

**Formulation and topical delivery of lidocaine and prilocaine with the use
of Pheroid™ technology**

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This dissertation is presented in the so-called article format, which includes an introductory chapter with sub-chapters, a full length article for publication in a pharmaceutical journal and appendixes containing experimental results and discussion. The article in this dissertation is to be submitted for publication in the International Journal of Pharmaceutics, of which the complete guide for authors is included in Appendix E.

ABSTRACT

Local anaesthetics are used regularly in the medical world for a variety of different procedures. Topical anaesthetics are used largely in minor skin breaking procedures, laceration repair and minor surgical procedures such as laryngoscopy, oesophagoscopy or urethroscopy (Franchi *et al.*, 2008:186e1). The topical means of application of a local anaesthetic is non-invasive and painless that results in a good patient acceptability profile (Little *et al.*, 2008:102). An existing commercial topical anaesthetic product contains a eutectic mixture of the amide-type local anaesthetics lidocaine hydrochloride (HCl) and prilocaine hydrochloride (HCl). This commercial product takes up to an hour to produce an anaesthetic effect. This is considered as a disadvantage in the use of topical anaesthetics, an hour waiting time is not always ideal in certain medical circumstances (Wahlgren & Quiding, 2000:584).

This study compared the lag times, transdermal and topical delivery of lidocaine HCl and prilocaine HCl from four different semi-solid formulations with the inclusion of a current commercial product. One of the formulated semi-solid formulations included Pheroid™ technology, a novel skin-friendly delivery system developed by the Unit for Drug Research and Development at the North-West University, Potchefstroom Campus, South Africa.

The skin is the body's first line of defence against noxious external stimuli. It is considered the largest organ in the body with an intensive and complex structure. It consists of five layers with the first outer layer, the stratum corneum, the most impermeable (Williams, 2003:1). The stratum corneum has excellent barrier function characteristics and is the cause for the time delay in the transdermal delivery of active pharmaceutical ingredients (API) (Barry, 2007:569). Local anaesthetics need to penetrate all the epidermal skin layers in order to reach their target site, the dermis. Skin appendages as well as blood vessels and skin nerve endings are located in the dermis. Local anaesthetics have to reach the free nerve endings in the dermis in order to cause a reversible block on these nerves for a local anaesthetic effect (Richards & McConachie, 1995:41).

Penetration enhancement strategies for the transdermal delivery of lidocaine and prilocaine have been investigated and include methods like liposomal entrapment (Franz-Montan *et al.*, 2010; Müller *et al.*, 2004), micellisation (Scherlund *et al.*, 2000), occlusive dressing (Astra Zeneca, 2006), heating techniques (Masud *et al.*, 2010) and iontophoresis (Brounéus *et al.*, 2000). The Pheroid™

delivery system has improved the transdermal delivery of several compounds with its enhanced entrapment capabilities. Pheroid™ consists mainly of unsaturated essential fatty-acids, non-harmful substances that are easily recognised by the body (Grobler *et al.*, 2008:285). The morphology and size of Pheroid™ is easily manipulated because it is a submicron emulsion type formulation which provides it with a vast flexibility profile (Grobler *et al.*, 2008:284). Vesicular entrapment was used to entrap lidocaine HCl and prilocaine HCl in the Pheroid™ and incorporated into an emulgel formulation. An emulgel without the inclusion of Pheroid™ was formulated for comparison with the Pheroid™ emulgel as well as with a hydrogel. Pheroid™ solution was prepared and compared to a phosphate buffer solution (PBS) without Pheroid™, both containing lidocaine HCl and prilocaine HCl as APIs.

Franz cell type transdermal diffusion studies were performed on the four semi-solid formulations (emulgel, Pheroid™ emulgel, hydrogel and the commercial product) and two solutions (PBS and Pheroid™). The diffusion studies were performed over a 12 h period followed by the tape stripping of the skin after each diffusion study. Caucasian female abdominal skin was obtained with consent from the donors. The skin for the diffusion cells were prepared by using a Zimmer Dermatome®. PBS (pH 7.4) was prepared as the receptor phase of the diffusion studies. The receptor phase was extracted at certain pre-determined time intervals and analysed with high performance liquid chromatography (HPLC) to determine the amount of API that had traversed the skin. Stratum corneum-epidermis samples and epidermis-dermis samples were prepared and left over night at 4 °C and analysed the next day with HPLC. This was done to determine the amount of API that accumulated in the epidermis-dermis and the amount of API that were left on the outer skin layers (stratum corneum-epidermis).

The results from the Franz cell diffusion studies indicated that the emulgel formulation without Pheroid™ shortened the lag time of lidocaine HCl and that the emulgel formulated with Pheroid™ shortened the lag time of prilocaine HCl, when compared to the commercial product. Pheroid™ did not enhance the flux of lidocaine HCl and prilocaine HCl into the skin. The hydrogel formulation demonstrated a high transdermal flux of prilocaine HCl due to the hydrating effect it had on the stratum corneum. The commercial product yielded high flux values for both APIs but it did not result in a high concentration of the APIs delivered to the epidermis-dermis. Pheroid™ technology did, however, enhance the epidermal-dermal delivery of lidocaine HCl and prilocaine HCl into the skin epidermis-dermis.

The stability of the emulgel formulation, Pheroid™ emulgel formulation and the hydrogel formulation was examined over a 6 month period. The formulations were stored at 25 °C/60% RH, 30 °C/60% RH and 40 °C/75% RH. The API concentration, mass, pH, zeta potential, particle size, viscosity and visual appearance for each formulation at the different storage conditions were noted and compared at month 0, 1, 2, 3 and 6 to determine if the formulations remained stable for 6 months. The results obtained from the stability study demonstrated that none of the formulations were stable for 6 months. The emulgel remained stable for the first 3 months. At 6 months, large decreases in API concentration and pH occurred which could cause a loss of anaesthetic action in the formulations. The Pheroid™ emulgel formulation did not remain stable for 6 months.

Keywords: lidocaine HCl, prilocaine HCl, Pheroid™, transdermal, local anaesthesia, dermis

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OPSOMMING

Lokale verdowers word gereeld gebruik in die mediese bedryf vir 'n verskeidenheid van mediese prosedures. Topikale verdowers word gebruik vir pynlike prosedures met betrekking tot die vel soos die herstel van minimale vel wonde en chirurgiese prosedures soos larigioskopie, esofagoskopie en uretraskopie (Franchi *et al.*, 2008:186e1). Die aanwending van topikale verdowers is maklik en pynloos en dit bevorder goeie pasiëntsamerwerking (Little *et al.*, 2008:102). 'n Huidige kommersiële topikale anestetiese produk bevat 'n eutektiese mengsel van die lokale verdowers, lidokaïenhydrochloried en prilokaïenhydrochloried. Hierdie kommersiële produk kan tot langer as 'n uur neem om 'n anestetiese effek in die vel uit te oefen. Hierdie is gesien as een van die nadele in die gebruik van topikale verdowers omdat dit in seker mediese omstansighede nie ideaal is om 'n uur te wag vir 'n effek nie (Wahlgren & Quiding, 2000:584).

Hierdie studie het die aanvangstyd en die transdermale aflewering van lidokaïenhydrochloried en prilokaïenhydrochloried in die dermislaag van die vel vanuit vier semi-soliede formulerings, insluitende 'n kommersiële produk, ondersoek. Een van die formulerings was geformuleer met Pheroid™ tegnologie, 'n nuwe velafleweringstelsel wat ontwikkel is deur die Eenheid van Geneesmiddelnavoring en -Ontwikkeling aan die Noordwes-Universiteit, Potchefstroom Kampus, Suid-Afrika.

Die vel is die liggaam se eerste linie van verdediging teen skadelike eksterne stimuli. Die vel is die grootste orgaan van die liggaam en beskik oor 'n baie komplekse struktuur. Die vel bestaan hoofsaaklik uit vyf lae waarvan die stratum corneum-laag as 'n hoogs effektiewe skans dien (Williams, 2003:1). Hierdie eienskap van die stratum corneum veroorsaak 'n vertraging in die aanvangstempo van geneesmiddels wat onderweg is vir transdermale aflewering (Barry, 2007:569). Lokale verdowers moet die boonste epidermislae van die vel penetreer om hulle teikengebied, die dermis laag, te bereik. Die dermislaag van die vel bevat velstrukture, bloedvate as ook senuwee-eindpunte. Die teikenarea vir 'n lokale verdower is die senuwee-eindpunte in dermis (Barry, 2007:570). Die lokale verdower bind aan die reseptor op die senuwee en veroorsaak 'n onomkeerbare blokkade van impulsgeleiding wat 'n verdowende effek tot gevolg het (Richards & McConachie, 1995:41).

Verskeie penetrasie bevorderende strategieë is al ondersoek insluitende metodes soos liposome (Franz-Montan *et al.*, 2010; Müller *et al.*, 2004), miselle (Scherlund *et al.*, 2000), digsluitende bedekkings (Astra Zeneca, 2006), verhittingstegnieke (Masud *et al.*, 2010) en iontoforetiese aflewing (Brounéus *et al.*, 2000) om die aflewering van lokale verdoewers te verbeter. Die Pheroid™ geneesmiddel afleweringstelsel het die transdermale aflewing van verskeie aktiewe farmaseutiese bestanddele verbeter deur gebruik te maak van 'n bevorderende onsluitingsvermoë. Pheroid™ bestaan hoofsaaklik uit onversadigde essensiële vetsure wat onskadelik is vir die menslike liggaam (Grobler *et al.*, 2008:285). Die grootte en morfologie van Pheroid™ is maklik manipuleerbaar omdat dit 'n submikron-tipe emulsie formulering is met 'n hoë aanpasbaarheidsprofiel (Grobler *et al.*, 2008:284). Lidokaïenhydrochloried en prilokaïenhydrochloried was omsluit in Pheroid™ vesikels wat geïnkorporeer was in 'n emulgelformulering. Daar was 'n emulgel geformuleer sonder Pheroid™ vir vergelying en ook 'n hidrogel. 'n Pheroid™ oplossing was vergelyk met 'n oplossing sonder Pheroid™.

Franz sel diffusiétudes was uitgevoer op die vier semi-soliede produkte (insluitende die kommersiële produk) en die twee oplossings. Die diffusiétudes was oor 'n 12 h tydperk uitgevoer waarna 'tape-stripping' met die vel gedoen was. Koukasiese vroulike abdominale vel was ontvang vanaf anonieme donors. Vel sirkels vir die diffusiétudes was voorberei met 'n Zimmer Dermatome®. 'n Fosfaatbufferoplossing (pH 7.4) was gebruik as die reseptorfase tydens die diffusiétudes. Die reseptorfase was onttrek tydens vooraf bepaalde tye en geanaliseer met hoë druk vloeistof chromatografie (HDVC) om te bepaal hoeveel van die aktiewe farmaseutiese bestanddele deur die vel beweeg het. 'Tape-strip' monsters en dermis monsters was voorberei na elke diffusie studie en oornag gebêre by 4 °C. Die monsters is geanaliseer met HDVC die volgende dag. Die konsentrasies van lidokaïenhydrochloried en prilokaïenhydrochloried in die dermis en die konsentrasies wat op die vel agtergebly het was op die manier bepaal.

Die resultate van die Franz sel diffusie studies het getoon dat die emulgelformulering sonder Pheroid™ die aanvangstyd van lidokaïen HCl verbeter het en dat die emulgelformulering met Pheroid™ die aanvangstyd van prilokaïen verbeter het wanneer dit vergelyk word met die kommersiële produk. Pheroid™ het nie die vloed van lidokaïen HCl en prilokaïen in die vel verbeter nie. Die hidrogelformulering het 'n hoë vloed van prilokaïen in die vel gehad as gevolg van die hidrasie effek wat dit op die stratum corneum uitgeoefen het. Die kommersiële produk het 'n hoë vloed van altwee aktiewe bestanddele in die vel gehad maar het nie hoë konsentrasies in die epidermis-dermis agter gelaat nie. Die gebruik van Pheroid™ tegnologie het wel die aflewering

van lidokaïenhydrochloried en prilokaïenhydrochloried in die epidermis-dermis gedeelte van die vel bevorder.

Die stabiliteit van die emulgelformulering, Pheroid™ emulgelformulering en die hidrogelformulering was ondersoek oor `n tydperk van 6 maande. Die formulering was gestoor by 25 °C/60% RH, 30 °C/60% RH en 40 °C/75% RH. Die chemiese konsentrasie bepaling, massa, pH, Zeta potensiaal, deeltjie grootte, viskositeit en visuele voorkoms vir elke formulering by die verskillende stoor kondisies was aangeteken en vergelyk op maand 0, 1, 2, 3 en 6 om te bepaal of die formulering stabiel gebly het vir ses maande. Die strabiliteitstudie het getoon dat geen van die formulering stabiel was vir 6 maande nie. Die emulgelformulering was stabiel vir 3 maande. Op 6 maande het groot dalings in die konsentrasie van lidokaïen HCl en prilokaïen en pH voorgekom wat die anestetiese effek van die formulering kon verminder. Die Pheroid™ emulgelformulering het nie stabiel gebly vir 6 maande nie.

Sleutelwoorde: lidokaïenhydrochloried, prilokaïenhydrochloried, Pheroid™, transdermale aflewering, lokale verdower, dermis

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CHAPTER 1

INTRODUCTION AND PROBLEM STATEMENT

The sensation of pain is a physical, sensory and emotional experience most humans learn in early life. Therefore, it can be said that attempts to relieve pain are as old as the human race itself. If the external factor causing the pain cannot be found or removed, the next logical reaction would be to desensitise the area of pain. This was most likely how the concept of a local pain killer or local anaesthetic was born. Various methods were tested over centuries. Aristotle (384 BC – 322 BC) referred to a fish, the torpedo ray that produced numbness, while the Roman Physician, Scribonius Largus, further advised that a patient had to put the ray under the feet until numbness up to the knee is obtained (Ring, 2007:275). Pressure anaesthesia was used by the Egyptians. By applying sufficient pressure to certain sensory nerves, the pain could be numbed because the pain pathway towards the brain became blocked (Ring, 2007:276). Refrigeration anaesthesia was used by Napoleon's surgeon while amputating the limbs of soldiers lying in the cold snow of Moscow during Napoleon's retreat. He found that the snow around the soldier's limbs numbed the pain of the amputation (Ring, 2007:276). Through the Middle Ages it was common practice to drug a patient before surgery with opium or mandogora (Ring, 2007:276). Humans have continued with their trial and error processes of developing sufficient anaesthesia, and today there are still new anaesthetic agents being developed with better pharmacological and physical profiles.

Cocaine was the first local anaesthetic that held promise. Its numbing effect had been discovered centuries ago by the Incas (Bovet & Michelson, 1971:14) but was only isolated by the German graduate Albert Niemann in 1860 after chewing the leaves and noticing the numbing of his tongue (Catterall & Mackie, 2006:369). Cocaine became a popular local anaesthetic especially in dentistry in the late 1800s and early 1900s. During this period doctors also started noticing the addictive and bizarre effects experienced by patients (Ring, 2007:280). This led to the research of other cocaine derivatives to produce local anaesthesia. In 1905, Einhorn synthesised procaine, a cocaine derivate with much less side effects than cocaine (Catterall & Mackie, 2006:418). The real breakthrough came when Nils Löfgren came across the substance 2-diethylamine-2',6'-acetoxylilide, known as lidocaine, in 1943 (Bovet & Michelson, 1971:31). Under the trade name of xylocaine, Astra, a Swedish pharmaceutical company, introduced lidocaine to the world (Ring, 2007:281). Lidocaine and prilocaine are lipophilic amide-type local anaesthetics that, when protonated, become more soluble in their

hydrochloride salt forms (Catterall & Mackie, 2006:418). These local anaesthetics directly inactivate the voltage-gated sodium channels responsible for the onset and transmission of electric impulses for nerve conduction (Richards & McConachie, 1995:41).

The application of topical anaesthetics is a non-invasive method to produce local anaesthesia (Little *et al.*, 2008:102). Ideal characteristics of a topical anaesthetic would be one that can be applied painlessly, provide rapid onset of anaesthesia and produces an anaesthetic effect for a reasonable amount of time with minimal side effects (Huang & Vidimos, 2000:286). This should be achievable but proves to be difficult due to the application of the topical anaesthetic on the largest organ in the body, the skin (Williams, 2003:1). The skin is the body's first line of defence against foreign organisms and the environment and has an excellent barrier function (Williams, 2003:1). The barrier function is provided by the stratum corneum, the top part of the epidermis. Substances applied to the skin must first traverse through this complex layer of keratinised cells and lipid bilayers (Williams, 2003:9). The permeability of the stratum corneum is about one thousand times less than other biological membranes, which causes it to become the biggest rate-limiting factor for transdermal delivery (Foldvari, 2000:418).

The objective for any substance to be delivered transdermally, is to cross the stratum corneum as rapidly as possible and travel from there into the dermis and enter the blood circulation at a therapeutic concentration (Sequeira, 1993:163). There are also numerous advantages in using transdermal delivery to deliver a therapeutic substance, the primary one being the bypassing of the hepatic first-pass metabolism (Cerchiara & Luppi, 2006:89). Transdermal delivery is also a non-invasive method which conducts better patient compliance and minimises the risk of trauma and infection at the site of application. Another advantage of transdermal delivery is enhancing the delivery of a therapeutic substance to a specific site because the substance can exert its action on the site of application (Pefile & Smith, 1997:147).

Factors that influence the transdermal diffusion process are biological factors such as skin age, skin disease, race, gender, skin hydration, body site, temperature, as well as physicochemical properties of the drug like aqueous solubility, partition coefficient, diffusion coefficient, ionisation, melting point and molecular size (Williams, 2003:14-18, 35-39). After examining the physicochemical properties of lidocaine and prilocaine it can be concluded that these APIs fall in range of the parameters necessary for ideal transdermal delivery. Lidocaine and prilocaine are prepared in their hydrochloride salt forms for better ionisation.

The strategy to shorten the delivery time and time of onset of the lidocaine and prilocaine in formulations is to encapsulate the molecules of the API with Pheroid™ vesicles. In this study a formulation containing Pheroid™ will be compared with formulations without Pheroid™.

Pheroid™ technology is a specially designed patented skin delivery system with enhanced entrapment capabilities (Grobler *et al.*, 2008:284). The Pheroid™ consists primarily of essential fatty acids like vitamin F and tocopherol. Pheroid™ technology is a skin-friendly delivery system that can deliver a minimal amount of the therapeutic substance to its target site by changing its size and morphology to suit the requirements of the substance to be entrapped (Grobler *et al.*, 2008:285). Previous studies (Kruger, 2008:57) on the evaluation of solutions containing lidocaine hydrochloride and prilocaine hydrochloride with and without Pheroid™ have shown that the Pheroid™ drastically reduces the lag time of the therapeutic substances through the skin (Kruger, 2008:57).

The hypothesis of this study was that Pheroid™ would shorten the time of onset of lidocaine and prilocaine in a semi-solid formulation and that a formulation containing lidocaine and prilocaine without Pheroid™ would permeate transdermally.

The main aim of this study was to determine whether a specially designed drug delivery system called Pheroid™ would shorten the lag time of local anaesthetics lidocaine and prilocaine when incorporated into a semi-solid formulation instead of solutions. The second aim of this study was to determine whether lidocaine and prilocaine with and without the use of Pheroid™ would permeate transdermally, although the target site for drug delivery of lidocaine and prilocaine is the dermis. The following were the objectives of this study:

- ❖ The development and validation of an HPLC method for use of the transdermal and assay analysis of the API.
- ❖ Development of different formulations e.g. emulgel without Pheroid™, emulgel containing Pheroid™, hydrogel not containing any Pheroid™ components.
- ❖ The accelerated stability testing of the formulated lidocaine and prilocaine formulations.
- ❖ Experimentally determining the transdermal permeation of the lidocaine and prilocaine formulations with the use of diffusion studies.
- ❖ Experimentally determining the topical delivery of lidocaine and prilocaine to the target site (dermis) by making use of tape stripping.

To study these objectives one semi-solid formulation incorporated Pheroid™ technology and one semi-solid formulation that was precisely the same as the Pheroid™ formulation, except all Pheroid™ components had been left out of the formulation, were used. The APIs are highly soluble in water and were incorporated in a hydrophilic semi-solid formulation in order to compare the results of a hydrophilic formulation to that of the lipophilic formulations. Control

sets were included in this study, namely: 1) a control with all Pheroid™ components without APIs, 2) a control without Pheroid™ and without APIs and 3) a control in a hydrophilic semi-solid formulation without APIs.

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CHAPTER 2

TRANSDERMAL DELIVERY OF LOCAL ANAESTHETICS LIDOCAINE AND PRILOCAINE

2 INTRODUCTION

The skin is the largest organ in the body and because of its accessibility, ideal for API delivery. API delivery through the skin, however, proves to be difficult as there are many factors to overcome first. In this chapter the elements, factors that influence transdermal API delivery and how to overcome them will be discussed. The actives used during this study were the local anaesthetics lidocaine and prilocaine and are discussed in section 2.2. Local anaesthetics are used to block pain sensation and cause numbness in the area of application. For this reason, the pain pathway and types of pain associated with local anaesthesia will also be discussed.

2.1 INTRODUCTION TO PAIN AND PAIN SENSATION

Pain is defined by the International Association for the Study of Pain (ISAP) as a sensory or emotional experience which is uncomfortable for the person experiencing it. This experience is associated with potential or actual tissue damage and describes the pain in terms of this damage (Steeds, 2009:507). The sensation of pain is a subjective experience that most people learn in early life. Each individual experiences pain in a different and unique way and the complex interactions between the sensory, emotional and behavioural factors can complicate pain treatment. It is important that pain is managed appropriately for each individual patient to ensure optimal recovery and relief (Serpell, 2005:7). Giving preoperative pain medications before a surgical procedure can provide effective control of pain, followed by anaesthesia of choice and postoperative pain medication. Local anaesthesia plays an important role in the management of pain during localised procedures (Woodward, 2008:106).

2.1.1 The pain pathway

Nociceptors are specialised peripheral sensory neurons that react to painful stimuli. These free nerve endings are present in most parts of the body, including the skin, deep somatic tissue and viscera areas (Moffat & Rae, 2011:12). The nociceptors carry noxious information received from chemical, mechanical or thermal stimuli, from the periphery nervous system to the central nervous system. Their pain pathway along the axons can be divided into five phases:

transduction, transmission, modulation, projection and perception (Steeds, 2009:507; Woodward, 2008:106). The first transduction phase results when the stimulus is converted to an afferent electrical impulse. Transduction is then followed by the transmission phase where the impulses are sent to the dorsal horn part of the spinal horn. In the modulation phase the nerve impulses can be moderated or amplified by receptors inside the dorsal horn as they are being regulated up or down. Projection is the second last phase as the nerve impulses are now in transmission towards the thalamus where the pain is perceived by the individual in the last perception phase (Woodward, 2008:106).

2.1.1.1 Impulse conduction

It is important to understand the process of impulse conduction as this process is targeted by the mechanism of action of local anaesthetics. The structure of the synapse and sodium channels as well as resting membrane potential and action potential, will briefly be discussed.

2.1.1.1.1 The synapse

The transmission of nerve impulses occurs between a sensory receptor neuron and a motor or effector neuron. The site of contact between the axon terminals of these presynaptic and postsynaptic neurons is termed the synapse (Figure 2.1) (Afifi & Bergman, 1998:21; Sukkar *et al.*, 1997:366). The axon of the presynaptic neuron branches out towards the end terminal and forms small knobs or boutons that contain synaptic vesicles. The synaptic terminal is in contact with dendrites, cell bodies and axons of the postsynaptic neuron while mitochondria and neurofilaments are also present in the terminal (Afifi & Bergman, 1998:21). Neurotransmitters are substances found in the synaptic vesicles and facilitate the transfer of impulses. Acetylcholine and catecholamine are two of the more common neurotransmitters and substances like glycine, gamma-Aminobutyric acid (GABA), glutamic acid and the monoamines dopamine, adrenaline, serotonin and noradrenalin have also been identified as neurotransmitters (Afifi & Bergman, 1998:21, 23).

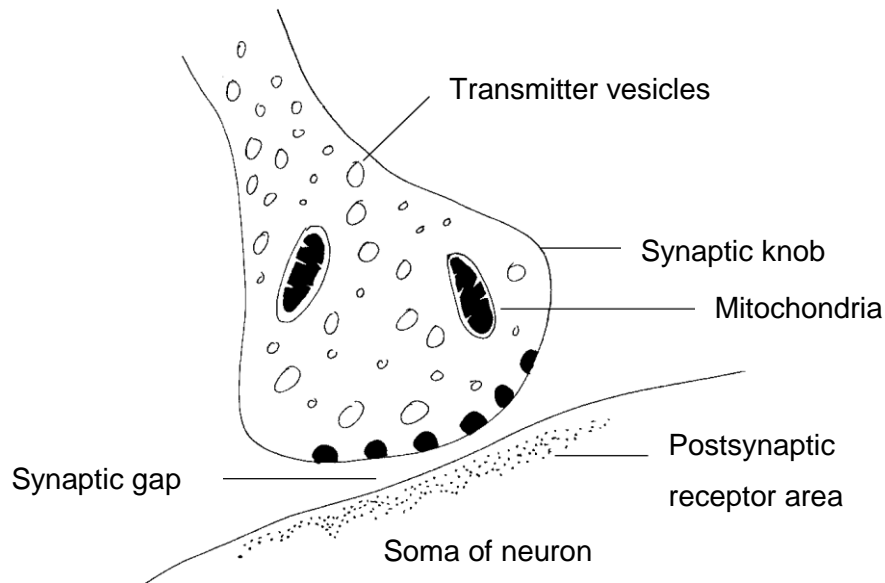


Figure 2.1: Anatomical structure of a synapse (Sukkar *et al.*, 1997:367)

The conduction of impulses at the synapse always takes place in a one-way direction. The impulse passes from the presynaptic neuron through the synapse to the postsynaptic neuron (Sukkar *et al.*, 1997:368). Synaptic transmission starts when the action potential reaches the synaptic knob. The action potential increases the permeability of the presynaptic membrane that allows an influx of calcium (Ca^{2+}) ions to enter the neuron through voltage-gated Ca^{2+} channels. A neurotransmitter like acetylcholine is then released through exocytosis into the synaptic gap (Figure 2.1) and diffuses across the gap to bind on the receptor molecules of the postsynaptic membrane (Sukkar *et al.*, 1997:367). The permeability and ionic permeability of the postsynaptic membrane increase and causes depolarisation so that an action potential is generated in the target postsynaptic cell (Afifi & Bergman, 1998:22). Separate channels for sodium (Na^+), potassium (K^+) and chloride (Cl^-) ions are present in the postsynaptic membrane. An excitatory transmitter opens the channels for Na^+ while an inhibitory transmitter that causes hyperpolarisation opens the channels for K^+ and Cl^- (Sukkar *et al.*, 1997:367).

2.1.1.1.2 The sodium channels

Voltage-dependent Na^+ channels are the main target for the mechanism of action of local anaesthetics. These Na^+ channels are found in the cell bodies and dendrites of neurons in the brain and axonal membranes. Na^+ channels play important roles in the conduction of an action potential of the axonal nerve fibres (Taylor & Narasimhan, 1997:47, 48). A group of therapeutic

substances called Na⁺ channel blockers interact with specific binding sites inside the Na⁺ channel and stop the generation of an action potential (Catterall & Mackie, 2006:371). Na⁺ channels propagate action potentials of 1 m/s when they are open for a short period and Na⁺ channel blocking APIs' therapeutic action is dependent on this depolarised or 'open' state of the cell (Taylor & Narasimhan, 1997:48, 51).

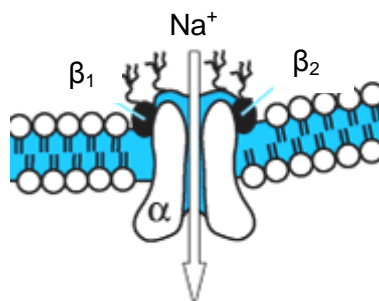


Figure 2.2: Structure of a voltage-gated sodium channel (Catterall & Mackie, 2006:148)

The structure of voltage-gated Na⁺ channels is described by Catterall & Mackie (2006:371) as complexes of glycosylated proteins of 300 kDa. They are divided into subunits with the main α subunit, 260 kDa, as the target for Na⁺ channel blockers (Figure 2.2). The β_1 to β_4 subunits range from 33 kDa to 38 kDa, respectively (Catterall & Mackie, 2006:371; Taylor & Narasimhan, 1997:51, 52). In each α subunit there are four homologous domains (I – IV) and each domain is divided into six transmembrane segments (S1 – S6) (Figure 2.3). The transmembrane segments are constructed out of an α helix of twenty-two amino acids and a re-entrant pore loop (Catterall & Mackie, 2006:371; Taylor & Narasimhan, 1997:51). The structure of the Na⁺ channel is symmetrical forming a special location in the centre of the channel for the Na⁺ selective transmembrane pore. The re-entrant pore loop is formed inside the transmembrane pore by the surrounding S5 and S6 transmembrane helices and their associated short membrane segments (Catterall & Mackie, 2006:371).

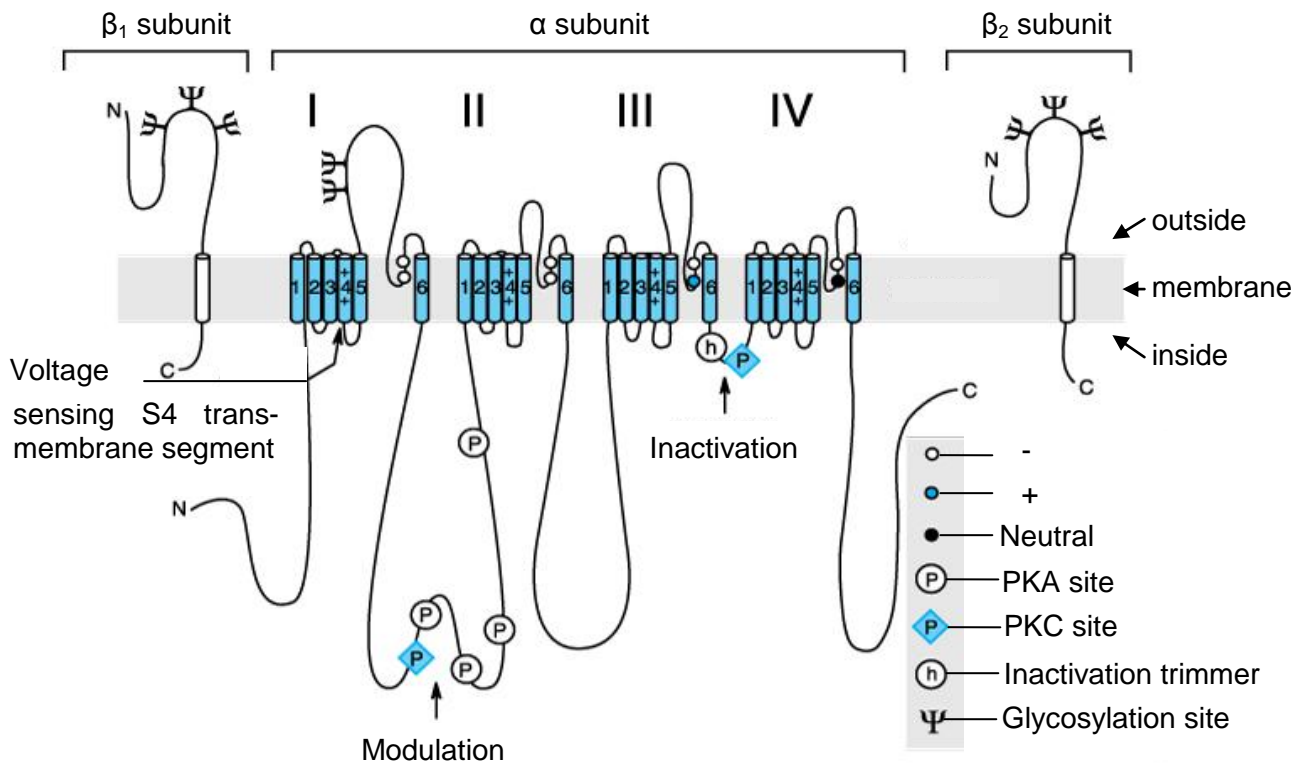


Figure 2.3: The function of voltage-gated sodium channels (Catterall & Mackie, 2006:372)

The open state of the voltage-gated Na^+ channel is initiated when a series of conformational changes take place in the four domains (Catterall & Mackie, 2006:371). These conformational changes are the result of the movement of 'gating charges' in the S4 domain where segments transduce voltage by moving across the electrical field of the transmembrane (Taylor & Narasimhan, 1997:51). These segments contain residues of arginine or lysine and the S4 helixes are hydrophobic and positively charged (Catterall & Mackie, 2006:371). When the Na^+ channel is open or in an activated state, sodium ions surge into the cell down the Na^+ concentration gradient and causes cell depolarisation (Taylor & Narasimham, 1997:51). Homologous domains III and IV are connected by a short intracellular loop of protein that forms an inactivation gate. This specialised gate mechanism binds to a special inactivation gate receptor and inactivates the Na^+ channel at a very rapid pace of 1 m/s. The surge of sodium ions is stopped and the cell returns to its hyperpolarised state (Catterall & Mackie, 2006:371).

2.1.1.1.3 Resting membrane potential

Resting membrane potential (RMP) can be described as a cell in steady-state with a negative potential of -70 mV in the intracellular liquid of the cell with respect to the extracellular liquid. The resting cell membrane is very permeable for K^+ ions which contribute to a positive charge in the intracellular liquid. When the leaking of K^+ ions down its concentration gradient out of the

cell persists, the positive charge inside the cell decreases and becomes more negative because of Cl^- ions that stay behind in the cell. A very small number of Na^+ ions are able to penetrate the membrane and if this happens together with the leakage of K^+ ions, a state of equilibrium between the intracellular and extracellular liquid will be reached. This, however, does not occur because of the functional sodium-potassium adenosine triphosphatase (Na^+/K^+ -ATPase) pump that returns two leaked K^+ ions to the intracellular liquid and removes three Na^+ ions to the extracellular liquid. The Na^+/K^+ -ATPase pump plays an important role in maintaining the concentration gradients on the inside and outside of the cell and electronegativity of -70 mV of the cell membrane. If the Na^+/K^+ -ATPase pump is blocked it leads to the decrease of RMP.

RMP can also occur because of non-diffusible anions like protein, sulphate and phosphate ions are unable to leave the cell. Respectively, there are more anions in the intracellular liquid leaving a negative charge and more cations in the extracellular liquid. Ions at rest do not prefer an inward or outward flux causing the resting membrane to stay polarised and maintain an equilibrium state (Sukkar *et al.*, 1997:48, 363).

2.1.1.1.4 Action potential

An action potential is responsible for the transmission of nerve impulses down an axon. After a chemical or physical stimulus excites the cell membrane it turns the stimulus into an electrical signal (action potential) ready for conduction (Sukkar *et al.*, 1997:48, 49). Action potentials not only transmits impulses but have other physiological effects like the release of neurosecretions or chemical transmitters in synapses, contractions of muscles and the activation or inhibition of glandular secretion (Sukkar *et al.*, 1997:49). The propagation of an action potential takes place in two types of fibres: unmyelinated and myelinated. The propagation of an action potential along unmyelinated C fibres is described as continuous with a velocity of $0.6 - 2.0$ m/s (Afifi & Bergman, 1998:16). The propagation of an action potential along the myelinated A fibres is described as jumping or salutatory conduction with a velocity of $5 - 120$ m/s caused by permeability changes at the nodes of Ranvier (Afifi & Bergman, 1998:16, 20; Catterall & Mackie, 2006:147).

The mechanism of action of an action potential starts with a chemical or physical stimulus that causes a change in the permeability of the cell membrane. This change causes an influx of Na^+ ions through the Na^+ channels into the cell and an efflux of K^+ ions. The cell membrane charge is reversed from -70 mV to $+35$ mV and the membrane now resides in a depolarised state (Afifi & Bergman, 1998:19; Sukkar *et al.*, 1997:49). The neuron will now become charged positively until it reaches the threshold point and releases the action potential (Sukkar *et al.*, 1997:364). After this happens inactivation of the Na^+ channel takes place while the K^+ channel

stays open to terminate depolarisation. The cell membrane is now repolarised to its original resting membrane potential of -70 mV (Figure 2.4) (Catterall & Mackie, 2006:147; Sukkar *et al.*, 1997:49). The opposite reaction of the cell to an increased negative charge is called hyperpolarisation. This happens when positive charge is completely moved out of the cell. A hyperpolarised membrane is very difficult to depolarise and the loss of K^+ ions in the body fluid causes hypocalcaemia. The hypocalcaemic state due to hyperpolarisation can cause periodic paralysis in muscles due to the effect on the muscle and nerve cells (Sukkar *et al.*, 1997:364).

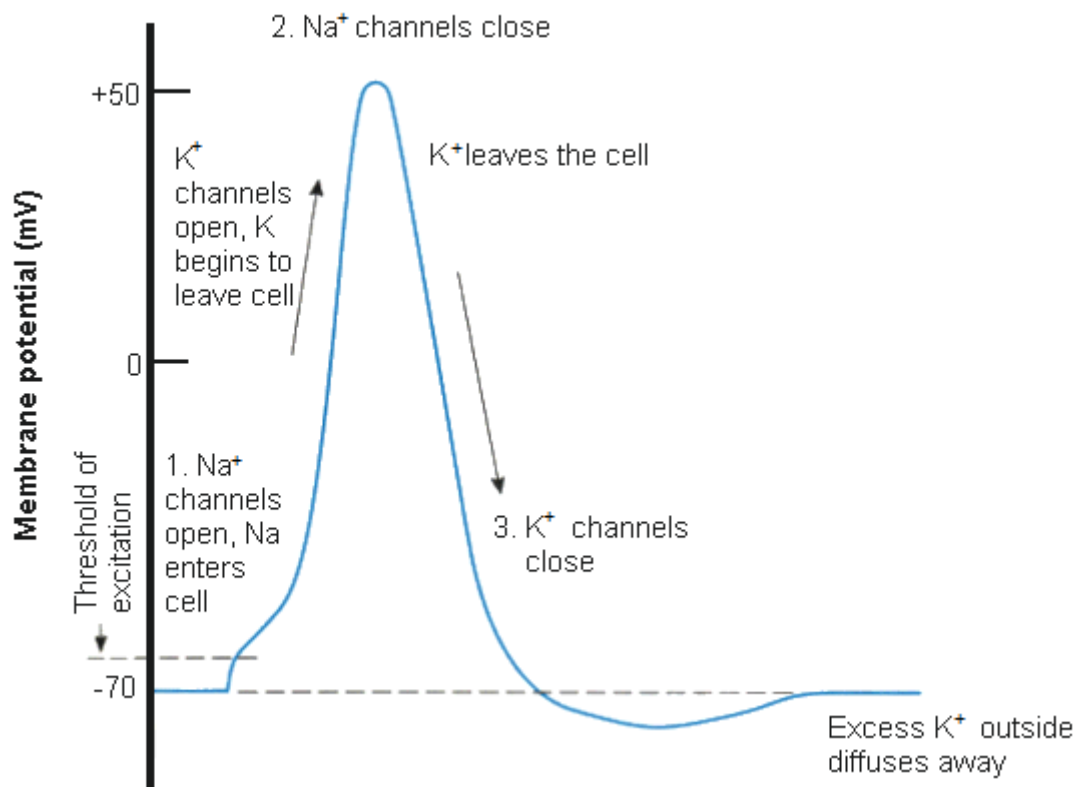


Figure 2.4: The ion flow during an action potential (Cofer, 2002)

2.1.2 Types of pain

It is an important concept to realise that pain is not only physical but also physiological. The interpretation of pain by an individual depends on past experiences, anxiety, injury or illness and belief in pain treatments and not only on the physical factor. Pain may occur for no reason or may be absent where a physical injury is obvious, it can continue after tissue healing or be unresponsive to treatment. It is a complex physiological and physical state and can present itself in different ways (Serpell, 2005:10).

Somatic pain can be described as fast or first physiological pain. Somatic pain causes the individual to react swiftly to quickly localise the pain and the pain stimuli and withdraw from it to

stop any further damage. The nociceptors produce a high stimulus threshold that leads to the rapid conducting of the impulse at a rate of 5 – 120 m/s via myelinated A δ fibres and a fast reaction (Afifi & Bergman, 1998:16; Serpell, 2005:7). When pain sensation is delayed after tissue injury, it is described as second or slow pain of pathophysiological origin. The stimulation of the high threshold polymodal nociceptors produces this type of slow pain as a response to mechanical, thermal or chemical stimuli and transmits the impulses along unmyelinated C fibres through slow conduction of 1 m/s (Afifi & Bergman, 1998:16; Serpell, 2005:7). The density of nociceptors is lower in the viscera and visceral pain, different than somatic pain, starts at the internal organs and is poorly localised (Steeds, 2009:509). The afferent visceral fibres do not respond to individual stimulating modalities, but to the intensity of the stimulation (Moffat & Rae, 2011:15). Neuropathic pain is the result of injury of nerve fibres or disease that causes abnormal pain sensation and dysfunction of the nervous system (Serpell, 2005:8; Steeds, 2009:510). Some of the factors that can cause paroxysmal or continuous neuropathic pain are crush injuries, surgery, traumatic injuries and infection. The pain can also be of ischemic, neoplastic or chemical origin and patients describe it as a burning, tingling, numbing, stabbing or shooting pain sensation (Moffat & Rae, 2011:15; Steeds, 2009:510). Evoked pain conditions like allodynia, hyperalgesia, hyperpathia, hyperaesthesia and dyesthesia also occur as sub parts of neuropathic pain (Steeds, 2009:510).

Pharmacologically pain is best treated with a multimodal analgesia program that benefits the patient with the minimum uncomfortable side-effects and therapeutic dosage for the most effective pain relief and pain control (Moffat & Rae, 2011:15). Non-steroidal anti-inflammatory drugs (NSAIDs), local anaesthetics, opioids, tricyclic anti-depressants, selective noradrenalin reuptake inhibitors and anticonvulsants are all drugs that can be included in a pain management program for a patient but it is also important to include the psychological factors of pain in the treatment plan, for optimal results (Moffat & Rae, 2011:15).

2.2 LOCAL ANAESTHETICS

Local anaesthesia can be described as substances that cause a temporally numbing sensation at the specific localised site of application. This abolition of sensation and pain at a localised area has made it possible for surgeons to perform smaller operations and examinations without having to put the patient under a complete unconscious state (Bovet & Michelson, 1971:IX). To block out the sensation of pain, local anaesthetics follow a certain mechanism. The therapeutically active ingredient reversibly binds and inactivates sodium channels at receptor sites that use these channels to generate action potentials for impulse conduction. This causes

a reverse block of impulse conduction which results in a temporarily loss of sensation at the application site (Catterall & Mackie, 2006:369; White & Katzung, 2004:418).

2.2.1 History of local anaesthesia

The history of local anaesthesia can be traced back as far as the 1500s and earlier when the Inca tribe living in the Andes Mountains of Peru were already using the pain numbing effect of coca leaves in various operational procedures (Bovet & Michelson, 1971:14). *Erythroxylon coca*, or the coca bush indigenous to the Andes Mountains of Peru, West Indies and Java, bore the first local anaesthetic of all, cocaine. Cocaine is a natural substance that forms in the leaves of the coca bush and the Incas discovered early on that conjuring an alkali extract of these leaves, left a person with an euphoric and over-stimulating sensory experience (Catterall & Mackie, 2006:369; Revis, 2005). The Spaniards conquered Peru in 1532 and with the drastic changes to the land it appeared that the Incas kept the secret of their natural stimulant to themselves or maybe it was because the Catholic Church declared the existence of such an euphoric drug as pure superstition which led to the prohibition of the chewing of coca leaves. In the 1800s the effects of cocaine were finally introduced into Europe (Bovet & Michelson, 1971:14, 15).

In 1860 cocaine was isolated by Niemann, while a Viennese physician, Carl Koller, also experimented with cocaine (Catterall & Mackie, 2006:369). He collaborated with famed psychiatrist, Sigmund Freud, to study the physiological effects of cocaine on muscle strength, but then finally, while working on his own in 1884, he introduced cocaine as a clinical topical anaesthetic to use on the eye during ophthalmic operations (Bovet & Michelson, 1971:17). It was also widely known that Freud had become addicted to the substance due to self-experimentation (Revis, 2005).

In 1905 Einhorn synthesised procaine, a large improvement on cocaine. It was not nearly as habit-forming as cocaine and replaced cocaine as local anaesthesia of choice in the early 1900s (White & Katzung, 2004:418). Isogramine was another substance which showed anaesthetic properties and was studied intensely by Erdtman and Löfgren. While investigating different compounds to try and reduce localised irritation effects, Löfgren finally came across the substance 2-diethylamine-2',6'-acetoxylilide, known as lidocaine, in 1943. Lidocaine or xylocaine was then recognised as a prototype agent for local anaesthesia as it was a great advance in the field. Lidocaine provided good anaesthetic effects without irritation and also showed great stability (Bovet & Michelson, 1971:31). Extensive pharmacological studies were done on lidocaine and it was quickly introduced to various medical practices, including, dentistry

(Bovet & Michelson, 1971:31) and is still the cocaine derivate most used for a wide range of indications over the world (Revis, 2005).

Many other synthetic local anaesthetics have been synthesised over the years, but some are more toxic than others. Certain local anaesthetics are prepared in different types of dosage forms to reduce toxic effects or ensure better stability. Table 2.1 gives a list of local anaesthetic preparations and their applications.

Table 2.1: A list of local anaesthetic preparations and their applications (Catterall & Mackie, 2007:377-379; White & Katzung, 2004:426)

Preparations for injection	Articaine Bupivacaine Chloroprocaine Etidocaine Lidocaine Mepivacaine Prilocaine Ropivacaine Procaine Tetracaine
Topical anaesthesia	Benzocaine Cocaine Dibucaine Dyclonine Lidocaine Pramoxine Tetracaine
Ophthalmological preparations	Proparacaine Tetracaine

2.2.2 Clinical use of local anaesthesia today

Local anaesthesia is used for different types of applications and plays an important role in hospitals, emergency rooms and operating rooms or facilities to provide sufficient anaesthesia to a patient undergoing a painful localised procedure. Various factors influence the state of anaesthesia in a patient, such as individual characteristics, the dose administered, speed of administration, technique of administration, blood flow and vascularisation of the local tissue, and the presence of adrenaline. The general rule that physicians follow for the administration of

local anaesthesia is to give the lowest dose possible over the longest period of time to achieve a sufficient level of anaesthesia (Revis, 2005).

2.2.2.1 Topical application

Topical anaesthesia is applied to the mucous membranes in the nose, mouth, throat, upper gastrointestinal tract and genitourinary tract for procedures such as a laryngoscopy, oesophagoscopy or urethroscopy (Catterall & Mackie, 2006:380). Topical anaesthesia is also used in ophthalmic operations and cosmetic surgeries. The primary advantage of the topical application of local anaesthetics is that it is painless. Tissue distortion does not occur with topical application as it might with infiltration anaesthesia. In emergency rooms it is used to prevent pain in minor skin-breaking procedures or while repairing lacerations (Young, 2007:232).

Lidocaine comes in a variety of formulations that can be applied topically. A lidocaine ointment is used for the anaesthesia of mucous membranes and skin while a lidocaine gel can be used for anaesthesia of the urinary tract or endoscopy. For anaesthesia of the mouth and upper gastrointestinal tract a topical solution of lidocaine is used. Lidocaine comes in the form of eye drops, suppositories and even transdermal patches. Lidocaine, together with prilocaine, is formulated in a eutectic mixture for skin anaesthesia under occlusive dressing (Sweetman, 2002:1315). Cocaine and tetracaine are two popular anaesthetics for topical use and pramoxine, dibucaine, benzocaine and dyclonine are other local anaesthetics with topical applications (White & Katzung, 2004:424). The primary disadvantage of topical anaesthesia is the time it takes to finally reach effective levels of anaesthesia. The eutectic mixture of 2.5% lidocaine and 2.5% prilocaine can take 45 – 60 min under occlusive dressing to produce skin anaesthesia to a depth of 5 mm (Young, 2007:233). Another problem that occurs is that of systemic toxicity. This happens when local anaesthetics are rapidly absorbed into the systemic circulation, especially through mucous membranes or abraded skin (Catterall & Mackie, 2006:380).

2.2.2.2 Local infiltration

Local infiltration anaesthesia occurs when the local anaesthetic is injected directly into the tissue. This type of anaesthesia can include deeper structures like organs or only the skin. It is a practical way of anaesthesia as it does not interrupt normal body functions. It can be a problem, however, that large amounts of the local anaesthetic are needed to provide effective anaesthesia for a small area. By adding adrenaline to the injection the duration of the infiltration anaesthesia can increase to twice as long (Catterall & Mackie, 2006:380), but there is a

possibility that necrosis due to the adrenaline can develop when injected into tissues containing arteries (Bovet & Michelson, 1971:350).

Lidocaine, procaine, prilocaine and bupivacaine are the local anaesthetics of choice for local infiltration anaesthesia. By blocking the nerves subcutaneously at the spinal root or at major nerves the amount of anaesthetic required can be reduced and the chances of systemic toxicity lessened (Catterall & Mackie, 2006:380).

2.2.2.3 Field block

A field block effect is achieved when the sensory nerve supply of an area is blocked by infiltrating the surrounding tissues with anaesthesia (Bovet & Michelson, 1971:351). Lidocaine and prilocaine solutions (Sweetman, 2002:1315, 1318), as well as some of the other local anaesthetics used for local infiltration, can be used for a field block effect. Field block anaesthesia is usually used when the local area is infected and normal local infiltration anaesthesia cannot be used. Some structures have a complicated nerve supply that makes the use of local infiltration anaesthesia difficult. An example of such procedure is when a hernial repair must be done. In these cases, where the use of local infiltration anaesthesia is difficult, field block anaesthesia is the method of choice (Bovet & Michelson, 1971:351). It is important though that the person responsible for giving the anaesthesia has a great and detailed knowledge of neuroanatomy. The advantage of field block anaesthesia is that a lower amount of the local anaesthetic is needed to provide a greater anaesthetic effect than with infiltration anaesthesia (Catterall & Mackie, 2006:380).

2.2.2.4 Nerve block

A nerve block is achieved when a solution of a local anaesthetic is injected into individual peripheral nerves or nerve plexuses. This technique can provide anaesthesia over a greater area of the body. This mixed nerve block can extend its effect to the somatic motor nerves and produce sufficient muscle relaxation crucial for surgical procedures. It is important that the local anaesthetic is injected as close to the nerve as possible and not directly into the nerve to avoid nerve damage (Catterall & Mackie, 2006:380).

Lidocaine is available in solutions for different types of nerve block, i.e., brachial plexus block, intercostal nerve block, paracervical block, paravertebral block, pudendal block and for retrobulbar block (Sweetman, 2002:1315). Lidocaine has a lower pK_a value which is important for the onset of the anaesthetic effect. It produces anaesthesia within 3 min while a bupivacaine solution with a higher pK_a value takes up to 15 min for the onset of action. It is also important to note that a more hydrophobic local anaesthetic will be more potent and more toxic (Catterall

& Mackie, 2006:381). The different local anaesthetics provide different effective anaesthetic duration times and can be divided accordingly:

- ❖ Short-acting (20 – 45 min): procaine and chlorprocaine
- ❖ Intermediate-acting (60 – 120 min): mepivacaine, lidocaine and prilocaine
- ❖ Long-acting (400 – 450 min): tetracaine, bupivacaine, levobupivacaine, etidocaine and ropivacaine

The duration of short-acting and intermediate-acting local anaesthesia can be increased by adding 5 µg/ml adrenaline to the solution (White & Katzung, 2004:423).

2.2.2.5 Intravenous regional anaesthesia

Intravenous regional anaesthesia, also known as Bier's block, is the anaesthesia of the arm or leg for short surgical procedures. A local anaesthetic is injected intravenously into a distal vein while blood flow to the limb is isolated with the use of a tourniquet placed in a proximal position (White & Katzung, 2004:423). The tourniquet should not be kept on for longer than 2 h, but should not be removed too early as it can cause a toxic amount of the local anaesthetic to enter the systemic circulation (Catterall & Mackie, 2006:381). A lidocaine solution without adrenaline is the local anaesthetic of choice for this procedure. Some physicians prefer a prilocaine solution because of its higher therapeutic index (Sweetman, 2002:1315, 1318). The more cardiotoxic local anaesthetics like bupivacaine and etidocaine are not recommended for this procedure (Catterall & Mackie, 2006:382).

2.2.2.6 Epidural anaesthesia

Epidural anaesthesia is achieved when a local anaesthetic is injected into the epidural space. The epidural space can be reached from different sites like the sacral hiatus, thoracic region, lumbar region or cervical region. The demand for this type of anaesthesia has become very high; since it can be administered by a continuous infusion or repeated bolus administration. The catheter is placed into the epidural space. The duration of the procedure determines the choice of local anaesthesia. Lidocaine is a popular anaesthetic for intermediate duration while bupivacaine is used for longer procedures. Chlorprocaine is short-acting and provides a short duration of anaesthesia for shorter procedures (Catterall & Mackie, 2006:383, 384).

2.2.2.7 Spinal anaesthesia

Spinal analgesia refers to the procedure where a local anaesthetic is injected directly into the cerebrospinal fluid (CSF) in the lumbar space of the spine where the lumbar and sacral roots are submerged in CSF to produce spinal anaesthesia. This is one of the most popular methods

for anaesthesia since an anaesthetic effect is achieved over a large part of the body with only minimum plasma concentration levels of the local anaesthetic (Catterall & Mackie, 2006:382). The most popular APIs used today for spinal anaesthesia are lidocaine, tetracaine and bupivacaine while procaine is sometimes used when anaesthesia is required for only a short time. Factors to consider that can influence the state of anaesthesia and the height of block is the amount, volume, speed of injection, patient position and baricity of the solution (Catterall & Mackie, 2006:382, 383). A hyperbaric solution of lidocaine hydrochloride in glucose solution is available for spinal anaesthesia. Higher concentrations of the solution can be used in normal delivery of a baby or a caesarean section (Sweetman, 2002:1315). There is a possibility of complications arising with spinal anaesthesia like possible trauma to the spinal cord, infection due to badly sterilised equipment, neurological complications like headache are common and possible cardiovascular and respiratory complications (Bovet & Michelson, 1971:353, 354). Even with the possibilities of complications this still remains one of the most popular methods of anaesthesia today and the chances of persistent neurological insufficiencies are extremely rare (Catterall & Mackie, 2006:383).

2.2.3 Mechanism of action of local anaesthesia

The primary initiative for the mechanism of local anaesthetics is that these APIs directly inactivate voltage-gated Na⁺ channels and prevent the onset or transmission of electrical impulses necessary for nerve conduction (Eappen & Datta, 1998:10; Richards & McConachie, 1995:41). The Na⁺ influx through these channels is responsible for causing the depolarisation of nerve cell membranes and the further propagation of an impulse. When the depolarisation is reversibly blocked by a local anaesthetic and no impulses are propagated, a loss of sensation will take effect in the area supplied by the nerve (Revis, 2005). The propagation of the impulse fails as the anaesthetic action in the nerve increases the threshold for electrical excitability, which causes a decrease in the rate of action potential that in turn slows down impulse conduction (Catterall & Mackie, 2006:369, 370).

When a local anaesthetic binds to a receptor the blockade of the Na⁺ channels will be voltage-dependent as well as time-dependent (White & Katzung, 2004:421). This means that at a site with a lower excitation rate or a rested channel, the binding affinity for the local anaesthetic will also be low. When the Na⁺ channels are open or in an activated state, the binding affinity will be much higher for the local anaesthetic. This activated state comes forth during high-frequency action potentials throughout any motor activity or sensory transmission (Ramamurthi & Krane, 2007:230). The binding affinity of the local anaesthetic is also higher at inactivated channels which present themselves at more positive membrane potentials (White & Katzung, 2004:421). The local anaesthetic molecule binds to the binding site within the pore of the

sodium channel in an open state while the local anaesthetic molecule is in the ionised form. These binding properties of local anaesthetics are dependent on their lipid solubility, molecular size and pK_a values. The rate of dissociation from the receptor binding site inside the Na^+ pore is of importance when it comes to the onset of action for the local anaesthetic. A high frequency of stimulation is usually required for API binding to exceed API dissociation during and between action potentials (Catterall & Mackie, 2006:370, 371). Phasic block is the phenomenon which results after the local anaesthetic has bound to the receptor on the inside of the channel and the anaesthetic effect has been enhanced by the stimulation of the nerve. If the ionised form of the API does not bind directly to the pore of the Na^+ channel, the unionised form can diffuse through the plasma membrane towards the inside of the cell. When this happens, the unionised form dissociates and starts to produce ionised species. Tonic block occurs when the local anaesthetic interacts with the Na^+ channels and binds with lower binding energy to the site. In this case, stimulating the nerve will not increase the binding energy and the site of action is less specific. The API diffuses through the lipid matrix of the cell membrane and causes conformational changes and channel inactivation once it has reached the Na^+ channel (Richards & McConachie, 1995:41, 42).

