TRANSDERMAL DELIVERY OF 5-FLUOROURACIL WITH PHEROID^{TM} TECHNOLOGY

C.P. VAN DYK

Philippians 4:6-7

"Be anxious for nothing,
but in everything by prayer and supplication,
with thanksgiving let your request be made known to God;
and the peace of God, which surpasses all understanding,
will guard your hearts and minds through Christ Jesus."

New King James Version

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B.Pharm

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ABSTRACT

5-Fluorouracil (5FU) is a pyrimidine analogue, indicated for the therapy of proliferative skin diseases such as actinic keratosis (AK), superficial basal cell carcinoma and psoriasis. It has also been used for the treatment of solid tumours like colorectal, breast and liver carcinomas for nearly 40 years.

Although 5FU has always been administered parenterally and orally, metabolism is rapid and absorption is erratic. Several severe side-effects are also commonly associated with 5FU therapy, including myelosuppression, hand-foot syndrome and gastrointestinal effects. Seeing that 5FU is an important part of the treatment of several malignant and pre-malignant disorders, it would be advantageous to find a delivery route and delivery system that negate absorption and metabolic variation and decrease side-effects.

The transdermal route provides a promising alternative to the above-mentioned conventional delivery routes, solving most of the problems associated with parenteral and oral administration. That being said, the formidable barrier situated in the skin is not easily breached. The stratum corneum, the outermost skin layer, is mostly lipophilic in nature, preventing hydrophilic molecules such as 5FU from entering.

5FU-containing creams and lotions are currently commercially available, but absorption is still very limited. The transdermal absorption from these formulations has been compared to that obtained with the use of new transdermal delivery vehicles, with the newer formulations proving to be promising.

It was decided to entrap 5FU in a novel therapeutic system, in the form of the Pheroid™ system, to increase its transdermal penetration.

Pheroid™ vesicles are stable spherical structures in a unique, emulsion-like formulation, and fall in the submicron range. The main components of the Pheroid™ system are the ethyl esters of the essential fatty acids linoleic acid and linolenic acid, as well as the *cys*-form of oleic acid, and water. The formulation is saturated with nitrous oxide (N_2O).

Although Pheroid™ vesicles may resemble other lipid-based vehicles, such as liposomes and micro-emulsions, they are unique in the sense that they have inherent therapeutic qualities as well. The Pheroid™ formulation can be specifically manipulated to yield

different types of vesicles, ensuring a fast transport rate, high entrapment efficiency, rapid delivery and stability of the delivery system for a specific drug.

In this study, 5FU was entrapped in the Pheroid™ formulation. Transdermal permeation studies were then performed to evaluate the influence of this delivery system on the transdermal flux of 5FU.

Vertical Franz diffusion cells were utilised to determine the transdermal penetration of 5FU. Only Caucasian female abdominal skin was used to minimise physiological variables. Diffusion studies were done over 12 hour periods, with the entire receptor phase being withdrawn at predetermined intervals. Samples were analysed using high performance liquid chromatography (HPLC), after which the cumulative concentration of active was plotted against time. The linear portion of this graph represents the flux of 5FU through the skin.

It was found that there were differences in the results between formulations containing 5FU in a phosphate buffer solution (PBS)-based Pheroid™ and water-based Pheroid™, though the difference was not statistically significant. The 0.5 % 5FU in water-based Pheroid™ resulted in a significantly bigger yield than the control (1 % 5FU in water) as well as a significant difference to the 1 % 5FU in PBS-based Pheroid™ formulation. In general the water-based Pheroid™ formulations had greater average cumulative concentrations, yields and fluxes than the other formulations.

The fluxes obtained with the water-based Pheroid™ formulations also correlated well with a previous study done by Kilian (2004).

Thus it can be concluded that the Pheroid™ therapeutic delivery system enhances the transdermal penetration of 5FU. Water-based Pheroid™ formulations proved to be more effective than PBS-based Pheroid™ formulations. It can also be concluded that a 0.5 % 5FU in water-based Pheroid™ formulation can be used instead of a 1 % formulation, because there were no statistically significant differences between the two formulations. This would be advantageous - patient compliance can be enhanced because of a more tolerable formulation with fewer side effects, while manufacturing cost is lowered by using a lower concentration of active.

It is recommended that some aspects of the study be investigated further to optimise the transdermal delivery of 5FU using the Pheroid™ therapeutic system. These aspects

include optimising the composition of the Pheroid^{TM} formulation, investigating the entrapment process of 5FU within Pheroid^{TM} spheres, the influence of PBS and water as basis of the Pheroid^{TM} formulation and the amount of 5FU remaining in the epidermis after the 12 hour period of the diffusion study.

Keywords: 5-Fluorouracil, Franz diffusion cell, Heat separated epidermis, Skin penetration, Transdermal, Drug delivery system, Pheroid^{TM}

UITTREKSEL

5-Fluorourasiel (5FU) is 'n pirimidien analoog, met indikasies vir die behandeling van proliferatiewe velsiektes soos aktiniese keratose (AK), oppervlakkige basaalselkarsinoom en psoriase. Dit word ook al vir byna 40 jaar gebruik as behandeling van soliede tumors soos kolorektale, bors- en lewerkarsinome.

Alhoewel 5FU gewoonlik parenteraal of oraal toegedien word, word dit vinnig gemetaboliseer en absorpsie is varierend. Verskeie ernstige newe-effekte word ook gereeld met 5FU terapie geassosieer, insluitende beenmurgonderdrukking, hand-voet sindroom en gastro-intestinale effekte. Siende dat 5FU 'n belangrike komponent in die behandeling van verskeie kwaadaardige toestande is, sal dit voordelig wees om 'n toedieningsroete en formulering te vind wat die variasie in absorpsie en metabolisme sowel as newe-effekte verminder.

Die transdermale roete bied 'n belowende alternatief vir bogenoemde konvensionele roetes, en los die meeste probleme geassosieer met parenterale en orale toediening op. Die formidabele skans geleë in die vel word egter nie maklik oorkom nie. Die buitenste laag, die stratum corneum, verhoed hidrofiele molekule soos 5FU om die liggaam binne te dring.

5FU-bevattende rome en aanwendings is tans kommersieel beskikbaar, maar daar is steeds 'n groot variasie in absorpsie. Die transdermale absorpsie vanuit hierdie formulerings is al vergelyk met die absorpsie verkry met die gebruik van nuwe transdermale afleweringsisteme, met die nuwe sisteme wat baie belowend lyk.

Daar is besluit om 5FU in 'n nuwe terapeutiese sisteem, naamlik die Pheroid™ sisteem, te inkorporeer, om die transdermale penetrasie van 5FU te verbeter.

Pheroid™ vesikels is stabiele, sferiese strukture in 'n unieke, emulsie-agtige formulering, en val in die sub-mikron grootte-area. Die hoofkomponente van die Pheroid™ sisteem is die etielesters van die essensiële vetsure linoliënsuur en linoleen suur, sowel as die *cys*-vorm van oleïensuur, en water. Die formulering is versadig met stikstofoksied (N_2O).

Alhoewel Pheroid™ vesikels lyk na ander lipied-gebasseerde sisteme, soos liposome en mikro-emulsies, is hulle uniek in die sin dat hulle inherente terapeutiese eienskappe

besit. Die Pheroid™ formulering kan spesifiek gemanupileer word om verskillende tipes vesikels te lewer, wat 'n vinnige transporttempo verseker, sowel as 'n hoë mate van geneesmiddelopname in die vesikels, vinnige aflewering en stabiliteit van die sisteem vir 'n spesifieke geneesmiddel.

In hierdie studie is 5FU opgeneem in die Pheroid™ formulering. Transdermale penetrasie studies is uitgevoer om die invloed van hierdie afleweringsisteem op die transdermale aflewering van 5FU te bestudeer.

Vertikale Franz diffusieselle is gebruik in die studies op die transdermale penetrasie van 5FU. Slegs blanke, vroulike abdominale vel is gebruik om fisiologiese veranderlikes te minimaliseer. Diffusiestudies is uitgevoer oor periodes van 12 uur, en die totale reseptorfase is onttrek op voorafbepaalde intervalle. Monsters is geanaliseer deur HPLC te gebruik, waarna die kumulatiewe konsentrasie van die aktiewe bestanddeel teen tyd gestip is. Die reguit deel van die grafiek verteenwoordig die fluks van 5FU deur die vel.

Daar is gevind dat die resultate van die 5FU in 'n fosfaatbufferoplossing (PBS)-gebasseerde Pheroid™ en water-gebasseerde Pheroid™ verskil, alhoewel nie statisties betekenisvol nie. Die 0.5 % 5FU in water-gebasseerde Pheroid™ het gelei tot 'n betekenisvolle hoër opbrengs as die kontrole (1 % 5FU in water), sowel as 'n betekenisvolle verskil van die 1 % 5FU in PBS-gebasseerde Pheroid™ formulering. Oor die algemeen het die water-gebasseerde Pheroid™ formulerings hoër gemiddelde kumulatiewe konsentrasies, opbrengste en flukse as die ander formulerings gelewer.

Die flukse verkry met die water-gebaseerde Pheroid™ formulerings korreleer ook goed met dié in 'n vorige studie deur Kilian (2004).

Daar kan dus tot die slotsom gekom word dat die Pheroid™ terapeutiese sisteem 'n positiewe effek het deur die transdermale penetrasie van 5FU te verbeter. Water-gebasseerde Pheroid™ formulerings het meer effektief voorgekom as PBS-gebasseerde Pheroid™ formulerings. Die gevolgtrekking kan ook gemaak word dat 'n 0.5 % 5FU in water-gebasseerde Pheroid™ formulering gebruik kan word in plaas van 'n 1 % formulering, omdat daar geen statisties betekenisvolle verskille tussen die twee formulerings was nie. Dit sal voordelig wees – pasiëntmeewerkendheid kan verbeter word omdat die formulering meer verdraagbaar is en minder newe-effekte het, terwyl vervaardigingskoste verlaag kan word deur 'n laer konsentrasie van die aktiewe bestanddeel te gebruik.

Sommige aspekte van die studie regverdig verdere ondersoek om die transdermale aflewering van 5FU deur middel van die Pheroid™ terapeutiese sisteem te optimaliseer. Hierdie aspekte sluit in die optimalisering van die samestelling van die Pheroid™ formulering, ondersoek na die proses van 5FU-inkorporering in die Pheroid™ vesikels, die invloed van PBS en water as basis van die Pheroid™ formulering en die hoeveelheid 5FU wat agterbly in die vel na die 12 uur periode van die diffusiestudie.

Sleutelwoorde: 5-Fluorourasiel, Franz diffusiesel, hitte-geskeide epidermis, velpenetrasie, transdermaal, geneesmiddelafleweringsisteem, Pheroid™

FOREWORD

The conductors of this study aimed to confirm a pilot study on the transdermal delivery of 5-fluorouracil (5FU) with the aid of the Pheroid^{TM} novel delivery system. This delivery system is already being applied in studies by the subprogram: Drug Delivery of the Unit for Drug Research and Development of the North-West University. These studies are carried out on various delivery routes and a wide variety of actives, ranging from simple molecules to actives as complex as peptide hormones.

This dissertation is written in a format that differs significantly from conventional dissertations, containing an introductory chapter (literature review) and a scientific article to be submitted for publication in a scientific journal. The journal chosen is the European Journal of Pharmaceutics and Biopharmaceutics; the guide for authors is also enclosed within this dissertation. Lastly the data procured during the studies are attached in appendices.

It has been an incredible experience to work on this study and I am convinced that it has helped me develop personally as well.

Christelle van Dyk
25 November 2007

THE TRANSDERMAL DELIVERY OF 5-FLUOROURACIL WITH PHEROID^{TM} TECHNOLOGY

1. Literature Review and Problem Statement

1.1. Introduction

5-Fluorouracil (5FU) is a pyrimidine analogue (Ghoshal & Jacob, 1997:1569). The function of pyrimidines is to combine with ribose or deoxyribose to form nucleosides (uridine) or deoxynucleosides (deoxyuridine) which in turn forms RNA and DNA. Uridine is important for the biosynthesis of DNA and thus for the communication and recording of genetic information (Material safety data sheet (MSDS) for 5FU).

As one of the first rationally-designed antimetabolites, synthesised for the first time in 1957 (Mader *et al.*, 1998:662), 5FU is indicated for the therapy of proliferative skin diseases such as actinic keratosis (AK), superficial basal cell carcinoma and psoriasis (Patrick *et al.*, 1997:40) and has been used for the treatment of solid tumours like colorectal, breast and liver carcinomas for nearly 40 years (Ghoshal & Jacob, 1997:1569).

1.2. Chemical and physical characteristics of 5FU

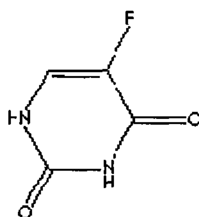


Figure 1: Chemical structure of 5-Fluorouracil (MSDS for 5FU)

Molecular Formula: $C_4H_3FN_2O_2$

Molecular Weight: 130.08 Dalton (Anon., 2005:http://pharmacology.unmc.edu/cancer/5fu).

Synonyms for 5-Fluorouracil: 5-FU, FU, 5-fluoro-2,4(1H,3H)-pyrimidinedione.

Tradenames: Timazin, Adrucil, Carzonal, Efudex, Efudix, Fluoroblastin, Queroplex and many others.

Appearance: White crystals or powder.

Melting point: Decomposes at 280 °C; sublimes at 195 °C (MSDS for 5FU).

5FU is poorly absorbed because of two functional groups contained in its structure: the imide and amide groups. These groups form intermolecular hydrogen bonds, leading to higher crystal lattice energy and a higher melting point. As a result, 5FU's general solubility is decreased as well (Patrick *et al.*, 1997:40).

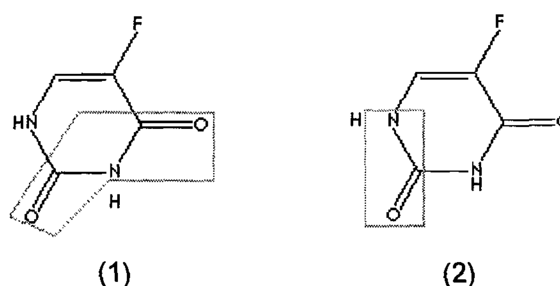


Figure 2: (1) Imide and (2) amide groups in 5-Fluorouracil

5FU is a hydrophilic, polar compound and is a diprotic acid, with pK_a values of 8.0 and 13.0 (Singh *et al.*, 2005:99).

The partition coefficient (K) of 5FU between octanol and water is 0.13 (Da Costa & Moraes, 2003:58), while the log $P_{oct/water}$-value is -0.824. This log P value indicates that 5FU is a hydrophilic substance (El Maghraby *et al.*, 2005:184).

The solubility of 5FU in a buffer solution (pH 4.0) at 23 °C has been found to be 11.1 mg/ml (Sloan & Beall, 1993:89) and the aqueous solubilities reported in the literature vary between 12.2 mg/ml (MSDS for 5FU) and 14.3 mg/ml (Yamane *et al.*, 1995:250).

1.3. Pharmacokinetic information on 5FU

The biological half-life of 5FU is short (10 to 15 minutes after short intravenous injection) (White, 2001:2970). 5FU is rapidly metabolised, especially in the liver (Gao & Singh, 1998:46), giving rise to active metabolites with anti-tumour activities, including 5-fluoro-uridine (FUrd), 5-fluoro-2'-deoxyuridine (FdUrd) and 5-fluoro-2'-deoxyuridine phosphate (FdUMP) (Del Nozal *et al.*, 1994:397; Barberi-Heyob *et al.*, 1992:247). The estimated clearance of 5FU is 0.6 to 2.3 L/min or 16 mL/min/kg (Anon., 2007:www.cancercare.on.ca).

5FU distributes into all body fluids via passive diffusion and crosses the placenta as well as the blood brain barrier. The volume of distribution (V_d) is estimated to be 0.25 L/kg or 8.84 L/m^2 (Anon., 2007:www.cancercare.on.ca).

1.4. Mechanism of action

The main mechanism of action of 5FU is still much debated, but it is said to be the inhibition of thymidylate synthetase (TS). This is the enzyme responsible for the *de novo* synthesis of thymidylic acid (Ghoshal & Jacob, 1997:1569). TS catalyses the conversion of deoxyuridine-5'-monophosphate (dUMP) to deoxythymidine-5'-monophosphate (dTMP), which is essential in DNA synthesis and thus cell replication (Backus *et al.*, 2001:209).

5FU itself is inactive, but is activated in the body in the same way as thymine, and FdUMP is formed (Mader *et al.*, 1998:662). FdUMP forms a stable complex with TS, inhibiting the enzyme and leading to growth arrest and cell death (Backus *et al.*, 2001:209). This phenomenon is often called "thymineless death", which entails inhibition of DNA synthesis. The fact that TS is indeed the main target of 5FU's metabolite, FdUMP, is proven by studies where thymidylate synthase (TS) is overproduced in 5FU-resistant cell lines (Ghoshal & Jacob, 1997:1569).

5FU's metabolites are related to uracil's physiological analogues. In Table 1 the names of the metabolites are given.

Table 1: Metabolites involved in 5FU's mechanism of action

Abbreviation	Name of metabolite
dUMP	Deoxyuridine monophosphate
dTMP	Deoxythymidine monophospate
dTDP	Deoxythymidine diphospate
dTTP	Deoxythymidine triphospate
FdURD	Fluorodeoxyuridine
FdUMP	Fluorodeoxyuridine monophosphate
FdUDP	Fluorodeoxyuridine diphosphate
FdUTP	Fluorodeoxyuridine triphosphate
FU-DNA	DNA containing 5FU
FUrd	Fluorouridine
FUMP	Fluorouridine monophosphate
FUDP	Fluorouridine diphosphate
FUTP	Fluorouridine triphosphate
FU-RNA	RNA containing RNA
URD	Uridine
UMP	Uridine monophosphate
UDP	Uridine diphosphate
UTP	Uridine triphosphate

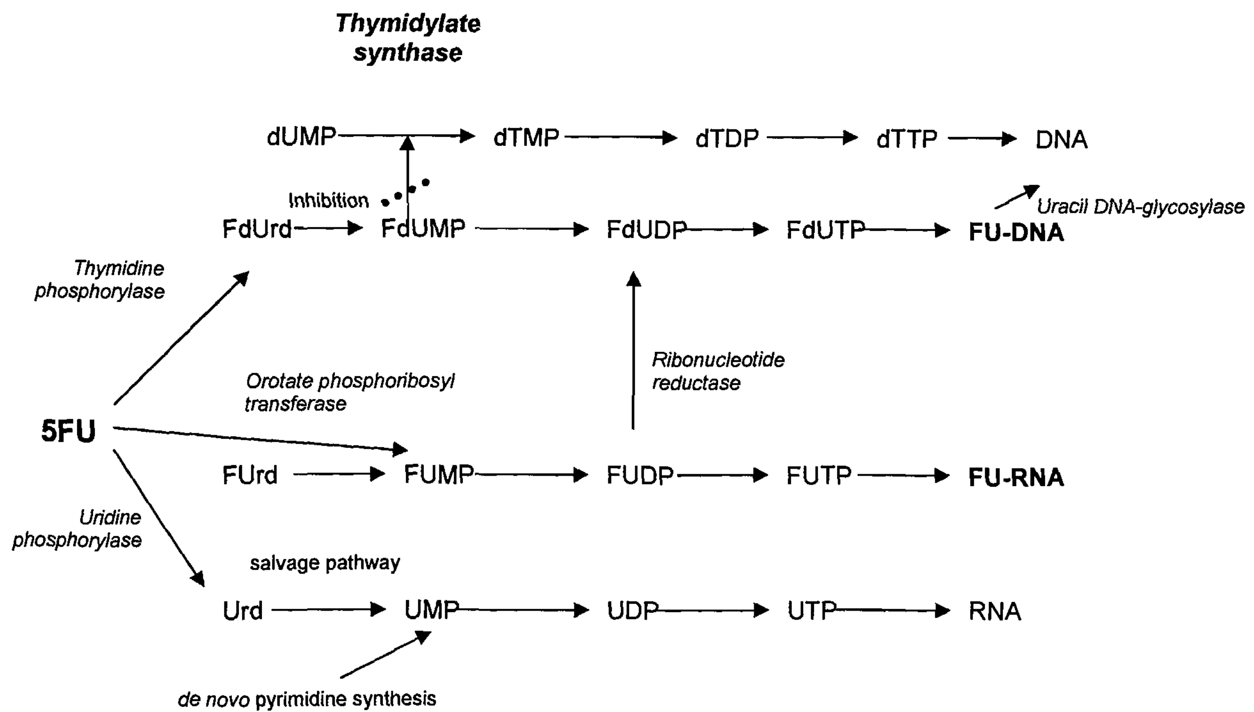


Figure 3: Activation of 5FU and its suggested mechanisms of action (Reproduced from Mader *et al.*, 1998:662)

Other mechanisms of action of 5FU are the incorporation of its metabolite, 5-FUrd, into RNA (forming fraudulent RNA) (Jung *et al.*, 1997:193) and the induction of G_1/S-phase cell cycle arrest in cancer cells. 5FU also increases the transcription of factors leading to possible apoptosis (cell death) (Li *et al.*, 2004:63).

1.5. Clinical uses

5FU is used in the therapy of proliferative skin diseases such as actinic keratosis (AK), superficial basal cell carcinoma and psoriasis (Patrick *et al.*, 1997:40), as well as the treatment of solid tumours like colorectal, breast, liver (Ghoshal & Jacob, 1997:1569), gastric, prostate, ovary, pancreatic and urinary bladder carcinomas (Anon., 2007:www.cancercare.on.za).

Actinic keratosis is the appearance of thick, scale-like growths (keratosis) caused by excessive exposure to sunlight (actinic). This condition has the potential to develop into invasive squamous cell carcinoma, and also has metastatic potential (Spencer, 2006).

The efficacy of 5FU in AK was first noticed when the actinic lesions of a patient receiving systemic 5FU for advanced carcinomatosis, improved significantly (Loven *et al.*, 2002:991). The safety and efficacy of topical 5FU in the treatment of AK lesions and psoriasis have since been proven (Singh *et al.*, 2005:99).

1.6. Transdermal delivery of 5FU

1.6.1. Introduction

Systemic 5FU (oral and parenteral) is effective in the treatment of a variety of malignant and pre-malignant conditions, but also presents an array of problems. When administered via the oral route, 5FU is poorly absorbed and its bioavailability is erratic. After parenteral administration it is swiftly eliminated (Singh *et al.*, 2005:99), with about 20 % of the intact drug appearing in the urine within 6 hours (Anon., 2007:www.cancercare.on.za). Several severe side-effects may occur when 5FU is administered systemically, including myelosuppression, mucositis, cardiac toxicity, gastro-intestinal side-effects and hand-foot syndrome (HFS) (Yen & McLeod, 2007:1011). A more detailed description of possible side-effects is given in Table 2, where "immediate" onset refers to side-effects developing within hours to days, and "early" within days to weeks.

Table 2: Side-effects caused by 5FU, with their time of onset (Reproduced from Anon., 2007:www.cancercare.on.ca)

Site	Side-effect	Onset of side-effect	
		Immediate	Early
Cardiovascular	Symptomatic ECG changes	✓	
	Myocardial ischemia	✓	
Dermatologic	Mild alopecia		✓
	Rash on extremities and trunk		✓
	Photosensitivity		✓
	Dry skin		✓
	Erythema and necrosis after topical application	✓	✓
	Palmar-plantar erythrodysesthesia (hand-foot syndrome)		✓
Gastrointestinal	Anorexia		✓
	Diarrhoea		✓
	Mild nausea and vomiting	✓	
	Stomatitis and esophagitis		✓
Hematologic	Immunosuppression		✓
	Megaloblastosis		✓
	Myelosuppression (very common)		✓
Injection site	Chemical phlebitis	✓	

Side-effects affect the cardiovascular and gastrointestinal systems, as well as other body sites, consequently making 5FU a probable candidate for topical and / or transdermal administration.

