DEVELOPMENT AND *IN VITRO* EVALUATION OF A CLOBETASOL 17-PROPIONATE TOPICAL CREAM FORMULATION

A Thesis Submitted in
Fulfilment of the Requirements for the Degree of

MASTER OF SCIENCE (PHARMACY)

of

RHODES UNIVERSITY

by

Kasongo Wa Kasongo

January 2007

Faculty of Pharmacy
Rhodes University
Grahamstown
South Africa

ABSTRACT

One of the primary contributing factors to the escalating costs of health care is the high cost of innovator pharmaceutical products. As a consequence, health authorities in various countries and in particular in the developing world have identified generic prescribing and generic substitution as possible strategies to contain the escalating costs of health care provision. There is therefore a need for formulation scientists in developing countries to invest more time in the research and development of generic formulations.

Clobetasol 17-propionate (CP) generic cream formulations containing 0.05% w/w of the drug were manufactured and characterized using *in vitro* testing. Formulation development studies were preceded by the development and validation of an RP-HPLC with UV detection for the quantitation and characterization of CP in innovator and generic cream formulations during formulation development and assessment studies. Furthermore the *in vitro* release rates of CP release from innovator and generic cream formulations were monitored using a validated *in vitr*o release test method developed in these studies.

The formulation of CP cream products was accomplished using a variety of commercially available mixed primary emulsifiers, such as Estol® 1474, Ritapro® 200, Emulcire® 61 WL and Gelot® 64. Successful formulations were selected based on their ability to remain physically stable immediately after manufacture and for 24 hours after storage at room temperature (22˚C). Estol® 1474 was found to produce an unstable cream and was therefore not investigated further.

The other three emulgents produced stable creams, but only the *in vitro* release profile of CP from a cream manufactured to contain Gelot® 64 was found to be statistically similar to that of the innovator formulation. Therefore the cream containing Gelot® 64 was selected as the most appropriate prototype generic cream formulation and was characterized *in vitro* in terms of CP content, viscosity, pH and *in vitro* release rate. Data generated from these studies were compared to those of the innovator product, Dermovate® cream, using statistical methods.

The CP content, pH and *in vitro* release rate data of the CP formulation were similar to those of the innovator product, however the intrinsic viscosity of Dermovate® cream was almost three (3) times greater than the intrinsic viscosity of the test formulation developed using Gelot® 64.

The CP cream formulation developed in these studies was stored for 4 weeks at 40 ± 2˚C and 25 ± 5% RH in an incubator and the formulation was found to be stable. A formulation has been developed and assessed and found to be suitable for use as a topical semi-solid dosage form for CP.

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to the following people:

My supervisor, Prof R.B. Walker for giving me the opportunity to be part of his Research Group and for his support, patience, guidance, understanding and assistance throughout the course of my studies and during the preparation of this thesis and also for providing me with laboratory facilities and financial assistance.

My co-supervisor, Dr M.F. Skinner for his availability and assistance in various ways.

The Dean and Head, Prof I. Kanfer and the staff of the Faculty of Pharmacy, for use of the facilities in the Faculty and Prof I. Kanfer for providing me with financial assistance.

Mr T. Samkange and Mr. L. Purdon for their technical expertise, assistance and advice.

Gattefossé SAS (Saint-Priest Cedex, France) for their donation of excipients.

My father for his love, encouragement, motivation, financial assistance, understanding and for always believing in me not only throughout the duration of this project, but also throughout my life. My mother, for her unfailing love and support. My sisters, brothers and Papa Andre for their understanding and encouragement.

My colleagues in the Biopharmaceutics Research Laboratory (BRG) for their congenial company, support, and encouragement throughout my study period.

Adrienne C. Muller for her support, encouragement and understanding throughout the course of my project and for showing interest in my research.

The Almighty God for giving me protection, strength and resolve to succeed throughout my life.

STUDY OBJECTIVES

Eczema or dermatitis is a common dermatological disorder affecting approximately one-third of a given population and is the most common skin condition in the Republic of South Africa (RSA). Topical corticosteroid formulations such as creams or ointments applied three (3) times daily are considered the most appropriate therapy for the treatment of eczema. Topical corticosteroids are however considered costly for the majority of patients and as a consequence the use of white petrolatum and hydrogenated vegetable oil as supplemental therapy is usually required. The high cost of therapy is a result of the use of innovator products of the super-potent topical corticosteroids such as clobetasol 17-propionate (CP) that are required for the treatment of severe or chronic eczema, especially of the hands and feet. The availability of generic formulations of innovator products will make medicinal products more affordable and accessible to a wider population.

The objectives of this study were:

1. To develop, optimize and validate a simple, selective, sensitive, precise, accurate and linear reversed phase high performance liquid chromatographic method that is suitable for the quantitative analysis of CP in cream formulations and for the assessment of CP release from topical formulations during *in vitro* testing.
2. To develop and validate a reliable, reproducible and discriminatory *in vitro* release test method for use in formulation development studies to assess product quality and ensure batch-to-batch consistency of topical formulations manufactured to contain 0.05% w/w CP.
3. To design and develop a generic version of Dermovate® cream and to evaluate the product in terms of several *in vitro* performance characteristics.
4. To determine the stability of the CP cream formulation developed in these studies at elevated temperatures.

TABLE OF CONTENTS

ABSTRACT ---------- II

ACKNOWLEDGEMENTS ---------- IV

STUDY OBJECTIVES ---------- V

LIST OF TABLES ---------- XIII

LIST OF FIGURES ---------- XV

CHAPTER ONE ---------- 1

CLOBETASOL 17-PROPIONATE ---------- 1

1.1. INTRODUCTION ---------- 1

1.2. DESCRIPTION ---------- 2

1.3. PHYSICOCHEMICAL PROPERTIES ---------- 2
- 1.3.1. Solubility ---------- 2
  - 1.3.1.1. Overview ---------- 2
  - 1.3.1.2. Solubility studies ---------- 3
    - 1.3.1.2.1. Overview ---------- 3
    - 1.3.1.2.2. Propylene glycol (PG) ---------- 3
    - 1.3.1.2.3. Acetonitrile (ACN) ---------- 3
- 1.3.2. Dissociation constant (pK_a) ---------- 4
- 1.3.3. Partition coefficient ---------- 4
- 1.3.4. Melting range ---------- 4
- 1.3.5. Optical rotation ---------- 4
- 1.3.6. Stability ---------- 5
- 1.3.7. Ultraviolet absorption spectrum ---------- 5
- 1.3.8. Infra-red Absorption Spectrum ---------- 6
- 1.3.9. Crystal Structure ---------- 7

1.4. STEREOCHEMISTRY AND STRUCTURE ACTIVITY RELATIONSHIP ---------- 8

1.5. CLINICAL PHARMACOLOGY ---------- 9
- 1.5.1. Mode of action ---------- 9
  - 1.5.1.1. Overview ---------- 9
  - 1.5.1.2. Anti-inflammatory action ---------- 10
  - 1.5.1.3. Immuno-suppressive effects ---------- 11
  - 1.5.1.4. Anti-mitotic and vasoconstrictive effects ---------- 12
- 1.5.2. Indications ---------- 12
- 1.5.3. Contra-indications ---------- 12
- 1.5.4. Adverse Effects ---------- 13

1.5.4.1. Overview ---- 13
1.5.4.2. Local side effects ---- 13
1.5.4.3. Systemic side effects ---- 14
1.5.5. High risk groups ---- 14
1.5.5.1. Pregnancy ---- 14
1.5.5.2. Lactation ---- 15
1.5.5.3. Paediatric use ---- 15
1.5.5.4. Geriatric use ---- 16

1.6. PHARMACOKINETICS ---- 16
1.6.1. Dosage and Administration ---- 16
1.6.2. Absorption ---- 17
1.6.3. Distribution ---- 17
1.6.4. Elimination ---- 18

1.7. CONCLUSIONS ---- 18

CHAPTER TWO ---- 21

DEVELOPMENT, OPTIMIZATION AND VALIDATION OF AN HPLC METHOD FOR THE ANALYSIS OF CLOBETASOL 17-PROPIONATE IN SEMI-SOLID DOSAGE FORMS ---- 21

2.1. INTRODUCTION ---- 21

2.2. PRINCIPLES OF RP-HPLC ---- 22

2.3. METHOD DEVELOPMENT ---- 24
2.3.1. Overview ---- 24
2.3.2. Experimental ---- 26
2.3.2.1. Chemicals ---- 26
2.3.2.2. Instrumentation ---- 26
2.3.2.3. UV detection of CP and internal standard ---- 27
2.3.2.4. Column selection ---- 28
2.3.2.5. Column efficiency ---- 31
2.3.2.6. Internal standard selection ---- 34
2.3.2.7. Mobile phase selection ---- 35
2.3.2.8. Preparation of mobile phase ---- 37
2.3.2.9. Preparation of stock solutions and calibration standards ---- 37
2.3.2.10. Effect of ACN concentration ---- 38
2.3.2.11. Effect of flow rate ---- 39
2.3.2.12. Optimal mobile phase composition and flow rate ---- 40
2.3.2.13. Chromatographic conditions ---- 42

2.4. METHOD VALIDATION ---- 42
2.4.1. Overview ---- 42
2.4.2. Linearity and Range ---- 43
2.4.3. Precision ---- 45
2.4.3.1. Repeatability ---- 45
2.4.3.2. Intermediate precision ---- 46

2.4.3.3. Reproducibility---------- 47
2.4.4. Accuracy---------- 47
2.4.5. Limit of quantitation (LOQ) and limit of detection (LOD)---------- 49
2.4.6. Specificity and selectivity ---------- 50
2.4.6.1. Overview---------- 50
2.4.6.2. Sample preparation ---------- 50
2.4.6.3. Extraction efficiency ---------- 53
2.4.6.4. Validation of the extraction procedure---------- 53
2.4.6.5. Selectivity studies---------- 54
2.4.7. Sample stability ---------- 56
2.4.7.1. Overview---------- 56
2.4.7.2. Stability data analysis ---------- 57
2.4.7.3. Stability of stock solutions ---------- 58
2.4.7.4. In-process sample stability ---------- 61

2.5. METHOD REVALIDATION ---------- 62
2.5.1. Overview ---------- 62
2.5.2. Linearity ---------- 63
2.5.3. Precision ---------- 64
2.5.3.1. Repeatability ---------- 64
2.5.3.2. Intermediate precision---------- 65
2.5.3.3. Reproducibility---------- 65
2.5.4. Accuracy---------- 66

2.6. APPLICATION OF THE ANALYTICAL METHOD ---------- 66

2.7. CONCLUSIONS---------- 67

CHAPTER THREE ---------- 70

DEVELOPMENT AND VALIDATION OF AN *IN VITRO* TEST METHOD FOR THE ASSESSMENT OF CLOBETASOL 17-PROPIONATE RELEASE FROM TOPICAL CREAM FORMULATIONS ---------- 70

3.1. INTRODUCTION---------- 70

3.2. METHOD DEVELOPMENT---------- 73
3.2.1. Overview ---------- 73
3.2.2. Diffusion cell test system---------- 73
3.2.2.1. Overview---------- 73
3.2.2.2. Franz diffusion cell ---------- 75
3.2.2.3. Modified Franz diffusion cell ---------- 76
3.2.2.4. Selection of diffusion cell test system ---------- 77
3.2.3. Number of samples---------- 78
3.2.4. Sampling times---------- 78
3.2.5. Temperature---------- 81
3.2.6. Receptor medium---------- 82
3.2.6.1. Overview---------- 82
3.2.6.2. Selection of receptor medium---------- 83

3.2.6.2.1. Aqueous systems ---------- 83
3.2.6.2.2. Water-immiscible systems ---------- 83
3.2.6.2.3. Water-miscible systems ---------- 83
3.2.6.2.3.1. Overview---------- 83
3.2.6.2.3.2. Alcohol/water mixtures ---------- 84
3.2.6.2.3.3. Propylene glycol/water mixtures ---------- 85
3.2.6.3. Saturation solubility ---------- 86
3.2.6.4. Preparation of the receptor medium---------- 89
3.2.7. Synthetic membranes ----------89
3.2.7.1. Overview---------- 89
3.2.7.2. Characteristics of membranes---------- 90
3.2.7.3. Assessment of membranes ---------- 91
3.2.7.4. Membrane selection ---------- 93
3.2.7.4.1. Silicone membrane---------- 94
3.2.7.4.2. Porous membranes ---------- 94
3.2.7.5. Membrane resistance ---------- 96
3.2.8. Sample Application ----------98
3.2.8.1. Overview---------- 98
3.2.8.2. Effects of sample application ---------- 98
3.2.9. Sample occlusion ---------- 100
3.2.9.1. Overview----------100
3.2.9.2. Effects of occlusion ----------101
3.2.10. Sample Analysis---------- 102
3.2.11. Comparison of diffusion or release rate profiles---------- 103
3.2.12. Optimal *in vitro* release test conditions---------- 104

3.3. METHOD VALIDATION ----------105
3.3.1. Overview ---------- 105
3.3.2. Changes in dosage strength ---------- 106
3.3.3. Changes in composition---------- 107
3.3.2.3. Changes in viscosity----------109
3.3.2.3.1. Overview ----------109
3.3.2.3.2. Determination of viscosity ----------110
3.3.2.3.3. Effects of viscosity----------110

3.4. CONCLUSIONS----------112

CHAPTER FOUR ----------114

DEVELOPMENT AND *IN VITRO* CHARACTERIZATION OF CLOBETASOL 17-PROPIONATE CREAM FORMULATIONS ----------114

4.1. INTRODUCTION ----------114

4.2. CREAM FORMULATIONS----------117
4.2.1. Overview ---------- 117
4.2.2. Instability mechanisms in creams---------- 120
4.2.2.1. Overview----------120
4.2.2.2. Flocculation ----------121

4.2.2.3. Coalescence----122
4.2.2.4. Creaming or sedimentation----122
4.2.2.5. Ostwald ripening----123
4.2.2.6. Phase inversion----124
4.2.3. Stabilization of creams----124
4.2.3.1. Surfactants----124
4.2.3.2. Mixed emulgents----125
4.2.3.3. Theory of cream emulsification----127
4.2.3.4. Hydrophilic-lipophilic balance (HLB)----130

4.3. EXPERIMENTAL----132
4.3.1. Characterization of CP creams----132
4.3.1.1. Overview----132
4.3.1.2. Assay of CP content----132
4.3.1.3. Intrinsic viscosity----133
4.3.1.4. pH-determination----133
4.3.1.5. In vitro release rate----134
4.3.2. Innovator product characterization----135
4.3.2.1. Overview----135
4.3.2.2. Qualitative composition----135
4.3.2.3. CP content----135
4.3.2.4. Intrinsic viscosity----136
4.3.2.5. pH-determination----136
4.3.2.6. In vitro release rate studies----136
4.3.3. Generic product development----138
4.3.3.1. Overview----138
4.3.3.2. Excipients----139
4.3.3.2.1. Overview----139
4.3.3.2.2. Clobetasol 17-propionate----139
4.3.3.2.3. Propylene glycol----139
4.3.3.2.4. Sodium citrate----140
4.3.3.2.5. Citric acid----140
4.3.3.2.6. Geleol®----140
4.3.3.2.7. Cetostearyl alcohol----141
4.3.3.2.8. White beeswax----142
4.3.3.2.9. Chlorocresol----142
4.3.3.2.10. Estol® 1474----143
4.3.3.2.11. Ritapro® 200----143
4.3.3.2.12. Emulcire® 61 WL----143
4.3.3.2.13. Gelot® 64----143
4.3.4. Formulation composition----144
4.3.5. Manufacturing methods----146
4.3.5.1. Overview----146
4.3.5.2. Aqueous phase----146
4.3.5.3. Oil phase----146
4.3.5.4. Dispersed phase----147
4.3.5.5. Drug phase----147
4.3.5.6. Cream formulation----147
4.3.6. Preliminary studies----149
4.5.7. *In vitro* release studies----151
4.5.7.1. Overview----151

4.5.7.2. Effects of Ritapro® 200 ---151
4.5.7.3. Effects of Emulcire® 61 WL---153
4.5.7.4. Effects of Gelot® 64 ---155
4.3.8. Generic product characterization ---157
4.3.8.1. Overview---157
4.3.8.2. CP content ---157
4.3.8.3. Intrinsic viscosity ---158
4.3.8.4. pH-determination ---161
4.3.8.5. In vitro release rate ---161

4.4. CONCLUSIONS---164

CHAPTER FIVE ---167

STABILITY OF CLOBETASOL 17-PROPIONATE CREAMS ---167

5.1. INTRODUCTION---167

5.2. EXPERIMENTAL---171
5.2.1. Overview ---171
5.2.2. Stability study protocol---172
5.2.2.1. Overview---172
5.2.2.2. Selection of batches ---172
5.2.2.3. Number of batches---173
5.2.2.4. Container-closure system---173
5.2.2.5. Sampling frequency ---173
5.2.2.6. Sampling plan ---174
5.2.2.7. Test storage conditions---174
5.2.2.8. Test specifications ---175
5.2.2.9. Product specifications---175
5.2.2.10. Methodology---176
5.2.2.10.1. Test procedure---176
5.2.2.10.2. Organoleptic appeal ---177
5.2.2.10.3. CP content ---177
5.2.2.10.4. Intrinsic viscosity ---177
5.2.2.10.5. pH-determination ---177
5.2.2.10.6. In vitro release test---177
5.2.2.11. Statistical evaluation---178
5.2.3. Results and discussion---179
5.2.3.1. Qualitative analysis---179
5.2.3.2. Quantitative analysis ---179
5.2.3.2.1. CP content ---180
5.2.3.3. Intrinsic viscosity ---181
5.2.3.4. Apparent intrinsic pH ---182
5.2.3.5. In vitro release rate testing ---183

5.3. CONCLUSIONS---186

CHAPTER SIX ---------- 189

CONCLUSIONS ---------- 189

APPENDIX ONE ---------- 197

APPENDIX TWO ---------- 198

APPENDIX THREE ---------- 199

APPENDIX FOUR ---------- 200

APPENDIX FIVE ---------- 201

APPENDIX SIX ---------- 208

APPENDIX SEVEN ---------- 215

REFERENCES ---------- 217

LIST OF TABLES

Table 1.1. Solubility of CP ... 3

Table 1.2. Major infra-red band assignment for CP ... 7

Table 2.1. RP-HPLC methods used for the analysis of CP in semi-solid dosage forms ... 25

Table 2.2. Retention times of CP and potential internal standards ... 34

Table 2.3. Chromatographic conditions for the analysis of CP ... 42

Table 2.4. Repeatability data for HPLC analysis of CP ... 46

Table 2.5. Intermediate precision data for HPLC analysis of CP ... 47

Table 2.6. Accuracy data for HPLC analysis of CP (n = 3) ... 48

Table 2.7. LOQ data for HPLC analysis of CP ... 50

Table 2.8. Extraction efficiency data following the extraction of CP from Dermovate® cream (n = 6) ... 53

Table 2.9. Extraction efficiency following extraction of 600 µl of a CP solution (0.05% w/w) (n = 3) ... 54

Table 2.10. Repeatability data for the revalidation of the HPLC method for CP ... 65

Table 2.11. Intermediate precision data for the revalidation of the HPLC method for CP ... 65

Table 2.12. Accuracy data for the revalidation of the HPLC method for CP (n = 3) ... 66

Table 3.1. Solubility data for CP in various concentrations of PG at 32˚C (n = 3) ... 87

Table 3.2. Summary of the characteristics of synthetic membranes ... 91

Table 3.3. Cumulative percentage CP released after 72 hour (n = 3) ... 95

Table 3.4. *In vitro* release characteristics from four loading doses applied to the test membrane ... 100

Table 3.5. Summary of optimal *in vitro* release test conditions ... 105

Table 3.6. Effect of changes in the CP concentration on the total cumulative amount released and the associated flux values (n = 3) ... 106

Table 3.7. Effect of changes in formulation composition on flux and cumulative amount of CP released (n = 3) ... 108

Table 3.8. Cumulative amount of CP released and the average *in vitro* release rate (flux) from CP cream formulations of different intrinsic viscosity (n = 3) ... 110

Table 4.1. Pharmacopoeial and commercially available emulsifying waxes [172, 173] ... 127

Table 4.2. Percentage composition of generic CP cream formulations developed and assessed in these studies ... 145

Table 4.3. Cumulative amount of CP released and the average *in vitro* release rate (flux) of CP from Batch CP002 and Dermovate® cream (n = 6) 151
Table 4.4. The lower limit (L.L.) and upper limit (U.L.) of the confidence interval (C.I) calculated using Batch CP002 (test) and Dermovate® cream (reference) 152
Table 4.5. Cumulative amount of CP released and the average *in vitro* release rate (flux) of CP from Batch CP003 and Dermovate® cream (n = 6) 153
Table 4.6. The lower limit (L.L.) and upper limit (U.L.) of the confidence interval (C.I.) calculated for using Batch CP003 (test) and Dermovate® cream (reference) 154
Table 4.7. Cumulative amount of CP released and the average *in vitro* release rate (flux) of CP from Batch CP004 and Dermovate® cream (n = 6) 155
Table 4.8. The lower limit (L.L.) and upper limit (U.L.) of the confidence interval (C.I) calculated using Dermovate® cream (reference) and Batch CP004 (test) 156
Table 4.9. CP content of Dermovate® cream and Batches of generic cream formulations (n = 3) 157
Table 4.10. Intrinsic viscosity readings for Dermovate® cream and generic cream products (n = 3) 158
Table 4.11. pH readings for Dermovate® cream and generic cream formulations (n = 3) 161
Table 4.12. Cumulative amount of CP released and the average *in vitro* release rate (flux) of CP from Dermovate® cream and generic cream formulations (n = 6) 162
Table 4.13. The lower limit (L.L.) and upper limit (U.L.) of the confidence interval (C.I.) calculated using Dermovate® cream (reference) and generic formulations (tests) 163

Table 5.1. Specifications for qualitative parameters of the test cream formulation 176
Table 5.2. Specifications for quantitative parameters of the test cream formulation 176
Table 5.3. Stability data generated for Batch CP004 after a four (4) week test period 180
Table 5.4. The lower limit (L.L.) and upper limit (U.L.) of the confidence intervals (C.I.) calculated using cream sample at week 0 (reference) and cream samples at weeks 1, 2, 3 and 4 (test) 186

LIST OF FIGURES

Figure 1.1. Chemical structure of clobetasol 17-propionate ($C_{25}H_{32}ClFO_5$, MW = 467.0) 2
Figure 1.2. UV absorption spectrum of CP in ACN:water (50:50).......... 6
Figure 1.3. IR absorption spectrum of CP (adapted from 22).......... 7

Figure 2.1. Typical chromatogram of a test mixture containing uracil (1), acetophenone (2), benzene (3), toluene (4) and naphthalene (5) after separation on a 4 μm Nova-Pak® C_{18} (150 mm x 3.9 i.d.) cartridge column.......... 33
Figure 2.2. Typical chromatograms generated using a binary mixture of MeOH and water (A) and a binary mixture of ACN and water (B) as mobile phase. 36
Figure 2.3. Effect of ACN concentration on the retention times of BV and CP.......... 38
Figure 2.4. Effect of mobile phase (50% v/v ACN) flow rate on the retention times of BV and CP 40
Figure 2.5. Typical chromatogram of a mixture of the internal standard, betamethasone 17-valerate (BV) and clobetasol 17-propionate (CP) using a mobile phase of 50% v/v ACN-water and a flow rate of 1.0 ml/min 41
Figure 2.6. Calibration curve constructed for CP following least squares linear regression analysis of peak height ratios of CP and IS versus concentration.......... 44
Figure 2.7. Schematic representation of the sample preparation procedure 52
Figure 2.8. Typical chromatograms obtained following the analysis of a sample of placebo cream without BV (A) and with BV (B) and Dermovate® cream without BV (C) and with BV (D) 55
Figure 2.9. Interpretation of stability data, as described by Timm *et al*, [97].......... 58
Figure 2.10. Stability of CP in ACN at two different concentrations, stored at + 4ºC for 1, 2, 3, 7, and 14 days.......... 60
Figure 2.11. Stability of CP in 50% v/v propylene glycol/water stored at + 22ºC for 1, 2 and 3 days.......... 62
Figure 2.12. Calibration curve for CP following revalidation of the method 64
Figure 2.13. *In vitro* release profile of CP 67

Figure 3.1. Schematic representation of an original Franz diffusion cell apparatus (redrawn from 127)...... 76
Figure 3.2. Schematic representation of a modified Kenshary-Chien Franz glass diffusion cell (redrawn from 127).......... 77
Figure 3.3. Schematic representation of a modified Franz cell multiple-cell drive unit.......... 78
Figure 3.4. *In vitro* release of CP from Dermovate® cream (n = 6) 80

Figure 3.5. Least squares linear regression best fit line of the *in vitro* release profile of CP from Dermovate® cream (n = 6) 81
Figure 3.6. *In vitro* release profile of CP from Dermovate® cream, using 30% v/v ethanol solution as receptor medium (n = 6) 85
Figure 3.7. *In vitro* release profile of CP from Dermovate ® cream using 30% v/v PG solution as receptor medium (n = 6) 86
Figure 3.8. Saturation solubility profile of CP in PG:water solutions of different proportions (n = 3) 88
Figure 3.9. Effect of membrane type on the *in vitro* release of CP from Dermovate® cream (n = 3) 93
Figure 3.10. Comparison of *in vitro* release of CP from a 0.05% v/v CP solution and Dermovate® cream using a 0.025 μm nitrocellulose membrane (n = 6) 97
Figure 3.11. Effect of sample loading on the *in vitro* release of CP from Dermovate® cream 99
Figure 3.12. Effect of occlusion on the *in vitro* release of CP from Dermovate® cream (n = 6)........ 101
Figure 3.13. CP calibration curve used to determine the amount of CP released in *in vitro* release experiments 103
Figure 3.14. Effect of changes in CP content on the *in vitro* release rate of CP from an extemperaneous cream base (n = 3) 107
Figure 3.15. Effect of changes in formulation composition on CP *in vitro* release rate (n = 3). 108
Figure 3.16. Effect of changes in the intrinsic viscosity of a formulation on the in *vitro* release rate of CP (n = 3). 111

Figure 4.1. Schematic representation of the principles of oil-in-water (o/w) and water-in-oil (w/o) emulsions (redrawn from 181). 119
Figure 4.2. Schematic representation of the mechanisms by which creams show instability (adapted from 184)........ 121
Figure 4.3. Illustration of the gel network theory (adapted from 171)........ 128
Figure 4.4. *In vitro* release rate profile for CP from Dermovate® cream (n = 6) 137
Figure 4.5. Schematic illustration of the manufacturing method for the CP cream formulation 148
Figure 4.6. *In vitro* release rate profile for CP from Batch CP002 and Dermovate® cream 152
Figure 4.7. *In vitro* release rate profile for CP from Batch CP003 and Dermovate® cream 154
Figure 4.8. *In vitro* release rate profile for CP from Batch CP004 and Dermovate® cream 156
Figure 4.9. *In vitro* release rate profile for CP release from CP generic cream products and Dermovate® cream 162

Figure 5.1. Effects of stability test conditions on CP content of the cream formulation after storage at 40 ± 2˚C and 25 ± 5% RH for 1, 2, 3 and 4 weeks. 181
Figure 5.2. Effects of stability test conditions on the apparent intrinsic viscosity of the CP cream formulation after storage at 40 ± 2˚C and 25 ± 5% RH for 1, 2, 3 and 4 weeks. 182
Figure 5.3. Effects of stability test conditions on the apparent intrinsic pH of the cream formulation after storage at 40 ± 2˚C and 25 ± 5% RH for 1, 2, 3 and 4 weeks. 183
Figure 5.4. Effects of stability test conditions on the *in vitro* release rates of CP from the cream formulation after storage at 40 ± 2˚C and 25 ± 5% RH for 1, 2, 3 and 4 weeks 184
Figure 5.5. *In vitro* release rate profiles for CP from CP cream samples at times 0, 1, 2, 3 and 4 weeks after storage 185

CHAPTER ONE

CLOBETASOL 17-PROPIONATE

1.1. INTRODUCTION

It is generally believed that the introduction of hydrocortisone in the early 1950s, for the treatment of patients with inflammatory conditions such as atopic dermatitis and other eczematous eruptions was a milestone in topical therapy of dermatological conditions [1, 2]. Subsequently, various topical corticosteroids of increasing potency have been synthesised and made available to dermatologists, whilst research has led toward the concomitant development of vehicles that enhance the activity of the glucocorticoid class of drugs [3-5].

The potency levels of the corticosteroids can be measured by use of the human vasoconstrictor [6] or skin blanching [7] assay, in which blanching is assessed in healthy volunteers after topical application of a corticosteroid formulation [6-8]. The blanching assay has been a reliable technique for the determination of the relative potency of topical corticosteroids, and has been shown to be effective for the correlation of potency and clinical effectiveness [8]. Based on the vasoconstrictor assay, topical corticosteroids are ranked according to their potency level, with the weakest class, including hydrocortisone, labelled as class VII compounds and the most potent steroids, including clobetasol 17-propionate, labelled as class I compounds [1, 2].

The chemical structure of clobetasol 17-propionate (CP) is depicted in Figure 1.1. CP is a class I or super-potent synthetic di-halogenated analogue of prednisolone [1, 9-11]. CP is 1800 times more potent than hydrocortisone when potency is measured using the human skin blanching assay [1, 7] and it is currently the most potent topical corticosteroid available on the market [2, 3, 9, 10, 12-14]. Since 1973, CP has been used for the short-term treatment of patients with inflammatory and pruritic manifestations of moderate-to-severe glucocorticoid-responsive dermatoses [1, 10, 11]. CP is currently available as a 0.05% w/w CP formulation in a variety of vehicles, including cream, ointment, gel, lotion and more recently foams [15-17].

1.2. DESCRIPTION

CP is 21-chloro-9α-fluoro-11β,17α-dihydroxy-16β-methylpregna-1,4-diene-3,20-dione 17-propionate[18-21].

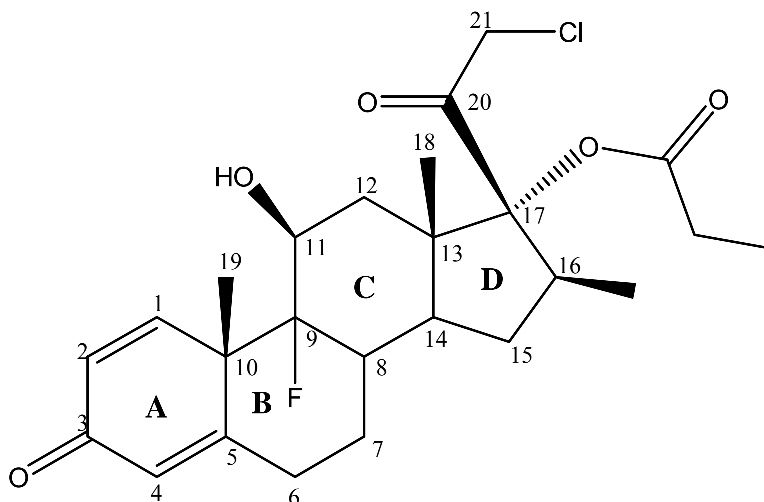


Figure 1.1. Chemical structure of clobetasol 17-propionate ($C_{25}H_{32}ClFO_5$, MW = 467.0)

CP occurs as a white, almost white or cream-coloured, crystalline powder [15, 18, 22] and is odourless [10, 14]. CP contains not less than 97.0 percent and not more than 102.0 percent $C_{25}H_{32}ClFO_5$, calculated with reference to a dry reference standard substance [22, 23].

1.3. PHYSICOCHEMICAL PROPERTIES

1.3.1. Solubility

1.3.1.1. Overview

The solubility data of CP in water, ethanol and other organic solvents at room temperature (22°C) are summarized in Table 1.1 [10, 14, 18, 20-22]. These data reveal that CP is practically insoluble in water, is sparingly soluble in ether, but is soluble in ethanol and is freely soluble in acetone, chloroform and dichloromethane [18, 20, 21, 23].

Table 1.1. Solubility of CP

Solvent	Solubility
Water	2 μg/ml
Ethanol	10 mg/ml
Ether	1 in 1000
Acetone	1 in 10
Chloroform	1 in 10
Dichloromethane	1 in 10

1.3.1.2. Solubility studies

1.3.1.2.1. Overview

The solubility of CP in propylene glycol (PG) and acetonitrile (ACN) has not yet been reported. Consequently solubility studies of CP in PG and ACN were undertaken. Since PG and ACN were used in the preparation of the receptor medium for use in *in vitro* release test studies (Section 3.2.6.4., Chapter 3) and in the preparation of the mobile phase for use in RP-HPLC studies (Section 2.3.2.8., Chapter 2) respectively, it was considered essential to evaluate the solubility of CP in these two solvents.

1.3.1.2.2. Propylene glycol (PG)

The solubility of CP in PG was determined experimentally as described and reported in Section 3.2.6.3., and was found to be 8.55 ± 2.51 mg/ml (n = 3) (Table 3.1, Section 3.2.6.3, Chapter 3).

1.3.1.2.3. Acetonitrile (ACN)

The solubility of CP in ACN was also determined experimentally following a similar procedure with minor modifications as described in Section 3.2.6.3. The procedure used when determining solubility was conducted at room temperature (22˚C) rather than at 32˚C, and samples were shaken at 120 rpm using a Model-3521 Junior Orbit Shaker (Lab-Line Instruments, Inc., Melrose Park, IL, USA). The solubility of CP in ACN was found to be 491.12 ± 0.15 mg/ml (n = 3).

1.3.2. Dissociation constant (pK_a)

CP does not have any ionisable functional groups and thus does not dissociate and therefore there is no reported dissociation constant [20].

1.3.3. Partition coefficient

The octanol/water partition coefficient (log $P_{o/w}$) of CP has been reported as 3.50 [21, 24]. The log $P_{o/w}$ is defined as the logarithm of the partitioning ratio of a substance between octanol and water, and is commonly used to quantitatively characterize the lipophilic nature of organic compounds [25, 26]. Similarly lipophilicity has been described as a molecular parameter, which may be used to describe the distribution equilibrium of a drug molecule between water and various water immiscible, lipid-like organic solvents or other solubilising media such as, for example, biological membranes [25, 27, 28].

Roberts *et al.,* [29] argued that the lipophilicity of a solute is the main determinant for solute partitioning into the *stratum corneum* from aqueous systems. Based on the log $P_{o/w}$ parameters, CP can therefore be considered more lipophilic than hydrocortisone, which has a log $P_{o/w}$ of 1.61 [25, 29], and will more than likely partition into the *stratum corneum* from aqueous based semi-solid formulations such as gels and creams faster than hydrocortisone would.

1.3.4. Melting range

CP has a melting range of approximately 195.5-197.0°C, at which temperature CP also decomposes [21-23].

1.3.5. Optical rotation

The specific optical rotation of CP in a 1% w/v solution in 1,4-dioxan is +96º to +104º, calculated with reference to a dry reference standard [22].

1.3.6. Stability

CP is unstable in the solid state and must be protected from light [22]. A solution or lotion of CP should be stored at temperatures of between 4-25°C, and should not be used near an open flame [14]. CP creams or ointments should be stored at a temperatures of between 5-30°C and the cream should not be refrigerated [14, 15].

CP gel formulations should be stored at temperatures of between 2-30°C [14, 16] and CP foams should be stored at controlled room temperatures of between 20-25°C and should not be exposed to heat or stored at temperatures exceeding 49°C [14, 17]. Since the contents of a foam product are usually under pressure, the container should also not be punctured, used or stored near heat or an open flame, or placed into a fire or incinerator for disposal [14].

1.3.7. Ultraviolet absorption spectrum

The ultraviolet (UV) absorption spectrum of CP, determined experimentally in a binary mixture of ACN:water (50:50) is depicted in Figure 1.2. The UV absorption spectrum was generated using a Model-GBC 916 UV-VIS Double Beam Spectrophotometer (GBC Scientific Equipment Pty. Ltd, Melbourne, Victoria, Australia), with the scanning range and speed set at between 200-800 nm and 600 nm/min, respectively.

The data revealed that CP has a wavelength of maximum absorption (λ-max) of 240 nm. Despite the use of different solvent systems, the absorption spectrum of CP obtained in these studies using ACN:water (50:50) as a vehicle was similar to that previously reported in the literature using methanol as the solvent or vehicle [21].

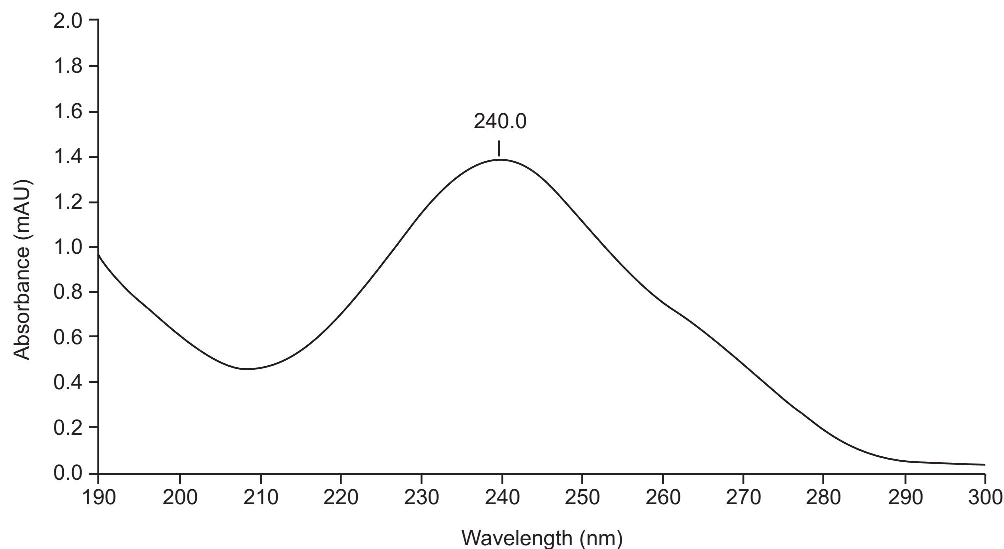


Figure 1.2. UV absorption spectrum of CP in ACN:water (50:50)

1.3.8. Infra-red Absorption Spectrum

The infra-red (IR) absorption spectrum of CP was determined using potassium bromide (KBr) pressed disks and the resultant spectrum is shown in Figure 1.3 [22]. The IR absorption spectrum of CP shows principal peaks at wave-numbers 1666, 1612, 1724, 1063 and 1010 cm^{-1} [20] and the relevant band assignments determined using theoretical concepts [30] are summarized in Table 1.2.

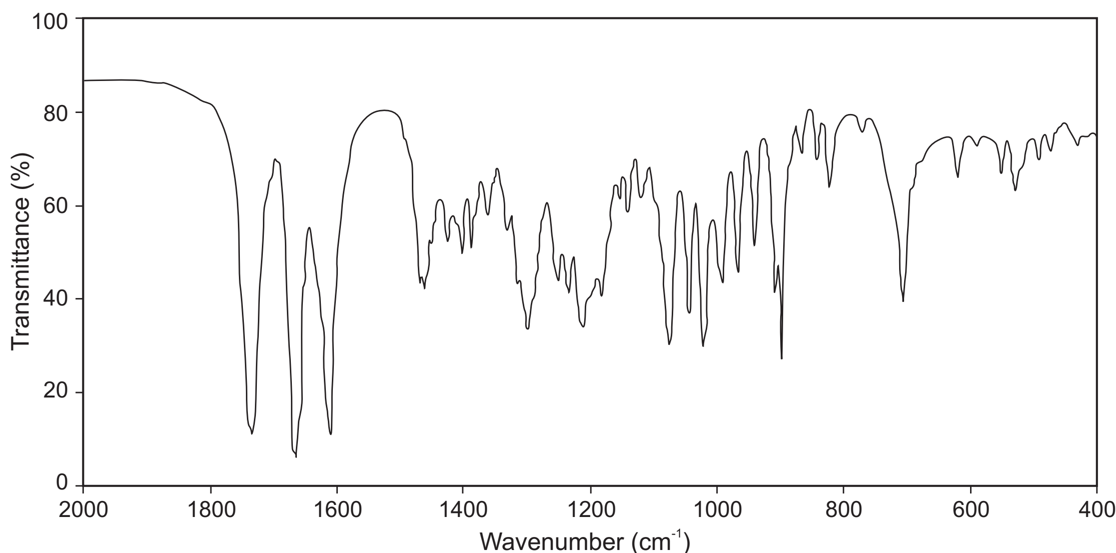


Figure 1.3. IR absorption spectrum of CP (adapted from 22)

Table 1.2. Major infra-red band assignment for CP

Band position (cm^{-1})	Assignment
1666	C=C stretching of the aliphatic non-conjugated alkene
1612	C=O stretching of the ketone
1724	C-Cl stretching of chlorine
1063	COO stretching of the ether
1010	O-H bending of the alcohol

1.3.9. Crystal Structure

Haramura *et al.,* [11] determined the crystal structure of CP using x-ray analysis and single crystals of CP, purified by recrystallization from a methanol-acetonitrile solution. They reported that the CP crystals belong to the group $P2_1$ and that the cell dimensions are a = 7.6961(3) Ǻ, b = 14.6036(5) Ǻ, c = 10.4355(5) Ǻ, β = 95.739(2) Ǻ and that the final discrepancy factor, R, is 0.038. Furthermore Harumara *et al.,* [11] postulated that in the pregna-1,4-diene-3-one skeleton, rings A, B, C (Figure 1.1) adopt planar, chair and chair conformations, respectively, whereas ring D takes on an envelope shape at C14.

Haramura *et al.,* [11] also argued that the stereochemistry of the methyl group at C16 is in the alpha (α) configuration, which may result in steric hindrance between this methyl group and the

methyl group at C13. Nevertheless, they also postulated that the non-bonded C18 to C23 distance of 4.103 Å is indicative of the fact that such steric hindrance may be relieved.

According to Haramura *et al.,* [11] the distance between the C20, carbonyl carbon and the O25 carbonyl oxygen is 2.742 Å. The torsion angle, C17-O24-C25-O25 of -7.2º reveals that this part of the molecule deviates significantly from the plane. The non-polarity of the CP is presumably due to the C20-O25 interaction [11].

Haramura *et al.,* [11] also reported that there are seven intermolecular interactions within the CP molecule for which the geometrical parameters indicate that the interactions are due to hydrogen bonds. Moreover Haramura *et al.,* [11] argued that there is a single intermolecular oxygen bond between the hydroxyl group and the carbonyl group attached to ring A.

1.4. STEREOCHEMISTRY AND STRUCTURE ACTIVITY RELATIONSHIP

All topical corticosteroids have a basic skeletal structure consisting of a fully reduced phenanthrene ring system fused to a five-membered ring, giving rise to a cyclopentanoperhydrophenanthrene nucleus, which is comprised of three six-membered and one five-membered rings [31]. A further common attribute of the corticosteroid class of drugs is the presence of methyl functional groups attached at positions 10, 13, 18 and 19 respectively (Figure 1.1).

The four rings of the corticosteroid skeleton do not exist in a flat place and the structure has no elements of symmetry with each of the 19 positions being chemically distinct from each of the other positions in the structure [31]. Furthermore, the corticosteroid skeleton is a rigid structure, and it has been suggested that small changes in the position of a substituent usually results in a significant change in the biological activity of the molecule [31].

Over the years, research has focused on strategies to optimise the potency and, in particular, the anti-inflammatory and immunosuppressive capacity of the corticosteroids, while minimizing adverse events and unwanted side effects associated with their use [9]. Although modifications to

the basic structure have led to the synthesis of topical corticosteroids with greater potency, these new highly potent molecules are often associated with a greater potential to precipitate adverse reactions to the molecule on administration [9].

As with all corticosteroids, CP consists of the basic four-ring 21-carbon structure. The 4,5 double bond in ring A in addition to the 3-ketone group are essential for anti-inflammatory activity, while the presence of a 1,2 double bond increases the glucocorticoid activity relative to mineralocorticoid effects [9, 18, 31, 32]. The 9α-fluoro group in ring B enhances both glucocorticoid and mineralocorticoid activity whereas the 11-hydroxy group in ring C is essential for anti-inflammatory and glucocorticoid activity but not for mineralocorticoid effects [9, 18, 32].

The addition of a methyl functional group at position C16 in ring D eliminates mineralocortucoid activity, while the presence of a propionate ester at position C17 and a chlorine atom at position C21 increases topical activity due to a considerable increase in the lipophilicity of the molecule [9, 18, 32]

1.5. CLINICAL PHARMACOLOGY

1.5.1. Mode of action

1.5.1.1. Overview

The physiological activity of corticosteroids can be divided into two broad categories, *viz.,* mineralocorticoid effects that control electrolyte and fluid balance in the body and glucocorticoid effects that influence carbohydrate, fat and protein metabolism [18, 33]. Although compounds that lack the unwanted mineralocorticoid activity have been produced, to date it has proved impossible to dissociate the glucocorticoid properties of synthetic topical corticosteroids from their anti-inflammatory activity [33].

Topical corticosteroids are generally believed to have anti-inflammatory, antiproliferative, antipuritic, and vasoconstrive properties [34]. However, it is through their anti-inflammatory action that these agents are thought to exert their main therapeutic effects in a wide range of acute and chronic inflammatory diseases, including most steroid-responsive dermatoses, irrespective of aetiology [35].

1.5.1.2. Anti-inflammatory action

The precise mechanism by which CP and other topical corticosteroids exert their anti-inflammatory effect in the treatment of steroid-responsive dermatoses is still uncertain [11, 15, 36]. However it is generally believed that at a cellular level, corticosteroids bind to specific glucocorticoid receptors (GR) that are 777 amino acid protein members of the superfamily of ligand-regulated nuclear receptors [9]. The GR have a modular structure and their principle functions of transactivation, DNA binding and ligand binding are localised to specific domains in that structure [36]. The GR are located and maintained in the cytoplasm as inactive multi-protein complexes of two heat shock proteins (hsp90), which promote glucocorticoid binding but prevent binding of the GR to DNA [9, 36].

The binding of a steroid to the GR is followed by dissociation of the hsp90 after which the glucocorticoid-GR complex migrates into the nucleus of the cell and binds to DNA at specific regions known as the glucocorticoid response elements on certain genes [34]. Successful binding results in an increase in the production of lipocortin-1 [36]. Lipocortin-1 is a protein that has been reported to belong to the annexin superfamily [9, 36], and its main function is to inhibit the activity of phospholipase A_2 directly, thereby decreasing the production of pro-inflammatory prostaglandins, leucotrines, thromboxanes and leucocyte migration [36].

Apart from the direct regulatory effect on gene transcription, it has also been reported that topical corticosteroids can regulate transcription of other transcription factors indirectly [34]. In particular, corticosteroids have been shown to increase cellular levels of an inhibitory nuclear factor (IκBα) by stimulating the expression of the IκBα gene [37]. IκBα then diffuses into the cytosol and binds to the nuclear factor-κ B (NF-κB) thereby preventing translocation of NF-κB

to the nucleus and suppression of various gene products regulated by NF-κB, such as the cytokines and adhesion molecules [37, 38]. Since NF-κB activates various immuno-regulatory genes in response to pro-inflammatory stimuli, the inhibition of its activity can be a major contributing factor in the anti-inflammatory activity of the glucocorticoids [38]. In this way, corticosteroids may affect the transcription of genes that do not contain a glucocorticoid-responsive receptor [34].

Topical corticosteroids have also been shown to inhibit the transcription of various pro-inflammatory cytokine genes involved in skin disease such as interleukin (IL)-1, IL-2, IL-6, interferon gamma (IFN-γ), and tumour necrosis factor-alpha [34]. Furthermore, corticosteroids appear to stimulate lymphocyte expression of genes for anti-inflammatory cytokines such as transforming growth factor-β and IL-10 [34]. Through regulation of cytokine production, it has been suggested that corticosteroids probably play a role in rebalancing the T-helper cell type 1 (T_H1) to T_H2 lymphocyte ratio in skin lesions [34]. The anti-inflammatory effects that have been associated with corticosteroid treatment include inhibition of capillary dilation and dermal oedema and the suppression of endothelial cell and lymphocyte function [34].

1.5.1.3. Immuno-suppressive effects

Corticosteroids have also been reported to inhibit the proliferation of various cell types, such as the T-lymphocytes [34]. However the anti-proliferative effects have not yet been clearly defined, although it has been suggested that the effects are more than likely a consequence of the blockade of cytokine expression and the suppression of cytokine effects [34]. Furthermore it has been reported that some of the anti-proliferative effects of the corticosteroids may be mediated through lipocortins which act as secondary messengers for corticosteroid compounds [34]. IL-10 and transforming growth factor-β1 have been shown to potentiate the inhibitory effects of the corticosteroids on T-lymphocyte proliferation [34].

1.5.1.4. Anti-mitotic and vasoconstrictive effects

It has also been reported that topical corticosteroids have anti-mitotic and vasoconstrictive activities in addition to their anti-inflammatory and immuno-suppressive actions [9]. The anti-mitotic effects of the corticosteroids are secondary to the general reduction of protein synthesis and may explain the therapeutic action of the corticosteroids in scaling dermatoses such as psoriasis [9]. Similarly, the vasoconstrictive activity of the corticosteroids as demonstrated in vascular beds may contribute to the anti-inflammatory activity of topical corticosteroids. Although the mechanism by which vasoconstriction is induced is not yet completely elucidated, it is thought to be related to the inhibition of natural vasodilators such as histamine, bradykinins and prostaglandins [9].

1.5.2. Indications

CP is usually indicated in the management of significant inflammation of the skin that is naturally thick or thickens as a result of disease, to such an extent that penetration of a less potent topical corticosteroid would be poor, thereby making treatment difficult or ineffective [39]. Typical indications for CP use include the treatment of psoriasis of the body, palmoplanter psoriasis, lichen planus, lichen simplex chronicus, lupus erythematosus, and acute exacerbations of atopic dermatitis in adults [39].

CP is also indicated in the management of severe and acute attacks of any type of eczema and chronic eczema, especially of the hands and feet where hyperkeratosis becomes an issue, chronic hyperkeratotic sporiasis of the hands and feet, localised bullous disorders, keloids, pretibial myoedema, vitiligo and also in the suppression of reaction after cryotherapy [20]. CP is occasionally used for the management of light or photo-sensitivity reactions [20].

1.5.3. Contra-indications

CP preparations are contraindicated in patients with a known history of hypersensitivity to CP and other related compounds or any excipients that may be used in the respective formulations

[15-17]. The use of CP in neonates and paediatric patients under the age of 12 is contraindicated due to the potent local and more importantly systemic effects should they be absorbed [1, 10, 15, 20]. CP formulations should not be used in the treatment of acne vulgaris, rosacea or perioral dermatitis or as monotherapy in the treatment of widespread plaque psoriasis [10, 14, 20].

CP should also not be used for the treatment of cutaneous infections caused by viruses, bacteria or fungi [20]. If concomitant skin infections develop during CP therapy, appropriate antifungal or antibacterial therapy must be initiated [14] and should the infection not respond to therapy, CP should be discontinued until the infection is adequately controlled [14]. Skin infestations such as scabies must not be managed with CP as exacerbations of the disease may occur or the disease may be disguised [20]. The use of CP on the face, groin, or axillae is also contraindicated [20].

1.5.4. Adverse Effects

1.5.4.1. Overview

Since the commercial introduction of CP in 1973, primarily for the treatment of specific dermatoses (Section 1.5.2), indications for the prescription of this potent corticosteroid have been modified due to potential deleterious side effects that have been reported [40]. Serious side effects nearly always follow the dispensing of uncontrolled repeat prescriptions [41] and have been reported more frequently for the treatment of psoriasis than in eczema, probably due to the fact that the parakeratotic keratin of psoriatic skin is more permeable than normal keratin [20]. The adverse effects are noticeable as localised skin reactions occurring at the site of application and generalised adverse effects arising from systemic absorption of the compound [2, 9].

1.5.4.2. Local side effects

Burning, stinging, irritation and itching sensations have been reported as the most frequent local cutaneous reactions in 1% of patients treated with CP creams in controlled clinical trials [15]. Less frequent local side effects include, cracking of the skin, erythema, folliculitis, numbness of fingers, skin atrophy and telangiectasia [15]. The use of CP may exacerbate pre-existing or

coexistent dermatoses, such as rosacea, perioral dermatitis, tinea infections and resistant psoriatic lesions [15].

1.5.4.3. Systemic side effects

Systemic side effects are usually not common but can arise when locally applied CP preparations are absorbed through the skin and enter the general circulatory system [2]. The occurrence of systemic absorption is rare and side effects are often clinically insignificant, particularly if the formulation is used on an *ad hoc* basis [2]. The greatest risk of systemic side effects occurs when large amounts of CP formulations are used over very large areas of the body for prolonged periods of time [2, 20].

CP has the potential to cause suppression of the hypothalamic-pituitary (HPA) axis, Cushing's syndrome, diabetes and hypertension [20]. Suppression of the HPA-axis has occurred following administration of topical dosages of as low as 2 g of the 0.05% w/w cream, ointment and gel or 7 g of the 0.05% w/w foam on a daily basis [15-17]. Cushing's syndrome has been reported in infants and adults following the prolonged use of topical CP formulations [15].

HPA-axis suppression, Cushing's syndrome, linear growth retardation, delayed weight gain and inter-cranial hypertension have also been reported in children being treated with topical corticosteroids [15]. Manifestations of adrenal suppression in children include low plasma levels and an absence of response to adrenocorticotropic hormone (ACTH) stimulation, whereas those of intracranial hypertension include bulging fontanelles, headaches and bilateral papilledema [15-17].

1.5.5. High risk groups

1.5.5.1. Pregnancy

The teratogenic potential of CP in humans is still unknown [14], however reproductive studies in mice and rabbits using subcutaneous dosages of CP of up to 1 mg/kg and 10 µg/kg, respectively

revealed substantial harm, including cleft palate, skeletal immaturity, increased rates of still birth and fetal resorption [14]. The administered doses were approximately 1.4 and 0.05 times those that would be typical of topical doses in humans of either the CP cream or ointment formulations [15-17].

Since CP can undergo percutaneous absorption [14] the data obtained from reproductive studies suggest that the use of CP in pregnancy should be avoided [14]. It is also worth noting that although no study has shown the absolute teratogenicity of CP, other potent corticosteroids have been shown to be teratogenic in animals following topical application [14]. Therefore the use of CP in pregnancy is contraindicated.

1.5.5.2. Lactation

Corticosteroids that are administered systemically are reportedly secreted in human milk and could, therefore, suppress growth and interfere with endogenous corticosteroid production in breast feeding infants [15]. It is unknown whether topical application of corticosteroids may result in sufficiently high systemic levels following absorption to produce detectable quantities in human milk. However since many drugs are excreted in breast milk, caution should be exercised when CP cream, ointment, gel or foam formulations are administered to nursing mothers [15-17].

1.5.5.3. Paediatric use

The safety and effectiveness of CP cream, ointment, gel and foam formulations in paediatric patients has not yet been established [15]. Consequently the use of CP in patients under the age of 12 years of age is not recommended. Paediatric patients are at a greater risk of HPA-axis suppression and Cushing's syndrome than adults when they are treated with topical corticosteroids [15]. This is a result of the fact that paediatric patients have a higher skin surface area to body mass ratio than adults [15-17].

1.5.5.4. Geriatric use

In clinical studies in which CP cream, ointment, gel and foam formulations were used, a sufficient number of patients over the age of 65 years were not included in the study to categorically conclude whether geriatric patients respond differently to topical steroid therapy than younger patients [15-17].

While other clinical experience has not revealed age-related differences in response, dosages should generally be titrated carefully in geriatric patients by initiating therapy at the low end of the dose range [15]. The greater frequency of decreased hepatic, renal or cardiac function and concomitant disease drug therapy suggest that care must observed when treating elderly patients with topical corticosteroid products [14]

1.6. PHARMACOKINETICS

1.6.1. Dosage and Administration

CP cream, ointment and gel should be applied sparingly as thin films and should be rubbed gently into the affected area twice daily, preferably in the morning and evening [14, 15]. CP foams and solutions should be applied to the affected areas of the scalp twice daily, in the morning and evening [14, 17]. Some patients may respond initially to once daily or intermittent therapy, for example, twice daily for three days per week [14].

The use of CP should be discontinued and a less potent topical corticosteroid preparation substituted as soon as it is clinically feasible to alter therapy [10]. CP dosage should not exceed 50g of a CP 0.05% w/w cream, ointment, gel and foam or 50 ml of CP 0.05% w/v lotion per week and the extended duration of a course of CP therapy should not exceed 14 days [10]. It has been reported that many clinicians have indicated that prolonged CP therapy may be necessary in rare cases in patients with resistant dermatological conditions, and careful monitoring of their use in these patients is essential [10, 14].

1.6.2. Absorption

Percutaneous penetration and absorption of CP varies between individuals and can be altered by using a variety of vehicles [14] and/or percutaneous penetration enhancers [42]. Absorption can be increased by use of occlusive dressings [14, 41, 43] and use of percutaneous penetration enhancers such as for example surface active agents, cyclodextrins or pyrrolidones [44, 45]. Following topical application of normal doses of CP to most areas of healthy skin, only small amounts of the drug reach the dermis and subsequently the systemic circulation [10]. Nevertheless, systemic absorption may be increased when the usual dosage is exceeded or when the skin is inflamed or diseased [10].

Mean peak plasma concentrations of CP of 0.63 ng/mL have been reported in one study, eight (8) hours following a second dose of 30 g of CP that was applied thirteen (13) hours after an initial dose of the 0.05% w/w CP ointment in healthy individuals with healthy skin. [14]. Mean peak plasma concentrations of CP were slightly higher and occurred ten (10) hours after a second dose of CP when a 0.05% w/w CP cream was administered to subjects [14]. Mean peak plasma concentrations of approximately 2.3 and 4.6 ng/mL respectively have been reported to occur approximately three (3) hours after a single application of a 25 g dose of a 0.05% w/w ointment in patients with psoriasis or eczema, respectively [14].

1.6.3. Distribution

Advances in vehicle technology have enhanced the distribution of topical corticosteroids within the skin structure [39] as illustrated by the results of an *in vitro* trial comparing the amount of drug recovered after the application of 0.05% w/w CP lotion, cream or emollient cream [39]. Human skin samples sectioned by surgical excision were treated with different formulations and the levels of CP in the skin were measured after centrifugation and extraction of the skin sample [39]. It was found that significantly more CP was recovered in the epidermis, including the *stratum corneum,* following administration of the lotion formulation as compared to that following administration of a cream [39]. Similarly significantly more drug was recovered from

the epidermis and dermis following administration of the lotion as compared to that following administration of an emollient cream [39].

1.6.4. Elimination

The absorption of CP into the systemic circulation seldom occurs (Section 1.6.2). Nevertheless when percutaneous penetration and subsequent absorption of CP occurs, it has been reported that the same metabolic pathways that are active when clearing systemically administered corticosteroids are involved in the removal of the topically administered corticosteroid [14]. Although the systemic metabolism of CP has not been fully characterized or quantified [14], it has been reported that the small amount of CP that may be absorbed following topical application is metabolized in the liver [21] and excreted in the bile [14] and in the urine [14, 21].

1.7. CONCLUSIONS

The description, physicochemical characteristics, structural activity relationships, clinical pharmacology and pharmacokinetics aspects of clobetasol 17-propionate (CP) have been presented. CP is chemically known as 21-chloro-9α-fluoro-11β, 17α-dihydroxy-16β-methylpregna-1,4-diene-3,20-dione 17-propionate. The empirical formula of CP is $C_{25}H_{32}ClFO_5$, and its molecular weight is 467.0 g/mole. CP is a synthetic di-halogenated analogue of prednisolone and occurs as a white or almost white or cream-coloured crystalline powder which is odourless.

Based on the human skin blanching assay CP has been labelled as a super-potent or class I topical corticosteroid and is one of the most potent topical corticosteroids currently available on the market. CP is marketed in a variety of vehicles such as creams, ointments, gels, lotions and foams each containing 0.05% w/w of the corticosteroid.

CP does not have any ionisable functional groups and thus does not dissociate. The lack of such functional groups results in CP having a relatively high octanol/water partition coefficient or log $P_{o/w}$ of 3.5, making it relatively more hydrophobic than other corticosteroids such as for example

hydrocortisone. CP is therefore practically insoluble in water, but soluble in water-miscible solvents such as acetonitrile or ethanol and freely soluble in some water-immiscible solvents such as acetone, chloroform, and dichloromethane. The hydrophobic nature of CP suggests that CP will partition into the *stratum corneum* from aqueous based topical formulations such as creams and gels at a much faster rate than hydrocortisone.

The melting range of CP is reported to be approximately 195.5-197.0°C at which temperature it also decomposes, while its specific optical rotation in a 1% w/v solution in 1,4-dioxan is between +96º to +104º. The ultraviolet (UV) absorption spectrum of CP reveals that CP has a wavelength of maximum absorption (λ-max) of 240 nm.

CP is unstable in the solid state and must be protected from light. Solutions and semi-solid formulations containing CP should be stored at suitable temperatures of between 4-25°C for solutions, 5-30°C for cream and ointments, 2-30°C for gels and 20-25°C for foams.