The speed of onset of a local anaesthetic is dependent on the lipid solubility of the unionised fraction of the API but it is important to note that the API binds to its receptor site in its ionised form. When the pH value of an API is equal to the pK_a value, the API will be 50% ionised. If the pK_a value is higher than the body fluid (pH 7.4) the ionised fraction will be predominant. An API with a low pK_a will have a lower concentration of the API in the ionised form and more of the API will be in the unionised, more lipid soluble form. Because the API molecule has to cross the lipid membrane to reach the receptor site, the more lipid soluble unionised form will reach the receptor site faster than the ionised form where it dissociates to produce its ionised fraction resulting in a faster onset of action (Richards & McConachie, 1995:41).

Due to the low solubility of unionised amines, the water soluble salts are used for formulation. This is the reason why local anaesthetics are sometimes prepared as the more soluble ionised form in mildly acidic solutions e.g. lidocaine hydrochloride, prilocaine hydrochloride. Depending upon the pH of the acidic solution and the pK_a of the API, all or most of the API will be ionised and dissolved (Richards & McConachie, 1995:43).

2.2.4 Basic pharmacology of local anaesthesia

Table 2.2 gives an indication of the onset time of action, duration of the therapeutic effect, the potency of the local anaesthetic agent, as well as the toxicity of the different APIs. The maximum safe dose of each local anaesthetic agent is also indicated.

Table 2.2: Clinical pharmacology aspects of local anaesthesia. Adapted from Richards & McConachie (1995:44).

Therapeutic API	Onset of action	Duration	Potency	Toxicity	Maximum safe dose (mg/kg)
Amethocaine	Slow	Medium	High	High	1.5
Benzocaine	Slow	Short	Weak	Low	2.0 – 3.0
Bupivacaine	Slow	Long	High	High	2.0
Chloroprocaine	Rapid	Short	Weak	Low	11.0
Cocaine	Slow	Medium	Medium	High	2.0
Etidocaine	Rapid	Long	High	Medium	4.0
Lidocaine	Rapid	Short	Medium	Medium	4.0
Mepivacaine	Rapid	Medium	Medium	Medium	6.0
Prilocaine	Rapid	Short	Medium	Low	6.0
Procaine	Rapid	Short	Weak	Low	14.0

2.2.4.1 Differential block

Some of the local anaesthetics possess a characteristic called differential block that allows them to selectively block either the sensory nerve fibres more than the motor nerve fibres or the motor nerve fibres more than the sensory nerve fibres (Eappen & Datta, 1998:14). Complete motor block is not always desired and can lead to complications during certain procedures. Respiratory impairment may occur, hypotension can follow nerve blockade and patient cooperation during obstetric delivery may be limited. But because the target nerve fibres for local anaesthetic differ in size, myelination, diameter and function the phenomenon of differential block can be very helpful (White & Katzung, 2004:422). Bupivacaine, for instance, produces effective anaesthesia with the minimal motor blockade which makes it a practical anaesthetic to use during obstetric delivery. It is possible that the different types of nerve fibres are differentially affected by comparable concentrations of local anaesthetics (Eappen & Datta, 1998:14). At a given concentration of a local anaesthetic API, the sensations carried by smaller axons like temperature and pain will be blocked first and more efficiently before sensations like touch and motor function. This means that the smaller type B fibres needed for preganglionic autonomic function and the type C pain fibres in the dorsal root are blocked first. The type A pain and temperature delta fibres are blocked before the alpha, beta and gamma fibres responsible for motor, touch and muscle functions, respectively (White & Katzung, 2004:422).

Minimum blocking concentration (C_m) can be used to measure the concentration of local anaesthetic *in vitro* that will block the specific nerve within a realistic time frame. A concentration higher than the C_m of the local anaesthetic will be needed to effectively

anaesthetise the nerve. Thickness and size of the fibres are important and the larger and thicker the fibre is, the greater the C_m concentration will be. It is also safe to assume that to acquire motor block in a larger fibre, a higher API concentration will be needed and clinically a higher concentration of the local anaesthetic needs to be administered, otherwise the concentration at the site of action will not be enough for effective anaesthesia (Richards & McConachie, 1995:43).

2.2.4.2 Periodic block

The process of periodic block takes place when the blocking effect of the local anaesthetic starts to wear off. The blocking effect starts to deteriorate from the outer nerve fibres and their anaesthetic concentration starts to fall below C_m . Even with the concentration lower than the C_m , a reservoir amount of the local anaesthetic starts to form within the fibres. When a small amount of new local anaesthetic is applied again at the original site of application, it is only necessary for the local anaesthetic to diffuse a small distance towards the outer fibres to quickly re-establish the blocking effect of the anaesthesia. A smaller amount of local anaesthetic that was originally applied, is now needed to obtain the anaesthetic effect (Richards & McConachie, 1995:43).

2.2.4.3 Duration

The duration of action of a local anaesthetic agent is linked to protein binding which is an important factor in the ease of receptor dissociation (Revis, 2005). Sodium channels are proteins and the local anaesthetic binds to these channels to deliver their effect. The stronger the protein binding of the local anaesthetic is with the Na^+ channel, the longer the duration will be. Procaine and chlorprocaine are local anaesthetics with weak protein bindings and shorter durations, while bupivacaine and etidocaine have strong protein binding properties and longer durations (Eappen & Datta, 1998:13).

Vascular uptake is another factor that influence the duration of a local anaesthetic. Local anaesthetics can also produce their own intrinsic vasoactivity. Lidocaine is a good example of this because at low concentrations lidocaine induces vasoconstriction and at a higher concentration it can produce vasodilatation and cause a shorter duration of action (Richards & McConachie, 1995:44). The greater part of the local anaesthetic agents has vasodilator properties which are caused by the direct relaxation of the peripheral arteriolar smooth muscle fibres. The absorption of the local anaesthetic is faster and the duration shorter. Because of this vasodilating property, a vasoconstrictor like adrenaline is usually added to prolong the action of the local anaesthetic with the nerve for a longer duration of anaesthesia (Revis, 2005). Cocaine has the strongest vasoconstrictor properties of all the local anaesthetics while

ropivacaine, bupivacaine and mepivacaine show mild vasoconstrictor properties (Catterall & Mackie, 2006:375). Ropivacaine reduces epidural and skin blood flow and its vasoconstrictor properties can be very helpful in situations where adrenaline is contraindicated (Richards & McConachie, 1995:44).

2.2.4.4 Potency

The cell membrane of a nerve is composed of lipids and it can be concluded that the lipid solubility of a local anaesthetic is an important factor to consider when it comes to the potency of the local anaesthetic. If a local anaesthetic agent has relatively high lipid solubility, it can penetrate faster towards the nerve and begin the blockade of the Na⁺ channels (Revis, 2005). The lipid solubility of a local anaesthetic agent is determined by its aqueous/organic partition coefficient. Lidocaine has a lipid solubility of 30 mg/ml and prilocaine's lipid solubility is 130 mg/ml. *In vitro* lipid solubility is a good indicator of a local anaesthetic's potency, but *in vivo* there are other factors that also influence the potency of a local anaesthetic. The vasodilatation and redistribution properties of the local anaesthetic are important and when lidocaine is compared with mepivacaine, with a higher lipid solubility of 90 mg/ml, their potencies are relatively the same due to lidocaine's more vasodilating properties (Eappen & Datta, 1998:11, 13).

2.2.4.5 Toxicity

Adverse effects of topical anaesthesia occur when too much of the API has been administered and is circulating in the blood (Revis, 2005). When the local anaesthetic is systemically absorbed the onset of central nervous system (CNS) toxicity may occur. A patient may experience common symptoms like light-headedness, headache, irritability, metallic taste, numbness and tingling of the mouth area and the tongue, blurred vision, tinnitus and seizures (Young, 2007:236). More adverse CNS symptoms are the development of nystagmus, anxiety and panic attacks and muscle tremors that can lead to a grand mal seizure (Revis, 2005). Lidocaine, prilocaine and mepivacaine can produce these toxic CNS effects at blood levels of 5 – 10 µg/ml. Bupivacaine is more toxic and can produce adverse CNS effects with blood levels as low as 4.5 – 5.5 µg/ml (Ramamurthi & Krane, 2007:232).

Local anaesthetics have effects on the cardiovascular system that can lead to cardio toxicity. Local anaesthetics have direct effects on the smooth muscle of the heart as they block the Na⁺ channels and cause the cardiac conductivity, excitability and heart pace to decrease. A very high concentration of a local anaesthetic can also block the Ca²⁺ channels. Weak cardiac contraction and arteriolar vasodilatation can lead to hypotension but cardiac related deaths are

rare. Bupivacaine is one of the more cardio toxic local anaesthetics (White & Katzung, 2004:425).

Hypersensitivity reactions to local anaesthetics may occur but are not the main concern as they present as skin dermatitis, oedema and irritation (Young, 2007:236). Preservatives in some of the commercial preparations can be the cause of hypersensitive reactions and not the local anaesthetic active ingredient itself. A person may also show similar hypersensitive reactions to similar types of local anaesthetics (Ramamurthi & Krane, 2007:232).

Methaemoglobinemia is an adverse haematological side-effect that is caused by some of the local anaesthetics. The production of methaemoglobin is increased with oxidants like lidocaine, prilocaine and benzocaine (Young, 2007:235). Prilocaine can cause serious methaemoglobinemia as it is metabolised to ortho-toluidine, the agent that oxidizes haemoglobin to methaemoglobin. The capacity of the blood to carry oxygen lowers dramatically as the methaemoglobin cannot bind to the oxygen in the blood and causes cyanosis and tissue hypoxia in worst cases. The effects of methaemoglobinemia can be reversed with the intravenous treatment of methylene blue (Ramamurthi & Krane, 2007:232).

2.2.5 Basic chemistry of local anaesthesia

The chemical structure of local anaesthetics can be broken down into three different groups. The first group is a lipophilic group, usually an aromatic ring, at one end of the chain. The aromatic ring is linked to an intermediate chain of an ester or amide that is linked to a hydrophobic group on the other end of the chain, usually a tertiary amine (White & Katzung, 2004:418).

The pK_a , potency, lipid solubility, rate of metabolism, toxicity and duration of action of the local anaesthetic agents can be modified by changing the length of the chain, increasing the carbon atoms on the aromatic ring or changing the chirality of the molecule (Ramamurthi & Krane, 2007:230). Ropivacaine is an example of a local anaesthetic with modified chirality. If a molecule is chiral it can exist in different three dimensional configurations that are mirror images of each other. These configurations are referred to as R- or S-isomers and ropivacaine is the first local anaesthetic to exist in only its S-isomeric configuration. The S-enantiomer of ropivacaine is three times less cardio toxic than the R-enantiomer (Eappen & Datta, 1998:10, 12).

Local anaesthetics are weak bases with pK_a values that range from 8 to 9. They are only slightly soluble and are formulated as water soluble salts (Catterall & Mackie, 2006:374). Once the local anaesthetic enters the body it exists in uncharged base form or charged cationic form

(White & Katzung, 2004: 418). The uncharged (unionised) form is important for the diffusion across the cellular membranes, while the charged (ionised) form provides the therapeutic action on the Na⁺ channel (Catterall & Mackie, 2006:374). The degree of the ionisation of the local anaesthetic is dependent on the p*K*_a of the local anaesthetic and the pH of body fluids like the blood. The ionisation fractions can be described by the Henderson-Hasselbach equation (White & Katzung, 2004:418):

$$\log \frac{[\text{Cationic form}]}{[\text{Uncharged form}]} = \text{p}K_a - \text{pH}$$

Equation 2.1

If the pH is equal to the p*K*_a of the local anaesthetic the API will be 50% ionised, but if it is higher than the body pH (7.4) the amount of ionised form will increase. As mentioned in Section 2.2.3 an API with a lower p*K*_a will be more lipid soluble and have a faster speed of onset (Richards & McConachie, 1995:41).

Molecular size has an influence on the dissociation rate of local anaesthetics. A smaller molecule can move faster to its receptor site than a larger molecule. It is important that the API molecule binds as fast as possible during an action potential to achieve the time- and voltage-dependent effect needed for therapeutic action (Catterall & Mackie, 2006:369).

The receptor site inside the Na⁺ channels is hydrophobic and a more hydrophobic local anaesthetic agent will have greater receptor affinity for the site than a hydrophilic agent (Catterall & Mackie, 2006:369).

2.2.5.1 Classification of local anaesthetics according to chemical structure

Local anaesthetics can be divided in two basic classes, based on their chemistry, i.e., amino amide local anaesthetics and ester amide local anaesthetics. Table 2.3 shows the classification of the different local anaesthetics.

Table 2.3: Classification of local anaesthetics (Sweetman, 2002:1302)

AMIDE TYPE	ESTER TYPE	OTHER
Articaine	Amethocaine	Diperodon
Bupivacaine	Amylocaine	Dyclonine
Cinchocaine	Benzocaine	Ethyl chloride
Etidocaine	Butacaine	Ketocaine
Levobupivacaine	Butoxycaine	Myrtecaine
Lidocaine	Butyl aminobenzoate	Octacaine
Mepivacaine	Chloroprocaine	Pramocaine
Oxetacaine	Cocaine	Propipocaine
Prilocaine	Oxybuprocaine	Quinisocaine
Ropivacaine	Parathoxycaine	
Tolycaine	Procaine	
Trimecaine	Propanocaine	
	Propoxycaine	
	Proxymetacaine	
	Tricaine	

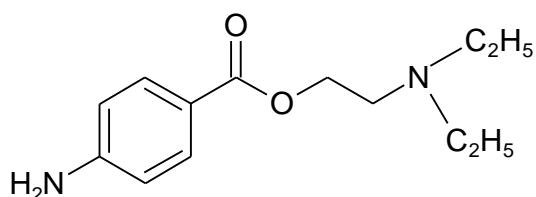


Figure 2.5: Chemical structure of the ester type local anaesthetic, procaine (White & Katzung, 2004:419)

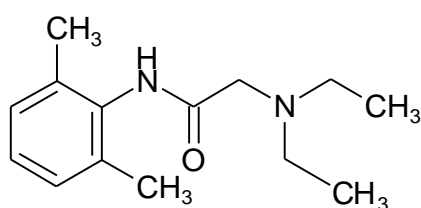


Figure 2.6: Chemical structure of the amide type local anaesthetic, lidocaine (BP, 2011b:1301)

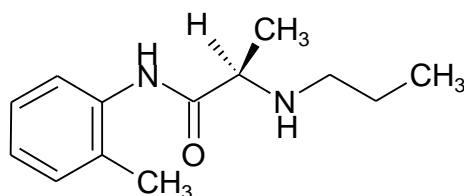


Figure 2.7: Chemical structure of the amide type local anaesthetic, prilocaine (BP, 2011b:1799)

The amide type local anaesthetics (Figures 2.6 and 2.7) are stable compounds and have longer shelf lives than the ester type local anaesthetics (Figure 2.5). They are also less likely to cause hypersensitivity reactions (Revis, 2005). The ester types are sensitive to high temperatures making them even more unstable. The amide types are metabolised in the liver and the ester types are rapidly hydrolysed in the plasma by cholinesterase enzymes. Para-aminobenzoic acid is a metabolite that forms during the hydrolysis of the ester types and causes allergic reactions in some patients (Eappen & Datta, 1998:12).

2.2.6 Physicochemical properties

The physicochemical properties of an active ingredient are important to consider before formulating any type of pharmaceutical product. When lidocaine and prilocaine are formulated into a mixture, the mixture will have a melting point lower than any of the two substances separately (Sweetman, 2002:1313). The physicochemical properties of lidocaine and prilocaine will be discussed next.

2.2.6.1 Lidocaine and lidocaine HCl

2-(Diethylamino)-N-(2,6-dimethylphenyl)acetamide, known as lidocaine or lignocaine (Figure 2.6) is classified as an amide type local anaesthetic agent with an intermediate duration of action of 60 – 120 min (BP, 2011b:1301; White & Katzung, 2004:423). The molecular mass (MM) of the lidocaine base form, $C_{14}H_{22}N_2O$, is 234.3 g/mol. The base form crystallizes from n-hexane as fine needles (Grönigsson *et al.*, 1979:210). A melting point between 66 °C and 70 °C has been determined for lidocaine, while lidocaine hydrochloride hydrate has a slightly higher melting point of between 75 °C and 79 °C. The MM of lidocaine hydrochloride, $C_{14}H_{22}N_2O \cdot HCl \cdot H_2O$, is 288.8 g/mol (BP, 2011b:1301, 1302) and is obtained from aqueous acetone as a microcrystalline powder (Grönigsson *et al.*, 1979:210). Lidocaine is described as an off-white to white crystalline powder with a characteristic odour and is nearly insoluble in water (BP, 2011b:1301; Lund, 1994:938). This minimal hygroscopic characteristic of lidocaine prevents it from absorbing water at as high as 92% relative humidity at 25 °C (Powell, 1986:770). It is soluble in ethanol, dichloromethane and chloroform and freely soluble in

benzene and ether (Lund, 1994:938). Lidocaine hydrochloride is a white crystalline, odourless powder with a vaguely bitter taste. This salt form of lidocaine is very soluble in water, freely soluble in ethanol, but insoluble in ether (BP, 2011b:1301; Lund, 1994:938). Lidocaine hydrochloride is a very stable compound in solid state or solution formulations. 2,6-Xylidine and N,N-diethylglycine are products of the hydrolysis of lidocaine hydrochloride. This is a slow process even at extreme temperatures and high pH levels (Lund, 1994:938). Lidocaine is metabolised in the liver by N-dealkylation, hydroxylation and hydrolysis to monoethylglycine xilidide and glycine xylidide and other therapeutic agents that decrease hepatic blood flow also decrease metabolism and clearance of lidocaine (O'Columb & Ramsaran, 2010:115; Grönigsson *et al.*, 1979:228).

2.2.6.2 Prilocaine and prilocaine

(RS)-N-(2-methylphenyl)-2-(propylamino)propanamide or prilocaine (Figure 2.7) is classified as an amide type local anaesthetic agent with an intermediate duration of action of 60 – 120 min (BP, 2011b:1799; White & Katzung, 2004:423). The prilocaine base form, C₁₃H₂₀N₂O, has a MM of 220.3 g/mol. It can be described as an off-white or white crystalline powder with a melting point between 36 °C and 39 °C. The water solubility of prilocaine is low, but it is very soluble in acetone and ethanol (BP, 2011b:1799). The salt form of prilocaine is prilocaine hydrochloride, C₁₃H₂₀N₂O.HCl, which has a MM of 256.8 g/mol. Prilocaine hydrochloride has a high melting point between 168 °C and 171 °C. The appearance of prilocaine hydrochloride is described as an off-white or white crystalline powder or as colourless crystals. Different to prilocaine, prilocaine hydrochloride is up to 96% freely soluble in water and ethanol, but only slightly soluble in acetone (BP, 2011b:1801). Prilocaine has a therapeutic action similar to that of lidocaine. It has a slower onset of action, a slightly longer duration than lidocaine, as well as less vasodilator activity. Prilocaine is metabolised in the liver and kidneys by amidases as different metabolites (Sweetman, 2002:1318). A widespread amount of prilocaine is taken up in the pulmonary system which leads to lower plasma concentration levels and a lower toxicity than lidocaine. The ortho-toluidine metabolite of prilocaine may cause methaemoglobinemia in some patients where the blood's capability to carry oxygen is dramatically decreased. Methylene blue is used to reverse the effect by reducing the ferric state (Fe³⁺) back to the ferrous (Fe²⁺) state and so methaemoglobin to haemoglobin (O'Columb & Ramsaran, 2010:1115,116).

2.3 TRANSDERMAL API DELIVERY

2.3.1 Introduction

The skin is the largest organ in the body. It is multifunctional with roles that vary from protection, mainly, to immunological, sensory and endocrine functions (Menon, 2002:S4). Despite its complex structure, the skin is the most accessible organ of the body for API delivery (Thomas & Finnin, 2004:697).

The skin is the body's first line of protection against injury of chemical, physical, pathogen or immunological origin. The skin also provides important protection against ultra violet (UV) rays by reflecting the UV rays and increasing melanin to decrease absorption of radiation caused by them. The production of antimicrobial peptides, Langerhans cells and T-cells functions as the skin's immunological defence mechanism (Venus, 2010:471). Ultimately the skin is a complex protective barrier that provides the formulator of transdermal products with exciting, yet difficult, challenges.

2.3.2 Anatomical structure and barrier function of the skin

The skin ultimately consists of two major layers: the epidermis and the dermis. The dermis is the inner layer of the skin and contains a large supply of capillaries (Marjukka Suhonen *et al.*, 1999:150). The dermis has a thickness of 3 – 5 mm containing nerves, sweat glands, sebaceous glands and hair follicles. Fibroblasts are specialised cells found in the dermis that synthesise collagen and elastin (Menon, 2002:S4). Collagen and elastin is connective tissue that provides the dermis with support and flexibility. The dermis is seen only as a minimal barrier in the transdermal delivery of APIs. It is the outer avascularised epidermal layer and its complex structure that provide the excellent barrier function most APIs have to overcome (Williams, 2003:3).

The epidermis consists of multiple layers (Figure 2.8). Each layer can be described as cells in a different stage of differentiation (Marjukka Suhonen *et al.*, 1999:150). The epidermis varies in thickness depending on the site. It has been noted that this layer is only 0.06 mm thick around the eyes but up to 0.80 mm thick on the soles of feet and palms. The site of transdermal API administration is thus an important factor for molecules crossing the epidermis in order to reach the systemic circulation (Williams, 2003:5).

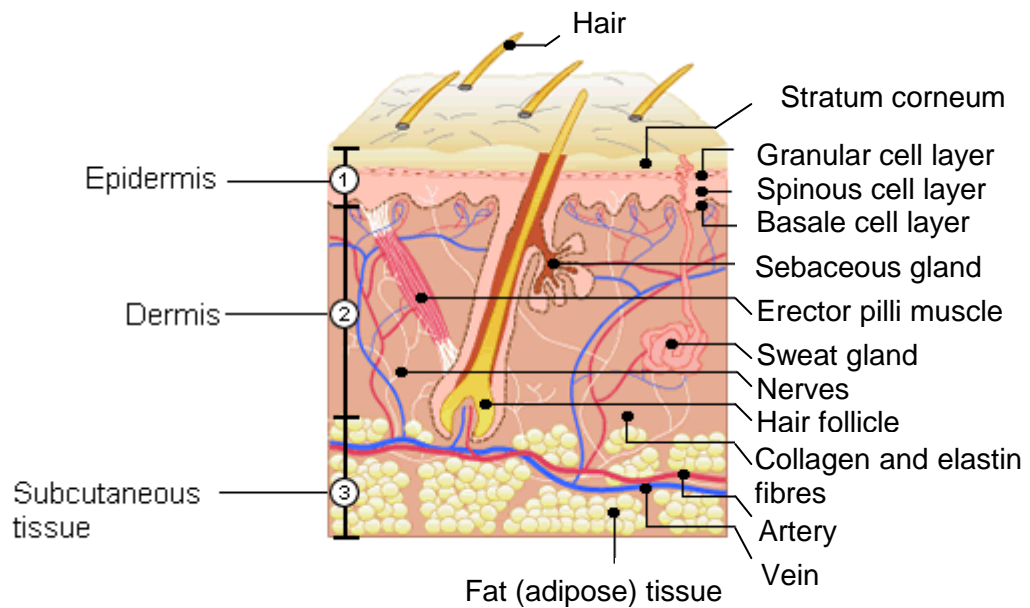


Figure 2.8: Anatomy of the skin (Melbourne, 2009)

As seen in Figure 2.8, the epidermis consists of four different cell layers. The single layer of metabolically active columnar basal cells is called the stratum basale (Menon, 2002:S4). In this layer there are keratinocytes that undergo cell division and melanocytes that synthesise skin pigment (melanin). Langerhans cells are found in this layer and play an important role in the immunological response of the skin while Merkel cells are specialised cells contributing to touch sensation (Williams, 2003:7, 8).

The stratum spinosum is the second layer of the epidermis and lies on top of the stratum basale. Keratins are synthesised in the upper part of this layer. The keratinocytes present in the stratum spinosum are differentiating and start their migration towards the next epidermal layer (Menon, 2002:S4).

When the keratinocytes reach the third layer, the stratum granulosum, the cells continue to produce keratin. The keratins inside cells are matured by special keratohyalin granules that characterise this layer. The keratinocytes start to elongate and flatten as they move towards the final stage of cell differentiation (Williams, 2003:8).

The stratum corneum, also named the horny layer, is the final layer of the epidermis. The cells are now in their final stage of differentiation. The stratum corneum consists of ten to fifteen layers of keratinised dead cells that lay embedded in a lipid matrix (Menon, 2002:S4). The structure of the stratum corneum has largely been referred to as a 'brick and mortar' model (Foldvari, 2000:418). This special structure of the stratum corneum is what makes it so difficult

for substances to move across the skin and provides excellent barrier function. The 'bricks' are represented by keratinised cells while the 'mortar' part is seen as the hydrophobic lipids. These lipids are organised in a tight lamellar structure that form lipid bilayers (Williams, 2003:9).

The stratum corneum contains a unique mixture of lipids without any phospholipids. Neutral lipids like cholesterol, fatty acids and cholesteryl esters are found in the stratum corneum together with ceramides. The ceramides form an important part of the composition of the stratum corneum. If taken on a dry weight basis the extracellular lipids make out roughly 10% of the stratum corneum while the other 90% consists of intracellular protein (Foldvari, 2000:418). Only 10 – 15% of the protein is water soluble while an unidentified substance has also been reported to make up another 5 – 10% of the final composition of the stratum corneum. All these components ensure the stratum corneum of a barrier function unlike any of the other biological membranes and form a tortuous path for any substance trying to cross it.

The stratum corneum is a surface layer with highly hydrophobic properties and has a water permeability a thousand times lower than some of the biological membranes (Marjukka Suhonen *et al.*, 1999:150). To polar molecules like water, K^+ , Na^+ and ion solutions the skin functions as an impermeable barrier but can be permeable to other substances such as aliphatic alcohols (Venus, 2010:471). The stratum corneum is rate-limiting to the penetration of therapeutic substances through the skin into the systemic circulation. The regulation of the API flux through the bilayered lipids is also problematic in transdermal delivery (Foldvari, 2000:418).

2.3.3 Transdermal permeation and permeation routes

Permeation, as described by Williams (2003:27), is the movement of an API through the membrane by partitioning of the drug molecules into the various domains present in the skin layers together with diffusion through these domains (Williams, 2003:27). The skin's lipid bilayers are the most important factor to determine the transdermal transport of any type of solute, especially those of hydrophobic character (Mitragotri, 2003:70). For most molecules the stratum corneum is the rate-limiting barrier to overcome for delivery.

A molecule follows a complex path from the moment of application on the skin until it reaches the systemic circulation. At first the process of permeation seems simple with the API partitioning on the stratum corneum's outer layer and then followed by diffusion through it. The diffusion step through the stratum corneum is, however, much more complex and consists of multiple steps the API molecules must follow before finally reaching the systemic circulation. Parallel pathways for permeation also exist. Partitioning occurs at the junction of the stratum corneum and epidermis, followed by diffusion through the membrane towards the junction of the

epidermis and dermis. Once there, another round of partitioning and diffusion occurs through the tissue of the dermis and into the capillaries. Finally, the process of permeation through the skin ends with the final partitioning of the API towards the blood vessels, to then be removed into the systemic circulation (Williams, 2003:30).

One of the possible problems that may occur during the permeation process is that of the possibility of molecules binding to various elements or structures in the skin. Binding to receptors in the skin may occur, as well as binding to keratins that can cause an API reservoir effect. It is also possible for the API to be degraded metabolically or get trapped in the subcutaneous fat layer. These are all hurdles that exist that can delay the API molecules from reaching the systemic circulation or cause them to not reach it at all (Williams, 2003:30). There are also API factors that influence transdermal permeation like the mobility of the vehicle carrying the API, the release of the API from its vehicle and of course its penetration through the skin (Kogan & Garti, 2006:372).

There are three possible permeation pathways a molecule can follow to reach the systemic circulation (Figure 2.9). It can move through 'shunt routes' formed by the hair follicles and their sebaceous glands and sweat ducts, or move across the stratum corneum navigating between these appendages (Barry, 2001:101). Molecules can also move through the intercellular lipids or follow the transcellular route across the stratum corneum. It has been found that most API molecules manage to pass through the stratum corneum by a combination of these pathways. Size and lipophilicity of the pathways are important contributors to skin permeation (Mitragotri, 2003:85).

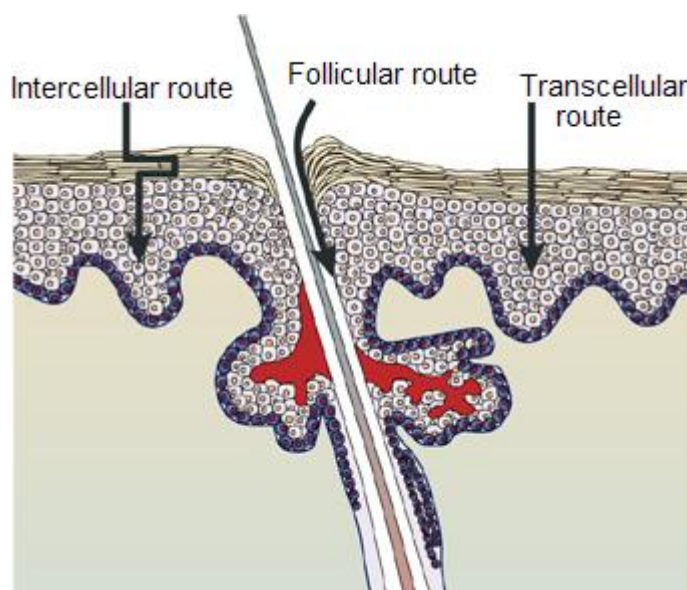


Figure 2.9: Permeation pathways across the skin (Adapted from Prausnitz *et al.*, 2004)

2.3.3.1 Transappendageal transport

It is possible that the transdermal transport of APIs can occur via the appendages of the skin. These appendages like the hair follicles and sweat glands are commonly referred to as 'shunt routes' (Mitragotri, 2003:79). This route may be important because it offers pores that the API molecules can use to avoid the stratum corneum barrier (Williams, 2003:31). It is also possible that these pores form because of imperfections that occur in the stratum corneum lipid bilayers (Mitragotri, 2003:76), but unfortunately these openings are only found on 0.1% of the skin's total surface.

The eccrine sweat glands are present in various areas of the body, especially on the palms and soles. It is unfortunate that their openings to the skin surface are smaller than desired. The active secretion of sweat in these ducts can also minimize inward diffusion of the applied topical agent. In contrast, the follicular opening is much larger than the opening of the eccrine gland, but considerably less is present on the body than the eccrine glands (Williams, 2003:31). Three parameters are of importance here: porosity, tortuosity and pore size distribution (Mitragotri, 2003:83).

Hydration of the tissue can cause the openings to the 'shunt routes' to close down (Williams, 2003:32) and because of small skin area coverage with appendages the transport through 'shunt routes' is not generally used (Mitragotri 2003:79), but may be important in the rapid transport of large polar molecules and ions (Barry, 2002:S32).

2.3.3.2 Transcellular transport

With transcellular transport the API molecule follows a pathway straight across the stratum corneum, meaning straight across the corneocytes and lipid matrix (Marjukka Suhonen *et al.*, 1999:151). It can also be seen as providing a polar route for the API through the membrane.

An API molecule has to overcome various hurdles standing in its way while traversing the stratum corneum by the transcellular route. The API molecules must first partition into the keratinocyte where they then diffuse through the hydrated keratin. In this aqueous environment hydrophilic molecules can diffuse with a rapid pace, but still have to face the intercellular bilayered lipid domains the keratinocytes are connected to. This diffusion from keratinocyte across the lipid bilayer towards the next keratinocyte makes for a difficult path to cross and further hurdles occur in the form of lamellae that the molecule must also partition and diffuse through (Williams, 2003:33).

The conclusion is that for this route, the multiple bilayered lipid between the keratinocytes is the rate-limiting factor for permeation. The thickness of the stratum corneum is used to describe the length of this pathway. As the molecules cross directly over the stratum corneum it can be seen as a shorter permeation route than the other routes. It is also not uncommon to use solvents that will remove lipids from the stratum corneum to increase the API flux of this route (Williams, 2003:33, 24).

2.3.3.3 Intercellular route

The intercellular route is the route of choice for the permeation of most APIs. Permeation occurs through the movement of the molecules in the lipid domains between the corneocytes (Marjukka Suhonen *et al.*, 1999:151). This route is highly tortuous and the movement between the lipid domains is the reason for a longer diffusional path length than the transcellular route. The pathway can be as long as 500 μm , which is much longer than just the length of the stratum corneum (Hadgraft, 2004:292).

As mentioned before, the lipid bilayers are a major barrier factor that causes a rate-limiting effect. Even though only 1% of the diffusion area of the stratum corneum consists of these bilayers, they are present as a continuous phase in the membrane (Williams, 2003:33). It is most likely small and uncharged molecules that choose this route of permeation with permeation distance ranging from 150 – 500 μm . The molecules' physicochemical properties play an important role when choosing a suitable permeation path (Williams, 2003:35). After consideration and study of the physicochemical properties lidocaine HCl and prilocaine HCl it is most likely that these substances will traverse the stratum corneum via the intercellular route.

2.3.4 Advantages and disadvantages of transdermal API delivery

Transdermal delivery is probably one of the easiest available routes for API administration. It is especially useful for delivering APIs that have poor bioavailability or side-effects caused by a too high API peak in the blood (Thomas & Finnin, 2004:697). The main factors formulators want to improve for this route are firstly, the delivery of the optimal API amount at specific sites, as well as ensuring the stability of the API with reduced skin irritation reactions. The presentation of an aesthetic acceptable product to the patient is also an important factor to improve patient compliance (Pefile, 1997:147).

2.3.4.1 Advantages of transdermal API delivery

The following advantages of the transdermal delivery route have been noted:

- ❖ The hepatic first-pass metabolism is avoided. This is especially useful for APIs with poor bioavailability properties.
- ❖ Patient friendly means of API delivering.
- ❖ It is a convenient way of dosing and makes self-regulation by the patient possible.
- ❖ Non-evasive methods cause no trauma to the skin and also lower the risk for infection (Thomas & Finnin, 2004:697, 698).
- ❖ Less frequent dosing intervals and the fact that this method is painless, lead to improved patient compliance.
- ❖ Acid hydrolysis in the stomach that degrades certain APIs is avoided.
- ❖ It is possible to stop therapy at any time if adverse reactions to the API occur.
- ❖ Side-effects are minimized with reduced concentrations and less frequent dosing while many of the transdermal delivery systems ensure the constant and controlled release of the API and steady plasma profiles (Danckwerts, 1991:315).
- ❖ It is possible to enhance the accuracy of delivering the API to a specific site (Pefile, 1997:147).

2.3.4.2 Disadvantages of transdermal delivery

The following disadvantages can make transdermal delivery of APIs less appropriate:

- ❖ Bacteria on the surface of the skin can metabolise the API.
- ❖ Problems can occur in maintaining contact between the skin and the transdermal device (Washington *et al.*, 2001:187).
- ❖ Skin irritation at the site of application may occur and cause discomfort.
- ❖ The high manufacturing costs of transdermal products is a problem that reduces the demand for these products.
- ❖ The cosmetic appearance of these products is not always ideal or appealing to the public (Thomas & Finnin, 2004:697).
- ❖ Allergic reactions to the adhesive of transdermal patches lead to the discontinuing of therapy.

- ❖ The barrier properties of the stratum corneum can cause permeation problems and cause only minimal API amounts to reach the systemic circulation (Danckwerts, 1991:315).

2.3.5 Physiological factors affecting transdermal API delivery

2.3.5.1 Skin age

Skin age is a factor that can affect the transdermal delivery of APIs as the structure and condition of the skin differ between age groups (Washington *et al.*, 2001:188). Older skin has been exposed to more environmental and chemical damage that causes the stratum corneum to thicken and increase its barrier function (Williams, 2003:14; Washington *et al.*, 2001:188). Older skin is less hydrated as the moisture content of the skin decreases with age and a loss of tissue hydration can influence transdermal delivery. Blood flow and dermal clearance of the skin reduce with age and can pose the problem of decreased transdermal API flux through the skin (Williams, 2003:14). The skin of a neonate has very little barrier function and exposes them to increased absorption of externally applied materials. The surface area to body weight ratio of an adult is four times that of a neonate and it is important that the transdermal dose is adjusted accordingly (Williams, 2003:15).

2.3.5.2 Body site

The permeation of a transdermal product is dependent on its site of application. The stratum corneum varies in thickness all over the body and it is only logical to apply a transdermal product to a site with increased permeability. Genital tissue is the most permeable tissue for transdermal application, followed by the head and neck area, the body trunk and then the arms and legs. The stratum corneum is the thickest on the soles of the feet and palms. The trunk is a popular site for the administration of transdermal products as it produces intermediate permeabilities for most APIs with general good patient compliance (Williams, 2003:16). Variation of permeation may occur between individuals at the same body site and it has been suggested that the application site of the transdermal device be varied. Varying of application site can also reduce local irritation and skin sensitivity (Washington *et al.*, 2001:188).

2.3.5.3 Skin hydration

The level of skin hydration is important and can have an important effect on API permeability. The more hydrated the skin is, the better the permeability will be and the transdermal delivery of the API will be increased (Williams, 2003:17). Occlusion is a method used to hydrate the stratum corneum and can improve the permeability of polar and non-polar APIs (Washington *et al.*, 2001:188). The occlusive dressing hydrates the stratum corneum by preventing the

evaporation of endogenous water from the surface. Areas with a high humidity can provide sufficient skin hydration. The measurement of transepidermal water loss (TEWL) can provide an assessment of the degree of hydration of the stratum corneum (Riviere, 1993:117). A eutectic mixture of lidocaine and prilocaine, 1,5 g/10 cm², can be applied under occlusive dressing for no longer than 5 h and provides an anaesthetic effect that lasts 1 – 2 h (Astra Zeneca, 2006).

2.3.5.4 Temperature

Temperature can effect API penetration. The temperature of the skin can vary from 20 °C cooler or 7 °C hotter than normal body temperature due to the surrounding environment (Washington *et al.*, 2001:188). If the skin temperature is largely increased it can cause the stratum corneum to undergo structural alterations. This leads to increased diffusion rates of the API (Williams, 2003:18). If disease is present, a higher body temperature can raise the absorption rate of the API and increase the risk of systemic toxicity (Washington *et al.*, 2001:189).

2.3.5.5 Disease

The skin is the body's first line of protection against harmful substances which can leave it damaged or irritated. Inflammation develops from infection, mechanical, thermal or chemical stimulation and reduces the barrier function of the stratum corneum. Dermatitis causes itching, pain, swelling, sweat retention and oozing that alter the barrier function of the stratum corneum. Under these conditions the skin becomes more permeable to APIs and higher API concentrations than necessary can pass through into the systemic circulation. UV rays and sunburn also alter the permeability of the skin, even if no visible damage is done and the skin layer remains unbroken. Allergic contact dermatitis is a common problem that emerges in patients using transdermal devices and poses an obstacle to the development of these devices (Washington *et al.*, 2001:189).

2.3.5.6 Race

Studies show that race only alters skin penetration to a minimal level. The TEWL between African, Asian and European skin shows no difference even if the skin contains darker pigments. The water content of the stratum corneum varies for different races and because of this difference in skin hydration there could be differences in API absorption (Williams, 2003:17). It has generally been determined that the stratum corneum of brown or black coloured persons has more layers, which makes it less permeable than Caucasian skin. Still no variation in the

thickness of the stratum corneum of darker coloured people and light coloured people has been found (Washington *et al.*, 2001:189).

2.3.6 Physicochemical factors affecting transdermal delivery

2.3.6.1 Partition coefficient (P)

The partitioning of a permeant molecule into the skin is an important step in the transdermal API delivery process. Unfortunately the partitioning of the permeant into the horny layer of the stratum corneum is rate-limiting to the whole permeation process. By measuring the partitioning coefficient (P), the redistribution of molecules between one domain and another can be determined. When doing a transdermal study the logarithm of the partition coefficient between octanol and water is used to guide the formulator as to how effectively a molecule will distribute itself between the lipids of the stratum corneum and water (Williams, 2003:27, 28). The horny layer has non-polar properties and therefore, the characteristics of the chosen solvent are important as they can greatly influence the partitioning coefficient (Zatz, 1993:25). Lipophilic molecules with a octanol-water partition coefficient ($\log P$) > 3 prefer to partition into the lipoidal domains and will most certainly use the intercellular route of permeation to cross the stratum corneum. Hydrophilic molecules with a $\log P < 1$ partition into the hydrated keratin cells and cross the stratum corneum through the transcellular route (Williams, 2003:35, 36). The ideal $\log P$ value for molecules is 1 – 3 (Hadgraft, 2004:292). Lidocaine HCl has a $\log P$ of 2.36 while the $\log P$ for prilocaine HCl is calculated at 2.09 (obtained from the ACD Labs prediction software). It is safe to say that molecules with their $\log P$ values in the ideal range of 1 – 3 will follow the intercellular route of permeation across the stratum corneum (Williams, 2003:36).

2.3.6.2 Diffusion coefficient (D)

Diffusion can easily be described as the movement of molecules through a domain from a high concentration to a low concentration. Diffusion is an important step in the permeation process and the diffusion coefficient of a molecule is a determination of how easily it will pass through tissue (Williams, 2003:27). The diffusion coefficient is a variable that is included in Fick's law (Equation 2.9) as an assumed linear concentration gradient of the permeant in the stratum corneum. The diffusion coefficient is calculated in cm^2/h or cm^2/s and can provide useful information about the transdermal permeation process (Rieger, 1993:38).

2.3.6.3 Ionisation

The permeation process depends on the degree of ionisation of the permeant. Most permeants exist in weak acidic or weak base forms but these ionisable APIs are usually poor transdermal permeants (Williams, 2003:38). Studies show that certain transdermal APIs are better applied

in their ionised form. The ionised form is more soluble with a lower permeability coefficient while the unionised form is less soluble with a much higher permeability coefficient (Hadgraft, 2004:292). The permeability coefficient (k_p) is described as the rate of permeant transport per unit of concentration with API flux as the product (Williams, 2003:27, 38). As the horny layer show non-polar properties it should be expected that ionised substances will have difficulty crossing this layer. By changing the pH of ionogenic substances the ratio of ionised to unionised form can be manipulated. A study conducted by Zatz (1993:28, 29) shows lidocaine permeation as a function of pH. He found that for 5% systems at pH 4.0, 6.0, 7.8 and 10.0 the permeation of the unionised form was significantly higher than the ionised form. The permeation coefficient of the unionised form was approximately 17×10^{-3} cm/h times higher than the ionised form at a 50 ratio (Zatz, 1993:28, 29). The speed of onset of a local anaesthetic is dependent on the lipid solubility and unionised form of the API. The pK_a value of the API will determine the degree of ionisation that will in turn determine the permeation of the permeant. Lidocaine and prilocaine are weak bases. They are prepared as their acidic forms, lidocaine HCl and prilocaine HCl, so that they will be more water soluble with increased ionisation (Richards & McConachie, 1995:43). The Henderson-Hasselbach equation (Equation 2.1) can be used to determine the extent of ionisation (White & Katzung, 2004:418).

2.3.6.4 Melting point and aqueous solubility

Organic materials with high melting points and high enthalpies show poor solubility under typical transdermal conditions. Lipophilicity is an important characteristic for most transdermal substances. It is also important that the molecules of these substances for transdermal use show aqueous solubility character as well. The general motivation for this is that most transdermal products are applied to the skin in an aqueous formulation. If an API formulated in an aqueous formulation has poor aqueous solubility the amount of API present will be minimal (Williams, 2003:37).

Most pharmaceutical compounds are lipophilic and processes to improve their aqueous solubility include forming salts of the basic API form. Hydrochlorides, sulphides, nitrates, maleates, citrates and tartarates are common salt forms used in the salt-forming process. Particle size reduction is another popular method to increase aqueous solubility (Kogan & Garti, 2006:370). Lidocaine in its base form has moderate aqueous and lipid solubility and penetrates relatively easily into the skin (Valenta *et al.*, 1999:77). The ionic form will, however, be dominant at skin pH and a slow onset of anaesthetic action will occur. By using the hydrochloride salt forms of both lidocaine and prilocaine, their aqueous solubility increases tremendously. This in turn increases ionisation. Adjusting the pH of a lidocaine HCl solution to 8 allows the unionised form to become dominant which leads to better skin penetration and a

faster time of onset of anaesthesia. Aqueous solubility experiments performed at 32 °C with both lidocaine HCl and prilocaine HCl solutions at pH 8, confirmed the high aqueous solubility characteristics of the salt forms. High values of 579.8 mg/ml for lidocaine HCl and 388.86 mg/ml prilocaine HCl were obtained (Appendix D.3.1).

There is a direct correlation between the melting point of a substance and the solubility of the substance. Melting point data can thus be used to predict solubility (Williams, 2003:37). The ideal melting point for a substance used in transdermal delivery is < 200 °C (Naik *et al.*, 2000:319). Lidocaine HCl has a melting point of 79 °C (BP, 2011b:1301) while the melting point of prilocaine HCl is measured at 171 °C (BP, 2011b:1801). These values are well within range of the ideal melting point for transdermal products. When lidocaine HCl and prilocaine HCl are formulated into a eutectic mixture it can produce a melting point lower than the melting points of the two separate compounds (Sweetman, 2002:1318).

2.3.6.5 Molecular size

Molecular size and shape are important variables when determining the API flux of a substance across the skin (Williams, 2003:36). Larger molecules diffuse slowly while smaller molecules cross the human skin much faster (Hadgraft, 2004:292; Williams, 2003:36). The ideal weight for molecules for transdermal delivery is determined by Naik *et al.* (2000:319) at < 500 Da. Lidocaine HCl and prilocaine HCl fall well into this range with their molecular weights of 288.0 Da and 256.8 Da, respectively (BP, 2011b:1301, 1801). If the molecular weight of transdermal APIs is lower than 500 Da, the effect of molecular size is minimal. For molecules larger than 500 Da, the influence on API flux is of greater importance (Williams, 2003:37).

2.3.7 The use of mathematics in determining skin permeation

API absorption across the human skin is usually described as a passive process and the process of diffusion is regularly equated with the transdermal permeation process. A more descriptive and precise definition of diffusion is that of matter transport resulting from the movement of a substance within a substrate. Because of the complex structure of the skin, diffusion and permeation through its different layers might seem difficult to describe mathematically. Realistically, simple mathematical concepts like the Laws of Fick can be applied to permeation data of the skin to establish the amounts of permeant passing through one or more stratum making up the skin (Rieger, 1993:28; Williams, 2003:41). The following equations will describe steady-state permeation at infinite dosing using Fick's Laws and variations thereof.

The first law of Fick states that the rate of transfer of a diffusing substance through a unit area of a section is proportional to the concentration gradient measured normal to the section (Williams, 2003:41). The API flux (J) is described by Equation 2.2:

$$J = -D \frac{dc}{dx}$$

Equation 2.2

J is the API flux, D the diffusion coefficient (Barry, 2002:S33), c is the concentration and x the space measured to the normal section; this makes dc/dx the concentration gradient (Williams, 2003:41). Unidirectional diffusion takes place as soon as a topical transdermal product is applied to the skin and the permeant enters the skin. The concentration gradient is determined as it moves from the outer surface into the skin, and if no API molecules are present in the skin prior to topical application, then the API movement into the skin can be described by Fick's second law of diffusion (Tojo, 1997:113; Williams, 2003:41):

$$\frac{\partial c}{\partial t} = D \frac{\partial^2 c}{\partial x^2}$$

Equation 2.3

The t describes time, x the distance from the surface of the skin and c is the concentration (Tojo, 1997:113). The rate of change in concentration with time at a point within a diffusional field is proportional to the rate of change in the concentration gradient at that point. Some additions to Fick's second law can be made as most *in vitro* experiments try to mimic *in vivo* conditions by using a two-compartment vehicle. One compartment is filled with permeant while the second compartment contains a receptor phase to provide skin conditions. Equation 2.3 can now be simplified as seen in Equation 2.4:

$$\frac{dM}{dt} = \frac{DC_0}{h}$$

Equation 2.4

M is described as the cumulative mass of permeant that passes through per unit area of the membrane in the time, t. C₀ is the concentration permeant in the first layer of the membrane. C₀ is related to the concentration permeant in the vehicle C_v and can be replaced by Equation 2.5 (Williams, 2003:42, 43):

$$C_0 = PC_v$$

Equation 2.5

P is the partition coefficient and by substituting Equation 2.4 with Equation 2.5, Equation 2.6 is formed (Barry, 2002:S33):

$$\frac{dM}{dt} = \frac{DPC_v}{h}$$

Equation 2.6

Lastly, another substitution follows with Equation 2.7 into Equation 2.6 to produce Equation 2.8 where k_p is the permeability coefficient:

$$K_p = \frac{PD}{h}$$

Equation 2.7

$$J = k_p C_v$$

Equation 2.8

Equation 2.8 can also be written as (Rieger, 1993:39) follows:

$$J = P\Delta C = \frac{KD\Delta C}{h}$$

Equation 2.9

J = API flux

P = permeability coefficient

ΔC = differences in concentration on each side of the membrane

K = partition coefficient

D = diffusion coefficient

h = thickness of membrane

Fick stated that API flux (J) should be proportional to the concentration differences (ΔC) between the two sides of a membrane and inversely proportional to the membrane thickness (h) (Rieger, 1993:39).

2.3.8 Penetration enhancement

The enhancement of API penetration through the skin has been widely studied. Methods for penetration enhancement include physical enhancement, chemical enhancement and the use of API delivery vehicles (Asbill & Michniak, 2000:37). A substance that can cause a temporarily decreased stratum corneum barrier function and enhanced API flux is termed an enhancer, accelerant or sorption promoter (Barry, 2001:106). It is important that enhancers show the following characteristics. They should:

- ❖ increase the diffusivity of an API through the skin;

- ❖ cause reversible stratum corneum lipid-fluidisation to decrease stratum corneum barrier function;
- ❖ increase and optimise the thermodynamic activity of the API in a vehicle and the skin;
- ❖ form a reservoir of the API in the skin; and
- ❖ have a positive effect on the partition coefficient of the skin that leads to increasing release of the API formulation into the upper layers of the skin (Shah, 1994:19, 20).

2.3.8.1 Chemical enhancers

Chemical enhancers are any substances that are pharmacologically inactive, non-damaging and promote hydration of the horny layer. Because penetration through the hydrated stratum corneum is much easier for an API, it is important that a chemical enhancer shows some form of hydration of the horny layer (Barry, 2001:106). The following desirable properties of chemical enhancers have been described (Barry, 2001:106; Williams, 2003:86, 87):

- ❖ A chemical enhancer should not be pharmacologically active in the body.
- ❖ A chemical enhancer should be non-toxic, non-irritating and non-allergenic.
- ❖ A rapid onset of action is important for a chemical enhancer and the duration of action should be predictable and reproducible.
- ❖ After the removal of the chemical enhancer off the skin, the barrier function of the stratum corneum should return to normal, to a full extent and at a rapid pace.
- ❖ A chemical enhancer should consist of a unidirectional mechanism that allows the API to enter the skin and not let any endogenous materials escape.
- ❖ The chemical enhancer should be compatible with the API and its properties and should be easily formulated into a topical product.
- ❖ A chemical enhancer should be cosmetically acceptable.
- ❖ A chemical enhancer should spread well onto the skin with an appropriate 'feel'.
- ❖ A chemical enhancer should be inexpensive, odourless, tasteless and colourless.

It is not possible for a chemical enhancer to possess all of these desirable properties, though many of them do display some of them (Kanikkannan *et al.*, 1999:596).

Chemical enhancers act through three mechanisms. Firstly, chemical enhancers can act via lipid action by causing the disruption of the structure of the highly ordered lipids of the stratum corneum to make it more permeable. The molecules of the chemical enhancer enter the lipid

bilayers, alter them and cause fluidisation of the pathway to increase diffusion (Kanikkannan *et al.*, 1999:596). Chemical enhancers can also cause protein modification when they interact with the keratin in the corneocytes. The protein structure opens and the density decreases, allowing the permeability to increase. Lastly, chemical enhancers promote the partitioning of the API into the stratum corneum by altering its chemical environment. It is also possible for some chemical enhancers to combine these mechanisms, like dimethyl sulfoxide (DMSO). At high concentrations it is possible for DMSO to disturb intercellular organisation by extracting lipids, interacting with the keratins and promoting API partitioning (Barry, 2001:106). Chemical penetration enhancers include water, hydrocarbons, fatty acids, surfactants, esters and alcohols, amides, ureas, amines and bases, sulphoxides, terpenes, steroids, dioxolanes, pyrrolidone and imidazole derivatives, azone, polyols, essential oils, oxazolidines, epidermal enzymes, polymers, lipid synthesis inhibitors, biodegradable enhancers and synergistic mixtures (Asbill & Michniak, 2000:37; Barry, 2001:106). Table 2.4 is a summary of the commonly used chemical enhancers and their mechanisms.

Table 2.4: Classification of chemical penetration enhancers and their mechanisms (Foldvari, 2000:420; Williams, 2003:87-102)

CLASS	EXAMPLE	MECHANISMS
Sulfoxides	Dimethyl sulfoxide (DMSO)	Denaturation of proteins and keratin interaction, increases lipid fluidity at high concentrations
Alcohols	Ethanol, Octanol	Transports API through lipid channels and causes lipid extraction, increases vehicular solubility of the API
Polyols	Propylene glycol	Increases solubility of the API, replaces bound water in the intercellular space, acts synergistically with other enhancers
Fatty acids	Oleic acid, Lauric acid, Linoleic acid	Increases fluidity of intercellular lipids, forms novel lipid domains in barrier lipids, these defects increase permeation
Fatty alcohols	Lauryl alcohol, Oleyl alcohol	Increases the solubility of the API and API partitioning, changes solvent properties of stratum corneum
Terpenes	L-menthol, D-limonene, Ascaridole	Modifies API diffusivity through the membrane, opens polar pathways, increases electrical conductivity, disrupts lipid structure
Pyrrolidones	2-pyrrolidone, N-methyl-2-pyrrolidone	Interacts with keratins and lipids and alter solvent nature of the tissue, forms a permeant reservoir inside the tissue
Surfactants	Anionic: sodium lauryl sulphate Cationic: cetyltrimethylammonium bromide Non-ionic: Polysorbate	Uncoil keratin fibres, extracts lipids from the stratum corneum, modifies water binding and increases TEWL, micellar solubilisation is caused by the non-ionic surfactants
Azone	1-dodecylhexahydro-2H-azepine-2-one	Disrupts lipid packing in the polar head group and tail group of the intercellular lipids
Urea	Dimethylacetamide	Causes skin hydration and is keratolytic, causing the modification of intercellular keratins

2.3.8.2 Physical enhancers

Physical methods to overcome the barrier function of the stratum corneum have been widely studied in the last decades. Most physical enhancers make use of an energy source to overcome the barrier function of the stratum corneum (Thomas & Finnin, 2004:699). It has been said that the transdermal route is a painless and simple way to administer APIs. The

problem that makes transdermal delivery difficult is the fact that most of the molecules meant to move through the skin are charged, large and polar (Naik *et al.*, 2000:322). Generally particles > 10 µm remain on the surface of the skin while particles < 3 µm penetrate the stratum corneum and hair follicles (Williams, 2003:139). Recent advances in physical enhancement strategies provide legitimate methods to overcome some of these problems (Naik *et al.*, 2000:322). Most physical enhancers work through a mechanism that involves forming a reservoir of the API on the skin surface so that the required levels for API delivery can be achieved (Thomas & Finnin, 2004:699). Electrical techniques, removal of the stratum corneum and the use of radio-waves as physical penetration enhancers will briefly be discussed next.

2.3.8.2.1 Removing or bypassing the stratum corneum with ablation techniques and microneedle array

Removing the stratum corneum before applying a transdermal product to the skin will increase the API flux of the permeating API through the skin. The stratum corneum can be removed through various methods such as tape stripping, the use of cyanoacrylate adhesives, chemical peeling, microdermabrasion and laser ablation (Barry, 2001:107; Williams, 2003:139). The technique of microdermabrasion uses a stream of aluminium oxide crystals together with dermabrasion as a motor-driven abrasive cylinder and provides a 'sandpaper-like' effect on the skin (Barry, 2001:108). Lasers provide a controlled and accurate method of removing the stratum corneum. The laser ablates the stratum corneum in a precise manner by removing approximately 1 µm of stratum corneum per laser pulse without damaging the underlying dermal tissue (Williams, 2003:139, 140).

Microneedles are used to painlessly penetrate the skin and increase API flux up 100 000-fold. Microneedles are manufactured as solid silicone needles coated with the API or as hollow metal needles with the API solution inside. Pain receptors are located in the deeper part of the skin so by applying microneedles only to the horny layer of the skin, nerve stimulation is avoided and no pain is felt (Barry, 2001:107). Microneedles can also be applied to the skin and then removed so that pores form within the stratum corneum before the API is topically applied to improve API flux (Williams, 2003:141).

