Formulation and evaluation of different transdermal delivery systems with flurbiprofen as marker

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This dissertation was written in the so-called article format. Lindi van Zyl was the primary author of this dissertation which includes an introductory chapter with sub-chapters, a full length article for publication in a pharmaceutical journal and annexures containing experimental results and discussions. The work was carried out under the supervision and assistance of Dr. J.M. Viljoen and Prof. J. du Plessis. The article contained in this dissertation, is to be submitted for publication in the International Journal of Pharmaceutics, of which the complete guide for authors is contained in Annexure D.

Love all, trust a few, do wrong to none.

William Shakespeare

ABSTRACT

The aim of this study was to investigate the effect of different penetration enhancers containing essential fatty acids (EFAs) on the transdermal delivery of flurbiprofen. Flurbiprofen was used as a marker / model compound. Fatty acids were chosen as penetration enhancers for their ability to reversibly increase skin permeability through entering the lipid bilayers and disrupting their ordered domains. Fatty acids are natural, non-toxic compounds (Karande & Mitragotri, 2009:2364). Evening primrose oil, vitamin F and Pheroid™ technology all contain fatty acids and were compared using a cream based-formulation. This selection was to ascertain whether EFAs exclusively, or EFAs in a delivery system, would have a significant increase in the transdermal delivery of a compound.

For an active pharmaceutical ingredient (API) to be effectively delivered transdermally, it has to be soluble in lipophilic, as well as hydrophilic mediums (Naik *et al.*, 2000:319; Swart *et al.*, 2005:72). This is due to the intricate structure of the skin, where the *stratum corneum* (outermost layer) is the primary barrier, which regulates skin transport (Barry, 2001:102; Moser *et al.*, 2001:103; Venus *et al.*, 2010:469). Flurbiprofen is highly lipophilic (log P = 4.24) with poor aqueous solubility. It has a molecular weight lower than 500 g/mol indicating that skin permeation may be possible, though the high log P indicates that some difficulty is to be expected (Dollery, 1999:F126; Hadgraft, 2004:292; Swart *et al.*, 2005:72; Karande & Mitragotri, 2009:2363; Drugbank, 2012).

In vitro transdermal diffusion studies (utilising vertical Franz diffusion cells) were conducted, using donated abdominal skin from Caucasian females. The studies were conducted over 12 h with extractions of the receptor phase every 2 h to ensure sink conditions. Prior to skin diffusion studies, membrane release studies were performed to determine whether the API was released from the formulation. Membrane release studies were conducted over 6 h and extractions done hourly. Tape stripping experiments were performed on the skin circles after 12 h diffusion studies to determine the concentration flurbiprofen present in the *stratum corneum* and dermis-epidermis. The flurbiprofen concentrations present in the samples were determined using high performance chromatography and a validated method.

Membrane release results indicated the following rank order for flurbiprofen from the different formulations: vitamin F > control > evening primrose oil (EPO) >> Pheroid™. The control formulation contained only flurbiprofen and no penetration enhancers. Skin diffusion results on

the other hand, indicated that flurbiprofen was present in the *stratum corneum* and the dermis-epidermis. The concentration flurbiprofen present in the receptor phase of the Franz cells (representing human blood) followed the subsequent rank order: EPO > control > vitamin F >> Pheroid™. All the formulations stipulated a lag time shorter than that of the control formulation (1.74 h), with the EPO formulation depicting the shortest (1.36 h). The control formulation presented the highest flux (8.41 µg/cm^2.h), with the EPO formulation following the closest (8.12 µg/cm^2.h).

It could thus be concluded that fatty acids exclusively, rather than in a delivery system, had a significant increase in the transdermal delivery of flurbiprofen.

Keywords: Fatty acid, transdermal, flurbiprofen, vitamin F, evening primrose oil, Pheroid™

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UITTREKSEL

Die doel van hierdie studie was om die effek van verskillende penetrasie-bevorderaars, wat essensiële vetsure (EFA's) bevat, op die transdermale aflewering van flurbiprofeen te ondersoek. Flurbiprofeen is as 'n merker- / model-geneesmiddel gebruik. Vetsure is gekies as penetrasie-bevorderaars aangesien hulle die vermoë het om die vel se deurlaatbaarheid omkeerbaar te verhoog deur in die lipied-dubbelvetlae in te dring en die geordende areas te ontwrig. Vetsure word beskou as ideale penetrasie-bevorderaars omrede hulle natuurlike, nie-toksiese middels is (Karande & Mitragotri, 2009:2364). Nagkersolie, vitamien F en Pheroid™-afleweringstegnologie bevat almal vetsure. Hierdie penetrasie-bevorderaars is vergelyk deur van 'n room-gebaseerde formulering gebruik te maak en is gebruik om vas te stel of die EFA's alleen, of EFA's in 'n afleweringsisteem 'n kenmerkende verhoging in die transdermale aflewering van 'n geneesmiddel sal toon.

Vir 'n geneesmiddel om effektief transdermaal afgelewer te word, moet dit in beide lipofiliese en hidrofiliese oplosmiddels oplosbaar wees (Naik *et al.*, 2000:319; Swart *et al.*, 2005:72). Dit is as gevolg van die ingewikkelde struktuur van die vel, met die *stratum corneum* (buitenste laag) as die primêre versperring wat deurgang van stowwe deur die vel reguleer (Barry, 2001:102; Moser *et al.*, 2001:103; Venus *et al.*, 2010:469). Flurbiprofeen is hoogs lipofilies (log P = 4.24) en swak wateroplosbaar. Dit het 'n molekulêre massa laer as 500 g/mol, wat daarop dui dat dit deur die vel kan diffundeer, alhoewel die hoë log P aandui dat probleme verwag kan word (Dollery, 1999:F126; Hadgraft, 2004:292; Swart *et al.*, 2005:72; Karande & Mitragotri, 2009:2363; Drugbank, 2012).

In vitro transdermale afleweringstudies (deur die gebruik van vertikale Franz-selle) is uitgevoer deur gebruik te maak van abdominale, vroulike, kaukasiese vel. Die studies is uitgevoer oor 'n tydperk van 12 h, met onttrekking van die reseptorfase elke 2 h, om "sink"-toestande te behou. Voordat vel-deurlaatbaarheidstudies uitgevoer is, is membraan-vrystellingstudies onderneem om vas te stel of die geneesmiddel vanuit die formulering vrygestel word. Membraan-vrystellingstudies is onderneem oor 'n tydperk van 6 h met ontrekkings elke uur. "Tape stripping"-eksperimente is uitgevoer op die velsirkel na afhandeling van die 12 h diffusiestudies om die konsentrasie flurbiprofeen in die *stratum corneum* en die dermis-epidermis vas te stel. Die flurbiprofeen-konsentrasies teenwoordig in die monsters is bepaal deur van hoëdruk-vloeistofchromatografie en 'n bevestigde metode gebruik te maak.

Membraan-vrystellingsresultate het die volgende orde vir flurbiprofeen vanuit die verskillende formulering getoon: vitamien F > kontrole > nagkersolie (EPO) >> Pheroid™. Die kontrole-formulering het slegs flurbiprofeen bevat en geen deurdringing-bevorderaars nie. Vel-diffusieresultate het egter getoon dat flurbiprofeen in die *stratum corneum* en die dermis-epidermis teenwoordig was. Die konsentrasie flurbiprofeen teenwoordig in die reseptorfase van die Franz-selle (verteenwoordigend van menslike bloed) het die volgende orde getoon: EPO > kontrole > vitamien F >> Pheroid™. Al die formulerings het 'n sloertyd korter as dié van die kontrole (1.74 h) getoon, terwyl die EPO-formulering die kortste was (1.36 h). Die kontrole het die grootste vloei getoon (8.41 µg/cm^2.h) met die EPO-formulering wat kort daarop volg (8.12 µg/cm^2.h).

Ten slotte kan dit dus gestel word dat vetsure alleen, eerder as in 'n afleweringsisteem, 'n noemenswaarlike toename in die transdermale aflewering van flurbiprofeen toon.

Sleutelwoorde: Vetsure, transdermaal, flurbiprofeen, vitamien F, nagkersolie, Pheroid™

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CHAPTER 1

INTRODUCTION AND PROBLEM STATEMENT

1.1 INTRODUCTION

Human skin consists mainly of two layers, namely the dermis and epidermis. The dermis is externally connected with the epidermis; and internally with the subcutaneous tissue. The epidermis can be divided mainly into four layers, namely: *stratum basale*, *stratum spinosum*, *stratum granulosum* and the *statum corneum* (Barry, 1983:2; Roy, 1997:140; Suhonen *et al.*, 1999:150; Hadgraft, 2001:1; Williams, 2003:5-9; Yamashita & Hashida, 2003:1187; Venus *et al.*, 2010:469).

For an active pharmaceutical ingredient (API) to be effectively delivered transdermally, it has to be soluble in both lipophilic and hydrophilic mediums (Naik *et al.*, 2000:319; Swart *et al.*, 2005:72). These specifications are due to the complicated structure of the skin, where the *stratum corneum* is the primary barrier which regulates skin transport (Barry, 2001:102; Moser *et al.*, 2001:103; Venus *et al.*, 2010:469). Generally, the accepted range of the partition coefficient (log P) for optimal permeation is between 1 and 3. The optimal molecular weight of an API to diffuse through the skin is less than 500 g/mol (Hadgraft, 2004:292; Swart *et al.*, 2005:72; Karande & Mitragotri, 2009:2363). Flurbiprofen has a molecular weight of 244.3 g/mol and a log P of 4.24 (Dollery, 1999:F126; Drugbank, 2012). This indicates that flurbiprofen should diffuse through the skin, but with some difficulty.

Flurbiprofen is an effective non-selective cyclooxygenase (COX) inhibitor, antipyretic and anti-inflammatory agent (Dollery, 1999:F126; Fang *et al.*, 2003:153; Swart *et al.,* 2005:71). The physicochemical properties of flurbiprofen are summarised in Table 1.1. Previous studies conducted on the transdermal delivery of flurbiprofen proved that it penetrated through the skin (Swart *et al.*, 2005:77; Ambade *et al.*, 2008:36).

Table 1.1: ***Chemistry and pharmacokinetics of flurbiprofen (Dollery, 1999:F126; van Sorge et al., 1999:91; Vallender, 2011; Drugbank, 2012)***

Physical and chemical characteristics of flurbiprofen	Value / description
IUPAC name	2-(3-fluoro-4-phenylphenyl)propanoic acid
Chemical name	2-Fluoro-α-methyl[1,1'-biphenyl]-4-acetic acid
Chemical formula	$C_{15}H_{13}FO_2$
Molecular weight	244.3 g/mol
Melting point	114 – 117 ℃
Log P	4.24
pK_a	4.22
Protein binding	> 99% (primarily to albumin)
Half life	6 h
Volume of distribution	0.12 ℓ/kg
Peak plasma concentration	1 – 2 h

Flurbiprofen, a propionic acid derivative (Figure 1.1), will operate as a marker or model compound during the transdermal delivery studies. In this study penetration enhancers will be included in the formulation to determine if there is a difference in the amount of drug diffusing through the skin, compared to when no penetration enhancers have been incorporated. The penetration enhancers that will be used are evening primrose oil, vitamin F and Pheroid™ technology, since they all contain essential fatty acids (EFAs) which are natural, non-toxic oils.

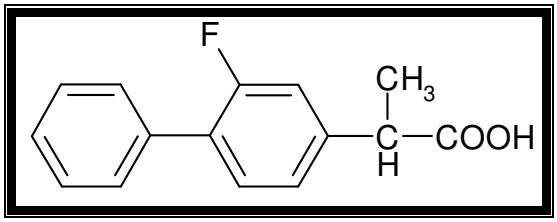

Figure 1.1: ***Chemical structure of flurbiprofen (modified from Dollery, 1999:F126; Burke et al., 2006:699)***

Evening primrose oil consists mainly of linoleic (65 - 80%), γ-linolenic (8 – 14%) and oleic acid (6 – 11%) which are collectively called fatty acids (Christie, 1999:74-75). Essential fatty acids (EFAs) are often referred to as vitamin F. These EFAs are unsaturated (contains one or more double bonds in the hydrocarbon chain) and can only be acquired through diet (Lockwood &

Kiselica, 2010:219). Vitamin F consists mainly of linoleic acid (35.5%), linolenic acid (30.5%) and oleic acid (21.4%) (Chemimpo, 2004).

Pheroid™ technology is a patented drug delivery system consisting of polyunsaturated fatty acids, which include omega-3 and omega-6 fatty acids but it excludes arachidonic acid. The fatty acids used to produce Pheroid™ are emulsified in nitrous oxide saturated water (Grobler *et* al., 2008:286; Du Plessis *et al.*, 2010:182), and dispersed in a dispersion medium. However, this technology also contains a dispersed gas phase (nitrous oxide), giving it an edge as it thus has three phases, namely a water phase, an oil phase, and a gas phase. The gas phase contributes to the stability and the self-assembly process of the Pheroid™ (Grobler *et al.*, 2008:288-289).

Fatty acids enhance permeation through entering the lipid bilayers and disrupting their ordered domains. They can improve API partitioning into the *stratum corneum* and can form lipophilic complexes with them (Karande & Mitragotri, 2009:2364). Fatty acids can be used to enhance the permeation of both lipophilic and hydrophilic APIs although the flux of polar compounds is improved to a larger degree (Williams, 2003:92).

It is clear that formulating a product to diffuse through the skin will present many difficulties. Penetration enhancers were chosen to minimise these difficulties. Essential fatty acids have a wide range of attributes and are non-toxic, making them optimal penetration enhancers. Even on their own, EFAs can be used for the treatment of many different diseases (e.g. eczema, rheumatoid arthritis, psoriasis, heart disease and high cholesterol). Pheroid™ technology also mainly consists of EFAs and has shown no immune responses in humans. Pheroids™ enhance bio-availability of various APIs and cause no cytotoxicity, also making it an optimal choice as drug delivery vehicle (Grobler, 2008:6).

1.2 RESEARCH PROBLEM, AIM AND OBJECTIVES

The key problem with effective transdermal delivery of an API is the excellent barrier function of the skin. This is a result of the complex route the permeant has to follow through the structured layers of the skin. Hydrophilic and lipophilic characteristics are necessary for optimal permeation through the skin, which not all compounds possess. Flurbiprofen is a lipophilic API, ensuring that some difficulty should be expected with transdermal delivery.

To improve flux, the barrier of the skin needs to be temporarily weakened (Barry, 2001:106). An increase in skin permeability can be achieved by reversibly damaging the stratum corneum, or by altering its physicochemical nature, through using penetration enhancers (Barry, 1983:161).

The aim of this study was to investigate the effect of different penetration enhancers in a cream formulation on the transdermal delivery of flurbiprofen.

Objectives for this study include the following:

- The choice of appropriate components for the different formulations that will be investigated.
- Determining the effect which a change in the composition of the different formulations will have on transdermal drug delivery through *in vitro* diffusion studies.
- Formulating a cream containing either evening primrose oil or vitamin F as penetration enhancer. There will also be one formulation containing Pheroid™ technology.
- Determining the release rate of flurbiprofen (model compound) from the cream formulation through membrane release studies prior to the skin diffusion studies.
- Determining whether the fatty acid content improved delivery of flurbiprofen into the skin (topical), through conducting tape stripping experiments.
- Comparing transdermal diffusion results obtained from the four formulations to select the optimal penetration enhancer.

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CHAPTER 2

THE TRANSDERMAL DELIVERY OF FLURBIPROFEN AS A MODEL COMPOUND USING A COMBINATION OF FATTY ACIDS

2.1 INTRODUCTION

Transdermal delivery of active pharmaceutical ingredients (APIs) has many advantages compared to, for example, the oral and parenteral route. Advantages include:

- Avoiding difficulties with API absorption due to pH and API interactions in the gastrointestinal tract.
- Sidestepping the first-pass effect.
- Less risk and better compliance due to lack of pain and inconvenience of parenteral therapy.
- Dose changes for specific patient needs and self-regulation of doses by patient are easier.
- Effects of the API can be terminated immediately if adverse effects occur.
- Sustained API release over a certain time period and thus, avoiding peaks and troughs in serum levels (Ansel & Popovich, 1990:311; Roy, 1997:139; Thomas & Finnin, 2004: 697-698; Swart *et al*., 2005:72; Li *et al,.* 2006:542).

Flurbiprofen is a nonsteroidal anti-inflammatory drug (NSAIDs) with many adverse reactions of which the gastrointestinal effects are potentially life-threatening (Dollery, 1999:F127). Therefore, flurbiprofen is an ideal candidate to be delivered transdermally to increase patient compliance and reduce adverse effects. However, flurbiprofen has a log P value of 4.24 and a molecular weight of 244.3 g/mol (Table 1.1), which are not optimal for transdermal delivery (Dollery, 1999:F126; Alsarra *et al*., 2010:233). For this reason, chemical penetration enhancers were chosen to aid in the transdermal delivery of flurbiprofen, which was used only as a model compound.

2.2 TRANSDERMAL API DELIVERY

2.2.1 ANATOMY AND FUNCTION OF HUMAN SKIN

Human skin, the largest organ of the human body, consists of mainly two layers, namely the dermis and epidermis. The dermis is externally connected with the epidermis and internally with the subcutaneous tissue. The epidermis is avascular and is a terminally differentiated stratified epithelium which can be divided into mainly four layers, as demonstrated in Figure 2.1 (Barry, 1983:2; Hadgraft, 2001:1; Williams, 2003:5-9; Yamashita & Hashida, 2003:1187; Venus *et al.*, 2010:469).

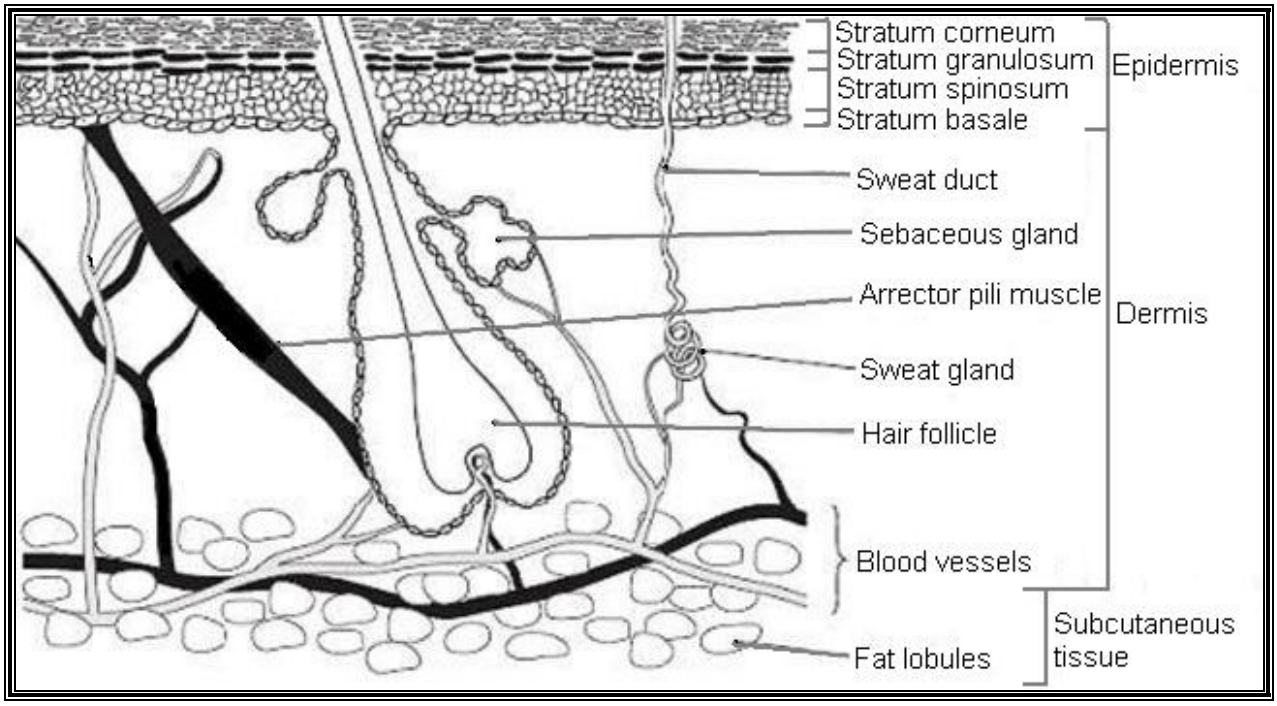

Figure 2.1: ***A diagrammatic representation of a cross-section through human skin (Williams, 2003:3)***

These layers, from the dermis outwards, are:

- The *stratum basale* (basal cell layer or stratum germinativum) which contains the only cells in the epidermis that undergo cell division and consist mainly of keratinocytes. Melanocytes (pigment cells) are also present in this layer (Williams, 2003:7; Venus *et al.*, 2010:469).
- The *stratum spinosum* (spinous layer or prickle cell layer) which consists of polyhedral cells that are connected by desmosomes (Venus *et al.*, 2010:469).
- The *stratum granulosum* (granular cell layer) that obtains keratinocytes from previous layers, which continue to differentiate and produce keratin and start to flatten. Enzymes

degrade viable cell components, for example, nuclei and organelles. These lipid components are discharged into the intercellular space which plays an important role in intercellular cohesion within the stratum corneum and also the barrier function of the skin (Williams, 2003:8; Venus *et al.*, 2010:469).

- The *stratum corneum* (horny layer) which is preceded by the *stratum lucidum* mainly on load-bearing areas such as the soles of the feet and palms of the hands. The *stratum corneum* is the outermost layer of the skin and is approximately only ten to fifteen cell layers thick (10 µm). The skin's barrier function is mainly ascribed to the *stratum corneum* because of its unique lipid composition and thus, its low water permeability (Roy, 1997:140; Suhonen *et al.*, 1999:150; Williams, 2003:9; Venus *et al.*, 2010:469).

Cells migrate from the *stratum granulosum* and are then called corneocytes since they have lost all their organelles, including their nuclei, and are cornified and condensed (Ghosh & Pfister, 1997:4-5). The corneocytes are connected by desmosomes and surrounded by multiple lipid bilayers, which are key in regulating API flux through the tissue (Williams, 2003:9-10; Venus *et al.*, 2010:469).

The dermis is connective tissue and consists of fibroblasts that synthesise collagen and elastin fibres. Collagen is a ground substance of polysaccharides and proteins which interact to produce hygroscopic proteoglycan macromolecules, whereas elastin fibres provide the skin with a degree of flexibility (Venus *et al.*, 2003:469). Williams (2003:4) stated that the dermis is vascular, which is essential in temperature regulation, oxygen and nutrients delivery to the tissue, and removal of waste products and toxins. Blood supply maintains the concentration gradient necessary to ensure constant API permeation through the skin (Yamashita & Hashida, 2003:1187). The sebaceous gland associated with hair follicles secretes sebum that lubricates and maintains the pH of the skin surface at approximately 5 (Williams, 2003:4). Some of the main functions of the skin are:

- Mechanical: It contains body fluids and tissues. The skin is elastic and can stretch from 1.1 to 1.5 times its original dimensions. However, the structural fibres change with age and the skin becomes rigid and wrinkled (Barry, 1983:15).
- Protective: The skin can function as a barrier against microorganisms, chemicals, radiation, heat, electricity and mechanical shock (Barry, 1983:16-20).
- Receives external stimuli to, for example, mediate sensation such as pain, pressure and heat.
- Regulates and maintains body temperature at 37℃ (Barry, 1983:14).

