

Formulation of 5-Fluorouracil for transdermal delivery

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ABSTRACT

Non-melanoma skin cancer (NMSC) is the most common human malignancy and it is estimated that over 1.3 million cases are diagnosed each year in the United States (Neville *et al.*, 2007:462). There are three main types of NMSC, which include basal-cell carcinoma (BCC), squamous-cell carcinoma (SCC) and cutaneous malignant melanoma (CMM). Exposure to ultra-violet (UV) radiation plays a major role in the aetiology of these three skin cancer types (Franceschi *et al.*, 1996:24).

5-Fluorouracil is an antineoplastic pyrimidine analogue that functions as an anti-metabolite. It interferes with DNA (deoxyribonucleic acid), and to a lesser extent, with RNA (ribonucleic acid) synthesis by blocking the methylation of deoxyuridylic acid into thymidylic acid. It is used in topical preparations for the treatment of actinic keratosis (AK) and NMSC. The cure rate with topical 5-fluorouracil is partly reflected by the degree of erythema, erosions, and eventual crusting which develop at the sites of treatment. This reaction often attains the best clinical response, but in turn, frustrates patients, which may lead to patient incompliance (McGillis & Fein, 2004:175). Due to the hydrophilic nature of 5-fluorouracil, the transdermal permeation through the lipophilic stratum corneum is very low and trivial (Singh *et al.*, 2005:99).

Transdermal drug delivery is the delivery of a chemical substance across the skin to reach the systemic circulation (Prausnitz *et al.*, 2004:115). This unique drug transport mechanism suggests many advantages that include safety, patient compliance, user-friendliness, efficiency and non-invasiveness (Fang *et al.*, 2004:241). The stratum corneum is a specialised structure that forms part of several anatomically distinct layers of the skin. Seeing that it is the outermost layer, it provides protection to the skin. It is known as the main barrier to percutaneous absorption of compounds, as well as water loss, through the skin (Bouwstra *et al.*, 2003:4).

This study focussed on the formulation of six different types of semisolid formulations, containing 0.5% 5-fluorouracil. The formulations included: a cream, Pheroid™ cream, emulgel, Pheroid™ emulgel, lotion and Pheroid™ lotion. Pheroid™ refers to a delivery system which was incorporated in the formulations in an attempt to enhance the penetration of 5-fluorouracil into the skin. This drug delivery system consists of unique and stable lipid-based submicron- and micron-sized structures, formulated in an emulsion. The dispersed Pheroid™ structures largely comprise of natural essential fatty acids, which have an affinity for the cell membranes of the human body (Grobler *et al.*, 2008:284-285).

These formulations were manufactured in large quantities and stored at three different

temperatures, each with their respective relative humidity (RH): 25 °C/60% RH, 30 °C/60% RH and 40 °C/70% RH, for a period of six months. Stability tests were conducted on each of these formulations on the day of manufacture (month 0), and then after 1, 2, 3 and 6 months. The tests included: determination of concentration of the analytes (assay) by means of high performance liquid chromatography (HPLC); determination of zeta-potential and droplet size; pH measurement; viscosity; mass loss determination; physical appearance; and particle size distribution.

Franz cell skin diffusion tests were performed with these six 5-fluorouracil containing semisolid formulations (0.5%), as well as with a 0.5% Pheroid™ solution, 0.5% non-Pheroid™ solution. A 5.0% Pheroid™ solution and a 5.0% non-Pheroid™ solution were also prepared in order to compare the skin diffusion test results to a 5.0% commercially available ointment. The data of the 0.5% formulations and solutions, as well as the 5.0% solutions and commercial ointment, were statistically compared and those formulations (and solutions) that yielded the best results, with regard to % diffused, epidermis and dermis concentrations, were identified.

Keywords: 5-fluorouracil, skin cancer, transdermal delivery, formulation, stability

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In die Verenigde State van Amerika kom nie-melanoomvelkanker (NMV) baie algemeen voor en na beraming is daar meer as 1.3 miljoen gevalle wat jaarliks gediagnoseer word (Neville *et al.*, 2007:462). NMV's kan verdeel word in drie tipes, naamlik: basaalselkarsinome (BSK), plaveisel-epiteelkarsinome (PEK) en kwaadaardige kutaneuse melanome (KKM). Die voorafgenoemde toestande word meestal geassosieër met 'n oormatige blootstelling van die vel aan ultraviolet (UV) bestraling (Franceschi *et al.*, 1996:24).

5-Fluorourasiel word as 'n antineoplastiese pirimidienanaloog met 'n uitgesproke anti-metaboliese funksie geklassifiseer. Die middel meng hoofsaaklik met DNS (deoksiribonukleïnsuur) sintese, maar ook tot 'n mindere mate met RNS (ribonukleïnsuur) sintese in, deurdat 5-fluorourasiel die metielering van deoksi-uridielsuur na timidielsuur verhoed. Die middel word algemeen in topikale preparate gebruik vir die behandeling van aktiniese keratinose (AK) en NMV. Die effektiwiteit van die behandeling word weerspieël deur die toestand van die area van aanwending met spesifieke verwysing na die graad van progressie vanaf eriteem na erosie en 'n daaropvolgende korsvorming. Die bovermelde tekens dui op 'n goeie kliniese reaksie, maar lei gereeld tot swak pasiëntmeewerkendheid (McGillis & Fein, 2004:175). Die lipofilliese aard van die stratum korneum veroorsaak dat deurlaatbaarheid van die hidrofilliese 5-fluorourasiel tot 'n groot mate beperk word (Singh *et al.*, 2005:99).

Transdermale geneesmiddelaflewering is die aflewering van 'n chemiese substans deur die vellae tot in die sistemiese sirkulasie (Prausnitz *et al.*, 2004:115). Hierdie manier van aflewering hou verskeie unieke voordele in soos verbeterde pasiëntmeewerkendheid, veiligheid, gebruikersvriendelikheid, effektiwiteit en verder is dit ook nie ingrypend van aard nie (Fang *et al.*, 2004:241). Die stratum korneum is die heel buitenste deel van verskeie, goed gedefiniëerde vellae en sy hoof funksie is om die vel te beskerm. Hierdie laag is bekend as die skans wat weerstand teen perkutaneuse absorpsie bied, sowel as waterverlies deur die vel bekamp (Bouwstra *et al.*, 2003).

Die studie het primêr op die formulering van ses verskillende semi-soliede formulerings met 5-fluorourasiel as hoofbestanddeel, gefokus. Hierdie formulerings sluit 'n room, Pheroid™ room, emulgel, Pheroid™ emulgel, huidmiddel en 'n Pheroid™ huidmiddel in. Die term Pheroid™ verwys na 'n afleweringssisteem wat by die 5-fluorourasielformulering gevoeg was, in 'n poging om velpenetrasie van die middel te verhoog. Die afleweringssisteem bestaan uit 'n emulsie wat unieke en stabiele lipied-gebaseerde strukture het met beide mikron en sub-mikron groottes.

Die gedispergeerde Pheroid™ strukture bestaan hoofsaaklik uit natuurlike essensiële vetsure wat met die selle en membrane in die menslike liggaam verenigbaar is (Grobler *et al.*, 2008:284-285).

Die formulerings is in grootmaat vervaardig en daarna vir ses maande by drie verskillende temperature met dienooreenkomstige relatiewe humiditeite (RH) geberg, nl. 25 °C/60% RH, 30 °C/60% RH en 40 °C/70% RH. Stabiliteitstoetse was vervolgens op die dag van vervaardiging (maand 0) uitgevoer asook 1, 2, 3, en 6 maande na vervaardiging. Die stabiliteitstoetse sluit onder andere die volgende in: die bepaling van die aktiewe bestandeelekonsentrasie met behulp van hoë-drukvloeistofchromatografie (HPLC), die bepaling van deeltjiegrootte en zeta-potensiaal, pH-meting, viskositeit, bepaling van massaverlies, fisiese voorkoms en deeltjiegrootteverspreiding.

Franz-diffusieseltoetse is uitgevoer op die voorafgenoemde ses 5-fluorourasiel- (0.5%) formulerings. Toetse is ook op 'n 0.5% Pheroid™-oplossing, 0.5% nie-Pheroid™-bevattende oplossing uitgevoer. 'n 5.0% Pheroid™-oplossing, asook 'n 5.0% nie-Pheroid™-bevattende oplossing is voorberei om sodoende die resultate van die veldiffusietoetse met 'n 5.0% kommersiële beskikbare salf te vergelyk. Die verkrygte data van die 0.5% formulerings en oplossings sowel as die 5.0% oplossings en kommersiële produk is statisties vergelyk. Die formulerings en/of oplossings wat die beste resultate gelever het in terme van gediffundeerde % , epidermis- en dermiskonsentrasies is vervolgens geïdentifiseer.

Sleutelwoorde: 5-fluorourasiel, velkanker, transdermale aflewering, formulering, stabiliteit

BRONNELYS

BOUWSTRA, J.A., HONEYWELL-NGUYEN, P.L., GOORIS, G.S. & PONEC, M. 2003. Structure of the skin barrier and its modulation by vesicular formulations. *Progress in lipid research*, 42:1-36.

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FOREWORD

Different types of semisolid formulations that contained the anticancer drug, 5-fluorouracil were formulated. The formulations were tested by transdermal diffusion experiments, in order to reveal the best delivery of 5-fluorouracil, for its intended purpose as a chemotherapeutic drug in the treatment of certain skin cancer types. Stability tests were conducted under three different temperature controlled conditions, for a period of six months.

This dissertation contains introductory chapters, a full length scientific article for publication in a pharmaceutical journal (Journal of Drug Delivery) and appendices that contain all experimental data obtained during this study. The complete guide for authors is included in Appendix E of the dissertation.

During this study, I have gained a lot of knowledge regarding this very unique route of drug delivery. I have learned that research requires a great deal of planning, effort and patience. I enjoyed working with my colleagues and study-leaders during this two-year period and I am looking forward to being part of innovative ways to develop the transdermal and cosmetic industry.

CHAPTER 1

INTRODUCTION AND PROBLEM STATEMENT

Non-melanoma skin cancer (NMSC) has an annual incidence of over one million cases in the United States of America. The majority of these cases consist of basal cell carcinoma (BCC) and squamous cell carcinoma (SCC). The incidence rates are increasing worldwide and are generally the result of increased cumulative ultraviolet (UV) exposure (Ridky, 2007:484). SCC can also be caused by other factors such as ionising radiation, human papillomavirus, chemicals, immunosuppression and chronically injured or inflamed skin can also lead to SCC (Hawrot *et al.*, 2003:91). Treatment options for NMSC include both surgical and non-surgical modalities. Regardless of the approach used, the goal is to remove the tumour, achieve a high cure rate, preserve the maximal amount of normal surrounding tissue, and provides an optimum cosmetic outcome (Neville *et al.*, 2007:462).

The topical chemotherapy drug most widely used in the treatment of cutaneous tumours is 5-fluorouracil. This drug interferes with DNA (deoxyribonucleic acid) synthesis in actively dividing cells, causing tumour death (Neville *et al.*, 2007:465). Although topical 5-fluorouracil has been used in the treatment of precancerous actinic keratosis lesions, which may develop to SCC, its usefulness in treating invasive NMSC is hindered by the insufficient depth of penetration into the dermis of the topically applied medication. For this reason, the topical application of 5-fluorouracil has been restricted to the treatment of superficial BCC or SCC (Neville *et al.*, 2007:466).

Human skin effectively inhibits drug permeation, mainly because of the horny layer of the skin, the stratum corneum (Barry, 2001:967). Properties that drugs should contain to penetrate this barrier, include: a low molecular weight (less than 600 Da); adequate solubility in oil and water; a high, but balanced, partition coefficient; and a low melting point (Barry, 2001:968). Hydration of the stratum corneum promotes the flux of nearly all drugs, by opening the compact structure of the cornified tissue (Barry, 2001:969). A number of physical, chemical and biochemical methods have been implied to improve the transportation of drug molecules through the skin. These attempts mainly aim at reducing the barrier properties of the stratum corneum or increasing the diffusion properties of the drug molecule (Gupta *et al.*, 2005:25).

Pheroid™ vesicles were incorporated into the formulations in an effort to improve the transdermal delivery of 5-fluorouracil in this study. The Pheroid™ delivery system is a patented (Pitney International NV Patent Portfolio, 1994), colloidal system that includes unique and

stable lipid-based submicron- and micron-sized structures. These structures are evenly distributed in a dispersion medium that may be adapted to the indication (Grobler *et al.*, 2008:284-285). The applicability of this pharmaceutical application (Pheroid™) lies in the improvement of the absorption of active ingredients into the viable epidermis or the underlying dermis.

In a previous study conducted at the North-West University (NWU), 5-fluorouracil was incorporated in different types of formulations (water, phosphate buffer solution (PBS)-based solutions) in concentrations ranging from 0.5% and 1.0% (Van Dyk, 2008:59). These formulations were tested on the heat separated epidermis by using Franz diffusion cells. The formulations included the Pheroid™ delivery system and were compared to their non-Pheroid™ equivalents. Van Dyk (2008:66) concluded that there were no statistically significant differences in the transdermal penetration of a 0.5% and a 1.0% 5-fluorouracil formulation and the water-based Pheroid™ formulation yielded the largest increase in penetration of 5-fluorouracil. For this reason, the formulations tested in this study contained 0.5% 5-fluorouracil.

This study focussed on the formulation of six different types of semisolid formulations for the transdermal delivery of 5-fluorouracil, which included the following:

- ❖ Cream
- ❖ Pheroid™ cream
- ❖ Emulgel
- ❖ Pheroid™ emulgel
- ❖ Lotion
- ❖ Pheroid™ lotion

Each of the above-mentioned formulations contained 0.5% 5-fluorouracil and were all tested by using the Franz cell skin diffusion method. A 0.5% 5-fluorouracil water solution, as well as a 0.5% Pheroid™ solution were formulated and compared to the six semisolid formulations. A commercially available ointment containing 5-fluorouracil was also tested by means of Franz cell skin diffusion, although, it contained 5.0% 5-fluorouracil. Thus, in order to compare the results of the commercial product, it was decided to formulate 5.0% solutions of 5-fluorouracil. These solutions were oversaturated and produced suspensions that consisted of 5.0% 5-fluorouracil in water, as well as Pheroid™ and were not included in the stability tests that were conducted as they were only prepared for the purpose of skin diffusion studies to compare with the commercially available product. Throughout the dissertation these 5.0% suspensions will be referred to as solutions.

The aims and objectives of this study include:

- ❖ Formulation of six semisolid formulations containing 0.5% 5-fluorouracil.
- ❖ Development and validation of an analytical method to determine the concentration of the analytes within the formulations, by means of HPLC (high performance liquid chromatography).
- ❖ Stability testing on these formulations over a period of six months.
- ❖ Determination of the aqueous solubility and octanol/buffer partition coefficient (log D) of 5-fluorouracil.
- ❖ Delivery of 5-fluorouracil to the underlying layers of the skin, by using the Franz cell skin diffusion method and determination of the influence of Pheroid™.
- ❖ Formulation of 5.0% solutions and comparing the transdermal diffusion to that of a 5.0% commercially available product.

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CHAPTER 2

TRANSDERMAL DELIVERY OF 5-FLUOROURACIL FOR USE IN SKIN CANCER

2.1 INTRODUCTION

The skin is the body's largest organ as it covers almost 2 m² of the total exterior area of an average human body. It receives approximately one-third of the circulating blood (Fang & Leu, 2006:212). The skin covers and isolates the body's internal structures and protects them from the external harmful environment. The main function of the skin is to serve as a barrier and while fulfilling this function; it keeps essential substances and water inside the body whilst keeping foreign objects out. A particular structure developed by the skin, called the stratum corneum (SC), comprises of unique and specialised physiochemical properties that make it possible for the skin to endure its harmful surroundings (Franz & Lehman, 2000:15).

Drug delivery across the skin has had many researchers investigating new and more advanced methods to improve this route of drug delivery. This unique drug transport mechanism suggests many advantages that include safety, patient compliance, user-friendliness, efficiency and non-invasiveness (Fang *et al.*, 2004:241). Transdermal drug delivery is defined as the delivery of a chemical substance across the skin to reach the systemic circulation (Prausnitz *et al.*, 2004:115).

Some other advantages of transdermal drug delivery include the escaping of the hepatic "first-pass" effect and gastrointestinal incongruity, less side effects as well as improved patient compliance because of the elimination of numerous dosing schedules, a reduction of the dosage frequency and the fact that most of the products can be administered by the patients themselves, is beneficial (Kydonieus *et al.*, 2000:3).

Although there are many advantages, one should bear in mind that the SC limits the clinical purpose of the skin, as it is a major obstacle, which has to be overcome before any drug can have an effect in the body.

The anticancer drug 5-fluorouracil has been used for more than 40 years in the treatment of diverse types of solid malignancies, which include cancer of the rectum, colon, stomach as well as breast and skin cancer (Kaiser *et al.*, 2003:123). The standard route of administering 5-fluorouracil is via the parental route (intravenous infusion) because of its short biological half-life. The oral route is not recommended as 5-fluorouracil is broken down strongly by the

enzyme dihydropyrimidine dehydrogenase that is present in the mucosa of the gut (Chu & Sartorelli, 2004:910).

This makes 5-fluorouracil a good candidate for topical or transdermal delivery by means of a gel or cream dosage form. 5-Fluorouracil has already been incorporated into an ointment for the treatment of certain skin cancers such as basal cell carcinoma (Chu & Sartorelli, 2004:910). Last mentioned will be the area of attention in this study as it will focus on the formulation and transdermal delivery of 5-fluorouracil (for the treatment of skin cancer) by means of a gel, cream and a lotion that will be formulated with and without the Pheroid™ technology.

To study this unique way of drug delivery it is important to understand certain aspects of the disease, the drug, as well as the skin itself, which acts as the route through which the drug is administered. These aspects, as well as the Pheroid™ technology, will be discussed in further detail in the following sections.

2.2 CARCINOMA (CANCER)

2.2.1 General introduction

Cancer is one of the most common diseases of our time and most people have been affected by it in some or other way – either by knowing about an acquaintance who has cancer or a very close relative that has been diagnosed with cancer. In most of the cases, cancer can only be cured when it is detected early; preferably, during the onset stages of its development. A person can obtain cancer at any stage of their lifetime and it can develop in any organ or tissue of the human body (Beers *et al.*, 2006:1143).

There is no major cause for cancer; it can develop because of a multiplicity of factors such as molecular abnormalities, genetic factors, environmental exposure, as well as immunologic disorders or illnesses, i.e. autoimmune diseases, hepatitis B and C or human immunodeficiency virus (HIV). In cases where there are malignancies, it can lead to nausea, pain, neuropathy, seizures, wasting, obstruction, anorexia, or hyperuricemia. Death generally arises due to rapid or progressive malfunction of one or numerous organ systems in the body. When taking a patient's history, there are some clear symptoms, which may imply occult cancer and they may include persistent pain, cough, haemoptysis (coughing of blood), fevers or night sweats, weight loss, fatigue, a change in bowel behaviour or haematemesis (Beers *et al.*, 2006:1147-1148).

2.2.2 Mechanism of the development of carcinoma

Beers *et al.* (2006:1143) stated that cancer is the uncontrolled production of cells in the human

body due to the loss of standard controls, which results in cell-growth that is unregulated, a shortage of differentiation between these cells, invasion of neighbouring tissue and usually metastasis.

As shown in Figure 2.1, cancer cells grow by means of a cell cycle, which is divided into different phases where specific metabolic reactions occur.

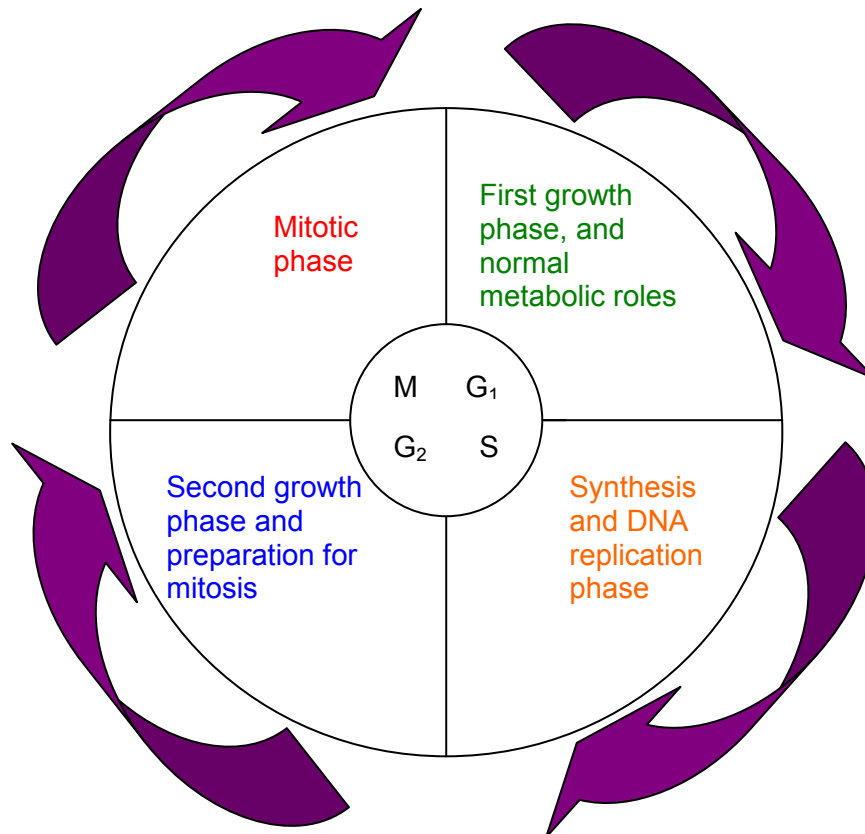


Figure 2.1: Cancer cell cycle (adapted from Wordpress.com, 2007) with G_1 – the phase before DNA synthesis occurs, this phase can last 12 h up to a few days; G_2 – the post-DNA synthesis phase, also ranges between 2 and 4 h; S – the DNA synthesis phase, which can range between 2 and 4 h and M – this is the mitoses-phase that lasts 1 – 2 h (Beers *et al.*, 2006:1144).

For the purpose of this study, the use of 5-fluorouracil and its use in cancerous conditions of the skin will be discussed. As mentioned, this anticancer drug is also used in the treatment of colon as well as breast cancer; these two types of cancer will only be discussed in short.

2.2.3 Cancer of the skin

Skin cancer is thought to be the type of cancer that is the most common, with more than one

million newly diagnosed cases in the United States, annually (Beers *et al.*, 2006:1023). Amongst these one million cases, different types of skin cancers can be distinguished, namely:

- ❖ Basal cell carcinoma (BCC) (80%)
- ❖ Squamous cell carcinoma (SCC) (16%)
- ❖ Melanoma (4%).

Skin cancer incidences are inversely correlated to the amount of skin pigmentation, melanin (Beers *et al.*, 2006:1023) and affect mostly people who have light skin, sunbathers, sportsmen, as well as persons who work outside. Melanin is a compound of the skin, which protects it from the sun, by turning the skin into a darker colour when it is exposed to the harmful UV-rays of the sun. Therefore, melanin has a significant function as the photoprotector of the skin, as skin pigmentation has a dramatic effect on the occurrence of skin cancer (Tadocoro *et al.*, 2003:1177).

Skin cancer often appears to be asymptomatic during the early stages and as time passes, a papule presents itself. Beers *et al.* (2006:932) described a papule as a prominent lesion generally smaller than 10 mm in width, which can be felt or palpated. This papule enlarges over time. Usually it changes in colour and is asymmetric, with or without mild tenderness. The two most common types of skin cancer are basal cell carcinoma and squamous cell carcinoma.

2.2.3.1 Basal cell carcinoma (BCC)



Figure 2.2: A photograph of a basal cell carcinoma tumour (Virtual cancer centre, 2008).

This type of skin cancer, seen in Figure 2.2, is known as the most frequent invasive malevolent cutaneous neoplasm found in human beings (Preston & Stern, 1992:1649). The nose area is mostly affected by basal cell carcinoma, with an occurrence of 25 - 35%. 85% of basal cell carcinoma becomes visible in areas of the head and neck. Tumours may also arise on body parts that are secluded from the sun. These sites include the breasts and genitals (Habif,

2004:724).

Most people affected by basal cell carcinoma complain of a lesion that starts to bleed or scab. After a while it heals, but then usually reappears. Basal cell carcinoma has the tendency to demolish normal tissue and when left untreated or incorrectly treated, it can destroy the entire area of the face; or infiltrate subcutaneous tissue and from there penetrate into the bone and also into the brain (Habif, 2004:724). A person of any age may develop a basal cell carcinoma tumour. However, the occurrence of basal cell carcinoma is higher at age 40 years or older. Recently, there has been an increase in younger patients diagnosed with basal cell carcinoma, probably due to an increase in sun exposure (Cox, 1992:26).

Treatment of these basal cell carcinoma tumours depends on the location as well as the size of the tumour. Usually there are specific techniques, which should be followed. These techniques include:

- ❖ electrodesiccation and curettage;
- ❖ excision surgery;
- ❖ radiation therapy;
- ❖ cryosurgery;
- ❖ topical 5-fluorouracil and
- ❖ mohs' micrographic surgery (Virtual cancer centre, 2008).

All of the above-mentioned techniques have been successfully applied. When appropriately selected by clinical specialists, they are linked to very high cure rates. Surgical excision is regarded as the preferred treatment when deciding between radiotherapy and excision therapy; and this account for almost all types of basal cell carcinoma (Virtual cancer centre, 2008).

2.2.3.2 Squamous cell carcinoma

According to Adam & Ratner (2001:975), squamous cell carcinoma (Figure 2.3) is the second most frequent diagnosed type of skin cancer. The highest incidence occurs in middle-aged and elderly white people. Based on the malignant potential, squamous cell carcinoma can be categorised into two main groups; firstly, those that are normally aggressive and have greater occurrence of metastasis (process that spreads to the neighbouring lymph nodes) – they usually start in regions of previous radiation or thermal injury as well as in chronic ulcers and draining sinuses. Secondly, are those that have a lower tendency of aggression and are not as likely to metastasise – they start in areas of the skin that are actinically damaged (Habif,

2004:744,747).



Figure 2.3: A photo of squamous cell carcinoma (Sperling, 2000).

As with most basal cell carcinomas, squamous cell carcinoma is often found in areas that are highly exposed to the sun, although their allocation is fairly different from that of basal cell carcinoma (Kwa *et al.*, 1992:1). The most common areas where squamous cell carcinomas start are on the scalp, the upper part of the pinna and also on the backside of the hands (Habif, 2004:744).

Squamous cells originate from the keratinocytes of the epidermal layer of the skin and proliferate for an indefinite period. The lesion set off by appearing flat and scaly after which it develops into an indurated SCC as the cells infiltrate the lower epidermal membrane and then thrive into the dermis (Habif, 2004:744). Squamous cell carcinomas that are small and have developed from actinic keratosis can be treated by means of electrodesiccation and curettage. It is best when the larger tumours are excised together with a piece of the subcutaneous fat (Kwa *et al.*, 1992:1).

2.2.3.3 Melanoma

Malignant melanoma is where a tumour occurs in the melanocyte cells of the skin as well as the mucosal membranes. Three types of malignant melanomas are differentiated on a histological level, namely:

- ❖ nodular melanomas,
- ❖ acral lentiginous, and
- ❖ superficial spreading (Gungor *et al.*, 2009:169).

According to Sperling (2000), the estimate for 2010 of the amount of deaths as well as new cases of people with melanoma of the skin would have been 8 700 (from which 5 670 are male

and 3 030 female) and 68 130 (from which 38 870 are male and 29 260 female), respectively. These amounts exclude basal cell and squamous cell carcinoma and account for all races. Melanoma is a malignant tumour derived from epidermal melanocytes and can occur in any tissue that contains these cells. These tissues include non-cutaneous sites such as the oral mucosa, nasopharynx, paranasal sinuses, tracheobronchial tree, vulva, vagina, anus, urinary tract, central nervous system, and eye. Fortunately, however, most melanomas arise on the skin surface and are therefore amenable to early detection (Cummins *et al.*, 2006:501). Cutaneous malignant melanoma (CMM) has a significantly higher morbidity and mortality. Although it is the third most common skin cancer, accounting for only 3% of all skin cancers, CMM accounts for 65% of all skin cancer deaths (Cummins *et al.*, 2006:500).

2.2.4 Colon cancer

Colorectal cancer is an extremely common type of cancer. It accounts for approximately 130 000 cases in the United States annually; 57 000 of these cases result in death (Beers *et al.*, 2006:175). At the age of 40, the occurrence begins to increase and reaches a peak in patients at the age of 50 years and older (Kerr *et al.*, 2001:1, 13).

When one distinguishes between colon and rectal cancer, women have a greater chance to get colon cancer, whereas men are more likely to be diagnosed with rectal cancer. In 5% of the cases there are more than one of these cancers present (Beers *et al.*, 2006:175).

Colorectal cancer is one of the types of cancer, which initially shows no symptoms; as it grows very slowly. In most cases, large lesions have already grown when the first symptoms appear. Common symptoms include changes in bowel behaviour or blood in the faeces (Beers *et al.*, 2006:175). The symptoms highly depend on the region of the colon or rectum that is affected (Bond & Tuckey, 2009:107).

Treatment includes chemotherapy with 5-fluorouracil and leucovorin as well as surgical resection of the tumour (Beers *et al.*, 2006:176).

2.2.5 Breast cancer

According to Nkondjock & Ghadirian (2004:1), breast cancer in women is considered the second most frequent cause of death that is related to cancer in the majority of industrialised states. They also stated that one woman out of every eight or nine (approximately 12%) has the risk to develop breast cancer at some stage in their lifetime.

Some of the risk factors for developing breast cancer include:

- ❖ Family history – when two or more relatives (a mother, daughter or sister) of the patient have breast cancer, the risk increases five - six times.
- ❖ Early menarche, late first pregnancies (after age 30) and late menopause may also increase the risk.
- ❖ Risk may be somewhat higher in women who use oral contraceptives, especially when they started using it before the age of 20.
- ❖ In postmenopausal women – hormone therapy with oestrogen and progestin may increase the risk after a 3-year period.
- ❖ There may be an increase in risk if the patient has been exposed to radiation therapy prior to the age of 30 (Beers *et al.*, 2006:2112).
- ❖ Where there are known gene mutations (breast cancer susceptibility gene 1 (BRCA1) and/or breast cancer susceptibility gene 2 (BRCA2)) for breast cancer – these women are considered to be at a high risk (Levy-Lahad & Friedman, 2007:11).

The most common sign that presents itself in the early stages of breast cancer is a lump that is usually asymptomatic and discovered with a mammogram or a physical examination of the breast. A biopsy is however the only confirmation of the diagnosis (Beers *et al.*, 2006:2113).

In most cases, the primary treatment consists of chemotherapy and/or surgery. Chemotherapy is considered as a reasonable pre-operative strategy to reduce the tumour size in breast cancer that is locally advanced, and thus, facilitates surgery to be less invasive, which in turn improves the outcome of the treatment (WHO, 2006:17).

Patients can be diagnosed with either LCIS (lobular carcinoma *in situ*) or DCIS (ductal carcinoma *in situ*). LCIS refers to a non-malignant lesion within the lobules of the breast that is non-palpable and often discovered via a biopsy. DCIS is cancer cells in the ducts of the breasts that is confined to a small area and can only be detected with a mammogram (Beers *et al.*, 2006:2113). LCIS can be treated with a daily dose of oral tamoxifen or a bilateral mastectomy when tamoxifen is refused by the patient or inappropriate. DCIS, on the other hand, is usually treated by means of a simple mastectomy (Beers *et al.*, 2006:2115, 2116).

2.3 5-FLUOROURACIL

5-Fluorouracil, in its raw material form, is a fragrance-free, white solid crystalline powder. It has optimum solubility in polar solvents, and is partially soluble in cold water, methanol and

insoluble in diethyl ether. This compound can be absorbed through skin, inhalation and ingestion (MSDS, 2008:3-4). It is used as an antineoplastic in the adjuvant treatment of breast and gastrointestinal malignancies and the palliation of various other malignant neoplasms particularly those of the gastrointestinal tract. It is given via intravenous injection or infusion, intra-arterial infusion, or by mouth. 5-Fluorouracil is also applied topically in the treatment of solar keratosis and superficial neoplasms of the skin. Toxic effects of 5-fluorouracil may be severe and sometimes fatal (Reynolds, 1993:480).



Figure 2.4: Chemical structure of 5-fluorouracil (adapted from Singh *et al.*, 2005:99)

2.3.1 Pharmacological classification of 5-fluorouracil

Fluorouracil is one of the cell cycle-specific (CCS) agents of the anticancer drugs. It is classified as an anti-metabolite and falls into the pyrimidine antagonists group. Some of the other anti-metabolites that fall in this group include capecitabine, cytarabine and gemcitabine. In Table 2.1 a thorough pharmacological classification of 5-fluorouracil will be given (Chu & Sartorelli, 2004:901, 909, 910).

Table 2.1: Pharmacological classification of 5-fluorouracil

Cell cycle-specific (CCS) agents		Cell cycle-non-specific (CCNS) agents
Anti-metabolites		Alkylating agents
Methotrexate		Busulfan
Thioguanine		Carmustine
Fludarabine		Cyclophosphamide
Cladribine		Lomustine
Fluorouracil		Mechlorethamine
Capecitabine		Melphalan
Cytarabine		Thiotepa
Gemcitabine		Anthracyclines
Mercaptopurine		Duanorubicin
Anti-tumour antibiotic		Doxorubicin
Bleomycin		Epirubicin
Epipodophyllotoxins		Idarubicin
Etoposide		Mitoxantrone
Teniposide		Anti-tumour antibiotic
Taxanes		Dactinomycin
Docetaxel		Mitomycin
Paclitaxel		Camptothecins
Vinca alkaloids		Irinotecan
Vinblastine		Topotecan
Vincristine		Platinum analogs
Vinorelbine		Carboplatin
		Cisplatin
		Oxaliplatin

2.3.2 Mechanism of action

5-Fluorouracil is known as a prodrug, which means that it must first undergo a series of reactions that transform it into its active metabolites: 5-fluorouridine-5'-triphosphate (FUTP), 5-fluoro-2'-deoxyuridine-5'-monophosphate (FdUMP) and 5-fluorodeoxyuridine-5'-triphosphate (FdUTP). These metabolites are responsible for its cytotoxic effects (Chu & Sartorelli, 2004:909). The mechanism of action of 5-fluorouracil is illustrated in Figure 2.5.

From Figure 2.5 it is clear that, by inhibiting the processes of both RNA - and DNA - mediated events, fluorouracil is cytotoxic, meaning that it prevents the spreading of cancerous cells by inhibiting the translation of RNA as well as the function, synthesis and repair of DNA (Chu &

Sartorelli, 2004:909). It also has immunosuppressant properties (Reynolds, 1993:480).

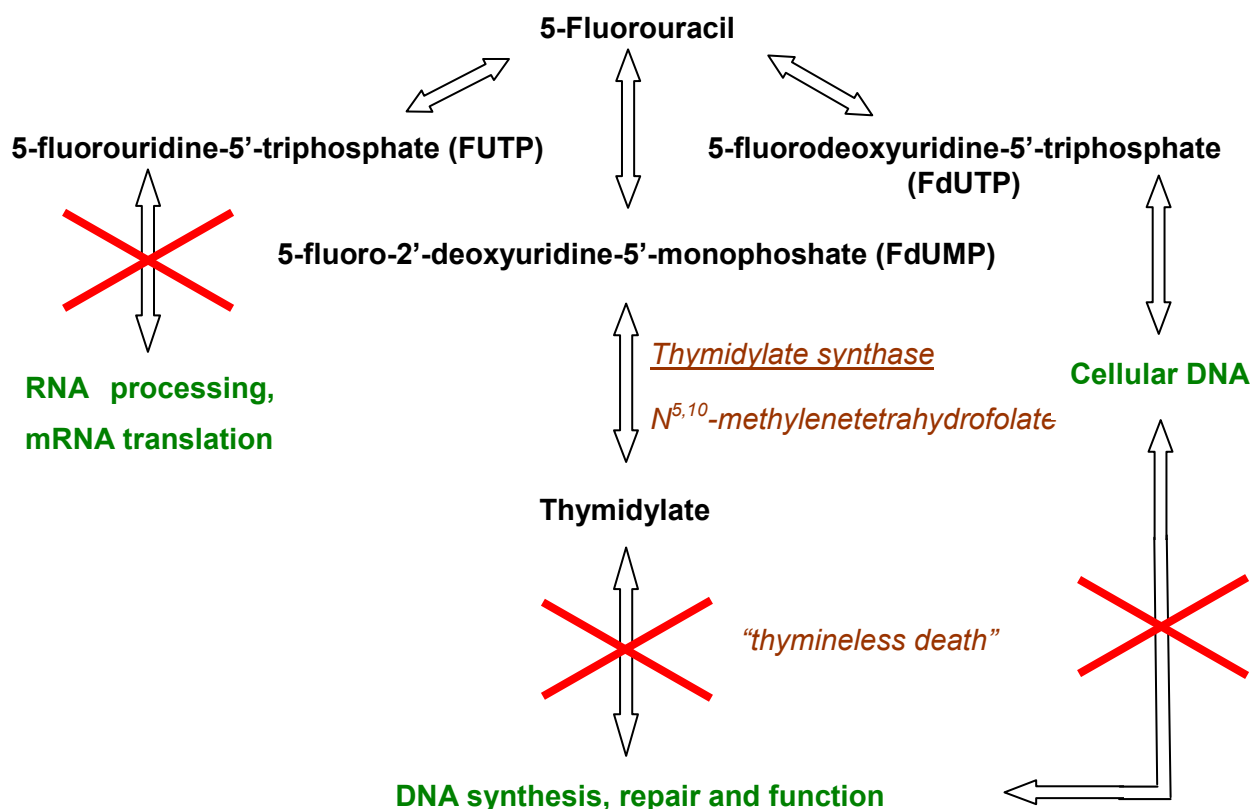


Figure 2.5: Schematic illustration of the mechanism of action of 5-fluorouracil

2.3.3 Clinical uses of 5-fluorouracil

5-Fluorouracil, the chemotherapy agent of fluorouracil, is used for a large variety of concrete malignancies, i.e., cancer of the gastrointestinal tract (colon, stomach and rectum) as well as breast cancer (Kaiser *et al.*, 2003:123). It can be used alone or in combination in the adjuvant therapies of the above-mentioned cancers, as well as palliation of inoperable malignant neoplasms, especially those of the gastrointestinal tract, breast, head and neck, liver, genito-urinary system and pancreas (Reynolds, 1993:480). It can also be used in the topical treatment of certain cancerous conditions of the skin similar to actinic keratosis, pre-malignant conditions and other neoplasms of the skin (Gibbon, 2005:333). 5-Fluorouracil shows potential for antineoplastic activity alongside numerous malignant and pre-malignant conditions of the skin. These conditions include Bowen's disease along with external basal cell carcinomas (Singh *et al.*, 2005:99).

5-Fluorouracil is frequently used as an anti-scarring agent during ophthalmic surgeries, such as a trabeculectomy where the intra-ocular pressure is being reduced in patients who suffer from glaucoma. 5-Fluorouracil is injected subconjunctivally over the scleral flap area to reduce the risk of surgery failure that could exist in cases where the trabeculectomy site is scarred. This

procedure seems to be a rather capable approach that has few problems and may conquer early as well as mid term failures of a trabeculectomy (Durak *et al.*, 2003:189).

2.3.4 Side effects and toxicities of 5-fluorouracil

Generally, 5-fluorouracil is administered by continuous infusion or as an intravenous (IV) bolus (Hamilton, 2003:607) due to its radically low bioavailability when given orally (Chu & Sartorelli, 2004:910). 5-Fluorouracil can also be administered topically by means of a cream, but in this form, it usually has fewer or very mild side effects. Extremely high toxicities are generally the result of a 5-fluorouracil IV bolus (15 mg/kg/week; 15 mg/kg/day for a period of 5 days, by 24 h infusion). The main adverse effects are on the bone marrow and the gastrointestinal tract (ulceration and bleeding). Reducing the rate of injection to a slow infusion over several hours can decrease haematological but not gastrointestinal toxicity, although last mentioned may be exacerbated if fluorouracil is given with folinic acid (Reynolds, 1993:480).

The following are major toxicities of 5-fluorouracil:

- ❖ diarrhoea;
- ❖ nausea;
- ❖ mucositis;
- ❖ myelosuppression; &
- ❖ hand and foot syndrome (Chu & Sartorelli, 2004:908).

Diarrhoea and stomatitis pose significant danger when it is the result of mucosal damage caused by continuous infusion. In these cases anti-diarrhoeal drugs, such as loperamide and octreotide should be used as intervention (Hamilton, 2003:607). Other common toxicities are listed in Table 2.2.

Table 2.2: Toxicities of 5-fluorouracil

Dermatological	Ocular
Alopecia	Itching
Hyper-pigmentation	Excessive tearing
Photosensitivity	Burning (Hamilton, 2003:603)
Nail banding (Hamilton, 2003:603)	
Redness, burning and scaling – topical 5-fluorouracil (Beers <i>et al.</i> , 2006:963)	

The toxicities mentioned in Table 2.2, all depend on the route, schedule, as well as the dose of

administration (Hamilton, 2003:607).

2.3.5 Contra-indications of 5-fluorouracil

The experience with 5-fluorouracil during pregnancy is limited but, following systemic therapy in the first trimester, multiple birth defects have been reported in an aborted fetus (Bawle *et al.*, 1998:54). Thus, it is very important that patients should take extra care while using 5-fluorouracil, by taking a contraceptive during, and for a few months after, the treatment.

A large quantity of 5-fluorouracil is degraded *in vivo* by an enzyme called dihydropyrimidine dehydrogenase (DPD) to fluorinated β -alanine. The level of DPD activity is a major determinant in the toxicity of 5-fluorouracil (Wei *et al.*, 1996:610). Patients with a deficiency can thus not use 5-fluorouracil-containing products, as it will reach toxic levels in the body resulting in a number of dangerous side effects.

2.3.6 Transdermal delivery of 5-fluorouracil

The SC is known as the major barrier that must be overcome by any drug to enter the body via the skin. Its hydrophobic nature and negative charge make it particularly difficult for drugs i.e., 5-fluorouracil to cross, which is hydrophilic in nature and also carry a negative charge (Prausnitz *et al.*, 1996:208).

5-Fluorouracil has pKa values of 8.0 and 13.0 (Singh *et al.*, 2005:99). It also has a octanol-water partition coefficient (log P) value of -0.89 (Quigley & Lloyd, 2002:244), this property suggests that it is a polar molecule, implicating that it has a hydrophilic nature, therefore it will have difficulty penetrating through the highly lipophilic SC (Singh *et al.*, 2005:99). These properties make it somewhat difficult to deliver 5-fluorouracil via the transdermal route and thus the need arises to assist this process by using certain alternative methods in reaching this goal. Some of the approaches that have been investigated in the past include electroporation, iontophoresis, laser treatment, phonophoresis, prodrugs and penetration enhancers (Singh *et al.*, 2005:99). However, in this study the use of Pheroid™ technology will be used in an attempt to optimise the process of penetrating the skin.

Except for the clinical value that topical preparations of 5-fluorouracil holds in the treatment of certain types of skin cancers, one should bear in mind that the transdermal delivery of this drug may be very useful. By incorporating the transdermal route, some of the well-known boundaries related to the oral and the parental routes of administration may be conquered (Singh *et al.*, 2005:99). These boundaries include poor absorption of 5-fluorouracil when administered orally, followed by an inconsistent bioavailability, with interpatient variations (Baccanari *et al.*,

1993:11067).

Guerquin-Kern *et al.*, (1991:5772) measured the terminal half-life of 5-fluorouracil in different types of tissue and in a pH range of 7.0 - 7.4. They concluded that 5-fluorouracil had a quick elimination due to its significantly short therapeutic half-life (in the region of approximately 30 min).

2.4 TRANSDERMAL

2.4.1 Structure of the skin

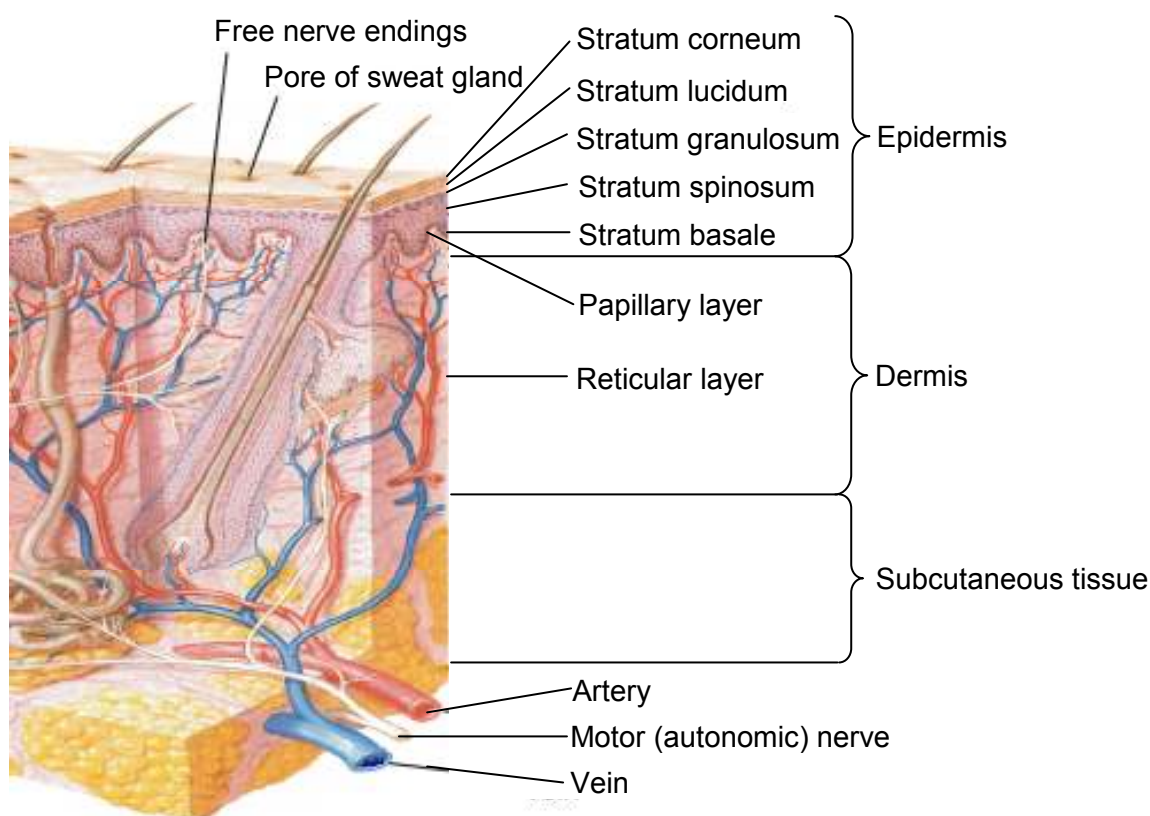


Figure 2.6: A cross-section of the human skin (Adapted from Habif, 2004:1).

To fulfil the main function of the skin, namely, to protect the human body from the harmful environment that surrounds it, it implies that the skin acts as a barrier, keeping water inside the body while it inhibits other harmful molecules from entering the body. Bearing in mind, the maturity of this uniquely developed structure; it can ease the challenges to improve percutaneous absorption of drug particles (Potts *et al.*, 1992:14).

The skin comprises of three major layers that contribute to its extensive barrier function, from top to bottom (as seen in Figure 2.6):

- ❖ Epidermis (avascular, cellular)
- ❖ Dermis (the connective tissue)
- ❖ Hypodermis (subcutaneous, fatty layer) (El Maghraby *et al.*, 2008:204).

Although there are three major layers of the skin, the epidermis can be divided into two layers, namely the SC as well as the viable epidermis. The viable epidermis consists of the basal, granular, malphigian and cell layers (Potts *et al.*, 1992:14). These layers are known as the stratum lucidum, stratum granulosum, stratum spinosum and the stratum germinativum (Fang & Leu, 2006:212).

2.4.1.1 Stratum corneum

This is the outermost or upper part of the epidermis, which consists of corneocytes. These corneocytes are keratinised cells, which are surrounded by lipids (Wickett & Visscher, 2006:98). The SC is arranged in 15 - 20 layers and has a thickness of 10 - 15 µm when it is dry. As the SC is hydrated, it swells and reaches a thickness of 40 µm (El Maghraby *et al.*, 2008:204). In various sources, the SC is referred to as the rate-limiting barrier in transdermal permeation of most molecules.

The structure of this unique layer is often described as a “brick and mortar array”. The so-called bricks are represented by the keratin corneocytes, which are entrenched in the mortar that is represented by the intercellular lipid-loaded mould (El Maghraby *et al.*, 2008:204).

Barry (1983:6) has often referred to the SC as the horny layer of the skin as it forms structures similar to hair and nails (Franz & Lehman, 2000:16). If one would have to weigh the SC, approximately 14% of the weight would consist of lipids. In spite of the SC being a dehydrated structure, it can take up a large amount of water (nearly 5 times its mass) when it is placed in an aqueous solution (Scheuplein & Morgan, 1969:456).

Other unique characteristics of the SC include its resistance, stability and its insolubility. These properties are a result of the bulky cell membrane as well as the cell matrix, which comprise of amorphous proteins that have high sulphur content and also lipids with numerous disulphide linkages (Fang & Leu, 2006:212).

2.4.1.2 Epidermis

The epidermis is the part of the skin that is on the outside and is described as a stratified squamous membrane (Habif, 2004:1). It consists of a few layers; one of which is made up by basal cells (columnar cells) arranged in a single row. Basal cells divide, and form keratinocytes that include the spinous layer. Intercellular bridges link cells from this layer and therefore appear as lines. Insoluble protein is synthesised by the keratinocytes and the protein lingers in the cell, as it becomes a major part of the outermost layer of the epidermis, the SC (Habif, 2004:1).

The epidermis is known as the avascular layer (no blood vessels) and thus derives its nutrients from the dermis underneath it, which is highly perfused. There are some biochemical processes that take place in the epidermal cells, i.e. proliferation and differentiation. These events push the cells to the surface forming the SC (Potts *et al.*, 1992:14). Cells of the SC are dead and therefore the epidermis (exclusive of the SC) is generally expressed as the viable epidermis (El Maghraby *et al.*, 2008:204). The epidermis wraps itself over the whole external surface of the human body with a thickness ranging from 0.06 - 0.10 mm, however, areas like the palms and soles can be significantly thicker due to the SC in these locations (Franz & Lehman, 2000:17).

2.4.1.3 Dermis

The dermis is the layer directly underneath the epidermis. According to Williams (2003:2), the dermis is the largest component of the skin and it mainly consists of water, which makes it easy for polar molecules to be delivered via the transdermal route. Barry (1983:7) stated that it has a thickness of approximately 3 - 5 mm and is made out of tough proteins, which include elastin and collagen; as well as an interfibrillar gel, consisting of glycosaminoglycans, water and salts.

Hair follicles and sweat glands are situated in the dermis. Furthermore, to distinguish it from the epidermis that is avascular; this layer encloses arterioles, capillaries and venules (Potts *et al.*, 1992:16) as well as lymphatics and sensory nerves (Fang & Leu, 2006:212).

The dermis consists of three different kinds of joined tissue, which are reticular fibres, elastic tissue and collagen. It has two layers – a thin higher layer and a thick lower layer. The thin upper layer is also known as the papillary layer, which is made up of collagen fibres that are irregularly arranged. The thick lower layer, also known as the reticular layer, spreads out from underneath the thin upper layer to connect with the subcutaneous tissue. This layer is also made up of collagen fibres, but is much thicker and more equivalently arranged to the exterior of the skin (Habif, 2004:1).

2.4.1.4 Hypodermis

The hypodermis is the subcutaneous fatty tissue underneath the dermis. It consists of a netting of fatty cells (adipocytes) that is grouped in lobules and parted by filamentous (collagen) clusters (Franz & Lehman, 2000:16).

According to Franz & Lehman (2000:16), the hypodermis plays a very important role in acting as a thermal isolator as well as a pillow to powerful forces. However, its significance to dermal or transdermal drug delivery is certainly not well known.

2.4.1.5 Skin appendages

The following act as appendages of the skin:

- ❖ eccrine and apocrine sweat glands;
- ❖ hair follicles; and
- ❖ sebaceous glands.

The ducts of the first two appendages mentioned above, open in the SC and their primary function is to operate as shunts during the diffusion process. The third appendage evacuates into the hair follicle's tube approximately 0.5 mm underneath the skin surface (Franz & Lehman, 2000:24).

The openings of these appendages take up 1% or less of the skin's surface area, although their contribution is very little, they do indeed contribute to absorption (Franz & Lehman, 2000:24).

2.4.2 Routes of drug permeation across the skin

The process for a molecule to penetrate the skin includes a series of happenings that starts with the partitioning of the molecules to the SC from the vehicle carrying it. After which the drug molecules must penetrate the SC, following diffusion across each skin layer to reach a network of capillaries at the place where the epidermis and the dermis meet. Lastly, the drug molecules are transported via the blood to reach the target area where it can attain its therapeutic action (Fang & Leu, 2006:212).

Drug particles may permeate the skin through two major pathways, namely: transappendageal and transepidermal. These pathways are illustrated in Figure 2.7.

