A COMPARATIVE STUDY BETWEEN TWO LAMELLAR GEL PHASE SYSTEMS AND EMZALOIDS® AS DELIVERY VEHICLES FOR THE TRANSDERMAL DELIVERY OF 5-FLUOROURACIL AND IDOXURIDINE

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All honour to God, my saviour, without His mercy love and guidance I would never have been able to complete this study.

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ABSTRACT

The distinctive architecture of the stratum corneum with its unique nature of an interstitial lipoidal environment plays the major role in regulating the barrier function of the skin.

The major problem with the transdermal delivery of 5-fluorouracil or idoxuridine is the permeation of sufficient amounts to the deeper layers of the skin and into the systemic circulation.

In an attempt to enhance the transdermal permeability of 5-fluorouracil and idoxuridine, the aim of this study was to evaluate two lamellar gel phase systems (Physiogel Dermaquadrille® and Physiogel NT®) and Emzaloids® as transdermal delivery vehicles for the two actives. Lamellar gel phase systems (LGPS) and Emzaloids® are both novel drug delivery systems.

The epidermis of female abdominal skin was used in vertically mounted Franz diffusion cell experiments. An average amount of 250 mg of the 1% m/m LGPS was applied to cover the entire diffusion area of 1,075 cm² of the skin, which contained 2.5 mg of the active. Samples of the actives in Emzaloids® were prepared and applied in the same way.

The control solutions of the actives in water were prepared so that 1 ml of the applied solution contained the same amount of drug that was applied to the experimental cells. The entire receptor phase of the cells was removed at 2, 4, 6, 8, 10, 12 and 24 hours and was replaced with fresh 37°C receptor phase. The amount of active in the receptor phase was determined by HPLC analysis. Graphs of the cumulative amount of the active that permeated the skin over the 24 hour period were drawn and the slope of the graphs represented the flux in μg/ml/h. The average flux values of six experimental cells and six control cells were compared. Entrapment of the actives in the Emzaloid® vesicles was confirmed with the use of confocal laser scanning microscopy.
Results for the LGPS indicate an enhancement ratio in the order of 4.2 for 5-fluorouracil and 1.7 for idoxuridine when compared to the control cells. There were no viscosity changes in the LGPS samples containing 1% m/m of the active when compared with the blank LGPS samples, suggesting that no change in the internal structure of the LGPS occurred after the addition of the actives to it. There were also no significant changes in the pH of the samples.

Entrapment of the actives in the Emzaloid® vesicles occurred readily. The Emzaloid® vehicle showed a lower rate of release for idoxuridine than the LGPS did during the VanKel dissolution experiments. This suggests that higher flux values would be obtained with the LGPS for idoxuridine than with the Emzaloid® formulation, since more drug was available for permeation through the skin.

This was, however, not the case. The Emzaloid® formulation showed much higher flux values, showing that even with a smaller amount of active available to permeate the skin higher flux values were obtained.

Enhancement ratios of 20.33 and 3.50 were achieved with the Emzaloid® formulation for 5-fluorouracil and idoxuridine respectively.

The internal LGPS structure which mimics the skin's lipid components remained unchanged after the addition of the actives. Greater success might be achieved with the LGPS for different model drugs, since the drugs' physicochemical properties play an important part in its permeation through the skin.

The Emzaloid® formulation, which is closely related to liposomes and transfersomes, showed great potential for commercially marketable formulations for the drugs tested but further research on the formulation has to be done.

**Keywords**
5-fluorouracil, idoxuridine, permeation, transdermal delivery, lamellar gel phase systems, Emzaloid®
Die kenmerkende struktuur van die stratum corneum met sy unieke lipofiele aard speel die hoofrol in die regulering van die vel se funksie as skans tussen die eksterne omgewing en die liggaam.

Die belangrikste probleem met die transdermale aflewing van 5-fluoorurasiel en jodoksuridien is die permeasie van voldoende hoeveelhede in die dieperliggende lae van die vel en in die sistemiese sirkulasie.

In 'n poging om die permeasie van 5-fluoorurasiel en jodoksuridien te verhoog, was die doel van die studie om twee lamellêre jelfasestelsels (Physiogel Dermaquadrille® en Physiogel NT®) en Emzaloids® met mekaar te vergelyk en as transdermale aflewingstelsels vir 5-fluoorurasiel en jodoksuridien te evalueer. Lamellêre jelfasestelsels (LJFS) en Emzaloids® is albei nuwe aflewingstelsels.

Die epidermis van vroulike abdominale vel is in eksperimente met vertikaal gemonteerde Franz-difisieselle gebruik. 'n Gemiddeld van 250 mg van die 1% m/m LJFS, wat 2,5 mg van die aktiewe middel bevat, is op die vel aangewend om die totale diffusie-area van 1,075 cm² te bedek. Monsters van die geneesmiddel in die Emzaloid®-formulering is op dieselfde wyse aangewend.

Die kontroles van die geneesmiddels in water is so voorberei dat 1 ml van die aangewende oplossing dieselfde hoeveelheid geneesmiddel bevat as wat in die eksperimentele selle aangewend is. Die totale reseptorfase van die selle is op 2, 4, 6, 8, 10, 12 en 24 uur onttrek en met vars reseptorfase by 37 °C vervang. Die hoeveelheid geneesmiddel in die reseptormedium is met behulp van HDVC bepaal. Grafieke van die kumulatiewe hoeveelheid geneesmiddel wat oor die 24 uur deur die vel gedring het, is getrek en die hellling van die lyn gee die fluk in μg/ml/h. Die gemiddelde flukswaardes van ses eksperimentele en ses kontrole selle is met mekaar vergelyk. Opname van die geneesmiddels in die Emzaloid®-vesikels is met konfokale laserskanderingsmikroskopie bevestig.
Die resultate vir die LJFS toon 'n verbetering in die orde van 4,2 vir 5-fluoorurasiel en 1,7 vir jodoksuridien vergeleke met die kontroles. Daar was geen verandering in die viskositeit van die LJFS met 1% geneesmiddel vergeleke met dié van blanko monsters nie. Dit dui daarop dat die toevoeging van 5-fluoorurasiel of jodoksuridien geen effek op die interne struktuur van die LJFS het nie. Daar was ook geen betekenisvolle verandering in pH nie.

Opname van die geneesmiddels in die Emzaloid®-vesikels het geredelik plaasgevind. In die VanKel-dissolusie-eksperimente het die Emzaloid®-formulering 'n laer tempo van vrystelling van jodoksuridien getoon as die LJFS. 'n Hoër fluks vir die jodoksuridien in die LJFS kon dus verwag word omdat meer daarvan beskikbaar was om deur die vel te dring.

Dit was egter nie die geval nie. Selfs met minder middel beskikbaar om die vel te deurdring het die Emzaloid®-formulering 'n hoër fluks gegee.

Verbeteringsfaktore van 20,33 en 3,50 is onderskeidelik vir 5-fluoorurasiel en jodoksuridien met die Emzaloid®-formulering behaal.

Die interne struktuur van die LJFS, wat die lipiedsamestelling van die vel naboots, het na toevoeging van die geneesmiddels tot die formulerings onveranderd gebly. Omdat die geneesmiddel se fisies-chemiese eienskappe 'n belangrike rol in transdermale aflewering speel, kan groter sukses dalk met die LJFS behaal word as ander geneesmiddels as modelle gebruik word.

Die Emzaloid® wat baie soos liposome en transfersome is, toon groot potensiaal vir kommersieel bemarkbare formulerings van die getoetsde geneesmiddels, maar meer navorsing oor die formulering moet gedoen word.

**Sleutelwoorde**

5-fluoorurasiel, jodoksuridien, permeasie, transdermale aflewering, lamellère jelfasestelsels, Emzaloids®
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Introduction and Problem Statement

The skin is the outer covering of the body and encapsulates the organs from the environment. It serves as a multifunctional membrane, not only protecting the body from physical, chemical and microbial attacks, but also functions as a homeostatic barrier against outward loss of water. Thus, the skin has evolved to limit molecular transport into and out of the body (Suhonen et al., 1999).

Avoidance of first-pass metabolism by the liver, reduced side effects, improved patient compliance, longer duration of action and more uniform plasma levels are some of the main advantages of transdermal drug delivery (Pfister, 1997).

Only a handful of drugs are suitable for transdermal administration, because in order to achieve significant plasma concentrations, drug absorption must be substantial. For this to occur, the drug should preferably have a low molecular weight, be lipophilic and unionized at physiological pH (Alexander-Williams & Rowbotham, 1998).

5-Fluorouracil (5-FU), first introduced as a rationally synthesised anticancer agent 30 years ago, continues to be widely used in the management of several common malignancies including cancer of the colon, breast and skin. 5-Fluorouracil is poorly absorbed after oral administration, with erratic bioavailability. The parenteral preparation is the major dosage form, used intravenously. In addition, 5-fluorouracil continues to be used in topical preparations for the treatment of malignant skin cancers (Diasio & Harris, 1989).

Idoxuridine is an iodinated analogue of thymidine that has antiviral properties. The drug is only licensed for topical use as an antiviral. The drug is active in vitro against
adenoviruses, vaccinia virus, herpes simplex virus, varicella-zoster virus, and cytomegalovirus (Dollery, 1999).

Neither 5-fluorouracil nor idoxuridine have the ideal physicochemical properties needed to be a good candidate for transdermal delivery. They were therefore chosen to act as model drugs for the comparative evaluation of two lamellar gel phase systems and an Emzaloid® cream formulation.

The main objective of this study was:

- To evaluate the two lamellar gel phase systems and Emzaloids® on a comparative basis as potential transdermal delivery vehicles for 5-fluorouracil and idoxuridine.

In order to achieve the main objective the following aims had to be reached:

- Viscosity and rheological studies were carried out to evaluate the integrity of the lamellar gel phase systems internal structure before and after the addition of the actives to it.
- Dissolution studies were done in order to determine whether the actives were in fact released from the delivery vehicles.
- In vitro transdermal permeation studies had to be done in order to determine the amount of each active that permeated the skin from each delivery vehicle.
The Skin as Barrier to Transdermal Drug Delivery

2 Introduction

The skin is the largest organ of the human body and acts as a protective barrier with sensory and immunological functions (Foldvari, 2000:417). The skin of an average human is approximately four kilograms in weight and has a surface area of about 1.8 m² (Bronough & Collier, 1993:98).

Human skin is made up of four main layers: the stratum corneum (sc), epidermis, dermis and the subcutaneous fat layer (hypodermis). On average human skin is 0.5 mm thick but ranges in thickness from 0.05 mm - 2 mm in different parts of the body.

The skin forms a virtually impenetrable barrier to the penetration of micro-organisms and chemicals into the body. The principle barrier to penetration and transdermal drug delivery in human skin is the stratum corneum (Foldvari, 2000:417).

Transdermal drug delivery involves the application of a drug to the skin to treat systemic disease and is aimed at achieving systemically active levels of the drug (Flynn & Weiner, 1993:36). While topical delivery can be defined as the application of a drug containing formulation to the skin to directly treat cutaneous disorders or the cutaneous manifestations of general disease, with the intent of confining the pharmacological or other effect of the drug to the surface of the skin or within the skin. Regional delivery, by contrast, involves the application of a drug to the skin for the purpose of treating disease
or alleviating disease symptoms in deep tissue beneath the application site (Flynn & Weiner, 1993:35).

Advantages of transdermal drug delivery as opposed to the most popular route for systemic drug delivery, the oral route, includes the circumvention of variables such as the drastic pH changes in the gastro-intestinal tract, changes in intestinal motility and the changes in absorption and bio-availability of the drug due to food in the stomach. It also eliminates systemic first pass hepatic enzyme metabolism by the liver.

2.1 Structure of the Skin

Microscopically, the skin is a multi-layered organ composed of many histological layers. It is generally described in terms of these major multilaminate layers: the epidermis, the dermis and the subcutaneous fatty layer or the hypodermis. The epidermis is further divided into five anatomical layers with the outermost layer, the stratum corneum or the horny layer exposed to the external environment (Chien, 1987:2).