2.2.2 PERCUTANEOUS ABSORPTION

The *stratum corneum* is the rate-limiting barrier to the delivery of most molecules, whereas the viable epidermal membrane is the principal barrier to permeation for some highly lipophilic APIs. The three routes by which a molecule can cross intact skin (Figure 2.2), are the transappendageal, transcellular and intercellular pathways (Hadgraft, 2001:1; Williams, 2003:30-31).

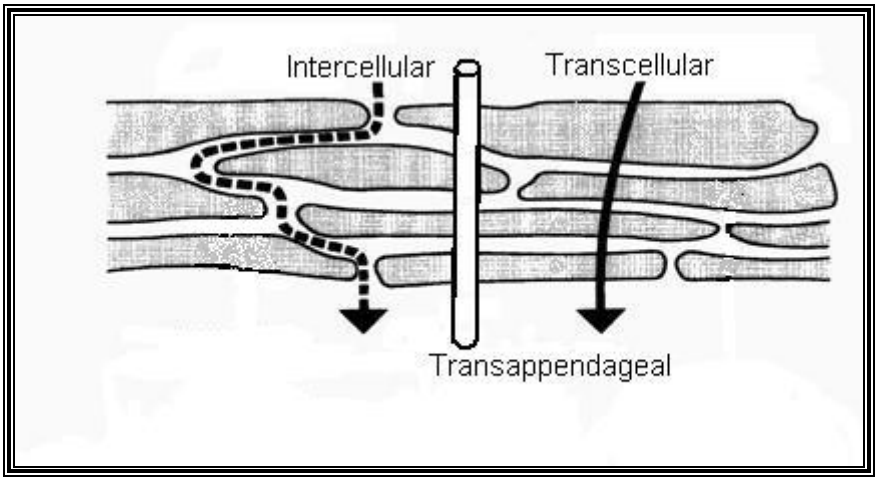

Figure 2.2: ***Permeation pathways through the skin (Modified from: Suhonen et al., 1999:152; Barry, 2001:102)***

Molecules following the transappendageal or shunt pathway, bypass the barrier of the *stratum corneum* by crossing appendages (hair follicles and sweat ducts). These openings only cover approximately 0.1% of the total skin surface and can, therefore, be regarded as insignificant, pertaining to their contribution to the total API flux at pseudo-steady state (Barry, 2001:101; Hadgraft, 2001:1; Moser *et al,* 2001:103-104; Williams, 2003:31). The shunt pathway can, nonetheless, be significant for molecules with a large molecular size when small doses are given (Suhonen *et al*., 1999:151; Williams, 2003:32).

The transcellular pathway is where molecules penetrate the corneocytes of the *stratum corneum* and it is often regarded as providing a polar route through the membrane (Roy, 1997:141; Williams, 2003:32). Diffusion of hydrophilic molecules following this route is swifter because of the aqueous environment the cellular components provide. This is essentially vastly hydrated keratin. However, the corneocytes are surrounded by lipid bilayers and that is why the substances crossing the skin must be partly hydrophilic and partly hydrophobic. Most of the steps needed for an API to cross hydrophilic and hydrophobic domains are unfavourable for various APIs (Williams, 2003:33).

Following the intercellular pathway, molecules have to diffuse through the lipid bilayers surrounding the corneocytes. This is believed to be the most popular route that small, uncharged molecules follow (Roy, 1997:141-142; Williams, 2003:34). The reason for this is because it provides the only continuous phase within the membrane. This pathway, however, is longer than the transcellular pathway, where the length is approximately the thickness of the *stratum corneum*. The intercellular pathway molecules follow, can be between 150 µm and 500 µm. This is dependent on the physicochemical properties of the substance (Williams, 2003:33-35).

2.2.2.1 FACTORS INFLUENCING PERCUTANEOUS PERMEATION

For a topically applied API to be able to produce the required effect, three processes are necessary. Firstly, the API has to be released from the API-vehicle. Secondly, it must penetrate through the barriers of the skin and thirdly, it must activate the desired pharmacological response (Barry, 1983:127).

2.2.2.1.1 Physiological factors

Changes caused to the skin by skin disorders will undoubtedly have a significant effect on the topical and transdermal delivery of APIs. However, some physiological factors will also affect the rate of API permeation in healthy skin (Williams, 2003:14).

It has been clear since the 1970s that the skin of a foetus, a young child and the elderly is more permeable than that of an adult (Barry, 1983:130). Skin of an elderly person may propose a less effective barrier, because it has more photo-damage and cuts, and bruises occur more easily. Aged skin furthermore heals slower than younger skin (Ademola & Maibach, 1997:203). During the ageing of human skin the moisture content decreases, altering API permeation (Williams, 2003:14-16).

Thickness of the *stratum corneum* can affect API permeation. It is not the sole parameter for determining skin permeability (Williams, 2003:16). Barry (1983:136) found, using the post-auricular (behind the ear) skin as a reference, that the following can affect the permeability of skin:

- Density and thickness of the *stratum corneum*.
- Amount of sebaceous and sweat glands per area.
- Proximity of capillaries to the surface of the skin.
- Temperature of the skin surface.

Variations do occur on the same body site between different individuals, and even on the same body site for one individual. For this reason, it is important to approach every patient as an individual (Williams, 2003:16-17). Enhanced skin permeation may also be induced through

- certain chemicals such as acids and alkalis;
- day to day cuts and bruises which injure the skin;
- diseased or damaged skin; as well as
- bacteria causing skin damage, including *Mycobacterium tuberculosis* and *Streptococcus pyogenes* (Barry, 1983:130; Ademola & Maibach, 1997:203-204; Williams, 2003:21).

Normal skin bacteria can decrease permeation due to API metabolism by the organisms on the skin before penetration can commence. This also depends on the area of the body. Bacterial species include Staphylococcal species, *micrococci*, *corynebacteria* and *propionibacteria* (Barry, 1983:130; Ademola & Maibach, 1997:203-204; Williams, 2003:21).

Metabolism of the API on the skin surface may occur which usually results in an inactive metabolite. Sometimes though, active compounds can be formed (Barry, 1983:136), for example batametasone-17-valerate from betametasone (Ademola & Maibach, 1997:204).

Changes in blood flow through the dermis can alter percutaneous absorption if transport across the *stratum corneum* is very swift, since the *stratum corneum* is the rate-limiting barrier. Vasoconstriction or reduced blood flow can theoretically decrease percutaneous absorption of the API (Barry, 1983:137; Ademola & Maibach, 1997:204).

2.2.2.1.2 Physicochemical factors

Many physicochemical factors affect skin permeation. Some of these factors are summarised in Table 2.1.

Table 2.1: ***The physicochemical factors influencing percutaneous absorption***

Physicochemical factor	Effect
Skin hydration	• Hydration of the stratum corneum promotes penetration of most APIs (Ademola &Maibach, 1997:204; Barry, 2001:105; Williams, 2003:17).
API-skin binding	• Some APIs may bind to components of the skin, hindering uptake into the circulation (Ademola & Maibach, 1997:204). • Interactions can vary from hydrogen bonding to Van der Waals forces. This is due to the diverse nature of skin components and possible variety within permeants (Williams, 2003:39).
Temperature	• Surface temperature of the skin is approximately 32°C (Ademola & Maibach, 1997:205; Williams, 2003:18). • The stratum corneum can withstand temperatures of 60 °C for a few hours, but temperatures above 65°C for longer than a minute can result in irreversible structural changes (Barry, 1983:159; Ademola & Maibach, 1997:205). These changes can increase diffusion through the skin (Williams, 2003:18).
pH	• A pH below 3 and above 9 can have irreversible structural damage to the stratum corneum, which in turn increases permeability (Barry, 1983:159; Naik *et al.*, 2000:319).
Molecular size and weight	• Small molecules permeate faster through the skin than larger molecules. • Molecules with a weight in the range of 100 – 500 g/mol have a minimal influence on API flux (Naik *et* al., 2000:319; Williams, 2003:36-37).
Diffusion coefficient	• Increased hydrogen bonding capacity can lead to decreased diffusivity (Barry, 1983:205; Williams, 2003:40).
Partition coefficient	• To obtain an acceptable steady-state penetration rate, the API must be readily soluble in water and *n*-octanol. • Molecules with a log $P_{(octanol/water)}$ of 1 to 3 have intermediate partition coefficients, therefore, have some solubility in the oil and water phases. • Molecules with a log P larger than 3 are highly lipophilic and will almost solely follow the intercellular route (Barry, 1983:207; Williams, 2003:36).
Melting point or Solubility	• Most organic molecules with high melting points have reasonably low aqueous solubilities at normal temperatures and pressures (Williams, 2003:37). A melting point lower than 200°C is preferred for skin permeation (Naik *et al.*, 2000:319).

2.2.2.2 MATHEMATICS OF SKIN PERMEATION

Skin diffusion is mostly passive, following random molecular movements, and predominantly following a route through the intercellular spaces. The skin is an extremely complex organ and it seems idealistic to describe this entire process with simple mathematical models. Using Fick's simple law, two main situations can be considered namely infinite and finite dosing (Barry, 1983:49-50; Williams, 2003:40-41; Yamashita & Hashida, 2003:1186; Hadgraft, 2004:292).

Infinite dosing is where the API concentration does not deplete and is applicable to a transdermal patch. This section will focus on finite dosing (transient permeation) where a pseudo-steady-state is not encountered and is applicable to semi-solids for local action (Williams, 2003: 41). Permeation through the skin is usually a passive diffusion process from a high concentration of API on the surface of the *stratum corneum* to a lower API concentration inside the dermis and epidermis. Mathematical models have been offered that suitably describe this kinetic process (Smith & Surber, 2000:23).

The most fundamental diffusion equation is Fick's law, which has regularly been applied to API permeation studies (Equation 2.1):

$$J = KD\frac{\Delta C}{h} \qquad \text{(Equation 2.1)}$$

Where

- J is the flux (µg/cm^2.h);
- K is the partition coefficient;
- D is the diffusion coefficient (cm^2/h);
- ΔC is the concentration difference (µg/cm^3) between the applied permeant in the vehicle and the permeant in the receptor phase; and
- h is the diffusional path length (cm) or membrane thickness (Smith & Surber, 2000:24; Wiliams, 2003:47; Hadgraft, 2004:292).

Fick's first law assumes that the rate of transfer of the diffusing substance through a unit area of a section, is proportional to the concentration gradient measured (Barry, 1983:50-51; Yamashita & Hashida, 2003:1187).

2.2.3 PENETRATION ENHANCERS

To improve the API flux, the barrier of the skin needs to be temporarily weakened (Barry, 2001:106). An increase in skin permeability can be achieved by reversibly damaging the *stratum corneum*, or by altering its physicochemical nature through using penetration enhancers (Barry, 1983:161).

2.2.3.1 DESIRABLE ATTRIBUTES OF PENETRATION ENHANCERS

Penetration enhancers should act temporarily and their effects on the *stratum corneum* should be reversible (Williams, 2003:86). Some of the ideal properties for penetration enhancers include:

- It should be pharmacologically inert (have no pharmacological activity).
- The enhancer should be non-toxic, non-allergenic and non-irritating.
- Onset of action should be immediate and the extent of the action should be predictable, reproducible and reversible.
- Barrier function should only decrease one-directionally; thus, endogenous materials should not be lost.
- It should be chemically and physically compatible with a wide range of APIs and it should be stable in a topical formulation.
- It should be cosmetically acceptable with a suitable skin “feel”.
- It should be tasteless, odourless and colourless.
- The agent should be released from the formulation and show a high degree of penetration enhancement.
- The compound should be inexpensive (Barry, 1983:160-161; Büyüktimkin *et al.*, 1997:359-360; Williams, 2003:86-87).

Though many chemicals have been evaluated as permeation enhancers, not one has proven to be ideal (Williams, 2003:86). Some permeation enhancers used in preparations are briefly described in the following sections.

2.2.3.2 CHEMICAL PENETRATION ENHANCERS

Chemical penetration enhancers assist the transfer of APIs through the skin by partitioning into the lipid bilayers and disrupting the lipid lamellae to bring forth temporary diminution of the barrier properties (Büyüktimkin *et al.*, 1997:358-359; Barry, 2001:106; Karande & Mitragotri, 2009:2364). This weakening of the *stratum corneum* is attributed to a subtle alteration of the solvent potential and provides an area of higher affinity for the permeant (Walker & Smith,

1996:299). Chemical penetration enhancers can also assist the transfer of APIs through the skin by extracting lipids from the skin, creating diffusion pathways (Karande & Mitragotri, 2009:2364). Since more than 300 chemical penetration enhancers have been studied, only a few will be discussed (Karande & Mitragotri, 2009:2363).

Water: Water is the most natural and safest of all the penetration enhancers with the least side effects (Williams, 2003:84; Karande & Mitragotri, 2009:2364). Reducing or preventing transepidermal water loss (TEWL) increases the water content of the *stratum corneum*, and thus increases API flux (Williams, 2003:84).

Hydrocarbons: Several hydrocarbons have been used as enhancers, including alkanes, alkenes, halogenated alkanes, mineral oil, squalane and squalene. These generally work through entering the *stratum corneum* and disrupting the ordered lipid bilayer structure (Karande & Mitragotri, 2009:2364).

Alcohols: Alcohols can be used as vehicles, solvents and penetration enhancers, and include alkanols (fatty alcohols), alkenols, glycerols, glycols and polyglycols. As solvents, alcohols can extract lipids and proteins from *stratum corneum* membranes (Suhonen, 1999:153; Williams, 2003:94-95; Karande & Mitragotri, 2009:2364).

Amines & amides: Primary, secondary, tertiary, cyclic and acyclic amines, as well as cyclic and acyclic amides can enhance API permeation by partitioning into the skin. Urea and its analogues usually act through the disruption of the lipid bilayers (Karande & Mitragotri, 2009:2364). It is also a hydrotrope and is keratolytic (Walker & Smith, 1996:298; Williams, 2003:98).

Azone: Azone was specifically designed as a penetration enhancer and displays many of the desirable attributes of an ideal penetration enhancer. It is the first synthetic permeation enhancer (Karande & Mitragotri, 2009:2364) and can be viewed as a hybrid between cyclic amide and alkylsulfoxide, but it does not have the disadvantages of dimethylsulfoxide (DMSO) due to the absent aprotic sulfoxide group (Williams, 2003:89).

Fatty acids: Fatty acids (such as oleic acid, lauric acid, linoleic acid and linolenic acid) enhance permeation through entering the lipid bilayers and disrupting their ordered domains. They can improve API partitioning into the *stratum corneum* and can form lipophilic complexes with APIs (Karande & Mitragotri, 2009:2364). Fatty acids can be used to enhance the permeation of both lipophilic and hydrophilic APIs though the flux of polar APIs is improved to a larger degree (Williams, 2003:92).

Esters: Esters of fatty acids, such as isopropyl myristate and ethyl acetate, show permeation enhancement to a wide variety of APIs. They generally work by partitioning into the ordered lipid domains of the *stratum corneum* and temporarily decreasing the barrier properties (Walker & Smith, 1996:298; Karande & Mitragotri, 2009:2364).

Surfactants: Anionic, cationic, zwitterionic and non-ionic surfactants have been pursued as skin penetration enhancers. Their activity, however, depends upon the hydrophilic to lipophilic balance, lipid tail length and charge (Büyüktimkin *et al.*, 1997:441; Karande & Mitragotri, 2009:2364). Cationic surfactants are more destructive to the skin, causing a higher API flux than anionic surfactants, whereas anionic surfactants cause a more significant increase in API flux than non-ionic surfactants (Walker & Smith, 1996:298). Surfactants are usually included in formulations to solubilise lipophilic active ingredients. They also have the ability to solubilise lipids in the *stratum corneum* (Williams, 2003:96).

Essential oils, terpenoids and terpenes: The effect of specific terpenes depends on their exact physicochemical properties, specifically their lipophilicity. Smaller terpenes with non-polar groups are generally better permeation enhancers (Karande & Mitragotri, 2009:2364). Terpenes increase permeation by increasing diffusivity of APIs into the *stratum corneum* (Walker & Smith, 1996:298). Essential oils (such as oils of eucalyptus, chenopodium and ylang-ylang for hydrophilic APIs) are complex combinations of aromatic and aliphatic chemicals containing several functional groups (Williams, 2003:99). For the increase in API flux of lipophilic APIs, the cyclic ethers and hydrocarbon terpenes such as d-limonene are effective (Williams, 2003:100).

Sulfoxides: Sulfoxides, of which DMSO is the earliest, improve API partitioning into the skin (Williams, 2003:87; Karande & Mitragotri, 2009:2365). It is believed that DMSO denatures the intercellular structural proteins of the *stratum corneum*, or promotes lipid fluidity by disruption of the ordered lipid bilayers (Walker & Smith, 1996:297). DMSO is effective as a penetration enhancer for both lipophilic and hydrophilic APIs. However, it is concentration-dependent and a concentration of 60% or more is required for optimum enhancement efficacy. Unfortunately, at these concentrations DMSO has overwhelming side effects reducing its clinical use (Williams, 2003:87-88). Decylmethylsulfoxide (DCMS) on the other hand, is a potent enhancer for hydrophilic APIs. Dimethylacetamide (DMAC) and dimethylformamide (DMF) have similar chemical structures as DMSO and thus have a wide range of activities. DMF however, causes irreversible membrane damage when used on human skin (Williams, 2003:88-89).

Phospholipids: Many studies have utilised phospholipids in the form of vesicles (liposomes), microemulsions or micellar systems to carry APIs into or through the skin (Williams, 2003:102; Karande & Mitragotri, 2009:2365). In these forms phospholipids can interact with the lipid bilayers, enhancing partitioning of encapsulated APIs, as well as disrupting the ordered bilayer structure (Karande & Mitragotri, 2009:2365).

Other groups of APIs which have been investigated as permeation enhancers include cyclic oligosaccharides (cyclodextrins), amino acids and thioacyl derivatives of amino acids, alkyl amino esters, oxazolidinones and ketones (Karande & Mitragotri, 2009:2365).

2.3 COMBINATION OF ESSENTIAL FATTY ACIDS USED AS CHEMICAL PENETRATION ENHANCERS

Essential fatty acids (EFAs) are excellent examples of fats that are beneficial to your health. The two main EFAs are linoleic (omega-6) and linolenic (omega-3) acid. Linoleic acid can be found in various plants and fish, whereas linolenic acid can be found in a variety of seeds. These fatty acids are called essential since they cannot be produced in the human body and need to be acquired through diet. Other fats that are beneficial include mono-unsaturated fats (olive oil, canola oil and peanut oil) and polyunsaturated fats (corn oil, safflower seed oil, sunflower seed oil and fish oil) (Di Pasquale, 2009:144-145).

2.3.1 EVENING PRIMROSE OIL

Evening primrose oil (EPO) consists of approximately 98% triacylglycerols (triglycerides), 0.05% phospholipids, and 1 – 2% unsaponifiable matter (sterols and tocopherols are of some importance). The typical fatty acid content of evening primrose oil is

- 65 – 80% linoleic acid ($C_{18:2n-6}$),
- 8 – 14% γ-linolenic acid (GLA) ($C_{18:3n-6}$),
- 6 – 11% oleic acid (omega-9) ($C_{18:1}$),
- 7 – 10% palmitic acid ($C_{16:0}$), and
- 1.5 – 3.5% stearic acid ($C_{18:0}$) (Christie, 1999:74-75).

Extensive crossbreeding of the varieties of evening primrose plants yielded a commercial variety that repeatedly offered oil with 72% linoleic acid and 9% GLA (Drug information online, 2012). Other minor fatty acids in evening primrose are myristic acid ($C_{14:0}$, 0.07%), palmitoleic acid (0.11%), vaccenic acid (0.84%), linolenic acid (0.18%), eicosanoic acid (0.31%), and eicosenoic acid (0.23%) (Christie, 1999:75). Some experts believe only linoleic acid is essential, since small amounts of linolenic acid can be produced from it. The GLA in EPO has a

wide variety of attributes making it an effective supplement. It aids skin, nails, and hair to appear healthier, though it works best on improving skin and hair dryness. GLA can assist in relieving PMS (pre-menstrual syndrome) symptoms, as well as mild eczema (Di Pasquale, 2009:147).

2.3.2 VITAMIN F

Essential fatty acids (EFAs) are often referred to as vitamin F. These EFAs are unsaturated, meaning it contains one or more double bonds in the hydrocarbon chain (Figures 2.3 and 2.4) (Lockwood & Kiselica, 2010:219). There are two basic categories of EFAs, namely omega-3 and omega-6. The name omega-3 is given to linolenic acid, since the first double carbon bond is at the third carbon atom (Figure 2.3) relative to the acid tail (named omega from the last letter of the Greek alphabet). Linoleic acid is named in the same way, where the first double carbon bond is at the sixth carbon atom (Figure 2.4). The omega-3 family (fatty acids derived from linolenic acid) include α-linolenic acid, eicosapentaenoic acid, and docosahexaenoic acid, whereas the omega-6 family (fatty acids derived from linoleic acid) include *cis*-linoleic acid, linoleic acid, and γ-linolenic acid (GLA) (Di Pasquale, 2009:144-152).

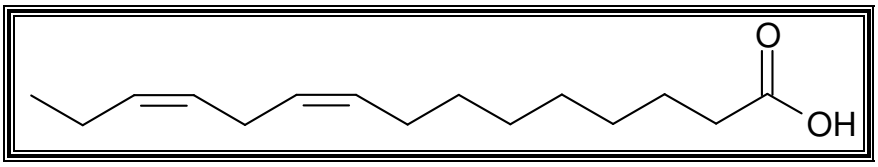

Figure 2.3: ***Chemical structure of linolenic acid (Omega-3) (Gunawardena, 2012).***

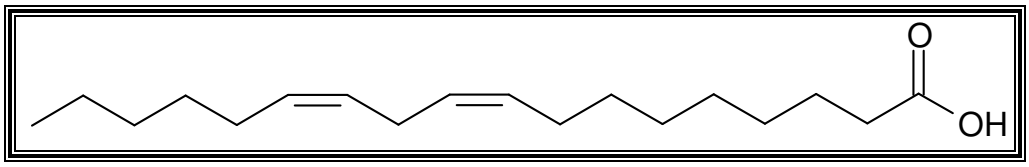

Figure 2.4: ***Chemical structure of linoleic acid (Omega-6) (Gunawardena, 2012).***

Vitamin F consists mainly of two EFAs, linoleic acid and α-linoleic acid (Table 2.2). Flaxseed oil, fish and seafood; vegetable oils such as canola oil and corn oil, avocados, raw nuts, and grape seed oil are some of the richest food sources containing vitamin F (Di Pasquale, 2009:145).

Table 2.2: ***Fatty acid content of vitamin F (Chemimpo, 2004).***

Fatty acid	Percentage (%)
< C_{18} + $C_{18:0}$	11.6
$C_{18:1}$ (Oleic acid)	21.4
$C_{18:2}$ (Linoleic acid)	35.5
$C_{18:3}$ (Linolenic acid)	30.5
> C_{18}	1

2.3.3 PHEROID™ TECHNOLOGY

Pheroid™ technology (based on Emzaloid™) is a patented API delivery system consisting of polyunsaturated fatty acids, which include omega-3 and omega-6 fatty acids, but it excludes arachidonic acid. The fatty acids used to produce Pheroid™ are emulsified in nitrous oxide saturated water (Grobler *et* al., 2008:286; Du Plessis *et al.*, 2010:182), and are dispersed in a dispersion medium. However, this technology also involves a dispersed gas phase (nitrous oxide), giving it an edge above other delivery systems, as it thus consists three phases namely, a water phase, an oil phase, and a gas phase. The gas phase contributes to the stability and the self-assembly process of the Pheroid™ (Grobler *et al.*, 2008:288-289).