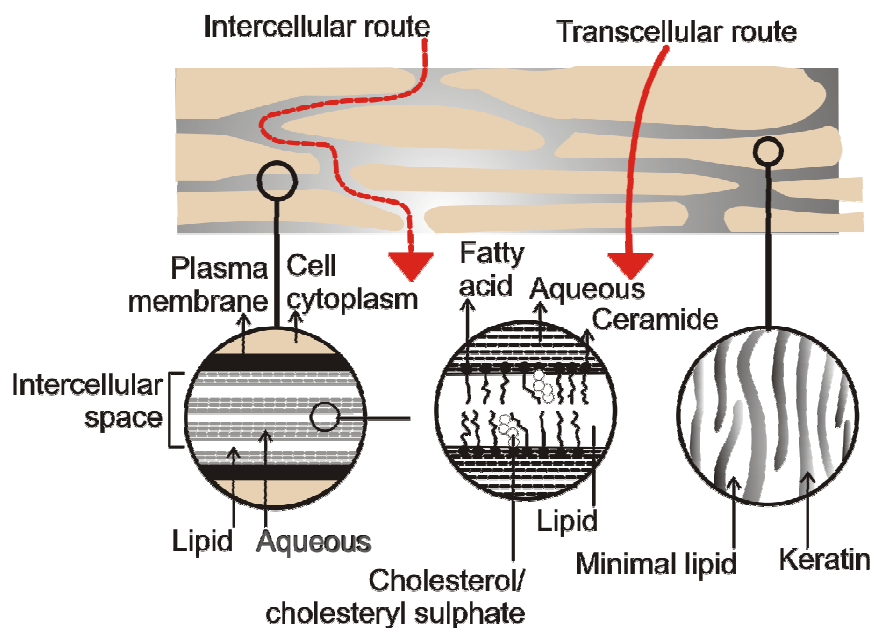


Figure 2.7: Pathways for drug delivery across the skin (Adapted from Fang & Leu, 2006:212).

The transappendageal route refers to permeation through the following two skin appendages: the hair follicles and the sweat glands, with their linked sebaceous glands (El Maghraby *et al.*, 2008:204).

El Maghraby *et al.* (2008:205) defined the transepidermal route as the pathway by which molecules filter through the unbroken, intact SC. This route is divided into two smaller pathways, namely the intercellular and the transcellular routes.

The intercellular pathway is considered as an unbroken and twisted route throughout the domains of the intercellular lipids and it is generally considered the main road of access for most drug molecules, regardless of the fairly small available surface area. By taking this into consideration, we realize that the intercellular lipids have a foremost function in the obstruction character of the SC (El Maghraby *et al.*, 2008:205).

On the other hand, the transcellular route goes right through the cells of the SC, called keratinocytes, after which it crosses the intercellular lipids. This pathway involves diffusion through as well as partitioning into the keratin bricks and it also requires permeation across and into the intercellular lipids (El Maghraby *et al.*, 2008:205).

All molecules permeate through a combination of all three of these routes, although their permeation still depends on the physiochemical properties of each particle (El Maghraby *et al.*, 2008:205).

2.4.3 Physiochemical properties influencing transdermal delivery

There are many important characteristics that transdermal drugs should have in common, but Prausnitz *et al.* (2004:116) defined three of the most important, which include:

- ❖ Molecular mass < 500 Da
- ❖ High oil solubility
- ❖ Small (milligrams) essential dose

Molecules should also have a melting point below 200 °C and a log P of round about 2 (Farahmand & Maibach, 2009:2). The following biopharmaceutical and pharmacokinetic attributes are also of great importance:

- ❖ A daily dose of smaller than 20 mg.
- ❖ Half-life of 10 h or less.
- ❖ The lipid solubility should be high, log P of = - 1.0 to 4.0.
- ❖ Skin permeability should be high, permeability coefficient of > 0.5 x 10⁻³ cm/hr.
- ❖ Non-sensitising and non-irritating to the skin.
- ❖ Oral bioavailability should be low.
- ❖ Therapeutic index should be low (Pfister, 1997:48).

The different physiochemical properties will be discussed in further detail.

2.4.3.1 Diffusion coefficient (D)

Diffusion is defined as the process where molecules are transferred in a system from one component to another. This process is a result of unsystematic movements of the molecules, which move from a high concentration to a low concentration (Crank, 1999:1). The unit that D is measured in is usually in an area per unit time, for example cm²/h or cm²/s (Williams, 2003:27).

In one study done by Sonavane *et al.* (2008:4), where they did *in vitro* tests to see what the effect of particle size was on the permeation of gold nanoparticles through the skin and intestine of rats, they calculated a theoretical diffusion coefficient (D_(T)) by using an equation called the “Stokes-Einstein” equation:

$$D_{(T)} = kT / 6 \pi \eta a \quad \text{Equation 2.1}$$

where: $D_{(T)}$ = the diffusion coefficient
 T = the temperature
 η = the medium's viscosity
 a = the nanoparticle's radius

There are many mathematical equations where the diffusion coefficient (D) is used. In some instances D may be taken as a constant (in diluted solutions) or in other cases it is dependent on the concentration of the diffusing substance (for example polymers) (Crank, 1999:2).

The diffusion coefficient will further be discussed under Fick's law of diffusion, where it is present in a mathematical equation. This equation is very often used in transdermal theory and therefore will be understood better when seen in context with the other entities.

2.4.3.2 Fick's law of diffusion

As previously discussed, diffusion is a process where molecules move from a higher to a lower concentration. Williams (2003:41) states that these molecules, which are constantly in motion, move about in reaction to a thermodynamic vigour that arises from a concentration gradient. Fick's first law declares that the transferring rate of the diffusing molecules through a unit area of a division is comparative to the concentration gradient, which is calculated normal to the division. Equation 2.2 represents the first law of Fick:

$$J = - D \, dC / dx \quad \text{Equation 2.2}$$

where: J = the flux of the permeant (rate of transfer per unit area), mol/cm²/s
 D = the diffusion coefficient of the permeant, cm²/s
 C = the concentration of the diffusing substance, mol/cm³
 x = the length coordinate measured normal to the division, cm

} Concentration
gradient

Equation 2.2 was derived from a combination of Williams (2003:41) and Sonavane *et al.* (2008:4). In conclusion, Fick's first law of diffusion can be used to calculate the rate of diffusion by which a particle diffuses across the skin. Another equation that represents the first law of Fick is as follows:

$$J_s = P_e \times \Delta C \quad \text{Equation 2.3}$$

where: J_s = the flux of the drug in the skin, µg/cm²/h
 P_e = the permeability coefficient of the drug, cm/h
 ΔC = the difference in the drug concentration across the skin, ng/cm³ (Roy, 1997:143)

2.4.3.3 Partition coefficient, log P and lipophilicity

For a drug to reach the viable tissue of the epidermis, it needs to undergo a phase change which implies that it should be transferred from the SC's lipophilic intercellular pathways to these living aqueous cells of the epidermis which are fundamentally buffered to a pH of 7.4 (Hadgraft & Wolff, 1993:165, 166). For this to occur the drug should have the necessary properties to cross the skin and enter the systemic circulation, considering this, lipophilic drugs may enter the aqueous viable epidermis at a slower rate than hydrophilic molecules. The partition coefficient should thus be among a suitable aqueous and lipid phase at pH 7.4 and is therefore considered a very important property as many drugs have ionisable groups (Hadgraft & Wolff, 1993:166). Usually the ionised form of a molecule will be more water-soluble, and in the unionised form more lipid-soluble (Hillery, 2001:23, 24). To add to this statement, Naik *et al.* (2000:319) came to a conclusion that the ideal would be for a drug to have both aqueous and lipoidal solubilities, because if the molecules are too hydrophilic, they will not be able to be transported into the lipid-rich SC and vice versa – if they are too lipophilic it will cause the molecules to stay behind in the layers of the SC.

The partition coefficient of a drug is usually referred to as the oil/water equilibrium partition coefficient and is therefore a measure of the drug's lipid solubility. This has been established by adding a drug to a combination of equivalent volumes of a lipophilic fluid (octanol) and water, the mixture is then shaken energetically to encourage the partitioning of the drug into each of the water and lipid phases. As equilibrium is reached between the lipophilic and hydrophilic phases, the two phases will separate and the drug can then be analysed (Hillery, 2001:21). The partition coefficient (P) can be calculated by using Equation 2.4:

$$P = C_{\text{oil}} / C_{\text{water}} \quad \text{Equation 2.4}$$

where: P = the partition coefficient

C_{oil} = the concentration of the drug in the lipid (oil) phase

C_{water} = the concentration of the drug in the aqueous (water) phase (Hillery, 2001:21)

For any drug to provide a maximum flux across the SC, it should have a combination of good water and lipid solubility as well as a reasonable partition coefficient (Hadgraft & Wolff, 1993:166). Usually there is referred to the logarithm of the partition coefficient (log P), and the following usually accounts for a specific drug:

- ❖ when $\log P = 0$, it suggests that the drug is equivalently divided into each of the two phases,

- ❖ when $\log P > 0$, it indicates that the drug is lipid soluble, and
- ❖ when $\log P < 0$, it is a water-soluble drug (Hillery, 2001:21)

After considering the ideal properties which a drug should consist of for transdermal delivery, drugs with a $\log P$ value of ≤ 2 and those that have a reasonable lipophilic/hydrophilic character are regarded as prospective candidates for this method (Guy & Hadgraft, 1989:70, 71).

Another way to calculate $\log P$ is by means of an equation developed by Potts & Guy (Mitragotri, 2003:70):

$$\log P = -2.7 + 0.71 \log K_{o/w} - 0.0061 M_w \quad \text{Equation 2.5}$$

The partition coefficient ($\log K_p$) of 5-fluorouracil was measured by Einmahl *et al.* (1999:193) with an octanol-phosphate buffer solution at a pH of 7.4 and 37 °C. A 1%-solution of 5-fluorouracil was used with a pH of 4.5, and the value obtained was -0.47. This partition coefficient value of 5-fluorouracil was under 0, indicating (according to the statements above) that 5-fluorouracil is indeed a water-soluble drug.

2.4.3.4 pKa, ionisation and physiological pH

The quantity of a substance that passes through the lipophilic SC layer can largely be affected by the level of ionisation (Williams, 2003:68). As previously mentioned the ionised form of a substance is more water-soluble and will not easily permeate through a lipid-rich membrane; whereas, the unionised form will dissolve more rapidly in the lipophilic membranes. The degree to which a drug ionises depends on certain other physiochemical properties such as the pK_a of the drug as well as the pH of the solution (Hillery, 2001:23, 24). There is an equation that can be used to calculate the degree of drug ionisation and it is called the Henderson-Hasselbach equation:

$$pK_a = pH + \log [HA]/[A^-] \quad \text{Equation 2.6}$$

where: $[A^-]$ = the concentration ionised form of the drug
 $[HA]$ = the concentration unionised form of the drug

From Equation 2.6 there can be distinguished between the ionisation of a weak acidic and weak basic substance that is present in the donor solution, thus the following two equations can be used and they apply to substances with only one ionisable group (Williams, 2003:69):

For a weak acidic drug:

$$\log [A^-]/[HA] = \text{pH} - \text{pKa} \quad \text{Equation 2.7}$$

For a weak basic substance:

$$\log [BH^+]/[B] = \text{pKa} - \text{pH} \quad \text{Equation 2.8}$$

The degree to which any drug is ionised should be controlled, as mentioned earlier, as it can have a large influence on the permeation through the skin, this necessitates the use of a drug vehicle that is buffered to keep the pH constant (Williams, 2003:70).

Williams (2003:70) has stated a common rule to create insignificant ionisation, this can be accomplished by selecting a buffer that is 2 - 3 pH units less than the weak acidic permeant's pKa-value or, on the other hand, one that is 2 - 3 pH units more than the weak basic substance's pKa-value.

One can conclude that when a pH of the same value as the pKa is used, it will indicate that there will be equal amounts of ionised and unionised quantities present. Thus, when taking the pKa-values of 5-fluorouracil into account, pKa1 = 8.0 and pKa2 = 13.0, these are the pH-values where there will be equal amounts of drug in the ionised as well as the unionised form.

2.4.3.5 Molecular weight and size (M)

The size of a molecule is one of the foremost important factors, which determines its fluctuation through the skin. The molecular size has a great influence on the permeation ability and thus it is most appropriate to use molecular volume as a measure when one has large quantities of the permeant (Williams, 2003:36). To simplify this statement, Williams (2003:36) has made the conclusion that molecular weight is usually used, as it is an estimation of the molecular volume, and this accounts for when one presumes that the molecules are in actual fact sphere-shaped. Most of the molecules that are chosen as usual therapeutic agents and used for delivery via the transdermal route are small molecules, which have molecular weights that range between 100 and 500 Da (Williams, 2003:37).

As mentioned before, the ideal molecular weight for any drug to be delivered via the transdermal route should be less than 500 Da (Naik *et al.*, 2000:31). This makes 5-fluorouracil a good candidate for the transdermal route, as it has a molecular weight of 130.08 Da (Drugbank, 2005). Thus, its relatively small size makes it easier for this molecule to cross the membranes of the skin.

2.4.3.6 Permeability coefficient (kp)

The permeability of a substance indicates how well the molecules permeate through the skin. By calculating the permeability coefficient one can determine the cumulative quantity of the substance that permeates through a component of the surface area of the skin and this is plotted against time (Cole & Heard, 2007:12). Equation 2.9 can be used to calculate this property:

$$K_p = J_s / C_v \quad \text{Equation 2.9}$$

where: K_p = permeability coefficient in cm.h^{-1}

J_s = the fluctuation of the drug, an amount permeating through a region per unit time (Williams, 2003:41), which can be obtained from the linear fraction of a concentration-time profile, in $\mu\text{g.cm}^{-2}.\text{h}^{-1}$

C_v = the total concentration of the drug solute, in $\mu\text{g.cm}^{-2}$ (Duracher *et al.*, 2009:3).

One can also calculate the permeability coefficient (P_e) by means of a different equation, by using some of the other physiochemical properties such as the partition coefficient (K_{sc}) of the drug molecule between the vehicle and the SC, the diffusion coefficient (D_{sc}) in the SC, as well as the thickness (h_{sc}) of the SC (Roy, 1997:146), as shown in Equation 2.10:

$$P_e = \frac{K_{sc} \times D_{sc}}{h_{sc}} \quad \text{Equation 2.10}$$

2.4.3.7 Solubility

The solubility of a drug in the SC as well as the amount of a drug applied to the skin will determine the degree to which the drug will be released from the formulation and accordingly partition into the external layers of the SC. A drug's solubility constraint can thus be seen as the probability it has to form a reservoir in the SC of the skin (Hadgraft & Wolff, 1993:162,163).

There are two different equations by which the solubility ($\sigma_{sc}/\mu\text{g.cm}^{-2}$) can be calculated:

$$\text{Log } \sigma_{sc} = 1.31 \log [\text{oct}] - 0.13 \quad \text{Equation 2.11}$$

$$\text{Log } \sigma_{sc} = 1.911 (10^3/\text{mp}) - 2.956 \quad \text{Equation 2.12}$$

where: $[\text{oct}]$ = the permeant's octanol solubility (g/l)

M_p = the permeant's melting point, Kelvin (Hadgraft & Wolff, 1993:162).

From the aforementioned equations, it is clear that a drug with a lower melting point will go through the skin more readily than that of a drug with a higher melting point.

The solubility of 5-fluorouracil in different solvents is shown in Table 2.3.

Table 2.3: Solubility of 5-fluorouracil in different solvents (NIH, 2007:2).

Solvent	Polarity	Solubility (g/l)
Dimethylformamide	Most polar	28.50
Water		13.26
Methanol		7.69
Ethanol		3.26
Benzene and chloroform	Least polar	virtually insoluble

Ideally, a drug should possess both lipoidal and aqueous solubilities, if it is too hydrophilic, the molecule will be unable to transfer into the stratum corneum; and if it is too lipophilic, the drug will tend to remain in the layers of the stratum corneum. A drug should have an aqueous solubility of more than 1 mg/ml to ideally be delivered by the transdermal route (Naik *et al.*, 2000:319).

2.4.3.8 Melting point (Mp)

According to Hadgraft & Wolff (1993:163), it can usually be assumed that drugs with low melting points enter the skin more easily than those with higher melting points. The melting point of a drug has a great influence on its solubility and thus also on its potential to penetrate the skin. The correlation between these two properties is described by means of the regular solution theory, which declares that, in a specified solvent, a substance with a lower melting point results in higher solubility (Benson, 2005:26). Thus, the higher the melting point of a given drug, the less soluble it becomes and this will make it more difficult to cross the SC, as previously mentioned.

Naik *et al.* (2000:319) state that there are certain aspects that should be taken into consideration before the formulation of a drug can be done, and one of these aspects is that the ideal melting point to reach passive transdermal drug delivery should be less than 200 °C. The melting point of 5-fluorouracil lies between 282 and 283 °C (Bayomi & Al-Badr, 1989:606). This is a bit higher than one should consider the ideal melting point due to certain functional groups on the 5-fluorouracil structure, which causes problems. The amide- and imide-like groups on the 5-fluorouracil structure usually have the tendency to develop intermolecular hydrogen bonds and these “links” enhance the molecule’s crystal lattice energy, which in turn leads to a higher

melting point and thus lower solubilities in general (Sloan & Beall, 1993:86).

Important aspects to consider during the formulation process with 5-fluorouracil:

- 1) Stability: In solid form, 5-fluorouracil is stable when stored in dark bottles at 25 °C. Solutions are stable at pH-levels under 9. High temperatures and alkaline conditions may contribute to instability.
- 2) Storage: solid 5-fluorouracil and solutions should be stored in dark-coloured containers, tightly closed and preferably in a refrigerator.
- 3) When exposed to the skin, it may cause erythema (NIH,200?:2-3).

2.4.4 Penetration enhancers

There are many different ways to enhance drug penetration across the skin and all of these methods can be classified under two main categories, namely chemical enhancement methods as well as physical enhancement methods. As far as the chemical method is concerned, this strategy makes use of certain chemicals to make the SC more porous, some of the substances include amines, amides, alcohols, polyalcohols, fatty acids, pyrrolidones, esters and sulphoxides. These chemicals have the power to compromise the purpose of the skin as barrier and thus allow other molecules that usually struggle to cross the skin, to enter the skin and reach the systemic circulation (Naik *et al.*, 2000:321).

Much progress has been made in the last few years to overcome certain challenges in transdermal delivery as many of the drugs are too large or in their charged (polar) state which make it difficult to deliver these drugs via the transdermal route. Hence, there have been advanced physical enhancement methods, which counter these challenges and thus contribute to a more effective drug delivery process (Naik *et al.*, 2000:322). Some of the physical penetration enhancement methods (iontophoresis and electroporation) as well as liposomes and a pharmaceutical application called Pheroid™ technology will be discussed in further detail.

2.4.4.1 Iontophoresis

This process is used to drive ionic drugs across the skin and into the body by means of a small quantity of an electric current (Banga *et al.*, 1999:2). This is said to be the most evolved technology of all the physical enhancement methods. The size of the current that is used to accomplish this is usually smaller than 500 $\mu\text{A}/\text{cm}^2$ (Naik *et al.*, 2000:322). Iontophoresis can be seen as a very exclusive prospect to accomplish drug delivery that is programmable, because the amount of the drug delivered is in ratio with the size of the current, which can also be readily adapted, producing the correct dose of the drug to any individual. This type of dosage form will

be developed to be the size of an average transdermal patch (Banga *et al.*, 1999:2).

2.4.4.2 Electroporation

Electroporation is a physical enhancement method where small pulses of high voltage (100 – 1000 V) are used to conquer the barrier properties of the lipophilic SC and thus produce aqueous pathways through which the drug can penetrate the skin (Jadoul *et al.*, 1998:265).

In a study conducted by Fang *et al.* (2004:246); they used this method in comparison with laser treatment as well as iontophoresis to deliver 5-fluorouracil via the transdermal route. What they found was that electroporation had created a reservoir-effect for 5-fluorouracil, the reason being that, even after the pulses had been removed, the quantity of 5-fluorouracil, which penetrated across the skin, still remained elevated. They came to the conclusion that electroporation caused a definite alteration in the structure of the skin which created these significant changes in the skin's permeative ability.

2.4.4.3 Liposomes

Liposomes have been used as a drug delivery system from the early 1980s to improve drug delivery via the skin. These lipid vesicles have many different functions when applied to the skin (El Maghraby *et al.*, 2008:206). Some of these functions are listed below:

- ❖ Provide localised effects, thus reducing systemic absorption and minimise unwanted side effects.
- ❖ Offer targeted delivery through skin appendages.
- ❖ Improve transdermal drug delivery.

2.4.4.4 Pheroid™

Pheroid™ technology is a patented (Pitmey International NV Patent Portfolio, 1994), delivery system that was previously known as Emzaloid™ technology. The property of this unique form of drug delivery has been purchased by the North-West University in 2003 from a company called MeyerZall (Pty) Ltd. Mr. Meyer developed the Pheroid™ technology to treat his own skin condition; psoriasis (Grobler *et al.*, 2008:284).

The Pheroid™ drug delivery system consists of stable, submicron structures that are formulated in an emulsion. These structures can be influenced and changed in terms of certain aspects i.e., size, structure, function and morphology. Pheroid™ structures largely comprise of natural essential fatty acids as well as lipid-rich acids in plants which has an affinity for the body's cell

membranes. They have the ability to enclose, transfer and set certain drug molecules free at their site of action; and all of this takes place at a much faster rate than drugs, which are not entrapped in a Pheroid™ (Grobler, 2004:4).

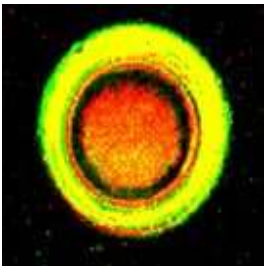
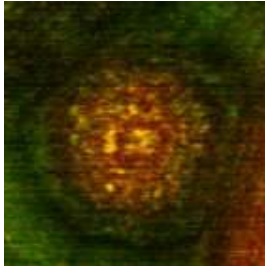
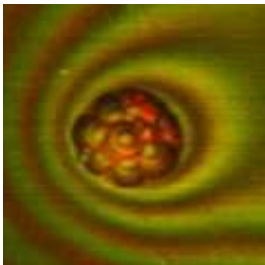
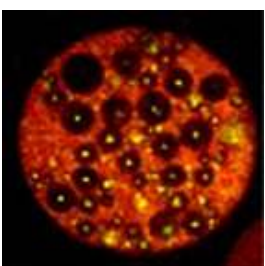
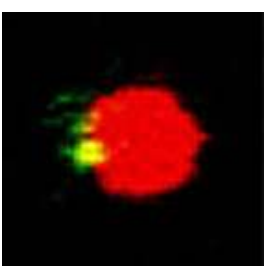
Some of the major advantages of this unique form of enhanced drug delivery system include the following:

- ❖ Improved therapeutic efficacy.
- ❖ Enhanced delivery of active drug molecules.
- ❖ Onset of therapeutic action in reduced time.
- ❖ Penetration of the majority of barriers that are known in the human body and cells.
- ❖ Reduced minimal effective concentration of the drug.
- ❖ Less cytotoxic side effects.
- ❖ Assists in maintaining the cell membranes in the body.
- ❖ Targeting ability for specific areas in the body.
- ❖ A reduced amount of drug resistance.
- ❖ No immunological response.
- ❖ Ability to transport genes to the nuclei of cells (Grobler, 2004:3).

The Pheroid™ can penetrate a range of different barriers in the body, which include skin, bacteria, parasites, fungi, and layers of the vascular system as well as the intestinal layers (Grobler, 2004:4). It has a very unique ingredient that accounts for the gas phase that is added to the dispersed water and oil phases, N₂O. This adds an extra dimension to the Pheroid™ formulation. This anaesthetic compound is soluble in water as well as lipids, which make it possible for N₂O to move freely across the skin's epidermis and dermis (Grobler *et al.*, 2008:289). Another huge advantage over other enhancement delivery systems, such as liposomes, is that Pheroid™ systems have a polyphilic nature. This enables it to entrap drugs with different solubilities (lipophilic and hydrophilic) and even drugs which are insoluble (Grobler, 2004:7).

The different types and shapes of Pheroid™ can be seen via confocal laser scanning microscopy (CLSM) and are illustrated in Table 2.4. These micrographs have been extracted from the confidential concept document of Anne Grobler (2004:5).

Table 2.4: Different shapes and types of Pheroid™ molecules

	1) Vesicle with a bilayer membrane. It has a diameter of 100 nm and contains the anti-tuberculosis drug, rifampicin. The average diameter for these vesicles is 100 nm.
	2) A bilayered vesicle that is highly elastic or fluid-like. It is packed with loose lipids and also contains the active substance, rifampicin.
	3) This micrograph illustrates how small pro-Emzaloid™ form. This is from a MeyerZall formulation and can be used for various oral administrations.
	4) A reservoir that contains many coal tar particles. This reservoir has a large loading capacity for Emzaloid™ on its surface area and can thus entrap insoluble compounds to a large extent. It has a general size of 1 µm.
	5) Here, the Emzaloid™ is in the process of entrapping water-soluble diclofenac that is fluorescently labelled. As can be seen, it is very small, with a diameter under 30 nm, and this is a sponge-like membrane packing.
	6) This illustrates a depot with a hydrophobic core that contains pro-Emzaloid™ formulation, surrounded by a hydrophilic zone and has an outer layer of vesicles. By selectively adding fluid, the vesicles will be released from a release zone. These depots may be used to get sustained release from a concentration gradient.

There is a general ingredient that all the topical Pheroid™-based formulations contain namely vitamin E (dl- α -tocopherol) or its derivatives. Vitamin E acts as an anti-oxidant as well as an emulsion stabiliser. This is a lipid-soluble vitamin, which can thus be widely distributed in the cell membranes of the body. Its major function is to prevent the process of oxidation in the membrane lipids. For the purpose of this study, where the active ingredient is entrapped in a Pheroid™ vesicle to be delivered across the SC and into the viable epidermis, there are a few vital processes, which take place. Firstly, the active ingredient must be captured in the Pheroid™. Secondly, the entrapped active compound must cross the SC and enter the viable epidermis. Then it must be taken up by the corneocytes and, lastly it must be released from this Pheroid™ vesicle, which acts as a carrier (Grobler *et al.*, 2008:293).

2.5 SUMMARY

Skin cancer is the most common type of cancer. Many different types of skin cancers, namely BCC, SCC and melanoma can be distinguished. The anticancer drug, 5-fluorouracil has been used for more than 40 years in human medicine as a remedy for many cancerous conditions, that include breast, colon as well as pancreatic cancer (alone or in conjunction with other anti-cancer drugs) and topical use in treating certain precancerous dermatoses. Except for the clinical value that topical preparations of 5-fluorouracil holds in the treatment of certain types of skin cancers, one should bear in mind that the transdermal delivery of this drug may be very useful. By incorporating the transdermal route, some of the well-known boundaries related to the oral and the parental routes of administration may be conquered. These boundaries include an inconsistent bioavailability when administered orally and interpatient variations. These factors make 5-fluorouracil a good candidate to be administered by the transdermal route, as this route has many advantages such as safety, patient compliance, efficiency and non-invasiveness. Except for the advantages, transdermal drug delivery has certain limitations. One of the main restrictions for drugs to be delivered by this route is the SC, which is often referred to as the rate limiting barrier of the skin, due to its lipophilic character.

5-Fluorouracil has been formulated in topical preparations, but this study focused on the formulation, stability testing and skin diffusion tests with different types of formulations, with and without the drug delivery system, Pheroid™ technology. These formulations included a cream, Pheroid™ cream, emulgel, Pheroid™ emulgel, lotion and Pheroid™ lotion containing 5-fluorouracil (0.5%). A method was developed and validated for the HPLC analysis of the formulations to determine the concentration of the different analytes in the formulations. Another method that was previously developed and validated for the purpose of another study was used to determine the concentration 5-fluorouracil that penetrated the skin, the concentration 5-fluorouracil that accumulated in the epidermis and dermis layers.

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CHAPTER 3

ARTICLE FOR PUBLICATION IN DRUG DELIVERY

Chapter 3 is written in an article format for the purpose of publication in Drug Delivery. The complete guide for authors of this journal is given in Appendix E and from there it is advised that the authors write in concise US English.

Application of Selected Formulations for Percutaneous Delivery of 5-Fluorouracil

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Abstract

Context: The anticancer chemotherapy drug, 5-fluorouracil, is widely used in the treatment of gastrointestinal, breast, and as topical treatment in certain types of skin cancers, including basal-cell carcinoma, squamous-cell carcinoma and actinic keratosis. The stratum corneum is the main barrier for drugs to penetrate the skin, due to its lipophilic character. **Objective:** This study focused mainly on the formulation and transdermal delivery of six different types of 5-fluorouracil topical formulations (0.5%), which were compared to 5-fluorouracil solutions (0.5%). Solutions (5.0%) were also prepared to compare the results to a 5-fluorouracil commercial product (5.0%). **Methods:** The vertical Franz cell skin diffusion method was used to test the formulations through the skin. Analyses of samples were performed by means of high performance liquid chromatography (HPLC). **Materials:** The formulations included an emulgel, cream and a lotion, with and without a drug delivery system, called Pheroid™ technology to determine if it will improve the delivery of 5-fluorouracil through the skin. **Results and discussion:** After 5% diffused and epidermis-dermis concentrations were compared the emulgel (0.5%) depicted the best results; the commercial product (although it contained 10-times more active) depicted the lowest % diffused and epidermis-dermis values. **Conclusion:** The results showed that an emulgel (0.5%) yielded the best transdermal delivery and accumulation in the epidermis-dermis and can thus be used to treat non-melanoma skin cancers and superficial actinic keratosis, which was contradictory to sources that stated only superficial actinic keratosis, could be cured with 0.5% preparations and non-melanoma skin cancers needed 5.0% 5-fluorouracil preparations.

1 Introduction

Cancer is the uncontrolled production of cells in the human body due to the loss of standard controls, which result in unregulated cell-growth, invasion of neighboring tissue, a shortage of differentiation between these cells and this process usually leads to metastasis (Beers *et al.*, 2006). There are many cancerous conditions of the skin, which include basal-cell carcinoma (BCC), squamous-cell carcinoma (SCC) and cutaneous malignant melanoma (CMM). Exposure to ultraviolet (UV) radiation plays a major role in the etiology of these three types of skin cancer (Franceschi *et al.*, 1996). These types of skin cancers can be ranked according to their sensitivity to solar UV-B irradiance, which is strong for SCC, intermediate for BCC, and weak for melanoma (Grant, 2008). Primary cutaneous SCC is a form of non-melanoma skin cancer (NMSC) that originates from epithelial keratinocytes or their appendages. It is locally aggressive and, without treatment, has the potential to metastasize to other parts of the body (Garcia-Zuazaga & Olbricht, 2008). BCC is the most common human cancer and although rarely metastatic, it is capable of extensive local growth that can result in significant cosmetic and functional impairment. A certain amount of UV light may significantly stimulate the process of BCC production, but additional exposure does not increase BCC incidence to the degree that it increases SCC incidence (Miller, 1995). CMM is the one type of skin cancer that has a significantly higher morbidity and mortality. Although it is the third most common skin cancer, accounting for only 3% of all skin cancers, CMM accounts for 65% of all skin cancer deaths. The importance of prevention and early detection of CMM cannot be overemphasized. Early detection of melanoma is crucial to long-term survival, because a direct and steep correlation exists between tumor thickness and mortality (Cummins *et al.*, 2006).

5-Fluorouracil is an antineoplastic (anticancer) agent of extensive use in clinical chemotherapy for the treatment of solid tumors (García *et al.*, 2000). It has promising antineoplastic activity against several pre-malignant and malignant conditions of the skin, which include Bowen's disease and superficial BCC. Its topical application has also been

proven to be a valuable and safe treatment for psoriasis and actinic keratosis (AK) (Singh *et al.*, 2005).

Numerous 5-fluorouracil schedules are currently in clinical use, but erratic oral bioavailability has historically mandated intravenous administration of this drug. When 5-fluorouracil is administered by the intravenous route, it produces severe systemic toxic effects that are of gastrointestinal, hematological, neural, cardiac and dermatological origin, which are caused by the cytotoxic effect of 5-fluorouracil after it reaches unwanted sites (Gupta *et al.*, 2005). This makes 5-fluorouracil a good candidate to be formulated in a dosage form that can be administered by the transdermal route.

From a chemical point of view, 5-fluorouracil is a diprotic acid with pK_a values of 8.0 and 13.0 and is a polar molecule. Due to the hydrophilic nature of 5-fluorouracil, the transdermal permeation through the lipophilic stratum corneum is very low and trivial (Singh *et al.*, 2005). The stratum corneum is a specialized structure that forms part of several anatomically distinct layers of the skin. It provides protection of the skin and is generally known as the primary barrier to the percutaneous absorption of compounds as well as water loss through the skin (Bouwstra *et al.*, 2003). Drug molecules can penetrate the skin by means of two major pathways, which include the transappendageal and transepidermal routes. The transappendageal route refers to permeation through the skin appendages (hair follicles and sweat glands), with their linked sebaceous glands and the transepidermal route is the pathway by which molecules filter through the unbroken, intact stratum corneum (El Maghraby *et al.*, 2008). A number of physical, chemical and biochemical methods have been implied to improve the transportation of drug molecules through the skin. These attempts mostly aim at reducing the barrier properties of the stratum corneum or either increasing the diffusion properties of the drug (Gupta *et al.*, 2005).

In this study a patented (Pitney International NV Patent Portfolio, 1994), drug delivery system, called Pheroid™ technology, was used in an attempt to improve the permeation of 5-fluorouracil through the stratum corneum into the underlying layers of the skin. Pheroid™

technology is a drug delivery system that consists of unique and stable lipid-based submicron- and micron-sized structures, formulated in an emulsion. The dispersed Pheroid™ structures largely comprise of natural essential fatty acids (Grobler *et al*, 2008). These acids have an affinity for the cell membranes of the human body and have the ability to enclose, transfer and set certain drug molecules free at their site of action; and this entire process take place at a much faster rate than drugs which are not entrapped in a Pheroid™ (Grobler, 2004). Prior studies with 5-fluorouracil conducted at the NWU include that of Van Dyk (2008), where different concentrations of 5-fluorouracil (0.5% and 1.0%) were incorporated in water, buffer or Pheroid™ solutions, the Pheroid™ solutions yielded the best results in comparison to the non-Pheroid™ equivalents. The 0.5% water-based Pheroid™ formulation yielded the best statistical results and did not show a significant statistical difference to that of the 1.0% water-based Pheroid™ formulation.

The main aim of this study was to prepare 5-fluorouracil semisolid formulations and compare the transdermal diffusion of each. These formulations consisted of a cream, emulgel and lotion containing 0.5% 5-fluorouracil, due to the above-mentioned data obtained from Van Dyk (2008). These formulations were prepared with and without the inclusion of Pheroid™ technology, in order to determine if it would improve the delivery of 5-fluorouracil. Two solutions containing 5-fluorouracil (0.5%) were also prepared, a non-Pheroid™ as well as a Pheroid™ solution.

The vertical Franz cell skin diffusion method was performed with the formulations and compared to a 5.0% commercial product (ointment) containing 5-fluorouracil. To make a direct comparison with the 5.0% commercial product containing 5-fluorouracil, which was an ointment, two solutions containing 5-fluorouracil (5.0%), non-Pheroid™ and Pheroid™ respectively, were prepared and tested by means of Franz cell skin diffusion.

2 Materials and Methods

2.1 Materials

5-Fluorouracil was obtained from DB Fine Chemicals. Cetyl alcohol, Tween-80, liquid paraffin, propyl and methyl paraben, orthophosphoric acid (H_3PO_4), potassium dihydrogen orthophosphate (KH_2PO_4), sodium hydroxide (NaOH) pearls that were used in the preparation of the phosphate buffer solution (PBS), as well as LiChrosolv[®] HPLC analytical grade methanol were obtained from Merck Laboratory Supplies. Span-60 was obtained from Brunel Manufacturers. Butylated hydroxytoluene (BHT) was obtained from SAFC and xanthan gum was obtained from Warren Chem Specialities. The dl- α -tocopherol (that formed part of the Pheroid[™] vesicles) was obtained from Chempure. Deionized HPLC-grade water which was used throughout the study was derived from a Milli-Q[®] water purification system (Millipore, Milford, USA). The skin was obtained from anonymous Caucasian (white) female patients after they had undergone abdominal plastic surgery. The process of obtaining and preparing the skin was approved by the Research Ethics Committee of the North-West University, reference number 04D08.

2.2 HPLC method

2.2.1 Determination of concentration 5-fluorouracil

The analytical instrument used, was an Agilent[®] 1100 series, which consisted of a degasser, a quaternary pump, an auto sampler and a diode array detector. Chemstation Rev. A.10.02 software was used for data acquisition and analysis (Agilent Technologies, Palo Alto, CA). A Phenomenex[®] Synergi Fusion 4 μm Reversed Phase 4.6 mm x 250.0 mm column (Phenomenex, Torrance, CA) was used. The mobile phase consisted of 3% acetonitrile solution which was prepared by mixing 30 mL of acetonitrile with 1 mL H_3PO_4 and adding sufficient HPLC-grade water to 1000 mL. The flow rate was set to 1 mL/min, the injection volume was 50 μL , and the UV-detector was set at a wavelength of 266 nm. The runtime of each sample was 8 min and the retention time of 5-fluorouracil was approximately 5 min.

2.2.2 Standard preparation

A standard solution of 5-fluorouracil was made to obtain a calibration curve for the samples derived from the skin diffusion studies. The standard solution was prepared by weighing

approximately 5 mg of 5-fluorouracil in a 50 mL volumetric flask and adding sufficient PBS (pH 7.4) to reach 50 mL, yielding a 100 µg/mL concentration. Approximately 2 mL of this solution was placed into an auto sampler vial and injected into the HPLC before the samples were injected.

2.3 Preparation of 5-fluorouracil solutions and semisolid formulations

2.3.1 Preparation of 5-fluorouracil solutions

Four solutions of 5-fluorouracil was prepared, they included a 0.5% non- Pheroid™ (water) and 0.5% Pheroid™ solution as well as a 5.0% water and 5.0% Pheroid™ solution.

The water solutions were prepared by weighing 0.5% and 5.0% 5-fluorouracil, respectively and adding sufficient HPLC-grade water to reach 100 mL solutions. The solutions were prepared the day before the experiment and stirred overnight.

The Pheroid™ solutions were prepared by heating the Pheroid™ ingredients for each solution as well as the water (sufficient to reach a 100 mL of each solution when 5-fluorouracil was added), separately to approximately 80 °C. The Pheroid™ ingredients were added to the water while homogenizing the mixture at 13 500 rpm until approximately 40 °C was reached, the mixtures were cooled to room temperature. 5-Fluorouracil was weighed (0.5 g and 5.0 g, respectively) and added to the Pheroid™ solutions. These solutions were also prepared the day before the experiments and left to stir overnight.

2.3.2 Ingredients and quantities used in preparing semisolid formulations

Ingredients used in the semisolid formulations and the function of each were as follows: 5-fluorouracil (active ingredient), cetyl alcohol (thickening agent), Tween-80 (surface active agent), Span-60 (emulsifier), liquid paraffin (oil-phase), propyl paraben and methyl paraben (preservatives), BHT (anti-oxidant), dl-α-tocopherol (stabilizing agent and anti-oxidant) and xanthan gum (thickening/gelling agent). The names of the additional two Pheroid™ ingredients may not be mentioned as they are proprietary, and for the sake of protecting the Intellectual Property (IP) (patent number SA93/3895, 1994) they will not be mentioned by name

2.3.2.1 Cream

The oil phase of the cream consisted of 10% cetyl alcohol, 1.5% Tween-80, 1.5% Span-60, 12.0% liquid paraffin, 0.04% propyl paraben and 0.2% BHT. These ingredients were added together and the mixture was heated to approximately 80 °C. Methyl paraben (0.2%) and 5-fluorouracil (0.5%) were added to 74.06 g distilled water, to produce the water-phase. This mixture was also heated to approximately 80 °C. When both phases reached approximately 80 °C, the oil phase was added to the water phase while homogenizing the mixture at 13 500 rpm, until approximately 40 °C was reached. The mixture was cooled down to approximately 25 °C while stirring at 200 rpm.

2.3.2.2 Pheroid™ cream

Exactly the same ingredients and quantities were used to prepare the vesicles for the Pheroid™ cream, except for the addition of dl- α -tocopherol and two other Pheroid™ ingredients, which were added to the oil-phase in the above-mentioned procedure (2.3.2.1). However, the addition of the Pheroid™ ingredients resulted in a smaller quantity of water used.

2.3.2.3 Emulgel

The oil phase of the emulgel consisted of 20.0% liquid paraffin, 4.5% Tween-80, 0.5% Span-60, 0.08% propyl paraben and 0.2% BHT. Methyl paraben (0.2%) and 5-fluorouracil (0.5%) were added to 72.32 g distilled water, to produce the water phase. This mixture was heated to approximately 40 °C, after which 1.5% xanthan gum was added little by little while homogenizing the mixture at 777 rpm. This mixture was also heated to approximately 80 °C. When both phases reached approximately 80 °C, the oil phase was added to the water phase while homogenizing the mixture at 13 500 rpm, until approximately 40 °C was reached. The mixture was cooled down to approximately 25 °C while stirring at 200 rpm.

2.3.2.4 Pheroid™ emulgel

Exactly the same ingredients and quantities were used to prepare the vesicles for the Pheroid™ emulgel, except for the addition of dl- α -tocopherol and two other Pheroid™

ingredients, which were added to the oil phase in the above-mentioned procedure (2.3.2.3). However, the addition of the Pheroid™ ingredients resulted in a smaller quantity of water used.

2.3.2.5 Lotion

The oil phase of the lotion consisted of 5.0% cetyl alcohol, 7.0% Tween-80, 2.0% Span-60, 20.0% liquid paraffin, 0.1% propyl paraben and 0.2% BHT. These ingredients were added together and the mixture was heated to approximately 80 °C. Methyl paraben (0.5%) and 5-fluorouracil (0.5%) were added to 64.7 g distilled water, to produce the water-phase. This mixture was also heated to approximately 80 °C. When both phases reached approximately 80 °C, the oil phase was added to the water phase while homogenizing the mixture at 13 500 rpm, until approximately 40 °C was reached. The mixture was cooled down to approximately 25 °C while stirring at 200 rpm.

2.3.2.6 Pheroid™ Lotion

Exactly the same ingredients and quantities were used to prepare the vesicles for the Pheroid™ lotion, except for the addition of dl- α -tocopherol and two other Pheroid™ ingredients, which were added to the oil phase in the above-mentioned procedure (2.3.2.5). However, the addition of the Pheroid™ ingredients resulted in a smaller quantity of water used.

2.4 Physicochemical properties of 5-fluorouracil

2.4.1 Determination of aqueous solubility of 5-fluorouracil

The aqueous solubility of 5-fluorouracil was determined by preparing three over saturated solutions of 5-fluorouracil in PBS (pH 7.4) in glass politop containers. These solutions were placed in a 32 °C water bath with magnetic stirrers and left to stir for 24 h. The solutions were filtered, diluted 100 times and analyzed by means of HPLC. Each sample was injected in triplicate.

2.4.2 Determination of octanol/buffer partition coefficient (log D)

To determine the log D value of 5-fluorouracil, equal amounts of *n*-octanol and PBS (pH 7.4) were added and the mixture was shaken. The phases were separated from each other and the internal layer was discarded. A 1000 µg/mL solution was made by weighing 2.5 mg of 5-fluorouracil and dissolving it in 2.5 mL of the saturated *n*-octanol. This was done in triplicate. Equal parts (2.5 mL) of the 5-fluorouracil/*n*-octanol solution and PBS (pH 7.4) were added to the 3 test tubes and left in a rotator for 24 h. The solutions were centrifuged for 15 min at 5 000 rpm with an Eppendorf Centrifuge 5804 R, before the PBS phases were extracted and analyzed by means of HPLC (in triplicate). The logarithmic ratio of the concentration in the *n*-octanol-phase relative to the concentration in the PBS phase was used to calculate the log D value.

2.5 Franz cell diffusion experiments

2.5.1 Skin preparation

The skin was kept frozen (at approximately -20 °C) until preparation. On the day of preparation, the skin was taken from the freezer and left to defrost a little. The easiest way to get rid of the largest part of the fat layer was to remove it with a scalpel while it was still frozen. The rest of the fatty layer, closest to the epidermis, was removed by scraping it with a blunt scalpel. The skin was wiped with a paper towel and placed on a plastic cutting board. Circles ($\pm$ 15 mm in diameter) were punched into the skin and placed on Whatman® filter paper. For one diffusion experiment, a total of 12 skin circles were required. Thus, it was ensured that 12 circles were derived from the same patient's skin. The filter paper was covered with aluminum foil and placed into the freezer until the day of the experiment.

2.5.2 Preparation of the receptor phase solution

The solution consisted of PBS at pH 7.4. It was prepared by adding 13.620 g of KH₂PO₄ to 500 mL HPLC-grade water and 3.1472 g of NaOH to 786.8 mL of HPLC-grade water. These two mixtures were stirred until the powder had fully dissolved, after which they were added to each other. The pH of the PBS was set to 7.4 by either using 10% NaOH or 10% H₃PO₄.

2.5.3 Procedure of Franz cell skin diffusion experiments

The apparatus used to conduct the skin diffusion experiments consisted of vertical Franz cells with a receptor phase capacity of ± 2 mL and a diffusion area of 1.075 cm^2 . The donor compartments were large enough to contain 1 mL of the formulation to be tested. Small magnetic stirrers were placed in the receptor compartment to stir the PBS (pH 7.4). Skin circles were placed on the surface of each receptor compartment, with the stratum corneum facing upwards and covered with the donor compartment. Sufficient Dow Corning[®] vacuum grease was rubbed on the sides of the Franz cells to prevent them from leaking. 1 mL of the formulation, which was pre-heated to $32\text{ }^{\circ}\text{C}$, was placed in each donor compartment of 10 Franz cells, another two were filled with PBS and a placebo formulation, respectively, these two Franz cells acted as control samples for the HPLC analysis. The 12 Franz cells were carefully placed in horse shoe clamps to ensure the skin circles are firmly mounted between the donor and receptor compartments. The donor compartments were covered with a piece of Parafilm[®] and a plastic cap to prevent the formulations from leaking. 2 mL of PBS (pH 7.4) were added to each receptor compartment, and the Franz cells were placed in a $37\text{ }^{\circ}\text{C}$ water bath, this was to attain a skin temperature of $32\text{ }^{\circ}\text{C}$. The 12 h period started at this point in time and extraction of the receptor phases were carried out every 2 h. The receptor compartments were immediately filled with fresh PBS (pH 7.4) after each extraction. The extracted receptor phases were placed in auto sampler vials and directly analyzed by HPLC to determine the concentration 5-fluorouracil. After the completion of the 12 h period, tape stripping was performed.

2.5.4 Tape stripping procedure

Tape stripping is a simple and efficient method to assess the quality and efficacy of cosmetic and dermatologic formulations. It is defined as the subsequent removal of the cell layers of the stratum corneum, after topical application and penetration of formulations by using adhesive tapes. The tape strips contain an amount of the formulation that penetrated the stratum corneum and can be determined by analytical chemical methods (Lademann *et al.*, 2009). At the end of the 12 h period of a skin diffusion study, the skin circles were carefully

removed by using tweezers. The circles were dabbed dry with clean paper towel to ensure that the excess formulations or solutions were removed from the skin surface. 3M Scotch[®] Magic[™] Tape was used to do this procedure. The tape was firmly placed in the centre of the skin circle and pulled off again. A total of sixteen tape strips were used on each circle, of which the first strip was discarded. The other 15 tape strips from each skin circle were placed in 5 mL PBS (pH 7.4) and placed in the refrigerator at 4 °C overnight. The part of the skin (epidermis-dermis) that was left after the tape stripping procedure was cut into pieces and also placed in 5 mL PBS (pH 7.4) in the refrigerator at 4 °C overnight.

The samples had to be prepared for injection on the HPLC the following morning. The 5 mL PBS solutions of the stratum corneum-epidermis and epidermis-dermis were extracted and filtered with Acrodisc[®] Premium 25 mm Syringe Filters, with GxP/0.45 µm GHP membranes (obtained from PALL Life Sciences). The solutions were transferred into Eppendorf vials and centrifuged for 7 min at 10 000 rpm and 4 °C in an Eppendorf Centrifuge 5804 R. Auto sampler vials were filled with these solutions and analyzed by means of HPLC.

2.5.5 Membrane diffusion experiments

Membrane diffusion experiments were carried out to determine if the six semisolid formulations released 5-fluorouracil. The exact same procedure was used as described in section 2.5.3, except that Polytetrafluoroethylene (PTFE) membrane filters, with a thickness of 0.45 µm, from PALL Corporation, (Ann Arbor Michigan) were used instead of skin. The receptor phases were extracted at 0.5, 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0 h and analyzed by means of HPLC.

2.5.6 Data analysis

The data obtained from the skin diffusion experiments, as well as the membrane diffusion experiments were processed and the average % diffused was determined. The % diffused was calculated by determining the amount of active ingredient that diffused through the skin as a percentage of the amount of active ingredient originally applied to the skin.

2.5.7 Statistical data analysis

The data analysis contains both descriptive and inferential statistical methods. For the descriptive analysis, we used medians as summary statistics and box-plots (with the data points and average superimposed) as graphical representation. The inferential analysis involved formal hypothesis testing to compare the methods (formulations and solutions) and Pheroid™ application, with respect to dermis concentrations and epidermis concentrations. Due to the heterogeneity of variance (for some of the variables), as well as small sample sizes, we used non-parametric methods throughout. In specific, we used the two-way test proposed by Brunner *et al.* (1997) to test the existence of an interaction effect between the methods (formulations and/or solutions) and the application of Pheroid™. To test the main effect of methods (formulations and/or solutions), we used the one-way test of Brunner *et al.* (1997), followed by post-hoc analysis, using a Bonferroni correction, as described in Dmitrienko *et al.* (2007). To test the main effect of Pheroid™ application, the Mann-Whitney test was used. The Brunner *et al.* (1997) test is henceforth referred to as the BDM-test. All inferential statistics were performed at the 5% level of significance. Differences were significant when the p-value was less than 0.05.

3 Results and discussion

3.1 Physicochemical properties of 5-fluorouracil

3.1.1 Aqueous solubility

The value obtained for the aqueous solubility of 5-fluorouracil at 32 °C in PBS (pH 7.4) was 0.56 mg/mL, which indicated poor transdermal permeation, as the ideal aqueous solubility for a drug to permeate the skin should be 1 mg/mL or more (Naik *et al.*, 2000). When compared to aqueous solubility value obtained during a previous study (14.3 mg/ml) conducted by Yamane *et al.* (1995:250), this value was very low. The possible reasons might be due to the fact that a different temperature, buffer or pH was used when compared to this study.

3.1.2 Octanol/buffer partition coefficient (log D)

When the ideal properties of a drug are taken into account for transdermal delivery, drugs with a reasonable lipophilic/hydrophilic character and a log P (octanol-water partition coefficient) of ≤ 2 are regarded as prospective candidates (Guy & Hadgraft, 1989). The log D value of 5-fluorouracil was determined as -0.98, and according to Hadgraft (2004), drugs with a log P value in the range of 1 – 3, are generally considered to have optimum partition behavior. Thus, the obtained value for 5-fluorouracil did not indicate optimum partitioning into the skin. Although, in a previous study conducted by Quigley & Lloyd (2002), the log P of 5-fluorouracil was determined as -0.83, which barely differs from the value obtained during this study.

3.2 Franz cell diffusion experiments

3.2.1 Membrane diffusion experiments

The highest % 5-fluorouracil diffused after 6 h was through Pheroid™ cream (0.025%) this was followed by Pheroid™ lotion (0.024%), Pheroid™ emulgel (0.023%), emulgel (0.020%), cream (0.013%) and lotion (0.012%). Hence, all the formulations released 5-fluorouracil and diffused through the membranes. In the above-mentioned, all Pheroid™ formulations in comparison to the non-Pheroid™ formulations released a higher % 5-fluorouracil.

3.2.2 Skin diffusion experiments

3.2.2.1 Comparison of % diffused values

The % diffused values of the formulations (0.5%) and solutions (0.5%) were compared in Figure 1. After comparing the average % diffused values of the 0.5% formulations and 0.5% solutions, the following was observed: emulgel (0.099%) achieved the highest average % diffused, followed by 0.5% water solution (0.093%), Pheroid™ lotion (0.090%), lotion (0.081%), Pheroid™ emulgel (0.079%), cream (0.070%), 0.5% Pheroid™ solution (0.066%), and lastly, Pheroid™ cream (0.063%).

When comparing the 0.5% formulations (cream, emulgel and lotion) and not taking into account the influence of Pheroid™ or solutions (0.5%), it was depicted that emulgel obtained the highest average % diffused value. The reason for this could be due to the high fluid

content of the emulgel that hydrated the skin and caused the stratum corneum to swell and open its structures, which in turn lead to an increase in penetration (Benson, 2005). Cream showed the poorest average % diffused value, which could be due to the high concentration of thickening agent within the cream that caused a stiff appearance and thus, less hydration on the skin, which resulted in a decreased penetration.

After the addition of Pheroid™ was taken into account, all formulations (0.5%) and solutions (0.5%) showed lower average % diffused values with Pheroid™, than without Pheroid™, except for Pheroid™ lotion, which depicted a higher average % diffused value than lotion. The reason for this phenomenon could be that the Pheroid™ lotion consisted of the largest total content of surface active ingredients, when compared to all the other formulations, which may have caused enhanced penetration of 5-fluorouracil by a mechanism involving interaction of the surfactant with the structured lipids of the stratum corneum (Wiechers & Watkinson, 2008).

When solutions (0.5%) were weighed against formulations (0.5%); it was observed that the water solution had a higher average % diffused value than Pheroid™ solution and all the formulations (0.5%), except emulgel. The aforementioned could be due to fact that Pheroid™ solution and the formulations consisted of oily ingredients; whereas the water solution consisted only of water and active ingredient and the emulgel being the most hydrophilic of all formulations had a sufficient amount of oil to increase diffusion through the lipophilic stratum corneum.

When Pheroid™ solution was compared to the other formulations and water solution; it achieved a lower average % diffused value than all the other formulations and solution, except for the Pheroid™ cream that yielded the lowest % diffused of all the 0.5% preparations, which was the most lipophilic of all formulations and solutions.

