Effect of Brij 97 in the presence and absence of carrageenan on the transdermal delivery of 5-fluorouracil

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ABSTRACT

Effect of Brij 97 in the presence and absence of carrageenan on the transdermal delivery of 5-fluorouracil

The skin is the largest and most easily accessible organ of the human body thus making it the ideal route for systemic drug delivery. The transdermal route of drug delivery offers several advantages compared to the traditional routes including elimination of first pass metabolism and higher patient compliance. However, many drugs are topically and systemically ineffective when applied onto the skin, due to their almost complete failure to penetrate the skin. The main limitation lies in the stratum corneum, the barrier of the skin, which prevent the drug from reaching the deeper skin strata.

5-Fluorouracil is a polar hydrophilic drug and is therefore not a good penetrant through skin. A popular technique to increase transdermal permeation is to use a penetration enhancer, which reversibly reduce the permeability barrier of the stratum corneum. The primary aim of this study was to determine the effect of Brij 97 in the presence and absence of carrageenan on the transdermal delivery of 5-fluorouracil.

The formulations were identified by means of confocal laser scanning microscopy and measurement of the particle size. The zeta-potential was measured to determine whether the formulations were stable and the pH was measured to determine if the internal structures of the formulations were affected by the drug. The drug released from the formulations was measured with a VanKel® dissolution apparatus. *In vitro* transdermal diffusion studies were performed using vertical Franz diffusion cells with human epidermal skin. Histopathological studies were carried out on human epidermis skin to determine if the surfactant, Brij 97, had any effect on the skin.

Through confocal laser scanning microscopy and particle size measurements, the 4 and 8% Brij 97 formulations without carrageenan could be identified as emulsions while the 15 and 25% Brij 97 formulations without carrageenan could be identified as microemulsions. The 4, 8, 15 and 25% Brij 97 formulations containing carrageenan could be identified as gels.

The results obtained from the zeta-potential analysis indicated that the 4 and 8% Brij 97 formulations without carrageenan and 4% Brij 97 formulation with carrageenan are the most electronegative and thus the most stable. The pH measurements confirmed that the internal structure of the formulations was not influenced by the drug.

5-Fluorouracil was released from the formulations. The 4 and 8% Brij 97 formulations without carrageenan had an enhancing effect on the penetration of 5-fluorouracil while the 4, 8, 15 and 25% Brij 97 formulations with carrageenan and the 15 and 25% Brij 97 formulations without carrageenan had an hindering effect on the penetration of 5-fluorouracil. Although carrageenan led to good adhesiveness of the formulation on the skin, it did not lead to the enhancement of the penetration of 5-fluorouracil through the skin.

When histopathological studies were carried out on female human abdominal skin, Brij 97, the surfactant, was found to have no damaging effect on the skin structure.

OPSOMMING

Effek van Brij 97 in die teenwoordigheid en afwesigheid van karrageen op die transdermale aflewering van 5-fluoorurasiel

Die vel is die grootste en mees toeganklike orgaan van die menslike liggaam wat dit die ideale roete vir sistemiese geneesmiddelaflewering maak. Die transdermale toedieningsroete het verskeie voordele in vergelyking met die tradisionele toedieningsroetes insluitend die uitskakeling van die eerstedeurgangseffek en beter pasïentmeewerkendheid. Baie geneesmiddels is topikaal en sistemies oneffektief wanneer dit op die vel aangewend word omdat dit die vel amper glad nie kan penetreer nie. Die grootste skans is die stratum corneum, die versperring van die vel, wat die geneesmiddel verhinder om die dieperliggende vellae te bereik.

5-Fluoorurasiel is 'n polêre hidrofiele geneesmiddel en penetreer die vel dus nie goed nie. 'n Aanvaarbare tegniek om transdermale penetrasie te verhoog, is om gebruik te maak van 'n penetrasiebevorderaar wat die penetrasieskans van die stratum corneum omkeerbaar sal verminder. Die hoofdoel van hierdie studie was om die effek van Brij 97 in die teenwoordigheid en afwesigheid van karrageen op die transdermale aflewering van 5-fluoorurasiel te bepaal.

Die formulerings was geïdentifiseer met behulp van konfokale laserskanderingmikroskopie en die bepaling van die deeltjiegrootte. Die zetapotensiaal is gemeet om te bepaal of die formulerings stabiel is en die pH is gemeet om te bepaal of die interne struktuur deur die geneesmiddel beïnvloed word. Die geneesmiddelvrystelling vanuit die formulerings is met behulp van die VanKel® dissolusie-apparaat bepaal. Die *in vitro*-diffusie deur menslike epidermis is met behulp van vertikale Franz-diffusieselle bepaal. Histopatologiese studies op menslike epidermisvel is uitgevoer om vas te stel of die surfaktant, Brij 97, enige effek op die vel gehad het.

Met behulp van konfokale laserskanderingmikroskopie en die deeltjiegroottebepaling is vasgestel dat die 4 en 8% Brij 97-formulerings sonder karrageen as emulsies geïdentifiseer kon word terwyl die 15 en 25% Brij 97-formulerings sonder karrageen as mikroemulsies geïdentifiseer kon word. Die 4, 8, 15 and 25% Brij 97-formulerings wat karrageen bevat, kon as jelle geïdentifiseer word.

Die resultate wat vanaf die zeta-potensiaalanalise verkry is dui daarop dat die 4 en 8% Brij 97-formulerings sonder karrageen en 4% Brij 97-formulering met karrageen die mees elektronegatiewe en dus die stabielste is. Die pH-metings het bevestig dat die interne struktuur van die formulerings nie deur die geneesmiddel beïnvloed is nie.

5-Fluoorurasiel word deur die formulerings vrygestel. Die 4 en 8% Brij 97-formulerings sonder karrageen het die penetrasie van 5-fluoorurasiel verhoog, terwyl die 4, 8, 15 en 25% Brij 97-formulerings met karrageen en die 15 en 25% Brij 97-formulerings sonder karrageen die penetrasie van 5-fluooruracil deur die vel verlaag het. Alhoewel karrageen tot 'n beter klewing van die formulering op die vel gelei het, het dit nie tot verhoging in die penetrasie van 5-fluoorurasiel deur die vel gelei nie.

Histopatologiese studies is uitgevoer op vroulike mensvel en daar is vasgestel dat Brij 97, die surfaktant, nie die vel beskadig het nie.

Sleutelwoorde:

5-Fluoorurasiel; Brij 97; karrageen; transdermale aflewering; permeasie; stratum corneum

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CHAPTER 1

INTRODUCTION AND PROBLEM STATEMENT

The skin, the interface between humans and their environment, is the largest organ in the body. It weighs an average of 4 kg and covers an area of 2 m^2 (Hunter *et al.*, 1996:5). Skin provides a painless and compliant interface for systemic drug administration (Prausnitz *et al.*, 2004:116). However, one of the major functions of skin is to prevent the body from losing water into the environment and to block the entry of exogenous agents, which means that it naturally has a very low permeability to the penetration of foreign molecules (Asbill & Mickniak, 2000:37; Prausnitz *et al.*, 2004:116). A unique hierarchical structure of lipid-rich matrix with embedded corneocytes in the upper strata (15 μm) of skin, the stratum corneum, is responsible for this barrier (Prausnitz *et al.*, 2004:117). Overcoming this barrier function then, for the purpose of transdermal drug delivery, has been a necessarily challenging task for the pharmaceutical scientist, and one that boasts significant progress (Naik *et al.*, 2000:318).

Besides the structure of the stratum corneum, the physicochemical properties of the penetrant also play an important role in determining its transdermal absorption (Wiechers, 1989:188). Factors involved include molecular weight and size, lipid/water partition coefficient (log P_{oct}), temperature and pH and melting point (Malan *et al.*, 2002:386). Small molecules penetrate faster than large ones (Barry, 2002:513). According to Guy, compounds with a log P_{oct} value between 1 and 3, with relatively low molecular weights and relatively low melting points are likely to display optimum passive skin permeation (Malan *et al.*, 2002:386). As temperature increases at the site of application, blood flow in the area increases, as does the rate of transdermal absorption (West & Nowakowski, 1996:540). According to the simple form of the pH-partition hypothesis, only unionized molecules pass readily across lipid membranes (Barry, 2002:511).

Transdermal drug delivery has several advantages over oral and parenteral delivery. They include avoiding hepatic first-pass metabolism, less gastrointestinal side effects, maintaining constant blood

levels for longer periods of time, improving bioavailability, decreasing the administered dose, easy to cease absorption in the event of an overdose or other problems and improved patient compliance (Mitragotri, 2000:1026).

5-Fluorouracil (5-FU) is used topically in the treatment of solar (actinic) keratoses and other superficial tumours and premalignant conditions of the skin including Bowen's disease and superficial basal cell carcinomas (Sweetman, 2002:541). The advantages of topical cytotoxic therapy include the possibility of repeated use where necessary and its value in the treatment of large areas of carcinoma *in situ* where other methods of treatment are unsuitable because of the carcinoma's extent (Reynolds, *et al.*, 1989:630). 5-Fluorouracil is a polar hydrophilic compound with pK_a values of 8 and 13 and a log P value of -0.89 (Rudy & Senkowski, 1973:224; Williams & Barry, 1991:166). Due to these characteristics, 5-fluorouracil itself is not a good penetrant through skin. However, it was found that hydrophilic drugs have great potential for enhancement of skin penetration because their permeability coefficients are low (Williams & Barry, 1991:166).

Many studies have indicated that microemulsion vehicles can increase transdermal delivery of lipophilic and hydrophilic drugs, compared to conventional vehicles, depending on the components used for the microemulsion vehicle (Kreilgaard *et al.*, 2000:422). Microemulsions are thermodynamically stable colloidal systems, clear or slightly opalescent, that are composed of an aqueous phase, lipophilic phase and a surfactant or a surfactant/cosurfactant mixture (Paolino *et al.*, 2002:22). Saito and Shinoda showed that microemulsion systems could be formulated by using non-ionic surfactants without the addition of a co-surfactant. Many co-surfactant free microemulsions were established on these investigations. This fact is very important for dermal application since many of the co-surfactants exhibit irritative effects. Thus, the less surfactants, the less irritation can be expected for topical use. However, most of the microemulsions are of very low viscosity and therefore their use may be restricted (Valenta & Schultz, 2004:1). Adding a polymeric gellating agent such as carrageenan offers the possibility of a fine tuning of the required consistency for better application on large skin areas (Valenta & Schultz, 2004:8). Carrageenan is a polysaccharide which is frequently used as a food additive and has properties like good adhesiveness on skin which can be a benefit for topical application (Valenta & Schultz, 2004:2).

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Aim and objectives of this study

The aim of this study was to determine the effect of Brij 97 in the presence and absence of carrageenan on the transdermal delivery of 5-fluorouracil.

Objectives of this study:

- ➢ To identify the formulations.
- ➢ To determine whether the formulations were stable.
- ➢ To determine if the internal structures of the formulations were affected by the drug.
- ➢ To determine if the drug was released from the formulations.
- ➢ To determine whether the formulations have any influence on the permeation of the drug through the skin.
- ➢ To determine if the surfactant, Brij 97, had any effect on the skin.

CHAPTER 2

TRANSDERMAL DELIVERY

2.1 Introduction

The skin is the largest organ of the body and is composed of several layers that protect the underlying tissues. It forms an attractive and accessible route for systemic drug delivery because of the problems associated with other methods of administration, such as oral and parenteral. However, few drugs are able to diffuse passively across the outermost layer of the skin, the stratum corneum, as a result of its effective barrier properties (Foldvari, 2000:417; Asbill & Michniak, 2000:36). Besides the stratum corneum, the physicochemical properties of the drug and the biological factors also play an important role in determining its transdermal delivery (Wester & Maibach, 1985:107).

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

2.2 The skin as barrier to transdermal drug delivery

The skin covers an area of approximately 2 m^2 and provides the contact between our bodies and the external environment. It prevents the loss of water and the ingress of foreign materials (Hadgraft, 2001:1). The skin receives about one-third of the blood circulating through the body and thus is one

of the most readily accessible organs of the human body (Chien, 1987:2). The average thickness of the skin is about 0,5mm (ranging from 0,05 mm to 2 mm) and is composed of three main layers: the epidermis, dermis and hypodermis as shown in Figure 2.1 (Foldvari, 2000:417). The epidermis is further divided into two principal layers: the stratum corneum and the viable epidermis (Danckwerts, 1991:315). An average square centimetre of skin contains 3 blood vessels, 10 hair follicles, 15 sebaceous glands, 12 nerves and 100 sweat glands (Asbill & Michniak, 2000:36).
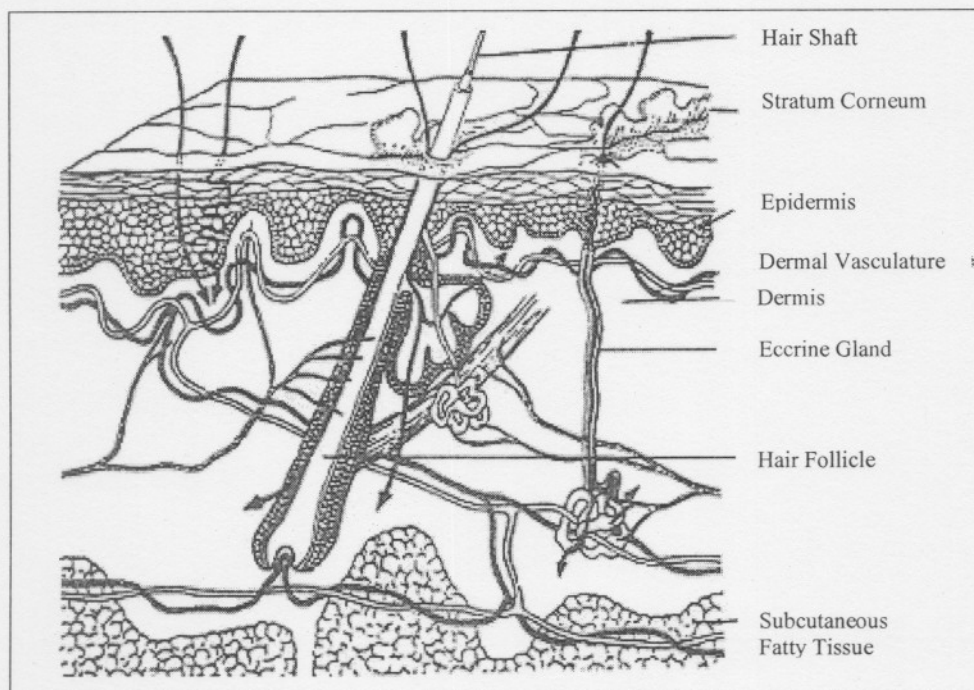
Figure 2.1: Schematic diagram of cross-section of human skin (Roy, 1997:141).

2.2.1 Stratum corneum

The outermost layer, the stratum corneum, is a highly hydrophobic, non-living layer of keratin-filled cells (corneocytes) surrounded by a lipid-rich extracellular matrix that provides the primary barrier to drug delivery into the skin (Prausnitz *et al.*, 2004:117). The thickness of the stratum corneum under normal non-hydrated conditions ranges from 10 – 20 μm and contains 10 to 15 layers of corneocytes (Foldvari, 2000:417). The stratum corneum has been represented as a 'brick and mortar' model (Figure 2.2) in which the keratin-filled corneocytes are the 'bricks' while the extracellular matrix represents the 'mortar' (Williams, 2003:9).
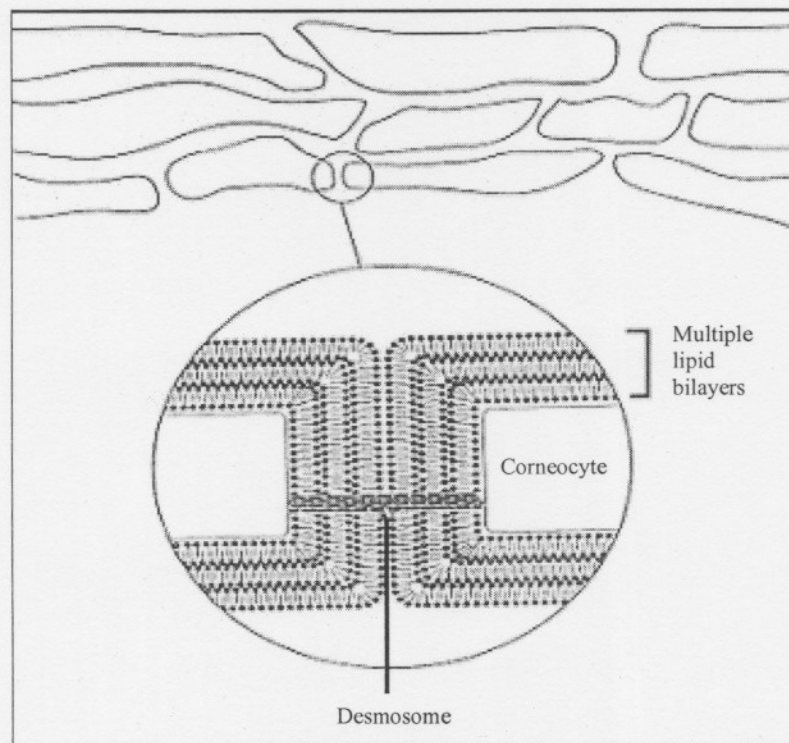
Figure 2.2: A representation of the 'brick and mortar' model of the stratum corneum (Williams, 2003:10).

The corneocytes, which comprise crosslinked keratin fibres, are about $0,2 - 0,4$ µm thick and about 40 µm wide. The corneocytes are held together by corneodesmosomes, which gives structural stability to the stratum corneum (Prausnitz *et al.*, 2004:117). The stratum corneum lipids are composed mainly of ceramides, cholesterol and fatty acids that are assembled into multi-lamellar bilayers. This unusual extracellular matrix of lipid bilayers serves the primary barrier function of the stratum corneum. The layer of lipids immediately next to each corneocyte is covalently bound to the corneocyte and is important in maintaining barrier function. The stratum corneum is continuously shed (desquamated), with a renewal period of two to four weeks. It is actively repaired by cellular secretion of lamellar bodies following the disruption of its barrier properties or other environmental insults (Prausnitz *et al.*, 2004:117).

2.2.2 Viable epidermis

The viable epidermis lies between the stratum corneum and the dermis and it has shown readily definable interfaces with each. The thickness of the viable epidermis ranges from about 40 to 50 µm up to 400 µm in the thickest portion of the skin and consists of multiple layers of keratinocytes at various stages of differentiation (Rieger, 1993:36). The basal layer contains actively dividing cells, which migrate upwards to successively form the spinous, granular and clear layers. As part of this process, the cells gradually lose their nuclei and undergo changes in composition.

The role of the viable epidermis in skin barrier function is mainly related to the intercellular lipid channels and to several partitioning phenomena (Foldvari, 2000:418). The cellular structure of the viable epidermis is mainly hydrophilic throughout its various layers and substances can be transported in its intercellular fluids. Especially for polar compounds, the resistance to penetrate is considerably lower than in the stratum corneum because the tightly packed alternating hydrophilic and lipophilic layers are no longer present (Wiechers, 1989:187). Thus, depending on their solubility, drugs can partition from layer to layer after diffusing through the stratum corneum.

Several other cells (e.g. melanocytes, Langerhans cells, dendritic T cells, epidermotropic lymphocytes and Merkel cells) are scattered throughout the viable epidermis, which also contains a variety of active catabolic enzymes (e.g. esterases, phosphatases, proteases, nucleotidases and lipases). Lipid catabolic enzymes (such as acid lipase, phospholipase, sphingomyelinase, steroid sulphatase), although mainly concentrated in the stratum corneum and granulosum, have been demonstrated throughout the epidermal layers. Although the basal and spinous layers are rich in phospholipids, as the cells differentiate during their migration to the surface, the phospholipid content decreases and the sphingolipid (glycosylceramide and ceramides) and cholesterol content simultaneously increases (Foldvari, 2000:418).

2.2.3 Dermis

The dermis (or corium) is typically 3-5 mm thick and is the main component of skin. It is composed of a network of connective tissue, predominantly collagen fibrils providing support and elastic tissue (elastin) giving flexibility, embedded in a mucopolysaccharide gel. In terms of transdermal drug

delivery, this layer is often viewed as essentially gelled water, and thus provides a minimal barrier to the delivery of most polar substances, although the dermal barrier may be significant when delivering highly lipophilic molecules (Williams, 2003:2). Fibroblasts, macrophages, mast cells and leukocytes are found throughout the dermis. A network of nerves and capillaries is found in the dermis. This network comprises the neurovascular supply to dermal appendages (hair follicles, sebaceous glands and sweat glands). The most important regions of the dermis are the papillary and reticular layers. The papillary layer, under the epidermis, is rich in blood vessels and the papillae probably assist in bringing nutrients to the avascular epidermis. Below the papillae, the reticular layer contains coarser tissue that connects the dermis with the hypodermis (West & Nowakowski, 1996:539).