CP has structural features that are common to all topical corticortisteroids including 3-keto and 11-hydroxy groups, which are essential for anti-inflammatory activity, a 1,2 double bond and a 16-methyl group, which increases its glucocorticoid activity relative to the unwanted mineralocorticoid effects. Functional groups that are unique to CP include a 9α-fluoro group that enhances glucocorticoid and mineralocorticoid activity and a 17-propionate ester and a 21-chlorine group, which increases the lipophilicity of the drug and hence its topical activity.

The mechanism of action of CP may involve anti-inflammatory, immuno-suppressive, anti-mitotic and vasoconstrictive effects. The anti-inflammatory action of CP may be due to its binding to specific glucocorticoid receptors (GR), which through a cascade of events decreases the production of pro-inflammatory prostaglandins, leucotrines and thromboxanes and leucocyte migration. The immunosuppressive effects of CP more than likely involve the blockade of cytokine expression and the suppression of cytokine effects. Whereas the anti-mitotic effects of CP may be secondary to a general reduction of protein synthesis the vasoconstrictive effects may be due to the inhibition of natural vasodilators such as histamine, bradykinins and/or prostaglandins.

CP is indicated for the treatment of significant inflammation of skin that is naturally thick or thickens as a result of disease and where penetration of a less potent topical corticosteroid would likely be poor. Some of the classical skin diseases that may require treatment with semi-solid formulations of CP include psoriasis of the body, palmoplanter psoriasis, lichen planus, lichen simplex chronicus, lupus erythematosus, and acute exacerbations of atopic dermatitis in adults.

Preparations containing CP are contraindicated in neonates and paediatric patients under the age of 12 and in pregnant women, and caution should be exercised when CP formulations are administered in nursing mothers as well as geriatric patients. As with most potent topical corticosteroids CP may be associated with local and very seldom systemic side effects. The most frequent local cutaneous reactions include burning, stinging, irritation and itching sensations and although systemic side effects are very rare, they may include the suppression of the hypothalamic-pituitary (HPA) axis, Cushing's syndrome, diabetes and hypertension.

CP semi-solid formulations should normally be applied sparingly as a thin film and rubbed gently into the affected area twice daily preferably in the morning and evening. CP dosage should not exceed 50g of CP 0.05% w/w cream, ointment, gel and foam or 50ml of CP 0.05% w/v lotion per week and the extended duration of a course of CP therapy should not exceed 14 days. Percutaneous penetration of CP is very rare when CP formulations are used appropriately as described above. However in the unlikely event of CP absorption into the circulatory system CP is likely metabolised in the liver and excreted in the bile and urine.

It is therefore, evident that CP has physicochemical and pharmacological properties, such as a relative high lipophilicty and local anti-inflammatory activity, respectively, which make the drug suitable for incorporation into semi-solid formulations, such as creams, ointments, gels and foams for topical administration. Consequently, CP was selected as an ideal candidate for inclusion into a generic cream formulation developed and assessed in these studies.

CHAPTER TWO

DEVELOPMENT, OPTIMIZATION AND VALIDATION OF AN HPLC METHOD FOR THE ANALYSIS OF CLOBETASOL 17-PROPIONATE IN SEMI-SOLID DOSAGE FORMS

2.1. INTRODUCTION

The quantitative analysis of clobetasol 17-propionate (CP) in semi-solid formulations has primarily been accomplished using reversed-phase high performance liquid chromatography (RP-HPLC) with ultraviolet detection [3, 10, 46-48]. Several other analytical techniques that have been used for the quantitation of CP in dosage forms include normal-phase HPLC, ultraviolet spectroscopy, liquid chromatography-mass spectrometry (LC-MS) and liquid chromatography-mass spectrometry-mass spectrometry (LC-MS-MS or LC-MS^n) [47].

The Food and Drug Administration (FDA) has published a guidance document [49] in which it recommends that an appropriate, specific and sensitive analytical procedure be used to analyse and determine drug concentrations and the amount of drug released from dosage forms during *in vitro* release studies [49]. It has been reported that a major difficulty encountered in the analysis of semi-solid dosage forms is the potential interference due to formulation adjuvants and preservatives that are usually present in what are relatively complex formulations [50]. Generally, only one or two components are required to be quantitated and these components must be adequately separated from formulation exicipients, which may interfere with the assay procedure [50].

RP-HPLC is a commonly used, powerful and reliable analytical tool that can be used for the *in vitro* analysis of formulations such as creams, ointments and gels that are of a complex nature, since HPLC not only provides separation and quantitative data but also has the ability to eliminate almost all interference problems [51]. The objective of these studies was therefore to develop, optimize and validate a simple, selective, sensitive, precise, accurate and linear RP-

HPLC method that is suitable for the quantitative analysis of CP in cream formulations and CP release during *in vitro* release studies.

2.2. PRINCIPLES OF RP-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 and percolates through a stationary phase, usually contained in a column [52]. Although various terms, including high-speed LC, high-efficiency LC and high-pressure LC, have been used to describe LC, high-performance liquid chromatography (HPLC) is now the generally accepted terminology [52].

In HPLC, a stationary phase is either coated onto a finely divided inert support or chemically bonded to a support material, contained within a stainless steel tube over which the mobile phase flows, thereby affecting the separation of individual components of a mixture [52, 53]. Coated phase LC uses a bulk liquid stationary phase, which is mechanically held to the support by adsorption [54], whereas in bonded phase chromatography (BPC), an organic stationary phase is chemically bonded to a support material rather than being held in place by mechanical means [55].

BPC packing materials have been reported to be more stable than coated phase materials due to the fact that a stationary phase that is chemically bound to a support cannot be easily removed or lost during normal use of the column [56]. However, the poor reproducibility of some packing materials that are commercially available has been mentioned as being one of the major disadvantages of using BPC [56]. Nevertheless, the success of BPC has virtually eliminated the use of coated stationary phases in chromatography [52].

In HPLC, the sample to be analysed is dispersed into a mobile phase and the analyte(s) of interest pass through the stationary phase by pumping the mobile phase through the stationary phase using a solvent delivery module [53, 55]. As the analyte molecules pass through the column, there is constant interaction between the solute molecules, the stationary phase and the

mobile phase [53, 55]. Separation of the various components of a mixture is reported to occur as a result of differences in equilibrium of distribution of different solute molecules in the sample undergoing analysis [53, 55].

Two principle modes of HPLC, *viz.,* normal-phase HLPC (NP-HPLC) and reversed-phase HPLC (RP-HPLC) can be distinguished by the relative differences in the nature of the stationary phases used to effect a separation in addition to the corresponding mobile phase composition and differences in the nature of the interaction of functional groups present in solute molecules with these phases [52, 53]. The nature of the functional groups of a molecule dictate the selectivity and specificity of an interaction between an analyte and the column support material or mobile phase, leading to the selectivity and specificity of a separation [53].

NP-HPLC requires the use of a polar stationary phase and a non-polar mobile phase in order to separate analyte(s) of interest [53, 57]. In NP-HPLC the stationary phase may be either an adsorbent, such as silica, or a liquid phase that is bonded to a solid support. The mobile phase may consist of either a single non-polar solvent, such as hexane, or a mixture of non-polar solvents or a non-polar solvent mixed with a small amount of a polar non-aqueous solvent, such as methanol [57].

NP-HPLC is most useful for the separation of compounds of moderate to strong polarity since non-polar solutes elute near the mobile phase front [53]. NP-HPLC is normally restricted to the separation of stereochemical isomers, diastereomers, low molecular weight aromatic compounds and long chain aliphatic compounds [53].

In contrast, RP-HPLC entails the use of a hydrophobic bonded stationary phase with a mobile phase that consists of polar solvents such as water, with or without buffers or mixtures of water and water-miscible organic solvents such as methanol and acetonitrile [53, 57, 58]. RP-HPLC is usually the first choice of method for use in most pharmaceutical applications and especially for the analysis of neutral or non-polar compounds that dissolve in water-organic solvent mixtures [59]. Since the majority of pharmaceutical compounds of interest are relatively non-polar, most HPLC analyses in pharmaceutical research are carried out using RP-HPLC techniques [57].

BPC columns for both NP-HPLC and RP-HPLC may be categorized according to the length and functionality of the organic side chain used to manufacture the column [53]. Typically carbon chains ranging in length from three (3) to eighteen (18) carbon atoms, *viz.,* C_3, C_4, C_8 and C_{18}, are chemically bonded to a silica support surface [53]. If the organic side chain is only alkyl in functionality, the resulting phase is hydrophobic and thus useful for the reversed -phase mode of analysis [53]. Consequently, stationary phases for RP-HPLC are prepared by reaction of residual silanol functional groups in a silica backbone with *n*-alkylchlorosilanes [58].

RP-HPLC is used extensively in various scientific fields *viz.,* pharmaceutical, agricultural and medical sciences, in addition to fundamental studies in the separation sciences [60]. However, despite its extensive application, the retention mechanism of molecules in RP chromatographic process has not yet been fully elucidated [56, 60]. This is more than likely due to the complexity of RP-HPLC systems, the properties of which have been reported to change dynamically with the composition of the mobile phase and type(s) of the stationary phase used in separations [61]. Nevertheless, some investigators have alluded to the fact that separation in RP-HPLC may be due to either adsorption effects or partitioning of a solute between a stationary phase and a mobile phase, or combinations thereof [53, 56].

2.3. METHOD DEVELOPMENT

2.3.1. Overview

A summary of RP-HPLC with UV detection methods that have been developed and used for the quantitative determination of CP in semisolid dosage forms is listed in Table 2.1. The data contained in these reports were used to set preliminary RP-HPLC conditions for the development of a suitable *in vitro* analytical method for the quantitation of CP in cream formulations and for the characterisation of release of CP from topical formulations developed in these studies.

Table 2.1. RP-HPLC methods used for the analysis of CP in semi-solid dosage forms

Column	Mobile phase	Flow rate (ml/min)	Detection method	Reference
µBondapak® C_{18} 300 mm x 3.9 i.d	methanol:water (75:25% v/v)	1.5	UV-240 nm	46
Nova-Pak® C_{18} 5-µm 150 mm x 3.9 mm i.d.	methanol:water (70:30% v/v)	1.0	UV-254 nm	10
Lichrospher® C_{18} 125 mm x 4.0 mm i.d.	methanol:aqueous phase pH 3.5 adjusted with 0.05% citric acid (65:35% v/v)	1.1	UV-240 nm	3
Nova-Pak® C_{18} 4-µm 150 mm x 3.9 mm i.d.	acetonitrile:water (50:50% v/v)	1.0	UV-240 nm	47
Purospher-Lichrocart® 5-µm C_{18} 250 mm x 4.0 mm i.d.	acetonitrile:water (40:60% v/v)	1.0	UV-237 nm	48
Phenomenex® Luna C_{18} 150 mm x 4.6 mm i.d.	methanol: 0.1M KH_2PO_4 pH 3: acetonitrile (10:40:50% v/v)	1.0	UV-239 nm	62
L1® 150 mm x 4.6 mm i.d.	acetonitrile:0.05M NaH_2PO_4 pH 2.5: methanol (95:85:20% v/v)	1.0	UV-240 nm	23
Spherisorb ODS® 2, 5µm, 50 mm x 4.6 mm i.d.	methanol:0.05M NaH_2PO_4 pH 2.5: acetonitrile (10:42.5:47.5% v/v)	1.0	UV-240 nm	22

2.3.2. Experimental

2.3.2.1. Chemicals

All chemicals used in these studies were at least of analytical reagent grade. HPLC-grade solvents, *viz.,* methanol (UV cutoff of 215 nm) and acetonitrile (UV cutoff of 200 nm) were purchased from Romil Ltd. (Waterbeach, Cambridge, UK). HPLC-grade water was prepared using a Milli-RO® 15 water purification system (Millipore Co., Bedford, MA, USA) that consisted 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® 40 stack filter (Millipore Co., Bedford, MA, USA) prior to use.

Clobetasol 17-propionate (CP) was purchased from Symbiotec Pharmalab P.V.T. Ltd (Pigdamber, Maharastra, India). Betamethasone 17-valerate (BV) was purchased from Sigma-Aldrich SA (Pty) Ltd (Brackenfell, Western Cape, RSA) and Dermovate® cream (Glaxo Wellcome SA (Pty) Ltd, Midrand, Gauteng, RSA) was purchased from Wallaces Pharmacy (Grahamstown, Eastern Cape, RSA).

2.3.2.2. Instrumentation

Two modular HLPC-UV chromatographic systems, *i.e.,* System 1 and System 2, were used in these studies. System 1 was used for the development, optimization and validation of the analytical method and System 2 for the characterisation of CP content and release in topical formulations during product development and assessment studies. A mini-revalidation of the analytical method was undertaken prior to using System 2 for quantitative purposes.

System 1

The modular HPLC-UV system consisted of a Beckman Model 112 Solvent Delivery Module (Beckman Instruments, Inc., San Ramon, CA, USA), a WISP™ Model 712 Autosampler (Millipore® Waters Associates, Milford, MA, USA) and a linear UV-100 detector

(Spectrachrom, NV, USA) set at λ = 240 nm. Data acquisition was performed using a dual-pen Model 561 strip chart recorder (Perkin-Elmer, Maywood, IL, USA) and separation was achieved on a Nova-Pak® 60 Å C_{18} 4-µm (3.9 i.d. x 150 mm) cartridge column (Millipore® Waters Associates, Milford, MA, USA).

System 2

The modular HPLC-UV differed from System 1 in that it consisted of an Isochrom LC dual piston solvent delivery module (Spectra-Physics, San Jose, CA, USA) and data acquisition was performed using an SP-4600 Integrator (Spectra-Physics, San Jose, CA, USA). All the other system components were the same as those used for System 1.

2.3.2.3. UV detection of CP and internal standard

The ideal detector for HPLC should be sensitive, respond to all solutes universally or have predictable specificity, have a linear response over several orders of concentration, possess a low dead volume, be non-destructive, be insensitive to changes in temperature and mobile phase velocity changes, operate continuously and be reliable and convenient to use [52]. Unfortunately, no single detector is able to satisfy all these criteria and consequently analytical methods have to be optimized for specific pieces of equipment.

In most cases, analytical method development for HPLC commences by use of ultraviolet (UV) detection with either a variable-wavelength spectrophotometric or diode-array detector [63]. UV detector technology has developed over the years and is associated with good sensitivity and linearity and can provide an adequate response for most samples, except when samples have little or no UV absorbance, analyte concentrations are too low for UV detection, sample interference is important, and/or qualitative structural information about a molecule is required [63].

It has been suggested that the manner in which a UV detector is used may affect the relative response of sample components and could potentially interfere with the selectivity, sensitivity

and baseline noise of an analytical method [63]. The achievement of adequate sensitivity is primarily dependent on the selection of an appropriate wavelength for analysis, which is chosen from knowledge of the UV absorption spectrum of the individual sample components to be evaluated [63]. The UV absorption spectrum of an analyte should therefore be evaluated prior to the development of a comprehensive HPLC method for analysis of that compound [63].

The analysis of CP by RP-HPLC has been accomplished using UV detection at 237 nm [64], 239 nm [62], 240 nm [3, 46, 47] and 254 nm [10]. An evaluation of the UV absorption spectrum of CP (Figure 1.2, Section 1.3.7.) reveals that the wavelength of maximum absorption (λ_{max}) is 240 nm. Consequently, the eluent in these studies was monitored at 240 nm. The detection of the internal standard does not have to occur at the λ_{max} of the compound in question, since enough material may be added to a sample to achieve an adequate response from the molecule, at the desired attenuation used for analysis of the compound of interest.

2.3.2.4. Column selection

It has been suggested that the column is the heart of any HPLC separation procedure [65, 66]. It follows therefore that the availability of a stable, high-performance column is vital for the development of a rugged, reproducible and reliable analytical method. The stability of a column is especially important in method development since once a desired separation is achieved the column characteristics should remain unchanged for as long as possible and excellent column stability minimizes the need for further adjustment of the separation conditions or replacement of a column during any specific application of the method [65].

Commercially available columns may differ in their reproducibility when purchased from different suppliers and in many cases differences exist in columns supplied from a single supplier [66]. Such differences can have a serious impact on developing a reproducible and useful HPLC method of analysis for a particular compound [65]. The decision to use a particular HPLC stationary phase is based on physicochemical characteristics such as the solubility, molecular weight and ionic nature of the analyte of interest, in addition to the column packing material and dimensions of the column [66, 67].

The majority of column materials used for HPLC separations make use of a silica particle base or support, although there are a number of columns that are now available that are packed with porous-polymeric support materials [65, 66, 68]. However, the majority of RP-HPLC separation techniques use silica-based bonded-phase chromatographic (BPC) columns [58, 66]. Such columns are made by reacting mono-functional chorodimethylsilanes with available silanol functional groups of a stationary phase material [58, 65, 66].

A variety of different alkyl and substituted alkyl silica materials are formed as a consequence of these reactions. Some of the examples of the types of bonded phase materials that have been produced include n-octadecylsilane (ODS or C_{18}), n-octylsilane (C_8), phenyl, dimethylsilane and dimethylamino functionalities [65, 66]. Separation of compounds on BPC columns in RP-HPLC, primarily depend on molecular interactions that occur between a solute and the components of the mobile and stationary phases being used for that separation [69]. The underlying mechanisms of a separation include hydrophobic and solute-solvent interactions that result in increased retention of compounds with large C-H or hydrophobic surface areas and shorter retention times for compounds containing polar or hydrophilic functional groups such as hydroxyl groups (OH) [68].

The retention process in RP-HPLC involves partitioning of solutes between a mobile phase and the bonded alkyl groups of a stationary phase that contain organic solvents extracted from the mobile phase in use [68]. The retention of an analyte may be affected by the composition of the mobile phase and the structure of the stationary phase. Consequently, the length and surface density of alkyl chains in a stationary phase have been reported to be the primary determinant of the phase ratio between the mobile and stationary phases of a column, the orderliness of alkyl chains in that column and the solvent content (or polarity) of the stationary phase [68] .

The stability of silica-based BPC columns depends on the types of silica support and bonded phases used to manufacture the column, the mobile phase pH and the type of the buffer and organic modifier(s) used to prepare the mobile phase. The loss of silane-bonded phases can occur due to hydrolysis of the siloxane bonds (Si-O-Si) that bind the silane to the support backbone of

the column. Furthermore, degradation of supports is accentuated at high temperatures, low pH and in highly aqueous mobile phases [65].

The most commonly used columns that have been reported for the analysis of CP in dosage forms are octadecylsilyl (C_{18}) based stationary phases[3, 10, 46-48, 70], which may be due to the fact that CP is a highly hydrophobic drug with an octanol-water partition coefficient of 3.5 [47]. Therefore, retention and separation of CP on such columns can be easily accomplished using a mixture of water and water-miscible solvents such as methanol and/or acetonitrile. Other common features of these columns are the average particle size of the packing materials, which are ≤ 5 µm, and the column dimensions, which are 150 mm (L) x 3.9-4.6 mm (i.d.) (Table 2.1, Section 2.3.1). It has been suggested that the retention of a compound on an RP-HLPC analytical column may be affected by the column packing materials as well as the column dimensions [65, 66].

It is generally believed that the efficiency of packed RP-HPLC columns is enhanced with a decrease in the particle diameter of the stationary phase, such that the smaller the particles the better the resolution and sensitivity that can be obtained with the column of interest [65, 66]. Most RP-HPLC columns are packed with particles with sizes ranging between 3-10 µm and although smaller particles will generally result in high theoretical plate numbers (Section 2.3.2.5) and better sensitivity, they more than likely lead to high column back-pressures than the packing materials with large sizes [65, 66]. Consequently, RP-HPLC columns packed with particles with an average diameter of 5 µm may represent a good compromise in terms of column efficiency, back-pressure and lifetime of use [65].

As far as column dimensions are concerned, it has been suggested that columns of lengths of between 100-1500 mm may be used for HPLC analysis, although most of the newer equipment allows for the use of columns with maximum lengths of only 250 mm [66]. Kromidas [71] suggested that the use of a longer column may improve resolution at the expense of retention times and column back-pressures. Similarly, the efficiency of well-packed columns of small particles of ≤ 10 µm in size has been reported to increase with an increase in the column internal diameter (i.d.) [66]. However, HPLC columns with i.d. of between 4-5 mm have been reported to

represent a good compromise of performance, convenience, amount of mobile phase used and column packing required in analytical applications [66].

A Nova-Pak® 4 µm (150 mm x 3.9 i.d.) cartridge column, packed with dimethyl octadecylsilyl (C_{18}) bonded amorphous silica was therefore selected for testing and thereafter for the development of an analytical method for the determination of CP in dosage forms.

2.3.2.5. Column efficiency

The efficiency of a chromatographic column can be expressed quantitatively as the number of theoretical plates (*N*) for a test substance conducted under favorable conditions and may be calculated using equation 2.1 [72].

$$N = 5.54\left(\frac{t_R}{W_{1/2}}\right)^2$$ **Equation 2.1**

Where,

t_R = retention time of a test peak

$W_{1/2}$ = peak width at half peak height.

The value obtained for *N* for a column increases as the mean diameter of the packing material decreases, due to an increase in the associated surface area of the stationary phase available for interaction with the mobile phase and the analyte of interest [57]. Particles with an average diameter of 10 µm are considered standard for RP-HPLC [57].

Such material provides excellent efficiency with an estimated theoretical plate count of approximately 8000 plates per column in addition to the ability to use relatively high solvent flow rates without exceeding the pressure limits of a system [57]. Packing particles of 5 µm diameter yield an approximate doubling of the column efficiency as compared to 10 µm packing materials but yield substantial increases in system back pressures at given mobile phase flow rates [57].

The efficiency of a column as measured by *N* is best determined with an ideal test system, rather than with the analyte for which the method is being developed and the conditions used during method development [72]. Column test systems may be comprised of a small, neutral test compound such as toluene or naphthalene, a flow rate of 1 ml/min and a mobile phase with a viscosity (η) of less than 1 cP, such as compositions of 0 to 100% acetonitrile-water mixtures maintained at temperatures of less than 20˚C [72].

The efficiency of the column selected for use in these studies, a Nova-Pak® C_{18} 4 µm (150 mm x 3.9 i.d.) was interrogated by injection (n = 6) of a test mixture containing uracil, acetophenone, benzene, toluene and naphthalene onto the column at room temperature (22˚C). The separation was achieved using a mobile phase composition of acetonitrile-water (65:35) at a flow rate of 1 mL/min. Detection of the compounds was conducted at 254 nm and a typical chromatogram of the separation of the test mixture under these conditions is depicted in Figure 2.1.

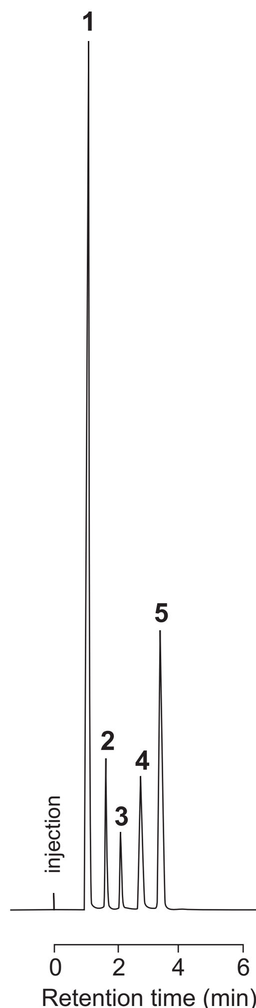


Figure 2.1. Typical chromatogram of a test mixture containing uracil (1), acetophenone (2), benzene (3), toluene (4) and naphthalene (5) after separation on a 4 µm Nova-Pak® C_{18} (150 mm x 3.9 i.d.) cartridge column.

The calculation of the theoretical plate number gives an indication of the efficiency and performance of a column. The Nova-Pak® C_{18} 4 µm (150 mm x 3.9 i.d.) cartridge column yielded an average efficiency of 6905 ± 777 theoretical plates (n = 6). Although ideally a column of such small particle diameter (4 µm) and length (150 mm) should give an N value of more than 10000 [65], the column was considered efficient and suitable to provide reproducible separations of CP while minimizing solvent usage at low flow rates, and it was therefore selected for method development and validation studies. The low column efficiency may have been due to the fact that the column was not new as it had been used for other applications in our laboratory.

2.3.2.6. Internal standard selection

An internal standard (IS) is a compound added in equal amounts to all standards and test samples to be analyzed [73] and is used to improve the accuracy of an analytical method by compensating for varying injection volumes and day-to-day instrumental changes [74, 75]. The physicochemical and analytical properties of an ideal IS should be similar to those of the analyte of interest [73]. Clobetasol butyrate [10], p-phenylphenol [3] and uracil [46] are some of the compounds that have used as IS for the analysis of CP.

Potential internal standards that were tested included uracil (UR), prednisone (PR), triamcinolone (TA), pregnenolone carbonitrile (PC), stilboestrol dipropionate (SD), hydrocortisone acetate (HA), betamethasone 17-valerate (BV) and mometasone furoate (MF). Separation was achieved using a mobile phase composition of acetonitrile-water (50:50) at a flow rate of 1 mL/min. Detection of compounds was conducted at 254 nm and the data generated in these studies are depicted in Table 2.2.

Table 2.2. Retention times of CP and potential internal standards

Compound	Retention time
Clobetasol 17-propionate (CP)	8.2
Uracil (UC)	1.0
Prednisone (PR)	1.0
Triamcinolone (TA)	1.2
pregnenolone carbonitrile (PC)	2.0
stilboestrol dipropionate (SD)	2.0
hydrocortisone acetate (HA)	2.2
betamethasone 17-valerate (BV)	5.4
mometasone furoate (MF)	8.8

The retention times of UR, PR, TA, PC, SD and HA were considered too short and therefore undesirable. Consequently, UR, PR, TA, PC, SD and HA were not considered as suitable for use as IS, since the short retention times could result in interference from the solvent front or any other peaks that elute close to the solvent front during HPLC analysis. Similarly, MF was not selected since it eluted close to the retention time of CP. Only BV was eluted at a suitable retention time and at a distance from the solvent front without interfering with the CP peak. Consequently BV was selected as the most suitable IS for the analysis of CP.

2.3.2.7. Mobile phase selection

It is worth noting that the development of a successful RP-HPLC separation does not rely solely on the selection of a suitable column, but also on matching an appropriate mobile phase to a specific column, analyte and sample matrix [76]. The retention times of compounds in RP-HPLC are adjusted by changing the mobile phase composition or solvent strength, which in turn depends on the choice of organic solvent and the concentration of that solvent in the mobile phase [67].

Conversely, the correct choice of organic solvent requires a knowledge of the physicochemical characteristics such as boiling point, viscosity and UV absorbance of that solvent [76, 77]. Furthermore, solvent purity, cost and the impact of the solvent on retention of the analyte, in addition to interactions with the analyte and the stationary phase, should be carefully considered when selecting an organic modifier for use in RP-HPLC analysis [76-78].

Methanol (MeOH) and acetonitrile (ACN) are the most commonly used organic modifiers in RP-HPLC [79]. Tan *et al.,* [80] reported that in hydro-organic mixtures, ACN and MeOH may self-associate or associate with water molecules to form clusters, albeit to different extents. As the polarity of methanol is greater than that of acetonitrile, the former forms hydrogen bonds by accepting or donating protons, whereas the latter being aprotic is unlikely to form hydrogen bonds [80]. Therefore the difference in hydrogen bonding ability may influence the adsorption of a modifier into a stationary phase as well as solute partitioning, thereby influencing a resultant separation [80].

CP is a hydrophobic compound and is practically insoluble in aqueous solutions, whereas it is freely soluble in water-miscible solvents, such as acetone, chloroform and dichloromethane (Table 1.1, Section 1.3.1.1). HPLC analysis of CP has therefore been achieved using bonded phase columns (C_{18}) and mobile phases containing either MeOH or ACN as the organic modifier of choice (Table 2.1). The use of MeOH and water as the mobile phase in this analytical method resulted in asymmetric peak shapes with a high degree of peak tailing and exceedingly high column back-pressures. In contrast, excellent peak symmetry, resolution, a reduction in peak

tailing and column back-pressure were observed when mobile phases of binary mixtures of ACN and water were used. Typical chromatograms generated using a binary mixture of MeOH and water and a binary mixture of ACN and water are shown in Figure 2.2.

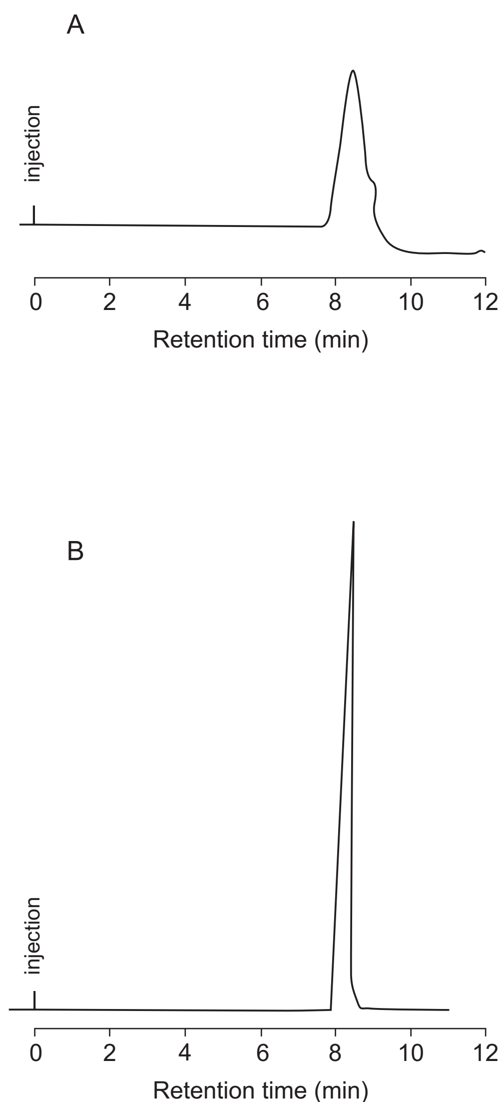


Figure 2.2. Typical chromatograms generated using a binary mixture of MeOH and water (A) and a binary mixture of ACN and water (B) as mobile phase (chromatograms graphically redrawn).

ACN-water mixtures have lower viscosities than the corresponding MeOH-water mobile phases, resulting in higher theoretical plate numbers and lower column back-pressures, which ultimately result in better peak shapes [67, 79]. Although MeOH is less expensive and apparently less damaging to the environment than ACN [79] the use of MeOH-water binary mixtures as mobile

phase for this analytical method was considered inappropriate. In order to prolong the lifespan of the column and develop an effective separation for the quantitation of CP in semi-solid dosage forms ACN was used as the organic modifier and was the only solvent evaluated in these studies.

2.3.2.8. Preparation of mobile phase

Mobile phases were prepared by adding equal parts by volume of HPLC-grade ACN and HPLC-grade water that had been prepared using a Milli-RO® 15 water purification system (Millipore Co., Bedford, MA, USA) to a glass Duran® Schott solvent mixing bottle (Schott Duran GmbH, Hattenbergstrasse, Germany). The mixture was allowed to equilibrate to room temperature and the mobile phase was filtered through a 0.45 μm Millipore® HVLP filter (Millipore, Bedford, MA, USA) and degassed under vacuum with the aid of a Model A-2S Eyela Aspirator (Rikakikai Co., Ltd, Tokyo, Japan) prior to use. Degassing of mobile phases is necessary to remove dissolved oxygen, which could lead to the formation of air bubbles in the flow cell of a detector or in the connecting tubing, thereby affecting the reproducibility and sensitivity of the detection system and the flow rate [77]. The mobile phase was freshly prepared daily and was not recycled during use.

2.3.2.9. Preparation of stock solutions and calibration standards

Standard stock solutions of CP (0.1 mg/ml) and BV (0.5 mg/ml) were prepared by accurately weighing 10 mg of CP and BV using a Model AG-135 Mettler Toledo top-loading analytical balance (Mettler Instruments, Zurich, Switzerland) into 100 ml and 20 ml A-grade volumetric flasks, and dissolving in 20 ml and 5 ml ACN, respectively. The stock solutions were placed in a Model 8845-30 ultrasonic bath (Cole-Parmer Instrument Comp. Chicago, IL, USA) for 5 min, in order to ensure complete dissolution of the drug, after which samples were made up to volume with ACN. Stock solutions were protected from light using aluminium foil and were, stored in a refrigerator at 4˚C. Stock solutions were used within a maximum period of two weeks based on stability study data generated as described in Section 2.4.7.3. Calibration standards of CP were prepared by serial dilution of the stock standard solution on a daily basis to produce solutions of concentration of 0.10, 0.50, 1.0, 3.0, 6.0, 12 and 18 μg/ml, using mobile phase as the solvent.

2.3.2.10. Effect of ACN concentration

The impact of ACN concentration on the retention times of CP and BV was evaluated using a variety of binary mixtures of ACN and water (ACN/water). The mixtures varied in composition from 40–65% ACN and the data generated in these studies are depicted in Figure 2.3.

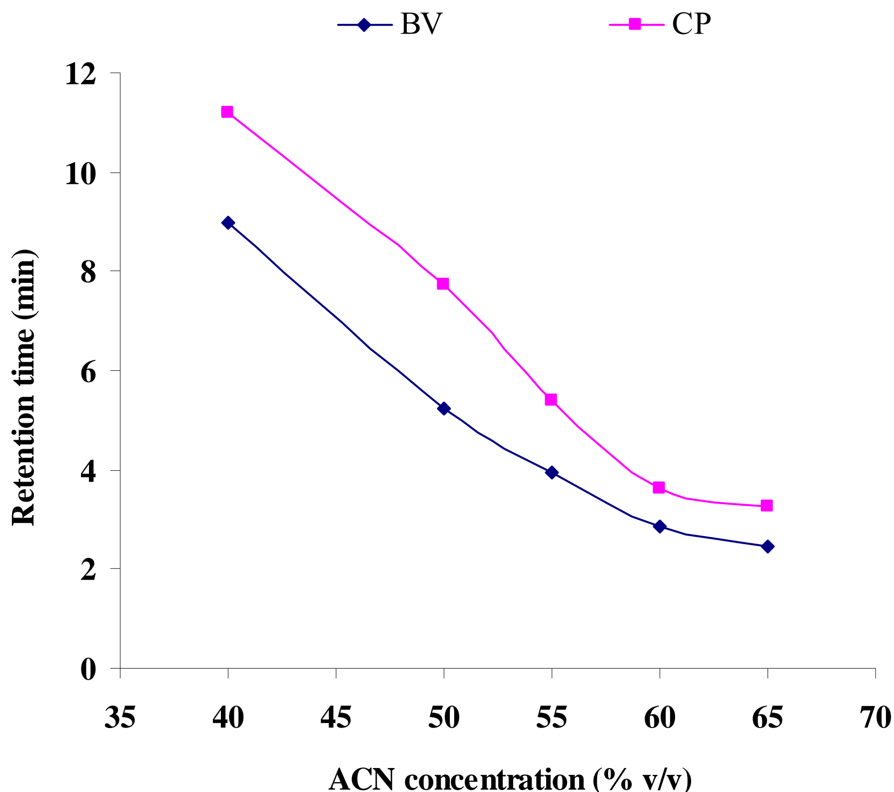


Figure 2.3. Effect of ACN concentration on the retention times of BV and CP

It is clearly evident that BV has a shorter retention time (R_t) than CP with any of the ACN/water binary mixtures tested. This is more than likely due to the fact that CP by virtue of its physicochemical characteristics is relatively more hydrophobic than BV and therefore interacts to a greater extent with the stationary phase than BV does.

The data also show that the retention times of BV and CP were inversely proportional to the ACN concentration in the mobile phase. In other words an increase in the ACN content of the mobile phase composition led to shorter retention times for both CP and BV. For example, at a

low ACN concentration of 40% v/v, BV had an R_t of 9.00 min, whereas CP had an R_t of 11.20 min. However, at a high ACN concentration of 60% v/v, BV had an R_t of 2.45, whereas CP had an R_t of 3.28 min. The decrease in R_t for both BV and CP with an increase in the ACN content is more than likely due to enhanced solute-solvent interactions and diminished solute-stationary phase interactions.

The aim of conducting these studies was to determine an optimal ACN concentration for use in the mobile phase that would produce acceptable retention times for both CP and BV. In our laboratory, retention times are considered acceptable when the first peak of interest, which is either the IS or the API, elutes 4 min after the solvent front, with the second peak of either the IS or the API eluting 2-4 min later, resulting in a maximum run time of approximately 10 min. Based on these criteria and the data illustrated in Figure 2.3 a binary mixture consisting of 50% w/w ACN-water was selected as the most suitable mobile phase for the separation of CP and BV.

2.3.2.11. Effect of flow rate

The effects of mobile phase flow rate on the retention times of CP and BV are illustrated in Figure 2.4. The results of these studies indicate that, as expected, the retention time of both CP and BV decrease as mobile phase flow rate was increased. A rapid HPLC separation with respect to a run time of approximately 10 min for the analysis of CP in pharmaceutical formulations was desired. A flow rate of less than 0.8 ml/min resulted in a CP retention time of greater than 10 min, which was considered unacceptable since the method would be time-consuming and result in long overnight analyses.

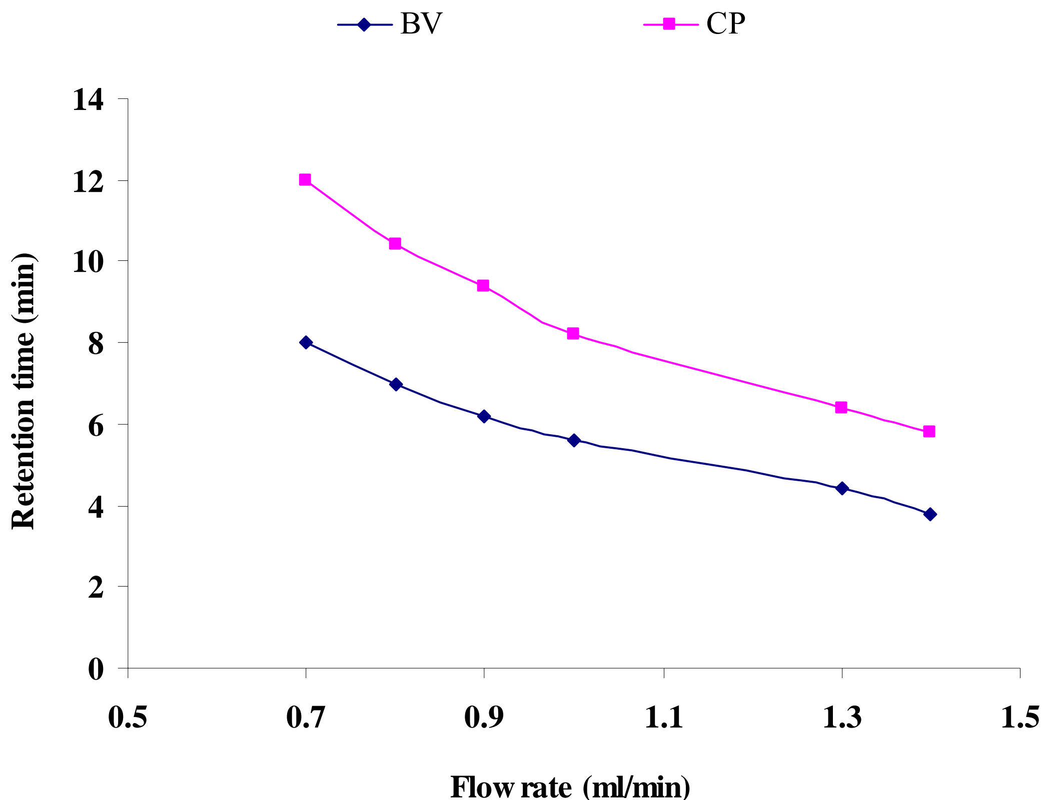


Figure 2.4. Effect of mobile phase (50 %v/v ACN) flow rate on the retention times of BV and CP

Although increasing the flow rate to 1.4 ml/min resulted in relatively short retention times for both CP and BV, closer inspection of the chromatogram revealed some peak shouldering, especially in the case of BV peak, which was possibly due to poor column performance at high flow rates. However, when the flow rate was set at 1.0 ml/min, sharp well-resolved peaks with the desired retention times for both BV and CP were observed (Figure 2.5). Consequently the mobile phase flow rate was set to 1.0 ml/min and this rate was used for all subsequent analyses.

2.3.2.12. Optimal mobile phase composition and flow rate

The final mobile phase selected for the analysis of CP using BV as the IS was a binary mixture of ACN and water in a ratio of 50:50 or 50 %v/v ACN. The peak shape and retention times were found to be suitable when this mobile phase composition was used with excellent resolution between CP and the IS. The mobile phase flow rate was set at 1.0 ml/min with resultant retention times of 5.6 min and 8.2 min for BV and CP respectively.

The total run time for sample analysis was 10 min and the use of this flow rate and resultant run time ensured that comparatively low volumes of mobile phase and solvents were consumed by the analysis of CP in test samples. A typical chromatogram of the separation achieved using this mobile phase and the reported chromatographic conditions, is shown in Figure 2.5.

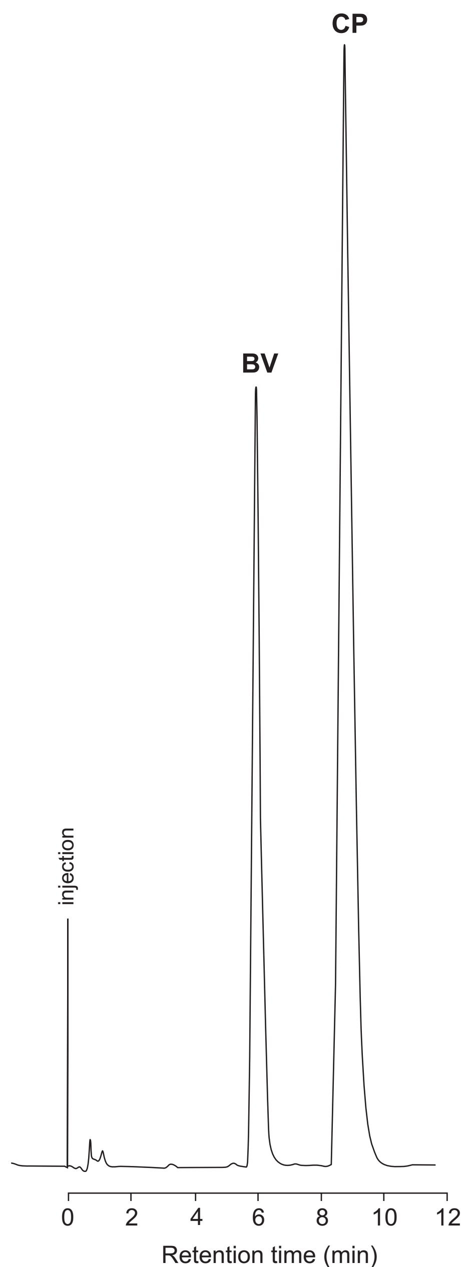


Figure 2.5. Typical chromatogram of a mixture of the internal standard, betamethasone 17-valerate (BV) and clobetasol 17-propionate (CP) using a mobile phase of 50% v/v ACN-water and a flow rate of 1.0 ml/min

2.3.2.13. Chromatographic conditions

The final chromatographic conditions established for the analysis of CP are summarized in Table 2.3.

Table 2.3. Chromatographic conditions for the analysis of CP

Column	Nova-Pak® C_{18} 150 mm x 3.9 mm i.d., 4 µm
Mobile phase	Acetonitrile : water (50:50)
Flow rate	1.0 ml/min
Retention times	5.6 min (BV) and 8.2 min (CP)
Column pressure	1300 psi
Column temperature	Ambient (22˚)
Injection volume	15 µl
Wavelength	240 nm
Sensitivity	0.005 AUFS
Integrator speed	0.5 mm/min
Recorder input	10 mV full scale

2.4. METHOD VALIDATION

2.4.1. Overview

One of the most critical factors in developing pharmaceutical drug substances and products is ensuring that the analytical test methods used to analyze fine chemicals and products generate valid and meaningful data in terms of reliability, accuracy and precision, regardless of whether it is intended for acceptance, release, stability or pharmacokinetic studies [81, 82]. Validation of an analytical method is a process that provides documented evidence that an analytical test method performs in an appropriate manner for the purposes for which it was intended [23, 83, 84].

The first step in the development of an HPLC method validation protocol is to determine the objective of the intended method [82]. A method is considered a Level I or quantitative assay method if it is intended to monitor blood levels in patients, for final product release, for the determination of potency, or for the assessment of levels of impurity or contaminants in human drug products [82]. If the method is to be used as a qualitative examination for identity, then it is

considered a Level II or qualitative assay method [81]. A variety of general validation protocols have been recommended by organizations such as the FDA [84], United States Pharmacopeia (USP) [23] and the International Conference on Harmonization (ICH) [85, 86].

The FDA [84], for example, requires a manufacturer of a pharmaceutical product to establish and document accuracy, sensitivity, specificity and reproducibility of the analytical method [81]. The USP [23] specifies typical performance characteristics such as accuracy, precision, specificity, limit of quantitation (LOQ), limit of detection (LOD), linearity, range and sample stability that should be considered in the validation of analytical methods intended for the analysis of active ingredients alone or in finished pharmaceutical products, *i.e.,* a Level I assay. Consequently, the validation of an RP-HPLC analytical method for the analysis and characterization of CP in multi-source topical products during formulation development and assessment was carried out as outlined by the FDA [84] and USP [23] guidelines with specific reference to the ICH recommendations [85, 86].

2.4.2. Linearity and Range

The linearity of an analytical method is an indication of the capability of a test method to produce test results that are directly proportional to the amount of analyte in a sample within a given concentration range [23, 83, 86]. Similarly, the working range of an analytical method defines the inclusive upper and lower concentrations of an analyte in a sample for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity [23, 83, 86].

The ICH guidelines [46] specify that a minimum of five (5) samples of increasing concentration along with certain minimum specified ranges should be used to establish the linearity of a test method [86]. The linearity of the analytical method was evaluated over the concentration range 0.10-18 μg/ml with the lower concentration representing the limit of quantitation and the upper concentration indicating 120% of the test target concentration (15 μg/ml) in the samples to be analyzed.

Calibration standards (0.10, 0.50, 1.0, 3.0, 6.0, 12 and 18 µg/ml) spiked with BV (IS) were prepared as described in Section 2.3.2.9 and were injected (n = 5) onto the chromatographic system described in Section 2.3.2.2 using the conditions described in Section 2.4.6. The peak height ratios of CP response to BV response were calculated and used to construct a calibration curve which was used to establish whether there was linearity of response of the analyte in relation to concentration. Linearity was tested using least squares linear regression analysis of the peak height ratios versus concentration data and the results of these studies are depicted in Figure 2.6.

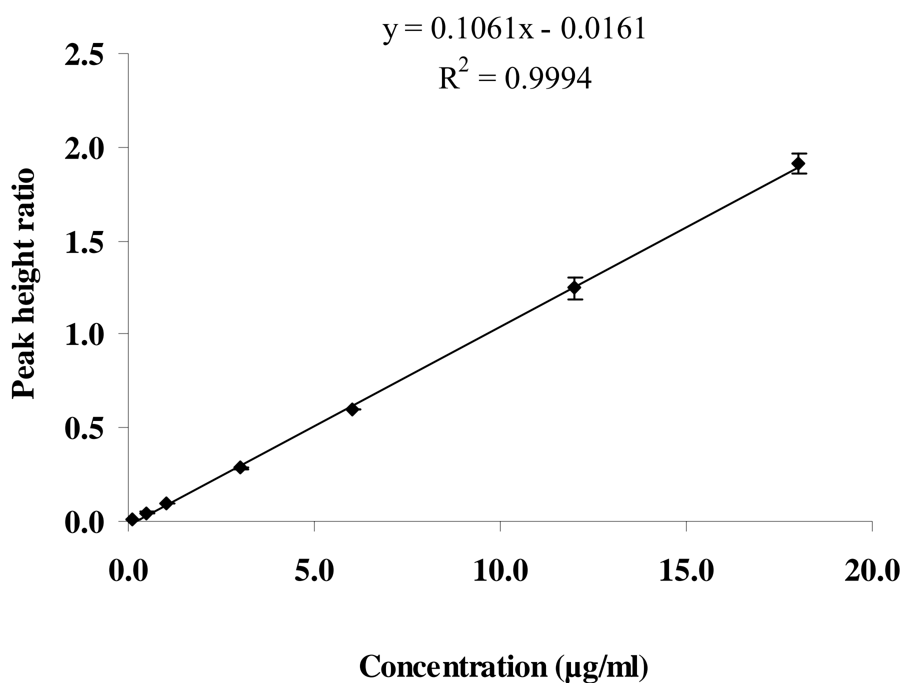


Figure 2.6. Calibration curve constructed for CP following least squares linear regression analysis of peak height ratios of CP and IS versus concentration.

The acceptability of linearity data is determined by evaluation of the coefficient of determination (R^2) of the best fit linear regression line for the response *vs.* concentration plot as shown (Figure 2.6). An R^2 value of greater than 0.999 is generally considered as evidence of an acceptable fit of the data to the regression line [81]. The calibration curve in these studies was found to be linear

with an R^2 value = 0.9994, a slope of 0.1061 and a y-intercept of -0.0161 yielding an equation for the calibration line of y = 0.1061x – 0.0161.

2.4.3. Precision

The precision of an analytical method is a measure of the degree of scatter or closeness of agreement among individual test results when the method is applied repeatedly to multiple aliquots of the same homogeneous sample under the prescribed test conditions [23, 85]. Analytical method precision provides an indication of the variability of analytical results as a function of the analyst, manipulation of samples and the day-to-day environment in which the method is applied [87]. The precision of an analytical method is usually expressed as coefficient of variation or percentage relative standard deviation (% RSD) of a series of measurements [23, 81, 85].

The FDA [84] recommends that the precision at each concentration level should not exceed 15% RSD except for the LOQ, where it should not exceed 20% RSD for biological assays. It was anticipated that the samples that would be collected in these studies would not be subject to high degrees of variability and interference as would be expected for biological matrices and therefore the acceptance criteria for precision studies was set at less than or equal to 5% RSD at each concentration level, as opposed to the 15% RSD recommended by the FDA [84]. The ICH [85, 86] recommends that precision should be performed at three different levels *i.e.,* repeatability, intermediate precision and reproducibility.

2.4.3.1. Repeatability

Repeatability or intra-day precision of an analytical method is an indication of the performance of an analytical procedure conducted within a laboratory over a short time interval using the same analyst with similar equipment [23, 85]. The repeatability of this method was assessed by calculating the % RSD of peak height ratios using five (5) replicates of the calibration standards run on a single day. The results from repeatability studies (n = 5) are summarized in Table 2.4.

The data show in all cases that the % RSD values were less than 5%, indicating that the analytical method for the analysis of CP was repeatable.

Table 2.4. Repeatability data for HPLC analysis of CP

Conc. (µg/ml)	**MPHR* (n = 5)**	**SD**	**% RSD**
0.500	0.0498	0.00200	4.02
1.00	0.107	0.000100	0.900
3.00	0.332	0.00310	0.940
6.00	0.654	0.00470	0.710
12.0	1.18	0.00570	0.480
18.0	1.87	0.00570	0.300

* Mean peak height ratio of CP/internal standard

2.4.3.2. Intermediate precision

Intermediate precision or inter-day variability of an analytical method is an indication of the variability in results obtained within a laboratory due to random events such as analysis on different days, using different analysts and/or equipment [23, 81, 85]. The intermediate precision of this method was assessed over a period of three consecutive days using five replicates of the calibration standard concentrations prepared as previously described in Section 2.3.2.9.

Intermediate precision (n = 5) data expressed as coefficient of variation (% RSD) of the peak height ratios of the calibration standards are shown in Table 2.5. The acceptance criteria for intermediate precision in these studies was set at less than or equal to 5% RSD at each concentration level and the data revealed that in all cases the % RSD values were less than 5%, indicating that the analytical method would be precise when employed to analyze CP in semi-solid dosage forms on different days.

Table 2.5. Intermediate precision data for HPLC analysis of CP

Day	Conc. (µg/ml)	MPHR* (n = 5)	SD	% RSD
1	0.500	0.0498	0.00200	4.02
	1.00	0.107	0.00100	0.900
	3.00	0.332	0.00310	0.940
	6.00	0.654	0.00470	0.710
	12.0	1.18	0.00570	0.480
	18.0	1.87	0.00570	0.300
2	0.500	0.0474	0.00145	3.06
	1.00	0.0974	0.000200	0.170
	3.00	0.305	0.000600	0.200
	6.00	0.561	0.00130	0.240
	12.0	1.12	0.00320	0.270
	18.0	1.81	0.00750	0.410
3	0.500	0.0569	0.00250	4.33
	1.00	0.114	0.00320	2.70
	3.00	0.292	0.000900	0.320
	6.00	0.648	0.00140	0.220
	12.0	1.24	0.00330	0.270
	18.0	1.96	0.00400	0.200

* Mean peak height ratio of CP/internal standard

2.4.3.3. Reproducibility

The reproducibility of an analytical test method refers to the precision of a method following application of that analytical procedure in different laboratories, and is often a part of inter-laboratory crossover studies [23, 81]. Reproducibility studies should only be considered in the case of standardized analytical procedures [86] and reproducibility studies are not normally expected if intermediate precision is performed as part of method validation studies [81]. Consequently, reproducibility studies were not conducted, as the data for repeatability and intermediate precision were considered sufficient to show that the analytical method was precise and appropriate for the intended purpose of analyzing CP in semi-solid dosage forms.

2.4.4. Accuracy

The accuracy of an analytical method or procedure is an indication of the closeness of agreement between a value measured or quantitated using the method and a value that is accepted to be either a conventional true value or an accepted reference value for that sample [85]. In

combination precision and accuracy data determine the error of an analysis, and accuracy is therefore an integral criterion in the validation of an analytical test method [83].

Accuracy may be determined in a number of different ways. For example, the accuracy of a method can be determined by analyzing a sample of known concentration and comparing the measured value to the true value or comparing test results from a new method with results from an existing alternate well-characterized procedure or spiking a known amount of analyte in blank matrices and calculating the percent recovery, [81]. Another approach used to assess the accuracy of a method is to perform a two-sided t-test to determine if any significant differences exist between the mean data generated by the test method and the nominal value, with a 95% level of confidence [83].

The accuracy of the analytical method was determined by replicate analysis of samples containing known amounts of CP. Three (3) samples representing low (1.5 μg/ml), medium (7.5 μg/ml) and high (15 μg/ml) concentrations prepared in mobile phase were injected in replicates of five (5). Accuracy was reported as the percent recovery, % RSD and % Bias. Bias is the difference between the mean value determined for an analyte of interest and the accepted true value for that sample. Bias assesses the influence of the analyst on method performance and accuracy measurements are designed to measure the effectiveness of sample preparation prior to analysis [88].

The results from accuracy studies for this procedure are listed in Table 2.6. The acceptance criteria for accuracy are that the mean percent recovered and the % RSD should be 100 ± 2% [81] and less than 2% [89, 90], respectively, at each concentration level. A percent Bias of less than 5% at each concentration level was considered as the test limit in our laboratory and based on these criteria, the data in Table 2.6 reveal that this analytical method is accurate.

Table 2.6. Accuracy data for HPLC analysis of CP (n = 3)

Theortical conc. (μg/ml)	Actual conc. (μg/ml)	SD	% RSD	% Bias	% Recovery
1.50	1.48	0.005000	0.360	-1.61	98.67
7.50	7.47	0.0160	0.210	-0.360	99.60
15.0	15.1	0.00300	0.0200	+0.460	100.7

2.4.5. Limit of quantitation (LOQ) and limit of detection (LOD)

The limit of quantitation (LOQ), sometimes referred to as the lower limit of quantitation (LLOQ), is the lowest concentration of an analyte in a sample that can be determined with acceptable precision and accuracy under the stated operational conditions of an analytical method [83, 91]. Similarly, the limit of detection (LOD) is the lowest concentration of analyte in the sample that can be detected but not necessarily quantitated under the stated experimental conditions of the analytical method [23, 83, 91]. The LOQ is normally taken as the lowest concentration point in the calibration curve [83] whereas the LOD is a limit test that merely specifies whether or not the amount of an analyte is above or below a certain level [23, 91].

Four methods are commonly used to determine the LOQ and LOD of an HPLC method [83, 91]. The ultimate method of choice is usually left to the discretion of the analyst or the standard operating procedures of a specific laboratory, since all four techniques essentially yield equivalent results and are suitable for satisfying the USP [23] and ICH [85, 86] requirements for the determination of the LOQ and LOD of an analytical method [91]. Thus, the LOQ in these studies was determined by evaluating the lowest concentration of analyte that resulted in a precision of less than 5% RSD [91], in other words the LOQ is the lowest concentration for which the % RSD of multiple injections of a sample ($n = 6$) was less than 5%. By convention the LOD value is taken as 30% of the LOQ value [91].

Five different concentrations of CP were evaluated as potential LOQ values and the data generated in these studies are depicted in Table 2.7. Based on these results the LOQ for this analytical test method was found to be 0.10 μg/ml, with a % RSD value of 1.82% and by convention, the LOD value was taken as 0.03 μg/ml, which when injected into the HPLC, resulted in a detectable but not quantifiable peak.

Table 2.7. LOQ data for HPLC analysis of CP

Conc. (µg/ml)	MPHR* (n = 6)	SD	% RSD
0.25	0.0312	0.000200	0.670
0.20	0.0261	0.000300	1.11
0.15	0.0210	0.000400	1.70
0.10	0.0154	0.000300	1.82
0.050	0.0133	0.00140	10.8

* Mean peak height ratio of CP/internal standard

2.4.6. Specificity and selectivity

2.4.6.1. Overview

Specificity and selectivity are relative terms, often used interchangeably to describe the ability of an analytical test method to measure accurately the analyte of interest in the presence of other components that may be present in a sample matrix [88, 92]. However, an analytical method is specific if it produces a response for only a single analyte, whereas selectivity describes a procedure that provides a response for the target compound that is distinguishable from all other responses that may be generated from a sample matrix [92, 93].

As most chromatographic procedures produce responses for other substances and not only for the analyte of interest, the term selectivity is more appropriate than specificity in this context [93]. Consequently, the term selectivity is applied for these studies. Selectivity was evaluated by analyzing a sample of a commercially available 0.05% w/w CP cream product (Dermovate®, Glaxo Wellcome SA (Pty) Ltd, Midrand, RSA) and an extemporaneous placebo cream formulation, using a simple liquid-liquid extraction procedure with hexane and acetonitrile, which is described in Section 2.4.6.2.

2.4.6.2. Sample preparation

A schematic representation of the sample preparation procedure is shown in Figure 2.7. Approximately 600 mg of a cream sample, equivalent to 0.30 mg of CP, was weighed and transferred into a 100 ml Duran® Schott round neck Erlenmeyer flask (Schott Duran GmbH,

Hattenbergstrasse, Germany). A standard stock solution for BV was prepared in ACN as previously described in Section 2.3.2.9 and used to prepare 7.5 μg/ml BV in ACN. A 20 ml aliquot of the BV solution was added to a stoppered flask containing the cream, followed by the addition of 30 ml of n-hexane (Associated Chemical Enterprises (Pty) Ltd, Johannesburg, Gauteng, RSA).

The mixture was shaken vigorously to disperse the semi-solid sample and sonicated for 10 minutes to aid the dissolution of CP in ACN using a Model 8845-30 ultrasonic bath (Cole-Parmer Instrument Comp., Chicago, IL, USA). The mixture was then transferred to a Model-NS 19/26, 1.5 x 12.5 mm stopcock separating funnel (VIT-LAB GmbH, Seeheim-Jugenheim, Germany) and was shaken vigorously while venting the funnel to avoid a potential build up of pressure within the funnel.

Following the separation of the immiscible solvents, the lower distinct ACN layer was withdrawn via the stopcock of the separating funnel and was collected in a clean Duran® Schott round neck Erlenmeyer flask. The sample was then filtered through a 0.22 μm Millipore filter (Millipore Co., Bedford, MA, USA) and an aliquot of the filtered sample was injected onto the chromatographic system in replicates of six (6). A calibration curve was constructed on the same day that the samples were prepared and analysed, and the concentration of CP in the sample filtrate was interpolated from the calibration curve.