The % diffused values of the commercial product (5.0%) and the solutions (5.0%) are shown in Figure 2. When comparing the average % diffused values the following was portrayed: 5.0% water solution (0.012%) achieved the highest average % diffused, followed by 5.0%

Pheroid™ solution (0.003%) and lastly, but close to the 5.0% Pheroid™ solution, the 5.0% commercial product (0.002%). Diffusion increased as hydrophilicity increased, which could be caused by the high fluid content that hydrated the skin and caused the stratum corneum to swell and open its structures, which in turn lead to an increase in penetration (Benson, 2005).

The % diffused values of all the formulations (0.5%) and solutions (0.5% and 5.0%) as well as the commercial product (5.0%) are shown in Figure 3. The emulgel attained the highest % diffused value of all the solutions and formulations whereas the commercial product had the lowest % diffused value of all the solutions and formulations.

The water solutions (0.5% and 5.0%) presented with an enhanced penetration when compared to the average % diffused values of the oily solutions (Pheroid™ solutions (0.5% and 5.0%)). As a result of the aforementioned phenomenon, it could be assumed that the hydrophilic nature of 5-fluorouracil caused it to have higher availability within a hydrophilic environment (water solutions (0.5% and 5.0%)), which in turn lead to elevated diffusion through the skin. Whereas, the oily preparations (Pheroid™ solution (0.5% and 5.0%)), did not result in a favorable environment for 5-fluorouracil, and thus caused less diffusion through the skin.

By taking this into account, the commercial product depicted the poorest % diffused, due to it being an ointment, which consisted of higher concentration oily ingredients than the Pheroid™ solutions (0.5% and 5.0%). When the commercial product was compared to all the solutions (0.5% and 5.0%), it achieved the lowest average % diffused values. The values of the commercial product were comparable to and just beneath those of 5.0% Pheroid™ solution, which could be due to the fact that the 5.0% Pheroid™ solution was also a fatty formulation and had the same concentration 5-fluorouracil.

Once the water solutions (0.5% and 5.0%) were compared with each other it was evident that 0.5% yielded a higher average % diffused than that of 5.0%. Although, the applied concentration (5-fluorouracil) of 5.0% was ten times higher than that of 0.5%, the 5.0% water

solution presented with a significantly decreased % diffused than 0.5%. Hence, it was clear that a 0.5% preparation will be more successful to use than a 5.0% preparation, as it will lead to less active ingredient used, which in turn will cause less side effects on the skin surface (as the active ingredient penetrates through the skin and into systemic circulation).

3.2.3 Tape stripping

3.2.3.1 Concentration in the epidermis

The epidermis concentration values of the formulations (0.5%) and solutions (0.5%) were compared in Figure 4a. When comparing the average epidermis concentrations, the following was observed: Pheroid™ emulgel (0.613 µg/ml) attained the highest average concentration 5-fluorouracil within the epidermis, followed by 0.5% water solution (0.229 µg/ml), emulgel (0.221 µg/ml), Pheroid™ lotion (0.182 µg/ml), cream (0.178 µg/ml), lotion (0.135 µg/ml), Pheroid™ cream (0.132 µg/ml) and lastly, 0.5% Pheroid™ solution (0.057 µg/ml).

The reason that Pheroid™ emulgel attained the highest epidermis concentration, could be due to a high oil content (Pheroid™) and the addition of a gelling agent (emulgel) that caused an adhesive residue on the skin surface, which lead to high concentration 5-fluorouracil in the lipophilic epidermis. Pheroid™ solution (0.5%) depicted the poorest epidermis concentration, due to a high fluid content when compared to the formulations (0.5%) that caused it to not be favorable for the lipophilic epidermis. Although it did not contain the highest fluid content when compared to water solution (0.5%), the addition of a small amount of oily ingredients (Pheroid™) lead to the residue of a slight amount within this layer, due to slow transfer from the lipophilic layer to the underlying aqueous layer (Potts & Guy, 1992).

When median epidermis values were compared with each other, Pheroid™ emulgel (0.279 µg/ml) depicted the highest median concentration, followed by 0.5% water solution (0.228 µg/ml), Pheroid™ lotion (0.183 µg/ml), emulgel (0.162 µg/ml), cream (0.145 µg/ml), lotion

(0.134 µg/ml), Pheroid™ cream (0.123 µg/ml) and lastly, the 0.5% Pheroid™ solution (0.021 µg/ml).

Once the median and average epidermis concentration values of formulations (0.5%) as well as solutions (0.5%) were compared, it was detected that cream, Pheroid™ cream, lotion, Pheroid™ lotion and water solution had approximately the same epidermis values.

Therefore both median and average can be used to determine concentration. However, emulgel, Pheroid™ emulgel and 0.5% Pheroid™ solution portrayed significant differences between the median and average epidermis concentration values. The median epidermis values gave a more accurate representation of the true concentration, since it took all the data into consideration and was not affected by a distortion in the spread of data, as in the case with average concentration values (Gerber *et al.*, 2008).

The epidermis concentration values of commercial product (5.0%) and the solutions (0.5% and 5.0%) were compared in Figure 4b. When comparing the average epidermis concentrations, the following was observed: 5.0% water solution (9.444 µg/ml) attained the highest average concentration 5-fluorouracil within the epidermis, followed by 0.5% water solution (0.229 µg/ml), commercial product (0.120 µg/ml), 5.0% Pheroid™ solution (0.116 µg/ml) and lastly, 0.5% Pheroid™ solution (0.057 µg/ml).

As the epidermis was hydrated for a period of 12 h, it caused the stratum corneum-epidermis to swell. It can swell to a thickness of 40 µm (El Maghraby *et al.*, 2008) and thus caused the aqueous 5.0% water solution to attain the highest concentration in the swollen epidermis.

Secondly, this could have been due to the over-saturated water solution (5.0%), which caused solid particles of 5-fluorouracil to saturate on this layer and thus lead to a very high concentration in the epidermis. The Pheroid™ solution (0.5%) consisted of a lower concentration 5-fluorouracil, which caused less saturation and the poorest epidermis concentration, which could also be related to the above-mentioned swollen stratum corneum-epidermis, but to a much lesser extent, due to the oily ingredients of Pheroid™ solution (0.5%).

When median epidermis values were compared with each other, 5.0% water solution (9.198 µg/ml) depicted the highest median concentration, followed by 0.5% water solution (0.228 µg/ml), 5.0% Pheroid™ solution (0.143 µg/ml), commercial product (0.122 µg/ml) and lastly, the 0.5% Pheroid™ solution (0.021 µg/ml).

Once the median and average epidermis concentration values of commercial product (5.0%) and the solutions (0.5% and 5.0%) were compared with one another, it was detected that only the Pheroid™ solutions (0.5% and 5.0%) showed significant differences between the median and average epidermis concentration values. The median epidermis values gave a more accurate representation of the true concentration, since it took all the data into consideration and was not affected by a distortion in the spread of data, as in the case with average concentration values (Gerber *et al.*, 2008).

3.2.3.2 Concentration in the dermis

The dermis concentration values of formulations (0.5%) and solutions (0.5%) were compared in Figure 5a. After comparing the average dermis concentrations, the following was detected: emulgel (0.650 µg/ml) depicted the highest average concentration 5-fluorouracil within the dermis, followed by (0.5% water solution) (0.552 µg/ml), Pheroid™ emulgel (0.472 µg/ml), lotion (0.424 µg/ml), Pheroid™ lotion (0.310 µg/ml), cream (0.139 µg/ml), Pheroid™ cream (0.136 µg/ml) and lastly, 0.5% Pheroid™ solution (0.005 µg/ml). After comparing all the formulations (0.5%) it was evident that emulgel was the most hydrophilic formulation with the least oil particles. Due to the hydrophilic nature of emulgel and 0.5% water solution, it depicted the highest concentration in the dermis, which was a hydrophilic layer. According to Williams (2003), the dermis mainly consists of water, which will lead to higher diffusion of hydrophilic compounds.

Once median dermis values were compared with each other, emulgel (0.554 µg/ml) attained the highest median concentration, followed by Pheroid™ emulgel (0.461 µg/ml), 0.5% water solution (0.438 µg/ml), lotion (0.430 µg/ml), Pheroid™ lotion (0.306 µg/ml), Pheroid™ cream (0.138 µg/ml), cream (0.131 µg/ml) and lastly, the 0.5% Pheroid™ solution (0.000 µg/ml).

When the median and average dermis concentration values of formulations (0.5%) as well as solutions (0.5%) were compared, it was detected that only emulgel and 0.5% water solution portrayed significant differences between the median and average dermis concentration values. The median dermis values gave a more accurate representation of the true concentration, since it took all the data into consideration and was not affected by a distortion in the spread of data, as in the case with average concentration values (Gerber *et al.*, 2008).

The dermis concentration values of commercial product (5.0%) and the solutions (0.5% and 5.0%) were compared in Figure 5b. After comparing the average dermis concentrations, the following was showed: 5.0% water solution (2.696 µg/ml) attained the highest average concentration 5-fluorouracil within the dermis, followed by 5.0% Pheroid™ solution (0.715 µg/ml), 0.5% water solution (0.552 µg/ml), commercial product (0.052 µg/ml) and lastly, 0.5% Pheroid™ solution (0.005 µg/ml).

The water solution (5.0%) attained the highest concentration 5-fluorouracil within the dermis, when compared to the rest of the solutions and the commercial product, could also have been due to Fick's law of diffusion. Williams (2003) stated that the transferring rate of the diffusing molecules through a unit area is comparative to the concentration gradient (a higher concentration will lead to higher diffusion), which caused 5-fluorouracil to penetrate the skin more readily than from the other solutions and the commercial product. Due to the hydrophilic nature of water solution (5.0%) when compared to Pheroid™ solution (5.0%), it depicted the highest concentration in the dermis, which is a hydrophilic layer. According to Williams (2003), the dermis mainly consists of water, which will lead to higher diffusion of hydrophilic compounds. The Pheroid™ solution (0.5%) consisted of a lower concentration 5-fluorouracil, which caused the least saturation and the poorest dermis concentration, which could also be related to the oily ingredients (Pheroid™).

After median dermis values were compared with each other, 5.0% water solution (2.212 µg/ml) attained the highest median concentration, followed by 5.0% Pheroid™ solution

(1.539 µg/ml), 0.5% water solution (0.438 µg/ml), commercial product (0.049 µg/ml) and lastly, the 0.5% Pheroid™ solution (0.000 µg/ml).

Once the median and average dermis concentration values of the commercial product (5.0%) and the solutions (0.5% and 5.0%) were compared, it was detected that only the solutions (5.0%) showed significant differences between the median and average dermis concentration values. The median dermis values gave a more accurate representation of the true concentration, since it took all the data into consideration and was not affected by a distortion in the spread of data, as in the case with average concentration values (Gerber *et al.*, 2008).

3.2.4 Statistical data analysis

3.2.4.1 Effect of Pheroid™ application to epidermis and dermis when examining formulations (0.5%) and solutions (0.5%)

3.2.4.1.1 Epidermis

The reader is referred to Figure 4a where **(A)** represents non-Pheroid™ and **(B)** represents Pheroid™.

The 2-way BDM-test found a significant interaction between **(A)** and **(B)** on the epidermis values. Hence, the effect of Pheroid™ application should be compared on different levels of the preparations and vice versa.

With the effect of application of Pheroid™ at different levels of the preparations (thus comparing each preparation in **(A)** with its respective preparation in **(B)**), a statistical significant difference was observed for lotion and Pheroid™ lotion as well as water solution (0.5%) and Pheroid™ solution (0.5%) although not visually observed in the box-plot.

With the effect of preparations on different levels of non-Pheroid™ **(A)** application, the one-way BDM-test showed significant differences. Subsequently, the post-hoc analysis only revealed significant differences between lotion and water solution (0.5%).

With the effect of preparations on different levels of Pheroid™ **(B)** application, the one-way BDM-test also showed differences. Post-hoc analysis revealed significant differences

between Pheroid™ emulgel and Pheroid™ cream; Pheroid™ emulgel and Pheroid™ solution (0.5%), as well as Pheroid™ lotion and Pheroid™ solution (0.5%).

3.2.4.1.2 Dermis

The reader is referred to Figure 5a where **(A)** represents non-Pheroid™ and **(B)** represents Pheroid™.

The 2-way BDM-test found a significant interaction between **(A)** and **(B)** on the dermis values. Hence, the effect of the Pheroid™ application should be compared on different levels of the preparations and vice versa.

With the effect of application of Pheroid™ at different levels of the preparations (thus comparing each preparation in **(A)** with its respective preparation in **(B)**), a statistical significant relationship was observed for cream and Pheroid™ cream, due to the fact that the p-value was above 0.05.

With the effect of preparations on different levels of non-Pheroid™ **(A)** application, the one-way BDM-test showed significant differences. Subsequently, the post-hoc analysis revealed significant differences between emulgel and cream, lotion and cream, as well as cream and water solution (0.5%).

With the effect of preparations on different levels of Pheroid™ **(B)** application, the one-way BDM-test also showed differences. Post-hoc analysis revealed significant differences between Pheroid™ emulgel and Pheroid™ cream, Pheroid™ emulgel and Pheroid™ solution (0.5%), as well as Pheroid™ lotion and Pheroid™ solution (0.5%).

3.2.4.2 Effect of Pheroid™ application to epidermis and dermis when examining solutions (5.0%) and commercial product (5.0%)

3.2.4.2.1 Epidermis

The reader is referred Figure 4b where **(C)** represents non-Pheroid™ and **(D)** represents Pheroid™.

The one-way BDM-test found a significant difference between the 5.0% solutions (non-Pheroid™ and Pheroid™) and the commercial product on the epidermis values. Post-hoc

tests revealed significant differences between the commercial product and the non-Pheroid™ 5.0% solution as well as between the non-Pheroid™ and Pheroid™ solutions.

3.2.4.2.2 Dermis

The reader is referred Figure 5b where **(C)** represents non-Pheroid™ and **(D)** represents Pheroid™.

The one-way BDM-test found a significant difference between the 5.0% solutions (non-Pheroid™ and Pheroid™) and the commercial product on the dermis values. Post-hoc tests revealed significant differences between the commercial product and the non-Pheroid™ 5.0% solution as well as between the non-Pheroid™ and Pheroid™ solutions.

4 Conclusions

The aqueous solubility of 5-fluorouracil was calculated to be 0.56 mg/ml, which did not indicate the ideal value (1 mg/ml or more, according to Naik *et al.*, (2000)) to permeate the skin. The log D value obtained for 5-fluorouracil, was -0.98, which indicated that it will have difficulty penetrating through the highly lipophilic stratum corneum, as the ideal log P should be between 1 and 3 (Hadgraft, 2004). After these physiochemical properties were determined, it showed that 5-fluorouracil did not consist of the ideal properties to be delivered by the transdermal route. Nevertheless, 5-fluorouracil proved the contrary, as it penetrated the stratum corneum and permeated through full-thickness skin to achieve % diffused, epidermis as well as dermis concentration values.

In previous studies conducted in the laboratories of NWU, 5-fluorouracil was incorporated in 0.5% and 1.0% of water and PBS-based Pheroid™ formulations and tested by means of Franz diffusion cells through epidermis. These studies concluded that the inclusion of Pheroid™ enhanced the penetration of 5-fluorouracil through the epidermis. The 0.5% and 1.0% water-based Pheroid™ formulations did not show significant statistical differences when yields, fluxes and cumulative concentrations were compared by means of ANNOVA analysis. It was concluded that a 0.5% formulation would be the most affordable to

manufacture and more effective in terms of patient compliance due to less side effects on the skin surface.

From the membrane studies data in this study, it was concluded that the highest % 5-fluorouracil was released by the Pheroid™ cream and the lowest by the cream.

Franz cell skin diffusion studies showed that the emulgel achieved the highest average % diffused value, when considering all formulations and solutions. The reason for this could have been due to the high fluid content of the emulgel that caused hydration of the skin, which in turn lead to swelling of the stratum corneum and opening of its structures. These actions lead to an increase in 5-fluorouracil penetration (Benson, 2005).

When all formulations and solutions were compared in terms of epidermis concentration, the 5.0% water solution depicted the highest average concentration 5-fluorouracil in the epidermis and dermis. This was an oversaturated solution of 5-fluorouracil, which caused solid particles to reside in the epidermis, consequently causing a high drive force for 5-fluorouracil into the dermis, when considering Fick's law of diffusion Williams (2003).

Even though the 5.0% water solution yielded the highest concentration in the epidermis and dermis, the 0.5% emulgel formulation would be more suitable to use. The emulgel contained one tenth of the active ingredient concentration, therefore, causing less side effects on the skin surface (such as skin irritation) and will be more profitable to manufacture. A 5.0% solution will leave a powder residue on the skin surface due to the high saturation, thus, not stick to the skin. The inclusion of Pheroid™ did not show an improved transdermal delivery of 5-fluorouracil within the 0.5% formulations when a head tot head comparison was made to the equally strong non-Pheroid™ formulations, except for the Pheroid™ cream, Pheroid™ emulgel and Pheroid™ lotion, which showed greater transdermal delivery than the 5.0% commercially available product.

It has only been approved by the FDA (Food and Drug Administration) that 0.5% formulations may cure AK, located on the face and anterior scalp; there has not yet been proof that it is used in the treatment of NMSC. Whereas, 5.0% formulations is an

appropriate treatment for NMSC, including superficial BCC, and in some cases, SCC (McGillis & Fein, 2004:176).

McGillis and Fein (2004:176) stated that both 0.5% and 5.0% 5-fluorouracil preparations have been used before in the treatment of AK. In a side-to-side study, a 0.5% cream compared favorably to a 5.0% cream in the reduction and clearance of AK. All patients experienced cutaneous irritation; however, the once daily application of the 0.5% formulation was preferred.

Throughout this study, the addition of Pheroid™ mostly resulted in lower % diffused values, as well as lower epidermis and dermis concentrations and should thus not be incorporated into a formulation that contains 5-fluorouracil, as it will not result in enhanced effects.

Although, the Pheroid™ cream, Pheroid™ emulgel and Pheroid™ lotion yielded greater transdermal delivery of 5-fluorouracil than the commercial product. Further study is encouraged to investigate these formulations in comparison with the commercial product. An emulgel is a lot more cosmetically acceptable, than any solution, as it will retain on the skin surface and lead to higher patient compliance. Possible further study is encouraged with the emulgel as it resulted in the best transdermal delivery of 5-fluorouracil throughout this study.

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Declaration of interest

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Figure legends

Figure 1: Comparison between average % diffused values of 0.5% formulations and 0.5% solutions

Figure 2: Comparison between average % diffused values of 5.0% solutions and 5.0% commercial product

Figure 3: Comparison between average % diffused values of 0.5% formulations, 0.5% solutions, 5.0% commercial product and 5.0% solutions

Figure 4: Box-plot representation of the epidermis concentration values for: **a) (A):** non-Pheroid™ (0.5%) formulations and solutions; **(B):** Pheroid™ (0.5%) formulations and solutions and **b) (C):** non-Pheroid™ (5.0%) solutions; **(D):** Pheroid™ (5.0%) solutions and *TM is the commercial product. The average and median epidermis concentrations are indicated by a dotted and solid line, respectively. The dots represent the epidermis data (µg/ml).

Figure 5: Box-plot representation of the dermis concentration values for: **a) (A):** non-Pheroid™ (0.5%) formulations and solutions; **(B):** Pheroid™ (0.5%) formulations and solutions and **b) (C):** non-Pheroid™ (5.0%) solutions; **(D):** Pheroid™ (5.0%) solutions and *TM is the commercial product. The average and median dermis concentrations are indicated by a dotted and solid line, respectively. The dots represent the dermis data (µg/ml).

Figures:

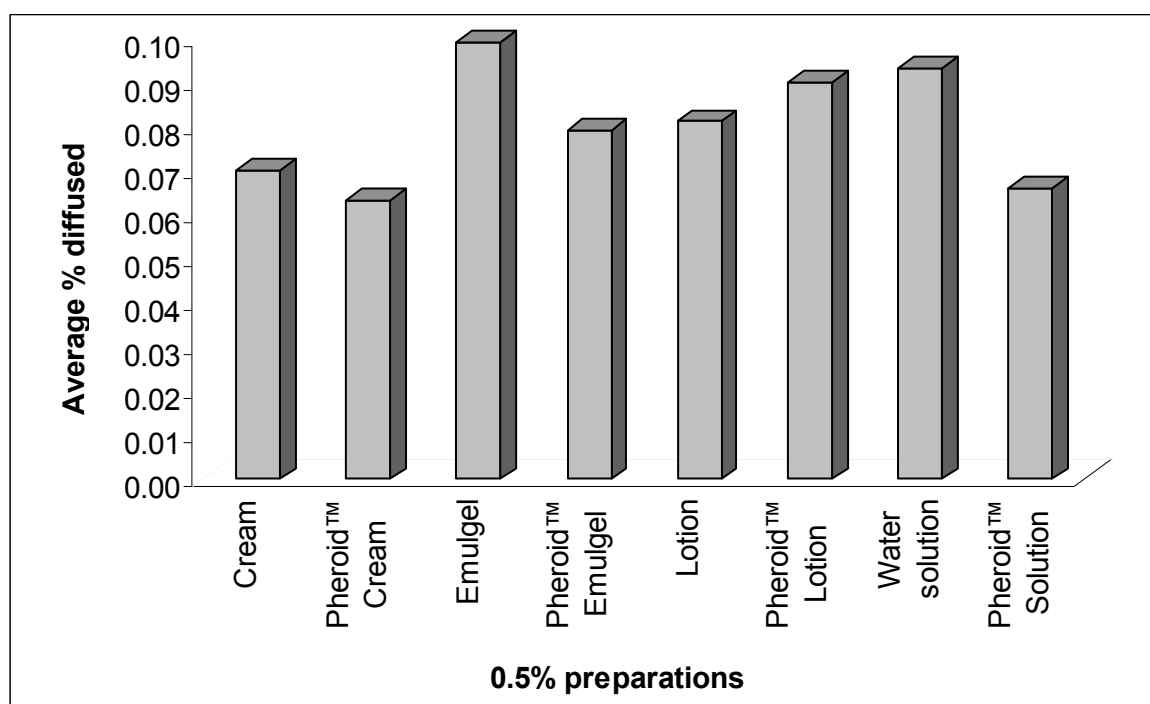


Figure 1: Comparison between average % diffused values of 0.5% formulations and 0.5% solutions

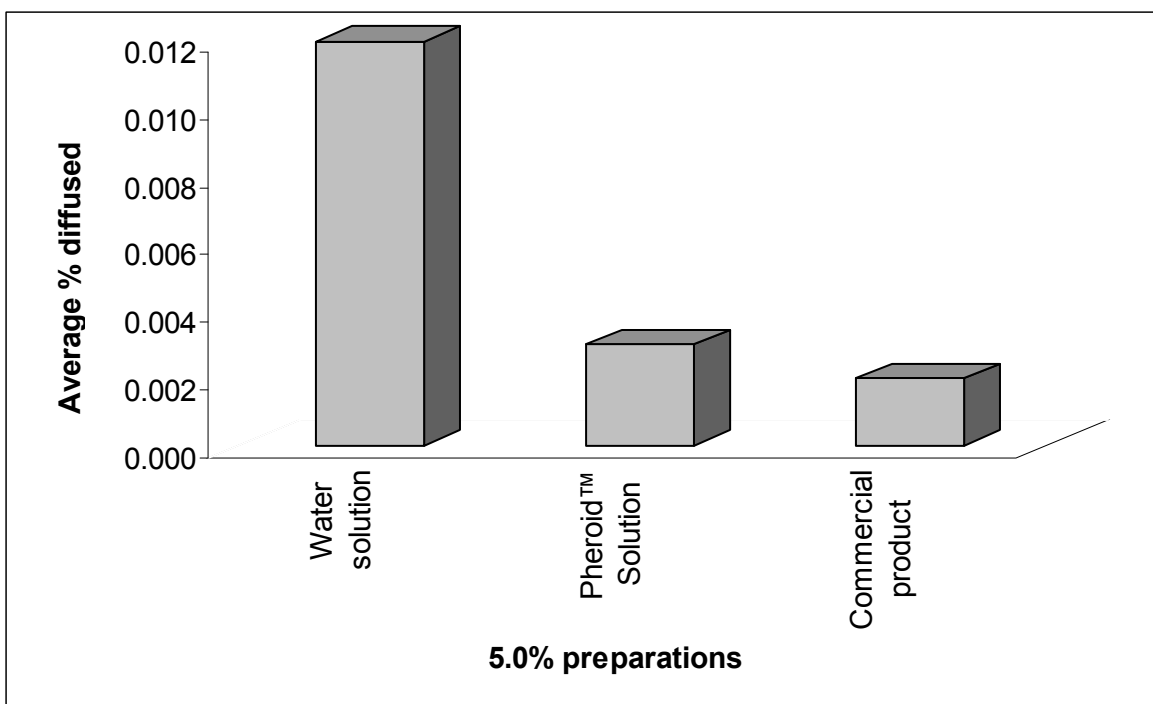


Figure 2: Comparison between average % diffused values of 5.0% solutions and 5.0% commercial product

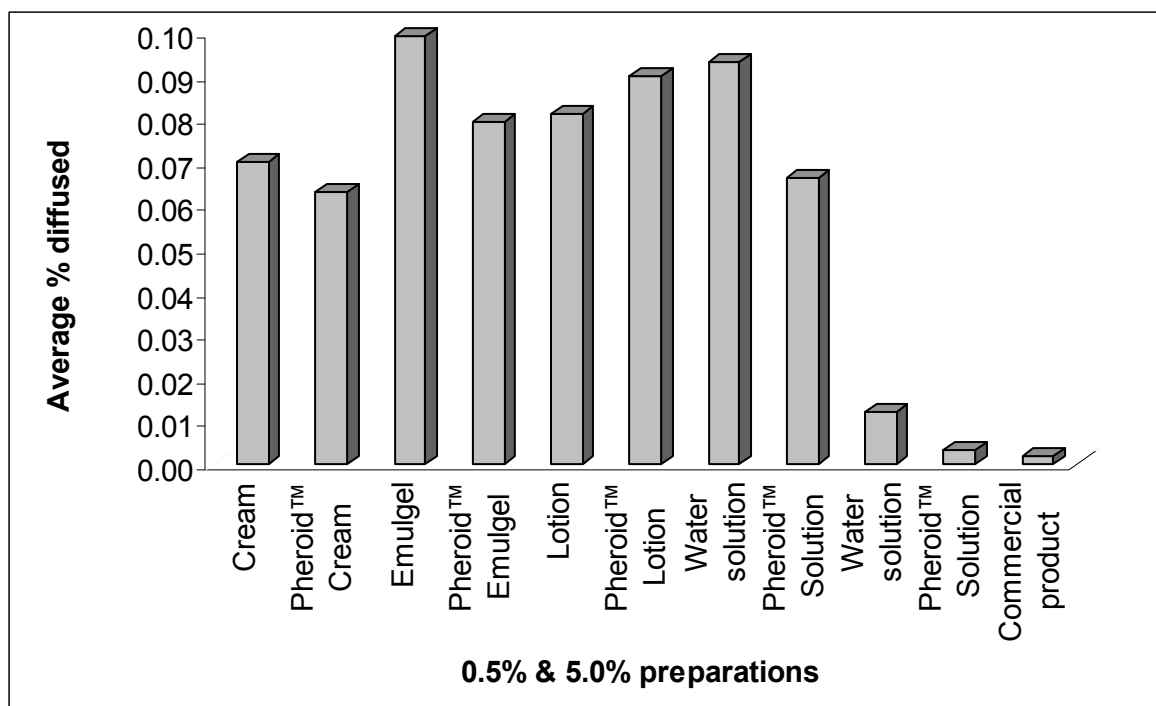


Figure 3: Comparison between average % diffused values of 0.5% formulations, 0.5% solutions, 5.0% commercial product and 5.0% solutions

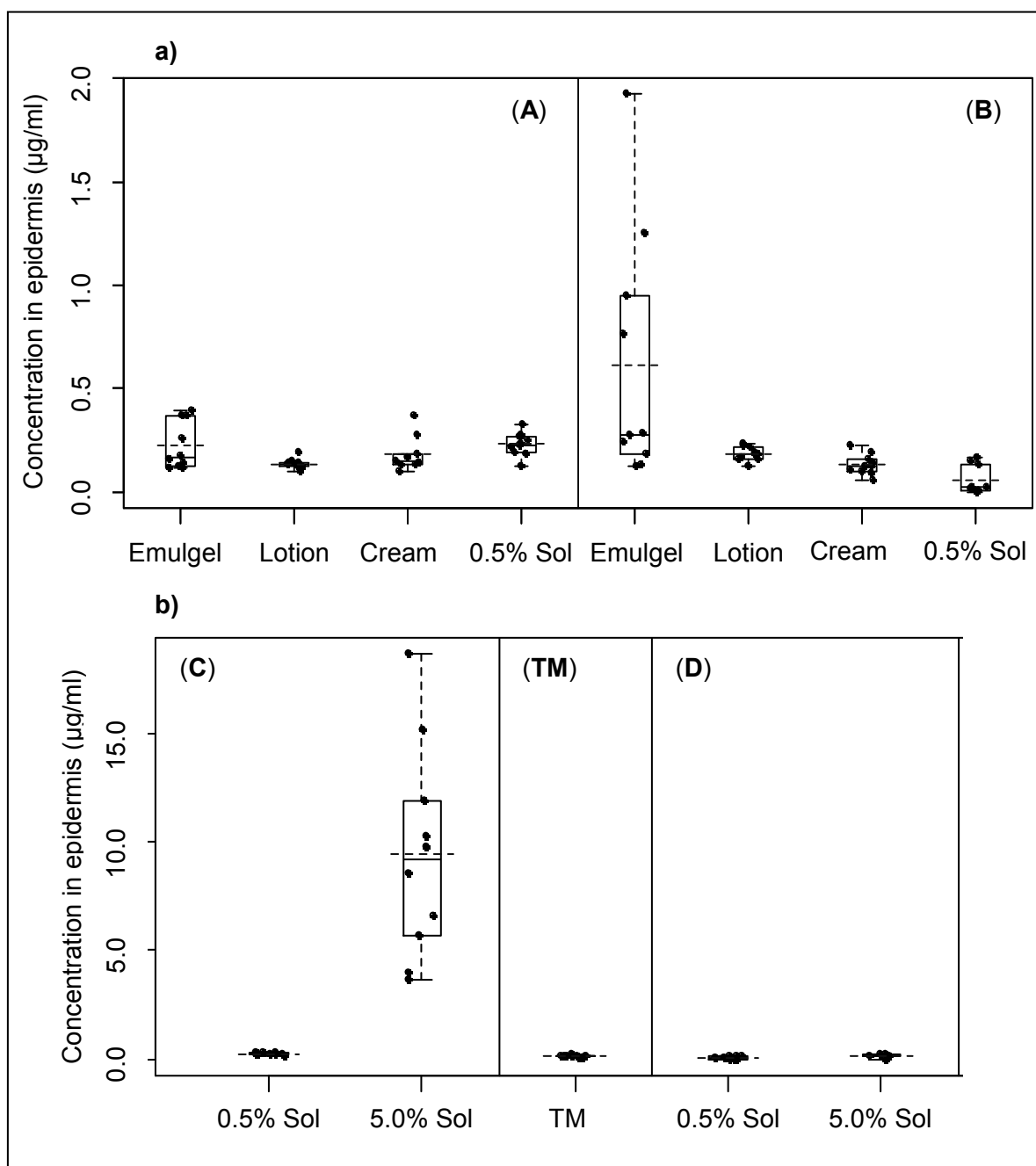


Figure 4: Box-plot representation of the epidermis concentration values for: **a) (A):** non-PheroidTM (0.5%) formulations and solutions; **(B):** PheroidTM (0.5%) formulations and solutions and **b) (C):** non-PheroidTM (5.0%) solutions; **(D):** PheroidTM (5.0%) solutions and *TM is the commercial product. The average and median epidermis concentrations are indicated by a dotted and solid line, respectively. The dots represent the epidermis data (µg/ml).

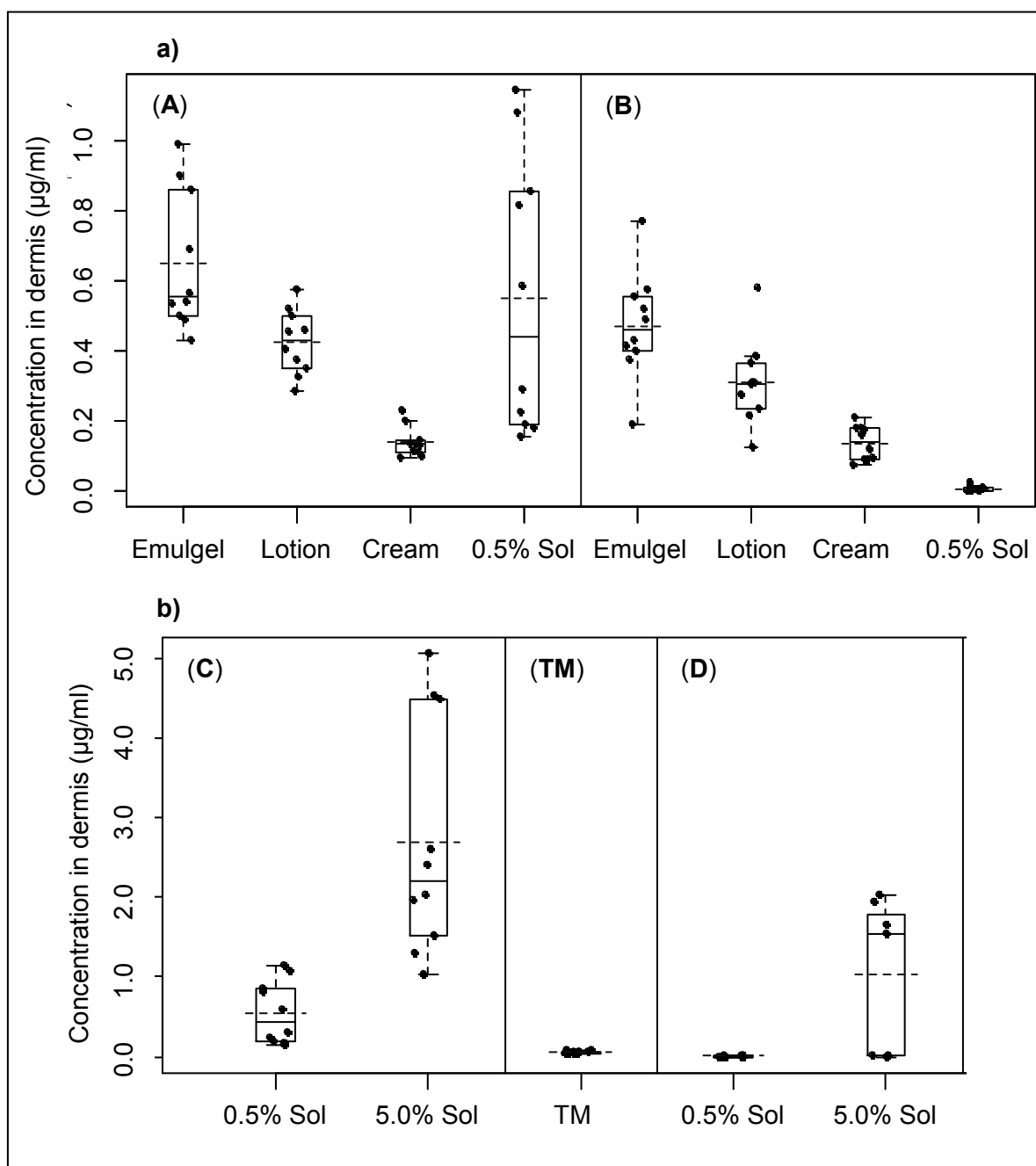


Figure 5: Box-plot representation of the dermis concentration values for: **a) (A):** non-Pheroid™ (0.5%) formulations and solutions; **(B):** Pheroid™ (0.5%) formulations and solutions and **b) (C):** non-Pheroid™ (5.0%) solutions; **(D):** Pheroid™ (5.0%) solutions and ***TM** is the commercial product. The average and median dermis concentrations are indicated by a dotted and solid line, respectively. The dots represent the dermis data (µg/ml).

CHAPTER 4

FINAL CONCLUSIONS AND FUTURE PROSPECTS

5-Fluorouracil is widely used as a chemotherapeutic drug in the treatment of proliferated skin diseases, including AK, BCC and psoriasis, by the inhibition of DNA synthesis. This in turn leads to the inhibition of cell replication (Patrick *et al.*, 1997:40).

The aims and objectives of this study include:

- ❖ Formulation of six semisolid formulations containing 5-fluorouracil (0.5%).
- ❖ Development and validation of an analytical method to determine the concentration of the analytes within the formulations, by means of HPLC.
- ❖ Stability testing on these formulations over a period of six months.
- ❖ Determination of the aqueous solubility and log D of 5-fluorouracil.
- ❖ Delivery of 5-fluorouracil to the underlying layers of the skin, by using the Franz cell skin diffusion method and determination of the influence of Pheroid™.
- ❖ Formulation of 5.0% solutions and comparing the transdermal diffusion to that of a 5.0% commercially available product.

The main purpose of this study was to manufacture a variety of 0.5% semisolid formulations containing 5-fluorouracil for transdermal delivery. The semisolid formulations consisted of a cream, Pheroid™ cream, emulgel, Pheroid™ emulgel, lotion and Pheroid™ lotion. These formulations were successfully manufactured.

The HPLC method used in this study for determination of concentration indicating assay, was successfully developed and validated for the use in the stability testing period of six months.

A series of stability tests was conducted after manufacturing and on months 1, 2, 3 and 6. The concentration indicating assay yielded the lotion as the most stable formulation. Zeta-potential values fluctuated for all formulations throughout the six months. When droplet size was evaluated, the emulgel showed the least fluctuation in droplet size throughout the stability period. The pH of the formulations mainly decreased with an increase in temperature and relative humidity; the cream showed the lowest % decrease in pH. Viscosity did not demonstrate a constant change throughout the stability period, although the Pheroid™ emulgel showed the lowest %RSD, regarding viscosity. The Pheroid™ emulgel depicted the lowest

% decrease in mass over the 6 month stability period. The physical appearance of the cream, emulgel and lotion did not show any significant change throughout the stability period, but all the Pheroid™ formulations became more yellow as time passed. When comparing the formulations at 25 °C/60% RH to those at 40 °C/75% RH over the stability period of 6 months, a slight change in particle size distribution was observed on CLSM. Particles of the emulgel and Pheroid™ emulgel formulations clogged together and creams showed an increase in particle size distribution.

When drug delivery through the skin is investigated, the outer layer of the skin (stratum corneum) is the most important consideration, as it acts as a barrier for any substance to enter or water to emit the skin. When the ideal properties of a drug are taken into account for transdermal delivery, drugs with a log P value in the range of 1 – 3, are generally considered to have optimum partition behaviour (Hadgraft, 2004:291-292). Thus, the obtained log D value for 5-fluorouracil (-0.98) did not indicate optimum partitioning into the skin.

The inclusion of a drug delivery system, known as Pheroid™ technology, was also investigated. A commercially available 5.0% 5-fluorouracil product was also tested by means of Franz cell diffusion, but due to the difference in concentration, it was decided that a 5.0% water solution and 5.0% Pheroid™ solution should be prepared in order to compare these results with the commercial product. The addition of Pheroid™ within the formulations and solutions did not show enhanced permeation of 5-fluorouracil; although the Pheroid™ cream, Pheroid™ emulgel as well as Pheroid™ lotion yielded greater transdermal penetration of 5-fluorouracil than the 5.0% commercial product.

After conducting the Franz cell skin diffusion experiments with the 0.5% semisolid formulations containing 5-fluorouracil, the emulgel depicted the highest average % diffused (0.099%) through the skin. This could have been due to the high fluid content of the emulgel that hydrated the skin and caused the stratum corneum to swell and open its structures, which in turn lead to an increase in penetration (Benson, 2005:28). The 5.0% commercial product depicted the poorest average % diffused (0.002%), that could be due to the ointment base, which caused the least hydration on the skin's surface and in turn prevented 5-fluorouracil to penetrate the stratum corneum. Further study should be conducted with the emulgel to investigate the influence of different components on Franz cell skin diffusion as well as stability of the product.

Tape stripping revealed that the water solution (5.0%) showed the highest average concentration in both epidermis (9.444 µg/ml) and dermis (2.696 µg/ml). This was an over-saturated solution of 5-fluorouracil, which caused solid particles to reside in the epidermis. As a

result, this caused a high drive force for 5-fluorouracil into the dermis, when considering Fick's law of diffusion Williams (2003:41).

Even though the water solution (5.0%) produced the highest concentration in the epidermis and dermis, from a practical point of view, the emulgel formulation (0.5%) would be more suitable to use. It will be more profitable to manufacture and cause fewer side effects on the surface of the skin, due to the ten times smaller concentration when compared to the water solution (5.0%). The water solution (5.0%) will leave a powder residue on the skin surface due to its high saturation; hence, it will not stick to the skin and cannot be used for the purpose of treating skin cancer.

Recommended future prospects include the following:

- ❖ Stability parameters, such as droplet size and zeta-potential should be investigated in detail, since no direct correlation could be yielded with these parameters.
- ❖ Attention should be given to the stability of the formulations, with regard to changes in viscosity and discolouration.
- ❖ Formulation of preparations which will produce targeted delivery of 5-fluorouracil within the dermis and consequently lead to lower % diffused values, owing to the fact that skin cancer should be treated topically and not systemically.
- ❖ Further investigation of a 5-fluorouracil emulgel is encouraged, with regard to different excipients, as it yielded the best results with regard to dermal delivery of 5-fluorouracil.

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APPENDIX A

METHOD VALIDATION FOR THE ASSAY OF THE FORMULATIONS

A.1 INTRODUCTION

In order to use a method of analysis on an instrument for example HPLC, it should first be validated to insure that the method is compelling and suitable for the purpose it is used for (ICH, 2005:1). The purpose of the method was to determine the concentrations of the different analytes in each formulation. The analytes include 5-fluorouracil (active ingredient); methyl and propyl paraben (preservatives); butylated hydroxytoluene (BHT) and dl- α -tocopherol (anti-oxidants). This is called the assay and was performed on the day the formulations were manufactured. The assay was repeated in 28-day cycles for the first three months, and then only after the sixth month of the stability period.

A.2 CHROMATOGRAPHIC CONDITIONS

The method used in this section of the study was created and developed, with the help and knowledge of Professor Jan du Preez of the North-West University (NWU), Potchefstroom. All procedures were carried out in the Analytical Technology Laboratory at NWU.

There are certain factors (chromatographic conditions) which should be taken into account when developing a method of analysis. These factors include: the choice of column to use; the type of mobile phase; the injection volume and flow rate; wave length where detection should take place as well as the stop time. The chromatographic conditions for the method were as follows:

Analytical instrument:	Agilent® 1100 series, which consisted of a degasser, a quaternary pump, an auto sampler and a diode array detector. Chemstation Rev. A.10.02 software was used for data acquisition and analysis (Agilent Technologies, Palo Alto, CA).
Column:	Phenomenex® Synergi Fusion 4 μ m Reversed Phase 4.6 mm x 250.0 mm (Phenomenex, Torrance, CA).
Mobile phase A:	Octanesulphonic acid solution was made by weighing 1 g of the octane-1-sulphonic acid sodium salt powder and adding 1000 ml of deionised HPLC-grade water. The pH of this solution was set to 3.5; this was done by means of adding either 10%

orthophosphoric acid (H₃PO₄) solution to lower the pH, or NH₄OH to achieve a higher pH.

Mobile phase B: LiChrosolv[®] methanol, obtained from Merck[™]. The mobile phases were injected with a gradient as described in Table A.1.

Flow rate: 1 ml/min

Injection volume: 5 µl

Detection wavelength: 210 nm

Stop time: 20 min

Solvent: Methanol was used for the preparation of standard solutions as well as samples.

Table A.1: Gradient for mobile phases

Time interval (min):	Octanesulphonic acid (A)	Methanol (B)
0 - 1.0	90%	10%
8.0 – 15.0	0%	100%
15.0 – 15.2	0%	100%
15.2 – 20.00	90%	10%

A chromatogram of the five different analytes is shown in Figure A.1. The order (and retention times) in which the analytes appeared on the chromatogram was as follows:

1. 5-Fluorouracil (4.7 min)
2. Methyl paraben (10.2 min)
3. Propyl paraben (11.2 min)
4. BHT (12.8 min)
5. dl-α-Tocopherol (17.7 min)

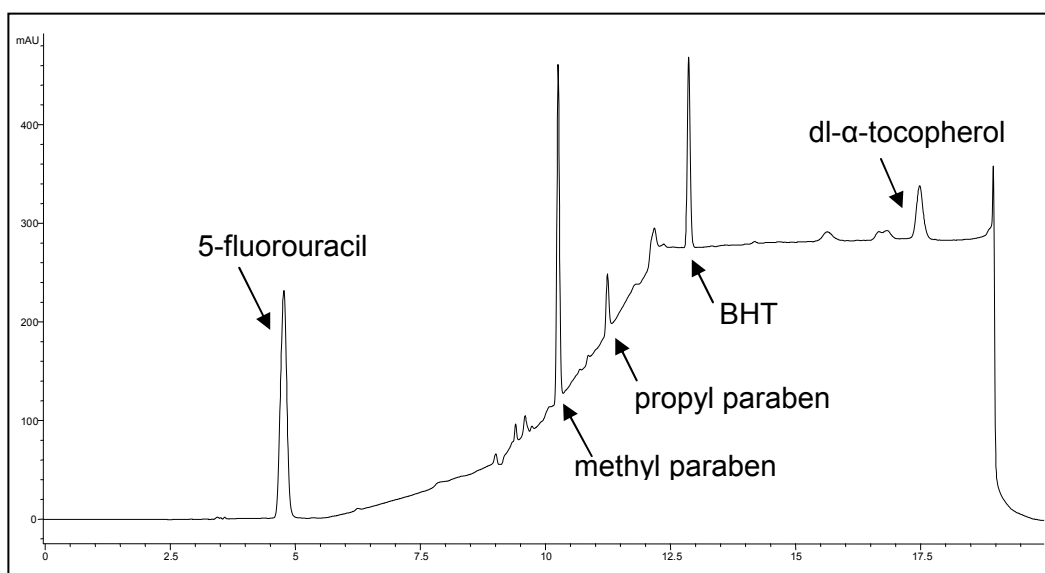


Figure A.1: Chromatogram to illustrate the order in which the analytes appeared

A.3 STANDARD PREPARATION

A.3.1. Standard preparation of the five analytes

The standard solution consisted of a combination of the five main ingredients used in the Pheroid™ cream formulation, as this was the most complicated formulation. These ingredients included the active ingredient (5-fluorouracil), the preservatives (methyl- and propyl paraben), an anti-oxidant (BHT) as well as a stabilising agent and anti-oxidant (dl-α-tocopherol). The quantities of each ingredient (analyte) were calculated for a 1 g formulation diluted in 50 ml solvent (methanol).

The quantity of the propyl paraben was too small to be weighed accurately, thus, a larger quantity had to be weighed and diluted to achieve the correct concentration. The method of preparation for this standard solution was as follows:

1. Weigh 5 mg 5-fluorouracil, 2 mg methyl paraben, 2 mg BHT and 2 mg dl-α-tocopherol in a glass weighing boat. Place in a 50 ml flask, using a small amount of methanol.
2. Weigh 2 mg propyl paraben in a separate 50 ml volumetric flask and dissolve in 50 ml of the same solvent, methanol.
3. Sonicate this solution of propyl paraben until fully dissolved and add 10 ml of this solution to the other substances mentioned in step 1.
4. Add sufficient methanol to the mixture in order to reach the 50 ml mark.

5. Sonicate the mixture (all the analytes) until they have completely dissolved and transfer approximately 2 ml of this standard solution to an auto sampler vial and analyse on the HPLC.

A.4 SAMPLE PREPARATION

For the purpose of this method, the samples were made by weighing exactly 1 g of the most complicated formulation, as this formulation contained all the possible ingredients which were present in the formulations and that had to be analysed. This formulation was the Pheroid™ cream, and was prepared as described in Section B.5.2. By using a syringe with a small rubber tube connected to the front, the cream was transferred into the syringe and directly weighed into a 50 ml volumetric flask, after which a small amount of HPLC-grade water was added (to achieve better solubility), and filled up with methanol. This solution had to be sonicated for a few minutes in order for the cream to fully dissolve in the methanol and form a homogenous solution. Approximately 2 ml of this solution were put into a vial and analysed by means of HPLC. The quantities of the samples differed for each validation parameter and will be specified accordingly in each section.

A.5 VALIDATION PARAMETERS

Linearity, accuracy, intra- and interday precision, ruggedness, sample stability and specificity are the validation parameters that will be discussed in the following section.

A.5.1 Linearity

The linearity of an analytical procedure is described as the ability to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample, measured within a given range of concentrations.

The concentrations (X-axis) of the analytes were plotted against the peak areas (Y-axis) derived from integrating each peak on the chromatogram. These values should give a straight line on a graph, giving the following linear equation:

$$y = mx + c$$

Equation A.1

where: y = peak area of the analyte
m = slope
x = concentration of the analyte
c = y intercept

When a linear regression analysis is done, it should yield a regression coefficient (r^2) of ≥ 0.99 .

A.5.1.1 Linearity method

To determine the linearity of the five main analytes, a 50 ml standard solution was prepared as described in Section A.3.1, and a range of 62.5 – 125.0% was used.

The above mentioned samples were analysed in duplicate by means of HPLC.

The peak areas of each analyte, in the dilutions made from the 125% standard solution, were integrated from the chromatograms and interpreted separately for each analyte as shown in Tables A.2 to A.6.

Table A.2: Results for linearity of 5-fluorouracil

Sample	Average peak area	Concentration ($\mu\text{g/ml}$)
62.5%	1008.5	81.3
75.0%	1204.7	97.5
87.5%	1438.3	113.8
100.0%	1659.0	130.0
112.5%	1813.1	146.3
125.0%	2026.7	162.5

The r^2 value for 5-fluorouracil was within the accepted criteria of 0.99, as seen in Figure A.2.

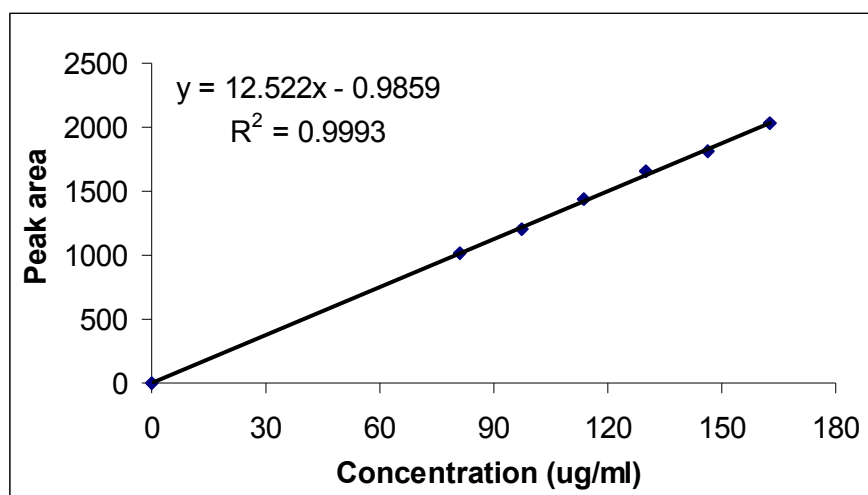
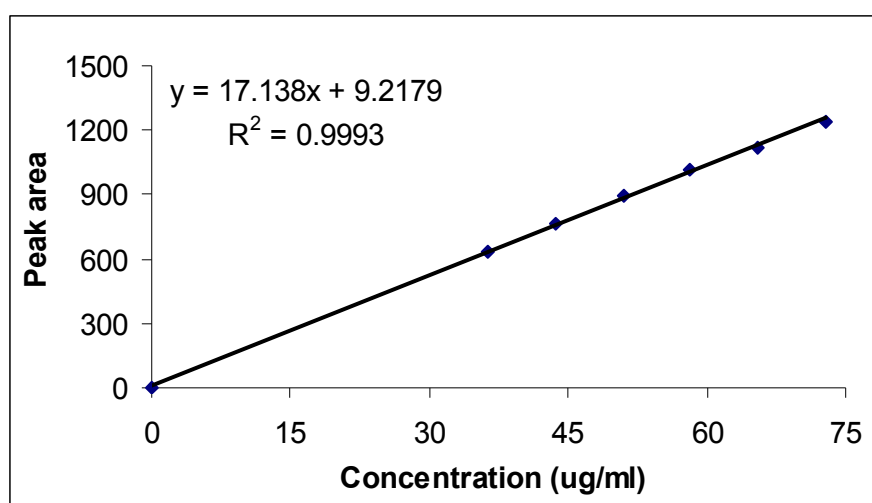


Figure A.2: Linearity graph for 5-fluorouracil

Table A.3: Results for linearity of methyl paraben

Sample	Average peak area	Concentration (µg/ml)
62.5%	637.7	36.4
75.0%	761.2	43.7
87.5%	896.0	50.9
100.0%	1016.7	58.2
112.5%	1120.9	65.5
125.0%	1242.8	72.8

The r^2 value for methyl paraben was within the acceptance criteria of 0.99, as seen in Figure A.3.

**Figure A.3:** Linearity graph for methyl paraben**Table A.4:** Results for linearity of propyl paraben

Sample	Average peak area	Concentration (µg/ml)
62.5%	108.1	6.6
75.0%	134.7	8.0
87.5%	152.3	9.3
100.0%	179.7	10.6
112.5%	189.2	11.9
125.0%	214.5	13.3

The r^2 value for propyl paraben, when taken to the next decimal, was within the acceptance criteria of 0.99, as seen in Figure A.4.