2.2.4 Hypodermis

The hypodermis, or subcutaneous fat layer, is the innermost layer of the skin. It acts as a heat insulator, a shock absorber and an energy storage region. This layer is a network of fat cells arranged in lobules and linked to the dermis by interconnecting collagen and elastin fibers. In addition to fat cells, probably 50% of the body's weight, the other main cell component in the hypodermis is fibroblasts and macrophages. One of the main functions of the hypodermis is to carry the vascular and neural systems for the skin. It also anchors the skin to underlying muscle (Walters & Roberts, 2002:12).

2.2.5 Skin appendages

In addition to the above three layers of the skin, the skin has other appendages that affect the transdermal delivery of drug compounds (Danckwerts, 1991:315). There are four skin appendages: the hair follicles with their associated sebaceous glands, eccrine sweat glands, apocrine sweat glands, and the nails (Walters & Roberts, 2002:12). They are derived from epithelial germs during embryogenesis and, excluding the nails, lie in the dermis (Hunter *et al.*, 1996:14). The sebaceous glands secretes sebum; this is composed of free fatty acids, waxes and triglycerides which lubricate the skin surface and help to maintain the surface pH at about 5 (Williams, 2003:4). Lipophilic drugs that are compatible with sebum will diffuse through the follicles, while hydrophilic drugs that are

incompatible with the sebaceous lipids will not be able to make use of this pathway for passive diffusion (Ramachandran & Fleisher, 2000:202).

2.3 The process of transdermal absorption

INTERFACIAL BOUNDERIES   PENETRATION ROUTES   LOSS PROCESSES

SURFACE

Drug dissolves, diffuses, releases from vehicle

Surface loss

TRANSEPIDERMAL

STRATUM CORNEUM

Partition/diffusion, stratum corneum

Metabolism and irreversible binding

TRANSAPPENDAGEAL

APPENDAGES

Pilosebaceous unit   Eccrine gland

VIABLE EPIDERMIS

Partition/diffusion, viable epidermis

Metabolism

DERMIS

Partition/diffusion, dermis

CIRCULATION

Removal via circulation

Figure 2.3:   The routes by which drugs penetrate the skin and the loss processes that a drug can experience (Barry, 2002:504).

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The process of transdermal absorption can be described as follows (Figure 2.3). When a drug system is applied topically, the drug diffuses passively out of its vehicle and, depending on where the molecules are placed, it partitions into either the stratum corneum or the eccrine glands or sebum-filled ducts of the pilosebaceous glands. Inward diffusive movement continues from these sites to the viable epidermal and dermal points of entry. In this way, a concentration gradient is created across the skin up to the outer reaches of the skin's microcirculation, where the drug is swept away by the capillary flow and rapidly distributed throughout the body (Flynn, 1995:260).

2.4 Routes of penetration

There are several routes by which a molecule can cross the stratum corneum; these are intercellular, transcellular (intracellular) and appendageal – through either the eccrine (sweat) glands or hair follicles with their associated sebaceous glands (Figure 2.4) (Hadgraft, 2001:1; Wiechers, 1989:186).
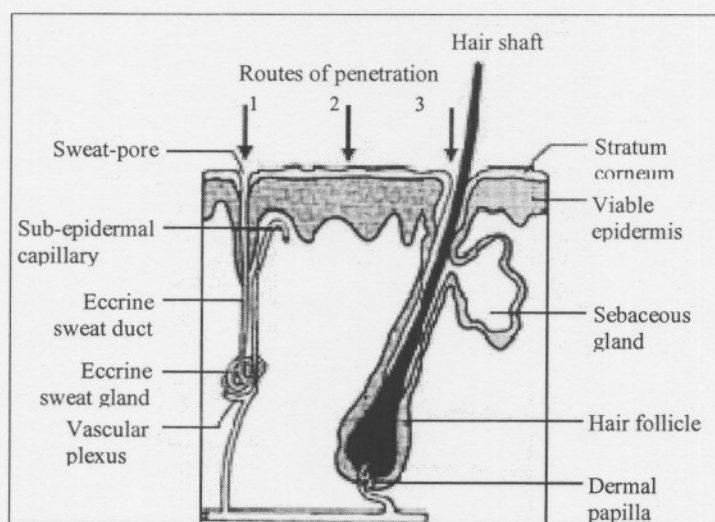
Figure 2.4: Simplified diagram of skin structure and macroroutes of drug penetration: (1) *via* the sweat ducts; (2) across the continuous stratum corneum or (3) through the hair follicles with their associated sebaceous glands (Barry, 2001:102).

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2.4.1 Transappendageal route

The fractional appendageal area available for transport is only about 0,1% of the total skin surface and hence this route usually contributes negligibly to steady state drug flux. The pathway may be important for ions and large polar molecules that struggle to cross intact stratum corneum. Appendages may also provide shunts, important at short times previous to steady state diffusion. Additionally, polymers and colloidal particles can target the follicle (Barry, 2001:101).

2.4.2 Transcellular route

The intercellular route comprises transport via the intercellular spaces and by the intracellular or transcellular route through the cells themselves as depicted in Figure 2.5 (Barry, 1987:86). The transcellular pathway for a molecule to pass through intact stratum corneum is often regarded as providing a polar route through the membrane. Indeed, the cellular components that the solute diffuses through, primarily highly hydrated keratin, do provide an essentially aqueous environment, and thus diffusion of hydrophilic molecules through these keratinocytes is rapid. However, the keratin-filled cells do not exist in isolation and they are bound to a lipid envelope that connects to the intercellular multiply bilayered lipid domains (Williams, 2003:33).

2.4.3 Intercellular route

The lipid bilayers cover around 1% of the stratum corneum's diffusional area, yet provide the only continuous phase within the membrane. There has been considerable debate over the past 20 years regarding the relative contributions of the intercellular and transcellular pathways for drug permeation. The significance of the stratum corneum lipids in regulating the loss of water from the body and in controlling the penetration of materials into the skin has long been established (Williams, 2003:33). It is now in general accepted that, except for some specific cases, the intercellular lipid route provides the principle pathway by which most small, uncharged molecules pass through the stratum corneum (Williams, 2003:34).
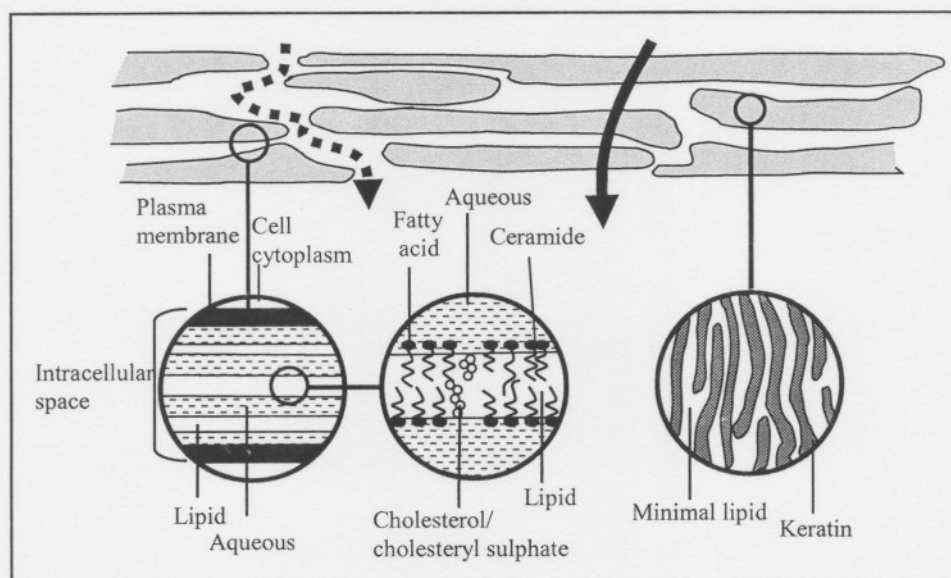
Figure 2.5: Simplified diagram of the stratum corneum and two microroutes for drug penetration (Barry, 2001:102).

2.5 Mathematics of skin permeation

There has been little evidence to suggest that there are any active processes involved in skin permeation and therefore the underlying transport process is controlled by simple passive diffusion (Hadgraft, 2001:2). The simplest way of modelling the process of skin permeation is to assume that Fick's first law of diffusion is applicable (Guy & Hadgraft, 1989:15). In terms of Fick's law of diffusion, the skin can be regarded as a composite membrane, keeping in mind that the effects of the circulation must be taken into account (Schalla & Schaefer, 1982:44). The amount of a material passing through a unit time is termed the flux (J) (Williams, 2003:41). In passive diffusion, matter moves from one region of a system to another, following random molecular motions. The basic hypothesis underlying the mathematical theory for isotropic materials (which have identical structural and diffusional properties in all directions) is that the rate of transfer of diffusing substance per unit area of a section is proportional to the concentration gradient measured normal to the section (Barry, 2002:506).

This is expressed as Fick's first law of diffusion:

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

(Equation 2.1)

where J is the flux of the permeant, dc/dx is the concentration gradient (c is the concentration and x is the space coordinate measured normal to the section) and D is the diffusion coefficient of the permeant (Williams, 2003:41). The negative sign indicates that the flux is in the direction of decreasing concentration, namely down the concentration gradient (Barry, 2002:506).

Fick's second law of diffusion can be derived from Equation 2.1. When a topically applied permeant enters the skin, it is usually assumed that diffusion is unidirectional; that is, the concentration gradient is only along the x-axis (form the outer surface into the tissue). Unidirectional diffusion in an isotropic medium is expressed mathematically by Fick's second law of diffusion:

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

(Equation 2.2)

where t is time. Thus, the rate of change in concentration with time at a point within a diffusional field ($\delta c/\delta t$) is proportional to the rate of change in the concentration gradient ($\delta^2 c/\delta x^2$) at that point (Williams, 2003:42).

Transformation shows that the quantity which diffuses from the drug depot up to a given distance is proportional to the square root of the diffusion time; this means that the substance spreads with a decreasing velocity. Over very short distances however, diffusion is constant.

The assumption that the diffusion coefficient is constant is only a good approximation. Furthermore, neither the horny layer nor the whole skin is a unique inert membrane. Therefore the drug concentrations in the formulation are not the same as at the skin surface but are related to them by the skin-vehicle partition coefficient K. When the difference between the concentration at the upper membrane surface and its lower surface is ΔC and the diffusional pathlength is h, then the

equation can be stated as follows (Fick's first law can be simplified to) (Schalla & Schaefer, 1982:45)

$$J = \frac{DK\Delta c}{h}$$ (Equation 2.3)

where J is the flux per unit area, D is the diffusion coefficient in the skin, K is the skin-vehicle partition coefficient, Δc is the concentration difference across the skin and h is the diffusional pathlength. Under normal circumstances the applied concentration (c_{app}) is very much larger than the concentration under the skin and Equation 2.3 is often simplified to

$$J = k_p \cdot c_{app}$$ (Equation 2.4)

where k_p is a permeability coefficient (= KD/h) and a heterogeneous rate constant with units, for example, $cm.h^{-1}$. It is often difficult to separate K and D and their calculated magnitude will depend on h. h cannot be accurately estimated as it measures the tortuosity of the intercellular channels, which is imprecise (Hadgraft, 2001:2). Therefore a composite parameter, permeability coefficient, is used (Barry, 2002:506). A plot of the cumulative amount of drug passing through a unit of area of membrane (e.g. $\mu g/cm^2$) against time gives the typical permeation profile, as shown in Figure 2.6.

The lag time can be obtained from extrapolation of the pseudo-steady-state section of the permeation profile to the intercept on the time axis. According to Crank (1975), the lag time (L) can be related to the diffusion coefficient by:

$$L = h^2/6D$$ (Equation 2.5)

From the equation it is apparent that the diffusion coefficient of a molecule in the membrane can be obtained by measuring the lag time (Roy, 1997:143).

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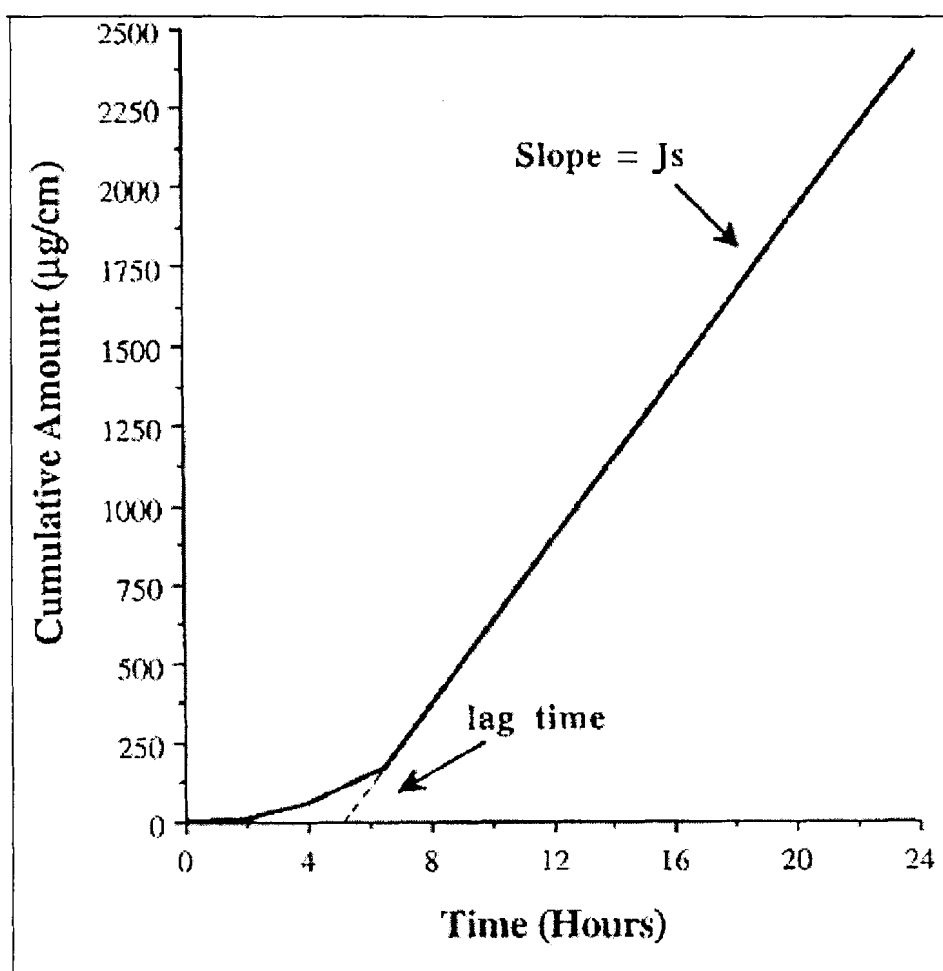
Figure 2.6: A typical cumulative amount of a drug permeated through the skin versus time plot. The slope of the linear section of the curve is the steady-state flux (J) and the x-intercept of the slope is the lag time (T_{lag}) (Roy, 1997:145).

2.6 Factors that influence transdermal delivery

The major factors that influence transdermal delivery can be broadly classified into two categories: biological and physicochemical (Mukhtar, 1992:23). However, because transdermal delivery is a dynamic process it should be borne in mind that, as one variable changes, it usually causes several effects on drug flux. The various factors are separated for convenience, but in practice this is an artificial distinction, useful for discussion (and learning) purposes (Barry, 2002:509).

2.6.1 Physicochemical factors

Besides the structure of the stratum corneum through which it has to permeate, the physicochemical properties of the penetrant also play an important role in determining its transdermal delivery. Factors involved include the partition coefficient and the diffusion coefficient. These factors in turn, depend on variables such as molecular weight, size and structure, and degree of ionization of the penetrant (Wester & Maibach, 1985:112).

2.6.1.1 Drug solubility

The solubility characteristics of a substance greatly influence its ability to penetrate biological membranes. In the formulation of preparations for topical application, it is profitable to select or prepare compounds having the required solubility characteristics before attempting to promote their penetration by pharmaceutical manipulation (Idson, 1975:912).

Essentially, the stratum corneum is lipophilic, with the intercellular lipid lamellae forming a conduit through which drugs must diffuse in order to reach the underlying vascular infrastructure and to ultimately access the systemic circulation. For this reason, lipophilic molecules are better accepted by the stratum corneum. A molecule must first be liberated from the formulation and partition into the uppermost stratum corneum layer, before diffusing through the entire thickness, and must then repartition into the more aqueous viable epidermis beneath. Ideally, a drug must possess both lipoidal and aqueous solubilities: if it is too hydrophilic, the molecule will be unable to transfer into the stratum corneum; if it is too lipophilic, the drug will tend to remain in the stratum corneum layers (Naik et al., 2000:319).

The thermodynamic activity of a drug in a particular vehicle indicates the potential of the active substance to become available for therapeutic purposes. A saturated solution is, therefore, preferable for a topical drug delivery system as it represents maximum thermodynamic activity (leaving potential) (Kemken et al., 1992 quoted by Malan et al., 2002:387). The level of saturation is dependent on the solubility of the drug in the delivery formulation (Danckwerts, 1991:316). Fewer drugs are released from sub-saturated solvents than from saturated ones (Pefile & Smith, 1997:147).

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The solubility constraint in the stratum corneum, $\sigma_{sc}/\mu g.cm^{-2}$, can be estimated using either equation 2.6 or 2.7 (Hadgraft & Wolff, 1993:162).

$$\text{Log } \sigma_{sc} = 1.31 \text{ log [oct]} - 0.13$$
$$\text{Log } \sigma_{sc} = 1.911 \ (10^3/mp) - 2.956$$

Where [oct] is the octanol solubility of the permeant (g/l) and mp is its melting point (Kelvin). The calculation of σ_{sc} and its subsequent use in predicting skin penetration assumes that it is not altered by the formulation components. Clearly, penetration enhancers which diffuse into the skin and act in a solvent capacity will modify σ_{sc}. (Hadgraft & Wolff, 1993:163).

The solubility parameter or cohesive energy density of a drug is synonymous with lipophilic/hydrophilic properties (Roy, 1997:148). The solubility parameter of the skin has been estimated as ~10 and therefore drugs which possess similar values would be expected to dissolve readily in the stratum corneum. Formulation components which can diffuse into the skin, e.g. propylene glycol, will tend to increase the value of the solubility parameter and would be expected to promote the solubility of polar drugs in the lipids.

The partitioning behaviour of the drug will be linked to its solubility characteristics and is an important factor that must be taken into account in any assessment of the feasibility of transdermal delivery (Hadgraft & Wolff, 1993:164).

2.6.1.2 Diffusion coefficient

The diffusion coefficient or diffusivity, D, is a rough measure of the ease with which a molecule can move about within a medium, in this case the stratum corneum, and is influenced by the molecular size of the drug and the viscosity of the surrounding medium (Smith, 1990; Idson, 1983 quoted by Gerber, 2003:24). It is dependent on the properties of both the drug and the medium and on the degree of interaction between compound and stratum corneum (Wiechers, 1989:190; Roy, 1997:143).

There appears to be an inverse relationship between the absorption rate and the molecular weight (Pauletti *et al.*, 1997 quoted by Malan *et al.*, 2002:387). For molecules of similar polarity, those having the lower molecular weight permeate faster. This might be explained by the observed decrease in diffusivity in liquid media with increasing molecular volume according to Equation 2.8.

$$D = A.V_m^{-1/3}$$

where D is the diffusivity of a spherical penetrant, A is a constant and V is the molecular volume (Wiechers, 1989:191).

The drug may bind non-specifically and specifically within the epidermis and dermis, thus reducing the diffusivity and decreasing skin permeability (Barry, 2002:512; Wiechers, 1989:190). Another important factor that influences the diffusion coefficient is the drug state, e.g. ionized or unionized, with unionized forms diffusing more freely than the ionized forms (Abdou, 1989 quoted by Gerber, 2003:25).

Other parameters include the affinity of the drug for the vehicle, the temperature of the vehicle and the viscosity. The lower the affinity of the drug is for the vehicle, the higher the diffusion coefficient (Baber *et al.*, 1990 quoted by Gerber, 2003:25). Diffusivity decreases with increasing viscosity and decreasing temperature of the vehicle (Pefile & Smith, 1997:148; Gerber, 2003:25)

The value of the diffusion coefficient, D, measures the penetration rate of a molecule under specified conditions and is therefore useful to know (Barry, 2002:512).