2.3.3.1 STRUCTURAL CHARACTERISTICS OF THE PHEROID™ DELIVERY SYSTEM

The Pheroid™ delivery system is a colloidal system containing lipid-based micron-sized and submicron-sized structures, hereafter referred to as Pheroids. The Pheroids are distributed (dispersed phase) in a dispersion medium (continuous phase) and can be manipulated in terms of structure, size, morphology, and function. A typical colloidal system has particles of 1 – 100 nm in diameter, whereas Pheroids are 200 nm to 2 µm in diameter. These Pheroid sizes are determined by the amount of APIs that need to be entrapped, the administration route, and the delivery rate (Grobler *et al.*, 2008:284-285).

2.3.3.2 ADVANTAGES OF PHEROIDS™

Pheroid™ technology is able to improve the absorption and efficacy of a variety of APIs and other components. This technology has the potential to entrap, protect and deliver both hydrophilic and lipophilic API molecules across a range of biological membranes (Grobler *et al.*, 2008:284; Du Plessis *et al.*, 2010:182).

2.4 FLURBIPROFEN AS A MODEL COMPOUND

Though NSAIDs are highly effective against pain and inflammation in various conditions, it has many side effects and should be used only if no other treatment options are suitable (Gibbon, 2008:369-370). For this reason other routes of delivery are pursued to minimise adverse effects caused by oral flurbiprofen therapy. Transdermal delivery is an effective alternative since it bypasses the gastrointestinal tract where most of this API's adverse effects occur.

2.4.1 PHYSICOCHEMICAL AND BIOPHARMACEUTICAL CHARACTERISTICS

Flurbiprofen is a propionic acid derivative and is an exceedingly effective NSAID (Dollery, 1999:F126; Burke *et al.*, 2006:698). Some of the physicochemical characteristics of flurbiprofen can be seen in Table 2.3 and the molecular structure in Figure 2.5.

Table 2.3: ***Physicochemical characteristics of flurbiprofen (Dollery, 1999:F126; Van Sorge et al., 1999:91; Vallender, 2011; Drugbank, 2012)***

Chemical characteristics	**Value**
Dissociation constant	pK_a 4.22 (van Sorge *et al.*, 1999:91)
Partition coefficient	Log P (octanol/water), 4.24 (Li *et al.*, 2006:542; Alsarra *et al.*, 2010:233)
Chemical name	2-Fluoro-αmethyl[1,1'-biphenyl]-4-acetic acid
Chemical formula	$C_{15}H_{13}FO_2$
Physical characteristics	**Value**
Molecular weight	244.3 g/mol
Solubility	Freely soluble in ethanol (96%) and methylene chloride. Practically insoluble in water.
Melting point	114℃ to 117℃.
Physical appearance	White or cream, crystalline powder.

A melting point lower than 200℃ and a solubility higher than 1 mg/ml are ideal for skin permeation (Naik *et al.*, 2000:319). Flurbiprofen has a very poor aqueous solubility which may not be ideal for skin permeation, though the melting point is within the stated parameters. The ideal partition coefficient is between 1 and 3, while flurbiprofen has a partition coefficient of 4.24. This is also not ideal for skin permeation (Hadgraft, 2004:292).

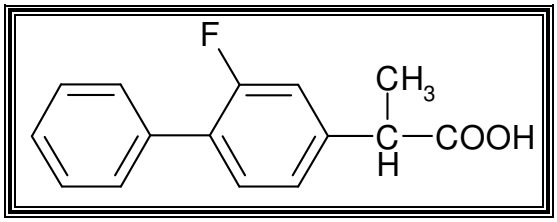

Figure 2.5: ***The molecular structure of flurbiprofen (modified from Dollery, 1999:F126; Burke et al., 2006:699)***

The biopharmaceutical characteristics can be seen in Table 2.4.

Table 2.4: ***Biopharmaceutical characteristics of flurbiprofen (Dollery, 1999:F126)***

Biopharmaceutical characteristics	Value
Bioavailability	> 95%
Half-life : range	2 – 6 h
Volume of distribution	0.1 ℓ.kg^{-1}
Clearance	95% excreted in urine within 24 h of administration, of which 25% is unchanged API.
Plasma protein binding	99%

2.4.2 MECHANISM OF ACTION

Flurbiprofen is a non-selective cyclooxygenase (COX) inhibitor which has potent anti-inflammatory, antipyretic and analgesic activity. A summarised representation of flurbiprofen's mechanism of action can be seen in Figure 2.6 (Dollery, 1999:F126; Trevor *et al*., 2005:307).

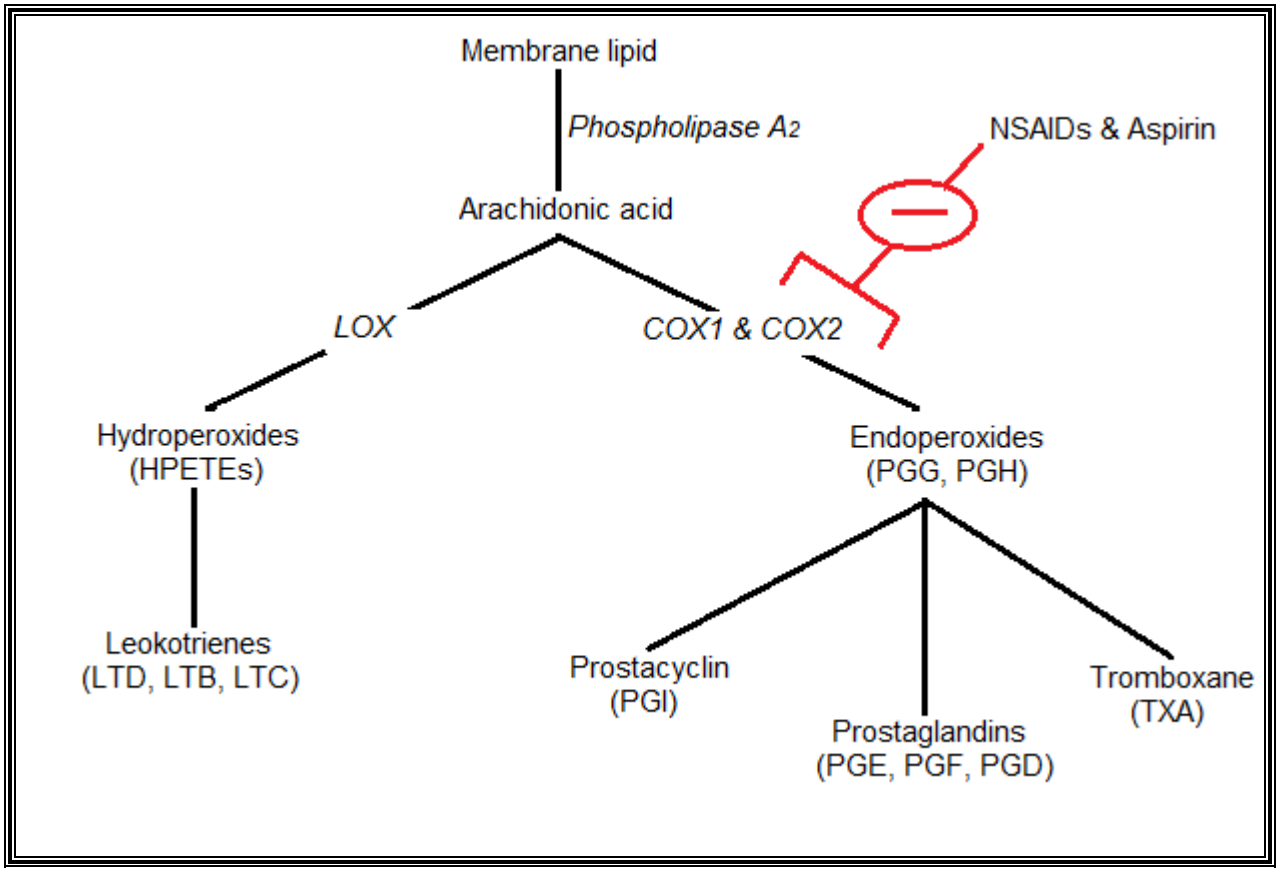

Figure 2.6: ***Simplified diagram of the metabolism of arachidonic acid (modified from Trevor et al., 2005:163)***

Tromboxane (TX), prostaglandins (PG) and prostacyclin (PGI) are produced from endoperoxide intermediates. Flurbiprofen inhibits the COX pathway and the metabolism directs to the lipoxygenase (LOX) pathway (Figure 2.6) and its effects may be more prominent (Dollery, 1999:F126; Burke *et al.*, 2006:673-674).

2.5 SUMMARY

The combination of the physicochemical properties of flurbiprofen and the barrier function of the *stratum corneum* indicates that flurbiprofen will permeate through the skin, but with some difficulty. Essential fatty acids (EFAs) were chosen for their ability to reversibly increase the skin permeability through entering the lipid bilayers and disrupting their ordered domains. Fatty acids are natural products with minimal side effects in humans.

It is clear that formulating a product to diffuse through the skin will present many difficulties. Penetration enhancers were chosen to minimise these difficulties. Essential fatty acids have a wide range of attributes and are non-toxic, making them optimal penetration enhancers. Even on their own, EFAs can be used in the treatment of many different diseases (e.g. eczema, rheumatoid arthritis, psoriasis, heart disease and high cholesterol) (Di Pasquale, 2009:147-

148). Pheroid™ technology consists mainly of EFAs and has shown no immune responses in humans. Pheroids™ enhance bio-availability and cause no cytotoxicity, also making it an optimal choice as API delivery vehicle (Grobler, 2008:306-309).

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CHAPTER 3

ARTICLE FOR PUBLICATION IN THE INTERNATIONAL JOURNAL OF PHARMACEUTICS

FORMULATION AND EVALUATION OF FLURBIPROFEN TRANSDERMAL DELIVERY SYSTEMS

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Abstract

The aim of this study was to investigate the effect of different penetration enhancers containing essential fatty acids (EFAs) on the transdermal delivery of flurbiprofen. Evening primrose oil (EPO), vitamin F and Pheroid™ technology all contain fatty acids and were compared using a cream-based formulation. This selection was to ascertain whether EFAs solely, or EFAs in a delivery system, would have a significant increase in the transdermal delivery of a compound. Membrane release studies were performed to determine whether the active pharmaceutical ingredient (API) would be released from the formulation. These release studies were performed on all the creams and the results indicated the following rank order for flurbiprofen concentrations released from the different formulations:
vitamin F > control > EPO >> Pheroid™. The control formulation contained only flurbiprofen and no penetration enhancers. Skin diffusion results, on the other hand, indicated that flurbiprofen was present in the *stratum corneum* and the dermis-epidermis. The concentration flurbiprofen present in the receptor phase (representing human blood) followed the subsequent rank order: EPO > control > vitamin F >> Pheroid™. All the formulations stipulated a lag time shorter than that of the control formulation (1.74 h), with the EPO formulation being the shortest (1.36 h). The control formulation presented the highest flux (8.41 µg/cm^2.h), with the EPO formulation following the closest (8.12 µg/cm^2.h). It could thus be concluded that EPO is the most acceptable chemical penetration enhancer when used in this cream formulation.

Keywords: Fatty acid, transdermal, flurbiprofen, vitamin F, evening primrose oil, Pheroid™

3.1 INTRODUCTION

The aim of this study was to investigate different penetration enhancers containing essential fatty acids (EFAs). EPO, vitamin F and Pheroid™ technology all contain fatty acids and were compared using cream-based formulations. This selection was to ascertain whether EFAs exclusively, or EFAs in a delivery system, would have a significant increase in the transdermal delivery of a compound.

Flurbiprofen, used as a reference, is a highly lipophilic compound with poor aqueous solubility. Generally, the accepted range of the partition coefficient (log P) for optimal permeation is between 1 and 3. The optimal weight of an API to diffuse through the skin is a molecular weight of less than 500 g/mol (Hadgraft, 2004; Karande & Mitragotri, 2009; Swart *et al.*, 2005). Flurbiprofen, with a molecular weight of 244.3 g/mol, has a log P of 4.24 (Dollery, 1999; Drugbank, 2012). This log P value indicates that the API is highly lipophilic. This is an implication that the API would diffuse through the skin, but with some difficulty, if applied onto the skin for transdermal delivery.

EPO consists of approximately 98% triacylglycerols (triglycerides), 0.05% phospholipids, and 1 – 2% unsaponifiable matter (sterols and tocopherols are of some importance). The key contents of EPO are linoleic acid, γ-linolenic acid (GLA), and oleic acid (Christie, 1999). Often EFAs are referred to as vitamin F. These EFAs are unsaturated (contain one or more double bonds in the hydrocarbon chain) and can only be acquired through diet (Lockwood & Kiselica, 2010). Vitamin F consists mainly of linoleic (35.5%), linolenic (30.5%), and oleic acid (21.4%) (Chemimpo, 2004).

Pheroid™ technology is a patented drug delivery system consisting of polyunsaturated fatty acids, which include omega-3 and omega-6 fatty acids, but it excludes arachidonic acid. The fatty acids used to produce Pheroid™ are emulsified in nitrous oxide saturated water and dispersed in a dispersion medium. However, this technology also contains a dispersed gas phase (nitrous oxide), giving it the edge as it thus has three phases, namely a water phase, an oil phase, and a gas phase. The gas phase contributes to the stability and the self-assembly process of the Pheroid™ (Du Plessis *et al.*, 2010; Grobler *et al.*, 2008).

Fatty acids enhance permeation through entering the lipid bilayers of the skin and disrupting their ordered domains. They can improve API partitioning into the *stratum corneum* (outmost layer of the skin) and can form lipophilic complexes with compounds (Karande & Mitragotri, 2009). Fatty acids can be used to enhance the permeation of both lipophilic and hydrophilic APIs, though the flux of polar APIs is improved to a larger degree (Williams, 2003:92).

Formulating a product to diffuse through the skin presented many difficulties. Penetration enhancers were chosen to minimise these difficulties. EFAs have a wide range of attributes and are non-toxic, making them optimal penetration enhancers. Even on their own, EFAs can be used in the treatment of many diseases (Di Pasquale, 2009). Pheroid™ technology consists mainly of EFAs and has shown no immune responses in humans. Pheroids™ have, furthermore, been shown to enhance bio-availability and cause no cytotoxicity, therefore making it an optimal choice as drug delivery vehicle (Du Plessis *et al.*, 2010; Grobler *et al.*, 2008).

3.2 MATERIALS AND METHODS

3.2.1 MATERIALS

Flurbiprofen was obtained from DB fine chemicals®, South Africa. Evening primrose oil (Windrose, South Africa), Pheroid™ and vitamin F (North-West University, Potchefstroom Campus, South Africa) were used as penetration enhancers. Other ingredients used in the formulation of the semi-solid products (liquid paraffin, tween, and cetyl alcohol) were obtained from Merck® Laboratory Supplies (Midrand, South Africa). Span 60 was obtained from Fluka® Analytical (Germany). Water used was of HPLC (high performance liquid chromatography) grade deionised with a Milli-Q® academic purification system (Millipore®, Milford, United States of America). Methanol AR grade (Merck®, South Africa) was used for sample preparation. Acetonitrile Lichrosolv, methanol Lichrosolv and octane-sulphonic acid (Merck®, South Africa) were used in the HPLC mobile phase preparation. Potassium dihydrogen orthophosphate and potassium hydroxide (Merck®, South Africa) were both used in the preparation of the phosphate buffer solution (pH 7.4). *n*-Octanol (Fluka®) was used in the determination of log D (octanol-buffer distribution coefficient).

3.2.2 METHODS

3.2.2.1 Formulation of semi-solid product

A cream-based formulation was used for the diffusion studies. The ingredients for this formulation were as follows: cetyl alcohol (10%), tween 80 (1.5%), span 60 (1.5%), liquid paraffin (12%), flurbiprofen (1%), methanol to dissolve flurbiprofen (6%) and the penetration enhancer used was kept at 5%. HPLC grade water was added to volume (63%).

3.2.2.2 Solubility and log D determination of flurbiprofen

The solubility of flurbiprofen was determined in Milli-Q® water and a phosphate buffer solution (pH 5). An excess amount of flurbiprofen was placed in a polytop containing 5 ml solvent in order to keep the solution saturated. The contents were continuously mixed using a magnetic

stirring bar and kept at 32℃ in a water bath for a period of 24 h. The solution was filtered and analysed using an HPLC. This experiment was performed in triplicate.

The experimental partition coefficient (log D) was determined by pre-saturating equal volumes (100 ml) *n*-octanol and a phosphate buffer solution (pH 5) for 24 h. The pH range acceptable for skin products is 5 - 8, since a pH higher or lower will irritate the skin. A pH of 5 was used due to the fact that flurbiprofen is predominantly unionised at this specific pH, which is still within the acceptable range. A higher degree of unionisation would have been preferred, since an unionised molecule will cross a membrane more readily (Lamb, 2012). After 24 h of pre-saturation, the phases were separated. Flurbiprofen (3 mg) was dissolved in 20 ml pre-saturated *n*-octanol. The flurbiprofen pre-saturated *n*-octanol (3 ml) was placed in a test tube and 3 ml pre-saturated phosphate buffer solution was added. The test tube was shaken continuously in a 32℃ water bath for 24 h after which the mixture was centrifuged at 5 000 rpm for 10 min, using an Eppendorf® centrifuge model 5804 R. Finally, the two phases in the test tube were separated by means of a pipette. The phosphate buffer solution was injected into the HPLC, while the *n*-octanol phase was diluted with methanol (1:4) before injection into the HPLC. This dilution was done to avoid band-broadening caused by pure octanol. This experiment was done in triplicate and the logarithmic ratio of the flurbiprofen concentration in the *n*-octanol phase to the flurbiprofen concentration in the phosphate buffer solution was used to calculate the log D value.

3.2.2.3 Franz cell diffusion studies

3.2.2.3.1 Preparing phosphate buffer solution

The following steps were used to prepare the phosphate buffer solution:

- Weigh 6.81 g potassium dihydrogen phosphate (KH_2PO_4) and dissolve in 250 ml deionised water.
- Weigh 1.571 g sodium hydroxide (NaOH) and dissolve in 393.4 ml deionised water.
- Slowly add the NaOH solution to the KH_2PO_4 solution.
- Measure the pH. If the pH is not at 7.4, it can be lowered with the addition of 2M H_3PO_4 or increased with the addition of 2M NaOH.
- Store in refrigerator (2 - 4℃) until utilised.

3.2.2.3.2 Skin preparation

Human Caucasian skin was used during skin permeation studies which were donated by anonymous female donors who underwent abdominoplastic surgery. Ethical approval for the procurement and preparation of the skin was provided by the Research Ethics Committee of the

North-West University under the reference number NWU-00114-11-A5. Full-thickness skin was removed from the abdomen of female patients which was subsequently frozen at -20°C for no longer than 6 months. Before the studies were conducted, the full-thickness skin had been taken out of the freezer and left to thaw. The skin was visually examined for any defects such as stretch marks and large hair follicles/holes before cutting it into pieces of approximately 2 cm in width, 4 cm in length and 400 µm in thickness with the Zimmer® electric dermatome model 8821. The prepared skin samples were put on Whatman® filter paper; wrapped in aluminium foil, and stored in a freezer at -20°C until utilised (within 24 h).

3.2.2.3.3 Membrane release and skin diffusion studies

Twelve amber vertical Franz diffusion cells (FCs), with a diffusion area of approximately 1.075 cm^2, were used during permeation studies. Amber cells were used to avoid any breakage of the API due to light sensitivity. Ten FCs contained one of a specific formulation with the API and the remaining two FCs contained placebo formulations (cream with no penetration enhancers or API). Skin or an artificial membrane was mounted between the two compartments of the diffusion apparatus, the skin with the *stratum corneum* facing upwards in the direction of the donor compartment.

Each receptor compartment was filled with 2 ml phosphate buffer solution (pH 7.4) and the temperature of the cell system was maintained at 37°C using a water bath. The pH was set to 7.4 since the receptor phase represented human blood of which the temperature is normally approximately 37°C. The receptor compartment was filled, ensuring that no air bubbles formed underneath the skin or membrane, which might impair permeation. A Teflon-coated magnetic stirring bar was placed in the receptor compartment to continuously mix the phosphate buffer solution. Enough of the formulation (1 ml at 32°C) to ensure skin saturation was added to the donor compartment of the Franz cells. The donor compartment was closed with Parafilm® and a cap to prevent evaporation of the formulation. Dow Corning® high vacuum grease was used to seal the Franz cells in order to avoid leakage and thereafter a horse-shoe clamp was used to secure the two compartments.

At pre-determined time intervals the entire receptor volume was withdrawn and replaced with fresh buffer (37°C). The time intervals for the membrane release studies were hourly up to 6 h followed by every two hours up to 12 h for the skin diffusion studies. This was to insure that sink conditions existed throughout the experiment. Samples were directly assayed, using an Agilent® isocratic system to determine the API concentration in the receiver fluid.

3.2.2.3.4 Tape stripping

All the skin circles were removed after each of the 12 h diffusion studies. The skin circles were fixed onto Whatman® filter paper which was placed on a flat surface. All excess formulation was dabbed from the skin with a clean paper towel. Circular imprints from the donor phase showed the diffusion area. To perform tape stripping, sixteen strips of 3M Scotch® Tape were needed. The first strip was discarded as it was considered part of the cleaning procedure. The remaining fifteen strips were used to obtain data. After stripping the *stratum corneum*-epidermis off the diffusion area, the fifteen tape strips were put into a polytop containing a 5 ml 40% methanol in phosphate buffer solution. The polytop was vigorously shaken to wet all the strips and left overnight (approximately 12 h) to be analysed by HPLC the following day.

The diffusion area of the remaining skin circle (dermis) was cut out and also cut into pieces before placing it into a polytop containing 5 ml of a 40% methanol in phosphate buffer solution. This area was cut into pieces in order to enlarge the surface area in contact with the methanol solution. The polytop was vigorously shaken and stored overnight to be analysed by HPLC the following day.

3.2.2.3.5 High performance liquid chromatography (HPLC) analysis

An HPLC method, to determine API concentrations, had already been developed and validated in the Analytical Technology Laboratory (ATL) in conjunction with Prof. J.L. du Preez at the North-West University, Potchefstroom Campus.

Concentration assays were conducted using an Agilent® isocratic system, Luna C_{18}-2 column, 150 x 4.6 mm, 5 µm, (Phenomenex, Torrance, CA). The flow rate was set at 1 ml/min and the detection wavelength at 247 nm. The mobile phase consisted of 300 ml Milli-Q® water, 700 ml Acetonitrile and 10 ml glacial acetic acid.

3.2.2.3.6 Data analysis

After conduction of the diffusion studies, the cumulative concentration (µg/cm^2) of the permeated API was plotted against time. The steady-state slope of the average cumulative concentration against time provided the flux (µg/cm^2.h) of the API, whereas the average cumulative API concentration versus square root of time (h$^{0.5}$) presented the lag time for each of the formulations. The percentage flurbiprofen yielded from the total amount of API applied in the formulations was presented for the skin (after 12 h) and membrane (after 6 h) studies.