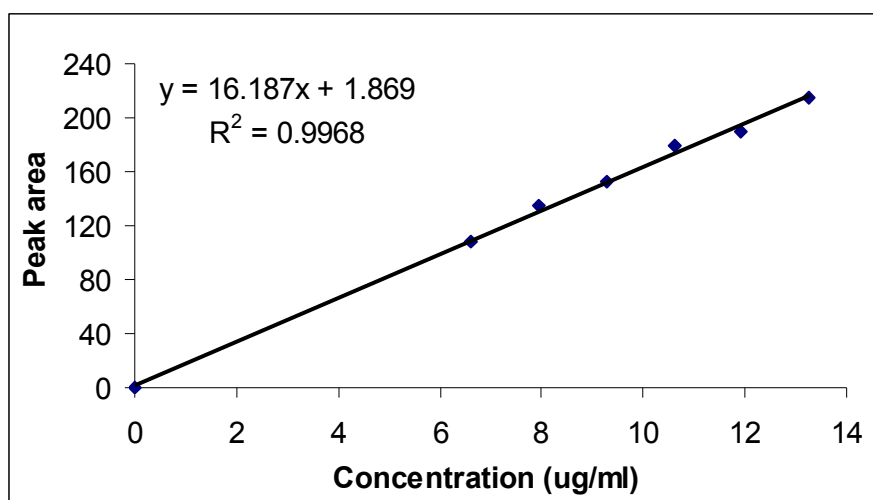


Figure A.4: Linearity graph for propyl paraben

Table A.5: Results for linearity of BHT

Sample	Average peak area	Concentration (µg/ml)
62.5%	398.9	37.3
75.0%	487.3	44.7
87.5%	578.2	52.2
100.0%	652.3	59.6
112.5%	714.6	67.1
125.0%	800.1	74.5

The r^2 value for BHT was within the acceptance criteria of 0.99, as seen in Figure A.5.

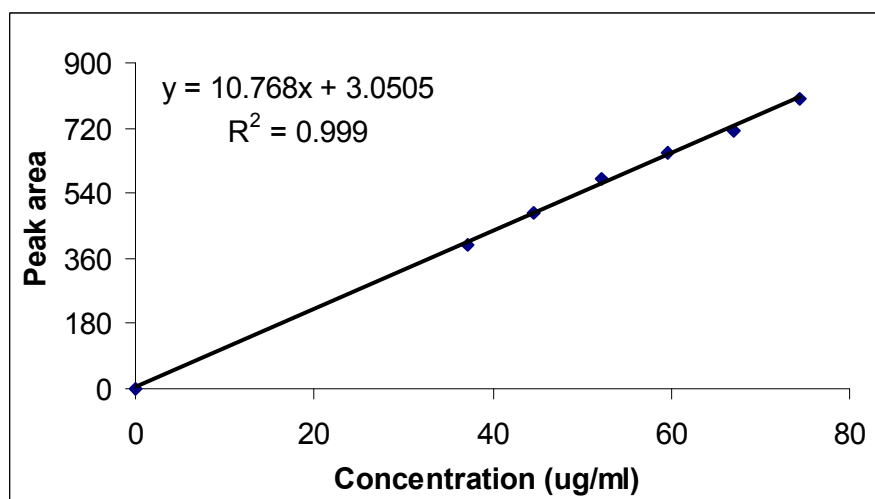


Figure A.5: Linearity graph for BHT

Table A.6: Results for linearity of dl- α -tocopherol

Sample	Average peak area	Concentration ($\mu\text{g/ml}$)
62.5%	192.4	34.0
75.0%	296.2	40.8
87.5%	368.8	47.6
100.0%	429.0	54.4
112.5%	488.5	61.2
125.0%	526.1	68.0

The r^2 value for dl- α -tocopherol was 0.97, and thus not within the acceptance criteria of 0.99, as seen in Figure A.6. This may be due to the fact that it degraded, as it is extremely light-sensitive.

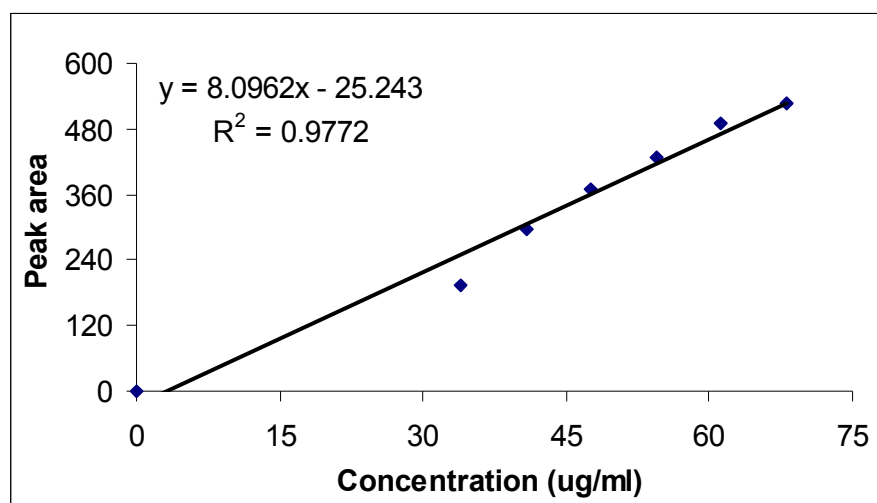


Figure A.6: Linearity graph for dl- α -tocopherol

A.5.2 Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found (ICH, 2005:4). It specifies the variation between the mean value and the exact value established. To determine the accuracy, the method is applied to samples to where identified quantities of analyte have been added. These should be analysed against standard and blank solutions to ensure that no interference exists. The accuracy is then calculated from the test results as a percentage of the analyte recovered by the assay (Van Iterson, 200?). The mean recovery of each analyte should be between 98-102% (Du Preez, 2008:5).

A.5.2.1 Accuracy method

Firstly, a ten-time stronger standard solution of the analytes was prepared as described in Section A.3.1. The quantities were respectively 50 mg of 5-fluorouracil, 20 mg of methyl paraben, 20 mg of BHT and 20 mg of dl- α -tocopherol. The propyl paraben was again diluted in the same manner as described in Section A.3.1, except 20 mg was used. This standard solution was used to place an amount of analytes in a placebo Pheroid™ cream standard solution.

Secondly, 0.8 g, 1.0 g and 1.2 g respectively, of a placebo Pheroid™ cream (without the five analytes) was weighed in triplicate, into 50 ml volumetric flasks, and filled up with methanol, leaving enough space for the amount of analytes to be added later. Thus, 3 x 80%, 3 x 100% and 3 x 120% solutions were prepared. The flasks were sonicated for a few minutes in order to homogenise the solutions.

The before mentioned ten-time stronger standard solution was used to transfer the correct amount of the analytes into placebo Pheroid™ cream solutions. This was done in the following manner:

- 4 ml of the ten-time stronger solution was added to all three of the 80% placebo solutions. ($4 \text{ ml} / 50 \text{ ml} \times 1000 = 80 \text{ } \mu\text{g/ml}$)
- 5 ml of the ten-time stronger solution was added to all three of the 100% placebo solutions. ($5 \text{ ml} / 50 \text{ ml} \times 1000 = 100 \text{ } \mu\text{g/ml}$)
- 6ml of the ten-time stronger solution was added to all three of the 120% placebo solutions. ($6 \text{ ml} / 50 \text{ ml} \times 1000 = 120 \text{ } \mu\text{g/ml}$)

The nine 50 ml flasks were filled up with methanol to reach the 50 ml mark.

All of the above samples were analysed in duplicate on the HPLC and data is shown in Tables A.7 to A.11.

Table A.7: Results for accuracy of 5-fluorouracil

Sample	Concentration (µg/ml)	Peak area 1	Peak area 2	Mean	Recovery (µg/ml)	Recovery (%)
80% (1)	80.8	1659.2	1623.4	1641.3	82.4	102.0
80% (2)	80.8	1591.9	1583.9	1587.9	79.7	98.7
80% (3)	80.8	1574.1	1597.5	1585.8	79.6	98.6
100% (1)	101.0	1954.8	1953.7	1954.3	98.1	97.2
100% (2)	101.0	2000.2	1986.0	1993.1	100.1	99.1
100% (3)	101.0	2015.2	1996.5	2005.9	100.7	99.7
120% (1)	121.2	2377.4	2398.8	2388.1	119.9	98.9
120% (2)	121.2	2393.5	2394.3	2393.9	120.2	99.1
120% (3)	121.2	2357.6	2374.7	2366.2	118.8	98.0
					Mean	99.0
					SD	1.3
					%RSD	1.3

Over the range of 80 – 120% of the sample concentration, the method yielded a mean recovery of 99.0% for 5-fluorouracil, which was within the accepted criteria.

Table A.8: Results for accuracy of methyl paraben

Sample	Concentration (µg/ml)	Peak area 1	Peak area 2	Mean	Recovery (µg/ml)	Recovery (%)
80% (1)	32.2	815.7	782.7	799.2	31.8	98.8
80% (2)	32.2	811.0	817.6	814.3	32.4	100.6
80% (3)	32.2	811.1	823.4	817.3	32.5	101.0
100% (1)	40.3	1021.0	1019.3	1020.2	40.6	100.7
100% (2)	40.3	1039.5	1036.3	1037.9	41.3	102.5
100% (3)	40.3	1051.7	1038.0	1044.9	41.6	103.2
120% (1)	48.4	1247.5	1256.9	1252.2	49.8	103.0
120% (2)	48.4	1256.8	1258.4	1257.6	50.1	103.4
120% (3)	48.4	1228.9	1247.8	1238.4	49.3	101.8
					Mean	101.7
					SD	1.4
					%RSD	1.4

Over the range of 80 – 120% of the sample concentration, the method yielded a mean recovery of 101.7% for methyl paraben, which was within the accepted criteria.

Table A.9: Results for accuracy of propyl paraben

Sample	Concentration (µg/ml)	Peak area 1	Peak area 2	Mean	Recovery (µg/ml)	Recovery (%)
80% (1)	6.1	124.2	94.0	109.1	5.4	89.5
80% (2)	6.1	96.2	97.3	96.8	4.8	79.4
80% (3)	6.1	97.8	101.0	99.4	5.0	81.5
100% (1)	7.6	120.9	120.7	120.8	6.0	79.3
100% (2)	7.6	124.0	122.6	123.3	6.1	80.9
100% (3)	7.6	125.6	124.4	125.0	6.2	82.0
120% (1)	9.1	150.9	156.7	153.8	7.7	84.1
120% (2)	9.1	150.6	152.5	151.6	7.6	82.9
120% (3)	9.1	149.9	152.9	151.4	7.6	82.8
					Mean	82.5
					SD	2.9
					%RSD	3.5

Over the range of 80 – 120% of the sample concentration, the method yielded a mean recovery of 82.5% for propyl paraben, which was not within the accepted criteria. This could be due to poor homogeneity of the samples.

Table A.10: Results for accuracy of BHT

Sample	Concentration (µg/ml)	Peak area 1	Peak area 2	Mean	Recovery (µg/ml)	Recovery (%)
80% (1)	33.6	557.9	528.5	543.2	32.0	95.2
80% (2)	33.6	535.7	550.9	543.3	32.0	95.2
80% (3)	33.6	501.1	508.7	504.9	29.7	88.5
100% (1)	42.0	665.2	660.3	662.8	39.0	92.9
100% (2)	42.0	635.9	634.0	635.0	37.4	89.0
100% (3)	42.0	691.5	683.1	687.3	40.5	96.3
120% (1)	50.4	809.8	815.2	812.5	47.8	94.9
120% (2)	50.4	812.9	811.2	812.1	47.8	94.9
120% (3)	50.4	803.4	818.6	811.0	47.7	94.7
					Mean	93.5
					SD	2.7
					%RSD	2.9

Over the range of 80 – 120% of the sample concentration, the method yielded a mean recovery of 93.5% for BHT, which was not within the accepted criteria. This could be due to poor homogeneity of the samples.

Table A.11: Results for accuracy of dl- α -tocopherol

Sample	Concentration ($\mu\text{g/ml}$)	Peak area 1	Peak area 2	Mean	Recovery ($\mu\text{g/ml}$)	Recovery (%)
80% (1)	34.0	503.8	511.2	507.5	32.8	96.6
80% (2)	34.0	528.0	519.0	523.5	33.9	99.6
80% (3)	34.0	514.6	508.8	511.7	33.1	97.4
100% (1)	42.5	650.4	660.5	655.5	42.4	99.8
100% (2)	42.5	649.3	642.4	645.9	41.8	98.3
100% (3)	42.5	622.4	633.9	628.2	40.6	95.6
120% (1)	51.0	763.6	763.4	763.5	49.4	96.9
120% (2)	51.0	775.5	776.7	776.1	50.2	98.5
120% (3)	51.0	752.9	771.8	762.4	49.3	96.7
					Mean	97.7
					SD	1.3
					%RSD	1.4

Over the range of 80 – 120% of the sample concentration, the method yielded a mean recovery of 97.7% for dl- α -tocopherol, which was not within the accepted criteria. This could be due to poor homogeneity of the samples.

A.5.3 Precision

The precision is described as the measurement of reproducibility of an analytical method and it consists of intraday and interday variability. It is established by the use of the method to assay the samples for a number of times in order to attain statistically compelling results. Precision is articulated as the relative standard deviation (RSD) and is calculated by the following equation (Van Iterson, 200?):

$$\%RSD = (\text{standard deviation} \times 100\%)/\text{mean}$$

Equation A.2

A.5.3.1 Intraday precision (within-day variation)

Intraday precision was conducted in order to see what the reproducibility of the method was on the same day. This was conducted with three samples of each of 80%, 100% and 120% of the Pheroid™ cream sample described in Section A.4. Thus, 0.8 g, 1.0 g and 1.2 g were weighed in triplicate and made up to 50 ml with methanol. The samples were injected in duplicate and analysed through HPLC and data is given in Tables A.12 to A.16.

The percentage relative standard deviation (%RSD) should be less than or 2%, if there are 9 samples (Du Preez, 2008:5).

Table A.12: Intraday precision values for 5-fluorouracil

Sample	Mass (g)	Peak area 1	Peak area 2	Mean	Concentration (µg/ml)	%
80% (1)	0.818	1826.1	1799.9	1813.0	86.6	104.0
80% (2)	0.805	1753.9	1758.9	1756.4	85.3	102.5
80% (3)	0.843	1839.2	1878.1	1858.7	86.1	103.5
100% (1)	1.009	2258.0	2273.7	2265.9	109.6	105.4
100% (2)	1.024	2253.0	2281.2	2267.1	108.1	103.9
100% (3)	1.027	2262.0	2260.6	2261.3	107.5	103.3
120% (1)	1.214	2699.4	2694.3	2696.9	130.2	104.3
120% (2)	1.222	2631.4	2631.6	2631.5	126.2	101.1
120% (3)	1.205	2638.5	2623.8	2631.2	127.9	102.5
					Mean	103.4
					SD	1.2
					%RSD	1.1

The precision of 5-fluorouracil was satisfactory, with a percentage RSD of 1.1%.

Table A.13: Intraday precision values for methyl paraben

Sample	Mass (g)	Peak area 1	Peak area 2	Mean	Concentration (µg/ml)	%
80% (1)	0.818	954.5	940.4	947.5	31.7	94.2
80% (2)	0.805	934.4	930.6	932.5	31.7	94.3
80% (3)	0.843	959.0	978.5	968.8	31.4	93.5
100% (1)	1.009	1111.4	1117.0	1114.2	37.7	89.8
100% (2)	1.024	1162.2	1177.6	1169.9	39.0	92.9
100% (3)	1.027	1160.3	1160.9	1160.6	38.6	91.9
120% (1)	1.214	1384.8	1377.6	1381.2	46.7	92.6
120% (2)	1.222	1385.3	1386.6	1386.0	46.5	92.3
120% (3)	1.205	1369.5	1357.0	1363.3	46.4	92.1
					Mean	92.6
					SD	1.3
					%RSD	1.4

The precision of methyl paraben was satisfactory, with a percentage RSD of 1.4%.

Table A.14: Intraday precision values for propyl paraben

Sample	Mass (g)	Peak area 1	Peak area 2	Mean	Concentration (µg/ml)	%
80% (1)	0.818	147.0	146.7	146.9	7.2	101.7
80% (2)	0.805	142.5	144.0	143.3	7.1	100.9
80% (3)	0.843	149.5	151.4	150.5	7.1	101.2
100% (1)	1.009	169.1	168.3	168.7	8.3	94.7
100% (2)	1.024	179.3	182.9	181.1	8.8	100.2
100% (3)	1.027	183.1	181.0	182.1	8.8	100.4
120% (1)	1.214	217.8	217.0	217.4	10.7	101.5
120% (2)	1.222	218.0	219.1	218.6	10.7	101.4
120% (3)	1.205	217.7	215.2	216.5	10.8	101.8
					Mean	100.4
					SD	2.1
					%RSD	2.1

The precision of propyl paraben was a little higher than the satisfactory specifications of 2%, with a %RSD of 2.1%, although still within an acceptable range, as this analyte is not an active ingredient and need not comply with the exact criteria as an active ingredient.

Table A.15: Intraday precision values for BHT

Sample	Mass (g)	Peak area 1	Peak area 2	Mean	Concentration (µg/ml)	%
80% (1)	0.818	595.0	575.9	585.5	30.2	85.7
80% (2)	0.805	576.2	579.6	577.9	30.3	86.0
80% (3)	0.843	598.5	605.6	602.1	30.1	85.6
100% (1)	1.009	704.3	709.4	706.9	36.9	83.9
100% (2)	1.024	720.8	733.1	727.0	37.4	85.0
100% (3)	1.027	731.2	728.0	729.6	37.4	85.1
120% (1)	1.214	866.2	862.4	864.3	45.0	85.3
120% (2)	1.222	858.1	864.7	861.4	44.6	84.4
120% (3)	1.205	855.3	845.0	850.2	44.6	84.5
					Mean	85.1
					SD	0.6
					%RSD	0.8

The precision of BHT was satisfactory with a percentage RSD of 0.8%.

Table A.16: Intraday precision values for dl- α -tocopherol

Sample	Mass (g)	Peak area 1	Peak area 2	Mean	Concentration ($\mu\text{g/ml}$)	%
80% (1)	0.818	502.7	492.1	497.4	38.3	110.4
80% (2)	0.805	487.4	493.4	490.4	38.4	110.7
80% (3)	0.843	492.9	504.4	498.7	37.3	107.4
100% (1)	1.009	619.7	631.5	625.6	48.9	112.6
100% (2)	1.024	609.5	618.9	614.2	47.3	108.9
100% (3)	1.027	630.8	621.7	621.3	47.7	109.8
120% (1)	1.214	723.7	721.9	722.8	56.3	108.1
120% (2)	1.222	721.0	720.4	720.7	55.8	107.1
120% (3)	1.205	707.3	708.6	708.0	55.6	106.7
					Mean	109.1
					SD	1.8
					%RSD	1.7

The precision of dl- α -tocopherol was satisfactory with a percentage RSD of 1.7%.

A.5.3.2 Interday precision (interlaboratory variation)

Interday precision is performed to determine the between-day variability of the analytical method and is usually performed on two or more different days, within the same laboratory conditions (Du Preez, 2008:5).

Interday precision was conducted on three consecutive days with three samples of a 100% standard solution, as described in Section A.4. These samples were injected in duplicate and analysed on the HPLC, the percentage recovery for each sample relative to a standard solution is given in Tables A.17 to A.21.

The samples should give a %RSD of less than or 5% (Du Preez, 2008:5).

Table A.17: Interday precision values for 5-fluorouracil

	Day 1	Day 2	Day 3	Between days
	104.3	104.6	104.4	
	101.7	104.6	102.4	
	100.5	99.6	102.0	
Mean	102.2	102.9	102.9	102.7
SD	1.6	2.4	1.0	1.7
%RSD	1.6	2.3	1.0	1.6

5-Fluorouracil had a %RSD of 1.6 which complied with the pharmaceutical acceptance criteria of less than or 5%.

Table A.18: Interday precision values for methyl paraben

	Day 1	Day 2	Day 3	Between days
	91.1	81.7	89.3	
	91.8	87.3	92.1	
	90.7	88.3	90.8	
Mean	91.2	85.8	90.7	89.2
SD	0.5	2.9	1.2	1.5
%RSD	0.5	3.4	1.3	1.7

The %RSD of 1.7 for methyl paraben complied with the pharmaceutical acceptance criteria of less than or 5%.

Table A.19: Interday precision values for propyl paraben

	Day 1	Day 2	Day 3	Between days
	91.1	80.5	92.6	
	94.2	87.0	95.2	
	93.3	89.5	96.0	
Mean	92.9	85.7	94.6	91.1
SD	1.3	3.8	1.5	2.2
%RSD	1.4	4.5	1.5	2.5

The %RSD of 2.5 for propyl paraben complied with the pharmaceutical acceptance criteria of less than or 5%.

Table A.20: Interday precision values for BHT

	Day 1	Day 2	Day 3	Between days
	87.4	80.6	87.6	
	87.9	88.3	89.2	
	85.7	84.5	87.2	
Mean	87.0	84.5	88.0	86.5
SD	0.9	3.1	0.9	1.6
%RSD	1.1	3.7	0.1	1.6

The %RSD of 1.6 for BHT complied with the pharmaceutical acceptance criteria of less than or 5%.

Table A.21: Interday precision values for dl- α -tocopherol

	Day 1	Day 2	Day 3	Between days
	110.0	111.3	111.5	
	109.5	108.4	101.8	
	101.9	100.4	103.8	
Mean	107.1	106.7	105.7	106.5
SD	3.7	4.6	4.2	4.2
%RSD	3.5	4.3	3.9	3.9

The %RSD of 3.9 for dl- α -tocopherol complied with the pharmaceutical acceptance criteria of less than or 5%.

A.5.4 Ruggedness

A.5.4.1 Stability of sample solutions

The stability of the sample solutions was conducted within a 24 h-period. The 100% standard solution with the five analytes as described in Section A.4 was used and injected by the HPLC at hourly intervals for 24 h. The peak areas were compared to the peak area derived at time 0.0 h in order to determine the stability of the samples. Samples should not be used for a period longer than it takes to degrade by 2% (Du Preez, 2008:6).

All of the analytes were stable for a period of 24 h as can be seen in Tables A.22 to A.26, except for dl- α -tocopherol, which depicted a %RSD of 3.1.

Table A.22: Stability of 5-fluorouracil

Time (hours)	Peak area	%
0	2400.8	100.0
1	2367.3	98.6
2	2366.9	98.6
3	2365.7	98.5
4	2341.5	97.5
5	2385.0	99.3
6	2362.4	98.4
7	2396.9	99.8
8	2419.7	100.8
9	2372.3	98.8
10	2375.3	89.9
11	2415.9	100.6
12	2424.2	101.0
13	2395.5	99.8
14	2432.8	101.3
15	2418.8	100.7
16	2434.7	101.4
17	2439.1	101.6
18	2447.5	101.9
19	2432.8	101.3
20	2393.0	99.7
21	2434.7	101.4
22	2436.1	101.5
23	2436.9	101.5
24	2430.9	101.3
Mean	2405.1	100.2
SD	30.3	1.3
%RSD	1.3	1.3

Table A.23: Stability of methyl paraben

Time (hours)	Peak area	%
0	1086.6	100.0
1	1081.3	99.5
2	1076.8	99.1
3	1078.0	99.2
4	1066.8	98.2
5	1094.6	100.7
6	1079.0	99.3
7	1093.9	100.7
8	1104.9	101.7
9	1082.4	99.6
10	1084.8	99.8
11	1111.9	102.3
12	1124.5	103.5
13	1110.3	102.2
14	1117.8	102.9
15	1110.0	102.2
16	1127.3	103.7
17	1136.8	104.6
18	1124.4	103.5
19	1115.5	102.7
20	1106.1	101.8
21	1116.8	102.8
22	1118.6	102.9
23	1118.0	102.9
24	1119.5	103.0
Mean	1103.5	101.6
SD	19.0	1.7
%RSD	1.7	1.7

Table A.24: Stability of propyl paraben

Time (hours)	Peak area	%
0	153.1	100.0
1	149.7	97.8
2	150.0	98.0
3	150.5	98.3
4	149.4	97.6
5	154.4	100.8
6	149.8	97.8
7	153.8	100.5
8	151.1	98.7
9	150.6	98.4
10	149.2	97.5
11	150.5	98.3
12	150.5	98.3
13	148.1	96.7
14	150.5	98.3
15	147.3	96.2
16	148.2	96.8
17	146.8	95.9
18	148.4	96.9
19	150.1	98.0
20	149.7	97.8
21	149.6	97.7
22	149.5	97.6
23	148.4	96.9
24	148.2	96.8
Mean	149.9	97.9
SD	1.9	1.2
%RSD	1.2	1.2

Table A.25: Stability of BHT

Time (hours)	Peak area	%
0	679.0	100.0
1	670.3	98.7
2	668.2	98.4
3	666.4	98.1
4	662.4	97.6
5	680.4	100.2
6	673.8	99.2
7	686.6	101.1
8	688.4	101.4
9	675.9	99.5
10	671.0	98.8
11	682.0	100.4
12	691.4	101.8
13	672.9	99.1
14	684.6	100.8
15	678.9	100.0
16	688.3	101.4
17	681.7	100.4
18	681.5	100.4
19	688.5	101.4
20	678.5	99.9
21	686.2	101.1
22	688.8	101.4
23	688.9	101.5
24	692.8	102.0
Mean	680.3	100.2
SD	8.2	1.2
%RSD	1.2	1.2

Table A.26: Stability of dl- α -tocopherol

Time (hours)	Peak area	%
0	1263	100.0
1	1190.8	94.3
2	1178.4	93.3
3	1174.1	93.0
4	1142.9	90.5
5	1230.4	97.4
6	1247.9	98.8
7	1262.4	100.0
8	1276.7	101.1
9	1206.2	95.5
10	1278.8	101.3
11	1248.5	98.9
12	1248.7	98.9
13	1212.7	96.0
14	1262.8	100.0
15	1192.9	94.4
16	1249.2	98.9
17	1269.1	100.5
18	1258.5	99.6
19	1222.8	96.8
20	1213.1	96.0
21	1280.5	101.4
22	1275.7	101.0
23	1259	99.7
24	1299.5	102.9
Mean	1237.8	98.0
SD	38.9	3.1
%RSD	3.1	3.1

A.5.4.2 System repeatability

A 100% standard solution of the analytes, as described in Section A.3.1.1, was prepared and injected six consecutive times in order to test the repeatability of the method. Peak areas and retention times were compared as given in Tables A.27 to A.31. Peak area and retention times should have a %RSD of 2% or less (Du Preez, 2008:6).

Table A.27: Repeatability of 5-fluorouracil

Injection number	Peak area	Retention time (min)
1	2083.5	4.6
2	2125.7	4.3
3	2124.2	4.6
4	2127.7	4.6
5	2129.7	4.6
6	2129.4	4.6
Mean	2120.0	4.6
SD	16.5	0.1
%RSD	0.8	3.0

The %RSD of 5-fluorouracil was within the accepted range for peak area (0.8%), but the retention time was not, with a %RSD of 3%. This tendency of 5-fluorouracil, to have a large range of retention times, was seen throughout this study, but it did not have a negative effect on the peak areas obtained during the analysis. This phenomenon may be due to the hydrophilic nature of 5-fluorouracil, which made it necessary to use a Fusion column with polar embedded groups. This was still acceptable, as the peak areas were reproducible and the retention times would not have a negative effect on the validity of the method.

Table A.28: Repeatability of methyl paraben

Injection number	Peak area	Retention time (min)
1	1180.3	10.0
2	1208.3	10.0
3	1206.2	10.0
4	1205.7	10.0
5	1205.8	10.0
6	1205.1	10.0
Mean	1201.9	10.0
SD	9.7	0.0
%RSD	0.8	0.0

The %RSD of methyl paraben was within the accepted range with a value of 0.8 for peak area, and with a value of 0.0 for retention time, this was very good as there were no relative standard deviation.

Table A.29: Repeatability of propyl paraben

Injection number	Peak area	Retention time (min)
1	202.0	11.1
2	208.1	11.1
3	206.5	11.1
4	206.0	11.1
5	207.3	11.1
6	208.6	11.1
Mean	206.4	11.1
SD	2.2	0.0
%RSD	1.0	0.0

The %RSD of propyl paraben was within the accepted range with a value of 1.0 for peak area, and with a value of 0.0 for retention time, this was very good as there was no relative standard deviation.

Table A.30: Repeatability of BHT

Injection number	Peak area	Retention time (min)
1	720.6	12.7
2	737.2	12.7
3	732.8	12.7
4	734.6	12.7
5	733.5	12.7
6	735.6	12.7
Mean	732.4	12.7
SD	5.5	0.0
%RSD	0.7	0.0

The %RSD of BHT was within the accepted range with a value of 0.7 for peak area, and with a value of 0.0 for retention time, this was very good as there was no relative standard deviation.

Table A.31: Repeatability of dl- α -tocopherol

Injection number	Peak area	Retention time (min)
1	281.1	17.0
2	277.9	17.0
3	273.2	16.9
4	270.0	17.0
5	267.9	17.0
6	265.7	17.0
Mean	272.6	17.0
SD	5.4	0.0
%RSD	2.0	0.1

The %RSD of dl- α -tocopherol was within the accepted range with a value of 2.0 for peak area, and with a value of 0.0 for retention time, this was very good as there was no relative standard deviation.

A.5.5 Specificity

Specificity of an analytical method is conducted to determine if the method is specific for a particular analyte (ICH, 2005:6).

For the purpose of determining the specificity, the 100% standard solution was used as described in Section A.3.1.1. 1 ml of this solution was placed in 4 test tubes and 1 ml sodium hydroxide (NaOH), water (H₂O), hydrochloric acid (HCl) and 10% hydrogen peroxide (H₂O₂) were added to the 4 test tubes, respectfully. The solutions were left overnight and injected the next day. Only 5-FU was stable in two of the solutions, NaOH and H₂O, but degraded in the other two solutions.

A.6 CONCLUSION

The HPLC method was reliable and suitable for its intended use of determining the concentration of the analytes in the samples. Although some of the analytes failed one or more of the validation tests, this method was still used as it was most suitable to analyse the active ingredient, 5-fluorouracil. As there is no stringent criteria for these analytes (as with an active ingredient) to comply with for a method validation, they need not comply with the exact criteria as set for the active ingredient. Their function is to prevent the process of oxidation and while fulfilling this purpose these analytes do break down. For the purpose of monitoring the breakdown of the formulation, this method was still satisfactory. This method was used for the purpose of the assay during stability testing, which will be discussed in Appendix C.

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APPENDIX B

FORMULATION OF COSMECEUTICAL SEMISOLIDS CONTAINING 5-FLUOROURACIL

B.1 INTRODUCTION

In general, the term “cosmetic” is defined as any object with the aim of application, either by rubbing or spraying to the skin for the purpose of cleaning, alternating, decorating and endorsing beauty of the human body. A cosmetic may also preserve the wellbeing of the hair and skin, provided that the physiological activities of the product on the human body are mild. Thus, cosmetics are used to support personal hygiene and good appearance. Whereas, pharmaceutical products (drugs) are used to cure or prevent a disease and they affect the constitution and functions of the human body (Mitsui, 1997:3-4). It can, therefore, be said that all cosmetics should be safe to use, have a high-quality of stability, and good usability and consistency; bearing in mind that everything should be supported by thorough research and awareness of the physiological roles of the human skin (Mitsui, 1997:319).

A “cosmeceutical” is defined as a cosmetic article which contains biologically active components and has drug-like or therapeutic benefits (MedicineNet, 2003).

The active ingredient 5-fluorouracil has been widely formulated in creams and ointments. Formulations of cosmeceutical semisolids containing 5-fluorouracil will be discussed in this chapter. The aim was to formulate pharmaceutically stable products and to test these products on the skin by means of skin diffusion studies. Stability of each of the prepared semisolid formulations will be discussed in Appendix C and the diffusion studies will be discussed in Appendix D.

B.2 SEMISOLID FORMULATIONS

According to Flynn (2002:213-214) all semisolids have a very distinctive rheological character. Semisolid systems maintain their original shape until an exterior force is acted upon them, whereas, unlike solids, they are easily deformed. In general, their rheological characteristics make it possible for them to be spread over the skin to form films that cling persistently. Thus, semisolids are very unique topical applications due to the fact that wherever they are applied to the skin, they stick to the skin’s surface until rubbed or washed off.

B.2.1 Pharmaceutically used semisolid systems

The following semisolid systems are used pharmaceutically:

- ❖ ointments;
- ❖ solid variants of ointments, such as water-in-oil (w/o) emulsions;
- ❖ pastes;
- ❖ oil-in-water (o/w) creams with solid internal phases;
- ❖ o/w creams with liquid internal phases;
- ❖ gels and
- ❖ rigid foams (Flynn, 2002:214).

All of the above-mentioned systems have extremely different underlying structures, but they have one property in common, which is, that the structures of these systems are effortlessly transformed, reorganised and broken down (Flynn, 2002:214).

When deciding on the type of formulation to use; for instance a cream, gel, lotion (emulsion) or an ointment, it depends on a number of factors, such as:

- ❖ the characteristics of the skin to which the product is being applied,
- ❖ the quantity of the active ingredient desired to penetrate the skin (Allen, 1998:188).

According to Allen (1998:188), creams are often used to apply to moist lesions of the skin, due to their drying effect, whereas ointments are commonly applied on dry and crusty lesions of the skin because of their rehydrating effect on the skin, and are formulated as topical, vaginal and rectal preparations. Lotions, on the other hand, have a lubricating effect and are normally applied to areas that are intertriginous – areas where skin draws closer together (between the thighs and fingers, as well as under the arms). Except for topical administration, gels have a wide variety of administration routes, such as oral, vaginal, intra-nasal, as well as rectal (Allen, 1998:203).

Semisolid formulations are seen as the desired vehicles when it comes to dermatological treatment due to their ability to deliver drugs over extensive periods of time and because they remain *in situ* (Walters & Brain, 2002:322).

B.2.1.1 Ointments

Ointments fall under the category of semisolid preparations and are formulated to be applied externally to mucous membranes or the surface of the skin. This type of formulation melts or becomes softer when exposed to body temperature and should be able to spread easily (Allen, 1998:187). Ointments may contain suspended or dissolved drugs and have a very specific crystalline matrix structure with a high molecular weight due to hydrocarbons. At room temperature, this structure precipitates to a large extent, forming the stiff appearance of an ointment. These preparations can be manufactured by adding high melting waxes to mineral oils and exposing the mixture to a high temperature. When cooled down the preparation hardens to form the solid matrix (Flynn, 2002:214).

B.2.1.2 Creams

Creams are thick liquids or bulky soft solids and are also intended to be applied externally. They are made up of drugs that are suspended or dissolved in a cream basis which is water soluble or that has evaporated. They can be classified as emulsions which can either be w/o- or o/w-type (Allen, 1998:187). Creams have their appearance due to the result of light reflection commencing from their emulsified phases, which distinguish them from translucent ointments. Creams are made by adding heated waxy components to an aqueous phase, forming an emulsion. The semisolid character of creams only forms as the liquid emulsion droplets cool down and solidify after being in contact with a high temperature (Flynn, 2002:215).

B.2.1.3 Types of gels

B.2.1.3.1 Regular gels

Gels are semisolid preparations which consist of an entrapped liquid phase surrounded by a three-dimensional, interconnected polymeric medium of a natural or artificial gum. This polymer matrix has a high degree of chemical or physical cross-linking. Only 0.5 – 2.0% of the gelling agent is needed for this system to set. Gel products are usually formulated for areas of the body where ointments and creams are too oily or greasy to use, for example the scalp, and thus bring about increased patient compliance (Flynn, 2002:215-216). Gels that contain inorganic materials consist of two phases – the first phase being the small, isolated particles that are dispersed throughout the second, which is an aqueous dispersion medium (Mahalingam *et al.*, 2008:288). In terms of clarity, there are two opposite appearances that gel systems can have. The first one is a completely clear gel (like water) and the other a turbid gel. The second type is formed due to incomplete molecular dispersion of the ingredients or the formation of aggregates that disperse light (Allen, 1998:201).

B.2.1.3.2 Emulgels

Emulgels are in fact emulsions that can either be of the w/o- or the o/w-emulsion sort. These types of formulations are prepared by mixing the ingredients of the emulsion in the company of a gelling agent. They have good patient acceptability due to the advantages of both emulsion as well as those of regular gels. These advantages include:

Emulgels:

- ❖ Elegant appearance
- ❖ Easy to wash off the skin
- ❖ High penetration ability

Regular gels:

- ❖ They are non-greasy
- ❖ Easy to spread on the skin
- ❖ They do not stain
- ❖ Easily removed from the skin
- ❖ Compatible with a wide range of excipients
- ❖ Thixotropic, emollient and water-soluble (Mohamed, 2004:1).

B.2.1.4 Lotions

Lotions are fluid emulsions which contain more aqueous components than oily ingredients, thus, playing an important role in retaining homeostasis of the skin as it supplies sufficient moisture and elasticity to the skin. Lotions are well tolerated on normal to oily types of skin and give a very light feeling due to the fact that they are not greasy and spread easily over the skin (Mitsui, 1997:335).

B.3 FORMULATION

Flynn (2002:217) states that “the formulation of topical dosage forms tends to be something of an art perfected through experience”. His motivation for this statement was that these products consist of a large quantity of ingredients and each one has a diverse range of physical properties.

When selecting the type of dermatological formulation for a certain drug, the decision is generally subjective to the character of the skin condition and the opinion of the medical practitioner (Walters & Brain, 2002:321).

B.3.1 Pre-formulation

Pre-formulation is the stage in the formulation process that includes studies that have to be conceded prior to the development of formulation. During the pre-formulation stage the main objective is to develop dosage forms that are safe, stable as well as effective. This is achieved through major consideration of the drug to be used and its physiochemical properties (Walters & Brain, 2002:321).

For the purpose of pre-formulation in this study, a comprehensive literature study was conducted that included information of skin cancer, the use of 5-fluorouracil, the important physiochemical properties thereof, as well as transdermal drug delivery.

B.3.2 General method for the formulation of creams

It is a general procedure that all the ingredients of the water phase are added together and heated to reach a temperature of approximately 70 °C. The oil phase is made up by adding the liquid oils, semisolid oils, solid oils (waxes), antioxidants and preservatives together and stirring the mixture while heating it to approximately 70 °C. If the formula contains a perfume, it is added to the oil phase prior to the emulsification process. When both phases reach 70 °C, the oil phase is slowly added to the water phase – this is referred to as the initial emulsification. The combination of the two phases should then be vigorously stirred with emulsification apparatus in order to form an emulsion with uniform particles. This procedure is followed by removing air from the emulsion, filtration and cooling, after which the cream is stored in appropriate containers (Mitsui, 1997:343-344).

B.3.3 General method for the formulation of gels

Due to the fact that gels have high viscosities, it is essential to keep this in mind when choosing the equipment to prepare them. The mixing equipment must be able to attain homogeneous mixing as well as remove air bubbles that might appear in the mixture. It should furthermore be suitable for the correct procedures in the transport, filter and cooling processes when it comes to the manufacturing of high viscosity preparations. Seeing that clearness is an important factor in preparing gels, specific attention should be paid to the uniformity and dissolution of the raw ingredients (Mitsui, 1997:353).

The method used in this study for the formulation of gel preparations, only differed in terms of the addition of a gelling agent to the water phase. This was done by means of high speed stirring with a homogeniser before adding the oil phase to the water phase.

B.3.4 General method for the formulation of lotions

When manufacturing a lotion there are many similarities to the manufacturing process of a cream. However, with milky lotions it is of high importance that the conditions of manufacture are properly selected due to physical properties and the viscosity which may be influenced by the manufacturing procedures, in terms of the following:

- ❖ The emulsification process – temperature and the order of adding together the materials
- ❖ The emulsification equipment
- ❖ Stirring
- ❖ Cooling procedure (Mitsui, 1997:338).

During the manufacturing procedure of an o/w-emulsion type lotion, the dispersion phase (oil) is added to the dispersion medium (water) and the particle size of the formed emulsion is made homogeneous by using powerful emulsification apparatus such as a homogeniser. This procedure is followed by removing air from the emulsion, filtration and cooling (Mitsui, 1997:339).

B.4 FORMULATION OF SEMISOLIDS CONTAINING 5-FLUOROURACIL

During this study the main purpose was to formulate different cosmeceutical semisolid formulations with the active ingredient, 5-fluorouracil. It was decided to formulate a cream, an emulgel, as well as a lotion. Each of these formulations was formulated by adding a drug delivery system, called Pheroid™ technology. Thus, in total, there were six formulations. These formulations included 0.5 % of the active ingredient, 5-fluorouracil. The decision to use 0.5% 5-fluorouracil was based on a previous study conducted by Van Dyk (2008:67) where the influence of Pheroid™ technology on the transdermal delivery of 5-fluorouracil was examined. Both a 0.5% and a 1% formulation were formulated in a water solution, a phosphate buffer solution (PBS) as well as in a water-based Pheroid™ solution and PBS-based Pheroid™ solution. The formulations were applied to the epidermis and allowed to permeate for a 12 h period. All of the Pheroid™ containing formulations depicted significantly higher permeation than those without the Pheroid™. However, the 0.5% water-based Pheroid™ proved to be the superior formulation (Van Dyk, 2008:66).

The formulations in this study were manufactured in large quantities and stored at three different temperatures (25, 30 and 40 °C) over a period of six months. Stability tests were conducted on each of the formulations at month 0, 1, 2, 3 and 6, which will be discussed in further detail in Appendix C.

Skin diffusion tests were conducted with all the above-mentioned formulations, with the addition of 5-fluorouracil water solutions (0.5 and 5%) as well as 5-fluorouracil containing Pheroid™ solutions (0.5 and 5%). The reason for the preparation of 5.0% solutions was to compare the results to a commercially available 5-fluorouracil ointment (5%); which will be discussed in detail in Appendix D.

B.4.1 Formulation process of semisolids containing 5-fluorouracil

As previously discussed, semisolid formulations consist of an oil phase as well as a water phase. The oil-phases of the formulations used in this study contained ingredients such as waxes and oils (cetyl alcohol, span-60, tween-80, liquid paraffin); whereas the aqueous phases contained water and water-soluble ingredients such as anti-oxidants and preservatives (BHT, propyl paraben and methyl paraben).

The early-formulation process started off by preparing different formulations on a trial-and-error basis in order to find the most appropriate and cosmetically acceptable formulations. This process consisted of constituting a few small quantities of each of the three types of formulations, including all the ingredients of the formulations, except for the active ingredient. This was done in order to examine the homogeneity, the overall appearance, thickness as well as the short-term stability of each of the three formulations. These smaller quantities had to be satisfactory in each of the above-mentioned fields before making larger quantities for the purpose of stability testing. The final formulations of the large batches for each of the cream, emulgel and lotion are shown in Tables B.2 - B.4.

The quantity of the large batches was calculated to be sufficient for all the stability tests that were performed during the 6-month period. A total of 2000 g of each of the formulations were prepared. Plastic storage containers were purchased from Brunel Manufacturers; they were all rinsed with 70% ethanol and dried in a flow cabinet prior to their use.

B.4.2 Ingredients used in the manufacturing of all the formulations

Table B.1: Batch numbers, suppliers and functions of the ingredients used in the formulations

Ingredient	Batch number	Supplier	Function
Cetyl alcohol	S5428004 951	Merck Chemicals	Thickening agent
Tween-80	1032991	Merck Chemicals	Surface active agent
Span-60	00259	Brunel	Emulsifier
Liquid paraffin	1034040	Merck Chemicals	Oil-phase (mineral oil)
Propyl paraben	GBGA 032949	Merck Chemicals	Preservative
Methyl paraben	GBG 000137	Merck Chemicals	Preservative
BHT	04416 KD-076	SAFC	Anti-oxidant
5-Fluorouracil	IF-FL-090612-DB276	DB Fine Chemicals	Active ingredient
Xanthan gum	4450902790	Warren Chem Specialities	Thickening/gelling agent
dl- α -Tocopherol	UT 090 I 0010	Chempure	Stabilising agent and anti-oxidant

B.4.2.1 Cetyl alcohol

Cetyl alcohol is a viscosity enhancer and is commercially available as white granules or flakes. Even though it is a water-insoluble substance, it has superior emulsifying and water-absorptive characteristics, which make it appropriate in the preparation of creams and emollient ointments. Concentrations in topical preparations vary from 2 – 10% which conveys thickening, emulsifying and water-absorptive properties (Mahalingam *et al.*, 2008:274).

B.4.2.2 Tween-80

The chemical identification name of this material is polyethylene glycol sorbitan monooleate, which is an odourless, viscous yellow liquid (MSDS, 200?). This is a non-ionic surface active ingredient which falls under the polyoxyethylene type. It has excellent solubilising and emulsifying capabilities, which makes its use in lotions and creams very popular in terms of the emulsifying ability; and in perfumes because of the solubilising effects (Mitsui, 1997:133).

B.4.2.3 Span-60

Span-60 is a sorbitan monostearate ester and is lipophilic. It is generally used in pharmaceutical products and cosmetics as a non-ionic surfactant with emulsifying properties. Span-60 is a solid wax with a cream colour (Kibbe, 2000:512).

B.4.2.4 Liquid paraffin

Liquid paraffin is an oily material which consists of a combination of saturated hydrocarbons that can have up to 15 – 30 carbon atoms, but is chemically inactive. This oil is a liquid at room temperature and is unscented and colourless. It is often used in the manufacturing of skin care products, for example milky lotions and creams; and can form an emulsion effortlessly. In these products liquid paraffin controls the loss of moisture and improves the feeling on the skin upon use (Mitsui, 1997:124).

B.4.2.5 Propyl paraben

Propyl paraben is used as a preservative in pharmaceutical products and cosmetics due to the antimicrobial activity. The concentrations used in topical preparations vary between 0.01 – 0.60%. It is presented as a white crystalline powder that is tasteless and odourless (Kibbe, 2000:450).

B.4.2.6 Methyl paraben

Similar to propyl paraben, methyl paraben is also used as a preservative in pharmaceutical products and cosmetics due to the antimicrobial activity. The concentrations used in topical preparations differ from that of propyl paraben, with a range of 0.02 – 0.30%. Methyl paraben can be presented as a white crystalline powder or colourless crystals. It has no odour and may cause a minor burning taste (Kibbe, 2000:340).

B.4.2.7 Butylated hydroxytoluene (BHT)

BHT is an anti-oxidant that is widely used in cosmetic preparations in concentrations ranging from 0.0002 – 0.5000%. The anti-oxidant activity of BHT causes it to form steady free radicals which disrupt the process of oxidation and in return improves the stability of products. It is a crystalline solid substance with a white to yellowish-white colour (Lanigan & Yamarik, 2002:19-20).

B.4.2.8 Xanthan gum

This is a naturally refined gum and acts as a thickening agent. The use of xanthan gum in pharmaceutical topical preparations (gels) is popular due to its small reliance on temperature and the fact that it is stable over a large pH-range (Mitsui, 1997:139-140).

B.4.2.9 dl- α -Tocopherol (Vitamin E)

Vitamin E forms part of the Pheroid™ vesicles in the Pheroid™ formulations and acts as a stabilising agent and an anti-oxidant. This is a lipid-soluble vitamin, which can thus be widely distributed in the cell membranes of the body. Its major function is to prevent oxidation in the membrane lipids (Grobler *et al.*, 2008:293).

B.5 FORMULATION OF CREAM AND PHEROID™ CREAM

Table B.2: Formula of the cream preparation

#	Ingredient	m/m (%)	Quantity (per 2000 g)	Purpose for use
1	Cetyl alcohol	10.00	200.0	Thickening agent
2	Tween-80	1.50	30.0	Surface active agent
3	Span-60	1.50	30.0	Emulsifier
4	Liquid paraffin	12.00	240.0	Oil phase
5	Propyl paraben	0.04	0.8	Preservative
6	BHT	0.20	4.0	Anti-oxidant
7	Methyl paraben	0.20	4.0	Preservative
8	5-fluorouracil	0.50	10.0	Active ingredient
9	dH ₂ O	74.06	1481.2	Solvent

B.5.1 Method for preparation of 5-fluorouracil cream

- ❖ Weigh and heat ingredients 1 – 6 to approximately 80 °C (oil phase).
- ❖ Weigh and heat the water.
- ❖ Add the methyl paraben and stir the solution while adding 5-fluorouracil until it is fully dissolved in the water.
- ❖ Heat to approximately 80 °C (water phase).
- ❖ When both solutions have reached 80 °C, add the oil phase to the water phase while homogenising the mixture at 13 500 rpm until 40 °C is reached.
- ❖ Cool the mixture down to approximately 25 °C while stirring at 200 rpm.

B.5.2 Method for preparation of 5-fluorouracil Pheroid™ cream

Exactly the same ingredients were used to prepare the Pheroid™ cream, except for the addition of dl- α -tocopherol and two other Pheroid™ ingredients, which may not be mentioned as they are proprietary, and for the sake of protecting the Intellectual Property (IP), these ingredients will

not be mentioned by name, as Pheroid™ is a patented technology, with patent number SA93/3895 (Pitney International NV Patent Portfolio, 1994). These three ingredients were added to the oil-phase in the above-mentioned procedure.

B.5.3 Findings

During the early-formulation process, 12% of cetyl alcohol was used as thickener for the cream. This resulted in a very thick and sticky cream preparation. It was decided to use less cetyl alcohol. After a trial-and-error period, 10% of cetyl alcohol resulted in a homogenous white cream, which was easy to apply and depicted a good overall appearance.

B.6 FORMULATION OF EMULGEL AND PHEROID™ EMULGEL

Table B.3: Formula of the emulgel preparation

#	Ingredient	m/m (%)	Quantity (per 2000 g)	Purpose for use
1	Liquid paraffin	20.00	400.0	Oil phase
2	Tween-80	4.50	90.0	Surface active agent
3	Span-60	0.50	10.0	Emulsifier
4	Propyl paraben	0.08	1.6	Preservative
5	BHT	0.20	4.0	Anti-oxidant
6	Methyl paraben	0.20	4.0	Preservative
7	Xanthan gum	1.50	30.0	Thickening/gelling agent
8	5-fluorouracil	0.50	10.0	Active ingredient
9	dH ₂ O	72.32	1446.4	Solvent

B.6.1 Method for preparation of 5-fluorouracil emulgel

- ❖ Weigh and heat ingredients 1 – 6 to approximately 80 °C (oil phase).
- ❖ Weigh and heat the water.
- ❖ Add the methyl paraben and stir the solution while adding 5-fluorouracil until it is fully dissolved in the water.
- ❖ Heat to approximately 40 °C (water phase).
- ❖ Weigh the xanthan gum and add little by little to the water phase while homogenising the mixture at 777 rpm.
- ❖ Heat to approximately 80 °C.

- ❖ When both solutions have reached approximately 80 °C, add the oil phase to the water phase while homogenising the mixture at 13 500 rpm until 40 °C is reached.
- ❖ Cool the mixture down to approximately 25 °C while stirring at 200 rpm.

B.6.2 Method for preparation of 5-fluorouracil Pheroid™ emulgel

Exactly the same ingredients were used to prepare the Pheroid™ emulgel, except for the addition of dl- α -tocopherol and two other Pheroid™ ingredients, which may not be mentioned as they are proprietary, and for the sake of protecting the IP, these ingredients will not be mentioned by name, as Pheroid™ is a patented technology, with patent number SA93/3895 (Pitney International NV Patent Portfolio, 1994). These three ingredients were added to the oil-phase in the above-mentioned procedure.

B.6.3 Findings

The emulgel depicted a very good appearance in terms of homogeneity. It was white in colour and rubbed easily into the skin. No alterations were necessary in terms of the quantities of the ingredients.

B.7 FORMULATION OF LOTION AND PHEROID™ LOTION

Table B.4: Formula of the lotion preparation

#	Ingredient	m/m (%)	Quantity (per 2000 g)	Purpose for use
1	Cetyl alcohol	5.0	100.0	Thickening agent
2	Tween-80	7.0	150.0	Surface active agent
3	Span-60	2.0	40.0	Emulsifier
4	Liquid paraffin	20.0	400.0	Oil phase
5	Propyl paraben	0.1	2.0	Preservative
6	BHT	0.2	4.0	Anti-oxidant
7	Methyl paraben	0.5	10.0	Preservative
8	5-fluorouracil	0.5	10.0	Active ingredient
9	dH ₂ O	64.7	1294.0	Solvent

B.7.1 Method for preparation of 5-fluorouracil lotion

- ❖ Weigh and heat ingredients 1 – 6 to approximately 80 °C (oil phase).
- ❖ Weigh and heat the water.

- ❖ Add the methyl paraben and stir the solution while adding 5-fluorouracil until it is fully dissolved in the water.
- ❖ Heat to approximately 80 °C (water phase).
- ❖ When both solutions have reached approximately 80 °C, add the oil phase to the water phase while homogenising the mixture at 13 500 rpm until approximately 40 °C is reached.
- ❖ Cool the mixture down to approximately 25 °C while stirring at 200 rpm.

B.7.2 Method for preparation of 5-fluorouracil Pheroid™ lotion

Exactly the same ingredients were used to prepare the Pheroid™ lotion, except for the addition of dl- α -tocopherol and two other Pheroid™ ingredients, which may not be mentioned as they are proprietary, and for the sake of protecting the IP, these ingredients will not be mentioned by name, as Pheroid™ is a patented technology, with patent number SA93/3895 (Pitney International NV Patent Portfolio, 1994). These three ingredients were added to the oil phase in the above-mentioned procedure.

B.7.3 Findings

At first, the lotion was too thick and needed to be liquefied. The quantity of cetyl alcohol was reduced from 7 to 5% and this provided a much better composition and appearance to the lotion. The ability of the lotion to rub into the skin as well as the fluidity of the lotion was satisfactory.

B.8 CONCLUSION

Six topical preparations of 0.5% (m/m) 5-fluorouracil were formulated, which consisted of a cream, a Pheroid™ cream, an emulgel, a Pheroid™ emulgel, a lotion and a Pheroid™ lotion.