2.6.1.3 Partition coefficient

The single most important compound characteristic influencing skin penetration is distribution into the stratum corneum (Zatz, 1993:25). Partition coefficients are the gate-keepers controlling the access of the compound to the stratum corneum. A compound's passage through the stratum corneum cannot begin until the compound has been transferred from the vehicle to one of the stratum corneum components. It is the partition coefficient (K) which controls this process (Rieger, 1993:43). It may be expected that a hydrophilic molecule will partition preferentially into the

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hydrated keratin-filled keratinocytes rather than into the lipid bilayers, while lipophilic permeants will preferentially partition into the lipoidal domains. Consequently, hydrophilic molecules are expected to permeate largely *via* the intracellular route while the intercellular route will dominate for lipophilic molecules (Williams, 2003:35).

The partition coefficient is routinely determined by analyzing a substance's concentrations in two immiscible solvents, in a solvent and a tissue or in two tissues at equilibrium. In the case of the stratum corneum, the partition coefficient K_1 is defined as:

$$K_1 = C_{sc} / C_v$$

where C_{sc} is the compound concentration in the stratum corneum and C_v is the compound concentration in the vehicle (Rieger, 1993:43).

The *n*-octanol-water two-phase system is a popular model for assessing partitioning at lipid membranes because of the similarities between the *n*-octanol, long hydrophobic chain and polar hydroxyl group, and membrane lipids (Malan *et al.*, 2002:386). Other examples of solvents include ether and isopropyl myristate (Zatz, 1993:25). The selection of such solvents is based on the hypothesis that the $K_{solvent/water}$ is a realistic parallel to the value of $K_{sc/water}$ (Rieger, 1993:43).

Molecules with intermediate partition coefficients, showing some solubility in both oil and water phases, probably predominates the intercellular route. This would typically include most molecules with a log P $_{(oct/water)}$ of 1 to 3. For more highly lipophilic molecules (log P > 3) the intercellular route will be almost exclusively the pathway used to pass through the stratum corneum.

The transcellular route becomes increasingly important for more hydrophilic molecules (log P < 1), yet there are still lipid bilayers to cross between the keratinocytes. For highly hydrophilic (and charged) molecules, the appendageal pathway may also become significant (Williams, 2003:36).

2.6.1.4 Ionization

Considering the nature of the stratum corneum barrier to transdermal delivery, residing largely in the lipid domains, it is widely believed that ionisable drugs are poor transdermal permeants. Many of the arguments against using weak acids and weak bases that will dissociate to varying degrees depending on the pH of the formulation and the ionization constant, pK_b value, are founded in the pH-partition hypothesis. According to this hypothesis only the unionized form of the drug can permeate through the lipid barrier in significant amounts. However, with the complex structure of human skin, this model cannot be rigidly applied.

As described above, permeation across human skin can occur *via* several pathways, all of which probably operate for most molecules passing through the skin. Some appendages offer an essentially aqueous pathway through the stratum corneum, although one of limited cross-sectional area. The transcellular route can be viewed as one of intermediate properties, whereas the intercellular pathway is essentially a lipophilic route. Thus it is likely that ionized drugs can cross the membrane by the appendageal route but that the amounts of these permeants may be somewhat less than if the species were unionized and were to pass largely *via* the lipoidal intercellular route.

Further complexity can be introduced if one considers the relative aqueous solubilities of the ionized and unionized species. As described above, drug flux is the product of the permeability coefficient and effective drug concentration in the vehicle. Whereas the permeability coefficient of the unionized species through the lipid membrane may be high, its aqueous solubility will be low. In contrast, for an ionized species, the permeability coefficient may be low but the solubility will be high. It is possible that the resultant fluxes from these two situations may be equivalent (Williams, 2003:38).

2.6.1.5 Molecular size

An important factor in determining the flux of a material through human skin is the size and the shape of the molecule. Molecular volume is the most appropriate measure of compound bulk when considering the influence of molecular size on permeation. However, for simplicity, the molecular weight is generally taken as an approximation of molecular volume, with an inherent assumption that most molecules are essentially spherical (Williams, 2003:36).

An inverse relationship exists between the absorption rate and the molecular weight of the compound (Idson, 1975:538). Potts & Guy (1995:1632) stated that increasing the molecular volume increases the hydrophobic surface area and this will enhance partitioning into and hence, permeability through, a lipid membrane. Conversely, larger molecules diffuse more slowly because they require more 'space' to be created in the medium, and this in turn leads to diminished permeability. Scheuplein et al. (1969) showed that small molecules cross human skin faster than large molecules. However, most small organic molecules that are selected as candidates for transdermal delivery lie within a relatively narrow range of molecular weights (100-500 Dalton). Within such narrow range, the influence of molecular weight on drug permeation appears to be relatively negligible if compared to the influence of changes in partition coefficient. When selecting much larger molecules as therapeutic agents the molecular weight dependency on transdermal permeation is much more apparent (Williams, 2003:37).

For compounds ranging from 18 to >750 in molecular weight and from -3 to +6 in log K_{oct}, the permeability through human skin can be predicted by Equation 2.9

$$\text{Log } k_p = -6.3 + 0.71 \log K_{oct} - 0.0061 \text{ MW}$$

where k_p is the permeability coefficient (cm.sec^{-1}), K_{oct} is the octanol/water partition coefficient and MW is the molecular weight (Potts & Guy, 1992:666). This equation, it should be noted, is used for predicting the permeability coefficient from an aqueous solution of the diffusant. The physical significance of this empirical equation is clear; as the molecule becomes more lipophilic its permeability increases due to better partitioning into the skin. However, as it becomes larger its diffusion in the skin is reduced (Hadgraft, 2001:11).

In conclusion, it was found that the apparently sigmoidal dependence of log k_p on log K_{oct} suggests a non-linear relationship between these parameters. When molecular volume is taken into account, the data lie on a three-dimensional surface defined by log k_p, log K_{oct} and molecular volume (Potts & Guy, 1992:668).

2.6.1.6 Skin hydration and temperature

When the skin is hydrated, the tissue swells and its permeability increases. Hydration of the stratum corneum is one of the most important factors in increasing the penetration rate of most substances that permeate skin (Barry, 2002:511). Thus, occlusive dressings and patches are highly effective strategies to increase transdermal drug delivery since they create elevated hydration of the stratum corneum (Williams, 2003:17).

Since diffusion through the stratum corneum is a passive process, increasing the temperature increases the diffusion coefficient at a fixed concentration gradient (Williams, 2003:18). As the surface temperature increases, the kinetic activity of the skin increases, thus resulting in enhanced drug permeability across the stratum corneum (Pefile & Smith, 1989:149). The human body maintains a temperature gradient across the skin from about 37°C inside to about 32°C at the outer surface. Greatly elevating the skin temperature (> 65°C) can result in structural alterations within the stratum corneum and these modifications can also increase the permeability through the tissue. However, compared to the effects of hydration, slight variations in skin or environmental temperatures tend to have minimal effects on drug permeation (Williams, 2003:18).

2.6.2 Biological factors

Physiological factors can influence the rate of drug delivery to and through skin. Disease, the age of skin and the site of drug application are some of the physiological factors that influence drug permeation (Williams, 2003:14; Pefile & Smith, 1989:148).

2.6.2.1 Skin age

The most widely investigated biological factor affecting the permeation of drugs is that of skin ageing. Specific structural and functional modifications occur to the membrane as it ages (Williams, 2003:14), although it is difficult to ascribe some of the age-related changes to intrinsic ageing processes or to cumulative environmental damages. The literature contains some controversy over slight alterations to the viable epidermis with ageing but it is generally recognized that the stratum corneum remains basically invariant during a normal lifespan. This may be expected, since an intact stratum corneum is essential for terrestrial life (Williams, 2003:15). Potts

et al. (1984) demonstrated that the moisture content of human skin decreases with age and this could alter drug permeation. However, other factors alter skin hydration and will probably have a greater influence than the age-related moister decrease (Williams, 2003:14).

Other than the skin membrane, there are some age-related modifications that can theoretically affect the amounts of a topically applied drug entering the systemic circulation. Blood flow or dermal clearance tends to decrease with age and this could, *in vivo*, reduce transdermal drug flux. However, for the most permeants dermal clearance tends not to be the rate-limiting factor in transdermal therapy.

There is good evidence in the literature for negligible differences in transdermal drug delivery on ageing of normal skin, though it has been suggested that risk estimations for use in children should be separate from those of adults; children have a higher surface area to weight ratio and may have different metabolic activities (Williams, 2003:15).

Although the ageing effects of normal skin on drug permeation are minimal, there are important morphological and hence permeability differences between mature skin and the skin of a neonate (pre-term infant). At birth the dermis is only about 60% of its adult thickness and the dermis takes 3-5 months after birth to mature. There are concerns associated with the imperfect skin barrier of the neonate: the surface area to body weight ratio may be four times that in an adult thereby causing difficulties with thermoregulation, infection, absorption of exogenous chemicals and transepidermal water loss. However, the reduction in skin barrier properties leads to an increase in drug permeation. Thus, it can be advantageous for the delivery of drugs to the neonate .

Conversely, it is not possible to provide a general transdermal formulation for drug delivery across neonatal skin because neonates vary in gestation time, and therefore in the degree of stratum corneum immaturity and consequently in the permeability of the skin, and the neonatal stratum corneum matures post delivery (Williams, 2003:15).

2.6.2.2 Skin conditions and sites

Intact skin presents a barrier to absorption that can be reduced considerably when the skin is damaged or is in a diseased state. Cuts, abrasions and skin diseases like atopic dermatitis and psoriasis enhance permeability while corns, calluses, warts and the skin disease ichthyosis reduce permeability (Pefile & Smith, 1989:148; Barry, 2002:510).

After injury or removal of the stratum corneum, the skin builds a temporary barrier within 3 days that persists until the regenerated epidermis can form normal keratinizing cells. Even the first complete layer of new stratum corneum cells formed over a healing layer can markedly reduce permeation (Barry, 2002:510).

Skin structure varies to some degree over the human body. The stratum corneum is thick on the palms of the hands and soles of the feet and much thinner on the lips and eyelids. However, the relative permeability of different skin sites is not simply a function of stratum corneum thickness since different permeants demonstrate varied rank orders through different skin sites. Wester & Maybach (1989) stated that variations in drug absorption can be seen for sites with similar thickness of stratum corneum and that some areas with different stratum corneum thickness provide similar levels of drug absorption (Williams, 2003:16).

It is valuable to put the regional variations in drug permeation into context with respect to variation found for the same site between different individuals. There is significant variation (up to 30%) in permeation across a given body site (for example the trunk) of an individual and also significant variation (up to about 40%) between the same body site on different individuals. Such variability can thus exceed that resulting from regional differences if using skin from, for example, the arm and the leg where the regional factor is small (Williams, 2003:17).

2.6.2.3 Skin metabolism

The skin is a metabolically active organ which has the ability to metabolize many drugs such as steroids and consequently reduce their therapeutic efficacy and absorption. Below the stratum corneum is the viable epidermis, the most metabolically active layer in the skin. Transdermal metabolism may reduce the pharmacological potential of the active drug through cutaneous first-

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pass effects. It is necessary to inquire into the extent of transdermal absorption which a topical drug undergoes since this will determine the deposition of the substance in other parts of the skin and delivery to the capillaries in the dermis (Pefile & Smith, 1989:149).

2.6.3 Physicochemical properties of the investigated drug

The transdermal delivery of drugs is only suitable for a limited number of drugs and it is therefore important to consider its physicochemical and pharmacokinetic properties. The physicochemical factors will determine the rate at which the drug can penetrate the skin. These must be related to the pharmacokinetic factors which control its clearance so that concentrations either in the lower regions of the skin or in the plasma can be estimated (Harrison *et al.*, 1996:283; Hadgraft & Wolff, 1993:161).

The ideal limits for passive transdermal delivery for any formulation are summarized in Table 2.1.

Table 2.1:   Ideal limits of physicochemical properties for the transdermal delivery of drugs (Naik *et al.*, 2000:319).

Aqueous solubility	$> 1 \text{mg.ml}^{-1}$
Lipophilicity	$10 < K_{o/w} < 1000$
Molecular weight	$< 500 \text{ Da}$
Melting point	$< 200°C$
pH of saturated aqueous solution	pH 5-9
Dose deliverable	$< 10 \text{ mg.day}^{-1}$

2.6.3.1 5-Fluorouracil

2.6.3.1.1   Structure

The chemical structure of 5-fluorouracil is given in Figure 2.7.
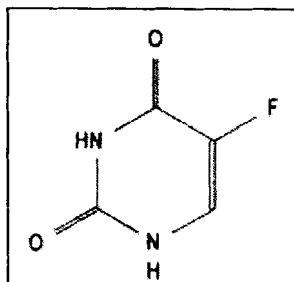
Figure 2.7:   Chemical structure of 5-fluorouracil

2.6.3.1.2   Physicochemical properties

The physicochemical properties of the drug, 5-fluorouracil, are given in Table 2.2.

Table 2.2:   Physicochemical properties of 5-fluorouracil.

	5-Fluorouracil	References
Characteristics	White to almost white, odourless, crystalline powder	Rudy & Senkowski, 1973:223
Empirical formula	$C_4H_3FN_2O_2$	Bayomi & Al-Badr, 1989:602.
Molecular weight	130.08 g/mol	Bayomi & Al-Badr, 1989:603.
Melting point	Between 282 and 283 °C	Bayomi & Al-Badr, 1989:603.
Dissociation constants (pK_a)	8 and 13	Rudy & Senkowski, 1973:224
Partition coefficient (log P)	- 0.89	Williams & Barry, 1991:166
Stability	Stable in solutions up to pH 9	Rudy & Senkowski, 1973:234
Solubility	Sparingly soluble in water, slightly soluble in alcohol and practically insoluble in ether	European Pharmacopoeia, 2002:1204

2.6.3.1.3 Pharmacology

2.6.3.1.3.1 Mechanism of action

5-Fluorouracil, a pyrimidine analogue, is an antineoplastic that acts as an antimetabolic to uracil. After intracellular conversion to the active deoxynucleotide, it interferes with the synthesis of DNA by blocking the conversion of deoxyuridylic acid to thymidylic acid by the cellular enzyme thymidylate synthetase. It can also interfere with RNA synthesis (Sweetman, 2002:541).

2.6.3.1.3.2 Therapeutic use

5-Fluorouracil is used topically in the treatment of solar (actinic) keratoses and other superficial tumours and premalignant conditions of the skin including Bowen's disease, leucoplakia, superficial basal cell carcinomas and squamous cell carcinomas (Sweetman, 2002:541). The advantages of topical cytotoxic therapy include the possibility of repeated use where necessary and its value in the treatment of large areas of carcinoma *in situ* where other methods of treatment are unsuitable because of the carcinoma's extent (Reynolds, *et al.*, 1989:630).

2.7 Penetration enhancers

Several physical (iontophoresis, sonophoresis, electroporation and temperature) and chemical methods have been reported in the literature and have successfully resulted in elevated levels of drugs delivered across and into the skin (Asbill & Michniak, 2000:37).

2.7.1 Physical enhancers

Although many different physical approaches to enhancing transdermal permeation have been attempted, the most notable approaches are iontophoresis, ultrasound (sonophoresis) and electroporation. None of these enhancement methods is passive in that they require the input of energy to achieve their effects. To date, these methods show the most promise for transdermal drug delivery systems that incorporate a large drug reservoir on the surface of the skin and that need to deliver very large molecular weight compounds in the kiloDalton range (Finnin & Morgan, 1999:956). Iontophoresis will be discussed further.

2.7.1.1 Iontophoresis

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

Iontophoresis uses a small electrical current (usually < 500 $\mu A.cm^{-2}$) to facilitate the transfer of drugs across the skin. Charged species are repelled into and through the skin as a result of an electrical potential across the membrane and the competence of this process is dependent on the polarity, valency and ionic mobility of the drug. Typically, two electrolyte chambers containing electrodes, one containing the ionized therapeutic molecule of similar polarity, that is, cationic drug in anodal chamber, are placed on the skin surface (Naik et al., 2000:322). When the current flows, the drug is repelled from the electrode of similar polarity and is attracted to the oppositely charged electrode, thus driving the drug into the skin as shown in Figure 2.8 (Williams, 2003:145). The magnitude of the current determines the amount of charge generated in the circuit and therefore the number of ions transported across the skin.

The anodal delivery of small cationic drugs is usually favoured because the skin carries a net negative charge at physiological pH, which makes it permselective to positively charged molecules under the imposition of an electrical field. As a result, the preferential transport of cations (Na^+) induces a net flow in the direction of cation movement (anode to catode) as these ions collide with solvent molecules in their pathway. The resulting electro-osmotic flow consequently enables the facilitated transport of neutral and positively charged molecules from the anode to the catode (Naik et al., 2000:323).

One other possibility is that the skin may be damaged or disrupted to some degree by the applied current. Most studies using human skin and 'acceptable' current densities up to $0,5$ $mA.cm^{-2}$ show no evidence of skin damage but the literature contains reports where extravagant current densities are applied to the more fragile membranes, whereupon enhanced flux could be attributed to skin damage (Williams, 2003:146).
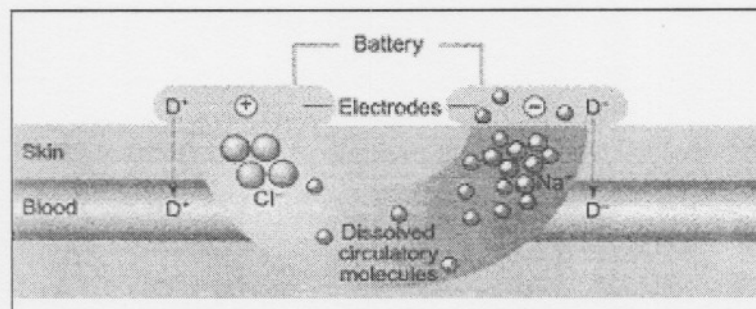
Figure 2.3: A schematic diagram of an iontophoretic device. An iontophoretic assembly consists of a pair of electrode chambers which are placed in contact with the skin surface (Naik *et al.,* 2000:322).

2.7.2 Chemical enhancers

Currently, the most widely used approach to drug permeation enhancement across the stratum corneum barrier is the use of chemical penetration enhancers also referred to as absorption promoters or accelerants (Asbill & Michniak, 2000:37). Chemical penetration enhancers generally partition into the skin and interact with different skin components to obtain temporary and, ideally, reversible reduction of barrier properties (Büyüktimkin *et al.*, 1997:358).

2.7.2.1 Ideal properties

Some properties of an ideal penetration enhancer have been described as follows (Williams, 2003:86):

- The enhancer should have no pharmacological activity in the body.
- It should be non-toxic, non-irritating and non-allergenic.
- It should work rapidly and the extent of action should be predictable and reproducible.
- When removed from the skin, the barrier resistance of the membrane should return rapidly and fully.
- The enhancer should work unidirectional, that is, it should allow therapeutic agents to enter the body while preventing the loss of fluids, electrolytes and endogenous materials from the body.

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- The substance should be suitable for formulation into topical preparations and so should be compatible with drugs and excipients.

- It should be cosmetically acceptable with appropriate skin 'feel' and ideally be tasteless, odourless and colourless.

No single penetration enhancer possesses all these desirable properties. However, some substances exhibit several of these attributes and they have been investigated clinically or in the laboratory (Barry, 1988).

2.7.2.2 Mechanisms of action

Barry (1991) discussed the mode of action of enhancers and described the lipid protein partition theory based on lipid interaction, protein modification and partition changes in the stratum corneum (Büyüktimkin et al., 1997:360).

2.7.2.2.1 Lipid interaction

The mode of action of lipophilic enhancers is based on their action on the lipid domain of the stratum corneum. Their most significant property is the ability to create a disorder between the lipid bilayers. These enhancers can solubilize or extract lipids from the bilayers and increase the fluidity. They may also partition into the lipid domain and increase water absorption or influence transepidermal water loss (TEWL). Various hydrocarbons, alkanols, alkenols, esters, lipophilic amines, terpenes, Azone$^{®}$ and its derivatives and some of the nonionic surfactants increase the permeation of drugs by one or more of these mechanisms.

2.7.2.2.2 Protein interaction

Hydrophilic enhancers are more active in the more hydrophilic domain and therefore they interact with the proteins of the stratum corneum. These compounds may solubilize some parts of the proteins and extract them from their matrix. Their interaction with proteins may disrupt S-S bonds and enhance the retention of water thereby increasing the permeation of drugs. Such enhancers may also open water channels between corneocytes and facilitate diffusion. TEWL or the reduction of the hydration capacity of the skin also occurs during this period. Short chain alcohols, various

sulfoxides, some mercapto derivatives, anionic or cationic surfactants and dialkylamino esters may increase transdermal delivery through one or more of these mechanisms.

2.7.2.2.3 Partitioning changes

Some of the enhancers show their activity in the vehicle itself by solubilizing the drugs in the formulation or increasing their thermodynamic activity. A decrease in the vehicle/skin partition coefficient of the drug may also account for their promoting behaviour. Some small chain esters, for example ethyl acetate, some cyclodextrins, various alcohols, N,N-diethyl-m-toluamide (DEET), dimethyl sulfoxide (DMSO), Azone® and cationic surfactants enhance the drug permeability through this mechanism (Büyüktimkin *et al.*, 1997:360).

