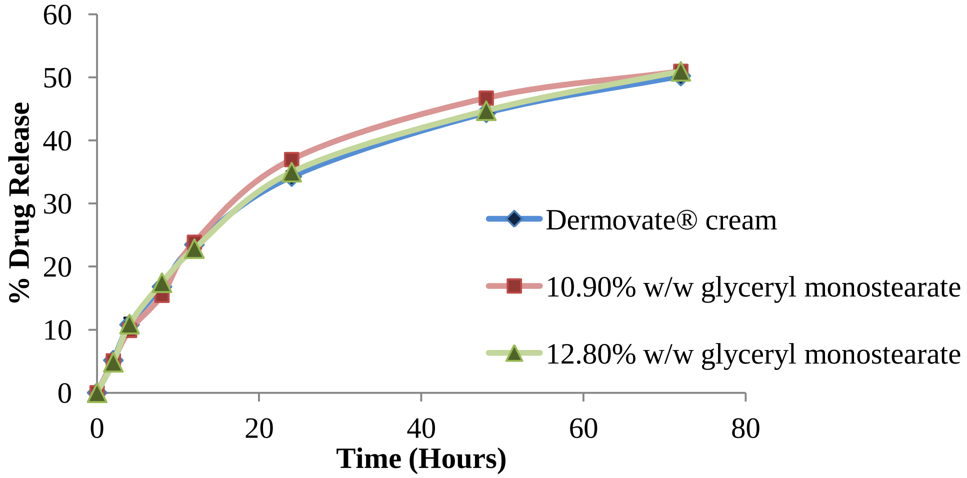


Figure 8.12 *In vitro* release profiles for the design space formulations containing acceptable lower and upper limits of glyceryl monostearate

The cumulative percent CP released from eight test formulations established from the design space was similar to that observed for Dermovate®. The *in vitro* release curves of Dermovate® and eight design space formulations were virtually superimposable. The difference and similarity factors (f_1, f_2, S_d) were used to compare the *in vitro* release profiles generated for CP from cream formulations of different composition and are summarized in Table 8.3.

Table 8.3 f_1, f_2 and S_d values for design space and Dermovate® creams

Comparison*	f_1	f_2	S_d
01	1.5	99.2	0.0001
02	0.5	99.9	0.0001
03	0.6	99.7	0.0001
04	0.5	99.9	0.0001
05	1.5	99.2	0.0001
06	4.5	94.1	0.0001
07	1.5	99.2	0.0001
08	1.5	99.1	0.0001

***Comparison = Dermovate® cream vs design space cream formulations (01-08)**

Consideration of the difference factor alone reveals that CP release from all the eight batches were similar to that of Dermovate® with f_1 values < 15 for each comparison. However batches 02 and 03 with a 46.40% v/v and 46.10% v/v PG showed the least difference for the *in vitro* release profile compared to Dermovate® as the f_1 factor is close to zero. Batch 06 showed the greatest value for f_1 but was still found to be similar to the *in vitro* release profile of the reference product with a value of 4.5 which is well below the limit of 15. The similarity factor for the comparison of the cream formulations established by the design space indicates that all batches showed similarity in their *in vitro* release profiles with f_2 and S_d values > 50 and close to zero. However, an f_2 value of close to 100 indicates that the *in vitro* profiles of a test and reference product are identical. The release profiles for batches 02 and 04 were most similar to that of Dermovate® with f_2 and S_d values of 99.9 and 0.0001.

8.3.3. Stability Assessment of Design Space CP creams

The eight CP creams formulations obtained from the design space network subjected to stability conditions *viz.*, 40 ± 2°C, 75 ± 5% RH and 25 ± 2°C, 60 ± 5% RH were evaluated in terms of viscosity, spreadability, pH, % CP content, extrudability, electrical conductivity and % CP released from these formulations over a period of 72 hours. The stability data are summarized in Tables 8.4 – 8.7.

Table 8.4 Stability data generated at 40 ± 2°C, 75 ± 5% RH and 25 ± 2°C, 60 ± 5% RH for the viscosity and spreadability of the design space cream formulations for a period of eight weeks

Sampling Time (Weeks)	**40 ± 2°C, 75 ± 5 % RH; Viscosity ± SD (cP)**								**40 ± 2°C, 75 ± 5% RH; Spreadability ± SD (cm^2)**							
	01	**02**	**03**	**04**	**05**	**06**	**07**	**08**	**01**	**02**	**03**	**04**	**05**	**06**	**07**	**08**
1	43 555 ± 896	43 100 ± 782	43 200 ± 1258	44 860 ± 2653	44 890 ± 567	42 890 ± 2567	43 120 ± 1892	43 650 ± 658	23.50 ± 1.86	22.25 ± 1.56	24.05 ± 1.56	24.87 ± 2.56	24.86 ± 2.83	24.52 ± 2.16	24.68 ± 1.23	24.05 ± 1.17
2	44 390 ± 1099	44 050 ± 2568	44 890 ± 1365	44 950 ± 782	43 836 ± 2895	42 562 ± 1607	44 180 ± 986	43 000 ± 968	23.31 ± 2.78	22.96 ± 1.83	24.45 ± 0.12	23.99 ± 0.58	24.00 ± 0.78	23.78 ± 2.36	25.78 ± 0.25	24.00 ± 0.23
4	44 000 ± 892	43 250 ± 894	44 100 ± 458	43 560 ± 756	42 450 ± 562	43 100 ± 1230	44 200 ± 1035	43 000 ± 452	23.00 ± 1.85	23.12 ± 1.08	23.26 ± 1.65	23.00 ± 1.78	24.96 ± 2.58	24.89 ± 1.84	24.10 ± 0.50	23.86 ± 0.85
8	42 500 ± 1056	44 050 ± 892	43 562 ± 785	42 890 ± 1056	43 780 ± 1789	43 050 ± 759	43 890 ± 1230	43 000 ± 985	22.89 ± 0.85	21.32 ± 1.25	24.00 ± 1.02	22.78 ± 1.23	23.85 ± 0.58	22.36 ± 2.56	24.99 ± 0.55	23.55 ± 2.50
Sampling Time (Weeks)	**25 ± 2°C, 60 ± 5% RH; Viscosity ± SD (cP)**								**25 ± 2°C, 60 ± 5% RH; Spreadability ± SD (cm^2)**							
	01	**02**	**03**	**04**	**05**	**06**	**07**	**08**	**01**	**02**	**03**	**04**	**05**	**06**	**07**	**08**
1	44 000 ± 2779	44 050 ± 1230	43 890 ± 2365	45 200 ± 1364	44 560 ± 1569	42 689 ± 687	44 200 ± 1785	43 650 ± 452	23.85 ± 1.52	23.26 ± 0.69	24.25 ± 0.56	24.96 ± 0.23	25.56 ± 2.33	24.12 ± 1.96	25.63 ± 2.96	24.45 ± 1.52
2	44 120 ± 1699	44 860 ± 1607	45 190 ± 1052	44 950 ± 3620	44 152 ± 895	43 986 ± 1365	44 200 ± 1025	43 000 ± 789	24.86 ± 0.70	23.85 ± 0.95	24.63 ± 2.22	24.00 ± 1.85	24.99 ± 1.56	24.86 ± 0.89	24.89 ± 3.86	24.50 ± 0.78
4	44 960 ± 563	44 560 ± 892	44 100 ± 1896	44 890 ± 789	44 892 ± 1536	44 652 ± 1458	43 200 ± 563	43 800 ± 1052	23.00 ± 0.85	23.99 ± 1.58	24.96 ± 2.85	24.12 ± 1.89	25.76 ± 0.88	24.58 ± 0.86	25.12 ± 0.78	24.00 ± 0.25
8	43 780 ± 1020	43 850 ± 1258	43 890 ± 569	43 920 ± 1028	43 000 ± 892	44 050 ± 589	42 900 ± 968	43 780 ± 1075	22.99 ± 0.27	23.52 ± 0.89	23.89 ± 2.78	23.12 ± 0.89	24.00 ± 2.56	23.78 ± 1.78	24.02 ± 0.75	23.29 ± 1.50

Table 8.5 Stability data generated at 75% RH, 40°C and 60% RH, 25°C for the % drug content and pH of the design space cream formulations for a period of eight weeks

Sampling Time (Weeks)	40 ± 2°C, 75 ± 5% RH; % CP Content ± SD (%)								40 ± 2°C, 75 ± 5% RH; pH ± SD							
	01	**02**	**03**	**04**	**05**	**06**	**07**	**08**	**01**	**02**	**03**	**04**	**05**	**06**	**07**	**08**
1	99.23 ± 0.24	99.02 ± 0.85	100.18 ± 0.84	102.96 ± 0.56	98.78 ± 0.56	99.23 ± 1.26	100.00 ± 1.56	98.45 ± 2.12	5.92 ± 0.01	5.94 ± 0.01	6.00 ± 0.02	6.01 ± 0.10	6.10 ± 0.02	6.00 ± 0.07	5.99 ± 0.02	6.01 ± 0.02
2	100.64 ± 0.78	99.87 ± 2.96	101.78 ± 0.78	101.00 ± 0.78	99.75 ± 2.78	98.12 ± 1.45	100.89 ± 1.25	99.00 ± 2.10	6.01 ± 0.01	6.02 ± 0.03	6.01 ± 0.05	6.01 ± 0.78	6.12 ± 0.05	6.02 ± 0.12	6.02 ± 0.02	6.01 ± 0.04
4	99.45 ± 1.70	100.02 ± 0.75	100.56 ± 0.77	101.72 ± 0.75	99.23 ± 2.58	99.54 ± 2.15	99.12 ± 1.11	100.86 ± 0.12	6.12 ± 0.03	6.15 ± 0.02	6.12 ± 0.02	6.02 ± 0.25	6.20 ± 0.05	6.00 ± 0.01	6.01 ± 0.05	6.01 ± 0.03
8	99.00 ± 1.11	99.85 ± 2.15	99.99 ± 1.89	100.15 ± 1.75	98.25 ± 0.75	98.20 ± 1.89	99.00 ± 1.02	99.12 ± 2.17	6.02 ± 0.04	6.00 ± 0.03	5.99 ± 0.01	6.00 ± 0.05	6.10 ± 0.01	5.98 ± 0.04	6.00 ± 0.01	6.00 ± 0.01
Sampling Time (Weeks)	**25 ± 2°C, 60 ± 5% RH; % CP Content ± SD (%)**								**25 ± 2°C, 60 ± 5% RH; pH ± SD**							
	01	**02**	**03**	**04**	**05**	**06**	**07**	**08**	**01**	**02**	**03**	**04**	**05**	**06**	**07**	**08**
1	100.99 ± 0.21	100.34 ± 2.36	101.25 ± 1.52	101.96 ± 1.85	99.78 ± 1.26	100.12 ± 0.56	100.63 ± 0.85	100.45 ± 0.79	6.00 ± 0.04	6.02 ± 0.02	6.03 ± 0.01	6.02 ± 0.05	6.05 ± 0.05	6.13 ± 0.05	6.10 ± 0.01	6.03 ± 0.02
2	100.25 ± 0.59	99.85 ± 2.96	100.75 ± 0.18	100.75 ± 2.15	100.24 ± 0.58	99.86 ± 0.14	101.89 ± 0.23	100.00 ± 1.23	6.04 ± 0.01	6.05 ± 0.04	6.01 ± 0.01	6.09 ± 0.05	6.05 ± 0.02	6.01 ± 0.03	6.02 ± 0.01	6.03 ± 0.03
4	101.23 ± 1.70	100.89 ± 1.50	101.22 ± 2.55	100.25 ± 1.56	101.25 ± 3.25	100.89 ± 0.33	100.12 ± 0.14	100.86 ± 1.56	6.15 ± 0.02	6.01 ± 0.01	6.20 ± 0.05	6.01 ± 0.01	6.15 ± 0.03	6.05 ± 0.01	6.05 ± 0.01	6.10 ± 0.03
8	99.99 ± 1.25	99.78 ± 2.01	100.41 ± 0.12	99.85 ± 0.99	100.01 ± 2.15	99.15 ± 2.01	100.00 ± 1.22	99.86 ± 2.12	6.00 ± 0.04	5.99 ± 0.02	6.00 ± 0.05	5.98 ± 0.02	6.10 ± 0.05	6.07 ± 0.05	5.99 ± 0.02	6.00 ± 0.01

Table 8.6 Stability data generated at 75% RH, 40°C and 60% RH, 25°C for the extrudability and electrical conductivity of the design space cream formulations for a period of eight weeks

Sampling Time (Weeks)	**40 ± 2°C, 75 ± 5% RH; Extrudability ± SD (g/cm^2)**								**40 ± 2°C, 75 ± 5% RH; Electrical Conductivity ± SD (μS/cm)**							
	01	**02**	**03**	**04**	**05**	**06**	**07**	**08**	**01**	**02**	**03**	**04**	**05**	**06**	**07**	**08**
1	77.52 ± 1.56	78.56 ± 0.16	76.25 ± 1.34	75.12 ± 1.52	76.05 ± 0.37	75.35 ± 5.35	77.99 ± 2.51	77.99 ± 1.52	198.81 ± 9.36	200.02 ± 2.54	198.12 ± 6.34	198.12 ± 8.52	201.05 ± 10.25	198.35 ± 7.35	202.86 ± 10.58	198.00 ± 6.52
2	77.78 ± 1.58	77.39 ± 0.27	76.89 ± 0.26	75.92 ± 0.89	75.10 ± 1.25	77.48 ± 5.53	76.99 ± 1.56	77.78 ± 1.25	198.07 ± 3.18	199.12 ± 2.52	198.99 ± 9.26	198.92 ± 9.89	198.10 ± 8.89	195.48 ± 9.53	200.87 ± 9.56	199.25 ± 4.75
4	76.96 ± 1.85	78.12 ± 1.02	76.22 ± 0.85	76.24 ± 0.12	76.20 ± 1.45	76.28 ± 2.58	76.54 ± 0.89	76.85 ± 1.14	196.78 ± 4.35	198.00 ± 5.23	198.53 ± 9.85	199.22 ± 8.12	198.20 ± 7.33	198.28 ± 9.58	201.23 ± 7.89	198.35 ± 7.52
8	75.98 ± 2.78	76.10 ± 1.00	75.99 ± 1.56	76.00 ± 2.32	75.99 ± 1.85	75.28 ± 2.36	77.00 ± 1.85	75.75 ± 2.15	197.26 ± 5.15	197.99 ± 8.89	197.00 ± 7.23	198.00 ± 10.52	197.00 ± 8.56	196.23 ± 10.26	199.56 ± 8.33	197.22 ± 8.75
Sampling Time (Weeks)	**25 ± 2°C, 60 ± 5% RH; Extrudability ± SD (g/cm^2)**								**25 ± 2°C, 60 ± 5% RH; Electrical Conductivity ± SD (μS/cm)**							
	01	**02**	**03**	**04**	**05**	**06**	**07**	**08**	**01**	**02**	**03**	**04**	**05**	**06**	**07**	**08**
1	77.85 ± 1.21	77.02 ± 0.96	77.25 ± 1.54	75.00 ± 1.44	75.00 ± 0.52	76.37 ± 1.45	77.81 ± 1.58	78.03 ± 1.52	199.99 ± 7.52	202.78 ± 8.16	200.84 ± 3.34	199.02 ± 6.21	200.05 ± 7.12	199.35 ± 5.35	199.86 ± 7.58	197.00 ± 7.45
2	78.56 ± 0.58	76.55 ± 1.27	76.99 ± 1.45	75.12 ± 1.58	75.25 ± 1.45	76.45 ± 3.23	76.89 ± 3.56	79.03 ± 2.75	199.04 ± 4.25	201.54 ± 4.33	199.99 ± 4.52	198.78 ± 7.85	200.12 ± 2.15	199.48 ± 7.53	198.87 ± 3.54	197.25 ± 5.25
4	77.00 ± 1.45	78.00 ± 1.12	76.77 ± 0.25	75.22 ± 0.85	75.20 ± 1.00	76.08 ± 1.08	77.23 ± 2.89	78.10 ± 1.52	198.58 ± 9.58	201.00 ± 6.14	199.23 ± 8.52	200.45 ± 5.14	201.25 ± 5.12	199.28 ± 9.58	199.23 ± 2.58	199.35 ± 7.02
8	76.00 ± 1.25	77.89 ± 2.59	76.02 ± 1.23	76.02 ± 1.89	77.20 ± 2.85	76.00 ± 1.85	76.02 ± 2.00	77.12 ± 1.85	197.23 ± 5.23	199.00 ± 6.89	198.23 ± 9.85	199.25 ± 8.25	199.23 ± 9.25	198.26 ± 7.25	197.23 ± 8.26	198.85 ± 8.23

Table 8.7 Stability data generated for the % CP released from the design space cream formulations after 72 hours for a period of eight weeks

Sampling Time (Weeks)	**40 ± 2°C, 75 ± 5% RH; % CP Released after 72 hours ± SD (%)**							
	01	**02**	**03**	**04**	**05**	**06**	**07**	**08**
1	50.96 ± 0.02	50.48 ± 0.02	49.86 ± 0.17	48.15 ± 0.15	50.12 ± 0.88	48.89 ± 1.05	52.41 ± 0.01	49.99 ± 1.26
2	50.00 ± 0.10	51.75 ± 0.04	50.12 ± 0.04	49.25 ± 1.10	51.23 ± 2.02	49.27 ± 0.03	51.23 ± 1.82	49.03 ± 0.03
4	49.44 ± 0.04	50.00 ± 0.32	49.00 ± 2.17	48.00 ± 0.05	49.98 ± 0.71	48.90 ± 0.01	51.53 ± 0.72	50.02 ± 0.15
8	48.26 ± 0.52	51.25 ± 0.78	49.23 ± 1.25	48.25 ± 0.03	48.00 ± 0.01	47.26 ± 0.11	50.25 ± 0.02	47.10 ± 0.03
Sampling Time (Weeks)	**25 ± 2°C, 60 ± 5% RH; % CP Released after 72 hours ± SD (%)**							
	01	**02**	**03**	**04**	**05**	**06**	**07**	**08**
1	51.22 ± 1.22	50.00 ± 1.12	50.86 ± 0.11	49.00 ± 1.27	51.12 ± 1.28	48.56 ± 0.05	50.41 ± 0.12	50.02 ± 1.52
2	50.56 ± 0.18	51.02 ± 1.44	51.12 ± 0.25	49.25 ± 0.15	51.74 ± 1.32	48.99 ± 0.03	50.23 ± 1.26	50.23 ± 1.35
4	50.20 ± 0.12	51.00 ± 0.02	50.00 ± 0.27	50.00 ± 0.03	50.99 ± 1.01	49.90 ± 1.35	49.53 ± 2.22	50.10 ± 1.53
8	49.99 ± 1.89	51.52 ± 2.02	52.00 ± 1.25	49.89 ± 1.89	49.12 ± 1.78	49.50 ± 1.20	48.00 ± 1.88	51.00 ± 1.89

The stability data for all responses showed that the viscosity, spreadability, pH, % CP content and % CP released from the formulations over a test period of eight weeks remained within the specifications established (Table 7.1, Chapter 7) for these formulations. To determine whether or not changes between the *in vitro* release rates of CP released from cream samples are statistically significant and/or relevant, the *in vitro* release rate of CP data were subjected to a statistical analysis in § 4.2.8, Chapter 4 *vide infra* and the data generated are shown in Figures 8.13 – 8.16.

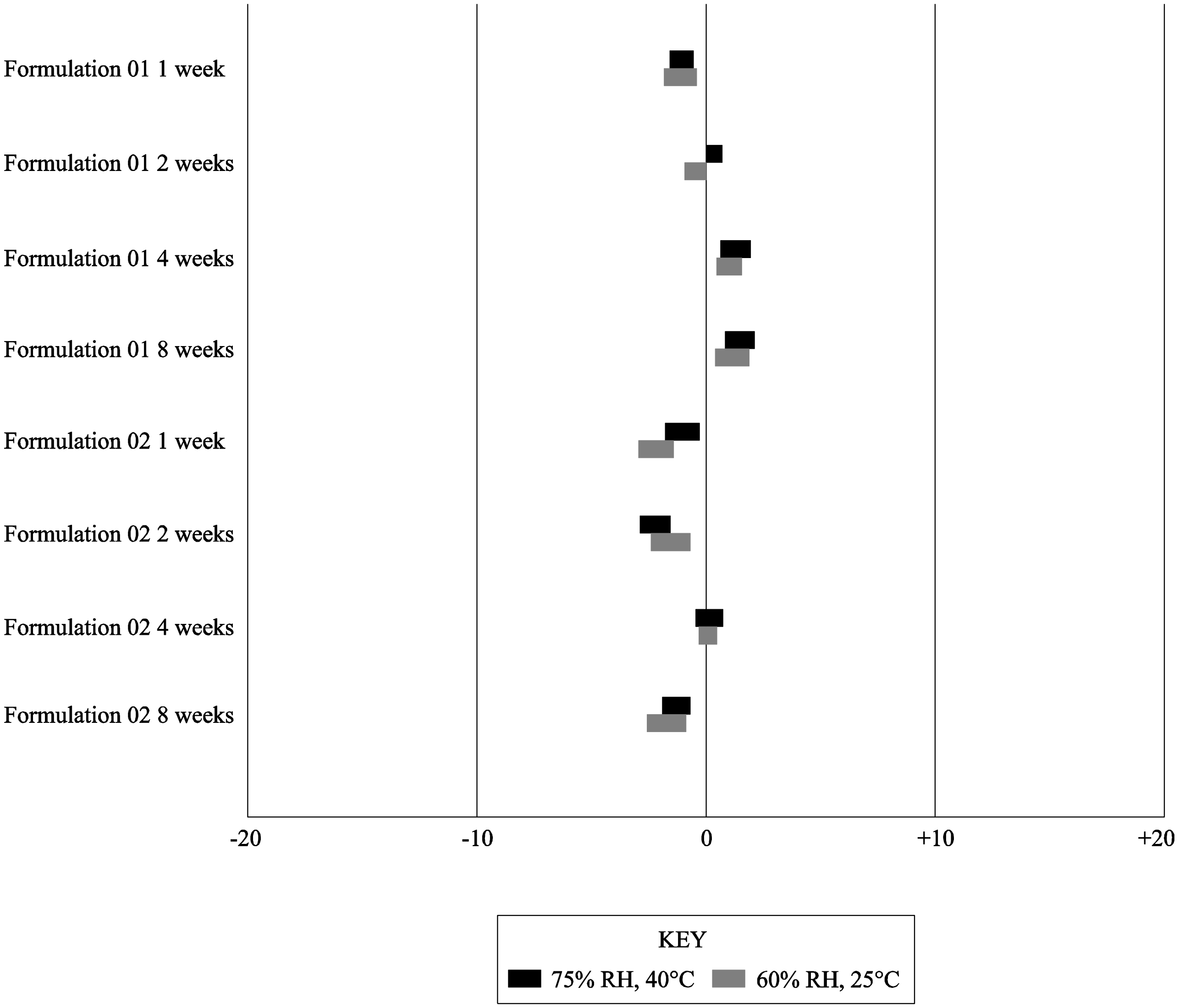


Figure 8.13 Effects of stability test conditions on the *in vitro* release rates of CP from cream formulations 01 and 02 over 72 hours, after storage in the two stability chambers

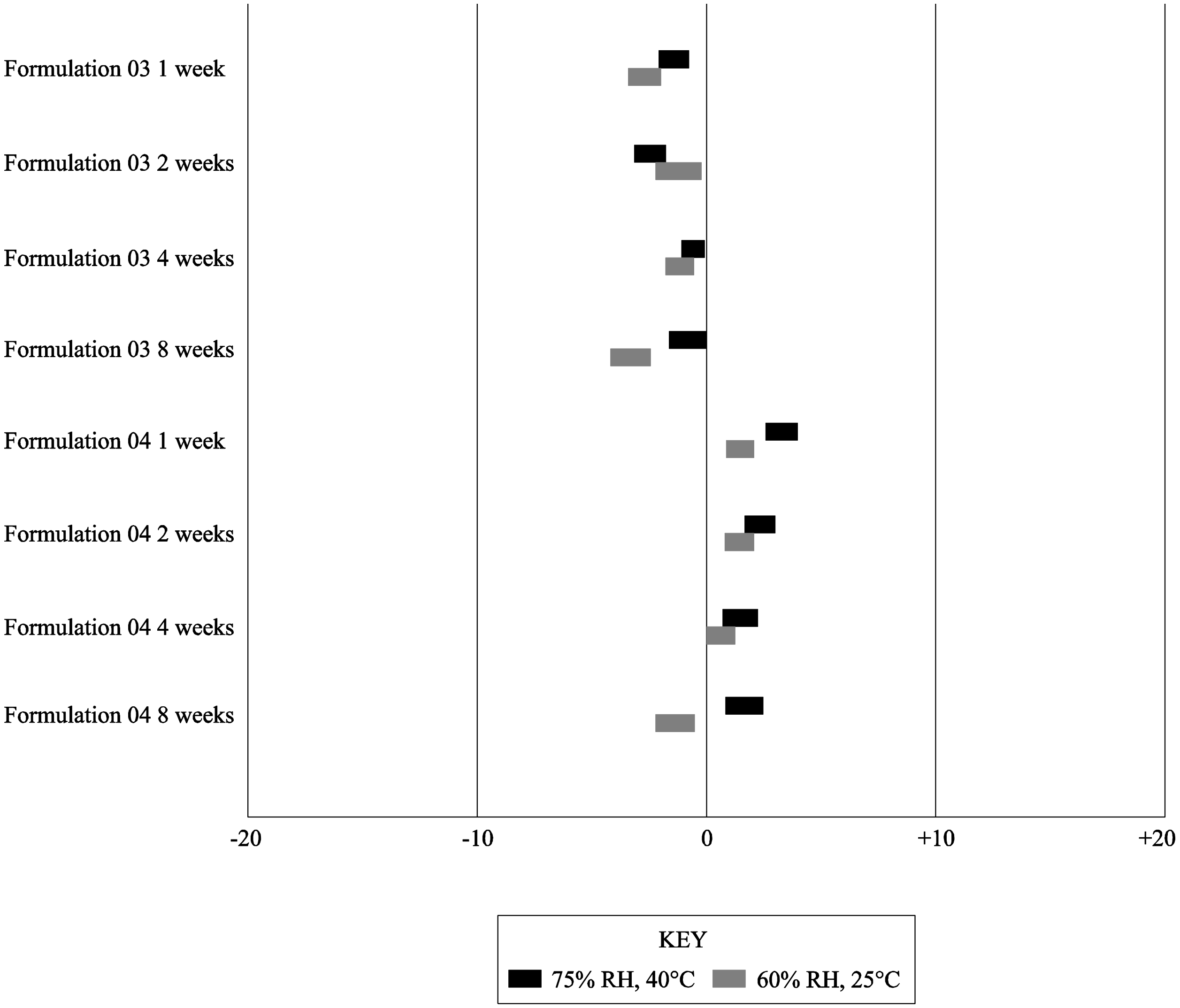


Figure 8.14 Effects of stability test conditions on the *in vitro* release rates of CP from cream formulations 03 and 04 over 72 hours, after storage in the two stability chambers

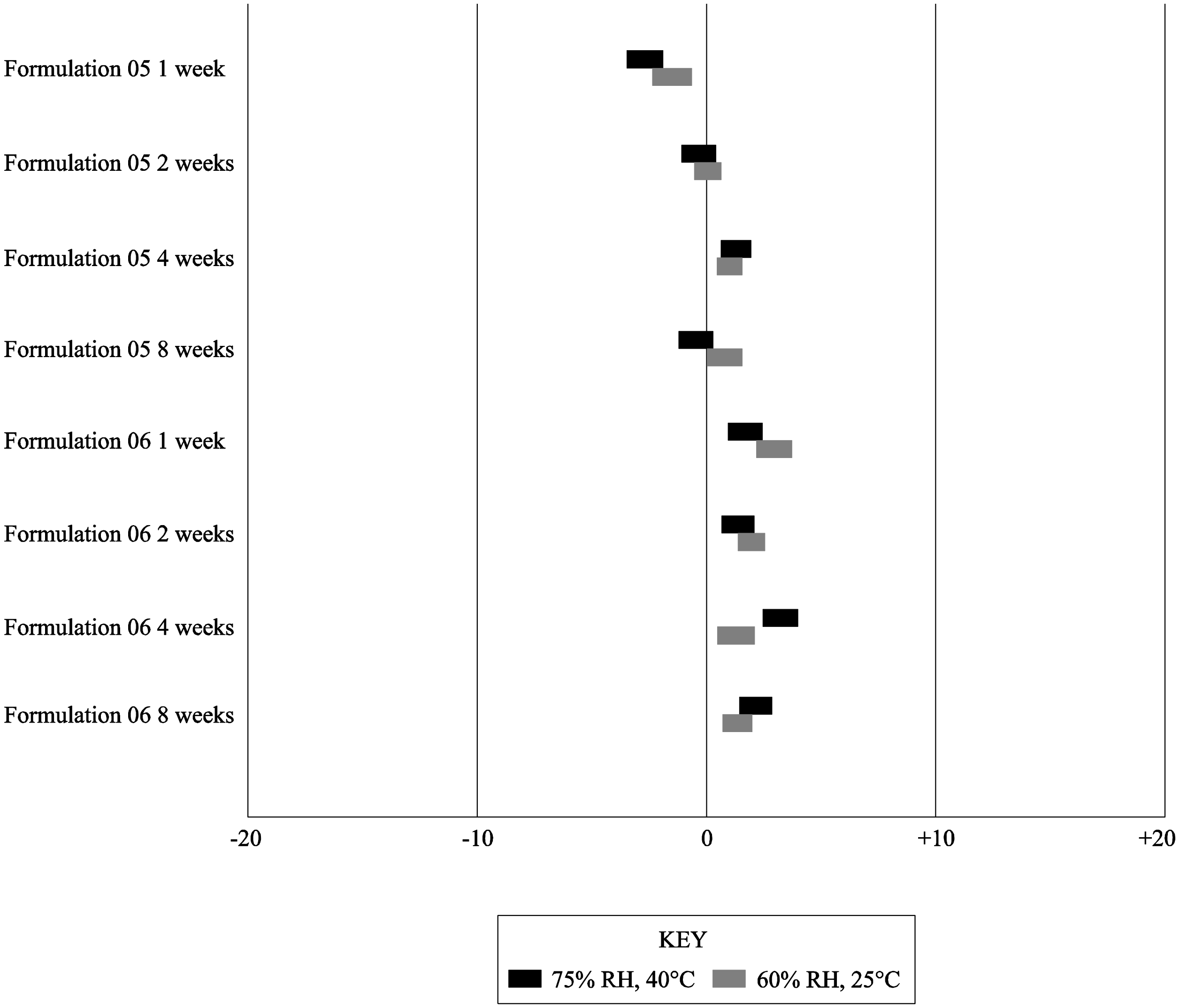


Figure 8.15 Effects of stability test conditions on the *in vitro* release rates of CP from cream formulations 05 and 06 over 72 hours, after storage in the two stability chambers

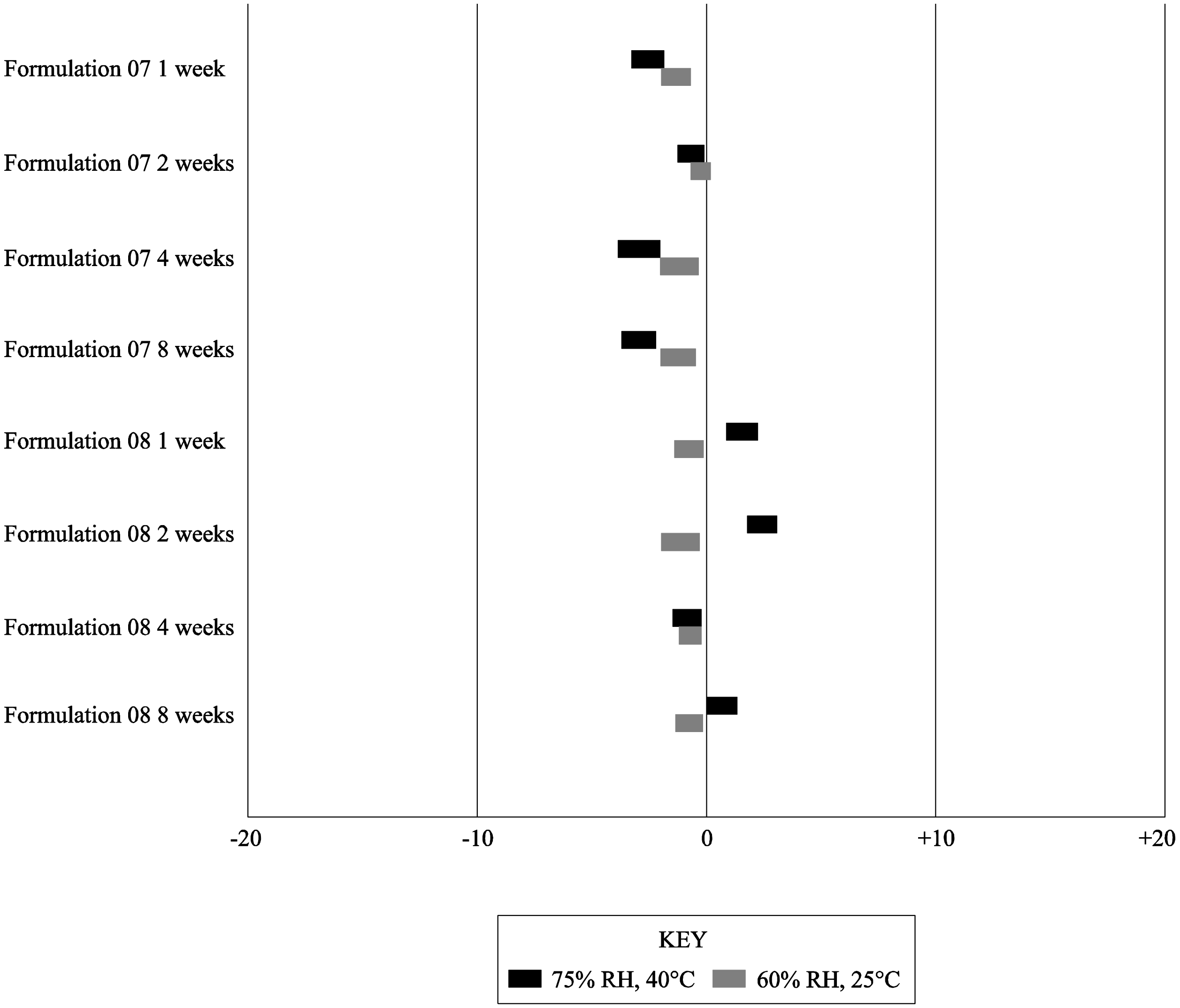


Figure 8.16 Effects of stability test conditions on the *in vitro* release rates of CP from cream formulations 07 and 08 over 72 hours, after storage in the two stability chambers

The graphical results indicate that the change of responses for the *in vitro* release of CP from the eight design space cream formulations over 72 hours at two stability conditions for eight weeks was not significant and not relevant. Both the upper and lower limits of the confidence interval in the graphs above showed values > -10% or < 10%, indicating the stability of the cream formulations. The CP cream formulations was therefore considered to be stable at 40 ± 2°C, 75 ± 5% RH and 25 ± 2°C, 60 ± 5% RH for a period of eight weeks.

8.4. CONCLUSIONS

The design space concept is one of the fundamental paradigms of the QbD framework and the description of that space is expected to be one of the results of pharmaceutical development investigations conducted according to a QbD approach. Operating within the design space provides flexibility in releasing the batch in real time and consequently enhances the confidence of the manufacturers in their products as well as relieving the work load of regulatory authorities such as FDA significantly, as quality is built into the system [119]. The ICH Q8 (R2) guideline [2] proposed the use of interaction, contour and response surface plots to establish and present the design space for a pharmaceutical formulation. Several approaches such as CCD, Box-Behnken and ANN strategies can be used to visualize, identify and define the design space [522,526]. Although there is no set way to represent a proposed pharmaceutical design space and a limited amount of work has been reported on this concept, a conventional approach using RSM and constraint plots generated from the statistical optimization of the formulation and processes was used to establish the design space limits for formulation variables that were investigated and found to be critical to the performance of the test formulations.

This study has showed that the extreme lower and upper limits of propylene glycol, Gelot® 64, cetostearyl alcohol and glyceryl monostearate content were 44.0% v/v and 46.4% v/v, 2.9% w/w and 3.8% w/w, 7.5% w/w and 10.0% w/w and 10.9% w/w and 12.8% w/w respectively. CP cream formulations manufactured within those extreme limits may show bioequivalence to Dermovate® cream. The cream formulations were also subjected to two stability conditions *viz.*, 40 ± 2°C, 75 ± 5% RH and 25 ± 2°C, 60 ± 5% RH for a period of eight weeks to assess short term stability. The design space batches were considered stable under the specified storage conditions for the period of testing and were further investigated in an *in vivo* human skin blanching assay to establish whether the formulations were bioequivalent to Dermovate®.

CHAPTER NINE

ASSESSMENT OF BIOEQUIVALENCE (BE) OF TOPICAL CP CREAMS USING THE HUMAN SKIN BLANCHING ASSAY (HSBA)

9.1. INTRODUCTION

9.1.1. Bioequivalence (BE)

“Bioequivalence” (BE) is a term used in pharmacokinetics to assess *in vivo* biological equivalence of two formulations containing the same API. Two products are said to be bioequivalent when they are expected to be, for all intents and purposes, the same [527]. The FDA has defined BE as “the absence of a significant difference in the rate and extent to which an API or active moiety in pharmaceutical equivalents dosage forms becomes available at the site of action when administered at the same molar dose under similar conditions in an appropriately designed study” [528]. In contrast to the approaches used to determine BE of oral dosage forms where the API is intended to be absorbed into the systemic circulation, BE assessment of topical dosage forms is a more difficult and daunting challenge [529]. These challenges are likely due to the complexity of drug transport through the skin from different formulations and the absence of harmonized guidance documents to facilitate the process [530].

The current approval strategy of FDA for most topical drug products whether a New Drug Application (NDA) or Abbreviated New Drug Application (ANDA) is usually based on clinical studies [531]. Clinical trials to prove BE often lack sensitivity, require large and costly trials and therefore may not be cost effective for generic drug approvals as manufacturers may have to conduct large clinical trials [79]. Pharmacodynamic approaches based on a pharmacological response have been found to be appropriate for dosage forms that deliver API intended for local action at the site of application. The FDA guidance [532] recommends demonstrating *in vivo* BE of topical dermatologic corticosteroids based on a pharmacodynamics approach first described by Stoughton and McKenzie as the vasoconstrictor assay [532]. This test uses readings of the human skin blanching effect resulting from the vaso-constrictive action of corticosteroids by use of visual observation or use of a chromameter [175]. Considerable effort has been channeled towards the development and validation of alternative approaches to demonstrate BE of other topical and transdermal products [533]. The critical opportunity pathways identified by the FDA for the industry with regards to topical BE include the use of surrogate methods *viz., in vitro* studies [516], dermatopharmacokinetic approaches, dermal microdialysis [336] and near infrared spectroscopy [533,534]. Although alternative methods including skin biopsy and suction blister have been developed, they are known to be considerably invasive and have failed to gain popularity in application [533].

Of all *in vivo* methods currently in use, the Human Skin Blanching Assay (HSBA) is the most frequently used bio-screening technique for assessing TC availability, potency and BE [336,535-537]. The objective of this study was to evaluate the *in vivo* BE of eight 0.05% w/w design space CP cream formulations with respect to Dermovate® in healthy human volunteers using the HSBA method. The creams to be tested were established through earlier studies and were selected as they corresponded to potential limits for formulation composition with respect to defining a define space for the optimized CP formulation developed in this research and described in Chapter 8 of this thesis.

9.1.2. The Mechanism of Skin Blanching

Topically applied corticosteroids elicit a blanching response after topical application [145,146,538]. This blanching response is important since this side effect relates to the relative potency and activity of corticosteroids and can be used to assess TC formulations [146]. However the mechanism of corticosteroid-induced blanching is not fully understood [145,539,540]. Fritz and Levine [541] demonstrated that the vaso-constrictive effect of noradrenaline on the mesoappendix of adrenalectomised rats does not occur unless the cortical extract is applied topically, suggesting that corticosteroids may support vascular tone by potentiating the pressor effects of adrenaline [536]. TC interact with vascular receptors, directly influencing vascular sensitivity to noradrenaline. Vascular smooth muscles contain glucocorticoids and mineralocorticoid receptors, however only glucocorticoid receptors appear to be involved in the vasoconstriction response. A report by Juhlin [542] revealed no evidence of such potentiation in the vascular reaction of normal skin when treated with fluocinolone acetonide. When TC are applied locally, local vaso-constrictive effects in the skin microvasculature are observed and consequently blood-flow is reduced [543]. However several factors influence vaso-constrictive effects of steroids including the concentration of the API, duration of application, nature of vehicle used, occlusion, posture and location of application [536,539,540].

9.2. TOPICAL BLANCHING RESPONSE ASSESSMENT

9.2.1. Visual Assessment

Since 1962 the assessment including BE of TC formulations has been successfully performed, using a visual observation approach [540,544,545]. The assessment of intensity of corticosteroid-induced skin blanching is performed visually by one or more trained investigators, using an ordinal data scale [537,545]. During the initial stages of visual assessment experiments, McKenzie and Stoughton [532] decided to grade the blanching response with a simple “yes or no” evaluation and merely indicated whether blanching was or was not evident at each application site. However this approach gave no indication as to the degree of blanching response observed. Sarkany *et al.* [546] were the first group of researchers that attempted to grade the blanching response using an arbitrary 0 - 3 intensity scale where 0 represents no blanching and 1 or 2 represented intermediate degrees of blanching, with the value 3 representing highly blanching. Another method that has been used involved a direct comparison of observed blanching at different application sites [540]. This method is useful for the direct comparison of different formulations applied in pairs however this method fails when one formulation elicits only slightly more blanching than the other [540].

Despite all the efforts put in the assessment of TC, some researchers still criticize the visual scoring approach as they appear to lack reproducibility or may be variable due to the subjective nature of visual observation for the HSBA. Criticisms about the subjective method include comments that suggest that subtle differences in the blanching response may be difficult to evaluate and attempts to estimate and assign a numerical value to the degree of skin blanching may lead to invalid conclusions [145,536,540]. However the visual assessment of skin blanching continues to appear more robust than the chromameter readings when evaluating different formulations containing the same corticosteroid, especially when only a few application sites are used [547]. A 0 - 4 graded scale was introduced by Barry and Woodford [548,549] and has been a sensitive approach for assessing TC BE [336,535,536] and the data are reported in terms of percent of a total possible score (% TPS) calculated using Equation 9.0.

$$\%\ \text{TPS} = \frac{\text{Actual Score}}{\text{TPS}} \text{ x } 100 \qquad \textbf{\textit{Equation 9.0}}$$

Where

Total possible score (TPS) = 4 x n x S x V
The maximum score per site = 4
The number of independent observers = n
The number of sites per preparation per arm = S
The number of subjects = V [550]

9.2.2. Chromameter Assessment

In contrast to the use of visual observation for the assessment of BE, the FDA [532] proposed the use of a chromameter for the quantitative assessment of the blanching response for BE purposes [537,545,547]. This instrument measures the intensity of skin blanching as an absolute color, based on tristimulus analysis of a reflected pulse of light from a xenon source. The result is expressed in a three-dimensional manner consisting of two color coordinates *viz.*, green-red or *a** scale and yellow-blue or *b** scale and a luminance coordinate *viz.*, black-white or *L** scale [545,547]. The three coordinates, *a*, *b* and *L* define the absolute color of a site at an observation time and will therefore change the blanching effect over time. A schematic of the coordinate colors is shown in Figure 9.1 [551].

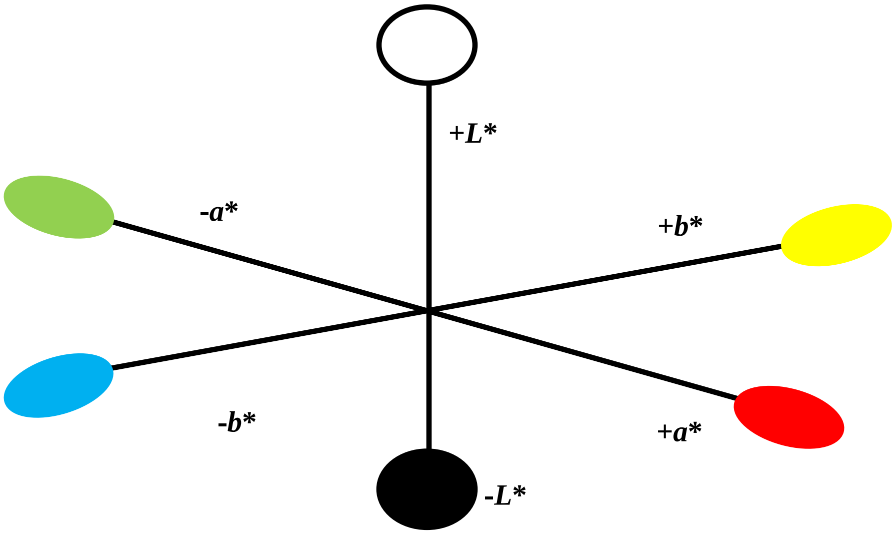


Figure 9.1 Schematic of the *L**, *a** and *b** color space, adapted and redrawn from [552]

The skin blanching response is measured relative to color change of the skin and as the skin blanching response develops, the skin becomes lighter and redness fades [529,553]. Therefore the *L** scale coordinate increases, the *a** scale coordinate decreases and the *b** scale coordinate may increase very slightly as the skin pales. It has been shown that the *L** and *a** coordinates are more discriminative than the *b** coordinate in determining skin blanching response and the *b** coordinate is usually omitted from any data analysis for BE [336,529,554]. The FDA [532] recommends that only the *a** scale data be used for statistical analysis of pilot and pivotal trials with baseline and unmedicated-site correction. The *a** scale coordinate value appears to have the greatest sensitivity to color change over the progression of the blanching response, followed by the *L** scale and *b** scale data [532,535,547] .

9.3. METHODS

9.3.1. Study Population and Criteria for Participation

9.3.1.1. Number of Subjects

The selection of subjects to be considered as volunteers for this study was based on pre-screening for a blanching response prior to the study. Two groups of ten subjects were enrolled and there were no dropouts. The volunteers were allocated into groups and each group was comprised of five male and female subjects.

9.3.1.2. Conditions of Participation in this Study

Subjects completed a written informed consent form indicating that they are willing to participate fully in the study. They underwent pre-screening to assess any possibility of sensitivity to CP and to evaluate whether they exhibited any blanching. Volunteers had to fulfil inclusion and exclusion criteria summarized in § 8.3 and § 8.4 of Appendix VIII. The volunteers had to be fully committed to the study and adhere to all restrictions required by subjects, as listed in § 8.5 of Appendix VIII.

9.3.2. Study Products

The test products for this study included eight 0.05% w/w CP cream formulations, the composition of which was determined following evaluation of data obtained from the design space network studies in Chapter 8. Dermovate® cream is a commercially available product in RSA. Dermovate® creams were purchased in a sufficient quantity from a local pharmacy and stored at ± 22°C. The first four CP test formulations *viz.*, T_1, T_2, T_3 and T_4 were manufactured at the extreme limits of propylene glycol and Gelot® 64 content *viz.*, 44.0% v/v and 46.4% v/v and 2.9% w/w and 3.8% w/w respectively. The last four test formulations *viz.*, T_5, T_6, T_7 and T_8 were manufactured at the extreme percent limits of cetostearyl alcohol and glyceryl monostearate content *viz.*, 7.5% w/w and 10.0% w/w and 10.9% w/w and 12.8% w/w respectively. The formulations were developed and manufactured specifically for the purposes of this research and produced similar formulation responses and *in vitro* release profiles to Dermovate®. The formulations were manufactured in the Faculty of Pharmacy at Rhodes University using the principles of cGMP. These formulations form the design space limits for the generic CP cream developed in this research and were packed into 50 g collapsible aluminium cream tubes and stored at ± 22°C prior to use. These test formulations were then subjected to two stability conditions *viz.*, 40 ± 2°C, 75 ± 5% RH and 25 ± 2°C, 60 ± 5% RH.

9.3.3. Study Design

Approval from the Rhodes University Ethical Standards Committee (PHARM 2013-26), Rhodes University, Grahamstown, RSA was obtained prior to commencing the study. The original signed copy of the ethical approval has been retained by the study investigator. A pre-screening test was conducted prior to the study to select subjects based on the definition of a "responder" as described in the FDA guidance [532]. A single application site located on the upper forearm was demarcated using a pre-punched adhesive template that exposed an area of skin equivalent to 121 mm^2. Approximately 10 μL equivalent to 11 mg of the reference cream was applied to the application site using an Eppendorf® micropipette (Merck® Chemicals Pty Ltd, Germiston, RSA) and was spread uniformly over the site using a glass rod. The site was exposed to the cream for 40 minutes after which the product was gently removed from the skin using three consecutive wet and dry cotton wool swabs. Assessment of blanching was performed visually approximately six to eight hours following product removal to establish if skin blanching had occurred. Subjects exhibiting a blanching response of 3 - 4 on a multiple unit scale of 1 - 4 where 1 and 2 represent intermediate grades of blanching and 4 represents intense blanching. Twenty healthy male (10) and female subjects (10) that exhibited a positive blanching response in the pre-screening test were included in the study. The study was split into two groups to accommodate the large number of products to be investigated. Four products *viz.*, formulations manufactured at the extreme limits of propylene glycol and Gelot® 64 content were tested in one group and four products *viz.*, formulations manufactured at the extreme limits of cetostearyl alcohol and glyceryl monostearate content were tested in the other group. The subjects were housed in the Biopharmaceutics Research Institute Clinic (BRI) at Rhodes University in which the ambient temperature was maintained at 22°C and had a 55 ± 3% RH. Standard overhead lightning was used during the pre-screening test and for the duration of the study.

Fourteen application sites per forearm were demarcated using pre-punched adhesive labels that exposed squares of 121 mm^2 for the application of reference and test products. Dermovate® (R_1) and eight test batches (T_1 - T_8) were used in this study. A staggered application approach was used and approximately 10 µL of cream was applied to designated application sites on each forearm using a 10 µL Eppendorf® micropipette. The cream was spread uniformly in the demarcated area using a glass rod. The glass rod tip was cleaned with a paper towel to remove product prior to use on the next application site. Three dose durations including the ED_{50}, D_1 and D_2 were used in accordance with the FDA guideline [532]. A dose duration (ED_{50}) of 40 minutes was used to assess the BE of CP topical creams. Dermovate® cream was applied to randomly assigned application sites demarcated D_1 (20 minutes) and D_2 (80 minutes) to establish the "detectors" amongst the study subjects. Four untreated control sites (UNT) were randomly assigned amongst the 14 application sites. A representative application chart for the BE assessment of the test creams for the individual groups is shown in Figures 9.2 and 9.3.

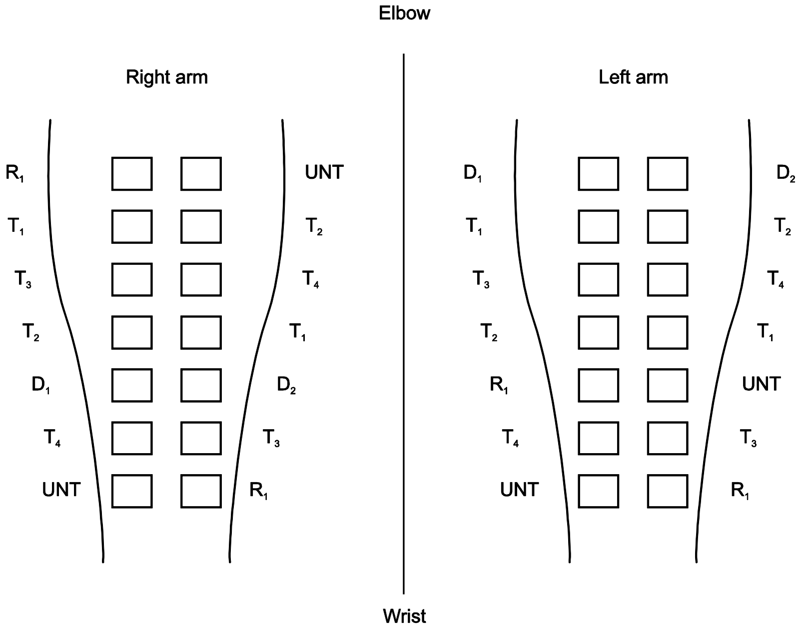


Figure 9.2 Schematic diagram indicating the application sites on both forearms for Group 1

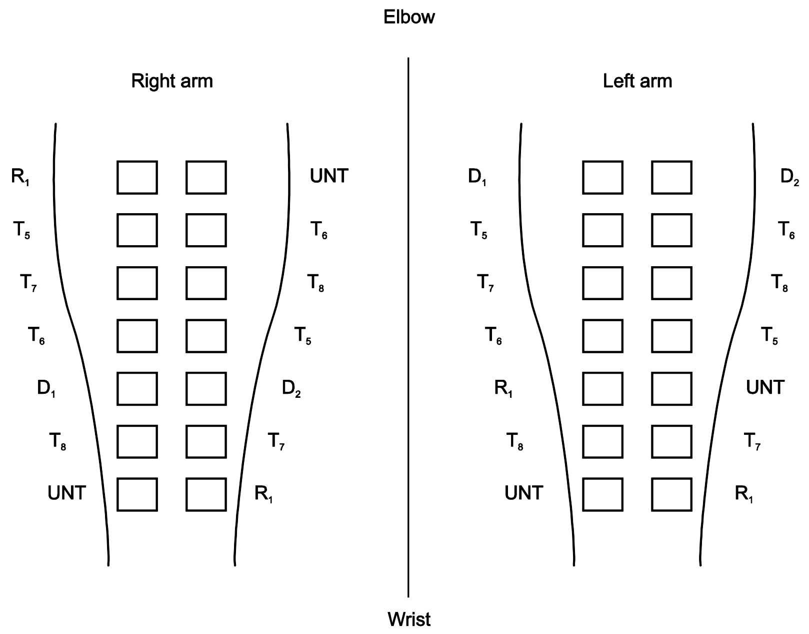


Figure 9.3 Schematic diagram indicating the application sites on both forearms for Group 2

Key		
	Formulation	**Duration**
R_1	Reference	40 minutes
D_1	Reference	20 minutes
D_2	Reference	80 minutes
T	Test	40 minutes
UNT	Unmedicated	-

The application site for each product on the ventral forearm was randomly assigned to prevent bias that may occur for visual assessment. The application sites on the forearms were protected with a non-occluding arm guard to prevent any loss of cream during the application period. The adhesive labels were removed gently prior to removal of the products. The remaining formulation was removed gently in a synchronize manner and each site was swabbed three consecutive times with wet and dry cotton swabs. Skin blanching was evaluated visually and with a chromameter at times = 0, 2, 4, 6, 8, 10, 12, 20 and 24 hours following removal of the products.

9.3.4 Skin Blanching Assessment

The blanching response on each was evaluated visually by two trained observers at designated time following removal of the products and the data were used to generate blanching profiles. Standard lighting using overhead fluorescent lamps were used throughout the study. The forearms were placed horizontally on a desk directly in front of each observer for the assessment. Visual assessment was scored on a 0 – 4 scale, where 0= insignificant or no blanching and the application site appeared identical to surrounding area of skin, 1= faint blanching observed, but the outline of the application square was not seen, 2= faint to moderate blanching with one or two sides of the application square observed, 3= moderate to strong blanching where the outline of the application square is clearly visible and 4= strong to intense blanching where intense pallor was observed over the entire square application site. Each site was assigned a blanching score by comparing the treated and untreated skin at the site of application to the surrounding. [336]. The blanching response results were recorded and the % TPS calculated for each formulation at each application site for each observer. A graph of the average % TPS against time was plotted to generate a blanching profile for each formulation. The trapezoidal rule was then used to calculate the area under the effect curve (AUEC) for each formulation.

Prior to color measurement using the chromameter, a mechanically-mounted Minolta® Model CR 400 chromameter (Minolta®, Osaka, Japan) was calibrated using a white calibration plate. This instrument was also used to assess skin blanching at the application sites at designated times following removal of the creams. The chromameter was aligned and placed in position directly over but covering the site of application with the measuring head of the device. The orientation of the chromameter was the same for each measurement so as to ensure consistent and accurate readings were recorded for each site. Only the *a** scale data for each application site was used for the BE assessment and these data was baseline-corrected by subtracting baseline or zero time values to yield baseline-corrected values *viz.*, Δa-values. The Δa-values of all formulations were calculated and plotted against time. The AUEC for the Δa-curve was calculated for each formulation using the trapezoidal rule. Only the data obtained from subjects defined as "detectors" *viz.*, individual subjects whose AUEC values at D_1 and D_2 are both negative and that meet the dose duration-response criterion, should be included in the data analysis [532,547]. Furthermore the FDA guidelines [532] state that a "detector" is a "responder" if the blanching data for the subjects meet the criterion established using Equation 9.1.

$$\frac{\text{AUEC at } D_2}{\text{AUEC at } D_1} \geq 1.25 \qquad \textbf{\textit{Equation 9.1}}$$

Where

AUEC at D_2 = 0.5 [AUEC at D_2 (left arm) + AUEC at D_2 (right arm)]
AUEC at D_1 = 0.5 [AUEC at D_1 (left arm) + AUEC at D_1 (right arm)]

9.3.5. Data and Statistical Analysis

The trapezoidal rule was used to calculate the AUEC from visual and chromameter data. The AUEC values calculated for the left and right forearms were averaged for both the visual and chromameter data. To assess the visual and chromameter data statistically, Locke's method was used to calculate a confidence interval (CI), based on Fieller's Theorem for the AUEC data as recommended in the FDA guidelines [532]. The AUEC data sets for all formulations *viz.*, reference (R_1) and the eight test formulations (T_1 - T_8) were compared to calculate an exact CI and to evaluate the BE status of the test formulations under investigation.

9.4. RESULTS AND DISCUSSION

9.4.1. Assessment of Test Formulations 1- 4

The first ten subjects include five females and five males of which nine were caucasian and one was indian. All ten subjects showed some degree of blanching through the study. No adverse drug reactions or other clinical events were observed however, some subjects experienced slight sensitivity to the adhesive labels for approximately 15 minutes following removal of the label *viz.,* slight redness and inflammation of the skin. This sensitivity reaction did not affect the visual assessment or chromameter data collection. The blanching response of one of the ten subjects following application of five CP cream formulations *viz.,* Dermovate® (R_1), T_1 - T_4 is shown in Figure 9.4.

Figure 9.4 Blanching response of a subject from Group 1 following application and removal of CP cream formulations

The mean visual and chromameter blanching profiles (n=10) for the reference product, Dermovate® using different dose durations *viz.,* D_2, ED_{50} and D_1 are shown in Figures 9.5 and 9.6.

