The Effect of Brij 97 and Carrageenan on the Transdermal Delivery of Acyclovir

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The Effect of Brij 97 and Carrageenan on the Transdermal Delivery of Acyclovir

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ABSTRACT

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The skin, by weight, is the largest organ of the body. Human skin serves to provide several important functions that may be classified, in a general context, as protective, maintaining homeostasis and sensing. The outermost layer of the skin, the stratum corneum, has an essential role as a barrier against the transport of water and of chemical and biological agents.

In this study acyclovir (ACV), an antiviral used for treating the varicella zoster virus, was used. It is sensible to say that a hydrophilic drug like acyclovir needs a delivery vehicle or penetration enhancer to permeate the skin with more ease.

In an attempt to enhance the permeation of acyclovir, it was formulated in a delivery vehicle with the same formulation as for a microemulsion. Increasing percentages of the surfactant, Brij 97, were incorporated in the formulation to determine which of the four formulations is indeed a microemulsion. A gelating agent, carrageenan, was used to make the emulsion transdermally more applicable; the influence of this component on the transdermal delivery of acyclovir was also determined.

Therefore the aim of this study was to determine:

- The effect of a drug delivery vehicle on the transdermal delivery of acyclovir;
- The specific formulation of a microemulsion and
- The influence of a gelating agent on the transdermal delivery of acyclovir.

Diffusion studies were performed in vertically mounted glass Franz diffusion cells. The epidermis of female abdominal skin, obtained after abdomeoplasty, was heat separated from the dermis. One millilitre of emulsion (0.1%: 1mg/ml ACV) was added to the skin sample in the donor side of the diffusion cell. The control solution had an equivalent amount of active in water and was added to the donor compartment in a separate experiment. The receptor phase was PBS (phosphate buffered solution). The entire receptor phase of the cells was removed every second hour and was replaced with fresh receptor phase at 37°C. The amount of acyclovir in the receptor phase was determined by HPLC analysis.

The cumulative amounts of the active that permeated the skin over the 24 hour period were plotted with the slope of the graphs representing the flux in ng/cm^2/h. The average flux values of the experimental cells and control cells were compared.

Results of the diffusion studies without carrageenan showed that increasing the concentration of the surfactant increased the diffusion of acyclovir. Permeation studies with carrageenan had a totally different outcome. The enhancement ratio of the experimental cells was much lower than that of the control cells. However the experimental cells showed a small increase as the concentration of the surfactant increased.

From VanKel dissolution studies it could be seen that release of acyclovir from the emulsion was not a problem and that the active was available for absorption.

Confocal studies were done to determine whether there were any vesicles in the emulsions. Vesicles were expected in the 25% Brij 97 emulsion because it was the same formulation as a microemulsion, but vesicles could only be found in the 4% and 8% Brij 97 emulsion.

A previous study with acyclovir and three different delivery vehicles gave enhancement ratios between 0.32 to 2.92. Values obtained in this study of the 4% and 8% Brij 97 emulsion without carrageenan were more or less the same but the 15% and 25% Brij 97 emulsion had a much higher enhancement ratio. For the emulsions with carrageenan not one exceeded an enhancement ratio of 0.57.

More studies still have to be done on microemulsions to determine which specific concentration of surfactant forms a microemulsion. The active itself and its physicochemical properties also play an important role in the diffusion studies with the specific delivery vehicle and further research has to be done with different model drugs.

Keywords
Acyclovir, penetration enhancer, Brij 97, transdermally, carrageenan, permeation, microemulsion, delivery vehicle.

OPSOMMING

Die Effek van Brij 97 en Karrageen op die Transdermale Aflewering van Asiklovir

Die vel, per gewig, is die grootste orgaan in die liggaam. Die menslike vel vervul talle belangrike funksies wat oor die algemeen as beskerming en behoud van homeostase en gevoel geklassifiseer kan word. Die buitenste laag van die vel, die stratum corneum, het 'n essensiële rol as 'n skans teen die transport van water en/of chemiese en biologiese middels.

In hierdie studie is asiklovir (ACV), 'n antivirale middel vir behandeling van varicella zoster-virus gebruik. Dit maak sin om te sê dat 'n hidrofiele geneesmiddel soos asiklovir 'n afleweringstelsel of penetrasieverhoger nodig het om permeasie deur die vel te vergemaklik.

In 'n poging om die deurgang van asiklovir te verhoog, is dit in 'n afleweringstelsel geïnkorporeer wat dieselfde formulering as 'n mikro-emulsie bevat. Toenemende konsentrasies van die surfaktant, Brij 97, is in die formulering geïnkorporeer om vas te stel watter een van die vier formulerings inderdaad 'n mikro-emulsie vorm. 'n Viskositeitsverhoger, naamlik karrageen, is gebruik om te verseker dat die emulsie transdermaal makliker aangewend kan word. Daar is ook bepaal wat die komponent se invloed op die transdermale aflewering van asiklovir is.

Die doel van hierdie studie was dus die volgende:

- Om die effek van 'n afleweringstelsel op die transdermale aflewering van asiklovir te bepaal ;
- Om die spesifieke formulering van 'n mikro-emulsie te bepaal en
- Om die invloed van 'n viskositietsverhoger op die transdermale aflewering van asiklovir vas te stel.

Diffusiestudies is met behulp van vertikale Franz-diffusieselle uitgevoer. Die epidermis van vroulike abdominale vel, verkry na abdominale sjirurgie, was m.b.v. hitte van die dermis geskei. Een milliliter van die emulsie (0.1%: 1mg/ml ACV) is in die donorkompartement van die diffusiesel gevoeg. Die kontroles het 'n ekwivalente hoeveelheid van die geneesmiddel in water gehad en is in 'n aparte eksperiment in die donorkompartement gesit. Die reseptorfase was PBS (fosfaatbufferoplossing). Die reseptorfase se totale inhoud is elke tweede uur onttrek en is met vars reseptorfase by

37°C vervang. Die hoeveelheid asiklovir in die reseptorfase is met behulp van HDVC bepaal.

Die kumulatiewe hoeveelheid geneesmiddel wat gedurende die 24 uur deur die vel gedring het, is op grafieke gestip en die helling van die lyn is bepaal om die fluks ($ng/cm^2/h$) te bereken. Die gemiddelde flukswaardes van die eksperimentele sowel as kontroleselle is met mekaar vergelyk.

Resultate van die transdermale studies sonder karrageen wys dat die verhoging van die konsentrasie van die surfaktant 'n gelyktydige verhogende effek op die diffusie van asiklovir gehad het. Permeasiestudies van emulsies met karrageen het 'n totaal ander effek gehad. Die verbetering in permeasie in die eksperimentele selle was heelwat laer as die van die kontroleselle, maar het ook verhoging van diffusie getoon soos wat die konsentrasie van die surfaktant toegeneem het.

Vanaf VanKel-dissolusie-eksperimente kon gesien word dat die vrystelling van asiklovir vanuit die emulsie nie 'n probleem was nie en dat die geneesmiddel vir absorpsie beskikbaar was.

Konfokale studies is gedoen om vas te stel of daar enige druppels in die emulsie gevorm het. Daar is verwag dat druppels sou voorkom in die emulsie met 25% Brij 97 omdat die formulering dieselfde is as 'n mikro-emulsie, maar druppels is slegs in die emulsie met 4% Brij 97 gevind.

'n Vorige studie met dieselfde geneesmiddel, asiklovir, wat in drie verskillende afleweringstelsels geïnkorporeer is, het 'n verbetering in permeasie met waardes tussen 0.32 tot 2.92 gelewer. Waardes gekry in hierdie studie van die 4%- en 8% Brij 97-emulsies sonder karrageen het min of meer dieselfde resultate getoon, maar die 15%- en 25% Brij 97-emulsies se verbetering was baie hoër. Vir die emulsie met karrageen het die verbetering nooit bo 0.57 gekom nie.

heelwat meer studies sal nog op mikro-emulsies gedoen moet word om die spesifieke konsentrasie van die surfaktant wat 'n mikro-emulsie vorm, vas te stel. Die aktiewe bestanddeel en sy fisies-chemise eienskappe speel 'n belangrike rol in die diffusiestudies met die spesifieke geneesmiddelafleweringstelsel en verdere navorsing met verskillende modelgeneesmiddels is nodig.

Sleutelwoorde
Asiklovir, penetrasiebevorderaars, Brij 97, transdermaal, karrageen, permeasie, mikro-emulsie, afleweringstelsel.