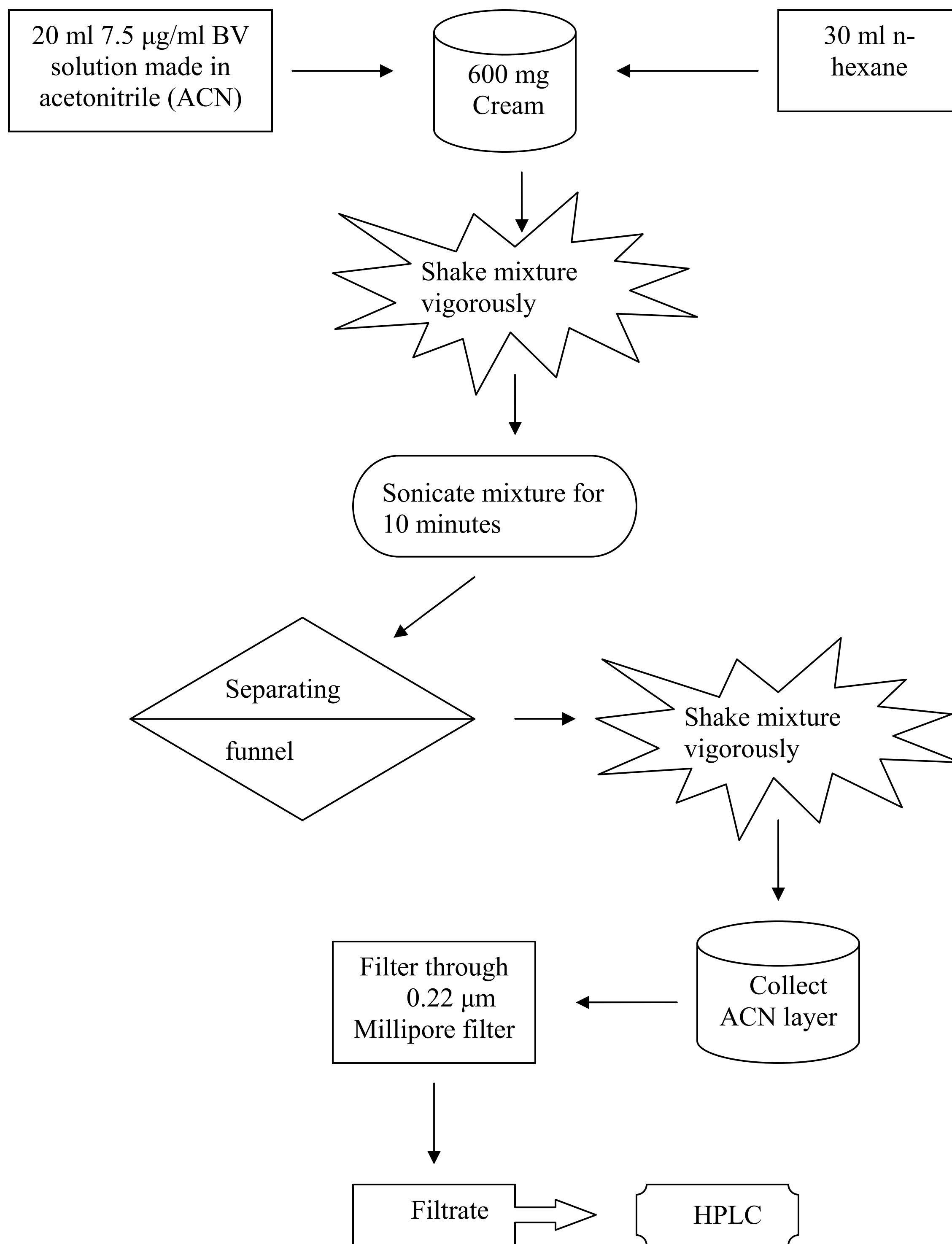


Figure 2.7. Schematic representation of the sample preparation procedure

2.4.6.3. Extraction efficiency

A commercially available 0.05% w/w CP cream product (Dermovate®, Glaxo Wellcome SA (Pty) Ltd, Midrand, SA) was used to evaluate the extraction efficiency or percentage recovery of the sample preparation procedure described in Section 2.4.6.2. The extraction efficiency was determined using 600 mg samples of the cream (n = 4). Based on the labelled amount of CP in the semi-solid dosage form (0.05% w/w CP) and assuming that the entire CP in the cream sample dissolved in the 20 ml ACN added, a theoretical concentration of 15 µg/ml was expected. The theoretical concentration was used as a reference sample to determine the actual concentration of CP in the sample filtrate for the calculation of the percentage recovery values.

The extraction efficiency and precision data generated from these studies are tabulated in Table 2.8. The United States Pharmacopoeia (USP) [23] specifies that CP cream formulations should contain not less than 90.0% and not more than 115.0% of the labelled amount of CP. The extraction efficiency data shown in Table 2.8 fall within this range with excellent precision and therefore, the cream formulation tested in these studies complied with the USP standards. Section 2.4.6.4 describes the validation of the extraction procedure.

Table 2.8. Extraction efficiency data following the extraction of CP from Dermovate® cream (n = 6)

Sample weight (mg)	Expected theoretical conc. (µg/ml)	Actual conc. (µg/ml)	SD	% RSD	% Recovery
608.9	15.22	14.85	0.04700	0.3200	97.53
600.6	15.01	14.23	0.1314	0.9200	94.77
601.8	15.05	14.43	0.08290	0.5700	95.94
594.1	14.85	14.35	0.1387	0.9700	96.60

2.4.6.4. Validation of the extraction procedure

In order to determine the authenticity of the sample preparation procedure described in Section 2.4.6.2 and to ensure that the data generated in the studies described in Section 2.4.6.3 and summarized in Table 2.8 were valid, the method was applied to the extraction of CP from a 0.05% w/w solution. The 600 mg sample of CP cream was replaced by 600 µl of a CP solution.

Samples (n = 3) of the CP solution were extracted and analysed and the data generated in these studies are summarized in Table 2.9.

Table 2.9. Extraction efficiency following extraction of 600 µl of a CP solution (0.05% w/w) (n = 3)

Sample volume (µl)	Expected theoretical conc. (µg/ml)	Actual conc. (µg/ml)	SD	% RSD	% Recovery
600.0	15.00	14.98	0.04800	0.3200	99.84
600.0	15.00	14.23	0.1314	0.9200	94.77
600.0	15.00	15.86	0.03352	0.1200	105.8

The data show excellent percentage recovery and precision values that are similar to those obtained when CP cream was used (Section 2.4.6.3) and are indicative of the suitability of the method used to prepare samples of the cream, for analysis. The extraction process was therefore used in subsequent studies to determine and confirm CP content in extemporaneously prepared 0.05% w/w creams during product development and assessment studies.

2.4.6.5. Selectivity studies

The selectivity of the analytical method was evaluated by analyzing a sample of an extemporaneous placebo cream formulation, extracted with:

a) ACN without BV, and
b) ACN spiked with the BV.

The selectivity was further evaluated by analyzing a sample of a Dermovate® cream extracted with:

c) ACN without BV, and
d) ACN with BV.

Typical chromatograms obtained in these studies are shown in Figure 2.8. Inspection of the resultant chromatograms reveal that the CP and BV peaks are free from interference from other formulation excipients, including chlorocresol (CH), which was the preservative in the commercially available CP cream. The method was therefore considered selective for the purposes of analyzing CP in semi-solid formulations.

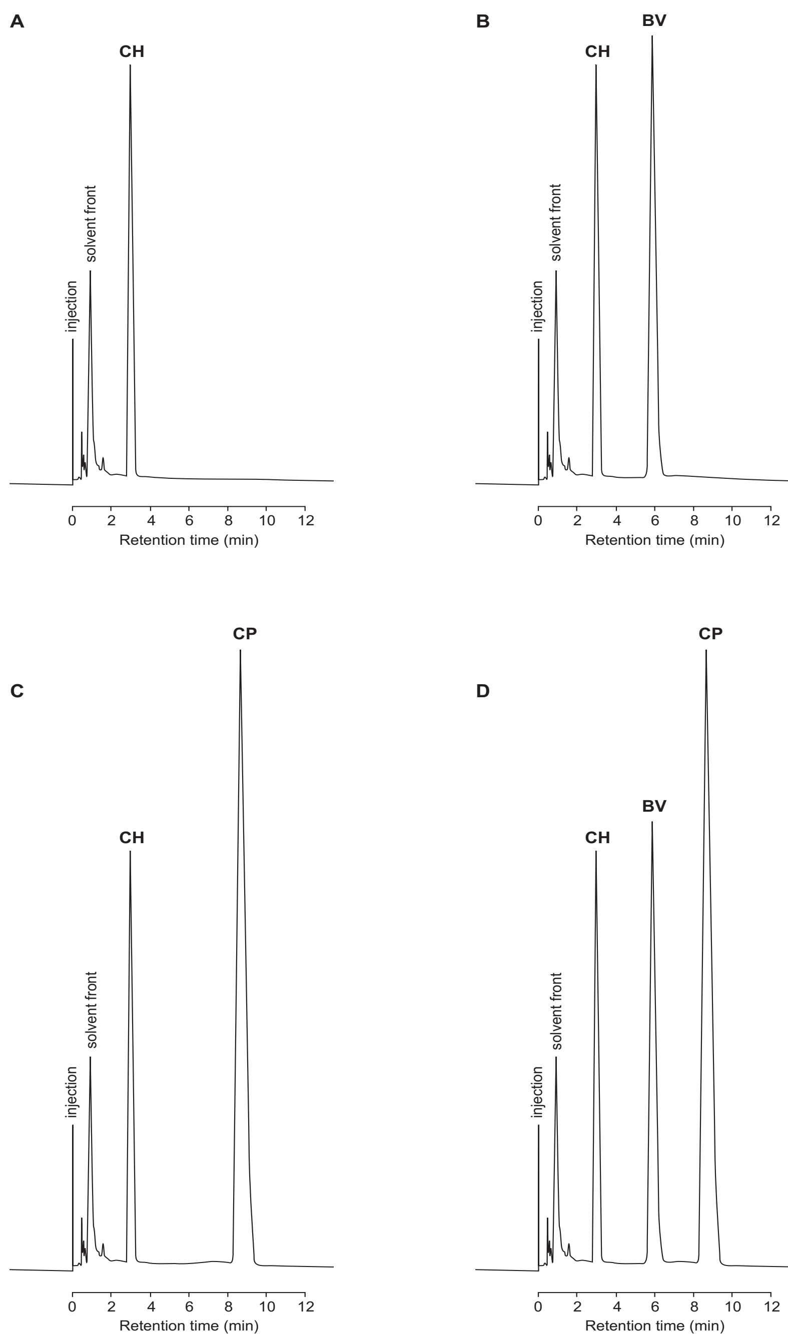


Figure 2.8. Typical chromatograms obtained following the analysis of a sample of placebo cream without BV (A) and with BV (B) and Dermovate® cream without BV (C) and with BV (D)

2.4.7. Sample stability

2.4.7.1. Overview

Another important consideration during method validation is the demonstration of stability of an analyte in a sample matrix and solvents used during the sample work-up, under the conditions to which the study samples will be subjected throughout the analytical procedure [93]. It has been reported that a compound may be considered stable under certain conditions for a certain period of time when there is no significant difference between the detector response for an analyte following analysis of solutions that have been stored, compared to the response generated from freshly prepared solutions of the analyte in similar matrices [83].

There are numerous areas that need to be identified and stability evaluations undertaken during sample analysis [83, 93]. In these studies, the stability of CP in the standard stock solutions (Section 2.3.2.9) used to prepare the calibration standards (Section 2.3.2.9) and the stability of CP in process samples used during *in vitro* release studies (Chapter 3) were evaluated. It has been recommended that the stability of standard stock solutions of an analyte used to prepare calibration curves be evaluated over the maximum time period for which the solutions will be stored prior to use [83, 93, 94]. Accordingly, the stability of the stock solutions must be determined under the same conditions of light or dark, at the same temperature(s), and in the same solvent(s) and container(s) as are used during analysis [83, 93].

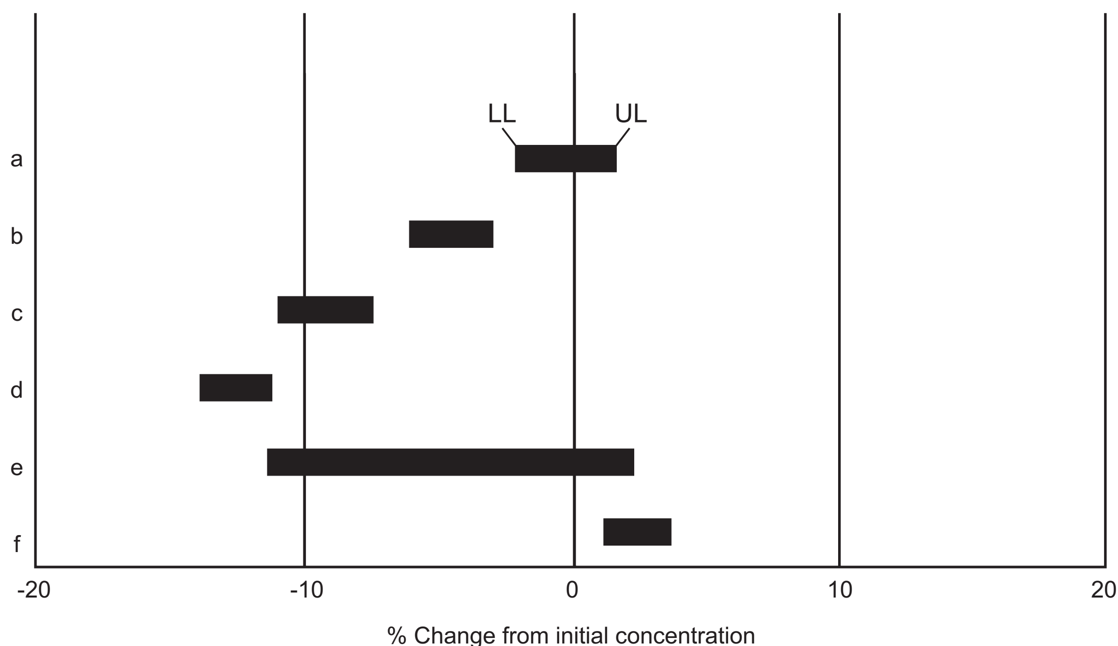
Buick *et al.,* [94] suggested that in the absence of stability data, standard stock solutions must be freshly prepared on a daily basis during sample analysis, since failure to do so may lead to the preparation of calibration standards that do not represent the true concentrations of the analyte, which may then result in the determination of incorrect concentrations in unknown samples. Similarly, in-process sample stability studies are required in order to determine the stability of a compound in a sample matrix under specific storage conditions over the time needed to store and analyze the samples [95, 96]. The objective of conducting these stability studies was to determine if any degradation of CP occurs during the entire period of sample collection, processing, storing, preparation and analysis [83]. Consequently, the data obtained from in-

process sample stability studies may be used to provide guidelines concerning the maximum times and conditions of sample storage prior to analysis and therefore to ensure that the integrity of samples is maintained up to the point of analysis [93, 94, 96].

2.4.7.2. Stability data analysis

Stability data generated in these studies were analysed using a statistical test method developed by Timm *et al*., [97]. The procedure was initially developed for the evaluation of stability of drugs in biological fluids and takes into account the quality of the experimental procedure including the precision of the method and the number of replicates used during analysis of the stored samples [97]. The statistical interpretation of the stability data as proposed by Timm *et al.,* [97] is based on the construction of a 90% confidence interval (C.I.) for the true percentage change (Δ) in concentration or detector response generated from stored and freshly prepared samples.

The lower limit (L.L.) and the upper limit (U.L.) of the C.I. are calculated using the measured percentage response difference (D) between stored and freshly prepared samples for replicate analyses and the true percentage change in response lies within that limit with 90% certainty [97]. According to Timm *et al.,* [97] a change of response during storage may be considered statistically relevant if the values for both the L.L. and U.L. of the C.I. are either < -10 or > 10. Figure 2.9 depicts the possible outcomes that could be generated when stability data are analyzed using the method described by Timm *et al.,* [97].



The bars above the axis depict the ranges of the 90% CI for the % Δ between stored and freshly prepared samples.

a) change of response, not significant and not relevant
b) decrease of response, significant, but not relevant
c) decrease of response, significant and possibly relevant
d) decrease of response, significant and relevant
e) decrease of response, not significant, but possibly relevant
f) increase of response, significant

Figure 2.9. Interpretation of stability data, as described by Timm *et al*, [97]

2.4.7.3. Stability of stock solutions

The stability of CP stock solutions prepared in ACN was evaluated following storage for two weeks at 4˚C. CP stock solutions were prepared as previously described in Section 2.3.2.9. 75 µl and 1500 µl aliquots of CP stock solutions were measured using single channel model electronic pipettes covering the volume range of 50-1200 µl and 500-5000 µl respectively (ePET Electronic Pipettes Biohit, Helsinki, Finland), and transferred to a 10 ml A-grade volumetric flask and made up to volume with ACN, yielding solutions of 0.75 µg/ml and 15 µg/ml, representing the lower and upper concentrations, respectively.

A maximum of five (5) replicate samples at each concentration studied were prepared for analysis on days 1, 2, 3, 7 and 14 after storage at 4˚C, resulting in a total of twenty-five (25) separate samples being analyzed. On each day of analysis fresh samples at each concentration were prepared from a freshly made CP stock solution (Section 2.3.2.7) and analysed together with the five (5) stored samples. Both freshly made and stored samples were spiked with 60 μl of a freshly prepared 0.5 mg/ml BV solution (measured using a 50-1200 electronic pipette) prior to analysis, and for each sample analyzed, the peak height was measured and the ratio of CP to BV was used as the response.

The results obtained from stability studies of CP stock solutions at both the lower and upper concentrations stored at 4˚C for 1, 2, 3, 7 and 14 days are depicted in Figure 2.10. These data reveal that at the lower (0.75 μg/ml) and upper (15 μg/ml) concentrations, stored at 4˚C for 14 days the change of response for CP was neither significant nor relevant. The wide confidence interval calculated for the 0.75 μg/ml sample on days 2 and 3 may be indicative of the poor precision of the assay procedure at these low levels [97]. Nevertheless, these studies reveal that CP was stable in acetonitrile at 4˚C following storage for two weeks. Consequently, stock solutions of CP were prepared in acetonitrile, stored at 4˚C and used within a period of two weeks, after which fresh solutions were made.

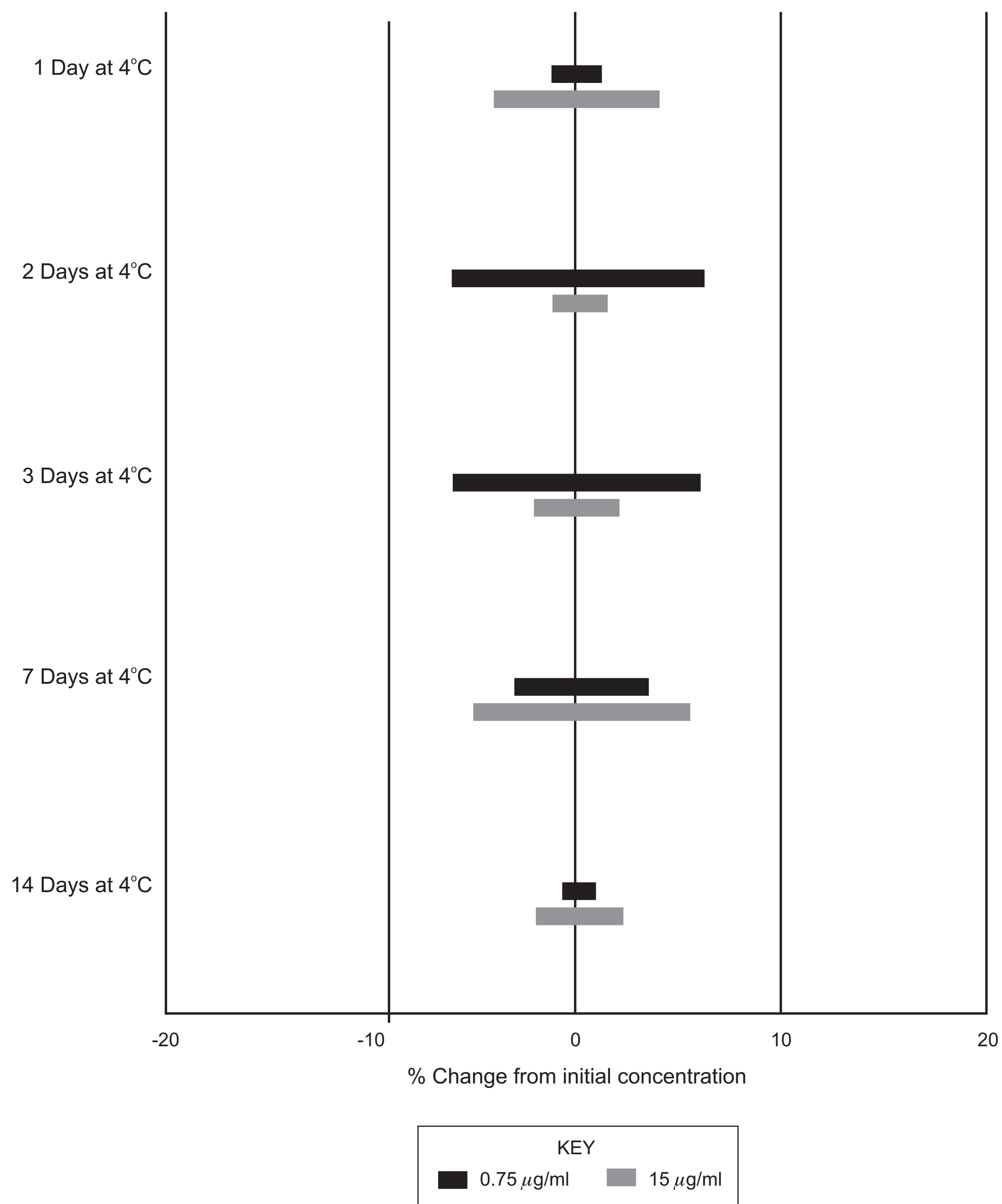


Figure 2.10. Stability of CP in ACN at two different concentrations, stored at + 4ºC for 1, 2, 3, 7, and 14 days

2.4.7.4. In-process sample stability

In-process sample stability studies were also conducted by evaluating CP stability in the receptor medium to be used for *in vitro* release studies. A receptor medium of 50% v/v propylene glycol/water (Chapter 3, Section 3.2.6.2.3.3) was to be used during the *in vitro* release studies at room temperature (22˚C). Stock solutions of CP were prepared as previously described in Section 2.3.2.9 but in this case propylene glycol rather than ACN was used as the solvent of choice. A 75 µl and 1500 µl aliquot of the CP stock solution measured as described in Section 2.5.7.3 was diluted and made up to 10 ml using the receptor phase, yielding concentrations of 0.75 µg/ml and 15 µg/ml concentrations as previously described in Section 2.5.7.3.

A maximum of five (5) replicate samples were prepared at both the lower and upper concentration and stored for 1, 2 and 3 days, resulting in a total of fifteen (15) separate samples for each concentration being studied. The samples were stored at room temperature (22˚C) and on each day of analysis stored and freshly prepared samples (n = 5) spiked with 60 µl of a freshly prepared 0.5 mg/ml internal standard (BV) were analysed. The percentage response differences between these samples were calculated and used to construct a 90% CI as described by Timm *et al.,* [97] and the data generated from these studies are depicted in Figure 2.11.

The results indicate that the change of response of CP at both the lower (0.75 µg/ml) and upper (15 µg/ml) concentrations prepared in 50% v/v propylene glycol/water and stored at room temperature (22˚C) for three (3) consecutive days was not significant and not relevant. CP was therefore considered to be stable when prepared in 50% v/v propylene glycol/water and stored at room temperature (22˚C) for three days. Consequently, CP samples from *in vitro* release studies were stored at 22˚C after removal of each sample and analyzed immediately at the conclusion of the *in vitro* release studies, *i.e.* within three days of the commencement of the study.

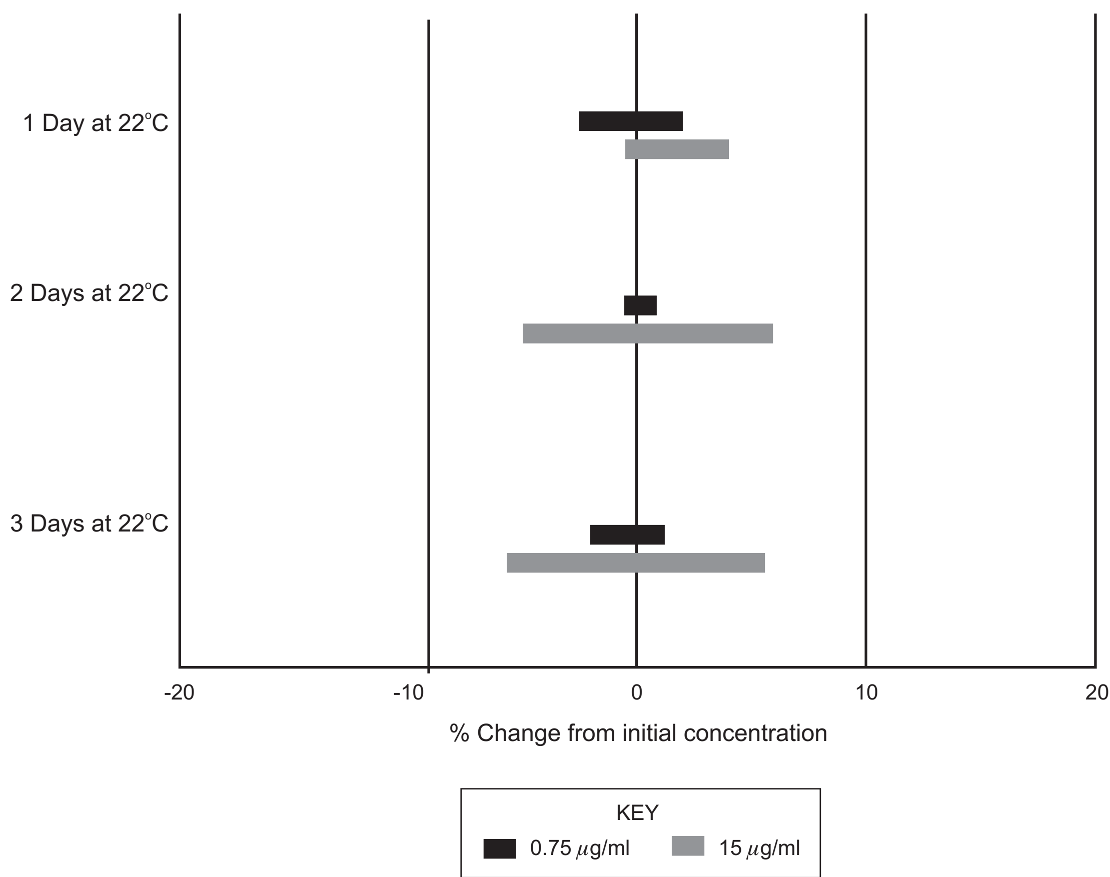


Figure 2.11. Stability of CP in *50* %v/v propylene glycol/water stored at + 22ºC for 1, 2 and 3 days

2.5. METHOD REVALIDATION

2.5.1. Overview

According to the FDA guidance document on Analytical procedures and methods validation: Chemistry, manufacturing and controls documentation [98], it is vital that an analytical method is revalidated when changes are made to an original procedure or to the original operating conditions of a validated method. Such revalidation studies are intended to ensure that the method maintains the appropriate performance characteristics as specified prior to the implementation of the changes to the method [95, 98].

Although the extent of the revalidation procedure to be undertaken depends on the nature of the change to a method, it is not necessary to conduct extensive revalidation studies when an

analytical method has been previously fully validated according to an international protocol [99]. Therefore, the decision concerning which parameters require revalidation is based on logical consideration of those specific validation attributes that are more than likely to be affected by the change in question [95]. However, as a minimum requirement, linearity, precision and accuracy studies should be undertaken in most cases [99].

The revalidation of this analytical method was necessary since a change was made to the solvent delivery module of the system as outlined in Section 2.3.2.2. The definitions and acceptance criteria of the relevant performance characteristics discussed below have been previously described in Section 2.4.2.

2.5.2. Linearity

A calibration curve was constructed by plotting the peak height ratios of CP to BV *versus* CP concentration within the concentration range 0.10-18 μg/ml and performing least squares linear regression of the constructed calibration curve. The data revealed that the calibration curve was linear with an R^2 value = 0.9999, a slope of 0.1093 and a y-intercept of 0.0026 yielding a curve of $y = 0.1357x + 0.0015$ and this is depicted in Figure 2.12.

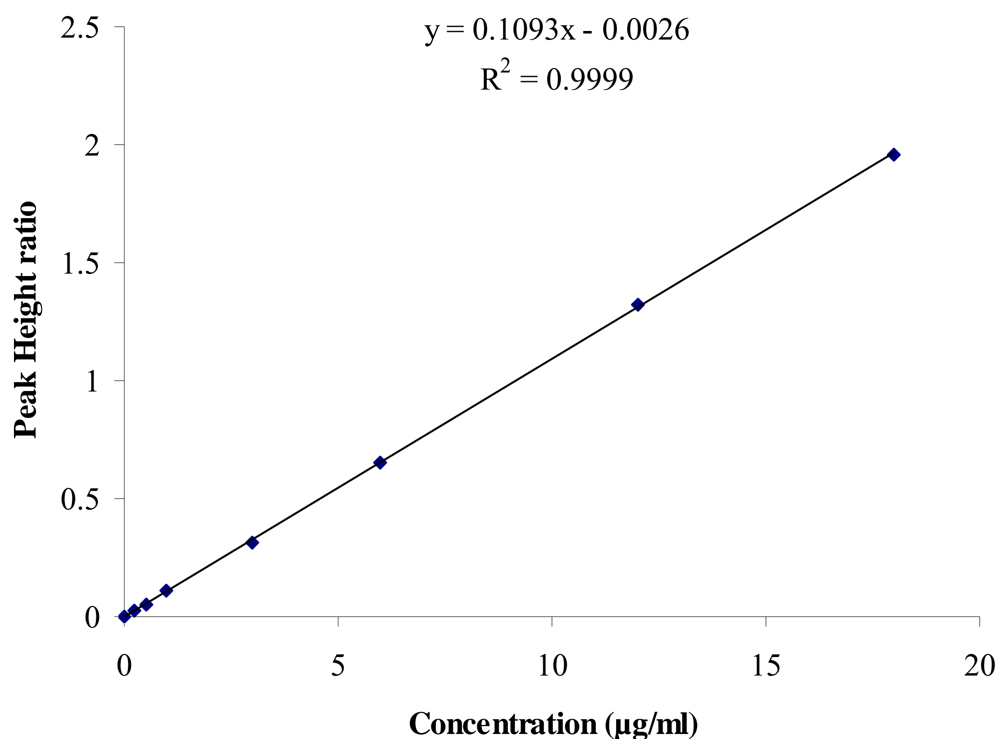


Figure 2.12. Calibration curve for CP following revalidation of the method

2.5.3. Precision

2.5.3.1. Repeatability

Repeatability was determined by calculating the coefficient of variation of the peak height ratios of CP to BV of the calibration standards (n =5). The data summarized in Table 2.10 revealed that in all cases, the relative standard deviations were less than 5%, indicating that the analytical method for the analysis of CP was precise in terms of the repeatability criterion.

Table 2.10. Repeatability data for the revalidation of the HPLC method for CP

Conc. (µg/ml)	MPHR* (n = 5)	SD	% RSD
0.250	0.0260	0.000400	1.47
1.00	0.127	0.000600	0.460
6.00	0.883	0.00180	0.200
12.0	1.56	0.00230	0.150
18.0	2.47	0.00580	0.230

* Mean peak height ratio of CP/internal standard

2.5.3.2. Intermediate precision

Intermediate precision was assessed over a period of three consecutive days and was expressed as coefficient of variation (% RSD) of the peak height ratios of the calibration standards (n = 5). The data summarized in Table 2.11 revealed that all % RSD values were less than 5%, indicating that the analytical method conforms to the requirements for intermediate precision.

Table 2.11. Intermediate precision data for the revalidation of the HPLC method for CP

Day	Conc. (µg/ml)	MPHR* (n = 5)	SD	% RSD
	0.250	0.0260	0.000400	1.47
	1.00	0.127	0.000600	0.460
1	6.00	0.883	0.00180	0.200
	12.0	1.57	0.00230	0.150
	18.0	2.47	0.00580	0.230
	0.250	0.0281	0.000400	1.59
	1.00	0.128	0.000400	0.330
2	6.00	0.720	0.000300	0.050
	12.0	1.64	0.00170	0.100
	18.0	2.52	0.00120	0.0500
	0.25	0.0326	0.000700	2.08
	1.00	0.133	0.000300	0.260
3	6.00	0.712	0.000200	0.0300
	12.0	0.542	0.00110	0.0700
	18.0	2.59	0.00350	0.140

* Mean peak height ratio of CP/internal standard

2.5.3.3. Reproducibility

Reproducibility studies were not conducted for the same reasons as discussed in Section 2.4.3.3.

2.5.4. Accuracy

Accuracy was assessed at three concentrations *viz.,* 1.5 μg/ml (low), 7.5 μg/ml (medium) and 15 μg/ml (high), (n = 5) and was reported as % recovery, % RSD and % Bias. The data summarized in Table 2.12 show that the analytical method can be considered accurate for the analysis of CP in these studies.

Table 2.12. Accuracy data for the revalidation of the HPLC method for CP (n = 3)

Theortical conc. (μg/ml)	Actual conc. (μg/ml)	SD	% RSD	% Bias	% Recovery
1.50	1.46	0.00200	0.120	-2.39	97.33
7.50	7.47	0.0140	0.230	-0.360	99.60
15.0	14.6	0.0270	0.190	+2.84	97.20

2.6. APPLICATION OF THE ANALYTICAL METHOD

Following development and validation studies, the RP-HPLC method was applied to the quantitative determination of CP in samples obtained following *in vitro* release testing of CP proprietary cream product (Dermovate®, Glaxo Wellcome SA (Pty) Ltd, Midrand, RSA). A typical *in vitro* release profile (n = 6) generated during these studies is depicted in Figure 2.13.

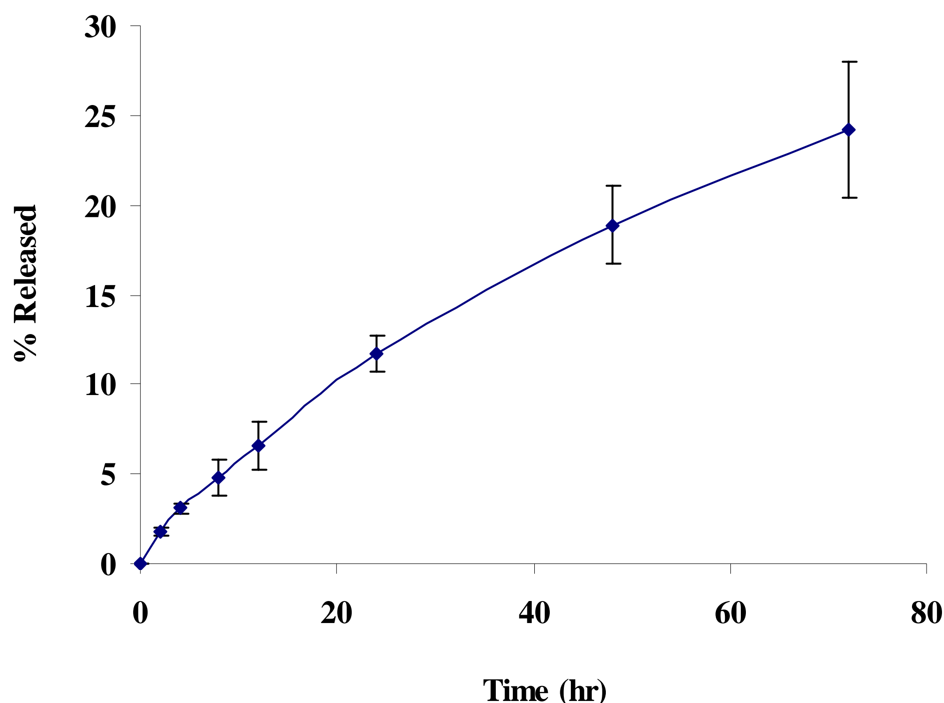


Figure 2.13. *In vitro* release profile of CP

2.7. CONCLUSIONS

A suitable RP-HPLC analytical method with UV detection at 240 nm has been developed, optimized and validated for use in the *in vitro* analysis of CP in topical formulations. Method development studies included the selection of a suitable detection wavelength, analytical column, and internal standard (I.S.), as well as the selection of mobile phase components.

The ultraviolet (UV) absorption spectrum of CP revealed that the wavelength of maximum absorption (λ_{max}) of CP is 240 nm. Consequently, the UV detector in these studies was set at 240 nm. The selection of the analytical column was based on the physicochemical properties of CP, the type and size of column packing materials, column dimensions and the column efficiency as determined by the number of theoretical plates (*N*). Based on these features a Nova-Pak® C_{18} 4 µm (150 mm x 3.9 i.d.) cartridge column was selected as a suitable column for the analysis of CP.

Betamethasone 17-valerate (BV) was selected as the most suitable I.S. from amongst the various compounds that were tested. Analysis using RP-HPLC is best achieved with non-polar stationary phases such as n-octadecylsilane (ODS or C_{18}) and with mobile phases consisting of a polar solvent *i.e.* water or a mixture of water and water-miscible organic modifier such as methanol (MeOH) and/or acetonitrile (ACN). A binary mixture of water and an organic modifier was considered the most suitable mobile phase composition and ACN was chosen over MeOH as the most appropriate organic modifier.

The method was optimized by manipulation of ACN concentration in the mobile phase and mobile phase flow rates of the optimal mobile phase composition selected. The effects of mobile phase composition and flow rate on retention time and resolution of CP and BV were assessed. The separation of CP and BV was best achieved using a mobile phase composition consisting of a binary mixture of ACN-water (50:50) and a mobile phase flow rate of 1 ml/min. These chromatographic conditions resulted in retention times of 5.6 min and 8.2 min for BV and CP respectively, and the total run time for sample analysis was within 10 min.

The RP-HPLC analytical method was validated in terms of the guidelines recommended by various organizations such as the United States Pharmacopeia (USP) [23], the United States Food and Drug Administration (FDA) [84] and the International Conference on Harmonization (ICH) [85, 86]. Based on these guidelines, the analytical method was found to be linear, precise, accurate, selective and sensitive and CP was found to be stable in acetonitrile and 50% w/w propylene glycol-water binary mixture following storage at 4˚C and room temperature (22˚C), respectively, for a maximum of 14 days and 3 days, respectively.

Following changes to the modular HPLC system used to develop, optimize and validate the RP-HPLC analytical method described herein, a mini-revalidation of the analytical method was carried out so as to ensure that the method maintained its performance characteristics as reported prior to the implementation of the changes. The data from revalidation studies revealed that the analytical method was linear, accurate, and precise for the *in vitro* analysis of CP in semi-solid dosage forms.

A RP-HPLC has been developed, optimized and validated RP-HPLC for the quantitation of CP in cream formulations and can be applied to the assessment of *in vitro* performance of CP in pharmaceutical semi-solid dosage forms

CHAPTER THREE

DEVELOPMENT AND VALIDATION OF AN *IN VITRO* TEST METHOD FOR THE ASSESSMENT OF CLOBETASOL 17-PROPIONATE RELEASE FROM TOPICAL CREAM FORMULATIONS

3.1. INTRODUCTION

In vitro dissolution testing of solid oral dosage forms is a well-established technique used to assess drug release from tablets and capsules for the purposes of assessing quality [100, 101]. Dissolution testing is considered the single most valuable *in vitro* test method that can be used to guide formulation development, assess product quality and ensure batch-to-batch uniformity [102]. In addition, *in vitro* dissolution testing may be used as a pre-formulation tool and it has been suggested that dissolution testing is a useful surrogate measure for the prediction of bioavailability for extended-release oral dosage forms using pre-established *in vitro-in vivo* correlations (IVIVC) [101, 103].

Official dissolution test methods have been developed and reported for *in vitro* dissolution studies of solid oral dosage forms and for the investigation of *in vitro* release characteristics of active pharmaceutical ingredients (API) from transdermal patches [22, 23]. However, there are currently no official guidelines or requirements for the performance evaluation of drug release from semi-solid dosage forms [104, 105]. Nevertheless, a variety of physical and chemical quality control tests have been traditionally used to provide reasonable evidence of consistent product performance [49]. These tests include the determination of solubility, particle size and size distribution and crystalline form of an API and evaluation of the intrinsic viscosity and homogeneity of a final product [49, 104-108].

These tests, however, provide little information about the drug release characteristics from the product or the effects of processing and manufacturing variables on the performance of a finished product [106]. As an API must first be released from a formulation and then permeate through the *stratum corneum* in order to exert a therapeutic effect [109], it may be more

appropriate to use *in vitro* drug release characterization in conjunction with traditional quality control tests to determine the quality and consistency of topical formulations that are manufactured during product development studies [109].

The FDA issued a Guidance document [49] in 1997, in which issues relating to scale-up and post-approval formulation changes of semi-solid dosage forms were addressed [49]. This guidance document outlines the requirements that the pharmaceutical industry must meet to maintain certification of previously approved semi-solid dosage forms, following post-approval formulation changes [49]. A key element in the guidance document is the requirement that an *in vitro* release test method be used to determine if the diffusional rate of release of a drug from a formulation is the same following changes to that formulation as it was prior to the implementation of the changes being made [49].

Despite the issuance of this Guidance document [49] by the FDA, an official *in vitro* release test is still not mandatory and is not applied industry-wide as a quality control test when compared to the utility and requirements for *in vitro* dissolution testing of solid and liquid oral dosage forms [107]. This is more than likely due to the fact that a universally acceptable *in vitro* release test protocol that can be applied to all semi-solid dosage forms has yet to be established [108, 110]. With the exception of the recommendations of the FDA, there are currently no apparatus, procedures or requirements that have been described in any pharmacopoeias for *in vitro* release test of topical semi-solid preparations [103, 107, 110, 111].

Another point relevant to *in vitro* release testing is that it cannot be considered as a surrogate test for establishing bioequivalence of a generic product relative to an innovator semi-solid formulation [49, 107, 112]. This is more than likely due to the fact that at this time there is no convincing evidence for IVIVC of release tests for semi-solid dosage forms as that for *in vitro* dissolution of tablets and capsules [49]. Moreover, measurement conditions during *in vitro* release testing do not usually mimic physiological reality since *in vitro* release test equipment does not include a membrane resembling the *stratum corneum* and the barrier functions of the skin, which are essential determinants of the skin penetration characteristics of an API of interest in semi-solid dosage form bases [113].

The type of membrane usually chosen for *in vitro* release testing should have the least possible diffusional resistance, whereas, clinically dosage forms are applied to the *stratum corneum,* a high resistance membrane that, when intact invariably controls the delivery rate of API from semi-solid dosage forms [112]. Furthermore it should be noted that *in vitro* release testing is performed in a manner in which the dosage form being tested is applied so that an infinitely thick layer is present, which is in stark contrast to the thin films usually applied during conventional clinical use of these products [114].

Nevertheless, many manufacturers of topical drug products have devoted significant resources to developing and validating *in vitro* release tests during the product development process [108]. Such tests can be used to detect the effects of changes in a formulation on the release rate of API from a dosage form in which the API is suspended and/or dissolved and therefore can be used to ensure that the manufacture of semi-solid products is consistent and fulfils a similar role as dissolution testing does for tablets and capsules [115].

The objective of these studies was therefore to develop and validate a reliable, reproducible and discriminatory *in vitro* release test method. The method would be applied in formulation development studies in order to assess product quality and ensure batch-to-batch consistency of topical formulations manufactured to contain 0.05% w/w clobetasol 17-propionate (CP).

3.2. METHOD DEVELOPMENT

3.2.1. Overview

Various protocols that may be used in the development of an *in vitro* release test method for a semi-solid drug product have been outlined and reported in the literature [108, 113, 115]. Essentially, method development studies are designed to:

a) facilitate the selection of a diffusion cell system,
b) determine an appropriate receptor medium with adequate sink characteristics,
c) evaluate synthetic support membranes with minimal reactivity with the drug and formulation components, and
d) determine appropriate sampling times [108, 113, 115].

In addition, conditions such as finite or infinite dosing and sample occlusion or non-occlusion that may have considerable effects on the *in vitro* release characteristics of a drug should be investigated and optimized during method development studies [115]. During *in vitro* release method development studies, the aforementioned criteria were evaluated to produce a test system for the assessment of CP release from topical semi-solid formulations.

3.2.2. Diffusion cell test system

3.2.2.1. Overview

In vitro dissolution testing in the pharmaceutical industry is a widely used tool in formulation development and quality control testing [111]. Consequently as dissolution testing has evolved different apparatus have become official and have been included in the United States Pharmacopoeia (USP) [23] and the British Pharmacopoeia (BP) [22] for such purposes. The USP [23] for example makes provision for the dissolution testing of tablets and capsules, specifying and describing official apparatus as USP Apparatus I (basket), USP Apparatus II (paddle), USP Apparatus III (reciprocating cylinder or Bio-Dis®) and USP Apparatus IV (flow-through). In

addition, the USP [23] specifies and describes methods and equipment for transdermal delivery patch systems such as USP Apparatus V (paddle over disk), USP Apparatus VI (cylinder) and USP Apparatus VII (reciprocating holder).

Although the FDA Guidance document [49] outlines the general methodology and description of diffusion systems, no single device has been universally accepted for measuring *in vitro* drug release rates from semi-solid dosage forms. Due to the variety of formulation type, sites of application and release rates required from semi-solid dosage forms, a single test method would not be appropriate for the development, biopharmaceutical characterization and quality control of such formulations, and hence the inclusion of a single apparatus in pharmacopoeias may not be possible [111].

Numerous apparatus of different design have been reported in the literature for studying API diffusion from semi-solid dosage forms [23, 49, 102-107, 109-131]. The general design of the test systems is such that they consist of a donor cell in which a semi-solid material is placed and a receptor compartment in which a chosen receptor medium is contained and from which samples are withdrawn for analysis at appropriate intervals. The system may or may not include a membrane that separates the two compartments and would also include a mechanism by which the receptor fluid is stirred and a means to control the temperature of the receptor medium [113].

Several reviews have alluded to the many significant considerations that must be taken into account when designing such test devices [113, 115, 119, 124]. An *in vitro* diffusion cell must be made from an inert, non-reactive material such as, for example, glass, stainless steel or Teflon® [122]. All components of a cell, including flow-through lines and the collection chambers, must have their inert nature established by experimental means and it should be shown that there is no loss of drug due to volatility during the permeation test procedure [122]. If volatility of an API or receptor and/or product being tested is an issue, a quantitative account of the volatility must be undertaken and reported [113, 122].

A receptor medium must provide effective sink conditions for the appropriate assessment of the rate of release of a permeant, penetrant or API [115]. The cell in which the medium is placed

should ideally contain a minimum volume of fluid to facilitate analysis, since the more concentrated a drug in the collection medium is, the easier the analytical process [122]. The design of a cell must allow for efficient mixing of the receptor fluid at a controlled temperature [113, 122] and the system should be versatile and accommodate a diverse range of membranes that are routinely used in diffusion studies [124]. The current trend is towards the development and use of systems with automated operations with better instrument control and minimal instrument-related variability [128].

3.2.2.2. Franz diffusion cell

Studies using a diverse range of *in vitro* release systems have been reported [23, 49, 102-107, 109-131]. Of those reported the vertical diffusion cell, commonly known as the Franz and/or modified Franz cell has shown the most potential for use as a standardized system that may be adapted for use as a compendial test method [111, 113]. Initially designed and described by Franz in 1975 [132], the vertical Franz diffusion cell has been widely used to study *in vitro* drug release from semi-solid dosage forms [102, 107, 114-118, 121, 123, 126, 127, 129, 133-135].

The original Franz cell has a bichamber arrangement that includes a dumb-bell-shaped receptor compartment [133] and a schematic of this apparatus is shown in Figure 3.1. An unstoppered sampling port is connected to the upper section of the receptor chamber and only the central, cylindrical portion of the receptor compartment is jacketed. A stirrer bar is placed in the lower, ellipsoid bulb chamber to provide a means of agitation of the receptor medium.

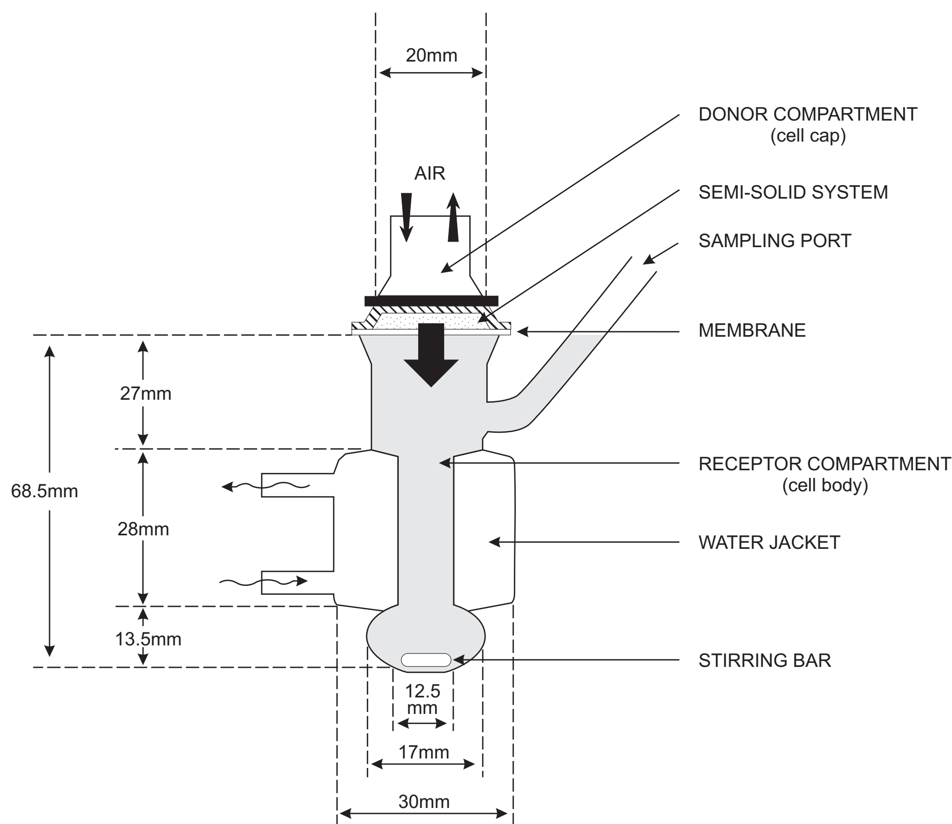


Figure 3.1. Schematic representation of an original Franz diffusion cell apparatus (redrawn from 127)

3.2.2.3. Modified Franz diffusion cell

Keshary and Chien [127, 136] identified and reported deficiencies associated with use of the original Franz diffusion cell. They reported that the design of the Franz cell did not provide for adequate solution hydrodynamics, mixing efficiency and temperature control that are necessary for quantitative permeation studies. Due to these shortcomings, Keshary and Chien [127, 136] proposed several modifications to the original design of the Franz cell. A schematic representation of a modified Franz diffusion cell is illustrated in Figure 3.2.

The diffusion cell has a cylindrical receptor compartment that is shorter than the receptor compartment of the original Franz cell (Figure 3.1) and is completely enclosed by a water jacket. A star-head magnetic stirrer as opposed to a stirrer bar is used to agitate the receptor fluid. As a result of these modifications, the equilibrium temperature of the receptor fluid is better

maintained, while solution-mixing efficiency is improved and the thickness of boundary diffusion layer is reduced [127].

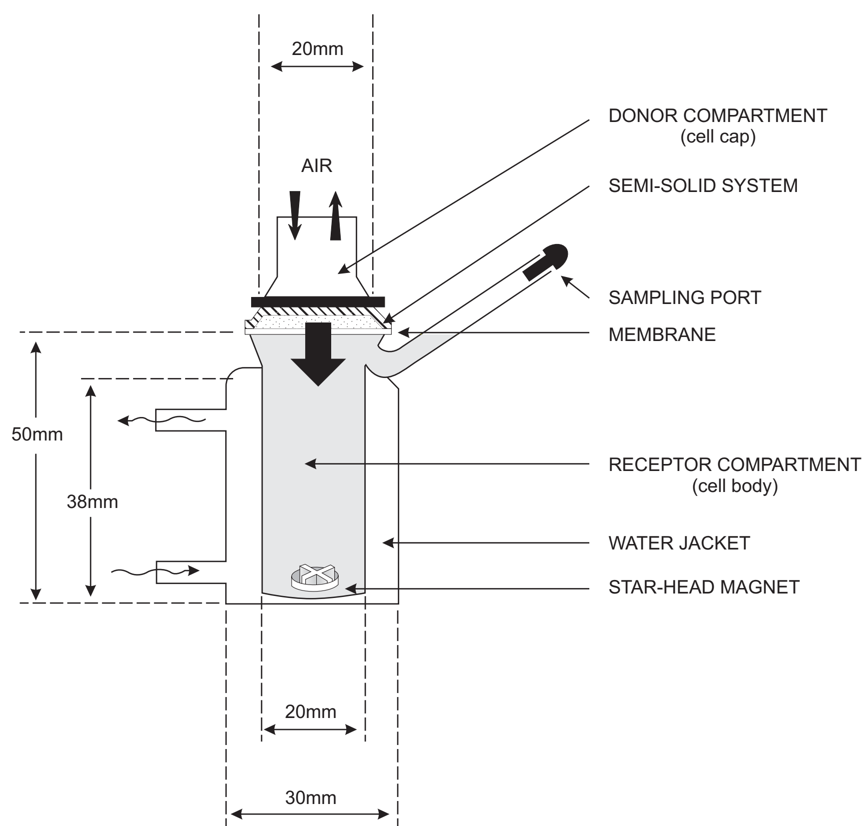


Figure 3.2. Schematic representation of a modified Kenshary-Chien Franz glass diffusion cell (redrawn from 127)

3.2.2.4. Selection of diffusion cell test system

A modified Franz glass diffusion cell system (Crown Glass Company Inc., Branchburg, NJ, USA) was selected for use in these studies. Each jacketed cell has an opening of 15 mm with an associated area available for diffusion of 1.767 cm^2 and a receptor fluid volume of 12.5 ml. The cells were positioned in a multiple-cell drive unit (Figure 3.3), which permitted efficient stirring with a 2x2 mm star head magnetic stirrer bar (Merck Chemicals (Pty) Ltd, Darmstadt, Germany) agitating the receptor medium at a controlled rate. The jacketed portions of the multiple cells were connected in series to a temperature-controlled circulating water bath (Grant Instruments Ltd, Cambridge, England) with Tygon® tubing (Eagle Electric, Cape Town, Western Cape, RSA).

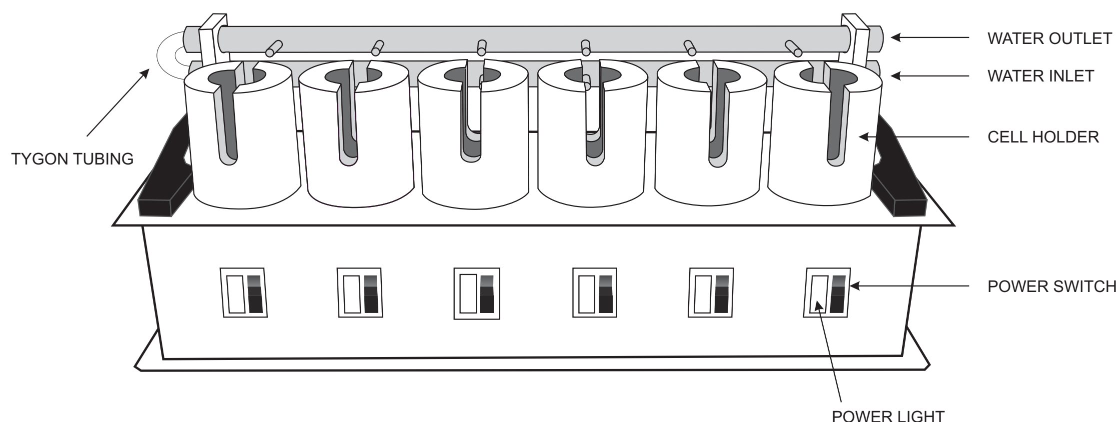


Figure 3.3. Schematic representation of a modified Franz cell multiple-cell drive unit

3.2.3. Number of samples

In vitro release testing of CP from topical formulations was conducted using a modified Franz glass diffusion cell system, containing housings for six cells (Figure 3.3). Although systems with three cells have been used [126, 129], a minimum of six cells or samples is recommended when characterizing release rate profiles of an API from semi-solid products [49]. The use of six samples is recommended so as to minimize the variability associated with individual sample application to the donor compartments of diffusion cell systems [108].

3.2.4. Sampling times

The FDA species that at least five sampling times must to be used over a suitable time period in order to ensure that an adequate *in vitro* release profile is generated and can be used to determine API release rates from semi-solid products [49]. Sampling times may be varied depending on the formulation matrix, but in most cases samples can be taken over the course of one day, unless the release of the drug from the formulation is extremely slow or a sustained or controlled-release profile is expected [115].

It has been reported that there is a specific time window during which samples for release experiments should be taken [113]. Ideally, samples should be removed at times when the influence of the membrane and its associated stagnant layer disappears and before excessive drug depletion from the semi-solid dosage form being tested has taken place [17]. Two main reasons

have been put forward in an attempt to explain the rationale behind withdrawing samples during this time window.

Firstly, it has been reported that the migration of an API from a semi-solid matrix to a receptor medium occurs as a series of successive diffusional steps [137, 138]. In the initial stages the membrane, together with an unstirred layer at its surface, have been reported to provide some resistance to the permeation of API and thus affect the release rate of an API [113]. However, over time, a diffusional layer is formed within the semi-solid matrix and thus becomes the rate-controlling element [113]. As a consequence, samples should be withdrawn when the influence of the membrane and its associated stagnant layer has disappeared [113], or otherwise the early sampling time points should be ignored when calculating API release rates derived from such systems [113].

Secondly, according to theoretical diffusion models a plot of the amount of API released *vs.* the square root of time should be linear if drug release from a semi-solid matrix is diffusion controlled or diffusion is the rate controlling element within the dosage form [137, 138]. However, it has been shown that API release from semi-solid dosage forms tends to deviate from linearity at extended times [113] and the deviation is usually observed when more than approximately 35-45% of the API in the dosage form has been released from the semi-solid sample placed in the donor chamber [113]. Therefore, when choosing the upper time limit for sample collection, this factor must be taken into consideration [113].

Preliminary *in vitro* release studies were undertaken over a three-day period using a commercially available CP cream product *viz.,* Dermovate® cream (Glaxo Wellcome SA (Pty) Ltd, Midrand, Gauteng, RSA), a 0.1 μm Polycarbonate membrane filter (Millipore Co., Bedford, MA, USA) and a receptor phase consisting of 50% v/v propylene glycol/water mixture (Section 3.2.6.2.3.3 and Section 3.2.6.3).

The data generated from these studies are shown in Figure 3.4. These data reveal that approximately 3 % of CP in the semi-solid sample applied to the membrane was released after 2 hours. Therefore, it appears that two (2) hours was an adequate time for the influence of the

membrane and its associated stagnant layer to disappear, as the plot does not appear to show a significant lag phase. Approximately 32% of CP contained in the formulation applied to the membrane was released at the end of the 72-hour test period and this value is below the 35-45%, after which non-linearity of release was expected to occur [17].

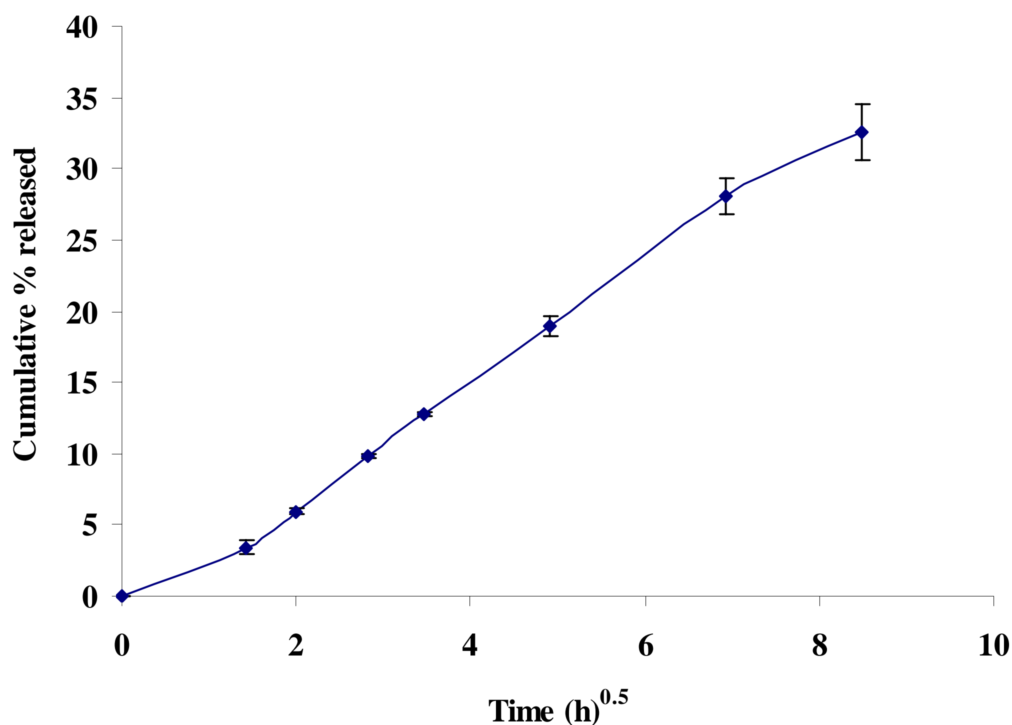


Figure 3.4. *In vitro* release of CP from Dermovate® cream (n = 6)

Least squares linear regression analysis was used to determine the linearity of the *in vitro* release profile for CP during the time period of 2 to 72 hours and the resultant coefficient of determination (R^2) of 0.9962, shown in Figure 3.5, reveals that a plot of % CP released *vs.* square root of time was linear. In light of the constraints relating to the monitoring of API release from semi-solid dosage forms, samples of the receptor medium were removed at 2, 4, 8, 12, 24, 48 and 72 hours in order to generate satisfactory CP release profiles and to characterize the mechanism and type of release of CP from semi-solid dosage forms.

