# *IN VITRO* RELEASE OF KETOPROFEN FROM PROPRIETARY AND EXTEMPORANEOUSLY MANUFACTURED GELS

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## ABSTRACT

Ketoprofen is a potent non-steroidal anti-inflammatory drug which is used for the treatment of rheumatoid arthritis. The oral administration of ketoprofen can cause gastric irritation and adverse renal effects. Transdermal delivery of the drug can bypass gastrointestinal disturbances and provide relatively consistent drug concentrations at the site of administration.

The release of ketoprofen from proprietary gel products from three different countries was evaluated by comparing the *in vitro* release profiles. Twenty extemporaneously prepared ketoprofen gel formulations using Carbopol® polymers were manufactured. The effect of polymer, drug concentration, pH and solvent systems on the *in vitro* release of ketoprofen from these formulations were investigated. The gels were evaluated for drug content and pH. The release of the drug from all the formulations obeyed the Higuchi principle.

Two static FDA approved diffusion cells, namely the modified Franz diffusion cell and the European Pharmacopoeia diffusion cell, were compared by measuring the *in vitro* release rate of ketoprofen from all the gel formulations through a synthetic silicone membrane.

High-performance liquid chromatography and ultraviolet spectrophotometric analytical techniques were both used for the analysis of ketoprofen. The validated methods were employed for the determination of ketoprofen in the sample solutions taken from the receptor fluid.

Two of the three proprietary products registered under the same manufacturing license exhibited similar results whereas the third product differed significantly. Among the variables investigated, the vehicle pH and solvent composition were found have the most significant effect on the *in vitro* release of ketoprofen from Carbopol® polymers. The different grades of Carbopol® polymers showed statistically significantly different release kinetics with respect to lag time.

When evaluating the proprietary products, both the modified Franz diffusion cell and the European Pharmacopoeia diffusion cell were deemed adequate although higher profiles were generally obtained from the European Pharmacopoeia diffusion cells.

Smoother diffusion profiles were obtained from samples analysed by high-performance liquid chromatography than by ultraviolet spectrophotometry in both diffusion cells. Sample solutions taken from Franz diffusion cells and analysed by ultraviolet spectrophotometry also produced smooth diffusion profiles. Erratic and higher diffusion profiles were observed with samples taken from the European Pharmacopoeia diffusion cell and analysed by ultraviolet spectrophotometry.

The choice of diffusion cells and analytical procedure in product development must be weighed against the relatively poor reproducibility as observed with the European Pharmacopoeia diffusion cell.

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## STUDY OBJECTIVES

Ketoprofen is a non-steroidal anti-inflammatory, analgesic and antipyretic drug used for the treatment of rheumatoid osteoarthritis, ankylosing spondylitis and gout. It is more potent than the other non-steroidal anti-inflammatory drugs (NSAIDs) with respect to some effects such as anti-inflammatory and analgesic activities.

Although ketoprofen is rapidly absorbed, metabolized and excreted, it causes some gastrointestinal complaints such as nausea, dyspepsia, diarrhoea, constipation and some renal side effects like other NSAIDs. Therefore, there is a great interest in developing topical dosage forms of these NSAIDs to avoid the oral side effects and provide relatively consistent drug concentrations at the application site for prolonged periods.

The objectives of this study were:

1. To develop and validate a suitable high-performance liquid chromatographic method for the determination of ketoprofen from topical gel formulations.
2. To develop and validate a suitable ultraviolet spectrophotometric method for the determination of ketoprofen from topical gel formulations.
3. To extemporaneously manufacture topical gel formulations using Carbopol® polymers and study the effect of polymer type, pH, loading concentration and solvent composition on the *in vitro* release of ketoprofen.
4. To compare and contrast the *in vitro* release rates of ketoprofen from proprietary gel products and extemporaneously prepared topical gel formulations using the Franz diffusion cell and the European Pharmacopoeia diffusion cell.
5. To compare and contrast the *in vitro* release rates of different proprietary gel products and extemporaneously prepared topical gel formulations using ultraviolet spectrophotometry and high-performance liquid chromatography utilizing both the Franz diffusion cell and the European Pharmacopoeia diffusion cell.

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# CHAPTER ONE
# TRANSDERMAL DRUG DELIVERY

## 1.1 PAST PROGRESS, CURRENT STATUS AND FUTURE PROSPECTS OF TRANSDERMAL DRUG DELIVERY

### *1.1.1 Introduction*

Human beings have been placing salves, lotions and potions on their skin from ancient times (1) and the concept of delivering drugs through the skin is a practice which dates as far back as the 16$^{th}$ century BC (2). The *Ebers Papyrus*, the oldest preserved medical document, recommended that the husk of the castor oil plant be crushed in water and placed on an aching head and 'the head will be cured at once, as though it had never ached' (2). In the late seventies transdermal drug delivery (TDD) was heralded as a methodology that could provide blood drug concentrations controlled by a device and there was an expectation that it could therefore develop into a universal strategy for the administration of medicines (3).

The transdermal route of controlled drug delivery is often dismissed as a relatively minor player in modern pharmaceutical sciences. One commonly hears that the skin is too good a barrier to permit the delivery of all but a few compounds and that transdermal transport is not even worth the consideration for new drugs of the biotechnology industry (4). This has however been disputed as today TDD is a well-accepted means of delivering many drugs to the systemic circulation (2) in order to achieve a desired pharmacological outcome. Traditional preparations used include ointments, gels, creams and medicinal plasters containing natural herbs and compounds. The development of the first pharmaceutical transdermal patch of scopolamine for motion sickness in the early 1980s heralded acceptance of the benefits and applicability of this method of administration of modern commercial products (4 - 6). The success of this approach is evidenced by the fact that there are currently more than 35 TDD products approved in the USA for the treatment of conditions including hypertension, angina, female menopause, severe pain states, nicotine dependence, male hypogonadism, local pain control and more recently, contraception and urinary incontinence (2 - 8, 55). Several products are in late-stage development that will further expand TDD usage into new therapeutic areas, including Parkinson's disease, attention deficit and hyperactivity disorder and female dysfunction (5, 6). New and improved TDD products are

also under development that will expand the number of therapeutic options in pain management, osteoporosis and hormone replacement (6). The current USA market for transdermal patches is over $3 billion annually and for testosterone gel is approximately $225 million (7, 8, 55) and represents the most successful non-oral systemic drug delivery system (27).

Clearly, the clinical benefits, industrial interest, strong market and regulatory precedence show why TDD has become a successful and viable dosage form (6).

### *1.1.2 Rationale for transdermal drug delivery*

Given that the skin offers such an excellent barrier to molecular transport, the rationale for this delivery strategy needs to be carefully identified. There are several instances in which the most convenient of drug intake methods (the oral route) is not feasible therefore alternative routes must be sought. Although intravenous introduction of the medicament avoids many of these shortfalls (such as gastrointestinal tract (GIT) and hepatic metabolism), its invasive nature (particularly for chronic administration) has encouraged the search for alternative strategies and few anatomical orifices have not been investigated for their potential as optional drug delivery routes. The implementation of TDD technology must be therapeutically justified. Drugs with high oral bioavailability and infrequent dosing regimens that are well accepted by patients do not warrant such measures. Similarly, transdermal administration is not a means to achieve rapid bolus-type drug inputs, rather it is usually designed to offer slow, sustained drug delivery over substantial periods of time and, as such, tolerance-inducing drugs or those (*e.g.,* hormones) requiring chronopharmacological management are, at least to date, not suitable. Nevertheless, there remains a large pool of drugs for which TDD is desirable but presently unfeasible. The nature of the stratum corneum (SC) is, in essence, the key to this problem. The excellent diffusional resistance offered by the membrane means that the daily drug dose that can be systematically delivered through a reasonable 'patch-size' area remains in the < 10 mg range (27). The structure and barrier property of the SC are discussed in sections 1.2.2.1 and 1.2.4 respectively.

### *1.1.3 Advantages and drawbacks of transdermal drug delivery*

The skin offers several advantages as a route for drug delivery and most of these have been well documented (2 - 8, 27). In most cases, although the skin itself controls drug input into

the systemic circulation, drug delivery can be controlled predictably and over a long period of time, from simple matrix-type transdermal patches (3). Transdermal drug systems provide constant concentrations in the plasma for drugs with a narrow therapeutic window, thus minimising the risk of toxic side effects or lack of efficacy associated with conventional oral dosing (2). This is of great value particularly for drugs with short half-lives to be administered at most once a day and which can result in improved patient compliance (2, 5). In clinical drug therapies, topical application allows localized drug delivery to the site of interest. This enhances the therapeutic effect of the drug while minimising systemic side effects (11). The problems associated with first-pass metabolism in the GIT and the liver are avoided with TDD and this allows drugs with poor oral bioavailability to be administered at most once a day and this can also result in improved patient compliance (2, 3, 12, 37). Transdermal administration avoids the vagaries of the GIT milieu and does not shunt the drug directly through the liver (1). How much of a problem exists is very dependant on the properties of the medicinal agent, but it should be remembered that the skin is capable of metabolising some permeants (3, 38). The deeper layers of the skin are metabolically more active than the SC. Although the SC is considered to be a dead layer, it has been established that microflora present on the skin surface are capable of metabolising drugs (9, 38). The GIT tract presents a fairly hostile environment to a drug molecule. The low gastric pH or enzymes may degrade a drug molecule, or the interaction with food, drinks and/or other drugs in the stomach may prevent the drug from permeating through the GIT wall (1). The circumvention of the drug from the hostile environment of the GIT minimises possible gastric irritation and chemical degradation or systemic deactivation of the drug (2, 11, 37). Unlike parenteral, subcutaneous and intramuscular formulations, a transdermal product does not have the stigma associated with needles nor does it require professional supervision for administration (1, 2, 27). This increases patient acceptance (1, 3) and allows ambulatory patients to leave the hospital while on medication. In the case of an adverse reaction or overdose, the patient can simply remove the transdermal device without undergoing the harsh antidote treatment of having the stomach pumped (1, 12, 37). An additional benefit that has been noted in hospitals is the ability of the nurse or physician to tell that the patient is on a particular drug, since the transdermal is worn on the person and can be identified by its label (1) although this does not hold if the dosage form is a semi-solid. Further benefits of TDD systems have emerged over the past few years as technologies have evolved. These include the potential for sustained release and controlled input kinetics which are particularly useful for drugs with narrow therapeutic indices (27). Transdermal applications are suitable for

patients who are unconscious or vomiting (2). Despite all these advantages, a timely warning to formulators was also issued in 1987 (2), 'TDD is not a subject which can be approached simplistically without a thorough understanding of the physicochemical and biological parameters of percutaneous absorption. Researchers who attempt TDD without appreciating this fact do so at their peril.'

As with the other routes of drug delivery, transport across the skin is also associated with several disadvantages, the main drawback being that not all compounds are suitable candidates (94). Since the inception of TDD there has only been a very limited number of products launched onto the market (3) and the considerable research and development expense in the transdermal product development and skin research field to bring more TDD products to the market has been slow (37). There are various reasons for this but the most likely is the rate-limiting factor of the skin (1). The rate-limiting resistance resides in the SC (26). The skin is a very effective barrier to the ingress of materials, allowing only small quantities of a drug to penetrate over a period of a day (3, 9). A typical drug that is incorporated into a dermal drug delivery system will exhibit a bioavailability of only a few percent and therefore the active has to have a very high potency. For transdermal delivery, as a rule of thumb, the maximum daily dose that can permeate the skin is of the order of a few milligrams. This further underscores the need for high potency drugs (3). As evidence of this, all of the drugs presently administered across the skin share constraining characteristics such as low molecular mass (< 500 Da), high lipophilicity ( $\log P$ in the range of 1 to 3), low melting point (< 200°C) and high potency (dose is less than 50 mg per day) (1, 2, 4, 6, 8, 10, 55). The smallest drug molecule presently formulated in a patch is nicotine (162 Da) and the largest is oxybutinin (359 Da). Opening the transdermal route to large hydrophilic drugs is one of the major challenges in the field of TDD (8). The required high potency can also mean that the drug has a high potential to be toxic to the skin causing irritation and/or sensitisation (1, 3, 7). If the barrier function of the skin is compromised in any way, some of the matrix-type delivery devices can deliver more of the active than necessary and the transdermal equivalent of 'dose dumping' can occur (3). Elevated drug concentrations can be attained if a transdermal system is repeatedly placed on the old site and this can lead to the possibility of enhanced skin toxicity. Other difficulties encountered with TDD are the variability in percutaneous absorption, the precision of dosing, the reservoir capacity of the skin, heterogeneity and inducibility of the skin in turnover and metabolism, inadequate

definition of bioequivalence criteria and an incomplete understanding of technologies that may be used to facilitate or retard percutaneous absorption (5, 12, 20).

### *1.1.4 Innovations in transdermal drug delivery*

TDD has been the subject of extensive research (11). The introduction of new transdermal technologies such as chemical penetration enhancement (2 - 6, 9, 11, 12, 18, 28, 34, 35), iontophoresis (2 - 6, 7, 11, 18, 19, 28, 34), sonophoresis (2 - 7, 10 - 12, 17, 18, 34), transferosomes (12), thermal energy (6, 8), magnetic energy (6), microneedle applications (6, 8), electroporation (3, 4, 7, 11, 12, 34) and high velocity jet injectors (8) challenge the paradigm that there are only a few drug candidates for TDD. Despite difficult issues related to skin tolerability and regulatory approval, most attention, at least until recently, has been directed at the use of chemical penetration enhancers. However, this focus is now shifting towards the development of novel vehicles comprising accepted excipients (including lipid vesicular-based systems, supersaturated formulations and microemulsions) and to the use of physical methods to overcome the barrier. In the latter category, iontophoresis is the dominant player and is by far the method furthest along the evaluatory path. Applications of electroporation, ultrasound and high pressure, *etc.,* remain at the research and feasibility stage of development. Interestingly, the level of endeavour devoted to either removal or perforation of the SC (*e.g.,* by laser ablation, or the use of microneedle arrays) has increased sharply, with these so-called 'minimally invasive' techniques essentially dispensing with the challenge of the barrier function of the skin (29). Physical methods have the advantage of decreased skin irritant/allergic responses, as well as no interaction with the drugs being delivered (11). The extent to which these are translated into practise will be defined by time (12). TDD is therefore a thriving area of research and product development, with many new diverse technology offerings both within and beyond traditional passive transdermal technologies (6).

## 1.2 PERCUTANEOUS ABSORPTION

### *1.2.1 Introduction*

Although the skin is the most accessible organ of the body to superficial investigations, the direct measurement of penetrating substances has long posed major hurdles for detailed mechanistic studies. In recent decades many investigators have studied the mechanisms, routes and time curves by which drugs and toxic compounds may penetrate the skin, which is of particular importance for many areas of medicine, pharmacy, toxicity assessment and the cosmetic industry (22). The introduction of chemicals into the body through the skin occurs by passive contact with the environment and direct application of chemicals on the body for the purposes of medical therapy in the management of skin diseases and in use in TDD devices and as cosmetics (40). Percutaneous absorption is a complex physicochemical and biological process. In addition to partition and diffusion processes, there are other potential fates for drug entities entering the skin which include irreversible binding to cutaneous proteins such as keratin, degradation by cutaneous enzymes and partition into subcutaneous fat (36, 39). Many *in vitro* and *in vivo* experimental methods for determining transdermal absorption have been used to understand and/or predict the delivery of drugs from the skin surface into the body of living animals or humans (36). The skin acts as a barrier to maintain the internal milieu, however, it is not a total barrier and many chemicals have been shown to penetrate into and through the skin (30). The release of a therapeutic agent from a formulation applied to the skin surface and its transport to the systemic circulation involves:

i. dissolution within and release from the formulation,
ii. partitioning into the outermost layer of the skin, SC,
iii. diffusion through the SC,
iv. partitioning from the SC into the aqueous viable epidermis,
v. diffusion through the viable epidermis and into the upper dermis and
vi. uptake into the local capillary network and eventually the systemic circulation (31).

In order to rationally design formulations for cosmetic or pharmaceutical purposes, a detailed knowledge of the human skin and its barrier function is imperative (28).

### *1.2.2 Human skin*

#### *1.2.2.1 Structure and functions of skin*

The skin is the largest organ of the body, accounting for more than 10% of body mass and the one that enables the body to interact most intimately with its environment (12, 32). It is one of the most extensive, readily accessible organs and is the heaviest single organ of the body which combines with the mucosal linings of the respiratory, digestive and urogenital tracts to form a capsule which separates the internal body structures from the external environment (14). It covers around 2 $m^2$ of an adult average body and receives approximately one-third of all blood circulating through the body (13, 82). A typical square centimetre of skin comprises 10 hair follicles, 12 nerves, 15 sebaceous glands, 100 sweat glands, 3 blood vessels with 92 cm total length, 360 cm of nerves and 3 x $10^6$ cells (14). Many of the functions of the skin can be classified as essential to the survival of animals in a relatively hostile environment (12). In terms of the number of functions performed, the skin outweighs any other organ. Its primary function is protection, which covers physical, chemical, immune, pathogen, uv radiation and free radical defences. It is also a major participant in thermoregulation, functions as a sensory organ, performs endocrine functions (vitamin D synthesis, peripheral conversion of prohormones), significant in reproduction (secondary sexual characteristics, pheromone production) and perpetuation of the species, human non-verbal communications (verbal signalling, emotions expressed), as well as a factor in xenophobia and bias against fellow humans that has shaped the destiny of humanity (12, 15, 16). The skin also serves as a barrier against the penetration of water-soluble substances and to reduce transepidermal water loss (TEWL) (23) and is also the basis of several billion-dollar industries such as the personal care, cosmetic and fashion industries. For pharmaceuticals, it is both a challenge (barrier) and an opportunity (large surface area) for delivering drugs (15). The skin is a multilayered organ composed of many histological layers (13). In essence, the skin consists of four layers namely the SC (non-viable epidermis), the remaining layers of the epidermis (viable epidermis), dermis and subcutaneous tissues (41). There are also several associated appendages such as hair follicles, sweat ducts, apocrine glands and nails (12).

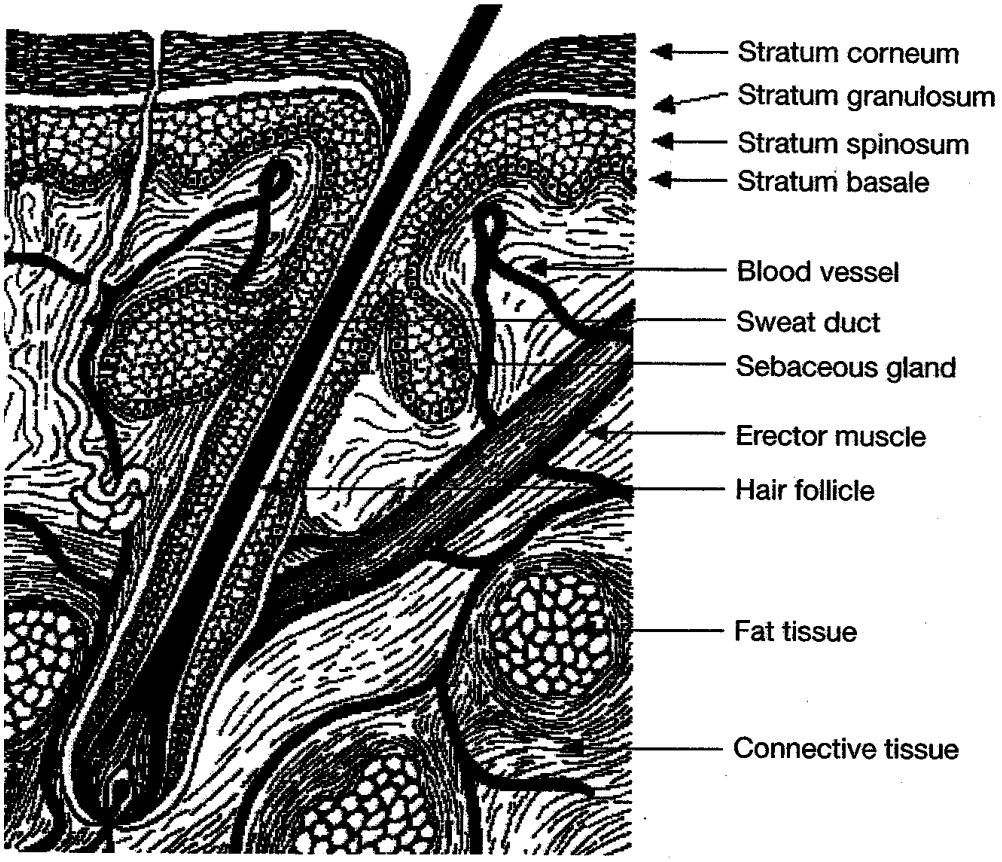



*Figure 1.1 Components of the epidermis and dermis of human skin (12)*

*1.2.2.2 The epidermis*

The epidermis is divided into four anatomical layers namely stratum basale (SB), stratum spinosum (SS), stratum granulosum (SG) and SC (13, 15, 21) as shown in Figure 1.1. The SC is the heterogeneous outermost layer of the epidermis and is approximately 10 - 20 μm thick. It is non-viable epidermis and consists of 15 - 25 flattened, stacked, hexagonal and cornified cells embedded in a mortar of intercellular lipid. Each cell is approximately 40 μm in diameter and 0.5 μm thick (12, 28, 39). The thickness varies and may be a magnitude of order larger in areas such as the palms of the hands and soles of the feet. These are areas of the body associated with frequent direct and substantial physical interaction with the physical environment. Not surprisingly, absorption is slower through these regions than through the skin of other parts of the body (12). The cells of the SC, keratinocytes, originate in the viable epidermis and undergo many morphological changes before desquamation. The keratinocytes are metabolically active and capable of mitotic division (39) and therefore the epidermis consists of several cell strata at varying levels of differentiation (Figure 1.2).

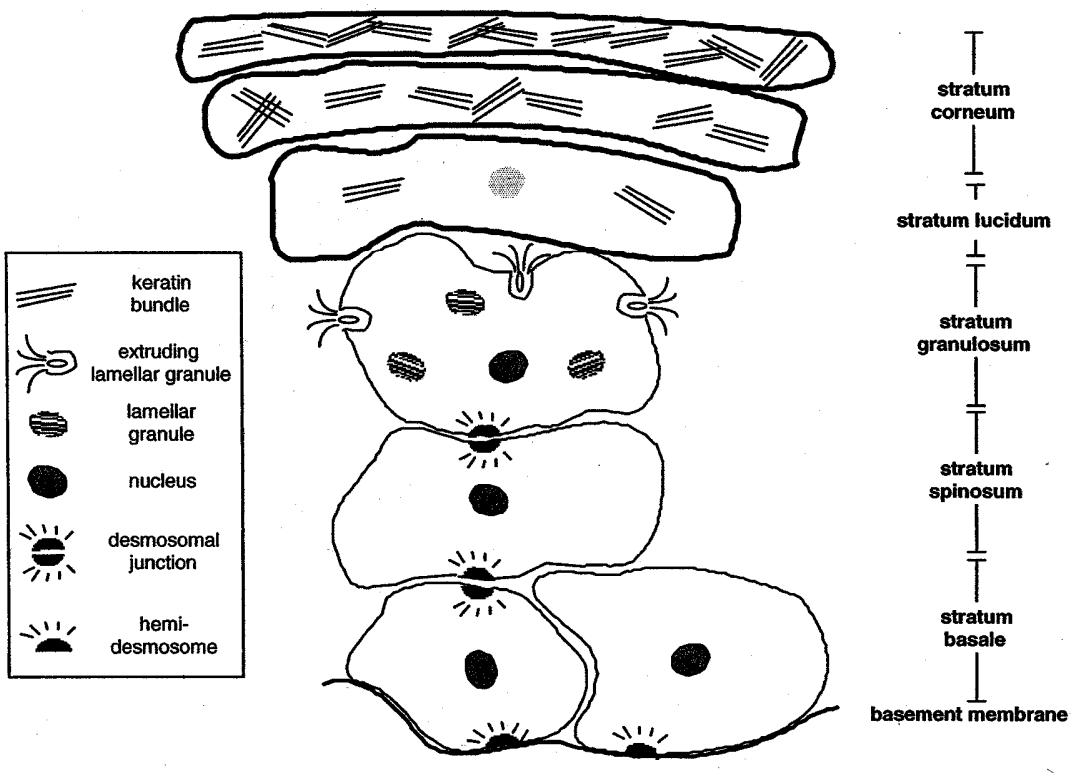



*Figure 1.2 Epidermal differentiation: major events include extrusion of lamellar bodies, loss of nucleus and increasing amount of keratin in the stratum corneum (12)*

The origins of the cells of the epidermis lie in the basal lamina between the dermis and viable epidermis (12). In the basal layer of the epidermis there is continuous renewal of cells. These cells are subsequently transported to the upper layers of the epidermis. The composition of lipids changes markedly during apical migration through successive epidermal layers. When the differentiation process is accomplished (*i.e.,* in the SC), lipid composition changes markedly, phospholipids are degraded enzymatically into glycerol and free fatty acids and glucosylceramides into ceramides. The main constituents of the SC lipids are cholesterol, free fatty acids and ceramides (26, 28). At physiological temperature, which is below the gel-to-liquid crystalline phase transition temperature, the lipids are highly ordered (26). The SC is a composite of corneocytes (terminally differentiated keratinocytes) and secreted contents of the lamellar bodies (elaborated by the keratinocytes), that give it a 'bricks-and-mortar' structure (15, 18, 42). This arrangement creates a tortuous path through which substances have to traverse in order to cross the SC. The classic 'bricks-and-mortar' structure is still the most simplistic organizational description. The protein-enriched corneocytes (bricks) impart a high degree of tortuosity to the path of water or any other molecule that traverses the SC, while the hydrophobic lipids, organised into tight lamellar

structures (mortar) provide a water-tight barrier property to the already tortuous route of permeation in the interfollicular domains (15).

#### *1.2.2.3 The viable epidermis*

The viable epidermis consists of multiple layers of keratinocytes at various stages of differentiation. The basal layer contains actively dividing cells, which migrate upwards to successively form the spinous, granular and clear layers. As part of this process, the cells gradually lose their nuclei and undergo changes in composition as shown in Figure 1.2. The role of the viable epidermis in skin barrier function is mainly related to the intercellular lipid channels and to several partitioning phenomena. Depending on their solubility, drugs can partition from layer to layer after diffusing through the SC. Several other cells (*e.g.,* melanocytes, Langerhans cells, dendritic T cells, epidermotropic lymphocyes and Merkel cells) are also scattered throughout the viable epidermis, which contain a variety of active catabolic enzymes (*e.g.,* esterases, phosphatates, proteases, nucleotidases and lipases) (24, 41).

#### *1.2.2.4 The dermis*

The dermis (or corium), at 3 to 5 mm thick, is much wider than the overlying epidermis which it supports and thus makes up the bulk of the skin (14). The dermis, which provides the elasticity of the skin, contains immune cells and has the vascular network that supplies the epidermis with nutrients that can carry absorbed substances into the body (30, 39). The dermis consists of a matrix of connective tissue woven from fibrous proteins (collagen 75%, elastin 4% and reticulin 0.4%) which is embedded in mucopolysaccharide providing about 2% of the mass. Blood vessels, nerves and lymphatic vessels cross this matrix and skin appendages (endocrine sweat glands, apocrine glands and pilosebaceous units) penetrate it. In man, the dermis divides into a superficial, thin image of the ridged lower surface of the epidermis and a thick underlying reticular layer made of wide collagen fibres (14). It also plays a role in temperature, pressure and pain regulation (12).

### *1.2.3 Routes of drug permeation across the skin*

For transdermal delivery to be effective, drugs have to enter into the viable skin in sufficient quantities to produce a therapeutic effect (11). The route through which permeation occurs is largely dependent on the physicochemical properties of the penetrant, the most important

being the relative ability to partition into each skin phase (18). Three possible pathways for TDD (Figure 1.3) have been reported (11, 24 - 26, 82, 84, 94). They are transport through appendages such as hair follicles, transcellular transport through the corneocytes and intercellular transport *via* the extracellular matrix.

#### *1.2.3.1 Transcellular pathway*

It was originally believed that transcellular diffusion mechanisms dominated over the intercellular and transappendageal routes during the passage of solutes through the SC (94). The permeant crosses the SC by the most direct route and repeatedly partitions between and diffuses through the cornified cells, the extracellular lipid bilayers (26), viable epidermis and papillary layer of the dermis, with the microcirculation usually providing an infinite sink (21). Although the transcellular route appears most favoured on geometric grounds, there has been no direct evidence presented to provide support for its participation in the SC penetration process. However the so-called 'protein domain' of the SC may represent a region into which topically applied molecules may partition and therefore act as a reservoir. Additionally, certain penetration enhancers (*e.g.,* anionic surfactants and alkyl sulphoxides) have been shown to interact with keratin and induce protein conformational changes. The presence of these materials could increase the likelihood that permeates access the transcellular route (26).

#### *1.2.3.2 Intercellular pathway*

The intracellular SC spaces were initially dismissed as a potentially significant diffusion pathway because of the small volume they occupy. However, the physical structure of the intracellular lipids was thought to be a significant factor in the barrier properties of the skin (94). The solute remains in the lipid domains and permeates *via* a tortuous pathway. Within this lipid domain, the drug has to cross repetitively complete lipid bilayers (26). Available evidence has shown (26) that there is a preponderance of support for the intercellular pathway and it has been identified as the major route of transport across the SC. The intracellular route is usually regarded as a pathway for polar (hydrophilic) molecules, since cellular components are predominantly aqueous in nature. Here the pathway is directly across the SC, the rate-limiting barrier being the multiple bilayered lipids that must also be crossed (39).

*1.2.3.3 Appendageal pathway*

The penetrant transverses the SC *via* a 'shunt' pathway: *e.g.,* a hair follicle or a sweat gland. These shunts are known to be important at short times prior to steady state diffusion. The available diffusional area of the shunt route is approximately 0.1% of the total skin area and therefore the contribution to drug permeation compared to the former is significantly less (21, 24, 26, 33, 82). Despite their small fractional area, the skin appendages may provide the main portal of entry into the subepidermal layers of the skin for ions and large polar molecules (21, 24, 26, 33). The appendageal pathway has been reported (26) to be the major contributor to the initial phase of SC permeation.

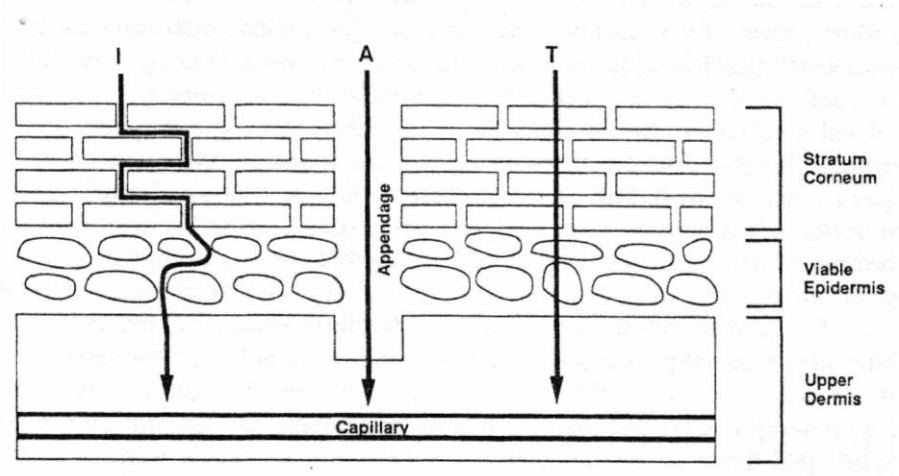



*Figure 1.3 Schematic diagram of the potential routes of drug penetration through the stratum corneum I = intercellular T = transcellular A = appendegeal (26)*

The precise mechanisms by which drugs permeate the SC are still under debate but there is substantial evidence that the route of permeation is a tortuous one following the intercellular channels (3, 9, 32). The transcellular pathway requires the substrates to travel through the corneocytes while the intercellular pathway is *via* the extracellular matrix between the corneocytes. For intercellular skin transport, hydrophilic substrates are rate limited by the lipid environment of the intercellular matrix of the SC. On the other hand, lipophilic substrates partition into the intercellular lipids of the SC. However the rate-limiting step is the partition into the epidermis, which is practically an aqueous environment. Molecular transport through the skin has been described by a solubility-diffusion model and a transfer free energy model (11). Hydrophilic substances prefer the transcellular route through the protein-enriched corneocytes. In general, molecules with amphoteric chemical properties are thought to be able to penetrate best. Additionally, the stereochemical characteristics and the molecular weight of a compound are of major interest (22). Although the intracellular route

has been identified as the major contributor to percutaneous permeation, it must be emphasised that the other pathways also contribute. The three pathways are not mutually exclusive and most molecules will pass through the SC by a combination of these routes (39). The existence of these pathways for permeation across skin has significant implications in the design, development and use of penetration enhancers. It is unlikely that an enhancer that acts primarily on one pathway, *e.g.,* by increasing the fluidity of the extracellular lipid, will have any great effect on the permeability rate of a compound whose route is primarily transcellular. Furthermore, it is entirely feasible that the presence of an enhancer will alter the thermodynamic activity of a penetrant in a formulation resulting in changes in partitioning tendencies (18).

### *1.2.4 Barrier function of the skin*

The natural function of the skin is the protection of the body against the loss of endogenous substances such as water and undesired influences from the environment caused by exogenous substances (28, 39). This implies that the skin acts as a barrier against diffusion of substances through the underlying tissue (28). The diffusional resistance of the SC is a challenge that has been accepted by the pharmaceutical scientist and considerable activity has been directed towards percutaneous penetration enhancement technologies (4). Overcoming this natural barrier is the main challenge in dermal or transdermal delivery of drugs (28).

The barrier function of the skin is accomplished by the outermost few microns of the skin, the SC, a compositionally and morphologically unique membrane (27, 28, 39, 47). This extremely thin, least permeable layer of skin is the ultimate stage in the epidermal differentiation process, forming a laminate of compressed keratin-filled corneocytes (terminally differentiated keratinocytes) anchored in a lipophilic matrix (27). The lipids of this extracellular matrix are distinctive in many respects. They provide the only continuous phase (and diffusion pathway) from the skin surface to the base of the SC, the composition (ceramides, free fatty acids and cholesterol) is unique among biomembranes and particularly noteworthy is the absence of phospholipids. Despite the deficit of polar bilayer-forming lipids, the SC lipids exist as multilamellar sheets and the predominantly saturated, long-chain hydrocarbon tails facilitate a highly ordered, interdigitated configuration and the formation of gel-phase membrane domains as opposed to the more usual (and more fluid and permeable) liquid crystalline membrane systems (18, 20, 22, 23, 27). However, the unusual lipid matrix

alone cannot entirely explain the outstanding resistivity of the membrane and the SC architecture as a whole has been proposed to play an instrumental role in the barrier function of the membrane (27). The staggered corneocyte arrangement in a lipid continuum (similar to the brick and mortar assembly) is suggested to bestow a highly tortuous lipoidal diffusion pathway rendering the membrane one thousand times less permeable to water relative to most other biomembranes. The transport role of this sinuous pathway is further supported by visualization studies localizing several permeants in the intercellular channels by kinetic analysis of the *in vivo* skin penetration rates of model compounds and by the evidence from thermotropic biophysical studies of lipid domains (27, 31). The impermeability is a considerable problem in the delivery of medicines both to and through the skin. It has been estimated that only a small percentage of the active material reaches its target site when it is delivered topically (82).

### *1.2.5 Enhancing transdermal drug delivery*

To produce a systemic effect, TDD requires that suitable quantities of drug be transported through the skin (11, 41). A disadvantage of this route for drug delivery is that a relatively high dose is required to deliver therapeutic amounts across the skin and therefore evaluation of the potential for enhancement of skin penetration is of great practical importance (43). This has proved to be a challenge and has led to the development of a large repertoire of penetration enhancer compounds and physical techniques that, to different degrees, facilitate drug penetration across the skin (41). Traditionally, enhanced TDD has been achieved with patch devices that occlude the skin. Occlusion traps the natural transepidermal moisture of the skin which increases the water content of the horny layer and swells the membranes, therefore compromising its barrier function. Prolonged occlusion of this nature can cause a 10 to 100-fold increase in drug permeability. However the tradeoff with these occlusive delivery systems is their propensity to cause local skin irritation (2).

#### *1.2.5.1 Chemical approach*

Much effort has been directed towards the search for specific chemicals, or combinations of chemicals, that can act as penetration enhancers (2, 4, 5) also known as accelerants or sorption promoters (24, 44). These chemicals interact with skin constituents to promote drug flux (44) by reversibly compromising the barrier function of the skin and consequently allowing the entry of otherwise poorly penetrating molecules into the membrane and through

to the systemic circulation (27). Diffusion of drugs across the skin is a passive process and compounds with low solubility and affinity for the hydrophilic and lipophilic components of the SC will partition at a slow rate. These difficulties may be overcome by addition of a chemical adjunct to the delivery system that would promote drug partitioning into the SC (35). The trend in recent years has been to identify substances that are categorized as 'generally recognised as safe' (GRAS), rather than the more difficult path of seeking regulatory approval for a newly synthesized enhancer (*i.e.,* a new chemical entity) (4, 82). However with limited success, attempts have been made to synthesize novel penetration enhancers *e.g.,* lauracapram (Azone) and 2-n-nonyl-1,3-dioxolane (SEPA) which are being evaluated for clinical applications (55). The ideal candidate would provide a reversible reduction in the barrier properties of the skin without long term damage to the viable cells (5). An expanded list of desirable attributes is as follows (45):

i. The material should be pharmacologically inert and it should possess no action of itself at receptor sites in the skin or in the body generally. In fact, the most widely studied penetration enhancer, dimethyl sulfoxide (DMSO), is clinically active in many disease states.
ii. The material should not be toxic, irritating or allergenic.
iii. On application, the onset of penetration-enhancing action should be immediate; the duration of the effect should be predictable and should be suitable.
iv. When the material is removed from the skin, the tissue should recover its normal barrier property.
v. The barrier function of the skin should reduce in one direction only, so as to promote penetration into the skin. Body fluids, electrolytes or other endogenous materials should not be lost to the atmosphere.
vi. The enhancer should be chemically and physically compatible with a wide range of drugs and pharmaceutical adjuvants.
vii. The substance should be an excellent solvent for drugs.
viii. The material should spread well on the skin and it should possess a suitable skin feel.
ix. The chemical should formulate into lotions, suspensions, ointments, creams, gels, aerosols and skin adhesives.
x. It should be inexpensive, odourless, tasteless and colourless so as to be cosmetically acceptable.

It is unlikely that any single material would possess such a formidable array of desirable properties (45) and compromises will have to be made with appropriate benefit to risk

calculations (46). However, some substances do possess several of these attributes and they have been investigated clinically or in the laboratory (45).

#### *1.2.5.1.1 Chemical penetration enhancers (CPE)*

The mechanisms by which CPE act have their basis in the underlying physical chemistry that controls percutaneous absorption (82). Permeation enhancers fall into two major categories: those that impact on diffusion across the SC and those that alter partitioning into the SC (5, 6). The former class generally comprises a long alkyl chain capable of interacting with long chains of the intercellular lipids, in addition to a polar head group that is capable of interacting with the lipid polar head groups (5). This serves to disrupt the ordered nature of the skin lipids (5) and renders the SC more fluid thereby increasing the diffusion co-efficient of the permeant (46). Substances reported to the render the SC more permeable include alcohols, polyalcohols, pyrrolidones, amines, amides, fatty acids, sulphoxides, esters, terpenes, alkanes, surfactants and phospholipids (27, 35, 46, 87 - 90). Water is perhaps the ideal enhancer, since hydrated skin is generally more permeable (47), however, it is not applicable to all permeants (46). The latter class of CPE modify skin permeability by shifting the solubility parameter of the skin in the direction of that of the permeant. The solubility of the permeant in the outer layers of the skin will be increased and this, in turn, improves the flux. Simple solvent type molecules, such as propylene glycol, ethanol, Transcutol®, and *N*-methyl pyrollidone are thought to act in this way (46). It is possible that both mechanisms may operate simultaneously, therefore an additive effect on the overall rate of drug delivery may then be expected. Other mechanisms of CPE have been reported (49). CPE may increase skin/vehicle partitioning of the drug and they may increase solvent transport into or across the skin. The results of increased penetration may include increased drug solubility in the skin and increased skin penetration of the drug if the drug has a high affinity for the solvent (49). If an enhancer, or combination of enhancers, affects both the solubility of the diffusant in the SC and reduces the rigidity of the lipid matrix, then the overall increase in the flux rate theoretically should approximate the product of the increases afforded by either enhancement method alone (48). Despite the extensive studies performed, few compounds have been successfully incorporated into marketed products, partly because of the difficulty in predicting *in vivo* behaviour under conditions of use from the *in vitro* permeation tests that are normally used as screens for enhancement. It has also proved a difficult task to balance the formulation characteristics to ensure that the drug retains its tendency to partition from

the vehicle in the presence of the CPE (5). Numerous articles in this field have been published in recent years. Sridevi *et al.* (50) discussed optimizing transdermal delivery of ketoprofen using pH and hydroxypropyl-β-cyclodextrin as co-enhancers. Mura *et al.* (51) reviewed the evaluation of Transcutol® as a clonazepam transdermal permeation enhancer from hydrophilic gel formulations. Ghafourian *et al.* (52) reviewed the effect of penetration enhancers on the drug delivery through skin. Godwin *et al.* (53) discussed the influence of Transcutol® CG on the skin accumulation and transdermal permeation of ultraviolet absorbers. Fang *et al.* (54) studied the effect of enhancers and retarders on percutaneous absorption of flurbiprofen from hydrogels among others. Although individual chemical enhancers have had limited success, combinations offer new opportunities in transdermal formulations. However, the rational design of enhancer combinations is limited by the lack of mechanistic information on the interactions between individual chemical enhancers and the SC (55). Synergistic interactions between CPE, ultrasound, iontophoresis and electroporation have been reported (25).

#### *1.2.5.2 Physical approach*

The passive delivery of most compounds across the skin is limited due to the barrier properties of the epidermis (86). An interesting area of research over the past 10 to 15 years has been focussed on developing transdermal technologies that utilise mechanical energy to increase the drug flux across the skin by either altering the skin barrier or increasing the energy of the drug molecules (6). The reason for such research is due to the newly emerging biotechnology drugs such as peptides, proteins and oligonucleotides. These drugs, although highly specific and potent, are usually large, polar and/or charged - characteristics that normally preclude TDD. Recent advances in physical enhancement technologies directly respond to these challenges and offer exciting and powerful strategies to resolve these delivery issues (27).

##### *1.2.5.2.1 Iontophoresis*

Iontophoresis has been the primary electrical approach studied and has been shown to provide enhanced transport for some low molecular weight molecules such as pain medications and even decapeptides (7). This technique has been known for many years with some of the pioneering studies conducted in the nineteenth century (3) due to the emergence of a large number of peptide drugs (60). Drugs in the ionic form, contained in some

reservoir, can be '*phoresed*' out with a small current and driven into the body through the skin (62). It involves the use of an electric field to move both charged and uncharged species across the skin (55, 85). The electric field imposes a force on the ion which adds to and often dominates the 'diffusion force' or concentration gradient. This additional force then drives the ion through the membrane far more efficiently than in the case of pure diffusion or 'passive' TDD (58). Charged species are repelled into and through the skin as a result of an electrical potential across the membrane. The amount of compound delivered is directly proportional to the quantity of charge passed and is dependent on the applied current, the duration of current application and the area of the skin surface in contact with the active electrode compartment (59).

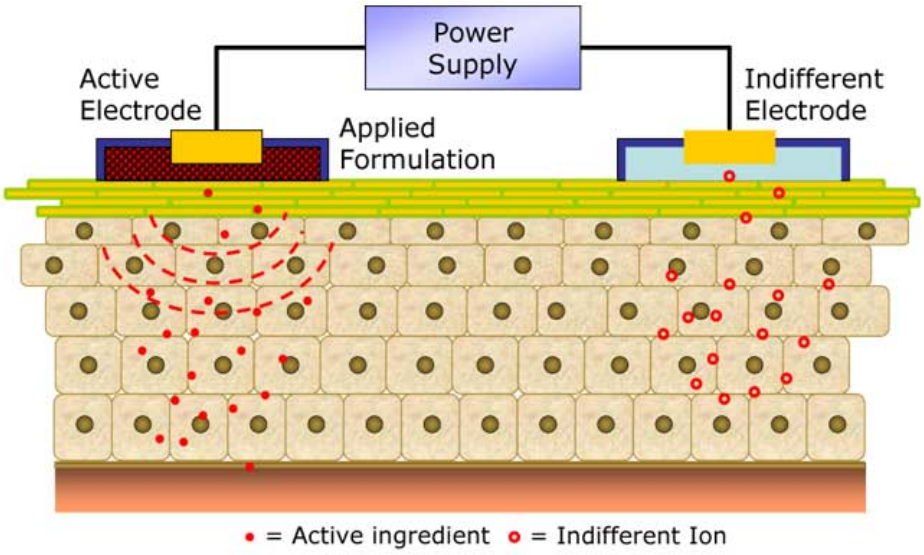



*Figure 1.4 Basic principle of iontophoresis. A current passed between the active electrode and the indifferent electrode repelling drug away from the active electrode and into the skin (63)*

Three main mechanisms enhance molecular transport (25, 33, 56):

i. charged species are driven, primarily by electrical repulsion, from the driving electrode,
ii. the flow of electric current may increase the permeability of skin,
iii. electroosmosis may affect uncharged molecules and large polar peptides.

The efficiency of this process is dependent on the polarity, valency and ionic mobility of the permeant as well as on the composition of the delivery formulation and the current profile (27). A particular advantage of the technique is that the rate of drug delivery may potentially

be regulated by controlling the current through the device. Furthermore, iontophoresis may serve to reduce the intra- and inter-subject variability in the rates of the drug delivery through the skin (56, 57).

#### *1.2.5.2.2 Electroporation*

Electroporation or electropermeabilization is the transitory structural perturbation of lipid bilayer membranes due to the application of high voltage pulses (67, 72) and is also described as the simultaneous creation of a transient, high permeability state and electrically driven transport in bilayer membranes by the application of high voltage for a short period of time (61). It is best known as a physical transfection method in which cells are exposed to a brief electrical pulse, thereby opening pores in the cell membrane, allowing DNA or other macromolecules to enter the cell. The technique of electroporation is normally used on the unilamellar phospholipid bilayers of cell membranes. However, it has been demonstrated that electroporation of skin is feasible, even though the SC contains multilamellar, intercellular lipid bilayers with few phospholipids and no living cells (66). Cell membrane electroporation is widely used to manipulate cells *in vitro*, usually for the introduction of genetic material (70).

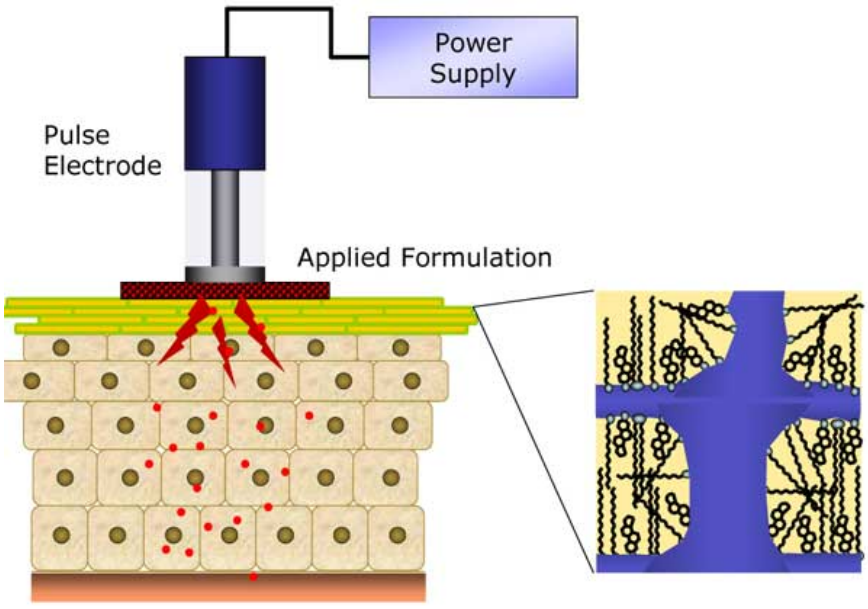



*Figure 1.5 Basic principle of electroporation. High voltage current is applied to the skin producing hydrophilic pores in the intercellular bilayers via momentary realignment of lipids (63)*

The application of high voltage pulses to skin induces the creation of new and/or the enlargement of existing aqueous pathways in the SC through which molecular transport

occurs (67 - 74). These pores allow the passage of macromolecules due to a combination of diffusion, electrophoresis and electroosmosis (63, 67). Permeability and electrical conductance of lipid bilayers can be rapidly and reversibly increased by many orders of magnitude. Electroporation occurs when the transmembrane voltage reaches a few hundred millivolts for electric field pulses, typically of 10 µs to 100 ms duration (63, 68, 69). Despite a high current density within a pore while a high electric field is present, electroporation is theoretically described as a non-thermic phenomenon (71).

#### *1.2.5.2.3 Phonophoresis*

Phonophoresis (or sonophoresis) uses ultrasound energy in order to enhance the skin penetration of active substances (63). It is the movement of drugs through living intact skin and into soft tissue under the influence of an ultrasonic perturbation (83). When skin is exposed to ultrasound, the waves propagate to a certain level and cause several effects that assist skin penetration (63). The propagation of an ultrasonic wave within the skin has two main physical consequences, namely heating and cavitation. These mechanisms may be linked as cavitation may cause heating (76). Although considerable attention has been given to the investigation of sonophoresis in the past years, its mechanisms are not clearly understood, reflecting the fact that several phenomena may occur in the skin upon ultrasound exposure (61).

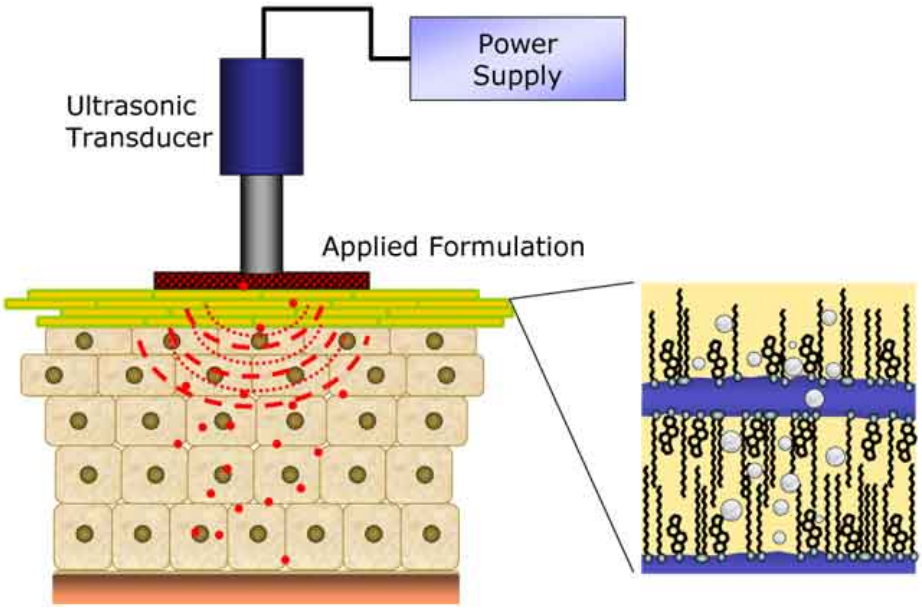



*Figure 1.6 Basic principle of phonophoresis. Ultrasound pulses are passed through the probe into the skin fluidizing the lipid bilayers by the formation of bubbles caused by cavitation (63)*

These include thermal effects due to absorption of ultrasound by the skin, acoustic streaming caused by development of time-independent fluid velocities in the skin due to ultrasound, cavitational effects due to the formation, oscillation and possible collapse of air bubbles in or next to the skin (17, 27, 55) and mechanical effects due to the occurrence of stresses from pressure variation induced by ultrasound (27, 61, 80). Among these, cavitation was found to be primarily responsible for sonophoresis (55, 61, 75, 78 - 80). Although literature supports the observation that increasing temperature leads to enhanced skin permeability (61, 75, 76, 81), recent studies indicate that thermal effects play an insignificant role in promoting transdermal drug transport that is effected using low-frequency (20 - 100 *kHz*) ultrasound and therefore the observed skin permeability is related to the non-thermal effect of ultrasound (79). The overall consequence is increased skin permeability due to increased fluidity of intercellular lipids by heating or mechanical stress and/or by enlarging intercellular space, or by creating permeant or transient holes through corneocytes and keratinocytes as a consequence of cavitation and/or by driving the drug and the vehicle through the permeabilized skin by convection (76, 77). The interest in ultrasound-mediated molecule delivery is based on two factors (10):

i. the capacity to enhance the efficacy of existing transdermal formulations (*e.g.,* anaesthetic and non-steroidal anti-inflammatory drugs) by improving the topical action of the drug and
ii. the potential of sonophoresis for the improvement of patient compliance in therapeutic domains such as diabetes and psychiatry and also in the delivery of vaccines.

Low frequency ultrasound has shown an enhancing effect on the transdermal delivery of various molecules, both *in vitro* and *in vivo*. These methods include *in vitro* and *in vivo* delivery of insulin, mannitol, glucose and heparin (17, 80). Phonophoresis may have an additional advantage in the transcutaneous permeation of non-polar agents due to the utilization of mechanical rather than electromotive force. Differential scanning calorimetry (DSC) and attenuated total reflectance-Fourier transform infrared (ATR-FTIR) studies suggested that there were no irreversible morphological changes in the SC due to ultrasound exposure (83).

*1.2.5.2.4 Microneedle*

Recently, several attempts have been made to enhance the transport of substances across the skin barrier using minimally invasive techniques. The proper function of an appropriate system requires that the SC has to be breached. More recent developments focus on the concepts of microneedles (63). Microneedles are needles that are 10 to 200 µm in length and 10 to 50 µm in width. They are solid or hollow and are connected to a reservoir which contains the active principle. Microneedle arrays are applied to the skin surface so that they pierce the upper epidermis far enough to increase skin permeability and allow drug delivery, but too short to cause any pain to the receptors in the dermis (5, 55, 63, 65).

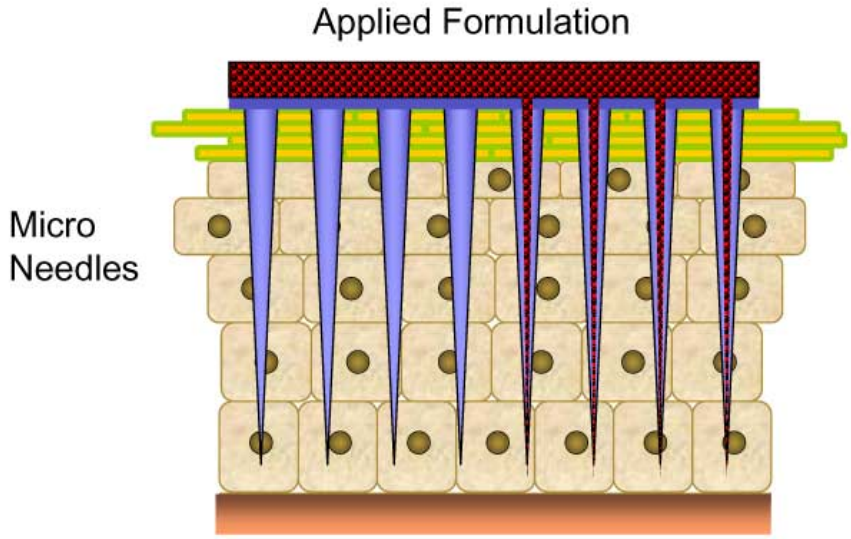



*Figure 1.7 Basic design of microneedle delivery system devices. Needles with or without hollow centre channels are placed onto the skin surface so that they penetrate the SC and epidermis without reaching the nerve endings present in the upper epidermis (63)*

Human studies have shown that microneedles are reported as painless when inserted into the skin of human subjects (55). Microneedles create larger transport pathways of micron dimensions. These pathways are orders of magnitude bigger than molecular dimension and therefore should readily permit transport of macromolecules as well as possibly supramolecular complexes and microparticles (34). Solid microneedles have increased skin permeability *in vitro* by up to four orders of magnitude for compounds ranging from small molecules to proteins to polystyrene nanospheres (55, 64). Therefore there is no limitation concerning polarity and molecular weight of the delivered molecules. The fabrication of such small structures became possible with the advent of micromachining technology which is an essential technology for the microelectronic industry (63).

*Table 1.1 Comparison of methods to enhance transdermal delivery*

| Delivery method | Increased transport | Sustained delivery | No pain/ irritation | Low cost/ complexity |
|---|---|---|---|---|
| Hypodermic needle | Good | Moderate | Limited | Good |
| Chemical enhancers | Limited | Good | Moderate | Good |
| Iontophoresis | Moderate | Good | Good | Limited |
| Electroporation | Moderate | Good | Moderate | Limited |
| Ultrasound | Moderate | Good | Good | Limited |
| Microneedles | Moderate | Good | Good | Limited |
| Jet injection | Good | Limited | Limited | Limited |
| Thermal poration | Moderate | Good | Good | Limited |

#### *1.2.5.2.5 Pressure waves*

Pressure waves (high amplitude pressure transients) generated by lasers is, perhaps, one of the latest platforms for drug delivery (11). They were described by Ogura *et al.* (84) as laser-induced stress waves (LISW). The LISW generated by high-power pulsed lasers are characterised by broadband, unipolar and compressive waves. The LISW interacts with tissues in ways that are different from those of ultrasound. Whereas the action of ultrasound is primarily mediated by heat and cavitation which is induced by a negative pressure, the effects of the LISW are caused by positive mechanical forces (11, 84). Currently the mechanism of permeabilization of the SC by applying a LISW is not well understood. Electron microscopy of the human SC exposed to a LISW showed that there was an expanded lacuna system with the SC although high peak pressures were needed to obtain deep penetration of the drug into the skin. However, increases in the peak pressure and the pulse width of the LISW result in mechanical damage in the tissue (84).

#### *1.2.5.2.6 Other approaches*

Similar to microneedles that pierce the holes into the surface of the skin, *thermal methods* have also been used to locally heat and ablate holes in the SC, thereby increasing skin permeability. This thermal poration approach has been used to deliver conventional drugs and DNA vaccines to animals and to extract interstitial fluid glucose from human subjects.

After a rise and fall in popularity in the mid-twentieth century, *high-velocity jet injectors* are receiving increased attention. The focus now is on improved device designs for controlled, needle-free injection of drug solutions across the skin and into deeper tissues. Insulin is delivered clinically by jet injection and jet injectors for other drugs are under development (55). There have been problems with bruising and particles bouncing off skin surfaces.

Regulatory authorities will need convincing that high velocity particles passing through the SC do no damage and they do not carry contaminants such as bacteria into viable skin layers (24).

Limited work probed the ability of magnetic fields (*magnetophoresis*) to move diamagnetic material through skin (24) although Murthy *et al.* (91, 92) has demonstrated the efficacy of a magnetic field to act as permeation enhancer.

#### *1.2.5.2.7 Synergistic effect of enhancers*

Although the various penetration enhancement methods discussed above have individually been shown to enhance transdermal drug transport, their combinations are often more effective. During the past ten years, several studies have supported this hypothesis, specially addressing combinations of chemicals and iontophoresis, chemicals and electroporation, chemicals and ultrasound, iontophoresis and ultrasound, electroporation and iontophoresis and electroporation and ultrasound. In addition to increasing transdermal transport in a possibly synergistic manner, a combination of enhancers can also reduce the required dose of each enhancer. In this way, combinations of enhancers could increase safety and efficacy. Although combinations offer opportunities, most commercial efforts have emphasized single enhancers, probably due to the complexity of combining multiple technologies (55).

### *1.2.6 Selection of drug candidates for transdermal drug delivery*

One important goal for the pharmaceutical industry is the identification of molecules with the potential for becoming approved drugs (96). The drug development process selects for molecules having the optimal pharmacological activity in the biological assay of choice (26). Although it may appear to be a simple task to select lead compounds for pharmaceutical product development based on therapeutic rationale and compound safety and efficacy, the practicalities of this procedure are somewhat more complex. For the most part, therapeutic efficacy is dependent on the ability of a compound to cross biological barriers, travel to the target site and interact with the receptors. However, it is often more appropriate in dermatological therapy to select compounds based on their inability to breach relevant biological barriers (93). Transport across the SC is largely a passive process and thus the physicochemical properties of a permeant are an important determinant of its ability to penetrate and diffuse across the membrane (94).

#### *1.2.6.1 Biological properties of the drug*

##### *1.2.6.1.1 Potency*

The skin is a very efficient barrier to the ingress of materials, allowing only small quantities of a drug to penetrate over a period of a day. Realistically, a transdermal delivery system should not cover an area much larger than 50 $cm^2$. Drugs such as nitroglycerin, which penetrate skin relatively rapid, do so at fluxes in the 10 - 15 $\mu g/cm^2/h$ range from saturated aqueous solution. Hence the total amount of nitroglycerin which can be delivered across the skin from a 50 $cm^2$ system in one day is approximately 15 mg. In general, therefore, TDD is suitable only for drugs for which the daily dose is of the order of a few milligrams (9).

##### *1.2.6.1.2 Half-life*

The biological half-life of the active is a factor that is often ignored in the selection and design of sustained and controlled drug delivery systems. It is pointless, for example, to produce a transdermal system for a drug which has a very long biological half-life (9).

##### *1.2.6.1.3 Toxicity*

Skin toxicity is another biological property that should be considered. If a pharmacologically active material is to be presented to the skin under an occlusive patch system over an extended period, the likelihood of an irritant or allergic response is significant. Limits will have to be determined for the acceptability of this undesired effect (9).

### *1.2.7 Physicochemical properties of the drug*

#### *1.2.7.1 Oil-water partition co-efficient*

It is generally accepted that the oil-water partitioning characteristics of a chemical are crucial to its ability to penetrate the skin (26) and can be used to predict the partition behaviour within the skin (5). Essentially, the SC barrier is lipophilic, with the intercellular lipid lamellae forming a conduit through which drugs must diffuse in order to reach the underlying vascular infrastructure and to ultimately access the systemic circulation. For this reason, lipophilic molecules are better accepted by the SC. A molecule must first be liberated from the formulation and partition into the uppermost SC layer, before diffusing through the entire thickness, and must then repartition into the more aqueous viable epidermis beneath (27). In general, the more lipophilic chemicals are absorbed more readily and hydrophilic chemicals

may penetrate only very slowly (30). There is often a parabolic relationship between the octanol-water partition co-efficient as expressed by $\log P$ and the penetration rate. Compounds with low $\log P$ exhibit low permeability because there is little partitioning into the skin lipids. However, compounds with high $\log P$ also give low permeability due to their inability to partition out of the SC (5). Thomas *et al.* (5) reported the accepted range of $\log P$ for maximum permeation between 1 and 3. Hadgraft *et al.* (148) in previous work showed that there was maximum percutaneous absorption for a series of NSAIDs and salicylates where the $\log P$ was between 2 and 3.

#### *1.2.7.2 Solubility and molecular dimensions*

The chemical structure of the drug also influences the diffusivity, due to interactions between the polar head groups of the intercellular lipids with hydrogen-bond-forming functional groups present in the drug structure. As a general rule, the number of hydrogen bonding groups in the permeant should not exceed two. Suitable candidates for transdermal permeation are small molecules with good water and lipid solubility. These solubility characteristics are often also indicated by the possession of a low melting point, typically less than 200°C (5). Mathematical models have been described to predict the permeability of the SC to hydrophobic drugs (55). Potts *et al.* (97) developed an equation that correlates skin permeability to a drug in aqueous solution with solute molecular mass and octanol-water partition co-efficient.

#### *1.2.7.3 Polarity and charge*

Polar and non-polar substances may diffuse through the skin by different mechanisms. The more polar substances may follow cellular surfaces or follicular pathways whereas the more non-polar substances may pass through the lipid matrix of the skin (30). Many permeants are weak acids or bases. Permeation will depend on the degree of ionisation and how ionisation influences the solubility in the applied phase and its partition into the skin. One of the problems involved in interpreting permeation data of ionised compounds is that the species that permeate will be a composite of free acid (or base) of the ionised material and ion pairs that can exist with counter ions present either in the formulation or in the skin (32).

## 1.3 MATHEMATICAL PRINCIPLES IN TRANSMEMBRANE DIFFUSION

### *1.3.1 Introduction*

A number of mathematical models have been used to describe percutaneous absorption kinetics. In general, most of these models use either diffusion-based or compartmental equations which suffer from being too complex to be practically useful (98) however sound knowledge of the underlying mathematical principles of membrane transport is essential if we are to expand our understanding of how membrane barriers fulfil their function and how we can alter their properties to our advantage. Much of the early mathematics relating to transmembrane diffusion had its origin in the theoretical description of heat transfer and conductance. Indeed, the most basic of the diffusion equations, Fick's first law, has its roots here (99).

### *1.3.2 Fickian model*

#### *1.3.2.1 Fick's first law of diffusion*

In transport, the flow (or flux, $J_i$ in mol $cm^{-2}$ $s^{-1}$) is related to the velocity of molecular movement ($\upsilon$ in cm $s^{-1}$) and the concentration ($C_i$ in mol $cm^{-3}$) of the molecules in motion in equation 1.1.

$$J_i = C_i \upsilon \tag{1.1}$$

A fundamental principle of irreversible thermodynamics is that the flow, at any point in the system, at any instant, is proportional to the appropriate potential gradient. It can be expressed mathematically for a species $i$ as shown in equation 1.2 where $\partial \mu_i / \partial x_i$ is the gradient and $L_i$ is the proportionality constant.

$$J_i = -L_i \left( \frac{\partial \mu_i}{\partial x} \right) \tag{1.2}$$

Equation 1.2 is the general form of Fick's first law of diffusion. If constant temperature and pressure is assumed equation 1.2 can be expressed as equation 1.3 where $D_i$ is the diffusion co-efficient.

$$J_i = -D_i\left(\frac{\partial C_i}{\partial x}\right) \tag{1.3}$$

*1.3.2.2 Fick's second law of diffusion*

Fick's second law relates the rate of change in concentration with time at a given point in a system to the rate of change in concentration gradient at that point. It is expressed in equation 1.4 where $x$ is the diffusion distance in the direction of the concentration gradient.

$$\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial x^2} \tag{1.4}$$

Fick's laws are more applicable if certain parameters or boundaries are specified. In this laboratory, the mathematical boundary conditions imposed are those of a well-designed diffusion experiment when the permeant is at a high, fixed activity on one side of an inert homogenous membrane through which it diffuses into a sink on the other side and before the start of the experiment the membrane is entirely devoid of permeant (99).