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CHAPTER 1

Introduction and Problem Statement

Orally administered acyclovir's absorption is slow, variable and incomplete (Von Plessing Rossel *et al.*, 2000:749); it has an oral bioavailability that ranges from 10-30% and the percentage decreases with increasing dose. Peak plasma concentrations average 0,4 to 0,8 μg/ml after 200 mg and 1,6 μg/ml after 800 mg dose (Bangaru, Bansal, Rao & Gandhi, 2000:231). Because of acyclovir's hydrophilicity it was necessary to incorporate the drug in a vehicle with a penetration enhancer to ease the penetration of the skin.

There are major advantages that the transdermal route offers above the oral and intravenous route. Just to name a few: the gastrointestinal and hepatic metabolism are being avoided, the skin presents a relatively large and readily accessible surface area for absorption and it is a non-invasive procedure that allows continuous intervention. The transdermal route includes the potential for sustained release and controlled input kinetics (Naik, Kalia & Guy, 2000:319).

The physicochemical properties of a drug have a great influence on the transdermal delivery of that specific drug. In this study where acyclovir was used this fact was proven yet again. Acyclovir, a hydrophilic drug with dissociation coefficient (pKa) values of 2.27 and 9.25, shows great difficulty on penetrating the skin, which is why the incorporation of the drug in a delivery system, such as an emulsion used in this study, was necessary as mentioned above.

The major limitation to transdermal drug delivery is the skin itself. The skin is the outermost layer of the human organism which separates the internal from the external environment and acts as a two-way barrier by preventing the ingress of foreign molecules and the egress of endogenous substances. The major barrier to penetration of matter through the skin is provided by a superficial layer of the skin, the stratum corneum and its compact structure (Suhonen, Bouwstra & Urtti, 1999:149). It has been found that the stratum corneum can incorporate water under swelling and that the permeability of drugs depends on the degree of hydration.

Furthermore, some substances with considerable polarities also enhance the permeability of the horny layer (Loth, 1991:3). The main interest in dermal absorption assessment is the application of compounds to the skin, for instance for local effects in dermatology, for transport through the skin for systematic effects, for surface effects, to target deeper tissues and unwanted absorption (Walters & Roberts, 2002:2).

It has been known, for almost a century now, that transdermal drug absorption is influenced by the vehicle in which the applied drug is incorporated. Furthermore, the use of certain substances which are able to enhance transdermal drug penetration has been practiced for a long time (Loth, 1991:1).

Surfactants are able to function as enhancers and are believed to penetrate the skin mainly in their monomer form. This form can diffuse through the skin surface and act as enhancers. They either disrupt the lipid structure of the stratum corneum, facilitating diffusion through the barrier phase, or increase the solubility of the drug in the skin, i.e., increasing the partition coefficient of the drug between the skin and the vehicle (Kreilgaard, 2002:S94). Nonionic surfactants are widely used in topical formulations as solubilizing agents but some recent results indicate that they may affect the skin barrier function (Peltola *et al.*, 2003:100). A nonionic surfactant, Brij 97 which is less toxic than ionic surfactants, was incorporated into the emulsions used in this study (Kreilgaard, 2002:S94).

κ-Carrageenan is an anionic sulfated polysaccharide extracted from certain species of red seaweed (algae). Its gelating occurs on cooling and is generally considered a two-step process. The gelling behaviour is strongly influenced by the nature and concentration of cations present in the solution as well as by the biopolymer concentration (Uruakpa & Arntfield, 2004:420). Most emulsions in general are of very low viscosity and therefore their use may be restricted. Carrageenan possesses the property of good adhesiveness on skin which can be a benefit for topical application (Valenta & Schultz, 2004:258).

Acyclovir is a synthetic analogue of 2'-deoxiguanosine and is one of the most effective and selective agents against viruses of the herpes group. Acyclovir is active against herpes simplex virus type 1 (HSV-1), herpes simplex virus type 2 (HSV-2), varicella zoster virus, and to a lesser extent against Epstein-Barr virus and cytomegalovirus. The mechanism of action of this drug is its antiviral activity and has been shown to be caused by the inhibition of the herpes virus DNA replication (De Jalón *et al.*, 2001:191).

The main objectives of this study were to determine:

- The transdermal delivery of acyclovir with the help of a drug delivery vehicle;
- The conformation of the formulation of a microemulsion;
- The influence of a gelating agent on the transdermal delivery of acyclovir.

CHAPTER 2

Transdermal Drug Delivery and Penetration Enhancement

2.1 INTRODUCTION

The skin, the heaviest single organ of the body (Barry, 1983:1), accounting for more than 10% of body mass (Walters & Roberts, 2002:1), combines with the mucosal linings of the respiratory, digestive and urogenital tracts to form a capsule that separates the internal body structures from the external environment (Barry, 1983:1).

In essence, the skin consists of four layers: the stratum corneum (nonviable epidermis), the remaining layers of the epidermis (viable epidermis), dermis and subcutaneous tissues. There are also several associated appendages: hair follicles, sweat ducts, apocrine glands and nails. The skin's function may be classified as protective, maintaining homeostasis, or sensing. The value of the protective and homeostatic role of the skin is illustrated in one context by its barrier property. This allows the survival of humans in an environment of variable temperature, water content (humidity and bathing) and the presence of environmental dangers, such as chemicals, bacteria, allergens, fungi and radiation. In a second context, the skin is a major organ for maintaining the homeostasis of the body, especially in terms of its composition, heat regulation, blood pressure control and excretory roles. In the third context, the skin is a major sensory organ in terms of sensing environmental influences, such as heat, pressure, pain, allergen and micro-organism entry. The skin is an organ that is in continual state of regeneration and repair. To fulfill each of these functions, the skin must be tough, robust and flexible, with effective communication between each of its intrinsic components (Walters & Roberts, 2002:1).

Many agents are applied to the skin either deliberately or accidentally (Walters & Roberts, 2002:2). We easily damage it, mechanically, chemically, biologically and by radiation. Thus we cut, bruise and burn it (Barry, 1983:2). The application of compounds to the skin is the main interest in dermal absorption assessment, for instance for local effects in dermatology, for transport through the skin for systematic effects, for surface effects, to target deeper tissues and unwanted absorption (Walters & Roberts, 2002:2).

The skin became popular as a potential site for systemic drug delivery because it was thought to:

- avoid the problems of stomach emptying,

- avoid enzyme deactivation associated with gastrointestinal passage,
- miss hepatic first-pass metabolism,
- avoid pH effects and
- enable control of input, as exemplified by termination of delivery through removal of the device.

There are various difficulties in delivery of solutes through the skin, such as:

- the variability in percutaneous absorption owing to site,
- disease,
- age and species differences,
- the skin's "first-pass" metabolic effect,
- the reservoir capacity of the skin,
- irritation and other toxicity caused by topical products,
- heterogeneity and educability of the skin in both turnover and metabolism,
- inadequate definition of bioequivalence criteria and
- an incomplete understanding of the technologies that may be used to facilitate or retard percutaneous absorption (Walters & Roberts, 2002:2).

In order to appreciate and control the biopharmaceutics of dermatological formulations and to answer questions regarding the therapeutic and cosmetic properties of the many topical preparations available in the market or on prescription, an understanding of the skin is necessary (Barry, 1983:2).

2.2 STRUCTURE OF THE SKIN

Histologically, the skin is a complex multilayered organ with a total thickness of 2-3 mm (Ghosh & Pfister, 1997). In figure 2-1 the layers of the skin can be seen which consist of the following: epidermis, dermis and skin appendages.

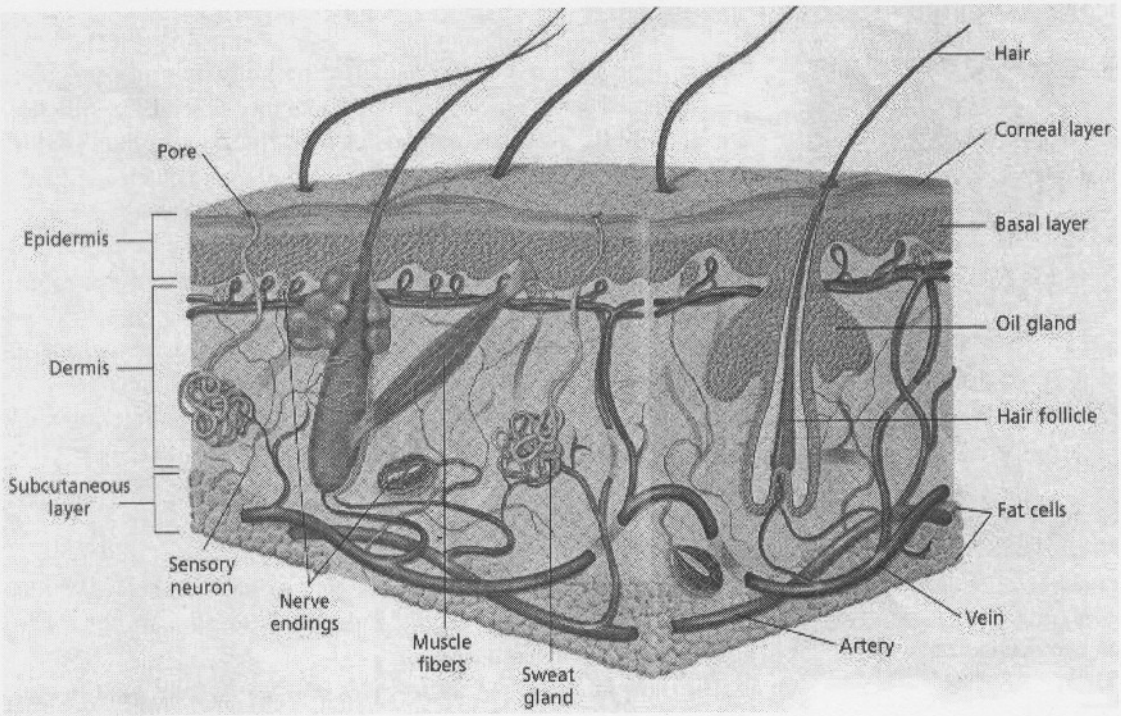

Figure 2-1: The skin (Dowshen & Hyde, 2004).