2.3.8.2.2 Iontophoresis, electroporation and ultrasound

Iontophoresis and electroporation are methods that have been proven valuable for the delivery of transdermal products, especially where the delivery of compounds with large molecular weight or high potency is required (Thomas & Finnin, 2004:700). Iontophoresis involves the electrical driving of charged molecules into the tissue through a small direct electrical current via an API containing electrode that is in contact with the skin. On another location on the body, a

grounding electrode is placed to complete the electrical circuit (Barry, 2001:108). When the electrical current is flowing, the API is repelled from the electrode of similar polarity and is attracted to the charged electrode of opposite polarity. This reaction drives the API molecules into the skin. It is known that small organic compounds like lidocaine have been successfully delivered through iontophoresis (Williams, 2003:145). Advantages of iontophoresis include the delivery of larger API amounts than with passive delivery systems, the delivery of larger API amounts of molecules with large molecular weight and better control over delivery profiles (Sarpotdar, 1993:240).

Electroporation forms transient aqueous pores in the stratum corneum lipid bilayers through high-voltage short-duration pulses (Barry, 2001:109; Naik *et al.*, 2000:324). These aqueous pores form pathways for APIs to penetrate the stratum corneum and move straight across the barrier. During the high voltage pulses of 100 – 1000 V, the API travels through means of iontophoresis or electro osmosis (Barry, 2001:109). Ultrasound is another method of enhancing API delivery. By applying ultrasound as low frequency sonophoresis transdermally, the structure and lipid packaging in the stratum corneum get interrupted by cavitation. The cavitation can increase free volume space that allows enhanced API penetration into tissue (Barry, 2001:108).

2.3.8.2.3 Penetration enhancement through radio-wave energy

Recently radio-wave energy has been used to cause thermal ablation of the stratum corneum through radio frequencies. The radio frequency energy forms micro-channels across the stratum corneum by microablating skin cells. This technique enhances the delivery of lipophilic APIs across the stratum corneum (Thomas & Finnin, 2004:700).

2.3.8.2.4 Combination strategies

The use of a combination of penetration enhancers has also been researched. Combinations include iontophoresis with chemical enhancers like terpenes, fatty acids and propylene glycol. The iontophoresis-propylene glycol combination increase API flux up to 30 times. Vasoactive chemicals applied with iontophoresis increases the API concentration at the localised site as seen with lidocaine and a vasoconstrictor agent applied together with iontophoresis (Williams, 2003:156, 157). Electroporation is also applied in combination with chemical enhancers. The main reason for this is to create an enhancement effect alongside the created pores, as well as to help expand the pores. Sodium thiosulphate is seen as a pathway-enlarging agent that dramatically increases API flux, especially of macromolecules (Williams, 2003:158). Electroporation can be used in combination with iontophoresis where iontophoresis drives the API molecules into the porous pathways created by electroporation. This combination of

iontophoresis and electroporation shows significance in the delivery of peptides like alpha-lipoic acid and macromolecules like vasopressin and calcitonin (Williams, 2003:158).

2.3.8.3 API delivery systems

Table 2.5: Summary and classification of API delivery systems (Purdon *et al.*, 2004:100; Williams, 2003:125-138)

CLASS	EXAMPLE	STRUCTURE	MECHANISMS
Lipid based delivery systems	Liposomes	Microscopic spherical vesicles formed by the hydration of lipids (phospholipids)	Interacts with lipids in the stratum corneum, trap hydrophilic molecules in the aqueous region, incorporate lipophilic molecules in the membrane
	Transfersomes	A phospholipid with a surfactant forms ultra flexible, highly deformable vesicles of 200 – 300 nm (Barry, 2001:104)	Squeezes through the pores of the stratum corneum or polar channels under a hydration gradient
	Ethosomes	Phospholipids that contain a high ethanol concentration, form a soft vesicle	Disrupts intercellular lipid domains to increase permeation, ethanol enhances penetration
	Niosomes	Preparation of non-ionic surfactants into vesicles	Penetration enhancing effect, penetration of vesicle in the outer skin layers, transfollicular penetration
	Pheroid™	Colloidal system with lipid-based micron particle vesicular system, dispersed in an adaptable medium and encapsulated by a porous membrane (Grobler <i>et al.</i> , 2008:289)	Interacts with skin through fatty acid membrane-binding proteins inside the lipid rafts in the cell membrane (Grobler <i>et al.</i> , 2008:299)
Needleless injections	Particles	API particles	Helium gas blasts API particles from two holding membranes inside a canister at a pressure of 20 atm and 500 m/sec into and across the stratum corneum
	Liquids	API or vaccine in liquid formation	Delivery via a spring powered jet-injector
API-vehicle interactions	Microemulsions	Liquid vesicle formed out of oil, surfactant, co-surfactant and aqueous constituents (Kogan & Garti, 2006:371, 382)	API partitioning into the skin is increased through the disruption of the stratum corneum lipids

The use of API delivery vehicles to enhance transdermal delivery has become an important point of study in the transdermal field. The use of lipid based API delivery systems is probably the most studied in the field and authors like Cerv (2004:675-711); Cerv & Blume (1992:226-232); El Maghraby *et al.* (2006:206-208); Grobler *et al.* (2008:283-311); and Touitou *et al.* (2000:403-418), to name a few, have written numerous articles on the subject. Lipid based API delivery systems range from traditional liposomes, transfersomes, ethosomes to niosomes. Other API delivery systems includes the use of needleless-injection methods and API vehicle interactions like microemulsions (Gillet *et al.*, 2011:1; Purdon *et al.*, 2004:100; Williams, 2003:124). Because of the multiple literature articles that exist on this subject, the most important points of API delivery systems is summarised in Table 2.5. Pheroid™ is the API vehicle of choice for this study and will be discussed in more detail in Section 2.3.8.4.

2.3.8.4 The use of Pheroid™ technology as API delivery vehicle for lidocaine and prilocaine

The Pheroid™ delivery system is a patented skin delivery system with enhanced entrapment capabilities. The Pheroid™ system increases the absorption of the active ingredient into the epidermal layers and the dermis. Enhanced absorption produces increased transport and delivery of the active ingredient and a faster time of onset. With Pheroid™ it is possible to deliver the minimal therapeutic concentration to the target site with reduced chances of toxicity. Pheroid™ technology is based on the previous Emzaloid™ technology and provides skin-friendly means of API delivery through the skin with applications in the pharmaceutical and cosmeceutical industries (Grobler *et al.*, 2008:284).

2.3.8.4.1 Structure

The Pheroid™ delivery system is described as a colloidal system. This colloidal system is formed by the uniform distribution of stable lipid-based submicron- and micron-sized structures in an adaptable dispersion medium (Grobler *et al.*, 2008:284, 285). Pheroid™ is formulated with a diameter range between 200 nm and 20 µm and consists mainly of fatty acids. Ethylated and pegylated polyunsaturated fatty acids are used as main ingredients with the inclusions of omega-3- and omega-6 fatty acids. Arachidonic acid, however, is not included in the formulation. The fatty acids are formulated in the special *cis*-formation so that it can be compatible with the fatty acids found in the human body (Grobler *et al.*, 2008:285). Pheroid™ is formed through a self-assembly process and contains lipid bilayers without cholesterol or phospholipids. Pheroid™ contains an oil and water phase, as well as a dispersed gas phase with the unique inclusion of nitrous oxide (N₂O). N₂O contributes to the miscibility of the fatty acids in their dispersal medium, the self-assembly process and to the stability of the Pheroid™ (Grobler *et al.*, 2008:288, 289). The N₂O gas is able to move freely through the skin layers

because of its water- and fat soluble characteristics. It thrives in lipid-rich membranes and dramatically increases the fluidity of cell membranes (Grobler *et al.*, 2008:290).

The sizes for the different Pheroid™ are usually pre-determined by the specific fatty acid, used as well as their ratios, saturation states, modification states and manufacturing procedures. The simplest Pheroid™ vesicles are those containing ethylated linolenic acid, ethylated linoleic acid and pegylated ricinoleic acid. These vesicles have a very ordered structure with their radii in multiples of approximately 28 nm. The molecular stacking of the fatty acids is determined by the molecule with the largest molecular radius (Grobler *et al.*, 2008:291). When the composition and the ratio of the fatty acids are changed, it is possible to manipulate the mean particle size repeatedly. Adaption of the surface charge of the Pheroid™ is possible through the degree of hydrogenation of the fatty acids (Grobler *et al.*, 2008:292).

One of the aspects that make Pheroid™ so versatile is the fact that it can be structurally and functionally manipulated by the following:

- ❖ Alteration of fatty acid composition or concentration.
- ❖ Inclusion of non-fatty acids or phospholipids like cholesterol.
- ❖ Inclusion of cryoprotectants.
- ❖ Inclusion of charge-inducing agents.
- ❖ Altering the preparation method.
- ❖ Alteration of the character and concentration of the active compound.
- ❖ Adding it to sunscreen formulations (Grobler *et al.*, 2008:292).

The topical formulations containing Pheroid™ at present also have the inclusion of a tocopherol or vitamin E molecule. This inclusion is useful because of its anti-oxidant and membrane stabilising effects (Grobler *et al.*, 2008:293).

2.3.8.4.2 Pliability

The extremely elastic Pheroid™ structures form due to the use of N₂O gas and the addition of pliable pegylated tails to the fatty acids. Pegylation sterically stabilises the Pheroid™ so that they can maintain their interior spaces. The Pheroid™ does not shatter under pressure or extravasation. The addition of polyethylene glycol has positive effects on the Pheroid™ such as increased bioavailability and API stability, extended circulating life, enhanced API solubility and lower toxicity (Grobler *et al.*, 2008:294).

2.3.8.4.3 Entrapment efficiency

Entrapment efficiency is the percentage of the initial compound added to the entrapped formulation. For Pheroid™ products the objective entrapment efficiency has been set at 90%. Confocal laser scanning microscopy (CLSM) is used to determine the entrapment efficiency of Pheroid™ and is visualised through fluorescence labelling. The amount of colloidal particles per volume can be changed to fit the required concentration of the active compound. The following factors influence the internal entrapment volume:

- ❖ Size of the vesicles.
- ❖ Character and concentration of the fatty acids.
- ❖ Character and concentration of the active compound.
- ❖ Hydration medium.

A number of molecules of the active ingredient can be entrapped in one Pheroid™ which is determined by the size, charge and solubility of the active compound (Grobler *et al.*, 2008:294). 150 Pheroid™/μl of formulation is the average amount of Pheroid™ contained in its simplest formulation (Grobler *et al.*, 2008:295).

2.3.8.4.4 Penetration efficiency

Penetration efficiency is influenced by various factors like molecular weight, water solubility, melting point and oil/water partition coefficient. Comparative investigation is the easiest way to measure penetration efficiency. The enhancement of the carrier is determined by comparing it to an already existing commercial product (Grobler *et al.*, 2008:296). CLSM can be used to determine the amount of fluorescent active compound that has penetrated through the skin at a specific depth. The compound can also be pre-labelled with a fluorescent marker to help determination (Grobler *et al.*, 2008:297).

2.3.8.4.5 Cellular uptake

Pheroid™ are stabilised by electro-chemical interaction that allows Pheroid™ to have an extremely elastic and vesicular structure. Pheroid™ cross capillary walls and the fluidity of the Pheroid™ membrane contributes to efficient transdermal delivery of an active compound through the stratum corneum and into the dermis (Grobler *et al.*, 2008:297). Cellular uptake is facilitated by fatty acid membrane binding proteins inside the lipid rafts of the cell (Grobler *et al.*, 2008:299).

Cellular uptake of Pheroid™ can be influenced by two factors, the formulation and the mechanism of uptake by the cells. Pheroid™ permeation can be influenced by the following factors:

- ❖ Pheroid™ size
- ❖ Pheroid™ morphology
- ❖ Molecular geometry of the fatty acids
- ❖ Fatty acid concentration and ratio
- ❖ Hydration medium
- ❖ Preparation pH
- ❖ Occurrence of charge-changing molecules
- ❖ Occurrence of molecules that influence the electrostatic milieu
- ❖ Pheroid™ state (gel state or fluid state)
- ❖ API character and concentration (Grobler *et al.*, 2008:297, 299).

A previous comparative study between a lidocaine and prilocaine Pheroid™ formulation and market product EMLA® cream has shown findings that the formulation with Pheroid™ delivered a much higher concentration and reduced lag time of the active ingredient than EMLA® (Kruger, 2008:56).

2.4 SUMMARY

Transdermal API delivery is a non-invasive method of API delivery that can be of great importance when administering local anaesthesia. The transdermal delivery of lidocaine and prilocaine will ensure better patient compliance as the application will be painless and uncomplicated. The long time of onset of transdermally applied local anaesthesia proposes a problem. The reason for this is the excellent barrier function of the skin managed by the stratum corneum. The stratum corneum's main purpose is to keep foreign substances from penetrating the skin, entering the systemic circulation and cause possible harm to the human body. This barrier function of the skin is an enormous challenge for the formulator to overcome.

Lidocaine HCl and prilocaine HCl fall in range of parameters set for transdermal delivery like melting point, molecular weight and log P value. After considering the properties of lidocaine HCl and prilocaine HCl it has been determined that these substances will most likely traverse the stratum corneum through the intercellular route. This route is the most tortuous route across

the skin barrier and it takes a long time to reach the blood vessels situated in the dermis. Different methods of penetration enhancement have been considered for this study and it has been decided that the Pheroid™ API delivery system could pose as a possible solution with a positive outcome. The Pheroid™ system uses its excellent entrapment capabilities to provide the best possible enhancing effect for its therapeutic substance and improves lag time and the toxicity profile of the chosen therapeutic substance.

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CHAPTER 3

ARTICLE FOR PUBLISHING IN THE INTERNATIONAL JOURNAL OF PHARMACEUTICS

Chapter 3 is written in article format in US English for publication in The International Journal of Pharmaceutics. The complete author's guide is given in Appendix E.

**Formulation and topical delivery of lidocaine and prilocaine with the use of Pheroid™
technology**

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Abstract

Background: Topical local anesthetics provide a non-invasive manner of application. An existing commercial topical anesthetic product contains a eutectic mixture of the local anesthetics lidocaine hydrochloride (HCl) and prilocaine hydrochloride (HCl). This commercial product takes an hour to produce effective anesthesia. **Objective:** The aim of this study was to conduct transdermal diffusion studies on three formulated topical products and a commercial product containing 2.5% (m/v) lidocaine HCl and 2.5% (m/v) prilocaine HCl in order to determine if the formulated products provided better lag times than that of the commercial product. One of the three products was formulated with Pheroid™ technology.

Methods: Franz cell type diffusion studies were performed over a 12 hour period on the three topical formulations, a commercial product and two solutions of which one was prepared with the active pharmaceutical ingredients (API) entrapped in Pheroid™ vesicles 8 h prior to the experiment. Tape stripping was performed after each diffusion study.

Results: The Pheroid™ formulation produced competitive lag times but did not increase dermal penetration when compared with the commercial product. The Pheroid™ solution showed increased dermal concentrations of the APIs. Six month stability testing showed that the three formulated products were not stable for the testing period.

Keywords: lidocaine HCl, prilocaine HCl, topical anesthesia, diffusion, lag time, Pheroid™

1 INTRODUCTION

Lidocaine and prilocaine are two amide type local anesthetics that, when used in combination form a binary eutectic mixture and exert a strong local anesthetic effect (Sweetman, 2002). A commercial product containing these two local anesthetics in a topical formulation is used for minor surgical procedures in pediatrics, dermatology, reconstructive surgery and medical procedures like laryngoscopy, oesophagoscopy or urethroscopy (Franchi et al., 2008). Topical anesthesia provides a method of anesthesia that is non-invasive and painless. This characteristic can be extremely helpful especially with pediatric patients or patients that have needle phobias (DeCou et al., 1999).

When a topical mixture of lidocaine and prilocaine is applied to the mucous membranes, the onset of anesthesia is rapid (5 – 10 min). When the same mixture is applied to intact skin the reaction time is much longer. The absorption of lidocaine and prilocaine into the skin is slow and it takes up to 60 min for the anesthetic effect to commence (DeCou et al., 1999; Franchi et al., 2008). The main contributor to this slow uptake of anesthesia is the excellent barrier function of the stratum corneum layer of the skin. The stratum corneum is the body's first line of defense against noxious stimuli (Barry, 2001). A topical anesthetic must traverse the stratum corneum and upper skin layers successfully in order to reach the nerve endings of the nociceptors situated in the viable dermis of the skin to produce an anesthetic effect (Welin-Berger et al., 2002).

The physicochemical properties of the active pharmaceutical ingredients (API) traversing the skin play an important role. Parameters like solubility, lipophilicity, molecular size, melting point and octanol/water partition coefficient (log P) can give a good indication on the manner of how the API will cross the skin (Welin-Berger et al., 2002). The ideal melting point for an API to cross the skin is described by Niak et al. (2000) as a melting point below 200 °C. A log P between 1 and 3 will provide an API with reasonable partitioning characteristics into the lipid skin layers (Williams, 2003). When examining the physicochemical properties of lidocaine hydrochloride (HCl) and prilocaine HCl, the results indicate that these two

compounds are ideally suited for transdermal delivery. Both lidocaine HCl and prilocaine HCl have low molecular masses of 288.82 g/mol and 220.3 g/mol, respectively (BP, 2011). The combination of these two APIs forms a binary eutectic mixture with a melting point lower than either API separately, making it an ideal combination for transdermal delivery (Sweetman, 2002). Lidocaine HCl has a log P value of 3.4 (Lund, 1994:938) and prilocaine HCl a log P of 2.1 (Analysis of Drugs and Poisons, 2010) which falls within range given by Williams (2003).

Many investigations into the transdermal delivery of a eutectic mixture of local anesthetics have been done in recent years. Subjects like the effects of eutectic formation on transmembrane transport (Fiala et al., 2010), the depth of anesthesia of a eutectic mixture (Wahlgren and Quiding, 2000; Gupta and Sibbald, 1995), the physicochemical interactions of the local anesthetics (Welin-Berger et al., 2002) and the topical delivery of an eutectic mixture of local anesthetics through mucous membranes (Franchi et al., 2008; Franz-Montan et al., 2010) have been well researched, to name a few.

This study concerned itself with shortening the lag time of the anesthetic ingredients while trying to enhance skin penetration of the anesthetic compounds into the skin dermis. This can be achieved by incorporating a special carrier system, Pheroid™ technology, into the topical formulation. The Pheroid™ delivery system is a submicron emulsion type formulation that has been patented for skin delivery. It consists mainly of fatty acids and has enhanced entrapment capabilities. The Pheroid™ delivery system is able to increase the absorption of the API entrapped in its vesicles into the epidermal and dermal skin layers while increasing the delivery time of the API for a faster time of onset. Pheroid™ can deliver APIs at their minimal therapeutic concentration and decreases the chance of toxicity (Grobler et al., 2008). Various methods to improve the transdermal penetration of lidocaine and prilocaine have been investigated in previous years. These include methods like formulating of lidocaine and prilocaine in a micro-emulsion (Kogan and Garti, 2006), entrapment in liposomes (Franz-Montan et al., 2010; Müller et al., 2004) and micelles (Scherlund et al.,

2000), iontophoresis (Brounéus et al., 2000) and heated anesthetic patches (Masud et al., 2010). Kruger (2008) previously investigated the delivery of lidocaine and prilocaine solutions containing Pheroid™ and found that the lag time of these APIs into the skin increased exceptionally.

For this study, three different types of topical products were formulated, one with the inclusion of Pheroid™ technology. Franz cell skin diffusion studies were performed to determine the release of lidocaine HCl and prilocaine HCl from these topical formulations into the skin dermis. The three formulations were prepared in bulk and placed in a stability program for six months following ICH Triplicate Guidelines (2003). Concentration assay, pH, mass, viscosity, particle size, zeta potential and visual appearance of each formulation at each temperature was noted at month 0, 1, 2, 3 and 6 to determine if they were stable for this time period.

2 MATERIALS AND METHODS

2.1 Chemical materials used in the formulation and analysis of topical products containing lidocaine HCl and prilocaine HCl as active ingredients

Lidocaine HCl was obtained from Konduskar Laboratories (India) and prilocaine HCl was obtained from DB Fine Chemicals (South Africa). Ingredients used in the formulation of the topical semi-solid products were propylene glycol diacetate and liquid paraffin, both obtained from Merck Laboratory Supplies (South Africa), Pluronic obtained from BASF (South Africa) and ethanol obtained from Rochelle Chemicals (South Africa). The water used during the preparation of all formulations and experiments was purified by a Milli-Q® water purification system from Millipore (United States of America). The phosphate buffer solution was prepared with potassium orthophosphate crystals and sodium hydroxide pearls obtained from Merck Laboratory Supplies (South Africa). During the high performance liquid chromatography (HPLC) analysis of the samples HPLC analytical grade acetonitrile (CH₃CN) supplied by Merck Laboratory Supplies (South Africa) and triethylamine (N(CH₂CH₃)₃) supplied by Sigma-Aldrich (Germany) were used. Pheroid™ vesicles were

prepared by the Unit for Drug Research and Development at the North-West University, South Africa.

2.2 Preparation of the phosphate buffer solution at a pH of 7.4

Phosphate buffer solution (PBS) was prepared by weighing and dissolving potassium orthophosphate crystals (6.81 g) in HPLC grade water (250 ml). A solution containing sodium hydroxide pearls (1.573 g) dissolved in HPLC grade water (393.5 ml) was added to the potassium orthophosphate solution. The pH was adjusted to 7.4, in accordance with the pH of the body fluids, with 10% phosphoric acid (H_3PO_4).

2.3 Semi-solid topical formulations containing lidocaine HCl and prilocaine HCl as active ingredients

Lidocaine HCl and prilocaine HCl were incorporated into three basic semi-solid formulations: an emulgel, Pheroid™ emulgel and hydrogel. The final formulations were prepared in bulk and placed in a stability program for six months. The stability program according to ICH triplicate guidelines (2003) was followed. The products were kept at 25 °C/60% RH (relative humidity), 30 °C/60% RH and 40 °C/75% RH in Labcon® humidity chambers (Labcon, United States of America).

2.3.1 Formulation of an emulgel containing lidocaine HCl and prilocaine HCl

Phase A of the formulation contained lidocaine HCl (2.5%) and prilocaine HCl (2.5%) dissolved in HPLC grade water (50%), propylene glycol diacetate (15%) as a thickening agent and alcohol (10%) as preservative. Phase B consisted of liquid paraffin (10%) as the oil phase. Phase A was added to Phase B. The mixture was heated to a regulated temperature of 70 °C while stirring continuously. The emulsifying agent (Pluronic, 10%) was added to the mixture in small proportions while maintaining the temperature at 70 °C and stirring continuously. When the emulsifying agent dissolved into the mixture, the mixture was homogenized at a speed of 13 500 rpm to obtain the full consistency and texture of an emulgel. The mixture was then cooled down in a container with cold water to 25 °C.

Formulation pH was set at 8 with sodium hydroxide (10%) to increase the unionized fractions of the APIs for better skin penetration.

2.3.2 Formulation of a Pheroid™ emulgel containing lidocaine HCl and prilocaine HCl

The same manufacturing method explained in Section 2.3.1 was used for the manufacturing of a Pheroid™ emulgel. The emulgel formula was adjusted to incorporate the ingredients for the Pheroid™ vesicles by the Unit of Drug Research and Development at the North-West University, South Africa.

2.3.3 Formulation of a placebo emulgel and placebo Pheroid™ emulgel as control sets

A placebo emulgel was prepared as described in Section 2.3.1 and a placebo Pheroid™ emulgel was prepared as described in Section 2.3.2 without the addition of the APIs lidocaine HCl and prilocaine HCl. The placebo formulations were prepared to act as control sets for the diffusion studies of the emulgel and Pheroid™ emulgel formulations containing lidocaine HCl and prilocaine HCl.

2.3.4 Formulation of a hydrogel containing lidocaine HCl and prilocaine HCl

Lidocaine HCl (2.5%) and prilocaine HCl (2.5%) were dissolved in HPLC grade water (60%) with the addition of alcohol (10%) as preservative. Propylene glycol diacetate (15%) was added to the API mixture as a thickening agent. The mixture was heated to a regulated temperature of 70 °C. An emulsifier (Pluronic 10%) was added in small proportions to the mixture while maintaining the temperature at 70 °C and stirring continuously. After the emulsifier had dissolved into the mixture it was homogenized at a speed of 13 500 rpm. The mixture was then cooled down in a container with cold water to 25 °C while stirring continuously until a gel state and smooth texture were obtained. Formulation pH was set at 8 with sodium hydroxide (10%) to increase the unionized fractions of the APIs for better skin penetration.

2.3.5 Formulation of a placebo hydrogel as a control set

A placebo hydrogel was prepared with the method described in Section 2.3.4 without the addition of the APIs. The placebo hydrogel was prepared to act as a control for the diffusion study of the hydrogel formulation containing lidocaine HCl and prilocaine HCl.

2.4 Solutions containing lidocaine HCl and prilocaine HCl as active ingredients

2.4.1 Solution containing lidocaine HCl and prilocaine HCl

A 100 ml solution was prepared by weighing and dissolving 2.5 g lidocaine HCl and 2.5 g prilocaine HCl in 60 ml PBS. The pH of the PBS had been adjusted to 8 with 10% phosphoric acid to increase the amount of unionized API fractions ready for skin penetration. After the lidocaine HCl and prilocaine HCl had completely dissolved the solution was filled to 100 ml with PBS (pH 8). A corresponding placebo solution was prepared and consisted of 100 ml PBS (pH 8) to act as a control for the diffusion study of the PBS solution containing lidocaine HCl and prilocaine HCl.

2.4.2 Pheroid™ solution containing lidocaine HCl and prilocaine HCl

The Pheroid™ solution was prepared by the Unit for Drug Research and Development at the North-West University, South Africa. Lidocaine HCl (2.5 g) and prilocaine HCl (2.5 g) were weighed and incorporated into the prepared Pheroid™ solution with a final volume of 100 ml. The Pheroid™ solution was shaken for 24 h and then refrigerated 8 h prior to the experiment to ensure the entrapment of the APIs inside the Pheroid™ vesicles. A corresponding placebo Pheroid™ solution was prepared without the inclusion of the APIs to act as a control for the diffusion study of the Pheroid™ solution containing lidocaine HCl and prilocaine HCl.

2.5 HPLC analysis method to determine the concentrations of lidocaine HCl and prilocaine HCl

A HPLC method was developed and validated for the analysis of lidocaine HCl and prilocaine HCl at the Analytical Technology Laboratory at the North-West University, Potchefstroom Campus, South Africa. An Agilent® 1200 series HPLC system (Agilent Technologies, United States of America) was used for concentration analysis. The system

was equipped with an Agilent® 1200 quaternary pump, auto sampler injection mechanism, vacuum degasser, solvent module and diode array detector. It comes equipped with Chemstation Rev. A.10.02 data acquisition and analysis software. A high performance silica based, reversed phase column (4.6 x 150 mm), with a 5 µm particle size was used for the concentration analysis (Phenomenex® Luna C₁₈ (2), Phenomenex, United states of America). Mobile phase A consisted of 2 ml triethylamine in 1000 ml of HPLC grade water. The pH of mobile phase A was set at 7 with 10% phosphoric acid (H₃PO₄). Mobile phase B consisted of 100% HPLC grade acetonitrile. The APIs were detected at 210 nm at a flow rate of 1 ml/min. The runtime for each sample injection was 6 min with a retention time for prilocaine HCl at 3.2 – 3.4 min and lidocaine HCl at 4.1 – 4.3 min.

2.6 The procedure and preparation for the Franz cell diffusion studies of topical formulations containing the local anesthetic agents lidocaine HCl and prilocaine HCl

2.6.1 The preparation of Caucasian skin for Franz cell diffusion studies

Ethical approval was given by the North-West University Research Ethics Committee under the reference number NWU-00114-11-A4, to obtain female Caucasian abdominal skin for the diffusion studies. The skin was obtained with consent from the anonymous patients who had undergone abdominoplastic surgery. After the skin was obtained it was frozen immediately at -20 °C within 24 h after the surgery. Dermatomed skin with a thickness of 400 µm and 2 cm in width and 4 cm in length was obtained by using a Dermatome™ (Zimmer® LTD, United Kingdom). The strips were placed on Whatman® filter paper and left to dry for 10 min before being cut into circles with a 15 mm diameter. The prepared skin samples was then wrapped in aluminum foil and sealed in plastic bags for storage in a freezer at -20 °C until utilized. The skin circles remain useable for six months, as the barrier function of the skin can stay intact under stable conditions (Leveque et al., 2004). Before the diffusion study the skin samples were thawed and examined to make sure that there were no abnormalities on the skin, like stretch marks, that could impact the permeation results of the study (Baert et al., 2011).

2.6.2 Preparation of the donor and receptor phase for Franz cell diffusion studies

Fresh donor phase formulations were prepared before each of their respective studies. An emulgel, emulgel containing Pheroid™, hydrogel, a PBS solution and a solution containing Pheroid™ were prepared as donor phases as described in Section 2.3. A commercial product containing the same concentration of lidocaine HCl and prilocaine HCl as the formulation was also included as a donor phase for one diffusion study in order to compare its results to that of the other formulations.

PBS (pH 7.4) was used as the receptor phase and freshly prepared before each diffusion study as described in Section 2.2.

2.6.3 Franz cell membrane release experiments with topical products containing lidocaine HCl and prilocaine HCl

Membrane release experiments were performed before the skin diffusion studies were initiated. The aim of these membrane release experiments was to determine if the lidocaine HCl and prilocaine HCl were released from the different topical formulations. The extraction of the receptor phase was done hourly over 6 h to determine the concentration of the APIs released from the formulations. The procedure described in Section 2.6.4 was followed with the exception of polytetrafluoroethylene (PTFE) membranes being used instead of human skin.

2.6.4 Procedure for Franz cell diffusion studies

Twelve 2 ml vertical Franz cells were prepared and used for each diffusion study. Two of the twelve Franz cells were used for controls, containing the placebo formulation as described in Section 2.3. The remaining ten Franz cells contained 1 ml of formulation (pre-heated to external skin temperature of 32 °C) in their donor compartments. The prepared skin circles were mounted onto the receptor compartment. The donor compartment was carefully placed on top of the skin circles before being filled with formulation. Dow Corning® vacuum grease was used to seal the donor and receptor compartments together to prevent any leakage. The two compartments were then further clamped tightly together with a horse

shoe clamp. The donor compartment was covered with a square of Parafilm[®] and capped with a polytop plastic cap. The receptor phase was filled with 2 ml PBS (pre-heated to blood phase temperature of 37 °C). A magnetic stirrer bar was placed into each receptor compartment. The Franz cells were then placed into a Grant[®] water bath at a temperature of 37 °C (normal body temperature) on top of a Variomag[®] magnetic stirrer plate. The PBS of the receptor compartment was withdrawn and replaced with 2 ml fresh heated PBS (37 °C) at intervals of 20; 40, 60, 80, 100 min and 2, 4, 6, 8, 10 and 12 h and analyzed on the HPLC.

2.7 Determination of the stratum corneum epidermis and epidermis-dermis concentrations of lidocaine HCl and prilocaine HCl after a twelve hour Franz cell skin diffusion study

To determine the stratum corneum-epidermis concentrations of the APIs after a 12 h diffusion study, the method of tape stripping was applied. Tape stripping is a technique applied to analyze the amount of the API in the outer skin layers (Pershing et al., 1993). Tape stripping also provides useful information on the efficacy with which the topical formulation delivers the API into the skin layers (Ademola, 1997). After the 12 h diffusion study the Franz cells were dismantled and the donor phase removed from the skin. The skin samples were removed and pinned to circles of Whatman[®] filter paper. The diffusion area of each piece of skin was dabbed dry with a paper towel. Sixteen strips of 3M Scotch[®] Magic[™] tape were cut for each piece of skin. The first tape strip was discarded where after the skin was stripped until the stratum corneum was completely removed and the viable epidermis glistened. The fifteen tape strips were placed in a polytop vial filled with 5 ml PBS (pH 7.4). The polytop vials containing the tape strips were kept at 4 °C overnight to ensure that the APIs dissolved into the PBS. The next morning samples were withdrawn to be analyzed with HPLC.

The concentration of the APIs in the epidermis-dermis were analyzed by cutting the diffusion area of the skin into smaller pieces and placing it inside a polytop vial containing 5 ml PBS

(pH 7.4). The skin was cut into smaller pieces to ensure that the APIs release into the PBS solution. The samples were kept overnight at 4 °C. The next morning samples were extracted and transferred into HPLC vials for analysis with HPLC. The aim of this study was to formulate products containing lidocaine HCl and prilocaine HCl and effectively deliver these APIs into the skin dermis. The skin dermis is the layer containing the nerve receptors topical local anesthetics target (Barry, 2007). Effective penetration of the local anesthetic agent into the skin dermis is an important characteristic a topical local anesthetic preparation must exhibit.

2.8 The statistical analysis of the data of lidocaine HCl and prilocaine HCl obtained from the Franz cell diffusion studies

All the data from the transdermal diffusion studies were statistically analyzed, using quantitative statistical methods. SPSS software (SPSS Inc., 2011) was utilized to perform the analysis. The average and median values of the data were determined. If a big variance between the data is observed, the median value is more accurate as it is not affected by the outliers in the data (Gerber et al., 2008). Parametric tests were fitted to the data where the mean values were used. The median values were still noted to be complete. Tests of normality were performed on the data utilizing the Kolmogorov-Smirnov and Shapiro-Wilk tests. Equal variance was determined by Levene's test and graphically inspected using box-plots. The data was considered as statistically significant where $p < 0.05$. Deviations were not severe; however, robust tests were also performed. Significant differences between the treatments for all measures were revealed through a one way ANOVA test. Post Hoc tests were used to reveal the occurrences of these differences. The Games-Howell test was performed to determine the statistical significant differences between treatments where $p < 0.05$ is statistically significant. Cohen's test was performed to determine the practical significant differences or effect size between treatments where $d > 0.8$ indicated a large practical significance in the data. A linear regression model was fitted to the flux data of each repeat (individual Franz cell) to obtain estimated y-intercepts and gradient (flux) values.

The r^2 values obtained from the linear regression was $r^2 > 0.8$ for all models and statistically significant where $p < 0.05$ for all models. A one-way ANOVA model was performed as mentioned above to obtain further statistical data.

2.9 The six month stability program for topical products containing lidocaine HCl and prilocaine HCl as active ingredients

A stability program according to the ICH triplicate guidelines (2003) was followed. The formulated emulgel, Pheroid™ emulgel and hydrogel products containing lidocaine HCl and prilocaine HCl were stored in airtight containers at 25 °C/60% RH, 30 °C/60% RH and 40 °C/75% RH in Labcon® humidity chambers.

2.9.1 Concentration assay of lidocaine HCl and prilocaine HCl

The concentration of lidocaine HCl and prilocaine HCl in the formulated products were determined at month 0, 1, 2, 3, and 6 with HPLC analysis. Formulation samples were prepared by weighing 1 g of formulation into a 100 ml volumetric flask. The flask was filled to 100 ml with methanol and placed on an ultrasonic bath for 5 min or until all of the formulation had completely dissolved. Samples were prepared in duplicate and transferred to HPLC vials for analysis.

2.9.2 Viscosity determination of the formulated topical products

A Brookfield Model DV – II+ viscometer (Brookfield, United States of America) was used to determine the viscosity of the products kept at 25 °C/60%RH over a six month period. Glass jars containing 125 g of each formulation were tested. A T-F helipath spindle was used to measure the viscosity. 32 readings were measured 10 sec apart by the rotating spindle at 0.3 rpm.

2.9.3 The pH determination of the formulated topical products

The pH of the formulations were measured in triplicate on month 0, 1, 2, 3 and 6 with a Mettler Toledo Seven Multi pH meter with an In Lab 410 NTC electrode 9823 (Mettler Toledo International Inc., United States of America). Calibration of the pH meter was performed before each monthly set of tests.

2.9.4 The mass variation of the topical formulated products

The mass variation or mass loss of the formulations was determined over the six month testing period. This was done by weighing the same container for each separate formulation at month 0, 1, 2, 3 and 6. Each measurement was done in triplicate to ensure accuracy.

2.9.5 The particle size variation of the formulated topical products

Variation in particle size was determined by the Malvern Mastersizer 2000 Hydro SM (Malvern Instruments Ltd., United Kingdom). Measurements were taken at three levels, d(0.1), d(0.5), d(0.9). Samples were prepared in triplicate and measurements were also done in triplicate to ensure accuracy.

2.9.6 The zeta potential measurement of the formulated topical products

The zeta potentials of the formulations were determined over the six month stability testing period. The Malvern Zetasizer Nano (Malvern Instruments Ltd., United Kingdom) was utilized to take these measurements. Samples were prepared by measuring 0.05 g of each formulation into a 20 ml volumetric flask. The volumetric flask was filled up to 20 ml with 0.01 M potassium chloride and shaken well. An almost clear solution had to be obtained. Samples were prepared in triplicate and three readings of each were taken.

2.9.7 Visual assessment of color, odor and texture of the formulated topical products

The formulations were visually inspected and any changes in color, odor or texture were noted over the six month period.

3 RESULTS

3.1 Formulation of semi-solid products and solutions containing lidocaine HCl and prilocaine HCl

A fresh donor phase formulation was prepared and the pH stabilized before each diffusion study as described in Section 2.3 and 2.4. The Pheroid™ emulgel and emulgel were not overly oily with a smooth homogeneous texture. The hydrogel had a light application and

watery feeling. The PBS solution was clear while the Pheroid™ solution was a milkier color and slightly more viscous.

3.2 Franz cell diffusion studies

3.2.1 Lag time determination of lidocaine HCl and prilocaine HCl

Table 1: The lag times of lidocaine HCl and prilocaine HCl in the different formulations. The lag times of lidocaine HCl and prilocaine HCl (Table 1) in the different formulations were determined by plotting the cumulative API concentrations obtained during the twelve hour period against time and extrapolating a straight line from the point where a steady state API flux was obtained. The time before the API reaches a steady-state flux is described as the lag time (Barry, 2007). The lag times of the different products were determined between 2 – 12 h and compared with each other and with that of the commercial product to determine if any of the formulations showed improved lag times.

The emulgel produced the shortest lag time for lidocaine HCl (1.65 h) but a much longer lag time for prilocaine HCl (1.93 h). The Pheroid™ emulgel displayed the shortest lag time for prilocaine HCl (1.41 h) but also the longest lag time for lidocaine HCl (1.99 h). This indicates that the Pheroid™ had an effect on the lag time of prilocaine HCl but not on the lag time of lidocaine HCl if the values are compared with the emulgel formulation without Pheroid™.

The emulgel and Pheroid™ emulgel formulations contained oils (liquid paraffin) that act as occlusives on the skin. The occlusive nature of the formulations prevented water loss from the skin and left the skin fully hydrated which could decrease the time it takes for skin penetration to commence (Barry, 2007:577).

The commercial product produced better lag times for lidocaine HCl and prilocaine HCl (1.70 h for lidocaine HCl; 1.53 h for prilocaine HCl) than the hydrogel formulation and the two solutions. The hydrogel and the two solutions contained higher water content than the three formulations which could increase skin hydration for better diffusion (Hadgraft, 1999). The longer lag times were observed because the APIs had trouble partitioning out of their more hydrophilic vehicles into the lipophilic stratum corneum and were influenced by the time it

took for the stratum corneum to become hydrated (Barry, 2007). The PBS solution reached a steady-state flux sooner than the Pheroid™ solution and produced better lag times as a result (1.76 h for lidocaine HCl and 1.60 h for prilocaine HCl). The commercial product is formulated at a pH of 9 where 90% of the API fractions are unionized and available for skin penetration. The formulated products were formulated at a less skin-irritating pH of 8 where only 52% of the API fractions were unionized. This could influence the lag times of lidocaine HCl and prilocaine HCl from the different formulated products because there were less unionized API fractions available for immediate skin penetration (Surber and Smith, 2000).

3.2.2 The steady state-flux of lidocaine HCl and prilocaine HCl and the average cumulative API concentration after twelve hours

Figure 1: Average cumulative concentration lidocaine HCl **(A)** and prilocaine HCl **(B)** that diffused through the skin as a function of time in the four formulated products and the two solutions.

The cumulative concentrations of lidocaine HCl and prilocaine HCl obtained from the separate 12 h diffusion studies were plotted against time (Figure 1). Both lidocaine HCl and prilocaine HCl showed simple zero-order skin diffusion behavior from all the products. Lidocaine HCl and prilocaine HCl reached a steady-state flux state after 2 h. The flux was determined by calculating the slope of the straight line between 2 – 12 h. The results obtained for lidocaine HCl indicated that the commercial product had the highest average flux value (108.98 $\mu\text{g}/\text{cm}^2\cdot\text{h}$) followed by the PBS solution (68.95 $\mu\text{g}/\text{cm}^2\cdot\text{h}$), Pheroid™ solution (54.14 $\mu\text{g}/\text{cm}^2\cdot\text{h}$), hydrogel (29.47 $\mu\text{g}/\text{cm}^2\cdot\text{h}$), emulgel (25.83 $\mu\text{g}/\text{cm}^2\cdot\text{h}$) and Pheroid™ emulgel (16.04 $\mu\text{g}/\text{cm}^2\cdot\text{h}$). The flux values obtained for prilocaine HCl from the different formulations indicated that the hydrogel obtained the highest average prilocaine HCl flux (96.49 $\mu\text{g}/\text{cm}^2\cdot\text{h}$) followed by the commercial product (60.96 $\mu\text{g}/\text{cm}^2\cdot\text{h}$), PBS solution (53.60 $\mu\text{g}/\text{cm}^2\cdot\text{h}$), Pheroid™ solution (44.75 $\mu\text{g}/\text{cm}^2\cdot\text{h}$), emulgel (33.29 $\mu\text{g}/\text{cm}^2\cdot\text{h}$) and Pheroid™ emulgel (30.69 $\mu\text{g}/\text{cm}^2\cdot\text{h}$).

The commercial product yielded the highest average concentration of lidocaine HCl that diffused through the skin after 12 h ($651.20 \mu\text{g}/\text{cm}^2$) as a result of the high lidocaine HCl flux followed by the average lidocaine HCl concentrations of the PBS solution ($411.38 \mu\text{g}/\text{cm}^2$), Pheroid™ solution ($263.38 \mu\text{g}/\text{cm}^2$), hydrogel ($173.86 \mu\text{g}/\text{cm}^2$), emulgel ($158.53 \mu\text{g}/\text{cm}^2$) and Pheroid™ emulgel ($92.37 \mu\text{g}/\text{cm}^2$). The Pheroid™ emulgel yielded only 14% of the amount of lidocaine HCl that diffused through the skin from the commercial product after 12 h. The highest average concentration of prilocaine HCl was delivered by the hydrogel ($545.25 \mu\text{g}/\text{cm}^2$) followed by the commercial product ($376.74 \mu\text{g}/\text{cm}^2$), PBS solution ($327.99 \mu\text{g}/\text{cm}^2$), Pheroid™ solution ($258.87 \mu\text{g}/\text{cm}^2$), emulgel ($193.72 \mu\text{g}/\text{cm}^2$) and Pheroid™ emulgel ($193.44 \mu\text{g}/\text{cm}^2$).

The average concentration of lidocaine HCl and prilocaine HCl from the different formulations and solutions were related to the average flux values obtained from the different formulations and solutions. The highest average flux and average concentration results for lidocaine HCl were achieved from the commercial product. This was as a result of the large amount of unionized, lipid soluble APIs fractions that were available for skin penetration into the lipid stratum corneum layer of the skin at a high pH (pH 9) (Richards and McConachie, 1995:41). Lidocaine HCl and prilocaine HCl is highly water soluble and has a high affinity for the hydrophilic environments of the PBS solution and the hydrogel formulation. Hydrophilic formulations can increase skin hydration that causes the stratum corneum to swell and soften. This allows easier passage for hydrophilic APIs across the epidermal layers and into the more water soluble viable dermis layers (Barry, 2007; Ghafourian, 2010; Morganti et al., 2001). The emulgel and Pheroid™ emulgel had higher lipophilic content than hydrophilic content and impaired the partitioning of the hydrophilic APIs from their lipophilic vehicles (Surber and Smith, 2000). The lidocaine HCl and prilocaine HCl had better affinity for the lipophilic environment of the Pheroid™ vesicles where a greater amount of water was present, e.g. the Pheroid™ solution and achieved higher flux values and diffused concentrations from the Pheroid™ solution than from the Pheroid™ emulgel.

3.2.3 Concentrations of lidocaine HCl and prilocaine HCl in the stratum corneum-epidermis obtained through utilizing the tape stripping technique

Table 2: The concentrations of lidocaine HCl and prilocaine HCl in the stratum corneum-epidermis and epidermis-dermis after 12 h

The use of the emulgel formulation did not result in any lidocaine HCl or prilocaine HCl concentrations in the stratum corneum-epidermis layer (Table 2). The use of the Pheroid™ emulgel formulation did not produce any prilocaine HCl concentrations in the stratum corneum-epidermis but a small concentration of lidocaine HCl was detected. The hydrophilic APIs did not have as high affinity for the lipophilic surroundings of the stratum corneum-epidermis as a lipophilic API would and did not accumulate in this layer but rather diffused into the more hydrophilic viable dermis (Barry, 2007; Ghafourian et al., 2010). The results obtained with the hydrogel formulation showed a small concentration of lidocaine HCl in the stratum corneum-epidermis and a higher concentration of prilocaine HCl in the stratum corneum-epidermis. The results obtained from the skin diffusion experiment with the commercial product showed the highest concentrations of lidocaine HCl and prilocaine HCl in the stratum corneum-epidermis layer. This may be because the large amount of lipid soluble unionized fractions of the APIs showed affinity to the lipophilic environment of the stratum corneum-epidermis and accumulated in this layer (Surber and Smith, 2000). The use of the PBS solution resulted in a higher concentration amount of lidocaine HCl in the stratum corneum-epidermis than the amount of prilocaine HCl. The use of the Pheroid™ solution led to the second highest stratum corneum-epidermis concentration of lidocaine HCl but a lower prilocaine HCl concentration. This concluded that the Pheroid™ vesicles increased the penetration into the stratum corneum epidermis (Grobler *et al.*, 2008).

3.2.4 The concentrations of lidocaine HCl and prilocaine HCl in the epidermis-dermis after a twelve hour diffusion experiment with the various topical products

The use of the Pheroid™ solution produced the highest epidermis-dermis concentrations of lidocaine HCl and prilocaine HCl. This indicates that the Pheroid™ vesicles encapsulating

the APIs increased their penetration into the skin dermis (Grobler *et al*, 2008). The Pheroid™ solution delivered two times more lidocaine HCl and three times more prilocaine HCl to the epidermis-dermis than the commercial product which could be attributed to the accumulation of the APIs in the stratum corneum-epidermis by the commercial product (Section 3.2.3). The delivery of the local anesthetic agents into the epidermis-dermis is crucial because this is where the free nerve endings, the site of action for local anesthesia, are situated (Welin-Berger *et al.*, 2002). The use of the PBS solution also produced higher epidermis-dermis concentrations of lidocaine HCl and prilocaine HCl than the commercial product. The three other formulated products did not produce high lidocaine HCl and prilocaine HCl concentrations in the epidermis-dermis. The Pheroid™ emulgel which displayed low API flux values and produced low diffused concentrations of APIs also produced low stratum corneum-epidermis and epidermis-dermis API concentrations. The epidermis-dermis concentrations from the Pheroid™ emulgel for lidocaine HCl and prilocaine HCl were 1.16 µg/ml and 2.46 µg/ml, respectively. The emulgel produced higher epidermis-dermis concentrations of lidocaine HCl and prilocaine HCl than the Pheroid™ emulgel. The Pheroid™ emulgel formulation contains more lipophilic substances than the emulgel which may have influenced the manner of partitioning of the hydrophilic APIs from their vehicle into the skin (Surber and Smith, 2000). The hydrogel had a high concentration of APIs that diffused through the skin after 12 h, but only managed to leave small concentrations of lidocaine HCl and prilocaine HCl behind in the epidermis-dermis. The high solubility of the APIs in the hydrogel allowed them to diffuse into the blood capillaries in the dermis layer where they were taken up into the blood circulation (Barry, 2007).

3.2.5 The statistical evaluation of the data of each of the formulations obtained during the diffusion experiments

The mixed model analysis of the different treatments depicted against time for lidocaine HCl and prilocaine HCl concluded that the lag times of the three formulations and two solutions only became statistically significant (where there is no zero in the confidence interval) after 4

h because of the small values in the first 4 h. The commercial product was the only product that provided statistically significant values for lidocaine HCl and prilocaine HCl below 4 h. The lidocaine HCl was statistically significant after 1.33 h and the prilocaine HCl after 1.67 h. This complies with existing data, namely that the commercial product has a time of onset of action only 60 min after application (Friedman et al., 2001).

There were no significant statistical differences between the lidocaine HCl flux values of the emulgel and the hydrogel ($p = 0.696$) and the hydrogel and the Pheroid™ solution ($p = 0.065$). Significant differences between the flux values of lidocaine HCl were revealed between the Pheroid™ emulgel and all the other formulated products and solutions ($p < 0.05$), the commercial product and all the other formulations and solutions ($p < 0.05$) and the PBS solution and all the other formulations and solutions ($p < 0.05$). The statistical analysis validated the conclusion that the lidocaine HCl in the Pheroid™ emulgel had the weakest skin penetration (lowest flux). For prilocaine HCl, no significant statistical differences were indicated between the flux values of prilocaine HCl of the emulgel and the Pheroid™ emulgel ($p = 1$) and the Pheroid™ solution ($p = 0.218$), the Pheroid™ emulgel and the Pheroid™ solution ($p = 0.203$), the commercial product and the PBS solution ($p = 0.599$) and the PBS solution and the Pheroid™ solution ($p = 0.588$). There was significant difference between the flux value of prilocaine HCl from the hydrogel and all the other formulations and solutions where $p < 0.05$.

Figure 2: Box-plots to illustrate the difference in lidocaine HCl concentration in the stratum corneum-epidermis. 1 = Emulgel, 2 = Pheroid™ emulgel, 3 = hydrogel, 4 = commercial product, 5 = PBS solution and 6 = Pheroid™ solution.

No statistical significant differences in lidocaine HCl concentration in the stratum corneum-epidermis were revealed between the emulgel and the hydrogel ($p = 0.339$), the hydrogel and the Pheroid™ emulgel ($p = 0.972$) and the commercial product and the two solutions (PBS solution $p = 0.214$; Pheroid™ solution $p = 0.230$). Practical significant differences in the lidocaine HCl concentration in the stratum corneum-epidermis were present between all

the products where $d > 0.8$, except between the Pheroid™ emulgel and the hydrogel ($d = 0.2$).

Figure 3: Box-plots to illustrate the difference in lidocaine HCl concentration in the epidermis-dermis. 1 = Emulgel, 2 = Pheroid™ emulgel, 3 = hydrogel, 4 = commercial product, 5 = PBS solution and 6 = Pheroid™ solution.

There were statistical significant differences between the epidermis-dermis concentration of lidocaine HCl of the emulgel and all the other formulations as well as the hydrogel and all the other formulations where $p < 0.05$. Practical significant differences were present between all the epidermis-dermis concentration values of lidocaine HCl of the products where $d > 0.8$ except between the commercial product and the PBS solution where $d = 0.1$.

Figure 4: Box-plots to illustrate the difference in prilocaine HCl concentration in the stratum corneum-epidermis. 1 = Emulgel, 2 = Pheroid™ emulgel, 3 = hydrogel, 4 = commercial product, 5 = PBS solution and 6 = Pheroid™ solution.

The emulgel and Pheroid™ emulgel had no prilocaine HCl concentration values in the stratum corneum-epidermis which led these formulations to have significant statistical differences with all the other tested products ($p < 0.05$). Practical significant differences were present where $d > 0.8$ between all the formulations except the Pheroid™ and PBS solution ($d = 0.02$).

Figure 5: Box-plots to illustrate the difference in epidermis-dermis prilocaine HCl concentration. 1 = Emulgel, 2 = Pheroid™ emulgel, 3 = hydrogel, 4 = commercial product, 5 = PBS solution and 6 = Pheroid™ solution.

The concentration of the prilocaine HCl in the epidermis-dermis produced from the use of the Pheroid™ emulgel showed significant difference to the hydrogel ($p = 0.021$), the commercial product ($p = 0.001$), the solution ($p = 0.036$) and the Pheroid™ solution ($p = 0.001$). This concludes that the Pheroid™ emulgel had the weakest penetration into the skin epidermis-dermis of all the formulations. No significant difference between the Pheroid™ emulgel and the emulgel's epidermis-dermis penetration ability ($p = 0.541$) were observed. Practical

significant differences existed between the epidermis-dermis concentration of prilocaine HCl in the different formulations where $d > 0.8$ except between the emulgel and Pheroid™ emulgel ($d = 0.03$).

3.3 Six month stability testing topical products containing lidocaine HCl and prilocaine HCl as active ingredients

3.3.1 Concentration assay of formulated topical products containing lidocaine HCl and prilocaine HCl over a six month period

The lidocaine HCl and prilocaine HCl concentration stayed stable in the emulgel formulation over a three month period at 25 °C/60% RH, 30 °C/60% RH and 40 °C/75% RH. After six months the concentrations of lidocaine HCl and prilocaine HCl decreased significantly.

Lidocaine HCl decreased 14% at 25 °C/60% RH, 6% at 30 °C/60% RH and 6% at 40 °C/75% RH. The prilocaine HCl decreased 21% at 25 °C/60% RH, 12% at 30 °C/60% RH and 14% at 40 °C/75% RH. The lidocaine HCl and prilocaine HCl stayed stable in the Pheroid™ emulgel for two months before their concentrations decreased. After six months the decrease in lidocaine HCl concentration in the Pheroid™ emulgel was 12% at 25 °C/60% RH, 16% at 30 °C/60% RH and 12% at 40 °C/75% RH. Prilocaine HCl concentration in the Pheroid™ emulgel decreased 23% at 25 °C/60% RH 29% at 30 °C/60% RH and 32% at 40 °C/75% RH. The lidocaine HCl stayed stable in the hydrogel formulation for three months. After six months the lidocaine HCl concentration in the hydrogel had decreased 7% at 25 °C/60% RH, 30 °C/60% RH and 40 °C/75% RH. The prilocaine HCl was less stable than the lidocaine HCl in the hydrogel over the stability testing period. After two months the prilocaine HCl concentration in the hydrogel had decreased 7% at 30 °C/60% RH and 40 °C/75% RH. After six months the prilocaine HCl concentrations decreased even more significantly with 19% at 25 °C/60% RH, 22% at 30 °C/60% RH and 21% at 40 °C/75% RH. The emulgel formulation exhibited the best concentration stability results with the lidocaine HCl and prilocaine HCl concentrations staying stable for a three

month period. None of the formulations, however, produced stable API assay concentrations over the six month testing period.

3.3.2 Viscosity of the formulated topical products over a six month period

The viscosities of the three formulations stored at 25 °C/60% RH were measured over a six month period. The emulgel had the biggest decrease in viscosity of 13%. The Pheroid™ emulgel decreased 6.2% in viscosity over six months and the hydrogel decreased 2.7% in viscosity.

3.3.3 Changes in the pH of the formulated topical products noted over a six month period

The pH of all the formulations decreased over time. The high temperatures and humidities had a great effect on all the formulations. The pH of the emulgel decreased 21.3% at 25 °C/60% RH after six months while at the higher temperatures of 30 °C/60% RH and 40 °C/75% RH bigger decreases in pH of 22.7% and 32.5%, respectively, were observed. The Pheroid™ emulgel displayed a similar decrease in pH over six months. After six months the pH decrease of the Pheroid™ emulgel at 25 °C/60% RH was 14.9%, at 30 °C/60 %RH 22.2% and at 40 °C/75% RH 31.5%. The pH of the hydrogel decreased 17% at 25 °C/60% RH after six months and 25% and 32% at 30 °C/60% RH and 40 °C/75% RH, respectively.

3.3.4 Mass variation of the formulated topical products over a six month period

There were no significant changes in the mass of the various formulations at the different temperatures.

3.3.5 Particle size variation of the formulated topical products over six month period

The particle size of the emulgel measured at d(0.5), where 50% of the particles were smaller than this value, stayed stable over the first three months at all temperatures. Only minor changes were noted and the particle size was below the suggested 3 µm parameter given by Barry (2002). After six months the particle size of the emulgel kept at 25 °C/60% RH and 30 °C/60% RH did not change significantly. The emulgel stored at 40 °C/75% RH underwent phase separation after six months and this caused an increase in particle size

where 90% of the particles were smaller than 10 μm . The particle size of the Pheroid™ emulgel varied slightly over the six month period but stayed relatively stable. The Pheroid™ emulgel kept at 40 °C/75% RH did, however, show a large increase in particle size of 12.17 μm from the initial size at d(0.9). This was an indication that flocculation had occurred inside the Pheroid™ emulgel after six months. The particle size of the hydrogel varied extensively over the six month period. Decrease and increase in the size was noted. The initial particle size of the hydrogel was also larger than the emulgel and Pheroid™ emulgel (93.29 μm at d(0.5)). It is possible that crystallization might have occurred in the hydrogel at the different temperatures, producing larger particle size values.