This implies that the diffusive flow begins at the high-concentration side (the donor side) of the membrane where $C = C_0$ and $x = h$ at all time intervals, $t$. There is no diffusant material within the membrane before ingress of the permeant being modelled, implying that at $t = 0$ we have $C = 0$ for all values of $x$. Diffusion occurs in the direction of decreasing $x$ toward the opposite side of the membrane where $x = 0$ and $C = 0$ (sink receptor phase) for all time intervals, $t$. The cumulative mass $Q$, of permeant that passes through a unit area of a membrane in a time $t$ is shown in equation 1.5 where $C_0$ is the concentration of diffusant in the membrane lamina juxtaposed to the donor vehicle, and h is the thickness of the membrane (99).

$$Q = C_o h\left[\frac{Dt}{h^2} - \frac{1}{6} - \frac{2}{\pi^2}\sum_{n=1}^{\infty}\frac{(-1)^n}{n^2}\exp\left(\frac{-Dn^2\pi^2 t}{h^2}\right)\right] \tag{1.5}$$

As $t \to \infty$, the exponential term tends to zero and Fick's second law has been expanded to become equation 1.6.

$$Q = \frac{DC_o}{h}\left[t - \frac{h^2}{6D}\right] \tag{1.6}$$

This equation is applicable to typical cumulative mass versus time diffusion plots that have an initial non-linear lag time followed by a linear steady state plot (100). From equation 1.6, we can solve for $t$ and this yields the lag time ($t_{lag}$) as described by equation 1.7 which relates inversely to the diffusion co-efficient and directly to the diffusional pathlength.

$$t_{lag} = \frac{h^2}{6D} \tag{1.7}$$

When equation 1.6 is differentiated relative to time we obtain equation 1.8, possibly the most well-known form of Fick's law of diffusion that describes the flux $J$, at steady state.

$$\frac{dQ}{dt} = J = \frac{DC_o}{h} \tag{1.8}$$

It is often impractical to use the forms of equations 1.6 and 1.8 as shown because they include a term $C_o$ (the concentration of permeant in the outer layer of the membrane), that is extremely difficult to measure. The value $C_o$ is replaced with a term that links it to the concentration in the vehicle $C_v$ through the partition co-efficient $K$, which rearranges to give equation 1.9.

$$\frac{dQ}{dt} = J = \frac{DKC_v}{h} \tag{1.9}$$

The product of the partition co-efficient and the donor vehicle concentration will yield the diffusant concentration in the membrane lamina. Therefore the main variables influencing the rate of diffusion are $D$, $K$, $C_v$ and $h$ (100). Frequently, particularly in biological membranes, there is a practical difficulty in measuring the diffusional pathlength and that the information concerning the individual effects of changes in $K$ and $D$ is often not required, a composite parameter is usually used to replace these values in equation 1.9. The permeability co-efficient $P$, is thus defined as $P = KD/h$, and this simplifies equation 1.9 to further give equation 1.10.

$$J = PC_v \tag{1.10}$$

Equation 1.10 is perhaps the most basic and frequently used expression in the routine assessment of membrane permeability. However the principles upon which this equation is based stipulate that the donor concentration is constant and that the diffusion has reached steady state (99).

### *1.3.3 Higuchi model*

Higuchi (101) describes drug release as a diffusion process based on Fick's law, square root time dependant. This relation can be used to describe the drug dissolution from several types of modified release pharmaceutical dosage forms, as in the case of some transdermal systems and matrix tablets with water soluble drugs (102). For drug release from an ointment in which the drug is initially uniformly dissolved is governed by equation 1.11 and 1.12 where $Q$ is the amount of drug released per unit area of application, $h$ is the thickness of layer, $C_o$ is the initial concentration of the drug in the ointment, $D$ is the diffusion co-efficient of drug in the ointment, $t$ is the time after application and $R$ is the percent of drug released (101, 104).

$$Q = 2C_o\left(\frac{Dt}{\pi}\right)^{\frac{1}{2}} \tag{1.11}$$

$$R = 200\left(\frac{Dt}{\pi h^2}\right)^{\frac{1}{2}} \tag{1.12}$$

If the rate of drug release obeys this law, the amount of drug released is a linear function of $t^{½}$, and $D$ can be calculated from the slope. The assumptions in this treatment are that the drug is the only component diffusing out of the vehicle, that sink conditions are maintained in the receptor phase and that $D$ is constant with respect to time and position in the vehicle (101, 103). Permeation of this nature has a characteristic curved profile, exhibiting relatively high flux at early contact times which decreases as the diffusant front regresses into the bulk vehicle, away from the membrane. The path is progressively more tortuous and it takes

longer for drug molecules to diffuse from the region of high concentration in the vehicle to replenish the drug molecules at the membrane interface that have partitioned into the membrane, therefore the flux rate decreases with time (100).

Equations 1.1 - 1.12 describe drug diffusion through a homogenous membrane with a constant activity difference and a constant diffusion co-efficient and these mathematical functions have been employed in the analysis of data in this laboratory.

It is evident that from sections 1.2 - 1.3 that the transport of drugs though the skin depends on a number of factors such as the characteristics of the permeant, condition and type of skin, other chemicals present in the topical formulation (*e.g.,* enhancers) and external conditions (*e.g.,* temperature). The factor with perhaps the greatest influence is the physicochemical character of the permeant. According to Fick's first law (section 2.3.2.1), the permeation of a drug through the SC depends on the permeability co-efficient and the concentration gradient of the permeant across the SC. The permeability co-efficient is the product of the partition and the diffusion co-efficient, divided by the length of the pathway through the SC. These factors, in turn, depend on variables such as molecular weight, size and structure and degree of ionization of the permeant (95).

## 1.4 METHODS FOR STUDYING PERCUTANEOUS ABSORPTION

### *1.4.1 Introduction*

There is an increasing demand for data describing the rate, degree and route of penetration of compounds across human skin. First, there is a requirement to optimise the delivery of dermatological drugs into various skin strata for maximum therapeutic effect. Second, the transdermal and topical routes have become popular alternatives to more traditional methods of drug delivery. A third stimulus has been the toxicological and risk assessment implications of the everyday use of a wide range of potentially harmful materials in the agrochemical, chemical, cosmetic, household and pharmaceutical sectors. This has been driven largely by regulatory and safety bodies and a perceived need for improved data on the permeability of the skin to xenobiotics (111). A key aspect of any new drug product is its safety and efficacy as demonstrated in controlled clinical trials. However the time and expense associated with such trials make them unsuitable as routine quality control methods to re-establish comparability in quality and performance following a change in formulation or method of manufacture. Therefore, *in vitro* and *in vivo* surrogate tests are often used to assure that product quality and performance are maintained over time in the presence or absence of change (107). At present, U. S. Food and Drug Administration (FDA) approval for transdermal dosage forms requires *in vivo* and where appropriate, *in vitro* data. *In vivo* studies include clinical safety and efficacy, local irritation, systemic toxicity and bioavailability. *In vitro* studies, on the other hand, include quality control procedures such as assay, content uniformity and drug release characteristics (112).

### *1.4.2 Diffusion cell design*

Numerous designs of apparatus for studying diffusion of active ingredients from semisolids have appeared in the literature (103 - 120). In general, these systems consist of a donor cell which holds the semisolid material and a receptor cell or compartment which holds the chosen receptor medium and from which samples are withdrawn at regular intervals. The system may or may not include a membrane separating the two phases and means of stirring and controlling the temperature of the receptor medium (116).

The *in vitro* diffusion cells should be made from inert, non-reactive materials (such as glass, stainless steel, Teflon). Inertness (lack of absorption) to all components of the cell, including

flow-through lines and the collection chambers themselves, should be demonstrated by the experiment. It should also be shown that there is no loss of drug through its volatility during the permeation procedure. If volatility is a problem, a quantitative accounting of this must be made. The receptor medium should provide an effective sink for the penetrant. Ideally, it should, at the same time, contain a minimum volume to facilitate analysis because, in general, the more concentrated the drug in the collection medium, the easier the assay procedure. The cell design should allow the receptor fluid to be well mixed and temperature controlled (116). *In vitro* systems range in complexity from a simple two-compartment static diffusion cell to multijacketed flow-through cells (111). Although numerous studies using modifications of the various systems described above have been reported (103 - 120), two design types have shown the highest potential for use as standardised, compendial methods. They are the vertical diffusion cells for measurement of percutaneous absorption (*i.e.,* Franz and modified Franz), which require the use of a membrane when measuring drug release and immersion cells for use with the standard USP dissolution apparatus (*i.e.,* the European Pharmacopoeia diffusion cell), which may be used with or without a membrane (116).

#### *1.4.2.1 Franz and modified Franz diffusion cell*

Most of the published work on *in vitro* release from semisolids used vertical Franz-type cells (115). Finite dose techniques and the design of a static one-chambered diffusion cell were described by Franz (121, 122). Shortcomings of the original Franz cells were identified and modifications were proposed to improve adequate solution hydrodynamics, mixing efficiency and temperature control (271). These modifications were employed in this study. The cell body consists of a jacketed glass receptor chamber, 12.5 ml in volume (modified cells exist with slightly different receptor volumes) and a glass sampling port. The membrane is placed horizontally over the receptor chamber, the cell cap is applied over that, and the components are held together with a metal clamp. The test formulation can then be applied to the surface of the membrane through the top of the cell cap, which is open to the atmosphere unless sealed by the user. A study in this laboratory by Pefile *et al.* (123) showed that the inability to control the evaporation process of the volatile components from the vehicle resulted in erratic drug release from the unoccluded bases with marked variability in drug release rates. Therefore the top of the cell cap was sealed during the diffusion run. A micro-magnetic stirrer is placed at the bottom of the receptor chamber.

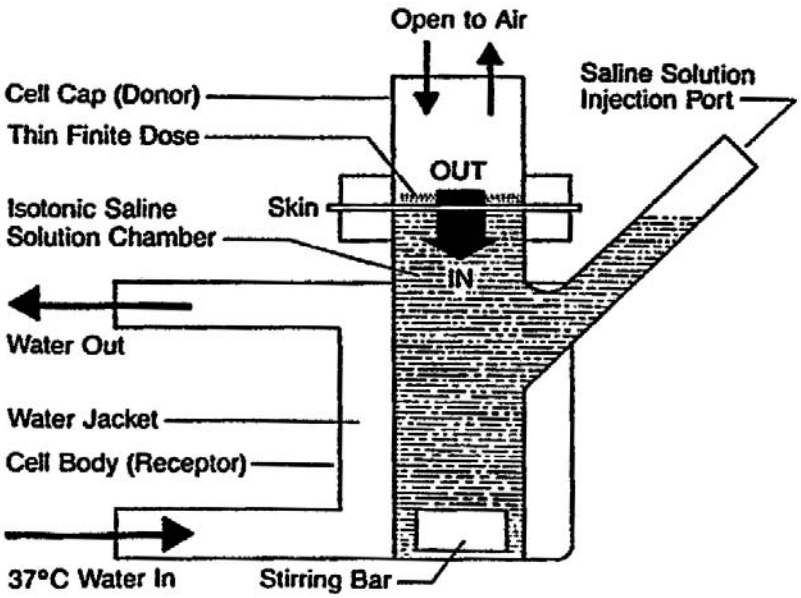



*Figure 1.8 Modified Franz diffusion cell (116)*

The temperature in the bulk of the receptor medium is maintained by circulating water through a water jacket that surrounds the receptor compartment (223). The entire cell is positioned in a multiple-cell drive unit which drives the magnetic stirrer to agitate the receptor medium at a controlled rate of 100 rpm. The jacketed portions of multiple cells are connected in series with tubing to a circulating temperature-controlled water bath (116).

### *1.4.2.2 European Pharmacopoeia diffusion cell*

An alternate to the Franz diffusion cell is the use of an immersion cell with a standard USP dissolution apparatus. The European Pharmacopoeia diffusion cell, although similar in design and operation to the VanKel enhancer cell and to the Hanson ointment cell (116), has some notable differences. The European Pharmacopoeia diffusion cell is described in detail in the European Pharmacopoeia (124). The diffusion cell consists of a support, a cover and a membrane. The support contains a central depression which acts as the reservoir for the transdermal dosage form. The depth of the central reservoir is 2.6 mm and the diameter 38 mm. The cover has a central aperture with a diameter of 32 mm and a corresponding surface area of 8.03 $cm^2$ through which the topical formulations diffuses. The cover is held firmly in place above the support by four screws extending into the support base.

The diffusion cell is assembled and placed into a cylindrical vessel with the diffusion surface facing upwards and parallel to the horizontal plane. The cylindrical vessel is made from

borosilicate glass or other suitable transparent material, with a hemispherical bottom and a nominal capacity of 1000 ml. The vessel has a flanged upper rim and is fitted with a lid that has a number of openings, one of which is central. The USP dissolution apparatus is equipped with a motor with a speed regulator capable of maintaining the speed of rotation of the paddle within ± 4% of 100 rpm. The motor is fitted with a stirring element which consists of a drive shaft and a paddle. The paddle is lowered to a depth corresponding to 10 ± 2 mm above the diffusion cell surface. The temperature of the receptor fluid is maintained at 32 ± 5 °C. Evaporation is prevented by the fitted lid. Samples are taken from the cylindrical vessel at suitable time intervals (124).

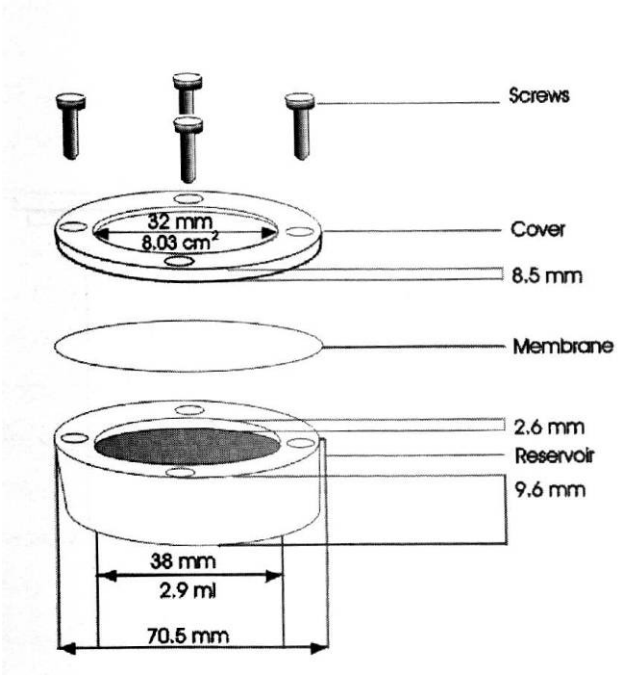



*Figure 1.9 European Pharmacopoeia diffusion cell (125)*

The diffusion cell is easy to load and most laboratories have a USP dissolution apparatus (115) and it does not have the serious drawback due to the presence of air bubbles at the membrane/liquid interface as commonly observed with the Franz diffusion cells (110). Fares *et al.* (115), Liebenberg *et al.* (105) and Sanghvi *et al.* (125) compared the rate of drug release from both the Franz cells and the enhancer cells and concluded that once the data is corrected for differing surface area, drug release is nearly superimposable.

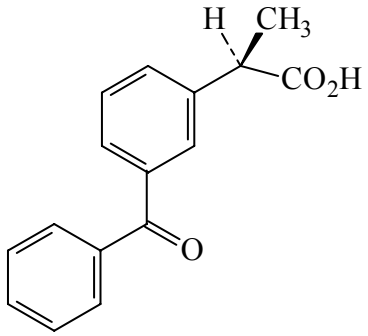
# CHAPTER TWO
# KETOPROFEN MONOGRAPH

## 2.1 PHYSICOCHEMICAL PROPERTIES OF KETOPROFEN

### *2.1.1 Introduction*

Ketoprofen is an anionic non-steroidal anti-inflammatory drug (NSAID). It is a derivative of propionic acid and widely used in the management and treatment of patients with rheumatic disease (131). It has been described chemically in a number of ways:

i. (2*RS*)-2-(3-benzoylphenyl) propanoic acid (132)
ii. 2-(3-benzoylphenyl) propionic acid (133, 134, 135, 138)
iii. 2-(benzoyl-3-phenyl) propionic acid (135)
iv. 3-benzoyl-α-methylbenzeneacetic acid (134, 138)
v. α-(benzoylphenyl) propionic acid (135)
vi. α-(3-benzoylphenyl) propionic acid (135)
vii. m-benzoylhydratropic acid (135, 138, 139)
viii. (*RS*)-2-(3-benzoylphenyl) propionic acid (131, 140).




$C_{16}H_{14}O_3$ MM 254.3 g/mol

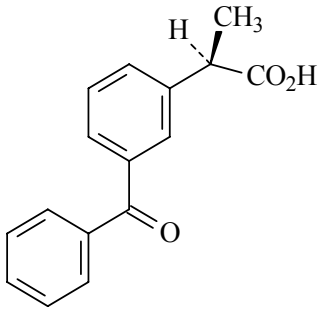
*Figure 2.1 Structure of ketoprofen*

### *2.1.2 Description*

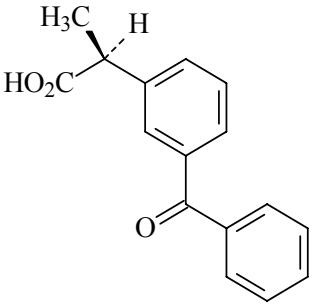
Ketoprofen is a white or almost white, crystalline odourless powder with a sharp bitter taste (131-134, 140). It is prepared by chemical synthesis as a racemate (131) and contains not less than 99.0% and not more than the equivalent of 100.5% of (2*RS*)-2-(3-benzoylphenyl) propanoic acid, calculated with reference to the dried substance (132).

### *2.1.3 Stereochemistry*

The presence of at least one asymmetric carbon atom in a chemical entity results in the existence of stereoisomers. Ketoprofen has one asymmetric carbon, also referred to as a chiral centre, which gives rise to two enantiomers (140). Both enantiomers possess different biological activities (142). The (*S*)-enantiomer reduces inflammation and relieves pain, whereas the (*R*)-enantiomer can be used as a toothpaste additive to prevent periodontal disease. The majority of synthetic chiral drugs are now marketed as racemates, but this situation is rapidly changing due to Food and Drug Administration (FDA) regulations and recent advances in biocatalytic methods (143, 144).




(*R*) - enantiomer




(*S*) - enantiomer

*Figure 2.2 Stereochemistry of ketoprofen*

### *2.1.4 Melting point*

Ketoprofen has been reported to melt in the range of 94°C to 97°C (132, 140), 93°C to 95°C, 94°C, 96°C, 92°C, 91°C (135) and 93°C to 96°C (134).

### *2.1.5 Solubility*

Ketoprofen is practically insoluble in water, freely soluble in acetone, ethanol and methylene chloride (132). It is also soluble in chloroform, ether and benzene (134). In ethanol, ketoprofen has a solubility of about 1 in 5 and in water < 1 in 10 000 (131). Adjusting the pH to a higher value can solubilize ketoprofen, as solubility increases at pH values above its p$K_a$ (141).

### *2.1.6 Dissociation constant*

Ketoprofen is a weak monocarboxylic acid (141, 145) and has reported dissociation constant values, p*Ka*, of 4.23 (147), 4.55 (131), 4.45 (134, 146), 4.60 (141, 145, 149) in water and 5.02 in aqueous solutions of pH 1.5 (148). The p*Ka* will be an important determinant in ionisation and hence permeation (150).

### *2.1.7 Maximum flux ($J_{max}$)*

The maximum flux through the skin is obtained by taking the permeability co-efficient and multiplying it by the aqueous solubility. The $J_{max}$ is reported as 0.75 μg/cm$^2$/h (147, 150).

### *2.1.8 Partition co-efficient and permeability co-efficient*

There is a linear free energy relationship between lipophilicity and biological activity. As a suitable measure of lipophilicity, the partition co-efficient, $P$, between 1-octanol and water was determined. The value of $P$ varies slightly with temperature and concentration of solute (151). The octanol-water partition co-efficient ($\log P$) has been reported as 3.12 (147, 150). Previous work by Hadgraft *et al.* (147) showed that there was maximum percutaneous absorption for a series of NSAIDs and salicylates where the $\log P$ was between 2 and 3. At low $\log P$, the permeability co-efficient is low but the aqueous solubility is high and at high $\log P$, the permeability co-efficient is high but the aqueous solubility is low. The reported permeability co-efficient is 5.01 x $10^{-3}$ (cm/h)$^2$ (147, 150). NSAIDs tend to have low solubilities and high permeabilities at low pH, but high solubilities and reduced effective permeabilities at higher pH (149).

### *2.1.9 Optical rotation*

Ketoprofen is a racemic mixture of (±) α-(3-benzoylphenyl) propionic acid. Both enantiomers show Cotton Effects at 223 nm. The (+)-enantiomer shows a positive Cotton Effect indicating an *S*-absolute configuration and interacts more strongly with human serum albumin as well as with biotransformation enzymes than the (-)-enantiomer (135).

(+)-enantiomer $[\alpha]_D^{23}$ + 57.1 o (C = 0.76 in $CH_2Cl_2$)

(-)-enantiomer $[\alpha]_D^{23}$ - 57.4 o (C = 0.88 in $CH_2Cl_2$)

*2.1.10 Synthesis*

Ketoprofen was synthesised by Rhône-Poulenc Research Laboratories, Paris in 1967 and was first approved for clinical use in France and the United Kingdom in 1973 (136, 137). Several methods for the synthesis of ketoprofen have been reported in the literature. Figures 2.3 - 2.5 show the synthesis starting from (3-carboxyl-phenyl)-2-propionitrile, 2-(4-aminophenyl)-propionic acid and (3-benzoylphenyl)-acetonitrile respectively.

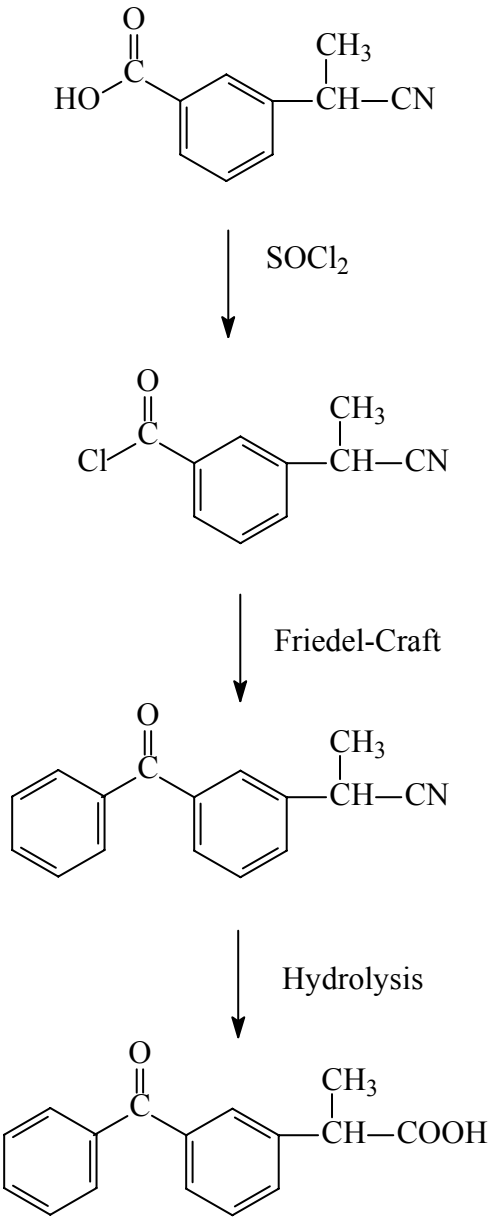



*Figure 2.3 Synthesis of ketoprofen starting from (3-carboxy-phenyl)-2-propionitrile (135)*

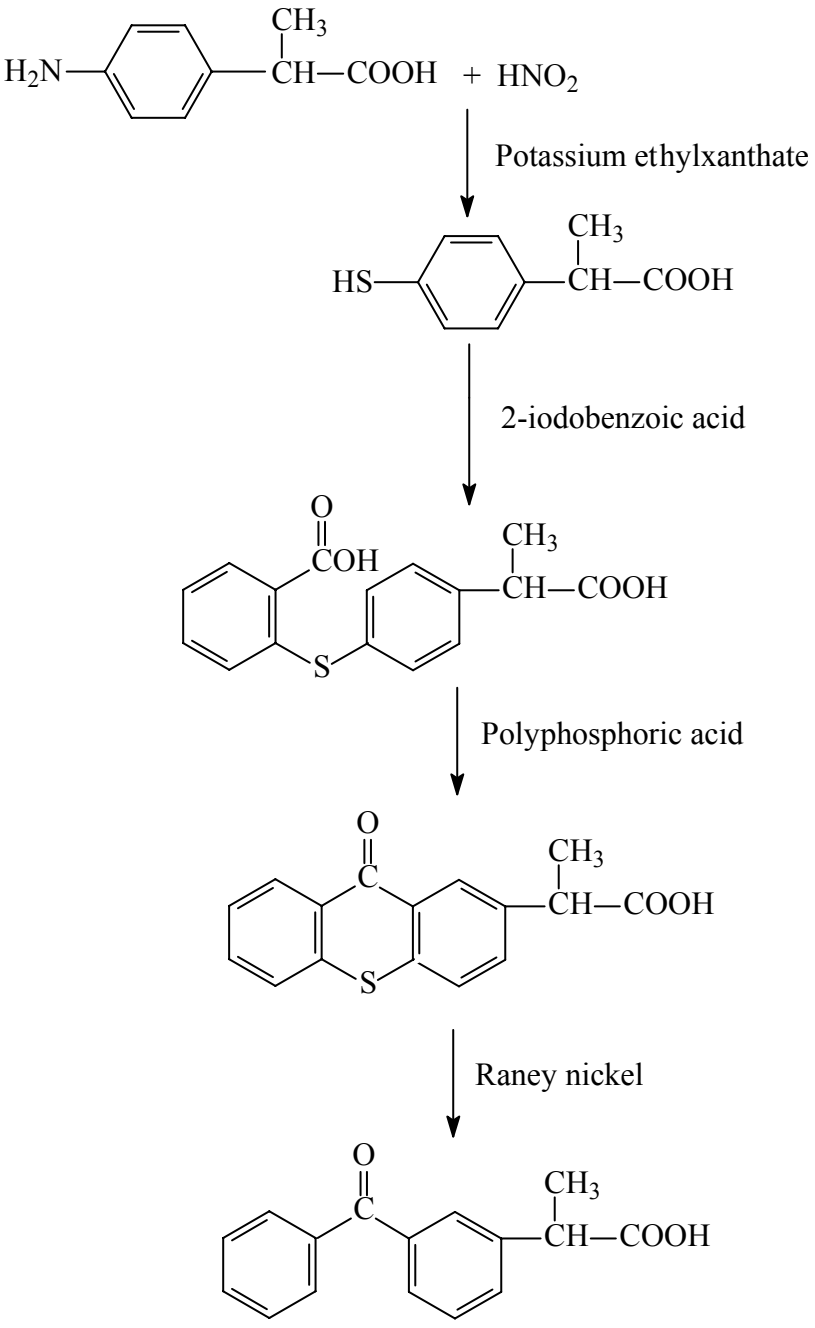



*Figure 2.4 Synthesis of ketoprofen starting from 2-(4-aminophenyl)-propionic acid (135)*

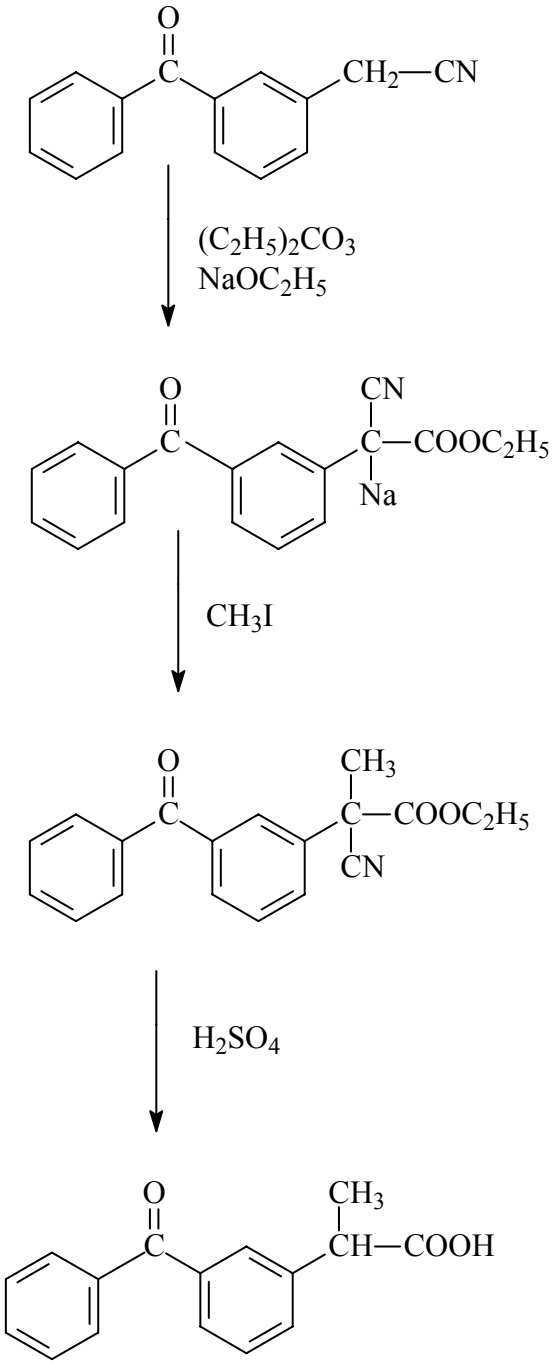
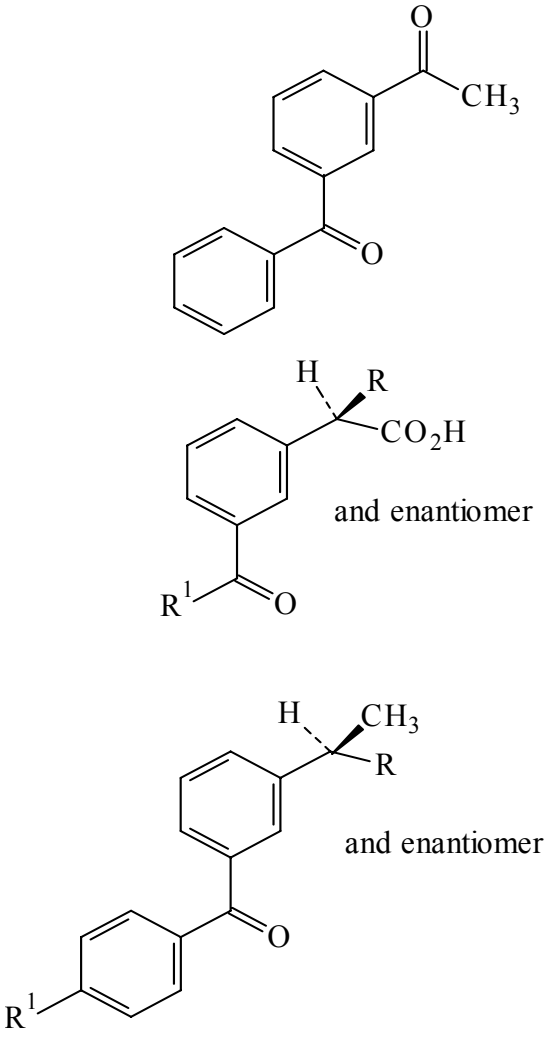



*Figure 2.5 Synthesis of ketoprofen starting from (3-benzoylphenyl)-acetonitrile (135)*

### *2.1.11 Stability*

Ketoprofen must be protected from light and moisture (135). Exposure of aqueous solutions of ketoprofen (as the sodium salt) to ultraviolet radiation at 254 nm or daylight, for one hour at room temperature, was reported (134) to yield (3-benzoylphenyl) ethane which was subsequently converted to (3-benzoylphenyl) ethanol and (3-benzoylphenyl) ethanone (analysis by thin layer chromatography and high-performance liquid chromatography). Samples that were protected from light showed negligible decomposition over 24 months.




1-(3-benzoylphenyl) ethanone

R = H, $R^1 = C_6H_5$:
(3-benzoylphenyl) acetic acid
R = $CH_3$, $R^1$ = OH:
(2*RS*)-2-(3-carboxyphenyl) propanoic acid

R = $CO_2H$, $R^1 = CH_3$:
(2*RS*)-2-[3-(4-methylbenzoyl) phenyl] propanoic acid
R = CO-$NH_2$, $R^1$ = H:
(2*RS*)-2-(3-benzoylphenyl) propanamide
R = CN, $R^1$ = H:
(2*RS*)-2-(3-benzoylphenyl) propanenitrile

*Figure 2.6 Ketoprofen impurities and photodegradation products (132)*

### *2.1.12 Ultraviolet absorption*

The ultraviolet (uv) spectrum of ketoprofen in 0.2 M phosphate buffer at pH 6.8 is depicted in Figure 2.7. The $\lambda_{max}$ is 260 nm. The spectrum was obtained using a double beam GBC UV/Vis 916 Spectrophotometer from GBC Scientific Equipment Pty Ltd (Victoria, Australia). The solvent also affects the $\lambda_{max}$. In acetonitrile the $\lambda_{max}$ is 254 nm. The $\lambda_{max}$ reported in alcohol is 255 nm (132, 135).

### *2.1.13 Infrared spectrum*

The major band assignments of ketoprofen are given in Table 2.1.

*Table 2.1 Major infrared band assignments of ketoprofen (135)*

| **Band position ($cm^{-1}$)** | **Assignment** |
|---|---|
| 3200 - 2500 | O - H stretching |
| 3020 | C - H stretching of aromatic groups |
| 2970, 2930 | C - H stretching of $CH_3$ group (asymmetric) |
| 2880 | C - H stretching of $CH_3$ group (symmetrical) |
| 1695 | C = O stretching of the acid |
| 1655 | C = O stretching of the ketone |
| 1595, 1580, 1455 | C = C stretching of the aromatic ring |
| 1440 | C – H deformation of $CH_3$ (asymmetrical) |
| 1370 | C – H deformation of $CH_3$ (symmetrical) |
| 860 - 690 | C – H deformation of aromatic rings |

### *2.1.14 Nuclear magnetic resonance spectrum*

The $H^1$-NMR spectrum of ketoprofen in $CDCl_3$ on an EM-360 6 MHz NMR spectrophotometer is shown in Figure 2.9. Table 2.2 compares published values for ketoprofen.

*Table 2.2 Published ketoprofen $H^1$-NMR spectrum values (135)*

| **Instrument used** | **Values** |
|---|---|
| Varian EM-360 | 1.53 (d, 3 H, J = 7 Hz), 3.80 (q, 1 H, J = 7 Hz), 7.20 – 7.90 (m, 9 H), 11.50 (s, 1 H) |
| Varian T60 & A60 | 1.52 (d, 3 H, J = 7 Hz), 3.76 (q, 1 H, J = 7 Hz), 7.2 – 7.8 (m, 9 H), 11.8 (s, 1 H) |

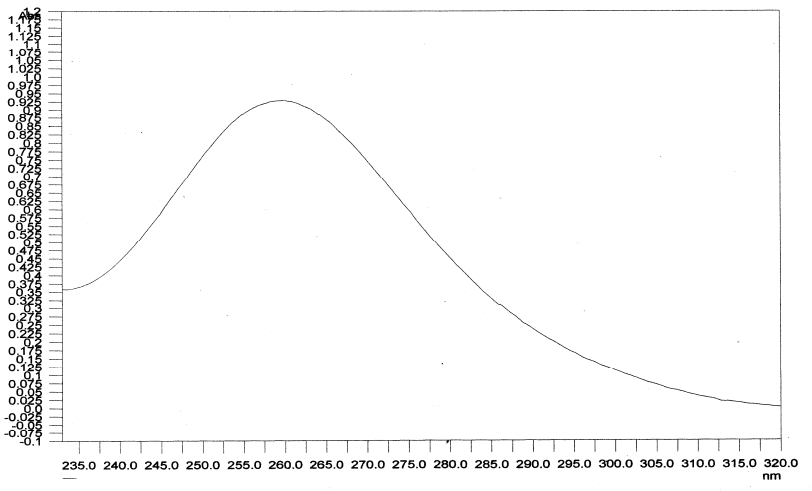



*Figure 2.7 Ultraviolet spectrum of ketoprofen standard in aqueous solution*

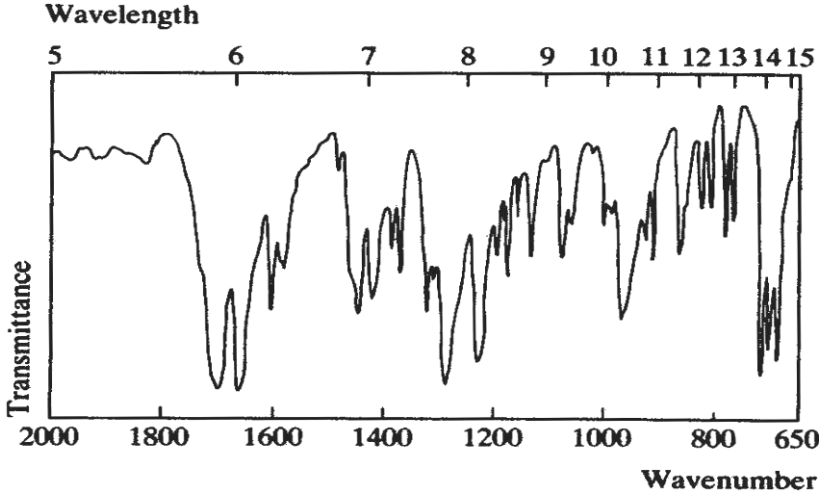



*Figure 2.8 Infrared spectrum of ketoprofen (135)*

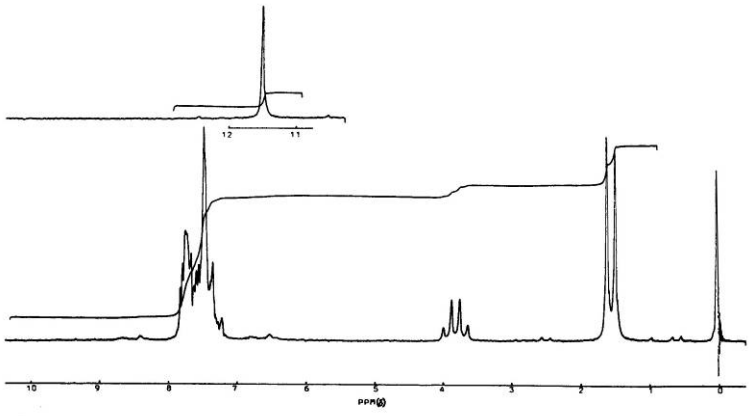


*Figure 2.9 Nuclear magnetic resonance spectrum of ketoprofen (135)*

## 2.2 CLINICAL PHARMACOLOGY OF KETOPROFEN

Ketoprofen is an effective anti-inflammatory and analgesic drug in clinical practice and is used in the treatment of rheumatoid arthritis and osteoarthritis. It is as effective in clinical trials as other NSAIDs such as naproxen from both the efficacy and side effect point of view (131). Like most NSAIDs, ketoprofen is advantageous because it lacks addictive potential and does not result in sedation or respiratory depression. In addition ketoprofen also exerts analgesic and antipyretic pharmacological properties (152).

### *2.2.1 Anti-inflammatory effects*

In several animal models (rats, mice, rabbits, guinea pigs and pigeons) ketoprofen displayed potent activity against acute inflammation (increased vascular permeability, oedema and erythema), sub acute inflammation (pleurisy, abscess and granuloma formation), and chronic inflammation (experimental arthritis and synovitis) (136). Its anti-inflammatory activity is 20 times more potent than ibuprofen, 80 times more potent than phenylbutazone and 160 times more potent than aspirin (131, 136).

### *2.2.2 Analgesic and antipyretic effects*

Ketoprofen was shown to be a potent, peripherally acting analgesic in two classical animal models of pain. It was also shown to be equivalent to indomethacin, slightly more potent than naproxen and 30 times more potent than aspirin in pain management. Like other NSAIDs, ketoprofen is inactive in assays measuring centrally mediated analgesia. It did not reduce basal temperature but decreased antigen-induced hyperthermia in rats and rabbits to a greater extent than any other NSAID tested, including indomethacin, naproxen, ibuprofen and phenylbutazone (136).

### *2.2.3 Mechanism of action*

As with all NSAIDs, the physiological basis of the pharmacodynamic activities of ketoprofen is presumed to be interference with arachidonic acid metabolism (136). Arachidonic acid is the most abundant and probably the most important of the precursors of the eicosanoids. It is a 20-carbon fatty acid that contains four double bonds beginning at the omega-6 position to yield 5, 8, 11, 14-eicosatetraenoic acid (153). Free release of arachidonic acid from membrane phospholipids is catalysed by the enzymatic activation of phospholipid $A_2$. It is

then converted to various forms of prostaglandins, including thromboxane $A_2$ ($TXA_2$), prostaglandin $D_2$ ($PGD_2$), prostaglandin $E_2$ ($PGE_2$), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and prostaglandin $I_2$ ($PGI_2$) by the cyclooxygenase activity of the COX enzyme. Prostaglandins have overlapping roles but co-ordinately regulate smooth muscle contractility, mediation of pain and fever, regulation of blood pressure and platelet aggregation (154, 174).

Cyclooxygenase (COX), a prostaglandin-endoperoxide synthase (PTGS), catalyses the formation of prostaglandins from arachidonic acid (155). COX is a dual function enzyme, incorporating both a cyclooxygenase and a peroxidase activity (174). There are principally two isozymes of COX, COX-1 (constitutive isozyme) and COX-2 (inducible isozyme) (152-156). Although a COX-3 isozyme has recently been identified (154, 157), its existence with respect to activity has been challenged (154, 158). These isozymes have diverse physiologic and pathophysiologic roles and exhibit pharmacologically important differences in structure and profiles of inhibition (156). The COX-1 isozyme is essential for the maintenance of normal physiologic states in many tissues including the kidneys, gastrointestinal tract and platelets. The COX-2 isozyme is induced by various inflammatory stimuli including cytokines, endotoxins and growth factors (155, 171, 174).

Ketoprofen is one of the most powerful inhibitors of cyclooxygenase at concentrations well within the range of therapeutic plasma concentrations (EC50 2 μg/l) (136). It produces reversible COX inhibition by competing with the substrate, arachidonic acid, for the active site of the enzyme (174). This inhibition results in a reduction in the tissue production of prostaglandins such as $PGE_2$ and $PGF_{2\alpha}$ (131). In addition to its effects on cyclooxygenase, ketoprofen inhibits the lipoxygenase pathway of the arachidonic acid cascade. This pathway produces non-cyclized monohydroxyl acids (HETE) and leukotrienes. Of these, only leukotrienes (B4, C4, and D4) are thought to increase vascular permeability, however, both HETE and leukotrienes synthesised within leukocytes are active in promoting leukocyte migration and activation. It has been suggested that lipoxygenase inhibitors may attenuate cell-mediated inflammation and thus retard the progression of tissue destruction in inflamed joints. Ketoprofen is also a powerful inhibitor of bradykinin, an important chemical mediator of pain and inflammation. It also stabilises lysosomal membranes against osmotic damage and prevents the release of lysosomal enzymes that mediate tissue destruction in inflammatory reactions (131, 136, 137, 159, 168).

### *2.2.4 Therapeutic use*

#### *2.2.4.1 Indications*

Ketoprofen is used for musculoskeletal and joint disorders such as ankylosing spondylitis, osteoarthritis, and rheumatoid arthritis, and in peri-articular disorders such as bursitis and tendinitis. It is also used for postoperative pain, painful and inflammatory conditions such as acute gout or soft tissue disorders and to reduce fever (131, 140, 148, 152). It is also indicated for the management of acute painful shoulder syndrome and juvenile rheumatoid arthritis (136). Ketoprofen can also be used in the following instances.

i. For prophylaxis and treatment of migraine headaches.
ii. In surgical and traumatic situations where analgesic action is required for sports injuries, orthopaedic manipulations, dental extraction.
iii. In infectious diseases which require analgesic, anti-inflammatory and anti-pyretic effects.
iv. In gynaecological conditions which involve the management of dysmenorrhoea following intra-uterine device (IUD) insertion and for uterine relaxation and analgesia in post-partum, non-nursing women (131).

#### *2.2.4.2 Contraindications*

Ketoprofen is contraindicated in the following medical conditions.

i. Bronchospasm

   Patients with rhinitis, nasal polyps and asthma associated with aspirin use may show cross-sensitivity with other non-steroidal anti-inflammatory drugs including ketoprofen (131). NSAIDs can affect the lungs through inhibition of prostaglandins and increased concentrations of leukotrienes, leading to asthma exacerbation in susceptible patients (152).

ii. Peptic ulceration

   Ketoprofen should not be administered to patients with active peptic ulceration or with a history of recurrent peptic ulceration or chronic dyspepsia (131). NSAIDs damage the gastrointestinal tract (GIT) through both local and systemic effects. At the superficial mucosal level, NSAIDs act as weak acids and because of the highly acidic milieu of the stomach, they remain in the non-ionised form that favours migration across cell membranes and into superficial epithelium. Once there, they are metabolised into their ionised form where they trap hydrogen ions. NSAIDs can also

attenuate the protective effects of the gastric mucosa, leading to epithelial damage. Although it has been theoretically possible to reduce upper GIT damage at the local level, systemic effects are responsible for injury *via* inhibition of protective prostaglandins (152).

iii. Severe renal insufficiency

Since prostaglandins synthesised in the kidneys are potent vasodilators that balance the effects of vasoconstrictive stimuli (norepinephrine, angiotensin II and renin) on renal blood flow, preventing their production will affect renal function in some situations. As expected, the presence of underlying pathologic conditions that cause renal ischaemia, such as congestive heart failure, high renal state, cirrhosis and renal disease predispose the patients to adverse renal effects during NSAID treatment (136, 152). Any patient with these risks is highly dependent on prostaglandins for renal flow. Renal functional changes induced by NSAIDs, whether asymptomatic or accompanied by oedema, are reversible on withdrawal of the drug (136).

### *2.2.5 Adverse reactions*

NSAIDs are very widely prescribed but they have a poor tolerability profile, with a range of potential adverse effects. NSAIDs that are used in topical formulations have been developed in the past 15 years and their use is increasing. The purpose is to achieve a high local concentration of the active ingredient at the effected site, with as low plasma concentration as possible to minimise possible systemic adverse effects (169).

The unwanted side effects of ketoprofen are due to inhibition of COX-1, while their therapeutic effects are due to inhibition of COX-2 (156, 160). Most of the ketoprofen adverse drug reactions (ADRs) are mild upper gastrointestinal complaints such as nausea, dyspepsia or epigastric discomfort. Less frequent are subjective nervous system symptoms (headache, drowsiness and dizziness) and complaints referable to the lower GIT (diarrhoea, constipation and flatulence) (131, 136, 137). The central nervous system (CNS) related side effects include headache, vertigo, dizziness, nervousness, tinnitus, depression, drowsiness and insomnia. Hypersensitivity reactions may occur occasionally and include fever, angio-oedema, bronchospasm and rashes (160, 162). Some patients may experience visual disturbance (160). Haematological adverse effects of ketoprofen include anaemias, thrombocytopenia, neutropenia, eosinophilia and agranulocytosis. Ketoprofen has been

associated with nephrotoxicity such as interstitial nephritis and nephrotic syndrome. NSAIDs may provoke renal failure especially in patients with pre-existing renal impairment. Fluid retention may occur, rarely precipitating heart failure in elderly patients (131, 160, 162, 169, 170). Similar to other NSAIDs, ketoprofen can produce increases in blood urea nitrogen and serum creatinine concentrations, mostly transient and asymptomatic (137). Other adverse effects include photosensitivity, alveolitis, pulmonary eosinophilia, pancreatitis, Stevens-Johnson syndrome and toxic epidermal necrolysis. Induction or exacerbation of colitis has also been reported (131, 140, 159, 160). Deaths caused by ketoprofen are rare and are probably mainly due to idiosyncrasy such as anaphylaxis rather than to the pharmacological effects of the drug, though occasionally gastrointestinal haemorrhage may have a fatal outcome (131).

For topical preparations, delayed hypersensitivity dermatitis may occur at the site of the application, but this is uncommon (162, 169). Cases of contact erythema and photocontact dermatitis have also been reported (170). It is advised that they should not be used on broken or inflamed skin (169). The skin reactions are reversible on discontinuation of therapy (168). Cross-sensitisation of topical ketoprofen and other propionic NSAIDs has been reported (162).

### *2.2.6 Toxicology*

Acute studies have been performed in mice, rats, guinea pigs and dogs and oral chronic studies in rats, dogs and monkeys. Rats exhibited toxic effects in the gastrointestinal and renal systems, a spectrum consistent with the typical NSAID profile (136). Dogs were also susceptible to ulceration however baboons had only minimal irritation of the gastrointestinal tract. There was no evidence of carcinogenicity or mutagenicity in standard screening assays, and the drug appears to have no effect on proteins, or on DNA or RNA synthesis. No embryotoxic or teratogenic effects have been demonstrated for ketoprofen and the drug has not been shown to affect foetal or postpartum development (131, 136). As with other NSAIDs, its use during pregnancy should be avoided since increased maternal toxicity and dystocic effects have been observed in rats (131, 136, 160).

### *2.2.7 Drug interactions*

i. Aspirin

Concomitant use of more than one NSAID should be avoided because of increased risk of adverse effects (160).

ii. Warfarin, sulphonylureas and hydantoins

Ketoprofen is highly protein-bound about 95% (131) 99% (136, 137, 140). In theory, interaction is possible following concomitant use of other protein-bound drugs, for example oral anti-diabetic agents, anticoagulants and hydantoins. In practice, such interactions seem extremely rare and there is just a single report of prolongation of the prothrombin time and gastrointestinal bleeding occurring in a patient taking several drugs following concomitant use of ketoprofen (131, 136).

iii. Methotrexate, lithium and cardiac glycosides

Ketoprofen reduced the clearance of methotrexate, lithium and cardiac glycosides leading to increased plasma concentrations (131, 136, 160).

iv. Furosemide and angiotensin converting enzyme (ACE) inhibitors

Like other NSAIDs, ketoprofen slightly inhibits the sodium diuresis induced by furosemide and other diuretics. It may increase the risk of hyperkalaemia with potassium-sparing diuretics and ACE inhibitors (131, 160, 161).

v. β-blockers

Ketoprofen may reduce the antihypertensive effect of β-blockers (131).

vi. Probenecid

The effects of probenecid on ketoprofen pharmacokinetics were investigated. Increases in the concentration of ketoprofen and its conjugates were observed (131).

vii. Zidovudine

There may be increased risk of haematotoxicity during concomitant use of zidovudine (160, 161).

The risk of gastrointestinal bleeding and ulceration associated with NSAIDs is increased when used with corticosteroids, the antiplatelets clopidogrel and ticlopidine, alcohol, bisphosphonate and oxpentifylline (160, 161).

### *2.2.8 Pharmaceutics*

Ketoprofen is currently marketed throughout the world in a variety of forms: capsules, tablets, injectable solutions, suppositories and gels (136). South Africa is the only country that markets ketoprofen in tablet dosage forms.

*Table 2.3 Ketoprofen formulations*

| **Route** | **Dosage form** | **Strength** |
|---|---|---|
| Oral | Tablets | 50 mg |
| | Enteric coated | 100 mg |
| | Capsules | 50 mg |
| | | 75 mg |
| | | 100 mg |
| | Extended (Controlled) Release | 100 mg |
| | | 200 mg |
| Parenteral | Intramuscular | 100 mg/2 ml |
| Rectal | Suppository | 100 mg |
| Topical | Gel | 2.5 g/100 g |

In the treatment of rheumatic disorders a usual daily dose of ketoprofen by mouth is 100 to 200 mg in 2 to 4 divided doses, modified-release formulations taken once daily may also be used. In the USA some manufacturers suggest initial oral doses of 75 mg three times daily or 50 mg four times daily increased as needed to a maximum of 300 mg daily in divided doses. Ketoprofen may also be administered rectally as suppositories in a dose of 100 mg at night. In the UK it is recommended that the total daily combined dose by mouth and by rectum should not exceed 200 mg. Ketoprofen may be given by deep intramuscular injection into the gluteal muscle for acute exacerbation of musculoskeletal, joint, peri-articular and soft tissue disorders and in the management of pain following orthopaedic surgery. Doses of 50 to 100 mg may be given every 4 hours, up to a maximum dose of 200 mg in 24 hours, for up to 3 days. Ketoprofen may be applied as a 2.5% m/m gel for local pain relief. Doses vary slightly between preparations; a typical regimen is application 2 or 3 times daily for up to 10 days (140). Gel preparations should not be diluted (134).

## 2.3 PHARMACOKINETICS OF TOPICAL KETOPROFEN

The introduction of a transdermal formulation for an NSAID raises the distinct possibility of achieving therapeutic benefit, without the risk of gastrointestinal or other side effects suffered by the oral route. To establish the validity of this hypothesis requires that:

i. percutaneous absorption occurs in sufficient amounts to achieve therapeutic concentrations in the target tissues,
ii. clinical efficacy is demonstrable in suitably controlled clinical trials,
iii. the safety profile of the topical agent is superior to that of the oral agent, particularly with regard to gastrointestinal side effects and
iv. the topical agents are cost-effective (162).

The pharmacokinetics of ketoprofen in man after repeated percutaneous administration has been investigated and compared to the results obtained after oral administration of ketoprofen (163). In this study, a 2.5% ketoprofen gel formulation was used and was applied over an area of 750 $cm^2$ for a period of 15 days. An average quantity of 375 mg was applied, and from the third day onward, the dose was divided into two applications for 10 consecutive days and for the remaining two days, only one of the divided doses was administered. Ten subjects (5 men and 5 women) with a mean age of 23.2 ± 2.5 years, a mean weight of 60.7 ± 9.5 kg and a mean height of 1.70 ± 0.09 m participated in the study. The peak plasma concentrations ($C_{max}$) obtained were 144 ± 91 ng/ml. The apparent absorption and elimination half-lives calculated respectively were 3.2 ± 2.4 h and 27.7 ± 18.0 h. The apparent total body clearance was 76.5 ± 39.9 l/h and the total quantities of ketoprofen eliminated in the urine were 9.7 ± 3.2 mg, representing about 2.6% of the dose applied. Although the inter-subject variability of the peak plasma concentration of ketoprofen after administration of the gel appears to be equivalent to that observed after oral administration, the extreme values for $C_{max}$ in this study differed by a factor of 1 to 5 as compared with 1 to 2.5 or 4 with the oral route. It reaches $C_{max}$ in the first hour of administration if taken orally and after six hours if applied topically (135). The area under the curves were much more widely scattered for the gel than for the oral route. Following cutaneous application of ketoprofen gel (375 mg of ketoprofen), the peak plasma concentration was about 100 times lower than that observed after administration of an oral dose of 150 mg. The apparent plasma elimination half-life was very much longer than that observed after oral administration ($t_{½}$ of

about 2 h). This apparent half-life was evaluated more accurately after stopping treatment (17.1 ± 9.1 h) than after the first administration (27.7 ± 18.0 h), as reflected by the values calculated from both the plasma data and the urinary data (17.2 ± 4.2 h). In contrast the apparent absorption half-life ($t_{½a}$ = 3.2 ± 2.4 h) was similar to the values usually recorded for elimination half-life after oral administration. Comparison of the values for these two parameters suggests that percutaneous administration corresponds to a flip-flop phenomenon. This phenomenon is observed when the absorption constant is very much smaller than the elimination constant, either in the case of very rapidly eliminated drugs or in the case of pharmaceutical formulations with very slow release according to the first order process. In this study (163), the dermis may be considered a reservoir from which the absorption of ketoprofen would be extremely slow and continuous. The ketoprofen value of the apparent elimination constant is 0.052 ± 0.029 $h^{-1}$. The quantity of ketoprofen eliminated in the urine in the 48 hours after the first dose only accounted for 2.9% of the dose administered, whereas urinary elimination over the same time period accounts for 60% of an oral or intramuscular dose. The relation between these percentages suggests that the bioavailability of the drug in gel form would be of the order of 5%.

Another study (164) compared the concentration of ketoprofen in intra-articular adipose tissue, capsular tissue and synovial fluid after topical administration in man. It showed that ketoprofen was detected from the second hour and reached its maximum in six hours. The concentration of ketoprofen in the tissues was about one hundred times higher than that found in the plasma and the concentration of ketoprofen in the synovial fluid is one hundred times higher than in the plasma (164, 168, 173). Ballerini *et al.* (164) and Rolf *et al.* (172) showed high concentrations of ketoprofen in intra-articular adipose tissues and in capsular tissue after gel and plaster applications respectively.

Pharmacokinetic data has demonstrated that, following percutaneous administration of ketoprofen gel, the anti-inflammatory agent penetrates into the general circulation slowly (163, 164) and reaches underlying tissues including the synovial fluid (168). This low systemic diffusion, combined with good local tolerance, would ensure an improved systemic tolerance for the local treatment of rheumatic conditions (163 - 170). However, it has been suggested that the gel formulation containing 2.5% ketoprofen should be evaluated further (166).

Ketoprofen is extensively metabolised in the liver. Only 1% of the dose is excreted unchanged in the urine. The main pathway of metabolism is glucuronic acid conjugation, with hydroxylation as a minor pathway (131, 135, 140, 168). The metabolites are excreted mainly in the urine with 80% of the dose as the glucuronide. 10 - 20% of the dose is excreted in the bile. Some enterohepatic circulation is probable. The metabolic products of ketoprofen appear to be pharmacologically inert (131). Figure 2.10 shows the metabolic products of ketoprofen.

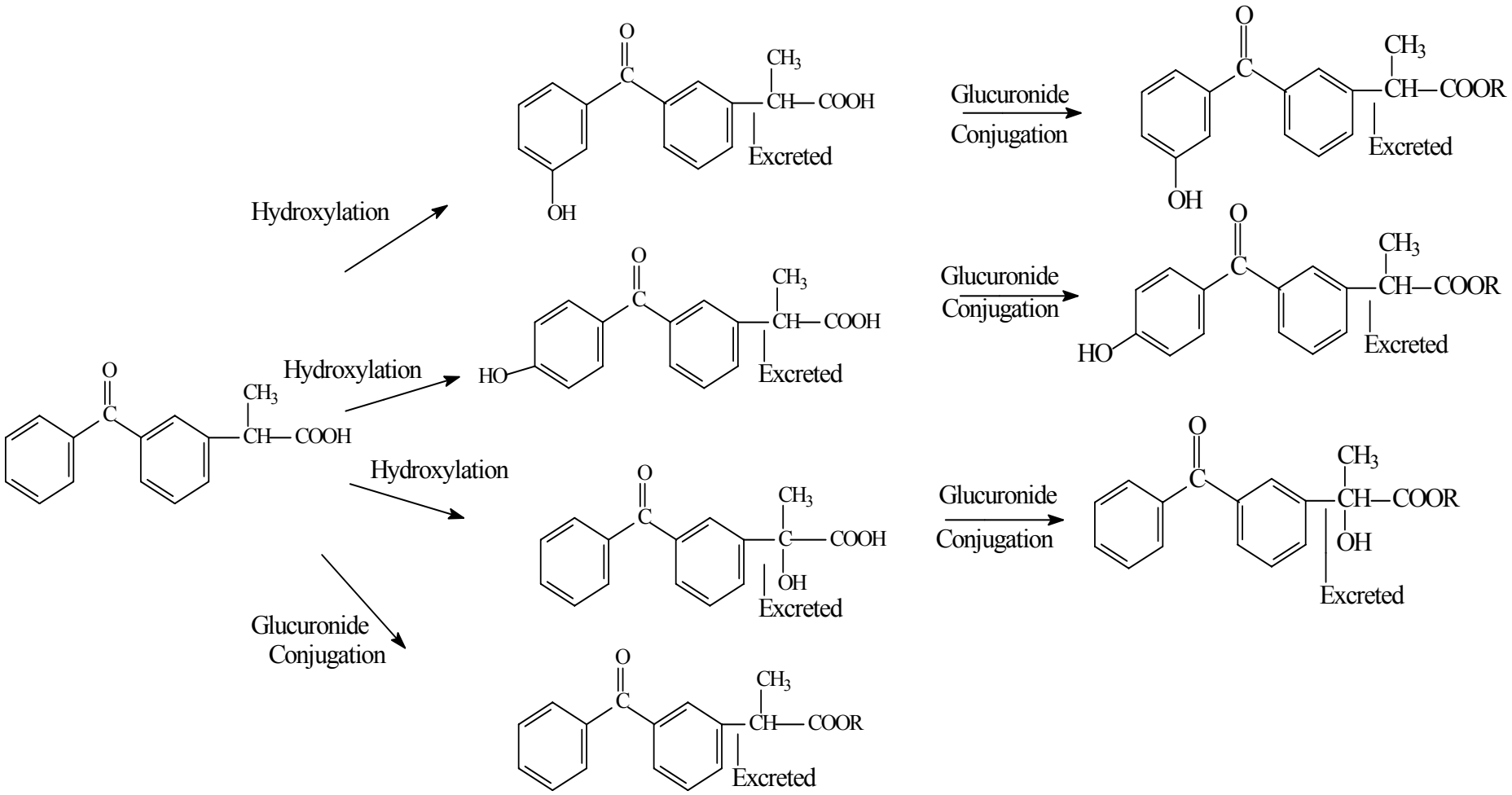



*Figure 2.10 Metabolism of ketoprofen (135)*

# CHAPTER THREE
# *IN VITRO* ANALYSIS OF KETOPROFEN

## 3.1 DEVELOPMENT AND VALIDATION OF AN HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR THE DETERMINATION OF KETOPROFEN

### *3.1.1 Method development*

#### *3.1.1.1 Introduction*

High-performance liquid chromatography (hplc) is a separation technique based on a solid stationary phase and a liquid mobile phase. Separations are achieved by partition, adsorption, or ion-exchange processes depending upon the type of stationary phase used. Hplc has distinct advantages over gas chromatography for the analysis of organic compounds. Compounds to be analysed are dissolved in a suitable solvent and most separations take place at room temperature. Thus most drugs, being non-volatile or thermally unstable compounds, can be chromatographed without decomposition or the necessity of making volatile derivatives (175).

Numerous chromatographic methods have been published for the quantification of ketoprofen in different media, serum (178), urine (179), plasma (179), and also in pharmaceuticals (185). The amount of drug in these samples was determined using double beam ultraviolet spectrophotometry (144, 183, 184, 187), thin-layer chromatography (181), capillary electrophoresis (178), gas chromatography (181), electrospray ionization mass spectrometry (180) and hplc (141 - 143, 145, 148, 182, 183, 186, 188, 189, 191, 192). Published analytical procedures for determining ketoprofen in topical preparations also include FT-near infrared spectroscopy (177) and a solid-phase extraction spectrophotometry (176). In general, hplc has been the most commonly employed method for the measurement of ketoprofen, although some published methods present insufficient sensitivity, inadequate reproducibility or chromatographic interferences and are not sufficiently sensitive for the determination of ketoprofen in topical preparations in the presence of other excipients and also in the presence of potential degradation products (190).

A simple, rapid and sensitive method with a relatively simple and robust sample preparation was required to analyse dissolution, stability and content uniformity of batches. Previously published methods shown in Table 3.1 were used as initial studies for the development of a rapid and specific hplc method for the analysis of ketoprofen.

### *3.1.1.2 Experimental*

#### *3.1.1.2.1 Reagents*

All reagents used were of analytical grade. Ketoprofen (K1751) was donated by Sigma-Aldrich (Atlasville, South Africa). Acetonitrile 200 far uv ROMIL-SpS™ Super Purity Solvent and Methanol 215 ROMIL-SpS™ Super Purity Solvent were purchased from ROMIL Ltd. (Cambridge, United Kingdom). Acetic acid glacial, sodium hydrogen pellets, potassium dihydrogen orthophosphate and ortho-phosphoric acid 85% v/v were obtained from MERCK (Wadeville, South Africa).

Hplc grade water was obtained from a Milli-Q® Academic A10 water purification system (Millipore, Molsheim, France) that consists of a Quantum™ EX ultrapure organex cartridge and a Q-Gard™ purification pack. The water was filtered through a 0.22 µm Millipak stack filter prior to use.

#### *3.1.1.2.2 Instrumentation*

The apparatus consisted of a binary pump solvent delivery system (SpectraSERIES P100), an ultraviolet-visible (UV-Vis) variable wavelength detector (Spectra 100) and an integrator (SP 4290) all from Spectra-Physics Inc. (California, USA). Samples were introduced into a Rheodyne 7120 20 µl fixed-loop injector from Catati (California, USA) with a 100 µl glass syringe (710 NR) from Hamilton Bonaduz AG (Bonaduz, Switzerland). Chromatographic separation was performed on a partition 5 µm pore size, 4.0 mm x 250 mm Waters Spherisorb® $C_{18}$ stainless steel analytical column from Waters Corporation (Massachusetts, USA) fitted with a 5 µm pore size, 4.6 mm x 30 mm Spheri-5 RP-18 guard column from Perkin Elmer Instruments (Norwalk, USA).