3.2.2.3.7 Statistical data analysis

Data gathered were analysed using descriptive and inferential statistics. Descriptive statistics involved the calculation of the mean, median and standard deviation (SD). Inferential statistics involved one-way analysis of variance (ANOVA) and also parametric assumption testing (omnibus tests for differences between group means). The mean (average or arithmetic average) is a summarising statistic and is a measure of the centre of distribution which is particularly meaningful if the data is symmetrically scattered around the mean. The median is less affected by extreme data points and represents the centre of a data set. The SD calculates the spread of data around the centre point (Bolton, 1997).

The first parametric assumption was the normality hypothesis. Statistical tests (Shapiro-Wilk and Kolmogorov-Smirnov) were used to determine whether the sample data have been drawn from a normally distributed population. The second assumption was the homogeneity of variance hypothesis. Levene's test was employed to determine whether the variances of the four formulation groups were equal. It was found that the deviation from normality and difference in variation were not severe, though one-way ANOVA, as well as more robust tests (Welch and Brown-Forsythe) were performed to access the differences. After performing the omnibus tests, *post hoc* follow-up tests (Games-Howell and Tukey HSD) were conducted to determine which formulation's average cumulative concentration varied from one other (SPSS, 2011).

Furthermore, since skin diffusion studies represented repeated measures taken over time, a mixed model analysis was fitted to the data to account for the dependence structure. The Type III test for fixed effects was applied as an omnibus test to indicate statistical significant difference between the levels of factors time and treatment (formulation). Pairwise comparisons of estimated marginal means were employed, after a Bonferroni adjustment for multiple comparisons was applied, to determine which levels differed significantly.

Statistical analyses were conducted by the Statistical Consultation Services of the North-West University, Potchefstroom Campus. The analyses were performed using SPSS (version 20, 2011).

3.3 RESULTS AND DISCUSSION

3.3.1 SOLUBILITY AND LOG D-VALUES OF FLURBIPROFEN

The ideal solubility of an API to permeate through the skin is > 1 mg/ml (Naik *et al.*, 2000; Williams, 2003). Experimental results delivered solubility values at a pH lower than five since flurbiprofen is an acidic compound that lowers the pH in solution. The solubility was determined

to be 0.057 mg/ml in water (pH 4.75, 32℃) and 0.0704 mg/ml in phosphate buffer solution (pH 4.69, 32℃). These results indicated that flurbiprofen was only slightly soluble and, therefore, it could be expected that it will experience some difficulty in permeating through the skin.

A log P value of 4.24 for flurbiprofen was found in literature (Alsarra *et al.*, 2010; Li *et al.*, 2006); however, experimental results delivered a log D of 3.41 at 32℃. Therefore, it was clear that flurbiprofen does not have an ideal log P-value for skin permeation.

3.3.2 MEMBRANE RELEASE STUDIES

The formulation that contained vitamin F depicted the highest average percentage flurbiprofen released (3.04%) from the total amount applied to the donor compartment of the Franz cells. Furthermore, this formulation also presented the highest average cumulative concentration released after 6 h (564.89 µg/cm^2). In fact, the vitamin F formulation was the only formulation that delivered results which were significantly higher ($p = 0.001$) than that of the control formulation. On the other hand, the Pheroid™ formulation depicted the lowest average percentage flurbiprofen released (1.81%), as well as the lowest average cumulative concentration released after 6 h (336.72 µg/cm^2).

3.3.3 SKIN DIFFUSION STUDIES

From the results it was clear that the EPO formulation depicted the highest average percentage flurbiprofen diffused (0.51%), even though it did not show the highest concentration released from the formulation. However, the differences between the average cumulative flurbiprofen concentration diffused after 12 h for the EPO (94.14 µg/cm^2) and the control formulation (91.53 µg/cm^2) were not found to be statistically significantly different ($p = 1$). The Games-Howell *post hoc* test also delivered p-values stating this fact ($p = 0.989$). The Pheroid™ formulation depicted the lowest average percentage flurbiprofen released (1.81%) and also illustrated the lowest average percentage flurbiprofen diffused (0.24%). This percentage flurbiprofen diffused was approximately half the percentage diffused through the skin compared to the control formulation (0.49%). Average cumulative concentrations of flurbiprofen diffused through the skin after 12 h, could be placed in the following rank order: EPO formulation > control formulation > vitamin F formulation >> Pheroid™ formulation.

A graphic representation of the average cumulative flurbiprofen concentration and the flux (slope of the regression line) of the different formulations can be seen in Figures 3.1, as well as a box-plot representation of the cumulative flurbiprofen concentration in Figure 3.2.

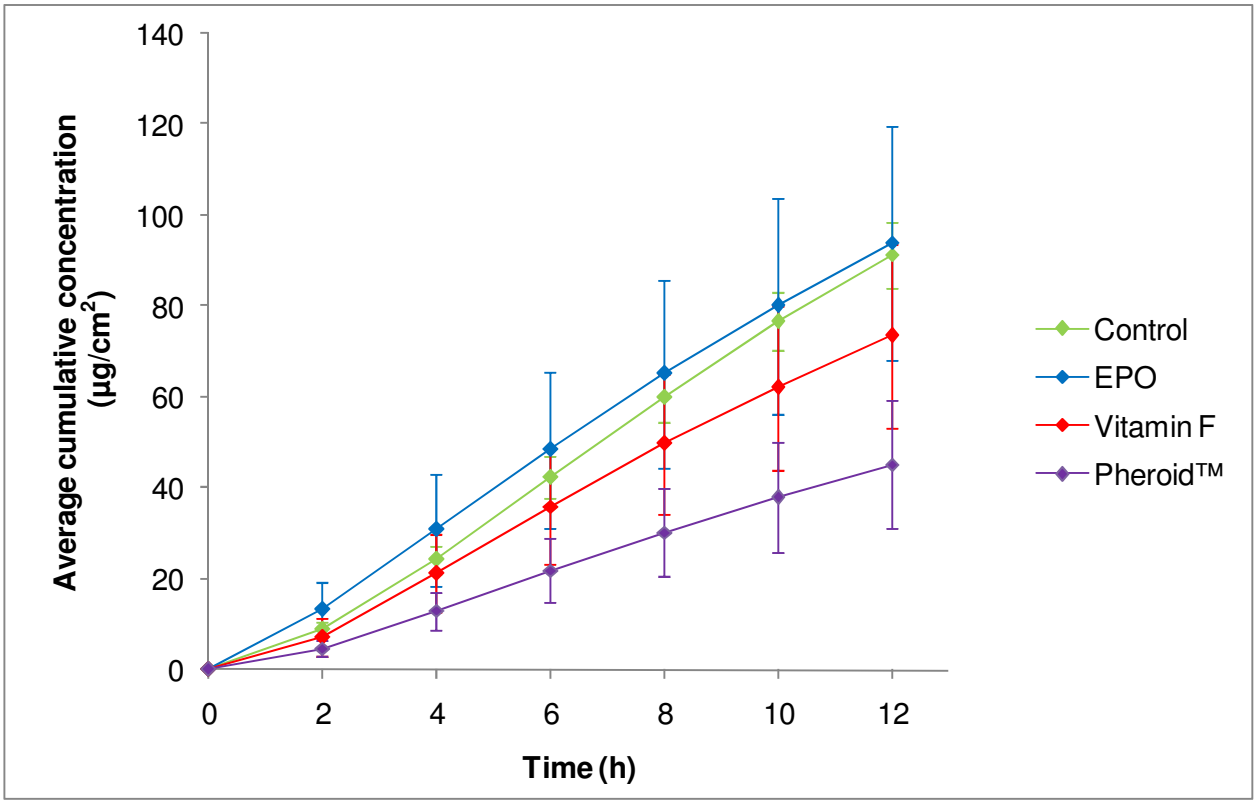

Figure 3.1: ***The average (n ≥ 9) cumulative concentration flurbiprofen diffused through the skin for the different formulations used.***

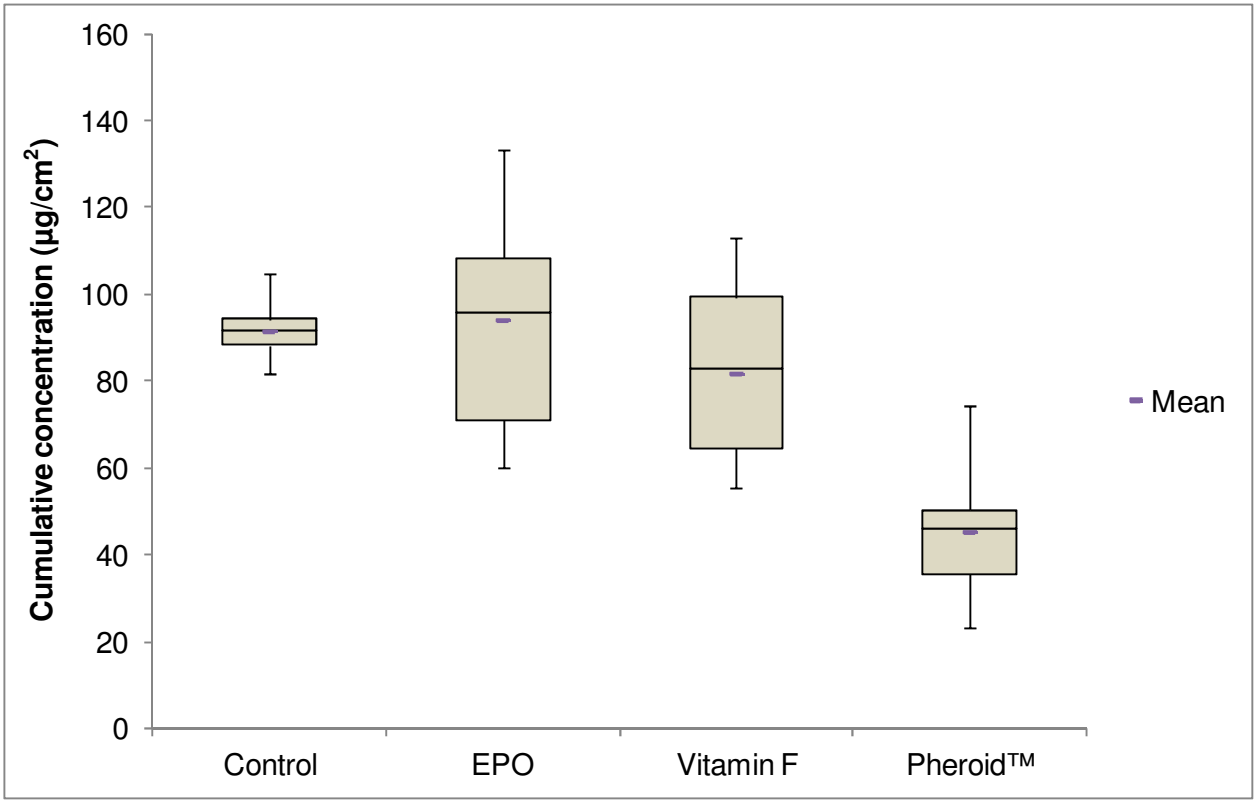

Figure 3.2: ***Box-plots representing the cumulative concentration (n ≥ 9) flurbiprofen of the four formulations used with the purple line as the mean and the line dividing the box as the median.***

Figure 3.1 and Figure 3.2 clearly indicated that the EPO formulation depicted the highest average cumulative concentration (94.14 µg/cm²) and the Pheroid™ formulation the lowest average cumulative concentration (45.32 µg/cm²). The mean and median, as indicated in Figure 3.2, do not differ significantly and may indicate relatively few outliers in the data points. The box-plot of the control and vitamin F formulation indicated a linear distribution of data, whereas the other two formulations depicted a skew distribution of data.

The flux of the control formulation (8.41 µg/cm^2.h) was the highest with the EPO formulation following closely (8.12 µg/cm^2.h). The Pheroid™ formulation depicted the lowest flux (4.08 µg/cm^2.h) at approximately half the rate of the control formulation, whereas the EPO formulation depicted the shortest lag time (1.36 h), though all the formulations showed shorter lag times compared to the control formulation (1.74 h).

3.3.3.1 Tape stripping

The control formulation depicted the highest flurbiprofen concentrations in the skin. A higher concentration was observed in the dermis-epidermis (0.45 µg/ml), whereas a concentration of 0.26 µg/ml was present in the *stratum corneum*. The EPO formulation presented the second highest concentration in the skin where a concentration of 0.37 µg/ml was obtained in the dermis-epidermis and 0.19 µg/ml in the *stratum corneum*. A graphical box-plot representation of this data can be seen in Figure 3.3.

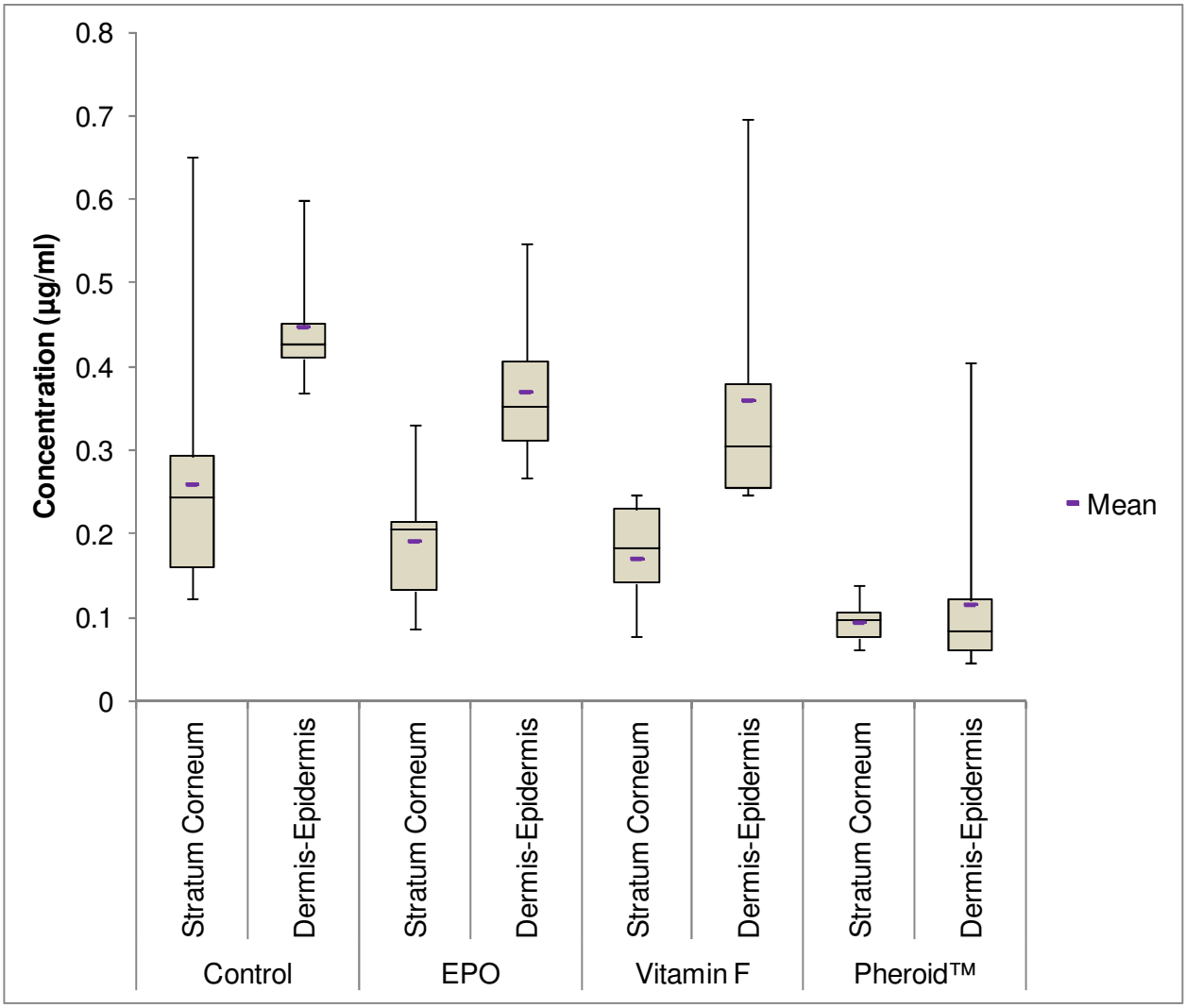

Figure 3.3: ***Box-plot representation of the concentration (n ≥ 9) flurbiprofen present in the skin after tape stripping, with the purple line as the mean and the line dividing the box as the median.***

After inspecting the box-plot representation (Figure 3.3) of the average concentration flurbiprofen present in the *stratum corneum* and dermis-epidermis, it was clear that higher concentrations flurbiprofen were present in the dermis-epidermis. All the formulations used delivered a distorted distribution of data with extreme outliers as observed from the error bars. This fact can also be seen in the higher variation between the mean and median as indicated by the purple and black line, respectively. It was also observed that although the control formulation depicted the highest concentration flurbiprofen present in the skin, there was no significant difference between the control, vitamin F and EPO formulation. The Pheroid™ formulation, however, were significantly lower than the other formulations.

The Games-Howell *post hoc* tests revealed a statistically significant difference for the flurbiprofen concentrations present in the *stratum corneum* for the Pheroid™ and EPO

formulations ($p = 0.01$), and the Pheroid™ and vitamin F formulation ($p = 0.032$). The difference between the control and Pheroid™ formulation delivered a p-value of 0.062. A statistically significant difference was also found for the concentrations present in the dermis-epidermis for the Pheroid™ and control formulations ($p < 0.05$), Pheroid™ and EPO formulations ($p < 0.05$), and Pheroid™ and vitamin F formulations ($p = 0.005$). Furthermore, a statistically significant difference was found between the concentration present in the receptor phase of the Franz cells (diffused through the skin) and the concentration API present in the skin (*stratum corneum* and dermis-epidermis) with $p < 0.05$.

3.4 CONCLUSIONS

Membrane release data revealed that the API was released from all of the formulations and was therefore available on the skin surface for possible diffusion through it. The vitamin F formulation depicted the highest flurbiprofen released and therefore this formulation provided the highest concentration available for diffusion compared to the other formulations. However, analysis of the skin diffusion data revealed the contrary. Results showed that the formulation containing EPO as penetration enhancer depicted the highest concentration delivered transdermally. This may be due to the fact that skin already consists of approximately 15% linoleic acid and 30% oleic acid and less obstruction is encountered. Previous studies showed that the enhancing effect of fatty acids follow the following rank order: linoleic acid > oleic acid > linolenic acid, though linolenic acid was proven to be more irritant to the skin (Fang *et al.*, 2003). EPO contain significantly high concentrations linoleic (65 - 80%) and oleic acid (6 - 11%), causing it to diffuse more readily through the skin. The *stratum corneum* consists of approximately 41% ceramides of which linoleic acid is a precursor, which may explain why higher linoleic concentrations have higher enhancing qualities (Hopkins, 2012; McCusker & Grant-Kels, 2010; Suhonen *et al.*, 1999).

The control formulation that contained no penetration enhancers provided the highest flux of flurbiprofen diffusing through the skin. However, again the EPO formulation illustrated a flux-value that did not significantly differ from the rate of flurbiprofen diffused through the skin as provided by the control formulation ($p > 0.05$). This formulation furthermore demonstrated the shortest lag time compared to the other formulations. A shorter lag time indicated that an API will more rapidly start to diffuse through the skin. Thus, the EPO formulation depicted a faster onset of diffusion of flurbiprofen through the skin, which may be explained due to the fact that the fatty acids present in EPO is also part of the *stratum corneum* structure and, thus, less obstruction is experienced by the *stratum corneum* permeability barrier (SCPB).

Tape stripping data suggested that this specific cream formulation containing fatty acids as penetration enhancers will be most effective if used for transdermal therapy rather than topical therapy, since very low concentrations were present within the skin (*stratum corneum* and dermis-epidermis), which might not have a significant therapeutic effect. Significantly higher concentrations were observed in the receptor phase which represented the concentration flurbiprofen which diffused through the skin.

Future prospects for further investigation and aspects that need to be considered include:

- Different semi-solid products containing varying concentrations fatty acids as penetration enhancers should be formulated in order to determine whether these formulations possess the ability to improve the transdermal delivery of flurbiprofen.
- Linoleic, palmitic and oleic acid are the fatty acids with the highest concentrations present in the skin. It could thus be hypothesised that these fatty acids, separately or in combination, would deliver the best transdermal delivery results.
- Formulations can be adjusted and evaluated to determine which combination of ingredients deliver the most effective formulation to deliver the API. Stability testing should be conducted on the formulations to determine which formulation is more stable.
- Compounds different from flurbiprofen, which have more preferable physicochemical properties for transdermal diffusion, can be used in a formulation to determine whether the fatty acids can improve the transdermal delivery of other compounds as well.
- After formulating an effective and stable product, clinical studies can be initiated to determine the effect of the formulation on a representative human group.

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CHAPTER 4

FINAL CONCLUSIONS AND FUTURE PROSPECTS

Flurbiprofen (model compound) was chosen to evaluate the penetration enhancing effects of fatty acids on the transdermal delivery of an active pharmaceutical ingredient (API). Fatty acids were chosen as penetration enhancers for their ability to reversibly increase the skin permeability through entering the lipid bilayers and disrupting their ordered domains. They are, furthermore, natural products which increase their popularity for inclusion in formulations and they present minimal known side effects in humans (Karande & Mitragotri, 2009:2364).

For an API to be effectively delivered transdermally, it has to be soluble in both lipophilic and hydrophilic mediums (Naik *et al.*, 2000:319; Swart *et al.*, 2005:72). This is due to the complex structure of the skin, in which the *stratum corneum* (outermost layer) is the primary barrier that regulates skin transport (Barry, 2001:102; Moser *et al.*, 2001:103; Venus *et al.*, 2010:469).

Flurbiprofen is highly lipophilic (log P = 4.24) and portrays poor aqueous solubility (0.005 mg/ml). A log P value of between 1 and 3; a molecular weight of less than 500 g/mol; and a solubility > 1 mg/ml are preferred for optimal skin permeation (Hadgraft, 2004:292; Karande & Mitragotri, 2009:2363; Swart *et al.*, 2005:72). In addition to the aforementioned characteristics, flurbiprofen has a molecular weight of 244.3 g/mol and a log P of 4.24, indicating that diffusion might occur with some difficulty (Dollery, 1999:F126; Drugbank, 2012).

Cream-based formulations containing 5% of a chosen penetration enhancer were prepared, utilising flurbiprofen as a model compound in order to determine whether any significant effects could be detected. Evening Primrose Oil, vitamin F and Pheroid™ technology all contain fatty acids that have been proven to enhance penetration. Therefore, these products were incorporated to act as penetration enhancers.

Transdermal permeation was evaluated by means of Franz cell diffusion studies conducted over a 12 h period. However, prior to the transdermal permeation studies, membrane permeation studies were performed over 6 h to determine if any release of the API from the formulation had occurred. Membrane release results obtained indicated that the API was indeed released from each of the formulations and, therefore, the API was available on the skin surface for possible

diffusion through the skin. The highest flurbiprofen concentration was released from the vitamin F formulation.