3.3.6 Zeta potential determination of the formulated topical products over a six month period

The zeta potential of the emulgel stayed stable over six months with only a small decrease noted at 30 °C/60% RH and 40 °C/75% RH. The zeta potential of the Pheroid™ emulgel stayed stable over the six month period with the Pheroid™ emulgel kept at 40 °C/75% RH displaying the largest decrease. The emulgel and Pheroid™ emulgel both displayed zeta potential values of 20.7 mV and 21.05 mV initially. An emulsion is considered stable when the zeta potential is bigger than +30 mV or smaller than -30 mV. The emulgel and Pheroid™ emulgel produced zeta potential values too weak to ward off particles of the opposite charge over the six month period. The hydrogel produced an initial high negative zeta potential of -113.1 mV. The zeta potential decreased extensively over the six month period. After six months the zeta potential of the hydrogel at 25 °C/60% RH was -48.8 mV, -55.2 mV at 30 °C/60% RH and -20.8 mV at 40 °C/75% RH.

3.3.7 Changes in the visual appearance of the formulated topical products over six months

No changes in the visual appearance of the emulgel formulation stored at 25 °C/60% RH and 30 °C/60% RH were noted over the six month test period. The emulgel stored at 40 °C/75% RH underwent phase separation after six months. The visual appearance of the

Pheroid™ emulgel changed over the six month period. The Pheroid™ emulgel had an initial milky white color but changed to light yellow at 25 °C/60% RH, darker yellow at 30 °C/60% RH and deep mustard yellow at 40 °C/75% RH after six months. The Pheroid™ is extremely light sensitive and discoloration can indicate that oxidation occurred within the formulation. The hydrogel was a cloudy white color after manufacturing. After six months the hydrogel stored at 25 °C/60% RH showed no change in visual appearance where the hydrogel stored at 30 °C/60% RH had become more transparent. The hydrogel stored at 40 °C/75% RH changed into a clear thick liquid.

4 CONCLUSION

The chemical assay results at each time interval showed that none of the formulations were stable over the six month period. The concentration of lidocaine HCl and prilocaine HCl in the emulgel formulation were the most stable, staying stable for the first three months. No major changes were noted in the mass loss of the formulations. The zeta potential of the emulgel and Pheroid™ emulgel was stable for six months but the zeta potential of the hydrogel was not. The emulgel showed the biggest decrease in viscosity after six months. Changes in the particle size for all the formulations were noted and indicated that flocculation in the two emulgel formulations and crystallization in the hydrogel formulation might have occurred. The visual appearance of all the formulations had all undergone drastic change after six months with the Pheroid™ emulgel displaying visual change at every temperature. The pH of all the formulations decreased more than 20% after six months. The decrease in pH will change the ratio of ionized/unionized fractions of the APIs which leads to decreasing anesthetic action. After six months it was clear that none of the formulations abided by the stability guidelines of the Medicine Control Council of South Africa (2006) or the ICH Triplicate Guidelines (2003).

An ideal topical local anesthetic product is one that applies easily, has a short time of onset and produces a sufficient anesthetic effect. The target site of action for a topical local anesthetic is the receptors of the nerve endings present in the skin epidermis-dermis (Barry,

2007). It would be ideal if a topical anesthetic formulation allowed the local anesthetic agent to cross the skin barrier rapidly and in large concentrations. The emulgel produced the shortest lag time for lidocaine HCl and the Pheroid™ emulgel produced the shortest lag time for prilocaine HCl. The stratum corneum is a lipophilic layer with an affinity for lipophilic API vehicles. The hydrophilic API can achieve maximum skin partitioning from a volatile solvent environment (Surber and Smith 2000). This aspect together with the occlusive nature of the formulations and the enhanced entrapment capabilities of the Pheroid™ vesicles (Grobler et al., 2008) had an effect on the initiation of skin penetration and portrayed shorter lag times. It is however, important to note that considering all the other results, the emulgel may have initiated skin partitioning quicker for lidocaine HCl and the Pheroid™ emulgel for prilocaine HCl, but by much smaller amounts and low steady state fluxes than the other formulations and solutions, indicating that the hydrophilic APIs does not perform optimally from its lipophilic surroundings into the lipophilic skin layer (Surber and Smith, 2000).

Compared to the commercial product, the Pheroid™ emulgel was not able to produce high concentrations of lidocaine HCl and prilocaine HCl that diffused through the skin after 12 h. This can be attributed to the poor flux values lidocaine HCl and prilocaine HCl displayed from the Pheroid™ emulgel formulation. The hydrogel produced long lag times for both lidocaine HCl and prilocaine HCl but higher flux values and average diffused concentration of the APIs after 12 h than the emulgel and Pheroid™ emulgel. This can be attributed to the fact that the hydrogel formulation initially struggled to penetrate the first lipophilic skin layer and first had to produce a hydrating effect on the stratum corneum for better penetration (Ghanfourian, 2010; Morganti et al., 2001). Once this skin hydration state was obtained, the APIs cleared the skin barrier and a large concentration amount of the APIs was released into the more water soluble dermis layers. The concentration of lidocaine HCl and prilocaine HCl that diffused through the skin after 12 h represents the concentrations of the APIs that entered the systemic circulation. The dermis layer of the skin contains a high amount of capillaries and causes APIs to be taken up into the systemic circulation and even more so if

the API is hydrophilic (Barry, 2007). The systemic circulation is not the target site for local anesthetics and if a large amount is absorbed into the systemic circulation it can cause dangerous side effects (Eappen and Datta, 1998). The evaluation of the concentrations of lidocaine HCl and prilocaine HCl delivered to the dermis layer of the skin give a better indication of which formulation was most effective in delivering the APIs to their target site. The Pheroid™ solution produced the highest concentrations of lidocaine HCl and prilocaine HCl in the skin epidermis-dermis. This indicated that the Pheroid™ vesicles increased the penetration of the APIs into the skin (Grobler et al., 2008). The commercial product delivered high concentrations of the APIs into the stratum corneum-epidermis layer but yielded lower concentrations in the dermis because of the high amount of lipid soluble unionized API fractions produced at a high pH, which have a higher affinity for the lipophilic environment of the stratum corneum-epidermis (Surber and Smith, 2000). The emulgel formulated without Pheroid™ yielded higher concentrations of both APIs in the dermis than the Pheroid™ emulgel. The hydrogel showed good skin diffusion of the APIs because of their high solubility state in the hydrogel and continued to diffuse into the capillaries in the papillary dermis, leaving only small concentration amounts of lidocaine HCl and prilocaine HCl behind in the epidermis-dermis.

The commercial product is formulated at pH 9 which produces high amounts of unionized API fractions (90%) that can penetrate the skin for an anesthetic effect. The commercial product had short lag times for both APIs, high flux values and a high average concentration of the APIs that diffused through the skin. Compared to the other 5 products formulated at a pH of 8, which is less skin irritating and more ideal for Pheroid™ technology, the commercial product had a slight advantage. The commercial product did, however, fail to produce high concentrations of lidocaine HCl and prilocaine HCl in the skin epidermis-dermis, the target site for local anesthetics. The penetration capabilities of the Pheroid™ vesicles into the skin layers were superior to the penetration of the APIs from all the other formulations. The Pheroid™ vesicles delivered two times the concentration of lidocaine HCl and three times

the concentration of prilocaine HCl into the skin epidermis-dermis compared to the commercial product. The Pheroid™ emulgel performed poorly compared to the Pheroid™ solution. This was an indication that the incorporation of the Pheroid™ encapsulated APIs into the chosen emulgel formulation was not ideal, as the same penetration enhancement was not noted from the Pheroid™ emulgel as with the Pheroid™ solution. This can be attributed to the greater amount of water that was present in the Pheroid™ solution than the Pheroid™ emulgel. The higher water amount increased the solubilization of the APIs and might have hydrated the skin more than the Pheroid™ emulgel, increasing the API skin penetration (Barry, 2007).

The six month stability testing also indicated that the Pheroid™ emulgel was not stable and oxidation of the Pheroid™ ingredients occurred after the first month (discoloration of the emulgel). The Pheroid™ emulgel did, however, display a shorter lag time than the other formulated products. In conclusion, Pheroid technology™ did enhance the penetration of lidocaine HCl and prilocaine HCl into the skin epidermis-dermis and shortened the lag time of prilocaine HCl. The effect of Pheroid™ technology on other local anesthetic topical formulations (cream, micro-emulsion) should be investigated in the future and formulation at a higher pH (i.e. 9) should also be considered in future studies.

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Table 1: The lag times of lidocaine HCl and prilocaine HCl in the different formulations

	Lag time (h)	
	Lidocaine HCl	Prilocaine HCl
Emulgel	1.65	1.93
Pheroid™ emulgel	1.99	1.41
Hydrogel	1.87	2.11
Commercial product	1.70	1.53
PBS solution	1.76	1.60
Pheroid™ solution	1.84	1.98

Table 2: The concentrations of lidocaine HCl and prilocaine HCl in the stratum corneum-epidermis and epidermis-dermis after 12 h

	Lidocaine HCl			
	Stratum corneum-epidermis		Epidermis-dermis	
	Average	Median	Average	Median
	concentration	concentration	concentration	concentration
	(µg/ml)	(µg/ml)	(µg/ml)	(µg/ml)
Emulgel	0	0	9.35	8.93
Pheroid™ emulgel	2.25	3.04	1.16	1.87
Hydrogel	1.57	1.98	2.91	2.62
Commercial	22.09	16.32	10.93	8.55
PBS solution	6.63	6.46	12.23	10.56
Pheroid™ solution	6.99	7.22	22.64	19.48
	Prilocaine HCl			
	Stratum corneum-epidermis		Epidermis-dermis	
	Average	Median	Average	Median
	concentration	concentration	concentration	concentration
	(µg/ml)	(µg/ml)	(µg/ml)	(µg/ml)
Emulgel	0	0	4.38	3.90
Pheroid™ emulgel	0	0	2.46	2.60
Hydrogel	3.33	4.02	4.00	2.80
Commercial	12.40	11.32	6.34	5.60
PBS solution	4.13	5.14	10.90	8.45
Pheroid™ solution	3.81	3.61	21.23	19.06

FIGURE LEGENDS

Figure 1: Average cumulative concentration lidocaine HCl **(A)** and prilocaine HCl **(B)** that diffused through the skin as a function of time in the four formulated products and the two solutions.

Figure 2: Box-plots to illustrate the difference in lidocaine HCl concentration in the stratum corneum-epidermis. 1 = Emulgel, 2 = Pheroid™ emulgel, 3 = hydrogel, 4 = commercial product, 5 = PBS solution and 6 = Pheroid™ solution.

Figure 3: Box-plots to illustrate the difference in lidocaine HCl concentration in the epidermis-dermis. 1 = Emulgel, 2 = Pheroid™ emulgel, 3 = hydrogel, 4 = commercial product, 5 = PBS solution and 6 = Pheroid™ solution.

Figure 4: Box-plots to illustrate the difference in prilocaine HCl concentration in the stratum corneum-epidermis. 1 = Emulgel, 2 = Pheroid™ emulgel, 3 = hydrogel, 4 = commercial product, 5 = PBS solution and 6 = Pheroid™ solution.

Figure 5: Box-plots to illustrate the difference in prilocaine HCl concentration in the epidermis-dermis. 1 = Emulgel, 2 = Pheroid™ emulgel, 3 = hydrogel, 4 = commercial product, 5 = PBS solution and 6 = Pheroid™ solution.

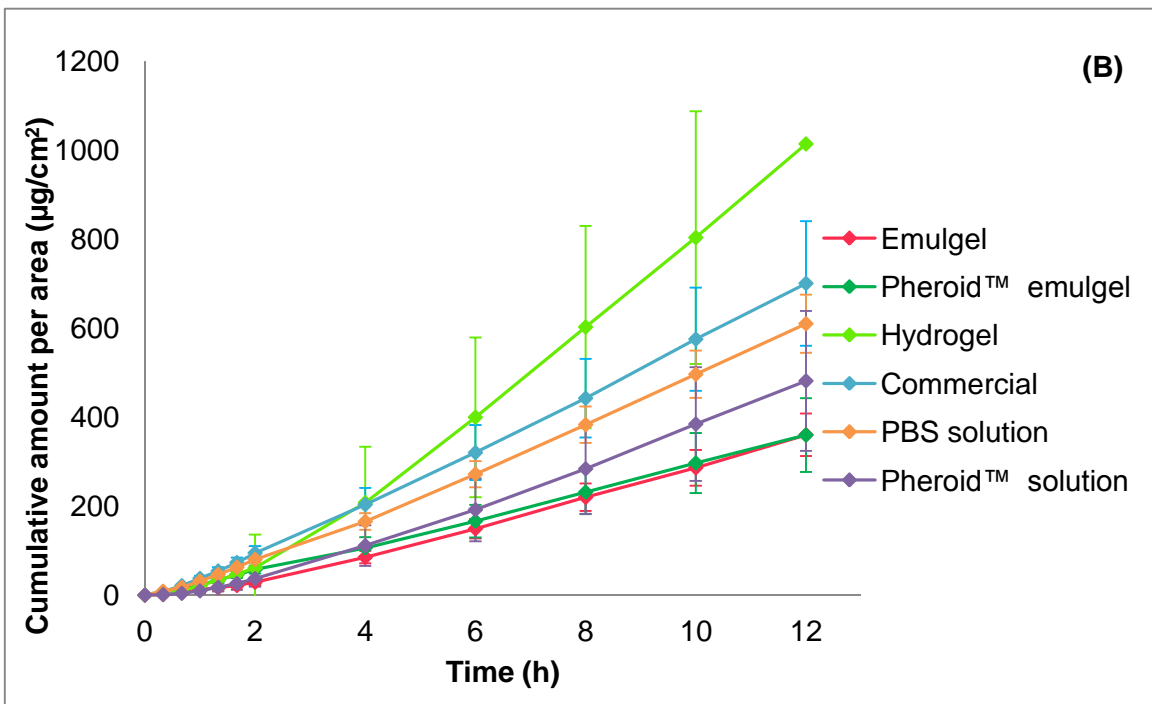
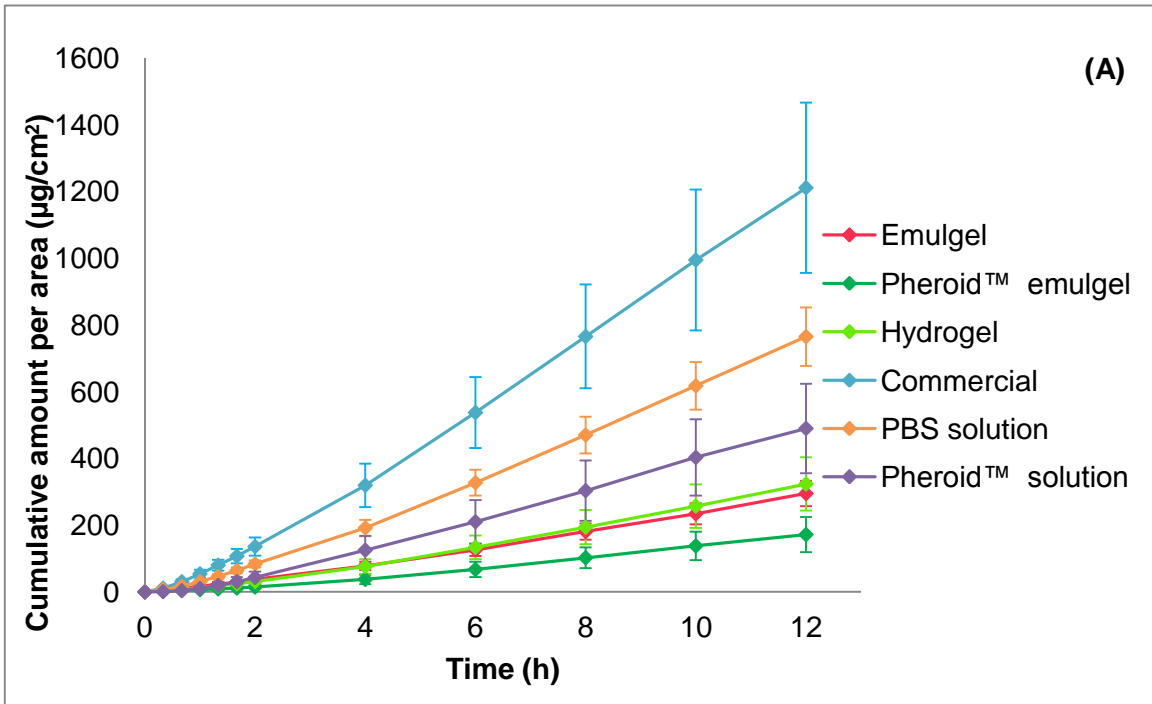


Figure 1: Average cumulative concentration lidocaine HCl **(A)** and prilocaine HCl **(B)** that diffused through the skin as a function of time in the four formulated products and the two solutions

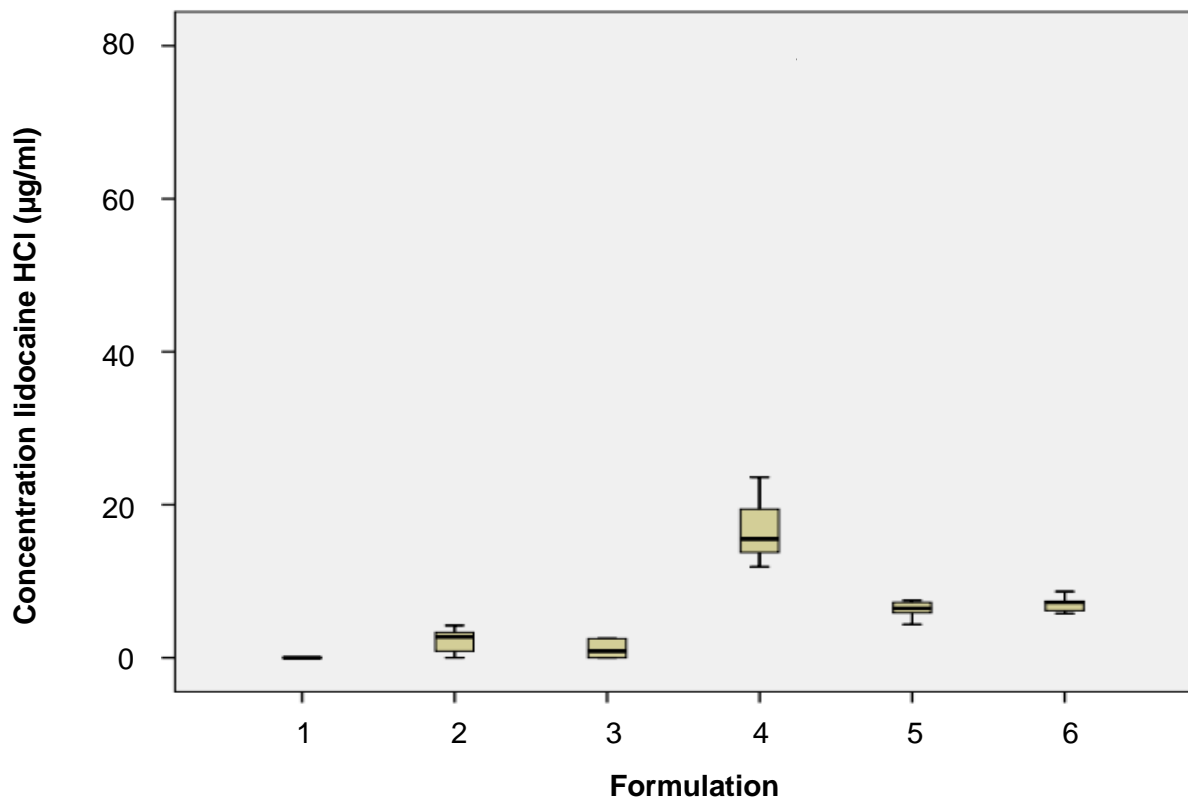


Figure 2: Box-plots to illustrate the difference in lidocaine HCl concentration in the stratum corneum-epidermis. 1 = Emulgel, 2 = Pheroid™ emulgel, 3 = hydrogel, 4 = commercial product, 5 = PBS solution and 6 = Pheroid™ solution.

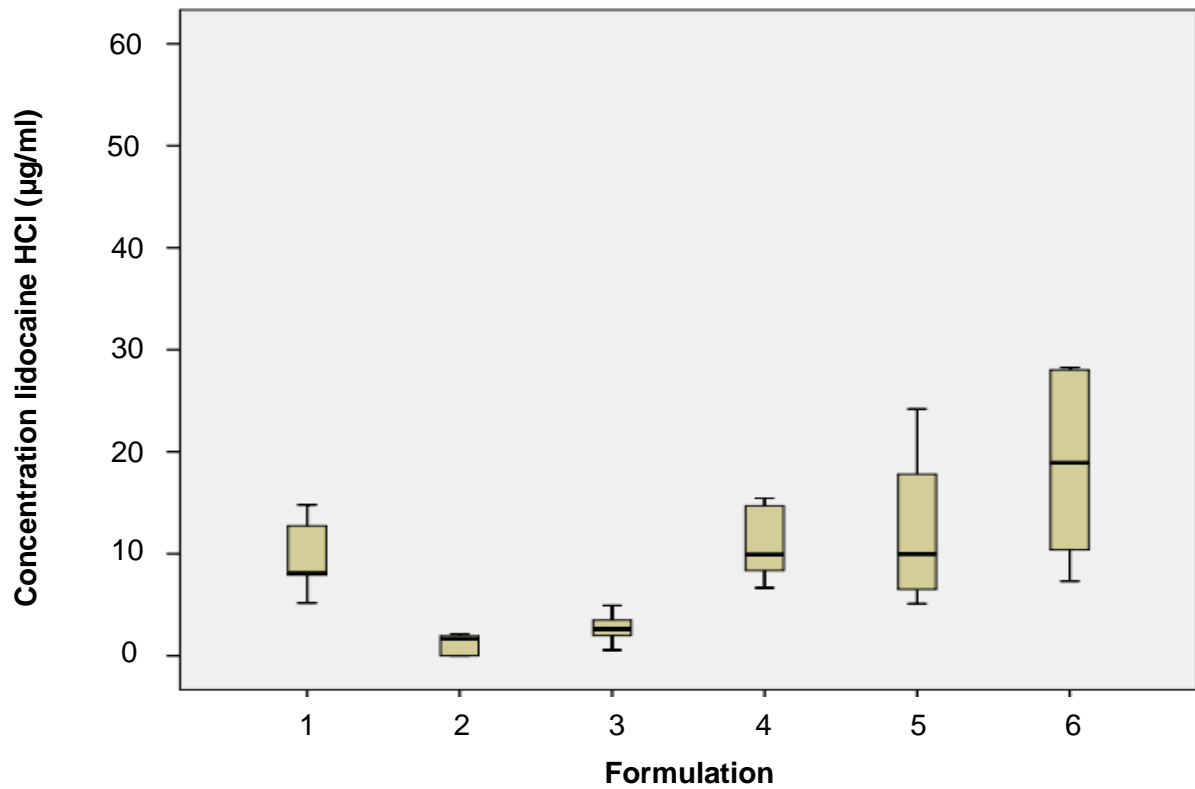


Figure 3: Box-plots to illustrate the difference in lidocaine HCl concentration in the epidermis-dermis. 1 = Emulgel, 2 = Pheroid™ emulgel, 3 = hydrogel, 4 = commercial product, 5 = PBS solution and 6 = Pheroid™ solution.

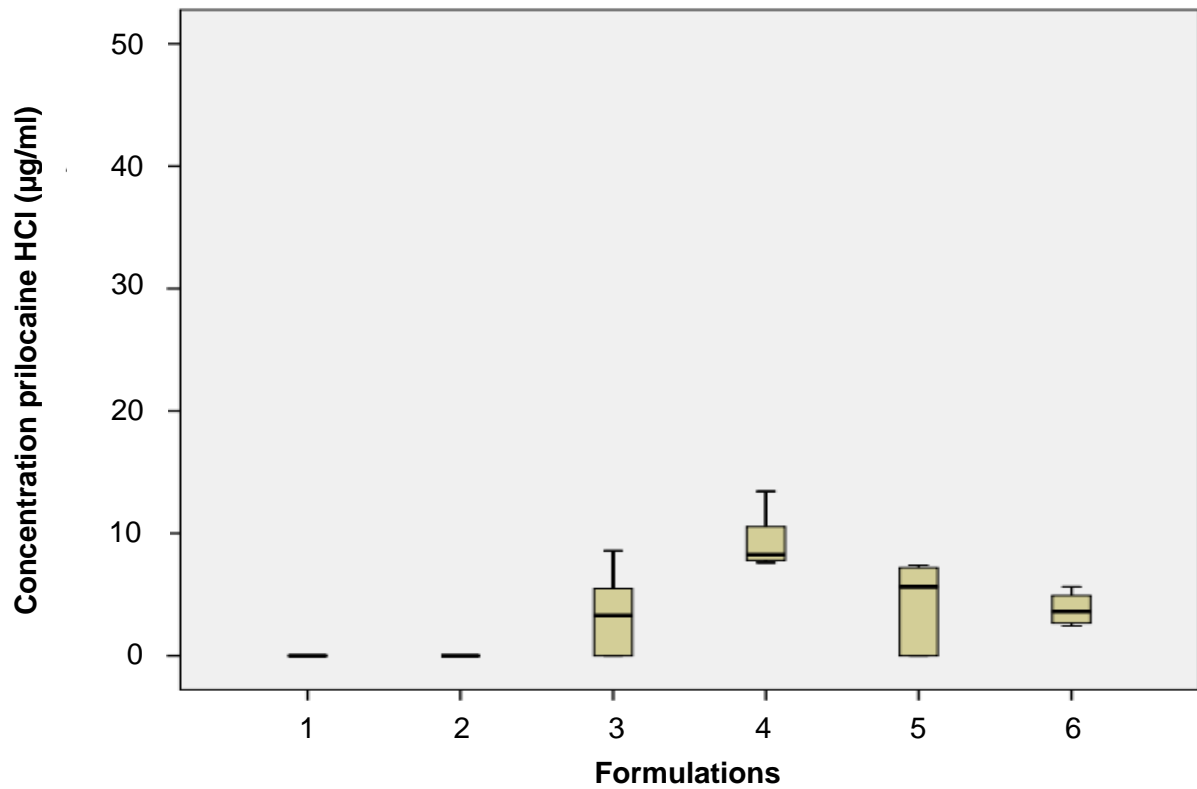


Figure 4: Box-plots to illustrate the difference in prilocaine HCl concentration in the stratum corneum-epidermis. 1 = Emulgel, 2 = Pheroid™ emulgel, 3 = hydrogel, 4 = commercial product, 5 = PBS solution and 6 = Pheroid™ solution.

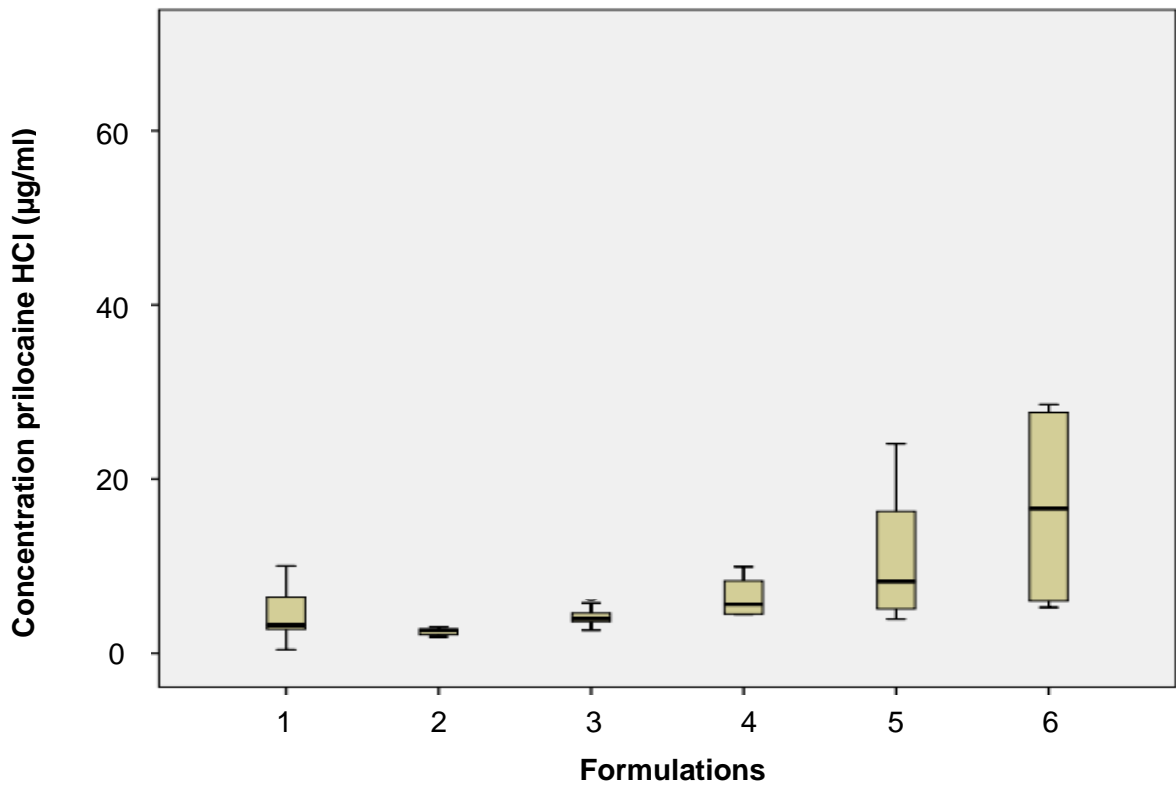


Figure 5: Box-plots to illustrate the difference in prilocaine HCl concentration in the epidermis-dermis. 1 = Emulgel, 2 = Pheroid™ emulgel, 3 = hydrogel, 4 = commercial product, 5 = PBS solution and 6 = Pheroid™ solution.

CHAPTER 4

FINAL CONCLUSION AND FUTURE PROSPECTS

Topical local anaesthetic products provide a painless manner of anaesthesia. An existing commercial product utilises the strong anaesthetic properties of a eutectic binary mixture of the local anaesthetics lidocaine HCl and prilocaine HCl (Sweetman, 2002:1315). The target site for local anaesthetics is the skin dermis where the nerve endings of the nociceptors are located. The local anaesthetic agents must traverse a torturous path through the skin barrier, known as the stratum corneum, and the upper skin layers in order to reach the viable dermis (Welin-Berger *et al.*, 2002:34). The barrier function of the skin is excellent and it can take more than 60 min before the anaesthetic effect initiates (DeCou *et al.*, 1999:946).

The hypothesis of this study was that Pheroid™ technology would shorten the time of onset of lidocaine HCl and prilocaine HCl and that the formulated topical products would permeate transdermally. Pheroid™ technology is an innovative skin delivery system with enhanced entrapment capabilities (Grobler *et al.*, 2008:293). The effect of the inclusion of Pheroid™ in an emulgel formulation and a solution on the skin delivery of lidocaine HCl and prilocaine HCl was investigated. *In vitro* transdermal studies and tape stripping were used to determine the effects of Pheroid™ in a local anaesthetic formulation on skin permeation.

The main aim of this study was to determine whether a specially designed drug delivery system called Pheroid™ shortens the lag time of local anaesthetics lidocaine and prilocaine when incorporated into a semi-solid formulation instead of solutions. The second aim of this study was to determine whether lidocaine and prilocaine with and without the use of Pheroid™ will permeate transdermally, although the target site for drug delivery of lidocaine and prilocaine is the dermis. The following were the objectives of this study:

- ❖ The development and validation of an HPLC method for use of the transdermal and assay analysis of the API.
- ❖ Development of different formulations e.g. emulgel without Pheroid™, emulgel containing Pheroid™, hydrogel not containing any Pheroid™ components.
- ❖ The accelerated stability testing of the formulated lidocaine and prilocaine formulations.

- ❖ Experimentally determining the transdermal permeation of the lidocaine and prilocaine formulations with the use of diffusion studies.
- ❖ Experimentally determining the topical delivery of lidocaine and prilocaine to the target site (dermis) by making use of tape stripping.

The samples obtained during the transdermal diffusion studies and the samples taken during the stability study were analysed with an HPLC method which was successfully developed and validated in a controlled laboratory environment at the North-West University Analytical Laboratory, Potchefstroom Campus, South Africa.

A eutectic mixture of lidocaine HCl and prilocaine HCl was formulated into an emulgel, emulgel containing Pheroid™ and a hydrogel. Emulsions consist of an oil phase and an aqueous phase which allows for optimal dissolution of the hydrophilic APIs in the aqueous phase (Ramchandani & Toddywala, 1997:543). Pheroid™ is a mixture of fats and oils and these ingredients were formulated into the oil phase of the emulgel. Lidocaine HCl and prilocaine HCl were formulated into a hydrogel due to their high solubility in a hydrophilic environment. The hydrogel formulation contained a large amount of water which could increase skin hydration and allow better skin penetration of the hydrophilic APIs (Barry, 2007:576).

The formulated emulgel, Pheroid™ emulgel and hydrogel formulations were placed in a 6 month stability program following ICH triplicate guidelines (2003) and MCC regulations (2006). The formulations were stored at 25 °C/60% RH, 30 °C/60% RH and 40 °C/75% RH. The API concentration, pH, viscosity, zeta potential, particle size, mass and visual appearance of the formulations at the different temperatures were noted initially and at months 1, 2, 3 and 6 to determine if the formulations had remained stable over 6 months. The results obtained indicated that none of the formulations had been stable over a 6 month period. All the formulations were exposed to significant pH and concentration decreases which could lead to a loss of local anaesthetic action. The Pheroid™ ingredients in the emulgel formulation oxidised and caused significant change in the visual appearance of the Pheroid™ emulgel formulation. The emulgel formulation displayed the best stability out of the three products and remained stable for the first three months.

In vitro Franz cell diffusion studies were conducted over a 12 h period. Female Caucasian abdominal skin was obtained from anonymous donors and used as permeation membranes. The Pheroid™ emulgel contained lidocaine HCl (2.5%) and prilocaine HCl (2.5%) entrapped in

Pheroid™ vesicles. The Pheroid™ emulgel was compared to an emulgel without Pheroid™, a hydrogel and a commercial product all containing lidocaine HCl (2.5%) and prilocaine HCl (2.5%). A Pheroid™ solution containing lidocaine HCl (2.5%) and prilocaine HCl (2.5%) was prepared by the Unit for Drug Research and Development at the North-West University and compared to a solution without Pheroid™ and containing the same concentration of the APIs as the Pheroid™ solution. The pH of all the formulations was set at 8 to increase the unionised/ionised API fractions. The commercial product had a pH of 9 and increased unionised API fractions that could penetrate the skin. A pH of 8 is less skin irritating and a more acceptable environment for the Pheroid™ vesicles.

Membrane (PFTE) diffusion studies were conducted on the emulgel, Pheroid™ emulgel, hydrogel and the commercial product before the skin diffusion studies to determine the API release from the different formulations. All the formulations released lidocaine HCl and prilocaine HCl effectively. The hydrogel yielded the highest concentration lidocaine HCl and prilocaine HCl that diffused through the PFTE membranes after 6 h. The Pheroid™ emulgel yielded the lowest diffused concentration of the APIs. The hydrophilic APIs released better into the membranes from the hydrophilic formulation than a more lipophilic formulation.

During the *in vitro* studies conducted on all the formulations, lidocaine HCl and prilocaine HCl displayed simple zero-order skin diffusion behaviour. Both lidocaine HCl and prilocaine HCl reached a steady-state flux after 2 h. The commercial product had the highest lidocaine HCl flux value when compared to the other products. This could be attributed to the high amount of lipid soluble unionised fractions (90%) which were present at the pH of the commercial product (pH 9) (Barry, 2007:576; Richards & McConachie, 1995:41; Surber & Smith, 2000:27). The lidocaine HCl flux from the Pheroid™ emulgel was not higher than the lidocaine HCl flux from the emulgel without Pheroid™ or the hydrogel formulation. This could be attributed to the higher oil content in the Pheroid™ emulgel, compared to the emulgel and hydrogel that impaired the ability of the hydrophilic APIs to partition into the stratum corneum (Thomas & Finnin, 2004:699). The lidocaine HCl flux obtained by the Pheroid™ solution was not higher than the lidocaine HCl flux obtained by the solution without Pheroid™. The highest prilocaine HCl flux was obtained from the hydrogel formulation. The hydrogel contained a large amount of water that altered the skin lipids of the stratum corneum for increased penetration of the hydrophilic APIs. The alteration of the hydration state of this skin had a direct impact on the skin permeability of the APIs (Morganti *et al.*, 2001:492). The Pheroid™ emulgel did not display a higher flux of prilocaine HCl than the emulgel

without Pheroid™ and the Pheroid™ solution did not have a higher prilocaine HCl flux than the solution without Pheroid™. The emulgel and solution without Pheroid™ had lower oil content than the emulgel and solution containing Pheroid™, which might have allowed better partitioning of the hydrophilic APIs out of less lipophilic vehicles into the skin which had been exposed to more hydration (Barry, 2007:576). This concluded that Pheroid™ technology did not increase the flux of neither lidocaine HCl nor prilocaine HCl into the skin. The average flux values for lidocaine HCl and prilocaine HCl did not vary extensively from their median flux values and indicated that only a small amount of outliers were present in the data.

The lag times of lidocaine HCl and prilocaine HCl in the different formulations were determined during the *in vitro* studies. Lidocaine HCl displayed the shortest lag time from the emulgel formulation without Pheroid™ and prilocaine HCl displayed the shortest lag time from the emulgel formulation with Pheroid™. According to Surber & Smith (2000:34) the maximal partitioning of an API is obtained if the API has low affinity for its surroundings in the delivery vehicle and higher affinity for the surroundings of the stratum corneum layer. The emulgel and Pheroid™ emulgel were more lipophilic than the hydrogel formulation and the solutions. This might have initiated skin partitioning rapidly, producing short lag times but if the flux values from the two more lipophilic formulations are taken into consideration it can be concluded that only minimal concentration amounts were able to partition out of the lipophilic vehicles into the lipophilic stratum corneum. Lidocaine HCl and prilocaine HCl both displayed shorter lag times from the formulated products than the commercial product.

The average concentration amount of the APIs that diffused through the skin after 12 h was in accordance with the formulations that displayed the highest flux values for lidocaine HCl and prilocaine HCl. The commercial product had the highest concentration of lidocaine HCl that diffused through the skin. The Pheroid™ emulgel had the lowest diffused concentration of lidocaine HCl. The Pheroid™ emulgel did not have the same skin hydration characteristics as the hydrogel and the hydrophilic APIs might have had difficulties clearing the lipophilic skin barrier (Thomas & Finnin, 2004:699). The hydrogel produced the highest diffused concentration prilocaine HCl. The emulgel with Pheroid™ and the emulgel without Pheroid™ had a similar concentration of diffused prilocaine HCl. The Pheroid™ did not enhance the flux of the APIs into the skin and low concentrations of the APIs diffused through the skin after 12 h as a result.

The target site for local anaesthetics is the skin dermis. The API delivery to this layer of the skin is of the utmost importance (Welin-Berger *et al.*, 2002). The commercial product left a high

concentration of lidocaine HCl and prilocaine HCl in the stratum corneum-epidermis of the skin. The large amount of unionised lipid soluble API fractions available for skin penetration at pH 9 seemed to have an affinity to the lipophilic surroundings in the stratum corneum (Struber & Smith, 2000:27). The highest concentration of lidocaine HCl and prilocaine HCl were delivered to the skin epidermis-dermis from the Pheroid™ solution. There was a distinct difference between the API concentrations delivered to the epidermis-dermis between the solution with Pheroid™ and the solution without Pheroid™. The Pheroid™ vesicles in the Pheroid™ solution enhanced the dermal delivery of both the APIs into the skin. The Pheroid™ solution had higher water content than the Pheroid™ emulgel which hydrated the stratum corneum to a certain beneficial extent. The hydrophilic APIs was encapsulated in the lipophilic Pheroid™ vesicles (volatile surrounding) and partitioned into a slightly hydrated stratum corneum (less volatile surroundings) (Surber & Smith, 2000:34) where the Pheroid™ vesicles carried the hydrophilic APIs into the more water soluble skin layers, the viable dermis, and the target site. The emulgel formulation without Pheroid™ delivered higher concentration amounts of the APIs into the epidermis-dermis than the emulgel formulation containing the Pheroid™ vesicles. This concluded that the chosen emulgel formulation with the inclusion of the Pheroid™ vesicles did not increase the dermal concentrations of the APIs. The Pheroid™ technology did enhance the dermal delivery of lidocaine HCl and prilocaine HCl from the Pheroid™ solution and this indicated that Pheroid™ technology could improve the dermal delivery of the local anaesthetics lidocaine HCl and prilocaine HCl into the skin dermis.

The final conclusion of this study is that Pheroid™ technology did have an effect on the topical delivery of the local anaesthetics lidocaine and prilocaine. Pheroid™ shortened the lag time of prilocaine HCl and the Pheroid™ vesicles in the Pheroid™ solution delivered more than twice the amount of APIs to the epidermis-dermis than the commercial product.

Future prospects for further investigation can include:

- ❖ The development of a stable semi-solid Pheroid™ formulation that enhances the topical delivery of lidocaine HCl and prilocaine HCl into the skin and that improves the lag time of these APIs.
- ❖ Investigating the effect of different pH ranges on the topical delivery of lidocaine HCl and prilocaine HCl in semi-solid formulations with and without Pheroid™ technology.

- ❖ Comparing the topical delivery of lidocaine HCl and prilocaine HCl in formulations containing Pheroid™ with formulations utilising other penetration enhancement strategies (liposomes, micellisation, iontophoresis).
- ❖ Clinical trials can be held to determine the depth of anaesthesia from topical formulations by making use of skin punching.

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APPENDIX A

VALIDATION OF THE HPLC ANALYTICAL METHOD FOR THE ANALYSIS OF LIDOCAINE HCl AND PRILOCAINE HCl

A.1 INTRODUCTION

The validation of a method ensures that the demonstrated analytical method is reliable and sensitive to determine the amount of API that has permeated through the skin. The validation process was done under strict laboratory conditions where a temperature of 25 °C was regulated. A validation method was developed for solutions containing both lidocaine HCl and prilocaine HCl and semi-solid products containing these two ingredients in collaboration.

A.2 VALIDATION OF ACTIVE INGREDIENTS

A.2.1 Chromatographic conditions

A.2.1.1 Analytical instrument

The analysis was done with an Agilent® 1100 Series HPLC system (Agilent Technologies, United States of America). The Agilent® 1100 Series comes fitted with an 1100 pump, autosampler injection mechanism, vacuum degasser, solvent module and diode array detector. It comes equipped with Chemstation Rev. A.10.02 data acquisition and analysis software.

A.2.1.2 Column

A high performance silica based, reversed phase column (4.6 x 150 mm), with a 5 µm particle size was used (Phenomenex® Luna C₁₈ (2), Phenomenex, United States of America).

A.2.1.3 Chromatographic conditions

Milli-Q, double deionised HPLC grade water was used throughout this study (Millipore, United States of America). HPLC grade acetonitrile (Merck Laboratory Supplies, South Africa) and triethylamine (Sigma-Aldrich, Germany) was used in this study. Signal detection was at 210 nm at a flow rate of 1.0 ml/min. The default injection volume was set at 20 µl. The retention times obtained for the two APIs were 4.2 min for lidocaine HCl and 3.3 min for prilocaine HCl. The run time for each sample was set at 6 min.

A.2.1.4 Mobile phase A

A degassed and filtered mixture containing 2 ml of triethylamine in 1000 ml of Milli-Q water was prepared. The pH of the mobile phase was set at 7 using 10% phosphoric acid.

A.2.1.5 Mobile phase B

100% acetonitrile

A.2.1.6 Elution

The elution was carried out isocratically with a mixture of 70% acetonitrile and 30% triethylamine and water solution.

A.2.2 Standard preparation

The standard preparation was prepared by dissolving 10 mg lidocaine HCl and 10 mg prilocaine HCl in PBS pH 8 in a 100 ml volumetric flask. The volumetric flask was filled to the 100 ml mark with PBS. Next, 10 ml of this solution was diluted to 20 ml in a 20 ml volumetric flask. Standard concentrations of 100 µg/ml and 50 µg/ml were prepared. Both standards were analysed with injection volumes of 2.5, 5, 10, 15 and 20 µl to obtain a calibration curve ranging from 6.25 – 100 µg/ml.

A.2.3 Sample preparation

The sample solution was prepared by weighing 10 mg of lidocaine HCl and 10 mg prilocaine HCl into a 100 ml volumetric flask. PBS, pH 8, was used as solvent to fill up to 100 ml. Ten millilitres was extracted and diluted to 20 ml with PBS. Ten millilitres from the second solution was then extracted and diluted to 20 ml with PBS. Concentrations of 100 µg/ml, 50 µg/ml and 2.5 µg/ml were obtained and injected in concentrations of 2.5 µg/ml, 5 µg/ml, 10 µg/ml, 15 µg/ml and 20 µg/ml respectively for each dilution.

A.2.4 Linearity

Linearity is an important method used to obtain test results that are directly proportional to the concentration (amount) of analyte present in the sample. By using linear regression analysis to plot peak area versus concentration, the linearity of lidocaine HCl and prilocaine HCl can be determined. A set of standard solutions were prepared by weighing 50 mg of each analyte and

diluting the solution to various concentrations between 5 – 500 µg/ml. The data produced by the regression can be described by the linear equation of $y = mx + c$ where:

y = peak area

m = slope

x = concentration

c = intercept on the y-axis

The regression coefficient (R^2) can be described as the straight line correlation between x (input) and y (output). The acceptance criterion for R^2 are a value ≥ 0.99 . Both lidocaine HCl and prilocaine HCl produced excellent values of 0.99 for this method. This indicated that the HPLC system was stable. The linearity of lidocaine HCl and prilocaine HCl is noted in Table A.1 and Table A.2. The regression curve of the linearity for lidocaine HCl and prilocaine HCl is shown in Figure A.1 and Figure A.2, respectively.

Table A.1: Linearity of lidocaine HCl

Standard (µg/ml)	Peak area
12.75	887.7
25.50	1764.4
38.25	2638.0
51.00	3501.1
25.50	1787.0
51.00	3539.72
76.50	5260.95
102.00	6966.28
Slope	76.033
y-intercept	0
r^2	0.999

Table A.2: Linearity of prilocaine HCl

Standard (µg/ml)	Peak area
12.81	986.8
25.62	1852.4
38.43	2699.6
51.25	3511.9
25.62	1823.27
51.25	3464.77
76.87	5025.32
102.50	6514.43
Slope	76.543
y-intercept	0
r²	0.998

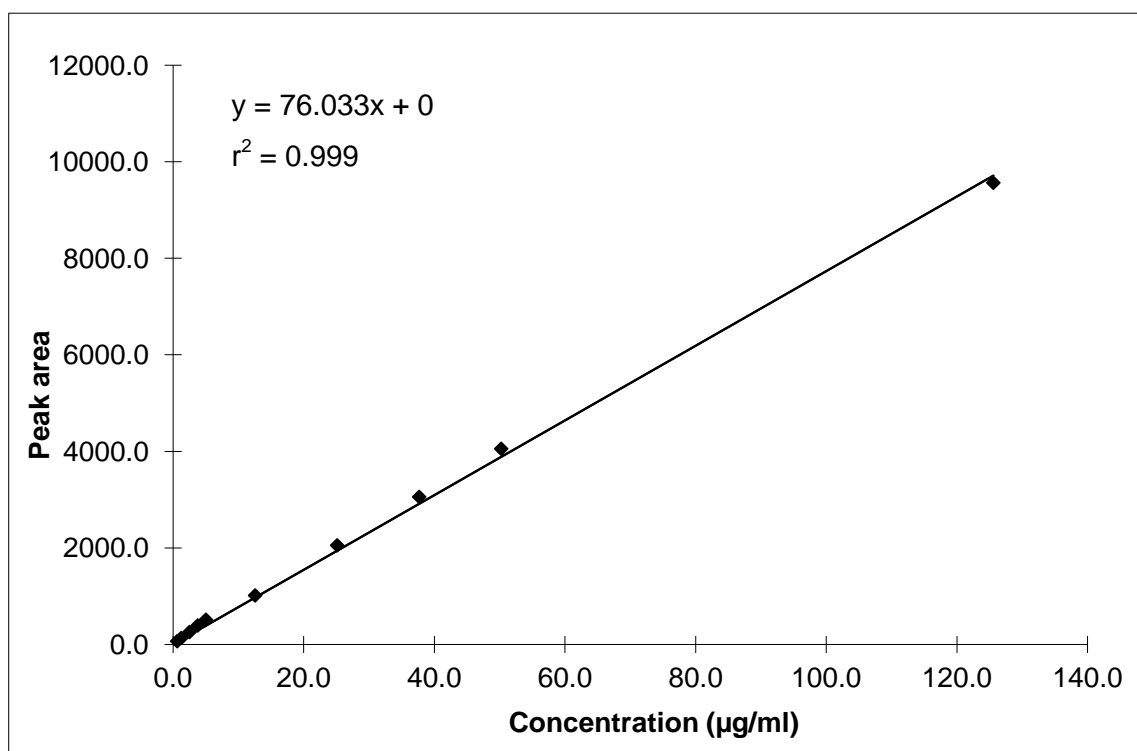


Figure A.1: Linear regression curve of lidocaine HCl

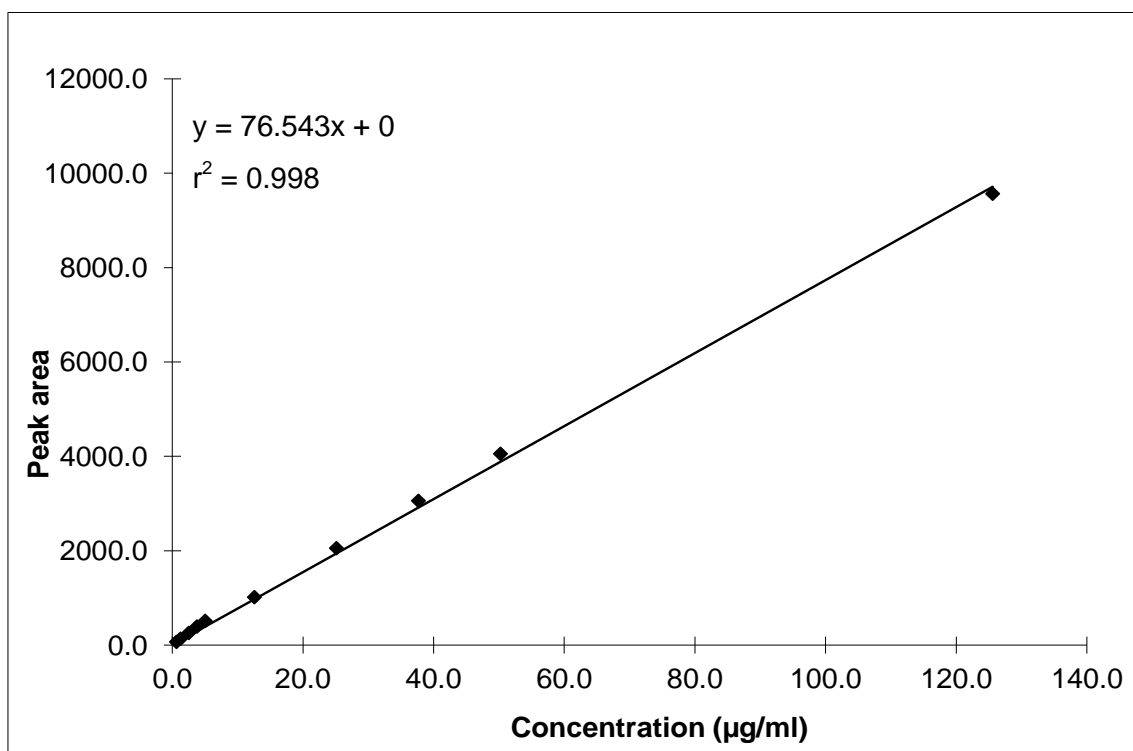


Figure A.2: Linear regression curve of prilocaine HCl

A.2.5 Accuracy and precision

The accuracy of an analytical method is described as the closeness of the experimental value to the true reference value. Accuracy is a crucial procedure in the validation of a method as it can determine how great the influence of systematic errors is on the analytical method being validated (Swartz & Krull, 1997:56). One standard solution and three sample solutions at three different concentrations (n=9) were prepared as described in Section A.2.2 and Section A.2.3. Three samples of each sample solution were carried over into HPLC vials and then injected in duplicate on the HPLC. The results are displayed in Table A.3 and Table A.4 for lidocaine HCl and prilocaine HCl respectively. Accuracy is expressed as percentage (%) recovery with an acceptance criterion of 98 – 102%.

Precision adds to the determination of error of the analytical measurement by expressing the agreeability between a series of measurements of the same homogeneous sample. Method repeatability and reproducibility can be determined this way (Swartz & Krull, 1997:57). A standard solution and a sample solution (50 µg/ml) were prepared as described in A.2.2 and A.2.3. Multiple

injections were made under the prescribed conditions. Precision is divided into intra-day variability (assessment of the same batch on the same day) and inter-day variability.

Table A.3: Accuracy and intra-day precision of lidocaine HCl

Concentration spiked (µg/ml)	Peak area 1	Peak area 2	Mean	Recovery (µg/ml)	%
26.0	1780.2	1783.8	1782	25.6	98.6
26.0	1785.13	1779.48	1782	25.6	98.6
26.0	1782.25	1778.68	1780	25.6	98.5
52.0	3591.38	3589.77	3591	52.2	100.3
52.0	3593.05	3589.811	3591	52.2	100.4
52.0	3589.47	3594.29	3592	52.2	100.4
104.0	7132.76	7137.15	7135	104.2	100.2
104.0	7194.21	7204.725	7199	105.2	101.1
104.0	7192.94	7190.84	7192	105.0	101.0
				Mean	99.9
				SD*	1.0
				% RSD**	1.0

*SD refers to standard deviation

*%RSD refers to relative standard deviation

The average recovery of the lidocaine HCl was 99.9% with an acceptable %RSD of 1.0. USP requires the RSD% value to be 2.0 or less.

Table A.4: Accuracy and intra-day precision of prilocaine HCl

Concentration spiked (µg/ml)	Peak area 1	Peak area 2	Mean	Recovery (µg/ml)	%
25.5	1906.2	1917.9	1912.1	26.4	103.6
25.5	1924.6	1916.2	1920.4	26.6	104.1
25.5	1936.4	1937.7	1937.0	26.8	105.2
51.0	3590.8	3584.7	3587.7	53.5	104.9
51.0	3589.5	3584.2	3586.8	53.5	104.9
51.0	3587.1	3576.3	3581.7	53.4	104.7
102.0	6653.9	6665.4	6659.7	103.2	101.2
102.0	6714.0	6711.4	6712.7	104.1	102.0
102.0	6690.3	6684.3	6687.3	103.6	101.6
				Mean	103.6
				SD*	1.5
				% RSD**	1.4

*SD refers to standard deviation

*%RSD refers to relative standard deviation

The average recovery of the prilocaine HCl was 103.6% with an acceptable %RSD of 1.4. The mean % is higher than the set standard of 98% - 102% but by a small amount with still good repeatability.

A.2.5.1 Inter-day precision

A standard solution (100 µg/ml) and a sample solution (50 µg/ml) were prepared as described in Section A.2.2 and Section A.2.3. Precision was determined by performing HPLC analysis on three samples of the solution over three consecutive days (Table A.5 and Table A.6). Acceptance criterion for inter-day repeatability is listed by the USP as a % RSD ≤ 5%.

Table A.5: Percentage recovery (%) for the inter-day repeatability of lidocaine HCl

	Day 1 (%)	Day 2 (%)	Day 3 (%)	Between days
	100.3	101.3	97.4	
	100.4	101.2	97.2	
	100.4	101.2	97.5	
Mean (%)	100.37	101.23	97.37	99.66
SD*	0.05	0.05	0.12	1.66
%RSD**	0.05	0.05	0.13	1.66

*SD refers to standard deviation

**%RSD refers to relative standard deviation

The % RSD of lidocaine HCl is 1.66 and complies with the USP standard.

Table A.6: Percentage recovery (%) for the inter-day repeatability of prilocaine HCl

	Day 1 (%)	Day 2 (%)	Day 3 (%)	Between days
	104.9	105.1	100.3	
	104.7	105.0	100.9	
	104.7	105.0	102.1	
Mean (%)	104.77	105.03	101.10	103.63
SD*	0.09	0.05	0.75	1.79
%RSD**	0.09	0.04	0.74	1.73

*SD refers to standard deviation

**%RSD refers to relative standard deviation

The % RSD of prilocaine HCl is 1.73 and complies with the USP standard.

A.2.6 Ruggedness

A.2.6.1 Stability of the sample solution

A standard (100 µg/ml) sample was prepared as described in Section A.2.2. The standard sample was injected in hourly intervals for 24 h to determine the sample stability. Both lidocaine HCl (Table A.7) and prilocaine HCl (Table A.8) proved to be stable over a 24 h period.