Because of its highly organized structure and hydrophobic nature the stratum corneum is widely regarded as the rate limiting factor in the penetration of therapeutic agents through the skin (Foldvari, 2000:418). For most practical purposes, removal of the stratum corneum by stripping it away with tape or other mechanical means eliminates the barrier properties of the skin and allows entry of foreign substances into the living tissue (Rieger, 1993:34).
2.1.1 The Stratum Corneum

The skin's barrier function is accomplished entirely and quite remarkably, by the highly hydrophobic outermost 10 μm to 20 μm of the skin, the stratum corneum (sc), a compositionally and morphologically unique biomembrane (Naik et al., 2000:318). This extremely thin (approximately one hundredth of a millimetre), least permeable of all the skin layers is the ultimate stage in the epidermal differentiation process, forming a laminate of compressed keratin filled corneocytes (terminally differentiated keratinocytes), anchored in a lipophilic matrix (Naik et al., 2000:318). The keratin deposited within the corneocytes provides strength and chemical resistance (Zats, 1993:12).

The stratum corneum lacks phospholipids, but is enriched in ceramides and neutral lipids like cholesterol, fatty acids and cholesterol esters that are arranged in a bilayer format and form so-called lipid channels (Foldvari, 2000:418). These lipid channels provide the only...
continuous phase and diffusion pathway from the skin's surface to the base of the stratum corneum (Naik et al., 2000:318). The ability of various agents to interact with the intercellular lipids therefore dictates the degree to which and the rate at which absorption takes place (Foldvari, 2000:418).

2.1.2 The Epidermis

The epidermis comprises of the viable epidermis and the stratum corneum (Walters, 1989:198). The viable epidermis is a layer of cells that undergo continuous differentiation to produce the stratum corneum, which is the outermost skin layer and principle barrier to penetration through the skin (Walters, 1989:198).

Ordinarily the viable tissue is not much of a diffusion impediment, and net drug passes by way of gradients through the living tissue towards the closest capillary bed, where it is taken up into systemic circulation (Flynn & Weiner, 1993:42).

2.1.3 The Dermis

Below the epidermis is the dermis or corium. Convolutions in the boundary between the epidermis and dermis with its numerous blood vessels, nerves, and lymphatics increase the area of contact between these two layers and bring the blood supply closer to the skin surface (Lund, 1994:137). The dermis provides physiological support for the epidermis and because the blood vessels approach the interface between the two layers very closely, the dermis cannot be considered as a significant barrier in vivo (Walters, 1989:198).

2.1.4 The Hypodermis

The final layer of skin, the hypodermis or subcutaneous fat layer contains adipose cells, which serves primarily as an energy source. Additionally, the tissue cushions the outer
skin layers from impact and its insulation properties contribute to the temperature regulation function of the skin (Lund, 1994:137).

2.1.5 Skin Appendages

The stratum corneum is breached by hair follicles and sweat ducts (Walters, 1989:198).

2.1.1.1 Hair Follicles

Hair follicles are sebum-filled openings from which keratinous hair filaments protrude. Follicles occupy about 0.1% of the skin surface area. They are, however, absent from plantar and palmar surfaces. Ducts into each hair follicle transport sebum secreted by one or more sebaceous glands. Collectively the follicle and gland make up a pilosebaceous unit. About 100 sebaceous glands per square centimetre is the usual level of distribution but on more hairy regions of the body they number between 400 and 900 per square centimetre (Lund, 1994:137).

2.1.1.2 Sweat Glands

Sweat glands are coiled tubules in the dermis, which open onto the skin surface; they can be subdivided in two classes; eccrine glands and the larger apocrine glands.

2.1.1.2.1 Eccrine Sweat Glands

Eccrine sweat glands are involved in the regulation of body temperature by water elimination. There are about two million eccrine sweat glands on the average human body. Sweat secreted by eccrine sweat glands varies in composition with the stimulus, the rate of sweating and the site. It is a clear watery liquid of acid pH containing electrolytes, trace elements and organic substances (Lund, 1994:137).
The acidic pH and electrolytes helps with the prevention of microbial infection of the skin.

2.1.1.2.2 Apocrine Sweat Glands

Apocrine sweat glands are larger than eccrine but fewer in number; they are mainly located in the hairier regions of the axillae and around the nipples. Apocrine sweat differs in composition from eccrine and may be cloudy and coloured (Lund, 1994:137).

2.2 Functions of the Skin

Mammalian skin is a dynamic organ with a myriad of biological functions. The most obvious is its barrier property, which is of primary relevance to percutaneous absorption. Another major function of mammalian skin is thermoregulation, since maintenance of body temperature is one of the defining characteristics distinguishing mammals from lower vertebrates. Control of water evaporation is perhaps the most important function of the skin (Riviere, 1993:113).

Other functions include excretion of wastes, receiving sensory stimuli and to separate and protect the sensitive protoplasmic jelly of the body's interior from the environment. The skin also prevents the intrusion of microbes, chemicals and various forms of radiation (Zats, 1993:12).

2.3 Routes of Transdermal Drug Delivery

At the skin surface, molecules contact cellular debris, micro-organisms, sebum and other materials, which negligibly affect permeation. The penetrant has three potential pathways to the viable tissue: through hair follicles with associated sebaceous glands, via sweat ducts or across the continuous stratum corneum between these appendages.
2.3.1 Transepidermal Route

The unbroken epidermis constitutes the larger surface for absorption and is widely regarded as the major, but not the exclusive, pathway for percutaneous absorption of many compounds.

There are two possible routes for the transepidermal absorption of drugs. The first involves a tortuous course between the cells of the stratum corneum and the second is the direct diffusion of the drug through the cells. These two pathways are respectively called the intercellular and intracellular or transcellular/transfollicular routes (Lund, 1994:138).
2.3.1.1 Intercellular Route

The intercellular spaces account for only a small proportion, up to 1%, of the stratum corneum. However, absorption by this route should be assisted by the lower resistance to diffusion in the intercellular spaces (Lund, 1994:138).

The simple “brick and mortar” model has in recent years evolved into a more complex “domain mosaic” model. In this model skin lipids are described as having domains of solid or gel-state lipids, bordered by lipids in a more fluid liquid crystalline state called a grain boundary. The fluid character of the grain boundaries represents areas where materials may diffuse in or out of the system. According to the domain mosaic model, lipids in the fluid grain boundaries can be lost through a process termed co-micellization detergency. Loss of any lipids from the grain boundaries disrupts the organization of stratum corneum lipids and leads to loss of barrier function (ISP, 2000:2).
Healthy skin requires optimal barrier function and maintenance of skin moisture for prevention of irritation and dryness. Lamellar gels, like Prolipid® 141, mimic the structure of lipids in the stratum corneum (ISP, 2000:2).

2.3.1.2 Intracellular/Transcellular Route

Many researchers now believe that the intracellular route is the dominant pathway. Their evidence supports an inverse relationship between the thickness of the stratum corneum and its permeability. Penetration rates progressively increase as layers of the stratum corneum are removed by stripping. The theory has also been supported by measurements of drug distribution in skin removed by adhesive tape stripping (Lund, 1994:138).

Figure 2-4: The grain boundaries of the “domain mosaic”. Such a grain boundary arrangement provides an effective barrier that prevents the indiscriminate loss of water, yet allows controlled evaporation to regulate temperature (ISP, 2000).
2.3.2 Transappendageal Route

The transappendageal theory proposes that the barrier afforded by the stratum corneum is circumvented and that there is relatively rapid ingress via eccrine sweat glands and hair follicles (Lund, 1994:138). The human skin surface is known to contain on average 10-70 hair follicles and 200-250 sweat ducts on every square centimetre of skin area. These appendages occupy, grossly, only 0.1% of the total human skin surface (Chien, 1987:4).

Of the two routes the eccrine sweat glands are probably the least important because increased permeability has not been demonstrated in areas where they predominate and they represent only a tiny proportion of the skin surface (Lund, 1994:138). The sebaceous glands as well as the upper sections of the hair follicles, which are filled with sebum, are potential locations for the uptake of lipiodal substances. The hair follicles extend down into the dermal region (Zats, 1993:13).

2.4 Mathematical Models

2.4.1 Fick's First Law

Fick's laws are generally viewed as the mathematical description of diffusion processes through membranes. Fick's laws are applicable whenever the chemical or physical nature of the membranes controls the rate of diffusion (Rieger, 1993:38).

Fick postulated that diffusive flow, which is the flux (J), through a membrane should be proportional to the concentration differences (Δc) between the two sides of the membrane and inversely proportional to the thickness (L) of the membrane. The proportionality constant is defined as the permeability coefficient (kp). It includes the differential diffusion coefficient (D) and the partition coefficient (K).
This relationship is known as Fick’s first law.

\[ J = kp\Delta c = \frac{KD\Delta c}{L} \]

Equation 2-1

The units of \( J \) are mole/cm\(^2\) sec, which clarifies the physical meaning: \( J \) is the quantity of solute passing through a unit area of the membrane in unit time. This is also known as flux.

In order for any measurable flux to occur, the solute molecules must first enter the stratum corneum (controlled by \( K \)). Next the entering solute must concentrate within the stratum corneum and begin its time dependant diffusion process (controlled by \( D \)) until the solute molecules reach the border between the stratum corneum and the viable epidermis (Rieger, 1993: 39).
2.4.2 Lag Time

Figure 2-5: A typical cumulative amount of a drug (µg/cm²) permeated through the skin versus time plot. The slope of the linear portion of the curve is $J$, while the x-axis intercept of the slope is the lag time ($t_{lag}$) (Roy, 1997).
2.4.3 Fick's Second Law

The homogeneous membrane model for describing the drug transport across the skin has been widely applied, since diffusion across the stratum corneum is a rate limiting step for most drugs.

If the skin contains no drug molecules prior to the application of the delivery device then drug movement in the skin, can be described by Fick's second law of diffusion as given in Equation 2.2.

\[
\frac{\partial C}{\partial t} = D \frac{\partial^2 C}{\partial x^2}
\]

Equation 2-2

where D is the diffusion coefficient in the stratum corneum and c is the concentration, \( t \) is the time and \( x \) is the distance from the surface of the skin (Tojo, 1997:115).

2.5 Physicochemical Properties Affecting Skin Permeation

The physicochemical properties of a drug substance are the most important determinants for its permeation through the skin. The molecular weight, water solubility, melting point and oil/water partition coefficient are some of the important physicochemical attributes that should be taken into account for selecting potential candidates for transdermal delivery (Roy, 1997:143).

In general, under the most ideal circumstances, only approximately 1 mg of a drug can be delivered across a 1 cm\(^2\) area of skin in 24 hours. A melting point above 150 °C and a
molecular weight higher than 500 daltons may reduce this amount of drug by 10 to 100-fold or even more (Ghosh & Pfister, 1997:8).

Considering the dosage requirements of various drugs, only less than 1 percent can be anticipated to be candidates for transdermal delivery (Ghosh & Pfister, 1997:8).

The molecular structures of the drugs that have been used in this study are given in figure 2-6 below.

![Figure 2-6: Idoxuridine (A) and 5-fluorouracil (B)](image)

The physicochemical properties of the drugs idoxuridine and 5-fluorouracil, which have been used in this study, are given in Table 2-1.
Table 2-1: Physicochemical properties of idoxuridine and 5-fluorouracil.

<table>
<thead>
<tr>
<th></th>
<th>Idoxuridine</th>
<th>5-Fluorouracil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Characteristics</td>
<td>A white or almost white, crystalline powder.</td>
<td>A white or almost white, crystalline powder.</td>
</tr>
<tr>
<td>Molecular Formula</td>
<td>C₆H₁₁IN₅O₅</td>
<td>C₄H₃FN₂O₂</td>
</tr>
<tr>
<td>Molecular Weight</td>
<td>354,1 g/mol</td>
<td>130,08 g/mol</td>
</tr>
<tr>
<td>Nominal Mass</td>
<td>354 Da</td>
<td>130 Da</td>
</tr>
<tr>
<td>Melting Point</td>
<td>Around 180 °C</td>
<td>Between 282 and 283 °C</td>
</tr>
<tr>
<td>log P</td>
<td>-0.39</td>
<td>-0.58</td>
</tr>
<tr>
<td>Solubility</td>
<td>Slightly soluble in water and alcohol, practically insoluble in ether. It dissolves in dilute solutions of alkali hydroxides.</td>
<td>Sparingly soluble in water, slightly soluble in alcohol, practically insoluble in ether.</td>
</tr>
<tr>
<td>pH</td>
<td>0.10 g in 100 ml CO₂ free water gives a pH of 5.5-6.5.</td>
<td>0.5 g in 50 ml CO₂ free water gives a pH of 4.5-5.0.</td>
</tr>
</tbody>
</table>

2.5.1 Molecular Weight And Size

Molecular weight below 500 g/mol, (5000 daltons) has no effect on percutaneous absorption. For such materials partition coefficient and lipid solubility are more important (Jackson, 1993:191).
2.5.2 Melting Point

Lipophilic compounds have fewer of the functional groups that are responsible for strong hydrogen and dipolar bonding within the crystalline state. Therefore they melt at lower temperatures and consume less energy per mole in doing so. Consequently, lipophilic compounds exhibit higher absolute solubilities in non-polar media, including the lipids of the skin (Flynn & Weiner, 1993:44). In general materials with low melting points penetrate the skin more readily (Hadgraft & Wolff, 1993:44).