Skin diffusion results indicated that a small amount of API was present in the *stratum corneum* and the dermis-epidermis, though higher API concentrations were present in the receptor phase of the Franz cells (representing human blood). This proved that transdermal delivery, rather than topical delivery, was achieved. By comparing membrane release and skin diffusion data, it was found (contradicting to the membrane release data) that the evening primrose oil (EPO) formulation, rather than the vitamin F formulation, depicted the best penetration enhancement of flurbiprofen when used in a cream-based formulation. This may be due to the fact that human skin already consists of approximately 15% linoleic acid and 30% oleic acid, which may result in less obstruction from the *stratum corneum* permeability barrier. Previous studies showed that the enhancing effect of fatty acids follow the following rank order: linoleic acid > oleic acid > linolenic acid, though linolenic acid was proven to be more irritant to the skin (Fang *et al.*, 2003:156-159). EPO contains significantly high concentrations linoleic (65 - 80%) and oleic acid (6 - 11%), causing it to diffuse more readily through the skin. The *stratum corneum* consists of approximately 41% ceramides of which linoleic acid is a precursor, which may also explain why higher linoleic acid concentrations may result in higher enhancing qualities (Hopkins, 2012; McCusker & Grant-Kels, 2010:442; Suhonen *et al.*, 1999:150).

The control formulation contained no penetration enhancers and provided the highest flux (8.41 µg/cm^2.h) of flurbiprofen diffusing through the skin. The EPO formulation illustrated a flux value (8.12 µg/cm^2.h) close to that of the control formulation, though no statistically significant difference was found ($p > 0.05$). The Pheroid™ formulation portrayed the lowest flux (4.08 µg/cm^2.h; $p < 0.05$), at approximately half the rate of the control formulation. Furthermore, the EPO formulation resulted in the shortest lag time (1.36 h), though all the formulations depicted a lag time shorter than that of the control formulation (1.74 h). A shorter lag time indicates that an API will more rapidly start to diffuse through the skin. Thus, the EPO formulation showed a faster onset of diffusion of flurbiprofen through the skin, which may be ascribed to the fact that the fatty acids present in EPO are also part of the *stratum corneum* structure and, thus, less obstruction is presented by the stratum corneum permeability barrier (SCPB). The Pheroid™ formulation displayed the lowest average cumulative concentration (45.32 µg/cm^2), diffusing through the skin ($p < 0.05$) at approximately half the concentration compared with that of the control formulation (91.53 µg/cm^2). Pheroid™ technology is highly lipophilic and also contains a lower concentration fatty acids than the other penetration enhancers used. Flurbiprofen is also highly lipophilic and, thus, the lower concentration flurbiprofen released from the formulation and diffused through the skin may be explain by the

fact that flurbiprofen will mostly reside in the lipid bilayer of the Pheroids™ and not diffuse into the skin (Grobler *et al.*, 2008:285-288). The EPO formulation, on the other hand, produced a cumulative concentration of 94.14 µg/cm^2 ($p > 0.05$), which was the highest concentration of all the formulations used.

Tape stripping data suggested that this specific cream formulation, containing fatty acids as penetration enhancers, will be most effective if used for transdermal therapy, rather than topical therapy, since very low concentrations were present within the skin (*stratum corneum* and dermis-epidermis) while significantly higher concentrations were observed in the receptor phase, which represented the concentration flurbiprofen which diffused through the skin.

Future prospects for further investigation and aspects that need to be considered include:

- Different semi-solid products containing varying concentrations fatty acids as penetration enhancers should be formulated in order to determine whether these formulations possess the ability to improve the transdermal delivery of flurbiprofen.
- Linoleic, palmitic and oleic acid are the fatty acids with the highest concentrations present in the skin. It could thus be hypothesised that these fatty acids, separately or in combination, would deliver the best transdermal delivery results.
- Formulations can be adjusted and evaluated to determine which combination of ingredients deliver the most effective formulation to deliver the API. Stability testing should be conducted on the formulations to determine which formulation is more stable.
- Compounds different from flurbiprofen, which have more preferable physicochemical properties for transdermal diffusion, can be used in a formulation to determine whether the fatty acids can improve the transdermal delivery of other compounds as well.
- After formulating an effective and stable product, clinical studies can be initiated to determine the effect of the formulation on a representative human group.

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ANNEXURE A

METHOD VALIDATION FOR THE HPLC DETERMINATION OF FLURBIPROFEN

A.1 INTRODUCTION

The Latin meaning of validation implies that something has been proved useful, true and of adequate standard. These requirements are fulfilled through the provision and the examination of objective evidence (Westgard, 2008:255; Araujo, 2009:2224). A chromatographic method was validated to ensure that this analytical method is reliable and sensitive when determining the active pharmaceutical ingredient (API) concentration obtained in membrane diffusion studies.

A.2 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY METHOD VALIDATION FOR FLURBIPROFEN

A.2.1 CHROMATOGRAPHIC CONDITIONS

Analytical instrument: An Agilent® 1100 series HPLC was used for the analysis (Agilent® Technologies, Palo Alto, CA). The instrument is equipped with a isocratic pump, autosampler injection mechanism, diode array detector, and Chemstation Rev. A.06.02 software for data acquisition and analysis.

Column: Luna C_{18}-2 column, 150 x 4.6 mm with a 5 µm particle size was used (Phenomenex, Torrance, CA).

Mobile phase: The mobile phase consisted of a filtered mixture of 700 ml acetonitrile, 10 ml glacial acetic acid and 300 ml HPLC-grade water.

Solvent: Methanol to a volume of 20% and phosphate buffer solution were used to prepare standard preparations.

Run time: 6 min.

Retention time: Approximately 4 min.

Flow rate: 1 ml/min.

Injection volume: 50 µl.

Detection wavelength: UV at 247 nm.

A.2.2 STANDARD AND SAMPLE PREPARATION

Approximately 25 mg flurbiprofen was accurately weighed in a 50 ml volumetric flask. Methanol (± 20 ml) was used to dissolve the flurbiprofen and the volumetric flask was filled up with phosphate buffer solution (pH 7.4) to 50 ml. Five millilitres of this solution was extracted and diluted to 50 ml with phosphate buffer solution. The diluted solution was transferred to autosampler vials and analysed using the liquid chromatography system.

A.2.3 VALIDATION OF TEST PROCEDURE AND ACCEPTANCE CRITERIA

A.2.3.1 LINEARITY

Snyder *et al.* (1997:644) described linearity as a measure of how well data fit to the linear equation (Equation A.1):

$$y = mx + c \qquad \text{(Equation A.1)}$$

Where:

- y is the peak area
- m is the slope
- x is the concentration
- c is the y-intercept.

Linearity can also be defined as the capability to obtain test results directly proportional to the concentration of analyte within a given range. Range is the interval between the upper and lower levels of analyte (including these levels) that are determined with precision, accuracy, and linearity, using the above mentioned method (Swartz & Krull, 1997:65; USP34 NF29, 2012).

Figure A.1 and Table A.1 demonstrate the linearity data of flurbiprofen. An acceptable value of the correlation coefficient (r^2) is > 0.999 and flurbiprofen displayed an acceptable r^2 value of 0.9999, which falls within the accepted criteria (International Pharmaceutical Operations:32). A minimum of 5 concentrations is necessary to ascertain linearity (ICH, 2005:8). The closer the correlation coefficient is to one, the stronger the positive linear relationship is (Snyder, 1997:691).

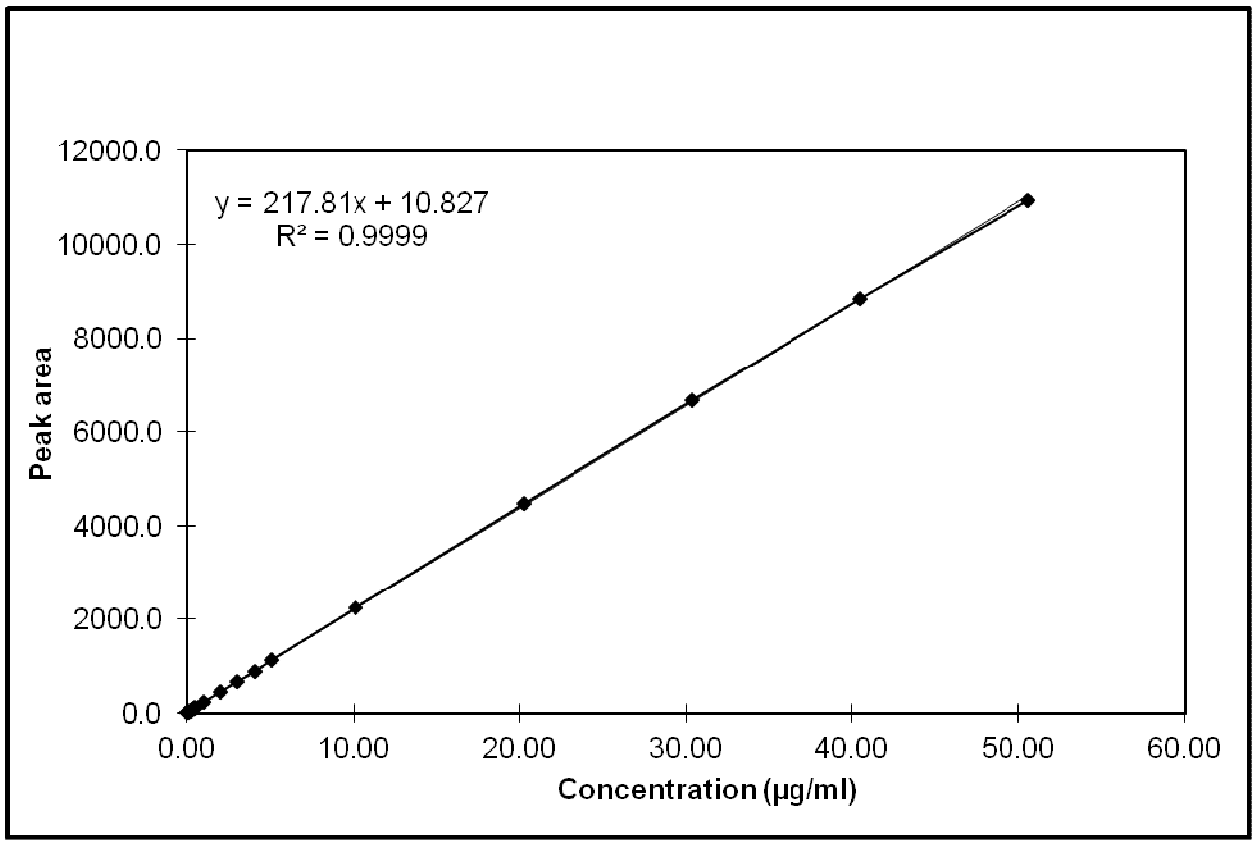

Figure A.1: ***Linear regression curve of flurbiprofen***

Table A.1: ***Linearity of flurbiprofen***

µg/ml	Peak area		Mean
0.03	5.9	5.9	5.9
0.05	12.1	12.9	12.5
0.1	22.7	23.6	23.2
0.2	45.9	45.9	45.9
0.3	68.8	70	69.4
0.4	91.1	90.9	91
0.51	116.5	114.3	115.4
0.25	54.9	55.3	55.1
0.51	110.9	109.8	110.4
1.01	222.7	221.1	221.9
2.02	449	446.2	447.6
3.03	672.4	668.5	670.4
4.04	894.8	894.2	894.5
5.06	1121.6	1116.6	1119.1
10.11	2241.4	2241.7	2241.6
20.22	4473.9	4468.3	4471.1
30.34	6670	6683	6676.5
40.45	8855.1	8847.8	8851.4
50.56	10954.4	10917.2	10935.8
R^2	0.9999155	Lower 95%	Upper 95%
Intercept	10.827041	-6.893194	28.547275
Slope	217.8084	216.78361	218.83319

A.2.3.2 ACCURACY

The accuracy of an analytical procedure is the proximity of the measured value to the true or accepted reference value that should be ascertained across its range (Snyder, 1997:644; Swartz & Krull, 1997:56; ICH, 2005:4; Westgard, 2008:257; USP34 NF29, 2012).

Table A 2 depicts the accuracy results of flurbiprofen. The International Pharmaceutical Operations stated that the percentage recovery should be between 98 and 102% with a %RSD (relative standard deviation) of ≤ 2%. The values obtained were within these parameters.

Table A.2: ***Accuracy of flurbiprofen***

Concentration spiked				Recovery	
µg/ml	**Peak area**		**Mean**	**µg/ml**	**%**
2.5	564.9	570.6	567.7	2.5	100.2
2.5	568.5	565.2	566.9	2.5	100.1
2.5	565.4	562.7	564.1	2.5	99.6
10.2	2261.6	2258.9	2260.3	10.4	102
10.2	2259.3	2269.4	2264.3	10.4	102.2
10.2	2268.1	2261.5	2264	10.4	102.2
40.6	8927	8927.3	8927.2	41.2	101.3
40.6	8903.7	8911.5	8907.6	41.1	101
40.6	8953.9	8939	8946	41.2	101.5
				Mean	101.1
				SD[1]	0.9
				%RSD[2]	0.9

1. Standard deviation (SD)
2. Relative standard deviation (%RSD)

A.2.3.3 PRECISION (INTRA-DAY VARIATION)

Precision is the measure of the degree of repeatability or reproducibility of multiple measurements of the analytical method, using a homogeneous sample. Intra-assay precision requires at least three samples to be injected over the duration of one day. This is usually expressed as the standard deviation (SD) or relative standard deviation (%RSD) of a statistically significant amount of samples (Snyder *et al.*, 1997:644; Swartz & Krull, 1997:57; USP34 NF29, 2012).

Data obtained for the precision (intra-assay precision) of flurbiprofen can be seen in Table A.3. An accepted %RSD value should be ≤ 2% when determining the precision (Beuving:37; Snyder *et al.*, 1997:710). All the data in Table A.3 adhered to these criteria.

Table A.3: ***Intra-day variation of flurbiprofen***

Concentration spiked				Recovery
µg/ml	Peak area		Mean	%
10.2	2261.6	2258.9	2260.3	102
10.2	2259.3	2269.4	2264.3	102.2
10.2	2268.1	2261.5	2264	102.2
			Mean	102.1
			%RSD	0.142

A.2.3.4 INTER-DAY VARIATION

Inter-day repeatability refers to the acquiring of test results with the analytical method over at least a few days and under the same conditions. The conditions are the same analyst with the same equipment. Reproducibility refers to the use of the analytical method in different laboratories (Snyder *et al.*, 1997:644; Swartz & Krull, 1997:57-60; USP34 NF29, 2012).

A.2.3.4.1 Day 2 inter-day repeatability

Table A.4 illustrates the precision data acquired on the second day, using the same equipment and the same analyst. The %RSD was ≤ 2%, thus adhering to the criterion (Beuving:37; Snyder *et al.*, 1997:710).

Table A.4: ***Precision day 2 of flurbiprofen***

Concentration spiked				Recovery	
µg/ml	Peak area		Mean	µg/ml	%
10.4	2334.2	2337.3	2335.8	10.5	101.2
10.4	2324.8	2331.8	2328.2	10.5	100.9
10.4	2331	2327.6	2329.3	10.5	101
				Mean	101.04
				SD	0.14
				%RSD	0.14

A.2.3.4.2 Day 3 inter-day repeatability

Table A.5 represents the precision done on the third day, using the same analyst and equipment. The %RSD was ≤ 2%, thus adhering to the criterion (Beuving:37; Snyder *et al.*, 1997:710).

Table A.5: ***Precision day 3 of flurbiprofen***

Concentration spiked				Recovery	
µg/ml	Peak area		Mean	µg/ml	%
9.48	2131.4	2122.4	2126.9	9.6	101.1
9.48	2134.2	2132.2	2133.2	9.6	101.4
9.48	2128.8	2134.1	2131.5	9.6	101.4
				Mean	101.31
				SD	0.13
				%RSD	0.12

Data presented in Table A.6 is a summary of the precision data collected over the three days, using the same equipment and analyst.

Table A.6: ***Precision of flurbiprofen between three days***

	Day 1	Day 2	Day 3	Between days
	102	101.2	101.1	
	102.2	100.9	101.4	
	102.2	101	101.4	
Mean	102.13	101.03	101.3	101.49
SD	0.09	0.12	0.14	0.48
%RSD	0.09	0.12	0.14	0.48

A.2.3.5 RUGGEDNESS (INTERMEDIATE PRECISION)

Intermediate precision refers to within-laboratory variations such as differences in experimental periods, analysts, equipment, columns, etc. The analytical method is done in the same laboratory, but all the other factors may vary (Snyder, 1997:701-702; Swartz & Krull, 1997:57-60; ICH, 2005:5; USP34 NF29, 2012).

Ruggedness of flurbiprofen can be seen in Table A.7. The criterion for the intermediate precision is %RSD ≤ 2.5 (Beuving: 38). Another source stated that the %RSD should be ≤ 2% (Snyder *et al.*, 1997:710). Results obtained for flurbiprofen met all standards set by different authors.

Table A.7: ***Sample stability of flurbiprofen***

Time (h)	Peak area	Percentage (%)
0	4684.2	100
1	4669	99.7
2	4662.8	99.5
3	4650.8	99.3
4	4656	99.4
5	4657.9	99.4
6	4658.9	99.5
7	4662.2	99.5
8	4666.6	99.6
9	4658.1	99.4
10	4665.6	99.6
11	4674.8	99.8
12	4672.6	99.8
13	4674.8	99.8
14	4680.1	99.9
15	4679.6	99.9
16	4679.3	99.9
17	4683.1	100
18	4678.4	99.9
19	4692.6	100.2
20	4690.2	100.1
21	4689.8	100.1
22	4698.3	100.3
23	4700.6	100.3
24	4695.5	100.2
Mean	4675.3	99.6
SD	14	0.2
%RSD	0.3	0.2

A.2.3.6 SYSTEM REPEATABILITY

This validation parameter is to ascertain whether the HPLC system and procedure are capable of providing adequate data quality whenever it is used (Snyder *et al.*, 1997:705-706; USP34 NF29, 2012). The %RSD should be ≤ 1.5 (Beuving:36). Data presented in Table A.8 depict the system repeatability for flurbiprofen, which is in accordance with the standards set.

Table A.8: ***System repeatability for flurbiprofen***

	Peak area	Retention time (min)
	4232.8	4.02
	4229.1	4.03
	4224.4	4.02
	4215.1	4.02
	4214.6	4.02
	4215.1	4.03
Mean	4221.8	4.02
SD	7.36	0.004
%RSD	0.17	0.106

A.3 CONCLUSION

The HPLC method for flurbiprofen was validated and found that it is reliable and sensitive in determining the API concentrations. Table A.1 through to Table A.8 show all the validation parameters determined and concluded that all the criteria were met.

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ANNEXURE B

FORMULATION OF CREAMS CONTAINING ESSENTIAL FATTY ACIDS AS PENETRATION ENHANCERS

B.1 INTRODUCTION

The aim of this study was to formulate and compare creams containing penetration enhancers. Essential fatty acids (EFAs) were chosen as penetration enhancers in the form of evening primrose oil (EPO), vitamin F, and Pheroid™ technology. The creams were each formulated with 1% of an active pharmaceutical ingredient (API) that served as a marker. Each cream furthermore contained 5% of a specific form of the penetration enhancer investigated.

B.2 MATERIALS AND METHODS

During the formulation process the following equipment were used:

- Mettler Toledo balance (Greifensee, Switzerland) (Figure B.1)
- Homogeniser (Figure B.2)
- Appropriate glassware
- Hotplate / magnetic stirrer (Figure B.3)

Flurbiprofen used (marker API) was obtained from DB Fine Chemicals (Pty) Ltd, South Africa. Evening primrose oil (Windrose, South Africa), Pheroid™ and vitamin F (North-West University, Potchefstroom Campus, South Africa) were incorporated as penetration enhancers. Other ingredients used in the formulation of the semi-solid products (liquid paraffin, tween, and cetyl alcohol) were obtained from Merck Laboratory Supplies (Midrand, South Africa). Span 60 was obtained from Fluka Analytical (Germany). Water used was HPLC (high performance liquid chromatography) grade, deionised with a Milli-Q® (Figure B.4) academic purification system (Millipore, Milford, United States of America).

B.3 FORMULATION OF NON-PHEROID™ CREAMS

B.3.1 PLACEBO CREAM

The ingredients for the placebo cream containing no penetration enhancers are given in Table B.1.

Table B.1: ***Ingredients of the placebo cream***

Ingredient	Amount (grams)
Cetyl alcohol	10
Span 60	1.5
Tween 80	1.5
Liquid Paraffin	12
Methanol	6
Water	69
Total:	100

Method:

1. Weigh Cetyl alcohol, span 60, tween 80, and liquid paraffin in a beaker and heat to 70℃ in order to produce the oil phase.
2. Weigh water and also heat to 70℃ (water phase).
3. Promptly add 6 g methanol to the oil phase, before adding the oil and water phases together.
4. Once the two phases are added together, mix with a homogeniser at 500 rpm until the cream reaches room temperature (25 ± 0.5℃).

B.3.2 CONTROL CREAM

The ingredients for the control cream containing no penetration enhancers are specified in Table B.2.

Table B.2: ***Ingredients of control cream***

Ingredient	Amount (grams)
Cetyl alcohol	10
Span 60	1.5
Tween 80	1.5
Liquid Paraffin	12
Methanol	6
Flurbiprofen	1
Water	68
Total:	100

Method:

1. Weigh Cetyl alcohol, span 60, tween 80, and liquid paraffin in a beaker and heat to 70℃ (oil phase).
2. Weigh water and also heat to 70℃ (water phase).
3. Weigh the flurbiprofen and dissolve it in the methanol.
4. Before the two phases are mixed, quickly add the flurbiprofen solution to the oil phase.
5. Immediately thereafter, add the water phase to the oil phase and mix with a homogeniser at 500 rpm until the cream reaches room temperature (25 ± 0.5℃).

B.3.3 CREAM CONTAINING EVENING PRIMROSE OIL

The ingredients for the cream containing evening primrose oil (EPO) as penetration enhancer are summarised in Table B.3.

Table B.3: ***Ingredients for the EPO cream***

Ingredient	Amount (grams)
Cetyl alcohol	10
Span 60	1.5
Tween 80	1.5
Liquid Paraffin	12
Methanol	6
Flurbiprofen	1
Evening primrose oil	5
Water	63
Total:	100

Method:

1. Weigh Cetyl alcohol, span 60, tween 80, and liquid paraffin in a beaker and heat to 70℃ (oil phase).
2. Weigh water and also heat to 70℃ (water phase).
3. Weigh the flurbiprofen and dissolve it in the 6 g methanol.
4. Rapidly add the flurbiprofen solution to the oil phase (70℃).
5. Add the water phase, immediately after adding the flurbiprofen solution, to the oil phase and mix with a homogeniser at 500 rpm until the cream reaches 45℃.
6. Once the cream is at 45℃, add the EPO and continue mixing with the homogeniser until the cream reaches room temperature (25 ± 0.5℃).

B.3.4 CREAM CONTAINING VITAMIN F

The ingredients for the cream containing vitamin F as penetration enhancer are presented in Table B.4.