*Table 3.1 Initial hplc studies employed in the method development for the analysis of ketoprofen*

| Stationary Phase | Mobile Phase | Wavelength (nm) | Flow rate (ml/min) | Retention time (mins) | Internal Standard | Reference |
|---|---|---|---|---|---|---|
| Shim-Pack $C_{18}$ (250 x 4.5 mm, 5 µm) | Methanol: 100 mM acetic acid (80:20) | 255 | 1.5 | 3.2 | | 141 |
| Silica gel $C_{18}$ | Methanol: Water (85:15) | 254 | 0.8 | | | 142 |
| Reverse-phase $C_{18}$ | Acetonitrile: Water (80:20) with 50 µl phosphoric acid | 254 | 1.0 | | | 143 |
| Eurospher $C_8$ (250 x 4 mm, 5 µm) | Acetonitrile: phosphate buffer pH 7.5 (15:85) | 262 | | | | 145 |
| Kromasil 100 $C_{18}$ (250 x 4 mm, 5 µm) | Acetonitrile: 10 mM phosphate buffer pH 1.5 with orthophosphoric acid (60:40) | 260 | 1.0 | | Ibuprofen | 148 |
| Phenomenex Luna $C_{18}$ (250 x 4.6 mm, 5 µm) | Acetonitrile: Methanol: Water (36:54:10) | 265 | 1.2 | 2.3 | Propylparaben | 182 |
| Zorbax ODS (150 x 4.6 mm) | Acetonitrile: 50 mM phosphate buffer pH 2.5 with glacial acetic acid (45:55) | 265 | 1.2 | 5.5 | Flurbiprofen | 182 |
| Microsorb-MV $C_{18}$ (150 x 4.6 mm, 5 µm) | Acetonitrile: 5 mM phosphate buffer pH 3.0 with glacial acetic acid (60:40) | 254 | 1.0 | 4.5 | Ibuprofen | 183 |
| Phenomenex Kingsorb $C_{18}$ (250 x 4.6 mm, 5 µm) | Acetonitrile: 20 mM phosphate buffer pH 3 (45:55) | 258 | 1.0 | 6.8 | | 186 |
| Alltech Alltima $C_{18}$ (150 x 4.6 mm, 3 µm) | Acetonitrile: 20 mM pH 4 acetate buffer (55:45) | 256 | 1.0 | 5.2 | | 188 |
| Lichrosorb RP-18 (150 x 4.6 mm) | Acetonitrile: 20 mM phosphate buffer pH 3.0 (35: 65) | 256 | 1.0 | | | 189 |
| Nucleosil 100-5$C_{18}$ (250 x 4 mm, 5 µm) | Acetonitrile: phosphate buffer pH 5.0 (40:60) | 260 | 1.0 | | | 191 |
| Shim-Pack $C_{18}$ (150 x 4.6 mm, 5 µm) | Acetonitrile: 10 mM phosphate buffer pH 7.0 (24:76) | 258 | 1.0 | 7.7 | Naproxen | 192 |

*3.1.1.2.3 Ultraviolet detection*

Detection by ultraviolet (uv) absorption is a convenient and effective technique that is readily combined with hplc. Uv spectrophotometers have a high sensitivity for many solutes but samples must absorb in the uv or visible region (190 - 600 nm) to be detected. It is the most commonly described method of detection for the analysis of ketoprofen in the literature (141 - 143, 145, 148, 182, 183, 186, 188, 189, 191, 192). There are essentially three types of uv detectors available that can be used with hplc namely fixed, multi-wavelength and variable wavelength detectors (175). A variable wavelength detector was employed in this laboratory for the analysis of ketoprofen. This type of detector permits a wavelength to be chosen where the solute absorbance is maximal and little interference is observed from additional solutes or the mobile phase. The absorption maximum of ketoprofen was measured at 255 nm. Published methods for the analysis of ketoprofen vary considerably, utilising wavelengths from 254 to 265 nm, but many of these methods are not only optimised for the detection of ketoprofen but for the presence of other excipients such as preservatives. The ability of the uv source to generate the required wavelength is important, particularly if impurities related to the solute are present and any error in wavelength may not be consistent over the range of the detector (193).

*3.1.1.2.4 Column selection*

The column is the heart of hplc separation processes. The availability of a stable, high-performance column is essential in developing a rugged, reproducible method. Commercial columns differ widely between suppliers and even between supposedly identical columns from a single source. Such differences can have a serious impact on developing the desired hplc method. Specifically, different columns can vary in plate number, band symmetry, retention, band spacing and lifetime (194).

Most column packings used for hplc separations make use of a silica particle or support although there are columns that can be packed with porous-polymer supports. Totally porous microspheres are most commonly used because of the favourable compromise of desired properties such as efficiency, sample loading, durability, convenience and availability. Micropellicular particles have a solid core with a very thin outer skin of interactive stationary phase. These silica- or polymer-based particles, usually available in 1.5 to 2.5 μm sizes, display outstanding efficiency for macromolecules because of fast mass transfer kinetics but

have limited sample load characteristics because of low surface areas and thus are best suited for analysis only. They are also very useful in guard columns. Perfusion particles contain very large pores (*e.g.,* 4000 to 8000 Å) throughout the support and also include a network of smaller interconnecting pores (*e.g.,* 300 to 1000 Å) between these large throughpores. Experiences with these particles are still limited, however applications appear to be best suited for the preparative isolation of macromolecules such as proteins. Particle size is very important in hplc. Particle diameters of about 5 μm represent a good compromise for analytical columns in terms of column efficiency, backpressure and lifetime. It is worth noting that smaller diameters such as 3 μm are available for faster separations and 1.5 μm for extremely rapid separations of macromolecules such as proteins (194).

Bonded phase columns are the most commonly used and these can be polar or non-polar. Non-polar bonded phases are used for partition hplc with polar solvents such as water, buffers, methanol and acetonitrile, while polar bonded phases are used in adsorption hplc usually with non-polar mobile phases. Non-polar bonded silica is frequently used as it is extremely stable and octadecasilane ($Si(CH_2)_{17}CH_3$) is the most common bonded phase. Partition chromatography is similar to the extraction of different molecules from water into an organic solvent such as octanol, where more hydrophobic (non-polar) compounds preferentially extract into the non-polar octanol phase. The column (typically a silica support modified with a $C_8$ or $C_{18}$ bonded support) is less polar than the water-organic mobile phase. Molecules partition between the polar mobile phase and non-polar $C_8$ or $C_{18}$ stationary phase and more hydrophobic (non-polar) compounds are retained more strongly (195).

The choice of the bonded phase will depend on the properties of the solute. As the separations obtained in hplc depend on the interactions between the solute, the bonded phase and the mobile phase, then the polarity of all three must be considered. Polar compounds are readily analysed by partition hplc and their retention time will depend on the degree to which they interact with the bonded phase which is influenced by molecular weight, ionisable groups and solubility of the solute. The most commonly used columns reported for the analysis of ketoprofen are octadecasilyl $C_{18}$ (141 - 143, 148, 182, 183, 186, 188, 189, 192) and octylsilane $C_8$ (145).

To prolong the life of analytical columns, guard columns can be inserted between the sample valve and the analytical column. The guard column captures the strongly retained sample

components and prevents them from contaminating the analytical column. Guard columns are usually relatively short (5 - 10 cm) and contain a stationary phase equivalent to that in the analytical column. These guard columns are replaced at required intervals to ensure constant performance of the more expensive analytical column (194).

*3.1.1.2.5 Mobile phase selection*

The design of a successful hplc separation depends on matching the right mobile phase to a given column and sample. Solvents used should be readily available, compatible with the detector, safe to use, pure and relatively unreactive. The solvent should be able to dissolve the sample. In cases where a photometric detector is used for detection, it is important to know the lowest wavelength at which the solvent transmits significant energy. Acetonitrile and methanol, the two commonly used solvents, have ultraviolet cut-off wavelength values of 190 and 205 nm respectively when highly purified. Many solvents are ruled out for most applications because of their tendency to react with the sample, or undergo polymerization in the presence of certain stationary phases. It is usually preferable that solvents boil at 20 - 50°C above the temperature of the column. Lower boiling solvents are difficult to use with reciprocating pumps, since they tend to form bubbles in the piston chamber, which adversely affect pumping precision and in extreme cases, lead to loss of pumping prime. Furthermore, the composition of mixtures of such solvents can readily change due to evaporation. Higher boiling solvents are usually excessively viscous, which reduces separation efficiency and results in higher back pressure (196).

The use of a mobile phase that interacts with the selected stationary phase can alter the properties or integrity of the stationary phase. The pH of mobile phases should be within 2 - 8 as hydrolysis of the bonded phase or dissolution of silica may occur. The mobile phase should be degassed before use to minimise the presence of oxidative species which can react with the drug of interest and also to reduce possible mechanical damage in relation to the pumping mechanism of the hplc system.

The initial mobile phases used were based on published data as shown in Table 3.1. The mobile phases used during method development and the corresponding retention times of ketoprofen are reported in Table 3.2 below. Where necessary, the pH correction was achieved with glacial acetic acid.

*Table 3.2 The effect of mobile phase composition on the retention time of ketoprofen*

| Reference | Mobile phase composition | Retention time (mins) | Comment |
|---|---|---|---|
| 142 | Methanol: Water (85:15) | 2.72 | Peak tailing barely resolved from solvent front. |
| 143 | Acetonitrile: Water (80:20) with 50 µl phosphoric acid | 2.70 | Sharp peak barely resolved front solvent front with peak tailing. |
| 182 | Acetonitrile: Methanol: Water (36:54:10) | 2.35 | No solvent front and excessive peak tailing. |
| 182 | Acetonitrile: 50 mM phosphate buffer pH 2.5 (45:55) | 7.76 | Sharp peak, well resolved from solvent front. |
| 183 | Acetonitrile: 5 mM phosphate buffer pH 3.0 (60:40) | 4.22 | Sharp peak, with peak tailing. |
| 186 | Acetonitrile: 20 mM phosphate buffer pH 3 (45:55) | 8.80 | Sharp peak, well resolved from solvent front. |
| 188 | Acetonitrile: 20 mM phosphate buffer pH 4 (55:45) | 5.11 | Sharp peak, well resolved from solvent front. |
| 192 | Acetonitrile: 10 mM phosphate buffer pH 7.0 (24:76) | 5.88 | Broad peak. |

Sharp peaks that were well resolved from the solvent front were observed with mobile phases comprising acetonitrile and phosphate buffer at low pH values. The mobile phase composition selected for further development and validation was acetonitrile and 20 mM phosphate buffer at pH 4.0 in a ratio of 55:45 because it was reported to have sharp peaks which were well resolved from the solvent front. It was noted that, although well resolved, sharp peaks were obtained from some of the mobile phases shown in Table 3.2, mobile phases should however be optimised to produce peaks with shorter retention times. Mobile phase selection was based on peak parameters (symmetry, tailing), run time, ease of preparation and cost.

#### *3.1.1.2.6 Preparation of selected mobile phase*

The buffer solution was prepared by weighing accurately 1.3609 g of potassium dihydrogen orthophosphate into a 500 ml A-grade volumetric flask and made up to volume with hplc grade water. The pH was then adjusted to 2.5 using glacial acetic acid. A Crison GLP 21 pH-meter from Crison Instruments, (Lasec, South Africa) was used for pH measurements. The buffer solution was then combined with acetonitrile in a ratio of 40:60 and filtered through a 0.5 µm FH filter from Millipore (Massachusetts, USA) under vacuum with an Eyela Aspirator A-2S from Tokyo Rikakikai Co., Ltd (Tokyo, Japan). The mobile phase was prepared daily and was not recycled during use.

*3.1.1.2.7 Preparation of stock solutions*

Stock solutions of ketoprofen were prepared by accurately weighing approximately 10 mg into an A-grade 100 ml volumetric flask and made up to volume with 0.2 M phosphate buffer at pH 6.8 prepared with hplc grade water. Standards ranging in concentration from 0.5 - 15.0 µg/ml were prepared by serial dilution of this stock solution using A-grade glassware.

*3.1.1.3 Optimisation of the chromatographic conditions*

*3.1.1.3.1 Detector wavelength (λ)*

Published methods from the literature, shown in Table 3.1, used different wavelengths for the analysis of ketoprofen. The wavelengths ranged from 255 nm (141), 254 nm (142, 143, 183), 256 nm (188, 189), 258 nm (186, 192), 260 nm (148, 191), 262 nm (145) and 265 nm (182). It is possible that the wavelengths of some methods were adjusted to accommodate the presence of excipients and other actives that may be present in the formulation. Consequently, the effect of different wavelengths on peak area was investigated.

*Table 3.3 Effect of wavelength on the relative percent peak area of ketoprofen*

| **Concentration (µg/ml)** | **254 nm** | **255 nm** | **260 nm** | **265 nm** |
|---|---|---|---|---|
| 0.5025 | 92.05 | 100 | 88.98 | 89.94 |
| 1.0050 | 93.19 | 100 | 83.33 | 98.74 |
| 5.0250 | 80.08 | 100 | 85.58 | 96.91 |
| 10.050 | 96.85 | 100 | 83.31 | 89.88 |
| 20.100 | 79.73 | 100 | 85.83 | 93.25 |
| Average | 88.38 | 100 | 85.41 | 93.74 |
| Standard Deviation | 7.94 | 0 | 2.33 | 4.02 |
| % RSD | 8.98 | 0 | 2.73 | 4.29 |

The results, as shown in Table 3.3, indicated that concentration was optimal at 255 nm, with decreasing concentration through 254 nm, 260 nm and 265 nm. Based on the results of this experiment, 255 nm was chosen as the wavelength of detection.

*3.1.1.3.2 Choice of column*

A partition 5 µm pore size, 4.0 mm x 250 mm Waters Spherisorb® $C_{18}$ stainless steel analytical column from Waters Corporation (Massachusetts, USA) was selected for the analysis of ketoprofen. Ketoprofen is a weak acid and is soluble in the aqueous-organic mobile phase combination, thus the retention time is expected to be short with a non-polar stationary phase, as ketoprofen will partition preferentially into the mobile phase. This is

desirable as rapid and selective analysis of ketoprofen in single component dosage forms was required.

*3.1.1.3.3 Mobile phase composition*

Manipulation of mobile phase composition is undoubtedly the most powerful means for adjusting both absolute and relative retentions in chromatography, particularly partition hplc. Not only can the water content be adjusted, but the nature of the organic modifier such as methanol, acetonitrile and tetrahydrofuran can also be changed. It is also possible to control the pH and ionic strength of the eluent, which has a large effect on the chromatographic behaviour of ionic compounds (197). As mentioned previously the mobile phase selected for further development comprised acetonitrile and 20 mM phosphate buffer at pH 4.0 in a ratio of 55:45. This mobile phase gave a satisfactory retention time and resolution, however further developments were performed to ascertain the effect of pH changes, ratio volumes and molarity on the retention time and nature of the peaks.

It was observed that lower pH values produced sharper peaks than higher pH values which produced broad peaks with much longer retention times. Changes in the pH of the mobile phase of partition systems will modify the retention time of weak acids and bases, as the unionised species is retained longer, being less polar (197). A pH value of 2.5 of the buffer was chosen. It was however noted later that the pH of the overall mobile phase was 3.9 which was well within the range of pH values of 2.0 - 8.0 thus not compromising the stability of the stationary phase. High concentrations of buffer salts are undesirable as there is a potential for precipitation with subsequent damage to pump heads, seals and pistons and blockages with consequent increases in back pressure. No statistically significant differences ($p > 0.05$, ANOVA) on the retention times were noted when the molarity of the buffer was altered. 20 mM was chosen as the optimum concentration as no apparent damage to the binary pump was observed. Changing the content of acetonitrile in the mobile phase produced significant changes in the retention time. Increasing the volume of acetonitrile produced sharp peaks which eluted quickly and in some cases were barely resolved from the solvent front. Ketoprofen becomes more soluble in the mobile phase with higher acetonitrile content and partitioned less in the stationary phase. The optimum content of acetonitrile should be one that elutes the peaks within a relatively short time but well resolved from the solvent front so that multiple analyses can be performed as rapidly as possible especially

where manual injection of samples is employed. The ratio of the final mobile phase was changed to 60% acetonitrile and 40% phosphate buffer at pH 2.5 to produce a well resolved peak with a retention time of 4.0 minutes.

### *3.1.1.4 Chromatographic conditions*

The optimal chromatographic conditions established during the method development are summarised below in Table 3.4 and Figure 3.1 shows a typical chromatogram obtained from the analysis of a standard solution of ketoprofen using the method described above.

*Table 3.4 Optimal chromatographic conditions applied*

| **Parameter** | **Conditions applied** |
|---|---|
| Mobile phase | 60% acetonitrile: 40% 20 mM phosphate buffer at pH 2.5 |
| Flow rate | 1.0 ml/min |
| Detection wavelength | 255 nm |
| Sensitivity | 0.2 AUFS |
| Injection volume | 20 µl |
| Retention time | 4.0 mins |
| Temperature | Ambient |

*Figure 3.1 Typical chromatogram of a standard solution of ketoprofen at 10 µg/ml obtained using the chromatographic conditions specified*

### *3.1.1.5 Conclusion*

The effects of altering system variables on the elution of ketoprofen were established in these preliminary investigations. By optimising the choice of mobile phase, detection wavelength and analytical column, an hplc method which provided well resolved symmetrical peaks, suitable retention times and minimal baseline noise was developed.

### *3.1.2 Method validation*

#### *3.1.2.1 Introduction*

Validation of analytical methodologies is widely accepted as pivotal before they are put into routine use (213, 214). A method must be tested for effectiveness and must be appropriate for the particular analysis to be undertaken (198). Method validation is defined as the process of proving, through scientific studies, that an analytical method is acceptable for its intended use (213, 199) and it instils confidence that the method can generate test data of acceptable quality (202).

Recent guidelines for methods development and validation for new non-compendial test methods are provided by the FDA draft document, 'Analytical Procedures and Method Validation: Chemistry, Manufacturing and Controls Documentation' (200). In recent years, a great deal of effort has been devoted to the harmonization of pharmaceutical regulatory requirements in the United States, Europe and Japan. As part of this initiative, the International Conference on Harmonization (ICH) has issued guidelines for analytical method validation. The recent FDA methods validation draft guidance document as well as the United States Pharmacopoeia (USP) (201) both refer to the ICH guidelines (199).

The validation process may vary slightly between laboratories (215), however, a number of general tests are usually performed; recovery, accuracy, precision, reproducibility, linearity, specificity, limit of detection and quantitation and ruggedness. Therefore method development is complete only when the method has been stringently tested and shown to demonstrate acceptable analytical performance (198). It is important to realise that method validation is not a stand-alone process and is rather a part of an overall validation process, which includes the validation of the hardware and software being used (installation, operation and performance qualifications should be performed for all equipment being utilized) and the verification of system suitability and performance (203).

#### *3.1.2.2 Accuracy and bias*

Accuracy is the measure of exactness of an analytical method, or the closeness of agreement between the measured value and the value that is accepted either as a conventional, true value or an accepted reference value (203). Bias assesses the influence of the analyst on the performance of the method. Accuracy and bias were determined by injecting three replicate

measurements each of three samples of varying concentration. According to the ICH guidelines, the measurements were performed in consecutive and non-consecutive sequences (203). It is desirable to have an injection precision, in terms of area unit relative standard deviation (RSD), of less than 2.0% for each standard solution and recovery within 98% and 102% (204).

*Table 3.5 Accuracy test results on blinded samples*

| **Concentration (µg/ml)** | **Mean experimental concentration (µg/ml)** | **Standard deviation** | **RSD (%)** | **Recovery (%)** | **Bias (%)** |
|---|---|---|---|---|---|
| 4.95 | 4.97 | 0.01 | 0.29 | 100.40 | -0.40 |
| 9.90 | 9.85 | 0.04 | 0.36 | 99.49 | 0.51 |
| 14.85 | 14.87 | 0.01 | 0.10 | 100.13 | -0.13 |

The accuracy results shown in Table 3.5 were within the limits indicating that the analytical method was accurate for the determination of ketoprofen.

### *3.1.2.3 Precision*

Precision quantifies the variability of an analytical result as a function of operator, method manipulations and day-to-day environment (205). It is also the measure of the degree of repeatability of an analytical method under normal operation and is expressed as the percent RSD for a statistically significant number of samples (201). Precision experiments give a good indication of the performance of the method and should be repeated regularly. Generally, any increase of the RSD above 2.0% should be investigated (198). According to ICH, three types of precision can be defined and should all be assessed as described below.

#### *3.1.2.3.1 Repeatability*

Repeatability refers to the results of the method operating over a short time interval under the same conditions (inter-assay precision). It expresses the degree of variation arising during replicate assays performed consecutively and non-consecutively but on the same day. Repeatability should be determined from a minimum of nine determinations covering the specified concentration range of the procedure (203).

*Table 3.6 Inter-day (repeatability) assessment on five concentrations*

| **Concentration (µg/ml)** | **Mean peak area** ($n = 3$) | **Standard deviation** | **% RSD** |
|---|---|---|---|
| 1.025 | 5670.67 | 19.22 | 0.34 |
| 2.050 | 11633.33 | 30.44 | 0.26 |
| 5.125 | 26639.00 | 130.05 | 0.49 |
| 10.250 | 52417.33 | 41.02 | 0.08 |
| 20.500 | 103074.00 | 205.67 | 0.20 |

The inter-day assay results shown in Table 3.6 were within the limits indicating that there was minimum variation of the analytical method with respect to the analyst and the equipment for the determination of ketoprofen.

*3.1.2.3.2 Intermediate precision (ruggedness)*

Intermediate precision refers to the results from laboratory variations due to random events such as differences in experimental periods, analysts and equipment (203). Intermediate precision was expressed in this laboratory on three different days with respect to the analyst and the equipment. Three replicates of the five standards analysed below were injected over a three day period and collected as the intra-day data.

*Table 3.7 Intra-day assessment of five concentrations*

| **Concentration (µg/ml)** | **Mean peak area** ($n = 3$) | **Standard deviation** | **% RSD** |
|---|---|---|---|
| 1.025 | 5335.44 | 14.18 | 0.27 |
| 2.050 | 11432.78 | 177.14 | 1.55 |
| 5.125 | 25995.67 | 730.42 | 2.81 |
| 10.250 | 51307.00 | 1542.81 | 3.01 |
| 20.500 | 101433.56 | 2801.00 | 2.76 |

The intra-day assay results shown in Table 3.7 produced percent RSD values greater than 0.2% but less than 3.1%. Although there was no evidence of official acceptance criteria or figures of merit found for intermediate precision these values were acceptable based on the literature (206 - 210).

*3.1.2.3.3 Reproducibility*

Reproducibility is an indication of the ability of the method to be transferred from one laboratory to another. This was not assessed.

### *3.1.2.4 Specificity and selectivity*

A method is specific if it produces a response for only one single solute. Since it is almost impossible to develop a chromatographic assay for a drug in a matrix that will respond to only the compound of interest, the term selectivity is more appropriate (214). Selectivity describes the ability of an analytical method to differentiate various substances in the sample and is applicable to methods in which two or more components are separated and quantitated in a complex matrix (211). It is a measure of degree of interference from such things as other active ingredients, excipients, impurities and degradation products, ensuring that a peak response is due only to a single component, *i.e.,* that no co-elution exists (203). Specificity was assessed by analysing a 2.5% m/m ketoprofen proprietary gel product (Fastum®, South Africa) and a placebo extemporaneous gel formulation in 0.2 M phosphate buffer at pH 6.8 after exposing it to light for two hours.


*Figure 3.2 Chromatographic representation of a buffered solution of Fastum® gel formulation after exposure to light*

The chromatogram shown in Figure 3.2 indicated that the hplc method developed was specific for the analysis of ketoprofen (peak C) as there were no interferences with either the presence of other excipients (peak A and B) in the proprietary gel product or degradation products (peak D) in solution. Analysis of an extemporaneous placebo gel formulation showed no peak at the retention time of 4.0 minutes. The retention time of the primary peak in the chromatogram of the proprietary gel formulation is the same as the retention time of the ketoprofen peak in the standard, indicating specificity of the procedure.

### *3.1.2.5 Limit of detection and limit of quantitation*

The USP requires that the limit of detection (LOD) and the limit of quantitation (LOQ) be determined for studies that involve the detection and quantitation of components at or near trace levels. Such studies include purity testing of active pharmaceutical ingredients, stability testing of dosage forms and the analysis of manufacturing equipment cleaning validation

samples (212). For many pharmaceutical applications the LOQ is generally a more useful parameter than the LOD (211). The LOD is defined as the lowest concentration of a solute in a sample that can be detected, though not necessarily quantitated and the LOQ is defined as the lowest concentration of a solute in a sample that can be determined with acceptable precision and accuracy under the stated operational conditions of the method (203, 214). Four techniques have been outlined by Paino *et al.* (212) for the determination of LOD and LOQ values of an hplc method. The lowest concentration for which RSD is less than 5% was the method employed in the determination of LOD and LOQ. This method involves choosing the LOQ as the lowest concentration for which the RSD of multiple injections is less than 5% and by convention, the LOD value is taken as $0.3 \times LOQ$.

*Table 3.8 Limit of quantification values assessed*

| **Concentration (µg/ml)** | **Mean peak area** $(n = 3)$ | **Standard deviation** | **% RSD** |
|---|---|---|---|
| 0.5075 | 2584.67 | 8.50 | 0.33 |
| 0.4060 | 2048.67 | 6.35 | 0.31 |
| 0.3045 | 1537.33 | 11.68 | 0.76 |
| 0.2030 | 1007.33 | 4.16 | 0.41 |
| 0.1015 | 502.33 | 29.87 | 5.95 |

The LOQ was found to be 0.2 µg/ml which had a percent RSD value of 0.41% and therefore by convention the LOD value is taken to be 0.06 µg/ml. It is worth noting that the LOD is not a very stable characteristic because of its susceptibility to minor changes in the conditions of the analytical method like temperature, purity of reagents, sample matrices and instrumental system changes. For this reason the LOD concentration level should not be included in the calibration curve (214).

### *3.1.2.6 Linearity and range*

Linearity defines the analytical response as a function of solute concentration and range prescribes a region over which acceptable linearity, precision and accuracy are achieved (205). Linearity is generally reported as the variance of the slope of the regression line. Range is the interval between the upper and lower concentrations of solute that have been demonstrated to be determined with precision, accuracy and linearity using the method. The ICH guidelines specify a minimum of five concentrations, along with certain minimum specified ranges (203). A calibration curve was constructed over the concentration range

0.5 - 15 μg/ml by linear regression of the peak areas obtained versus the concentration. Three replicate determinations of each of five concentrations were used. The calibration curve shown in Figure 3.3 was linear over the concentration range studied with $r^2 = 0.9999$. The equation of the regression line is $y = 4856.9x + 818.71$.

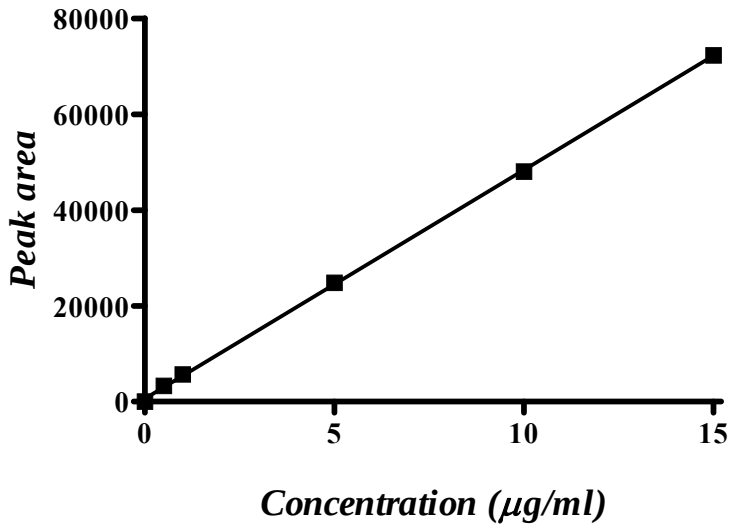



*Figure 3.3 Calibration curve of ketoprofen*

*3.1.2.7 Sample solution stability*

In order to design a chromatographic system for the analysis of an active component of a pharmaceutical product it is essential to have a good knowledge of susceptibility of the drug to degradation and its degradation pathway, assay interference by possible degradants or synthesis precursors and assay interference by chemicals employed in sample preparation and excipients present in the formulation (198). The aim of a stability test is to detect any degradation of the solute of interest during the entire period of sample collection, processing, storing, preparing and analysis (214). The stability of a 10 μg/ml solution of ketoprofen was evaluated under two storage conditions over a period of five days. The results were expressed as percent peak area relative to initial (recovery). Although a recovery of 100% is most desirable, in practice solute recoveries higher than 70% with a variation of 15% are accepted (214).

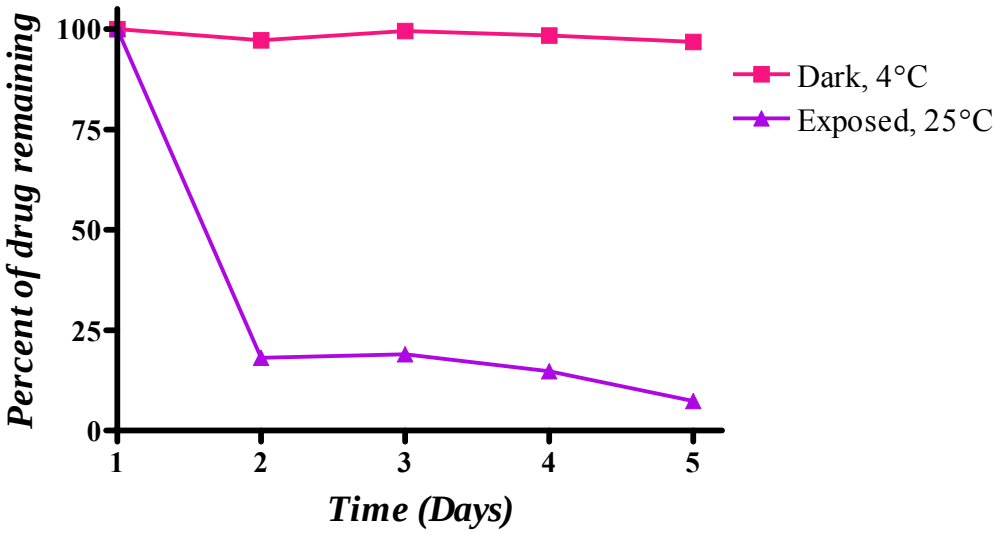



*Figure 3.4 Curves of ketoprofen aqueous solution (10 µg/ml) stability stored in the dark at 4°C and on exposure to light at 25°C analysed by hplc*

Figure 3.4 shows that the sample solutions were stable when stored in the dark at 4°C but were found to be photolabile in the presence of light. Ketoprofen was less stable on exposure to light at 25°C with a degradation of 81.9% on the second day. All samples were stored in vials wrapped with aluminium foil and kept in the fridge. Bempong *et al.* (190) reported that there were no significant changes in the chromatograms obtained with ketoprofen subjected to oxidative, heat, acid or base stress, when compared to the chromatograms obtained with the non-stressed samples.

### *3.1.2.8 Conclusion*

This report presents development and validation of a simple isocratic hplc procedure suitable for the analysis of ketoprofen in solution. The current hplc procedure separated the excipients and potential degradant peaks from the ketoprofen peak and has been demonstrated to be sufficiently accurate, specific, precise and stability indicating.

## 3.2 DEVELOPMENT AND VALIDATION OF AN ULTRAVIOLET SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF KETOPROFEN

### *3.2.1 Method development*

#### *3.2.1.1 Introduction*

The technique of ultraviolet-visible spectroscopy is one of the most frequently employed in pharmaceutical analysis. It involves the measurement of the amount of ultraviolet-visible (190 - 600 nm) radiation absorbed by a substance in solution. Instruments which measure the ratio, or a function of the ratio, of the intensity of two beams of radiation in the ultraviolet-visible region are called ultraviolet-visible spectrophotometers. Absorption of radiation in both the ultraviolet and visible regions of the electromagnetic spectrum occurs when the energy of the radiation matches that required to induce in the molecule an electronic transition and its associated vibrational and rotational transitions. It is therefore convenient to consider the techniques of ultraviolet spectrophotometry and visible spectrophotometry together (216).

The use of ultraviolet spectrophotometry as an analytical procedure has become less popular due to the advent of more sophisticated techniques such as chromatographic separation techniques resulting in limited literature been available for the determination of ketoprofen in pharmaceutical formulations. Nevertheless there are some publications that have used ultraviolet spectrophotometry in the analysis of ketoprofen (144, 183, 184, 187). An attempt was made to develop and validate an ultraviolet absorption spectrophotometric method for the determination of ketoprofen in transdermal formulations and compare the outcomes to those obtained from hplc.

#### *3.2.1.2 Principles of ultraviolet-visible absorption spectroscopy*

##### *3.2.1.2.1 Beer-Lambert law*

When a beam of radiation is passed through a transparent cuvette containing a solution of an absorbing substance, reduction of the intensity of the radiation may occur (216). The reduction is due in part to reflections at the surfaces and to scattering by any suspended particles present but in clear liquids is primarily accounted for by the absorption of radiation

by the liquid (220). The Beer-Lambert law states that successive increments in the number of identical absorbing molecules in the path of a beam of monochromatic radiation absorb equal fractions of the radiation traversing them. Equation 3.1 expresses the mathematical relationship between the absorbance $A$ of a 1 g/1000 ml solution, the incident radiation $I_o$, the transmitted radiation $I_T$, the absorptivity $a$, the pathlength of the cuvette $b$ and the concentration of the solute $c$.

$$A = \log \frac{I_o}{I_T} = abc \tag{3.1}$$

The absorptivity is a characteristic of a particular combination of solute and solvent for a given wavelength. The name and value of $a$ depend on the units of concentration. When $c$ is in moles per litre, the constant is called the molar absorptivity and has the symbol $\varepsilon$ expressed in equation 3.2.

$$A = \varepsilon bc \tag{3.2}$$

It must be noted that absorptivity is a property of a substance (an intensive property), while absorbance is a property of a particular sample (an extensive property) and will therefore vary with the concentration and dimensions of the container. The absorbance $A$ or the absorptivity $a$ is useful as a measure of the degree of absorption of radiation. The molar absorptivity $\varepsilon$ is preferable if it is desired to compare quantitatively the absorption of various substances of known molecular mass (220). Another form of the Beer-Lambert proportionality constant is the specific absorbance as outlined in the British Pharmacopoeia (226), which is the absorbance of a specified concentration in a cuvette of specified pathlength. The most common form in pharmaceutical analysis is the $A$(1%, 1 cm), which is the absorbance of a 1 g/100 ml (1% m/v) solution in a 1 cm cuvette (216).

The Beer-Lambert law indicates that the absorptivity is a constant independent of concentration, length of path and intensity of incident radiation. The law provides no hint of the effect of temperature, the nature of the solvent or the wavelength. For much practical work temperature effects may be disregarded, especially when the absorption of an unknown is directly compared with a standard at the same temperature. The effect of changing the solvent on the absorption of a given solute cannot be predicted in any general way. The

analyst is frequently limited to a particular solvent or class of solvents in which the material is soluble, so that the question may not arise. A further restriction applies particularly to work in the ultraviolet range, where many common solvents absorb radiation. Even at constant temperature and in a specified solvent, it is sometimes found that the absorptivity may not be constant. If the absorbance $A$ is plotted against concentration, a straight line through the origin should result, according to the prediction of equation 3.1. Deviations from the law are designated positive or negative, according to whether the observed curve is concave upward or downward (220). Deviation from Beer-Lambert law may be observed in graphs of absorbance versus concentration (Beer's Law plot) or of absorbance versus pathlength (Lambert's Law plots). These deviations may be primarily due to two reasons namely the use of non-monochromatic radiation or chemical effects. A requirement for adherence to the Beer-Lambert law is that the radiation incident on the sample is monochromatic. In normal circumstances, radiation of spectral bandwidth less than one-tenth the natural bandwidth of the substance, gives linear Beer's Law and Lambert's Law graphs if stray light is absent. If, however, the radiation incident on the sample is insufficiently monochromatic due to the presence of stray light or the use of a wide slitwidth and if the absorptivity of the substance at these extraneous wavelengths is less than that at the nominal wavelength of measurement, the measured absorbance will be less than the true absorbance and negative deviation from linearity, particularly at high absorbances, will be observed in Beer's Law and Lambert's Law plots. Conversely, if the absorptivity at the extraneous wavelengths is greater than that at the wavelength of measurement, then a positive deviation will be observed. Deviation from the Beer-Lambert Law may also occur if the substance undergoes chemical changes (*e.g.,* dissociation, association, polymerization, complex formation) as a result of the variation of concentration (216) and the law only holds for very dilute solutions. Once a curve corresponding to equation 3.1 is established for the material under specified conditions, it may be used as a calibration curve. The concentration of an unknown may then be read off from the curve as soon as its absorbance is found by observation (220).

#### *3.2.1.3 Experimental*

##### *3.2.1.3.1 Reagents*

All reagents used were of analytical grade. Ketoprofen (K1751) was donated by Sigma-Aldrich (Atlasville, South Africa). Sodium hydrogen pellets and potassium dihydrogen orthophosphate were obtained from MERCK (Wadeville, South Africa).

##### *3.2.1.3.2 Instrumentation*

The ultraviolet spectrophotometric analysis was performed on a double beam (GBC UV-Visible 916) spectrophotometer from GBC Scientific Equipment Pty Ltd (Victoria, Australia) equipped with a computer station with the appropriate software. It was equipped with a deuterium lamp as the radiation source for measurements in the ultraviolet region. Samples were held in identical 10 mm quartz cuvettes. The slit width was set at 2 nm with an integration time of 1 s.

##### *3.2.1.3.3 Preparation of stock solutions*

Stock solutions of ketoprofen were prepared by accurately weighing approximately 10 mg into an A-grade 100 ml volumetric flask and made up to volume with 0.2 M phosphate buffer at pH 6.8. Standards ranging in concentration from 0.5 - 15.0 µg/ml were prepared by serial dilution of this stock solution using A-grade glassware.

#### *3.2.1.4 Optimization of spectrophotometric conditions*

##### *3.2.1.4.1 Solvent*

The choice of solvent is governed by the solubility of the absorbing substance and by the absorption of the solvent at the analytical wavelength. Although water is the ideal solvent as it is transparent at all wavelengths in the visible and ultraviolet regions above 180 nm, it cannot be used as a solvent for the simple reason that ketoprofen is insoluble in water. Organic solvents on the other hand are restricted to measurements at wavelengths where the solvents are reasonably transparent. The choice of solvent was based on the receptor fluid from the *in vitro* experiments of the hplc analytical procedure. A 0.2 M phosphate buffer solution at pH 6.8 was chosen as the solvent. This solvent showed the cut-off wavelength at approximately 200 nm making it ideal for the analysis of ketoprofen. The blank solvent without active agent served as a reference in the uv analytical measurements.

*3.2.1.4.2 Ultraviolet detection*

The absorption maximum of ketoprofen was measured at 255 nm as shown in Figure 2.7 in the chosen solvent. This wavelength was used in the analysis of ketoprofen.

*3.2.1.4.3 Concentration of solute*

The intensity of radiation transmitted by an absorbing solution will show small random fluctuations due to small variations in the radiation source intensity, detector and amplifier noise. Consequently, every absorbance value will have a small random error associated with it. For modern instruments equipped with a phototube detector, it may be shown that the relative error is at a minimum when absorbance is 0.869. The optimum accuracy and precision are therefore obtained when the absorbance is around 0.9. However, in practice absorbances in the range 0.3 to 1.5 are sufficiently reliable and the concentration of the solute should be adjusted to give an absorbance within this range (216). Sample solutions of higher concentrations were diluted where necessary with solvent to produce absorbance readings of less than 1.

*3.2.1.4.4 Spectrophotometric conditions*

The optimal spectrophotometric conditions established during the method development are summarised below in Table 3.9.

*Table 3.9 Optimal spectrophotometric conditions applied*

| **Parameter** | **Settings employed** |
|---|---|
| Measurement mode | Absorbance |
| Solvent | 0.2 M phosphate buffer at pH 6.8 |
| Wavelength | 255 nm |
| Integration time | 1.0 s |
| Slit width | 2.0 nm |
| Beam mode | Double beam |

*3.2.1.5 Conclusion*

The effects of altering system variables on the detection of ketoprofen were established in these preliminary investigations. By optimising the choice of the solvent, detection wavelength and concentration of the solute, an ultraviolet spectrophotometric method was developed.

### *3.2.2 Method validation*

The conditions outlined below have been previously addressed in sections 3.1.2.2 - 3.1.2.7.

#### *3.2.2.1 Accuracy and bias*

*Table 3.10 Accuracy test results on blinded samples of ketoprofen by uv analysis*

| **Concentration (µg/ml)** | **Mean experimental concentration (µg/ml)** | **Standard deviation** | **RSD (%)** | **Recovery (%)** | **Bias (%)** |
|---|---|---|---|---|---|
| 0.81 | 0.80 | 0.01 | 0.91 | 98.72 | 1.28 |
| 7.07 | 7.09 | 0.01 | 0.18 | 100.26 | -0.26 |
| 12.12 | 11.91 | 0.15 | 1.27 | 98.23 | 1.77 |

The accuracy results shown in Table 3.10 were within the limits indicating that the analytical method was accurate for the determination of ketoprofen.

#### *3.2.2.2 Precision*

##### *3.2.2.2.1 Repeatability*

*Table 3.11 Inter-day (repeatability) assessment on five concentrations of ketoprofen by uv analysis*

| **Concentration (µg/ml)** | **Mean absorbance** $(n = 5)$ | **Standard deviation** | **% RSD** | **Absorptivity** |
|---|---|---|---|---|
| 1.01 | 0.06565 | 0.00003 | 0.05 | 65.00 |
| 2.02 | 0.13554 | 0.00019 | 0.14 | 67.10 |
| 5.05 | 0.33338 | 0.00004 | 0.01 | 66.02 |
| 10.10 | 0.66864 | 0.00012 | 0.02 | 66.20 |
| 15.15 | 0.99767 | 0.00014 | 0.01 | 65.85 |

The inter-day assay results shown in Table 3.11 were within the limits indicating that there was minimum variation of the analytical method with respect to the analyst and the equipment for the determination of ketoprofen.

##### *3.2.2.2.2 Intermediate precision (ruggedness)*

Intermediate precision was determined in this laboratory on three different days with respect to the analyst and the equipment. Five replicates of the five standards analysed below were injected over a three day period and collected as the intra-day data.

*Table 3.12 Intra-day assessment of five concentrations of ketoprofen by uv analysis*

| **Concentration (µg/ml)** | **Mean absorbance** ($n = 5$) | **Standard deviation** | **% RSD** | **Absorptivity** |
|---|---|---|---|---|
| 1.01 | 0.06512 | 0.00248 | 3.80 | 64.48 |
| 2.02 | 0.13904 | 0.00620 | 4.46 | 68.83 |
| 5.05 | 0.33260 | 0.00310 | 0.93 | 65.86 |
| 10.10 | 0.67383 | 0.00510 | 0.76 | 66.72 |
| 15.15 | 1.01511 | 0.01511 | 1.49 | 67.00 |

The intra-day assay results shown in Table 3.12 produced percent RSD values less than 5.0% which were acceptable based on literature (206 - 210).

*3.2.2.2.3 Reproducibility*

This was not assessed.

*3.2.2.3 Limit of detection and limit of quantitation*

The LOQ and LOD for ketoprofen were found to be 0.9 µg/ml and 0.3 µg/ml respectively.

*3.2.2.4 Linearity and range*

Calibration curves were constructed in the range of concentrations of 0.5 - 15.0 µg/ml for ketoprofen. The Beer-Lambert law is obeyed over these concentration ranges. The linear regression equation obtained was $y = 0.60657x + 0.0025$, with a correlation co-efficient of 0.9999 for ketoprofen.

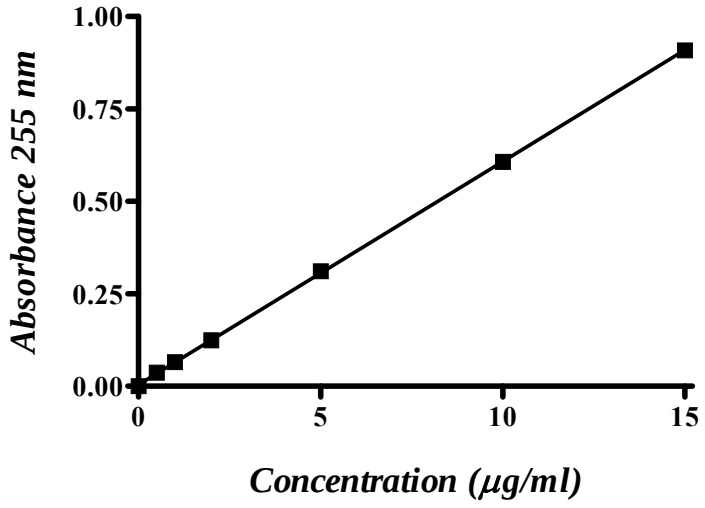



*Figure 3.5 Calibration curve of ketoprofen by uv analysis*

*3.2.2.5 Sample solution stability*

The stability of ketoprofen sample solutions were evaluated by observing for changes in the absorbance readings at the analytical wavelength under two storage conditions over a five day period. The results were expressed as percent absorbance relative to initial (recovery).

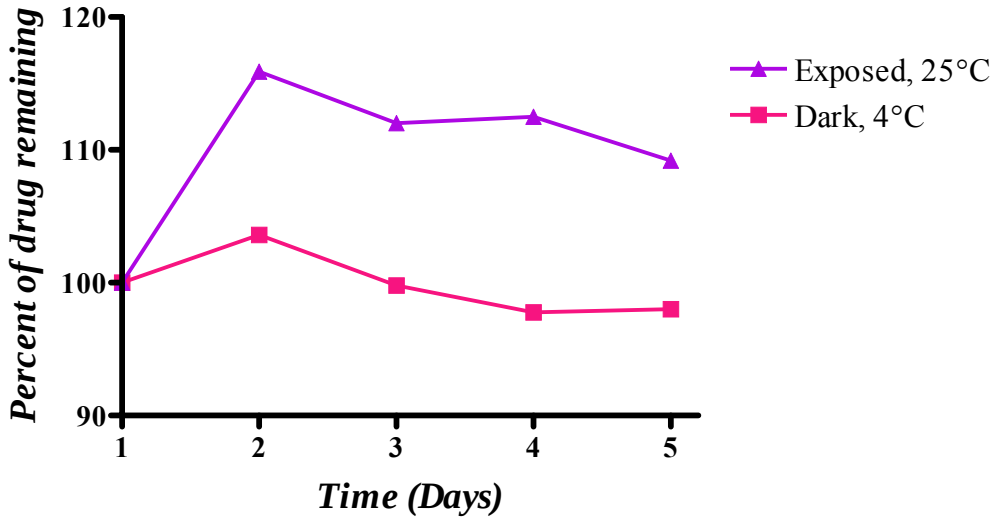



*Figure 3.6 Curves of ketoprofen aqueous solution (10 µg/ml) stored in the dark at 4°C and on exposure to light at 25°C analysed by uv*

Figure 3.6 showed that the solution was stable when stored in the dark at 4°C. There was less than 5% variation confirming the hplc results. The exposed solutions at 25°C indicated higher absorbance readings which could be due to degration products absorbing at 255 nm.

*3.1.2.6 Conclusion*

The proposed uv method allows a rapid and economical quantitation of ketoprofen without any time consuming sample preparation. Moreover, the spectrophotometric methods involve simple instrumentation compared with other instrumental techniques.

# CHAPTER FOUR
# THE *IN VITRO* RELEASE OF KETOPROFEN

## 4.1 *IN VITRO* DISSOLUTION METHODOLOGY

### *4.1.1 Introduction*

The evaluation of *in vitro* release of actives from semisolid preparations has received much attention in recent years. Release is a function of several physicochemical characteristics within the semisolid, so that constancy of release from one batch to another implies that the manufacturing process is the same (115). A bioavailability study of a topical formulation begins with the *in vitro* investigation of the drug release from the formulation under evaluation (106). Historically, although *in vitro* release rate testing from semisolids could potentially provide valuable information about product performance, it is not an industry wide quality control test requirement as compared to the utility of *in vitro* dissolution testing of oral dosage forms (105). To change this situation the extension of *in vitro* dissolution methodology to semisolid dosage forms has been the subject of substantial effort and debate. Similar to the dissolution testing of oral dosage forms, a simple, reliable and reproducible release rate method can guide formulation development, help to monitor batch-to-batch quality and stability and control the manufacturing process. It is particularly useful for detecting the effect of product changes including drug substance, excipients and manufacturing processes (105, 107, 112, 113). The measurement of *in vitro* release from semisolids attempts to measure changes in important physical properties that may be related to topical bioavailability. However, measurement conditions do not usually mimic physiological reality. In particular, the *in vitro* release methodology does not include a membrane resembling the SC of the skin, an essential determinant of skin penetration characteristics (116). Therefore, *in vitro* release is not a surrogate for *in vivo* bioequivalency testing, but is intended to compare a product following scale-up or post approval manufacturing changes (105). *In vitro* release testing cannot also, on first principles, be considered as a test for establishing the bioequivalence of a product relative to an innovator formulation, however such testing does appear to have value in the following instances:

i. in formulation design and optimization,
ii. for determining the likelihood that changes in composition and/or processing of a formulation might impact on its function and

iii. for qualifying a new manufacturing method or site (235).

### *4.1.2 In vitro release testing*

Release testing can, in principle, reveal much about the physical attributes (solubility, microscopic viscosity, emulsion state, particle size) of a semisolid dosage form (235). *In vitro* release is one of several standard methods which can be used to characterise performance characteristics of a topical dosage form, *i.e.,* semisolids such as creams, gels and ointments. Important changes in the characteristics of a drug product formula or the thermodynamic properties of the drug it contains will be seen as a difference in drug release (108).

Recommendations on *in vitro* methodologies have been collated as guidelines by regulatory bodies. The FDA Scale-Up And Post Approval Changes (SUPAC-SS) and the International Pharmaceutical Federation in collaboration with the American Association of Pharmaceutical Sciences (FIP/AAPS) have guidelines requiring the performance of release testing from semisolid dosage forms after formulation changes (108, 109). In particular, *in vitro* dissolution of pre- and post-change formulations must be compared whenever changes are made to the composition of the product, manufacturing equipment, or process (105, 235). Although the FDA SUPAC-SS guidance includes general methodology descriptions of diffusion systems, it does not specify a particular test methodology because no compendial apparatus, procedures or requirement for *in vitro* release testing of semisolids topical dosage forms have been described in relevant Pharmacopoeias to date (105, 109, 110, 114). An *in vitro* release study design recommended by FDA SUPAC-SS is employed in this laboratory.

#### *4.1.2.1 Diffusion cell system*

The modified Franz diffusion cells and the European Pharmacopoeia diffusion cells were employed in the comparative diffusion study on ketoprofen containing topical formulations. These cells have been described in section 1.4.2. The Franz diffusion cells are more frequently employed in the measurement of *in vitro* release from semisolid formulations than the European Pharmacopoeia diffusion cells (105, 223).

#### *4.1.2.2 Synthetic membrane*

Synthetic membranes selected for use in *in vitro* diffusion experiments should usually be commercially available, have little capacity to bind to the drug, have little tendency to interact with the releasing medium and offer the least possible diffusional resistance (113, 235). Commercially available synthetic membranes have the added benefit of ensuring batch-to-batch homogeneity and uniformity, a property that is lacking with biological membranes. Silatos™ silicone sheeting REF 7458 from Atos Medical (Hőrby, Sweden) with a 0.12 mm thickness was used in both types of diffusion cell systems. Its relatively inert, lipophilic nature makes it an ideal environment for partitioning and permeation of drugs while its aporosity provides some rate-limiting function to this process. Pieces of the membrane were cut to fit the aperture through which diffusion occurs in both the Franz diffusion cell and the European Pharmacopoeia diffusion cell.

#### *4.1.2.3 Receptor medium*

A receiving medium that is similar to the physiological condition of the skin is indicated, including the practical consideration to choose a receiving medium that allows sufficient amounts of active ingredient released within a reasonable time period to ensure accurate analysis. To achieve sink conditions, the receptor medium must have a high capacity to dissolve or carry away the drug in question. This is accomplished by keeping the thermodynamic activity of the drug in the receiver medium at a small fraction of that initially found in the semisolid. It is desirable to minimise the capacity of the receptor medium to elute ingredients from the semisolid matrix other than the drug. A receptor medium is chosen which is compatible with the membrane and formulation (235). The thermodynamic activity of the drug in the receptor medium should not exceed 10% of its thermodynamic activity in the donor medium so as to maintain a favourable driving force for permeation and assure reasonable and efficient collection of the permeant (236). The choice of the appropriate receptor medium was primarily based on the physicochemical properties of ketoprofen. Ketoprofen is practically insoluble in water but soluble in alcoholic media. A previous study in this laboratory (100) indicated that alcoholic solutions produced excess bubble formations on the under surface of the membrane using the Franz diffusion cells which reduced the surface area available for diffusion, therefore phosphate buffers were chosen. An attempt was made to evaluate the effect of molarity and pH on the release rate of ketoprofen.

The *in vitro* diffusion was performed in modified Franz diffusion cells using Silatos™ silicone sheeting membrane with 100 mg of 2.5% m/m ketoprofen gel (Fastum®, South Africa) in the donor compartment. The cells were maintained at 32 ± 0.5°C and the samples were analysed for ketoprofen at 2, 4, 8, 24, 48 and 72 hours by the hplc method described in the previous chapter. The diffusion profile is illustrated in Figure 4.1.

Generally there was an effect of the molarity and pH of the receptor medium on the release of ketoprofen. The highest release profile was observed with 0.5 M phosphate buffer and this could be attributed to its high buffering capacity. These buffers were not considered further because most of the drug had been released within 24 hours and moreover 0.5 M was considered too high a concentration which could reduce the life span of the hplc analytical column. The 0.2 M phosphate buffer was chosen for further development as it is easier to prepare in small quantities and the diffusion profiles were smooth with gradual drug release over the diffusion run time. It is also important to appreciate that the pH of an aqueous buffered receptor solution may markedly affect the apparent flux of a permeating weakly ionisable compound (111). The pH value of 6.8 for all three concentrations produced higher diffusion profiles than pH 7.2. Although some studies have reported (236) a pH value of 7.2 as the standard receptor medium pH value, this laboratory chose to use pH 6.8 because the skin is reported to have a slightly acidic pH (105, 224).

The buffer solution was prepared by accurately weighing 136.09 g of potassium dihydrogen orthophosphate into a 5 l A-grade volumetric flask and made up to volume with hplc grade water. The pH was then adjusted to 6.8 using sodium hydroxide pellets. The solution was filtered through a 0.44 μm hvlp filter from Millipore (Massachusetts, USA) and degassed under vacuum with an Eyela Aspirator A-2S from Tokyo Rikakikai Co., Ltd (Tokyo, Japan) before use. The receptor medium indicated for use with the Franz diffusion cells was degassed 10 minutes prior to filling because of the greater possibility of the formation of air bubbles under the membrane.

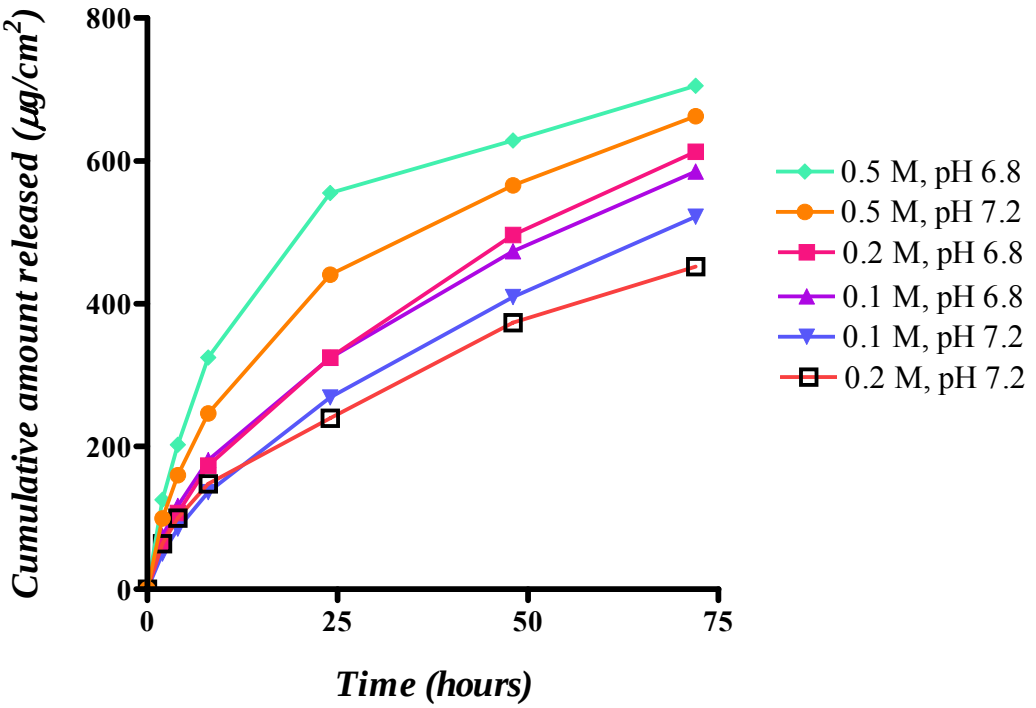



*Figure 4.1 Effect of molarity and pH on the diffusion profile of ketoprofen*

The temperature of the receptor medium can also affect the release profile of ketoprofen from the formulations. Higher temperatures produce faster release of the active from topical formulations although excessively high temperatures that would melt the formulation or otherwise cause significant physical changes should be avoided (116). Figure 4.2 illustrates the effect of receptor medium temperature on the *in vitro* release of ketoprofen from a proprietary formulation. Although the receptor temperature is generally set to 32 ± 0.5°C to approximate skin surface temperature (116, 223) some studies have indicated the use of 37 ± 0.5°C. An attempt was made to evaluate the difference in the diffusion profiles at 32 ± 0.5°C and 37 ± 0.5°C using the European Pharmacopoeia diffusion cells with approximately 500 mg of 2.5% m/m ketoprofen gel (Fastum®, South Africa) in each reservoir. Although there is no significant difference in the diffusion profiles in Figure 4.2, it can be noted that at 37 ± 0.5°C ketoprofen is released much faster compared to that at 32 ± 0.5°C. The temperature of the water bath in the USP dissolution apparatus was maintained at 32 ± 0.5°C for all European Pharmacopoeia diffusion experiments. However, it was observed with the Franz diffusion apparatus that the temperature had to be set at 37 ± 0.5°C to produce a temperature reading of the formulation in the donor compartment of 32 ± 0.5°C. This suggests that there is some inevitable heat loss through the plastic tubes that connect the Franz diffusion cells in series. The open end of the donor chamber of the Franz diffusion cell was covered with

PARAFILM 'M'® American National Can™ (Chicago, USA), an impermeable film, and then with aluminium foil to exclude the evaporation of volatile components of the formulations and to maintain constant temperature readings.

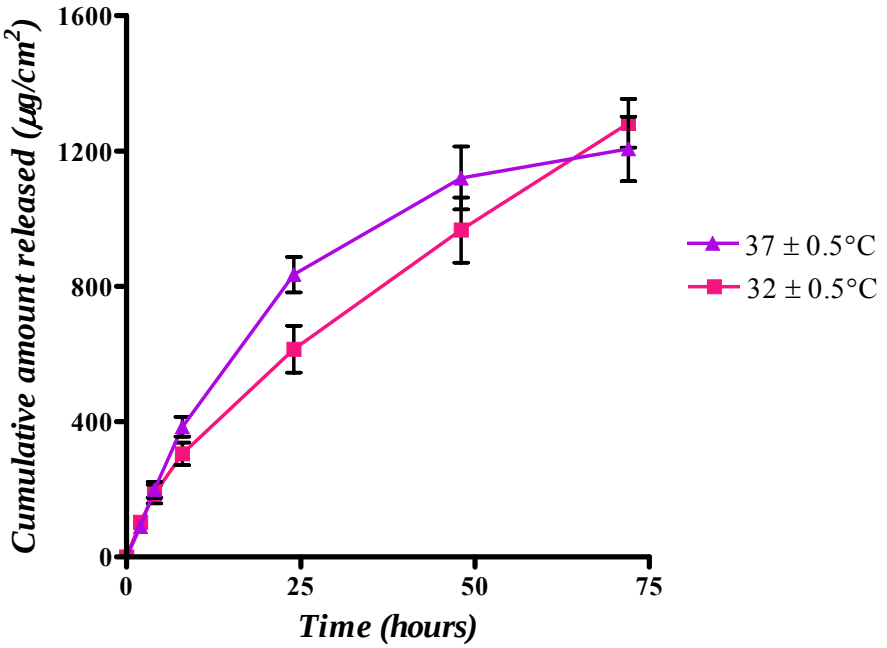



*Figure 4.2 Effect of temperature on the diffusion profile of ketoprofen*

*4.1.2.4 Sample applications*

Thick applications are applied to the test membrane and the diffusion cell system is capped or completely submerged in the receptor medium to prevent volatile substances from evaporating (235). The effect of mass of the sample application was investigated in modified Franz diffusion cells using Silatos™ silicone sheeting membrane with 50 mg, 100 mg and 200 mg of 2.5% m/m ketoprofen gel (Fastum®, South Africa) in the donor compartment.

Figure 4.3 illustrates the effect of mass on the *in vitro* release of ketoprofen from a proprietary product. The 200 mg diffusion profile was significantly different from the 50 mg and 100 mg profiles. 200 mg was considered too excessive as such amounts could lead to saturation kinetics. Although the diffusion profiles of 50 mg and 100 mg were not significantly different, 100 mg was chosen over 50 mg because it was easier to measure from the bulk formulation.

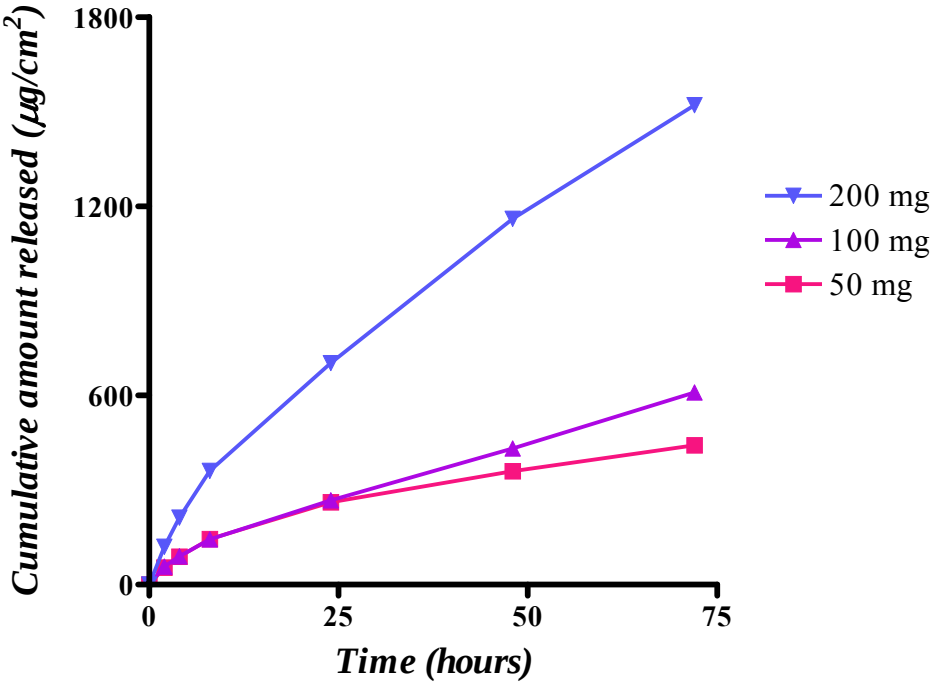



*Figure 4.3 Effect of mass on the diffusion profile of ketoprofen*

Approximately 100 mg of the ketoprofen containing topical formulation was uniformly placed on the membrane within the donor compartment of the Franz diffusion cells. The compartment was kept occluded to prevent solvent evaporation and any significant compositional changes. The amount of topical formulation per unit membrane area available to the diffusant in the donor chamber of the Franz diffusion cell was calculated to be 60.63 mg/cm$^2$. From this, the corresponding sample application was calculated for the European Pharmacopoeia diffusion cell. Approximately 500 mg of the ketoprofen containing topical formulation was uniformly placed within the reservoir of each cell and the membrane placed over it ensuring the absence of air bubbles between the formulation and the membrane. Direct comparisons of the diffusion profiles obtained from both the Franz diffusion cells and the European Pharmacopoeia diffusion cells were possible because the mass of the topical formulation to the unit surface area available was identical.

### *4.1.2.5 Number of samples*

A minimum of six samples is recommended to determine the release rate (profile) of a topical formulation (108). The recommended number of six samples was based on the imperial system and therefore had six sample stations. The imperial numerical system has been replaced by the metric numerical system and five samples taken at each sampling time were

deemed to be sufficient to give a diffusion profile from which the kinetics of the diffusion process could be calculated (100).

#### *4.1.2.6 Sampling time*

Samples of receptor fluid were taken at 2, 4, 8, 24, 48 and 72 hours. These multiple sampling times were chosen over an appropriate time period to generate an adequate diffusion profile and to determine the drug release rate. The Franz diffusion cells were completely emptied at each sampling time and refilled with fresh degassed receptor fluid. Aliquots of 5 ml were taken from the European Pharmacopoeia diffusion cells at each sampling time before analysis using both the hplc and the uv spectrophotometric method developed in chapter three. The total percentage of receptor fluid removed for sample analysis was 3% and considered insignificant to disrupt sink conditions.

#### *4.1.2.7 Sample analysis*

Samples withdrawn from the receptor chambers of both the Franz diffusion cell and the European Pharmacopoeia diffusion cell were analysed by validated hplc and uv spectrophotometric procedures as outlined in chapter three. Samples from the Franz cell diffusion apparatus for uv analysis were diluted to obtain absorbance readings in the range of 0.2 - 0.8. Five ml of these samples were diluted to 25 ml with solvent in an A-grade volumetric flask. The data obtained from both diffusion cell experiments were manipulated using a designed Microsoft® Office Excel 2003 spreadsheet (appendix I).

#### *4.1.2.8 Diffusion profile comparison*

Comparing the *in vitro* diffusion profiles provides the formulator with critical information necessary to screen formulations during product development, evaluate stability and optimise dosage forms. Curve comparisons produce the means to evaluate the effect that changing a process variable has upon dissolution and are useful as a quality assurance tool to measure batch-to-batch uniformity (225). The diffusion profiles of the three proprietary ketoprofen-containing gel products from three different manufacturing sites and a number of extemporaneously prepared gel formulations were compared. The diffusion profiles for each formulation were obtained using both the Franz diffusion cells and the European Pharmacopoeia diffusion cells with hplc and uv analysis and plotted to the model of Higuchi (101). For each diffusion cell, drug release as a function of square root of time was

determined. The drug release profile was best fitted by least squares linear regression using the time points over the square root time period of 72 hours. The mean ± SD of the slope of ketoprofen release from five cells for each formulation was determined. The rate of drug released and extent of drug released in each of the cells was statistically evaluated for significance between formulations by the ANOVA test using GraphPad PRISM® version 4.00 from GraphPad Software Inc. (California, USA). A statistical significant difference was accepted at $p < 0.05$.

