PHEROID™ TECHNOLOGY FOR THE TRANSDERMAL DELIVERY OF LIDOCAINE AND PRILOCAINE

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ABSTRACT

Local anaesthetics have been implemented extensively in the case of a variety of painful superficial procedures, venipuncture, skin graft harvesting, anal or genital pruritus, poison ivy rashes, postherpetic neuralgia and several other dermatoses. The dilemma with commercially available local acting anaesthetics is that it may take well up to an hour to produce an anaesthetic effect. Anaesthetics have to traverse the highly efficient barrier, the stratum corneum, in order to reach the intended target site which is the free nerve endings located in the dermis.

The objective of this study was to compare the transdermal delivery of an eutectic combination of two ionisable amide types of local anaesthetics, lidocaine HCl and prilocaine HCl, delivered with the novel Pheroid™ technology to that of a commercially available product in order to establish whether the lag time could be significantly reduced.

Several techniques of promoting the penetration of these anaesthetics have previously been employed, including occlusive dressing, entrapment in liposomes and miscelles, iontophoretic delivery and so forth. The Pheroid™ delivery system is novel technology that entails improved delivery of several active compounds. It is a submicron emulsion type formulation that possesses the ability to be transformed in morphology and size, thereby affording it tremendous flexibility. Since it primarily consists of unsaturated essential fatty acids, it is not seen as foreign to the body but rather as a skin-friendly carrier.

Vertical Franz cell diffusion studies were performed over a 12 hour period using Caucasian female abdominal skin obtained, with the consent of the donor, from abdominoplastic surgery. Comparison was made between the commercial product EMLA® cream, the active local anaesthetics dissolved in phosphate buffered solution (PBS) and the active ingredients entrapped within Pheroid™ vesicles. Distinct entrapment could be ascertained visually by confocal laser scanning microscopy (CLSM). The amount of drug that traversed the epidermal membrane into the receptor phase was then assayed by high performance liquid chromatography (HPLC).

The results obtained with the Pheroid™ vesicles revealed a biphasic character with rapid permeation during the first two hours, followed by a plateau between 3 to 12 hours. The initial dramatic increase in percentage yield and flux indicates that the Pheroid™ carrier enhances the transdermal delivery of the actives in order to accelerate the onset of action.

Keywords: transdermal delivery, Pheroid™, lidocaine hydrochloride, prilocaine hydrochloride, local anaesthesia

OPSOMMING

Lokale verdowers word grootskaals gebruik vir 'n verskeidenheid pynlike oppervlakkige prosedures, venipunkture, versameling van vel vir oorplantings, anale of genitale pruritus, veluitslag, post-herpetiese neuralgie en talle ander dermatoses. Die dilemma met kommersieel beskikbare lokale verdowers is dat dit tot 'n uur kan neem om 'n anestetiese effek uit te oefen. Anestetika moet die hoogs effektiewe skans, die stratum corneum, deurdring om die teikengebied te bereik wat die senu-eindpunte in die dermis is.

Die doel van hierdie studie was om die transdermale aflewering van 'n eutektiese mengsel van twee ioniseerbare amiedtipe lokale verdowers, lidokaïen HCl en prilokaïen HCl, afgelewer met die nuwe Pheroid™-tegnologie te vergelyk met dié van 'n kommersieel beskikbare produk ten einde te bepaal of die vertragingstyd beduidend verkort kan word.

Talle tegnieke om die penetrasie van hierdie anestetika te bevorder, is voorheen gebruik, waaronder digsluitende bedekkings, insluiting in liposome en miselle, iontoforetiese aflewering en so meer. Die Pheroid™-afleweringstelsel is nuwe tegnologie wat beter aflewering van talle aktiewe verbindings gee. Dit is 'n tipe formulering van 'n emulsie op submikronvlak wat die vermoë besit om in morfologie en grootte te verander wat geweldige buigsaamheid daaraan gee. Omdat dit hoofsaaklik uit onversadigde essensiële vetsure bestaan, word dit nie as vreemd deur die liggaam beskou nie, maar eerder as 'n velvriendelike draer.

Diffusiestudies met koukasiese vroulike abdominale vel, met die toestemming van die skenker na abdominoplastiese chirurgie verkry, is oor 12 uur in vertikale Franz-selle gedoen. 'n Vergelyking van die kommersiële produk, EMLA®-room, die aktiewe lokale verdowers opgelos in fosfaatbuffer en die aktiewe bestanddele vasgevang in Pheroid™-vesikels is gemaak. Duidelike insluiting in die vesikels kon visueel met konfokale laserskandeer-mikroskopie bevestig word. Die hoeveelheid middel wat deur die epidermale membraan tot in die reseptorfase gedring het, is met hoëdoeltreffendheidvloeistofchromatografie (HDVC) bepaal.

Die resultate met die Pheroid™-vesikels verkry, toon 'n bifasige profiel met vinnige permeasie in die eerste twee uur gevolg deur 'n plato tussen 3 en 12 uur. Die aanvanklike dramatiese toename in persentasie opbrengs en vloed toon dat die Pheroid™-draer die transdermale aflewering van die aktiewe stowwe bevorder deur die aanvang van werking te versnel.

Sleutelwoorde: transdermale aflewering, Pheroid™, lidokaïenhidrochloried, prilokaïenhidrochloried, lokale verdowing

CHAPTER 1: INTRODUCTION AND PROBLEM STATEMENT

Attempts to relief pain must certainly be as old as humankind itself. From as long as 2500 years ago, narcotic substances like alcohol, cannabis, mandrake and opium had been taken to provide pain relief. The ancient Indian work Sushruta Samhita, which dates back as far as 400 BC, advised the use of alcohol to numb pain. The Greek physician Dioscorides in 58 AD recommended that patients swallow a blend of mandrake and wine before limb amputation and Celsus (37 AD) suggested the use of opium before surgery. Theodoric, a monk and physician in the 13th century described the *spongia somnifera* which is a brew of opium and mandrake amongst others, boiled within a sponge and then inhaled to provide general anaesthesia (Hamilton & Baskett, 2000:368). As time progressed, researchers have investigated and enhanced the pharmacological and physiological actions of new anaesthetic agents and the administration thereof.

Cocaine was the first local anaesthetic that was brought to use but when it became clear that the unwanted addiction exceeded its advantageous local anaesthetic effect, it led to the development of other derivatives. Lidocaine was developed by Nils Löfgren in 1943 and was the most widely used local anaesthetic during World War II (Calatayud & Gonzalez 2003:1507; White & Katzung, 2004:418). Lidocaine and prilocaine are both lipophilic amide type local anaesthetics (Conley & Brammar, 1999:816) that can be protonated to become more soluble hydrochloride salt forms (White & Katzung, 2004:418).

Transdermal delivery offers several advantages like bypassing first-pass metabolism, exerting its action locally on the site of application, enhancing patient compliance and reducing the risk of trauma and infection since it is a non-invasive method (Cerchiara & Luppi, 2006:89). The objective of transdermal delivery is to circumvent the excellent protective skin barrier in order to deliver therapeutic concentrations of a drug beyond the stratum corneum within reasonable time and without significant systemic effects (Sequeira, 1993:163).

Numerous physiological and structural factors such as skin age, anatomic variation, cutaneous metabolism, humidity, gender, race etc. can influence transdermal diffusion (Williams, 2003:14-18). The physicochemical properties of the permeant also play a fundamental role. Lidocaine and prilocaine both exhibit ideal properties for transdermal delivery; they have low molecular masses of 288.82 and 220.3 and low melting points of 79°C and 171°C respectively (BP, 2007) and moreover forms a binary eutectic mixture

(Sweetman, 2002:1318). The octanol-water partition coefficient (log P) values of these anaesthetics (2.36 ± 0.26 and 2.09 ± 0.49, calculated by ACD/lab ChemScketch Freeware 11.0) afford it the ability to partition well between the hydrophilic and lipophilic domains of the skin.

Several techniques have previously been employed to promote the penetration of lidocaine and prilocaine, for instance occlusive dressing (Astra Zeneca, 2004:2), entrapment in liposomes (Müller *et al.*, 2004:139) and miscelles (Scherlund *et al.*, 2000:37), and iontophoretic delivery, etc. (Abla *et al.*, 2006:185). Pheroid™ technology is a patented novel delivery system that is composed mainly of essential fatty acids. It is a pliable entity of which the morphology and size can be modified to best suit the specifications of the entrapped compound that needs to be delivered (Grobler *et al.*, 2008:285).

The objective was to determine the transdermal permeation of lidocaine and prilocaine with the use of Pheroid™ and EMLA® as delivery systems and to reduce the lag time of the currently available commercial product that take up to an hour to generate an anaesthetic effect.

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CHAPTER 2: TRANSDERMAL DELIVERY OF LOCAL ANAESTHETICS LIDOCAINE AND PRILOCAINE WITH ADRENALINE AS VASOCONSTRICTOR

1 INTRODUCTION

Local anaesthesia can be defined as the condition that results when sensory transmission of a circumscribed area of the body to the central nervous system is temporarily blocked, thereby resulting in reversible loss of sensation (Trevor *et al.*, 2002:240). These agents have proved to offer effective analgesia in the symptomatic treatment of well-defined regions of the body, for instance in the case of a variety of painful superficial procedures, venipuncture, skin graft harvesting, anal or genital pruritus, poison ivy rashes, postherpetic neuralgia, leg ulcers and several other dermatoses (Catterall & Mackie, 2006:378; Sweetman, 2002:1304; Astra Zeneca, 2004a). Currently there are numerous preparations of local anaesthetics available, e.g. various combinations of lidocaine, prilocaine and adrenaline injections, jellies, oral topical solutions, oral solutions, inhalation aerosols, nasal solutions, ophthalmic solutions, ointments and topical solutions (USP, 2007:2471).

Were it not for the significant barrier properties of the skin, many more drugs would be available on the market for transdermal administration (Behl *et al.*, 1994:107). Invasive methods of delivery have a more rapid onset of action, yet it is the least favourable because of its apprehensive nature (pain, needle phobia, repetitive injections and possible cross-contamination are contributing factors) (Williams, 2003:141). Topical local anaesthetic applications such as EMLA® cream can take up to an hour to cross the highly efficient protective stratum corneum in the skin in order to produce a therapeutic effect (Astra Zeneca, 2004b:2).

This chapter concerns itself with an overview of local anaesthesia, the anatomy of the skin, the various factors that influence transdermal penetration and the prospective ways in which the delivery of local anaesthetics can significantly be enhanced.

2 LOCAL ANAESTHETICS DELINEATED

2.1 HISTORY

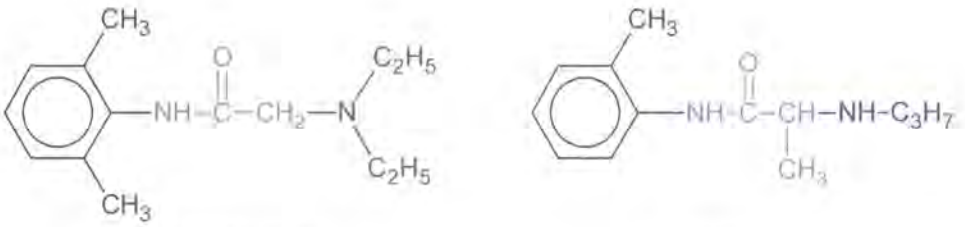
Cocaine was the first local anaesthetic that was discovered by Albert Niemann in Germany in the 1860s. It was isolated from the leaves of the coca plant which are indigenous to the

Andes Mountains in the West Indies and Java. The eminent Austrian psychoanalyst Sigmund Freud was the first to use cocaine clinically for the purpose of weaning a patient who was addicted to morphine. He and his colleague, Karl Koller, noticed the local anaesthetic effect of cocaine in the 1880s and Koller first introduced it to ophthalmology as a topical ocular anaesthetic. In 1884, Dr. William Stewart Halsted described the injection of cocaine into a sensory nerve trunk to produce surgical anaesthesia. Fatefully both Freud and Halsted became addicted through self-experimentation (White & Katzung, 2004:418; Catterall & Mackie, 2006:369; Revis, 2005; Spiller, 2000).

As time progressed it became apparent that the euphoria and consequent unsolicited addiction of cocaine exceeded its advantageous local anaesthetic effect. The development of modern organic chemistry led to the synthesis of the first derivative, procaine, which was developed by Einhorn in 1905 (White & Katzung, 2004:418). Procaine, however, was not as potent as cocaine, had a long time of onset, wore off rapidly and was classified as an ester, which has a high tendency to cause allergic reactions. Therefore dentists of the day preferred to work without the use of any anaesthetic (except for nitrous oxide gas). Lidocaine was developed by Nils Löfgren in 1943 and was the most widely used local anaesthetic during World War II (Calatayud & Gonzalez 2003:1507; White & Katzung, 2004:418).

2.2 CHEMISTRY AND STRUCTURE-ACTIVITY RELATIONSHIP OF LOCAL ANAESTHETICS

Clinically active local anaesthetics share the same general chemical configuration of an ionisable hydrophilic group (usually a tertiary amine portion) connected by an intermediate chain, such as an ester or amide, to a lipophilic aromatic residue. This intermediate linker region plays an important role in determining the pharmacological properties of the drug (Sweetman, 2002:1302; Catterall & Mackie, 2006:369). Local anaesthetics with an ester link are hydrolysed readily by plasma esterases and result in shorter duration of action (Catterall & Mackie, 2006:369; Torrens & Castellano, 2006:22). Hypersensitivity can also be related to the ester type (Sweetman, 2002:1302). Lidocaine and prilocaine are both ionisable, lipid soluble tertiary amine compounds (Conley & Brammar, 1999:816; Hille, 1992:404) and the solubility and stability of these weak bases are greatly increased when made available in the form of its salt (White & Katzung, 2004:418). Figure 2.1 illustrates the junction of the local anaesthetic moiety.



LIDOCAINE PRILOCAINE

Figure 2.1: Structure of lidocaine and prilocaine with aromatic ring connected to amine moiety by amide chain (Catterall & Mackie, 2006:370)

Local anaesthetics exist as either the uncharged base or protonated cation depending on their pK_a and the pH of the biological environment. At physiological pH it can diffuse through connective tissue and cellular membranes to reach the nerve fibre where ionisation occurs (Sweetman, 2002:1303). These forms are in rapid equilibrium with each other (White & Katzung, 2004:418; Hille, 1992:405) and the relative proportions can be calculated by the Henderson-Hasselbalch equation.

$$\log \frac{[\text{Protonated form}]}{[\text{Unprotonated form}]} = pK_a - pH$$

Equation 2.1: Henderson-Hasselbalch equation (White & Katzung, 2004:418)

Physiologically both the protonated and unprotonated forms are important in a neural block. The non-ionised base form is hydrophobic (Hille, 1992:405) and plays an important role in rapid penetration of biological membranes. The lipid insoluble ionised form (such as the quaternary amine analogue of lidocaine) conversely cannot cross the cell membrane, making it ineffectual when applied outside the axon, but actively exerts its action once applied inside the cell at receptor site (White & Katzung, 2004:418; Conley & Brammar, 1999:817; Revis, 2005).

Potency and duration of action both increase linearly with hydrophobicity. This arises because the drug cannot readily exit closed sodium channels (Catterall & Mackie, 2006:369) since the receptor site on the sodium channel is hydrophobic and thus receptor affinity for anaesthetic agents is greater for more hydrophobic drugs. Regrettably the toxicity also increases to bring about a decrease in the therapeutic index (Catterall & Mackie, 2006:369; White & Katzung, 2004:418).

Local anaesthetics have intrinsic vasoactivity which can also influence its rate of removal from the site of action and therefore its duration of action. Ester-type local anaesthetics have a tendency to produce vasodilatation where as local anaesthetics of the amide types tend to bring about vasoconstriction (Sweetman, 2002:1304).

2.3 CLASSIFICATION OF LOCAL ANAESTHETICS

A broad classification of local anaesthetics is given in Table 2.1 below.

Table 2.1: Classification of local anaesthetics (Sweetman *et al.*, 2002:1302)

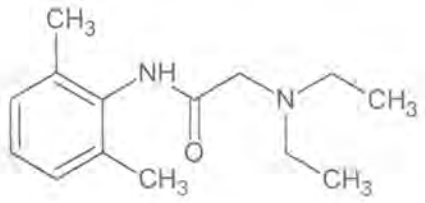
AMIDE TYPE	ESTER TYPE	MISCELLANEOUS
Articaine	Amethocaine	Diperodon
Bupivacaine	Amylocaine	Dyclonine
Cinchocaine	Benzocaine	Ethyl chloride
Etidocaine	Butacaine	Ketocaine
Levobupivacaine	Butoxycaine	Myrtecaine
Lidocaine	Butyl aminobenzoate	Octacaine
Mepivacaine	Chloroprocaine	Pramocaine
Oxetacaine	Cocaine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

2.4 PHYSICOCHEMICAL PROPERTIES

The physicochemical properties of lidocaine HCl and prilocaine HCl will be discussed in this section.

2.4.1 LIDOCAINE AND LIDOCAINE HCL

The name "lignocaine" was formerly used in the United Kingdom. Lidocaine or 2-(diethylamino)-N-(2,6-dimethylphenyl)acetamide (Figure 2.2) is an intermediate-acting amino amide. It is a white, odourless substance (BP, 2007:1243) of which the base form ($C_{14}H_{22}N_2O$) has a relative molecular mass (MM) of 234.34 and a melting point of 66 - 69°C. It is practically insoluble in water, but soluble in ethanol, dichloromethane, chloroform, benzene and ether (Lund, 1994:938; Ganellin, 1996). The hydrochloride hydrate ($C_{14}H_{22}N_2O \cdot HCl \cdot H_2O$) measures at 288.82 and melts at 74 - 79°C (BP, 2007:1244). The anhydrous hydrochloride melts at 125°C (Grönigsson *et al.*, 1979:210).



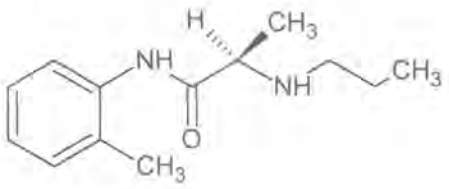
Lidocaine

Figure 2.2: Structure of lidocaine (BP, 2007:1243)

The base crystallises as fine needles (from n-hexane) and the hydrochloride forms a micro-crystalline powder (from aqueous acetone) (Grönigsson *et al.*, 1979:210). Lidocaine is more readily hydrolysed by acid than by alkali and can thus not be used orally because of the extensive first-pass metabolism (Grönigsson *et al.*, 1979:226). Lidocaine is rapidly and extensively metabolised predominantly by N-dealkylation, hydroxylation in the aromatic ring and amide hydrolysis. The formed metabolites, monoethylglycylxylidide (MEGX) and glycylxylidide (GX) (Grönigsson *et al.*, 1979:228), both retain anaesthetic activity that is less potent than that of lidocaine. The activity of the metabolite 2,6-xylidine is unknown (Astra Zeneca, 2004b:4).

2.4.2 PRILOCAINE AND PRILOCAINE HCL

Prilocaine or N-(2-methylphenyl)-2-(propylamino)propranamide base ($C_{13}H_{20}N_2O$) (Figure 2.3) is an almost white, crystalline powder that is slightly soluble in water, but very soluble in acetone and alcohol. It has a melting point of 36°C - 39°C and molecular mass of 220.3 (BP, 2007:1724). Prilocaine hydrochloride ($C_{13}H_{20}N_2O \cdot HCl$) with a molecular mass of 256.8, is however freely soluble in water and alcohol, but only slightly soluble in acetone. The melting point of 168 - 171°C is significantly higher than that of the base form (BP, 2007:1726).



Prilocaine

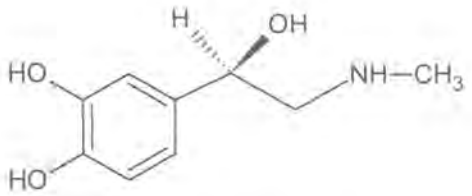
Figure 2.3: Structure of prilocaine (BP, 2007:1725)

Prilocaine is metabolised by amidases in both the liver and kidneys. Its metabolites include N-n-propylalanine and ortho-toluidine. The latter mentioned metabolite has led to carcinogenic effects in several animal models as well as methaemoglobinemia where the maximum daily dose was exceeded (Astra Zeneca, 2004b:4).

2.4.3 ADRENALINE

Adrenaline (Figure 2.4) or chemical name 1-1-(3,4-dihydroxyphenyl)-2-methylaminoethanol ($C_9H_{13}NO_3$, MM = 183.2) (BP, 2007:64) is an endogenous catecholamine (secreted by the adrenal medulla) that was solely added to the formulation for its arterial vasoconstrictive properties. Stimulation of α_1 adrenoceptors lead to constriction of skin and splanchnic blood vessels (Trevor *et al.*, 2002:80). In doing so, the duration of action is prolonged and the rate of absorption is decreased leading to decreased systemic toxicity (Catterall & Mackie, 2006:377). Adrenaline illustrates poor dermal penetration (Catterall & Mackie, 2006:380).

Adrenaline powder has a white or creamy white sphaero-crystalline appearance with a melting point established at 212°C. It is insoluble in ethanol and ether, sparingly soluble in water and soluble in solutions of mineral acids such as sodium hydroxide. Adrenaline is rapidly metabolised by catechol-O-methyltransferase and monoamine oxidase. It rapidly degrades and becomes red upon exposure to air and light, especially where the temperature is elevated. Adrenaline solutions are most stable at pH 3.2 to 3.6 and are unstable in neutral or alkaline solutions (BP, 2007:64; Lund, 1994:714).



Adrenaline

Figure 2.4: Structure of adrenaline (BP, 2007:64)

2.5 MECHANISM OF ACTION OF LOCAL ANAESTHETICS

The core of the mechanism of action for lidocaine and prilocaine is the reversible interruption of intricate neural traffic in peripheral nerves by preventing the generation and conduction of nerve impulse (White & Katzung, 2004:418; Hille, 1992:403).