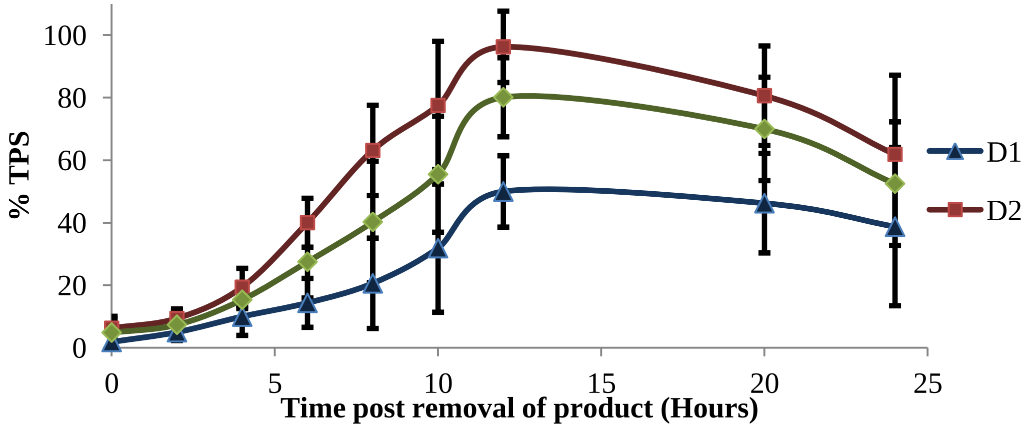


Figure 9.5 Mean visual blanching profiles ± SD (n=10) for the reference product, Dermovate® at dose durations of D_2 (80 minutes), ED_{50} (40 minutes) and D_1 (20 minutes) for Group 1

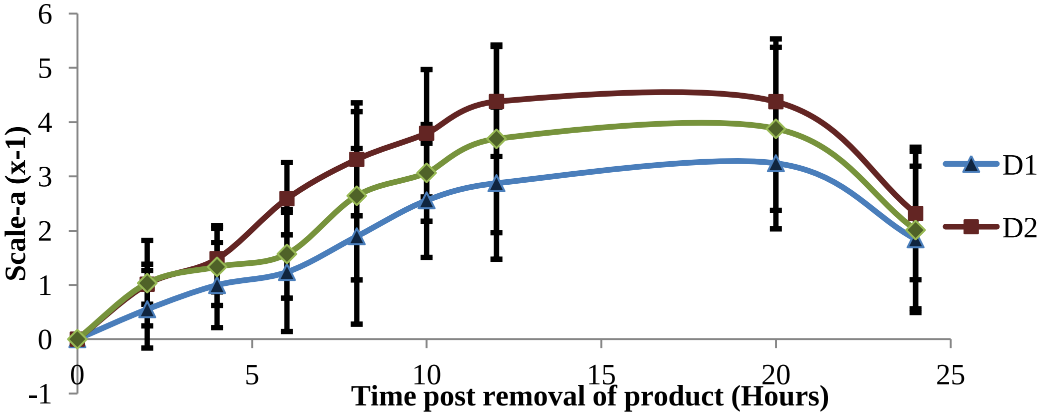


Figure 9.6 Mean chromameter profiles ± SD (n=10) for the reference product, Dermovate® at dose durations of D_2 (80 minutes), ED_{50} (40 minutes) and D_1 (20 minutes) for Group 1

The visual and chromameter assessment data shown in Figures 9.5 and 9.6 indicate that the blanching profile for D_2 was of a higher intensity of blanching than the ED_{50} due to exposure of the subject to a relatively long dose duration of 80 minutes. A lower blanching intensity was observed for D_1 which is a relatively short dose duration of 20 minutes. The blanching profiles for a dose duration at the ED_{50} showed an intermediate blanching profile that fell between the profiles for D_1 and D_2 as the dose duration was 40 minutes. The graphs reveal that as the dose duration increases, there is a corresponding increase in the blanching response [61]. However at a certain point in time following application, the skin blanching response reaches a maximum and thereafter any increase in dose duration will not result in any change in the blanching response and the AUEC. Consequently any discriminatory power of the BE test may be lost and the ability differentiate between products, if indeed differences do exist will not be possible. The use of an appropriate dose duration is therefore extremely important for use in a pivotal study to establish BE between topically applied products [336,529]. The AUEC of the dose duration profiles was calculated using the trapezoidal rule for individual subject and the ratio of $\frac{\text{AUEC } D_2}{\text{AUEC } D_1}$ was calculated to establish which subjects could be defined as "detectors". These data are listed in Table 9.1.

Table 9.1 Determination of "Detectors" for the 10 subjects included in Group 1

Subjects	Visual Ratio	Summary	Subjects	Chromameter Ratio	Summary
1	3.242	Detector	**1**	3.242	Detector
2	3.085	Detector	**2**	3.085	Detector
3	1.660	Detector	**3**	1.660	Detector
4	3.158	Detector	**4**	3.158	Detector
5	1.620	Detector	**5**	1.620	Detector
6	2.211	Detector	**6**	2.211	Detector
7	1.628	Detector	**7**	1.628	Detector
8	2.869	Detector	**8**	2.869	Detector
9	1.767	Detector	**9**	1.767	Detector
10	2.597	Detector	**10**	2.597	Detector

The ten subjects in Group 1 were all found to be "detectors" as defined as the ratios of $\frac{\text{AUEC } D_2}{\text{AUEC } D_1}$ were ≥ 1.25 for the visual and chromameter data. The mean visual and chromameter blanching profiles for T_1 – T_4 formulations for all subjects in Group 1 (n=10) are shown in Figures 9.7 - 9.10 respectively.

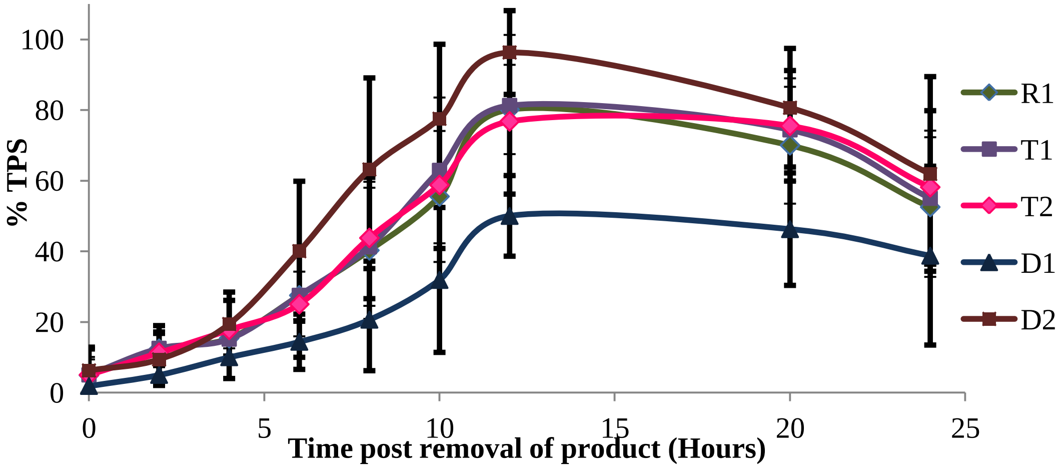


Figure 9.7 Average blanching profiles (visual data) for formulations T_1 and T_2 for all subjects (n=10) in Group 1

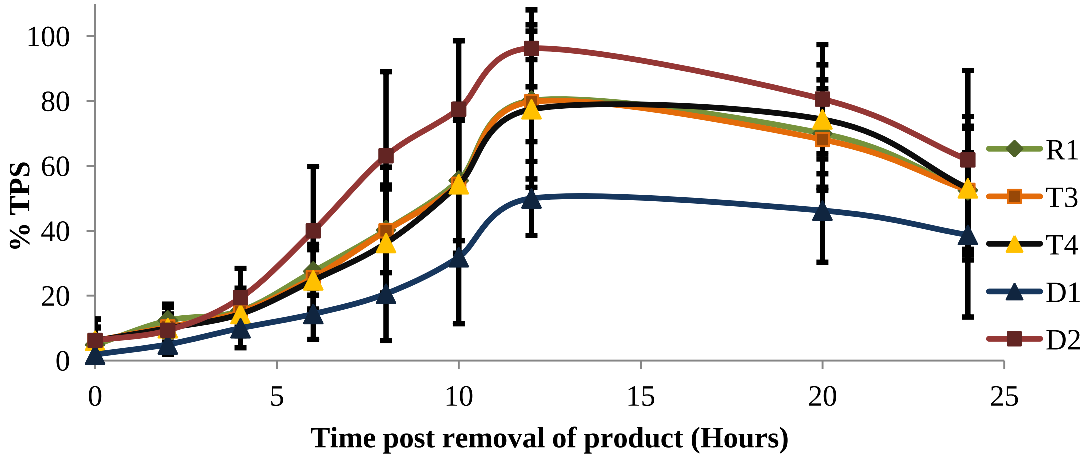


Figure 9.8 Average blanching profiles (visual data) for formulations T_3 and T_4 for all subjects (n=10) in Group 1

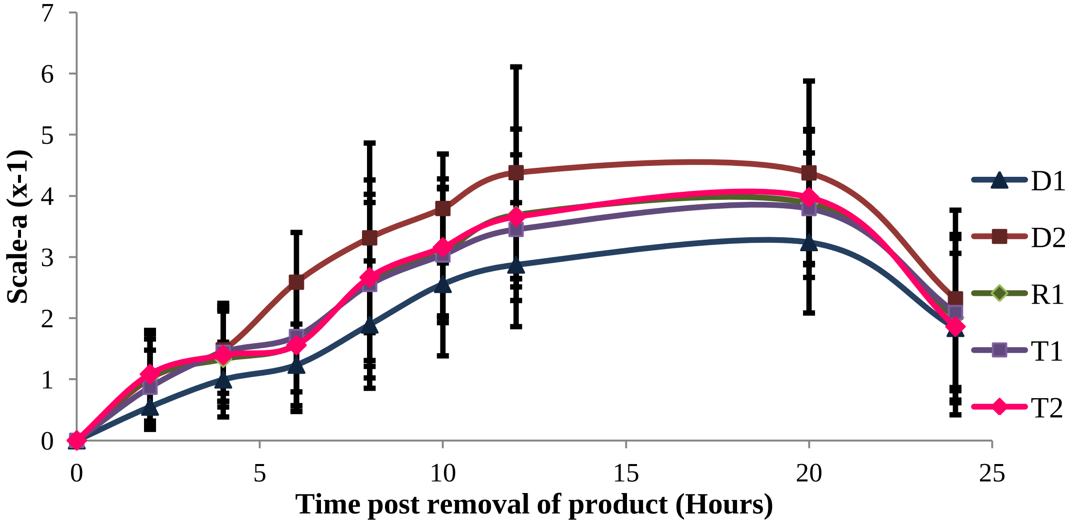


Figure 9.9 Average blanching profiles (chromameter data) for formulations T_1 and T_2 for all the subjects (n=10) in Group 1

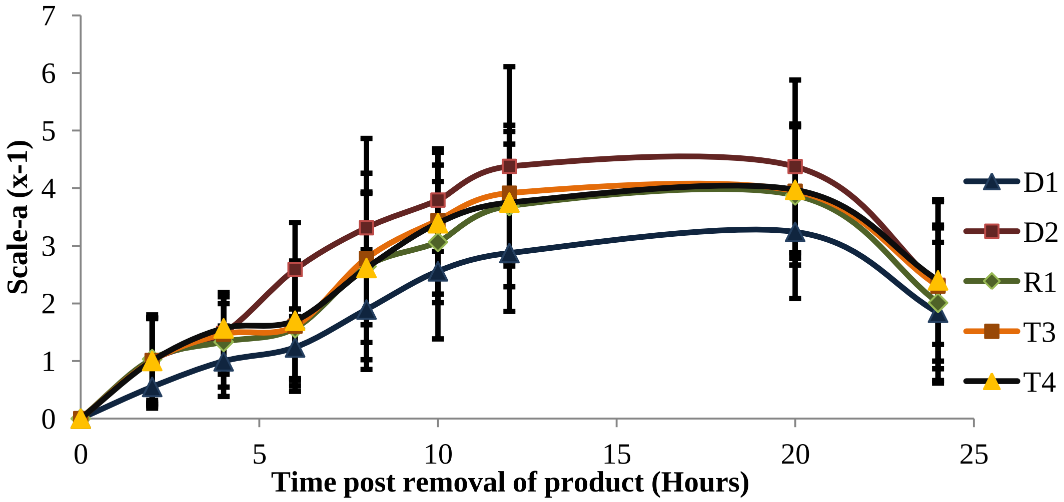


Figure 9.10 Average blanching profiles (chromameter data) for formulations T_3 and T_4 for all the subjects (n=10) in Group 1

The mean visual and chromameter blanching profiles for CP cream formulations clearly indicate that peak blanching occurred at approximately 12 hours following product removal. The distinct peak blanching at 12 hours was more visible for the visual blanching profile as compared to the chromameter profile even though the human eye is regarded as a subjective method of assessment [537,545]. Both the chromameter and visual profiles reveal relatively large standard deviations and this might be due to the small sample size used in this study. For the mean visual and chromameter profiles, formulation R_1 might demonstrate a similar blanching effect to formulations T_1 – T_4. Graphically, it can be seen that these four test formulations manufactured at the extreme limits of propylene glycol and Gelot® 64 *viz.*, 44.0% v/v and 46.4% v/v and 2.9% w/w and 3.8% w/w content might be equivalent to the reference product, Dermovate®. The visual and chromameter AUEC data confirm that these formulations T_1 – T_4 exhibited a similar blanching effect to the reference formulation R_1 as can be seen from the data summarized in Table 9.2.

Table 9.2 AUEC data for the visual and chromameter profiles for "detectors"

	Visual (n=10)					Chromameter (n=10)				
	T_1	**T_2**	**T_3**	**T_4**	**R_1**	**T_1**	**T_2**	**T_3**	**T_4**	**R_1**
Mean AUEC	534.443	535.018	534.058	535.591	534.531	31.120	31.4911	30.825	31.422	31.61
SD	109.256	110.677	106.246	107.482	110.083	9.379	9.858	9.996	9.909	9.72
C.V%	20.44	20.68	19.89	20.06	20.59	30.13	31.31	32.42	31.33	30.76

A Locke's method was used to calculate a CI based on Fieller's Theorem for the visual and chromameter AUEC data as described in the FDA guidance document [532]. A 90% CI was calculated for the ratio of the average AUEC response test products versus the reference product using Locke's method. The exact CI calculated for each of the CP formulations *viz.*, R_1, T_1 –T_4 was then used to evaluate the BE status of each formulation and these results are listed in Table 9.3.

Table 9.3 90% CI for visual and chromameter AUEC data calculated using Locke's method

	Locke's Method (90% CI) (n=10)							
Formulation	**Visual**				**Chromameter**			
	% Mean Ratio	**%C.V**	**90% CI**	**Summary**	**% Mean Ratio**	**%C.V**	**90% CI**	**Summary**
T_1/R_1	99.9	0.9	99.46 – 100.54	BE	100.6	4.5	97.40 – 103.80	BE
T_2/R_1	100.1	0.8	99.60 – 100.59	BE	101.5	4.5	98.34 – 104.52	BE
T_3/R_1	99.9	2.1	98.74 – 101.16	BE	99.1	5.0	96.53 – 101.07	BE
T_4/R_1	110.3	2.5	99.11 – 101.66	BE	102.3	3.9	98.79 – 105.42	BE
T_1/T_2	99.8	0.8	99.43 – 100.38	BE	99.1	4.2	96.59 – 101.98	BE
T_1/T_3	100.1	2.4	98.59 – 101.53	BE	101.6	4.5	99.23 – 104.58	BE
T_1/T_4	99.6	2.5	98.31 – 100.95	BE	98.4	4.4	96.57 – 100.67	BE
T_2/T_3	100.2	2.6	98.68 – 101.63	BE	102.5	3.9	99.83 – 105.53	BE
T_3/T_4	99.5	3.8	97.47 – 101.72	BE	96.8	3.2	94.09 – 99.56	BE
T_2/T_4	99.7	2.5	98.38 – 101.07	BE	99.3	3.6	95.79 – 103.02	BE

The FDA guidance [522] mentions that a CI between 80% and 125% can be used as the standard for the assessment of BE of TC products [532]. The visual and chromameter data for the ten "detectors" in Group 1 revealed that all cream formulations tested in this group had a point estimate for the ratio of AUEC of the test and reference product fell in the 80% - 125% range and the test formulations met BE to Dermovate® cream. A comparison between test products also demonstrated that the point estimate for the test products *viz.*, comparing T_1 to T_2 also fell into the CI to establish BE.

9.4.2. Assessment of Test Formulations 5 - 8

The second ten subject group was comprised of five females and five males of which six were caucasian and four were indian. All subjects exhibited some degree of blanching throughout the study. No adverse drug reactions or other clinical events were recorded and the blanching response of one of the ten subjects following application of CP formulations *viz.*, Dermovate® (R_1) and $T_5 – T_8$ is shown in Figure 9.11. The mean blanching profiles (n=10) (visual and chromameter) for the reference product, Dermovate® dosed for durations D_2, ED_{50} and D_1 are shown in Figures 9.12 and 9.13.

Figure 9.11 Blanching response of a subject from Group 2 following application and removal of cream formulations

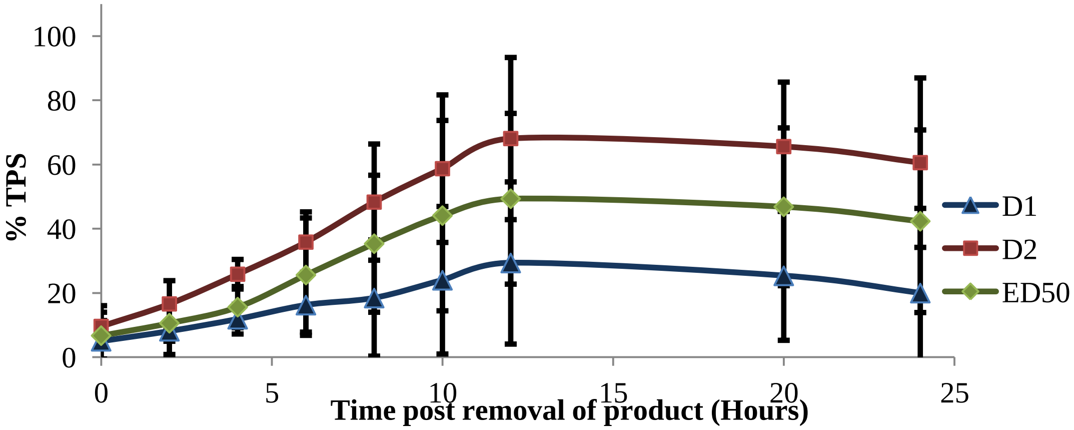


Figure 9.12 Mean visual blanching profiles ± SD (n=10) for the reference product, Dermovate® at dose durations of D_2 (80 minutes), ED_{50} (40 minutes) and D_1 (20 minutes) for Group 2

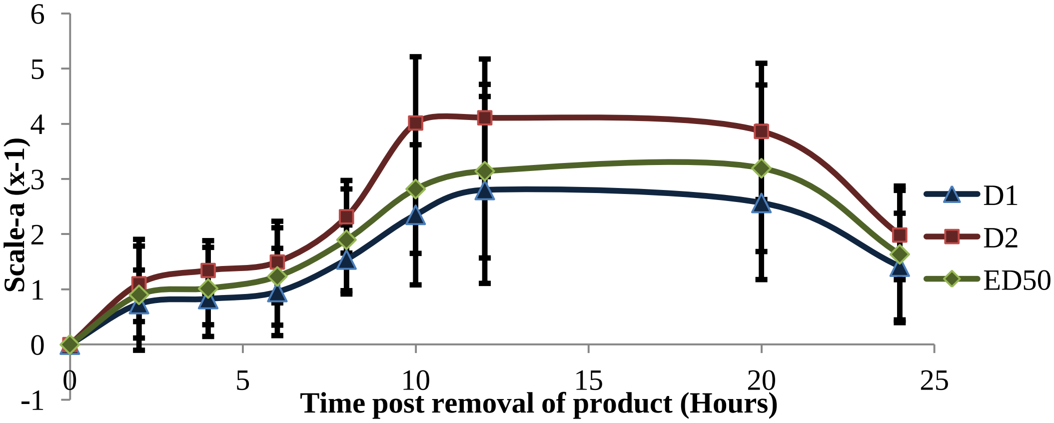


Figure 9.13 Mean chromameter profiles ± SD (n=10) for the reference product, Dermovate® at dose durations D_2 (80 minutes), ED_{50} (40 minutes) and D_1 (20 minutes) for Group 2

For Group 2, the blanching profiles reveal that an increase in dose duration from 20 minutes to 80 minutes resulted in an increase in the intensity of blanching. The ratio of $\frac{\mathrm{AUEC\ D_2}}{\mathrm{AUEC\ D_1}}$ was calculated to establish which subjects could be defined as "detectors" and these data are summarized in Table 9.4.

Table 9.4 Determination of "Detectors" for the 10 subjects included in Group 2

Subjects	Visual Ratio	Summary	Subjects	Chromameter Ratio	Summary
11	5.079	Detector	**11**	1.683	Detector
12	3.326	Detector	**12**	1.526	Detector
13	1.647	Detector	**13**	1.475	Detector
14	3.453	Detector	**14**	1.867	Detector
15	1.412	Detector	**15**	1.779	Detector
16	1.889	Detector	**16**	1.317	Detector
17	1.111	Non Detector	**17**	1.190	Non Detector
18	3.666	Detector	**18**	1.738	Detector
19	2.810	Detector	**19**	1.421	Detector
20	2.034	Detector	**20**	1.696	Detector

Nine of the ten subjects were determined to be "detectors" as the ratio of $\frac{\mathrm{AUEC\ D_2}}{\mathrm{AUEC\ D_1}}$ was ≥ 1.25 for the visual and chromameter evaluation. Volunteer No 17 was established as "non detector" due to the ratio of $\frac{\mathrm{AUEC\ D_2}}{\mathrm{AUEC\ D_1}}$ being < 1.25. The average visual and chromameter profiles for formulations T_5 – T_8 for all detector subjects in Group 2 (n=9) are shown in Figures 9.14 - 9.17 respectively.

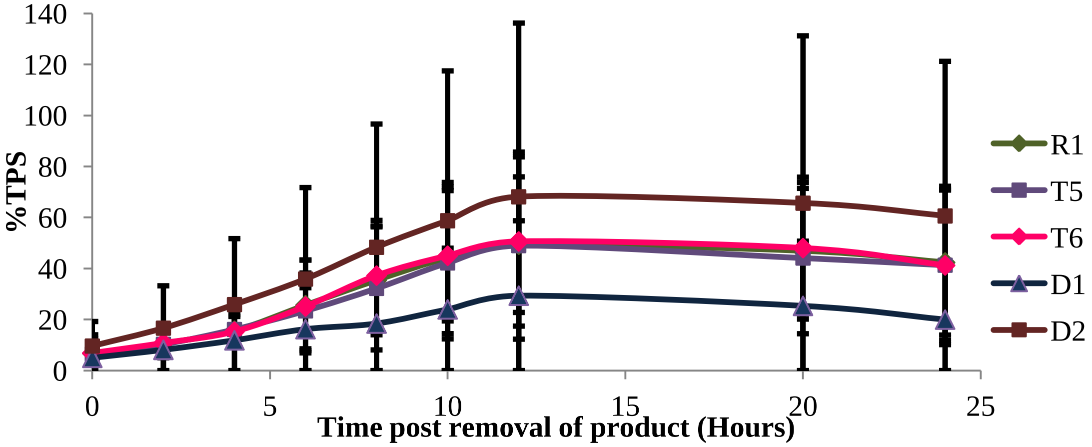


Figure 9.14 Average blanching profiles (visual data) for formulations T_5 and T_6 for "Detectors" (n=9) in Group 2

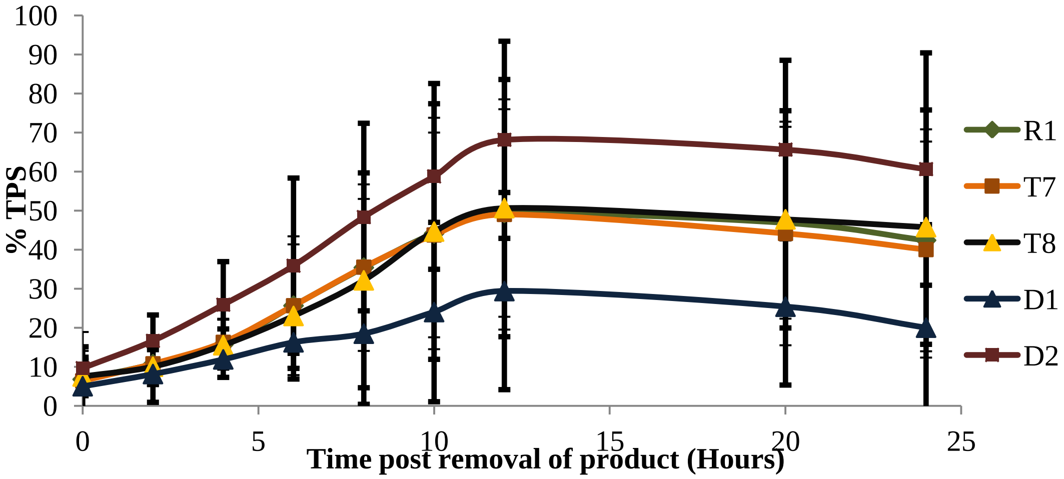


Figure 9.15 Average blanching profiles (visual data) for formulations T_7 and T_8 for “Detectors” (n=9) in Group 2

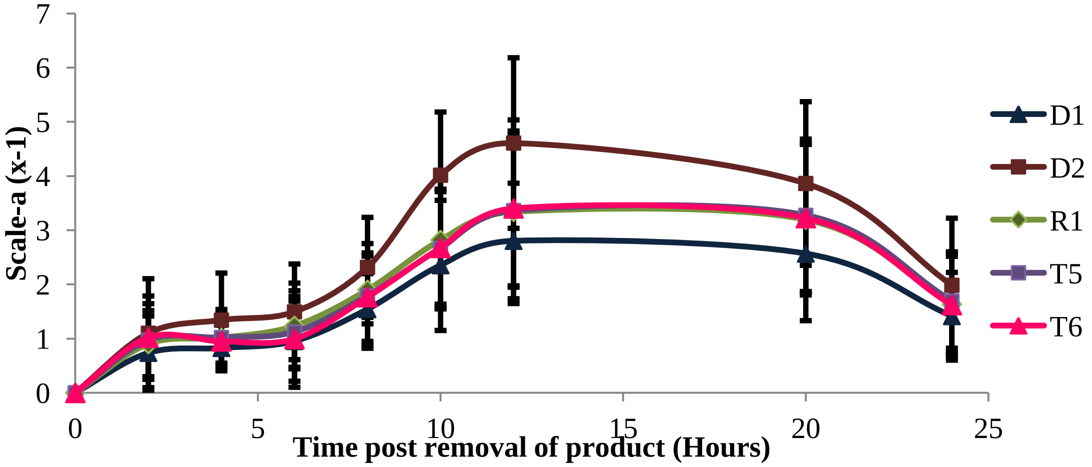


Figure 9.16 Blanching profiles (chromameter data) for formulations T_5 and T_6 for “Detectors” (n=9) in Group 2

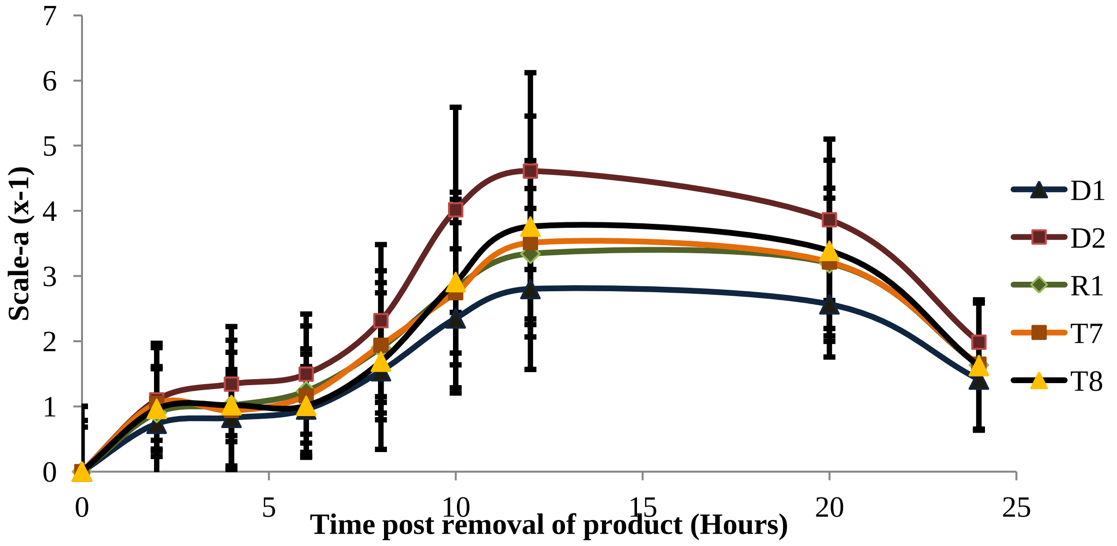


Figure 9.17 Blanching profiles (chromameter data) for formulations T_7 and T_8 for "Detectors" (n=9) in Group 2

The average blanching profiles (visual and chromameter) for CP formulations tested using Group 2 indicates that from 2 hours following product removal, blanching commenced and increased until maximum blanching peak at 12 hours following product removal. The blanching response faded slowly up to 24 hours following product removal. The large standard deviation bars on the profiles may be due a consequence of the small number of subjects used in the study. This study was undertaken to establish whether a design space could be established *in vivo* and the small sample size used should be adequate to determine whether the cream formulations tend towards BE against the reference product. The mean visual and chromameter profiles reveal that formulations $T_5 – T_8$ exhibited a similar blanching profile to formulation $R_{1.}$ The test formulations were manufactured at the extreme limits of cetostearyl alcohol and glyceryl monostearate content of 7.5% w/w and 10.0% w/w and 10.9% w/w and 12.8% w/w respectively to establish whether they were to Dermovate®. The AUEC data summarized in Table 9.5 reveal that these formulations $T_5 – T_8$ exhibited similar blanching effects to formulation R_1.

Table 9.5 AUEC data for the visual and chromameter profiles for "detectors"

	Visual (n=9)					Chromameter (n=9)				
	T_5	**T_6**	**T_7**	**T_8**	**R_1**	**T_5**	**T_6**	**T_7**	**T_8**	**R_1**
Mean AUEC	425.416	425.138	419.153	423.201	420.139	24.694	25.136	25.016	24.931	25.081
SD	190.614	193.960	190.400	184.23	188.40	8.429	9.053	8.577	8.424	8.59
C.V%	44.82	45.62	45.42	43.53	44.84	34.14	36.01	34.29	33.79	34.23

Locke's method was used to calculate a CI based on Fieller's Theorem for the visual and chromameter AUEC data for Group 2. The 90% CI was calculated for the point estimate of the ratio of the average

AUEC response of the test products versus the reference product calculated using Locke's method, as are shown in Table 9.6.

Table 9.6 90% CI for visual and chromameter AUEC data using Locke's method

	Locke's Method (90% CI) (n=9)							
Formulation	**Visual**				**Chromameter**			
	% Mean Ratio	**%C.V**	**90% CI**	**Summary**	**% Mean Ratio**	**%C.V**	**90% CI**	**Summary**
T_5/R_1	101.3	3.09	99.65 – 102.89	BE	99.36	6.2	96.34 – 102.67	BE
T_6/R_1	101.2	3.24	99.25 – 102.88	BE	99.0	4.8	96.36 – 101.69	BE
T_7/R_1	99.8	1.05	98.99 – 100.33	BE	101.3	3.4	97.92 – 104.04	BE
T_8/R_1	103.8	2.61	96.56 – 114.31	BE	100.5	3.5	97.95 – 102.93	BE
T_5/T_6	100.0	5.74	97.42 – 103.06	BE	98.4	2.9	96.79 – 100.14	BE
T_5/T_7	101.5	3.44	99.63 – 103.61	BE	99.3	5.3	97.12 – 102.18	BE
T_5/T_8	96.3	3.78	87.48 – 104.55	BE	100.1	4.1	98.10 – 102.23	BE
T_6/T_7	101.4	3.53	99.51 – 103.23	BE	97.7	4.4	95.60 – 100.62	BE
T_7/T_8	94.90	3.22	86.24 – 102.74	BE	100.4	3.8	86.24 – 102.74	BE
T_6/T_8	96.3	3.33	87.09 – 104.59	BE	98.6	2.4	97.48 – 99.72	BE

The visual and chromameter data for the nine "detectors" revealed that all cream formulations had a point estimate that fell in the range 80% - 125% and therefore all four test formulations manufactured at the extreme limits of cetostearyl alcohol and glyceryl monostearate content met the BE limits and were therefore bioequivalent to Dermovate® cream. A comparison between test products also demonstrated that the point estimate for the test products *viz.*, comparing T_5 to T_6 also fell into the CI to establish BE.

9.5. CONCLUSIONS

TC have the ability to induce blanching following application and through the use of this pharmacodynamics response, the HSBA can be used to demonstrate *in vivo* BE of topical formulation using this approach as recommended by FDA [5,7]. The HSBA was used to collect data using visual and chromameter approaches to assess the BE to Dermovate® of the eight test formulations manufactured from information generated using design space network. *In vitro* release studies showed that test formulations with different propylene glycol, Gelot® 64, cetostearyl alcohol and glyceryl monostearate content exhibited responses and *in vitro* release characteristic that were similar to the reference product.

BE assessment was undertaken in two study groups *viz.*, Group 1 and Group 2. Four formulations manufactured at extreme excipient limits *viz.*, propylene glycol and Gelot® 64 at 44.0% v/v and 46.4% v/v) and 2.9% w/w and 3.8% w/w were tested using subjects in Group 1 whereas the other test formulations with cetostearyl alcohol content of 7.5% w/w and 10.0% w/w and glyceryl monostearate content of 10.9% w/w and 12.8% w/w were tested using subjects in Group 2. Each group consisted of 10 subjects who had 14 application sites demarcated on each arm of which two were control sites. The small sample size of subjects might have led to variability in the data but the focus of this study was essentially a proof of concept of establishing a design space for BE for formulations generated using design space studies. The dose durations for Dermovate® were 20 minutes (D_1), 40 minutes (ED_{50}) and 80 minutes (D_2). For both groups, the mean visual and chromameter blanching response profiles for different dose durations revealed that as the dose duration increased, so did the intensity of blanching observed. The blanching profiles for ED_{50} showed an intermediate blanching response that fell between those observed for D_1 and D_2. The AUEC for the dose duration profiles was calculated using the trapezoidal rule for individual subjects and the ratio of $\frac{\mathrm{AUEC}\ \mathrm{D}_2}{\mathrm{AUEC}\ \mathrm{D}_1}$ for subjects in Group 1 indicated that the ten subjects were "detectors" as defined as the ratio for this parameter was ≥ 1.25. However for Group 2, volunteer 17 was a "non detector" with ratio < 1.25.

The mean visual and chromameter blanching profiles observed for both groups revealed that maximum blanching occurred at 12 hours following product removal. The graphical representation of the blanching curves for formulations T_1 –T_8 reveal that all test formulations exhibited similar blanching profiles to the reference product, Dermovate®. This indicates that the eight formulations manufactured using the limits of excipient levels may be bioequivalent to Dermovate®. Locke's method was used to calculate the 90% CI to confirm BE of the test formulations established through the development of a design space. The visual and chromameter AUEC data generated for "detector" subjects confirmed that the eight design space formulations were bioequivalent to Dermovate®. This BE study has therefore confirmed that the formulation limits generated from the design space network developed using RSM was accurate. The findings of this research on design space development for CP cream formulations has clearly indicated that high quality CP cream formulations can be manufactured within an acceptable range for key excipients and will still be bioequivalent to Dermovate®. It is therefore feasible that this design space network can facilitate the production of products of high quality with limited wastage of materials, thereby reducing cost and time with minimal product failure.

CHAPTER TEN

CONCLUSIONS

The pharmaceutical industry is under pressure as a consequence of numerous factors including pricing, demands and control, regulatory constraints and interference, shrinking research pipelines due to less innovation, outsourcing, investment challenges, increasing research and development costs, long approval times, growth of the generic industry, parallel importation and increasing counterfeiting of medicines [11,12,555]. The reduced spend on research and development together with severe financial constraints such as those imposed by government-run healthcare systems, health insurance and the government debt crisis present a perfect opportunity for generic companies to expand and build their capability to facilitate seizure of large sectors of the global market [24]. Access to affordable medicines especially generic medications therefore play an important role in assisting developing nations deal with non-communicable infectious and related diseases. However increasing availability and accessibility to affordable pharmaceuticals for populations in developing countries is still a key challenge for many governments [44,49].

Due to the numerous challenges faced by the pharmaceutical industry, FDA decided to focus on "Pharmaceutical Quality for the 21st Century: A Risk-Based Approach" in a report published in 2007 [6]. For several years, FDA has implemented the concept of QbD into its pre-market processes since this approach ought to reduce the amount of product waste as a result of errors that occur frequently in the manufacturing sector, thereby ultimately reducing the cost and time of manufacturing. This idea ought to achieve reasonable access to product quality with a minimal risk of failure. QbD is a systematic approach for the development of high quality pharmaceutical dosage forms that begins with establishing predefined objectives based on the premise that quality must be built and not tested into a product [127]. QbD together with design space establishment for dosage forms is fairly new concept [101]. There is a dearth of information on the concept of QbD that has been published and that has evaluated the entire process of identifying CQA, designing formulation and processes to meet product CQA, understanding the impact of material attributes and process parameters on product CQA, identifying and controlling sources of variability in material and process product CQA and finally establishing, evaluating and testing a design space using *in vitro* and *in vivo* assessments to assure that a product of consistent quality can be manufactured. The overall objective of this research was to implement a QbD approach to establish a design space for the manufacture of safe, effective, stable generic topical formulation containing 0.05% w/w clobetasol 17-propionate that has similar *in vitro* and *in vivo* characteristics to Dermovate® (Sekpharma® Pty Ltd, Sandton, Gauteng, RSA), the innovator product commercially available in RSA. The production of such a product would pose minimal risk of failure and would be suitable for the treatment of severe skin disorders such as seborrhoeic dermatitis, extreme photodermatitis and/or severe psoriasis that is often observed in HIV/AIDS patients in Southern Africa.

CP is an ultra-high-potency compound that is used to treat numerous HIV-related skin disorders such as HIV-associated psoriasis, atopic eczema and extreme photodermatitis [3,193]. CP possesses anti-inflammatory, anti-pruritic and anti-proliferative properties and is usually formulated at a dose of 0.05% w/w in topical formulations [556]. CP is commercially available in cream, ointment, gel, solution, foam and emollient cream. CP creams that are commercially available on the South African market include Dermovate® (reference product), Dovate® (generic product) and Xenovate® (generic product) [557].

A RP-HPLC method for the quantitation of CP in cream formulations was developed and the optimal conditions for a separation were achieved with the aid of CCD and RSM. The RP-HPLC method was validated according to ICH guidelines and used for *in vitro* assessment of CP in topical dosage forms. ANOVA at a 95% level of significance was used to study the effects of MeOH content, flow rate and column temperature on the retention time of CP and BV, the asymmetry factor of CP, the resolution factor for CP and BV and HPLC run time. These studies were necessary to establish the optimal conditions that would result in an appropriate chromatographic separation with a short analytical run time. The quadratic response surface model that was used to describe the relationship between input variables and the chromatographic responses revealed that MeOH content and flow rate had a significant effect on HPLC responses with MeOH content, the most significant factor. An increase in MeOH concentration resulted in a decrease in the retention time of CP and BV, the asymmetry factor for CP, the resolution factor for CP and BV and the run time. An increase in flow rate resulted in a slight decrease in the HPLC responses that were monitored. Column temperature on the other hand has almost no effect on HPLC responses for CP and BV. These relationships were confirmed through the use of response surface plots *viz.,* contour and 3-Dimensional plots. The optimal conditions for HPLC analysis of CP were predicted using the optimization function of Design-Expert® software and the experimental responses were found to be close to the predicted values for the optimized HPLC method. The optimum HPLC conditions were a MeOH content of 65% v/v, a flow rate of 1.0 mL/min and a column temperature of 25ºC. The use of these conditions yielded a retention time of 6.00 minutes for CP and 8.00 minutes for BV, an asymmetry factor of 1.05 for CP, a resolution factor of 2.00 with an analytical run time of 10 minutes.

The validated RP-HPLC method was linear over the range of 0.25 - 15 µg/mL with a coefficient of determination of 1.000. Statistical analysis confirmed that the method was precise and reproducible with adequate inter- or intra-day precision with % RSD values < 2% for these parameters, indicating that there was no significant difference between CP:BV PHR for low, medium and high concentration samples analyzed over three consecutive days. The RP-HPLC method was found to be accurate with a % bias ranging between -2.79% and 0.58% for all samples tested. CP was found to be stable in MeOH at 4ºC and 22ºC for 14 days, permitting the storage of samples in this solvent at these temperatures for up to 14 days prior to analysis. The RP-HPLC method developed and validated was used to establish whether formulation decisions made in the establishment of a design space for generic CP cream formulations were appropriate.

QbD requires a scientific approach to identify desired dosage form and performance attributes through establishing a TPP and through use of the TPP, an initial list of CQA was developed. A risk assessment was undertaken to identify possible formulation variables and unit operations that were most likely to have an impact on the CQA of the product and/or manufacturing process. This data was then used to focus formulation and process development activities on potential high risk areas. An Ishikawa diagram was used to conduct risk assessment evaluations and this allowed for an exhaustive analysis to be conducted that included all factors that could have had an impact on product quality to be considered. Risk analysis commenced with an assessment of the physico-chemical characteristics of CP that led to the identification of a viable formulation and manufacturing approach. It was established that the impact of excipient attributes of glyceryl monostearate, cetostearyl alcohol, Gelot® 64, propylene glycol and manufacturing parameters such as homogenization speed, homogenization time, anchor speed, mixing time, heating temperature, batch size and cooling time could have a significant impact on the ultimate quality of the cream formulations that were manufactured.

Process validation according to ICH and FDA guidelines was undertaken as a means of ensuring that manufacturing processes were operated within specified design parameters and that they were capable of reliably producing a finished product of the desired quality on a repetitive basis. The critical parameters evaluated during process validation of the manufacturing process of pilot scale cream formulations were the mixing, homogenization and cooling unit operations. It was observed that the % CP content for three batches of cream were between > 90% and < 115% which is well within the acceptance criteria following mixing. The homogenization step for the three batches was validated for high, low and optimum rpm and the physical parameters for the creams were found to be within tight limits and accurate with % RSD < 5% for all test results. Therefore the homogenization process was considered validated and appropriate for this product. The cooling periods of 60 and 120 minutes revealed that there was no formation of congealed product materials at any location sampled in the mixing bowl and the % CP content for the batches was well within the predefined acceptance limits. Therefore it was established that cooling did not appear to have an impact on content uniformity of CP creams.

Due to the large number of excipient and process parameters that may impact quality of cream formulations, experimental screening designs were used to identify significant factors from a long list of potential variables that may impact product quality. Three screening designs commonly used in the pharmaceutical industry *viz.*, Taguchi, Plackett-Burman and 2-Level Fractional Factorial DOE were evaluated to compare their effectiveness for optimization of formulations and processing for cream manufacture. The TD and PBD designs required 12 runs whereas the 2-LFFD required a minimum of 16 runs. The eleven variables investigated were % v/v propylene glycol, % w/w cetostearyl alcohol, % w/w glyceryl monostearate, % w/w Gelot® 64, mixing time, anchor speed, homogenization time and speed, heating temperature, batch size and cooling time. The viscosity, spreadability, pH, % CP content, tube extrudability, electrical conductivity and *in vitro* CP released over 2, 4, 8, 12, 24, 48 and 72 hours were the thirteen response variables monitored in these studies. The formulations were manufactured using a Wintech® cream/ointment plant (Wintech® Pharmachem Equipment PVT, Ltd, Mumbai, India). ANOVA revealed that a correlation between the formulation and process variables and the cream responses was most suitably described by use of linear and two factorial models. Linear and two factorial model equations were generated for each response observed from the screening designs and statistical analysis was undertaken using ANOVA, evaluation of the regression coefficient and by evaluation of Main Effect and Pareto plots. Of the thirteen formulation responses, spreadability, electrical conductivity and cumulative % CP released over 2, 12, 48 and 72 hours were found to be significant with P-values < 0.05. However each statistical design used generated different significant responses with the TD identifying at least ten significant responses and this approach was found to be a less selective statistical model, in comparison to the other two designs which revealed only eight significant responses. Furthermore the PBD and 2-LFFD designs generated low coefficients of variation for most of responses monitored suggesting that these approaches may generate more accurate models in comparison to a TD approach. The Main Effect and Pareto plots confirmed that formulation parameters had the most significant effect on creams when compared to process related parameters. Cetostearyl alcohol was the formulation variable that had the most significant effect on the quality of the resultant formulations. An increase in cetostearyl alcohol content resulted in an increase in viscosity, a decrease in spreadability and a decrease in the cumulative % CP released over time. Gelot® 64 and glyceryl monostearate had secondary yet significant effects on formulation responses and propylene glycol showed a slight effect for all three designs. Of all manufacturing and mixer variables evaluated, homogenization and mixing times had at least some significant effect on the responses monitored.

A CCD in conjunction with a RSM approach was used to further investigate the formulation variables for product design optimization. The % v/v propylene glycol, % w/w Gelot® 64, % w/w cetostearyl alcohol and % w/w glyceryl monostearate were the formulation variables selected to establish their impact on the formulation responses monitored *viz.*, the viscosity, spreadability, pH, % CP content, tube extrudability, electrical conductivity and *in vitro* CP released over 2, 4, 8, 12, 24, 48 and 72 hours. The thirty cream batches were manufactured using a Wintech® cream/ointment plant (Wintech® Pharmachem Equipment PVT, Ltd, Mumbai, India). A correlation between formulation variables and cream responses that was best described using quadratic polynomial models was found to exist. The most significant responses were viscosity, spreadability, CP released at 12, 24, 48 and 72 hours with P-values < 0.05. The 2-D and 3-D plots confirmed that cetostearyl alcohol was the formulation variable that had the most significant effect on the ultimate quality of the resultant cream formulations. The other formulation variable that had a similar significant effect was glyceryl monostearate. These two formulation variables usually exhibit dual roles as they can act as emulsifiers and/or viscosity modifying agents. Propylene glycol was found to have the least significant effect on formulation responses, however propylene glycol cannot be excluded from these formulations as it is vital for solubility of CP and acts as a penetration enhancer for topical formulations. The pH of the creams ranged between 4 and 7 suggesting that it is unlikely that any skin irritation would occur during use of this product. The content uniformity of the 30 batches of product produced in this study ranged between 98% and 104% and therefore met compendial limits for this parameter.

Design-Expert® software was used to determine the composition of the optimized formulation that would have similar *in vitro* release profiles and formulation responses to those observed for Dermovate®. The difference, f_1 and similarity, f_2 and S_d factors were used to compare the release rate profiles of CP cream formulations to that generated for the reference product and the values of f_1, f_2 and S_d = 0.5, 99.9 and 0.0001 calculated for the optimized formulation respectively clearly indicate that the optimized *in vitro* release profile is similar to that of the reference product. The optimized formulation consisted of approximately 46.0% v/v propylene glycol, 8.6% w/w cetostearyl alcohol, 10.5% w/w glyceryl monostearate and 3.8% w/w Gelot® 64. CP release from the optimized formulation followed Higuchi release kinetics with a R^2 value of > 0.99 that was similar to that of Dermovate® and therefore a diffusion-controlled release mechanism appears to be the dominant factor controlling CP release from this formulation.

RSM was further used to establish a design space for CP cream formulations. The *in vitro* release profile of Dermovate® was used to establish the constraints for this study and to determine the limits of the level of each key excipient that would result in the manufacture of a cream that was similar to Dermovate® in terms of formulation responses and bioequivalence. The impact of the levels of % v/v propylene glycol, % w/w Gelot® 64, % w/w cetostearyl alcohol and % w/w glyceryl monostearate on the extent of CP released from the cream formulations at different times was determined from constraint plots generated through RSM procedures. The lower and upper limits of propylene glycol, Gelot® 64, cetostearyl alcohol and glyceryl monostearate content were established as 44.0% v/v and 46.4% v/v, 2.9% w/w and 3.8% w/w, 7.5% w/w and 10.0% w/w and 10.9% w/w and 12.8% w/w respectively. The products manufactured with excipient levels at either the extreme lower and upper limits produced *in vitro* release profiles similar to that of reference product and suggest that these test products may be bioequivalent to Dermovate®. The creams were also placed on stability at 40 ± 2°C, 75 ± 5% RH and 25 ± 2°C, 60 ± 5% RH for a period of eight weeks to assess short term stability. These design space batches were found to be stable over a period of eight weeks, exhibited formulation responses similar to the Dermovate® for that period of time and were therefore selected for evaluation in *in vivo* studies to confirm BE.

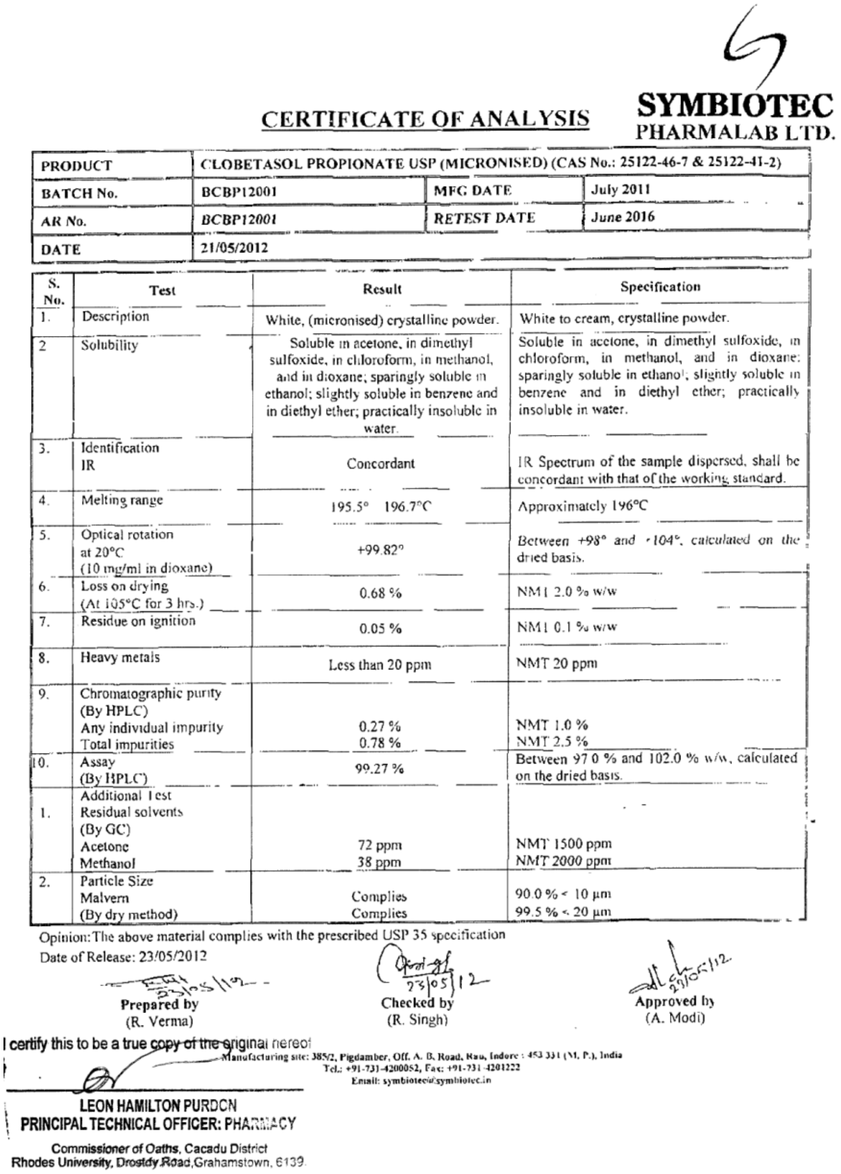
The HSBA using visual and chromameter data collection was used to establish BE of the test formulations (to Dermovate®) developed and produced by use of the design space network reported in this thesis. Eight formulations containing either 44.0% v/v or 46.4% v/v propylene glycol, 2.9% w/w or 3.8% w/w Gelot® 64, 7.5% w/w or 10.0% w/w cetostearyl alcohol and 10.9% w/w or 12.8% w/w glyceryl monostearate were manufactured and compared on the basis of product characteristics and *in vitro* release profiles to those of the reference product. Two groups, each containing ten subjects participated in the BE study. Group 1 was used to assess the formulations containing different levels of propylene glycol and Gelot® 64 and Group 2 was used to assess formulations containing different levels of cetostearyl alcohol and glyceryl monostearate. All participants from Group 1 were classified as "detectors" with a ratio $\frac{\text{AUEC D}_2}{\text{AUEC D}_1} \geq 1.25$ and for those of Group 2, one of the ten subjects was classified as "non detector" and the data for that subject were not used for analysis. The mean AUEC visual and chromameter profiles for the CP cream formulations for both groups revealed that maximum blanching occurred at 12 hours following product removal. Graphical representation of the blanching curves for formulations T_1 –T_8 reveal that the test formulations exhibited similar blanching profiles to Dermovate® suggesting that the eight formulations manufactured were bioequivalent to Dermovate®. Locke's method was used to calculate the 90% CI to confirm BE of the CP formulations manufactured according to the established design space. The visual and chromameter data generated for the "detectors" confirmed that the eight design space formulations were BE to Dermovate®. This BE study therefore confirmed that the excipient limits generated from the design space network using RSM did not affect BE suggesting that products manufactured with a variable content of these excipients within the defined limits are unlikely to affect the efficacy of the product.

The studies conducted and reported in this thesis define a route for the design, development and assessment of high quality cream formulations using a QbD approach. This path introduced the concept of quality built into a topical pharmaceutical product and process by design, based on scientific understanding and management of sources of variability. The QbD approach was found to be proactive and involved experiments using multivariate approaches with subsequent optimization of the formulation using several different statistical approaches. Risk assessment and analysis was found to be fundamentally crucial approach to reduce possible failure and to meet quality targets. The design space established using a RSM technique resulted in the development of acceptable limits for the range of content of excipients and that would still produce *in vitro* release profiles similar to the reference product. The eight formulations identified and manufactured using extreme limits of key excipients were tested *in vitro* and *in vivo* and exhibited BE characteristics similar to that of the reference product. Therefore cream formulations manufactured within the limits of this design space should always be BE to Dermovate®. The value of such data generated using a QbD approach in the commercial sector would ensure regulatory flexibility and minimize delays in making formulation and process changes within defined limits and would result in the production of high quality cream formulations with less wastage, cost, time and minimal product failure. The generic formulations produced in these studies high quality, are stable and would be suitable for the treatment of HIV-related skin disorders in patients. Future studies would include the assessment of other statistical designs and/or artificial neural network simulation to establish and validate a design space for topical formulations, to test the design space formulations both *in vitro* and *in vivo* and to identify limitations with regards to different statistical modeling.

APPENDIX I

CERTIFICATES OF ANALYSIS AND QUALITY CONTROL DOCUMENTS

The Certificates Of Analysis (COA) and Quality Control (QC) documents for clobetasol 17-propionate are included in this appendix. The COA and QC documents for propylene glycol, sodium citrate, anhydrous citric acid, white beeswax, glyceryl monostearate, cetostearyl alcohol, Gelot® 64 and chlorocresol have not been included in this appendix but are included on the accompanying electronic file "Appendices-CD" on the CD. The COA for clobetasol 17-propionate was obtained from Symbiotec PharmaLab® Ltd (Indore, India) in May 2012 when the raw material was purchases. QC testing was performed for CP in November - December 2012.



CERTIFICATE OF ANALYSIS

SYMBIOTEC PHARMALAB LTD.

PRODUCT	CLOBETASOL PROPIONATE USP (MICRONISED) (CAS No.: 25122-46-7 & 25122-41-2)		
BATCH No.	BCBP12001	MFG DATE	July 2011
AR No.	*BCBP12001*	*RETEST DATE*	*June 2016*
DATE	21/05/2012		

S. No.	Test	Result	Specification
1.	Description	White, (micronised) crystalline powder.	White to cream, crystalline powder.
2	Solubility	Soluble in acetone, in dimethyl sulfoxide, in chloroform, in methanol, and in dioxane; sparingly soluble in ethanol; slightly soluble in benzene and in diethyl ether; practically insoluble in water.	Soluble in acetone, in dimethyl sulfoxide, in chloroform, in methanol, and in dioxane; sparingly soluble in ethanol; slightly soluble in benzene and in diethyl ether; practically insoluble in water.
3.	Identification IR	Concordant	IR Spectrum of the sample dispersed, shall be concordant with that of the working standard.
4.	Melting range	195.5° 196.7°C	Approximately 196°C
5.	Optical rotation at 20°C (10 mg/ml in dioxane)	+99.82°	*Between +98° and +104°, calculated on the* dried basis.
6.	Loss on drying (At 105°C for 3 hrs.)	0.68 %	NMT 2.0 % w/w
7.	Residue on ignition	0.05 %	NMT 0.1 % w/w
8.	Heavy metals	Less than 20 ppm	NMT 20 ppm
9.	Chromatographic purity (By HPLC) Any individual impurity Total impurities	 0.27 % 0.78 %	 NMT 1.0 % NMT 2.5 %
10.	Assay (By HPLC)	99.27 %	Between 97.0 % and 102.0 % w/w, calculated on the dried basis.
	Additional Test		
1.	Residual solvents (By GC) Acetone Methanol	 72 ppm 38 ppm	 NMT 1500 ppm *NMT 2000 ppm*
2.	Particle Size Malvern (By dry method)	 Complies Complies	 90.0 % < 10 µm 99.5 % < 20 µm

Opinion: The above material complies with the prescribed USP 35 specification

Date of Release: 23/05/2012

23/05/12
Prepared by
(R. Verma)

23/05/12
Checked by
(R. Singh)

23/05/12
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I certify this to be a true copy of the original hereof

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RHODES UNIVERSITY, Faculty of Pharmacy,
Department of Pharmaceutics & Pharmaceutical Chemistry
Grahamstown 6140, REPUBLIC OF SOUTH AFRICA

Quality Control Document

Document Number: CP_QC_Version 1 **Effective Date: 30 May 2012 08:30:00**

Title: Clobetasol 17-propionate_2012

Item Name	Clobetasol 17-propionate (CP)		
Item Number	RM000150		
Batch Number	BCBP12001		
Analysis Start Date	29 May 2012	**Analysis End Date**	29 May 2012

*	Test	Specification	Result	Analyst Name & Initials	Checked by Name & Initials
	Characters				
*	Appearance	White (micronized) crystalline powder	Complies		
	Identification				
*	Infra Red Spectroscopy	-	Complies		
	Optical Rotation	98º-104º	+99.82º		
*	**Loss on Drying**	NMT 2.0% w/w	0.68%		
	Residue on Ignition	NMT 0.1% w/w	0.05%		
	Heavy Metals	NMT 20 ppm	< 20 ppm		
	Chromatographic Purity **Any Individual Impurity** **Total Impurities**	NMT 1.0% NMT 2.5%	0.27% 0.78%		
*	**Assay by HPLC**	97.0%-102.0%	99.8%		
	Particle Size **Malvern** **Dry Method**	NMT 1500 ppm NMT 2000 ppm	72 ppm 38 ppm		

1	Description	Identification	Infra Red	Loss on Drying	Assay by HPLC	Certificate of Analysis	BP Validation	USP Validation
CP	Complies	Complies	Complies	Complies	Complies	√	-	-

- Rhodes University, Faculty of Pharmacy, Department of Pharmaceutics and Pharmaceutical Chemistry to identify with * the tests to be performed
- Tests marked with * to be performed by Rhodes University Faculty of Pharmacy, Department of Pharmaceutics and Pharmaceutical Chemistry
- Tests not marked with * may have not been able to be performed due to lack of equipment in the facility or may be taken from the certificate of analysis

APPENDIX II

BATCH PRODUCTION RECORDS

SCREENING DESIGN MANUFACTURE

TAGUCHI, PLACKETT-BURMAN AND 2-LEVEL FRACTIONAL FACTORIAL DESIGNS

A representative of a sample of the batch production record for the Taguchi design *viz.*, Batch TD-CP-01 manufactured using a Wintech® cream/ointment mixer for CP screening design cream formulations is included in this appendix. The samples for the batch production records for the Plackett-Burman and 2-Level Fractional Factorial designs *viz.,* PBD-CP-01 and 2-LFFD-CP-01 have not been included in this appendix but are included on the accompanying electronic file "Appendices-CD" on the CD. The batch production records for all the other batches *viz.*, TD-CP-02 – TD-CP-12, PBD-CP-02 – PBD-CP-12 and 2-LFFD-CP-02 – 2-LFFD-CP-16 are available on request.