In 1994, Shah outlined the general effects of various enhancers on the skin, formulation and drug (Asbill & Michniak, 2000:37). According to Shah, enhancers:

- Increase the diffusivity of the drug in the stratum corneum.

- Cause stratum corneum lipid-fluidization, which leads to decreased barrier function (a reversible action).

- Increase and optimize the thermodynamic activity of the drug in the vehicle and the skin.

- Result in a reservoir of drug within the skin.

- Modify the partition coefficient of the drug, increasing its release from the formulation into the upper layers of the skin.

2.7.2.3 Pharmaceutical acceptable enhancers

A selected group of pharmaceutically acceptable penetration enhancers and their proposed mechanisms of action are summarized in Table 2.3. Many current penetration enhancers influence the properties of the various pathways by which drugs can cross the stratum corneum, including the lipoidal intercellular pathway, the inter- and intracellular polar pathways and the appendageal pathways (Foldvari, 2000:419).

Table 2.3: Selected skin penetration enhancers (Foldvari, 2000:420).

Class	Representative compounds	Mechanism of interaction with skin and enhancement of drug permeability
Water	Hydrating and occlusive topical preparations Occlusive dressings	Hydrates the stratum corneum (SC), evidence for increasing permeability of both hydrophilic and lipophilic compounds, increases fluidity or disorder of intercellular bilayers; occlusive dressings and vehicles prevent water loss from skin and provide full hydration
Organic solvents	Alcohols (ethanol) Polyols (PG) Sulfoxides (DMSO) Pyrrolidones	Cotransports with the drug through the lipid channels, partial extraction of lipids Replaces bound water in the intercellular space, enhances penetration of lipophilic drugs Partition into the corneocyte, binds keratin; at higher concentration increases lipid fluidity and disrupts lipid packing Interacts with both the keratin and lipid component of the SC
Fatty acids	Oleic acid	Increases fluidity of the intercellular lipids: shorter chain (C10-12) and branched or unsaturated chain fatty acids are more effective than longer chain saturated fatty acids
Terpenes	Ascaridole 1,8-Cineole L-Menthol D-Limonene	Disrupts intercellular lipid order; increases electrical conductivity, indicates the opening of polar pathways in SC
Surfactants (nonionic, cationic and anionic)	Polysorbates (Tween) Polyoxyethylene alkylethers (Brij) Dodecyltrimethyl ammonium bromide (DTA-B) Sodium lauryl sulfate	Penetrates into skin, micellar solubilization of SC Penetrates into skin, extracts lipid from SC Binds to intercellular keratin in corneocytes, removes some of the intercellular lipid, increases transepidermal water loss (TEWL), alters processing of epidermal lipids
Azone®	1-Dodecylhexahydro-2H-azepine-2-one and certain derivates	Disrupts skin lipids in the headgroup and tail regions
Phospholipids (liposomes)	Phosphatidylcholine from soybean or egg yolk	Diffuses into SC, disturb intercellular lipids, enhances drug partitioning into the skin

2.7.2.4 Surfactants

Surfactants have traditionally been used in topical pharmaceutical formulations and cosmetic products, including hydrophilic ointments, absorption bases, emulsion-type creams and lotions. Their usual role is to aid in drug solubilization and to attribute water washability to the vehicle for cosmetic appeal. More recently, surfactants have been evaluated as components of transdermal drug delivery systems (Foldvari, 2000:421).

Surfactant molecules consist of hydrophobic moieties, usually alkyl or aryl chains, and a hydrophilic head group. Depending on the nature of the hydrophilic moiety, surfactants can be classified into four groups namely nonionic, cationic, anionic and zwitterionic (Wiechers, 1989:193). In contrast to the penetration enhancers above, surfactants have the added ability to form micelles and to reduce surface tension. Because of the similarity in structure of surfactants and membranes, such as the intracellular bilayer in the stratum corneum, adsorption at interfaces readily occurs, leading to a reduction in interfacial tension (Wiechers, 1989:193).

The effect on the barrier function of the skin depends on the chemical structure of the surfactant and on its concentration in the vehicle (Wiechers, 1989:193). The enhancement of membrane transport occurs at low concentrations of the surfactant but this is seen to decrease at higher concentrations, generally above the critical micelle concentration of the surfactant. The increase in transdermal flux at low surfactant concentrations is normally attributed to the ability of the surfactant molecule to penetrate the stratum corneum and increase its permeability. Reduction of the permeation rate of a drug present in surfactant systems is normally ascribed to the ability of the surfactant to form micelles above the critical micelle concentration and is normally observed only if interaction between the micelle and drug occurs. Solubilization of active drug species by surfactants micelles decreases the thermodynamic activity of the drug and, thus, decreases the driving force of drug absorption. Therefore, the overall effect of a surfactant on the drug permeation rate will be a combination of the influence of these two opposing effects (French et al., 1993:117).

2.7.2.4.1 Nonionic surfactants

The four best-known groups of nonionic surfactants include the polysorbates (polyoxoethylene sorbitan esters; for example Tween®), the polyethoxylated alkyl ethers and esters (for example Brij® and Myrj®), the polyethoxylated alkyl phenols (for example Triton X-100®) and poloxamers (polyoxyethylene-polyoxypropylene block co-polymers; for example Pluronic®).

The nonionic surfactants have two possible modes of action. Firstly, they may increase membrane fluidity by penetrating into the intercellular regions of the stratum corneum (Brij®), after penetration, they may solubilize and extract lipid components (Triton X-100® and poloxamers). Secondly, but of minor importance, these surfactants may penetrate into the intracellular matrix and interact and bind with keratin filaments, resulting in a disruption of order within the corneocyte (Wiechers, 1989:194). Polyoxyethylene alkyl ethers and esters (Brij® and Myrj®) enhance the transdermal delivery of many compounds and appear to be the most potent enhancers among the nonionic surfactant classes (French et al., 1993:131). Their relatively low toxicity and irritation potential make these compounds good candidates as potential penetration enhancers for use in transdermal drug delivery systems (French et al., 1993:113).

2.8 Gelling agents

2.8.1 Carrageenan

Carrageenan is a collective term for polysaccharides prepared by alkaline extraction (and modification) from species of red seaweed. Carrageenan consists of alternating 3-linked-β-D-galactopyranose and 4-linked-α-D-galactopyranose. Carrageenans are linear polymers of about 25,000 galactose derivatives with regular but imprecise structures, dependent on the source and extraction conditions (Valenta & Schultz, 2004:5). The main differences in structure of carrageenans are caused by the degree of sulphate esters, ranging from 15 to 40%.

K-Carrageenan is widely employed in the food, pharmaceutical and cosmetic industry as a thickening, gelling and stabilizing agent because of its ability to form thermoreversible gels, in the presence of gel-promoting cations such as Ca^{2+}, K^+ and Na^+ (Roesch et al., 2004:429). Properties of

carrageenan, such as good adhesiveness on skin, can be a benefit for topical application (Valenta & Schultz, 2004:2).

2.9 Microemulsions

Hydrophilic drugs are known to be of the most problematic in dermal administration. It is therefore important to develop vehicles that will facilitate the transport of these compounds by affecting lipid barrier properties of the stratum corneum regarding increased penetration (Schmalfuβ *et al.*, 1997:280). Many studies have indicated that microemulsion vehicles can increase transdermal delivery of lipophilic and hydrophilic drugs, compared to conventional vehicles, depending on the components used for the microemulsion vehicle (Kreilgaard *et al.*, 2000:422).

The use of a microemulsion as vehicle can enhance transdermal penetration by different mechanisms. A large amount of drug can be incorporated in the formulation due to the high solubilization capacity. Additionally, microemulsions induce a change in the thermodynamic activity of the drugs they contain, modifying their partition coefficient, and thereby favouring penetration of the stratum corneum. Furthermore, the surfactants in the microemulsion may reduce the functional barrier of the stratum corneum and thus increase the permeation into the skin. This last function may be more or less important depending on the nature of the surfactant used (Alverez-Figueroa & Blanco-Méndez, 2001:57).

2.9.1 Theory and properties of microemulsions

Microemulsions are thermodynamically stable colloidal systems, clear or slightly opalescent, that are composed of an aqueous phase, lipophilic phase and a surfactant or a surfactant/cosurfactant mixture (Paolino *et al.*, 2002:22).

2.9.1.1 Formation

Microemulsions can potentially appear over a wide range of oil-water-surfactant compositions, depending on the properties of the involved components. However, with given oil-water-surfactant compositions, microemulsions are usually formed in narrow specific concentration ranges.

The most important determinant for the range of microemulsion formation is the physicochemical properties of the aqueous phase, oil phase and surfactants. The physicochemical interaction between the constituents is too complex to provide a functional general mathematical guideline for prediction of microemulsion formation as a function of constituent properties. However, a few necessary conditions have been described by Schulman et al., 1959:

- Production of a very low interfacial tension at the water-oil interface.
- Formation of a highly fluid interfacial surfactant film.
- Penetration and association of the molecules of the oil phase with the interfacial surfactant film.

Lowering of the interfacial tension and fluidization of the interfacial surfactant film of a single surfactant system, can be done when the lipophilic chains of the surfactant is sufficiently short or contain fluidizing groups, for example unsaturated bonds. To enable integration of the oil with the interfacial film, the size of the oil molecules should not be too large (Kreilgaard, 2002:S79).

2.9.1.2 Structure

The mixture of oil, water and surfactants is able to form a wide range of structures and phases. Besides microemulsions, structural examinations can reveal the existence of regular emulsions, anisotropic crystalline hexagonal or cubic phases and lamellar structures depending on the ratio of the components. Most of these different structures and phases are easily recognized by simple visual inspection of the components due to their physical appearance (for example, emulsions are nontransparent and phases separate after a while; lamellar structures and cubic phases are highly viscous) or can be revealed by inspection with polar light (crystalline phases) and thus be distinguished from actual microemulsions (Kreilgaard, 2002:S79). A comparison between the physical characteristics of micellular systems, microemulsions and emulsions is given in Table 2.4 (Cortesi & Nastruzzi, 1999:291).

Table 2.4: Comparison of the physical characteristics of micellular solutions, microemulsions and emulsions (Cortesi & Nastruzzi, 1999:291).

Micellular solutions	Microemulsions	Emulsions
Spontaneously obtained	Spontaneously obtained	Mechanically obtained
Stable systems	Stable systems	Unstable systems
Optically transparent	Optically transparent	Optically turbid
Colloidal dimensions (<0.1μm)	Colloidal dimensions (<0.1μm)	Dimensions range (<0.5-5μm)
No cosurfactants	Cosurfactants	No cosurfactants
Dispersed phase <10%	Dispersed phase >10%	Dispersed phase >10%
Micellular structure	Bicontinuous structure	Globular structure

However, even within the microemulsion regions, numerous different internal structures can form from the immiscible water and oil phase, and the interfacial surfactant film. The microemulsion's structure is greatly influenced by the physicochemical properties of the components used and the ratios between the components. The term 'microemulsions' is in some way deceiving since the structures often differ from the static spherical droplet-shapes of regular emulsions. Microemulsions are dynamic systems in which the interface is continuously and spontaneously fluctuating (Kreilgaard, 2002:S79). In very dilute systems, with only a few percentage of oil or water phase, microemulsion structures may approach regular or reverse 'swollen micelle' droplet-like shapes. Though, in between these extremes, the microemulsion components typically form non-spherical aggregates, which may be approximately continuous in the phase with highest volume fraction. For the majority of microemulsion systems, these aggregates fluently change into bicontinuous structures by titration with the phase of the lowest volume fraction and through these structures, fluently invert to 'reversed' aggregates (Figure 2.9).
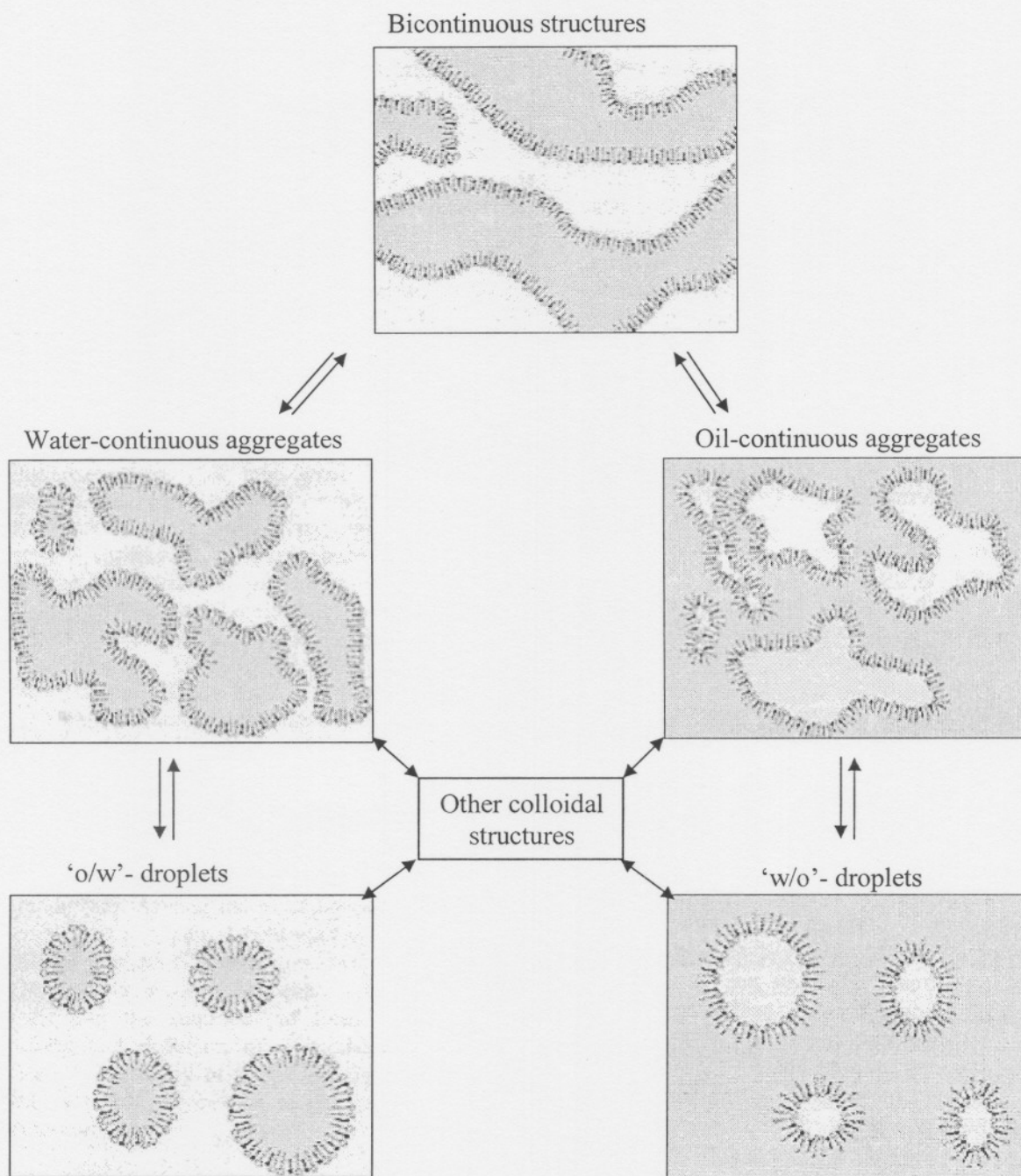
Figure 2.4: Basic dynamic microemulsion structure formed by oil phase (grey), aqueous phase (white) and surfactant/co-surfactant interfacial film, and credible transitions between the structures by increase of oil fraction (clockwise from left to right) and water fraction (anti-clockwise from right to left), respectively (Kreilgaard, 2002:S81).

Therefore, microemulsion systems often do not display emulsion-like behaviour with sudden inversion of the 'swollen micelle'. The emulsion technology of characterizing the systems as oil-in-water (o/w), or water-in-oil (w/o), is consequently in many situations not applicable to microemulsions (Kreilgaard, 2002:S80).

Some microemulsion systems have, however, been suggested to display typical emulsion-like behaviour, forming small droplet-like 'swollen micelle' structures with a dispersed and continuous phase. By continuous addition of the dispersed phase to this system, the droplets can either swell and form other colloidal structures (normally regular macroemulsions) and hence lose the microemulsion structure and characteristics, or they can invert to reverse 'swollen micelles', changing the dispersed and continuous phase in the microemulsion. Separate regions of existence for o/w and w/o droplet-like microemulsion can also be observed for some systems. The precise mechanism behind the structural formulations and conversions, and the relation to the physicochemical properties of the components is not yet well established. Most likely, the flexibility of the surfactant film, which determines the possible structures and ways of structural conversions by changes in component ratios, is an important factor for a given microemulsion system. Very rigid surfactant films will likely result in droplet-like shapes and will not enable the existence of bicontinuous structures. This will hinder the range of existence and microemulsions will only form in very narrow composition ranges. A more flexible surfactant film will most likely enable the existence of several different structures like aggregates and bicontinuous structures and thus broaden the range of existence, enabling formation of microemulsion with a wide diversity of compositions.

The internal structure of a microemulsion vehicle is very important for the diffusivity of the phases and thereby also for the diffusion of a drug in the respective phases (Kreilgaard, 2002:S80).

2.9.1.2.1 Characterization

Microemulsion structures can vary from emulsion-like normal or reverse swollen micelles, to aggregates with typical sizes of 10-100 nm, over bicontinuous structures, depending on the ratio of the components (Kreilgaard et al., 2000:422).

2.9.1.3 Pharmaceutical considerations

2.9.1.3.1   Choice of microemulsion components

The choice of components for pharmaceutical microemulsions is often a balance between compounds which are able to form microemulsions, are nontoxic and are able to fulfil the requirements of a good vehicle for optimal dermal absorption that is, high solubility and a high thermodynamic activity of the drug of interest. A lot of characterization studies of microemulsions have been done with medium chain alcohols as co-surfactant since they can form microemulsions with many surfactants and oils. However, they are generally recognized to induce skin irritation (Kreilgaard, 2002:S82) and therefore, the removal of such a cosurfactant from microemulsions would be preferred for pharmaceutical applications. Fortunately, this can be achieved by the use of nonionic surfactants such as the monoalkyl polyoxyethylene ethers (Brij®), which are known to form microemulsions without the addition of a co-surfactant. These surfactants have the added advantage of being less toxic than their ionic equivalents (Malcolmson & Lawrence, 1995:98).

Depending on the physicochemical properties of the components and composition, the stability of the microemulsion can be affected by the addition of buffers, electrolytes, preservatives, polymers and drugs which may alter the microemulsion structure and region of existence. Microemulsions based on nonionic surfactants are normally less affected by additives and changes in pH than ionic surfactants (Kreilgaard, 2002:S83).

2.9.1.3.2   Formulation

In contrast to regular emulsions, microemulsion vehicles are formed spontaneously when mixing the suitable quantities of the components, without requiring additional mechanical energy, and they are physically stable due to their thermodynamic nature. Furthermore, they are clear or slightly opalescent with a low viscosity and can be thickened by a suitable noninteracting polymer, such as carrageenan, or a gel forming surfactant. These characteristics of microemulsions make them very easy to prepare for pharmaceutical formulations. The wide range of oil-water-surfactant compositions, which can form microemulsions, enable solubilization of a wide range of lipophilic and hydrophilic drugs (Kreilgaard, 2002:S83).

2.9.2 Mechanism of action

A schematic diagram of the variety of possible events involved in transdermal drug delivery from a microemulsion formulation is represented in Figure 2.9. There are different partitioning processes occurring, namely, between the internal and external phases of the microemulsion, and between the internal or external phase of the microemulsion and the skin (steps a, b, c). Drug transport can be controlled by any of these processes and the thermodynamic driving force for release will reflect the relative activities of the drug in the different phases. Furthermore, it is possible that one or more components of the microemulsion enters the skin and interacts with the stratum corneum to alter the barrier properties, thereby increasing the permeability (step d). Additionally, there is the possibility that lipids of the skin are extracted by the microemulsion leading to a new physical entity from which some drug delivery now takes place (step e) (Delgado-Charro et al., 1997:41).
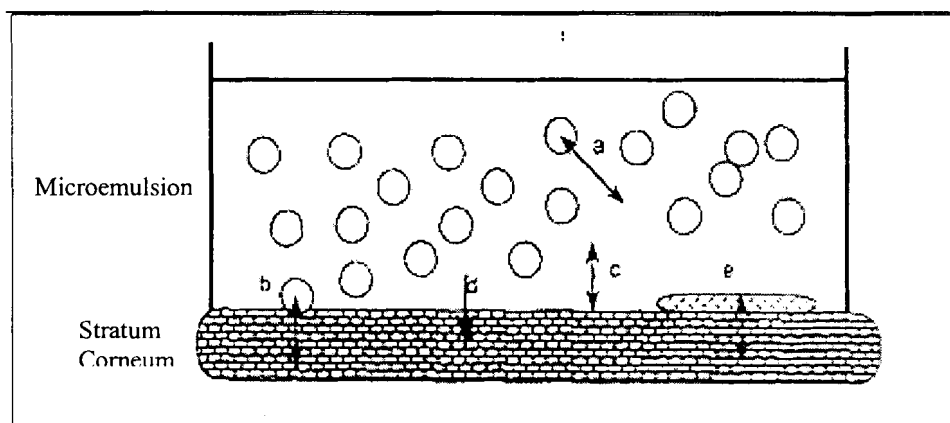
Figure 2.5: Schematic diagram of the possible events involved in transdermal drug delivery from a microemulsion (Delgado-Charro et al., 1997:41).