The poor topical absorption of 5-fluorouracil can be attributed to its physicochemical properties. 5-Fluorouracil contains two functional groups, an amide and an imide, which results in the high crystal lattice energy and high melting point. This also accounts for the low water and lipid solubility of 5-fluorouracil (Patrick et al., 1997:40).

2.5.3 Ionization

The non-polar nature of the horny layer suggests that charged compounds should encounter high resistance to permeation through it (Zats, 1993:28).

2.5.4 Partition Coefficient

The single most important permeant characteristic influencing skin penetration is distribution into the horny layer. The horny layer or stratum corneum has for many years been identified as a non-polar membrane. Its “solvent” properties have therefore been mimicked by various non-polar liquids including ether, octanol and isopropyl myristate, usually expressed through an organic solvent (or oil)/aqueous solution partition coefficient (Zats, 1993:25). Octanol is the best of the common solvents for modelling partitioning of penetrant into the stratum corneum.
The partition coefficient can be calculated by Equation 2-3 (Pugh et al., 1996:164).

\[ \log K_{sc} = -0.024 + 0.59 \log K_{oct} \]

Equation 2-3

The magnitude of the partition of a compound between the stratum corneum and delivery vehicle is affected by the composition of the vehicle, the chemical structure of the penetrant and the charge (or distribution within) the penetrant (Wiechers, 1989:189).

2.5.5 Permeability Coefficient

The extend of the permeability is expressed in the permeability coefficient (kp), which is the product of the partition coefficient (K), and the diffusion coefficient (D), divided by the thickness of the stratum corneum (L) (Wiechers, 1989:189).

2.5.6 Diffusion Coefficient

The diffusion coefficient can be defined as the transport of matter resulting from movement of a substance within a substrate, from a high to a low concentration (Rieger, 1993:38).

The diffusion coefficient across the stratum corneum is influenced by various factors, including the molecular weight and molecular structure of the penetrant, additives and penetration enhancers (Tojo, 1997:116).
2.6 Biological Factors

2.6.1 Anatomical Site

There are significant differences in the structure and chemistry of the stratum corneum from one region of the body to another that are reflected in the drug's permeability through the skin (Gupta et al., 1997:224). Studies have indicated scrotal skin and forehead skin to be the most permeable.

2.6.2 Micro Circulation (Blood Flow)

Normal resting blood flow in human skin ranges from 3-10 ml/min/100 g (Riviere, 1993:118). The effect of blood circulation is not a major factor for controlling percutaneous absorption. However, if the drug is highly lipophilic and dissolution into the blood is very minimal, the rate of absorption into the blood circulation may become a rate-limiting step. The effect of blood flow on the rate of absorption can be described by the following boundary condition (Tojo, 1997:121).

\[
\frac{dC}{dx} = \frac{D_m k_2 C}{D(\partial k + D_m K_2)} - \frac{D_m k_2 C_0}{D(\partial k + D_m K_2)}
\]

Equation 2-4

where
- \(C_0\) is the concentration of the drug in the blood
- \(D\) is the diffusion coefficient in the stratum corneum
- \(D_m\) is the diffusion coefficient in the capillary wall
- \(K\) is the mass transfer coefficient in the blood
- \(K_1\) and \(K_2\) are the partition coefficient in the skin/capillary wall and in blood/skin respectively
- \(\delta\) is the thickness of the capillary wall (Tojo, 1997:121).
2.6.3 Skin Metabolism

The skin has the potential for both metabolism and elimination of drugs (Gupta et al., 1997:216). The stratum corneum controls the percutaneous absorption of most drugs that are stable in the skin. For drugs that undergo biotransformation, however, skin metabolism may become a rate-limiting step in percutaneous absorption. Recently bioconversion by enzymatic activity in the skin has been exploited for the transdermal delivery of prodrugs. In general, bioconversion takes place in the viable skin (Tojo, 1997:122).

The entire skin-to-liver enzyme activity ratio has been suggested to be 0.8-2.4 for different enzyme systems. However, in the case of skin the enzyme activity is distributed over a total area of approximately 2 m². Because the drug is delivered transdermally from a comparatively small area (10-100 cm²), it is expected that skin metabolism per unit area would be negligible compared to drug metabolism in the liver (Gupta et al., 1997:222).

2.6.4 Skin Age and Race

The basic skin structure is the same across races, but the morphological features and physiological responses are quite different. Race could, therefore, be a factor in the percutaneous absorption of drugs. Overall, black skin appears to be the least permeable to chemical compounds and the most resistance to allergens (Gupta et al., 1997:225).

Although the ultra structure of infant skin is indistinguishable from that of an adult, blood concentrations of topically applied drugs can be much higher. This difference is because the skin is a much larger organ, relatively in infants than in adults and because the epidermal enzymes capable of metabolising applied medicaments may not be fully developed. The skin of pre-term infants may be even more permeable as the stratum corneum is not completely formed until the end of gestation (Lund, 1994:139).
Old age can also affect permeability of the skin through changes in the elasticity, ultrastructure, chemical composition and barrier properties (Lund, 1994:140).

2.6.5 Skin Condition

Diseased skin is damaged skin. Diseased skin increases the potential for percutaneous absorption precisely because it is inflamed. Whether the skin is diseased by a transient infection or a chronic condition such as acne or psoriasis, it is more penetrable by environmental, occupational or topical product exposures than healthy, normal skin (Jackson, 1993:178).

Another form of damaged skin is skin which has been breached by an epidermal break, which may penetrate into the dermis, the subcutaneous layer or even the muscle tissue. Any cut, scratch, crack or split from excessive dryness, opens the system up to virtually instantaneous exposure through this cutaneous breach (Jackson, 1993:178).

2.6.6 Skin Hydration

Hydrated skin increases percutaneous absorption potential. The very fact that skin swells or plumps is itself a demonstration of increased water absorption which increases the partitioning of certain materials into the skin (Jackson, 1993:179).

Hydration of skin occurs through bathing, sweating, being in an area of high humidity, occlusion or application of a film forming product such a moisturizer (Jackson, 1993:179).

2.6.7 Drug Skin Binding

Chemical binding to surface stratum corneum may be lost by exfoliation and this fraction of the applied will not penetrate the skin (Riviere, 1993:113).
2.6.8 Other Factors

2.6.8.1 Temperature

In thermodynamic terms heat is the internal energy that a body possesses. High temperature means an elevated internal energy, which implies accelerated thermal movement of particles, resulting in an increased drug diffusion coefficient. The high temperature of a system also influences the drug concentration gradient by improving the slow kinetic processes of drug dissolution and by increasing drug solubility in the donor solution (Sun, 1997:329).

The skin responds to applied heat or elevated environmental temperature by dilating blood vessels and increasing blood flow, which accelerates the removal of drug in the skin, thus leading to increased drug permeation (Sun, 1997:329).

2.7 Methods Used To Enhance Skin Permeability

2.7.1 Iontophoresis

As it became clear that most drugs cannot permeate through human skin in therapeutic quantities by passive diffusion alone, and almost all peptide and protein drugs cannot permeate into the skin at all because of their large molecular size and hydrophilicity, the need for permeation techniques brought iontophoresis research to the front line (Sun, 1997: 345).

When a potential difference is applied across the skin an external force is applied to solute molecules present in the system, which facilitates their transport across the skin when compared to passive diffusion alone. The easiest external force to visualize is that of direct electrostatic repulsion. For example, if one is interested in transporting a positively charged drug across skin, one would place the anode (positive electrode) in electrical contact with a solution containing the drug. When a voltage is applied, the
positively charged drug will be propelled from the anode, through the skin and into the systemic circulation.

Negative idoxuridine ions are repelled from the cathode (negative electrode) and attracted to the anode (positive electrode) (Gangarosa & Hill, 1995:160).

It is pertinent to note that the extracellular fluid and the blood contain electrolytes (e.g. \( \text{Na}^+ \) and \( \text{Cl}^- \)) that make these fluids highly conductive. In contrast, the stratum corneum is a rather nonconductive barrier (Burnette, 1989:249).

**Figure 2-7:** Schematic of an iontophoretic device. An iontophoretic assembly principally consists of a pair of electrode chambers, which are placed in contact with the skin surface (Naik et al., 2000).
2.7.2 Penetration Enhancers

Hadgraft defined the term penetration enhancer as a substance that increases the permeability of the skin without severe irritation or damage to its structure (Boddé et al., 1989:94).

In order to increase the flux of drugs across the stratum corneum it is necessary to decrease the diffusional resistance in the structured lipids by making them more fluid. This can be achieved by the use of penetration enhancers (Hadgraft & Wolff, 1993:164).

2.7.2.1 Ideal Properties of a Penetration Enhancer

Some of the properties that an ideal penetration enhancer should have are given below.

- Elicit no pharmacological response
- Specific in its action
- Immediate action with a predictable duration, its action should be reversible
- Chemically and physically stable and compatible with formulation
- Odourless, colourless and tasteless
- Non-toxic, non-allergic and non-irritant (Boddé et al., 1989:94).

An important factor that determines the effectiveness of the barrier is the hydration of the stratum corneum. The hydration has, for example, a profound effect on the lipid fluidity within the stratum corneum, thus reducing the barrier capacity. Hence, water can be considered as an ideal, natural, non-toxic penetration enhancer (Boddé et al., 1989:94).
2.7.3 Mechanisms of action of Chemical Permeation enhancers

2.7.3.1 Lipid Action

The enhancer disrupts stratum corneum lipid organization, making it permeable. The essential action increases the drug’s diffusion coefficient. The accelerant molecules jump into the bilayer, rotating, vibrating and translocating, forming micro cavities and increasing the free volume available for drug diffusion (Barry, 2001:106).

2.7.3.2 Protein modification

Ionic surfactants, decymethylsulphoxide and DMSO (dimethyl sulphoxide) interact well with keratin in corneocytes, opening up the dense protein structure, making it more permeable. However, the intercellular route is not usually important in drug permeation, although drastic reductions to this route’s resistance could open up an alternative pathway (Barry, 2001:106).

2.7.3.3 Partitioning Promotion

Many solvents enter the stratum corneum, change its solution properties by altering the chemical environment and thus increase partitioning of a second molecule into the horny layer (Barry, 2001:106).
2.7.4 Delivery Vehicles

2.7.4.1 Liposomes

Liposomes or lipid vesicles are spherical self closed structures composed of concentric lipid bilayers that entrap part of the vehicle or active in the centre core.

![Diagram of a liposome](image)

Figure 2-8: Diagram of a liposome interacting with hydrophilic (in centre core or surface) and hydrophobic (dissolved in bilayer) molecules (Liu & Wisniewski, 1997).

They may consist of one or several membranes (i.e. unilamellar or multilamellar). The size of liposomes ranges from 20 nanometres (nm) to 100 micrometers (μm), of which the thickness of each membrane is around 4 nm. Liposomes help to dissolve and formulate water insoluble or hydrophobic ingredients, they can encapsulate water soluble or hydrophilic drugs and they enhance water retention in the skin via their bilayer
structure, resulting in improved skin elasticity and barrier function (Liu & Wisniewski, 1997:593).

Phospholipids are the major components of biological membranes and they are the most commonly used lipid to manufacture liposomes (Liu & Wisniewski, 1997:594).

2.7.4.2 Lamellar Gel Phase Systems

Lamellar gel phase systems (LGPS) or Derma membrane structures (DMS) are composed of hydrogenated phosphatidyl choline, lipids, polyol and water. Unlike liposomes, DMS has a bilayer structure in a flat sheet form. Creams made from DMS do not exhibit oil-in-water or water-in-oil characteristics (Liu & Wisniewski, 1997:598).

Figure 2-9 Under high power magnification two forms of lamellar materials are observed. The primary form of organization is extended multilamellar sheets. These have a three-dimensional organization that extends for hundreds of microns, with a bilayer thickness estimated at ten nanometers. Multilamellar spherical vesicles are also present (ISP, 2000).
Figure 2-10: The micrograph of the cross section of a spherical vesicle clearly demonstrates that lamellar bilayer organization is present throughout the vesicle. No evidence of a discrete oil phase is seen indicating that the oil phase is dispersed throughout the hydrocarbon chains of the bilayers (ISP, 2000:6).