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140, REPUBLIC OF SOUTH AFRICA

BATCH RECORD PRODUCTION

Product name: Taguchi Design Clobetasol 17-Propionate Cream Page 1 of 3
Batch number: TD-CP-01 **Batch Size**: 6000 g

MANUFACTURING APPROVALS

Batch record issued by:_________________ **Date:_________________**

Master record issued by:_________________ **Date:_________________**

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics
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BATCH PRODUCTION RECORD

Product name: Taguchi Design Clobetasol 17-Propionate Cream Page 2 of 3
Batch number: TD-CP-01 **Batch Size**: 6000 g

MASTER FORMULA AND BATCH FORMULA							
Item no.	**Material**	**Rhodes No.**	**Quantity (% w/w)**	**Amount/ Batch**	**Amount Dispensed**	**Dispensed by**	**Checked by**
1	CP	RM000150	0.05	4.50 g			
2	Propylene glycol	RM000181	51.00	2870.05 mL			
3	Sodium citrate	RM000183	0.05	4.50 g			
4	Citric acid	RM000185	0.05	4.50 g			
5	Gelot® 64	RM000177	5.00	281.40 g			
6	Glyceryl monostearate	RM000182	16.00	900.56 g			
7	Cetostearyl alcohol	RM000184	3.00	168.85 g			
8	White beeswax	RM000142	1.15	64.72 g			
9	Chlorocresol	RM000186	0.075	4.22 g			
10	Distilled water	N/A	30.225	1701.21 mL			

WINTECH® CREAM/OINTMENT MIXER VERIFICATION			
Description	**Type**	**Verified by**	**Confirmed by**
Bowl vessel	Stainless steel		
Co-centric Homogenizer	Teflon and stainless steel		
Anchor and impellers	Stainless steel		
Water inlet	Stainless steel		
Water Jacket	Stainless steel		
Charging port	Stainless steel		
Water immersion heater	NA		
PT-100 sensor	NA		
Operating panel touch screen	LCD		

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics
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BATCH PRODUCTION RECORD

Product name: Taguchi Design Clobetasol 17-Propionate Cream Page 3 of 3
Batch number: TD-CP-01 **Batch Size:** 6000 g

MANUFACTURING PROCEDURE				
Steps	**Procedure**	**Time**	**Done by**	**Checked by**
1	Clean the bowl with Liquid K® detergent and distilled water. Allow the bowl to dry and equilibrate at room temperature Room temperature: _____°C Mixer temperature: _____°C			
2	Fill the jacket with distilled water until the jacket is 30 Liters			
3	Weigh all the materials accurately using a Model AE 163 Mettler® analytical balance (Mettler® Inc., Zurich, Switzerland)			
4	Introduce 2870.05 mL propylene glycol and 1701.21 mL distilled water, followed by 4.50 g sodium citrate, 4.50 g citric acid, 4.50 g clobetasol 17-propionate, 900.56 g glyceryl monostearate, 168.85 g cetostearyl alcohol, 64.72 g white beeswax, 281.40 g Gelot® 64 and 4.22 g chlorocresol through the charging port			
5	Allow mixture of excipients to stir at an anchor speed of 20 rpm for 60 minutes at 65°C			
6	Following mixing, allow the blend to be homogenized at 1000 rpm for 120 minutes			
7	Allow the molten mixture to cool by passing distilled water through the heating jacket for 120 minutes Temperature of cold water=____°C			
8	After cooling, close the water inlet and allow slow continuous agitation of the anchor until the temperature on the operating panel displays 30°C - 35°C			
9	Stop the anchor and release the pressure built up in the bowl vessel by the use of the pressure vent			
10	Store the final CP cream in 10 kg opaque containers until quality control tests are completed and then package into 50 g collapsible aluminium cream tubes			

SIGNATURE AND INITIAL REFERENCE			
Full name (Print)	**Signature**	**Initials**	**Date**

APPENDIX III

BATCH RECORD REPORTS

A representative of a sample of the batch record report for each screening design *viz.,* Batches TD-CP-01, PBD-CP-01 and 2-LFFD-CP-01 manufactured using a Wintech® cream/ointment mixer for the CP screening design cream formulations is included in this appendix. The batch record reports for all the other formulation batches, *viz.,* TD-CP-02 – TD-CP-12, PBD-CP-02 – PBD-CP-12 and 2-LFFD-CP-02 – 2-LFFD-CP-16 have not been included in this appendix but are included on the accompanying electronic file “Appendices-CD” on the CD.

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140, REPUBLIC OF SOUTH AFRICA

Batch Record Summary

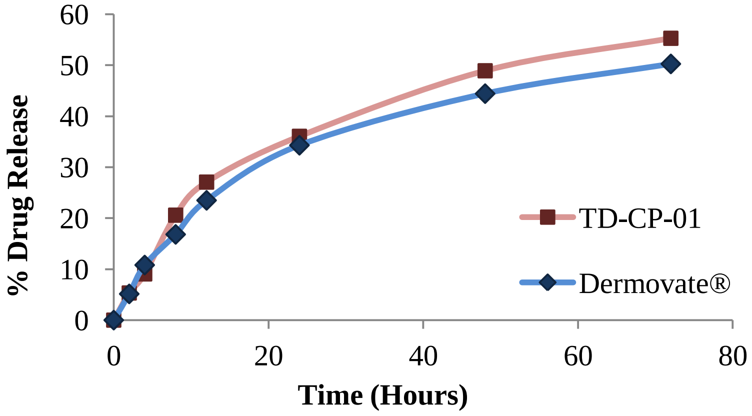
Formulator : Ayeshah Fauzee
Product : Taguchi Design Clobetasol 17-Propionate Cream
Batch Number: TD-CP-01

Date of Manufacture :10/04/2013
Melting Temperature :65 °C
Batch Size : 6000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	4.50 g	RM000150
Propylene glycol	51.00	2870.05 mL	RM000181
Sodium citrate	0.05	4.50 g	RM000183
Citric acid	0.05	4.50 g	RM000185
Gelot® 64	5.00	281.40 g	RM000177
Glyceryl monostearate	16.00	900.56 g	RM000182
Ceteostearyl alcohol	3.00	168.85 g	RM000184
White beeswax	1.15	64.72 g	RM000142
Chlorocresol	0.075	4.22 g	RM000186
Distilled water	30.225	1701.21 mL	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenization Speed (rpm)	1000
Homogenization Time (min)	120
Anchor Speed (rpm)	20
Mixing Time (min)	60
Heating Temperature (°C)	65
Cooling time (min)	120

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	37000.00 ± 550	1.49
Spreadability (cm^2)	27.00 ± 0.60	2.22
CP Content (%)	100.10 ± 1.50	1.50
pH	6.54 ± 0.08	1.22
Extrudability (g/cm^2)	352.40 ± 1.52	0.43
Electrical Conductivity (µS/cm)	490.30 ± 7.30	1.48



OBSERVATIONS/COMMENTS

-A homogeneous smooth white cream was produced

- No sign of cracking or bleeding was observed during or after manufacture

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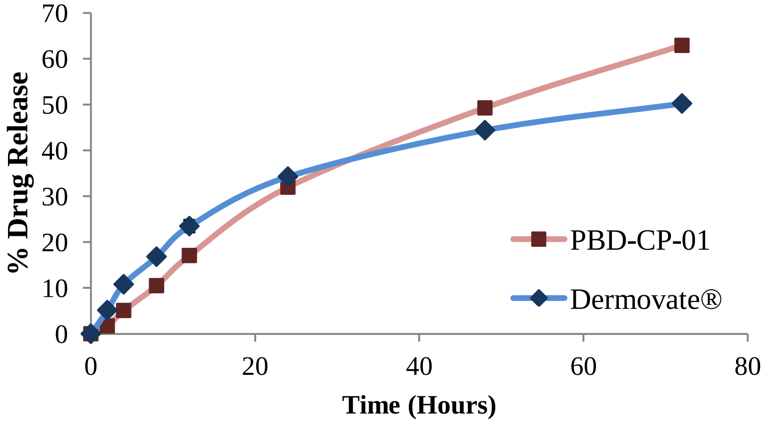
Batch Record Summary

Formulator : Ayeshah Fauzee **Date of Manufacture** :20/04/2013
Product : Plackett-Burman Design Clobetasol 17-Propionate Cream **Melting Temperature** :65 °C
Batch Number: PBD-CP-01 **Batch Size :** 6000 g

Excipient Name	**Original Formula(% w/w)**	**Working Formula**	**Rhodes Batch Number**
CP	0.05	3.24 g	RM000150
Propylene glycol	51.00	3304.54 mL	RM000181
Sodium citrate	0.05	3.24 g	RM000183
Citric acid	0.05	3.24 g	RM000185
Gelot® 64	1.00	64.79 g	RM000177
Glyceryl monostearate	6.00	388.77 g	RM000182
Ceteostearyl alcohol	3.00	194.38 g	RM000184
White beeswax	1.15	74.51 g	RM000142
Chlorocresol	0.075	4.89 g	RM000186
Distilled water	30.225	1958.42 mL	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenization Speed (rpm)	2000
Homogenization Time (min)	60
Anchor Speed (rpm)	40
Mixing Time (min)	60
Heating Temperature (°C)	65
Cooling time (min)	120

Tests Performed	**Mean ± SD (n=3)**	**%RSD**
Viscosity (cP)	5633.00 ± 75	1.33
Spreadability (cm^2)	58.10 ± 0.10	0.17
CP Content (%)	100.38 ± 1.02	1.02
pH	6.65 ± 0.06	0.90
Extrudability (g/cm^2)	57.66 ± 1.02	1.77
Electrical Conductivity (µS/cm)	599.66 ± 4.20	0.70



OBSERVATIONS/COMMENTS

-A homogeneous smooth white cream was produced

- No sign of cracking but slight bleeding after manufacture

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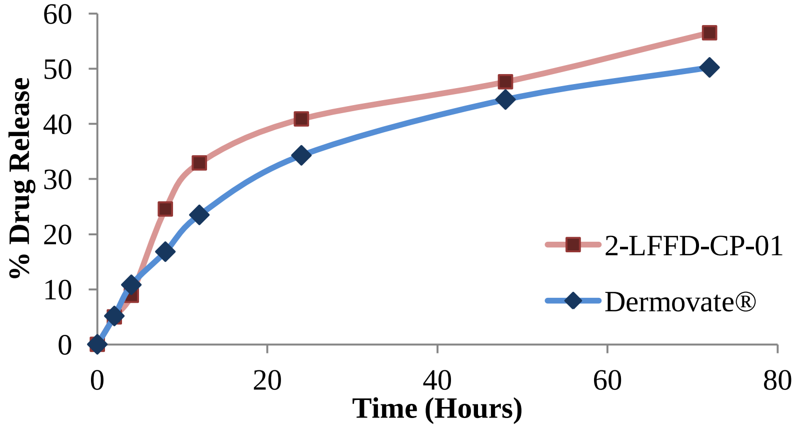
Batch Record Summary

Formulator : Ayeshah Fauzee **Date of Manufacture** :01/05/2013
Product : 2-Level Fractional Factorial Design Clobetasol 17-Propionate Cream
Melting Temperature :75 °C
Batch Number: 2-LFFD-CP-01 **Batch Size :** 6000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	3.24 g	RM000150
Propylene glycol	51.00	3304.54 mL	RM000181
Sodium citrate	0.05	3.24 g	RM000183
Citric acid	0.05	3.24 g	RM000185
Gelot® 64	1.00	64.79 g	RM000177
Glyceryl monostearate	16.00	388.77 g	RM000182
Ceteostearyl alcohol	3.00	194.38 g	RM000184
White beeswax	1.15	74.51 g	RM000142
Chlorocresol	0.075	4.89 g	RM000186
Distilled water	30.225	1958.42 mL	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenization Speed (rpm)	2000
Homogenization Time (min)	120
Anchor Speed (rpm)	20
Mixing Time (min)	60
Heating Temperature (°C)	75
Cooling time (min)	60

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	24780.00 ± 720	2.91
Spreadability (cm^2)	21.51 ± 0.25	1.16
CP Content (%)	99.84 ± 1.02	1.02
pH	6.24 ± 0.01	0.16
Extrudability (g/cm^2)	72.02 ± 2.50	3.47
Electrical Conductivity (µS/cm)	913.66 ± 9.00	0.99



OBSERVATIONS/COMMENTS

-A homogeneous smooth white cream was produced

- No sign of cracking or bleeding was observed during and after manufacture

APPENDIX IV

BATCH PRODUCTION RECORDS

OPTIMIZATION DESIGN MANUFACTURE

CENTRAL COMPOSITE DESIGN

A representative of a sample of the batch production record for the central composite design *viz.*, Batch CCD-CP-01 manufactured using a Wintech® cream/ointment mixer for the optimized CP cream formulation is included in this appendix. The batch production records for all the other batches, *viz.*, CCD-CP-02 – CCD-CP-30 are available on request.

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BATCH RECORD PRODUCTION

Product name: Central Composite Design Clobetasol 17-Propionate Cream Page 1 of 3
Batch number: CCD-CP-01 **Batch Size**: 6000 g

MANUFACTURING APPROVALS

Batch record issued by:_________________ **Date:_________________**

Master record issued by:_________________ **Date:_________________**

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BATCH PRODUCTION RECORD

Product name: Central Composite Design Clobetasol 17-Propionate Cream Page 2 of 3

Batch number: CCD-CP-01 **Batch Size**: 6000 g

MASTER FORMULA AND BATCH FORMULA							
Item no.	**Material**	**Rhodes No.**	**Quantity (% w/w)**	**Amount/ Batch**	**Amount Dispensed**	**Dispensed by**	**Checked by**
1	CP	RM000150	0.05	3.01 g			
2	Propylene glycol	RM000181	46.00	2771.08 mL			
3	Sodium citrate	RM000183	0.05	3.01 g			
4	Citric acid	RM000185	0.05	3.01 g			
5	Gelot® 64	RM000177	3.00	180.72 g			
6	Glyceryl monostearate	RM000182	11.00	662.65 g			
7	Cetostearyl alcohol	RM000184	8.00	481.92 g			
8	White beeswax	RM000142	1.15	69.27 g			
9	Chlorocresol	RM000186	0.075	4.52 g			
10	Distilled water	N/A	30.225	1820.78 mL			

WINTECH® CREAM/OINTMENT MIXER VERIFICATION			
Description	**Type**	**Verified by**	**Confirmed by**
Bowl vessel	Stainless steel		
Co-centric Homogenizer	Teflon and stainless steel		
Anchor and impellers	Stainless steel		
Water inlet	Stainless steel		
Water Jacket	Stainless steel		
Charging port	Stainless steel		
Water immersion heater	NA		
PT-100 sensor	NA		
Operating panel touch screen	LCD		

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BATCH PRODUCTION RECORD

Product name: Central Composite Design Clobetasol 17-Propionate Cream Page 3 of 3
Batch number: CCD-CP-01 **Batch Size**: 6000 g

MANUFACTURING PROCEDURE				
Steps	**Procedure**	**Time**	**Done by**	**Checked by**
1	Clean the bowl with Liquid K® detergent and distilled water. Allow the bowl to dry and equilibrate at room temperature Room temperature: _____°C Mixer temperature: _____°C			
2	Fill the jacket with distilled water until the jacket is 30 Liters			
3	Weigh all the materials accurately using a Model AE 163 Mettler® analytical balance (Mettler® Inc., Zurich, Switzerland)			
4	Introduce 2771.08 mL propylene glycol and 1820.78 mL distilled water, followed by 3.01 g sodium citrate, 3.01 g citric acid, 3.01 g clobetasol 17-propionate, 662.65 g glyceryl monostearate, 481.92 g cetostearyl alcohol, 69.27 g white beeswax, 180.72 g Gelot® 64 and 4.52 g chlorocresol through the charging port			
5	Allow mixture of excipients to stir at 40 rpm for 120 minutes at 75°C			
6	Following mixing, allow the blend to be homogenized at 2000 rpm for 120 minutes			
7	Allow the molten mixture to cool by passing distilled water through the heating jacket for 120 minutes. Temperature of cold water=____°C			
8	After cooling, close the water inlet and allow slow continuous agitation of the anchor until the temperature on the operating panel displays 30°C - 35°C			
9	Stop the anchor and release the pressure built up in the bowl vessel by the use of the pressure vent			
10	Store the final cream in 10 kg opaque containers until quality control tests are completed and then package into 50 g collapsible aluminium cream tubes			

SIGNATURE AND INITIAL REFERENCE			
Full name (Print)	**Signature**	**Initials**	**Date**

APPENDIX V

BATCH RECORD REPORTS

A representative of a sample of the batch record report for the optimization design *viz.*, Batch CCD-CP-01 manufactured using a Wintech® cream/ointment mixer for the CP cream formulations is included in this appendix. The batch record reports for the other optimization design cream formulation batches, *viz.*, CCD-CP-02 – CCD-CP-30 have not been included in this appendix but are included on the accompanying electronic file "Appendices-CD" on the CD.

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140, REPUBLIC OF SOUTH AFRICA

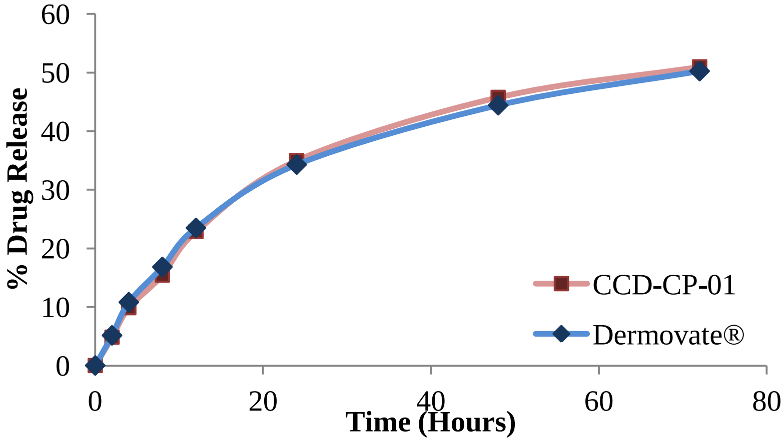
Batch Record Summary

Formulator : Ayeshah Fauzee **Date of Manufacture :** 05/06/2013
Product : Central Composite Design Clobetasol 17-Propionate Cream
Melting Temperature : 75 °C
Batch Number: CCD-CP-01 **Batch Size :** 6000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	3.01 g	RM000150
Propylene glycol	46.00	2771.08 mL	RM000181
Sodium citrate	0.05	3.201g	RM000183
Citric acid	0.05	3.201 g	RM000185
Gelot® 64	3.00	180.72 g	RM000177
Glyceryl monostearate	11.00	662.65 g	RM000182
Ceteostearyl alcohol	8.00	481.92 g	RM000184
White beeswax	1.15	69.27 g	RM000142
Chlorocresol	0.075	4.52 g	RM000186
Distilled water	30.225	1820.78 mL	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenization Speed (rpm)	2000
Homogenization Time (min)	120
Anchor Speed (rpm)	40
Mixing Time (min)	120
Heating Temperature (°C)	75
Cooling time (min)	120

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	44066.00 ± 1520	3.45
Spreadability (cm^2)	24.34 ± 1.20	4.93
CP Content (%)	100.80 ± 1.50	1.48
pH	6.15 ± 0.06	0.98
Extrudability (g/cm^2)	72.02 ± 3.50	4.86
Electrical Conductivity (µS/cm)	200.02 ± 10.20	5.00



OBSERVATIONS/COMMENTS

- A homogeneous smooth white cream was produced

- No sign of cracking or bleeding was observed during and after manufacture

APPENDIX VI

BATCH PRODUCTION RECORDS

DESIGN SPACE MANUFACTURE

A representative of a sample of the batch production record for design space *viz.*, Batch DS-CP-01 manufactured using a Wintech® cream/ointment mixer for the design space CP cream formulations is included in this appendix. The batch production records for the other design space cream formulation batches, *viz.*, DS-CP-02 – DS-CP-08 are available on request.

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140, REPUBLIC OF SOUTH AFRICA

BATCH RECORD PRODUCTION

Product name: Design Space Clobetasol 17-Propionate Cream Page 1 of 3
Batch number: DS-CP-01 **Batch size** : 6000 g

MANUFACTURING APPROVALS

Batch record issued by:__________________ **Date:__________________**

Master record issued by:__________________ **Date:__________________**

BATCH PRODUCTION RECORD

Product name: Design Space Clobetasol 17-Propionate Cream Page 2 of 3
Batch number: DS-CP-01 **Batch size** : 6000 g

MASTER FORMULA AND BATCH FORMULA							
Item no.	**Material**	**Rhodes No.**	**Quantity (% w/w)**	**Amount/ Batch**	**Amount Dispensed**	**Dispensed by**	**Checked by**
1	CP	RM000150	0.05	3.34 g			
2	Propylene glycol	RM000181	44.00	2936.59 mL			
3	Sodium citrate	RM000183	0.05	3.34g			
4	Citric acid	RM000185	0.05	3.34 g			
5	Gelot® 64	RM000177	3.80	253.62 g			
6	Glyceryl monostearate	RM000182	10.50	700.78 g			
7	Cetostearyl alcohol	RM000184	8.60	573.97 g			
8	White beeswax	RM000142	1.15	76.75 g			
9	Chlorocresol	RM000186	0.075	5.01 g			
10	Distilled water	N/A	30.225	2017.24 mL			

WINTECH® CREAM/OINTMENT MIXER VERIFICATION			
Description	**Type**	**Verified by**	**Confirmed by**
Bowl vessel	Stainless steel		
Co-centric Homogenizer	Teflon and stainless steel		
Anchor and impellers	Stainless steel		
Water inlet	Stainless steel		
Water Jacket	Stainless steel		
Charging port	Stainless steel		
Water immersion heater	NA		
PT-100 sensor	NA		
Operating panel touch screen	LCD		

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BATCH PRODUCTION RECORD

Product name: Design Space Clobetasol 17-Propionate Cream Page 3 of 3
Batch number: DS-CP-01 **Batch size** : 6000 g

MANUFACTURING PROCEDURE

Steps	Procedure	Time	Done by	Checked by
1	Clean the bowl with Liquid K® detergent and distilled water. Allow the bowl to dry and equilibrate at room temperature Room temperature: _____°C Mixer temperature: _____°C			
2	Fill the jacket with distilled water until the jacket is 30 Liters			
3	Weigh all the materials accurately using a Model AE 163 Mettler® analytical balance (Mettler® Inc., Zurich, Switzerland)			
4	Introduce 2936.59 mL propylene glycol and 2017.24 mL distilled water, followed by 3.34 g sodium citrate, 3.34 g citric acid, 3.34 g clobetasol 17-propionate, 700.78 g glyceryl monostearate, 573.97 g cetostearyl alcohol, 76.75 g white beeswax, 253.62 g Gelot® 64 and 5.01 g chlorocresol through the charging port			
5	Allow mixture of excipients to stir at 40 rpm for 120 minutes at75°C			
6	Following mixing, allow the blend to be homogenized at 2000 rpm for 120 minutes			
7	Allow the molten mixture to cool by passing distilled water through the heating jacket for 120 minutes Temperature of cold water=____°C			
8	After cooling, close the water inlet and allow slow continuous agitation of the anchor until the temperature on the operating panel displays 30°C - 35°C			
9	Stop the anchor and release the pressure built up in the bowl by the use of the pressure vent			
10	Store the final CP cream in 10 kg opaque containers until quality control tests are completed and then package into 50 g collapsible aluminium cream tubes			

SIGNATURE AND INITIAL REFERENCE

Full name (Print)	Signature	Initials	Date

APPENDIX VII

BATCH RECORD REPORTS

A representative of a sample of the batch record report for the design space network *viz.*, Batch DS-CP-01 manufactured using a Wintech® cream/ointment mixer for the CP cream formulation is included in this appendix. The batch record reports for the other design space cream batches *viz.*, DS-CP-02 – DS-CP-08 have not been included in this appendix but are included on the accompanying electronic file “Appendices-CD” on the CD.

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140, REPUBLIC OF SOUTH AFRICA

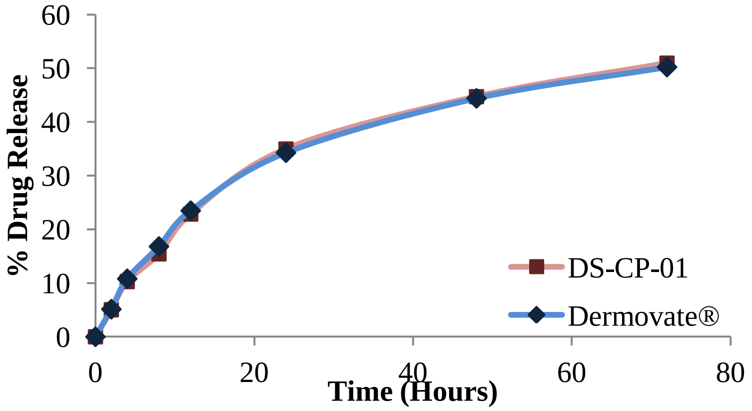
Batch Record Summary

Formulator : Ayeshah Fauzee **Date of Manufacture :** 15/10/2013
Product : Design Space Clobetasol 17-Propionate Cream
Melting Temperature : 75 °C
Batch Number: DS-CP-01 **Batch Size :** 6000 g

Excipient Name	Original Formula (% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	3.34 g	RM000150
Propylene glycol	44.00	2936.59 mL	RM000181
Sodium citrate	0.05	3.34g	RM000183
Citric acid	0.05	3.34 g	RM000185
Gelot® 64	3.80	253.62 g	RM000177
Glyceryl monostearate	10.50	700.78 g	RM000182
Ceteostearyl alcohol	8.60	573.97 g	RM000184
White beeswax	1.15	76.75 g	RM000142
Chlorocresol	0.075	5.01 g	RM000186
Distilled water	30.225	2017.24 mL	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenization Speed (rpm)	2000
Homogenization Time (min)	120
Anchor Speed (rpm)	40
Mixing Time (min)	120
Heating Temperature (°C)	75
Cooling time (min)	120

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	43750.00 ± 540	1.23
Spreadability (cm^2)	23.45 ± 0.50	2.13
CP Content (%)	100.00 ± 0.15	0.15
pH	5.95 ± 0.01	0.16
Extrudability (g/cm^2)	77.50 ± 1.20	1.55
Electrical Conductivity (µS/cm)	198.20 ± 2.00	1.01



OBSERVATIONS/COMMENTS

- A homogeneous smooth white cream was produced

- No sign of cracking or bleeding was observed during and after manufacture

APPENDIX VIII

RESEARCH PROTOCOL

APPLICATION OF SKIN BLANCHING STUDY FOR THE ASSESSMENT OF THE *IN VIVO* BIOEQUIVALENCE OF CLOBETASOL 17-PROPIONATE (CP) 0.05% W/W CREAM FORMULATIONS WITH RESPECT TO DERMOVATE® IN HEALTHY HUMAN VOLUNTEERS

STUDY NUMBER: DMV/CP 01-2013
PROTOCOL VERSION 2 (12th September 2013)
BROCHURE VERSION: 12th SEPT 2013

Reference Product	Dermovate® 0.05% w/w cream
Test Products	Clobetasol 17-propionate 0.05% w/w creams (Between 2 and 8 Formulations)
Site	Faculty of Pharmacy, Rhodes University, Grahamstown 6140, Republic of South Africa

1. STUDY PROTOCOL

Study Number	DMV/CP 01-2013
Study Title	The application of skin blanching study for the assessment of the in vivo bioequivalence of clobetasol 17-propionate (CP) 0.05% w/w cream formulations with respect to Dermovate® in healthy human volunteers
Reference Product	Dermovate® 0.05% w/w cream
Test Products	Clobetasol 17-propionate 0.05% w/w creams (Between 2 and 8 formulations)

Principal Investigator **Prof. Roderick Walker** Faculty of Pharmacy, Rhodes University, Grahamstown 6140, Republic of South Africa *Tel No:* 072 225 6365	**Signature:-----------------------------** **Date:-----------------------------------**
Co-Investigator/Study Investigator **Ms. Ayeshah Fauzee** Faculty of Pharmacy, Rhodes University, Grahamstown 6140, Republic of South Africa *Tel No:* 072 239 5718	**Signature:-----------------------------** **Date:-----------------------------------**
Study Visual Observer 1 **Prof. Roderick Walker** Faculty of Pharmacy, Rhodes University, Grahamstown 6140, Republic of South Africa *Tel No:* 072 225 6365	**Signature:-----------------------------** **Date:-----------------------------------**
Study Visual Observer 2 **Mr. Leon Purdon** Faculty of Pharmacy, Rhodes University Grahamstown 6140, Republic of South Africa *Tel No:* 046 603 8397	**Signature:---------------------------** **Date:---------------------------------**
Chromameter Assessor **Ms. Ayeshah Fateemah B. Fauzee** Faculty of Pharmacy, Rhodes University, Grahamstown 6140, Republic of South Africa *Tel No:* 072 239 5718	**Signature:---------------------------** **Date:---------------------------------**

2. STUDY SUMMARY

2.1 Title: The application of skin blanching study for the assessment of the *in vivo* bioequivalence of clobetasol 17-propionate (CP) 0.05% w/w cream formulations with respect to Dermovate® in healthy human volunteers.

2.2 Objective: To evaluate the *in vivo* bioequivalence of CP 0.05% w/w cream formulations with respect to Dermovate® in healthy human volunteers using the skin blanching effect assessed by a vasoconstrictor assay technique. A vasoconstrictor assay is a highly reliable method to determine bioequivalence of generic formulations through vasoconstriction characteristics. This assay uses the skin pallor induced at the application site as an indicator of the drug potency.

2.3 Products:-
Reference product: Dermovate ® 0.05% w/w cream
Test products: Between two and eight clobetasol 17-propionate 0.05% w/w creams

2.4 Facilities: The pre-screening test will be conducted in the Biopharmaceutics Research Group (BRG) facility and the research study will be conducted in the Biopharmaceutics Research Institute (BRI) clinic, situated at Rhodes University, Grahamstown 6140. The BRI clinic will be staffed by the principal investigator, co-investigator, visual observers and chromameter assessor.

2.5 Study design: The study will consist of a pivotal *in vivo* bioequivalence study to compare the reference and the test products. A pilot dose duration-response study has already been determined by Au *et al.* [558]. Prior to the pivotal study, a pre-screening test will be done to allow the selection of volunteers for inclusion in the pivotal study.

2.6 Date and duration of study: The study will consist of around 1-3 groups depending on the number of formulations, a pivotal study which will be carried out over a period of one weekend.

2.7 Subject number: Around sixty (~60) volunteers will be chosen for the pre-screening test in which between twelve and thirty six (~12-36) subjects will be enrolled for the pivotal study. Dropouts will not be replaced during the study.

2.8 Subject characteristics: Healthy non-smoking male/female volunteers aged 18-60 years who have not been treated with any topical corticosteroids for at least two (2) months prior to the study trial.

2.9 Pre-screening test: Volunteers will be selected according to the stated inclusion and exclusion criteria as mentioned in Sections 8.3 and 8.4. Subjects will have to carry out a pre-screening questionnaire in which they will be asked general questions regarding their health condition as well as topical drug related questions. Subjects must look healthy and will be asked whether they suffer from any chronic or skin conditions. The pre-screening test will include subjects that demonstrate adequate vasoconstriction to topical corticosteroids.

2.10 Study procedure: After the pre-screening selection is done, the chosen subjects will be asked to read the "Information for volunteers brochure" which includes the background information of clobetasol 17-propionate, the study formulations, the objective of this study, the study design, the skin blanching assessment methods, the study population and the criteria for participation, ethical considerations and practice, confidentiality and remuneration as shown in Sections 3, 4, 5, 6, 7, 8, 9, 10 and 11. Subjects will be asked to provide their written consent to participate in the pivotal study. The subjects will check into the research facility at 18 00 the day before the pivotal study and will remain in the facility from check in until 30 hour assessment time. Subjects will be asked a brief medical history by the co-investigator at the check-in, inclusion and exclusion criteria check and study restrictions check as mentioned in Sections 8.3, 8.4 and 8.5.

2.11 Duration of application: $ED_{50:}$ 40 minutes, $D_{1:}$ 20 minutes and D_2: 80 minutes.

2.12 Skin blanching assessment methods: Visual observation will be performed by two (2) trained observers on a multiple unit scale of 0-4 and chromametric assessment will be done using a Minolta® Chromameter (Model CR 400, Minolta, Osaka, Japan).

2.13 Skin blanching assessment times: At t = 0, 2, 4, 6, 8, 10, 12, 22 and 24 hours

2.14 Statistical analysis: For visual assessment, a graph of a percentage total possible score (%TPS) will be plotted against time in hours whereas for chromametric assessment, an average of *a*-scale values against time in hours will be plotted respectively for each formulation. The area under the blanching curve (AUBC) for each formulation will be calculated using the trapezoidal rule. Statistical analysis will be carried out using a Locke's method to determine the bioequivalence of the formulations using data for all subjects.

3. BACKGROUND INFORMATION

3.1 Structure of Clobetasol 17-propionate (CP)

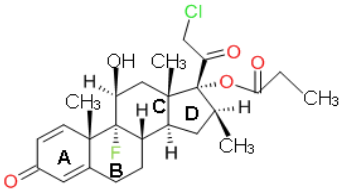


Figure 1. Molecular Structure of Clobetasol 17-propionate [559]

3.2 Mode of Action

CP is a potent steroid which has high glucocorticoid and low mineralocorticoid activity. Since CP has high glucocorticoid activity, it can bind with high affinity to glucocorticoid receptors following intravenous administration. CP has both catabolic and anti-anabolic effects on proteins in the peripheral tissues and may also cause insulin resistance and impair peripheral glucose utilization.The use of CP as the propionate salt results in both anti-inflammatory and immunosuppressive responses following topical administration [205]. CP seems to bind effectively to glucocorticoid receptors that are located in the skin and has other therapeutic activities that are not clearly defined. CP passes readily through cellular membranes and causes immediate vasoconstriction in the vascular tissues but however this mechanism of action is not really known. CP also inhibits the adherence of neutrophils and monocyte-macrophages to endothelial cells of capillaries at the site of inflammation and blocks the effect of macrophage migration inhibitory factor whilst decreasing the conversion of inactive plasminogen to active plasmin. CP therefore has immunosuppressive anti-proliferative, anti-pruritic, vasoconstrictive and anti-inflammatory effects [560,561].

3.3 Uses

CP topicals are used for the initial control of all forms of hyper acute eczema in all age groups, however only for a few days in paediatric patients, chronic hyperkeratonic eczema of the hands and feet, and patches of chronic lichen simplex, chronic hyperkeratotic psoriasis of any area of the body, severe acute photosensitivity, acute contact dermatitis, hypertrophic lichen planus, localised bullous planus, keloid scarring, pretibial myxoedema, vitiligo and for the suppression of a reaction following cryotherapy [205]. CP cream is also indicated for use in the short-term topical treatment of inflammatory and pruritic manifestations of moderate-to-severe corticosteroid-responsive dermatoses of the skin areas and for short-term topical treatment of mild-to-moderate plaque-type psoriasis of non-scalp regions excluding the face and other areas of the body such as the axilla and groin [562].

3.4 Dose

The action of corticosteroids usually lasts for six to eight hours only and therefore formulations are applications are best applied three times a day [563]. Since CP is a super potent topical corticosteroid, 0.05% w/w CP cream should be applied in a thin layer to the affected areas of the skin twice daily, preferably in the morning and evening with gentle and complete rubbing [564]. The frequency of application is dependent on the severity of the condition undergoing therapy. The use of more than a thin layer of formulation provides no beneficial therapeutic effect if excess formulation is applied. However, the potential for local and systemic side effects is enhanced. Treatment with corticosteroid formulation beyond two (2) consecutive weeks is not recommended due to the potent nature of many of the compounds, particularly CP. Due to potential for CP to suppress the hypothalamic-pituitary-adrenal (HPA) axis the total dose should not exceed 50 g per week [421].

3.5 Pharmacokinetic Parameters

The rate and extent of percutaneous absorption of CP is dependent on multiple factors such as the vehicle of the formulation, the severity of disease state, the thickness of the stratum corneum and the anatomical site amongst others [565]. The absorption of an active pharmaceutical ingredient (API) can be assessed using either *in vitro* or in *vivo* techniques. *In vitro* studies are usually performed using human, animal or synthetic membranes and are based on the Franz diffusion cell approach that has found widespread application in pharmaceutical research [566]. However, *in vivo* studies must be performed to establish the amount of API levels in the stratum corneum. The *in vivo* bioequivalence of CP can be assessed using the vasoconstrictor/skin blanching assay of topical corticosteroids. An *in vivo* study usually consists of a pilot dose duration-response study and a pivotal *in vivo* bioequivalence study comparing the test and the reference product [558]. No human data regarding the distribution of corticosteroids to organs following topical administration are available. Once percutaneous absorption has occurred a systemically absorbed topical corticosteroid undergoes elimination via pharmacokinetic pathways similar to those systemically administered corticosteroids. Since the circulating levels of topically administered compounds are usually below the level of detection of many analytical methods, no data that related to the metabolism of CP following topical administration is available as yet. The small amount of CP that may be absorbed is metabolized in the liver. Clobetasol and its metabolites are excreted in bile and urine of animals [205].

3.6 Contraindications

CP is a high potent corticosteroid and it may exhibit several topical and systemic side effects. The use of topical corticosteroids is absolutely contraindicated in patients with primary bacterial infections and may mask bacterial infections due to their vasoconstrictive and anti-inflammatory properties, thus making diagnosis and treatment difficult. Impetigo, furuncles and carbuncles, paronychia, ecthyma, erysipelas, cellulitis, lymphagitis and erythrasma are all conditions in which the use of topical steroids must be avoided [567]. Patients suffering from cutaneous infections that are caused by viruses, bacteria or fungi should not be treated with topical corticosteroids. Such infections may be exacerbated or, in the case of fungal infections may mask the inflammatory response. In addition infestations such as scabies should not be treated with topical corticosteroids as the condition may be exacerbated or even disguised. The use of topical CP in patients with acne vulgaris can result in suppression of the inflammatory response but that may be followed by serious rebound if the therapy is stopped. In the majority of patients, rosacea responds dramatically to topical CP preparations but breakthrough occurs rapidly [205]. If potent topical corticosteroids are applied to gravitational ulcers the resulting vasoconstriction will delay the healing process and may exacerbate infections that may already be present in the ulcer. Additional contraindications include the use of steroids in the treatment of Candida and dermatophytic infections as the organisms thrive on macerated skin. The use of topical corticosteroids in conjunction with an appropriate anti-infective agent may occasionally be appropriate. In order to treat herpes simplex and zoster-customary, the virus itself must be subjected to aggressive treatment. High potent steroids such as CP may have a tendency to cause topical side effects such as atrophy, stria, purpura or even ulceration, particularly when used on skin areas that are naturally thin such as the periorbital and groin regions, under occlusion or in paediatric patients. Special precautions must be taken when applying topical steroids around the eye as their use may precipitate serious glaucoma. CP topical formulations are highly contraindicated for use in patients with a history of hypersensitivity to any of the components of the formulations [568].

3.7 Drug Interactions

Some effects of CP have been reported with other high potency topical steroids such as acneiform eruptions, allergic contact dermatitis, hypertrichosis, hypopigmentation, maceration of the skin, perioral dermatitis and secondary infection [569].

3.8 Adverse Effects

Most common observed local side effects of CP are burning, itching, dryness, irritation in particular if applied to bare skin, purpura, acne and telangietasias. Less commonly observed local side effects include local hypertrichosis, hypopigmentation, glaucoma and allergic contact dermatitis. However, reports of contact dermatitis to topical corticosteroids are increasing [570]. Skin atrophy and thinning of the skin, the development of stria, telangiectasias, subcutaneous haemorrhage and easy bruising and bleeding may occur as a consequence of the long-term use of topical corticosteroids [200]. Topical corticosteroids may worsen pre-existing or co-existent dermatoses, such as rosacea, perioral dermatitis, and tinea infections. Patients presenting with psoriasis may become prone to papulopustular flare following withdrawal of topical corticosteroid therapy if large areas are treated with high potency agent for prolonged periods of time. Although systemic adverse effects are uncommon following topical corticosteroid use, some locally applied corticosteroids can be absorbed through the skin and enter the general circulatory system. The greatest risk of systemic reactions or potentially life-threatening effects can occur when ultra-high or high potent agents such as CP are used in large quantities over very large areas of the body, leading to sufficient systemic absorption to produce adrenal suppression, Cushing's syndrome, diabetes and hypertension. It has also been reported that the misuse of super potent topical corticosteroids has resulted in death due to Addisonian type crises [214].

4. STUDY DRUGS

4.1 Description

Table 1. *The details regarding the different CP products used*

	Reference Product	**Test Products**
Name	Dermovate® 0.05% w/w	Clobetasol 17-propionate 0.05% w/w creams
Active Ingredient	Clobetasol 17-propionate	Clobetasol 17-propionate
Dosage Form	Topical cream	Topical creams
Manufacturer	Sekpharma Pty Ltd,Gauteng, Republic of South Africa	Rhodes University, Faculty of Pharmacy, Grahamstown 6140, Republic of South Africa
Date of Manufacture	-	Three weeks before study
Expiry Date	05/2014	-
Batch Number	305628	T

4.2 Manufacturing Procedure, Supply and Storage

An adequate amount of the reference product and the test products will be provided during the study. The reference product, Dermovate® 0.05% w/w cream will be purchased in sufficient amount from the respective pharmaceutical manufacturers and will be kept at a temperature of 22°C. The test products will be manufactured in a Rhodes University cream manufacturing facility a week before the study will take place. It will be manufactured under good manufacturing practice (GMP) principles and stored at 22°C in an opaque container. Each product quantity, batch number and expiry dates will be recorded appropriately in a register and the quantity of cream used will also be noted. Unused product will be kept for a maximum of a year after the final report has been submitted and will then be disposed accordingly in the appropriate waste disposal container.

5. OBJECTIVE

CP is a highly potent corticosteroid often classified as a Class 1 agent [61]. It is usually formulated as a 0.05% concentration in topicals such as creams, ointments or gels [205]. A series of batches of CP cream formulations was manufactured using a cream/ointment mixer (Wintech® Pharmachem Equipments Ltd, Maharashtra, India) according to an established design space. Formulation optimization of CP cream formulations and the establishment of a design space were carried out using Response Surface Methodology (RSM). Between two and eight formulations that gave the most desirable *in vitro* release profile similar to Dermovate® will be chosen to be the test products for this pivotal study. Therefore it was necessary to evaluate the *in vivo* bioequivalence of CP 0.05% w/w cream formulations with respect to Dermovate® in healthy human volunteers using the skin blanching effect.

6. STUDY DESIGN

Prior to the pivotal study, a pre-screening test will be conducted to allow the selection of volunteers for inclusion in the pivotal study. One (1) application site on the upper forearm will be demarcated using a pre-punched adhesive template exposing a 1.1 x 1.1 cm square for the application of the reference product, Dermovate®. Approximately 10 µl which is equivalent to 11 mg will be applied on the site of application using a 10 µL Eppendorf® micropipette and will be uniformly spread using a glass rod. The dose duration will be 40 minutes. Prior to the measurement of the pharmacodynamic response at the end of the application, the remaining topical corticosteroid will be gently removed from the skin by three consecutive swabbings using dry cotton swabs. A visual assessment evaluation will be done six (6) hours after the drug product removal to see if any skin blanching effect has taken place. Any volunteer who will experience a degree of blanching on a multiple unit scale of 1-4, where 1 is intermediate degree of blanching and 4 is intense blanching will be selected for the pivotal study. The pivotal study will consist of between twelve and thirty-six (~12-36) healthy human subjects who showed positive skin blanching response in the pre-screening test conducted and according to the FDA guidelines [532] both visual and chromametric assessment methods will be used to evaluate the degree of skin blanching on the subjects. Fourteen (14) application sites will be used per forearm and will be demarcated using a pre-punched adhesive label template exposing a 1.1 x 1.1 cm square for the application of the reference and three CP creams. One cream formulation of 0.05% w/w CP, Dermovate® (reference product) and three (3) CP creams (test products) will be used during one phase of this study. Approximately 10 µL, equivalent to 11 mg of creams will be applied to the designated application sites on each forearm using 10 µL Eppendorf® micropipettes and will be uniformly spread using a glass rod. The FDA guidance [532]mentions three different dose durations (ED_{50}, D_1, and D2) to be used. ED_{50} is the dose duration at which half of the skin blanching effect is achieved. D_1 is the dose duration equal to half of the ED_{50} whereas D_2 is the dose duration that doubles that of $ED_{50.}$ The dose duration values for CP cream were determined by Au et al. [558] where a pilot study was conducted. ED_{50} chosen for both reference (R_1) and three test products (T_1, $T_{2,}$ T_3) will be 40 minutes and will assess the bioequivalence of the CP topical creams. Only the reference product will be applied to application sites demarcated as D_1 (20 minutes) and D_2 (80 minutes) for the determination of “detectors” among subjects. Two (2) control sites will be randomly selected from the fourteen (14) application sites and will be demarcated as (UNT). The application of the dose duration to the sites on the ventral forearms will be randomly assigned and the sites will be secluded using guards. Prior to the evaluation of the pharmacodynamic response at the end of the application period, the remaining cream will be gently removed by three (3) consecutive swabbings at each application site by using wet and dried using two dry cotton swabs at each site. The skin blanching response will then be evaluated both visually and using a chromameter at t = 0, 2, 4, 8, 10, 12, 22 and 24 hours after the removal of the products. The facility will be maintained at a room temperature of 22°C. A standard lighting by the overhead lamp will be used for the study. T_1= Test product 1 (40 minutes), T_2= Test product 2 (40 minutes), T_3= Test product 3 (40 minutes), D_1=Reference product (20 minutes), D_2=Reference product at (80 minutes), R_1=Reference product (40 minutes), UNT= Control (Application site without cream)

7. SKIN BLANCHING ASSESSMENTS

7.1 Visual Assessment

The degree of blanching will be measured visually by two (2) trained observers using a multiple unit scale of 0-4 where 0 indicates no blanching, 4 indicates intense blanching and 1, 2, 3 represents intermediate grades. Each application site will be assigned a blanching score by comparing the extent of blanching of the treated skin at the site to the surrounding skin. The blanching response results will be stored as % TPS (percentage total possible score). The %TPS will be calculated for each formulation and will then be plotted against time in hours after product application to produce the blanching profiles for each individual observer. The trapezoidal rule will be used to calculate the area under the blanching curve (AUBC) for each formulation.

7.2 Chromametric Assessment

The chromametric assessment will be done using a Minolta® Chromameter (Model CR 400, Minolta, Osaka, Japan) for the quantification of the intensity of blanching. The instrument will be calibrated using the white calibration plate immediately before the study. Baseline readings (zero time) will be taken at all times (including the untreated control sites).The chromameter provides readings based on three scales: L-scale, a-scale and b-scale. The L-value is the lightness variable and the a- and b-values are the chromaticity co-ordinates (red-green and blue-yellow, respectively). These three values can be used to define a point in three-dimensional space that characterizes a colour in absolute terms. The FDA guidelines [532] indicate that only an a-scale data can be used to calculate the area under the effect curve (AUEC). The FDA guidance [532] states that a “detector” is a “responder” whose blanching data must meet the following criterion, AUEC at D2 /AUEC at D1 $\geq$ 1.25. At each observation, the recorded a-value for each application site will be corrected by subtracting the baseline (time zero) values to yield baseline-corrected values, Δa-values. The mean of Δa-values of all formulations will be calculated and plotted against time in hours. The areas under the Δa-curve (AUBC) will be calculated for each formulation using the trapezoidal rule.

7.3 Data Analysis

The AUBC values obtained from the left and right forearms will be averaged for both visual and chromameter data. Statistical analysis will be carried out using Locke’s method to calculate the confidence intervals from the visual and chromameter AUBC data according to the directive of the FDA guidance [532]. AUBC data sets for all the formulations (reference (R_1) and the two test formulations (T_1 and T_2)) will be compared to obtain an exact confidence interval.

8. STUDY POPULATION AND CRITERIA FOR PARTICIPATION

8.1 Number of Subjects

Around twelve (~12) subjects will be enrolled for the first phase of the pivotal study. Around twelve (~12) subjects will be enrolled for the second phase of the pivotal study and another twelve (~12) subjects will be enrolled for the third phase of the pivotal study if necessary. Dropouts will not be replaced during the study. All subjects who complete the study will be included in the statistical analysis.

8.2 Conditions for Participation in This Study

1. Volunteers must be able to complete a written consent that they will be willing to participate fully in this study.
2. Volunteers must undergo a pre-screening test to assess any possibility or sensitivity to CP and to evaluate the degree of blanching.
3. Subjects must be able to fulfil the inclusion and exclusion criteria which have been set out in Sections 8.3 and 8.4 in this research protocol.
4. Subjects must be able to be fully committed in this study and abide to all the restrictions require of the subject in Section 8.5.

8.3 Subject Inclusion Criteria

Only subjects that are able to meet the following criteria will be able to participate in this study.

1. Subjects must be physically and mentally healthy and must be available for the entire period of the study.
2. Subjects can be male or female, between the ages of 18-60.
3. Subjects must not suffer from any chronic or skin conditions.
4. Subjects must not be hairy on the ventral forearms.
5. Subjects must demonstrate adequate vasoconstriction to topical corticosteroids.

8.4 Subject Exclusion Criteria

Subjects will not participate if they:

1. Have any mental or physical handicap/inability.
2. Are currently pregnant or breast feeding.
3. Suffer clinically significant hypertension or circulatory disease.
4. Smoke within one week of study.
5. Caffeine intake greater than 500 mg per day (500 mg of caffeine is equivalent to 3 (three) cups of instant coffee or tea) prior to or during the study.
6. Have clinically significant history of alcoholism or drug abuse.
7. Have participated in another skin blanching study within two (2) months of the study date.
8. Have been exposed or willing to be exposed to any sun tanning during or a week prior to the study.
9. Use topical dermatologic drug therapy on ventral forearms, including prior dosing of a topical corticosteroid in a pharmacodynamic study to a particular skin site, within one month prior to the study.
10. Have any adverse reactions to topical or systemic corticosteroids.
11. Suffer from any allergic conditions (allergic rashes, dermatitis and eczema).
12. Have any current or past skin medical condition, including active dermatitis, or any other dermatologic condition, which might significantly affect the pharmacodynamic response to the administered drug.
13. Have hairy ventral forearms surfaces and/or any abrasions on the fore arms and would require shaving ventral forearms to ensure consistent dose on skin surface.
14. Would use any vasoactive (constrictor/dilator) medication, prescription or OTC that would modulate blood flow. Examples of such drugs include nitroglycerin, anti-hypertensives, anti-histamines, NSAIDs, aspirin and OTC cough/cold products containing anti-histamines, and/or either phenypropanolamine or phentolamine.
15. Any obvious difference in skin colour between arms.

8.5 Study Restrictions

Restriction	Duration of restriction	Examples of restriction	Comment
Medications	No systemically or locally applied corticosteroids, no vasoactive (dilator/ constrictor) prescription or OTC medication are allowed a week prior to the start of the study until a week after the end of the study.	Betamethasone cream/ointment/ gel, hydrocortisone cream/ointment/gel, nitroglycerine, anti-hypertensives, NSAIDS, aspirin, OTC cough/cold products containing anti-histamines and/or phenypropanolamine or phentolamine.	Some effects of CP with other high potency topical steroids have been reported such as acneiform eruptions, allergic contact dermatitis, hypertrichosis, hypopigmentation, maceration of the skin, perioral dermatitis and secondary infection.
Moisturising creams	No use of creams, emollients or similar products to forearms for 24 hours prior to and throughout the study	All skin creams for example vaseline, aqueous creams, tanning creams, sunscreen creams, medicated topical dosage forms.	It can interfere with the absorption of the API from the appropriate cream through the stratum corneum.
Bathing/Showering	No bathing or showering during the periods of drug application and assessment of skin blanching.	Water.	If water gets in contact with the application sites during the dose duration period, negative results will be obtained.
Smoking	No smoking will be allowed one (1) day prior to the study and throughout the study.	Cigarettes, pipe smoking, cigars.	Tobacco can cause interactions with the drug, preventing the drug from getting absorbed.
Alcohol	No alcohol may be taken by subjects two (2) days prior to the study and throughout the study.	All alcoholic drinks for example whiskey, rum, beer and alcohol containing food products.	Alcohol may affect the absorption effect of clobetasol 17-propionate
Exercise	No exercise with both arm and no strenuous exercise, overall for study duration.	Badminton, squash, rugby, volley ball, soccer, tennis/tennis table, rowing, gym.	Walking only will be permitted.
Caffeine	No caffeine intake greater than 500 mg per day will be allowed.	More than three (3) cups of instant coffee or tea	A lot of caffeine can affect the action of the corticosteroid.

Subjects will be properly informed of all the details of the inclusion/exclusion criteria as well as the study restrictions. Random checks will be carried out to confirm that subjects have adhered to the above criteria. If subjects are unable to carry out the appropriate study restrictions, they must inform the principal or co-investigator immediately so that a decision about their participation in the entire study can be made.

8.6 Criteria for Removal from the Study

Subjects may withdraw from the study if they:

1. Suffer from any adverse reaction or sign of toxicity during the study period.
2. Have not been able to abide to the study restrictions.
3. Feel that they are not able to continue participating due to any reason.
4. Suffer from any illness or injury during the study if regarded as clinically significant by the principal investigator/co-investigator.

9. STUDY PROCEDURE

9.1 Pre-Screening Test

The pre-screening test will be done prior to the pivotal study.

9.2 Facility Check-in

On the Friday prior to the study, subjects will check in the Biopharmaceutical Research Institute (BRI) clinic (Rhodes University, Grahamstown 6140) at 18h00. Subjects will be asked a brief medical history by the principal/subject investigator at the check-in, inclusion and exclusion criteria check and study restrictions check as mentioned in Sections 8.3, 8.4 and 8.5. A pregnancy test will be done to the female volunteer in order to check for pregnancy. The study will start at 06h00 where the application of the creams on the sites will take place. Blanching assessments will be made on subjects in the same order in which the applications will be performed, thus ensuring equal time differences. Subjects accepted in this study will remain in the clinic from check in until 30 hour assessment time. Subjects will then be allowed to leave the clinic provided that there are no significant symptoms or adverse effects present.

9.3 Posture and Physical Activity

At the time of application, subjects will be seated exposing their forearms on the table where pre-punched adhesive templates exposing 1.1 x 1.1 cm squares will be placed on both of the ventral forearms respectively. Subjects will have to remain seated until the removal of the remaining cream after the dose duration time. Subjects will be allowed to lie down after the removal of the cream, however at each blanching assessment, subjects will be requested to be seated. Subjects will be required to adhere to restrictions on physical activity during the study.

9.4 Refreshments

Any food or non-alcoholic drink will be allowed during the study period. Subjects will be allowed to bring their own meals but however snacks such as finger food snacks and non-alcoholic drinks such as water and juice will be provided during the study.

9.5 Subject Monitoring

The co-investigator as well as the principal investigator will be in the clinic from the time of check-in till the time the subjects will be allowed to leave. Subjects will be asked open-ended questions about their health and if they are comfortable with the study.

9.6 Adverse Effects

Subjects will be monitored for any adverse events during the study. If any adverse effects are reported, whether or not they are thought to be related to the investigational products or procedures, the principal investigator/co-investigator will monitor the adverse effect and will seek medical advice from a medical practitioner if necessary. All adverse events will be treated accordingly until a satisfactory result is attained. However if the adverse event persists, a decision will be made by the principal investigator as to whether or not to withdraw the subject from the study.

10. ETHICAL CONSIDERATIONS AND PRACTICE

10.1 Ethical Review

Approval by The Faculty of Pharmacy's Ethics Committee will be obtained prior to the skin blanching study. The study will be conducted in accordance with the recommendation of the Food and Drug Administration (FDA) guidelines (1995). Several topical CP formulations have been used in the past for treating skin conditions such as atopic dermatitis, eczema, *lichen planus* and psoriasis. Similar bioequivalence studies of CP including a pilot study were conducted by Wai Ling Au and Ayeshah Fauzee at Rhodes University in 2008 and 2010.

10.2 Written Consent Form

Preceding the study, the nature, purpose and the risk in participating in the study will be explained to all volunteers. If the volunteers desire, they will be given time overnight to consider the information and any questions that they might have will be answered. If they decide to participate in the study, they must be fully committed in completing the study. However they will also be informed that they may withdraw from the study at any time without penalty, other than a reduced remuneration. They will sign a consent form in the presence of a witness and they are encouraged to consult their parents or a personal medical doctor for approval in this study.

10.3 Confidentiality

All medical histories and physical examination records and any other information regarding volunteers in this study will be kept confidential. However volunteers must agree that all documentation or data can be released for any lawful purpose and for publication purposes in scientific journals and/or presentation in a thesis submitted to Rhodes University in fulfilment of the requirement for a degree of Doctor of Philosophy (Pharmacy) after the completion of the study. In those cases, subject's name will be removed from all documentation to ensure adequate anonymity.

11. VOLUNTEER PAYMENT

Subjects will be paid R 800.00 for full participation in the study. The payment following withdrawal from the study will be calculated on a pro-rated basis from the beginning of the study to the end of the study.

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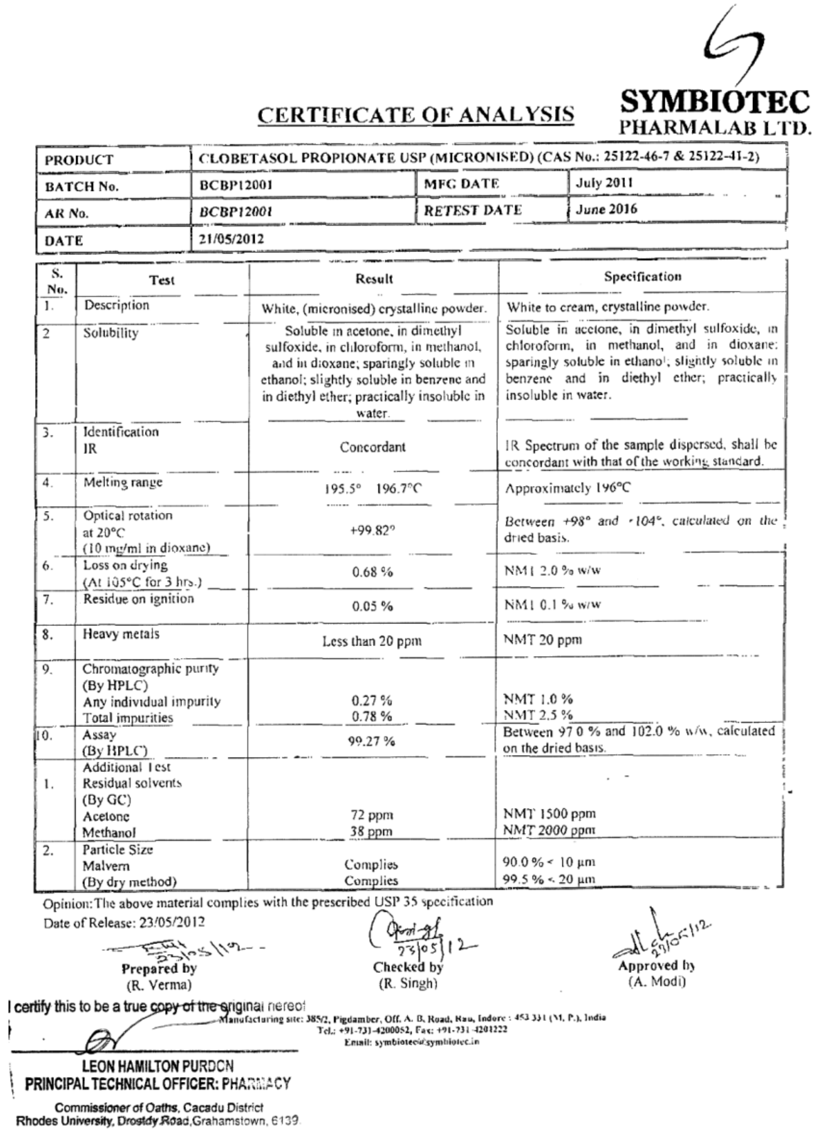
APPENDIX I

CERTIFICATES OF ANALYSIS AND QUALITY CONTROL DOCUMENTS

The Certificates of Analysis (COA) and Quality Control (QC) documents for clobetasol 17-propionate, propylene glycol, sodium citrate, anhydrous citric acid, white beeswax, glyceryl monostearate, cetostearyl alcohol, Gelot® 64 and chlorocresol are included on this accompanying electronic file “Appendices-CD” on the CD. The COA for clobetasol 17-propionate was obtained from Symbiotec PharmaLab® Ltd (Indore, India) in May 2012 when the raw material was purchased. QC testing was performed for CP in November-December 2012.