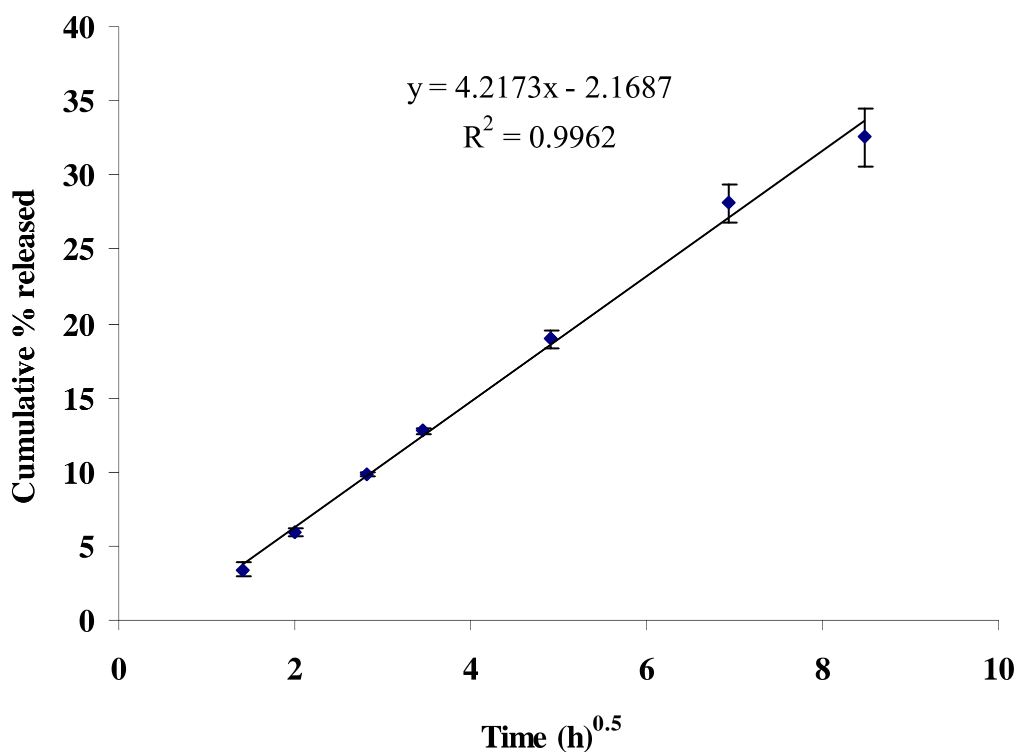


Figure 3.5. Least squares linear regression best fit line of the *in vitro* release profile of CP from Dermovate® cream (n = 6)

3.2.5. Temperature

The temperature of the receptor medium is generally set to 32 ± 0.5˚C in an attempt to approximate the natural surface temperature of the skin [102, 107, 111, 113, 115, 122, 123, 126]. An increase in temperature normally results in an increase in drug release rate and hence some deviation from using 32˚C, for example the use of 37˚± 0.5˚C may be justifiable. However the use of excessively high temperatures may melt the base or the product being tested or cause significant physical changes to such a product, which in turn may change the resistance of the matrix to drug diffusion into the receptor medium and should therefore, be avoided [113].

In vitro release experiments conducted at 32°C and 37˚C showed no significant differences in the *in vitro* release rates of triamcinolone acetonide from various commercial cream and ointment formulations [106]. Therefore the *in vitro* release profile characterization of CP release from semi-solid formulations manufactured in these studies was conducted at 32° ± 0.5˚C.

3.2.6. Receptor medium

3.2.6.1. Overview

One of the most important considerations when developing an *in vitro* release test method is the selection of a receptor medium that has adequate sink characteristics throughout the course of an experiment [115]. In order to achieve favourable sink conditions and to ensure that the lower surface of the membrane remained in contact with the receptor phase over the entire experimental time period, each diffusion cell was completely emptied and refilled immediately with fresh receptor fluid at each sampling time.

However, it is also worth noting that a receptor medium must have a high capacity to dissolve the active ingredient that has been released from the formulation being tested [112]. This may be accomplished by maintaining the thermodynamic activity of an API in a receptor fluid at less than 10% of its thermodynamic activity in a donor medium, thereby maintaining a favourable driving force for permeation and ensuring reasonable and efficient transfer and collection of the permeant of interest [122]. Therefore, another important factor to consider when selecting a receptor fluid is the solubility of an API being tested in a receptor fluid or medium [108]. It is therefore critical that a drug substance has sufficient solubility in a receptor medium without impacting on the sink conditions of that system [121].

Ideally, receptor media should be aqueous systems [115] and for most studies isotonic solutions, buffered to a pH of 7.4 are preferred for use as receptor fluids [122]. However, for products formulated with water-insoluble drugs, the selection of an appropriate receptor medium to maintain sink characteristics is a challenge [121], and in order to facilitate and monitor drug release from such topical formulations it may be necessary to alter receptor fluid pH, add surfactants and/or complexing agents such as cyclodextrins [115] or use non-aqueous media in which the drug is more soluble to efficiently dissolve the API that has been released from the matrix during release studies [122].

3.2.6.2. Selection of receptor medium

3.2.6.2.1. Aqueous systems

CP is a hydrophobic corticosteroid with a limited aqueous solubility (Chapter 1, Section 1.3.1.1). Consequently, the use of a purely aqueous receptor fluid was not considered. In addition, since the drug is non-ionisable (Chapter 1, Section 1.3.2) altering the pH of an aqueous receptor solution would not have an effect on its aqueous solubility and therefore the use of other solvent systems was considered appropriate.

3.2.6.2.2. Water-immiscible systems

The hydrophobic nature of CP and the associated difficulties in selecting an appropriate receptor medium might persuade researchers to use non-aqueous solvents such as acetone, chloroform or dichloromethane in which the drug would be freely soluble as the receptor medium (Chapter 1, Section 1.3.1.1). However, the use of such media in release studies is undesirable since these solvents are likely to interfere with the analytical method of choice, especially if the analytical procedure requires direct injection of the receptor medium onto the HPLC system as was desired in these studies.

3.2.6.2.3. Water-miscible systems

3.2.6.2.3.1. Overview

A further practical consideration in the selection of an appropriate receptor medium is to consider the use of water-miscible solvents such as ethanol and propylene glycol (PG) or propane-1,2-diol. Ethanol, PG and aqueous solutions of various concentrations are widely used as solvents in pharmaceutical formulations and cosmetics [139, 140]. Ethanol is a clear, colourless, mobile and volatile liquid with a slight characteristic odour and burning taste [139] and PG is a clear, colourless, viscous, practically odourless solvent with a sweet, slightly acrid taste resembling that of glycerine [140].

Ethanol has a specific gravity or density of 0.8119-0.8139 g/cm^3 at 20˚C [139] and the specific gravity of PG is 1.038 g/cm^3 at 20˚C [140]. These materials are generally regarded as relatively non-toxic and chemically stable when stored in well-closed containers at cool temperatures [139, 140]. Since CP is relatively soluble in ethanol and PG (Sections 1.3.1.1 and 1.3.1.2.1, Chapter 1), the use of ethanol and PG aqueous mixtures as potential receptor fluids for CP was considered in preliminary studies.

3.2.6.2.3.2. Alcohol/water mixtures

The use of hydro-alcoholic solutions as receptor media for lipophilic drugs such as triamcinolone acetate [106, 126], betamethasone dipropionate [121] and rooperol tetra-acetate [123] has been reported. Consequently, ethanol ABS (Protea Chemicals, Port Elizabeth, Eastern Cape, RSA) at a concentration of 30% v/v was assessed as a receptor medium for CP, using Silatos™ silicone sheeting, REF 7458 (Atos Medical, Hőrby, Sweden) as a supporting membrane.

The data generated from these studies are illustrated in Figure 3.6., and are reported as the cumulative amount of CP released per unit area (Q) *vs.* time (t). However the use of aqueous ethanol solutions as a receptor medium for CP were not considered ideal because of the presence of excessive air bubbles that were observed beneath the synthetic support membrane. Air bubbles have been reported to interfere with the contact between the receptor medium and the supporting membrane that is required, resulting in a reduced surface area available for drug diffusion to occur [121].

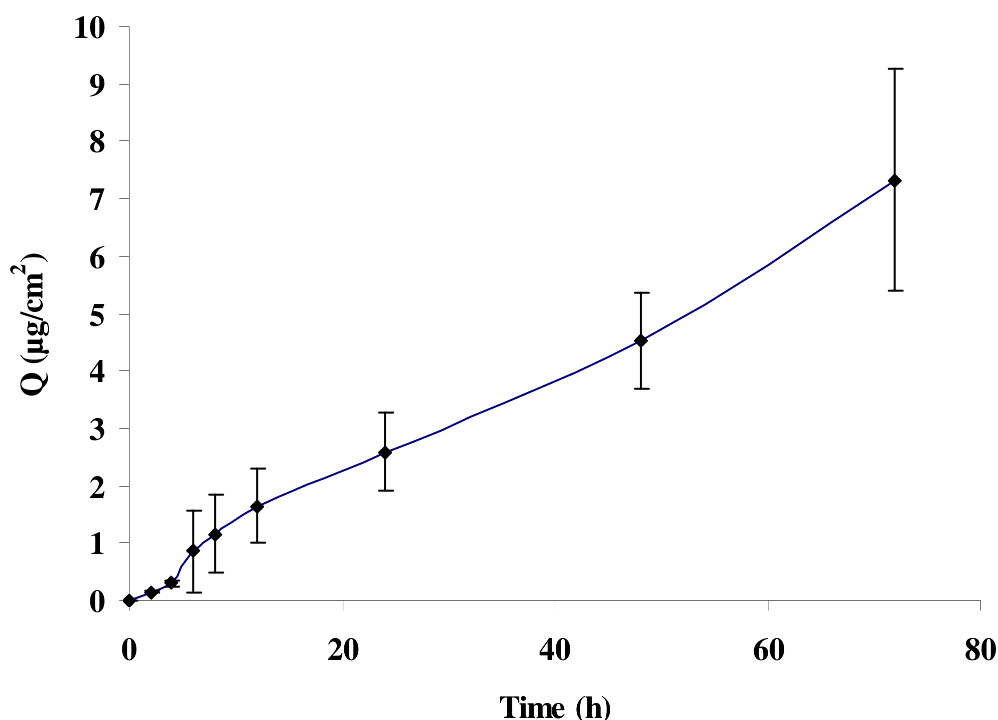


Figure 3.6. *In vitro* release profile of CP from Dermovate® cream, using 30% v/v ethanol solution as receptor medium (n = 6)

Visual inspection of the Franz cells at the end of the experiment also revealed noticeable evaporation of the receptor medium, which resulted in a decrease in the volume of the receptor fluid in some cells. The extent of evaporation of the receptor medium might have contributed to the large standard deviations observed at the longer sample times such as 48 and 72 hours.

3.2.6.2.3.3. Propylene glycol/water mixtures

The use of PG as a receptor fluid has also been reported [141]. Therefore in preliminary studies the *in vitro* release of CP through Silatos™ silicone sheeting, REF 7458 (Atos Medical, Hőrby, Sweden) into a 30% v/v PG:water receptor medium was evaluated and the data from these studies are shown in Figure 3.7. Unlike aqueous ethanol, the use of 30% v/v PG did not lead to the formation of excessive of air bubbles beneath the surface of the membrane used in these studies. Inspection of the Franz cells at the end of the experiment also revealed little evidence of

receptor medium evaporation and therefore this solution was considered promising as an appropriate receptor medium for CP release studies.

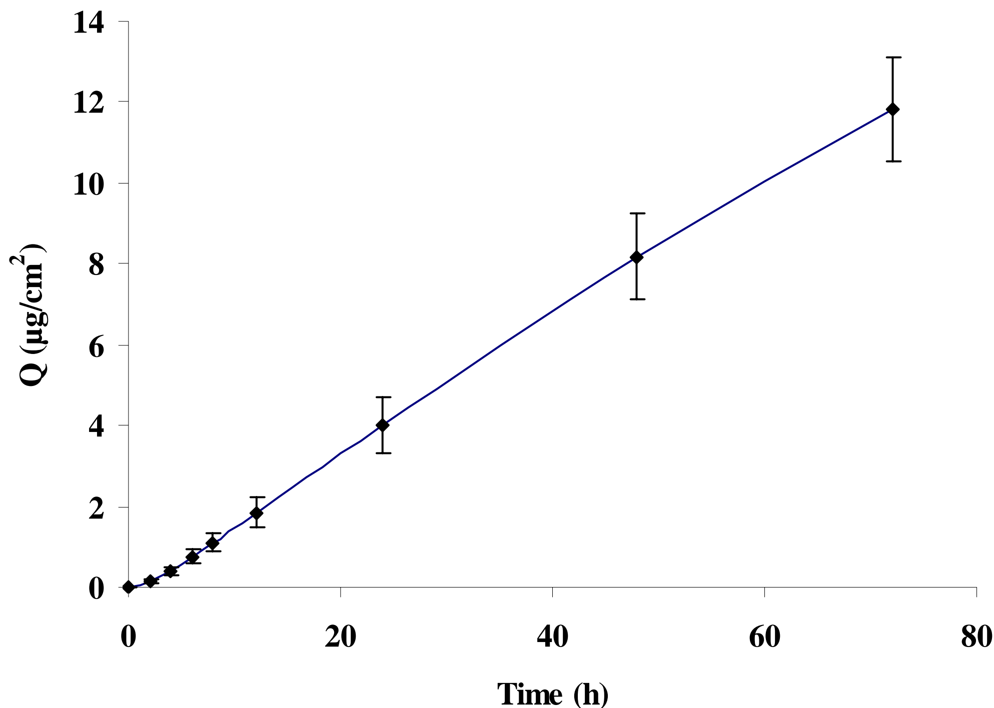


Figure 3.7. *In vitro* release profile of CP from Dermovate ® cream using 30% v/v PG solution as receptor medium (n = 6)

3.2.6.3. Saturation solubility

Following the selection of 30% v/v PG:70% v/v water as the most promising receptor medium for CP release studies, saturation solubility studies of CP in water, PG and PG:water binary mixtures were conducted in order to determine the optimal concentration of PG for use in a receptor medium for CP.

The saturation solubility of CP was determined in 0, 30, 40, 50, 60 and 100% v/v PG. Approximately 50 mg of CP was accurately weighed and placed into a test tube and 2.0 ml of the different solvents to be tested were added. Samples were agitated at 120 oscillations per minute

in a Model-123 Labotec Oscillating water bath (Labotec (Pty) Ltd, Johannesburg, Gauteng, RSA), maintained at 32°C for 48 hours.

The solutions were then filtered through a 0.22 μm Millipore filter (Millipore Co., Bedford, MA, USA) and a 1.0 ml aliquot of the filtrate was diluted to 10 ml with acetonitrile and analysed using a specific and sensitive, previously validated HPLC method with UV detection at 240 nm (Chapter 2, *vide infra*).

A list of the experimentally determined saturation solubility values for CP in various solutions of different concentrations of PG concentrations is listed in Table 3.1, and the saturation solubility profile of CP in the various solutions, plotted as log solubility *vs.* PG concentration, is depicted in Figure 3.8.

Table 3.1. Solubility data for CP in various concentrations of PG at 32˚C (n = 3)

PG concentration (%v/v)	Mean saturation solubility (mg/ml)	SD
0	0.003600	0.0009000
30	0.01760	0.001680
40	0.05880	0.0007800
50	0.1983	0.0002900
60	1.334	0.2300
100	8.550	2.500

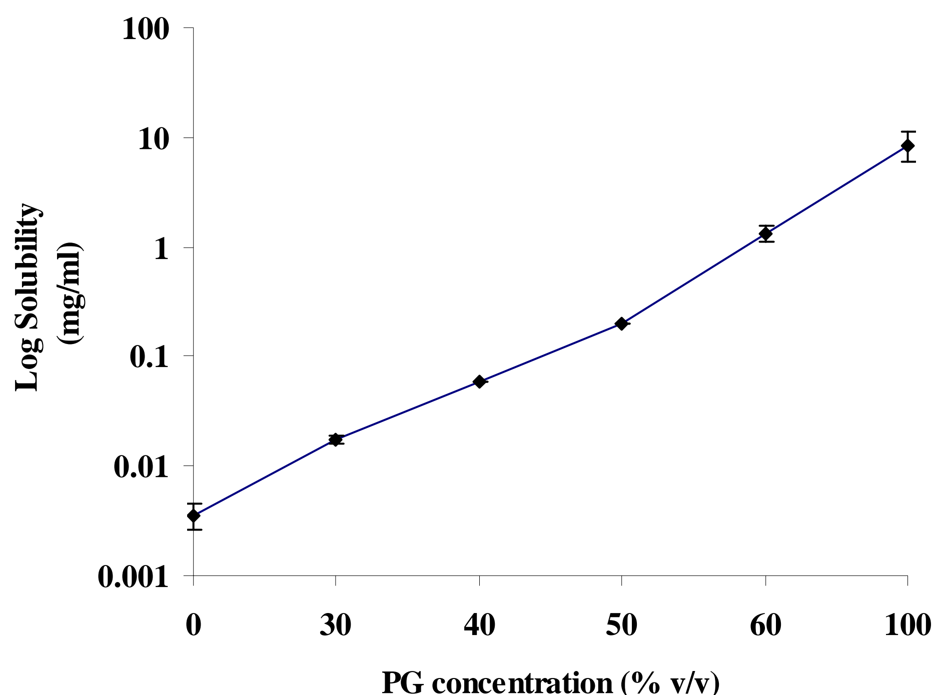


Figure 3.8. Saturation solubility profile of CP in PG:water solutions of different proportions (n = 3)

The resultant data show that CP is poorly soluble in water, but relatively soluble in PG. It is clear that as the PG content of the dissolution or receptor medium is increased, the saturation solubility of CP also increases. The objective of conducting these studies was to determine the saturation solubility of CP in a PG concentration that was greater than ten (10) times that of the maximum achievable concentration that may be attained during the course of *in vitro* release experiments.

In the pilot study conducted and reported in Section 3.2.6.2.3.3 (Figure 3.7), approximately 300 mg of 0.05% w/w Dermovate® cream was applied to the synthetic membrane and the volume of the receptor medium used was 12.5ml. Assuming that CP is freely soluble in the receptor medium, the theoretical maximum concentration that would be achieved during the course of an *in vitro* release study is calculated to be 12 µg/ml.

The saturation solubility data summarized in Table 3.1 reveal that CP has a saturation solubility of 198.27 ± 0.29 µg/ml in a 50% v/v PG:water solution, corresponding to nearly 16.5 times the estimated maximum concentration of 12 µg/ml that could be achieved. Therefore based on the

saturation solubility of CP in this solvent system and the fact that the receptor medium was replaced at each sampling time during the course of the experiment, it was considered that adequate sink conditions would prevail for the duration of all studies. Therefore a 50% v/v PG:water solution was selected as an appropriate receptor phase for use in future *in vitro* release studies of CP creams.

3.2.6.4. Preparation of the receptor medium

The receptor medium was prepared by carefully adding equal parts by volume of propane-1,2-diol (Merck Chemicals (Pty) Ltd, Darmstadt, Germany) and HPLC-grade water generated from a Milli-RO® 15 water purification system (Millipore Co., Bedford, MA, USA), to a Schott Duran® glass solvent mixing bottle (Schott Duran GmbH, Hattenbergstrasse, Germany) using a measuring cylinder. Following mixing, the mixture was allowed to equilibrate to room temperature and then the receptor phase was degassed under vacuum with the aid of a Model A-2S Eyela Aspirator (Rikakikai Co., Ltd, Tokyo, Japan) and filtered through a 0.45 µm Millipore® HVLP filter (Millipore Co., Bedford, MA, USA) prior to use. The receptor phase was degassed in order to remove dissolved air, thereby minimising the potential formation of air bubbles beneath the supporting membrane, which have been reported to adversely affect the *in vitro* release of an API from semi-solid dosage forms [122].

3.2.7. Synthetic membranes

3.2.7.1. Overview

Synthetic membranes have been used extensively to determine the *in vitro* release characteristics of an API from topical formulations [102, 115, 117, 121, 134, 142]. These membranes are not intended to mimic the barrier properties or the heterogeneous nature of the skin [113]. Instead, they are designed to provide a physical support and maintain constant contact between the formulation and dissolution medium and to prevent bulk transfer of the dosage form, whilst allowing for the monitoring of API release from a formulation into a receptor medium [113, 115].

It has been reported that it is possible to study the *in vitro* release of a drug from rigid topical formulations such as ointments into an aqueous medium in the absence of a separating membrane [142]. However, creams and gels invariably contain an aqueous phase and/or adjuvant components that are aqueous in nature, are water-miscible or water-soluble. Therefore a membrane must be placed between the formulation being tested and the receptor medium in order to maintain the physical integrity of individual formulations and/or dosage forms [112].

3.2.7.2. Characteristics of membranes

Synthetic membranes that have been commonly used for *in vitro* release studies are those with porous characteristics such as cellulose acetate [102, 103, 107, 134], nitrocellulose [105, 121] and polycarbonate membrane filters [104, 105 123], or alternatively homogeneous permeable polymers such as silicone based filter membranes [102, 107, 129, 134].

Important considerations that must be taken into account when selecting a synthetic membrane for use in *in vitro* drug release experiments include the requirement for the membrane to be commercially available and have little or no capacity to bind the API of interest [112, 113]. In addition, the membrane should have low reactivity with formulation components, be compatible with the receptor medium and offer the least possible diffusional resistance to the permeant of interest [112, 113, 119, 125]. In short, the membrane of choice should be inert and provide a holding surface without barrier properties for the active ingredient and test formulation(s) [108].

The effects of several membranes on the *in vitro* release of CP from Dermovate® cream were investigated. The membranes tested were a non-porous synthetic membrane, Silatos™ silicone sheeting REF 7458 (Atos Medical, Hőrby, Sweden) and porous synthetic membranes such as Isopore™ Membrane Filters (Millipore Co., Bedford, MA, USA) and MF-Millipore™ Membrane Filters (Millipore Co., Bedford, MA, USA). The characteristics of the membranes used in these studies are summarized in Table 3.2.

Table 3.2. Summary of the characteristics of synthetic membranes

Membrane	Pore size (µm)	Thickness (µm)	Nature
Silatos™ (Silicone)	NA	120	Hydrophobic
Isopore™ (Polycarbonate)	0.10	20	Hydrophilic
Isopore™ (Polycarbonate)	0.40	20	Hydrophilic
MF-Millipore™ (Nitrocellulose)	0.025	105	Hydrophilic
MF-Millipore™ (Nitrocellulose)	0.45	150	Hydrophilic

3.2.7.3. Assessment of membranes

The effects on *in vitro* release of CP of each membrane were assessed using a modified Franz diffusion cell system (Section 3.2.2) and a receptor medium comprised of 50% v/v PG:water prepared as described in Section 3.2.6.3. The purpose of these studies was to determine the most suitable membrane to be used as a support synthetic medium for the assessment of semi-solid products containing 0.05% w/w CP. Each membrane was cut to approximately 30 mm in diameter to fit the circumference of the membrane holder on the diffusion cell system and was soaked in the receptor medium prior to use.

Cellulose membranes are believed to contain a number of water-soluble softeners, preservative and plasticizer additives which may affect drug permeation [143]. To ensure the removal of these substances, pieces of cellulose-based membranes were rinsed with distilled water prior to soaking in the receptor fluid. After one hour, the membranes were carefully removed from the receptor medium with the aid of a pair of tweezers, wiped with adsorbent tissue to remove excess surface liquid and mounted between the donor and the receptor compartments of the Franz cell assembly.

Parafilm 'M'® Laboratory Film (Pechiney Plastic Packaging, Chicago, IL, USA) was used to ensure that a leak-proof seal was formed between the flanges of the upper and lower components of the diffusion cell assembly that was held together with a screw clamp. Following equilibration of the cell to 32˚C an infinite dose of approximately 300 mg of the test formulation was applied evenly to the entire surface of the membrane with the aid of a glass rod and the final dose was determined by weighing the rod before and after application. The donor compartment was covered with Parafilm® in order to achieve occlusive conditions throughout the experiment.

The receptor chamber of each diffusion cell was filled with 12.5 ml of the receptor medium and stirred by means of a 2x2 mm star-head magnetic stirrer (Merck Chemicals (Pty) Ltd, Darmstadt, Germany). The receptor compartment was examined for the presence of air bubbles and if present bubbles were removed by manually tapping or rotating the diffusion cell apparatus assembly. Aliquots of receptor medium were removed at the appropriate sampling times as discussed in Section 3.2.4.

Following the removal of a sample to be analyzed, each diffusion cell was completely emptied and refilled immediately with fresh receptor fluid. Samples were stored at room temperature (22˚C) until analyzed. The samples were analyzed immediately after the conclusion of the release studies *i.e.* within three days (72 hours) of the commencement of the study. These samples were found to be stable at these storage conditions as investigated and reported in Chapter 2, Section 2.5.2.6.3.2.

At the conclusion of release experiments, no physical changes to any of the membranes tested were observed following exposure of the membrane to the PG-water receptor medium or the semi-solid formulation applied to the membrane. These results were considered indicative of the compatibility between the membranes and the receptor medium used, as well as the components of the topical formulation containing CP. A specific, sensitive and validated HPLC method with UV detection at 240 nm (Chapter 2) was used to analyze the samples and to determine the amount of drug released per unit area. The cumulative amount of CP released (Q) over the 72 hour time period was plotted against time.

The results obtained following membrane selection studies are depicted in Figure 3.9. It is immediately apparent that the *in vitro* release rate of CP through the silicone membrane was significantly slower than through any of the nitrocellulose and polycarbonate membranes tested. The highest amount of CP that was released was 56.37 ± 5.02 $\mu g/cm^2$ and was released through the 0.45 μm nitrocellulose membrane, whereas the lowest amount of 6.69 ± 1.11 $\mu g/cm^2$ was released through the silicone membrane. These data are discussed further in Section 3.2.7.4.

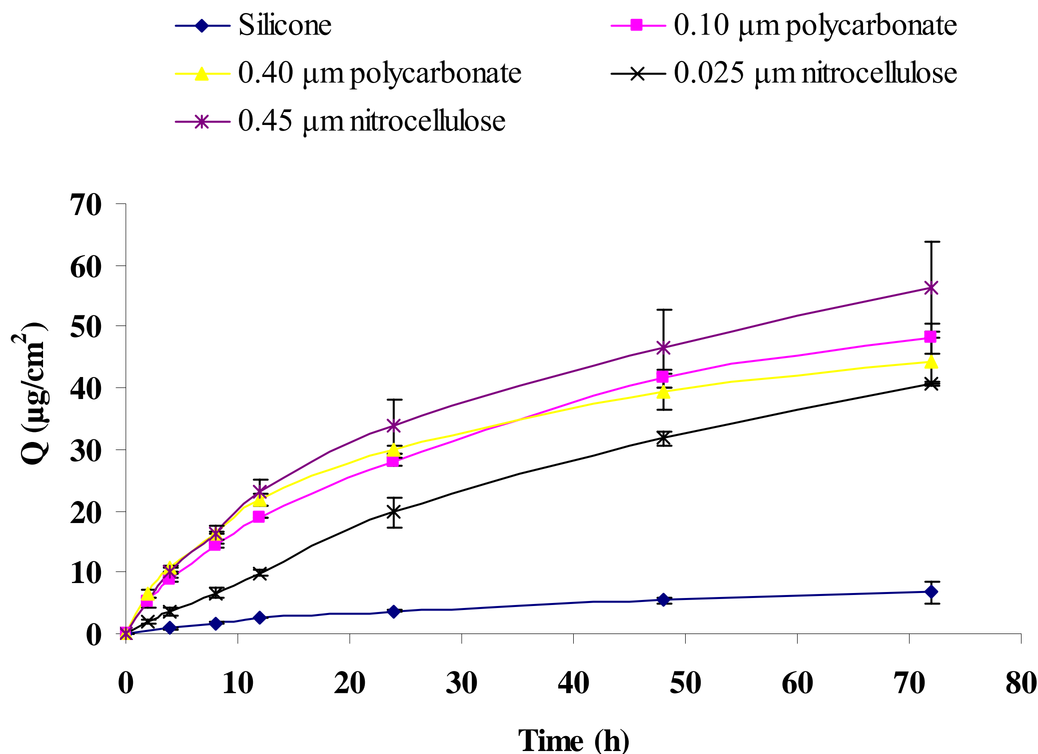


Figure 3.9. Effect of membrane type on the *in vitro* release of CP from Dermovate® cream (n = 3)

3.2.7.4. Membrane selection

The migration of a drug from a semi-solid matrix into a receptor medium is essentially a function of one or a combination of the following three processes:

a) drug release from the semi-solid matrix itself,
b) passage of the drug through the membrane, and,
c) clearance of the drug from below the membrane.

In cases where neither of the last two processes are rate-limiting it is only the thermodynamic activity of the drug in the semi-solid matrix that is an important determinant of drug release [144]. Since the physical properties of the product itself are of interest, diffusion through the base should be the rate-limiting step [113]. It is therefore critical that the membrane and the receptor medium be highly permeable and accessible to the drug in the formulation to effect drug release.

3.2.7.4.1. Silicone membrane

Silicone is a relatively inert, lipophilic and non-porous membrane as summarized in Table 3.2. The lipophilic nature of silcone provides an ideal environment for partitioning and permeation of lipophilic drugs, whilst its aporosity provides some rate-limiting function to this process [143]. Consequently, these membranes are useful for use as surrogate membranes for human skin when evaluating *in vitro* release characteristics of a drug from topical or transdermal formulations.

The *in vitro* release profile of CP release generated through the silicone membrane suggested that the membrane behaves as a rate-limiting component of the test system, since the passage of only 4.42 ± 1.11% of the applied dose into the receptor medium over the 72 hour test period was allowed. This percentage release was considered low and appeared to indicate the lack of free passage of CP through the membrane into the receptor medium.

The objective of these studies was to find a synthetic membrane with the least possible resistance to CP diffusion. Therefore the use of a silicone membrane in this test system was considered inappropriate for these studies as the use of this membrane would not provide relevant information pertaining to CP release from test formulations but would inhibit release and provide information pertaining to the flux of the drug molecule via membrane-limited release.

3.2.7.4.2. Porous membranes

Cellulose membranes have been used extensively in diffusion cell test systems for quality control studies [102, 103, 105, 107, 121, 134]. It is worth noting that pure cellulose acetate membranes are no longer commercially available, due to an Environmental Protection Agency (EPA) ban on one of the chemicals needed to manufacture the membranes [102]. The membranes currently referred to as cellulose acetate membranes contain a mixture of mixed esters of cellulose acetate and cellulose nitrate, unlike the pure cellulose acetate membrane which contained cellulose acetate only. Generally these membranes are reported to be more permeable than biological membranes or aporous synthetic media and to allow the passage of diffusing molecule(s) irrespective of the physicochemical characteristics of the compound under investigation [143].

Similarly porous polycarbonate filter membranes generally appear to be useful for use as a dividing medium or as a supporting screen in test systems, where the release rate of a drug from a delivery system is under investigation, as opposed to the evaluation of the kinetics of release of a permeant from a formulation [143]. These filter media do not simulate the skin and provide no significant barrier to the diffusion of a drug molecule but rather provide a relevant support for a test formulation as is required for these types of studies.

The highest rate and amount of CP release was observed when using a 0.45 μm nitrocellulose membrane, implying that it had the lowest resistance to the diffusion of CP. It would therefore seem appropriate to select a 0.45 μm nitrocellulose membrane as the support membrane of choice. However, a series of assumptions have to be made when selecting a membrane that offers little resistance to drug penetration for use in *in vitro* release studies [138]. Among these assumptions is the fact that there should be no more than 30% of the total amount of an applied dose released into a receptor medium at the end of an experiment [138]. The cumulative percentage CP released through each of the hydrophilic membranes studied over the 72 hour period is summarized in Table 3.3.

Table 3.3. Cumulative percentage CP released after 72 hour (n = 3)

Membrane	Pore size (μm)	Mean % CP released/cm^2	SD
Nitrocellulose	0.025	26.75	0.6100
Polycarbonate	0.40	29.53	2.720
Polycarbonate	0.10	32.53	1.970
Nitrocellulose	0.45	37.38	5.020

It is evident from the data summarized in Table 3.3 that the 0.45 μm nitrocellulose membrane allowed for the passage of more than 30% of the drug placed in the donor chamber and therefore, based on the assumption that less than 30% should be released as reported by Higuchi [138], this membrane was deemed inappropriate for use. Similarly the 0.1 μm and 0.4 μm polycarbonate membranes were also deemed inappropriate for the assessment of CP release from semi-solid formulations. The cumulative percent CP released during *in vitro* release testing with a 0.025 μm nitrocellulose was approximately 27% and therefore the 0.025 μm nitrocellulose membrane was considered the membrane of choice for further studies (Section 3.2.7.5).

3.2.7.5. Membrane resistance

Following selection of the 0.025 μm nitrocellulose membrane as the most suitable synthetic membrane for formulation development studies, membrane resistance to free permeation of CP was evaluated by assessing the release of CP from a 0.05% w/v solution of CP made up in the receptor medium (50% v/v PG: 50% v/v water). In addition 300 μl of the 0.05% w/v solution was substituted for 300 mg of semi-solid formulation in the donor compartment. The release profile generated from the CP solution was then compared to the profile generated following testing CP release from Dermovate® cream.

The data generated in these studies are depicted in Figure 3.10. The results revealed that the *in vitro* release of CP through a 0.025 μm nitrocellulose membrane was significantly slower from the cream formulation than from the 0.05% w/v CP solution. Within the first 8 hours of the release experiment, 4.82 ± 0.98% and 29.16 ± 1.92% of CP had been released from the cream and solution respectively. A total of 24.4 ± 3.75% and 46.04 ± 2.07% of CP were released from the cream and solution, respectively, at the end of the 72 hour experiment.

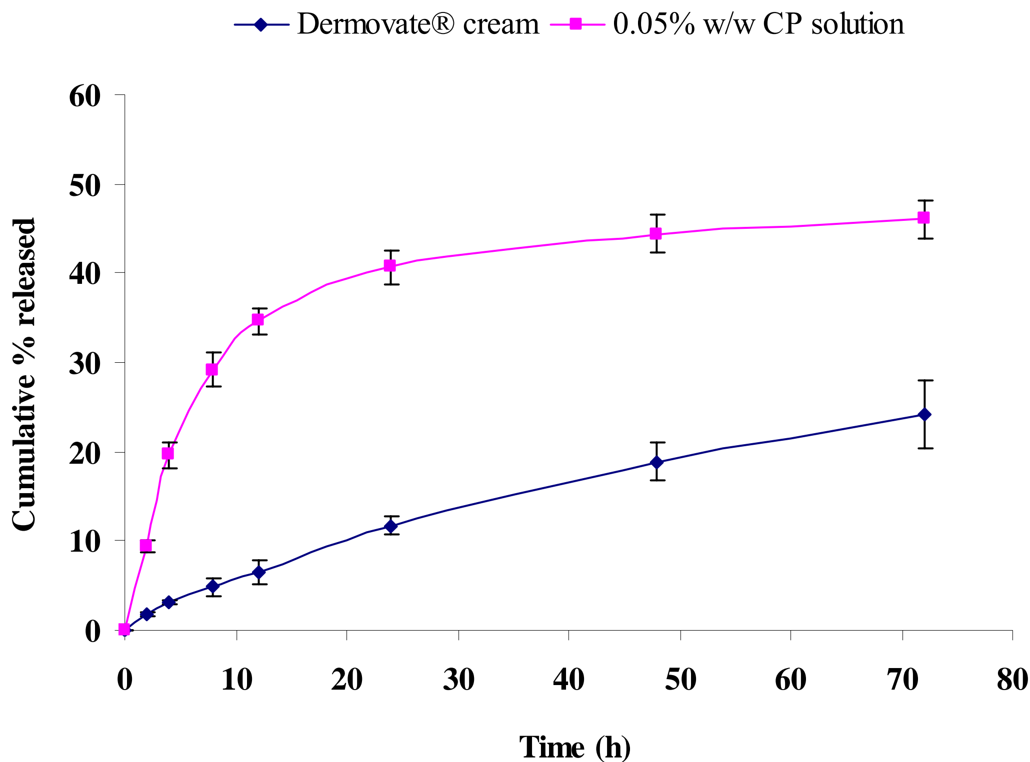


Figure 3.10. Comparison of *in vitro* release of CP from a 0.05% v/v CP solution and Dermovate® cream using a 0.025 µm nitrocellulose membrane (n = 6)

The fact that the permeation of CP across the 0.025 µm nitrocellulose membrane was faster from the CP solution than from the cream suggested that the membrane was not a rate-limiting factor in diffusion and did not affect CP release from the cream formulation and test solution. The differences observed in the release rate profiles for CP could therefore be directly attributed to the characteristics of the semi-solid formulation and not the membrane used in the release rate studies. Consequently the 0.025 µm nitrocellulose membrane was selected as an appropriate inert, porous and commercially available synthetic membrane for the assessment of *in vitro* release from topical formulations containing 0.05% w/w CP.

3.2.8. Sample Application

3.2.8.1. Overview

Depending on the method used to interpret the release data, either a finite or infinite dose may be applied to the donor compartment of a test system [115]. The use of finite dosing results in the release of the majority of the drug applied to the surface of a membrane into the receptor medium, whereas with infinite dosing only a small percentage of the drug that is applied to the membrane is released into the receptor medium over the course of the experiment [115].

Generally, data analysis is far less complicated when using infinite dosing or in situations in which less than 30% of the applied dose is released during a test period [115]. Consequently the effects of the amount of formulation applied to a membrane on the *in vitro* release rate of CP were investigated. The objective was to determine an appropriate amount of formulation to be applied to the membrane in order to correspond to an infinite dose condition or application.

3.2.8.2. Effects of sample application

The effect of applying different amounts of a sample to be tested into the donor compartment was studied using a modified Franz glass diffusion cell system (Section 3.2.2.4), a 50% v/v PG:water receptor medium (Section 3.2.6.3) and a 0.025 μm nitrocellulose membrane (Section 3.2.7.2). Approximately 50 mg, 100 mg, 200 mg and 300 mg of 0.05% Dermovate® cream was accurately weighed and spread evenly over the entire surface of the membrane using a glass-stirring rod. The glass rod was weighed before and after application and an accurate weight of cream applied to the membrane was recorded. This technique was found to be reproducible with respect to the sample weight applied to the membrane in all tests. The results obtained from these studies are shown in Figure 3.11.

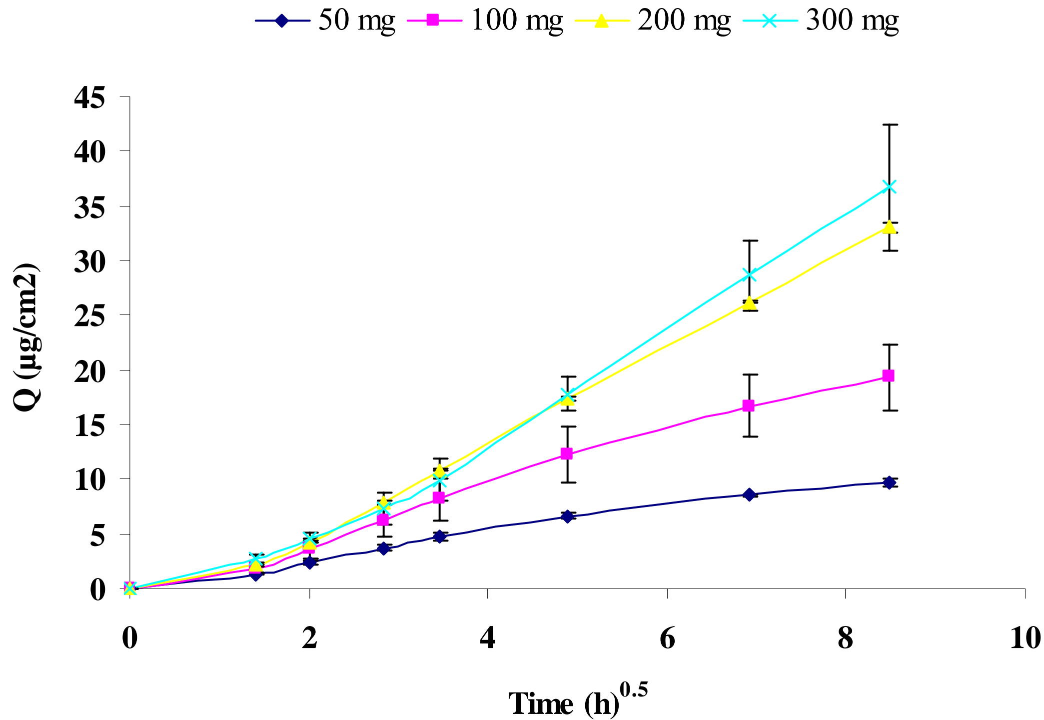


Figure 3.11. Effect of sample loading on the *in vitro* release of CP from Dermovate® cream

The results shown in Figure 3.11 are reported as the amount released per unit area (Q) *vs.* the square root of time ($t^{1/2}$) as opposed to time. In this manner the effect of sample amount on *in vitro* release rates of CP may be explained using theoretical diffusion models. The data reveal that the amount of drug released (Q) vs. $t^{1/2}$ increased as the amount of semi-solid applied to the membrane was increased. The Higuchi diffusion model [137, 144] which can be described using Equation 3.1 may be used to describe the release characteristics of a drug from a suspension or in solution in a semi-solid base.

$$Q = \left[(2A - C_s)C_s D_s t\right]^{1/2} \qquad \textbf{Equation 3.1}$$

Where:

Q = the cumulative amount of drug released,

D_s = the diffusivity of drug in the semi-solid matrix,

A = the amount of drug in the formulation, and

C_s = the saturation solubility of the drug in the semi-solid matrix.

t = time

The Higuchi equation predicts that a plot of Q *vs.* $t^{1/2}$ would be linear for a drug in suspension in a semi-solid matrix with an infinite dose application provided that such release is diffusion-controlled by the matrix material that comprises the semi-solid dosage form [137].

Least squares linear regression analysis was used to determine the degree of linearity of the plots, and the resultant coefficients of determination (R^2) indicate that all the plots were linear over the time period studied. The data are summarized in Table 3.4.

Table 3.4. *In vitro* release characteristics from four loading doses applied to the test membrane

Loading dose (mg)	R^2
50	0.9846
100	0.9896
200	0.9873
300	0.9881

The data shown in Table 3.3 imply that any of the amounts of semi-solid (50, 100, 200 and 300 mg) applied to the membrane in these studies conformed to Higuchi kinetics and although the results suggest that an infinite dose condition was achieved with all four amounts of formulation tested, 300 mg was selected for use in these studies due to the ease of experimental set-up, for example weighing 300 mg by difference was considered much quicker and easier than weighing smaller quantities.

3.2.9. Sample occlusion

3.2.9.1. Overview

The FDA guidance document [49] recommends that the amount of the semi-solid preparation applied uniformly onto a membrane should be kept occluded during the assessment of the *in vitro* release characteristics of a drug from that formulation. Occlusion is preferred in order to prevent solvent evaporation and compositional changes of a formulation that is applied to the test membrane [49].

The term occlusion refers to the use of a moderately impermeable barrier to cover the donor compartment of a diffusion cell assembly [115]. Experiments were performed in the sample

occluded or non-occluded mode to determine whether the degree of occlusion affects the *in vitro* release of CP and to select the appropriate conditions for future studies.

3.2.9.2. Effects of occlusion

The effect of occlusion on the *in vitro* release of CP from Dermovate® cream was investigated using approximately 300 mg of the cream formulation. Diffusion cells were occluded by placing a piece of Parafilm® over the donor compartment of the diffusion cell and sealing the cell.

The effects of occlusion and non-occlusion of the donor cell on the *in vitro* release characteristics of CP from an infinite dose condition are shown in Figure 3.12. These data reveal that the amount of CP released in the *in vitro* release profiles from an infinite dose condition under occlusion was greater than from an un-occluded test system.

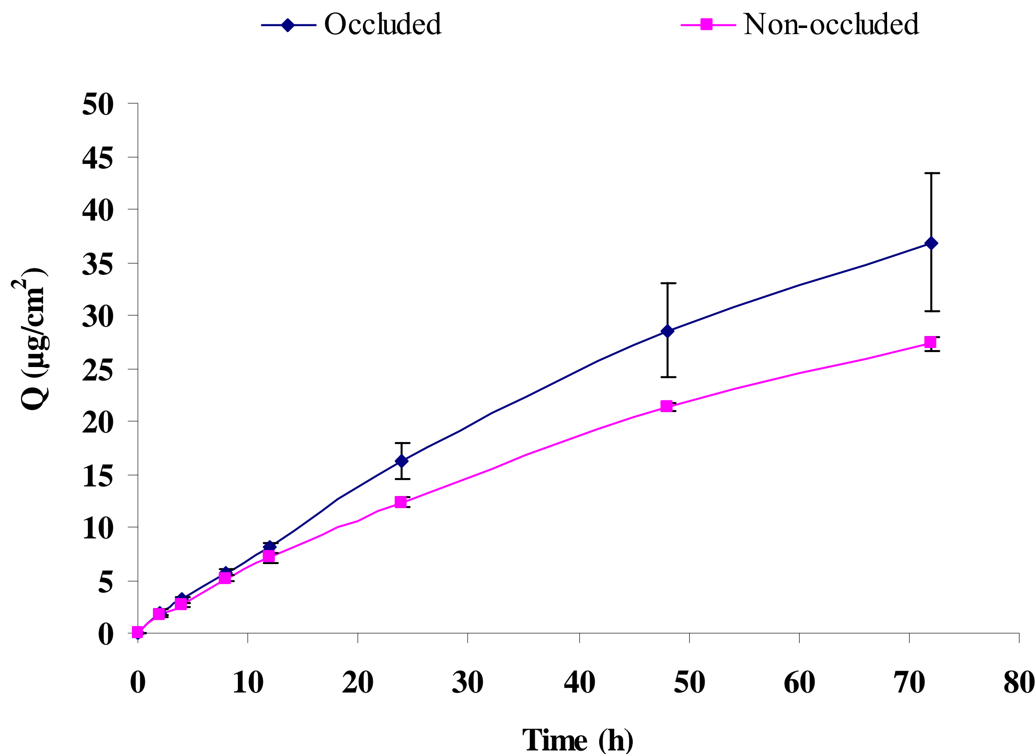


Figure 3.12. Effect of occlusion on the *in vitro* release of CP from Dermovate® cream (n = 6)

Many semi-solid formulations contain solvents such as ethanol, water and propylene glycol and it has been reported that ethanol and water evaporate rapidly, whereas propylene glycol only evaporates appreciably over a 24 hour period [112]. The composition of a semi-solid formulation being tested would therefore change over the experimental period, should the test system not be occluded for the duration of the experiment.

It is possible that the use of non-occluded cells resulted in the evaporation of water as well as propylene glycol over 24 hours which might have led to an increase in the intrinsic viscosity of the formulation, thereby causing an increased resistance to the diffusion of CP and a subsequent lower overall release rate as shown in Figure 3.12. Consequently the *in vitro* release profile of CP release from the topical formulation was markedly decreased and the deviation between the two curves was more noticeable for the period 24-72 hours of the diffusion experiment.

As a result of the improved extent of release observed when using occluded cells, all future *in vitro* release experiments for the assessment of CP release from semi-solid formulations were carried out under occlusive conditions to prevent solvent evaporation and compositional changes of the formulations being tested as such changes may influence the overall cumulative amount of CP released during testing.

3.2.10. Sample Analysis

A sensitive, selective, accurate and precise previously validated HPLC method with UV detection at 240 nm (Chapter 2) was used to analyze the samples withdrawn from the receptor chambers at each time-point. The concentration of CP in each receptor cell was determined by interpolation of the peak height ratios of CP to I.S. from a calibration curve. A typical calibration curve is shown in Figure 3.13 and each calibration curve was prepared on the day on which samples were analysed.

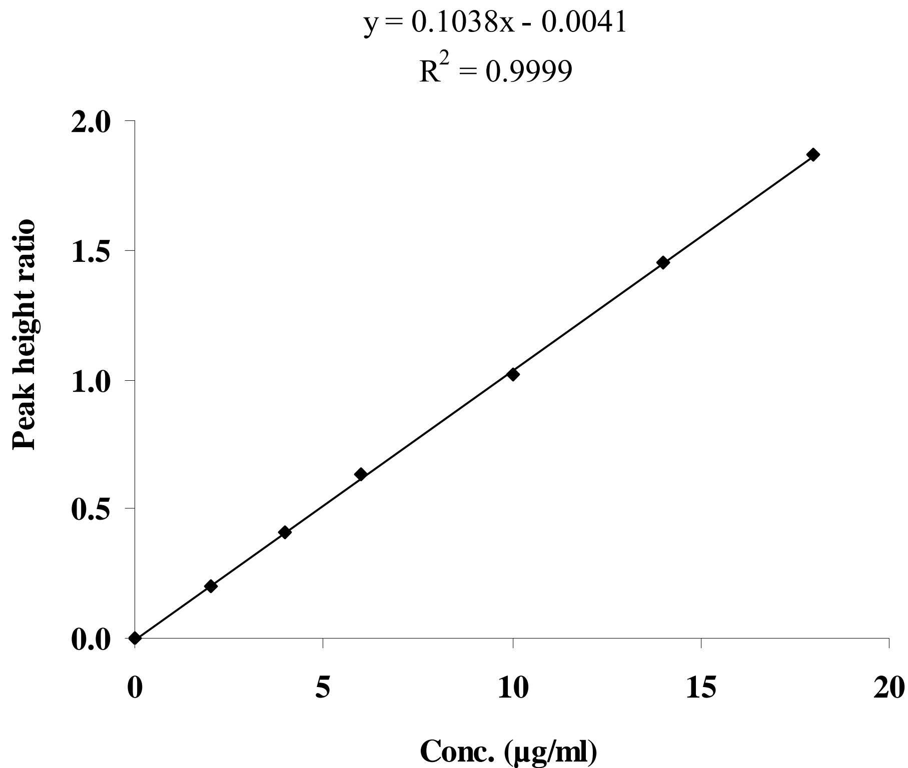


Figure 3.13. CP calibration curve used to determine the amount of CP released in *in vitro* release experiments

3.2.11. Comparison of diffusion or release rate profiles

The *in vitro* release profiles generated from two semi-solid products (test and reference) were compared using a statistical test recommended by the FDA to assess product sameness when changes are made to an approved topical semi-solid product [49]. The analysis involves non-parametric assessment of release rate profiles by statistical means and is based on the calculation of an appropriate confidence interval (C.I.).

The test is related to the Wilcoxon Rank Sum/Mann-Whitney Rank Test that is applied to the log of the slope of the release profiles or the release rate. The *in vitro* release profiles of two formulations are generated for a test and a reference (T and R) product (n = 6) and the resulting slopes of the profiles or estimated *in vitro* release rates for each of the formulations are calculated. A 90% C.I. for the ratio of the median *in vitro* release rate or population for the test

product over the *in vitro* release rate or population for the reference product is computed and expressed in percentage terms.

The computation of the C.I. is achieved by evaluation of 36 (6 x 6) individual ratios of T/R *in vitro* release rates and are ordered in sequence, *i.e.* all possible T/R ratios (36 in total) are arranged in ascending order from lowest to highest with respect to relative release rates. The eighth (8th) and twenty-ninth (29th) individual ratios are then established and are assigned as the lower and upper limits of the 90% C.I. for the T/R release rate ratio respectively and converted into percentages. In order to accept that the *in vitro* release rate of a test product (T) is within the 90% C.I. of a reference product (R), the values for T/R ratios should fall within the limits of a 0.75 or 75% to 1.33 or 133% C.I.

An example of the calculation of the confidence interval is shown in Appendix 1. The data shown were generated in *in vitro* release studies in which CP release from an extemporaneously manufactured CP cream (T) and from Dermovate® cream (R) were assessed (Chapter 4, *vide infra*). The eighth and twenty-ninth ordered individual T/R ratios are 101.6% and 125.3%, which fall within the 75% -133% limits. Therefore the release rates from the two semi-solid formulations in this example are considered identical and as a result the two formulations can be considered equivalent.

3.2.12. Optimal *in vitro* release test conditions

The optimal *in vitro* release test conditions established for the assessment of CP release from topical formulations are summarised in Table 3.5.

Table 3.5. Summary of optimal *in vitro* release test conditions

Parameter	Condition
Apparatus	Vertical glass Franz cell diffusion cell
Sample number	6 cells
Average diffusional surface area	1.921 ± 0.056 cm^2
Average receptor volume	12.50 ± 0.38 ml
Receptor medium	50% propylene glycol:50% water
Temperature	32 ± 0.5˚C.
Synthetic membrane	0.025 µm nitrocellulose
Dosing conditions	Infinite (300 mg sample application)
Sample occlusion/non-occlusion	Occlusion
Magnetic stirrers	2x2 mm star-headed magnetic stirrers
Sampling time	0, 2, 4, 8, 12, 24, 48, and 72 hours
Sample analysis	HPLC with UV detection at 240 nm
Comparison of diffusion profiles	Non-parametric statistical test

3.3. METHOD VALIDATION

3.3.1. Overview

The fundamental principle on which the validation of an *in vitro* release test method is based is that following validation there is an assurance that the test method is performing as expected and that when changes are made to the formulation composition, batches or sources of ingredients and method of manufacture, changes in drug release rates can be detected [113, 115].

The integrity of this *in vitro* release method was therefore interrogated by investigating the effect of changing various formulation attributes on the CP release rate from a prototype extemporaneous cream. The variables validated in these studies were changes in:

a) dose strength,
b) composition, and,
c) viscosity of the semi-solid dosage form.

The use of these attributes as parameters in validation protocols for *in vitro* release test methods have been previously reported [108, 113, 115].

The slope of the linear portion of the cumulative amount of drug released *vs.* the square root of time (Q *vs.* $t^{1/2}$) plot has been reported as the most logical, meaningful and accurate parameter for comparing *in vitro* release characteristics of a drug from different topical formulations [113]. Thakker and Wendy [108] used flux values in the validation of a typical *in vitro* release test method and reported flux values as the average slope of the best fit line of a Q *vs.* $t^{1/2}$ plot [108].

The approach adopted by Thakker and Wendy [108] was used to calculate flux values for CP release from the test product and flux values were used to confirm the ability of the *in vitro* release test method to differentiate between semi-solid formulations of CP that differed in strength, excipient composition and viscosity.

3.3.2. Changes in dosage strength

The effects of changes in strength of CP in the formulation on *in vitro* release rate of CP were evaluated using extemporaneously manufactured cream formulations at three different concentrations levels, specifically, *0.01*, 0.025 and 0.05% w/w of CP.

According to theoretical principles, the release rate and ultimate amount of drug released from a specific formulation should be proportional to the amount of drug in the formulation if the diffusion of the drug within the semi-solid matrix is the rate-controlling mechanism in drug release [138, 144]. Data generated in these studies are summarized in Table 3.6 and shown in Figure 3.14 and are in agreement with the theory. The higher the concentration of CP in the semi-solid formulation, the greater the flux and ultimately the total cumulative amount of CP release per unit area.

Table 3.6. Effect of changes in the CP concentration on the total cumulative amount released and the associated flux values (n = 3)

Dosage strength (%w/w)	Q (µg/cm^2)	Flux (µg/cm^2/h$^{1/2}$)
0.010	11.22	1.470
0.025	26.09	3.540
0.050	41.81	5.410

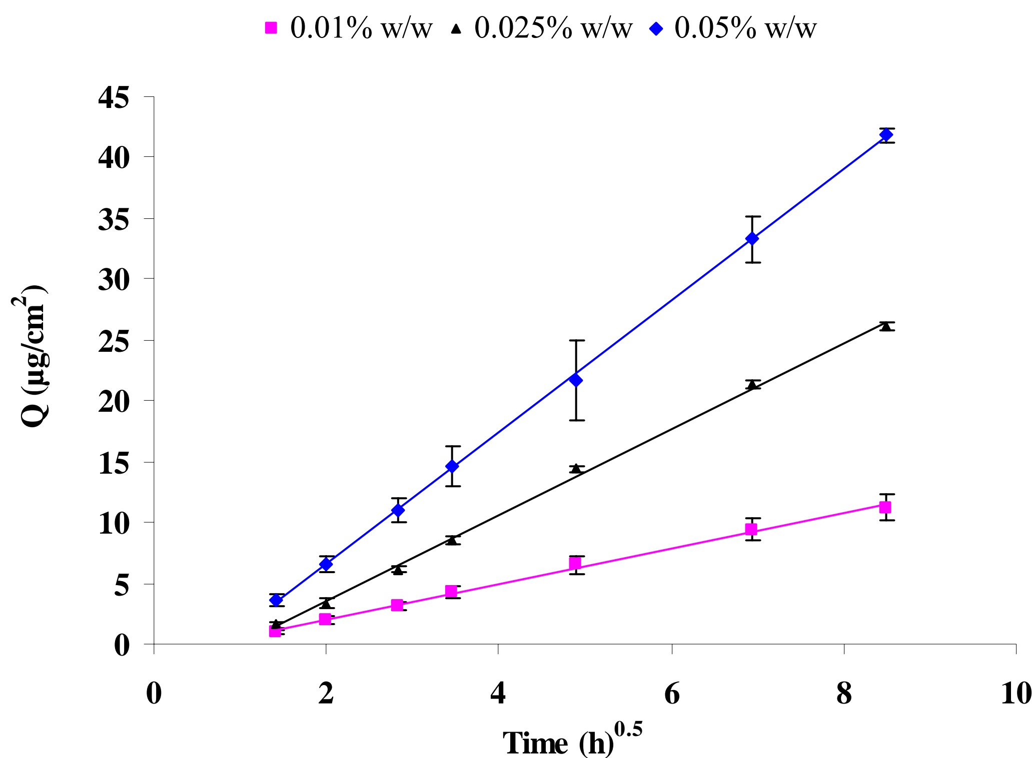


Figure 3.14. Effect of changes in CP content on the *in vitro* release rate of CP from an extemperaneous cream base (n = 3)

The data suggest that the selected receptor phase, support membrane and other experimental conditions are suitable to show discrimination between different formulations since the rate-controlling mechanism for drug release is diffusion of CP within the semi-solid base and not through the membrane used in the cell assembly. Consequently, the impact of formulation changes that may result in drug loading differences can be assessed using this *in vitro* release method.

3.3.3. Changes in composition

The effect of changes in formulation composition on the *in vitro* release rate of CP was examined using two extemporaneously manufactured creams formulations (Formulations 1 and 2). Both formulations were manufactured using the same method of manufacture and contained the same amount of CP (0.05% w/w). The difference between the two formulations was that a different

primary emulsifier was used in each case. Formulation 1 contained 1% w/w Ritapro® 200 (Rita, Crystal Lake, IL, USA) and Formulation 2 contained 1% w/w Emulcire® 61 WL (Gattefossé SAS, Saint-Priest Cedex, France) as the primary emulsifier. The data generated from these studies are summarized in Table 3.7 and shown in Figure 3.15 and reveal that alterations in the composition of the CP formulation do in fact result in changes in flux in addition to the total cumulative amount of CP released. These data confirm the ability of this *in vitro* release method to detect the impact of changes of formulation constituents on the *in vitro* release of CP from such formulations.

Table 3.7. Effect of changes in formulation composition on flux and cumulative amount of CP released (n = 3)

CP cream Formulation	Dose strength (%w/w)	Primary Emulsifier	Q (μg/cm^2)	Flux (μg/cm^2/h$^{1/2}$)
1	0.05	Ritapro® 200	63.06 ± 4.84	8.63
2	0.05	Emulcire®	44.47 ± 3.63	6.14

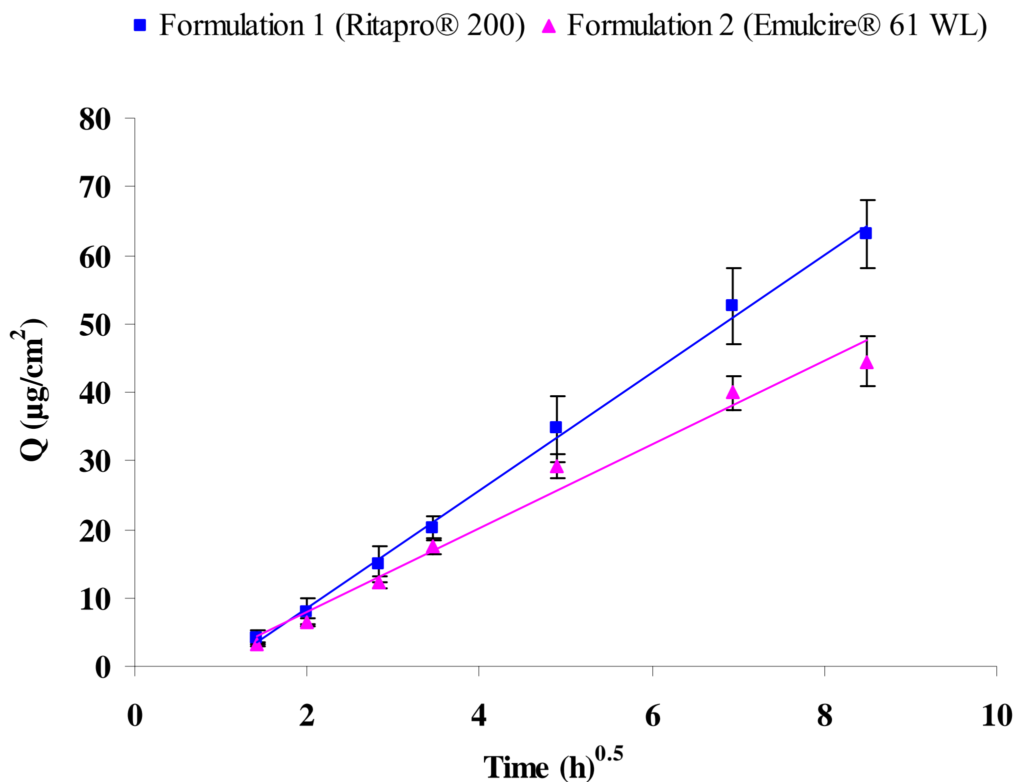


Figure 3.15. Effect of changes in formulation composition on CP *in vitro* release rate (n = 3).