When present in oil-in-water or in water-in-oil systems, conventional emulsifiers function by forming an interface with their hydrophobic portions in the oil and their hydrophilic portions in the water. Regardless of whether the emulsion is oil or water continuous, the system can be fundamentally described as having two phases and one interface.

In contrast, the emulsifier composition is balanced to produce a complex bilayer lamellar gel system. The bilayer gels herein advantageously stabilize emulsions by forming a discrete third phase between the oil and water phases. The result is a non-traditional system, which can be described as having three phases and two interfaces, which is fundamentally different from the traditional two phases and one interface systems.

The lamellar gel stabilization network that is formed in the skin care composition herein thus is naturally compatible with the lamellar structure of the stratum corneum lipids (Rerek et al., 1998:3).
The influence of a w/o cream and a lamellar cream on the degree of order of the skin lipid film and on skin moisture was investigated by FT-IR (Fourier transform infrared spectroscopy) measurements on the lower arm before and after cream treatment. Six hours after cream application, the cream components had penetrated the stratum corneum; this could be demonstrated by the lack of characteristic IR-bands of the applied cream on the skin print. Whereas the w/o cream reduced the degree of order of the lipids, the lamellar cream increased the degree of order as illustrated in figure 2-12 below.

Figure 2-11: The structure of a DMS cream in relation to the structure of the lipid barrier of the skin. The difference between an oil-in-water and a DMS cream is also shown (Dermocosmetics, 2001).
Figure 2-12: Degree of order of the lipid alkyl chains after use of a w/o cream and a lamellar cream on the forearm, measured as the band position of antisymmetric CH₂ stretching vibrations on 14 volunteers for the w/o cream and the lamellar cream, respectively. Error bars indicate SEM. The difference after 6 h is highly significant with $p \leq 0.01$ (Prasch et al., 2000:381).

Six hours after treatment, these opposing effects are highly significant ($p \leq 0.01$). After 12 hours, the degree of skin lipid order approaches the starting value again. This means that the lipid-rich lamellar cream increases the conformational degree of order of the alkyl chains of the lamellar lipid film in the stratum corneum, whereas the liquid oil of the w/o cream actually reduces the degree of order. The lamellar cream therefore strengthens the skin lipid film in a biomimetic manner (Prasch et al., 2000:382).
2.7.4.3 Emzaloid®

Emzaloid® is a patented system comprising of a unique submicron emulsion type formulation. An Emzaloid® is a stable structure within a novel therapeutic system that can be manipulated in terms of morphology, structure, size and function. Emzaloid® consists mainly of plant and essential fatty acids and can entrap, transport and deliver pharmacologically active compounds and other useful molecules (MZL, 2002:8).

There are various types of Emzaloid® A lipid bilayer vesicles in both the nano- and micrometer size ranges, micro-sponges and depots or reservoirs that contain pro-Emzaloids®. Each type of Emzaloid® has a specific composition (MZL, 2002:8).

Although there are many lipid based delivery systems, the Emzaloid® is unique among these in that its composition, the essential fatty acids, are manipulated in a very specific manner to ensure its high entrapment capabilities, very fast rate of transport, delivery and stability (MZL, 2002:9).
Figure 2-13: The micrographs illustrates some of the basic Emzaloid\textsuperscript{®} types (MZL, 2002).

Micrograph (A) shows a bilayer membrane vesicle containing rifampicin with a diameter of 100 nm. (B) shows a highly elastic or fluid bilayer vesicle with loose lipid packing, containing rifampicin. (C) illustrates a small pro-Emzaloid\textsuperscript{®}. (D) shows a reservoir with multiple particles of coal tar. The reservoir has a large loading capacity to surface area and is a good entrapper of insoluble compounds. General size is 1 \(\mu\)m. (E) shows the Emzaloid\textsuperscript{®} in the process of entrapping fluorescently labelled water soluble diclofenac. It is very small (diameter less than 30 nm) and the membrane packing is sponge-like. (F) depicts a depot with a hydrophobic core containing pro-Emzaloid\textsuperscript{®} formulation, a surrounding hydrophilic zone and an outer vesicle containing zone. Selective addition of fluid by a flow cell results in the release of vesicles from a release zone. The depots are
used for sustained release according to a concentration gradient and can range in size from 5 to 100 μm (MZL, 2002:9).

2.8 Summary

Transdermal drug delivery poses a great challenge. This is due to the skin's unique structure, especially that of the stratum corneum. Lamellar Gel Phase systems Physiogel NT® and Physiogel Dermaquadriile® as well as Emzaloids® will be used in a comparative study with 5-fluorouracil and idoxuridine as model drugs. This in vitro study will be conducted with the employment of Franz diffusion cells. The cumulative amount of the drug that permeated the stratum corneum into the receptor phase will be determined over a twenty four hour period, utilising HPLC analysis.
CHAPTER 3

Effects of Selected Delivery Vehicles on the Transdermal Delivery of 5-Fluorouracil and Idoxuridine

3 Introduction

The hypothesis for the use of the LGPS as a transdermal delivery vehicle lies in the fact that the internal structure it possesses mimics the lipid channels found in the skin. The LGPS and the lipids in the skin should therefore interact well with each other and so facilitate the transdermal delivery of the drugs through the lipid channels.

The Emzaloid® formulation is a novel delivery system and its mechanism of transdermal drug delivery is expected to be very similar to that of liposomes.

3.1 Methods

3.1.1 Materials

5-Fluorouracil was obtained from Fluka (Steinheim, Switzerland). Idoxuridine was purchased from Sigma-Aldrich Corporation (Johannesburg South Africa). Octane-1-sulphonic acid sodium salt was obtained from Romil Ltd (Cambridge, England). Analytical grade methanol and phosphoric acid as well as sodium chloride (NaCl), disodium orthophosphate dehydrate (Na₂PO₄·2H₂O), sodium dihydrogen orthophosphate dehydrate (NaH₂PO₄·2H₂O) and dipotassium hydrogen orthophosphate anhydrous (K₂HPO₄) were supplied by Merck Laboratory Supplies (Midrand, South Africa).
Double distilled deionised water was prepared by a Milli-Q water purification system (Millipore, Milford, USA). HPLC grade water was used throughout the study.

The lamellar gel phase systems that were used in this study were donated by Kuhs GmbH, Germany. The Emzaloids® used were donated by the Department of Pharmaceutics of the North-West University.

3.1.2 High pressure liquid chromatography (HPLC)

3.1.2.1 Apparatus

The HPLC system used for the analysis of both idoxuridine and 5-FU was an Agilent 1100 series equipped with a variable wavelength UV detector, isocratic pump, autosampler and Chemstation Rev. A.09.01 (1206) data acquisition and analysis software. All analysis for both idoxuridine and 5-FU were done using HPLC grade water and reactants. The temperature of the columns was kept at 25 °C throughout the entire analysis.

3.1.2.2 Chromatographic conditions

3.1.2.2.1 Idoxuridine

All analyses of idoxuridine were performed using a Phenomenex® Luna 5μ C_{18} (250 x 4,60 mm) column at a flow rate of 1 ml/min and a wavelength of 300 nm. The column was also fitted with a HPLC Guard Cartridge system. The mobile phase was made up by dissolving 6,96 g of K₃HPO₄ (di-potassium hydrogen orthophosphate anhydrous) in 1 litre HPLC grade water. 300 ml methanol for HPLC was added to 700 ml of the K₃HPO₄ solution to make 1 litre of the mobile phase. The pH was adjusted to 7,4 with 85% orthophosphoric acid. The mobile phase was then filtered through a 0,45 μm HV
filter (Millipore, Milford USA) with the aid of a BUCHI (model B-169, Switzerland) vacuum system to remove any solid particles and gasses from the mobile phase.

The receptor phase for idoxuridine in the receptor compartment of the Franz diffusion cell was phosphate buffered saline (PBS) at physiological pH, consisting of 4,4 g sodium chloride (NaCl), 9,2 g disodium orthophosphate dehydrate (Na2PO4.2H2O) and 2,1 g sodium dihydrogen orthophosphate dehydrate (NaH2PO4.2H2O) in water to 1000 ml.

Known standard concentrations of idoxuridine were made up with PBS.

The following chromatograms show the peaks obtained for known concentrations of idoxuridine in PBS.

Figure 3-1: The idoxuridine peak (A) was obtained with a 0,05 µg/ml standard solution of idoxuridine in phosphate buffered saline (PBS). The AUC was 4,89 mAU and the retention time was 5,21 min.
Figure 3-2: The idoxuridine peak (A) was obtained with a 10 µg/ml standard solution of idoxuridine in PBS. The AUC was 1051.63 mAU and the retention time was 5.27 min.

3.1.2.2.2 5-Fluorouracil

All analyses of 5-FU were performed using a Phenomenex® Synergi 4µ Hydro-RP 80A (250 x 4.60 mm) column at a flow rate of 1 ml/min and at a wavelength of 266 nm. The column was also fitted with a HPLC Guard Cartridge system. The mobile phase was made up by dissolving 1 g sodium 1-octanesulfonate monohydrate (C₈H₁₇NaO₃S.H₂O) in 970 ml HPLC grade water. 30 ml acetonitrile for HPLC was added to the 970 ml solution and made up to 1000 ml with HPLC grade water. The pH was adjusted to 3.5 with 85% orthophosphoric acid. The mobile phase was then filtered through a 0.45 µm HV filter (Millipore, Milford USA) with the aid of a BUCHI (model B-169, Switzerland) vacuum system to degas and to remove any solid particles from the mobile phase.

The following chromatograms shows the peaks obtained for 5-FU when known concentrations were dissolved in PBS solution. The pH of the PBS solution had to be lowered to 3.5 with 85% orthophosphoric acid because at a pH of 7.4 the PBS solution components had retention times very close to that of 5-FU and definite identification of the 5-FU peak between the PBS component peaks was not possible.
Figure 3-3: The 5-fluorouracil peak (A) was obtained with a 0,1 µg/ml standard solution of 5-FU in PBS with a pH of 3,5. The AUC was 30,35 mAU and the retention time was 5,00 min. Peaks B and C were from components in the PBS with AUC of 130,13 and 154,71 mAU, respectively. Retention times for peak B was 12,67 min and for peak C 33,27 min.

Figure 3-4: The 5-fluorouracil peak (A) was obtained with a 10 µg/ml standard solution of 5-FU in PBS with a pH of 3,5. The AUC for peaks A, B and C were 3216,14; 122,97 and 142,06 mAU respectively and the retention times for peaks A, B and C were 5,01; 12,70 and 33,11 min respectively.
The phosphate buffered saline was broken up into its comprising components and analysed on the HPLC to give peaks B and C.

Figure 3-5: The sodium chloride (NaCl) component of the PBS. Peaks B and C had AUCs of 142.88 and 121.27 mAU and retention times of 11.99 and 31.50 min, respectively.

Figure 3-6: The disodium hydrogen orthophosphate dehydrate component of the PBS. Peak B had an AUC of 201.69 mAU and a retention time of 14.94 min.
Figure 3-7: The sodium dihydrogen orthophosphate dehydrate component of the PBS. Peak B had an AUC of 88.62 mAUC and that of C was 222.45 mAU, the retention times for Peaks B and C were 12.28 and 32.91 min, respectively.

Due to the very long retention times of peaks B and C after the adjusting of the pH of the PBS from 7.4 to 3.5 to give better peak definition, it was decided not to use the PBS as a receptor phase in the Franz diffusion studies for 5-fluorouracil.

If the phosphate buffered saline were to be used and the analysis time per injection were shortened, since a stop time of 35 minutes would be unacceptable, one would get carry over from the first injection to the third or fourth injection in a sample sequence. Thus the possibility of peaks B and C overlapping the peak of 5-FU and giving a cumulative AUC during analysis of the experimental samples especially at low concentrations of 5-FU was a big concern.

It was thus decided to use HPLC grade water as receptor medium in the Franz diffusion cell studies for 5-fluorouracil. The peaks from standard solutions of 5-FU in HPLC grade water are given below.
Figure 3-8: The 5-fluorouracil peak (A) was obtained with a 0.1 μg/ml standard solution of 5-FU in HPLC grade water. The AUC was 28.86 mAU and the retention time was 4.99 min.