Table B.4: ***Ingredients for the cream containing vitamin F***

Ingredient	Amount (grams)
Cetyl alcohol	10
Span 60	1.5
Tween 80	1.5
Liquid Paraffin	12
Methanol	6
Flurbiprofen	1
Vitamin F	5
Water	63
Total:	100

Method:

1. Weigh Cetyl alcohol, span 60, tween 80, and liquid paraffin in a beaker and heat to 70℃ (oil phase).
2. Weigh water and also heat to 70℃ (water phase).
3. Weigh 1 g flurbiprofen and dissolve in 6 g methanol.
4. Promptly add the flurbiprofen solution to the oil phase (70℃).
5. Immediately add the water phase to the oil phase and mix with a homogeniser at 500 rpm until the cream reaches 45℃.
6. Once the cream has reached 45℃, add the vitamin F and continue mixing with the homogeniser until the cream reaches room temperature (25 ± 0.5℃).

B.4 FORMULATION OF PHEROID™ CREAM

Pheroid™ preparations were produced in the formulation facility at the North-West University. Due to the confidential nature of the Pheroid™ formulation, the entrapment procedure of the Pheroid™ components cannot be stipulated. The oil phase of the Pheroid™ was adjusted to 5%, in order to be equivalent to the penetration enhancers in the non-Pheroid™ formulations. Table B.5 depicts the content of the Pheroid™ cream.

Table B.5: ***List of ingredients used in the Pheroid™ cream***

Ingredient	Amount (grams)
Cetyl alcohol	10
Span 60	1.5
Tween 80	1.5
Liquid Paraffin	12
Methanol	6
Flurbiprofen	1
Pheroid™ components	68
Total:	100

Method:

1. Weigh Cetyl alcohol, span 60, tween 80, liquid paraffin, and the oil phase of the Pheroid™ in a beaker and heat to 70°C (oil phase).
2. Weigh water phase of the Pheroid™ and also heat to 70°C (water phase).
3. Weigh 1 g flurbiprofen and dissolve in 6 g methanol.
4. Rapidly add the flurbiprofen solution to the oil phase (70°C).
5. Cool the oil phase to 55°C; add the final oily part of the Pheroid™ oil phase; and stir well.
6. Immediately add the water phase to the oil phase and mix with a homogeniser at 13 500 rpm until the cream reaches room temperature (25 ± 0.5°C).

B.5 SUMMARY

Both the Pheroid™ and non-Pheroid™ creams were of a pearly-white colour. No preservatives were added to the Pheroid™ cream to minimise variations in the four formulations, thus it was kept for no longer than two weeks before use.

B.6 PHOTOS OF APPARATUS USED DURING FORMULATION OF CREAMS

Figure B.1: ***Metler Toledo balance***

Figure B.2: ***Heidolph® homogenisers***

Figure B.3: ***Labcon® hotplate and magnetic stirrer***

Figure B.4: ***Milli-Q® academic water purification system***

ANNEXURE C

TRANSDERMAL DIFFUSION STUDIES OF FORMULATIONS CONTAINING FATTY ACIDS

C.1 INTRODUCTION

Transdermal active pharmaceutical ingredient (API) delivery has received increased attention over the past few decades since it proposes an attractive alternative to the accustomed delivery routes (Boucaud *et al*., 2001:70; Baert *et al*., 2011:472). Vertical Franz diffusion cells (Figure C.1) were used for the *in vitro* transdermal studies. These *in vitro* transdermal studies evaluate the permeation of APIs through dermatomed female Caucasian skin (obtained after abdominoplastic surgery) or an artificial membrane over a specified time period (12 h and 6 h, respectively). Franz cells comprise donor (top) and receptor (bottom) compartments. The skin (or membrane) used, is clamped between the two compartments with the *stratum corneum* facing the donor compartment. A phosphate buffer solution is placed in the receptor compartment and withdrawn at regular predetermined time intervals, whereas the formulation to be analysed is placed in the donor compartment.

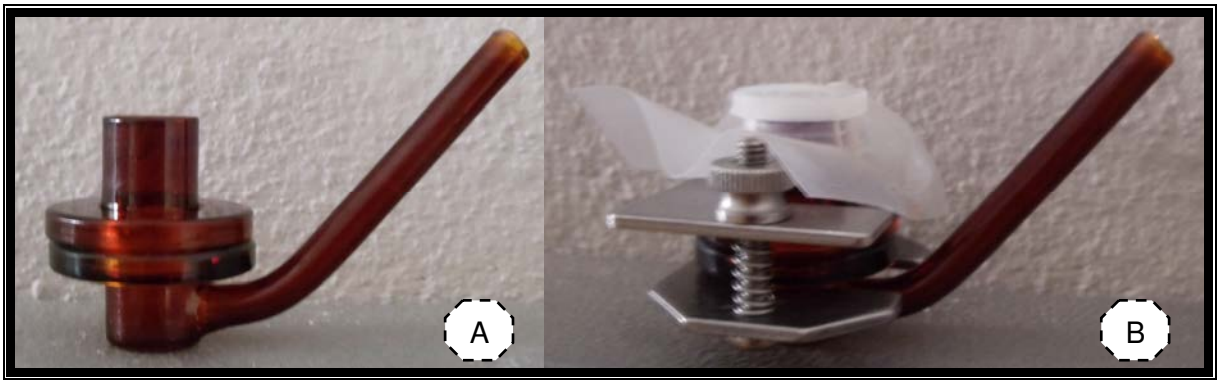

Figure C.1: ***A: Compartments of a vertical Franz diffusion cell, B: Assembled Franz cell.***

C.2 METHODS AND MATERIALS

C.2.1 PREPARATION OF PHOSPHATE BUFFER SOLUTION

The following steps were used to prepare the phosphate buffer solution:

- Weigh 6.81 g potassium dihydrogen phosphate (KH_2PO_4) and dissolve it in 250 ml deionised water.
- Weigh 1.571 g sodium hydroxide (NaOH) and dissolve it in 393.4 ml deionised water.
- Slowly add the NaOH solution to the KH_2PO_4 solution.
- Measure the pH. If the pH is not at 7.4, it can be lowered with the addition of 2M H_3PO_4 or increased with the addition of 2M NaOH. Solubility and the partition coefficient of flurbiprofen were determined at pH 5 for the purpose of unionisation. This adjustment is done simply by adding more H_3PO_4.
- Store in a refrigerator (2 - 4°C) until utilised.

C.2.2 SOLUBILITY OF FLURBIPROFEN IN WATER AND PHOSPHATE BUFFER SOLUTION

Prior to the commencement of any transdermal diffusion studies, the solubility and partition coefficient (log D) of flurbiprofen were determined as relevant physicochemical properties that influence drug permeation (Swart *et al.*, 2005:72). The solubility of flurbiprofen was determined in Milli-Q® water and phosphate buffer solution. An excess amount of flurbiprofen was placed in a polytop containing 5 ml solvent in order to keep the solution saturated. The contents was continuously mixed, using a magnetic stirring bar and maintained at 32°C in a water bath (Figure C.2) for a period of 24 h. Thereafter, the solution was filtered and injected into the HPLC (Figure C.7) to be analysed. This experiment was performed in triplicate.

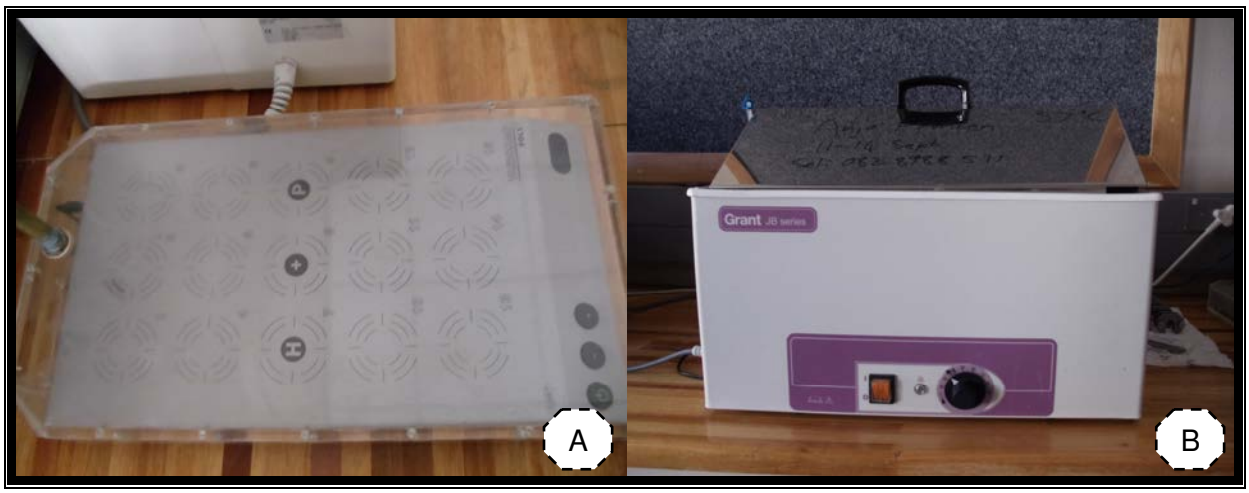

Figure C.2: ***A: Magnetic stirrer plate placed in the water bath, and B: Grant® JB series water bath.***

C.2.3 OCTANOL-BUFFER PARTITIONING COEFFICIENT (LOG D)

The experimental partition coefficient (log D) was determined using pre-saturated *n*-octanol and phosphate buffer solution (pH 5). The pH range acceptable for skin products is between 5 and 8, since a pH higher or lower than this will irritate the skin. The pH was prepared at 5 since this is the pH at which flurbiprofen is at the highest percentage unionised within the acceptable pH range. A higher degree of unionisation is preferred, since an unionised molecule will cross a membrane more readily (Lamb, 2012:1).

Equal volumes of *n*-octanol and phosphate buffer solution (pH 5) were equilibrated in a pear-shaped separatory funnel for at least 24 h in order to co-saturate the two phases. The phases were left to separate and stored. To determine the log D, 3 mg flurbiprofen was dissolved in 20 ml of the pre-saturated *n*-octanol. 3 ml of the flurbiprofen pre-saturated *n*-octanol was placed in a test-tube and 3 ml pre-saturated phosphate buffer solution was added. The test-tube was shaken continuously in a 32°C water bath for 24 h and the mixture was centrifuged at 5 000 rpm for 10 min using an Eppendorf® centrifuge model 5804 R (Figure C.3). Finally, the two phases in the test-tube were separated by means of a pipette. The phosphate buffer solution was injected in the HPLC as prepared, and the *n*-octanol phase was diluted with methanol (1:4) before injection into the HPLC. This dilution was necessary since pure octanol causes severe band-broadening, resulting in unusable chromatographic peaks. The experiment was done in triplicate and the logarithmic ratio of the flurbiprofen concentration in the *n*-octanol phase to the flurbiprofen concentration in the phosphate buffer solution was used to calculate the log D value.

Figure C.3: ***Eppendorf centrifuge, model 5804 R.***

C.2.4 SKIN PREPARATION

During skin permeation studies dermatomed Caucasian human skin was used which was donated by anonymous female donors who underwent abdominoplastic (tummy tuck) surgery. Ethical approval for the procurement and preparation of the skin was provided by the Research Ethics committee of the North-West University under the reference number NWU-00114-11-A5. Full-thickness skin was removed from the abdomen of female patients which was frozen at -20°C for no longer than 6 months. Before the studies were conducted, the full-thickness skin was taken from the freezer and left to thaw. The skin was visually examined for any defects such as stretch marks and large hair follicles/holes before cutting it into pieces with the Zimmer® electric dermatome model 8821 (Figure C.4). After the skin had been fully thawed, the dermatome was used to cut the skin into pieces of approximately 2 cm in width, 4 cm in length and 400 µm in thickness. All prepared skin samples were placed on Whatman® filter paper, wrapped in aluminium foil, and stored in a freezer at -20°C until utilised (within 24 h).

Figure C.4: ***Zimmer® electric dermatome.***

C.2.5 MEMBRANE RELEASE AND SKIN DIFFUSION STUDIES

Amber vertical Franz diffusion cells (Figure C.1 A) with a diffusion area of approximately 1.075 cm^2 were used during permeation studies. Twelve Franz cells were used, of which ten contained a specific formulation that included the API, whereas the remaining two contained placebo formulations (cream with no penetration enhancers or API). Either a membrane or skin was mounted between each of the two compartments of the diffusion apparatus, the skin with the *stratum corneum* facing upwards in the direction of the donor compartment.

Each receptor compartment was filled with 2 ml phosphate buffer solution (pH 7.4) and the temperature of the cell system was maintained at 37℃, using a water bath. The pH was set to 7.4 since the receptor phase represents human blood of which the temperature is approximately 37℃. The receptor compartment was filled, ensuring that no air bubbles formed underneath the skin or membrane, which might impair permeation. A Teflon-coated magnetic stirring bar was placed in the receptor compartment to continuously mix the phosphate buffer solution. To ensure skin saturation, enough of the formulation (1 ml at 32℃) was added to the donor compartment of the Franz cells. The applied formulation was maintained at 32℃ since that is the temperature of the skin surface. The donor compartment was closed with Parafilm® and a cap (Figure C.1 B) to prevent evaporation of the formulation. Dow Corning® high vacuum grease (Figure C.5) was used to seal the Franz cells in order to avoid leakage and thereafter a horse-shoe clamp (Figure C.6) was used to secure the two compartments.

Figure C.5: ***Dow Corning® high vacuum grease.***

Figure C.6: ***A horse-shoe clamp.***

At pre-determined time intervals the entire receptor volume was withdrawn and replaced with fresh buffer (37℃). During the membrane release studies, samples were withdrawn every hour for 6 h, whereas samples were withdrawn every 2 hours up to 12 h during skin diffusion studies. This was to insure that sink conditions existed throughout the experiment. Samples were directly assayed using an Agilent® isocratic system (Figure C.7) to determine the drug concentration in the receptor fluid.

Figure C.7: ***Agilent Technologies®, 1100 series High Performance Liquid Chromatography.***

C.2.6 TAPE STRIPPING

All the skin circles were removed after the 12 h diffusion studies. They were fixed onto Whatman® filter paper which was set to a flat surface. All excess formulation was dabbed from the skin with a clean paper towel. Circular imprints from the donor phase showed the diffusion area. To perform tape stripping, sixteen strips of 3M Scotch® Tape were needed. The first strip was discarded as it was considered part of the cleaning procedure. The remaining fifteen strips were used to obtain data.

After stripping the *stratum corneum*-epidermis off the diffusion area, the fifteen tape strips were put into a polytop containing a 5 ml 40% methanol in phosphate buffer solution. The polytop was vigorously shaken to wet all the strips and left overnight (approximately 12 h) to be analysed by HPLC the following day.

The diffusion area of the remaining skin circle (dermis) was first cut out and then cut into pieces which were placed in a polytop containing 5 ml of a 40% methanol in phosphate buffer solution. This area was cut into pieces in order to enlarge the surface area in contact with the methanol solution. The polytop was furthermore vigorously shaken and left overnight to be analysed by HPLC the following day.

C.2.7 HPLC ANALYSIS

An HPLC method had already been developed and validated in the Analytical Technology Laboratory (ATL) in conjunction with Prof. J.L. du Preez at the North-West University, Potchefstroom Campus. The method and instrumentation used, was described in Section A.2.1.

C.2.8 DATA ANALYSIS

C.2.8.1 TRANSDERMAL DATA ANALYSIS

After conduction of the diffusion studies, the cumulative concentration (µg/cm^2) of the permeated API was plotted against time. The steady-state slope of the average cumulative concentration against time provided the flux (µg/cm^2.h) of the API, whereas the average cumulative API concentration versus square root of time (h$^{0.5}$) presented the lag time for each of the formulations. The percentage flurbiprofen yielded from the total amount of API applied in the formulations was calculated for the skin (after 12 h) and membrane (after 6 h) studies.

Ten Franz diffusion cells were originally employed for each study in order to determine the flurbiprofen concentration diffused from the specific formulation. Two Franz cells (FCs) contained a placebo formulation to ensure results gathered from the flurbiprofen-containing formulations did indeed represent flurbiprofen concentrations and not ingredients from the cream formulation. From the ten active Franz cells some FCs were omitted from the results (outliers) due to abnormal data distribution. This abnormal data distribution were due to leakage from the FCs and early depletion of the formulation applied to that specific FC. The amount (n) of relevant FCs finally used in each study is noted in Section C.3.

C.2.8.2 STATISTICAL DATA ANALYSIS

Data gathered were analysed, using descriptive and inferential statistics. Descriptive statistics involved the calculation of the mean, median and standard deviation (SD). Inferential statistics involved one-way analysis of variance (ANOVA) and also parametric assumption testing (omnibus tests for differences between group means). The mean (average or arithmetic average) is a summarising statistic and is a measure of the centre of distribution which is particularly meaningful if the data is symmetrically scattered around the mean. The median is less affected by extreme data points and represents the centre of a data set (Bolton, 1997:14-18). The SD calculates the spread of data around the centre point (Bolton, 1997:20).

The first parametric assumption was the normality hypothesis. Statistical tests (Shapiro-Wilk and Kolmogorov-Smirnov) were used to determine whether the sample data had been drawn

from a normally distributed population. The second assumption was the homogeneity of variance hypothesis. Levene's test was employed to determine whether the variances of the four formulation groups were equal. Thereafter, the data was graphically inspected using QQ-plots and box-plots. It was found that the deviation from normality and difference in variation were not severe, though one-way ANOVA, as well as more robust tests (Welch and Brown-Forsythe) were performed to access the differences. After performing the omnibus tests, *post hoc* follow-up tests (Games-Howell and Tukey HSD) were conducted to determine which formulation's average cumulative concentration varied from one another (SPSS, 2011).

Furthermore, since skin diffusion studies represented repeated measures taken over time, a mixed model analysis was fitted to the data to account for the dependence structure. An AR(1) covariance structure was specified. The Type III test for fixed effects was applied as an omnibus test to indicate statistically significant differences between the levels of factors time and treatment (formulation). Pairwise comparisons of estimated marginal means were employed, after a Bonferroni adjustment for multiple comparisons had been applied to determine which levels differed significantly.

Statistical analyses were conducted by the Statistical Consultation Services of the North-West University, Potchefstroom Campus. The analyses were performed using SPSS (version 20, 2011).

C.3 RESULTS AND DISCUSSION

C.3.1 SOLUBILITY OF FLURBIPROFEN

The ideal solubility of an API to permeate through the skin is >1 mg/ml (Naik *et al.*, 2000:319; Williams, 2003:187). Literature suggests that flurbiprofen is characterised by significantly poor aqueous solubility of approximately 0.07 mg/ml in phosphate buffer solution (pH 4, 32°C) (Dollery, 1999:F126; Swart *et al.*, 2005:76) and 0.005 to 1.306 mg/ml in water (Ambade *et al.*, 2008:37; Baek *et al.*, 2011:393).

Experimental results delivered solubility values at a pH lower than 5 since flurbiprofen is an acidic compound that lowers the pH after dissolution. The solubility was estimated at 0.057 mg/ml in water (pH 4.75, 32°C) and 0.0704 mg/ml in phosphate buffer solution (pH 4.69, 32°C). These results indicated that flurbiprofen was only slightly soluble and, therefore, it could be expected that it would encounter some difficulty in permeating through the skin.

C.3.2 LOG D OF FLURBIPROFEN

A partition coefficient of an API between 1 and 3 is preferred for optimal skin permeation (Hadgraft, 2004:292; Swart *et al.*, 2005:72; Karande & Mitragotri, 2009:2363). Literature values vary in respect of the documented log P values for flurbiprofen. Drugbank (2012) stated that the log P is 3.8, whereas other literature claimed a log P value of 4.24 for flurbiprofen (Li *et al.*, 2006:542; Alsarra *et al.*, 2010:233). Swart *et al.* (2005:76) provided an experimental log P of 3.34 compared to a value from the literature of 4.16 (Hadgraft *et al.*, 2000:33).

Experimental results delivered a log D of 3.41 at 32°C. It was, therefore, clear that flurbiprofen does not have an ideal log P value for skin permeation.

C.3.3 MEMBRANE RELEASE STUDIES

Data obtained from the membrane release studies is expressed as the percentage of the average amount released from the total amount applied to the donor compartment. The data also includes the average and median cumulative amount released per unit area (Table C.1).

Table C.1: ***Membrane release results for flurbiprofen after 6 h.***

Formulation	Average % released	Average cumulative concentration (µg/cm^2)	Median cumulative concentration (µg/cm^2)
Control	2.69 ± 0.4	499.27 ± 74.76	466.13
Evening Primrose Oil	2.45 ± 0.21	455.79 ± 38.52	459.28
Vitamin F	3.04 ± 0.24	564.89 ± 43.79	521.73
Pheroid™	1.81 ± 0.18	336.72 ± 33.09	321.88

As seen in Table C.1, the formulation that contained vitamin F displayed the highest average percentage flurbiprofen released (3.04%) from the total amount applied to the donor compartment of the Franz cells. Furthermore, this formulation also presented the highest average cumulative concentration released after 6 h (564.89 µg/cm^2). In fact, the vitamin F formulation was the only formulation that delivered results which were significantly higher than that of the control formulation. On the other hand, the Pheroid™ formulation depicted the lowest average percentage flurbiprofen released (1.81%), as well as the lowest average cumulative concentration released after 6 h (336.72 µg/cm^2).

The smallest differences between the mean (455.79 µg/cm^2) and median (459.28 µg/cm^2) cumulative concentration of flurbiprofen were found in the EPO formulation and the Pheroid™

formulation (mean = 336.72 µg/cm^2, median = 321.88 µg/cm^2) as seen in Table C.1. This may indicate that the outliers in the data points were minimal for each Franz cell during membrane release studies.

After the development of a mixed model and applying the mixed model to assess the Type III tests of fixed effects (Section C.2.8.2), a statistically significant difference was found between the levels of time, treatment, and the time/treatment interactions with p-values < 0.05. Pairwise comparisons displayed a statistically significant difference between the vitamin F and control formulation with a p-value of 0.001. All the other combinations of treatments also depicted a statistical significant difference ($p < 0.05$), except for the difference between the control and EPO formulations, which displayed a p-value of 0.054 (Table C.2), though this value was significantly close to 0.05 so that it could be said that a statistically significant difference was found between the control and EPO formulation.

Table C.2: ***P-values between different formulations for membrane release (6 h) after employing pairwise comparisons and applying Bonferroni adjustments for multiple comparisons.***

	Pheroid™	Vitamin F	EPO
Control	0.000001	0.001	0.054
EPO	0.000001	0.000001	
Vitamin F	0.000001		

C.3.4 SKIN DIFFUSION STUDIES

The data reflecting the average percentage diffused of the applied dose, as well as the average and median cumulative concentration diffused per unit area is presented in Table C.3 and Figures C.8 - C.15.

Table C.3: ***The average percentage API diffused and the average and median cumulative concentration diffused through the skin diffusion area after 12 h.***

Formulation	Ave. % diffused	Ave. Cumulative concentration (µg/cm^2)	Median cumulative concentration (µg/cm^2)
Control	0.49 ± 0.039	91.53 ± 7.24	91.43
Evening Primrose Oil	0.51 ± 0.139	94.14 ± 25.78	95.76
Vitamin F	0.44 ± 0.108	81.80 ± 20.15	82.72
Pheroid™	0.24 ± 0.077	45.32 ± 14.26	46.14

It was clear from Table C.3 that the EPO formulation delivered the highest average percentage flurbiprofen diffused, even though it did not show the highest concentration released from the formulation. However, the differences between the average cumulative flurbiprofen concentration diffused after 12 h for the EPO and control formulation were not found to be statistically significant (Table C.4), after employing a mixed model with pairwise comparisons for estimated marginal means and a Bonferroni adjustment for multiple comparisons (Section C.2.8.2). After applying an ANOVA with Games-Howell *post hoc* tests, this fact was supported and can be seen in Section C.3.4.1, Table C.7. The Pheroid™ formulation depicted the lowest average percentage flurbiprofen released (1.81%) and also illustrated the lowest average percentage flurbiprofen diffused (0.24%). This percentage flurbiprofen diffused was approximately half diffused through the skin from the control formulation (0.49%). Average cumulative concentrations of flurbiprofen, diffused through the skin after 12 h, could be placed in the following rank order: EPO formulation > control formulation > vitamin F formulation >> Pheroid™ formulation.