CERTIFICATE OF ANALYSIS

SYMBIOTEC
PHARMALAB LTD.

PRODUCT	**CLOBETASOL PROPIONATE USP (MICRONISED) (CAS No.: 25122-46-7 & 25122-41-2)**		
BATCH No.	**BCBP12001**	**MFG DATE**	**July 2011**
AR No.	***BCBP12001***	**RETEST DATE**	***June 2016***
DATE	**21/05/2012**		

S. No.	Test	Result	Specification
1.	Description	White, (micronised) crystalline powder.	White to cream, crystalline powder.
2	Solubility	Soluble in acetone, in dimethyl sulfoxide, in chloroform, in methanol, and in dioxane; sparingly soluble in ethanol; slightly soluble in benzene and in diethyl ether; practically insoluble in water.	Soluble in acetone, in dimethyl sulfoxide, in chloroform, in methanol, and in dioxane: sparingly soluble in ethanol; slightly soluble in benzene and in diethyl ether; practically insoluble in water.
3.	Identification IR	Concordant	IR Spectrum of the sample dispersed, shall be concordant with that of the working standard.
4.	Melting range	195.5° 196.7°C	Approximately 196°C
5.	Optical rotation at 20°C (10 mg/ml in dioxane)	+99.82°	*Between +98° and +104°, calculated on the dried basis.*
6.	Loss on drying (At 105°C for 3 hrs.)	0.68 %	NMT 2.0 % w/w
7.	Residue on ignition	0.05 %	NMT 0.1 % w/w
8.	Heavy metals	Less than 20 ppm	NMT 20 ppm
9.	Chromatographic purity (By HPLC) Any individual impurity Total impurities	 0.27 % 0.78 %	 NMT 1.0 % NMT 2.5 %
10.	Assay (By HPLC)	99.27 %	Between 97.0 % and 102.0 % w/w, calculated on the dried basis.
	Additional Test		
1.	Residual solvents (By GC) Acetone Methanol	 72 ppm 38 ppm	 NMT 1500 ppm NMT 2000 ppm
2.	Particle Size Malvern (By dry method)	Complies Complies	90.0 % < 10 µm 99.5 % < 20 µm

Opinion: The above material complies with the prescribed USP 35 specification

Date of Release: 23/05/2012

23/05/12	23/05/12	23/05/12
Prepared by (R. Verma)	**Checked by** (R. Singh)	**Approved by** (A. Modi)

Manufacturing site: 385/2, Pigdamber, Off. A. B. Road, Rau, Indore : 453 331 (M. P.), India
Tel.: +91-731-4200052, Fax: +91-731-4201222
Email: symbiotec@symbiotec.in

I certify this to be a true copy of the original hereof

LEON HAMILTON PURDON
PRINCIPAL TECHNICAL OFFICER: PHARMACY

Commissioner of Oaths, Cacadu District
Rhodes University, Drostdy Road, Grahamstown, 6139

RHODES UNIVERSITY, Faculty of Pharmacy,
Department of Pharmaceutics
Grahamstown 6140, REPUBLIC OF SOUTH AFRICA

Quality Control Document

Document Number: CP_QC_Version 1 **Effective Date: 1 December 2012**

Title: Clobetasol 17-propionate_2012

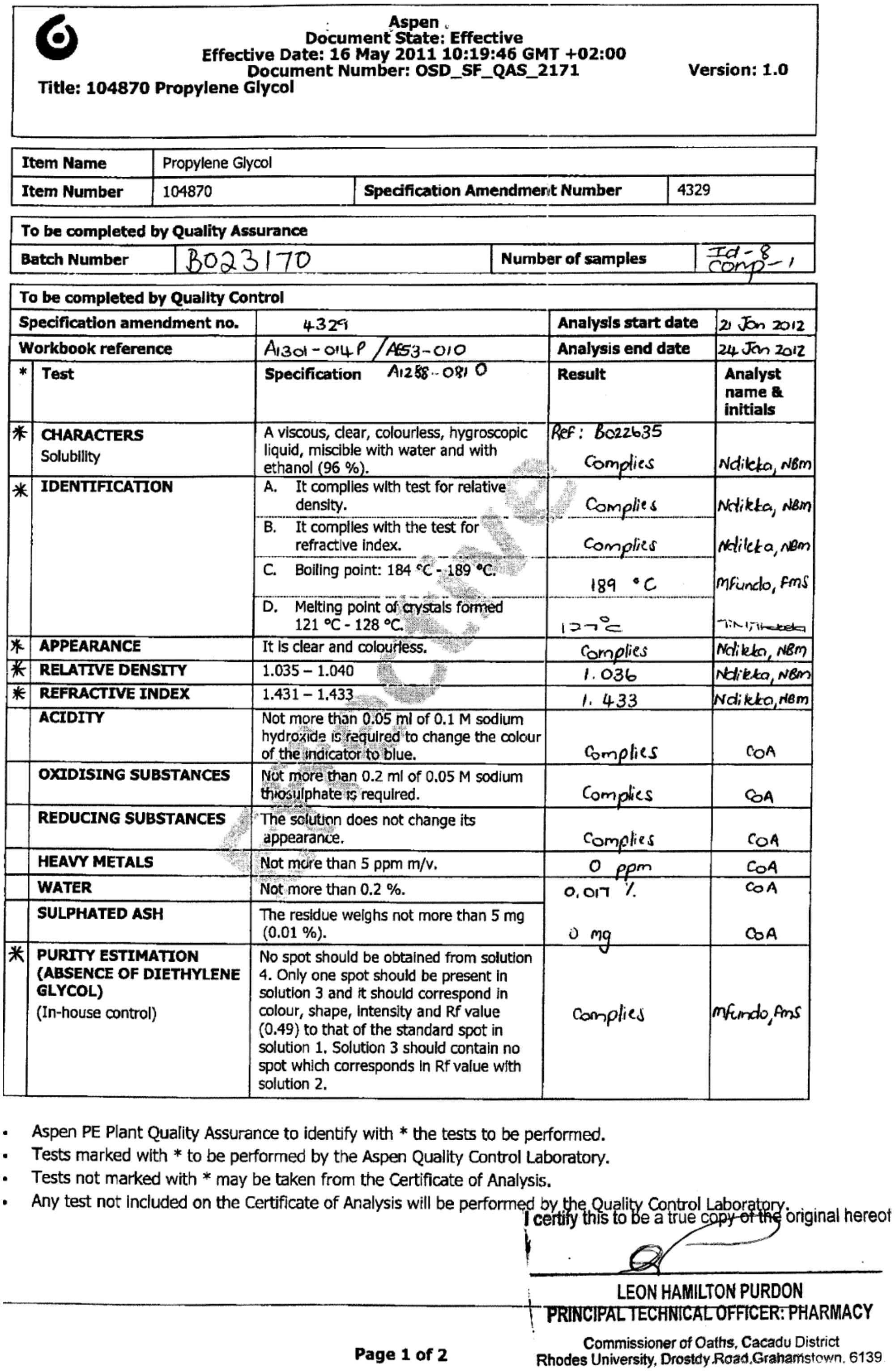
Item Name	Clobetasol 17-propionate (CP)		
Raw Material Number	RM000150		
Batch Number	BCBP12001		
Analysis Start Date	30 November 2012	**Analysis End Date**	30 November 2012

*	Test	Specification	Result	Analyst	Checked By
	Characters				
*	Appearance	White (micronized) crystalline powder	Complies	A. FAUZEE	M.T CHIWAKATA
	Identification				
*	Infra Red Spectroscopy	-	Complies	A. FAUZEE	M.T CHIWAKATA
	Optical Rotation	98°-104°	+99.82°	A. FAUZEE	M.T CHIWAKATA
*	**Loss on Drying**	NMT 2.0% w/w	0.68%	A. FAUZEE	M.T CHIWAKATA
	Residue on Ignition	NMT 0.1% w/w	0.05%	A. FAUZEE	M.T CHIWAKATA
	Heavy Metals	NMT 20 ppm	< 20 ppm	A. FAUZEE	M.T CHIWAKATA
	Chromatographic Purity				
	Any Individual Impurity	NMT 1.0%	0.27%	A. FAUZEE	M.T CHIWAKATA
	Total Impurities	NMT 2.5%	0.78%	A. FAUZEE	M.T CHIWAKATA
*	**Assay by HPLC**	97.0%-102.0%	99.8%	A. FAUZEE.	M.T CHIWAKATA
	Particle Size				
	Malvern	NMT 1500 ppm	72 ppm	A. FAUZEE	M.T CHIWAKATA
	Dry Method	NMT 2000 ppm	38 ppm	A. FAUZEE	M.T CHIWAKATA

Excipient	Description	Identification	Infra Red	Loss on Drying	Assay by HPLC	Certificate of Analysis	BP Validation	USP Validation
CP	Complies	Complies	Complies	Complies	Complies	√	-	-

- Department of Pharmaceutics, Faculty of Pharmacy, Rhodes University to identify the tests (*) to be performed
- Tests marked with * to be performed by Faculty of Pharmacy, Rhodes University
- Tests not marked with * were not performed and taken as per the COA

The COA for propylene glycol was obtained from Aspen Pharmacare® (Port Elizabeth, RSA) in January 2012. A QC analysis was perfomed in January 2013.



Aspen
Document State: Effective
Effective Date: 16 May 2011 10:19:46 GMT +02:00
Document Number: OSD_SF_QAS_2171 **Version: 1.0**
Title: 104870 Propylene Glycol

Item Name	Propylene Glycol		
Item Number	104870	**Specification Amendment Number**	4329

To be completed by Quality Assurance			
Batch Number	B023170	**Number of samples**	Id - 8 comp - 1

To be completed by Quality Control			
Specification amendment no.	4329	**Analysis start date**	21 Jan 2012
Workbook reference	A1301 - 014 P / A553 - 010	**Analysis end date**	24 Jan 2012

*	Test	Specification A1288 - 081 0	Result	Analyst name & initials
*	**CHARACTERS** Solubility	A viscous, clear, colourless, hygroscopic liquid, miscible with water and with ethanol (96 %).	Ref: B022635 Complies	Ndikka, NBm
*	**IDENTIFICATION**	A. It complies with test for relative density.	Complies	Ndikka, NBm
		B. It complies with the test for refractive index.	Complies	Ndikka, NBm
		C. Boiling point: 184 °C - 189 °C.	189 °C	Mfundo, FmS
		D. Melting point of crystals formed 121 °C - 128 °C.	127°C	[illegible]
*	**APPEARANCE**	It is clear and colourless.	Complies	Ndikka, NBm
*	**RELATIVE DENSITY**	1.035 – 1.040	1.036	Ndikka, NBm
*	**REFRACTIVE INDEX**	1.431 – 1.433	1.433	Ndikka, NBm
	ACIDITY	Not more than 0.05 ml of 0.1 M sodium hydroxide is required to change the colour of the indicator to blue.	Complies	CoA
	OXIDISING SUBSTANCES	Not more than 0.2 ml of 0.05 M sodium thiosulphate is required.	Complies	CoA
	REDUCING SUBSTANCES	The solution does not change its appearance.	Complies	CoA
	HEAVY METALS	Not more than 5 ppm m/v.	0 ppm	CoA
	WATER	Not more than 0.2 %.	0,017 %	CoA
	SULPHATED ASH	The residue weighs not more than 5 mg (0.01 %).	0 mg	CoA
*	**PURITY ESTIMATION (ABSENCE OF DIETHYLENE GLYCOL)** (In-house control)	No spot should be obtained from solution 4. Only one spot should be present in solution 3 and it should correspond in colour, shape, intensity and Rf value (0.49) to that of the standard spot in solution 1. Solution 3 should contain no spot which corresponds in Rf value with solution 2.	Complies	Mfundo, FmS

- Aspen PE Plant Quality Assurance to identify with * the tests to be performed.
- Tests marked with * to be performed by the Aspen Quality Control Laboratory.
- Tests not marked with * may be taken from the Certificate of Analysis.
- Any test not included on the Certificate of Analysis will be performed by the Quality Control Laboratory.

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Department of Pharmaceutics
Grahamstown 6140, REPUBLIC OF SOUTH AFRICA

Quality Control Document

Document Number: PG_QCD_Version 1 **Effective Date: 07 January 2013**

Title: Propylene Glycol_2013

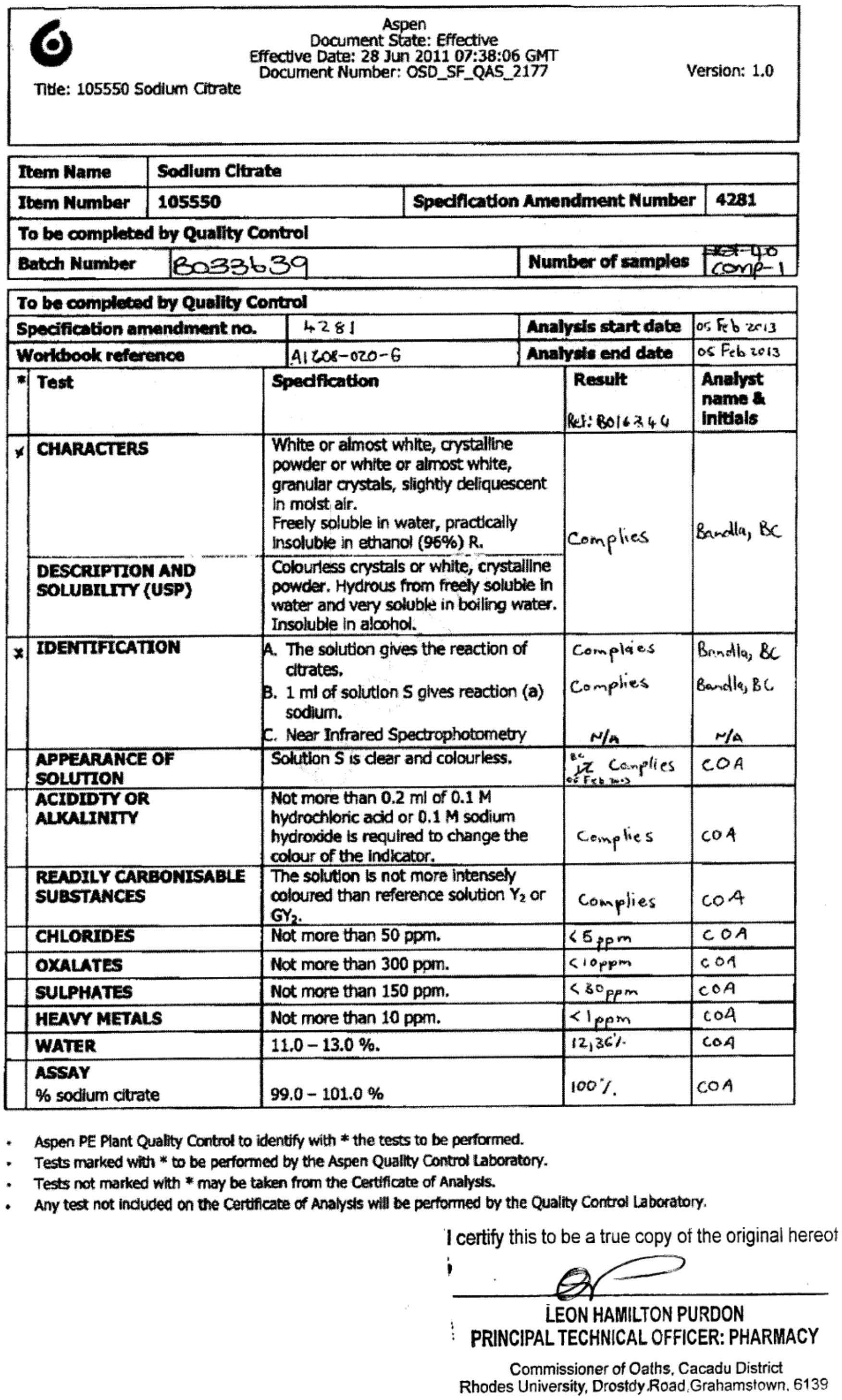
Item Name	Propylene Glycol (PG)		
Raw Material Number	RM000181		
Batch Number	B023170		
Analysis Start Date	06 January 2013	**Analysis End Date**	06 January 2013

*	Test	Specification	Result	Analyst Name & Initials	Checked by Name & Initials
	Characters				
*	Appearance	A viscous, clear, colorless, hygroscopic liquid	Complies	A. FAUZEE	M.T CHIWAKATA
*	Solubility	Miscible with water and with 96% v/v ethanol	Complies	A. FAUZEE	M.T CHIWAKATA
	Identification				
*	Relative Density	1.035-1.040 g/mL	1.037 g/mL	A. FAUZEE	M.T CHIWAKATA
*	Boiling Point	184°C-189°C	187°C-189°C	A. FAUZEE	M.T CHIWAKATA
*	Infra Red Spectroscopy	-	Complies	A. FAUZEE	M.T CHIWAKATA
*	Nuclear Magnetic Resonance Spectroscopy	-	Done	A. FAUZEE	M.T CHIWAKATA
*	**Appearance of Solution**	Clear and colorless	Complies	A. FAUZEE	M.T CHIWAKATA
*	**Acidity**	Absent	Complies	A. FAUZEE	M.T CHIWAKATA
*	**Oxidizing Agents**	Absent	Complies	A. FAUZEE	M.T CHIWAKATA
*	**Reducing Agents**	Absent	Complies	A. FAUZEE	M.T CHIWAKATA
	Heavy Metals	Not more than 5 ppm	-	A. FAUZEE	M.T CHIWAKATA
*	**% Water Content**	< 0.2%	0.018%	A. FAUZEE	M.T CHIWAKATA
*	**Sulfated Ash**	< 0.01%	0.008%	A. FAUZEE	M.T CHIWAKATA

Excipient	Description	Identification	Appearance of solution	Acidity	% water content	Sulfated Ash	Certificate of Analysis	BP Validation	USP Validatio
Propylene Glycol	Complies	Complies	Complies	Absent	Complies	Complies	-	√	√

- Department of Pharmaceutics, Faculty of Pharmacy, Rhodes University to identify the tests (*) to be performed
- Tests marked with * to be performed by Faculty of Pharmacy, Rhodes University
- Tests not marked with * were not performed and taken as per the COA

The COA for sodium citrate was obtained from Aspen Pharmacare® (Port Elizabeth, RSA) in 2012/2013. A QC analysis was performed in March 2013.



Aspen
Document State: Effective
Effective Date: 28 Jun 2011 07:38:06 GMT
Document Number: OSD_SF_QAS_2177
Version: 1.0
Title: 105550 Sodium Citrate

Item Name	**Sodium Citrate**		
Item Number	**105550**	**Specification Amendment Number**	**4281**
To be completed by Quality Control			
Batch Number	B033639	**Number of samples**	[illegible]-40 comp-1

To be completed by Quality Control			
Specification amendment no.	4281	**Analysis start date**	05 Feb 2013
Workbook reference	A1208-020-6	**Analysis end date**	05 Feb 2013

*	Test	Specification	Result Ref: B016344	Analyst name & initials
✓	**CHARACTERS**	White or almost white, crystalline powder or white or almost white, granular crystals, slightly deliquescent in moist air. Freely soluble in water, practically insoluble in ethanol (96%) R.	Complies	Bandla, BC
	DESCRIPTION AND SOLUBILITY (USP)	Colourless crystals or white, crystalline powder. Hydrous from freely soluble in water and very soluble in boiling water. Insoluble in alcohol.		
✗	**IDENTIFICATION**	A. The solution gives the reaction of citrates. B. 1 ml of solution S gives reaction (a) sodium. C. Near Infrared Spectrophotometry	Complies Complies N/A	Bandla, BC Bandla, BC N/A
	APPEARANCE OF SOLUTION	Solution S is clear and colourless.	BC JZ 05 Feb 2013 Complies	COA
	ACIDIDTY OR ALKALINITY	Not more than 0.2 ml of 0.1 M hydrochloric acid or 0.1 M sodium hydroxide is required to change the colour of the indicator.	Complies	COA
	READILY CARBONISABLE SUBSTANCES	The solution is not more intensely coloured than reference solution Y_2 or GY_2.	Complies	COA
	CHLORIDES	Not more than 50 ppm.	< 5 ppm	COA
	OXALATES	Not more than 300 ppm.	< 10 ppm	COA
	SULPHATES	Not more than 150 ppm.	< 30 ppm	COA
	HEAVY METALS	Not more than 10 ppm.	< 1 ppm	COA
	WATER	11.0 – 13.0 %.	12,36%	COA
	ASSAY % sodium citrate	99.0 – 101.0 %	100%.	COA

- Aspen PE Plant Quality Control to identify with * the tests to be performed.
- Tests marked with * to be performed by the Aspen Quality Control Laboratory.
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Quality Control Document

Document Number: SC_QCD_Version 1 **Effective Date: 08 March 2013**

Title: Sodium Citrate_2013

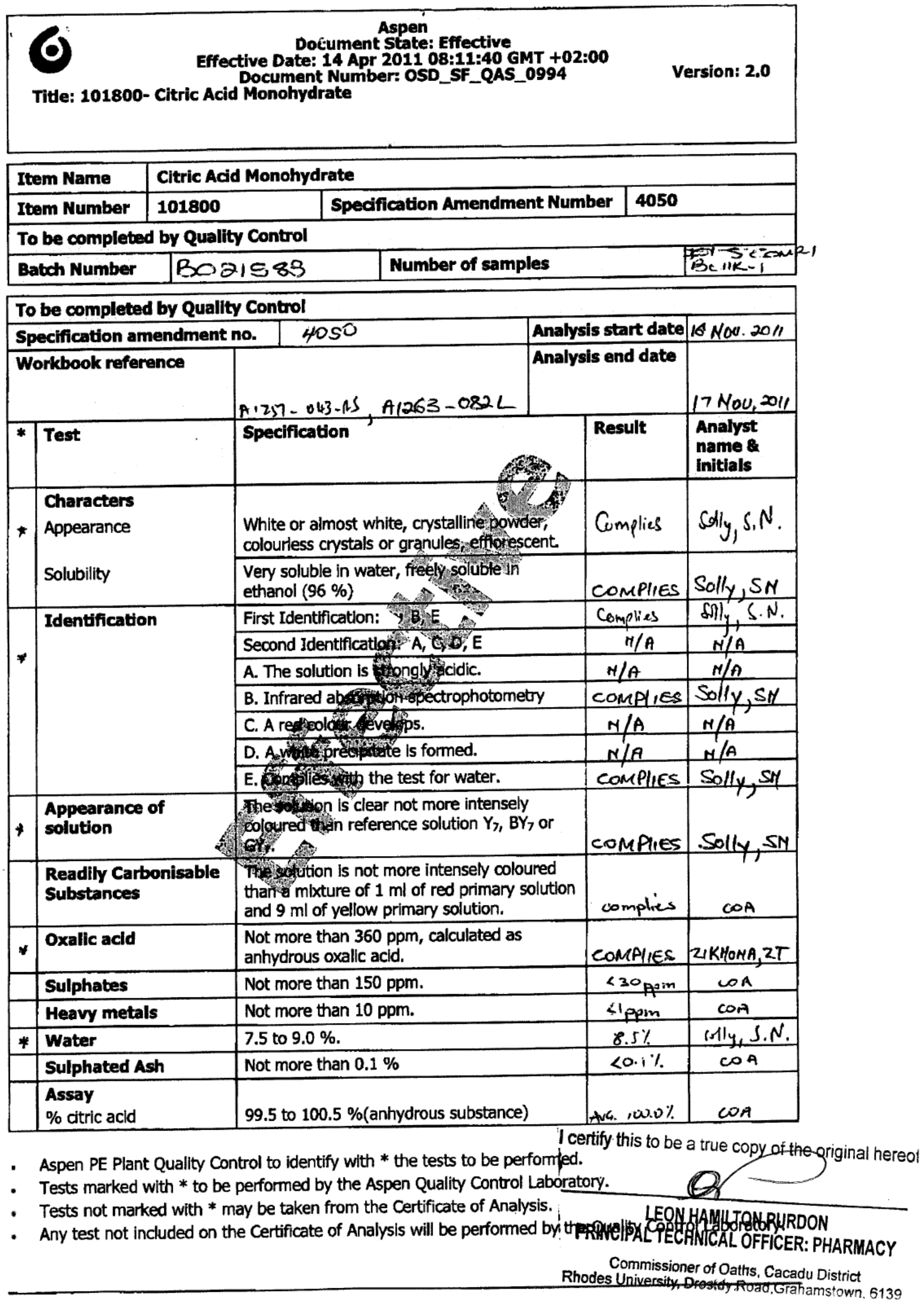
Item Name	Sodium Citrate (SC)		
Raw Material Number	RM000183		
Batch Number	X074210		
Analysis Start Date	07 March 2013	**Analysis End Date**	07 March 2013

*	Test	Specification	Result	Analyst Name & Initials	Checked by Name & Initials
	Characters				
*	Appearance	White or almost white, crystalline powder or white or almost white, granular crystals	Complies	A· FAUZEE	M.T CHIWAKATA
*	Solubility	Freely soluble in water	Complies	A· FAUZEE	M.T CHIWAKATA
	Identification				
*	Infra Red Spectroscopy	-	Complies	A· FAUZEE	M.T CHIWAKATA
*	Nuclear Magnetic Resonance Spectroscopy	-	Done	A· FAUZEE	M.T CHIWAKATA
*	**Appearance of Solution**	Clear and colorless	Complies	A· FAUZEE	M.T CHIWAKATA
*	**Alkalinity**	Present (pH 8.0)	Complies	A· FAUZEE	M.T CHIWAKATA
*	**Readily Carbonisable Substances**	Absent	Complies	A· FAUZEE	M.T CHIWAKATA
	Chlorides	< 50 ppm	-	A· FAUZEE	M.T CHIWAKATA
	Oxalates	< 300 ppm	-	A· FAUZEE	M.T CHIWAKATA
	Sulfates	< 150 ppm	-	A· FAUZEE	M.T CHIWAKATA
	Heavy Metals	< 10 ppm	-	A· FAUZEE	M.T CHIWAKATA
*	**% Water Content**	11.0%-13.0%	12.6%	A· FAUZEE	M.T CHIWAKATA
*	**Assay**	99.0%-101.0%	99.9%	A· FAUZEE	M.T CHIWAKATA

Excipient	**Description**	**Identification**	**Appearance of Solution**	**Alkalinity**	**% water content**	**Assay**	**Certificate of Analysis**	**BP Validation**	**USP Validatio**
Sodium Citrate	Complies	Complies	Complies	Present	Complies	Complies	-	√	√

- Department of Pharmaceutics, Faculty of Pharmacy, Rhodes University to identify the tests (*) to be performed
- Tests marked with * to be performed by Faculty of Pharmacy, Rhodes University
- Tests not marked with * were not performed and taken as per the COA

The COA for citric acid monohydrate was obtained from Aspen Pharmacare® (Port Elizabeth, RSA) in 2012. A QC analysis was done in March 2013.



Aspen
Document State: Effective
Effective Date: 14 Apr 2011 08:11:40 GMT +02:00
Document Number: OSD_SF_QAS_0994
Version: 2.0
Title: 101800- Citric Acid Monohydrate

Item Name	**Citric Acid Monohydrate**		
Item Number	**101800**	**Specification Amendment Number**	**4050**
To be completed by Quality Control			
Batch Number	B021583	**Number of samples**	Test - S COMP-1 BULK-1

To be completed by Quality Control			
Specification amendment no.	4050	**Analysis start date**	18 NOV. 2011
Workbook reference	A1257-043-AS, A1263-082L	**Analysis end date**	17 NOV, 2011

*	Test	Specification	Result	Analyst name & initials
*	**Characters** Appearance	White or almost white, crystalline powder, colourless crystals or granules, efflorescent.	Complies	Solly, S.N.
	Solubility	Very soluble in water, freely soluble in ethanol (96 %)	COMPLIES	Solly, SN
*	**Identification**	First Identification: B, E	Complies	Solly, S.N.
		Second Identification: A, C, D, E	N/A	N/A
		A. The solution is strongly acidic.	N/A	N/A
		B. Infrared absorption spectrophotometry	COMPLIES	Solly, SN
		C. A red colour develops.	N/A	N/A
		D. A white precipitate is formed.	N/A	N/A
		E. Complies with the test for water.	COMPLIES	Solly, SN
*	**Appearance of solution**	The solution is clear not more intensely coloured than reference solution Y_7, BY_7 or GY_7.	COMPLIES	Solly, SN
	Readily Carbonisable Substances	The solution is not more intensely coloured than a mixture of 1 ml of red primary solution and 9 ml of yellow primary solution.	complies	COA
*	**Oxalic acid**	Not more than 360 ppm, calculated as anhydrous oxalic acid.	COMPLIES	ZIKHONA, ZT
	Sulphates	Not more than 150 ppm.	<30 ppm	COA
	Heavy metals	Not more than 10 ppm.	<1 ppm	COA
*	**Water**	7.5 to 9.0 %.	8.5%	Solly, S.N.
	Sulphated Ash	Not more than 0.1 %	<0.1%	COA
	Assay % citric acid	99.5 to 100.5 %(anhydrous substance)	AVG. 100.0%	COA

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Department of Pharmaceutics
Grahamstown 6140, REPUBLIC OF SOUTH AFRICA

Quality Control Document

Document Number: CA-Mono_QCD_Version 1 **Effective Date: 31 March 2013**

Title: Citric Acid Monohydrate_2012

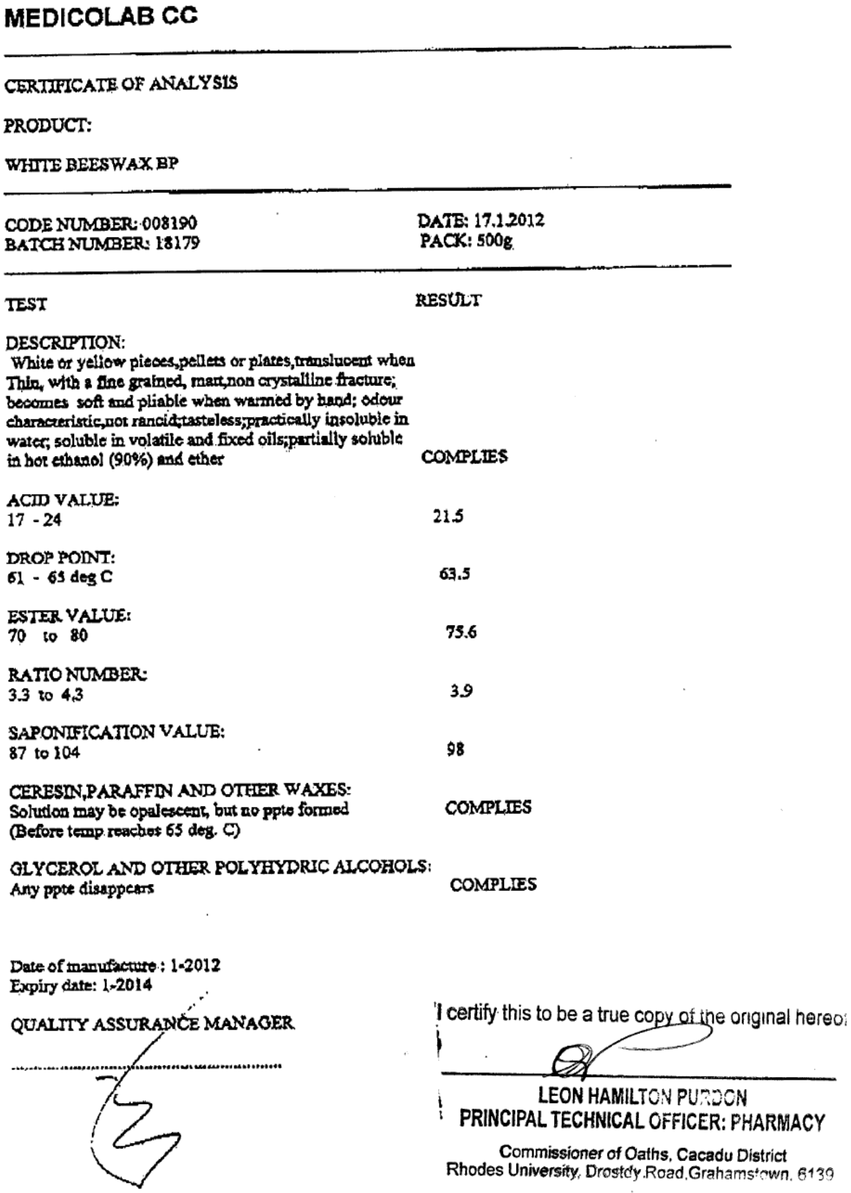
Item Name	Citric Acid Monohydrate (CA-Mono)		
Raw Material Number	RM000185		
Batch Number	X073900		
Analysis Start Date	30 March 2013	**Analysis End Date**	30 March 2013

*	Test	Specification	Result	Analyst Name & Initials	Checked by Name & Initials
	Characters				
*	Appearance	White or almost white, crystalline powder, colorless crystals	Complies	A· FAUZEE	M.T CHIWAKATA
*	Solubility	Very soluble in water	Complies	A. FAUZEE	M.T CHIWAKATA
	Identification				
*	Acidity	Highly Present (pH 3.0)	Complies	A. FAUZEE	M.T CHIWAKATA
*	Infra Red Spectroscopy	-	Complies	A. FAUZEE	M.T CHIWAKATA
*	Nuclear Magnetic Resonance Spectroscopy	-	Done	A. FAUZEE	M.T CHIWAKATA
*	**Appearance of Solution**	Clear and colorless	Complies	A. FAUZEE	M.T CHIWAKATA
*	**Readily Carbonisable Substances**	Absent	Complies	A. FAUZEE	M.T CHIWAKATA
	Oxalic Acid	< 360 ppm	Complies	A. FAUZEE	M.T CHIWAKATA
	Sulfates	< 150 ppm	< 30 ppm	A. FAUZEE	M.T CHIWAKATA
	Heavy Metals	< 10 ppm	< 1 ppm	A. FAUZEE	M.T CHIWAKATA
*	**% Water Content**	11.0%-13.0%	12.6%	A. FAUZEE	M.T CHIWAKATA
*	**Sulfated Ash**	< 0.1%	0.09%	A. FAUZEE	M.T CHIWAKATA
*	**Assay**	99.5%-100.5%	99.9%	A. FAUZEE	M.T CHIWAKATA

Excipient	**Description**	**Identification**	**Appearance of Solution**	**Acidity**	**% Water Content**	**Assay**	**Certificate of Analysis**	**BP Validation**	**USP Validatio**
Citric acid nonohydrate	Complies	Complies	Complies	Present	Complies	Complies	√	√	√

- Department of Pharmaceutics, Faculty of Pharmacy, Rhodes University to identify the tests (*) to be performed
- Tests marked with * to be performed by Faculty of Pharmacy, Rhodes University
- Tests not marked with * were not performed and taken as per the COA

The COA for white beeswax was obtained from Medicolab CC® (Johannesburg, RSA) in January 2012. A QC analysis was performed in March 2013.



MEDICOLAB CC

CERTIFICATE OF ANALYSIS

PRODUCT:

WHITE BEESWAX BP

CODE NUMBER: 008190
BATCH NUMBER: 18179

DATE: 17.1.2012
PACK: 500g

TEST	RESULT
DESCRIPTION: White or yellow pieces,pellets or plates,translucent when Thin, with a fine grained, matt,non crystalline fracture; becomes soft and pliable when warmed by hand; odour characteristic,not rancid;tasteless;practically insoluble in water; soluble in volatile and fixed oils;partially soluble in hot ethanol (90%) and ether	COMPLIES
ACID VALUE: 17 - 24	21.5
DROP POINT: 61 - 65 deg C	63.5
ESTER VALUE: 70 to 80	75.6
RATIO NUMBER: 3.3 to 4.3	3.9
SAPONIFICATION VALUE: 87 to 104	98
CERESIN,PARAFFIN AND OTHER WAXES: Solution may be opalescent, but no ppte formed (Before temp reaches 65 deg. C)	COMPLIES
GLYCEROL AND OTHER POLYHYDRIC ALCOHOLS: Any ppte disappears	COMPLIES

Date of manufacture : 1-2012
Expiry date: 1-2014

QUALITY ASSURANCE MANAGER

....

I certify this to be a true copy of the original hereo

LEON HAMILTON PURDON
PRINCIPAL TECHNICAL OFFICER: PHARMACY

Commissioner of Oaths, Cacadu District
Rhodes University, Drostdy Road, Grahamstown, 6139

RHODES UNIVERSITY, Faculty of Pharmacy,
Department of Pharmaceutics
Grahamstown 6140, REPUBLIC OF SOUTH AFRICA

Quality Control Document

Document Number: BW_QAS_Version 1 **Effective Date: 16 March 2013**

Title: White Beeswax_2013

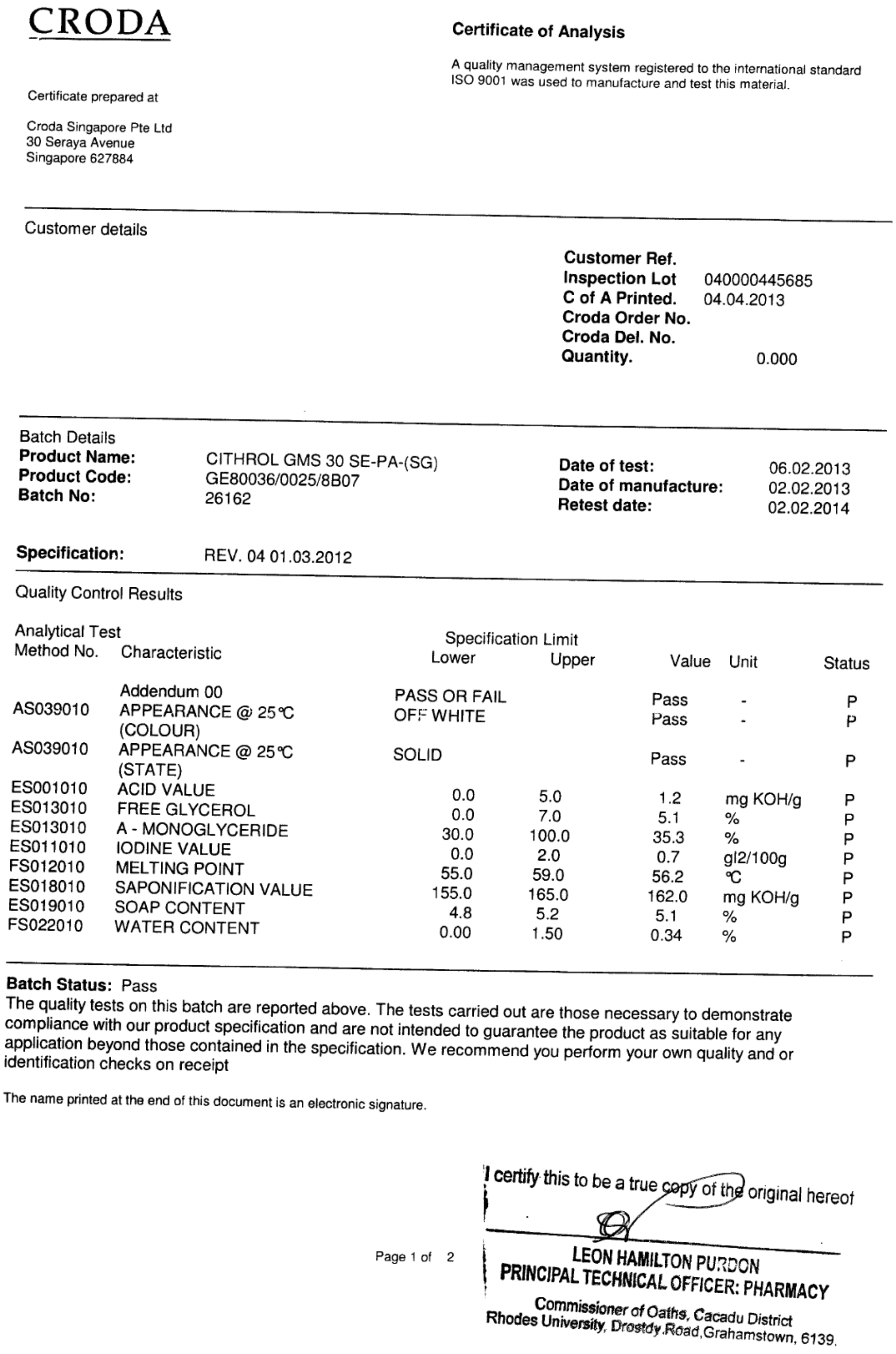
Item Name	White Beeswax (WB)		
Raw Material Number	RM000142		
Batch Number	18179		
Analysis Start Date	15 March 2013	**Analysis End Date**	15 March 2013

*	Test	Specification	Result	Analyst Name & Initials	Checked by Name & Initials
	Characters				
*	Appearance	White or yellowish-white pieces or plates, with a fine-grained, matt, or non-crystalline fracture	Complies	A - FAUZEE	M.T CHIWAKATA
*	Solubility	Practically insoluble in water, partially soluble in hot 96% v/v ethanol	Complies	A FAUZEE	M.T CHIWAKATA
	Identification				
	Relative Density	~0.960	Complies	A FAUZEE	M.T CHIWAKATA
*	Infra Red Spectroscopy	-	Complies	A. FAUZEE	M.T CHIWAKATA
*	Nuclear Magnetic Resonance Spectroscopy	-	Done	A. FAUZEE	M.T CHIWAKATA
*	**Drop Point**	61°C-66°C	62°C-64°C	A. FAUZEE	M.T CHIWAKATA
*	**Acid Value**	17.0-24.0 mgKOH/g	19.4 mgKOH/g	A. FAUZEE	M.T CHIWAKATA
	Ester Value	70.0-80.0 mgKOH/g	-	A. FAUZEE	M.T CHIWAKATA
*	**Saponification Value**	87.0-104.0 mgKOH/g	97.7 mgKOH/g	A. FAUZEE	M.T CHIWAKATA
*	**Ceresin, paraffins and other waxes**	Present	Complies	A. FAUZEE	M.T CHIWAKATA

Excipient	Description	Identification	Drop Point	Acid Value	Saponification Value	Certificate of Analysis	BP Validation	USP Validation
White Beeswax	Complies	Complies	Complies	Complies	Complies	√	√	√

- Department of Pharmaceutics, Faculty of Pharmacy, Rhodes University to identify the tests (*) to be performed
- Tests marked with * to be performed by Faculty of Pharmacy, Rhodes University
- Tests not marked with * were not performed and taken as per the COA

The COA for glyceryl monostearate was obtained from Croda (SA)® Ltd (Johannesburg, RSA) in January 2012. A QC analysis was performed in March 2013.



CRODA

Certificate of Analysis

A quality management system registered to the international standard ISO 9001 was used to manufacture and test this material.

Certificate prepared at

Croda Singapore Pte Ltd
30 Seraya Avenue
Singapore 627884

Customer details

Customer Ref.	
Inspection Lot	040000445685
C of A Printed.	04.04.2013
Croda Order No.	
Croda Del. No.	
Quantity.	0.000

Batch Details

Product Name:	CITHROL GMS 30 SE-PA-(SG)	**Date of test:**	06.02.2013
Product Code:	GE80036/0025/8B07	**Date of manufacture:**	02.02.2013
Batch No:	26162	**Retest date:**	02.02.2014

Specification: REV. 04 01.03.2012

Quality Control Results

Analytical Test Method No.	Characteristic	Specification Limit Lower	Specification Limit Upper	Value	Unit	Status
	Addendum 00	PASS OR FAIL		Pass	-	P
AS039010	APPEARANCE @ 25℃ (COLOUR)	OFF WHITE		Pass	-	P
AS039010	APPEARANCE @ 25℃ (STATE)	SOLID		Pass	-	P
ES001010	ACID VALUE	0.0	5.0	1.2	mg KOH/g	P
ES013010	FREE GLYCEROL	0.0	7.0	5.1	%	P
ES013010	A - MONOGLYCERIDE	30.0	100.0	35.3	%	P
ES011010	IODINE VALUE	0.0	2.0	0.7	gI2/100g	P
FS012010	MELTING POINT	55.0	59.0	56.2	℃	P
ES018010	SAPONIFICATION VALUE	155.0	165.0	162.0	mg KOH/g	P
ES019010	SOAP CONTENT	4.8	5.2	5.1	%	P
FS022010	WATER CONTENT	0.00	1.50	0.34	%	P

Batch Status: Pass

The quality tests on this batch are reported above. The tests carried out are those necessary to demonstrate compliance with our product specification and are not intended to guarantee the product as suitable for any application beyond those contained in the specification. We recommend you perform your own quality and or identification checks on receipt

The name printed at the end of this document is an electronic signature.

I certify this to be a true copy of the original hereof

LEON HAMILTON PURDON
PRINCIPAL TECHNICAL OFFICER: PHARMACY
Commissioner of Oaths, Cacadu District
Rhodes University, Drostdy Road, Grahamstown, 6139.

Page 1 of 2

RHODES UNIVERSITY, Faculty of Pharmacy,
Department of Pharmaceutics
Grahamstown 6140, REPUBLIC OF SOUTH AFRICA

Quality Control Document

Document Number: GMS_QC_Version 1 **Effective Date: 21 March 2013**

Title: Glyceryl Monostearate_2013

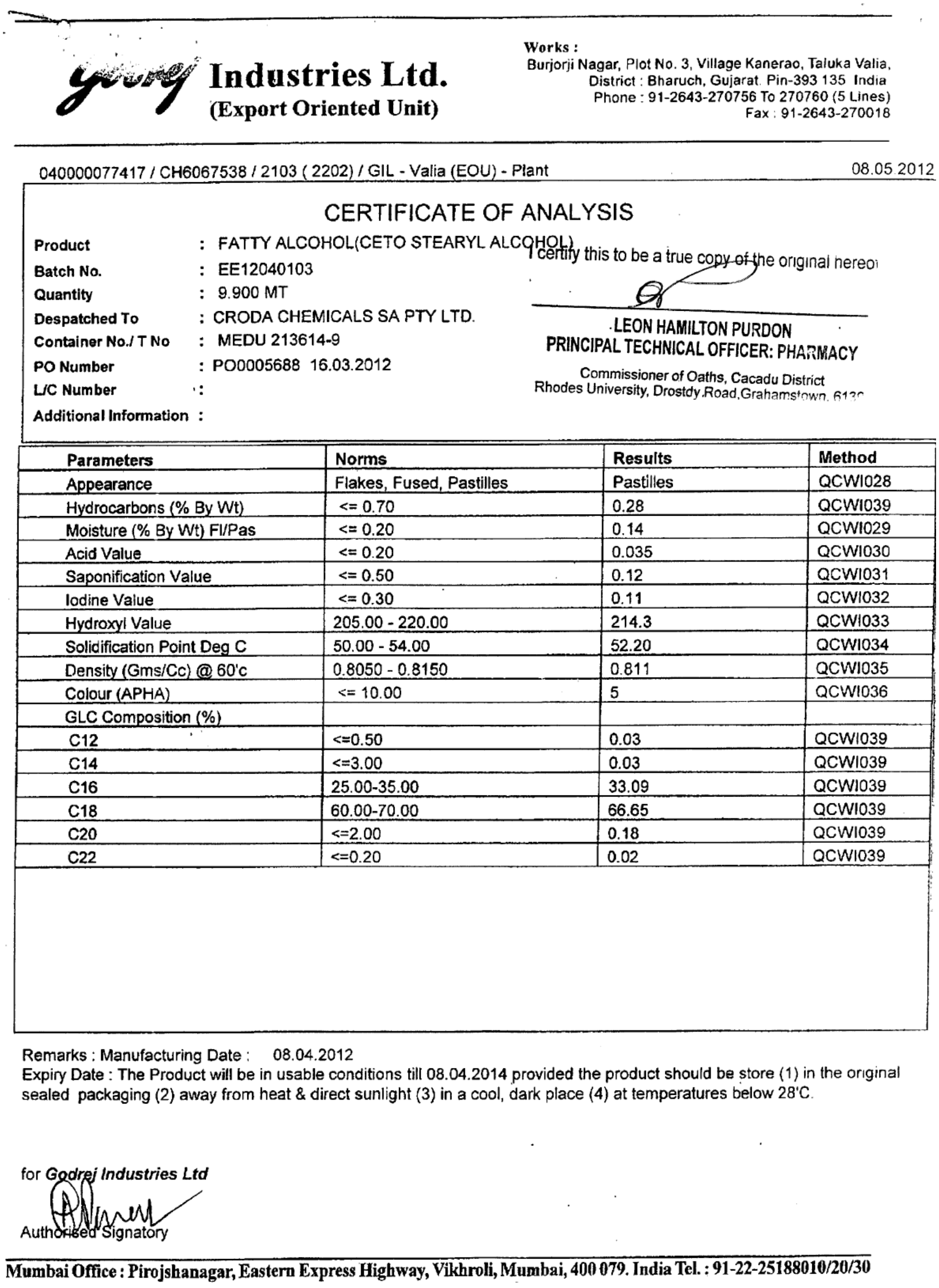
Item Name	Glyceryl Monostearate (GMS)		
Raw Material Number	RM000182		
Batch Number	18111		
Analysis Start Date	20 March 2013	**Analysis End Date**	20 March 2013

*	Test	Specification	Result	Analyst Name & Initials	Checked by Name & Initials
	Characters				
*	Appearance	White to cream hard waxy solid	Complies	A- FAUZEE	M.T CHIWAKATA
*	Solubility	Dispersible in hot water	Complies	A. FAUZEE	M.T CHIWAKATA
	Identification				
*	Melting Point	55°C-59 °C	56.5°C	A- FAUZEE	M.T CHIWAKATA
*	Infra Red Spectroscopy	-	Complies	A. FAUZEE	M.T CHIWAKATA
*	Nuclear Magnetic Resonance Spectroscopy	-	Done	A. FAUZEE	M.T CHIWAKATA
*	**Acid Value**	< 6.0 mgNaOH/g	5.4 mgNaOH/g	A. FAUZEE	M.T CHIWAKATA
	Iodine Value	< 3.0 mgI_2/g	0.6 $gI_2/100g$	A. FAUZEE	M.T CHIWAKATA
	Saponification value	155-165 mgKOH/g	162.1 mgKOH/g	A. FAUZEE	M.T CHIWAKATA
*	**Alkalinity**	8.0-10.0	pH 8.0	A. FAUZEE	M.T CHIWAKATA
	Heavy Metals	< 10 ppm	-	A. FAUZEE	M.T CHIWAKATA
	% Water content	< 2.0% w/w	0.48%	A. FAUZEE	M.T CHIWAKATA
*	Assay				
	Free Glycerol	< 7.0%	4.6%	A. FAUZEE	M.T CHIWAKATA
	Monoglycerides	< 30.0%	29.5%	A. FAUZEE	M.T CHIWAKATA
	Soap	< 6.0%	4.62%	A. FAUZEE	M.T CHIWAKATA

Excipient	Description	Identification	Acid Value	Alkalinity	Assay	Certificate of Analysis	BP Validation	USP Validation
Glyceryl Monostearate	Complies	Complies	Complies	Complies	Complies	√	√	√

- Department of Pharmaceutics, Faculty of Pharmacy, Rhodes University to identify the tests (*) to be performed
- Tests marked with * to be performed by Faculty of Pharmacy, Rhodes University
- Tests not marked with * were not performed and taken as per the COA

The COA for cetostearyl alcohol was obtained from Croda (SA)® Ltd (Johannesburg, RSA) in 2012. A QC analysis was done in March 2013.



Industries Ltd.
(Export Oriented Unit)

Works :
Burjorji Nagar, Plot No. 3, Village Kanerao, Taluka Valia,
District : Bharuch, Gujarat. Pin-393 135 India
Phone : 91-2643-270756 To 270760 (5 Lines)
Fax : 91-2643-270018

040000077417 / CH6067538 / 2103 (2202) / GIL - Valia (EOU) - Plant 08.05.2012

CERTIFICATE OF ANALYSIS

Product : FATTY ALCOHOL(CETO STEARYL ALCOHOL)
Batch No. : EE12040103
Quantity : 9.900 MT
Despatched To : CRODA CHEMICALS SA PTY LTD.
Container No./ T No : MEDU 213614-9
PO Number : PO0005688 16.03.2012
L/C Number :
Additional Information :

I certify this to be a true copy of the original hereo
LEON HAMILTON PURDON
PRINCIPAL TECHNICAL OFFICER: PHARMACY
Commissioner of Oaths, Cacadu District
Rhodes University, Drostdy Road,Grahamstown, 613

Parameters	Norms	Results	Method
Appearance	Flakes, Fused, Pastilles	Pastilles	QCWI028
Hydrocarbons (% By Wt)	<= 0.70	0.28	QCWI039
Moisture (% By Wt) Fl/Pas	<= 0.20	0.14	QCWI029
Acid Value	<= 0.20	0.035	QCWI030
Saponification Value	<= 0.50	0.12	QCWI031
Iodine Value	<= 0.30	0.11	QCWI032
Hydroxyl Value	205.00 - 220.00	214.3	QCWI033
Solidification Point Deg C	50.00 - 54.00	52.20	QCWI034
Density (Gms/Cc) @ 60'c	0.8050 - 0.8150	0.811	QCWI035
Colour (APHA)	<= 10.00	5	QCWI036
GLC Composition (%)			
C12	<=0.50	0.03	QCWI039
C14	<=3.00	0.03	QCWI039
C16	25.00-35.00	33.09	QCWI039
C18	60.00-70.00	66.65	QCWI039
C20	<=2.00	0.18	QCWI039
C22	<=0.20	0.02	QCWI039

Remarks : Manufacturing Date : 08.04.2012
Expiry Date : The Product will be in usable conditions till 08.04.2014 provided the product should be store (1) in the original sealed packaging (2) away from heat & direct sunlight (3) in a cool, dark place (4) at temperatures below 28'C.

for *Godrej Industries Ltd*
Authorised Signatory

Mumbai Office : Pirojshanagar, Eastern Express Highway, Vikhroli, Mumbai, 400 079. India Tel. : 91-22-25188010/20/30

RHODES UNIVERSITY, Faculty of Pharmacy,
Department of Pharmaceutics
Grahamstown 6140, REPUBLIC OF SOUTH AFRICA

Quality Control Document

Document Number: CetoA_QAS_Version 1 **Effective Date: 26 March 2013**

Title: Cetostearyl Alcohol_2013

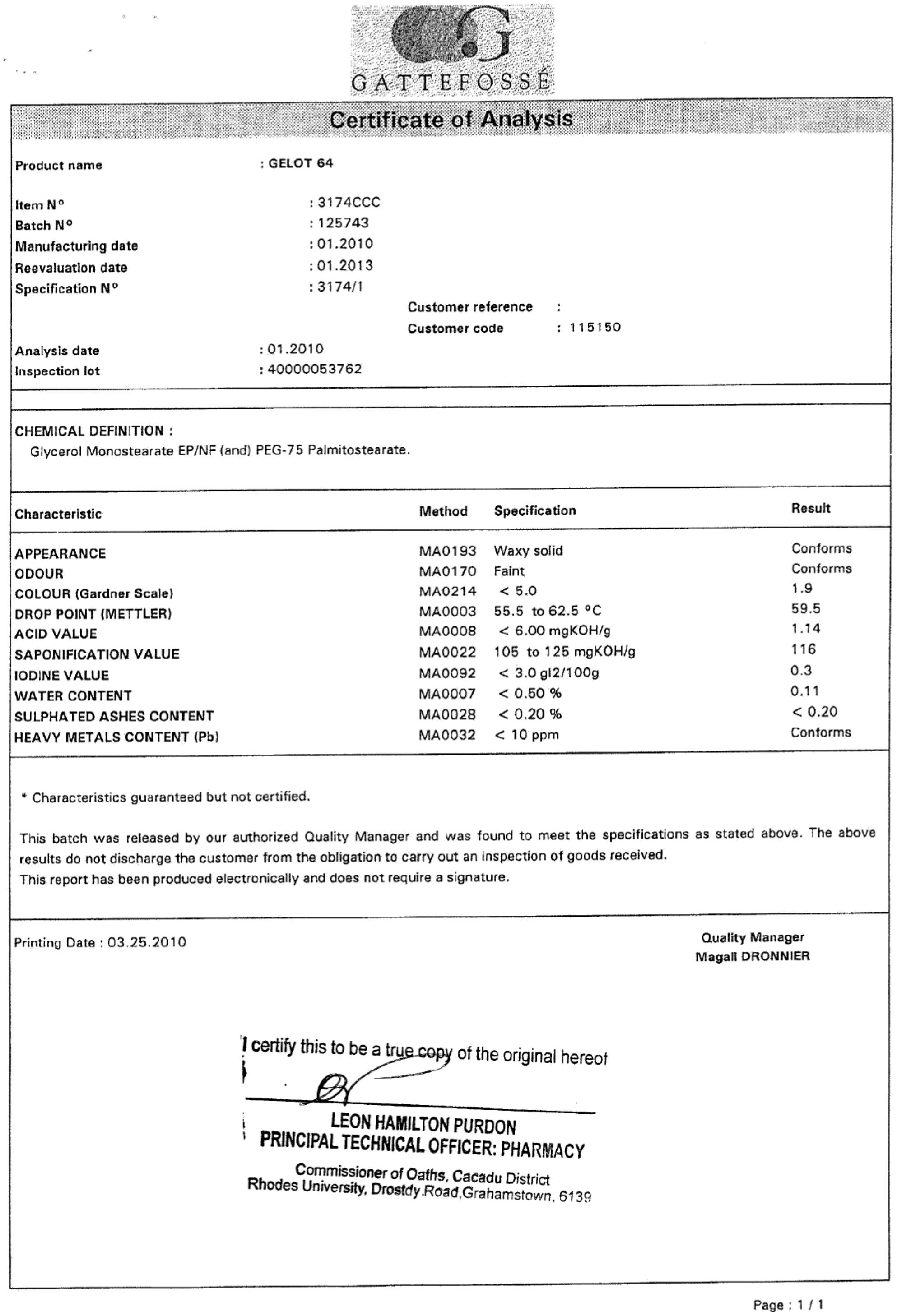
Item Name	Cetostearyl Alcohol (CA)		
Raw Material Number	RM000184		
Batch Number	EE11060146		
Analysis Start Date	25 March 2013	**Analysis End Date**	25 March 2013

*	Test	Specification	Result	Analyst Name & Initials	Checked by Name & Initials
	Characters				
*	Appearance	White or pale yellow, wax like mass, plates or flakes	Complies	A. FAUZEE	M.T CHIWAKATA
*	Solubility	Practically insoluble in water, soluble in 96% v/v ethanol	Complies	A. FAUZEE	M.T CHIWAKATA
	Identification				
*	Infra Red Spectroscopy	-	Complies	A. FAUZEE	M.T CHIWAKATA
*	Nuclear Magnetic Resonance Spectroscopy	-	Done	A. FAUZEE	M.T CHIWAKATA
*	**Appearance of Solution**	Clear and colorless	Complies	A. FAUZEE	M.T CHIWAKATA
*	**Melting Point**	49°C-56°C	Complies	A. FAUZEE	M.T CHIWAKATA
*	**Acid Value**	Max 1.0 mgNaOH/g	0.95 mgNaOH/g	A. FAUZEE	M.T CHIWAKATA
*	**Saponification Value**	Max 2.0 mgKOH/g	0.70 mgKOH/g	A. FAUZEE	M.T CHIWAKATA
	Hydroxyl value	208.0-228.0 mgKOH/g	214.2 mgKOH/g	A. FAUZEE	M.T CHIWAKATA
	Iodine Value	Max 2.0 mgI$_2$/g	0.12 mgKOH/g	A. FAUZEE	M.T CHIWAKATA
	Moisture Content	< 0.20%	0.14%	A. FAUZEE	M.T CHIWAKATA

Excipient	Description	Identification	Appearance of Solution	Acid Value	Saponification Value	Certificate of Analysis	BP Validation	USP Validation
Cetostearyl Alcohol	Complies	Complies	Complies	Complies	Complies	√	√	√

- Department of Pharmaceutics, Faculty of Pharmacy, Rhodes University to identify the tests (*) to be performed
- Tests marked with * to be performed by Faculty of Pharmacy, Rhodes University
- Tests not marked with * were not performed and taken as per the COA

The COA for Gelot® 64 was obtained from Gattefossé (Saint-Priest Cedex, France) in 2011. A QC analysis was done in March 2013.