1.6.2. Previous studies on the transdermal delivery of 5FU

5FU is already being marketed in creams of different strengths (0.5 %, 1 %, and 5 %), as well as solutions (2 % and 5 %) (Anon., 2007:www.drugs.com).

In one study, a 5 % 5FU cream was compared to a 0.5 % cream containing 5FU entrapped in microsponges (as described by Embil & Nacht in 1996). The results indicated that the 0.5 % cream was as effective as the 5 % cream, with less systemic side-effects, suggesting better targeted delivery of the drug (Levy *et al.*, 2001:906).

Another study compared the tolerability and efficacy of a 0.5 % 5FU cream with that of a 5 % cream by applying it to both sides of patients' faces. The conclusions made included that the 0.5 % cream (one tenth the strength of the 5 % cream) was at least as effective as the 5 % formulation. Though no statistically significant differences in occurrence of side-effects were present, a lower proportion of patients complained about these symptoms when using the 0.5 % cream. The 0.5 % formulation was also found to be easier to apply (Loven *et al.*, 2002:995,999), which will be beneficial for patient compliance.

A study was also conducted at the North-West University (South Africa) where the efficacy of two lamellar gel phase systems was compared to that of the Pheroid™ system in the transdermal delivery of 5FU. It was concluded that the Pheroid™ system is indeed effective in delivering 5FU transdermally (Kilian, 2004).

Several studies have investigated liposomes in the transdermal delivery of 5FU, *e.g.* Da Costa & Moraes, 2003 and El Maghraby *et al.*, 2001. The aims of these studies were rather the optimising of entrapment and release of 5FU from liposomes than increasing the transdermal penetration of 5FU.

1.6.3. Limitations to transdermal delivery

As with any other delivery route, there are also some problems associated with the transdermal delivery of 5FU.

According to the values in Table 3 the hydrophilic nature of 5FU seems to be the main obstacle in transdermal penetration. The reason for this is that the stratum corneum, the outermost skin layer and main barrier to penetration, is lipophilic in nature (Guy & Hadgraft, 1988:753). Thus 5FU does not have a great affinity for the stratum corneum. Furthermore, 5FU has a high melting point, caused by high crystal lattice energy, leading to poor solubility (Patrick *et al.*, 1997:40).

In Table 3 5FU's physicochemical characteristics are compared to the ideal values for transdermal delivery.

Table 3: Ideal physicochemical values for transdermal penetration

Property	Ideal value(s)	5-Fluorouracil
Molecular weight	< 600 Dalton[1]	130.08 Dalton[2]
Aqueous solubility	> 1 $mg.ml^{-1}$ [3]	14.3 $mg.ml^{-1}$ [4] 12.2 $mg.ml^{-1}$ [2]
Lipophilicity (partition coefficient)	$10 < K_{o/w} < 1000$ [5] Log $P_{oct/water} = 2$ [5]	$K_{oct/w} = 0.13$ [6] Log $P_{oct/water} = -0.824$ [7]
Melting point	< 200 °C [3]	280 °C [8]

(1) Barry, 2002:513; (2) Anon., 2005:http://pharmacology.unmc.edu/cancer/5fu; (3) Naik *et al.*, 2000:319; (4) Yamane *et al.*, 1995:250;

(5) Malan *et al.*,2002:386; (6) Da Costa & Moraes, 2003:58; (7) El Maghraby *et al.*, 2005:184; (8) MSDS for 5FU

1.6.4. Advantages of transdermal delivery

The transdermal delivery of drugs has several advantages over conventional delivery routes. The skin offers a considerable surface area for drug absorption and is easy to access (Naik *et al.*, 2000:319). In the gastrointestinal tract there are several variables present which influence drug absorption, including drastic changes in pH, the presence of food and changes in intestinal motility. These factors may decrease bioavailability and cause unwanted side-effects due to metabolic products (Wiechers, 1989:185), but are side-stepped by the transdermal route.

Transdermal delivery from controlled release systems (*e.g.* transdermal patches) further reduces side-effects by providing constant, controlled and repeatable drug release from the delivery vehicle into central circulation. Thus, constant blood levels are maintained, eliminating the peak and trough levels that might cause side-effects. This is also beneficial for the delivery of drugs with a short biological half-life (Kydonius *et al.*, 2000:2-5) like 5FU.

Another advantage of transdermal delivery is improved patient compliance, because dosing frequency is reduced and self-administration is made possible (Kydonius *et al.*, 2000:2-5). This is especially comfortable for patients undergoing cancer treatment, as is the case with 5FU. Compliance is further improved in cases where the patient cannot tolerate oral or parenteral dosing (Wilkosz & Bogner, 2003).

In the next section, the barrier function of the skin will be explained by giving an overview of the skin structure.

1.6.5. Barrier function of the skin – skin structure

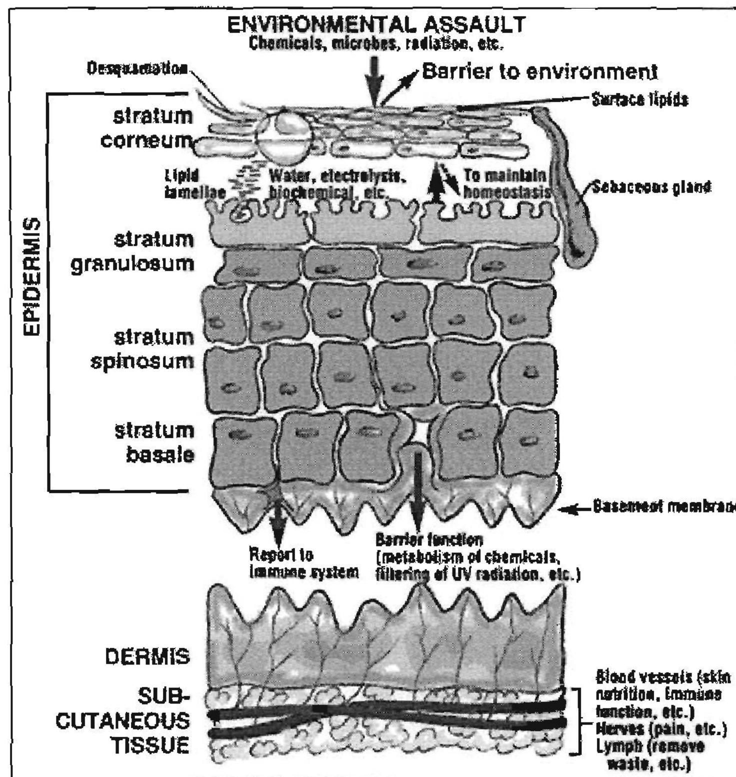


Figure 4: Skin components and functions performed
(Reprinted from Bogner & Wilkosz, 2003)

1.6.5.1. Stratum corneum

The outermost layer of the epidermis, the stratum corneum, is a specialised layer (Franz & Lehman, 2000:15) considered to be solely responsible for the barrier function of the skin (Naik *et al.*, 2000:318).

The stratum corneum consists of about 15 to 25 layers of cells that are arranged parallel to the skin surface (Wiechers, 1989:186). A simplified model of the structure of the stratum corneum is the "bricks and mortar" model, where the bricks represent the cells and the mortar is representative of the intercellular spaces (Franz & Lehman, 2000:25).

Lipid bilayers fill the intercellular spaces of the stratum corneum, constituting about 14% of the stratum corneum's weight (Franz & Lehman, 2000:21). Extraction of the lipids

shows a significant increase in skin permeability, leading to the conclusion that the lipids are largely responsible for the barrier function of the stratum corneum (Wiechers, 1989:186).

The distinguishing attribute that makes the bilayers so exceptionally impermeable, is that the hydrocarbon chains are nearly totally saturated, tightly packed in the intercellular spaces and are extremely ordered. The hydrophobic chains alternate with water-filled channels, which provide a polar pathway for permeants (Wiechers, 1989:186).

This lipid component of the stratum corneum is continuous and complex, with a long tortuous lipid pathway as result, while the protein compartment (consisting mainly of corneocytes) is interrupted by the lipid bilayers (Wiechers, 1989:186). Thus, the diffusion path length is much longer than would have been the case if such a structure would not have been present (Anon., 2005:www.nmsl.chem.ccu.edu.tw).

1.6.5.2. Other skin layers

Underneath the stratum corneum lies the more hydrophilic viable epidermis (Wiechers, 1989:187). The primary function of the viable epidermis is to provide the stratum corneum with terminally differentiated cells as the process of desquamation removes cells from the skin surface (Franz & Lehman, 2000:16). The viable epidermis does not possess any blood vessels; and all nutrients must diffuse into the epidermis from the underlying dermis (Zatz, 1993:13). This layer lacks the tightly packed cell layers of the stratum corneum, as well as the intercellular lipids, resulting in a mostly hydrophilic nature. Thus, resistance to penetration of polar compounds, such as 5FU, is greatly reduced compared to the stratum corneum (Wiechers, 1989:187).

The dermis, lying underneath the viable epidermis, constitutes the largest section of the skin, with the epidermal-dermal interface convoluted, creating the dermal papillae. These papillae contain capillary loops that reach to just beneath the interface with the epidermis (Franz & Lehman, 2000:24). Functions of the dermis include supplying the epidermis with nutrients, regulating body temperature and pressure, assisting the immune system to activate its defence forces and providing the skin with its mechanical strength. Lymph vessels in the dermis remove waste products (Barry 2002:502). The dermis also gives resilience to the skin, enabling it to return to its normal form after external forces have deformed it (Franz & Lehman, 2000:24). Compounds reaching the dermis are readily absorbed into the systemic circulation, encountering little resistance to

permeation (Wiechers, 1989:187), except for the obstacle posed by capillary walls that have to be crossed.

Along with the dermis, the underlying hypodermis (fatty layer) performs a mechanical function, cushioning and insulating the body against external forces (Zatz, 1993:24).

The skin also contains appendages, with the following types that can be distinguished: hair follicles, sebaceous glands, sweat glands (eccrine and apocrine) and nails (Zatz, 1993:13). These appendages do not play a great role in the transdermal absorption of 5FU.

As can be seen from the structure of the skin it is difficult for a compound, especially one as hydrophilic as 5FU, to cross the skin and reach the systemic circulation. It would, however, be advantageous to deliver 5FU transdermally, for reasons explained earlier. In addition, there are a number of physicochemical and physiological factors influencing the transdermal delivery of drugs that must be taken into account when attempting to deliver a drug transdermally. These will be discussed in the next section.

1.6.6. Fick's law of diffusion

Several factors indicate that the barrier function of the skin has a solely physicochemical basis, totally independent from living cells' activity (Hunter, 1973:342). Absorption through the skin is generally a passive process, with diffusion of penetrants taking place down a concentration gradient (Smith & Surber, 2000:23). Thus, physicochemical laws of penetration apply, with the stratum corneum acting as resistance to diffusion (Hunter, 1973:342). Fick's first law expresses the theory that the transfer rate of a penetrant per unit area is proportional to the concentration gradient (Barry, 2002:506). Solving this equation given by Fick's law could indicate mechanisms of skin penetration (Howes *et al.*, 1996:5) as well as suggest ways to optimise transdermal bioavailability (Naik *et al.*, 2000:320).

At steady state, the flux (transfer rate per unit of surface area) is given by the following equation (Wiechers, 1989:188):

$$J = \frac{(D.K_P)}{l}.\Delta C$$ **Equation 1**

where:

J	= flux, in $\mu g.cm^{-2}h^{-1}$
D	= diffusion coefficient, in cm^2h^{-1}
K_P	= partition coefficient between the vehicle and stratum corneum
ΔC	= concentration gradient across the stratum corneum, in $\mu g.cm^{-3}$
l	= thickness of membrane, in cm

According to this equation, flux across the skin can be enhanced by:

- Increasing drug diffusivity (D)
- Increasing the drug concentration in the applied vehicle
- Increasing the drug's partitioning into the stratum corneum (K_P)

In the described study, drug concentrations are constant in the formulations being compared, therefore any enhancement must be the result of a change in D or K_p.

Penetration enhancement will be discussed in more detail further on in this dissertation.

1.6.7. Factors influencing transdermal delivery

There is a variety of factors influencing the transdermal delivery of drugs. In the following table, some of these factors are named (Howes *et al.*, 1996:17), and some factors will be discussed in more detail.

Table 4: Factors influencing the transdermal delivery of drugs

	Factor(s)
Penetrant	• Partition coefficient • Molecular weight • Ionization state
Skin	• Species • Anatomical site of application • Temperature (skin and environment) • Age, gender and race • Hydration and state of stratum corneum • Metabolism in skin
Formulation	• Concentration of drug contained in formulation • Solubility / affinity of carrier for drug • pH

	• Volatility • Excipients included
Application of formulation	• Dose per unit skin area (influenced by the thickness of application) • Total area of skin in contact with formulation • Time of exposure

1.6.7.1. Physicochemical factors

The physicochemical properties of a penetrant have a very important influence on its penetration rate. As the thickness of the stratum corneum must be taken into account (see Equations 1 and 2), the thoroughfare of a penetrant through this layer will generally encompass partitioning from the vehicle into the stratum corneum, diffusion through the stratum corneum and finally partitioning into the underlying viable epidermis (Wiechers, 1989:188). These physicochemical characteristics are all inter-related.

The ideal values for the various parameters, as well as 5FU's values, have been stated in Table 3.

(a) *Partition coefficient (P or K)*

For a penetrant where the stratum corneum comprises the rate-limiting step (*i.e.* the most important barrier) in transdermal permeation, as is the case with hydrophilic drugs like 5FU, the partition coefficient between the delivery vehicle and the stratum corneum generally plays a major role in ascertaining flux through the skin (Barry, 2002:511). Drug molecules usually have to be freed from the delivery vehicle and partition into the lipophilic stratum corneum, before it can move through the skin (Naik *et al.*, 2000:319).

The partition coefficient tends to be lower when drug solubility in the vehicle is high (Barry, 2002:513). The relative affinities of the drug for the vehicle and for the stratum corneum play an important role in its partitioning into the stratum corneum. If the drug is soluble enough in the stratum corneum, it will ensure a net flow of drug molecules down the concentration gradient. When the drug's solubility in the stratum corneum is low or insignificant, there will be no or negligible flux. It can be concluded that, with increasing solubility in the stratum corneum, the partition coefficient will increase and so will the permeation rate (Smith & Surber, 2000:28). After crossing the stratum corneum, drug molecules must once again partition from the stratum corneum into the more hydrophilic

viable epidermis (Naik *et al.*, 2000:319). Therefore the partition coefficient must ideally be balanced to allow partitioning into and from both hydrophilic and lipophilic regions (Naik *et al.*, 2000:320).

The most frequently used expression of lipophilicity is the logarithm of the *n*-octanol-water partition coefficient (log $P_{oct/water}$). The reason for the popularity of this system is the likeness between the structure of *n*-octanol (a compound with a long hydrophobic chain and a polar hydroxyl group) and lipids found within cell membranes. As a result, the lipid/water partition coefficient is a fundamental parameter in determining drug permeability through the skin (Malan *et al.*, 2002:386).

It is estimated that a drug with a log $P_{oct/water}$ value of approximately 2, will be a good choice for potential delivery through the skin (Malan *et al.*, 2002:386). According to El Maghraby *et al.*, 5-FU has a log *P* value of -0.824 (El Maghraby *et al.*, 2005:184). Therefore, 5-FU might have difficulty partitioning into the skin on its own and additional methods for increasing skin penetration will have to be implemented.

(b) *Diffusion coefficient (D)*

The diffusion coefficient indicates the ability of a molecule to diffuse through a solvent system (Smith & Surber, 2000:29). The magnitude of this parameter (at constant temperature) is influenced by other physicochemical properties of the penetrant and solvent (in the case of transdermal delivery, the stratum corneum) and the degree to which the penetrant and the solvent interact with each other (Barry, 2002:511).

Phenolic compounds, such as 5FU, have experimental diffusion coefficients in the stratum corneum that are up to 10 000 times smaller than that of these compounds in water (Roberts *et al.*, 1978:488); thus not a very favourable diffusion coefficient for transdermal penetration.

After the penetrant has dissolved in the viable epidermis, it moves down the concentration gradient to the more aqueous dermis, where diffusion will be rapid compared to the tortuous pathways through the lipoidal stratum corneum (Hadgraft & Wolff, 1993:165).

(c) *Permeability coefficient (k)*

The permeability coefficient expresses the degree of permeability and is defined by the following equation (Wiechers, 1989:188):

$$k = \frac{K_P . D}{l}$$ **Equation 2**

where:

k = permeability coefficient

K_P = partition coefficient

D = diffusion coefficient

l = thickness of the stratum corneum

The permeability coefficient is influenced by the vehicle composition, chemical structure of the drug and the net charge of the molecule (or the distribution of the charge on the penetrating molecule) (Wiechers, 1989:188).

5-FU is a hydrophilic compound and has experimental permeation coefficients through untreated human stratum corneum ranging between 2.46 x 10^{-5} cm^{-1} and 3.50 x 10^{-5} cm^{-1} (Yamane *et al.*, 1995:237, 241). The major obstruction to permeation of hydrophilic drugs through the skin is usually inadequate partitioning from the vehicle into the stratum corneum, because it is difficult for polar molecules to penetrate the lipophilic domain found in the stratum corneum. Generally permeation increases with an increase in lipophilicity (*e.g.* an increase in chain length) (Wiechers, 1989:188).

(d) *Thermodynamic activity and concentration gradient*

Thermodynamic activity (leaving potential) is the inclination of a drug molecule to leave the delivery vehicle and partition into the stratum corneum. This is the main driving force for the partitioning of drug molecules (Smith & Surber, 2000:31).

It has been found that maximal flux of drug molecules is attained in a system that is thermodynamically stable (Barry, 2002:511). A saturated vehicle or solution is one such example. Maximal thermodynamic activity results in a maximal concentration gradient (Smith & Surber, 2000:32), and is independent of the type of vehicle and the drug's solubility there-in (Naik *et al.*, 2000:320).

Drug solubility in the delivery vehicle can be altered by using alternative solvents or a combination of solvents. Thermodynamic activity is influenced by changes in vehicle pH, the formation of complexes or micelles and the presence of surfactants and co-solvents (Barry, 2002:511). Once again, a balance between solubility in the vehicle and the stratum corneum must be maintained to ensure favourable partitioning of the drug into the stratum corneum (Naik *et al.*, 2000:320).

Apart from a saturated donor solution, sink conditions is another requirement for a maximal concentration gradient over the membrane. This ensures that the drug does not accumulate in the skin and the concentration gradient is maintained (Wiechers, 1989:188). Sink conditions were maintained in the present study by removing the entire receptor phase from the Franz diffusion cells on pre-determined time intervals and replacing it with fresh receptor solution.

(e) *Solubility*

Transcutaneous flux will increase with an increase in drug concentration in the applied vehicle. This can be done by using a vehicle with a high affinity (solubility) for the drug (Naik *et al.*, 2000:320).

A penetrant's solubility is influenced by various factors, including the amount of formulation applied, the solubility limit in the stratum corneum (Hadgraft & Wolff, 1993:161) and the physicochemical characteristics of the drug such as partition coefficient and the molecule's surface qualities. Seeing that these factors also influence absorption, there is a connection between the solubility of a penetrant and its absorption (Malan *et al.*, 2002:387).

5-FU is a polar molecule owing to its polar surface area of 65.7 Å. This value is calculated from the total sum of the areas of polar atoms or groups in the molecule's chemical structure. The amide and imide groups are the main contributors to 5FU's polar surface area (El Maghraby *et al.*, 2005:180).

Resistance to the solubility of a penetrant in the stratum corneum is dependent on its $\log P_{oct/water}$-value and melting point (Hadgraft & Wolff, 1993:161). According to Table 3, 5FU has an unfavourable $\log P_{oct/water}$-value and melting point, so it can be deduced that there will be considerable resistance to its solubility in the stratum corneum.

The higher the drug concentration in the vehicle the higher the diffusion gradient will be. Chemical properties of the penetrant and solvent will determine the extent of the penetrant's saturation solubility. A substance will be more soluble in a solvent with similar chemical properties than in one with contrasting properties (Smith & Surber, 2000:26-27).

A penetrant with a balanced solubility in both oil and water will be able to penetrate the stratum corneum to a greater extent than a penetrant with either high oil- or water solubility (Malan *et al.*, 2002:387). 5-FU has relatively low solubilities in both oil and water (Patrick *et al.*, 1997:40). Therefore a method must be found to enhance its transdermal absorption.

Another factor influencing the solubility of penetrants in the stratum corneum is co-diffusing components, such as penetration enhancers (*e.g.* propylene glycol and oleic acid). These components may increase the solubility parameter of the skin – this effect results in increased solubility of polar penetrants (such as 5-FU (Sloan & Beall, 1993:86)) (Hadgraft & Wolff, 1993:194).

(f) *Melting point (M_p)*

The melting point of a compound influences its solubility (Hadgraft & Wolff, 1993:194). It has been found that a lower melting point (lower than 200 °C) correlates with better solubility and penetration of the skin (Daniels, 2004). 5-FU's imide and amide groups form intermolecular hydrogen bonds, which leads to a high crystal lattice energy and gives rise to its high melting point (Sloan & Beall, 1993:86) of 280 °C (MSDS of 5FU). This in turn lowers solubility in the stratum corneum.

(g) *Molecular weight (M) and size*

Generally molecular weight is inversely related to the molecule's absorption rate. It is known that smaller molecules penetrate the skin faster than bigger molecules. This is because bigger molecules require larger openings to be created in the stratum corneum for it to diffuse into, with a reduction in permeability as result (Malan *et al.*, 2002:387).

There is also a relationship between the diffusion coefficient (*D*) and molecular weight (*M*) (Malan *et al.*, 2002:387):

$$D(M_t) = const$$ **Equation 3**

Within a small range of molecular size there is little correlation between molecular size and penetration rate (Malan *et al.,* 2002:387). The absorption rate of molecules with sizes up to 500 Dalton, and even molecules as big as 5000 Dalton, is insignificantly affected by molecular size (Wiechers, 1989, 190). Therefore 5-FU, with a molecular weight of only 130.08 Dalton (MSDS of 5FU), shouldn't be hampered by its size in penetrating the skin.

(h) *Ionization and pK*

Most drugs are weak acids or bases. Their aqueous solubility is a function of the pK_a or pK_b-value (ionization constant) and the pH at the vehicle-membrane interface (Malan *et al.*, 2002:388). Unionized molecules are more lipophilic in nature and ionized molecules tend to be more hydrophilic. Therefore unionized molecules penetrate the lipid membrane of the skin with greater ease (Smith & Surber, 2000:27). Ionized molecules have a propensity to use the intercellular spaces to diffuse through the stratum corneum, while unionized molecules more often diffuse via the intracellular route (Malan *et al.*, 2002:388). 5-FU is a diprotic acid and has pK_a-values of 8.0 and 13.0 (Singh *et al.*, 2005:99). It occurs in the neutral form at a pH of 7.4 (Da Costa & Moraes, 2003:58).

Not only physicochemical characteristics of drug molecules play a role in transdermal absorption, but physiological factors also have an important impact on absorption. These will be discussed in section 1.6.7.2.

1.6.7.2. Physiological factors influencing transdermal delivery

Physiological factors that play an important role in transdermal delivery of drugs include anatomical site, species variation, integrity of the skin, microcirculation and temperature, skin hydration (Wiechers, 1989:187-195) and skin metabolism (Barry, 2002:509-510).