2.2.1 Epidermis

The epidermis is approximately 100 μm thick in man and may be further classified into a number of layers. The stratum germinativum is the basal layer of the epidermis. Above the basal layer are the stratum spinosum, the stratum granulosum, the stratum lucidum and, lastly, the stratum corneum (Ghosh & Pfister, 1997:3). The epidermis contains a variety of other cell types that have their own specific biological functions. These include the melanocytes, Langerhans cells and Merkle cells (Wertz & Downing, 1989:2).

2.2.1.1 The Basal layer (Stratum germinativum)

The basal cells are nucleated, columnar and about 6 μm wide, with their long axis at right angles to the dermo-epidermal junction; they connect by cytoplasmic intercellular bridges. Mitosis of the basal cells constantly renew the epidermis and in healthy skin this proliferation balances the loss of dead horny cells from the skin surface. The epidermis thus remains constant in thickness. Although there are difficulties in calculating epidermal turnover times, researchers use tritiated thymidine to selectively label nuclear DNA. They could thereby estimate that a cell from the basal layer takes at least 14 days to reach the stratum corneum. In the rapidly proliferating epidermis of psoriasis patients, the transit time is only 2 days (Barry, 1983:4).

The basal layer consists of a continuous carpet of stem cells (basal cells) that reside along the basal lamina (the border between the epidermis and dermis). These cells are relatively undifferentiated, columnar epithelial cells that are linked to the basal lamina by

hemidesmosomes and to each other by desmosomes (Eckert, 1992:4). The basal cell layer includes melanocytes, which produce and distribute melanin granules to the keratinocytes in a complex interaction. The skin requires melanin for pigmentation, a protective measure against radiation (Barry, 1983:4).

2.2.1.2 The dermo-epidermal junction

The complex dermo-epidermal junction lays just below the basal cell layer. In electron micrographs, the junction spans four components: firstly the basal cell plasma membrane with its specialized attachment devices, the hemidesmosomes, secondly the lamina lucida, thirdly the basal lamina and lastly the fibrous components below the basal lamina, which include anchoring fibrils, dermal microfibril bundles and collagen fibrils (Barry, 1983:4).

The junction serves the two functions of dermal-epidermal adherence: mechanical support for the epidermis and control of the passage of cells and some large molecules across the junction. Thus, the adhesion of the epidermis to the dermis can markedly be reduced by diseases which operate at this level and by some experimental techniques (Barry, 1983:4).

The barrier function of the junction can best be considered in terms of three species: small molecules, large molecules and cells. There is no evidence that the junction significantly inhibits the passage of water, electrolytes and other low molecular weight materials. Large molecules also cross the junction. However, an even larger substance such as thorotrast mainly stays beneath the basal lamina. It is well established that dermal cellular elements traverse the junction in normal skin and that passage is pronounced in some pathological conditions. The sequence of events is that an area of basal lamina disintegrates, a gap forms in the junction between adjacent cells and the invading cell penetrates. In the end basal cells on either side close the gap (Barry, 1983:5).

2.2.1.3 The prickle cell layer: the keratinocytes (stratum germinativum)

The major cell type of the epidermis is the keratinocytes. It comprises more than 90% of the cells of the epidermal layer (Eckert, 1992:4). As the cells produced by the basal layer move outward, they change morphologically and histochemically. The cells flatten and their nuclei shrink. These polygonal cells are called prickle cells because they interconnect by fine prickles. Each prickle encloses an extension of the cytoplasm and the opposing tips of the prickles of adjacent cells adhere to form intercellular bridges: the desmosomes. These links maintain the integrity of the epidermis (Barry, 1983:5).

Keratinocytes is the cell type responsible for formation of the protective sheath (epidermis) that repels pathogens, guards against fluid loss and is abrasion resistant. To accomplish this, keratinocytes undergo a programmed process of differentiation in which proliferative, undifferentiated cells are converted to highly differentiated, nondividing cells (Eckert, 1992:4).

2.2.1.4 The granular layer (stratum granulosum)

As the keratinocytes approach the surface, they manufacture basic-staining particles, the keratohyalin granules. It was suggested that these granules represent an early form of keratin, but they may be cell organelles partially destroyed by hydrolytic enzymes. A dynamic operation manufactures the keratin to form the horny layer by an active rather than by a degenerative process (Barry, 1983:6).

2.2.1.5 The stratum lucidum

On the sole of the foot and in the palm of the hand, an anatomically distinct, poorly staining hyaline zone forms a thin, translucent layer immediately above the granular layer. This region is the stratum lucidum (Barry, 1983:6).

2.2.1.6 The horny layer (stratum corneum)

The stratum corneum or the horny layer consists of flattened keratin-filled cells (e.g. corneocytes) (Ghosh & Pfister, 1997:4). This stratum of the epidermis serves as a barrier which both prevents dessiccation of the underlying tissues and excludes the entry of noxious substances from the environment (Wertz & Downing, 1989:2).

The barrier properties of the stratum corneum may be related to its very high density (1.4 g/cm^3 in the dry state), its low hydration of 15-20%, compared with the usual 70% for the body and its low surface area for solute transport (it is now recognized that most solutes enter the body through the less than 0.1 μm wide intercellular regions of the stratum corneum) (Walters & Roberts, 2002:4).

The production of this protective covering is the principal function of the living epidermis (Wertz & Downing, 1989:2). New epidermal cells are constantly formed in the basal layer and slowly move upward away from their source of oxygen and nourishment. Upon reaching the stratum corneum, these cells are cornified and flatten. The corneocytes are then sloughed off the skin at a rate of about one cell layer per day, a process called desquamation. The main source of resistance to penetration and permeation through the skin is the stratum corneum. There is a remarkable histologic difference between the stratum corneum and other layers of the epidermis (Ghosh & Pfister, 1997:5).

Examination of the skin surface reveals that the stratum corneum is neither continuous nor homogeneous. Some regions, most notably the fingertips, bottoms of the toes and the palmer and planter surfaces, display extensive systems of lines and ridges, or dermatoglyphics, whereas the remainder of the skin surface is relatively smooth. Close examination reveals sweat pores and hair penetrating through the stratum corneum. Although it has occasionally been suggested that these various openings through the stratum corneum could be exploited to bypass the barrier, it appears that the cross sectional area of the pores is so small as to be negligible. Furthermore, the outward movement of sweat or sebum would tend to flush out anything which did penetrate (Wertz & Downing, 1989:2).

The stratum corneum is approximately 15-20 μm thick over much of the human body and the corneocytes are composed of cytoplasmic protein matrices comprising keratin embedded in the extracellular lipid. The keratin-containing cells are arranged in a brick and mortar configuration (Ghosh & Pfister, 1997:5). The flattened, stacked, hexagonal cells of the stratum corneum is approximately 40 μm in diameter and 0.5 μm thick. The thickness varies (Walters & Roberts, 2002:4) and is viewed as the bricks that provide strength to the barrier membrane. They contain fewer lipids, and their main structural components are aggregates of keratins arranged as bundles of individual keratin filaments. The majority of human stratum corneum lipids consists of ceramides and neutral lipids, such as free sterols, free fatty acids and triglycerides. Phospholipids, glycosphingolipids and cholesterol sulphate constitutes the remaining groups of lipids (Ghosh & Pfister, 1997:5).