Each of the six formulations was successfully manufactured and placed in adequate numbers of containers ready for stability testing. The containers were stored in controlled climate rooms at three different temperatures; 25, 30 and 40 °C, with respective relative humidities (RH) of 60, 60 and 75%. The stability period was six months and a large range of stability tests were conducted. The tests were done immediately after the manufacturing of the formulations, followed by 1, 2, 3 and 6 months after manufacturing. The stability outcomes of the formulations will be discussed in Appendix C.

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APPENDIX C

STABILITY OF COSMESEUTICAL SEMISOLID FORMULATIONS

C.1 INTRODUCTION

The stability of a pharmaceutical product is defined as the potential of a particular formulation in a specific container or closure system to remain within its physical, chemical, therapeutic, toxicological, microbiological, informational and protective specifications. It is the ability of the product to contain the same properties and characteristics that it possessed at the time of manufacture, within specified limits and throughout the storage period and use of the product. Stability testing of pharmaceutical drugs and products is a routine procedure that should be performed at different stages of the development of a product. The reason to perform stability tests is to guarantee that these products preserve their condition for use until the end of their expiry dates (Kommanaboyina & Rhodes, 1999:858).

The most important function of stability testing is the provision of evidence on how the quality of a drug substance or product varies over a period of time. These products should be exposed to a range of environmental factors, such as temperature, humidity and light. Secondly, is to create a retest period for the drug substance or a shelf life for the drug product as well as the recommendation of the correct storage conditions (Lin & Chen, 2003:338).

When designing a stability study, there are basic considerations to be made with regards to the design. The stability program should always start with a well planned protocol that should be strictly followed. The following aspects should be clearly specified in the protocol:

- ❖ The study objective
- ❖ The design of the study
- ❖ Scientific details of the drug substance and excipients
- ❖ Batch and packaging information
- ❖ Specifications
- ❖ Time points
- ❖ Storage conditions
- ❖ Sampling plan
- ❖ Planned statistical analysis (Lin & Chen, 2003:344).

The chemical and physical integrity of semisolid topical preparations are of high importance and all factors concerning their stability should initially be satisfactory and preferably remain so over a specified period of time (Flynn, 2002:229).

There are a large number of factors that can influence the stability of a drug and dosage form. These factors include pH, temperature, light, air (oxygen), solvent, carbon dioxide, particle size, moisture and/or humidity (Allen, 1998:32). Amongst all these factors that may cause drug degradation, temperature is said to be the most important one as it cannot be controlled by package selection. An increase in temperature is one of the most common methods to achieve accelerated stability testing of pharmaceutical products, as this procedure calculates the product stability by exposing it to a condition that will accelerate the degradation process (Kommanaboyina & Rhodes, 1999:859-860).

C.1.1 Storage conditions

The storage conditions for the manufactured products during this study consisted of three climate rooms of different temperatures, each maintained a specific relative humidity (RH):

- ❖ 25 °C with a RH of 60%;
- ❖ 30 °C with a RH of 60% and
- ❖ 40 °C with a RH of 75%.

C.1.2 Storage period and frequency of stability testing

The manufactured formulations were all stored under the above-mentioned conditions for a period of 6 months, where a 28-day cycle was referred to as 1 month. The stability tests were performed on the day of manufacture (month 0), and then after 1, 2, 3 and 6 months.

C.1.3 Types of stability tests

The types of stability tests that were conducted during this study were as follows:

- ❖ Determination of concentration of the analytes (assay)
- ❖ Determination of zeta-potential and droplet size
- ❖ pH measurement
- ❖ Viscosity
- ❖ Mass loss determination
- ❖ Physical appearance
- ❖ Particle size distribution

C.1.4 Quantity and packing of the manufactured formulations

The quantity of each of the formulations was calculated to be sufficient for each of the above-mentioned stability tests to be carried out on the specified points in time. The total of each of the six formulations that were manufactured at the commencement of the stability period was 2000 g. The formulations were stored in plastic containers that were obtained from Brunel Manufacturers and cleaned with a 70% ethanol/water solution and dried in a laminar flow cabinet. The glass jars that were used for the viscosity tests were cleaned in the same manner as the plastic containers, except for drying them in an oven at 180 °C for 30 min.

C.2 STABILITY TESTING METHODS

C.2.1 Assay by means of HPLC analysis

A stability-indicating assay is an analytical procedure that can detect the changes within the pertinent properties of a drug substance or product over a period of time. This analytical procedure can accurately determine the percentage active ingredient within a sample. The use of an assay analytical procedure should be an indicator of stability for the purpose of stability studies (FDA, 2000:4).

The method used for the assay of the manufactured formulations was validated and the chromatographic conditions were as described in Appendix A.

C.2.1.1 Preparation of standard solutions

The standard solution for each formulation was prepared by weighing the analytes in the quantities as there would be present in 1 g of the specific formulation; and adding sufficient methanol to obtain a 50 ml solution. The reason for the standard solution was to act as a control solution in order to compare the samples with. Three standard solutions were freshly prepared at the beginning of each assay, one for the cream and Pheroid™ cream, one for the emulgel & Pheroid™ emulgel, and lastly, one for the lotion & Pheroid™ lotion. Although dl- α -tocopherol was only present in the Pheroid™ formulations, it was included in the collective standard solution for the sake of determining the concentrations in the Pheroid™ formulations. Table C.1 depicts the quantities of each analyte used for preparing the standard solutions.

Table C.1: Analytes for preparing standard solutions

Analytes	Cream & Pheroid™ cream (mg)	Emulgel & Pheroid™ emulgel (mg)	Lotion & Pheroid™ lotion (mg)
5-Fluorouracil	5.0	5.0	5.0
Methyl paraben	2.0	4.0	5.0
Propyl paraben	0.4	0.8	1.0
BHT	2.0	2.0	2.0
dl- α -Tocopherol	2.0	2.0	2.0

C.2.1.2 Preparation of sample solutions

The samples of each formulation for the purpose of the assay were prepared by weighing 1 g of each formulation in a 50 ml volumetric flask with a syringe (attached to a rubber tube) and dissolving it in a few drops of water before filling the flask to volume with methanol. This was done with each of the six formulations that were stored at the three different temperatures.

C.2.1.3 Results for assay of the formulations containing 5-fluorouracil

The initial assay (month 0) was only conducted with the six formulations manufactured at room temperature (25 °C), as they have not been stored at the other two temperatures yet. From month 1 onwards, stability testing could commence for all three temperatures at their respective relative humidity.

During accelerated stability testing, it is recommended that a minimum of three time points should be completed during a study of 6 months. These points must include the initial, as well as final time points (ICH, 2003:7). During stability testing in this study, complications occurred with the specific HPLC-column used. These complications resulted in inadequate data during the second month of testing. Therefore, data could only be collected for months 0, 1, 3 and 6.

According to the ICH (2003:13) a noteworthy change for a drug product can be seen as a 5% change in assay from the initial value obtained.

Tables C.2 to C.6 depict the percentage recoveries of each analyte at months 0, 1, 3 and 6, relative to a standard solution.

C.2.1.3.1 Cream

Table C.2: Percentage recovery of each analyte in cream at month 0, 1, 3 and 6, relative to a standard solution

	Month 0	Month 1	Month 3	Month 6
25 °C/60% RH				
5-Fluorouracil	112.5%	111.2%	111.2%	116.0%
Methyl paraben	115.1%	114.8%	112.9%	60.5%
Propyl paraben	109.3%	107.1%	105.0%	78.7%
BHT	110.4%	107.3%	106.1%	96.7%
30 °C/60% RH				
5-Fluorouracil	X*	111.5%	111.8%	113.6%
Methyl paraben	X*	114.0%	112.8%	62.6%
Propyl paraben	X*	103.8%	100.3%	78.2%
BHT	X*	108.5%	105.7%	89.1%
40 °C/75% RH				
5-Fluorouracil	X*	111.7%	110.4%	110.9%
Methyl paraben	X*	105.2%	102.3%	58.7%
Propyl paraben	X*	105.8%	101.5%	77.4%
BHT	X*	100.8%	97.3%	76.9%

*X = No data collected for these temperatures

The 5-fluorouracil concentration in the cream was extremely stable throughout the 6 month period when compared to the other 5 formulations, due to the fact that it stayed within the acceptable 5% limit from the initial percentage for the duration of 6 months at all three temperature controlled conditions. Within the first 3 months, the 5-fluorouracil concentration did not differ significantly, however from months 3 to 6 it showed an increase in concentration which could be due to an increase in vaporisation. Vaporisation would have resulted in loss of solvent or liquid within the formulation. In return, this caused the concentration of the product to increase (Allen, 1998:33).

Methyl paraben, propyl paraben and BHT stayed within the acceptable 5% limit from the initial value within the first 3 months. During the last 3 months, these 3 analytes showed a significant decrease that was due to decomposition of the cream. These analytes were clearly not as stable as 5-fluorouracil. The loss in solvent did not have an effect on them. The reason for the decrease in concentration of the excipients could also have been due to adsorption of the drug or excipients to the container (Allen, 1998:33), causing a lower concentration in the formulation over a period of time.

C.2.1.3.2 Pheroid™ cream

Table C.3: Percentage recovery of each analyte in Pheroid™ cream at month 0, 1, 3 and 6, relative to a standard solution

	Month 0	Month 1	Month 3	Month 6
25 °C/60% RH				
5-Fluorouracil	110.5%	107.2%	108.2%	114.2%
Methyl paraben	100.7%	105.3%	105.3%	55.2%
Propyl paraben	96.3%	96.4%	100.7%	74.3%
BHT	108.2%	105.5%	111.8%	80.4%
dl-α-Tocopherol	106.2%	96.0%	93.3%	80.2%
30 °C/60% RH				
5-Fluorouracil	X*	107.0%	107.4%	111.8%
Methyl paraben	X*	104.9%	105.3%	54.8%
Propyl paraben	X*	94.8%	99.3%	73.9%
BHT	X*	104.9%	106.1%	78.7%
dl-α-Tocopherol	X*	93.6%	85.3%	72.7%
40 °C/75% RH				
5-Fluorouracil	X*	106.3%	108.3%	112.0%
Methyl paraben	X*	106.9%	104.0%	52.6%
Propyl paraben	X*	99.8%	98.6%	69.6%
BHT	X*	100.3%	97.1%	74.4%
dl-α-Tocopherol	X*	91.8%	79.8%	60.6%

*X = No data collected for these temperatures

The 5-fluorouracil concentration in the Pheroid™ cream stayed within the acceptable 5% limit from the initial percentage throughout the 6 month stability period, at all three temperature controlled conditions. From months 3 to 6 there was an increase in concentration, which could have been due to an increase in vaporisation. Again, vaporisation would have resulted in loss of solvent or liquid within the formulation. In return this caused the concentration of the product to increase (Allen, 1998:33).

This aforementioned phenomenon could also be seen from months 1 to 3 for all the analytes in the Pheroid™ cream formulation at 25 °C/60% RH and 30 °C/60% RH, except for dl-α-tocopherol, which decreased. The analytes depicted the opposite reaction at these two temperatures from months 3 to 6 and at 40 °C/75% RH, where they degraded from months 1 to 6, which could be due to degrading of the Pheroid™ cream at a higher temperature.

Methyl paraben, propyl paraben and BHT also stayed within the acceptable 5% limit from the initial value within the first 3 months at all three temperature controlled conditions, except for BHT at 40 °C/75% RH for month 1 and 3, as well as methyl paraben at 40 °C/75% RH for month 1. dl- α -Tocopherol showed a significant decrease from the initial values at all three temperature controlled conditions, which was due to degradation of the Pheroid™ cream and the sensitivity of dl- α -tocopherol to heat and light. During the last 3 months methyl paraben, propyl paraben and BHT showed a significant decrease due to decomposition of the Pheroid™ cream, as these analytes were not as stable as 5-fluorouracil. The loss in solvent did not have an effect on them.

C.2.1.3.3 Emulgel

Table C.4: Percentage recovery of each analyte in emulgel at month 0, 1, 3 and 6, relative to a standard solution

	Month 0	Month 1	Month 3	Month 6
25 °C/60% RH				
5-Fluorouracil	107.1%	107.6%	101.5%	101.4%
Methyl paraben	104.6%	103.7%	99.9%	98.6%
Propyl paraben	103.2%	100.7%	99.3%	95.1%
BHT	106.6%	106.1%	91.8%	89.7%
30 °C/60% RH				
5-Fluorouracil	X*	106.2%	103.1%	100.5%
Methyl paraben	X*	106.4%	102.2%	101.8%
Propyl paraben	X*	100.5%	98.2%	96.5%
BHT	X*	105.7%	92.4%	90.2%
40 °C/75% RH				
5-Fluorouracil	X*	108.3%	106.0%	100.1%
Methyl paraben	X*	107.8%	105.5%	100.9%
Propyl paraben	X*	101.1%	100.2%	101.6%
BHT	X*	107.3%	81.0%	78.9%

*X = No data collected for these temperatures

The 5-fluorouracil concentration in the emulgel showed a considerable change (decrease) from months 1 to 3 at 25 °C/60% RH, but stayed within the acceptable 5% limit from the initial value at 30 °C/60% RH and 40 °C/75% RH. Although, at month 6 the recovery of 5-fluorouracil was not within the 5% limit for each of the three storage temperatures.

Methyl paraben and propyl paraben stayed within the acceptable 5% limit from the initial value at all three temperature controlled conditions, up to month 3, but the concentration BHT

decreased significantly from the initial values to month 3. When the initial concentrations were compared to month 6, methyl paraben, propyl paraben and BHT depicted a significant decrease in concentration, more than the acceptable limit of 5%, except for propyl paraben, which stayed within the 5% limit at 30 °C/60% RH and 40 °C/75% RH. The reason for the decrease in concentration of the excipients could have been due to adsorption of the drug or excipients to the container (Allen, 1998:33), causing a lower concentration in the formulation over a period of time. Degradation of the emulgel could also have contributed to the decrease in concentration. This phenomenon could be seen for all the analytes in the emulgel, as they showed a significant decrease from their initial concentrations to month 6.

C.2.1.3.4 Pheroid™ emulgel

Table C.5: Percentage recovery of each analyte in Pheroid™ emulgel at month 0, 1, 3 and 6, relative to a standard solution

	Month 0	Month 1	Month 3	Month 6
25 °C/60% RH				
5-Fluorouracil	107.0%	105.3%	104.0%	106.8%
Methyl paraben	105.3%	104.5%	101.8%	100.7%
Propyl paraben	106.9%	103.0%	99.1%	97.5%
BHT	111.2%	114.3%	96.2%	91.0%
dl-α-Tocopherol	105.2%	96.6%	92.7%	78.4%
30 °C/60% RH				
5-Fluorouracil	X*	105.5%	102.8%	100.2%
Methyl paraben	X*	108.8%	99.9%	97.8%
Propyl paraben	X*	102.8%	99.4%	96.3%
BHT	X*	109.5%	95.2%	94.7%
dl-α-Tocopherol	X*	93.3%	96.3%	62.8%
40 °C/75% RH				
5-Fluorouracil	X*	108.1%	106.5%	102.7%
Methyl paraben	X*	111.3%	103.8%	92.6%
Propyl paraben	X*	105.2%	102.8%	91.8%
BHT	X*	104.7%	83.5%	80.9%
dl-α-Tocopherol	X*	86.1%	39.5%	25.2%

*X = No data collected for these temperatures

The 5-fluorouracil concentration in the Pheroid™ emulgel stayed within the acceptable 5% range from the initial value throughout the 6 month stability period, at all three temperature controlled conditions. Although, at month 6 the recovery of 5-fluorouracil was not within the 5% limit at 30 °C/60% RH.

At higher temperatures, vaporisation increases which will result in loss of solvent or liquid within a formulation. This in return usually causes the concentration of a product to increase (Allen, 1998:33). The phenomenon could be seen for 5-fluorouracil (month 1 at 40 °C/75% RH), methyl paraben (month 1 at 30 °C/60% RH and 40 °C/75% RH) and BHT (month 1 at 25 °C/60% RH) in the Pheroid™ emulgel formulation.

The concentration of the excipients decreased properly due to adsorption of the drug or excipients to the container (Allen, 1998:33), which caused a lower concentration in the formulation over a period of time. Degradation of the formulation could have been another factor. This phenomenon could be seen with methyl paraben (month 6 at 30 °C/60% RH and 40 °C/75% RH), propyl paraben (month 3 and 6 at 25 °C/60% RH and 30 °C/60% RH, as well as month 6 at 40 °C/75% RH), and BHT (month 3 and 6 at all three temperature controlled conditions, as well as month 1 at 40 °C/75% RH). dl- α -Tocopherol did not stay within the acceptable 5% limit from the initial values at all three temperature controlled conditions throughout the stability period.

C.2.1.3.5 Lotion

Table C.6: Percentage recovery of each analyte in lotion at month 0, 1, 3 and 6, relative to a standard solution

	Month 0	Month 1	Month 3	Month 6
25 °C/60% RH				
5-Fluorouracil	102.8%	101.0%	106.1%	108.6%
Methyl paraben	103.5%	102.6%	104.8%	105.3%
Propyl paraben	99.9%	100.8%	101.8%	107.8%
BHT	109.4%	109.5%	83.9%	96.9%
30 °C/60% RH				
5-Fluorouracil	X*	103.4%	104.4%	108.1%
Methyl paraben	X*	102.0%	101.8%	102.7%
Propyl paraben	X*	108.5%	100.8%	101.3%
BHT	X*	108.2%	86.8%	96.5%
40 °C/75% RH				
5-Fluorouracil	X*	102.1%	103.6%	107.9%
Methyl paraben	X*	101.2%	99.9%	100.2%
Propyl paraben	X*	103.3%	100.3%	102.5%
BHT	X*	106.6%	69.8%	92.8%

*X = No data collected for these temperatures

The 5-fluorouracil concentration in the lotion stayed within the acceptable 5% range from the initial value at 25 °C/60% RH, 30 °C/60% RH and 40 °C/75% RH throughout months 0 to 3. 5-Fluorouracil depicted an increase in concentration from months 1 to 3 and months 3 to 6, which could be due to the loss of fluid within the lotion. However, at month 6 the recovery of 5-fluorouracil was higher than the 5% limit at 25 °C/60% RH and 30 °C/60% RH.

Methyl paraben and propyl paraben stayed within the acceptable 5% limit at all three temperature controlled conditions from months 0 to 6. Although, at month 6 the concentration propyl paraben increased and was higher than the 5% limit at 25 °C/60% RH. BHT did not stay within the acceptable 5% range at month 3 to 6 at all three temperature controlled conditions.

All the analytes of the lotion increased in concentration from months 3 to 6, which could be due to the loss of fluid within the lotion during this period.

C.2.1.3.6 Pheroid™ lotion

Table C.7: Percentage recovery of each analyte in Pheroid™ lotion at month 0, 1, 3 and 6, relative to a standard solution

	Month 0	Month 1	Month 3	Month 6
25 °C/60% RH				
5-Fluorouracil	105.6%	105.8%	108.3%	110.8%
Methyl paraben	104.7%	106.1%	103.3%	106.7%
Propyl paraben	106.4%	108.0%	102.2%	111.7%
BHT	110.1%	112.7%	84.1%	98.3%
dl-α-Tocopherol	103.4%	93.5%	82.1%	71.6%
30 °C/60% RH				
5-Fluorouracil	X*	103.9%	105.5%	114.3%
Methyl paraben	X*	105.0%	101.2%	104.5%
Propyl paraben	X*	106.9%	101.7%	101.3%
BHT	X*	111.3%	91.5%	87.3%
dl-α-Tocopherol	X*	89.3%	78.3%	68.6%
40 °C/75% RH				
5-Fluorouracil	X*	103.9%	106.0%	111.1%
Methyl paraben	X*	105.9%	103.9%	101.2%
Propyl paraben	X*	102.4%	97.7%	90.6%
BHT	X*	108.8%	86.5%	81.7%
dl-α-Tocopherol	X*	81.2%	62.3%	50.8%

*X = No data collected for these temperatures

The 5-fluorouracil concentration in the Pheroid™ lotion stayed within the acceptable 5% range from the initial value at 25 °C/60% RH, 30 °C/60% RH and 40 °C/75% RH throughout months 0 to 3 with an increase in concentration at all three temperature controlled conditions. This could have been due to the loss of fluid within the Pheroid™ lotion. The increase was higher than 5% from months 3 to 6 at 30 °C/60% RH and 40 °C/75% RH. This phenomenon could also be observed from a physical point of view, as the Pheroid™ lotion thickened (higher viscosity can be seen in Figure C.12) throughout the stability period when compared to its initial appearance.

Methyl paraben, propyl paraben and BHT showed a slight increase in concentration from their initial values to month 1 at 25 °C/60% RH and 30 °C/60% RH, which could have been due to an increased vaporisation. Vaporisation would result in loss of solvent or liquid within a formulation. In return, this would cause the concentrations of the products to increase (Allen, 1998:33). Only methyl paraben increased in concentration from month 0 to 1 at 40 °C/75% RH, whereas propyl paraben and BHT decreased at this higher temperature.

Throughout the 6 month period, methyl paraben and propyl paraben were the only two analytes that increased within the Pheroid™ lotion at 25 °C/60% RH, whereas the rest of the analytes decreased at all three temperature controlled conditions.

There was no constant change or phenomenon seen throughout the 6 month period with regards to the concentrations of these 6 formulations. All 6 formulations showed a unique degradation, which could not be related. When evaluating all 6 formulations with regard to the most stable analyte within each formulation, 5-fluorouracil was most stable within the cream. The reason for this was that it showed the lowest change in concentration within the cream. Methyl paraben, propyl paraben and BHT were the most stable analyte within the lotion and dl- α -tocopherol was most stable within the Pheroid™ cream.

C.2.2 Zeta-potential and droplet size with dispersion technology-1200 (DT-1200)

This instrument is a combination of an acoustic and electro acoustic spectrometer that was developed by Dispersion Technology Inc. The DT-1200 uses a pulse technique by means of two separate sensors that measure acoustic and electro acoustic signals (Dukhin & Goetz, 1998:50). The interaction of sound with the dispersed particles offers useful information in both these methods, but the set of parameters measured by each method differ from one another (Dukhin & Goetz, 1998:49). Acoustics is able to provide reliable information about the particle size of undiluted concentrated dispersions (Dukhin *et al.*, 2000:128).

The acoustic spectroscopy is able to measure attenuation (sound energy losses) and sound speed (Dukhin *et al.*, 2000:128) that are macroscopic properties. The microscopic properties include the electric surface properties, structure, composition as well as particle size distribution (PSD) of a heterogeneous system (Dispersion Technology, 200?b).

The electro-acoustic spectroscopy measures the colloid vibration potential/current (CVP/CVI) or the electrokinetic sonic amplitude (ESA) (Dukhin & Goetz, 1998:49). Electro-acoustic properties such as zeta-potential and particle size can be provided by electro acoustics (Dukhin *et al.*, 2000:128).

All of the above-mentioned experimental data are stored in an Access database after which a special analyses program calculates PSD's from the attenuation spectra and the zeta-potentials from the CVI (Dukhin & Goetz, 1998:50).

The sample volume that was needed for analysis on the DT-1200 was approximately 100 ml.

C.2.2.1 Zeta-potential analysis

In between the oil droplet and the surrounding medium of a o/w-emulsion there is an interfacial layer, where properties such as viscosity and density vary from one phase to the other. These electrochemical aspects of the interfacial layer are combined to form a concept called the "electrical double layer" (DL). The reason for the formation of the DL is the formation of an electric surface charge between ions on the surface of the particles that creates an electrostatic field. When combining this electrostatic field with the thermal motion of the ions, this action creates a counter-charge; which results in the screening of the electric surface (Dispersion Technology, 200?a).

Zeta-potential is defined as the potential at the shear plane (interface separating layers that move with the particle from layers that do not move with the particle) and can be regarded as the "apparent" or "active" charge of the particle (Im-Emsap *et al.*, 2002:243).

According to Silver-colloids (2010), the magnitude of the zeta-potential provides an indication of the potential stability of the colloidal system. Zeta-potential indicating stable particles is when all the particles have a negative or positive zeta-potential, the particles will ward off each other and this results in dispersion stability. Particles with zeta-potentials that are more positive than +30 mV or less negative than -30 mV are generally considered stable.

Zeta-potential is unstable when the particles have low zeta-potential values, then there is no force to prevent the particles coming together, and this causes dispersion instability.

Considering the above-mentioned statement, particles that have zeta-potentials of between -30 mV and +30 mV are considered to be unstable (Silver-colloids, 2010).

C.2.2.1.1 Results for zeta-potential of the formulations containing 5-fluorouracil

No constant change in zeta-potential for the formulations was observed over the 6 month period. The data of most of the formulations (shown in Tables C.8 to C.13), depicted zeta-potential values that were either less than -30 mV or more than +30 mV, which indicated stable particles within a dispersion.

C.2.2.1.1.1 Cream

Table C.8: Zeta-potential values (mV) of 5-fluorouracil containing cream

Month 0	Month 1	Month 2	Month 3	Month 6
25 °C/60% RH				
443125.3	-469201	-487900	-429145	-568467
30 °C/60% RH				
X*	-1.25004	-636194	-772854	-553783
40 °C/75% RH				
X*	-7.69526	-948124	587428.8	452600.4

*X = No data collected for these temperatures

Zeta-potential of the cream particles at 25 °C/60% RH stayed within the range of less than -30 mV or more than +30 mV from months 0 to 6. This indicated stable particles, as they consisted of large enough negative or positive values to repel each other. After 1 month, the zeta-potential values at 30 °C/60% RH and 40 °C/75% RH were between -30 mV and +30 mV which indicated extremely unstable particles, but this changed at months 2, 3 and 6 at these two temperature controlled conditions, when the particles returned to a stabilised state.

C.2.2.1.1.2 Pheroid™ cream

Table C.9: Zeta-potential values (mV) of 5-fluorouracil containing Pheroid™ cream

Month 0	Month 1	Month 2	Month 3	Month 6
25 °C/60% RH				
441611	377.5167	-516204	-540780	316.5
30 °C/60% RH				
X*	287.4067	-551688	-465765	256.2
40 °C/75% RH				
X*	196.98	460993.9	446969.3	206.2

*X = No data collected for these temperatures

Zeta-potential of the Pheroid™ cream particles at all three temperature controlled conditions, stayed within the given range from months 0 to 6. This indicated stable particles, as the particles consisted of large enough negative or positive values to repel each other. Although, at month 6, the zeta-potential values were much less than the previous readings, which indicated that the Pheroid™ cream particles did not exert such a high repelling force and were thus not as stable as at months 2 and 3.

C.2.2.1.1.3 Emulgel

Table C.10: Zeta-potential values (mV) of 5-fluorouracil containing emulgel

Month 0	Month 1	Month 2	Month 3	Month 6
25 °C/60% RH				
-3897.07	40371.67	36478.67	51505.23	39317.8
30 °C/60% RH				
X*	42584.57	39353.1	37496.07	44436.9
40 °C/75% RH				
X*	43711.67	49258.37	48708.93	49188.4

*X = No data collected for these temperatures

Zeta-potential of the emulgel particles at all three temperature controlled conditions, stayed within the given range from months 0 to 6. This indicated stable particles, as the particles consisted of large enough negative or positive values to repel each other. At month 6 the zeta-potential values of the emulgel were still acceptable with a high enough charge to repel each other.

C.2.2.1.1.4 Pheroid™ emulgel

Table C.11: Zeta-potential values (mV) of 5-fluorouracil containing Pheroid™ emulgel

Month 0	Month 1	Month 2	Month 3	Month 6
25 °C/60% RH				
-4813.26	17185.07	38940	5031.003	22699.97
30 °C/60% RH				
X*	17182.77	40802.03	4731.917	4745.59
40 °C/75% RH				
X*	18242.67	40241.07	6151.283	4537.383

*X = No data collected for these temperatures

Zeta-potential of the Pheroid™ emulgel particles at all three temperature controlled conditions, stayed within the given range from months 0 to 6. This indicated stable particles, as the particles consisted of large enough negative or positive values to repel each other. At month 6

the zeta-potential values of the Pheroid™ emulgel were still acceptable with a high enough charge to repel each other.

C.2.2.1.1.5 Lotion

Table C.12: Zeta-potential values (mV) of 5-fluorouracil containing lotion

Month 0	Month 1	Month 2	Month 3	Month 6
25 °C/60% RH				
-2735	383377.1	78790.33	96828.03	57616.47
30 °C/60% RH				
X*	357829.1	67963.03	81587.07	52340.17
40 °C/75% RH				
X*	340511	70166.63	3214.67	-11786.4

*X = No data collected for these temperatures

Zeta-potential of the lotion particles at all three temperature controlled conditions, stayed within the given range from months 0 to 6. This indicated stable particles, as the particles consisted of large enough negative or positive values to repel each other. At month 6 the zeta-potential values of the lotion were still acceptable with a high enough charge to repel each other.

C.2.2.1.1.6 Pheroid™ lotion

Table C.13: Zeta-potential values (mV) of 5-fluorouracil containing Pheroid™ lotion

Month 0	Month 1	Month 2	Month 3	Month 6
25 °C/60% RH				
X*	1149.58	64067.03	658.36	-27410.8
30 °C/60% RH				
X*	1149.077	66727.07	516.6533	-56.8233
40 °C/75% RH				
X*	1145	71552.67	564.62	-63.74

*X = No data collected for these temperatures

Zeta-potential of the Pheroid™ lotion particles at all three temperature controlled conditions, stayed within the given range from months 0 to 6. This indicated stable particles, as the particles consisted of large enough negative or positive values to repel each other. At month 6 the zeta-potential values of the Pheroid™ emulgel were still acceptable at 25 °C/60% RH, with a high enough charge to repel each other, but the charges at 30 °C/60% RH and 40 °C/75% RH were significantly lower, which indicated less stable particles than those at 25 °C/60% RH.

C.2.2.2 Droplet size analysis

The droplet size of the oil phase in each formulation was measured and will be given in the data as shown in Tables C.14 to C.19. The droplet size distribution within the formulations was a quantitative analysis.

Colloidal systems generally have a polydisperse nature, which means that the particles within a particular sample fluctuate in size. With the aim of characterising this polydisperse nature, the term particle size distribution (PSD) is used (Dispersion Technology, 2007b).

The particle size data can be presented by graphical and digital methods. When the number or weight of particles lying within a certain size range is plotted against the size range or mean particle size, a bar graph (histogram) or a frequency distribution curve is obtained. Alternatively, a cumulative percentage over or under a particular size can be plotted against the particular size, which results in a typical sigmoidal curve called a cumulative frequency plot. From these data, the mean particle size, standard deviation, and the extent of polydispersity may be determined (Im-Emsap *et al.*, 2002:241).

C.2.2.2.1 Results for droplet size of the formulations containing 5-fluorouracil

Changes in droplet size, size distribution or particulate nature of semisolid preparations are the consequence of factors, i.e., crystal growth, changes in crystalline habit, or the reversion of the crystalline materials to a more stable polymorphic form. Any one of these crystalline alterations may lead to a definite decrease in drug delivery capabilities and therapeutic efficacy of a formulation. Thus, when products show signs of such changes they are seriously physically unstable and unusable (Flynn, 2002:230).

For the purpose of this study the term “droplet size” will be used, as this was the parameter that was measured by the DT-1200.

The droplet size was only measured as from month 1 of the stability due to instrumentation that was not working properly at this point in time. Thus, data presented in Tables C.14 to C.19 include droplet sizes for months 1, 2, 3 and 6 and the final tests should thus be compared to month 1 as this was the first time point of this particular stability test, and not month 0.

C.2.2.2.1.1 Cream

Table C.14: Droplet size (μm) of 5-fluorouracil containing cream over 6 months

	25 °C/60% RH			
Month	1	2	3	6
Size median	0.356	91.344	90.761	91.270
Standard deviation	0.088	0.050	0.050	0.051
	30 °C/60% RH			
Month	1	2	3	6
Size median	0.500	90.305	93.112	91.344
Standard deviation	0.100	0.052	0.050	0.051
	40 °C/75% RH			
Month	1	2	3	6
Size median	0.338	98.336	106.510	106.343
Standard deviation	0.100	0.053	0.120	0.093

The droplet size of the cream varied throughout the 6 month period. There was a definite increase from the initial size to month 2 at 25 °C/60% RH, 30 °C/60% RH and 40 °C/75% RH. The cream stored at 40 °C/75% RH depicted the largest droplet size after the 6 month stability period. This increase in droplet size could be due to coalescence, which occurs when the mechanical or electrical barrier is insufficient to prevent the formation of progressively larger droplets (Im-Emsap *et al.*, 2002:266).

It could also have been due to a type of instability called creaming. Creaming occurs when the disperse droplets separate from the disperse medium under the influence of gravitational force. Larger droplets cream more rapidly than smaller droplets (Im-Emsap *et al.*, 2002:266). This phenomenon could be seen from month 3, where the oil phase separated from the rest of the formulation.

A high viscosity reduces creaming and also lessens the tendency of particles to coalescence and produce phase separation (Im-Emsap *et al.*, 2002:265).

The opposite of the above-mentioned statement was observed from a physical point of view, as the initial thick appearance of the cream changed to a thin, less viscous cream at month 3. This attributed to an increase in creaming and coalescence of the droplets which also produced a phase separation. The decrease in viscosity of the cream from months 0 to 3 is shown in Figure C.7.

C.2.2.2.1.2 Pheroid™ cream

Table C.15: Droplet size (µm) of 5-fluorouracil containing Pheroid™ cream over 6 months

	25 °C/60% RH			
Month	1	2	3	6
Size median	0.097267	95.89367	90.807	0.82225
Standard deviation	0.033667	0.050333	0.05	0.0475
	30 °C/60% RH			
Month	1	2	3	6
Size median	0.925233	90.95767	90.567	0.2455
Standard deviation	0.049	0.05	0.05	0.033
	40 °C/75% RH			
Month	1	2	3	6
Size median	0.007333	99.79533	99.645	0.007833
Standard deviation	0.04	0.052667	0.071	0.050333

The droplet size of the Pheroid™ cream varied throughout the 6 month period. There was a definite increase from the initial size to months 2 and 3 at all three temperature controlled conditions. This could have been due to the instability type called coalescence, followed by creaming, as explained previously. Phase separation was also seen at month 3, with a decrease in viscosity at month 3 as shown in Figure C.8. The droplet size showed a significant decrease from months 3 to 6 at all three temperature controlled conditions, which was due to decomposition of the Pheroid™ cream.

C.2.2.2.1.3 Emulgel

Table C.16: Droplet size (µm) of 5-fluorouracil containing emulgel over 6 months

	25 °C/60% RH			
Month	1	2	3	6
Size median	90.28	89.452	89.974	89.974
Standard deviation	0.05	0.053667	0.05	0.05
	30 °C/60% RH			
Month	1	2	3	6
Size median	89.82467	89.30133	89.37667	89.974
Standard deviation	0.053667	0.054333	0.054667	0.05
	40 °C/75% RH			
Month	1	2	3	6
Size median	89.974	89.526	44.67533	7.6072
Standard deviation	0.051333	0.052	0.049667	0.05

The droplet size of the emulgel did not show a significant change with time at 25 °C/60% RH and 30 °C/60% RH. Thus, it could be said that the emulgel stayed relatively stable at these two temperature conditions with regard to droplet size, up to month 6. At 40 °C/75% RH the droplet size decreased significantly from months 2 to 3 and again from months 3 to 6. This could be due to decomposition of the emulgel at this high temperature.

C.2.2.2.1.4 Pheroid™ emulgel

Table C.17: Droplet size (µm) of 5-fluorouracil containing Pheroid™ emulgel over 6 months

	25 °C/60% RH			
Month	1	2	3	6
Size median	90.123	92.952	25.094	21.082
Standard deviation	0.050333	0.05	0.048333	0.059
	30 °C/60% RH			
Month	1	2	3	6
Size median	59.91767	90.076	23.06667	19.0913
Standard deviation	0.052333	0.050333	0.051667	0.056
	40 °C/75% RH			
Month	1	2	3	6
Size median	62.64233	107.5	23.145	6.6667
Standard deviation	0.049	0.087	0.048667	0.049

The droplet size of the Pheroid™ emulgel showed a slight increase from the initial value to month 2 at 25 °C/60% RH. A larger increase at 30 °C/60% RH and 40 °C/75% RH was depicted, after which the droplet size decreased significantly at all three temperature controlled conditions, from months 2 to 3; and again from months 3 to 6 due to decomposition of the Pheroid™ emulgel.

C.2.2.2.1.5 Lotion

Table C.18: Droplet size (μm) of 5-fluorouracil containing lotion over 6 months

	25 °C/60% RH			
Month	1	2	3	6
Size median	89.148	15.026	20.21933	11.45567
Standard deviation	0.049	0.052667	0.055667	0.052667
	30 °C/60% RH			
Month	1	2	3	6
Size median	89.148	12.827	14.71733	4.4437
Standard deviation	0.05	0.049333	0.05	0.053333
	40 °C/75% RH			
Month	1	2	3	6
Size median	97.382	67.716	89.148	0.077
Standard deviation	0.050667	0.049	0.051	0.216667

The droplet size of the lotion decreased significantly from the initial value to month 2 at all three temperature controlled conditions. There was a slight increase to month 3 at all three temperature controlled conditions. The droplet size decreased again from months 3 to 6, which could be due to decomposition of the lotion.

C.2.2.2.1.6 Pheroid™ lotion

Table C.19: Droplet size (μm) of 5-fluorouracil containing Pheroid™ lotion over 6 months

	25 °C/60% RH			
Month	1	2	3	6
Size median	57.13233	21.68067	88.705	7.84
Standard deviation	0.053	0.048667	0.06	0.3633
	30 °C/60% RH			
Month	1	2	3	6
Size median	38.18233	5.663333	48.962	39.437
Standard deviation	0.049333	0.05	0.049333	0.05
	40 °C/75% RH			
Month	1	2	3	6
Size median	64.935	0.11	90.62633	51.431
Standard deviation	0.069667	0.3	0.063333	0.049

The droplet size of the Pheroid™ lotion significantly decreased from the initial value to month 2 and showed a significant increase (higher than the initial values) from months 2 to 3. This was

observed at all three temperature controlled conditions. The droplet size decreased again from months 3 to 6, which could be due to decomposition of the Pheroid™ lotion.

C.2.3 pH measurement

The pH of a given sample can be defined as a measure of the hydrogen ion (H^+) concentration in a given solution. It is a measure of the acidity or alkalinity of a solution. For example, any aqueous solution at 25 °C, with a pH less than 7.0, is acidic. Whereas one with a pH higher than 7.0 is alkaline (or basic). A “neutral” pH-level is 7.0 at 25 °C; the reason being that the concentration of H_3O^+ is equal to the concentration of OH^- in pure water (Helmenstine, 2010).

The apparatus used in this study was a Mettler Toledo Inlab® 410 electrode. The pH meter was calibrated each time before the measurements were taken.

The pH of all 6 formulations was measured once at 25 °C at the start of the 6 month stability period and thereafter at 25 °C/60% RH; 30 °C/60% RH and 40 °C/75% RH at the end of months 1, 2, 3 and 6.

C.2.3.1 Results for pH of the formulations containing 5-fluorouracil

The pH of all the formulations decreased with an increase in temperature of the climate rooms over the period of 6 months. According to Flynn (2002:230), changes in the pH of products indicate chemical decomposition that are most probably of a hydrolytic nature. The cream showed the lowest % decrease in pH and the Pheroid™ lotion the highest. The results for pH are shown in Tables C.20 to C.25.

C.2.3.1.1 Cream

Table C.20: pH of the cream over the 6 month period at three different conditions

Month 0	Month 1	Month 2	Month 3	Month 6	% decrease
25 °C/60% RH					
5.04	4.84	4.76	4.58	4.49	10.9
30 °C/60% RH					
X*	4.56	4.51	4.38	4.29	14.9
40 °C/75% RH					
X*	4.44	4.42	4.27	3.96	21.4

*X = No data collected for these temperatures

The pH of the cream declined with an increase in temperature of the climate rooms over the 6 month period, as shown in Figure C.1. This was probably due to chemical decomposition, and thus, instability of the product (Flynn, 2002:230).

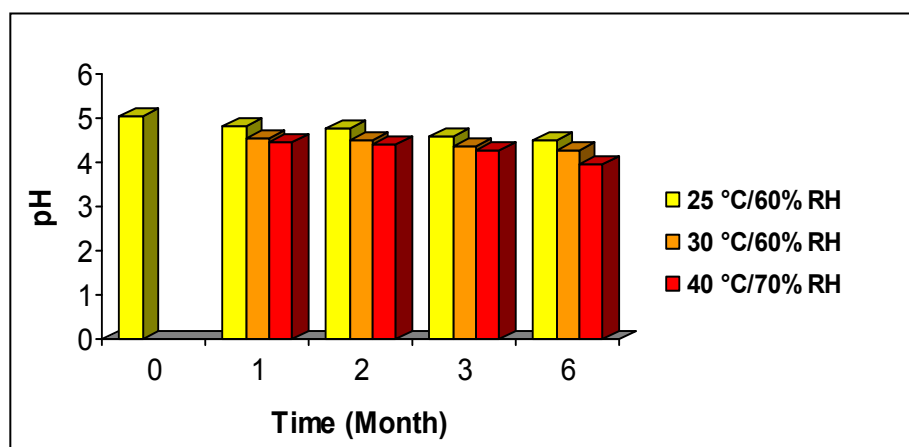


Figure C.1: pH of the cream over the 6 month period at three different conditions

C.2.3.1.2 Pheroid™ cream

Table C.21: pH of the Pheroid™ cream over the 6 month period at three different conditions

Month 0	Month 1	Month 2	Month 3	Month 6	% decrease
25 °C/60% RH					
4.83	4.67	4.52	4.32	4.18	13.5
30 °C/60% RH					
X*	4.53	4.29	4.16	3.96	18.0
40 °C/75% RH					
X*	4.34	4.03	3.68	3.64	24.6

*X = No data collected for these temperatures

The pH of the Pheroid™ cream declined with an increase in temperature of the climate rooms over the 6 month period as shown in Figure C.2. This was probably due to chemical decomposition, and thus, instability of the product (Flynn, 2002:230).

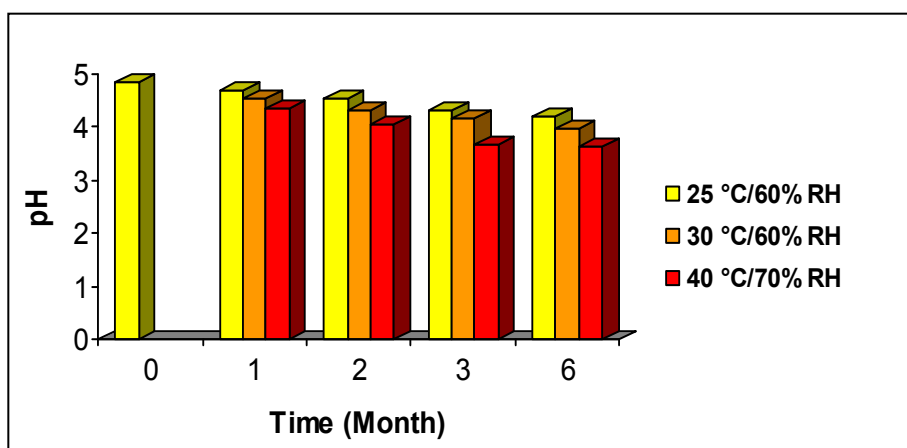


Figure C.2: pH of the Pheroid™ cream over the 6 month period at three different conditions

C.2.3.1.3 Emulgel

Table C.22: pH of the emulgel over the 6 month period at three different conditions

Month 0	Month 1	Month 2	Month 3	Month 6	% decrease
25 °C/60% RH					
5.7	5.31	5.04	4.96	4.70	17.5
30 °C/60% RH					
X*	5.12	4.91	4.74	4.47	21.6
40 °C/75% RH					
X*	5.06	4.64	4.41	4.12	27.7

*X = No data collected for these temperatures

The pH of the emulgel declined with an increase in temperature of the climate rooms over the 6 month period as shown in Figure C.3. This was probably due to chemical decomposition, and thus, instability of the product (Flynn, 2002:230).

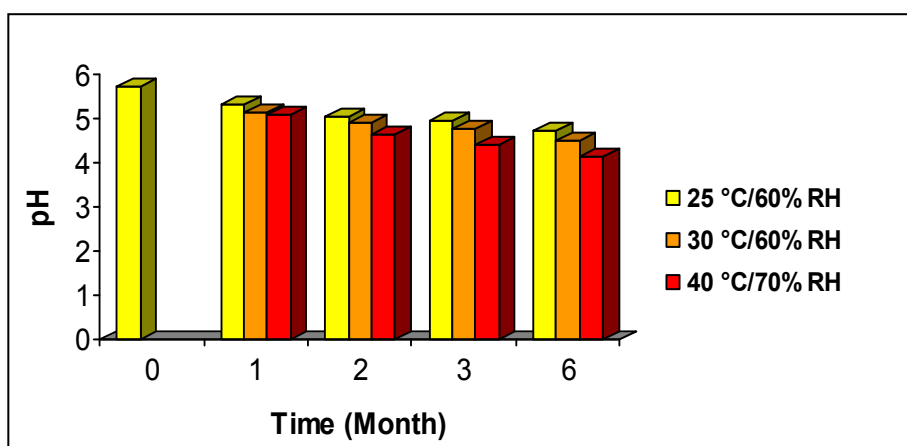


Figure C.3: pH of the emulgel over the 6 month period at three different conditions

C.2.3.1.4 Pheroid™ emulgel

Table C.23: pH of the Pheroid™ emulgel over the 6 month period at three different conditions

Month 0	Month 1	Month 2	Month 3	Month 6	% decrease
25 °C/60% RH					
5.93	5.34	5.10	4.89	4.72	20.4
30 °C/60% RH					
X*	5.22	5.10	4.76	4.45	25.0
40 °C/75% RH					
X*	5.11	4.66	4.40	4.30	27.5

*X = No data collected for these temperatures

The pH of the Pheroid™ emulgel declined with an increase in temperature of the climate rooms over the 6 month period as shown in Figure C.4. This was probably due to chemical decomposition, and thus, instability of the product (Flynn, 2002:230).

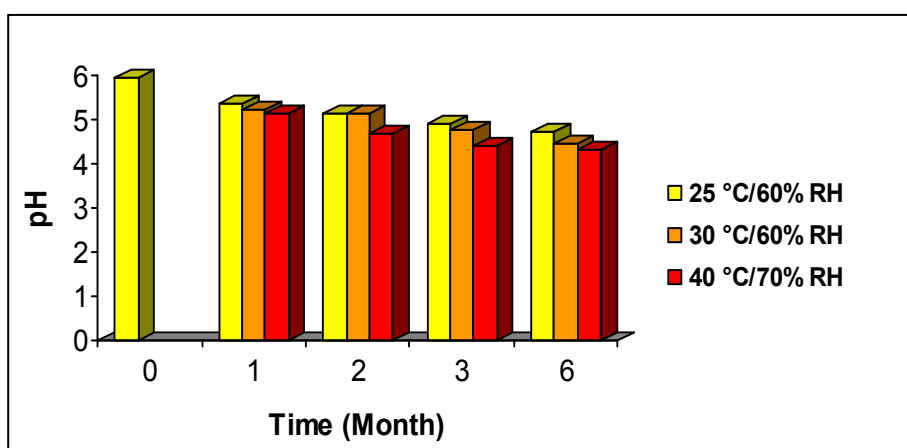


Figure C.4: pH of the Pheroid™ emulgel over the 6 month period at three different conditions

C.2.3.1.5 Lotion

Table C.24: pH of the lotion over the 6 month period at three different conditions

Month 0	Month 1	Month 2	Month 3	Month 6	% decrease
25 °C/60% RH					
5.97	4.62	4.45	4.31	4.19	29.8
30 °C/60% RH					
X*	4.71	4.49	4.23	4.13	30.8
40 °C/75% RH					
X*	4.60	4.41	4.03	3.95	33.8

*X = No data collected for these temperatures

The pH of the lotion declined with an increase in temperature of the climate rooms over the 6 month period, except at 30 °C/60% RH on months 1 and 2, where the pH was higher than at 25 °C/60% RH and 40 °C/75% RH, as shown in Figure C.5. This was probably due to chemical decomposition, and thus, instability of the product (Flynn, 2002:230).

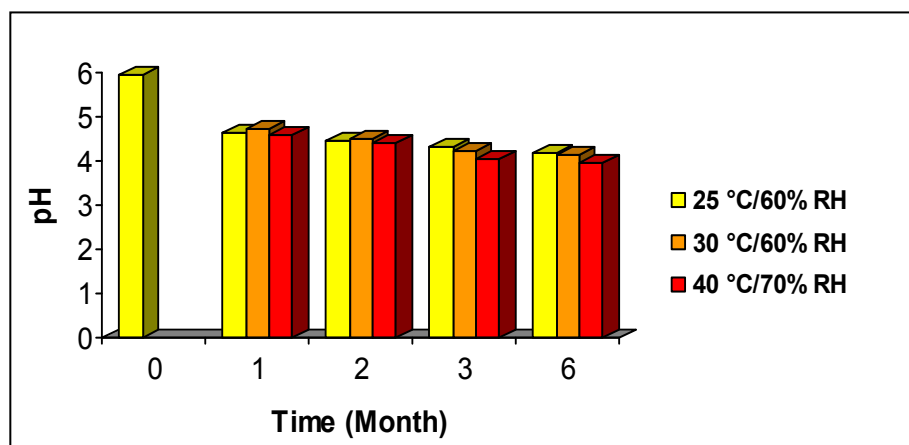


Figure C.5: pH of the lotion over the 6 month period at three different conditions

C.2.3.1.6 Pheroid™ lotion

Table C.25: pH of the Pheroid™ lotion over the 6 month period at three different conditions

Month 0	Month 1	Month 2	Month 3	Month 6	% decrease
25 °C/60% RH					
6.17	4.57	4.48	4.47	4.30	30.3
30 °C/60% RH					
X*	4.55	4.42	4.34	4.22	31.6
40 °C/75% RH					
X*	4.50	4.37	3.99	4.12	33.2

*X = No data collected for these temperatures

The pH of the Pheroid™ emulgel declined with an increase in temperature of the climate rooms over the 6 month period as shown in Figure C.6. However, the pH increased slightly from months 3 to 6 at 40 °C/75% RH. This was probably due to chemical decomposition, and thus, instability of the product (Flynn, 2002:230).

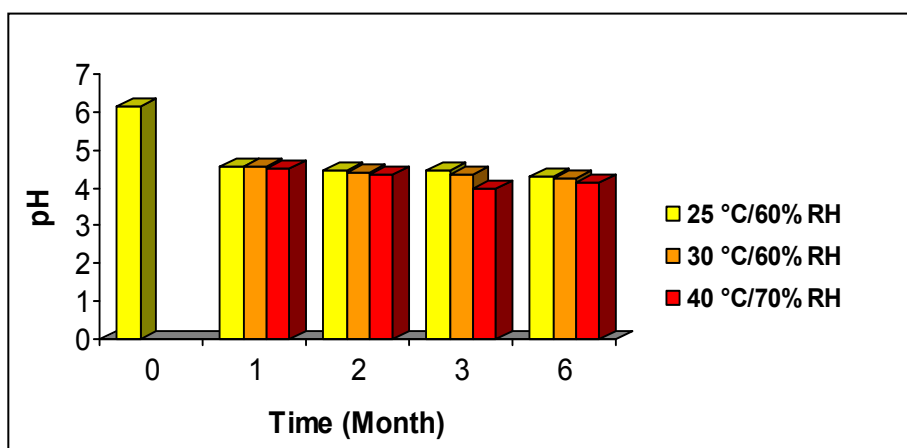


Figure C.6: pH of the Pheroid™ lotion over the 6 month period at three different conditions

C.2.4 Viscosity determination

Rheology is defined as the study of the change in form and the flow of matter, embracing elasticity, viscosity and plasticity. Viscosity can further be defined as the internal friction of a fluid, caused by molecular attraction, which makes it resist a tendency to flow (Brookfield Engineering, 2005).

The viscosity of the 6 formulations was measured using a Brookfield Viscometer (model DV II, Stoughton, Massachusetts, USA). It uses a rotating spindle and helipath attachments. The spindle was put into the sample formulation after which the speed was specified. The viscosity was measured by determining the resistance to the rotating spindle. Data was collected on Wingather™ software that was programmed to take 32 readings at 10 second intervals. The average viscosity was calculated as shown in Table C.26.

Due to the fact that the viscosity meter required a large quantity of the formulation to be measured (cost implications), the test was only conducted at one of the temperature controlled storage conditions, namely 25 °C/60% RH. The viscosity of all 6 formulations was measured at this condition on months 0, 1, 2, 3 and 6.

C.2.4.1 Results for viscosity of the formulations containing 5-fluorouracil

Increases or decreases in viscosity of semisolid formulations indicate changes in the structural elements of the formulation. If these rheological changes are irreversible, it is a sign of poor physical stability (Flynn, 2002:230).

Table C.26: Viscosity values (cP) over 6 months of the formulations containing 5-fluorouracil at 25 °C/60% RH

	Month 0	Month 1	Month 2	Month 3	Month 6	%RSD*
Cream	386685.1	275380.7	370380.0	181738.6	532.5	58.4%
Pheroid™ cream	71400.8	248365.0	235906.7	185273.0	754.7	65.2%
Emulgel	67231.7	74730.1	84942.8	44053.1	1162.8	54.8%
Pheroid™ emulgel	75569.8	85235.7	98299.3	49471.9	115453.9	26.1%
Lotion	64810.4	3015.4	104.5	93.4	727.6	185.8%
Pheroid™ lotion	295.6	1100.3	1279.3	1970.3	687.9	53.0%

*%RSD = % Relative Standard Deviation

C.2.4.1.1 Cream

As seen in Figure C.7, the viscosity of the cream did not have a constant change during the first 2 months, although from months 2 to 6, it showed a definite decrease. The initial viscosity was much higher than at month 6, which is an indication of instability of the product.