Different anatomical sites on the body have varying permeabilities. This is due to the fact that the stratum corneum thickness varies from site to site and the spreading and density of appendages also vary (Wiechers, 1989:187-188). In this study, we used only Caucasian, female abdominal skin to exclude as many physiological variables as possible. Another variable compensated for in this study is temperature. Thermal analysis of epidermal lipids suggests that the enhancement of absorption with an

increase in temperature is a result of disordering in the skin's lipid structure (Wiechers, 1989:194). The diffusion cells in our studies were kept in a water bath, with the skin temperature maintained at approximately 32 °C. The water bath also helped to keep the skin hydration constant, eliminating another variable.

The skin's barrier function is primarily dependent on its integrity to function normally. Permeability through the skin can be increased by breaching its integrity by physical or chemical means, or because of a pathological condition (Wiechers, 1989:188). This physiological factor can be exploited to enhance the transdermal delivery of a chosen drug.

Therefore scientists have developed an array of alternative ways to increase the transdermal permeation of drugs by breaching the integrity of the stratum corneum. In the next section a few of these ways will be discussed.

1.7. Penetration enhancement

The formidable barrier of the stratum corneum is not easily breached. Scientists have implemented techniques to reversibly compromise the barrier for a certain period in order to increase drug permeation across the skin. Even so, the drugs able to penetrate the skin successfully are still limited to those within a narrow range of physicochemical properties (see Table 3). These properties usually correlate with an acceptable solubility (Daniels, 2004).

In order to minimize irritation and damage to the skin, the ideal properties of a penetration enhancer can be summarized as follows (Barry, 2002:522):

- Pharmacologically inactive.
- Compatible with all drugs and excipients.
- Able to formulate into different topical preparations.
- Non-toxic, non-allergenic and non-irritating.
- Takes effect immediately, with the required and predicted effect.
- Effect reversible when removed. Skin should recover its normal barrier function at once upon removal of the preparation.
- Good solvent for drugs.
- Colourless, odourless and tasteless.
- Affordable.

- Do not cause loss of body fluids, electrolytes or other endogenous materials.
- Cosmetically acceptable with regard to how it feels and its ability to spread.

Penetration enhancers can be sorted into three broad categories, depending on their mechanisms of action. Firstly, systems where there is an interaction between the penetrant and the formulation vehicle (Barry, 2002:521-523); secondly, physical ways to bypass the stratum corneum (electrically assisted and bypassing the stratum corneum) and lastly penetration enhancers that use chemical means to overcome the barrier function of the stratum corneum (Bogner & Wilkosz, 2003).

1.7.1. Interaction between penetrant and vehicle

1.7.1.1. Ion pairs

When ion pairs are used to enhance the penetration of a charged drug molecule, the drug is coupled with a molecule with an opposite charge, rendering the complex neutral. This complex penetrates the lipophilic stratum corneum easier than a charged molecule, where after the complex dissociates into the original molecules (Barry, 2002:521).

1.7.1.2. Supersaturated formulations

Supersaturated formulations employ another form of drug-vehicle interaction. A saturated formulation enhances transdermal flux by providing maximum thermodynamic activity and a favourable concentration gradient (Naik *et al.*, 2000:320).

1.7.2. Physical penetration enhancement

When using physical means to bypass the barrier situated in the stratum corneum, alternative energy sources are usually applied to drive the drug molecules through or past the stratum corneum (Bogner & Wilkosz, 2003). These methods are particularly useful in the case of larger, charged molecules (Daniels, 2004).

1.7.2.1. Electrically assisted

(a) *Iontophoresis*

This technique employs a low (approximately 0.5 $mA.cm^{-2}$), constant electrical current to force charged molecules through the stratum corneum. An electrode with a similar

charge as the penetrant is placed over a drug reservoir, with a grounding electrode somewhere else on the body to complete the circuit. The penetrant is forced into the underlying tissues by electro-repulsion (Barry, 2002:521). The amount of penetrant molecules crossing the membrane can be controlled by regulating the applied current (Daniels, 2004).

Another approach is to use electro-osmosis. Here, a flow of solvent across the membrane carries larger, uncharged or polar penetrant molecules along with it (Daniels, 2004).

Both electro-repulsion and electro-osmosis have been used to enhance the transdermal penetration of 5-fluorouracil's anionic and neutral forms (Fang *et al.*, 2004; Merino *et al.*, 1999).

(b) *Electroporation*

Electroporation is based on the creation of aqueous pathways because of short electric pulses. The pulses generate potentials of approximately 1 $V.cm^{-2}$ and last about 10 μs to 10 ms. Pores created like this are about 10 nm in diameter and can be present for several hours, but are not permanent. This creates a passage for big molecules to diffuse through (Kydonieus *et al.*, 2000:6). 5-Fluorouracil delivery has been improved by using electroporation combined with iontophoresis (Fang *et al.*, 2004).

(c) *Phonophoresis (ultrasound)*

When phonophoresis is employed to increase transdermal delivery of a drug, an ultrasonic device is used to massage the application site. The ultrasound disturbs the stratum corneum lipids and decreases the membrane's activation energy. There are two mechanisms by which the lipids are disturbed: cavitation and heating (Barry, 2002:521).

Cavitation is the process of creating gas bubbles in the membrane liquid, where after the bubbles collapse, causing holes to form between the corneocytes. As a result the intercellular spaces in the stratum corneum become larger and the lipid bilayers are disrupted, making it easier for molecules to penetrate the barrier (Daniels, 2004).

As the ultrasound wave propagates through the membrane, it loses energy to the skin through absorption and scattering. This causes the skin temperature to rise with several

degrees centigrade, increasing the fluidity of the stratum corneum, with an associated increase in the diffusion of molecules (Daniels, 2004).

1.7.2.2. Bypassing the stratum corneum

(a) *Microporation*

Microporation utilizes solid silica needles, coated with the drug, or hollow drug-filled metal needles. These needles only pierce the upper 10 μm of the stratum corneum, thus not reaching and stimulating the underlying, pain-registering nerves situated in the dermis (Bogner & Wilkosz, 2003).

(b) *Needleless jet injectors*

Needleless jet injectors use high pressure helium gas to force solid, fine drug particles through the stratum corneum. This method effectively combines the advantages of both the parenteral and transdermal delivery routes (Bogner & Wilkosz, 2003).

(c) *Other*

Other physical ways of bypassing the stratum corneum's barrier include high frequency oscillating needle bundles and epidermal erosion (Naik *et al.*, 2000:324).

1.7.3. Chemical penetration enhancement

Chemical penetration enhancers are substances that modify the skin's barrier to allow specific compounds to penetrate the skin faster and / or to a greater extent (Kydonieus *et al.*, 2000:6).

1.7.3.1. Partitioning promotion

The partitioning of molecules from the delivery vehicle into the stratum corneum generally plays an important role in the transdermal absorption of drugs, especially hydrophilic drugs such as 5FU. Solvents like alcohols, alkyl methyl sulfoxide and polyols (Daniels, 2004) increase a molecule's solubility in the stratum corneum by altering the stratum corneum's solvent characteristics. Thus, penetration is increased by a higher

drug concentration in the stratum corneum, brought about by an increase in partitioning from the vehicle (Barry, 2002:523).

1.7.3.2. Lipid, protein and corneocyte action

In Figure 5 it is shown how penetration enhancers disrupt the stratum corneum's highly ordered intercellular lipids (Barry, 2004:165-167). The structured lipid bilayers in these intercellular spaces present the primary barrier to the penetration of drug molecules. It also constitutes the major pathway through the stratum corneum. Various agents interfere with these layers, decreasing the efficiency of the barrier (Riviere, 1993:123).

Some solvents (*e.g.* dimethyl sulphate (DMSO), ethanol or a mixture of ethanol and methanol) extract the stratum corneum lipids, creating unnatural openings for molecules to penetrate into (Daniels, 2004).

Other substances, like oleic acid, isopropyl myristate (Daniels, 2004) or Azone® (Naik *et al.*, 2000:322) enter the structured bilayers and disrupt them, making the stratum corneum more fluid and thus decreasing the barrier function (Daniels, 2004).

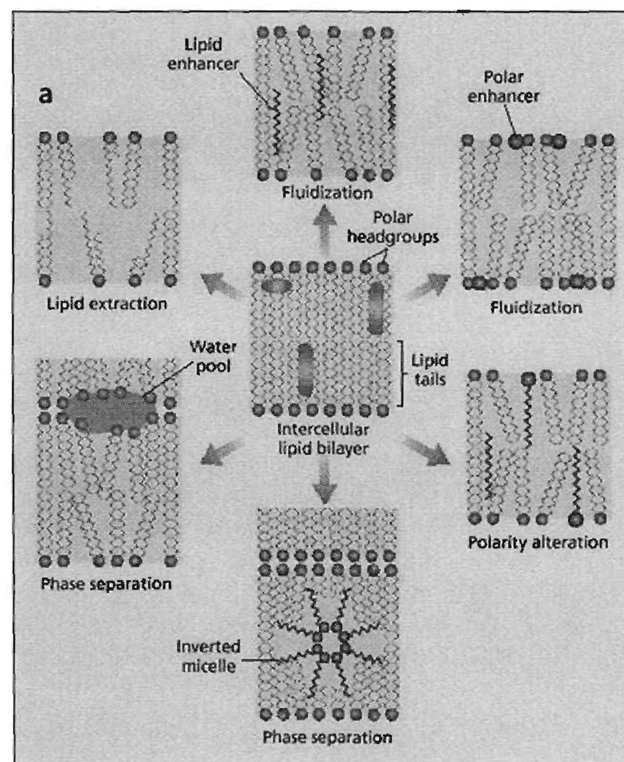


Figure 5: Chemical penetration enhancers affecting the stratum corneum lipids

(Reprinted from Barry, 2004:165-167)

Another mechanism affecting the lipid double layers is phase separation, as is the case with oleic acid. Oleic acid is in the liquid state midst in the lipid bilayers, creating permeable defects for polar compounds (such as 5-fluorouracil) at the interface between the lipids and the liquid oleic acid pools. The resistance to diffusion is lower at these interfacial defects, but pores aren't necessarily formed (Ongpipattanakul *et al.*, 1991:350). This could be advantageous because foreign molecules, for example viruses, will not be able to penetrate the skin along with the active.

Oleic acid

Oleic acid is a *cys*-unsaturated fatty acid, with a structure as depicted below (Figure 6):

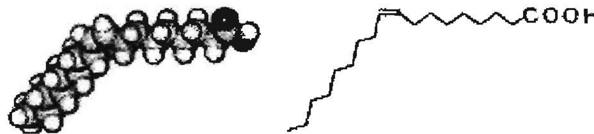


Figure 6: Chemical structure of oleic acid
(Reprinted from Yamane *et al.*, 1995:238)

This compound is known to enhance the penetration of polar to relatively polar substances (Ongpipattanakul *et al.*, 1991:350). It has been demonstrated on numerous occasions, by Bond & Barry (1988), Goodman & Barry (1989) and Turunen (1993) amongst others, that oleic acid also enhances 5-fluorouracil's penetration (Gao & Singh, 1998:46).

Oleic acid has two main mechanisms of action, both of which affect the stratum corneum lipids. Firstly it induces conformational changes in the lipids, and secondly it disrupts the highly ordered lipids through phase separation (Naik *et al.*, 1995:300).

The disruption of stratum corneum lipids is brought about by oleic acid lowering the lipid transition temperature (T_m) (Ongpipattanakul *et al.*, 1991:350). This means that the temperature where the lipids' physical form is changed from the ordered gel phase to the disordered liquid crystalline phase (Leekumjorn, 2004:5) is lowered.

Phase separation occurs because oleic acid exists in a nearly completely disordered state at the T_m of endogenic lipids. Therefore it is in a liquid state, forming pools

between the solid stratum corneum lipids. At the interfaces between these pools and the solid lipid phase, permeable interfacial defects are created, presenting shorter diffusion pathways for permeants, with less resistance (Ongpipattanakul *et al.*, 1991:350).

Protein and desmosome action:

Figure 7 shows the dramatic action that some penetration enhancers can have on protein structures and desmosomes. This, however, isn't clinically acceptable, because the stratum corneum is split into additional squamous cells (Barry, 2004:165-167).

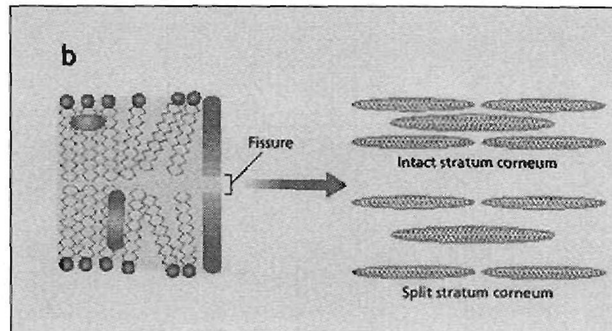


Figure 7: Chemical penetration enhancers affecting the stratum corneum proteins and desmosomes (Reprinted from Barry, 2004:165-167)

Figure 8 shows the action of penetration enhancers inside corneocytes. Although not so drastic, this is also not cosmetically acceptable (Barry, 2004:165-167). When proteins are denatured, damage to the skin is permanent. Ionic surface active agents like DMSO and urea are examples of such penetration enhancers. They open the complex packing of keratin inside the corneocytes, making it more permeable. These substances mainly benefit molecules utilizing the transcellular route (Daniels, 2004).

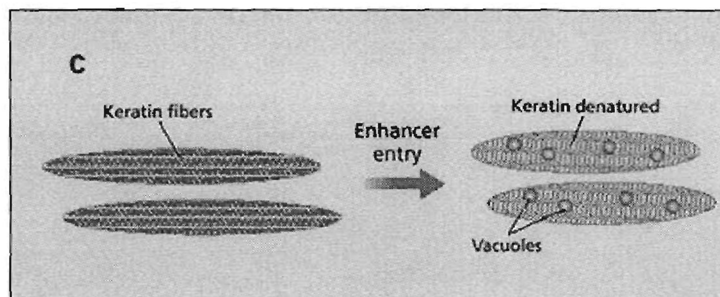


Figure 8: Chemical penetration enhancers affecting the stratum corneum keratin (Reprinted from Barry, 2004:165-167)

1.7.3.3. Hydration (occlusion)

The stratum corneum is a relatively dry tissue. When occluded, water from the underlying viable epidermis continues to diffuse upwards, but does not evaporate from the skin surface. This results in a hydrated stratum corneum, with a possibly increased reservoir for certain penetrants. The water opens up the structure of the stratum corneum, affecting the penetration of especially non-polar substances (Riviere, 1993:115). Water is the ideal penetration enhancer when it comes to efficacy and safety (Wilkosz & Bogner, 2003), because it is an endemic substance to the skin. The efficacy of 5-fluorouracil in the treatment of severe psoriasis has been found to increase significantly with occlusion (Patrick *et al.*, 1997:40), although there is a possibility of developing an inflammatory reaction (Spencer, 2006).

1.7.3.4. Vesicles and particles (colloidal systems)

Colloidal particles in the submicron range are used to deliver entrapped drug molecules into the skin (Daniels, 2004). They are used for optimized and controlled drug delivery (Daniels, 2001), by providing enhanced efficacy and a reduced side-effect profile of entrapped drugs (Grobler *et al.*, 2006). These particles include liposomes, Transfersomes®, niosomes, sphingosomes, micro-emulsions, lipid nanoparticles and the Pheroid™.

Possible mechanisms of action of colloidal carriers are thought to be the following:

- Intact colloidal particles penetrate the skin and carry the entrapped drug with them.
- Colloidal particles dissociate when in close proximity of the skin surface. The entrapped actives are then absorbed into the skin together with components of the colloidal particles, or
- After the colloidal particle-containing vehicle is applied, the entrapped active partitions out of the vehicle into the skin on its own, crossing the barrier situated in the stratum corneum (Alvarez-Román *et al.*, 2004:309).

(a) *Liposomes*

Liposomes are spherical phospoholipid vesicles that form spontaneously, consisting of a single, or multiple lipid bilayer membranes. These bilayers encapsulate a portion of the solution that they are suspended in. Liposomes occur naturally, but can also be manufactured (Lasic, 1998:307-309). They can entrap hydrophilic drugs in their

aqueous core, or lipophilic drugs in the lipid bilayer membrane (Elishalom, 2005). Several groups have studied the entrapment of 5FU in liposomes for transdermal delivery, *e.g.* Da Costa & Moraes, 2003 and El Maghraby *et al.*, 2001.

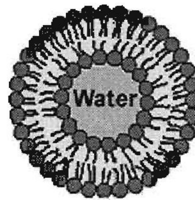


Figure 9: Schematic illustration of a liposome (Daniels, 2001)

(b) *Transfersomes®*

Similar to liposomes, Transfersomes® also consist of lipid bilayers, encapsulating a hydrophilic core (Gevc, 2005). It is claimed that Transfersomes® are ultra-deformable liposomes (Barry, 2002:523). They have extremely flexible, self-regulating membranes, able to retain their integrity amidst extreme changes in shape as they move through the intercellular spaces in the stratum corneum (Gevc, 2005).

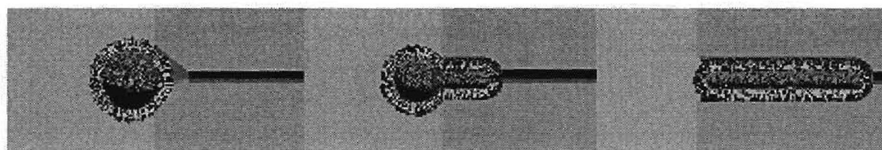


Figure 10: Illustration of a Transfersome® moving through an intercellular space (Gevc, 2005)

(c) *Niosomes and sphingosomes*

Niosomes and sphingosomes are modifications of liposomes, containing alternative bilayer-forming substances instead of phospholipids (Leekumjorn, 2004:10; Daniels, 2001). In niosomes, non-ionic surfactants are responsible for the formation of the bilayers (Leekumjorn, 2004:10) together with, or without, cholesterol and dicetyl phosphate (Shahiwala & Misra, 2002:220), while sphingolipids form the bilayers in sphingosomes (Daniels, 2001).

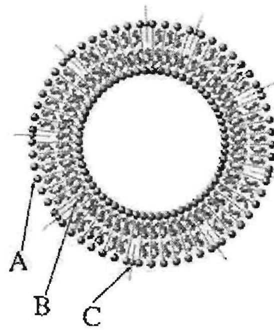


Figure 11: A schematic illustration of a niosome, where A is SPAN, B is Cholesterol and C is Dicetyl Phosphate (Reprinted from Leekumjorn, 2004:17)

(d) *Micro-emulsions*

Also referred to as nano-emulsions, these are oil in water emulsions, where a single lipid layer encloses a fluid lipid centre, in contrast to a liposome's bilayer-enclosed hydrophilic core. Therefore, micro-emulsions might be more suitable for the delivery of lipophilic drugs. Drop diameter ranges between 50 and 1000 nm, with an average of 100-500 nm (Daniels, 2001).

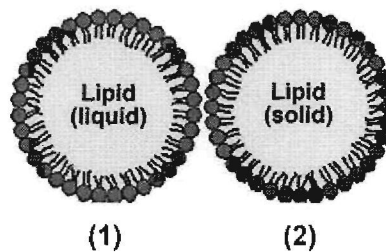


Figure 12: A schematic illustration of (1) a micro-emulsion drop and (2) a lipid nanoparticle (Daniels, 2001)

(e) *Lipid nanoparticles*

Lipid nanoparticles are similar to nano-emulsions, differing only in the fact that nanoparticles have solid lipid centres, as opposed to the fluid cores in nano-emulsions (Daniels, 2001).

(f) *Pheroid™ delivery system*

Pheroid™ particles are stable circular structures in a unique, emulsion-like formulation, and fall in the submicron range. Plant- and essential fatty acids are the main

components in the Pheroid™. Although Pheroid™ particles may resemble other lipid-based vehicles, such as the afore-mentioned, they are unique in the sense that they have inherent therapeutic qualities as well (Grobler, 2005:4).

It was decided to study the influence of the Pheroid™ on the transdermal delivery of 5FU. In section 1.7.4 a more detailed description of the Pheroid™ system will be given.

1.7.4. Pheroid™ delivery system

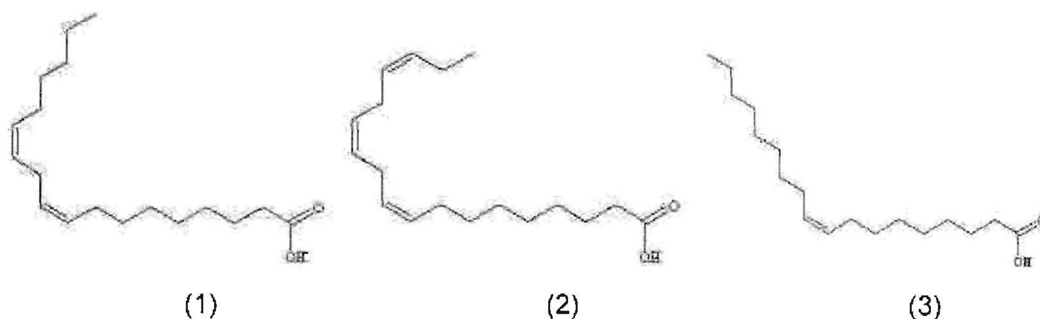
The Pheroid™ delivery system, formerly known as Emzaloid™, is patented in South Africa, China, Europe and the USA (Grobler et al., 2006).

Piet Meyer, founder of MeyerZall (Pty) Ltd., developed Pheroid™ technology to treat the psoriasis he suffered from. In December 2003, the North-West University (South Africa) procured the intellectual property for this technology from MeyerZall (Grobler et al., 2006).

1.7.4.1. Composition

Pheroid™ particles do not contain cholesterol or phospholipids, as is the case with liposomes. Furthermore the Pheroid™ system is comparable to micro-emulsions. It does not only have two liquid phases, though – it has a third phase, namely the dispersed N_2O-gas phase associated with the fatty acids in the dispersed phase (Grobler *et al.*, 2006).

The main components of these structures are the ethyl esters of the essential fatty acids linoleic acid and linolenic acid, as well as the *cys*-form of oleic acid and water. The formulation is saturated with nitrous oxide (N_2O) (Saunders *et al.*, 1999:99).



(1) (2) (3)

Figure 13: The kinked structures of the essential fatty acids linoleic acid (1), linolenic acid (2), as well as oleic acid (3)
(Reprinted from http://www.supplementquality.com/news/fatty_acid_structure.html)

The essential fatty acids used in the Pheroid™ formulation are important for cellular functions but cannot be manufactured by the body. Their functions include homeostasis of energy, modulating the immune system, maintaining the integrity of cell membranes and regulating some components of programmed cell death. Thus the Pheroid™ delivery system contributes greatly to the normal functioning of cells as well (Grobler, 2005). The cys-form of the fatty acids (the "kinked" form) is responsible for membrane flexibility (Anon, 2007:www.supplementquality.com).

N_2O, an anaesthetic that is both lipid and water soluble, has at least three known functions in the formulation, namely helping with the self-assembly of Pheroid™ vesicles and the miscibility of the fatty acids, as well as playing a role in the stability of Pheroid™ vesicles or microsponges that are formed (Grobler *et al.*, 2006).

1.7.4.2. Structure and types of Pheroid™ particles

The Pheroid™ formulation can be specifically manipulated to yield different types of vesicles, ensuring a fast transport rate, high encapsulation efficiency, rapid delivery and stability of the delivery system for a specific drug (Grobler, 2005). The manipulation is done in terms of size, function, morphology and structure of the vesicles (Grobler *et al.*, 2006).