# CHAPTER FIVE
# FORMULATIONS OF PROPRIETARY AND EXTEMPORANEOUS TOPICAL KETOPROFEN GEL PREPARATIONS USING CARBOPOL® POLYMERS AND CO-POLYMERS

## 5.1 DERMATOLOGICAL FORMULATIONS

### *5.1.1 Introduction*

Dermatological prescriptions are among those most frequently compounded. Many pharmacists who seldom compound are often asked to prepare ointments and creams. Generally, this may include something as relatively basic as the addition of an active drug to a topical vehicle. However, many dermatological products are much more complex, reflecting at the same time the experience and requests of the physician, the disorders experienced by the patient and the ability and facilities of the compounding pharmacist (130).

Over the past few decades there have been many advances in the understanding of the physicochemical properties of both formulation systems and their ingredients. These have led to the ability to develop physically, chemically and biologically stable products. There has also been a significant increase in our knowledge of the properties of the skin and the processes that control skin permeation. It has been established that the permeation of compounds across intact skin is controlled fundamentally by the SC and it is the chemical composition and morphology of this layer that usually determines the rate and extent of absorption. Similarly, section 1.2.5 shows how to modify this barrier by chemical or physical means and thereby alter the rate of diffusion of many permeating molecules. A basic deficiency, however, in the application of our understanding of the barrier properties of the skin to dermatological and transdermal therapy, is that this knowledge has largely been generated by investigations on normal skin, rather than pathological skin. The relevance of such information to diseased skin, for which permeation characteristics are probably significantly altered, has yet to be fully established. In modern-day pharmaceutical practice, therapeutic compounds are applied to the skin for dermatological (within the skin), local (regional) and for transdermal (systemic) delivery. However, whatever the target site or organ, it is usually a prerequisite that the drug crosses the outermost layer of the skin (93).

### *5.1.2 Formulation of dermatological products*

The selection of formulation type for dermatological products is usually influenced by the nature of the skin lesion and the opinion of the medical practitioner. To this day a practising dermatologist would prefer to apply a wet formulation (ranging from simple tap-water to complex emulsion formulations with or without drug) to a wet lesion and a dry formulation (*e.g.,* petrolatum) to a dry lesion. Solutions and powders lack staying power (retention time) on the skin and can afford only transient relief. In modern-day pharmaceutical practice, semisolid formulations are the preferred vehicles for dermatological therapy because they remain *in situ* and deliver the drug over extended time periods (93). The term vehicle is very common for a complex system and implies a differentiation between active and inactive principles, whereby the active principle is embedded into a matrix, the vehicle. With the aid of the vehicle the active principle is delivered to the application site or target organ, where the desired effect is achieved (126). In most cases, the developed formulation will be an ointment, emulsion or gel.

#### *5.1.2.1 Ointments*

Ointments are greasy, semisolid preparations which are often anhydrous and which contain the medicament either dissolved or dispersed in the vehicle (127). There are four types of ointment bases namely hydrocarbon base, absorption base, water removable base and water-soluble base. Only the hydrocarbon bases are completely anhydrous. A typical formulation contains fluid hydrocarbons (minerals oils and liquid paraffins) mixed with a longer alkyl chain, higher-melting point, hydrocarbons (white and yellow paraffin and petroleum jelly). The difference between the white and yellow paraffin is simply that the yellow paraffin has been bleached. Although the non-medicated anhydrous ointments are extremely useful as emollients, their value as topical drug delivery systems is limited by the relative insolubility of many drugs in hydrocarbon oils. It is possible to increase drug solubility within a formulation by incorporation of hydrocarbon-miscible solvents (section 1.2.5.1.1), such as isopropyl myristate or propylene glycol, into the ointment. Although increasing the solubility of a drug within a formulation may often decrease the release rate, it does not necessarily decrease the therapeutic effect. The preparation of ointment formulations may appear to be a simple matter of heating all of the constituents to a temperature higher than the melting point of all of the excipients and then cooling with constant mixing. The reality, however, is that the process is somewhat more complex and requires careful control over various parameters,

particularly the cooling rate. Rapid cooling creates stiffer formulations in which there are numerous small crystallites, whereas a slow-cooling rate results in the formulation of fewer, but larger, crystallites and a more fluid product (93).

#### *5.1.2.2 Gels*

Gels have a variety of applications in the administration of medications orally, topically, intranasally, vaginally and rectally. Some gel systems are transparent and others are translucent, since the ingredients involved may not be completely dispersed or they may form aggregates which disperse light. Gels are semisolid systems consisting of suspensions made up of small inorganic particles or large organic molecules interpenetrated by a liquid (128). The common characteristic of all gels is that they contain continuous structures that provide solid-like properties (93). Where the gel mass consists of a network of small, discrete particles, the gel is classified as a two-phase system. In these two-phase systems, if the particle size of the dispersed phase is large, the product is referred to as a magma. Single-phase gels consist of organic macromolecules uniformly distributed throughout a liquid in such a manner that no apparent boundaries exist between the dispersed macromolecules and the liquid. Single-phase gels may be made from synthetic macromolecules or from natural gums (mucilages). The continuous phase is usually aqueous, but it can also be alcoholic or oleaginous. Gels exhibit a number of different characteristics, including imbibition, swelling, syneresis and thixotropy (128, 129).

*Table 5.1 General classification and description of gels (128)*

| **Class** | **Description** | **Examples** |
|---|---|---|
| Inorganic | Usually two-phase systems | Aluminium hydroxide gel, bentonite magma |
| Organic | Usually single-phase systems | Carbopol®, tragacanth |
| Hydrogels | Contains water | Silica, bentonite, pectin, sodium alginate, methylcellulose, alumina |
| Organogels | Hydrocarbon type | Petrolatum, mineral oil/polyethylene gel, Plastibase |
| | Animal/vegetable fats | Lard, cocoa butter |
| | Soap-base greases | Aluminium stearate with heavy mineral-oil gel |
| | Hydrophilic organogels | Carbowax bases (PEG ointment) |
| Hydrogels | Organic hydrogels | Pectin paste, tragacanth jelly |
| | Natural and synthetic gums | Methylcellulose, sodium carboxymethylcellulose, Pluronic® F-127 |
| | Inorganic hydrogels | Bentonite gel (10% to 25%), Veegum® |

There are a variety of semi-synthetic celluloses in use as thickeners in gel formulations. These include methylcellulose, carboxymethylcellulose, hydroxyethylcellulose,

hydroxypropylcellulose and hydroxypropylmethylcellulose (93, 126). Branched-chain polysaccharide gums, such as tragacanth, pectin, carrageenan and agar, are of naturally occurring plant origin, therefore they can have widely varying physical properties, depending on their source. By far the most extensively employed gelling agents in the pharmaceutical and cosmetic industries are the carboxyvinyl polymers known as carbomers (93).

#### *5.1.2.3 Emulsions*

The most common emulsions used in dermatological therapy are creams. These are two-phase preparations in which one phase (the dispersed or internal phase) is finely dispersed in the other (the continuous or external phase). The dispersed phase can have either a hydrophobic base (oil-in-water creams, o/w), or be aqueous based (water-in-oil creams, w/o). Whether a cream is o/w or w/o depends on the properties of the system used to stabilize the interface between the phases. Emulsions have two incompatible phases in close conjunction and therefore the physical stability of creams is always tenuous, although it may be maximized by the judicious selection of an appropriate emulsion-stabilizing system. In most pharmaceutical emulsions, the stabilizing systems comprise either surfactants (ionic or non-ionic), polymers (non-ionic polymers, polyelectrolytes, or biopolymers), or mixtures of these. The most commonly used surfactant systems are sodium alkyl sulphates (anionic), alkyl ammonium halides (cationic) and polyoxyethylene alkyl ethers or polysorbates (non-ionic). These are often used alone or in conjunction with non-ionic polymeric substances, such as polyvinyl alcohol or poloxamer block co-polymers or polyelectrolytes, such as polyacrylic-polymethacrylic acids (93). Modern formulations, which are called emulsifier free, are composed of polymers, water and oil. Several cosmetic and pharmaceutical preparations have been developed containing sodium polyacrylate dispersed in the oil phase as the main emulsifier or co-emulsifier for topical application. These formulations have very high skin compatibility as they are free of surfactant or with low emulsifier content. The o/w emulsions have a velvet-like and soft touch skin feeling, which does not display any tackiness compared to traditional thickeners and are suitable to realise light gel/cream textures (126).

## 5.2 EXCIPIENTS

### *5.2.1 Gelling agents*

The in-house gelling agents used in the extemporaneous manufacture of gels are Carbopol® polymers and co-polymers. The generic (*i.e.,* non-proprietary) name adopted by USP-NF for various Carbopol® polymers is carbomer. Carbopol® polymers are synthetic high molecular weight polymers of acrylic acid cross-linked with either allylsucrose or allylethers of pentaerythritol whereas Carbopol® co-polymers are synthetic high molecular weight polymers of acrylic acid with small amounts of long chain alkyl acrylate co-monomers cross-linked with allylpentaerythritol. All these polymers have the same acrylic acid backbone (227, 229). These polymers contain between 56.0 - 68.0% of carboxylic acid (COOH) groups, calculated on the dry basis (238). They are fluffy, white, mildly acidic flocculated powders averaging 2 to 7 microns in diameter. Each primary particle can be viewed as a network structure of linear polymer chains interconnected by cross-links. These linear polymers are soluble in a polar solvent, such as water. Carbopol® polymers, along with Pemulen® polymeric emulsifiers are all cross-linked. They swell in water up to 1000 times their original volume (and ten times their original diameter) to form a gel when exposed to a pH environment between 4.0 - 6.0. Since the p$K_a$ of these polymers is 6.0 ± 0.5, the carboxylate groups on the polymer backbone ionize, resulting in repulsion between the negative charges, which adds to the swelling of the polymer. Cross-linked polymers do not dissolve in water. The glass transition temperature of Carbopol® polymer is 105°C in powder form. However, the glass transition temperature drops dramatically as the polymer comes into contact with water. The polymer chains starts gyrating and the radius of gyration becomes larger. Macroscopically, this phenomenon manifests itself as swelling (228).

Carbopol® polymers and co-polymers are used mainly in liquid or semisolid pharmaceutical formulations as suspending or viscosity increasing agents. Formulations include creams, gels and ointments. Carbopol® polymers are also employed as emulsifying agents in the preparation of o/w emulsions for external use and are also employed in cosmetics (238).

In the dry state, a carbomer molecule is tightly coiled, but when dispersed in water the molecule begins to hydrate and partially uncoil, exposing free acidic moieties. To attain maximum thickening effect the carbomer molecule must be fully uncoiled and this can be

achieved by one of two mechanisms. The most common method is to convert the acidic molecule to a salt, by the addition of an appropriate neutralizing agent. For formulations containing aqueous or polar solvents, carbomer gellation can be induced by the addition of simple inorganic bases such as sodium or potassium hydroxide. Less polar or non-polar solvent systems can be neutralised with amines such as triethanolamine or diethanolamine. Neutralization ionises the carbomer molecule, generating negative charges along the polymer backbone, and the resultant electrostatic repulsion creates an extended three-dimensional structure. It is important to add a sufficient quantity of the neutralising agent to commence gellation. Insufficient or excess amounts of the neutralising agent will result in viscosity or thixotropic changes (93). The use of a strong base such as sodium hydroxide is not recommended since there is a greater risk of overshooting the desired pH range thus causing loss of the gel integral network.

A number of different Carbopol® polymer grades are commercially available which vary in molecular weight, degree of cross-linking and polymer structure and therefore they may not have identical properties with respect to their use for specific pharmaceutical purposes, *e.g.,* as controlled-release agents, bioadhesives, topical gels, thickening agents and emulsifying agents. Carbopol® polymers should not be interchanged unless performance equivalency has been ascertained (227). Four different types of Carbopol® polymers namely 974 PNF, 980 NF, 981 NF and Ultrez™ 10 NF with one co-polymer namely Pemulen® polymeric emulsifier, Pemulen® TR1 NF, were used in this laboratory.

Carbopol® 974 PNF polymer was introduced specifically for use in oral and mucoadhesive contact applications such as controlled release tablets, oral suspensions and bioadhesives. In addition Carbopol® 974 PNF provides thickening, suspending and emulsification properties to high viscosity systems for topical applications (227).

Carbopol® 980 NF polymer forms clear hydroalcoholic gels. This polymer is the key to obtaining clear gels. Carbopol® 980 NF polymer is also the most efficient thickener of all the Carbopol® polymers and has extremely slow flow properties suitable for spray-on applications (227).

Carbopol® 981 NF polymer provides permanent emulsions and suspensions with low viscosities. Gels produced with this polymer have excellent clarity. In ionic systems, this

polymer performs better than most of the other Carbopol® polymers. Carbopol® 981 NF polymer produces higher viscosities than Carbopol® 980 NF at concentrations below 0.1% in water systems and at concentrations below 1.5% in solvent systems (227).

Carbopol® Ultrez™ 10 NF polymer is a new member of the Carbopol® family. It is a dispersable polymer that offers a wide range of performance properties and can be used in a variety of personal care applications (231). The unique dispersion performance of Carbopol® Ultrez™ 10 NF polymer allows it to wet quickly, yet hydrate slowly. This property helps minimise lumping, which can be troublesome when turbulent mixing is not available during dispersion (227).

Pemulen® TR1 NF is a versatile primary polymeric emulsifier which can emulsify up to 30% oil by weight, within a pH range of 4.0 - 5.5 and up to 20% oil over the pH range of 3.0 - 11.0. 'Primary' indicates that it is an emulsion former, not an emulsion stabiliser, as many of the Carbopol® polymers are. Pemulen® TR1 NF can enable formulation of permanent emulsions, even at elevated temperatures without the use of irritating surfactants. In addition, the hydrophobic phase of emulsions formed with Pemulen® polymeric emulsifiers do not re-wet in the presence of water as traditional surfactants-based emulsions do. Instead, emulsions based on Pemulen® polymers deposit an occlusive layer on the skin delivering the topical medication in the form of low irritancy lotions and creams with elegant skin feel. Pemulen® TR1 NF can also be used for high clarity topical gels with hydrophobic or highly ionic components (227). While Pemulen® TR1 NF thickens the water, Carbopol® polymers should be used with Pemulen® TR1 NF to provide greater thickening properties where higher viscosity emulsions are required. Pemulen® polymeric emulsifiers can actually form o/w emulsions. The lipophilic portion adsorbs at the oil-water interface and the hydrophilic portion swells in the water forming a gel network around oil droplets to provide exceptional emulsion stability to a broad range of oils (230). Pemulen® polymeric emulsifiers have been designed to act both as primary emulsifiers and viscosity increasing agents (126).

It is worth noting that other grades of Carbopol® polymers and co-polymers that can be used in the preparation of topical formulations, namely 934 NF, 934 PNF, 940 NF, 941 NF, 971 PNF, 71G NF, 1342 NF and Pemulen® TR2 NF, are available but were not used in this study.

### *5.2.2 Triethanolamine*

Triethanolamine, also chemically known as triethylolamine, trihydroxytriethylamine or tris(hydroxyethyl)amine is widely used in topical pharmaceutical formulations primarily in the formation of emulsions. It is a clear, colourless to pale yellow-coloured viscous liquid having a slight ammoniacal odour. In this study triethanolamine is used as a weak neutralizing agent to commence the gelling process by converting the free acidic hydrogens into amine salts. Triethanolamine may turn brown on exposure to air and light and thus should be stored in an airtight container, protected from light, in a cool, dry, place (256).

### *5.2.3 Propylene glycol*

Propylene glycol, chemically known as 1,2-propanediol, is widely used as a solvent extractant and preservative in a variety of parenteral and non-parenteral pharmaceutical formulations. It is a clear, colourless, viscous, practically odourless liquid with a sweet acrid taste resembling glycerine. Propylene glycol can also be used as an antimicrobial preservative, disinfectant, humectant, plasticizer and water-miscible co-solvent. Propylene glycol is chemically stable when mixed with ethanol 95% v/v or water. Propylene glycol is hygroscopic and should be stored in an airtight container, protected from light, in a cool, dry, place (257). For the purposes of this research propylene glycol was mainly employed as a water-miscible co-solvent to aid in the dissolution of ketoprofen during the manufacture and as a chemical penetration enhancer.

### *5.2.4 Ethanol*

Ethanol is a colourless, clear, volatile, flammable liquid (277) and employed in the manufacture of topical formulation primarily as a solvent. However, although alcohols are useful to increase solubility of non-polar drugs, their use as co-solvents with hydrophilic polymers is often limited.

### *5.2.5 Transcutol® HP*

Transcutol® HP is highly purified diethylene glycol monoethyl ether. It is a hydroscopic liquid that is freely miscible with both polar and non-polar solvents (53). Transcutol® HP has unique solubilizing properties. It has the ability to solubilize both hydrophilic and hydrophobic materials. Transcutol® HP was traditionally used as an industrial solvent. Since then it has been used in a wide variety of pharmaceutical and personal care products.

Transcutol® HP can be incorporated into all types of topical emulsions (creams and lotions), solutions, gels and ointments. Transcutol® HP has been recognised as a potential transdermal permeation enhancer due to its non-toxicity, biocompatibility with skin and excellent solubilizing properties (53). Recent studies (51) have shown that Transcutol® HP significantly increases the percutaneous penetration of various active substances particularly if used in combination with suitable co-solvents. In this study, propylene glycol and ethanol were selected for a possible synergistic enhancer effect on account of their satisfactory solvent power for ketoprofen.

*Table 5.2 Common excipients employed and their sources*

| **Excipients** | **Manufacturer** |
|---|---|
| *Active principal ingredient* | |
| Ketoprofen | Sigma-Aldrich (Atlasville, SOUTH AFRICA) |
| *Carbopol® polymers* | |
| Carbopol® 974 PNF | Noveon Inc. (Cleveland, USA) |
| Carbopol® 980 NF | Noveon Inc. (Cleveland, USA) |
| Carbopol® 981 NF | Noveon Inc. (Cleveland, USA) |
| Carbopol® Ultrez™ 10 NF | Noveon Inc. (Cleveland, USA) |
| *Carbopol® co-polymer* | |
| Pemulen® TR1 NF | Noveon Inc. (Cleveland, USA) |
| *Neutralizing agent* | |
| Triethanolamine | Aspen Pharmacare (Port Elizabeth, SOUTH AFRICA) |
| *Co-solvent/solubilizing agent* | |
| Ethanol | MERCK (Wadeville, SOUTH AFRICA) |
| Transcutol® HP | Gattefossé (Saint-Priest, FRANCE) |
| Propylene glycol | MERCK (Wadeville, SOUTH AFRICA) |

## 5.3 EXPERIMENTAL

### *5.3.1 Proposed design*

The aim of this study was to statistically assess ($p < 0.05$, ANOVA) the *in vitro* release rate of ketoprofen from three proprietary products from three different countries and also from a series of extemporaneously prepared hydroalcoholic topical gel formulations using Carbopol® polymers and co-polymers as the gelling agents. The investigation was further extended to assess the *in vitro* release of ketoprofen from these hydroalcoholic gels as a function of variables such as pH, different grades of Carbopol® polymers, drug/polymer ratio and drug/CPE ratio.

### *5.3.2 Preliminary studies*

Initial studies pertaining to the manufacture of extemporaneous Carbopol® gel formulations containing an NSAID were sourced from Macedo *et al.* (232). Low concentrations of Carbopol® polymers *i.e.,* 0.5% m/m, 0.75% m/m and 1.0% m/m resulted in formulations which did not gel. Very poor release was observed from all preliminary formulations mainly due to the amount of triethanolamine required in the preparation of these gels although justifiable due to the type of the Carbopol® polymer, Carbopol® 934 NF, initially used. The amount of the organic base was too high and it is possible that salts of ketoprofen were formed in gel formulations which could not easily partition across the lipophilic silicone membrane. Another possible explanation was that the pH of the gel formulations was too high mainly because of the large amount of organic base, triethanolamine, added. The next step was to identify how much organic base was needed to effectively induce the gelling mechanism without having a significant effect on the release of ketoprofen.

### *5.3.3 Preparation of extemporaneous topical gel formulations*

The gelling agent used in the preparation of the formulations was taken from the range of different grades of the Carbopol® polymers and co-polymers as indicated above. The alcoholic and/or co-solvent mixture comprised varying amounts of propylene glycol, ethanol and Transcutol® HP. The gels were prepared with different grades of Carbopol® polymers, with varying amount of the polymers. Extemporaneous topical gels containing different amounts of ketoprofen and excipients were also prepared. The required amount of ketoprofen was dissolved in the alcoholic and/or co-solvent mixtures in a 100 ml beaker

containing a magnetic bar placed on a LABCON MSH 10 magnetic stirrer/hot plate from LABMARK (Maraisburg, South Africa). The open end of the beaker was covered with PARAFILM 'M'® from American National Can™ (Chicago, USA) to minimise the evaporation of volatile components of the alcoholic and/or co-solvent mixture. The required amount of Carbopol® polymer was slowly dispersed in the ketoprofen/co-solvent mixture with vigorous mixing at 1800 rpm. The beaker was covered with aluminium foil and left mixing for approximately 60 minutes. The mixture was also homogenised with an Ultra-turrax® from Janke & Kunkel (Essex, UK) for 5 minutes at low speed. After complete addition of the polymer and mixing, the gels were spontaneously formed with the addition of triethanolamine in a 1:1.5 ratio of polymer to base unless otherwise indicated. Gels with partially wetted polymer lumps were discarded. The composition of each extemporaneously prepared gel is shown in Tables 5.3 and 5.4.

### *5.3.4 Physical characterization of extemporaneous topical gel formulations*

#### *5.3.4.1 Drug content*

Approximately 800 mg of extemporaneous topical gel, equivalent to 20 mg of ketoprofen unless otherwise indicated, from each batch was weighed into a 50 ml Quickfit® Erlenmeyer flask on a Sartorius 1474 MP8-2 top load balance from Zeiss West Germany Optical Instruments (Pty) Ltd. (Port Elizabeth, South Africa). Approximately 40 ml of mobile phase for hplc analysis or receptor phase for uv analysis was added to the gel and shaken vigorously until the gel dissolved. The gel-liquid solution was then transferred to an A-grade 100 ml volumetric flask. The procedure was repeated with either more mobile phase or receptor phase until the volume of the mixture was made up to the mark. An aliquot of 2.5 ml of the solution was accurately transferred with a pipette to an A-grade 50 ml volumetric flask and made up to volume with either mobile phase or receptor phase. The solution was filtered through a 0.45 µm hydrophilic pvdf membrane from Millipore® Millex-HV, Millipore Corporation (Bedford, MA, USA) prior to both hplc and uv analysis. Individual concentrations were calculated from a standard calibration curve and the average values calculated for each batch.

*Table 5.3 Summary of formulae used in the extemporaneous manufacture of ketoprofen gels KET001 - KET010*

| EXCIPIENTS | KET001 | KET002 | KET003 | KET004 | KET005 | KET006 | KET007 | KET008 | KET009 | KET010 |
|---|---|---|---|---|---|---|---|---|---|---|
| Ketoprofen | 2.50 | 2.50 | 2.50 | 2.50 | 2.50 | 2.50 | 2.50 | 2.50 | 2.50 | 2.50 |
| Carbopol® 974 PNF | | | | 1.50 | | | | | | |
| Carbopol® 980 NF | | | | | 1.50 | | | 1.00 | 1.00 | 1.00 |
| Carbopol® 981 PNF | | | | | | 1.50 | | | | |
| Carbopol® Ultrez™ 10 NF | 1.00 | 1.50 | 2.00 | | | | | | | |
| Pemulen® TR1 NF | | | | | | | 1.50 | 0.10 | 0.30 | 0.50 |
| Triethanolamine | 1.50 | 2.25 | 3.00 | 2.25 | 2.25 | 2.25 | 2.25 | 1.65 | 1.95 | 2.25 |
| Ethanol | 20.00 | 20.00 | 20.00 | 20.00 | 20.00 | 20.00 | 20.00 | 20.00 | 20.00 | 20.00 |
| Transcutol® HP | | | | | | | | | | |
| Propylene glycol | 20.00 | 20.00 | 20.00 | 20.00 | 20.00 | 20.00 | 20.00 | 20.00 | 20.00 | 20.00 |
| Distilled water ad | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |

*Table 5.4 Summary of formulae used in the extemporaneous manufacture of ketoprofen gels KET011 - KET020*

| EXCIPIENTS | KET011 | KET012 | KET013 | KET014 | KET015 | KET016 | KET017 | KET018 | KET019 | KET020 |
|---|---|---|---|---|---|---|---|---|---|---|
| Ketoprofen | 2.50 | 2.50 | 2.50 | 2.50 | 1.50 | 5.50 | 2.50 | 2.50 | 2.50 | 2.50 |
| Carbopol® 974 PNF | | | | | | | | | | |
| Carbopol® 980 NF | 1.00 | | | | | | | | | |
| Carbopol® 981 PNF | | | | | | | | | | |
| Carbopol® Ultrez™ 10 NF | | | 1.50 | 1.50 | 1.50 | 1.50 | 1.50 | 1.50 | 1.50 | 1.50 |
| Pemulen® TR1 NF | 1.00 | 1.00 | | | | | | | | |
| Triethanolamine | 3.00 | 1.15 | 4.26 | 3.00 | 2.25 | 2.25 | 2.25 | 2.25 | 2.25 | 2.25 |
| Ethanol | 20.00 | 20.00 | 20.00 | 20.00 | 20.00 | 20.00 | | 20.00 | 40.00 | |
| Transcutol® HP | | | | | | | 20.00 | 20.00 | | 40.00 |
| Propylene glycol | 20.00 | 20.00 | 20.00 | 20.00 | 20.00 | 20.00 | 20.00 | | | |
| Distilled water ad | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |

#### *5.3.4.2 pH*

The pH of each extemporaneous topical gel formulation was measured with a Crison GLP 21 pH-meter from Crison Instruments (Lasec, South Africa). About 20 g of the gel was subjected to pH measurement within 24 hours of manufacture. An average pH reading of three readings was recorded.

#### *5.3.4.3 Viscosity*

This was not assessed.

#### *5.3.4.4 In vitro dissolution studies*

The *in vitro* dissolution methodology employed in this laboratory to assess the rate of drug release from the extemporaneous topical gel formulations as well as the proprietary products has been outlined in chapter four. A summary of experimental conditions and settings are listed in Table 5.5 below. The use of a finite dose technique was chosen for its similarity to the clinical use of topical formulations (183). The diffusion profiles for each formulation are reported in appendix II.

*Table 5.5 Summary of in vitro experimental conditions*

| | |
|---|---|
| Receptor medium | 0.2 M phosphate buffer at pH 6.8 |
| Temperature | 32 ± 0.5°C |
| Run time | 72 hours |
| Agitation | 100 rpm |
| Franz diffusion volume | 9 ml |
| European Pharmacopoeia diffusion volume | 1000 ml |
| Sample application | |
| Franz diffusion cell | 100 mg |
| European Pharmacopoeia diffusion cell | 500 mg |
| Membrane | Silatos™ silicone sheeting REF 7458 |
| Analytical procedure | Hplc and uv spectrophotometric analysis |

## 5.4 DIFFUSION PROFILES AND RELEASE KINETIC DATA OF PROPRIETARY KETOPROFEN CONTAINING TOPICAL GEL PREPARATIONS FROM THREE COUNTRIES

### *5.4.1 Introduction*

There has been growing interest in percutaneous administration of NSAIDs for the treatment of local conditions (163, 172). NSAIDs are one of the most widely used groups of agents both on prescription and over the counter for their analgesic, anti-inflammatory and antipyretic properties (170). The rationale behind using NSAIDs in topical formulations is to achieve a high local concentration of the active ingredient at the affected site, with as low a plasma concentration as possible in order to minimise systemic adverse effects. The short distance from the site of application (the skin) to the target (the joint) and the avoidance of the enterohepatic cycle is an attractive concept (169).

Studies to evaluate the clinical efficacy and safety of various topical NSAIDs compared to oral NSAIDs have been reported (162, 169, 170) but little research (234) has been conducted to evaluate the efficacy of topical NSAIDs from different manufacturers containing the same concentration of medicament. This gives rise to a potential problem when researchers conduct clinical trials using a particular brand of a proprietary product and publish results with the assumption that the results will be similar to other proprietary products which contain the same medicinal agent in the same concentration. Another drawback in such research studies is that some authors fail to indicate the name of the proprietary product used in a study (106, 163) which makes it difficult to undertake inter-laboratory comparisons. Proprietary products may contain different vehicle types and numerous studies have reported (218, 233, 239 - 242, 248, 260, 262, 264 - 267) on the influence of the vehicle composition on the *in vitro* and *in vivo* release of various medicaments.

Proprietary products usually undergo stringent processes and have to pass various tests as set out by the relevant regulatory body before they can be put on the market for use by the general public. It would be expected that a proprietary product purchased in one country would be as efficacious as another proprietary product purchased in another country. Studies have shown that formulations manufactured in different parts of the world have different efficacies and this maybe attributed to a number of reasons. One reason is the climatic

conditions under which the formulations are manufactured and subjected to during storage. Although formulations are usually prepared in temperature controlled laboratories, sometimes differences with respect to formulation viscosity, pH and drug release kinetics do occur. Another problem is how the formulation is transported to the distribution sites or to other countries. Most pharmaceutical products are manufactured in temperate climates and transported for use in tropical third world countries. High temperatures in tropical countries can affect the efficacy of a formulation (100).

Three different proprietary products each containing 2.5% m/m ketoprofen from South Africa, United Kingdom and France were used in this study. The proprietary products from the United Kingdom and France were transported to South Africa in the hand luggage on a commercial flight while the South African product was purchased from a local pharmacy. The aim of this study was to investigate any possible differences with respect to the kinetics and release pattern of three proprietary products from three different countries.

### *5.4.2 Results*

#### *5.4.2.1 Composition of proprietary products*

All three proprietary products indicated a 2.5% m/m ketoprofen content and were manufactured containing the same type of excipients but no concentrations were indicated on the label. However the South African manufacturers did not state on the product what type of excipients were used but indicated that the product contained *p*-hydroxybenzoic acid esters 0.1% m/m as preservatives.

*Table 5.6 Detailed compositions of proprietary products as indicated on package*

| Proprietary product | Manufactured | Excipients | End use date |
|---|---|---|---|
| Fastum® Gel | South Africa | Not stated | October 2008 |
| Ketum® Gel | France | p-hydroxybenzoic acid esters<br>Carbomer 940<br>Ethanol<br>Lavender oil<br>Diethanolamine<br>Purified water | December 2006 |
| Oruvail® Gel | United Kingdom | Carboxypolymethylene 940<br>Triethanolamine<br>Lavender oil<br>Ethanol<br>Purified water | January 2006 |

The presence of the preservatives was evident during the hplc analytical procedure which showed two additional peaks (A and B, section 3.1.2.4, Figure 3.2) next to the principal ketoprofen peak. The chromatograms for the other products only contained the principal peak. All three products were packaged in tubes. The products were also well within their end use date suggesting that neither the excipients nor the active principal had undergone changes which could have an effect on the results. It was also interesting to note that the South African product and the French product were both registered under the same license and the British product was registered under a separate license. Similar results from both the South African and the French product were to be expected. The composition of each of the proprietary products is listed in Table 5.6.

### *5.4.2.2 Drug content and pH readings*

An assay procedure and pH determination as outlined in sections 5.3.4.1 and 5.3.4.2 respectively confirmed that each proprietary product contained approximately 2.5% m/m ketoprofen and the pH readings ranged from 5.44 to 5.61. The drug content and pH readings are reported in Table 5.7. There was no statistical significant difference ($p > 0.05$, ANOVA) in the drug content and pH reading among the proprietary products.

*Table 5.7 Drug content uniformity and pH values of proprietary products*

| **Proprietary product** | **Actual percentage (% m/m) (*n* = 5 )** | **Percentage RSD** | **Percentage deviation** | **pH** |
|---|---|---|---|---|
| Fastum® Gel | 2.46 | 0.24 | 1.60 | 5.44 |
| Ketum® Gel | 2.45 | 0.29 | 2.00 | 5.51 |
| Oruvail® Gel | 2.45 | 0.26 | 2.00 | 5.61 |

It was observed that the drug content of the proprietary products was less than the indicated value of 2.5% m/m on the products. This finding was in agreement with a previous study (100) in this laboratory which showed that the actual drug content of proprietary topical ibuprofen products was less than that indicated on the products. Although this finding may not be statistically significantly different ($p > 0.05$, ANOVA) from the indicated amount, it may further compound the lack of efficacy of the topical formulation when other formulation factors are taken into consideration. Less than 2.5% m/m ketoprofen drug content can be mainly attributed to manufacturing processes. The manufacturing process may allow for loss of drug and should be investigated. Studies have reported (233) minimum interaction of ketoprofen with the Carbopol® polymer by differential scanning calorimetry and so the loss

of ketoprofen cannot be attributed to the gelling agent. The pH of the formulation is normally achieved by the addition of a weak base, usually diethanolamine or triethanolamine, which consequently effects the gelling process. The pH readings shown in Table 5.7 play an important role with respect to drug permeation. The reported p*Ka* of ketoprofen (section 2.1.6) is 4.60 and by application of the Henderson-Hasselbach equation about 12.60%, 10.95% and 8.90% of the unionised form of ketoprofen for Fastum® Gel, Ketum® Gel and Oruvail® Gel respectively is present in the neutral undissociated form. From the pH data in Table 5.7, it can be inferred that Oruvail® Gel should produce the lowest diffusion profile whilst Fastum® Gel should produce the highest. The neutral undissociated form of ketoprofen is more likely to cross the synthetic lipophilic membrane during the diffusion run than the ionised molecules of ketoprofen.

### *5.4.2.3 In vitro release of ketoprofen*

The *in vitro* release profiles of the different proprietary products measured through a synthetic membrane using the Franz diffusion cell are illustrated below. Figures 5.1 and 5.2 illustrate the cumulative amount of ketoprofen released plotted against $t$ and $t^{1/2}$ respectively.

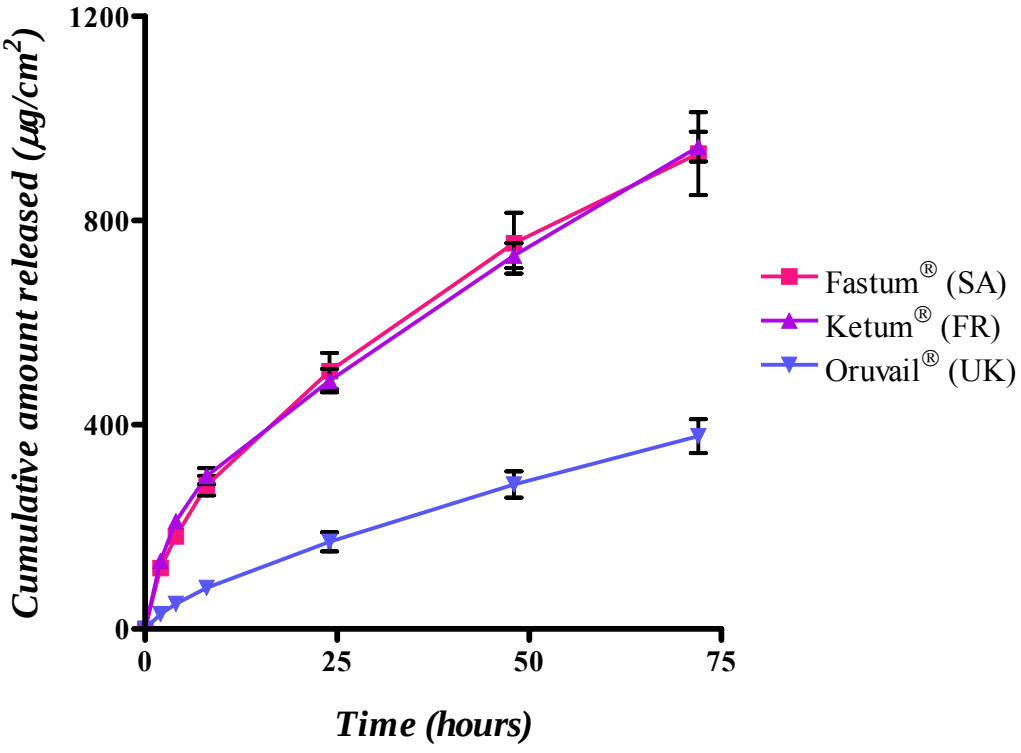



*Figure 5.1 Diffusion profiles of proprietary products* ($n = 5$)

Fastum® Gel and Ketum® Gel produced very similar diffusion profiles and thus as expected there was no statistically significant difference ($p > 0.05$, ANOVA) between the two proprietary products. The incorporation of the preservatives in Fastum® Gel did not appear to affect the release rate of ketoprofen from that proprietary product. Oruvail® Gel produced a very low diffusion profile despite the fact that this proprietary product contained the same type of excipients and similar results with respect to drug content and pH reading. There was a statistically significant difference ($p < 0.05$, ANOVA) between Oruvail® Gel and the other two proprietary products.

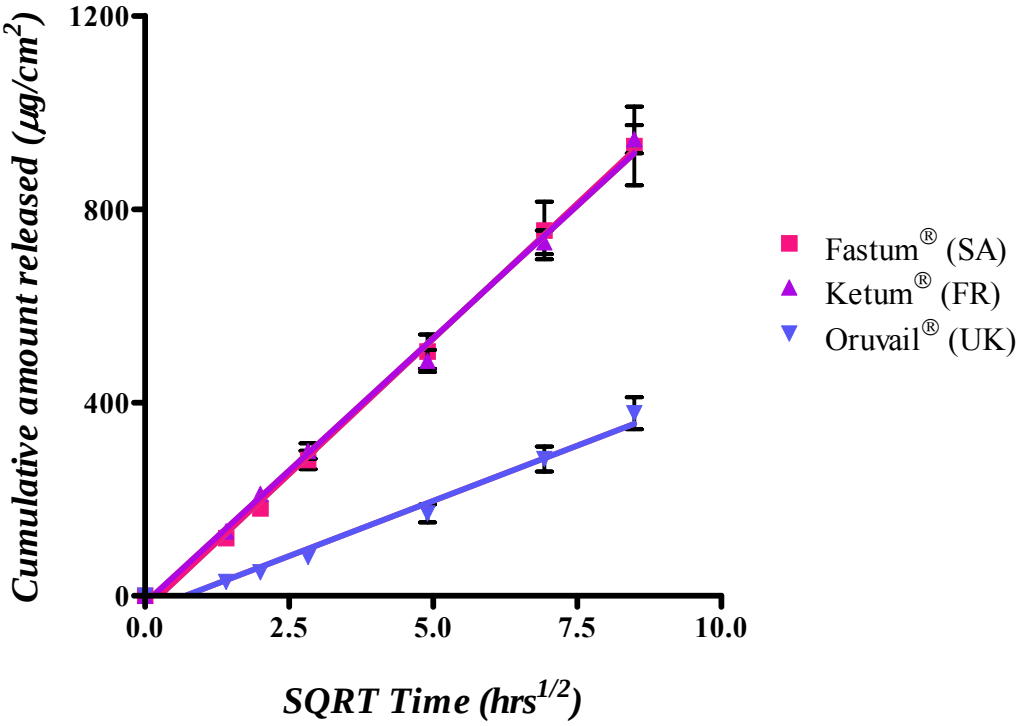



*Figure 5.2 Higuchi plots of proprietary products* ($n = 5$)

The mean *in vitro* ketoprofen release across a synthetic silicone membrane increased with the square root of time for all proprietary products. At the end of the 72 hour run, Oruvail® Gel produced a cumulative amount of 40% less compared to Ketum® Gel and Fastum® Gel. Fastum® Gel and Ketum® Gel produced similar initial fluxes of 59.72 ± 58.99 µg/cm$^2$/h and 66.56 ± 66.40 µg/cm$^2$/h respectively although the initial flux produced by Ketum® Gel was slightly higher. Oruvail® Gel produced an initial flux of 12.43 ± 12.63 µg/cm$^2$/h which is 80% less than that obtained from both Ketum® Gel and Fastum® Gel. The individual fluxes are presented in appendix III. At the end of the experiment, the gel matrix was still intact,

indicating that the process by which the drug was present in the receptor phase was diffusion controlled.

The linear regression analysis performed on the square root data is shown in the Figure 5.2. The regression lines produced by both Ketum® Gel and Fastum® Gel are almost identical with the line produced by Oruvail® Gel being statistically significantly different ($p < 0.05$, ANOVA).

Table 5.8 summaries the kinetic data obtained from the Higuchi plots of each proprietary product. The linear regression correlation co-efficient ($r^2$) obtained was greater than 0.91 and the slope of each regression line displayed a significant deviation from zero ($p < 0.05$, ANOVA) indicating all three proprietary products conformed to the Higuchi principle.

*Table 5.8 In vitro ketoprofen release kinetic data of proprietary products*

| **Proprietary product** | **Apparent release constant (μg/cm$^2$/h$^{½}$)** | **Lag time (minutes)** | $r^2$ |
|---|---|---|---|
| Fastum® (SA) | 112.0 ± 5.111 | 3.91 | 0.9357 |
| Ketum® (FR) | 109.6 ± 2.538 | 1.10 | 0.9826 |
| Oruvail® (UK) | 45.63 ± 2.433 | 28.89 | 0.9142 |

The highest apparent flux over 72 hours of 112.0 ± 5.111 μg/cm$^2$/h$^{½}$ was achieved with Fastum® Gel, with Ketum® Gel producing an apparent flux of 109.6 ± 2.538 μg/cm$^2$/h$^{½}$ and the lowest apparent flux of 45.63 ± 2.433 μg/cm$^2$/h$^{½}$ was obtained with Oruvail® Gel. Although the highest apparent flux was obtained with Fastum® Gel over a 72 hour period, Ketum® Gel had a shorter lag time compared to Fastum® Gel, implying that it would take Ketum® Gel approximately 3 minutes more to achieve the desired pharmacological action compared to Fastum® Gel. Oruvail® Gel once again had a very long lag time close to half an hour.

### *5.4.3 Discussion*

A number of studies have reported (100, 233, 234, 239 - 242, 248, 260, 262, 264 - 267) the effect of different vehicles on the release of a principal active ingredient in topical formulations. There is however limited study on the effect of the same vehicle type on the release of the active ingredient. This study has demonstrated that proprietary products

containing the same amount of drug and excipients can differ in the extent to which the drug is released. The two products under the same manufacturing license produced differed significantly from the third product. Fastum® Gel and Ketum® Gel proved to be much more efficient than Oruvail® Gel at each time interval measured even though all three proprietary products contained the same vehicle type.

The results obtained from both Fastum® Gel and Ketum® Gel are very similar because they are both under the same licence implying that similar manufacturing processes may have been employed. Although the results are almost identical they are not exactly the same and this is mainly attributed to difference in manufacturing processes between the two countries.

The extremely low release profile of Oruvail® Gel could be attributed to a number of reasons. Although a viscosity assessment was not performed in this study, the Oruvail® Gel was subjectively thicker than the gel of the other two proprietary products. The gelling process of Carbopol® polymers and the viscosity are indicative of how much triethanolamine is added and the type of Carbopol® polymer used. The pH of the Oruvail® Gel was the highest and it may be that two phenomena are occurring. Firstly, there is an increased viscosity of the formulation which invariably lengthens the lag time of ketoprofen release and secondly, ketoprofen is a weak acid with a p*Ka* of 4.60 (section 2.1.6) and the pH of the formulation although acidic by virtue of having a pH value less than 7, is relatively basic to ketoprofen and thus this environment will make ketoprofen more favourable in the formulation and less likely to diffusive out. The effect of pH of the formulation will be discussed later in section 5.53.

It was also interesting to note that less than 20% of ketoprofen present in the formulation is made available to diffuse out of the formulation into the receptor phase in the unionised form. Ionic molecules are not generally favoured to cross a silicone membrane, or the SC, and as a result approximately 80% of ketoprofen has not been used. There are also a number of fates of active ingredients from topical formulations before they reach the site of action. One noticeable fate is that drugs are also subjected to skin flora metabolism (36) which further reduces that amount of active drug that reaches the active site. Generally there is low bioavailability of active ketoprofen in these formulations and this may explain why there are so few of these products on the market. The use of Carbopol® polymers which require raising the pH to effect gelation is the main problem. A possible suggestion is the use of

Carbopol® co-polymers which are viscosity increasers and may not need a large amount of base to effect gelation. The results of this study will be discussed in section 5.5.3. Research studies have been conducted (237) which have successfully employed other gelling agents such as Pluronic® F127 in the manufacture of topical preparations containing medicinal agents notably the NSAIDs with the added advantage of controlling the pH of the formulation which inevitable can improve the bioavailability of the drug into the skin. However the costs of large scale manufacture of these topical products have made their use uncommon. The use of soya-lecithin as another vehicle type for the transdermal delivery of ketoprofen has been conducted (191).

### *5.4.4 Conclusion*

This data indicates that the simple denotation of 'ketoprofen 2.5% m/m gel' is inadequate as a means of distinguishing between similar formulations that might contain the same types of excipients or manufactured under the same licence. This finding is very similar to a study demonstrated by Pershing *et al.* (255) which reports that drug release comparisons across all topical drug products between manufacturers can be different when the vehicle composition is not qualitatively and quantitatively the same and therefore cannot be assumed to be the same as a function of labelled strength. This highlights the potential problems that may arise from the mistaken assumption that generic prescriptions and formulations are pharmaceutically and clinically equivalent and interchangeable (234).

## 5.5 DIFFUSION PROFILES AND RELEASE KINETIC DATA OF EXTEMPORANEOUS TOPICAL KETOPROFEN GEL PREPARATIONS USING CARBOPOL® POLYMERS AND CO-POLYMERS

### *5.5.1 Introduction*

Gel preparations using Carbopol® polymers and co-polymers are classified as hydrogels (250). Hydrogels are three-dimensional macromolecular networks that contain a large fraction of water within their structure, do not dissolve and are soft and pliable. These properties are similar to natural tissue and therefore, hydrogels are particularly useful in biomedical and pharmaceutical applications (254). The use of Carbopol® polymers and co-polymers as vehicles for the *in vitro* and *in vivo* transdermal delivery of various medicinal agents has been extensively researched (51, 106, 148, 184, 232, 237, 239 - 253, 263) due to their ability to exhibit high viscosities at low concentrations. Moreover, they are quite stable to heat with negligible batch-to-batch variability. They are also unaffected by aging, do not support bacterial or fungal growth and are non-toxic and non-irritating (232). Polyacrylic acid has been widely used as a bioadhesive agent in formulations to enhance bioavailability. It has been reported that the bioadhesiveness of polyacrylic acid gel is pH and ionic strength dependent (249).

Section 5.4 reported different results from three proprietary products using the same vehicle composition and as a result this laboratory investigated the effect of altering a number of manufacturing variables on the *in vitro* release of ketoprofen from Carbopol® polymers and co-polymers and by so doing offer suggestions to improve topical bioavailability. A number of new grades of these polymers have been synthesized which offer better dispersion profiles, optical clarity and improved stability. Different grades of Carbopol® polymers and co-polymers were sourced and used in this study.

The aim of this investigation was to study the effect of varying solvent compositions, Carbopol® polymer type, Carbopol® polymer concentration, drug concentration and the incorporation of the co-polymer in the polymer matrix on the *in vitro* drug release from gels prepared using four different Carbopol® polymers and a Carbopol® co-polymer.

*5.5.2 Results*

The composition of the formulations is shown in Tables 5.3 and 5.4. All the gels formed were elegant and transparent with a few translucent gels mainly due to the evaporation of the volatile components during manufacture. Some of the gels were not devoid of air bubbles. The air bubbles were incorporated during mixing after neutralisation had occurred. KET017, KET018 and KET020 had an aromatic scent due to the incorporation of Transcutol® HP in the formula. All the gels maintained their structural integrity after overnight storage prior to any other evaluation. Preliminary studies showed that a minimum of 20% m/m alcoholic content in the formulation was required to dissolve ketoprofen in the hydrogel. The alcoholic content upper limit for obtaining acceptable non-liquefied gel formulations was 40% m/m.

*Table 5.9 Drug content uniformity and pH values obtained for KET001 - KET020*

| **Formulation** | **Drug content (% m/m) (** $n = 5$ **)** | **RSD (%)** | **Deviation (%)** | **pH** |
|---|---|---|---|---|
| KET001 | 2.49 | 0.21 | 0.40 | 5.46 |
| KET002 | 2.51 | 0.21 | 0.40 | 5.85 |
| KET003 | 2.51 | 0.20 | 0.40 | 5.91 |
| KET004 | 2.51 | 0.19 | 0.40 | 5.91 |
| KET005 | 2.52 | 0.22 | 0.80 | 5.73 |
| KET006 | 2.53 | 0.21 | 1.20 | 5.81 |
| KET007 | 2.54 | 0.23 | 0.80 | 5.81 |
| KET008 | 2.52 | 0.21 | 0.80 | 5.62 |
| KET009 | 2.51 | 0.22 | 0.40 | 5.64 |
| KET010 | 2.51 | 0.24 | 0.40 | 5.71 |
| KET011 | 2.52 | 0.21 | 0.80 | 5.41 |
| KET012 | 2.53 | 0.20 | 1.20 | 5.41 |
| KET013 | 2.50 | 0.19 | 0.00 | 7.21 |
| KET014 | 2.51 | 0.23 | 0.40 | 6.51 |
| KET015 | 1.51 | 0.21 | 0.00 | 6.40 |
| KET016 | 5.55 | 0.22 | 0.90 | 5.57 |
| KET017 | 2.51 | 0.24 | 0.40 | 5.76 |
| KET018 | 2.51 | 0.21 | 0.40 | 5.80 |
| KET019 | 2.51 | 0.19 | 0.40 | 5.94 |
| KET020 | 2.51 | 0.20 | 0.40 | 6.25 |

The drug content uniformity and pH values of all the extemporaneous formulations prepared in this laboratory are reported in Table 5.9. The assay values for all the formulations with the exception of KET015 and KET016 which were deliberately altered were within the limit of 2.31 - 2.69% m/m as indicated in the 2002 edition of the British Pharmacopoeia (185). The percentage deviation of all the extemporaneous formulations were less than those obtained from the proprietary products in Table 5.7. The pH values were within limits of 5.41 - 6.25.

The pH of the vehicle of KET013 and KET014 was deliberately altered. The pH value for KET015 was not deliberately altered. The pH values indicated in Table 5.9 were very similar to those obtained from the proprietary products in Table 5.7.

*Table 5.10 In vitro ketoprofen release kinetic data for KET001 - KET020*

| **Formulation** | **Apparent release constant ($\mu g/cm^2/h^{½}$)** | **Lag time (minutes)** | $r^2$ |
|---|---|---|---|
| KET001 | 139.6 ± 5.109 | 6.78 | 0.9577 |
| KET002 | 95.75 ± 2.180 | 25.67 | 0.9832 |
| KET003 | 94.47 ± 3.225 | 34.56 | 0.9630 |
| KET004 | 104.4 ± 2.694 | 24.99 | 0.9785 |
| KET005 | 124.4 ± 4.364 | 25.17 | 0.9610 |
| KET006 | 133.3 ± 5.253 | 39.50 | 0.9513 |
| KET007 | 86.60 ± 3.744 | 24.06 | 0.9419 |
| KET008 | 116.6 ± 4.650 | 21.62 | 0.9501 |
| KET009 | 101.5 ± 2.944 | 38.73 | 0.9730 |
| KET010 | 96.80 ± 4.047 | 56.89 | 0.9455 |
| KET011 | 60.04 ± 5.217 | 65.90 | 0.8006 |
| KET012 | 136.1 ± 5.604 | 21.23 | 0.9470 |
| KET013 | 7.57 ± 0.7859 | 103.44 | 0.7380 |
| KET014 | 35.29 ± 3.014 | 62.06 | 0.8061 |
| KET015 | 43.96 ± 3.143 | 47.18 | 0.8556 |
| KET016 | 154.7 ± 9.051 | 39.70 | 0.8985 |
| KET017 | 75.56 ± 4.401 | 85.39 | 0.8993 |
| KET018 | 142.5 ± 8.035 | 59.23 | 0.9051 |
| KET019 | 98.83 ± 6.622 | 11.34 | 0.8710 |
| KET020 | 101.4 ± 6.352 | 115.43 | 0.8853 |

Table 5.10 summaries the kinetic data obtained from the Higuchi plots for all the extemporaneous gel preparations. In these preliminary studies, the mean *in vitro* ketoprofen release across a synthetic membrane increased with the square root of time for the gel preparations. The linear regression correlation co-efficients range from 0.7380 - 0.9832. Although the linear regression correlation co-efficients expressed by the formulations were not all greater than 0.9, the slope of each regression line displayed a significant deviation from zero ($p < 0.05$, ANOVA) indicating conformation to the Higuchi principle. KET013 produced the lowest apparent flux of 7.578 ± 0.7859 $\mu g/cm^2/h^{½}$ whilst the highest apparent flux of 154.7 ± 9.051 $\mu g/cm^2/h^{½}$ was produced by KET016. KET001 produced the shortest lag time of approximately 6 minutes and KET020 produced the longest lag time of approximately 2 hours. No direct correlation was observed between the lag time and the apparent flux released. KET020 produced the longest lag time of approximately 2 hours and a flux of 101.4 ± 6.352 $\mu g/cm^2/h^{½}$ and KET001 with the shortest lag time did not produce the maximum apparent flux.

### 5.5.2.1 *Effect of different grades of Carbopol® polymers and co-polymer*

Five 2.5% m/m ketoprofen gels (KET002, KET004, KET005, KET006 and KET007) each containing a different grade (Carbopol® Ultrez™ 10 NF, Carbopol® 974 PNF, Carbopol® 980 NF, Carbopol® 981 PNF and Pemulen® TR1 NF) of Carbopol® polymer or co-polymer were prepared and evaluated.

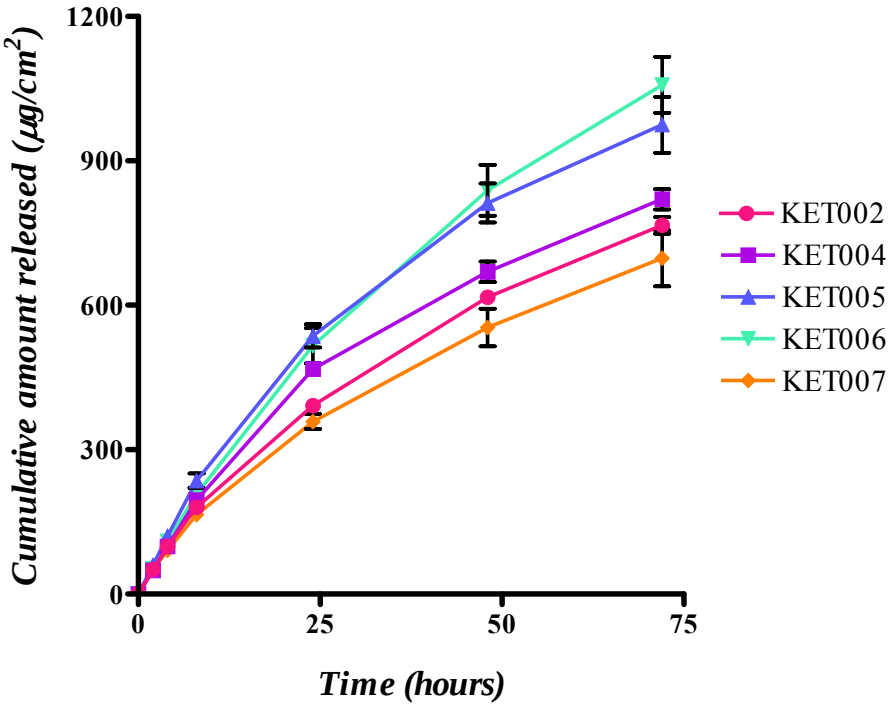



*Figure 5.3 Diffusion profiles showing the effect of different grades of Carbopol® polymers on the release of ketoprofen* ($n = 5$)

The amount of ketoprofen released from the different grades of Carbopol® polymers or co-polymer is shown in Figure 5.3 and 5.4. It can be seen that the amount of drug released was lowest from KET007 and highest from KET006. The rank order of the drug release from these formulations was KET006 > KET005 > KET004 > KET002 > KET007. The differences between the released amounts of drug from the different grades were not statistically significant ($p > 0.05$, ANOVA). The calculated apparent fluxes and the lag times for these formulations from Table 5.10 are graphically represented in Figure 5.5. The pH values for these formulations were within the range of 5.70 - 5.95 and were not statistically significantly different ($p > 0.05$, ANOVA). The minor changes in pH between the formulations did not have a significant effect on the release of drug.

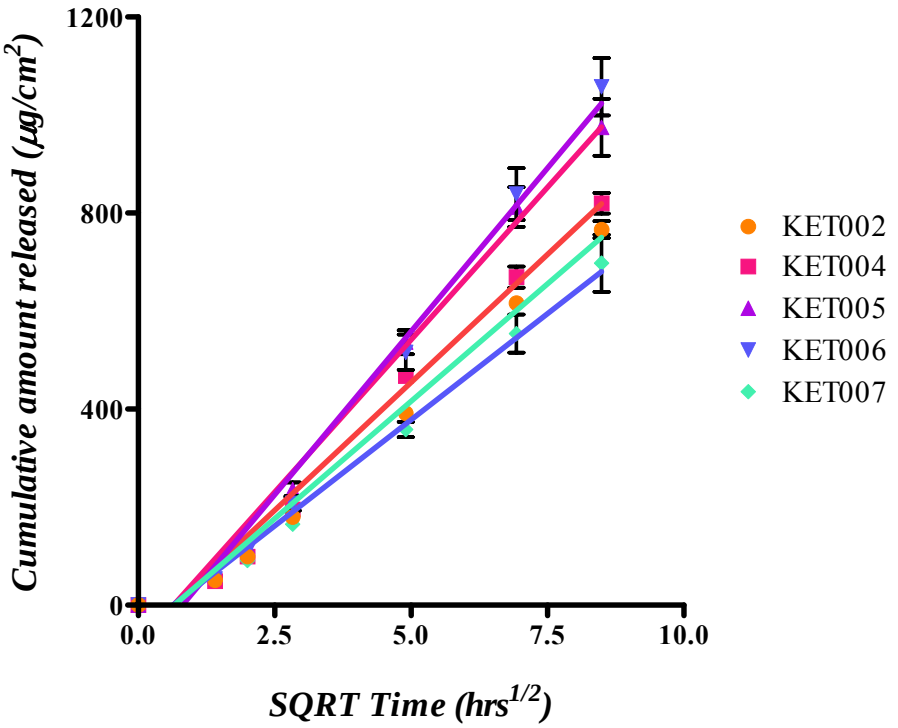



*Figure 5.4 Higuchi plots showing the effect of different grades of Carbopol® polymers* ($n = 5$)

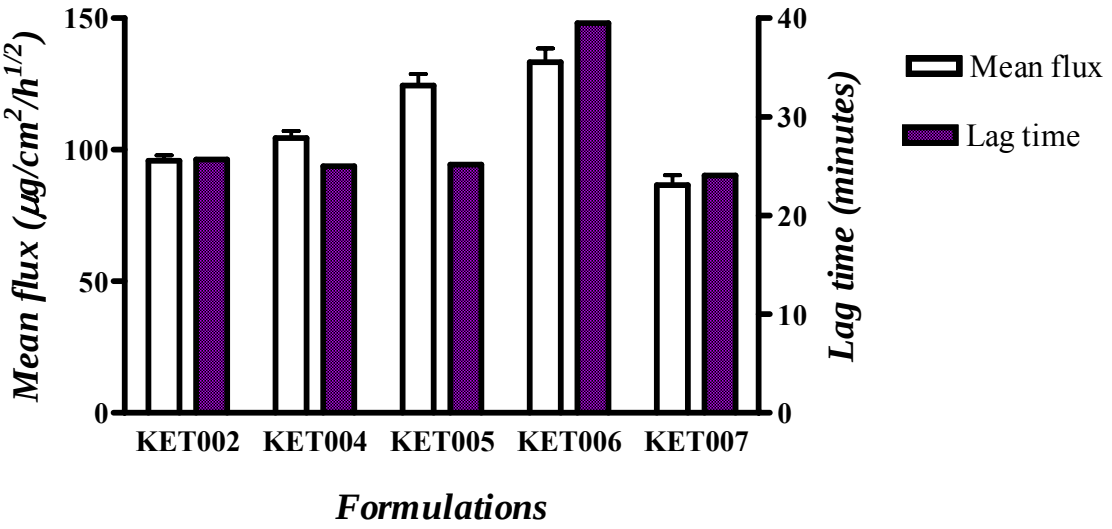



*Figure 5.5 Mean maximum fluxes and lag times obtained from the release kinetics of ketoprofen from different grades of Carbopol® polymers* ($n = 5$)

Although KET006 produced the maximum apparent flux, it had the longest lag time. There is an approximately a 1.5-fold increase in terms of drug release and the time it takes the drug to leave the formulation from KET006 in comparison to KET007. Interestingly the amount of drug release from KET005 and KET006 was not statistically significantly different ($p > 0.05$, ANOVA) but the time it takes for drug release from KET006 is approximately 1.6 times slower than KET005. The lag times produced by KET002, KET004, KET005 and KET007 were not statistically significantly different ($p > 0.05$, ANOVA).

### *5.5.2.2 Effect of polymer concentration*

Three approximately 2.5% m/m ketoprofen gels (KET001, KET002 and KET003) each containing a different concentration (1.0% m/m, 1.5% m/m and 2.0% m/m) of Carbopol® Ultrez™ 10 NF polymer were prepared and evaluated. Figure 5.6 and Figure 5.7 both show that the results can be divided into two groups.

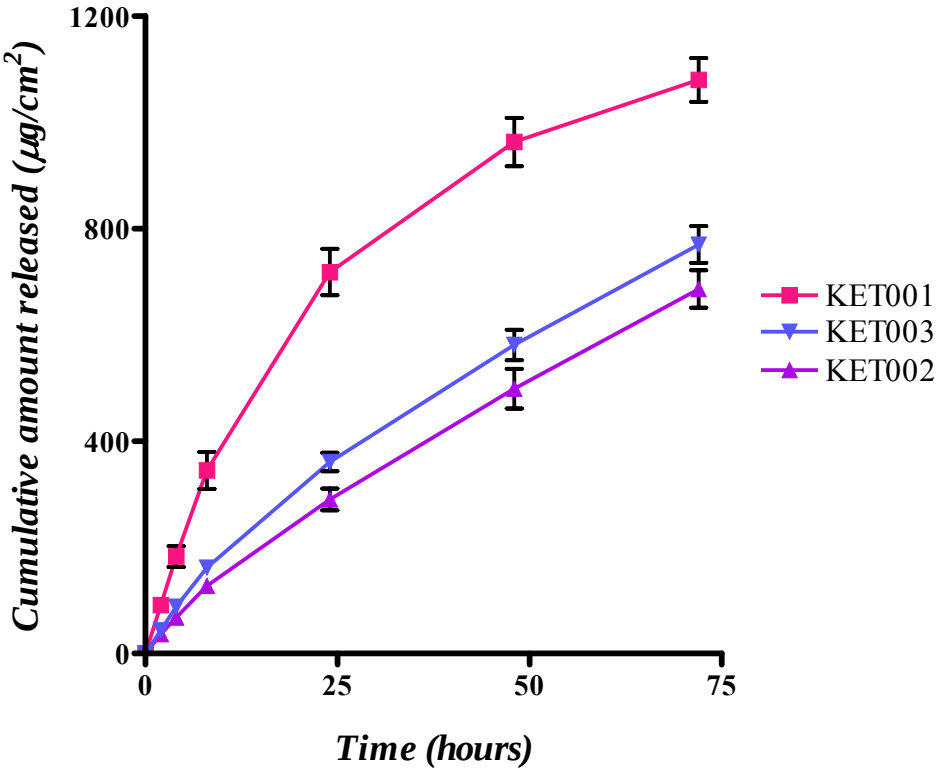



*Figure 5.6 Diffusion profiles showing the effect of different concentrations of Carbopol® Ultrez™ 10 NF polymer on the release of ketoprofen* ($n = 5$)

A statistical significant difference ($p < 0.05$, ANOVA) between KET001, KET002 and KET003 with a rank order increase of KET001 > KET003 > KET002 was observed. KET001 produced an apparent flux approximately 1.5 times higher than KET002 and KET003. The lag time produced by KET001 was approximately 4 times less than KET002 and 5 times less than KET003.

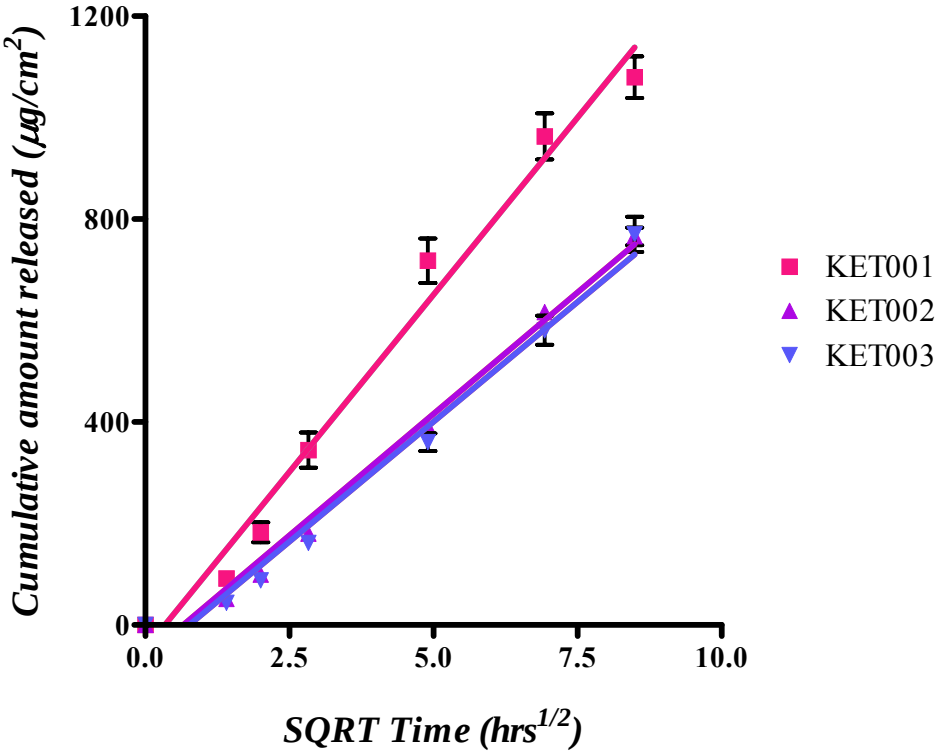



*Figure 5.7 Higuchi plots showing the effect of different concentrations of Carbopol® Ultrez™ 10 NF polymer* $(n = 5)$

The pH of the gels produced was within 5.45 - 5.92. Although the pH values were not significantly different ($p > 0.05$, ANOVA), there seemed to be a direct correlation between the pH value and the release of ketoprofen from these formulations. The lowest pH value from KET001 produced the highest apparent flux of 139.6 ± 5.109 µg/cm$^2$/h$^{½}$ while the highest pH value from KET003 produced the lowest apparent flux of 94.47 ± 3.225 µg/cm$^2$/h$^{½}$. The amount of polymer used showed an inverse relationship to the apparent flux produced and a direct relationship to the lag time.