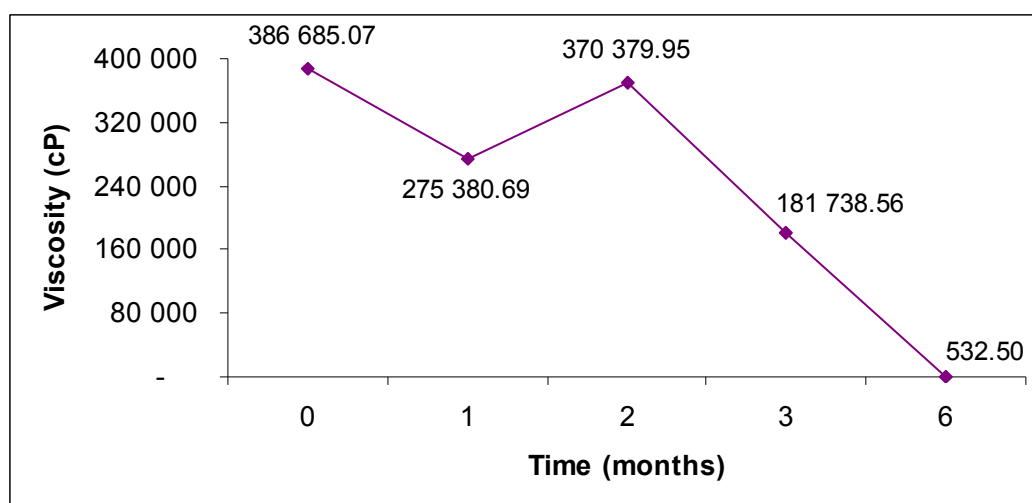


Figure C.7: Viscosity of cream over a 6 month period

C.2.4.1.2 Pheroid™ cream

As seen in Figure C.8, the viscosity of the Pheroid™ cream increased from its initial value to month 1, and after that it gradually decreased until month 6, which is an indication of instability of the product.

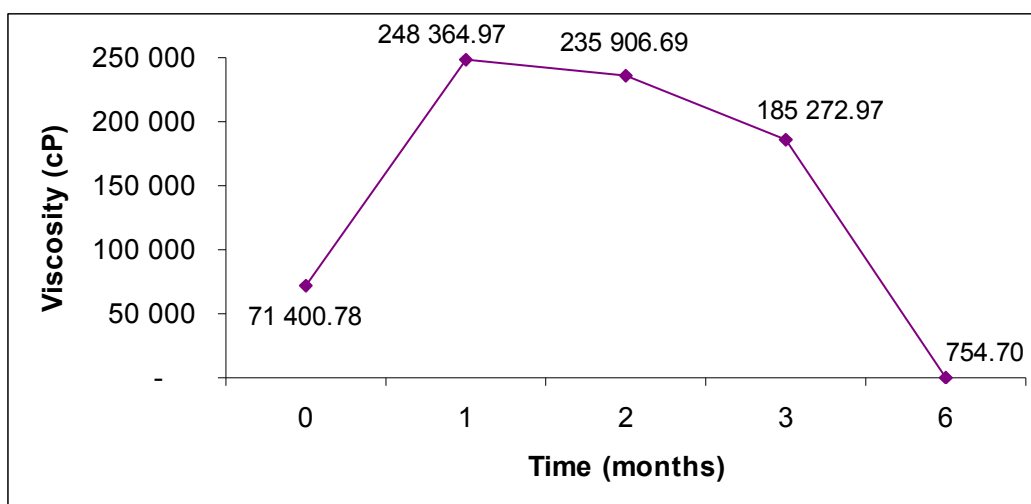


Figure C.8: Viscosity of Pheroid™ cream over a 6 month period

C.2.4.1.3 Emulgel

As seen in Figure C.9, the viscosity of the emulgel increased from the initial measurement up to month 2, but decreased from months 2 to 6. This could be an indication of instability of the product.

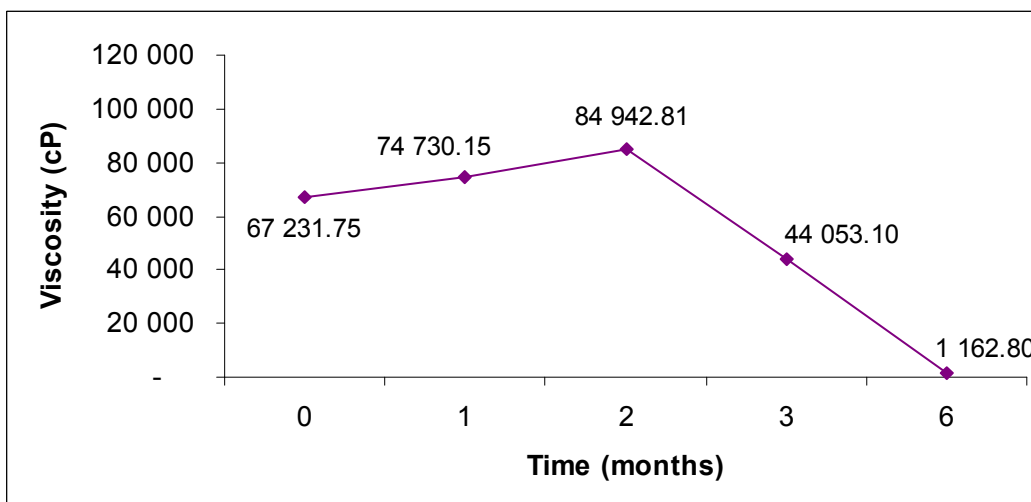


Figure C.9: Viscosity of emulgel over a 6 month period

C.2.4.1.4 Pheroid™ emulgel

As seen in Figure C.10, the viscosity of the Pheroid™ emulgel increased from the initial measurement up to month 2, but decreased from months 2 to 3, after which it increased to a value higher than the initial value. This could be an indication of instability of the product. Though, when all the formulations were compared, the Pheroid™ emulgel showed the lowest

%RSD (26.1%) throughout the 6 month period, thus, it could be concluded that this was the most stable formulation with reference to viscosity.

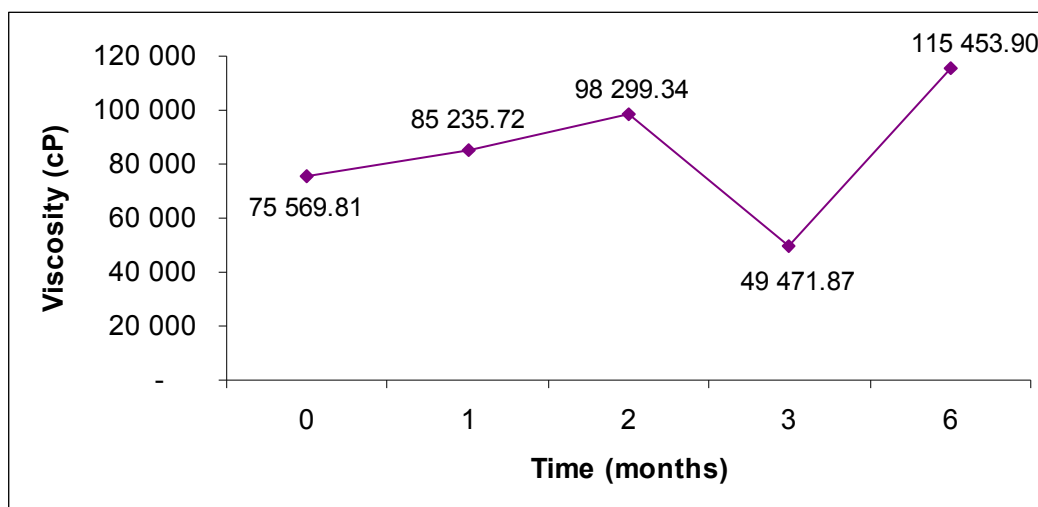


Figure C.10: Viscosity of Pheroid™ emulgel over a 6 month period

C.2.4.1.5 Lotion

As seen in Figure C.11, the viscosity of the lotion showed a significant decrease from the initial value to month 1 and depicted values close to zero for months 2 and 3, and showed a slight increase to month 6. It appeared as a liquid from month 2 onward, which showed little or no viscosity. When all the formulations were compared, the lotion showed the highest %RSD (185.8%) throughout the 6 month period, thus, it could be concluded that this was the least stable formulation regarding viscosity.

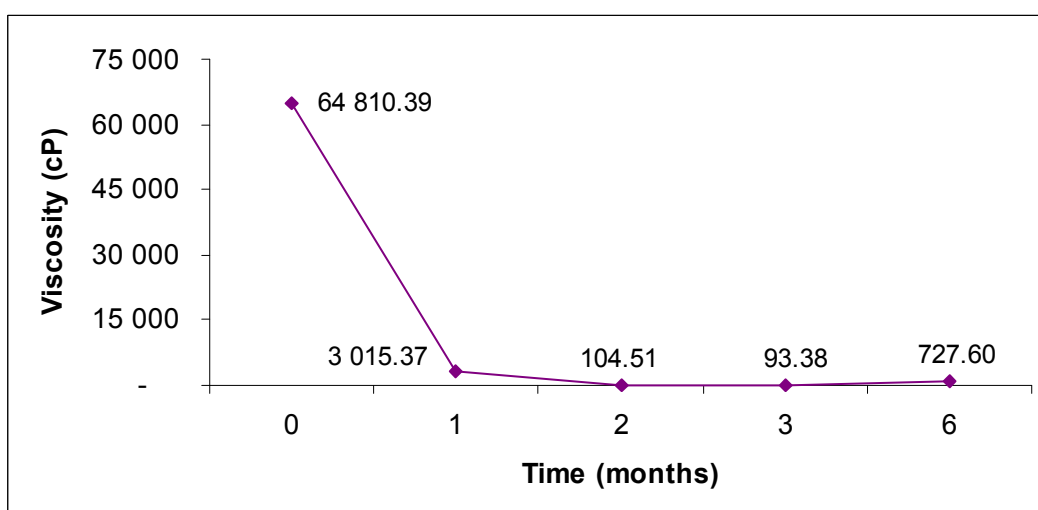


Figure C.11: Viscosity of lotion over a 6 month period

C.2.4.1.6 Pheroid™ lotion

As seen in Figure C.12, the viscosity of the Pheroid™ lotion depicted a constant change (increase) during the first 3 months, which could have been due to the loss of fluid within the product. At month 6 it showed a decrease.

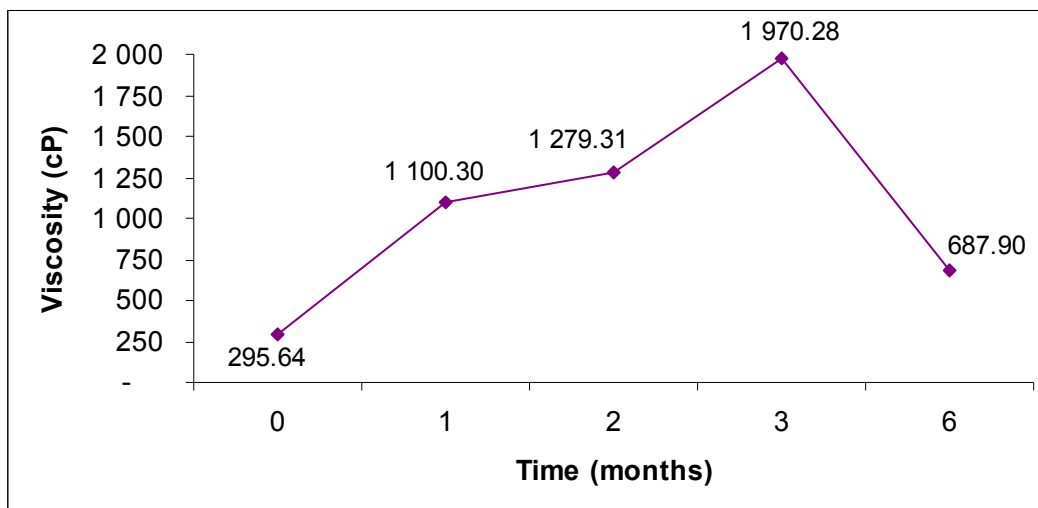


Figure C.12: Viscosity of Pheroid™ lotion over a 6 month period

C.2.5 Mass loss determination

To determine the mass loss of the formulations over a period of 6 months, approximately 50 g of each formulation was stored in separate containers and marked for each temperature condition. Thus, each temperature condition was evaluated separately, due to the fact that the containers did not have an identical initial mass. The apparatus used was a Shimadzu (Japan) scale. The initial weight of each container was noted after manufacture. From month 1, 2, 3 and 6 the containers, stored at 25 °C/60% RH, 30 °C/60% RH and 40 °C/75% RH, were weighed precisely, to determine the mass variation over time. The Pheroid™ emulgel showed the lowest % decrease in mass over the 6 month stability period and the Pheroid™ lotion the highest. The mass variation of the formulations over 6 months are shown in Tables C.27 to C.31.

C.2.5.1 Results for mass loss of the formulations containing 5-fluorouracil

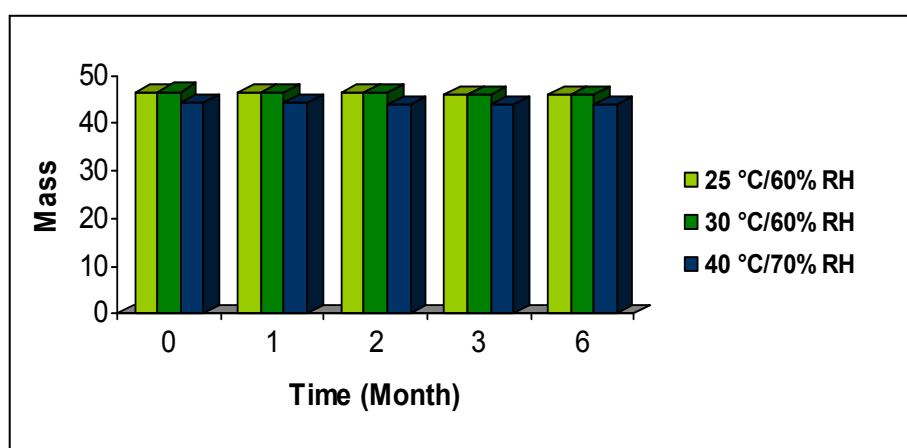
C.2.5.1.1 Cream

The mass of the cream decreased with time at all three temperature controlled conditions. This could have been due to an increase in vaporisation, as the temperatures rose within each of the three storage conditions separately, which resulted in loss of solvent or liquid within the formulation (Allen, 1998:33). Mass loss of the cream is shown in Figure C.13.

Table C.27: Mass loss of the cream over the 6 month period at three different conditions

Month 0	Month 1	Month 2	Month 3	Month 6	% decrease*
25 °C/60% RH					
46.4643	46.3731	46.2746	46.2007	46.0855	0.8
30 °C/60% RH					
46.6680	46.5902	46.4006	46.1513	46.1257	1.2
40 °C/75% RH					
44.2320	44.2175	44.0847	44.0461	43.8152	0.9

* % decrease = Percentage decrease in mass from month 0 to month 6

**Figure C.13:** Mass loss of the cream over the 6 month period at three different conditions

C.2.5.1.2 Pheroid™ cream

The mass of the Pheroid™ cream decreased with time at all three temperature controlled conditions. This could have been due to an increase in vaporisation, as the temperatures rose within each of the three storage conditions separately, which resulted in loss of solvent or liquid within the formulation (Allen, 1998:33). This in turn might have caused mass loss of the Pheroid™ cream, as seen in Figure C.14.

Table C.28: Mass loss of the Pheroid™ cream over the 6 month period at three different conditions

Month 0	Month 1	Month 2	Month 3	Month 6	% decrease*
25 °C/60% RH					
49.6966	49.6231	49.5095	49.4551	49.3700	0.7
30 °C/60% RH					
47.6112	47.5678	47.4661	47.3953	47.2985	0.7
40 °C/75% RH					
50.0927	49.9694	49.8277	49.7369	49.6241	0.9

*% decrease = Percentage decrease in mass from month 0 to month 6

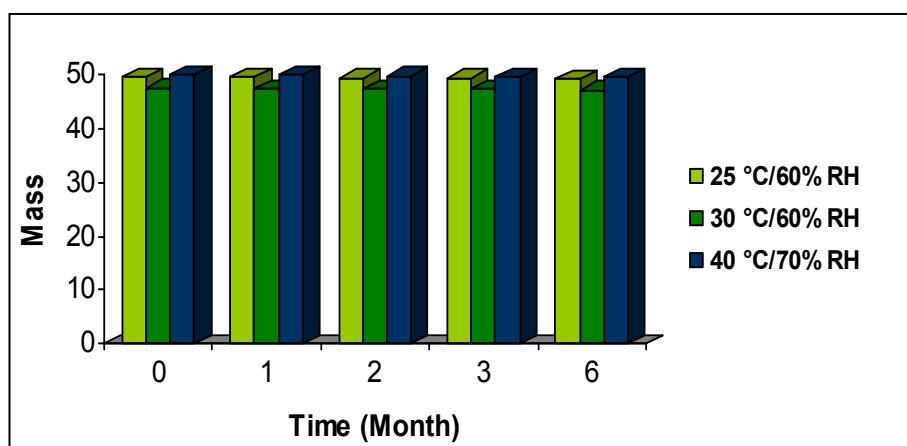


Figure C.14: Mass loss of the Pheroid™ cream over the 6 month period at three different conditions

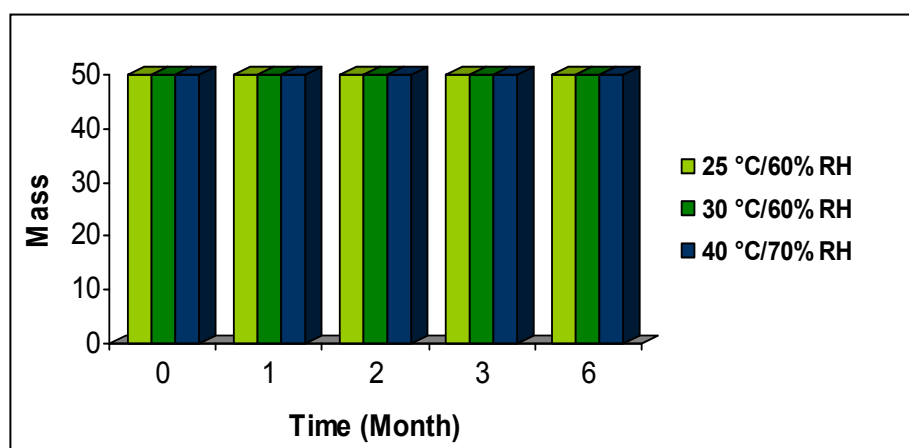
C.2.5.1.3 Emulgel

The mass of the emulgel also decreased with time at all three temperature controlled conditions as shown in Figure C.15. This could have been due to an increase in vaporisation, as the temperatures rose within each of the three storage conditions separately, which resulted in loss of solvent or liquid within the formulation (Allen, 1998:33). This phenomenon may only have slightly contributed to the mass loss due to the fact that the concentration of excipients in the emulgel did not increase as they did in the cream and Pheroid™ cream due to the above-mentioned reason. The degradation of the excipients in the emulgel may also have contributed to the mass loss.

Table C.29: Mass loss of the emulgel over the 6 month period at three different conditions

Month 0	Month 1	Month 2	Month 3	Month 6	% decrease*
25 °C/60% RH					
51.4213	51.1852	51.1815	51.0527	50.8933	1.0
30 °C/60% RH					
51.4281	51.3828	51.2431	51.1228	50.8225	1.2
40 °C/75% RH					
51.4744	51.4033	51.2193	51.0749	51.0418	0.8

*% decrease = Percentage decrease in mass from month 0 to month 6

**Figure C.15:** Mass loss of the emulgel over the 6 month period at three different conditions

C.2.5.1.4 Pheroid™ emulgel

The mass of the Pheroid™ emulgel increased from the initial value to month 1 at all three temperature controlled conditions as seen in Figure C.16. As from month 1 it gradually decreased to months 2, 3, and 6. The Pheroid™ emulgel showed the lowest % decrease in mass over the 6 month stability period.

Table C.30: Mass loss of the Pheroid™ emulgel over the 6 month period at three different conditions

Month 0	Month 1	Month 2	Month 3	Month 6	% decrease*
25 °C/60% RH					
54.6556	54.8159	54.7349	54.6194	54.4581	0.4
30 °C/60% RH					
52.4882	52.7189	52.6711	52.5441	52.4731	0.02
40 °C/75% RH					
55.0870	55.2610	55.0859	54.7116	54.6951	0.7

*% decrease = Percentage decrease in mass from month 0 to month 6

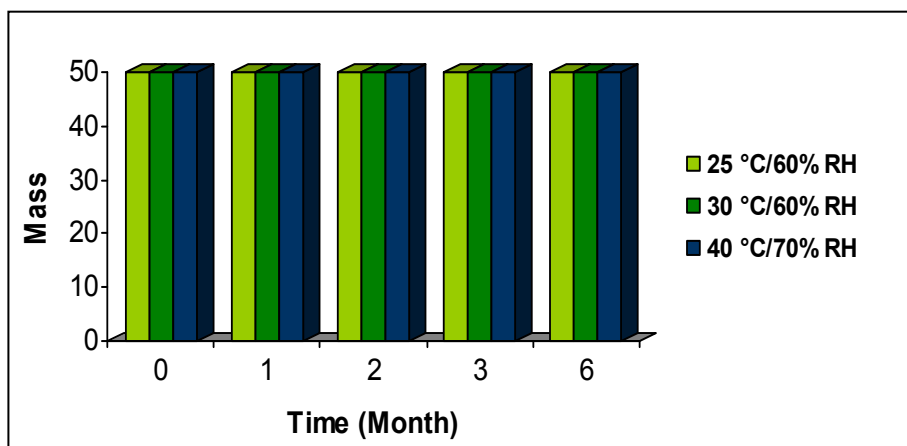


Figure C.16: Mass loss of the Pheroid™ emulgel over the 6 month period at three different conditions

C.2.5.1.5 Lotion

The mass of the lotion decreased with time at all three temperature controlled conditions. This could be due to increase in vaporisation, as the temperatures rose within each of the three storage conditions separately, which resulted in loss of solvent or liquid within the formulation (Allen, 1998:33). This in turn might have caused mass loss of the cream, as seen in Figure C.17.

Table C.31: Mass loss of the lotion over the 6 month period at three different conditions

Month 0	Month 1	Month 2	Month 3	Month 6	% decrease*
25 °C/60% RH					
53.5604	53.4935	53.3441	53.0779	52.9719	1.1
30 °C/60% RH					
49.0479	48.9492	48.8113	48.5728	48.5568	1.0
40 °C/75% RH					
50.7437	50.6963	50.5358	50.5022	50.3725	0.7

*% decrease = Percentage decrease in mass from month 0 to month 6

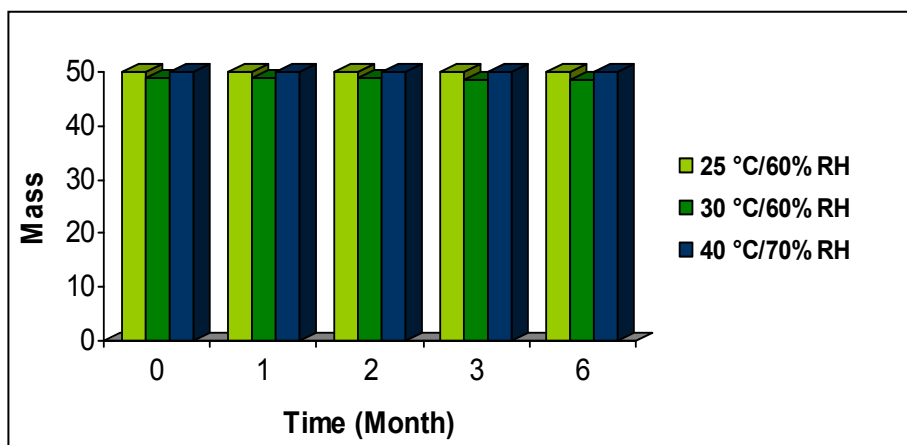


Figure C.17: Mass loss of the lotion over the 6 month period at three different conditions

C.2.5.1.6 Pheroid™ lotion

The mass of the Pheroid™ lotion increased from the initial value to month 1 at 25 °C/60% RH, although it decreased with time at all three temperature controlled conditions. This could have been due to an increase in vaporisation, as the temperatures rose within each of the three storage conditions separately, which resulted in loss of solvent or liquid within the formulation (Allen, 1998:33). This in turn may have caused mass loss of the cream as seen in Figure C.18.

Table C.32: Mass loss of the Pheroid™ lotion over the 6 month period at three different conditions

Month 0	Month 1	Month 2	Month 3	Month 6	% decrease*
25 °C/60% RH					
48.0407	48.0579	47.8431	47.7063	47.5155	1.1
30 °C/60% RH					
41.3799	41.2729	41.0898	41.0347	40.9863	1.0
40 °C/75% RH					
48.9074	48.8088	48.5803	48.5241	48.0877	1.7

*% decrease = Percentage decrease in mass from month 0 to month 6

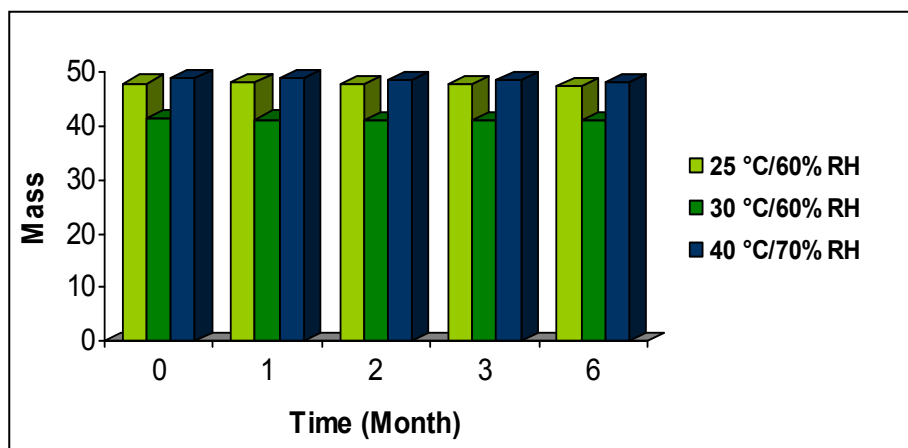


Figure C.18: Mass loss of the Pheroid™ lotion over the 6 month period at three different conditions

C.2.6 Physical appearance

To determine if there were any changes in the physical appearance of the formulations over the 6 month period, photos were taken of each formulation initially, at 25 °C; and compared to the photos obtained at months 1, 2, 3 and 6 for the three temperature conditions.

The images shown in Tables C.33 to C.38 compare the initial appearance to that of month 3 and 6 at 25 °C/60% RH, 30 °C/60% RH and 40 °C/75% RH.

C.2.6.1 Cream

Table C.33: Physical appearance of the cream initially and after 3 and 6 months

	Month 0	Month 3	Month 6
25 °C/60% RH			
30 °C/60% RH			
40 °C/75% RH			

The cream showed no significant change in colour from the initial appearance to month 6.

C.2.6.2 Pheroid™ cream

Table C.34: Physical appearance of the Pheroid™ cream initially and after 3 and 6 months

	Month 0	Month 3	Month 6
25 °C/60% RH			
30 °C/60% RH			
40 °C/75% RH			

The Pheroid™ cream showed a slight change in colour from the initial appearance to month 3 at 25 °C/60% RH and 30 °C/60% RH, it changed to a slight yellowish colour. At 40 °C/75% RH it showed a more significant change in colour (to a brighter yellow) from the initial appearance to months 3 and 6. The products developed an off-odour and showed phase changes at the end of the stability period, especially at 40 °C/75% RH. The reason for this phenomenon could be due to oxidative reactions occurring in the base of the products, which often cause them to change colour (yellow or brown) with age. These kinds of discolorations are often seen when natural fats and oils are used to build the vehicle. The development of a disagreeable odour is due to extensive oxidation of natural fatty minerals (Flynn, 2002:229-230). To substantiate the above-mentioned, all the Pheroid™ formulations contained several natural oils and anti-

oxidants (dl- α -tocopherol and BHT). The concentrations of the anti-oxidant used in the Pheroid™ formulations might have been too little to stop the oxidation reaction from taking place.

C.2.6.3 Emulgel

Table C.35: Physical appearance of the emulgel initially and after 3 and 6 months

	Month 0	Month 3	Month 6
25 °C/60% RH			
30 °C/60% RH			
40 °C/75% RH			

The emulgel showed no significant change in colour from the initial appearance to month 3, but showed a slight light yellow colour at month 6. Products may often yellow or brown with age due to oxidative reactions occurring in the base (Flynn, 2002:229-230).

C.2.6.4 Pheroid™ emulgel

Table C.36: Physical appearance of the Pheroid™ emulgel initially and after 3 and 6 months

	Month 0	Month 3	Month 6
25 °C/60% RH			
30 °C/60% RH			
40 °C/75% RH			

The Pheroid™ emulgel showed a very slight change in colour from the initial appearance to month 3 at 25 °C/60% RH and 30 °C/60% RH. It depicted a slight off-white colour. At 40 °C/75% RH it showed a more significant change in colour from the initial appearance and changed to a light yellow colour at month 3. These changes were more significant at month 6, where they appeared more yellow. The products also developed an off-odour at the end of month 6, but did not show phase changes at the end of the stability period, as was the case with the Pheroid™ cream.

The reason for the slight change in colour and disagreeable odour of the Pheroid™ emulgel was due to the same reasons as mentioned under Section C.2.6.2 with the Pheroid™ cream.

C.2.6.5 Lotion

Table C.37: Physical appearance of the lotion initially and after 3 and 6 months

	Month 0	Month 3	Month 6
25 °C/60% RH			
30 °C/60% RH			
40 °C/75% RH			

The lotion showed no significant change in colour from the initial appearance to month 3, but showed a slight change at month 6 (light yellow). Products may often colour yellow or brown with age due to oxidative reactions occurring in the base (Flynn, 2002:229-230).

C.2.6.6 Pheroid™ Lotion

Table C.38: Physical appearance of the Pheroid™ lotion initially and after 3 and 6 months

	Month 0	Month 3	Month 6
25 °C/60% RH			
30 °C/60% RH			
40 °C/75% RH			

The Pheroid™ lotion showed a slight change in colour from the initial appearance to month 3 at 25 °C/60% RH and 30 °C/60% RH, it depicted a light yellow colour. At 40 °C/75% RH it showed a more significant change in colour (to a brighter yellow) from the initial appearance to month 3. These changes were more significant at month 6, where they appeared a much brighter yellow. The products also developed an off-odour at the end of month 3 and showed phase changes at the end of the stability period, especially at 40 °C/75% RH. The reason for the slight change in colour and disagreeable odour of the Pheroid™ lotion was due to the same reasons as mentioned under Section C.2.6.2 with the Pheroid™ cream.

C.2.7 Visual particle size distribution measurement with confocal laser scanning microscopy

The best way to evaluate the stability of an emulsion is probably to measure its particle size distribution. Optical microscopy, although a time-consuming technique, is a direct way of measuring droplet sizes larger than 1 μm (Im-Emsap *et al.*, 2002:268).

In this study, CLSM provided a qualitative indication of the particle size distribution of each formulation.

The CLSM provides confocal reflectance images (micrographs) of a given sample. A Nikon PCM 2000 CLSM with a He/Ne laser-543 nm and an argon ion laser 457 – 517 nm was used.

All the formulations that were stored at 25 °C/60% RH, 30 °C/60% RH and 40 °C/75% RH were analysed on the CLSM from months 1, 2, 3 and 6.

The samples for this stability test were prepared by weighing 0.1 g of each formulation in an Eppendorf vial and adding 2 μl Nile-red fluorescent solution (dye) and 500 μl of HPLC-grade water. These Eppendorf vials were vortexed until the formulations were fully dissolved in the solution. 25 μl of each solution was placed on a microscope plate and covered with a cover slip after which the microscope plates were placed in a dark area for 15 min before analysis commenced.

The following images shown below are the CLSM micrographs on months 1 and 6 at 25 °C/60% RH and at 40 °C/75% RH. The samples stored at 30 °C/60% RH are not included in the images below, as they did not differ significantly from those at 25 °C/60% RH.

There were no significant changes in any of the lotions and Pheroid™ lotions CLSM micrographs, but the changes were more significant with the cream, Pheroid™ cream, emulgel and Pheroid™ emulgel formulations.

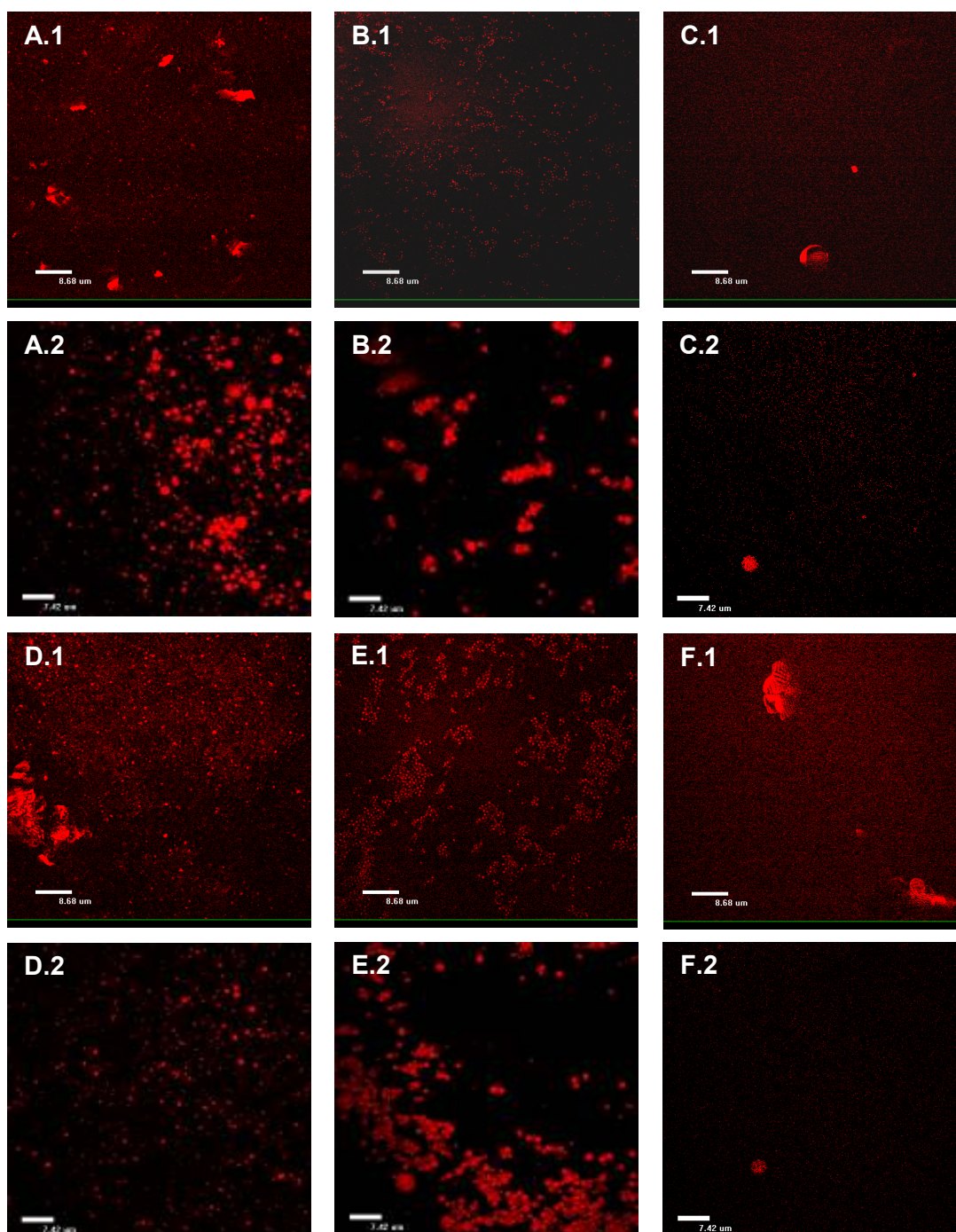


Figure C.19: The confocal laser scanning micrographs of the different formulations. The capital letters are used to illustrate the type of formulation (**A**: cream, **B**: emulgel, **C**: lotion, **D**: Pheroid™ cream, **E**: Pheroid™ emulgel and **F**: Pheroid™ lotion) and the numeral is used to illustrate the month and temperature conditions (**1**: after 1 month at 25 °C/60% RH and **2**: after 6 months at 25 °C/60% RH).

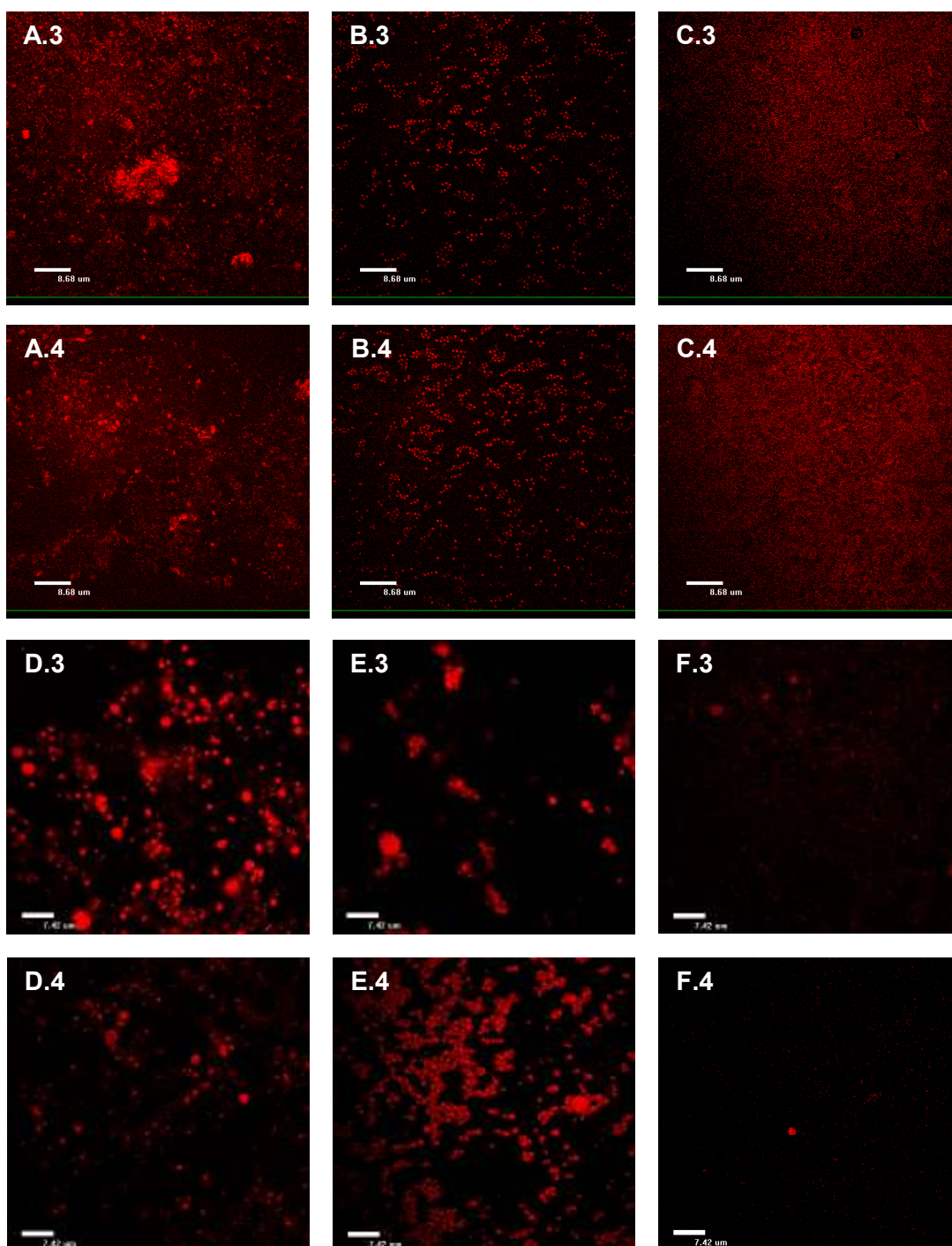


Figure C.20: The confocal laser scanning micrographs of the different formulations. The capital letters are used to illustrate the type of formulation (**A**: cream, **B**: emulgel, **C**: lotion, **D**: Pheroid™ cream, **E**: Pheroid™ emulgel and **F**: Pheroid™ lotion) and the numeral is used to illustrate the month and temperature conditions (**3**: after 1 month at 40 °C/75% RH and **4**: after 6 months at 40 °C/75% RH).

Comparing these images, it could be said that the initial cream and emulgel formulations at storage conditions 25 °C/60% RH and 40 °C/75% RH, differed from those after 6 months, at the

same conditions. The lotions stayed relatively the same, and thus, depicted the best results concerning change in particle size distribution. Differences in the cream and emulgel formulations could be observed through the increase in droplet size over the specific time period. The droplets of the emulgel and Pheroid™ emulgel formulations clogged together after 6 months. The same changes could be seen for the Pheroid™ creams over the period of 6 months.

C.3 CONCLUSION

Stability tests were conducted on 6 different formulations. The tests included an assay, zeta-potential, droplet size, pH, viscosity, mass loss, physical appearance and particle size distribution. These stability tests were conducted on a cream, Pheroid™ cream, emulgel, Pheroid™ emulgel, lotion and Pheroid™ lotion containing 0.5% 5-fluorouracil. The formulations were stored at three different temperature controlled conditions: 25 °C/60% RH, 30 °C/60% RH and 40 °C/75% RH. All tests were conducted on at least 4 of the time points during the 6 month stability period (initially and/or after 1, 2, 3 and 6 months).

The assay yielded increases as well as decreases in the concentrations of the analytes. 5-Fluorouracil concentration remained within the acceptable 5% limit from the initial value, within the cream and Pheroid™ cream during the 6 month stability period. The emulgel, Pheroid™ emulgel, lotion and Pheroid™ lotion did not yield an acceptable 5% limit from the initial values throughout the 6 months. Out of all 6 formulations, the lotion was the most stable due to the fact that 3 of the analytes within the lotion yielded most stable within the lotion, for the period of 6 months.

Zeta-potential and droplet size of all the formulations fluctuated during the 6 month stability period and there was no direct correlation to the results obtained. The zeta-potential values mostly indicated stable particles; due to high enough forces between the particles which made them repel each other. This was observed for all formulations, except for the cream that showed zeta-potential values of between -30 mV and +30 mV at 30 °C/60% RH and 40 °C/75% RH after 1 month, which indicated unstable particles. The zeta-potential of the Pheroid™ cream and Pheroid™ lotion also showed a significant change at month 6; where the forces were much lower than the previous months. This indicated less stable particles as they could not repel each other as much as earlier in the stability period.

The droplet size of the cream fluctuated throughout the stability period, although, at month 6 it showed a significant increase compared to the initial droplet size; this was observed with an increase in temperature. The droplet size of the Pheroid™ cream also fluctuated throughout the stability period, but at month 6 the size did not differ significantly from the initial droplet size; and

the size decreased with an increase in temperature at month 6. The emulgel showed the least fluctuation in droplet size throughout the stability period, and depicted almost the same droplet size at month 6 when compared to the initial droplet size. This phenomenon was not observed at 40 °C/75% RH, where the size decreased significantly at month 6, in comparison to the initial droplet size. The droplet size of the Pheroid™ emulgel also fluctuated throughout the stability period, but when the values at month 6 were compared to the initial values, a decrease in droplet size was observed, and the droplet size at 40 °C/75% RH was less than at the other two temperature controlled conditions at the end of the stability period. The lotion showed the same phenomenon as the Pheroid™ emulgel. The Pheroid™ lotion also fluctuated throughout the stability period. When the values at month 6 were compared to the initial values, the droplet size showed a definite decrease at 25 °C/60% RH, a very slight increase at 30 °C/60% RH, and a decrease at 40 °C/75% RH; overall, the droplet size increased with an increase in temperature at month 6.

The pH of all the formulations, except the lotion, decreased with an increase in temperature over the period of 6 months. The lotion showed the before mentioned phenomenon, except at 30 °C/60% RH on months 1 and 2, where the pH was higher than at 25 °C/60% RH and 40 °C/75% RH. The cream showed the lowest % decrease in pH and the Pheroid™ lotion the highest.

Viscosity did not show a constant change throughout the stability period. The values fluctuated for all the formulations, except for the emulgel and Pheroid™ emulgel. These 2 formulations showed a definite increase from the initial viscosity up to month 2, and then decreased after month 3 and 6. The Pheroid™ emulgel depicted the lowest %RSD, which indicated that it was the most stable formulation concerning viscosity. Whereas the lotion depicted the largest %RSD, which indicated that it was the least stable formulation regarding viscosity.

The mass of all the formulations decreased with time at all three temperature controlled conditions, except for the Pheroid™ emulgel and Pheroid™ lotion. The mass of the Pheroid™ emulgel increased from the initial value to month 1 at all three temperature controlled conditions. From month 1 it gradually decreased to months 2, 3 and 6. The mass of the Pheroid™ lotion only showed an increase from the initial value to month 1 at 25 °C/60% RH, and then decreased with time at all three temperature controlled conditions. The Pheroid™ emulgel showed the lowest % decrease in mass over the 6 month stability period and the Pheroid™ lotion the highest.

The physical appearance of the cream, emulgel and lotion did not show any significant change throughout the stability period. However, all the Pheroid™ formulations showed more

considerable changes in colour. The initial colours were white and became more yellow with an increase in temperature over the period of 6 months. The Pheroid™ lotion showed the most significant change in colour, followed by the Pheroid™ cream and lastly the Pheroid™ emulgel that showed the least significant change of all the Pheroid™ formulations.

CLSM did not show any significant change in particle size distribution when comparing the formulations at 25 °C/60% RH to those at 30 °C/60% RH. Although, it showed a slight change in particle size distribution when comparing the formulations at 25 °C/60% RH to those at 40 °C/75% RH over the stability period of 6 months. Some of the particles clogged together, this phenomenon was especially seen with the emulgel and Pheroid™ emulgel formulations. The creams showed an increase in particle size distribution.

Overall, attention should be given to the stability of such semisolid formulations investigated in this study; and further study is recommended for this purpose.

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APPENDIX D

DIFFUSION STUDIES

D.1 INTRODUCTION

Transdermal drug delivery offers a non-invasive route for drug administration that has many advantages i.e., the potential to continuously control the delivery rate of drugs and avoiding the first-pass effect of the liver (Park *et al.*, 2008:94). This method has been used from as early as the start of the twentieth century, when dimethyl sulphoxide reached the systemic circulation when applied to the skin. This proved that transdermal delivery was possible for externally applied drugs to permeate across the skin (El-Kattan *et al.*, 2000:426). There are a number of events that govern percutaneous absorption following the application of a drug in a thin vehicle film (Flynn, 2002:204); these events are displayed in Figure D.1.

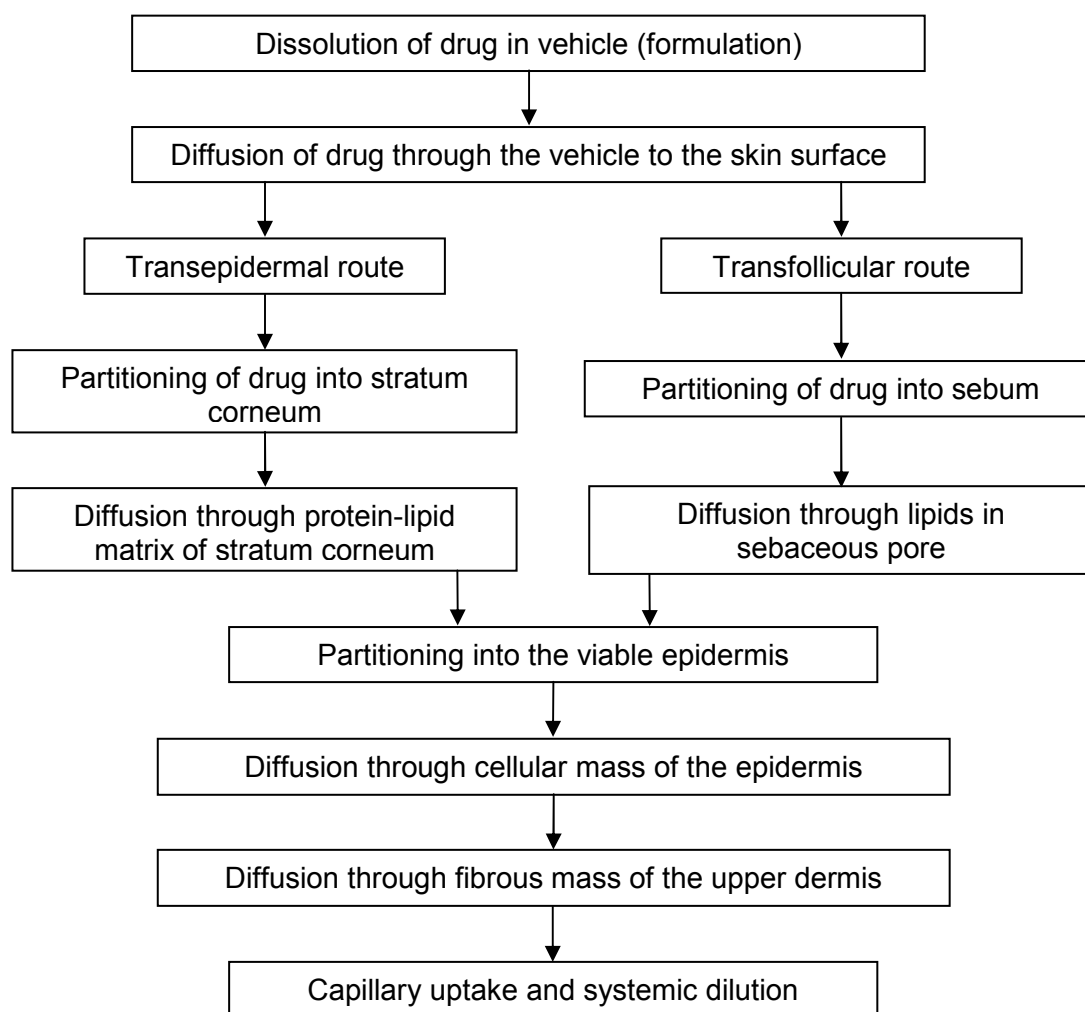


Figure D.1: Events governing the percutaneous absorption of a drug (adapted from Flynn, 2002:205).

In situ and *in vivo* techniques make use of the skin of living humans or experimental animals, whereas *in vitro* methods use excised human or animal skin. This method mimics the living skin *in vivo*, in order to determine percutaneous absorption, which may lead to the development of possible variation in outcomes (Venter *et al.*, 2001:169).

Drug permeation across the skin can be evaluated by using different *in vitro* models, which include vertical Franz diffusion cells; horizontal-type skin permeation systems and flow-through diffusion cells (El-Kattan *et al.*, 2000:428).

In this study, the vertical Franz diffusion cell method was used to conduct the skin diffusion studies and will be explained in Section D.2.8. A total of 11 skin diffusion studies were conducted.

Six studies were with semi-solid formulations; i.e.:

- ❖ cream **(1)**,
- ❖ Pheroid™ cream **(2)**,
- ❖ emulgel **(3)**,
- ❖ Pheroid™ emulgel **(4)**,
- ❖ lotion **(5)** and
- ❖ Pheroid™ lotion **(6)** containing 5-fluorouracil (0.5%).

One study was performed on a commercial product (ointment) **(7)** containing 5-fluorouracil (5%).

Another four studies were carried out with solutions of 5-fluorouracil, i.e.:

- ❖ 0.5% water solution **(8)**,
- ❖ 0.5% Pheroid™ solution **(9)**,
- ❖ 5.0% water solution **(10)** and
- ❖ 5.0% Pheroid™ solution **(11)**.

The reason for preparing 0.5% formulations was based on a study that was previously conducted by Van Dyk (2008:67) on 5-fluorouracil where the influence of the Pheroid™ delivery system on the transdermal delivery of 0.5% and 1.0% 5-fluorouracil preparations was determined. The overall conclusion was that 0.5% 5-fluorouracil in water-based Pheroid™ depicted the best results in terms of transdermal permeation and can be used instead of a 1% preparation, because there were no statistically significant differences in the transdermal penetration between these two preparations. A 0.5% formulation will lead to fewer side effects

and will be more economic to manufacture, hence, it was decided to formulate 0.5% formulations in this study. In order to compare the results with the only commercially available ointment, which consists of 5.0% 5-fluorouracil, 5.0% solutions were also prepared.

D.2 MATERIALS AND METHODS

D.2.1 Analysis of samples by HPLC

The method used for this analysis was developed by Professor Jan du Preez at the North-West University (NWU), Potchefstroom, in the Analytical Technology Laboratory. It has been used and validated in a previous study on 5-fluorouracil conducted by Van Dyk (2008:64). An Agilent® 1100 Series, which consisted of a degasser, a quaternary pump, an auto sampler and a diode array detector, was implemented for analysis. Chemstation Rev. A.06.02 software was used for data acquisition and analysis. A Phenomenex® Synergi Fusion 4 µm Reversed Phase 4.6 mm × 250.0 mm column was used.

The mobile phase consisted of 3% acetonitrile solution which was prepared by mixing 30 ml of acetonitrile with 1 ml orthophosphoric acid; and adding sufficient HPLC-grade water to 1000 ml. The flow rate was set to 1 ml/min, the injection volume was 50 µl, and the UV-detector was set at a wavelength of 266 nm in order to detect 5-fluorouracil. The runtime of each sample was 8 min and the retention time of 5-fluorouracil was approximately 5 min. However, during the analysis, it was a continuous tendency that the retention time of 5-fluorouracil varied throughout the study, as previously mentioned in Appendix A.