Table C.4: ***P-values between different formulations for skin diffusion (12 h) after employing pairwise comparisons and applying Bonferroni adjustments for multiple comparisons. (Statistically significant differences indicated in red).***

	Pheroid	Vitamin F	EPO
Control	0.001	1	1
EPO	0.000001	0.824	
Vitamin F	0.008		

The control and Pheroid™ formulation exhibited the smallest difference between the average and median cumulative concentration, which may indicate the smallest number of outliers in the data for these formulations. This can be visually confirmed in Figures C.9 and C.15, though after visual inspection it was found that the Pheroid™ formulation did show many outliers. Figures C.8, C.10, C.12, and C.15 illustrate the average cumulative concentrations diffused over a 12 h period for the various formulations.

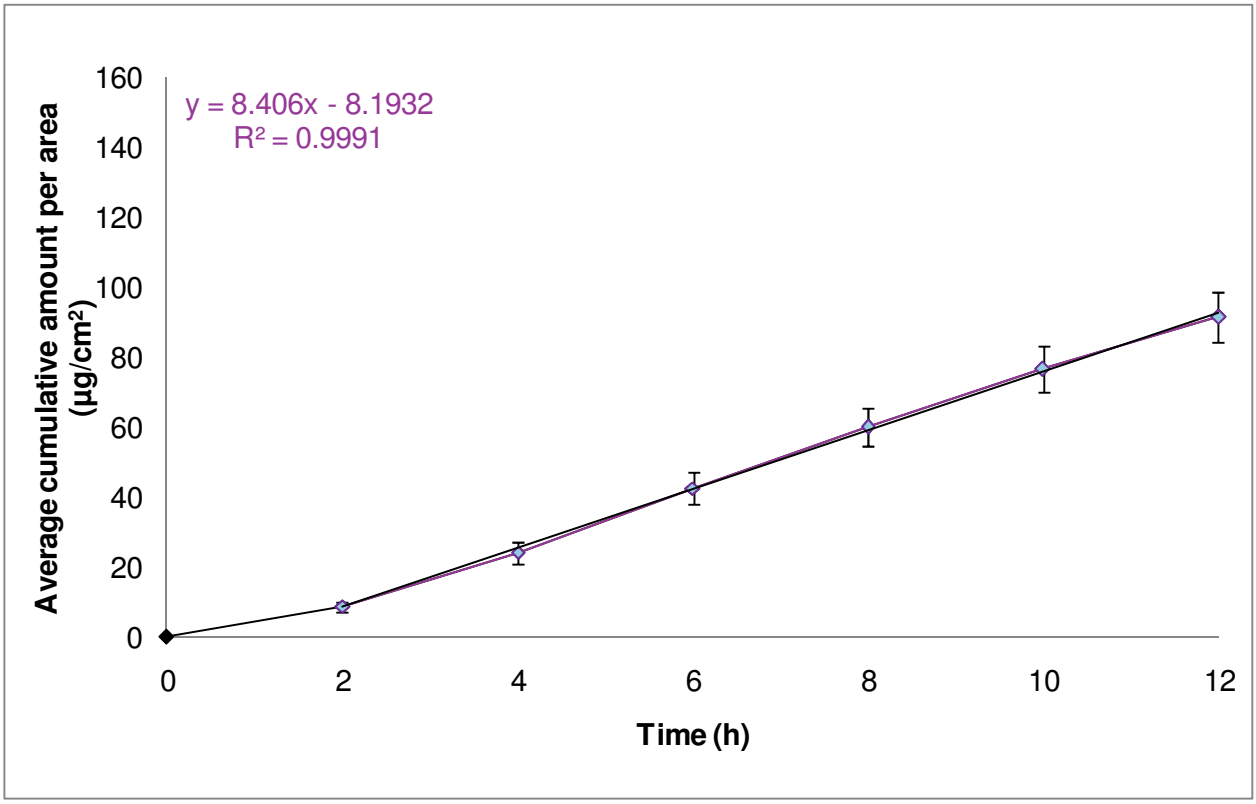

Figure C.8: ***The average (n=9) cumulative concentration flurbiprofen diffused through the skin for the control formulation.***

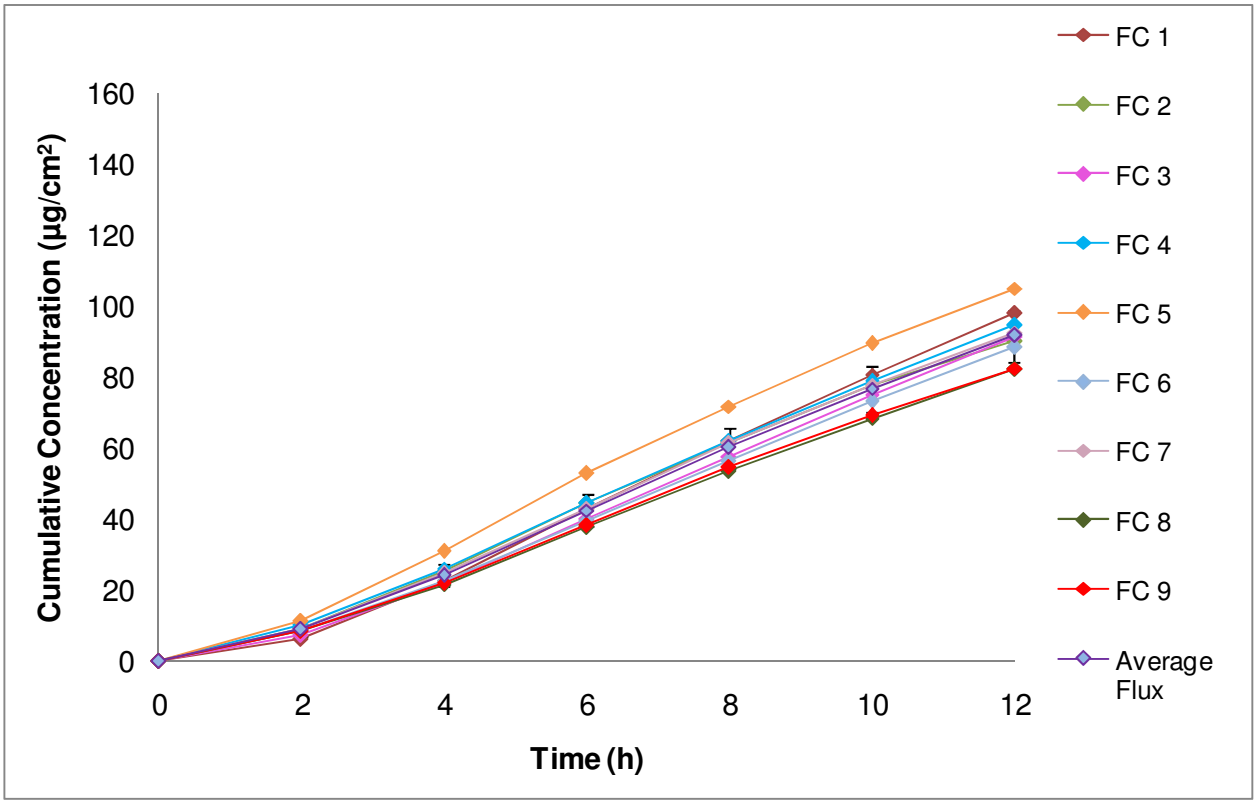

Figure C.9: ***Cumulative concentration flurbiprofen diffused through the skin for the assorted Franz cell (FC) repeats used for the control formulation.***

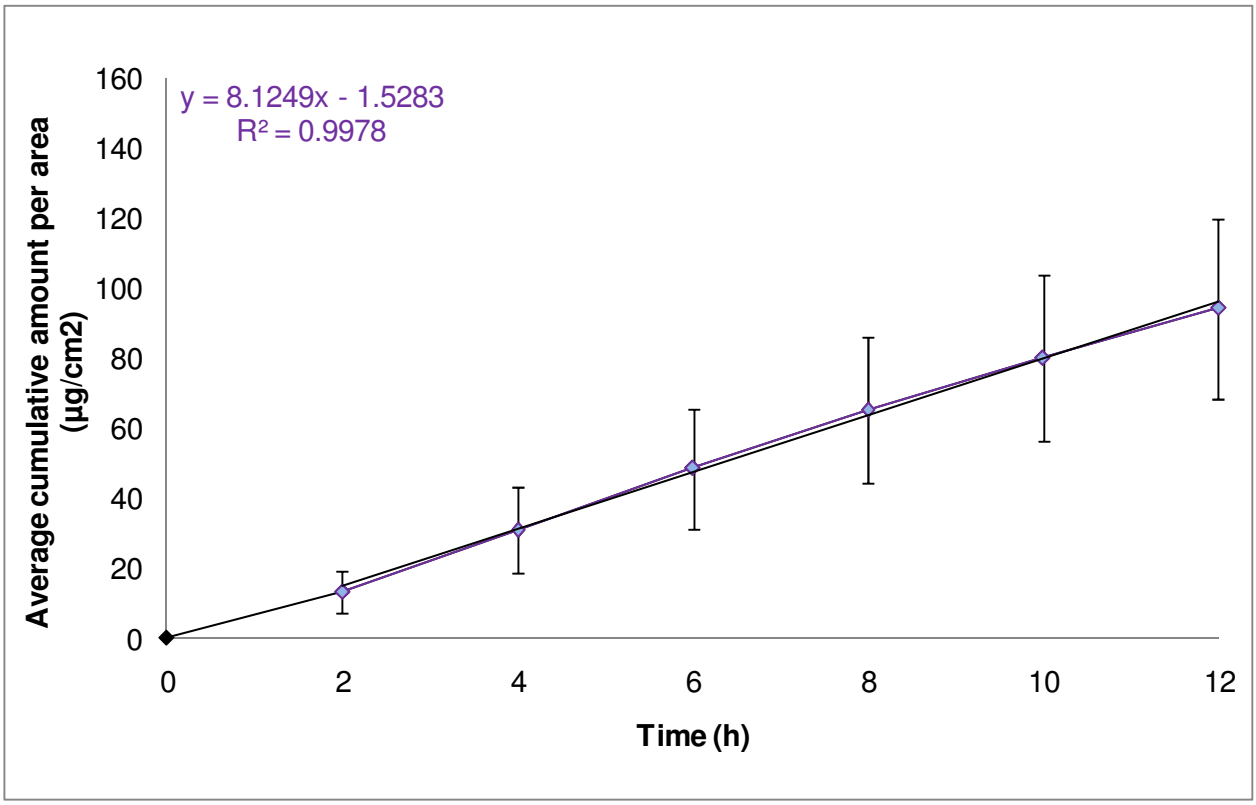

Figure C.10: ***The average (n=10) cumulative concentration flurbiprofen diffused through the skin for the evening primrose oil (EPO) formulation.***

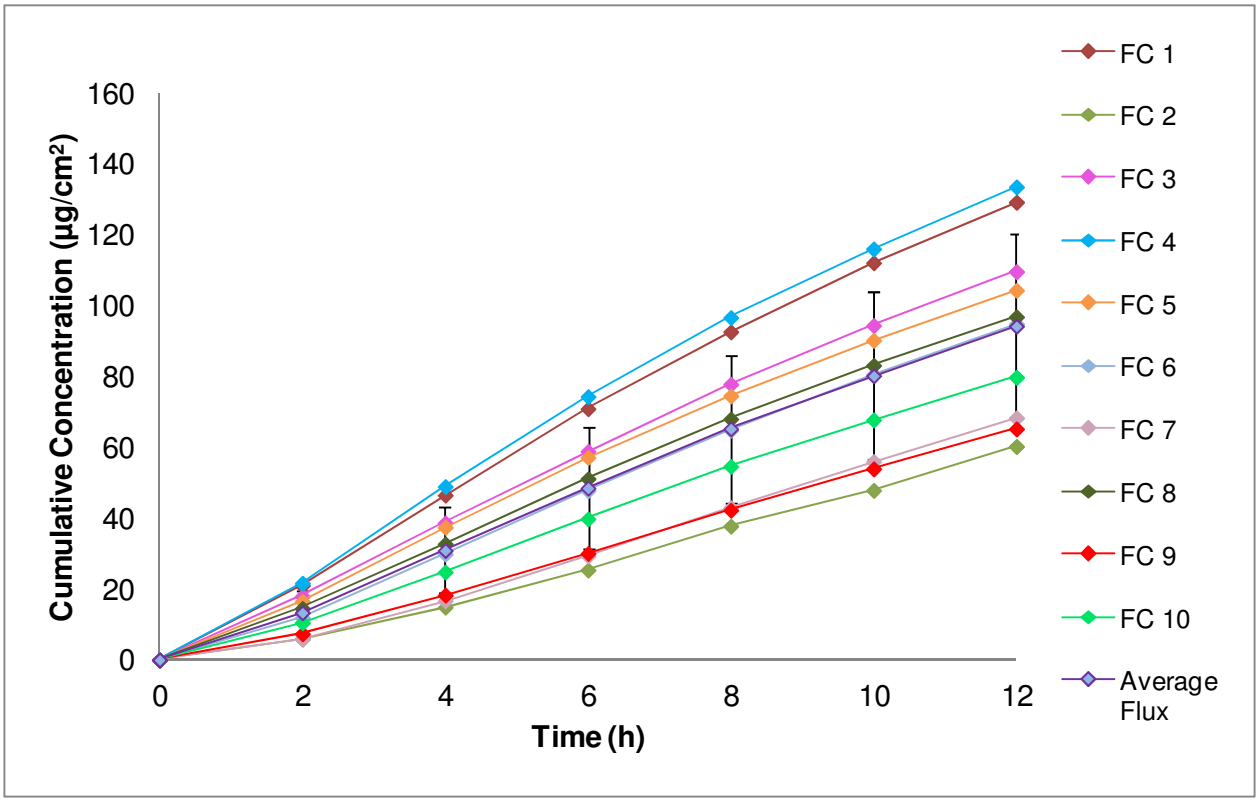

Figure C.11: ***Cumulative concentration flurbiprofen diffused through the skin for the assorted Franz cell (FC) repeats used for the EPO formulation.***

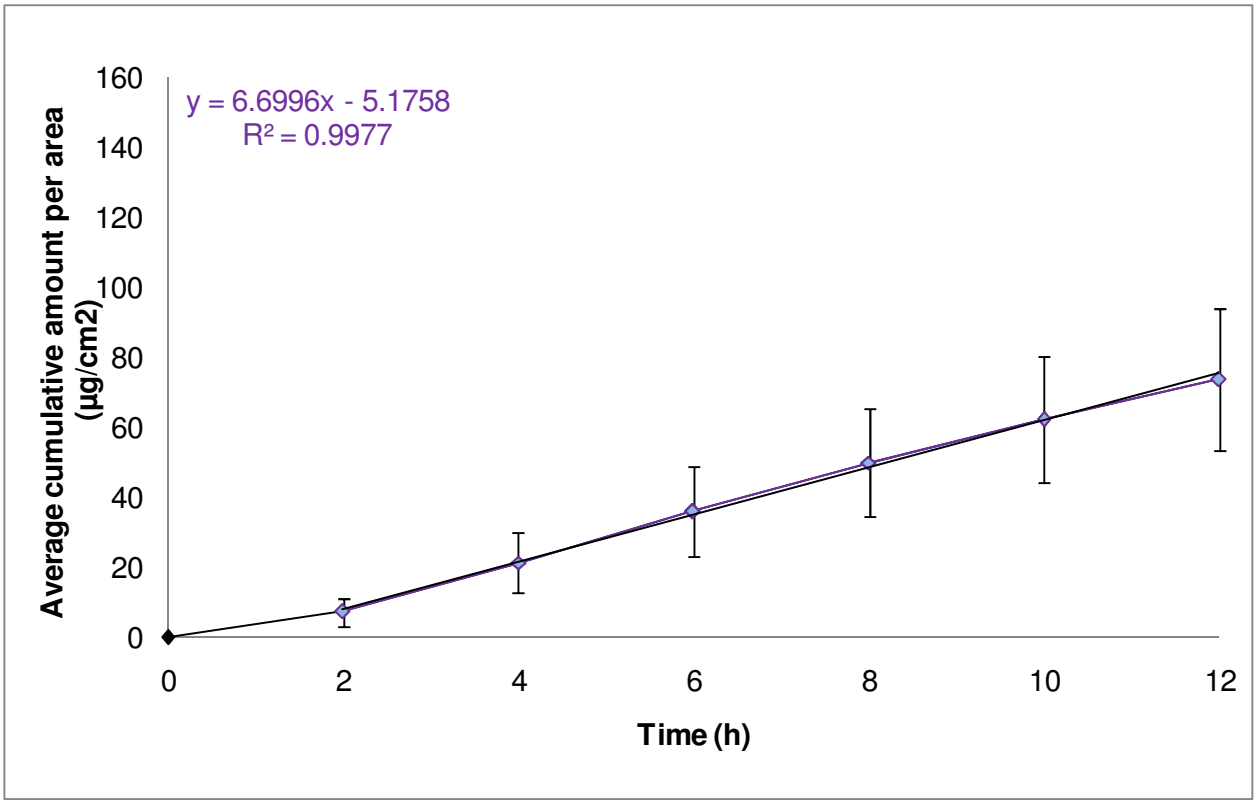

Figure C.12: ***The average (n=9) cumulative concentration flurbiprofen diffused through the skin for the vitamin F formulation.***

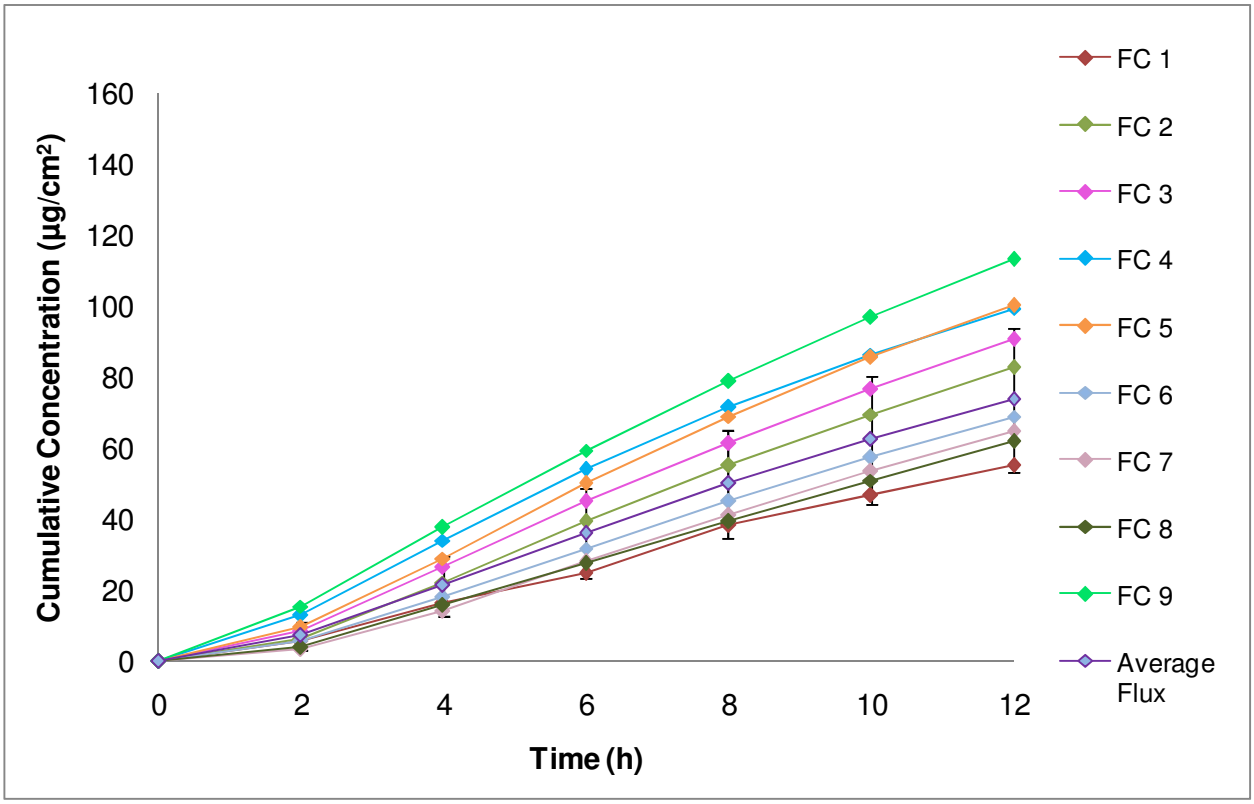

Figure C.13: ***Cumulative concentration flurbiprofen diffused through the skin for the assorted Franz cell (FC) repeats used for the vitamin F formulation.***

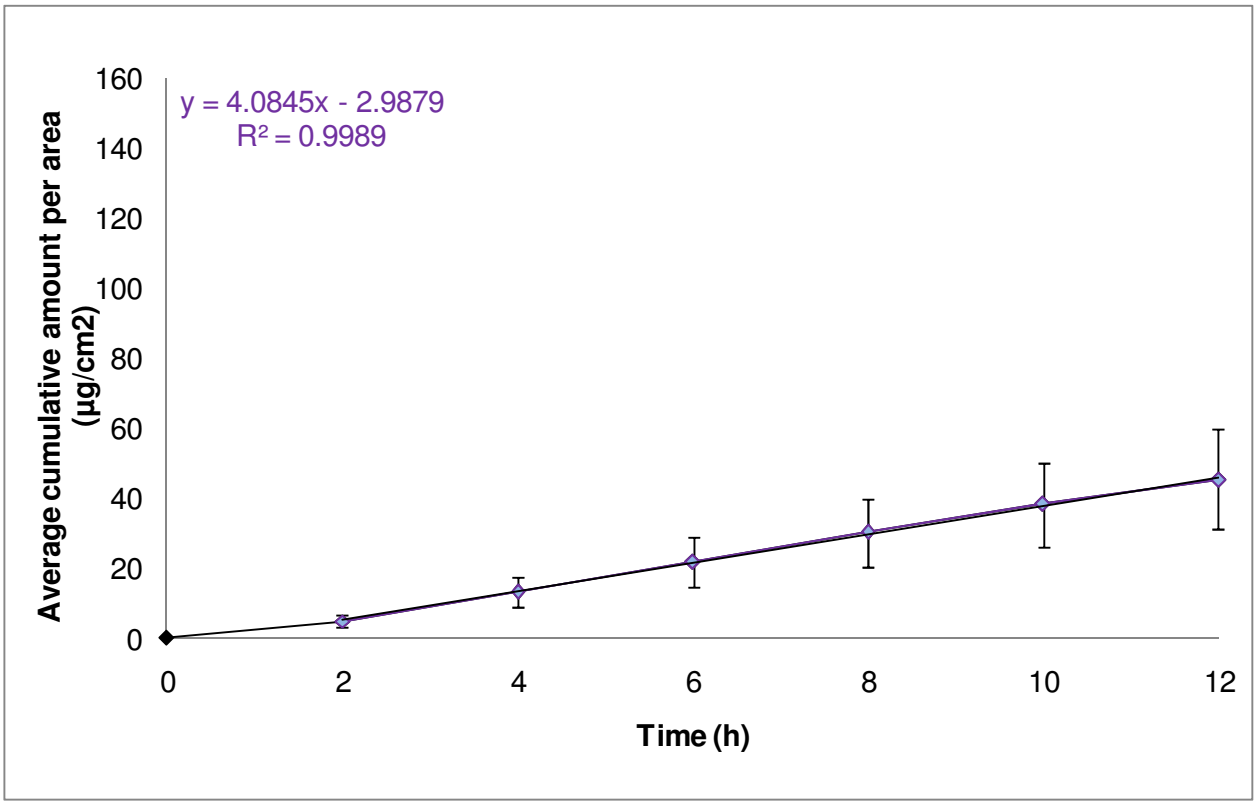

Figure C.14: ***The average (n=10) cumulative concentration flurbiprofen diffused through the skin for the Pheroid™ formulation.***