Three types of Pheroid™ particles can be classified:

- Basic Pheroid™ vesicles with an average diameter of 200-440 nm (Grobler *et al.*, 2006). The vesicle consists of a highly elastic double layer membrane with the lipids packed loosely (Grobler, 2005).
- Very small, sponge-like vesicles with a diameter of less than 30 nm (Grobler, 2005). These nanosponges can entrap hydrophobic molecules in their membrane, while small hydrophilic molecules can be entrapped within the aqueous central area (Grobler *et al.*, 2006).
- Pro-Pheroid™ spheres, or reservoirs, ranging from 5 to 100 µm in diameter, serving as a depot. They have a hydrophobic centre, where the Pheroid™ lipid phase is entrapped, surrounded by a hydrophilic area. The hydrophilic area serves as a vesicle forming and release zone. The vesicles are gradually released to attain continuous release (Grobler *et al.*, 2006).

1.7.4.3. Interaction of Pheroid™ particles with the skin

There are two possible mechanisms suggested for the interaction between the Pheroid™ delivery system and human skin.

Firstly it is proposed that the N_2O and unsaturated fatty acids in the formulation increase membrane and intercellular lipid fluidity, resulting in an increased transdermal transport rate of entrapped substances (Saunders *et al.*, 1999:105). Cell membranes in the skin are rich in lipids, providing an ideal place for N_2O to accumulate, leading to the increase in fluidity. With the increase in fluidity, there is also an increase in lateral movement of lipophilic molecules, or hydrophilic molecules that are associated with the fatty acids, into neighbouring cells (Grobler *et al.*, 2006). The N_2O also causes an increase in Pheroid™ membrane elasticity, enabling pheroid vesicles to penetrate the intercellular spaces of the stratum corneum as intact structures and thereby delivering entrapped substances to the dermis (Saunders *et al.*, 1999:105).

In the second place, freeze fracture electron microscopy has shown both small and big vesicles present within human stratum corneum. This has led to the hypothesis that separate molecules present in Pheroid™ vesicles, cross the intercellular spaces in dispersed form and reassemble in favourable spaces within the stratum corneum to form vesicles again (Saunders *et al.*, 1999:105).

Furthermore the possibility exists that oleic acid also plays a role in the increased transdermal delivery of substances entrapped within Pheroid™ vesicles, although it makes up only as little as 0.02% of the formulation. Oleic acid is a known penetration enhancer, described earlier in this dissertation.

1.7.4.4. Comparison of the Pheroid™ system with other delivery systems

Table 5: Comparison of the Pheroid™ system to other delivery systems (Modified from Grobler, 2005)

Pheroid™	Other lipid-based delivery systems
Comprises mainly essential fatty acids – a natural and important part of the human body.	Usually contain substances foreign to the human body, such as lysolecithin, artificial polymers or phosphatidylcholine.
Various types of Pheroid™ vesicles can be formulated, depending on the method of manufacturing. There are three main types distinguished: • Lamellar vesicles • Depots • Microsponges with the latter two types providing extended release according to a concentration gradient.	Different types of liposomes have been achieved, including: • Single lamellar vesicles • Multi-lamellar vesicles • Multivesicular vesicles • Nanosomes • Transfersomes® • Spingosomes
Can be manipulated in terms of size, lipid composition, membrane packing and charge. Can be optimised for a specific drug and / or indication and can be reproduced in a repeatable manner.	Liposomal systems, sizes and types are not easily repeatable. Big size variations have to be limited by filtration.
Components form part of the natural biochemical pathways, thus the Pheroid™ system is not cytotoxic and helps with maintenance of cell membranes.	Cytotoxic reactions to liposomes have been described, although liposomes decreasing cytotoxicity of drugs have also been described.

Pheroid™ vesicles consist of fatty acids, and therefore have an affinity for cell membranes. Cell membranes have a specific group of proteins responsible to bind fatty acids and absorb them into the cell. This ensures that Pheroid™ vesicles follow the endosome sorting mechanism and move through the cell membrane. The result is a fast binding and uptake rate of the Pheroid™ compared to diffusion over cell membranes.	Binding and uptake mechanisms into cells have not yet been clarified for other delivery systems, including liposomes. The mechanism is thought to be mostly by diffusion.
Aids in reducing and / or eliminating drug resistance, via a mechanism still being explored. The mechanism is possibly by releasing entrapped drugs inside cells, avoiding drug efflux pumps situated in resistant cells' membranes (Grobler, 2005). This mechanism of resistance is known to be a great obstacle in the efficacy of 5FU therapy (Van Triest, *et al.*, 1997:1856).	Many delivery systems are known to induce adverse reactions or drug resistance, mainly because drugs are still subjected to efflux pumps situated in the cell membranes, which are responsible for resistance.
Entrapment efficiency of all substances tested ranged between 85-100 %. Thus very efficient.	Other delivery systems often have problems entrapping some actives, as result of charge and steric limitations associated with the delivery system.
Pheroid™ vesicles are already sterically stabilized, because of their composition, without loss of elasticity or increase in vesicle size.	In most cases, delivery systems such liposomes, have to be sterically stabilized by means of pegylation for example. This usually results in loss of elasticity and an increase in vesicle size. Transfersomes® were developed to address this problem, but the manufacturing process is very complicated.
The formulation contains no cholesterol, but still maintains a favourable inner volume. Pheroid™ membranes have a high grade of elasticity and are able to extravasate to or from the vascular system, making this a useful delivery system for antineoplastic drugs. Pheroid™ particles have a relatively high transition temperature, without compromising inner volume, stability or entrapment efficiency. As a result Pheroid™ vesicles are ideal for rapid release, while the possibility for sustained release is still available with pro-Pheroid™ depots.	Cholesterol and phospholipids are important components of most other lipid-based delivery systems. Cholesterol serves to maintain the inside of vesicles and stabilize vesicles. These components cause the liposomal systems to become less fluid and elastic. Phospholipids have a higher phase transition temperature than the fatty acids used in the Pheroid™, causing even more loss in fluidity and elasticity – the result is more rigid vesicles, unable to change shape and move through intercellular spaces.

Proof has been presented that the bioavailability of drugs entrapped in the Pheroid™ system increase after oral, buccal and topical administration	Absorption through most biological barriers has been increased by some liposomal systems, although some systems have decreased absorption.
The loading capacity of Pheroid™ vesicles is determined by the type of Pheroid™ formulated to entrap a certain active. Micro- and nanosponges have especially high loading capacities.	In general other lipid-based systems have loading capacities dependent on the interior volume or volume inside the membrane, limiting their use.
The Pheroid™ vesicle is poliphilic, which means actives with differing solubilities, or even insoluble substances, can be entrapped.	Most other systems are either hydrophilic or lipophilic, limiting their use as well.
Drugs entrapped in Pheroid™ vesicles tend to have a smaller volume of distribution than expected from small molecules, leading to a higher concentration at the intended place of action. This means an enhanced, narrow therapeutic index can be reached and toxic effects on other, non-targeted tissues are reduced. When delivering a cytotoxic drug such as 5FU, this is very important.	Liposomes act in a similar way to the Pheroid™, resulting in a higher concentration of drug in tumors (in the case of cytotoxic agents) and a reduction in toxic effects on other tissues.

1.7.4.5. Advantages of the Pheroid™ system as transdermal drug delivery system for 5FU

5FU is an anticancer drug, with severe systemic side-effects (Yen & McLeod, 2007:1011) and is subjected to extensive hepatic metabolism when administered parenterally (Gao & Singh, 1998:46). Furthermore 5FU has erratic bioavailability after oral administration (Singh *et al.*, 2005:99). It would therefore be advantageous to deliver 5FU via the transdermal route. When drugs are delivered transdermally, variation in bioavailability and metabolism is decreased and systemic side-effects are also reduced (Wiechers, 1989:185). 5FU is a polar, hydrophilic compound, having difficulty in crossing the skin barrier (Singh *et al.*, 2005:99). Therefore it is necessary to make use of a penetration enhancer in its transdermal delivery. In the light of the new technology available in the form of the Pheroid™ system, it was decided to explore the effect of the Pheroid™ on the transdermal delivery of 5FU.

There are some advantages of using the Pheroid™ system as transdermal delivery system of 5FU:

- A lower concentration of active is needed for the required response (Saunders *et al.*, 1999:106) possibly lowering the occurrence of side-effects as well as limiting associated costs (Grobler, 2005).
- Application to the skin isn't limited to topical effects – transdermal effects are also made possible by using Pheroid™ technology (Grobler, 2005). This helps to overcome the problems encountered with other delivery routes.
- The added advantage of topical application lies in the fact that lipids in the skin are restored by the fatty acids in the Pheroid™ formulation, without the irritation caused by a number of other penetration enhancers; the skin's permeation barrier is maintained (Saunders *et al.*, 1999:106).
- Reduced toxicity as result of effects on non-targeted tissues, because the Pheroid™ delivery system provides targeted delivery to some extent (Grobler, 2005).
- Pheroid™ vesicles have been shown to penetrate most barrier cells, the stratum corneum, as well as keratinised tissues (Grobler, 2005).
- The pro-Pheroid™ option allows sustained release (Grobler, 2005).
- Pheroid™ technology reduces and even eliminates drug resistance (Grobler, 2005), which is a great obstacle in the success of 5FU treatment.
- Poorly soluble or insoluble actives can be effectively entrapped and delivered by Pheroid™ vesicles (Grobler, 2005).

2. Summary

The main objective of this study was to improve the transdermal penetration of 5-fluorouracil (5FU), a pyrimidyne analogue used in the treatment of several solid tumours and skin malignancies.

5FU is currently available in oral and parenteral dosage forms, but there are several problems associated with these delivery routes. When administered parenterally 5FU is rapidly metabolised in the liver and can cause severe side-effects, mainly because of toxicity caused in non-targeted organs and tissues. Parenteral delivery is also very invasive, decreasing patient compliance. After oral administration, bioavailability is erratic and thus cannot be trusted. 5FU is also swiftly metabolised in the liver after oral administration, via the first-pass effect. Transdermal delivery is therefore a viable option.

Some transdermal 5FU products are available commercially, but their use is limited because of insufficient patient compliance caused by the severe irritation that develops at the site of application. Currently, the success of topical 5FU therapy is measured by the degree of erythema and crust formation, with these signs indicating successful therapy. As a result, patients seldom complete therapy and drug resistance is increased.

Transdermal delivery of any drug, especially one as hydrophilic as 5FU, is a troublesome process, due to the nearly impenetrable barrier situated in the stratum corneum. Furthermore, 5FU has a high melting point and relatively low general solubility, which cause more problems when attempting to deliver it via the transdermal route.

The new technology situated in the Pheroid™ formulation presents a solution to the problems stated above. Pheroid™ vesicles are stable, spherical structures in a submicron emulsion-like system. They consist of lipid bilayer membranes encapsulating a hydrophilic interior. The main components are the ethyl esters of linoleic and linolenic acid (two essential fatty acids that cannot be manufactured in the body), as well as the known penetration enhancer oleic acid. A third, dispersed N_2O-gas phase adds another dimension to Pheroid™ technology. Pheroid™ vesicles are able to entrap hydrophilic or even insoluble drugs in their interior, as well as lipophilic drugs that associate with the lipid bilayer, while being compatible with cell membranes in the skin. A previous study (Kilian, 2004) indicated that the Pheroid™ delivery system might be effective in the

transdermal delivery of 5FU). Therefore the hypothesis was formulated that the Pheroid™ delivery system could increase the transdermal delivery of 5FU.

The main objective of this study can be stated as follows:

- To determine the influence of the Pheroid™ delivery system on the transdermal delivery of 5-fluorouracil.

A journal article is included in this dissertation, wherein a summary of methods, results and conclusions concerning this study can be found.

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TRANSDERMAL DELIVERY OF 5-FLUOROURACIL WITH PHEROID^{TM} TECHNOLOGY.

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ABSTRACT

In this study the aim was to examine the influence of the novel drug delivery system, Pheroid™ technology, on the transdermal permeation of 5-fluorouracil (5FU). Pheroid™ vesicles are stable spherical structures in the submicron range. The investigation was performed by *in vitro* diffusion studies utilising Franz diffusion cells and heat separated epidermis. Formulations containing either 0.5 % or 1 % 5FU in a phosphate buffer solution, water or Pheroid™ solution were applied to the epidermis and allowed to permeate for a period of 12 hours. Samples taken were then analysed by high performance liquid chromatography. The results indicated that the Pheroid™ system indeed improves transdermal permeation of 5FU.

5-Fluorouracil
Franz diffusion cell
Heat separated epidermis
Skin penetration
Transdermal
Drug delivery system
Pheroid™

1. Introduction

5-Fluorouracil (5FU) is a pyrimidine analogue (1). As one of the first rationally-designed antimetabolites, synthesised for the first time in 1957 (2), 5FU is indicated for the therapy of proliferative skin diseases such as actinic keratosis (AK), superficial basal cell carcinoma and psoriasis (3) and has been used for the treatment of solid tumours like colorectal, breast and liver carcinomas for nearly 40 years (1).

Although 5FU has always been administered parenterally and orally, metabolism is rapid and absorption is erratic (4). Several severe side-effects are also commonly associated with 5FU therapy, including myelosuppression, hand-foot syndrome and gastro-intestinal effects (5). Seeing that 5FU is an important part of the treatment of several malignant and pre-malignant disorders (1, 3), it would be advantageous to find a delivery route and delivery system that can replace existing parenteral and oral dosage forms and decrease side-effects.

The transdermal route provides a promising alternative to the above-mentioned conventional delivery routes, solving most of the problems associated with parenteral and oral administration (6). That being said, the formidable barrier situated in the skin is not easily breached. The stratum corneum, the outermost skin layer, is mostly lipophilic in nature (7), preventing hydrophilic molecules such as 5FU (4) from entering.

5FU-containing creams and lotions are currently commercially available, but absorption is still very limited. Transdermal delivery from these formulations has been compared to delivery by new transdermal delivery vehicles, with the newer formulations proving to be promising. One such study was done by Levy *et al.*, where a commercially available 5 % 5FU cream's transdermal permeation was compared to that of three 0.5 % creams where 5FU was incorporated into a porous microsphere system. The researchers concluded that the 0.5 % creams offered better targeted drug delivery (8). Another clinical study was conducted by Loven *et al.* (9), comparing the efficacy and tolerability of a 5 % 5FU cream to that of a newly-developed 0.5 % 5FU cream. It was found that the 0.5 % cream was at least as effective as the 5 % cream, while being more tolerable to patients (9). Another study done on 5FU was by Kilian, who investigated 5FU in lamellar gel phase systems and 5FU entrapped in Emzaloid™ spheres (now called Pheroid™). It was found that both the delivery systems might increase the transdermal permeation of 5FU, with the Pheroid™ formulation providing the highest average flux of 5FU (10).

Pheroid™ vesicles are stable spherical structures in a unique, emulsion-like formulation, and fall in the submicron range. This delivery system is patented in Europe, China, USA and South Africa (11).

The main components of the Pheroid™ system are the ethyl esters of the essential fatty acids linoleic acid and linolenic acid, as well as the *cys*-form of oleic acid, and water. The formulation is saturated with nitrous oxide (N_2O) (12).

Although Pheroid™ vesicles may resemble other lipid-based vehicles, such as liposomes and micro-emulsions, they are unique in the sense that they have inherent therapeutic qualities as well. The Pheroid™ formulation can be specifically manipulated to yield different types of vesicles, ensuring a fast transport rate, high entrapment efficiency, rapid delivery and stability of the delivery system for a specific drug (11). The manipulation is done in terms of size, function, morphology and structure of the vesicles (13).

In this study, 5FU was entrapped in a Pheroid™ formulation. Transdermal permeation studies were then performed to evaluate the influence of this delivery system on the transdermal flux of 5FU. The influence of phosphate buffer solution (PBS) as receptor solution and basis for the Pheroid™ formulation as compared to that of water was also investigated. At first PBS was used as receptor phase, but it was later discovered that PBS might interfere with the high performance liquid chromatography analysis of 5FU. PBS was then replaced with deionised water.

2. Materials and methods

2.1. Materials

5-Fluorouracil (5FU; MW = 130.08) was purchased from Fluka (Steinheim, Switzerland). HPLC-grade water (double distilled, deionised) was prepared using a Milli-Q purification system (Millipore, Milford, USA) and was used in all studies conducted as well as all analyses done. Phosphate buffer solution (PBS) was made using the salts disodium orthophosphate dehydrate ($Na_2HPO_4.2H_2O$) and sodium dihydrogen orthophosphate dehydrate ($NaH_2PO_4.2H_2O$). These were obtained from Merck Laboratories, Midrand, South Africa. Solvents and analytes used were all of HPLC-grade. Phosphoric acid, acetonitrile (ACN) and isopropyl alcohol used in the HPLC analyses were obtained from VWR International, Poole, England.

Pheroid™ solutions were prepared by the subprogram: Drug Delivery of the Unit for Drug Research and Development, North-West University, South Africa.

Excised abdominal skin was obtained from Caucasian female patients (Pretoria, Boksburg, Johannesburg – South Africa) having undergone cosmetic surgery. Skin was frozen at -20°C within 2 hours of excision. Studies were carried out under approval from the ethics committee of the North-West University (reference number 04D08).

2.2. Methods

2.2.1. Skin preparation:

Excised skin was thawed overnight at room temperature before further preparations. Excess adipose tissue was removed with a scalpel, yielding full thickness skin. Skin was then immersed in 60 °C water for 60 seconds (according to the heat separation technique described by Howes *et al.* (14)). The epidermal layer was gently teased from the underlying skin layers, where after it was floated on HPLC-grade water with the stratum corneum facing upwards, and secured on Whatman® filter paper for ease of handling. The epidermis was left to air dry overnight before being stored in aluminium foil and airtight plastic bags at -20 °C until further use. Before commencement of diffusion studies, prepared skin was allowed to thaw at room temperature and then cut into circles with an approximate radius of 75 mm. Skin was inspected prior to diffusion studies to ensure that integrity was maintained, taking care that no holes or flaws were present in skin pieces used.

The skin of 5 different donors was used throughout all the studies, with each study having at least 3 Franz cells with skin from every specific donor. Thus there was continuity through the studies in so far as skin differences were concerned.

2.2.2. Entrapment of 5FU in Pheroid™ vesicles

Entrapment of 5FU was affirmed by inspection using confocal laser scanning microscopy (CLSM). Fig. 1 shows confocal inmages of the Pheroid™ vesicles with and without entrapped 5FU. The microscope acts as an optical knife through the use of laser as an energy source. The sample can be optically sectioned at different depths (12).

Pheroid™ vesicles were marked with fluorophore Nile red, which associates readily with lipid rich areas, such as the Pheroid™ bilayer and emits at a wavelength of between 568 nm and 650 nm (12). There was no need to label 5FU with a fluorescent marker, because 5FU is an auto-fluorescent molecule when using reflectance mode in association with a short wavelength laser.

The confocal system used was a Nikon PCM 2000, equipped with a Hamamadzu CCD camera and two lasers – Helium/Neon (red) and Krypton/Argon (green). Excitation wavelengths were 505 nm and 488 nm for the red and green lasers, respectively, and emission wavelengths were 568 nm and 515 nm, respectively. The microscopic system also utilised real-time imaging, a 60x 1.4D ApoPlanar oil immersion objective and a medium pin-hole (10 µm).

2.2.3. Diffusion studies

2.2.3.1. Preparation

PBS was prepared by accurately weighing 9.2 g $Na_2HPO_4.2H_2O$ and 2.1 g $NaH_2PO_4.2H_2O$. These were then made up to 1000 ml with HPLC-grade water and sonicated to ensure that no air was trapped in the liquid.

For the positive control studies 5FU was accurately weighed and added to the PBS or HPLC-grade water to make up 1 % 5FU solutions.

For the other diffusion studies, 5FU was weighed and combined with prepared Pheroid™ solution. The solution was left to shake gently for 24 h to ensure acceptable entrapment of 5FU in Pheroid™ vesicles.

Glass Franz diffusion cells (Perme Gear Inc., Bethlehem, PA, USA) were used in the diffusion studies. Skin samples were clamped between the donor and receptor compartments with the stratum corneum facing the donor compartment. High vacuum grease was applied to the junction of the donor and receptor compartments to ensure that it is properly sealed.

2.2.3.2. Equilibration

Diffusion cells were placed in a water bath and maintained at approximately 37 °C. This resulted in a skin temperature of approximately 32 °C, simulating the skin's normal physiological temperature (15). The receptor liquid used was either PBS or HPLC-grade water, depending on the donor phase. The receptor phase was continuously stirred by a magnetic bar at 950 rpm (using a Variomag® stirrer plate). Skin samples were hydrated (equilibrated) for an hour by applying 1 ml PBS or HPLC-grade water to the donor compartment and 2 ml in the receptor compartment, using an Eppendorf® micropipette.

2.2.3.3. Franz cell diffusion study

The liquid in the donor compartment was removed after an hour and replaced with 1 ml 5FU-containing solutions, at the same temperature as the Franz diffusion cells. Donor solutions were 5FU dissolved or entrapped in one of the following: PBS, water, PBS-based Pheroid™ solution or water-based Pheroid™ solution. Donor compartments were occluded with Parafilm® to prevent evaporation of donor solution.

Liquid in the receptor compartment was replaced with fresh PBS or water, while ensuring no air pockets were trapped underneath the skin. Receptor phases were withdrawn at predetermined intervals (15, 30, 45 min; 1, 2, 3, 4, 5, 6, 8, 10, 12 h) and replaced with 2 ml fresh PBS or water (at the same temperature) to maintain sink conditions. Samples were stored at 5-8 °C until HPLC analysis was performed.

2.2.4. High performance liquid chromatography (HPLC) analysis:

An Agilent® 1100-series HPLC system was used to perform HPLC analysis. The system utilises an isocratic pump, a variable wavelength UV detector, autosampler and Chemstation® Rev. A08.03 software for data acquisition and analysis. The HPLC method was developed with the help of Prof. Jan du Preez (Analytical and Technical Laboratory, North-West University) and was the same as the method used by Kilian (10).

Samples were analysed using a Phenomenex® Synergi 4u Fusion-RP 80 column with a mobile phase flow rate of 1 ml/min. The wavelength of detection was set at 266 nm. The mobile phase consisted of 30 ml acetonitrile and 1 ml 10 % ortophosphoric acid in a final volume of 1000 ml, the diluent being HPLC-grade water. The solution had a pH of approximately 3 and was sonicated for 15 min to remove air trapped in the liquid.

The injection volume was set at 100 µl per sample and each sample was analysed once. Standard solutions of 5FU in PBS or water with concentrations ranging from 0.1µg/ml to 1.0 µg/ml were analysed three times: at the start of the analysis, in the middle, as well as at the end of the analysis.

After analysing all samples, the column was rinsed with 70 % acetonitrile in water for 3 hours at a flow rate of 1 ml/min to remove all impurities, where after it was rinsed with 100 % isopropyl alcohol for 3 hours to prolong column life.

2.2.5. Analysis of data

Data was expressed as the cumulative concentration 5FU that had permeated in time in relation to the area of exposed skin. These values were expressed in graphs of cumulative concentration (µg/ml) against time (h). The linear portion of the graph represents the flux (µg/cm^2/h) of 5FU. The yield was calculated by determining the percentage of applied 5FU that had permeated into the receptor compartment in the 12 hour period. An ANOVA analysis was done on the data to determine statistical significance of differences between the formulations.

3. Results and discussion

Skin diffusion studies were carried out to determine the influence of the Pheroid™ delivery on the transdermal delivery of 5FU.

In the positive control studies, which represent passive flux, 5FU was only dissolved in PBS or water. The plots (fig. 4, plots 1 and 2) exhibit a lag time, where after there is a small increase in permeation. These controls exhibit a slight biphasic character, which can be seen between 1 and 4 hours, as well as 4 and 12 hours. There is a slightly higher permeation of 5FU dissolved in PBS than in water, although not statistically significant.