The lipid structures are rearranged to form the multiple lipid bilayers of the stratum corneum (Loth, 1991:3). The lipids from which the intercellular lamellae are composed are highly unusual. Unlike all other biological membranes, those in the stratum corneum do not contain phospholipids. Instead, they are mainly composed of ceramides, cholesterol, fatty acids and cholesteryl esters (Wertz & Downing, 1989:10).

For many years it has been recognized that keratin is a complex mixture of proteins having an excess of the sulfur-containing diamino acid cystine. The flat stratum corneum cells are in interdigitated stacks that are replaced from underneath. Full thickness skin derives its elasticity and compliance from the water-rich hydrogel lining structure of its proteinaceous matrix (Ghosh & Pfister, 1997:5).

2.2.1.7 Other cells of the epidermis

Langerhans' cells are dentritic cells with a lobular nucleus, a clear cytoplasm containing characteristic Langerhans' cell granules, and well-developed endoplasmic reticulum, Golgi complex, and lysosomes. In recent years, evidence has been presented that Langerhans' cells are also involved in the immune response in the skin; thus they bind antigens, probably modify them and transport them to the lymph nodes for lymphocyte activation. Merkel's corpuscles attached to adjacent epidermal cells by numerous desmosomes are associated with the sensation of touch (Barry, 1983:7).

2.2.2 Dermis

The second layer, the dermis, provides nutritive, immune and other support systems to the epidermis. It plays a critical role in temperature, pressure and pain regulation. The main structural component of the dermis is referred to as a coarse reticular layer. The dermis is about 0.1-0.5 cm thick and consists of collagenous fibers (70%), providing a scaffold of support and cushioning, and elastic connective tissue, providing elasticity, in a semi-gel matrix of mucopolysaccharides (see table 2-1). In general, the dermis has a sparse cell population. The main cells present are the fibroblasts, which produce the connective tissue components of collagen, laminin, fibronectin and vitronectin; mast

cells, which are involved in the immune and inflammatory responses; and melanocytes involved in the production of the pigment melanin (Walters & Roberts, 2002:11).

Table 2-1: Composition of the dermis (Flynn, 1990:271).

Extra Cellular Components	**Approximate percentage composition**
Collagen	75.0
Elastin	4.0
Reticulin	0.4
Ground substance	20.0
Cellular Components	**Function**
Melanocytes	Pigment synthesis
Fibroblasts	Fiber synthesis
Mast cells	Synthesis of ground substance

Mast cells produce granules that are packed with factors that are vasoactive or are chemoattractant for neutrophilis and eosinophilis. These cells respond to light, cold, acute trauma, vibration and pressure, as well as chemical and immunologic stimuli. When triggered by these stimuli, they release the contents of the granules initiating chemotaxis or vasodilatation (Eckert, 1992:7).

Other components also situated in the dermis are the roots of the body hair and the secretory coils of the sweat glands. In contrast to the avascular epidermis, the dermis is pervaded with a mass of arterioles, venules and capillaries. Permeants which are transported through the stratum corneum and epidermis are ultimately removed by this dermal vasculature (Potts *et al.*, 1992:16). The dermis is divided into a superficial, thin, papillary layer (composed of narrow fibers) which forms a negative image of the ridged lower surface of the epidermis and a thick underlying reticular layer made of wide collagen fibers (Barry, 1983:8).

The blood flow rate to the skin is about 0.05 ml min^{-1} cm^{-3} of skin, providing a vascular exchange area equivalent to that of the skin surface area. Skin blood vessels derive from those in the subcutaneous tissues, with an arterial network supplying the papillary layer, the hair follicles, the sweat and apocrine glands, the subcutaneous area, as well as the dermis. Of particular significance in this vascular network is the presence of arteriovenous anastomoses at all levels in the skin. Blood flow changes are most evident in the skin in relation to various physiological responses and include psychological effects, such as shock ("draining of color from the skin") and embarrassment ("blushing"); temperature effects; and physiological responses to exercise, hemorrhage and alcohol consumption (Walters & Roberts, 2002:11).

The lymphatic system is the component of the skin that regulates its interstitial pressure, mobilization of defense mechanisms and waste removal. It exists as a dense, flat meshwork in the papillary layers of the dermis and extends into the deeper regions of the dermis (Walters & Roberts, 2002:12).

2.2.3 Skin Appendages

The last component of the skin is the appendages. There are four skin appendages: the hair follicles with their associated sebaceous glands, eccrine sweat glands, apocrine sweat glands and the nails. Each appendage has a different function summarized in table 2-2. The hair follicles are distributed across the entire skin surface with the exception of the soles of the feet, the palms of the hand and the lips. A smooth muscle, the erector pilorum, attaches the follicle to the dermal tissue and enables hair to stand up in response to fear,cold and certain chemicals. Each follicle is associated with a sebaceous gland that varies in size from 200 to 2000 μm in diameter. The sebum secreted by this gland, consisting of triglycerides, free fatty acids and waxes, protects and lubricates the skin as well as maintains a pH of about 5 (Walters & Roberts, 2002:12).

Sebaceous glands are largest and most numerous on the forehead, face, anogenital surfaces, in the ear and on the midline of the back. The palms and the soles are usually free of them and glands are sparse on the dorsal surfaces of the hand and the foot (Barry, 1983:10).

Table 2-2: Appendages associated with the skin (Walters & Roberts, 2002:13).

	Appendage			
Parameter	**Hair follicle and sebaceous gland**	**Eccrine gland**	**Apocrine gland**	**Nails**
Function	Protection (hair) and lubrication (sebum)	Cooling	Vestigal secondary sex gland?	Protection
Distribution	Most of the body	Most of the body	Axillae, nipples, anogenital	Ends of fingers and toes
Average/cm^2	57-100	100-200	Variable	-
Fractional area	2.7×10^3	10^{-4}	Variable	-
Secretions	Sebum	Sweat (dilute saline)	"Milk" protein, lipoproteins, lipid	Nil
Secretions stimulated by	Heat (minor)	Heat, cholinergic	Heat	-
Biochemical innervation of gland response	-	Cholinergic	Cholinergic (?)	-
Control	Hormonal	Sympathic nerves	Sympathic nerves	-

The apocrine and eccrine glands account for about one-third and two-thirds of all glands, respectively. Located in the lower dermis are the eccrine glands that are epidermal structures. They are simple, coiled tubes arising from a coiled ball, of approximately 100 μm in diameter (Walters & Roberts, 2002:12).

The gland density varies greatly with skin site, for example, the thighs possess about 120 glands per cm^2 and the soles of the feet have about 620 per cm^2. The average fractional surface area which these glands occupy is only of the order of 10^{-5} or less (Barry, 1983:10). It secretes a dilute salt solution with a pH of about 5, this secretion being stimulated by temperature-controlling determinants, such as exercise and high environmental temperature, as well as emotional stress through the autonomic (sympathic) nervous system (see table 2-2). These glands have a total surface area of about 1/10 000 of the total body surface (Walters & Roberts, 2002:12).

The apocrine glands are limited to specific body regions and are also coiled tubes. These glands are about ten times the size of the eccrine ducts, extend as low as the subcutaneous tissues and are paired with hair follicles (Walters & Roberts, 2002:12). Each structure consists of a tubule and a duct, most ducts open into the neck of a hair follicle above the sebaceous gland, but a few exit onto the surface of the skin. Small quantities of a milky or oily fluid which may be coloured are secreted by the apocrine gland. The secretion contains lipids, proteins, lipoproteins and saccharides (Barry, 1983:13).

In many respects the nail may be considered as vestigial in humans. However, some manipulative and protection functions can be ascribed (Walters & Roberts, 2002:12). The nail plate, like hair, consists of "hard" keratin, with relatively high sulfur content, mainly in the form of the amino acid cysteine, which constitutes 9.4% by weight of the nail (Barry, 1983:14). The cells of the nail plate originate in the nail matrix and grow distally at a rate of about 0.1 mm/day. In the keratinization process the cells undergo shape and other changes, similar to those experienced by the epidermal cells forming the stratum corneum. This is not surprising because the nail matrix basement membrane shows many biochemical similarities to the epidermal basement membrane. The structure of the keratinized layers is very tightly knit but, unlike the stratum corneum, no exfoliating of cells occurs (Walters & Roberts, 2002:14).

The nail plate comprises two major layers (the dorsal and intermediate layer) with, possibly, a third layer adjacent to the nail bed. The dorsal nail plate is harder and thinner than the intermediate plate, suggesting that there are differences in the chemical composition of the two layers. This suggests that applied drugs may possess differing partitioning tendencies between the layers. The latter is a particularly important consideration for the topical treatment of fungal infections of the nail (Walters & Roberts, 2002:14).