3.3.2.3. Changes in viscosity

3.3.2.3.1. Overview

In order for a drug molecule to be released across a membrane from a semi-solid vehicle, it has to reach the membrane surface prior to partitioning and mass transport of the drug from the formulation. Mass transport occurs by diffusion of drug molecules through the formulation base [145] and API diffusion depends on the properties of the drug, the diffusion medium and the extent of vehicle-drug interactions that may be present [146]. The extent and degree of these interactions will in turn determine the rate and extent of drug release and the overall shape of the drug release profile.

It is well established that the diffusivity of a drug molecule through a semi-solid material decreases progressively as the viscosity of the base material increases [146]. The converse is also true and the implication is that any alteration in the intrinsic viscosity of a topical formulation would have an effect on the rate and extent of release of an active ingredient from a vehicle. It follows therefore that the intrinsic viscosity of a formulation is a key attribute of semi-solid dosage forms and a parameter that can be monitored for quality control purposes during and after formulation development.

The effect of changes in the intrinsic viscosity of the semi-solid formulation base on the *in vitro* release of CP was therefore investigated using formulations of different viscosity. Two prototype cream formulations (Formulations A and B) were manufactured extemporaneously using similar exicipients and method of manufacture. The formulation compositions differed in the amount of consistency modifier, *viz.,* white beeswax that was added to each formulation. Formulation A was manufactured using 0.6% w/w white beeswax whereas formulation B contained 2% w/w white beeswax. The exact intrinsic viscosity of each formulation was ascertained as described in Section 3.3.2.3.2, *vide infra*.

3.3.2.3.2. Determination of viscosity

The intrinsic viscosity of Formulations A and B were measured using a Model-RVDI+ Brookfield Viscometer (Brookfield ENG Labs Inc., Stoughton, MA, USA). The viscometer was operated at 100 rpm using a T-F (code 96) spindle and a Helipath stand and the spindle was chosen to maintain a torque between 10% and 90%. In order to obtain stable display readings, all measurements were recorded 60 sec after the commencement of spindle rotation and a maximum of three (3) readings were taken to obtain an average viscosity value.

The intrinsic viscosity readings obtained were 14.83 ± 0.05773 KcP and 21.37 ± 0.1071 KcP for Formulations A and B, respectively. These results indicate that Formulation B has a higher intrinsic viscosity than Formulation A. *In vitro* release studies were conducted as previously described in order to ascertain whether the intrinsic viscosity resulted in a different flux for CP in addition to total cumulative amount CP released.

3.3.2.3.3. Effects of viscosity

The effects of viscosity of the semi-solid formulation on the *in vitro* release of CP are tabulated in Table 3.8 and shown in Figure 3.16.

Table 3.8. Cumulative amount of CP released and the average *in vitro* release rate (flux) from CP cream formulations of different intrinsic viscosity (n = 3)

CP cream Formulation	Dose strength (%w/w)	Viscosity (KcP)	Q ($\mu g/cm^2$)	Flux ($\mu g/cm^2/h^{1/2}$)
A	0.05	14.83 ± 0.05773	41.81 ± 0.54	5.41
B	0.05	21.37 ± 0.1071	36.21 ± 1.71	4.95

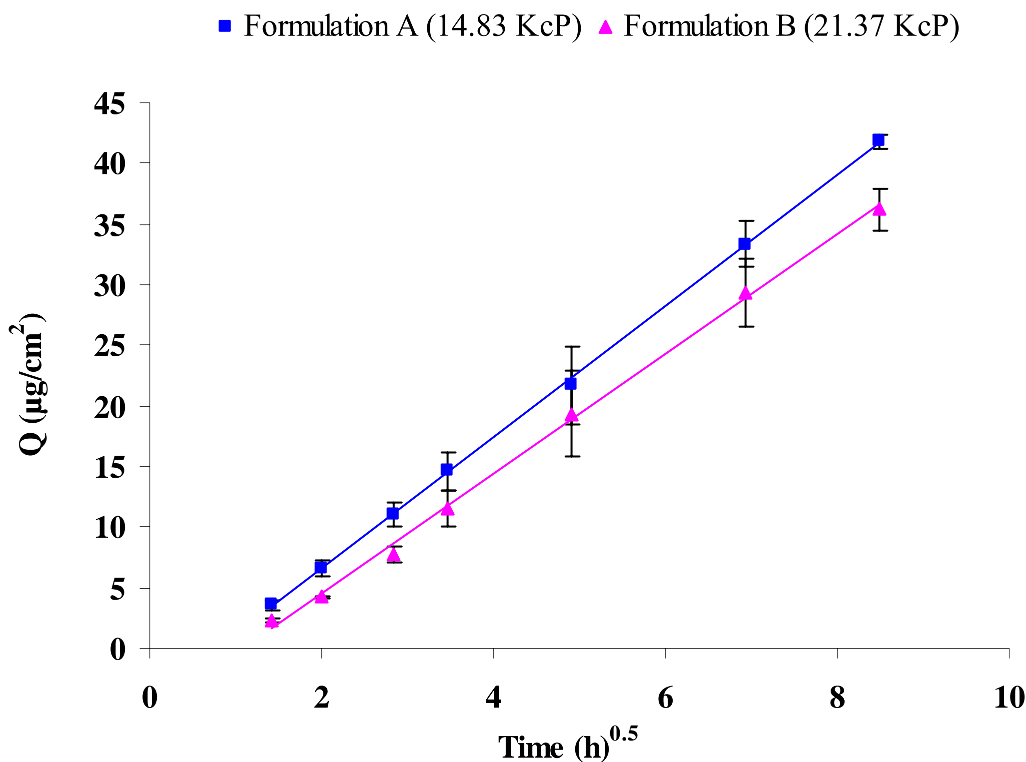


Figure 3.16. Effect of changes in the intrinsic viscosity of a formulation on the in *vitro* release rate of CP (n = 3).

These data indicate that there was a decrease in CP flux as well as the total cumulative amount of CP released from the semi-solid formulation of higher viscosity. However, the CP release rates from Formulation A and Formulation B, respectively, appear to be similar, probably more than likely due to the small difference in the intrinsic viscosity values obtained for these two formulations. Nevertheless, the results show that this *in vitro* release method is suitable and capable of detecting the impact of small changes of the physical properties of CP semi-solid formulations on the rate and extent of CP release from these products.

3.4. CONCLUSIONS

An *in vitro* release method for the assessment of clobetasol 17-propionate (CP) release from topical semi-solid dosage forms has been developed and validated in terms of protocols reported [108, 113, 115]. Method development studies entailed the selection of a suitable diffusion cell system, appropriate sampling times, receptor medium and appropriate synthetic membranes in addition to investigating the effects of sample application and sample occlusion or non-occlusion on the *in vitro* release of CP from semi-solid dosage forms.

A modified Franz glass diffusion cell system (n = 6) was selected and used in these studies since it has the most potential for use as a standardized test system that may be adapted for use as a compendial method. Various solvent systems and compositions were investigated for use as receptor fluids during the course of these experiments and a 50% propylene glycol: 50% water mixture was deemed to be the optimal receptor medium as it was shown to have appropriate sink characteristics for CP following saturation solubility studies.

There are certain constraints that are associated with the selection of sampling times and these must be considered and investigated when monitoring the rate and extent of API release from semi-solid dosage forms. Samples of the receptor medium were withdrawn at 2, 4, 8, 12, 24, 48 and 72 hours in order to generate a satisfactory CP release profile and to characterize the release of CP from semi-solid topical formulations.

Various synthetic membranes were evaluated as potential physical barriers between the semi-solid sample being tested in the donor chamber and the receptor medium in the receptor chamber of the modified Franz cell assembly. A 0.025 μm nitrocellulose membrane was found to be the most appropriate, inert, porous and commercially available synthetic membrane for the assessment of *in vitro* release of CP from topical formulations. This synthetic membrane was shown to have no resistance to the free diffusion of CP when tested using a solution of CP in a propylene glycol water mixture.

The temperature of the test system was set at 32° ± 0.5˚C to approximate the usual surface temperature of the skin and a 2 x 2 mm star head magnetic stirrer was used to agitate the receptor medium. About 300 mg of the semi-solid preparation, which corresponded to an infinite dose, was applied uniformly to the membrane, and the formulation was occluded for the duration of testing, to prevent solvent evaporation and compositional changes to the formulation.

At the conclusion of studies to define the optimal parameters for *in vitro* release testing, the method was assessed for its ability to detect the effects of changes in formulation characteristics on CP release. The effects of changes of strength, composition and intrinsic viscosity of topical formulations on *in vitro* release of CP were then evaluated. The results obtained indicated that the *in vitro* release method that has been developed is able to detect the effects of changes in formulation in which CP is suspended and/or dissolved on CP release and was therefore applied in all future formulation development studies.

It is evident, from the data presented that the *in vitro* release test method is simple, reliable, reproducible and discriminatory. It can therefore be used in conjunction with traditional quality control tests to determine the quality and consistency of extemporaneously manufactured CP topical formulations in addition to the assessment of the associated rate and extent of release of the API during product development studies.

CHAPTER FOUR

DEVELOPMENT AND *IN VITRO* CHARACTERIZATION OF CLOBETASOL 17-PROPIONATE CREAM FORMULATIONS

4.1. INTRODUCTION

The elevated costs of brand name or innovator pharmaceutical products has been reported as one of the major contributing factors to the rapidly escalating costs of health care services worldwide [147-150]. As a consequence, there has been a growing international trend for the demand of safe, effective and, more importantly, affordable medicines [151]. A number of mechanisms have been evaluated in an attempt to make pharmaceutical products more affordable and more accessible, especially in the developing world where lifesaving medicines are reported to be financially out of reach of the wider population [152-154].

In the Republic of South Africa (RSA) for example, generic prescribing and generic substitution have been identified and targeted as some of the possible strategies to contain the escalating costs of brand name pharmaceutical products, and these are currently being implemented [147, 149, 155, 156]. Generic prescribing refers to the prescription of multi-source (generic) medicines by authorized prescribers, such as for example, medical doctors [155], whereas generic substitution refers to the replacement of an innovator product with a multi-source (generic) product by pharmacists [155].

Generic medicinal products have been described as products that are equivalent to brand name products in terms of the strength of active pharmaceutical ingredients (API), dosage form, route of administration, quality, safety, efficacy, performance characteristics and therapeutic indication [151, 157]. However, generic products are typically sold at much lower prices than brand name products and therefore are more affordable than innovator products [151]. It has been reported that the low cost of generic pharmaceutical products is usually attributed to the fact that an Abbreviated New Drug Application (ANDA) process [151, 157] does not require the generic product manufacturer or sponsor to repeat costly and time-consuming clinical and non-clinical

studies on an API or dosage form (s) in order to demonstrate the safety and efficacy of the drug or drug product(s) in question [151, 157].

Sponsors of generic drug products are required to satisfy specific criteria for the registration of generic drug products as set by relevant regulatory authorities such as, for example, the office of generic drugs of the Food and Drug Administration in the United States (FDA) [157], the European Agency for the Evaluation of Medicinal Products (EMEA) [158] and the Medicines Control Council (MCC) in South Africa [159].

It has been argued that the time, risk and cost associated with the development of brand name pharmaceutical products are responsible for the high costs of innovator medicinal products [160]. According to a report compiled by the European Federation of Pharmaceutical Industries and Associations (EFPIA) [161], an average of between 12-13 years is required for a medicinal product to reach pharmacy shelves from discovery, costing an estimated € 870 million during the research and development (R&D) phase for a product [161]. Furthermore, the EFPIA report [161] alludes to the fact that only one (1) or two (2) out of ten thousand (10000) potential drug substances synthesized in laboratories are able to pass the extensive testing in the R&D stages of drug development to produce a marketable pharmaceutical product [161].

As a consequence, innovator medicinal products are developed under patent protection, which protects the investment in the development of the drug product by giving the innovator company the sole right to sell the product while the patent is in effect [151, 157]. As a matter of interest, innovator pharmaceutical companies may apply and obtain patent rights from the United States Patent and Trademark Office at anytime during the lifetime of the drug or drug product [151]. When the intellectual property rights for a product have been exhausted, normally after a period of twenty (20) years from the date of filing for the patent [151, 160] generic medicinal product manufacturers may then apply to the relevant authorities, such as the FDA for permission to produce and sell a generic version of the branded product [151, 157].

Shargel and Kanfer [151] discussed various aspects that should be considered when selecting a generic drug product for manufacture. They [151] argue that the estimated sales volume for the

innovator product and the potential market share the manufacturer of the generic product expects to achieve once the multi-source product is manufactured and approved for marketing is the main driving force for the selection of drug candidates for generic product manufacture [151].

Shargel and Kanfer [151] also argue that patent expiration and exclusivity as well as other legal issues must be considered carefully prior to selecting an innovator drug and/or drug product for generic manufacture. The manufacturers of a generic drug product will continue to face various legal and patent challenges from innovator companies, which in the opinion of Shargel and Kanfer hinder the entry of generic products into the marketplace [151].

Eczema or dermatitis is a common dermatological disorder affecting approximately one-third of a given population [162] and may be defined as superficial inflammation of the skin, that is characterized histologically by epidermal oedema and clinically by vesicles, poorly marginated redness, oedema, oozing, crusting, scaling, pruritis and lichenification and that is usually caused by scratching or rubbing of the skin [163]. Hartshorne [162] reported that eczema is the most common skin condition in the RSA accounting for approximately 34.5%, 32.7%, 30.4% and 17.8% of the dermatological outpatients in the coloured, black, Indian and white populations, respectively. Topical corticosteroid formulations such as creams or ointments applied three (3) times daily have been reported to be the primary therapy for the treatment of eczema [163, 164].

Topical corticosteroids are, however, reportedly considered expensive for the majority of the population, and as a consequence the supplemental use of other substances such as white petrolatum and hydrogenated vegetable oil has been reported [163]. The high costs of therapy are especially a result of the use of super-potent topical corticosteroids such as clobetasol 17-propionate (CP) that are required for the treatment of severe or chronic eczema, especially of the hands and feet (Section 1.5.2, Chapter 1). The elevated costs of super-potent topical corticosteroid products, such as CP, especially in developing countries such as the RSA, may be ascribed to the lack of a variety of generic versions of the product on the local market. For example, despite the expiration of the patent for CP in 1990 [165], only one generic version of Dermovate® cream and Dermovate® ointment (GlaxoSmithKline plc, Brentford, Middlesex, U.K) has been registered for marketing in the RSA [166].

The objective of these studies was therefore to design and develop a generic version of Dermovate® cream and to evaluate the product in terms of a number of *in vitro* performance characteristics. The successful development and subsequent determination of equivalence between the generic product and the innovator formulation in terms of *in vitro* performance characteristics would form the basis for future studies, such as the evaluation of bioequivalence of the generic CP product relative to Dermovate® cream, potentially resulting in the development of a marketable generic CP cream.

It has been reported that pharmaceutical manufacturers frequently manufacture topical preparations of a drug in both cream and ointment vehicles to satisfy the preference of the patient and dermatologist [167]. It has been argued that many patients and physicians prefer creams to ointments, because the former are easier to apply and remove and may have a cooling sensation on the skin [167-170]. Ointments are usually more difficult to apply or to remove, but are expected to act as a barrier and increase the hydration of the skin [168].

The development of a generic version of Dermovate® cream rather than Dermovate® ointment was considered most appropriate, since it was assumed that a generic version of Dermovate® cream would have a larger market share than a generic Dermovate® ointment, based on patient preference for cream rather than ointment formulations.

4.2. CREAM FORMULATIONS

4.2.1. Overview

Pharmaceutical emulsions have been described as systems that contain at least two immiscible liquids, such as an oil and water and in which one of the phases is dispersed as globules *viz.,* the internal or dispersed phase, within the other phase *viz.,* the external or continuous phase [168, 171, 172]. Since emulsions are formed from two incompatible liquids, they are considered to be thermodynamically unstable colloidal systems and therefore usually require the addition of a

third component such as an emulsifying agent or surfactant to impart stability to the system [171, 172].

Emulsions are thermodynamically unstable since the two immiscible phases that exist in close conjunction impart a positive interfacial free energy to the emulsion system [171-173]. As a consequence of the positive interfacial free energy, the two immiscible liquids will always tend to divide into their separate components in an attempt to achieve thermodynamic equilibrium [173]. Therefore by use of an emulsifying agent, that is a compound possessing both polar and non-polar characteristics that aligns itself at the interface between the oil and water phases, the interfacial free energy between the phases can be reduced [171, 174]. The film structures formed by an emulsifying agent at the interface between phases forms the basis of homogenous and stable emulsions [171, 174].

Emulsions may be classed as either oil-in-water (o/w) or water-in-oil (w/o) systems [168, 171-173] and a schematic representation of such emulsions is depicted in Figure 4.1. Other types of emulsions that have been reported are the so-called multiple emulsions such as, for example, water-in-oil-in-water (w/o/w) emulsions [175-178] and oil-in-water-in-oil (o/w/o) emulsions [177-180]. Essentially, o/w emulsions contain oil droplets dispersed in an aqueous continuous phase, whereas w/o emulsions contain aqueous or polar droplets dispersed in an oily continuous phase [168, 171-173]. Molecules of an emulsifying agent assemble at the oil/water interface and the hydrophilic or polar group of the emulsifying agent is orientated towards the water phase and the hydrophobic or non-polar tail aligns itself towards the oil phase [171] as depicted in Figure 4.1.

Multiple emulsions such as w/o/w emulsions are emulsions in which a w/o emulsion is dispersed as droplets in an aqueous phase [175, 176, 178], whereas o/w/o emulsions occur as emulsions in which an o/w emulsion is dispersed in an oil phase [178-180]. In order to stabilize multiple emulsions, two surfactants are invariably used, with a hydrophobic surfactant designed to stabilize the interface of the w/o internal emulsion and a hydrophilic surfactant to stabilize the external interface of the oil globules for w/o/w multiple emulsions, and the converse is true for o/w/o multiple emulsions [178].

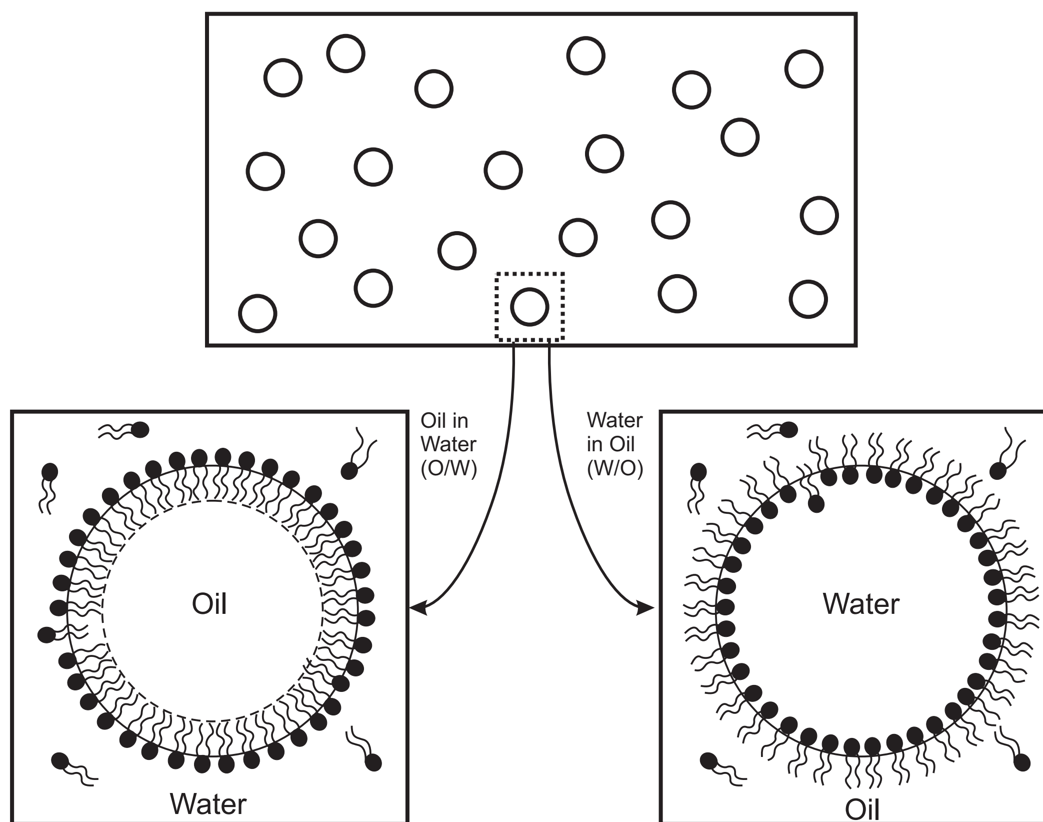


Figure 4.1. Schematic representation of the principles of oil-in-water (o/w) and water-in-oil (w/o) emulsions (redrawn from 181).

The most common type of emulsions used in dermatological therapy are creams [171]. Creams are described as emulsions of a semi-solid consistency [172, 182] or emulsions of a high apparent viscosity [172, 182] with a typical creamy white appearance [183] that are manufactured for topical application [172, 182]. However, more recently Buhse et *al.* [168] classified and reported creams as semisolid dosage forms that contain >20% water and volatile ingredients and/or <50% of hydrocarbon, wax or polyethylene glycol constituents as a vehicle and that are intended for external application to the skin.

Buhse et *al.,* [168] also described creams as semi-solid systems that display plastic flow behaviour and in total contrast to other semi-solid formulations such as ointment bases, creams exhibit two or more transition states when evaluated using thermogravimetric analysis (TGA). The transition states are indicative of a system composed of at least two phases and cream formulations can be classified as semi-solid systems that appear opaque, are viscous and have a

non-greasy to mildly greasy texture and tend to evaporate or get absorbed when rubbed onto the skin [168].

Similar to liquid emulsions, creams usually contain a third component *viz.,* an emulsion-stabilizing system or an emulsifier [171]. However, in contrast to liquid emulsions, creams are reported to contain more emulsifier than that required to form a condensed monomolecular surfactant film at a droplet interface in liquid emulsions [172]. It has been argued that excess emulsifiers in cream formulations interact with other components of these formulations either at the droplet interface or in the bulk phase to produce complex, multiphase structures and these complex multiphase structures are reported to be essential for the formation of creams that are stable for extended periods of time [172] (Section 4.2.3.3., *vide infra*).

Pharmaceutical creams may contain one or more APIs dissolved or dispersed in either an o/w or a w/o system [167]. Oil-in-water creams are usually referred to as "vanishing creams" as when rubbed into the skin the formulation disappears without leaving any trace of their presence on the skin [172].

4.2.2. Instability mechanisms in creams

4.2.2.1. Overview

The physical instability of creams has been reported to occur through various time- and temperature-dependent physicochemical destabilizing mechanisms [171, 184-189] and these mechanisms are summarized schematically in Figure 4.2.

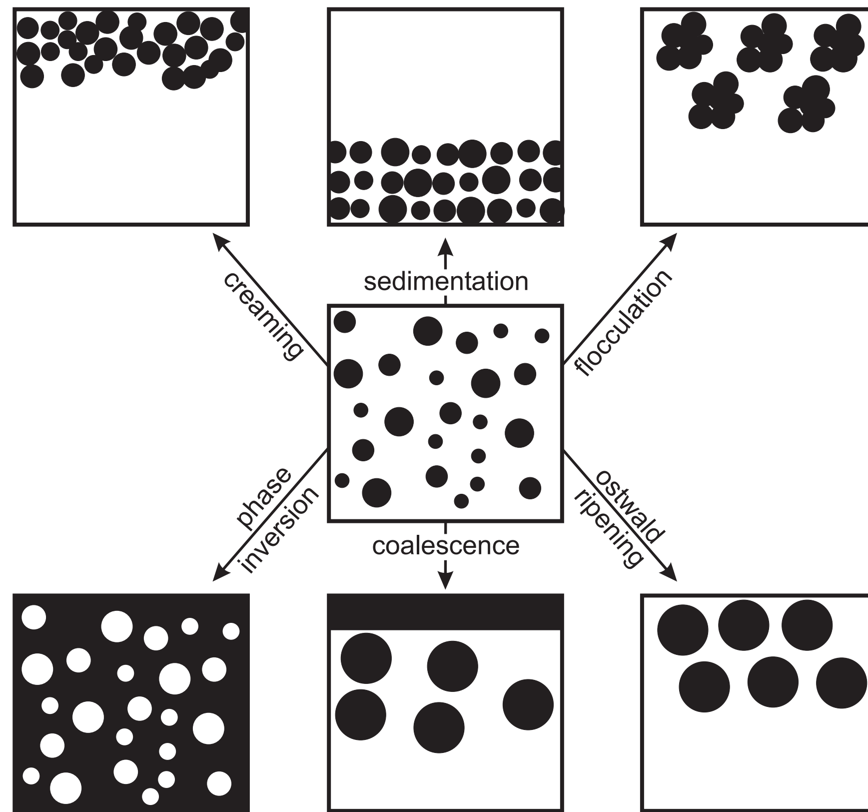


Figure 4.2. Schematic representation of the mechanisms by which creams show instability (adapted from 184)

4.2.2.2. Flocculation

In order for a cream to form, two immiscible liquid phases must be mechanically agitated in the presence of an emulgent [171]. It has been reported that when agitation occurs in the absence of any form of interfacial stabilization both phases of the cream will form droplets that will rapidly flocculate and separate into two distinct phases [171, 184]. Flocculation may therefore be described as the close accumulation of two or more individual droplets of a dispersed phase to form loose assemblies or flocs, without loss of the interfacial film [171, 184]. In other words, in flocculated cream systems the single droplets of the dispersed phase become replaced by twin droplets or multiple flocs separated by a thin interfacial film [171, 184].

It has been reported that flocculation occurs as a result of van der Waal's attractive forces that take place in the absence of adequate repulsion between the droplets of the dispersed phase [171, 184]. Usually, individual dispersed phase droplets move through the external phase due to

diffusion or agitation [184]. If resistance between the droplets is not sufficient, flocculation occurs and for example individual droplets may aggregate to form flocs [184] Flocculation does not lead to an increase in the average size of the emulsion droplets, indicating that individual droplets do not lose their integrity [186, 190].

4.2.2.3. Coalescence

The term coalescence is used to describe the aggregation of flocculated droplets into one large droplet [171]. It has been argued that thinning and/or disruption or loss of the interfacial film between approaching droplets in a cream layer is the main driving force for coalescence [171, 184]. Tadros [184] reported that when two flocculated droplets are in close proximity to each other, the liquid surfaces undergo fluctuations forming what Tadros referred to as "waves".

The apices of these fluctuations become the point at which strong van der Waal's forces of attraction are prevalent and when these fluctuations grow in amplitude the distance separating the apices of the interfacial film may reach a critical value that will cause the film to collapse [184]. Subsequently the two flocculated droplets combine to form a single larger droplet [184]. Unlike flocculation coalescence invariably leads to an increase in the average size of the droplets [186, 190] as seen in Figure 4.2.

4.2.2.4. Creaming or sedimentation

Creaming refers to the process by which buoyant droplets of a dispersed phase rise to the top of a container [184, 188, 189], and sedimentation takes place when dispersed droplets sink to the bottom of a container [184]. Creaming or sedimentation occurs due to differences in the densities of the dispersed and continuous phases [184, 188]. The dispersed phase is usually less dense than the continuous phase and therefore droplets will cream or sediment in a gravitational field [184, 188]. Creaming or sedimentation does not involve an increase in the average size of the dispersed droplets, although both creaming and sedimentation may occur prior to coalescence, since coalescence requires the droplets of the dispersed phase to be in close proximity [186].

4.2.2.5. Ostwald ripening

Another relatively important but often neglected cream or emulsion instability is known as Ostwald ripening [184-186, 190]. Ostwald ripening has been described as the growth of large emulsion droplets at the expense of smaller ones due to differences in the solubility [184] or the chemical potential [185, 186, 190] of small and large droplets.

The difference in chemical potential arises from the difference in the radius of curvature of the droplets [184-186, 190]. Ostwald ripening may also be described as a mass transfer between drops of different curvatures through a surrounding continuous phase [185]. The chemical potential of a droplet is reported to increase with a decreasing radius of curvature and, as a consequence, the solubility of the dispersed material at the surface of the droplet also increases [185, 190].

It follows that materials at the surface of smaller droplets tend to dissolve and subsequently diffuse through the continuous phase down a concentration gradient and are deposited on the surface of larger droplets [185, 186, 190]. As a result of mass transfer from small droplets to large droplets the small droplets shrink and ultimately disappear, whereas large droplets grow eventually leading to the formation of a cracked or separated cream [185, 186, 190].

Ostwald ripening does not require the droplets to be in close proximity, since the process takes place by transport of dissolved matter from one droplet to another, through the external phase [186]. Ostwald ripening generally proceeds with the cube of the average radius of a droplet varying linearly with time and this is one of the reasons why Ostwald ripening is usually not considered as being an important phenomenon when considering macroemulsions, since droplets in macroemulsions have radii in excess of between 1-2 μm [190]. Nevertheless, Ostwald ripening is an important cause of instability in creams.

4.2.2.6. Phase inversion

Phase inversion is a phenomenon that occurs when one of the two phases of a cream, for example the internal phase, becomes the external phase as the droplets of the internal phase coalesce faster than the droplets of the external phase [171]. Phase inversion may also be caused by an increase in the volume fraction of a dispersed phase or by a transition produced by changing temperature and/or the addition of an electrolyte to a previously stable cream formulation [184].

4.2.3. Stabilization of creams

4.2.3.1. Surfactants

The physical instability of creams invariably occurs due to the tendency of formulations to revert back to the original distinct two phase systems that have a minimum interfacial free energy (Section 4.2.2) [186]. Since interfacial free energy is the driving force for the irreversible fusion of droplets as seen with flocculation and coalescence (Section 4.2.2), creams may be stabilized by the inclusion of an appropriate emulsion-stabilizing system that will concentrate at the oil-water interface [172, 173].. The use of emulsion stabilization results in a lowering of the interfacial tension between oil and water phases [171, 182]. The stabilizing systems used in most creams consist of surfactants or surface active agents (SAA) or amphiphiles [191].

SAA tend to settle at the boundary between two immiscible phases due to their chemical structure [182]. SAA are characterized by having two distinct regions in their chemical structure *viz.,* a hydrophilic and hydrophobic region [182] as shown in Figure 4.1. The hydrophilic portions may be ionic or non-ionic, whereas hydrophobic regions are invariably saturated or unsaturated hydrocarbon chains or, less commonly, heterocyclic or aromatic ring structures [182]. Generally, SAA are classified according to the nature of the hydrophilic group, therefore SAA can be anionic, such as for example sodium alkyl sulphates, cationic, such as for example alkylammonium halides, or non-ionic, such as polyoxyethylene alkyl ethers or polysorbates [182, 191].

Non-ionic SAA are reported to be the most widely used group of surfactants in cosmetic and pharmaceutical creams [182]. Non-ionic surfactants do not possess a charged group in their hydrophilic polar group, and therefore have a greater degree of compatibility with other components of cream formulations than do the ionic surfactants [182]. In addition non-ionic surfactants are reportedly less sensitive to changes in pH or to the addition of electrolytes, and when used in topical cream formulations non-ionic surfactants tend to cause less skin irritation than ionic surfactants [182]. However, it has been suggested that the main disadvantage of using non-ionic surfactants is that they tend to be more expensive than ionic surfactants [182].

When incorporated into a cream formulation, the hydrophilic region of an SAA is orientated towards the aqueous phase and the hydrophobic portion towards the oil phase [171] (Figure 4.1). The resultant cream that is formed is either an o/w or a w/o emulsion and the specific configuration that is formed depends on the properties of the emulgent system used to stabilize the interface between the dispersed droplets and the continuous phase [171, 191]. The emulsification capacity of SAA is reported to be determined by the relative difference in the size and strength of the polar and non-polar groups that make up the molecule [182].

Oil in water creams are prepared if the hydrophilic characteristics of a SAA are slightly more dominant than the hydrophobic characteristics since the SAA molecule will orientate at the interface in such a way that the hydrophobic portion is forced to the centre of the unit [171, 182] (Figure 4.1). Similarly, it has been reported that w/o creams are prepared if the hydrophobic properties of an amphiphile are slightly more dominant than the hydrophilic characteristics of that molecule [171, 182] (Figure 4.1).

4.2.3.2. Mixed emulgents

In the preparation of simple liquid emulsions, it has been suggested that surfactants alone can stabilize such formulations and mixtures of surfactants have the ability to form more stable emulsions than individual surfactants [171]. However the manufacture of a consistent cream product with a realistic shelf-life may only be achieved by incorporating a specific mixed emulsifying system into the cream formulation [173]. By definition, a mixed emulsifier is an

emulsifying system that consists of a combination of an ionic or non-ionic SAA and a fatty amphiphile, such as a fatty alcohol, fatty acid or monoglyceride [173].

The combination of a surfactant with a fatty amphiphile in the correct ratio is able to produce a powerful emulsifying system of the o/w type with excellent stabilization and thickening properties [172]. It has been suggested that nine (9) parts by weight of fatty alcohol to one (1) part by weight of an ionic surfactant (12:1 molar ratio) or four (4) parts by weight of a fatty alcohol to one (1) part by weight of a non-ionic surfactant (20:1 molar ratio) may also be used to prepare appropriate emulsifying blends for use in o/w creams [173]. The components of mixed emulsifying systems may be added to a cream formulation separately during the manufacturing process or alternatively as a previously blended mixture of emulsifying wax [172, 173].

In its simplest form a cream consists of an oil phase, a water phase and a mixed emulsifying system [171, 192]. Commercial pharmaceutical o/w creams, however, are complex polydispersed systems usually manufactured with several emulgents which complement the properties of each other [172, 173]. Mixed emulgents that are used in o/w creams are usually water-soluble and may consist of anionic or cationic SAA, such as sodium lauryl sulphate or cetrimide respectively, and/or a non-ionic SAA, such as cetomacrogol 1000 in combination with fatty amphiphiles [173].

The amphiphiles are usually higher fatty alcohols having chain lengths of between fourteen (14) and eighteen (18) carbon atoms (C_{14} to C_{18}) and may include substances such as cetyl, stearyl and cetostearyl alcohols, glycerol monosterate and stearic acid [172, 173]. Typical pharmacopoeial and commercially available emulsifying waxes and their respective compositions are summarized in Table 4.1 [172, 173].

Table 4.1. Pharmacopoeial and commercially available emulsifying waxes [172, 173]

Emulsifying wax	Constituents
Emulsifying wax BP	Cetostearyl alcohol and sodium lauryl sulphate
Emulsifying wax USNF	Cetostearyl alcohol and polysorbate-60
Cationic emulsifying wax BPC	Cetostearyl alcohol and cetrimide
Cetomacrogol emulsifying wax BPC	Cetostearyl alcohol and cetomacrogol-1000
Glyceryl monostearate S.E.	Glycerol monostearate and sodium citrate
Lecithin	Phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol and phosphatic acid

4.2.3.3. Theory of cream emulsification

It has been argued that a reasonable and coherent explanation for the manner in which mixed emulsifiers or emulsifying waxes stabilize oil droplets and control the consistency of o/w creams may be given using the gel network theory of emulsion stability [171-173]. The gel network theory relates the consistencies and stabilities of o/w creams to the presence or absence of crystalline viscoelastic gel networks in the external phase of cream formulations [171-173].

The amount of mixed emulgent added to an o/w semi-solid pharmaceutical cream is usually in excess of that required to form a monomolecular interfacial film in a simple model emulsion [171, 173]. The surplus emulsifying wax in a cream formulation interacts with the aqueous continuous phase to form strong crystalline viscoelastic gel networks at the oil-water interface of the dispersed droplet [171, 173]. In general, the gel network theory illustrated schematically in Figure 4.3 implies that a cream formulated to contain a mixed emulsifier system consisting of combination of a fatty amphiphile, such as cetostearyl alcohol, and an ionic or a non-ionic surfactant may be composed of at least four (4) phases [171, 173] *viz.*:

a) a bulk water phase,
b) a dispersed oil phase,
c) a crystalline hydrate phase,
d) a crystalline gel phase.

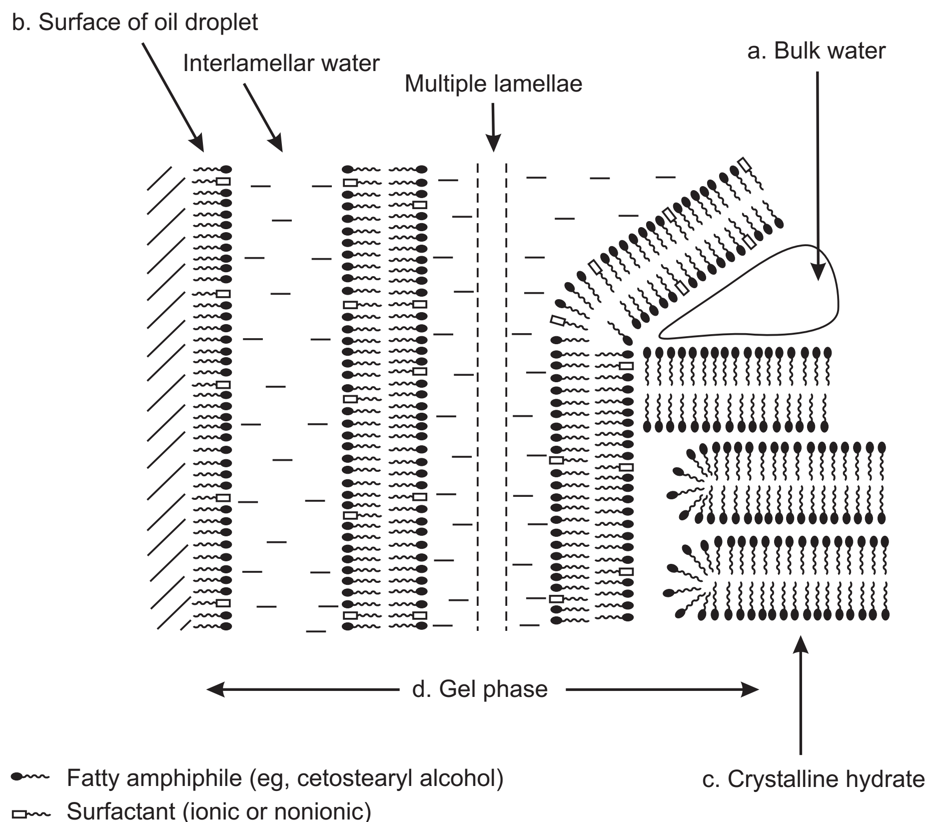


Figure 4.3. Illustration of the gel network theory (adapted from 171).

Eccleston [173] reported that fatty amphiphiles such as cetostearyl alcohol usually exhibit marked crystalline polymorphism and have the capacity to form an α-crystalline polymorph under specific conditions. Eccleston [173] argued that in the α-form, the hydrocarbon chains of the amphiphile are hexagonally packed and there is a rotation about the long axis of the molecule. Eccleston [173] further argued that in the presence of water (a) the α-crystalline polymorph will form hydrated crystalline phase (b) in which the thickness of the water layers incorporated in the polar groups of the amphiphile is approximately eighteen (18) Å and is limited by the considerable strength of the van der Waal's attractive forces balancing osmotic repulsion in the system.

The presence of very small quantities of ionic or non-ionic surfactant affect the degree of swelling in the system and the swelling is enhanced giving rise to a swollen crystalline gel phase (d) [172].. The swelling capacity of the crystalline gel phase depends on the hydrophilic properties of the intrinsic polar groups of the amphiphile used in the emulsifier system [171]. It

has been suggested that the gel phase is characterized by a lamellar structure of alternating bilayers of fatty amphiphile and surfactant separated by layers of interlamellar and fixed water [171, 173].

The swelling properties and the concentration of the crystalline gel phase are factors that dictate the overall consistency of a cream structure [171, 172]. The interlamellar-fixed water located between the bilayers of the gel and crystalline hydrate phases enhances the volume ratio of the dispersed phase to the free bulk water phase, resulting in stiffening of the system [173]. In order to maintain the stability of a system it is vital to maintain a dynamic equilibrium between the fixed water in the interlamellar gel and the bulk water phases [171, 172].

At low mixed emulsifier concentrations, such as for example 2-4% w/w, stiffening is sufficient to produce a structured lotion [173]. However, at high mixed emulsifier concentrations, such as for example > 4% w/w, the crystalline hydrate and the gel phases link to form strong a crystalline viscoelastic gel phase and the system becomes a structured semi-solid cream [173].

The swelling capacity of the α-crystalline polymorphic form of an amphiphile that leads to the formation of the crystalline viscoelastic gel phase is greater in the presence of ionic surfactants than in the presence of non-ionic surfactants [171]. This has been ascribed to that fact that swelling in the presence of an ionic surfactant is due to an electrostatic phenomenon, whereas in the presence of a non-ionic surfactant, swelling is attributed to hydration of the polyoxythylene chains of the surfactant and is usually limited by the length of the chain [171]. Nevertheless, the underlying principles of the gel network theory apply whichever surfactant and fatty amphiphile is used, albeit to different extents [173].

The gel network theory reveals that mixed emulsifiers through swelling of the α-crystalline form of an amphiphile and subsequent formation of crystalline viscoelastic gel networks introduce an electrostatic repulsive force between dispersed droplets [173]. Consequently, there is a reduction in van der Waal's forces of attraction between dispersed oil droplets which subsequently prevents or delays the processes of flocculation, coalescence and/or Ostwald ripening (Section 4.2.2) from occurring [173]. Furthermore, the resultant stiffening of a cream structure due to the

formation of a crystalline viscoelatic gel phase invariably inhibits or reduces creaming or sedimentation (Section 4.2.2) in a cream formulation [184].

The formation of a crystalline viscoelastic gel network phase in a cream formulation is dependent on the type of amphiphile and the nature of the ionic or non-ionic surfactant, the concentration of the mixed emulsifier and the molar ratios of amphiphile to surfactant used in that formulation [173].

4.2.3.4. Hydrophilic-lipophilic balance (HLB)

Sections 4.2.3.2 and 4.2.3.3 report that the physical stability of pharmaceutical creams may be best achieved using mixed emulsifiers rather than by use of a single SAA. In addition the components of a mixed emulsifier may be added separately to a cream formulation during manufacture, or alternatively as a previously blended emulsifying wax (Table 4.1). When the components of a mixed emulsifier system are added separately, it is vital that the formulation scientist determines the appropriate ratio of each component necessary to produce a physically stable cream [182].

The relative quantities of individual emulgents may be calculated using a tool referred to as the hydrophilic-lipophilic balance (HLB) [171, 182]. Essentially each emulgent is allocated an HLB number representing the relative balance between the hydrophilic and lipophilic characteristics within a SAA molecule [171, 182]. The HLB number is an arbitrary value ranging between 0-20, which is then assigned to a particular SAA [171].

According to the HLB system, hydrophilic surfactants have high HLB values, whereas hydrophobic surfactants have low HLB values [171, 182]. Generally emulgents with HLB values ranging between 4-6 are w/o emulsifiers and the use of these emulsifiers tends to favour the formation of w/o creams [171, 182]. SAA with HLB values ranging between 8-18 are reported to be o/w emulsifiers and their use will favour the formation of o/w creams [171, 182]. Although the HLB system was originally applied to non-ionic SAA its successful use has been extended and applied to ionic emulgents [182].

The HLB number of a polyoxyethylene-based non-ionic SAA may be calculated using Equation 4.1 [171]:

$$HLB = \frac{mol\%hydrophobicgroup}{5} \qquad \textbf{Equation 4.1.}$$

The HLB value of a polyhydric alcohol fatty acid ester, such as glyceryl monostearate may be derived from Equation 4.2 [171]:

$$HLB = 20\left(\frac{1-S}{A}\right) \qquad \textbf{Equation 4.2.}$$

Where,

S = the saponification value of the ester

A = the acid value of the fatty acid

When it is impossible to obtain a saponification value of a compound as is the case with lanolin derivatives, the HLB may be calculated using Equation 4.3 [171]:

$$HLB = \left(\frac{E+P}{5}\right) \qquad \textbf{Equation 4.3.}$$

Where,

E = the weight percent (wt %) of the polyoxyethylene chain

P = the wt % of the polyhydric alcohol group

When using a mixed emulsifier system, the overall HLB value of a surfactant mixture (HLB_M) may be calculated using Equation 4.4 [171]:

$$HLB_M = fHLB_A + (1-f)HLB_B \qquad \textbf{Equation 4.4.}$$

Where,

f = the weight fraction of emulsifier A.

The HLB system and theory may be used as an approximation in the design of a cream formulation, however the stability of a cream may not be guaranteed by the use of a mixed emulsifier system with an appropriate HLB value [171]. This is more than likely due to the fact that a typical destabilization process, such as for example creaming, may be much more dependent on other variables, such as viscosity of the continuous phase, rather than the characteristics of the interfacial film [171]. Thus such factors should be taken into consideration when designing complex pharmaceutical cream formulations.

4.3. EXPERIMENTAL

4.3.1. Characterization of CP creams

4.3.1.1. Overview

Prior to the development of a generic CP cream formulation, it was considered essential to select physicochemical quality control tests for use in conjunction with an *in vitro* release test method (Chapter 3) to characterize the commercial and extemporaneously manufactured CP cream formulations. In addition the assessment of quality and consistency of extemporaneously manufactured CP topical formulations form an integral part of formulation development studies.

Physicochemical tests such as the determination of solubility, particle size, size distribution and crystalline form of an API and evaluation of the intrinsic viscosity and homogeneity of cream products, have been traditionally used to provide reasonable evidence of consistent product performance (Chapter 3). However, for the purposes of these studies, innovator and generic cream products were characterized for CP content, apparent intrinsic viscosity, pH and *in vitro* release.

4.3.1.2. Assay of CP content

The United States Pharmacopoeia (USP) [23] specifies that CP cream formulations should contain not less than 90.0% and not more than 115.0% of the labelled amount of CP. As a consequence, it was considered essential to assay CP content in all generic formulations

manufactured extemporaneously in these studies in order to ensure that the formulations manufactured contained CP within this specified range. The CP content of all extemporaneously manufactured and innovator cream products was assayed using the sample preparation procedure described in Section 2.4.6.2 (Chapter 2) and as illustrated in Figure 2.7 (Chapter 2).

4.3.1.3. Intrinsic viscosity

The intrinsic viscosity of a semi-solid formulation invariably affects the rate and extent of release of an active pharmaceutical ingredient (API) from a vehicle (Section 3.3.2.3). Therefore, the evaluation of the intrinsic viscosity values of extemporaneously manufactured generic CP cream products and the intrinsic viscosity value of the innovator product was considered essential in these studies.

Any similarity or difference in the intrinsic viscosity values between the innovator and extemporaneously manufactured CP cream products may be used as a basis for the discussion of similarity or differences in the *in vitro* release rates of CP from the generic and innovator cream products.

The intrinsic viscosity values of all extemporaneously manufactured CP products and the intrinsic viscosity value of the innovator formulation were measured as described in Section 3.3.2.3.2.

4.3.1.4. pH-determination

The pH of dermatological semi-solid vehicles has been reported to affect the extent of dissociation of ionisable API molecules and therefore the thermodynamic activity of the API, partitioning and skin penetration [193]. CP is a non-ionisable molecule and does not dissociate (Section 1.3.2). Therefore, it is highly unlikely that the pH of a CP topical formulation would affect the release rate of CP from the vehicle or penetration and partitioning of CP into the skin.

Healthy human skin reportedly has a surface pH that ranges between 4-6 [193, 194] and a pH gradient exists within the skin [193]. Although the pH of a topical formulation of CP may not have any influence on the performance characteristics of the product, it was nevertheless considered vital to develop a drug delivery system that would be pH-sensitive to the human skin. Consequently the pH of the innovator and extemporaneously manufactured cream formulations was determined to ensure that the manufactured products had an appropriate pH.

The pH of extemporaneously manufactured CP cream formulations and the innovator product was measured using a Model GLP 21 Crison pH meter (Crison Instruments, Barcelona, Spain). The pH was evaluated using 100 g of the cream and the measurements were taken within twenty-four (24) hours of manufacture of the extemporaneous formulations. The pH measurements were recorded in triplicate to generate an average pH value for each formulation.

4.3.1.5. In vitro release rate

The role of *in vitro* release rate testing during the development of topical semi-solid formulations has been discussed in Chapter 3. In this chapter the equipment and conditions suitable for assessing the *in vitro* release rate of CP from cream formulations were developed and validated. The *in vitro* release test was essentially used as a marker for the successful development of the generic cream formulation relative to the commercially available innovator product (Dermovate® cream). Furthermore, the *in vitro* release test was used to determine batch-to-batch consistency as well as in the evaluation of cream formulations used in accelerated stability studies (Chapter 5).

The *in vitro* release studies were conducted using the *in vitro* release test conditions presented in Table 3.5 (Section 3.2.12). *In vitro* release rate profiles of CP were plotted as cumulative amount of CP released per unit area (Q) *vs.* the square root of time ($t^{1/2}$) or Q *vs.* $t^{1/2}$ (Section 3.3.1). Similarities or differences in the *in vitro* release rates of CP from the test formulations (generic products) *vs.* Dermovate® cream were determined theoretically using Q and flux values (Section 3.3.1) and statistically using a non-parametric test which was described and reported in Section 3.2.11.

4.3.2. Innovator product characterization

4.3.2.1. Overview

Kanfer e*t al.* [195] suggest that during the development of a generic drug product, it may be beneficial to critically evaluate the innovator product with respect to qualitative and/or quantitative assessments. Thus, prior to the development of a generic CP cream formulation, the *in vitro* performance characteristics of the innovator product were evaluated and the data generated from these studies were used as a general guide for the successful development of a generic formulation. The purpose of conducting these studies was to ensure that the *in vitro* performance characteristics of the generic CP cream formulations produced during the development phase were equivalent to those of the innovator product.

4.3.2.2. Qualitative composition

The formulator of a generic product may consider using the same or similar inactive excipients as in the innovator product [151]. Consequently, as part of the characterization phase of Dermovate® cream, it was considered essential to evaluate the qualitative composition of the product, as this could give an indication of the inactive excipients that could be considered for use in the formulation of a generic CP cream product. The qualitative composition of Dermovate® cream [196] is listed in Appendix 2.

4.3.2.3. CP content

Dermovate® cream was assayed for CP content using the sample preparation procedure described and reported in Section 2.4.6.2 and from these studies, Dermovate® cream was found to have a CP content of 103.9 ± 2.797% (2.69% RSD) (n = 3). These data reveal that Dermovate® cream complies with the USP specifications for clobetasol proprionate cream [23], which states that formulations should contain not less than 90.0% and not more than 115.0% of the labelled amount of CP. The small % RSD is indicative of the homogeneity of the product and the precision of the analytical method.

4.3.2.4. Intrinsic viscosity

The intrinsic viscosity of Dermovate® cream was measured as described in Section 3.3.2.3.2 and it was found that Dermovate® cream has an apparent intrinsic viscosity value of 44.467 ± 1.026 KcP (2.31% RSD) (n = 3). The measurements were recorded at room temperature (22˚C).

4.3.2.5. pH-determination

The apparent pH of Dermovate® cream was determined at room temperature (22˚C) as described in Section 4.3.1.4 and from these studies, it was found that Dermovate® cream has an apparent pH value of 5.05 ± 0.0321 (0.00637% RSD) (n =3). These data reveal that the apparent pH of Dermovate® cream lies within a pH range of between 4-6, which is considered to be the pH range of a healthy human skin (Section 4.3.1.4).

4.3.2.6. In vitro release rate studies

The *in vitro* release rate of CP from Dermovate® cream was assessed using the *in vitro* release test conditions described in Table 3.5 and the resultant *in vitro* release rate profile of CP from Dermovate® cream plotted as Q vs $t^{½}$ is shown in Figure 4.4. These data reveal that the cumulative amount of CP released (Q) over the 72-hour *in vitro* release studies was 36.69 ± 5.720 $\mu g/cm^2$, which was equivalent to a cumulative % release of 24.19 ± 3.750%. The average in vitro release rate of CP from Dermovate® cream or flux (n = 6) was found to be 4.924 $\mu g/cm^2/hr^{1/2}$.

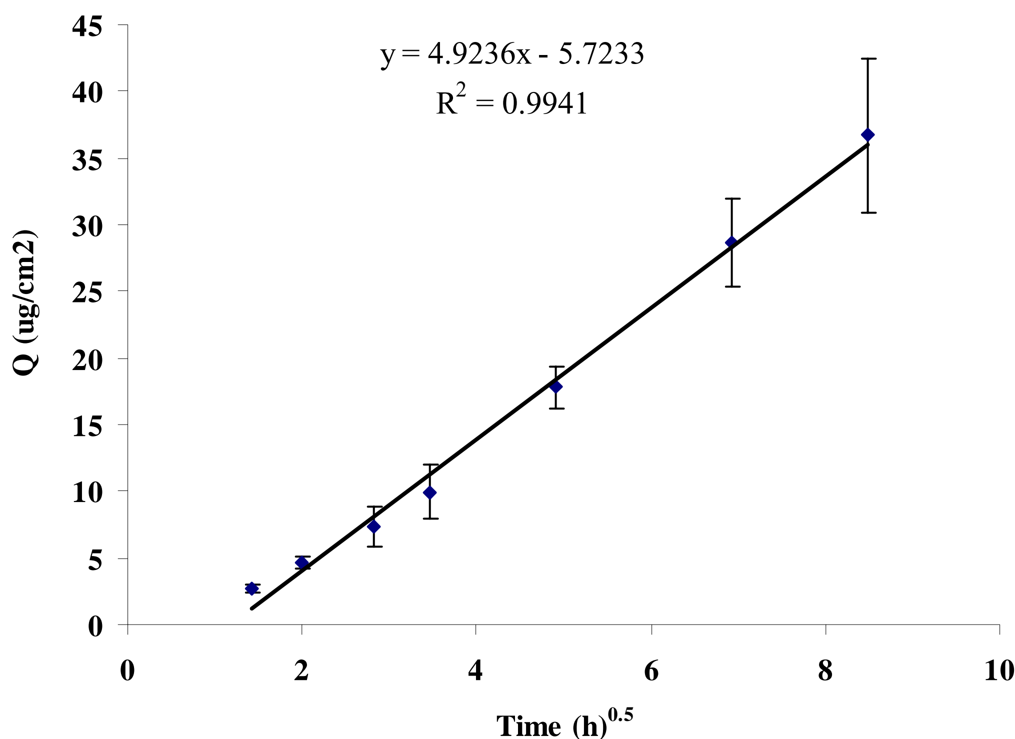


Figure 4.4. *In vitro* release rate profile for CP from Dermovate® cream (n = 6)

The *in vitro* release rate or flux of CP from Dermovate® cream of 4.924 $\mu g/cm^2/h^{1/2}$ appears to be fairly slow. Moreover the extent of CP release of 36.69 ± 5.720 $\mu g/cm^2$ or 24.19 ± 3.750% seems to be low considering the fact that the experiments were conducted over a 72-hour time period. The apparent slow *in vitro* release rate of CP from Dermovate® cream and the relatively small cumulative amount of CP released over the 72-hour experiment may be attributed to the seemingly high apparent intrinsic viscosity value of Dermovate® cream (Section 4.3.2.4).

CP release from Dermovate® cream formulation, however, appears to fit the Higuchi model described and reported in Section 3.2.8.2. According to the Higuchi model, a plot of Q *vs.* $t^{1/2}$ would be linear for a drug in suspension in a semi-solid matrix with an infinite dose application provided that such release is diffusion-controlled by the matrix material that comprises the semi-solid dosage form. Therefore, judging from the R^2 value *viz.,* $R^2 = 0.9941$ shown in Figure 4.4, CP release from Dermovate® cream may be diffusion-controlled.

4.3.3. Generic product development

4.3.3.1. Overview

In order to gain market approval, a generic product cannot be superior to or better than the innovator product against which it is tested and the product must meet certain criteria [151] to be considered bioequivalent and therefore therapeutically equivalent to the brand name product [151]. During the development of generic drug product formulations, it is vital to ensure that the developed formulation will have the same therapeutic efficacy, safety and performance characteristics as the innovator product it is tested against [151].

Although the objective of the current research work was not to determine bioequivalence or therapeutic equivalence of the extemporaneously manufactured generic CP cream formulation relative to Dermovate® cream, it was vital to ensure that the generic formulation was at least equivalent to the innovator cream product in terms of the *in vitro* tests conducted on these products.

Essentially formulation development studies for the generic CP cream product were comprised of three stages:

a) Preliminary studies for which the objective was to manufacture a formulation that remained physically stable for at least twenty-four (24) hours after manufacture.
b) *In vitro* release studies for which the objective was to establish whether the formulation tested had an *in vitro* release rate that was similar to that of the innovator product.
c) Characterization of the generic formulation for that formulation that had an *in vitro* release rate profile similar to that of the branded product.

In addition, the best generic CP formulation was then manufactured on a relatively larger scale to produce a batch with a sufficient number of samples for accelerated stability studies. The purpose of the latter studies was to determine whether the formulation development process was

successful in producing a stable generic CP cream product. The data generated from these studies are described and reported in Chapter 5 *vide infra*.

4.3.3.2. Excipients

4.3.3.2.1. Overview

The excipients used in formulation development studies of generic CP cream products and their sources are listed in Appendix 3. The physicochemical characteristics and functions of each excipient used in these formulation development studies are described in detail in the following Sections.

4.3.3.2.2. Clobetasol 17-propionate

The description, physicochemical characteristics, structural activity relationships, clinical pharmacology and pharmacokinetic aspects of clobetasol 17-propionate (CP) have been reported and described previously (Chapter 1).

4.3.3.2.3. Propylene glycol

Propylene glycol, chemically known as 1,2-propanediol, has been described as a clear, colourless, viscous liquid with a sweet, slightly acrid taste resembling that of glycerin [19, 140, 197, 198]. Propylene glycol may be used as a preservative, humectant, plasticizer, solvent or stabilizer in a variety of pharmaceutical formulations [19, 140]. However in these studies propylene glycol was used mainly as a water-miscible co-solvent for CP. Propylene glycol has a better ability than glycerine to dissolve drugs such as the corticosteroids [140, 198]. Propylene glycol is generally regarded as a relatively non-toxic material and is considered to be minimally irritant when used in topical formulations [19, 140]. Propylene glycol has also been reported to be chemically stable when stored in well-closed containers at cool temperatures [197, 140].

4.3.3.2.4. Sodium citrate

Sodium citrate is the non-proprietary name for sodium citrate dihydrate [199] and occurs as odourless, colourless, monoclinic crystals or as a white crystalline powder with a cooling saline taste [19, 199]. The solubility of sodium citrate dihydrate has been reported to be 1 in 1.5 parts of water at room temperature (20˚C) [19, 197, 199] and 1 in 0.6 parts of boiling water (100˚C) [19, 199]. Sodium citrate is generally regarded as a stable, non-toxic and non-irritant excipient that can be used as a pH adjusting agent [199]. Sodium citrate was used as a pH adjusting agent to adjust the pH of the CP cream formulations.

4.3.3.2.5. Citric acid

Citric acid is also known as citric acid monohydrate and occurs as colourless or translucent crystals, or as a white crystalline efflorescent powder [19, 200]. Citric acid is odourless [19, 200] and has a strong acidic taste and an orthorhombic crystal structure [200]. The solubility of citric acid is 1 in less than 1 part of water at room temperature (20˚C) [19, 197, 200]. Citric acid was used in conjunction with sodium citrate as a buffering agent [200].

4.3.3.2.6. Geleol®

Geleol® (Gattefossé SAS, Saint-Priest Cedex, France) is the proprietary name for glyceryl monostearate. Glyceryl monostearate is a white or almost white to cream-coloured, wax-like solid in the form of beads, flakes or powder [19, 201]. Glyceryl monostearate is waxy to the touch and has a slight fatty odour and taste [19, 201]. Commercially available glyceryl monostearate contains a mixture of variable proportions of glyceryl monostearate and glyceryl monopalmitate [19, 201]. Glyceryl monstearate is insoluble in water but is soluble in mineral and fixed oils [19, 201]. Glyceryl monostearate can be used as an emollient, emulsifying agent, solubilising agent and/or stabilizing agent in topical pharmaceutical formulations, [201].