GATTEFOSSÉ

Certificate of Analysis

Product name : GELOT 64

Item N° : 3174CCC
Batch N° : 125743
Manufacturing date : 01.2010
Reevaluation date : 01.2013
Specification N° : 3174/1
Customer reference :
Customer code : 115150
Analysis date : 01.2010
Inspection lot : 40000053762

CHEMICAL DEFINITION :
Glycerol Monostearate EP/NF (and) PEG-75 Palmitostearate.

Characteristic	Method	Specification	Result
APPEARANCE	MA0193	Waxy solid	Conforms
ODOUR	MA0170	Faint	Conforms
COLOUR (Gardner Scale)	MA0214	< 5.0	1.9
DROP POINT (METTLER)	MA0003	55.5 to 62.5 °C	59.5
ACID VALUE	MA0008	< 6.00 mgKOH/g	1.14
SAPONIFICATION VALUE	MA0022	105 to 125 mgKOH/g	116
IODINE VALUE	MA0092	< 3.0 gI2/100g	0.3
WATER CONTENT	MA0007	< 0.50 %	0.11
SULPHATED ASHES CONTENT	MA0028	< 0.20 %	< 0.20
HEAVY METALS CONTENT (Pb)	MA0032	< 10 ppm	Conforms

* Characteristics guaranteed but not certified.

This batch was released by our authorized Quality Manager and was found to meet the specifications as stated above. The above results do not discharge the customer from the obligation to carry out an inspection of goods received.
This report has been produced electronically and does not require a signature.

Printing Date : 03.25.2010

Quality Manager
Magali DRONNIER

I certify this to be a true copy of the original hereof

LEON HAMILTON PURDON
PRINCIPAL TECHNICAL OFFICER: PHARMACY
Commissioner of Oaths, Cacadu District
Rhodes University, Drostdy Road, Grahamstown, 6139

Page : 1 / 1

RHODES UNIVERSITY, Faculty of Pharmacy,
Department of Pharmaceutics
Grahamstown 6140, REPUBLIC OF SOUTH AFRICA

Quality Control Document

Document Number: G-64_QC_Version 1 **Effective Date: 9 March 2013**

Title: Gelot 64®_2013

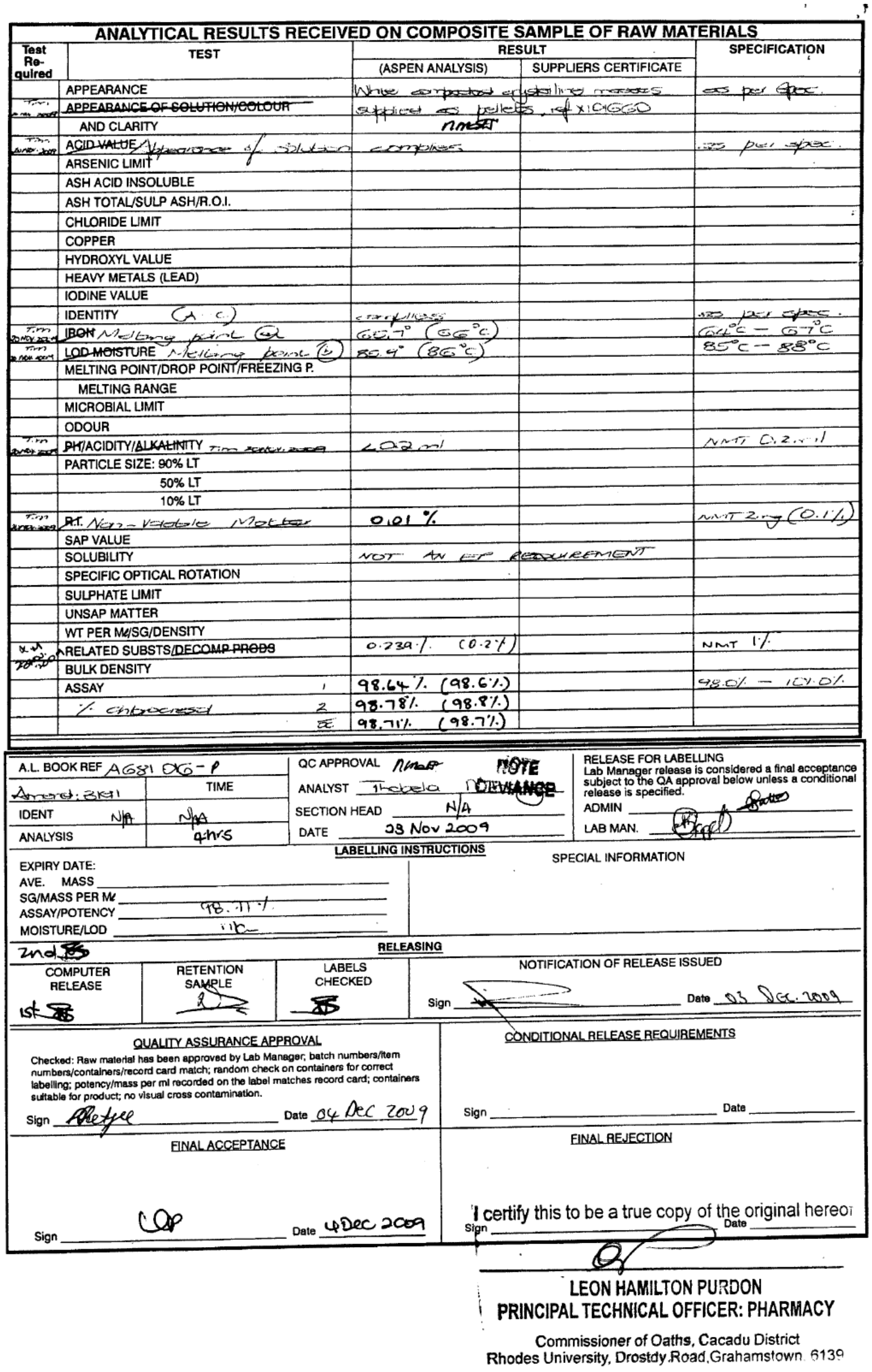
Item Name	Gelot 64® (G-64)		
Raw Material Number	RM000177		
Batch Number	125743		
Analysis Start Date	8 March 2013	**Analysis End Date**	8 March 2013

*	Test	Specification	Result	Analyst Name & Initials	Checked by Name & Initials
	Characters				
*	Appearance	White waxy solid	Complies	A. FAUZEE	M.T CHIWAKATA
	Identification				
*	Infra Red Spectroscopy	-	Complies	A. FAUZEE	M.T CHIWAKATA
*	Nuclear Magnetic Resonance Spectroscopy	-	Done	A. FAUZEE	M.T CHIWAKATA
*	**Drop Point**	55.5°C-62.5°C	57.4°C-59.9°C	A. FAUZEE	M.T CHIWAKATA
*	**Acid Value**	< 6.00 mgKOH/g	2.80 mgKOH/g	A. FAUZEE	M.T CHIWAKATA
*	**Saponification Value**	105-123 mgKOH/g	105.1 mgKOH/g	A. FAUZEE	M.T CHIWAKATA
	Iodine Value	< 3.0 gI_2/100g	0.3 gI_2/100g	A. FAUZEE	M.T CHIWAKATA
	% Water content	< 0.60%	0.11%	A. FAUZEE	M.T CHIWAKATA
*	**Sulfated Ash**	< 0.20%	< 0.20%	A. FAUZEE	M.T CHIWAKATA
	Heavy Metals	< 10 ppm	Complies	A. FAUZEE	M.T CHIWAKATA

Excipient	Description	Identification	Drop Point	Acid Value	Saponification Value	Sulfated Ash	Certificate of Analysis	BP Validation	USP Validation
Gelot 64®	Complies	Complies	Complies	Complies	Complies	Complies	√	-	-

- Department of Pharmaceutics, Faculty of Pharmacy, Rhodes University to identify the tests (*) to be performed
- Tests marked with * to be performed by Faculty of Pharmacy, Rhodes University
- Tests not marked with * were not performed and taken as per the COA

The COA for Chlorocresol was obtained from Aspen Pharmacare® (Port Elizabeth, RSA) in 2011. A QC analysis was performed in March 2013.



ANALYTICAL RESULTS RECEIVED ON COMPOSITE SAMPLE OF RAW MATERIALS

Test Required	TEST	RESULT (ASPEN ANALYSIS)	RESULT SUPPLIERS CERTIFICATE	SPECIFICATION
	APPEARANCE	White compacted crystalline masses		as per spec.
Tim [illegible]	~~APPEARANCE OF SOLUTION/COLOUR~~	supplied as pellets, ref X10660		
	AND CLARITY	nmsa		
Tim [illegible]	~~ACID VALUE~~ Appearance of solution	complies		as per spec.
	ARSENIC LIMIT			
	ASH ACID INSOLUBLE			
	ASH TOTAL/SULP ASH/R.O.I.			
	CHLORIDE LIMIT			
	COPPER			
	HYDROXYL VALUE			
	HEAVY METALS (LEAD)			
	IODINE VALUE			
	IDENTITY (A C)	complies		as per spec.
Tim 20 Nov 2009	~~IRON~~ Melting point (a)	66.7° (66°C)		64°C – 67°C
Tim [illegible]	~~LOD MOISTURE~~ Melting point (b)	85.9° (86°C)		85°C – 88°C
	MELTING POINT/DROP POINT/FREEZING P.			
	MELTING RANGE			
	MICROBIAL LIMIT			
	ODOUR			
Tim [illegible]	~~PH~~/ACIDITY/~~ALKALINITY~~ Tim 20 Nov 2009	<0.2 ml		NMT 0.2 ml
	PARTICLE SIZE: 90% LT			
	50% LT			
	10% LT			
Tim [illegible]	~~R.I.~~ Non-Volatile Matter	0.01 %		NMT 2 mg (0.1%)
	SAP VALUE			
	SOLUBILITY	NOT AN EP REQUIREMENT		
	SPECIFIC OPTICAL ROTATION			
	SULPHATE LIMIT			
	UNSAP MATTER			
	WT PER Mℓ/SG/DENSITY			
[illegible]	RELATED SUBSTS/~~DECOMP PRODS~~	0.239 % (0.2%)		NMT 1%
	BULK DENSITY			
	ASSAY 1	98.64% (98.6%)		98.0% – 101.0%
	% chlorocresol 2	98.78% (98.8%)		
	x̄	98.71% (98.7%)		

A.L. BOOK REF A681 06-P

Amend: 3191

	TIME
IDENT N/A	N/A
ANALYSIS	4hrs

QC APPROVAL nmsa

NOTE DEVIANCE

ANALYST Thabela

SECTION HEAD N/A

DATE 23 Nov 2009

RELEASE FOR LABELLING
Lab Manager release is considered a final acceptance subject to the QA approval below unless a conditional release is specified.

ADMIN

LAB MAN.

LABELLING INSTRUCTIONS

EXPIRY DATE:

AVE. MASS

SG/MASS PER Mℓ

ASSAY/POTENCY 98.71%

MOISTURE/LOD n/a

SPECIAL INFORMATION

2nd

RELEASING

COMPUTER RELEASE	RETENTION SAMPLE	LABELS CHECKED
1st		

NOTIFICATION OF RELEASE ISSUED

Sign Date 03 Dec. 2009

QUALITY ASSURANCE APPROVAL

Checked: Raw material has been approved by Lab Manager; batch numbers/item numbers/containers/record card match; random check on containers for correct labelling; potency/mass per ml recorded on the label matches record card; containers suitable for product; no visual cross contamination.

Sign Date 04 Dec 2009

CONDITIONAL RELEASE REQUIREMENTS

Sign Date

FINAL ACCEPTANCE

Sign Date 4 Dec 2009

FINAL REJECTION

Sign Date

I certify this to be a true copy of the original hereof

LEON HAMILTON PURDON
PRINCIPAL TECHNICAL OFFICER: PHARMACY

Commissioner of Oaths, Cacadu District
Rhodes University, Drostdy Road, Grahamstown, 6139

RHODES UNIVERSITY, Faculty of Pharmacy,
Department of Pharmaceutics
Grahamstown 6140, REPUBLIC OF SOUTH AFRICA

Quality Control Document

Document Number: CH_QCD_Version 1 **Effective Date: 2 March 2013**

Title: Chlorocresol_2013

Item Name	Chlorocresol (CH)		
Raw Material Number	RM000_86		
Batch Number	X109689		
Analysis Start Date	1 March 2013	**Analysis End Date**	1 March 2013

*	Test	Specification	Result	Analyst Name & Initials	Checked by Name & Initials
	Characters				
*	Appearance	White or almost white, crystalline powder or compacted crystalline masses, pellets	Complies	A. FAUZEE	M.T CHIWAKATA
*	Solubility	Slightly soluble in water, very soluble in 96% v/v ethanol	Complies	A. FAUZEE	M.T CHIWAKATA
	Identification				
*	Melting Point	64°C-67°C	66.7°C	A. FAUZEE	M.T CHIWAKATA
*	4 chloro-3-methylphenyl-benzoate Formation	85°C-88°C	86.9°C	A. FAUZEE	M.T CHIWAKATA
*	Presence of Phenolic	Present	Complies	A. FAUZEE	M.T CHIWAKATA
*	Infra Red Spectroscopy	-	Complies	A. FAUZEE	M.T CHIWAKATA
*	Nuclear Magnetic Resonance Spectroscopy	-	Done	A. FAUZEE	M.T CHIWAKATA
*	Appearance of Solution	Clear and colorless	Complies	A. FAUZEE	M.T CHIWAKATA
*	Non-volatile Matter	< 0. %	0.09%	A. FAUZEE	M.T CHIWAKATA
*	Assay	98.0%-101.0%	99.3%	A. FAUZEE	M.T CHIWAKATA

Excipient	Description	Identification	Appearance of Solution	Non-Volatile Matter	Assay	Certificate of Analysis	BP Validation	USP Validation
Chlorocresol	Complies	Complies	Complies	Complies	Complies	√	√	√

- Department of Pharmaceutics, Faculty of Pharmacy, Rhodes University to identify the tests (*) to be performed
- Tests marked with * to be performed by Faculty of Pharmacy, Rhodes University
- Tests not marked with * were not performed and taken as per the COA

APPENDIX II

BATCH PRODUCTION RECORDS

SCREENING DESIGN MANUFACTURE

2-LEVEL FRACTIONAL FACTORIAL DESIGN

A sample of a batch production record for the 2-Level Fractional Factorial design *viz.*, Batches 2-LFFD-CP-01 manufactured using a Wintech® cream/ointment mixer for CP screening design cream formulations is included in this accompanying electronic file "Appendices-CD" on the CD. The batch production records for all batches *viz.*, 2-LFFD-CP-02 – 2-LFFD-CP-12 are available on request.

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140, REPUBLIC OF SOUTH AFRICA

BATCH RECORD PRODUCTION

Product name: 2-Level Fractional Factorial Design Clobetasol 17-Propionate Cream Page 1 of 3
Batch number: 2-LFFD-CP-01 **Batch Size**: 6000 g

MANUFACTURING APPROVALS

Batch record issued by:_________________ **Date:_________________**

Master record issued by:_________________ **Date:_________________**

Product name: 2-Level Fractional Factorial Design Clobetasol 17-Propionate Cream
Batch number: 2-LFFD-CP-01 **Batch Size**: 6000 g

MASTER FORMULA AND BATCH FORMULA							
Item no.	**Material**	**Rhodes No.**	**Quantity (% w/w)**	**Amount/ Batch**	**Amount Dispensed**	**Dispensed by**	**Checked by**
1	CP	RM000150	0.05	3.24 g			
2	Propylene glycol	RM000181	51.00	3304.54 mL			
3	Sodium citrate	RM000183	0.05	3.24 g			
4	Citric acid	RM000185	0.05	3.24 g			
5	Gelot® 64	RM000177	1.00	64.79 g			
6	Glyceryl monostearate	RM000182	16.00	388.77 g			
7	Cetostearyl alcohol	RM000184	3.00	194.38 g			
8	White beeswax	RM000142	1.15	74.51 g			
9	Chlorocresol	RM000186	0.075	4.89 g			
10	Distilled water	N/A	30.225	1958.42 mL			

WINTECH® CREAM/OINTMENT MIXER VERIFICATION			
Description	**Type**	**Verified by**	**Confirmed by**
Bowl vessel	Stainless steel		
Co-centric Homogenizer	Teflon and stainless steel		
Anchor and impellers	Stainless steel		
Water inlet	Stainless steel		
Water Jacket	Stainless steel		
Charging port	Stainless steel		
Water immersion heater	NA		
PT-100 sensor	NA		
Operating panel touch screen	LCD		

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BATCH PRODUCTION RECORD

Product name: 2-Level Fractional Factorial Design Clobetasol 17-Propionate Cream Page 3 of 3
Batch number: 2-LFFD-CP-01 **Batch Size**: 6000 g

MANUFACTURING PROCEDURE				
Steps	**Procedure**	**Time**	**Done by**	**Checked by**
1	Clean the bowl with Liquid K® detergent and distilled water. Allow the bowl to dry and equilibrate at room temperature Room temperature: _____°C Mixer temperature: _____°C			
2	Fill the jacket with distilled water until the jacket is 30 Litres			
3	Weigh all the materials accurately using a Model AE 163 Mettler® analytical balance (Mettler® Inc., Zurich, Switzerland)			
4	Introduce 3304.54 mL propylene glycol and 1958.42 mL distilled water, followed by 3.24 g sodium citrate, 3.24 g citric acid, 3.24 g clobetasol 17-propionate, 388.77 g glyceryl monostearate, 194.38 g cetostearyl alcohol, 74.51 g white beeswax, 64.79 g Gelot® 64 and 4.89 g chlorocresol through the charging port			
5	Allow mixture of excipients to stir at an anchor speed of 20 rpm for 60 minutes at 75°C			
6	Following mixing, allow the blend to be homogenized at 2000 rpm for 120 minutes			
7	Allow the molten mixture to cool by passing distilled water through the heating jacket for 60 minutes Temperature of cold water=____°C			
8	After cooling, close the water inlet and allow slow continuous agitation of the anchor until the temperature on the operating panel displays 30°C - 35°C			
9	Stop the anchor and release the pressure built up in the bowl vessel by the use of the pressure vent			
10	Store the final CP cream in 10 kg opaque containers until quality control tests are completed and then package into 50 g collapsible aluminium cream tubes			

SIGNATURE AND INITIAL REFERENCE			
Full name (Print)	**Signature**	**Initials**	**Date**

APPENDIX III

BATCH RECORD REPORTS

The batch record reports for all the 2-Level Fractional Factorial design formulation batches, *viz.*, 2-LFFD-CP-01 – 2-LFFD-CP-12 are included on this accompanying electronic file “Appendices-CD” on the CD.

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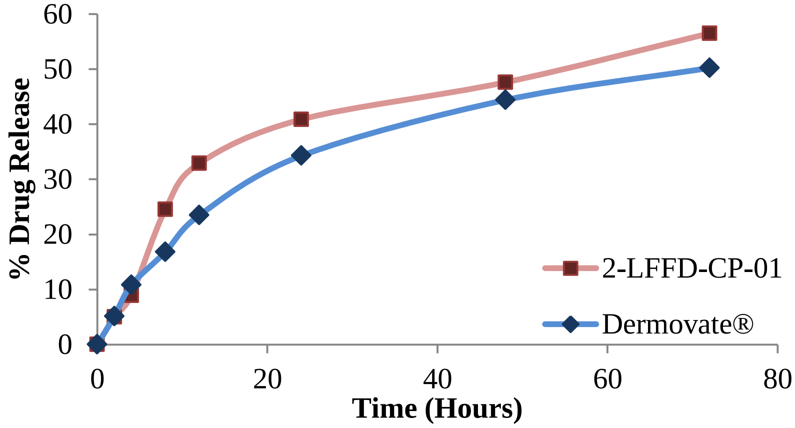
Batch Record Summary

Formulator : Ayeshah Fauzee **Date of Manufacture** :12/05/2013
Product : 2-Level Fractional Factorial Design Clobetasol 17-Propionate Cream
Melting Temperature :75 °C
Batch Number: 2-LFFD-CP-01 **Batch Size :** 6000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	3.24 g	RM000150
Propylene glycol	51.00	3304.54 mL	RM000181
Sodium citrate	0.05	3.24 g	RM000183
Citric acid	0.05	3.24 g	RM000185
Gelot® 64	1.00	64.79 g	RM000177
Glyceryl monostearate	16.00	388.77 g	RM000182
Ceteostearyl alcohol	3.00	194.38 g	RM000184
White beeswax	1.15	74.51 g	RM000142
Chlorocresol	0.075	4.89 g	RM000186
Distilled water	30.225	1958.42 mL	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenization Speed (rpm)	2000
Homogenization Time (min)	120
Anchor Speed (rpm)	20
Mixing Time (min)	60
Heating Temperature (°C)	75
Cooling time (min)	60

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	24780.00 ± 720	2.91
Spreadability (cm^2)	21.51 ± 0.25	1.16
CP Content (%)	99.84 ± 1.02	1.02
pH	6.24 ± 0.01	0.16
Extrudability (g/cm^2)	72.02 ± 2.50	3.47
Electrical Conductivity (µS/cm)	913.66 ± 9.00	0.99



OBSERVATIONS/COMMENTS

-A homogeneous smooth white cream was produced

- No sign of cracking or bleeding was observed during or after manufacture

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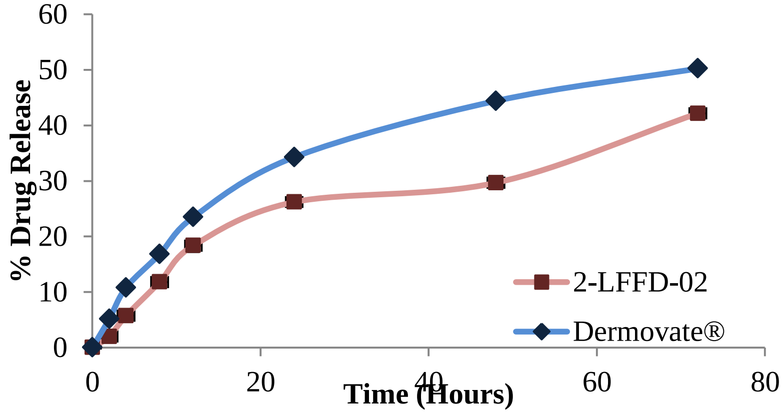
Batch Record Summary

Formulator : Ayeshah Fauzee **Date of Manufacture** :13/05/2013
Product : 2-Level Fractional Factorial Design Clobetasol 17-Propionate Cream
Melting Temperature :75 °C
Batch Number: 2-LFFD-CP-02 **Batch Size :** 6000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	3.11 g	RM000150
Propylene glycol	41.00	1273.29 mL	RM000181
Sodium citrate	0.05	3.11 g	RM000183
Citric acid	0.05	3.11 g	RM000185
Gelot® 64	5.00	64.79 g	RM000177
Glyceryl monostearate	16.00	310.56 g	RM000182
Ceteostearyl alcohol	3.00	186.34 g	RM000184
White beeswax	1.15	71.43 g	RM000142
Chlorocresol	0.075	4.66 g	RM000186
Distilled water	30.225	1877.33 mL	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenization Speed (rpm)	2000
Homogenization Time (min)	60
Anchor Speed (rpm)	40
Mixing Time (min)	60
Heating Temperature (°C)	75
Cooling time (min)	60

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	18806.00 ± 560	2.97
Spreadability (cm^2)	14.30 ± 1.20	8.39
CP Content (%)	100.50 ± 0.02	0.02
pH	6.25 ± 0.09	1.44
Extrudability (g/cm^2)	244.17 ± 2.85	1.17
Electrical Conductivity (µS/cm)	978.66 ± 10.50	1.07



OBSERVATIONS/COMMENTS

-A homogeneous smooth white cream was produced

- No sign of cracking or bleeding was observed during or after manufacture

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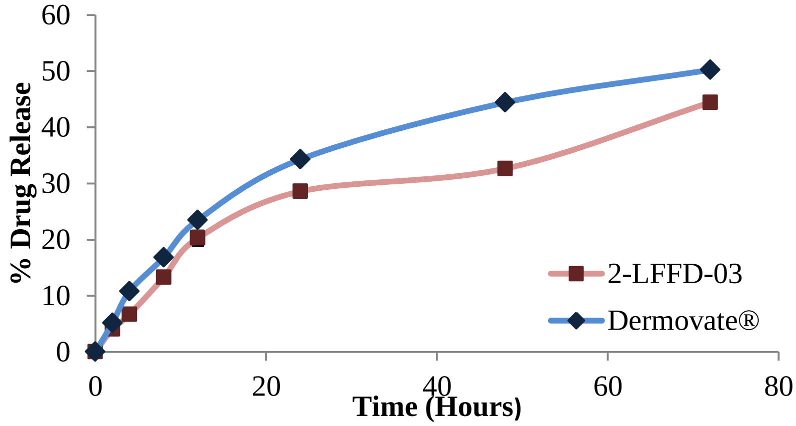
Batch Record Summary

Formulator : Ayeshah Fauzee **Date of Manufacture** :14/05/2013
Product : 2-Level Fractional Factorial Design Clobetasol 17-Propionate Cream
Melting Temperature :75 °C
Batch Number: 2-LFFD-CP-03 **Batch Size :** 6000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	2.93 g	RM000150
Propylene glycol	51.00	2982.45 mL	RM000181
Sodium citrate	0.05	2.93 g	RM000183
Citric acid	0.05	2.93 g	RM000185
Gelot® 64	1.00	58.48 g	RM000177
Glyceryl monostearate	6.00	350.88 g	RM000182
Ceteostearyl alcohol	13.00	760.23 g	RM000184
White beeswax	1.15	67.25 g	RM000142
Chlorocresol	0.075	4.38 g	RM000186
Distilled water	30.225	1767.54 mL	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenization Speed (rpm)	1000
Homogenization Time (min)	120
Anchor Speed (rpm)	20
Mixing Time (min)	120
Heating Temperature (°C)	75
Cooling time (min)	60

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	16106.00 ± 980	6.08
Spreadability (cm^2)	26.73 ± 1.00	3.74
CP Content (%)	100.47 ± 1.50	1.49
pH	6.06 ± 0.05	0.83
Extrudability (g/cm^2)	203.20 ± 4.75	2.34
Electrical Conductivity (µS/cm)	504.33 ± 11.25	2.23



OBSERVATIONS/COMMENTS

-A homogeneous smooth white cream was produced

- No sign of cracking or bleeding was observed during or after manufacture

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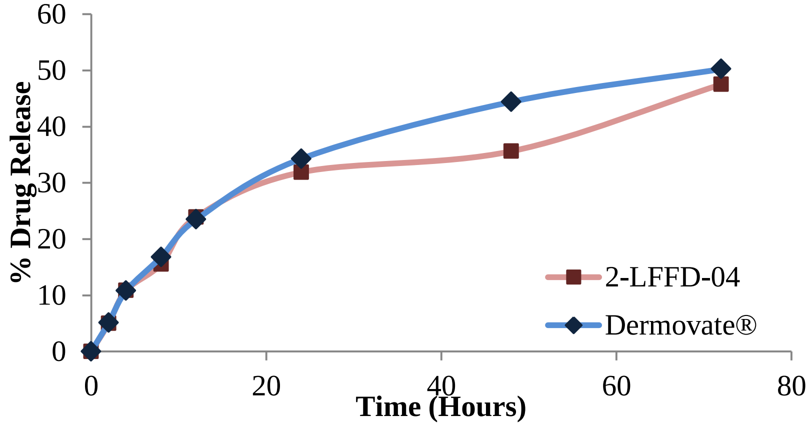
Batch Record Summary

Formulator : Ayeshah Fauzee **Date of Manufacture** :15/05/2013
Product : 2-Level Fractional Factorial Design Clobetasol 17-Propionate Cream
Melting Temperature :75 °C
Batch Number: 2-LFFD-CP-04 **Batch Size :** 6000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	3.63 g	RM000150
Propylene glycol	41.00	2978.21 mL	RM000181
Sodium citrate	0.05	3.63 g	RM000183
Citric acid	0.05	3.63 g	RM000185
Gelot® 64	1.00	72.64 g	RM000177
Glyceryl monostearate	6.00	435.84 g	RM000182
Ceteostearyl alcohol	3.00	217.92 g	RM000184
White beeswax	1.15	83.54 g	RM000142
Chlorocresol	0.075	5.44 g	RM000186
Distilled water	30.225	2195.52 mL	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenization Speed (rpm)	1000
Homogenization Time (min)	60
Anchor Speed (rpm)	20
Mixing Time (min)	60
Heating Temperature (°C)	75
Cooling time (min)	120

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	7773.00 ± 450	5.79
Spreadability (cm^2)	33.18 ± 0.03	0.09
CP Content (%)	99.84 ± 0.9	0.89
pH	5.08 ± 0.02	0.39
Extrudability (g/cm^2)	67.40 ± 2.80	4.15
Electrical Conductivity (µS/cm)	203.66 ± 5.24	2.57



OBSERVATIONS/COMMENTS

-A homogeneous smooth white cream was produced

- No sign of cracking or bleeding was observed during or after manufacture

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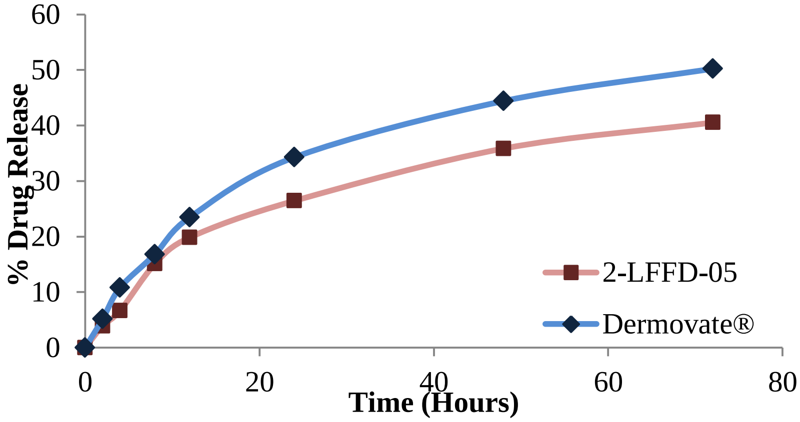
Batch Record Summary

Formulator : Ayeshah Fauzee **Date of Manufacture** :16/05/2013
Product : 2-Level Fractional Factorial Design Clobetasol 17-Propionate Cream
Melting Temperature :65 °C
Batch Number: 2-LFFD-CP-05 **Batch Size :** 3000 g

Excipient Name	**Original Formula(% w/w)**	**Working Formula**	**Rhodes Batch Number**
CP	0.05	1.41 g	RM000150
Propylene glycol	41.00	1153.85 mL	RM000181
Sodium citrate	0.05	1.41 g	RM000183
Citric acid	0.05	1.41 g	RM000185
Gelot® 64	5.00	140.72 g	RM000177
Glyceryl monostearate	16.00	450.28 g	RM000182
Ceteostearyl alcohol	13.00	365.85 g	RM000184
White beeswax	1.15	32.36 g	RM000142
Chlorocresol	0.075	2.11 g	RM000186
Distilled water	30.225	883.77 mL	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenization Speed (rpm)	1000
Homogenization Time (min)	120
Anchor Speed (rpm)	20
Mixing Time (min)	60
Heating Temperature (°C)	65
Cooling time (min)	60

Tests Performed	**Mean ± SD (n=3)**	**%RSD**
Viscosity (cP)	23913.00 ± 970	4.06
Spreadability (cm^2)	9.26 ± 0.20	2.16
CP Content (%)	100.25 ± 1.10	1.10
pH	5.44 ± 0.05	0.92
Extrudability (g/cm^2)	337.25 ± 1.23	0.36
Electrical Conductivity (µS/cm)	98.33 ± 2.30	2.34



OBSERVATIONS/COMMENTS

-A homogeneous smooth white cream was produced

- No sign of cracking or bleeding was observed during or after manufacture

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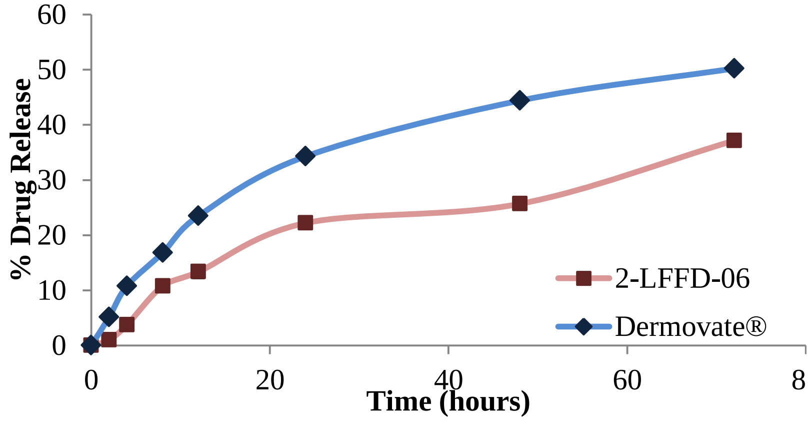
Batch Record Summary

Formulator : Ayeshah Fauzee **Date of Manufacture** :17/05/2013
Product : 2-Level Fractional Factorial Design Clobetasol 17-Propionate Cream
Melting Temperature :75 °C
Batch Number: 2-LFFD-CP-06 **Batch Size :** 3000 g

Excipient Name	**Original Formula(% w/w)**	**Working Formula**	**Rhodes Batch Number**
CP	0.05	1.46 g	RM000150
Propylene glycol	41.00	1198.83 mL	RM000181
Sodium citrate	0.05	1.46 g	RM000183
Citric acid	0.05	1.46 g	RM000185
Gelot® 64	1.00	29.24 g	RM000177
Glyceryl monostearate	16.00	467.84 g	RM000182
Ceteostearyl alcohol	13.00	380.11 g	RM000184
White beeswax	1.15	33.63 g	RM000142
Chlorocresol	0.075	2.19 g	RM000186
Distilled water	30.225	883.77 mL	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenization Speed (rpm)	2000
Homogenization Time (min)	60
Anchor Speed (rpm)	20
Mixing Time (min)	120
Heating Temperature (°C)	75
Cooling time (min)	120

Tests Performed	**Mean ± SD (n=3)**	**%RSD**
Viscosity (cP)	34900.00 ± 920	2.64
Spreadability (cm^2)	7.71 ± 0.15	1.95
CP Content (%)	100.43 ± 1.00	1.00
pH	6.26 ± 0.09	1.44
Extrudability (g/cm^2)	352.35 ± 1.20	0.34
Electrical Conductivity (µS/cm)	283.66 ± 7.56	2.66



OBSERVATIONS/COMMENTS

-A homogeneous smooth white cream was produced

- No sign of cracking or bleeding was observed during or after manufacture

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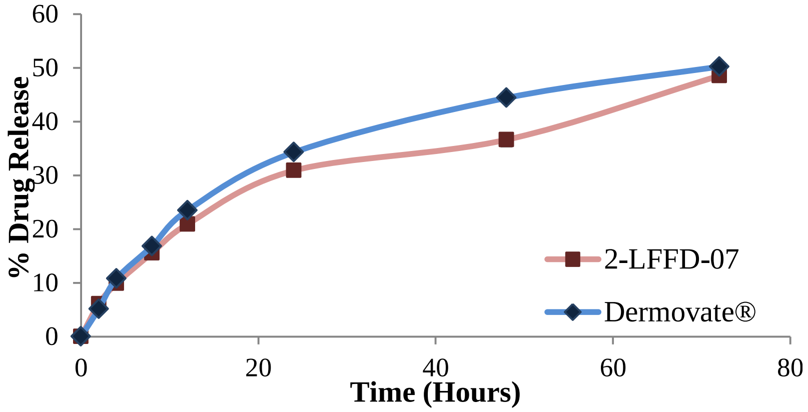
Batch Record Summary

Formulator : Ayeshah Fauzee **Date of Manufacture** :18/05/2013
Product : 2-Level Fractional Factorial Design Clobetasol 17-Propionate Cream
Melting Temperature :65 °C
Batch Number: 2-LFFD-CP-07 **Batch Size :** 3000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	1.62 g	RM000150
Propylene glycol	51.00	1652.27 mL	RM000181
Sodium citrate	0.05	1.62 g	RM000183
Citric acid	0.05	1.62 g	RM000185
Gelot® 64	1.00	32.39 g	RM000177
Glyceryl monostearate	6.00	194.38 g	RM000182
Ceteostearyl alcohol	3.00	97.19 g	RM000184
White beeswax	1.15	37.25 g	RM000142
Chlorocresol	0.075	2.43 g	RM000186
Distilled water	30.225	979.21 mL	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenization Speed (rpm)	2000
Homogenization Time (min)	60
Anchor Speed (rpm)	40
Mixing Time (min)	120
Heating Temperature (°C)	65
Cooling time (min)	60

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	6000.00 ± 240	4.00
Spreadability (cm^2)	24.04 ± 1.00	4.16
CP Content (%)	100.47 ± 0.09	0.89
pH	5.68 ± 0.10	1.76
Extrudability (g/cm^2)	63.32 ± 1.25	1.97
Electrical Conductivity (µS/cm)	529.00 ± 5.25	0.99



OBSERVATIONS/COMMENTS

-A homogeneous smooth white cream was produced

- No sign of cracking or bleeding was observed during or after manufacture

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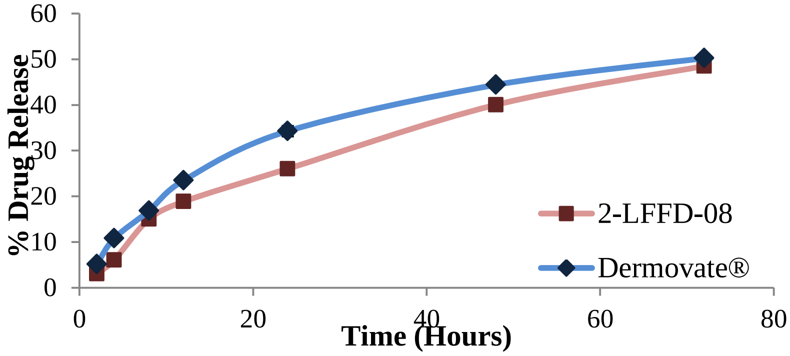
Batch Record Summary

Formulator : Ayeshah Fauzee **Date of Manufacture** :19/05/2013
Product : 2-Level Fractional Factorial Design Clobetasol 17-Propionate Cream
Melting Temperature :65 °C
Batch Number: 2-LFFD-CP-08 **Batch Size :** 6000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	3.46 g	RM000150
Propylene glycol	41.00	2840.64 mL	RM000181
Sodium citrate	0.05	3.46 g	RM000183
Citric acid	0.05	3.46 g	RM000185
Gelot® 64	5.00	346.42 g	RM000177
Glyceryl monostearate	6.00	415.70 g	RM000182
Ceteostearyl alcohol	3.00	207.86 g	RM000184
White beeswax	1.15	77.68 g	RM000142
Chlorocresol	0.075	5.19 g	RM000186
Distilled water	30.225	2094.11 mL	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenization Speed (rpm)	2000
Homogenization Time (min)	120
Anchor Speed (rpm)	20
Mixing Time (min)	120
Heating Temperature (°C)	65
Cooling time (min)	60

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	24710.00 ± 900	3.64
Spreadability (cm^2)	16.62 ± 1.20	7.22
CP Content (%)	100.38 ± 0.06	0.06
pH	5.60 ± 0.02	0.36
Extrudability (g/cm^2)	313.53 ± 1.20	0.38
Electrical Conductivity (μS/cm)	518.33 ± 2.10	0.41



OBSERVATIONS/COMMENTS

-A homogeneous smooth white cream was produced

- No sign of cracking or bleeding was observed during or after manufacture

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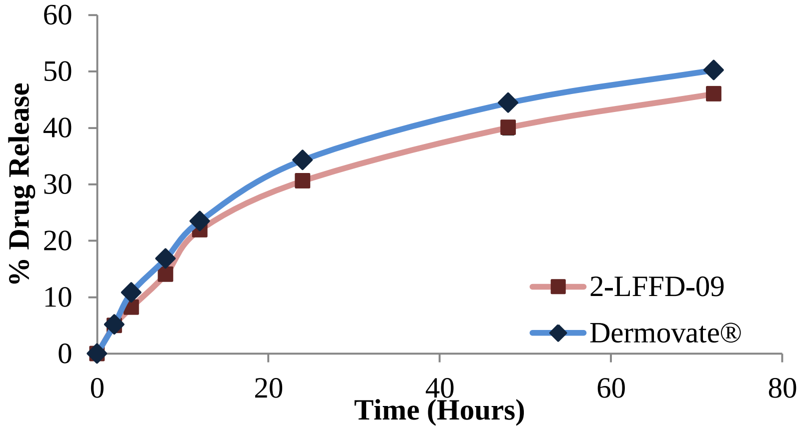
Batch Record Summary

Formulator : Ayeshah Fauzee **Date of Manufacture** :20/05/2013
Product : 2-Level Fractional Factorial Design Clobetasol 17-Propionate Cream
Melting Temperature :65 °C
Batch Number: 2-LFFD-CP-09 **Batch Size :** 6000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	3.24 g	RM000150
Propylene glycol	41.00	2656.58 mL	RM000181
Sodium citrate	0.05	3.24 g	RM000183
Citric acid	0.05	3.24 g	RM000185
Gelot® 64	1.00	64.79 g	RM000177
Glyceryl monostearate	16.00	1036.71 g	RM000182
Ceteostearyl alcohol	3.00	194.38 g	RM000184
White beeswax	1.15	74.51 g	RM000142
Chlorocresol	0.075	4.89 g	RM000186
Distilled water	30.225	1958.42 mL	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenization Speed (rpm)	1000
Homogenization Time (min)	120
Anchor Speed (rpm)	40
Mixing Time (min)	120
Heating Temperature (°C)	65
Cooling time (min)	120

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	8826.00 ± 500	5.66
Spreadability (cm^2)	28.29 ± 0.85	3.00
CP Content (%)	100.52 ± 0.90	0.89
pH	6.23 ± 0.03	0.48
Extrudability (g/cm^2)	72.37 ± 1.20	1.65
Electrical Conductivity (µS/cm)	1578.00 ± 5.62	0.36



OBSERVATIONS/COMMENTS

-A homogeneous smooth white cream was produced

- No sign of cracking or bleeding was observed during or after manufacture

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Grahamstown 6140, REPUBLIC OF SOUTH AFRICA

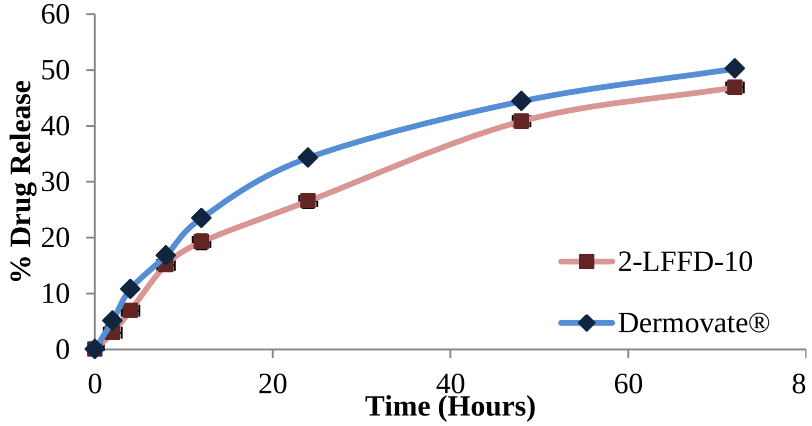
Batch Record Summary

Formulator : Ayeshah Fauzee **Date of Manufacture** :21/05/2013
Product : 2-Level Fractional Factorial Design Clobetasol 17-Propionate Cream
Melting Temperature :65 °C
Batch Number: 2-LFFD-CP-10 **Batch Size :** 6000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	2.81 g	RM000150
Propylene glycol	51.00	2870.54 mL	RM000181
Sodium citrate	0.05	2.81 g	RM000183
Citric acid	0.05	2.81 g	RM000185
Gelot® 64	5.00	281.43 g	RM000177
Glyceryl monostearate	6.00	337.71 g	RM000182
Ceteostearyl alcohol	13.00	731.71 g	RM000184
White beeswax	1.15	64.73 g	RM000142
Chlorocresol	0.075	4.22 g	RM000186
Distilled water	30.225	1701.21 mL	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenization Speed (rpm)	2000
Homogenization Time (min)	60
Anchor Speed (rpm)	20
Mixing Time (min)	60
Heating Temperature (°C)	65
Cooling time (min)	120

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	24266.00 ± 1020	4.20
Spreadability (cm^2)	12.99 ± 0.15	1.15
CP Content (%)	101.46 ± 0.05	0.05
pH	5.53 ± 0.08	1.44
Extrudability (g/cm^2)	313.56 ± 3.75	1.20
Electrical Conductivity (µS/cm)	148.33 ± 9.50	6.41



OBSERVATIONS/COMMENTS

-A homogeneous smooth white cream was produced

- No sign of cracking or bleeding was observed during or after manufacture

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Grahamstown 6140, REPUBLIC OF SOUTH AFRICA

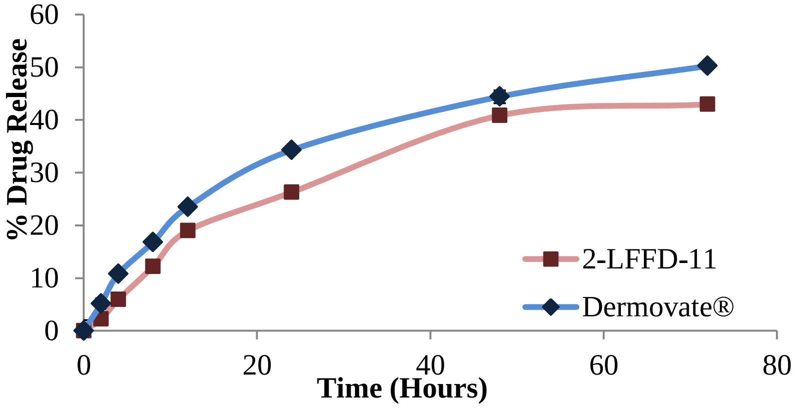
Batch Record Summary

Formulator : Ayeshah Fauzee **Date of Manufacture** :22/05/2013
Product : 2-Level Fractional Factorial Design Clobetasol 17-Propionate Cream
Melting Temperature :65 °C
Batch Number: 2-LFFD-CP-11 **Batch Size :** 3000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	1.41 g	RM000150
Propylene glycol	51.00	1435.27 mL	RM000181
Sodium citrate	0.05	1.41 g	RM000183
Citric acid	0.05	1.41 g	RM000185
Gelot® 64	5.00	140.72 g	RM000177
Glyceryl monostearate	16.00	450.28 g	RM000182
Ceteostearyl alcohol	3.00	84.43 g	RM000184
White beeswax	1.15	32.36 g	RM000142
Chlorocresol	0.075	2.11 g	RM000186
Distilled water	30.225	850.61 mL	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenization Speed (rpm)	1000
Homogenization Time (min)	60
Anchor Speed (rpm)	20
Mixing Time (min)	120
Heating Temperature (°C)	65
Cooling time (min)	120

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	18540.00 ± 1025	5.53
Spreadability (cm^2)	15.68 ± 0.10	0.63
CP Content (%)	99.14 ± 0.09	0.09
pH	6.27 ± 0.02	0.31
Extrudability (g/cm^2)	180.49 ± 1.35	0.75
Electrical Conductivity (µS/cm)	687.66 ± 10.25	1.49



OBSERVATIONS/COMMENTS

-A homogeneous smooth white cream was produced

- No sign of cracking or bleeding was observed during or after manufacture

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140, REPUBLIC OF SOUTH AFRICA

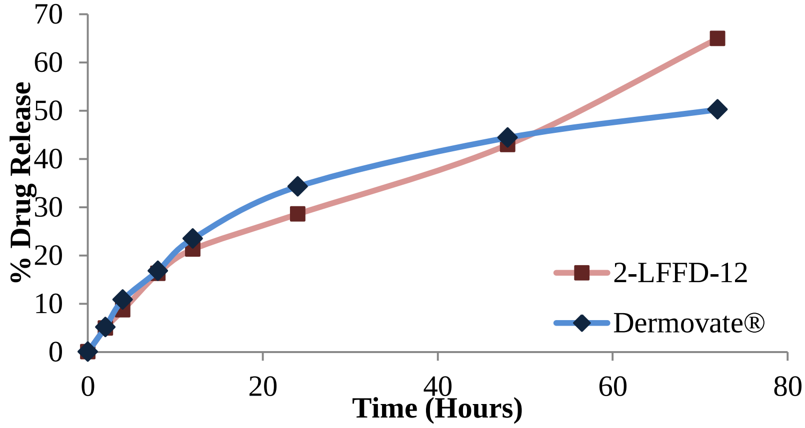
Batch Record Summary

Formulator : Ayeshah Fauzee **Date of Manufacture** :23/05/2013
Product : 2-Level Fractional Factorial Design Clobetasol 17-Propionate Cream
Melting Temperature :65 °C
Batch Number: 2-LFFD-CP-12 **Batch Size :** 3000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	1.62 g	RM000150
Propylene glycol	41.00	1328.29 mL	RM000181
Sodium citrate	0.05	1.62 g	RM000183
Citric acid	0.05	1.62 g	RM000185
Gelot® 64	1.00	32.40 g	RM000177
Glyceryl monostearate	6.00	194.38 g	RM000182
Ceteostearyl alcohol	13.00	421.17 g	RM000184
White beeswax	1.15	3.75 g	RM000142
Chlorocresol	0.075	2.43 g	RM000186
Distilled water	30.225	979.21 mL	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenization Speed (rpm)	2000
Homogenization Time (min)	120
Anchor Speed (rpm)	40
Mixing Time (min)	60
Heating Temperature (°C)	65
Cooling time (min)	120

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	12266.00 ± 540	4.40
Spreadability (cm^2)	29.24 ± 0.61	2.10
CP Content (%)	100.50 ± 1.00	1.00
pH	5.57 ± 0.03	0.53
Extrudability (g/cm^2)	98.58 ± 3.00	3.04
Electrical Conductivity (µS/cm)	308.33 ± 4.80	1.55



OBSERVATIONS/COMMENTS

-A homogeneous smooth white cream was produced

- No sign of cracking or bleeding was observed during or after manufacture

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140, REPUBLIC OF SOUTH AFRICA

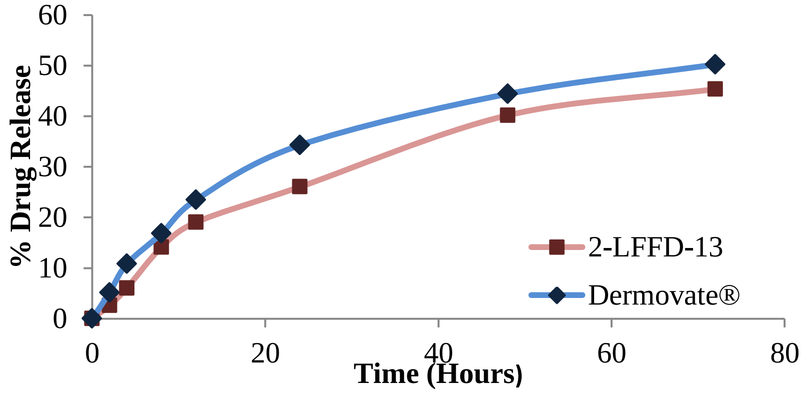
Batch Record Summary

Formulator : Ayeshah Fauzee **Date of Manufacture** :24/05/2013
Product : 2-Level Fractional Factorial Design Clobetasol 17-Propionate Cream
Melting Temperature :75 °C
Batch Number: 2-LFFD-CP-13 **Batch Size :** 3000 g

Excipient Name	**Original Formula(% w/w)**	**Working Formula**	**Rhodes Batch Number**
CP	0.05	1.55 g	RM000150
Propylene glycol	41.00	1273.29 mL	RM000181
Sodium citrate	0.05	1.55 g	RM000183
Citric acid	0.05	1.55 g	RM000185
Gelot® 64	5.00	155.28 g	RM000177
Glyceryl monostearate	6.00	186.34 g	RM000182
Ceteostearyl alcohol	13.00	403.73 g	RM000184
White beeswax	1.15	35.72 g	RM000142
Chlorocresol	0.075	2.33 g	RM000186
Distilled water	30.225	938.66 mL	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenization Speed (rpm)	1000
Homogenization Time (min)	60
Anchor Speed (rpm)	40
Mixing Time (min)	120
Heating Temperature (°C)	75
Cooling time (min)	60

Tests Performed	**Mean ± SD (n=3)**	**%RSD**
Viscosity (cP)	28000.00 ± 840	3.00
Spreadability (cm^2)	16.37 ± 0.10	0.61
CP Content (%)	100.15 ± 1.00	1.00
pH	5.67 ± 0.05	0.88
Extrudability (g/cm^2)	323.61 ± 3.00	0.93
Electrical Conductivity (µS/cm)	129.00 ± 8.40	6.51



OBSERVATIONS/COMMENTS

-A homogeneous smooth white cream was produced

- No sign of cracking or bleeding was observed during or after manufacture

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140, REPUBLIC OF SOUTH AFRICA

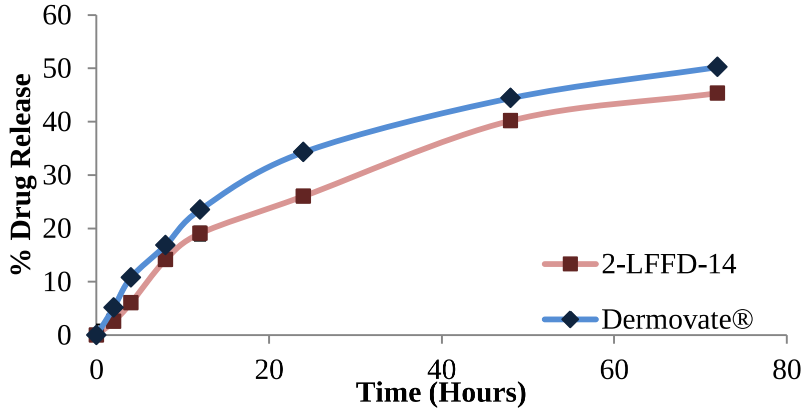
Batch Record Summary

Formulator : Ayeshah Fauzee **Date of Manufacture** :25/05/2013
Product : 2-Level Fractional Factorial Design Clobetasol 17-Propionate Cream
Melting Temperature :65 °C
Batch Number: 2-LFFD-CP-14 **Batch Size :** 6000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	2.66 g	RM000150
Propylene glycol	51.00	2715.58 mL	RM000181
Sodium citrate	0.05	2.66 g	RM000183
Citric acid	0.05	2.66 g	RM000185
Gelot® 64	1.00	53.29 g	RM000177
Glyceryl monostearate	16.00	852.58 g	RM000182
Ceteostearyl alcohol	13.00	692.72 g	RM000184
White beeswax	1.15	61.28 g	RM000142
Chlorocresol	0.075	3.99 g	RM000186
Distilled water	30.225	1610.57 mL	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenization Speed (rpm)	1000
Homogenization Time (min)	60
Anchor Speed (rpm)	40
Mixing Time (min)	60
Heating Temperature (°C)	65
Cooling time (min)	60

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	23040.00 ± 550	2.39
Spreadability (cm^2)	20.93 ± 0.25	1.19
CP Content (%)	99.71 ± 1.00	1.00
pH	6.06 ± 0.05	0.83
Extrudability (g/cm^2)	314.52 ± 1.25	0.39
Electrical Conductivity (µS/cm)	309.00 ± 5.15	1.66



OBSERVATIONS/COMMENTS

-A homogeneous smooth white cream was produced

- No sign of cracking or bleeding was observed during or after manufacture

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Grahamstown 6140, REPUBLIC OF SOUTH AFRICA

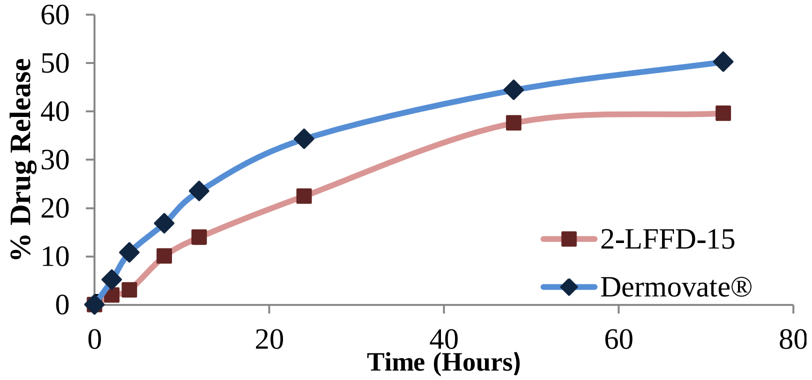
Batch Record Summary

Formulator : Ayeshah Fauzee **Date of Manufacture** :26/05/2013
Product : 2-Level Fractional Factorial Design Clobetasol 17-Propionate Cream
Melting Temperature :75 °C
Batch Number: 2-LFFD-CP-15 **Batch Size :** 6000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	2.57 g	RM000150
Propylene glycol	51.00	2624.35 mL	RM000181
Sodium citrate	0.05	2.57 g	RM000183
Citric acid	0.05	2.57 g	RM000185
Gelot® 64	5.00	257.29 g	RM000177
Glyceryl monostearate	16.00	823.33 g	RM000182
Ceteostearyl alcohol	13.00	668.96 g	RM000184
White beeswax	1.15	59.18 g	RM000142
Chlorocresol	0.075	3.86 g	RM000186
Distilled water	30.225	1555.32 mL	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenization Speed (rpm)	2000
Homogenization Time (min)	120
Anchor Speed (rpm)	40
Mixing Time (min)	120
Heating Temperature (°C)	75
Cooling time (min)	120

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	35023.00 ± 560	1.59
Spreadability (cm^2)	7.22 ± 0.21	2.91
CP Content (%)	99.71 ± 0.05	0.05
pH	5.27 ± 0.05	0.95
Extrudability (g/cm^2)	352.37 ± 3.20	0.91
Electrical Conductivity (µS/cm)	128.00 ± 5.50	4.29



OBSERVATIONS/COMMENTS

-A homogeneous smooth white cream was produced

- No sign of cracking or bleeding was observed during or after manufacture

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140, REPUBLIC OF SOUTH AFRICA

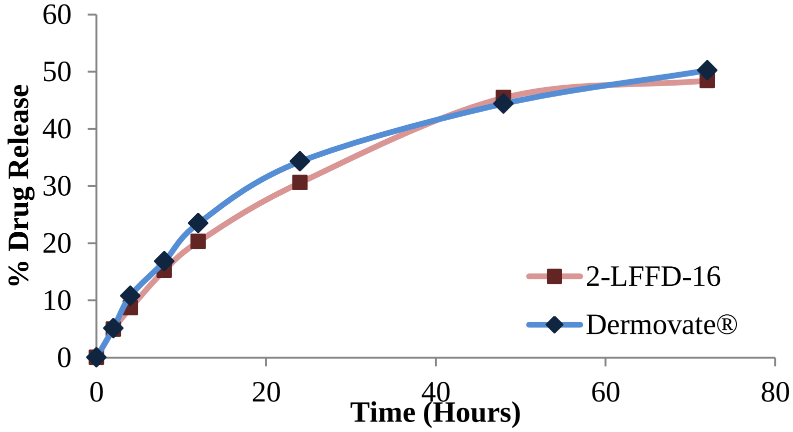
Batch Record Summary

Formulator : Ayeshah Fauzee **Date of Manufacture** :27/05/2013
Product : 2-Level Fractional Factorial Design Clobetasol 17-Propionate Cream
Melting Temperature :75 °C
Batch Number: 2-LFFD-CP-16 **Batch Size :** 3000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	1.55 g	RM000150
Propylene glycol	51.00	1583.85 mL	RM000181
Sodium citrate	0.05	1.55 g	RM000183
Citric acid	0.05	1.55 g	RM000185
Gelot® 64	5.00	155.28 g	RM000177
Glyceryl monostearate	6.00	186.34 g	RM000182
Ceteostearyl alcohol	3.00	93.17 g	RM000184
White beeswax	1.15	35.71 g	RM000142
Chlorocresol	0.075	2.33 g	RM000186
Distilled water	30.225	938.66 mL	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenization Speed (rpm)	1000
Homogenization Time (min)	120
Anchor Speed (rpm)	40
Mixing Time (min)	60
Heating Temperature (°C)	75
Cooling time (min)	120

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	11000.00 ± 800	7.28
Spreadability (cm^2)	28.00 ± 0.15	0.54
CP Content (%)	100.55 ± 1.00	1.00
pH	6.04 ± 0.06	0.99
Extrudability (g/cm^2)	98.58 ± 3.05	3.09
Electrical Conductivity (µS/cm)	392.66 ± 10.75	2.72



OBSERVATIONS/COMMENTS

-A homogeneous smooth white cream was produced

- No sign of cracking or bleeding was observed during or after manufacture

APPENDIX II

BATCH PRODUCTION RECORDS

SCREENING DESIGN MANUFACTURE

PLACKETT-BURMAN DESIGN

A sample of a batch production record for the Plackett-Burman design *viz.*, Batches PBD-CP-01 manufactured using a Wintech® cream/ointment mixer for CP screening design cream formulations is included in this accompanying electronic file “Appendices-CD” on the CD. The batch production records for all batches *viz.*, PBD-CP-02 – PBD-CP-12 are available on request.