*5.5.2.3 Effect of ketoprofen concentration*

Three 1.5% m/m Carbopol® Ultrez™ 10 NF polymer gels (KET015, KET002 and KET016) each containing a different concentration (1.5% m/m, 2.5% m/m and 5.5% m/m) of drug were prepared and evaluated. The release rate of ketoprofen from these formulations as shown in Figures 5.8 and 5.9 showed concentration dependency. There was a statistically significant difference ($p < 0.05$, ANOVA) between the formulations.

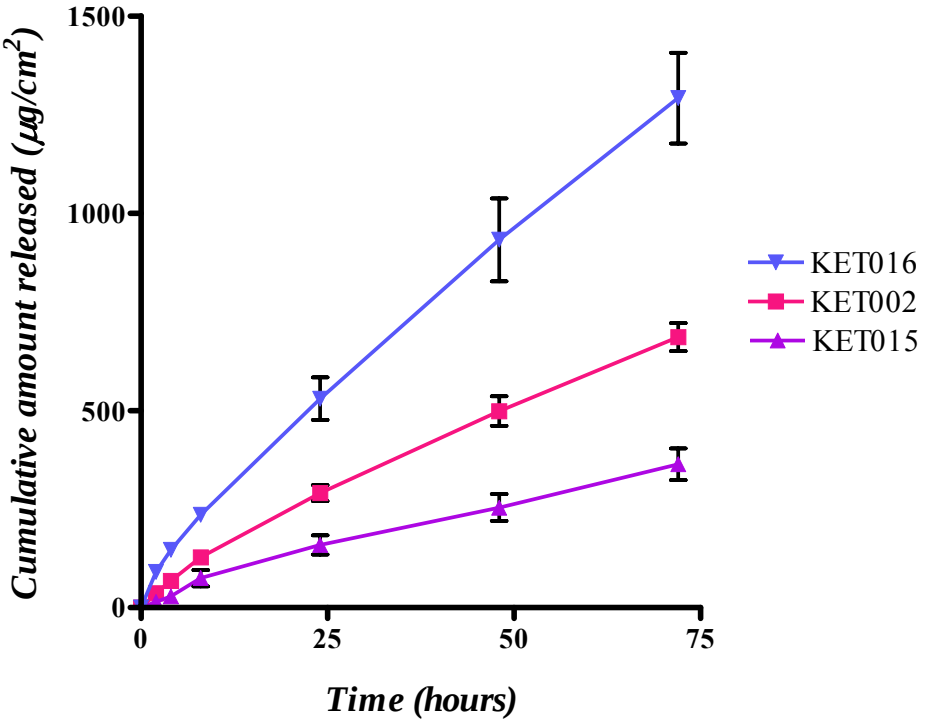



*Figure 5.8 Diffusion profiles showing the effect of drug concentration on the release rate of ketoprofen* ($n = 5$)

There was a good correlation between the concentration of ketoprofen and release rate for all the formulations however no correlation was observed between the concentration of ketoprofen and the lag times produced. The rate of drug released, however, was not consistently proportional to the increase in drug concentration in the formulation. For example, increasing the ketoprofen concentration 4-fold from 1.5% m/m to 5.5% m/m in the formulations produced a 3.5-fold increase in the rate of drug released. The pH values of the formulations were within the range 5.56 - 6.40. An inverse relationship between the drug concentration and the pH value of the formulation was observed. KET015 with the drug concentration of 1.5% m/m ketoprofen produced the highest pH value of 6.40 while the lowest pH value of 5.57 was obtained from KET016 with a drug concentration of 5.5% m/m.

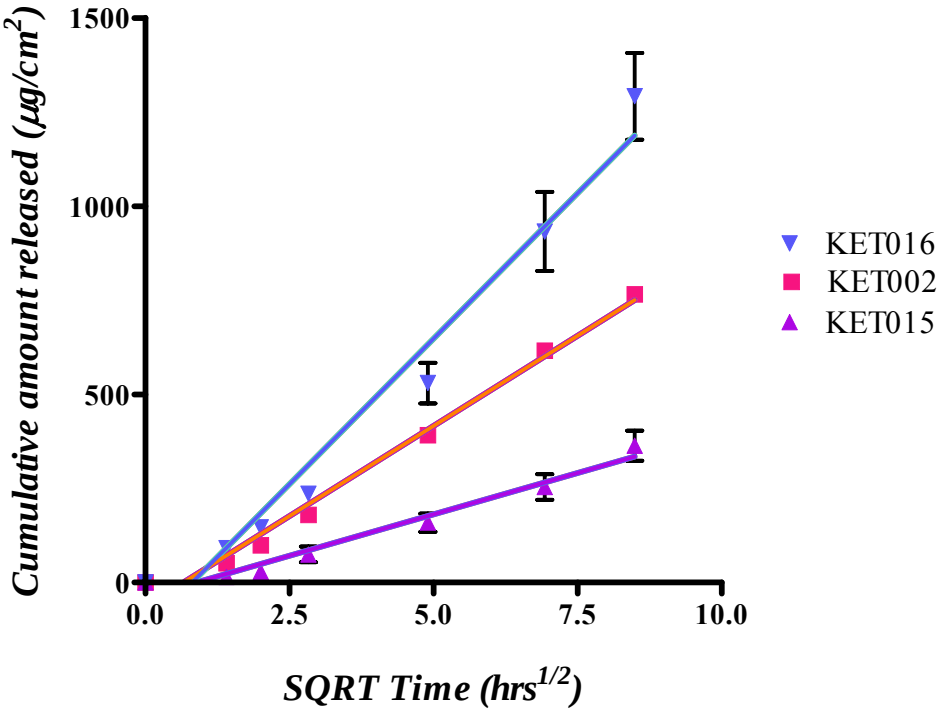



*Figure 5.9 Higuchi plots showing the effect of drug concentration on the release rate of ketoprofen* ($n = 5$)

### *5.5.2.4 Effect of vehicle pH*

The effects of vehicle pH (5.85, 7.21 and 6.51) were investigated for three formulations (KET002, KET013 and KET014) each containing 2.5% m/m ketoprofen in a Carbopol® Ultrez™ 10 NF polymer. The change in pH was achieved by altering the amount of triethanolamine required to neutralise the polymer to bring about gelling. There was a statistically significant difference ($p < 0.05$, ANOVA) between the release rates obtained from the different formulations. The effect of pH was significant on the release of ketoprofen from the formulations. It was apparent that as the pH was increased, the steady state flux falls substantially which was also accompanied by the expected large increase in the solubility of the drug in the formulation. A plot of flux against the fraction of drug in the unionised (Figure 5.12) form revealed a direct linear dependence. A p*Ka* value of 4.60 for ketoprofen was used in these calculations (141, 145, 149). An inverse correlation between the pH of the vehicle and the lag time was observed.

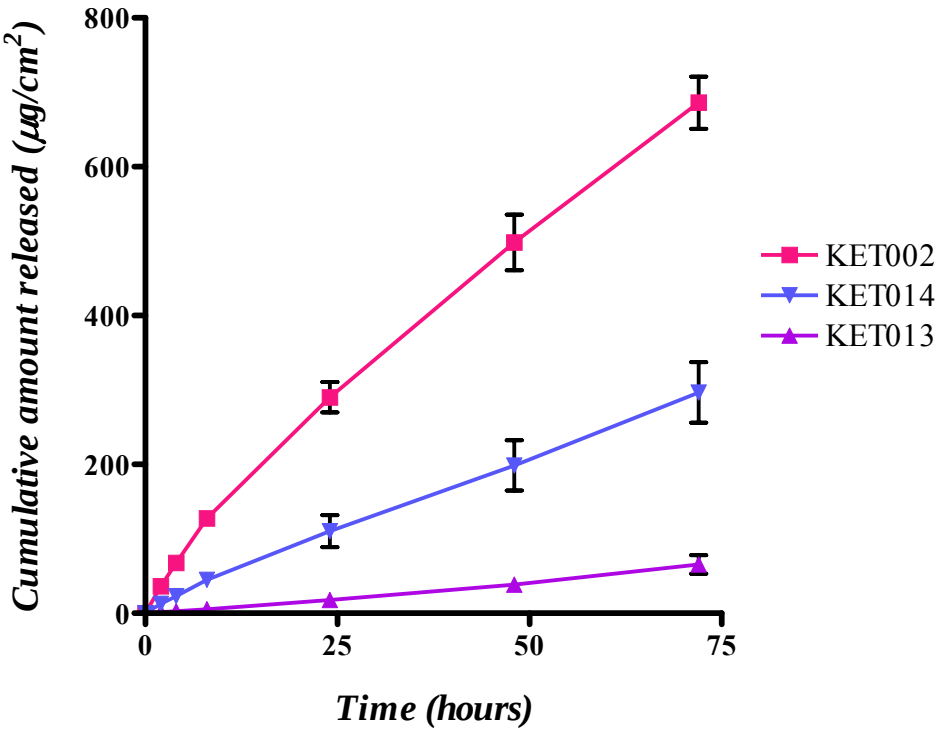



*Figure 5.10 Diffusion profiles showing the effect of pH on the release rate of ketoprofen* $(n = 5)$

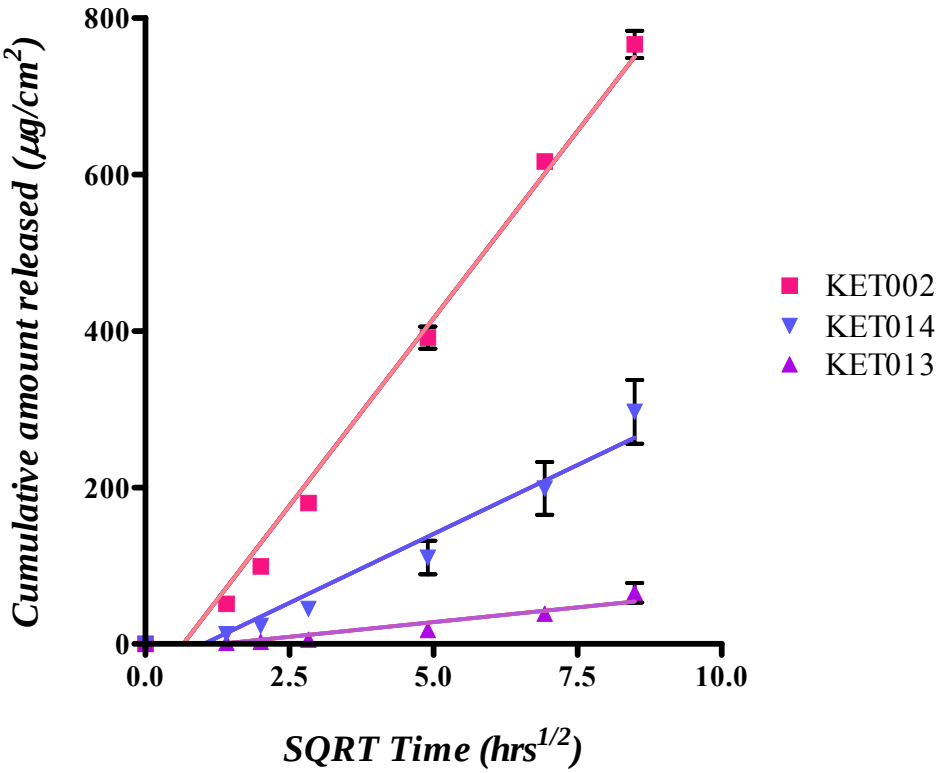



*Figure 5.11 Higuchi plots showing the effect of pH on the release rate of ketoprofen* $(n = 5)$

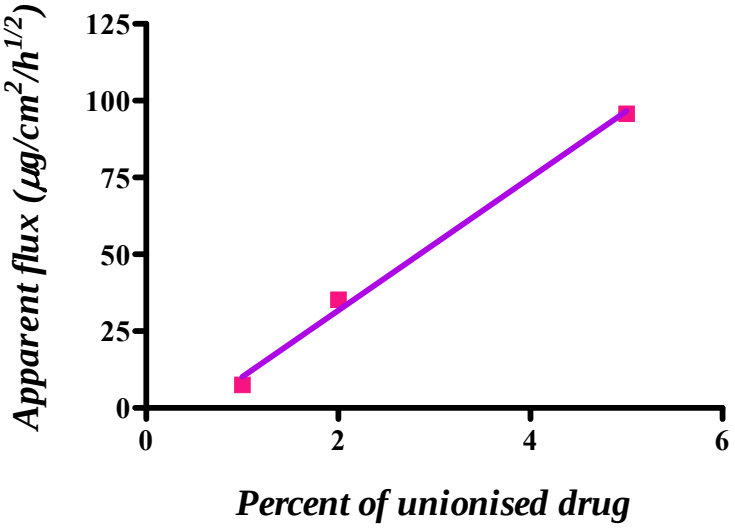



*Figure 5.12 Relationship between the apparent fluxes of the formulations to the amount of unionised drug present in each formulation* $(n = 5)$

### *5.5.2.5 Effect of co-polymer concentration*

The effect of incorporating small amounts of Pemulen® TR1 NF into Carbopol® 980 NF formulations on the *in vitro* release of ketoprofen was investigated. Four gels (KET008, KET009, KET010 and KET011) each containing 2.5% m/m ketoprofen, 1.0% m/m Carbopol® 980 NF and varying amounts (0.1% m/m, 0.3% m/m, 0.5% m/m and 1.0% m/m) of Pemulen® TR1 NF were prepared and evaluated. Figures 5.13 and 5.14 illustrate the diffusion profiles obtained for these formulations. Although no statistically significant difference ($p > 0.05$, ANOVA) among these profiles existed, the rank order increase of release of ketoprofen from these formulations is KET008 > KET009 > KET010 > KET011.

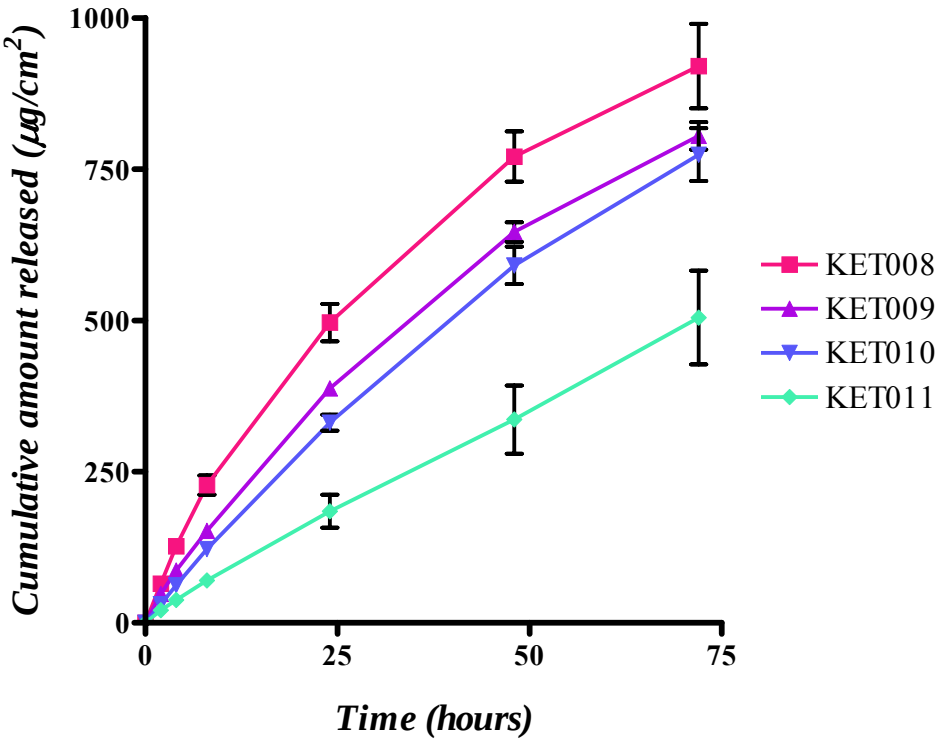



*Figure 5.13 Diffusion profiles showing the effect of Pemulen® TR1 NF into Carbopol® 980 NF formulations on the release rate of ketoprofen* $(n = 5)$

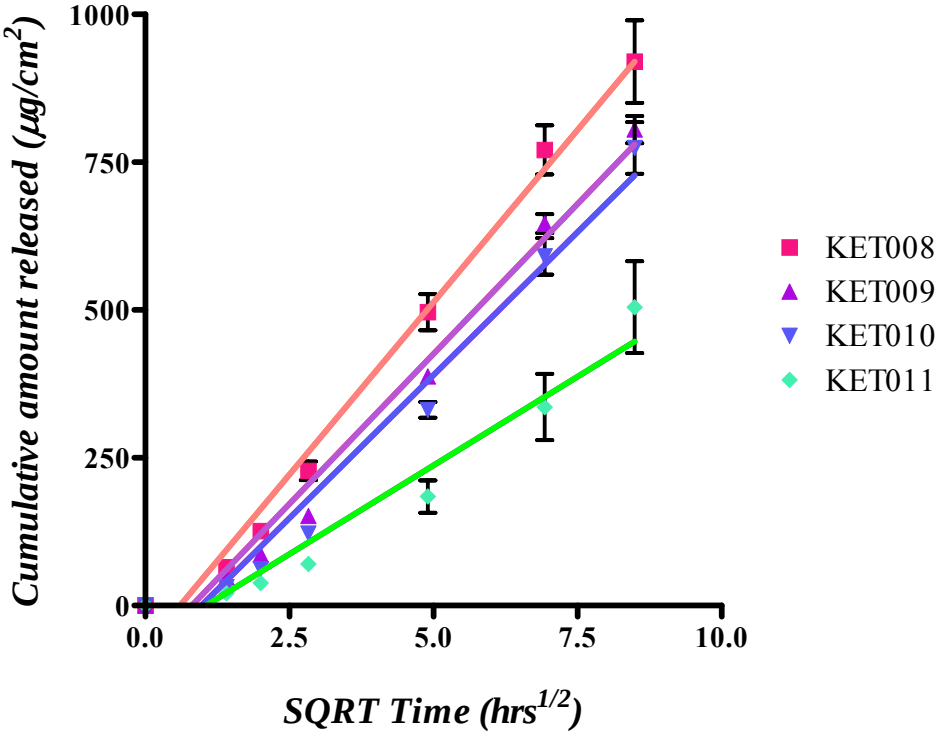



*Figure 5.14 Higuchi plots showing the effect of Pemulen® TR1 NF into Carbopol® 980 NF formulations on the release rate of ketoprofen* $(n = 5)$

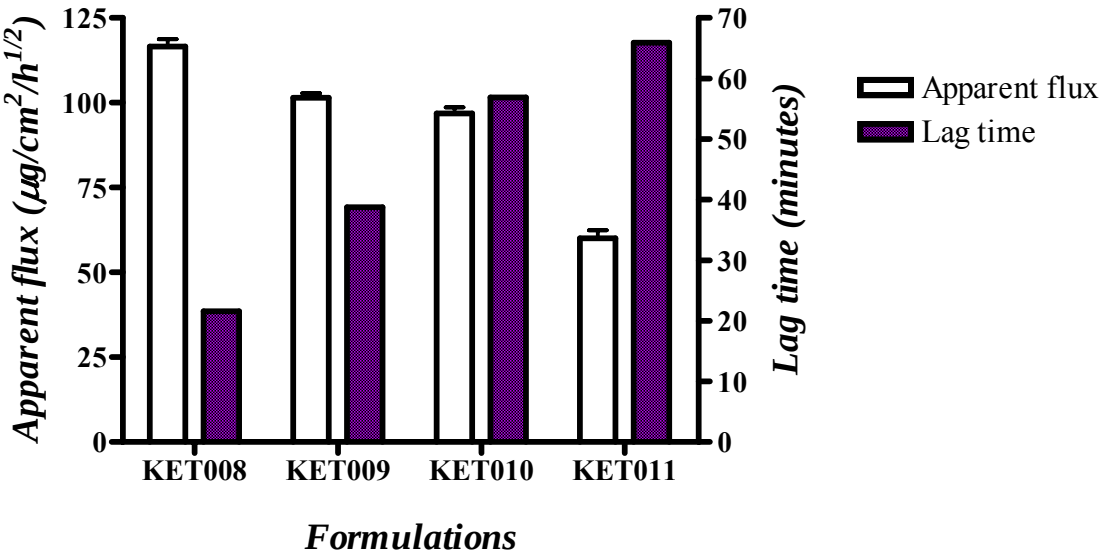



*Figure 5.15 Mean maximum fluxes and lag times obtained from the effect of Pemulen® TR1 NF incorporated in Carbopol® 980 NF formulations* $(n = 5)$

Increasing the amount of the co-polymer in the formulations decreased the apparent flux from the formulations but increased the time it took for the drug to leave the formulation and diffuse into the receptor medium (Figure 5.15).

The amount of flux produced and the lag time was not in direct proportion to increasing the amount of the co-polymer. A 10-fold increase in Pemulen® TR1 NF produced a 2-fold decrease in apparent flux and a 2.3-fold increase in lag time whereas a 5-fold increase in Pemulen® TR1 NF produced a 1.2-fold decrease in apparent flux and an approximately 3-fold increase in lag time. It was interesting to note that KET008 produced an identical profile to the proprietary product Fastum® Gel (Figure 5.16).

Although there is no significant difference ($p > 0.05$, ANOVA) with respect to the apparent flux and the lag time of the drug produced, KET008 compared to the proprietary product, Fastum® Gel, produced a higher apparent flux of 116.6 ± 4.650 μg/cm$^2$/h$^{½}$ compared to that produced by Fastum® Gel of 112.0 ± 5.111 μg/cm$^2$/h$^{½}$ but more importantly, the lag time produced by KET008 was 5.5 times more than Fastum® Gel. There was no correlation between the vehicle pH of the formulations and the release pattern of ketoprofen.

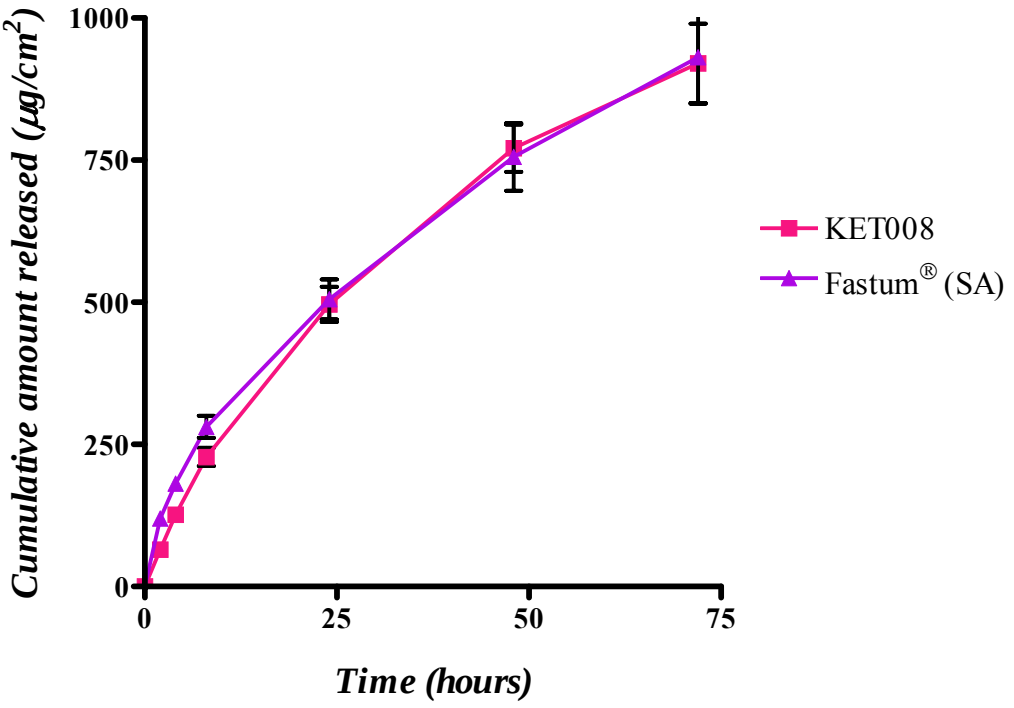



*Figure 5.16 Diffusion profiles comparing KET008 and Fastum® Gel* ($n = 5$)

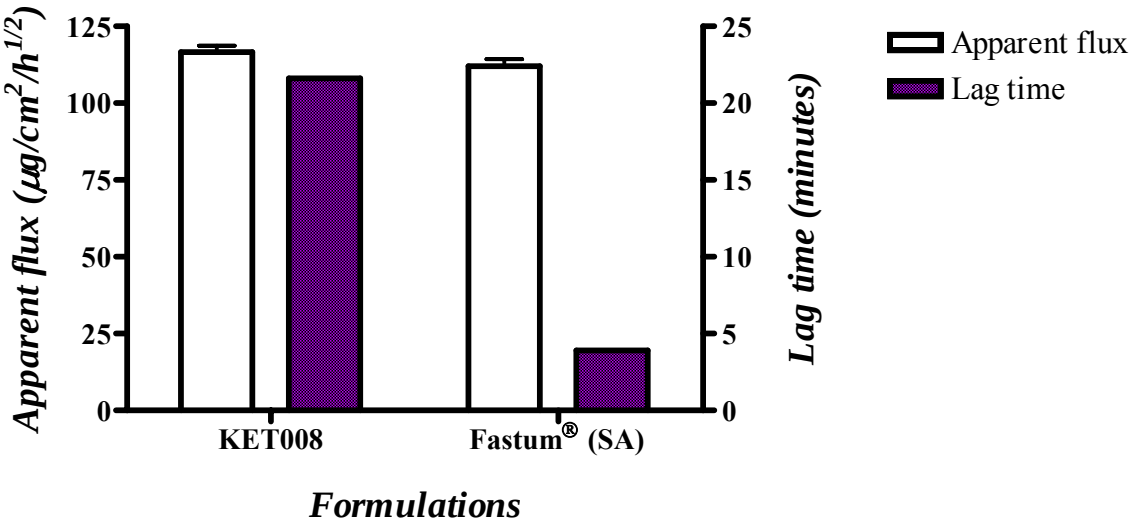



*Figure 5.17 Comparisons of apparent fluxes and lag times obtained from KET008 and Fastum® Gel* ($n = 5$)

*5.5.2.6 Effect of solvent systems*

The effect of employing different solvents on the release rate and lag time of ketoprofen was investigated. Transcutol® HP, ethanol and propylene glycol were the solvent systems studied. Figures 5.18 and 5.19 illustrate the effect of the various solvent systems on the release rate of drug from the formulations. The effect of Transcutol® HP was initially investigated. Three gel formulations (KET017, KET018 and KET020) containing varying amounts of Transcutol® HP in the presence and absence of other solvent systems were prepared and evaluated. KET017 and KET018 each contained 20.0% m/m Transcutol® HP with a composite solvent system of propylene glycol and ethanol respectively. KET020 was formulated with 40.0% m/m Transcutol® HP. The diffusion profiles are illustrated in Figures 5.18 and 5.19. Although no statistically significant difference ($p > 0.05$, ANOVA) was observed, the rank order increase is KET018 > KET020 > KET017. Unusually long lag times were observed in these formulations as seen in Figure 5.20. KET018 produced a lag time of approximately an hour and KET020 produced a lag time of almost 2 hours. KET018 containing ethanol produced better release kinetics compared to KET017 which contains propylene glycol. A larger amount of Transcutol® HP produced a relatively large apparent flux but with an extremely long lag time.

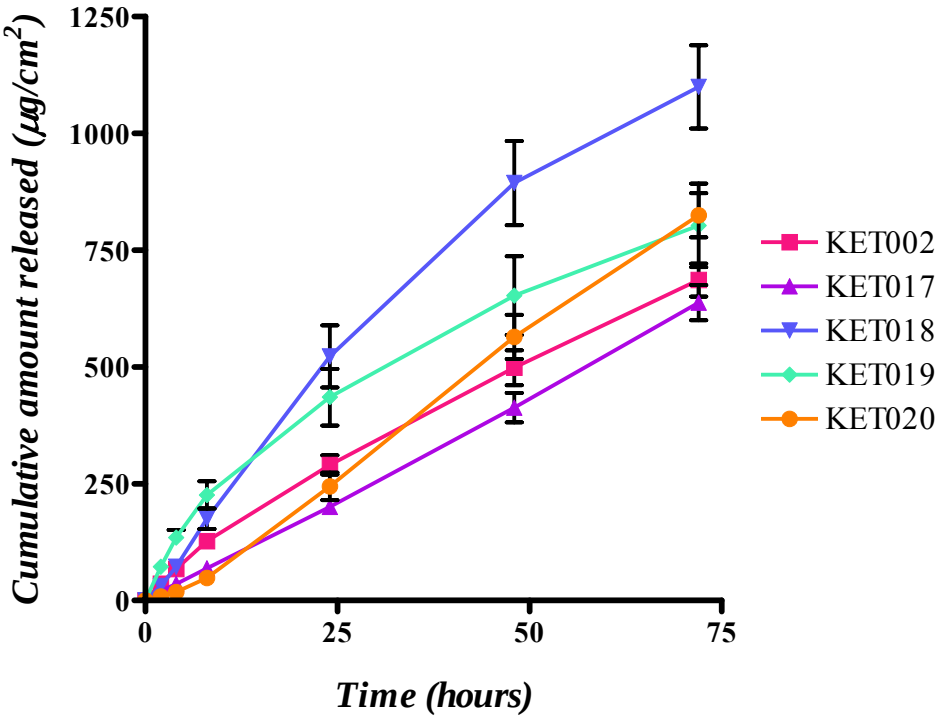



*Figure 5.18 Diffusion profiles showing the effect of solvent systems* ($n = 5$)

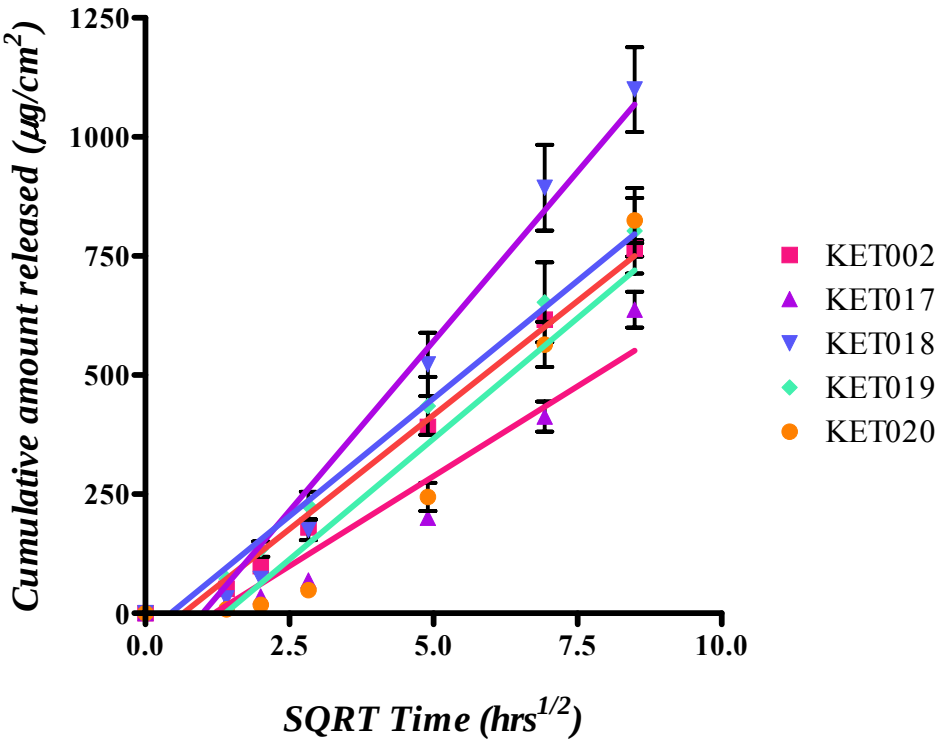



*Figure 5.19 Higuchi plots showing the effect of solvent systems* ($n = 5$)

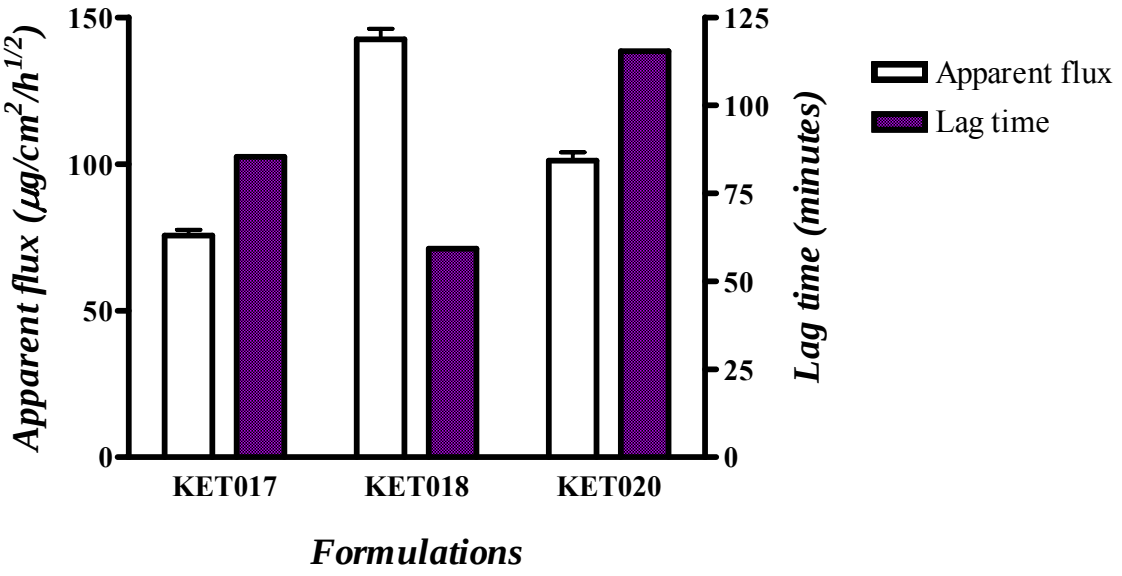



*Figure 5.20 Mean apparent fluxes and lag times obtained from the Transcutol® HP formulations* ($n = 5$)

The effect of Transcutol® HP and ethanol as single solvent systems on the release rate of ketoprofen was also evaluated. Two gel preparations (KET019 and KET020) both containing 2.5% m/m ketoprofen in a 1.5% m/m Carbopol® Ultrez™ 10 NF polymer and 40.0% m/m ethanol or Transcutol® HP respectively were evaluated. The diffusion profiles of KET019 and KET020 are illustrated in Figures 5.18 and 5.19 above. The initial flux from KET020 was extremely low compared to KET019. KET019 and KET020 both produced the same apparent flux but KET020 produced a 10.2-fold increase in lag time.

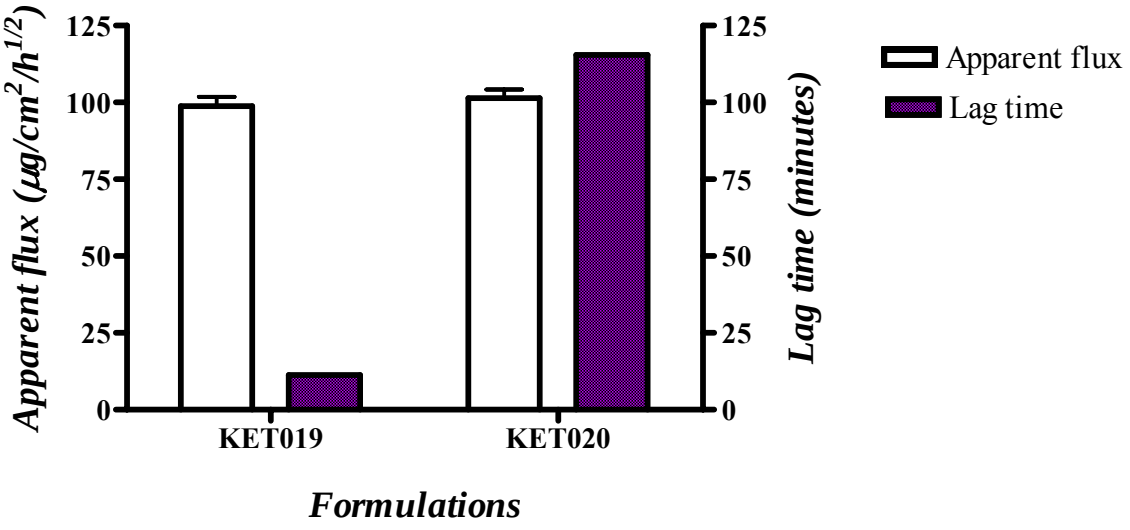



*Figure 5.21 Mean apparent fluxes and lag times obtained from KET019 and KET020* ($n = 5$)

Co-solvents systems were also employed in the preparations of the gels to evaluate their effect on the release of ketoprofen. Transcutol® HP/ethanol (KET018) and propylene glycol/ethanol (KET002) systems were used. The diffusion profiles of these formulations are illustrated in Figures 5.18 and 5.19. Although KET018 produced a 1.5-fold increase in apparent flux, it also produced a 2.3-fold increase in lag time. Formulations containing Transcutol® HP produce large apparent fluxes but relatively long lag times.

Transcutol® HP/propylene glycol (KET017) and ethanol/propylene glycol (KET002) systems were also compared. The diffusion profiles of these formulations are illustrated in Figures 5.18 and 5.19. KET017 produced a smaller apparent flux compared to KET002, a finding contrary to the results obtained when KET002 and KET018 were compared. In Figures 5.18 and 5.19 KET017 produced the lowest diffusion profile. From Figure 5.23 KET017 produced a 1.3-fold decrease in apparent flux but produced a 3.3-fold increase in lag time.

The lag time of the Transcutol® HP containing formulation was higher and this finding was similar to that observed in Figure 5.22.

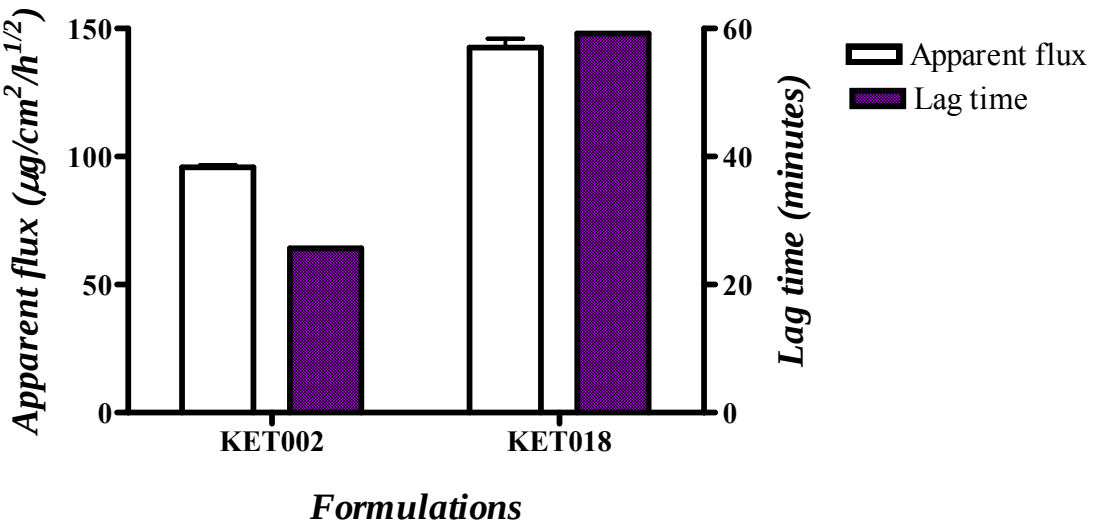



*Figure 5.22 Mean apparent fluxes and lag times obtained from KET002 and KET018* ($n = 5$)

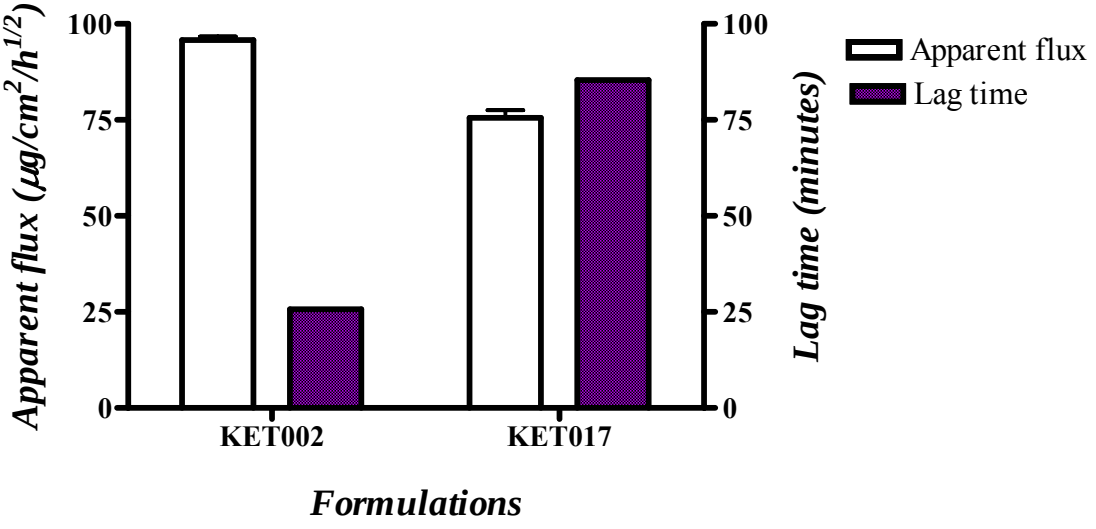



*Figure 5.23 Mean apparent fluxes and lag times obtained from KET002 and KET017* ($n = 5$)

### *5.5.3 Discussion*

To develop effective topical formulations, it is important to determine the diffusion properties of drugs in the semisolid vehicles, especially when the release of drugs at the application site is likely to be rate-limited by the diffusion of drugs from those vehicles (245). The drug must have some and preferably greater affinity for the membrane than for the vehicle, thereby maximising the thermodynamic leaving potential (260). Although most topical formulations consist of simple components, the ability of a vehicle to release drugs at the local site is limited by numerous factors such as drug-vehicle, drug-skin and vehicle-skin interaction (245).

In the preliminary studies, Carbopol® 934 NF was initially used as the test polymer for the manufacture of extemporaneous topical gels. This polymer was unsuitable for the preparation of ketoprofen gels because the gel with optimum characteristics could only be formed at a pH range of 7.5 to 8.5 (258). This pH range will make it impossible for any drug release to occur and therefore this polymer was not investigated any further. Another finding during the preliminary studies was a decrease in consistency of the gel network with an increase in alcoholic content above 40% m/m. This was probably due to competition for water molecules between the polymer and the alcohol which may lead to poor hydration and therefore reduced swelling of the polymer molecules so that they adopt a tighter more close-coiled configuration. Proniuk *et al.* (237) and Díez-Sales *et al.* (239) both found a similar decrease in gel integrity on increasing the alcoholic content.

The choice of polymer for the manufacture of topical formulations should be one that produces the highest drug flux with the lowest lag time. The effect of Carbopol® polymer type on the release of drug is illustrated in Figures 5.3 and 5.4. Although no significant difference ($p > 0.05$, ANOVA) in drug release characteristics were observed for all five gels, some types of polymers were more efficient at releasing the drug than others. Similar results were reported by Macedo *et al.* (232) and Edsman *et al.* (247) who worked with more than one Carbopol® polymer type and noticed differences in drug release rates. Carbopol® polymers and co-polymers have the same acrylic acid backbone. The main differences are related to the presence of co-monomer and cross-link density. Minor adjustments in the cross-link density and co-monomer concentration can produce a large number of different polymers engineered to provide specific properties (228). Therefore the differences observed

could be attributed to the differences in cross-link density as noted above. Increasing the cross-link density of the polymer increases the tortuosity of the matrix through which the drug has to diffuse, thus decreasing drug release and increasing lag time (232).

It is has been reported (251) that the main barrier to the release of drugs from aqueous Carbopol® polymer gels is a mechanical layer formed by the random network of polymer molecules, which binds and entraps surrounding water. This aqueous phase in the polymer network may be the region responsible for diffusion of the drug from the gel. A change in polymer concentration can affect the diffusional pathway and thus drug release. Schantz *et al.* (259) showed that when diffusion of drugs occurs primarily through the aqueous channels in a gel, the diffusivity is an inverse function of polymer content. Although statistical differences ($p > 0.05$, ANOVA) in Carbopol® polymer content were observed from Figure 5.6, the apparent flux values of ketoprofen calculated in Table 5.10 did not decrease linearly with increased Carbopol® polymer concentration. This finding was not in agreement with a study by Lu *et al.* (245). These authors found that the effect of Carbopol® polymer content on diffusivity of methotrexate decreased linearly with increasing polymer content. The observed decrease in apparent flux with increasing polymer concentration may be attributed to an increase in tortuosity of the formulations making it difficult for the drug to be released and therefore increasing the lag time.

The effect of drug loading on release was evaluated and the results are illustrated in Figure 5.8. The increase in release rate with increasing concentration is due to the increasing thermodynamic activity of the drug which is related to its concentration in the base. As pointed out in section 5.5.2.3 the release of ketoprofen from the polymer was not linearly related to the drug concentration. This finding was not in agreement with El Gendy *et al.* (251) who found that the release of flurbiprofen from Carbopol® 934P and Pluronic® 407 polymers was linearly related to the loading concentration in the base. This may be due to the amount of drug that was used in the formulation of KET016. The 5.5% m/m ketoprofen was not soluble in the alcoholic co-solvent mixture. The initial drug release was mainly due to the drug molecules at the surface of the vehicle which were quickly released due to the slow molecular diffusion in the internal phase. The diffusion profile obtained was a summation of both phenomena whereas with the other formulations the release was principally due to the diffusion from the core of the vehicle to the vehicle/membrane interface. Gürol *et al.* (233) showed by differential scanning colorimetry, a strong hydrogen

bond formation between ketoprofen, Carbopol® polymer and triethanolamine at high concentrations of the drug. This results in ketoprofen having high partition selectivity for the base and thus a lower than expected apparent flux was generated.

The pH of the vehicle has been shown to be one of the major variables that could influence diffusion of drugs from semisolid vehicles (245). Theoretically, the pH value of the vehicle, the drug solubility in the vehicle and the viscosity of the gel matrix are three important factors to consider in the evaluation of drug diffusion from a gel dosage form across the membrane or the skin (239, 240). Therefore the pH values were adjusted to obtain values within limits of 5.00 - 6.00 unless where deliberately altered in KET013 and KET014 to investigate the effect of pH on ketoprofen release. KET013 demonstrates a long lag time (approximately one half of an hour) before steady state diffusion is seen at a relatively low initial delivery rate of 0.78 $\mu g/cm^2/h$. This would suggest that chemical (dissolution and membrane partitioning) parameters are controlling the rate of delivery. These delivery rates can be explained by examining Fick's law of diffusion (section 1.3.2). The flux is proportional to the partition co-efficient, the diffusion co-efficient and the donor concentration and inversely proportional to the thickness of the membrane. In this study the intrinsic diffusion concentration, the membrane thickness and the donor concentration were constant. Therefore in the absence of physical mechanisms, the rate of delivery will be dictated by the partition co-efficient between the donor formulation and the membrane (260). Numerous drugs are weak organic electrolytes (224) and therefore the ionization depends on the pH of the delivery medium. Consideration of this pH, as well as of the drug dissociation constant (p*Ka*), allow some degree of diffusion to be predicted (262). The larger partition towards the donor formulation than to the membrane can be explained in terms of the percentage of ionised and unionised species present in the formulation in accordance with the pH-partition hypothesis. An increase in formulation pH above the p*Ka* of the drug significantly decreases the amount of unionised species available for diffusion. The ionised species does not penetrate the membrane to any significant extent and when the drug is totally ionised the diffusion rate should be negligible, as is apparent in this case.

The Carbopol® polymers and co-polymers are also affected by pH values. The different grades of Carbopol® polymers have different effective pH ranges below and above which there is a loss of viscosity and between these two pH ranges, viscosity is optimum. However, within the effective range, differences in viscosity of the gel formulation can still occur

depending on how much base is added. The viscosity of a Carbopol® polymer based gel formulation begins to decrease after pH 9.0 and will continue to decrease if the pH is increased. This is due to the dampening of the electrostatic repulsion caused by the presence of excess electrolytes. It is possible to achieve high viscosity systems at pH values below 5.0 and above 9.0, but the concentration of Carbopol® polymer must be increased (261). The viscosity of the vehicle can hinder the diffusion of a drug across the membrane. The removal of ketoprofen from the vehicle/membrane interface creates a drug depletion zone which is slowly replenished by further diffusion of ketoprofen from the core of the gel to the vehicle/membrane interface. This diffusion is hindered by the viscosity of the vehicle, hence the decrease in ketoprofen delivery rate with time.

Carbopol® polymers form transparent elegant hydrogels at concentrations as low as 0.5% m/m. This concentration will however cease to hold when other excipients are present in the formulation. The presence of ions decreases the efficiency of mucilages of Carbopol® polymers (258). A formulation of ketoprofen gel requires 2.5% m/m of drug. Another challenge is that ketoprofen is poorly soluble in water. Less than 1 g is soluble in 10 l of water (section 2.1.5). The manufacturing technology employed to circumvent this problem is the use of co-solvent systems to dissolve the drug so that a transparent elegant gel can be formulated. However the use of alcohols as solvents in the manufacture of Carbopol® polymer based gels also reduce the viscosity of the formulation. To circumvent these two challenges, Pemulen® TR1 NF co-polymers have been identified and can be added to the Carbopol® polymer vehicle. Pemulen® TR1 NF co-polymers are indicated for use in the manufacture of transdermal and topical preparations as viscosity increasing agents. They are used in conjunction with Carbopol® polymers where high viscosity vehicles are required. Concentrations as small as 0.1% m/m of Pemulen® TR1 NF co-polymers can produce significant differences in the viscosity of the formulations and ultimately the release profile of the drug. This is evident in Figure 5.13. With increasing concentration of the co-polymer there was a corresponding decrease in apparent flux with increasing lag times (Table 5.10). The difference is mainly due to increasing viscosity of the formulations. It may also be that Pemulen® TR1 NF contains both hydrophobic and hydrophilic entities hence its ability to form emulsion systems. Increasing the concentration of the co-polymer in the formulation increases the hydrophobic content and will result in a decrease in drug release. Ketoprofen is a hydrophobic compound and will partition more in the formulation in the presence of increasing amounts of Pemulen® TR1 NF than in the vehicle/membrane interface. Figures

5.13 and 5.14 both illustrate the efficacy of Pemulen® TR1 NF co-polymer in the manufacture of ketoprofen gels. A 0.1% m/m Pemulen® TR1 NF co-polymer concentration produced a larger apparent flux and shorter lag time.

As described above, due to the poor aqueous solubility of ketoprofen, alcoholic solvent or co-solvent systems are needed to be able dissolve the drug in order to formulate an elegant transparent gel. Co-solvents have been widely used as vehicles as well as penetration enhancers in topical formulations (240). The effects of various solvent and co-solvent systems on the *in vitro* release of ketoprofen have been illustrated in Figures 5.18 - 5.23. Transcutol® HP, propylene glycol and ethanol were studied. All three solvents have been actively researched for their role as penetration enhancers. Transcutol® HP has been reported (51, 53, 262, 264, 266) to significantly increase the percutaneous penetration of various active substances particularly if used in combination with suitable co-solvents. Mura *et al.* (51) studied the effect of propylene glycol as a co-solvent with Transcutol® HP and found that the combination significantly increases the flux of clonazepam. Godwin *et al.* (53) reported that Transcutol® HP has been shown to increase the skin accumulation of topically applied compounds with a concomitant increase in transdermal permeation. Studies on propylene glycol as the principal solvent system have shown (239, 266) an increase in drug flux with increasing amounts of propylene glycol however this was not the case with Velissaratou *et al.* (265). They found that the release of chlorpheniramine maleate from ointment bases did not increase significantly when propylene glycol was incorporated. Co-solvent systems with isopropyl myristate (240), Transcutol® HP (51, 264), Azone (248), D-limonene (248) and propylene glycol have all shown an increase in drug flux. Studies on ethanol as a penetration enhancer have shown a modification of the SC as the principal mechanism. Low concentrations affect only the lipid pathway, while the polar pathway is also affected at higher concentrations (25, 267).

Ethanol, as a single solvent, was the most efficient system for the dissolution of the drug prior to the gelling process. High flux values with short lag times were observed with ethanol when incorporated into the formulation as the only solvent. This could be as a result of the extremely volatile nature of ethanol. Ketoprofen is readily soluble in ethanol but ethanol volatises rapidly from the formulation when applied leaving behind a high concentration of drug which will be made available for diffusion. This was in contrast to the findings of Suwanpidokkul *et al.* (267) who demonstrated that increasing the amount of ethanol was

associated with an increase in both flux values and lag times of zidovudine. A co-solvent system of ethanol and Transcutol® HP produced a synergistic effect with respect to the amount of flux produced but an increase in lag time was noted. Transcutol® HP has been indicated as a powerful solubilising agent (51) but is less volatile than ethanol. The increase in lag time of KET018 in Figure 5.20 is due to the lower volatile nature of the co-solvent system thus it takes more time for the drug to diffuse from the core of the gel to the membrane. This was evident in Figure 5.20 with the use of Transcutol® HP as a single solvent system. Lafforgue *et al.* (264) also found reduced flux values of methyl nicotinate with Transcutol® HP, similar results were observed by Mayorga *et al.* (266). Propylene glycol as a co-solvent failed to increase flux in ethanol or Transcutol® HP containing formulations to any significant extent. The lag times produced were also increased. These results were also observed by Mura *et al.* (51) and Arellano *et al.* (240). Although propylene glycol produced an *in vitro* decrease in drug flux when used with a synthetic membrane, it has been reported (240) that there is an *in vitro* and *in vivo* increase in drug flux with human or animal skin. This is because propylene glycol diffuses into the skin and enhances the partition of the drug into it, thus producing higher permeability co-efficients (239).

### *5.5.4 Conclusion*

It is clear that many factors can influence the release of drug from a topical semisolid formulation. Variables such as grade of polymer, polymer concentration, drug loading in donor chamber, vehicle pH and solvent systems can all influence the thermodynamic activity of the solute for the vehicle in question. It is the combined effect of these factors that influence the release rate of the drug, the parameter most useful for screening of formulations during the early stages of product development.

## 5.6 COMPARISON OF DIFFUSION STUDIES OF KETOPROFEN BETWEEN THE FRANZ DIFFUSION CELL AND THE EUROPEAN PHARMACOPOEIA DIFFUSION CELL

### *5.6.1 Introduction*

A number of different diffusion cells have been reported (110, 114 - 116, 270) in the literature for the measurement of drug release from topical formulations. Some of these diffusion cells are commercially available and others are laboratory designed (268). This diversity of apparatus has complicated the inter-laboratory comparisons of results and the extrapolation of data to the *in vivo* situation (268). Chilcott *et al.* (269) studied the inter- and intra-laboratory variation in diffusion cell measurements from 18 laboratories and reported significantly different results on the *in vitro* release measurements of methyl paraben through a synthetic membrane. Significantly different statistical results were also reported for similar diffusion cells from different laboratories.

Nearly all the published work on the *in vitro* release of drugs from semisolids was reported using vertical Franz diffusion cells. An alternative is the USP tablet dissolution apparatus modified to accommodate the European Pharmacopoeia diffusion cell. Figures 1.8 and 1.9 in section 1.4.2 present schematic diagrams of the modified Franz diffusion cell and the European Pharmacopoeia diffusion cell. In a recent study (100) in this laboratory the release of ibuprofen from various proprietary products through biological and synthetic membranes was greater from the European Pharmacopoeia diffusion cell than the vertical modified Franz diffusion cell, however the data was not corrected for membrane temperature which was different in the two diffusion systems.

The purpose of this study was to compare the more commonly used modified Franz diffusion cell to the European Pharmacopoeia diffusion cell and note any significant differences ($p < 0.05$, ANOVA) of *in vitro* release from semisolids while keeping the membrane temperature in both systems constant. Three proprietary 2.5% m/m ketoprofen gel products and a number of extemporaneously manufactured gel formulations were evaluated using both diffusion cells and the results compared. The use of a synthetic membrane in this study further minimises the variability associated with biological membranes.

### *5.6.2 Results*

The results presented in this study represent data obtained from hplc analysis.

*Table 5.11 In vitro release data comparison between Franz and European Pharmacopoeia diffusion cells*

| Formulation | Franz diffusion cell | | | European Pharmacopoeia diffusion cell | | |
|---|---|---|---|---|---|---|
| | **Apparent release constant ($\mu g/cm^2/h^{1/2}$)** | **Lag time (minutes)** | $r^2$ | **Apparent release constant ($\mu g/cm^2/h^{1/2}$)** | **Lag time (minutes)** | $r^2$ |
| Fastum® Gel | 112.0 ± 5.111 | 3.91 | 0.9357 | 154.4 ± 4.319 | 21.31 | 0.9748 |
| Ketum® Gel | 109.6 ± 2.538 | 1.10 | 0.9826 | 158.6 ± 7.260 | 18.07 | 0.9353 |
| Oruvail® Gel | 45.63 ± 2.433 | 28.89 | 0.9142 | 81.05 ± 3.859 | 97.23 | 0.9304 |
| KET001 | 139.6 ± 5.109 | 6.78 | 0.9577 | 157.3 ± 7.027 | 54.81 | 0.9382 |
| KET002 | 95.75 ± 2.180 | 25.67 | 0.9832 | 167.2 ± 5.682 | 44.69 | 0.9633 |
| KET003 | 94.47 ± 3.225 | 34.56 | 0.9630 | 108.8 ± 5.914 | 106.13 | 0.9111 |
| KET004 | 104.4 ± 2.694 | 24.99 | 0.9785 | 130.8 ± 6.046 | 63.28 | 0.9342 |
| KET005 | 124.4 ± 4.364 | 25.17 | 0.9610 | 154.4 ± 5.684 | 59.60 | 0.9572 |
| KET006 | 133.3 ± 5.253 | 39.50 | 0.9513 | 158.8 ± 6.083 | 48.21 | 0.9538 |
| KET007 | 86.60 ± 3.744 | 24.06 | 0.9419 | 166.1 ± 5.330 | 51.10 | 0.9671 |
| KET008 | 116.6 ± 4.650 | 21.62 | 0.9501 | 145.0 ± 6.179 | 46.86 | 0.9435 |
| KET009 | 101.5 ± 2.944 | 38.73 | 0.9730 | 169.5 ± 5.696 | 31.00 | 0.9641 |
| KET010 | 96.80 ± 4.047 | 56.89 | 0.9455 | 146.8 ± 5.021 | 52.99 | 0.9628 |
| KET011 | 60.04 ± 5.217 | 65.90 | 0.8006 | 118.9 ± 6.026 | 81.43 | 0.9219 |
| KET012 | 136.1 ± 5.604 | 21.23 | 0.9470 | 160.9 ± 10.33 | 68.31 | 0.8803 |
| KET013 | 7.57 ± 0.7859 | 103.44 | 0.7380 | - | - | - |
| KET014 | 35.29 ± 3.014 | 62.06 | 0.8061 | 83.09 ± 4.320 | 102.02 | 0.9181 |
| KET015 | 43.96 ± 3.143 | 47.18 | 0.8556 | 58.23 ± 3.557 | 112.78 | 0.8903 |
| KET016 | 154.7 ± 9.051 | 39.70 | 0.8985 | 179.0 ± 11.09 | 80.87 | 0.8876 |
| KET017 | 75.56 ± 4.401 | 85.39 | 0.8993 | 138.2 ± 6.902 | 103.28 | 0.9239 |
| KET018 | 142.5 ± 8.035 | 59.23 | 0.9051 | 213.8 ± 7.850 | 57.27 | 0.9574 |
| KET019 | 98.83 ± 6.622 | 11.34 | 0.8710 | 180.4 ± 6.881 | 20.56 | 0.9542 |
| KET020 | 101.4 ± 6.352 | 115.43 | 0.8853 | 160.2 ± 9.997 | 129.13 | 0.8861 |

Table 5.11 summarizes the kinetic data obtained from the linear regression analysis performed at a 95% confidence interval for both the Franz diffusion cell and the European Pharmacopoeia diffusion cell. For both cells the mean *in vitro* ketoprofen release across the synthetic membrane increased with the square root of time for all formulations. The linear correlation co-efficient ranges from 0.7380 - 0.9832 for the Franz diffusion cell and 0.8803 - 0.9748 for the European Pharmacopoeia diffusion cell. Although the linear correlation co-efficients expressed by some of the formulations in both diffusion cells were less than 0.9, the slope of each regression line displayed a significant deviation from zero ($p < 0.05$, ANOVA) indicating conformation to the Higuchi principle. Higher flux values and longer lag times were observed with the European Pharmacopoeia diffusion cell for all formulations. The

lowest flux value was produced by KET013 in the Franz diffusion cell but the concentrations were too small to be detected in the receptor fluid into which the European Pharmacopoeia diffusion cell had been submerged. There was no correlation observed between the two diffusion cells with respect to lag time. KET016 produced the highest flux value of 154.7 ± 9.051 $\mu g/cm^2/h^{½}$ in the Franz diffusion cell while KET018 produced a flux value of 213.8 ± 7.850 $\mu g/cm^2/h^{½}$ in the European Pharmacopoeia diffusion cell. KET013 would probably have produced the lowest flux value in the European Pharmacopoeia diffusion cell if quantification was possible by a more sensitive analytical procedure.

The individual diffusion profiles of the proprietary products and all the extemporaneous formulations are presented in appendix II. Group representation of results from Franz diffusion cells and European Pharmacopoeia diffusion cells based on the experimental manipulation of formulations are illustrated below. Figures 5.24 - 5.29 illustrate the comparison of the Franz diffusion cell and the European Pharmacopoeia diffusion cell for the *in vitro* release of ketoprofen from various formulations including the proprietary formulations. The graphs show that the release of ketoprofen was somewhat higher in the case of the European Pharmacopoeia diffusion cells ($p < 0.05$, ANOVA).