Figure 3-9: The 5-fluorouracil peak (A) was obtained with a 10 μg/ml standard solution of 5-FU in HPLC grade water. The AUC was 3082.15 mAU and the retention time was 5.04 min.
3.1.2.3 Column maintenance

After each analysis HPLC grade water was passed through the columns for 30 min at a flow rate of 1 ml/min, it was then rinsed with a mixture of 70% acetonitrile in HPLC grade water for at least 30 min at a flow rate of 1 ml/min. This was done to prolong column life and to remove any impurities from the column and HPLC system. HPLC grade water was then again rinsed through the columns for 30 min at a flow rate of 1 ml/min. It was then rinsed for 30 minutes with the storage solution comprising of a 50% methanol/water for HPLC mixture.

3.1.2.4 Preparation of standard solutions

3.1.2.4.1 Idoxuridine

Ten milligrams of idoxuridine was weighed and transferred to a 100 ml volumetric flask and made up to volume with PBS with a pH of 7.4 to produce a 100 μg/ml stock solution. Dilutions with concentrations of 0.05; 0.1; 0.5; 1; 5 and 10 μg/ml were made from the 100 μg/ml stock solution. These dilutions were used for the validation of the HPLC procedure for idoxuridine.

3.1.2.4.2 5-Fluorouracil

Ten milligrams of 5-FU was weighed and transferred to a 100 ml volumetric flask and made up to volume with HPLC grade water to give a 100 μg/ml stock solution. Dilutions with concentrations of 0.05; 0.1; 0.5; 1; 5; and 10 μg/ml were made from the 100 μg/ml stock solution. These dilutions were used for the validation of the HPLC procedure for 5-fluorouracil.
3.1.3 Validation of the HPLC Method

3.1.3.1 Idoxuridine

3.1.3.1.1 Linearity

The linearity for idoxuridine was determined by performing linear regression analysis on the plot of the peak AUC versus concentration. Five standard solutions were prepared as described in § 3.2.2.4, to obtain concentrations ranging from 0.05 to 10 µg/ml. The regression value ($r^2$) was greater than 0.99998 and the Y-intercept was 0.66301.

3.1.3.1.2 Precision

- **Intra-day precision**

  Precision (repeatability) was determined by performing HPLC analyses ($n = 3$) of a low, medium and a high concentration (0.05 µg/ml, 0.5 µg/ml and 10 µg/ml) of idoxuridine on the same day. The intra-day precision complied with pharmaceutical standards (see Table 3-1).

- **Inter-day precision**

  The inter-day precision was determined by performing HPLC analyses ($n = 3$) of a low, medium and a high concentration (0.05 µg/ml, 0.5 µg/ml and 10 µg/ml) of idoxuridine on three consecutive days. The intra-day precision complied with pharmaceutical standards (see Table 3-2).
Table 3-1: The mean, percentage (%) of idoxuridine recovered, standard deviation (SD) and percentage relative standard deviation (%RSD) for idoxuridine by analyzing three sets of samples on the same day.

<table>
<thead>
<tr>
<th>Idoxuridine concentrations (µg/ml)</th>
<th>Mean % recovered</th>
<th>Standard deviation</th>
<th>%RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>0,1</td>
<td>101,34</td>
<td>0,07</td>
<td>0,71</td>
</tr>
<tr>
<td>0,5</td>
<td>99,46</td>
<td>0,43</td>
<td>0,83</td>
</tr>
<tr>
<td>10,0</td>
<td>99,91</td>
<td>0,71</td>
<td>0,07</td>
</tr>
</tbody>
</table>

Table 3-2: The mean, percentage (%) of idoxuridine recovered, standard deviation (SD) and percentage relative standard deviation (%RSD) for idoxuridine by analyzing three sets of samples on three consecutive days.

<table>
<thead>
<tr>
<th>Idoxuridine concentrations (µg/ml)</th>
<th>Mean % recovered</th>
<th>Standard deviation</th>
<th>%RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>0,1</td>
<td>97,10</td>
<td>0,05</td>
<td>1,90</td>
</tr>
<tr>
<td>0,5</td>
<td>100,20</td>
<td>0,11</td>
<td>0,24</td>
</tr>
<tr>
<td>10,0</td>
<td>102,16</td>
<td>0,67</td>
<td>0,07</td>
</tr>
</tbody>
</table>

3.1.3.1.3 Sensitivity

The sensitivity of an analytical method can be measured by determining the limit of quantification and limit of detection. The limit of quantification is defined as the lowest concentration of an analyte in a sample that can be quantitatively determined with acceptable precision and accuracy (% RSD < 15%). The limit of detection on the other hand, is defined as the lowest concentration that of an analyte in a sample that can be detected, but not necessarily quantified as an exact value. The limit of quantification for the idoxuridine studied was 0,0742 µg/ml and the limit of detection was 0,0245 µg/ml.
3.1.3.1.4 Selectivity

Selectivity is the ability to detect and analyse a specific compound in the presence of other compounds. The components of the (PBS) pH 7,4 and the mobile phase were separately analysed by HPLC. This method was selective since there were no interfering peaks with the same retention time as idoxuridine. There was also no interference from the components of the LGPS or Emzaloid® formulation that permeated the skin with the peak of idoxuridine with a retention time of ± 5.27 min.

3.1.3.1.5 System repeatability

In order to evaluate the repeatability of the peak area and the retention time, samples of idoxuridine with known concentrations (0.8 µg/ml) were injected six times. The variation in the response (% RSD) of the detection system when six determinations of idoxuridine were made on the same day, and under the same conditions, was found to be 1.49% for the peak area and 0.37% for the retention time. The system repeatability for idoxuridine was well within acceptable criteria.

3.1.3.2 5-Fluorouracil

3.1.3.2.1 Linearity

The linearity for 5-fluorouracil was determined by performing linear regression analysis on the plot of the peak AUC versus concentration. Five standard solutions were prepared as described in § 3.2.2.4, to obtain concentrations ranging from 0.05 to 10 µg/ml. The regression value ($r^2$) was greater than 0.999997 and the Y-intercept was 0.392975.
3.1.3.2.2 Precision

- Intra-day precision

Precision (repeatability) was determined by performing HPLC analyses (n = 3) of a low, medium and a high concentration (0.05 µg/ml, 0.5 µg/ml and 10 µg/ml) of 5-fluorouracil on the same day. The intra-day precision complied with pharmaceutical standards (see Table 3-3).

- Inter-day precision

The inter-day precision was determined by performing HPLC analyses (n = 3) of a low, medium and a high concentration (0.05 µg/ml, 0.5 µg/ml and 10 µg/ml) of 5-fluorouracil on three consecutive days. The inter-day precision complied with pharmaceutical standards (see Table 3-4).

Table 3-3: The mean, percentage (%) recovered, standard deviation (SD) and percentage relative standard deviation (%RSD) for 5-fluorouracil by analyzing three sets of samples on the same day.

<table>
<thead>
<tr>
<th>5-Fluorouracil concentrations (µg/ml)</th>
<th>Mean % recovered</th>
<th>Standard deviation</th>
<th>%RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.02</td>
<td>98.33</td>
<td>0.03</td>
<td>0.42</td>
</tr>
<tr>
<td>0.10</td>
<td>99.11</td>
<td>0.03</td>
<td>0.10</td>
</tr>
<tr>
<td>1.00</td>
<td>100.19</td>
<td>0.09</td>
<td>0.03</td>
</tr>
</tbody>
</table>
Table 3-4: The mean, percentage (%) of 5-fluorouracil recovered, standard deviation (SD) and percentage relative standard deviation (%RSD) for 5-fluorouracil by analyzing three sets of samples on three consecutive days.

<table>
<thead>
<tr>
<th>5-Fluorouracil concentrations (μg/ml)</th>
<th>Mean % recovered</th>
<th>Standard deviation</th>
<th>%RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.02</td>
<td>98.42</td>
<td>0.03</td>
<td>0.46</td>
</tr>
<tr>
<td>0.10</td>
<td>99.42</td>
<td>0.08</td>
<td>0.25</td>
</tr>
<tr>
<td>1.00</td>
<td>100.19</td>
<td>0.10</td>
<td>0.03</td>
</tr>
</tbody>
</table>

3.1.3.2.3 Sensitivity

The sensitivity of an analytical method can be measured by determining the limit of quantification and limit of detection. The limit of quantification is defined as the lowest concentration of an analyte in a sample that can be quantitatively determined with acceptable precision and accuracy (%RSD < 15%). The limit of detection on the other hand, is defined as the lowest concentration that of an analyte in a sample that can be detected, but not necessarily quantified as an exact value. The limit of quantification for the 5-fluorouracil studied was 0.0163 μg/ml and the limit of detection of the 5-fluorouracil studied was 0.0054 μg/ml.

3.1.3.2.4 Selectivity

Selectivity is the ability to detect and analyse a specific compound in the presence of other compounds. The phosphate buffer solution (PBS) was not used as the receptor phase for 5-fluorouracil for reasons stated in 3.2.2.2.2. The components of the mobile phase was also separately analysed by HPLC. This method was selective since there were no interfering peaks with the same retention time as 5-fluorouracil. There was also
no interference from the components of the LGPS or Emzaloid® formulations that permeated the skin with the peak of 5-fluorouracil with a retention time of ± 5.00 min.

3.1.3.2.5 System repeatability

In order to evaluate the repeatability of the peak area and the retention time, samples of 5-fluorouracil with known concentrations (0.03 μg/ml) were injected six times. The variation in the response (%RSD) of the detection system when six determinations of 5-fluorouracil were made on the same day, and under the same conditions, was found to be 1.73% for the peak area and 0.02% for the retention time. The system repeatability for 5-fluorouracil, was well within acceptable criteria.

3.1.4 Preparation of the experimental samples

3.1.4.1 Drug containing LGPS samples

The LGPS were used as received; it was stored at 25 °C for the entire duration of the study. A sufficient amount of idoxuridine was weighed and mixed in with each LGPS to obtain a 1% strength idoxuridine sample of each LGPS. The incorporation of the idoxuridine into the LGPS was done by means of manual stirring with a stainless steel spatula on a glass plate. Each LGPS drug sample was prepared one week before it was used for the transdermal diffusion studies. The LGPS samples were stirred daily to ensure homogeneous distribution of the drug throughout the whole prepared sample. The same method was used to prepare the 5-fluorouracil containing LGPS samples.
3.1.4.2 Drug containing Emzaloid® samples

A 1% (m/m) cream Emzaloid® formulation was made for each of the actives.

The 5-fluorouracil and the idoxuridine were entrapped in the Emzaloid® formulations during the production process of the Emzaloids®. This is in contrast to the LGPS drug containing samples, where the actives were added to the already finished lamellar gel phase systems.

The drug containing Emzaloid® samples were viewed with the use of confocal laser scanning microscopy (CLSM). The use of a laser as an energy source enables the microscope to act as an optical knife that can optically section a sample at varying depths. Due to the addition of a Hamamadzu CCD camera, dynamic changes can also be visualised in real time. The confocal microscope system used was a Nikon PCM 2000 equipped with a Krypton/Argon and Helium/Neon lasers. A 60x Plan Achromat oil immersion objective with a numerical aperture of 1.4 was used to confirm that the actives were indeed entrapped inside the Emzaloid® vesicles. This is achieved by labelling the sample with a fluorescent marker, a molecule that enters an excited state during laser exposure (excitation) and emits photons at a specific wavelength (emission) (Saunders et al., 1999:2).

In the case of 5-fluorouracil and idoxuridine the addition of a fluorescent marker was not necessary, since both actives were spontaneously fluorescent during laser interaction. Nile red was chosen as the fluorophore as it predominantly associates with lipids, and therefore readily stains the fatty acid components of the formulations.
Figure 3-10: 5-Fluorouracil in a cream formulation of the Emzaloids®. Micrograph (A) shows the active (green) inside and around the Emzaloid® vesicle (red). The black space around the vesicle is the water phase. Micrograph (B) shows the association of the active with the Emzaloid® vesicles in greater detail. The white bar in (A) represents 3,05 μm and in (B) 5,08 μm.

The yellow areas on the micrographs are formed from the overlapping of the red and green light. It is especially visible where the actives are in the core of the Emzaloid® vesicle.
Figure 3-11: Idoxuridine in a cream formulation of the Emzaloids®. Micrograph (A) shows the active (green) inside and around the Emzaloid® vesicle (red). The black space around the vesicle is the water phase. Micrograph (B) shows the association of the active with the Emzaloid® vesicles in greater detail. The white bar in (A) represents 5.02 μm and in (B) 5.08 μm.