With this general overview of skin structure, one can begin to understand how the complex biology of the skin is directed toward the establishment of an effective transport barrier. It should be recognized, however, that subtle structural differences are apparent at different anatomic sites (e.g., thicker stratum corneum on the palms, higher follicular

density on the scalp). It follows that percutaneous absorption should not be expected to be similar at all anatomic sites (Potts *et al.*, 1992:16).

2.3 TRANSDERMAL DRUG DELIVERY AND DRUG PERMEATION ENHANCEMENT

The internal living human being is separated from the external environment by the skin. The skin has a complex structure (as described above in § 2.2) and performs many physiological functions such as metabolism, synthesis, temperature regulation and excretion. The outermost layer of this organ, the stratum corneum, is considered to be the main barrier to the percutaneous absorption of exogenous materials. In the maintenance of water within the body and ingress of compounds, the skin barrier is an essential component. This is of particular importance from an occupational viewpoint for workers in the cosmetic and agrochemical industries (Roberts *et al.* 2002:89).

2.3.1 Advantages of Transdermal Delivery

Given that the skin offers such an excellent barrier to molecular transport, the rationale for this delivery strategy needs to be carefully identified. Clearly, there are several instances in which the most convenient of drug intake methods (the oral route) is not feasible and when alternative routes must be sought (Naik, Kalia & Guy, 2000:319). A major advantage is the first-pass metabolism that is minimized, which can often limit the tolerability and efficacy of many orally and parenterally delivered drugs. Furthermore, some drugs degrade in the acidic environment of the stomach. The mixing of drugs with food in the stomach and the pulsed, often erratic delivery of drugs to the intestine leads to variability in the plasma concentration-time profiles achieved for many drugs (Roberts *et al.* 2002:90).

More distinct advantages that is offered *via* the transdermal mode is that the skin presents a relatively large and readily accessible surface area (1-2 m^2) for absorption (Naik, Kalia & Guy, 2000), the transdermal route provides a more-controlled, non-invasive method of delivery, with the added advantage of being able to cease absorption in the event of an overdose or other problems and furthermore, patient compliance may be improved because of the reduced frequency of administration for short half-life medications or avoidance of the trauma associated with parenteral therapy (Roberts *et al.* 2002:90).

2.3.2 Disadvantages of Transdermal Delivery

The main disadvantage of transdermal delivery is that not all compounds are suitable candidates. A number of physicochemical parameters have been identified that influence the diffusion process, and variations in permeation rates can occur between individuals, different races and between the old and young. Furthermore, diseased skin, as well as the extent of the disease, can affect permeation rates. The metabolic enzymes in the skin can pose a problem and some drugs are almost completely metabolized before they reach the

cutaneous vasculature. Another problem that can arise, which is sometimes overlooked, is that some drugs can be broken down by the bacteria that live on the skin surface before penetration through the stratum corneum (Roberts *et al.* 2002:90).

Transdermal administration is not a way to achieve rapid bolus-type drug inputs. Lower plasma levels are rather possible to offer slow, sustained drug delivery over substantial periods of time. There remains a large pool of drugs for which transdermal drug delivery is desirable but presently unfeasible. The nature of the stratum corneum is, in essence, the key to this problem (Naik, Kalia & Guy, 2000:319).

2.3.3 Transport Pathways Through the Stratum Corneum

According to Roy (1997) there are two major diffusion pathways through the stratum corneum for the transdermal delivery of drugs: the transcellular pathway and the intercellular pathway (Roy, 1997:141). Earlier Guy and Hadgraft (1989b) proposed that three possible pathways across the stratum corneum exist: transcellular, intercellular and appendageal (as demonstrated in figure 2-2). In figure 2-3 Barry (1983) demonstrates alternative pathways for percutaneous absorption. It now appears that the intercellular route predominates. Suhonen *et al.* (1999) wrote that the skin appendages only occupies 0.1% of the total human skin surface and, therefore, it is now widely believed that the transepidermal pathway of passive diffusion is the principal pathway associated with the permeation of drugs through the skin (Suhonen, Bouwstra & Urtti, 1999:151). The transcellular path, although maximizing the surface area, requires multiple partitioning steps between the densely packed corneocytes and the intercellular lipids (Guy & Hadgraft, 1989b:96).

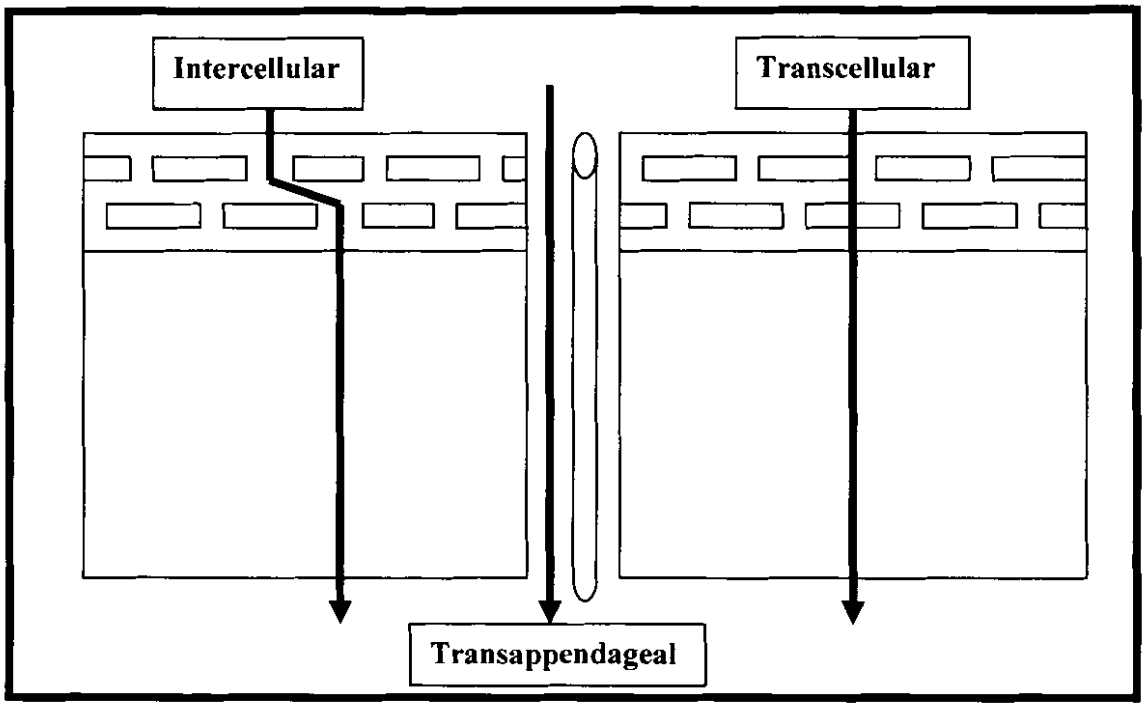

Figure 2-2: Proposed pathways of drug penetration through the skin (Guy & Hadgraft, 1989b).

Roy's explanation of the proposed pathways was that the transcellular route involves the penetration of substances through the flat cells of the stratum corneum. The intercellular pathway involves the diffusion of substances through intercellular lipids that essentially glue the flat squamous cells of the stratum corneum. The appendageal route composed of hair follicles, sebaceous glands and sweat glands is considered to be substantially less important for drug transport. Nonetheless, this route may be of some importance for large ionic molecules (see figure 2-3) (Roy, 1997:141).