Table A.7: Sample stability parameters of lidocaine HCl

Time (h)	Peak Area	% Recovery
0	3478.1	100.0
1	3471.8	99.8
2	3470.0	99.8
3	3467.9	99.7
4	3485.0	100.2
5	3482.2	100.1
6	3473.0	99.9
7	3476.0	99.9
8	3483.0	100.1
9	3489.8	100.3
10	3485.7	100.2
11	3485.1	100.2
12	3487.2	100.3
13	3490.8	100.4
14	3486.3	100.2
15	3496.4	100.5
16	3500.8	100.7
17	3505.7	100.8
18	3499.0	100.6
19	3504.1	100.8
20	3513.6	101.0
21	3506.1	100.8
22	3514.4	101.0
23	3497.6	100.6
24	3497.6	100.6
Mean	3489.9	100.1
SD*	13.06	0.21
%RSD*	0.37	0.21

*SD refers to standard deviation

**%RSD refers to relative standard deviation

Table A.8: Sample stability parameters of prilocaine HCl

Time (h)	Peak Area	% Recovery
0	3071.6	100.0
1	3068.6	99.9
2	3068.3	99.9
3	3072.4	100.0
4	3066.2	99.8
5	3068.4	99.9
6	3070.1	100.0
7	3069.2	99.9
8	3072.8	100.0
9	3073.7	100.1
10	3078.6	100.2
11	3067.9	99.9
12	3070.2	100.0
13	3073.5	100.1
14	3074.2	100.1
15	3071.0	100.0
16	3071.1	100.0
17	3069.9	99.9
18	3071.3	100.0
19	3074.4	100.1
20	3070.7	100.0
21	3073.8	100.1
22	3076.7	100.2
23	3067.6	99.9
24	3068.3	99.9
Mean	3071.2	100.0
*SD	2.94	0.10
**%RSD	0.10	0.10

*SD refers to standard deviation

**%RSD refers to relative standard deviation

A.2.6.2 System repeatability

A standard solution (100 µg/ml) was prepared as described in Section A.2.2 and injected repeatedly six times to assess the repeatability of the HPLC method. This was done on the same

day under the same conditions. The system repeatability for lidocaine HCl is displayed in Table A.9 and for prilocaine HCl in Table A.10

Table A.9: System repeatability for the retention time of lidocaine HCl

	Peak area	Retention times (minutes)
	6843.37	4.252
	6848.76	4.249
	6844.99	4.250
	6842.00	4.254
	6853.41	4.251
	6842.69	4.251
Mean	6845.87	4.251
SD*	4.03	0.002
%RSD**	0.06	0.037

*SD refers to standard deviation

**%RSD refers to relative standard deviation

The % RSD produced excellent values of 0.06 for peak area and 0.037 for retention time.

Table A.10 System repeatability for the retention time of prilocaine HCl

	Peak area	Retention times (minutes)
	5903	3.393
	5899	3.388
	5902	3.389
	5911	3.393
	5911	3.390
	5902	3.389
Mean	5904.61	3.390
SD*	4.48	0.002
%RSD**	0.08	0.058

*SD refers to standard deviation

**%RSD refers to relative standard deviation

The % RSD produced excellent values of 0.08 for peak area and 0.058 for retention time.

A.2.7 Specificity

A standard solution (100 µg/ml) was prepared as described in Section A.2.2 and injected in duplicate. One millilitre of the standard solution was transferred into four separate test tubes. One millilitre of a 10% 0.1 M HCl solution was added to the first test tube, 1 ml of 10% 0.1 M NaOH solution to the second and 1 ml of a 10% solution of H₂O₂ to the third test tube. The fourth test tube was filled with 1 ml HPLC grade water. The test tubes were kept overnight and analysed the next day on the HPLC. Both the lidocaine HCl and prilocaine HCl provided unstable peaks after being exposed to these conditions. The sample with the HPLC water provided the most stable peaks. None of the additional peaks interfered with the determination of the actives.

A.3 ASSAY

This method was used for the assay of the ingredients lidocaine HCl and prilocaine HCl in three different semi-solid formulations. Samples of each semi-solid formulation were prepared by carefully weighing 1 g of each formulation into a volumetric flask. The volumetric flask was filled up to a volume of 100 ml with methanol. The sample solution was placed on a sonic bath for 5 minutes to ensure that the formulation is completely dissolved before being placed into HPLC vials. A standard solution was prepared by weighing 25 mg of lidocaine HCl and prilocaine HCl into a 100 ml volumetric flask. The flask was made up to volume with methanol. A standard curve was constructed by injecting 5 µl, 10 µl, 15 µl, 20 µl and 25 µl followed by injecting 5 µl of the semi-solid formulation samples. These samples were analysed in duplicate. The following chromatograms were obtained which showed that the method was stable to use on semi-solid formulations dissolved in methanol (Figure A.3).

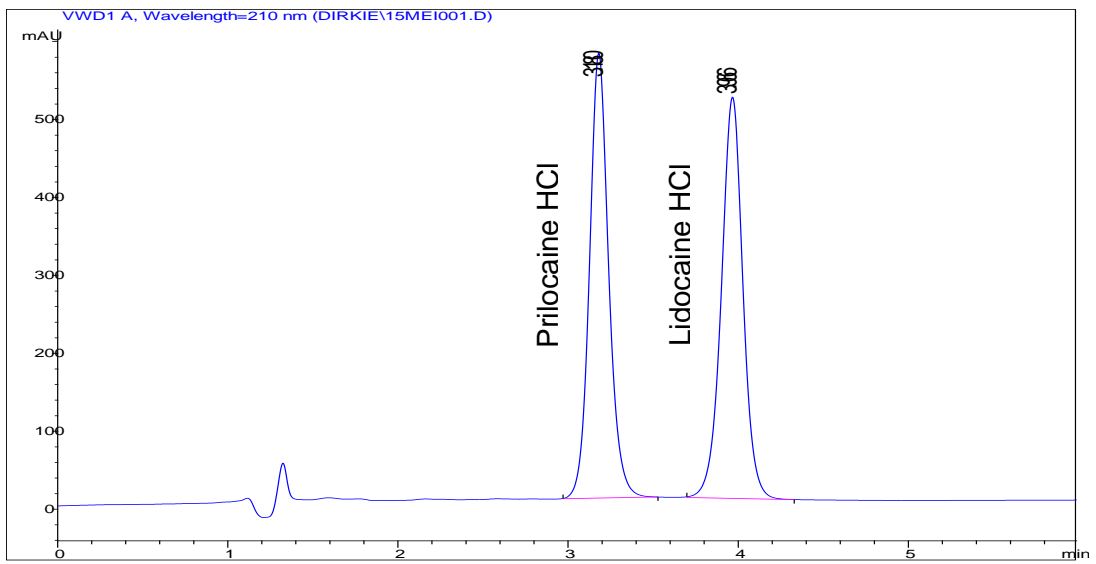


Figure A.3: HPLC chromatograms of lidocaine HCl (3.18 min) and prilocaine HCl (3.96 min)

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APPENDIX B

THE FORMULATION OF SEMISOLID PREPARATIONS CONTAINING LOCAL ANAESTHETICS LIDOCAINE AND PRILOCAINE

B.1 INTRODUCTION

Topical drug delivery systems are described as formulations intended for local effect on the skin rather than systemic delivery. Topical products are divided into pharmaceutical topical products and cosmetic topical products (Kligman, 2002:5). A pharmaceutical topical product is an API containing a formulation that is applied to the skin to directly treat cutaneous disorders or manifestations of general disease. The pharmacological effect of the API is then contained to the local area of application, which is the skin surface or the deeper skin layers (Ademola, 1997:511). Topical products include creams, ointments, lotions, pastes, powders, gels and gel creams (Surber & Smith, 2000:10).

The use of topical anaesthesia has become increasingly popular due to its non-invasive manner of application. It is used in minor skin-breaking repair procedures, reduction of cutaneous pain and diagnostic procedures (Little *et al.*, 2008:100; 102). Lidocaine and prilocaine are two pharmaceutical agents used to produce local anaesthesia. They alter the function of the body by interrupting impulse conduction and can be clearly categorised as pharmaceutical topical agents.

An emulgel and a hydrogel were the formulations of choice for a eutectic mixture of the local anaesthetics lidocaine and prilocaine in their hydrochloride salt forms. Emulsions consist of an oil phase and aqueous phase (Attwood, 2007:92). The water soluble active ingredients, lidocaine HCl and prilocaine HCl, are easily dissolved in the aqueous phase of the formulation while the Pheroid™ ingredients are added to the oil phase. An emulgel with and without Pheroid™ was formulated. A watery gel formulation contains a great amount of water which is suitable for the water soluble active ingredients. Hydrogels can cause the skin to become more hydrated, which will benefit the water soluble active ingredients when traversing the skin (Barry, 2007:539). A hydrogel formulation was also formulated for this study.

B.2 THE FORMULATION OF PHARMACEUTICAL SEMI-SOLIDS

B.2.1 Preformulation

The term preformulation is used to describe the steps taken before formulation (Wells & Aulton, 2007:337). These steps include thorough research on the character and physicochemical properties of the APIs being formulated into topical products. The target site for API delivery of local anaesthetics is the dermis. Preformulation includes a planning process for the formulation that insures that the formulation chosen is used optimally and to the advantage of the delivery of the API (Ademola, 1997:511, 517). When choosing the dosage form it is important to consider the physicochemical properties of the API, stability of the API in the formulation, release of the API from the formulation, available manufacturing equipment and cost constraints (Ramchandani & Toddywala, 1997:542).

Criteria taken into consideration from a pharmaceutical technological point of view during preformulation are listed below (Surber & Smith, 2000:8):

- ❖ The stability of the APIs
- ❖ The stability of other ingredients in the formulation
- ❖ Rheological properties like consistency and extrudability
- ❖ Water loss and loss of other volatile components
- ❖ Phase changes like homogeneity or phase separation and bleeding
- ❖ The particle size and particle size distribution of the dispersed phase
- ❖ pH
- ❖ Sterility and microbial contamination
- ❖ The enhanced or controlled release of the API from its API vehicle/dosage form

Criteria taken into consideration from a cosmetic point of view during preformulation can be summarised as (Surber & Smith, 2000:8):

- ❖ The visual appearance of the product
- ❖ The colour and odour of the product

- ❖ The sampling and dispensing characteristics of the product like how easily it can be removed from its container
- ❖ Application properties
- ❖ Texture like stiffness, grittiness, greasiness and adhesiveness
- ❖ The residual impression after application and permanency on the skin

Criteria taken into consideration from a biopharmaceutical point of view during preformulation, are (Surber & Smith, 2000:8):

- ❖ Targeted API delivery and API retention in the skin
- ❖ Enhanced API delivery and API retention in the skin
- ❖ Controlled API delivery and API retention in the skin

Enhanced API delivery and API retention in the skin are especially important when formulating with local anaesthetics. It is necessary for the enhancement of the local anaesthetic effect at the site of application. An optimal local anaesthetic formulation would be one that enhances API delivery over a shorter time period and retains the API in the skin for the anaesthetic effect. During the preformulation stage formulations were developed through trial-and-error where various different formulations were tried and tested. The final formulations were decided on after examining the different formulations by taking light microscope photos to rule out crystallisation, determining viscosity and observing the feel, colour and odour of the formulations. The formulations were then manufactured in bulk for stability testing over a period of six months.

B.2.2 Formulation of a gel

A gel can be described as a two compartment semi-solid system consisting of a suspension of either small inorganic particles or large macromolecules interpenetrated with liquid (Ramchandani & Toddywala, 1997:547). In a polar gel a three dimensional matrix is built throughout the hydrophilic liquid by a natural or synthetic polymer (Barry, 2007:593).

B.2.2.1 Function and purpose of a gel

A gel is rich in liquid and one of its main characteristics is the presence of a continuous structure that provides it with solid-like properties (Barry, 2007:593). Consumers find gel dosage forms very acceptable because it provides a soothing and cooling feeling with its easy application. A gel

makes the skin feel moist and light and it is applied for skin cleansing, make-up removal and moisturising. An aqueous gel contains a high percentage of moisture and is preferably used in summer. An oil-based gel will be used for dry skin in the winter as it supplies the skin with oil (Mitsui, 1997:351).

B.2.2.2 Ingredients used to manufacture gels

The main ingredients used in gels include a gel base, surfactants, humectants, preservatives, pharmaceutical agents, colouring agents and perfumes (Mitsui, 1997:351). Typical polymers used in gel formulations are natural gums (i.e., alginates, pectin, xanthan, chitosan), cellulose derivatives (i.e. methyl cellulose, carboxymethyl cellulose, hydroxyethyl cellulose, hydroxypropyl cellulose, hydroxypropylmethyl cellulose), acrylic acid polymers (i.e. carbopol and polycarbophil), colloidal solids (i.e. silica and clay), thermoreversible polymers (i.e. poloxamers), surfactants and polyethylenes (Ramchandani & Toddywala, 1997:548). The type of solvent used will determine the type of gel. A hydrogel is a gel that contains a large amount of water as solvent. If the solvent is a polyethylene-containing mineral oil, an organogel will form (Ramchandani & Toddywala, 1997:547). A gel can be a transparent colour or a cloudy white, depending on the other ingredients of the formulation. If the API does not bind to the polymer the gel will release the active ingredient sufficiently (Barry, 2007:593).

B.2.3 Method for the manufacturing of a gel

B.2.3.1 Emulgel formulation

Emulsions are used in various cream, gel and lotion type formulations as a basis. Emulsions consist of an oil phase and an aqueous phase and an emulsifier (Ramchandani & Toddywala, 1997:543). An emulgel is an oil-in-water (O/W) emulsion that is integrated into a gel formulation (Lopez-Cervantez *et al.*, 2009:1). The aqueous phase and the oil phase are mixed together and then homogenised to attain a uniform product. In this study the emulgel formulation was homogenised at 13 500 rpm (revolutions per minute). If the components of the oil phase are solid or have a very high viscosity, they have to be melted before being added to the water phase. The water phase should be heated to the same temperature as the oil phase before being added together. Water soluble active ingredients can be dissolved in the aqueous phase. It is important to continuously stir the formulation after the two phases have been combined and during cooling to prevent de-emulsification. Other ingredients that are added to an emulgel are emulsifiers, viscosity

builders, preservatives, antioxidants, pH adjusting agents and chelating agents (Ramchandani & Toddywala, 1997:544). The final formula of a lidocaine and prilocaine emulgel is given in Table B.1

Table B.1: Ingredients used in the emulgel formulation

Ingredients	%m/m	Function
Lidocaine HCl	2.5%	Active ingredient
Prilocaine HCl	2.5%	Active ingredient
Propylene glycol diacetate	15.0%	Thickening agent
Liquid paraffin	10.0%	Oil phase of emulsion
Pluronic	10.0%	Emulsifier
Alcohol	10.0%	Preservative
Distilled water	To 100.0%	Solvent

For Phase A of the formulation the lidocaine HCl and prilocaine HCl were weighed and dissolved in the dH₂O. The propylene glycol diacetate was added to the API mixture. The alcohol was added last to Phase A. Phase B consisted of the liquid paraffin. Phase A was added to Phase B and mixed at a high speed of 13 500 rpm. The mixture was then heated to 70 °C. The pluronic was added to the mixture while maintaining the temperature at 70 °C and stirring continuously. When most of the pluronic was dissolved the mixture was homogenised at a speed of 13 500 rpm until full thickness and texture was reached. The mixture was then cooled down in a container with cold water.

B.2.3.2 Pheroid™ emulgel formulation

The same manufacturing method was used for both the emulgel and the Pheroid™ emulgel. Pheroid™ ingredients were added to the basic emulgel formula. Pheroid™ vesicles were used to encapsulate the API in the formulation.

B.2.3.3 Gel formulation

A typical gel formulation was developed containing a large amount of water. Because lidocaine HCl and prilocaine HCl are both very highly water soluble, a water-based formulation was tested. A clear water-based gel formulation was obtained but during pH adjustment (see Section B.2.3.4) the gel became a cloudy white colour. The final formulation of a lidocaine and prilocaine gel are given in Table B.2

Table B.2: Ingredients used in the gel formulation

Ingredients	%m/m	Function
Lidocaine HCl	2.5%	Active ingredient
Prilocaine HCl	2.5%	Active ingredient
Propylene glycol diacetate	15.0%	Thickening agent
Pluronic	10.0%	Gelling agent
Alcohol	10.0%	Preservative
Distilled water	To 100.0%	Solvent

The lidocaine HCl and prilocaine HCl were weighed and dissolved in the dH₂O. The propylene glycol diacetate was added to the API mixture. The alcohol was added last to the API mixture whereafter the mixture was heated to 70 °C. The pluronic was added to the mixture while maintaining the temperature at 70 °C and stirring continuously. When most of the pluronic was dissolved the mixture was homogenised at a speed of 13 500 rpm until full thickness and texture were reached. The mixture was then cooled down in a container with cold water.

B.2.3.4 pH adjustment

Acids, bases and buffering agents are pH adjusters used on the finished product to control the pH of the formulation. The reason for adjusting the pH of a formulation can be to lower skin irritation, to create a pH closer to that of the skin and to change the ionisation of the API (Ramchandani & Toddywala, 1997:555). The pKa values of lidocaine HCl and prilocaine HCl are 7.89 and 7.90, respectively. This means that both APIs are 50% ionised and 50% unionised at a pH of 7.9. It was decided that the pH of the formulations would be between 7.9 and 8.1. When using a pH higher than 8.4 the risk of skin irritation can be increased. The pH was set using sodium hydroxide (10%).

B.2.3.5 Preservation

Preservatives are added to topical formulations for long-term protection against microbial contamination. The ingredients in the oily and aqueous phases can include carbohydrates and proteins that provide an optimal environment for bacteria, fungi or yeasts to grow because of the supply of carbon and nitrogen (Barry, 2007:596, Mitsui, 1997:199). Preservation plays an important role in maintaining formulation stability and to prevent infection in the consumer. Microbial contamination can occur in the raw materials used, equipment used, packaging used or from the workers handling the products in the manufacturing process (Barry, 2007:596). It is important to

keep in mind the stability and compatibility of a preservative in a formulation and in its intended container. In emulsion formulations the partitioning of the preservative in the two phases can occur and this has to be brought into consideration when choosing an appropriate preservative (Ramchandani & Toddywala, 1997:556). The chosen preservative should be compatible with all the formulation ingredients, effective in low concentrations, heat-stable, pH-stable, stable over long-period storage, non-toxic, non-irritant and non-sensitising to human skin (Barry, 2007:596). Types of preservatives include alcohols (ethanol), quaternary amines (benzalkonium chloride), acids (benzoic acid), parabens (methyl and propyl paraben), phenols (triclosan), and anti-oxidants (tocopherols and ascorbic acid) (Ramchandani & Toddywala, 1997:551). The chosen preservative for the lidocaine and prilocaine formulations after multiple considerations and tests was ethanol. It was compatible with all the ingredients in the formulations.

B.3 RAW MATERIALS USED DURING FORMULATION

- ❖ Lidocaine HCl obtained from Konduskar Laboratories, India. Batch number, Lid-03-1-001.
- ❖ Prilocaine HCl obtained from DB Fine Chemicals, South Africa. Batch number, 11051007.
- ❖ Propylene glycol diacetate obtained from Merck Laboratory Supplies, South Africa. Batch number, 5543368898.
- ❖ Liquid paraffin obtained from Merck Laboratory Supplies, South Africa. Batch number, 45035.
- ❖ Pluronic obtained from BASF, South Africa. Batch number, WPDD556B.
- ❖ Ethanol obtained from Rochelle Chemicals, South Africa. Batch number, 230811EX.

B.4 CONCLUSION

The two emulgel formulations manufactured were non-sticky, milky white and smooth emulgel formulations. The formulations contained no crystals and only minor problems were encountered during preformulation regarding different possibilities of emulsifier and oil phase. The hydrogel formulation was a cloudy white colour with smooth application. It also had a cooling effect on the skin. The problems encountered were during early preformulation regarding the large amount of water and pH adjustment. The amount of water had to be adjusted to stop the gel from breaking

during pH adjustment. During the manufacturing of the master batches no problems were encountered.

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APPENDIX C

STABILITY TESTING OF COSMECEUTICALS

C.1 INTRODUCTION

The South African Medicines Control Council (MCC) defines the stability of a product as the capacity of an active pharmaceutical ingredient (API) or dosage form to remain within specifications established to assure its identity, purity, strength and critical physicochemical characteristics (MCC, 2011:18). Because of the mass development of products for the cosmetic market an effective and rapid method of predicting a product's stability is needed (Guaratini *et al.*, 2006:12).

The stability of a cosmeceutical product is important as it can affect product quality and efficacy. The stability is influenced by chemical, physical and microbial factors that cause the product to deteriorate rapidly (Barnes, 2007:665). The aim is to develop a product that will not lose its chemical, physical and microbial stability under the correct storage conditions, thus providing it with an optimal shelf life (Barnes, 2007:650). Emulsions are of the most popular dosage forms for cosmeceuticals but have an unstable nature. Emulsions are thermodynamically unstable and can easily split into two phases. Creaming and cracking of emulsions are common (Masmoudi, 2004:118).

Accelerated stability testing or stress testing is the method described by the MCC for stability testing of new API substances and products. They follow the triplicate guidelines provided by the ICH (ICH Triplicate Guidelines, 2003). These accelerated studies are designed to increase the rate of chemical degradation and physical change of a product (MCC, 2011:16). The formulations are stored at different temperatures and humidities. A series of different stability tests are performed on formulations at certain time intervals and the noticeable changes are noted. Accelerated testing is performed over six months (MCC, 2011:5). Three formulations (as described in Appendix B) were prepared for this study and then placed in a stability testing procedure for six months at 25 °C/60% RH (relative humidity), 30 °C/60% RH and 40 °C/75% RH in Labcon[®] humidity chambers (Labcon, United States of America). Analysis was done at months 0, 1, 2, 3 and 6.

C.2 STABILITY OF COSMECEUTICAL FORMULATIONS

A total amount of 2000 g of each of the formulations were prepared and then packaged in glass containers obtained from Consol[®], South Africa. Jars containing 50 g and 125 g of the formulation were used.

The following stability tests were conducted:

- ❖ Assay of concentration of active ingredient
- ❖ Viscosity determination
- ❖ pH determination
- ❖ Mass variation
- ❖ Particle size variation
- ❖ Zeta potential
- ❖ Physical assessment

C.2.1 Assay

The concentration of the APIs, namely lidocaine HCl and prilocaine HCl in the formulated products was determined by HPLC analysis after each month. The validated HPLC method described in Appendix A was used. A reduction of API concentration to 90% is acceptable (Barnes, 2007:650). The analysis was done with an Agilent[®] 1100 Series HPLC system (Agilent Technologies, United States of America), fitted with an 1100 pump, autosampler injection mechanism, vacuum degasser, solvent module and diode array detector. It comes equipped with Chemstation Rev. A.06.02 data acquisition and analysis software. The same analytical column, mobile phases, gradients and chromatic conditions were used as discussed in Section A.1.1.

C.2.1.1 Standard preparation

A standard solution was prepared by mixing lidocaine HCl (25 mg) and prilocaine HCl (25 mg) and dissolving the mixture in methanol in a 100 ml volumetric flask. The flask was filled to volume with methanol. A fresh standard was prepared each month and analysed before the formulation samples. The standard was injected in duplicate at different concentrations to obtain a standard curve. The injection volume was set at 5 µl.

C.2.1.2 Sample preparation

Duplicate samples of the emulgel, Pheroid™ emulgel and the hydrogel were prepared by weighing 1 g of each of the various formulations in separate 100 ml volumetric flasks. The volumetric flask was filled up to volume with methanol and then placed on a sonic bath for 5 min to ensure that all of the formulation had been dissolved. Samples were analysed in duplicate on the HPLC apparatus to ensure accuracy.

C.2.2 Viscosity determination

The viscosity of a topical semi-solid product is described as its ability to produce resistance against flow or movement (Marriot, 2007:42). It gives an indication of the stability and effectiveness of the product (Ramchandani & Toddywala, 1997:557). The rheology of topical products is known to be complex and most of them do not follow the Newtonian flow model. Semi-solid emulsions exhibit non-Newtonian plastic flow (Marriot, 2007:57). Viscosity testing of products over a period of time is important because changes in the viscosity indicate changes in the spreadability and smoothness of the product and also indicate if creaming has occurred. Consumers prefer formulations with medium to high viscosity for external application. These formulations should not take long to be absorbed and should have ideal spreadability properties (Billany, 2007:392). This can be achieved by studying the rheology of a topical product.

For this study a Brookfield Model DV – II+ viscometer (Brookfield, United States of America) was used. The temperature was controlled at 25 °C by placing the formulations in a Brookfield circulating water bath. 125 g of each formulation was tested. A Helipath spindle set (T-bar spindle and chuck) was used to measure the viscosity. The T-F spindle was chosen and used for each formulation. The viscometer was moved up and down by a Helipath D20733. 32 readings were measured 10 sec apart by the rotating spindle at 0.3 rpm (revolutions per minute).

C.2.3 pH determination

The pH of the skin surface is between 4 and 6 and topical products are formulated taking this into consideration (Ramchandani & Toddywala, 1997:557). However, this is not the only factor that should be taken into consideration when determining the pH of a product. The ratio of ionised to unionised molecules is crucial. The unionised form of the API penetrates the stratum corneum effectively, but it is the ionised form of the local anaesthetic that binds to the receptor (Section 2.2.3). The pH of the formulations was maintained at 8. This ensures that more than 50%

of the molecules are unionised and ready to cross the stratum corneum. The skin can tolerate pH ranges of 3-9 (Barry, 2007:565). The pH of the formulations was tested in triplicate each month by using a Mettler Toledo Seven Multi pH meter with an In Lab 410 NTC electrode 9823 (Mettler Toledo International Inc., United States of America). The pH meter was calibrated before each monthly series of tests.

C.2.4 Mass variation

The mass variation or mass loss of the different formulations was determined by weighing the same container of each formulation every month for the six month trial period. A Shimadzu AUW 120 D scale (Shimadzu Corporation, Japan) was used to weigh the containers in triplicate. The scale was calibrated before each monthly series of tests.

C.2.5 Particle size variation

The control over an API's particle size is becoming increasingly important during the manufacturing process of pharmaceutical products. Pharmaceutical companies make use of particle size reduction of APIs that are less water soluble so that they are easier to formulate into a specific dosage form. During the shelf-life of a topical product the increase or decrease in the particle size of the API can cause variations in the *in vitro* release and bioavailability of the API. It is recommended that stability tests are performed on the particle size of a formulation over a period of time and on different batches (Ramchandani & Toddywala, 1997:558). Monitoring the particle size of emulsions can alert the formulator of any stability problems in the early stages of product development. The droplets of the internal phase of an emulsion can be a good indication of the stability of the emulsion. The emulsion droplets or globules can increase in size because of coalescence. Breakage of the gel network of the emulsion can occur over time while crystal structure can increase or change completely. If these changes occur, it drastically alters the therapeutic action of the formulation (Barry, 2007:596). Particle size also plays a role in the grittiness and texture of topical products and can influence the manner of application of the product (Aulton & Staniforth, 2007:10). For transdermal and topical delivery the particle size of the API has an effect on the API flux of the API into the skin because smaller particles cross the skin more easily and increases diffusion. Particle size can also influence the permeation pathway the API molecules take. Particles larger than 10 μm get left behind on the skin, particles between 3 – 10 μm move through the skin appendages and particles smaller than 3 μm move through stratum corneum and follicles (Barry, 2002:36).

The particle size of the three lidocaine HCl and prilocaine HCl formulations tested over a period of six months. Analysis was done with a Malver Mastersizer 2000 Hydro SM (Malvern Instruments Ltd., United Kingdom). The Hydro SM unit of the Mastersizer measures samples in wet dispersions. It consists of a tank with 120 ml capacity with a centrifugal pump and stirrer. The speed of the stirrer was set to 2 750 rpm. Because the Mastersizer 2000 uses the light scattering technique for measuring particle size, low concentration samples were prepared and diluted. Laser diffraction works on the principle that the particles moving through the laser beam will scatter light at an angle related directly to their size. In wet dispersions the sample is dispersed into a liquid and will circulate over a laser-illuminated quartz cell. Laser diffraction is described by the Mie theory and Fraunhofer approximation. The Mastersizer 2000 can measure a range of particles between 0.02 – 2 000 μm (Balsamo & Storti, 2010:25). Samples were prepared in triplicate and three measurements of each were done. The average was calculated and noted. The Mastersizer takes readings at three levels: d(0.1), d(0.5) and d(0.9). The d(0.1) level determines that 10% of the particles in the sample are below the determined level. The d(0.5) level is the is median particle size level in the sample with 50% of the particle sizes below this particle size level. The d(0.9) particle size level indicates that 90% of the particle sizes are below this particle size level.

C.2.6 Zeta potential

Measurement of the zeta potential of a colloidal system can give a good indication to the system's stability. A colloidal system can be either a gas, liquid or a solid dispersed in one of each other. An emulsion is described as a liquid dispersed in a liquid (Malvern Instruments 2009:16.2). Emulsions can undergo various destabilising processes over time that can cause the emulsion to break. Creaming, coalescence, rupture, flocculation and phase separation are the main causes of emulsion instability (Roland *et al.*, 2003:85). By measuring the zeta potential of the sample it can be determined if the particles in the liquid phases flocculate together. This can give a good indication of the stability of the emulsion (Bhatt *et al.*, 2010:512).

Zeta potential is defined as the difference in the potential that exists between the electroneutral region of a solution and the tightly bound layer of ions on the particle surface (Roland *et al.*, 2003:87). The ions on the particle surface are sensitive to the development of a net charge on the particle surface. The net charge influences the distribution of the ions in such a manner that ions of opposite charge increase at the surface. The increase in counter ions causes an electrical double layer to form around each particle. This double layer can then be described in two parts, namely the Stern, or inner layer and an outer diffusive part. Ions are bound strongly to the Stern layer. In

the diffuse layer it is possible for the ions and particles to form a stable entity because a notional boundary exists inside the layer. Ions within this boundary can move together with the particle but ions that fall outside of this boundary do not move with it. This boundary in the diffusive layer is described by the term 'slipping plane'. The zeta potential is the potential that exists in the slipping plane. A zeta potential bigger than +30 mV or smaller than -30 mV is an indication that the emulsion is stable. The larger positive or negative value the zeta potential of the particles, the better they will be able to ward off particles of the same charge to avoid flocculation (Malvern Instruments 2009:16.1, 2; Billany, 2007:388).

pH has a big effect on zeta potential. The more alkaline the pH is, the more negative the zeta potential will be. An acid pH tends to produce positive zeta potential values. It is, however, important to note that the solvent used in preparing samples may alter the pH of the sample. If an acid solvent is added to a slightly alkali sample, the negative charge may be neutralised or become positive (Malvern Instruments 2009:16.1, 2; Attwood, 2007:76).

A Malvern Zeta-sizer Nano (Malvern Instruments Ltd., United Kingdom) was used to measure the zeta potentials of the formulations over a six month period. Samples were prepared by measuring 0.05 g of each formulation into a 20 ml volumetric flask. The volumetric flask was filled up to 20 ml with 0.01 M potassium chloride and shaken well. An almost clear solution must be obtained. Samples were prepared in triplicate and three readings of each were taken. The Zeta-sizer Nano determines zeta potential by determining the electrophoretic mobility of the particles by means of electrophoresis and Laser Doppler Velocimetry (LDV) of the sample and then applying the Henry equation.

C.2.7 Physical assessment

The physical assessment of topical products includes the assessment of appearance, colour, odour and texture. Oxidation can cause the colour and odour of topical products to change and shorten the shelf life of the product (Ramchandani & Toddywala, 1997:557). The emulgel and Pheroid™ emulgel were a slightly off-white colour after formulation while the hydrogel was a cloudy white colour. The colour of the formulations was documented by taking photos over the six month period and comparing them with the photos taken right after formulation.

C.3 RESULTS

C.3.1 Assay of lidocaine HCl and prilocaine HCl

The concentration of lidocaine HCl and prilocaine HCl increased in month 1 from the initial concentration which indicates that the emulgel still had to settle (Table C.1). The concentration of the APIs varied slightly over the three month period and decreased more than 5% after six months. Prilocaine HCl was stable for the first three months but decreased in concentration from month 3 to month 6. According to the MCC, a 5% potency loss from the initial assay percentage is considered as a significant change in formulation and indicates instability (MCC, 2011:12).

Table C.1: Percentages of lidocaine HCl and prilocaine HCl present in the emulgel formulation

API	Temperature (°C)	Month (%)				
		0	1	2	3	6
Lidocaine HCl	25	107.15	108.30	107.42	107.13	93.64
	30	-	112.98	113.19	108.20	101.94
	40	-	108.11	112.51	106.45	101.39
Prilocaine HCl	25	104.36	105.01	104.83	103.95	83.29
	30	-	104.57	105.15	105.63	92.08
	40	-	101.15	104.27	103.95	89.87

The lidocaine HCl and prilocaine HCl in the Pheroid™ emulgel formulation experienced a decrease in active concentration over six months (Table C.2). The Pheroid™ emulgel exposed to 30 °C/60% RH and 40 °C/75% RH experienced the biggest decrease in concentration. The prilocaine HCl decreased more than lidocaine HCl. The prilocaine HCl decreased more than 20% at 30 °C/60% RH and 40 °C/75% RH which places its concentration values outside of the acceptable range. The prilocaine HCl concentration decreased significantly in the last three months from 13.03% at 25 °C/60% RH to 18.49% at 40 °C/75% RH. According to Barnes (2007:650) a concentration of up to 90% is still acceptable but a concentration decrease of 10% is not. The two API were not stable in combination with the Pheroid™ ingredients.

Table C.2: Percentages of lidocaine HCl and prilocaine HCl present in the emulgel formulation with Pheroid™

API	Temperature (°C)	Month (%)				
		0	1	2	3	6
Lidocaine HCl	25	101.12	102.98	100.09	95.35	89.33
	30	-	102.85	100.16	93.45	85.95
	40	-	105.76	97.89	95.59	89.74
Prilocaine HCl	25	105.57	98.39	98.82	95.10	82.07
	30	-	96.85	97.92	93.48	76.60
	40	-	96.69	93.63	92.33	73.84

The prilocaine HCl experienced a bigger decrease in concentration than the lidocaine HCl (Table C.3). The biggest decrease in prilocaine HCl concentration was observed at 30 °C/60% RH and 40 °C/75% RH. The concentration decrease was less than 20% after six months from which it was concluded that the concentration of the APIs was not acceptable in the hydrogel formulation for the duration of this study. The prilocaine HCl concentration decreased significantly from month 3 to month 6 with a percentage fall of 14.35% in this period. The lidocaine HCl was more stable in the hydrogel with only 7% decrease after 6 months.

Table C.3: Percentages of lidocaine HCl and prilocaine HCl present in the hydrogel formulation

API	Temperature (°C)	Month (%)				
		0	1	2	3	6
Lidocaine HCl	25	98.52	96.75	101.62	96.82	91.11
	30	-	99.98	98.68	94.77	91.27
	40	-	102.41	102.06	95.05	91.23
Prilocaine HCl	25	99.93	98.06	98.98	95.77	80.86
	30	-	92.28	92.38	92.39	77.63
	40	-	92.94	92.24	92.39	78.04

Lidocaine exhibited a higher stability in all the formulations over the six month period. The prilocaine concentration decreased after the three month period to unacceptable low levels in the emulgel with Pheroid™ and the hydrogel. The concentration varied from 82.07% to 73.84% at the end of the six month testing period.

C.3.2 Viscosity determination

C.3.2.1 Viscosity of emulgel

The viscosity of the emulgel over six months is depicted in Figure C.1. Viscosity measurements were done on an emulgel containing lidocaine HCl and prilocaine HCl exposed to 25 °C/60% RH.

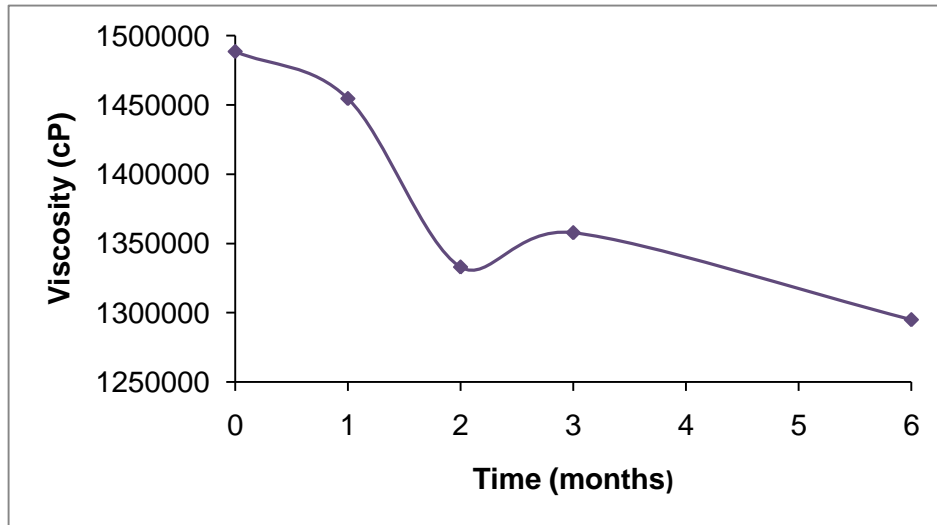


Figure C.1: Viscosity (cP) of the emulgel over a six month period

The viscosity of the emulgel over a six month period decreased slightly. Overall there was a 193 485 cP difference ($\pm 13\%$) between the initial viscosity and the viscosity at month 6. This amount is acceptable and the emulgel can be considered as stable. The decrease from the initial viscosity may be attributed to the formulation still settling before it reached its final resting stage. The Van der Waals forces between the emulgel molecules might have been disturbed during previous measurements of the same emulgel which caused a decrease in viscosity.

C.3.2.2 Viscosity of emulgel containing Pheroid™

The viscosity of the emulgel containing Pheroid™ over six months is depicted in Figure C.2. Viscosity measurements were done on a Pheroid™ emulgel containing lidocaine HCl and prilocaine HCl exposed to 25 °C/60% RH.

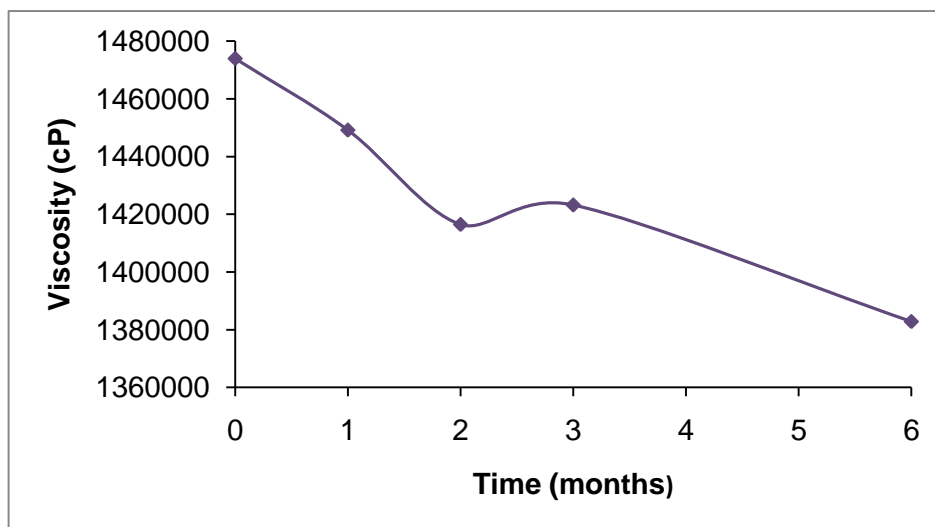


Figure C.2: Viscosity (cP) of the Pheroid™ emulgel over a six month period

A slight decrease in viscosity of 91 000 cP ($\pm 6.2\%$) was observed over the six month stability testing period. The Pheroid™ emulgel experienced the biggest drop in viscosity in the first two months due to the Pheroid™ emulgel still settling or broken Van der Waals forces. The decrease in viscosity was not significant and still within the acceptable range. The Pheroid™ emulgel was deemed stable after a six month period.

C.3.2.3 Viscosity of hydrogel

The viscosity of the hydrogel over six months is depicted in Figure C.3. Viscosity measurements were done on a hydrogel containing lidocaine HCl and prilocaine HCl exposed to 25 °C/60% RH.

The hydrogel experienced an increase in viscosity of 150 800 cP from the initial viscosity up to month 3. Due to the large amount of water in the hydrogel it is possible that swelling of the gel occurred as the gelling agent absorbed more water during the first three months (Ramchandani & Toddywala, 1997:547). The hydrogel decreased in viscosity towards month 6, which can be explained by the possible breaking of the Van der Waals forces between the molecules due to earlier viscosity measurements. After six months the hydrogel had decreased a small amount of 30 900 cP ($\pm 2.7\%$) below the initial viscosity. Due to more fluctuations in the viscosity the hydrogel appears to be less stable than the other two formulations.

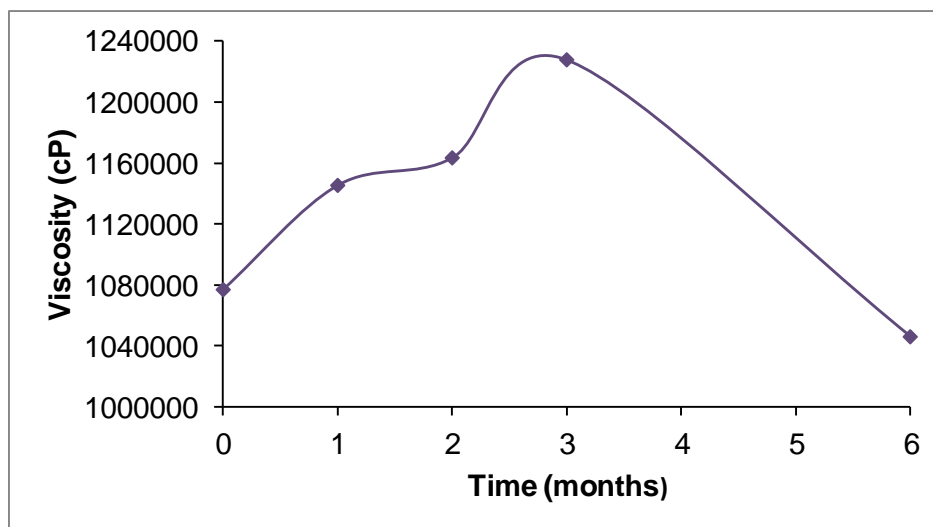


Figure C.3: Viscosity (cP) of the hydrogel over a six month period

C.3.3 pH

C.3.3.1 pH determination of emulgel

Table C.4: pH values of the emulgel formulation over 6 months

Storage temperature	Initial pH	Month 1	Month 2	Month 3	Month 6	pH decrease
25 °C/60% RH	8.25	7.44	7.18	7.08	6.74	1.51
30 °C/60% RH	8.25	7.20	6.91	6.83	6.38	1.87
40 °C/75% RH	8.25	6.83	6.57	6.40	5.57	2.68

During the first month of stability testing the highest decrease in pH value for the emulgel at all temperatures was observed (Table C.4). This can be attributed to the emulgel settling. The emulgel kept at 40 °C/75% RH had the largest decrease in pH. For the period of the second month of stability testing, another decrease in pH was observed. The pH of the emulgel formulation decreased at all temperatures with ± 0.25 . At month 3 another lowering in pH was noted. This time the decrease of pH was smaller than the previous months with ± 0.1 for all the temperatures. When compared with the initial pH it is clear that the emulgel at 40 °C/75% RH experienced the biggest decrease in pH because of the higher instability at this temperature and humidity. Because the emulgel formulations experienced the same amount of pH lowering over month 2 and 3 they can be considered as stable. After six months the pH continued to decrease but still produced

values within the recommended pH for topical formulations of 3 – 9. It is, however, important to note that a change in pH may drastically alter the ionisation of the lidocaine HCl and prilocaine HCl which will lead to ineffective anaesthetic action. A decrease or alteration in pharmacological action because of pH is not acceptable and indicates instability aspects in the emulgel formulation (Ramchandani & Toddywala, 1997:557). The storage temperature had a significant influence on the pH of the emulgel. At 25 °C/60% RH the decrease of pH value over a six month period was 21.3%, while at the higher temperatures of 30 °C/60% RH and 40 °C/75% RH the decrease in pH values were 22.7% and 32.5%, respectively.

C.3.3.2 pH determination of emulgel containing Pheroid™

Table C.5: pH values of the emulgel formulation containing Pheroid™ over 6 months

Storage temperature	Initial pH	Month 1	Month 2	Month 3	Month 6	pH decrease
25 °C/60% RH	8.15	7.45	7.18	7.04	6.71	1.44
30 °C/60% RH	8.15	7.19	6.90	6.79	6.34	1.81
40 °C/75% RH	8.15	6.81	6.56	6.40	5.58	2.57

The Pheroid™ emulgel experienced a decrease in pH during the first month of stability testing (Table C.5). As seen with the emulgel, the Pheroid™ emulgel kept at 40 °C/75% RH experienced the highest decrease in pH. Over month 2 the pH decreased with ± 0.25 ; this was noted for all temperatures, followed by a decrease in pH of ± 0.16 for month 3. Formulation stability may be suggested by the relatively equal amounts of pH decreases during month 2 and 3, respectively, but the fact that the pH decreases each month may point to instabilities in the formulation. The Pheroid™ emulgel decreased in pH value with increasing temperature. Lowering of the pH value at 25 °C/60% RH was 14.9%, at 30 °C/60% RH was 22.2% and at 40 °C/75% RH was 31.5% over the six month period. The decrease in pH causes a decrease in anaesthetic action and depicts the Pheroid™ emulgel to be unstable.

C.3.3.3 pH determination of hydrogel

Table C.6: pH values of the hydrogel formulation over 6 months

Storage temperature	Initial pH	Month 1	Month 2	Month 3	Month 6	pH decrease
25 °C/60% RH	7.99	7.48	7.22	7.08	6.63	1.36
30 °C/60% RH	7.99	7.22	6.92	6.80	6.15	1.84
40 °C/75% RH	7.99	6.83	6.52	6.36	5.37	2.62

The behaviour of the hydrogel is similar to that of the emulgel and Pheroid™ emulgel over the six month stability testing period (Table C.6). The initial decrease in pH during month 1 was less for the hydrogel when compared with the other two formulations. After month 1 a decrease of ± 0.3 in pH was noted. A decrease of ± 0.15 was seen in month 3. A bigger decrease in pH was observed after six months, indicating that the hydrogel systematically decreased in pH each month. This would cause the anaesthetic action to also decrease each month which is not acceptable.

The following observation was made over the six month stability testing period. The pH values for the different formulations at their different temperatures corresponded with each other (± 0.03). This leads to the conclusion that the different topical vehicles (emulgel, Pheroid™ emulgel, hydrogel) remained stable and that it was the APIs that caused the decrease in pH. This can be because of the acidic nature of the hydrochloride salt forms of lidocaine and prilocaine. These APIs do not seem to respond well to the high pH of the formulation. In the future different ways to counter this problem in the stability of the topical lidocaine and prilocaine formulations can be investigated. Different methods to set a stable pH after formulation need to be implemented.

In all the formulations the pH value decreased significantly at the highest temperature indicating that storage should take place at the lower temperatures.

C.3.4 Mass variation

C.3.4.1 Mass variation of emulgel

No significant mass loss for the emulgel was observed over a six month period (Table C.7). The slight changes in mass can be due to the water loss of the formulation which is an effect of the different temperatures. The emulgel exposed to 25 °C/60% RH showed the smallest amount of mass loss and the emulgel kept at 40 °C/75% RH showed the greatest mass loss.

Table C.7: Mass variation (g) of the emulgel formulation over 6 months

Storage temperature	Initial (g)	Month 1 (g)	Month 2 (g)	Month 3 (g)	Month 6 (g)	Mass decrease (g)
25 °C/60% RH	110.46	110.43	110.38	110.37	110.31	0.09
30 °C/60% RH	113.03	112.99	112.96	112.93	112.90	0.13
40 °C/75% RH	112.70	112.71	112.56	112.50	112.45	0.25

C.3.4.2 Mass variation of emulgel containing Pheroid™

Table C.8: Mass variation (g) of the emulgel formulation containing Pheroid™ over 6 months

Storage temperature	Initial (g)	Month 1 (g)	Month 2 (g)	Month 3 (g)	Month 6 (g)	Mass decrease (g)
25 °C/60% RH	114.39	114.36	114.34	114.30	114.24	0.15
30 °C/60% RH	113.61	113.57	113.50	113.46	113.35	0.26
40 °C/75% RH	111.40	111.32	111.22	111.16	111.20	0.20

Relative small amounts of mass loss were observed for the Pheroid™ emulgel over the six month stability testing period (Table C.8). The mass variation is attributed to the loss of water from the formulations at their different temperatures with the formulation at 25 °C/60% RH showing the smallest, and the formulation at 40 °C/75% RH the biggest change.

C.3.4.3 Mass variation of hydrogel

Table C.9: Mass variation (g) of the hydrogel formulation over 6 months

Storage temperature	Initial (g)	Month 1 (g)	Month 2 (g)	Month 3 (g)	Month 6 (g)	Mass decrease (g)
25 °C/60% RH	116.64	116.79	116.72	116.70	116.59	0.05
30 °C/60% RH	118.56	118.76	118.67	118.64	118.55	0.01
40 °C/75% RH	117.69	117.80	117.65	117.57	107.22	10.47

The hydrogel showed signs of mass variation during the first three months, but none that significant to note as drastic (Table C.9). After month 1 an increase in mass was noted. This can be because of the settling of the hydrogel or possible swelling of the hydrogel. After month 1 the mass decreased slightly due to water loss of the formulation at all temperatures. At month 6 the

hydrogels exposed to 25 °C/60% RH and 30 °C/60% RH showed slight mass loss but the hydrogel exposed to 40 °C/75% RH decreased with 10 g. The hydrogel degenerated after six months at 40 °C/75%RH and turned into a thick liquid. The hydrogel showed signs of being thermally sensitive to the increase in temperature for such a long period. The mass of hydrogel was only stable for the first three months.

C.3.5 Particle size variation

C.3.5.1 Particle variation of the emulgel

Table C.10: Particle size variation of the emulgel over 6 months

Storage temperature	(μm)	Initial	Month 1	Month 2	Month 3	Month 6
25 °C/60% RH	d(0.1)	1.39	1.41	1.35	1.42	1.45
	d(0.5)	1.99	2.25	2.12	2.85	2.54
	d(0.9)	3.04	3.15	3.54	3.64	3.71
30 °C/60% RH	d(0.1)	1.39	1.42	1.40	1.40	1.37
	d(0.5)	1.99	2.20	2.20	1.76	2.05
	d(0.9)	3.04	3.07	3.06	2.99	3.30
40 °C/75% RH	d(0.1)	1.39	1.38	1.39	1.37	1.41
	d(0.5)	1.99	1.97	2.04	2.00	2.01
	d(0.9)	3.04	2.99	3.16	3.11	10.90

The d(0.5) values were compared at each month (Table C.10) at each temperature to determine the stability of the emulgel over six months. The d(0.5) value is an indication that 50% of the particles are smaller than this value. Over the first three months small changes in particle size were observed. At the end of three months the emulgel kept at 25 °C/60% RH had the biggest increase in particle size (0.86 μm). The emulgel kept at 30 °C/60% RH experienced a small drop in particle size at the end of month 3 (0.23 μm). These changes in particle size are relatively small and the emulgel can be considered as stable over three months. The particle size for 50% of the particles at each temperature was smaller than 3 μm which falls within the parameters given by Barry (2002:36). After six months the particle size d(0.5) values did not fluctuate significantly. It was noted that the d(0.9) value for the emulgel exposed to 40 °C/75% RH had tripled in size from the initial 3.04 μm to 10.90 μm . This meant that 90% of the particles were smaller than 10.90 μm .

The increase in particle size was because the emulgel underwent phase separation after six months at 40 °C/75% RH and flocculation of the particles had occurred.

C.3.5.2 Particle variation of the emulgel containing Pheroid™

Table C.11: Particle size variation of the Pheroid™ emulgel over 6 months

Storage temperature	(µm)	Initial	Month 1	Month 2	Month 3	Month 6
25 °C/60% RH	d(0.1)	0.07	0.19	0.09	0.10	0.09
	d(0.5)	0.43	1.24	0.76	1.20	0.35
	d(0.9)	2.45	3.35	2.84	3.01	2.21
30 °C/60% RH	d(0.1)	0.07	0.19	0.09	0.09	0.14
	d(0.5)	0.43	1.27	0.54	0.72	1.62
	d(0.9)	2.45	3.38	2.47	2.96	3.11
40 °C/75% RH	d(0.1)	0.07	0.08	0.09	0.12	0.09
	d(0.5)	0.43	0.56	0.72	1.20	0.45
	d(0.9)	2.45	2.65	2.35	2.65	14.62

The d(0.5) values were compared at each month (Table C.11) at each temperature to determine the stability of the Pheroid™ emulgel over six months. Each month, for the first 3 months, the particle size of the formulations at all temperatures varied. At the end of month 1 all the d(0.5) values increased, indicating that the Pheroid™ emulgel had to settle first. The Pheroid™ emulgel kept at 40 °C/75% RH showed an increase in particle size each month while the Pheroid™ emulgels kept at the other two temperatures gave varied increased and decreased particle size values. The changes were not that significant and indicated that 50% of the particles stayed relatively small and within the size parameters given by Barry (2002:36). After six months the Pheroid™ emulgel exposed to 25 °C/60% RH experienced a decrease in particle size while the Pheroid™ emulgel exposed to 30 °C/60% RH increased in size (0.9 µm). The particle size of emulgel exposed to 40 °C/75% RH decreased in size at d(0.5) but increased significantly in size at d(0.9), indicating that flocculation of the particles inside the Pheroid™ emulgel had occurred.

C.3.5.3 Particle variation of the hydrogel

Table C.12: Particle size variation of the hydrogel over 6 months

Storage temperature	(μm)	Initial	Month 1	Month 2	Month 3	Month 6
25 °C/60% RH	d(0.1)	56.27	50.42	54.00	57.14	52.89
	d(0.5)	93.29	80.90	90.67	83.31	88.91
	d(0.9)	153.78	128.75	138.70	137.28	149.19
30 °C/60% RH	d(0.1)	56.27	51.05	58.48	49.00	47.55
	d(0.5)	93.29	86.39	93.69	81.13	79.53
	d(0.9)	153.79	145.00	138.29	135.00	130.45
40 °C/75% RH	d(0.1)	56.27	47.63	54.32	48.82	52.209
	d(0.5)	93.29	87.65	88.50	81.04	89.85
	d(0.9)	153.78	136.08	146.23	134.59	154.28

The d(0.5) values of the hydrogel showed an initial decrease in particle size after month 1 ($\pm 13 \mu\text{m}$) due to the settling of the hydrogel (Table C.12). At month 2 the particle sizes increased where after it decreased at month 3. The particles were larger than $10 \mu\text{m}$ indicating that a large amount would not penetrate the skin (Barry, 2002:36). The particle size values determined at month 6 showed increased particle sizes at the 25 °C/60% RH and 40 °C/75% RH temperatures and a decrease at 30 °C/60% RH. The particle sizes over the six month period produced large values indicating that crystallisation might have occurred in the emulgel formulation which produced particles too big for skin penetration (Barry, 2002:36).

C.3.6 Zeta potential

C.3.6.1 Zeta potential of the emulgel

Table C.13: Zeta potential measurements of the emulgel over 6 months

Storage temperature	Initial (mV)	Month 1	Month 2	Month 3	Month 6	Average
25°C/60% RH	20.7	20.6	21.1	20.8	20.3	20.7
30°C/60% RH	20.7	17.1	22.2	19.4	17.5	19.4
40°C/75% RH	20.7	20.6	18.4	21.6	17.5	19.8

The zeta potential of the emulgel kept at 25 °C/60% RH stayed stable over the six month period (Table C.13). The zeta potential of the formulations kept at 30 °C/60% RH and 40 °C/75% RH experienced small changes but stayed relatively close to their initial values. Because the zeta potential values had slight changes over the six month period, the formulation appeared to be stable. According to Malvern Instruments (2009:16.1, 2), an emulsion is only stable when the zeta potential is bigger than +30 mV or smaller than -30 mV. The zeta potential values of the emulgel do not fall within this range, which indicates that the particles might not sufficiently ward off particles of the same charge. This leads to emulsion instability.

C.3.6.2 Zeta potential of the Pheroid™ emulgel

Table C.14: Zeta potential measurements of the Pheroid™ emulgel over 6 months

Storage temperature	Initial (mV)	Month 1	Month 2	Month 3	Month 6	Average
25°C/60% RH	21.05	22.3	20.2	22.6	20.4	21.3
30°C/60% RH	21.05	20.1	22.4	20.9	19.5	20.8
40°C/75% RH	21.05	18.7	19.6	21.2	18.0	19.7

The Pheroid™ emulgel produced relatively stable, but weak zeta potential values over the six month period (Table C.14). The Pheroid™ emulgel exposed to 40 °C/75% RH showed the biggest decrease in zeta potential. The zeta potential of the Pheroid™ emulgel stayed stable but portrayed weak values which might cause flocculation and formulation instabilities.

C.3.6.3 Zeta potential of the hydrogel

Table C.15: Zeta potential measurements of the hydrogel over 6 months

Storage temperature	Initial (mV)	Month 1	Month 2	Month 3	Month 6	Average
25°C/60% RH	-113.1	-33.9	-43.9	-19.5	-31.8	-48.4
30°C/60% RH	-113.1	-105.4	-37.8	-14.1	-5.7	-55.2
40°C/75% RH	-113.1	-20.7	-16.3	22.1	23.9	-20.8

The hydrogel showed a very unstable zeta potential profile over six months (Table C.14). A big decrease was seen in month 1 for the hydrogel at all temperatures. The zeta potential decreased

even more with the hydrogel at 40 °C/75% RH, producing a positive value at the end of month 6. These changes in zeta potential can be due to the varying pH of the hydrogel at each month (Malvern Instruments 2009:16.1, 2). As the pH became less alkaline each month, the zeta potential of the hydrogel responded accordingly with lower and lower negative values each month.