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2.10 Summary

The skin forms a complex barrier to the external environment, thus protecting us against harmful substances and maintaining bodily fluids within our system (Guy & Hadgraft, 1989:13). The stratum corneum is the main barrier to transdermal delivery of most drugs. The transport of drugs is primarily through two pathways, the intercellular or transcellular route, depending on the physicochemical properties of the drug.

There are many variables involved in transdermal drug delivery, which are strongly interrelated. These variables relate to the skin, the penetrating drug and the vehicle containing the drug. Transdermal delivery can be considered to be the result of two opposing effects, namely the lipophilicity and hydrophilicity of the penetrating drug. This phenomenon makes the skin a unique barrier. Given this barrier function, the key requirements for transdermal application of a therapeutic agent are a good solubility in lipoidal and aqueous systems, low molecular weight and volume, high diffusivity in the stratum corneum and little or no binding in the stratum corneum (Wiechers, 1989:195).

Although there are many biological and physicochemical factors that influence transdermal drug delivery, it can be promoted by penetration enhancers, such as physical- and chemical enhancers. Chemical penetration enhancers present an effective alternative to other more complex and expensive methods, including iontophoresis (Büyüktimkin et al., 1997:442). The use of penetration enhancers provides an opportunity to reduce the barrier properties of the skin temporarily and reversibly, and to enhance and control the delivery of drugs through the largest and most easily accessible entrance to systemic circulation. However, it also presents great challenges to control the delivery of drugs efficiently, without side effects and in therapeutic amounts from a transdermal delivery system (Büyüktimkin et al., 1997:446).

CHAPTER 3

EFFECT OF BRIJ 97 IN THE PRESENCE AND ABSENCE OF CARRAGEENAN ON THE TRANSDERMAL DELIVERY OF 5-FLUOROURACIL

3.1 Introduction

The outermost layer of the skin, the stratum corneum, is highly lipophilic and consists of a non-living layer of corneocytes surrounded by a lipid-rich extracellular matrix (Prausnitz *et al.*, 2004:115). Because of its highly organized structure, the stratum corneum is the main permeability barrier to external materials and is regarded as the rate-limiting factor in the permeation of therapeutic agents through the skin. Thus, the ability of various agents to interact with the intercellular lipid dictates the degree to which absorption is enhanced (Foldvari, 2000:418).

5-Fluorouracil is a hydrophilic compound with a log P value of -0,89 and is therefore not a good penetrant through the stratum corneum (Williams & Barry, 1991:166) (Tables 2.1 and 2.2). Hydrophilic drugs are known as some of the most problematic in dermal administration. In order to obtain increased penetration, it is therefore necessary to develop vehicles that will facilitate the transport of these compounds by affecting lipid barrier properties of the stratum corneum (Schmalfuß *et al.*, 1997:280). According to Flynn & Stewart (1988) hydrophilic drugs have great potential for enhancement because of their low permeability coefficients (Williams & Barry, 1991:166). Currently, the most widely used approach to drug permeation enhancement across the stratum corneum barrier is the use of chemical penetration enhancers (Asbill & Michniak, 2000:37). They generally partition into the skin and interact with different skin components to obtain temporary and, ideally, reversible reduction of barrier properties (Büyüktimkin *et al.*, 1997:358).

The purpose of this study was to investigate the penetration enhancing effect of Brij 97 in the presence and absence of carrageenan on the transdermal delivery of 5-fluorouracil.

3.2 Analytical methods

3.2.1 Materials

5-Fluorouracil was obtained from Fluka (Steinheim, Switzerland). Brij 97, soybean oil, carrageenan as well as sodium 1-octanesulfonate monohydrate were purchased from Sigma-Aldrich (Steinheim, Germany). HPLC analytical grade acetonitrile (VWR International Ltd Poole, England) was used in the experiments. Orthophosphoric acid 85% AR, sodium chloride (NaCl), sodium dihydrogen orthophosphate dihydrate (NaH$_2$PO$_4$.2H$_2$O) and disodium hydrogen orthophosphate dihydrate (Na$_2$HPO$_4$.2H$_2$O) were supplied by Merck Laboratory Supplies (Midrand, South Africa). HPLC grade water (double distilled deionized) prepared by a Milli-Q 50 water purification system (Millipore, Milford, USA) was used throughout the study.

3.2.2 High pressure liquid chromatography (HPLC)

3.2.2.1 Apparatus

The HPLC system used for the analysis of 5-fluorouracil was an Agilent 1100 series equipped with a variable wavelength UV detector, isocratic pump, autosampler and Chem-station.Rev.A.06.02 data acquisition and analysis software. A Phenomenex® Synergi 4μ Hydro-RP 80A (250 x 4.6mm) column together with a Phenomenex® Security Guard precolumn (C-18.4 x 3mm) insert were used; the last had the purpose of prolonging column life.

3.2.2.2 Chromatographic conditions

All analyses were performed with a mobile phase comprising of 3% acetonitrile, 1g sodium 1-octanesulfonate monohydrate and HPLC grade water. The pH was adjusted to 3,5 with a 10% orthophosphoric acid solution. The mobile phase was then filtered through a 0,45 μm HV filter (Millipore, Milford, USA) with a BUCHI (model B-169, Switzerland) vacuum system to degas and to remove any solid particles from the mobile phase.

The samples were eluted at a flow rate of 1,0 ml/min with retention times ranging from 4-5 minutes. Analyses were performed at a detection wavelength of 266 nm at room temperature (25 ± 1 °C).

The injection volume for all the samples was 100 μl and the stop time was 15 minutes for each sample.

3.2.2.3 Preparation of standard solutions

Ten milligrams of 5-fluorouracil was weighed, transferred to a 100 ml volumetric flask and made up to volume with HPLC grade water to produce a 100 μg/ml stock solution. Standard solutions with concentrations of 25, 50, 100, 200, 300, 500, 1000 and 2000 ng/ml were prepared from the stock solution. These dilutions were used for the validation of the HPLC.

3.2.2.4 Validation of the HPLC analytical method

3.2.2.4.1 Linearity

The linearity of an analytical method is its ability (within a given range) to obtain test results which are directly proportional to the concentration of analyte in the sample. The linearity for 5-fluorouracil was determined by performing a linear regression analysis on the plot of peak area under the curve (AUC) against concentration (Figure 3.1). Eight standard solutions were prepared, as described in § 3.2.2.4, to obtain concentrations ranging from 25 to 2000 ng/ml. The regression value (r^2) was greater than 0,9999 and the y-intercept was 0,8376.
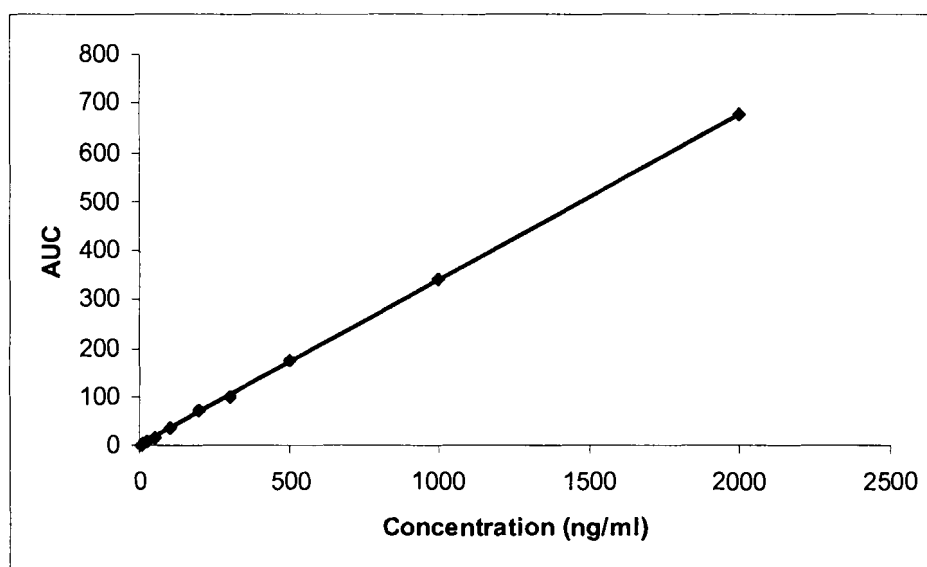
Figure 3.1: Linear regression curve of 5-fluorouracil standards.

3.2.2.4.2   Precision

The precision of an analytical method expresses the closeness of agreement (degree of scatter) between sequences of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision was studied in terms of intra-day (repeatability) and inter-day (reproducibility) variation.

- **Intra-day precision**

The intra-day precision was determined by performing HPLC analyses (n = 3) of eight known standard solutions of 5-fluorouracil on the same day. The results can be seen in Table 3.1 and were within acceptable limits.

Table 3.1:   The mean area under curve (AUC), standard deviation (S.D.) and percentage relative standard deviation (%RSD) for 5-fluorouracil after analysis of three sets of samples on the same day.

Standards (ng/ml)	AUC Mean	S.D.	%RSD
25	8,85	0,04	0,51
50	18,33	1,26	6,86
100	34,70	0,98	2,83
200	70,26	0,56	0,80
300	103,66	2,38	2,30
500	174,63	0,75	0,43
1000	337,48	20,71	6,14
2000	669,37	30,18	4,51

- **Inter-day precision**

The inter-day precision was determined by performing HPLC analyses (n = 3) of eight different standard solutions of 5-fluorouracil on three consecutive days. The results can be seen in Table 3.2 and were within acceptable limits.

Table 3.2:   The mean area under curve (AUC), standard deviation (S.D.) and percentage relative standard deviation (%RSD) for 5-fluorouracil after analysis of three sets of samples on three consecutive days.

Standards (ng/ml)	AUC Mean	S.D.	%RSD
25	8,29	0,65	7,89
50	17,29	0,16	0,90
100	34,54	0,13	0,37
200	69,60	0,77	1,10
300	93,57	7,28	7,78
500	172,60	2,33	1,35
1000	344,25	1,59	0,46
2000	686,53	2,29	0,33

3.2.2.4.3   Selectivity

Selectivity is the ability of the analytical method to detect and analyse a specific compound in the presence of other components. The mobile phase, phosphate buffer solution (PBS) pH 7,4 and the formulation containing carrageenan were separately analysed by HPLC. The method that was used proved to be selective, since there were no interfering peaks with the same retention time as 5-fluorouracil.

3.3   Solubility determination

A saturated solution of 5-fluorouracil was prepared by adding an excess amount of 5-fluorouracil to HPLC grade water in order to determine the aqueous solubility of this compound. The 5-FU/HPLC water solution was equilibrated at a constant temperature of 37 °C. The temperature was held constant by means of a water bath (Grand Instruments, UK). A magnetic stirring bar was added to the solution and stirring was maintained by a submersible magnetic stirring bed. Equilibrium of 5-fluorouracil was reached within 24 hours. After 24 hours, the solution was filtered through a 0,45 μm filter (Type HV, Millipore). The filtrate was appropriately diluted with HPLC grade water prior to its assay and the concentration of 5-fluorouracil was determined by HPLC. The experiment was done in triplicate (Steenekamp, 2003:46; Gerber, 2003:42).

The determined aqueous solubility of 5-fluorouracil was 13,71 mg/ml. Experiments showed that crystals formed when the amount of compound was incorporated into the formulation and left to cool to room temperature (25 °C). Therefore, it was suggested that we do not use a saturated solution but a concentration of 0,1% (m/m).

3.4 Preparation of experimental samples

The formulation consisted of a specific amount (4; 8; 15 and 25% m/m) polyoxyethylene-10-oleyl ether (Brij 97), 7,88% (m/m) soybean oil, distilled water and 0,1% (m/m) 5-fluorouracil. A sample was typically prepared by weighing the Brij 97 and soybean oil in the same beaker. The water was weighed in another beaker and the 5-fluorouracil dissolved in the water. The two beakers and their contents were heated to 80 °C and the oil and surfactant (Brij 97) mixture was poured into the heated water while stirring. In order to test the influence of carrageenan, the formulation was subsequently mixed at room temperature (25 °C) with 1,5% (m/m) carrageenan.

3.5 Experimental methods

3.5.1 Confocal laser scanning microscopy

To identify the formulations, the drug containing samples were viewed with the use of a confocal laser scanning microscope (CLSM). The use of a laser as an energy source enables the microscope to act as an optical knife that can optically section a sample at varying depths. Due to the addition of a Hamamadzu CCD camera, dynamic changes can also be visualized in real time. The confocal microscope system used was a Nikon PCM 2000 equipped with krypton and helium/neon lasers. A 60x Plan Achromat oil immersion objective with a numerical aperture of 1,4 was used for identification of the different formulations (Saunders et al., 1999:2). Nile red was chosen as the fluorophore as it primarily associates with lipids and thus readily stains the fatty acid components of the formulations. Excitation of Nile red was at 505 nm and emmision of Nile red was monitored at a wavelenght of 568 nm.

3.5.2 Particle size analysis

Particle size analysis of the samples was carried out with a Mastersizer Micro (Malvern Instruments Ltd., Sparing Lane South, Worcs, England) equipped with a helium/neon laser (633 nm wavelength). A 1000 ml glass beaker containing ± 800 ml water as disperse medium was used and the rotating speed was set at 2500 rpm. The real and imaginary refractive indexes were set at 1,4564; 0,1 in 1,33 and 0,0, respectively. Two measurements were carried out for each sample. Data are expressed as mean value ± standard deviation (S.D.).

3.5.3 Zeta potential analysis

Zeta potential distribution was measured with a Zetasizer (Malvern Instruments Ltd., Grovewood Road, Enigma Business Park, England) equipped with a neon/helium laser (633 nm wavelength). Samples were diluted with distilled water to produce a 0,002 µl/ml solution. Ten measurements were carried out for each sample. Data are expressed as mean value ± standard deviation (SD).

3.5.4 pH Measurements

The pH of the formulations in the presence and absence of carrageenan was measured before and after the addition of 5-fluorouracil with the Metrohm Autotitrate 785 DMP Titrino pH meter. This was done to evaluate the integrity of the internal structure of the formulations after the addition of 5-fluorouracil. The pH measurements obtained were also used to determine the state of ionization of 5-fluorouracil at each formulation's specific pH.

3.5.5 Dissolution of 5-fluorouracil from the formulations

The *in vitro* release rate of 5-fluorouracil from the different percentages of Brij formulations containing carrageenan was measured with a release unit that fits directly onto the VanKel® VK 700 six station dissolution apparatus (Figure 3.2). The release experiment was carried out in six fold for each preparation. In each experiment the reservoir of the dissolution cell was filled with the formulation containing carrageenan and covered with a membrane (0,45 µm pore size), taking care to exclude air bubbles between the formulation and the membrane. The cell was capped and placed in the dissolution vessel that contained 190ml of the receptor medium (phosphate buffer solution).

The paddle speed was set at 150 rpm and the temperature at 32 °C. Samples of 200 μl were withdrawn with a micropipette at time intervals of 30, 60, 120, 180, 240, 300 and 360 minutes. The samples were analysed by the described HPLC method to determine the amount of 5-fluorouracil which have been released by the formulation (Reynecke, 2004:44).
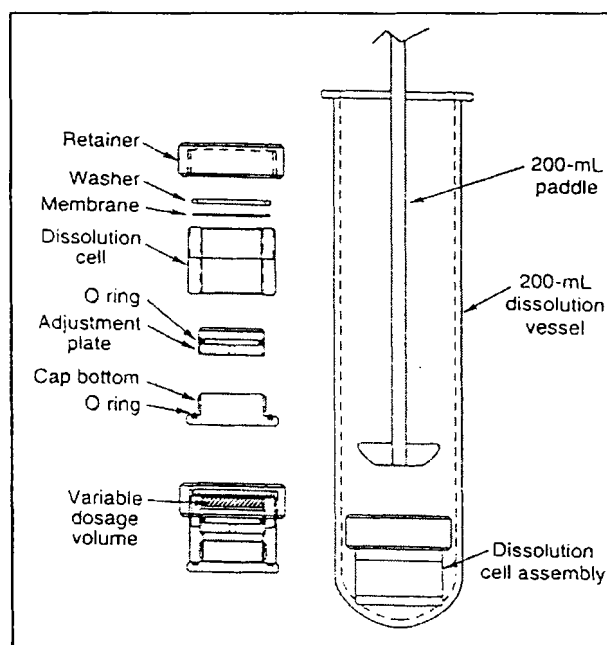
Figure 3.2: The dissolution cell and dissolution vessel for the VanKel® dissolution apparatus.

3.5.6 *In vitro* transdermal diffusion studies

3.5.6.1 Skin preparation

The skin used in the permeation studies was female human abdominal skin and was obtained after cosmetic surgery. The full-thickness skin was frozen at -20 °C within 24 hours after excision. Before preparation, the skin was thawed at room temperature. The adipose tissue was removed by blunt dissection and the epidermal layers were separated by immersing the skin in 60 °C water for one minute. The epidermis was placed in a bath filled with distilled water and carefully set on Whatman® filterpaper and left to dry. When dry it was wrapped in aluminium foil and stored in a freezer at -20 °C until used. Prior to a diffusion study, the skin sections were examined for any defects and then cut into circles with a diameter of approximately 10 mm, before it was mounted on the Franz diffusion cells (Steenekamp, 2003:47).

3.5.6.2 Diffusion studies

Vertical Franz diffusion cells (Figure 3.3) with receptor capacities varying from 1,9 to 2,3 ml and a $1,075$ cm^2 diffusion area were used in the diffusion studies. The epidermal skin layer, prepared as described previously, was mounted on the lower half (receptor compartment) of the diffusion cell with the stratum corneum facing upwards. A metal clamp was used to fasten the upper and lower parts of the Franz cell together, with the prepared epidermal layer separating the donor and receptor compartments. A small magnetic stirring bar was placed in each receptor compartment to accomplish stirring and rotated at a speed of 500 rpm.

The receptor compartments were filled with phosphate buffer solution (PBS). Care was taken to ensure that no air bubbles were trapped in the compartment or underneath the skin. The diffusion cells were placed in a water bath at a constant temperature of 37 °C, on a submersible magnetic stirring bed and stirring was continued throughout the entire experiment. Only the receptor compartments were submerged in the water. The diffusion cells, containing PBS, were equilibrated for one hour. After a period of one hour, 1 ml of the Brij 97 formulation with and without carrageenan or 1 ml of aqueous solution containing 0,1% (m/m) 5-fluorouracil (control) was then applied onto the stratum corneum in the donor compartment and covered immediately with Parafilm® to prevent evaporation from the donor compartment for the duration of the experiment. An excess amount of formulation was present in the donor compartment at all times during the experimental procedures (Steenekamp, 2003:48)
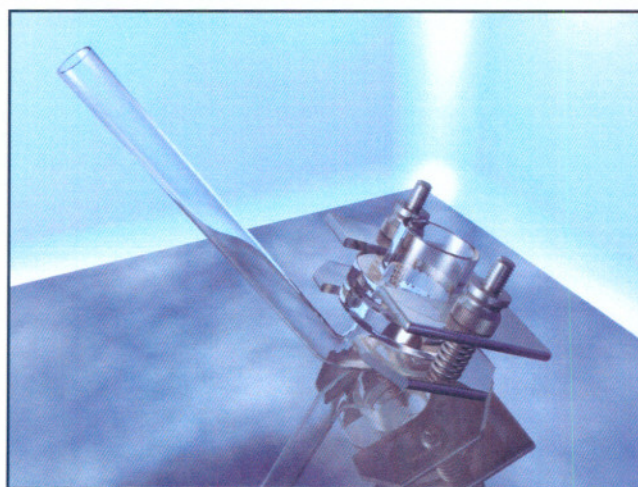

Figure 3.3:   Vertical Franz diffusion cell.

The receptor compartment contained phosphate buffer solution (PBS) at physiological pH 7,20, consisting of 4,4 g sodium chloride (NaCl), 2,1 g sodium dihydrogen orthophosphate ($NaH_2PO_4 \cdot 2H_2O$) and 9,2 g disodium hydrogen orthophosphate ($Na_2HPO_4 \cdot 2H_2O$) in HPLC grade water to 1000 ml (Steenekamp, 2003:46).