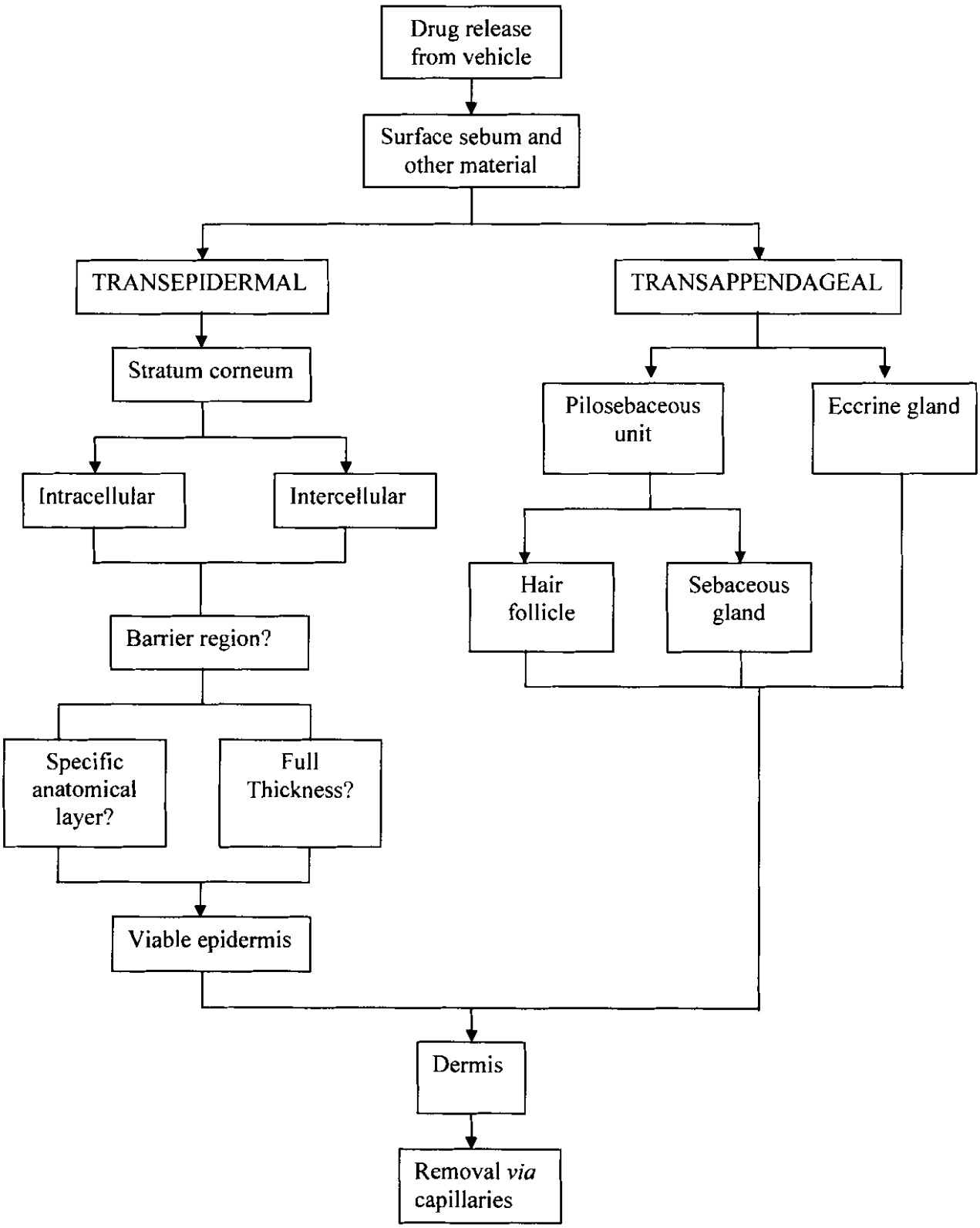

Figure 2-3: Network of alternative pathways for percutaneous absorption (Barry, 1983:32).

The percutaneous absorption of most drugs that are stable in the skin is controlled by the stratum corneum. However, skin metabolism may become a rate-limiting step in percutaneous absorption for drugs that undergo biotransformation. Recently, bioconversion by enzymatic activity in the skin has been exploited for the transdermal delivery of prodrugs (Tojo, 1997:113).

Without significant modification of the stratum corneum, the application of transdermal drug delivery is rather limited due to the difficulty of large and polar molecules in penetrating the skin. The barrier function of the skin may be overcome by the proper selection of drug candidates that favors skin transport and possible modification of the upper horny layers of the skin (i.e. stratum corneum) in order to facilitate the transport of drug molecules across the skin (Roy, 1997:142).

2.3.4 Percutaneous Absorption: The Process

When a delivery system is applied topically, the drug should diffuse out of its carrier or vehicle and partitions into either the stratum corneum or the sebum-filled ducts of the pilosebaceous glands. Inward diffusive movement continues from these locations to the viable epidermal and dermal points of entry. In this way, a concentration gradient is established 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.

In figure 2-4 the events governing percutaneous absorption after application of a drug in a thin vehicle film can be seen. There are two principal absorption routes as described in 2.3.3. Percutaneous absorption is a spontaneous, passive diffusional process that takes the path of least resistance, and therefore, either or both routes can be important (Flynn, 1996:261).

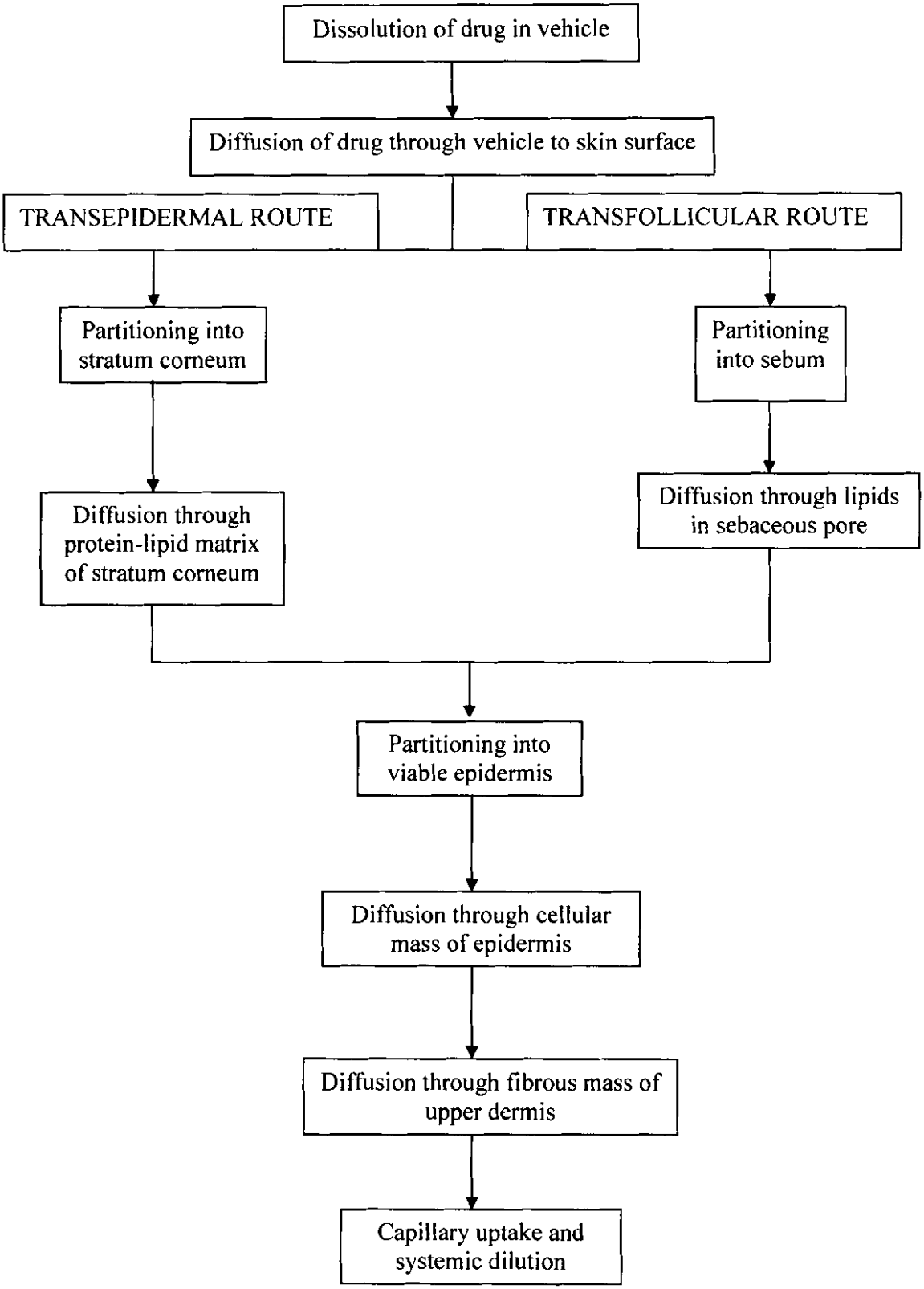

Figure 2-4: Events governing percutaneous absorption (Flynn, 1996:261).

2.3.5 Factors that Affect Percutaneous Absorption

Transdermal kinetics appear to follow Fick's first law of diffusion

$$Js = \frac{KmDCs}{E} \qquad \text{(Equation 2-1)}$$

Where

Js	=	steady state flux of solute
Km	=	distribution coefficient of the drug between the solvent or vehicle and the stratum corneum.
Cs	=	concentration difference of solute across the membrane
E	=	thickness of the stratum corneum.
D	=	average membrane diffusion coefficient for the solute in the stratum corneum (Lund, 1994:139)

The factors controlling percutaneous absorption and gastro-intestinal absorption are essentially the same. Skin penetration can be considered under three main headings: condition of the skin; physicochemical characteristics of the active substances and the effects due to the vehicle (Lund, 1994:139).