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BATCH RECORD PRODUCTION

Product name: Plackett-Burman Design Clobetasol 17-Propionate Cream Page 1 of 3
Batch number: PBD-CP-01 **Batch Size**: 6000 g

MANUFACTURING APPROVALS

Batch record issued by:_________________ **Date:_________________**

Master record issued by:_________________ **Date:_________________**

Product name: Plackett-Burman Design Clobetasol 17-Propionate Cream
Batch number: PBD-CP-01 **Batch Size**: 6000 g

MASTER FORMULA AND BATCH FORMULA							
Item no.	**Material**	**Rhodes No.**	**Quantity (% w/w)**	**Amount/ Batch**	**Amount Dispensed**	**Dispensed by**	**Checked by**
1	CP	RM000150	0.05	3.24 g			
2	Propylene glycol	RM000181	51.00	3304.54 mL			
3	Sodium citrate	RM000183	0.05	3.24 g			
4	Citric acid	RM000185	0.05	3.24 g			
5	Gelot® 64	RM000177	1.00	64.79 g			
6	Glyceryl monostearate	RM000182	6.00	388.77 g			
7	Cetostearyl alcohol	RM000184	3.00	194.38 g			
8	White beeswax	RM000142	1.15	74.51 g			
9	Chlorocresol	RM000186	0.075	4.89 g			
10	Distilled water	N/A	30.225	1958.42 mL			

WINTECH® CREAM/OINTMENT MIXER VERIFICATION			
Description	**Type**	**Verified by**	**Confirmed by**
Bowl vessel	Stainless steel		
Co-centric Homogenizer	Teflon and stainless steel		
Anchor and impellers	Stainless steel		
Water inlet	Stainless steel		
Water Jacket	Stainless steel		
Charging port	Stainless steel		
Water immersion heater	NA		
PT-100 sensor	NA		
Operating panel touch screen	LCD		

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Grahamstown 6140, REPUBLIC OF SOUTH AFRICA

BATCH PRODUCTION RECORD

Product name: Plackett-Burman Design Clobetasol 17-Propionate Cream Page 3 of 3
Batch number: PBD-CP-01 **Batch Size**: 6000 g

MANUFACTURING PROCEDURE				
Steps	**Procedure**	**Time**	**Done by**	**Checked by**
1	Clean the bowl with Liquid K® detergent and distilled water. Allow the bowl to dry and equilibrate at room temperature Room temperature: _____°C Mixer temperature: _____°C			
2	Fill the jacket with distilled water until the jacket is 30 Liters			
3	Weigh all the materials accurately using a Model AE 163 Mettler® analytical balance (Mettler® Inc., Zurich, Switzerland)			
4	Introduce 3304.54 mL propylene glycol and 1958.42 mL distilled water, followed by 3.24 g sodium citrate, 3.24 g citric acid, 3.24 g clobetasol 17-propionate, 388.77 g glyceryl monostearate, 194.38 g cetostearyl alcohol, 74.51 g white beeswax, 64.79 g Gelot® 64 and 4.89 g chlorocresol through the charging port			
5	Allow mixture of excipients to stir at an anchor speed of 40 rpm for 60 minutes at 65°C			
6	Following mixing, allow the blend to be homogenized at 2000 rpm for 60 minutes			
7	Allow the molten mixture to cool by passing distilled water through the heating jacket for 120 minutes Temperature of cold water=____°C			
8	After cooling, close the water inlet and allow slow continuous agitation of the anchor until the temperature on the operating panel displays 30°C - 35°C			
9	Stop the anchor and release the pressure built up in the bowl vessel by the use of the pressure vent			
10	Store the final CP cream in 10 kg opaque containers until quality control tests are completed and then package into 50 g collapsible aluminium cream tubes			

SIGNATURE AND INITIAL REFERENCE			
Full name (Print)	**Signature**	**Initials**	**Date**

APPENDIX III

BATCH RECORD REPORTS

The batch record reports for all the Plackett-Burman design formulation batches, *viz.*, PBD-CP-01 – PBD-CP-12 are included on this accompanying electronic file “Appendices-CD” on the CD.

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140, REPUBLIC OF SOUTH AFRICA

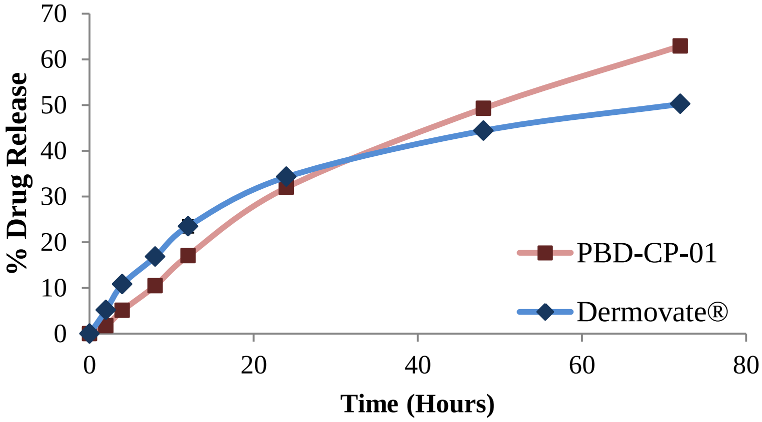
Batch Record Summary

Formulator : Ayeshah Fauzee
Date of Manufacture :25/04/2013
Product : Plackett-Burman Design Clobetasol 17-Propionate Cream
Melting Temperature :65 °C
Batch Number: PBD-CP-01
Batch Size : 6000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	3.24 g	RM000150
Propylene glycol	51.00	3304.54 mL	RM000181
Sodium citrate	0.05	3.24 g	RM000183
Citric acid	0.05	3.24 g	RM000185
Gelot® 64	1.00	64.79 g	RM000177
Glyceryl monostearate	6.00	388.77 g	RM000182
Ceteostearyl alcohol	3.00	194.38 g	RM000184
White beeswax	1.15	74.51 g	RM000142
Chlorocresol	0.075	4.89 g	RM000186
Distilled water	30.225	1958.42 mL	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenization Speed (rpm)	2000
Homogenization Time (min)	60
Anchor Speed (rpm)	40
Mixing Time (min)	60
Heating Temperature (°C)	65
Cooling time (min)	120

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	5633.00 ± 75	1.33
Spreadability (cm^2)	58.10 ± 0.10	0.17
CP Content (%)	100.38 ± 1.02	1.02
pH	6.65 ± 0.06	0.90
Extrudability (g/cm^2)	57.66 ± 1.02	1.77
Electrical Conductivity (μS/cm)	599.66 ± 4.20	0.70



OBSERVATIONS/COMMENTS

-A homogeneous smooth white cream was produced

- No sign of cracking but slight bleeding after manufacture

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140, REPUBLIC OF SOUTH AFRICA

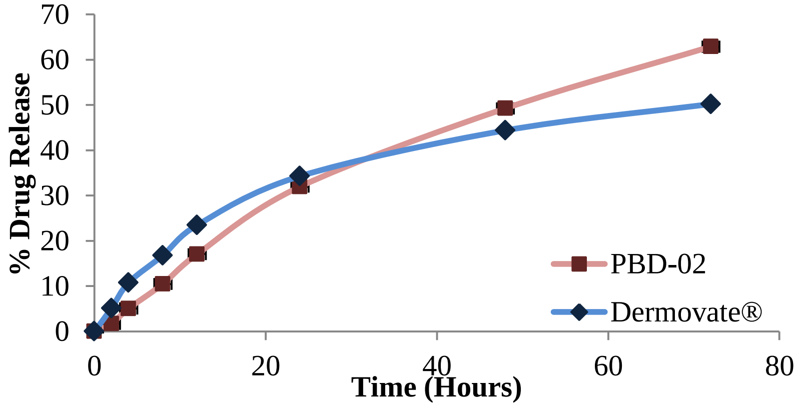
Batch Record Summary

Formulator : Ayeshah Fauzee
Date of Manufacture :26/04/2013
Product : Plackett-Burman Design Clobetasol 17-Propionate Cream
Melting Temperature :65 °C
Batch Number: PBD-CP-02
Batch Size : 3000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	1.33 g	RM000150
Propylene glycol	51.00	1358.79 mL	RM000181
Sodium citrate	0.05	1.33 g	RM000183
Citric acid	0.05	1.33 g	RM000185
Gelot® 64	1.00	26.65 g	RM000177
Glyceryl monostearate	16.00	426.17 g	RM000182
Ceteostearyl alcohol	13.00	346.36 g	RM000184
White beeswax	1.15	30.64 g	RM000142
Chlorocresol	0.075	1.89 g	RM000186
Distilled water	30.225	805.28 mL	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenization Speed (rpm)	1000
Homogenization Time (min)	120
Anchor Speed (rpm)	40
Mixing Time (min)	120
Heating Temperature (°C)	65
Cooling time (min)	60

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	51166.00 ± 2015	3.93
Spreadability (cm^2)	18.17 ± 1.20	6.60
CP Content (%)	100.52 ± 1.50	1.49
pH	6.67 ± 0.02	1.12
Extrudability (g/cm^2)	116.60 ± 2.12	1.81
Electrical Conductivity (µS/cm)	327.66 ± 7.56	2.30



OBSERVATIONS/COMMENTS

-A homogeneous smooth white cream was produced

- No sign of cracking or bleeding during and after manufacture

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140, REPUBLIC OF SOUTH AFRICA

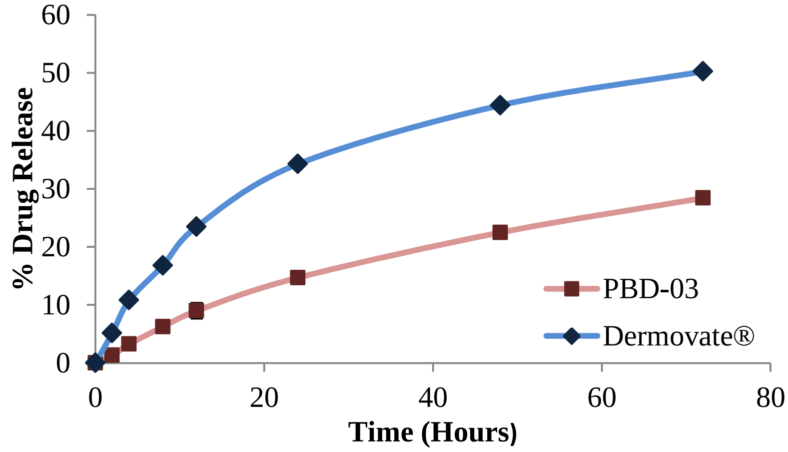
Batch Record Summary

Formulator : Ayeshah Fauzee
Date of Manufacture :27/04/2013
Product : Plackett-Burman Design Clobetasol 17-Propionate Cream
Melting Temperature :65 °C
Batch Number: PBD-CP-03
Batch Size : 3000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	1.41 g	RM000150
Propylene glycol	51.00	1435.27 mL	RM000181
Sodium citrate	0.05	1.41 g	RM000183
Citric acid	0.05	1.41 g	RM000185
Gelot® 64	5.00	140.71 g	RM000177
Glyceryl monostearate	6.00	168.85 g	RM000182
Ceteostearyl alcohol	13.00	365.85 g	RM000184
White beeswax	1.15	32.36 g	RM000142
Chlorocresol	0.075	2.11 g	RM000186
Distilled water	30.225	850.61 mL	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenization Speed (rpm)	1000
Homogenization Time (min)	120
Anchor Speed (rpm)	40
Mixing Time (min)	60
Heating Temperature (°C)	75
Cooling time (min)	120

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	58133.00 ±1289	2.22
Spreadability (cm^2)	21.77 ± 1.25	5.74
CP Content (%)	101.46 ± 1.02	1.02
pH	6.55 ± 0.05	0.76
Extrudability (g/cm^2)	141.51 ± 2.10	1.48
Electrical Conductivity (µS/cm)	132.83 ± 5.23	3.91



OBSERVATIONS/COMMENTS

-A homogeneous smooth white cream was produced

- No sign of cracking or bleeding during and after manufacture

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140, REPUBLIC OF SOUTH AFRICA

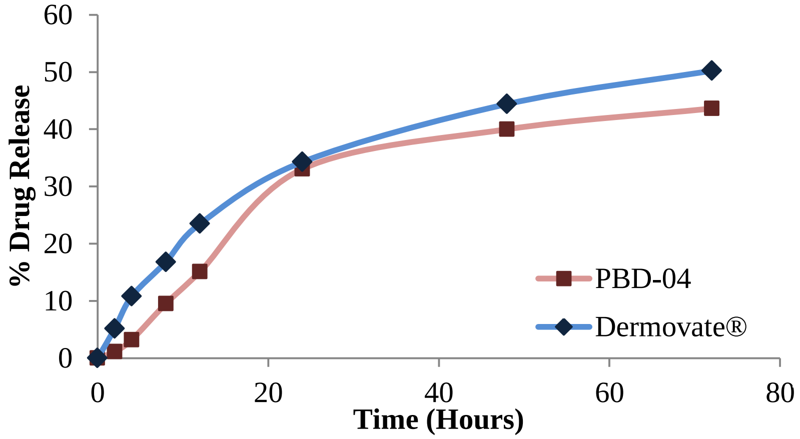
Batch Record Summary

Formulator : Ayeshah Fauzee **Date of Manufacture** :28/04/2013
Product : Plackett-Burman Design Clobetasol 17-Propionate Cream **Melting Temperature** :75 °C
Batch Number: PBD-CP-04 **Batch Size :** 6000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	3.24 g	RM000150
Propylene glycol	41.00	2656.58 mL	RM000181
Sodium citrate	0.05	3.24 g	RM000183
Citric acid	0.05	3.24 g	RM000185
Gelot® 64	1.00	64.79 g	RM000177
Glyceryl monostearate	16.00	1036.74 g	RM000182
Ceteostearyl alcohol	3.00	194.38 g	RM000184
White beeswax	1.15	74.51 g	RM000142
Chlorocresol	0.075	4.89 g	RM000186
Distilled water	30.225	1958.42 mL	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenization Speed (rpm)	1000
Homogenization Time (min)	120
Anchor Speed (rpm)	40
Mixing Time (min)	60
Heating Temperature (°C)	75
Cooling time (min)	120

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	16466.00 ± 960	5.83
Spreadability (cm^2)	26.11 ± 1.02	3.91
CP Content (%)	99.14 ± 1.90	1.91
pH	6.33 ± 0.07	1.11
Extrudability (g/cm^2)	288.93 ± 2.10	0.73
Electrical Conductivity (µS/cm)	826.00 ± 8.50	1.03



OBSERVATIONS/COMMENTS

-A homogeneous smooth white cream was produced

- No sign of cracking or bleeding was observed during or after manufacture

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Grahamstown 6140, REPUBLIC OF SOUTH AFRICA

Batch Record Summary

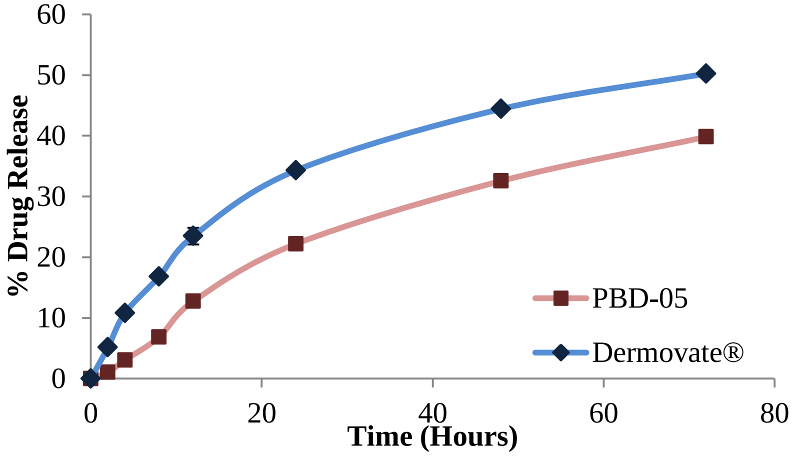
Formulator : Ayeshah Fauzee
Product : Plackett-Burman Design Clobetasol 17-Propionate Cream
Batch Number: PBD-CP-05

Date of Manufacture :29/04/2013
Melting Temperature :65 °C
Batch Size : 6000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	2.81 g	RM000150
Propylene glycol	41.00	2307.69 mL	RM000181
Sodium citrate	0.05	2.81 g	RM000183
Citric acid	0.05	2.81 g	RM000185
Gelot® 64	5.00	281.43 g	RM000177
Glyceryl monostearate	16.00	900.56 g	RM000182
Ceteostearyl alcohol	13.00	731.71 g	RM000184
White beeswax	1.15	64.72 g	RM000142
Chlorocresol	0.075	4.22 g	RM000186
Distilled water	30.225	1701.22 mL	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenization Speed (rpm)	1000
Homogenization Time (min)	60
Anchor Speed (rpm)	20
Mixing Time (min)	120
Heating Temperature (°C)	65
Cooling time (min)	120

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	81466.00 ± 2132	2.62
Spreadability (cm^2)	12.51 ± 0.98	7.83
CP Content (%)	100.25 ± 1.50	1.50
pH	6.97 ± 0.04	0.57
Extrudability (g/cm^2)	497.42 ± 2.10	0.42
Electrical Conductivity (µS/cm)	312.00 ± 8.52	2.73



OBSERVATIONS/COMMENTS

-A homogeneous smooth white cream was produced

- No sign of cracking or bleeding was observed during or after manufacture

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140, REPUBLIC OF SOUTH AFRICA

Batch Record Summary

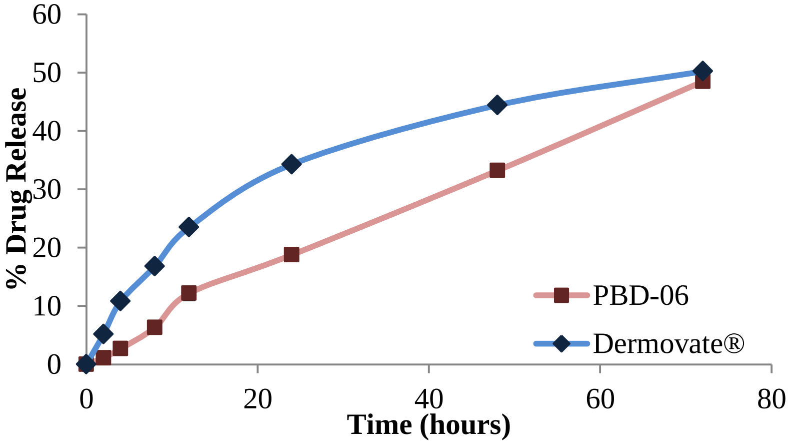
Formulator : Ayeshah Fauzee
Product : Plackett-Burman Design Clobetasol 17-Propionate Cream
Batch Number: PBD-CP-06

Date of Manufacture :01/05/2013
Melting Temperature :65 °C
Batch Size : 6000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	3.11 g	RM000150
Propylene glycol	41.00	2546.58 mL	RM000181
Sodium citrate	0.05	3.11 g	RM000183
Citric acid	0.05	3.11 g	RM000185
Gelot® 64	5.00	310.59 g	RM000177
Glyceryl monostearate	6.00	372.67 g	RM000182
Ceteostearyl alcohol	13.00	807.45 g	RM000184
White beeswax	1.15	71.42 g	RM000142
Chlorocresol	0.075	4.66 g	RM000186
Distilled water	30.225	1877.33 mL	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenization Speed (rpm)	2000
Homogenization Time (min)	120
Anchor Speed (rpm)	40
Mixing Time (min)	60
Heating Temperature (°C)	65
Cooling time (min)	60

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	95933.00 ± 2350	2.45
Spreadability (cm^2)	17.17 ± 1.20	6.98
CP Content (%)	100.79 ± 2.03	2.01
pH	6.36 ± 0.10	1.57
Extrudability (g/cm^2)	221.64 ± 1.23	0.55
Electrical Conductivity (µS/cm)	124.50 ± 10.56	8.52



OBSERVATIONS/COMMENTS

-A homogeneous smooth white cream was produced

- No sign of cracking or bleeding was observed during or after manufacture

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140, REPUBLIC OF SOUTH AFRICA

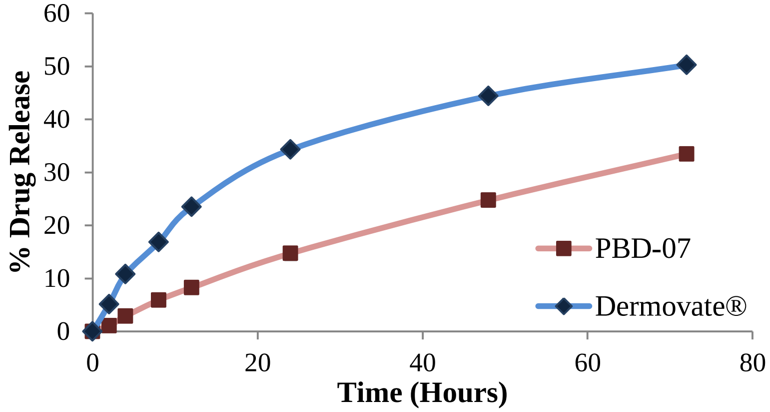
Batch Record Summary

Formulator : Ayeshah Fauzee **Date of Manufacture** :02/05/2013
Product : Plackett-Burman Design Clobetasol 17-Propionate Cream **Melting Temperature** :75 °C
Batch Number: PBD-CP-07 **Batch Size :** 6000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	2.66 g	RM000150
Propylene glycol	51.00	2717.58 mL	RM000181
Sodium citrate	0.05	2.66 g	RM000183
Citric acid	0.05	2.66 g	RM000185
Gelot® 64	1.00	53.29 g	RM000177
Glyceryl monostearate	16.00	852.57 g	RM000182
Ceteostearyl alcohol	13.00	692.71 g	RM000184
White beeswax	1.15	61.28 g	RM000142
Chlorocresol	0.075	3.99 g	RM000186
Distilled water	30.225	1610.57 mL	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenization Speed (rpm)	2000
Homogenization Time (min)	60
Anchor Speed (rpm)	20
Mixing Time (min)	60
Heating Temperature (°C)	75
Cooling time (min)	60

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	62066.00 ± 1125	1.81
Spreadability (cm^2)	19.88 ± 0.29	1.46
CP Content (%)	99.08 ± 0.12	0.12
pH	6.08 ± 0.02	0.33
Extrudability (g/cm^2)	263.33 ± 2.15	0.82
Electrical Conductivity (µS/cm)	328.77 ± 8.90	2.71



OBSERVATIONS/COMMENTS

-A homogeneous smooth white cream was produced

- No sign of cracking or bleeding was observed during or after manufacture

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140, REPUBLIC OF SOUTH AFRICA

Batch Record Summary

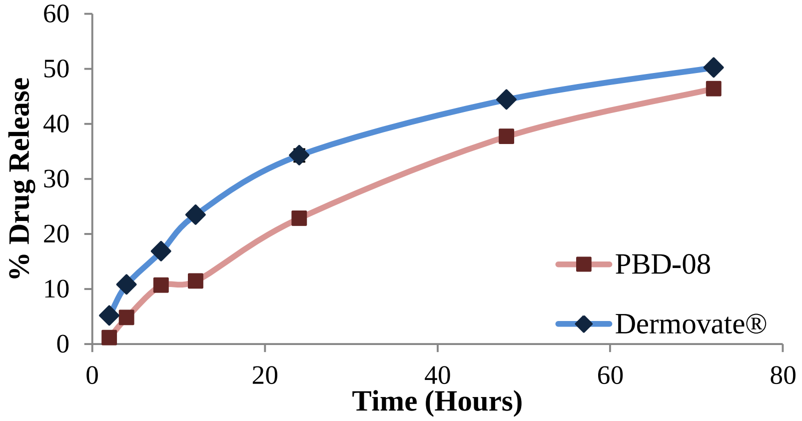
Formulator : Ayeshah Fauzee
Product : Plackett-Burman Design Clobetasol 17-Propionate Cream
Batch Number: PBD-CP-08

Date of Manufacture :03/05/2013
Melting Temperature :65 °C
Batch Size : 3000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	1.41 g	RM000150
Propylene glycol	51.00	1435.27 mL	RM000181
Sodium citrate	0.05	1.41 g	RM000183
Citric acid	0.05	1.41 g	RM000185
Gelot® 64	5.00	140.72 g	RM000177
Glyceryl monostearate	16.00	450.28 g	RM000182
Ceteostearyl alcohol	3.00	84.42 g	RM000184
White beeswax	1.15	32.36 g	RM000142
Chlorocresol	0.075	2.11 g	RM000186
Distilled water	30.225	850.61 mL	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenization Speed (rpm)	2000
Homogenization Time (min)	120
Anchor Speed (rpm)	20
Mixing Time (min)	60
Heating Temperature (°C)	65
Cooling time (min)	120

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	43866.00 ± 750	1.71
Spreadability (cm^2)	16.37 ± 1.00	6.11
CP Content (%)	99.19 ± 1.59	1.60
pH	6.46 ± 0.04	0.62
Extrudability (g/cm^2)	318.71 ± 2.15	0.67
Electrical Conductivity (µS/cm)	705.66 ± 8.56	1.22



OBSERVATIONS/COMMENTS

-A homogeneous smooth white cream was produced

- No sign of cracking or bleeding was observed during or after manufacture

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Grahamstown 6140, REPUBLIC OF SOUTH AFRICA

Batch Record Summary

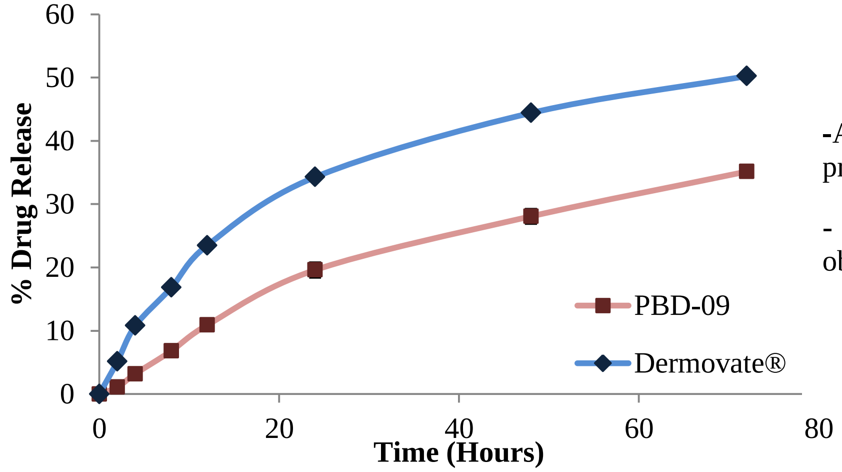
Formulator : Ayeshah Fauzee
Product : Plackett-Burman Design Clobetasol 17-Propionate Cream
Batch Number: PBD-CP-09

Date of Manufacture :04/05/2013
Melting Temperature :75 °C
Batch Size : 3000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	1.64 g	RM000150
Propylene glycol	41.00	1342.79 mL	RM000181
Sodium citrate	0.05	1.64 g	RM000183
Citric acid	0.05	1.64 g	RM000185
Gelot® 64	1.00	32.75 g	RM000177
Glyceryl monostearate	6.00	196.51 g	RM000182
Ceteostearyl alcohol	13.00	425.76 g	RM000184
White beeswax	1.15	37.66 g	RM000142
Chlorocresol	0.075	2.45 g	RM000186
Distilled water	30.225	989.90 mL	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenization Speed (rpm)	2000
Homogenization Time (min)	120
Anchor Speed (rpm)	20
Mixing Time (min)	120
Heating Temperature (°C)	75
Cooling time (min)	120

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	38300.00 ± 1050	2.74
Spreadability (cm^2)	26.11 ± 0.89	3.41
CP Content (%)	100.98 ± 1.80	1.78
pH	6.68 ± 0.04	0.60
Extrudability (g/cm^2)	392.97 ± 2.50	0.64
Electrical Conductivity (µS/cm)	265.00 ± 10.57	3.98



OBSERVATIONS/COMMENTS

-A homogeneous smooth white cream was produced

- No sign of cracking or bleeding was observed during or after manufacture

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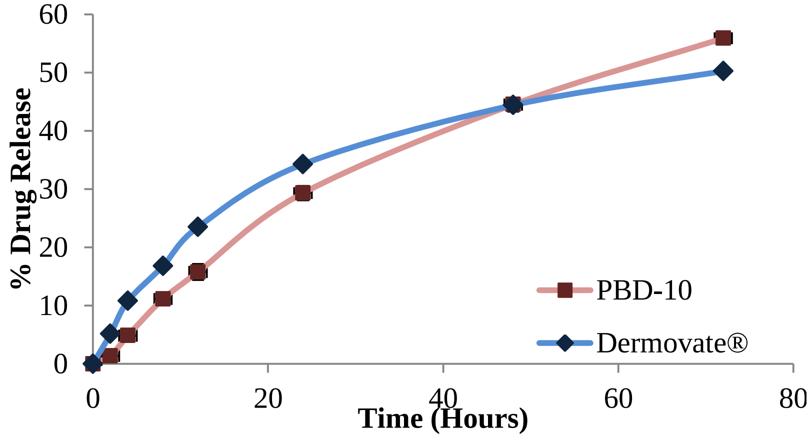
Batch Record Summary

Formulator : Ayeshah Fauzee **Date of Manufacture** :05/05/2013
Product : Plackett-Burman Design Clobetasol 17-Propionate Cream **Melting Temperature** :65 °C
Batch Number: PBD-CP-10 **Batch Size :** 3000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	1.82 g	RM000150
Propylene glycol	41.00	1489.10 mL	RM000181
Sodium citrate	0.05	1.82 g	RM000183
Citric acid	0.05	1.82 g	RM000185
Gelot® 64	1.00	36.32 g	RM000177
Glyceryl monostearate	6.00	217.92 g	RM000182
Ceteostearyl alcohol	3.00	108.95 g	RM000184
White beeswax	1.15	47.77 g	RM000142
Chlorocresol	0.075	2.73 g	RM000186
Distilled water	30.225	1098.76 mL	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenization Speed (rpm)	1000
Homogenization Time (min)	60
Anchor Speed (rpm)	20
Mixing Time (min)	60
Heating Temperature (°C)	65
Cooling time (min)	60

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	5566.00 ± 530	9.52
Spreadability (cm^2)	43.40 ± 1.25	2.88
CP Content (%)	100.20 ± 1.00	1.00
pH	6.71 ± 0.08	1.19
Extrudability (g/cm^2)	50.56 ± 2.96	5.85
Electrical Conductivity (µS/cm)	703.66 ± 5.25	0.75



OBSERVATIONS/COMMENTS

-A homogeneous smooth white cream was produced

- No sign of cracking or bleeding was observed during or after manufacture

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Grahamstown 6140, REPUBLIC OF SOUTH AFRICA

Batch Record Summary

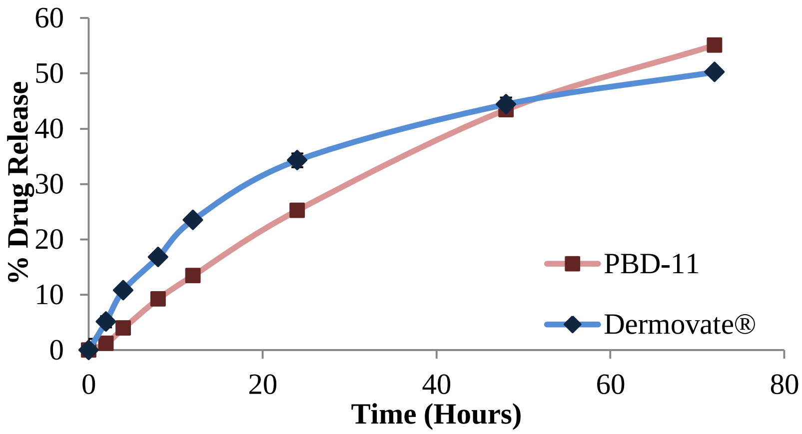
Formulator : Ayeshah Fauzee
Product : Plackett-Burman Design Clobetasol 17-Propionate Cream
Batch Number: PBD-CP-11

Date of Manufacture :06/05/2013
Melting Temperature :75 °C
Batch Size : 3000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	1.55 g	RM000150
Propylene glycol	41.00	1273.29 mL	RM000181
Sodium citrate	0.05	1.55 g	RM000183
Citric acid	0.05	1.55 g	RM000185
Gelot® 64	5.00	64.79 g	RM000177
Glyceryl monostearate	16.00	155.28 g	RM000182
Ceteostearyl alcohol	3.00	93.17 g	RM000184
White beeswax	1.15	35.71 g	RM000142
Chlorocresol	0.075	2.33 g	RM000186
Distilled water	30.225	938.67 mL	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenization Speed (rpm)	2000
Homogenization Time (min)	60
Anchor Speed (rpm)	40
Mixing Time (min)	120
Heating Temperature (°C)	75
Cooling time (min)	60

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	48933.00 ± 1123	2.29
Spreadability (cm^2)	14.33 ± 0.15	1.05
CP Content (%)	100.72 ± 1.10	1.10
pH	6.36 ± 0.03	0.47
Extrudability (g/cm^2)	203.64 ± 2.56	1.26
Electrical Conductivity (µS/cm)	212.76 ± 8.90	4.18



OBSERVATIONS/COMMENTS

-A homogeneous smooth white cream was produced

- No sign of cracking or bleeding was observed during or after manufacture

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140, REPUBLIC OF SOUTH AFRICA

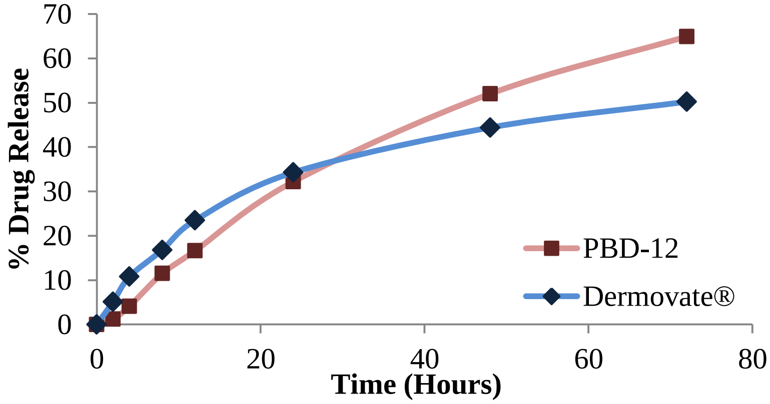
Batch Record Summary

Formulator : Ayeshah Fauzee
Date of Manufacture :07/05/2013
Product : Plackett-Burman Design Clobetasol 17-Propionate Cream
Melting Temperature :75 °C
Batch Number: PBD-CP-12
Batch Size : 6000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	3.11 g	RM000150
Propylene glycol	51.00	3167.70 mL	RM000181
Sodium citrate	0.05	3.11 g	RM000183
Citric acid	0.05	3.11 g	RM000185
Gelot® 64	5.00	310.56 g	RM000177
Glyceryl monostearate	6.00	372.67 g	RM000182
Ceteostearyl alcohol	3.00	186.34 g	RM000184
White beeswax	1.15	71.43 g	RM000142
Chlorocresol	0.075	4.66 g	RM000186
Distilled water	30.225	1877.33 mL	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenization Speed (rpm)	1000
Homogenization Time (min)	120
Anchor Speed (rpm)	20
Mixing Time (min)	120
Heating Temperature (°C)	75
Cooling time (min)	60

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	23800.00 ± 980	4.12
Spreadability (cm^2)	28.97 ± 1.10	3.79
CP Content (%)	100.47 ± 1.00	1.00
pH	6.83 ± 0.08	1.17
Extrudability (g/cm^2)	80.03 ± 2.20	2.75
Electrical Conductivity (µS/cm)	375.00 ± 5.50	1.47



OBSERVATIONS/COMMENTS

-A homogeneous smooth white cream was produced

- No sign of cracking or bleeding was observed during or after manufacture

APPENDIX II

BATCH PRODUCTION RECORDS

SCREENING DESIGN MANUFACTURE

TAGUCHI DESIGN

A sample of a batch production record for the Taguchi design *viz.*, Batches TD-CP-01 manufactured using a Wintech® cream/ointment mixer for CP screening design cream formulations is included in this accompanying electronic file "Appendices-CD" on the CD. The batch production records for all batches *viz.*, TD-CP-02 – TD-CP-12 are available on request.

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140, REPUBLIC OF SOUTH AFRICA

BATCH RECORD PRODUCTION

Product name: Taguchi Design Clobetasol 17-Propionate Cream Page 1 of 3
Batch number: TD-CP-01 **Batch Size**: 6000 g

MANUFACTURING APPROVALS

Batch record issued by:_________________ **Date:_________________**

Master record issued by:_________________ **Date:_________________**

BATCH PRODUCTION RECORD

Product name: Taguchi Design Clobetasol 17-Propionate Cream Page 2 of 3
Batch number: TD-CP-01 **Batch Size**: 6000 g

MASTER FORMULA AND BATCH FORMULA							
Item no.	**Material**	**Rhodes No.**	**Quantity (% w/w)**	**Amount/ Batch**	**Amount Dispensed**	**Dispensed by**	**Checked by**
1	CP	RM000150	0.05	4.50 g			
2	Propylene glycol	RM000181	51.00	2870.05 mL			
3	Sodium citrate	RM000183	0.05	4.50 g			
4	Citric acid	RM000185	0.05	4.50 g			
5	Gelot® 64	RM000177	5.00	281.40 g			
6	Glyceryl monostearate	RM000182	16.00	900.56 g			
7	Cetostearyl alcohol	RM000184	3.00	168.85 g			
8	White beeswax	RM000142	1.15	64.72 g			
9	Chlorocresol	RM000186	0.075	4.22 g			
10	Distilled water	N/A	30.225	1701.21 mL			

WINTECH® CREAM/OINTMENT MIXER VERIFICATION			
Description	**Type**	**Verified by**	**Confirmed by**
Bowl vessel	Stainless steel		
Co-centric Homogenizer	Teflon and stainless steel		
Anchor and impellers	Stainless steel		
Water inlet	Stainless steel		
Water Jacket	Stainless steel		
Charging port	Stainless steel		
Water immersion heater	NA		
PT-100 sensor	NA		
Operating panel touch screen	LCD		

BATCH PRODUCTION RECORD

Product name: Taguchi Design Clobetasol 17-Propionate Cream
Batch number: TD-CP-01 **Batch Size:** 6000 g

MANUFACTURING PROCEDURE

Steps	Procedure	Time	Done by	Checked by
1	Clean the bowl with Liquid K$^{®}$ detergent and distilled water. Allow the bowl to dry and equilibrate at room temperature Room temperature: _____°C Mixer temperature: _____°C			
2	Fill the jacket with distilled water until the jacket is 30 Liters			
3	Weigh all the materials accurately using a Model AE 163 Mettler$^{®}$ analytical balance (Mettler$^{®}$ Inc., Zurich, Switzerland)			
4	Introduce 2870.05 mL propylene glycol and 1701.21 mL distilled water, followed by 4.50 g sodium citrate, 4.50 g citric acid, 4.50 g clobetasol 17-propionate, 900.56 g glyceryl monostearate, 168.85 g cetostearyl alcohol, 64.72 g white beeswax, 281.40 g Gelot$^{®}$ 64 and 4.22 g chlorocresol through the charging port			
5	Allow mixture of excipients to stir at an anchor speed of 20 rpm for 60 minutes at 65°C			
6	Following mixing, allow the blend to be homogenized at 1000 rpm for 120 minutes			
7	Allow the molten mixture to cool by passing distilled water through the heating jacket for 120 minutes Temperature of cold water=____°C			
8	After cooling, close the water inlet and allow slow continuous agitation of the anchor until the temperature on the operating panel displays 30°C - 35°C			
9	Stop the anchor and release the pressure built up in the bowl vessel by the use of the pressure vent			
10	Store the final CP cream in 10 kg opaque containers until quality control tests are completed and then package into 50 g collapsible aluminium cream tubes			

SIGNATURE AND INITIAL REFERENCE

Full name (Print)	Signature	Initials	Date

APPENDIX III

BATCH RECORD REPORTS

The batch record reports for the Taguchi screening design for all formulation batches, *viz.*, TD-CP-01 – TD-CP-12 are included on this accompanying electronic file “Appendices-CD” on the CD.

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140, REPUBLIC OF SOUTH AFRICA

Batch Record Summary

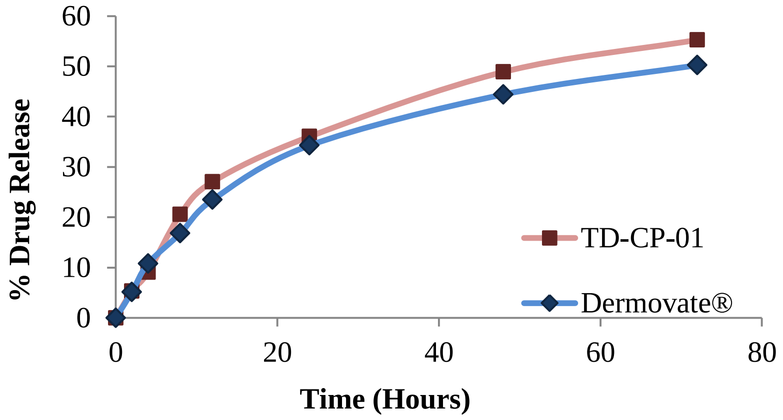
Formulator : Ayeshah Fauzee
Product : Taguchi Design Clobetasol 17-Propionate Cream
Batch Number: TD-CP-01

Date of Manufacture :10/04/2013
Melting Temperature :65 °C
Batch Size : 6000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	4.50 g	RM000150
Propylene glycol	51.00	2870.05 mL	RM000181
Sodium citrate	0.05	4.50 g	RM000183
Citric acid	0.05	4.50 g	RM000185
Gelot® 64	5.00	281.40 g	RM000177
Glyceryl monostearate	16.00	900.56 g	RM000182
Ceteostearyl alcohol	3.00	168.85 g	RM000184
White beeswax	1.15	64.72 g	RM000142
Chlorocresol	0.075	4.22 g	RM000186
Distilled water	30.225	1701.21 mL	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenization Speed (rpm)	1000
Homogenization Time (min)	120
Anchor Speed (rpm)	20
Mixing Time (min)	60
Heating Temperature (°C)	65
Cooling time (min)	120

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	37000.00 ± 550	1.49
Spreadability (cm^2)	27.00 ± 0.60	2.22
CP Content (%)	100.10 ± 1.50	1.50
pH	6.54 ± 0.08	1.22
Extrudability (g/cm^2)	352.40 ± 1.52	0.43
Electrical Conductivity (µS/cm)	490.30 ± 7.30	1.48



OBSERVATIONS/COMMENTS

-A homogeneous smooth white cream was produced

- No sign of cracking or bleeding was observed during or after manufacture

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140, REPUBLIC OF SOUTH AFRICA

Batch Record Summary

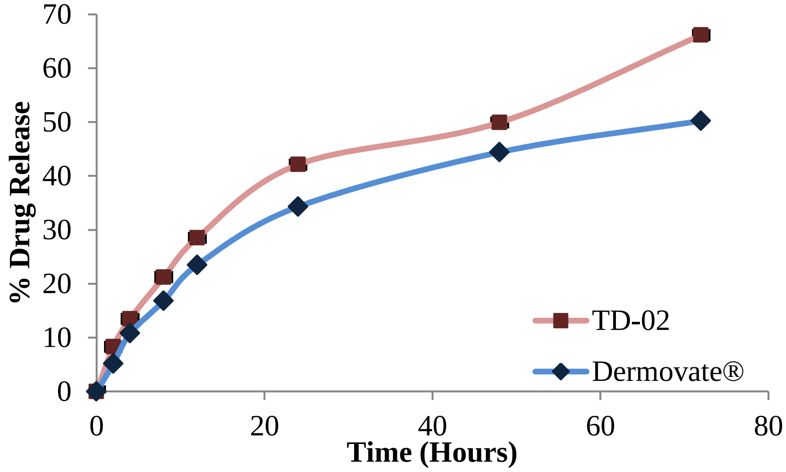
Formulator : Ayeshah Fauzee
Product : Taguchi Design Clobetasol 17-Propionate Cream
Batch Number: TD-CP-02

Date of Manufacture :11/04/2013
Melting Temperature :75 °C
Batch Size : 6000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	3.63 g	RM000150
Propylene glycol	41.00	2978.20 mL	RM000181
Sodium citrate	0.05	3.63 g	RM000183
Citric acid	0.05	3.63 g	RM000185
Gelot® 64	1.00	72.63 g	RM000177
Glyceryl monostearate	6.00	435.84 g	RM000182
Ceteostearyl alcohol	3.00	217.91 g	RM000184
White beeswax	1.15	83.54 g	RM000142
Chlorocresol	0.075	5.44 g	RM000186
Distilled water	30.225	2195.52 mL	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenization Speed (rpm)	1000
Homogenization Time (min)	120
Anchor Speed (rpm)	40
Mixing Time (min)	120
Heating Temperature (°C)	75
Cooling time (min)	120

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	6500.00 ± 450	6.92
Spreadability (cm^2)	47.82 ± 1.20	2.50
CP Content (%)	99.74 ± 2.10	2.10
pH	5.74 ± 0.02	0.34
Extrudability (g/cm^2)	72.02 ± 2.50	3.47
Electrical Conductivity (µS/cm)	899.70 ± 10.25	1.13



OBSERVATIONS/COMMENTS

-A homogeneous smooth white cream was produced

- No sign of cracking or bleeding was observed during or after manufacture

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Grahamstown 6140, REPUBLIC OF SOUTH AFRICA

Batch Record Summary

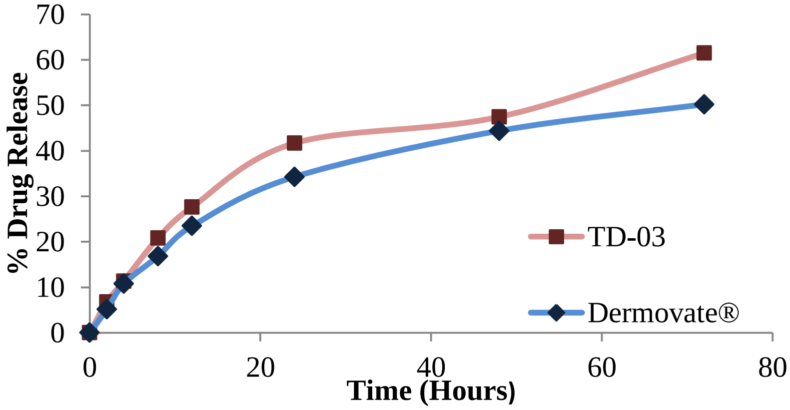
Formulator : Ayeshah Fauzee
Product : Taguchi Design Clobetasol 17-Propionate Cream
Batch Number: TD-CP-03

Date of Manufacture :12/04/2013
Melting Temperature :65 °C
Batch Size : 3000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	1.81 g	RM000150
Propylene glycol	41.00	1489.10 mL	RM000181
Sodium citrate	0.05	1.81 g	RM000183
Citric acid	0.05	1.81 g	RM000185
Gelot® 64	1.00	36.32 g	RM000177
Glyceryl monostearate	6.00	217.92 g	RM000182
Ceteostearyl alcohol	3.00	108.96 g	RM000184
White beeswax	1.15	41.77 g	RM000142
Chlorocresol	0.075	2.72 g	RM000186
Distilled water	30.225	1097.76 mL	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenization Speed (rpm)	1000
Homogenization Time (min)	60
Anchor Speed (rpm)	20
Mixing Time (min)	60
Heating Temperature (°C)	65
Cooling time (min)	60

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	11900.00 ± 890	7.47
Spreadability (cm^2)	37.75 ± 1.00	2.65
CP Content (%)	99.71 ± 1.86	1.87
pH	6.14 ± 0.01	0.16
Extrudability (g/cm^2)	98.59 ± 2.50	2.54
Electrical Conductivity (µS/cm)	719.70 ± 9.85	1.37



OBSERVATIONS/COMMENTS

-A homogeneous smooth white cream was produced

- No sign of cracking or bleeding was observed during or after manufacture

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140, REPUBLIC OF SOUTH AFRICA

Batch Record Summary

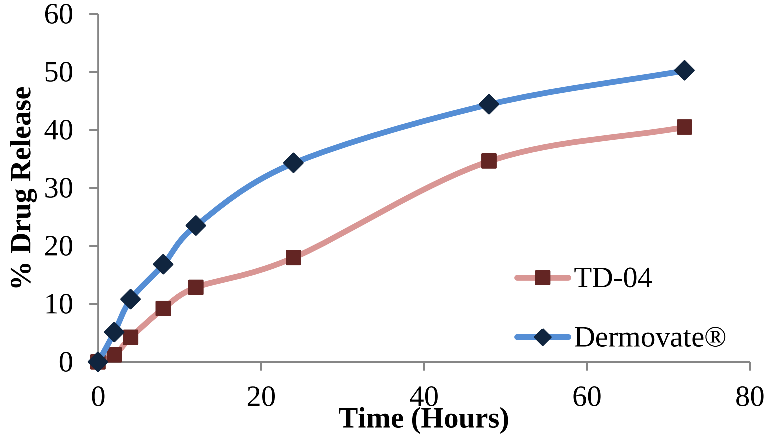
Formulator : Ayeshah Fauzee
Product : Taguchi Design Clobetasol 17-Propionate Cream
Batch Number: TD-CP-04

Date of Manufacture :13/04/2013
Melting Temperature :75 °C
Batch Size : 3000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	1.95 g	RM000150
Propylene glycol	41.00	1605.74 mL	RM000181
Sodium citrate	0.05	1.95 g	RM000183
Citric acid	0.05	1.95 g	RM000185
Gelot® 64	5.00	195.82 g	RM000177
Glyceryl monostearate	16.00	626.63 g	RM000182
Ceteostearyl alcohol	13.00	509.14 g	RM000184
White beeswax	1.15	45.04 g	RM000142
Chlorocresol	0.075	2.94 g	RM000186
Distilled water	30.225	1183.75 mL	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenization Speed (rpm)	1000
Homogenization Time (min)	120
Anchor Speed (rpm)	40
Mixing Time (min)	60
Heating Temperature (°C)	75
Cooling time (min)	60

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	53467.00 ± 550	1.02
Spreadability (cm^2)	20.16 ± 0.60	2.97
CP Content (%)	100.60 ± 1.50	1.49
pH	6.10 ± 0.08	1.31
Extrudability (g/cm^2)	884.80 ± 1.52	0.17
Electrical Conductivity (μS/cm)	408.70 ± 7.30	1.77



OBSERVATIONS/COMMENTS

-A homogeneous smooth white cream was produced

- No sign of cracking or bleeding was observed during or after manufacture

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140, REPUBLIC OF SOUTH AFRICA

Batch Record Summary

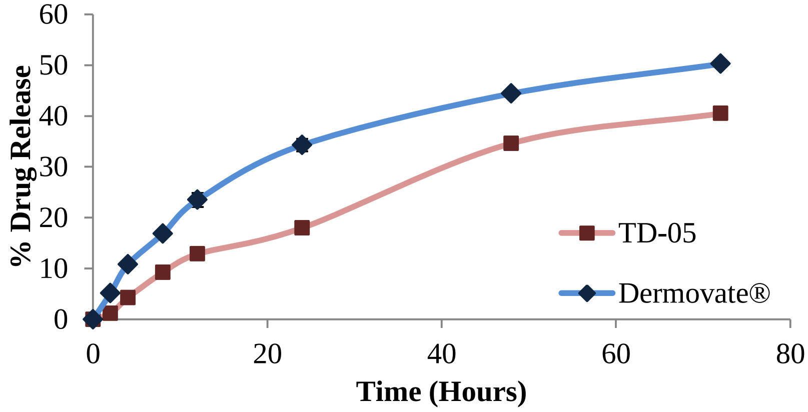
Formulator : Ayeshah Fauzee
Product : Taguchi Design Clobetasol 17-Propionate Cream
Batch Number: TD-CP-05

Date of Manufacture :14/04/2013
Melting Temperature :65 °C
Batch Size : 3000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	1.56 g	RM000150
Propylene glycol	41.00	1273.29 mL	RM000181
Sodium citrate	0.05	1.56 g	RM000183
Citric acid	0.05	1.56 g	RM000185
Gelot® 64	5.00	155.27 g	RM000177
Glyceryl monostearate	6.00	186.35 g	RM000182
Ceteostearyl alcohol	13.00	403.73 g	RM000184
White beeswax	1.15	35.72 g	RM000142
Chlorocresol	0.075	2.33 g	RM000186
Distilled water	30.225	938.66 mL	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenization Speed (rpm)	2000
Homogenization Time (min)	120
Anchor Speed (rpm)	20
Mixing Time (min)	120
Heating Temperature (°C)	65
Cooling time (min)	120

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	35700.00 ± 1205	3.38
Spreadability (cm^2)	27.37 ± 1.05	3.83
CP Content (%)	100.51 ± 0.95	0.94
pH	5.33 ± 0.02	0.37
Extrudability (g/cm^2)	321.31 ± 2.85	0.88
Electrical Conductivity (µS/cm)	150.10 ± 10.59	7.05



OBSERVATIONS/COMMENTS

-A homogeneous smooth white cream was produced

- No sign of cracking or bleeding was observed during or after manufacture

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140, REPUBLIC OF SOUTH AFRICA

Batch Record Summary

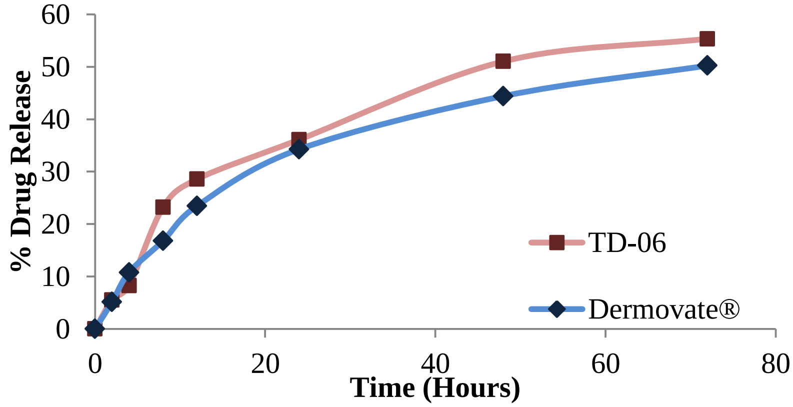
Formulator : Ayeshah Fauzee
Product : Taguchi Design Clobetasol 17-Propionate Cream
Batch Number: TD-CP-06

Date of Manufacture :15/04/2013
Melting Temperature :75 °C
Batch Size : 3000 g

Excipient Name	**Original Formula(% w/w)**	**Working Formula**	**Rhodes Batch Number**
CP	0.05	1.46 g	RM000150
Propylene glycol	51.00	1491.22 mL	RM000181
Sodium citrate	0.05	1.46 g	RM000183
Citric acid	0.05	1.46 g	RM000185
Gelot® 64	1.00	29.23 g	RM000177
Glyceryl monostearate	16.00	467.83 g	RM000182
Ceteostearyl alcohol	3.00	87.72 g	RM000184
White beeswax	1.15	33.63 g	RM000142
Chlorocresol	0.075	2.19 g	RM000186
Distilled water	30.225	883.77 mL	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenization Speed (rpm)	2000
Homogenization Time (min)	120
Anchor Speed (rpm)	20
Mixing Time (min)	120
Heating Temperature (°C)	75
Cooling time (min)	60

Tests Performed	**Mean ± SD (n=3)**	**%RSD**
Viscosity (cP)	19400.00 ± 850	4.38
Spreadability (cm^2)	32.92 ± 1.20	3.65
CP Content (%)	100.72 ± 2.10	2.08
pH	6.40 ± 0.02	0.78
Extrudability (g/cm^2)	203.12 ± 3.25	1.60
Electrical Conductivity (µS/cm)	916.70 ± 8.20	0.89



OBSERVATIONS/COMMENTS

-A homogeneous smooth white cream was produced

- No sign of cracking or bleeding was observed during or after manufacture

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Grahamstown 6140, REPUBLIC OF SOUTH AFRICA

Batch Record Summary

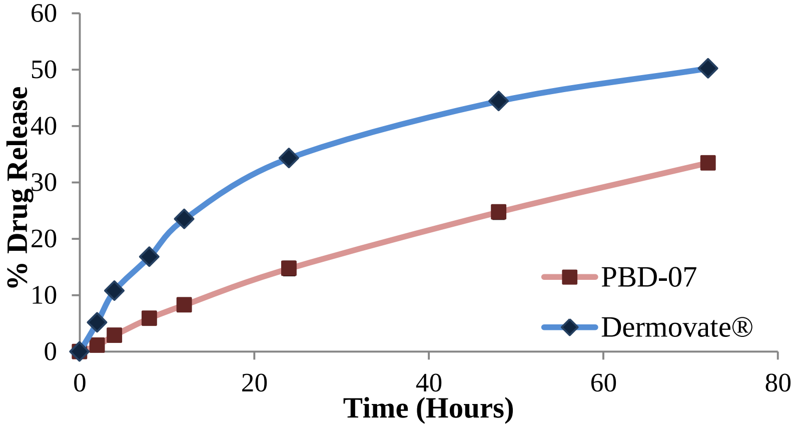
Formulator : Ayeshah Fauzee
Product : Taguchi Design Clobetasol 17-Propionate Cream
Batch Number: TD-CP-07

Date of Manufacture :16/04/2013
Melting Temperature :65 °C
Batch Size : 3000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	1.33 g	RM000150
Propylene glycol	51.00	1358.79 mL	RM000181
Sodium citrate	0.05	1.33 g	RM000183
Citric acid	0.05	1.33 g	RM000185
Gelot® 64	1.00	26.64 g	RM000177
Glyceryl monostearate	16.00	426.28 g	RM000182
Ceteostearyl alcohol	13.00	346.36 g	RM000184
White beeswax	1.15	30.64 g	RM000142
Chlorocresol	0.075	1.99 g	RM000186
Distilled water	30.225	805.28 mL	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenization Speed (rpm)	1000
Homogenization Time (min)	60
Anchor Speed (rpm)	40
Mixing Time (min)	120
Heating Temperature (°C)	65
Cooling time (min)	120

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	72733.00 ± 2130	2.92
Spreadability (cm^2)	15.61 ± 1.20	7.68
CP Content (%)	100.51 ± 1.05	1.04
pH	6.25 ± 0.03	0.48
Extrudability (g/cm^2)	1052.00 ± 2.15	0.20
Electrical Conductivity (µS/cm)	367.00 ± 10.75	2.92



OBSERVATIONS/COMMENTS

-A homogeneous smooth white cream was produced

- No sign of cracking or bleeding was observed during or after manufacture

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Grahamstown 6140, REPUBLIC OF SOUTH AFRICA

Batch Record Summary

Formulator : Ayeshah Fauzee
Product : Taguchi Design Clobetasol 17-Propionate Cream
Batch Number: TD-CP-08