2.5.1 UNDERSTANDING IMPULSE CONDUCTION

Resting membrane potential, action potential, synapses and the sodium channel will be discussed briefly in this section.

2.5.1.1 Resting Membrane Potential (RMP)

Resting Membrane Potential (RMP) is a steady electrical potential caused by the imbalanced dispersion of ions across either sides of the neuronal membrane as is the case with

extracellular fluid that contains essentially more sodium (Na^+) and chloride (Cl^-) whilst intracellular fluid contains potassium (K^+) and protein. The RMP is maintained intracellularly by an active, energy-consuming Na^+, K^+ pump (the Na^+K^+ATPase pump) at a magnitude of -70 mV. Changes in normal threshold stimuli instigate the process of conduction (Sukkar *et al.*, 1997:48).

2.5.1.2 Action Potential

An action potential is the signal that is propagated down an axon. It is caused by an abrupt overturn of membrane polarity generated by a physical or chemical stimulus. Action potentials move down both myelinated and unmyelinated axons (Giuliodori & DiCarlo, 2004:80). Along a myelinated axon, propagation is known as *saltatory (*or *jumping) propagation* which occur only at the nodes of Ranvier whilst an unmyelinated axon accommodates *continuous propagation* (Sukkar *et al.*, 1997:48; Afifi & Bergman, 1998:20). Please refer to the table below for a summary of the types of action potentials.

Table 2.2: Types of action potentials (Afifi & Bergman, 1998:10,16)

Fibre	Conduction Type	Diameter	AP Velocity
Unmyelinated	Continuous Conduction	< 2.0 µm	0.6 - 2 m/sec
Myelinated	Saltatory Conduction	1 - 20 µm	5 - 120 m/sec

Because of their ability to convert electrical into chemical signals, action potentials produce a scope of diverse cellular effects such as transmission of impulse, release of chemical transmitters, muscle contraction and regulation of glandular secretion (Sukkar *et al.*, 1997:48).

Stimulation of the membrane significantly increases the permeability of Na^+. Voltage gated Na^+ channels open resulting in the flow of Na^+ ions into the inside of the cell. This large influx of positive charge into the cells causes depolarisation to a threshold point at a level of -55 mV. Beyond the threshold point an all-or-nothing response called the action potential occurs (Sukkar *et al.*, 1997:52,364). The membrane potential has now become reversed and displays +35 mV.

At the end of depolarisation the K^+ ions rush down the concentration gradient to the outside of the cell, causing rapid repolarisation of the intracellular environment. The relative refractory period occurs when K^+ channels are open and the movement of positive charge out of cells causes hyperpolarisation. A hyperpolarised membrane (-80 mV) is less excitable

relative to normal resting membrane potential (-70 mV), because it is slightly more difficult to depolarise. The duration of an action potential in skeletal muscle and nerves for instance, completes in an astounding 1 to 5 milliseconds. Finally, the energy consuming Na^+-K^+ pump shuttles Na^+ sodium ions out and potassium ions back into the cell in order to re-establish Resting Potential distribution of the Na^+ and K^+ (Sukkar *et al.*, 1997:49; Matthews, G.G., 2000). The conduction process is graphically illustrated in Figure 2.5.

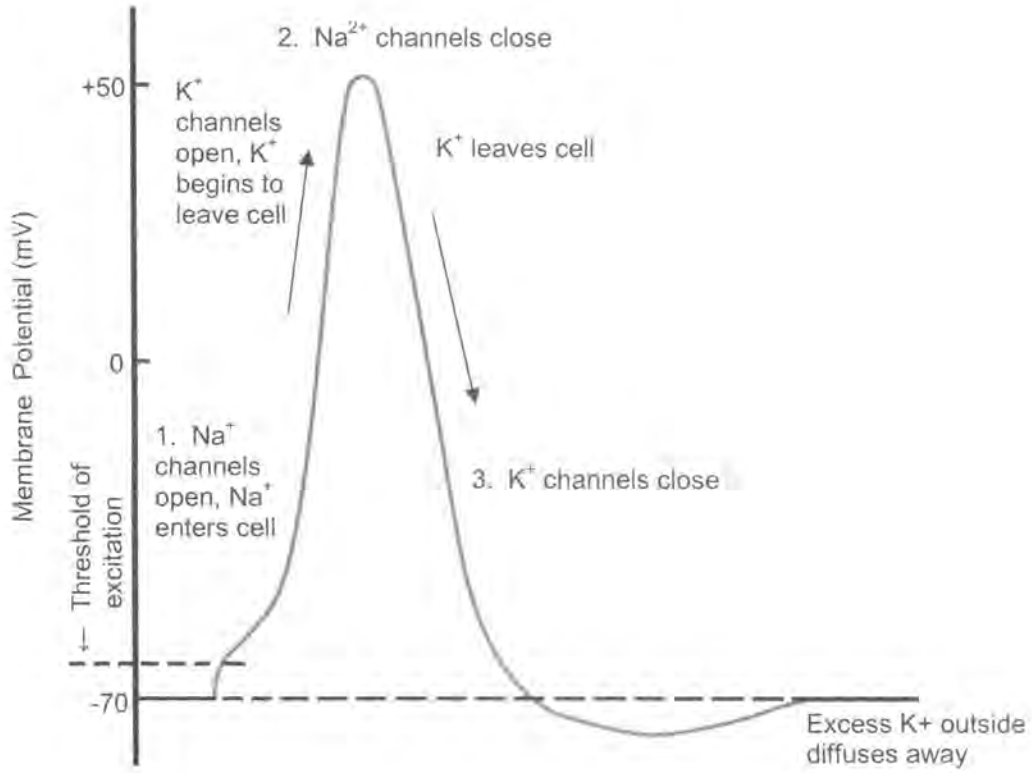


Figure 2.5: Ion flow in action potential (Cofer, 2002)

2.5.1.3 Synapse

A synapse is the junction between two neurones: a pre-synaptic sensory receptor neuron and postsynaptic effector neuron. The terminal endings are slightly dilated into a knob called the boutons terminaux (Afifi & Bergman, 1998:21). The area between cells at the distal ends where the myelin sheath is interposed is known as the node of Ranvier. This is the site of ionic displacement involved in impulse conduction through action potentials. The electric impulse progress saltatory along a myelinated axon from node to node and is considerably faster than the process of continuous conduction found in the smaller non-myelinated nerve fibres. The rate of impulse conduction is proportional to the size of the nerve fibre. Larger myelinated nerve fibres with a diameter of 1 to 20 μm, conduct impulses at a faster rate than the small non-myelinated axons that are no larger than 2 μm (Afifi & Bergman, 1998:16).

When an action potential arrives at an axon terminal the membrane becomes depolarised. Calcium (Ca^{2+}) ions penetrate the neuron through the opening of voltage-gated Ca^{2+} channels and fusion of synaptic vesicles with the pre-synaptic membrane occurs. Neurotransmitters contained within the synaptic vesicle are then released into the synaptic cleft through exocytosis in an amount proportional to the Ca^{2+} influx. The neurotransmitters bind to specific receptors on the post-synaptic membrane to increase the permeability thereby leading to depolarisation and generation of an action potential (Sukkar et al., 1997:366; Afifi & Bergman, 1998:21).

2.5.1.4 Structure of the sodium channel

Sodium channels are hetero-oligomeric glycosylated protein complexes with designated alpha (α) (260 000 daltons) and β_1 to β_4 (up to 38 000 daltons) subunits. The α subunit is the receptor site for local anaesthetics. It contains four homologous domains (I to IV), each comprised of six α-helical transmembrane segments and a membrane-reentrant pore loop. The centre of this symmetrical configuration encompasses the Na^+-selective transmembrane pore (Catterall & Mackie, 2006:371).

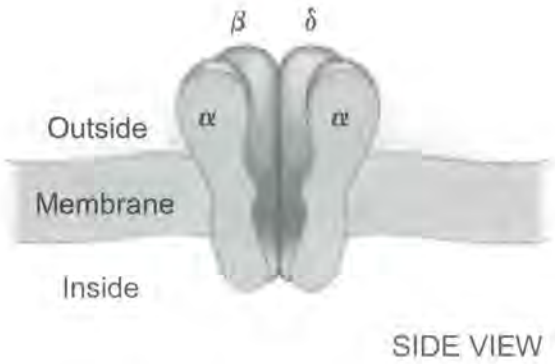


Figure 2.6: Structure of the sodium channel (Matthews, 2000)

Variation of transmembrane potential and the accompanying movement of voltage sensors (so called "gating charges" located in the S4 transmembrane helix) lead to conformational changes. This is also suggested by the voltage dependence of channel opening.

The S4 transmembrane helices are hydrophobic and positively charged. They contain lysine or arginine residues at every third position. It is hypothesised that, under the influence of transmembrane potential, these residues move perpendicular to the plane of the membrane to initiate a series of conformational changes in all four domains in order to open the channel. Amino acid residues in the short segments in between S5 and S6 (the openings that are visible in Figure 2.7) determine the ion conductance and selectivity of the channel. Closure

of an inactivation gate causes the open Na^+ channel to close within milliseconds (Catterall & Mackie, 2006:372).

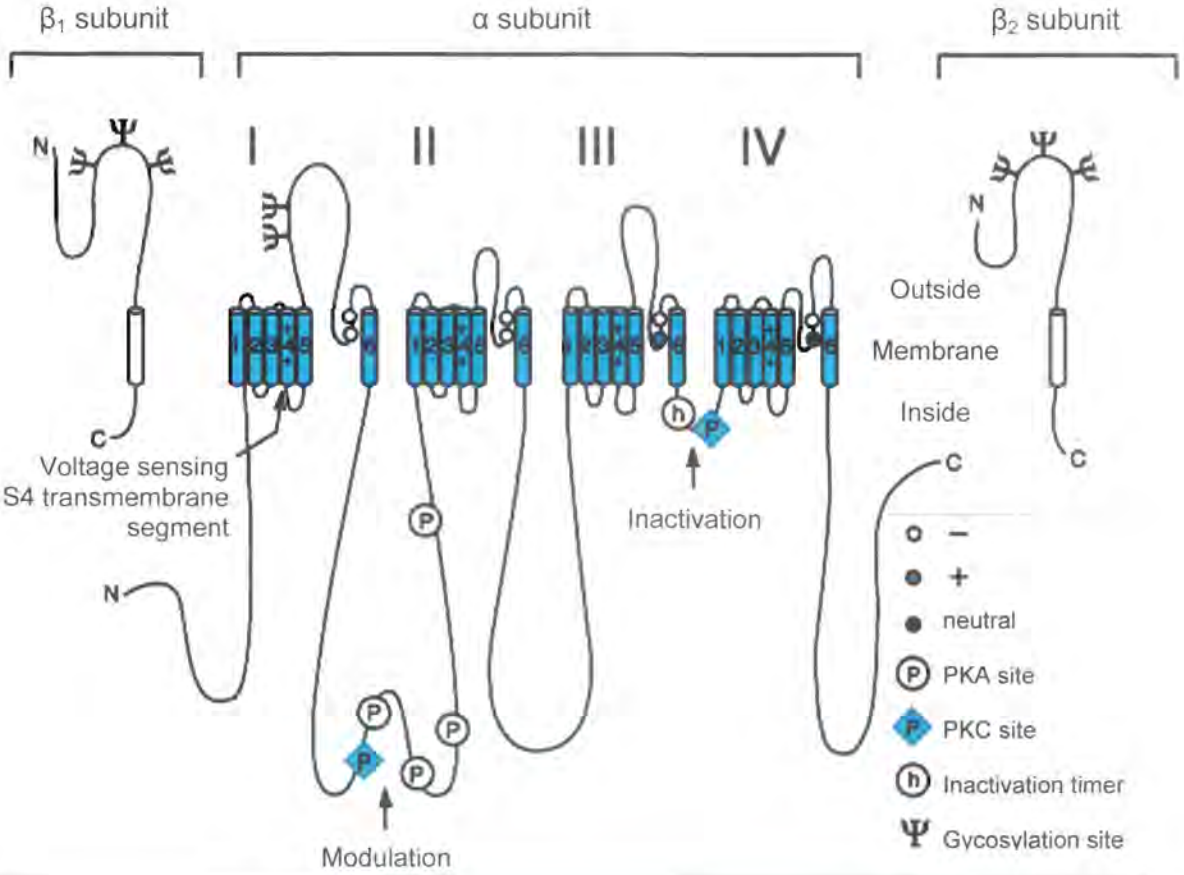


Figure 2.7: Function of voltage gated sodium channels (Catterall & Mackie, 2006:372)

2.5.2 PAIN SENSATION

Pain is defined by the International Association for the Study of Pain (IASP) as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage" (Merskey & Bogduk, 1994:209). This sensation is generated by noxious stimuli that threaten to cause damage to tissue. Pain involves motor reactions (for instance the withdrawal reflex), emotional reactions (such as anxiety, anguish, crying and depression) and autonomic reactions (including tachycardia, peripheral vasoconstriction, alterations in blood pressure, pupillodilatation and sweating) (Sukkar *et al.*, 1997:383).

The nociceptors (derived from the latin word "noci" that means harm or injury) are free nerve endings that primarily sense mechanical and chemical tissue damage. Stimulation thereof leads to depolarisation of the nerve membrane, Na^+ channels open, an action potential rises and, through transmission to the spine and brain, leads to the perception of pain (Sukkar *et al.*, 1997:383; Venugopal & Swamy, 2006:2).

Painful stimuli evoke two distinct sensations referred to as first and second pain. First pain occurs within 0.1 seconds after stimulus and is transmitted via myelinated Aδ fibres at a rate of 5 - 120 m/s (Afifi & Bergman, 1998:16). It is an acute, sharp and well localised pricking pain sensation for instance when skin is cut with a knife. Second pain is a duller, often poorly localised burning or aching sensation that develops over a slower period of time, for example toothache. Such a pain sensation will be conducted along unmyelinated type C fibres that conduct at a rate of about 1 m/s (Afifi & Bergman, 1998:16). Both of these pain fibres enter the spinal cord along the lateral division of the dorsal nerve roots from where it is signalled to the brain. Pain sensation is experienced as soon as the impulse reaches the thalamus, whilst the cerebral cortex is involved in the localisation and interpretation of the stimulus (Venugopal & Swamy, 2006:3; Sukkar *et al.*, 1997:383).

Cutaneous pain is elicited by stimulation of C-type nociceptors in the skin. It can be accurately localised due to the density of receptors in the skin and also by the aid of vision and touch (Sukkar *et al.*, 1997:385).

2.5.3 INTERRUPTION OF NERVE CONDUCTION

Local anaesthetics act at the cell membrane to produce a time- and voltage-dependant anaesthetic effect through inhibiting excitation of nerve endings or propagation of action potentials in peripheral nerves. These nerve endings appear in a penicillate fashion within the upper dermis (Tschachler, 2004:178).

The local anaesthetic binds to the α-subunit of the Na^+ channel which leads to inactivation thereof. As the anaesthetic action progressively develops in a nerve, the electric excitability threshold steadily increases, the rate of rise of action potential declines, impulse conduction slows, capacity to propagate the action potential decreases until nerve conduction eventually fails and causes the individual to lose sensation in the area supplied by the nerve (Catterall & Mackie, 2006:371; Tuckley,1994).

3 TRANSDERMAL DRUG DELIVERY OUTLINED

The following section describes the anatomy of the skin, advantages and disadvantages of transdermal delivery, the pathways in which permeation can take place, governing factors of transdermal delivery as well as a few penetration enhancers.

3.1 ANATOMY AND FUNCTIONS OF HUMAN SKIN

The skin is one of the largest organs in the body that can cover approximately 1.8 m^2 and weigh up to 4 kg in an adult male (Sukkar *et al.*, 1997:308). It is a tough yet flexible

protective barrier that varies in thickness from approximately ½ mm on the eyelids to 4 mm on palms and soles of the feet. The skin shields the body from external agents, extremes of temperature and invading organisms such as viruses, bacteria, fungi and parasites. Specialised nerve receptors allow the body to sense pain, temperature, touch and pressure. It plays an important role in the regulation of body temperature, protection from harmful ultraviolet light, storing fat, producing vitamin D and also to attract the opposite sex (Parker, 2005:346).

The three main layers comprise the outermost epidermis, the dermis in the middle and the innermost hypodermis as illustrated in Figure 3.1.

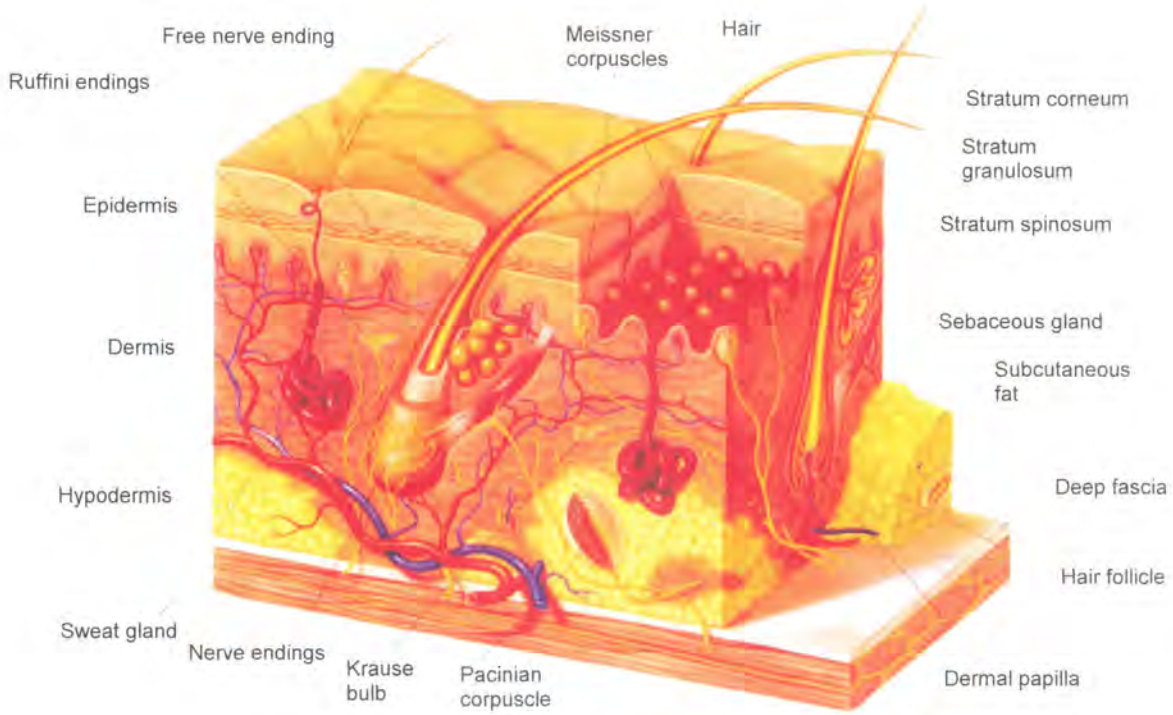


Figure 3.1: Anatomy of the skin (Parker, 2005:346)

The dermis (or corium) is the most significant target area of this study since it contains the nerve endings and blood vessels. It is the largest of the three skin layers (Lund, 1994:137) and is typically 3 - 5 mm thick. It mainly provides tensile strength, support and elasticity. It is composed of two layers: the more superficial papillary dermis and the deeper reticular dermis (Revis, 2006). These layers contain a network of connective tissue, fibroblast cells, collagen fibres, blood and lymphatic vessels, pilosebaceous units, sweat glands and Pacinian corpuscles (pressure receptors) (Williams, 2003:2; Zatz, 1994:13). The dermis is connected to the epidermis by an undulated basement membrane called the dermo-epidermal layer (Revis, 2006).

The epidermis is a complex, metabolically active membrane that is composed of multiple layers. This layer which is approximately 200 μm thick, has the ability to completely regenerate itself on average every 45 days (Bronaugh & Collier, 1993:98). It is largely aqueous in nature and can impede the delivery of lipophilic molecules (Williams, 2003:5; Hadgraft & Finnin, 2006:362).

The epidermis contains five histologically distinct layers, namely the stratum basale (a singe layer of cells lying directly above the dermis that is also referred to as the basal layer or stratum germinivatum), stratum spinosum, stratum granulosum, stratum licidum (lower layers of the stratum corneum) and the stratum corneum (Jones & Williams, 2007:143). Mitosing keratinocyte cells from the stratum basale migrate slowly upwards to the stratum granulosum where they flatten and their content becomes granular (Lund, 1994:137).

The stratum corneum is believed to be predominantly responsible for the impenetrability of the skin (Lund 1994:136). It is a lipohilic barrier (Guy, 1996:1766) of around 15 - 20 μm thick and comprises 10 - 15 cell layers of dead, anucleate keratinised cells (Hsieh, 1994:6) embedded in a lipid matrix that are arranged in a brick-and-mortar fashion. The lipids, found mainly in the intercellular region, consist of ceramides, cholesterol, free sterols, free fatty acids and triglycerides (Walters, 1989:198; Lund 1994:136; Williams, 2003:5). Nutrients have to diffuse into the epidermal tissue since it contains no blood vessels (Zatz, 1994:13) but deprivation of nutrients within the upper layers of the stratum corneum eventually causes cells to shrink, die and excoriate (Lund 1994:137). The underlying layers are referred to as the "viable epidermis" although the viability is debateable since the cell components degrade during differentiation (Williams, 2003:5).

3.2 TRANSDERMAL DELIVERY

The objective of local therapy is to produce adequate levels of the drug in the confined area while simultaneously producing low systemic circulation in order to limit pharmacological effects and toxicity in different regions of the body. Not only might ample blood circulation cause untoward effects as a result of systemic uptake, but it might also cause rapid clearance of the drug. The optimal outcome would be to have proper skin absorption with limited penetration into capillaries (Zatz, 1993:14).