At the start of each analysis on the HPLC, a standard solution of 5-fluorouracil was prepared to act as a control for the samples derived from the skin diffusion studies. The standard solution was prepared by weighing approximately 5 mg of 5-fluorouracil in a 50 ml volumetric flask and adding sufficient PBS (pH 7.4) to reach 50 ml, which provided a concentration of 100 µg/ml. Approximately 2 ml of this solution was placed into an auto sampler vial and injected into the HPLC before the samples were injected. Injection volumes varied between 1 and 15 µl, in order to establish a range of concentrations from 1 – 15 µg/ml. The values of the obtained peak areas were plotted against concentration in order to achieve a linear regression line, which was used to calculate the concentration values.

D.2.2 Aqueous solubility

The aqueous solubility of 5-fluorouracil was determined by preparing three over saturated solutions of 5-fluorouracil in PBS (pH 7.4) in glass politop containers. These solutions were placed in a 32 °C water bath with magnetic stirrers and left to stir for 24 h. The solutions were

filtered, diluted a 100 times and analysed by means of HPLC. Each sample was injected in triplicate. A standard solution was prepared in order to act as a control (linearity). It was injected in a concentration range of 0.32 – 4.83 µg/ml.

D.2.3 *n*-Octanol-buffer partition coefficient (log D)

To determine the log D value of 5-fluorouracil, equal amounts of *n*-octanol and PBS (pH 7.4) were added and the mixture shaken. The phases were separated and the internal layer was discarded. A 1000 µg/ml solution was prepared by weighing 2.5 mg 5-fluorouracil and dissolving it in 2.5 ml of the saturated *n*-octanol. This was done in triplicate. 5-Fluorouracil was dissolved in *n*-octanol, because this is the phase in which 5-fluorouracil will not likely dissolve, as it is a water-soluble drug. Equal parts (2.5 ml) of the 5-fluorouracil/*n*-octanol solution and PBS (pH 7.4) were added to the 3 test tubes and left in a rotator for 24 h. All solutions were centrifuged for 15 min at 5 000 rpm with an Eppendorf Centrifuge 5804 R, before the PBS phases were extracted and analysed by means of HPLC (in triplicate). The logarithmic ratio of the concentration in the *n*-octanol-phase relative to the concentration in the PBS phase was used to calculate the log D value.

D.2.4 Preparation of skin

Abdominal skin of anonymous Caucasian (white) female patients was obtained after they had undergone abdominal plastic surgery. Permission from the doctor was given and the skin was collected at the hospital theatre and transported to the university in a cooler box. The process of obtaining and preparing the skin was ethically approved by the Research Ethics Committee of the North-West University; the reference number is 04D08. A copy of the approval from the Ethics Committee is attached in Appendix F. Upon arrival at the transdermal laboratory of the university, the skin was frozen (within 24 h from the operation) at -20 °C until the skin was prepared. The preparation of the skin consisted of the following steps:

- ❖ Skin was taken out of the freezer and left in the preparation area to defrost a little.
- ❖ The majority of the hypodermis (fat) layer was carefully removed with a scalpel.
- ❖ The rest of the fat layer was scraped off with a blunt scalpel to ensure that the entire hypodermis was removed.
- ❖ Skin was gently wiped with a paper towel to ensure no fatty fluid was left underneath or on top of the skin due to handling.
- ❖ Skin was placed on Whatman® filter paper (with the stratum corneum facing upwards) on a hard plastic board.

- ❖ A punch with a diameter of approximately 15 mm was placed on the skin and circles were punched into the skin.
- ❖ These circles were stored on a clean piece of Whatman[®] filter paper and covered with foil, to ensure that no air could reach the skin as this would cause it to dry out.
- ❖ The circles were kept frozen at -20 °C until the day of the experiment.
- ❖ On the day of the experiment, the circles were left outside the freezer to defrost before they were placed on the Franz cells.

D.2.5 Preparation of receptor phase solution

Typical solutions that are used as the receptor phase in permeation studies are physiological saline or PBS (El-Kattan *et al.*, 2000:428). In this study PBS (pH 7.4) was used in order to reproduce the pH of the blood. It was prepared by the following procedure:

- ❖ 13.620 g potassium dihydrogen orthophosphate (KH_2PO_4) was weighed and dissolved in 500 ml HPLC-grade water.
- ❖ 3.1472 g sodium hydroxide (NaOH) pearls was weighed and dissolved in 786.8 ml HPLC-grade water.
- ❖ These two solutions were added together and stirred with a magnetic stirrer until the KH_2PO_4 and NaOH were fully dissolved.
- ❖ The pH was measured and set to 7.4 with 10% NaOH or 10% orthophosphoric acid solution.

D.2.6 Preparation of 5-fluorouracil containing solutions for donor phase

The water solutions were prepared by weighing 5-fluorouracil (0.5 g or 5.0 g) in respective glass containers and adding sufficient HPLC-grade water to obtain a 100 g solution of each. These solutions were stirred with a magnetic stirrer overnight and used the following morning to conduct the Franz cell diffusion studies.

Pheroid[™] solutions were prepared by weighing the Pheroid[™] ingredients in two separate containers (one for the 0.5% and the other for the 5.0% solution). This will be referred to as Phase A. Sufficient water for each of the solutions (to obtain a 100 g solution of each) was also weighed and will be referred to as Phase B. Both Phase A (oil) and Phase B (water) were heated to 80 °C. After Phase A and Phase B reached 80 °C, the oil-phases were added to the water whilst the mixture was homogenised at 13 500 rpm until 40 °C was reached. The mixtures were left to cool down to room temperature. 5-Fluorouracil (0.5 g and 5.0 g) was

weighed in respective glass containers and added to the mixed Phase A and B solutions. Solutions were stirred with a magnetic stirrer overnight and used the following morning to conduct the Franz cell diffusion studies.

D.2.7 Diffusion studies

Franz cells comprise of two compartments, a donor and a receptor compartment (El-Kattan *et al.*, 2000:428). The donor compartment was the upper part of the Franz cell that contained the donor phase which was the formulation or solution that had to be tested. Donor compartments were filled with 1 ml of each formulation. The receptor compartment had a volume capacity of ± 2 ml and a diffusion area of 1.075 cm^2 . It contained the receptor phase that consisted of PBS with pH 7.4 (prepared as described in Section D.2.5). Figure D.2 illustrates a Franz cell.

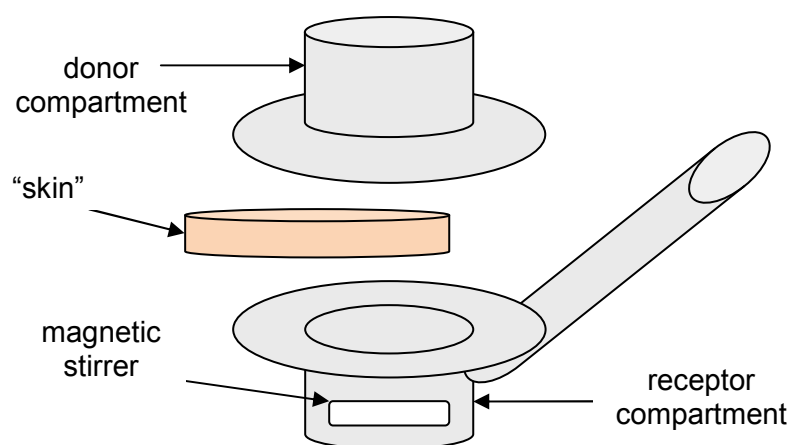


Figure D.2: Representation of a Franz cell

D.2.7.1 Membrane diffusion studies

Membrane diffusion studies were conducted prior to the skin diffusion studies. This was conducted with the six semisolid formulations in order to determine if 5-fluorouracil was released from each formulation. The same method was used as with the Franz cell skin diffusion studies (as discussed in Section D.2.8), except for using membranes, instead of skin. Polytetrafluoroethylene (PTFE) membrane filters with a thickness of $0.45 \mu\text{m}$ from PALL Corporation, Ann Arbor Michigan, were used. Membrane studies were conducted within 6 h, with extraction times at 0.5, 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0 h.

D.2.7.2 Skin diffusion studies

A total of 12 Franz cells were used for one skin diffusion study. Ten cells contained the formulation, 1 cell contained a placebo formulation (without the active ingredient, 5-fluorouracil) and 1 cell contained PBS, the last two cells mentioned were prepared to act as control samples

for the HPLC analysis. The preparation and process of a complete skin diffusion study will briefly follow in Section D.2.8. Tape stripping was performed after the 12 h period of each skin diffusion study, as described in Section D.2.9.

D.2.8 Preparation process and proceedings of a complete skin diffusion study

- ❖ Place small magnetic stirrers in each receptor phase.
- ❖ Rub the surface of both receptor and donor compartments with a thin layer of Dow Corning[®] vacuum grease.
- ❖ Place the skin circles (with stratum corneum facing upwards) on the surface of each receptor compartment and cover with the donor compartment.
- ❖ Seal the Franz cells with sufficient Dow Corning[®] vacuum grease to prevent leakage.
- ❖ Secure the Franz cells in a horse shoe clamp to insure that the skin is firmly mounted between the donor and receptor compartments.
- ❖ Carefully place 2 ml of the receptor phase (PBS, 37 °C) in each receptor compartment, ensuring that there are no air bubbles present.
- ❖ Place 1 ml of the donor phase (formulation or solution, 32 °C) in each donor compartment and seal with a piece of Parafilm[®] and a plastic cap to prevent evaporation.
- ❖ Place the 12 Franz cells in a Franz cell tray and place the tray in a water bath at 37 °C. This point in time is the commencement of the 12 h period of the study.
- ❖ Withdraw the entire volume of each receptor phase at 2 h intervals and replace the receptor compartments with fresh PBS that was preheated to 37 °C.
- ❖ Fill HPLC vials with the withdrawn samples from the receptor compartments and immediately analyse the samples by means of HPLC to determine the concentration 5-fluorouracil that permeated through the skin.

D.2.9 Tape stripping

Tape stripping is the subsequent removal of the cell layers of the stratum corneum-epidermis by using adhesive tapes, after topical application and penetration of formulations. The tape strips contain an amount of penetrated formulation that can be determined by analytical chemical methods. This method is simple and efficient in the assessment of quality and efficacy of cosmetic and dermatologic formulations (Lademann *et al.*, 2009:317).

After the removal of the skin circles from the Franz cells, the areas of diffusion were dabbed with a paper towel to clear the excess formulations or solutions on the surface. 3M Scotch[®]

Magic™ Tape was cut into pieces (± 3 cm in length). The first piece of tape was firmly pressed on and removed from the diffusion area, after which it was discarded. This process was repeated on all 12 skin circles with 15 tape strips each after which the strips (stratum corneum-epidermis) were placed in a glass sample holder containing 5 ml PBS (pH 7.4). These solutions were kept in a refrigerator overnight at 4 °C and analysed the following day. The diffusion area of the remaining skin circles were cut into pieces (epidermis-dermis) and placed in 5 ml PBS (pH 7.4) and left overnight. The preparation of the samples for analysis on the HPLC, involved the extraction of the PBS with a syringe, filtration, using Acrodisc® Premium 25 mm syringe filters, with GxP/0.45 µm GHP membranes, from PALL Life Sciences, into Eppendorf vials; and centrifugating all the samples for 7 min at 10 000 rpm and 4 °C on an Eppendorf Centrifuge 5804 R. After centrifugation, auto sampler vials were filled with the solutions and analysed by means of HPLC.

D.2.10 Data analysis

The data obtained from the skin diffusion experiments, as well as the membrane diffusion experiments were processed and the average % diffused and average cumulative amount/area ($\mu\text{g}/\text{cm}^2$) for 5-fluorouracil was determined. The % diffused was calculated by determining the amount of active ingredient that diffused through the skin as a percentage of the amount of active ingredient originally applied to the skin.

D.2.11 Statistical data analysis of Franz cell diffusion studies and tape stripping

The data analysis contained both descriptive and inferential statistical methods. For the descriptive analysis, medians were used as summary statistics and box-plots (with the data points and average superimposed) as graphical representation.

The inferential analysis involved formal hypothesis testing to compare the preparations (formulations and solutions) and Pheroid™ application, with respect to dermis concentrations and epidermis concentrations. Due to the skewness and heterogeneity of variance (for some of the variables), as well as small sample sizes, non-parametric methods were used throughout. More specific, the two-way test proposed by Brunner *et al.* (1997) to test the existence of an interaction effect between the preparations (formulations and/or solutions) and the application of Pheroid™ was used. To test the main effect of preparations (formulations and/or solutions), a one-way test of Brunner *et al.* (1997) was used, followed by post-hoc analysis, using a Bonferroni correction, as described in Dmitrienko *et al.* (2007). To test the main effect of Pheroid™ application, the Mann-Whitney test was used. The Brunner *et al.* (1997) test is, henceforth, referred to as the BDM-test. All inferential statistics were performed at the 5% level of significance. Differences were significant when the p-value was less than 0.05. An

illustration of the two-way design for statistical significance is given in Figure D.3., which was used for comparing all formulations and solutions.

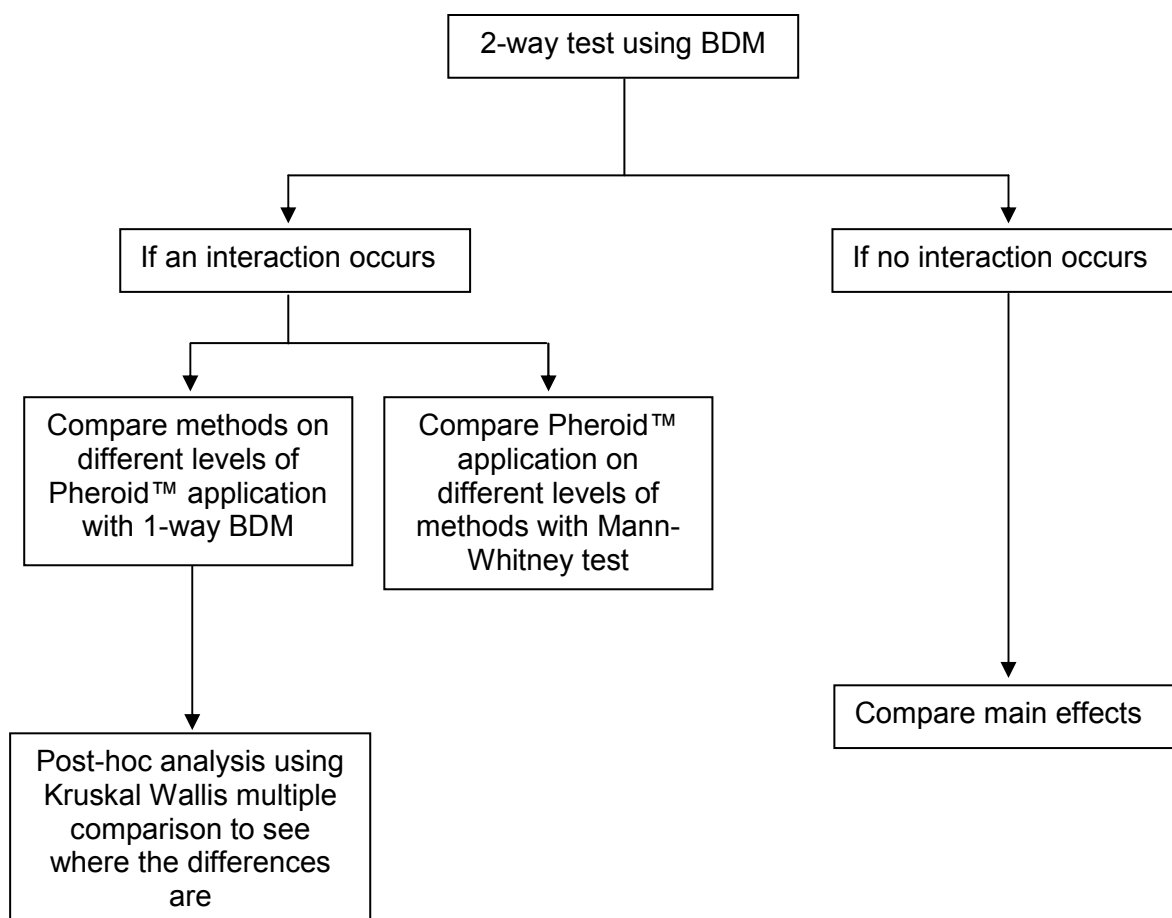


Figure D.3: Illustration of the 2-way design method used to yield statistical significance

All inferential statistics were performed at the 5% level of significance. Differences were significant when the p-value was less than 0.05. The statistical analysis of the procured data was conducted by Statistical Consultation Services, North-West University, Potchefstroom Campus, using the SPSS and R statistical software.

D.3 RESULTS AND DISCUSSION

D.3.1 Aqueous solubility

Ideally, a drug should possess both lipoidal and aqueous solubility values. If it is too hydrophilic, the molecule will be unable to transfer into the stratum corneum; and if it is too lipophilic, the drug will tend to remain in the layers of the stratum corneum. A drug should have an aqueous solubility of more than 1 mg/ml to ideally be delivered by the transdermal route (Naik *et al.*, 2000:319).

The solubility of 5-fluorouracil was determined to be 0.56 mg/ml at 32 °C in PBS (pH 7.4), which did not indicate ideal delivery by the transdermal route. When compared to a previous study, the value obtained was much lower than the 14.3 mg/ml obtained by Yamane *et al.* (1995:250). The variation between the solubility values of this study and the study conducted by Yamane *et al.* (1995:250) might be due to different temperatures, buffers or pH's used between the two studies.

D.3.2 *n*-Octanol-buffer partition coefficient (log D)

The partition coefficient of a drug is usually referred to as the oil/water equilibrium partition coefficient and is therefore a measure of the drug's lipid solubility. This has been established by adding a drug to a combination of equivalent volumes of a lipophilic fluid (octanol) and a hydrophilic fluid (water/buffer). The mixture is then shaken energetically to encourage the partitioning of the drug into each of the water and lipid phases. Two phases will separate after equilibrium has been reached and thereafter the drug can then be analysed (Hillery, 2001:21). When one considers the ideal properties any drug should have for transdermal delivery, drugs with a log P (octanol-water partition coefficient) of ≤ 2 and that have a reasonable lipophilic/hydrophilic character, are regarded as prospective candidates for this method (Guy & Hadgraft, 1989:70, 71).

The experimental log D value obtained for 5-fluorouracil was -0.98, which indicated that it is a polar molecule. This implicated that it has a hydrophilic nature; therefore, it will have difficulty penetrating through the highly lipophilic stratum corneum (Singh *et al.*, 2005:99). Generally, drugs with a log P value in the range of 1 – 3, are considered to have optimum partition behaviour (Hadgraft, 2004:292). When compared to a previous study conducted by Quigley & Lloyd (2002:244), the log P of 5-fluorouracil was determined as -0.83, which barely differed from the value obtained during this study.

D.3.3 Membrane diffusion studies

The average % 5-fluorouracil that diffused, as well as the average cumulative amount per area of 5-fluorouracil within each 0.5% semisolid formulation, derived from the membrane diffusion studies, are shown in Table D.1.

Table D.1: Data obtained from membrane diffusion studies after 6 h

Formulation	Average % diffused	Average cumulative amount/area ($\mu\text{g}/\text{cm}^2$)
Cream (1)	0.013	1.232
Pheroid™ cream (2)	0.025	2.350
Emulgel (3)	0.020	1.762
Pheroid™ emulgel (4)	0.023	2.060
Lotion (5)	0.012	1.155
Pheroid™ lotion (6)	0.024	2.186

The highest % 5-fluorouracil diffused after 6 h was through **(2)** (0.025%) this was followed by **(6)** (0.024%), **(4)** (0.023%), **(3)** (0.020%), **(1)** (0.013%) and lastly, **(5)** (0.012%). In each of the parameters shown in Table D.1 the Pheroid™ formulations achieved higher values than those without the addition of Pheroid™.

D.3.4 Franz cell skin diffusion studies

Table D.2 shows the average cumulative concentration ($\mu\text{g}/\text{cm}^2$) and average % diffused values for all the formulations **((1) – (6))**, the commercial product **(7)** and the solutions **((8) – (11))**.

Table D.2: Data obtained from skin diffusion studies after 12 h for **(1) – (11)**

Formulation	Average cumulative concentration ($\mu\text{g}/\text{cm}^2$)	Average %diffused (%)
Cream (1)	7.593	0.070
Pheroid™ cream (2)	7.068	0.063
Emulgel (3)	10.855	0.099
Pheroid™ emulgel (4)	8.973	0.079
Lotion (5)	9.060	0.081
Pheroid™ lotion (6)	10.014	0.090
Commercial product (7)	2.516	0.002
0.5% water solution (8)	9.631	0.093
0.5% Pheroid™ solution (9)	6.184	0.066
5.0% water solution (10)	11.158	0.012
5.0% Pheroid™ solution (11)	2.583	0.003

The average cumulative amount per area ($\mu\text{g}/\text{cm}^2$) for each Franz cell was plotted against time for the formulations **((1) – (6))** in Figures D.4, D.5, D.6, D.7, D.8 and D.9; for **(7)** in Figure D.10; and for the solutions **((8) – (11))** in Figures D.11, D.12, D.13 and D.14, respectively.

D.3.4.1 Cream (1)

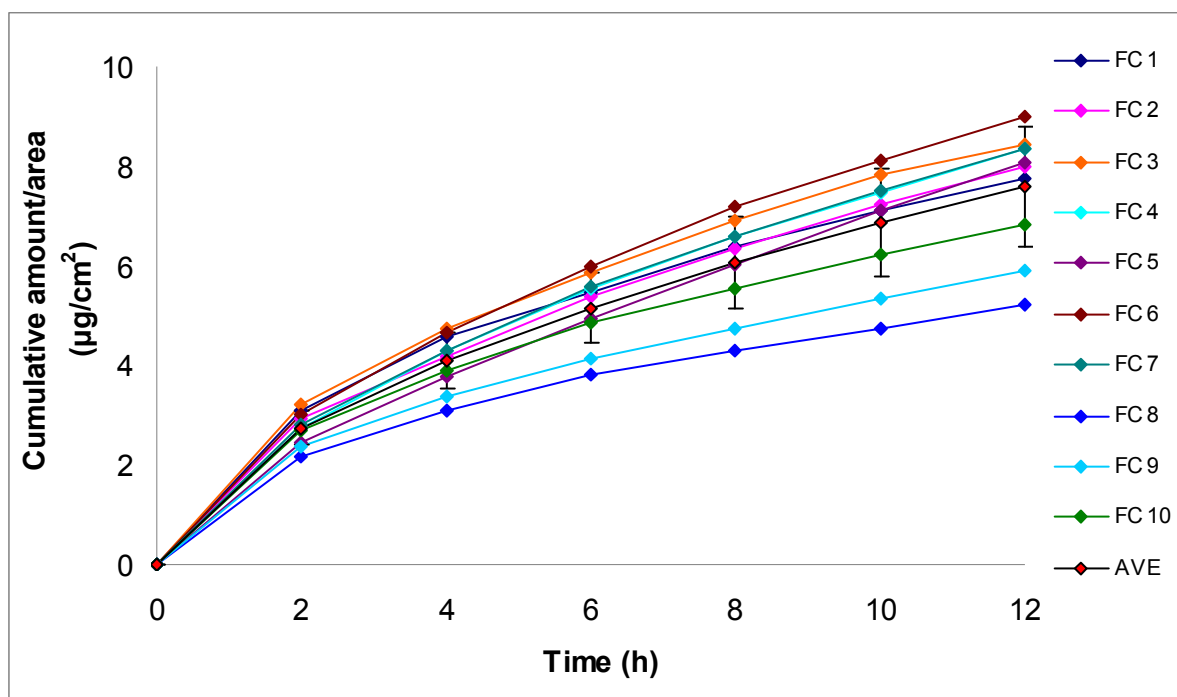


Figure D.4: Cumulative amount per area ($\mu\text{g}/\text{cm}^2$) for cream that penetrated the skin as a function of time

D.3.4.2 Pheroid™ cream (2)

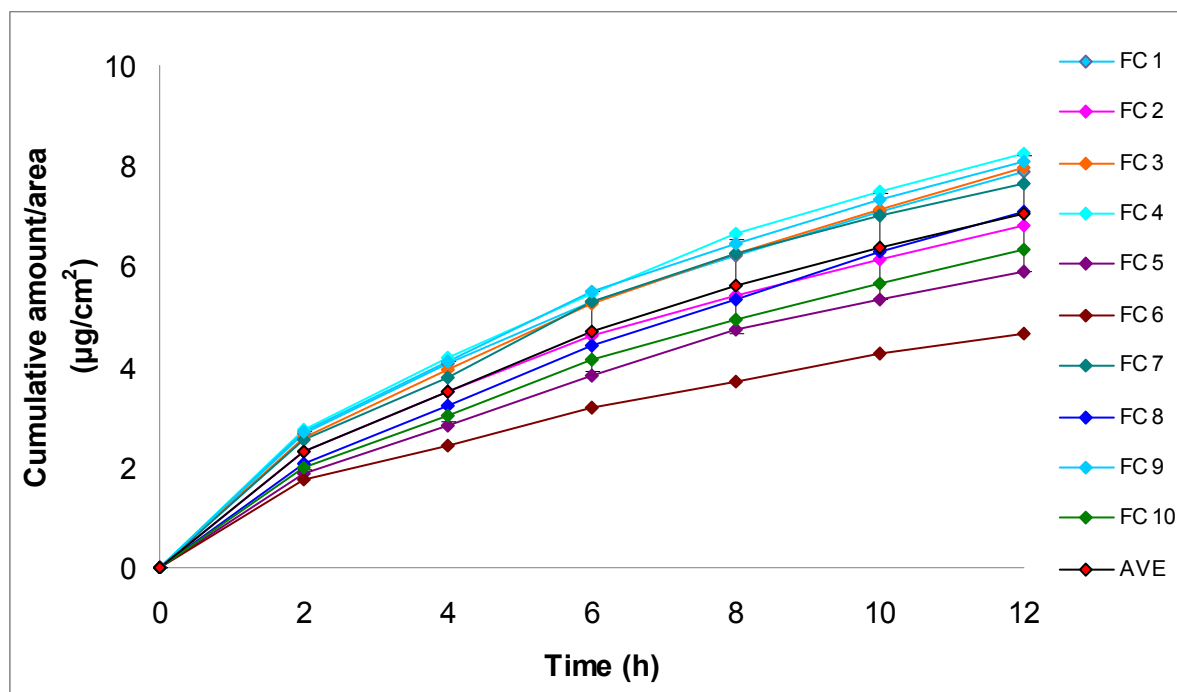


Figure D.5: Cumulative amount per area ($\mu\text{g}/\text{cm}^2$) for Pheroid™ cream that penetrated the skin as a function of time

D.3.4.3 Emulgel (3)

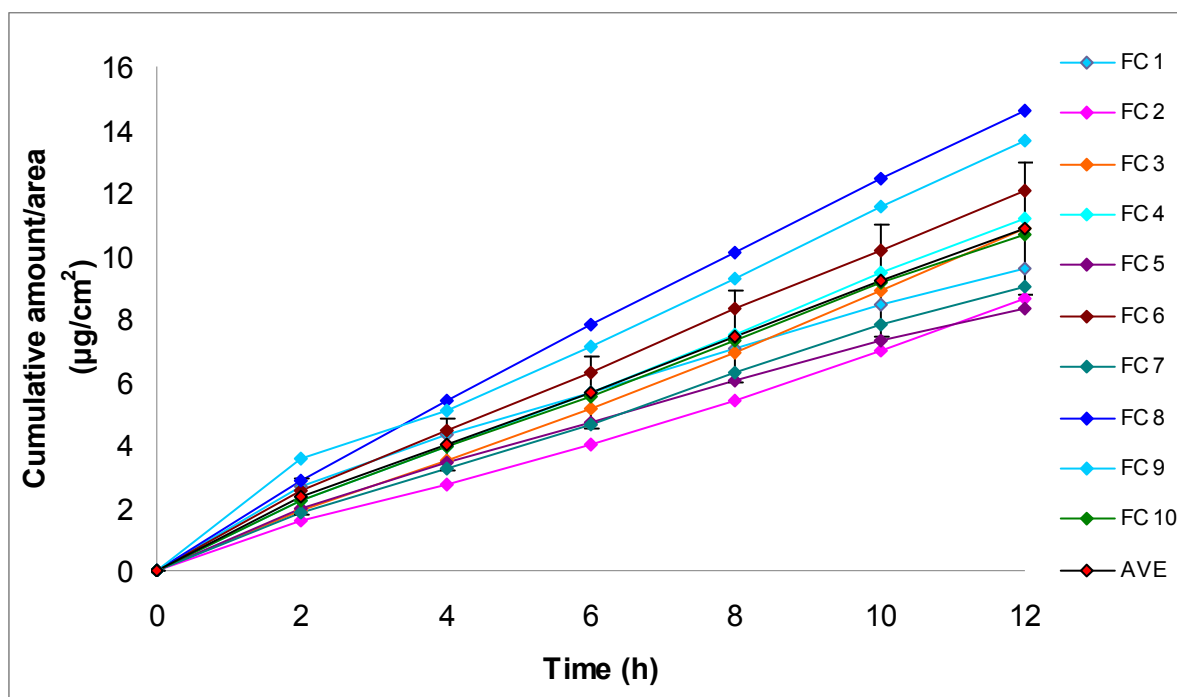


Figure D.6: Cumulative amount per area ($\mu\text{g}/\text{cm}^2$) for emulgel that penetrated the skin as a function of time

D.3.4.4 Pheroid™ emulgel (4)

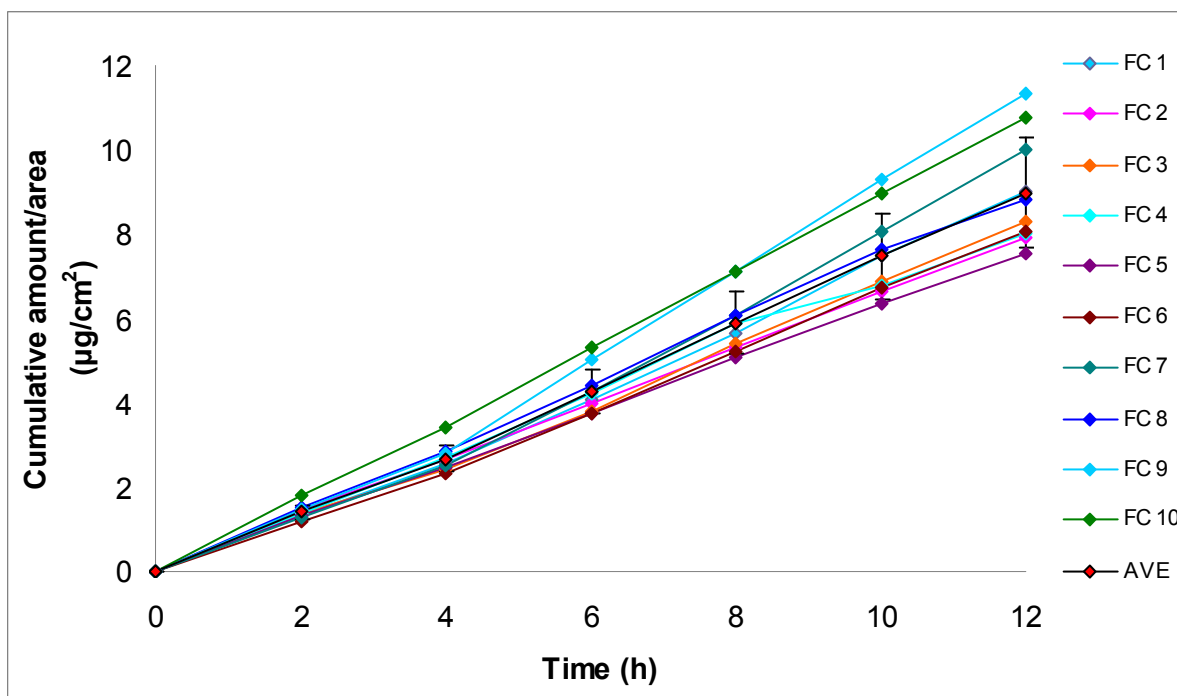


Figure D.7: Cumulative amount per area ($\mu\text{g}/\text{cm}^2$) for Pheroid™ emulgel that penetrated the skin as a function of time

D.3.4.5 Lotion (5)

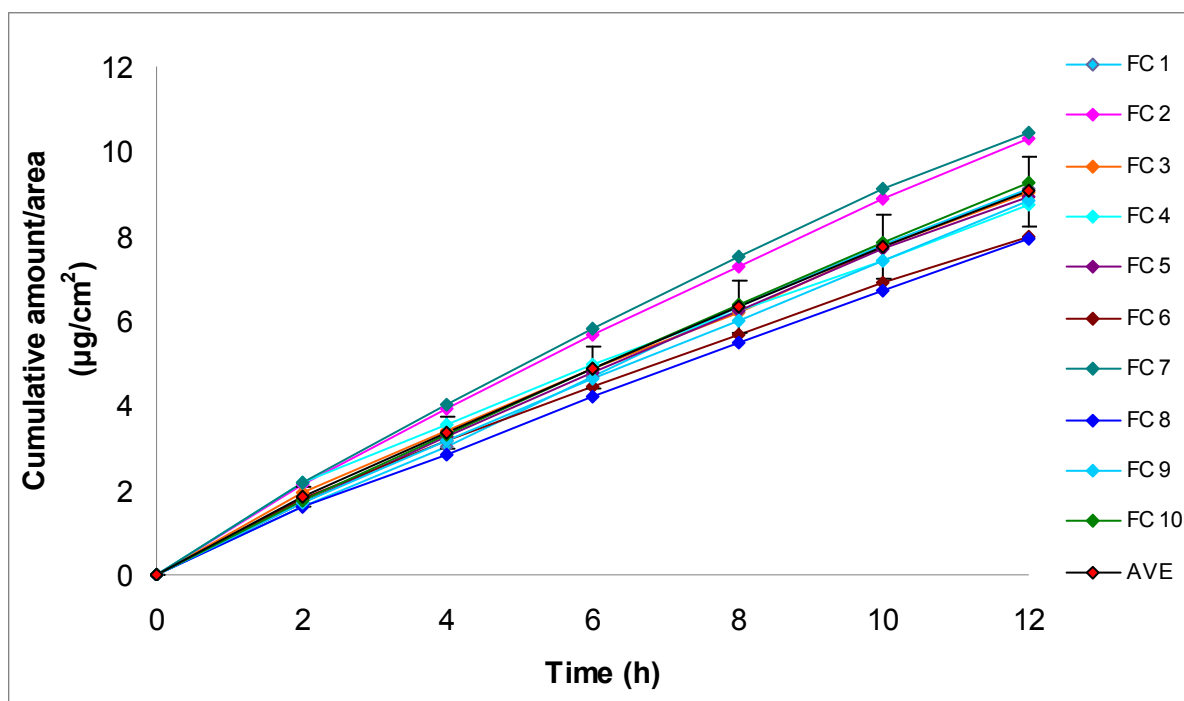


Figure D.8 Cumulative amount per area ($\mu\text{g}/\text{cm}^2$) for lotion that penetrated the skin as a function of time

D.3.4.6 Pheroid™ lotion (6)

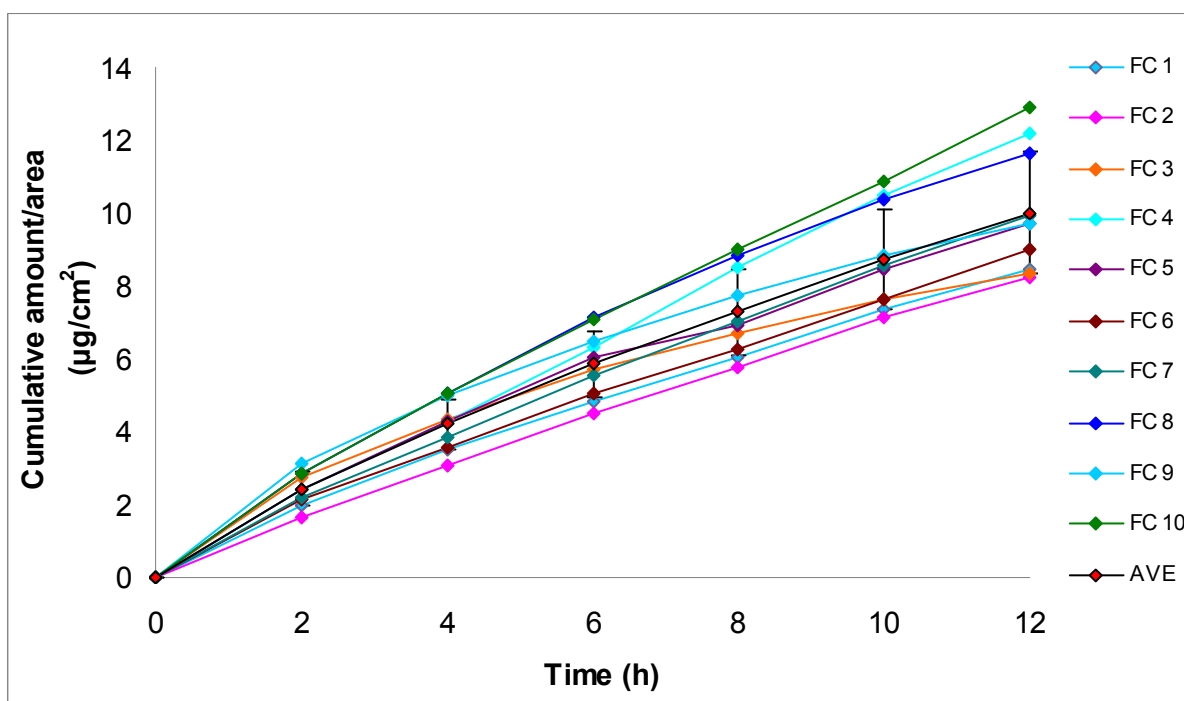


Figure D.9: Cumulative amount per area ($\mu\text{g}/\text{cm}^2$) for Pheroid™ lotion that penetrated the skin as a function of time

D.3.4.7 Commercial product (7)

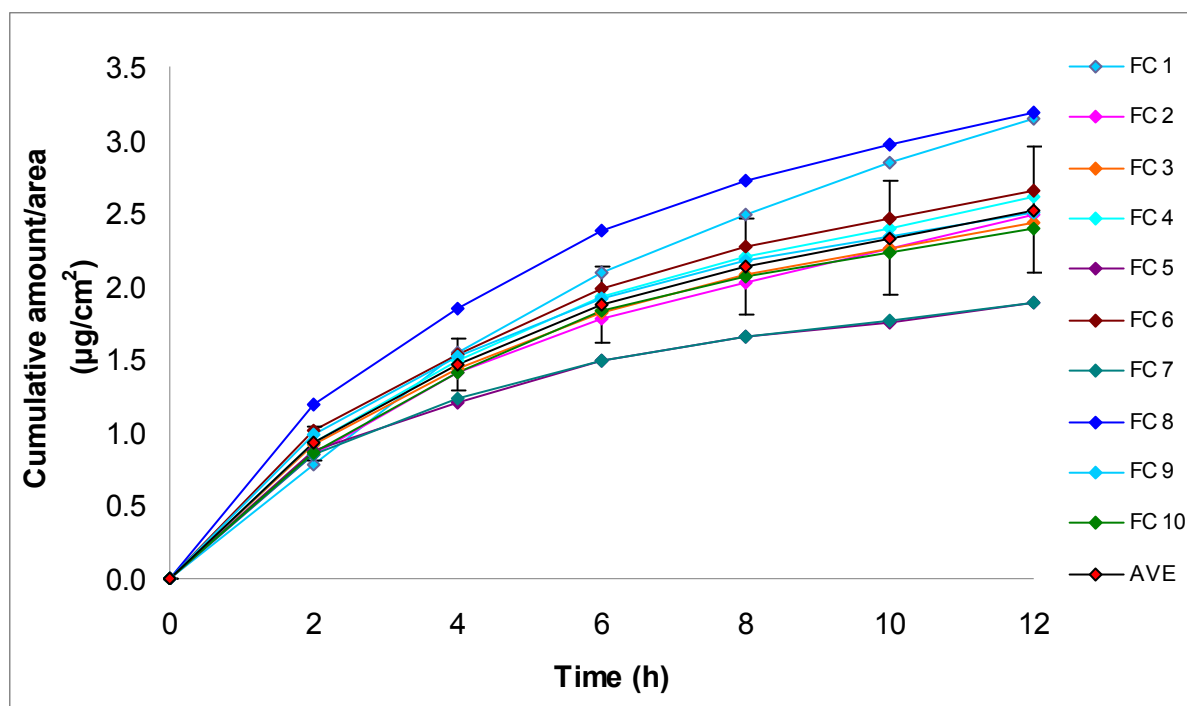


Figure D.10: Cumulative amount per area ($\mu\text{g}/\text{cm}^2$) for the commercial product that penetrated the skin as a function of time

D.3.4.8 0.5% Water solution (8)

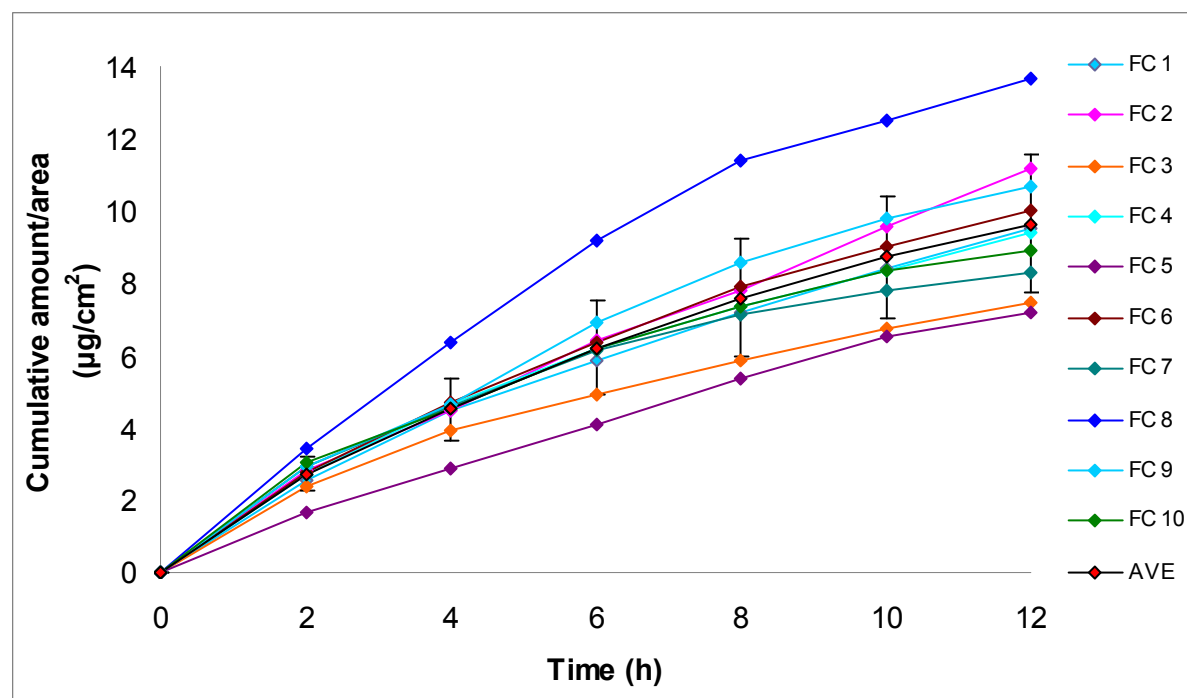


Figure D.11: Cumulative amount per area ($\mu\text{g}/\text{cm}^2$) for 0.5% non-Pheroid™ (water) solutions that penetrated the skin as a function of time

D.3.4.9 0.5% Pheroid™ solution (9)

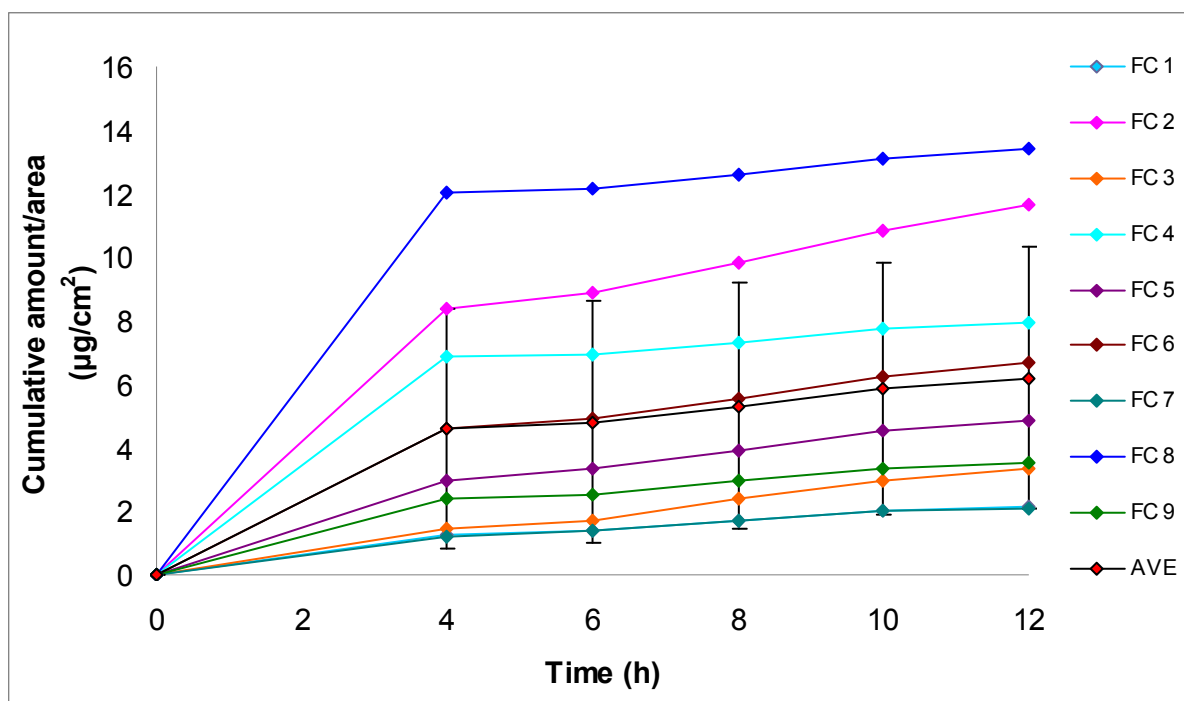


Figure D.12: Cumulative amount per area ($\mu\text{g}/\text{cm}^2$) for 0.5% Pheroid™ solutions that penetrated the skin as a function of time

D.3.4.10 5.0% Water solution (10)

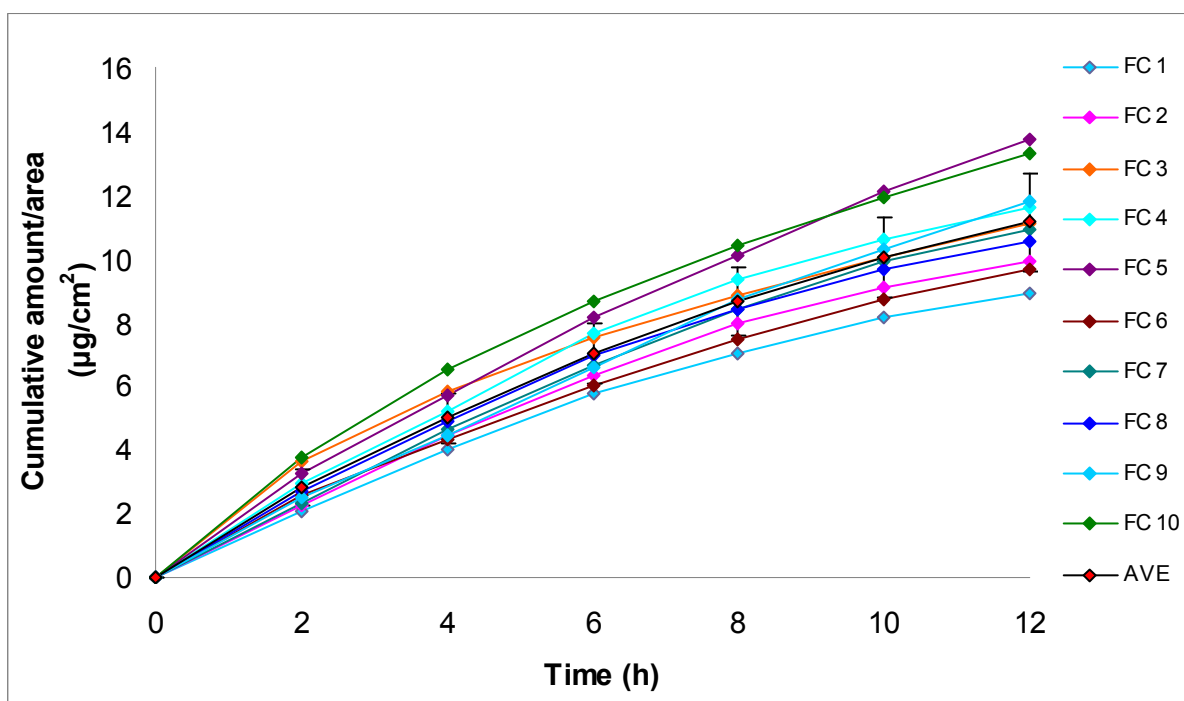


Figure D.13: Cumulative amount per area ($\mu\text{g}/\text{cm}^2$) for 5.0% non-Pheroid™ (water) solutions that penetrated the skin as a function of time

D.3.4.11 5.0% Pheroid™ solution (11)

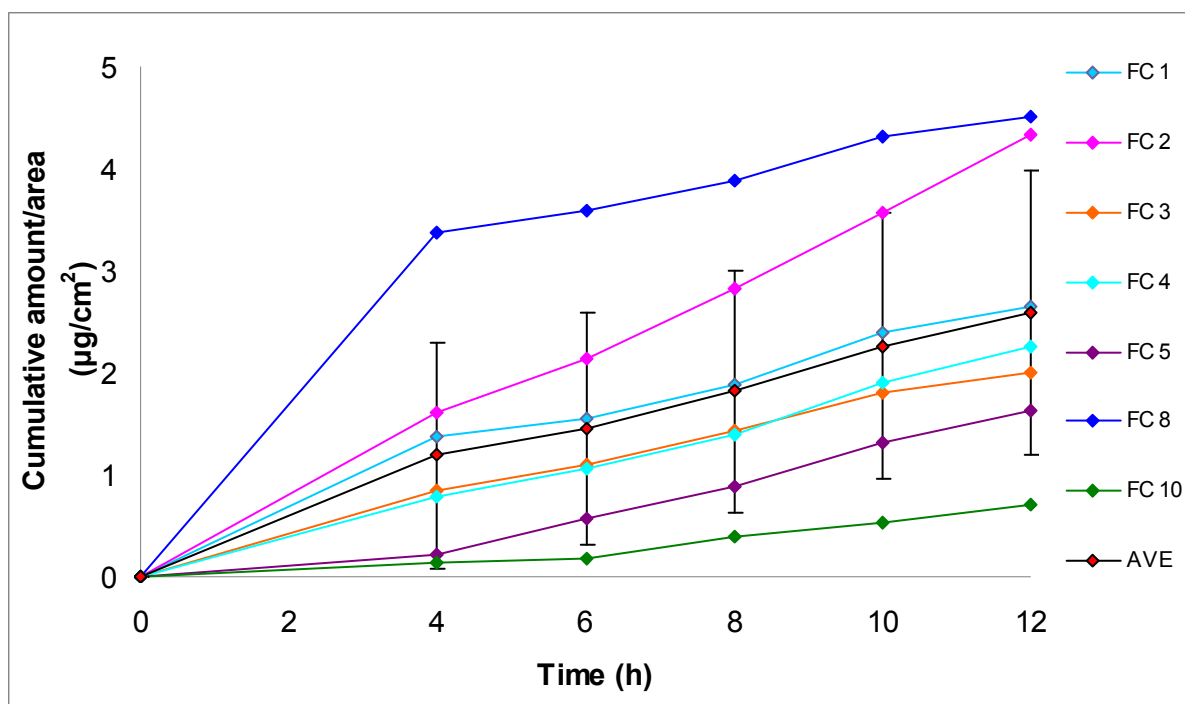


Figure D.14: Cumulative amount per area ($\mu\text{g}/\text{cm}^2$) for 5.0% Pheroid™ solutions that penetrated the skin as a function of time

When average cumulative concentration of the 0.5% formulations ((1) – (6)) were compared, (3) ($10.855 \mu\text{g}/\text{cm}^2$) depicted the highest average cumulative concentration, followed by (6) ($10.014 \mu\text{g}/\text{cm}^2$), (5) ($9.060 \mu\text{g}/\text{cm}^2$), (4) ($8.973 \mu\text{g}/\text{cm}^2$), (1) ($7.593 \mu\text{g}/\text{cm}^2$) and lastly, (2) ($7.068 \mu\text{g}/\text{cm}^2$).

The average % diffused values of the 0.5% formulations ((1) – (6)) and 0.5% solutions ((8) – (9)) are shown in Figure D.15.