A few conditions of the skin that can affect percutaneous absorption are:

- damage and disease – skin can be damaged by dryness, irritation, allergic reactions or by abrasion.
- age – an infant's blood concentrations of topically applied drugs can be much higher than an adults. In infants the skin is a much larger organ, relatively, than in adults and the epidermal enzymes capable of metabolizing applied medicaments may not be fully developed. The skin of pre-term infants may be even more permeable as the stratum corneum is not completely formed until the end of gestation.
- temperature and humidity
- skin site
- hydration – absorption of active substances is enhanced as the skin becomes more hydrated.
- sex and race
- miscellaneous aspects (Lund, 1994:139).

The physicochemical characteristics of the active substances that can also affect percutaneous absorption are:

- drug lipophilicity – 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;
- drug mobility - after the drug has partitioned into the membrane, it must be sufficiently "mobile" to diffuse across the stratum corneum and

- optimizing passive drug diffusion (these are only applicable to passive diffusion) (Naik, Kalia & Guy, 2000:319).

The mechanisms by which percutaneous absorption takes place are not yet fully understood and the processes involved are still the subject of argument and debate. Of the many theories for drug penetration that have been advanced, the two (as previously mentioned in § 2.3.3) that are most widely accepted are the transappendageal theory and the transepidermal theory (Lund, 1994:140).

2.4 THE INFLUENCE OF PERMEATION ENHANCERS ON TRANSDERMAL DELIVERY

Several methods have been reported in the literature that has successfully resulted in elevated levels of drugs delivered across and into the skin. The most commonly used approach to drug permeation enhancement across the stratum corneum barrier, is the use of chemical penetration enhancers (Asbill & Michniak, 2000:37). This is currently the most cost-effective approach to optimize the delivery of active agents into or through the skin.

Chemical modification of the barrier properties of the skin is now recognized as a safe, effective and practical method for optimizing the local and systematically targeted delivery of active agents. Ideally, permeation enhancers should be pharmacologically inert, nontoxic, nonirritating and nonallergenic, have a rapid and reversible onset of action, be compatible with the formulation components and be cosmetically acceptable (Ghosh & Pfister, 1997:21).

Under normal conditions the stratum corneum contains about 45% water and upon contact with water it can absorb up to five times its dry weight. Dry stratum corneum is about ten times less permeable to polar molecules than normal stratum corneum and hydration increases the permeability some two- to three-fold (Dennis, 1990:27).

In 1994, Shah outlined the general effects of various enhancers on the skin, formulations and the drug. Enhancers:

- increase the diffusivity of the drug in the skin;
- 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;
- affect the partition coefficient of the drug, increasing its release from the formulation into the upper layers of the skin (Shah, 1994:20).

The outcome of enhancer action is usually a result of one or more of the mechanisms outlined. In order for permeation enhancers to be considered as acceptable agents in transdermal devices and topical products, more work is needed in evaluating the systemic

and local toxicity of the enhancers, as well as their mechanisms of action (Asbill & Michniak, 2000:37).

2.5 PHYSICOCHEMICAL PROPERTIES

Physicochemical properties of a drug have an impact in transdermal drug delivery. To appreciate this, a basic knowledge of the transfer process from the device into and through the skin is required (Guy & Hadgraft, 1989a:62). The physicochemical properties will determine the rate at which the drug can penetrate. These properties must be equated to the pharmacokinetic factors which control its clearance so that concentrations either in the lower regions of the skin or the plasma can be estimated (Hadgraft & Wolff, 1993:161).

The stratum corneum can be idealized as a "bricks and mortar" structure. Penetration of many drugs appears to be through the intercellular channels, the mortar, which consists of a complex mixture of lipids. Underlying the lipid matrix in the stratum corneum is the viable tissue. This is primarily aqueous in nature and its diffusional resistance resembles an aqueous protein gel. The drug reaches the dermal circulation once it has diffused through this region. It is then distributed throughout the body after rapid uptake into the systemic blood pool as if it were an intravenous infusion. From a physicochemical stand-point the most important processes to consider are therefore the partitioning and diffusion steps that occur in the transport into, through and out of the stratum corneum (Hadgraft & Wolff, 1993:161).

2.5.1 Diffusion Coefficient

The transport of matter resulting from passive movement of a substance within a substrate can be defined as diffusion. Diffusion through skin can also be the transport from one medium to another through restricting partially permeable membranes. Models have been created of this complex system by investigators to describe the passage of a permeant (or solute) from one compartment (the donor) through the stratum corneum to a second compartment (the receptor). The ability to diffuse depends critically on the capability of the substance to enter a particular skin layer or reach a specific site. The process is called penetration (Rieger, 1993:34).

The diffusional constant is influenced by the molecular volume of the drug and the viscosity of the surrounding medium. For ideal molecules, roughly similar in size and spherical in shape, the Stokes-Einstein equation can be applied (equation 2-2; T is the absolute temperature, r is the hydrodynamic radius of the drug molecule, k is the distribution coefficient and η is the viscosity of the environment) (Dennis, 1990:27).

$$D = kT/6\pi r\eta \qquad \text{(Equation 2-2)}$$

In absorption across a membrane, the current or flux refers to matter or molecules, rather than electrons, and the driving force is a concentration gradient, rather than a voltage drop. In general, an individual resistance (*Ri)* in a set may be represented by:

$$Ri = \frac{hi}{fiDiKi} \qquad \text{(Equation 2-3)}$$

The resistance of a layer is proportional to the diffusive mobility of a substance within it, as reflected in a diffusion coefficient (*Di*); inversely proportional to the capacity of the layer to solubilize the substance relative to all other layers, as expressed in a partition coefficient (*Ki*) and inversely proportional to the fractional area of the membrane occupied by the diffusion route (*fi*) if there is more than one route in question (Flynn, 1996:263).

Diffusion within the confines of the stratum corneum has been modeled with the aid of three simplifying modeling processes:

1. the particle must pass through the vehicle (donor compartment) to the surface of the stratum corneum. The step controlling this process is diffusion.
2. the passage into the stratum corneum, is controlled by the distribution coefficient, k.
3. the permeant diffuses through the stratum corneum.

This is generally the rate-determining step as shown by extensive experimentation in the study of skin permeation (Rieger, 1993:34). According to Fick's law of diffusion, once the drug has dissolved in the outer skin lipids it will diffuse down its concentration gradient. The rate constant k_1 (h^{-1}) is a first order approximation for diffusion and its magnitude is related to the molecular size through the molecular weight M by the equation:

$$k_1 = 0{,}9M^{-0.33} \qquad \text{(Equation 2-4)}$$

It is necessary to decrease the diffusional resistance in the structured lipids by making them more fluid in order to increase the flux of drugs across the stratum corneum. This can be achieved by the use of penetration enhancers (Hadgraft & Wolff, 1993:164). Also, to pass from the solvent (or vehicle) to the skin, the diffusing solute molecule must have some affinity for the stratum corneum (Rieger, 1993:34).

2.5.2 Partition Coefficient

When the drug reaches the viable tissue it encounters a phase change, it has to transfer from the predominantly lipophilic intercellular channels of the stratum corneum into the living cells of the epidermis which is largely aqueous in nature and essentially buffered to pH 7.4. Below the optimum log P of ~2.5, the absorption rate increases with P as a result of the higher partition coefficient providing a larger concentration gradient across the stratum corneum. At steady state the flux, J, across the stratum corneum will be given by:

$$J = \frac{KD_{co}}{l} \qquad \text{(Equation 2-5)}$$

D = diffusion coefficient in the stratum corneum of thickness l

$_{co}$ = applied drug concentration.

As K increases, the resistance created by the stratum corneum decreases and the resistance in the viable tissue becomes more dominant (Hadgraft & Wolff, 1993:164).

The partition coefficient of a compound that exists as a monomer in two solvents is given by:

$$K = \frac{C_1}{C_2} \qquad \text{(Equation 2-6)}$$

If it exists as an n-mer in one of the phases, the equation becomes:

$$K = \frac{(C_1)^n}{C_2} \qquad \text{(Equation 2-7)}$$

or

$$\log k = n \log C_1 - \log C_2 \qquad \text{(Equation 2-8)}$$

The easiest way to determine the partition coefficient is to extract V_1 ml of saturated aqueous solution with V_2 ml of solvent and determine the concentration C_2 in the latter. The amount left in the aqueous phase is $(C_1V_1 - C_2V_2) = M_1$ so that the partition coefficient becomes the ratio of the solubilities and it is sufficient to simply determine the solubility of the drug substance in the solvent (since it is assumed that the solubility is already known in water) (Carstensen, 1996:223).

$$K = \frac{S_1}{S_2} \qquad \text{(Equation 2-9)}$$

It has been postulated that two parallel routes of absorption exist through the stratum corneum. The lipid route would be between the columns of dead cells and therefore through the intercellular lipid material. The polar route would be through the cellular material and thus through the hydrated protein mass of the keratinocytes. Permeability coefficient for polar molecules are in the region of 10^{-5} to 10^{-6} cm.h^{-1}, which is some 1000-fold lower than for reasonably lipid soluble molecules (Dennis, 1990:26).