3.2.1 ADVANTAGES OF TRANSDERMAL DELIVERY

Transdermal drug delivery offers several advantages over conventional routes:

- First-pass metabolism in the liver is avoided since the drug is directly circulated into the main venous return.

- Frequent dosing is decreased since medication can be delivered over several days. This enhances patient compliance.
- Sustained plasma profile of the drug is supported by continuous delivery of the drug (Washington *et al.,* 2001:187; Cerchiara & Luppi, 2006:89).
- Degradation by gastric acid and enzymes in the gastrointestinal environment are bypassed.
- The transdermal route would be more favourable should gastrointestinal distress occur.
- Being a non-invasive method, both trauma and the risk of infection is eliminated.
- The delivery of a drug can be interrupted at any time (Cerchiara & Luppi, 2006:89).

3.2.2 DISADVANTAGES OF TRANSDERMAL DELIVERY

Several disadvantages might be encountered in the study of skin permeation:

- Transdermal devices or their adhesive might cause irritation to the skin (Washington *et al.,* 2001:187; Cerchiara & Luppi, 2006:89).
- Few preparations have successfully been delivered across the uncompromising stratum corneum (Cerchiara & Luppi, 2006:89).
- Bacteria on the skin surface and epithelial activity can influence the metabolism of drugs and might cause unpredicted degradation of compounds.
- Drug sensitisation may occur.
- Transdermal technology is often more expensive compared to a plain oral tablet (Washington *et al.,* 2001:187).

3.3 PATHWAYS OF TRANSDERMAL PENETRATION

Structurally skin permeation can take place across the skin's dual membrane system that is perforated with shunts such as hair follicles and eccrine sweat ducts. The stratum corneum of the epidermis is the first rate limiting barrier and the second is the epidermal-dermal junction or basement membrane although the stratum corneum controls percutaneous absorption to a greater extent (Jackson, 1993:177). Local anaesthetics can only function when they are able to reach the viable tissue in the dermis (Zatz, 1993:14). Figure 3.2 illustrates the pathways through which penetration can take place.

3.3.1 TRANSAPPENDAGEAL ROUTE OF TRANSPORT (SHUNT PATHWAY)

The transappendageal route offers a pathway through the stratum corneum by means of the transeccrine route (through eccrine sweat ducts), the transsebaceous route (through sebaceous glands) and transfollicular route (through hair follicles). It has been estimated

that shunts occupy less than 1 % of the total surface area of human skin and therefore contribute only minimally to the overall kinetic profile of passive permeation (Junginger *et al.*, 1994:59; Zatz, 1993:16; Banga, 1998:5). However the follicular pathway proved to be somewhat significant in topical delivery of large polar permeants during a study performed by du Plessis *et al.* (1994:281) and could therefore prove to be the most probable route of permeation for lidocaine HCl and prilocaine HCl. Iontophoretic delivery occurs primarily through these hydrophilic follicular pores since they offer the least resistance to electric current (Sarpotdar, 1993:242).

3.3.2 TRANSEPIDERMAL ROUTE OF TRANSPORT

3.3.2.1 Transcellular route

The transcellular route provides a direct pathway across the corneocytes and intercellular bilayers of the stratum corneum that is merely 20 μm thick. Since a molecule traversing the heterogeneous intact stratum corneum faces numerous diffusion and partitioning obstacles, the transcellular route is trivial compared to the intercellular route (Williams 2003:33,227).

3.3.2.2 Intercellular route

According to Hadgraft (2004:292) experimental evidence has proven the intercellular route of delivery to be dominant despite the molecule permeation path length estimated at 150 to 500 μm through tortuous lipid domains, considerably longer than the mere 20 μm thickness of the stratum corneum. Most small, uncharged molecules would cross the stratum corneum through this route (Williams, 2003:34). The stratum corneum is capable of forming lipid bilayers despite the absence of phospholipids and a variety of ceramides present in the skin. This suggests that the intercellular space consist of lamellar granules which are extruded in the intercellular space and these lipid sheets form the key barrier to hydrophilic permeants (Walters, 1989:199).

1. INTRACELLULAR ROUTE 2. INTERCELLULAR ROUTE 3. SHUNT ROUTES

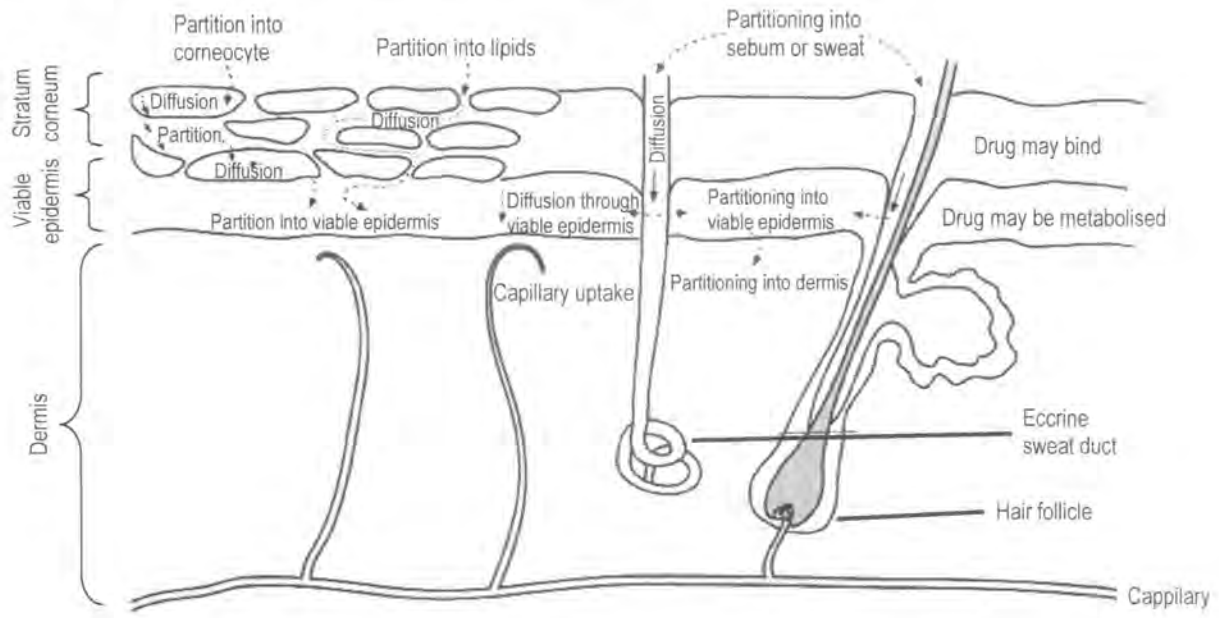


Figure 3.2: Schematic representation of the principal pathways of transdermal drug delivery (Williams, 2003:29)

3.4 TRANSDERMAL KINETICS

It is known that diffusion takes place from a region of high to a region of lower concentration. Under passive conditions it is generally accepted that the steady state diffusion of molecules across a membrane is governed by Fick's first law of diffusive flow. Fick postulated that flux (J) should be proportional to the concentration differential (ΔC) across a plane and inversely

proportional to the thickness of the membrane (*h*). The permeability coefficient (P) defines proportionality. This equation also includes the distribution coefficient of the drug (K) between the solvent or vehicle and stratum corneum and the average membrane diffusion coefficient (D) for the solute in the stratum corneum (Rieger, 1994:39).

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

Equation 3.1: Fick's first law of diffusion (Rieger, 1994:39)

The following factors have an influence on the quantity of drug absorbed over a specific area in a particular time (Lund 1994:139):

- Solubility and distribution characteristics of the solute.
- The concentration difference of the drug across the membrane.
- The nature of the vehicle in which the drug is transported.
- The thickness of the stratum corneum.

3.5 FACTORS THAT INFLUENCE PERMEATION ACROSS THE SKIN

The factors that govern the pathway and rate of transdermal penetration are complex and variable (Lund 1994:138). Several physiological, structural and physicochemical characteristics may have a considerable effect.

3.5.1 PHYSIOLOGICAL FACTORS

3.5.1.1 Skin age

Clear structural and functional alterations occur with skin ageing, such as atrophy, decreased blood circulation, reduced elasticity, lower moisture content and changes in chemical composition and barrier properties (Lund, 1994:141). There is controversy in the literature on whether or not these changes have a significant effect on skin permeability since the stratum corneum still remains intact (Williams, 2003:14). Certainly the skin of a neonate is much more permeable due to its immaturely developed stratum corneum that is only 60 % of its adult thickness at birth (Guy & Hadgraft, 1989:59; Williams, 2003:15). It should further be taken into consideration that children may have an up to four times higher surface area to body weight ratio than those of adults (Williams, 2003:15; Fox *et al.*, 2006:1681) and the dosage (maximum dose of 20 g of a 5 % ointment equivalent to 1 g lidocaine base in 24 hours for adults) (Sweetman, 2002:1315) should be adapted accordingly.

3.5.1.2 Regional anatomic variation

Skin location has proven to have a significant effect on permeation variance, for instance the genitals are much more permeable than areas of the head and neck which are in turn more permeable than the trunk and limbs (Zatz, 1993:13; Williams, 2003:16). This is supported by Fick's law which states that flux is inversely proportional to the diffusion path length. Absorption rates are slower in the load-bearing plantar and palmar areas of the body (such as the soles of the feet) (Monteiro-Riviere, 2004:47) where the stratum corneum is thicker than on the face (particularly on the lips and eyelids and behind the ears) (Lund, 1994:140).

Furthermore, factors such as lipid composition, size of the cells, number of layers, their associated stacking pattern and the density of skin appendages such as hair follicles in the stratum corneum all have an influence on permeation (Lund, 1994:140). To limit regional variations on transdermal delivery during this specific study, only skin obtained from the abdominal section of the trunk of adult female patients were used.

3.5.1.3 Cutaneous metabolism

The skin contains enzymes which catalyse both Phase 1 (functionalisation) and Phase 2 (conjugation) reactions (Sartorelli *et al.*, 2000:147). It possesses the ability to metabolise several topically applied xenobiotics (Ademola & Maibach, 1997:204; Bronaugh & Collier, 1993:98). Enzyme systems including CYPs, epoxide hydrolase, transferases such as N-acetyl-transferase and diverse enzymes including glucoronyl transferases, sulfatases, esterase, oxidase and reductase distributed throughout the skin can biotransform drugs and their metabolites (Brunton *et.al.*, 2006:1; Riviere, 1993:123). Potentially high numbers of topical microbial flora, including bacteria and yeasts, may also cause applied drugs to be metabolised prior to even penetrating the tissue (Williams, 2003:21). Even inert compounds may be toxicologically converted into active species (Sartorelli *et al.*, 2000:147). It is not known whether lidocaine and prilocaine are metabolised in the skin (Astra Zeneca, 2004b:4).

3.5.1.4 Temperature and humidity

Environmental factors such as skin temperature and surface humidity have an influence on percutaneous absorption. The temperature of the epidermis is typically between 30 - 37°C (Ademola & Maibach, 1997:205). Transient rise in temperature causes accelerated diffusion (Lund, 1994:140) by altering the physiology of the skin or by an increase in the physicochemical diffusion rate. It also has an effect on blood flow in the surface vasculature (Washington *et al.*, 2001:189). Normal blood flow in human skin ranges from 3 - 10 ml/min/100 g but can increase up to ten-fold when ambient temperature exceeds 43°C (Riviere, 1993:118). Occlusion prevents transepidermal water loss (TEWL) through surface evaporation. This leads to increased hydration and improved permeability of both polar and non-polar drugs (Riviere, 1993:117; Washington *et al.*, 2001:188; Williams & Barry, 2004:606). It is recommended that EMLA® cream should also be occluded after application with the enclosed dressing (Astra Zeneca, 2004b:18) to enhance absorption.

3.5.1.5 Pathological disorders

Skin that is damaged or in diseased state is prone to reduce barrier function and subsequently lead to increased permeability (Washington *et al.*, 2001:189). Irritation,

inflammation, dryness, abrasion, allergic reactions and ultraviolet irradiation all compromise barrier action even though the skin layer remains intact (Lund, 1994:139). Care was taken to ensure that the skin used during this diffusion study did not include areas affected by striae.

Bacterial, viral and fungal infections may cause metabolic degradation of topically active substances. The damage that they might cause to skin barrier integrity vary in severity but one fact is clear, and that is that they all conjointly diminish effective therapy (Williams, 2003:22). Dentists and surgeons have observed that local anaesthetics are much less effective when applied to infected tissue. The infected area has a low extracellular pH (may be as low as 6.4 as opposed to physiological pH of 7.4) and therefore a very low fraction of non-ionised local anaesthetics will be available for diffusion into the cell (White & Katzung, 2004:420; Trevor *et al.*, 2006:238).

Conditions such as eczema, atopic and contact dermatitis, lichenoid eruptions, tumours, ichthyoses and psoriasis may also influence drug delivery (Williams, 2003:20). It is surprising that skin permeability can be increased by these conditions since many have a thickening effect on the epidermis, but it is possibly due to compromised structural integrity of the stratum corneum (Washington *et al.*, 2001:189).

3.5.1.6 Gender and race

Other than the fact that keratinocytes are apparently slightly larger in females (37 - 46 μm) than in males (34-44 μm), there are no further supporting evidence of significant differences in drug delivery between the two genders (Williams, 2003:17). According to Washington *et al.* (2002:189) Negroid stratum corneum generally have more layers and are less permeable than Caucasian stratum corneum.

3.5.1.7 Miscellaneous aspects

It must be taken into consideration that application of a vasoconstrictor such as adrenaline will delay the penetration of topically applied substances such as local anaesthetics, because of a reduction in blood circulation (Lund, 1994:140).

3.5.2 PHYSICOCHEMICAL FACTORS

3.5.2.1 Partition coefficient (P)

A partition coefficient gives an indication of the ratio of molecular distribution between two phases. For transdermal studies, the logarithm of the partition coefficient between water and octanol is often used to measure how well a molecule will distribute between the stratum corneum lipids and water and will govern which pathway a permeant will follow through the

skin (Williams, 2003:27). The partition coefficient is also dependant on the solvent properties of the vehicle (Zatz, 1993:26). Lipophilicity is generally a desired feature of transdermal candidates in order to penetrate the stratum corneum lipids, but the molecule needs to exhibit aqueous solubility also to partition out of the stratum corneum into the essentially aqueous viable tissues (Williams, 2003:36).

To enable a compound to partition reasonably well between these hydrophilic and lipophilic domains in human skin, it needs to have a log $P_{(octanol/water)}$ in the region of 1 to 3 (Williams, 2003:36; Hadgraft, 2004:292). Lidocaine HCl possesses an octanol-water partition coefficient (log P) of 2.36 ± 0.26 (calculated by ACD/lab ChemScketch Freeware 11.0). Prilocaine has a pharmacological profile similar to that of lidocaine (Catterall & Mackie, 2006:378) and its log P calculated to be 2.09 ± 0.49. Thus, it is safe to assume that the intercellular route would be the most probable route these anaesthetics would traverse through. More lipohilic compounds (log P > 3) will exclusively follow the intercellular route whilst hydrophilic molecules (log P < 1) might prefer the transcellular or appendageal pathways (Williams, 2003:36).

3.5.2.2 Diffusion coefficient (D)

The diffusion coefficient defines the transport of matter resulting in movement of a substance within a substrate (Rieger, 1993:38). It relates to the ability of the permeant to traverse through tissue and is expressed in a unit of area/time (cm^2/h or cm^2/s) (Williams, 2003:27). This parameter included in Fick's law is assumed to create a linear concentration gradient of the permeant within the stratum corneum (Rieger, 1993:38).

3.5.2.3 Permeability coefficient (k_p)

The partition coefficient dictates the rate in which the permeant will be transported across a membrane as a unit of distance/time (cm/h or cm/s) (Williams, 2003:226). The permeability coefficient is influenced by hydrophobicity, size of the penetrant, degree of ionisation and other characteristics of the application area (Morganti *et al.*, 2001:494). For unionised species that traverse through the lipid membrane, the permeability coefficient might be high and its aqueous solubility low, whilst the opposite is true for ionised species (Williams, 2003:39).

3.5.2.4 Ionisation, pH and pKa

Lidocaine and prilocaine are both weak bases (pKa values range from 8 to 9) owing to the presence of the nitrogen atom of the aliphatic amine group (Woolfson & McCafferty, 1993:63) and their state of ionisation have an effect on their partitioning into the skin. Their

hydrochloride salts are mildly acidic (Catterall & Mackie, 2006:374). In the ionised form, they will be more soluble but will have a lower permeability coefficient (Hadgraft, 2004:292). The non-polar nature of the horny layer might cause lidocaine and prilocaine in their charged form to encounter high resistance to permeation but it should not be assumed that charged molecules are locked out of the skin.

Cutaneous pH is regulated between 5.5 and 6.5. It seems that the acidic pH suppresses growth of opportunistic bacteria, fungi and yeasts (Morganti *et al.*, 2001:501). Alteration of the pH of the vehicle can manipulate the ratio of charged to uncharged species (Zatz, 1993:28). A study conducted by Zatz (1993:29) shows that after flux normalised to saturation, a significant amount of ionised lidocaine could permeate the skin (refer to Figure 3.3).

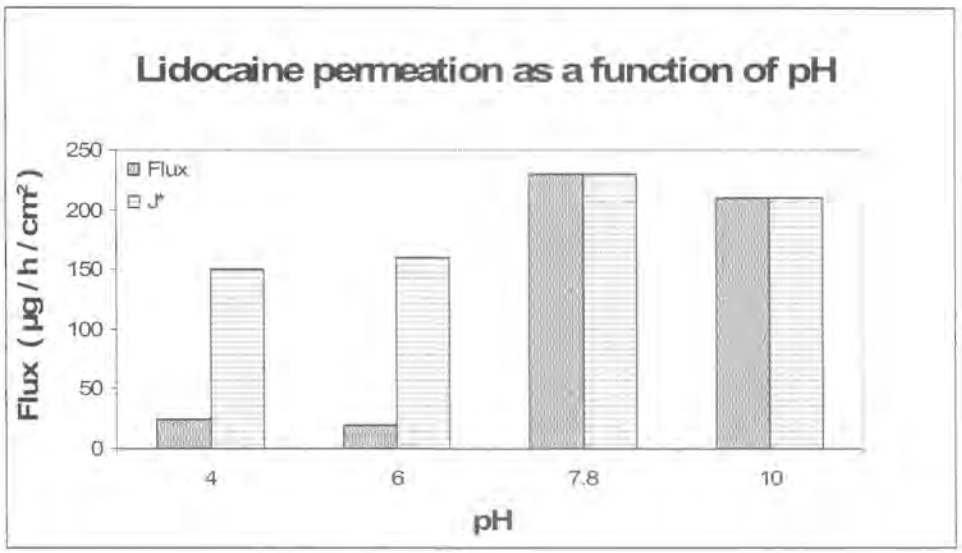


Figure 3.3: Lidocaine permeation through excised human skin as a function of pH. Shown are the actual flux for 5 % systems and the maximal flux (J*) for a saturated solution (Zatz, 1993:28)

The extent of ionisation of a weakly acidic or basic permeant in a donor solution can be determined by substituting pH and pKa in the Henderson-Hasselbalch equation (Williams, 2003:69). The pKa value of lidocaine HCl is 7.86 at 25°C (Dollery, 1999:L52; Lund 1994:938) and that of prilocaine HCl is 7.89 (Woolfson & McCafferty, 1993:63; Dollery, 1999:P198). Dissociation constants for the three enantiomers of adrenaline at 20°C were measured at 8.7, 10.2 and 12.0 respectively (Lund, 1994:714).

$$\log \frac{[BH^+]}{[B]} = pK_a - pH$$

Equation 3.2: Henderson-Hasselbalch equation (Williams, 2003:69)

3.5.2.5 Solubility and melting point

There is a clear relationship between melting point and solubility: organic materials with high enthalpies of melting have relatively low aqueous solubility at normal temperature and pressure (Williams, 2003:37). Fortunately both lidocaine HCl and prilocaine HCl exhibit relatively low melting points of 79°C (BP, 2007:1244) and 171°C (BP, 2007:1725) respectively, indicating good solubility for both substances. Furthermore when combined, lidocaine and prilocaine forms a binary eutectic mixture that has a lower melting point than that of the separate substances (Sweetman, 2002:1318). Naik *et al.* (2000:319) recommends that the ideal limit for passive transdermal delivery is below 200°C. Adrenaline has a slightly higher melting point of 212°C (Lund, 1994:714).

3.5.2.6 Molecular modification through altering functional groups

Changes in chemical structure have marked effects on the activity of active substances (Lund, 1994:140). Since local anaesthetics are only slightly soluble in the unprotonated amine form, they can be modified to become their more acidic water soluble hydrochloride salts. This contributes to the stability of the catecholamine vasoconstrictor adrenaline which require an acidic pH (Caterrall & Mackie, 2006:20; Sweetman, 2002:1304) (refer to § 2.4.3).

3.5.2.7 Molecular size and shape

Molecular size and shape influence permeability across cell monolayers to have an effect on drug absorption (Krämer, 1999:379). Smaller molecules in higher concentrations tend to penetrate more readily into the skin than larger molecules (Lund, 1994:141; Hadgraft, 2004:292). Williams (2003:36) stated that the most probable organic candidates for transdermal delivery lie within 100 to 500 Dalton. This range well includes the low molecular weight of lidocaine HCl at 288 Da, prilocaine HCl at 256.8 Da and adrenaline at 183.2 Da (refer to §2.4).