In the diffusion studies where 5FU was entrapped in Pheroid™ vesicles (fig. 4, plots 3-5), a comparison was made between the influence of PBS- and water-based Pheroid™ formulations on 5FU flux, as well as the difference between 1 % and 0.5 % 5FU water-based Pheroid™ formulations. These were compared to control Franz cells included in

the study, where there was no 5FU in the donor solutions, as well as to the positive control studies (passive 5FU flux).

A comparison of average cumulative concentrations, average fluxes and average yields between the different 5FU solutions can be seen in Table 1 and fig. 2 and 3. Fig. 4 shows the comparison of average cumulative concentrations between the different solutions. Both the PBS-based and water-based Pheroid™ formulations of 5FU had a greater permeation of 5FU than 5FU in PBS or water alone, although only the 0.5 % water-based Pheroid™ formulation had a statistically significant increase as result. Thus it can already be deduced that the Pheroid™ system increases 5FU permeation.

When the 1 % 5FU in the water-based Pheroid™ formulation (plot 3) is compared to the 1 % 5FU in the PBS-based Pheroid™ formulation (plot 5), it can be seen that the water-based Pheroid™ formulation facilitates a greater cumulative concentration than the PBS-based Pheroid™ formulation. Both the 0.5 % and the 1% water-based Pheroid™ formulations also resulted in a greater yield and average flux than the 1 % PBS-based Pheroid™ solution, while only the 0.5 % formulation's yield was significantly greater.

Furthermore, the 0.5 % 5FU in the water-based Pheroid™ formulation (plot 4) resulted in a greater average yield than the other formulations, while yielding an average cumulative concentration and average flux similar to that of the 1 % formulation. According to the ANOVA analysis, there are no statistically significant differences between the 1 % and 0.5 % water-based Pheroid™ formulations' yields, fluxes or cumulative concentrations. It can therefore be hypothesised that the 0.5 % formulation will be at least as effective as the 1 % formulation. The added benefits of using a 0.5 % formulation instead of a 1 % formulation might include it being more cost-effective to manufacture and more tolerable to patients. This finding correlates to previous studies comparing 0.5 % formulations to 5 % formulations (8, 9).

The Pheroid™ formulations lack obvious lag times. The 0.5 % formulation shows the biggest initial permeation. The reason for the slightly smaller average cumulative concentration of the 0.5 % formulation after 12 hours when compared to the 1 % water-based Pheroid™ formulation might simply be that it contained half the dosage of active in the donor phase.

At 12 hours both the 1 % formulations seem to have reached a plateau, while the 0.5 % formulation still hasn't. This might indicate that better sustained release of the active is possible with the 0.5 % formulation.

When compared to the study by Kilian (10), the results of this study correlate with the previous findings. In Kilian's study, the average flux of 5FU in an Emzaloid™ (now Pheroid™) formulation was 0.63 ± 0.13 $\mu g/cm^2/h$ (10), while in the current study Pheroid™ formulations resulted in fluxes of 0.89 ± 1.58, 0.73 ± 1.08 and 0.41 ± 0.82 respectively (see Table 2). Kilian's study was only a pilot study, and this study was meant to confirm the preliminary findings, which it did.

4. Conclusions

- This study has proven that the novel delivery system presented by Pheroid™ technology does indeed increase the transdermal delivery of 5FU.
- It might enable the use of lower concentrations of the active in a transdermal product.
- A 0.5 % 5FU in water-based Pheroid™ can be used instead of a 1 % formulation, because there were no statistically significant differences in the transdermal penetration of these two formulations.
- When a lower concentration of active is used, it will enhance patient compliance because of fewer side-effects and thus a better tolerability. Skin integrity might also be maintained to a greater degree.
- A lower concentration of active in the formulation will also lower production costs.
- It can be concluded that it would be more advantageous to use water-based Pheroid™ formulations than PBS-based formulations.
- The absence of obvious lag-times indicates a faster therapeutic response with the use of the Pheroid™ delivery system.

Acknowledgements:

The conductors of this study would like to acknowledge the monetary grant supplied by the National Research Foundation (NRF) of South Africa, as well as everything supplied by the Unit for Drug Research and Development, North-West University, South Africa. A further word of gratitude to Prof. Jan du Preez for his help with the development and implementation of the analytical methods used. Thanks to Liezl-Marié Niewoudt and Dale Elgar for the preparation of the Pheroid™ system used, and to Liezl-Marié for the confocal microscopy as well. Thank you to Dr. Suria Ellis, at the Statistical Consultation Services of the North-West University, for performing the statistical analysis.

Tables

Table 1: Comparison of Average Cumulative Concentration (µg/ml), Average Flux (µg/cm^2/h), Mean Flux (µg/cm^2/h) and Average Yield (%) of the different 5FU formulations

Formulation	Number of cells (*n*)	Ave. Cum. Conc. (µg/ml)	Ave. Flux (µg/cm^2/h)	Mean flux (µg/cm^2/h)	Ave. Yield (%)
1% 5FU in Water	14	0.898768	0.074677	0.02850	0.008783
1% 5FU in PBS	14	1.193784	0.121829	0.03785	0.011900
1% 5FU in Water-Pheroid™	12	6.659802	0.890927	0.02000	0.890927
1% 5FU in PBS-Pheroid™	25	3.699159	0.412268	0.07180	0.037016
0.5% 5FU in Water-Pheroid™	11	6.079822	0.729010	0.03810	1.214022

Table 2: Average Cumulative Concentrations (µg/ml) and Average fluxes (µg/cm^2/h) with the respective standard deviations of the different 5-FU formulations

Formulation	**Ave. Cum. Conc. (µg/ml) ± Standard deviation**	**Ave. flux (µg/cm^2/h) ± Standard deviation**
1% 5FU in Water	0.898767545 ± 0.77316	0.074676923 ± 0.102781
1% 5FU in PBS	1.19378444 ± 1.42812	0.121828571 ± 0.210049
1% 5FU in Water-Pheroid™	6.659802104 ± 11.88919	0.890927273 ± 1.576919
0.5% 5FU in Water-Pheroid™	6.079822145 ± 8.94095	0.72901 ± 1.08305
1% 5FU in PBS-Pheroid™	3.699159345 ± 6.69281	0.412268 ± 0.823512

Figures

Figure 1: Confocal laser scanning micrographs of Pheroid™ formulations. Images A and B show the basic Pheroid™ formulation containing fluorophore Nilered®, emitting in the red spectrum. Images C and D show the 5FU-containing Pheroid™ formulation. 5FU is an auto-fluorescent molecule, emitting in the green spectrum. It can be clearly seen that 5FU is indeed entrapped within the Pheroid™ spheres.

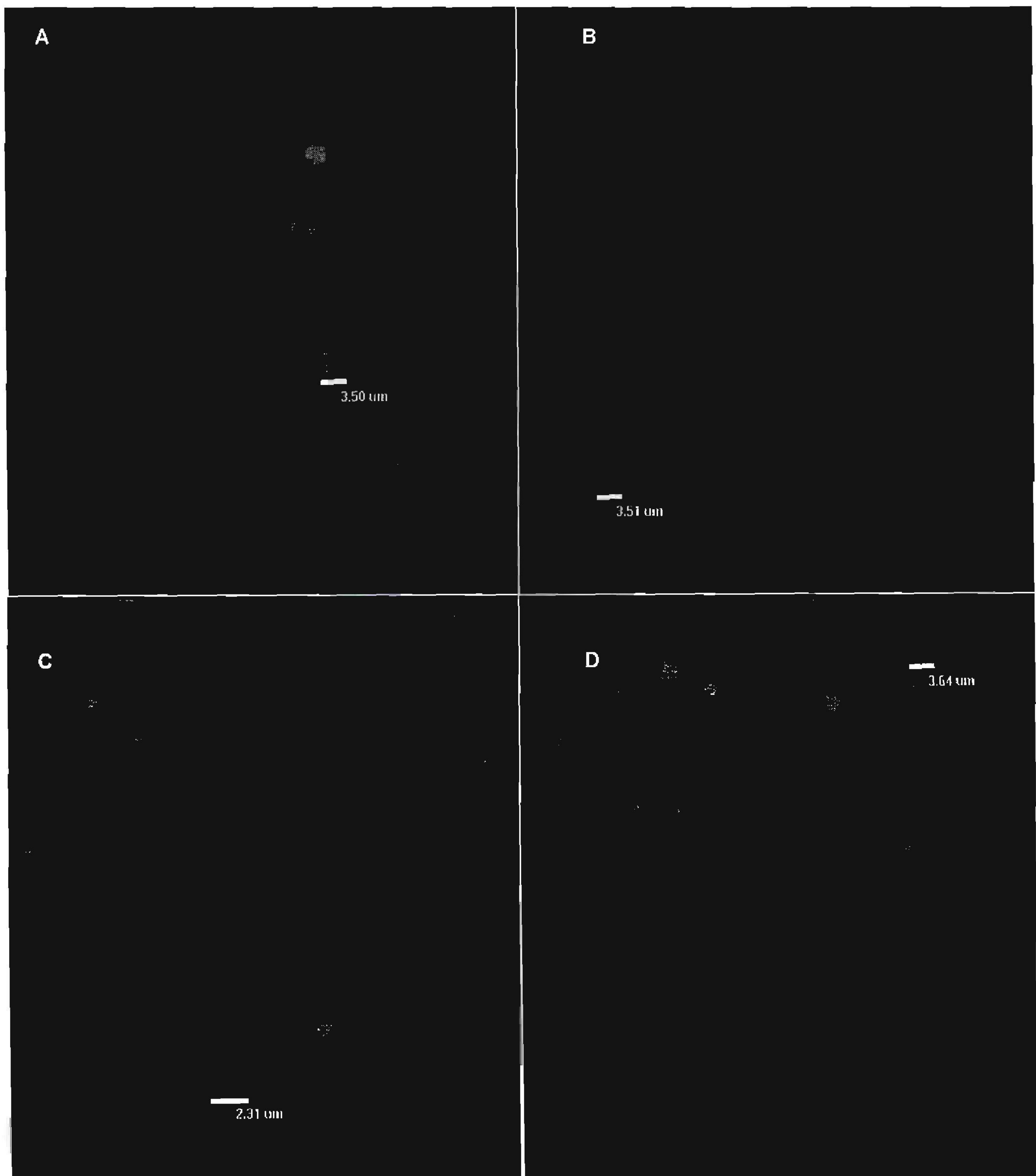


Figure 2: Comparison of Average Flux (µg/cm²/h), Mean Flux (µg/cm²/h) and Average Yield (%) of the different 5FU formulations.

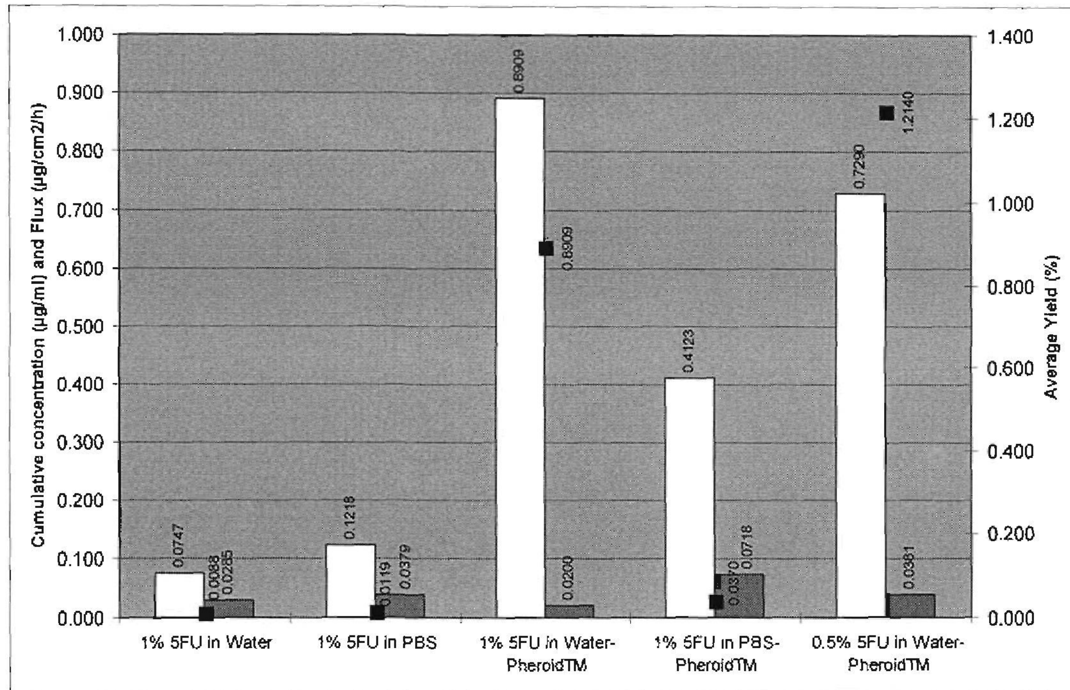


Figure 3: Comparison of Average Cumulative Concentration (µg/ml) of the different 5FU-formulations.

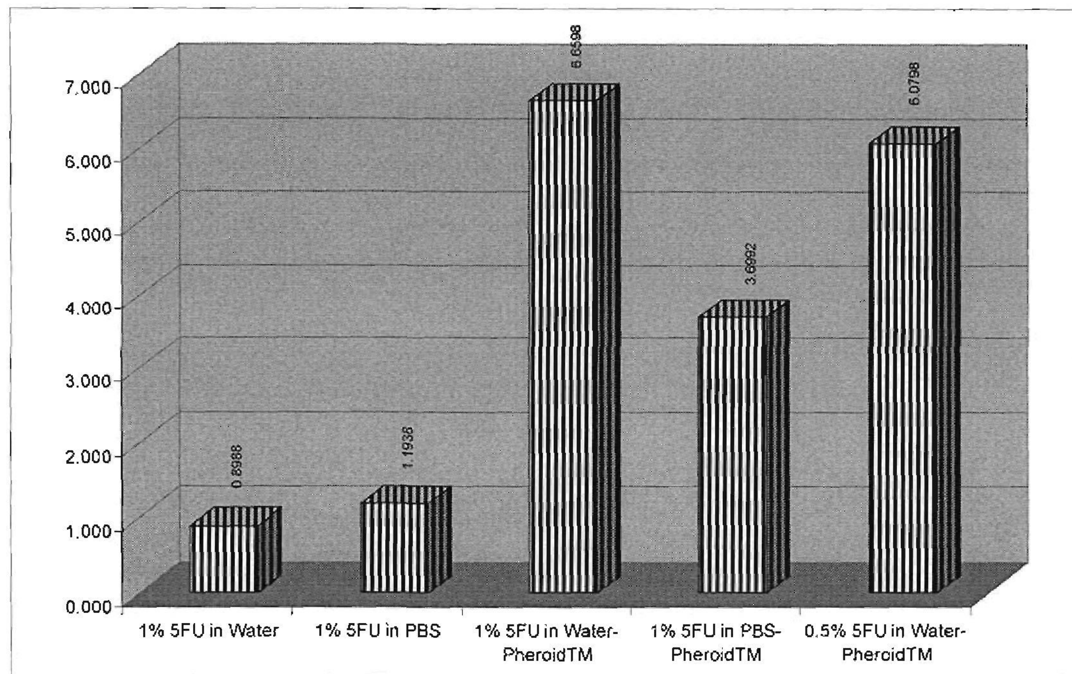
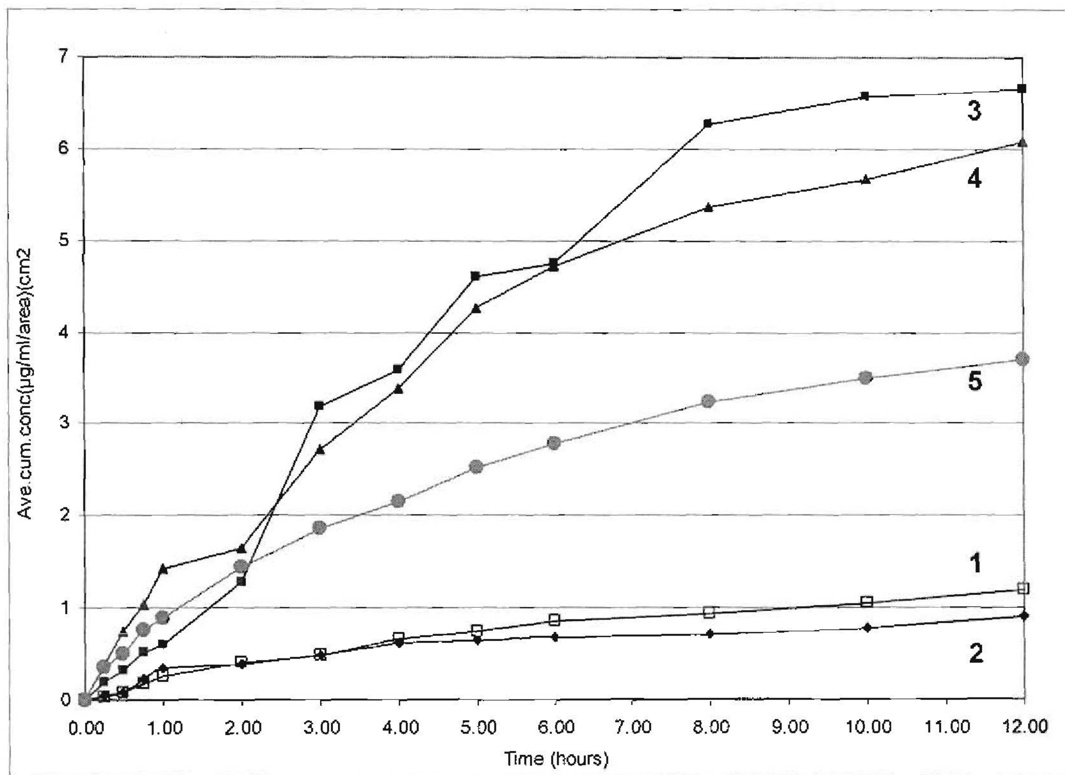


Figure 4: Comparison of the Average Cumulative Concentrations (µg/ml) / area (cm^2) of the different 5FU formulations over a 12 h period.



Legends of figures:

Figure 2:

□ Average Flux (µg/cm²/h)

■ Mean Flux (µg/cm²/h)

■ Average Yield (%)

Figure 3:

◘ Average Cumulative Concentration (µg/ml)

Figure 4:

□ 1% 5FU in PBS (1)

♦ 1 % 5FU in Water (2)

■ 1 % 5FU in Water-Pheroid™ (3)

▲ 0.5 % 5FU in Water- Pheroid™ (4)

● 1 % 5FU in PBS- Pheroid™ (5)

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FINAL CONCLUSIONS

The aim of this study was to investigate the transdermal penetration of 5-Fluorouracil (5FU), a relatively small, hydrophilic anti-cancer drug. The Pheroid™ therapeutic system was utilised in an effort to enhance the transdermal penetration.

5FU is often used as a model drug for small hydrophilic molecules and has a molecular weight of 130.08 Dalton. When delivered via the transdermal route, hydrophilic drugs have trouble penetrating the lipophilic stratum corneum, where the main barrier to penetration is situated. Specifically, 5FU's high melting point and crystal lattice energy further complicate its transdermal delivery.

The Pheroid™ delivery system has proven successful in the delivery of several actives through different delivery routes. Furthermore, it yielded promising results in a previous study of the transdermal delivery of 5FU. The Pheroid™ system might assist in the transdermal delivery of 5FU, because of its lipophilic nature and its high entrapment efficiency of a wide variety of actives.

In this study, the *in vitro* transdermal penetration of 5FU was investigated through diffusion studies utilising vertical Franz diffusion cells. Abdominal skin of Caucasian females was used in order to determine the penetration of 5FU. Studies were conducted over 12 hour periods, with samples being withdrawn on predetermined intervals.

Confocal laser scanning and reflectance microscopy was employed to confirm entrapment of 5FU in the Pheroid™ vesicles. This investigation proved positive.

Conclusions to be made from this study include the following:

- 5FU was definitely entrapped within Pheroid™ vesicles, as proven by confocal laser scanning and reflectance microscopy (see Figure 1 in enclosed journal article).
- The Pheroid™ therapeutic system caused a definite increase in the transdermal penetration of 5FU.
- Obvious lag-times in the transdermal penetration of 5FU were absent when the active was entrapped in the Pheroid™ system.

Recommendations for future studies on this subject:

- The influence of phosphate buffer solution and water as basis for the Pheroid™ delivery system might warrant some more attention.
- The Pheroid™ formulation must be optimised for the entrapment of 5FU.
- Entrapment time for 5FU within Pheroid™ vesicles can be optimised.
- A cream or lotion formulation must be applied to investigate the influence of other formulation ingredients on the transdermal penetration of 5FU.
- The amount of 5FU still present in the epidermis after the 12 hour period should be determined, to investigate possible targeted delivery to the skin.
- The impact of 5FU on the skin structure and skin components warrants investigation.

APPENDICES

APPENDIX I: VALIDATION OF HIGH PERFORMANCE LIQUID CHROMATOGRAPHY ANALYTICAL METHOD

The HPCL analytical method was not validated again, because the same method was used as by Kilian[1].

To ensure that the method was applicable and reliable in analysing the amount of 5FU that permeates skin, a linearity test was performed.

1.1. Linearity of analytical method

An analytical method must be able to produce results that are directly proportional to the concentration of the substance analysed. The linearity of this method was assessed by plotting the area under the curve (AUC, mAUC) versus the concentration (µg/ml). Standard solutions were prepared as previously described (Kilian, 2004).

The resulting plot can be described by the equation of a straight line, being:

$$y = mx + c$$ **Equation 4**

where:

y = peak area ratio (area under the curve) of 5FU
m = slope
x = 5FU concentration
c = y-axis intercept.

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[1] **KILIAN, D.** 2004. A comparative study between two lamellar gel phase systems and Emzaloids® as delivery vehicles for the transdermal delivery of 5-fluorouracil and idoxuridine.

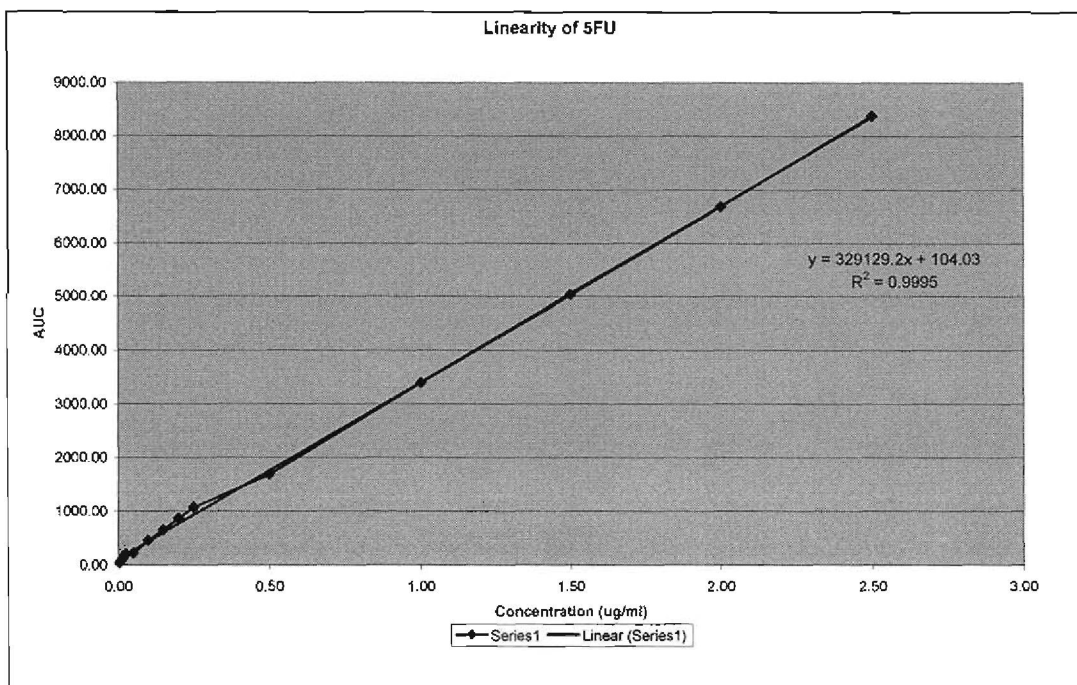

Figure 14: Linear regression curve of 5FU

The regression value (r^2) obtained was 0.9995, indicating a very good degree of linearity. Thus it can be accepted that the analytical method will be trustworthy.