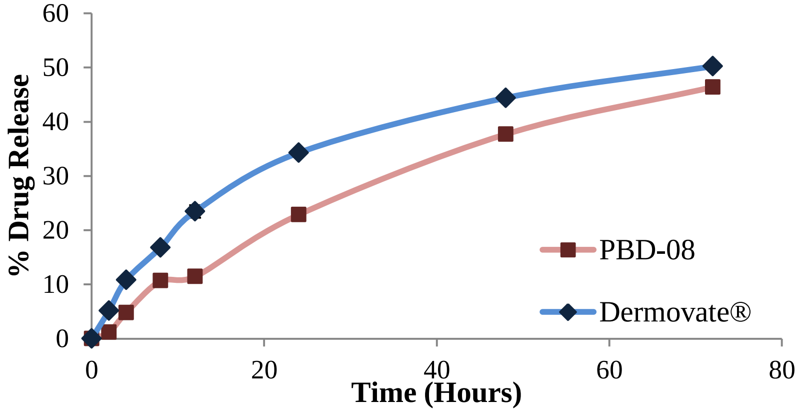
Date of Manufacture :17/04/2013
Melting Temperature :75 °C
Batch Size : 6000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	2.81 g	RM000150
Propylene glycol	51.00	2870.54 mL	RM000181
Sodium citrate	0.05	2.81 g	RM000183
Citric acid	0.05	2.81 g	RM000185
Gelot® 64	5.00	281.40 g	RM000177
Glyceryl monostearate	6.00	337.71 g	RM000182
Ceteostearyl alcohol	13.00	731.70 g	RM000184
White beeswax	1.15	64.72 g	RM000142
Chlorocresol	0.075	4.22 g	RM000186
Distilled water	30.225	1701.21 mL	N/A

Wintech® Cream/Ointment Mixer Parameters

Homogenization Speed (rpm)	1000
Homogenization Time (min)	60
Anchor Speed (rpm)	20
Mixing Time (min)	120
Heating Temperature (°C)	75
Cooling time (min)	120

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	66700.00 ± 860	1.29
Spreadability (cm^2)	17.17 ± 0.78	4.54
CP Content (%)	100.20 ± 1.25	1.24
pH	5.74 ± 0.04	0.69
Extrudability (g/cm^2)	898.21 ± 2.16	0.24
Electrical Conductivity (µS/cm)	114.70 ± 10.86	9.46



OBSERVATIONS/COMMENTS

-A homogeneous smooth white cream was produced

- No sign of cracking or bleeding was observed during or after manufacture

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Grahamstown 6140, REPUBLIC OF SOUTH AFRICA

Batch Record Summary

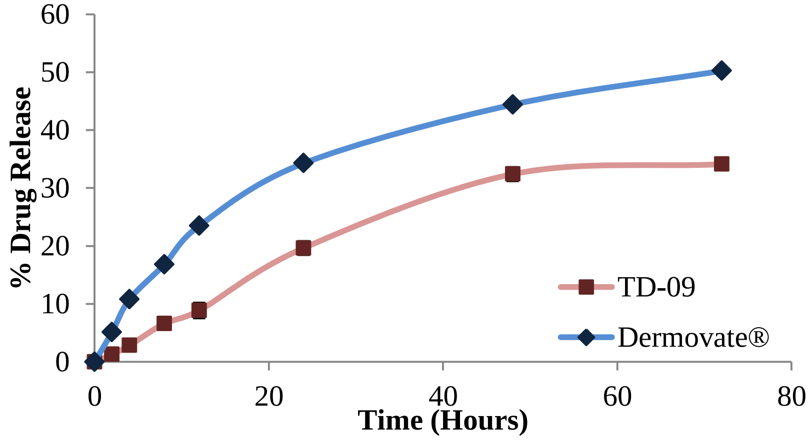
Formulator : Ayeshah Fauzee
Product : Taguchi Design Clobetasol 17-Propionate Cream
Batch Number: TD-CP-09

Date of Manufacture :18/04/2013
Melting Temperature :75 °C
Batch Size : 3000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	1.55 g	RM000150
Propylene glycol	51.00	1583.85 mL	RM000181
Sodium citrate	0.05	1.55 g	RM000183
Citric acid	0.05	1.55 g	RM000185
Gelot® 64	5.00	155.28 g	RM000177
Glyceryl monostearate	6.00	186.33 g	RM000182
Ceteostearyl alcohol	3.00	93.17 g	RM000184
White beeswax	1.15	35.741 g	RM000142
Chlorocresol	0.075	2.35 g	RM000186
Distilled water	30.225	938.66 mL	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenization Speed (rpm)	2000
Homogenization Time (min)	60
Anchor Speed (rpm)	40
Mixing Time (min)	60
Heating Temperature (°C)	75
Cooling time (min)	120

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	45800.00 ± 1025	2.24
Spreadability (cm^2)	24.28 ± 1.20	4.94
CP Content (%)	99.08 ± 2.10	2.11
pH	5.91 ± 0.05	0.84
Extrudability (g/cm^2)	393.10 ± 3.12	0.79
Electrical Conductivity (µS/cm)	164.80 ± 9.35	5.67



OBSERVATIONS/COMMENTS

-A homogeneous smooth white cream was produced

- No sign of cracking or bleeding was observed during or after manufacture

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Grahamstown 6140, REPUBLIC OF SOUTH AFRICA

Batch Record Summary

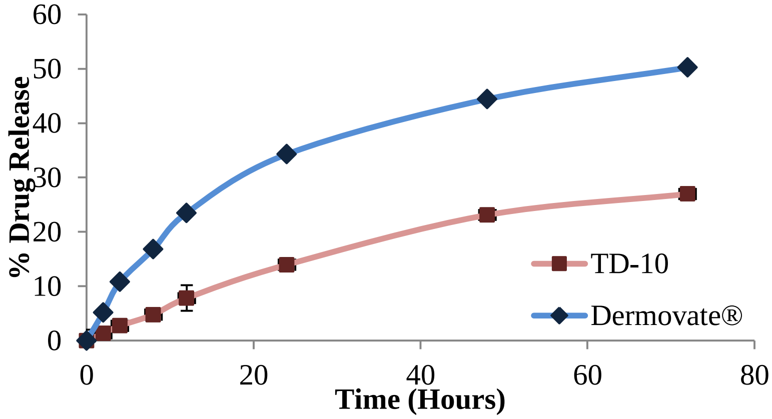
Formulator : Ayeshah Fauzee
Product : Taguchi Design Clobetasol 17-Propionate Cream
Batch Number: TD-CP-10

Date of Manufacture :19/04/2013
Melting Temperature :65 °C
Batch Size : 6000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	3.10 g	RM000150
Propylene glycol	41.00	2546.58 mL	RM000181
Sodium citrate	0.05	3.10 g	RM000183
Citric acid	0.05	3.10 g	RM000185
Gelot® 64	5.00	310.59 g	RM000177
Glyceryl monostearate	16.00	993.78 g	RM000182
Ceteostearyl alcohol	3.00	186.33 g	RM000184
White beeswax	1.15	71.43 g	RM000142
Chlorocresol	0.075	4.66 g	RM000186
Distilled water	30.225	1877.33 mL	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenization Speed (rpm)	2000
Homogenization Time (min)	120
Anchor Speed (rpm)	40
Mixing Time (min)	120
Heating Temperature (°C)	65
Cooling time (min)	60

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	84267.00 ± 1025	1.21
Spreadability (cm^2)	12.67 ± 1.10	8.68
CP Content (%)	99.84 ± 2.15	2.15
pH	6.03 ± 0.01	0.14
Extrudability (g/cm^2)	1084.00 ± 2.59	0.23
Electrical Conductivity (µS/cm)	392.00 ± 9.35	2.39



OBSERVATIONS/COMMENTS

-A homogeneous smooth white cream was produced

- No sign of cracking or bleeding was observed during or after manufacture

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140, REPUBLIC OF SOUTH AFRICA

Batch Record Summary

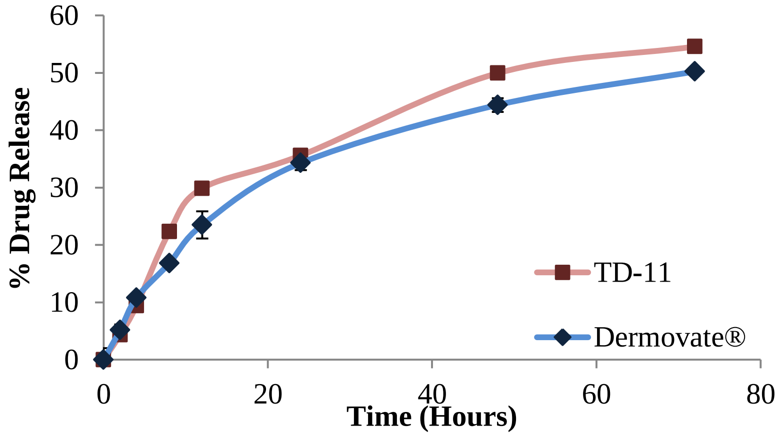
Formulator : Ayeshah Fauzee
Product : Taguchi Design Clobetasol 17-Propionate Cream
Batch Number: TD-CP-11

Date of Manufacture :20/04/2013
Melting Temperature :65 °C
Batch Size : 6000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	2.81 g	RM000150
Propylene glycol	51.00	2870.05 mL	RM000181
Sodium citrate	0.05	2.81 g	RM000183
Citric acid	0.05	2.81 g	RM000185
Gelot® 64	5.00	281.40 g	RM000177
Glyceryl monostearate	6.00	337.71 g	RM000182
Ceteostearyl alcohol	13.00	731.70 g	RM000184
White beeswax	1.15	64.72 g	RM000142
Chlorocresol	0.075	4.22 g	RM000186
Distilled water	30.225	1701.21 mL	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenization Speed (rpm)	2000
Homogenization Time (min)	120
Anchor Speed (rpm)	40
Mixing Time (min)	60
Heating Temperature (°C)	65
Cooling time (min)	60

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	32466.00 ± 890	2.64
Spreadability (cm^2)	27.82 ± 1.20	4.31
CP Content (%)	100.45 ± 2.32	2.31
pH	5.82 ± 0.02	0.34
Extrudability (g/cm^2)	302.71 ± 1.42	0.46
Electrical Conductivity (µS/cm)	189.50 ± 8.50	4.48



OBSERVATIONS/COMMENTS

-A homogeneous smooth white cream was produced

- No sign of cracking or bleeding was observed during or after manufacture

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Grahamstown 6140, REPUBLIC OF SOUTH AFRICA

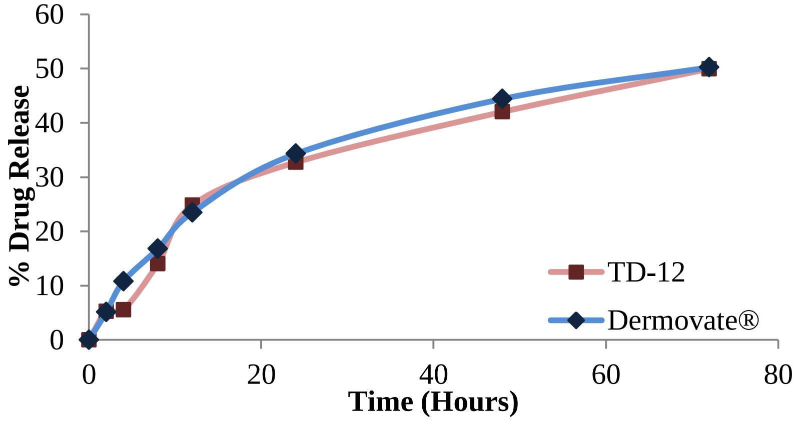
Batch Record Summary

Formulator : Ayeshah Fauzee	**Date of Manufacture** :21/04/2013
Product : Taguchi Design Clobetasol 17-Propionate Cream	**Melting Temperature** :75 °C
Batch Number: TD-CP-12	**Batch Size :** 6000 g

Excipient Name	**Original Formula(% w/w)**	**Working Formula**	**Rhodes Batch Number**
CP	0.05	2.92 g	RM000150
Propylene glycol	41.00	2397.66 mL	RM000181
Sodium citrate	0.05	2.92 g	RM000183
Citric acid	0.05	2.92 g	RM000185
Gelot® 64	1.00	58.47 g	RM000177
Glyceryl monostearate	16.00	935.67 g	RM000182
Ceteostearyl alcohol	13.00	760.23 g	RM000184
White beeswax	1.15	67.25 g	RM000142
Chlorocresol	0.075	4.39 g	RM000186
Distilled water	30.225	1767.54 mL	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenization Speed (rpm)	2000
Homogenization Time (min)	60
Anchor Speed (rpm)	20
Mixing Time (min)	60
Heating Temperature (°C)	75
Cooling time (min)	120

Tests Performed	**Mean ± SD (n=3)**	**%RSD**
Viscosity (cP)	46100.00 ± 1250	2.71
Spreadability (cm^2)	24.04 ± 1.10	4.58
CP Content (%)	100.51 ± 1.96	1.95
pH	5.89 ± 0.05	0.84
Extrudability (g/cm^2)	450.00 ± 1.02	0.23
Electrical Conductivity (µS/cm)	202.30 ± 9.45	4.67



OBSERVATIONS/COMMENTS

-A homogeneous smooth white cream was produced

- No sign of cracking or bleeding was observed during or after manufacture

APPENDIX IV

BATCH PRODUCTION RECORDS

OPTIMIZATION DESIGN MANUFACTURE

CENTRAL COMPOSITE DESIGN

A sample of a batch production record for the central composite design *viz.*, Batch CCD-CP-01 manufactured using a Wintech® cream/ointment mixer for the optimized CP cream formulation is included is included on this accompanying electronic file "Appendices-CD" on the CD. The batch production records for all the other batches, *viz.*, CCD-CP-02 – CCD-CP-30 are available on request.

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BATCH RECORD PRODUCTION

Product name: Central Composite Design Clobetasol 17-Propionate Cream Page 1 of 3
Batch number: CCD-CP-01 **Batch Size**: 6000 g

MANUFACTURING APPROVALS

Batch record issued by:_________________ **Date:_________________**

Master record issued by:_________________ **Date:_________________**

BATCH PRODUCTION RECORD

Product name: Central Composite Design Clobetasol 17-Propionate Cream

Batch number: CCD-CP-01 **Batch Size**: 6000 g

MASTER FORMULA AND BATCH FORMULA							
Item no.	**Material**	**Rhodes No.**	**Quantity (% w/w)**	**Amount/ Batch**	**Amount Dispensed**	**Dispensed by**	**Checked by**
1	CP	RM000150	0.05	3.01 g			
2	Propylene glycol	RM000181	46.00	2771.08 mL			
3	Sodium citrate	RM000183	0.05	3.01 g			
4	Citric acid	RM000185	0.05	3.01 g			
5	Gelot® 64	RM000177	3.00	180.72 g			
6	Glyceryl monostearate	RM000182	11.00	662.65 g			
7	Cetostearyl alcohol	RM000184	8.00	481.92 g			
8	White beeswax	RM000142	1.15	69.27 g			
9	Chlorocresol	RM000186	0.075	4.52 g			
10	Distilled water	N/A	30.225	1820.78 mL			

WINTECH® CREAM/OINTMENT MIXER VERIFICATION			
Description	**Type**	**Verified by**	**Confirmed by**
Bowl vessel	Stainless steel		
Co-centric Homogenizer	Teflon and stainless steel		
Anchor and impellers	Stainless steel		
Water inlet	Stainless steel		
Water Jacket	Stainless steel		
Charging port	Stainless steel		
Water immersion heater	NA		
PT-100 sensor	NA		
Operating panel touch screen	LCD		

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BATCH PRODUCTION RECORD

Product name: Central Composite Design Clobetasol 17-Propionate Cream Page 3 of 3
Batch number: CCD-CP-01 **Batch Size**: 6000 g

MANUFACTURING PROCEDURE

Steps	Procedure	Time	Done by	Checked by
1	Clean the bowl with Liquid K® detergent and distilled water. Allow the bowl to dry and equilibrate at room temperature Room temperature: _____°C Mixer temperature: _____°C			
2	Fill the jacket with distilled water until the jacket is 30 Liters			
3	Weigh all the materials accurately using a Model AE 163 Mettler® analytical balance (Mettler® Inc., Zurich, Switzerland)			
4	Introduce 2771.08 mL propylene glycol and 1820.78 mL distilled water, followed by 3.01 g sodium citrate, 3.01 g citric acid, 3.01 g clobetasol 17-propionate, 662.65 g glyceryl monostearate, 481.92 g cetostearyl alcohol, 69.27 g white beeswax, 180.72 g Gelot® 64 and 4.52 g chlorocresol through the charging port			
5	Allow mixture of excipients to stir at 40 rpms for 120 minutes at 75℃			
6	Following mixing, allow the blend to be homogenized at 2000 rpms for 120 minutes			
7	Allow the molten mixture to cool by passing distilled water through the heating jacket for 120 minutes. Temperature of cold water=____°C			
8	After cooling, close the water inlet and allow slow continuous agitation of the anchor until the temperature on the operating panel displays 30°C - 35°C			
9	Stop the anchor and release the pressure built up in the bowl vessel by the use of the pressure vent			
10	Store the final cream in 10 kg opaque containers until quality control tests are completed and then package into 50 g collapsible aluminium cream tubes			

SIGNATURE AND INITIAL REFERENCE

Full name (Print)	Signature	Initials	Date

APPENDIX V

BATCH RECORD REPORTS

The batch record reports for all the optimization design cream formulation batches, *viz.*, CCD-CP-01 – CCD-CP-30 are included on this accompanying electronic file “Appendices-CD” on the CD.

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140, REPUBLIC OF SOUTH AFRICA

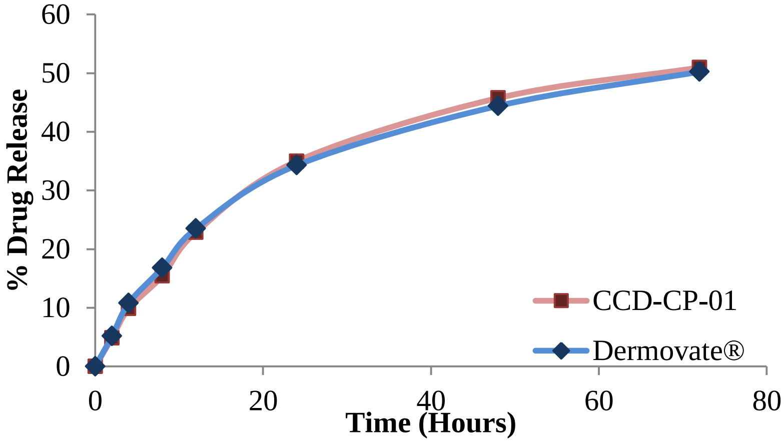
Batch Record Summary

Formulator : Ayeshah Fauzee **Date of Manufacture** :05/06/2013
Product : Central Composite Design Clobetasol 17-Propionate Cream
Melting Temperature :75 °C
Batch Number: CCD-CP-01 **Batch Size :** 6000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	3.01 g	RM000150
Propylene glycol	46.00	2771.08 mL	RM000181
Sodium citrate	0.05	3.20 g	RM000183
Citric acid	0.05	3.20 g	RM000185
Gelot® 64	3.00	180.72 g	RM000177
Glyceryl monostearate	11.00	662.65 g	RM000182
Ceteostearyl alcohol	8.00	481.92 g	RM000184
White beeswax	1.15	69.27 g	RM000142
Chlorocresol	0.075	4.52 g	RM000186
Distilled water	30.225	1820.78 mL	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenization Speed (rpm)	2000
Homogenization Time (min)	120
Anchor Speed (rpm)	40
Mixing Time (min)	120
Heating Temperature (°C)	75
Cooling time (min)	120

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	44066.00 ± 1520	3.45
Spreadability (cm²)	24.34 ± 1.20	4.93
CP Content (%)	100.80 ± 1.50	1.48
pH	6.15 ± 0.06	0.98
Extrudability (g/cm²)	72.02 ± 3.50	4.86
Electrical Conductivity (µS/cm)	200.02 ± 10.20	5.00



OBSERVATIONS/COMMENTS

- A homogeneous smooth white cream was produced

- No sign of cracking or bleeding was observed during and after manufacture

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140, REPUBLIC OF SOUTH AFRICA

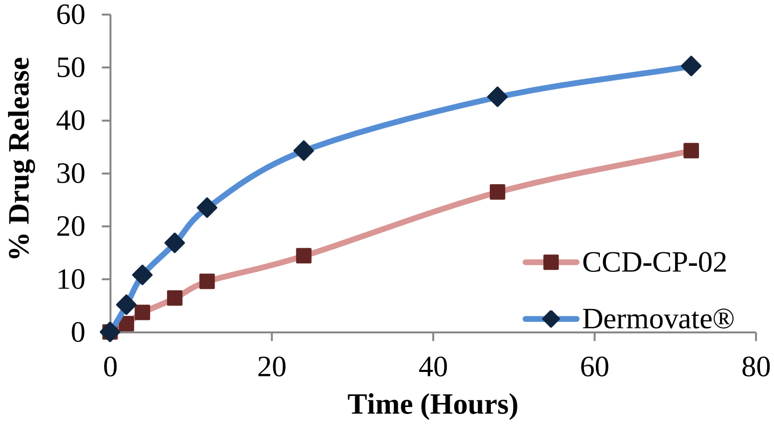
Batch Record Summary

Formulator : Ayeshah Fauzee **Date of Manufacture** :06/06/2013
Product : Central Composite Design Clobetasol 17-Propionate Cream
Melting Temperature :75 °C
Batch Number: CCD-CP-02 **Batch Size :** 6000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	2.74 g	RM000150
Propylene glycol	46.00	2518.25 mL	RM000181
Sodium citrate	0.05	2.74 g	RM000183
Citric acid	0.05	2.74 g	RM000185
Gelot® 64	3.00	164.24 g	RM000177
Glyceryl monostearate	11.00	602.19 g	RM000182
Ceteostearyl alcohol	18.00	985.40 g	RM000184
White beeswax	1.15	62.96 g	RM000142
Chlorocresol	0.075	4.11 g	RM000186
Distilled water	30.225	1654.65 mL	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenization Speed (rpm)	2000
Homogenization Time (min)	120
Anchor Speed (rpm)	40
Mixing Time (min)	120
Heating Temperature (°C)	75
Cooling time (min)	120

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	89633.00 ± 1020	1.14
Spreadability (cm^2)	12.15 ± 0.50	4.11
CP Content (%)	99.72 ± 1.25	1.25
pH	6.62 ± 0.01	0.01
Extrudability (g/cm^2)	244.17 ± 4.25	1.74
Electrical Conductivity (µS/cm)	101.50 ± 9.50	9.36



OBSERVATIONS/COMMENTS

- A homogeneous smooth white cream was produced

- No sign of cracking or bleeding was observed during and after manufacture

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140, REPUBLIC OF SOUTH AFRICA

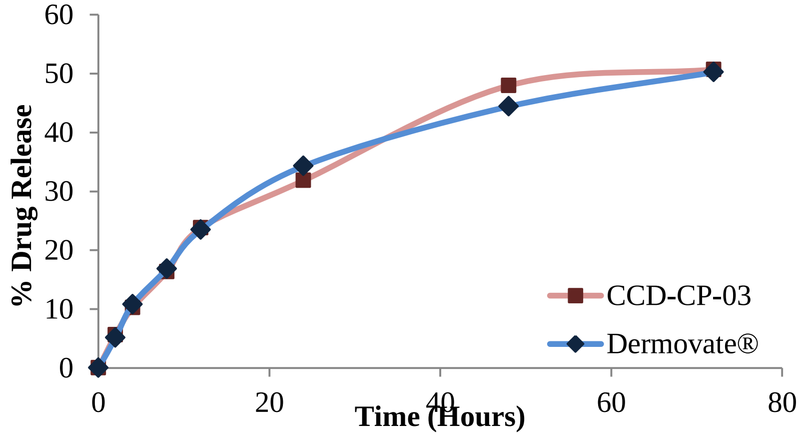
Batch Record Summary

Formulator : Ayeshah Fauzee **Date of Manufacture** :07/06/2013
Product : Central Composite Design Clobetasol 17-Propionate Cream
Melting Temperature :75 °C
Batch Number: CCD-CP-03 **Batch Size :** 6000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	2.92 g	RM000150
Propylene glycol	51.00	2982.45 mL	RM000181
Sodium citrate	0.05	2.92 g	RM000183
Citric acid	0.05	2.92 g	RM000185
Gelot® 64	1.00	58.48 g	RM000177
Glyceryl monostearate	6.00	350.88 g	RM000182
Ceteostearyl alcohol	13.00	760.23 g	RM000184
White beeswax	1.15	64.92 g	RM000142
Chlorocresol	0.075	4.39 g	RM000186
Distilled water	30.225	1767.54 ml	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenization Speed (rpm)	2000
Homogenization Time (min)	120
Anchor Speed (rpm)	40
Mixing Time (min)	120
Heating Temperature (°C)	75
Cooling time (min)	120

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	46333.00 ± 980	2.12
Spreadability (cm^2)	22.07 ± 1.00	4.59
CP Content (%)	102.33 ± 1.25	1.25
pH	6.63 ± 0.09	1.35
Extrudability (g/cm^2)	77.45 ± 5.75	7.42
Electrical Conductivity (µS/cm)	191.33 ± 8.50	4.44



OBSERVATIONS/COMMENTS

- A homogeneous smooth white cream was produced

- No sign of cracking or bleeding was observed during and after manufacture

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140, REPUBLIC OF SOUTH AFRICA

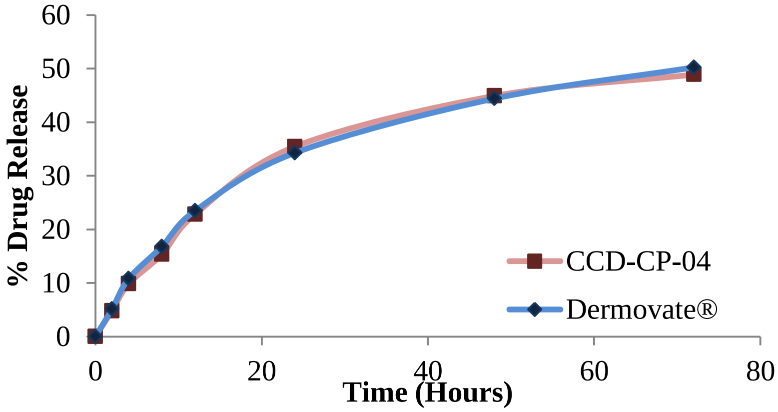
Batch Record Summary

Formulator : Ayeshah Fauzee **Date of Manufacture** :08/06/2013
Product : Central Composite Design Clobetasol 17-Propionate Cream
Melting Temperature :75 °C
Batch Number: CCD-CP-04 **Batch Size :** 6000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	3.01 g	RM000150
Propylene glycol	46.00	2771.08 mL	RM000181
Sodium citrate	0.05	3.201g	RM000183
Citric acid	0.05	3.201 g	RM000185
Gelot® 64	3.00	180.72 g	RM000177
Glyceryl monostearate	11.00	662.65 g	RM000182
Ceteostearyl alcohol	8.00	481.92 g	RM000184
White beeswax	1.15	69.27 g	RM000142
Chlorocresol	0.075	4.52 g	RM000186
Distilled water	30.225	1820.78 mL	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenization Speed (rpm)	2000
Homogenization Time (min)	120
Anchor Speed (rpm)	40
Mixing Time (min)	120
Heating Temperature (°C)	75
Cooling time (min)	120

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	45500.00 ± 980	2.15
Spreadability (cm^2)	25.68 ± 1.50	5.84
CP Content (%)	100.64 ± 0.01	0.01
pH	6.03 ± 0.01	0.16
Extrudability (g/cm^2)	76.51 ± 2.56	3.34
Electrical Conductivity (µS/cm)	198.30 ± 7.50	3.78



OBSERVATIONS/COMMENTS

- A homogeneous smooth white cream was produced

- No sign of cracking or bleeding was observed during and after manufacture

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Grahamstown 6140, REPUBLIC OF SOUTH AFRICA

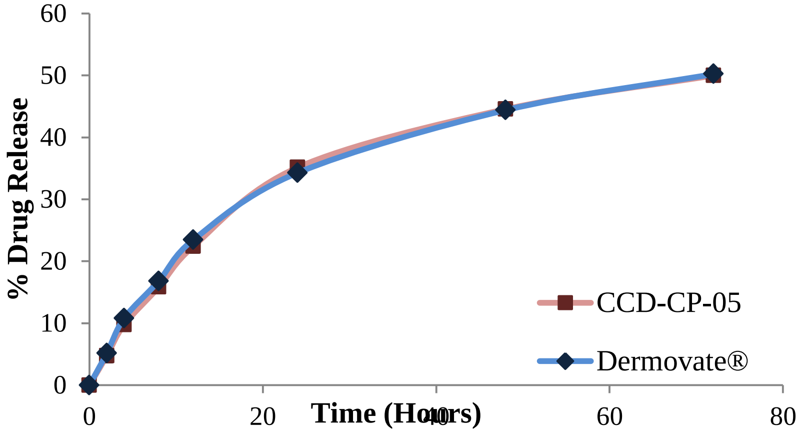
Batch Record Summary

Formulator : Ayeshah Fauzee **Date of Manufacture** :09/06/2013
Product : Central Composite Design Clobetasol 17-Propionate Cream
Melting Temperature :75 °C
Batch Number: CCD-CP-05 **Batch Size :** 6000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	3.01 g	RM000150
Propylene glycol	46.00	2771.08 ml	RM000181
Sodium citrate	0.05	3.201g	RM000183
Citric acid	0.05	3.201 g	RM000185
Gelot® 64	3.00	180.72 g	RM000177
Glyceryl monostearate	11.00	662.65 g	RM000182
Ceteostearyl alcohol	8.00	481.92 g	RM000184
White beeswax	1.15	69.27 g	RM000142
Chlorocresol	0.075	4.52 g	RM000186
Distilled water	30.225	1820.78 ml	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenization Speed (rpm)	2000
Homogenization Time (min)	120
Anchor Speed (rpm)	40
Mixing Time (min)	120
Heating Temperature (°C)	75
Cooling time (min)	120

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	44066.00 ± 1520	3.45
Spreadability (cm^2)	24.34 ± 1.20	4.93
CP Content (%)	100.80 ± 1.50	1.48
pH	6.15 ± 0.06	0.98
Extrudability (g/cm^2)	72.02 ± 3.50	4.86
Electrical Conductivity (µS/cm)	200.02 ± 10.20	5.00



OBSERVATIONS/COMMENTS

- A homogeneous smooth white cream was produced

- No sign of cracking or bleeding was observed during and after manufacture

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140, REPUBLIC OF SOUTH AFRICA

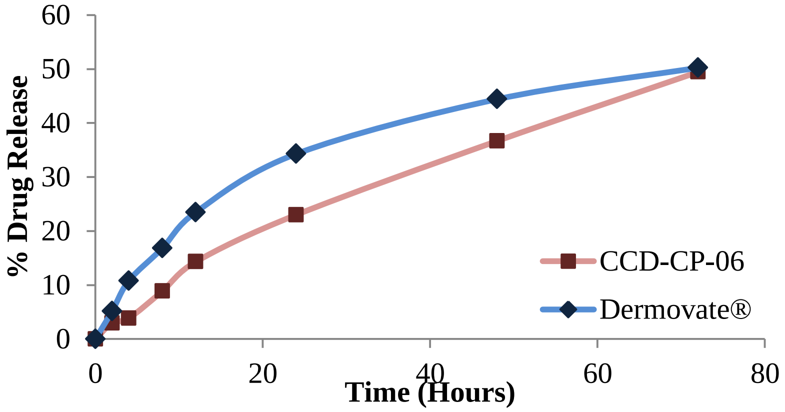
Batch Record Summary

Formulator : Ayeshah Fauzee **Date of Manufacture** :10/06/2013
Product : Central Composite Design Clobetasol 17-Propionate Cream
Melting Temperature :75 °C
Batch Number: CCD-CP-06 **Batch Size :** 6000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	2.81 g	RM000150
Propylene glycol	51.00	2870.54 mL	RM000181
Sodium citrate	0.05	2.81 g	RM000183
Citric acid	0.05	2.81 g	RM000185
Gelot® 64	5.00	281.43 g	RM000177
Glyceryl monostearate	16.00	900.56 g	RM000182
Ceteostearyl alcohol	3.00	168.85 g	RM000184
White beeswax	1.15	64.72 g	RM000142
Chlorocresol	0.075	4.22 g	RM000186
Distilled water	30.225	1701.22 mL	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenization Speed (rpm)	2000
Homogenization Time (min)	120
Anchor Speed (rpm)	40
Mixing Time (min)	120
Heating Temperature (°C)	75
Cooling time (min)	120

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	55700.00 ± 1150	2.06
Spreadability (cm^2)	13.89 ± 0.90	6.49
CP Content (%)	98.76 ± 0.01	0.01
pH	6.35 ± 0.08	1.25
Extrudability (g/cm^2)	107.69 ± 5.00	4.64
Electrical Conductivity (µS/cm)	391.56 ± 7.50	1.91



OBSERVATIONS/COMMENTS

- A homogeneous smooth white cream was produced

- No sign of cracking or bleeding was observed during and after manufacture

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140, REPUBLIC OF SOUTH AFRICA

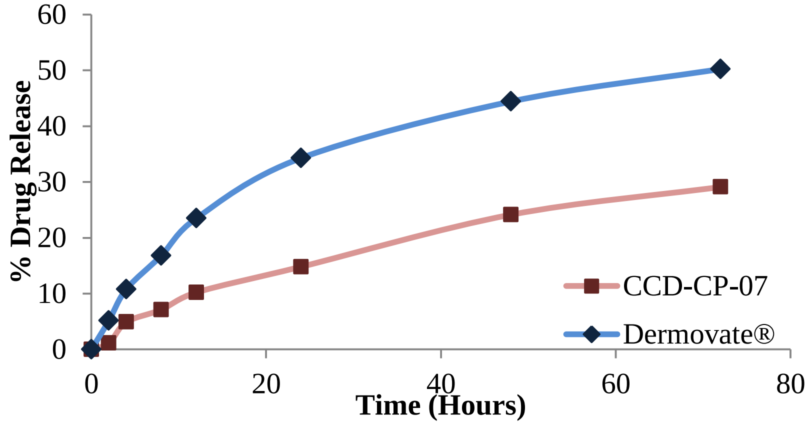
Batch Record Summary

Formulator : Ayeshah Fauzee **Date of Manufacture** :11/06/2013
Product : Central Composite Design Clobetasol 17-Propionate Cream
Melting Temperature :75 °C
Batch Number: CCD-CP-07 **Batch Size :** 6000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	2.89 g	RM000150
Propylene glycol	46.00	2664.09 mL	RM000181
Sodium citrate	0.05	2.89 g	RM000183
Citric acid	0.05	2.89 g	RM000185
Gelot® 64	7.00	405.40 g	RM000177
Glyceryl monostearate	11.00	637.06 g	RM000182
Ceteostearyl alcohol	8.00	463.32 g	RM000184
White beeswax	1.15	66.60 g	RM000142
Chlorocresol	0.075	4.22 g	RM000186
Distilled water	30.225	1701.21 mL	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenization Speed (rpm)	2000
Homogenization Time (min)	120
Anchor Speed (rpm)	40
Mixing Time (min)	120
Heating Temperature (°C)	75
Cooling time (min)	120

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	87766.00 ± 980	1.11
Spreadability (cm^2)	13.44 ± 0.50	3.72
CP Content (%)	102.54 ± 1.80	1.79
pH	6.03 ± 0.05	0.83
Extrudability (g/cm^2)	204.96 ± 1.25	0.60
Electrical Conductivity (µS/cm)	229.93 ± 7.45	3.24



OBSERVATIONS/COMMENTS

- A homogeneous smooth white cream was produced

- No sign of cracking or bleeding was observed during and after manufacture

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140, REPUBLIC OF SOUTH AFRICA

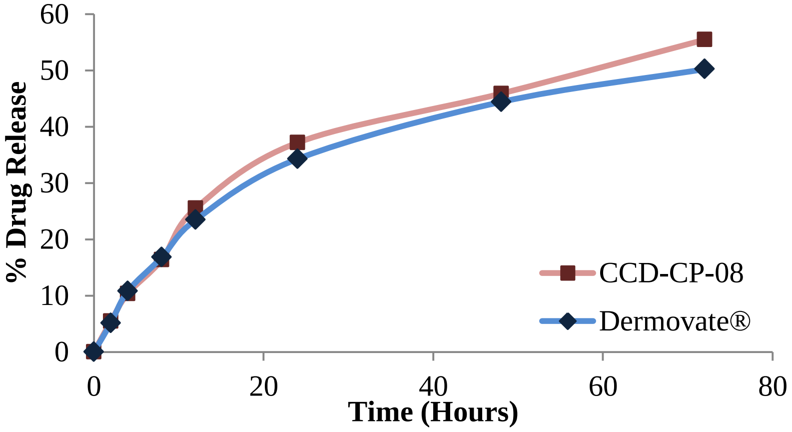
Batch Record Summary

Formulator : Ayeshah Fauzee **Date of Manufacture** :12/06/2013
Product : Central Composite Design Clobetasol 17-Propionate Cream
Melting Temperature :75 °C
Batch Number: CCD-CP-08 **Batch Size :** 6000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	3.11 g	RM000150
Propylene glycol	46.00	2857.14 mL	RM000181
Sodium citrate	0.05	3.11 g	RM000183
Citric acid	0.05	3.11 g	RM000185
Gelot® 64	0.00	0.00 g	RM000177
Glyceryl monostearate	11.00	683.22 g	RM000182
Ceteostearyl alcohol	8.00	496.89 g	RM000184
White beeswax	1.15	71.43 g	RM000142
Chlorocresol	0.075	4.66 g	RM000186
Distilled water	30.225	1877.33 mL	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenization Speed (rpm)	2000
Homogenization Time (min)	120
Anchor Speed (rpm)	40
Mixing Time (min)	120
Heating Temperature (°C)	75
Cooling time (min)	120

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	44500.00 ± 850	1.91
Spreadability (cm^2)	17.14 ± 1.03	6.00
CP Content (%)	100.18 ± 0.10	0.10
pH	6.22 ± 0.02	0.32
Extrudability (g/cm^2)	79.44 ± 2.95	3.71
Electrical Conductivity (μS/cm)	198.06 ± 2.90	1.46



OBSERVATIONS/COMMENTS

- A homogeneous smooth white cream was produced

- No sign of cracking or bleeding was observed during and after manufacture

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140, REPUBLIC OF SOUTH AFRICA

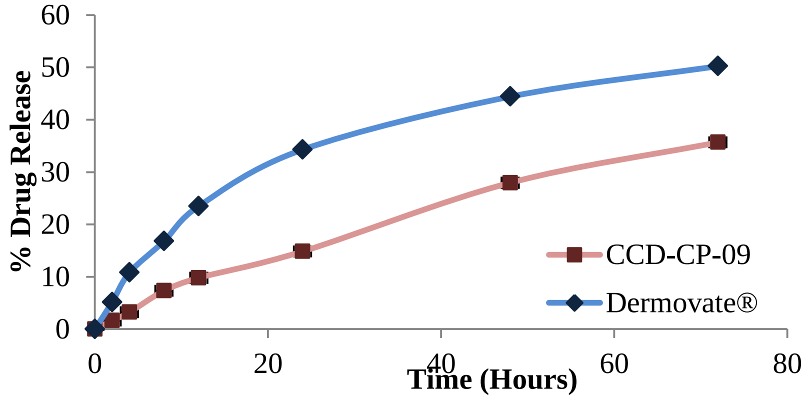
Batch Record Summary

Formulator : Ayeshah Fauzee **Date of Manufacture** :13/06/2013
Product : Central Composite Design Clobetasol 17-Propionate Cream
Melting Temperature :75 °C
Batch Number: CCD-CP-09 **Batch Size :** 6000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	3.35 g	RM000150
Propylene glycol	36.00	2410.71 mL	RM000181
Sodium citrate	0.05	3.35 g	RM000183
Citric acid	0.05	3.35 g	RM000185
Gelot® 64	3.00	200.89 g	RM000177
Glyceryl monostearate	11.00	736.61 g	RM000182
Ceteostearyl alcohol	8.00	535.71 g	RM000184
White beeswax	1.15	77.00 g	RM000142
Chlorocresol	0.075	5.02 g	RM000186
Distilled water	30.225	2023.99 mL	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenization Speed (rpm)	2000
Homogenization Time (min)	120
Anchor Speed (rpm)	40
Mixing Time (min)	120
Heating Temperature (°C)	75
Cooling time (min)	120

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	68600.00 ± 1056	1.54
Spreadability (cm^2)	12.57± 1.10	8.75
CP Content (%)	101.80 ± 0.09	0.09
pH	6.20 ± 0.01	0.16
Extrudability (g/cm^2)	115.47 ± 4.75	4.11
Electrical Conductivity (µS/cm)	285.36 ± 8.50	2.98



OBSERVATIONS/COMMENTS

- A homogeneous smooth white cream was produced

- No sign of cracking or bleeding was observed during and after manufacture

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140, REPUBLIC OF SOUTH AFRICA

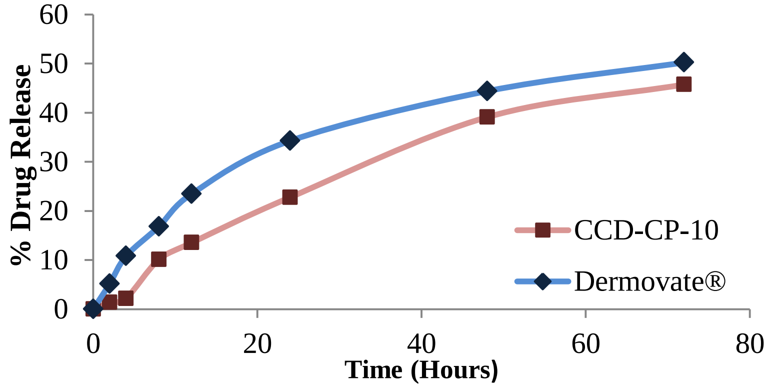
Batch Record Summary

Formulator : Ayeshah Fauzee **Date of Manufacture** :14/06/2013
Product : Central Composite Design Clobetasol 17-Propionate Cream
Melting Temperature :75 °C
Batch Number: CCD-CP-10 **Batch Size :** 6000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	2.93 g	RM000150
Propylene glycol	41.00	2397.66 mL	RM000181
Sodium citrate	0.05	2.93 g	RM000183
Citric acid	0.05	2.93 g	RM000185
Gelot® 64	1.00	58.47 g	RM000177
Glyceryl monostearate	16.00	935.67 g	RM000182
Ceteostearyl alcohol	13.00	760.23 g	RM000184
White beeswax	1.15	67.25 g	RM000142
Chlorocresol	0.075	4.39 g	RM000186
Distilled water	30.225	1767.54 mL	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenization Speed (rpm)	2000
Homogenization Time (min)	120
Anchor Speed (rpm)	40
Mixing Time (min)	120
Heating Temperature (°C)	75
Cooling time (min)	120

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	84900.00 ± 1600	1.88
Spreadability (cm^2)	16.46 ± 1.00	6.07
CP Content (%)	103.47 ± 0.50	0.50
pH	6.26 ± 0.01	0.15
Extrudability (g/cm^2)	313.56 ± 4.45	1.42
Electrical Conductivity (µS/cm)	315.33 ± 12.30	3.90



OBSERVATIONS/COMMENTS

- A homogeneous smooth white cream was produced

- No sign of cracking or bleeding was observed during and after manufacture

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140, REPUBLIC OF SOUTH AFRICA

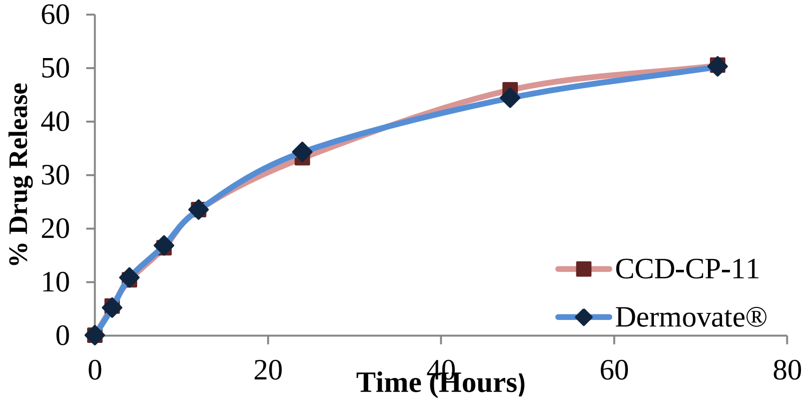
Batch Record Summary

Formulator : Ayeshah Fauzee **Date of Manufacture** :15/06/2013
Product : Central Composite Design Clobetasol 17-Propionate Cream
Melting Temperature :75 °C
Batch Number: CCD-CP-11 **Batch Size :** 6000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	3.23 g	RM000150
Propylene glycol	41.00	2656.58 mL	RM000181
Sodium citrate	0.05	3.23 g	RM000183
Citric acid	0.05	3.23 g	RM000185
Gelot® 64	1.00	64.79 g	RM000177
Glyceryl monostearate	16.00	1036.72 g	RM000182
Ceteostearyl alcohol	3.00	194.38 g	RM000184
White beeswax	1.15	74.51 g	RM000142
Chlorocresol	0.075	4.86 g	RM000186
Distilled water	30.225	1958.42 mL	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenization Speed (rpm)	2000
Homogenization Time (min)	120
Anchor Speed (rpm)	40
Mixing Time (min)	120
Heating Temperature (°C)	75
Cooling time (min)	120

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	46466.00 ± 890	1.91
Spreadability (cm^2)	18.87 ± 0.50	2.65
CP Content (%)	104.33 ± 0.05	0.05
pH	6.27 ± 0.01	0.16
Extrudability (g/cm^2)	80.22 ± 0.40	0.50
Electrical Conductivity (µS/cm)	1066.33 ± 6.80	0.64



OBSERVATIONS/COMMENTS

- A homogeneous smooth white cream was produced

- No sign of cracking or bleeding was observed during and after manufacture

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140, REPUBLIC OF SOUTH AFRICA

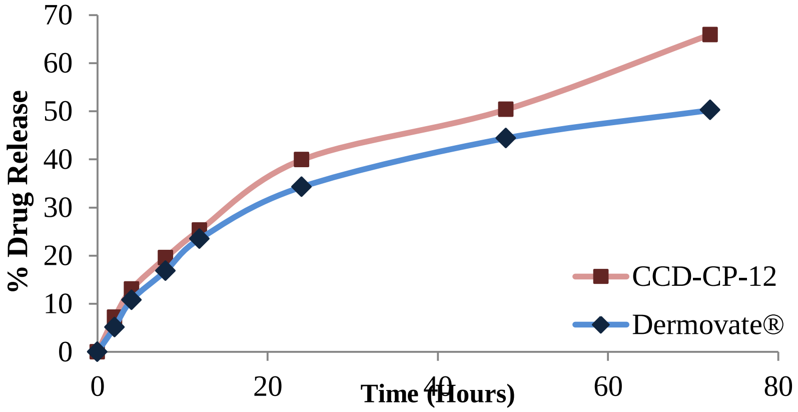
Batch Record Summary

Formulator : Ayeshah Fauzee **Date of Manufacture** :16/06/2013
Product : Central Composite Design Clobetasol 17-Propionate Cream
Melting Temperature :75 °C
Batch Number: CCD-CP-12 **Batch Size :** 6000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	2.74 g	RM000150
Propylene glycol	56.00	3065.69 mL	RM000181
Sodium citrate	0.05	2.74 g	RM000183
Citric acid	0.05	2.74 g	RM000185
Gelot® 64	3.00	164.23 g	RM000177
Glyceryl monostearate	11.00	602.19 g	RM000182
Ceteostearyl alcohol	8.00	437.96 g	RM000184
White beeswax	1.15	62.96 g	RM000142
Chlorocresol	0.075	4.11 g	RM000186
Distilled water	30.225	1654.65 mL	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenization Speed (rpm)	2000
Homogenization Time (min)	120
Anchor Speed (rpm)	40
Mixing Time (min)	120
Heating Temperature (°C)	75
Cooling time (min)	120

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	35766.00 ± 750	2.09
Spreadability (cm²)	16.13 ± 0.90	5.58
CP Content (%)	102.56 ± 0.20	0.20
pH	6.41 ± 0.01	0.15
Extrudability (g/cm²)	70.34 ± 1.25	1.77
Electrical Conductivity (μS/cm)	288.66 ± 3.80	1.32



OBSERVATIONS/COMMENTS

- A homogeneous smooth white cream was produced

- No sign of cracking or bleeding was observed during and after manufacture

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140, REPUBLIC OF SOUTH AFRICA

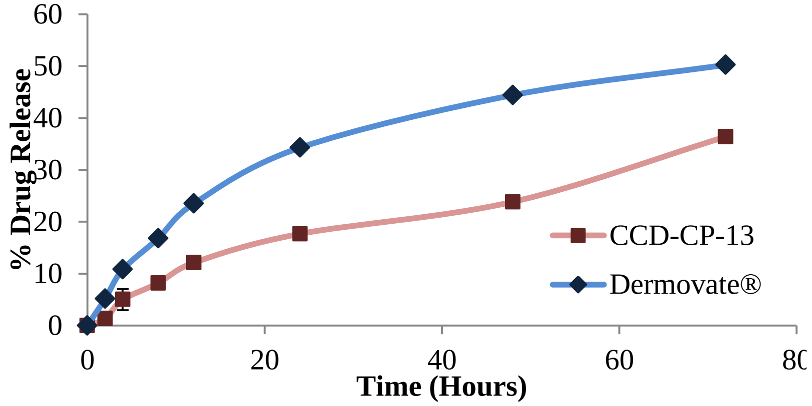
Batch Record Summary

Formulator : Ayeshah Fauzee **Date of Manufacture** :17/06/2013
Product : Central Composite Design Clobetasol 17-Propionate Cream
Melting Temperature :75 °C
Batch Number: CCD-CP-13 **Batch Size :** 6000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	3.11 g	RM000150
Propylene glycol	41.00	2546.58 mL	RM000181
Sodium citrate	0.05	3.11g	RM000183
Citric acid	0.05	3.11 g	RM000185
Gelot® 64	5.00	310.56 g	RM000177
Glyceryl monostearate	16.00	993.79 g	RM000182
Ceteostearyl alcohol	3.00	186.34 g	RM000184
White beeswax	1.15	71.43 g	RM000142
Chlorocresol	0.075	4.66 g	RM000186
Distilled water	30.225	1877.33 mL	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenization Speed (rpm)	2000
Homogenization Time (min)	120
Anchor Speed (rpm)	40
Mixing Time (min)	120
Heating Temperature (°C)	75
Cooling time (min)	120

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	82300.00 ± 1250	1.52
Spreadability (cm^2)	12.38 ± 0.50	4.04
CP Content (%)	100.48 ± 0.10	0.10
pH	6.83 ± 0.05	0.73
Extrudability (g/cm^2)	323.61 ± 2.75	0.85
Electrical Conductivity (µS/cm)	898.33 ± 6.40	0.71



OBSERVATIONS/COMMENTS

- A homogeneous smooth white cream was produced

- No sign of cracking or bleeding was observed during and after manufacture

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140, REPUBLIC OF SOUTH AFRICA

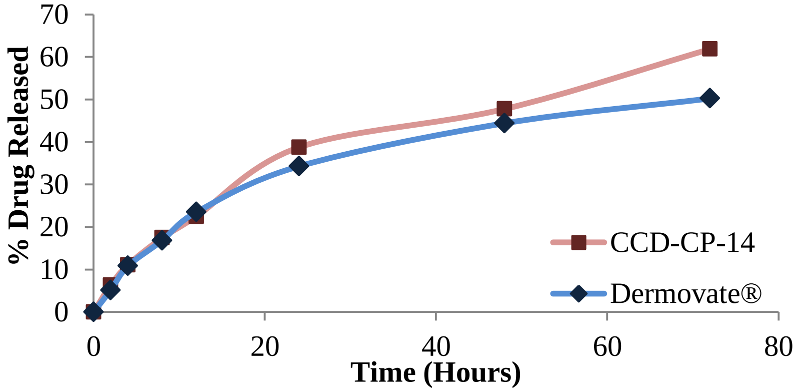
Batch Record Summary

Formulator : Ayeshah Fauzee **Date of Manufacture** :18/06/2013
Product : Central Composite Design Clobetasol 17-Propionate Cream
Melting Temperature :75 °C
Batch Number: CCD-CP-14 **Batch Size :** 6000 g

Excipient Name	**Original Formula(% w/w)**	**Working Formula**	**Rhodes Batch Number**
CP	0.05	3.35 g	RM000150
Propylene glycol	46.00	3080.35 mL	RM000181
Sodium citrate	0.05	3.35 g	RM000183
Citric acid	0.05	3.35 g	RM000185
Gelot® 64	3.00	200.89 g	RM000177
Glyceryl monostearate	1.00	66.96 g	RM000182
Ceteostearyl alcohol	8.00	535.71 g	RM000184
White beeswax	1.15	77.00 g	RM000142
Chlorocresol	0.075	5.02 g	RM000186
Distilled water	30.225	2023.99 mL	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenization Speed (rpm)	2000
Homogenization Time (min)	120
Anchor Speed (rpm)	40
Mixing Time (min)	120
Heating Temperature (°C)	75
Cooling time (min)	120

Tests Performed	**Mean ± SD (n=3)**	**%RSD**
Viscosity (cP)	41133.00 ± 1020	2.48
Spreadability (cm^2)	21.91 ± 0.50	2.28
CP Content (%)	100.75 ± 0.02	0.02
pH	6.40 ± 0.01	9.37
Extrudability (g/cm^2)	83.67 ± 4.82	5.76
Electrical Conductivity (µS/cm)	177.63 ± 1.25	0.70



OBSERVATIONS/COMMENTS

- A homogeneous smooth white cream was produced

- No sign of cracking or bleeding was observed during and after manufacture

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140, REPUBLIC OF SOUTH AFRICA

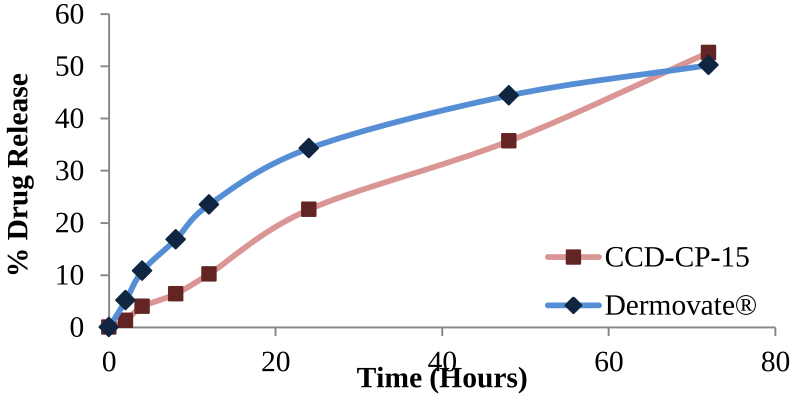
Batch Record Summary

Formulator : Ayeshah Fauzee **Date of Manufacture** :19/06/2013
Product : Central Composite Design Clobetasol 17-Propionate Cream
Melting Temperature :75 °C
Batch Number: CCD-CP-15 **Batch Size :** 6000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	3.46 g	RM000150
Propylene glycol	41.00	2840.65 mL	RM000181
Sodium citrate	0.05	3.46 g	RM000183
Citric acid	0.05	3.46 g	RM000185
Gelot$^{®}$ 64	5.00	180.72 g	RM000177
Glyceryl monostearate	6.00	346.42 g	RM000182
Ceteostearyl alcohol	3.00	207.85 g	RM000184
White beeswax	1.15	79.68 g	RM000142
Chlorocresol	0.075	5.19 g	RM000186
Distilled water	30.225	2093.76 mL	N/A

Wintech$^{®}$ Cream/Ointment Mixer Parameters	
Homogenization Speed (rpm)	2000
Homogenization Time (min)	120
Anchor Speed (rpm)	40
Mixing Time (min)	120
Heating Temperature (°C)	75
Cooling time (min)	120

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	54800.00 ± 650	1.19
Spreadability (cm^2)	18.35 ± 1.00	5.44
CP Content (%)	100.36 ± 0.05	0.05
pH	6.98 ± 0.01	0.14
Extrudability (g/cm^2)	102.01 ± 2.55	2.49
Electrical Conductivity (µS/cm)	311.20 ± 1.25	0.40



OBSERVATIONS/COMMENTS

- A homogeneous smooth white cream was produced

- No sign of cracking or bleeding was observed during and after manufacture

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140, REPUBLIC OF SOUTH AFRICA

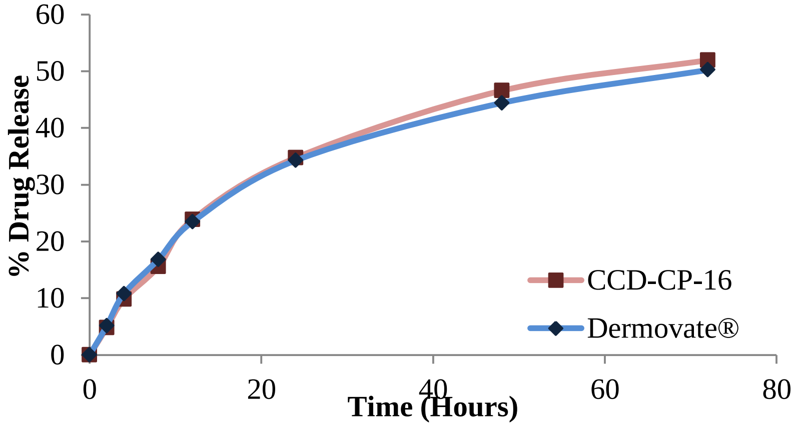
Batch Record Summary

Formulator : Ayeshah Fauzee **Date of Manufacture** :20/06/2013
Product : Central Composite Design Clobetasol 17-Propionate Cream
Melting Temperature :75 °C
Batch Number: CCD-CP-16 **Batch Size :** 6000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	3.01 g	RM000150
Propylene glycol	46.00	2771.08 mL	RM000181
Sodium citrate	0.05	3.20 g	RM000183
Citric acid	0.05	3.20 g	RM000185
Gelot® 64	3.00	180.72 g	RM000177
Glyceryl monostearate	11.00	662.65 g	RM000182
Ceteostearyl alcohol	8.00	481.92 g	RM000184
White beeswax	1.15	69.27 g	RM000142
Chlorocresol	0.075	4.52 g	RM000186
Distilled water	30.225	1820.78 mL	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenization Speed (rpm)	2000
Homogenization Time (min)	120
Anchor Speed (rpm)	40
Mixing Time (min)	120
Heating Temperature (°C)	75
Cooling time (min)	120

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	44400.00 ± 1020	2.29
Spreadability (cm^2)	24.90 ± 0.90	3.61
CP Content (%)	100.41 ± 0.50	0.50
pH	6.00 ± 0.02	0.33
Extrudability (g/cm^2)	77.80 ± 1.50	1.93
Electrical Conductivity (µS/cm)	182.00 ± 3.58	1.96



OBSERVATIONS/COMMENTS

- A homogeneous smooth white cream was produced

- No sign of cracking or bleeding was observed during and after manufacture

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140, REPUBLIC OF SOUTH AFRICA

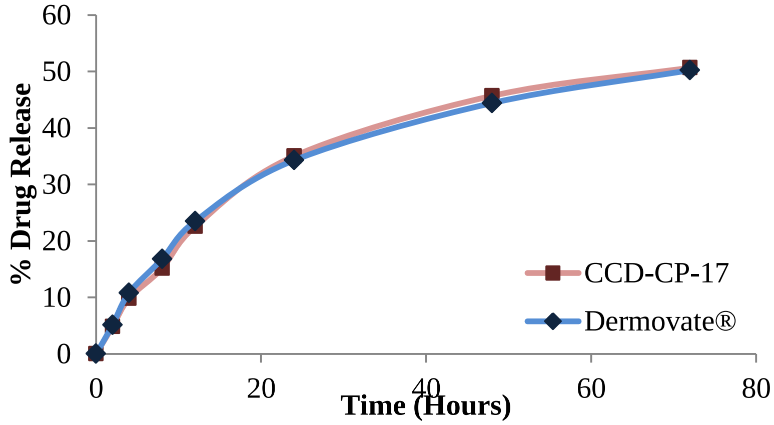
Batch Record Summary

Formulator : Ayeshah Fauzee **Date of Manufacture** :21/06/2013
Product : Central Composite Design Clobetasol 17-Propionate Cream
Melting Temperature :75 °C
Batch Number: CCD-CP-17 **Batch Size :** 6000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	3.01 g	RM000150
Propylene glycol	46.00	2771.08 mL	RM000181
Sodium citrate	0.05	3.20 g	RM000183
Citric acid	0.05	3.20 g	RM000185
Gelot® 64	3.00	180.72 g	RM000177
Glyceryl monostearate	11.00	662.65 g	RM000182
Ceteostearyl alcohol	8.00	481.92 g	RM000184
White beeswax	1.15	69.27 g	RM000142
Chlorocresol	0.075	4.52 g	RM000186
Distilled water	30.225	1820.78 mL	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenization Speed (rpm)	2000
Homogenization Time (min)	120
Anchor Speed (rpm)	40
Mixing Time (min)	120
Heating Temperature (°C)	75
Cooling time (min)	120