3.6 ENHANCING SKIN PENETRATION

A large variety of compounds have been studied for their potential to enhance skin penetrability. Principal characteristics of an ideal enhancer would include the following:

- It should be pharmacologically inert with no inherent action at receptor site on the skin surface or in the body.
- It should be non-toxic, non-irritating and non-allergenic.

- Onset of action should be immediate and effective.
- Duration of the effect should be suitable, predictable and reversible.
- Upon removal of the enhancer from the skin, the exposed tissue should instantly regain its normal barrier properties.
- Penetration should take place in one direction so that penetration can only occur into the skin and body fluids are not lost to the environment.
- The enhancer should be chemically and physically compatible with a wide range of drugs as well as pharmaceutical adjuvants.
- The enhancer should spread well on the skin and have superb solvent properties so that minimal quantities of the drug are required.
- It must be able to be formulated readily into lotions, suspensions, ointments, creams, gels, aerosols and skin adhesives.
- Finally, it should be inexpensive, odourless, colourless and tasteless (Hadgraft, *et al.*, 1993:175; Behl *et al.*, 1994:108).

Types of enhancers include chemical and physical enhancers as well as delivery systems.

3.6.1 CHEMICAL ENHANCERS

The objective of chemical enhancers is to increase drug absorption by using chemicals that are able to reversibly compromise the barrier function of the stratum corneum without damaging the delicate underlying tissue (Shah, 1994:20). According to the lipid protein partitioning (LPP) theory, these mechanisms of action would fall into any of the following categories which include: (a) disruption of the lipid matrix of the stratum corneum; (b) interaction with intracellular protein; (c) improvement in partitioning of a substance into the stratum corneum and was recently extended to also acknowledge: (d) disruption of the corneocyte envelope; (e) manipulate protein junctions such as desmosomes; (f) change in the partitioning between stratum corneum components and the diffusion pathway lipids (Kanikkannan *et al.*, 2006:18; Barry: 2006:9).

Examples of chemical enhancers include water, hydrocarbons, sulfoxides (especially dimethylsulfoxide [DMSO]), pyrrolidones, fatty acids, esters and alcohols, azone and its derivatives, various surfactants, amides, polyols, essential oils, terpenes, oxazolidines, epidermal enzymes, polymers, lipid synthesis inhibitors, bio-degradable enhancers and synergistic mixtures (Barry, 2006:9). Table 3-1 portrays the above mentioned chemical penetration enhancers as summarised by Purdon *et al.* (2004:100)

Table 3.1: Summary of the classification of chemical penetration enhancers (Purdon *et al.*, 2004:100)

CLASS		EXAMPLES	MECHANISMS
Sulphoxides		Dimethylsulfoxide (DMSO) (Sinha & Kaur, 2000,1135)	Extracting soluble components of the stratum corneum (SC), delaminating the horny layer, denaturing the proteins
Alcohols		Ethanol, octanol (Yum *et al.*, 1994:143)	Enhancing drug solubility, disruption of the SC integrity through biochemical extraction
Polyols		Propylene glycol	Solubility modification
Fatty alcohols		Lauryl & oleyl alcohol	Increase in drug solubility and partitioning, disruption of skin barrier function, ion-pair formation
Fatty acids		Oleic acid, lauric acid, linoleic acid, linolenic acid, capric acid	Same as for fatty alcohols
Esters		Ethyl acetate, octyl acetate (Walker & Smith, 1996:297)	Disruption of lipid packing, influence partitioning between vehicle and skin
Amines & amides	Urea	Dimethylacetamide, 1-alkyl-4-imidazolin-2-one	Facilitate hydration of the SC, disruption of lipids
	Pyrrolidones	1-dodecyl-azacycloheptan-2-one, 2-pyrrolidone-1-acetic acid dodecyl ester	Fluidising the lipids along the intercellular lipid domains in the SC, changing the lipid arrangement
Terpenes		D-limonene, menthol	Disruption of bilayered lipid packing, disturbance in the stacking
Surfactants	Anionic	Sodium laurate, sodium cholate	Alter the barrier function of the SC, removal of water-soluble agents, emulsifying sebum
	Cationic	Cetyltrimethyl ammonium bromide	
	Non-ionic	Poloxamer, lecithin	
Cyclodextrins		α-, β- and γ-cyclodextrin	Inclusion of drugs into molecular cavity
Oxazolidinones		4-decyloxazolidin-2-one	Localising drugs in skin layers

Chitosan	Poly[b-(1-4)-2-amino-2-deoxy-D-glucopyranose]	Ionic interactions with negatively charged sites on the cell surface and tight junctions

3.6.2 PHYSICAL ENHANCERS

Ultrasound (phonophoresis and sonophoresis), iontophoresis, electroporation, magnetophoresis, microneedle array, radio waves and photomechanical wave are means of physical enhancement (Barry, 2006:13) which may provide an effective alternative or synergistic method to other methods for improved permeation (Purdon *et al.*, 2004:119).

Different physical penetration enhancers are summarised below in Table 3.2.

Table 3.2: Summary of the classification of physical penetration enhancers (Purdon *et al.*, 2004:101)

CLASS	EXAMPLES	MECHANISMS
Circumventing the stratum corneum (SC)	Microneedle array	Painlessly penetrates the epidermal layer (Prausnitz, 2006:239)
	SC ablation	Ablating the SC e.g. skin stripping
Electrical techniques	Iontophoresis	Driving charged species by electrical repulsion, increasing permeability of skin by the flow of electric current, affecting the flux of uncharged molecules and large polar peptides by electroosmosis
	Ultrasound	Cavitation disturbs lipid-packing in the SC and the increased free volume enhances drug permeation
	Electroporation	Creating transient pores through the SC by electrical pulse
Radio-wave energy	Radiofrequency thermal ablation	Creating an array of small microchannels across the SC by microablating skin cells using radiofrequency energy

3.6.3 DELIVERY SYSTEMS

Various formulations for the topical delivery of therapeutic agents have been mentioned by Grobler *et al.* (2008:286) including liposomes, microemulsions, microspheres and Pheroids™. Table 3.3 summarises their mechanism of action.

Table 3.3: Summary of the classification of delivery systems (Purdon *et al.*, 2004:100)

CLASS	EXAMPLES	MECHANISMS
Lipid based delivery systems	Liposomes	Interacting with skin lipids
	Transfersomes	Squeezing through polar channels under a hydration gradient
	Ethosomes	Ethanol fluidises both ethosomal lipids and bilayers of the SC intercellular lipid (
	Pheroids™ (Grobler *et al.*, 2008:299)	Colloidal system that interacts with skin, facilitated through fatty acid membrane-binding proteins within lipid rafts in cell membrane
Drug-vehicle interactions	Supersaturated solution	Elevating thermodynamic activity of drug
	Microemulsions	Disruption of SC lipids, increasing the partitioning of the drug into the skin

In the following section the Pheroid™ delivery system will be emphasised.

4 PHEROID™ TECHNOLOGY IN AID OF OPTIMAL THERAPEUTIC DELIVERY OF LIDOCAINE AND PRILOCAINE

4.1 INTRODUCTION

The patented Pheroid™ delivery system is a skin-friendly carrier that mainly consists of plant and fatty acids. This novel delivery system proved to have infinite advantages in the effective topical delivery of administered compounds. It is a pliant yet stable and pharmaceutically safe structure of which the form and function can be manipulated to ensure versatile and effective therapy. Key advantages include enhanced entrapment capability, increased rate of transport and delivery of active compounds, decreased time of onset of action, reduction of minimal effective concentration and thus also reduced toxicity, ability to target treatment areas, lack of immunological response and reduced drug resistance. Ongoing research and development is currently being conducted to unlock the possibly limitless application of such extraordinary technology in the pharmaceutical industry (Grobler *et al.*, 2008:284; Grobler, 2004:3).

4.2 PHYSIOGNOMY OF PHEROIDS™

4.2.1 STRUCTURAL CHARACTERISTICS

The Pheroid™ delivery system is a colloidal system with a vesicular structure consisting of stable lipid-based sub-micron- and micron-sized particles dispersed in an adaptable medium and encapsulated by a porous membrane (Figure 4.1).

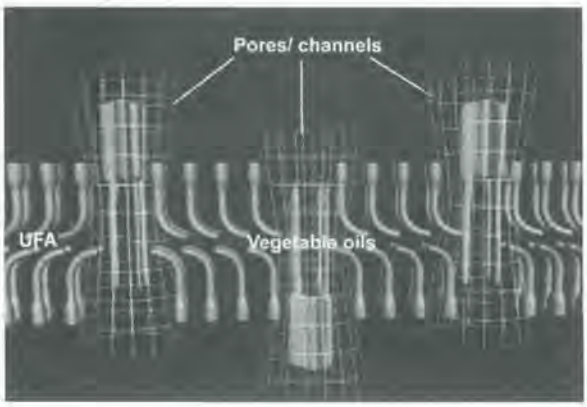


Figure 4.1: A section of the membrane of the Pheroid™ as calculated by molecular modeling, based on ab initio, forcefield and energy theory . The number of unsaturated fatty acids (UFAs) between the pores has not been determined and is used for illustration purposes only (Grobler *et al.*, 2008:289)

Pheroids™ are formed by a self-assembly process similar to that of low-energy emulsions. Apart from the two liquid phases (oil and water) it also contains a dispersed gas phase containing nitrous oxide (N_2O) gas (Grobler *et al.*, 2008:288). The Pheroid™ particles consists of ethylated and pegylated polyunsaturated omega-3 and -6 fatty acids (excluding the prostaglandin precursor, arachidonic acid) with a diameter ranging between 200 nm and 2 μm (Grobler *et al.*, 2008:283;285). The lipid bilayer of the cellular membrane contains no phospholipids or cholesterol (Grobler *et al.*, 2008:288). It is a dynamic system where proteins and lipids continuously move laterally on and molecules in and out of the membrane (Grobler *et al.*, 2008:290). Because the structure is in the cis-formation, it is compatible with the orientation of human fatty acids (Grobler *et al.*, 2008:285).

The dispersed phase can be manipulated in terms of morphology, structure, size and function to formulate various types of Pheroid™ structures that suits diverse therapeutic needs. Three main types are shown by confocal laser scanning micrographs in Figure 4.2.

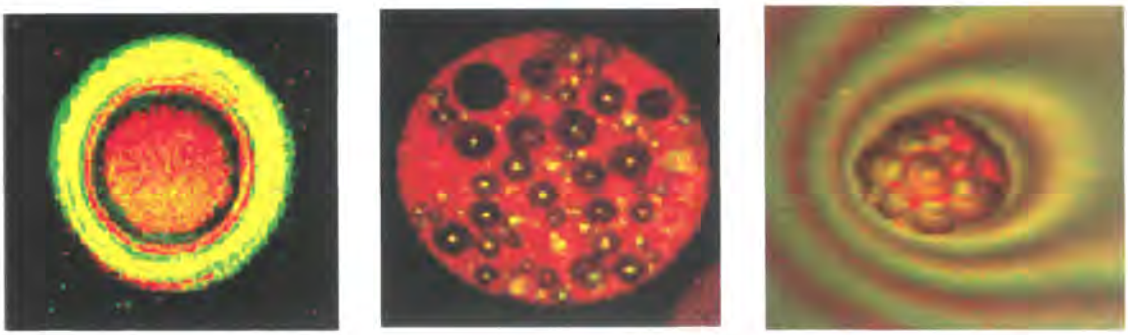


Figure 4.2: Confocal laser scanning microscopy (CLSM) micrograph illustrating various Pheroid™ types: (a) highly elastic or fluid bilayered vesicle, (b) micro-sponge with 1 - 10 µm diameter and (c) reservoir containing small pro-Pheroids™ for oral use (Grobler, 2004:5)

When deciding on the type and diameter of Pheroids™ to be used, the following parameters should be taken into account:

- The amount and particle size of the active compound to be entrapped
- Physicochemical properties of the system
- Inherent particle characteristics such as elasticity
- The rate of delivery
- Administration route (Grobler *et al.*, 2008:285).

The design of the Pheroid™ allows for manipulation of both its structural and functional features through the following:

- Altering the fatty acid composition or concentration
- Addition of non-fatty acids or phospholipids such as cholesterol
- Adding cryo-protectants
- Changes in the hydration medium (ionic strength, pH)
- Different characteristics of active compounds with
- Adding sunscreen formulations (Grobler *et al.*, 2008:292).

Making use of specific fatty acids, their modification state and the manufacturing process determines the size and pores of the Pheroids™. Mean particle size can be reproducibly manipulated by changing the ratio and composition of fatty acids. The simplest Pheroid™ vesicles containing ethylated linolenic acid, ethylated linoleic acid and pegylated ricinoleic acid have an incredibly ordered structure with radii of multiples of ± 28 nm and it is believed that the molecule with the largest molecular radius probably determines the packing (Grobler *et al.*, 2008:291). The degree of hydrogenation of the fatty acids can alter the surface charge. These modifications are an inherent feature of the Pheroid™ system (Grobler *et al.*,

2008:292). All topical Pheroid™ formulations contain tocopherol (vitamin E) that acts as both an anti-oxidant and membrane stabiliser (Grobler *et al.*, 2008:293).

4.2.2 FUNCTIONAL CHARACTERISTICS

Molecular modelling indicates some interaction between the fatty acids and the N_2O that promotes a stable vesicular Pheroid™ structure. The nitrous oxide essential fatty acid (NOEFA) matrix provides a functional model for the transport of both hydrophobic and hydrophilic drugs. Controlled experiments on various formulations showed a dramatic decrease in efficacy and stability if either the N_2O or the EFAs were omitted from the formulation (Grobler *et al.*, 2008:290). Nitrous oxide is an amphiphilic volatile anaesthetic compound. The association of this component with the dispersed phase contributes at least in three ways:

- It adds to the miscibility of the fatty acids in the dispersal medium.
- It contributes to the self-assembly process of the Pheroids™.
- It promotes the stability of the formed Pheroids™ even at high and low pH (Grobler *et al.*, 2008:289).

4.2.2.1 Pliability

The combination of a gas in adjunction with the pliable pegylated tails added to the fatty acid confers extreme elasticity upon the Pheroid™ structure. The addition of polyethylene glycol (PEG) increases bioavailability, drug stability, lowers toxicity and enhances solubility of the drug (Grobler *et al.*, 2008:294).

4.2.2.1.1 Entrapment efficiency

The objective is to achieve an entrapment efficiency of more than 90 %. The entrapment efficiency of the Pheroid™-based products is generally determined by confocal laser scanning microscopy (CLSM) and visualised through fluorescence labelling. The amount of colloidal particles per volume can be adapted to suit the required concentration of the compound. This depends mainly on the size, charge and solubility of the active compound (Grobler *et al.*, 2008:294). Figure 4.3 is a micrograph illustration of a Pheroid™ vesicle that contains 2.5 % lidocaine HCl, 2.5 % prilocaine HCl and 0.25 % adrenaline.

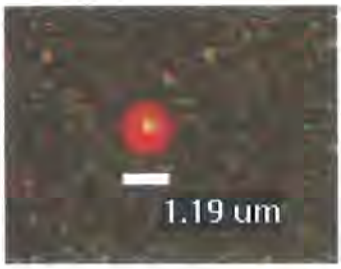


Figure 4.3: Pheroid vesicle containing 2.5 % lidocaine HCl and 2.5 % prilocaine HCl and 0.25 % adrenaline

4.2.2.2 Penetration efficiency

The efficiency of penetration can easily be measured by comparative investigation where the enhancement caused by the carrier could be compared to an existing commercial product such as EMLA® cream. CLSM can accurately determine the amount of active fluorescent compound that penetrated the skin at a specific depth (Grobler *et al.*, 2008:295).

4.3 CELLULAR UPTAKE OF PHEROIDS™ AND ENTRAPPED COMPOUNDS

Since Pheroid™ is stabilised by electro-chemical interaction and not by cholesterol, it allows for a very elastic structure that is able to cross even capillary walls (Grobler *et al.*, 2008:297). Preliminary evidence suggests that uptake is actively facilitated by the fatty-acid membrane-binding proteins (FABs) present within the lipid rafts of the cell membrane that efficiently mediates trans-bilayer movement of fatty acids.

Factors that influence cellular uptake include

- the size and morphology of the Pheroid™,
- molecular geometry of fatty acids,
- concentration ratio of the fatty acids,
- hydration medium (ionic strength etc.),
- the pH of the preparation,
- the presence of ionisable molecules or molecule that influence the electrostatic environment,
- character and concentration of the active, and
- gel or fluid state of Pheroid™ (Grobler *et al.*, 2008:297).

5 SUMMARY

Transdermal delivery of anaesthetics boasts several advantages in comparison to invasive methods. Rapid target delivery is however not easily accomplished since the formidable

barrier of human skin, the stratum corneum, is not easily overcome. The long time of onset of action proposes an enormous challenge.

When taking into consideration the physicochemical characteristics of both lidocaine HCl and prilocaine HCl, it can be assumed that these drugs would penetrate the skin through the intercellular route – a tortuous route that is approximately 150 to 500 μm long and takes a long time to traverse en route to the dermis. Various means of penetration enhancement and delivery have been considered during this study. Upon assessment, the Pheroid™ therapeutic drug delivery system proposed a possible solution. Being a pliable system, the boundless entrapment capabilities could lead to greatly enhanced therapeutic efficacy, reduced toxicity and dramatically reduced lag time.

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CHAPTER 3: ARTICLE FOR PUBLICATION IN THE INTERNATIONAL JOURNAL OF PHARMACEUTICS

PHEROID™ TECHNOLOGY FOR OPTIMISED TRANSDERMAL DELIVERY OF LOCAL ANAESTHETICS

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ABSTRACT

Existing commercial local anaesthetics may take well up to an hour to produce an anaesthetic effect. The objective of this study was to significantly reduce lag time in which a combination of local anaesthetics, lidocaine hydrochloride (LiHCl) and prilocaine hydrochloride (PrHCl), with a vasoconstrictor adrenaline, could rapidly be delivered transdermally. Pheroid™ technology was employed as carrier system. A concentration of 2.5 % (m/v) of each of the active drugs was entrapped in Pheroid™ vesicles, 8 hours prior to the experiment. Distinct entrapment could be established visually by means of confocal laser scanning microscopy (CLSM). Vertical Franz cell diffusion studies were performed over a 12 hour period using Caucasian female abdominal skin obtained from abdominoplastic surgery. Comparison was made between the commercial product, the active local anaesthetics dissolved in phosphate buffered solution (PBS) and the active ingredients entrapped within the Pheroid™ vesicles. The amount of drug that traversed the epidermal membrane into the receptor phase was then assayed by high performance liquid chromatography (HPLC). The results indicated a dramatic increase in percentage yield and flux during the first two hours.

Keywords: Transdermal delivery, Pheroid™, lidocaine hydrochloride, prilocaine hydrochloride, local anaesthesia

1 INTRODUCTION

The eutectic combination of the two lipid soluble and ionisable amide types of local anaesthetics (Conley & Brammar, 1999:816) in discussion, lidocaine and prilocaine, have been extensively implemented for topical anaesthesia in the past. It proved to be useful in the case of a variety of painful superficial procedures, venipuncture, skin graft harvesting, anal or genital pruritus, poison ivy rashes, postherpetic neuralgia and several other dermatoses (Catterall & Mackie, 2006; Sweetman, 2002). The transdermal route of drug delivery proves to be significant, since it is a non-invasive method that acts locally and reduces trauma and the risk of infection (Cerchiara & Luppi, 2006). Local anaesthetics possess intrinsic vasoactivity. The amide types tend to bring about vasoconstriction (Sweetman, 2002:1304). Adrenaline was additionally included in the formulation for the purpose of minimising systemic absorption and localising the anaesthetic effect.

The dilemma with commercially available, local acting anaesthetics is that it may take well up to an hour to produce an anaesthetic effect (Astra Zeneca, 2004). This is the result of the highly efficient barrier function the stratum corneum (SC) provides against noxious stressors (Tobin, 2005). Anaesthetics must traverse the top skin layers in order to reach the intended target site, which is the free nerve endings located in the dermis. The lipid insoluble ionised forms cannot cross the cell membrane, making it ineffectual when applied outside the axon, but actively exerts its action once applied inside the cell at receptor site (Conley & Brammar, 1999; White & Katzung, 2004).

Lidocaine HCl and prilocaine HCl both exhibit ideal properties for transdermal delivery: they have low molecular masses (LiHCl = 288.82, PrHCl = 220.3) and low melting points (LiHCl = 79°C, PrHCl = 171°C) (BP, 2007). Furthermore when combined, lidocaine and prilocaine forms a binary eutectic mixture that has a lower melting point than that of either component (Sweetman, 2002). Naik et al. (2000) recommends that the ideal melting point limit for passive transdermal delivery is below 200°C. Smaller molecules in higher concentrations also tend to penetrate more readily into the skin than larger molecules (Lund, 1994; Hadgraft,

2004). Williams (2003) stated that the most probable organic candidates for transdermal delivery lie within 100 to 500 Dalton; a range that well includes the low molecular weights of these anaesthetics. An octanol-water partition coefficient (log P) in the region of 1 to 3 will enable a compound to partition reasonably well between the hydrophilic and lipophilic domains in human skin (Williams, 2003; Hadgraft, 2004). The log P values of lidocaine HCl and prilocaine HCl are suitable at 2.36 ± 0.26 (LiHCl) and 2.09 ± 0.49 (PrHCl) respectively (calculated by ACD/lab ChemScketch 11.0).

Upon examining the physicochemical properties of the local anaesthetics, lidocaine HCl and prilocaine HCl, it is reasonable to assume that these compounds may possibly penetrate the skin through the intercellular route – a tortuous route with an estimated path length of 150 – 500 µm. Little wonder that the onset of action is so dawdling.