APPENDIX II: DATA PROCURED DURING FRANZ CELL DIFFUSION STUDIES

1.1. Removal of data from calculations

After completion of each diffusion study, samples were visually inspected and awarded a percentage of turbidity. The percentages were awarded according to a chart digitally generated. Franz cells with a percentage of turbidity above 80 % for 3 or more of the intervals were removed from calculations. This was done to eliminate data gathered from Franz cells that were leaking during the study, or where skin integrity was not maintained, although this is only a guide.

1.2. 1 % 5-Fluorouracil in Water (Positive Control)

In this study, 1 ml 1 % 5FU in HPCL-grade water solution was applied to each of the donor compartments of 14 Franz diffusion cells. The transdermal penetration of 5FU over a 12 hour period was noted. This study served as a positive control study.

Table 6: Cumulative concentration (µg/ml), Yield (%) and Flux (µg/cm^2/h) of Franz cells with 1 % 5FU in water as donor solution

Cell nr.	Cum. Conc. (µg/ml)	Yield (%)	Flux(µg/cm^2/h)
1	0.670272	0.006550	0.0267
2	0.000000	0.000000	0.0000
3	0.454403	0.004441	0.0300
4	2.505137	0.024482	0.3529
6	2.201390	0.021514	0.2455
7	0.474072	0.004633	0.0285
8	0.375682	0.003671	0.0232
9	0.416053	0.004066	0.0276
10	0.569898	0.005569	0.0436
11	1.990061	0.019448	0.0498
12	0.414044	0.004046	0.0262
13	0.412553	0.004032	0.0256
14	0.673403	0.006581	0.0662
15	0.527011	0.005150	0.0250

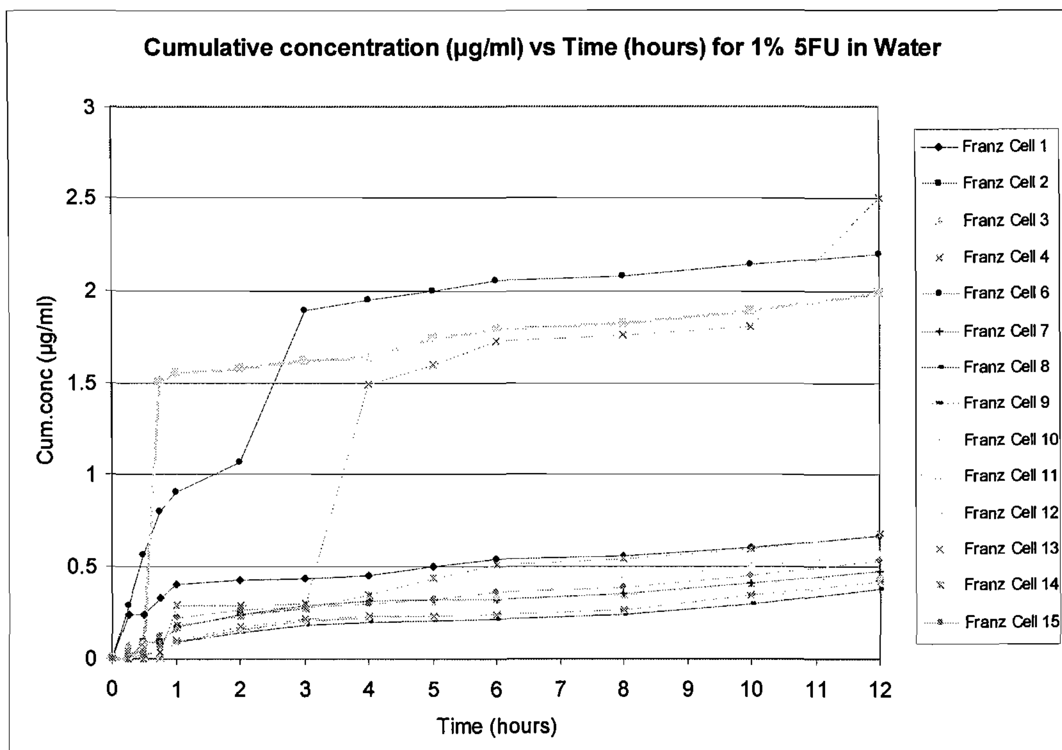

Figure 15: Cumulative concentrations (µg/ml) of Franz cells containing 1 % 5FU in water

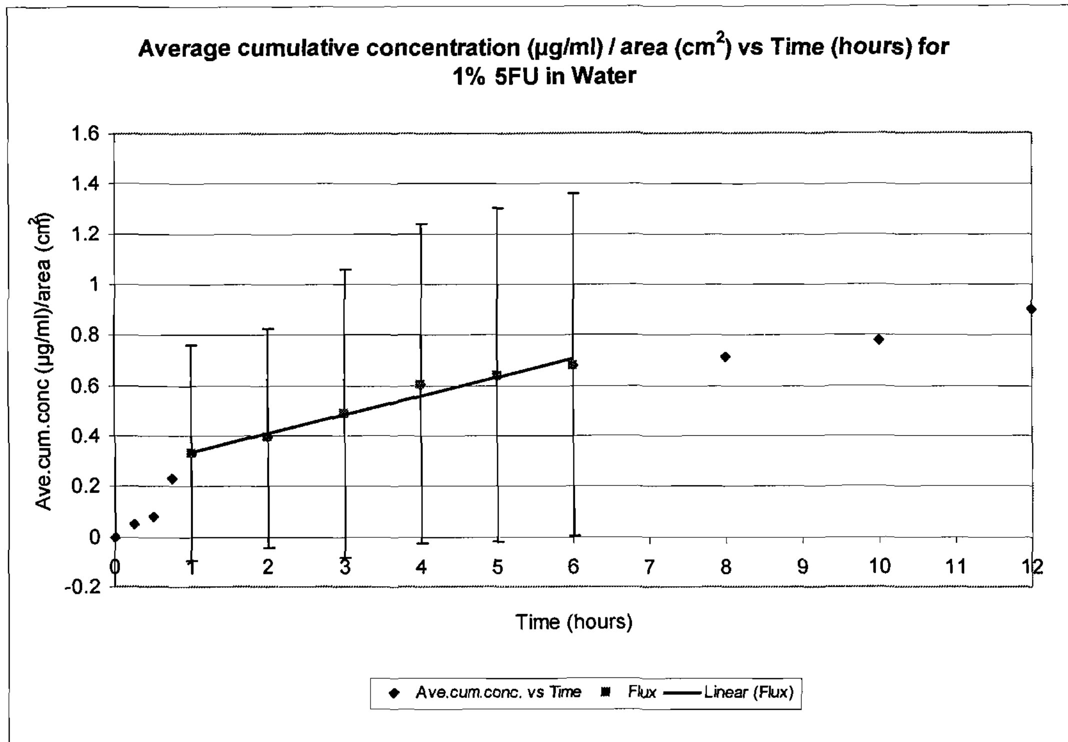

Figure 16: Average cumulative concentration (µg/ml) per area (cm^2) over time for a 1 % 5FU in water formulation, with standard deviation indicated

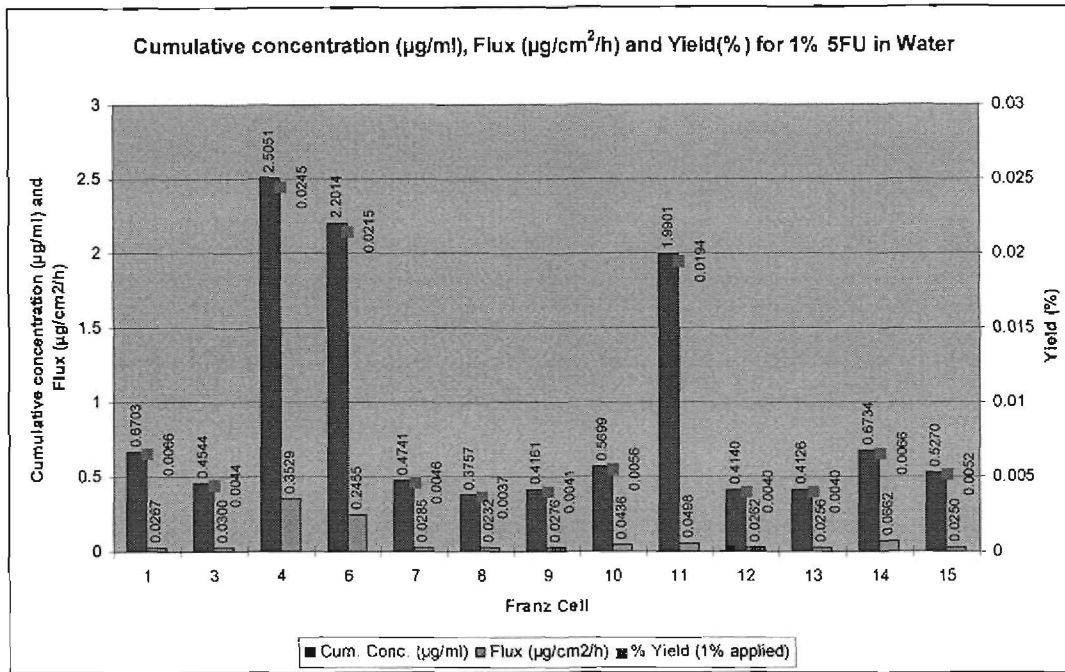

Figure 17: Cumulative Concentrations (µg/ml), Fluxes and Yields (%) for Franz cells containing 1 % 5FU in water

1.3. 1 % 5-Fluorouracil in PBS (Positive Control)

In this study, 1 ml 1 % 5FU in PBS solution was applied to each of the donor compartments of 14 Franz diffusion cells. The transdermal penetration of 5FU over a 12 hour period was noted. This study served as a positive control study.

Table 7: Cumulative concentration (µg/ml), Yield (%) and Flux (µg/cm²/h) of Franz cells with 1 % 5FU in PBS as donor solution

Cell	Cum. Conc. (µg/ml)	Yield (%)	Flux(µg/cm²/h)
1	0.501374238	0.004998	0.0282
2	0.493940329	0.004924	0.0281
3	0.488667141	0.004871	0.0244
4	0.674544001	0.006724	0.0314
5	1.109723608	1.109724	0.1324
6	1.633643738	0.016285	0.1343
7	0.397235379	0.00396	0.022
8	5.791295554	0.057731	0.8049
10	0.760882934	0.007585	0.0562
11	2.347214413	0.023398	0.2919
12	0.673854514	0.006717	0.0464
13	0.608473965	0.006066	0.0297
14	0.677548082	0.006754	0.0405
15	0.554584268	0.005528	0.0352

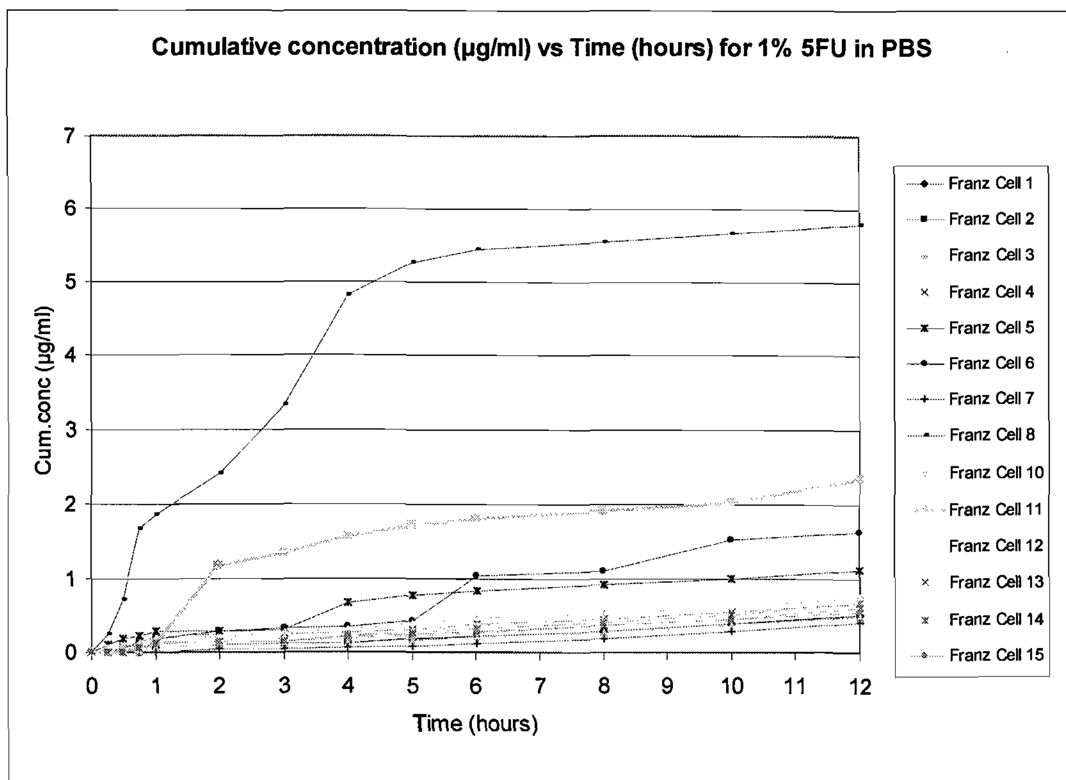

Figure 18: Cumulative concentrations (µg/ml) of Franz cells containing 1 % 5FU in PBS

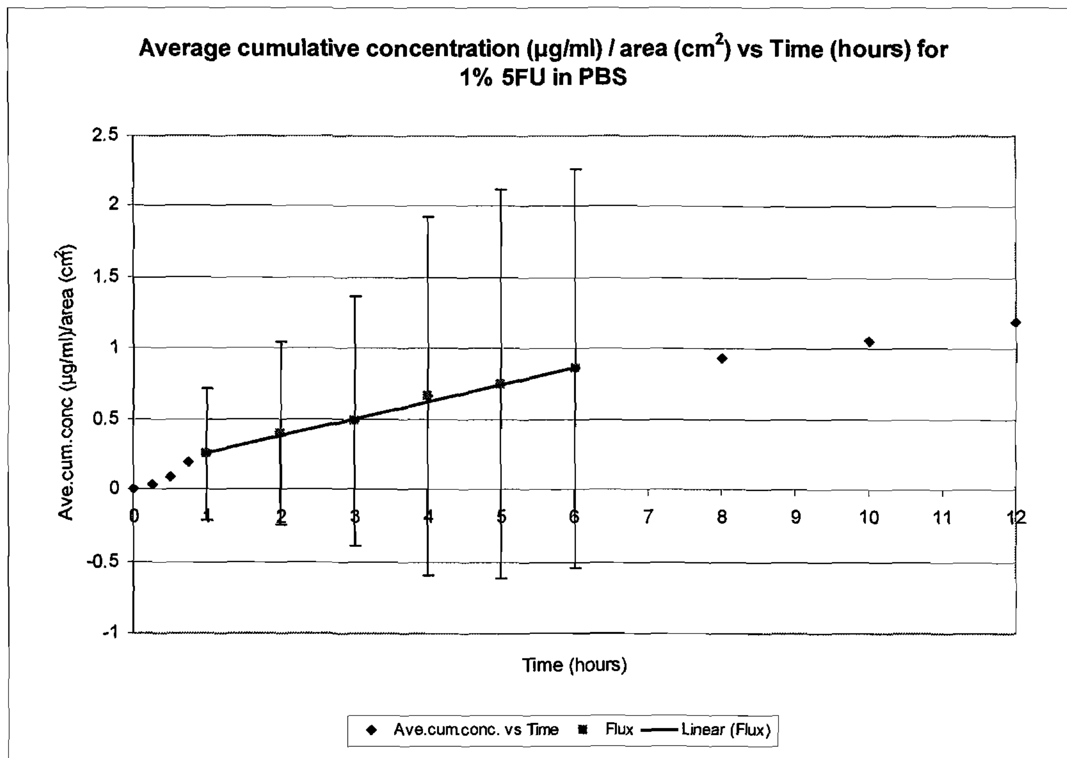

Figure 19: Average cumulative concentration (µg/ml) per area (cm^2) over time for a 1 % 5FU in PBS formulation, with standard deviation indicated

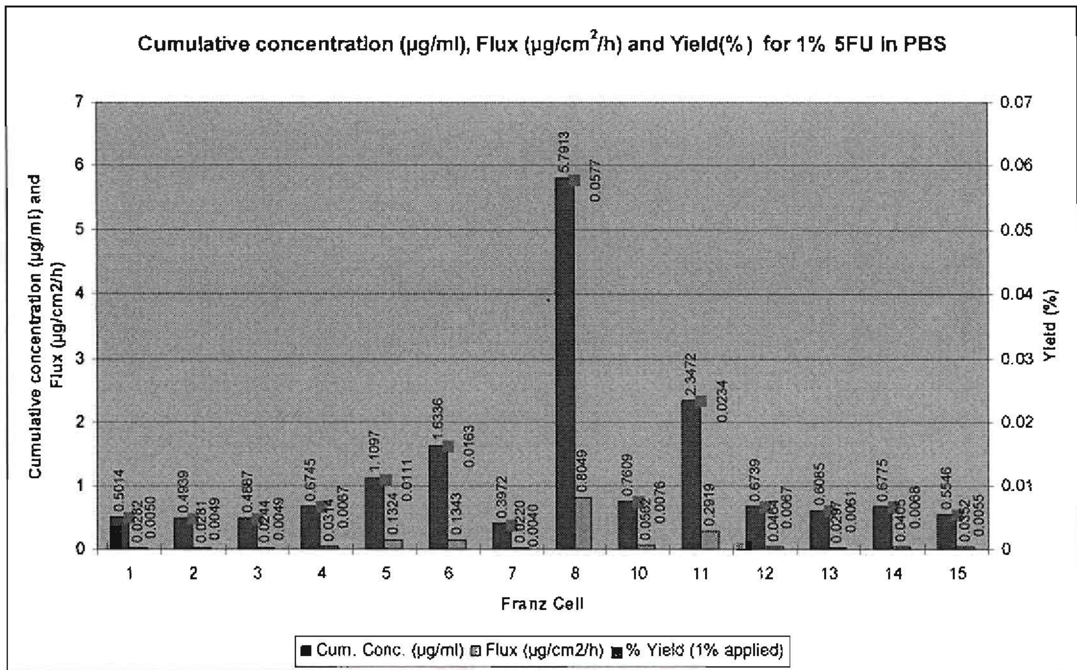

Figure 20: Cumulative Concentrations (µg/ml), Fluxes (µg/cm²/h) and Yields (%) for Franz cells containing 1 % 5FU in PBS

1.4. 1 % 5-Fluorouracil in Water-based Pheroid™

In this study, 1 ml 1 % 5FU in water-based Pheroid™ solution was applied to each of the donor compartments of 12 Franz diffusion cells. The transdermal penetration of 5FU over a 12 hour period was noted. The donor compartments of Franz cells 3 and 10 contained only basic Pheroid™ solution and HPLC-grade water respectively, serving as in-study controls.

Table 8: Cumulative concentration (µg/ml), Yield (%) and Flux (µg/cm²/h) of Franz cells with 1 % 5FU in water-based Pheroid™ as donor solution

Cell	Cum. Conc. (µg/ml)	Yield (%)	Flux(µg/cm²/h)
1	0.296644	0.029628	0.0067
2	0.168976	0.016877	0.0070
3 (Pheroid™)	*0.259770*	*0.025945*	*0.0346*
4	0.195508	0.019527	0.0380
5	0.342069	0.034165	0.0200
6	0.268948	0.026861	0.0321
7	0.149853	0.014967	0.0047
8	0.196684	0.019644	0.0163
9	0.141193	0.014102	0.0099
10 (Water)	*0.267205*	*0.026687*	*0.0267*
11	22.057404	2.203009	4.4408
12	0.000000	0.000000	0.0000
13	14.915357	1.489689	2.9479
14	34.525187	3.448243	2.2768

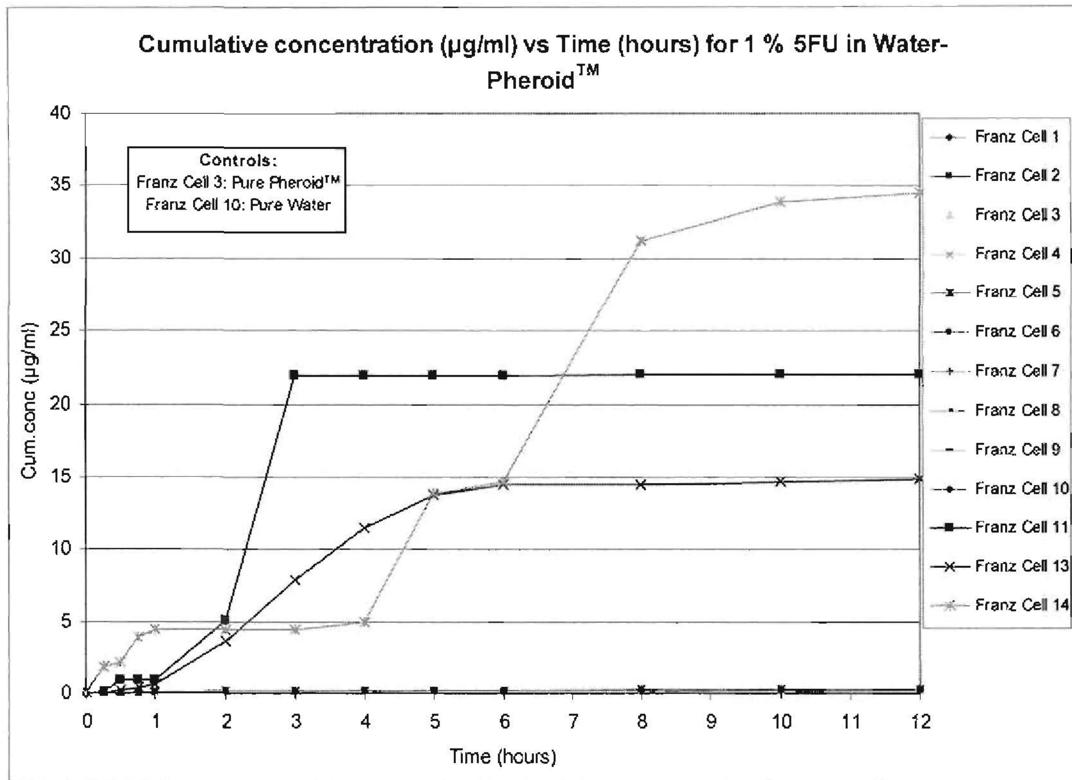

Figure 21: Cumulative concentrations (µg/ml) of Franz cells containing 1 % 5FU in water-based Pheroid™