C.3.7 Physical assessment

C.3.6.1 Visual appearance of the emulgel

No significant change was noted in the visual appearance of the emulgel stored at 25 °C/60% RH and 30 °C/90% RH over the six month period. The visual appearance of the emulgel stored at 40 °C/75% RH changed over the six month period. Phase separation (Figure C.4.d) occurred after three months of exposure to the high temperature and humidity (40 °C/75% RH).

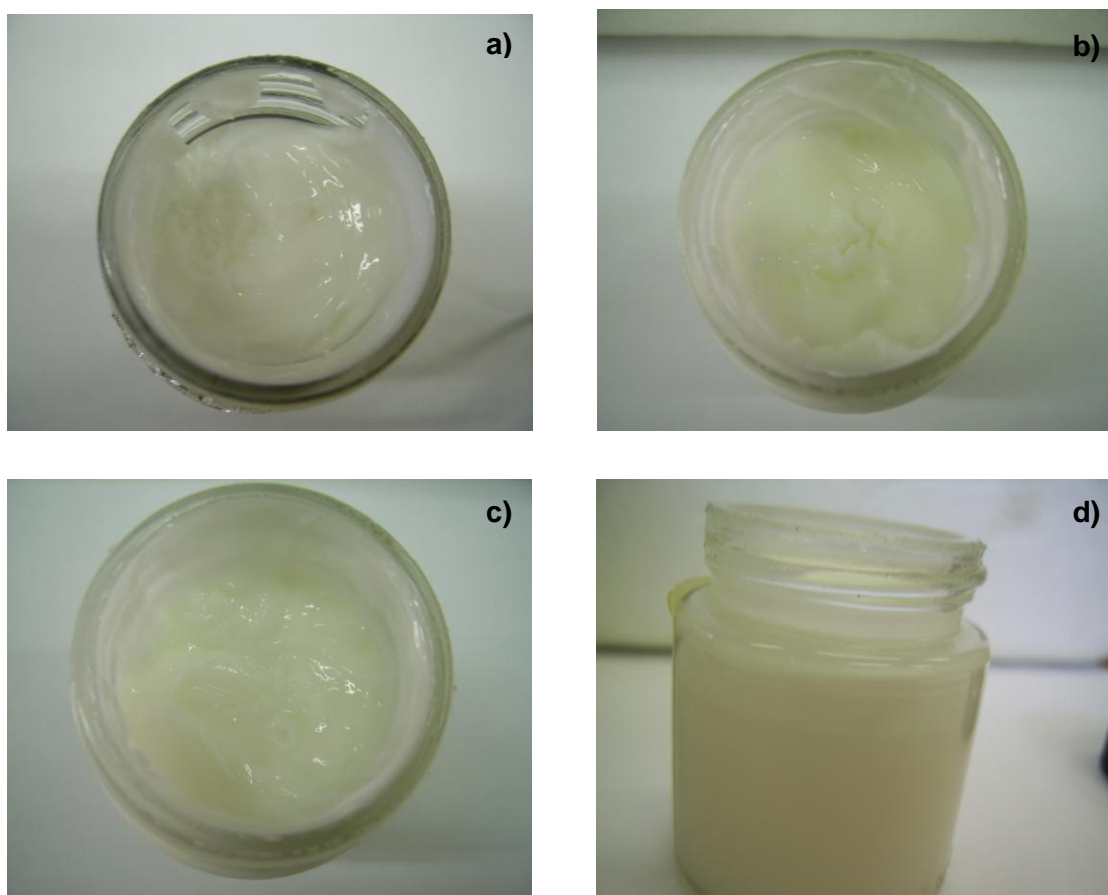


Figure C.4: Change in visual appearance for emulgel after 6 months: **a)** the initial visual appearance, **b)** 25 °C/60% RH, **c)** 30 °C/60% RH and **d)** 40 °C/75% RH

C.3.6.2 Visual appearance of the Pheroid™ emulgel

The visual appearance Pheroid™ emulgel changed significantly over the six month period. The Pheroid™ emulgel stored at 40 °C/75% RH showed the most drastic change over six months. The colour of the formulation changed from milky white to a creamy dark yellow. The Pheroid™ emulgel stored at 25 °C/60% RH discoloured to an off-white colour, while the 30 °C/60% RH Pheroid™ emulgel turned light yellow. This discolouration can be contributed to the oxidation of the light-sensitive Pheroid™ ingredients by atmospheric oxygen. The drastic change in visual appearance proved that the formulation was not stable and the discolouration could influence consumer acceptability.

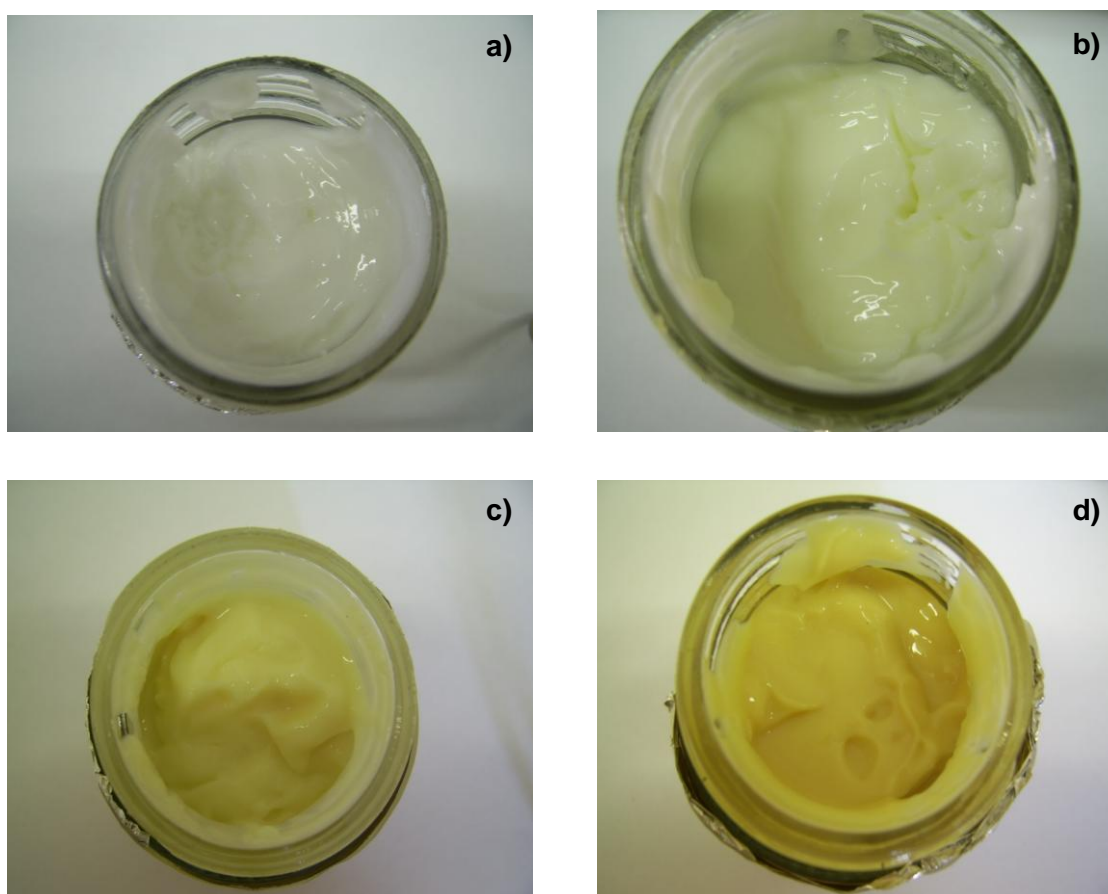


Figure C.5: Change in visual appearance for Pheroid™ emulgel after 6 months: **a)** the initial visual appearance, **b)** 25 °C/60% RH, **c)** 30 °C/60% RH and **d)** 40 °C/75% RH

C.3.6.3 Visual appearance of the hydrogel

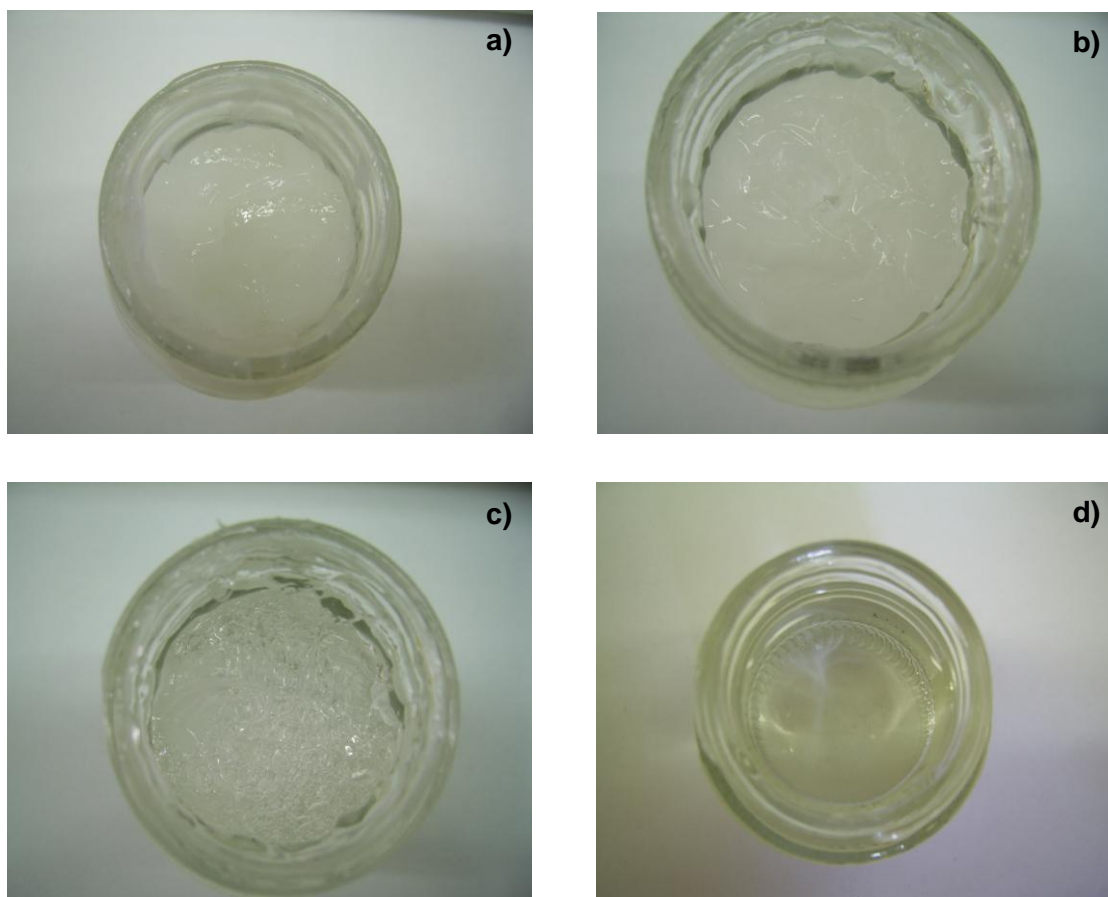


Figure C.6: Change in visual appearance for hydrogel after 6 months: **a)** the initial visual appearance, **b)** 25 °C/60% RH, **c)** 30 °C/60% RH and **d)** 40 °C/75% RH

The initial visual appearance of the hydrogel right after formulation and pH adjustment was a cloudy white colour. Over six months the visual appearance of the hydrogel changed significantly. The hydrogel became more transparent at each higher temperature and relative humidity. Both the hydrogel stored at 30 °C/60% RH and 40 °C/75% RH produced air bubbles in the formulation after three months. This can be attributed to the hydrogel not settling properly at the higher temperatures after it had been placed in the humidity chambers after formulation. The initial cloudy white colour was obtained after pH adjustment and the fact that the hydrogels at the two higher temperatures became more transparent indicated a possible change in pH. This was verified after pH measurements were taken and indicated a decrease in pH. The hydrogel stored at 40 °C/75% RH underwent a phase change because of stress caused by the high storage temperature after six months. It changed from a semi-solid to a thick transparent fluid

(Figure C.6.d). It can be concluded that the hydrogel stored at 25 °C/60% RH were more stable than the hydrogels stored at 30 °C/60% RH and 40 °C/75% RH.

C.4 CONCLUSION

The formulated products underwent stability testing for a period of six months. The outcomes of the stability tests showed that the lidocaine HCl was the more stable API in all of the formulations. The prilocaine HCl concentration decreased significantly in all of the formulations after six months. The pH of all the formulations varied extensively over the six month period and could cause a change in the anaesthetic effectiveness of the products. None of the formulations exhibited perfect stability over the six month period. The results of the chemical assay concluded that the APIs were more stable in the emulgel formulation. Significant changes in pH and particle size were noted for all the formulations after six months. Mass loss and viscosity measurements produced the most stable values over the six month testing period. The zeta potential for the hydrogel had undergone the largest change over six months while the zeta potential values for the emulgel and Pheroid™ emulgel stayed stable. The visual appearance of the Pheroid™ emulgel underwent severe changes over six months, indicating instability. All of the formulated products failed to comply with the product stability guidelines of the MCC.

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APPENDIX D

TRANSDERMAL DIFFUSION STUDIES

D.1 INTRODUCTION

Transdermal API delivery has become an increasingly popular method for delivering APIs for the treatment of various conditions. Transdermal delivery is a field that has been advancing at a rapid pace due to its advantages over the traditional oral route of administration. It avoids the hepatic metabolism, provides better control over plasma levels of potent drugs and patients have higher acceptability for the product which in turn improves patient compliance (Kogan & Garti, 2006:371).

Topical formulations like creams, gels and emulsions are used to deliver drugs into the local tissues directly at the site of application (Gosh & Pfister, 1997:7). Topical anaesthesia is commonly used on intact skin to provide anaesthesia for minor skin-breaking procedures. The local anaesthetic agents' target site is the dermis of the skin where the free nerve endings are situated. The only disadvantage with the topical use of anaesthetics is the time it takes for the anaesthetic effect to commence (Young, 2007:232,234).

In vitro diffusion studies were conducted during this research project to determine if the local anaesthetics lidocaine HCl and prilocaine HCl in three different formulations penetrated the dermis effectively. Vertical Franz diffusion cells were used for the diffusion studies, as well as for the membrane release studies. The membrane release studies were performed to determine if the two APIs were released from their topical formulations. Two emulgel formulations and one hydrogel formulation were tested. Pheroid™ was incorporated into one emulgel formulation in order to compare the permeation results with the emulgel formulation without Pheroid™. During this study dermatomed Caucasian skin with a thickness of 400 µm was used. Diffusion studies on a commercial product containing the same concentration of APIs as the prepared formulations were included in this study. The commercial product's diffusion rate through the skin was then compared with the diffusion rate of the three formulated products in this study. In order to determine the dermal delivery of the APIs, tape stripping techniques were utilised. The aim of this study was to determine if the incorporation of Pheroid™ into a semi-solid formulation containing lidocaine HCl

and prilocaine HCl shortened the lag time of the APIs and to determine if lidocaine HCl and prilocaine HCl in semi-solid formulations penetrated transdermally.

D.2 METHODS

D.2.1 Determination of the concentration of lidocaine HCl and prilocaine HCl

The concentration of lidocaine HCl and prilocaine HCl in the receptor phase, stratum corneum-epidermis and epidermis-dermis samples were determined with HPLC. A HPLC method was validated for this analysis, as described in Appendix A. A controlled laboratory environment where the temperature was regulated at 25 °C was maintained at the Analytical Laboratory, North-West University, Potchefstroom Campus, South Africa during the concentration analysis.

The samples collected from the receptor phase of the diffusion experiments were analysed using an Agilent® 1200 series HPLC system (Agilent Technologies, United States of America). The system was equipped with an Agilent® 1200 quaternary pump, autosampler injection mechanism, vacuum degasser, solvent module and diode array detector. The HPLC system is specially equipped with Chemstation Rev. A.06.02 data acquisition and analysis software. A High performance silica based, reversible Phenomenex® Luna C₁₈ (2) column (4.6 x 150 mm), with a 5 µm particle size was used during the concentration analysis (Phenomenex, United States of America).

The mobile phase consisted of 100% analytical HPLC grade acetonitrile. The sample injection volume was set at 20 µl at a flow rate of 1 ml/min. The sample runtime was 6 min with the retention times for prilocaine HCl at 3.2 – 3.4 min and lidocaine HCl at 4.1 – 4.3 min (see Appendix A for the full description of the analytical method).

D.2.2 Method for determining the solubility of lidocaine HCl and prilocaine HCl

Solubility experiments were conducted on lidocaine HCl and prilocaine HCl, both highly soluble in water. The experiments were done over a 24 h period in a water bath at 32 °C. Oversaturated solutions of each API were prepared separately with PBS (pH 8) as solvent. The samples were stirred continuously at 750 rpm. The samples were monitored and more API was added if the entire amount of API had dissolved. The API solutions were prepared in triplicate. After 24 h the samples were filtered with a pre-filter/PVDF 0.45 µm filter and centrifuged before analysis by HPLC.

D.2.3 Preparation of the donor and receptor phases for the Franz cell diffusion studies of lidocaine HCl and prilocaine HCl formulations

An emulgel, Pheroid™ emulgel, hydrogel and a commercial product containing 2.5% lidocaine HCl and 2.5% prilocaine HCl as the APIs were used as donor phases for both the membrane release studies and skin diffusion studies. Formulations were prepared as described in Section B.2.3.

Except for the four semi-solid formulations, two solutions were also formulated and used in the donor phase during skin diffusion studies, namely a PBS solution and a Pheroid™ solution. The PBS solution and the Pheroid™ solution consisted of lidocaine HCl (2.5%) and prilocaine HCl (2.5%) as APIs. The solution was prepared by weighing 2.5 g of lidocaine HCl and 2.5 g of prilocaine HCl and transferring it to a 100 ml volumetric flask. The volumetric flask was filled to volume with PBS at a pH of 8 for increased unionised/ionised fractions of the APIs. The Pheroid™ solution was prepared by weighing 2.5 g of lidocaine HCl and 2.5 g prilocaine HCl and transferring it to a 100 ml volumetric flask. The volumetric flask was filled to volume with Pheroid™ solution containing Pheroid™ ingredients. The Pheroid™ solution was then placed on a stirrer for 18 h prior to the diffusion study. This was done to ensure the entrapment of the APIs in the Pheroid™ vesicles.

The receptor phase consisted of PBS prepared at the same pH as that of the body fluids, namely 7.4. The PBS was prepared by carefully measuring 6.810 g potassium orthophosphate and dissolving it in 250.0 ml HPLC grade water. The solution was placed on a mixing plate while a solution containing 1.573 g of sodium hydroxide dissolved in 393.5 ml HPLC grade water was added. The pH was adjusted to 7.4 with 10% phosphoric acid (H₃PO₄). The pH of all the donor phases prepared for the diffusion studies was adjusted to 8 with 10% sodium hydroxide. This was done in order to increase the unionised fraction of the APIs for better skin penetration. The pH of the commercial product had a high pH value of 9, which indicated that a large amount of the APIs were in their unionised fraction.

D.2.4 Preparation of lidocaine HCl and prilocaine HCl standard preparations for concentration analysis

Lidocaine HCl (25 mg) and prilocaine HCl (25 mg) were carefully transferred into a 100 ml volumetric flask and filled up to volume with PBS (pH 7.4). The standard solution (10 ml) was extracted and diluted in a 20 ml volumetric flask with PBS to prepare a second standard solution.

Thereafter, 10 ml of the second standard solution was extracted and transferred into a 20 ml volumetric flask and again filled to volume with PBS to obtain a third standard solution. Both the second and third standard solutions were injected at the following concentrations: 5 µg/ml, 10 µg/ml, 15 µg/ml, 20 µg/ml and 25 µg/ml, to obtain a standard curve. Fresh standard samples were prepared before each diffusion study.

D.2.5 Membrane release studies

Membrane diffusion studies were performed on the two emulgel formulations, one hydrogel formulation and a commercial product. This was done to determine if the APIs were released from the different formulations. If no API concentrations were obtained during the membrane studies, it indicated that the APIs were not released effectively from their various formulations and diffusion through the skin would most probably not be possible. The Franz cells were assembled in the exact same manner as described in Section D.2.7, except that the skin circles had been replaced with polytetrafluoroethylene (PTFE) membranes. These PTFE membranes were 0.4 µm thick. Sink conditions were maintained by monitoring the water bath temperature at 37 °C. The receptor compartment withdrawal times were set at 1, 2, 3, 4, 5 and 6 h. The receptor compartments were immediately filled with 2 ml fresh PBS (37 °C) after each withdrawal. The withdrawn receptor compartment samples were then transferred to HPLC vials and analysed immediately with HPLC.

D.2.6 Preparation of Caucasian skin for Franz cell diffusion studies

The skin used for the permeation studies was obtained under ethical approval from the North-West University's Research Ethics Committee, reference number NWU-00114-11-A4. Caucasian abdominal female skin was donated by female patients who had undergone abdominoplastic cosmetic surgery. Informed consent was given by all donors before undergoing surgery and their identities were not disclosed.

Full-thickness skin was removed from the abdomen of the Caucasian female patients and was frozen at -20 °C for no longer than 24 h. Dermatomed skin strips of about 2 cm in width and 4 cm in length were obtained by using a Dermatome™ (Zimmer® LTD, United Kingdom). These skin strips were 400 µm thick. The strips were placed on Whatman® filter paper and left to dry for approximately 10 min and thereafter cut into circles with a 15 mm diameter. The prepared skin samples were then wrapped in aluminium foil and sealed in plastic bags for storage in a freezer at -20 °C until utilised. It is possible for the skin circles to remain useable and for the barrier function to

stay intact for 6 months under stable conditions (Leveque *et al.*, 2004:324). The frozen skin samples were thawed and visually examined for defects like stretch marks or hair before mounting them onto the diffusion apparatus (Baert *et al.*, 2011:472).

D.2.7 Procedure for transdermal Franz cell diffusion studies with formulations containing lidocaine HCl and prilocaine HCl as active pharmaceutical ingredients

Vertical Franz diffusion cells were used to determine the permeation of the APIs through the skin. The Franz cells consist of a donor (top) compartment and a receptor (bottom) compartment (Figure D.1). The receptor compartment has a capacity of 2 ml and the donor compartment provides a diffusion area of 1.075 cm². The receptor compartments were filled with 2 ml PBS (at body fluid pH 7.4) and the donor compartments with 1 ml of the formulation or solution. One diffusion study was performed for each formulation. Six diffusion studies were performed in total. Twelve Franz cells were utilised per study. Ten cells were filled with the API containing formulation or solution and the remaining two cells were filled with placebo formulation or solution to provide a control set.

The Franz cells were assembled in the following manner:

- ❖ The dermatomed skin circles were thawed and mounted between the receptor and donor compartments with the stratum corneum facing upwards.
- ❖ The donor and receptor compartments were then sealed using Dow Corning[®] vacuum grease.
- ❖ Metal horseshoe clamps (Figure D.2) was utilised to clamp the two compartments tightly together to prevent leakage.
- ❖ A magnetic stirrer bar was inserted into the receptor compartment.
- ❖ 1 ml of formulation or solution was inserted carefully into the donor compartment. The formulation or solution was preheated in a water bath at 32 °C as this corresponds to normal exposed skin temperature.
- ❖ A square of Parafilm[®] was then used to cover the top part of the donor compartment to prevent evaporation of the formulation or solution during the 12 h experiment.
- ❖ A plastic cap was used to keep the Parafilm[®] tightly in place.
- ❖ The PBS (pH 7.4) was placed in a 37 °C water bath so that it mimics blood temperature.

- ❖ 2 ml of the PBS was then inserted into the receptor compartment. This is done in a careful manner to insure that no air bubbles forms beneath the skin.
- ❖ The assembled Franz cell is shown in Figure D.3.

After the twelve Franz cells had been assembled they were placed inside a Grant[®] water bath (Grant Instruments, United Kingdom) (Figure D.4) on a Variomag[®] magnetic stirrer plate (Variomag, United States of America). The water bath temperature was set at body temperature, 37 °C, and the stirring speed at 750 rpm. The Franz cells were placed inside the water bath with the receptor compartments completely submerged under water. The regulated temperature of the receptor compartments (37 °C) resulted in a donor compartment skin surface temperature of 32 °C (Williams, 2003:62). The content of the receptor compartments were withdrawn at predetermined time intervals of 20; 40, 60, 80, 100 min and 2, 4, 6, 8, 10 and 12 h. The receptor compartments were then immediately replaced with 2 ml fresh PBS (pH 7.4) that had been preheated to 37 °C. This ensured that sink conditions persisted for the whole 12 h experiment. The samples were transferred to HPLC vials and analysed immediately. The extraction times were used with the objective to determine the time of onset of anaesthesia.

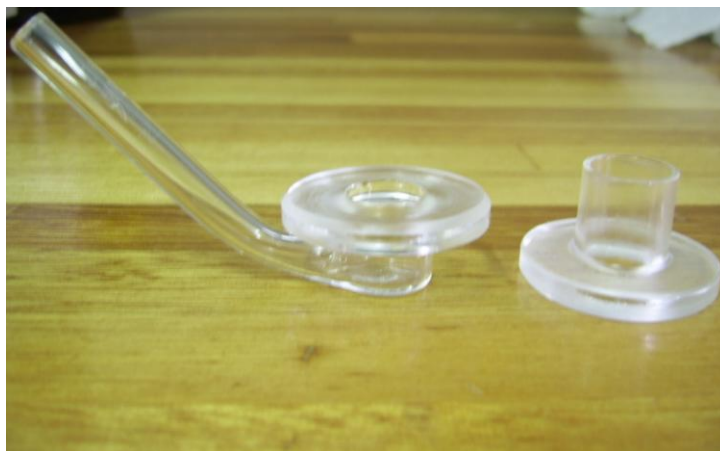


Figure D.1: Receptor and donor compartments of a Franz diffusion cell



Figure D.2: Horseshoe clamp



Figure D.3: Assembled Franz diffusion cell



Figure D.4: Grant[®] water bath

D.2.8 Analysing API concentrations in the epidermal skin layers utilising the tape stripping technique

The technique of tape stripping is used to analyse the amount of the API in the outer layers of the skin (Pershing *et al.*, 1993:352). Tape stripping can help determine the efficacy with which the API is delivered from its topical formulation (Ademola, 1997:532).

After the completion of each 12 h diffusion study the donor and receptor compartments of the Franz cells were carefully separated. The skin samples were removed and pinned to circles of Whatman[®] filter paper. The diffusion area of each piece of skin, which was clearly visible, was dabbed dry with a paper towel. Sixteen strips of 3M Scotch[®] Magic[™] tape were cut for each piece of skin. The strips were cut big enough to fit over the diffusion area. The first strip of each piece of skin was discarded as it could be contaminated with formulation leftover on the skin. The skin was then stripped with the remaining fifteen tape strips. The skin was stripped until the stratum corneum-epidermis was removed and the viable epidermis started to glisten. The fifteen tape strips were placed in a polytop vial filled with 5 ml PBS (pH 7.4). The polytop vials containing the tape strips were kept at 4 °C overnight to ensure the release of the APIs from the tape strips into the PBS solution.

The excess skin was removed from the remaining skin circles so that only the diffused skin remained (epidermis-dermis). The epidermis-dermis parts obtained from the Franz diffusion cells were then shredded into smaller pieces and placed inside a polytop vial filled with 5 ml PBS (pH 7.4). The skin was shredded into smaller pieces to ensure that the APIs would be released into the solution. The polytop vials with the epidermis-dermis samples were kept overnight at 4 °C to ensure the release of the APIs from the skin epidermis-dermis into the PBS solution. The next morning tape strip (stratum corneum-epidermis) and epidermis-dermis samples were extracted and transferred into HPLC vials for analysis on the HPLC.

D.2.9 Statistical analysis of the data obtained from the Franz cell diffusion studies

The data from the transdermal diffusion studies were statistically analysed, using quantitative statistical methods. SPSS software (SPSS Inc., 2011) was used to perform the analysis. Statistically it is important to take note of the following points:

- ❖ Availability samples were taken but the data could not be generalised because skin samples for one diffusion study were only attained from one donor, so the focus of the analysis

shifted to the practical significance of the data. The p-values were still noted to be complete.

- ❖ The confounding factors included the manner in which the skin samples were generated. Because each formulation was tested only on the skin from a single donor, n is too small to apply statistical methods. However, the confounding impact of the skin was ignored to show how the data could be statistically analysed.
- ❖ Parametric tests of normality and equal variances were fitted to the data where the mean values were used instead of the median values. The median values are still noted together with the transdermal data to be complete.

The stratum corneum-epidermis data obtained from tape stripping, the epidermis-dermis data and cumulative concentrations for each formulation were compared to determine if there were any differences between the averages of the different treatment groups. The stratum corneum-epidermis and epidermis-dermis data were compared with the cumulative concentration at 12 h in order to determine if any significant differences in the data were present. The flux data was also analysed by means of descriptive statistics and to determine the differences between the flux values of the different treatment groups. The mean, median and standard deviation (SD) were calculated as part of descriptive statistics. A linear regression model was fitted to the flux data of each repeat (individual Franz cell) to obtain estimated y-intercepts and gradient (flux) values. The r^2 values obtained from the linear regression were $r^2 > 0.8$ for all models and statistically significant where $p < 0.05$ for all models. A one-way ANOVA model was performed to obtain further statistical data.

A one-way variance analysis (ANOVA) was performed on the stratum corneum-epidermis, epidermis-dermis and cumulative concentration data set, as well as on the flux value data set. The Kolmogorov-Smirnov test and Shapiro-Wilk test were performed and graphically inspected using QQ-plots to determine the normal variance of the data in both data sets. Levene's test was utilised and graphically inspected, using box-plots to determine the equal variance of the data in both data sets. The data was considered statistically significant where $p < 0.05$. Deviations were not severe; however, robust tests were also performed. The one-way ANOVA tests revealed that there were significant differences between the treatments for all measures of both data sets. Post hoc tests were used to reveal where these differences occurred. The Games-Howell test was performed to determine the statistically significant differences between treatments where $p < 0.05$ is statistically

significant. Cohen's test was performed to determine the practically significant differences or effect size between treatments where $d > 0.8$ indicated a large practical significance in the data.

A mixed model was fitted to the data to determine if significant differences exist between the treatments (formulations) or times, or the interaction between the treatments and times. An AR(1) covariance structure was specified for repeated measures over time. The type III tests for fixed effects indicated that time and treatment, as well as the interaction between time and treatment all had a significant influence where $p < 0.05$. The significance of the lag times of lidocaine HCl and prilocaine HCl was included in the mixed model data.

D.3 RESULTS AND DISCUSSION

D.3.1 Physicochemical properties of lidocaine HCl and prilocaine HCl

The physicochemical properties of an API formulated for skin delivery is extremely important and have been discussed in Section 2.3.6. There are, however, two physicochemical properties which play a crucial role in the prediction of the transdermal diffusion of an API, namely, the log P value (octanol/water coefficient) and the aqueous solubility.

The log P value (octanol/water partition coefficient) is an important indication of how an API will penetrate the skin and how a local anaesthetic will diffuse across the lipid-rich membrane of the nerve to reach the target nerve endings (McLure & Rubin, 2005:63). A log P value between 1 and 3 will insure that an API can permeate the skin relatively fast (Williams, 2003:36). Lidocaine HCl has a log P value of 3.4 (Lund, 1994:938) and prilocaine HCl a log P of 2.1 (Analysis of Drugs and Poisons, 2010). The partition coefficient of lidocaine HCl in octanol/buffer at pH 7.4 is 43 and for prilocaine HCl is 25 (O'Columb & Ramsaran, 2010:115; McLure & Rubin, 2005:64). With log P values of 3.4 and 2.1 for lidocaine HCl and prilocaine HCl, respectively, we can conclude that these APIs will permeate the skin efficiently; however, no single characteristic can be a complete indication of the ability to diffuse or not.

The aqueous solubility of an API is an important physicochemical property to consider during formulation. The ideal value for an API considered for transdermal delivery is an aqueous solubility larger than 1 mg/ml (Naik *et al.*, 2000:319). The solubility experiments of lidocaine HCl and prilocaine HCl yielded high values of 579.8 mg/ml for lidocaine HCl and 388.86 mg/ml for prilocaine HCl. The high solubility values of lidocaine HCl and prilocaine HCl indicate that these two APIs are ideally suited for transdermal delivery.

D.3.2 Membrane release experiments of lidocaine HCl and prilocaine HCl

Membrane release studies were performed on the emulgel, Pheroid™ emulgel, hydrogel and a commercial product to determine how lidocaine HCl and prilocaine HCl were released from these formulations. The percentage lidocaine HCl that diffused through the membrane after 6 h, as well as the cumulative concentration ($\mu\text{g}/\text{cm}^2$) is given in Table D.1.

Table D.1: The amount of lidocaine HCl that diffused through PTFE membranes after 6 h

Formulation	Average % lidocaine HCl released after 6 h	Average cumulative concentration lidocaine HCl after 6 h ($\mu\text{g}/\text{cm}^2$)	Median cumulative concentration lidocaine HCl after 6 h ($\mu\text{g}/\text{cm}^2$)
Emulgel	1.47	366.94	377.43
Pheroid™	1.18	295.84	313.82
Hydrogel	3.91	977.73	899.68
Commercial	1.71	428.56	467.99

The average cumulative concentration of lidocaine HCl released from the hydrogel formulation was the highest with $977.73 \mu\text{g}/\text{cm}^2$ followed by the commercial product with $428.56 \mu\text{g}/\text{cm}^2$, the emulgel with $366.94 \mu\text{g}/\text{cm}^2$ and lastly the Pheroid™ emulgel with $295.84 \mu\text{g}/\text{cm}^2$. From these results we can deduce that the lidocaine HCl released poorly from the Pheroid™ emulgel formulation.

The difference between the average cumulative concentration and the median cumulative concentration of lidocaine HCl after 6 h for the emulgel and Pheroid™ emulgel did not vary notably and this can be attributed to a small amount of outliers in the data. The hydrogel and commercial product showed larger differences between the average and median cumulative concentration values after 6 h. The results had more prominent outliers in the data. The cumulative lidocaine HCl concentration against time for each Franz cell differed notably. The median value is determined by taking the centre point of the data while the average is the sum of the data divided by the number of data points. If a huge variation between the average and median value is observed, it would be more accurate to use the median value as it is not affected by outliers in the data (Gerber *et al.*, 2008:190).

Table D.2 is a summary of the percentage prilocaine HCl that diffused through the membrane and the cumulative concentration of prilocaine HCl after 6 h.

Table D.2: The amount of prilocaine HCl that diffused through PTFE membranes after 6 h

Formulation	Average % prilocaine HCl released after 6 h	Average cumulative concentration prilocaine HCl after 6 h ($\mu\text{g}/\text{cm}^2$)	Median cumulative concentration prilocaine HCl after 6 h ($\mu\text{g}/\text{cm}^2$)
Emulgel	1.66	414.36	413.83
Pheroid™	1.19	296.33	314.19
Hydrogel	3.94	985.21	879.85
Commercial	1.51	376.20	423.58

The release profile for prilocaine HCl followed the same pattern as lidocaine HCl with the percentage prilocaine HCl released and cumulative concentration prilocaine HCl slightly higher for each formulation except the commercial product. The hydrogel gave the best prilocaine HCl release with an average cumulative concentration of $985.21 \mu\text{g}/\text{cm}^2$ after 6 h. The Pheroid™ emulgel produced the lowest amount of API with an average cumulative concentration of $296.33 \mu\text{g}/\text{cm}^2$. Both the APIs formulated easily in the water-based formula because of their hydrophilic nature. The results from the membrane studies indicated that the hydrogel formulation allowed higher concentrations of the APIs to diffuse through the artificial membranes. It is, however, important to note that artificial membranes are poor representatives of human skin and their characteristics cannot come close to the complicated layers of the skin which are more lipophilic and may prove advantageous to the more lipophilic emulgel and Pheroid™ emulgel formulations (Williams, 2003:57).

The variations between the average cumulative concentration prilocaine HCl and median cumulative concentration prilocaine HCl after 6 h in the emulgel and Pheroid™ emulgel did not vary notably. The commercial product and hydrogel showed greater variations in the median and average cumulative concentration and had more notable outliers in the data and greater concentration variation for each Franz cell against time.

The purpose of the membrane release studies was to evaluate the release of the APIs from their formulations and positive results were obtained. All formulations released lidocaine HCl and prilocaine HCl effectively. It is important to mention that a direct correlation is not expected between the release of the API from the formulation through PTFE membranes and diffusion through the skin.

D.3.3 Franz cell skin diffusion of formulations containing lidocaine HCl and prilocaine HCl as active ingredients

D.3.3.1 Determining the lag time of lidocaine HCl and prilocaine HCl

The cumulative concentration of the APIs obtained during the 12 h diffusion study was plotted against time. Both APIs depicted simple zero-order flux behaviour. The lag time of an API can be described as the time it takes for the API to reach a steady-state flux (Barry, 2006:572). The lag time can then be determined by extrapolating a straight line from the steady-state flux or where the plot becomes linear (Barry, 2006:572). One of the disadvantages of topical anaesthesia is the long time it takes to produce an anaesthetic effect in the skin dermis. Pheroid™ has proved to increase the lag time and skin delivery of several substances into the skin (Grobler *et al.*, 2008:293). The lag times of the three formulations were compared with each other and to that of the commercial product to determine if any of the formulations produced better results. The lag times were determined between 2 - 12 h. The lag times of the two solutions (Section D.2.2) are also included in Tables D.3 and D.4.

D.3.3.1.1 Lidocaine hydrochloride

The lag times of lidocaine HCl are shown in Table D.3 with the percentage lidocaine HCl that diffused through the skin after 12 h. The shortest lag time of lidocaine HCl was observed from the use of the emulgel (1.65 h). After 12 h 0.63% of the lidocaine HCl had diffused through the skin from the emulgel formulation. The lidocaine HCl reached a steady-state flux after 1.70 h in the commercial formulation and produced a total percentage of lidocaine HCl diffused that was four times the amount produced than that of the emulgel after 12 h. The Pheroid™ solution (1.84 h) and the Pheroid™ emulgel (1.99 h) had longer lag times, which meant that the lidocaine HCl was slower to produce a steady-state flux from these formulations. After 12 h the Pheroid™ solution yielded 75% more lidocaine HCl that diffused through the skin than the Pheroid™ emulgel. In comparison with the commercial product's lag time and total percentage lidocaine HCl that diffused through the skin after 12 h, the lidocaine HCl performed poorly from the Pheroid™ emulgel. The lidocaine HCl released from the Pheroid™ emulgel took 0.29 h longer than the commercial product to reach a steady-state of API flux and did not improve the lag time of lidocaine HCl. The lag times of the different formulations did not vary extensively from the lag time of the commercial product.

Table D.3: Percentage lidocaine HCl diffused after 12 h and the lag time of lidocaine HCl from each formulation

	Lag time (h)	%Diffused lidocaine HCl after 12 h
Emulgel	1.65	0.63%
Pheroid™ emulgel	1.99	0.37%
Hydrogel	1.87	0.69%
Commercial product	1.70	2.61%
PBS solution	1.76	1.65%
Pheroid™ solution	1.84	1.05%

The lag times of lidocaine HCl from the different formulations in order of magnitude are: emulgel < commercial < PBS solution < Pheroid™ solution < hydrogel < Pheroid™ emulgel. The total percentage of lidocaine HCl that diffused through the skin from each formulation is commercial > PBS solution > Pheroid™ solution > hydrogel > emulgel > Pheroid™ emulgel. The emulgel formulation produced a shorter lag time for lidocaine HCl than all the other products, but did not have a high percentage of lidocaine HCl that diffused after 12 h.

D.3.3.1.2 Prilocaine hydrochloride

The lag time profiles and percentage of prilocaine HCl that diffused through the skin after 12 h differed slightly from that of lidocaine HCl and are given in Table D.4.

Table D.4: Percentage prilocaine HCl diffused after 12 h and the lag time of prilocaine HCl from each formulation

	Lag time (h)	%Diffused prilocaine HCl after 12 h
Emulgel	1.93	0.76%
Pheroid™ emulgel	1.41	0.77%
Hydrogel	2.11	2.18%
Commercial product	1.53	1.51%
PBS solution	1.60	1.31%
Pheroid™ solution	1.98	1.04%

A short lag time of 1.41 h for prilocaine HCl was obtained from the Pheroid™ emulgel. The prilocaine HCl in the Pheroid™ emulgel reached a steady-state flux 0.52 h before lidocaine HCl in the same formulation. The percentage prilocaine HCl that diffused through the skin from the Pheroid™ emulgel was low (0.77%), compared with that of the hydrogel (2.18%) and the

commercial product (1.51%). The prilocaine HCl in the hydrogel formulation took the longest time to reach a steady-state flux and had the longest lag time as a result (2.11 h). The lag time of prilocaine HCl from the commercial product was shorter (1.53 h) than the lag time of lidocaine HCl (1.70 h) in the same formulation. The lag time of prilocaine HCl from the Pheroid™ solution (1.98 h) was longer than the lag time of prilocaine HCl from the Pheroid™ emulgel but the Pheroid™ solution had a higher percentage of prilocaine HCl that diffused through the skin after 12 h (1.04%).

The lag times of prilocaine HCl from the different formulations in order of magnitude are: Pheroid™ emulgel < commercial < PBS solution < emulgel < Pheroid™ solution < hydrogel. The total percentage of prilocaine HCl that diffused through the skin from each formulation is hydrogel > commercial > PBS solution > Pheroid™ solution > Pheroid™ emulgel > emulgel.

The commercial product produced better results than the formulated products. It is important to note that the pH of the commercial product is 9 which makes a considerable difference in the amount of unionised/ionised fractions. More of the APIs are unionised at a pH of 9 which contributes to the better skin penetration and faster time of onset. The stratum corneum can endure pH adjustments as high as 9. Very few topical products are formulated at such a high basic pH as this may cause damage to the skin (Barry, 2007:576). The formulated products were formulated at a lower pH of 8 where only 52% of the APIs are unionised. This explains the lower amount of total diffused API after 12 h and slower time of onset. Overall the Pheroid™ emulgel produced a shorter lag time for prilocaine HCl than the emulgel, hydrogel and Pheroid™ solution but produced the lowest percentage of APIs that diffused through the skin.

D.3.3.1.3 Statistical analysis of the correlation between time and the active pharmaceutical ingredients in the different formulations

The mixed model statistical results between time and treatment for lidocaine HCl and prilocaine HCl are given in Tables D.5 and D.6, respectively. Lag time indicated in red is where the value is statistically significant. If the lower or upper bound value includes a zero, the confidence interval becomes statistically insignificant.

Table D.5: Mixed model statistical analysis indicating the mean lag time of lidocaine HCl

Treatment	Time	Mean	Std. Error	Deg. freedom (df)	95% Confidence Interval	
					Lower Bound	Upper Bound
Emulgel	0.00	-2.84 x10 ⁻¹²	29.91	50.61	-60.06	60.06
	0.33	2.88	29.91	50.61	-57.18	62.95
	0.67	8.55	29.91	50.61	-51.51	68.61
	1.00	16.94	29.91	50.61	-43.12	77.01
	1.33	22.99	29.91	50.61	-37.08	83.05
	1.67	29.49	29.91	50.61	-30.57	89.55
	2.00	37.72	29.91	50.61	-22.34	97.78
	4.00	78.19	29.91	50.61	18.13	138.25
	6.00	126.04	29.91	50.61	65.98	186.10
	8.00	180.85	29.91	50.61	120.79	240.91
	10.00	234.04	29.91	50.61	173.98	294.10
	12.00	294.79	29.91	50.61	234.73	354.85
Pheroid™ emulgel	0.00	-2.70 x10 ⁻¹²	31.73	50.61	-63.70	63.70
	0.33	2.19	31.73	50.61	-61.51	65.89
	0.67	4.30	31.73	50.61	-59.41	68.00
	1.00	6.79	31.73	50.61	-56.92	70.49
	1.33	9.26	31.73	50.61	-54.44	72.97
	1.67	11.67	31.73	50.61	-52.04	75.37
	2.00	14.48	31.73	50.61	-49.22	78.19
	4.00	37.42	31.73	50.61	-26.28	101.13
	6.00	67.39	31.73	50.61	3.69	131.10
	8.00	102.27	31.73	50.61	38.57	165.97
	10.00	137.87	31.73	50.61	74.16	201.57
	12.00	171.77	31.73	50.61	108.07	235.47
Hydrogel	0.00	-1.17 x10 ⁻¹²	28.38	50.61	-56.98	56.98
	0.33	1.93	28.38	50.61	-55.05	58.91
	0.67	6.43	28.38	50.61	-50.55	63.41
	1.00	11.99	28.38	50.61	-44.99	68.96
	1.33	18.17	28.38	50.61	-38.81	75.15
	1.67	24.67	28.38	50.61	-32.31	81.65
	2.00	31.49	28.38	50.61	-25.49	88.46
	4.00	75.93	28.38	50.61	18.95	132.91
	6.00	133.60	28.38	50.61	76.61	190.57
	8.00	194.01	28.38	50.61	137.03	250.99
	10.00	257.05	28.38	50.61	200.08	314.03
	12.00	323.29	28.38	50.61	266.31	380.27

Commercial product	0.00	-1.46 x10 ⁻¹¹	28.38	50.61	-56.98	56.98
	0.33	11.01	28.38	50.61	-45.97	67.99
	0.67	30.48	28.38	50.61	-26.50	87.46
	1.00	54.07	28.38	50.61	-2.91	111.05
	1.33	80.00	28.38	50.61	23.02	136.98
	1.67	107.23	28.38	50.61	50.25	164.20
	2.00	135.91	28.38	50.61	78.93	192.88
	4.00	319.34	28.38	50.61	262.36	376.32
	6.00	537.33	28.38	50.61	480.35	594.31
	8.00	765.44	28.38	50.61	708.46	822.42
	10.00	994.51	28.38	50.61	937.53	1051.48
	12.00	1210.90	28.38	50.61	1153.93	1267.88
PBS solution	0.00	-1.11 x10 ⁻¹¹	31.73	50.61	-63.70	63.70
	0.33	6.82	31.73	50.61	-56.89	70.52
	0.67	16.68	31.73	50.61	-47.03	80.38
	1.00	30.65	31.73	50.61	-33.05	94.36
	1.33	46.08	31.73	50.61	-17.62	109.78
	1.67	64.21	31.73	50.61	0.51	127.91
	2.00	83.66	31.73	50.61	19.96	147.36
	4.00	191.62	31.73	50.61	127.92	255.33
	6.00	327.20	31.73	50.61	263.49	390.90
	8.00	470.10	31.73	50.61	406.40	533.80
	10.00	617.44	31.73	50.61	553.74	681.15
	12.00	764.93	31.73	50.61	701.23	828.64
Pheroid™ solution	0.00	-1.30 x10 ⁻¹¹	31.73	50.61	-63.70	63.70
	0.33	0.84	31.73	50.61	-62.86	64.54
	0.67	3.76	31.73	50.61	-59.95	67.46
	1.00	10.30	31.73	50.61	-53.41	74.00
	1.33	19.64	31.73	50.61	-44.06	83.34
	1.67	30.53	31.73	50.61	-33.17	94.23
	2.00	43.19	31.73	50.61	-20.51	106.90
	4.00	125.14	31.73	50.61	61.44	188.85
	6.00	210.28	31.73	50.61	146.57	273.98
	8.00	303.36	31.73	50.61	239.66	367.06
	10.00	403.08	31.73	50.61	339.37	466.78
	12.00	489.75	31.73	50.61	426.05	553.46

Table D.6: Mixed model statistical analysis indicating the mean lag time of prilocaine HCl

Treatment	Time	Mean	Std. Error	Deg. freedom (df)	95% Confidence Interval	
					Lower Bound	Upper Bound
Emulgel	0.00	4.55 x10 ⁻¹³	35.11	50.44	-70.50	70.50
	0.33	1.25	35.11	50.44	-69.25	71.76
	0.67	5.01	35.11	50.44	-65.49	75.51
	1.00	12.20	35.11	50.44	-58.30	82.70
	1.33	16.57	35.11	50.44	-53.94	87.07
	1.67	21.61	35.11	50.44	-48.89	92.11
	2.00	28.91	35.11	50.44	-41.59	99.41
	4.00	85.10	35.11	50.44	14.60	155.60
	6.00	149.17	35.11	50.44	78.67	219.67
	8.00	219.79	35.11	50.44	149.29	290.29
	10.00	286.09	35.11	50.44	215.58	356.59
12.00	360.22	35.11	50.44	289.72	430.72	
Pheroid™ emulgel	0.00	-6.59x10 ⁻¹²	37.24	50.44	-74.78	74.78
	0.33	5.98	37.24	50.44	-68.80	80.76
	0.67	13.08	37.24	50.44	-61.70	87.85
	1.00	22.36	37.24	50.44	-52.42	97.14
	1.33	33.90	37.24	50.44	-40.88	108.68
	1.67	45.64	37.24	50.44	-29.14	120.42
	2.00	57.60	37.24	50.44	-17.18	132.37
	4.00	106.17	37.24	50.44	31.40	180.95
	6.00	166.20	37.24	50.44	91.42	240.97
	8.00	231.50	37.24	50.44	156.72	306.28
	10.00	296.91	37.24	50.44	222.13	371.69
12.00	359.71	37.24	50.44	284.93	434.49	
Hydrogel	0.00	-3.01 x10 ⁻¹²	33.31	50.44	-66.88	66.88
	0.33	2.29	33.31	50.44	-64.60	69.17
	0.67	10.15	33.31	50.44	-56.73	77.03
	1.00	20.88	33.31	50.44	-46.00	87.77
	1.33	33.66	33.31	50.44	-33.22	100.54
	1.67	47.07	33.31	50.44	-19.82	113.95
	2.00	60.97	33.31	50.44	-5.91	127.86
	4.00	207.77	33.31	50.44	140.89	274.65
	6.00	399.75	33.31	50.44	332.86	466.63
	8.00	602.51	33.31	50.44	535.62	669.39
	10.00	803.55	33.31	50.44	736.67	870.43
12.00	1013.89	33.31	50.44	947.01	1080.77	

Commercial product	0.00	-2.50×10^{-12}	33.31	50.44	-66.88	66.88
	0.33	7.89	33.31	50.44	-58.99	74.78
	0.67	21.23	33.31	50.44	-45.65	88.11
	1.00	37.52	33.31	50.44	-29.37	104.40
	1.33	54.18	33.31	50.44	-12.70	121.06
	1.67	73.14	33.31	50.44	6.26	140.02
	2.00	94.58	33.31	50.44	27.70	161.47
	4.00	203.44	33.31	50.44	136.55	270.32
	6.00	320.30	33.31	50.44	253.42	387.19
	8.00	442.43	33.31	50.44	375.55	509.32
	10.00	575.25	33.31	50.44	508.37	642.14
	12.00	700.55	33.31	50.44	633.67	767.44
PBS solution	0.00	-4.01×10^{-12}	37.24	50.44	-74.78	74.78
	0.33	8.57	37.24	50.44	-66.21	83.34
	0.67	17.56	37.24	50.44	-57.22	92.34
	1.00	31.19	37.24	50.44	-43.59	105.97
	1.33	46.27	37.24	50.44	-28.51	121.04
	1.67	62.53	37.24	50.44	-12.25	137.30
	2.00	80.28	37.24	50.44	5.50	155.05
	4.00	165.44	37.24	50.44	90.66	240.22
	6.00	271.91	37.24	50.44	197.14	346.69
	8.00	383.06	37.24	50.44	308.29	457.84
	10.00	496.31	37.24	50.44	421.57	571.09
	12.00	609.89	37.24	50.44	535.12	684.67
Pheroid™ solution	0.00	9.21×10^{-11}	37.24	50.44	-74.78	74.78
	0.33	1.26	37.24	50.44	-73.52	76.04
	0.67	4.04	37.24	50.44	-70.74	78.82
	1.00	9.62	37.24	50.44	-65.17	84.40
	1.33	17.52	37.24	50.44	-57.26	92.29
	1.67	26.22	37.24	50.44	-48.56	100.99
	2.00	37.16	37.24	50.44	-37.61	111.94
	4.00	111.24	37.24	50.44	36.46	186.01
	6.00	191.82	37.24	50.44	117.04	266.59
	8.00	283.81	37.24	50.44	209.03	358.59
	10.00	384.52	37.24	50.44	309.75	459.30
	12.00	481.36	37.24	50.44	406.58	556.14

The statistical analysis shows that the lag times for lidocaine HCl and prilocaine HCl from all the formulations were extremely poor when compared with the commercial product, which was the only formulation that was statistically relevant below 2 h. The data from the diffusion studies showed

extremely small values statistically in the first 2 h, which caused the data to become statistically irrelevant. The commercial product's mean lag time was at 1.33 h (lidocaine HCl) and 1.67 h (prilocaine HCl), which is in agreement with the existing data, namely that the onset of action of this product is 60 min after application (Friedman *et al.*, 2001:1020). The mean lag times for the three formulated products and the two solutions for both APIs only became statistically relevant after 4 h.

D.3.3.2 Steady-state flux of lidocaine HCl and prilocaine HCl

The flux of an API is the product of the rate of permeant transport per unit of concentration through the skin (Williams, 2003:27, 38). Lidocaine HCl and prilocaine HCl showed simple zero-order skin diffusion behaviour from all the formulations and produced a steady-state API flux after 2 h. The flux was determined by calculating the slope of the straight line between 2 - 12 h. This is discussed in the next section, as well as the concentration of API yielded after 12 h. The results were noted in Table D.7 and Table D.8.

D.3.3.2.1 Lidocaine hydrochloride

The average and median flux values of lidocaine HCl are noted in Table D.7. Figure D.5 - D.16 depicts the cumulative concentration lidocaine HCl that diffused through the skin of each Franz cell and the average flux value of lidocaine HCl in each formulation.