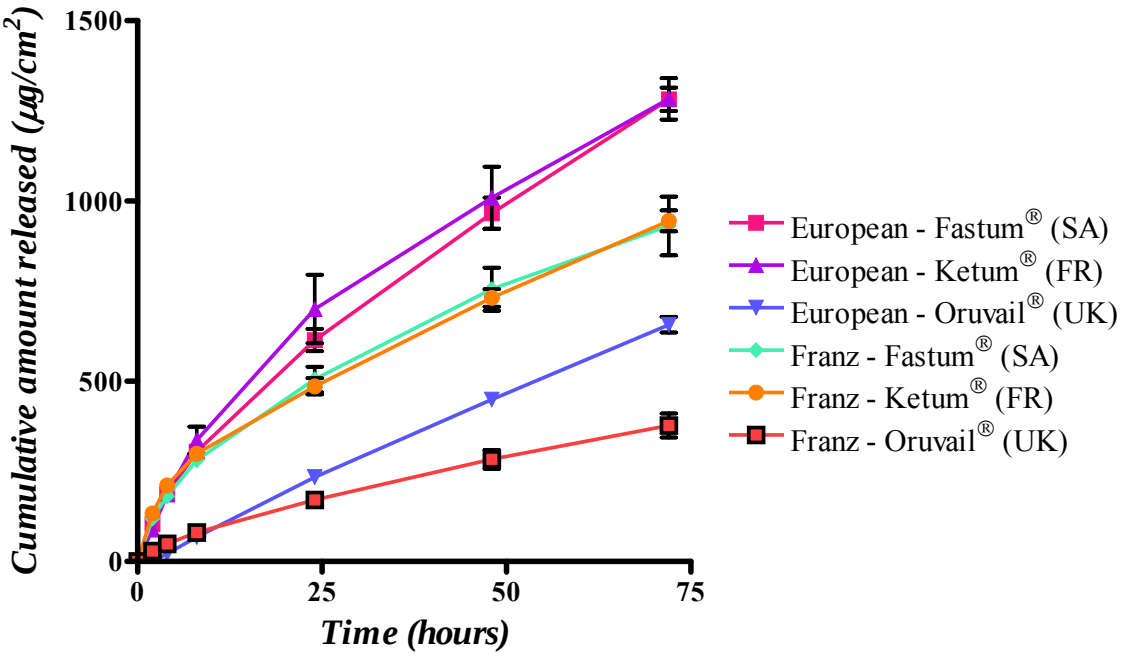



*Figure 5.24 Franz diffusion cell and European Pharmacopoeia diffusion cell comparison of the in vitro release of ketoprofen from proprietary formulations* ($n = 5$)

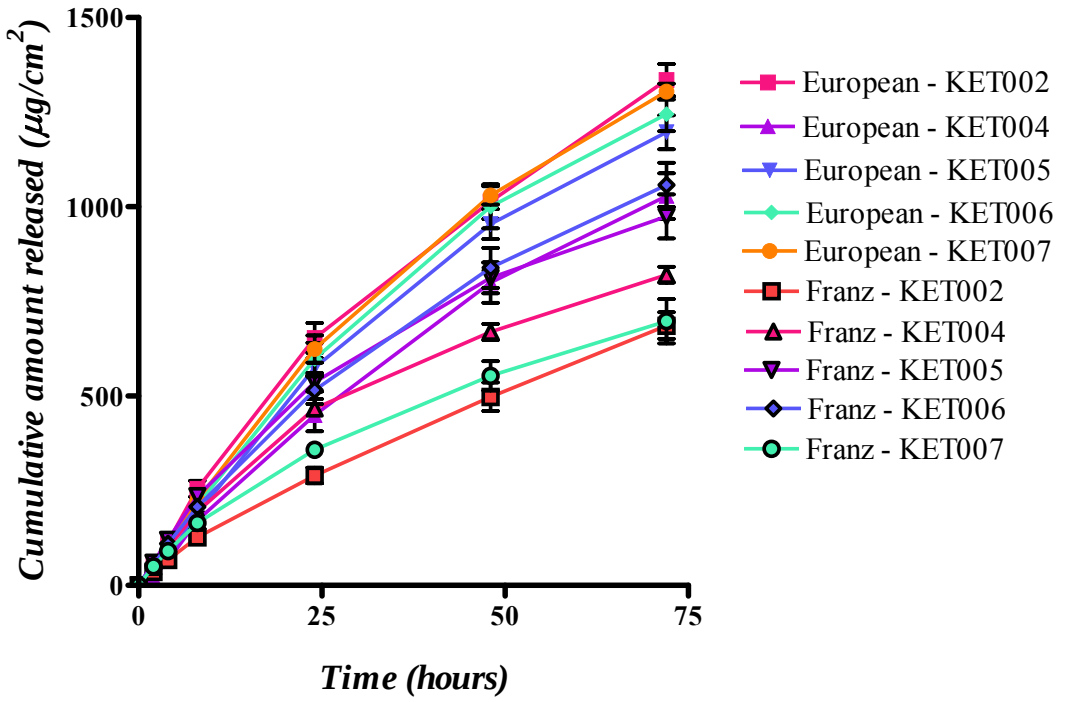



*Figure 5.25 Effect of different grades of Carbopol® polymers on the release of ketoprofen from Franz diffusion cells and European Pharmacopoeia diffusion cells* $(n = 5)$

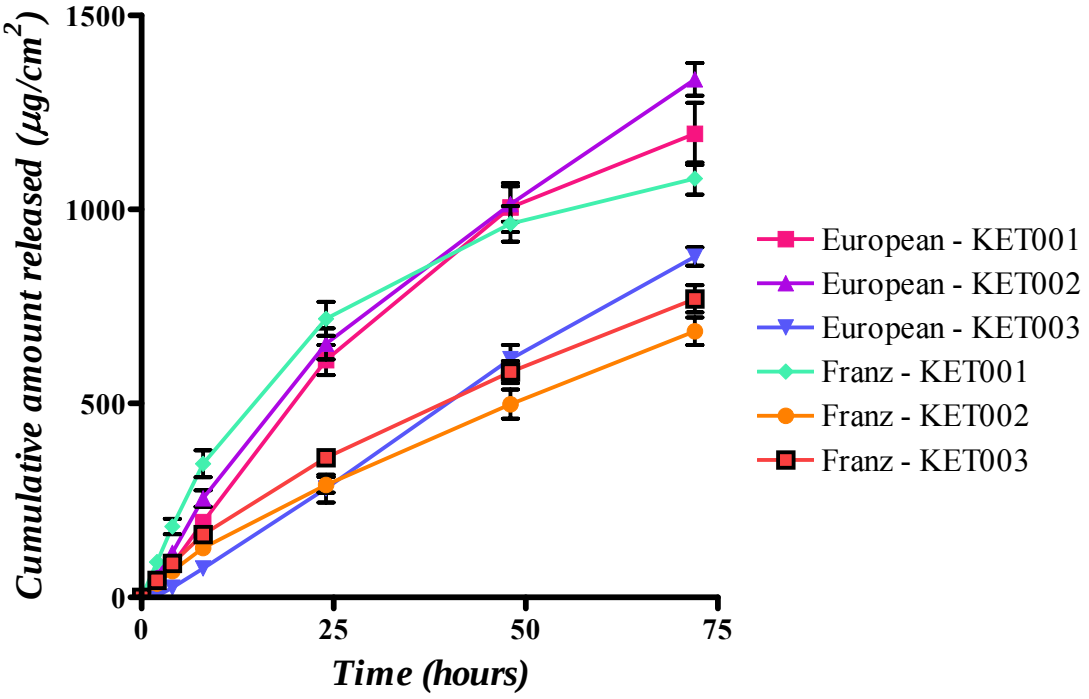



*Figure 5.26 Effect of different concentration of Carbopol® Ultrez™ 10 NF polymer on the release of ketoprofen from Franz diffusion cells and European Pharmacopoeia diffusion cells* $(n = 5)$

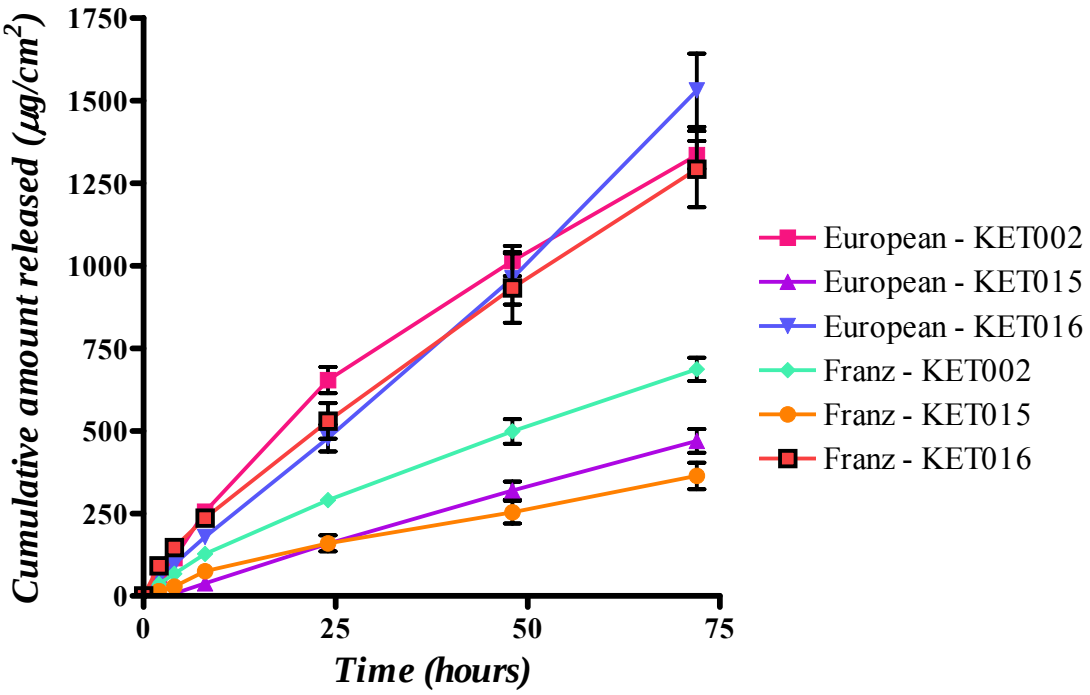



*Figure 5.27 Effect of drug concentration on the release of ketoprofen from Franz diffusion cells and European Pharmacopoeia diffusion cells* $(n = 5)$

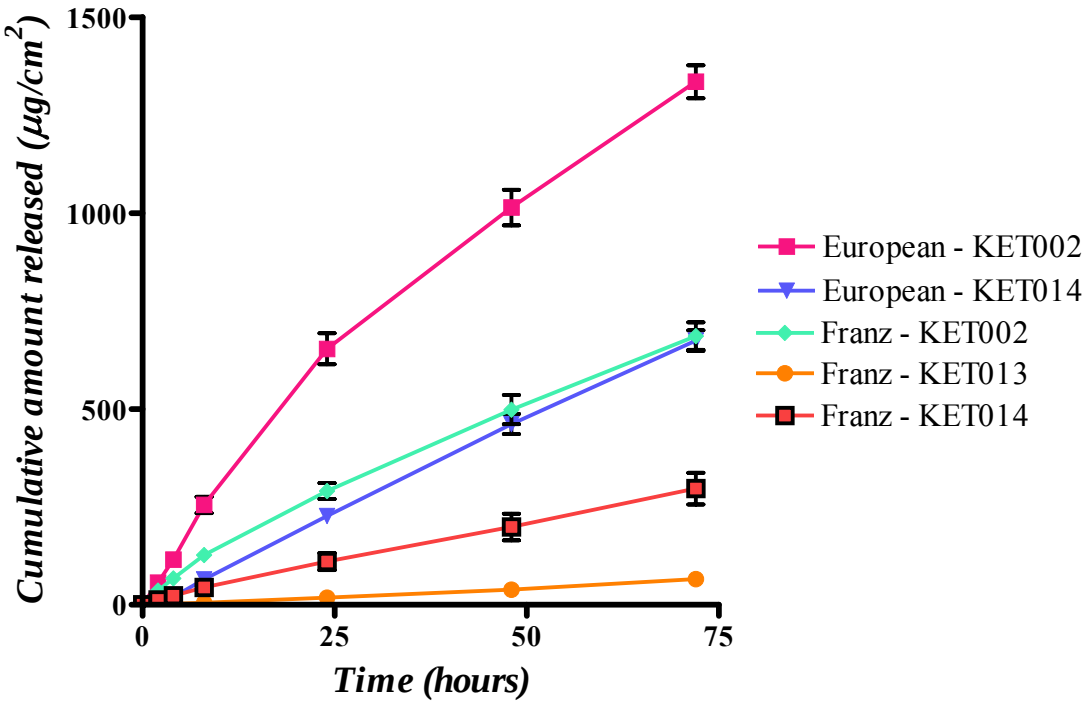



*Figure 5.28 Effect of pH on the release of ketoprofen from Franz diffusion cells and European Pharmacopoeia diffusion cells* $(n = 5)$

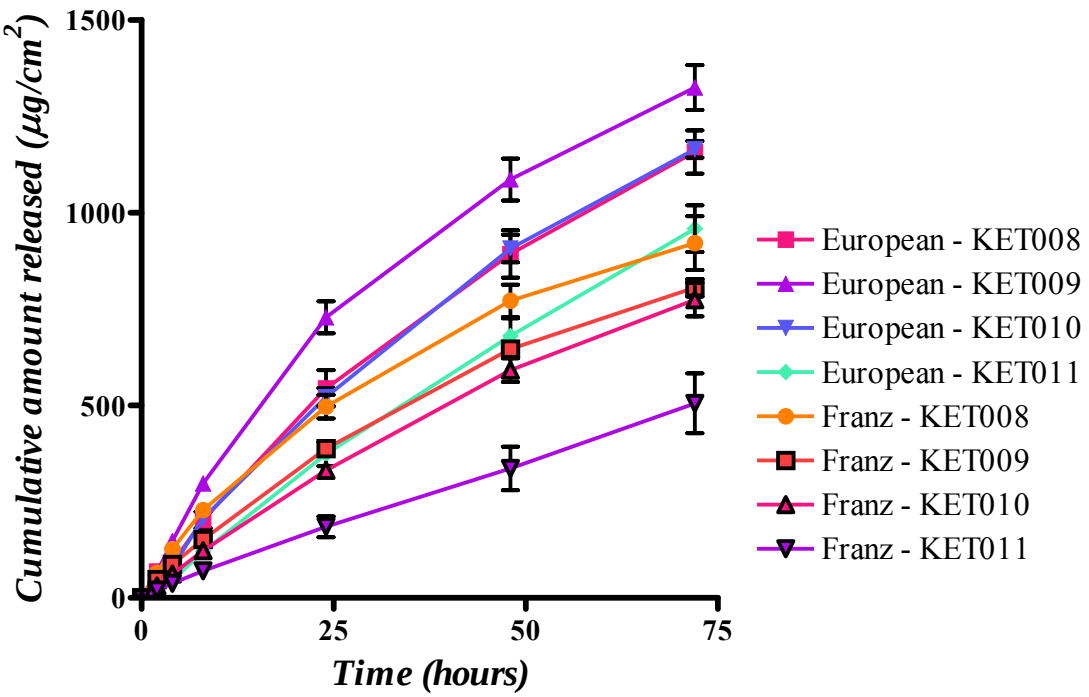



*Figure 5.29 Effect of Pemulen® TR1 NF into Carbopol® 980 NF formulations on the release of ketoprofen from Franz diffusion cells and European Pharmacopoeia diffusion cells* ($n = 5$)

### *5.6.3 Discussion*

The general setup of the diffusion apparatus was more tedious with the USP dissolution apparatus compared to the Franz apparatus. Approximately 6 l of degassed receptor fluid was required for the USP dissolution apparatus whereas 500 ml was more than enough for a dissolution run time of 72 hours using the Franz diffusion cells. The larger surface area of European Pharmacopoeia diffusion cell required more silicone membrane. The loading of the gel formulation into the European Pharmacopoeia diffusion cell was much easier as compared to the Franz diffusion cell. A direct weighing on a top loader analytical balance sufficed to transfer 500 mg of the gel into the donor chamber of the European Pharmacopoeia diffusion cell but a more tedious procedure was required for transferring 100 mg of gel into the donor chamber of the Franz diffusion cell. It was easier to attain a more uniform spread of the gel in the European Pharmacopoeia diffusion cell compared to the Franz diffusion cell where obtaining a uniform layer was not always possible. The manual manipulation of the receptor phase to avoid air bubbles during the dissolution run became very tedious in the Franz diffusion cells. Since the Franz diffusion cells are made of glass they were more susceptible to breakages while the European Pharmacopoeia diffusion cells were made from teflon and not easily broken.

The observed differences in the diffusion rate observed with the European Pharmacopoeia diffusion cell in comparison to the Franz diffusion cell can be explained in terms of the design of the diffusion apparatus, experimental conditions and the intrinsic nature of the gelling agent.

Although Keshary *et al.* (271) identified some shortfalls with respect to solution hydrodynamics, mixing efficiency and temperature control in the design of the original Franz diffusion cell, the modified Franz diffusion cell still leaves much to be desired in comparison to the European Pharmacopoeia diffusion cell. A suggestion would be to further extend the water jacket circulating around the receptor chamber in the Franz diffusion cell to the donor chamber in order to maintain the same temperature in both chambers.

The membrane temperature in both diffusion cells was adjusted in order to have the same temperature reading. This was not a problem in the USP dissolution apparatus because when the European Pharmacopoeia diffusion cell is immersed in the receptor fluid, the temperature of the membrane will equilibrate with the temperature of the water bath. This was somewhat of a challenge in the Franz diffusion apparatus. An experiment conducted in this study, on the temperature of the membrane in the donor chamber of the Franz diffusion cells, confirmed that a membrane temperature of 32 ± 0.5°C was only possible when the temperature of the heating element was set to 37 ± 0.5°C. Two problems were encountered as a result of this temperature manipulation. There was a substantial amount of receptor fluid evaporation through the sampling port from the receptor chamber of the Franz diffusion cell and there was some heat loss from the plastic tubing connecting the pump to the Franz diffusion cells. The evaporation from the receptor chamber meant that the lost fluids had to be replaced with fresh degassed receptor fluid which inevitably reduces the concentration of the permeant in the receptor chamber. More serious though with regards to the evaporation of receptor fluid is the formation of air bubbles on the underside of the membrane which reduces diffusion. The problem is exacerbated during the overnight run times when the Franz diffusion cell could not be manually tipped to remove the air bubbles even though the open end of the sampling ports were sealed. The problem of air bubbles has been identified by a number of authors (110, 114, 118, 120) and still remains the major drawback to the use of the Franz diffusion cell.

The loss of receptor fluid containing diffused drug was inevitable during complete emptying of the Franz diffusion cell at sampling times. This may have resulted in inaccurate drug quantification thus producing lower diffusion profiles as compared to those obtained from the European Pharmacopoeia diffusion cell. The non-continuous process of the Franz diffusion cell may result in absolute sink conditions not being maintained during the entire diffusion run especially after 8 hours. The concentration of the drug in the receptor fluid increases with time. This increase decreases the concentration gradient of the drug between the donor chamber and the receptor chamber resulting in lower drug diffusion before the next sampling time. In the case of the European Pharmacopoeia diffusion cell, absolute sink conditions were maintained at all times due to the large volume of the receptor phase and the continuous process which allowed for minimum drug loss during sampling times. Reference to the diffusion profile of KET016 in appendix II shows that a nearly superimposable profile was observed until about 24 hours for both diffusion cells after which the rate of release from the Franz diffusion cell began to decrease. Sink conditions were not maintained in the Franz diffusion cells after 24 hours.

Another possible reason to explain the high release from the European Pharmacopoeia diffusion cell compared to the Franz diffusion cell is the nature of the gelling agent employed in the manufacture of the gels. Carbopol® polymers, as with most other gelling agents, exhibit an intrinsic property known as thixotropy. This is where the gel has the ability to exhibit gel-sol transitions when subjected to external conditions such as increase in shear rate or an increase in temperature (129). In the European Pharmacopoeia diffusion cell, the temperature may be high enough for the gel to change into a slightly viscous liquid which will increase the kinetic energy of the drug within the formulation and therefore increase the rate of diffusion into the receptor fluid. An increase in temperature is associated with an increase in kinetic energy and a decrease in activation energy. This finding may not be the same for that obtained from the Franz diffusion cell. Increasing the temperature in the Franz diffusion apparatus would lead to an inevitable temperature loss through the plastic tubing and thus the temperature of the donor chamber would not be high enough to change the physical state of the gel under evaluation. Thixotropy would therefore not occur under such experimental conditions and fewer drug molecules will diffuse into the receptor phase.

### *5.6.4 Conclusion*

In product development, the large volume of the European Pharmacopoeia diffusion cell does not allow for the detection of very small quantities of diffused drug whereas it may be more than adequate to compare dissolution profiles of established finished products. Conversely, the Franz diffusion cells will be the best diffusion cell to employ in the initial stages of product development because the small volume produces high concentrations of the diffused drug which can easily be quantified. Formulations containing a large amount of drug may show reduced diffusion over long sampling times due to sink conditions not being maintained.

## 5.7 COMPARISON OF DIFFUSION STUDIES OF KETOPROFEN BETWEEN HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY AND ULTRAVIOLET SPECTROPHOTOMETRY

### *5.7.1 Introduction*

A survey of the literature (148, 183, 184, 186, 187, 190, 192, 233, 272 - 276) has revealed several analytical procedures for the determination of ketoprofen in a number of formulations including topical gel preparations with hplc and uv spectrophotometric procedures the most commonly employed. Most laboratories are equipped with a uv spectrophotometer primarily because it is simple and easy to use. Major drawbacks associated with the use of a uv spectrophotometer are its inability to measure very dilute concentrations, differentiate between compounds absorbing at the same wavelength and its use with photolabile agents. The use of an hplc system, although more expensive and cumbersome to setup, is on the increase primarily because it overcomes the problems associated with the uv spectrophotometer and automated systems are available.

A number of validated hplc analytical procedures for the determination of ketoprofen in the literature have been identified in section 3.1 but there seems to be little information on validated uv spectrophotometric procedures even though the uv spectrophotometer is the most common analytical apparatus used in laboratories. This has resulted in some uncertainty when inter-laboratory comparisons are conducted and different authors present their results using different analytical methods. Chilcott *et al.* (269) reported an international multicenter study on the *in vitro* release of methyl paraben with the choice of employing either an hplc or a uv spectrophotometric analytical procedure. Although significantly different results were obtained with reference to the use of different diffusion cells, the results were not analysed for the effect of the choice of the analytical procedure employed in each laboratory.

The purpose of this study was to compare the *in vitro* diffusion profiles obtained by utilising both the Franz diffusion cell and the European Pharmacopoeia diffusion cell under hplc and uv spectrophotometric analytical procedures. Three proprietary 2.5% m/m ketoprofen gel products and a number of extemporaneously manufactured gel formulations were evaluated.

*5.7.2 Results*

The flux values and lag times for all the formulations obtained from both the Franz diffusion cells and the European Pharmacopoeia diffusion cells using hplc and uv spectrophotometric analysis are reported in Tables 5.12 and 5.13. The results were obtained from a linear regression of the data at a 95% confidence interval. Generally higher flux values were obtained from both diffusion cells by the uv spectrophotometric analytical procedure although no significant differences ($p > 0.05$, ANOVA) were noted. The regression co-efficients obtained by uv analysis were generally somewhat higher than those obtained by hplc analysis. No distinct relationship was noted with respect to the lag times although generally shorter times were observed by uv analysis compared to hplc analysis.

*Table 5.12 Comparison of analytic procedure using Franz diffusion cells*

| | **High-performance liquid chromatographic analysis** | | | **Ultraviolet spectrophotometric analysis** | | |
|---|---|---|---|---|---|---|
| **Formulation** | **Apparent release constant ($\mu g/cm^2/h^{1/2}$)** | **Lag time (minutes)** | $r^2$ | **Apparent release constant ($\mu g/cm^2/h^{1/2}$)** | **Lag time (minutes)** | $r^2$ |
| Fastum® Gel | 112.0 ± 5.111 | 3.91 | 0.9357 | 126.3 ± 5.238 | 0.57 | 0.9463 |
| Ketum® Gel | 109.6 ± 2.538 | 1.10 | 0.9826 | 113.5 ± 2.862 | 3.16 | 0.9794 |
| Oruvail® Gel | 45.63 ± 2.433 | 28.89 | 0.9142 | 43.32 ± 2.437 | 39.83 | 0.9054 |
| KET001 | 139.6 ± 5.109 | 6.78 | 0.9577 | 152.1 ± 5.020 | 5.72 | 0.9653 |
| KET002 | 95.75 ± 2.180 | 25.67 | 0.9832 | 88.39 ± 3.506 | 34.84 | 0.9506 |
| KET003 | 94.47 ± 3.225 | 34.56 | 0.9630 | 100.7 ± 3.465 | 28.79 | 0.9624 |
| KET004 | 104.4 ± 2.694 | 24.99 | 0.9785 | 109.6 ± 2.815 | 25.76 | 0.9787 |
| KET005 | 124.4 ± 4.364 | 25.17 | 0.9610 | 117.7 ± 3.911 | 20.53 | 0.9648 |
| KET006 | 133.3 ± 5.253 | 39.50 | 0.9513 | 153.3 ± 5.009 | 15.45 | 0.9660 |
| KET007 | 86.60 ± 3.744 | 24.06 | 0.9419 | 100.2 ± 3.868 | 3.33 | 0.9531 |
| KET008 | 116.6 ± 4.650 | 21.62 | 0.9501 | 136.0 ± 4.950 | 10.13 | 0.9581 |
| KET009 | 101.5 ± 2.944 | 38.73 | 0.9730 | 105.5 ± 3.369 | 30.86 | 0.9674 |
| KET010 | 96.80 ± 4.047 | 56.89 | 0.9455 | 100.5 ± 4.113 | 56.79 | 0.9476 |
| KET011 | 60.04 ± 5.217 | 65.90 | 0.8006 | 65.02 ± 5.234 | 63.41 | 0.8238 |
| KET012 | 136.1 ± 5.604 | 21.23 | 0.9470 | 105.9 ± 6.158 | 59.57 | 0.8996 |
| KET013 | 7.57 ± 0.7859 | 103.44 | 0.7380 | 12.01 ± 1.033 | 21.58 | 0.8038 |
| KET014 | 35.29 ± 3.014 | 62.06 | 0.8061 | 41.57 ± 3.390 | 34.69 | 0.8201 |
| KET015 | 43.96 ± 3.143 | 47.18 | 0.8556 | 46.54 ± 2.705 | 39.51 | 0.8997 |
| KET016 | 154.7 ± 9.051 | 39.70 | 0.8985 | 145.8 ± 8.682 | 33.04 | 0.8953 |
| KET017 | 75.56 ± 4.401 | 85.39 | 0.8993 | 80.76 ± 4.641 | 82.13 | 0.9017 |
| KET018 | 142.5 ± 8.035 | 59.23 | 0.9051 | 149.8 ± 8.208 | 59.03 | 0.9098 |
| KET019 | 98.83 ± 6.622 | 11.34 | 0.8710 | 103.6 ± 6.482 | 10.04 | 0.8856 |
| KET020 | 101.4 ± 6.352 | 115.43 | 0.8853 | 93.85 ± 6.670 | 142.67 | 0.8571 |

No data was recorded for KET013 in Table 5.13 because the amount of ketoprofen in the receptor fluid was too small to be accurately quantified.

*Table 5.13 Comparison of analytical procedure using European Pharmacopoeia diffusion cells*

| Formulation | High-performance liquid chromatographic analysis | | | Ultraviolet spectrophotometric analysis | | |
|---|---|---|---|---|---|---|
| | **Apparent release constant ($\mu g/cm^2/h^{1/2}$)** | **Lag time (minutes)** | $r^2$ | **Apparent release constant ($\mu g/cm^2/h^{1/2}$)** | **Lag time (minutes)** | $r^2$ |
| Fastum® Gel | 154.4 ± 4.319 | 21.31 | 0.9748 | 174.0 ± 3.958 | 7.31 | 0.9832 |
| Ketum® Gel | 158.6 ± 7.260 | 18.07 | 0.9353 | 168.7 ± 7.892 | 10.70 | 0.9326 |
| Oruvail® Gel | 81.05 ± 3.859 | 97.23 | 0.9304 | 88.36 ± 4.419 | 99.07 | 0.9238 |
| KET001 | 157.3 ± 7.027 | 54.81 | 0.9382 | 172.4 ± 6.500 | 25.90 | 0.9552 |
| KET002 | 167.2 ± 5.682 | 44.69 | 0.9633 | 187.5 ± 5.861 | 28.00 | 0.9688 |
| KET003 | 108.8 ± 5.914 | 106.13 | 0.9111 | 126.8 ± 5.960 | 55.22 | 0.9320 |
| KET004 | 130.8 ± 6.046 | 63.28 | 0.9342 | 154.0 ± 6.843 | 66.78 | 0.9388 |
| KET005 | 154.4 ± 5.684 | 59.60 | 0.9572 | 165.8 ± 5.495 | 12.29 | 0.9650 |
| KET006 | 158.8 ± 6.083 | 48.21 | 0.9538 | 185.8 ± 6.078 | 2.84 | 0.9659 |
| KET007 | 166.1 ± 5.330 | 51.10 | 0.9671 | 193.9 ± 5.183 | 17.75 | 0.9770 |
| KET008 | 145.0 ± 6.179 | 46.86 | 0.9435 | 162.9 ± 7.261 | 75.67 | 0.9385 |
| KET009 | 169.5 ± 5.696 | 31.00 | 0.9641 | 182.1 ± 6.848 | 59.82 | 0.9554 |
| KET010 | 146.8 ± 5.021 | 52.99 | 0.9628 | 173.9 ± 5.259 | 37.66 | 0.9707 |
| KET011 | 118.9 ± 6.026 | 81.43 | 0.9219 | 132.5 ± 6.462 | 48.60 | 0.9272 |
| KET012 | 160.9 ± 10.33 | 68.31 | 0.8803 | 158.6 ± 12.49 | 151.69 | 0.8300 |
| KET013 | - | - | - | - | - | - |
| KET014 | 83.09 ± 4.320 | 102.02 | 0.9181 | 108.8 ± 5.947 | 100.09 | 0.9103 |
| KET015 | 58.23 ± 3.557 | 112.78 | 0.8903 | 77.52 ± 5.636 | 120.16 | 0.8515 |
| KET016 | 179.0 ± 11.09 | 80.87 | 0.8876 | 190.4 ± 11.21 | 44.53 | 0.8973 |
| KET017 | 138.2 ± 6.902 | 103.28 | 0.9239 | 153.5 ± 6.710 | 61.00 | 0.9407 |
| KET018 | 213.8 ± 7.850 | 57.27 | 0.9574 | 234.5 ± 7.300 | 40.06 | 0.9690 |
| KET019 | 180.4 ± 6.881 | 20.56 | 0.9542 | 200.7 ± 6.655 | 10.19 | 0.9650 |
| KET020 | 160.2 ± 9.997 | 129.13 | 0.8861 | 159.5 ± 10.61 | 146.02 | 0.8725 |

Figures 5.30 - 5.41 illustrate the diffusion profiles of the proprietary formulations and the extemporaneous formulation using Franz diffusion cells and European Pharmacopoeia diffusion cells using hplc and uv spectrophotometric analysis. The individual diffusion profiles of each formulation are presented in appendix II. Although no statistically significant difference ($p > 0.05$, ANOVA) was obtained between the diffusion profiles using hplc and uv spectrophotometric analysis, there was generally a rank order increase with respect to data obtained using uv analysis in comparison to those obtained using hplc analysis. The diffusion profiles produced by hplc analysis were generally smooth but some erratic profiles have been observed with uv analysis for KET012, KET014 and KET016 using European Pharmacopoeia diffusion cells. The diffusion profiles using hplc and uv analysis were generally superimposable where the Franz diffusion cell was utilised but more erratic profiles were observed with the use of the European Pharmacopoeia diffusion cell indicating that the concentration of the drug may play an important factor. KET016 displayed nearly superimposable diffusion profiles for both the Franz and European Pharmacopoeia diffusion cells.

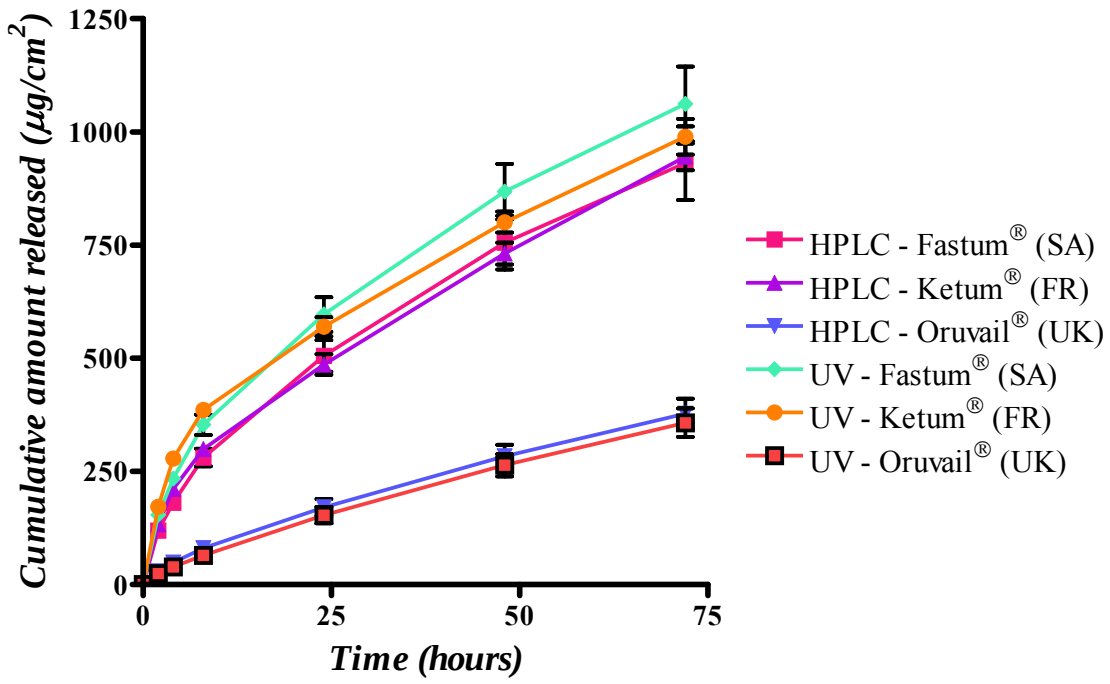



*Figure 5.30 In vitro Franz cell diffusion profiles of proprietary products using hplc and uv spectrophotometric analysis* $(n = 5)$

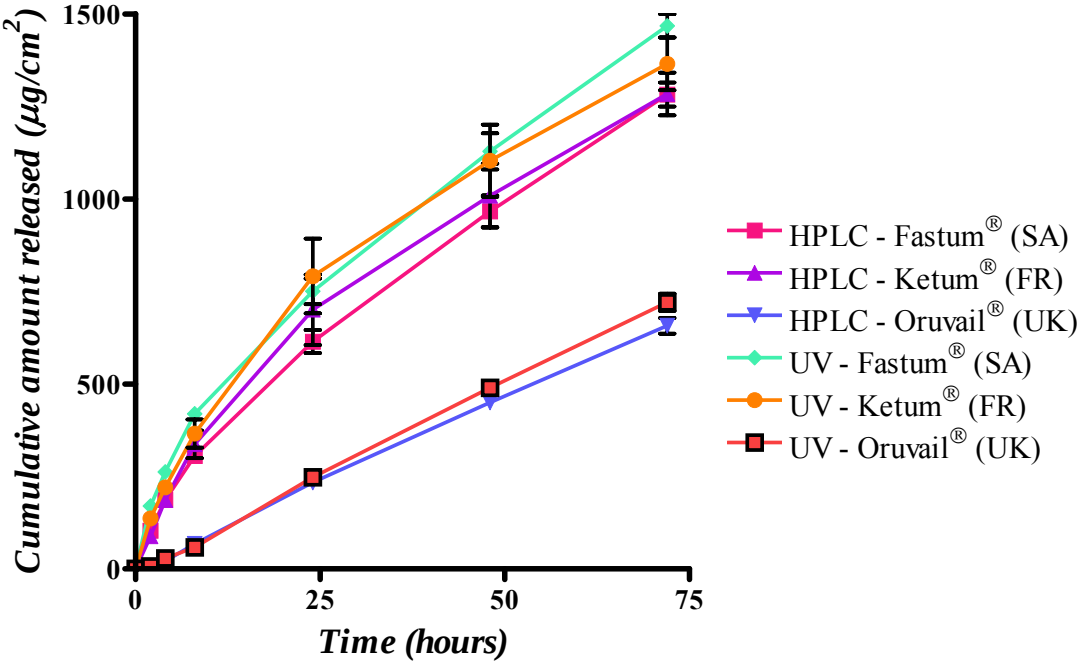



*Figure 5.31 In vitro European Pharmacopoeia cell diffusion profiles of proprietary products using hplc and uv spectrophotometric analysis* $(n = 5)$

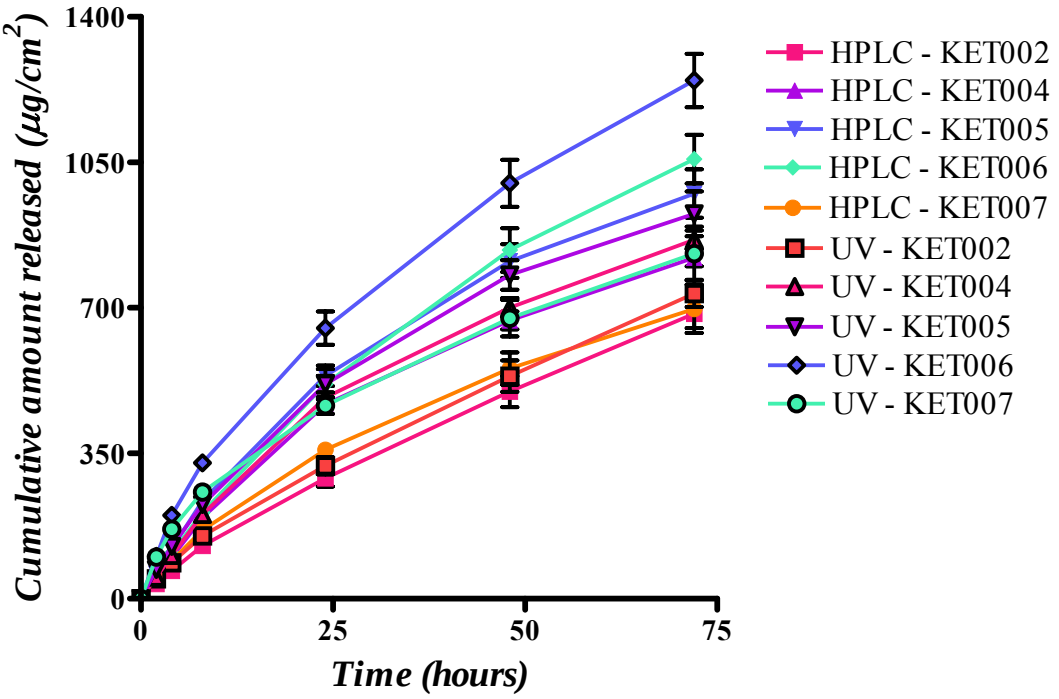



*Figure 5.32 Effect of different grades of Carbopol® polymers on the release of ketoprofen using Franz diffusion cells with hplc and uv spectrophotometric analysis* $(n = 5)$

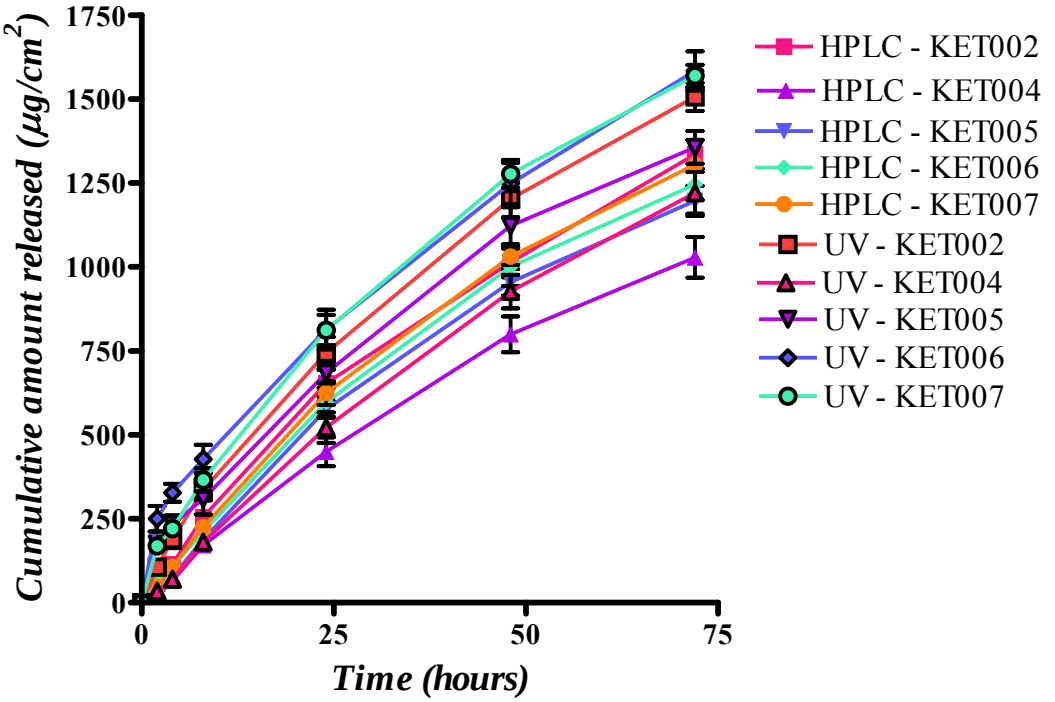



*Figure 5.33 Effect of different grades of Carbopol® polymers on the release of ketoprofen using European Pharmacopoeia diffusion cells with hplc and uv spectrophotometric analysis* $(n = 5)$

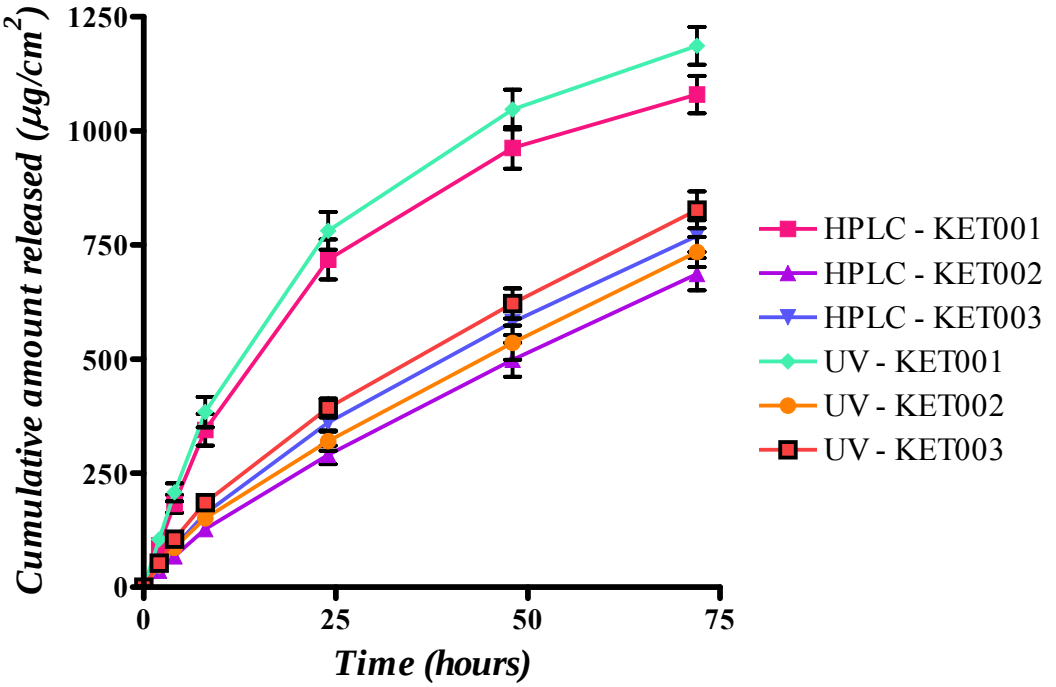



*Figure 5.34 Effect of different concentration of Carbopol® Ultrez™ 10 NF polymer on the release of ketoprofen using Franz diffusion cells with hplc and uv spectrophotometric analysis* $(n = 5)$

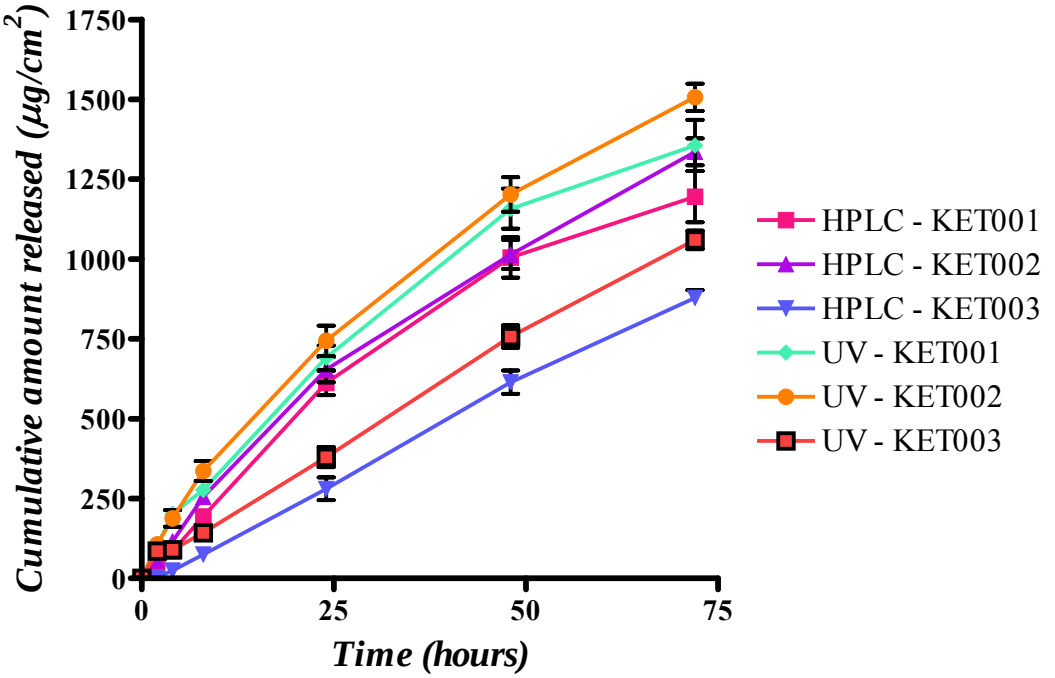



*Figure 5.35 Effect of different concentration of Carbopol® Ultrez™ 10 NF polymer on the release of ketoprofen using European Pharmacopoeia diffusion cells with hplc and uv spectrophotometric analysis* $(n = 5)$

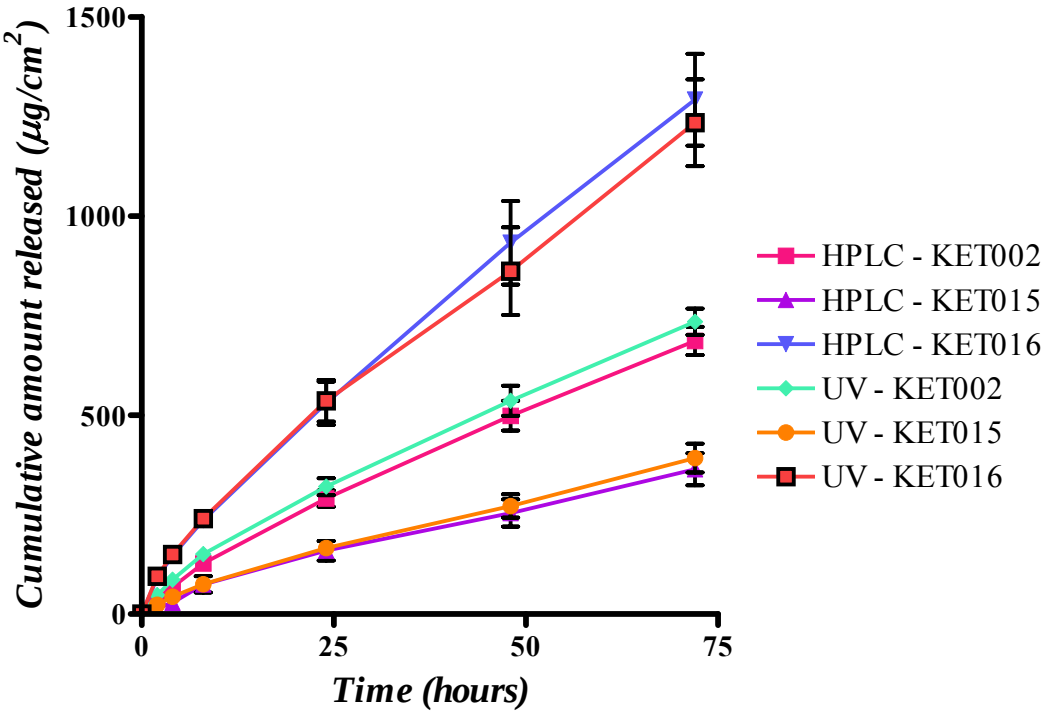



*Figure 5.36 Effect of drug concentration on the release of ketoprofen using Franz diffusion cells with hplc and uv spectrophotometric analysis* $(n = 5)$

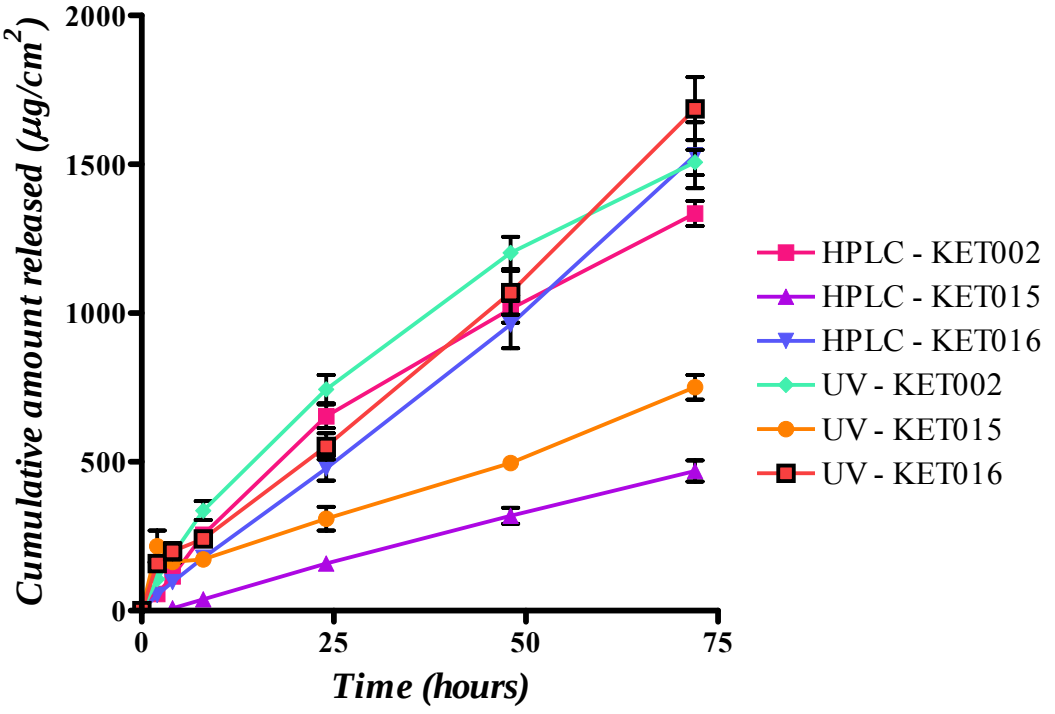



*Figure 5.37 Effect of drug concentration on the release of ketoprofen using European Pharmacopoeia diffusion cells with hplc and uv spectrophotometric analysis* $(n = 5)$

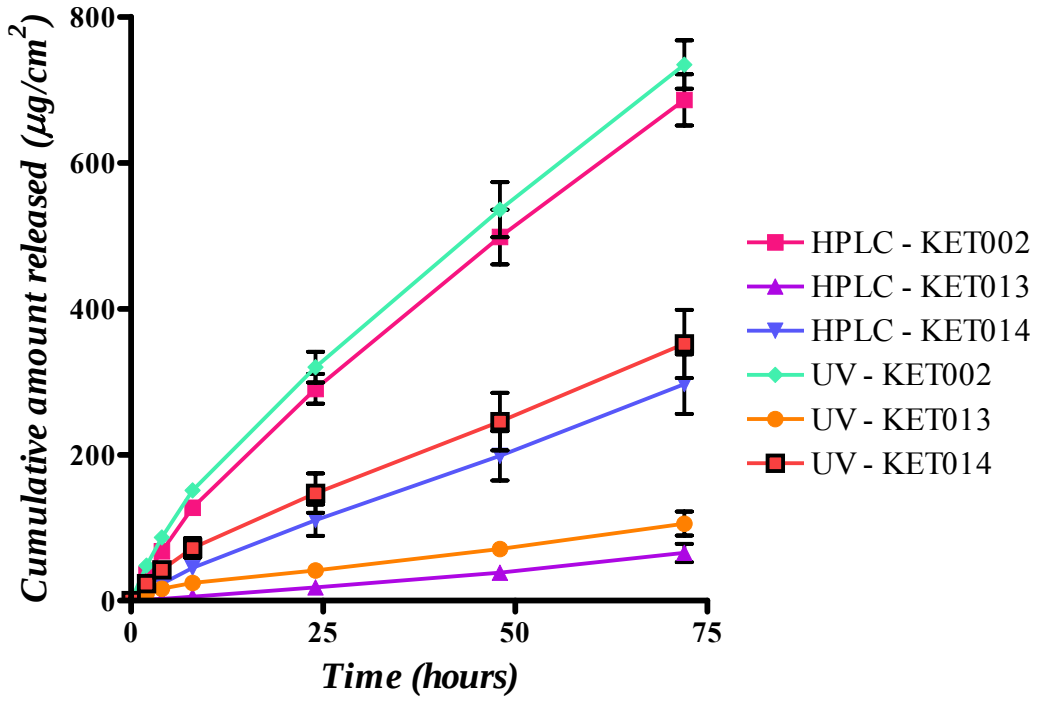



*Figure 5.38 Effect of pH on the release of ketoprofen using Franz diffusion cells with hplc and uv spectrophotometric analysis* $(n = 5)$

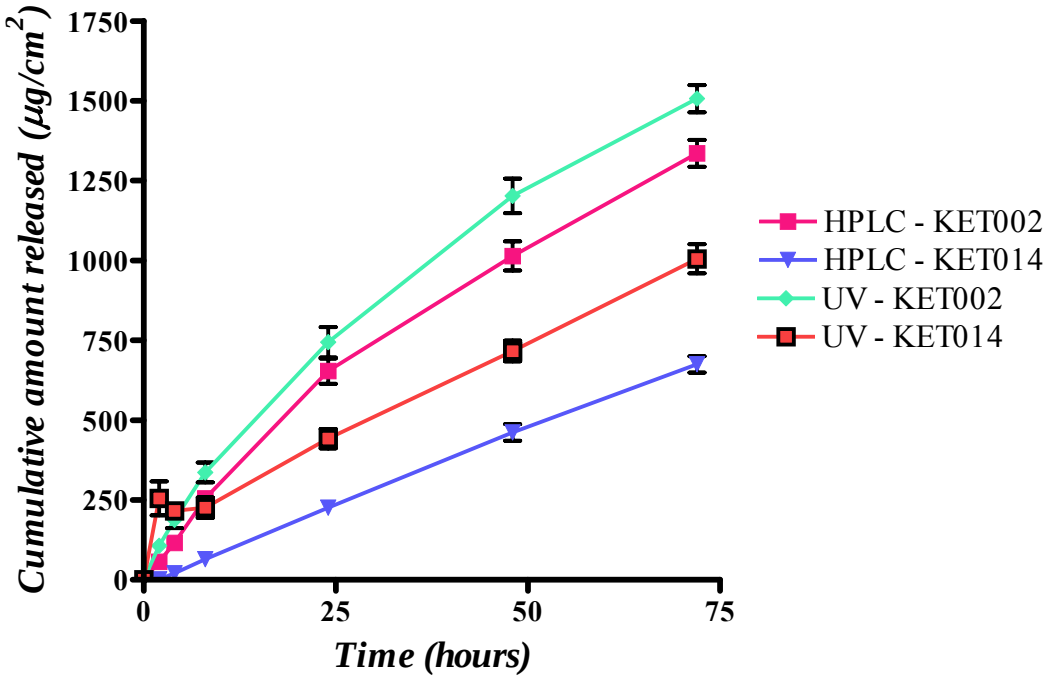



*Figure 5.39 Effect of pH on the release of ketoprofen using European Pharmacopoeia diffusion cells with hplc and uv spectrophotometric analysis* $(n = 5)$

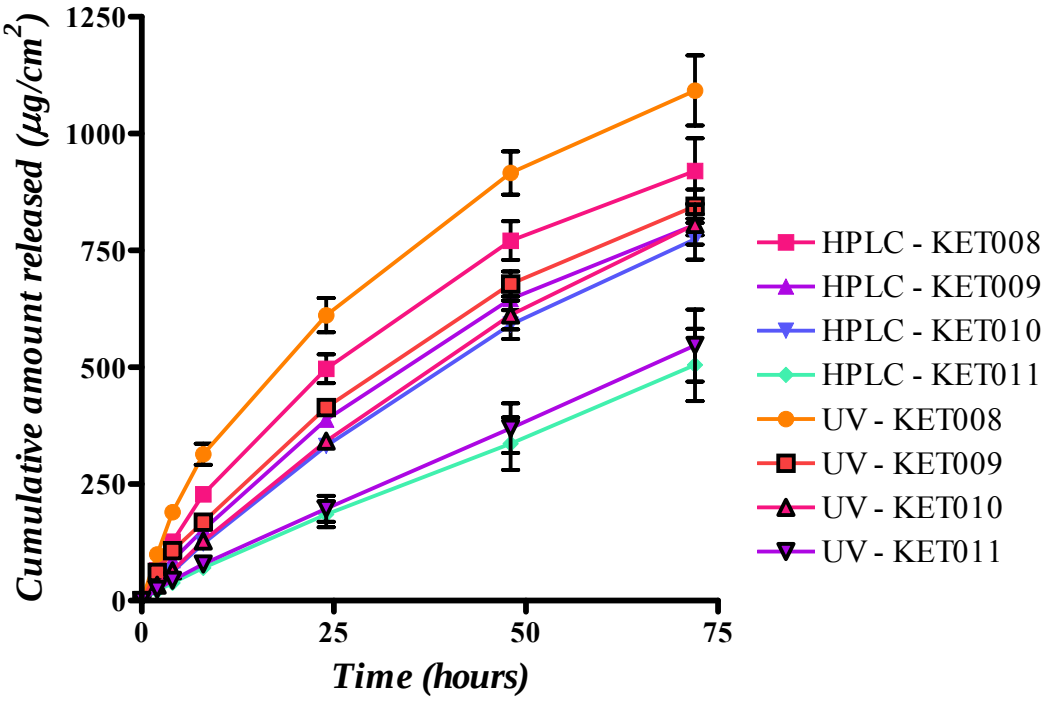



*Figure 5.40 Effect of incorporation of Pemulen® TR1 NF into Carbopol® 980 NF formulations on the release of ketoprofen using Franz diffusion cells with hplc and uv spectrophotometric analysis* ($n = 5$)

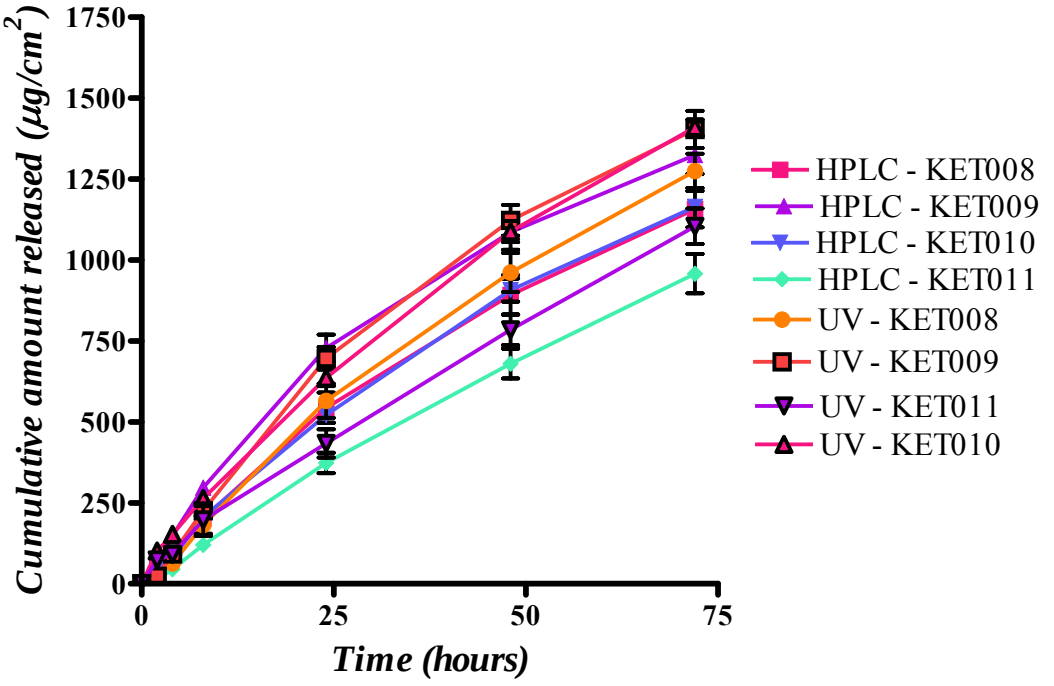



*Figure 5.41 Effect of incorporation of Pemulen® TR1 NF into Carbopol® 980 NF formulations on the release of ketoprofen using European Pharmacopoeia diffusion cells with hplc and uv spectrophotometric analysis* ($n = 5$)

### *5.7.3 Discussion*

The larger and sometimes more erratic diffusion profiles observed more commonly using the European Pharmacopoeia diffusion cell on uv analysis can be explained by non-conformity to the Beer-Lambert Law as outlined in section 3.2.1.2.1. The law holds for concentrations high enough to attain absorbance values in the region of 0.2 to 0.8. The European Pharmacopoeia apparatus receptor fluid concentrations obtained from KET001, KET002, KET003, KET005, KET006, KET007, KET010, KET012, KET013, KET014 and KET015 were too low to be accurately quantified by the uv spectrophotometer. This is because these formulations produced absorbance readings less than 0.2 which implied non-conformity with the Beer-Lambert Law. As a result erratic absorbance readings were obtained especially from the first 8 hours of the diffusion run. This was however not the case with the use of the Franz diffusion cells. The relatively small volumes of the receptor chamber produced high concentrations of ketoprofen which had to be diluted to obtain absorbance values within the recommended range therefore producing diffusion profiles very similar to those obtained from hplc analysis.

Another possible reason for the higher diffusion profiles obtained using uv analysis may be due to the photolability of ketoprofen. Ketoprofen is photolabile and uv radiation converts ketoprofen into an excited state, and the energy can be dissipated as heat, light or be transferred to the surrounding molecules. If the excitation energy cannot be lost then the molecule may rearrange and form decomposition products (272). These decomposition products may also absorb in the same region as ketoprofen and may result in exaggerated absorbance values which would distort the diffusion profiles. Although ketoprofen samples were stored in the dark at 4 ± 0.5°C, exposing the drug sample to uv light during spectrophotometric analysis could also result in the formation of decomposition products. It was noted that a number of researchers (184, 187, 217, 219, 221, 222, 233, 274 - 276) who utilised uv analysis for the quantification of active pharmaceutical ingredients did not indicate the integration time. This is very important as this is the time the drug sample is exposed to uv radiation. An increased exposure may produce more decomposition products which may then produce elevated absorbance readings.

### *5.7.4 Conclusion*

During product development of topical formulations, hplc and uv analysis can be safely employed with the utilisation of the Franz diffusion cell. The spectrophotometric method is however less sensitive in the initial stages of the diffusion experiment, especially where the European Pharmacopoeia diffusion cell is employed, due to its inability to accurately detect low concentrations. Although the hplc system is a more expensive analytical apparatus, it is more sensitive to the detection of small concentrations which can provide vital information for the optimization of topical dermatological formulations.