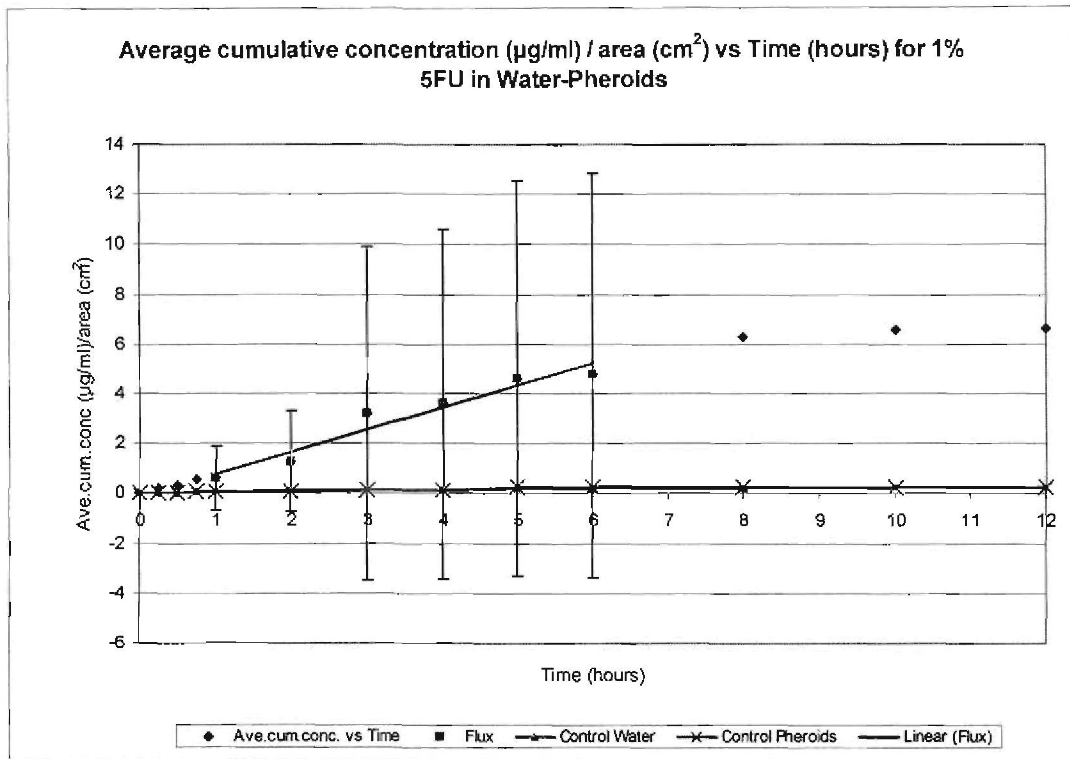

Figure 22: Average cumulative concentration (µg/ml) per area (cm^2) over time for a 1 % 5FU in water-based Pheroid™ formulation, with standard deviation and controls

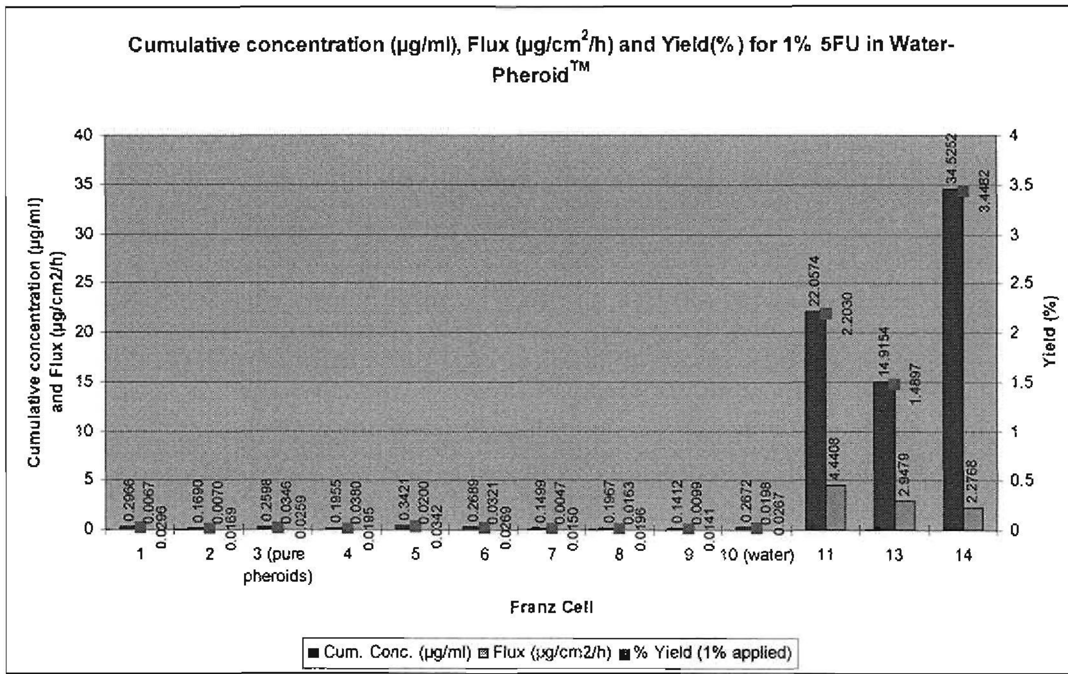

Figure 23: Cumulative Concentrations (µg/ml), Fluxes (µg/cm²/h) and Yields (%) for Franz cells containing 1 % 5FU in water-based Pheroid™

1.5. 0.5 % 5-Fluorouracil in Water-Based Pheroid™

In this study, 1 ml 0.5 % 5FU in water-based Pheroid™ solution was applied to each of the donor compartments of 11 Franz diffusion cells. The transdermal penetration of 5FU over a 12 hour period was noted. The donor compartments of Franz cells 4 and 7 contained only basic Pheroid™ solution and HPLC-grade water respectively, serving as in-study controls.

Table 9: Cumulative concentration (µg/ml), Yield (%) and Flux (µg/cm²/h) of Franz cells with 0.5 % 5FU in water-based Pheroid™ as donor solution

Cell	Cum. Conc. (µg/ml)	Yield (%)	Flux(µg/cm²/h)
1	0.280800	0.056070	0.0238
2	0.000000	0.000000	0.0000
3	0.153571	0.030665	0.0063
4 (Pheroid™)	*0.225778*	*0.045083*	*0.0191*
5	0.155560	0.031062	0.0034
6	20.489993	4.091452	2.8339
7 (Water)	*0.215891*	*0.043109*	*0.0146*
8	0.258515	0.051620	0.0210
9	1.267541	0.253103	0.0381
10	4.208936	0.840442	0.5847
12	0.186895	0.037319	0.0154
13	23.167959	4.626190	2.3367
14	10.628452	2.122295	1.4268

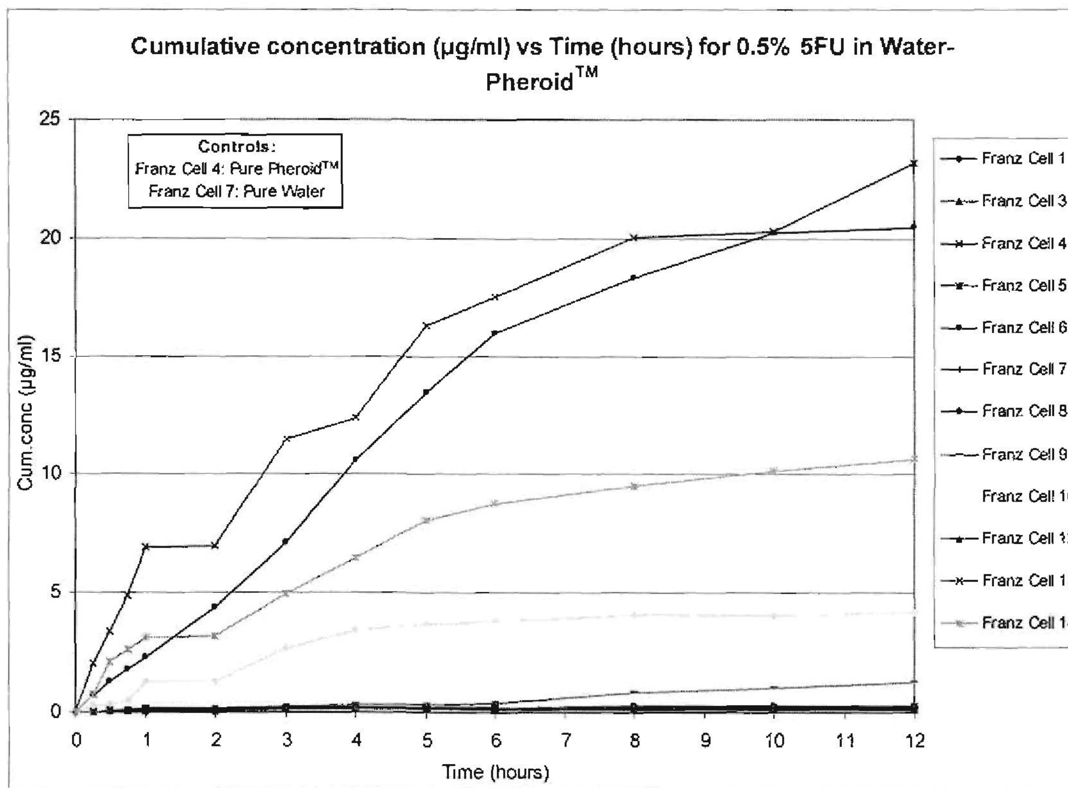

Figure 24: Cumulative concentrations (µg/ml) of Franz cells containing 0.5 % 5FU in water-based Pheroid™

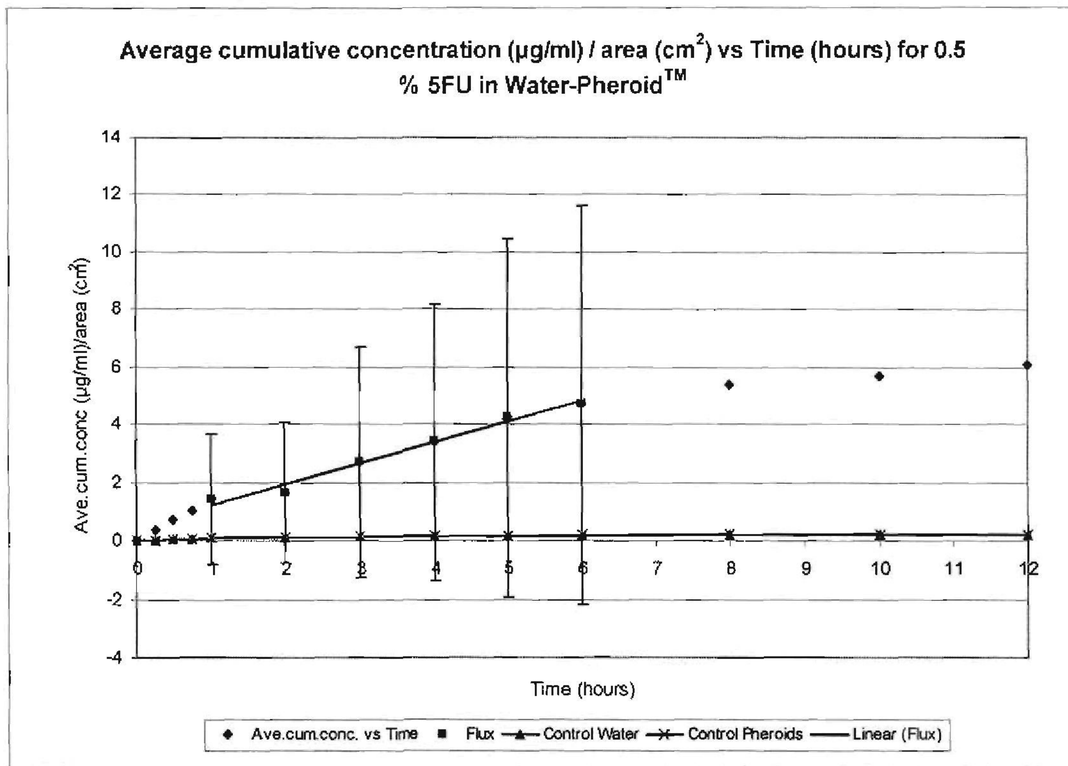

Figure 25: Average cumulative concentration (µg/ml) per area (cm^2) over time for a 0.5 % 5FU in water-based Pheroid™ formulation, with standard deviation and controls

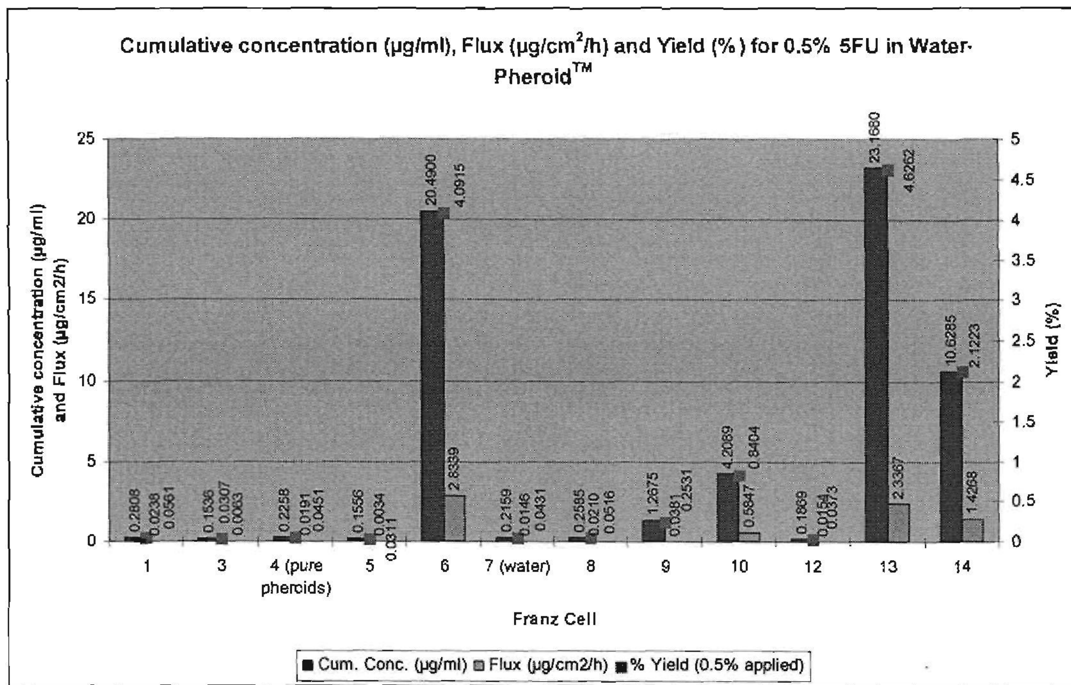

Figure 26: Cumulative Concentrations (µg/ml), Fluxes (µg/cm²/h) and Yields (%) for Franz cells containing 0.5 % 5FU in water-based Pheroid™

1.6. 1 % 5-Fluorouracil in PBS-based Pheroid™

In this study, 1 ml 1 % 5FU in PBS-based Pheroid™ solution was applied to each of the donor compartments of 25 Franz diffusion cells. The transdermal penetration of 5FU over a 12 hour period was noted. The donor compartments of Franz cells A12 and B12 contained only basic Pheroid™ solution and Franz cells A15 and B15 contained only PBS, serving as in-study controls.

Table 10: Cumulative concentration (µg/ml), Yield (%) and Flux (µg/cm²/h) of Franz cells with 1 % 5FU in PBS-based Pheroid™ as donor solution

Cell	Cum. Conc. (µg/ml)	Yield (%)	Flux(µg/cm²/h)
A1	0.972509	0.009731	0.0821
A2	0.987037	0.009877	0.0846
A3	15.441086	0.154512	1.9888
A4	0.838237	0.008388	0.0708
A5	0.866406	0.008670	0.0718
A6	3.623683	0.036261	0.1949
A7	0.864011	0.008646	0.0745
A8	0.515639	0.005160	0.0234
A9	0.781913	0.007824	0.0689
A10	0.677820	0.006783	0.0608
A12 (Pheroid™)	*0.834345*	*0.008349*	*12.1010*
A13	1.006456	0.010071	0.0806
A14	0.636932	0.006374	0.0193
A15 (PBS)	*1.052296*	*0.010530*	*0.0854*
B1	0.755188	0.007557	0.0517
B2	0.882959	0.008835	0.0589
B3	7.865106	0.078703	0.2140
B4	2.105229	0.021066	0.7946
B5	0.936358	0.009370	0.0205
B6	2.701043	0.027028	0.4600
B7	0.736053	0.007365	0.0617
B8	0.706587	0.007071	0.0696
B9	0.873969	0.008745	0.0797
B10	20.717302	0.207309	3.0836
B11	25.510236	0.255270	2.4615
B12 (Pheroid™)	*0.782422*	*0.007829*	*0.0665*
B13	0.633614	0.006340	0.0620
B14	0.843610	0.008442	0.0684
B15 (PBS)	*0.954035*	*0.009547*	*0.0808*

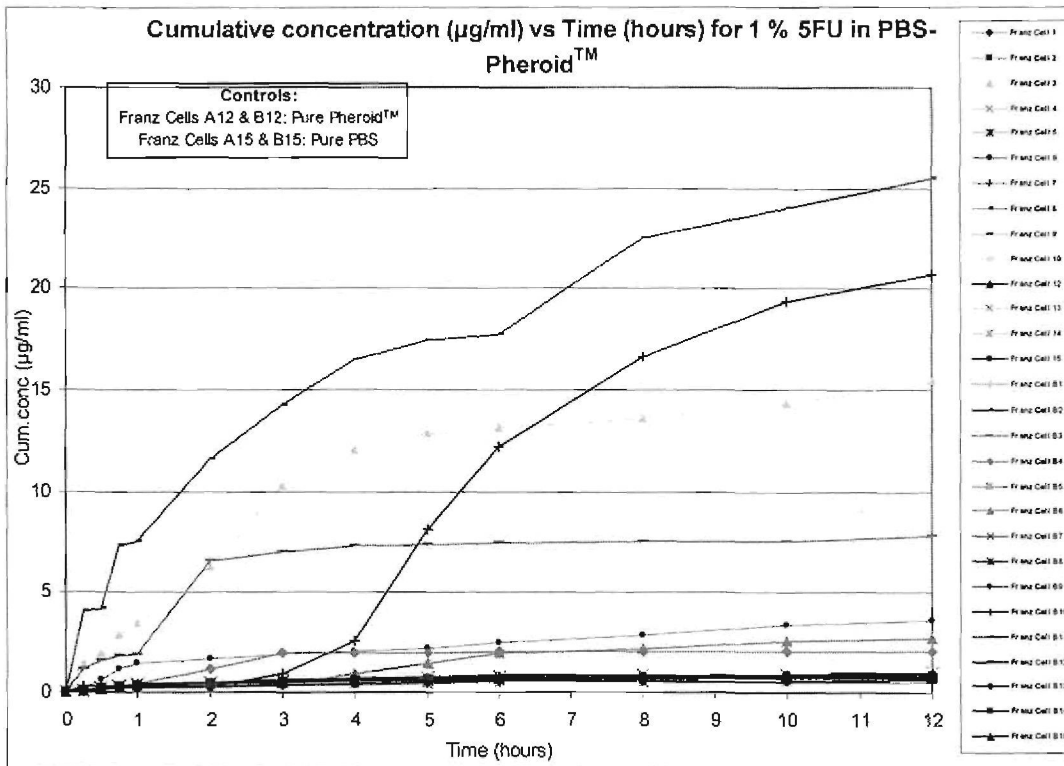

Figure 27: Cumulative concentrations (µg/ml) of Franz cells containing 1 % 5FU in PBS-based Pheroid™

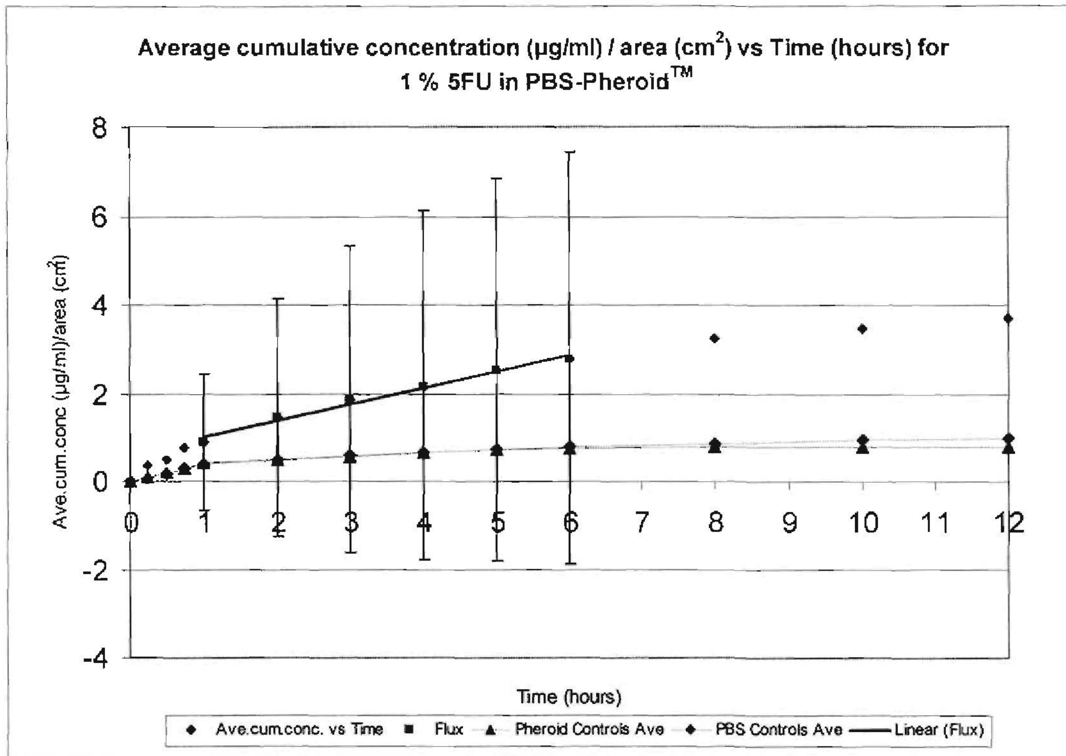

Figure 28: Average cumulative concentration (µg/ml) per area (cm²) over time for a 1 % 5FU in PBS-based Pheroid™ formulation, with standard deviation and controls

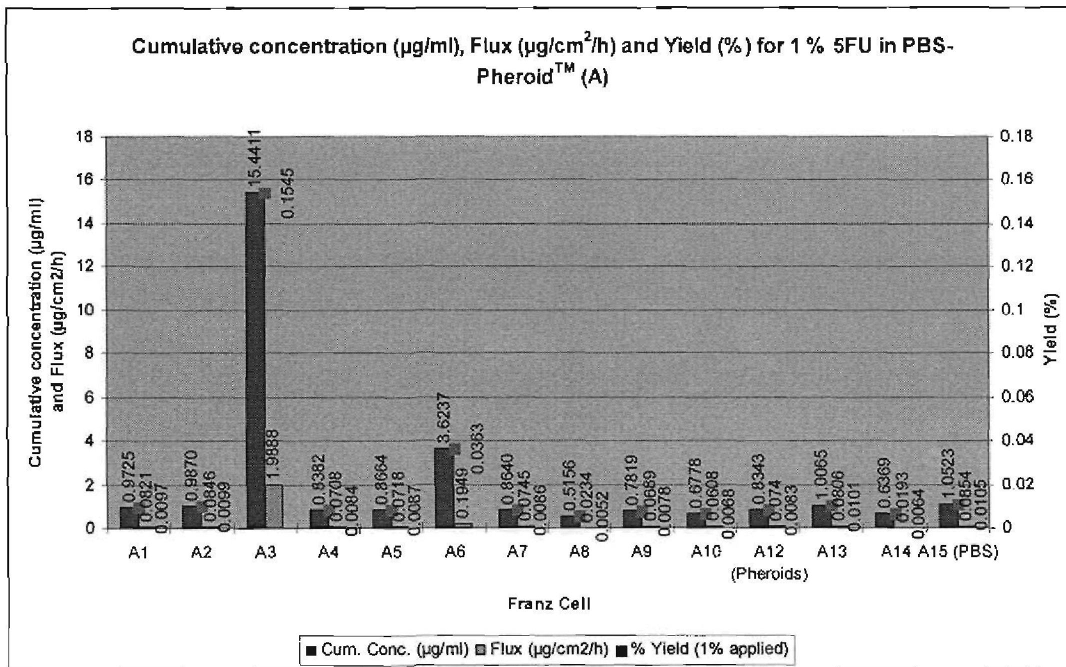

Figure 29: (A) Cumulative Concentrations (µg/ml), Flux (µg/cm²/h) and Yield (%) for Franz cells containing 1 % 5FU in PBS-based Pheroid™