Several techniques of promoting the penetration of lidocaine and prilocaine have been previously employed, for instance, occlusive dressing (Astra Zeneca, 2004:2), entrapment in liposomes (Müller *et al.*, 2004) and miscelles (Scherlund *et al.*, 2000), anaesthetic patches (Kundu & Achar, 2002) and iotophoretic delivery, etc. (Abla et al., 2006:185). The Pheroid™ carrier system is novel technology that entails improved delivery of several active compounds. It is a submicron emulsion type formulation (Grobler, 2004) that possesses the ability to be transformed in morphology and size (Grobler et al., 2008), affording it tremendous flexibility. Since it consists mainly of unsaturated essential fatty acids (including omega-3 and -6 fatty acids but auspiciously excluding arachidonic acid) (Grobler et al., 2008) it is not seen as foreign to the body but rather as a skin-friendly carrier.

This study concerned itself with the possible enhanced skin penetration by making use of the previously mentioned carrier in order to significantly reduce lag time.

2 MATERIALS & METHODS

2.1 MATERIALS

The active compounds adrenaline (alias (−)-adrenaline) $(HO)_2C_6H_3CH(OH)CH_2NHCH_3$), lidocaine hydrochloride monohydrate ($C_{14}H_{22}N_2O{\cdot}HCl{\cdot}H_2O$) and prilocaine hydrochloride ($C_{13}H_{20}N_2O{\cdot}HCl$) were obtained from Sigma-Aldrich Corporation (Johannesburg, South Africa) accompanied by Certificates of Analysis. Nile Red ($C_{20}H_{18}N_2O_2$) and triethylamine ($C_6H_{15}N$) were also purchased from the same company. Sodium chloride (NaCl), sodium dihydrogen orthophosphate dihydrate ($NaH_2PO_4{\cdot}2H_2O$), disodium hydrogen orthophosphate dihydrate ($Na_2HPO4{\cdot}2H_2O$) and potassium dihydrogen phosphate (KH_2PO_4) were supplied by Merck Laboratory Supplies (Midrand, South Africa). Ammonium hydroxide (NH_4OH) was ordered from Saarchem, (E. Merck, Johannesburg, South Africa). HPLC analytical grade acetonitrile (CH_3CN) supplied by Acros Organics (New Jersey, United States of America) and isopropanol ($(CH_3)_2CHOH$) from BDH Laboratory Supplies (Poole, England), were used. Deionised HPLC grade water prepared with a Milli-Q water purification system (Millipore, Milford, USA) was used throughout the entire study. Pheroid™ vesicles were prepared by the Unit for Drug Research and Development at the North-West University.

2.2 METHODS

2.2.1 Encapsulation of the actives in Pheroid™ vesicles

An amount of active was weighed and dissolved in the prepared Pheroid™ solution. It was shaken for approximately 30 minutes and then kept refrigerated for 8 hours prior the commencement of the experiment to ensure inclusion inside the Pheroid™ vesicle.

Figure 1: CLSM micrographs of placebo Pheroid™ vesicles **(a)** and active local anaesthetics entrapped in Pheroid™ vesicles **(b)**

Entrapment could be visualised clearly by means of confocal laser scanning microscopy (CLSM) (Figure 1). The phenoxazine dye nile red was used as a fluorescent probe (Haugland, 2005) to stain the phospholipid components of the Pheroid™ vesicles. Fluorophore (5 µl) was added to a solution containing 2.5 % lidocaine HCl and prilocaine HCl in Pheroid™, vortexed and allowed to penetrate for 15 minutes. The sample was then placed on a glass slide and covered with a glass cover-slip. A Nikon PCM 2000 confocal laser scanning microscope with a Nikon DXM1200 camera for realtime imaging and Nikon 60 x Planar Apochromate oil immersion objective with numerical aperture (NA) of 1.4 was used to capture the images. The microscope is equipped with a green argon laser (with a wavelength of 488 nm for excitation and of 515 nm for emission) and red helium/neon laser (excitation 505 nm and emission 568 nm).

2.2.2 Preparation of donor solutions

The donor solutions applied included solutions of lidocaine HCl (2.5 %) and prilocaine HCl (2.5 %), dissolved in PBS and Pheroid™ solutions, respectively, as well as 2.5 % lidocaine HCl and prilocaine HCl in EMLA® cream. Adrenaline was used throughout the study in a concentration of 1:100 to the active local anaesthetics. The active compounds were carefully weighed and dissolved in their distinct solutions and sonicated for 1 min. PBS with a physiological pH of 7.4 was prepared from 3.35 g disodium hydrogen orthophosphate dihydrate ($Na_2HPO4{\cdot}2H_2O$), 2.2 g sodium chloride (NaCl) and 1.05 g sodium dihydrogen orthophosphate dihydrate ($NaH_2PO_4{\cdot}2H_2O$) dissolved in HPLC grade water and made up to a final volume of 500 ml. The EMLA® cream was transferred into a 10 ml syringe from where it could be accurately measured in equal parts of 1 ml to be applied to the donor phase.

2.2.3 Skin preparation

Human skin was obtained (with the consent of the donor) from the abdomen of Caucasian females who underwent cosmetic abdominoplastic surgery. Approval for the procurement and utilisation of the skin was granted by the Research Ethics Committee of the North-West

University under reference number 04D08. The full-thickness skin was frozen at -20°C within 24 hours after excision. Prior to preparation, the skin was thawed to room temperature. Adipose tissue was removed with a scalpel and then the remaining layers were immersed in water at 60°C for 1 minute (Bronaugh & Collier, 1993). The fragile hydrated epidermis could then be stripped cautiously from the dermis using forceps and then be positioned onto Whatman® filter paper, with the SC facing upwards, in order to dry. The prepared samples were kept frozen in aluminium foil sheets until the diffusion study was due, defrosted and cut into circles with a diameter of ± 10 mm. The epidermal skin circles were examined thoroughly and any perforated sections discarded.

2.2.4 Skin permeation

Amber coloured vertical Franz diffusion cells (obtained from PermeGear Inc., Bethlehem, PA, USA) with a diffusion area of 1.075 cm^2 and receptor capacity of approximately 2 ml were used during the study. A small magnetic stirring bar was placed inside the orifice of the receptor compartment of the Franz cell. The epidermal skin circles were mounted on top with the SC facing upwards and roofed with the donor compartment. It was then sealed with Dow Corning high vacuum grease and secured with a horseshoe clamp. Both compartments were filled with PBS (pH 7.4) taking care not to allow any air bubbles in the receptor phase. The cells were then equilibrated in a water bath at 37°C for 1 hour on a Variomag® stirrer plate at 750 rpm. The PBS in the donor compartment was discarded prior to onset of the diffusion study and replaced by 1 ml of the donor solution that was covered with Parafilm® in order to prevent evaporation for the duration of the experiment. The entire volume of the receptor compartments were emptied at 20 min, 40 min, 1, 1.5, 2, 3, 4, 6, 8, 10 and 12 hours and replaced with fresh PBS. This was done in order to maintain sink conditions similar to that of the human body. The withdrawn samples were immediately assayed by HPLC to determine the drug concentration that had permeated through the epidermal membrane.

2.2.5 Chromatographic Procedure

A high performance liquid chromatography (HPLC) method had to be developed and validated for the assay of the active drugs. An Agilent® 1100 Series HPLC equipped with a quaternary pump, diode array detector, autosampler injection mechanism, vacuum degasser, solvent module and interfaced with Agilent Chemstation Rev. A.08.03 software for data acquisition and analysis was used. A silica based, reversed phase column was used (Luna C18 (2), 5 μm particle size, 150 x 4.6 mm, Phenomenex® Torrance, CA). To maintain the condition of the column, it was rinsed each time before storage with HPLC grade water at a flow rate of 1 ml/min for 20 minutes, then with 70 % CH_3CN for a further 20 minutes and finally with $(CH_3)_2CHOH$ for the last 20 minutes. Lidocaine HCl and prilocaine HCl were separated by gradient elution. The mobile phase consisted of two components, namely CH_3CN and a degassed mixture of 6.8012 g KH_2PO_4 and 1 ml $C_6H_{15}N$ dissolved in 1 litre of HPLC grade water. Linear gradient was run from 5 % CH_3CN at 1 min to 40 % after 6 min. After 12 min the system was re-equilibrated to the starting conditions. The pH was set at 7.1 by using 10 % NH_4OH. Refer to Table 1 for the mobile phase composition. The flow rate during analysis was set at 1 ml/min with a total run time of 16 min and an injection volume of 100 µl. Retention times were 2.3, 8.0 and 9.4 for adrenaline, lidocaine HCl and prilocaine HCl, respectively. Analysis was performed in a controlled laboratory environment at 25°C.

Table 1: Mobile phase composition of A (CH_3CN) and B (KH_2PO_4 and $C_6H_{15}N$) for gradient elution

2.2.6 Transdermal and statistical data analysis

The cumulative concentration of the drug that permeated through the epidermis was plotted against time. Average flux values were obtained by the slope of the straight line. This was done with the data for 2.5 % of the active local anaesthetics dissolved in Pheroid™ solution as well as for EMLA® cream. The profiles of the Pheroid™ charts exhibited biphasic

character with a clear steady-state flux between 0 and 2 hours and then again between 3 and 12 hours. The percentage yield could be expressed relative to the applied concentration. The spread was interpreted in terms of standard deviation (SD) and % SD. The % SD was calculated by dividing the SD by the average cumulative concentration at a specific time, and multiplying with 100.

For statistical analysis, both the mean (synonym for average used to distinguish between the transdermal and statistical analysis) and median (statistically calculated 50 percentile or centre of a given set of data) of the flux and % yield values were examined. A Shapiro-Wilk test of normality was performed to determine whether the data was distributed normally and the Levene test to test equality of variances. A p-value less than 0.05 would indicate a statistical significance between the data in the two groups. Subsequently the T-test was performed to evaluate the differences in mean values for the two groups, Pheroid™ and EMLA®, as well as the nonparametric alternative, the Mann-Whitney U test, to asses the median values. The relationship could be visualised by means of box and whisker plots (Field, 2005).

Figure 2: Average flux values for 2.5 % lidocaine and prilocaine in PBS, EMLA® and Pheroid™

The compared average flux data for 2.5 % of the active anaesthetics in PBS, EMLA® and Pheroid™ medium (Figure 2) gives a clear visual indication that the flux values obtained with the water soluble PBS was significantly higher in comparison to the latter mentioned preparations. This is possibly the result of rapid penetration into the skin via the hydrophilic transappendageal route (Williams, 2003). Since the essence of this study is captured in the comparison between the flux values obtained for commercially available EMLA® cream and the equivalent amount of active local anaesthetics in the Pheroid™ carrier system, these results will be elaborated upon.

Table 2: Summary of average, mean and median flux values (µg/cm².h) obtained with 2.5 % lidocaine and 2.5 % prilocaine in Pheroid™ and EMLA®

Pheroid™ entrapped actives showed an 8 fold increase in average flux (617.12 µg/cm².h LiHCl, 699.49 µg/cm².h PrHCl) in the first two hours for the same concentration as EMLA® (73.81µg/cm².h LiHCl, 53.93 µg/cm².h PrHCl). Fatty acids such as linolenic and linoleic acid (both components included in the Pheroid™ formulation) act as permeation enhancers by disrupting the SC (Purdon et al., 2004). The cellular uptake of Pheroid™ is actively facilitated by fatty acid membrane binding proteins within the lipid rafts of the cell membrane (Grobler et al., 2008). This may suggest that the Pheroid™ vesicles traverse the SC through the lipophilic transcellular route, which has a considerable shorter path to cross since its thickness is merely 20 µm (Williams, 2003). In addition, Pheroid™ vesicles are the smallest form of Pheroid™ and will therefore be able to readily penetrate the skin.

The average flux values between 3 - 12 hours obtained with Pheroid™ (106.42 µg/cm².h LiHCl, 118.89 µg/cm².h PrHCl) was only slightly higher than that of EMLA® (99.43 µg/cm².h LiHCl, 101.50 µg/cm².h PrHCl).

Upon comparing the average flux values between 0 - 2 and 3 - 12 hours, it is evident that the Pheroid™ show a definite decrease that may be ascribed to the fact that the membranes that were in contact with Pheroid™ have reached a point of saturation after the rapid flux within the first two hours. In contrast, the average flux values for EMLA® increased to 99.43 µg/cm².h for LiHCl and 101.50 µg/cm².h for PrHCl during 3 - 12 hours since the epidermis was probably still unsaturated.

Figure 3: Mean flux values of 2.5 % lidocaine & 2.5 % prilocaine in Pheroid™ and Emla® within **(a)** 0 - 2 hours and **(b)** 3 - 12 hours

There is a strong correlation between the average flux values and the mean values (shown in Figure 3). The mean flux values obtained with Pheroid™ was higher between 0 - 2 hours (LiHCl = 617.12 µg/cm².h; PrHCl = 699.49 µg/cm².h) than between 3 - 12 hours (LiHCl = 106.42 µg/cm².h; PrHCl = 112.89 µg/cm².h). They were also significantly superior to the mean flux values obtained with EMLA®. EMLA® increased over time with 3 - 12 hours (LiHCl = 99.42 µg/cm².h; PrHCl = 101.50 µg/cm².h) being somewhat higher than 0 - 2 hours (LiHCl = 73.55 µg/cm².h; PrHCl = 53.93 µg/cm².h).

Figure 4: Box and whisker plots of the median flux values for 2.5 % lidocaine & 2.5 % prilocaine in Pheroid™ and Emla® within **(a)** 0 - 2 hours and **(b)** 3 - 12 hours

The median flux profiles (Figure 4) strongly compare to that of the average and mean flux values except for lidocaine in Pheroid™ at 3 - 12 hours where the median (96.81 µg/cm².h) exhibit a lower flux value than EMLA® (103.14 µg/cm².h). Median flux values give a more

accurate representation of the true flux since it takes all the data into consideration and is not affected by a distortion in the spread of the data, as may be the case with mean flux (Gerber, 2008).

Larger variance (SD) was observed in the data for the Pheroid™ (0 - 2 hours) when compared to the variance in data for EMLA® however. This is possibly due to the homogenous dispersion of the active compound in EMLA® cream, which is a more viscous preparation than the Pheroid™ formulation.

Since the p-values of the Shapiro-Wilk test were greater than 0.05, it could be concluded that the sample was distributed normally. Levene's test revealed significant differences in spread for both the % yield ($p = 0.028$ for lidocaine and 0.027 for prilocaine) and the flux ($p = 0.023$ for lidocaine and 0.024 for prilocaine) between the Pheroid™ and EMLA® groups at 0 - 2 hours but no significant spread difference between the groups at 3 - 12 hours. The Levene test was used to choose an appropriate t-test, i.e. equal variances assumed or alternatively equal variances not assumed. The t-test indicated that there is a statistically significant difference ($p = 0.001$) between the dependant variables, % yield and flux, for both lidocaine and prilocaine at 0 - 2 hours. Table 3 summarises the obtained values for these variables although the study focused on the flux values rather than % yield. The output from the non-parametric Mann-Whitney U test showed similar results to that obtained with the t-test.

Table 3: % Yield and flux data for lidocaine and prilocaine

The % SD (SD divided by the average cumulative concentration at a specific time and multiplied with 100) for the cells at the first withdrawal after 20 min was higher than what could normally be expected for biological material. The values ranged between 56.57 and 70.18. This could be explained since the denominator in the equation (average cumulative concentration) is a smaller value that lies closer to zero. The values % SD declined proportional to the progression of time. From a pharmaceutical point of view, it is also

possible that the skin membrane in the first diffusion cell might be somewhat more hydrated than the skin membrane in cell 15 since the time taken to apply the donor phase accumulates, so that the first cell will be exposed to the active for a slightly longer period of time. Increased hydration leads to improved permeability of both polar and non-polar drugs (Riviere, 1993:117). The % RSD for the flux during the first 2 hours lay well within the limits with values ranging from 22.49 to 38.29.

Table 4: Average cumulative concentration (µg/ml) for lidocaine and prilocaine within the first hour

Table 4 gives a comparison of average cumulative concentration (µg/ml) for lidocaine and prilocaine within the first hour. It is evident that the Pheroid™ delivery system was able to deliver a much higher concentration (155.20 µg/ml of lidocaine and 180.15 µg/ml prilocaine) than EMLA® (13.41µg/ml of lidocaine and 7.82 µg/ml prilocaine) from as early as 20 minutes after application. Thus Pheroid™ delivery technology proved to be efficient in reducing the lag time of commercially available, EMLA® cream, which is currently established at 1 hour following application.

CONCLUSION

The Pheroid™ vesicle illustrated definite entrapment of the active local anaesthetics as could clearly be seen in the confocal micrographs. Furthermore this delivery system indicated an improvement in transdermal flux and in the % yield values during the first two hours. This improvement could be observed from as early as 20 min into the diffusion study, which gives an indication that the Pheroid™ carrier is likely to be efficient in reducing the lag time currently determined at 1 hour for EMLA® cream (Astra Zeneca, 2004). Future investigation may focus on the mechanism of action and route of permeation of these Pheroid™ vesicles by means of realtime confocal microscopy. Clinical studies should be undertaken in order to test these findings.

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FIGURE LEGENDS:

Figure 1: CLSM micrographs of placebo Pheroid™ vesicles **(a)** and active local anaesthetics entrapped in Pheroid™ vesicles **(b)**

Figure 2: Average flux values for 2.5 % lidocaine and prilocaine in PBS, EMLA® and Pheroid™

Figure 3: Mean flux values of 2.5 % lidocaine & 2.5 % prilocaine in Pheroid™ and Emla® within **(a)** 0 - 2 hours and **(b)** 3 - 12 hours

Figure 4: Box and whisker plots of the median flux values for 2.5 % lidocaine & 2.5 % prilocaine in Pheroid™ and Emla® within **(a)** 0 - 2 hours and **(b)** 3 - 12 hours

TABLES:

Table 1: Mobile phase composition of A (CH_3CN) and B (KH_2PO_4 and $C_6H_{15}N$) for gradient elution

Time (min)	% Mobile Phase A	% Mobile Phase B
0 - 1	5.0	95.0
6 -12	40.0	60.0
12.1 - 16	5.0	95.0

Table 2: Summary of average, mean and median flux values (µg/cm².h) obtained with 2.5 % lidocaine and 2.5 % prilocaine in Pheroid™ and EMLA®

Flux (µg/cm^2.h)	Product	Lidocaine		Prilocaine	
		0-2 hours	3-12 hours	0-2 hours	3-12 hours
Average	EMLA®	73.81	99.43	53.93	101.50
	Pheroid™	617.12	106.42	699.49	118.89
Mean	EMLA®	73.55	99.42	53.93	101.50
	Pheroid™	617.12	106.422	699.49	112.89
Median	EMLA®	69.17	103.14	58.89	105.33
	Pheroid™	606.53	96.81	694.61	113.97

Table 3: % Yield and flux data for lidocaine and prilocaine

Lidocaine in Pheroid™				
Cell	% Yield 0 - 2 (hour)	% Yield 3 - 12 (hour)	Flux ($\mu g/cm^2.h$)	Flux ($\mu g/cm^2.h$)
1	5.07	4.33	646.95	78.42
2	4.73	5.99	606.53	121.38
3	4.01	6.15	513.68	125.38
4	1.61	0.87	213.90	21.78
5	4.14	3.31	541.43	67.26
6	6.12	4.70	818.88	96.81
7	7.79	10.23	978.47	233.92
Lidocaine in EMLA®				
Cell	% Yield 0 - 2 (hour)	% Yield 3 - 12 (hour)	Flux ($\mu g/cm^2.h$)	Flux ($\mu g/cm^2.h$)
1	0.44	2.89	56.37	74.10
2	0.55	4.32	69.17	110.59
3	0.76	4.38	96.20	112.18
4	0.40	3.17	52.84	82.06
5	0.54	3.92	67.62	101.08
6	0.66	4.39	83.02	112.84
7	0.73	4.04	89.68	103.14
Prilocaine in Pheroid™				
Cell	% Yield 0 - 2 (hour)	% Yield 3 - 12 (hour)	Flux ($\mu g/cm^2.h$)	Flux ($\mu g/cm^2.h$)
1	5.83	4.92	742.83	90.22
2	5.41	6.88	694.61	140.84
3	4.57	6.00	584.80	126.57
4	1.78	0.90	239.00	23.31
5	4.82	3.91	627.90	38.08
6	6.92	5.47	922.49	113.97
7	8.64	11.27	1084.80	257.27
Prilocaine in EMLA®				
Cell	% Yield 0 - 2 (hour)	% Yield 3 - 12 (hour)	Flux ($\mu g/cm^2.h$)	Flux ($\mu g/cm^2.h$)
1	0.28	2.91	35.05	75.44
2	0.48	4.31	62.95	111.97
3	0.59	4.47	74.67	115.00
4	0.28	3.18	34.23	83.21
5	0.35	4.02	44.57	104.22
6	0.47	4.46	58.89	115.33
7	0.55	4.10	67.21	105.33

Table 4: Average cumulative concentration (µg/ml) for lidocaine and prilocaine within the first hour

	Lidocaine		**Prilocaine**	
Time (hour)	Average cumulative concentration (µg/ml)		Average cumulative concentration (µg/ml)	
	EMLA®	Pheroid™	EMLA®	Pheroid™
0	0.00	0.00	0.00	0.00
0.33	13.41	155.20	7.82	180.15
0.66	31.48	430.41	15.33	489.73
1	51.75	682.00	28.75	777.80

FIGURES:

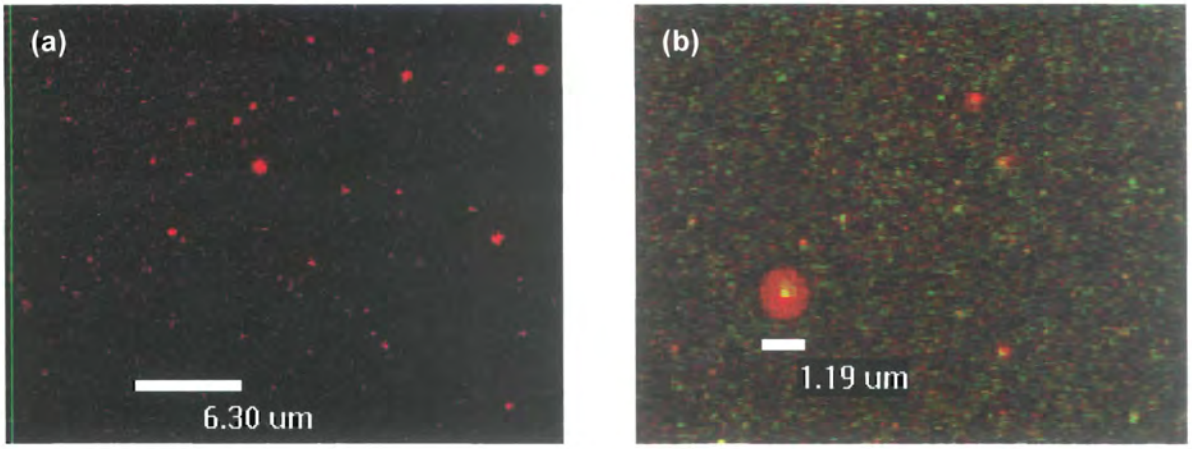
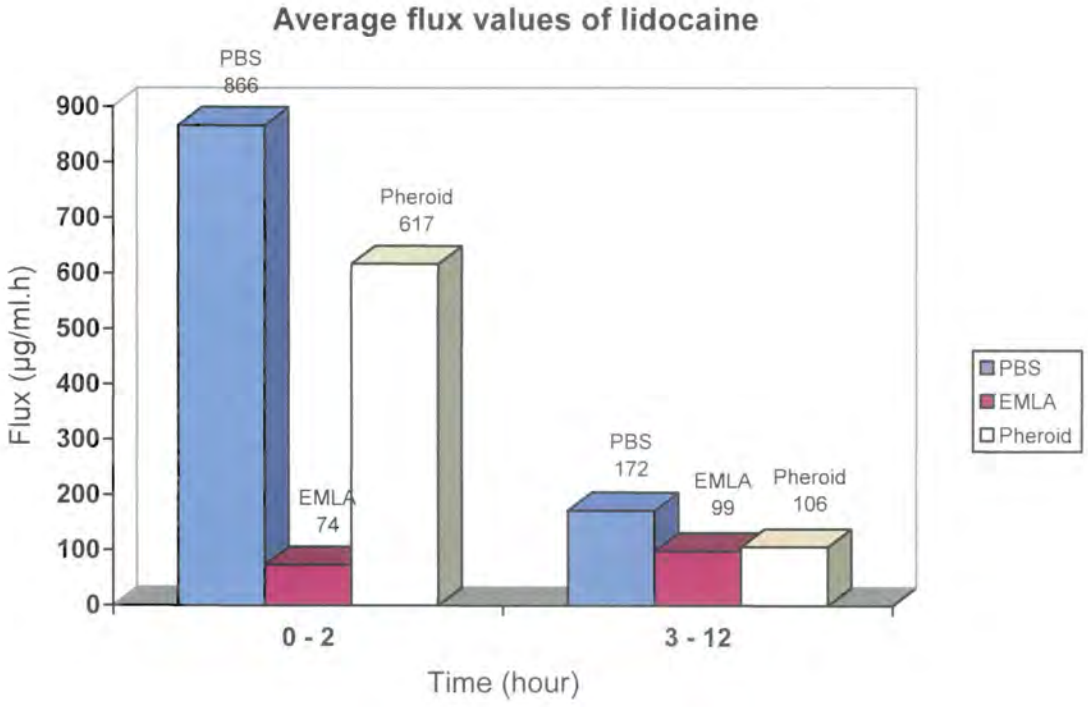


Figure 1: CLSM micrographs of placebo Pheroid™ vesicles **(a)** and active local anaesthetics entrapped in Pheroid™ vesicles **(b)**



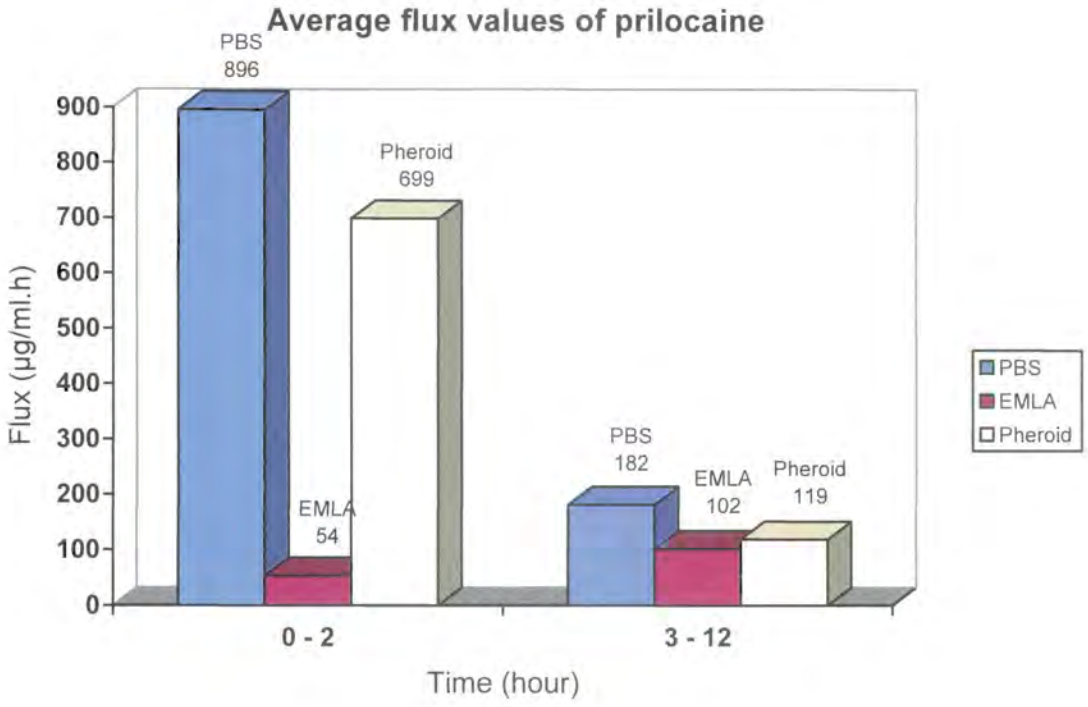


Figure 2: Average flux values (µg/cm^2.h) for 2.5 % lidocaine and prilocaine in PBS, EMLA® and Pheroid™

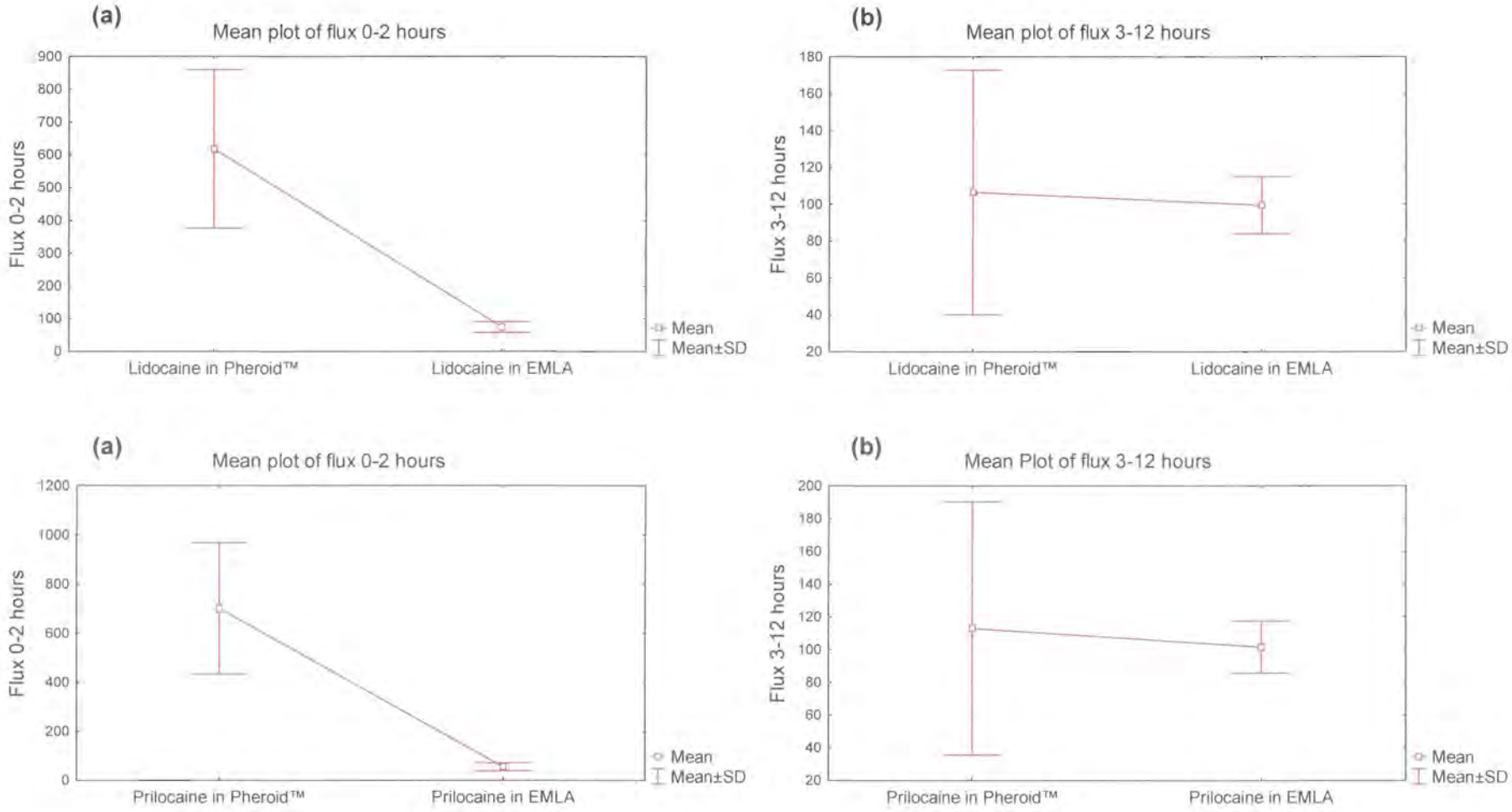


Figure 3: Mean flux values (µg/cm^2.h) of 2.5 % lidocaine & 2.5 % prilocaine in Pheroid™ and EMLA® within **(a)** 0 - 2 hours and **(b)** 3 - 12 hours

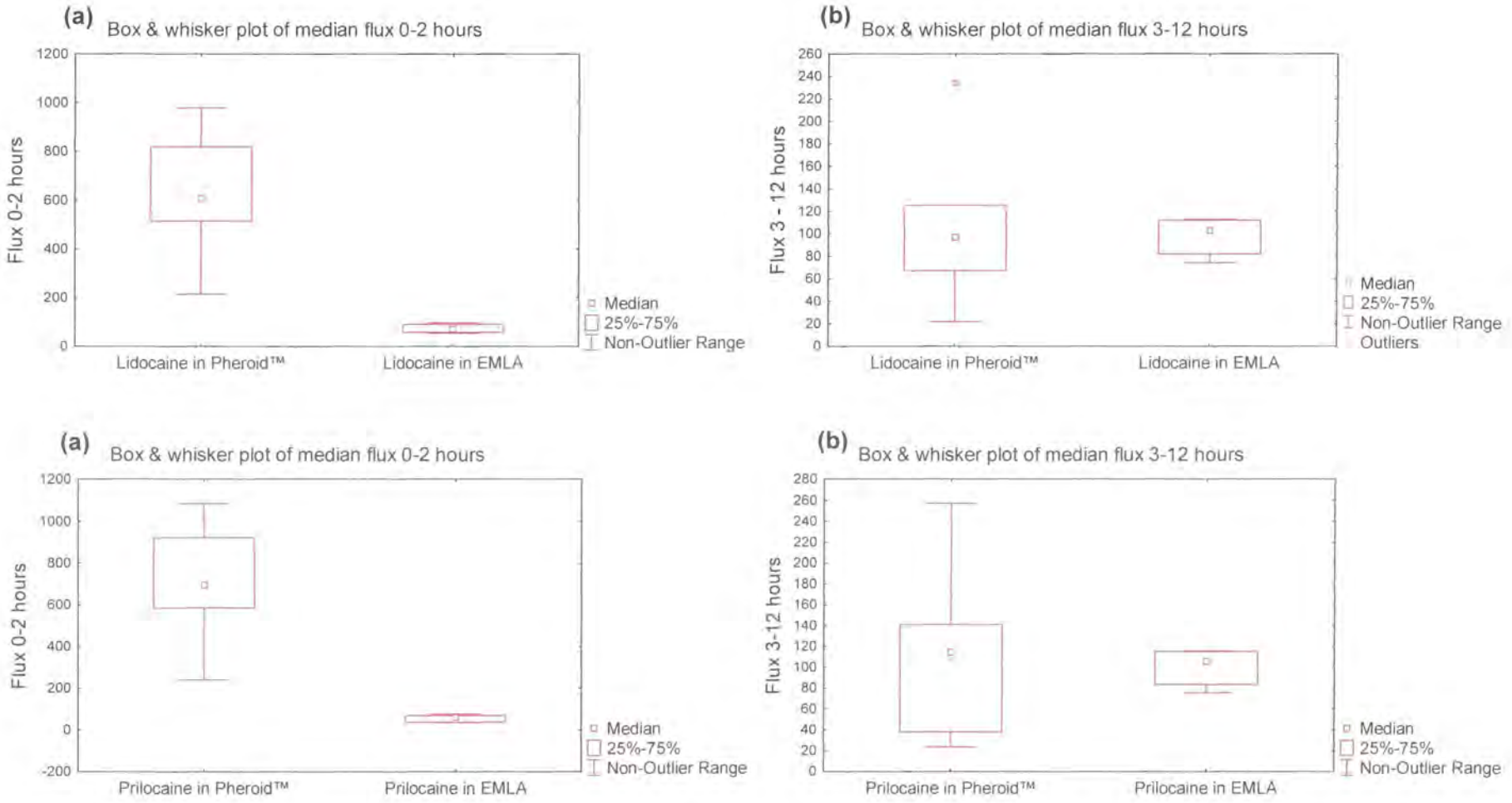


Figure 4: Box and whisker plots of the median flux values (µg/cm^2.h) for 2.5 % lidocaine & 2.5 % prilocaine in Pheroid™ and EMLA® within **(a)** 0 - 2 hours and **(b)** 3 - 12 hours

CHAPTER 4: FINAL CONCLUSIONS AND FUTURE PROSPECTS

This study mainly concerned itself with an investigation into the possibility of shortening the lag time of local anaesthetics, lidocaine HCl and prilocaine HCl, by making use of the innovative delivery system, Pheroid™ technology. The commercially available comparable preparation, EMLA® cream, effectively produces an anaesthetic effect only within 60 min following application (Astra Zeneca, 2004).

Human skin, being such an intricate protective organ, provides quite a challenge in overcoming the barrier to transdermal penetration. The stratum corneum was identified as the principal resistant layer (Langer, 2004:557). However once this has been achieved, the skin is the ideal location for drug administration. Local anaesthetics need to traverse into the dermal layers of the skin in order to reach the intended site of action which is the nerves and blood vessels (Tobin, 2006:58).

The physicochemical properties of compound greatly influence its penetration through the skin (Williams, 2003:35). Actives with balanced log P values in the range of 1 to 3 (Hadgraft, 2004:292) will partition reasonably well between the hydrophilic and lipophilic domains in the skin. Smaller molecules with low molecular weights (MM > 500 Dalton) penetrate the skin more readily than larger ones (Hadgraft, 2004:292; Williams, 2003:36). The melting point of a substance has a direct relation to its solubility (Williams, 2003:37).

Solutions of 2.5 % lidocaine HCl and prilocaine HCl each dissolved in PBS, Pheroid™ and EMLA® were compared during permeation studies. The essence of the study was however captured in the comparison between the data of Pheroid™ and EMLA®. The permeation profiles obtained from the experimental data exhibited clear biphasic character for the Pheroid™ entrapped local anaesthetics with a great increase in the flux values obtained within 0 to 2 hours. Statistical comparison of mean and median flux values for both these components seemed to prove that the Pheroid™ delivery system is more efficient for the transdermal delivery of the local anaesthetics within a given amount of time. Entrapment of the actives inside the Pheroid™ vesicles could be established visually by means of confocal laser scanning microscopy (CLSM).

The vasoconstrictor, adrenaline, that was included in the formulation in an attempt to localise the action of the anaesthetics proved to have no significant effect. This is probably due to poor transdermal penetration (Catterall & Mackie, 2006:380).

In future it may be sensible to render further investigation into the following aspects:

- Tape-stripping may be performed in order to determine the quantity of the active local anaesthetics that is distributed within the various layers of the skin.
- Real-time confocal laser scanning microscopy may be employed to track the penetration of the Pheroid™ vesicles in order to determine the pathway by which it enters the skin.
- Clinical trials can be performed to test whether the results obtained with the mimicking transdermal diffusion studies really do bring about a quicker onset of action.

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APPENDIX 1: VALIDATION OF EXPERIMENTAL METHODS

METHOD VALIDATION FOR THE HPLC ASSAY OF LIDOCAINE HYDROCHLORIDE, PRILOCAINE HYDROCHLORIDE AND ADRENALINE

1 INTRODUCTION

The purpose of method validation is to demonstrate that the established method is reliable and sensitive in verifying the quantities of analyte that permeates the skin. Since there were only official analytical methods available for lidocaine, prilocaine and adrenaline separately, a method had to be structured and validated for the coalescence of these three drugs.

2 ANALYTICAL METHOD

2.1 MATERIAL

The active compounds adrenaline hydrochloride (alias (±)-adrenaline hydrochloride) ($C_9H_{13}NO_3 \cdot HCl$), lidocaine hydrochloride monohydrate ($C_{14}H_{22}N_2O \cdot HCl \cdot H_2O$) and prilocaine hydrochloride ($C_{13}H_{20}N_2O \cdot HCl$) were obtained from Sigma-Aldrich Corporation (Johannesburg, South Africa) accompanied by certificates of analysis. Nile red ($C_{20}H_{18}N_2O_2$) and triethylamine ($C_6H_{15}N$) were also purchased from the same company. Sodium chloride (NaCl), sodium dihydrogen orthophosphate dihydrate ($NaH_2PO_4 \cdot 2H_2O$), disodium hydrogen orthophosphate dihydrate ($Na_2HPO4 \cdot 2H_2O$) and potassium dihydrogen phosphate (KH_2PO_4) were supplied by Merck Laboratory Supplies (Midrand, South Africa). Ammonium hydroxide (NH_4OH) was ordered from Saarchem (Johannesburg, South Africa). HPLC analytical grade acetonitrile (CH_3CN) supplied by Acros Organics (New Jersey, United States of America) and isopropanol ($(CH_3)_2CHOH$) from BDH Laboratory Supplies (Poole, England), was used. Double distilled, deionised HPLC grade water prepared by a Milli-Q water purification system (from Millipore, Milford, USA) was used throughout the entire study.

2.2 CHROMATOGRAPHIC CONDITIONS

Analytical instrument: An Agilent® 1100 Series HPLC (High Performance Liquid Chromatograph) system was used for the analysis. The machine is designed with a G1311A quaternary pump, G1315A diode array detector, G1313A autosampler injection

mechanism, G1322A vacuum degasser, solvent module and HP Chemstation Software for data acquisition and analysis. Analysis was performed in a controlled laboratory environment at 25°C.

Column: A high performance silica based, reversed phase Phenomenex® Luna C18 (2) column, (150 x 4.6 mm) with a 5 μm particle size was used. To maintain the condition of the column, it was rinsed each time before storage with HPLC grade water at a flow rate of 1.0 ml/min for 20 minutes, then with 70 % CH_3CN for a further 20 minutes and finally with $(CH_3)_2CHOH$ for the last 20 minutes.

Mobile phase A: CH_3CN.

Mobile phase B: Prepare a filtered and degassed mixture of 6.8012 g KH_2PO_4 and 1 ml $C_6H_{15}N$ in 1 L of HPLC water. The pH was set at 7.1 by using 10 % NH_4OH.

Gradient Table:

Time (min)	% Mobile Phase A	% Mobile Phase B
0 - 6	5.0	95.0
6 - 12	40.0	60.0
12 - 16	5.0	95.0

Flow rate: 1.0 ml/min.

Injection volume: 100 µl.

Detection: Diode array detector at 220 nm.

Retention time: Adrenaline elutes at 2.3 minutes, lidocaine HCl at 8.0 and prilocaine HCl at 9.4 minutes.