Once again as was the case with the 5-fluorouracil the yellow areas on the micrographs are formed from the overlapping of the red and green light. In micrograph (A) the close association of the active with the Emzaloid® vesicles is clearly visible.
3.1.5 Viscosity and Rheology

Rheology is the science of the flow of matter and its study begins with gathering of data on a fluid’s viscosity – its resistance to flow caused by its internal friction. Knowledge of a material’s rheological characteristics is valuable to predict its pourability, its performance in dipping or coating operation, or the ease with which it may be handled, processed or used. Viscosity data provides an accurate reference point in the formulation of materials, facilitating the achievement of consistency from batch to batch (Brookfield, 1998:2).

Viscosity measurements were done in order to determine whether the internal structure of the LGPS which mimic the lipids found in the skin, remained intact and unchanged after the addition of the actives. Rheological graphs were drawn to evaluate the change in flow characteristics and viscosity of the samples before and after the addition of the actives.

The viscosity of the LGPS samples was determined with the use of a Brookfield Model DV-II+ Viscometer (see figure 3-12) and a Brookfield small sample adapter. The Brookfield small sample adapter was used for its rheological evaluation of materials where sample volume is limited (see figure 3-13). The flow jacket allows for accurate temperature control and simultaneous sample temperature measurement (Brookfield, 1998:22).
Figure 3-12: The small sample adapter is shown here mounted on a Brookfield DV-II+ Viscometer, with a Brookfield Model TC-200 constant temperature bath (Brookfield, 1998:2).

Figure 3-13: Easily removable sample chamber is insulated for safe handling (Brookfield, 1998:2).
The viscosimeter was calibrated every three months by the operator. Spindle number SC4-34 was used for the Physiogel Dermaquadrille® and spindle number SC4-25 for the Physiogel NT®. The samples were transferred to the sample chamber and left to stand for twelve hours to assure that no air bubbles were trapped in the sample. The sample chamber was then placed in the water jacket and left to attain the desired temperature. All measurements were done at 25 °C.

At every minute time interval viscosity, shear stress, shear rate, torque and temperature were measured at a specific revolution per minute (rpm), of the rotating spindle in the sample, which was then increased after each minute interval. Graphs of the viscosity in centipoise versus rpm of the samples containing actives and those without were drawn as well as graphs of shear stress in dynes per square centimetre (dynes/cm²) versus shear rate, its unit of measure is called the reciprocal second (sec⁻¹), were also drawn for the samples and evaluated for changes before and after the addition of the actives to it.

3.1.6 Dissolution from Delivery Vehicles

Dissolution testing was done in order to determine whether 5-fluorouracil and idoxuridine were released from the lamellar gel phase system and Emzaloid® formulations. The apparatus used was a USP six spindle dissolution tester (VanKel 700) modified by the addition of the enhancer cell (VanKel Industries, Edison, NJ).

The enhancer cell consisted of a cap, a washer, a membrane (cellulose-acetate membrane, 0.45 μm pore size and 30 mm diameter), a ring and a body which is the reservoir for the sample to be tested (Figure 3-14).
A sample of the drug containing LGPS was placed in each of the six enhancer cells. Excess LGPS was removed with a stainless steel spatula. Membranes were placed on top of the cells containing the product. The washer was placed over the membrane, and the cells were capped and placed inside the dissolution vessel containing 190 ml of PBS for idoxuridine and 190 ml of HPLC grade water for 5-fluorouracil. The dissolution paddles rotated at a speed of 150 rpm and were adjusted to remain 1 cm from the top of the cells throughout the experiment of six hours. Every hour 500 µl were withdrawn from all six dissolution vessels and transferred to HPLC vials for HPLC analysis.

The corrected amount of drug released was calculated, with the use of standard curve drawn up from standard solutions, prior to the start of the analysis of the dissolution samples.

The results were plotted as the corrected amount of drug released per square centimetre (µg/cm²) versus the squared time in minutes. This was done for Physiogel Dermaquadrille®, Physiogel NT® and Emzaloid® samples containing 1% (m/m) 5-fluorouracil. The same was done for all three delivery vehicles containing 1% (m/m)
idoxuridine. The graphs obtained from the data gave a straight line and this was used to compare the release of the two drugs from the three delivery vehicles. The temperature of the receptor phase was kept at a constant 32°C throughout the course of the experiment.

3.1.7 pH Measurements

The pH of the LGPS and Emzaloid® formulations were measured before and after the addition of the actives using the Metrohm Autotitrator 785 DMP Titrino pH meter (figure 3-15). This was done to evaluate the integrity of the internal structure of the LGPS after the addition of the actives. The pH measurements obtained was then also use to determine the state of ionization of the actives at the formulations specific pH.

![Figure 3-15: Metrohm Autotitrator 785 DMP Titrino pH meter](image)
3.1.8  *In vitro* Transdermal Permeation Studies

3.1.8.1  Preparation of skin

Only female abdominal skin was used, obtained from cosmetic surgery, in order to minimize the variability in the skin permeability properties between different anatomical sites (which have varying thicknesses of stratum corneum and follicle densities). The full thickness skin was frozen at -20 °C not longer than 24 h after removal. Excess fatty and connective tissue were removed after which the skin was immersed in 60 °C water for 1 minute. The epidermis was then carefully removed and placed on Whatman® filter paper and left to dry. The prepared skin were wrapped in aluminium foil and sealed in airtight plastic bags. The prepared skin was stored at -20 °C for a maximum of two months before use. The frozen prepared skin pieces were visually examined for defects before mounting them in the diffusion apparatus.

3.1.8.2  Diffusion studies

Vertically mounted Franz diffusion cells with a donor capacity of 1 ml and receptor capacities varying from 1.9 to 2.3 ml were used (see figure 3-16). The epidermis were cut into circles with a diameter of about 2 cm and placed in between the donor and receptor compartments with the stratum corneum side facing the donor compartment side. The effective diffusion area is 1,0751 cm². One hour prior to the application of the drug containing delivery vehicle the receptor phase was placed into the receptor compartment and left to rehydrate the epidermis. Care was taken to ensure that no air bubbles were trapped in the compartment or under the skin as this would have decreased the effective diffusion area. The content of the receptor medium was continuously stirred with a small magnetic bar at 500 rpm and the temperature maintained at 37 °C by means of a water bath. This kept the skin surface at approximately 32 °C, which simulates the temperature of human skin.
The receptor phase for the diffusion studies with 5-fluorouracil was HPLC grade water with a pH of 7 and for the idoxuridine it was phosphate buffered saline (PBS) at physiological pH, consisting of 4.4 g sodium chloride (NaCl), 9.2 g disodium orthophosphate dehydrate (Na₂PO₄·2H₂O) and 2.1 g sodium dihydrogen orthophosphate dehydrate (NaH₂PO₄·2H₂O) in water to 1000 ml.

![Diagram of diffusion cell](image)

**Figure 3-16:** Schematic representation of the standard original diffusion cell developed by Franz (Bronaugh & Collier, 1993).

Just enough of the drug containing delivery vehicle was placed on to the skin in the donor compartment in order to cover the entire diffusion area of 1,0751 cm². On average 0.24 g of the delivery vehicle was used to accomplish this. 0.24 g of the delivery vehicle contained 0.0024 g (2.4 mg) of the active. 0.06 g (60 mg) of the active was then weighed and dissolved in 25 ml of HPLC grade water. One millilitre of this solution was then placed in the donor compartment of the control cells. One millilitre contained 0.0024 g (2.4 mg) of the active. The donor compartment was covered with Parafilm® in order to prevent evaporation after the donor vehicles were applied to the skin.
3.1.8.3 Sample collection

The entire contents of the receptor compartment from each diffusion cell was withdrawn at 2, 4, 6, 8, 10, 12, and 24 hours and immediately replaced with fresh 37 °C receptor medium in order to maintain sink conditions. The amount of active that penetrated the skin was recovered in the receptor compartment and analyzed by HPLC.

3.1.8.4 Calibration curves

The concentration of the active retrieved from the receptor compartment was determined by comparison to a calibration curve that was drawn up from standard solutions for each of the actives, with concentrations ranging from 0.05 μg/ml - 10 μg/ml as described in § 3.1.2.4 prior to each experiment.

3.2 Results

3.2.1 Viscosity and Rheology

No viscosity measurements were done for the Emzaloid® formulations since there was no internal structure that had to remain unchanged after the addition of the actives, as was the case for the LGPS formulations.

The results for the LGPS viscosity measurements are given below. The control represents the LGPS viscosity before the addition of the actives to it as described in § 3.2.4.1.
Figure 3-17: Viscosity plotted against RPM for the control which is the Physiogel Dermaquadrille® before the addition of actives to it. 5-Fluorouracil represents the Physiogel Dermaquadrille® after the addition of 1% (ml/m) 5-Fluorouracil to it. Idoxuridine shows the viscosity of Physiogel Dermaquadrille® after the addition of 1% (m/m) idoxuridine to it.

When the shear rate versus the shear stress is plotted against each other the resulting graph shows the type of rheological flow characteristic of the sample.

A graph of the shear rate versus the shear stress was plotted and is given (see Figure 3-18). From this graph it can be seen that Physiogel Dermaquadrille® shows pseudo-plastic flow characteristics.
Figure 3-18: Shear stress plotted against shear rate for the control which is the Physiogel Dermaquadrille® before the addition of actives to it. 5-Fluorouracil represents the Physiogel Dermaquadrille® after the addition of 1% (m/m) 5-fluorouracil to it. Idoxuridine shows the viscosity of Physiogel Dermaquadrille® after the addition of 1% (m/m) idoxuridine to it.
Figure 3-19: Viscosity plotted against rpm for the control which is the Physiogel NT® before the addition of actives to it. 5-Fluorouracil represents the Physiogel NT® after the addition of 1% (m/m) 5-fluorouracil to it. Idoxuridine shows the viscosity of Physiogel NT® after the addition of 1% (m/m) idoxuridine to it.

When a graph is plotted of the shear stress versus the shear rate of the control and the two actives in the Physiogel NT® it can be seen that the Physiogel NT® can also be characterised as having pseudo-plastic flow characteristics.
Figure 3-20: Shear stress plotted against shear rate for the control which is the Physiogel NT before the addition of actives to it. 5-Fluorouracil represents the Physiogel NT after the addition of 1% (m/m) 5-Fluorouracil to it. Idoxuridine shows the viscosity of Physiogel NT after the addition of 1% (m/m) idoxuridine to it.

3.2.1.1 Statistical data

The log values were determined for the viscosity data of the control and the actives in each of the two lamellar gel phase systems and were plotted against the log rpm values, which resulted in straight lines. Confidence bands of 97.5% were calculated for each straight line and were fitted over the lines. This resulted in Bon Ferroni-corrected
confidence bands with an overall level of 95%. Non-overlapping of the calculated confidence bands would have indicated a significant difference in the viscosity values on a 5% level. No significant differences were found. It can thus be said that no change in viscosity took place.

3.2.2 Dissolution from Delivery Vehicles

The results for the release of 5-fluorouracil from the three delivery vehicles during the dissolution studies are given below.

![Graph](image)

**Figure 3-21:** The amount of 5-FU released from the three delivery vehicles in $\mu$g/cm$^2$ against the squared time.
The results for the idoxuridine containing vehicles are given in figure 3-22.

![Figure 3-22](image)

**Figure 3-22:** The amount of idoxuridine released from the three delivery vehicles in $\mu g/cm^2$ against the squared time.

### 3.2.2.1 Statistical data

The log values were determined for the amount of active released from the delivery vehicles. The log values were then plotted against the squared time, this resulted in a straight line. Confidence bands of 98.3% were calculated for each straight line and were fitted over the lines. This resulted in Bon Ferroni-corrected confidence bands with an overall level of 95%. Non-overlapping of the calculated confidence bands would have indicated a significant difference in the release rate of the actives from the delivery systems on a 5% level. The release of 5-fluorouracil from the two LGPS showed no
statistically significant difference, a statistically significant higher release rate was seen for 5-fluorouracil from the Emzaloid® vehicle.

As with 5-fluorouracil no significant differences was seen for idoxuridine from the two lamellar gel phase systems. The release rate of idoxuridine from the Emzaloid® delivery vehicle was statistical significantly lower the lamellar gel phase systems.

### 3.2.3 pH measurements

The non-polar nature of the horny layer suggests that charged compounds should encounter high resistance to permeation through it (Zats, 1993:28).