# APPENDIX I

# DESIGNED SPREADSHEET

**Rhodes University**
**Faculty of Pharmacy**
**Transdermal Laboratory**
**Grahamstown, South Africa**

RHODES UNIVERSITY
*Where leaders learn*

# DESIGNED SPREADSHEET

| | | | | | |
|---|---|---|---|---|---|
| ***Analyst*** | | | ***Filename*** | | |
| ***Formulation*** | | ***Batch ID*** | | ***Run date*** | |
| ***Strength*** | | ***Run No.*** | | ***Number of repeats*** | |

| **Analytical summary** | | | |
|---|---|---|---|
| ***High-performance liquid chromatography*** | | ***Ultraviolet spectrophotometry*** | |
| *Parameter* | *Settings employed* | *Parameter* | *Settings employed* |
| Mobile phase | 60% ACN: 40% 20 mM PB at pH 2.5 | Measurement mode | Absorbance |
| Stationary phase | Spherisorb S5 ODS2 4.0 mm x 250 mm | Solvent | 0.2 M PB at pH 6.8 |
| Receptor phase | 0.2 M PB at pH 6.8 | Wavelength | 255 nm |
| Flow rate | 1.0 ml/min | Integration time | 1.0 s |
| Detection wavelength | 255 nm | Slit width | 2.0 nm |
| Injection volume | 20 µl | Beam mode | Double beam |
| AUFS | 0.2 | Lamp change | When necessary |
| Date of analysis | | Date of analysis | |

| **Calibration data** | | | | |
|---|---|---|---|---|
| ***Concentration (µg/ml)*** | ***Peak area/Absorbance (1)*** | ***Peak area/Absorbance (2)*** | ***Average*** | ***Standard deviation*** |
| | | | #DIV/0! | #DIV/0! |
| | | | #DIV/0! | #DIV/0! |
| | | | #DIV/0! | #DIV/0! |
| | | | #DIV/0! | #DIV/0! |
| | | | #DIV/0! | #DIV/0! |
| | | | #DIV/0! | #DIV/0! |
| | | | #DIV/0! | #DIV/0! |
| | | | #DIV/0! | #DIV/0! |
| Calibration date | | | | |
| Equation of linear regression | | | | |
| Correlation co-efficient | | | | |

| | **Diffusion cell chamber** | | | | |
|---|---|---|---|---|---|
| | ***1*** | ***2*** | ***3*** | ***4*** | ***5*** |
| ***Surface area ($cm^2$)*** | | | | | |
| ***Weighing*** | | | | | |

| **Peak Area/Absorbance** | | | | | | | |
|---|---|---|---|---|---|---|---|
| | ***Cell 1*** | ***Cell 2*** | ***Cell 3*** | ***Cell 4*** | ***Cell 5*** | | |
| ***Time (hours)*** | | | | | | ***Average*** | ***Standard deviation*** |
| 0 | | | | | | #DIV/0! | #DIV/0! |
| 2 | | | | | | #DIV/0! | #DIV/0! |
| 4 | | | | | | #DIV/0! | #DIV/0! |
| 8 | | | | | | #DIV/0! | #DIV/0! |
| 24 | | | | | | #DIV/0! | #DIV/0! |
| 48 | | | | | | #DIV/0! | #DIV/0! |
| 72 | | | | | | #DIV/0! | #DIV/0! |

| Volume recovered (ml) | | | | | | | |
|---|---|---|---|---|---|---|---|
| *Time (hours)* | *Cell 1* | *Cell 2* | *Cell 3* | *Cell 4* | *Cell 5* | *Average* | *Standard deviation* |
| 0 | | | | | | #DIV/0! | #DIV/0! |
| 2 | | | | | | #DIV/0! | #DIV/0! |
| 4 | | | | | | #DIV/0! | #DIV/0! |
| 8 | | | | | | #DIV/0! | #DIV/0! |
| 24 | | | | | | #DIV/0! | #DIV/0! |
| 48 | | | | | | #DIV/0! | #DIV/0! |
| 72 | | | | | | #DIV/0! | #DIV/0! |

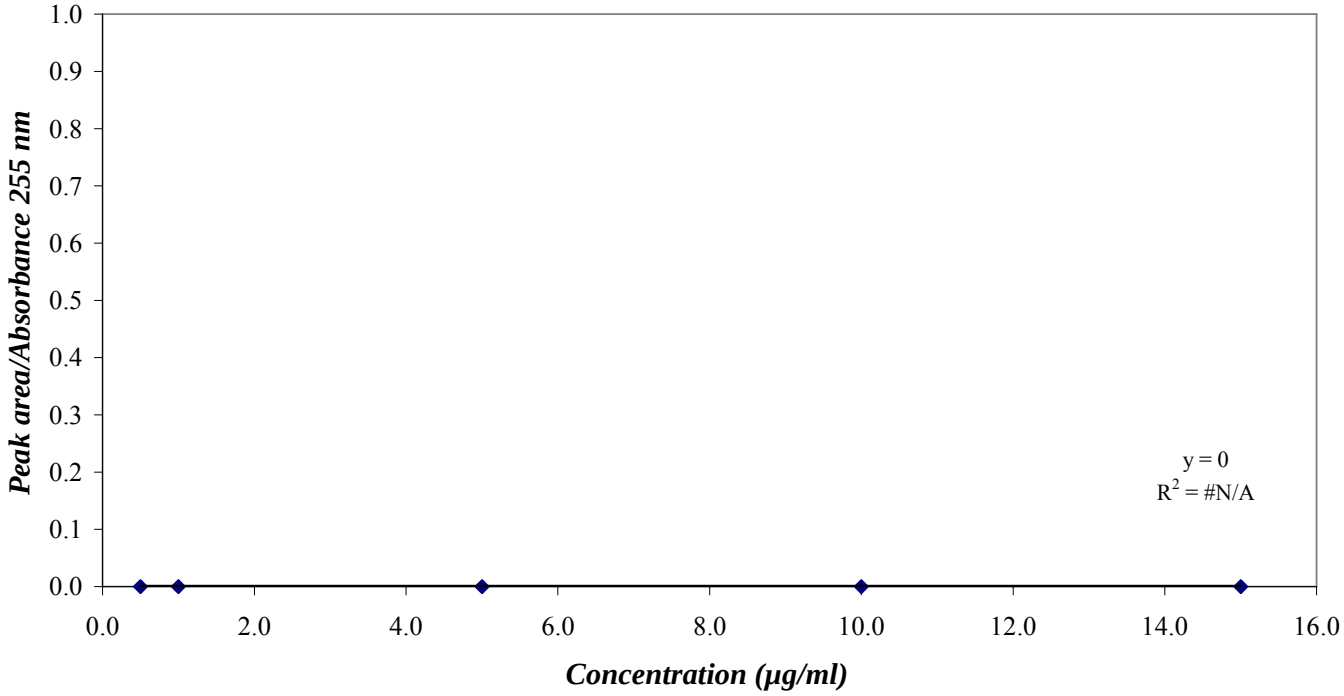
| Concentration (µg/ml) | | | | | | | |
|---|---|---|---|---|---|---|---|
| *Time (hours)* | *Cell 1* | *Cell 2* | *Cell 3* | *Cell 4* | *Cell 5* | *Average* | *Standard deviation* |
| 0 | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! |
| 2 | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! |
| 4 | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! |
| 8 | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! |
| 24 | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! |
| 48 | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! |
| 72 | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! |

| Amount liberated (µg) | | | | | | | |
|---|---|---|---|---|---|---|---|
| *Time (hours)* | *Cell 1* | *Cell 2* | *Cell 3* | *Cell 4* | *Cell 5* | *Average* | *Standard deviation* |
| 0 | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! |
| 2 | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! |
| 4 | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! |
| 8 | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! |
| 24 | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! |
| 48 | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! |
| 72 | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! |

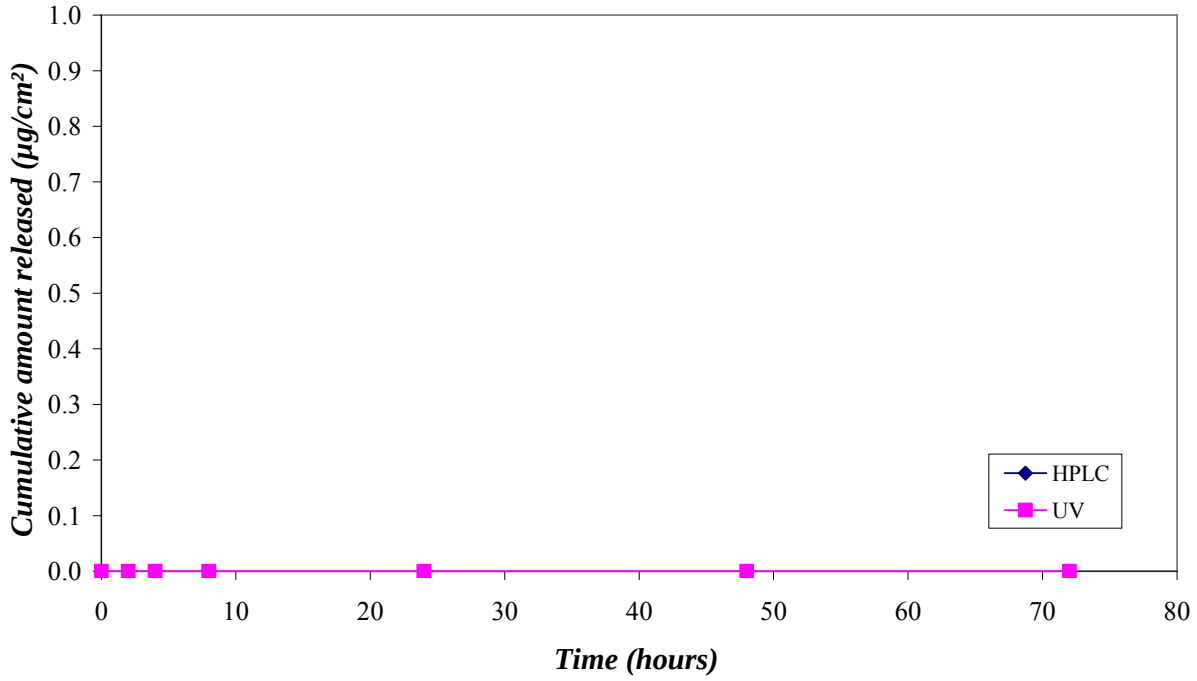
| Amount liberated (µg/cm$^2$) | | | | | | | |
|---|---|---|---|---|---|---|---|
| *Time (hours)* | *Cell 1* | *Cell 2* | *Cell 3* | *Cell 4* | *Cell 5* | *Average* | *Standard deviation* |
| 0 | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! |
| 2 | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! |
| 4 | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! |
| 8 | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! |
| 24 | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! |
| 48 | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! |
| 72 | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! |

| Cumulative amount liberated (µg/cm$^2$) | | | | | | | |
|---|---|---|---|---|---|---|---|
| *Time (hours)* | *Cell 1* | *Cell 2* | *Cell 3* | *Cell 4* | *Cell 5* | *Average* | *Standard deviation* |
| 0 | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! |
| 2 | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! |
| 4 | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! |
| 8 | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! |
| 24 | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! |
| 48 | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! |
| 72 | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! |

## *Calibration Analysis*




## *In vitro release of ketoprofen*




***An electronic version of this spreadsheet is included in the cd located at the back of the thesis***.

# APPENDIX II

# BATCH PRODUCTION RECORD

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**Grahamstown, South Africa**

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# BATCH PRODUCTION RECORD

| | | | | | |
|---|---|---|---|---|---|
| ***Formulator*** | Adcock Ingram Limited, Private Bag X69, Bryanston, 2021, South Africa | | | | |
| ***Product*** | Fastum® gel | ***Batch ID*** | 76 | ***Batch size*** | 50 g |
| ***Strength*** | 2.5% m/m ketoprofen | ***Date of issue*** | 05/10/2004 | ***Issued by*** | RNO Tettey-Amlalo |

| **Materials** | **Formulae** | | **Batch number** | **Signatures** | |
|---|---|---|---|---|---|
| | *Percent m/m* | *Actually added* | | *Dispensed by* | *Checked by* |
| Ketoprofen<br>Carbopol® 940<br>Triethanolamine<br>Lavender oil<br>Ethanol<br>Purified water | **PROPRIETARY FORMULATION** | | | | |

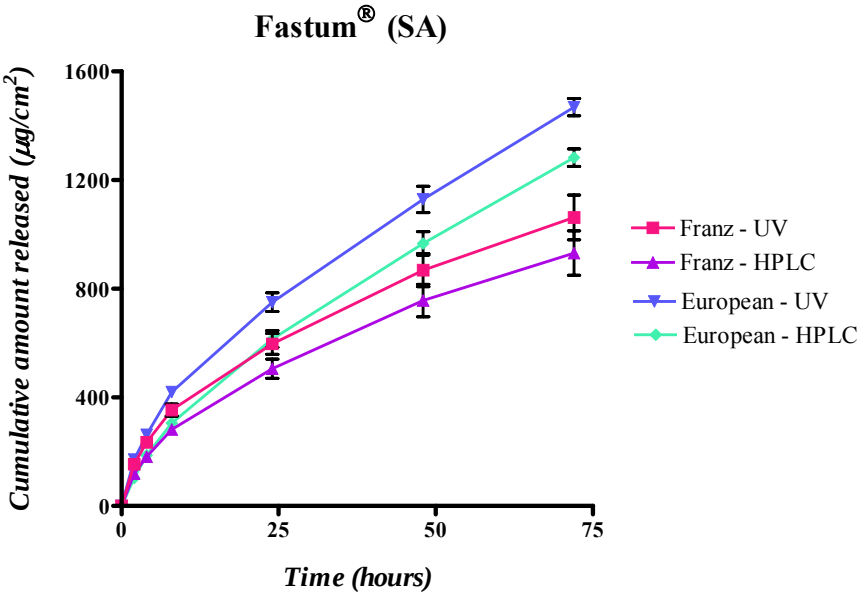
| | | | | | | | |
|---|---|---|---|---|---|---|---|
| ***Manufacturing date*** | 10/2002 | ***Homogenising time*** | | ***Primary packing*** | Tube | ***Storage*** | 25°C |
| ***Mixing time*** | | ***Ambient temperature*** | | ***Secondary packing*** | Box | ***Use by date*** | 09/2007 |

| **Production Equipment** | **Manufacturer** | **Model number** | **Dissolution Equipment** | **Manufacturer** | **Model number** |
|---|---|---|---|---|---|
| Analytical balance | Sartorius | 2403 | Franz diffusion cell | RU | 37058-8 |
| Top load balance | Sartorius | 1474 MP8-2 | European Pharmacopoeia diffusion cell | RU | |
| Magnetic stirrer | Labcon | MSH 10 | Franz heating element | Grant | TM |
| pH meter | Crison | GLP 21 | USP dissolution apparatus | Pharma Test | PTWS H-425/D |
| Ultra-turrax® | Janke & Kunkel | TP18-10 | Membrane | Silatos™ | REF 7458 |

**Certificate of Analysis**

| *Test* | *Result* | *Limits* | *Observation/Comment/Description* |
|---|---|---|---|
| pH | 5.44 | 5.0 - 6.0 | A transparent gel with no air bubbles was noted. The primary peak eluted at 4.00 mins with two other peaks eluting at 2.84 and 3.28 mins during the hplc analytical procedure. These peaks are suspected to be hydroxybenzoic acid esters which were present at 0.1% m/m in the formulation. |
| Drug content | | | |
| 1 HPLC analysis | 2.46% m/m | 2.31 - 2.69% m/m | |
| 2 UV analysis | 2.55% m/m | 2.31 - 2.69% m/m | |

*Dissolution Profile*

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# BATCH PRODUCTION RECORD

***Formulator*** Menarini France, 1/7, rue du Jura, Silic 528, 94633 Rungis Cedex
***Product*** Ketum® gel ***Batch ID*** 04 22 ***Batch size*** 60 g
***Strength*** 2.5% m/m ketoprofen ***Date of issue*** 15/10/2004 ***Issued by*** RNO Tettey-Amlalo

| **Materials** | **Formulae** | | **Batch number** | **Signatures** | |
|---|---|---|---|---|---|
| | *Percent m/m* | *Actually added* | | *Dispensed by* | *Checked by* |
| Ketoprofen | | | | | |
| Carbopol® 940 | | | | | |
| Diethanolamine | | | | | |
| Lavender oil | | | | | |
| Ethanol | | | | | |
| Purified water | | | | | |

**PROPRIETARY FORMULATION**

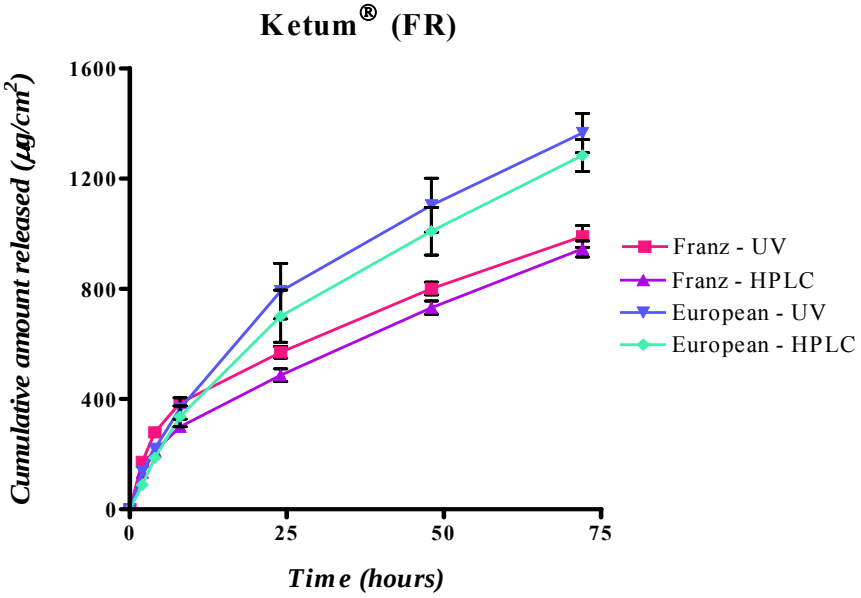
| | | | | | |
|---|---|---|---|---|---|
| ***Manufacturing date*** | | ***Homogenising time*** | | ***Primary packing*** Tube | ***Storage*** 25°C |
| ***Mixing time*** | | ***Ambient temperature*** | | ***Secondary packing*** Box | ***Use by date*** 12/2006 |

| **Production Equipment** | **Manufacturer** | **Model number** | **Dissolution Equipment** | **Manufacturer** | **Model number** |
|---|---|---|---|---|---|
| Analytical balance | Sartorius | 2403 | Franz diffusion cell | RU | 37058-8 |
| Top load balance | Sartorius | 1474 MP8-2 | European Pharmacopoeia diffusion cell | RU | |
| Magnetic stirrer | Labcon | MSH 10 | Franz heating element | Grant | TM |
| pH meter | Crison | GLP 21 | USP dissolution apparatus | Pharma Test | PTWS H-425/D |
| Ultra-turrax® | Janke & Kunkel | TP18-10 | Membrane | Silatos™ | REF 7458 |

**Certificate of Analysis**

| *Test* | *Result* | *Limits* | *Observation/Comment/Description* |
|---|---|---|---|
| pH | 5.51 | 5.0 - 6.0 | A transparent gel was noted and there were no air bubbles present. |
| Drug content | | | The primary peak eluted at 4.00 mins which is similar to the |
| 1 HPLC analysis | 2.45% m/m | 2.31 - 2.69% m/m | retention time of a standard sample of ketoprofen. There were no |
| 2 UV analysis | 2.59% m/m | 2.31 - 2.69% m/m | other peaks observed during the hplc analytical procedure. |

*Dissolution Profile*

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# BATCH PRODUCTION RECORD

***Formulator*** Rhone-Poulenc Rorer, 50 Kings Hill Avenue, Kings Hill, West Malling, Kent ME 19 4 AH, United Kingdom
***Product*** Oruvail® gel ***Batch ID*** 30104 ***Batch size*** 30 g
***Strength*** 2.5% m/m ketoprofen ***Date of issue*** 24/10/04 ***Issued by*** RNO Tettey-Amlalo

| **Materials** | **Formulae** | | **Batch number** | **Signatures** | |
|---|---|---|---|---|---|
| | *Percent m/m* | *Actually added* | | *Dispensed by* | *Checked by* |
| Ketoprofen<br>Carbopol® 940<br>Triethanolamine<br>Lavender oil<br>Ethanol<br>Purified water | **PROPRIETARY FORMULATION** | | | | |

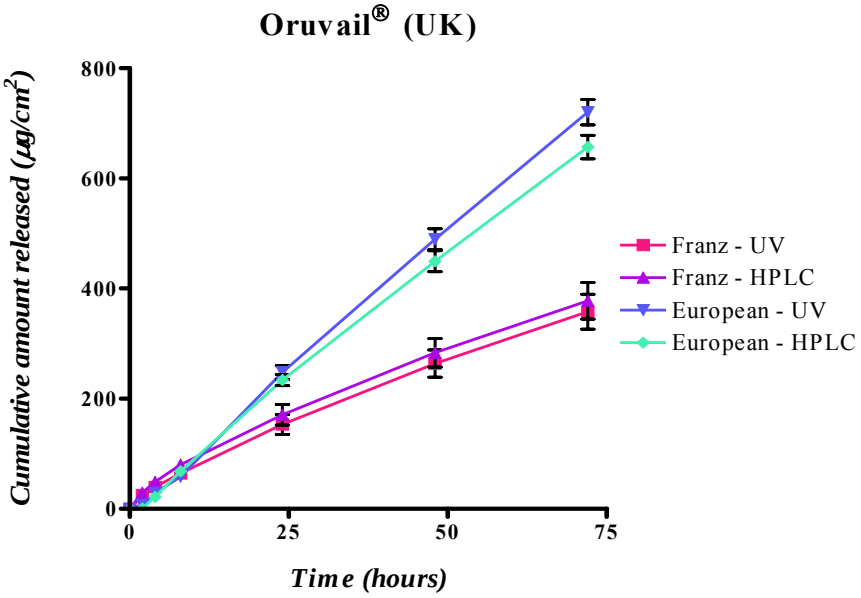
| | | | | | |
|---|---|---|---|---|---|
| ***Manufacturing date*** | 03/2000 | ***Homogenising time*** | | ***Primary packing*** | Tube |
| ***Mixing time*** | | ***Ambient temperature*** | | ***Secondary packing*** | Box |
| ***Storage*** | 25°C | | | | |
| ***Use by date*** | 01/2006 | | | | |

| **Production Equipment** | **Manufacturer** | **Model number** | **Dissolution Equipment** | **Manufacturer** | **Model number** |
|---|---|---|---|---|---|
| Analytical balance | Sartorius | 2403 | Franz diffusion cell | RU | 37058-8 |
| Top load balance | Sartorius | 1474 MP8-2 | European Pharmacopoeia diffusion cell | RU | |
| Magnetic stirrer | Labcon | MSH 10 | Franz heating element | Grant | TM |
| pH meter | Crison | GLP 21 | USP dissolution apparatus | Pharma Test | PTWS H-425/D |
| Ultra-turrax® | Janke & Kunkel | TP18-10 | Membrane | Silatos™ | REF 7458 |

**Certificate of Analysis**

| *Test* | *Result* | *Limits* | *Observation/Comment/Description* |
|---|---|---|---|
| pH | 5.56 | 5.0 - 6.0 | A clear transparent gel was noted although the gel seemed to be too viscous compared to Fastum® (SA) and Ketum® (FR). There were no air bubbles observed. The primary peak eluted at 4.00 and there were no other peaks observed during the hplc analytical procedure. |
| Drug content | | | |
| 1 HPLC analysis | 2.45% m/m | 2.31 - 2.69% m/m | |
| 2 UV analysis | 2.59% m/m | 2.31 - 2.69% m/m | |

*Dissolution Profile*

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# BATCH PRODUCTION RECORD

***Formulator*** Ralph Nii Okai Tettey-Amlalo
***Polymer*** Carbopol® Ultrez™ NF 10 ***Batch ID*** KET001 ***Batch size*** 50 g
***Strength*** 2.5% m/m ketoprofen ***Date of issue*** 17/02/2005 ***Issued by*** RNO Tettey-Amlalo

| **Materials** | **Formulae** | | **Batch number** | **Signatures** | |
|---|---|---|---|---|---|
| | *Percent m/m* | *Actually added* | | *Dispensed by* | *Checked by* |
| Ketoprofen | 2.50 | 1.25 g | 093K1522 | RNO | JMH |
| Carbopol® Ultrez™ NF 10 | 1.00 | 0.50 g | CC2NLZG148 | RNO | JMH |
| Triethanolamine | 1.50 | 0.75 g | 1024801 | RNO | JMH |
| Ethanol | 20.00 | 12.50 ml | L601707 | RNO | JMH |
| Propylene glycol | 20.00 | 10.00 g | 1026325 | RNO | JMH |
| Purified water | 55.00 | 27.50 ml | | RNO | JMH |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| ***Manufacturing date*** | 17/02/05 | ***Homogenising time*** | - | ***Primary packing*** | Jar | ***Storage*** | 25°C |
| ***Mixing time*** | 60 mins | ***Ambient temperature*** | 27°C | ***Secondary packing*** | - | ***Use by date*** | 19/02/05 |

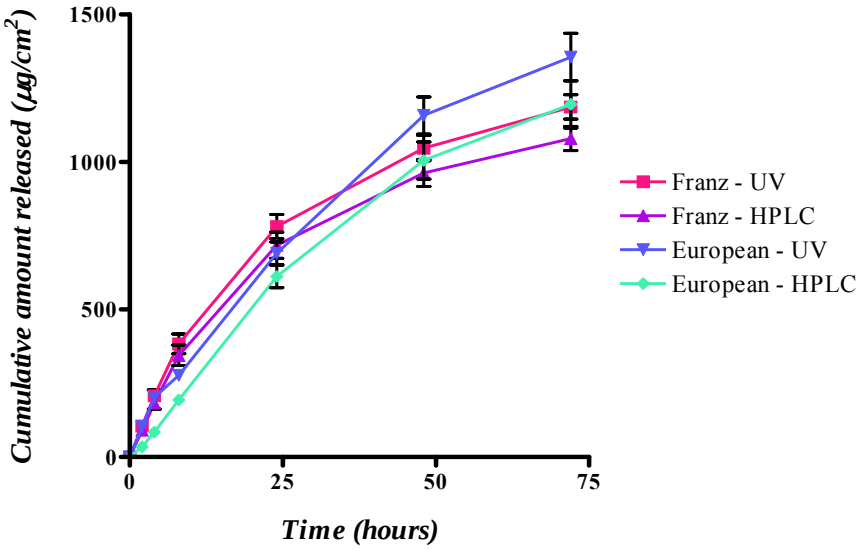
| **Production Equipment** | **Manufacturer** | **Model number** | **Dissolution Equipment** | **Manufacturer** | **Model number** |
|---|---|---|---|---|---|
| Analytical balance | Sartorius | 2403 | Franz diffusion cell | RU | 37058-8 |
| Top load balance | Sartorius | 1474 MP8-2 | European Pharmacopoeia diffusion cell | RU | |
| Magnetic stirrer | Labcon | MSH 10 | Franz heating element | Grant | TM |
| pH meter | Crison | GLP 21 | USP dissolution apparatus | Pharma Test | PTWS H-425/D |
| Ultra-turrax® | Janke & Kunkel | TP18-10 | Membrane | Silatos™ | REF 7458 |

**Certificate of Analysis**

| *Test* | *Result* | *Limits* | *Observation/Comment/Description* |
|---|---|---|---|
| pH | 5.46 | 5.0 - 6.0 | A slightly translucent gel was formed. Air bubbles were incorporated during the neutralisation stage. |
| Drug content | | | |
| 1 HPLC analysis | 2.49% m/m | 2.31 - 2.69% m/m | |
| 2 UV analysis | 2.61% m/m | 2.31 - 2.69% m/m | |

*Dissolution Profile*

**KET001**

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# BATCH PRODUCTION RECORD

***Formulator*** Ralph Nii Okai Tettey-Amlalo
***Polymer*** Carbopol® Ultrez™ 10 NF ***Batch ID*** KET002 ***Batch size*** 50 g
***Strength*** 2.5% m/m ketoprofen ***Date of issue*** 21/02/2005 ***Issued by*** RNO Tettey-Amlalo

| **Materials** | **Formulae** | | **Batch number** | **Signatures** | |
|---|---|---|---|---|---|
| | *Percent m/m* | *Actually added* | | *Dispensed by* | *Checked by* |
| Ketoprofen | 2.50 | 1.25 g | 093K1522 | RNO | JMH |
| Carbopol® Ultrez™ 10 NF | 1.50 | 0.75 g | CC2NLZG148 | RNO | JMH |
| Triethanolamine | 2.25 | 1.13 g | 1024801 | RNO | JMH |
| Ethanol | 20.00 | 12.50 ml | L601707 | RNO | JMH |
| Propylene glycol | 20.00 | 10.00 g | 1026325 | RNO | JMH |
| Purified water | 53.75 | 26.88 ml | | RNO | JMH |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| ***Manufacturing date*** | 21/02/05 | ***Homogenising time*** | - | ***Primary packing*** | Jar | ***Storage*** | 24°C |
| ***Mixing time*** | 60 mins | ***Ambient temperature*** | 27°C | ***Secondary packing*** | - | ***Use by date*** | 23/02/05 |

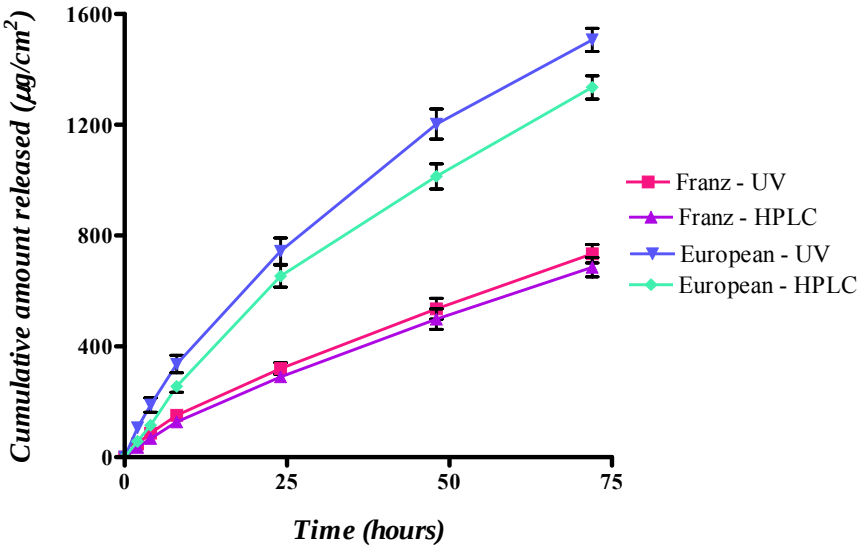
| **Production Equipment** | **Manufacturer** | **Model number** | **Dissolution Equipment** | **Manufacturer** | **Model number** |
|---|---|---|---|---|---|
| Analytical balance | Sartorius | 2403 | Franz diffusion cell | RU | 37058-8 |
| Top load balance | Sartorius | 1474 MP8-2 | European Pharmacopoeia diffusion cell | RU | |
| Magnetic stirrer | Labcon | MSH 10 | Franz heating element | Grant | TM |
| pH meter | Crison | GLP 21 | USP dissolution apparatus | Pharma Test | PTWS H-425/D |
| Ultra-turrax® | Janke & Kunkel | TP18-10 | Membrane | Silatos™ | REF 7458 |

**Certificate of Analysis**

| *Test* | *Result* | *Limits* | *Observation/Comment/Description* |
|---|---|---|---|
| pH | 5.85 | 5.0 - 6.0 | An elegant gel was formed. The gel was slightly viscous and not as runny as KET001. |
| Drug content | | | |
| 1 HPLC analysis | 2.51% m/m | 2.31 - 2.69% m/m | |
| 2 UV analysis | 2.63% m/m | 2.31 - 2.69% m/m | |

*Dissolution Profile*

**KET002**

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# BATCH PRODUCTION RECORD

***Formulator*** Ralph Nii Okai Tettey-Amlalo
***Polymer*** Carbopol® Ultrez™ 10 NF ***Batch ID*** KET003 ***Batch size*** 50 g
***Strength*** 2.5% m/m ketoprofen ***Date of issue*** 28/02/2005 ***Issued by*** RNO Tettey-Amlalo

| **Materials** | **Formulae** | | **Batch number** | **Signatures** | |
|---|---|---|---|---|---|
| | *Percent m/m* | *Actually added* | | *Dispensed by* | *Checked by* |
| Ketoprofen | 2.50 | 1.25 g | 093K1522 | RNO | JMH |
| Carbopol® Ultrez™ 10 NF | 2.00 | 1.00 g | CC2NLZG148 | RNO | JMH |
| Triethanolamine | 3.00 | 1.50 g | 1024801 | RNO | JMH |
| Ethanol | 20.00 | 12.50 ml | L601707 | RNO | JMH |
| Propylene glycol | 20.00 | 10.00 g | 1026325 | RNO | JMH |
| Purified water | 52.50 | 26.25 ml | | RNO | JMH |

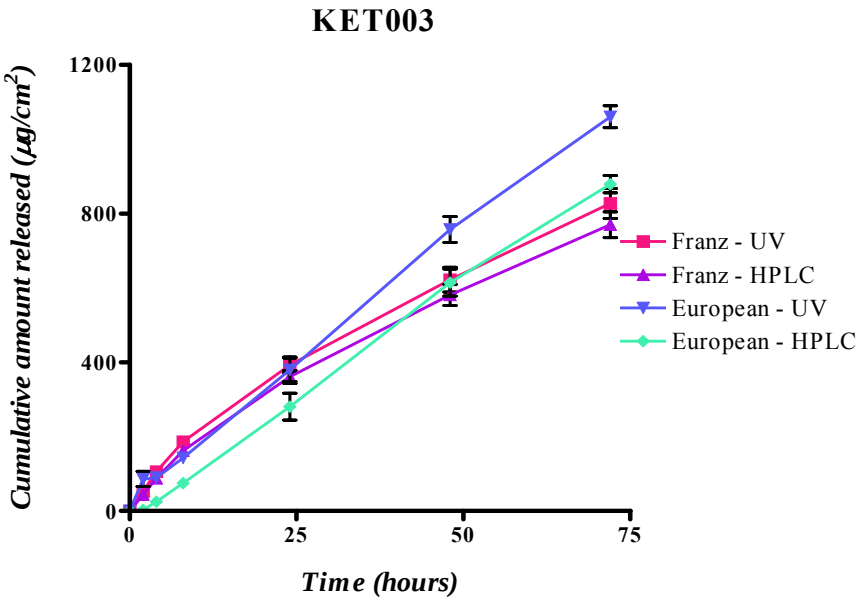
| | | | | | | | |
|---|---|---|---|---|---|---|---|
| ***Manufacturing date*** | 28/02/05 | ***Homogenising time*** | - | ***Primary packing*** | Jar | ***Storage*** | 25°C |
| ***Mixing time*** | 60 mins | ***Ambient temperature*** | 27°C | ***Secondary packing*** | - | ***Use by date*** | 02/03/05 |

| **Production Equipment** | **Manufacturer** | **Model number** | **Dissolution Equipment** | **Manufacturer** | **Model number** |
|---|---|---|---|---|---|
| Analytical balance | Sartorius | 2403 | Franz diffusion cell | RU | 37058-8 |
| Top load balance | Sartorius | 1474 MP8-2 | European Pharmacopoeia diffusion cell | RU | |
| Magnetic stirrer | Labcon | MSH 10 | Franz heating element | Grant | TM |
| pH meter | Crison | GLP 21 | USP dissolution apparatus | Pharma Test | PTWS H-425/D |
| Ultra-turrax® | Janke & Kunkel | TP18-10 | Membrane | Silatos™ | REF 7458 |

**Certificate of Analysis**

| *Test* | *Result* | *Limits* | *Observation/Comment/Description* |
|---|---|---|---|
| pH | 5.91 | 5.0 - 6.0 | An elegant gel was formed. The gel was slightly viscous and not as runny as KET001 but similar to KET002. |
| Drug content | | | |
| 1 HPLC analysis | 2.51% m/m | 2.31 - 2.69% m/m | |
| 2 UV analysis | 2.62% m/m | 2.31 - 2.69% m/m | |

*Dissolution Profile*

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# BATCH PRODUCTION RECORD

***Formulator*** Ralph Nii Okai Tettey-Amlalo
***Polymer*** Carbopol® 974P NF ***Batch ID*** KET004 ***Batch size*** 50 g
***Strength*** 2.5% m/m ketoprofen ***Date of issue*** 08/03/2005 ***Issued by*** RNO Tettey-Amlalo

| **Materials** | **Formulae** | | **Batch number** | **Signatures** | |
|---|---|---|---|---|---|
| | *Percent m/m* | *Actually added* | | *Dispensed by* | *Checked by* |
| Ketoprofen | 2.50 | 1.25 g | 093K1522 | RNO | JMH |
| Carbopol® 974 PNF | 1.50 | 0.75 g | CC47LAB329 | RNO | JMH |
| Triethanolamine | 2.25 | 1.13 g | 1024801 | RNO | JMH |
| Ethanol | 20.00 | 12.50 ml | L601707 | RNO | JMH |
| Propylene glycol | 20.00 | 10.00 g | 1026325 | RNO | JMH |
| Purified water | 53.75 | 26.88 ml | | RNO | JMH |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| ***Manufacturing date*** | 08/03/05 | ***Homogenising time*** | - | ***Primary packing*** | Jar | ***Storage*** | 25°C |
| ***Mixing time*** | 60 mins | ***Ambient temperature*** | 29°C | ***Secondary packing*** | - | ***Use by date*** | 10/03/05 |

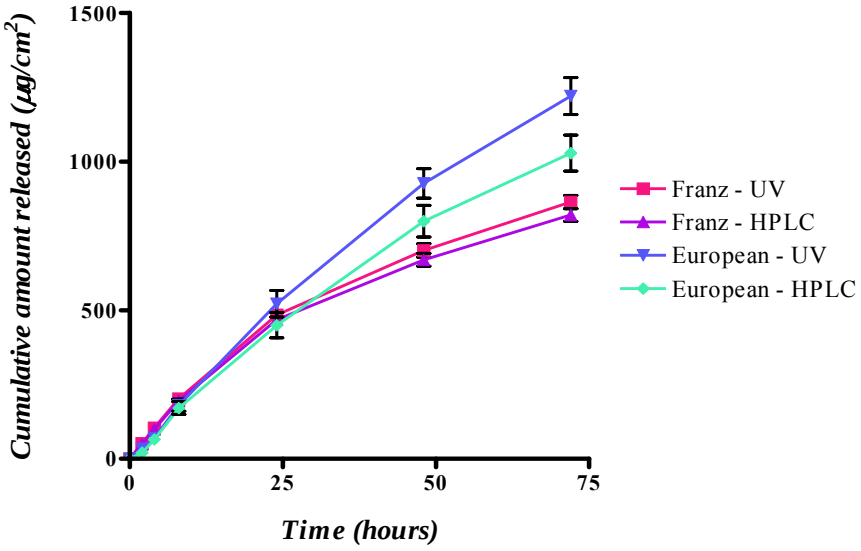
| **Production Equipment** | **Manufacturer** | **Model number** | **Dissolution Equipment** | **Manufacturer** | **Model number** |
|---|---|---|---|---|---|
| Analytical balance | Sartorius | 2403 | Franz diffusion cell | RU | 37058-8 |
| Top load balance | Sartorius | 1474 MP8-2 | European Pharmacopoeia diffusion cell | RU | |
| Magnetic stirrer | Labcon | MSH 10 | Franz heating element | Grant | TM |
| pH meter | Crison | GLP 21 | USP dissolution apparatus | Pharma Test | PTWS H-425/D |
| Ultra-turrax® | Janke & Kunkel | TP18-10 | Membrane | Silatos™ | REF 7458 |

**Certificate of Analysis**

| *Test* | *Result* | *Limits* | *Observation/Comment/Description* |
|---|---|---|---|
| pH | 5.91 | 5.0 - 6.0 | Gel formed, although slightly translucent. It was as elegant as KET003. |
| Drug content | | | |
| 1 HPLC analysis | 2.51% m/m | 2.31 - 2.69% m/m | |
| 2 UV analysis | 2.56% m/m | 2.31 - 2.69% m/m | |

*Dissolution Profile*

**KET004**

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# BATCH PRODUCTION RECORD

***Formulator*** Ralph Nii Okai Tettey-Amlalo
***Polymer*** Carbopol® 980 NF ***Batch ID*** KET005 ***Batch size*** 50 g
***Strength*** 2.5% m/m ketoprofen ***Date of issue*** 17/03/2005 ***Issued by*** RNO Tettey-Amlalo

| **Materials** | **Formulae** | | **Batch number** | **Signatures** | |
|---|---|---|---|---|---|
| | *Percent m/m* | *Actually added* | | *Dispensed by* | *Checked by* |
| Ketoprofen | 2.50 | 1.25 g | 093K1522 | RNO | JMH |
| Carbopol® 980 NF | 1.50 | 0.75 g | CC216CC696 | RNO | JMH |
| Triethanolamine | 2.25 | 1.13 g | 1024801 | RNO | JMH |
| Ethanol | 20.00 | 12.50 ml | L601707 | RNO | JMH |
| Propylene glycol | 20.00 | 10.00 g | 1026325 | RNO | JMH |
| Purified water | 53.75 | 26.88 ml | | RNO | JMH |

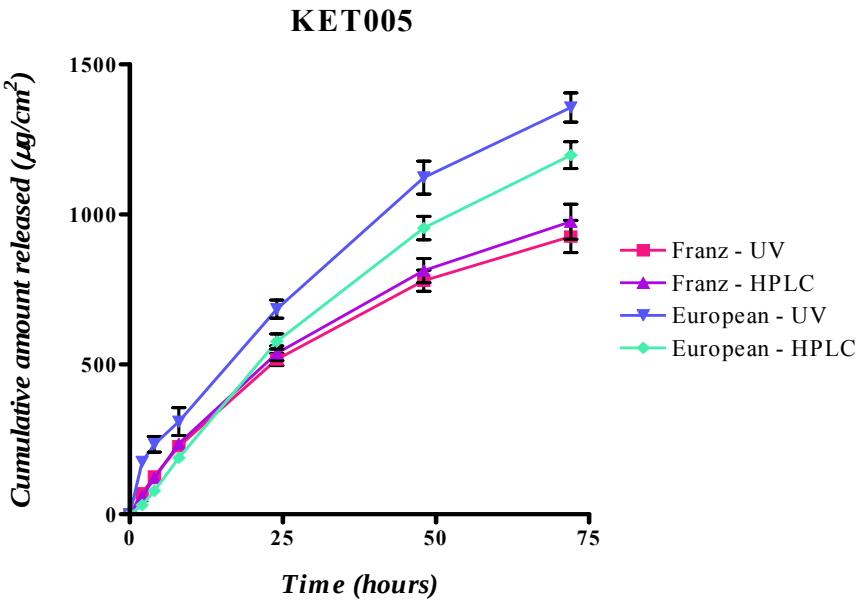
| | | | | | | | |
|---|---|---|---|---|---|---|---|
| ***Manufacturing date*** | 17/03/05 | ***Homogenising time*** | - | ***Primary packing*** | Jar | ***Storage*** | 26°C |
| ***Mixing time*** | 60 mins | ***Ambient temperature*** | 28°C | ***Secondary packing*** | - | ***Use by date*** | 19/03/05 |

| **Production Equipment** | **Manufacturer** | **Model number** | **Dissolution Equipment** | **Manufacturer** | **Model number** |
|---|---|---|---|---|---|
| Analytical balance | Sartorius | 2403 | Franz diffusion cell | RU | 37058-8 |
| Top load balance | Sartorius | 1474 MP8-2 | European Pharmacopoeia diffusion cell | RU | |
| Magnetic stirrer | Labcon | MSH 10 | Franz heating element | Grant | TM |
| pH meter | Crison | GLP 21 | USP dissolution apparatus | Pharma Test | PTWS H-425/D |
| Ultra-turrax® | Janke & Kunkel | TP18-10 | Membrane | Silatos™ | REF 7458 |

**Certificate of Analysis**

| *Test* | *Result* | *Limits* | *Observation/Comment/Description* |
|---|---|---|---|
| pH | 5.73 | 5.0 - 6.0 | Gel formed similar to KET004. Air bubbles were present. |
| Drug content | | | |
| 1 HPLC analysis | 2.52% m/m | 2.31 - 2.69% m/m | |
| 2 UV analysis | 2.58% m/m | 2.31 - 2.69% m/m | |

*Dissolution Profile*

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# BATCH PRODUCTION RECORD

***Formulator*** Ralph Nii Okai Tettey-Amlalo
***Polymer*** Carbopol® 981P NF ***Batch ID*** KET006 ***Batch size*** 50 g
***Strength*** 2.5% m/m ketoprofen ***Date of issue*** 14/04/2005 ***Issued by*** RNO Tettey-Amlalo

| **Materials** | **Formulae** | | **Batch number** | **Signatures** | |
|---|---|---|---|---|---|
| | *Percent m/m* | *Actually added* | | *Dispensed by* | *Checked by* |
| Ketoprofen | 2.50 | 1.25 g | 093K1522 | RNO | JMH |
| Carbopol® 981 PNF | 1.50 | 0.75 g | CC485CD381 | RNO | JMH |
| Triethanolamine | 2.25 | 1.13 g | 1024801 | RNO | JMH |
| Ethanol | 20.00 | 12.50 ml | L601707 | RNO | JMH |
| Propylene glycol | 20.00 | 10.00 g | 1026325 | RNO | JMH |
| Purified water | 53.75 | 26.88 ml | | RNO | JMH |

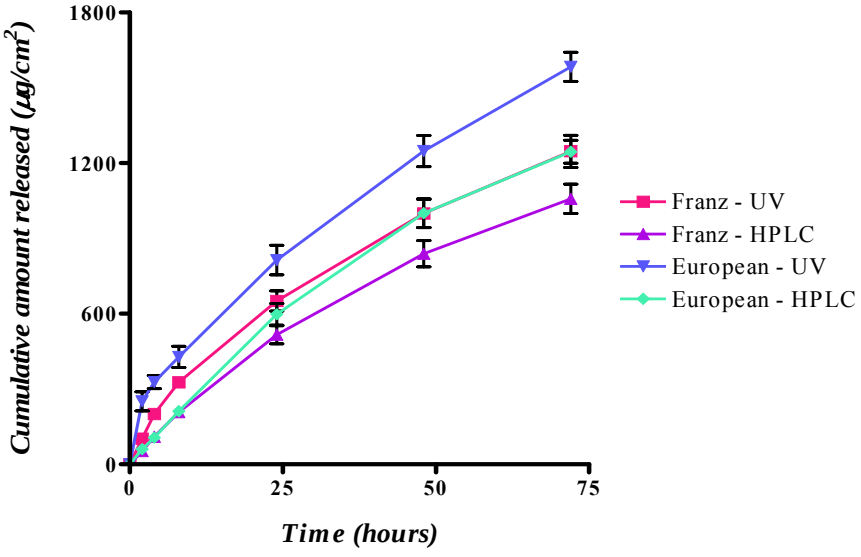
| | | | | | | | |
|---|---|---|---|---|---|---|---|
| ***Manufacturing date*** | 14/04/05 | ***Homogenising time*** | - | ***Primary packing*** | Jar | ***Storage*** | 20°C |
| ***Mixing time*** | 60 mins | ***Ambient temperature*** | 22°C | ***Secondary packing*** | - | ***Use by date*** | 16/04/05 |

| **Production Equipment** | **Manufacturer** | **Model number** | **Dissolution Equipment** | **Manufacturer** | **Model number** |
|---|---|---|---|---|---|
| Analytical balance | Sartorius | 2403 | Franz diffusion cell | RU | 37058-8 |
| Top load balance | Sartorius | 1474 MP8-2 | European Pharmacopoeia diffusion cell | RU | |
| Magnetic stirrer | Labcon | MSH 10 | Franz heating element | Grant | TM |
| pH meter | Crison | GLP 21 | USP dissolution apparatus | Pharma Test | PTWS H-425/D |
| Ultra-turrax® | Janke & Kunkel | TP18-10 | Membrane | Silatos™ | REF 7458 |

**Certificate of Analysis**

| *Test* | *Result* | *Limits* | *Observation/Comment/Description* |
|---|---|---|---|
| pH | 5.81 | 5.0 - 6.0 | Transparent elegant gel formed. Air bubbles incorporated during neutralization. |
| Drug content | | | |
| 1 HPLC analysis | 2.53% m/m | 2.31 - 2.69% m/m | |
| 2 UV analysis | 2.61% m/m | 2.31 - 2.69% m/m | |
| | | *Dissolution Profile* | |

**KET006**

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# BATCH PRODUCTION RECORD

***Formulator*** Ralph Nii Okai Tettey-Amlalo
***Polymer*** Pemulen® TR1 NF ***Batch ID*** KET007 ***Batch size*** 50 g
***Strength*** 2.5% m/m ketoprofen ***Date of issue*** 20/04/2005 ***Issued by*** RNO Tettey-Amlalo

| **Materials** | **Formulae** | | **Batch number** | **Signatures** | |
|---|---|---|---|---|---|
| | *Percent m/m* | *Actually added* | | *Dispensed by* | *Checked by* |
| Ketoprofen | 2.50 | 1.25 g | 093K1522 | RNO | JMH |
| Pemulen® TR1 NF | 1.50 | 0.75 g | CC3DACT860 | RNO | JMH |
| Triethanolamine | 2.25 | 1.13 g | 1024801 | RNO | JMH |
| Ethanol | 20.00 | 12.50 ml | L601707 | RNO | JMH |
| Propylene glycol | 20.00 | 10.00 g | 1026325 | RNO | JMH |
| Purified water | 53.75 | 26.88 ml | | RNO | JMH |

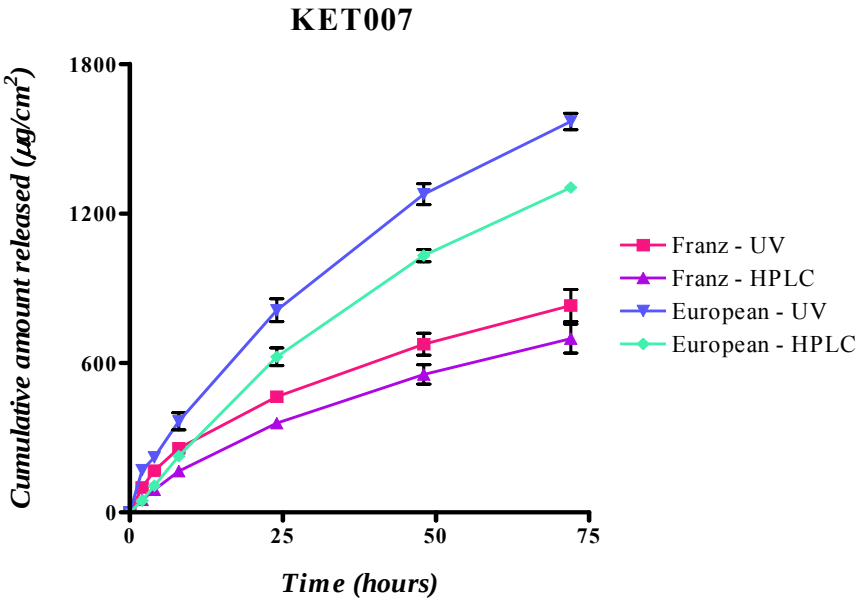
| | | | | | | | |
|---|---|---|---|---|---|---|---|
| ***Manufacturing date*** | 20/04/05 | ***Homogenising time*** | - | ***Primary packing*** | Jar | ***Storage*** | 18°C |
| ***Mixing time*** | 60 mins | ***Ambient temperature*** | 22°C | ***Secondary packing*** | - | ***Use by date*** | 22/04/05 |

| **Production Equipment** | **Manufacturer** | **Model number** | **Dissolution Equipment** | **Manufacturer** | **Model number** |
|---|---|---|---|---|---|
| Analytical balance | Sartorius | 2403 | Franz diffusion cell | RU | 37058-8 |
| Top load balance | Sartorius | 1474 MP8-2 | European Pharmacopoeia diffusion cell | RU | |
| Magnetic stirrer | Labcon | MSH 10 | Franz heating element | Grant | TM |
| pH meter | Crison | GLP 21 | USP dissolution apparatus | Pharma Test | PTWS H-425/D |
| Ultra-turrax® | Janke & Kunkel | TP18-10 | Membrane | Silatos™ | REF 7458 |

**Certificate of Analysis**

| *Test* | *Result* | *Limits* | *Observation/Comment/Description* |
|---|---|---|---|
| pH | 5.81 | 5.0 - 6.0 | A very thick gel was formed. The mixture prior to neutralization was not homogenised as the mixture did not resemble an emulsion. |
| Drug content | | | |
| 1 HPLC analysis | 2.54% m/m | 2.31 - 2.69% m/m | |
| 2 UV analysis | 2.61% m/m | 2.31 - 2.69% m/m | |

*Dissolution Profile*

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# BATCH PRODUCTION RECORD

***Formulator*** Ralph Nii Okai Tettey-Amlalo
***Polymer*** Carbopol® 980 NF, Pemulen® TR1 NF ***Batch ID*** KET008 ***Batch size*** 50 g
***Strength*** 2.5% m/m ketoprofen ***Date of issue*** 26/04/2005 ***Issued by*** RNO Tettey-Amlalo

| **Materials** | **Formulae** | | **Batch number** | **Signatures** | |
|---|---|---|---|---|---|
| | *Percent m/m* | *Actually added* | | *Dispensed by* | *Checked by* |
| Ketoprofen | 2.50 | 1.25 g | 093K1522 | RNO | JMH |
| Carbopol® 980 NF | 1.00 | 0.50 g | CC216CC696 | RNO | JMH |
| Pemulen® TR1 NF | 0.10 | 0.05 g | CC3DACT860 | RNO | JMH |
| Triethanolamine | 1.65 | 0.83 g | 1024801 | RNO | JMH |
| Ethanol | 20.00 | 12.50 ml | L601707 | RNO | JMH |
| Propylene glycol | 20.00 | 10.00 g | 1026325 | RNO | JMH |
| Purified water | 54.75 | 27.38 ml | | RNO | JMH |

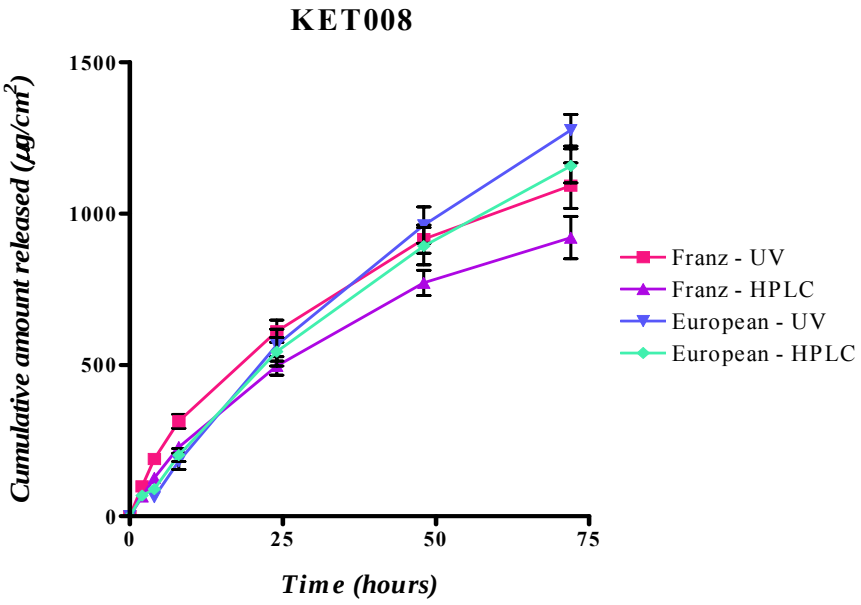
| | | | | | | | |
|---|---|---|---|---|---|---|---|
| ***Manufacturing date*** | 26/04/05 | ***Homogenising time*** | 5 mins | ***Primary packing*** | Jar | ***Storage*** | 25°C |
| ***Mixing time*** | 60 mins | ***Ambient temperature*** | 27°C | ***Secondary packing*** | - | ***Use by date*** | 28/04/05 |

| **Production Equipment** | **Manufacturer** | **Model number** | **Dissolution Equipment** | **Manufacturer** | **Model number** |
|---|---|---|---|---|---|
| Analytical balance | Sartorius | 2403 | Franz diffusion cell | RU | 37058-8 |
| Top load balance | Sartorius | 1474 MP8-2 | European Pharmacopoeia diffusion cell | RU | |
| Magnetic stirrer | Labcon | MSH 10 | Franz heating element | Grant | TM |
| pH meter | Crison | GLP 21 | USP dissolution apparatus | Pharma Test | PTWS H-425/D |
| Ultra-turrax® | Janke & Kunkel | TP18-10 | Membrane | Silatos™ | REF 7458 |

**Certificate of Analysis**

| *Test* | *Result* | *Limits* | *Observation/Comment/Description* |
|---|---|---|---|
| pH | 5.62 | 5.0 - 6.0 | On addition of Pemulen® the mixture turned milky. Surprisingly after neutralisation, the mixture assumed a transparent appearance. |
| Drug content | | | |
| 1 HPLC analysis | 2.52% m/m | 2.31 - 2.69% m/m | |
| 2 UV analysis | 2.61% m/m | 2.31 - 2.69% m/m | |

*Dissolution Profile*

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# BATCH PRODUCTION RECORD

***Formulator*** Ralph Nii Okai Tettey-Amlalo
***Polymer*** Carbopol® 980 NF, Pemulen® TR1 NF ***Batch ID*** KET009 ***Batch size*** 50 g
***Strength*** 2.5% m/m ketoprofen ***Date of issue*** 05/05/2005 ***Issued by*** RNO Tettey-Amlalo

| **Materials** | **Formulae** | | **Batch number** | **Signatures** | |
|---|---|---|---|---|---|
| | *Percent m/m* | *Actually added* | | *Dispensed by* | *Checked by* |
| Ketoprofen | 2.50 | 1.25 g | 093K1522 | RNO | JMH |
| Carbopol® 980 NF | 1.00 | 0.50 g | CC216CC696 | RNO | JMH |
| Pemulen® TR1 NF | 0.30 | 0.15 g | CC3DACT860 | RNO | JMH |
| Triethanolamine | 1.95 | 0.98 g | 1024801 | RNO | JMH |
| Ethanol | 20.00 | 12.50 ml | L601707 | RNO | JMH |
| Propylene glycol | 20.00 | 10.00 g | 1026325 | RNO | JMH |
| Purified water | 54.25 | 27.13 ml | | RNO | JMH |

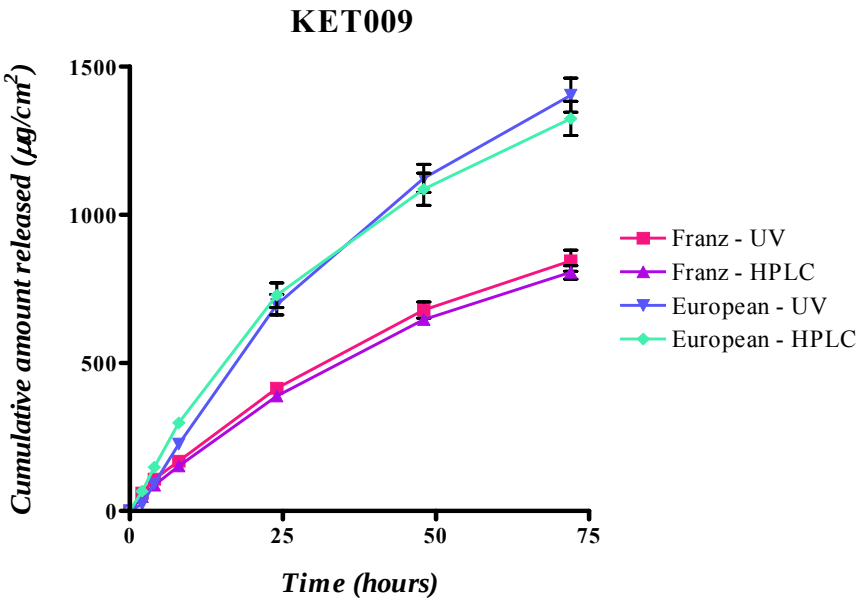
| | | | | | | | |
|---|---|---|---|---|---|---|---|
| ***Manufacturing date*** | 05/05/05 | ***Homogenising time*** | 5 mins | ***Primary packing*** | Jar | ***Storage*** | 23°C |
| ***Mixing time*** | 60 mins | ***Ambient temperature*** | 26°C | ***Secondary packing*** | - | ***Use by date*** | 07/05/05 |

| **Production Equipment** | **Manufacturer** | **Model number** | **Dissolution Equipment** | **Manufacturer** | **Model number** |
|---|---|---|---|---|---|
| Analytical balance | Sartorius | 2403 | Franz diffusion cell | RU | 37058-8 |
| Top load balance | Sartorius | 1474 MP8-2 | European Pharmacopoeia diffusion cell | RU | |
| Magnetic stirrer | Labcon | MSH 10 | Franz heating element | Grant | TM |
| pH meter | Crison | GLP 21 | USP dissolution apparatus | Pharma Test | PTWS H-425/D |
| Ultra-turrax® | Janke & Kunkel | TP18-10 | Membrane | Silatos™ | REF 7458 |

**Certificate of Analysis**

| *Test* | *Result* | *Limits* | *Observation/Comment/Description* |
|---|---|---|---|
| pH | 5.64 | 5.0 - 6.0 | Emulsion like mixture prior to neutralization. |
| Drug content | | | |
| 1 HPLC analysis | 2.51% m/m | 2.31 - 2.69% m/m | |
| 2 UV analysis | 2.58% m/m | 2.31 - 2.69% m/m | |

*Dissolution Profile*

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# BATCH PRODUCTION RECORD

***Formulator*** Ralph Nii Okai Tettey-Amlalo
***Polymer*** Carbopol® 980 NF, Pemulen® TR1 NF ***Batch ID*** KET010 ***Batch size*** 50 g
***Strength*** 2.5% m/m ketoprofen ***Date of issue*** 04/06/2005 ***Issued by*** RNO Tettey-Amlalo

| **Materials** | **Formulae** | | **Batch number** | **Signatures** | |
|---|---|---|---|---|---|
| | *Percent m/m* | *Actually added* | | *Dispensed by* | *Checked by* |
| Ketoprofen | 2.50 | 1.25 g | 093K1522 | RNO | JMH |
| Carbopol® 980 NF | 1.00 | 0.50 g | CC216CC696 | RNO | JMH |
| Pemulen® TR1 NF | 0.50 | 0.25 g | CC3DACT860 | RNO | JMH |
| Triethanolamine | 2.25 | 1.13 g | 1024801 | RNO | JMH |
| Ethanol | 20.00 | 12.50 ml | L601707 | RNO | JMH |
| Propylene glycol | 20.00 | 10.00 g | 1026325 | RNO | JMH |
| Purified water | 53.75 | 26.88 ml | | RNO | JMH |

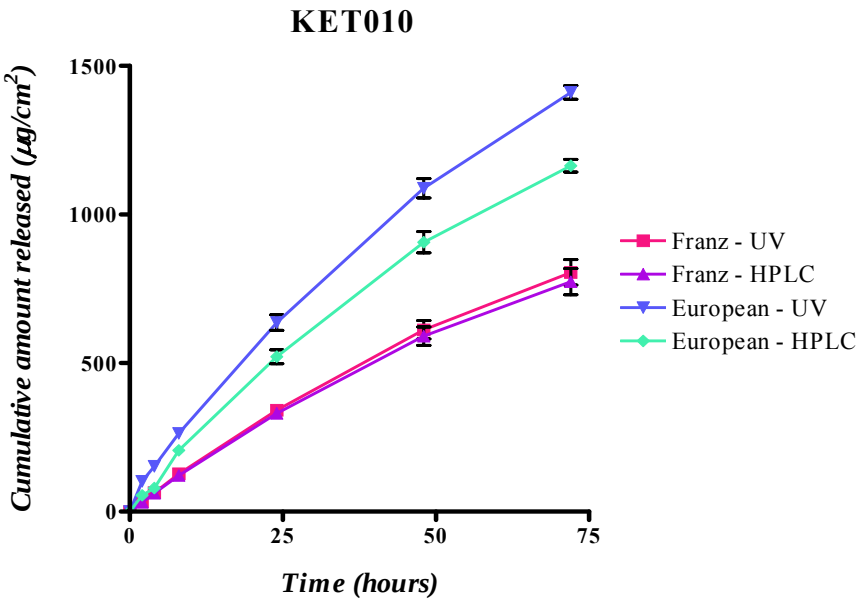
| | | | | | | | |
|---|---|---|---|---|---|---|---|
| ***Manufacturing date*** | 04/06/05 | ***Homogenising time*** | 5 mins | ***Primary packing*** | Jar | ***Storage*** | 18°C |
| ***Mixing time*** | 60 mins | ***Ambient temperature*** | 20°C | ***Secondary packing*** | - | ***Use by date*** | 06/06/05 |

| **Production Equipment** | **Manufacturer** | **Model number** | **Dissolution Equipment** | **Manufacturer** | **Model number** |
|---|---|---|---|---|---|
| Analytical balance | Sartorius | 2403 | Franz diffusion cell | RU | 37058-8 |
| Top load balance | Sartorius | 1474 MP8-2 | European Pharmacopoeia diffusion cell | RU | |
| Magnetic stirrer | Labcon | MSH 10 | Franz heating element | Grant | TM |
| pH meter | Crison | GLP 21 | USP dissolution apparatus | Pharma Test | PTWS H-425/D |
| Ultra-turrax® | Janke & Kunkel | TP18-10 | Membrane | Silatos™ | REF 7458 |

**Certificate of Analysis**

| *Test* | *Result* | *Limits* | *Observation/Comment/Description* |
|---|---|---|---|
| pH | 5.71 | 5.0 - 6.0 | Emulsion like mixture prior to neutralisation. |
| Drug content | | | |
| 1 HPLC analysis | 2.51% m/m | 2.31 - 2.69% m/m | |
| 2 UV analysis | 2.59% m/m | 2.31 - 2.69% m/m | |

*Dissolution Profile*

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# BATCH PRODUCTION RECORD

***Formulator*** Ralph Nii Okai Tettey-Amlalo
***Polymer*** Carbopol® 980 NF, Pemulen® TR1 NF ***Batch ID*** KET011 ***Batch size*** 50 g
***Strength*** 2.5% m/m ketoprofen ***Date of issue*** 09/06/2005 ***Issued by*** RNO Tettey-Amlalo

| **Materials** | **Formulae** | | **Batch number** | **Signatures** | |
|---|---|---|---|---|---|
| | *Percent m/m* | *Actually added* | | *Dispensed by* | *Checked by* |
| Ketoprofen | 2.50 | 1.25 g | 093K1522 | RNO | JMH |
| Carbopol® 980 NF | 1.00 | 0.50 g | CC216CC696 | RNO | JMH |
| Pemulen® TR1 NF | 1.00 | 0.50 g | CC3DACT860 | RNO | JMH |
| Triethanolamine | 3.00 | 1.50 g | 1024801 | RNO | JMH |
| Ethanol | 20.00 | 12.50 ml | L601707 | RNO | JMH |
| Propylene glycol | 20.00 | 10.00 g | 1026325 | RNO | JMH |
| Purified water | 52.50 | 26.25 ml | | RNO | JMH |

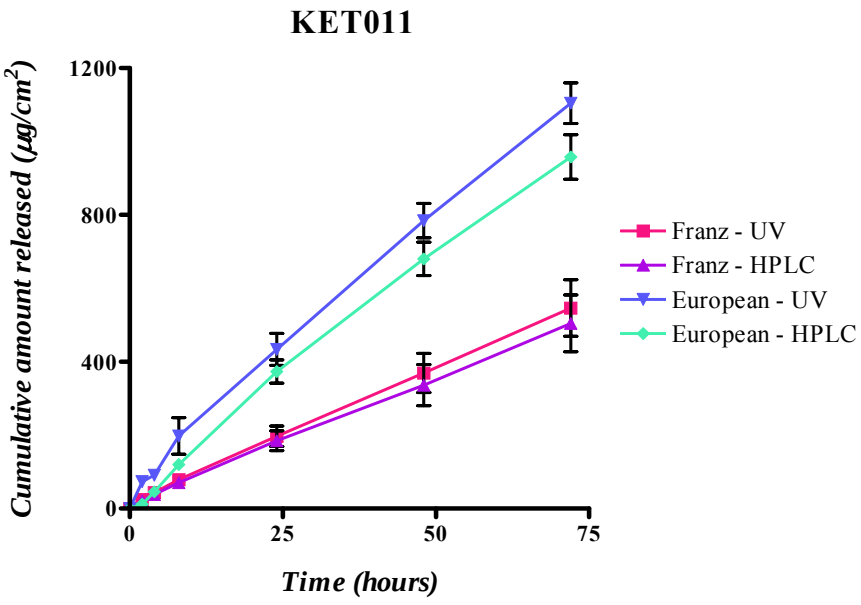
| | | | | | | | |
|---|---|---|---|---|---|---|---|
| ***Manufacturing date*** | 09/06/05 | ***Homogenising time*** | 5 mins | ***Primary packing*** | Jar | ***Storage*** | 18°C |
| ***Mixing time*** | 60 mins | ***Ambient temperature*** | 21°C | ***Secondary packing*** | - | ***Use by date*** | 11/06/05 |

| **Production Equipment** | **Manufacturer** | **Model number** | **Dissolution Equipment** | **Manufacturer** | **Model number** |
|---|---|---|---|---|---|
| Analytical balance | Sartorius | 2403 | Franz diffusion cell | RU | 37058-8 |
| Top load balance | Sartorius | 1474 MP8-2 | European Pharmacopoeia diffusion cell | RU | |
| Magnetic stirrer | Labcon | MSH 10 | Franz heating element | Grant | TM |
| pH meter | Crison | GLP 21 | USP dissolution apparatus | Pharma Test | PTWS H-425/D |
| Ultra-turrax® | Janke & Kunkel | TP18-10 | Membrane | Silatos™ | REF 7458 |

**Certificate of Analysis**

| *Test* | *Result* | *Limits* | *Observation/Comment/Description* |
|---|---|---|---|
| *pH* | 5.41 | 5.0 - 6.0 | A very thick, sticky gel was formed. There were small clumps of polymer present in the final product. |
| Drug content | | | |
| 1 HPLC analysis | 2.52% m/m | 2.31 - 2.69% m/m | |
| 2 UV analysis | 2.58% m/m | 2.31 - 2.69% m/m | |

*Dissolution Profile*

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# BATCH PRODUCTION RECORD

***Formulator*** Ralph Nii Okai Tettey-Amlalo
***Polymer*** Pemulen® TR1 NF ***Batch ID*** KET012 ***Batch size*** 50 g
***Strength*** 2.5% m/m ketoprofen ***Date of issue*** 13/06/2005 ***Issued by*** RNO Tettey-Amlalo

| **Materials** | **Formulae** | | **Batch number** | **Signatures** | |
|---|---|---|---|---|---|
| | *Percent m/m* | *Actually added* | | *Dispensed by* | *Checked by* |
| Ketoprofen | 2.50 | 1.25 g | 093K1522 | RNO | JMH |
| Pemulen® TR1 NF | 1.00 | 0.50 g | CC3DACT860 | RNO | JMH |
| Triethanolamine | 1.15 | 0.58 g | 1024801 | RNO | JMH |
| Ethanol | 20.00 | 12.50 ml | L601707 | RNO | JMH |
| Propylene glycol | 20.00 | 10.00 g | 1026325 | RNO | JMH |
| Purified water | 55.35 | 27.68 ml | | RNO | JMH |