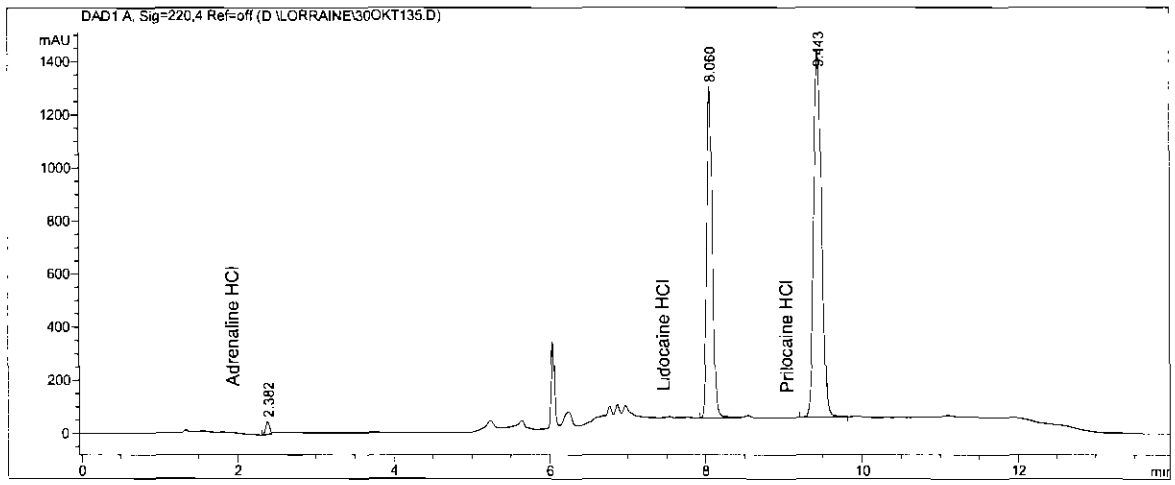

Figure 2.12: Chromatogram illustrating the retention times for adrenaline HCl, lidocaine HCl and prilocaine HCl respectively

2.3 STANDARD PREPARATION

Weigh approximately 5 mg of adrenaline accurately and dissolve in 100 ml HPLC water. Subsequently transfer 5 ml of the above mentioned solution into a 100 ml volumetric flask containing 25 mg of both lidocaine HCl and prilocaine HCl. Make up to volume with HPLC grade water. The concentration range includes standard solutions of 250, 175, 125, 75, 37.5, 15, 7.5, 1.5 and 0.15 µg/ml. The standard can be transferred to the autosampler vial for analysis.

2.4 SAMPLE PREPARATION

Weigh approximately 1250 mg lidocaine HCl and prilocaine HCl to constitute a 5% solution, and 12.5 mg adrenaline HCl (0.05%) into a 25 ml volumetric flask. Fill to volume with Pheroid™ solvent. Entrap the active compounds overnight for application to the donor compartment of the Franz cell.

2.5 VALIDATION PARAMETERS FOR LIDOCAINE HCL

2.5.1 SPECIFICITY

A standard solution of 250 µg/ml was prepared as described in § 2.3 and injected into the chromatograph in duplicate. An equal amount of the standard solution was transferred to four test tubes that each contained respectively 1 ml of a 10 % 0.1 M HCl solution, 10 % 0.1 M NaOH solution, 10 % H_2O_2 solution and HPLC grade water. The solutions were kept overnight in an oven at 40°C. Upon sample determination no additional peaks interfered with the active compound.

2.5.2 LINEARITY

Linear regression analysis was performed by injecting eight concentrations ranging from 0.15 to 175 µg/ml into the chromatograph. Concentrations were prepared as described in § 2.3 of standard preparation. The regression value (R^2) was established to be 0.9957.

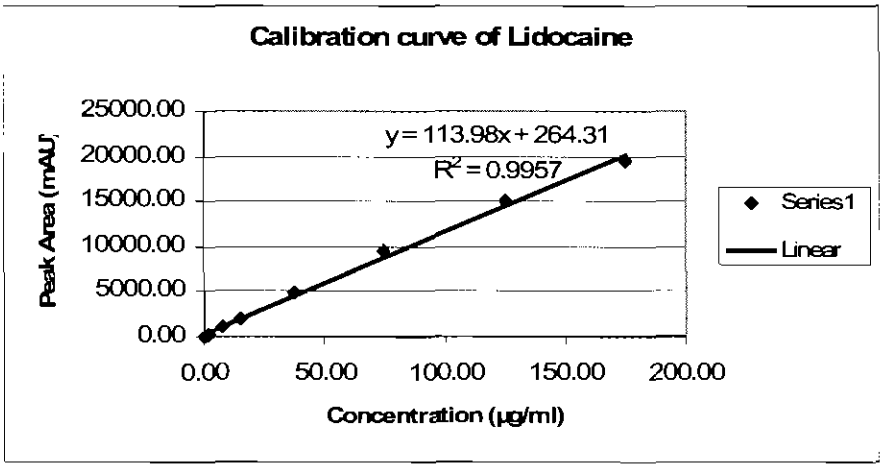

Figure 2.2: Lidocaine HCl linear regression curve of peak area versus concentration

2.5.3 ACCURACY

Concentrations of 175, 75 and 15 µg/ml were prepared from a 250 µg/ml standard solution and injected in duplicate into the chromatograph. Recovery proved to be 99.1 - 100.73 %.

Table 2.1: Accuracy parameters of lidocaine HCl standard

Conc. spiked (µg/ml)	AUC* 1	AUC* 2	Mean	Recovery (µg/ml)	%
15.03	1940.20	1935.60	1937.90	14.89	99.10
15.03	1942.33	1935.51	1938.92	14.90	99.15
15.03	1933.13	1932.51	1932.82	14.85	98.83
37.58	4829.32	4842.69	4836.00	37.77	100.52
37.58	4829.97	4837.00	4833.49	37.75	100.46
37.58	4822.79	4837.36	4830.07	37.72	100.39
75.15	9642.42	9639.94	9641.18	75.69	100.73
75.15	9552.19	9545.25	9548.72	74.97	99.75
75.15	9585.16	9595.78	9590.47	75.29	100.19

* AUC refers to the area under the curve

2.5.4 PRECISION

2.5.4.1 Intra-assay precision (Repeatability)

Concentrations of 75 µg/ml, 37.5 µg/ml and 15 µg/ml was prepared from a 250 µg/ml standard solution and injected in triplicate into the chromatograph on the same day (n = 3). The % recovery was found to be between 99.1 and 100.73 % with a standard deviation (SD) of 0.7. Repeatability complies with the pharmaceutical standard that allows a SD of 2.

Table 2.2: Intra-day precision parameters of lidocaine HCl standard

Mass (mg)	AUC 1	AUC 2	Mean	Conc. Spiked (µg/ml)	Conc. (µg/ml)	%
25.05	1940.20	1935.60	1937.90	15.03	14.89	99.10
25.05	1942.33	1935.51	1938.92	15.03	14.90	99.15
25.05	1933.13	1932.51	1932.82	15.03	14.85	98.83
25.05	4829.32	4842.69	4836.00	37.58	37.77	100.52
25.05	4829.97	4837.00	4833.49	37.58	37.75	100.46
25.05	4822.79	4837.36	4830.07	37.58	37.72	100.39
25.05	9642.42	9639.94	9641.18	75.15	75.69	100.73
25.05	9552.19	9545.25	9548.72	75.15	74.97	99.75
25.05	9585.16	9595.78	9590.47	75.15	75.29	100.19
				Mean	42.65	99.90
				SD	24.92	0.67
				RSD %	58.42	0.67

2.5.4.2 Inter-assay precision

A concentration of 37.5 µg/ml was prepared from a 250 µg/ml standard solution and injected in triplicate into the chromatograph (n = 3). The % recovery was found to be between 100.03

and 100.46 % with an SD of 0.02. Inter-day repeatability complies with the pharmaceutical standard that allows a SD of 5.

Table 2.3: Inter-day precision parameters of lidocaine HCl standard

	Day 1	Day 2	Day 3	Between days
Mean	100.46	100.69	100.03	100.39
SD	0.05	0.09	0.10	0.02
RSD %	0.05	0.09	0.10	0.02

2.5.5 SENSITIVITY

2.5.5.1 Limit of Detection (LOD)

The limit of detection is an indication of the lowest amount of analyte in a sample which can be detected but not necessarily quantified. Based on a signal to noise ratio of 3:1, the LOD for lidocaine HCl was determined to be 0.05 μg/ml.

2.5.5.2 Limit of Quantification (LOQ)

The limit of quantification is the lowest concentration of analyte in a sample that can be quantitatively determined with acceptable precision and accuracy (% RSD < 15 %). Based on a signal to noise ratio of 10:1, the LOQ for lidocaine HCl was established at 0.44 μg/ml.

2.5.6 RUGGEDNESS

2.5.6.1 Sample stability

A concentration of 37.5 µg/ml was prepared from a standard solution of 250 µg/ml. The sample vial was loaded into the autosampler tray and analysed at hourly intervals for twelve hours. The results complied with pharmaceutical standards.

Table 2.4: Sample stability parameters for lidocaine HCl

Time (hours)	Peak Area	%
0	4898.15	100.0
1	5052.54	103.2
2	4894.48	99.9
3	4897.96	100.0
4	4857.32	99.2
5	4852.62	99.1
6	4863.32	99.3
7	4867.35	99.4
8	4862.79	99.3
9	4886.89	99.8
10	4889.36	99.8
11	4914.89	100.3
12	4938.23	100.8
Mean	4898.1	100.0
SD	50.45	1.03
RSD %	1.03	1.03

2.5.6.2 System repeatability

A concentration of 37.5 µg/ml was prepared from a standard solution of 250 µg/ml and injected six consecutive times into the chromatograph. The variation in the response (% RSD) proved to be excellent with a value of 0.13 for peak area and 0.08 for retention time.

Table 2.5: System repeatability parameters for lidocaine HCl

	Peak Area	Retention Time (min)
	4805.79	7.93
	4801.11	7.93
	4813.38	7.94
	4811.63	7.94
	4820.30	7.94
Mean	4810.44	7.93
SD	6.57	0.01
RSD %	0.14	0.08

2.5.7 ROBUSTNESS

A concentration of 37.5 µg/ml was prepared from a standard solution of 250 µg/ml. The flow rate was altered with ± 2.0 ml/min and wavelength with ± 2 nm. No significant effect was observed.

Table 2.6: Flow rate and wavelength parameters of lidocaine HCl

Flow Rate	Peak Area	Wavelength	Peak Area
0.8 ml/min	6005.09	218 nm	5464.45
0.9 ml/min	5369.86	219 nm	5040.76
1.0 ml/min	4798.51	220 nm	4798.51
1.1 ml/min	4379.13	221 nm	4675.17
1.2 ml/min	3993.06	222 nm	4628.16

2.6 VALIDATION PROCEDURE FOR PRILOCAINE HCL

2.6.1 SPECIFICITY

A standard solution of 250 µg/ml was prepared as described in § 2.3 and injected into the chromatograph in duplicate. An equal amount of the standard solution was transferred to four test tubes that each contained respectively 1 ml of a 10 % 0.1 M HCl solution, 10 % 0.1 M NaOH solution, 10 % H_2O_2 solution and HPLC grade water. The solutions were kept overnight in an oven at 40°C. Upon sample determination no additional peaks interfered with the active compound.

2.6.2 LINEARITY

Linear regression analysis was performed by injecting seven concentrations ranging from 0.15 to 125 µg/ml into the chromatograph. Concentrations were prepared as described in § 2.3 of standard preparation. The regression value (R^2) was established to be 0.996.

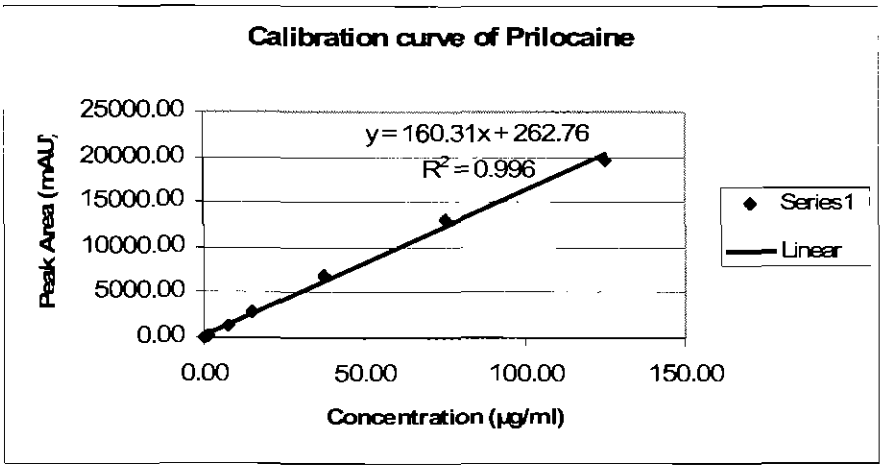

Figure 2.3: Prilocaine HCl linearity curve of peak area versus concentration

2.6.3 ACCURACY

Concentrations of 175 µg/ml, 75 µg/ml and 15 µg/ml was prepared from a 250 µg/ml standard solution and injected in duplicate into the chromatograph. Recovery proved to be 98.93 – 102.90 %.

Table 2.7: Accuracy parameters of prilocaine HCl standard

Conc. spiked (µg/ml)	Area 1	Area 2	Mean	Recovery (µg/ml)	%
15.024	2750.07	2749.22	2749.64	15.24	101.43
15.02	2743.83	2765.33	2754.58	15.27	101.62
15.02	2751.00	2750.11	2750.55	15.24	101.46
37.58	6791.91	6817.82	6804.87	38.55	102.63
37.58	6813.53	6831.47	6822.50	38.65	102.90
37.58	6814.96	6797.33	6806.14	38.55	102.65
75.15	13190.70	13204.30	13197.50	75.29	100.22
75.15	13022.20	13063.00	13042.60	74.40	99.04
75.15	13033.30	13022.40	13027.85	74.31	98.93

2.6.4 PRECISION

2.6.4.1 Intra-assay precision (Repeatability)

Concentrations of 75, 37.5 and 15 µg/ml was prepared from a 250 µg/ml standard solution and injected in triplicate into the chromatograph on the same day (n = 3). The % recovery was found to be between 99.69 and 102.90 % with an SD of 1.16. Repeatability complies with the pharmaceutical standard that allows a SD of 2.

Table 2.8: Intra-day precision parameters of prilocaine HCl standard

Mass (mg)	AUC 1	AUC 2	Mean	Conc. Spiked (µg/ml)	Conc. µg/ml	%
25.04	2750.07	2749.22	2749.64	15.02	15.24	101.43
25.04	2743.83	2765.33	2754.58	15.02	15.27	101.62
25.04	2751.00	2750.11	2750.55	15.02	15.24	101.46
25.04	6791.91	6817.82	6804.87	37.56	38.55	102.63
25.04	6813.53	6831.47	6822.50	37.56	38.65	102.90
25.04	6814.96	6797.33	6806.14	37.56	38.55	102.65
25.04	13190.70	13204.30	13197.50	75.12	75.29	100.22
25.04	13122.20	13163.00	13142.60	75.12	74.97	99.80
25.04	13133.30	13122.40	13127.85	75.12	74.89	99.69
				Mean	42.96	101.38
				SD	24.61	1.16
				RSD %	57.28	1.15

2.6.4.2 Inter-assay precision

A concentration of 37.5 µg/ml was prepared from a 250 µg/ml standard solution and injected in triplicate into the chromatograph (n = 3). The % recovery was found to be between 99.8 % and 130.6 % with an SD of 0.22. Inter-day repeatability complies with the pharmaceutical standard that allows a SD of 5.

Table 2.9: Inter-day precision parameters of prilocaine HCl standard

	Day 1	Day 2	Day 3	Between days
Mean	102.72	99.24	99.98	100.65
SD	0.12	0.50	0.17	0.17
RSD %	0.12	0.50	0.17	0.17

2.6.5 SENSITIVITY

2.6.5.1 Limit of Detection (LOD)

Based on a signal to noise ratio of 3:1, the LOD for prilocaine HCl was determined to be 0.06 µg/ml.

2.6.5.2 Limit of Quantification (LOQ)

Based on a signal to noise ratio of 10:1, the LOQ for prilocaine HCl was determined to be 0.52 µg/ml.

2.6.6 RUGGEDNESS

2.6.6.1 Sample stability

A concentration of 37.5 µg/ml was prepared from a standard solution of 250 µg/ml. The sample vial was loaded into the autosampler tray and analysed at hourly intervals for 12 hours. The results complied with pharmaceutical standards.

Table 2.10: Sample stability parameters for prilocaine HCl

Time (hours)	AUC	%
0.00	7059.18	100.00
1.00	7001.42	99.18
2.00	6987.87	98.99
3.00	6990.96	99.03
4.00	7022.21	99.48
5.00	7025.59	99.52
6.00	7051.16	99.89
7.00	7056.18	99.96
8.00	7071.70	100.18
9.00	7089.03	100.42
10.00	7112.89	100.76
11.00	7131.98	101.03
12.00	7169.15	101.56
Mean	7059.18	100.00
SD	53.33	0.76
RSD %	0.76	0.76

2.6.6.2 System repeatability

A concentration of 37.5 µg/ml was prepared from a standard solution of 250 µg/ml and injected six consecutive times into the chromatograph. The variation in the response (% RSD) proved to be excellent with a value of 0.3 for peak area and 0.05 for retention time.

Table 2.11: System repeatability parameters for prilocaine HCl

	Peak Area	Retention Time (minutes)
	6954.49	9.37
	7024.23	9.36
	6998.12	9.36
	6980.85	9.37
	6987.23	9.36
	6989.67	9.38
Mean	6989.10	9.37
SD	20.76	0.01
RSD %	0.30	0.05

2.6.7 ROBUSTNESS

A concentration of 37.5 µg/ml was prepared from a standard solution of 250 µg/ml. The flow rate was altered with ± 2.0 ml/min and wavelength with ± 2 nm. No significant effect was observed.

Table 2.12: Flow rate and wavelength parameters of prilocaine HCl

Flow Rate	Peak Area	Wavelength	Peak Area
0.8 ml/min	8565.58	218 nm	8124.00
0.9 ml/min	7763.16	219 nm	7550.84
1.0 ml/min	6992.11	220 nm	6992.11
1.1 ml/min	6371.49	221 nm	6460.26
1.2 ml/min	5823.54	222 nm	5955.22

2.7 VALIDATION PARAMETERS FOR ADRENALINE

2.7.1 LINEARITY

Linear regression analysis was performed by injecting thirteen concentrations ranging from 0.01 to 50 µg/ml into the chromatograph. Concentrations were prepared as described in § 2.3 of standard preparation. The regression value (R^2) was established to be 0.994.

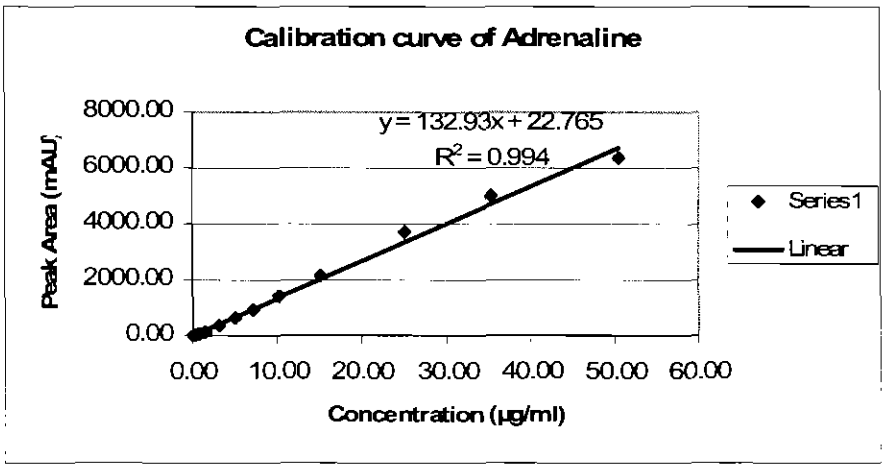

Figure 2.4: Adrenaline linearity curve of peak area versus concentration

During the HPLC assay of adrenaline, I was able to detect but not quantify adrenaline since the concentrations used are so low.

APPENDIX 2:

INTERNATIONAL JOURNAL OF PHARMACEUTICS

GUIDE FOR AUTHORS

The arrangement of full length papers should accord with the following:

(a) Title

The full title should not exceed 85 characters including spaces between words.

(b) List of Authors

Initial(s) (one given name may be used) followed by the surname of author(s) together with their affiliations. When the work has been carried out at more than one address, the affiliation of each author should be clearly indicated using superscript, lower-case letters. The author to whom correspondence should be directed must be indicated with an asterisk.

(c) Affiliation(s)

Name(s) and address(es) of the establishment(s) where the work was done, designated by superscript, lower-case letters where appropriate.

(d) Abstract

An Abstract not exceeding 200 words (a single paragraph) should be provided typed on a separate sheet.

(e) Keywords

A maximum of 6 keywords or short phrases suitable for indexing should be supplied. If possible keywords should be selected from Index Medicus or Excerpta Medica Index. Authors may also wish to refer to the Subject Index published in International Journal of Pharmaceutics, for example, Vol. 287/1-2, pp. 205-219.

(f) Corresponding Author

The author to whom correspondence should be directed should be designated with an asterisk (do not include the address unless different from that indicated by the author's affiliation). Telephone, fax and e-mail address of the corresponding author must be provided.

(g) Text

The text should be divided into main sections, such as the following: 1. Introduction. 2. Materials and methods. 3. Results. 4. Discussion. Acknowledgements. References, figure legends, tables and figures. These sections must be numbered consecutively as indicated. Subdivisions of a section should also be numbered within that section, for example, 2.1. Materials, 2.2. Relative humidity measurement, 2.3. Sample preparation, etc.

(h) Nomenclature

Standard nomenclature should be used throughout; unfamiliar or new terms and arbitrary abbreviations should be defined when first used. Unnecessary or ambiguous abbreviations and symbols are to be avoided. Data should be expressed in SI units.

(i) Figure Legends, Table Legends, Footnotes

Figure legends, tables and footnotes should be typed on separate sheets, lines double spaced. Footnotes, to be numbered consecutively in superscript throughout the text, should be used as little as possible.

References

(a) Text citation

The Harvard system of citation must be used. References should be cited in the text within parentheses: where several citations are given within a single set of parentheses, they should be arranged in ascending order of year of publication; where more than one reference with the same year of publication is cited, they should be arranged in alphabetical order of the first authors' names. When referring to a work of more than two authors, the name of the first author should be given, followed by et al.