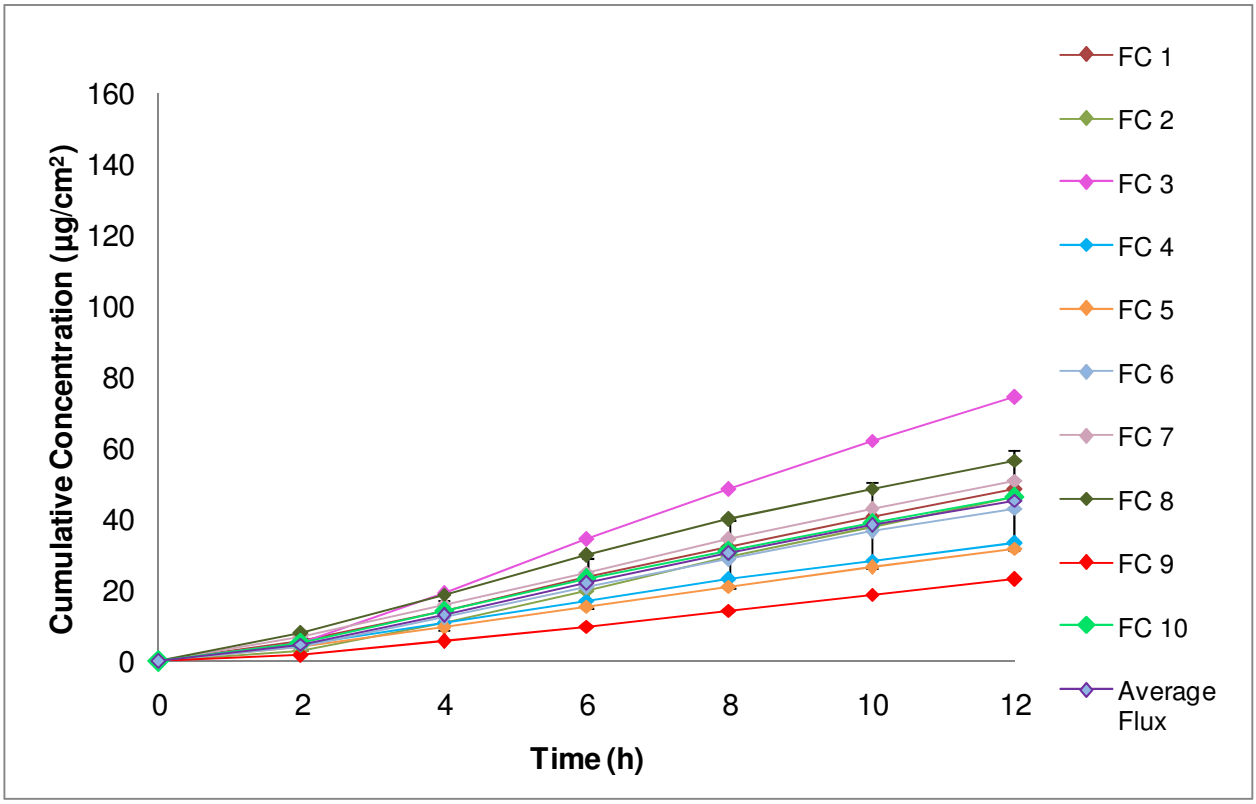

Figure C.15: ***Cumulative concentration flurbiprofen diffused through the skin for the assorted Franz cell (FC) repeats used for the Pheroid™ formulation.***

Subsequent application of a mixed model (Section C.2.8.2) to the data, statistically significant differences were found between the level of time, treatment, and the time/treatment interaction with p-values of 0.000001. Pairwise comparisons displayed a statistically significant difference between the Pheroid™ and control formulation ($p = 0.01$), Pheroid™ and vitamin F formulation ($p = 0.008$), and Pheroid™ and EPO formulation ($p = 0.000001$), as indicated in red in Table C.4. All the other combinations of treatment interactions did not portray a statistically significant difference ($p > 0.05$). A statistically significant difference was, however, found for the time/treatment interaction only after 2 h elapsed ($CI_{95\%}$ did not include zero). Figure C.16 is a box-plot representation of the cumulative concentrations of all the formulations used, with the line dividing the box as the median and the purple line as the mean.

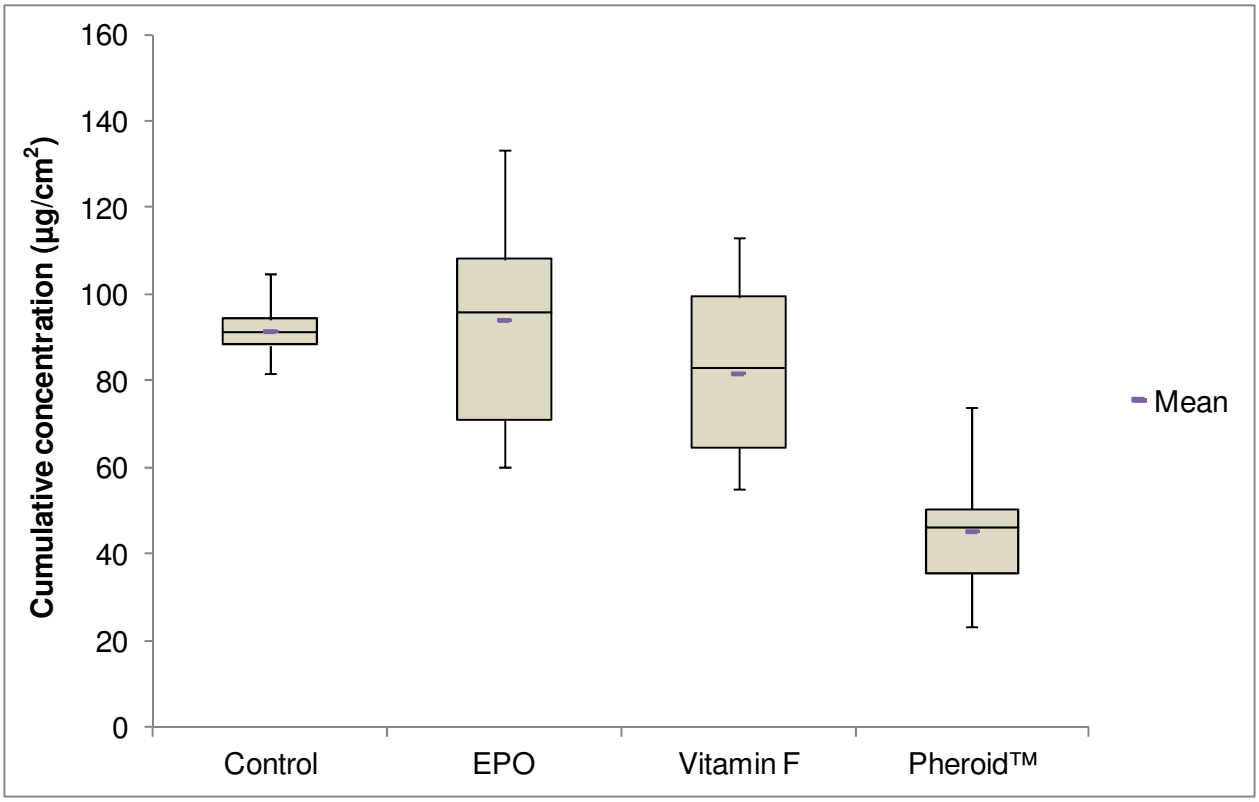

Figure C.16: ***Box-plots representing the cumulative concentration of the four formulations used, with the purple line as the mean and the line dividing the box as the median.***

After inspection of the box-plot representation of the average cumulative flurbiprofen concentration diffused through the skin, it was clear that the EPO formulation depicted the highest concentration diffused, followed by the control and vitamin F formulation, respectively. The control and vitamin F formulation indicated a linear distribution of data and, therefore, the smallest error bars. It could also be seen that the Pheroid™ formulation delivered a significantly lower average cumulative flurbiprofen concentration diffused through the skin. A low variation between the mean and median were observed in Table C.3 that was confirmed in Figure C.16, where the purple line indicated the mean and the black line dividing the box, the median.

Figures C.8, C.10, C.12 and C.14 present the flux of the formulations which can be seen in Table C.5, together with the lag time. The flux is obtained from the slope of the regression line, representing the average cumulative concentration over time. The lag time was calculated by plotting the square root of time ($h^{0.5}$) against the average cumulative concentration. The lag time is the time at which the regression line crosses the horizontal axis.

Table C.5: ***The flux and lag time of the different formulations after 12 h.***

Formulation	Flux (µg/cm^2.h)	Lag time (h)
Control	8.41	1.74
Evening Primrose Oil	8.12	1.36
Vitamin F	6.70	1.65
Pheroid™	4.08	1.62

The flux of the control formulation (8.41 µg/cm^2.h) was the highest, with the evening primrose oil (EPO) formulation following closely (8.12 µg/cm^2.h). On the other hand, the Pheroid™ formulation exhibited the lowest flux (4.08 µg/cm^2.h) at approximately half the rate of the control formulation. The EPO formulation delivered the shortest lag time (1.36 h), though all the formulations illustrated a shorter lag time than the control formulation.

C.3.4.1 TAPE STRIPPING

Tape stripping was conducted on the skin circles post removing it from the Franz cells after the 12 h diffusion studies (Section C.2.6). The data obtained is reported in Table C.6.

Table C.6: **Average c*oncentration API present in the stratum corneum and dermis-epidermis after 12 h.***

Formulation	*Stratum corneum* (µg/ml)	Dermis-epidermis (µg/ml)
Control	0.26 ± 0.16	0.45 ± 0.07
Evening Primrose Oil	0.19 ± 0.07	0.37 ± 0.08
Vitamin F	0.17 ± 0.06	0.36 ± 0.15
Pheroid™	0.09 ± 0.02	0.12 ± 0.11

The control formulation exhibited the highest concentrations in the skin with the dermis-epidermis concentration the highest. The EPO formulation presented the second highest concentration in the dermis-epidermis and the *stratum corneum*. The following rank order for the concentration flurbiprofen present in the skin could be seen: control > EPO ≈ vitamin F >> Pheroid™. Figure C.17 is a box-plot representation of the concentration flurbiprofen present in the *stratum corneum* and the dermis-epidermis after tape stripping experiments were conducted.

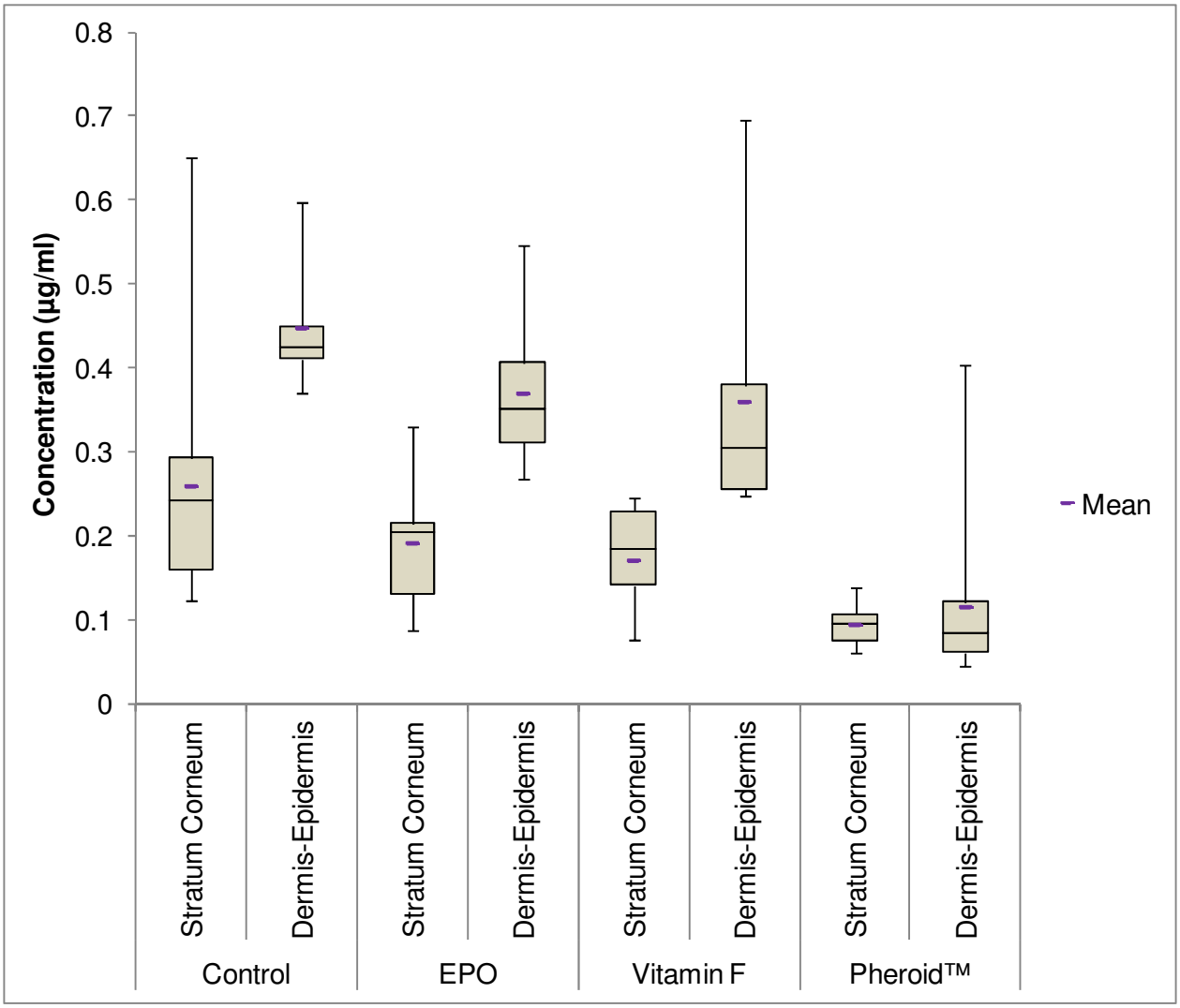

Figure C.17: ***Box-plots representing the average concentration flurbiprofen present in the stratum corneum and dermis-epidermis of the four formulations used, with the purple line as the mean and the line dividing the box as the median.***

After inspecting the box-plot representation (Figure C.17) of the average concentration flurbiprofen present in the *stratum corneum* and dermis-epidermis, it was clear that higher concentrations flurbiprofen were present in the dermis-epidermis. All the formulations used delivered a skew distribution of data with most of the outliers extending to the outer quartile. This fact can also be seen in the higher variation between the mean- and median values as indicated by the purple and black line, respectively. It was furthermore observed that although the control formulation depicted the highest concentration flurbiprofen present in the skin, there was no significant difference between the control, vitamin F and EPO formulation. The Pheroid™ formulation, however, were significantly lower than the other formulations.

One-way ANOVA of equality of means was employed by means of Brown-Forsythe and Welsh tests. Both of these tests indicated a statistically significant difference ($p < 0.05$) between the average cumulative concentration, the concentration present in the *stratum corneum* and the concentration flurbiprofen present in the dermis-epidermis. Games-Howell *post hoc* tests (Section C.2.8.2) were applied to the tape stripping data after 12 h and revealed a statistically significant difference (Table C.7) for the concentrations present in the *stratum corneum* for the Pheroid™ and EPO formulations ($p = 0.01$), and the Pheroid™ and vitamin F formulations ($p = 0.032$). The difference between the control and Pheroid™ formulation delivered a p-value of 0.062. A statistically significant difference was also found for the concentrations present in the dermis-epidermis for the Pheroid™ and control formulations ($p < 0.05$), Pheroid™ and EPO formulations ($p < 0.05$), and Pheroid™ and vitamin F formulations ($p = 0.005$). A statistically significant difference ($p < 0.05$) was obtained between the concentration present in the receptor phase of the Franz cells (diffused through the skin) and the concentration API present in the skin (*stratum corneum* and dermis-epidermis).

Table C.7: ***P-values for Games-Howell post hoc tests for multiple comparisons for the skin diffusion data obtained after 12 h. (p < 0.05 then the difference between the data points is statistically significant and is indicated in red).***

Formulation comparisons	Average cumulative concentration (µg/cm^2)	Concentration in stratum corneum (µg/ml)	Concentration in dermis-epidermis (µg/ml)
Control vs. EPO	0.989	0.664	0.158
Control vs. Pheroid™	0.000001	0.062	0.000001
Control vs. Vitamin F	0.547	0.458	0.403
EPO vs. Pheroid™	0.001	0.01	0.000001
EPO vs. Vitamin F	0.654	0.912	0.998
Pheroid™ vs. Vitamin F	0.002	0.032	0.005

C.4 CONCLUSIONS

Cream-based formulations containing 5% of a chosen penetration enhancer were prepared, utilising flurbiprofen as a model compound in order to be able to determine whether any significant effects could be detected. Evening primrose oil, vitamin F and Pheroid™ technology all contain fatty acids that have been proven to enhance penetration. Therefore, these products were incorporated to act as penetration enhancers.

Membrane release studies were performed over 6 h to determine if any release of the API from the formulation occurred. Results obtained from these studies indicated that the API was indeed released from each of the formulations and, therefore, it was available on the skin surface for possible diffusion through the skin. The highest flurbiprofen concentration was released from the vitamin F formulation.

By comparing membrane release and skin diffusion data, it was found that the EPO formulation, rather than the vitamin F formulation displayed better penetration enhancement of flurbiprofen when used in a cream-based formulation. This may be due to the fact that human skin already consists of approximately 15% linoleic acid and 30% oleic acid, which may result in less obstruction from the *stratum corneum* permeability barrier. Average cumulative concentrations of flurbiprofen, diffused through the skin after 12 h, could be placed in the following rank order: EPO formulation > control formulation > vitamin F formulation >> Pheroid™ formulation. Previous studies showed that the enhancing effect of fatty acids follow the following rank order: linoleic acid > oleic acid > linolenic acid, though linolenic acid was proven to be more of an irritant to the skin (Fang *et al.*, 2003:156-159). EPO contains significantly high concentrations linoleic (65 - 80%) and oleic acid (6 - 11%), causing flurbiprofen to diffuse more readily through the skin. The *stratum corneum* consists of approximately 41% ceramides of which linoleic acid is a precursor, which may also begin to explain why higher linoleic concentrations may result in higher enhancing qualities (Hopkins, 2012; McCusker & Grant-Kels, 2010:442; Suhonen *et al.*, 1999:150).

The control formulation containing no penetration enhancers provided the highest flux (8.41 µg/cm^2.h) of flurbiprofen diffusing through the skin. The EPO formulation illustrated a flux value (8.12 µg/cm^2.h) close to that of the control formulation, though no statistically significant difference was found ($p > 0.05$). The Pheroid™ formulation portrayed the lowest flux (4.08 µg/cm^2.h; $p < 0.05$), at approximately half the rate of the control formulation. Furthermore, the EPO formulation depicted the shortest lag time (1.36 h), though all the formulations exhibited a lag time shorter than that of the control formulation (1.74 h). A shorter lag time indicates that an API will more rapidly start to diffuse through the skin. Thus, the EPO formulation delivered a faster onset of diffusion of flurbiprofen through the skin, which may be due to the fact that the fatty acids present in EPO are also part of the *stratum corneum* structure and, thus, less obstruction is presented by the *stratum corneum* permeability barrier (SCPB).

The Pheroid™ formulation exhibited the lowest average cumulative concentration (45.32 µg/cm^2) diffused through the skin ($p < 0.05$) at approximately half the concentration compared with that of the control formulation (91.53 µg/cm^2). Pheroid™ technology is highly lipophilic and also

contains a lower concentration fatty acids than the other penetration enhancers used. Flurbiprofen is also highly lipophilic and, thus, the lower concentration flurbiprofen released from the formulation and diffused through the skin may be explained by the fact that flurbiprofen will reside mostly in the lipid bilayer of the Pheroids™ and not diffuse into the skin (Grobler *et al.*, 2008:285-288). The EPO formulation, on the other hand, produced a cumulative concentration of 94.14 µg/cm^2 ($p > 0.05$), which was the highest concentration of all the formulations used.

Tape stripping data suggested that, since this fatty acid containing cream illustrated an overall low concentration flurbiprofen present in the skin, it will be most effective if used for transdermal therapy rather than topical therapy, since the low concentrations flurbiprofen present within the skin (*stratum corneum* and dermis-epidermis) may not have a significant therapeutic effect. The following rank order, however, were observed for the concentration flurbiprofen present in the skin: control > EPO ≈ vitamin F >> Pheroid™. However, significantly higher concentrations flurbiprofen were observed in the receptor phase, which represented the concentration diffused through the skin.

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ANNEXURE D

AUTHOR'S GUIDE TO THE INTERNATIONAL JOURNAL OF PHARMACEUTICS

D.1 DESCRIPTION

The ***International Journal of Pharmaceutics*** is the journal for **pharmaceutical scientists** concerned with the **physical, chemical** and **biological** properties of **devices** and **delivery systems** for **drugs, vaccines** and **biologicals**, including their **design, manufacture** and **evaluation.** This includes evaluation of the properties of **drugs, excipients** such as **surfactants** and **polymers** and **novel materials.** The journal has special sections on **pharmaceutical nanotechnology and personalised medicines**, and publishes research papers, reviews, commentaries and letters to the editor as well as special issues.

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D.2 GUIDE FOR AUTHORS

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Areas of particular interest include: pharmaceutical nanotechnology; physical pharmacy; polymer chemistry and physical chemistry as applied to pharmaceutics; excipient function and characterisation; biopharmaceutics; absorption mechanisms; membrane function and transport; novel routes and modes of delivery; responsive delivery systems, feedback and control mechanisms including biosensors; applications of cell and molecular biology to drug delivery; prodrug design; bioadhesion (carrier-ligand interactions); and biotechnology (protein and peptide formulation and delivery).

Note: For details on pharmaceutical nanotechnology, see Editorials in 279/1-2 281/1, and 288/1.

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- **Rapid Communications**

(a) These articles should not exceed 1500 words or equivalent space.
(b) Figures should not be included otherwise delay in publication will be incurred.
(c) Do not subdivide the text into sections. An Abstract should be included as well as a full reference list.

- **Notes**

Should be prepared as described for full length manuscripts, except for the following:

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(b) Do not subdivide the text into sections. An Abstract and reference list should be included.

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