Glyceryl monostearate and other fatty acid monoesters are however reported to be inefficient emulsifiers, but may be useful emollients that can be readily emulsified by common emulsifying

agents by the addition of other fatty materials to a formulation [19, 201]. When incorporated in topical formulations, glyceryl monostearate is generally regarded as a non-toxic and non-irritant material [201] and should be stored in a tightly closed container in a cool, dry place protected from light [201]. Self-emulsifying grades of glyceryl monostearate are available and have been reported to be incompatible with acidic substances [201]. Glyceryl monostearate has a melting point of ≥ 55˚C [19, 201].

4.3.3.2.7. Cetostearyl alcohol

Cetostearyl alcohol occurs as a white or cream-coloured unctuous mass or as almost white flakes or granules and has a faint characteristic sweet odour [19, 202]. When heated, cetostearyl alcohol melts to a clear, colourless or pale yellow-coloured liquid that is free of suspended matter [19, 202]. Cetostearyl alcohol is practically insoluble in water and is soluble in oils [19, 197, 202]. Cetostearyl alcohol may be used as an emollient, emulsifying agent or as a viscosity-increasing agent in semi-solid formulations [19, 202].

Cetostearyl alcohol has been used to increase the viscosity of topical pharmaceutical formulations and imparts body to both w/o and o/w creams [19, 202]. Furthermore cetostearyl alcohol stabilizes creams and can act as a co-emulsifier, thereby reducing the amount of surfactant required to form a stable emulsion [202]. When used in combination with surfactants, cetostearyl alcohol forms creams with very complex microstructures, including liquid crystals, lamellar structures and gel phases (Section 4.2.3.3) [202].

Cetostearyl alcohol is stable when stored in a well-closed container in a cool, dry place [202]. Cetostearyl alcohol is incompatible with strong oxidizing agents and metal salts, but is generally a non-toxic material [202] and has a melting range of between 49-56˚C [19, 202] or between 48-55˚C [19].

4.3.3.2.8. White beeswax

White beeswax is a chemically bleached form of yellow beeswax [19, 197, 203] and occurs as tasteless, white or yellow or slightly yellow-coloured sheets or fine granules with some translucence [203]. White beeswax has an odour similar to that of yellow beeswax although the odour is less intense [203]. White beeswax is practically insoluble in water, but soluble in fixed and volatile oils and can be used as a stabilizing and stiffening agent in semi-solid formulations [19, 197, 203]. White beeswax stabilizes w/o emulsions [203] and increases the consistency of semi-solid formulations, such as creams and ointments [19, 197, 203].

White beeswax is a non-toxic and non-irritant material [203], however hypersensitivity which has been ascribed to contaminants in the wax has been reported [19, 203]. White beeswax is incompatible with strong oxidizing agents and is stable when stored in well-closed containers protected from light [203]. White beeswax has a melting point range of between 61-65˚C [19, 197, 203] and when heated to above 150˚C esterification occurs with a consequent lowering of the acid value and elevation of the melting point of the material [203].

4.3.3.2.9. Chlorocresol

Chlorocresol occurs as colourless or almost colourless dimorphous crystals or as a crystalline powder with a characteristic phenolic odour [19, 197, 204]. Chlorocresol is soluble as 1 in 260 parts of water at room temperature (20˚C) [19, 197, 204] and 1 in 50 parts of boiling water [19, 204] and is soluble in fixed oils [19, 197, 204]. Chlorocresol is primarily used as a preservative in topical formulations and has bactericidal activity against both Gram-positive and Gram-negative organisms, spores, moulds and yeasts [19, 197, 204]. Chlorocresol is stable at room temperature (22˚C) and should be stored in a well-closed container, protected from light, in a cool, dry place [204]. Chlorocresol on contact with strong alkali agents produces heat and fumes that ignite explosively [204]. The melting point range of chlorocresol is between 55.5-65˚C [204] or between 63-67˚C [19]

4.3.3.2.10. Estol® 1474

Estol® 1474 (Uniqema (Pty) Ltd, Bryanston, Johannesburg, Gauteng SA) is the proprietary name for glyceryl stearate. Estol® 1474 is a white powder at 25˚C [205] and is reported to be oil soluble and has an HLB value of 2.0-3.0 [205, 206]. According to the manufacturers of Estol® 1474 the material may be used as an emulsifier for o/w or w/o creams and can modify the consistency of such formulations thereby increasing the stability of cream products [205].

4.3.3.2.11. Ritapro® 200

Ritapro® 200 (Rita, Crystal Lake, IL, USA) is the proprietary name for an emulsifying wax containing a mixture of stearyl alcohol and ceteareth-20 [207]. Ritapro® 200 is sold as a white flaky or wax-like material and is odourless [207]. Ritapro® 200 may be used as an emulsifier for o/w creams and has a melting range of between 55-63˚C [207].

4.3.3.2.12. Emulcire® 61 WL

Emulcire® 61 WL (Gattefossé SAS, Saint-Priest Cedex, France) is the proprietary name for a self-emulsifying wax containing a mixture of cetyl alcohol, ceteth-20 and steareth-20 [208, 209]. Emulcire® 61 WL is sold as white pellets [209] and has a melting range of between 45.6-50.5˚C [208]. Emulcire® 61 WL is considered to be an o/w emulsifier that may be used for emulsifying difficult-to-formulate, unstable active ingredients and can yield creams with excellent heat stability [208].

4.3.3.2.13. Gelot® 64

Gelot® 64 (Gattefossé SAS, Saint-Priest Cedex, France) is the proprietary name of a self-emulsifying base containing a mixture of glyceryl monostearate and polyethylene glycol-75 stearate (PEG-75 stearate) [208]. The physical appearance of Gelot® 64 has not been reported but visual observation of Gelot® 64 reveals that the material occurs as yellow or off-yellow pellets. Gelot® 64 has a melting range of 55.5-62.5˚C [208] and is an o/w emulsifier that may be used for

emulsifying difficult-to-formulate, unstable active ingredients and can yield creams with excellent heat stability [208].

4.3.4. Formulation composition

All formulation compositions developed and tested during the development of generic CP cream are listed in Table 4.2. The qualitative and quantitative formulae were adapted from a published formulation [210] which is listed in Appendix 4. The main modification made to the formulation summarized in Appendix 4 was the use of different primary mixed emulsifiers.

Table 4.2. Percentage composition of generic CP cream formulations developed and assessed in these studies

Component #	Excipient	CP001	CP002	CP003	CP004	CP005	CP006
1	Clobetasol 17-propionate	0.05000	0.05000	0.05000	0.05000	0.0500	0.0500
2	Propylene glycol	44.50	44.50	44.50	44.50	44.50	44.50
3	Sodium citrate	0.05000	0.05000	0.05000	0.05000	0.0500	0.0500
4	Citric acid	0.050000	0.050000	0.050000	0.050000	0.0500	0.0500
5	Geleol® pastilles	5.000	5.000	5.000	5.000	5.000	5.000
6	Cetostearyl alcohol	4.000	4.000	4.000	4.000	4.000	4.000
7	White beeswax BP	0.6000	0.6000	0.6000	0.6000	0.6000	0.6000
8	Chlorocresol	0.07500	0.07500	0.07500	0.07500	0.07500	0.07500
9	Estol® 1474*	1.000	-	-	-	-	-
10	Ritapro® 200*	-	1.000	-	-	-	-
11	Emulcire® 61 WL*	-	-	1.000	-	-	-
12	Gelot® 64*	-	-	-	1.000	1.000	1.000
13	Propylene glycol	7.000	7.000	7.000	7.000	7.000	7.000
14	Propylene glycol	2.675	2.675	2.675	2.675	2.675	2.675
15	Purified water	35.00	35.00	35.00	35.00	35.00	35.00

* Primary emulsifier

4.3.5. Manufacturing methods

4.3.5.1. Overview

Generic CP cream formulations were manufactured using a manufacturing process adapted from a manufacturing method that has been published [210]. This method of manufacture was used for the preparation of all CP cream formulations developed and tested in these studies and is described in the following sections and illustrated in Figure 4.5. All items were weighed using a Model AE-163 Mettler top-loading analytical balance (Mettler Instruments, Zurich, Switzerland).

4.3.5.2. Aqueous phase

Purified water (item 15) was heated to 90˚C in a beaker using a Model RCH IKA-Combimag hotplate magnetic stirrer (Jankel & Kunkel KG, Staufen, Germany). The temperature of the heated purified water was brought down to and maintained at 60˚C using a Model NB-34980 Colora Ultra-Thermostat water bath (Colora, Lorch, Germany) that had been previously set at 60˚C. Sodium citrate and citric acid (items 3 and 4) were dissolved in item 15 at 60˚C and the resultant solution was mixed with propylene glycol (item 2). The temperature of the aqueous phase was maintained at 60˚C using the water bath.

4.3.5.3. Oil phase

Geleol® pastilles (item 5) and Estol® 1474 (item 9) or Ritapro® 200 (item 10) or Emulcire® 61 WL (item 11) or Gelot® 64 (item 12) were melted together with cetostearyl alcohol (item 6), white beeswax BP (item 7) and chlorocresol (item 8) while stirring in a beaker previously heated to and maintained at 75˚C using the IKA-combimag hotplate magnetic stirrer (Section 4.3.5.2). Once melted, the temperature of the molten oil phase was allowed to cool to 60°C and was maintained at that temperature using a Colora Ultra-Thermostat water bath (Section 4.3.5.2).

4.3.5.4. Dispersed phase

The aqueous phase (Section 4.3.5.2) was then transferred to the oil phase (Section 4.5.5.3) maintained at 60˚C. The mixture was stirred manually with a glass stirring rod for ten (10) minutes at that temperature and was then homogenized at approximately 15,000 rpm using a Model 6-105 AF Virtis homogenizer (Virtis Co., Gardiner, N.Y. USA) for five (5) minutes. The temperature of the mixture was allowed to cool to 50˚C while stirring manually with the manufacturing beaker placed in a water bath at 20˚C.

4.3.5.5. Drug phase

Clobetasol (item 1) was mixed with propylene glycol (item 13) in a beaker and sonicated using a Model 8845-30 ultrasonic bath (Cole-Parmer Instrument Co., Chicago, IL, USA) for approximately twenty-five (25) minutes or until a clear solution was obtained. The solution was heated to and maintained at 50˚C using a Colora water bath. Once the temperature of the solution had reached 50˚C the solution was added to the dispersed phase (Section 4.3.5.4) whilst maintaining the temperature at 50˚C. The beaker that had previously contained the drug phase was rinsed with propylene glycol (item 14), which had been heated and maintained at 50˚C. The rinse solution was added to the dispersed phase (Section 4.3.5.4) and the mixture was manually mixed for a further ten (10) minutes and then homogenized at 15,000 rpm for an additional five (5) minutes at 50˚C.

4.3.5.6. Cream formulation

The formulation prepared as described in Section 4.3.5.5 was cooled to 30˚C with continual manual stirring with the manufacturing beaker placed in a water bath at 20˚C. After cooling, the cream was passed through a Model-HO valve type of homogenizer (Erweka-Apparatebau, G.m.b.H, Heusenstamm, Germany) in order to generate a smooth formulation of improved consistency. The cream was then packaged into 100 g ointment jars and stored at room temperature (22˚C) until required for further analysis.

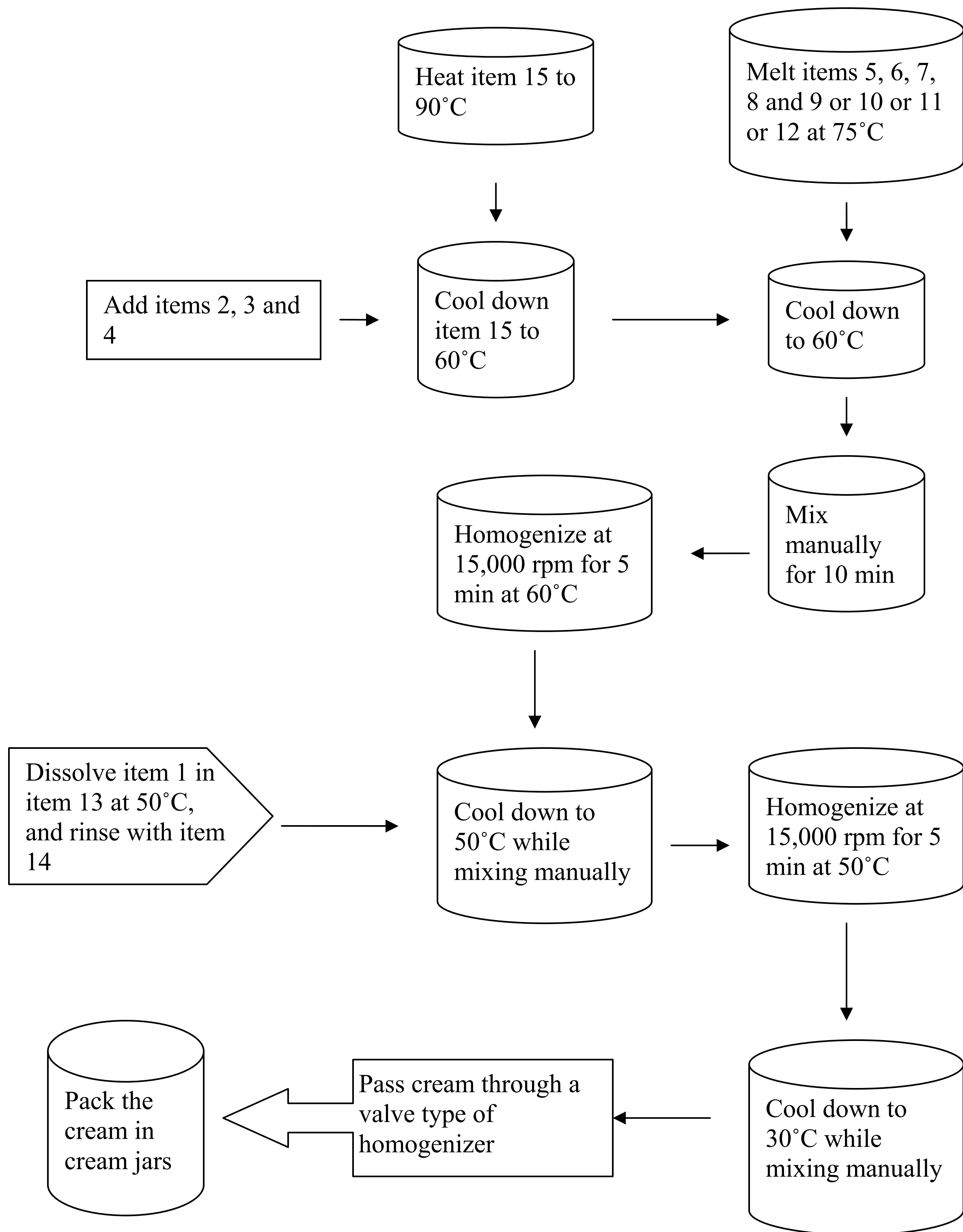


Figure 4.5. Schematic illustration of the manufacturing method for the CP cream formulation

4.3.6. Preliminary studies

In the preliminary stages of these studies, the objective was to manufacture a CP cream formulation extemporaneously that showed no visible signs of physical instability, such as, for example, cracking, creaming, phase inversion and/or bleeding of the cream base from the container. Physical instability was evaluated immediately after manufacture and then twenty-four (24) hours after manufacture and storage at room temperature (22˚C). Initial formulation development was undertaken on small batches of only 200 g and any formulation that showed signs of physical instability immediately and/or after twenty-four (24) hours of storage at room temperature (22˚C) was considered unsuitable and therefore not considered for further investigation.

The initial prototype cream formulation, Batch CP001 containing Estol® 1474 as the primary emulsifier, was extemporaneously manufactured. However, although Batch CP001 appeared homogenous, smooth and physically stable immediately after manufacture, signs of instability, such as phase separation or cracking and bleeding of the base from the container were observed twenty-four (24) hours after storage at room temperature (22˚C). Consequently, Batch CP001 was not characterized further in these studies. A batch summary record for Batch CP001 is reported in Appendix 4.

It is possible that Batch CP001 failed to remain physically stable over the twenty-four (24) hour storage period because Estol® 1474 is not a suitable primary emulsifier for CP cream formulation. Despite the manufacturers of Estol® 1474 [205] stating that the emulsifier may be used for the preparation of w/o and o/w in emulsions, the compound may only be useful for the manufacture of liquid emulsions and not semi-solid emulsions, such as creams. This characteristic may be due to the fact that Estol® 1474 contains no surfactant but only a single amphiphile, glyceryl stearate (Section 4.3.3.2.10).

Estol® 1474 therefore cannot be considered as a mixed emulsifier and considering the fact that no surfactant was incorporated into Batch CP001 as a separate ingredient, Estol® 1474 was not able to form a mixed emulsification system *in situ*. Consequently, viscoelastic gel networks at the oil-

water interface that are reported to provide and maintain the stability of o/w creams (Section 4.2.3.3) were not formed. The apparent stability of Batch CP001 observed immediately following manufacture may possibly be attributed to the presence of amphiphiles in the formulation that thickened the formulation immediately on production. However, on standing the formulation was not sufficiently viscous to maintain the physical stability over a prolonged period due to the fact that no surfactant was included in the formulation.

In order to manufacture a physically stable generic CP cream extemporaneously that would remain stable over a longer period of time, it may be necessary to add an appropriate surfactant to the formulation or substitute Estol® 1474 for a commercially available mixed emulsifier system, which would be the ideal approach. Consequently, three (3) batches of cream, Batches CP002, CP003 and CP004 were manufactured using the commercially available mixed emulsifiers Ritapro® 200 (Section 4.3.3.2.11), Emulcire® 61 WL (Section 4.3.3.2.12) and Gelot® 64 (Section 4.3.3.2.13). The quantitative compositions of these three batches are shown in Table 4.7 and the batch summary records for these formulations are shown in Appendix 4.

Batches CP002, CP003 and CP004 also appeared to be homogenous, smooth and physically stable immediately after manufacture as had been observed for Batch CP001. However, unlike Batch CP001 all three batches in which mixed emulsifiers were incorporated showed no signs of physical instability twenty-four (24) hours after manufacture and storage at room temperature (22˚C). A possible explanation for the immediate prolonged stability of these formulations could be that Ritapro® 200, Emulcire® 61 WL and Gelot® 64 are all emulsifying waxes, containing mixtures of fatty amphiphiles and surfactants. Therefore Ritapro® 200, Emulcire® 61 WL and Gelot® 64 provided consistency and stability to the creams due to their ability to form crystalline viscoelastic gel networks in the external phase of the cream formulations.

4.5.7. *In vitro* release studies

4.5.7.1. Overview

Once physically stable creams had been manufactured using Ritapro® 200 (Batch CP002), Emulcire® 61 WL (Batch CP003) and Gelot® 64 (Batch CP004), the effects of these commercially available synthetic mixed primary emulsifiers on the *in vitro* release rate of CP from the creams were investigated. The purpose of conducting these studies was to determine which of the cream formulations produced an *in vitro* release rate profile for CP that is similar to that of Dermovate® cream. The similarity assessment was based on a statistical evaluation using a non-parametric test described and reported in Section 3.2.11. Based on the results of these *in vitro* release studies the most suitable mixed primary emulsifier system was selected and used to manufacture additional generic CP cream formulations.

4.5.7.2. Effects of Ritapro® 200

The *in vitro* release of CP from a prototype formulation, Batch CP002, was evaluated and the results are summarised in Table 4.3 and compared with the *in vitro* release data obtained for Dermovate® cream. The resultant *in vitro* release rate profile of CP from Batch CP002 and from Dermovate® cream is depicted in Figure 4.6.

Table 4.3. Cumulative amount of CP released and the average *in vitro* release rate (flux) of CP from Batch CP002 and Dermovate® cream (n = 6)

Product	Q ($\mu g/cm^2$)	Cumulative % released	Flux ($\mu g/cm^2/hr^{1/2}$)
Batch CP002	63.54 ± 7.050	42.09 ± 4.700	8.681
Dermovate® cream	42.50 ± 6.540	27.50 ± 4.660	5.888

*In vitro r*elease rate data summarised in Table 4.3 and depicted in Figure 4.6 reveal that the *in vitro* release rate of CP from Batch CP002 may not be considered equivalent to the *in vitro* release of CP from Dermovate® cream.

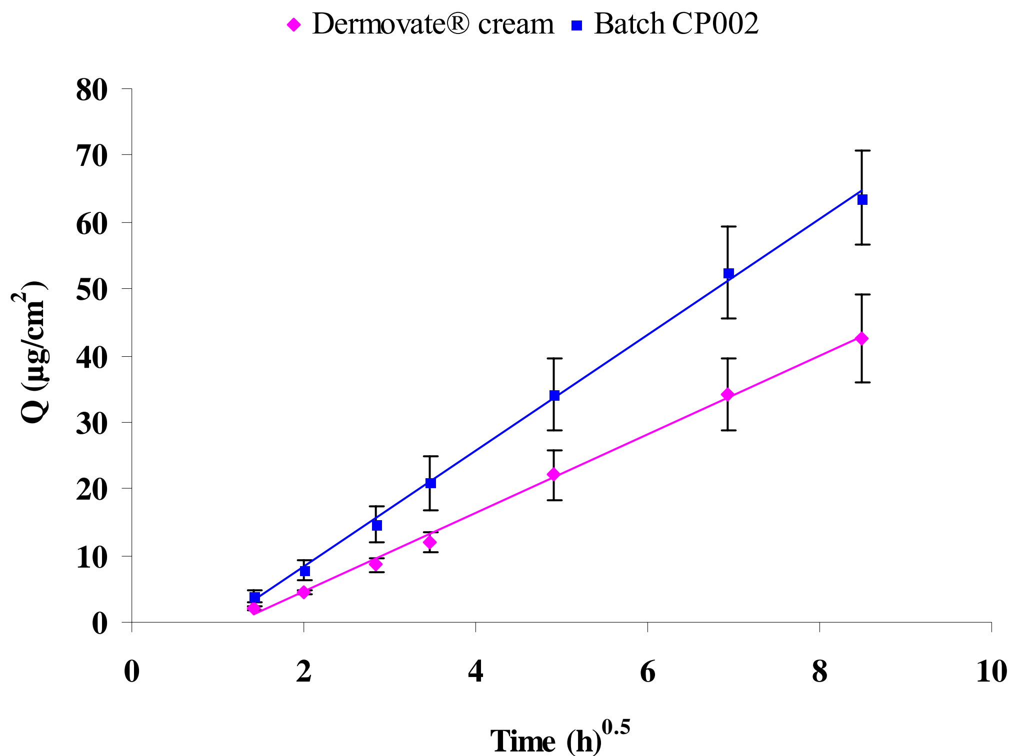


Figure 4.6. *In vitro* release rate profile for CP from Batch CP002 and Dermovate® cream

The lower limit (L.L.) and upper limit (U.L.) of a confidence interval (C.I.) calculated as described in Section 3.2.11 (Chapter 3) with Batch CP002 (test) and Dermovate® cream (reference) are summarised in Table 4.4. If the products are to be considered equivalent then the resultant limits for the C.I. calculated from experimental data should lie within the 75%-133% limits for the products to be considered equivalent [49].

Table 4.4. The lower limit (L.L.) and upper limit (U.L.) of the confidence interval (C.I) calculated using Batch CP002 (test) and Dermovate® cream (reference)

	Confidence intervals (C.I.)	
	L.L. (%)	**U.L. (%)**
C.I. limits	75.00	133.00
Dermovate® cream *vs.* Batch CP002	123.85	166.36

The data summarised in Table 4.4 reveal that the L.L. and U.L. of the C.I. calculated for Batch CP002 do not fall within the 75% -133% C.I. limits. Consequently, the *in vitro* release rate profile of CP for Batch CP002 is not equivalent to that of CP release from Dermovate® cream.

Based on these findings, Ritapro® 200 was not considered to be an ideal mixed emulsifier for use in manufacturing CP cream formulations.

4.5.7.3. Effects of Emulcire® 61 WL

The *in vitro* release rate of CP from a prototype formulation, Batch CP003, was assessed and the results are summarised in Table 4.5 and compared with the *in vitro* release data obtained for Dermovate® cream. The resultant *in vitro* release rate profile of CP from Batch CP003 and from Dermovate® cream is depicted in Figure 4.7.

Table 4.5. Cumulative amount of CP released and the average *in vitro* release rate (flux) of CP from Batch CP003 and Dermovate® cream (n = 6)

Product	**Q ($\mu g/cm^2$)**	**Cumulative % released**	**Flux ($\mu g/cm^2/hr^{1/2}$)**
Batch CP003	67.28 ± 5.040	51.04 ± 3.010	8.334
Dermovate® cream	42.50 ± 6.540	27.50 ± 4.660	5.888

*In vitro r*elease rate data summarised in Table 4.5 and depicted in Figure 4.7 reveal that the *in vitro* release rate of CP from Batch CP003 may not be considered equivalent to the *in vitro* release of CP from Dermovate® cream.

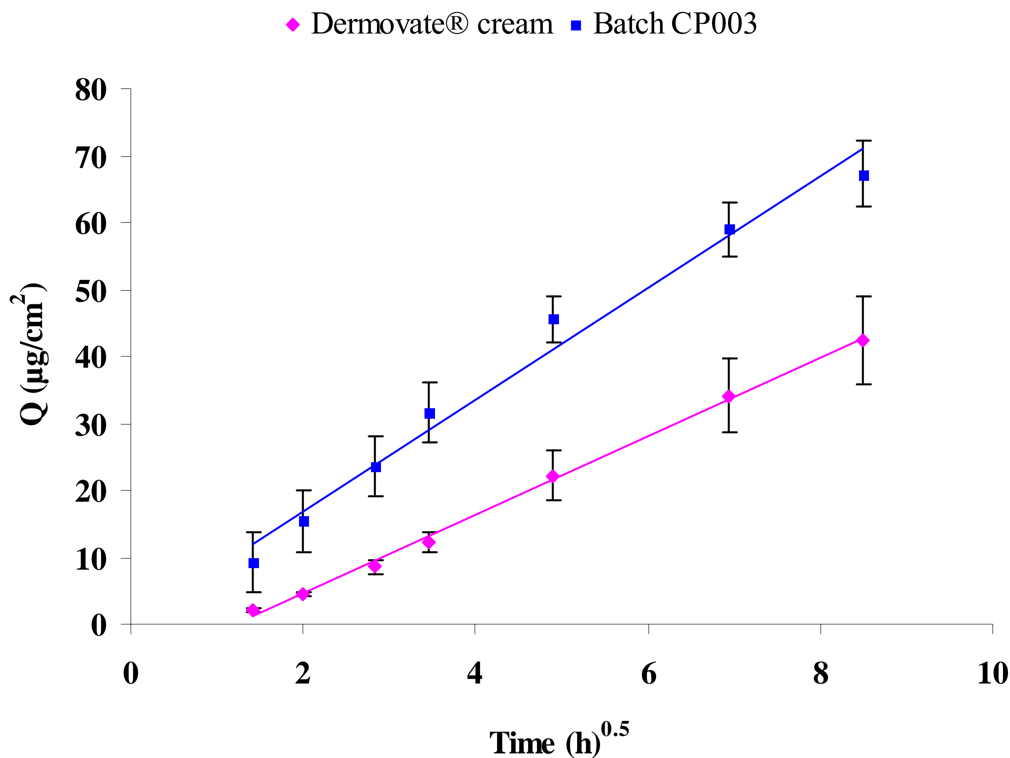


Figure 4.7. *In vitro* release rate profile for CP from Batch CP003 and Dermovate® cream

The lower limit (L.L.) and upper limit (U.L.) of a confidence interval (C.I.) calculated with Batch CP003 (test) and Dermovate® cream (reference) are summarised in Table 4.6. If the products are to be considered equivalent then the resultant limits for the C.I. calculated from experimental data should lie within the 75%-133% limits for the products to be considered equivalent [49]

Table 4.6. The lower limit (L.L.) and upper limit (U.L.) of the confidence interval (C.I.) calculated for using Batch CP003 (test) and Dermovate® cream (reference)

	Confidence intervals (C.I.)	
	L.L.(%)	**U.L. (%)**
C.I. limits	75.00	133.00
Dermovate® cream *vs.* Batch CP003	122.68	153.74

The data summarised in Table 4.6 reveal that although the L.L. the C.I. calculated for Batch CP003 does fall within the 75% -133% C.I. limits, the U.L. does not lie within these limits. Consequently, the *in vitro* release rate profile of CP for Batch CP003 is not equivalent to that of

CP release from Dermovate® cream. Based on these findings, Emulcire® 61 WL was not considered to be an ideal mixed emulsifier for use in manufacturing CP cream formulations.

4.5.7.4. Effects of Gelot® 64

The *in vitro* release rate of CP from a prototype formulation, Batch CP004, was evaluated and the results are summarised in Table 4.7 and compared with the *in vitro* release data obtained for Dermovate® cream. The resultant *in vitro* release rate profile of CP from Batch CP004 and from Dermovate® cream is depicted in Figure 4.8.

Table 4.7. Cumulative amount of CP released and the average *in vitro* release rate (flux) of CP from Batch CP004 and Dermovate® cream (n = 6)

Product	**Q ($\mu g/cm^2$)**	**Cumulative % released**	**Flux ($\mu g/cm^2/hr^{1/2}$**
Batch CP004	49.46 ± 4.010	32.98 ± 2.640	6.515
Dermovate® cream	42.50 ± 6.540	27.50 ± 4.660	5.888

*In vitro r*elease rate data summarised in Table 4.7 and depicted in Figure 4.8 reveal that the *in vitro* release rate of CP from Batch CP004 may be considered equivalent to the *in vitro* release of CP from Dermovate® cream.

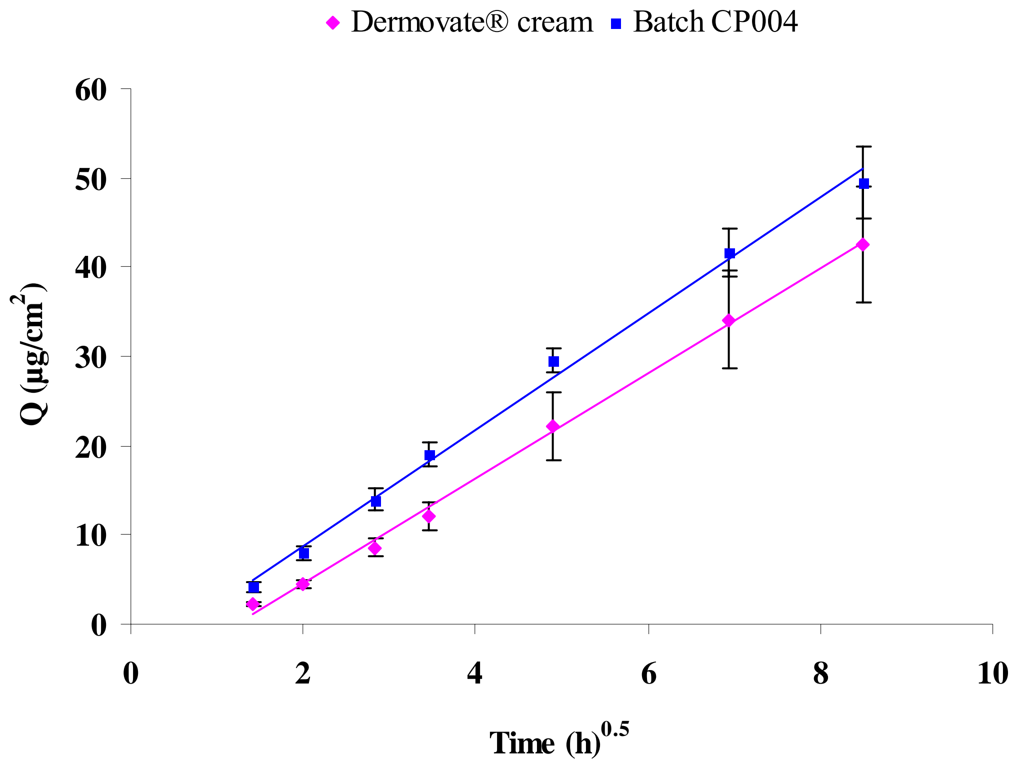


Figure 4.8. *In vitro* release rate profile for CP from Batch CP004 and Dermovate® cream

The lower limit (L.L.) and upper limit (U.L.) of a confidence interval (C.I.) calculated with Batch CP004 (test) and Dermovate® cream (reference) are summarised in Table 4.8. If the products are to be considered equivalent then the resultant limits for the C.I. calculated from experimental data should lie within the 75%-133% limits for the products to be considered equivalent [49].

Table 4.8. The lower limit (L.L.) and upper limit (U.L.) of the confidence interval (C.I) calculated using Dermovate® cream (reference) and Batch CP004 (test)

	Confidence intervals (C.I.)	
	L.L. (%)	**U.L. (%)**
C.I. limits	75.00	133.00
Dermovate® cream *vs.* Batch CP004	95.50	126.12

The data summarised in Table 4.8 reveal that the L.L. and U.L. of the C.I. calculated for Batch CP004 do fall within the 75% -133% C.I. limits. Consequently, the *in vitro* release rate profile of CP for Batch CP004 is equivalent to that of CP release from Dermovate® cream. Based on these

findings, Gelot® 64 was considered to be a suitable mixed emulgent for use in manufacturing CP cream formulations.

4.3.8. Generic product characterization

4.3.8.1. Overview

Following the selection of the most suitable synthetic mixed emulsifier for use and the development of a generic cream formulation containing CP which had the desirable *in vitro* release rate profile, the next step was to characterize the physico-chemical properties of the generic formulation and compare the data to those obtained for Dermovate® cream. Therefore three (3) batches of 500 g *viz.,* Batches CP004, CP005 and CP006, all containing equal amounts of Gelot® 64 and manufactured using the manufacturing method described in Section 4.5.5, were evaluated for CP content, viscosity, pH and *in vitro* release rate. The data generated from these studies were also used to determine batch-to-batch uniformity and therefore the consistency of the manufacturing method that had been developed. Batch summary records for Batches CP005 and CP006 are shown in Appendix 4.

4.3.8.2. CP content

The assay for CP content in Batches CP004, CP005 and CP006 was evaluated using the sample preparation procedure described in Section 2.4.6.2 and the resultant data are listed in Table 4.9 and compared to the CP content of the innovator product Dermovate® cream.

Table 4.9. CP content of Dermovate® cream and Batches of generic cream formulations (n = 3)

Product	CP content (%)	SD	% RSD
Dermovate® cream	103.9	2.797	2.69
Batch CP004	109.8	1.114	1.06
Batch CP005	109.8	2.474	2.25
Batch CP006	113.1	2.605	2.30

The data reveal that the CP content in all the three generic batches manufactured comply with the USP specifications [23] as does Dermovate® cream. Therefore the manufacturing process is suitable for the production of the generic cream.

4.3.8.3. Intrinsic viscosity

The intrinsic viscosity of Batches CP004, CP005 and CP006 was investigated using the procedure described and reported in Section 3.3.2.3.2 (Chapter 3). The results generated from these studies are summarised in Table 4.10 and compared to the intrinsic viscosity data of Dermovate® cream. It appears that there is little difference in the intrinsic viscosity values among the three batches manufactured, implying that the method of manufactured was consistent.

Table 4.10. Intrinsic viscosity readings for Dermovate® cream and generic cream products (n = 3)

Product	Intrinsic viscosity (KcP)	SD	% RSD
Dermovate® cream	44.47	1.026	2.31
Batch CP004	15.50	4.359	2.81
Batch CP005	12.37	0.3214	2.60
Batch CP006	11.43	0.05774	0.505

The data in shown in Table 4.10 clearly reveal a marked difference between the intrinsic viscosity of Dermovate® cream and of any of the three extemporaneously manufactured generic batches.

The intrinsic viscosity of Dermovate® cream is approximately three (3) times greater than the intrinsic viscosity of Batches CP004, CP005 and CP006. The difference in the intrinsic viscosity of the innovator formulation and of the three extemporaneously manufactured batches may be attributed to either the gel network theory or to manufacturing process variables such as homogenization speed and mode of cooling.

The gel network theory relates the consistency and stability of o/w creams to the presence or absence of viscoelastic gel networks in the external phase of cream formulations. The crystalline viscoelastic gel networks form in the continuous phase of a cream formulation when a fatty

amphiphile in a mixed emulsifier swells in the presence of water and in the presence of a surfactant in a cream formulation. The presence of swollen crystalline gel networks and their concentration in the continuous phase of a cream are reported to be responsible for the overall consistency of a cream structure [171, 172] as well as the self-bodying action of the mixed emulsifier [173].

Although an amphiphile will swell in the presence of any type of surfactant to form gel networks, the amount of swelling of an amphiphile is reportedly greater in the presence of ionic surfactants than in the presence of non-ionic surfactants [171]. In the presence of a non-ionic surfactant, swelling has been reported to be attributed to the hydration of hydrophilic chains of the non-ionic surfactant and is usually limited by the length of the chain [171].

The composition of the innovator formulation (Appendix 2) reveals that Arlacel® 165 is the mixed emulsifier in Dermovate® cream. The mixed emulsifier in the extemporaneously manufactured CP generic formulations is Gelot® 64. Both Arlacel® 165 [206] and Gelot® 64 (Section 4.3.3.2.13) contain a mixture of an amphiphile and a non-ionic surfactant. Essentially, Arlacel® 165 is a mixture of glyceryl monostearate and polyethylene glycol-100 stearate or PEG-100 stearate [206] whereas Gelot® 64 consists of glyceryl monostearate and polyethylene glycol-75 stearate or PEG-75 stearate (Section 4.3.3.2.13).

It is clearly evident that both Arlacel® 165 and Gelot® 64 are comprised of similar ingredients, the only difference being attributed to the chain length of the non-ionic surfactant. It is therefore possible that the higher viscosity of Dermovate® cream compared to that of the generic CP creams may be due to the increased length of the chain of the non-ionic surfactant in Arlacel® 165. The increased chain length more than likely resulted in greater swelling of the glyceryl monostearate and subsequent formation of a large amount of the crystalline viscoelastic gel phases in the continuous aqueous phase of the cream markedly increasing the viscosity of the formulation.

The apparent high intrinsic viscosity of Dermovate® cream may also be attributed to manufacturing process variables, such as the homogenization speed and mode of cooling. It has

been reported [170, 192] that the apparent intrinsic viscosity of a semi-solid formulation is influenced by the size of the droplets of the dispersed phase and it follows that droplets of small sizes will produce creams of higher viscosity than those of dispersed phases with large droplet sizes [170, 192, 211].

A reduction in the size of dispersed droplets of a dispersed phase may be achieved by increasing the speed of homogenization during manufacture [170, 192, 211]. The higher shearing forces provided by increased homogenization speeds disrupts the hydrocarbon chains of the oil and wax droplets, thereby exposing surfactant chains to water which in turn results in the formation of an additional gel phase and subsequently further increases the intrinsic viscosity of a cream formulation [170].

Although the speed of the laboratory homogenizer used in the manufacture of generic CP batches (Section 3.3.5) was set to approximately 15,000 rpm it is likely that the laboratory homogenizer does not provide the same shear as would be expected from high speed homogenizers used in the large scale manufacture of cream products. Consequently, the apparent intrinsic viscosity values of the extemporaneously manufactured CP batches did not match the apparent intrinsic viscosity value of the commercially available Dermovate® cream.

As far as mode of cooling is concerned, Niellound *et al,* [212] established and reported that a progressive mode of cooling of cream formulations at room temperature (25˚C), corresponding to 45 minutes of homogenization time, appears to lead to the production of creams with higher viscosity, better homogeneity and better stability than what Niellound *et al,* [212] referred to as brutal mode of cooling with a water bath at 15˚C, corresponding to 30 minutes of homogenization time.

It possible that at an industrial scale, a progressive mode of cooling corresponding to longer homogenization time is adopted, resulting in cream formulations with higher viscosity as seen in the case of Dermovate® cream. However, progressive cooling at a laboratory scale would be a time-consuming and laborious process, especially in these studies where manual mixing while cooling was used. Nevertheless, it is probable that the rapid cooling with a water bath at 20˚C as

was done in these studies and which corresponded to approximately 20 minutes of manual mixing may have contributed to the formation of less viscous creams compared to Dermovate® cream.

4.3.8.4. pH-determination

The pH of Batches CP004, CP005 and CP006 cream products was determined at room temperature (22˚C) as described in Section 4.3.4. The data generated from these studies are listed in Table 4.11 and compared to the apparent pH determined for Dermovate® cream. The pH values for all three CP cream batches fall within the pH range of 4-6 of a healthy human skin and therefore all the three formulations are unlikely to exert pH effects on pH-sensitive human skin. The pH data of all three batches is consistent, implying that the method of manufacture was consistent. In addition the pH of the creams is similar to that of Dermovate®.

Table 4.11. pH readings for Dermovate® cream and generic cream formulations (n = 3)

Product	pH	SD	% RSD
Dermovate® cream	5.05	0.0321	0.00637
Batch CP004	5.20	0.01527	0.294
Batch CP005	5.11	0.005770	0.113
Batch CP006	5.15	0.0264	0.514

4.3.8.5. In vitro release rate

The *in vitro* release of CP from Batches CP004, CP005 and CP006 was assessed and the resultant data generated from these *in vitro* release studies are summarised in Table 4.12 and are compared with the *in vitro* release data generated for Dermovate® cream. The resultant *in vitro* release rate profiles of CP from Batches CP004, CP005 and CP006 and from Dermovate® cream are depicted in Figure 4.9.

Table 4.12. Cumulative amount of CP released and the average *in vitro* release rate (flux) of CP from Dermovate® cream and generic cream formulations (n = 6)

Product	Q ($\mu g/cm^2$)	Cumulative % released	Flux ($\mu g/cm^2/hr^{1/2}$)
Dermovate® cream	42.50 ± 6.540	27.50 ± 4.660	5.888
Batch CP004	49.46 ± 4.010	32.98 ± 2.640	6.515
Batch CP005	56.73 ± 2.82	37.83 ± 3.39	7.532
Batch CP006	43.99 ± 5.270	29.36 ± 3.740	5.796

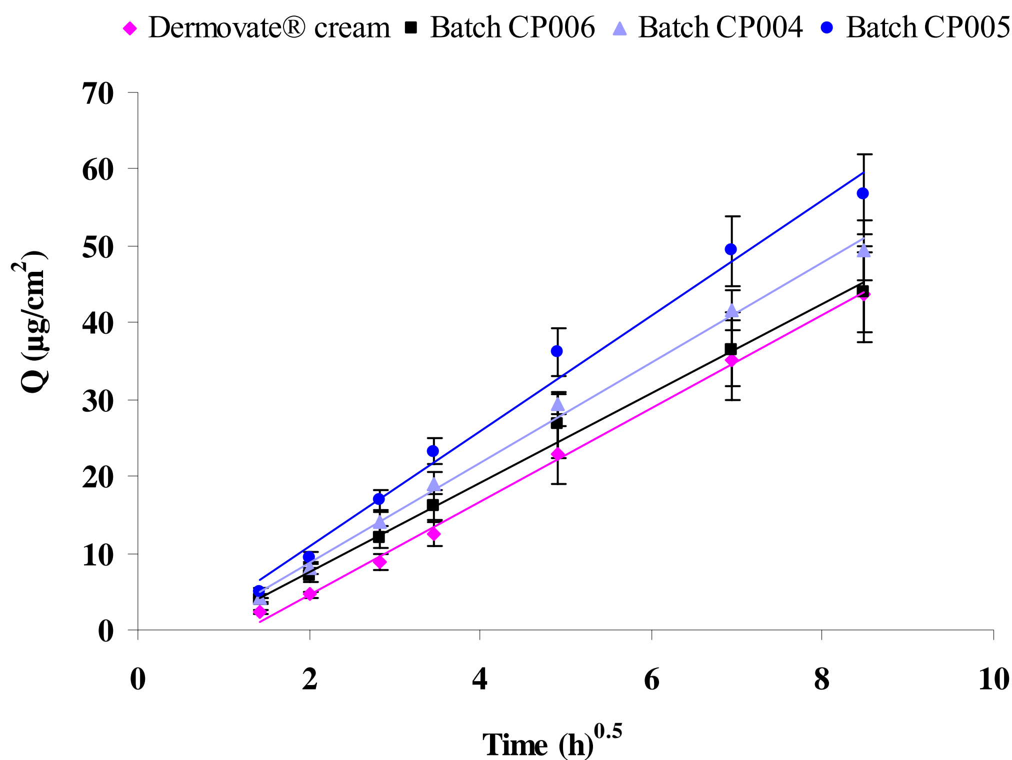


Figure 4.9. *In vitro* release rate profile for CP release from CP generic cream products and Dermovate® cream

From the *in vitro* release rate profile data for CP depicted in Figure 4.8 and summarised in Table 4.12, it appears that there is no significant difference in the *in vitro* rate of release of CP from Batches CP004, CP005 and C006 and the release rate of CP from the innovator product, Dermovate® cream. These data are consistent with the results of the non-parametric statistical test (Table 4.13), which reveal that all the three manufactured batches are equivalent to Dermovate® cream in terms of their CP *in vitro* release rate profiles.

Table 4.13. The lower limit (L.L.) and upper limit (U.L.) of the confidence interval (C.I.) calculated using Dermovate® cream (reference) and generic formulations (tests)

	Confidence intervals (C.I.)	
	L.L. (%)	**U.L. (%)**
C.I. limits	75.00	133.0
Dermovate® cream *vs.* Batch CP004	95.50	126.1
Dermovate® cream *vs.* Batch CP005	107.9	131.9
Dermovate® cream *vs.* Batch CP006	81.40	113.4

It is interesting to note that the *in vitro* release profile for CP from all three generic batches appears to be similar to that of CP release from Dermovate® cream despite the apparent viscosity of the innovator formulation being three times higher than the viscosity of any of the three generic Batches (Table 4.10). From these observations, it may be implied that the intrinsic viscosity of the cream formulation does not influence the *in vitro* release rate of CP in these formulations significantly, which contradicts well-established theories on drug release from semi-solid dosage forms [213].

It has been established that the rate and extent of release of an active pharmaceutical ingredient (API) from a semi-solid vehicle decreases progressively as the intrinsic viscosity of the base material increases, and the converse is also true [213]. Therefore it was expected that the difference in the intrinsic viscosity of Dermovate® cream and the extemporaneously manufactured CP cream batches would result in significantly different *in vitro* release rates for CP from each of the products.

It has also been reported that for an API molecule to be released from a semi-solid vehicle, it first has to reach the surface of the formulation and that mass transport in the cream formulation may occur by diffusion of the API molecules or by diffusion and convection of oil droplets [214].

The apparent intrinsic viscosity or macro-viscosity of a semi-solid product influences the diffusion of oil droplets in the dosage form when the diffusion length is greater than the diffusion length scale of the structure elements of a formulation [214]. In addition it has been suggested that diffusion of API molecules through a semi-solid formulation is also influenced by the micro-viscosity of a topical formulation and that the formulation environment may affect an API

molecule, such as for example the physical properties of the emulsifier used to manufacture a stable dosage form [214].

It is possible that the *in vitro* release of CP from cream formulations may to a large extent be affected by the physical properties of the mixed emulsifier system used in the formulation rather than the apparent intrinsic viscosity of the cream formulation. The components of the mixed emulsifier (Arlacel® 165) used in Dermovate® cream are essentially similar to the components of the mixed emulsifier (Gelot® 64) used in the manufacture of the generic batches. Therefore the behaviour of the mixed emulsifier in the cream formulation may have a dominant effect on the *in vitro* release rate of CP from semi-solid cream formulations, rather than the macro-viscosity of the semi-solid base.

4.4. CONCLUSIONS

The objective of these studies was to develop and assess a generic cream formulation for the delivery of clobetasol 17-propionate (CP) for use in the treatment of patients with inflammatory skin conditions, such as acute and chronic attacks of any type of eczema.

Initial experiments were designed to facilitate the characterization of the innovator product, Dermovate® cream. Consequently, the innovator product was characterized in terms of clobetasol 17-propionate (CP) content, apparent intrinsic viscosity, pH and *in vitro* release rate of CP. The data generated from these studies were used as a benchmark for the development of extemporaneously manufactured generic CP cream batches. The CP content of Dermovate® cream was found to fall within the USP specifications, and the pH of the product was found to lie in the pH range of the human skin.

Preliminary studies in the development of generic formulations were designed to facilitate the manufacture of a prototype generic formulation that showed no signs of physical instability, such as, for example, cracking, creaming, phase inversion and/or bleeding of the cream base from the container, immediately after the manufacture as well as twenty-four (24) hours after manufacture and storage at room temperature (22˚C).

Although a prototype formulation (Batch CP001) manufactured with Estol® 1474 as the primary emulsifier showed evidence of physical stability immediately after manufacture, the formulation was found to be physically unstable twenty-four (24) hours after manufacture and storage at room temperature (22˚C) and was therefore not considered for further investigation.

Prototype formulations containing commercially available mixed emulsifiers, such as Ritapro® 200 (Batch CP002), Emulcire® 61 WL (Batch CP003) and Gelot® 64 (Batch CP004) were found to be physically stable immediately after manufacture as well as twenty-four (24) hours after manufacture and storage at room temperature (22˚C) and were therefore considered for further evaluation. Once physically stable creams had been manufactured the *in vitro* release rate of CP from Batches CP002, CP003 and CP004 and Dermovate® cream was evaluated.

The purpose of these studies was to identify a prototype batch with a similar *in vitro* release profile for CP that was similar to that for Dermovate® cream. It was found that Batch CP004 was the only batch with an *in vitro* release profile for CP that was similar to that of Dermovate® cream. The similarity of release profiles was assessed using a non-parametric statistical test recommended by the Food and Drug Administration (FDA).

The successful manufacture of Batch CP004 necessitated the production of two additional batches of cream. Batches CP005 and CP006 were manufactured using Gelot® 64 as a mixed emulsifier with a composition exactly the same as that used to manufacture Batch CP004. Batches CP004, CP005 and CP006 were characterised in terms of CP content, apparent intrinsic viscosity, pH and *in vitro* release rate testing. The CP content of all three extemporaneously manufactured CP batches was within the specified USP limits and all the three batches had pH values within the pH range of healthy human skin.

However, it was found that the apparent intrinsic viscosity of the innovator product was almost three (3) times greater than that of all three generic cream batches. The difference in intrinsic viscosity of Dermovate® cream was attributed to the physicochemical characteristics of the mixed emulsifiers used in the innovator product and possibly to manufacturing process variables, such as homogenization speed and mode of cooling.

Despite the difference in the apparent intrinsic viscosity of Dermovate® cream and the CP batches, the *in vitro* release rate of CP was found to be statistically similar to the innovator product for all three generic batches tested. It was concluded that the behaviour of the mixed emulsifier in both the commercially available cream and the extemporaneously manufactured creams, and not the apparent intrinsic viscosity of the formulations, has an impact on the *in vitro* release rate of CP from these formulations.

Generic CP cream formulations have been successfully developed and characterized *in vitro* in terms of CP content, apparent intrinsic viscosity, pH and *in vitro* release rate testing. Although the viscosity of the generic CP cream formulations did not match the apparent viscosity of Dermovate® cream, the generic CP batches were equivalent to the innovator cream product in terms of other *in vitro* performance characteristics such as CP content, pH and *in vitro* release rate.

The data generated from these studies formed the basis for manufacture of a larger batch of cream that was subjected to accelerated stability studies at 40°C/25% RH (Chapter 5).

CHAPTER FIVE

STABILITY OF CLOBETASOL 17-PROPIONATE CREAMS

5.1. INTRODUCTION

Every medicinal product on the market is required to have an expiry date allocated by the regulatory authorities in a particular country and the expiration date must be presented on the label of the container in which the product is stored [215, 216]. The expiration date can be defined as the time interval over which a pharmaceutical product remains within the specifications established for product strength, quality and purity [215-217]. The expiration date of a product may be determined by adding a product's shelf-life period to the date of manufacture [216, 217]. The shelf-life of a product specifically refers to the time period during which the strength of the active pharmaceutical ingredient (API) in a product remains ≥ 90% of the original label claim and is therefore the period for which a product will be suitable for use by a patient [215, 217].

In order to determine the shelf-life of a medical product, pharmaceutical manufacturers must conduct appropriate stability studies [216]. In addition to generating data on which to base proposals for a product's shelf-life, stability studies may also reveal information that is vital for the selection of product packaging and storage conditions [217-219]. Stability studies may also provide evidence on how the quality of a drug product varies with time under the influence of a variety of different environmental factors, such as, for example, temperature, humidity and light [219-221]. Evaluation of the stability of a pharmaceutical product is therefore considered an integral part of formulation development studies [217, 218].

Most API molecules that are used in medicinal products are inherently unstable compounds [218]. This can be ascribed to the presence of reactive functional groups, which, in addition to providing reactive sites necessary to produce a therapeutic effect *in vivo*, may also increase the susceptibility of a drug molecule to chemical reactions outside the body, leading to degradation and subsequent loss of potency of a drug product [215]. Pharmaceutical products have been

reported to be susceptible to degradation via three (3) primary mechanisms: chemical, physical and biological. These degradation mechanisms may occur in isolation or in combination [215, 222].

Chemical degradation in pharmaceutical systems invariably results in chemical changes to the product. Such changes include for example the formation of degradation products, loss of API potency and/or loss of activity of excipients such as anti-microbial preservatives and/or anti-oxidants [219]. These changes may occur as a result of intrinsic chemical reactions, such as hydrolysis, photolysis, oxidation, reduction and/or racemisation reactions [215, 222]. It has been suggested that intrinsic chemical reactions may be triggered by extrinsic factors and therefore the chemical stability of medicinal products may be regarded as the ability of a drug product to withstand the effects of moisture, light, humidity and heat [219].

The physical degradation of pharmaceutical products may be caused by a range of external factors, such as, impact, vibration, abrasion and/or temperature fluctuations [222]. Physical degradation of a drug product may lead to changes in product appearance, consistency, uniformity, dissolution rate, colour, odour, moisture content and/or pH amongst others and such changes are usually dependent on the dosage form under investigation [219]. Biological and/or microbiological degradation in medicinal products may lead to the proliferation of micro-organisms in non-sterile products and changes in the efficacy of preservatives [219] and may be caused by microbiological and/or non-microbiological organisms [222].

Whether a change in a delivery system is chemical, physical or microbiological such changes are likely to adversely affect any attribute of the quality of the product in terms of its fitness for use by a patient [215]. Therefore the stability of a pharmaceutical product may be defined as the ability of a formulation in a specific container to remain within the physical, chemical, microbiological and hence therapeutic, toxicological, protective and informational specifications for that product [215, 217, 221]. In other words, the stability of a pharmaceutical product is the extent to which a pharmaceutical product retains the same properties and characteristics that the product possessed at the time of manufacture, throughout the shelf-life of the product within specified limits [215, 217].

The factors that may adversely affect the stability of a pharmaceutical product include for example the [215, 217]:

1) stability of the API,
2) potential interaction between an API and inactive excipients,
3) manufacturing process,
4) dosage form,
5) container/closure system,
6) environmental conditions the product is exposed to during shipment,
7) storage conditions,
8) handling, and,
9) length of time between manufacture and use.

During the formulation development process and manufacturing procedure a formulation scientist may be able to control the external factors to which a dosage form or the components of the dosage form may be exposed and that may affect product stability, and by control of these parameters, minimize or prevent product instability [223]. However once a dosage form has left a manufacturing plant, the formulation scientist has little or no control if any over the variables that the dosage form in question may be exposed to during distribution, storage and use [223].

It may be possible to minimize the potential hazards to which the dosage form may be exposed to during distribution, storage and use by selecting an appropriate package or packaging configuration for the product [223]. However of all the environmental attributes that may be involved in the degradation of an API in a dosage form, temperature is the most important factor and cannot be controlled by the selection of specific packaging [215], and it has been argued that the selection of a specific packaging configuration will not protect a pharmaceutical formulation form from the detrimental and adverse effects associated with the use of elevated temperatures [224]. The lack of protection from elevated temperatures afforded by packaging is problematic for pharmaceutical products destined for used in high temperature zones, such as for example Zones III or IV [218, 225].

In order to assess how a newly developed dosage form behaves under the influence of different environmental conditions and to minimize or avoid unforeseen adverse effects it is essential to assess the stability of the new pharmaceutical product in the desirable packaging configuration that is intended for use during marketing of the product. Stability studies are usually conducted under ambient and accelerated conditions [215-218, 221] and three different conditions for stability studies have been reported [217, 219, 221]. However only two types of stability studies *viz.,* short-term and long-term have been widely reported [215-218, 221].

Long-term stability studies are usually conducted under ambient storage conditions [215, 216, 218, 221] and are defined as experiments for the evaluation of the physical, chemical, biological, biopharmaceutical and microbiological characteristics of a drug substance or product, during and beyond the expected shelf-life and the storage of samples at the recommended storage conditions [217, 219]. Stability data generated from long-term stability studies are invariably the primary stability data used to establish the shelf-life of a product and to confirm a projected shelf-life and/or the recommended storage conditions for a product [216, 217, 219].

In contrast short-term or accelerated stability studies are experiments conducted under exaggerated storage conditions, such as, for example, high temperature and/or relative humidities [215-218, 221]. Accelerated stability studies are designed to increase the rate of a potential chemical and/or physical change of an API or medicinal product such that significant degradation, if it occurs, can be observed in a relatively short period of time [216, 219]. Data generated from short-tem stability studies may be used in conjunction with long-term stability studies to assess the longer term chemical effects at non-accelerated conditions and to evaluate the impact of short term deviations outside the appropriate storage conditions of a product, such as might occur during transportation, on product quality [217, 219, 226].

The objective of these studies was to conduct stability studies at elevated temperatures on the clobetasol 17-propionate (CP) cream formulation developed and characterized as described and reported in Chapter 4. Although laboratory-scale stability data are not normally acceptable as primary stability data for use to establish product shelf-life and/or storage conditions, such data may be submitted to regulatory agencies to support primary stability data [218, 219].

It is however important to note that the purpose of these stability studies was not to generate primary stability data for use to estimate a shelf-life for the CP cream product but rather to determine whether or not the formulation development process was successful in producing a potentially stable CP cream product. The stability data generated for CP generic cream from these studies would then form the basis for further more comprehensive studies on the stability of the formulation.

5.2. EXPERIMENTAL

5.2.1. Overview

The initial step in the generation of stability data requires that a stability protocol or a detailed stability testing plan is documented [218]. Since stability testing conditions may vary depending on the inherent stability of a drug compound, type of the dosage form and/or the proposed container-closure configuration to be used, the final stability protocol invariably relies on the type of drug substance or drug product being tested [218].

The ultimate stability protocol will also depend on whether a medicinal product containing a specific API is already on the market [218]. As a consequence some guidelines on stability studies may require less stringent stability evaluations for drug substances and/or drug products that have been on the market for extended period of time [218]. In addition, the target market for a medicinal product must also be taken into account when establishing a stability protocol as room temperature conditions vary from location to location and will affect the storage condition requirements in addition to other test parameters [217, 218].

The World Health Organization (WHO) [217], International Conference on Harmonisation (ICH) [221] and the Food and Drug Administration (FDA) [227] have published general guidelines pertaining to the assessment of stability of drug substances and/or drug products. The guidelines were used as a template to design a stability protocol for use in the assessment of stability of the CP topical cream formulation developed in these studies.

5.2.2. Stability study protocol

5.2.2.1. Overview

The stability protocol developed for use in these studies was designed to include the following information:

1) selection of batches,
2) number of batches,
3) container closure system,
4) sampling frequency,
5) sampling plan,
6) test storage conditions,
7) test specifications,
8) product specifications,
9) test procedure, and
10) statistical evaluation of the data.

A stability test summary sheet for the CP cream formulation used in these studies is shown in Appendix 7.

5.2.2.2. Selection of batches

Specification requirements with respect to the selection of batches for stability testing have been reported [217, 221]. According to the WHO [217] and the ICH [221] it is largely irrelevant whether batches to be tested are of a pilot scale or large scale. However, the batches to be sampled and tested should be representative of the manufacturing procedure used to produce the product. Therefore the batches of CP cream that were subjected to stability testing were selected from amongst the best prototype batches *viz.,* Batches CP004, CP005 and CP006 (Table 4.2), which were manufactured using the same procedure as described in Section 4.3.5. The size of each prototype batch that was produced was 500 g.

5.2.2.3. Number of batches

Current international guidelines on stability evaluation of drug substances and/or drug products recommend the testing of a minimum of three (3) batches [217, 221, 227]. The use of three (3) batches may be a compromise between statistical and practical considerations [218, 227]. The testing of fewer batches may not allow for a reliable statistical estimate of batch-to-batch variability and the impact on product stability [218, 227]. However, practical considerations may prevent the collection of large amounts of data, since the generation of excessive data may cause stress on analytical and other facilities within a company [218, 227]. Testing of fewer batches such as, for example, one (1) batch may be allowed for stable and well-established products produced on a pilot scale [218]. As a consequence, one (1) batch of the three best CP cream products *viz.,* Batch CP004 was used in these studies.

5.2.2.4. Container-closure system

According to the current guidelines [217, 221, 227] stability testing should be conducted using containers and closures that will be used for the marketing of the medicinal product. However, as part of the conditions for the accelerated stability testing of a dosage form, the ICH guidelines [221] consider the use of other packaging materials to be appropriate to generate supporting stability data for a product produced at a pilot scale level. As a consequence, the CP cream formulation was packed into 100 g glass ointment jars with tight-fitting closures. These containers were selected for use since it was easy to measure formulation parameters such as viscosity and pH directly from the ointment jar following storage of the samples for the requisite times (Section 5.2.2.8).