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	44666.00 ± 1230	2.75
Spreadability (cm^2)	24.38 ± 0.50	2.05
CP Content (%)	101.92 ± 0.02	0.02
pH	6.00 ± 0.01	0.16
Extrudability (g/cm^2)	75.40 ± 1.60	2.12
Electrical Conductivity (µS/cm)	198.66 ± 4.35	2.19



OBSERVATIONS/COMMENTS

- A homogeneous smooth white cream was produced

- No sign of cracking or bleeding was observed during and after manufacture

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Grahamstown 6140, REPUBLIC OF SOUTH AFRICA

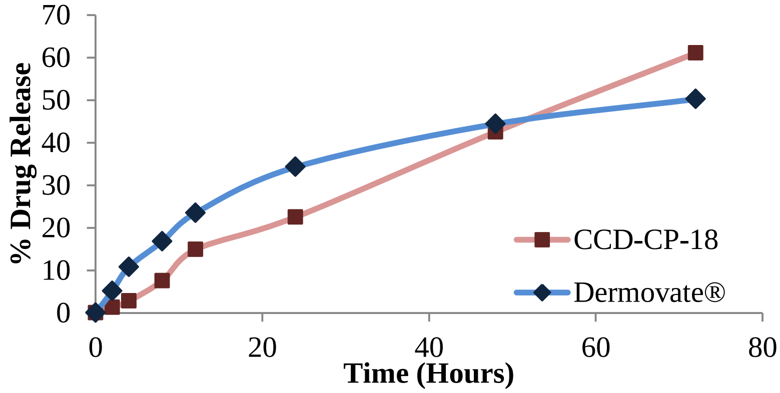
Batch Record Summary

Formulator : Ayeshah Fauzee **Date of Manufacture** :22/06/2013
Product : Central Composite Design Clobetasol 17-Propionate Cream
Melting Temperature :75 °C
Batch Number: CCD-CP-18 **Batch Size :** 6000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	2.81 g	RM000150
Propylene glycol	51.00	2791.97 mL	RM000181
Sodium citrate	0.05	2.81 g	RM000183
Citric acid	0.05	2.81 g	RM000185
Gelot® 64	5.00	273.72 g	RM000177
Glyceryl monostearate	6.00	328.46 g	RM000182
Ceteostearyl alcohol	13.00	711.68 g	RM000184
White beeswax	1.15	62.96 g	RM000142
Chlorocresol	0.075	4.11 g	RM000186
Distilled water	30.225	1654.65 mL	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenization Speed (rpm)	2000
Homogenization Time (min)	120
Anchor Speed (rpm)	40
Mixing Time (min)	120
Heating Temperature (°C)	75
Cooling time (min)	120

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	717660.00 ± 1810	0.25
Spreadability (cm^2)	14.44 ± 1.00	6.93
CP Content (%)	101.66 ± 0.90	0.90
pH	6.11 ± 0.02	0.33
Extrudability (g/cm^2)	179.23 ± 4.50	2.51
Electrical Conductivity (µS/cm)	272.33 ± 8.25	3.03



OBSERVATIONS/COMMENTS

- A homogeneous smooth white cream was produced

- No sign of cracking or bleeding was observed during and after manufacture

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140, REPUBLIC OF SOUTH AFRICA

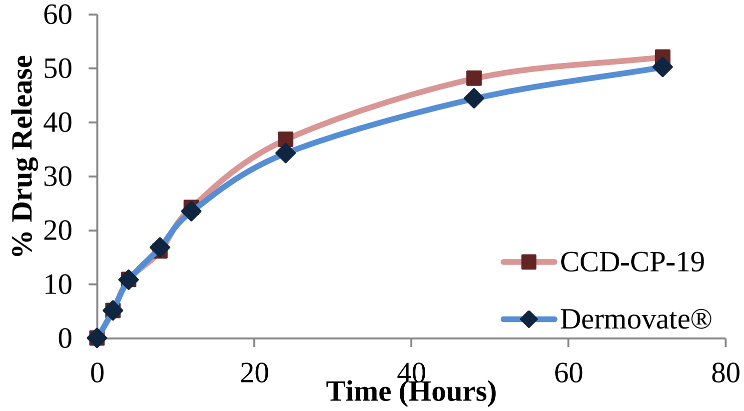
Batch Record Summary

Formulator : Ayeshah Fauzee **Date of Manufacture** :23/06/2013
Product : Central Composite Design Clobetasol 17-Propionate Cream
Melting Temperature :75 °C
Batch Number: CCD-CP-19 **Batch Size :** 6000 g

Excipient Name	**Original Formula(% w/w)**	**Working Formula**	**Rhodes Batch Number**
CP	0.05	3.46 g	RM000150
Propylene glycol	46.00	3187.07 mL	RM000181
Sodium citrate	0.05	3.46 g	RM000183
Citric acid	0.05	3.46 g	RM000185
Gelot® 64	3.00	207.96 g	RM000177
Glyceryl monostearate	11.00	762.12 g	RM000182
Ceteostearyl alcohol	0.00	0 g	RM000184
White beeswax	1.15	79.68 g	RM000142
Chlorocresol	0.075	5.19 g	RM000186
Distilled water	30.225	1820.78 mL	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenization Speed (rpm)	2000
Homogenization Time (min)	120
Anchor Speed (rpm)	40
Mixing Time (min)	120
Heating Temperature (°C)	75
Cooling time (min)	120

Tests Performed	**Mean ± SD (n=3)**	**%RSD**
Viscosity (cP)	71433.00 ± 1480	2.07
Spreadability (cm^2)	16.15 ± 0.50	3.09
CP Content (%)	103.79 ± 1.10	1.10
pH	6.05 ± 0.04	0.66
Extrudability (g/cm^2)	176.27 ± 1.25	0.70
Electrical Conductivity (µS/cm)	188.83 ± 5.50	2.93



OBSERVATIONS/COMMENTS

- A homogeneous smooth white cream was produced

- No sign of cracking or bleeding was observed during and after manufacture

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140, REPUBLIC OF SOUTH AFRICA

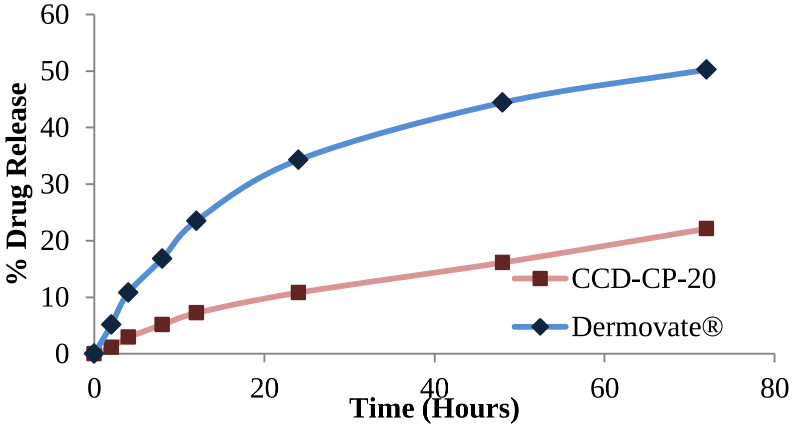
Batch Record Summary

Formulator : Ayeshah Fauzee **Date of Manufacture** :24/06/2013
Product : Central Composite Design Clobetasol 17-Propionate Cream
Melting Temperature :75 °C
Batch Number: CCD-CP-20 **Batch Size :** 6000 g

Excipient Name	**Original Formula(% w/w)**	**Working Formula**	**Rhodes Batch Number**
CP	0.05	2.78 g	RM000150
Propylene glycol	46.00	2565.06 mL	RM000181
Sodium citrate	0.05	2.78 g	RM000183
Citric acid	0.05	2.78 g	RM000185
Gelot® 64	1.00	55.76 g	RM000177
Glyceryl monostearate	16.00	892.19 g	RM000182
Ceteostearyl alcohol	13.00	724.91 g	RM000184
White beeswax	1.15	64.13 g	RM000142
Chlorocresol	0.075	4.18 g	RM000186
Distilled water	30.225	1685.41 mL	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenization Speed (rpm)	2000
Homogenization Time (min)	120
Anchor Speed (rpm)	40
Mixing Time (min)	120
Heating Temperature (°C)	75
Cooling time (min)	120

Tests Performed	**Mean ± SD (n=3)**	**%RSD**
Viscosity (cP)	92900.00 ± 1840	1.98
Spreadability (cm^2)	13.67 ± 0.90	6.58
CP Content (%)	101.76 ± 0.80	0.79
pH	6.35 ± 0.05	0.79
Extrudability (g/cm^2)	462.59 ± 5.25	1.13
Electrical Conductivity (µS/cm)	98.36 ± 3.45	3.51



OBSERVATIONS/COMMENTS

- A homogeneous smooth white cream was produced

- No sign of cracking or bleeding was observed during and after manufacture

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140, REPUBLIC OF SOUTH AFRICA

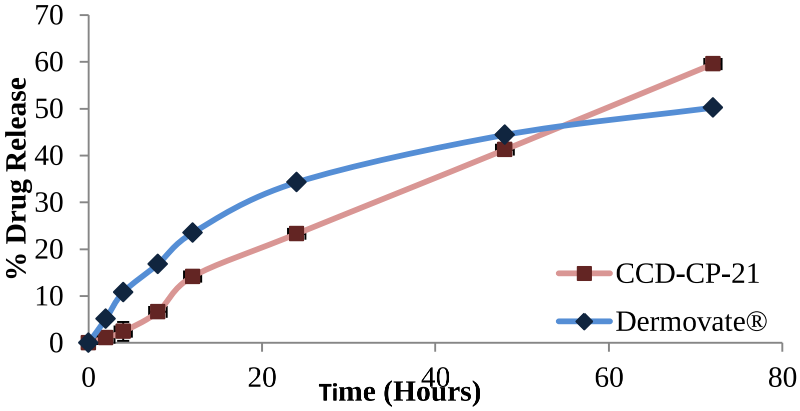
Batch Record Summary

Formulator : Ayeshah Fauzee **Date of Manufacture** :25/06/2013
Product : Central Composite Design Clobetasol 17-Propionate Cream
Melting Temperature :75 °C
Batch Number: CCD-CP-21 **Batch Size :** 6000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	3.24 g	RM000150
Propylene glycol	41.00	2656.58 mL	RM000181
Sodium citrate	0.05	3.24 g	RM000183
Citric acid	0.05	3.24 g	RM000185
Gelot® 64	1.00	64.79 g	RM000177
Glyceryl monostearate	6.00	388.76 g	RM000182
Ceteostearyl alcohol	13.00	842.33 g	RM000184
White beeswax	1.15	71.51 g	RM000142
Chlorocresol	0.075	4.86 g	RM000186
Distilled water	30.225	1958.42 mL	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenization Speed (rpm)	2000
Homogenization Time (min)	120
Anchor Speed (rpm)	40
Mixing Time (min)	120
Heating Temperature (°C)	75
Cooling time (min)	120

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	72000.00 ± 1025	0.15
Spreadability (cm^2)	22.38 ± 0.89	3.98
CP Content (%)	100.55 ± 0.65	0.65
pH	6.84 ± 0.02	0.29
Extrudability (g/cm^2)	191.50 ± 1.56	0.81
Electrical Conductivity (µS/cm)	209.66 ± 3.50	1.66



OBSERVATIONS/COMMENTS

- A homogeneous smooth white cream was produced

- No sign of cracking or bleeding was observed during and after manufacture

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140, REPUBLIC OF SOUTH AFRICA

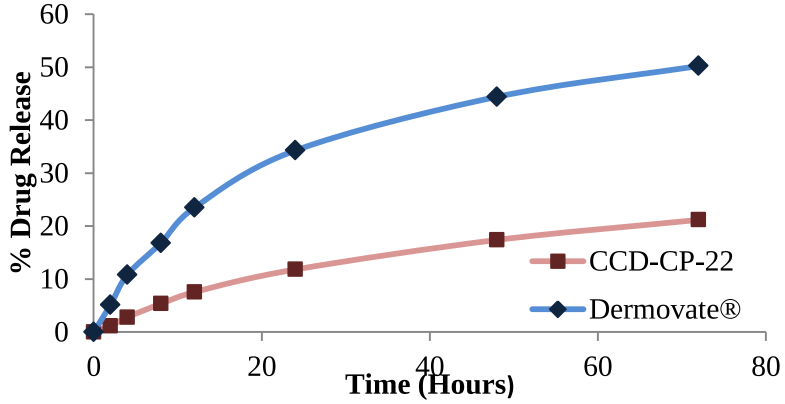
Batch Record Summary

Formulator : Ayeshah Fauzee **Date of Manufacture** :26/06/2013
Product : Central Composite Design Clobetasol 17-Propionate Cream
Melting Temperature :75 °C
Batch Number: CCD-CP-22 **Batch Size :** 6000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	3.10 g	RM000150
Propylene glycol	41.00	2546.58 mL	RM000181
Sodium citrate	0.05	3.10 g	RM000183
Citric acid	0.05	3.10 g	RM000185
Gelot® 64	5.00	310.56 g	RM000177
Glyceryl monostearate	6.00	372.67 g	RM000182
Ceteostearyl alcohol	13.00	807.45 g	RM000184
White beeswax	1.15	71.43 g	RM000142
Chlorocresol	0.075	4.66 g	RM000186
Distilled water	30.225	1877.33 mL	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenization Speed (rpm)	2000
Homogenization Time (min)	120
Anchor Speed (rpm)	40
Mixing Time (min)	120
Heating Temperature (°C)	75
Cooling time (min)	120

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	90600.00 ± 1900	2.09
Spreadability (cm^2)	9.99 ± 0.20	2.00
CP Content (%)	98.65 ± 0.68	0.68
pH	6.70 ± 0.04	0.59
Extrudability (g/cm^2)	445.63 ± 2.50	0.56
Electrical Conductivity (µS/cm)	143.80 ± 8.50	5.91



OBSERVATIONS/COMMENTS

- A homogeneous smooth white cream was produced

- No sign of cracking or bleeding was observed during and after manufacture

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140, REPUBLIC OF SOUTH AFRICA

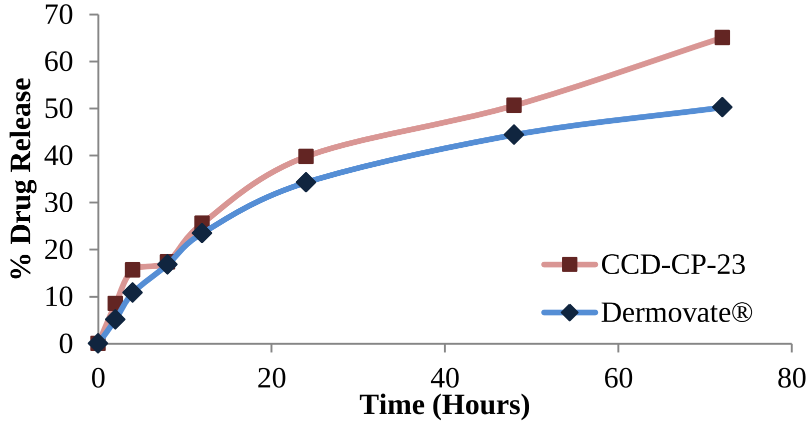
Batch Record Summary

Formulator : Ayeshah Fauzee **Date of Manufacture** :27/06/2013
Product : Central Composite Design Clobetasol 17-Propionate Cream
Melting Temperature :75 °C
Batch Number: CCD-CP-23 **Batch Size :** 6000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	2.92 g	RM000150
Propylene glycol	51.00	2982.45 mL	RM000181
Sodium citrate	0.05	2.92 g	RM000183
Citric acid	0.05	2.92 g	RM000185
Gelot® 64	1.00	58.48 g	RM000177
Glyceryl monostearate	16.00	935.67 g	RM000182
Ceteostearyl alcohol	3.00	175.44 g	RM000184
White beeswax	1.15	67.25 g	RM000142
Chlorocresol	0.075	4.39 g	RM000186
Distilled water	30.225	1767.54 mL	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenization Speed (rpm)	2000
Homogenization Time (min)	120
Anchor Speed (rpm)	40
Mixing Time (min)	120
Heating Temperature (°C)	75
Cooling time (min)	120

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	35066.00 ± 1085	3.09
Spreadability (cm^2)	19.63 ± 0.75	3.82
CP Content (%)	102.53 ± 0.25	0.25
pH	6.35 ± 0.04	0.62
Extrudability (g/cm^2)	49.13 ± 2.35	4.78
Electrical Conductivity (µS/cm)	963.00 ± 2.50	0.25



OBSERVATIONS/COMMENTS

- A homogeneous smooth white cream was produced

- No sign of cracking or bleeding was observed during and after manufacture

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140, REPUBLIC OF SOUTH AFRICA

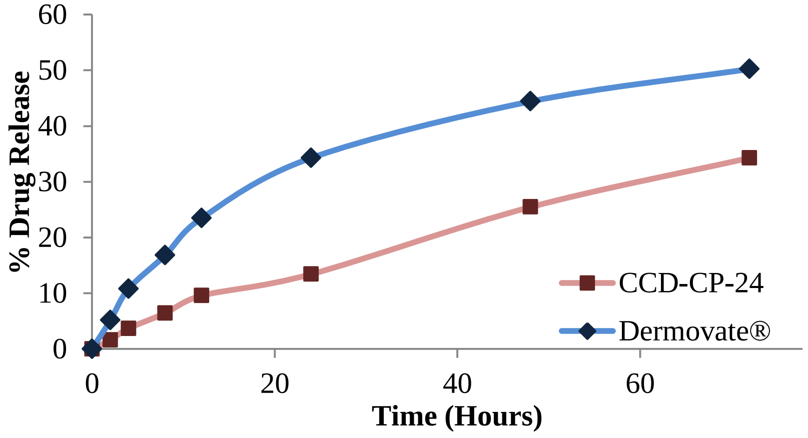
Batch Record Summary

Formulator : Ayeshah Fauzee **Date of Manufacture** :28/06/2013
Product : Central Composite Design Clobetasol 17-Propionate Cream
Melting Temperature :75 °C
Batch Number: CCD-CP-24 **Batch Size :** 6000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	2.74 g	RM000150
Propylene glycol	46.00	2244.53 mL	RM000181
Sodium citrate	0.05	2.74 g	RM000183
Citric acid	0.05	2.74 g	RM000185
Gelot® 64	3.00	164.23 g	RM000177
Glyceryl monostearate	21.00	1149.63 g	RM000182
Ceteostearyl alcohol	8.00	437.96 g	RM000184
White beeswax	1.15	62.96 g	RM000142
Chlorocresol	0.075	4.11 g	RM000186
Distilled water	30.225	1654.65 mL	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenization Speed (rpm)	2000
Homogenization Time (min)	120
Anchor Speed (rpm)	40
Mixing Time (min)	120
Heating Temperature (°C)	75
Cooling time (min)	120

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	89500.00 ± 1900	2.13
Spreadability (cm^2)	11.80 ± 0.85	7.20
CP Content (%)	98.76 ± 0.50	0.50
pH	6.68 ± 0.01	0.15
Extrudability (g/cm^2)	180.57 ± 1.25	0.69
Electrical Conductivity (μS/cm)	112.03 ± 2.95	2.63



OBSERVATIONS/COMMENTS

- A homogeneous smooth white cream was produced

- No sign of cracking or bleeding was observed during and after manufacture

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Grahamstown 6140, REPUBLIC OF SOUTH AFRICA

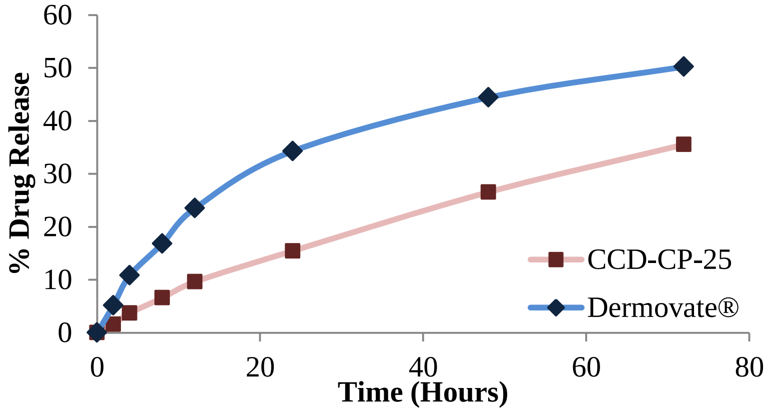
Batch Record Summary

Formulator : Ayeshah Fauzee **Date of Manufacture** :29/06/2013
Product : Central Composite Design Clobetasol 17-Propionate Cream
Melting Temperature :75 °C
Batch Number: CCD-CP-25 **Batch Size :** 6000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	3.11 g	RM000150
Propylene glycol	41.00	2546.58 mL	RM000181
Sodium citrate	0.05	3.11g	RM000183
Citric acid	0.05	3.11 g	RM000185
Gelot® 64	5.00	310.56 g	RM000177
Glyceryl monostearate	16.00	993.79 g	RM000182
Ceteostearyl alcohol	13.00	807.45 g	RM000184
White beeswax	1.15	71.43 g	RM000142
Chlorocresol	0.075	4.66 g	RM000186
Distilled water	30.225	1877.33 mL	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenization Speed (rpm)	2000
Homogenization Time (min)	120
Anchor Speed (rpm)	40
Mixing Time (min)	120
Heating Temperature (°C)	75
Cooling time (min)	120

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	88633.00 ± 1025	1.16
Spreadability (cm^2)	9.86 ± 0.25	2.03
CP Content (%)	99.77 ± 0.08	0.08
pH	6.37 ± 0.02	0.31
Extrudability (g/cm^2)	195.32 ± 1.50	0.76
Electrical Conductivity (µS/cm)	31.13 ± 0.35	1.12



OBSERVATIONS/COMMENTS

- A homogeneous smooth white cream was produced

- No sign of cracking or bleeding was observed during and after manufacture

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Grahamstown 6140, REPUBLIC OF SOUTH AFRICA

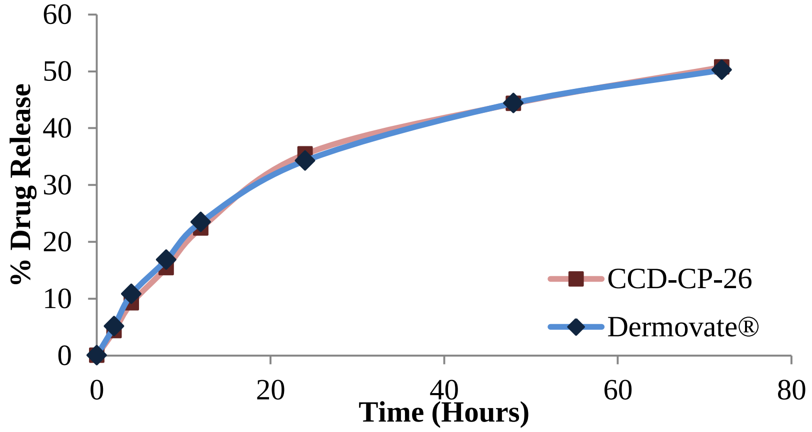
Batch Record Summary

Formulator : Ayeshah Fauzee **Date of Manufacture** :30/06/2013
Product : Central Composite Design Clobetasol 17-Propionate Cream
Melting Temperature :75 °C
Batch Number: CCD-CP-26 **Batch Size :** 6000 g

Excipient Name	**Original Formula(% w/w)**	**Working Formula**	**Rhodes Batch Number**
CP	0.05	3.01 g	RM000150
Propylene glycol	46.00	2771.08 mL	RM000181
Sodium citrate	0.05	3.20 g	RM000183
Citric acid	0.05	3.20 g	RM000185
Gelot® 64	3.00	180.72 g	RM000177
Glyceryl monostearate	11.00	662.65 g	RM000182
Ceteostearyl alcohol	8.00	481.92 g	RM000184
White beeswax	1.15	69.27 g	RM000142
Chlorocresol	0.075	4.52 g	RM000186
Distilled water	30.225	1820.78 mL	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenization Speed (rpm)	2000
Homogenization Time (min)	120
Anchor Speed (rpm)	40
Mixing Time (min)	120
Heating Temperature (°C)	75
Cooling time (min)	120

Tests Performed	**Mean ± SD (n=3)**	**%RSD**
Viscosity (cP)	44800.00 ± 1000	2.23
Spreadability (cm^2)	24.22 ± 0.50	2.06
CP Content (%)	100.10 ± 0.05	0.05
pH	6.07 ± 0.01	0.16
Extrudability (g/cm^2)	76.48 ± 2.75	3.60
Electrical Conductivity (μS/cm)	198.63 ± 3.50	1.76



OBSERVATIONS/COMMENTS

- A homogeneous smooth white cream was produced

- No sign of cracking or bleeding was observed during and after manufacture

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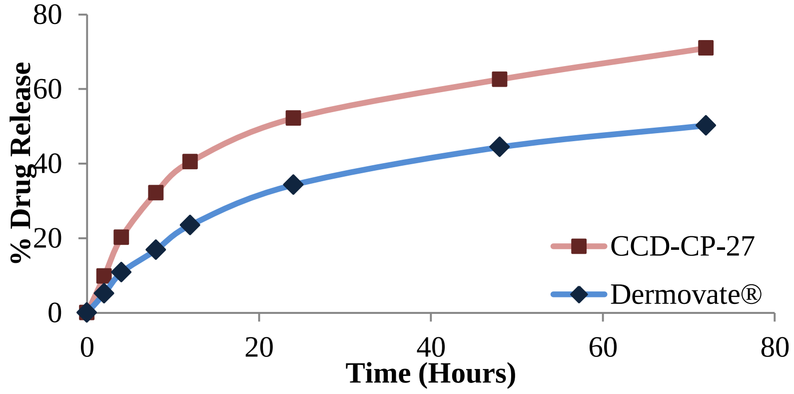
Batch Record Summary

Formulator : Ayeshah Fauzee **Date of Manufacture** :01/07/2013
Product : Central Composite Design Clobetasol 17-Propionate Cream
Melting Temperature :75 °C
Batch Number: CCD-CP-27 **Batch Size :** 6000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	3.24 g	RM000150
Propylene glycol	51.00	3304.53 mL	RM000181
Sodium citrate	0.05	3.24 g	RM000183
Citric acid	0.05	3.24 g	RM000185
Gelot® 64	1.00	64.80 g	RM000177
Glyceryl monostearate	6.00	388.77 g	RM000182
Ceteostearyl alcohol	3.00	194.38 g	RM000184
White beeswax	1.15	72.57 g	RM000142
Chlorocresol	0.075	4.86 g	RM000186
Distilled water	30.225	1958.42 mL	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenization Speed (rpm)	2000
Homogenization Time (min)	120
Anchor Speed (rpm)	40
Mixing Time (min)	120
Heating Temperature (°C)	75
Cooling time (min)	120

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	16600.00 ± 925	5.57
Spreadability (cm^2)	27.68 ± 0.75	2.71
CP Content (%)	99.78 ± 0.05	0.05
pH	6.21 ± 0.02	0.33
Extrudability (g/cm^2)	54.42 ± 2.45	4.50
Electrical Conductivity (µS/cm)	433.00 ± 2.86	0.66



OBSERVATIONS/COMMENTS

- A homogeneous smooth white cream was produced

- No sign of cracking or bleeding was observed during and after manufacture

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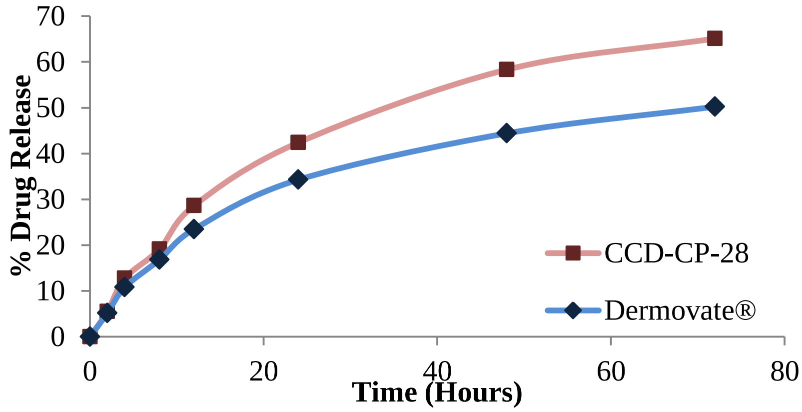
Batch Record Summary

Formulator : Ayeshah Fauzee
Date of Manufacture :02/07/2013
Product : Central Composite Design Clobetasol 17-Propionate Cream
Melting Temperature :75 °C
Batch Number: CCD-CP-28
Batch Size : 6000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	3.63 g	RM000150
Propylene glycol	41.00	2978.21 mL	RM000181
Sodium citrate	0.05	3.63 g	RM000183
Citric acid	0.05	3.63 g	RM000185
Gelot® 64	1.00	72.64 g	RM000177
Glyceryl monostearate	6.00	435.54 g	RM000182
Ceteostearyl alcohol	3.00	217.92 g	RM000184
White beeswax	1.15	83.54 g	RM000142
Chlorocresol	0.075	5.44 g	RM000186
Distilled water	30.225	2195.52 mL	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenization Speed (rpm)	2000
Homogenization Time (min)	120
Anchor Speed (rpm)	40
Mixing Time (min)	120
Heating Temperature (°C)	75
Cooling time (min)	120

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	27433.00 ± 875	3.19
Spreadability (cm^2)	22.62 ± 1.00	4.42
CP Content (%)	101.73 ± 0.75	0.75
pH	6.03 ± 0.10	1.65
Extrudability (g/cm^2)	65.35 ± 2.15	3.29
Electrical Conductivity (µS/cm)	734.00 ± 3.50	0.48



OBSERVATIONS/COMMENTS

- A homogeneous smooth white cream was produced

- No sign of cracking or bleeding was observed during and after manufacture

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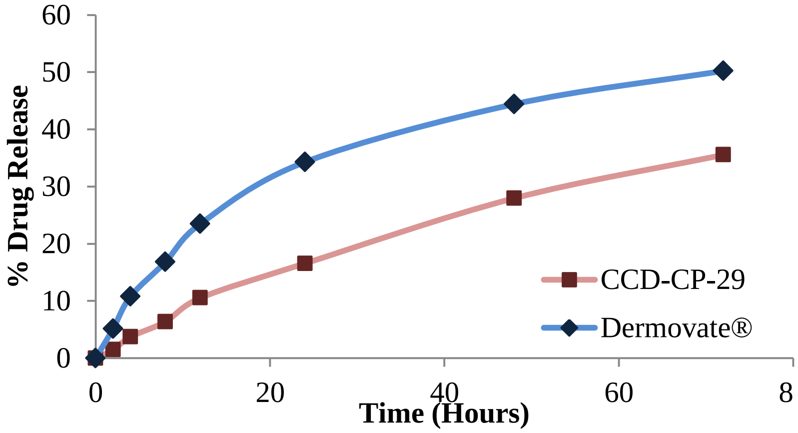
Batch Record Summary

Formulator : Ayeshah Fauzee **Date of Manufacture** :03/07/2013
Product : Central Composite Design Clobetasol 17-Propionate Cream
Melting Temperature :75 °C
Batch Number: CCD-CP-29 **Batch Size :** 6000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	2.57 g	RM000150
Propylene glycol	51.00	2624.36 mL	RM000181
Sodium citrate	0.05	2.57 g	RM000183
Citric acid	0.05	2.57 g	RM000185
Gelot® 64	5.00	257.29 g	RM000177
Glyceryl monostearate	16.00	823.33 g	RM000182
Ceteostearyl alcohol	13.00	668.95 g	RM000184
White beeswax	1.15	29.18 g	RM000142
Chlorocresol	0.075	3.85 g	RM000186
Distilled water	30.225	1555.32 mL	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenization Speed (rpm)	2000
Homogenization Time (min)	120
Anchor Speed (rpm)	40
Mixing Time (min)	120
Heating Temperature (°C)	75
Cooling time (min)	120

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	88367.00 ± 1200	1.36
Spreadability (cm^2)	12.35 ± 0.30	2.43
CP Content (%)	104.15 ± 0.02	0.02
pH	6.09 ± 0.01	0.16
Extrudability (g/cm^2)	194.29 ± 2.30	1.18
Electrical Conductivity (μS/cm)	252.66 ± 1.25	0.49



OBSERVATIONS/COMMENTS

- A homogeneous smooth white cream was produced

- No sign of cracking or bleeding was observed during and after manufacture

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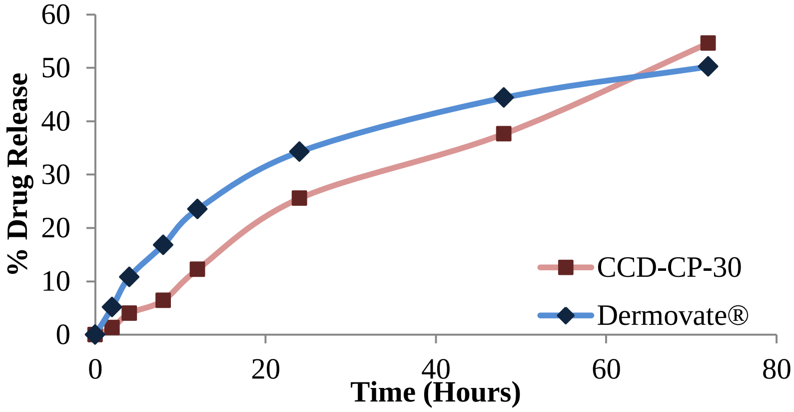
Batch Record Summary

Formulator : Ayeshah Fauzee **Date of Manufacture** :04/07/2013
Product : Central Composite Design Clobetasol 17-Propionate Cream
Melting Temperature :75 °C
Batch Number: CCD-CP-30 **Batch Size :** 6000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	3.11 g	RM000150
Propylene glycol	51.00	3167.70 mL	RM000181
Sodium citrate	0.05	3.11g	RM000183
Citric acid	0.05	3.11 g	RM000185
Gelot® 64	5.00	310.56 g	RM000177
Glyceryl monostearate	6.00	372.67 g	RM000182
Ceteostearyl alcohol	3.00	186.34 g	RM000184
White beeswax	1.15	71.43 g	RM000142
Chlorocresol	0.075	4.66 g	RM000186
Distilled water	30.225	1877.33 mL	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenization Speed (rpm)	2000
Homogenization Time (min)	120
Anchor Speed (rpm)	40
Mixing Time (min)	120
Heating Temperature (°C)	75
Cooling time (min)	120

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	50800.00 ± 1100	2.16
Spreadability (cm^2)	16.14 ± 0.75	4.65
CP Content (%)	100.55 ± 0.25	0.25
pH	6.13 ± 0.02	0.33
Extrudability (g/cm^2)	165.56 ± 1.50	0.91
Electrical Conductivity (µS/cm)	307.33 ± 2.30	0.74



OBSERVATIONS/COMMENTS

- A homogeneous smooth white cream was produced

- No sign of cracking or bleeding was observed during and after manufacture

APPENDIX VI

BATCH PRODUCTION RECORDS

DESIGN SPACE MANUFACTURE

A sample of a batch production record for design space *viz.*, Batch DS-CP-01 manufactured using a Wintech® cream/ointment mixer for the design space CP cream formulations is included in this appendix. The batch production records for the other design space cream formulation batches, *viz.*, DS-CP-02 – DS-CP-08 are available on request.

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BATCH RECORD PRODUCTION

Product name: Design Space Clobetasol 17-Propionate Cream Page 1 of 3
Batch number: DS-CP-01 **Batch size** : 6000 g

MANUFACTURING APPROVALS

Batch record issued by:_________________ **Date:_________________**

Master record issued by:_________________ **Date:_________________**

BATCH PRODUCTION RECORD

Product name: Design Space Clobetasol 17-Propionate Cream
Batch number: DS-CP-01 **Batch size** : 6000 g

MASTER FORMULA AND BATCH FORMULA							
Item no.	**Material**	**Rhodes No.**	**Quantity (% w/w)**	**Amount/ Batch**	**Amount Dispensed**	**Dispensed by**	**Checked by**
1	CP	RM000150	0.05	3.34 g			
2	Propylene glycol	RM000181	44.00	2936.59 mL			
3	Sodium citrate	RM000183	0.05	3.34g			
4	Citric acid	RM000185	0.05	3.34 g			
5	Gelot® 64	RM000177	3.80	253.62 g			
6	Glyceryl monostearate	RM000182	10.50	700.78 g			
7	Cetostearyl alcohol	RM000184	8.60	573.97 g			
8	White beeswax	RM000142	1.15	76.75 g			
9	Chlorocresol	RM000186	0.075	5.01 g			
10	Distilled water	N/A	30.225	2017.24 mL			

WINTECH® CREAM/OINTMENT MIXER VERIFICATION			
Description	**Type**	**Verified by**	**Confirmed by**
Bowl vessel	Stainless steel		
Co-centric Homogenizer	Teflon and stainless steel		
Anchor and impellers	Stainless steel		
Water inlet	Stainless steel		
Water Jacket	Stainless steel		
Charging port	Stainless steel		
Water immersion heater	NA		
PT-100 sensor	NA		
Operating panel touch screen	LCD		

BATCH PRODUCTION RECORD

Product name: Design Space Clobetasol 17-Propionate Cream

Batch number: DS-CP-01 **Batch size** : 6000 g

MANUFACTURING PROCEDURE

Steps	Procedure	Time	Done by	Checked by
1	Clean the bowl with Liquid K® detergent and distilled water. Allow the bowl to dry and equilibrate at room temperature Room temperature: _____°C Mixer temperature: _____°C			
2	Fill the jacket with distilled water until the jacket is 30 Liters			
3	Weigh all the materials accurately using a Model AE 163 Mettler® analytical balance (Mettler® Inc., Zurich, Switzerland)			
4	Introduce 2936.59 mL propylene glycol and 2017.24 mL distilled water, followed by 3.34 g sodium citrate, 3.34 g citric acid, 3.34 g clobetasol 17-propionate, 700.78 g glyceryl monostearate, 573.97 g cetostearyl alcohol, 76.75 g white beeswax, 253.62 g Gelot® 64 and 5.01 g chlorocresol through the charging port			
5	Allow mixture of excipients to stir at 40 rpms for 120 minutes at75°C			
6	Following mixing, allow the blend to be homogenized at 2000 rpms for 120 minutes			
7	Allow the molten mixture to cool by passing distilled water through the heating jacket for 120 minutes. Temperature of cold water=____°C			
8	After cooling, close the water inlet and allow slow continuous agitation of the anchor until the temperature on the operating panel displays 30°C - 35°C			
9	Stop the anchor and release the pressure built up in the bowl by the use of the pressure vent			
10	Store the final CP cream in 10 kg opaque containers until quality control tests are completed and then package into 50 g collapsible aluminium cream tubes			

SIGNATURE AND INITIAL REFERENCE

Full name (Print)	Signature	Initials	Date

APPENDIX VII

BATCH RECORD REPORTS

The batch record reports for the design space cream formulation batches, *viz.*, DS-CP-01 – DS-CP-08 are included on this accompanying electronic file “Appendices-CD” on the CD.

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140, REPUBLIC OF SOUTH AFRICA

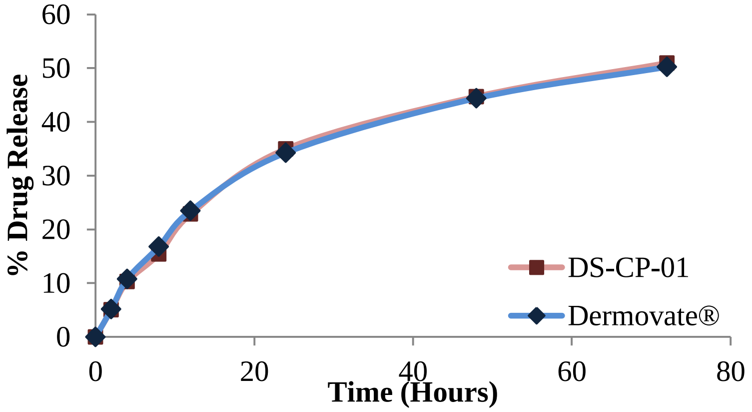
Batch Record Summary

Formulator : Ayeshah Fauzee
Date of Manufacture :15/10/2013
Product : Design Space Clobetasol 17-Propionate Cream
Melting Temperature :75 °C
Batch Number: DS-CP-01
Batch Size : 6000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	3.34 g	RM000150
Propylene glycol	44.00	2936.59 mL	RM000181
Sodium citrate	0.05	3.34g	RM000183
Citric acid	0.05	3.34 g	RM000185
Gelot® 64	3.80	253.62 g	RM000177
Glyceryl monostearate	10.50	700.78 g	RM000182
Ceteostearyl alcohol	8.60	573.97 g	RM000184
White beeswax	1.15	76.75 g	RM000142
Chlorocresol	0.075	5.01 g	RM000186
Distilled water	30.225	2017.24 mL	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenization Speed (rpm)	2000
Homogenization Time (min)	120
Anchor Speed (rpm)	40
Mixing Time (min)	120
Heating Temperature (°C)	75
Cooling time (min)	120

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	43750.00 ± 540	1.23
Spreadability (cm^2)	23.45 ± 0.50	2.13
CP Content (%)	100.00 ± 0.15	0.15
pH	5.95 ± 0.01	0.16
Extrudability (g/cm^2)	77.50 ± 1.20	1.55
Electrical Conductivity (μS/cm)	198.20 ± 2.00	1.01



OBSERVATIONS/COMMENTS

- A homogeneous smooth white cream was produced

- No sign of cracking or bleeding was observed during and after manufacture

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Grahamstown 6140, REPUBLIC OF SOUTH AFRICA

Batch Record Summary

Formulator : Ayeshah Fauzee

Date of Manufacture :16/10/2013

Product : Design Space Clobetasol 17-Propionate Cream

Melting Temperature :75 °C

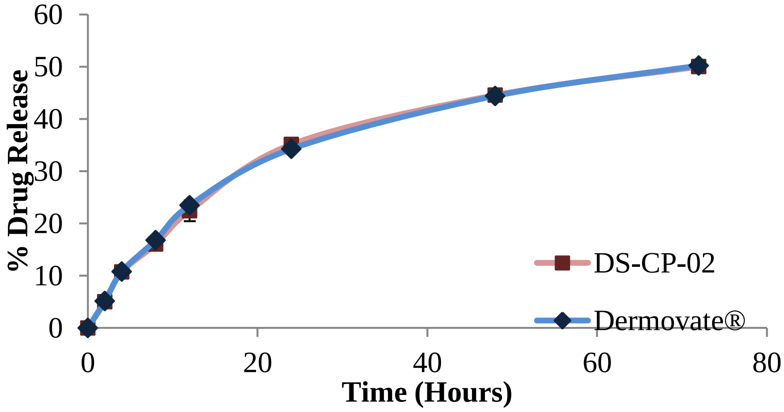
Batch Number: DS-CP-02

Batch Size : 6000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	2.97 g	RM000150
Propylene glycol	46.40	2759.16 mL	RM000181
Sodium citrate	0.05	2.97 g	RM000183
Citric acid	0.05	2.97 g	RM000185
Gelot® 64	3.80	225.97 g	RM000177
Glyceryl monostearate	10.50	624.38 g	RM000182
Ceteostearyl alcohol	8.60	511.38 g	RM000184
White beeswax	1.15	68.38 g	RM000142
Chlorocresol	0.075	4.46 g	RM000186
Distilled water	30.225	1797.32 mL	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenization Speed (rpm)	2000
Homogenization Time (min)	120
Anchor Speed (rpm)	40
Mixing Time (min)	120
Heating Temperature (°C)	75
Cooling time (min)	120

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	43200.00 ± 780	1.81
Spreadability (cm^2)	23.20 ± 1.10	4.74
CP Content (%)	100.00 ± 0.10	0.10
pH	5.75 ± 0.04	0.70
Extrudability (g/cm^2)	78.50 ± 0.12	0.15
Electrical Conductivity (µS/cm)	200.00 ± 3.15	1.57



OBSERVATIONS/COMMENTS

- A homogeneous smooth white cream was produced

- No sign of cracking or bleeding was observed during and after manufacture

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Grahamstown 6140, REPUBLIC OF SOUTH AFRICA

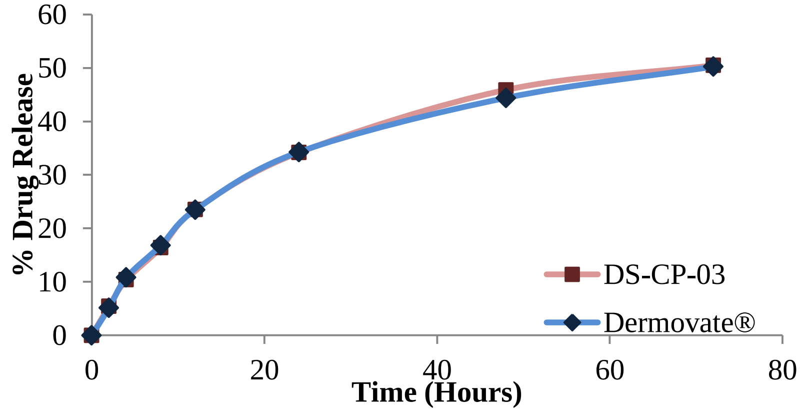
Batch Record Summary

Formulator : Ayeshah Fauzee
Date of Manufacture :17/10/2013
Product : Design Space Clobetasol 17-Propionate Cream
Melting Temperature :75 °C
Batch Number: DS-CP-03
Batch Size : 6000 g

Excipient Name	**Original Formula(% w/w)**	**Working Formula**	**Rhodes Batch Number**
CP	0.05	3.01 g	RM000150
Propylene glycol	46.10	2774.32 mL	RM000181
Sodium citrate	0.05	3.01 g	RM000183
Citric acid	0.05	3.01 g	RM000185
Gelot® 64	2.90	174.52 g	RM000177
Glyceryl monostearate	10.50	631.89 g	RM000182
Ceteostearyl alcohol	8.60	517.55 g	RM000184
White beeswax	1.15	69.21 g	RM000142
Chlorocresol	0.075	4.52 g	RM000186
Distilled water	30.225	1818.95 mL	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenization Speed (rpm)	2000
Homogenization Time (min)	120
Anchor Speed (rpm)	40
Mixing Time (min)	120
Heating Temperature (°C)	75
Cooling time (min)	120

Tests Performed	**Mean ± SD (n=3)**	**%RSD**
Viscosity (cP)	43150.00 ± 780	1.81
Spreadability (cm²)	24.00 ± 1.10	4.58
CP Content (%)	99.50 ± 0.10	0.10
pH	6.10 ± 0.04	0.66
Extrudability (g/cm²)	75.10 ± 0.12	9.59
Electrical Conductivity (µS/cm)	198.02 ± 3.15	1.59



OBSERVATIONS/COMMENTS

- A homogeneous smooth white cream was produced

- No sign of cracking or bleeding was observed during and after manufacture

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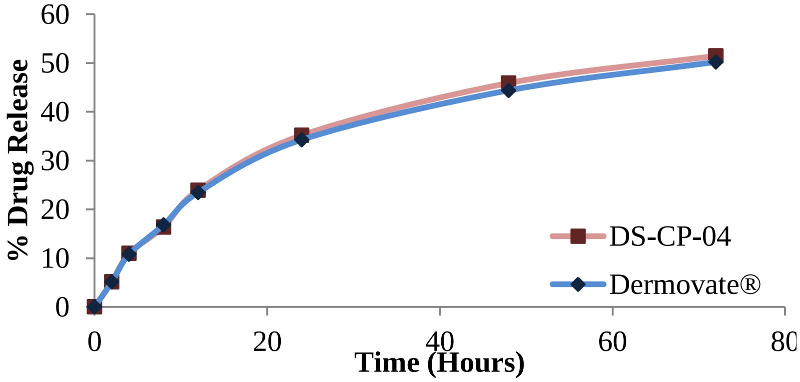
Batch Record Summary

Formulator : Ayeshah Fauzee **Date of Manufacture** :18/10/2013
Product : Design Space Clobetasol 17-Propionate Cream
Melting Temperature :75 °C
Batch Number: DS-CP-04 **Batch Size :** 6000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	2.98 g	RM000150
Propylene glycol	46.10	249.50 mL	RM000181
Sodium citrate	0.05	2.98 g	RM000183
Citric acid	0.05	2.98 g	RM000185
Gelot® 64	3.80	226.64 g	RM000177
Glyceryl monostearate	10.50	626.24 g	RM000182
Ceteostearyl alcohol	8.60	512.92 g	RM000184
White beeswax	1.15	68.59 g	RM000142
Chlorocresol	0.075	4.47 g	RM000186
Distilled water	30.225	1802.68 mL	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenization Speed (rpm)	2000
Homogenization Time (min)	120
Anchor Speed (rpm)	40
Mixing Time (min)	120
Heating Temperature (°C)	75
Cooling time (min)	120

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	44500.00 ± 950	2.13
Spreadability (cm^2)	24.50 ± 1.50	6.12
CP Content (%)	100.05 ± 0.10	0.10
pH	6.09 ± 0.02	0.32
Extrudability (g/cm^2)	75.00 ± 1.60	2.13
Electrical Conductivity (µS/cm)	199.20 ± 2.80	1.41



OBSERVATIONS/COMMENTS

- A homogeneous smooth white cream was produced

- No sign of cracking or bleeding was observed during and after manufacture

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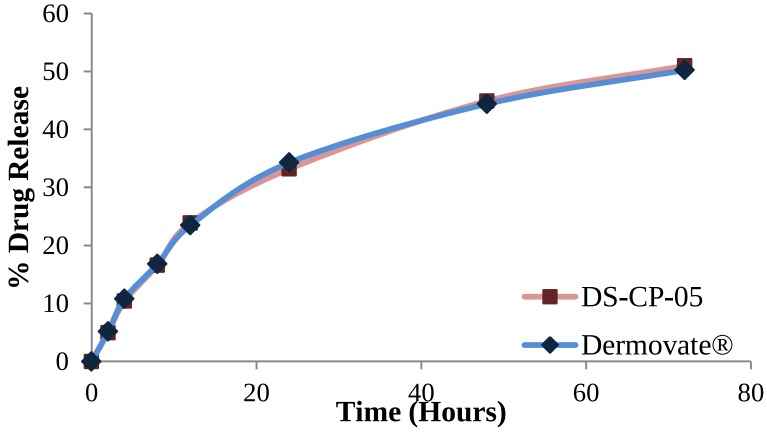
Batch Record Summary

Formulator : Ayeshah Fauzee
Date of Manufacture :19/10/2013
Product : Design Space Clobetasol 17-Propionate Cream
Melting Temperature :75 °C
Batch Number: DS-CP-05
Batch Size : 6000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	3.02 g	RM000150
Propylene glycol	46.10	2779.90 mL	RM000181
Sodium citrate	0.05	3.02 g	RM000183
Citric acid	0.05	3.02 g	RM000185
Gelot® 64	3.80	229.15 g	RM000177
Glyceryl monostearate	10.50	632.53 g	RM000182
Ceteostearyl alcohol	7.50	452.26 g	RM000184
White beeswax	1.15	69.35 g	RM000142
Chlorocresol	0.075	4.52 g	RM000186
Distilled water	30.225	1822.61 mL	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenization Speed (rpm)	2000
Homogenization Time (min)	120
Anchor Speed (rpm)	40
Mixing Time (min)	120
Heating Temperature (°C)	75
Cooling time (min)	120

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	44800.00 ± 800	1.78
Spreadability (cm^2)	24.80 ± 1.25	5.04
CP Content (%)	99.00 ± 0.05	0.05
pH	6.00 ± 0.05	0.83
Extrudability (g/cm^2)	75.15 ± 2.10	2.79
Electrical Conductivity (µS/cm)	198.00 ± 3.00	1.51



OBSERVATIONS/COMMENTS

- A homogeneous smooth white cream was produced

- No sign of cracking or bleeding was observed during and after manufacture

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140, REPUBLIC OF SOUTH AFRICA

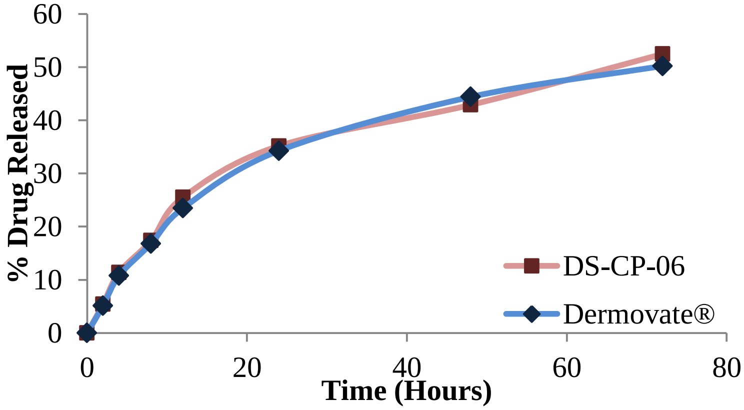
Batch Record Summary

Formulator : Ayeshah Fauzee
Date of Manufacture :20/10/2013
Product : Design Space Clobetasol 17-Propionate Cream
Melting Temperature :75 °C
Batch Number: DS-CP-06
Batch Size : 6000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	2.94 g	RM000150
Propylene glycol	46.10	2695.91 mL	RM000181
Sodium citrate	0.05	2.94 g	RM000183
Citric acid	0.05	2.94 g	RM000185
Gelot® 64	3.80	222.22 g	RM000177
Glyceryl monostearate	10.50	614.04 g	RM000182
Ceteostearyl alcohol	10.00	584.79 g	RM000184
White beeswax	1.15	67.25 g	RM000142
Chlorocresol	0.075	4.39 g	RM000186
Distilled water	30.225	1767.54 mL	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenization Speed (rpm)	2000
Homogenization Time (min)	120
Anchor Speed (rpm)	40
Mixing Time (min)	120
Heating Temperature (°C)	75
Cooling time (min)	120

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	43000.00 ± 1025	2.38
Spreadability (cm^2)	24.00 ± 1.05	4.38
CP Content (%)	99.50 ± 0.03	0.03
pH	6.25 ± 0.01	0.16
Extrudability (g/cm^2)	75.15 ± 1.30	1.73
Electrical Conductivity (μS/cm)	198.50 ± 2.95	1.49



OBSERVATIONS/COMMENTS

- A homogeneous smooth white cream was produced

- No sign of cracking or bleeding was observed during and after manufacture

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140, REPUBLIC OF SOUTH AFRICA

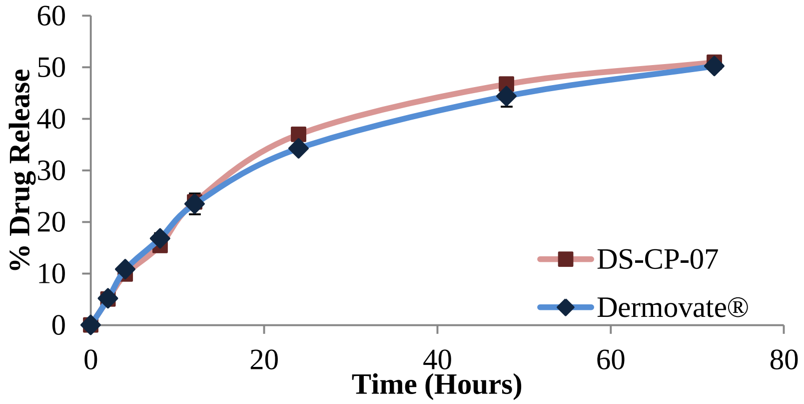
Batch Record Summary

Formulator : Ayeshah Fauzee
Date of Manufacture :21/10/2013
Product : Design Space Clobetasol 17-Propionate Cream
Melting Temperature :75 °C
Batch Number: DS-CP-07
Batch Size : 6000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	2.97 g	RM000150
Propylene glycol	46.10	2738.61 mL	RM000181
Sodium citrate	0.05	2.97 g	RM000183
Citric acid	0.05	2.97 g	RM000185
Gelot® 64	3.80	225.74 g	RM000177
Glyceryl monostearate	10.90	647.52 g	RM000182
Ceteostearyl alcohol	8.60	510.89 g	RM000184
White beeswax	1.15	68.32 g	RM000142
Chlorocresol	0.075	4.46 g	RM000186
Distilled water	30.225	1795.54 mL	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenization Speed (rpm)	2000
Homogenization Time (min)	120
Anchor Speed (rpm)	40
Mixing Time (min)	120
Heating Temperature (°C)	75
Cooling time (min)	120

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	43200.00 ± 950	2.19
Spreadability (cm^2)	24.50 ± 1.10	4.49
CP Content (%)	100.01 ± 0.01	0.01
pH	6.00 ± 0.01	0.16
Extrudability (g/cm^2)	78.00 ± 1.25	1.60
Electrical Conductivity (µS/cm)	200.20 ± 1.74	0.87



OBSERVATIONS/COMMENTS

- A homogeneous smooth white cream was produced

- No sign of cracking or bleeding was observed during and after manufacture

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140, REPUBLIC OF SOUTH AFRICA

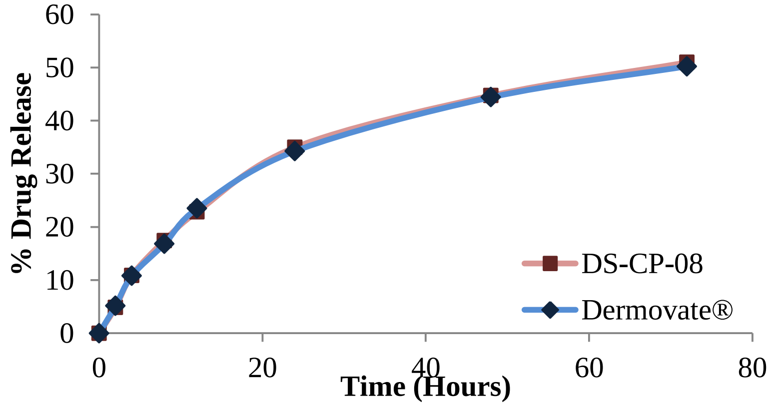
Batch Record Summary

Formulator : Ayeshah Fauzee
Date of Manufacture :22/10/2013
Product : Design Space Clobetasol 17-Propionate Cream
Melting Temperature :75 °C
Batch Number: DS-CP-08
Batch Size : 6000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	2.92 g	RM000150
Propylene glycol	46.10	2688.05 mL	RM000181
Sodium citrate	0.05	2.92 g	RM000183
Citric acid	0.05	2.92 g	RM000185
Gelot® 64	3.80	221.57 g	RM000177
Glyceryl monostearate	12.80	746.35 g	RM000182
Ceteostearyl alcohol	8.60	510.46 g	RM000184
White beeswax	1.15	67.06 g	RM000142
Chlorocresol	0.075	4.40 g	RM000186
Distilled water	30.225	1767.54 mL	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenization Speed (rpm)	2000
Homogenization Time (min)	120
Anchor Speed (rpm)	40
Mixing Time (min)	120
Heating Temperature (°C)	75
Cooling time (min)	120

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	43500.00 ± 950	2.18
Spreadability (cm^2)	24.00 ± 1.10	4.58
CP Content (%)	98.95 ± 0.01	0.01
pH	6.05 ± 0.01	0.02
Extrudability (g/cm^2)	78.00 ± 1.25	1.60
Electrical Conductivity (µS/cm)	199.50 ± 1.74	0.87



OBSERVATIONS/COMMENTS

- A homogeneous smooth white cream was produced

- No sign of cracking or bleeding was observed during and after manufacture