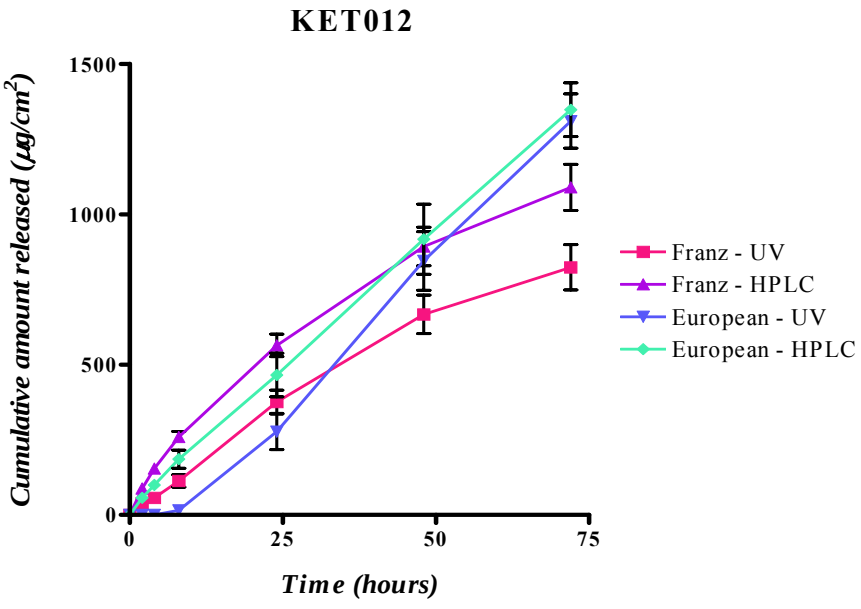
| | | | | | | | |
|---|---|---|---|---|---|---|---|
| ***Manufacturing date*** | 13/06/05 | ***Homogenising time*** | 5 mins | ***Primary packing*** | Jar | ***Storage*** | 18°C |
| ***Mixing time*** | 60 mins | ***Ambient temperature*** | 24°C | ***Secondary packing*** | - | ***Use by date*** | 15/06/05 |

| **Production Equipment** | **Manufacturer** | **Model number** | **Dissolution Equipment** | **Manufacturer** | **Model number** |
|---|---|---|---|---|---|
| Analytical balance | Sartorius | 2403 | Franz diffusion cell | RU | 37058-8 |
| Top load balance | Sartorius | 1474 MP8-2 | European Pharmacopoeia diffusion cell | RU | |
| Magnetic stirrer | Labcon | MSH 10 | Franz heating element | Grant | TM |
| pH meter | Crison | GLP 21 | USP dissolution apparatus | Pharma Test | PTWS H-425/D |
| Ultra-turrax® | Janke & Kunkel | TP18-10 | Membrane | Silatos™ | REF 7458 |

**Certificate of Analysis**

| *Test* | *Result* | *Limits* | *Observation/Comment/Description* |
|---|---|---|---|
| pH | 5.41 | 5.0 - 6.0 | Mixture was emulsion like prior to neutralization. On neutralization, the mixture was very opaque and not translucent like the previous formulations with Pemulen®. |
| Drug content | | | |
| 1 HPLC analysis | 2.53% m/m | 2.31 - 2.69% m/m | |
| 2 UV analysis | 2.61% m/m | 2.31 - 2.69% m/m | |

*Dissolution Profile*

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# BATCH PRODUCTION RECORD

***Formulator*** Ralph Nii Okai Tettey-Amlalo
***Polymer*** Carbopol® Ultrez™ 10 NF ***Batch ID*** KET013 ***Batch size*** 50 g
***Strength*** 2.5% m/m ketoprofen ***Date of issue*** 28/06/2005 ***Issued by*** RNO Tettey-Amlalo

| **Materials** | **Formulae** | | **Batch number** | **Signatures** | |
|---|---|---|---|---|---|
| | *Percent m/m* | *Actually added* | | *Dispensed by* | *Checked by* |
| Ketoprofen | 2.50 | 1.25 g | 093K1522 | RNO | JMH |
| Carbopol® Ultrez™ 10 NF | 1.50 | 0.75 g | CC2NLZG148 | RNO | JMH |
| Triethanolamine | 4.26 | 2.13 g | 1024801 | RNO | JMH |
| Ethanol | 20.00 | 12.50 ml | L601707 | RNO | JMH |
| Propylene glycol | 20.00 | 10.00 g | 1026325 | RNO | JMH |
| Purified water | 51.76 | 25.88 ml | | RNO | JMH |

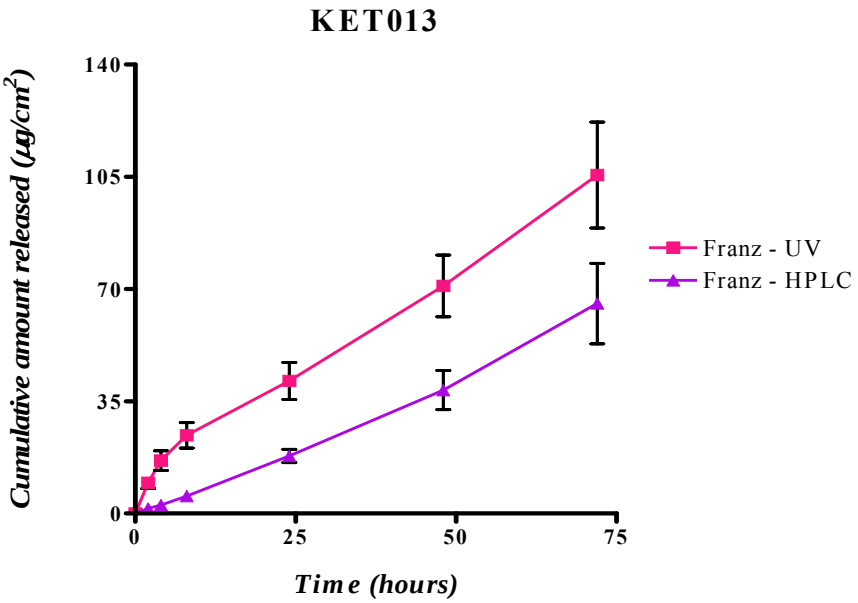
| | | | | | | | |
|---|---|---|---|---|---|---|---|
| ***Manufacturing date*** | 28/06/05 | ***Homogenising time*** | 5 mins | ***Primary packing*** | Jar | ***Storage*** | 18°C |
| ***Mixing time*** | 60 mins | ***Ambient temperature*** | 24.5°C | ***Secondary packing*** | - | ***Use by date*** | 01/07/05 |

| **Production Equipment** | **Manufacturer** | **Model number** | **Dissolution Equipment** | **Manufacturer** | **Model number** |
|---|---|---|---|---|---|
| Analytical balance | Sartorius | 2403 | Franz diffusion cell | RU | 37058-8 |
| Top load balance | Sartorius | 1474 MP8-2 | European Pharmacopoeia diffusion cell | RU | |
| Magnetic stirrer | Labcon | MSH 10 | Franz heating element | Grant | TM |
| pH meter | Crison | GLP 21 | USP dissolution apparatus | Pharma Test | PTWS H-425/D |
| Ultra-turrax® | Janke & Kunkel | TP18-10 | Membrane | Silatos™ | REF 7458 |

**Certificate of Analysis**

| *Test* | *Result* | *Limits* | *Observation/Comment/Description* |
|---|---|---|---|
| pH | 7.21 | 5.0 - 6.0 | An elegant gel was formed. Presence of air bubbles on mixing. |
| Drug content | | | |
| 1 HPLC analysis | 2.50% m/m | 2.31 - 2.69% m/m | |
| 2 UV analysis | 2.52% m/m | 2.31 - 2.69% m/m | |

*Dissolution Profile*

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# BATCH PRODUCTION RECORD

***Formulator*** Ralph Nii Okai Tettey-Amlalo
***Polymer*** Carbopol® Ultrez™ 10 NF ***Batch ID*** KET014 ***Batch size*** 50 g
***Strength*** 2.5% m/m ketoprofen ***Date of issue*** 05/07/2005 ***Issued by*** RNO Tettey-Amlalo

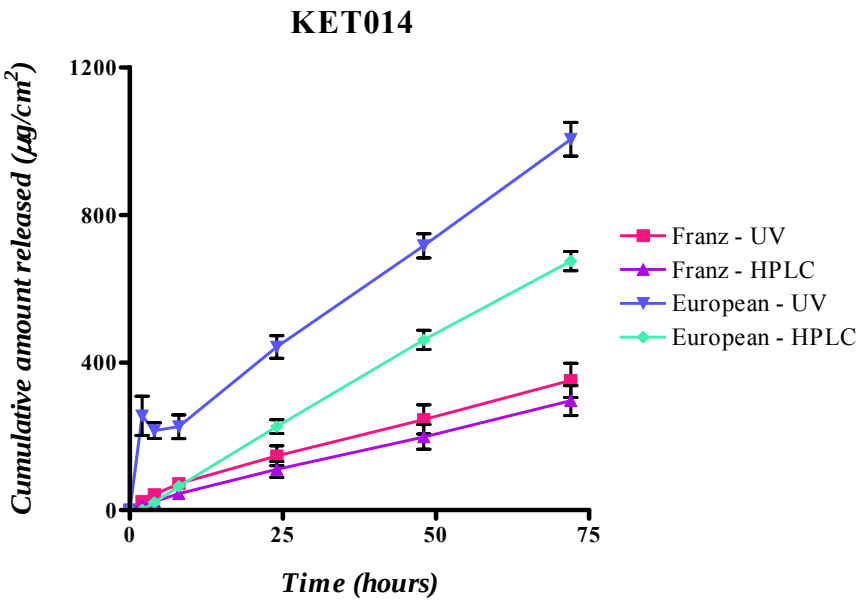
| **Materials** | **Formulae** | | **Batch number** | **Signatures** | |
|---|---|---|---|---|---|
| | *Percent m/m* | *Actually added* | | *Dispensed by* | *Checked by* |
| Ketoprofen | 2.50 | 1.25 g | 093K1522 | RNO | JMH |
| Carbopol® Ultrez™ 10 NF | 1.50 | 0.75 g | CC2NLZG148 | RNO | JMH |
| Triethanolamine | 3.00 | 1.50 g | 1024801 | RNO | JMH |
| Ethanol | 20.00 | 12.50 ml | L601707 | RNO | JMH |
| Propylene glycol | 20.00 | 10.00 g | 1026325 | RNO | JMH |
| Purified water | 52.76 | 26.38 ml | | RNO | JMH |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| ***Manufacturing date*** | 05/07/05 | ***Homogenising time*** | 5 mins | ***Primary packing*** | Jar | ***Storage*** | 18°C |
| ***Mixing time*** | 60 mins | ***Ambient temperature*** | 25°C | ***Secondary packing*** | - | ***Use by date*** | 07/07/05 |

| **Production Equipment** | **Manufacturer** | **Model number** | **Dissolution Equipment** | **Manufacturer** | **Model number** |
|---|---|---|---|---|---|
| Analytical balance | Sartorius | 2403 | Franz diffusion cell | RU | 37058-8 |
| Top load balance | Sartorius | 1474 MP8-2 | European Pharmacopoeia diffusion cell | RU | |
| Magnetic stirrer | Labcon | MSH 10 | Franz heating element | Grant | TM |
| pH meter | Crison | GLP 21 | USP dissolution apparatus | Pharma Test | PTWS H-425/D |
| Ultra-turrax® | Janke & Kunkel | TP18-10 | Membrane | Silatos™ | REF 7458 |

**Certificate of Analysis**

| *Test* | *Result* | *Limits* | *Observation/Comment/Description* |
|---|---|---|---|
| pH | 6.51 | 5.0 - 6.0 | An elegant gel was formed. Presence of air bubbles on mixing. The gel was thicker than KET013 and KET014. |
| Drug content | | | |
| 1 HPLC analysis | 2.51% m/m | 2.31 - 2.69% m/m | |
| 2 UV analysis | 2.52% m/m | 2.31 - 2.69% m/m | |
| | | *Dissolution Profile* | |

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***Production Pharmacist***

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**Faculty of Pharmacy**
**Transdermal Laboratory**
**Grahamstown, South Africa**

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# BATCH PRODUCTION RECORD

***Formulator*** Ralph Nii Okai Tettey-Amlalo
***Polymer*** Carbopol® Ultrez™ 10 NF ***Batch ID*** KET015 ***Batch size*** 50 g
***Strength*** 1.5% m/m ketoprofen ***Date of issue*** 18/06/05 ***Issued by*** RNO Tettey-Amlalo

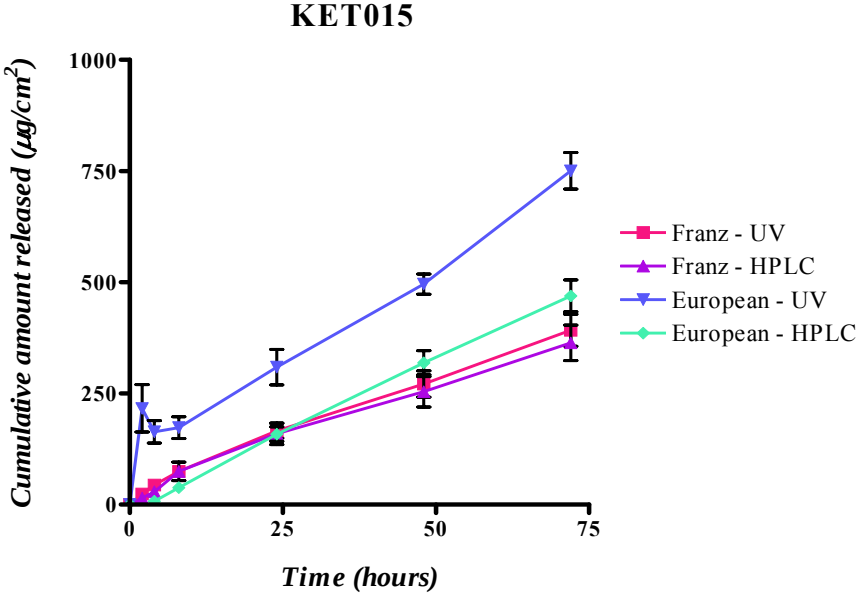
| **Materials** | **Formulae** | | **Batch number** | **Signatures** | |
|---|---|---|---|---|---|
| | *Percent m/m* | *Actually added* | | *Dispensed by* | *Checked by* |
| Ketoprofen | 1.50 | 0.75 g | 093K1522 | RNO | JMH |
| Carbopol® Ultrez™ 10 NF | 1.50 | 0.75 g | CC2NLZG148 | RNO | JMH |
| Triethanolamine | 2.25 | 1.13 g | 1024801 | RNO | JMH |
| Ethanol | 20.00 | 12.50 ml | L601707 | RNO | JMH |
| Propylene glycol | 20.00 | 10.00 g | 1026325 | RNO | JMH |
| Purified water | 54.75 | 27.38 ml | | RNO | JMH |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| ***Manufacturing date*** | 18/06/05 | ***Homogenising time*** | 5 mins | ***Primary packing*** | Jar | ***Storage*** | 18°C |
| ***Mixing time*** | 60 mins | ***Ambient temperature*** | 25°C | ***Secondary packing*** | - | ***Use by date*** | 20/06/05 |

| **Production Equipment** | **Manufacturer** | **Model number** | **Dissolution Equipment** | **Manufacturer** | **Model number** |
|---|---|---|---|---|---|
| Analytical balance | Sartorius | 2403 | Franz diffusion cell | RU | 37058-8 |
| Top load balance | Sartorius | 1474 MP8-2 | European Pharmacopoeia diffusion cell | RU | |
| Magnetic stirrer | Labcon | MSH 10 | Franz heating element | Grant | TM |
| pH meter | Crison | GLP 21 | USP dissolution apparatus | Pharma Test | PTWS H-425/D |
| Ultra-turrax® | Janke & Kunkel | TP18-10 | Membrane | Silatos™ | REF 7458 |

**Certificate of Analysis**

| *Test* | *Result* | *Limits* | *Observation/Comment/Description* |
|---|---|---|---|
| pH | 6.40 | 5.0 - 6.0 | An elegant gel was formed. Presence of air bubbles on mixing. |
| Drug content | | | |
| 1 HPLC analysis | 1.51% m/m | 1.31 - 1.69% m/m | |
| 2 UV analysis | 1.52% m/m | 1.31 - 1.69% m/m | |
| | | *Dissolution Profile* | |

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# BATCH PRODUCTION RECORD

***Formulator*** Ralph Nii Okai Tettey-Amlalo
***Polymer*** Carbopol® Ultrez™ 10 NF ***Batch ID*** KET016 ***Batch size*** 50 g
***Strength*** 5.5% m/m ketoprofen ***Date of issue*** 04/08/2005 ***Issued by*** RNO Tettey-Amlalo

| **Materials** | **Formulae** | | **Batch number** | **Signatures** | |
|---|---|---|---|---|---|
| | *Percent m/m* | *Actually added* | | *Dispensed by* | *Checked by* |
| Ketoprofen | 5.50 | 2.75 g | 093K1522 | RNO | JMH |
| Carbopol® Ultrez™ 10 NF | 1.50 | 0.75 g | CC2NLZG148 | RNO | JMH |
| Triethanolamine | 2.25 | 1.13 g | 1024801 | RNO | JMH |
| Ethanol | 20.00 | 12.50 ml | L601707 | RNO | JMH |
| Propylene glycol | 20.00 | 10.00 g | 1026325 | RNO | JMH |
| Purified water | 50.75 | 25.38 ml | | RNO | JMH |

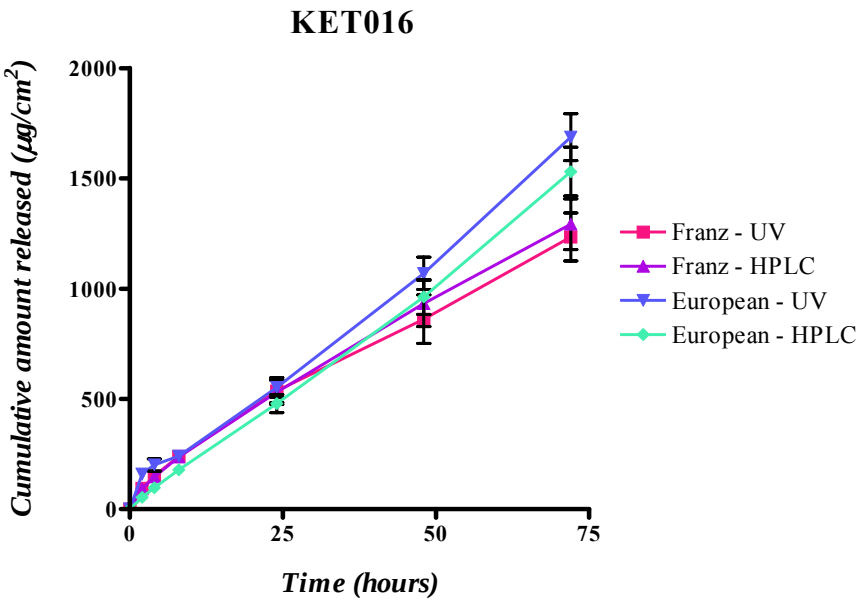
| | | | | | | | |
|---|---|---|---|---|---|---|---|
| ***Manufacturing date*** | 03/08/05 | ***Homogenising time*** | 5 mins | ***Primary packing*** | Jar | ***Storage*** | 18.5°C |
| ***Mixing time*** | 60 mins | ***Ambient temperature*** | 24°C | ***Secondary packing*** | - | ***Use by date*** | 06/08/05 |

| **Production Equipment** | **Manufacturer** | **Model number** | **Dissolution Equipment** | **Manufacturer** | **Model number** |
|---|---|---|---|---|---|
| Analytical balance | Sartorius | 2403 | Franz diffusion cell | RU | 37058-8 |
| Top load balance | Sartorius | 1474 MP8-2 | European Pharmacopoeia diffusion cell | RU | |
| Magnetic stirrer | Labcon | MSH 10 | Franz heating element | Grant | TM |
| pH meter | Crison | GLP 21 | USP dissolution apparatus | Pharma Test | PTWS H-425/D |
| Ultra-turrax® | Janke & Kunkel | TP18-10 | Membrane | Silatos™ | REF 7458 |

**Certificate of Analysis**

| *Test* | *Result* | *Limits* | *Observation/Comment/Description* |
|---|---|---|---|
| pH | 5.57 | 5.0 - 6.0 | The gel formed was not as elegant as the others. The ketoprofen came out of solution and precipitated due to the amount incorporated. The gel was gritty and not attractive. |
| Drug content | | | |
| 1 HPLC analysis | 5.55% m/m | 5.31 - 5.69% m/m | |
| 2 UV analysis | 5.60% m/m | 5.31 - 5.69% m/m | |

*Dissolution Profile*

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# BATCH PRODUCTION RECORD

***Formulator*** Ralph Nii Okai Tettey-Amlalo
***Polymer*** Carbopol® Ultrez™ 10 NF ***Batch ID*** KET017 ***Batch size*** 50 g
***Strength*** 2.5% m/m ketoprofen ***Date of issue*** 11/08/2005 ***Issued by*** RNO Tettey-Amlalo

| **Materials** | **Formulae** | | **Batch number** | **Signatures** | |
|---|---|---|---|---|---|
| | *Percent m/m* | *Actually added* | | *Dispensed by* | *Checked by* |
| Ketoprofen | 2.50 | 1.25 g | 093K1522 | RNO | JMH |
| Carbopol® Ultrez™ 10 NF | 1.50 | 0.75 g | CC2NLZG148 | RNO | JMH |
| Triethanolamine | 2.25 | 1.13 g | 1024801 | RNO | JMH |
| Transcutol® HP | 20.00 | 10.00 g | 0339024 | RNO | JMH |
| Propylene glycol | 20.00 | 10.00 g | 1026325 | RNO | JMH |
| Purified water | 53.75 | 26.88 ml | | RNO | JMH |

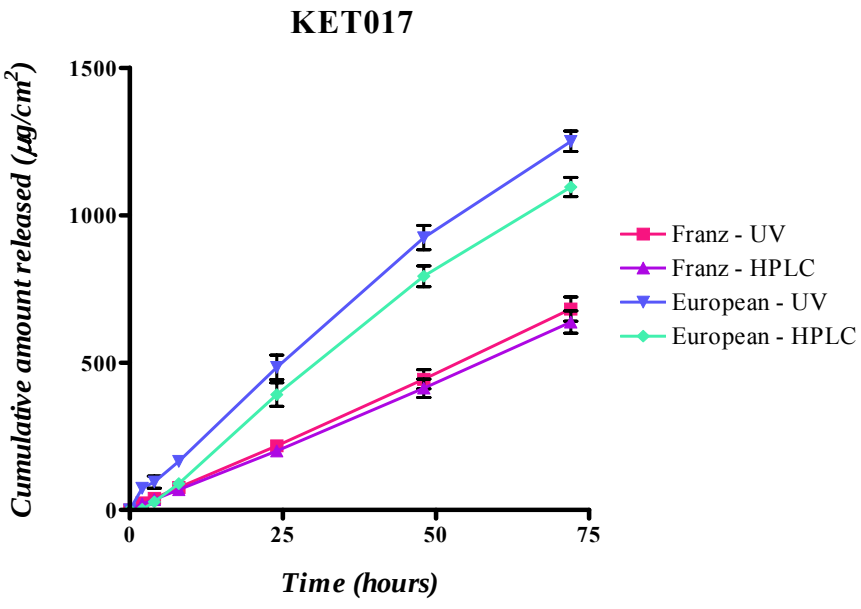
| | | | | | | | |
|---|---|---|---|---|---|---|---|
| ***Manufacturing date*** | 11/08/05 | ***Homogenising time*** | 5 mins | ***Primary packing*** | Jar | ***Storage*** | 18°C |
| ***Mixing time*** | 60 mins | ***Ambient temperature*** | 25°C | ***Secondary packing*** | - | ***Use by date*** | 13/08/05 |

| **Production Equipment** | **Manufacturer** | **Model number** | **Dissolution Equipment** | **Manufacturer** | **Model number** |
|---|---|---|---|---|---|
| Analytical balance | Sartorius | 2403 | Franz diffusion cell | RU | 37058-8 |
| Top load balance | Sartorius | 1474 MP8-2 | European Pharmacopoeia diffusion cell | RU | |
| Magnetic stirrer | Labcon | MSH 10 | Franz heating element | Grant | TM |
| pH meter | Crison | GLP 21 | USP dissolution apparatus | Pharma Test | PTWS H-425/D |
| Ultra-turrax® | Janke & Kunkel | TP18-10 | Membrane | Silatos™ | REF 7458 |

**Certificate of Analysis**

| *Test* | *Result* | *Limits* | *Observation/Comment/Description* |
|---|---|---|---|
| pH | 5.76 | 5.0 - 6.0 | An elegant sweet smelling gel was formed. Devoid of air bubbles. |
| Drug content | | | |
| 1 HPLC analysis | 2.51% m/m | 2.31 - 2.69% m/m | |
| 2 UV analysis | 2.52% m/m | 2.31 - 2.69% m/m | |

*Dissolution Profile*

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# BATCH PRODUCTION RECORD

***Formulator*** Ralph Nii Okai Tettey-Amlalo
***Polymer*** Carbopol® Ultrez™ 10 NF ***Batch ID*** KET018 ***Batch size*** 50 g
***Strength*** 2.5% m/m ketoprofen ***Date of issue*** 14/08/2005 ***Issued by*** RNO Tettey-Amlalo

| **Materials** | **Formulae** | | **Batch number** | **Signatures** | |
|---|---|---|---|---|---|
| | *Percent m/m* | *Actually added* | | *Dispensed by* | *Checked by* |
| Ketoprofen | 2.50 | 1.25 g | 093K1522 | RNO | JMH |
| Carbopol® Ultrez™ 10 NF | 1.50 | 0.75 g | CC2NLZG148 | RNO | JMH |
| Triethanolamine | 2.25 | 1.13 g | 1024801 | RNO | JMH |
| Ethanol | 20.00 | 12.50 ml | L601707 | RNO | JMH |
| Transcutol® HP | 20.00 | 10.00 g | 0339024 | RNO | JMH |
| Purified water | 53.75 | 26.88 ml | | RNO | JMH |

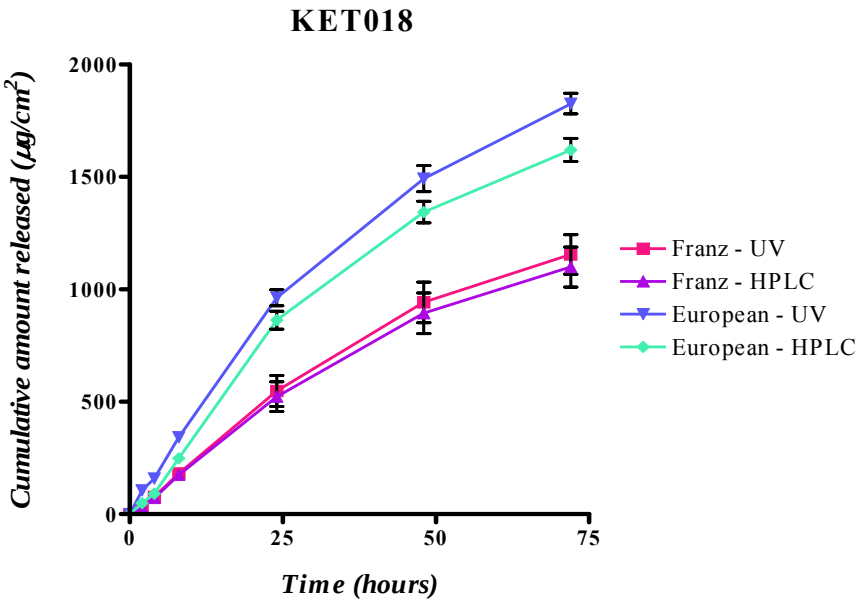
| | | | | | | | |
|---|---|---|---|---|---|---|---|
| ***Manufacturing date*** | 14/08/05 | ***Homogenising time*** | 5 mins | ***Primary packing*** | Jar | ***Storage*** | 18°C |
| ***Mixing time*** | 60 mins | ***Ambient temperature*** | 24°C | ***Secondary packing*** | - | ***Use by date*** | 16/08/05 |

| **Production Equipment** | **Manufacturer** | **Model number** | **Dissolution Equipment** | **Manufacturer** | **Model number** |
|---|---|---|---|---|---|
| Analytical balance | Sartorius | 2403 | Franz diffusion cell | RU | 37058-8 |
| Top load balance | Sartorius | 1474 MP8-2 | European Pharmacopoeia diffusion cell | RU | |
| Magnetic stirrer | Labcon | MSH 10 | Franz heating element | Grant | TM |
| pH meter | Crison | GLP 21 | USP dissolution apparatus | Pharma Test | PTWS H-425/D |
| Ultra-turrax® | Janke & Kunkel | TP18-10 | Membrane | Silatos™ | REF 7458 |

**Certificate of Analysis**

| *Test* | *Result* | *Limits* | *Observation/Comment/Description* |
|---|---|---|---|
| pH | 5.80 | 5.0 - 6.0 | An elegant sweet smelling gel was formed. Devoid of air bubbles. |
| Drug content | | | |
| 1 HPLC analysis | 2.51% m/m | 2.31 - 2.69% m/m | |
| 2 UV analysis | 2.52% m/m | 2.31 - 2.69% m/m | |

*Dissolution Profile*

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# BATCH PRODUCTION RECORD

***Formulator*** Ralph Nii Okai Tettey-Amlalo
***Polymer*** Carbopol® Ultrez™ 10 NF ***Batch ID*** KET019 ***Batch size*** 50 g
***Strength*** 2.5% m/m ketoprofen ***Date of issue*** 23/08/2005 ***Issued by*** RNO Tettey-Amlalo

| **Materials** | **Formulae** | | **Batch number** | **Signatures** | |
|---|---|---|---|---|---|
| | *Percent m/m* | *Actually added* | | *Dispensed by* | *Checked by* |
| Ketoprofen | 2.50 | 1.25 g | 093K1522 | RNO | JMH |
| Carbopol® Ultrez™ 10 NF | 1.50 | 0.75 g | CC2NLZG148 | RNO | JMH |
| Triethanolamine | 2.25 | 1.13 g | 1024801 | RNO | JMH |
| Ethanol | 40.00 | 25.00 ml | L601707 | RNO | JMH |
| Purified water | 53.75 | 26.88 ml | | RNO | JMH |

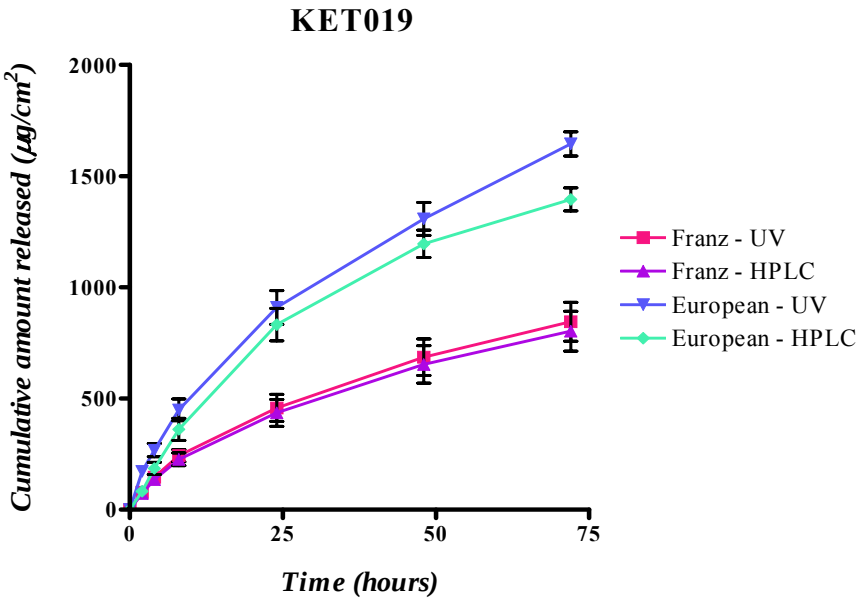
| | | | | | | | |
|---|---|---|---|---|---|---|---|
| ***Manufacturing date*** | 22/08/05 | ***Homogenising time*** | 5 mins | ***Primary packing*** | Jar | ***Storage*** | 18°C |
| ***Mixing time*** | 60 mins | ***Ambient temperature*** | 24°C | ***Secondary packing*** | - | ***Use by date*** | 25/08/05 |

| **Production Equipment** | **Manufacturer** | **Model number** | **Dissolution Equipment** | **Manufacturer** | **Model number** |
|---|---|---|---|---|---|
| Analytical balance | Sartorius | 2403 | Franz diffusion cell | RU | 37058-8 |
| Top load balance | Sartorius | 1474 MP8-2 | European Pharmacopoeia diffusion cell | RU | |
| Magnetic stirrer | Labcon | MSH 10 | Franz heating element | Grant | TM |
| pH meter | Crison | GLP 21 | USP dissolution apparatus | Pharma Test | PTWS H-425/D |
| Ultra-turrax® | Janke & Kunkel | TP18-10 | Membrane | Silatos™ | REF 7458 |

**Certificate of Analysis**

| *Test* | *Result* | *Limits* | *Observation/Comment/Description* |
|---|---|---|---|
| pH | 5.94 | 5.0 - 6.0 | An elegant gel was formed. Devoid of air bubbles. |
| Drug content | | | |
| 1 HPLC analysis | 2.51% m/m | 2.31 - 2.69% m/m | |
| 2 UV analysis | 2.53% m/m | 2.31 - 2.69% m/m | |

*Dissolution Profile*

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# BATCH PRODUCTION RECORD

***Formulator*** Ralph Nii Okai Tettey-Amlalo
***Polymer*** Carbopol® Ultrez™ 10 NF ***Batch ID*** KET020 ***Batch size*** 50 g
***Strength*** 2.5% m/m ketoprofen ***Date of issue*** 29/08/2005 ***Issued by*** RNO Tettey-Amlalo

| **Materials** | **Formulae** | | **Batch number** | **Signatures** | |
|---|---|---|---|---|---|
| | *Percent m/m* | *Actually added* | | *Dispensed by* | *Checked by* |
| Ketoprofen | 2.50 | 1.25 g | 093K1522 | RNO | JMH |
| Carbopol® Ultrez™ 10 NF | 1.50 | 0.75 g | CC2NLZG148 | RNO | JMH |
| Triethanolamine | 2.25 | 1.13 g | 1024801 | RNO | JMH |
| Transcutol® HP | 40.00 | 20.00 g | 0339024 | RNO | JMH |
| Purified water | 53.75 | 26.88 ml | | RNO | JMH |

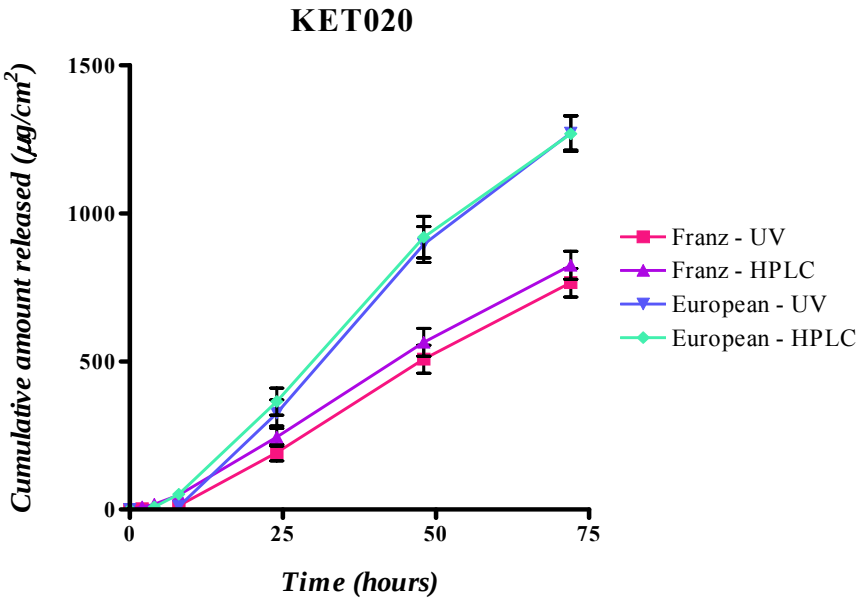
| | | | | | | | |
|---|---|---|---|---|---|---|---|
| ***Manufacturing date*** | 29/08/05 | ***Homogenising time*** | 5 mins | ***Primary packing*** | Jar | ***Storage*** | 18°C |
| ***Mixing time*** | 60 mins | ***Ambient temperature*** | 25°C | ***Secondary packing*** | - | ***Use by date*** | 31/08/05 |

| **Production Equipment** | **Manufacturer** | **Model number** | **Dissolution Equipment** | **Manufacturer** | **Model number** |
|---|---|---|---|---|---|
| Analytical balance | Sartorius | 2403 | Franz diffusion cell | RU | 37058-8 |
| Top load balance | Sartorius | 1474 MP8-2 | European Pharmacopoeia diffusion cell | RU | |
| Magnetic stirrer | Labcon | MSH 10 | Franz heating element | Grant | TM |
| pH meter | Crison | GLP 21 | USP dissolution apparatus | Pharma Test | PTWS H-425/D |
| Ultra-turrax® | Janke & Kunkel | TP18-10 | Membrane | Silatos™ | REF 7458 |

**Certificate of Analysis**

| *Test* | *Result* | *Limits* | *Observation/Comment/Description* |
|---|---|---|---|
| pH | 6.25 | 5.0 - 6.0 | An elegant sweet smelling gel was formed devoid of air bubbles. |
| Drug content | | | |
| 1 HPLC analysis | 2.51% m/m | 2.31 - 2.69% m/m | |
| 2 UV analysis | 2.52% m/m | 2.31 - 2.69% m/m | |

*Dissolution Profile*

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# APPENDIX III
# RAW DATA

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# R A W  D A T A

| Summary of methodology | |
|---|---|
| Receptor medium | 0.2 M phosphate buffer at pH 6.8 |
| Temperature | 32 ± 0.5°C |
| Run time | 72 hours |
| Agitation | 100 rpm |
| Franz diffusion volume | 9 ml |
| European diffusion volume | 1000 ml |
| Sample application | |
| Franz diffusion cell | 100 mg |
| European Pharmacopoeia diffusion cell | 500 mg |
| Membrane | Silatos™ silicone sheeting |
| Analytical procedure | Hplc and uv spectrophotometric analysis |

**Abbreviation**

$A_{CUM}$ Cumulative Amount Released (µg/cm$^2$)
SD Standard deviation

**Fastum® (SA)**

| | *Franz diffusion apparatus* | | | | *European diffusion apparatus* | | | |
|---|---|---|---|---|---|---|---|---|
| Time | HPLC | | UV | | HPLC | | UV | |
| (hours) | $A_{CUM}$ | SD | $A_{CUM}$ | SD | $A_{CUM}$ | SD | $A_{CUM}$ | SD |
| 0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 2 | 119.44 | 14.65 | 153.80 | 19.95 | 103.55 | 18.12 | 170.23 | 25.41 |
| 4 | 181.20 | 25.42 | 234.27 | 32.48 | 186.23 | 27.19 | 261.68 | 27.63 |
| 8 | 280.91 | 43.15 | 353.15 | 50.22 | 305.02 | 33.29 | 419.30 | 31.11 |
| 24 | 505.30 | 78.73 | 596.65 | 86.11 | 614.23 | 69.60 | 750.21 | 77.23 |
| 48 | 755.72 | 133.26 | 868.01 | 136.41 | 966.55 | 95.98 | 1128.34 | 109.70 |
| 72 | 931.04 | 182.15 | 1061.86 | 185.07 | 1281.96 | 72.26 | 1467.49 | 70.92 |

**Ketum® (FR)**

| | *Franz diffusion apparatus* | | | | *European diffusion apparatus* | | | |
|---|---|---|---|---|---|---|---|---|
| Time | HPLC | | UV | | HPLC | | UV | |
| (hours) | $A_{CUM}$ | SD | $A_{CUM}$ | SD | $A_{CUM}$ | SD | $A_{CUM}$ | SD |
| 0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 2 | 98.46 | 26.14 | 114.61 | 24.14 | 88.07 | 23.05 | 136.46 | 26.30 |
| 4 | 164.77 | 39.72 | 183.32 | 37.27 | 186.57 | 37.97 | 220.10 | 42.29 |
| 8 | 262.93 | 52.96 | 287.83 | 57.09 | 336.63 | 84.03 | 365.59 | 85.55 |
| 24 | 492.84 | 108.45 | 553.99 | 124.67 | 700.31 | 212.35 | 791.37 | 225.21 |
| 48 | 647.41 | 162.86 | 740.29 | 186.13 | 1008.82 | 193.43 | 1103.02 | 217.95 |
| 72 | 753.93 | 205.70 | 888.91 | 245.79 | 1283.49 | 129.19 | 1365.29 | 159.42 |

**Oruvail® (UK)**

| | *Franz diffusion apparatus* | | | | *European diffusion apparatus* | | | |
|---|---|---|---|---|---|---|---|---|
| Time | HPLC | | UV | | HPLC | | UV | |
| (hours) | $A_{CUM}$ | SD | $A_{CUM}$ | SD | $A_{CUM}$ | SD | $A_{CUM}$ | SD |
| 0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 2 | 29.11 | 5.73 | 24.87 | 4.91 | 0.00 | 2.03 | 5.48 | 11.66 |
| 4 | 48.94 | 10.50 | 39.45 | 9.74 | 21.41 | 3.82 | 28.69 | 14.66 |
| 8 | 80.37 | 19.42 | 64.52 | 18.14 | 68.09 | 6.80 | 58.44 | 10.48 |
| 24 | 170.83 | 41.53 | 153.23 | 40.43 | 233.81 | 23.01 | 247.73 | 28.12 |
| 48 | 283.28 | 57.64 | 263.73 | 55.36 | 449.58 | 42.54 | 489.52 | 41.93 |
| 72 | 377.73 | 74.11 | 357.70 | 70.83 | 656.99 | 47.86 | 720.33 | 51.86 |

**KET001**

| | *Franz diffusion apparatus* | | | | *European diffusion apparatus* | | | |
|---|---|---|---|---|---|---|---|---|
| Time | HPLC | | UV | | HPLC | | UV | |
| (hours) | $A_{CUM}$ | SD | $A_{CUM}$ | SD | $A_{CUM}$ | SD | $A_{CUM}$ | SD |
| 0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 2 | 91.50 | 21.78 | 105.21 | 21.30 | 34.57 | 6.20 | 103.00 | 6.28 |
| 4 | 182.58 | 44.17 | 208.23 | 44.10 | 84.60 | 14.23 | 202.00 | 14.04 |
| 8 | 344.81 | 77.56 | 383.91 | 74.56 | 194.13 | 31.63 | 277.00 | 31.61 |
| 24 | 718.04 | 98.20 | 781.13 | 92.22 | 611.90 | 86.12 | 690.60 | 86.16 |
| 48 | 962.91 | 101.65 | 1046.79 | 96.85 | 1005.09 | 141.08 | 1157.60 | 141.09 |
| 72 | 1079.60 | 91.93 | 1186.52 | 91.81 | 1195.17 | 179.22 | 1355.80 | 179.07 |

**KET002**

| | *Franz diffusion apparatus* | | | | *European diffusion apparatus* | | | |
|---|---|---|---|---|---|---|---|---|
| Time | HPLC | | UV | | HPLC | | UV | |
| (hours) | $A_{CUM}$ | SD | $A_{CUM}$ | SD | $A_{CUM}$ | SD | $A_{CUM}$ | SD |
| 0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 2 | 36.19 | 4.07 | 48.07 | 3.16 | 56.66 | 7.75 | 106.34 | 15.45 |
| 4 | 67.71 | 8.86 | 86.58 | 11.60 | 115.27 | 23.94 | 188.16 | 58.27 |
| 8 | 127.41 | 18.68 | 151.09 | 21.01 | 255.21 | 46.73 | 336.36 | 70.34 |
| 24 | 290.26 | 45.61 | 320.00 | 47.28 | 653.59 | 88.99 | 743.98 | 106.62 |
| 48 | 498.25 | 83.45 | 535.74 | 83.98 | 1014.00 | 101.81 | 1202.42 | 121.63 |
| 72 | 686.05 | 78.50 | 734.62 | 73.87 | 1335.25 | 94.14 | 1506.47 | 94.20 |

**KET003**

| | *Franz diffusion apparatus* | | | | *European diffusion apparatus* | | | |
|---|---|---|---|---|---|---|---|---|
| Time | HPLC | | UV | | HPLC | | UV | |
| (hours) | $A_{CUM}$ | SD | $A_{CUM}$ | SD | $A_{CUM}$ | SD | $A_{CUM}$ | SD |
| 0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 2 | 43.87 | 6.20 | 52.72 | 5.95 | 3.05 | 6.73 | 86.05 | 45.44 |
| 4 | 87.99 | 12.43 | 105.76 | 13.86 | 24.89 | 13.75 | 89.88 | 32.70 |
| 8 | 161.91 | 19.02 | 185.81 | 20.70 | 75.05 | 28.43 | 142.73 | 26.69 |
| 24 | 360.56 | 38.81 | 392.87 | 46.87 | 280.58 | 80.45 | 379.73 | 70.86 |
| 48 | 580.90 | 63.95 | 621.79 | 74.32 | 614.06 | 81.58 | 757.16 | 78.23 |
| 72 | 769.79 | 77.55 | 826.97 | 90.16 | 878.67 | 52.69 | 1060.15 | 65.45 |

**KET004**

| | *Franz diffusion apparatus* | | | | *European diffusion apparatus* | | | |
|---|---|---|---|---|---|---|---|---|
| Time | HPLC | | UV | | HPLC | | UV | |
| (hours) | $A_{CUM}$ | SD | $A_{CUM}$ | SD | $A_{CUM}$ | SD | $A_{CUM}$ | SD |
| 0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 2 | 49.17 | 5.05 | 51.98 | 6.88 | 20.77 | 10.74 | 33.96 | 33.79 |
| 4 | 99.22 | 9.30 | 104.13 | 11.01 | 65.17 | 21.02 | 70.26 | 22.60 |
| 8 | 195.07 | 14.19 | 202.79 | 15.62 | 171.23 | 47.99 | 180.52 | 45.30 |
| 24 | 467.65 | 31.75 | 484.19 | 32.13 | 449.60 | 95.81 | 521.53 | 100.68 |
| 48 | 669.48 | 47.83 | 700.72 | 50.69 | 799.48 | 119.17 | 926.60 | 111.26 |
| 72 | 820.35 | 46.92 | 863.74 | 49.80 | 1028.31 | 134.68 | 1220.17 | 138.80 |

**KET005**

| | *Franz diffusion apparatus* | | | | *European diffusion apparatus* | | | |
|---|---|---|---|---|---|---|---|---|
| Time | HPLC | | UV | | HPLC | | UV | |
| (hours) | $A_{CUM}$ | SD | $A_{CUM}$ | SD | $A_{CUM}$ | SD | $A_{CUM}$ | SD |
| 0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 2 | 60.08 | 9.31 | 70.49 | 12.27 | 30.90 | 10.49 | 174.95 | 33.54 |
| 4 | 120.42 | 18.99 | 125.83 | 19.05 | 78.05 | 14.06 | 233.43 | 59.59 |
| 8 | 235.54 | 33.41 | 226.83 | 28.54 | 188.19 | 28.22 | 308.85 | 103.79 |
| 24 | 536.43 | 54.66 | 516.20 | 45.12 | 575.78 | 55.65 | 683.95 | 67.65 |
| 48 | 812.29 | 90.65 | 778.94 | 79.06 | 954.17 | 88.25 | 1122.17 | 123.54 |
| 72 | 974.88 | 130.07 | 925.91 | 120.55 | 1196.81 | 100.59 | 1355.94 | 108.75 |

**KET006**

| Time | *Franz diffusion apparatus* | | | | *European diffusion apparatus* | | | |
|---|---|---|---|---|---|---|---|---|
| | HPLC | | UV | | HPLC | | UV | |
| (hours) | $A_{CUM}$ | SD | $A_{CUM}$ | SD | $A_{CUM}$ | SD | $A_{CUM}$ | SD |
| 0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 2 | 53.12 | 7.09 | 102.09 | 18.38 | 60.62 | 13.33 | 250.47 | 85.14 |
| 4 | 109.96 | 16.43 | 200.86 | 10.58 | 106.08 | 21.28 | 327.64 | 59.29 |
| 8 | 207.88 | 32.94 | 326.84 | 37.27 | 211.64 | 39.38 | 427.97 | 95.02 |
| 24 | 516.04 | 81.09 | 650.97 | 89.83 | 597.59 | 96.00 | 813.58 | 132.15 |
| 48 | 838.85 | 117.83 | 999.63 | 126.07 | 1000.23 | 128.28 | 1247.84 | 138.59 |
| 72 | 1057.80 | 130.64 | 1246.78 | 143.89 | 1245.11 | 102.27 | 1583.55 | 130.33 |

**KET007**

| Time | *Franz diffusion apparatus* | | | | *European diffusion apparatus* | | | |
|---|---|---|---|---|---|---|---|---|
| | HPLC | | UV | | HPLC | | UV | |
| (hours) | $A_{CUM}$ | SD | $A_{CUM}$ | SD | $A_{CUM}$ | SD | $A_{CUM}$ | SD |
| 0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 2 | 50.54 | 8.98 | 100.22 | 19.47 | 47.22 | 10.65 | 168.74 | 42.81 |
| 4 | 90.96 | 17.67 | 167.49 | 17.51 | 106.69 | 21.63 | 220.58 | 23.12 |
| 8 | 165.11 | 25.69 | 257.17 | 31.81 | 225.73 | 39.98 | 365.53 | 76.80 |
| 24 | 358.37 | 34.85 | 464.66 | 44.62 | 624.42 | 79.99 | 811.40 | 102.67 |
| 48 | 553.77 | 86.82 | 675.25 | 98.02 | 1030.43 | 54.55 | 1277.19 | 93.68 |
| 72 | 697.59 | 129.99 | 830.31 | 144.36 | 1304.19 | 47.05 | 1569.05 | 73.79 |

**KET008**

| Time | *Franz diffusion apparatus* | | | | *European diffusion apparatus* | | | |
|---|---|---|---|---|---|---|---|---|
| | HPLC | | UV | | HPLC | | UV | |
| (hours) | $A_{CUM}$ | SD | $A_{CUM}$ | SD | $A_{CUM}$ | SD | $A_{CUM}$ | SD |
| 0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 2 | 65.06 | 11.53 | 98.70 | 25.54 | 68.74 | 23.82 | 0.00 | 6.17 |
| 4 | 126.52 | 20.59 | 189.57 | 32.92 | 89.81 | 25.33 | 62.99 | 24.91 |
| 8 | 227.98 | 35.41 | 313.74 | 50.74 | 202.30 | 48.31 | 181.78 | 60.09 |
| 24 | 496.32 | 68.70 | 611.26 | 82.27 | 543.63 | 105.60 | 565.41 | 117.77 |
| 48 | 770.88 | 93.15 | 915.60 | 104.06 | 892.28 | 137.54 | 961.65 | 134.23 |
| 72 | 920.37 | 156.19 | 1092.23 | 167.75 | 1157.32 | 125.49 | 1275.32 | 117.66 |

**KET009**

| Time | *Franz diffusion apparatus* | | | | *European diffusion apparatus* | | | |
|---|---|---|---|---|---|---|---|---|
| | HPLC | | UV | | HPLC | | UV | |
| (hours) | $A_{CUM}$ | SD | $A_{CUM}$ | SD | $A_{CUM}$ | SD | $A_{CUM}$ | SD |
| 0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 2 | 47.84 | 2.45 | 60.66 | 9.39 | 66.24 | 13.83 | 23.12 | 31.17 |
| 4 | 86.52 | 6.92 | 107.52 | 26.09 | 147.66 | 20.00 | 92.84 | 26.77 |
| 8 | 151.86 | 12.25 | 168.05 | 30.61 | 297.29 | 40.28 | 225.62 | 40.81 |
| 24 | 387.62 | 14.48 | 413.90 | 27.61 | 728.10 | 92.65 | 696.23 | 77.45 |
| 48 | 646.09 | 35.74 | 678.07 | 60.20 | 1085.89 | 121.72 | 1122.63 | 106.26 |
| 72 | 805.20 | 51.06 | 844.38 | 79.73 | 1324.47 | 129.57 | 1403.56 | 128.65 |

**KET010**

| Time | *Franz diffusion apparatus* | | | | *European diffusion apparatus* | | | |
|---|---|---|---|---|---|---|---|---|
| | HPLC | | UV | | HPLC | | UV | |
| (hours) | $A_{CUM}$ | SD | $A_{CUM}$ | SD | $A_{CUM}$ | SD | $A_{CUM}$ | SD |
| 0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 2 | 30.95 | 8.80 | 33.46 | 2.92 | 54.69 | 1.64 | 101.83 | 16.67 |
| 4 | 62.21 | 11.83 | 64.21 | 5.47 | 79.06 | 9.03 | 153.07 | 22.83 |
| 8 | 122.12 | 16.62 | 127.48 | 12.49 | 206.58 | 33.44 | 263.38 | 23.17 |
| 24 | 331.10 | 29.48 | 341.83 | 26.13 | 521.18 | 52.70 | 636.49 | 58.01 |
| 48 | 590.83 | 69.12 | 611.77 | 69.39 | 906.95 | 79.86 | 1088.22 | 72.69 |
| 72 | 774.00 | 97.61 | 805.34 | 95.65 | 1164.01 | 47.45 | 1410.67 | 51.47 |

**KET011**

| | *Franz diffusion apparatus* | | | | *European diffusion apparatus* | | | |
|---|---|---|---|---|---|---|---|---|
| Time | HPLC | | UV | | HPLC | | UV | |
| (hours) | $A_{CUM}$ | SD | $A_{CUM}$ | SD | $A_{CUM}$ | SD | $A_{CUM}$ | SD |
| 0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 2 | 20.76 | 5.03 | 24.54 | 5.53 | 11.93 | 5.56 | 73.35 | 14.68 |
| 4 | 37.92 | 9.60 | 43.83 | 10.50 | 45.31 | 11.44 | 90.79 | 18.14 |
| 8 | 70.35 | 17.91 | 78.52 | 19.10 | 119.74 | 20.74 | 197.74 | 111.23 |
| 24 | 184.61 | 60.77 | 196.73 | 62.12 | 373.47 | 71.09 | 433.66 | 97.81 |
| 48 | 336.03 | 125.55 | 369.21 | 118.76 | 680.16 | 102.02 | 784.40 | 105.53 |
| 72 | 504.73 | 173.49 | 546.40 | 172.25 | 957.91 | 135.91 | 1104.37 | 123.75 |

**KET012**

| | *Franz diffusion apparatus* | | | | *European diffusion apparatus* | | | |
|---|---|---|---|---|---|---|---|---|
| Time | HPLC | | UV | | HPLC | | UV | |
| (hours) | $A_{CUM}$ | SD | $A_{CUM}$ | SD | $A_{CUM}$ | SD | $A_{CUM}$ | SD |
| 0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 2 | 87.59 | 12.82 | 38.68 | 12.57 | 56.57 | 34.63 | 0.00 | 0.00 |
| 4 | 154.68 | 23.40 | 56.56 | 24.70 | 99.77 | 34.44 | 0.00 | 0.00 |
| 8 | 259.22 | 41.54 | 112.57 | 45.57 | 185.24 | 66.90 | 15.37 | 34.38 |
| 24 | 563.87 | 84.03 | 375.46 | 88.39 | 465.15 | 162.11 | 277.25 | 134.87 |
| 48 | 892.66 | 143.04 | 666.92 | 142.81 | 917.04 | 260.61 | 844.86 | 217.47 |
| 72 | 1089.29 | 170.85 | 823.32 | 168.82 | 1347.58 | 200.22 | 1310.14 | 201.54 |

**KET013**

| | *Franz diffusion apparatus* | | | | *European diffusion apparatus* | | | |
|---|---|---|---|---|---|---|---|---|
| Time | HPLC | | UV | | HPLC | | UV | |
| (hours) | $A_{CUM}$ | SD | $A_{CUM}$ | SD | $A_{CUM}$ | SD | $A_{CUM}$ | SD |
| 0 | 0.00 | 0.00 | 0.00 | 0.00 | | | | |
| 2 | 1.58 | 0.46 | 9.56 | 3.84 | | | | |
| 4 | 2.61 | 0.76 | 16.54 | 6.91 | | | | |
| 8 | 5.43 | 1.48 | 24.39 | 8.89 | | | | |
| 24 | 17.99 | 4.61 | 41.38 | 12.97 | | | | |
| 48 | 38.51 | 13.66 | 71.02 | 21.51 | | | | |
| 72 | 65.50 | 28.11 | 105.62 | 36.96 | | | | |

**KET014**

| | *Franz diffusion apparatus* | | | | *European diffusion apparatus* | | | |
|---|---|---|---|---|---|---|---|---|
| Time | HPLC | | UV | | HPLC | | UV | |
| (hours) | $A_{CUM}$ | SD | $A_{CUM}$ | SD | $A_{CUM}$ | SD | $A_{CUM}$ | SD |
| 0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 2 | 12.53 | 5.03 | 23.59 | 7.84 | 1.21 | 3.64 | 255.40 | 119.11 |
| 4 | 23.08 | 10.57 | 42.29 | 14.33 | 20.09 | 6.73 | 215.59 | 47.32 |
| 8 | 44.87 | 21.07 | 72.22 | 30.01 | 64.81 | 15.35 | 226.10 | 72.31 |
| 24 | 110.31 | 48.10 | 147.32 | 60.21 | 226.35 | 41.15 | 442.20 | 68.35 |
| 48 | 198.62 | 75.44 | 245.59 | 88.25 | 461.50 | 57.71 | 716.36 | 73.06 |
| 72 | 296.71 | 90.97 | 351.91 | 104.42 | 674.62 | 58.06 | 1004.90 | 102.05 |

**KET015**

| | *Franz diffusion apparatus* | | | | *European diffusion apparatus* | | | |
|---|---|---|---|---|---|---|---|---|
| Time | HPLC | | UV | | HPLC | | UV | |
| (hours) | $A_{CUM}$ | SD | $A_{CUM}$ | SD | $A_{CUM}$ | SD | $A_{CUM}$ | SD |
| 0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 2 | 14.71 | 5.01 | 23.66 | 3.14 | 0.00 | 1.07 | 216.27 | 119.46 |
| 4 | 28.69 | 9.92 | 43.78 | 2.65 | 7.57 | 5.19 | 163.66 | 55.78 |
| 8 | 74.73 | 46.39 | 74.81 | 11.65 | 38.06 | 11.13 | 173.05 | 54.89 |
| 24 | 159.23 | 54.40 | 166.11 | 37.66 | 158.26 | 35.96 | 308.96 | 89.00 |
| 48 | 253.70 | 76.48 | 271.40 | 65.62 | 318.91 | 61.08 | 495.77 | 50.80 |
| 72 | 363.61 | 89.54 | 392.07 | 80.90 | 469.31 | 79.78 | 750.57 | 92.14 |

**KET016**

| Time | *Franz diffusion apparatus* | | | | *European diffusion apparatus* | | | |
|---|---|---|---|---|---|---|---|---|
| | HPLC | | UV | | HPLC | | UV | |
| (hours) | $A_{CUM}$ | SD | $A_{CUM}$ | SD | $A_{CUM}$ | SD | $A_{CUM}$ | SD |
| 0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 2 | 91.09 | 15.09 | 94.94 | 12.97 | 52.93 | 13.40 | 158.98 | 46.86 |
| 4 | 146.50 | 21.72 | 150.14 | 17.34 | 96.61 | 21.31 | 199.79 | 60.83 |
| 8 | 235.81 | 35.19 | 239.65 | 33.13 | 178.30 | 35.44 | 241.10 | 41.03 |
| 24 | 529.85 | 120.87 | 535.59 | 116.88 | 478.19 | 91.55 | 552.15 | 98.17 |
| 48 | 932.48 | 234.93 | 861.41 | 246.19 | 961.85 | 178.01 | 1069.24 | 164.51 |
| 72 | 1291.90 | 257.54 | 1234.17 | 243.26 | 1530.81 | 247.50 | 1687.03 | 237.02 |

**KET017**

| Time | *Franz diffusion apparatus* | | | | *European diffusion apparatus* | | | |
|---|---|---|---|---|---|---|---|---|
| | HPLC | | UV | | HPLC | | UV | |
| (hours) | $A_{CUM}$ | SD | $A_{CUM}$ | SD | $A_{CUM}$ | SD | $A_{CUM}$ | SD |
| 0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 2 | 20.65 | 5.51 | 24.44 | 2.21 | 2.14 | 4.17 | 73.61 | 34.59 |
| 4 | 35.18 | 5.76 | 39.69 | 3.53 | 28.03 | 11.51 | 94.68 | 45.93 |
| 8 | 68.78 | 9.67 | 76.27 | 10.12 | 90.17 | 24.84 | 165.41 | 16.06 |
| 24 | 200.53 | 32.29 | 217.70 | 37.17 | 391.47 | 89.47 | 484.06 | 92.01 |
| 48 | 413.12 | 70.44 | 443.56 | 72.01 | 792.85 | 79.60 | 923.62 | 92.36 |
| 72 | 637.57 | 84.55 | 681.91 | 91.36 | 1095.63 | 73.55 | 1250.62 | 77.35 |

**KET018**

| Time | *Franz diffusion apparatus* | | | | *European diffusion apparatus* | | | |
|---|---|---|---|---|---|---|---|---|
| | HPLC | | UV | | HPLC | | UV | |
| (hours) | $A_{CUM}$ | SD | $A_{CUM}$ | SD | $A_{CUM}$ | SD | $A_{CUM}$ | SD |
| 0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 2 | 31.12 | 9.75 | 34.51 | 9.62 | 48.62 | 11.50 | 106.26 | 10.41 |
| 4 | 72.11 | 23.40 | 77.72 | 23.60 | 91.64 | 13.10 | 158.90 | 17.38 |
| 8 | 174.90 | 48.40 | 180.95 | 54.34 | 248.37 | 35.51 | 342.46 | 36.91 |
| 24 | 522.56 | 148.76 | 547.77 | 153.52 | 862.98 | 88.52 | 962.35 | 79.49 |
| 48 | 893.75 | 201.50 | 941.99 | 201.88 | 1343.83 | 106.85 | 1492.07 | 128.72 |
| 72 | 1099.57 | 199.09 | 1155.07 | 196.23 | 1620.25 | 114.85 | 1825.72 | 102.00 |

**KET019**

| Time | *Franz diffusion apparatus* | | | | *European diffusion apparatus* | | | |
|---|---|---|---|---|---|---|---|---|
| | HPLC | | UV | | HPLC | | UV | |
| (hours) | $A_{CUM}$ | SD | $A_{CUM}$ | SD | $A_{CUM}$ | SD | $A_{CUM}$ | SD |
| 0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 2 | 72.30 | 19.10 | 78.65 | 17.95 | 82.93 | 32.06 | 172.59 | 44.39 |
| 4 | 134.86 | 36.22 | 145.58 | 34.19 | 185.36 | 61.70 | 267.40 | 67.02 |
| 8 | 225.99 | 64.72 | 242.68 | 62.60 | 360.79 | 110.36 | 448.99 | 110.00 |
| 24 | 435.38 | 135.74 | 457.86 | 134.89 | 832.21 | 164.34 | 909.70 | 169.76 |
| 48 | 652.75 | 188.27 | 685.61 | 183.43 | 1195.60 | 137.12 | 1308.18 | 166.19 |
| 72 | 803.03 | 200.14 | 844.92 | 195.61 | 1395.59 | 115.04 | 1644.93 | 120.36 |

**KET020**

| Time | *Franz diffusion apparatus* | | | | *European diffusion apparatus* | | | |
|---|---|---|---|---|---|---|---|---|
| | HPLC | | UV | | HPLC | | UV | |
| (hours) | $A_{CUM}$ | SD | $A_{CUM}$ | SD | $A_{CUM}$ | SD | $A_{CUM}$ | SD |
| 0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 2 | 8.60 | 0.46 | 2.53 | 5.46 | 0.00 | 4.71 | 0.00 | 0.03 |
| 4 | 17.90 | 2.11 | -0.13 | 5.55 | 8.72 | 10.97 | 0.00 | 0.03 |
| 8 | 48.58 | 11.78 | 12.34 | 8.47 | 51.11 | 24.29 | 10.21 | 26.27 |
| 24 | 244.35 | 66.49 | 191.86 | 61.77 | 364.40 | 101.61 | 325.63 | 99.17 |
| 48 | 564.48 | 105.50 | 507.41 | 105.70 | 919.33 | 156.81 | 895.11 | 135.09 |
| 72 | 824.78 | 106.10 | 765.68 | 106.96 | 1268.45 | 134.70 | 1271.25 | 129.35 |

# APPENDIX IV
# PODIUM AND POSTER PRESENTATIONS

## PODIUM PRESENTATION AT CONFERENCES

1. The effect of propylene glycol on the *in vitro* release of ibuprofen from extemporaneous cream formulations.
   RNO Tettey-Amlalo and JM Haigh.
   25th Annual Academy Pharmaceutical Congress, Grahamstown, South Africa, 2004.
2. An investigation into the effect of Pemulen® TR1 NF on the *in vitro* release rate of ketoprofen from extemporaneous Carbopol® 980 NF formulations.
   RNO Tettey-Amlalo and JM Haigh.
   26th Annual Academy Pharmaceutical Congress, Port Elizabeth, South Africa, 2005.

## POSTER PRESENTATION AT CONFERENCES

1. Comparison of the *in vitro* release of ketoprofen from extemporaneously prepared gels containing different concentrations of Carbopol® polymers.
   RNO Tettey-Amlalo and JM Haigh.
   65th International Congress of the International Pharmaceutical Federation, Cairo, Egypt, 2005.
2. Comparative *in vitro* release of ketoprofen from commercial gel formulations through silicone membrane utilizing European Pharmacopoeia and Franz diffusion cells.
   RNO Tettey-Amlalo and JM Haigh.
   65th International Congress of the International Pharmaceutical Federation, Cairo, Egypt, 2005.
3. Comparative *in vitro* release of ketoprofen and ibuprofen from commercially available topical gels from three countries.
   RNO Tettey-Amlalo, CH Purdon and JM Haigh.
   65th International Congress of the International Pharmaceutical Federation, Cairo, Egypt, 2005.
4. An investigation into the *in vitro* release rate of ketoprofen utilising different grades of Carbopol® polymers in extemporaneous formulations.
   RNO Tettey-Amlalo and JM Haigh.
   26th Annual Academy Pharmaceutical Congress, Port Elizabeth, South Africa, 2005.

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