Examples of text citations:

(Gesztes et al., 1988; Chestnut et al., 1989; Legros et al., 1990; Mhando and Li Wan Po, 1990; Korsten et al., 1991; Langerman et al., 1991, 1992a,b; Masters et al., 1991; Bonhomme et al., 1992; Kolli et al., 1992).

(Shaw et al., 1978; Nakano and Arita 1990b; Nakano et al., 1990a,b; Bone et al., 1992)

(b) Reference list

All references cited in the text should be listed at the end of the paper (typed with double spacing) and assembled alphabetically. More than one paper from the same author(s) in the same year must be identified by the letters a b c, etc. placed after the year of publication.

References must consist of names and initials of all authors, year, title of paper, abbreviated title of periodical, and volume and first and last page numbers. 'Personal communication' and 'unpublished data' should be cited in the text only. Papers referred to as 'submitted for publication' must include the name of the journal to which submission has been made. Journal titles should be abbreviated according to the 'List of Serial Title Word Abbreviations' (available from International Serials Data System, 20, rue Bachaumont, 75002 Paris, France. ISBN 2-904939-02-8).

Example of arrangement in the reference list:

Crowe, J.H., Crowe, L.M., Chapman, D., 1984a. Infrared spectroscopic studies on interactions of water and carbohydrates with a biological membrane. Arch Biochem. Biophys., 232, 400-407.

Crowe, J.H., Crowe, L.M., Hoekstra, F.A., 1989. Phase transitions and permeability changes in dry membranes during rehydration. J. Bioenerg. Biomembr., 21, 77-92.

Crowe, J.H., Crowe, L.M., Carpenter, J.F., Aurell Wistrom, C., 1987. Stabilization of dry phospholipid bilayers and proteins by sugars. Biochem. J., 242, 1-10.

Crowe, J.H., Crowe, L.M., Carpenter, J.F., Rudolph, A.S., Wistrom, C.A., Spargo, B.J., Anchordoguy, T.J., 1988. Interactions of sugars with membranes. Biochim. Biophys. Acta, 947, 367-384.

Crowe, L.M., Crowe, J.H., Womersley, C., Reid, D., Appel, L., Rudolph, A., 1986. Prevention of fusion and leakage in freeze-dried liposomes by carbohydrates. Biochim. Biophys. Acta, 861, 131-140.

Crowe, L.M., Mouradian, R., Crowe, J.H., Jackson, S.A., Womersley, C., 1984b. Effects of carbohydrates on membrane stability at low water activities. Biochim. Biophys. Acta, 769, 141-150.

Examples of presentation for various types of publication:

Langerman, L., Chaimsky, G., Golomb, E., Tverskoy, M., Kook, A.I., Benita, S., 1990. A rabbit model for evaluation of spinal anesthesia: chronic cannulation of the subarachnoid space. Anesth. Analg., 71, 529-535.

Timsina, M.P., Martin, G.P., Marriott, C., Ganderton, D., Yianneskis, M., 1994. Drug delivery to the respiratory tract using dry powder inhalers. Int. J. Pharm., 101, 1-13.

Gibaldi, M. and Perrier, D., 1982. Pharmacokinetics, 2nd Ed., Dekker, New York.

Deppeler, H.P., 1981. Hydrochlorothiazide. In: Florey, K. (Ed.), Analytical Profiles of Drug Substances, Vol. 10, Academic Press, New York, pp. 405-441.

US Pharmacopeia XXII, 1990. US Pharmacopeial Convention, Rockville, MD, pp. 1434-1435.

Mueller, L.G., 1988. Novel anti-inflammatory esters, pharmaceutical compositions and methods for reducing inflammation. UK Patent GB 2 204 869 A, 23 Nov.

Du Plessis, J., 1992. Topical liposomal delivery of biologically active peptides. Ph.D Thesis, Potchefstroom University for CHE, South Africa.

(c) Use of Digital Object Identifier (DOI)

The digital object identifier (DOI) may be used to cite and link to electronic documents. The DOI consists of a unique alpha-numeric character string which is assigned to a document by the publisher upon the initial electronic publication. The assigned DOI never changes. Therefore, it is an ideal medium for citing a document particularly "Articles in press" because they have not yet received their full bibliographic information.

The correct format for citing a DOI is shown as follows: doi:10.1016/j.ijpharm.2005.01.041

Articles in Special Issues: Please ensure that the words 'this issue' are added (in the list and text) to any references to other articles in this Special Issue.

Figures and Tables

Figures

Line drawings (including graphs) should be drawn in black ink on white paper or on tracing paper with blue or faint grey rulings; graduation will not be reproduced. Lettering should be large enough to permit photographic reduction. If figures are not to be reduced, their format should not exceed 16 x 20 cm. Photographs (or half-tone illustrations) must be of good quality, submitted as black and white prints on glossy paper, and have as much contrast as possible. The magnification of micrographs should be indicated by a scale bar in the figure. Figures should be clearly marked on the reverse side with the number, orientation (top) and author's name; a soft pencil or a felt-tipped pen should be used for marking photographs. The illustrations should be numbered with Arabic numerals. The legends should be typed separately with double spacing.

Colour illustrations should be submitted as original photographs, high-quality computer prints or transparencies, close to the size expected in publication, or as 35 mm slides. Polaroid colour prints are not suitable. If, together with your accepted article, you submit usable colour figures then Elsevier will ensure, at no additional charge, that these figures will appear in colour on the web (e.g., ScienceDirect and other sites) regardless of whether or not these illustrations are reproduced in colour in the printed version. For colour reproduction in print, you will receive information regarding the total cost from Elsevier after receipt of your accepted article. The 2008 price for color figures is EUR 285 for the first page and EUR 191 for subsequent pages. For further information on the preparation of electronic artwork, please see http://www.elsevier.com/artworkinstructions.

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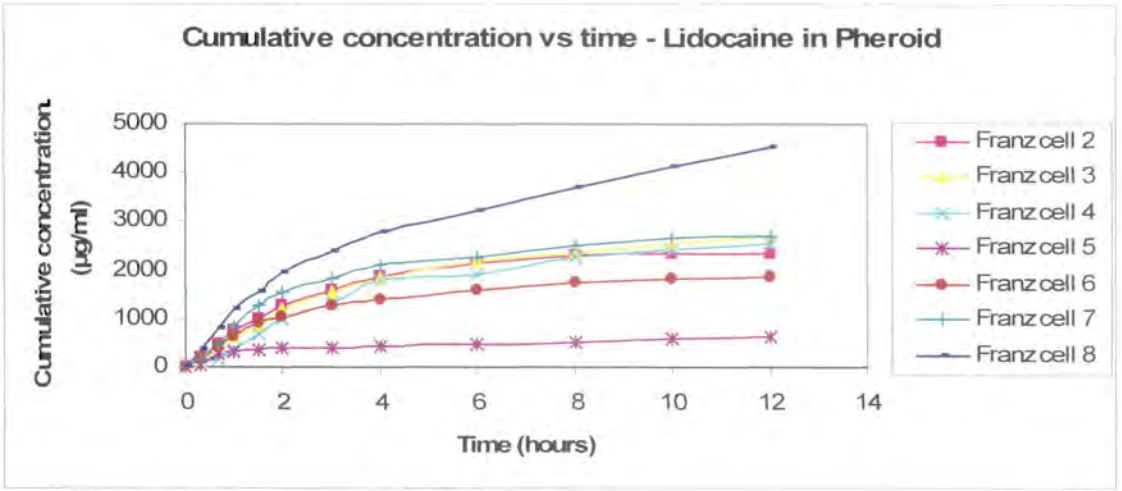
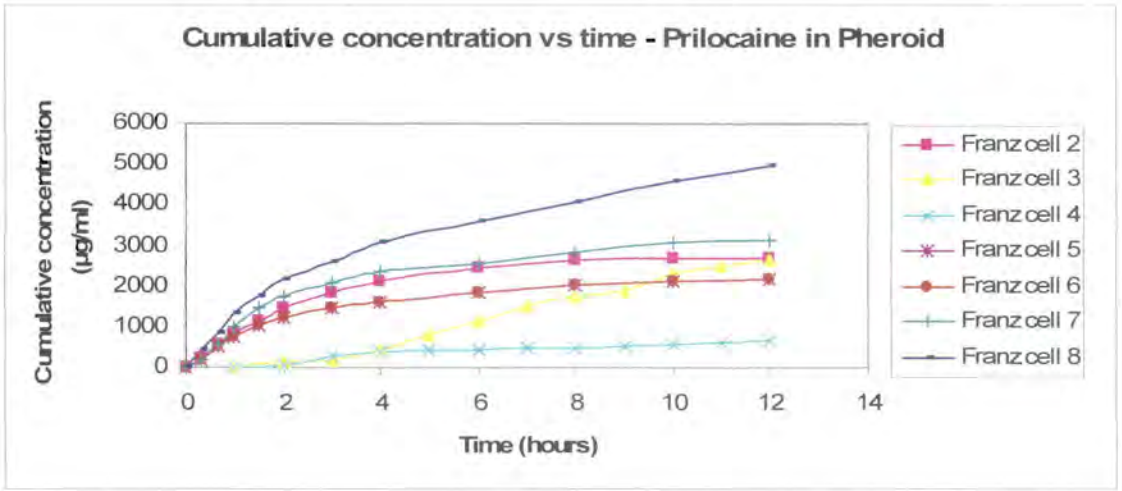
Tables

All tables must be numbered consecutively (with Arabic numerals) and be cited in the text. Titles should be short but descriptive. Tables should be compiled on separate sheets, together with a legend and/or footnotes identified by superscripts a.b.c, etc. Do not use vertical lines and keep horizontal rules to a minimum.

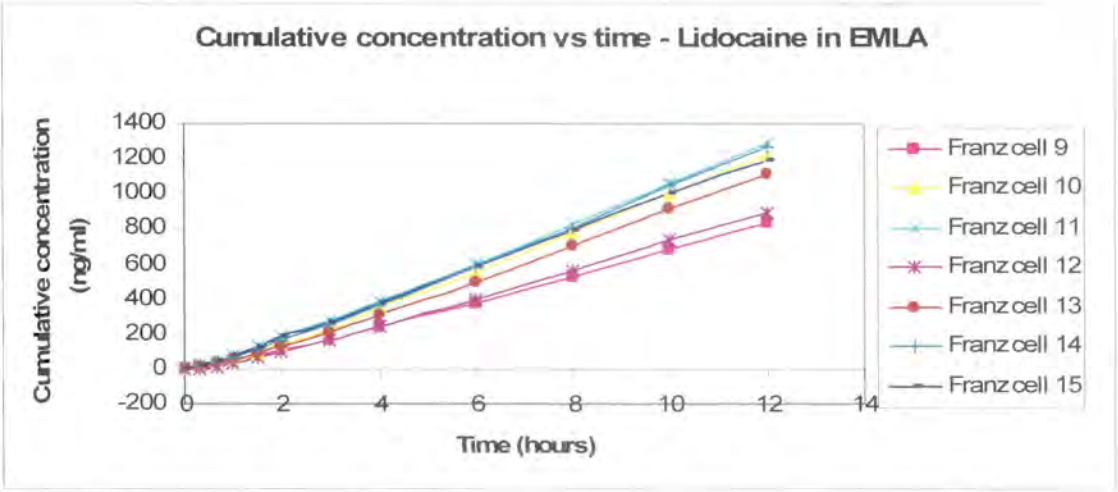
APPENDIX 3: DIFFUSION STUDY DATA

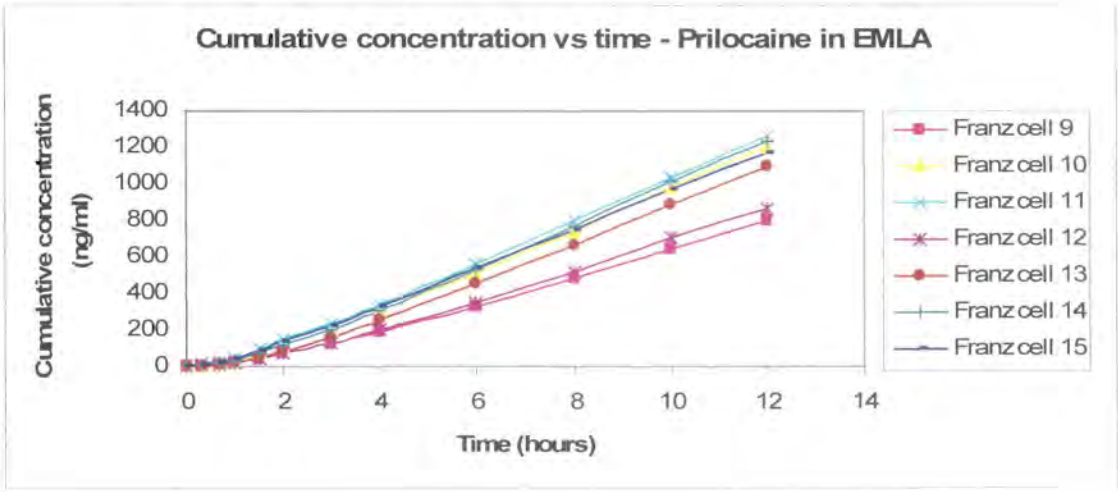
1. Permeation profiles for 7 cells containing 2.5 % lidocaine, 2.5 % prilocaine and 0.025 % adrenaline in Pheroid™:



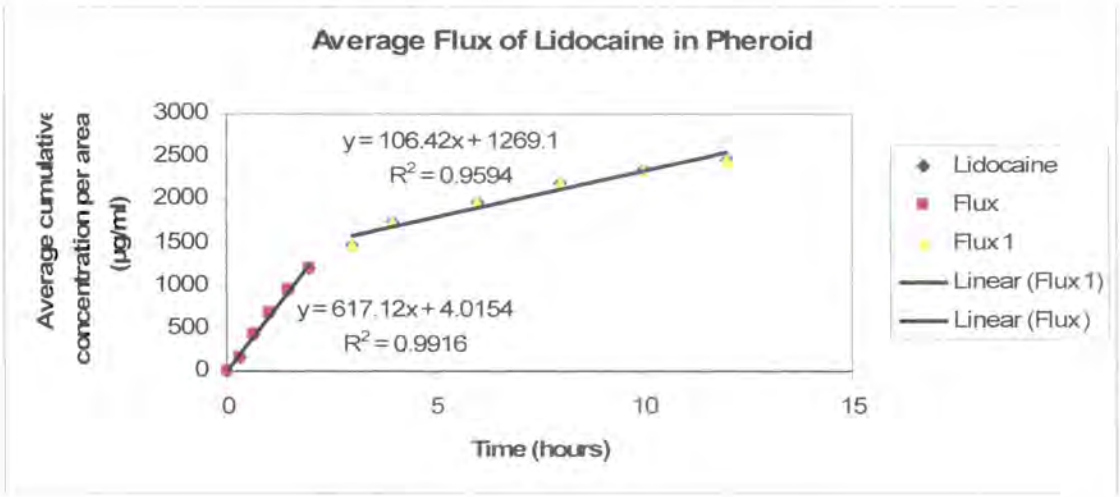


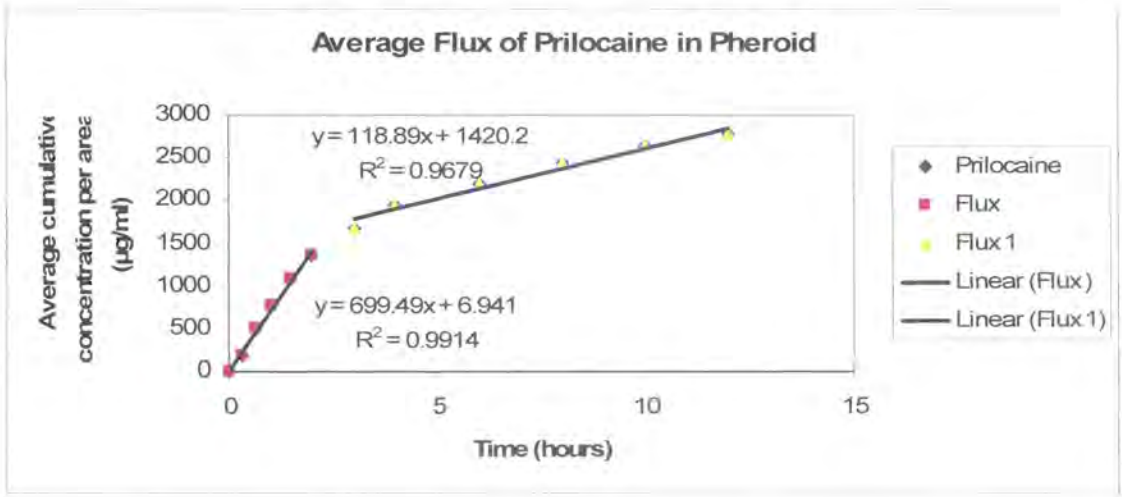
2. Permeation profiles for 7 diffusion cells containing 2.5 % lidocaine and 2.5 % prilocaine in EMLA® cream:



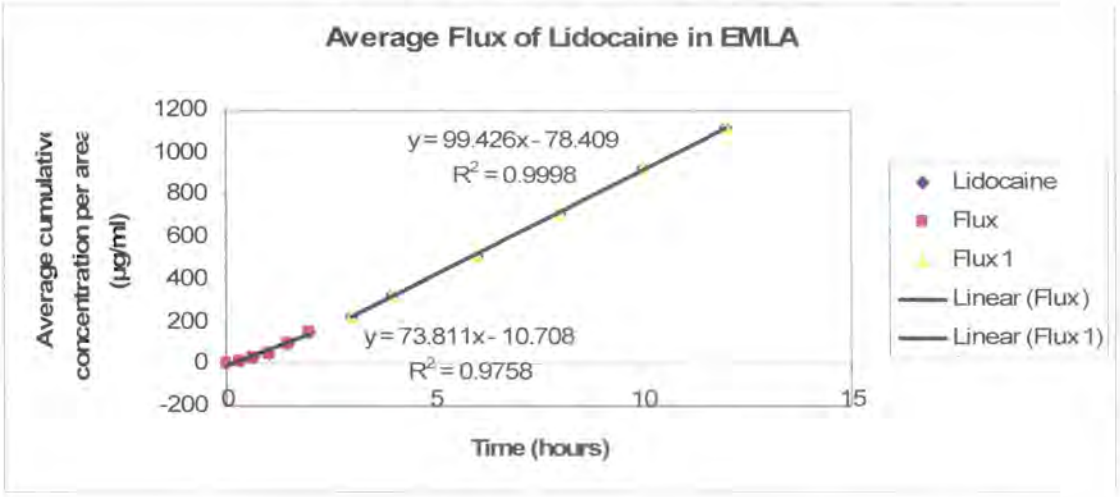


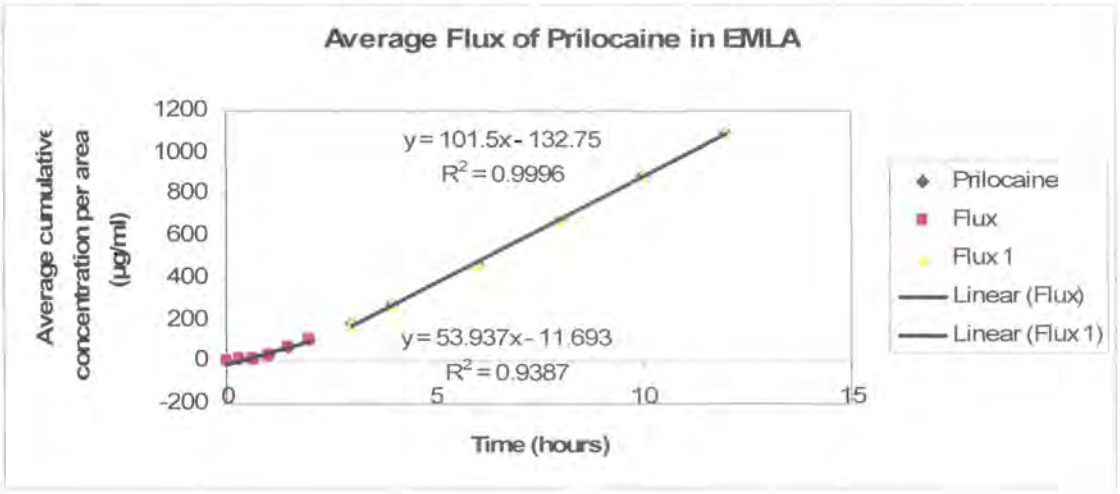
3. Average flux profiles of cumulative concentration obtained with Pheroid™:





4. Average flux profiles of cumulative concentration obtained with EMLA® cream:





APPENDIX 4: PHOTOS OF APPARATUS USED DURING VERTICAL FRANZ DIFFUSION STUDIES

Photo 1: Amber coloured vertical Franz diffusion cell with donor and receptor compartments

Photo 2: Process of withdrawal of donor compartment content

Photo 3: Milli-Q water purifying system

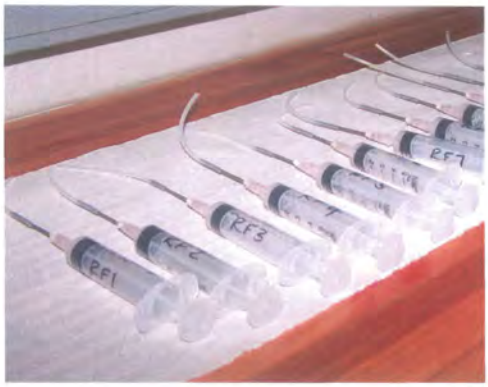


Photo 4: Syringes used to withdraw the contents of the receptor phase

Photo 5: HPLC vials used to contain samples during assay

Photo 6: Grant water bath

Photo 7: Agilent® 1100 series HPLC

Photo 8: Variomag® magnetic stirrer plate