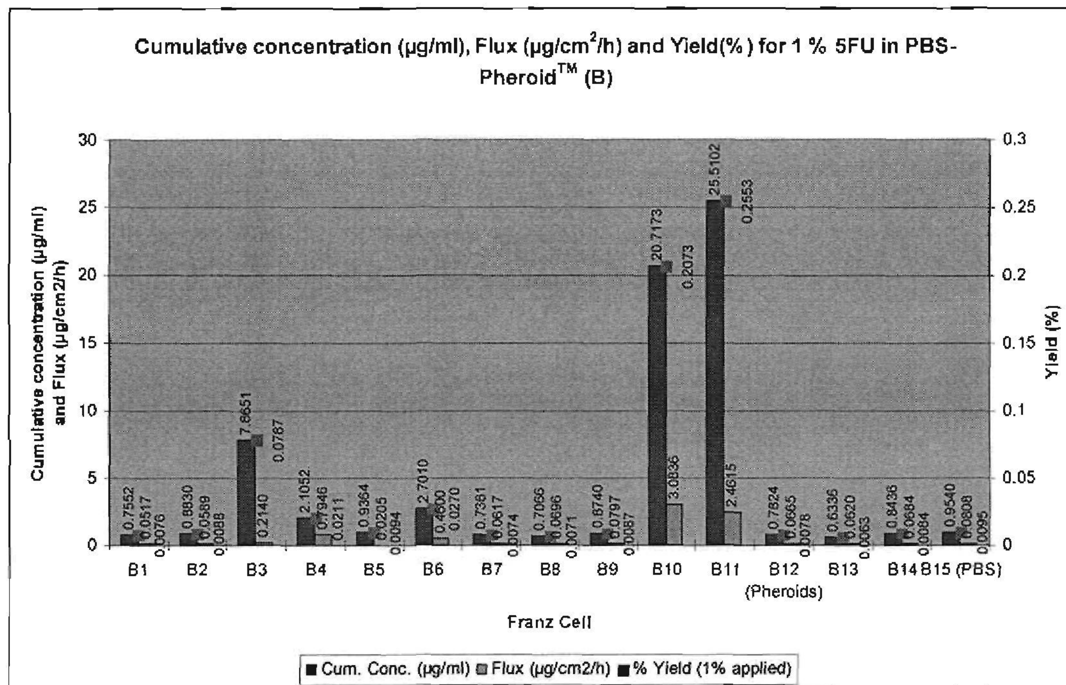

Figure 30: (B) Cumulative Concentrations (µg/ml), Flux (µg/cm²/h) and Yield (%) for Franz cells containing 1 % 5FU in PBS-based Pheroid™

1.7. Comparisons between the Cumulative Concentrations of Different 5-Fluorouracil Formulations

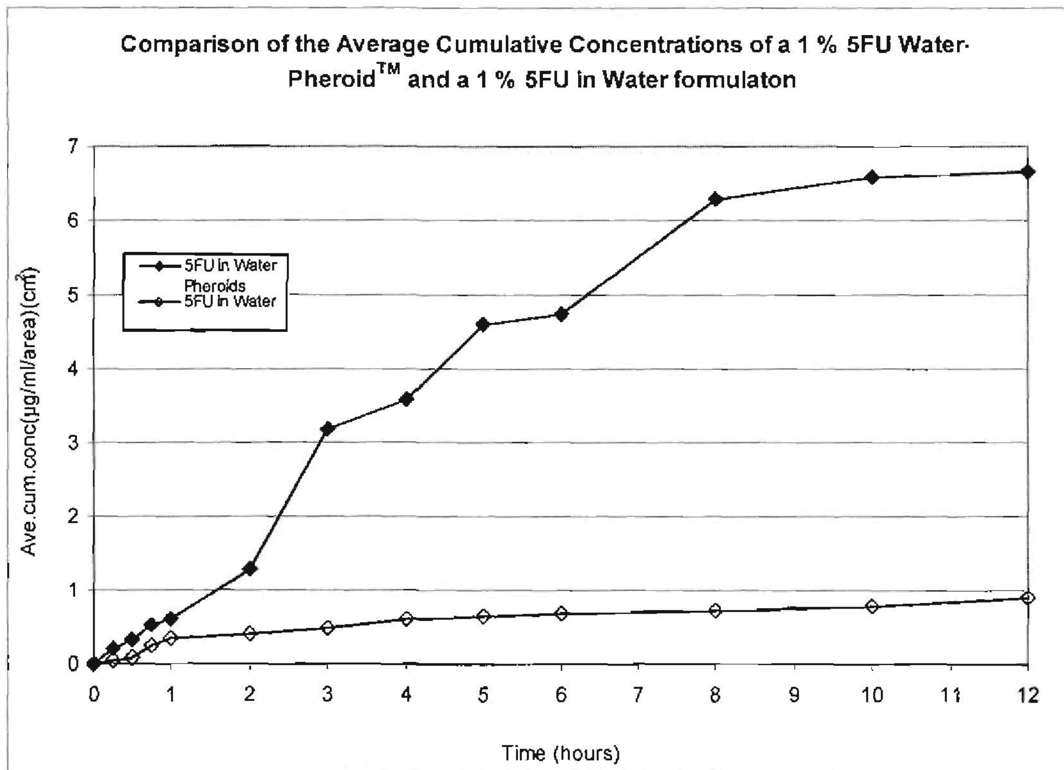

Figure 31: Average cumulative concentration (µg/ml) of a 1 % 5FU in water-based Pheroid™ formulation compared to a 1 % 5FU in water formulation

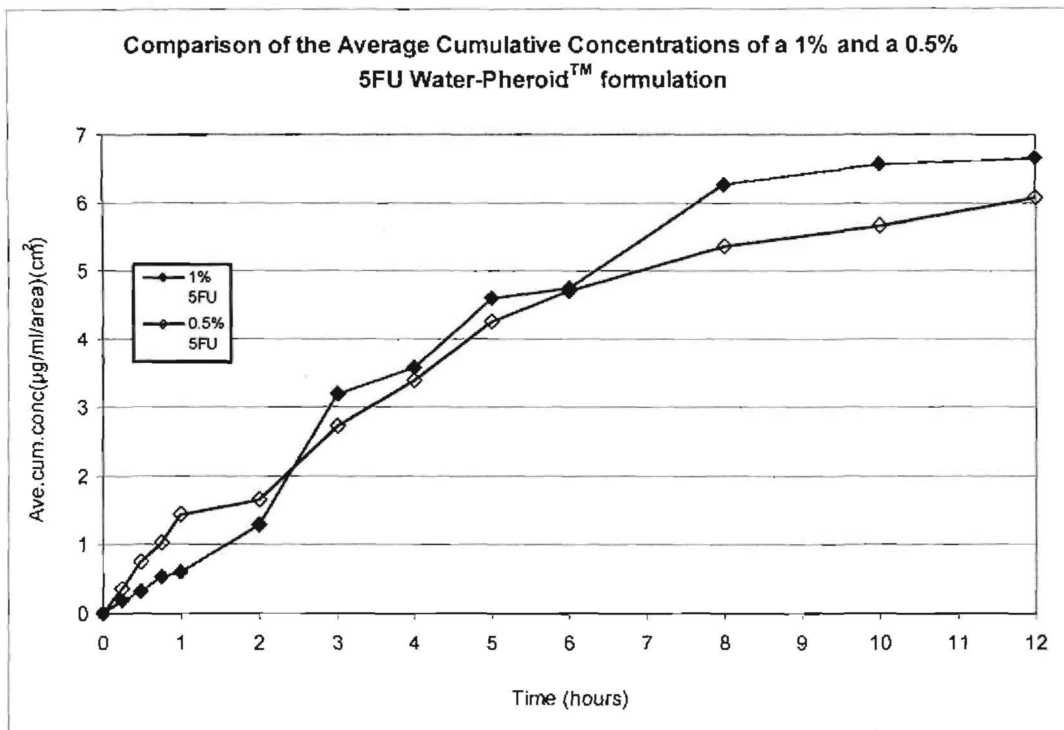

Figure 32: Average cumulative concentration (µg/ml) of a 1 % and a 0.5 % 5FU in water-based Pheroid™ formulation

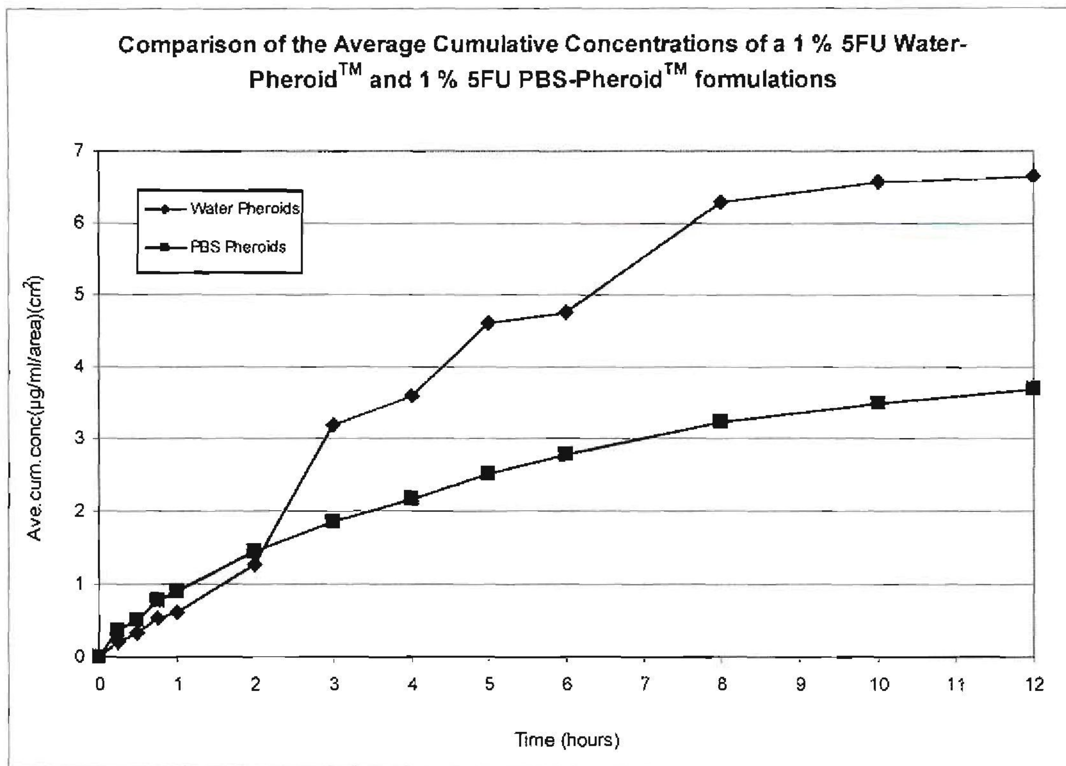

Figure 33: Average cumulative concentration (µg/ml) of a 1 % 5FU in water-based Pheroid™ formulation compared to a 1 % 5FU in PBS-based Pheroid™ formulation

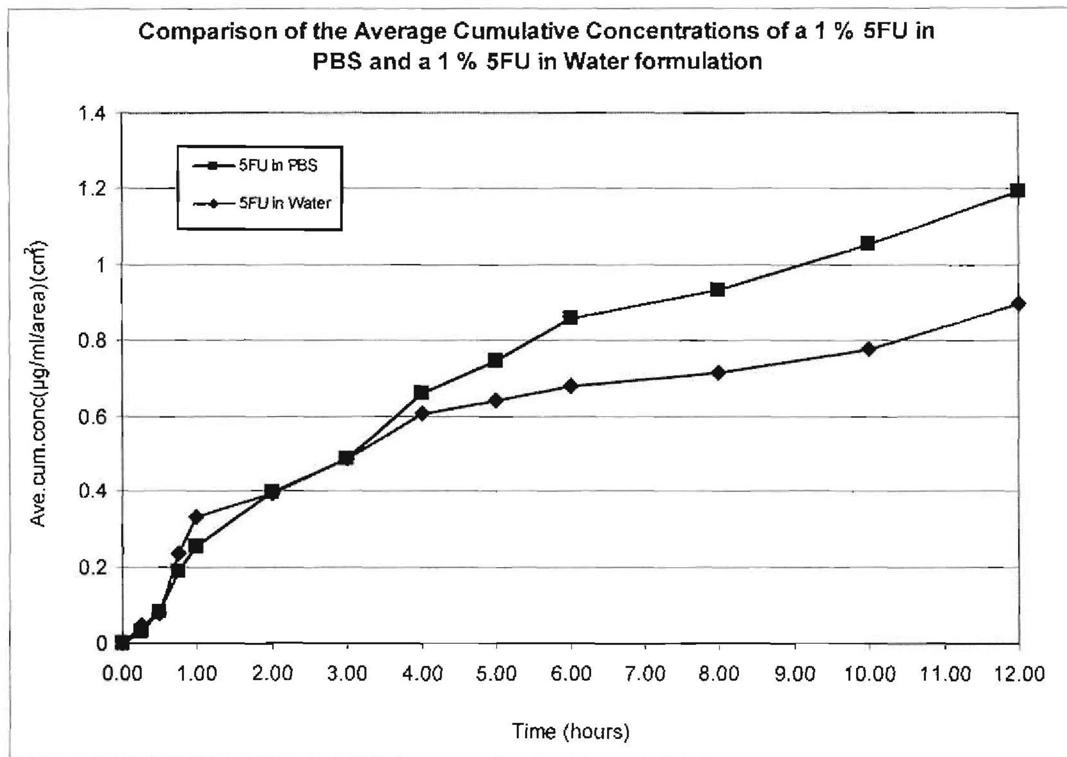

Figure 34: Average cumulative concentration (µg/ml) of a 1 % 5FU PBS formulation compared to a 1 % 5FU in water formulation

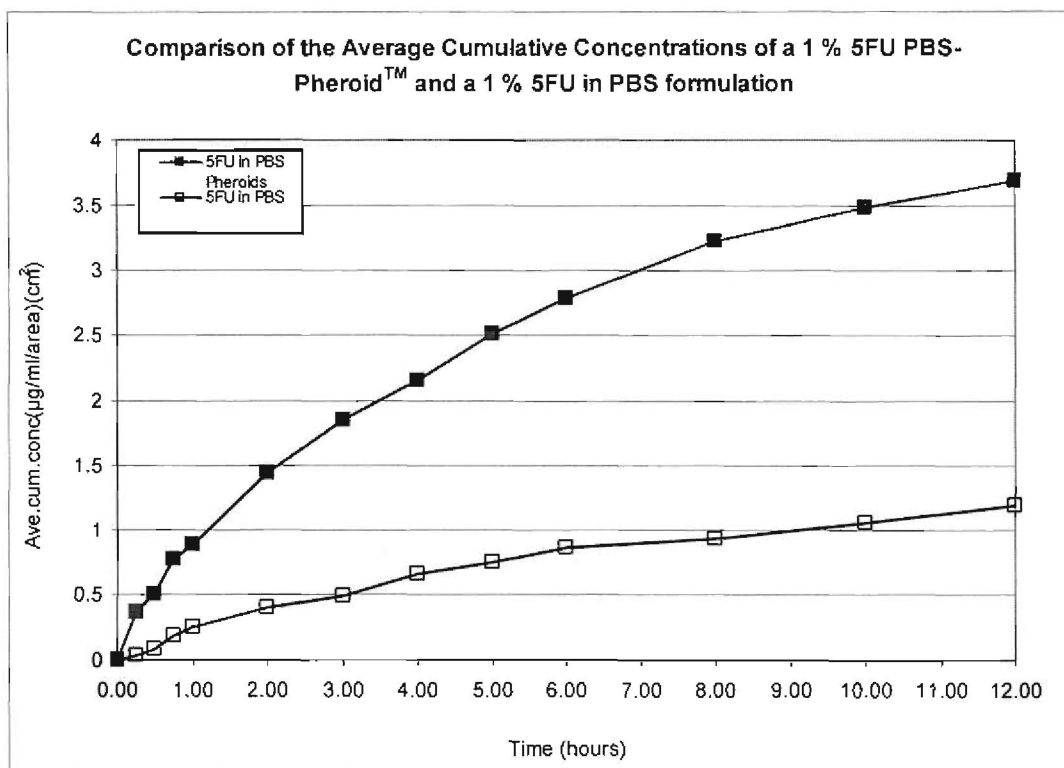

Figure 35: Average cumulative concentration (µg/ml) of a 1 % 5FU in PBS-based Pheroid™ formulation compared to a 1 % 5FU in PBS formulation

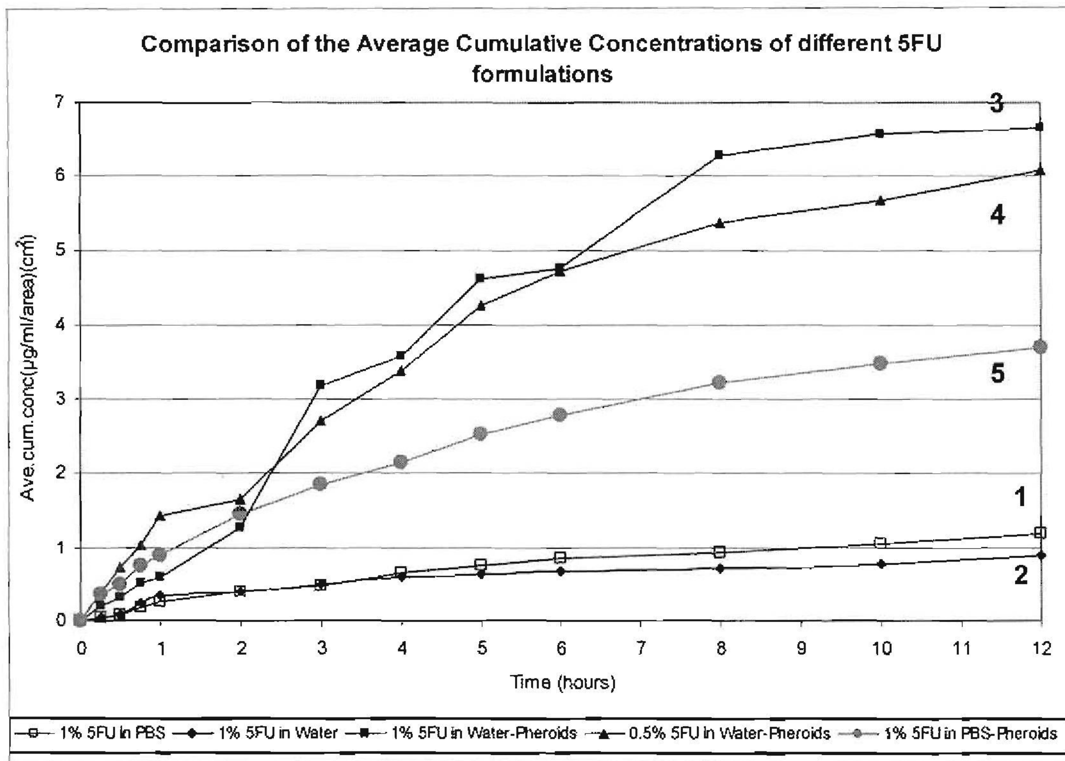

Figure 36: Average cumulative concentrations (µg/ml) of the different 5FU formulations

- ⊟ 1% 5FU in PBS (1)
- ♦ 1 % 5FU in Water (2)
- ■ 1 % 5FU in Water-Pheroid™ (3)
- ▲ 0.5 % 5FU in Water- Pheroid™ (4)
- ● 1 % 5FU in PBS- Pheroid™ (5)

APPENDIX III: STATISTICAL ANALYSIS OF THE DIFFERENT 5FU FORMULATIONS

The statistical analysis of procured data was done by the Statistical Consultation Services of the Potchefstroom campus of the North-West University. An ANOVA analysis was performed.

In Table 11, the differences in Average Cumulative Concentrations (µg/ml) between the different 5FU formulations are given. There were no statistically significant differences.

Table 11: Statistical differences between the *Average Cumulative Concentrations* (µg/ml) of the different 5FU formulations (Differences are significant when $p < 0.05000$)

	1 % 5FU in water-based Pheroid™	**1 % 5FU in Water**	**1 % 5FU in PBS-based Pheroid™**	**0.5 % 5FU in water-based Pheroid™**	**1 % 5FU in PBS**
1 % 5FU in water-based Pheroid™		0.298233	0.851986	0.999746	0.350599
1 % 5FU in Water	0.298233		0.838749	0.454337	0.999971
1 % 5FU in PBS-based Pheroid™	0.851986	0.838749		0.938137	0.871986
0.5 % 5FU in water-based Pheroid™	0.999746	0.454337	0.938137		0.513752
1 % 5FU in PBS	0.350599	0.999971	0.871986	0.513752	

In Table 12, the differences in Average Yield (%) between the different 5FU formulations are given. The 0.5 % 5FU in water-based Pheroid™ formulation differed significantly from the 1 % 5FU in water, 1 % 5FU in PBS-based Pheroid™ and 1 % 5FU in PBS formulations.

Table 12: Statistical differences between the *Average Yields* (%) of the different 5FU formulations (Differences are significant when p < 0.05000)

	1 % 5FU in water-based Pheroid™	**1 % 5FU in Water**	**1 % 5FU in PBS-based Pheroid™**	**0.5 % 5FU in water-based Pheroid™**	**1 % 5FU in PBS**
1 % 5FU in water-based Pheroid™		0.320405	0.364713	0.549887	0.455852
1 % 5FU in Water	0.320405		0.999987	0.011308	0.999047
1 % 5FU in PBS-based Pheroid™	0.364713	0.999948		0.014173	0.999806
0.5 % 5FU in water-based Pheroid™	0.549887	0.011308	0.014173		0.021501
1 % 5FU in PBS	0.455852	0.99047	0.999806	0.21501	

In Table 13, the differences in Average Flux (μg/cm²/h) between the different 5FU formulations are given. There were no statistically significant differences.

Table 13: Statistical differences between the *Average Fluxes* (μg/cm²/h) of the different 5FU formulations (Differences are significant when p < 0.05000)

	1 % 5FU in water-based Pheroid™	**1 % 5FU in Water**	**1 % 5FU in PBS-based Pheroid™**	**0.5 % 5FU in water-based Pheroid™**	**1 % 5FU in PBS**
1 % 5FU in water-based Pheroid™		0.199560	0.705049	0.993847	0.251884
1 % 5FU in Water	0.199560		0.863208	0.461165	0.999928
1 % 5FU in PBS-based Pheroid™	0.705049	0.863208		0.927720	0.905020
0.5 % 5FU in water-based Pheroid™	0.993847	0461165	0.927720		0.536386
1 % 5FU in PBS	0.251884	0.999928	0.905020	0.536386	