5.2.2.5. Sampling frequency

Accelerated stability studies may be undertaken for a minimum of six (6) months [217, 221, 227]. During the six (6) month test period, the ICH suggests that samples should be removed at a minimum of three (3) time points including the initial and final time points, for example, at 0, 3, and 6 months [221]. The FDA [227] however sets the sampling frequency at a minimum of four

(4) time points, for example at 0, 2, 4 and 6, whereas the WHO [217] has set the sampling frequency at a minimum of five (5) time points such as, for example, at 0, 1, 2 and 3 and 6 months (WHO). Since the objective of the current work was to determine whether the CP cream formulation is a potentially stable system, stability studies were conducted for a period of one (1) month and the sampling frequency in these studies was set at 1, 2, 3 and 4 weeks.

5.2.2.6. Sampling plan

Following completion of a sampling time plan, the total number of containers required for stability testing must be determined [218]. The primary requirement for determination of sample numbers is that the containers that are selected must represent the batch as a whole [218]. The formulation to be tested was packed into five (5) different 100 g glass ointment jars, resulting in the production of five (5) different test samples. The use of five (5) test samples was necessary to facilitate sampling at the different time points of 0, 1, 2, 3, and 4 weeks such that at the appropriate time, a jar could be removed without interfering with the remaining samples retained in the stability oven.

5.2.2.7. Test storage conditions

Haynes [228] gathered climatic data in various cities worldwide and has proposed a formula for the determination of the mean kinetic temperature (MKT) in these locations by taking into consideration variations in storage temperature. As a consequence different countries around the world have been distributed into four (4) different climatic zones, based on the prevailing annual climatic conditions in those regions [217-219]. The Republic of South Africa (RSA) falls into either Zones I or II [218, 225].

For countries located in Zones I and II accelerated stability studies for pharmaceutical products should be conducted at 40 ± 2˚C and 75 ± 5% RH [217, 221, 227]. Due to lack of a controlled humidity stability chamber in our laboratory, stability studies on the CP cream formulation were conducted at 40 ± 2˚C and 25 ± 5% RH, which were the test storage conditions that could be achieved with the facilities available in our laboratory. Whilst saturated salt chambers [229] may

be used to achieve the desired degree of humidity, the excessive salt required may affect the stability of the dosage form and containers and closures or cause corrosion and therefore these were not considered suitable for use.

5.2.2.8. Test specifications

The specifications for a stability test are designed to facilitate the selection of test parameters, analytical procedures and proposed acceptance criteria for a specific product [221]. Stability studies require that parameters of a product that may be susceptible to change during storage and that are more than likely to influence the quality, safety and/or efficacy of a product be tested [221].

For topical formulations such as creams, attributes that should be investigated in stability studies may include the organoleptic properties and homogeneity of the dosage form, pH, viscosity, particle size distribution, strength, weight loss and API release rate [227, 230]. For the purpose of these studies, the CP cream formulation was evaluated in terms of its organoleptic attributes, CP content, consistency, pH and *in vitro* release rate of CP. The analytical procedures and acceptance criteria used in these studies are discussed in Sections 5.2.2.9 and 5.2.2.10, respectively, *vide infra*.

5.2.2.9. Product specifications

The test product specifications for the qualitative and quantitative parameters tested in these studies are listed in Table 5.1 and Table 5.2 respectively. The objective of these stability studies was to determine whether or not the organoleptic properties (Table 5.1) and the physico-chemical parameters (Table 5.2) of the test formulation (Batch CP004) would remain within the specifications set prior to testing following exposure of the product to the stability test conditions used in these studies (Section 5.2.2.7). These specifications were determined for Batch CP004 based on the data generated at the time of manufacture of that batch (Section 4.3.8).

Table 5.1. Specifications for qualitative parameters of the test cream formulation

Product parameter	Specification
Appearance	Shiny
Colour	White
Odour	Odourless
Signs of physical instability	None

Table 5.2. Specifications for quantitative parameters of the test cream formulation

Product parameter	Specification
CP content	90-115 %
Intrinsic viscosity	13-18 KcP
Intrinsic pH	4-6
In vitro release rate (flux)	5-8 $\mu g/cm^2/hr^{1/2}$

5.2.2.10. Methodology

5.2.2.10.1. Test procedure

Stability studies were conducted using a Model L.T.I.E. Labcon low temperature incubator (Labcon (Pty) Ltd, Krugerdorp, Gauteng, RSA). A week prior to use, the incubator was allowed to equilibrate to the temperature and relative humidity conditions set for these studies and these test conditions were monitored using a Thermo-Hygro pen (Beijing Gaupu Automation Control Co., Ltd, Beijing, China). Four (4) containers containing 100 g of cream were placed in the equilibrated incubator and at weeks 1, 2, 3 and 4 a 100 g sample was removed from the incubator and immediately analysed in terms of the following test parameters:

a) organoleptic appeal,
b) CP content,
c) viscosity,
d) pH, and
e) CP release rate.

The data generated in these tests were subjected to statistical analysis where appropriate.

5.2.2.10.2. Organoleptic appeal

The appearance, odour and colour of the cream in each container was visually evaluated. Samples were also assessed for any signs of physical instability, such as for example, cracking and/or bleeding of the cream base from the container.

5.2.2.10.3. CP content

The CP content of the cream was evaluated using the sample preparation procedure described in Section 2.4.6.2 at each sampling time and a maximum of three (3) cream samples were used obtain an average value of CP. CP content was determined using a validated HPLC method with UV detection at 240 nm (Chapter 2). These samples were assessed immediately after removing the cream sample container from the incubator.

5.2.2.10.4. Intrinsic viscosity

The apparent intrinsic viscosity of the creams was measured as described in Section 3.3.2.3.2 at each sampling time and a maximum of three (3) readings were taken to obtain an average apparent intrinsic viscosity value. Intrinsic viscosity readings were taken once, immediately after removing the cream sample from the incubator.

5.2.2.10.5. pH-determination

The apparent pH was determined as described in Section 4.3.1.4 at each sampling time and a maximum of three (3) readings were taken to obtain an average apparent pH value. The apparent pH readings were taken once, immediately after removing the cream sample from the incubator.

5.2.2.10.6. In vitro release test

The *in vitro* release rate of CP was assessed using the in *vitro* release test conditions described in Table 3.5, and were assessed following removal of the sample at each sampling time. A

maximum of six (6) Franz glass diffusion cells were used to determine the average *in vitro* release rate of CP (flux). *In vitro* release rate studies were conducted immediately after removing the cream sample from the incubator.

5.2.2.11. Statistical evaluation

In addition to describing how a stability study is to be designed and undertaken, the stability study protocol must also establish the statistical procedures to be used to analyse the stability data generated in the stability studies [227]. Stability data evaluation methods proposed in the current international guidelines [217, 221, 227] and in some published reports [218, 231-233] are intended to establish an expiration dating period during which the average strength of a batch of drug product may be expected to remain within the specifications for that product. The guidelines [217, 221, 227] and reports [231, 232] do not specify the statistical methods to be used to predict the stability of a drug product in terms of the physical attributes of a formulation such as viscosity, pH and *in vitro* release rates as were determined in these studies.

The purpose of conducting these studies was to assess whether or not exposing the CP cream formulation to the test conditions reported in Section 5.2.2.7 would cause any relevant and/or significant changes to the organoleptic and physico-chemical properties of the cream. In terms of the qualitative tests, *i.e*. organoleptic properties, the formulation was considered to be stable if no changes were observed in terms of appearance, colour and odour of the product and if signs of physical instability, such as cracking and/or bleeding were absent during the test period. The organoleptic appeal was assessed qualitatively.

The physico-chemical attributes of the formulation were evaluated quantitatively. The formulation was considered to be stable if there was no statistical relevant and/or significant change between the test results of the 100 g unit assessed immediately after manufacture (time 0 weeks) and the 100 g units stored at accelerated test conditions and analysed at 1, 2, 3 and 4 weeks after storage. In order to assess whether or not these changes were statistically relevant and/or significant, the statistical procedure described by Timm *et al*., [97] as described in Section 2.4.7.2 was used.

The responses used to calculate the confidence intervals as described by Timm *et al*., [97] were the CP content, apparent intrinsic viscosity, apparent pH and CP *in vitro* release rates. The percentage difference in response between the initial sample (at time 0 weeks) and stored samples (at times 1, 2, 3 and 4 weeks) were calculated and used to construct a 90% C.I.. The data are reported as confidence intervals depicting the percentage change from the initial reading for CP content in the formulation (n =3), intrinsic viscosity (n = 3) and apparent pH (n = 3) of the product and CP *in vitro* release rates from the formulation (n = 6).

In addition, similarities or differences in the *in vitro* release rates of CP from each of the stability samples *vs.* the *in vitro* release rate of CP from the reference sample were evaluated using a non-parametric test, which was described and reported in Section 3.2.11.

5.2.3. Results and discussion

5.2.3.1. Qualitative analysis

There were no noticeable changes in the organoleptic properties of Batch CP004 in terms of appearance, colour and odour (Table 4.1) over the entire stability test period. In addition the formulation did not show any signs of physical instability such as phase separation or cracking and bleeding of the cream base from the container at any of the sampling times. Based on the organoleptic data observed in these studies the CP cream formulation may be considered to be potentially stable.

5.2.3.2. Quantitative analysis

The stability data for Batch CP004 evaluated in terms of CP content, intrinsic viscosity, intrinsic pH and *in vitro* release rate (flux) of CP from the cream formulation, generated over the entire stability test period are summarized in Table 5.3.

Table 5.3. Stability data generated for Batch CP004 after a four (4) week test period

	Stability test parameters			
Sampling time (week)	**CP content (%)**	**Intrinsic viscosity (KcP)**	**Intrinsic pH**	**Flux ($\mu g/cm^2/h^{1/2}$)**
0	111.7 ± 1.906	15.13 ± 0.7023	5.11 ± 0.00577	7.532 ± 0.8056
1	110.4 ± 2.871	16.27 ± 1.115	5.15 ± 0.0265	7.800 ± 0.6016
2	109.6 ± 2.474	17.17 ± 0.4163	5.21 ± 0.0568	7.480 ± 0.3761
3	103.7 ± 4.418	17.70 ± 0.6080	5.17 ± 0.0550	7.626 ± 0.08207
4	101.7 ± 4.073	17.23 ± 0.1528	5.25 ± 0.0608	6.727 ± 1.468

5.2.3.2.1. CP content

The data shown in Table 5.3 indicate a progressive decrease in the amount of CP in the formation as the time of exposure of the formulation to the stability test conditions increased. This may be due to degradation of the CP in the formulation and the longer the formulation remained exposed to the test conditions, the greater is the amount of CP that degrades. However, studies to establish whether or not the degradation of CP in the cream base occurred were not undertaken. Nevertheless, despite the progressive decrease in the amount of CP in Batch CP004 after exposure to stability test conditions, the CP content remained within the specifications established for the formulation (Table 5.2) at all sampling times.

The CP content data generated in these tests were subjected to a statistical analysis (Section 2.4.7.2) and the results obtained from this test are shown in Figure 5.1. These results indicate that the percentage change from the initial amount of CP in the formulation after storage at 40 ± 2˚C and 25 ± 5% RH for 1, 2, 3, and 4 weeks is not statistically significant or relevant. Consequently, the CP cream can be considered as stable for the time period under investigation.

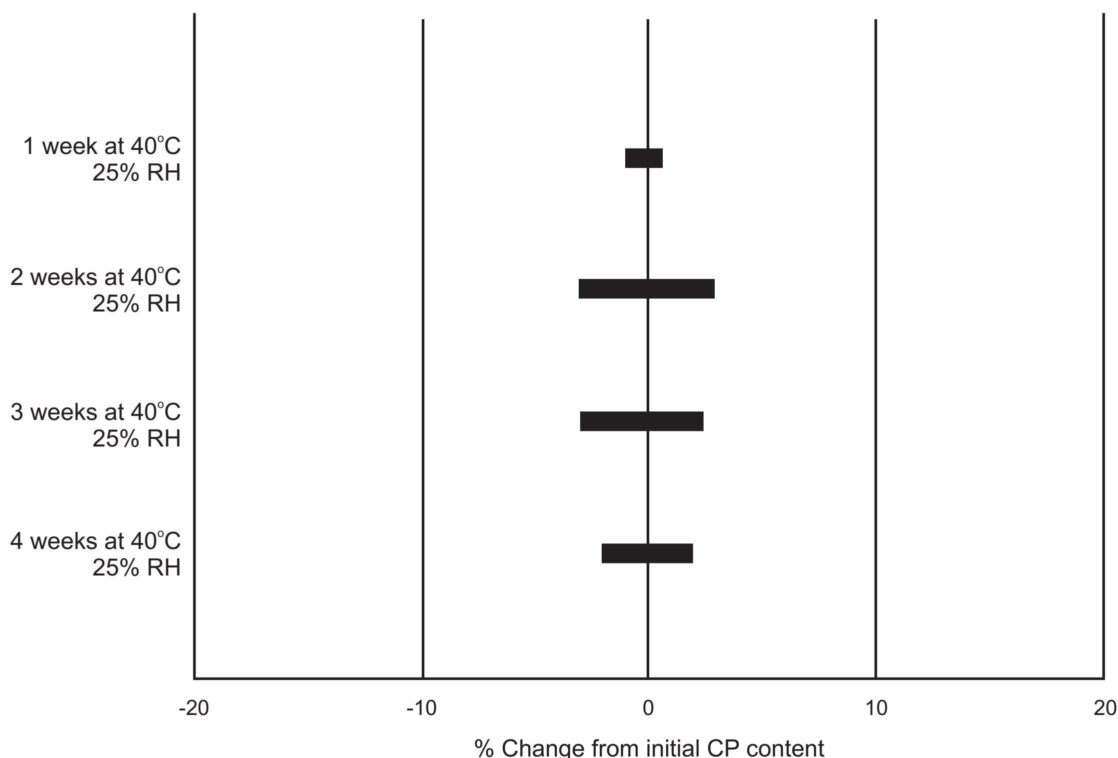


Figure 5.1. Effects of stability test conditions on CP content of the cream formulation after storage at 40 ± 2˚C and 25 ± 5% RH for 1, 2, 3 and 4 weeks**.**

5.2.3.3. Intrinsic viscosity

The data shown in Table 5.3 showing the apparent intrinsic viscosity indicate that the apparent intrinsic viscosity of Batch CP004 remained within the established specifications (Table 4.2) over the stability test period.

There seem to be a noticeable increase in the apparent intrinsic viscosity value of all the test samples when compared to the viscosity of the cream sample observed at time 0 weeks. The apparent intrinsic viscosity data generated in these tests were subjected to a statistical analysis (Section 2.4.7.2) to determine whether or not the increase was significant and/or relevant and the results obtained from this test are illustrated in Figure 5.2.

These data reveal that the percentage change from the initial apparent intrinsic viscosity of the CP cream formulation after storage at 40 ± 2˚C and 25 ± 5% RH for 1, 2, 3, and 4 weeks is not

statistically significant or relevant. Based on these results, the CP cream formulation is considered stable for the 1 month test period under investigation.

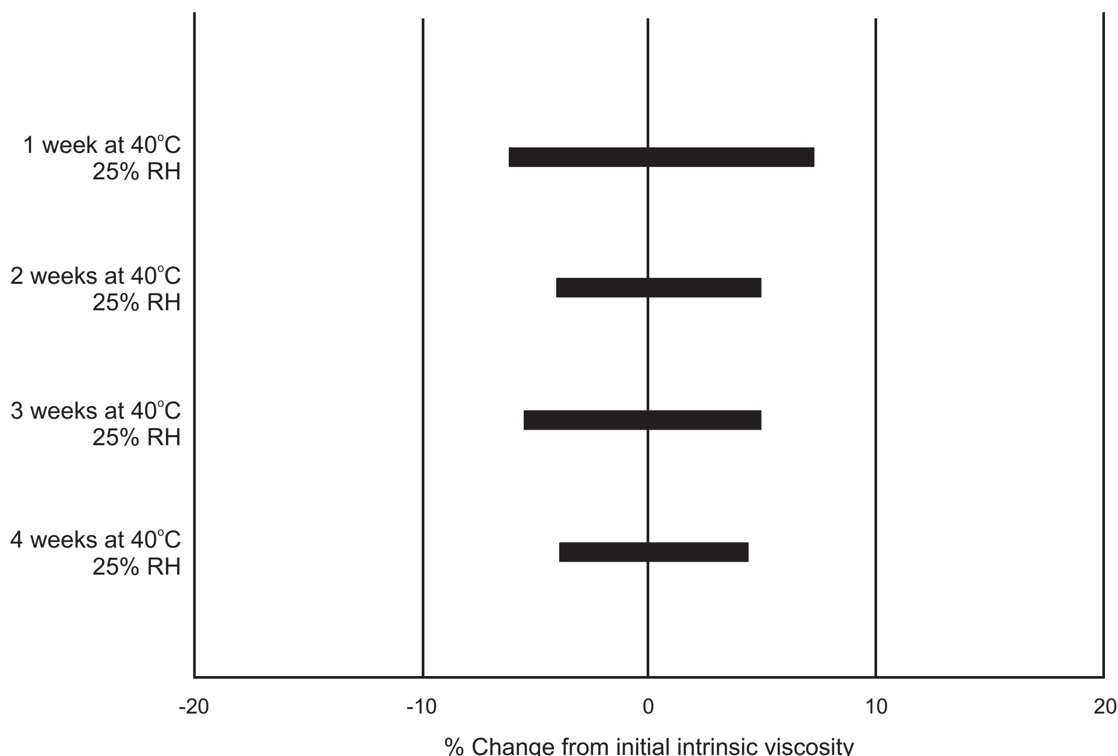


Figure 5.2. Effects of stability test conditions on the apparent intrinsic viscosity of the CP cream formulation after storage at 40 ± 2°C and 25 ± 5% RH for 1, 2, 3 and 4 weeks.

5.2.3.4. Apparent intrinsic pH

The data shown in Table 5.3 showing the apparent pH of the cream samples indicate that the apparent pH of the cream formulation remained within the established specifications (Table 4.2) over the entire test period. It appears that the apparent pH of the formulation increased slightly after exposure to the stability test conditions and this increase was proportional to the time of exposure.

In order to determine whether or not the increase in the apparent intrinsic pH of the formulation was statistically significant and/or relevant, the apparent intrinsic pH data were subjected to a statistical analysis (Section 2.4.7.2) and the results generated from this test are illustrated in Figure 5.3.

These data reveal that the percentage change from the initial apparent pH of the CP cream formulation after storage at 40 ± 2˚C and 25 ± 5% RH for 1, 2, 3, and 4 weeks is not statistically significant or relevant. As a consequence, these results reveal that the pH of the product is still within the specified pH range for topical formulations [193, 194] and within the range set for this product (Table 5.2). The CP cream formulation can be considered stable in terms of maintaining the pH within the specified limits during the time period the formulation was tested.

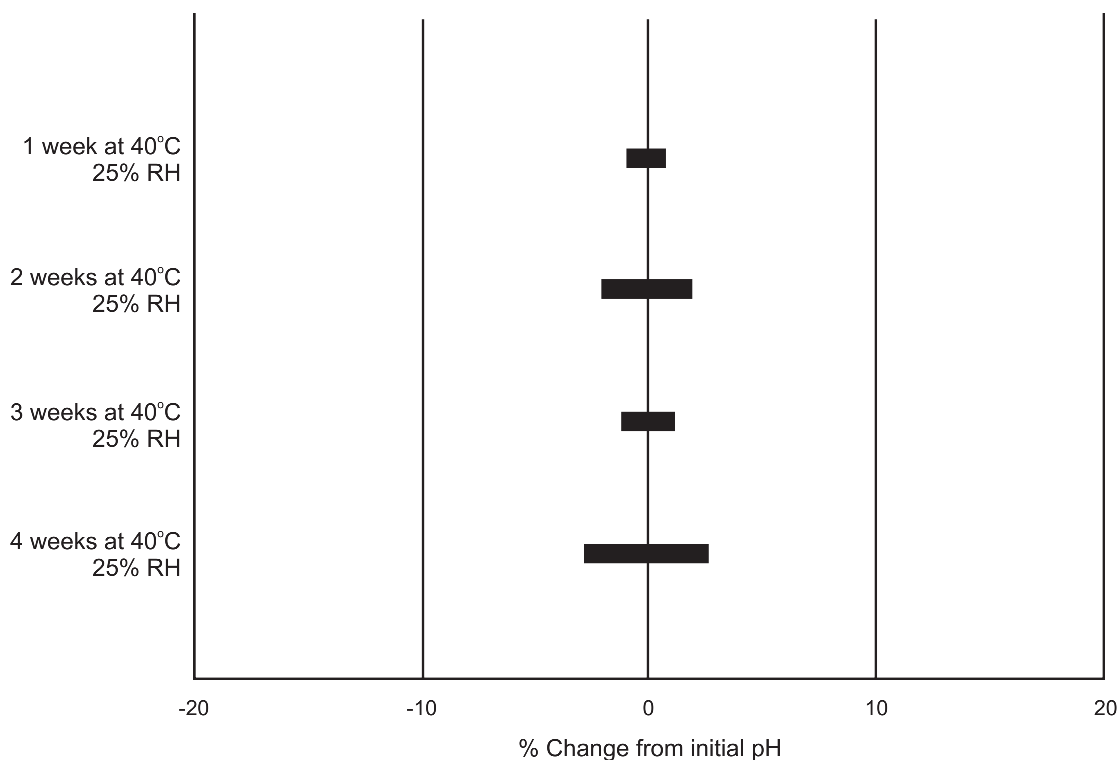


Figure 5.3. Effects of stability test conditions on the apparent intrinsic pH of the cream formulation after storage at 40 ± 2˚C and 25 ± 5% RH for 1, 2, 3 and 4 weeks.

.

5.2.3.5. In vitro release rate testing

The data shown in Table 5.3 for the *in vitro* release rate (flux) of CP from the cream reveal that the *in vitro* release rate (flux value) of CP from the CP cream remains relatively constant but shows a slight decrease in flux for the 4 weeks sample that appears significant. Nevertheless, all *in vitro* release rates (flux values) calculated in these studies fall within the *in vitro* release rate specifications established for the cream formulation (Table 5.3). There appears to be a

correlation between this slight decrease in flux and the slight increase in the viscosity of the formulation following four weeks of storage.

In order to determine whether or not changes between the *in vitro* release rates of CP release from cream samples are statistically significant and/or relevant, the *in vitro* release rate of CP data were subjected to a statistical analysis (Section 2.4.7.2) and the data generated are shown in Figure 5.4.

These data indicate that the percentage change from the initial CP *in vitro* release rate of CP release from the cream formulation after storage at 40 ± 2˚C and 25 ± 5% RH for weeks 1, 2, 3, and 4 is not statistically significant or relevant. These results indicate that the cream formulation is stable over the period tested.

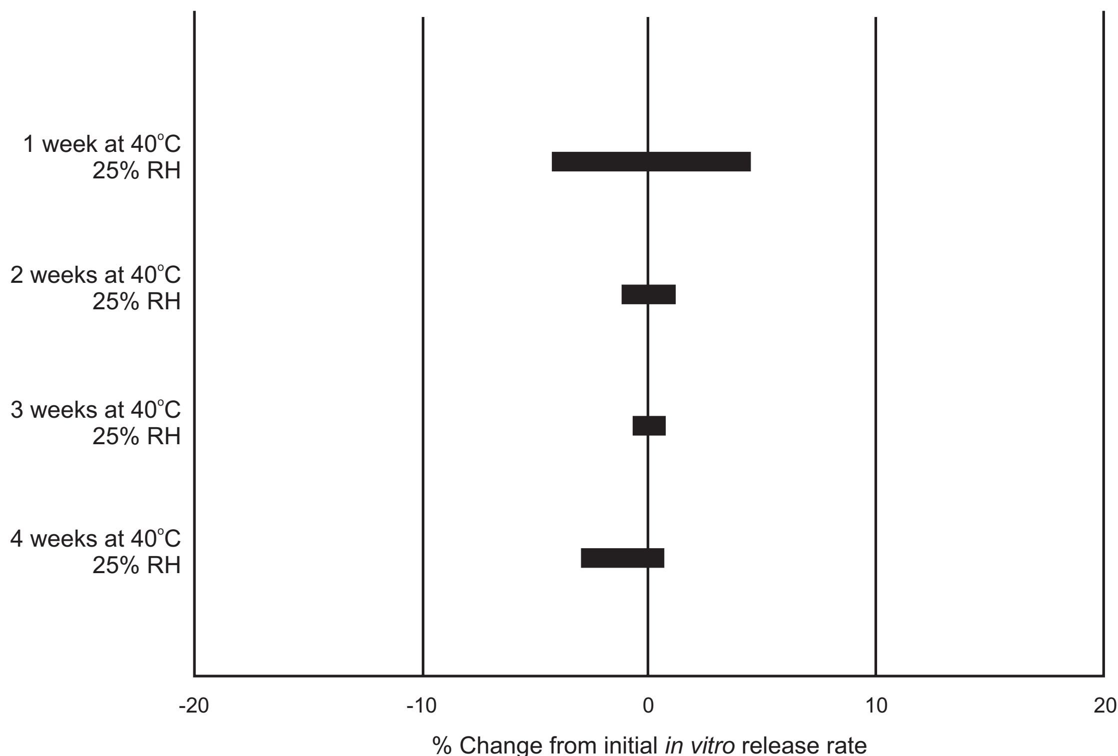


Figure 5.4. Effects of stability test conditions on the *in vitro* release rates of CP from the cream formulation after storage at 40 ± 2˚C and 25 ± 5% RH for 1, 2, 3 and 4 weeks

The data collected during *in vitro* release rate studies were also used to generate *in vitro* release rate profiles for CP and the data are plotted in Figure 5.5. These data are plotted as the cumulative amount of CP released per unit area (Q) *vs.* square root of time ($t^{1/2}$). From these results, it is evident that there is no difference in the *in vitro* release rate profiles of CP from the cream samples tested over the entire test period.

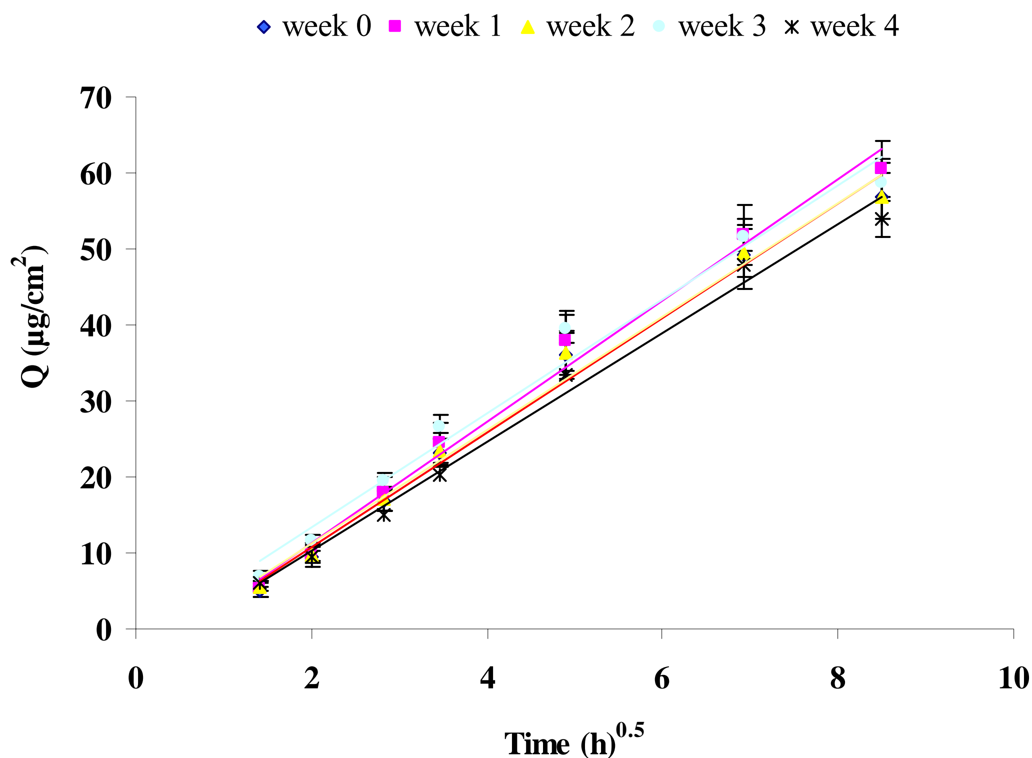


Figure 5.5. *In vitro* release rate profiles for CP from CP cream samples at times 0, 1, 2, 3 and 4 weeks after storage

In order to establish whether the apparent similarity in the *in vitro* release rate profiles for CP following storage of creams and the cream tested immediately after manufacture was real the data were tested using non-parametric statistical analysis (Section 3.2.11).

The lower limit (L.L.) and upper limit (U.L.) of a confidence interval (C.I.) calculated as described in Section 3.2.11 (Chapter 3) with the cream sample at week 0 (reference) and the cream sample at weeks 1, 2, 3 or 4 (test) are summarised in Table 5.4. If the *in vitro* release rate profile of CP release from the reference sample is to be considered equivalent to the *in vitro*

release rate profile for CP release from the test sample then the limits for the C.I. calculated from experimental data should lie within the 75%-133% limits [49].

Table 5.4. The lower limit (L.L.) and upper limit (U.L.) of the confidence intervals (C.I.) calculated using cream sample at week 0 (reference) and cream samples at weeks 1, 2, 3 and 4 (test)

	Confidence intervals (C.I.)	
	L.L. (%)	**U.L. (%)**
C.I. limits	75.00	133.0
Week 0 *vs.* week 1	93.97	114.0
Week 0 *vs.* week 2	89.43	110.9
Week 0 *vs.* week 3	93.88	110.0
Week 0 *vs.* week 4	86.02	108.1

From the results summarised in Table 5.4 it is clear that there is no statistically significant difference in the *in vitro* rate of release of CP release from creams subjected to stability test conditions. These data are consistent with the results generated using the statistical test described by Timm *et al*., [97] (Figure 5.5), which reveal that the *in vitro* release rates of CP release from the tested cream samples at weeks 1, 2, 3 and 4 are equivalent to the *in vitro* release rate of CP release from the cream sample tested at week 0. Consequently Batch CP004 can be considered stable over the time period the formulation was tested

5.3. CONCLUSIONS

The stability of the CP cream formulation developed, assessed and reported in Chapter 4 has been evaluated using accelerated stability test conditions. The initial step taken in these studies was to develop a stability study protocol using currently published international guidelines for the stability testing of drug substances and drug products. Essentially, the stability protocol used in these studies was designed to facilitate the selection of batches, number of batches, container closure system, sampling frequency, sampling plan, test storage conditions, test specifications, test procedure and stability data evaluation procedure.

One of the best prototype formulations, Batch CP004, was selected for use in stability testing studies. Based on the recommendations in the guideline documents a stability study on a single batch of the generic formulation was considered to be adequate for the purposes of these

investigations. The formulation was packed into four (4) 100 g glass ointment jars, to facilitate ease of sampling at the four (4) time points, 1, 2, 3, and 4 weeks.

The stability study was conducted under accelerated conditions of 40 ± 2˚C and 25 ± 5% RH. Internal guidelines on stability studies specify that accelerated stability studies be conducted at 40 ± 2˚C and 75 ± 5% RH. However, these conditions were slightly modified due to the availability of facilities in our laboratory. The main objective of these studies was to determine whether or not the modified accelerated stability conditions would have an effect on specific formulation attributes such as organoleptic appeal, CP content, apparent intrinsic viscosity, apparent pH and CP *in vitro* release rates.

Sample were removed from the storage incubator at weeks 1, 2, 3 and 4 and immediately tested for organoleptic appeal, CP content, apparent intrinsic viscosity, apparent intrinsic pH and the CP *in vitro* release rates.

Organoleptic data obtained from these studies were evaluated qualitatively, whereas quantitative data were generated for CP content, apparent intrinsic viscosity and pH and CP *in vitro* release rates. The data generated at each sample time were assessed using a statistical test procedure to determine whether a relevant and/or significant change occurred in the product following storage for up to four weeks as compared to that evaluated immediately after manufacture.

The original appearance of the cream formulation did not change and the formulation did not show signs of physical instability, such as phase separation or cracking and bleeding of the cream base from the container at any of the sampling times. Quantitative analysis revealed that the percentage change from the initial product specifications such as CP content, apparent intrinsic viscosity and pH and CP *in vitro* release rates was in all cases not statistically significant or relevant.

Based on the data generated in this pilot stability study, the CP cream formulation appears to be stable. However, it will be necessary to conduct additional studies of the cream formulation in conventional cream packaging and a stability chamber using the established accelerated stability

test conditions for longer periods to fully characterize the short-term and long-term stability of this formulation. Furthermore, it will be essential in future studies to conduct stability studies on the innovator product (Dermovate® cream) and compare the data generated from these studies to those obtained for the generic formulation.

It is evident that the formulation development process was successful in producing a potentially stable CP cream product and these studies could form the basis for further studies on the formulation that has been developed.

CHAPTER SIX

CONCLUSIONS

Clobetasol 17-propionate (CP) has been labelled as a class I topical corticosteroid and is currently one of the most potent topical corticosteroids available on the market. CP is used for the short-term treatment of inflammatory and pruritic manifestations of moderate-to-severe glucocorticoid-responsive dermatoses. CP has physicochemical and pharmacological properties such as a relative high lipophilicity and local anti-inflammatory activity which make it an ideal candidate for incorporation into topical semi-solid formulations. The objective of this research project was to develop and characterize a CP topical cream formulation containing 0.05% w/w of the drug.

Prior to initiating formulation development studies, it is vital that a suitable analytical method is developed and validated for the quantitation of drug and the characterization of dosage forms during the development and assessment process. A major difficulty often encountered in the analysis of semi-solid dosage forms is interference due to formulation adjuvants and preservatives that are usually present in what are relatively complex formulations. RP-HPLC is a commonly used, powerful and reliable analytical tool that can be used for the *in vitro* analysis of formulations of a complex nature, such as for example creams, gels and ointments.

HPLC not only provides separation and quantitative data but also has the ability to eliminate almost all interference challenges. The initial phases of this project entailed the development and validation of a suitable RP-HPLC method that was suitable for the quantitative analysis of CP in cream formulations and CP release during *in vitro* release studies. Separation of CP and the internal standard (BV) was achieved on a Nova-Pak® C_{18} cartridge column (3.9 x 150 mm, 4μm) using a mobile phase consisting of acetonitrile-water (50:50) at a flow rate of 1.0ml/min with UV detection at 240 nm. The retention time of BV and CP were 5.6 and 8.2 min respectively and the total run time for analysis was 10 min.

The RP-HPLC analytical method was validated according to USP [23], FDA [98] and ICH [86] guidelines. The analytical method was found to be linear, precise, accurate, selective and sensitive and suitable for the intended purpose of CP quantitation. CP was found to be stable in the mobile phase following storage at 4˚C for a maximum of 14 days, and thus all calibration standards, which were made in the mobile phase, were stored at 4˚C and used within a period of 14 days, after which fresh calibration standards were prepared.

In vitro dissolution testing of solid oral dosage forms is a well-established technique used to guide the formulation development process, assess product quality and ensure batch-to-batch uniformity. However, as far as semi-solid formulations are concerned, only quality control tests, such as for example the determination of solubility, particle size and size distribution and crystalline form of an API, and evaluation of the intrinsic viscosity and homogeneity of a final product has been traditionally used to provide reasonable evidence of consistent product manufacture and performance. The main drawback with these quality control tests is that they provide little information about drug release characteristics of a product or the effects of processing and manufacturing variables on the performance of a finished product.

In recent years, some international guidelines [49] have recommended the use of *in vitro* release test methods to determine if the diffusional rate of release of a drug from a formulation is the same following any post-approval formulation changes, as it was prior to the changes. Such tests can be used to detect the effects of changes in formulation composition on the rate of release of an API from a dosage form in which that API is suspended and/or dissolved and can therefore be used to ensure that the manufacture of semi-solid products is consistent. Therefore such tests may fulfil a similar role as dissolution testing does for tablets and capsules in evaluating topical semi-solid dosage forms.

In contrast to dissolution testing, for which official test methods have been developed and reported for use in *in vitro* dissolution studies of solid oral dosage forms, there are currently no official guidelines or requirements for the performance evaluation of drug release from semi-solid dosage forms. The onus is left to a formulation scientist to develop and validate specific *in vitro* release methods for the assessment of drug release from semi-solid products. Therefore, it

was essential to develop and validate an *in vitro* release test method for use in conjunction with traditional quality control tests to determine the quality and consistency of CP topical cream formulations that were to be manufactured during product development studies. The *in vitro* release method was also used to characterize formulations and evaluate batch-to-batch consistency, and in assessing the impact of storage at elevated temperatures on product performance.

The *in vitro* release test method development studies entailed the selection of a suitable diffusion cell system, appropriate sampling times, receptor medium, temperature of the receptor medium and appropriate synthetic membranes. In addition the effects of sample application and sample occlusion or non-occlusion on *in vitro* release of CP from semi-solid dosage forms were also investigated. A modified Franz glass diffusion cell system was selected and used in these studies since it has the most potential for use as a standardized test system that may be adapted for use as a compendial method.

The *in vitro* release of CP from 0.05% w/w cream formulations was tested using a receptor medium consisting of a binary mixture of water:propylene glycol (50:50) and a 0.025 μm nitrocellulose membrane. The temperature of the test system was set at 32° ± 0.5°C to approximate the usual surface temperature of the skin and a 2 x 2 mm star head magnetic stirrer was used to agitate the receptor medium.

About 300 mg of the semi-solid preparation that corresponded to an infinite dose was applied uniformly to the membrane and the formulation was occluded for the duration of testing, to prevent solvent evaporation and compositional changes to the formulation. Samples of the receptor medium were withdrawn at 2, 4, 8, 12, 24, 48 and 72 hours in order to generate a satisfactory CP release profile and to characterize the release of CP from semi-solid topical formulations.

The *in vitro* release test method was validated using published protocols. Method validation studies involved assessing the ability of the test method to detect the effects of changes in

formulation characteristics on CP release rates. The effects of changes of strength, composition and intrinsic viscosity of topical formulations on *in vitro* release of CP were investigated.

It was determined that the *in vitro* release method was able to detect the effects on CP release rates of changes in formulation in which CP is suspended and/or dissolved. The *in vitro* release test was used in conjunction with traditional quality control tests to determine the quality and consistency of CP topical formulations and the associated release characteristics of CP from the product during formulation development and assessment studies.

In generic formulation development studies it is important to characterize the innovator product to generate data, which can be used as a benchmark for the development of the generic formulation. The initial experiments in these studies were designed to facilitate the characterization of the innovator formulation, Dermovate® cream. CP content, apparent intrinsic viscosity, apparent pH and *in vitro* release rates of CP from the formulation were determined and the data obtained from these studies were used as reference specifications for the development of a generic formulation.

Preliminary studies were designed to facilitate the manufacture of a prototype generic formulation that showed no signs of physical instability, such as, for example, cracking, creaming, phase inversion and/or bleeding of the cream base from the container, immediately after the manufacture as well as twenty-four (24) hours after manufacture and storage at room temperature (22˚C). As a consequence, a prototype formulation, Batch CP001 was extemporaneously manufactured with Estol® 1474 as the primary emulsifier. Although Batch CP001 showed no signs of physical instability immediately after manufacture, the formulation had cracked twenty-four (24) hours after manufacture and storage at room temperature (22˚C) and was therefore not considered for further development.

Three additional prototype formulations containing commercially available mixed emulgents, Ritapro® 200 (Batch CP002), Emulcire® 61 WL (Batch CP003) and Gelot® 64 (Batch CP004) were manufactured. All three formulations were found to be physically stable immediately after

manufacture as well as twenty-four (24) hours after manufacture and storage at room temperature (22˚C) and were therefore considered for further development.

Following the manufacture of stable batches, the next phase of the formulation development studies was to investigate the *in vitro* release rate of CP release from Batches CP002, CP003 and CP004 and Dermovate® cream. These studies were conducted in order to identify a prototype batch with a similar *in vitro* release rate profile for CP similar to that of Dermovate® cream.

The similarities or differences between the *in vitro* release rates of CP release from the prototype cream formulations and Dermovate® cream were statistically determined using a non-parametric test recommended by the FDA [49]. It was found that only the *in vitro* release rate of CP release from Batch CP004 was found to be equivalent to the *in vitro* release rate of CP release from the innovator formulation. Consequently, only Batch CP004 was developed and investigated further.

Two additional batches, Batches CP005 and CP006, having similar a composition to Batch CP004, were manufactured using the same manufacturing procedure as that used to produce Batch CP004. The three formulations were then characterised for CP content, apparent intrinsic viscosity, pH and *in vitro* release rate and the data generated from these studies were compared to the data generated for the innovator product.

The CP content of all generic formulations and the Dermovate® cream were comparable and fell within the USP limits for CP content in topical semi-solid formulations. In addition, the apparent pH values of Batches CP004, CP005 and CP006 were comparable to that of the innovator product and fell within the pH range of healthy human skin.

The apparent intrinsic viscosity of Dermovate® cream was found to be almost three (3) times greater than that of generic cream batches and the difference in intrinsic viscosity of Dermovate® cream was attributed to the physicochemical characteristics of the mixed emulsifiers used in the innovator product and to manufacturing process variables, such as for example speed of homogenization speed and mode of cooling.

Despite the differences in apparent intrinsic viscosity the *in vitro* release rate of CP from Batches CP004, CP005 and CP006 was found to be statistically similar to the *in vitro* release rate of CP from the innovator product. It was concluded that the behaviour of the mixed emulsifier in both the commercially available cream and the extemporaneously manufactured creams, and not only the apparent intrinsic viscosity of the formulations, had e an impact on the *in vitro* release rate of CP from these formulations.

Based on the comparative data generated for the generic cream and those for the innovator formulation, the formulation development process was considered to have been successful. Despite the fact that the apparent intrinsic viscosity of the generic formulations was significantly different to that of the Dermovate® cream, Batches CP004, CP005 and CP006 were found to be equivalent to the innovator cream product in terms of other *in vitro* performance characteristics such as CP content, pH and *in vitro* release rates.

Following the development of batches of cream that were considered equivalent to the innovator formulation in terms of *in vitro* performance characteristics, three 500 g batches were produced for use in accelerated stability studies. The stability studies aimed to determine whether or not the formulation development process was successful in producing a stable CP cream product.

Therefore the stability study was designed to facilitate the selection of batches, number of batches, container closure system, sampling frequency, sampling plan, test storage conditions, test specifications, product specifications, test procedure and stability data evaluation procedure. Batch CP004 was selected for testing and the formulation was packed into five (5) 100 g glass ointment jars, to facilitate ease of sampling at the five (5) time points, 0, 1, 2, 3, and 4 weeks, and a stability test was conducted under conditions of 40 ± 2˚C and 25 ± 5% RH in an incubator.

Cream samples were tested for qualitative and quantitative attributes such as organoleptic appeal, CP content, apparent intrinsic viscosity, apparent pH and *in vitro* release rate. The product specifications for all parameters were set prior to initiating stability testing and qualitative data were generated by visual observation, whereas quantitative data were analysed and evaluated

using statistical testing. The cream samples were found to be physically stable over the entire test period and the samples did not change in appearance, colour or odour.

Although, there appeared to be a progressive decrease in CP content of the cream as the time of cream exposure to the stability test conditions increased, the decrease was not statistically significant or relevant. The apparent intrinsic viscosity and pH values of the formulation increased slightly over the test period, but again these increases were found to be neither statistically significant nor relevant.

The *in vitro* release rate of CP from the cream formulation appeared to remain constant, after 4 weeks a decrease from the initial *in vitro* release rate of CP was observed. Nevertheless, statistical analyses indicated that the changes in *in vitro* release rates were not significant or relevant.

The data generated during accelerated stability studies indicate that the CP cream formulation manufactured is potentially stable. However, it will be necessary to conduct additional studies on the cream formulation stored in conventional cream packaging and using a stability chamber with the ability to create established accelerated stability test conditions. Furthermore the product would have to be tested for longer periods to fully characterize the short-term and long-term stability of this formulation. In addition, stability studies would need to be conducted on the innovator product (Dermovate® cream) in order to provide comparative data for the assessment of the generic formulation.

Future studies would entail an investigation of the effects of formulation and process variables on *in vitro* performance of the dosage forms. The apparent intrinsic viscosity of the formulations must be increased to match the viscosity of the innovator product. Intrinsic viscosity invariably affects drug release from a semi-solid formulation and although the viscosity does not appear to affect the *in vitro* release of CP from the semi-solid formulations developed in these studies, the viscosity may have a significant effect when testing the products *in vivo*.

The formulation appears stable at high temperatures following pilot-scale manufacture and therefore scale-up of the process is necessary and challenges associated with formulation scale-up require investigation.

In vivo studies could be conducted using the the human skin blanching assay [7] to determine whether the generic formulation developed in these studies is bioequivalent to the innovator product. *In vitro* release rate testing with rate limiting membranes, such as for example a silastic membrane or membranes derived from animal models, may assist in the development of a test model to establish an *in vitro in vivo* correlation (IVIVC) for CP release from topical formulations.

A stable cream formulation has been developed and assessed and the data generated reveal that the dosage form behaves similarly to Dermovate® during *in vitro* studies. The results obtained provide a suitable platform from which a bioequivalent CP semi-solid cream can be developed and commercialised.

APPENDIX ONE

EXAMPLE OF THE CALCULATION OF A 95% CONFIDENCE INTERVAL FOR THE RATIO OF T/R RELEASE RATES

A. Release rates (slopes) for reference system (R) and test system (T)

Cell No.	Test	Reference
1	5.4050	5.1894
2	6.0153	4.3467
3	6.1577	5.4699
4	6.2852	5.9206
5	5.3946	5.2937
6	5.3641	4.3055

B. Computation of 36 individual T/R ratios

	(R) slopes					
(T)	**5.1894**	**4.3467**	**5.4699**	**5.9206**	**5.2937**	**4.3055**
5.4050	1.0415	1.2435	0.9881	0.9129	1.0210	1.2554
6.0153	1.1592	1.3839	1.0997	1.0160	1.1363	1.3971
6.1577	1.1866	1.4166	1.1257	1.0400	1.1632	1.4302
6.2852	1.2112	1.4460	1.1491	1.0616	1.1873	1.4598
5.3946	1.0395	1.2411	0.9862	0.9112	1.0191	1.2530
5.3641	1.0337	1.2341	0.9807	0.9060	1.0133	1.2459

C. Arrangement T/R ratios from the lowest to highest (counting from left to right)

0.9060	0.9112	0.9129	0.9807	0.9862	0.9881	1.0133	1.0160
1.0191	1.0210	1.0337	1.0395	1.0400	1.0415	1.0616	1.0997
1.1257	1.1363	1.1491	1.1592	1.1632	1.1866	1.1873	1.2112
1.2341	1.2411	1.2435	1.2459	1.2530	1.2554	1.3839	1.3971
1.4166	1.4302	1.4460	1.4598				

D. The eighth T/R ratio converted to % is 101.6% and the twenty-ninth T/R ratio converted to % is 125.3%, which lies between 75% and 133.3%. The test product is therefore equivalent to the reference product

APPENDIX TWO

QUALITATIVE COMPOSITION OF DERMOVATE® CREAM

Qualitative composition of Dermovate® cream [196]

Excipients
Clobetasol propionate
Chrorocresol
Cetostearyl alcohol
Glyceryl monostearate
Arlacel® 165
Beeswax substitute
Propylene glycol
Sodium citrate
Citric acid
Purified water

APPENDIX THREE

EXCIPIENTS USED IN CP CREAM FORMULATION DEVELOPMENT STUDIES

Excipients	Trade name	Manufacturer/Donor
Clobetasol 17-propionate	Clobetasol propionate	Symbiotec Pharmalab P.V.T. Ltd (Pigdamber, Maharastra, India
Propylene glycol	propane-1,2-diol	Merck Chemicals (pty) Ltd, Darmstadt, Germany
Sodium citrate	N/A	Aspen Pharmacare, Port Elizabeth, Eastern Cape, SA
Citric acid	N/A	Aspen Pharmacare, Port Elizabeth, Eastern Cape, SA
Glyceryl monostearate	Geleol®	Gattefosse SAS, Saint-Priest Cedex, France
Cetostearyl alcohol	N/A	Aspen Pharmacare, Port Elizabeth, Eastern Cape, SA
White beeswax BP	N/A	Croda Chemicals (SA) (Pty) Ltd, Johannesburg, Gauteng, SA
Chlorocresol	N/A	Aspen Pharmacare, Port Elizabeth, Eastern Cape, SA
Glyceryl stearate	Estol® 1474	Uniqema (Pty) Ltd, Bryanstone, Gauteng, SA
Stearyl alcohol and steareth-20	Ritapro® 200	Rita, Crystal Lake, IL, USA
Cetyl alcohol, ceteth-20 and steareth-20	Emulcire® 61 WL	Gattefosse SAS, Saint-Priest Cedex, France
Glyceryl stearate and PEG-75 stearate	Gelot® 64	Gattefosse SAS, Saint-Priest Cedex, France

APPENDIX FOUR

QUALITATIVE AND QUANTITATIVE FORMULAE USED TO MANUFACTURE CP CREAM FORMULATIONS IN THESE STUDIES

Material	Composition (% w/w)
Clobetasol propionate	0.05000
Propylene glycol	44.50
Sodium citrate	0.05000
Citric acid	0.05000
Glyceryl monostearate A/S	5.000
Cetostearyl alcohol	4.000
White wax (beeswax bleeched)	0.6000
Chlorocresol	0.0750
Glyceryl monostearate SE	1.000
Propylene glycol	7.000
Propylene glycol	2.675
Purified water	35.00

APPENDIX FIVE

BATCH SUMMARY REPORTS

RHODES UNIVERSITY, Faculty of Pharmacy, Grahamstown, SOUTH AFRICA

BATCH SUMMARY REPORT

Formulator: Kasongo Wa Kasongo
Product: Clobetasol 17-propionate cream
Batch ID: CP001 **Batch Size:** 200 g
Manufacturing Date: 04-04-2006 **Manual mixing time:** 10 min
High speed homogenization time: 5 min

Formula

Material	% (w/w)	Amount added (g)	Rhodes #
CP	0.05000	0.1023	RM000150
Propylene glycol	44.50	89.04	RM000181
Sodium citrate	0.05000	0.1019	RM000183
Citric acid	0.05000	0.1026	RM000185
Geleol® pastilles	5.000	10.05	RM000182
Cetostearyl alcohol	4.000	8.053	RM000184
White beeswax BP	0.6000	1.020	RM000142
Chlorocresol	0.07500	0.1508	RM000186
Estol® 1474	1.000	2.005	RM000160
Propylene glycol	7.000	14.10	RM000181
Propylene glycol	2.675	5.350	RM000181
Purified water	35.00	70.07	N/A

Production equipment used:
Water bath: Model NB-34980 Colora Ultra-Thermostat water bath
Hotplate: Model RCH IKA-Combimag hotplate magnetic stirrer
Homogenizer: Model 6-105 AF Virtis homogenizer

Parameters evaluated:

	Mean	SD
CP content	Not evaluated	Not evaluated
Viscosity	Not evaluated	Not evaluated
pH	Not evaluated	Not evaluated

***In vitro* release rate**	**Comments / Observations**
Not evaluated	• Shiny, white, odourless cream was produced • The cream was physically stable immediately after manufacture • The cream cracked 24 hours after manufacture • Physico-chemical attributes of the cream were not evaluated

RHODES UNIVERSITY, Faculty of Pharmacy, Grahamstown, SOUTH AFRICA

BATCH SUMMARY REPORT

Formulator: Kasongo Wa Kasongo
Product: Clobetasol 17-propionate cream
Batch ID: CP002 **Batch Size:** 200 g
Manufacturing Date: 11-05-2006 **Manual mixing time:** 10 min
High speed homogenization time: 5 min

Formula

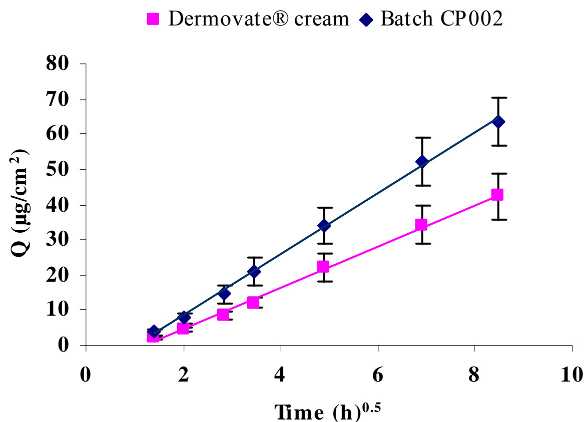
Material	% (w/w)	Amount added (g)	Rhodes #
CP	0.05000	0.1033	RM000150
Propylene glycol	44.50	89.10	RM000181
Sodium citrate	0.05000	0.1055	RM000183
Citric acid	0.05000	0.1008	RM000185
Geleol® pastilles	5.000	10.07	RM000182
Cetostearyl alcohol	4.000	8.071	RM000184
White beeswax BP	0.6000	1.218	RM000142
Chlorocresol	0.07500	0.1527	RM000186
Ritapro® 200	1.000	2.019	RM000140
Propylene glycol	7.000	14.01	RM000181
Propylene glycol	2.675	5.450	RM000181
Purified water	35.00	70.03	N/A

Production equipment used:
Water bath: Model NB-34980 Colora Ultra-Thermostat water bath
Hotplate: Model RCH IKA-Combimag hotplate magnetic stirrer
Homogenizer: Model 6-105 AF Virtis homogenizer

Parameters evaluated:

	Mean	SD
CP content (%)	104.6	3.722
Viscosity (KcP)	23.07	0.9741
pH	5.20	0.0152

***In vitro* release rate**



Comments / Observations

- Shiny, white, odourless cream was produced
- The cream was physically stable immediately after manufacture
- The cream remained physically stable 24 hours after manufacture
- Physico-chemical attributes of the cream were evaluated

RHODES UNIVERSITY, Faculty of Pharmacy, Grahamstown, SOUTH AFRICA

BATCH SUMMARY REPORT

Formulator: Kasongo Wa Kasongo
Product: Clobetasol 17-propionate cream
Batch ID: CP003 **Batch Size:** 200 g
Manufacturing Date: 21-06-2006 **Manual mixing time:** 10 min
High speed homogenization time: 5 min

Formula

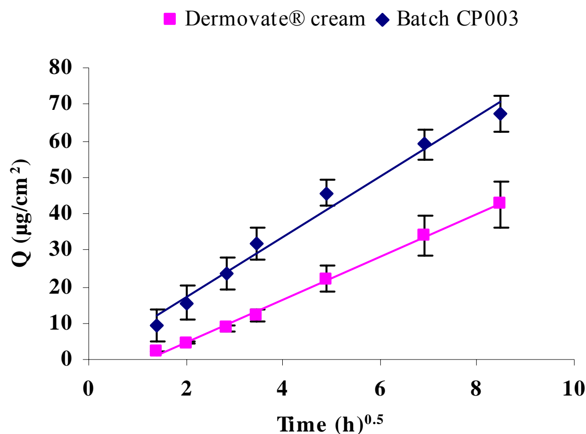
Material	% (w/w)	Amount added (g)	Rhodes #
CP	0.05000	0.1018	RM000150
Propylene glycol	44.50	89.00	RM000181
Sodium citrate	0.05000	0.1019	RM000183
Citric acid	0.05000	0.1026	RM000185
Geleol® pastilles	5.000	10.05	RM000182
Cetostearyl alcohol	4.000	8.053	RM000184
White beeswax BP	0.6000	1.220	RM000142
Chlorocresol	0.07500	0.1528	RM000186
Emulcire® 61 WL	1.000	2.005	RM000178
Propylene glycol	7.000	14.00	RM000181
Propylene glycol	2.675	5.350	RM000181
Purified water	35.00	70.00	N/A

Production equipment used:
Water bath: Model NB-34980 Colora Ultra-Thermostat water bath
Hotplate: Model RCH IKA-Combimag hotplate magnetic stirrer
Homogenizer: Model 6-105 AF Virtis homogenizer

Parameters evaluated:

	Mean	SD
CP content	113.1	2.605
Viscosity	11.43	0.05774
pH	5.15	0.0264

***In vitro* release rate**



Comments / Observations

- Shiny, white, odourless cream was produced
- The cream was physically stable immediately after manufacture
- The cream remained physically stable 24 hours after manufacture
- Physico-chemical attributes of the cream were evaluated

RHODES UNIVERSITY, Faculty of Pharmacy, Grahamstown, SOUTH AFRICA

BATCH SUMMARY REPORT

Formulator: Kasongo Wa Kasongo
Product: Clobetasol 17-propionate cream
Batch ID: CP004 **Batch Size:** 500 g
Manufacturing Date: 10-10-2006 **Manual mixing time:** 10 min
High speed homogenization time: 5 min

Formula

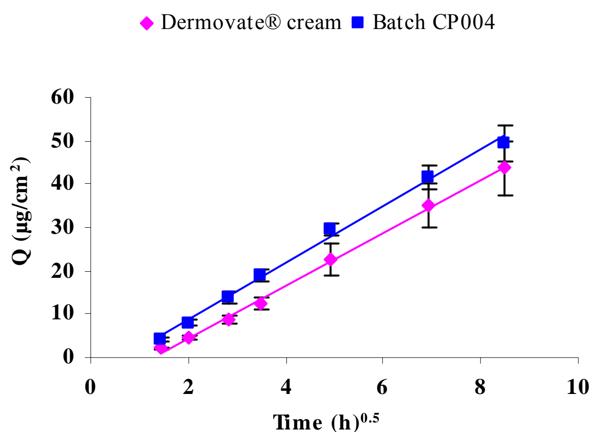
Material	% (w/w)	Amount added (g)	Rhodes #
CP	0.05000	0.2538	RM000150
Propylene glycol	44.50	222.8	RM000181
Sodium citrate	0.05000	0.2596	RM000183
Citric acid	0.05000	0.2548	RM000185
Geleol® pastilles	5.000	25.01	RM000182
Cetostearyl alcohol	4.000	20.13	RM000184
White beeswax BP	0.6000	3.01	RM000142
Chlorocresol	0.07500	0.3759	RM000142
Gelot® 64	1.000	5.100	RM000177
Propylene glycol	7.000	35.44	RM000181
Propylene glycol	2.675	13.68	RM000181
Purified water	35.00	175.0	N/A

Production equipment used:
Water bath: Model NB-34980 Colora Ultra-Thermostat water bath
Hotplate: Model RCH IKA-Combimag hotplate magnetic stirrer
Homogenizer: Model 6-105 AF Virtis homogenizer

Parameters evaluated:

	Mean	SD
CP content	109.8	1.114
Viscosity	15.50	4.359
pH	5.15	0.0264

***In vitro* release rate**



Comments / Observations

- Shiny, white, odourless cream was produced
- The cream was physically stable immediately after manufacture
- The cream remained physically stable 24 hours after manufacture
- Physico-chemical attributes of the cream were evaluated

RHODES UNIVERSITY, Faculty of Pharmacy, Grahamstown, SOUTH AFRICA

BATCH SUMMARY REPORT

Formulator: Kasongo Wa Kasongo
Product: Clobetasol 17-propionate cream
Batch ID: CP005 **Batch Size:** 500 g
Manufacturing Date: 14-10-2006 **Manual mixing time:** 10 min
High speed homogenization time: 5 min

Formula

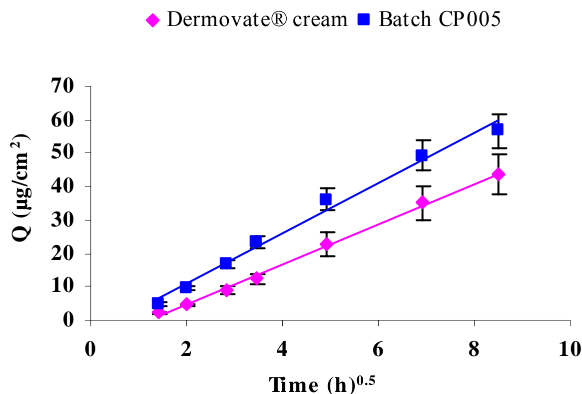
Material	% (w/w)	Amount added (g)	Rhodes #
CP	0.05000	0.2524	RM000150
Propylene glycol	44.50	222.8	RM000181
Sodium citrate	0.05000	0.2539	RM000183
Citric acid	0.05000	0.2533	RM000185
Geleol® pastilles	5.000	25.02	RM000182
Cetostearyl alcohol	4.000	20.07	RM000184
White beeswax BP	0.6000	3.02	RM000142
Chlorocresol	0.07500	0.3778	RM000142
Gelot® 64	1.000	5.030	RM000177
Propylene glycol	7.000	35.03	RM000181
Propylene glycol	2.675	13.46	RM000181
Purified water	35.00	175.2	N/A

Production equipment used:
Water bath: Model NB-34980 Colora Ultra-Thermostat water bath
Hotplate: Model RCH IKA-Combimag hotplate magnetic stirrer
Homogenizer: Model 6-105 AF Virtis homogenizer

Parameters evaluated:

	Mean	SD
CP content	109.8	2.474
Viscosity	12.37	0.03214
pH	5.11	0.05770

***In vitro* release rate**



Comments / Observations

- Shiny, white, odourless cream was produced
- The cream was physically stable immediately after manufacture
- The cream remained physically stable 24 hours after manufacture
- Physico-chemical attributes of the cream were evaluated

RHODES UNIVERSITY, Faculty of Pharmacy, Grahamstown, SOUTH AFRICA

BATCH SUMMARY

Formulator: Kasongo Wa Kasongo
Product: Clobetasol 17-propionate cream
Batch ID: CP006 **Batch Size:** 500 g
Manufacturing Date: 24-10-2004 **Manual mixing time:** 10 min
High speed homogenization time: 5 min

Formula

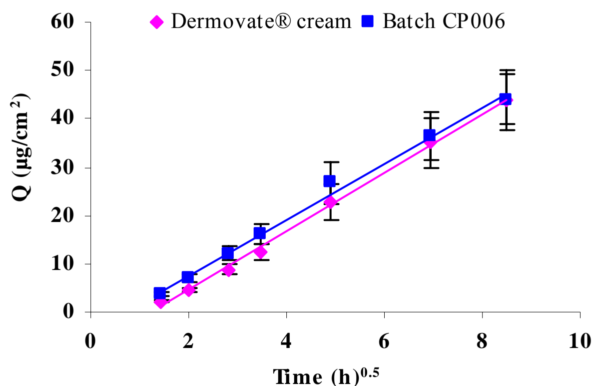
Material	% (w/w)	Amount added (g)	Rhodes #
CP	0.05000	0.2498	RM000150
Propylene glycol	44.50	222.55	RM000181
Sodium citrate	0.05000	0.2490	RM000183
Citric acid	0.05000	0.2523	RM000185
Geleol® pastilles	5.000	25.01	RM000182
Cetostearyl alcohol	4.000	20.09	RM000184
White beeswax BP	0.6000	3.000	RM000142
Chlorocresol	0.07500	0.3765	RM000142
Gelot® 64	1.000	5.060	RM000177
Propylene glycol	7.000	35.10	RM000181
Propylene glycol	2.675	13.47	RM000181
Purified water	35.00	175.40	N/A

Production equipment used:
Water bath: Model NB-34980 Colora Ultra-Thermostat water bath
Hotplate: Model RCH IKA-Combimag hotplate magnetic stirrer
Homogenizer: Model 6-105 AF Virtis homogenizer

Parameters evaluated :

	Mean	SD
CP content (%)	113.1	2.605
Viscosity (KcP)	11.43	0.05774
pH	5.15	0.0264

***In vitro* release rate**



Comments / Observations

- Shiny, white, odourless cream was produced
- The cream was physically stable immediately after manufacture
- The cream remained physically stable 24 hours after manufacture
- Physico-chemical attributes of the cream were evaluated

APPENDIX SIX

BATCH PRODUCTION RECORDS

Note that only one (1) batch production record for the CP cream formulations *viz.,* production record for Batch CP001 is included here. The batch production records for the other five (5) batches, *viz.,* Batches CP002, CP003, CP004, CP005 and CP006 are available on request.