3.5.6.3 Sample collection

The entire receptor volumes was withdrawn and replaced with 37 °C fresh PBS after 2, 6, 8, 10, 12, 14, 16, 18, 20 and 24 hours. This was done to mimic sink conditions as they occur in the human body. The experiments were conducted over 24-hour periods. The amount of 5-fluorouracil that permeated through the epidermal layer was recovered in the receptor compartment and was directly analysed by HPLC (Steenekamp, 2003:48).

3.5.6.4 Calibration curves

The concentration of 5-fluorouracil that permeated through the epidermal layer was determined by comparison to a calibration curve that was drawn up from standard solutions, with concentrations ranging from 5 to 2000 ng/ml as described in § 3.2.2.4, prior to each experiment.

3.5.7 Histopathological studies

Histopathological studies were carried out on female human abdominal skin (epidermis) in order to determine whether Brij 97 had an effect on the skin. Topically treated skin pieces were collected and fixated with Todd's solution (Todd, 1986). It was then dehydrated with acetone and inbedded in Spurr's resin. Sections of 100 nm were cut on the Ultra Microtome-Reichert-Jung Ultracut E and stained with 2% uranyl acetate and lead citrate. Micrographs were taken with a Philips CM 10 transmission electron microscope and compared to the control skin. The topical treatment consisted of the formulations being applied to the skin sections in the Franz diffusion cells for 24 hours. To maintain the uniformity of the experiment, 1 ml of Brij 97 (4; 8; 15 and 25%) formulation was applied on a 1,075 cm^2 area of the skin.

3.6 Results and discussion

3.6.1 Confocal laser scanning microscopy

3.6.1.1 Results

The confocal micrographs of the Brij 97 formulations with and without carrageenan are depicted in Figures 3.4 and 3.5.

(a)   (b)

(c)   (d)
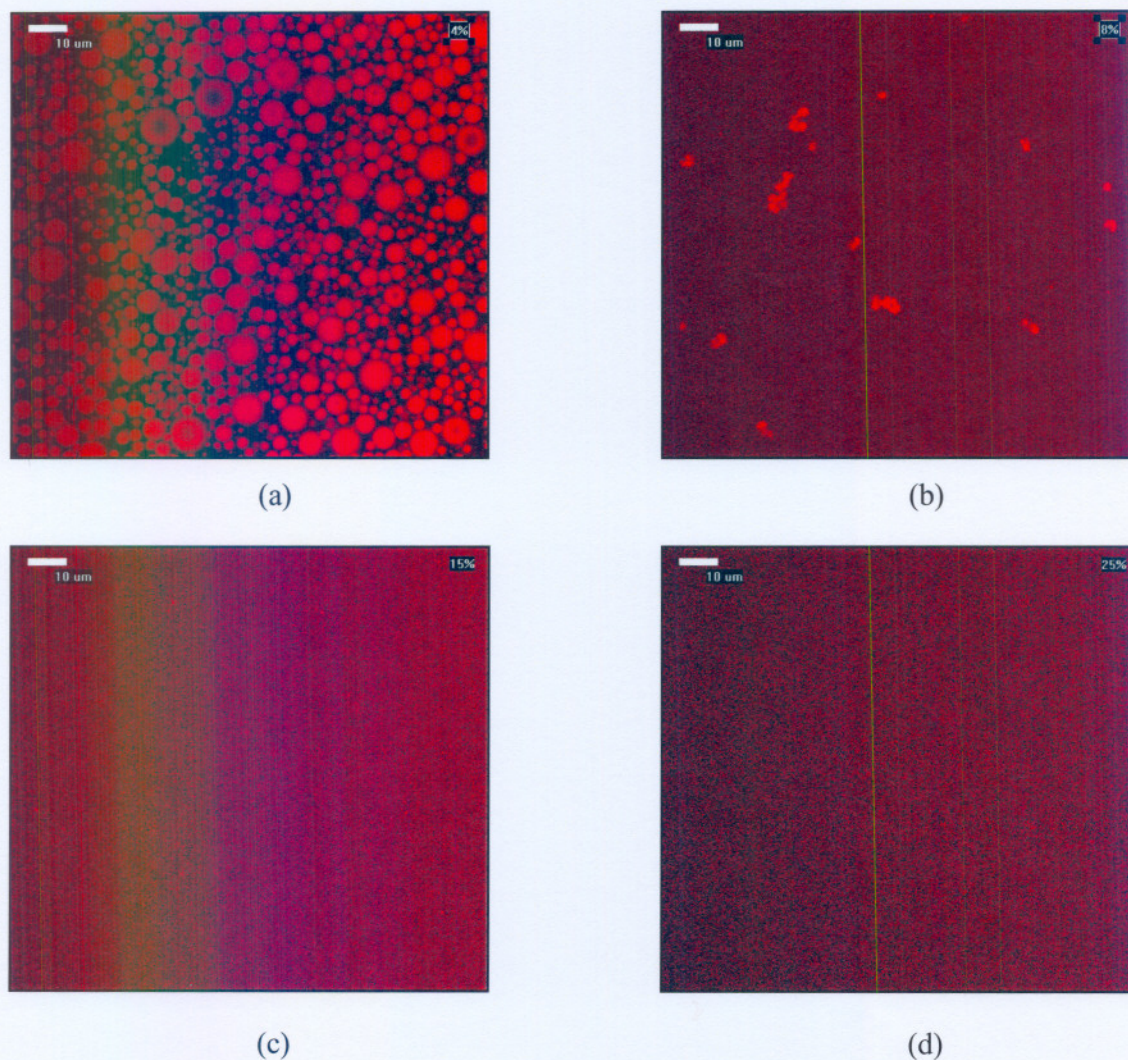
Figure 3.4: Confocal micrographs of the 4, 8, 15 and 25% Brij 97 formulations in the absence of carrageenan from a – d, respectively. The vesicles are indicated in red and the black space within and around the vesicle is the water phase. The white bar in each micrograph represents 10 μm.

(a)

(b)

(c)

(d)
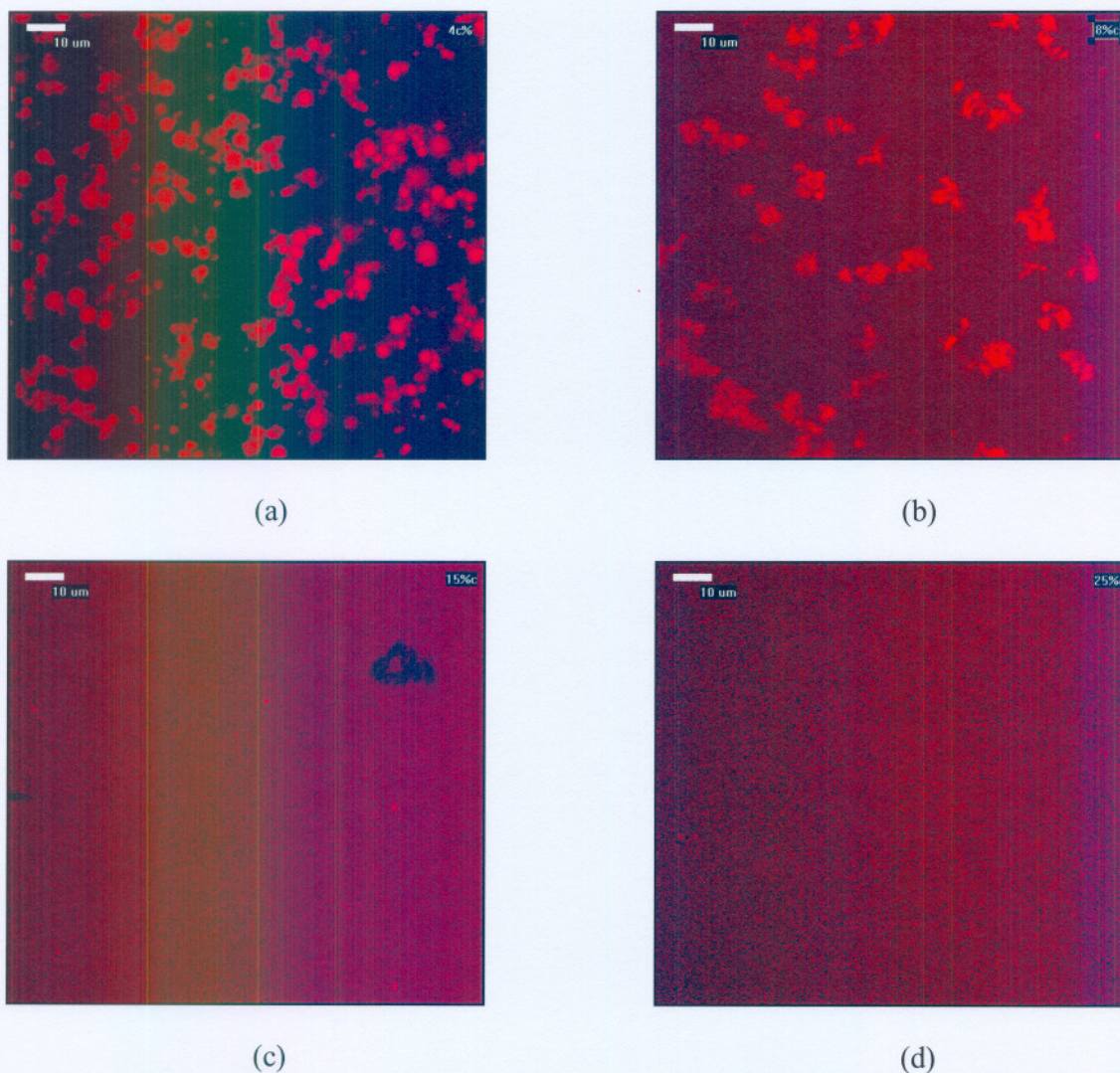
Figure 3.5:   Confocal micrographs of the 4, 8, 15 and 25% Brij 97 formulations in the presence of carrageenan from a – d, respectively. The vesicles are indicated in red and the black space around the vesicle is the water phase. The white bar in each micrograph represents 10 μm.

3.6.2 Particle size analysis

3.6.2.1 Results

A particle size analysis was carried out on the Brij 97 formulations in the presence and absence of carrageenan. The following results were obtained (Figures 3.6 to 3.8).
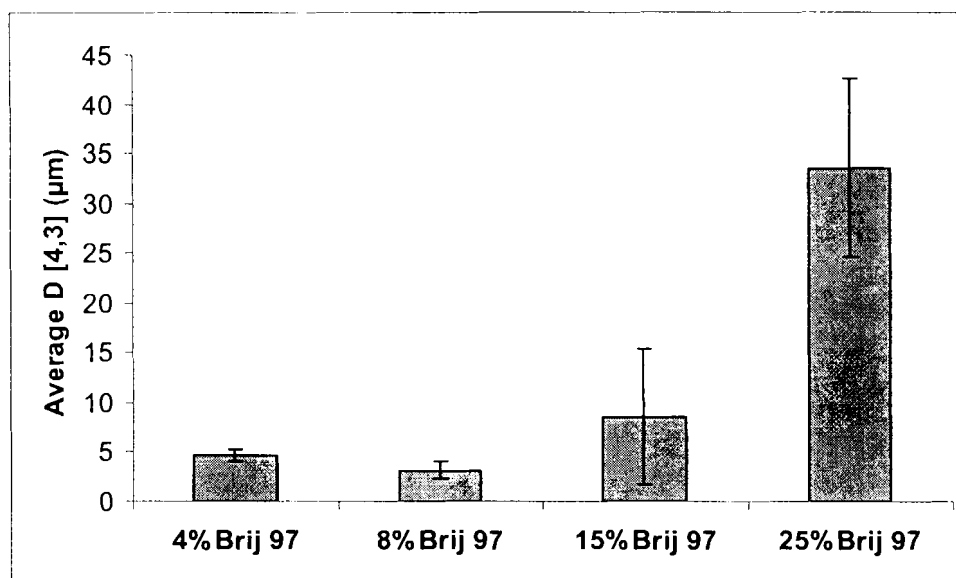
Figure 3.6: The average ± S.D. of the particle size of the 4, 8, 15 and 25% Brij 97 formulation in the absence of carrageenan.
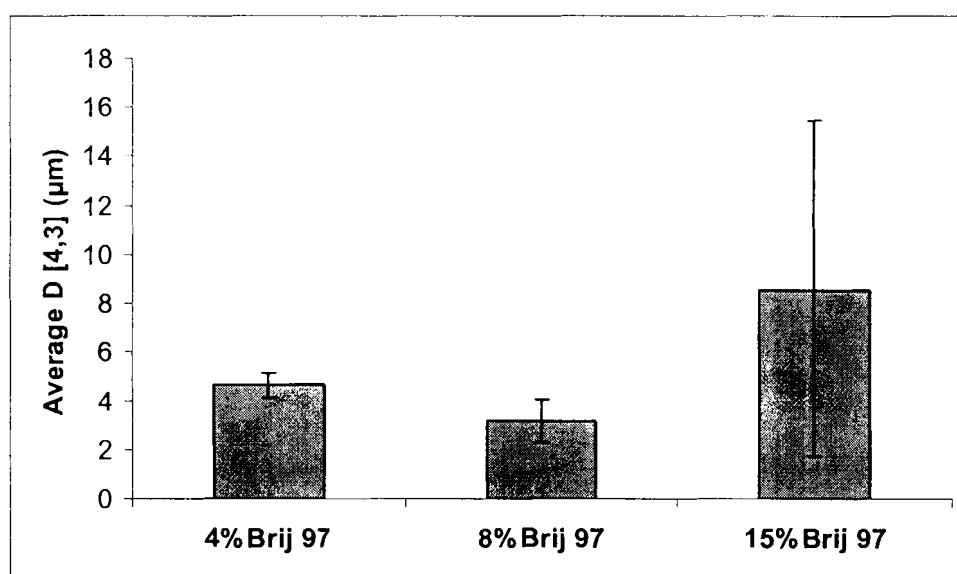
Figure 3.7: The average ± S.D. of the particle size of the 4, 8 and 15% Brij 97 formulation in the absence of carrageenan.
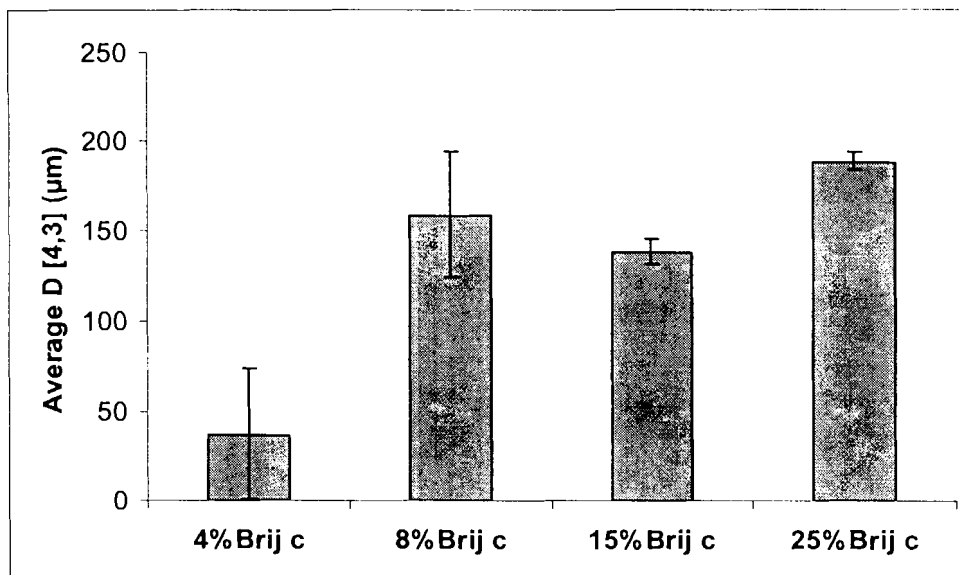
Figure 3.8: The average ± S.D. of the particle size of the 4, 8, 15 and 25% Brij 97 formulation in the presence of carrageenan (carrageenan is indicated with c).

3.6.2.2 Discussion and conclusions

As depicted in the confocal micrographs, the 4% Brij 97 formulation without carrageenan has large and small vesicles, while the 8% Brij 97 formulation without carrageenan has small vesicles and vesicles which are aggregated. The vesicles of the 4% and that of the 8% Brij 97 formulations containing carrageenan are aggregated with the 4% having smaller vesicles than the 8% Brij 97 formulation. The 15% Brij 97 formulation with and without carrageenan has either no or else very tiny vesicles and the 25% Brij 97 formulation has very small vesicles (Figure 3.4 and 3.5). The aggregation of the vesicles in the 4 and 8% Brij 97 formulations containing carrageenan could be explained by the presence of the carrageenan. K-Carrageenan is widely employed in the food, pharmaceutical and cosmetic industry as a thickening, gelling and stabilizing agent because of its ability to form thermoreversible gels (Roesch *et al.*, 2004:429).

The average particle size of the Brij 97 formulations without carrageenan decreased with an increase in the percentage surfactant up to a certain percentage (8% Brij 97). A further increase of the surfactant led to an increase in the average particle size.

The average particle size of the Brij 97 formulations containing carrageenan increased with an increase of the percentage surfactant when the standard deviation of the 8% Brij 97 formulation is taken into account.

When comparing the confocal micrographs with the corresponding average particle size of the Brij 97 formulations with and without carrageenan, the following conclusions could be made: The average particle size of the 4 and 8% Brij 97 formulations without carrageenan reflects the results of the corresponding confocal micrographs, namely, the 4% Brij 97 formulation has larger vesicles than the 8% Brij 97 formulation. The average particle size of the 4 and 8% Brij 97 formulations with carrageenan also reflects the results of the corresponding confocal micrographs, namely, the 4% Brij 97 formulation has smaller vesicles than the 8% Brij 97 formulation. Conversely, there is no correlation between the average particle size of the 15 and 25% Brij 97 formulations with and without carrageenan and the confocal micrographs. As depicted in the confocal micrographs, there were no or exceptionally small vesicles in the 15% Brij 97 formulations with and without carrageenan while the particle size analysis indicated that there were fairly large (> 3 μm) vesicles. Furthermore, the confocal micrographs of the 25% Brij 97 formulation with and without carrageenan showed that the vesicles were very small, whereas the particle size analysis indicated that the vesicles were large (> 30 μm and > 150 μm). The Mastersizer Micro can only analyse particle sizes that range between 0,31 and 301 μm. Thus, it could be that only the few large vesicles were analysed in the case of the 15 and 25% Brij 97 formulations without carrageenan while only the few large carrageenan-surfactant-aggregates could have been analysed in the case of the 15 and 25% Brij 97 formulations with carrageenan. Thus the small vesicles (< 0,31 μm) were not detected by the Mastersizer Micro.

The particle size of an emulsion normally ranges between 0,5 and 5 μm whereas the particle size of a microemulsion is generally smaller than 0,1μm (Table 2.4). The average particle size of the 4% Brij 97 formulation without carrageenan is 4,655 μm ± 0,53 and that of the 8% Brij 97 formulation without carrageenan 3,145 μm ± 0,88. The particle size of both the formulations ranges between 0,5 and 5 μm and, therefore, 4 and 8% Brij 97 formulations can be classified as emulsions. The vesicles of the 15 and 25% Brij 97 formulations without carrageenan are, according to CLSM, much smaller than the vesicles of the 4 and 8% Brij 97 formulations without carrageenan and, therefore, the 15

and 25% Brij 97 formulations can be identified as microemulsions. When the carrageenan was added to the four formulations, it became more viscous and caused cohesion of the vesicle. Thus, the four formulations containing carrageenan can be identified as gels.

3.6.3 Zeta potential analysis

3.6.3.1 Results

The changes in potential with distance from the surface of the particle may be represented as shown in Figure 3.9. The zeta potential is defined as the difference in electrical charge (potential) between the surface of the dense layer of ions surrounding the particle (tightly bound layer or shear plane) and the electroneutral region of the solution (Martin, 1993:387; Zeta potential instruments, inc., 2004:1). Zeta potential is measured to determine the stability of the formulations. Zeta potential values of the Brij 97 formulations in the presence of 5-fluorouracil are given in Table 3.3.
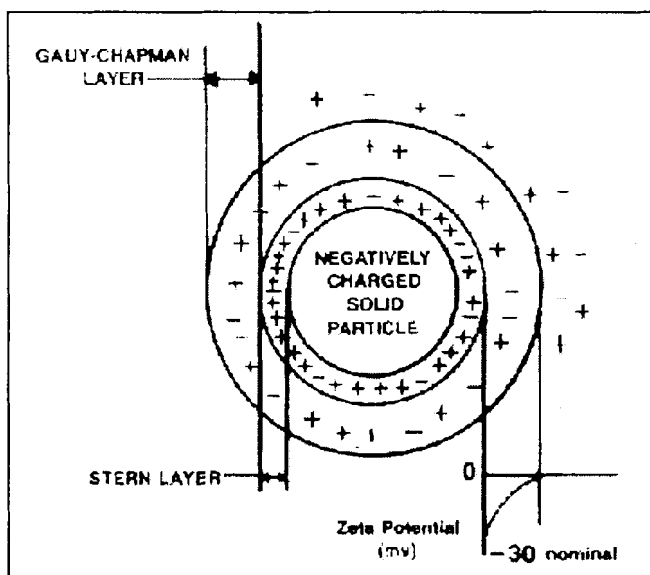

Figure 3.9: The electrical double layer at the surface of separation between two phases, showing distribution of ions. The system as a whole is electrically neutral (Zeta potential instruments, inc., 2004:1).