It is much more favourable to have as much of the drug in your formulation in a unionized state to promote greater permeation through the stratum corneum. Because of this the pH of the formulation is very important.

5-Fluorouracil has dissociation coefficients (pKa) values of 8 and 13 (Rudy & Senkowski, 1973). Idoxuridine has pKa value of 7.91 (Dollery, 1999).

**Table 3-5:** The pH values for the delivery systems before and after the addition of the actives to it.

<table>
<thead>
<tr>
<th></th>
<th>Physiogel</th>
<th>Physiogel</th>
<th>Emzaloids®</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-Fluorouracil</td>
<td>5,65</td>
<td>5-FU</td>
<td>5,68</td>
</tr>
<tr>
<td>Idoxuridine</td>
<td>5,64</td>
<td>Idoxuridine</td>
<td>5,45</td>
</tr>
</tbody>
</table>

The percentage ionized drug was calculated with the Henderson-Hasselbach equation (Ritschel, 1988).
The Henderson-Hasselbach equation for acidic compounds is given in equation 3-1.

\[
\frac{100}{1 + anti\log(pKa - pH)}
\]

Equation 3-1

The % unionized drug = 100 - % ionized drug.

**Table 3-6:** The percentage of the actives in the unionized state at the delivery systems specific pH. The percentages for 5-fluorouracil were calculated using a pKa value of 13.

<table>
<thead>
<tr>
<th></th>
<th>Physiogel Dermaquadrille®</th>
<th>Physiogel NT®</th>
<th>Emzaloids®</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-Fluorouracil</td>
<td>99,99%</td>
<td>99,99%</td>
<td>99,99%</td>
</tr>
<tr>
<td>Idoxuridine</td>
<td>99,47%</td>
<td>99,56%</td>
<td>99,59%</td>
</tr>
</tbody>
</table>

### 3.2.4 *In vitro* Transdermal Permeation Studies

The *in vitro* permeation studies were carried out as described in § 3.1.8.

The following graphs shows the cumulative amount of each active found over time in the receptor compartments of the Franz diffusion cells, after the application of the drug containing delivery vehicle to the skin.
Figure 3-23: 5-FU in Physiogel Dermaquadrille®. Mean ± SD, n = 6

Figure 3-24: 5-FU in Physiogel NT®. Mean ± SD, n = 6
Figure 3-25: Idoxuridine in Physiogel Dermaquadrille®. Mean ± SD, n = 6

Figure 3-26: Idoxuridine in Physiogel NT®. Mean ± SD, n = 6
Figure 3-27: 5-Fluorouracil in Emzaloids®. Mean ± SD, n = 6

Figure 3-28: Idoxuridine in Emzaloids®. Mean ± SD, n = 6
Table 3-7: Average flux values (µg/cm²/h) and standard deviations for each of the actives from the delivery systems (n = 6)

<table>
<thead>
<tr>
<th></th>
<th>5-Fluorouracil</th>
<th>Idoxuridine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.03 ± 0.01</td>
<td>0.016 ± 0.0019</td>
</tr>
<tr>
<td>Physiogel</td>
<td>0.15 ± 0.02</td>
<td>0.022 ± 0.0042</td>
</tr>
<tr>
<td>Dermaquadrielle*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physiogel NT*</td>
<td>0.14 ± 0.03</td>
<td>0.028 ± 0.0016</td>
</tr>
<tr>
<td>Emzaloid*</td>
<td>0.63 ± 0.13</td>
<td>0.07 ± 0.0180</td>
</tr>
</tbody>
</table>
The permeation enhancing activities of the delivery vehicles were expressed as enhancement ratios of flux (E.R.) according to equation.

\[ E.R. = \frac{\text{Flux}_{\text{Experimental}}}{\text{Flux}_{\text{Control}}} \]

Equation 3-2

<table>
<thead>
<tr>
<th>5-Fluorouracil</th>
<th>E.R.</th>
<th>Idoxuridine</th>
<th>E.R.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physiogel</td>
<td>4.67</td>
<td>Physiogel</td>
<td>1.31</td>
</tr>
<tr>
<td>Dermaquadrille®</td>
<td></td>
<td>Dermaquadrille®</td>
<td></td>
</tr>
<tr>
<td>Physiogel NT®</td>
<td>5.00</td>
<td>Physiogel NT®</td>
<td>1.75</td>
</tr>
<tr>
<td>Emzaloid®</td>
<td>20.33</td>
<td>Emzaloid®</td>
<td>3.50</td>
</tr>
</tbody>
</table>

Table 3-8: Enhancement ratios for 5-fluorouracil and idoxuridine in the LGPS and Emzaloid® delivery systems.

3.2.4.1 Statistical Data

A one way analysis of variance was done on the permeation and flux values in order to compare the delivery vehicles with each other and with the control cells for each active. This was followed by a multiple comparison (Post hoc test) using the Tukey method.

Statistical significant differences for 5-fluorouracil were obtained between the Physiogel Dermaquadrille® and the control for 5-fluorouracil (\( p = 0.041 \)) as well as between Physiogel Dermaquadrille® and Emzaloids® (\( p = 0.00002 \)), Physiogel NT® and Emzaloids® (\( p < 0.001 \)) and between Emzaloids® and the control (\( p < 0.001 \)).

No significant statistical differences were seen between the delivery systems for idoxuridine.
3.3 Discussion and Conclusions

3.3.1 Viscosity and Rheology

The results for the viscosity measurements of the lamellar gel phase systems before and after the addition of the actives to it suggest that no change in the viscosity took place. It can thus be concluded that no change in the internal structure of the LGPS took place, since the destruction or alteration of the internal lamellar structure would have resulted in a dramatic decrease in viscosity.

There was also no change in the rheological flow characteristics of the LGPS. Both exhibited pseudo-plastic flow.

It can thus be said that the lower flux values obtained with the LGPS when compared to the Emzaloid® formulations during the in vitro permeation studies were not as a result of the actives added to it which may have altered the LGPS delivery systems.

3.3.2 Dissolution from Delivery Vehicles

The dissolution from the Physiogel NT® was very closely related for each of the two actives. The same was seen for Physiogel Dermaquadille® suggesting that the two lamellar gel phase systems were very similar in composition and in its interaction with the synthetic membrane.

It has been shown that the Emzaloids® are capable of penetrating skin, keratinized tissue, intestinal epithelium, vascular walls and subcellular organelles (MZL, 2002:18).

For 5-fluorouracil the Emzaloid® formulation showed a statistically significant higher release than Physiogel Dermaquadille® and Physiogel NT®. With idoxuridine the LGPS showed a statistically significant higher release than the Emzaloid® formulation during the VanKel dissolution studies. The two lamellar gel phase systems showed no
Statistically significant difference in the release of idoxuridine. This may be attributed to the lipophilic nature of idoxuridine. It would much rather stay in the lipid rich vesicle environment before diffusing through the synthetic membrane and into the phosphate buffered saline receptor medium.

One would expect to see a greater flux for idoxuridine through the skin with the LGPS than with the Emzaloid® formulation, since there is more of the active released from the formulation, increasing the amount of active available for diffusion. This may however not be the case since Hadgraft (1999) stated that it is a common misconception that an increase in the applied concentration of a drug always results in an increase of drug flux.

The release of 5-fluorouracil from all three the delivery vehicles were far greater than for idoxuridine. This may be attributed to the physicochemical differences (see Table 2-1) of the two actives. The physicochemical properties of a drug substance are the most important determinants for its permeation through the skin (Roy, 1997:143). Idoxuridine is a much larger molecule with a molecular weight of 354,1g/mol as opposed to 5-fluorouracil with a molecular weight of 130,08g/mol.

3.3.3 pH measurements

There was no significant change in the pH of the LGPS before and after the addition of the actives to it, another indication that the actives did not alter the lamellar gel phase systems.

According to the pH-partition simple hypothesis, only the non-ionized forms of drugs are able to pass through lipoidal membranes in significant amounts (Banerjee & Ritschel, 1989:189). Both actives were more than 99% unionized in all three of the delivery vehicles.

The largely polar nature of idoxuridine may also have played a big part in its poor permeation through the skin.
3.3.4 *In vitro* Transdermal Permeation Studies

The lipid composition in the stratum corneum is unique. Unlike biological membranes the stratum corneum is almost devoid of phospholipids and the major constituents are ceramides, cholesterol and long-chain fatty acids (de Jager *et al.*, 2003). Both the Emzaloid® and LGPS formulations contain ceramides.

Since the Emzaloid® formulation is a novel delivery system and closely related to that of liposomes it can be expected that the same forces governing the permeation of liposomes through skin would also have the same or a similar effect on the Emzaloid® vesicles. Several factors such as lamellarity, lipid composition, charge on the liposomal surface and the total lipid concentration have been proven to influence drug deposition into the deeper skin strata (Weiner *et al.*, 1989).

Du Plessis and co-workers (1994) showed that a smaller liposomal particle size (0.06 μm) did not result in higher levels of cyclosporine A in the deeper skin strata. Their results suggested that topical delivery is influenced by the particle size of the vesicles of the liposomal suspension.

They also postulated that there might be an optimum particle size for optimal drug delivery. This is very important since there was a rather big variation in vesicle size between the idoxuridine and the 5-fluorouracil Emzaloid® formulations. Vesicle sizes of 3.05 μm and greater than 5.08 μm were observed for 5-fluorouracil and idoxuridine respectively. Further research would have to be done in order to determine the magnitude of the effect that vesicle size variations play in the Emzaloid® formulation.

The permeation of cyproterone acetate (CPA) from Derma Membrane Structure (DMS) and liposomal formulations was investigated by Valenta & Janisch (2003). By addition of phospholipid concentrate, the CPA permeation could be 2.6 fold further increased compared to the control DMS. In the case of the liposome formulations, the higher the lipid content, the higher was the CPA permeation. Extruding procedures for decreasing
the particle size of the liposomes resulted in a two-fold increase in CPA permeation compared to the unextruded liposomes. This is in agreement with what du Plessis and co-workers (1994) found.

The extrusion procedure of the multilamellar liposomes resulted in a unilamellar shape. Thus the extruded liposomes comprised of a single bilayer which could have been altered more easily than those of multilamellar vesicles with the same composition. The vesicles in the Emzaloid® formulation were also unilamellar, suggesting that the deformation by the vesicle during the permeation of the skin could also occur easier in unilamellar vesicles such as the Emzaloids® than in conventional multilamellar liposomes and so increase the rate of permeation. The use of nitrous oxide gas during the manufacturing process of the Emzaloids® is also believed to increase membrane fluidity and so increase the permeation potential of the vesicles through the stratum corneum.

The greater success of the Emzaloid® may also be attributed to the formulation's excipients permeating into the stratum corneum where according to Hadgraft (1999) two major effects can be obtained. It may intercalate into the structured lipids of the skin where it can disrupt the packing and the effect may render them more fluid thereby increasing the diffusion coefficient of the permeant. The second way in which excipients can modify skin permeability is to shift the solubility parameter of the skin in the direction of that of the permeant, the solubility of the permeant in the outer layers of the skin will increase and this, in turn, improves the flux.

The packing nature of unsaturated fatty acids have been proven, by Yokomizo & Sagitani (1996), to disrupt the stratum corneum lipid structure and enhance the percutaneous penetration of drugs by increasing the fluidity of the lipids. It is believed that the greater success of the Emzaloid® formulation may be attributed to the following proposed mechanism of action. The fatty acids found in the formulation intercalated into the structured lipids of the skin where it disrupted the packing, making them more fluid. The unilamellar vesicles of the Emzaloid® formulation with its high membrane fluidity, due to the nitrous oxide gas used during the manufacturing process could then pass through
these newly formed more fluid lipid channels to a much greater extend than would be expected from ordinary liposome formulations.

The lower success rate of the idoxuridine in the Emzaloid® formulation may be attributed to the fact that the enhancing effects of various fatty acids depends on the physicochemical properties of the active.

3.4 Final conclusions

The Emzaloid® formulation shows the potential for a decrease in the amount of active in the formulation without a reduction in efficacy.

Although the Emzaloid® formulation gave greater flux and enhancement factors than the lamellar gel phase systems for the two actives tested as model drugs, it should be kept in mind that greater success might be achieved with this delivery system for other drugs.

The Emzaloid® formulation has great potential as a commercially marketable delivery system especially for the transdermal delivery of 5-fluorouracil, but much research still has to be done on the Emzaloid® formulation.