Table D.7: Steady-state flux values ($\mu\text{g}/\text{cm}^2\cdot\text{h}$) of lidocaine HCl and average lidocaine HCl concentration ($\mu\text{g}/\text{cm}^2$) that diffused through the skin after 12 h

	Average flux ($\mu\text{g}/\text{cm}^2\cdot\text{h}$)	Median flux ($\mu\text{g}/\text{cm}^2\cdot\text{h}$)	Average concentration after 12 h ($\mu\text{g}/\text{cm}^2$)	Franz cells used per diffusion study (n)
Pheroid™ emulgel	16.04 \pm 4.9	14.52	92.37	8
Emulgel	25.83 \pm 3.2	24.01	158.53	9
Hydrogel	29.47 \pm 7.2	23.91	173.86	10
Pheroid™ solution	54.14 \pm 12.0	41.38	263.38	8
PBS solution	68.95 \pm 7.9	63.42	411.38	8
Commercial	108.98 \pm 23.8	108.63	651.20	10

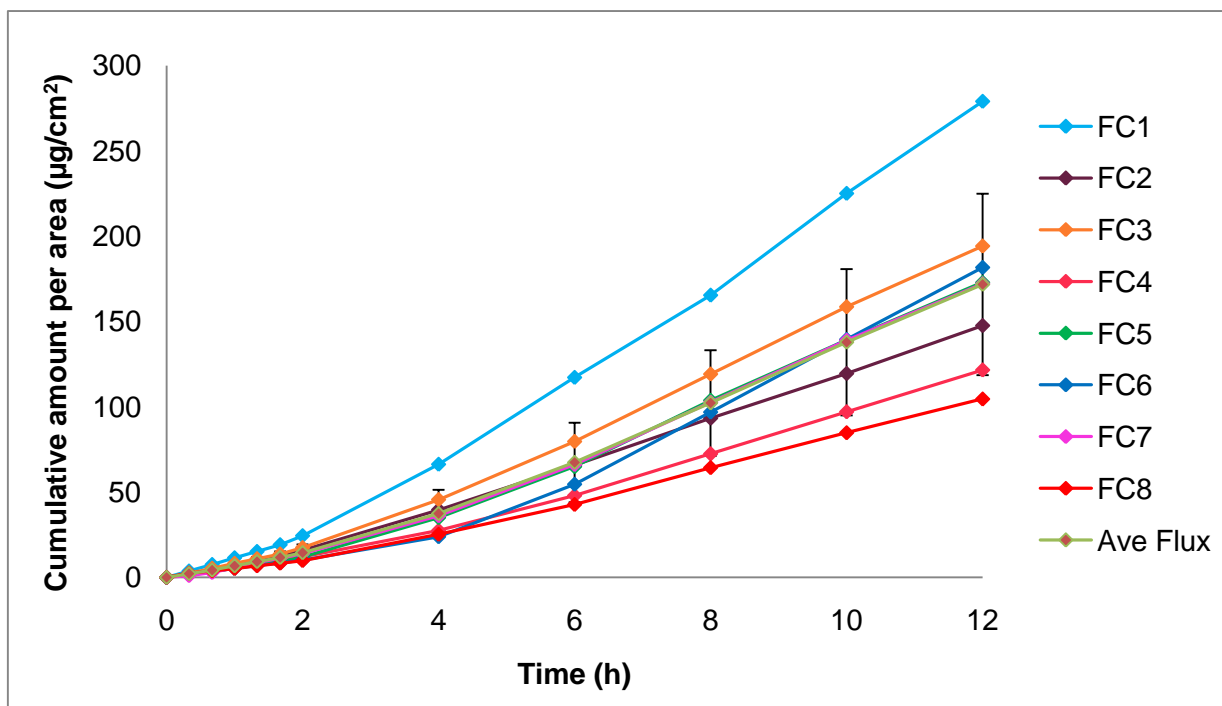


Figure D.5: Cumulative lidocaine HCl amount/area ($\mu\text{g}/\text{cm}^2$) of each individual Franz cell that diffused through the skin as a function of time for the Pheroid™ emulgel

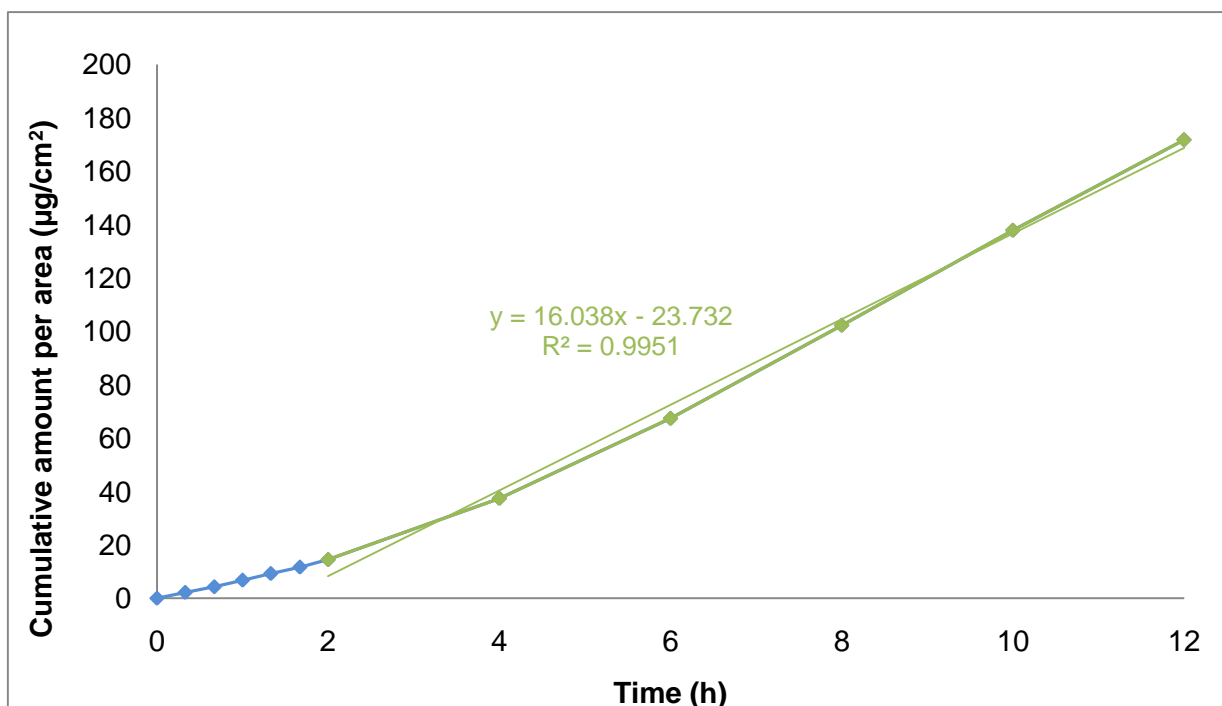


Figure D.6: Average cumulative concentration lidocaine HCl that diffused through the skin as a function of time in the Pheroid™ emulgel formulation

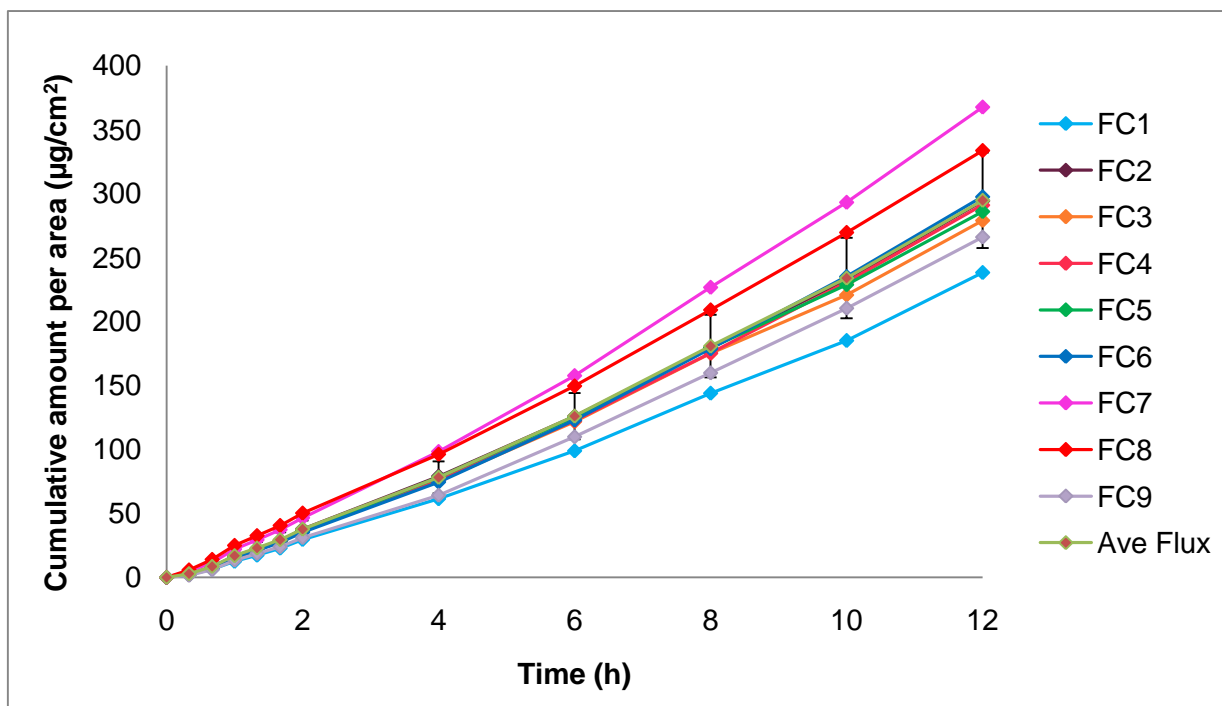


Figure D.7: Cumulative lidocaine HCl amount/area ($\mu\text{g}/\text{cm}^2$) of each individual Franz cell that diffused through the skin as a function of time for the emulgel

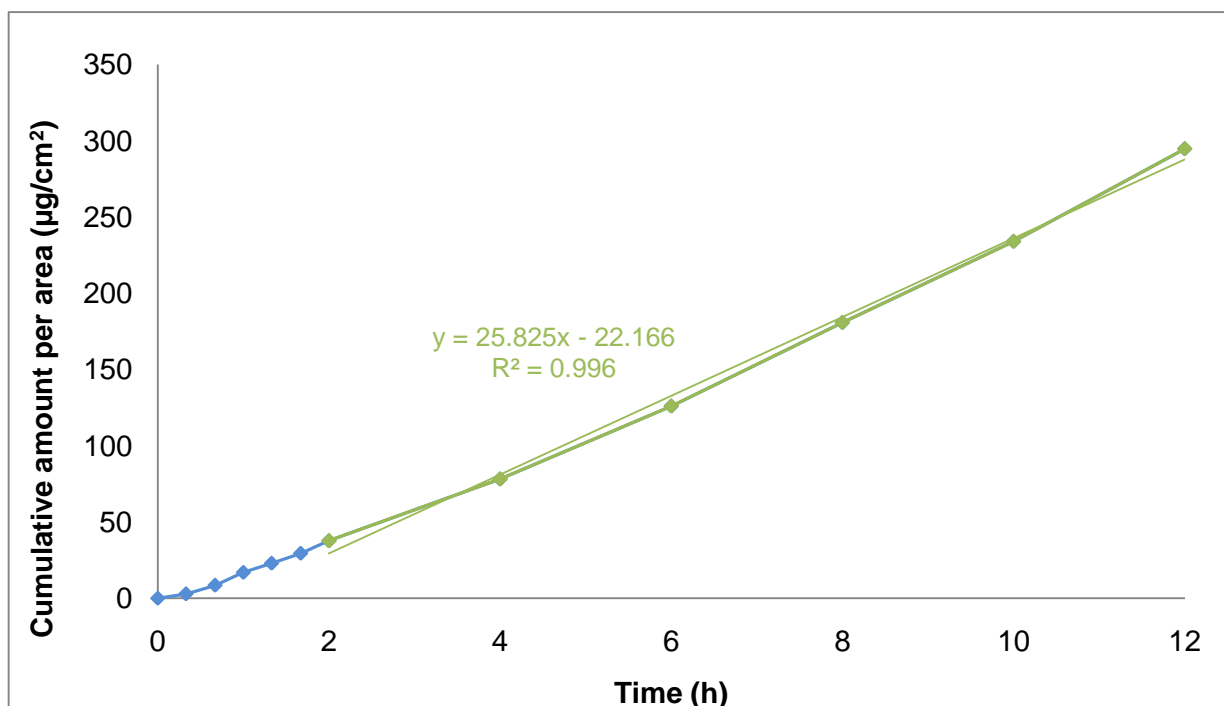


Figure D.8: Average cumulative concentration lidocaine HCl that diffused through the skin as a function of time in the emulgel formulation

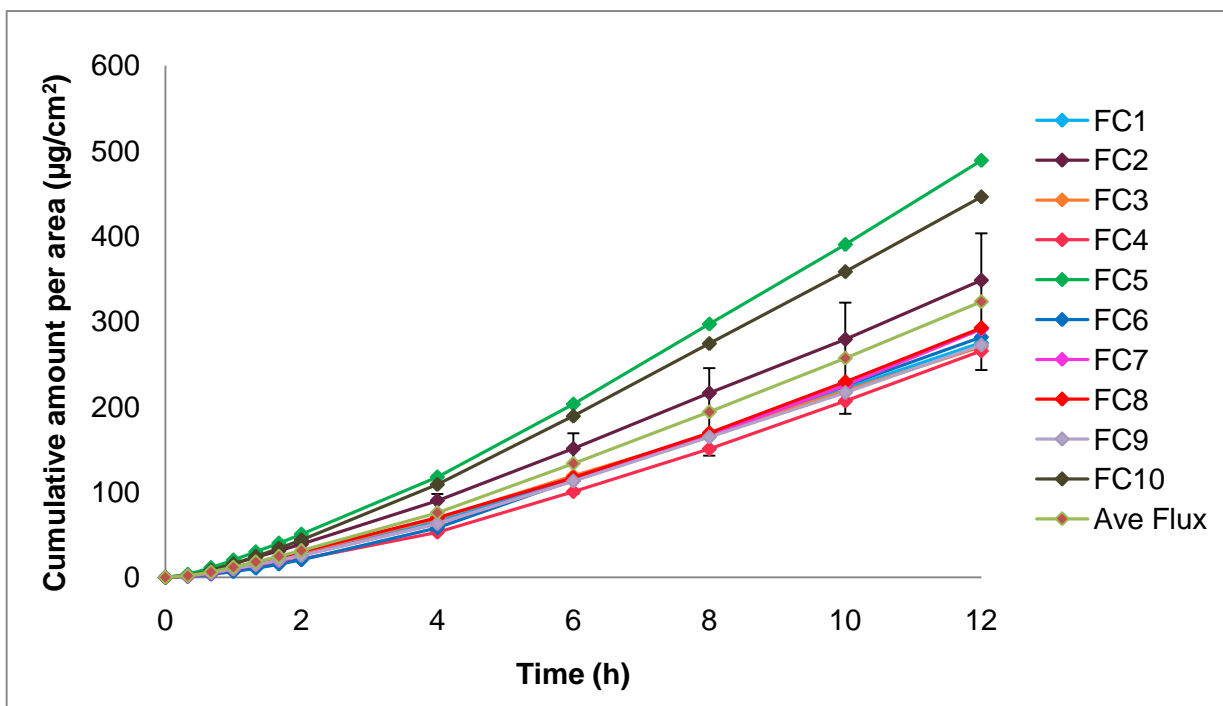


Figure D.9: Cumulative lidocaine HCl amount/area ($\mu\text{g}/\text{cm}^2$) of each individual Franz cell that diffused through the skin as a function of time for the hydrogel

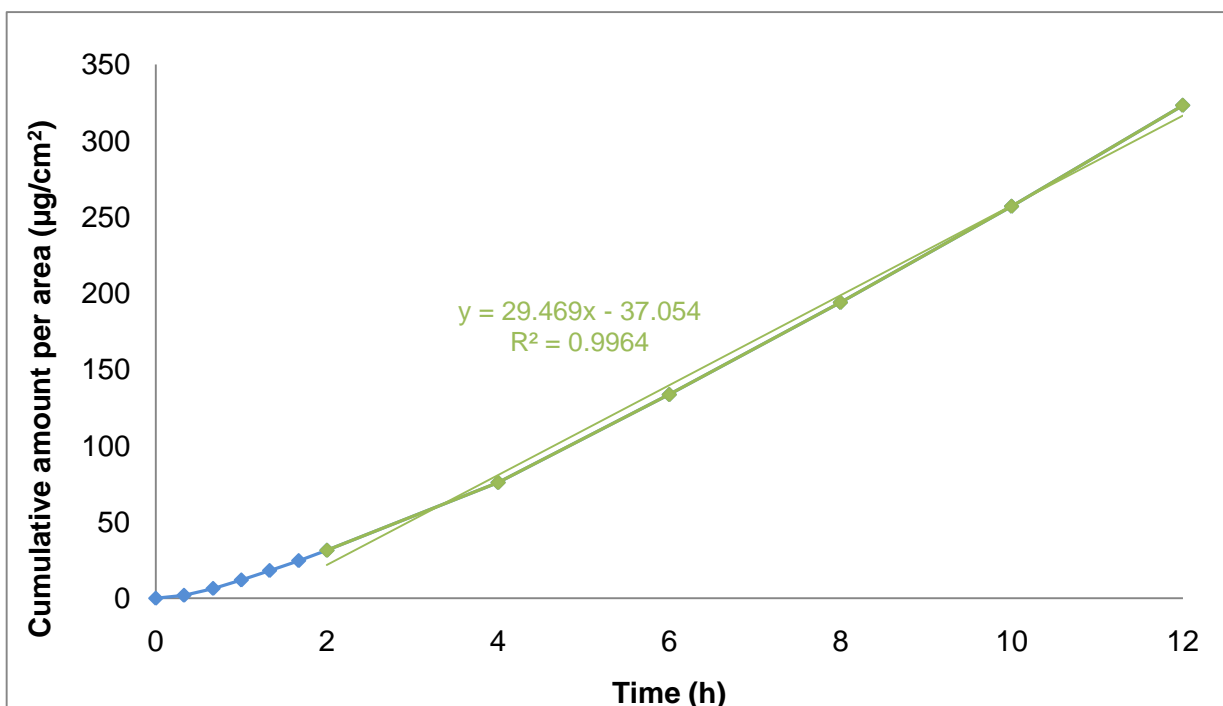


Figure D.10: Average cumulative concentration lidocaine HCl that diffused through the skin as a function of time in the hydrogel formulation

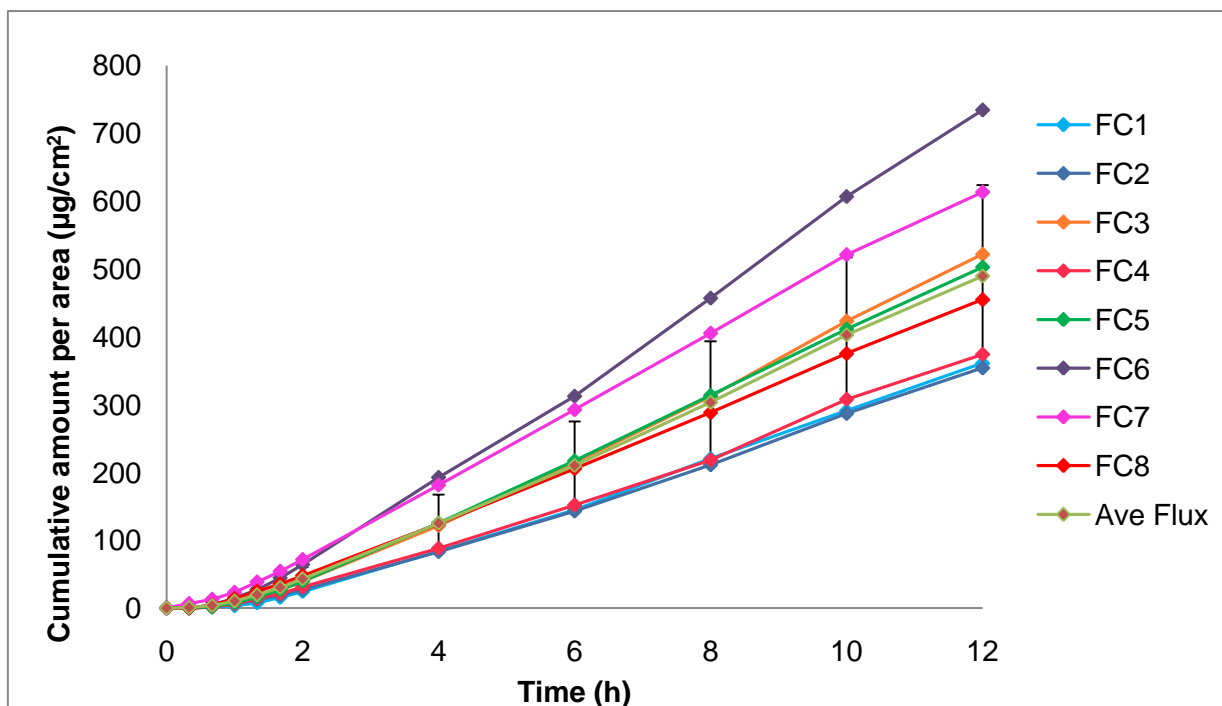


Figure D.11: Cumulative lidocaine HCl amount/area ($\mu\text{g}/\text{cm}^2$) of each individual Franz cell that diffused through the skin as a function of time for the Pheroid™ solution

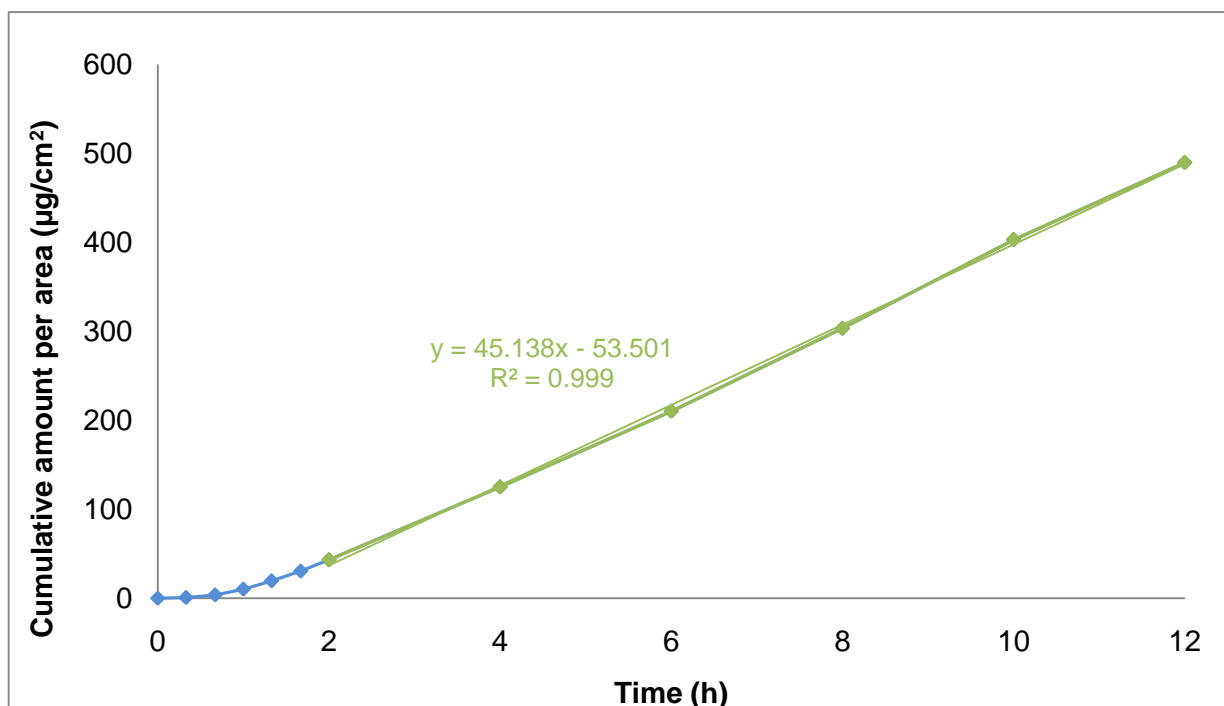


Figure D.12: Average cumulative concentration lidocaine HCl that diffused through the skin as a function of time in the Pheroid™ solution

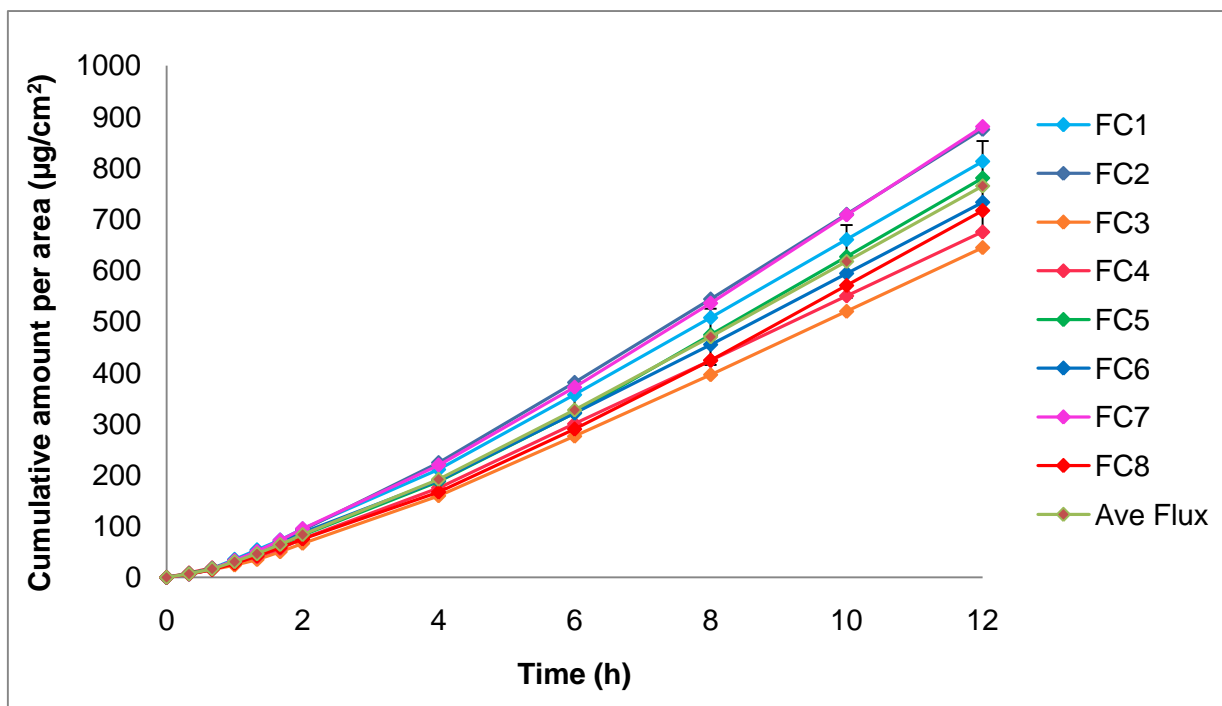


Figure D.13: Cumulative lidocaine HCl amount/area ($\mu\text{g}/\text{cm}^2$) of each individual Franz cell that diffused through the skin as a function of time for the PBS solution

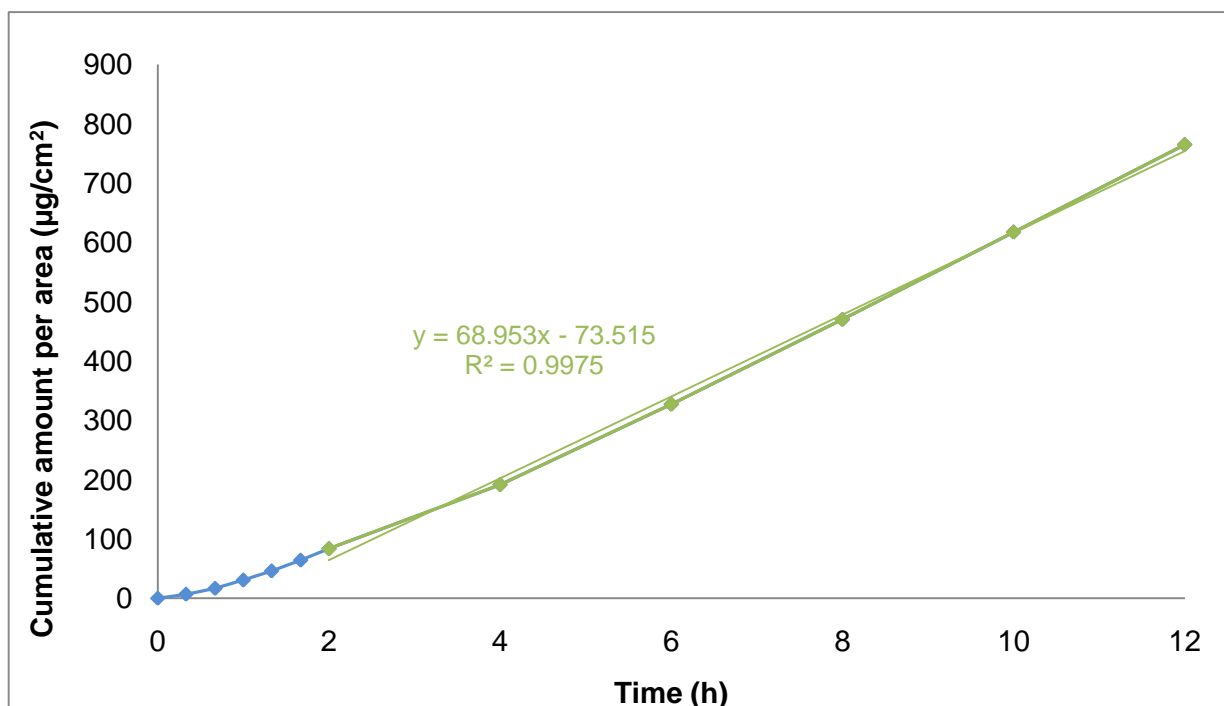


Figure D.14: Average cumulative concentration lidocaine HCl that diffused through the skin as a function of time in the PBS solution

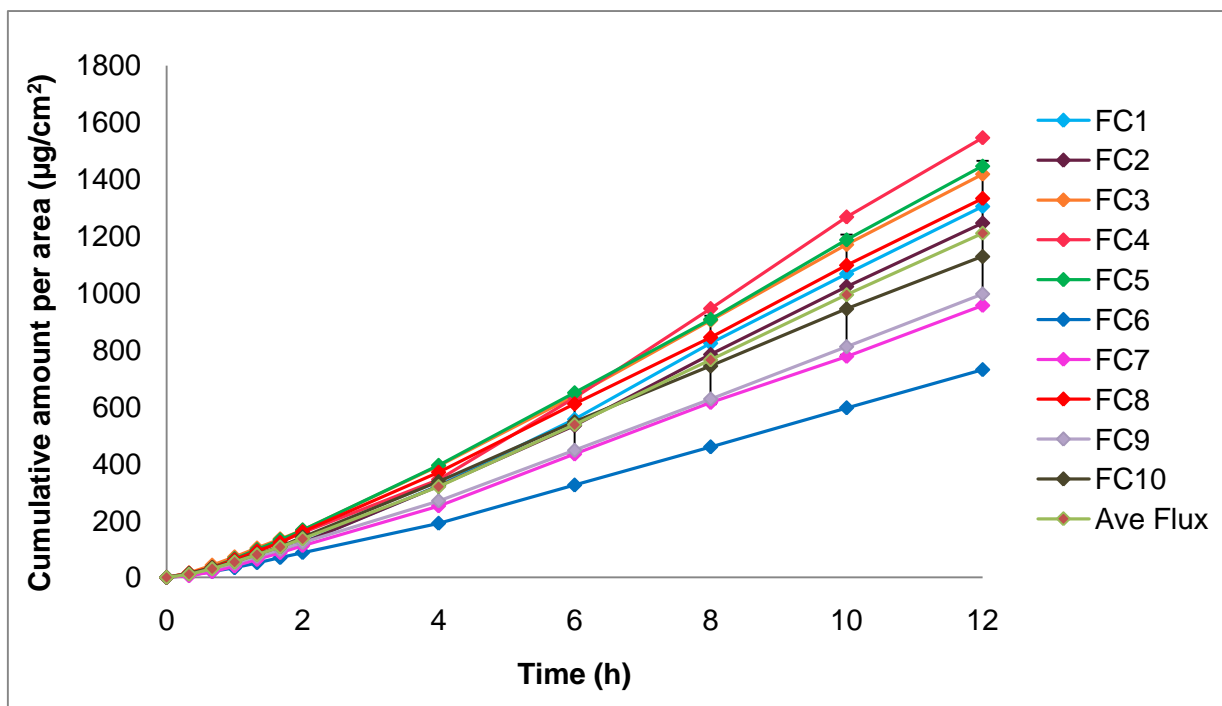


Figure D.15: Cumulative lidocaine HCl amount/area ($\mu\text{g}/\text{cm}^2$) of each individual Franz cell that diffused through the skin as a function of time for the commercial product

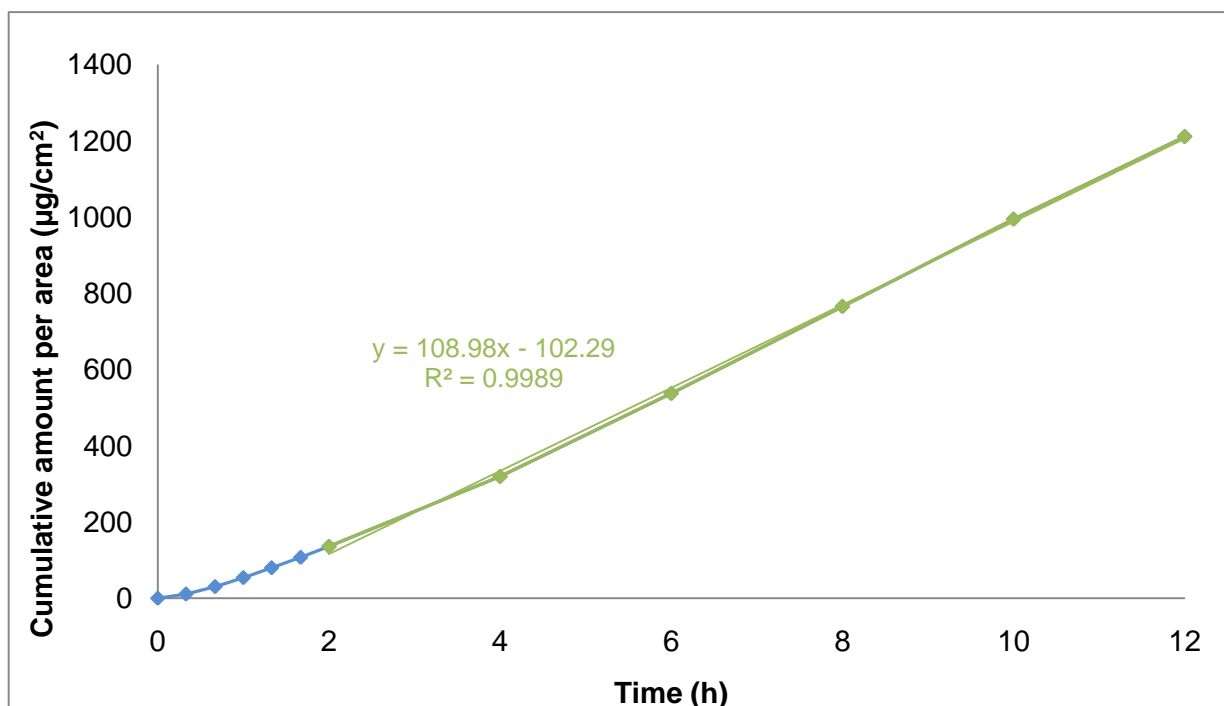


Figure D.16: Average cumulative concentration lidocaine HCl that diffused through the skin as a function of time in the commercial product

The average flux values for lidocaine HCl in the different formulations and solutions (Table D.7) were compared with one another and it was found that the commercial product had the highest average flux value (108.98 $\mu\text{g}/\text{cm}^2\cdot\text{h}$), followed by the PBS solution (68.95 $\mu\text{g}/\text{cm}^2\cdot\text{h}$), Pheroid™ solution (54.14 $\mu\text{g}/\text{cm}^2\cdot\text{h}$), hydrogel (29.47 $\mu\text{g}/\text{cm}^2\cdot\text{h}$), emulgel (25.83 $\mu\text{g}/\text{cm}^2\cdot\text{h}$) and Pheroid™ emulgel (16.04 $\mu\text{g}/\text{cm}^2\cdot\text{h}$). More lidocaine HCl was able to penetrate the skin through passive diffusion from the commercial product. The median flux values of the formulations and solutions did not vary drastically from the average flux values as seen in Table D.7. The Pheroid™ solution displayed the largest variance between the average and median flux values, which indicates that more outliers were present in the data. If a large variation is present between the average and median flux values, it is recommended that median values would provide a more accurate representation of the true flux value as they are not affected by the outliers present in the data (Gerber *et al.*, 2008:190).

The average concentration of lidocaine HCl that diffused through the skin after 12 h was in accordance with the flux values of lidocaine HCl from the different formulations and solutions. The commercial product which achieved the highest flux of lidocaine HCl, yielded the highest average diffused concentration as a result (651.2 $\mu\text{g}/\text{cm}^2$). The Pheroid™ emulgel displayed a poor flux into the skin layers, which ultimately allowed only 92.37 $\mu\text{g}/\text{cm}^2$ lidocaine HCl to diffuse through the skin. This was only 14% of the concentration amount of lidocaine HCl that diffused through the skin of the commercial product.

The pH difference between the commercial product (pH 9) and the other formulated products (pH 8) seems to have an extensive effect on the flux values and average diffused concentration of lidocaine HCl and prilocaine HCl. At a pH of 9 there are more lipid soluble unionised fractions of the APIs able to penetrate the skin and reach the skin nerves faster and in larger amounts where it can immediately start to produce its ionised fraction that binds to the inside of the Na^+ channel for anaesthetic effect (Richards & McConachie, 1995:41). The PBS solution, Pheroid™ solution and the hydrogel had higher lidocaine HCl flux values than the emulgel and the Pheroid™ emulgel. The two solutions and the hydrogel have higher water content if compared with the two emulgel formulations. The water promotes skin hydration and disrupts the lipid bilayers which allowed more of the hydrophilic API to cross the stratum corneum via the intercellular route at a higher flux (Barry, 2007:576). The lag times of the solutions and the hydrogel were longer than the lag times of the emulgel and Pheroid™ emulgel. This indicated that it took longer for the skin to become hydrated and produce but produced high flux values of lidocaine HCl as a result (Hadgraft, 1999:4).

D.3.3.2.2 Prilocaine hydrochloride

The average and median flux values of lidocaine HCl are noted in Table D.8. Figure D.17 - D.28 depicts the cumulative concentration lidocaine HCl that diffused through the skin of each Franz cell and the average flux value of lidocaine HCl in each formulation.

Table D.8: Steady-state flux values ($\mu\text{g}/\text{cm}^2\cdot\text{h}$) of prilocaine HCl and average prilocaine HCl concentration ($\mu\text{g}/\text{cm}^2$) that diffused through the skin after 12 h

	Average flux ($\mu\text{g}/\text{cm}^2\cdot\text{h}$)	Median flux ($\mu\text{g}/\text{cm}^2\cdot\text{h}$)	Average concentration after 12 h ($\mu\text{g}/\text{cm}^2$)	Franz cells used per diffusion study (n)
Pheroid™ emulgel	30.69 \pm 7.5	29.24	193.44	8
Emulgel	33.29 \pm 11.8	30.22	193.72	9
Pheroid™ solution	44.75 \pm 14.0	40.24	258.87	8
PBS solution	53.60 \pm 5.8	50.24	327.99	8
Commercial	60.96 \pm 12.8	61.31	376.74	10
Hydrogel	96.49 \pm 25.8	74.00	545.25	10

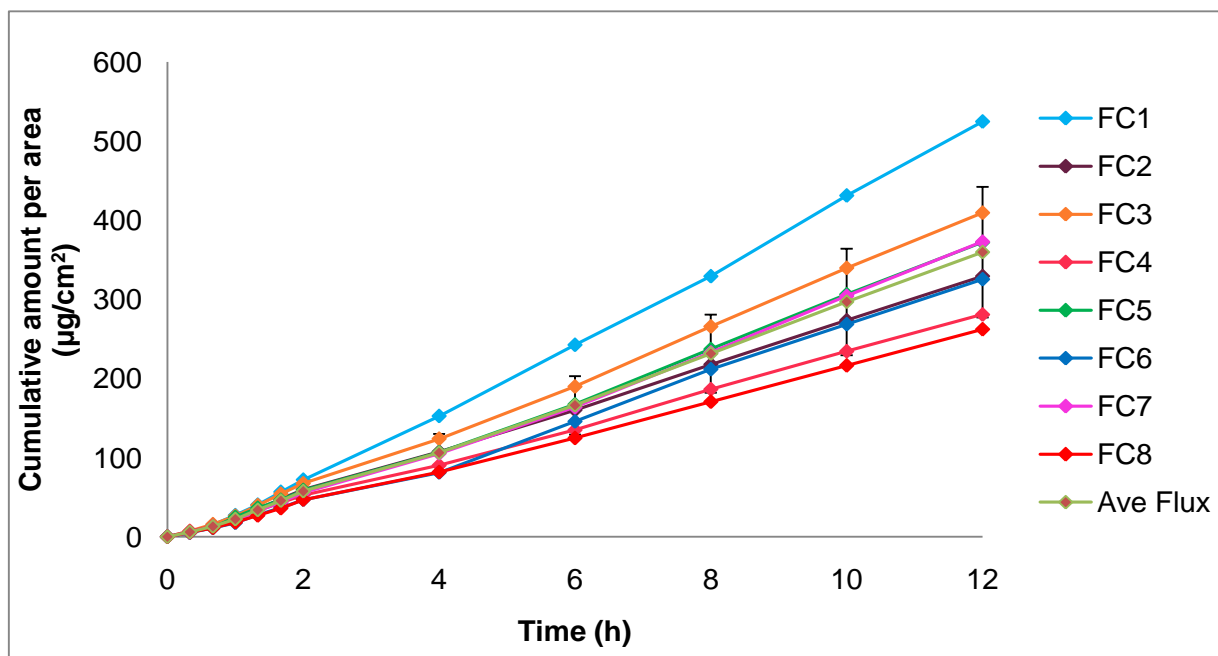


Figure D.17: Cumulative prilocaine HCl amount/area ($\mu\text{g}/\text{cm}^2$) of each individual Franz cell that diffused through the skin as a function of time for the Pheroid™ emulgel

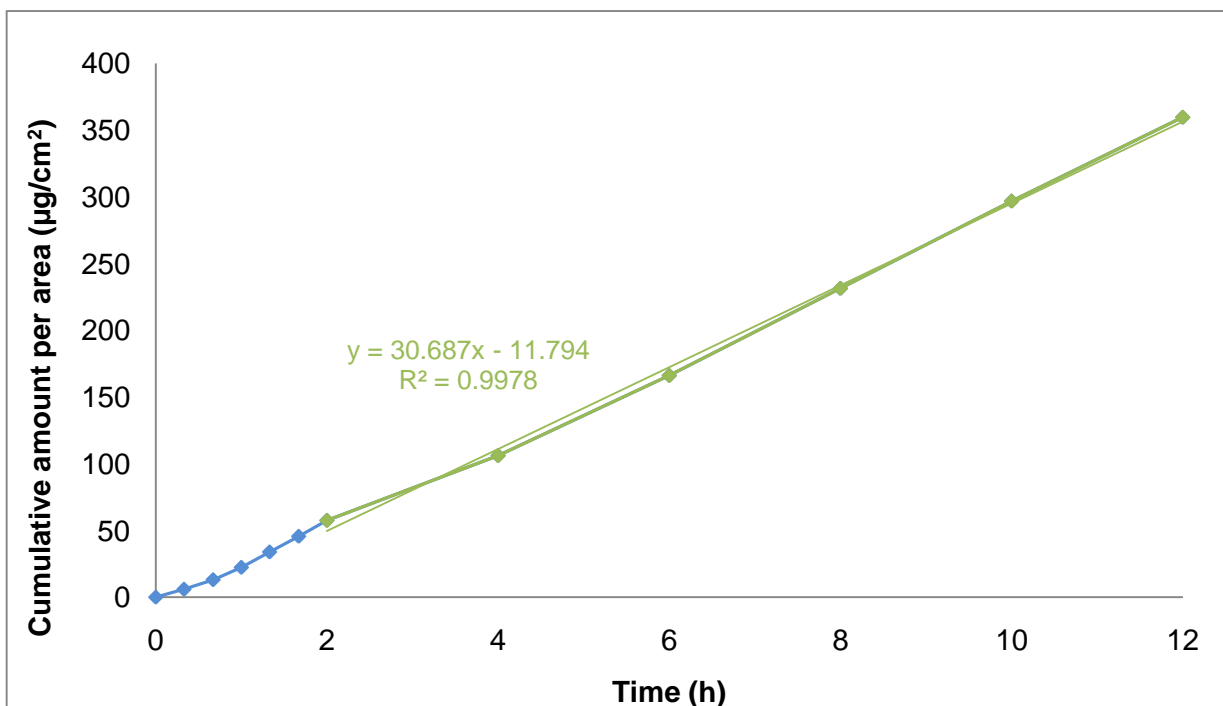


Figure D.18 Average cumulative concentration prilocaine HCl that diffused through the skin as a function of time in the Pheroid™ emulgel

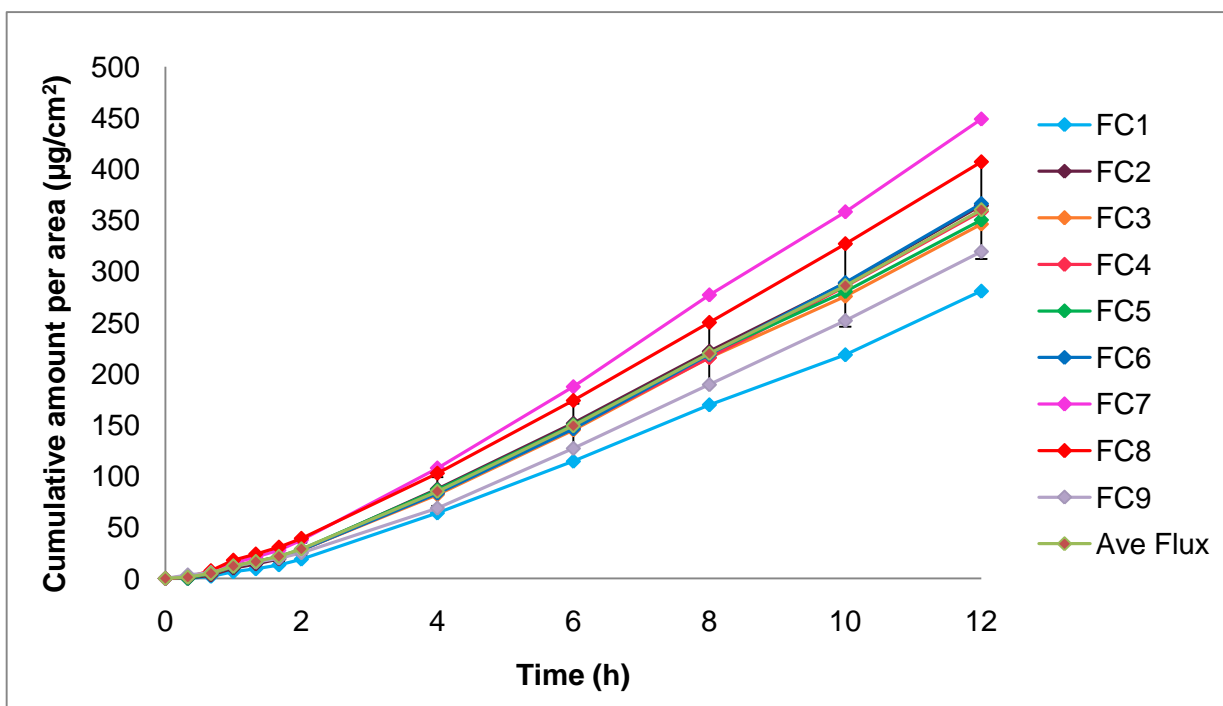


Figure D.19: Cumulative prilocaine HCl amount/area (µg/cm²) of each individual Franz cell that diffused through the skin as a function of time for the emulgel

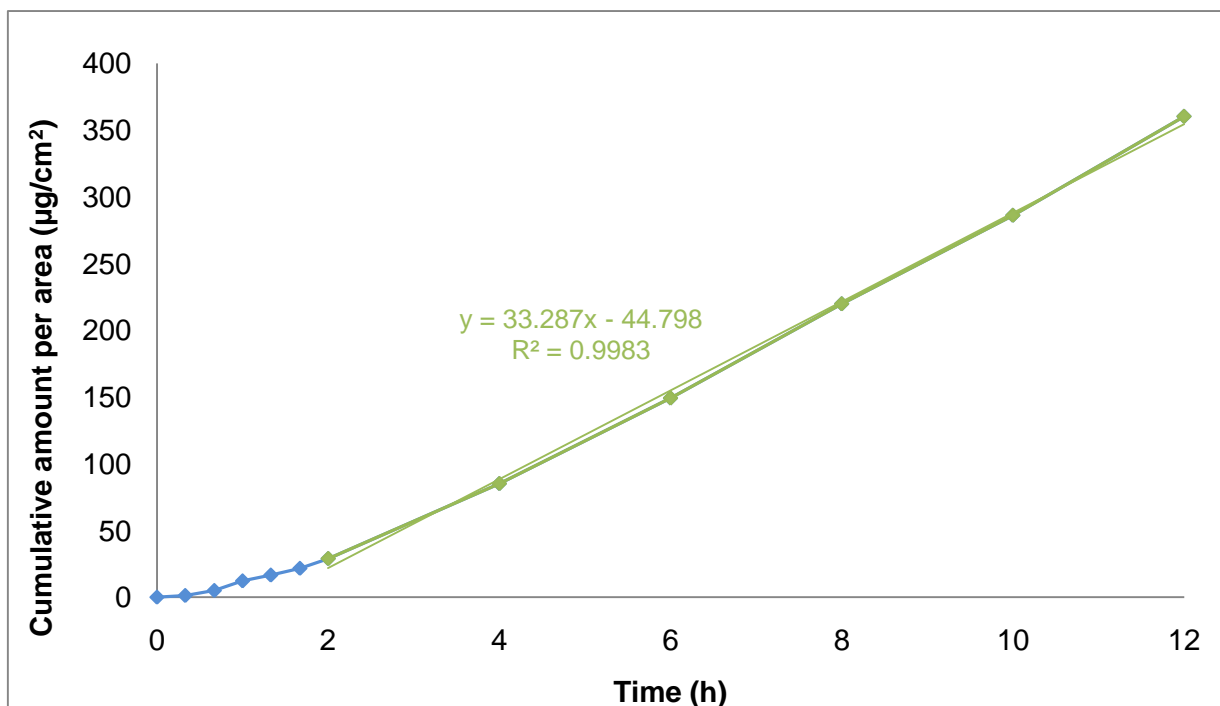


Figure D.20: Average cumulative concentration pilocaine HCl that diffused through the skin as a function of time in the emulgel

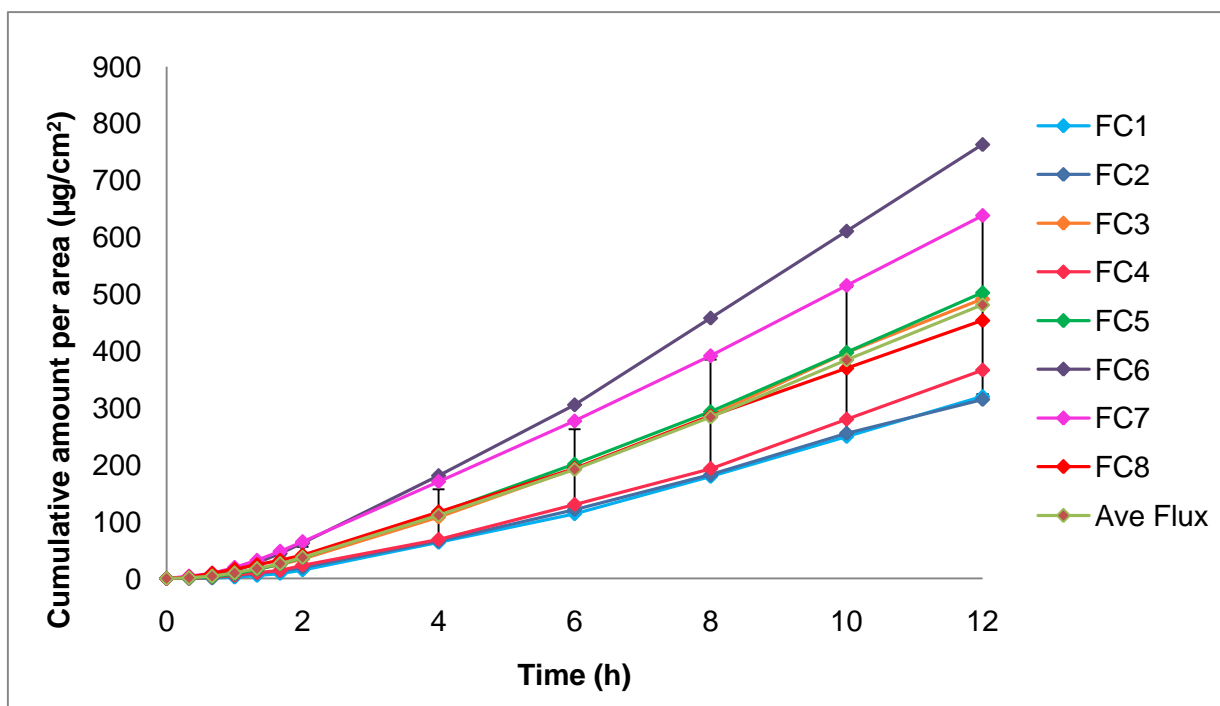


Figure D.21: Cumulative pilocaine HCl amount/area ($\mu\text{g}/\text{cm}^2$) of each individual Franz cell that diffused through the skin as a function of time for the Pheroid™ solution

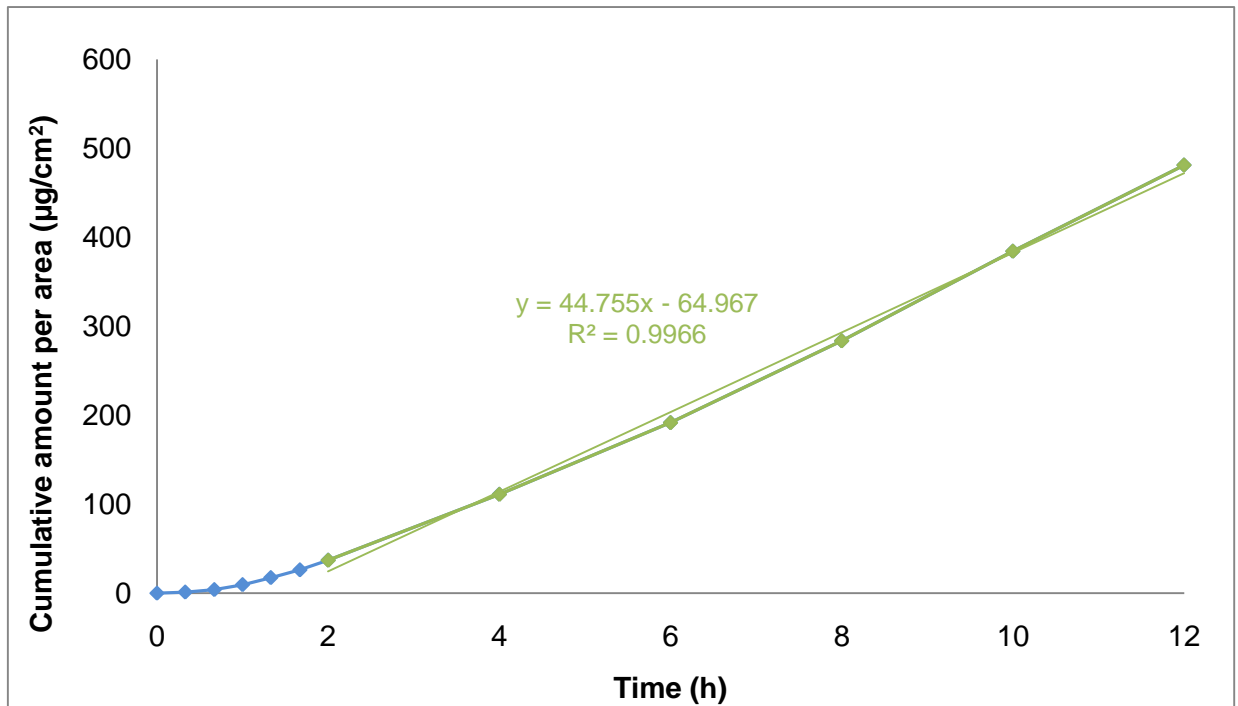


Figure D.22: Average cumulative concentration pilocaine HCl that diffused through the skin as a function of time in the Pheroid™ solution

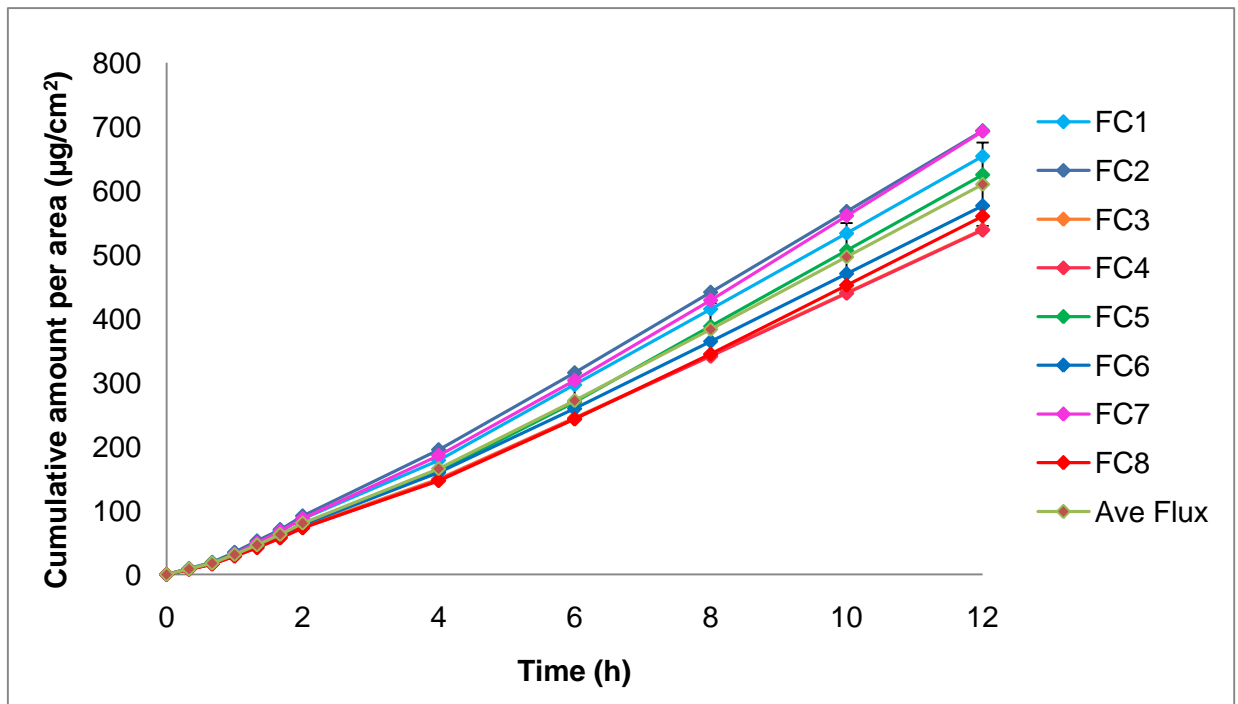


Figure D.23: Cumulative pilocaine HCl amount/area (µg/cm²) of each individual Franz cell that diffused through the skin as a function of time for the PBS solution