Table 3.3: Zeta potential values of the Brij 97 formulations in the presence and absence of carrageenan containing 5-fluorouracil (carrageenan is indicated by c). Mean ± S.D., n = 10.

Formulations without carrageenan	Zeta potential (mV)	Formulations with carrageenan	Zeta potential (mV)
4% Brij 97	-33,7 ± 1,5	4% Brij 97 c	-31,1 ± 2,7
8% Brij 97	-28,1 ± 1	8% Brij 97 c	-23,5 ± 0,4
15% Brij 97	-20,2 ± 2,6	15% Brij 97 c	-19,8 ± 1
25% Brij 97	-23,7 ± 6,2	25% Brij 97 c	-27,6 ± 5,6

3.6.3.2 Discussion and conclusions

When the zeta potential of the different Brij 97 formulations was determined, the results indicated the following. As the percentage Brij 97 increased, the zeta potential values became less electronegative up to a certain percentage (15% Brij 97) (Table 3.3). However, when the zeta potential of the formulation containing 25% Brij 97 was determined, the value was found to be more electronegative in comparison with the 15% Brij 97 formulation. This trend can be observed for both the formulations with and without carrageenan.

When comparing the zeta potential values of the corresponding formulations with and without carrageenan, the values of the formulations without carrageenan are consistently more electronegative than the values of the formulations with carrageenan. However, the zeta potential of the 25% Brij 97 formulation with carrageenan is more electronegative than the formulation without carrageenan.

The zeta potential generally ranges between -14 and -30 millivolts for a colloidal system. Values more electronegative than -30 mV normally represent adequate mutual repulsion to result in stability (Zeta potential instruments, inc., 2004:1). The 4% Brij 97 formulation with and without carrageenan is the most stable since the zeta potential is the most electronegative.

3.6.4 pH measurements

3.6.4.1 Results

The nonpolar nature of the stratum corneum suggests that charged molecules should encounter high resistance to permeation (Zatz, 1993: 28). The pH of the vehicle, in which the compound is present, in combination with the compound's ionization constant, pK_a, will determine the actual concentrations of the ionized and unionized species (Wiechers, 1989: 190) and thus it is important to measure the pH of the formulations.

5-Fluorouracil has dissociation constants (pK_a values) of 8 and 13 (Rudy & Senkowski, 1973). The percentage unionized 5-fluorouracil was calculated with the Henderson-Hasselbach equation (Ritschel, 1988 quoted by Steenekamp, 2003:49).

The Henderson-Hasselbach equation for acidic compounds is given in the following equation:

$$\frac{100}{1 + anti\log(pKa - pH)} \qquad \text{Equation 3.1}$$

The % unionized drug = 100 - % ionized drug.

The pH values for the Brij 97 formulations in the presence and absence of carrageenan before and after the addition of 5-fluorouracil and the percentage of 5-fluorouracil in the unionized state at the formulations' specific pH are shown in Table 3.4 and 3.5.

Table 3.4: The pH values for the Brij 97 formulations in the presence and absence of carrageenan before and after the addition of 5-fluorouracil, n = 3.

Formulations without carrageenan	Without 5-fluorouracil	With 5-fluorouracil
4% Brij 97	6,22	5,70
8% Brij 97	5,59	5,29
15% Brij 97	5,05	5,00
25% Brij 97	5,17	5,18
4% Brij 97 c	6,64	7,01
8% Brij 97 c	6,45	6,25
15% Brij 97 c	6,55	6,50
25% Brij 97 c	5,93	6,15

Table 3.5: The percentage of 5-fluorouracil in the unionized state at the formulations' specific pH. Percentages for 5-fluorouracil were calculated using a pK_a value of 8.

Formulations without carrageenan	% Unionized	Formulations with carrageenan	% Unionized
4% Brij 97	99,50	4% Brij 97 c	90,72
8% Brij 97	99,81	8% Brij 97 c	98,25
15% Brij 97	99,90	15% Brij 97 c	96,93
25% Brij 97	99,85	25% Brij 97 c	98,61

3.6.4.2 Discussion and conclusions

There was no significant change in the pH of the Brij 97 formulations in the presence and absence of carrageenan before and after the addition of 5-fluorouracil, an indication that 5-fluorouracil did not alter the internal structure of the formulations.

The active (5-fluorouracil) in the Brij 97 formulations without carrageenan were more than 99% unionized while the active in the Brij 97 formulations with carrageenan were between 90 and 98% unionized. Thus, the percentage unionized 5-fluorouracil in the Brij 97 formulations without carrageenan was higher than that of the Brij 97 formulations with carrageenan. This might be attributed to the addition of carrageenan, the gellating agent, which increased the pH of the

formulations and decreased the percentage unionized 5-fluorouracil, especially in the case of low surfactant concentrations (4% Brij 97).

According to the pH-partition hypothesis only the unionized form of the drug can permeate through the lipid barrier in significant amounts (Williams, 2003:380). The formulations without carrageenan might therefore lead to a better permeation of 5-fluorouracil than the formulations with carrageenan.

3.6.5 Dissolution of 5-fluorouracil from the formulations

3.6.5.1 Results

The release rate of 5-fluorouracil from the four Brij 97 formulations containing carrageenan were measured over a six hour period using membrane diffusion. The release experiment was done six fold and the average release was calculated for each analysis point. According to theory a plot of concentration in µg 5-fluorouracil per square cm membrane against the square root of time in minutes should produce a straight line. This is indeed the case (Figure 3.10), with excellent correlation coefficients of 0,9993, 0,9994, 0,9991 and 0,9995 for the 4, 8, 15 and 25% Brij 97 formulations, respectively. The slope of the line represents per definition the release rate. The results are given in Table 3.6.
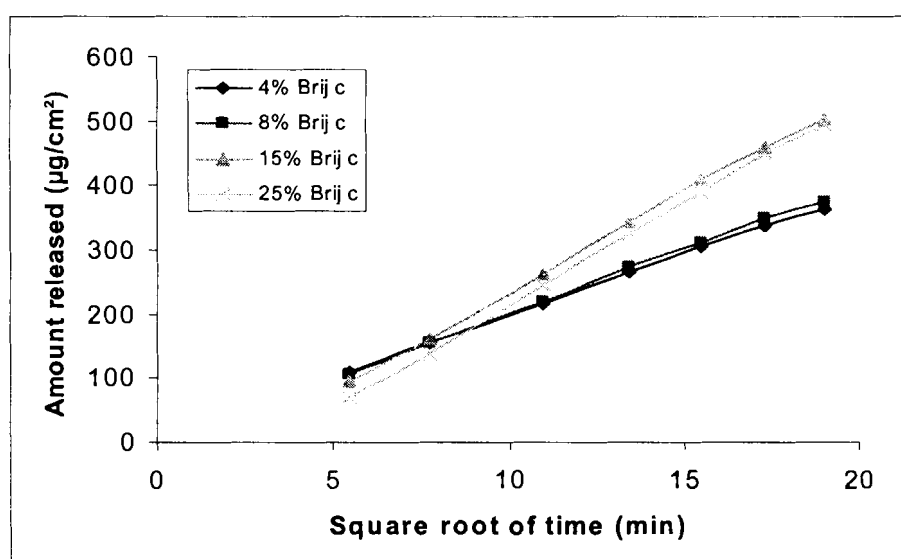

Figure 3.10: The amount of 5-fluorouracil released from the four Brij 97 formulations containing carrageenan (n = 6) in µg/cm^2 against the square root of time.

Table 3.6: The release rates of the four Brij 97 formulations containing carrageenan (indicated with c).

Formulation	Release rate $(\mu g/cm^2/min^{0.5})$
4% Brij 97 c	19,075
8% Brij 97 c	20,015
15% Brij 97 c	30,557
25% Brij 97 c	31,828

3.6.5.2 Discussion and conclusions

The amount of 5-fluorouracil released from the 4 and 8% Brij 97 formulations are lower than from the 15 and 25% Brij 97. The dissolution from the 4 and 8% Brij 97 formulations (19,075 and 20,015 $\mu g/cm^2$ respectively) were closely related while the dissolution from the 15 and 25% Brij 97 formulations (30,557 and 31,828 $\mu g/cm^2$ respectively) were also closely related (Table 3.6). No data are available without carrageenan.

One would expect to see a greater flux for 5-fluorouracil through the skin with the 15 and 25% Brij 97 formulations than with the 4 and 8% Brij 97 formulations, since there is more of the compound released from the formulation, increasing the amount of compound available for diffusion. This may however not be the case since Hadgraft (1999) stated that it is a common misconception that an increase in the applied concentration of a substance always results in an increase of drug permeation.

3.6.6 *In vitro* transdermal diffusion studies

3.6.6.1 Results

Permeation of 5-fluorouracil from the Brij 97 formulations in the presence and absence of carrageenan were measured to determine if it had any effect on the penetration of 5-fluorouracil through the skin. The *in vitro* transdermal permeation data obtained were graphically plotted as the cumulative amount of 5-fluorouracil that permeated through the skin against time (Figures 3.11 – 3.18).

Figure 3.11: Cumulative amount of 5-fluorouracil that permeated from the 4% Brij 97 formulation without carrageenan through the skin against time. Mean ± SD, n = 6.

Figure 3.12: Cumulative amount of 5-fluorouracil that permeated from the 8% Brij 97 formulation without carrageenan through the skin against time. Mean ± SD, n = 6.

Figure 3.13: Cumulative amount of 5-fluorouracil that permeated from the 15% Brij 97 formulation without carrageenan through the skin against time. Mean ± SD, n = 6.

Figure 3.14: Cumulative amount of 5-fluorouracil that permeated from the 25% Brij 97 formulation without carrageenan through the skin against time. Mean ± SD, n = 6.
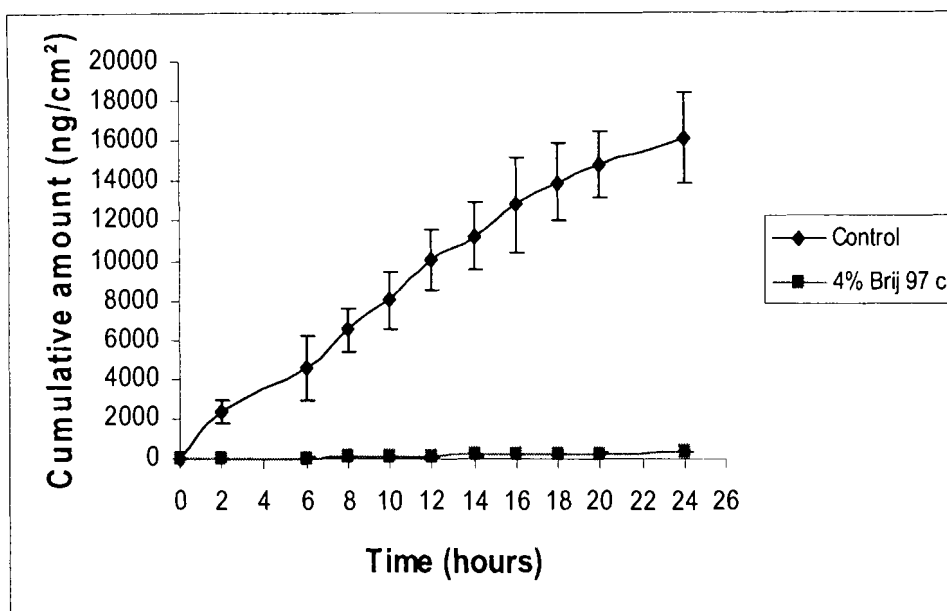
Figure 3.15: Cumulative amount of 5-fluorouracil that permeated from the 4% Brij 97 formulation with carrageenan through the skin against time. Mean ± SD, n = 6.

Figure 3.16: Cumulative amount of 5-fluorouracil that permeated from the 8% Brij 97 formulation with carrageenan through the skin against time. Mean ± SD, n = 6.
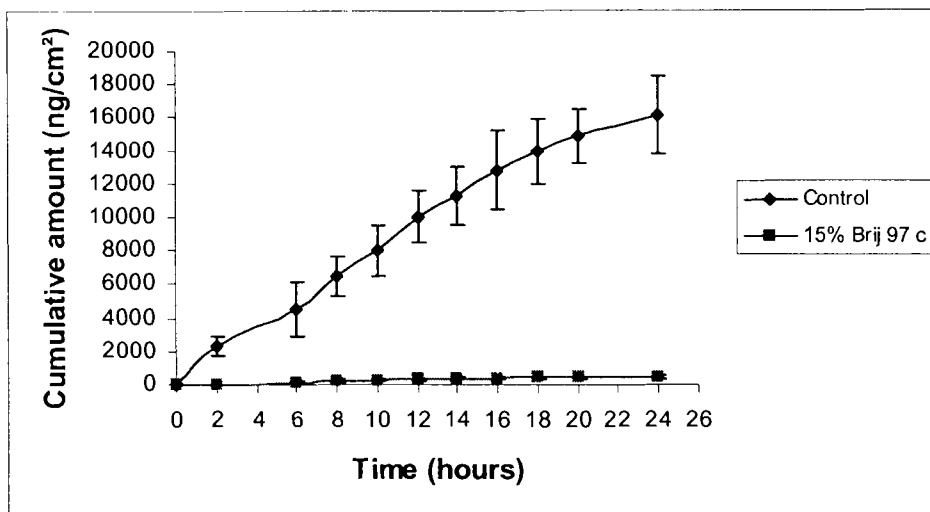
Figure 3.17: Cumulative amount of 5-fluorouracil that permeated from the 15% Brij 97 formulation with carrageenan through the skin against time. Mean ± SD, n = 6.
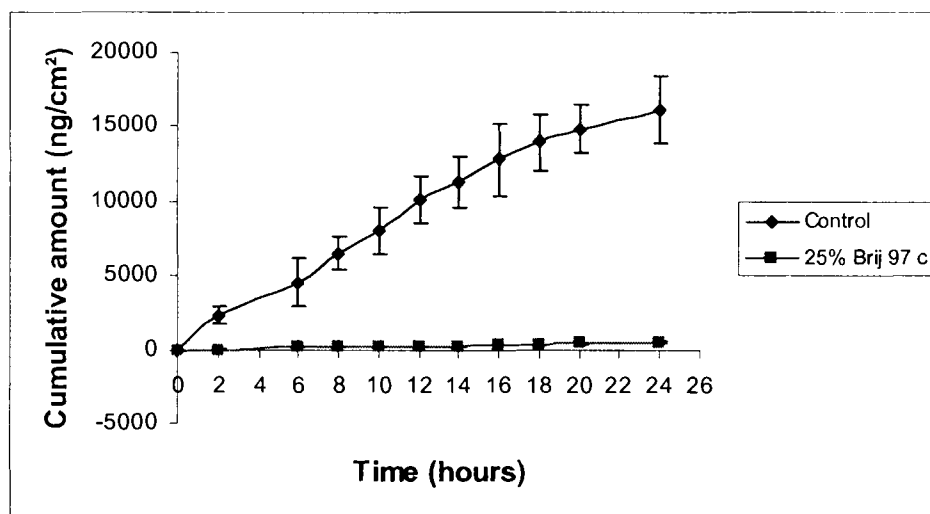
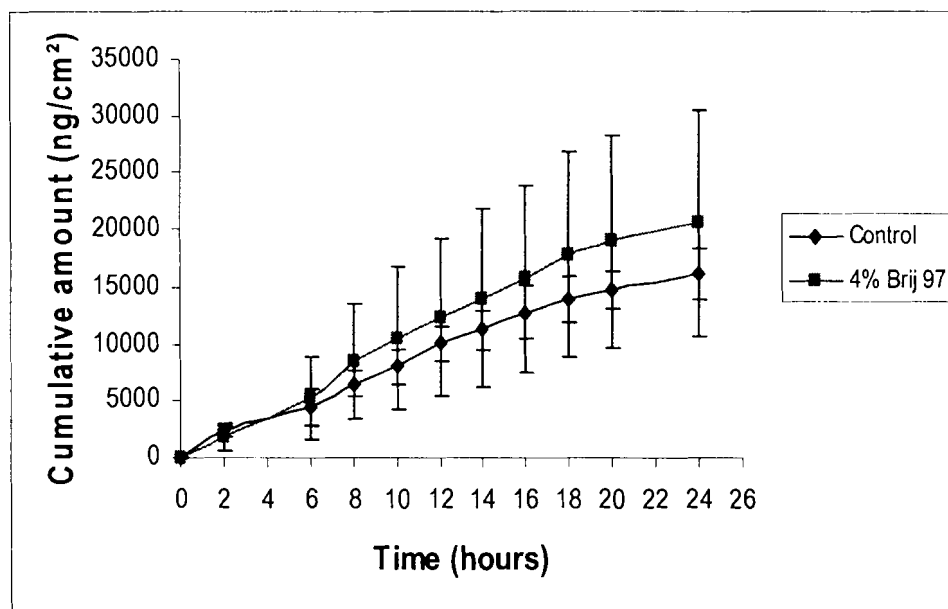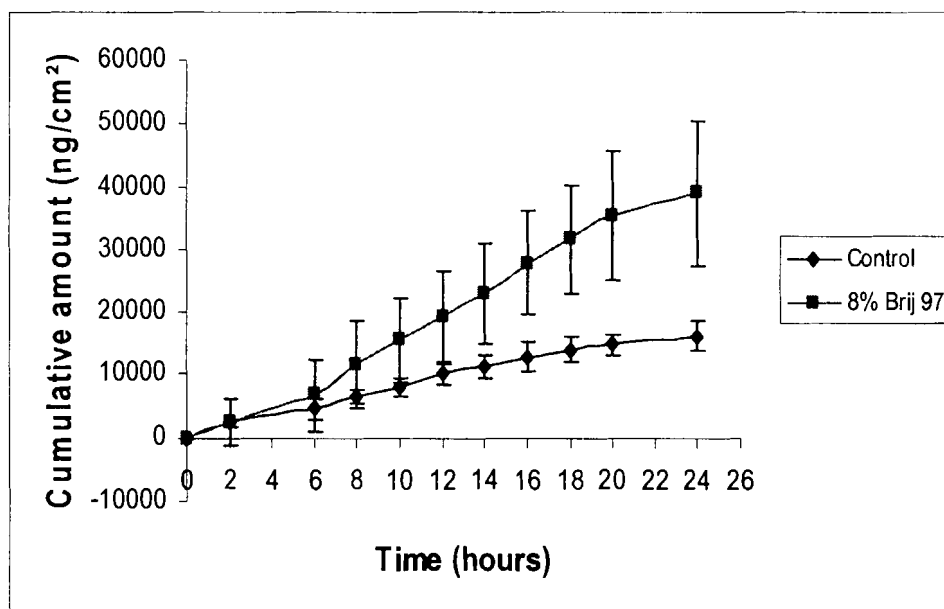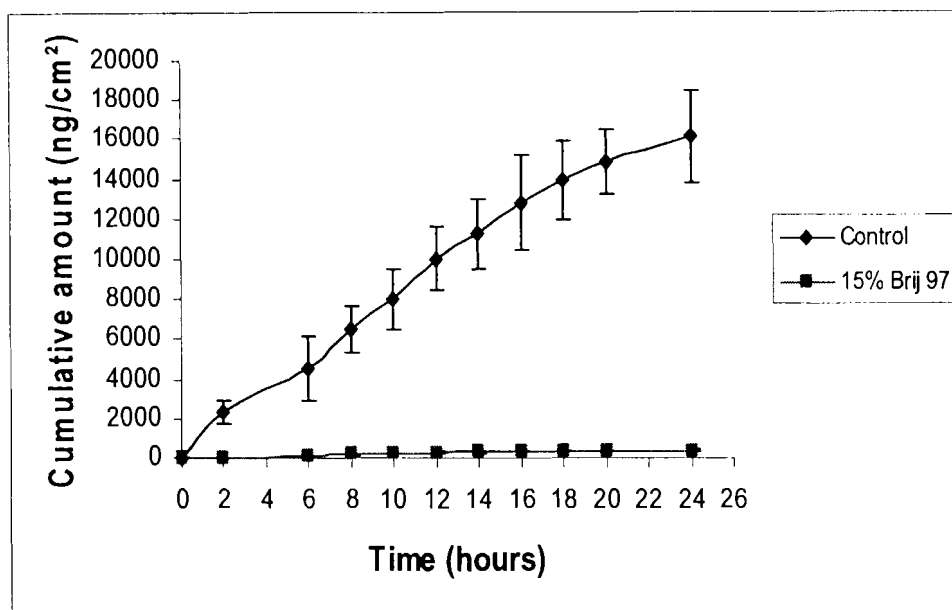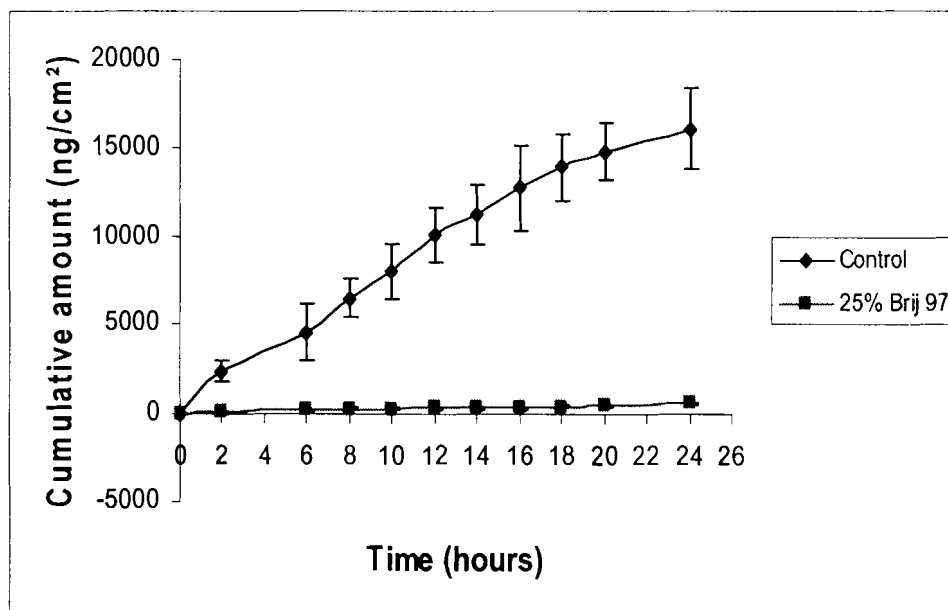**Figure 3.18:** Cumulative amount of 5-fluorouracil that permeated from the 25% Brij 97 formulation with carrageenan through the skin against time. Mean ± SD, n = 6.
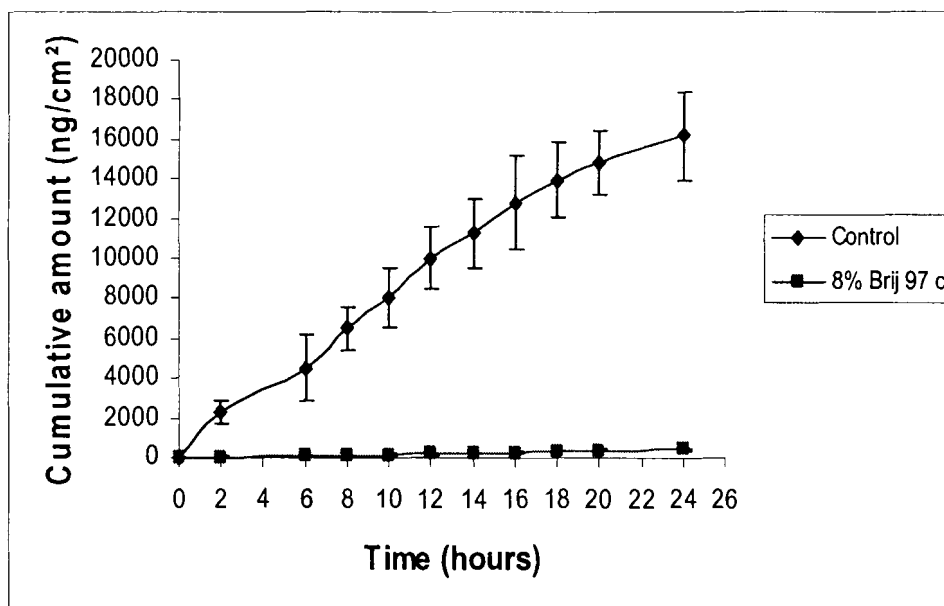
Flux (J_s) was determined from the slope of the linear section of the cumulative amount against time plot. The permeation enhancing effect of the formulations in the presence and absence of carrageenan was expressed as the enhancement ratios of flux (E.R.) and was calculated by using the following equation:

$$E.R. = \frac{Flux^{Experimental}}{Flux_{Control}}$$
Equation 3.2

The flux values (± S.D.) and enhancement ratios (E.R.) are given in Table 3.7.

An E.R. of smaller than 1 is an indication of retardation.

Table 3.7: The effect of Brij 97 in the absence (a) and presence (b) of carrageenan on the permeation of 5-fluorouracil.

Formulations without carrageenan	Flux (ng.cm^{-2}.h^{-1}), n = 6	E.R.
Control	792,01 ± 219,86	–
4% Brij 97	900,20 ± 476,59	1,14
8% Brij 97	2035,18 ± 458,43	2,57
15% Brij 97	17,88 ± 2,05	0,02
25% Brij 97	8,78 ± 0,98	0,01

a

Formulations with carrageenan	Flux (ng.cm^{-2}.h^{-1}), n = 6	E.R.
4% Brij 97 c	16,94 ± 0,84	0,02
8% Brij 97 c	25,96 ± 1,63	0,03
15% Brij 97 c	22,31 ± 4,13	0,03
25% Brij 97 c	9,63 ± 0,85	0,01

b

3.6.6.2 Statistical analysis

A one-way analysis of variance (ANOVA), followed by the Dunnett test, was done on the flux values (ng.cm^{-2}.h^{-1}) of 5-fluorouracil in order to compare the formulations of Brij 97 in the presence and absence of carrageenan with each other and with the control experiment. Thereafter, the Student t-test with unequal variances was done.

Data with p < 0,05 were considered statistically significant. Highly significant differences (p < 0,05) for 5-fluorouracil were obtained between all the Brij 97 formulations except the 4% Brij 97 formulation without carrageenan (8, 15 and 25% Brij 97 formulations without carrageenan and 4, 8, 15 and 25% Brij 97 formulations with carrageenan (p < 0,00001)) and the control for 5-fluorouracil. There were no significant differences between the 4% Brij 97 formulation without carrageenan and the control (p = 0,96).

Statistically significant differences (p < 0,05) for 5-fluorouracil were indicated for the 4% Brij 97 formulation without carrageenan in comparison to the 4% Brij 97 formulation with carrageenan (t (s) = 4,54 and p = 0,0062), the 8% Brij 97 formulation without carrageenan in comparison to the 8% Brij 97 formulation with carrageenan (t (s) = 10,74 and p = 0,00012) and the 15% Brij 97 formulation without carrageenan in comparison to the 15% Brij 97 formulation with carrageenan (t (s) = -2,36 and p = 0,049). There were no significant differences between the 25% Brij 97 formulation without carrageenan and the 25% Brij 97 formulation with carrageenan (t (s) = -1,62 and p = 0,14).

3.6.6.3 Discussion and conclusions

Results obtained showed that, in comparison to the control, the transdermal flux value of the 4% (42,54 $ng.cm^{-2}.h^{-1}$) formulation is slightly higher, the 8% (95,89 $ng.cm^{-2}.h^{-1}$) formulation much higher and the 15 (1,07 $ng.cm^{-2}.h^{-1}$) and 25% Brij 97 (0,58 $ng.cm^{-2}.h^{-1}$) formulations much lower. The flux values of the formulations containing carrageenan and that of the 15 and 25% Brij 97 formulations were very low in comparison to the flux values of the 4 and 8% Brij 97 formulations without carrageenan.

When comparing the enhancement ratios of the formulations with and without carrageenan, the 4 and 8% Brij 97 formulations were seen to have an increasing effect on the penetration of 5-fluorouracil while the 15 and 25% Brij 97 formulations hindered penetration of 5-fluorouracil. The formulations with carrageenan had a decreasing effect on the penetration of 5-fluorouracil in comparison to the 4 and 8% Brij 97 formulations without carrageenan.

Small molecules penetrate more rapidly than large molecules, but within a narrow range of molecular size there is little correlation between the molecular size and permeation rate (Liron & Cohen, 1984:538). The molecular weight of 5-fluorouracil is 130,08 g/mol and falls in the range of ideal molecular weight for transdermal delivery (Tables 2.1 and 2.2).

Drugs must preferably possess balanced lipophilic/hydrophilic characteristics and drugs with a log P_{oct} of ~ 2 is considered to be potential candidates for transdermal delivery (Guy & Hadgraft, 1989:59; Guy & Hadgraft, 1989 quoted by Malan et al., 2002:386). Since 5-fluorouracil has a log P value of - 0,89 (Table 2.2) it is believed that 5-fluorouracil is not a good candidate for transdermal delivery. However, according to Flynn & Stewart (1988) hydrophilic drugs have great potential for enhancement because of their low permeability coefficients (Williams & Barry, 1991:166).

Hydrophilic drugs are known as some of the most problematic in dermal administration. In order to obtain increased penetration, it is therefore necessary to develop vehicles that will facilitate the transport of these compounds by affecting lipid barrier properties of the stratum corneum (Schmalfuβ et al., 1997:280).

By increasing the percentage surfactant (Brij 97), the permeation of 5-fluorouracil is increased up to a certain percentage; a further increase of the surfactant led to a decrease of permeation in the skin despite a higher release rate of the 5-fluorouracil as shown by the dissolution. This is in agreement with what Levy and co-workers (1966) and Florence & Gillan (1975) found when increasing the percentage surfactant (French et al., 1993:117).

The formulations containing carrageenan have very low flux values. This is probably due to the gelling agent, carrageenan, which would increase the formulations' viscosity and further decrease the permeation in the skin (Peltola et al., 2003:99).

3.6.7 Histopathological studies

3.6.7.1 Results

Histopathological studies were carried out on female human abdominal skin (epidermis) in order to determine whether the Brij 97 formulations, at 4, 8, 15 and 25%, have any effect on the skin. Figure

3.19 represents the transmission electron micrograph of untreated human epidermis. Figures 3.20 a to 3.20 d represent the transmission electron micrographs of human epidermis after 24 hours of treatment with 4, 8, 15 and 25% Brij 97 formulations respectively.

Figure 3.19: Transmission electron micrograph of untreated human epidermis.

(a)

(b)

(c)

(d)
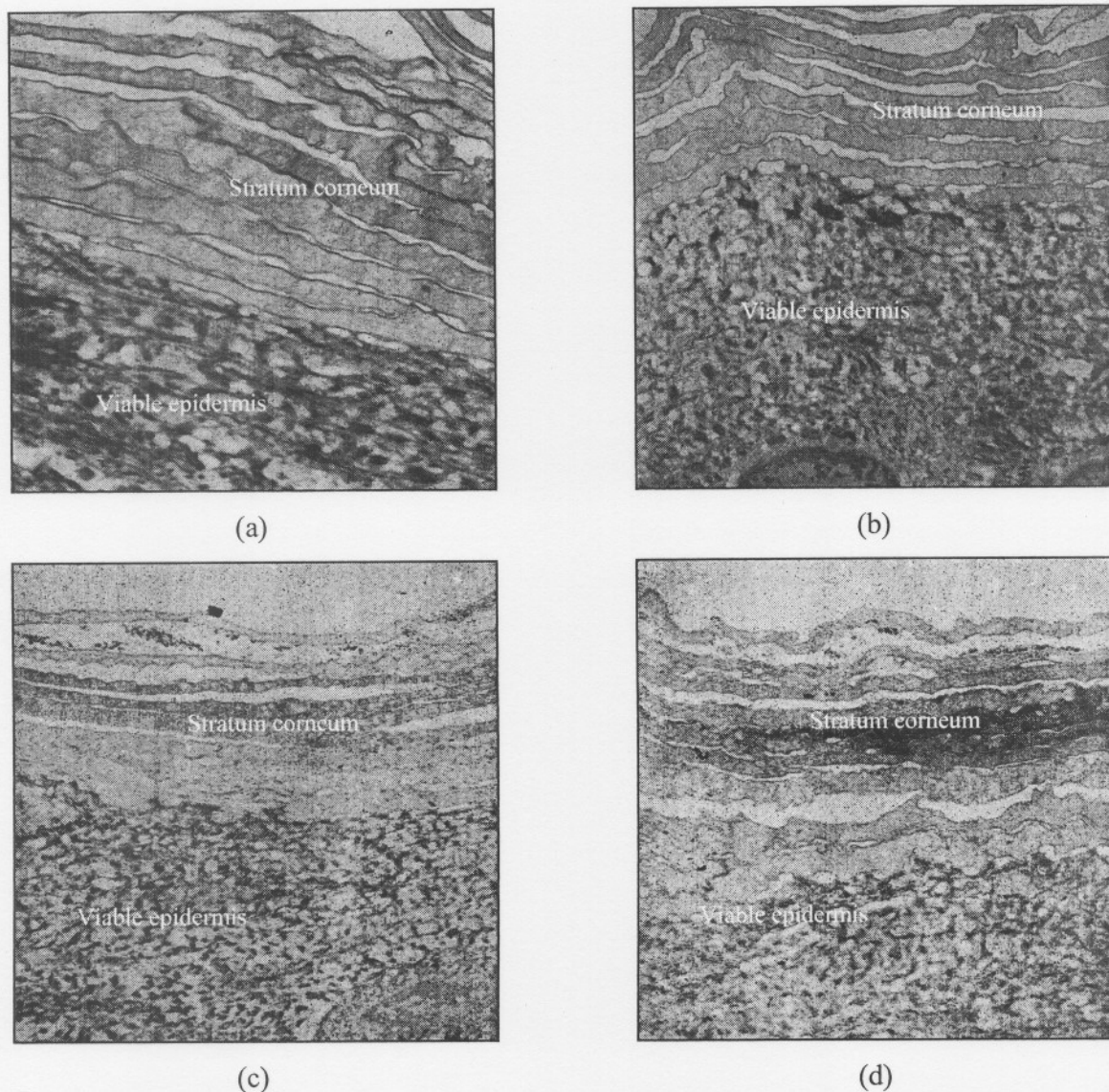
Figure 3.20: Transmission electron micrographs of human epidermis after 24 hours of treatment with 4, 8, 15 and 25% Brij 97 formulation from a – d, respectively.

3.6.7.2 Discussion and conclusions

When comparing the figure of the untreated human epidermis with the figures of the treated human epidermis with the 4, 8, 15 and 25% Brij 97 formulations, it could be seen that there was no significant change in the skin structure, where the stratum corneum was almost intact to the inner layer of the skin and not knocked off or damaged.

The reason for this could be that the nonionic surfactants have a relatively low toxicity and irritation potential. Thus, these compounds could make good candidates as potential penetration enhancers for use in transdermal drug delivery systems (French *et al.*, 1993:113).

3.7 Conclusions

The effect of Brij 97 in the presence and absence of carrageenan on the transdermal delivery of 5-Fluorouracil was investigated. Several experiments were done and the following observations were made.

The 4 and 8% Brij 97 formulations without carrageenan can be classified as emulsions while the 15 and 25% Brij 97 formulations without carrageenan could be identified as microemulsions by CLSM but not by particle size analysis. The 4, 8, 15 and 25% Brij 97 formulations containing carrageenan could be identified as gels. The zeta potential of the 4 and 8% Brij 97 formulation without carrageenan and 4% Brij 97 formulation with carrageenan is the most electronegative and thus it is the most stable. The percentage unionized 5-fluorouracil in the Brij 97 formulations without carrageenan was higher than that of the Brij 97 formulations with carrageenan. The better permeation without carrageenan might be the result of a higher percentage of unionized 5-fluorouracil.

A consistent amount of 5-fluorouracil was released from the Brij 97 formulations with carrageenan over a period of time. Significant enhancement of the transdermal flux of 5-fluorouracil was observed for the 4 and 8% Brij 97 formulations without carrageenan. When histopathological studies were carried out on female human abdominal skin, Brij 97, the surfactant, was found to have no significant effect on the skin structure.

CHAPTER 4

SUMMARY AND FINAL CONCLUSIONS

The transdermal route for the delivery of drugs can be considered as not only one of the safest and most accessible but also the most challenging route of administration. This can mostly be ascribed to the excellent barrier properties of the skin and also the limited number of available drugs that display certain physicochemical properties, making them suitable for transdermal drug delivery (Bonina *et al.*, 1995 quoted by Steenekamp, 2003:67).

The aim of this study was to investigate the effect of Brij 97 in the presence and absence of carrageenan on the transdermal delivery of 5-fluorouracil. The objectives of this study were:

- To identify the formulations by the means of the confocal laser scanning microscope and measurement of the particle size.
- To determine by the measurement of the zeta-potential whether the formulations were stable.
- To determine by the measurement of the pH if the internal structures of the formulations were affected by the drug.
- To determine if the drug was released from the formulations.
- To determine the influence of the formulations on the permeation of the drug through the skin.
- To determine by histopathological studies if the surfactant, Brij 97, had any effect on the skin.

The barrier function is attributed to the outermost layer, the stratum corneum (Sun, 1997:328). Its structure has been represented as a 'brick and mortar' model in which the keratinized cells are embedded in a mortar of lipid bilayers (Williams, 2003a:9). Drugs must thus diffuse through the intercellular lipid matrix, and to reduce temporarily and reversibly the resistance of this pathway,

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researchers use penetration enhancers (Williams & Barry, 1991:157). 5-Fluorouracil does not appear to cross the human epidermis primarily through aqueous shunt routes (Cornwell & Barry, 1993 quoted by Steenekamp, 2003:67). It is thus suggested that 5-fluorouracil travels across the intercellular lipid bilayers in the stratum corneum. Therefore, when the penetration enhancer interacts with the stratum corneum, it may create new polar pathways through which 5-fluorouracil can pass (Steenekamp, 2003:67).

Through the confocal laser scanning microscopy and particle size measurements, the 4 and 8% Brij 97 formulations without carrageenan could be identified as emulsions while the 15 and 25% Brij 97 formulations without carrageenan could be identified as microemulsions by CLSM. The 4, 8, 15 and 25% Brij 97 formulations containing carrageenan could be identified as gels.

The results obtained from the zeta-potential analysis indicated that the 4 and 8% Brij 97 formulation without carrageenan and 4% Brij 97 formulation with carrageenan is the most electronegative and thus it is the most stable. Through the pH measurements, it was determined that the drug did not alter the internal structures of the formulations and that the percentage of unionized drug, 5-fluorouracil, in the Brij 97 formulations without carrageenan were more than that of the Brij 97 formulations with carrageenan. The flux values of 5-fluorouracil from the 4 and 8% Brij 97 formulations without carrageenan showed an enhancement while the 15 and 25% Brij 97 formulations with and without carrageenan showed retardation.

Therefore, there seems to be a correlation between the results obtained from the confocal laser scanning microscopy, particle size measurements, zeta-potential analysis, pH measurements and the *in vitro* permeation studies. The 4 and 8% Brij 97 formulations without carrageenan could be identified as emulsions, were the most stable formulations, had the greatest percentage unionized drug and, thus, showed the most significant enhancement. Although carrageenan led to good adhesiveness on skin, it did not lead to the enhancement of the penetration of 5-fluorouracil through the skin. On the contrary, the penetration of 5-fluorouracil was hindered by the carrageenan in the formulations. Although many studies have indicated that microemulsion vehicles can increase transdermal delivery of hydrophilic drugs, in this study it could be seen that microemulsions are not necessarily better penetration enhancers and hindered permeation of 5-fluorouracil through the skin.

Even though 5-fluorouracil was released from all the Brij 97 formulations containing carrageenan, the Brij 97 formulations containing carrageenan did not permeate the skin very well. This is probably due to the permeability differences between the synthetic membrane that was used in the dissolution studies and the skin that was used in the *in vitro* permeation studies. Dissolution rate is thus not an indicator of the rate of transdermal penetration.

When histopathological studies were carried out on female human abdominal skin, Brij 97, the surfactant, was found to have no damaging effect on the skin structure. Thus, although the surfactant may create new polar pathways through which 5-fluorouracil could pass, it reduced the barrier properties of the skin temporarily and reversibly which led to no skin damage.

In conclusion, the following investigations are proposed:

- The enhancement effect of Brij 97 in different concentrations, ranging from 4-15% to determine the optimum concentration with reference to the permeation enhancement of 5-fluorouracil.
- More stability tests can be performed on the Brij 97 formulations.

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