Future researcher should include an investigation into the exact mechanism of interaction between the Emzaloid® vesicles and the skin. The employment of differential scanning calorimetry (DSC), fourier transform infrared spectroscopy (FTIR) and confocal laser scanning microscopy techniques may be useful to accomplish this. Efforts should be made to optimize the composition of the Emzaloid® formulation in order to try and get greater flux values for the drugs tested. Further studies should include the testing of other model drugs.

A detailed quantitative assessment of the Emzaloid® formulation and the effects of the addition of actives to it have to be done, in order to select actives which have the optimum physicochemical characteristics to be used in the Emzaloid® formulation.
4 Bibliography


ISP see International Specialty Products


MZL see MeyerZall Laboratories.


**Table A:** The results of viscosity measurements for Physiogel Dermaquadrille®.

The control is the viscosity of Physiogel Dermaquadrille® before the addition of the actives 5-Fluorouracil and Idoxuridine to it.

<table>
<thead>
<tr>
<th>RPM</th>
<th>Control</th>
<th>5-Fluorouracil</th>
<th>Idoxuridine</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.5</td>
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<td>577000</td>
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</tr>
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<td>5</td>
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</tr>
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<td>39232</td>
<td>33088</td>
<td>35840</td>
</tr>
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<td>19456</td>
</tr>
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<td>9011</td>
</tr>
<tr>
<td>100</td>
<td>5267</td>
<td>4723</td>
<td>5114</td>
</tr>
</tbody>
</table>

The rheological data results of the control of the Physiogel Dermaquadrille® without any actives and the 5-Fluorouracil and Idoxuridine containing samples and the RPM at which it was measured are given in table B.
Table B: The control represents the rheological data for the Physiogel Dermaquadrille® without the actives. The 5-Fluorouracil and Idoxuridine represent the data for the LGPS with a 1% (m/m) concentration of each active added to it.

<table>
<thead>
<tr>
<th>RPM</th>
<th>Control</th>
<th>5-Fluorouracil</th>
<th>Idoxuridine</th>
<th>Control</th>
<th>5-Fluorouracil</th>
<th>Idoxuridine</th>
</tr>
</thead>
<tbody>
<tr>
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<td>0</td>
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<td>940.8</td>
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<td>2.5</td>
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<td>924.7</td>
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<tr>
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<td>1475</td>
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</tr>
</tbody>
</table>
Table C: The results of viscosity measurements for Physiogel NT®. The control is the viscosity of Physiogel NT® before the addition of the actives 5-Fluorouracil and Idoxuridine to it.

<table>
<thead>
<tr>
<th>RPM</th>
<th>Control</th>
<th>5-Fluorouracil</th>
<th>Idoxuridine</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.5</td>
<td>1000000</td>
<td>1430000</td>
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<tr>
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<td>12288</td>
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</tr>
</tbody>
</table>
Table D: The control represents the viscosity and rheological data for the Physiogel NT® without the actives. The 5-Fluorouracil and Idoxuridine represent the data for the LGPS with a 1% (m/m) concentration of each active added to it.

<table>
<thead>
<tr>
<th>RPM</th>
<th>Shear rate (sec⁻¹)</th>
<th>Shear stress (dynes/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>5-Fluorouracil</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.5</td>
<td>0.11</td>
<td>0.11</td>
</tr>
<tr>
<td>1</td>
<td>0.22</td>
<td>0.22</td>
</tr>
<tr>
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<td>0.55</td>
<td>0.55</td>
</tr>
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<td>1.1</td>
<td>1.1</td>
</tr>
<tr>
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<td>11</td>
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<tr>
<td>100</td>
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<td>22</td>
</tr>
</tbody>
</table>
Table E: Transdermal permeation data of the amount of actives found in the receptor compartments at specific time intervals for the experimental and control cells for 5-Fluorouracil in Physiogel Dermaquadrille®.

<table>
<thead>
<tr>
<th>Hours</th>
<th>Experimental Mean (µg/ml) (n = 6)</th>
<th>Experimental SD (n = 6)</th>
<th>Control Mean (µg/ml) (n = 6)</th>
<th>Control SD (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
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<td>0.23</td>
<td>0.15</td>
<td>0.06</td>
<td>0.07</td>
</tr>
<tr>
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<td>0.45</td>
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<td>0.16</td>
<td>0.10</td>
</tr>
<tr>
<td>6</td>
<td>0.76</td>
<td>0.16</td>
<td>0.21</td>
<td>0.11</td>
</tr>
<tr>
<td>8</td>
<td>1.06</td>
<td>0.20</td>
<td>0.27</td>
<td>0.13</td>
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<td>10</td>
<td>1.33</td>
<td>0.30</td>
<td>0.34</td>
<td>0.15</td>
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</tr>
<tr>
<td>Slope</td>
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<td>Slope</td>
<td>0.03</td>
<td></td>
</tr>
</tbody>
</table>
### Table F: Transdermal permeation data for the experimental and control cells of 5-Fluorouracil in Physiogel NT® found in the receptor compartments of the Franz diffusion cells after specific time intervals.

<table>
<thead>
<tr>
<th>Hours</th>
<th>Experimental Mean (µg/ml) (n = 6)</th>
<th>Experimental SD (n = 6)</th>
<th>Control Mean (µg/ml) (n = 6)</th>
<th>Control SD (n = 6)</th>
</tr>
</thead>
<tbody>
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<td>0.03</td>
<td>0.06</td>
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</tr>
<tr>
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<td>0.11</td>
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<td>8</td>
<td>0.98</td>
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</tr>
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<td>10</td>
<td>1.26</td>
<td>0.27</td>
<td>0.34</td>
<td>0.15</td>
</tr>
<tr>
<td>12</td>
<td>1.57</td>
<td>0.34</td>
<td>0.39</td>
<td>0.16</td>
</tr>
<tr>
<td>24</td>
<td>3.43</td>
<td>1.31</td>
<td>0.59</td>
<td>0.28</td>
</tr>
<tr>
<td>Slope</td>
<td>0.15</td>
<td>Slope</td>
<td></td>
<td>0.03</td>
</tr>
</tbody>
</table>
Table G: Transdermal permeation data for the experimental and control cells for Idoxuridine in Physiogel Dermaquadille® found in the receptor compartments of the Franz diffusion cells after specific time intervals.

<table>
<thead>
<tr>
<th>Hours</th>
<th>Experimental Mean (µg/ml) (n = 6)</th>
<th>Control Mean (µg/ml) (n = 6)</th>
<th>Experimental SD (n = 6)</th>
<th>Control SD (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>0.11</td>
<td>0.07</td>
<td>0.04</td>
<td>0.03</td>
</tr>
<tr>
<td>4</td>
<td>0.15</td>
<td>0.10</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>6</td>
<td>0.19</td>
<td>0.13</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>8</td>
<td>0.23</td>
<td>0.16</td>
<td>0.05</td>
<td>0.04</td>
</tr>
<tr>
<td>10</td>
<td>0.28</td>
<td>0.19</td>
<td>0.05</td>
<td>0.04</td>
</tr>
<tr>
<td>12</td>
<td>0.32</td>
<td>0.23</td>
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<tr>
<td>24</td>
<td>0.45</td>
<td>0.31</td>
<td>0.08</td>
<td>0.05</td>
</tr>
<tr>
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<td>Slope</td>
<td></td>
<td>0.016</td>
</tr>
</tbody>
</table>
Table H: Transdermal permeation data for the experimental and control cells for Idoxuridine in Physiogel NT\textsuperscript{®} found in the receptor compartments of the Franz diffusion cells after specific time intervals.

<table>
<thead>
<tr>
<th>Hours</th>
<th>Experimental</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (µg/ml) (n = 6)</td>
<td>SD (n = 6)</td>
</tr>
<tr>
<td>2</td>
<td>0.10</td>
<td>0.02</td>
</tr>
<tr>
<td>4</td>
<td>0.15</td>
<td>0.03</td>
</tr>
<tr>
<td>6</td>
<td>0.20</td>
<td>0.03</td>
</tr>
<tr>
<td>8</td>
<td>0.25</td>
<td>0.03</td>
</tr>
<tr>
<td>10</td>
<td>0.31</td>
<td>0.03</td>
</tr>
<tr>
<td>12</td>
<td>0.37</td>
<td>0.04</td>
</tr>
<tr>
<td>24</td>
<td>0.54</td>
<td>0.06</td>
</tr>
<tr>
<td>Slope</td>
<td>0.028</td>
<td>Slope</td>
</tr>
</tbody>
</table>
Table I: Transdermal permeation data for the experimental and control cells for 5-Fluorouracil in Emzaloids® found in the receptor compartments of the Franz diffusion cells after specific time intervals.

<table>
<thead>
<tr>
<th>Hours</th>
<th>Experimental Mean (µg/ml) (n = 6)</th>
<th>Experimental SD (n = 6)</th>
<th>Control Mean (µg/ml) (n = 6)</th>
<th>Control SD (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>1.56</td>
<td>0.69</td>
<td>0.06</td>
<td>0.07</td>
</tr>
<tr>
<td>4</td>
<td>3.05</td>
<td>1.10</td>
<td>0.16</td>
<td>0.10</td>
</tr>
<tr>
<td>6</td>
<td>4.38</td>
<td>1.50</td>
<td>0.21</td>
<td>0.11</td>
</tr>
<tr>
<td>8</td>
<td>5.51</td>
<td>1.78</td>
<td>0.27</td>
<td>0.13</td>
</tr>
<tr>
<td>10</td>
<td>6.58</td>
<td>2.10</td>
<td>0.34</td>
<td>0.15</td>
</tr>
<tr>
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<td>7.70</td>
<td>2.12</td>
<td>0.39</td>
<td>0.16</td>
</tr>
<tr>
<td>24</td>
<td>13.52</td>
<td>1.95</td>
<td>0.59</td>
<td>0.28</td>
</tr>
<tr>
<td>Slope</td>
<td>0.61</td>
<td></td>
<td>Slope</td>
<td>0.03</td>
</tr>
</tbody>
</table>
Table J: Data for the experimental and control cells for Idoxuridine in Emzaloids®

<table>
<thead>
<tr>
<th>Hours</th>
<th>Mean (µg/ml) (n = 6)</th>
<th>SD (n = 6)</th>
<th>Mean (µg/ml) (n = 6)</th>
<th>SD (n = 6)</th>
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</thead>
<tbody>
<tr>
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<td>0.06</td>
<td>0.07</td>
<td>0.03</td>
</tr>
<tr>
<td>4</td>
<td>0.25</td>
<td>0.07</td>
<td>0.10</td>
<td>0.04</td>
</tr>
<tr>
<td>6</td>
<td>0.39</td>
<td>0.08</td>
<td>0.13</td>
<td>0.04</td>
</tr>
<tr>
<td>8</td>
<td>0.54</td>
<td>0.11</td>
<td>0.16</td>
<td>0.04</td>
</tr>
<tr>
<td>10</td>
<td>0.69</td>
<td>0.14</td>
<td>0.19</td>
<td>0.04</td>
</tr>
<tr>
<td>12</td>
<td>0.86</td>
<td>0.17</td>
<td>0.23</td>
<td>0.04</td>
</tr>
<tr>
<td>24</td>
<td>1.07</td>
<td>0.14</td>
<td>0.31</td>
<td>0.05</td>
</tr>
<tr>
<td>Slope</td>
<td>0.07</td>
<td></td>
<td>Slope</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Figure I: The VanKel dissolution data of 5-fluorouracil from the three delivery vehicles. The dotted lines indicate the Bon Ferroni-corrected confidence bands.
**Figure II:** The VanKel dissolution data of idoxuridine from the three delivery vehicles. The dotted lines indicate the Bon Ferroni-corrected confidence bands. As can be seen the Emzaloid® line falls far below the Lamellar Gel Phase Systems lines.

**Figure III:** The log values of both the viscosity and the RPM for the Physiogel Dermaquadrille® before and after the addition of 5-fluorouracil to it. The dotted lines indicate the Bon Ferroni-corrected confidence bands.
Figure IV: The log values of both the viscosity and the RPM for the Physiogel NT® before and after the addition of 5-fluorouracil to it. The dotted lines indicate the Bon Ferroni-corrected confidence bands.

Figure V: The log values of both the viscosity and the RPM for the Physiogel Dermaquadriile® before and after the addition of idoxuridine to it. The dotted lines indicate the Bon Ferroni-corrected confidence bands.
Figure VI: The log values of both the viscosity and the RPM for the Physiogel NT\textsuperscript{®} before and after the addition of idoxuridine to it. The dotted lines indicate the Bon Ferroni-corrected confidence bands.