RHODES UNIVERSITY, Faculty of Pharmacy, Grahamstown, SOUTH AFRICA

BATCH PRODUCTION RECORD

Product name: Clobetasol 17-propionate cream **Page:** 1 of 6

Batch ID: CP001 **Batch size:** 200 g

MANUFACTURING APPROVALS

Batch record issued by___________________________ **Date_______________________**

Master record issued by___________________________ **Date_______________________**

RHODES UNIVERSITY, Faculty of Pharmacy, Grahamstown, SOUTH AFRICA

BATCH PRODUCTION RECORD

Product name: Clobetasol 17-propionate cream **Page:** 2 of 6

Batch ID: CP001 **Batch size:** 200 g

MASTER FORMULA AND BATCH FORMULA							
Item	**Material**	**Quantity (% w/w)**	**Rhodes #**	**Amount/ Batch (g)**	**Amount dispensed (g)**	**Dispensed by**	**Checked by**
1	CP	0.05000	RM000150	0.1000	0.1023		
2	Propylene glycol	44.50	RM000181	89.00	89.04		
3	Sodium citrate	0.05000	RM000183	0.100	0.1029		
4	Citric acid	0.05000	RM000185	0.100	0.1026		
5	Geleol® pastilles	5.000	RM000182	10.00	10.05		
6	Cetostearyl alcohol	4.000	RM000184	8.000	8.053		
7	White beeswax BP	0.6000	RM000142	1.200	1.020		
8	Chlorocresol	0.07500	RM000186	0.1500	0.1508		
9	Estol® 1474	1.000	RM000160	2.000	2.005		
10	Propylene glycol	7.000	RM000181	14.00	14.10		
11	Propylene glycol	2.675	RM000181	5.350	5.350		
12	Purified water	35.00	N/A	70.00	70.07		

RHODES UNIVERSITY, Faculty of Pharmacy, Grahamstown, SOUTH AFRICA

BATCH PRODUCTION RECORD

Product name: Clobetasol 17-propionate cream **Page:** 3 of 6

Batch ID: CP001 **Batch size:** 200 g

EQUIPMENT VERIFICATION			
Description	**Type**	**Verified by**	**Confirmed by**
Weighing scale	Model AE-163 Mettler		
Hot plate	Model RCH IKA-Combimag		
Water bath	Model NB-34980 Colora Ultra-Thermostat		
Sonicator	Model 8845-30 ultrasonic bath		
Homogenizer	Model 6-105 AF Virtis		
Homoginizer	Model-HO valve type		

RHODES UNIVERSITY, Faculty of Pharmacy, Grahamstown, SOUTH AFRICA

BATCH PRODUCTION RECORD

Product name: Clobetasol 17-propionate cream **Page:** 4 of 6

Batch ID: CP001 **Batch size:** 200 g

	MANUFACTURING PROCEDURE			
Step	**Procedure**	**Time**	**Done by**	**Checked by**
1	Weigh all the materials			
2	Heat water (item 12) to 90˚C in a beaker. Bring down the temperature to 60˚C. Dissolve sodium citrate (item 3) and citric acid (item 4) to a clear solution. Mix the resultant solution with propylene glycol (item 2). Maintain the temperature of the resultant aqueous phase (step 4) at 60˚C.			
3	Melt Geleol® pastilles (item 5) and Estol® 1474 (item 9) together with cetostearyl alcohol (item 6), white beeswax BP (item 7) and chlorocresol (item 8) while stirring in a beaker previously heated to 75˚C using a hotplate. Cool the resultant oil phase to 60°C in a water bath and maintain the temperature at 60°C.			
4	Mix CP (item 1) in propylene glycol (item 10) and sonicate for approximately twenty-five (25) minutes or until a clear solution is obtained. Heat the resultant drug phase to 50˚C and maintained at 50˚C.			
5	Transfer the aqueous phase (step 2) to the oil phase (step 3) at 60˚C. Stir the mixture manually with a glass stirring rod for ten (10) minutes at 60˚C. Homogenize the mixture at 15,000 rpm for five (5) minutes at 60˚C.			
6	Place the beaker containing the mixture (step 5) in a water bath at 20˚C and cool down the temperature of the resultant dispersed phase to 50˚C while stirring manually			

RHODES UNIVERSITY, Faculty of Pharmacy, Grahamstown, SOUTH AFRICA

BATCH PRODUCTION RECORD

Product name: Clobetasol 17-propionate cream **Page:** 5 of 6

Batch ID: CP001 **Batch size:** 200 g

	MANUFACTURING PROCEDURE			
Step	**Procedure**	**Time**	**Done by**	**Checked by**
7	Add the drug phase (step 4) to the dispersed phase (step 7). Rinse the beaker previously containing the drug phase with propylene glycol (item 11) and add to the dispersed phase. Mix manually for ten (10) minutes at 50˚C. Homogenize the resultant cream at 15,000 rpm for five (5) minutes at 50˚C.			
8	Cool the resultant cream (step 7) to 30˚C with continual manual stirring and with the manufacturing beaker placed in a water bath at 20˚C.			
9	Pass the cooled formulation (step 8) through a valve type of homogenizer, to generate a smooth cream of improved consistency.			
10	Pack the resultant cream (step 9) into 100 g ointment jars and store the cream at room temperature (22˚C) until required for further analysis.			

BATCH PRODUCTION RECORD

Product name: Clobetasol 17-propionate cream **Page:** 6 of 6

Batch ID: CP001 **Batch size:** 200 g

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

APPENDIX SEVEN

STABILITY TEST SUMMARY SHEET

RHODES UNIVERSITY, Faculty of Pharmacy, Grahamstown, SOUTH AFRICA

STABILITY TEST SUMMARY SHEET

ACCELERATED STABILITY STUDIES	
Product name: Clobetasol 17-propionate cream	
Manufacturer: Kasongo Wa Kasongo	
Address: Rhodes University, Faculty of Pharmacy, Division of Pharmaceutics, Grahamstown, South Africa	
Active ingredient: Clobetasol 17-propionate	
Dosage form: Cream	
Batch ID	**Date of manufacture**
CP004	10-10-2006
Batch size	**Type of batch**
500 g	Experimental
Samples tested (per batch): Four (4) samples	
Test period: Four (4) weeks	
Storage/test conditions	
Temperature (˚C)	**Humidity (% RH)**
40.0	25.0
RESULTS	
Chemical findings: • The percentage change from the initial amount of CP in the cream formulation after storage at 40 ± 2˚C and 25 ± 5% RH for 1, 2, 3, and 4 weeks was not statistically significant or relevant. • The percentage change from the initial apparent pH of the cream formulation after storage at 40 ± 2˚C and 25 ± 5% RH for 1, 2, 3, and 4 weeks is not statistically significant or relevant	
Physical findings	
• No noticeable change in the organoleptic properties of the cream formulation in terms of appearance, colour and odour over the entire stability test period. No signs of physical instability such as phase separation or cracking and bleeding of the cream base from the container at any of the sampling times. • The percentage change from the initial apparent intrinsic viscosity of the CP cream formulation after storage at 40 ± 2˚C and 25 ± 5% RH for 1, 2, 3, and 4 weeks is not statistically significant or relevant. • The percentage change from the initial CP *in vitro* release rate of CP release from the cream formulation after storage at 40 ± 2˚C and 25 ± 5% RH for weeks 1, 2, 3, and 4 was not statistically significant or relevant	
Conclusions: The CP cream formulation (Batch CP004) was stable for the 1 month test period under investigation.	
Responsible Officer:	**Date:**

REFERENCES

1. M.L. Gordon. The role of clobetasol propionate emollient 0.05% in the treatment of patients with dry, scaly, corticosteroid-responsive dermatoses. *Clinical Therapeutics* 1998; **20**(1):26-38.

2. J.D. Rosso, S.F. Friendlander. Corticosteroids: Options in the era of steroi-sparing therapy. *Journal of American Academy of Dermatology* 2005; **53**(1):S50-S58.

3. J.Y. Fang, K.L. Shen, P.C.W. Huang, Y.H. Tsai. Evaluation of topical application of clobetasol 17-propionate from various cream bases. *Drug Development and Industrial Pharmacy* 1999; **25**(1):7-14.

4. X. Huang, H. Tanojo, J. Lenn, H. Deng, L. Krochmal. A novel foam vehicle for delivery of topical corticosteroids. *Journal of American Academy of Dermatology* 2005; **53**(1):S27-S38.

5. C. Purdon, J.M. Haigh, C. Surber, E.W. Smith. Foam drug delivery in dermatology. *American Journal of Drug Delivery* 2003; **1**(1):71-75.

6. A.W. McKenzie, R.B. Stoughton. Method for comparing percutaneous absorption of steroids. *Archives of Dermatology* 1962; **86**:608-610.

7. J.M. Haigh, I. Kanfer. Assessment of topical corticosteroid preparations: the human skin blanching assay. *International Journal of Pharmaceutics* 1984; **19**(3):245-262.

8. E. Meyer, A.D. Magnus, J.M. Haigh, I. Kanfer. Comparison of the blanching activities of Dermovate, Betnovate and Eumovate creams and ointments. *International Journal of Pharmaceutics* 1988; **41**:63-66.

9. B. Brazzini, N. Pimpinelli. New and established topical corticosteroids in dermatology; clinical pharmacology and therapeutic use. *American Journal of Clinical Dermatology* 2002; **3**(1):47-58.

10. S. Dyderski, E. Grzeskowiak, A. Mrzyglod. Pharmaceutical availability of clobetasol 17-propionate from cream and ointment. *Acta Poloniae Pharmaceutica-Drug Research* 2001; **58**(6):435-438.

11. M. Haramura, A. Tanaka, T. Kimoto, N. Hirayama. Crystal structure of clobetasol propionate. *Analytical Sciences* 2003; **19**:x37-x38.

12. J.Y. Fang, C.L. Fang, K.C. Sung, H.Y. Chen. Effect of low frequency ultrasound on the *in vitro* percutaneous absorption of clobetasol 17-propionate. *International Journal of Pharmaceutics* 1999; **191**(33):42.

13. J.C. Tsai, C.L. Cheng, Y.F. Tsai, H.M. Sheu, C.H. Chou. Evaluation of *in vivo* bioequivalence methodology for topical clobetasol 17-propionate based on

pharmacodynamic modeling using Chinese skin. *Journal of Pharmaceutical Sciences* 2004; **93**(1):207-217.

14. Drug information fulltext [database online]. McEvoy GK, ed. Clobetasol propionate. American Society of Health-System Pharmacists, Bethesda, 2006. Updated Dec 2003. http://library.dialog.com/bluesheets/pdf/bl0229.pdf. Retrieved 24/06/2005.

15. Temovate (clobetasol propionate) cream and ointment 0.05% prescribing information, GlaxoSmithKline Consumer Healthcare LP. Updated 2002. http://us.gsk.com/products/assets/us_temovate.pdf. Retrieved 06/10/2006.

16. Temovate (clobetasol propionate) gel 0.05% prescribing information. GlaxoSmithKline Consumer HealthcareLP. Updated2002 http://us.gsk.com/products/assets/us_temovate_gel.pdf. Retrieved 8/02/2006.

17. Olux (Clobetasol propionate) foam 0.05% patient information. CCL Pharmaceuticals, Updated 2000. http://www.fda.gov/cder/foi/label/2000/21142lbl.pdf. Retrieved 02/08/2006.

18. S.C. Sweetman (editor): *Martindale, "The Complete Drug Reference"*. 33rd Edition, The Pharmaceutical Press; London, 2002, pp. 1039-1065.

19. J.E.F. Reynolds (editor): *Martindale, "The Extra Pharmacopoeia"*.30th Edition, The Pharmaceutical Press, London, 1993, pp. 712-740, 792, 851, 1028, 1108, 1355, 1406.

20. C. Dollery: Clobetasol propionate. In: C. Dollery (editor): *Therapeutic Drugs*. Churchill Livingstone; Edinburgh, 1991, pp. C272-C274.

21. A.C. Moffat, M.D. Osselton, B.Widdop (editors): *Clarkes's Analysis of Drugs and Poisons: in pharmaceuticals, body fluids and post-mortem material.* 3rd Edition, Pharmaceutical Press; London, 2003, pp. 821.

22. *The British Pharmacopoeia incorporating the requirements of the 5th of the European Pharmacopoeia 2004 as amended by Supplements 5.1 and 5.2*. The Stationery Office; London, 2005, pp. 506-507, 2749-2757, A167-A168, A272-A277.

23. *The United States Pharmacopoeia incorporating "The National Formulary"*.The United States Pharmacopoeial Convention Inc.; Rockville, 2006, pp. 546-548, 2644-2646, 2682-2693, 3050-3053.

24. Data from SRC physprop database, CAS Number: 25122-46-7, Syracuse Research Corporation, 2004, http://www.syrres.com/esc/physdemo.htm. Retrieved 13/02/206.

25. S.F. Donovan, M.C. Pescatore. Method for measuring the logarithm of the octanol-water partition coefficient by using short octadecyl-poly(vinyl alcohol) high-performance liquid chromatography columns. *Journal of Chromatography A* 2002; **952**(1-2):47-61.

26. J.J. Ellington, T.L. Floyd. Measuring octanol/water partition coefficients by the "slow-stirring" method. Environmental Research Brief, EPA/600/S-96/005, United States Environmental Protection Agency, Research and Development, Athens, 1995, pp. 1-4.

27. S.K. Poole, C.F. Poole. Separation methods for estimating octanol-water partition coefficients. *Journal of Chromatography B* 2003; **797**(1-2):3-19.

28. J.T. Andersson, W. Schrader. A method for measuring 1-octanol-water partition coefficients. *Analytical Chemistry* 1999; **71**(16):3610-3614.

29. M.S. Roberts, W.J. Pugh, J. Hadgraft. Epidermal permeability: Penetrant structure relationships. 2. The effect of h-bonding groups in penetrants on their diffusion through the stratum corneum. *International Journal of Pharmaceutics* 1996; **132**(1-2):23-32.

30. R.M. Silverstein, G.C. Bassler, T.C .Morrill. Infrared spectrometry. In: R.M. Silverstein, G.C. Bassler, T.C. Morrill (editors): *Spectrometric Identification of Organic Compounds*. John Wiley & Sons, Inc.; New York, 1981, pp. 95-180.

31. E. Meyer. A critical evaluation of the human skin blanching assay and comparative bioavailability studies on topical corticosteroids preparations. Rhodes University, Grahamstown, 1998, pp. 1-282.

32. B.B. Michniak. Advances in topical corticosteroid therapy. *International Journal of Cosmetic Sciences* 1983; **5**:277-291.

33. E.S. Snell. Mechanisms of glucocorticoid activity-a review. In: L. Wilson, R. Marks (editors): *Mechanisms of topical corticosteroid activity-a Glaxo symposium*.: Churchill Livingstone, Edinburgh, 1976, pp. 136-145.

34. D.A. Norris. Mechanisms of action of topical therapies and the rationale for combination therapy. *Journal of the American Academy of Dermatology* 2005; **53**(1, Supplement 1):S17-S25.

35. E.S. Snell. The pharmacological properties of corticosteroids in relation to clinical efficacy. In: D.D. Munro, L. Wilson (editors): *Steroid and the skin*. Blackwell Scientific Publications and Glaxo Laboratories Ltd; London, 1976, pp. 15-23.

36. A. Prakash, P. Benfield. Topical mometasone, a review of its pharmacological properties and therapeutic use in the treatment of dermatlogical disorders. *Drugs* 1998; **55**(1):145-163.

37. R.I. Scheinman, P.C. Cogswell, A.K. Lofquist, A.S.jr. Baldwin. Role of transcriptional activation of IκBα in mediation of immunosuppression by glucocorticoids. *Science* 1995; **270**(5234):283-286.

38. N. Auphan, J.A. DiDonato, C. Rosette, A. Helmberg, M. Karin. Immunosuppression by glucocorticoids: Inhibition of NF-κB activity through induction of IκB synthesis. *Science* 1995; **270**(5234):286-290.

39. S.R. Feldman. Relative efficacy and interchangeability of various clobetasol propionate vehicles in the management of steroid-responsive dermatoses. *Current Therapeutic Research* 2005; **66**(3):154-171.

40. F.M. Keane, S.E. Munn, N.F. Taylor, A.W.P.D.U. Vivier. Unregulated use of clobetasol propionate. *British Association of Dermatologists, British Journal of Dermatology* 2001; **144**:1095-1096.

41. A. Tosti, B.M. Piraccini, M. Pazzaglia, C. Vincenzi. Clobetasol propionate 0.05% under occlusion in the treatment of alopecia totalis/universalis. *Journal of the American Academy of Dermatology* 2003; **49**(1):96-98.

42. E.W. Smith, H.I. Maibach. Percutaneous penetration enhancers; the fundamentals. In: E.W. Smith, H.I. Maibach (editors): *Percutaneous Penetration Enhancers*. CRC Press, Inc., New York 1995, pp. 1-4.

43. B.T. Gjertsen, J. Korsvold. Clobetasol propionate in psoriasis: monotherapy and in combinations. *Journal of the European Academy of Dermatology and Venereology* 1997; **9**(Supplement 1):S173-S174.

44. S.C. Chattaraj, R.B. Walker. Penetration enhancer classification. In: E.W. Smith (editor): *Percutaneous Penetration Enhancers*. H.I. Maibach: CRC Press, Inc.; New York, 1995, pp. 5-20.

45. R.B. Walker, E.W. Smith. The role of percutaneous penetration enhancers. *Advanced Drug Delivery Reviews* 1996; **18**(3):295-301.

46. S. Yateen, K.S. Dighe. High performance liquid chromatographic method for clobetasol propionate in dosage forms. *Indian Drugs* 1989; **27**(2):132-133.

47. J.C. Reepmeyer, L.K. Revelle, I. Vidavsky. Detection of clobetasol propionate as an undeclared steroid in zinc pyritione formulations by high-performance liquid chromatography with rapid-scanning ultraviolet spectroscopy and mass spectrometry. *Journal of Chromatography A* 1998; **828**:239-246.

48. L. Gagliardi, D. De Orsi, D. Tonelli. HPLC determination of clobetasol propionate in cosmetic products. *Journal of Liquid Chromatography & Related Technologies* 2000; **23**(3):355-362.

49. Guidance for Industry: SUPAC-SS Non-sterile Semi-solid Dosage Forms. Scale-Up and Post-approval Changes: Chemistry, Manufacturing, and Controls; In Vitro Release Testing and In Vivo Bioequivalence Documentation. SUPAC-SS CMC 7, U.S. Department of Health and Human Services, Food and Drug Administration, Centre for Drug Evaluation and Research, Rockville, 1997, 1-36.

50. E.W. Smith, J.M. Haigh, I. Kanfer. A stability-indicating HPLC assay with on-line clean-up for betamathanose 17-valerate in topical dosage forms. *International Journal of Pharmaceutics* 1985; **27**:185-192.

51. C.V. Garcia, A.R. Breier, B.M. Steppe, E.E.S. Schapoval, T.P. Oppe. Determination of dexamethasone acetate in cream by HPLC. *Journal of Pharmaceutical and Biomedical Analysis* 2003; **31**(597):600.

52. R.J. Hamilton, P.A. Sewell. Introduction to high performance liquid chromatography. In: R.J. Hamilton, P.A. Sewell (editors): *Introduction to high performance liquid chromatography*. Chapman and Hall; New York, 1981, pp. 1-12.

53. R. Raghavan, J.C. Joseph. Chromatographic methods of analysis: high-performance liquid chromatography. In: J. Swarbrick, J.C. Boylan (editors): *Encyclopedia of Pharmaceutical Technology*. Marcel Dekker, Inc.; New York, 2002, pp. 414-425.

54. L.R. Snyder, J.J. Kirkland. Liquid-liquid chromatography. In: L.R. Snyder, J.J. Kirkland (editors): *Introduction to modern liquid chromatography*. John Wiley & Sons, Inc.; New York: 1979, pp. 323-348.

55. L.R. Snyder, J.J. Kirkland. Basic concepts and control of separation. In: L.R. Snyder, J.J. Kirkland (editors): *Introduction to modern liquid chromatography*. Jonh Wiley & Sons, Inc.; New York,1979, pp. 15-82.

56. L.R. Snyder, J.J. Kirkland. Bonded-phase chromatography. In: L.R. Snyder, J.J. Kirkland (editors): *Introduction to modern liquid chromatography*. John Wiley & Sons, Inc.; New York, 1979, pp. 269-322.

57. G.M. Pollack. Chromatographic Analysis. In: J. Swarbrick (editor): *Encyclopedia of pharmaceutical technology*. Marcel Dekker; New York,1987, pp. 419-445.

58. R.E. Majors. Practical operation of bonded-phase columns in high-performance liquid chromatography. In: C. Horvath (editor): *High-performance liquid chromatography: Advances and perspectives*. Academic Press; New York, 1980, pp. 75-111.

59. L.R. Snyder, J.J. Kirkland, J.L. Glajch. Getting started. In: L.R. Snyder, J.J. Kirkland, J.L. Glajch (editors): *Practical HPLC method development*. 2nd Edition. John Wiley & Sons, Inc; New York, 1997, p. 10.

60. M.M. Rahman, M. Takafuji, H. Ihara. Retention mechanism of L-glutamide-derived noncrystalline stationary phases in reversed-phase high-performance liquid chromatography and application for separation of nucleic acid constituents. *Journal of Chromatography A* 2006; **1119**(1-2):105-114.

61. T.H Dzido, T.E. Kossowski, D. Matosiuk. Comparison of retention of aromatic hydrocarbons with polar groups in binary reversed-phase high-performance liquid chromatography systems. *Journal of Chromatography A* 2002; **947**(2):167-183.

62. B. Mueller, Y. G.Anissimov, M.S. Roberts. Unexpected clobetasol propionate profile in human stratum corneum after topical application *in vitro*. *Pharmaceutical Research* 2003; **20**:1835-1837.

63. L.R. Snyder, J.J. Kirkland, J.L. Glajch. Detection sensitivity and selectivity. In: L.R. Snyder, J.J. Kirkland, J.L. Glajch (editors): *Practical HPLC method development*. 2nd Edition. John Wiley and Sons, Inc.; New York: 1997, pp. 59-99.

64. L. Gagliardi, D. De Orsi, F. Manna, D. Tonelli. HPLC determination of clobetasol propionate in cosmetic products. *Journal of Liquid Chromatography & Related Technologies* 2000; **23**(3):355-362.

65. L.R. Snyder, J.J. Kirkland, J.L. Glajch. The column. In: L.R. Snyder, J.J. Kirkland, J.L. Glajch (editors): *Practical HPLC method development*. 2nd Edition. John Wiley and Sons, Inc.; New York, 1997, pp. 174-230.

66. L.R.Snyder, J.J.Kirkland. The column. In: L.R.Snyder, J.J.Kirkland (editors): *Introduction to modern liquid chromatography*. New York: John Wiley & Sons, Inc.; 1979, pp. 168-245.

67. L.R. Snyder, J.J. Kirkland, J.L. Glajch. Non-ionic sample: reversed and normal-phase HPLC. In: L.R. Snyder, J.J. Kirkland, J.L. Glajch (editors): *Practical HPLC method development*. 2nd Edition. John Wiley and Sons, Inc.; New York, 1997, pp. 223-291.

68. N. Tanaka, K. Kimata, K. Hosoya, H. Miyanishi, T. Araki. Stationary phase effects in reversed-phase liquid chromatography. *Journal of Chromatography A* 1993; **656**(1-2):265-287.

69. P. Zhuang, R.A. Thompson, T.P. O'Brien. A retention model for polar selectivity in reversed phase chromatography as a function of mobile phase organic modifier type. *Journal of Liquid Chromatography & Related Technologies* 2005; **28**:1357-1365.

70. J.K. Fang, K.L. Shen, Y.B. Huang, P.C. Wu, Y.H. Tsai. Evaluation of Topical Application of Clobetasol 17-Propionate from Various Cream Bases. *Drug Development and Industrial Pharmacy* 1999; **25**(1):7-14.

71. S. Kromidas. Optimization via column parameters: what works best? In: S. Kromidas (editor): *More practical problem solving in HPLC*. Wiley-VCH Verlag GmbH & Co. KGaA; Weinheim, 2005, pp. 14-16.

72. L.R. Snyder, J.J. Kirkland, J.L. Glajch. Basics of separation. In: L.R. Snyder, J.J. Kirkland, J.L. Glajch (editors): *Practical HPLC method development*. 2nd Edition. John Wiley and Sons, Inc.; New York, 1997, pp. 20-58.

73. C.M. Riley. Statistical parameters. In: C.M. Riley, T.W. Rosanske (editors): *Development and validation of analytical methods*. Elsevier Science Inc.; New York, 1996, pp. 44-45.

74. K. Hammarstrand. Internal standard in gas chromatography. *Varian Instrument Applications* 1976; **10**:10-11.

75. J. Lindholm, M. Johansson, T. Fornstedt. Guidelines for analytical method development and validation of biotechnological synthesis of drugs: Production of a hydroxyprogesterone as model. *Journal of Chromatography B* 2003; **791**(1-2):323-336.

76. L.R. Snyder, J.J. Kirkland, J.L. Glajch. Solvents. In: L.R. Snyder, J.J. Kirkland, J.L. Glajch (editors): *Introduction to modern liquid chromatography*. 2nd Edition John Wiley and Sons, Inc.; New York: 1979, pp. 246-268.

77. R.J. Hamilton, P.A. Sewell. Mobile phases in liquid chromatography. In: R.J. Hamilton, P.A. Sewell (editors): *Introductio to high performance liquid chromatography*. Chapman and Hall; New York, 1982, pp. 127-144.

78. Y. Sun, A.M. Stalcup. Mobile phase effects on retention on a new butylimidazolium-based high-performance liquid chromatographic stationary phase. *Journal of Chromatography A* 2006; **1126**(1-2):276-282.

79. S. Kromidas. Methanol versus acetonitrile. In: S. Kromidas (editor): *More practical solving in HPLC*. Wiley-VCH Verlag GmbH & Co. KGaA; Weinheim: 2005, pp. 180-182.

80. L.C. Tan, P.W. Carr. Study of retention in reversed-phase liquid chromatography using linear solvation energy relationships: II: the mobile phase. *Journal of Chromatography A* 1998; **799**(1-2):1-19.

81. G.A. Shabir. Validation of high-performance liquid chromatography methods for pharmaceutical analysis: Understanding the differences and similarities between validation requirements of the US Food and Drug Administration, the US Pharmacopeia and the International Conference on Harmonization. *Journal of Chromatography A* 2003; **987**(1-2):57-66.

82. Reviewer Guidance: Validation of chromatographic methods. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research and Center for Veterinary Medicine. Rockville, 1994, pp. 1-30.

83. H. Rosing, W.Y. Man., E. Doyle, A. Bult, J.H. Beijnen. Bioanalytical liquid chromatographic method validation: a review of current practices and procedures. *Journal of Liquid Chromatography and Related Technologies* 2000; **23**(3):329-354.

84. Guidance for industry: Bioanalytical methods validation. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research and Center for Veterinary Medicine.Rockville, 2001, pp. 1-22.

85. ICH Harmonised Tripartite Guidelines: validation of analytical methods: definitions and terminology. ICH Topic Q 2 A. International Conference on Harmonization, London, 1995, pp. 1-5.

86. ICH Harmonised Tripartite Guidelines: validation of analytical procedures: Methodology. ICH Topic Q 2 B. International Conference on Harmonization, London, 1996, pp. 1-9. 1996.

87. E. McGonogle. Assay validation and inter-laboratory transfer. In: C.M. Riley, T.W. Rosanske (editors): *Development and validation of analytical methods*. Elsevier Science Inc.; New York, 1996, pp. 3-13.

88. G.C. Hokanson. A life cycle approach to the development of analytical methods during pharmaceutical product development Part I: The initial method validation process. *Pharmaceutical Technology* 1994; **18**(9):118-130.

89. G.S. Clarke. The validation of analytical methods for drug substances and drug products in UK pharmaceutical laboratories. *Journal of Pharmaceutical and Biomedical Analysis* 1994; **12**(5):643-652.

90. G.P. Carr, J.C. Wahlich. A practical approach to method validation in pharmaceutical analysis. *Journal of Pharmaceutical and Biomedical Analysis* 1990; **8**(8-12):613-618.

91. T.C. Paino, A.D. Moore. Determination of the LOD and LOQ of an HPLC method using four different techniques. *Pharmaceutical Technology* 1999; **23**(10):82-90.

92. M.E. Swartz, I.S. Krull. Validation of chromatographic methods. *Pharmaceutical Technology* 1998; **22**(3):104-118.

93. D. Dadgar, P.E. Burnett. Issues in evaluation of bioanalytical method selectivity and drug stability. *Journal of Pharmaceutical and Biomedical Analysis* 1995; **14**(1-2):23-31.

94. A.R. Buick, M.V. Doig, S.C. Jeal, G.S. Land, R.D. McDowall. Method validation in the bioanalytical laboratory. *Journal of Pharmaceutical and Biomedical Analysis* 1990; **8**(8-12):629-637.

95. D. Dadgar, P.E. Burnett, M.G. Choc, K. Gallicano, J.W. Hooper. Application issues in bioanalytical method validation, sample analysis and data reporting. *Journal of Pharmaceutical and Biomedical Analysis* 1995; **13**(2):89-97.

96. S.R.J. Braggio, R.J. Barnaby, P. Grossi, M. Cugola. A strategy for validation of bioanalytical methods. *Journal of Pharmaceutical and Biomedical Analysis* 1996; **14**(4):375-388.

97. U. Timm, M. Wall, D. Dell. A new approach for dealing with the stability of drugs in biological fluids. *Journal of Pharmaceutical Sciences* 1985; **74**(9):972-977.

98. Guidance for industry: Analytical procedures and methods validations; Chemistry, manufacturing and controls documentation. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research and Center for Biologics Evaluation and Research. Rockville, 2000, pp. 1-33.

99. I. Taverniers, M. De Loose, E. Van Bockstaele. Trends in quality in the analytical laboratory. II. Analytical method validation and quality assurance. *Trends in Analytical Chemistry* 2004; **23**(8):535-552.

100. P.M. Sathe, A.S. Raw, L.A. Ouderkirk, L.X. Yu, A.S. Husain. Drug product performance, *in vitro*. In: L. Shargel, I. Kanfer (editors): *Generic drug product development: Solid oral dosage forms*. Marcel Dekker, Inc.; New York, 2005, pp. 187-209.

101. W.A. Hanson. Overall considerations. In: W.A. Hanson (editor): *Handbook of dissolution testing*. Pharmaceutical Technology Publications; Oregon, 1982, pp. 1-11.

102. V.P Shah, J. Elkins, J. Hanus, C. Noorizadeh, J.P. Skelly. *In vitro* release of hydrocortisone from topical preparations and automated procedure. *International Journal of Pharmaceutics* 1989; **53**(1):53-59.

103. S.C. Chattaraj, I. Kanfer. 'The “insertion cell”: a novel approach to monitor drug release from semi-solid dosage forms. *International Journal of Pharmaceutics* 1996; **133**(1):59-63.

104. J. Klimundova, H. Sklenarova, U.F. Schaefer, P. Solich. Automated system for release studies of salicylic acid based on a SIA method. *Journal of Pharmaceutical and BIomedical Analysis* 2005; **37**(5):893-898.

105. J. Klimundova, D. Satinsky, H. Sklenarova, P. Solich. Automation of simultaneous release tests of two substances by sequential injection chromatography coupled with a Franz cell. *Talanta* 2006; **69**(3):730-735.

106. P.R. Rege, V.D. Vilivalam, C.C. Collins. Development in release testing of topical dosage forms: use of the Enhancer Cell^{TM} with automated sampling. *Journal of Pharmaceutical and BIomedical Analysis* 1998; **17**(8):1225-1233.

107. W. Liebenberg, E. Engelbrecht, A. Wessels, B. Evarakonda, W. Yang, M.M. De Villie. A comparative study of the release of active ingredients from semisolid cosmeceuticals measured with Franz, Enhancer or flow-through cell diffusion apparatus. *Journal of Food and Drug Analysis* 2004; **12**(1):19-28.

108. K.D. Thakker, W.H. Chern. Development and validation of *in vitro* release tests for semisolid dosage forms-case study. *Dissolution Technologies* 2003; **10**(5):10-16.

109. V.P. Shah, C.R. Behl, G. Flynn, W.I. Higuchi, H. Schaefer. Principles and criteria in the development and optimization of topical therapeutic products. *International Journal of Pharmaceutics* 1992; **82**(1):21-28.

110. S.C. Chattaraj, J. Swarbrick, I. Kanfer. A simple diffusion cell to monitor drug release from semi-solid dosage forms. *International Journal of Pharmaceutics* 1995; **120**(1):119-124.

111. M. Siewett, J. Dressman, C.K. Brown, V.P. Shah. FIP/AAPS Guidelines to dissolution/*in vitro* release testing of novel/special dosage forms. *American Association of Pharmaceutical Scientists PharmSciTech* 2003; **4**(1):1-10.

112. G.L. Flynn, V.P. Shah, S.N. Tenjarla, M. Corbo, D. DeMagistris, T.G. Feldman, *et al,*. Assessment of value and applications of *in vitro* testing of topical dermatological drug products. *Pharmaceutical Research* 1999; **16**(9):1325-1329.

113. J.L. Zatz, J.D. Segers. Techniques for measuring in vitro release from semisolids. *Dissolution Technologies* 1998; **5**(2):3-13.

114. R. Pillai, V.P. Shah, C.F. Albrecht, S.C. Chattaraj, G. Flynn. Release of hydrocortisone from a cream matrix: dependency of release on suspension concentration and measurement of solubility and diffusity. *Pharmaceutical Development and Technology* 2001; **6**(3):373-384.

115. M. Corbo, T.W. Shultz, G.K. Wong, G.A. Van Buskirk. Development and validation of in vitro release testing methods for semisolid formulations. *Pharmaceutical Technology* 1993; **17**(9):112-128.

116. P.P. Sanghvi, C.C. Collins. Comparison of diffusion studies of hydrocortisone between the Franz cell and the Enhancer cell. *Drug Development and Industrial Pharmacy* 1993; **19**(13):1573-1585.

117. V.P Shah, J. Elkins, S.Y. Lam, J.P. Skelly. Determination of in vitro drug release from hydrocortisone creams. *International Journal of Pharmaceutics* 1989; **53**(1):53-59.

118. M.D. Vlachou, D.M. Rekkas, P.P. Dallas, N.H.Choulis. Development and *in vitro* evaluation of griseofulvin gels using Franz diffusion cells. *International Journal of Pharmaceutics* 1992; **82**(1-2):47-52.

119. L.J. Zatz. Drug release from semisolids: Effects of membrane permeability on sensitivity to product parameters. *Pharmaceutical Research* 1995; **12**(5):787-789.

120. T.X. Viegas, L.L.V. Winkle, P.A. Lehman, S.F. Franz, T.J. Franz. Evaluation of creams and ointments as suitable formulations for peldesine. *International Journal of Pharmaceutics* 2001; **219**(1-2):73-80.

121. V.P. Shah, J. Elkins, R.L. Williams. Evaluation of the test system used for *in vitro* release of drugs for topical dermatological drug products. *Pharmaceutical Development and Technology* 1999; **4**(3):377-385.

122. J.P. Skelly, V.P. Shah, H.I. Maibach, R.H. Guy, R.C. Wester, G. Flynn, *et al*., FDA and AAPS report of the workshop on principles and practices of in vitro percutaneous penetration studies: relevance to bioavailability and bioequivalence. *Pharmaceutical Research* 1987; **4**(3):265-267.

123. I. Csoka, E. Csanyi, G. Zapantis, E. Nagy, A. Feher-Kiss, G. Horvath, *et al*., *In vitro* and *in vivo* percutaneous absorption of topical dosage forms: case studies. *International Journal of Pharmaceutics* 2005; **291**(1-2):11-19.

124. E.W. Smith, J.M. Haigh. *In vitro* diffusion cell design and validation II. Temperature, agitation and membrane effects on betamethasone 17-valerate permeation. *Acta Pharmareutica Nordica* 1992; **4**(3):171-178.

125. V.P. Shah, J. Elkins, S. Shaw, R. Hanson. *In vitro* release: comparative evaluation of vertical diffusion cell system and automated procedure. *Pharmaceutical Development and Technology* 2003; **8**(1):97-102.

126. M.H. Fares, L.J Zatz. Measurement of drug release from topical gels using two types of apparatus. *Pharmaceutical Technology* 1995; **19**(1):53-58.

127. P.R. Keshary, Y.W. Chien. Mechanisms of transdermal controlled nitroglycerin administration (I): development of a finite-dosing skin permeation system. *Drug Development and Industrial Pharmacy* 1984; **10**(6):883-913.

128. P. Gupta, S. Garg. Recent advances in semisolid dosage forms for dermatological application. *Pharmaceutical Technology* 2002; **26**(3):144-162.

129. S.C. Pefile, E.W. Smith, C.F. Albrecht, P.B. Kruger. Release of rooperol tetra-acetate from topical bases: in vitro studies using silicone membrane. *International Journal of Pharmaceutics* 1998; **161**(2):237-243.

130. E.J. Ricci, L.O. Lunardi, D.M.A. Nanclares, J.M. Marchetti. Sustained release of lidocaine from Poloxamer 407 gels. *International Journal of Pharmaceutics* 2005; **288**(2):235-244.

131. B.W. Barry. Methods for Studying Percutaneous Absorption. In: B.W. Barry (editor): *Dermatological Formulations-Percutaneous Absorption*. Marcel Dekker, Inc.; New York: 1983, pp. 234-295.

132. T.J. Franz. On the relevance of *in vitro* data. *Journal of Investigative Dermatology* 1975; **64**:190-195.

133. T.J. Franz. The finite dose technique as a valid *in vivo* model for the study of percutaneous absorption. *Current Problems in Dermatology* 1978; **7**:58-68.

134. M. Dias, A. Farinha, E. Faustino, J. Hadgraft, J. Pais, C. Toscano. Topical delivery of caffeine from some commercial formulations. *International Journal of Pharmaceutics* 1999; **182**(1):41-47.

135. L. Montenegro, A.M. Panico, A. Ventimiglia, F.P. Bonina. *In vitro* retinoic acid release and skin permeation from different liposome formulations. *International Journal of Pharmaceutics* 1996; **133**(1):89-96.

136. L.G. Flynn, P.R. Keshary, Y.C. Huang. Comparative controlled skin permeation of nitroglycerin from markted transdermal delivery systems. *Journal of Pharmaceutical Sciences* 1983; **72**(8):968-970.

137. T. Higuchi. Mechanism of sustained-action medication. Theoretical analysis of rate of release of solid drugs dispersed in solid matrices. *Journal of Pharmaceutical Sciences* 1963; **52**(12):1145-1149.

138. W.I. Higuchi. Analysis of data on the medicament release from ointments. *Journal of Pharmaceutical Sciences* 1962; **51**(8):802-804.

139. S.C. Owen. Alcohol. In: R.C. Rowe, P.J. Sheskey, P.J. Weller (editors): *Handbook of Pharmaceutical Excipients*. American Pharmaceutical Association; Washington, DC, 2003, pp. 13-15.

140. P.J. Weller. Propylene glycol. In: R.C. Rowe, P.J. Sheskey, P.J. Weller (editors): *Handbook of Pharmaceutical Excipients*. American Pharmaceutical Association, Washington, DC, 2003, pp. 521-523.

141. A. Rolland, G. Demichelis, J. Jamoulle, B. Shroot. Influence of formulation, receptor fluid and occlusion on *in vitro* release from topical dosage forms using an automated flow-through diffusion cell. *Pharmaceutical Research* 1992; **9**(1):82-86.

142. V.P. Shah, J.S. Elkins. *In vitro* release from corticosteroid ointments. *Journal of Pharmaceutical Sciences* 1995; **84**(9):1139-1140.

143. J.M. Haigh, E.W. Smith. The selection and use of natural and synthetic membranes for *in vitro* diffusion experiments. *European Journal of Pharmaceutical Sciences* 1994; **2**(5-6):311-330.

144. T. Higuchi. Rate of release of medicaments from ointment bases containing drugs in suspension. *Journal of Pharmaceutical Sciences* 1961; **50**(10):874-875.

145. K. Welin-Berger, J.A.M. Neelissen, B. Bergenstahl. The effect of rheological behaviour of a topical anaesthetic formulation on the release and permeation rates of the active compound. *European Journal of Pharmaceutical Sciences* 2001; **13**(3):309-318.

146. B.W. Barry. Basic principles of diffusion through membranes. In: B.W. Barry (editor): *Dermatological Formulations, Percutaneous Absorption*. Mercel Dekker, Inc.; New York, 1983, pp. 49-94.

147. A. Karim, G. Pillai, T.T. Ziqubu-page, M.H. Cassimjee, M.S. Morar. Potential savings from generic prescribing and generic substitution in South Africa. *Health Policy and Planning* 1996; **11**(2):198-205.

148. D. Boyce, G. Bartlett. The maximum medical aid price programme: a review of the concept and its ability to reduce expenditure on medicines. *South African Medical Journal* 1990; **78**(4):147-151.

149. D.R. Scott, W.D. Reekie. Savings from generic drug substitution in the RSA- is its cost justified? *South African Medical Journal* 1987; **71**(3):314-316.

150. G. Hukins, D. Boyce. Containing medicines costs in the private sector-the example of a medical benefit scheme. *South African Medical Journal* 1990; **78**(5):152-157.

151. L. Shargel, I. Kanfer. Introduction to generic drug product development. In: L. Shargel, I. Kanfer (editors): *Generic Drug Product Development: Solid Oral Dosage Forms*. Marcel Dekker; New York, 2005, pp. 1-16.

152. 9 countries from the WHO African Region meet to analyse data from their medicines prices surveys 2006, pp. 1-2. http://www.who.int/medicines/areas/access/AFROmedicinesPricesShortArticle.pdf. Retrieved 01/11/2006

153. M.N.G. Dukes. Accountability of the pharmaceutical industry. *The Lancet* 2002; **360**(11):1682-1684.

154. J.K. Wang'ombe, G.M. Mwabu. Economics of essential drugs schemes: the perspectives of the developing countries. *Social Science and Medicine* 1987; **25**(6):625-630.

155. G.G. Djolov. Savings from generic drug substitution in South Africa: an arithmetical view. *South African Medical Journal* 2003; **93**(8):583-584.

156. Medicines and Related Susbstances Act 101 of 1965 (Act 101 of 1965) as amended. *PSSA Pharmacy Law Compendium*. LexisNexix Butterworths; Johannesburg, 2003, pp. MAE-30.

157. Office of generic drugs. The United States Food and Drug and Administration's Office of Generic Drug. 2006 http://www.fda.gov/cder/ogd/index.htm. Retrieved 01/11/2006.

158. The European Agency for the Evaluation of Medicinal Products (EMEA). http://www.emea.eu.int/. Retrieved 08/11/2006.

159. The Medical Control Council. http://www.mccza.com/main.asp. Accessed 08/11/2006.

160. D. Henry, J. Lexchin. The pharmaceutical industry as a medicines provider. *The Lancet* 2002; **360**(9345):1590-1595.

161. The pharmaceutical industry in figures. The European Federation of Pharmaceutical Industries and Associations (EFPIA). http://www.efpia.org/6_publ/infigures2005.pdf, 2005, pp 1-28. Retrieved 08/11/2006.

162. S.T. Hartshorne. Dermatological disorders in Johannesburg, South Africa. *Clinical and Experimental Dermatology* 2003; **28**(6):661-665.

163. Dermatologic disorders. In: M.H. Beers, R. Berkow (editors): *The Merck Manual of Diagnosis and Therapy*. 17th Edition, Whitehouse Station, Merck Research Laboratories, 1999, pp. 777-846.

164. A. Billich, H. Aschauer, A. Aszodi, A. Stuetz. Percutaneous absorption of drugs used in atopic eczema: pimecrolimus permeates less through skin than corticosteroids and tacrolimus. *International Journal of Pharmaceutics* 2004; **269**(1):29-35.

165. Pharmaceutical Price Regulation Scheme (PPRS): The study into the extent of competition in the supply of branded medicines to the NHS. The United Kingdom Department of Health [Component3]. London, 2006, pp. 121-143. http://www.dh.gov.uk/PublicationsAndStatistics/Publications/PublicationsPolicyAndGuidance/PublicationsPAmpGBrowsableDocument/fs/en?CONTENT_ID=4099371&MULTIPAGE_ID=5024085&chk=dwu6lz. Retrieved 08/11/2006.

166. Corticosteroids, dermatological preparations. In: C.J. Gibbon (editor): *South African Medicines Formulary*. Pinelands: South African Medical Association: Health and Medical Publishing Group; Cape Town, 2003, pp. 188-190.

167. L.V. Allen J, N.G. Popovich, H.C. Ansel. Semi-solid dosage forms and transdermal systems: ointments, creams, and gels. In: L.V. Allen Jr, N.G. Popovich, H.C. Ansel (editors): *Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems*. Lippincott Williams & Wilkins; Baltimore, 2005, pp. 276-297.

168. L. Buhse, R. Kolinski, B. Westenberger, *et a.,l*. Topical drug classification. *International Journal of Pharmaceutics* 2005; **295**(1-2):101-112.

169. N. Realdon, F. Perin, M. Morpurgo, E. Ragazzi. Influence of processing conditions in the manufacture of o/w creams: I. Effect on dispersion grade and rheological characteristics. *Il Farmaco* 2002; **57**(5):341-347.

170. U.T. Lashmar, J.P. Richardson, A. Erbod. Correlation of physical parameters of an oil in water emulsion with manufacturing procedures and stability. *International Journal of Pharmaceutics* 1995; **125**(2):315-325.

171. K.A. Walters, K.R. Brain. Dermatological formulation and transdermal systems. In: K.A. Walters (editor): *Dermatological and Transdermal Formulations*.: Mercel Dekker, Inc.; New York , 2002, pp. 319-399.

172. B.W. Barry. Formulation of dermatological vehicles. In: B.W. Barry (editor): *Dermatological Formulations: Percutaneous Absorption*. Mercel Dekker, Inc.; New York, 1983, pp. 296-350.

173. G.M. Eccleston. Functions of mixed emulsifiers and emulsifying waxes in dermatological lotions and creams. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* 1997; **123-124**:169-182.

174. M. Korhonen, J. Lehtonen, L. Hellen, J. Hirvonen, J. Yliruusi. Rheological properties of three component creams containing sorbitan monoesters as surfactants. *International Journal of Pharmaceutics* 2002; **247**(1-2):103-114.

175. H. Okochi, M. Nakano. Preparation and evaluation of w/o/w type emulsions containing vancomycin. *Advanced Drug Delivery Reviews* 2000; **45**(1):5-26.

176. T. Hino, Y. Kawashima, S. Shimabayashi. Basic study for stabilization of w/o/w emulsion and its application to transcatheter arterial embolization therapy. *Advanced Drug Delivery Reviews* 2000; **45**(1):27-45.

177. M. Nakano. Places of emulsions in drug delivery. *Advanced Drug Delivery Reviews* 2000; **45**(1):1-4.

178. S. Van der Graaf, C.G.P.H. Schroen, R.M. Boom. Preparation of double emulsions by membrane emulsification: a review. *Journal of Membrane Science* 2005; **251**(1-2):7-15.

179. S. Tamilvanan. Oil-in-water lipid emulsions: implications for parenteral and ocular delivering systems. *Progress in Lipid Research* 2004; **43**(6):489-533.

180. F.V. DiStefano, O.M. Shaffer, M.S. El-Aasser, J.W. Vanderhoff. Multiple oil-in-water-in-oil emulsions of extremely fine droplet size. *Journal of Colloid and Interface Science* 1983; **92**(1):269-272.

181. M. Korhonen. Rheological properties of pharmaceutical creams containing sorbitan fatty acid ester surfactant. University of Helsink Helsinki, 2004, 1-58. http://ethesis.helsinki.fi/julkaisut/mat/farma/vk/korhonen/. Retrieved 10/12/2006.

182. M. Billany. Suspensions and emulsions. In: M.E. Aulton (editor): *Pharmaceutics: The Science of Dosage Form Design*. Churchill Livingstone; New York: 2002, pp. 334-359.

183. G.L. Flynn. Topical Drug Absorption and Topical Pharmaceutical Systems. In: G.S. Banker, C.T. Rhodes (editors): *Modern Pharmaceutics*. Marcel Dekker, Inc.; New York, 1990, pp. 298-302.

184. T. Tadros. Application of rheology for assessment and prediction of the long-term physical stability of emulsions. *Advances in Colloid and Interface Science* 2004; **108-109**:227-258.

185. H.W. Yarranton, J.H. Masliyah. Numerical Simulation of Ostwald Ripening in Emulsions. *Journal of Colloid and Interface Science* 1997; **196**(2):157-169.

186. P. Taylor. Ostwald ripening in emulsions. *Advances in Colloid and Interface Science* 1998; **75**(2):107-163.

187. H. Masmoudi, Y. Le Dreau, P. Piccerelle, J. Kister. The evaluation of cosmetic and pharmaceutical emulsions aging process using classical techniques and a new method: FTIR. *International Journal of Pharmaceutics* 2005; **289**(1-2):117-131.

188. M.M. Robins, A.D. Watson, P.J. Wilde. Emulsions: creaming and rheology. *Current Opinion in Colloid & Interface Science* 2002; **7**(5-6):419-425.

189. M.M. Robins. Emulsions: creaming phenomena. *Current Opinion in Colloid & Interface Science* 2000; **5**(5-6):265-272.

190. P. Taylor. Ostwald ripening in emulsions. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* 1995; **99**(2-3):175-185.

191. G.A. Buskirk, V.P. Shah, D. Adair, *et al.,* Workshop III report: scale-up of liquid and semi-solid disperse systems. *Pharmaceutical Research* 1994; **11**(8):1216-1219.

192. U.T. Lashmar, J. Beesley. Correlation of rheological properties of an oil-in-water emulsion with manufacturing procedures and stability. *International Journal of Pharmaceutics* 1993; **91**(1):59-67.

193. C. Surber, A.F. Davis. Bioavailability and bioequivalence of dermatological formulations. In: K.A. Walters (editor): *Dermatological and Transdermal Formulations*. Marcel Dekker, Inc.; New York, 2002, pp. 401-498.

194. G. Yosipovitch, H.I. Maibach. Skin surface pH: a protective acid mantle. *Cosmetics and Toiletries* 1996; **111**(12):101-102.

195. I. Kanfer, R.B. Walker, P. Persicaner. Experimental formulation development. In: L. Shargel, I. Kanfer (editors): *Generic Drug Product Development: Solid Oral Dosage Forms*. Mercel Dekker; New York, 2005, pp. 53-93.

196. Patient information leaflet: Dermivate® cream: clobetasol propionate, GlaxoSmithKline plc.http://emc.medicines.org.uk/emc/assets/c/html/displaydoc.asp?documentid=3829. Retrieved 12/12/2006.

197. *British Pharmaceutical Codex*. The Pharmaceutical Press; London, 1973, pp. 40, 88, 100, 114, 413, 449. .

198. Propylene glycol. In: *The British Pharmacopoeia*. The Pharmaceutical Press, London, 2005, pp. 1680-1681.

199. G.E. Amidon. Sodium citrate dihydrate. In: R.C. Rowe, P.J. Sheskey, P.J. Weller (editors): *Handbook of Pharmaceutical Excipients*.: American Pharmaceutical Association; Washington, D.C. , 2003, pp. 560-562.

200. G.E. Amidon. Citric acid monohydrate. In: R.C. Rowe, P.J. Sheskey, P.J. Weller (editors): *Handbook of Pharmaceutical Excipients*. American Pharmaceutical Association; Washington, D.C., 2003, pp. 158-160.

201. A.K. Taylor. Glyceryl monostearate. In: R.C. Rowe, P.J. Sheskey, P.J. Weller (editors): *Handbook of Pharmaceutical Excipients*. American Pharmaceutical Association; Washington, D.C., 2003, pp. 264-266.

202. G. Frunzi, B. Sarsfield. Cetostearyl alcohol. In: R.C. Rowe, P.J. Sheskey, P.J. Weller (editors): *Handbook of Pharmaceutical Excipients*. American Pharmaceutical Association; Washington, D.C., 2003, pp. 125-126.

203. A.H. Kibbe. Wax, white. In: R.C. Rowe, P.J. Sheskey, P.J. Weller (editors): *Handbook of Pharmaceutical Excipients*. American Pharmaceutical Association; Washington, DC, 2003, pp. 687-688.

204. P.J. Weller. Chlorocresol. In: R.C. Rowe, P.J. Sheskey, P.J. Weller (editors): *Handbook of Pharmaceutical Excipients*. American Pharmaceutical Association; Washington, DC 2003, pp. 144-146.

205. Uniqema: Estol® 1474. http://www.uniqema.com/irj/portal?NavigationTarget=navurl://8975db69ba619ede9ec8a256b362aded. Retrieved 05/11/2006.

206. Masso: personal care ingredients-emulsifiers. http://www.cqmasso.com/fcr/cosmetica/catalogo/Type_of_Product/General%20Catalogue%20-%20Emulsifiers%20(M&Q).pdf. Retrieved 05/11/2006.

. 207. SpecialChem: innovation and solutions in cosmetics: Ritapro® 200. http://www.specialchem4cosmetics.com/common/COS/services/ingredients/displayproduct.aspx?or=docindex&menu=true&id=5149. Retrieved 05/11/2006.

. 208. Gattefossé: dermal route excipients; enhancing pharmaceutical performance. 3rd Edition, Saint-Priest Cedex, 1999. Production information sheet.

209. Chemical drug information: Emulcire® 61 WL.ttp://www.kreglinger-europe.com/eng/product?21202. Kreglinger Europe.

210. S.K. Niazi. Formulations of semi-solid drugs: Clobetasol propionate cream. In: S.K. Niazi (editor): *Handbook of Pharmaceutical Manufacturing Formulations: Semi-solid products*. CRC Press; Boca Raton, 2004, pp. 136-137.

211. L.M. Spalton. Formulation and preparation of emulsions. In: L.M. Spalton (editor): *Pharmaceutical Emulsions and emulsifying agents*. The chemist and Druggist; London: 1959, pp. 29-49.

212. F. Niellound, G. Marti-Mestres, J.P. Laget, C. Fernandez, H. Maillols. Emulsion formulations: study of the influence of parameters with experimental designs. *Drug development and Industrial Pharmacy* 1996; **22**(2):156-166.

213. B.W. Barry. Basic principles of diffusion through membranes. In: B.W. Barry (editor): *Dermatological Formulations, Percutaneous Absorption*. Mercel Dekker, Inc.; New York: 1983, pp. 49-94.

214. K.Welin-Berger J.A.M. Neelissen, B. Bergenstahl. The effect of rheological behaviour of a topical anaesthetic formulation on the release and permeation rates of the active compound. *European Journal of Pharmaceutical Sciences* 2001; **13**(3):309-318.

215. B. Kommanaboyina, C.T. Rhodes. Trends in stability testing with emphasis on stability during distribution and storage. *Drug Development and Industrial Pharmacy* 1999; **25**(7):857-868.

216. K. Komka, S.Kemeny. A modified error model for the assessment of stability of pharmaceutical products. *Chemometrics and Intelligent Laboratory Systems* 2004; **72**(2):161-165.

217. WHO expert committee on specifications for pharmaceutical preparations (WHO technical report, series 863). 34, 65-76. 1996. Geneva, World Health Organization.

218. S. Singh. Drug stability testing and shelf-life determination according to international guidelines. *Pharmaceutical Technology* 1999; **23**(6):68-88.

219. B.R.Matthews. Regulatory aspects of stability testing in Europe. *Drug Development and Industrial Pharmacy* 1999; **25**(7):831-856.

220. P.K. Bhattacharyya. Drug stability. In: L. Shargel, I. Kanfer (editors): *Generic Drug Product Development: Solid Oral Dosage Forms*. Marcel Dekker; New York, 2005, pp. 137-172.

221. ICH harmonised tripartite guideline: stability testing of new drug substances and products: Q1A (R2). CPMP/ICH/2736/99. International Conference on Harmonisation, London, 2003, pp.1-20.

222. C.T. Rhodes. Introductory overview. In: J.T. Carstensen, C.T. Rhodes (editors): *Drug Stability: Principles and Practices, Third Edition, Revised and Expanded*. Marcel Dekker; New York, 2000, pp. 2-18.

223. J.J. Verner. Formulation and dissolution assessment of a novel repeat action tablet containing a decongestant and an antihistamine. Rhodes University. Grahamstown, 2003, pp. 136-137.

224. H. Forcinio. Choosing a blister material. *Pharmaceutical Technology* 2000; **24**(5):26-30.

225. S. Singh. Stability test storage conditions for zones III and IV: some unresolved issues. *Pharmaceutical Technology* 1999; **23**(10):130-142.

226. D. Pecanac, F. Van Houtte, E. Roets, J. Hoogmartens. A stability study of ticlopidine products from 18 countries. *Drug Development and Industrial Pharmacy* 2000; **26**(4):391-401.

227. Guidance for industry: guideline for submitting documentation for the stability of human drugs and biologics. Food and Drug Administration, Rockville, 1987, 1-56.

228. J.D. Haynes. Worldwide virtual temperatures for pharmaceutical products stability testing. *Journal of Pharmaceutical Sciences* 1971; **60**(6):927-919.

229. S.C. Owen. Sodium chloride. In: R.C. Rowe, P.J. Sheskey, P.J. Weller (editors): *Handbook of Pharmaceutical Excipients*. American Pharmaceutical Association; Washington, D.C., 2003, pp. 556-559.

230. J.T. Carstensen. Physical testing. In: J.T. Carstensen, C.T. Rhodes (editors): *Drug Stability: Principles and Practices: Third Edition, Revised and Expanded*. Mercel Dekker; New York, 2000, pp. 261-328.

231. N.D. Ramirez-Beltran, L.A. Olivares. Statistical analysis of drug stability. *Computers & Industrial Engineering* 1999; **37**(1-2):351-354.

232. M.E. Gil-Alegre, J.A. Bernabeu, M.A. Camacho, A.I. Torres-Suarez. Statistical evaluation for stability studies under stress storage conditions. *Il Farmaco* 2001; **56**(11):877-883.

233. B. Kommanaboyina, C.T. Rhodes. Effects of temperature excursions on mean kinetic temperature and shelf-life. *Drug Development and Industrial Pharmacy* 1999; **25**(12):1301-1306.