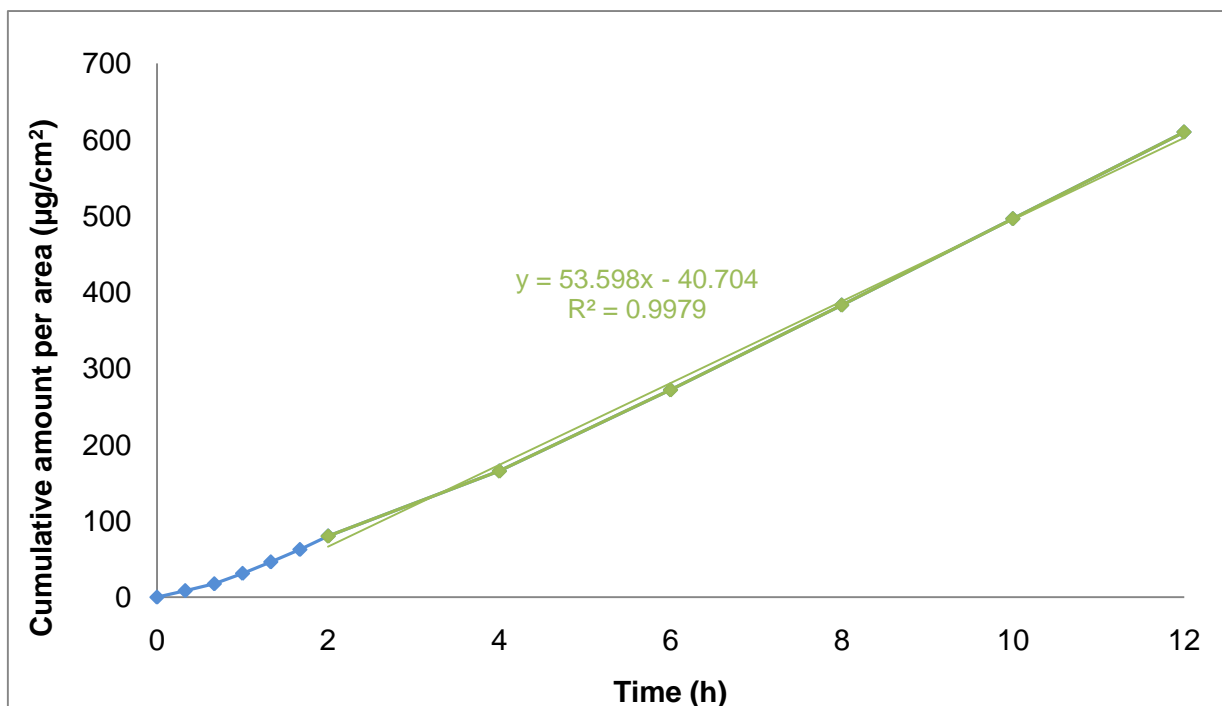


Figure D.24: Average cumulative concentration pilocaine HCl that diffused through the skin as a function of time in the PBS solution

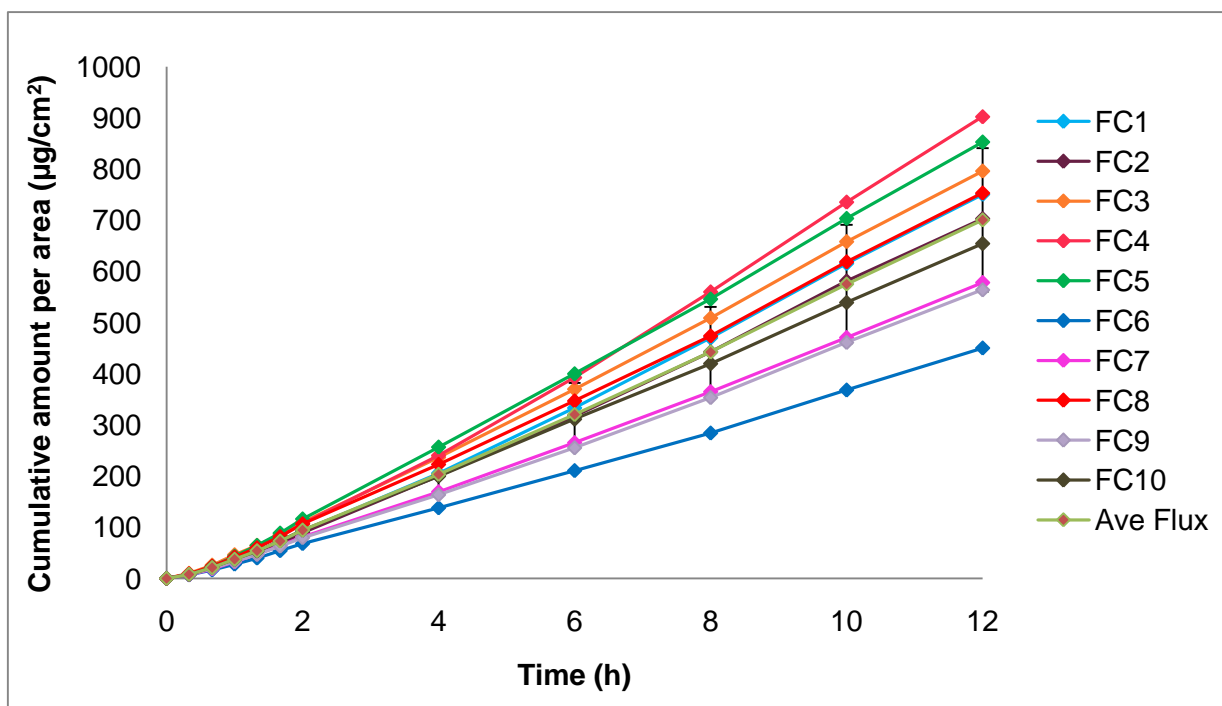


Figure D.25: Cumulative pilocaine HCl amount/area ($\mu\text{g}/\text{cm}^2$) of each individual Franz cell that diffused through the skin as a function of time for the commercial product

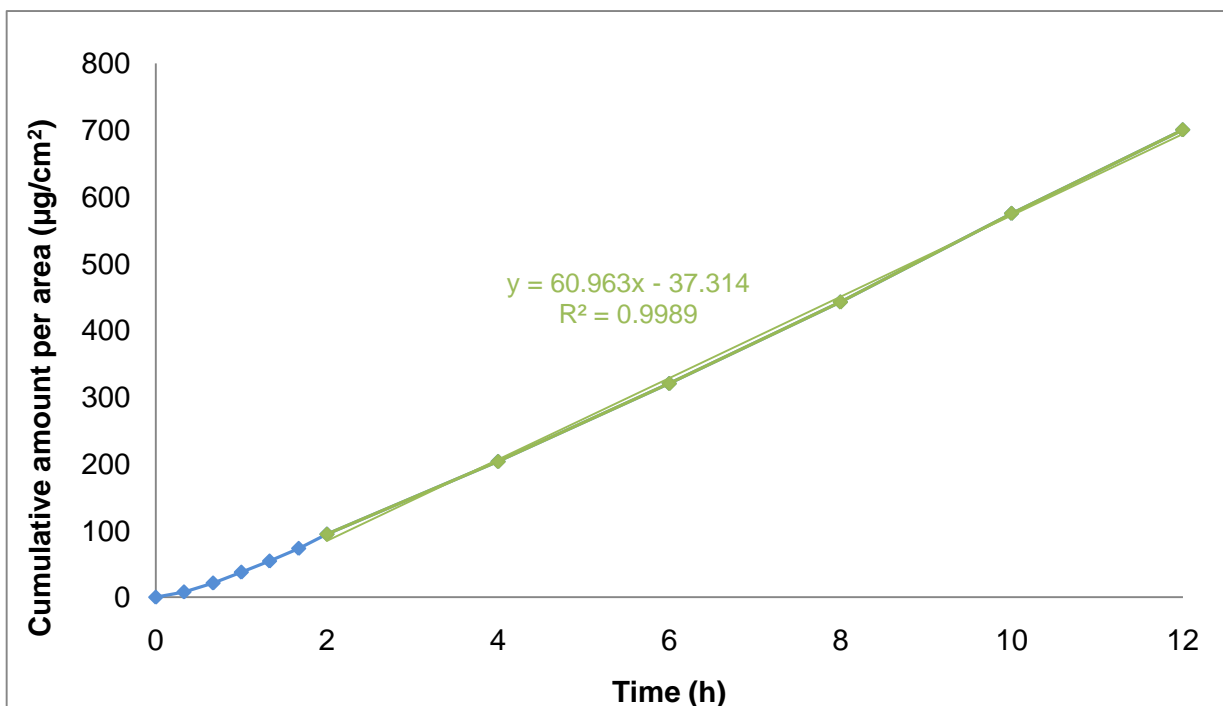


Figure D.26: Average cumulative concentration prilocaine HCl that diffused through the skin as a function of time in the commercial product

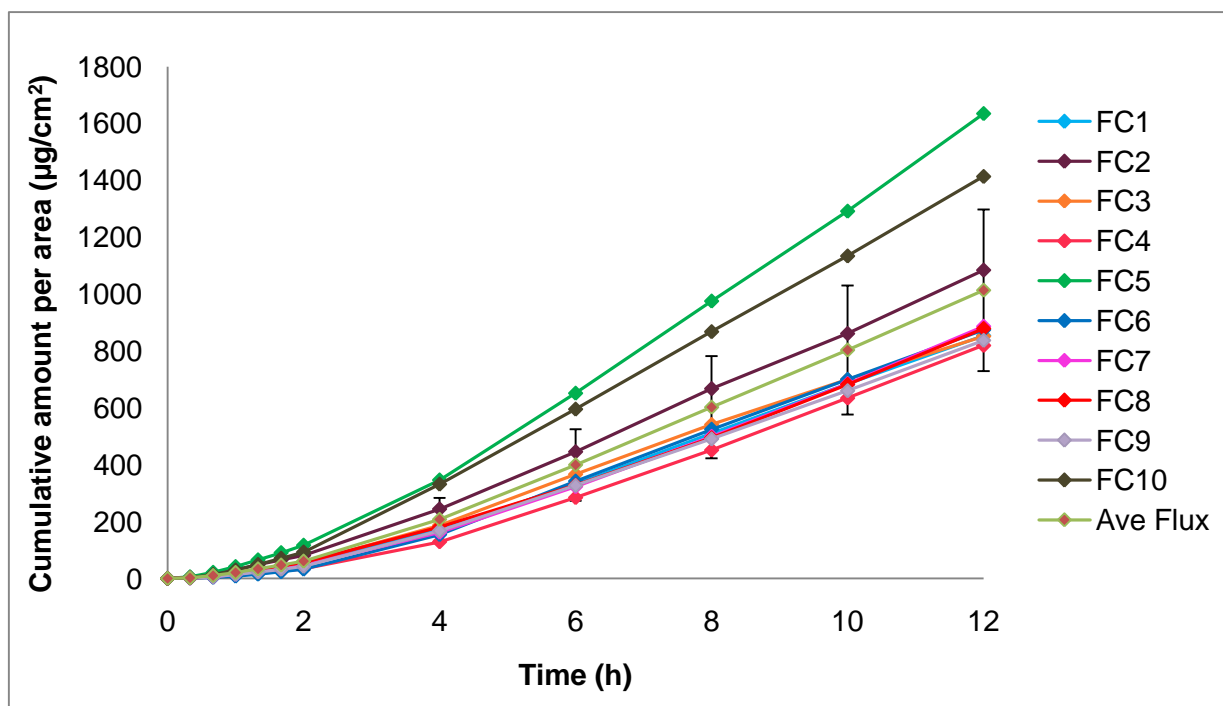


Figure D.27: Cumulative prilocaine HCl amount/area ($\mu\text{g}/\text{cm}^2$) of each individual Franz cell that diffused through the skin as a function of time for the hydrogel

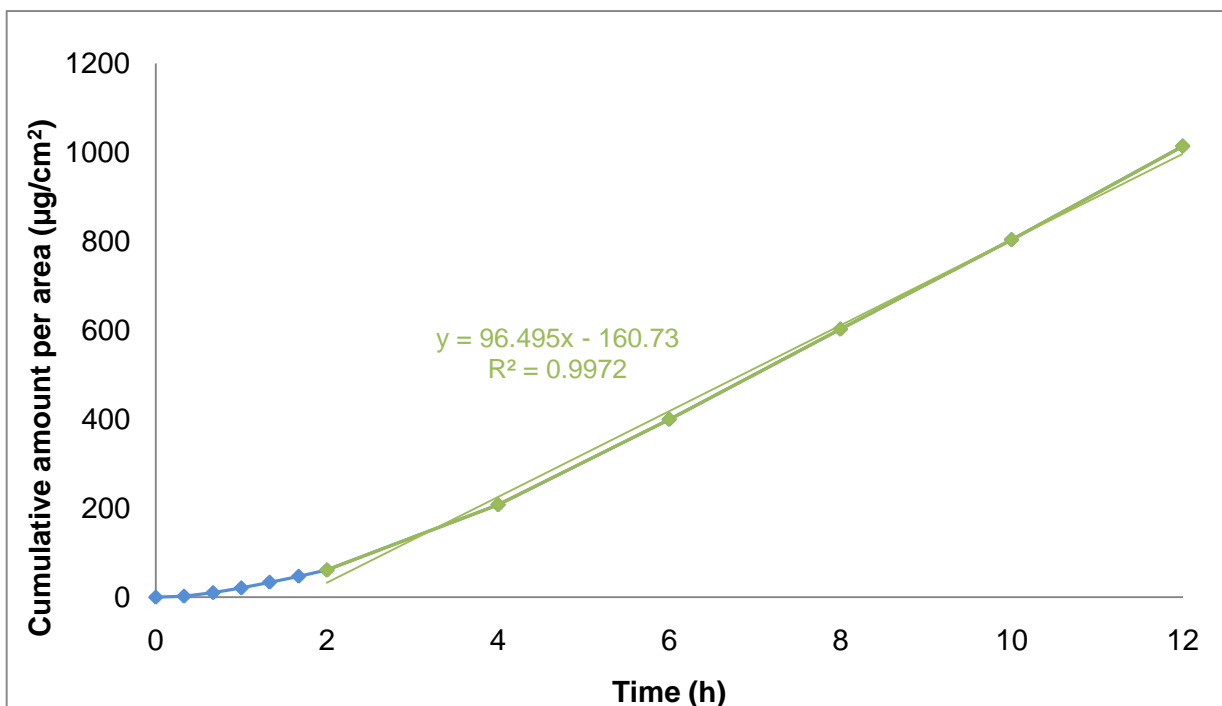


Figure D.28: Average cumulative concentration prilocaine HCl that diffused through the skin as a function of time in the hydrogel

The comparison of the average flux values for prilocaine HCl (Table D.8) indicated that the hydrogel achieved the highest average prilocaine HCl flux ($96.49 \mu\text{g}/\text{cm}^2\cdot\text{h}$), followed by the commercial product ($60.96 \mu\text{g}/\text{cm}^2\cdot\text{h}$), PBS solution ($53.60 \mu\text{g}/\text{cm}^2\cdot\text{h}$), Pheroid™ solution ($44.75 \mu\text{g}/\text{cm}^2\cdot\text{h}$), emulgel ($33.29 \mu\text{g}/\text{cm}^2\cdot\text{h}$) and Pheroid™ emulgel ($30.69 \mu\text{g}/\text{cm}^2\cdot\text{h}$). The three formulated products achieved higher average flux values for prilocaine HCl than for lidocaine HCl. Prilocaine HCl has a slightly lower log P value (2.1) than lidocaine HCl (3.4) that might partition better into the skin (Williams, 2003:36) and increase the flux value of prilocaine HCl. The Pheroid™ emulgel yielded an average steady-state flux for prilocaine HCl twice as high as for lidocaine HCl. This indicated that the prilocaine HCl diffused easier into the skin from the Pheroid™ emulgel than the lidocaine HCl. The PBS solution, the Pheroid™ solution and the commercial product had lower average steady-state flux values for prilocaine HCl than for lidocaine HCl (Table D.7). The hydrogel, PBS solution and Pheroid™ solution hydrated the stratum corneum and was able to produce higher flux values for the hydrophilic API than the more lipophilic formulations (emulgel, Pheroid™ emulgel) (Hadgraft, 1999:4). The median flux values for prilocaine HCl did not vary extensively from the average flux values which indicates that a small amount of outliers were present in the data. The hydrogel displayed the largest difference between

the average and median flux values and indicates that more outliers were present in the data. If there are a large number of outliers in the data it is better to use the median flux value (Gerber *et al.*, 2008:190).

The average prilocaine HCl concentration that diffused through the skin after 12 h was in accordance with the pattern displayed by the average flux values. The high average flux value of prilocaine HCl produced by the hydrogel yielded a high average concentration of diffused prilocaine HCl, followed by the commercial product, PBS solution, Pheroid™ solution, emulgel and Pheroid™ emulgel. Prilocaine HCl benefits more from a lower log P value (2.1) than lidocaine HCl (3.4), which falls in the ideal range described by Williams (2003:36) that indicated that prilocaine HCl partitioned slightly better into the skin layers from the formulated products than lidocaine HCl.

Table D.9: The total concentration amount ($\mu\text{g}/\text{cm}^2$) of local anaesthetic APIs that diffused through the skin after 12 h

	Average concentration APIs after 12 h ($\mu\text{g}/\text{cm}^2$)
Commercial	1027.94
PBS solution	739.37
Hydrogel	719.11
Pheroid™ solution	522.25
Emulgel	351.25
Pheroid™ emulgel	285.81

The use of the commercial product resulted in the highest concentration of diffused local anaesthetic APIs through the skin after 12 h ($1027.94 \mu\text{g}/\text{cm}^2$), followed by the hydrogel ($719.11 \mu\text{g}/\text{cm}^2$), PBS solution ($739.37 \mu\text{g}/\text{cm}^2$), Pheroid™ solution ($522.25 \mu\text{g}/\text{cm}^2$), emulgel ($351.25 \mu\text{g}/\text{cm}^2$) and lastly the Pheroid™ emulgel ($285.81 \mu\text{g}/\text{cm}^2$). The more hydrophilic formulation (hydrogel) and more hydrophilic solution (PBS solution) had higher concentration amounts of local anaesthetic APIs that diffused through the skin than the more lipophilic formulations (emulgel, Pheroid™ emulgel) and solution (Pheroid™ solution). This can be attributed to the fact that the hydrogel and PBS solution contains large amounts of water which hydrates the skin and allows more API to penetrate and diffuse through the skin via the intercellular route (Barry, 2007:576). The salt forms of lidocaine and prilocaine are highly water soluble and have a higher affinity for a more hydrophilic environment. The lipophilic formulations contain a lower amount of water and may not increase skin hydration as much as the hydrophilic formulations. The commercial product is formulated at a pH of 9, which affects the ionisation ratio of the APIs and

gives it an advantage over the other products formulated at a lower and less skin-harming pH of 8 (Richards & McConachie, 1995:41).

D.3.3.2.3 Statistical analysis of the steady-state flux data

A one-way ANOVA test concluded that there were significant statistical differences between the flux values of lidocaine HCl in the different treatment groups ($p < 0.05$). The Games-Howell test revealed no significant differences between the lidocaine HCl flux values of the emulgel and the hydrogel ($p = 0.696$) and the hydrogel and the Pheroid™ solution ($p = 0.065$). Significant differences between the flux values of lidocaine HCl were present between the Pheroid™ emulgel and all the other formulated products and solutions ($p < 0.05$), the commercial product and all the other formulations and solutions ($p < 0.05$) and the PBS solution and all the other formulations and solutions ($p < 0.05$). The statistical analysis validates the conclusion that the lidocaine HCl in the Pheroid™ emulgel had the weakest skin penetration (lowest flux). Figure D.29 graphically illustrates the differences between the lidocaine HCl flux values obtained from the various treatments.

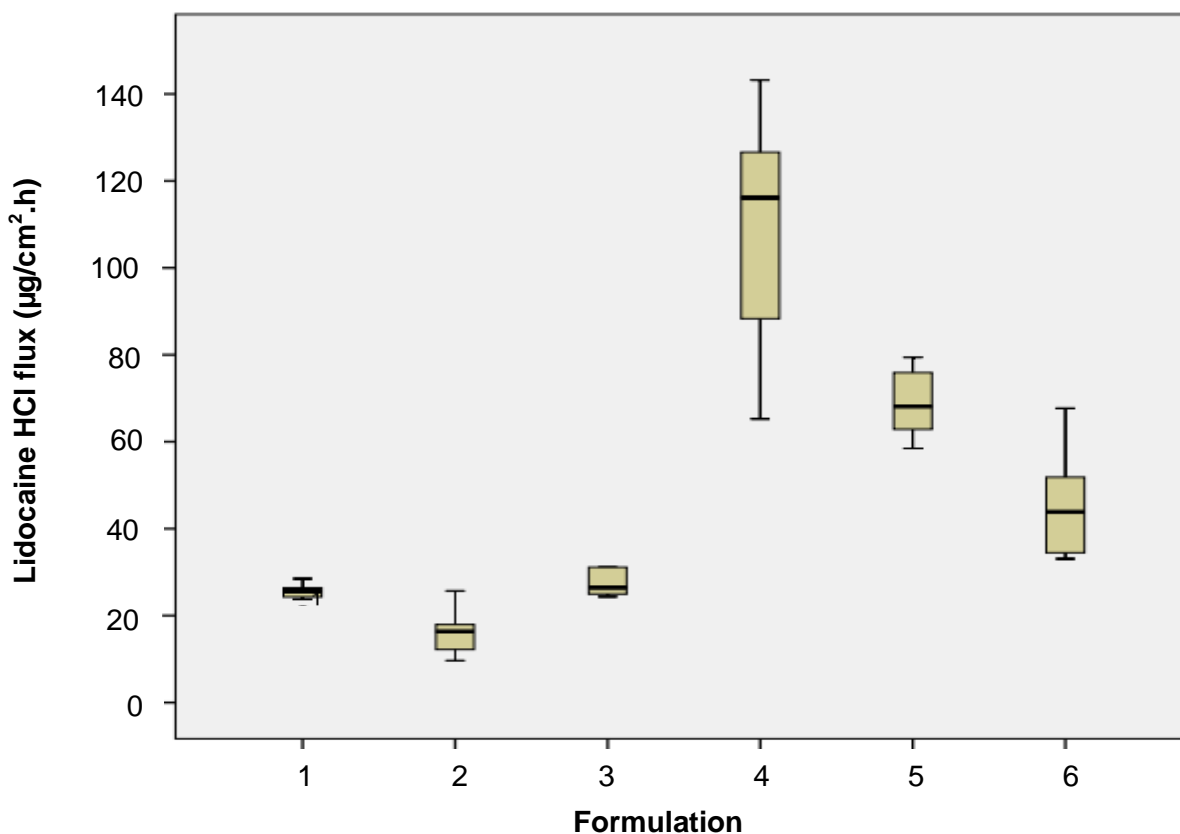


Figure D.29: Box-plot to illustrate the difference in mean flux values of lidocaine HCl. 1 = Emulgel, 2 = Pheroid™ emulgel, 3 = hydrogel, 4 = commercial product, 5 = PBS solution and 6 = Pheroid™ solution.

The one-way ANOVA test revealed that statistical significant differences were present between the flux values of prilocaine HCl of the different treatment groups ($p < 0.05$). The Games-Howell test then further concluded that there was no significant difference between the flux values of prilocaine HCl of the emulgel and the Pheroid™ emulgel ($p = 1$) and the Pheroid™ solution ($p = 0.218$), the Pheroid™ emulgel and the Pheroid™ solution ($p = 0.203$), the commercial product and the PBS solution ($p = 0.599$) and the PBS solution and the Pheroid™ solution ($p = 0.588$). There was a significant difference between the flux value of prilocaine HCl from the hydrogel and all the other formulations and solutions where $p < 0.05$. Figure D.30 illustrates the differences between the flux values of prilocaine HCl obtained from the various treatments.

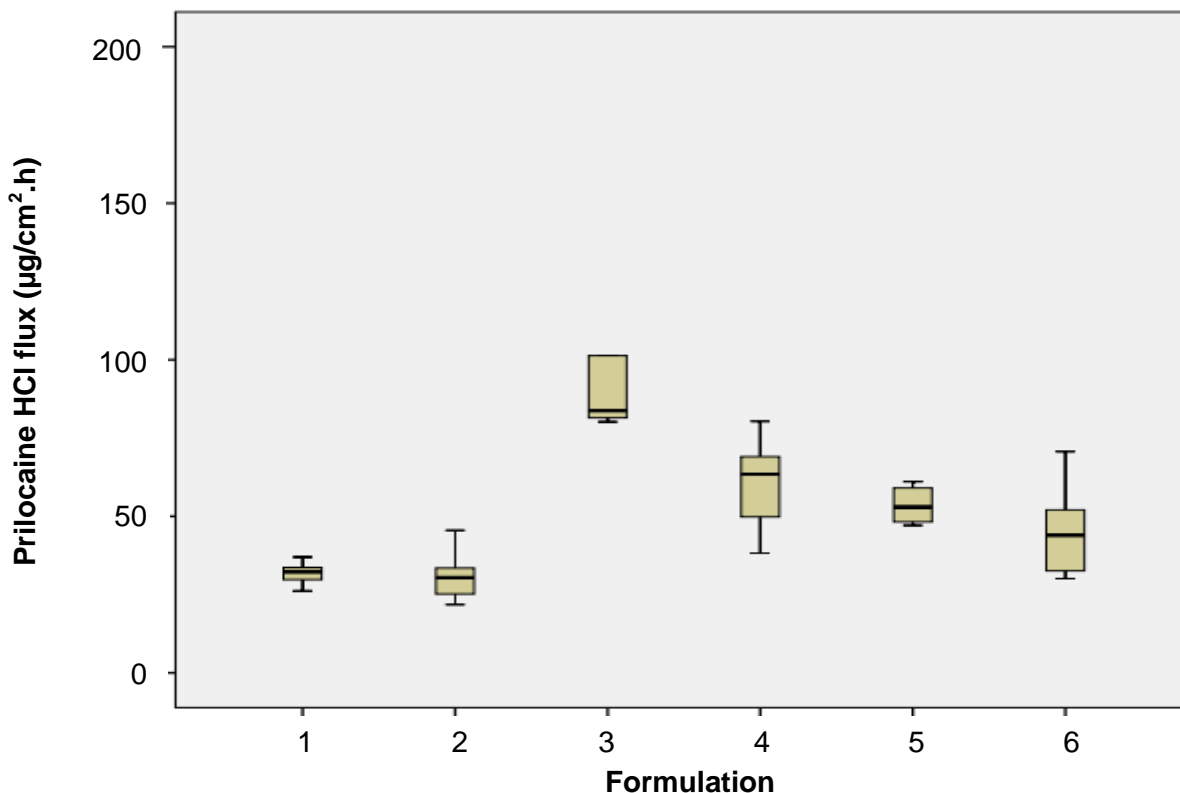


Figure D.30: Box-plot to illustrate the difference in mean flux values of prilocaine HCl. 1 = Emulgel, 2 = Pheroid™ emulgel, 3 = hydrogel, 4 = commercial product, 5 = PBS solution and 6 = Pheroid™ solution.

D.3.3.2 Concentration amounts of lidocaine HCl and prilocaine HCl present in the stratum corneum-epidermis and the epidermis-dermis layers

After the completion of the 12 h diffusion experiments, tape stripping was performed on the skin (Section D.2.3). For local anaesthetics to have an anaesthetic effect it is crucial that they penetrate the skin dermis to reach the free nerve endings located there (Welin-Berger *et al.*, 2002). The following section notes the average and median concentration amounts of both APIs in the stratum corneum-epidermis and epidermis-dermis layers in Table D.10 and Table D.11.

D.3.3.3.1 Lidocaine hydrochloride

The average and median concentration amounts of lidocaine HCl in the stratum corneum-epidermis and epidermis-dermis layers are noted in Table D.10.

Table D.10: Average concentration amounts of lidocaine HCl present in the stratum corneum-epidermis and epidermis-dermis layers of the skin

	Stratum corneum-epidermis		Epidermis-dermis	
	Average concentration (µg/ml)	Median concentration (µg/ml)	Average concentration (µg/ml)	Median concentration (µg/ml)
Emulgel	0.00	0.00	9.35 ±3.2	8.93
Pheroid™ emulgel	2.25 ±1.5	3.04	1.16 ±0.9	1.87
Hydrogel	1.57 ±2.2	1.98	2.91 ±1.7	2.62
Commercial	22.09 ±7.5	16.32	10.93 ±3.3	8.55
PBS solution	6.63 ±1.5	6.46	12.23 ±7.1	10.56
Pheroid™ solution	6.99 ±0.9	7.22	22.64 ±17.1	19.48

The use of the commercial product produced the highest average concentration lidocaine HCl in the stratum corneum-epidermis (22.09 µg/ml), followed by the Pheroid™ solution (6.99 µg/ml), PBS solution (6.63 µg/ml), the Pheroid™ emulgel (2.25 µg/ml) and the hydrogel (2.25 µg/ml). The use of the formulated emulgel produced no lidocaine HCl in the stratum corneum-epidermis. The average stratum corneum-epidermis concentration (22.09 µg/ml) and the median stratum corneum-epidermis concentration (16.32 µg/ml) for the commercial product's diffusion experiment varied notably, which is attributed to numerous outliers in the data. It would be more accurate to use the median value as it is less affected by the outliers in the data (Gerber *et al.*, 2008:190). The use of the Pheroid™ solution produced the second highest average lidocaine HCl concentration in the stratum corneum-epidermis, indicating that the Pheroid™ vesicles encapsulating the lidocaine HCl increased the stratum corneum-epidermis penetration (Grobler *et al.*, 2008:293). The Pheroid™ emulgel, however, showed poor epidermal penetration. Surber & Smith (2000:34) explain that maximum skin partitioning of an API occurs when the API is formulated in a poor solvent system but has better solubility in the barrier membrane (stratum corneum-epidermis). The Pheroid™ emulgel contains more oil components (liquid paraffin) than the Pheroid™ solution. Lidocaine HCl is hydrophilic and formulated within a more lipophilic formulation (emulgel). The stratum corneum-epidermis is also lipophilic and the conclusion was made that the lidocaine HCl would struggle to partition from the lipophilic vehicle into a lipophilic layer (Surber & Smith, 2000:34). The API characteristics and the oil/water ratio of emulsions should be carefully considered for optimal API delivery into the skin (Ramchandani & Toddywala, 1997:544).

The use of the Pheroid™ solution resulted in the highest average concentration of lidocaine HCl in the epidermis-dermis (22.64 µg/ml), indicating that the Pheroid™ vesicles encapsulating lidocaine

HCl had increased the penetration into the epidermis-dermis with a greater amount if compared with the other formulations. The average concentration of lidocaine HCl in the epidermis-dermis varied slightly from the median concentration, indicating that a small number of outliers were present in the data. The use of the PBS solution produced the second highest average concentration of lidocaine HCl (12.23 µg/ml) in the epidermis-dermis, followed by the commercial product (10.93 µg/ml). The Pheroid™ emulgel produced exceptionally low penetration into the epidermis-dermis (1.16 µg/ml). When the Pheroid™ emulgel is compared with the Pheroid™ solution, the difference in stratum corneum-epidermis and epidermis-dermis concentration is 2.25 µg/ml compared with 6.99 µg/ml and 1.16 µg/ml compared with 22.64 µg/ml, respectively. This may indicate that the chosen emulgel formula was not ideally suited for the Pheroid™ and lidocaine HCl as mentioned previously. The Pheroid™ solution consisted of a larger amount of water than the Pheroid™ emulgel which might have caused the stratum-corneum to become hydrated and allow a larger amount of hydrophilic API molecules to partition through the stratum corneum-epidermis and into the more hydrophilic viable dermis layer of the skin (Barry, 2007:576)

D.3.3.3.2 Prilocaine hydrochloride

The average and median concentration amounts of lidocaine HCl in the stratum corneum-epidermis and epidermis-dermis layers are noted in Table D.10.

Table D.11: Average concentration amounts of prilocaine HCl present in the stratum corneum-epidermis and epidermis-dermis layers of the skin

	Stratum corneum-epidermis		Epidermis-dermis	
	Average concentration (µg/ml)	Median concentration (µg/ml)	Average concentration (µg/ml)	Median concentration (µg/ml)
Emulgel	0	0	4.38 ±3.2	3.90
Pheroid™ emulgel	0	0	2.46 ±0.4	2.60
Hydrogel	3.33 ±3.0	4.02	4.00 ±1.2	3.80
Commercial	12.40 ±10.6	11.32	6.34 ±1.9	5.60
PBS solution	4.13 ±5.3	5.14	10.90 ±7.3	8.45
Pheroid™ solution	3.81 ±1.2	3.61	21.32 ±16.7	19.06

The commercial product produced the highest average concentration of prilocaine HCl in the stratum corneum-epidermis (12.40 µg/ml), followed by the PBS solution (4.13 µg/ml), Pheroid™ solution (3.81 µg/ml) and the hydrogel (3.33 µg/ml). The emulgel and Pheroid™ emulgel produced no prilocaine HCl values in the stratum corneum-epidermis, but concentration amounts of prilocaine

HCl from the emulgel and Pheroid™ emulgel were present in the epidermis-dermis. This can be attributed to the fact that the hydrophilic APIs did not have high affinity for the lipophilic surroundings of the stratum corneum-epidermis and did not accumulate in this layer but diffused into the more hydrophilic viable dermis (Barry, 2007:574; Ghafourian *et al.*, 2010:612).

The Pheroid™ solution had the highest average concentration of prilocaine HCl (21.32 µg/ml) in the epidermis-dermis, indicating excellent dermal penetration by the Pheroid™ vesicles. The PBS solution had the second best average concentration in the epidermis-dermis (10.90 µg/ml), followed by the commercial product (6.34 µg/ml), emulgel (4.38 µg/ml), hydrogel (4.00 µg/ml) and the Pheroid™ emulgel (2.46 µg/ml). The Pheroid™ solution contains more water than the Pheroid™ emulgel which may have increased the hydration of the stratum corneum-epidermis, making it easier for the hydrophilic APIs to penetrate into the hydrophilic viable epidermis (Barry, 2007:574, 576). The average concentration values for prilocaine HCl in the epidermis-dermis varied notably from the median concentrations because of outliers in the data. The median value is unaffected by data outliers and displays more accurate epidermis-dermis concentration values (Gerber *et al.*, 2008:190).

D.3.3.3.3 Statistical analysis of the stratum corneum-epidermal and epidermal-dermal concentrations of lidocaine HCl and prilocaine HCl

A one-way ANOVA test concluded that there was a significant statistical difference between the stratum corneum-epidermis and epidermis-dermis data of the different formulations ($p < 0.05$). The Games-Howell test further concluded that there was no significant difference in the lidocaine HCl concentration in the stratum corneum-epidermis of the emulgel and the hydrogel ($p = 0.339$), the hydrogel and the Pheroid™ emulgel ($p = 0.972$) and the commercial product and the two solutions (PBS solution $p = 0.214$; Pheroid™ solution $p = 0.230$). Figure D.31 illustrates the difference in lidocaine HCl concentration in the stratum corneum-epidermis between the formulations and solutions. Cohen's test revealed that there were practical significant differences between the concentration of lidocaine HCl in the stratum corneum-epidermis of the formulations where $d > 0.8$, except between the Pheroid™ emulgel and hydrogel ($d = 0.2$). The concentration of lidocaine HCl in the epidermis-dermis showed significant statistical differences between the emulgel and all the other formulations, as well as the hydrogel and all the other formulations where $p < 0.05$. Figure D.32 illustrates the differences in the concentration of lidocaine HCl in the epidermis-dermis. There were practical significant differences between all the concentration values of lidocaine HCl in

the epidermis-dermis of all the formulations where $d > 0.8$, except between the commercial product and the PBS solution where $d = 0.1$.

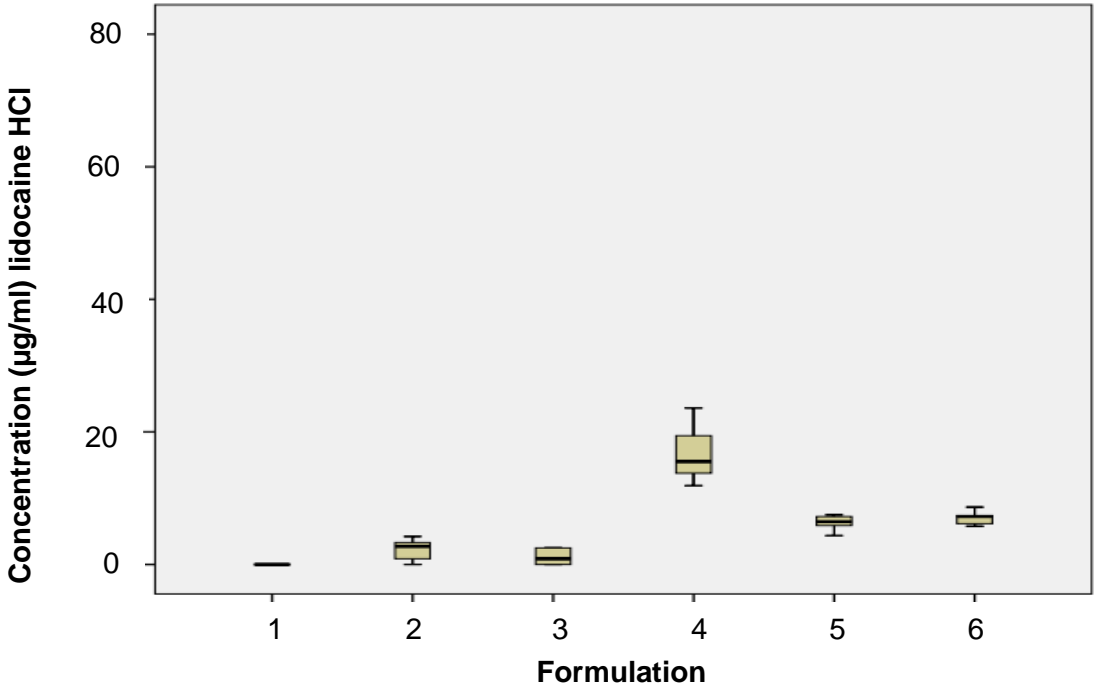


Figure D.31: Box-plot to illustrate the difference in stratum corneum-epidermis lidocaine HCl concentration. 1 = Emulgel, 2 = Pheroid™ emulgel, 3 = hydrogel, 4 = commercial product, 5 = PBS solution and 6 = Pheroid™ solution.

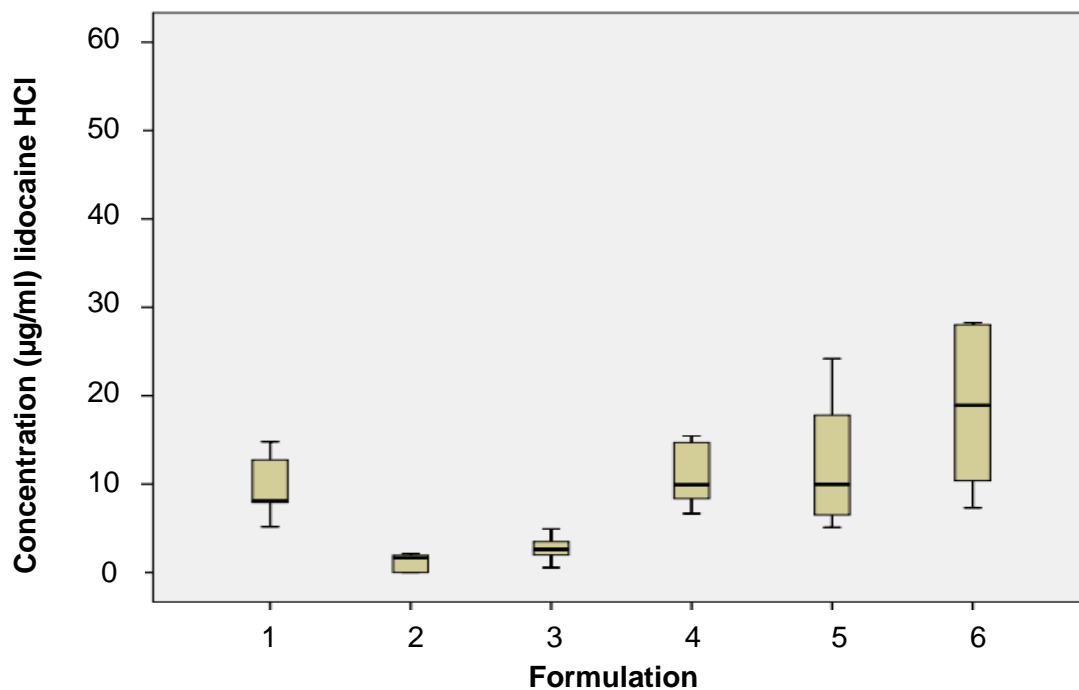


Figure D.32: Box-plot to illustrate the difference in epidermis-dermis lidocaine HCl concentration. 1 = Emulgel, 2 = Pheroid™ emulgel, 3 = hydrogel, 4 = commercial product, 5 = PBS solution and 6 = Pheroid™ solution.

The stratum corneum-epidermis and epidermis-dermis concentrations of prilocaine HCl in the different formulations revealed statistical significant differences between the formulations in the ANOVA test ($p < 0.05$). To determine the differences the Games-Howell test was utilised and graphically inspected using box-plots (Figures D.33 and D.34). The emulgel and Pheroid™ emulgel had no concentrations of prilocaine HCl in the stratum corneum-epidermis which varied significantly from the prilocaine HCl concentrations of the other formulations in the stratum corneum-epidermis. Cohen's test revealed practically significant differences between the concentrations of prilocaine HCl in the stratum corneum-epidermis, between all the formulations where $d > 0.8$, except between the solution and the Pheroid™ solution ($d = 0.09$). The epidermis-dermis concentration of the prilocaine HCl in the Pheroid™ emulgel showed a significant difference to the hydrogel ($p = 0.021$), the commercial product ($p = 0.001$), the PBS solution ($p = 0.036$) and the Pheroid™ solution ($p = 0.001$). This led to the conclusion that the Pheroid™ emulgel had the weakest penetration into the epidermis-dermis of all the formulations. There was no significant difference between the Pheroid™ emulgel and the emulgel's penetration ability ($p = 0.541$) into the

epidermis-dermis. Practically significant differences between the epidermis-dermis concentration of prilocaine HCl in the different formulations were present where $d > 0.8$ except between the emulgel and Pheroid™ emulgel ($d = 0.03$).

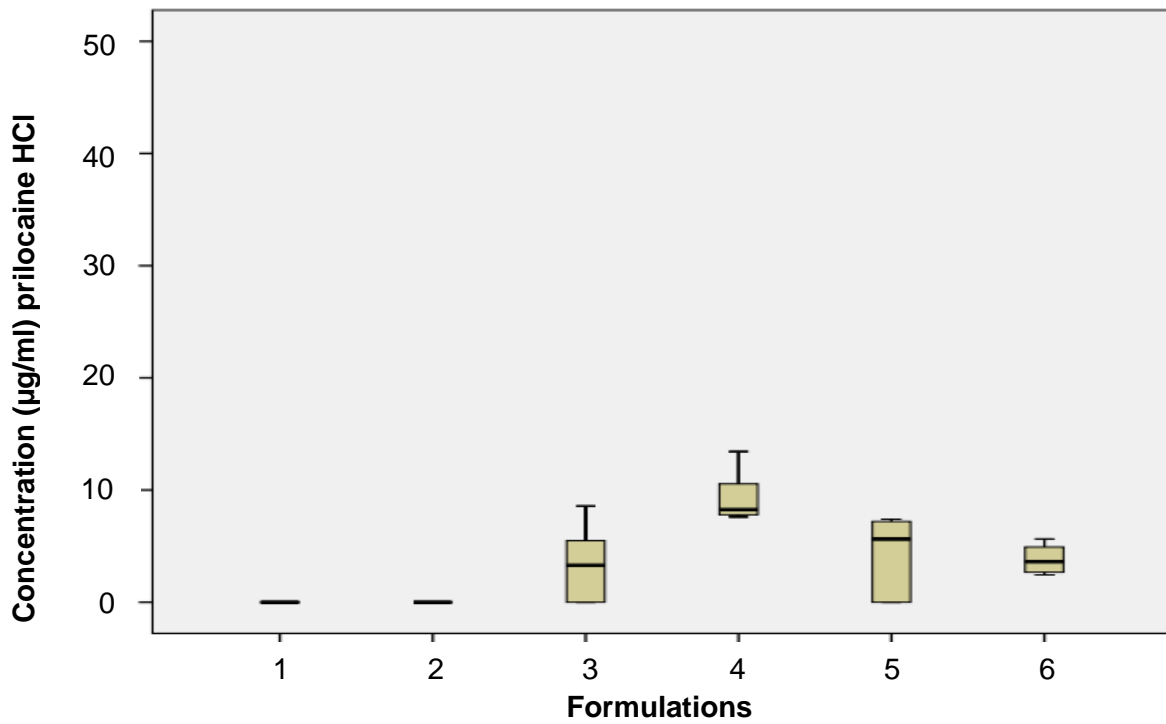


Figure D.33: Box-plot to illustrate the difference in stratum corneum-epidermis prilocaine HCl concentration. 1 = Emulgel, 2 = Pheroid™ emulgel, 3 = hydrogel, 4 = commercial product, 5 = PBS solution and 6 = Pheroid™ solution.

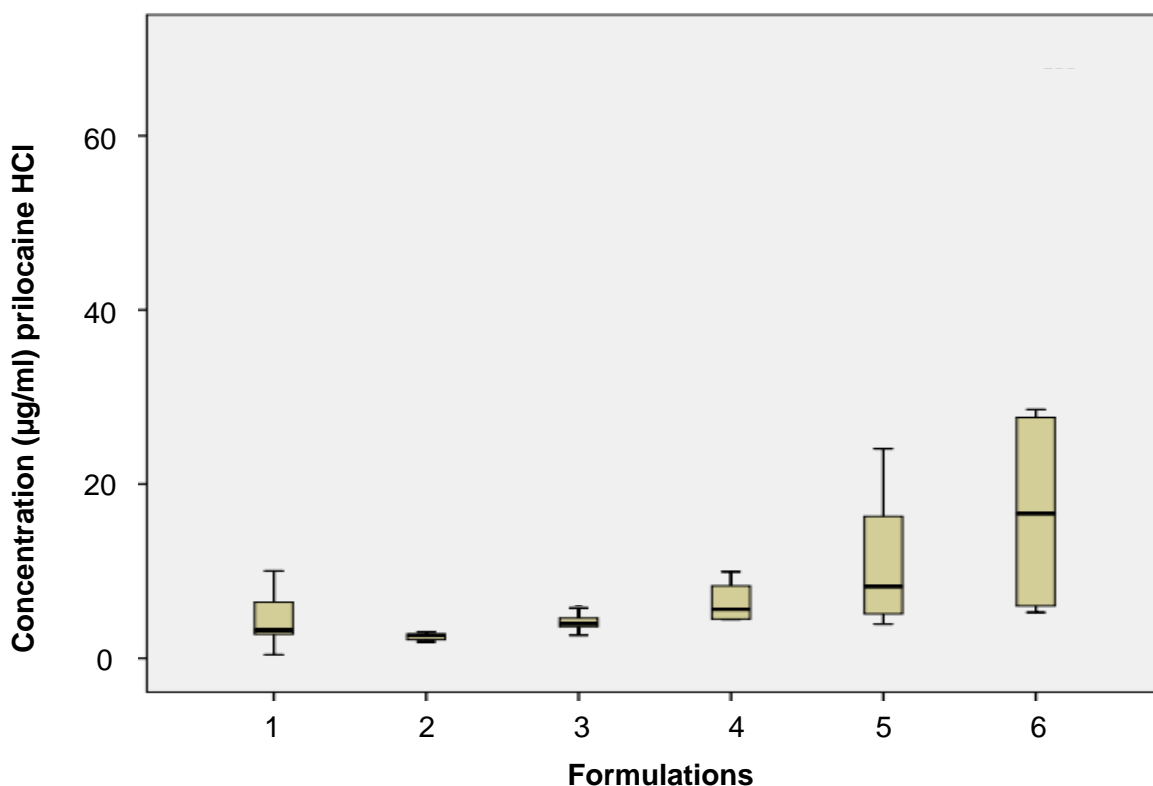


Figure D.34: Box-plot to illustrate the difference in epidermis-dermis prilocaine HCl concentration. 1 = Emulgel, 2 = Pheroid™ emulgel, 3 = hydrogel, 4 = commercial product, 5 = PBS solution and 6 = Pheroid™ solution.

D.4 CONCLUSION

If the physicochemical properties of lidocaine HCl and prilocaine HCl are taken into consideration, they show characteristics of substances ideally suited for transdermal delivery. Both lidocaine HCl and prilocaine HCl have melting points lower than 200 °C, log D values of 3.4 and 2.1, respectively, and good aqueous solubility of more than 1 mg/ml. The challenge is to overcome the ionisation characteristics of the two APIs. Lidocaine and prilocaine are amide type local anaesthetics with poor solubility and are used in formulations in their water soluble salt forms. These salt forms have high pKa values (7.9) which means they have to be formulated at a high pH for increased ionisation (Richards & McConachie, 1995:41). This has an effect on the number of API molecules that are able to penetrate the skin layers. The aim of this study was to investigate the transdermal penetration into the skin dermis of these local anaesthetics in different formulations, with the assistance of Pheroid™ technology, an API-enhancing delivery system. The results were compared with that of a commercial product formulated at a high pH of 9.

During the membrane studies all the formulations released lidocaine HCl and prilocaine HCl effectively. The hydrogel released the highest concentration of both APIs. This can be due to the high water contents of the hydrogel which increases solubility and permeation through the artificial membranes.

The commercial local anaesthetic product takes up to 60 min to produce an anaesthetic effect (Wahlgren & Quiding, 2000:586). The lag times of the different formulations were determined and compared with that of the commercial product. The emulgel produced the best lag time for lidocaine HCl (1.65 h) but a longer lag time for prilocaine HCl (1.93 h). The Pheroid™ emulgel produced a long lag time of 1.99 h for lidocaine HCl, but produced the shortest lag time of 1.41 h for prilocaine HCl of all the products tested. Both the emulgel and Pheroid™ emulgel contain oils that can act as occlusives on the skin. The oil content prevents water loss from the skin and leaves the skin fully hydrated (Barry, 2007:577). The lipophilic nature of the formulations allowed skin penetration to initiate rapidly and produced short lag times as a result. Wahlgren & Quiding (2000:586-587) also proved that the depth of anaesthesia in the skin layers increases with increased application time (longer than 60 min). The results of this study showed that the commercial product and the hydrogel had the highest percentage diffused local anaesthetic APIs through the skin over time, which might have provided the best anaesthetic effect if the diffused percentage is the only parameter taken into consideration.

The commercial product had the highest flux value for lidocaine HCl and reached a steady-state flux quicker than the other formulations. This can be attributed to the high formulation pH of the commercial product (pH = 9) where 90% of the APIs were in their unionised fraction. The unionised form of an API is more lipid soluble than the ionised form and penetrates easier into the lipophilic stratum corneum-epidermis (Surber & Smith, 2000:27). The formulated products had a pH of 8, which is less skin irritating and more ideal for Pheroid™. At this pH only 52% of the APIs are in their lipid soluble fraction, which indicates that there were 38% less unionised API fractions available for skin penetration and the reason why the commercial product had a greater lidocaine HCl flux. The highest flux for prilocaine HCl was, however, not achieved by the commercial product. The hydrogel produced the highest prilocaine HCl flux value. The hydrogel contains 70% water, which disrupts the lipid bilayers in the stratum corneum and causes it to swell and soften which makes it possible for large amounts of hydrophilic APIs to move through the stratum corneum-epidermis and into the hydrophilic viable epidermis at an increased concentration gradient (Ghafourian, 2010:612; Surber & Smith, 2000:28). Prilocaine HCl had a smaller partitioning value

(log P = 2.1) than lidocaine HCl (log P = 3.4) which might have been the reason why the prilocaine HCl was able to achieve a higher flux from the hydrogel than the lidocaine HCl. The hydrogel had long lag times, which indicate that it took long for the hydrogel formulation to hydrate the skin. This contributes to the conclusion that the hydrogel will perform better with a longer application time (2 h).

The Pheroid™ solution provided better flux values for both lidocaine HCl and prilocaine HCl than the Pheroid™ emulgel. This indicated that the lidocaine HCl and prilocaine HCl took a longer time to reach a steady-state flux in the Pheroid™ emulgel and that the release for these two APIs was not optimal from the lipophilic emulgel vehicle. Kruger (2008:57) found that a Pheroid™ solution containing lidocaine HCl and prilocaine HCl increased in transdermal flux compared with a solution without Pheroid™. In this study the solution without Pheroid™ (PBS solution) had slightly better flux values for both APIs than the solution with Pheroid™. Kruger (2008:49) does not mention at what pH the donor phase solutions were prepared. The results were directly compared with a commercial product and the assumption was made that the donor phase solutions were prepared at the same pH as the commercial product (pH 9) in which high unionised fractions of the APIs were present as explained in the previous paragraph. The donor solutions included a vasoconstrictor agent, adrenaline, which reduced the systemic uptake of the APIs and localised the anaesthetic effect (Kruger, 2008:46).

After 12 h the commercial product's average local anaesthetic API concentration was 72% higher than the Pheroid™ emulgel (28%), 65% more than the emulgel (35%), 51% more than the Pheroid™ solution (49%) and 30% more than the hydrogel (70%) (Table D.9).

The target site for local anaesthetics is the nerve endings located in the epidermis-dermis and not systemic delivery (Barry, 2007:570). Thus, the amount of APIs that penetrated and stays in the epidermis-dermis is crucial for an anaesthetic effect. The Pheroid™ solution had the highest concentration lidocaine HCl and prilocaine HCl in the epidermis-dermis. This proves that the encapsulating technique of the Pheroid™ vesicles to improve penetration into the skin was successful for lidocaine HCl and prilocaine HCl (Grobler *et al.*, 2008:293). The Pheroid™ emulgel had the weakest epidermis-dermis penetration. As mentioned in Section D.3.3.3.1, the extra oil components in the Pheroid™ emulgel (liquid paraffin) might have delayed the partitioning of the hydrophilic APIs from their delivery vehicle when compared with the Pheroid™ solution. The Pheroid™ solution allowed more skin hydration than the Pheroid™ emulgel and had the benefit of

the penetration enhancing effect of the Pheroid™ vesicles which allowed high amounts of the APIs to penetrate the hydrophilic epidermis-dermis (Barry, 2007:576; Grobler *et al.*, 2008:293).

The commercial product had high API concentrations in the stratum corneum-epidermis, but only produced average epidermis-dermis concentrations. At a pH of 9, the commercial product benefits from the high lipid soluble unionised fractions that can partition into the skin. Once this large number of unionised API molecules reaches the stratum corneum-epidermis via a high steady-state flux, the lipid soluble unionised fractions show a high affinity to the lipophilic surroundings and accumulate in the stratum corneum-epidermis (Surber & Smith, 2000:27). Surber & Smith (2000:27) state that the pH of optimum API solubility is not always the pH of maximum stability of a topical formulation. This leads to the conclusion that the commercial product, at a higher pH, failed to provide dermal concentrations of the APIs as high as the Pheroid™ solution. The hydrogel, which showed a high diffused percentage local anaesthetic APIs, showed extremely low concentration values in the stratum corneum-epidermis and epidermis-dermis. This indicates that more of the APIs was absorbed systemically because of their high solubility in the hydrogel. The hydrophilic API molecules continued to diffuse through the water soluble dermis layer into the blood circulation because of the high solubilisation state it was able to achieve through the hydrophilic formulation and the highly hydrated state of the stratum corneum (Surber & Smith, 2000:27). Systemic absorption is not the target for local anaesthesia and can cause dangerous side effects if too much of the APIs are absorbed into the blood circulation.

There is a clear indication that the Pheroid™ made a difference in the topical delivery of lidocaine HCl and prilocaine HCl. Compared with the commercial product it produced competitive lag times and high epidermis-dermis penetration. The problem is to choose the right formulation which, combined with Pheroid™, is not too lipophilic that it drastically depletes the barrier clearance ability of the APIs into the horny aqueous layer of the skin and in doing so depletes the dermal penetration of the hydrophilic actives lidocaine HCl and prilocaine HCl.

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APPENDIX E

AUTHOR'S GUIDELINES FOR THE INTERNATIONAL JOURNAL OF PHARMACEUTICS

E.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 personalized medicines, and publishes research papers, reviews, commentaries and letters to the editor as well as special issues.

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Pharmaceutical Scientists, Clinical Pharmacologists, Chemical Engineers, Biotechnologists.

E.3 IMPACT FACTOR

2011: 3.350 © Thomson Reuters Journal Citation Reports 2012.

E.4 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).

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(2) Rapid Communications

- (a) These articles should not exceed 1 500 words or an 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.

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those individuals who provided help during the research (e.g., providing language help, writing assistance or proof reading the article, etc.).

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Strunk Jr., W., White, E.B., 2000. *The Elements of Style*, fourth ed. Longman, New York.

Reference to a chapter in an edited book:

Mettam, G.R., Adams, L.B., 2009. How to prepare an electronic version of your article, in: Jones, B.S., Smith, R.Z. (Eds.), *Introduction to the Electronic Age*. E-Publishing Inc., New York, pp. 281–304.

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