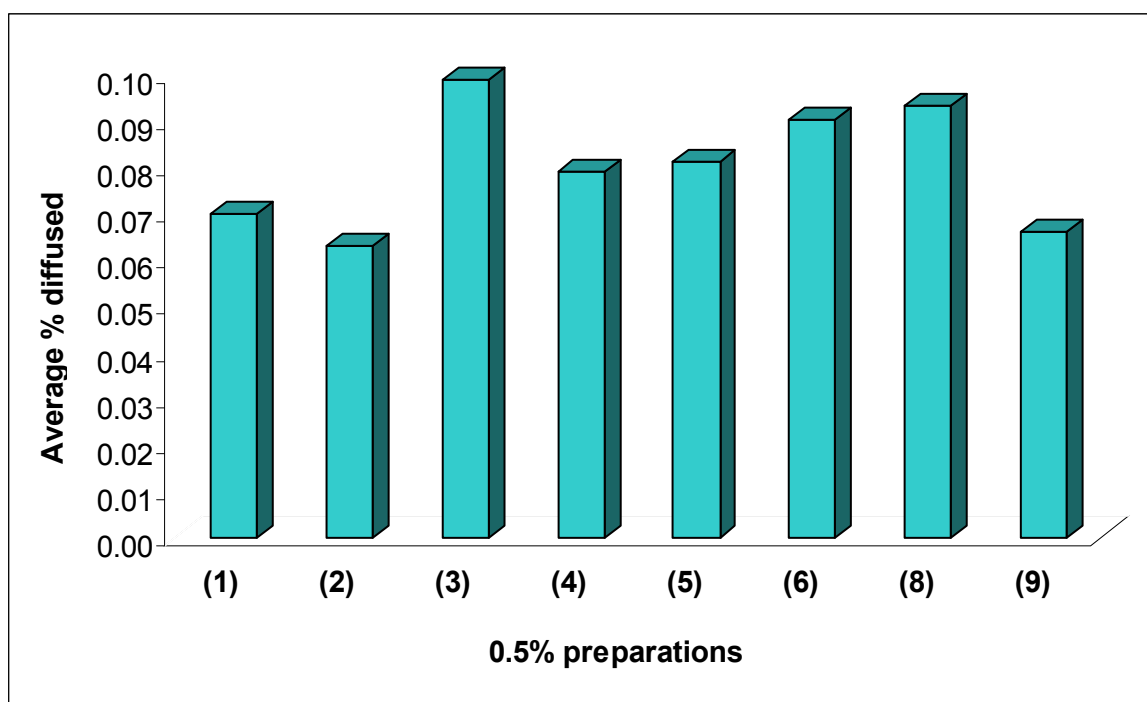


Figure D.15: Comparison of average % diffused for 0.5% formulations ((1) – (6)) and 0.5% solutions ((8) – (9))

Comparing the average % diffused values, the following was observed: **(3)** (0.099%) achieved the highest average % diffused, followed by **(8)** (0.093%), **(6)** (0.090%), **(5)** (0.081%), **(4)** (0.079%), **(1)** (0.070%), **(9)** (0.066%) and lastly, **(2)** (0.063%).

When comparing the non-Pheroid™ 0.5% formulations ((1), (3) and (5)) and not taking into account the influence of Pheroid™ ((2), (4) and (6)) or solutions ((8) and (9)), it was depicted that **(3)** had the highest average % diffused value. The reason for this could be due to the high fluid content of the emulgel that hydrated the skin and caused the stratum corneum to swell and open its structures, which in turn lead to an increase in penetration (Benson, 2005:28). **(1)** showed the poorest average % diffused value, which could be due to the high concentration of thickening agent within the cream that caused a stiff appearance and thus, less hydration on the skin, which resulted in a decreased penetration.

After the addition of Pheroid™ formulations ((2), (4) and (6)) and solutions ((8) and (9)) was taken into account, and comparing them to the non-Pheroid™ equivalent, all 0.5% formulations and solutions showed lower average % diffused values with Pheroid™, than without Pheroid™, except for **(6)**, which depicted a higher average % diffused value than **(5)**. The reason for this phenomenon could be that the Pheroid™ lotion consisted of the largest total content of surface active ingredients, when compared to all the other formulations, which may have caused

enhanced penetration of 5-fluorouracil by a mechanism involving interaction of the surfactant with the structured lipids of the stratum corneum (Wiechers & Watkinson, 2008:77).

When solutions ((8) and (9)) were weighed against formulations ((1) – (6)); (8) achieved a higher average % diffused value than (1) and (2), which could also be due to fact that the last two formulations mentioned, were creams that consisted of oily ingredients, whereas this solution consisted only of water and active ingredient. Comparing (9) to the other formulations and solution, it achieved the second poorest average % diffused value and (2) depicted the lowest % diffused, as this was a very thick and oily formulation.

The average % diffused values of the 5.0% solutions ((10) – (11)) and the 5.0% commercial product (7) are shown in Figure D.16.

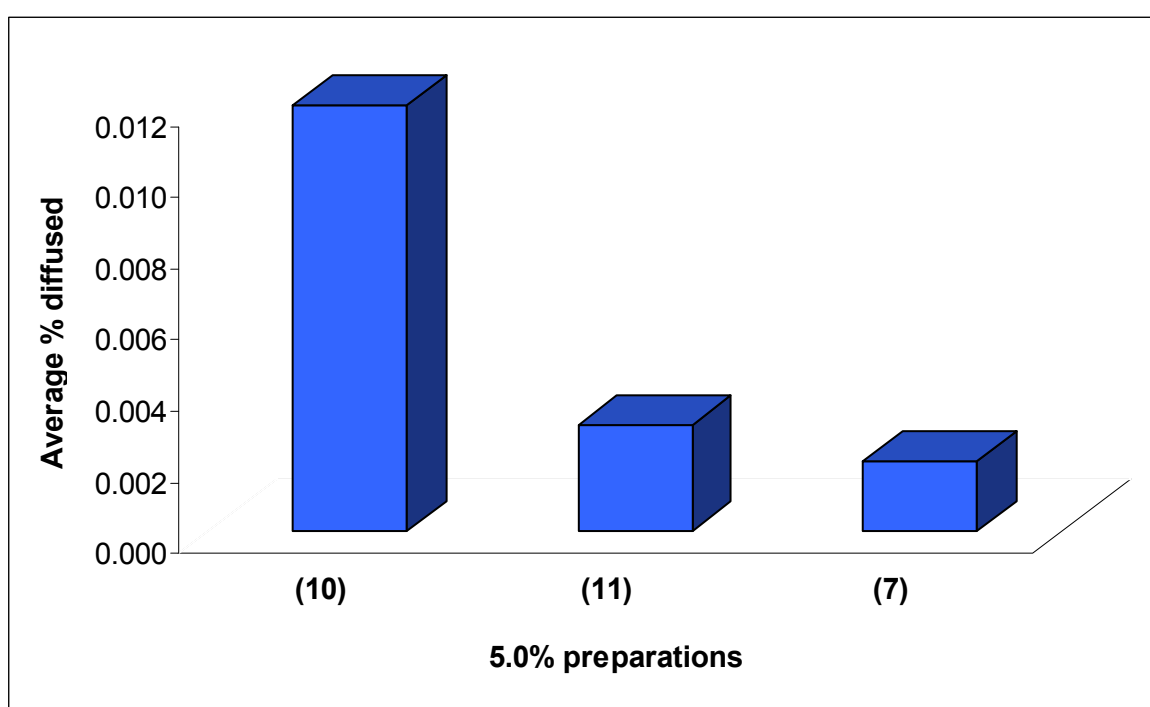


Figure D.16: Comparison of average % diffused for 5.0% solutions ((10) – (11)) and 5.0% commercial product (7)

After the average cumulative concentration of the 5.0% solutions ((10) – (11)) and the 5.0% commercial product (7) were compared, (10) depicted the highest cumulative concentration ($11.158 \mu\text{g}/\text{cm}^2$), followed by (11) ($2.583 \mu\text{g}/\text{cm}^2$) and (7) the lowest ($2.516 \mu\text{g}/\text{cm}^2$).

Comparing the average % diffused values, the following was portrayed: (10) (0.012%) achieved the highest average % diffused, followed by (11) (0.003%) and lastly, (7) (0.002%).

The water solution ((10)) provided enhanced penetration when compared to the average % diffused values of both oily solution (11) and formulation (7). The preparation with the highest oil concentration (7) obtained the lowest average % diffused value. As a result of the aforementioned phenomenon, it could be assumed that the hydrophilic nature of 5-fluorouracil caused it to have higher availability within a hydrophilic environment (water solution (10)), which in turn lead to elevated diffusion through the skin. The oily preparation (Pheroid™ (11)), on the other hand, did not result in a favourable environment for 5-fluorouracil, and thus caused less diffusion through the skin. By taking this into account, (7) depicted the poorest % diffused, due to it being an ointment, which was even oilier than (11).

The average % diffused values of the 0.5% formulations ((1) – (6)), 0.5% solutions ((8) – (9)), 5.0% solutions ((10) – (11)) as well as the 5.0% commercial product (7), are shown in Figure D.17.

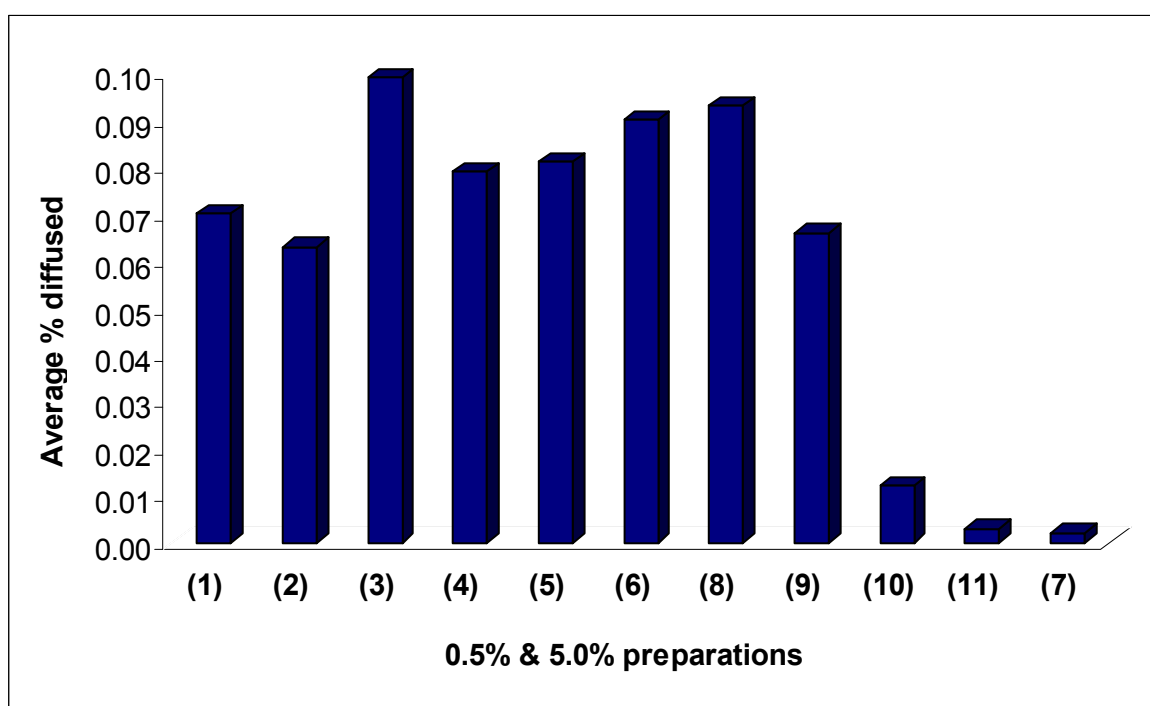


Figure D.17: Comparison of average % diffused for 0.5% formulations ((1) – (6)), 0.5% solutions ((8) – (9)), 5.0% solutions ((10) – (11)) as well as 5.0% commercial product (7)

When (7) was compared to all of the solutions ((8) – (11)), it achieved the lowest average % diffused value and average cumulative concentration. These values of (7) were comparable to the values of (11). It was slightly lower than the values obtained by (11), which could be due to the fact that the 5.0% Pheroid™ solution was also a fatty formulation, (as the commercial ointment), and contained the same concentration 5-fluorouracil.

Once the water solutions ((8) and (10)) were compared with each other, it was evident that (8) yielded a higher average % diffused than (10). Although the applied concentration (5-fluorouracil) of (10) was ten times higher than (8); (10) showed a significant decrease in % diffused than (8). Hence, it was clear that a 0.5% preparation will be more successful to use than a 5.0% preparation, as it will lead to less active ingredient used, which in turn will cause less side effects on the skin surface, as it yields in a larger % diffused than that of a 5.0% preparation. When all the 0.5% formulations ((1) – (6)) were compared to the commercially available 5.0% formulation ((7)), it was evident that ((1) – (6)) yielded in much higher % of active ingredient diffused than the commercial product, even though they were 10 times weaker. Therefore it will be useful to manufacture 0.5% formulations instead of 5.0% formulations as it will lead to less active ingredient used, causing fewer side effects on the skin surface and it will be a lot more cost-effective to manufacture.

D.3.5 Tape stripping

For the ease of reading, the stratum corneum-epidermis was referred to as “epidermis” and the epidermis-dermis was referred to as the “dermis”, in the rest of Appendix D.

The average concentrations of 5-fluorouracil in the epidermis and dermis, for the formulations ((1) – (6)), the commercial product (7) and the solutions ((8) – (11)) are shown in Table D.3.

Table D.3: Average concentration of 5-fluorouracil in the epidermis and the dermis for (1) – (11)

Formulation	Average concentration in epidermis (µg/ml)	Median concentration in epidermis (µg/ml)	Average concentration in dermis (µg/ml)	Median concentration in dermis (µg/ml)
(1)	0.178	0.145	0.139	0.131
(2)	0.132	0.123	0.136	0.138
(3)	0.221	0.162	0.650	0.554
(4)	0.613	0.279	0.472	0.461
(5)	0.135	0.134	0.424	0.430
(6)	0.182	0.183	0.310	0.306
(7)	0.120	0.122	0.052	0.049
(8)	0.229	0.228	0.552	0.438
(9)	0.057	0.021	0.005	0.000
(10)	9.444	9.198	2.696	2.212
(11)	0.116	0.143	0.715	1.539

D.3.5.1 Concentration in the epidermis for 0.5% formulations and solutions

The epidermis concentration values of the 0.5% formulations ((1) – (6)) and 0.5% solutions ((8) – (9)) were statistically compared in Figure D.28.

Comparing the average epidermis concentrations, the following was observed: (4) (0.613 µg/ml) attained the highest average concentration 5-fluorouracil within the epidermis, followed by (8) (0.229 µg/ml), (3) (0.221 µg/ml), (6) (0.182 µg/ml), (1) (0.178 µg/ml), (5) (0.135 µg/ml), (2) (0.132 µg/ml) and lastly, (9) (0.057 µg/ml). The reason that (4) attained the highest epidermis concentration, could be due to high oil content (Pheroid™) and the addition of a gelling agent (emulgel) that caused an adhesive residue on the skin surface, which in turn lead to high concentration 5-fluorouracil in the lipophilic epidermis. (9) depicted the lowest epidermis concentration, due to a high fluid content when compared to the formulations ((1) – (6)). This high fluid content caused it to be unfavourable for the lipophilic epidermis. Although it did not contain the highest fluid content when compared to (8), the addition of a small amount of oily ingredients (Pheroid™ ingredients) leads to the residue of a slight amount within this layer. This was due to slow transfer from the lipophilic layer to the underlying aqueous layer (Potts & Guy, 1992:667).

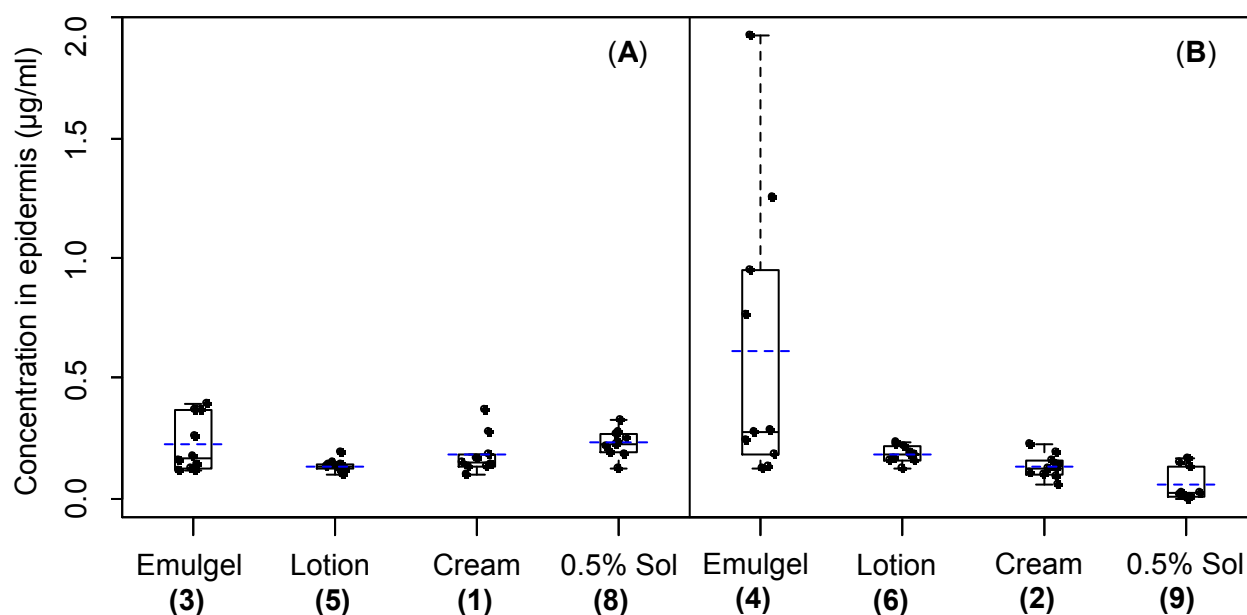


Figure D.18: Box-plot representation of the epidermis concentration values for different preparations. (A): 0.5% non-Pheroid™; (B): Pheroid™ formulations and solutions. The average and median epidermis concentrations are indicated by a dotted and solid line, respectively. The black dots represent the epidermis data (µg/ml).

Comparing the median epidermis values with each other, **(4)** (0.279 µg/ml) depicted the highest median concentration, followed by **(8)** (0.228 µg/ml), **(6)** (0.183 µg/ml), **(3)** (0.162 µg/ml), **(1)** (0.145 µg/ml), **(5)** (0.134 µg/ml), **(2)** (0.123 µg/ml) and lastly, the **(9)** (0.021 µg/ml).

Once the median and average epidermis concentration values of **((1) – (6))** as well as **(8)** and **(9)** were compared; it was detected that **(1)**, **(2)**, **(5)**, **(6)** and **(8)** depicted approximately the same epidermis values. Therefore, both median and average can be used to determine concentration. However, **(3)**, **(4)** and **(9)** portrayed significant differences between the median and average epidermis concentration values. The median epidermis values provided a more accurate representation of the true concentration, since it took all the data into consideration and was not affected by a distortion in the spread of data, as in the case with average concentration values (Gerber *et al.*, 2008:190).

D.3.5.2 Concentration in the dermis for 0.5% formulations and solutions

The dermis concentration values of the 0.5% formulations **((1) – (6))** and 0.5% solutions **((8) – (9))** were statistically compared in Figure D.29.

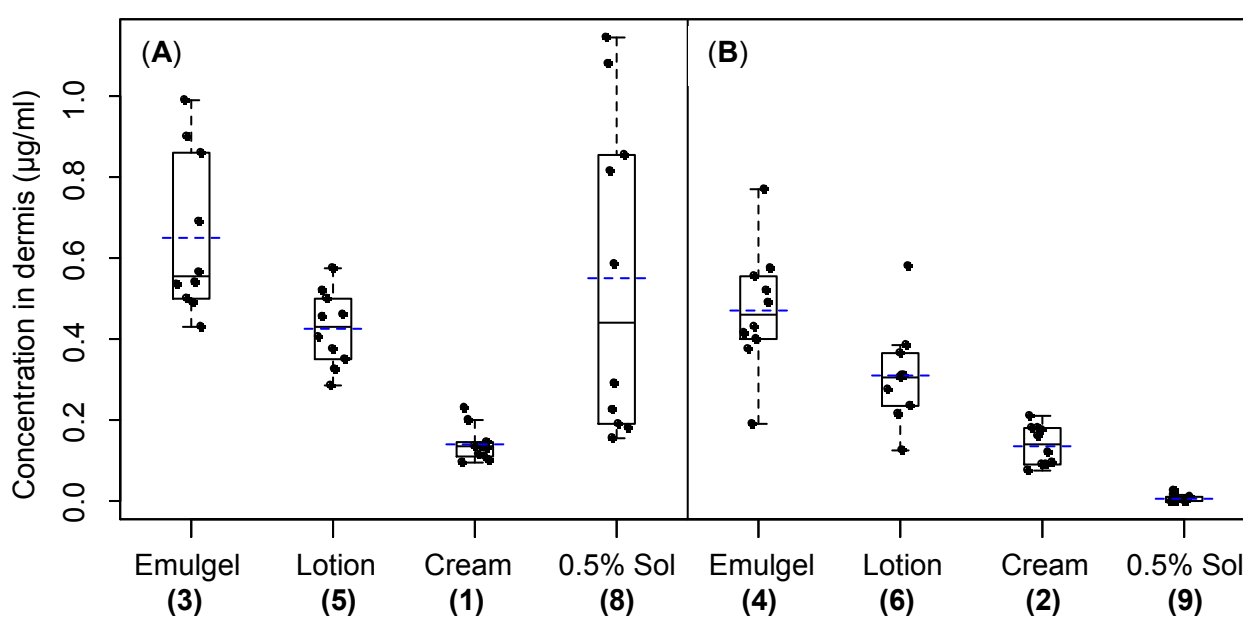


Figure D.19: Box-plot representation of the dermis concentration values for different preparations. **(A)**: 0.5% non-Pheroid™; **(B)**: Pheroid™ formulations and solutions. The average and median dermis concentrations are indicated by a dotted and solid line, respectively. The black dots represent the dermis data (µg/ml).

Comparing the average dermis concentrations, the following was detected: **(3)** (0.650 µg/ml) depicted the highest average concentration 5-fluorouracil within the dermis, followed by

(**8**) (0.552 µg/ml), (**4**) (0.472 µg/ml), (**5**) (0.424 µg/ml), (**6**) (0.310 µg/ml), (**1**) (0.139 µg/ml), (**2**) (0.136 µg/ml) and lastly, (**9**) (0.005 µg/ml). After comparing all the formulations ((**1**) – (**6**)) it is evident that (**3**) is the most hydrophilic formulation with the least oil particles. Due to the hydrophilic nature of (**3**) and (**8**), it depicted the highest concentration in the dermis, which is a hydrophilic layer. According to Williams (2003:2), the dermis mainly consists of water, which will lead to higher diffusion of hydrophilic compounds.

Once median dermis values were compared, (**3**) (0.554 µg/ml) attained the highest median concentration, followed by (**4**) (0.461 µg/ml), (**8**) (0.438 µg/ml), (**5**) (0.430 µg/ml), (**6**) (0.306 µg/ml), (**2**) (0.138 µg/ml), (**1**) (0.131 µg/ml) and lastly, the (**9**) (0.000 µg/ml).

When the median and average dermis concentration values of ((**1**) – (**6**)) as well as (**8**) and (**9**) were compared with one another; it was detected that only (**3**) and (**8**) portrayed significant differences between the median and average dermis concentration values. The median dermis values provided a more accurate representation of the true concentration, since it took all the data into consideration and was not affected by a distortion in the spread of data, as in the case with average concentration values (Gerber *et al.*, 2008:190).

D.3.5.3 Concentration in the epidermis for solutions and the commercial product

The epidermis concentration values of the 5.0% commercial product (**7**), the 0.5% solutions ((**8**) – (**9**)) and 5.0% solutions ((**10**) – (**11**)) were statistically compared in Figure D.30.

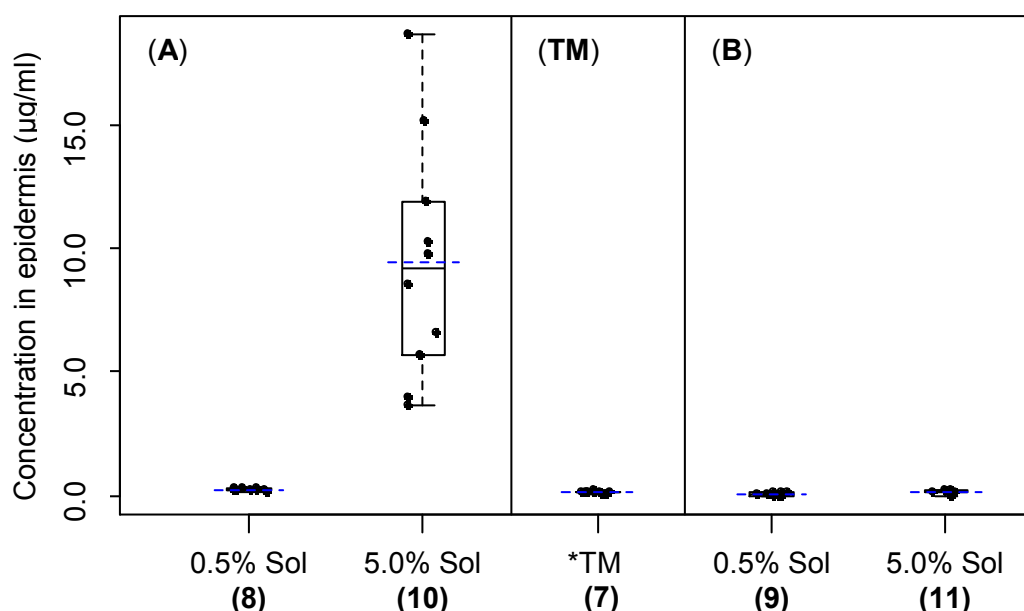


Figure D.20: Box-plot representation of the epidermis concentration values for different preparations. (**A**): 0.5% and 5.0% non-Pheroid™; (**B**): 0.5% and 5.0% Pheroid™ solutions and *TM: Commercial product. The average and median epidermis

concentrations are indicated by a dotted and solid line, respectively. The black dots represent the epidermis data ($\mu\text{g/ml}$).

Comparing the average epidermis concentrations, the following was observed: **(10)** ($9.444 \mu\text{g/ml}$) attained the highest average concentration 5-fluorouracil within the epidermis, followed by **(8)** ($0.229 \mu\text{g/ml}$), **(7)** ($0.120 \mu\text{g/ml}$), **(11)** ($0.116 \mu\text{g/ml}$) and lastly, **(9)** ($0.057 \mu\text{g/ml}$).

As the epidermis was hydrated for a period of 12 h, it caused the stratum corneum-epidermis to swell. It can swell to a thickness of $40 \mu\text{m}$ (El Maghraby *et al.*, 2008:204) and thus caused the aqueous **(10)** to attain the highest concentration in the swollen epidermis. Secondly, this could also have been due to the over-saturation of **(10)**, that caused solid particles of 5-fluorouracil to saturate on this layer; and thus, lead to a very high concentration in the epidermis. Whereas **(9)** consisted of a lower concentration 5-fluorouracil, which caused the least saturation and the poorest epidermis concentration, which could also be related to the above-mentioned swollen stratum corneum-epidermis, but to a much lesser extent, due to the oily ingredients of Pheroid™ in **(9)**.

When median epidermis values were compared with each other, **(10)** ($9.198 \mu\text{g/ml}$) depicted the highest median concentration, followed by **(8)** ($0.228 \mu\text{g/ml}$), **(11)** ($0.143 \mu\text{g/ml}$), **(7)** ($0.122 \mu\text{g/ml}$) and lastly, **(9)** ($0.021 \mu\text{g/ml}$).

Once the median and average epidermis concentration values of **((7) – (11))** were compared with one another, it was detected that only **(9)** and **(11)** showed significant differences between the median and average epidermis concentration values. The median epidermis values provided a more accurate representation of the true concentration, since it took all the data into consideration and was not affected by a distortion in the spread of data, as in the case with average concentration values (Gerber *et al.*, 2008:190).

D.3.5.4 Concentration in the dermis for solutions and the commercial product

The dermis concentration values of the commercial product **(7)**, the 0.5% solutions **((8) – (9))** and 5.0% solutions **((10) – (11))** were statistically compared in Figure D.31.

Comparing the average dermis concentrations, the following was showed: **(10)** ($2.696 \mu\text{g/ml}$) attained the highest average concentration 5-fluorouracil within the dermis, followed by **(11)** ($0.715 \mu\text{g/ml}$), **(8)** ($0.552 \mu\text{g/ml}$), **(7)** ($0.052 \mu\text{g/ml}$) and lastly, **(9)** ($0.005 \mu\text{g/ml}$).

The significantly high concentration of **(10)** within the dermis, could also have been due to the fact that it was an over-saturated solution, which caused 5-fluorouracil to penetrate the skin

more readily compared to the other solutions and the commercial product. Due to the hydrophilic nature of **(10)** when compared to **(11)**, it depicted the highest concentration in the dermis, which is a hydrophilic layer. According to Williams (2003:2), the dermis mainly consists of water, which will lead to higher diffusion of hydrophilic compounds. **(9)** consisted of a lower concentration 5-fluorouracil, which caused the least saturation and the poorest dermis concentration, which could also be related to the oily ingredients of Pheroid™ in **(9)**.

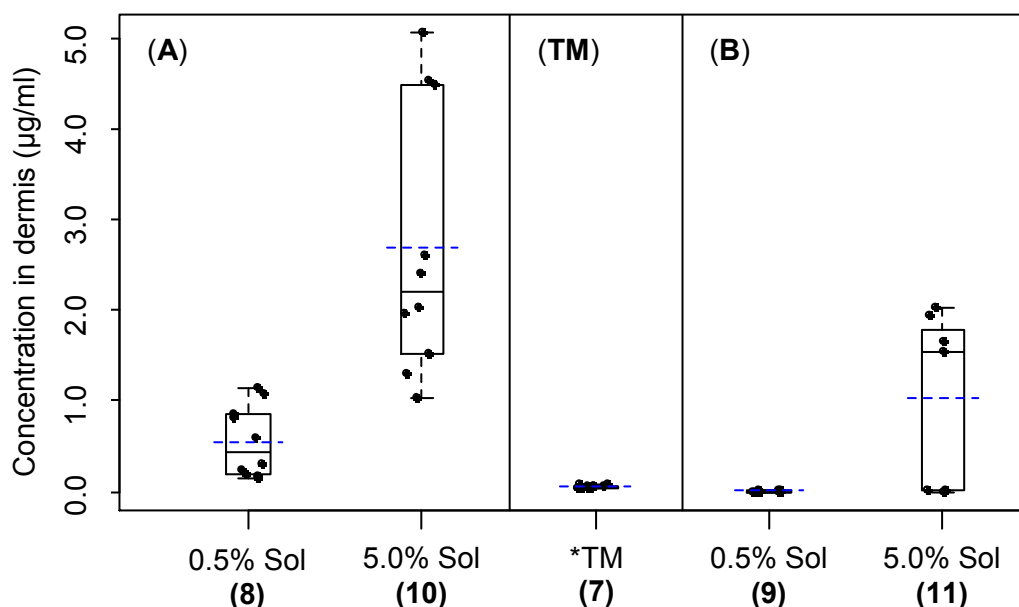


Figure D.21: Box-plot representation of the dermis concentration values for different preparations. **(A)**: 0.5% and 5.0% non-Pheroid™; **(B)**: 0.5% and 5.0% Pheroid™ solutions and *TM: Commercial product. The average and median dermis concentrations are indicated by a dotted and solid line, respectively. The black dots represent the dermis data (µg/ml).

After median dermis values were compared with each other, it was clear that **(10)** (2.212 µg/ml) attained the highest median concentration, followed by **(11)** (1.539 µg/ml), **(8)** (0.438 µg/ml), **(7)** (0.049 µg/ml) and lastly, **(9)** (0.000 µg/ml).

Once the median and average dermis concentration values of **((7) – (11))** were compared with one another, it was detected that only **(10)** and **(11)** showed significant differences between the median and average dermis concentration values. The median dermis values provided a more accurate representation of the true concentration, since it took all the data into consideration and was not affected by a distortion in the spread of data, as in the case with average concentration values (Gerber *et al.*, 2008:190).

D.3.6 STATISTICAL DATA ANALYSIS

D.3.6.1 Effect of Pheroid™ application to the epidermis and the dermis when examining formulations (0.5%) and solutions (0.5%)

D.3.6.1.1 Epidermis

The reader is referred to Section D.3.5, Figure D.28 where **(A)** represented non-Pheroid™ and **(B)** represented Pheroid™.

The 2-way BDM-test found a significant interaction between **(A)** and **(B)** on the epidermis values. Hence, the effect of Pheroid™ application should be compared on different levels of the preparations and vice versa.

- ❖ *With the effect of application of Pheroid™ at different levels of the preparations* (thus comparing each preparation in **(A)** with its respective preparation in **(B)**), a statistical significant difference was observed for **(5)** and **(6)** as well as **(8)** and **(9)** although not visually observed in the box-plot.
- ❖ *With the effect of preparations on different levels of non-Pheroid™ **(A)** application*, the one-way BDM-test showed significant differences. Subsequently, the post-hoc analysis only revealed significant differences between **(5)** and **(8)**.
- ❖ *With the effect of preparations on different levels of Pheroid™ **(B)** application*, the one-way BDM-test also showed differences. Post-hoc analysis revealed significant differences between the **(4)** and **(2)**, **(4)** and **(9)**, as well as **(6)** and **(9)**.

D.3.6.1.2 Dermis

The reader is referred to Section D.3.5, Figure D.29 where **(A)** represented non-Pheroid™ and **(B)** represented Pheroid™.

The 2-way BDM-test found a significant interaction between **(A)** and **(B)** on the dermis values. Hence, the effect of Pheroid™ application should be compared on different levels of the preparations and vice versa.

- ❖ *With the effect of application of Pheroid™ at different levels of the preparations* (thus comparing each preparation in **(A)** with its respective preparation in **(B)**), a statistical significant relationship was observed for **(1)** and **(2)**, due to the fact that the p-value was above 0.05.

- ❖ *With the effect of preparations on different levels of non-Pheroid™ (A) application*, the one-way BDM-test showed significant differences. Subsequently, the post-hoc analysis revealed significant differences between (3) and (1), (5) and (1), as well as (1) and (8).
- ❖ *With the effect of preparations on different levels of Pheroid™ (B) application*, the one-way BDM-test also showed differences. Post-hoc analysis revealed significant differences between (4) and (2), (4) and (6), as well as (6) and (9).

D.3.6.2 Effect of Pheroid™ application to the epidermis and the dermis when examining solutions (5.0%) and commercial product (5.0%)

D.3.6.2.1 Epidermis

The reader is referred to Section D.3.5, Figure D.30.

The one-way BDM-test found a significant difference between (10), (11) and (7) on the epidermis values. Post-hoc tests revealed significant differences between (7) and (10); as well as (10) and (11).

D.3.6.2.2 Dermis

The reader is referred to Section D.3.5, Figure D.31.

The one-way BDM-test found a significant difference between (10), (11) and (7) on the dermis values. Post-hoc tests revealed significant differences between (7) and (10); as well as (10) and (11).

D.4 PREVIOUS STUDIES CONDUCTED ON 5-FLUOROURACIL

Various studies have been conducted on 5-fluorouracil at the North-West University (NWU) campus in Potchefstroom. Kilian (2004) evaluated two novel drug delivery systems in an effort to improve the transdermal delivery of two active ingredients: 5-fluorouracil and idoxuridine. The first system was called a lamellar gel phase system (LGPS) and the second, Emzaloid™ (now referred to as Pheroid™) technology. It was found that the Pheroid™ produced a more significant flux and enhancement factors than the LGPS. Furthermore, the Pheroid™ systems depicted a more significant potential to use less active ingredient without influencing the efficacy of a formulation. Another, more recent study, conducted by Van Dyk (2008:66) examined the influence of Pheroid™ technology on the transdermal delivery of 5-fluorouracil. Both a 0.5% and a 1% formulation were formulated in a water solution, PBS, a water-based Pheroid™ solution, and in a PBS-based Pheroid™ solution. The formulations were applied to the epidermis and allowed to permeate for a 12 h period. All of the Pheroid™ containing

formulations showed a significantly higher permeation than those without Pheroid™. However, the 0.5% water-based Pheroid™ depicted the highest statistical increased results.

Van Ruth (2006:161) conducted a study where two patients with extensive non-melanoma skin cancer were treated with a 5 % topical 5-fluorouracil preparation on the total body. They found that most of the lesions healed in both the patients. The first patient was treated twice weekly and the second, twice daily, because they found that there were negligible systemic absorption, and thus very little systemic side effects. There was, however, irritation during the course of the treatment; particularly in combination with the erosions which also occurred. There were some secondary infections and pain, but overall, the total body treatment of these two patients with extensive non-melanoma skin cancer, was successful.

D.5 CONCLUSION

The aqueous solubility of 5-fluorouracil was determined to be 0.56 mg/ml, which did not indicate the ideal value (1 mg/ml or more) to permeate the skin (Naik *et al.*, 2000:319). The log D value obtained for 5-fluorouracil, was -0.98, which indicated that it will have difficulty penetrating through the highly lipophilic stratum corneum, as the ideal log P should be between 1 and 3 (Hadgraft, 2004:292). After these physiochemical properties were determined, it showed that 5-fluorouracil did not consist of the ideal properties to be delivered by the transdermal route. Nevertheless, 5-fluorouracil proved the contrary, as it penetrated the stratum corneum and permeated through full-thickness skin to achieve % diffused, epidermis, as well as dermis concentration values.

From the membrane studies data, it was concluded that the highest % 5-fluorouracil was released by **(2)** and the lowest by **(1)**.

Franz cell skin diffusion studies showed that **(3)** achieved the highest average % diffused value (0.099%), when considering all formulations and solutions. The reason for this could have been due to the high fluid content of the emulgel that caused hydration of the skin, which in turn lead to swelling of the stratum corneum and opening of its structures. These actions lead to an increase in 5-fluorouracil penetration (Benson, 2005:28). Further study is encouraged with the emulgel as it yielded the best transdermal delivery of 5-fluorouracil.

When all formulations and solutions were compared in terms of epidermis and dermis concentrations, **(10)** depicted the highest average concentration 5-fluorouracil in the epidermis (9.444 µg/ml) and dermis (2.696 µg/ml). This was an oversaturated solution of 5-fluorouracil, which caused solid particles to reside in the epidermis, consequently causing a high drive force for 5-fluorouracil to the dermis.

Even though **(10)** yielded the highest concentration in the epidermis and dermis; **(3)** would be more suitable to use as it contained one tenth of the active ingredient concentration when compared to **(10)**. Hence, causing less side effects on the skin surface and will be more profitable to manufacture. A 5.0% solution will leave a powder residue on the skin surface due to the high saturation, thus, not stick to the skin, which in turn may lead to serious side effects on the skin surface.

However, a 0.5% formulation has only been approved by the FDA (Food and Drug Administration) to cure AK, located on the face and anterior scalp; there has not yet been proof that it is used in the treatment of NMSC. Whereas, 5.0% formulations is an appropriate treatment for NMSC, including superficial BCC, and in some cases, SCC (McGillis & Fein, 2004:176).

McGillis and Fein (2004:176) stated that both 0.5% and 5.0% 5-fluorouracil preparations have been used before in the treatment of AK. In a side-to-side study, a 0.5% cream compared favourably to a 5.0% cream in the reduction and clearance of AK. All patients experienced cutaneous irritation; however, the once daily application of the 0.5% formulation was preferred.

Throughout this study it was observed that the Pheroid™ drug delivery system did not enhance the penetration of 5-fluorouracil into or through the skin when compared to the same non-Pheroid™ formulations. Although, formulations **(2)**, **(4)** and **(6)** did in fact yield greater penetration of 5-fluorouracil when compared to the commercial product **(7)**.

It was evident that a 0.5% formulation is more efficient in delivering 5-fluorouracil through the skin than a 5.0% formulation. It can be concluded that a 0.5% formulation will lead to less side effects on the skin surface, since more active ingredient penetrates through the skin and into the systemic circulation, whereas the 5.0% formulations might form a depot-effect in the skin layers (causing more side effects) as they did not yield in a large amount of 5-fluorouracil penetrating through the skin. As the 0.5% formulations delivered more 5-fluorouracil through the skin, they will in all likelihood yield greater systemic side effects than the 5.0% formulations, but the concentrations of 5-fluorouracil that reaches systemic circulation is too small to cause any toxic side effects. Toxic effects (stomatitis, diarrhea and leukopenia) are mostly caused by systemic AUC levels above 30 mg.h/L (Alnaim, 2010:7), and none of the formulations tested in this study depicted such high levels of 5-fluorouracil when tested through the transdermal route.

It was clear that the 0.5% semi-solid formulations that were prepared and tested in this study lead to significantly higher percentages of 5-fluorouracil diffused than any of the 5.0% preparations. It will lead to fewer side effects on the skin surface, increasing patient

compliance. This phenomenon also holds great economical value as it will be less expensive to manufacture a 0.5% formulation than a 5.0% formulation.

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APPENDIX E

JOURNAL OF DRUG DELIVERY: GUIDE FOR AUTHORS

E.1 ABOUT THE JOURNAL

E.1.1 Aims and scope

Drug Delivery serves the academic and industrial communities with peer reviewed coverage of basic research, development, and application principles of drug delivery and targeting at molecular, cellular, and higher levels. Topics covered include all delivery systems and modes of entry, such as controlled release systems; microcapsules, liposomes, vesicles, and macromolecular conjugates; antibody targeting; protein/peptide delivery. Papers on drug dosage forms and their optimization will not be considered unless they directly relate to the original drug delivery issues. Published articles present original research and critical reviews.

E.1.2 Editors-in-chief

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E.2 MANUSCRIPT SUBMISSION

All submissions should be made online at Drug Delivery's ScholarOne Manuscripts site. New users should first create an account. Once a user is logged onto the site, submissions should be made via the Author Centre. If you experience any problems with your submission or with the site, please contact ScholarOne support through the "get help now" link.

All submissions to the journal must include full disclosure of all relationships that could be viewed as presenting a potential conflict of interest. If there are no conflicts of interest, authors should state that there are none. This must be stated at the point of submission (within the manuscript, after the main text under a subheading "Declaration of interest", and, where available within the appropriate field on the journal's ScholarOne Manuscripts site).

Please see our full Declaration of Interest Policy for further information.

E.3 MANUSCRIPT PREPARATION

E.3.1 File preparation and types

Manuscripts are preferred in Microsoft Word format (.doc files). Documents must be double-spaced, with margins of one inch on all sides. Tables and figures should not appear in the main text, but should be submitted as separate digital files and designated with the appropriate file type on ScholarOne Manuscripts. References should be given in Harvard style (see References section for example).

Manuscripts should be compiled in the following order: title page; abstract; main text; acknowledgments; Declaration of Interest statement; appendices (as appropriate); references; tables with captions (on separate pages); figures; figure captions (as a list).

Drug Delivery publishes the following manuscript types: Original papers, Reviews and Book reviews.

E.3.2 Title page

A title page should be provided comprising the manuscript title plus the full names and affiliations of all authors involved in the preparation of the manuscript. One author should be clearly designated as the corresponding author and full contact information, including phone number and email address, provided for this person. Five key terms that are not in the title should also be included on the title page. The keywords will assist indexers in cross indexing your article. The title page should be uploaded separately to the main manuscript and designated as “title page – not for review” on ScholarOne Manuscripts.

E.3.3 Abstract

All original articles and reviews should start with an abstract of 250 or fewer words, summarising the central core of knowledge that is the focus of the paper. The recommended format is as a structured abstract, with the following headings for an original article: context, objective, materials and methods, results, discussion and conclusion. For a review article, it should be structured as follows: context, objective, methods (including data sources, study selection and data extraction), results and conclusion. It should be written in an informative style permitting its use, without revision, by abstracting services, give essential details of research findings without further reference to the text, and avoid generalisations and nonessential information.

E.3.4 Main text

E.3.4.1 Original articles

The body of the article should include the following sections: introduction; methods; results; discussion; conclusions.

Introduction: This section should state the relevance and background to the study, and its rationale and purpose.

Methods: This section should include only information that was available at the time the plan or protocol for the study was being written. You should describe your selection of the observational or experimental participants, identify the methods, apparatus and procedures in sufficient detail to allow others to reproduce the results, and describe statistical methods with enough detail to enable a knowledgeable reader with access to the original data to verify the reported results. Drug Delivery requires that studies involving humans, both volunteers and patients, or animals be approved by an institutional review board, in accordance with approved published guidelines, prior to actually performing the research and publishing the data. Details including clinical trial registration number must be provided in the methods section if research includes studies conducted on human volunteers.

Results: Present your results in logical sequence in the text, tables, and illustrations.

Discussion: This should include implications of the findings and their limitations, with reference to all other relevant studies and the possibilities these suggest for future research.

Conclusions: This must summarize the main paper. Ensure that extrapolations are reasonable and that conclusions are justified by the data presented, and indicate if the study design can be generalized to a broader study population.

E.3.4.2 Reviews

The body of a review article should be a comprehensive, scholarly evidence-based review of the literature, accompanied by critical analysis and leading to reasonable conclusions. Wherever appropriate details of the literature search methodology should be provided, i.e. the databases searched (normally Medline and at least one or two other databases), the search terms and inclusive dates, and any selectivity criteria imposed.

Wherever possible, use primary resources, avoiding “Data on File”, “Poster” or other unpublished references.

E.3.5 Acknowledgments and declaration of interest sections

Acknowledgments and Declaration of interest sections are different, and each has a specific purpose. The Acknowledgments section details special thanks, personal assistance, and dedications. Contributions from individuals who do not qualify for authorship should also be acknowledged here.

Declarations of interest, however, refer to statements of financial support and/or statements of potential conflict of interest. Within this section also belongs disclosure of scientific writing assistance (use of an agency or agency/ freelance writer), grant support and numbers, and statements of employment, if applicable. For a more detailed list of points to include, please see “Declaration of Interest section” below.

E.3.5.1 Acknowledgments section

Any acknowledgments authors wish to make should be included in a separate headed section at the end of the manuscript preceding any appendices, and before the references section. Please do not incorporate acknowledgments into notes or biographical notes.

Declaration of Interest section: All declarations of interest must be outlined under the subheading “Declaration of interest”. If authors have no declarations of interest to report, this must be explicitly stated. The suggested, but not mandatory, wording in such an instance is: The authors report no declarations of interest. When submitting a paper via ScholarOne Manuscripts, the “Declaration of interest” field is compulsory (authors must either state the disclosures or report that there are none). If this section is left empty authors will not be able to progress with the submission.

Please see our full Declaration of Interest Policy for further information.

Please note: for NIH/Wellcome-funded papers, the grant number(s) must be included in the Declaration of Interest statement.

E.3.6 References

References should be given in the Harvard style. Citation in the text is by author and date (Smith, 2001). The list of references appears alphabetically by primary author’s last name.

Examples:

Journal: Iyengar BS, Dorr RT, Remers WA. (2004). Chemical basis for the biological activity of Imexon and related Cyanaziridines. J Med Chem, 47, 218-23.

Book: Vyas SP, Khar RK. (2001). Targeted and controlled drug delivery. New Delhi, India: CBS Publisher and Distributor.

Contribution to a Book: Chandrasekaran SK, Benson H, Urquhart J. (1978). Methods to achieve controlled drug delivery: The biomedical engineering approach. In: Robinson JR, ed. Sustained and Controlled Release Drug Delivery Systems. New York: Marcel Dekker, 557-93.

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Periodical abbreviations should follow the style given by Index Medicus.

E.3.7 Tables

Tables should be used only when they can present information more efficiently than running text. Care should be taken to avoid any arrangement that unduly increases the depth of a table, and the column heads should be made as brief as possible, using abbreviations liberally. Lines of data should not be numbered nor run numbers given unless those numbers are needed for reference in the text. Columns should not contain only one or two entries, nor should the same entry be repeated numerous times consecutively. Tables should be grouped at the end of the manuscript on separate pages.

E.3.8 Illustrations

Illustrations (line drawings, halftones, photos, photomicrographs, etc.) should be submitted as digital files for highest quality reproduction and should follow these guidelines:

300 dpi or higher Sized to fit on journal page EPS, JPG, TIFF, or PSD format only Submitted as separate files, not embedded in the text Legends or captions for figures should be listed on a separate page, double spaced

For information on submitting animations, movie files and sound files or any additional information including indexes and calendars please click [here](#). All declarations of interest must be outlined under the subheading "Declaration of interest". If authors have no declarations of interest to report, this must be explicitly stated. The suggested, but not mandatory, wording in such an instance is: The authors report no declarations of interest. When submitting a paper via ScholarOne Manuscripts, the "Declaration of interest" field is compulsory (authors must

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Please see our full Declaration of Interest Policy for further information.

Please note: for NIH/Wellcome-funded papers, the grant number(s) must be included in the Declaration of Interest statement.

E.3.9 Notes on style

E.3.9.1 General Style

Authors are asked to take into account the diverse audience of the journal. Please avoid the use of terms that might be meaningful only to a local or national audience, or provide a clear explanation where this is unavoidable. However, papers that reflect the particularities of a social and cultural system are acceptable. Some specific points on style follow:

- 1) Authors should write in clear, concise US English. Language and grammar should be consistent with Fowler's English Usage; spelling and meaning of words should conform to Webster's Dictionary. If English is not your native language please ensure the manuscript has been reviewed by a native speaker. Please note: extensive rewriting of the text will not be undertaken by the editorial staff.
- 2) Latin terminology, including microbiological and species nomenclature, should be italicized.
- 3) Use standard convention for human and animal genes and proteins: italics for genes and regular font for proteins, and upper case for human products and lower case for animal products.
- 4) "US" is preferred to "American", "USA" to "United States", and "UK" to "United Kingdom".
- 5) Double quotation marks rather than single are used unless the "quotation is "within another".
- 6) Punctuation of common abbreviations should adhere to the following conventions: "e.g."; "i.e."; "cf.". Note that such abbreviations should not generally be followed by a comma or a (double) point/period.
- 7) Upper case characters in headings and references should be used sparingly, e.g. only the first word of paper titles, subheadings and any proper nouns begin upper case; similarly for the titles of papers from journals in the references and elsewhere.

- 8) Apostrophes should be used sparingly. Thus, decades should be referred to as follows: “The 1980s [not the 1980.s] saw...”. Possessives associated with acronyms (e.g. APU), should be written as follows: “The APU’s findings that...” but note that the plural is “APUs”.
- 9) All acronyms for national agencies, examinations, etc., should be spelled out the first time they are introduced in text or references. Thereafter the acronym can be used if appropriate, e.g. “The work of the Assessment of Performance Unit (APU) in the early 1980s...” and subsequently, “The APU studies of achievement...”, in a reference “(Department of Education and Science [DES] 1989a)”.
- 10) Brief biographical details of significant national figures should be outlined in the text unless it is quite clear that the person concerned would be known internationally. Some suggested editorial comments in a typical text are indicated in the following with square brackets: “From the time of H. E. Armstrong [in the 19th century] to the curriculum development work associated with the Nuffield Foundation [in the 1960s], there has been a shift from constructivism to heurism in the design of [British] science courses”.
- 11) The preferred local (national) usage for ethnic and other minorities should be used in all papers. For the USA, “African-American”, “Hispanic” and “Native American” are used, e.g. “The African-American presidential candidate, Jesse Jackson...”; for the UK, “Afro-Caribbean” (not “West Indian”), etc.
- 12) Material to be emphasised by italicisation in the printed version should be italicized in the typescript rather than underlined. Please use such emphasis sparingly.
- 13) Numbers in text should take the following forms: 300, 3000, 30 000 (not 30,000). Spell out numbers under 10 unless used with a unit of measure, e.g. nine pupils but 9 mm (do not use full stops (periods) within units). For decimals, use the form 0.05 (not .05, × 05 or 0× 05). “%” (not “per cent”) should be used in typescripts.
- 14) Appendices should appear before the references section and after any acknowledgments section. The style of the title is shown by the following example:

“Appendix C: The random network generator”. For information on colour figures and charges please click [here](#).

“Appendix C: The random network generator”. Figures and tables within appendices should continue the sequence of numbering from the main body of the text. Sections within appendices should be numbered, for example, C.1, C.2. Equations in appendices should be numbered, for example, (C 1), (C 2). If there is only one appendix, it is referred to as “the appendix” and not called “Appendix A”.

E.3.9.2 Abbreviations and nomenclature

For abbreviations and nomenclature, authors should consult the latest edition of the CSE Style Manual available from the Council of Science Editors, 60 Revue Drive, Suite 500 Northbrook, IL, 60062, USA.

E.3.9.3 Mathematics

Please click [here](#) for more information on the presentation of mathematical text.

E.3.9.4 Footnotes

Footnotes are not to be used except for designation of the corresponding author of the paper or current address information for an author (if different from that shown in the affiliation). Information concerning grant support of research should appear in a separate Declaration of interest section at the end of the paper. Acknowledgements of the assistance of colleagues or similar notes of appreciation belong in a separate Acknowledgements section.

Footnotes to tables should be typed directly below the table and are indicated by the following symbols: * (asterisk or star), † (dagger), ‡ (double dagger), ¶ (paragraph mark), § (section mark), || (parallels), # (number sign). Reinitialize symbol sequence within tables.

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E.4.3 Peer review

1 Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Writing and Editing for Biomedical Publication. Available at: <http://www.icmje.org> All manuscripts will be subjected to confidential peer review by experts in the field and, on the basis of reviewers' feedback papers, will be accepted unconditionally, accepted subject to revision or rejected.

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If there are no declarations, authors should explicitly state that there are none. This must be stated at the point of submission (within the manuscript, after the main text, under a subheading "Declaration of interest", and within the appropriate field on the journal's ScholarOne

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E.4.8 Additional Information

E.4.8.1 Proofs

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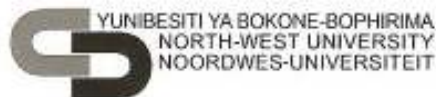
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APPENDIX F

COPY OF ETHICAL APPROVAL



Prof J du Plessis
Postal Cubicle 36
North-West University

Ethics Committee
Tel (018) 299 2558
Fax (018) 297 5308
Email drvealn@puk.ac.za

15 June 2006

Dear Prof Du Plessis

APPLICATION FOR THE USE OF LIVE VERTEBRATES IN EXPERIMENTS

This is to confirm that approval has been granted for your project "*In vitro transdermale aflewering van geneesmiddels*". The reference number 04D08 must be mentioned in all correspondence regarding this project.

It is expected from project leaders to submit annual reports on the ethical aspects of their projects, as well as of publications resulting from the projects. Such document will be forwarded during May 2007.

In terms of a decision made by the University's Senate on November 4, 1992 (Article 9.13.2), approval by the Ethics Committee is valid for 5 years where after a new application must be submitted.

Yours sincerely

PROF NT MALAN
CHAIRMAN



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