2.5.3 Solubility

A dominant factor in skin penetration is solubility. Its importance was recognized when it was found that compounds soluble in both lipid and water penetrate better than substances manifesting either high water or high lipid solubility. A substance's solubility greatly influences its ability to penetrate biological membranes (Malan, Chetty & Du Plessis, 2002:387). Only the dissolved fraction of a drug in a vehicle can enter the skin, so that solubility becomes one of the initial objectives for a novel pharmaceutical formulation (Kreilgaard, 2002:S83).

The aqueous solubility of a drug molecule is partly reliant on other physicochemical properties, for example, partition coefficient and molecular surface features that are relevant to drug absorption. As a result, a correlation between poor absorption and poor solubility can be expected and has been demonstrated (Malan, Chetty & Du Plessis, 2002:387).

Solubility and partitioning can be described in terms of the energy required to convert from a solid solute to a molecular form, the energy of dissolution in a vehicle and the energy of dissolution in the stratum corneum. It is necessary to express solubilities and partition coefficients in terms of the pure solid, also referred to as its ideal solubility (x_i°). This ideal solubility varies with the nature of the solute crystal and is related to the energy associated with the formation of the pure liquid form by melting of the crystals at a melting point (Tm)

$$\ln x_i^\circ = \frac{-\Delta H_f}{RT}\left[\frac{1}{T} - \frac{1}{T_m}\right] + \frac{\Delta C_p}{R}\left[\frac{T_m - T}{T} - \ln\frac{T_m}{T}\right] \qquad \text{(Equation 2-10)}$$

Where

x_i°	=	ideal solubility
T_m	=	melting point
ΔH_f	=	molar heat of fusion
R	=	gas constant
T	=	room temperature
ΔC_p	=	difference in heat capacity of the crystalline and molten state (Roberts *et al.* 2002:104).

Because it is such an important property an insight into the solubility of a drug can be regarded as the most important aspect of preformulation testing. It is often desirable to limit aqueous solubility in a liquid dosage form because drugs are generally less stable when in solution (Lund, 1994:185).

2.5.4 Molecular Weight and Size

Considering that the stratum corneum is a compact membrane and that diffusing molecules follow a tortuous path through it, it might seem obvious that the diffusion coefficient would be inversely related to molecular weight or some other measure of molecular size (Zatz, 1993:28).

A clear relationship between the diffusion coefficient (D) and molecular weight (M_r) exists; (M_r): D $(M_r)^{-½} \approx$ constant. While molecular charge is the rate-determining factor in the permeability of small molecules through mucosal membranes, with increasing molecular size, the "sieving effect" of the pores becomes increasingly discriminating and more important than electrical field interactions (Malan, Chetty & Du Plessis, 2002:387).

According to Lien and Tong (1973) as quoted by Roberts *et al.* (1995), the penetration has been related to partition (usually octanol/water) coefficients in many studies. It was shown that, in some cases the inclusion of other electronic forces or steric terms, such as molecular weight, significantly improved the correlations (Roberts *et al.* 1995:219).

Potts and Guy's (1992) work summarized the relationship between the permeability coefficient of human stratum corneum (k_p), the octanol/water partition coefficient ($K_{octanol}$) and solute size expressed as molecular weight. They differed from earlier studies in that log k_p was related directly to molecular weight and not the logarithm of the molecular size as originally reported by Lien and Tong (Roberts *et al.* 1995:220).

Partition coefficients represent not only solute polarity but also molecular size, since the bulk of most molecules are in the lipophilic carbon skeleton. The results of studies done on the nature of the epidermal barrier for various penetrant structures are difficult to interpret due to the large number of confounding variables. For instance, the variation in the choice of penetrants can lead to discrepancies in the apparent dependency of epidermal penetration on molecular size, according to (Roberts *et al.* 1995:220).

Diffusion is related to size by:

$$D = D_0 (MW)^b \qquad \text{(Equation 2-11)}$$

Where

D = diffusion coefficient (cm^2/h)
D_0 = diffusion of an infinitely small molecule.

Scheuplein, Blank and Roberts used values for *b* of -0.5 and -0.33, but this assumes the stratum corneum is an isotropic fluid medium that does not interact, apart from by physical obstruction, with the diffusant. In fact, the stratum corneum is an anisotropic, liquid crystalline structure, and the evidence already described suggests powerful

interaction *via* H-bonding. Diffusion should then be more accurately written as (Pugh, 1999:187)

$$D = D_0.(binding)^a.(MW)^b \qquad \text{(Equation 2-12)}$$

Potts and Guy (1992) suggested that, for compounds ranging in molecular weight from 18 to >750 and in log k_{oct} from -3 to +6, the permeability through human skin can be predicted by the following equation:

$$\log k_p = -6{,}3 + 0{,}71 \times \log k_{oct} - 0{,}0061 \times MW \qquad \text{(Equation 2-13)}$$

Where

k_p	=	permeability coefficient (cm/sec)
k_{oct}	=	octanol/water partition coefficient
MW	=	molecular weight.

For many compounds, molecular weight (MW) is often a reasonable approximation of molecular volume. Molecular volume was substituted with molecular weight to provide an equivalent fit in the model (Potts & Guy, 1992:664).

2.5.5 Hydrogen Bonding

The value of hydrogen bonding as a determinant of drug permeation across absorbing membranes has been recognized in several studies. There is a close relationship between the permeability coefficient of solutes that penetrate the stratum corneum and the presence of hydrogen-bonding groups on the solute molecule (Malan, Chetty & Du Plessis, 2002:386).

The equation

$$\log\left(\frac{D}{h}\right) = \log k_p - \log k_{SC} \qquad \text{(Equation 2-14)}$$

was used to determine the diffusion (D), using the permeability coefficient (k_p) and stratum corneum/water partition coefficient (k_{SC}). D is the diffusion coefficient and h the path length for diffusion (Roberts *et al.* 1996:25).

Hydrogen-bonding donor (alpha, α) and acceptor (beta, β) parameters are generally derived from substructure summation and have been successfully used in relation to steroid drugs to provide predictive algorithms for transdermal permeability (Malan, Chetty & Du Plessis, 2002). Using the lipid composition of the stratum corneum given by Wertz and the α and β values of Abraham, Pugh *et al.* (Pugh, 1999) calculated the H-

bonding effects of the stratum corneum to be in the ratio $\alpha_{SC} : \beta_{SC} = 0.4: 0.6$. This would suggest that stratum corneum is predominantly a H-bond acceptor environment (Pugh, 1999:183).

In other skin permeability studies the significance of H-bonding as distinct from lipophilicity has also been recognized. Roberts showed that after allowing for partitioning into the stratum corneum and molecular size, the permeability coefficient was related to the number of H-bonding groups in the penetrant. According to Anderson and Raykar quoted by Roberts the stratum corneum barrier micro-environment resembled a hydrogen bonding organic solvent. El Tayar and coworkers suggested that the H-bond donor potential of the solute was the dominant feature in epidermal penetration transport. In contrast, Roberts suggested that both H-bonding donor and acceptor potential of a solute governed its transport through the epidermis. Abraham has applied the solvatochromic approach to describe some of these and other permeation data (Roberts *et al.* 1995:221).

Abraham, Potts and Guy and Roberts as quoted by Hadgraft, suggested that the H-bonding power of the penetrant is a major determinant of penetration. The modification of H-bonding within the natural stratum corneum lipids is a possible mode of action of modifiers. According to Wertz ceramide 6 is the most powerful H-bonding lipid. It consists of four secondary alcohol and one secondary amide groups. Binding between ceremide 6 molecules should represent the strongest intermolecular binding possible between stratum corneum lipids (Hadgraft *et al.*, 1996:23).

2.5.6 Ionization

The ionization of a molecule generally decreases absorption through the skin (Dennis, 1990:27). This statement was confirmed by Zatz (1993) when he said that the charged compounds should encounter high resistance to permeation because of the nonpolar nature of the horny layer. This proposition is most easily studied by use of ionigenic compounds for which the ratio of charged to uncharged species can be manipulated by changing the pH of the vehicle. The two species should have the same diffusion coefficient value because they are both of the same size. In every case studied, the permeability coefficient of the unionized form exceeds that of the charged species, in some cases by two or three orders of magnitude. The first reason is the greater solubility of the unionized compound in the horny layer, the second is its poorer solubility in the aqueous donor solvent (Zatz, 1993:29).

The ionized molecule is believed to pass through the intercellular spaces, whereas the non-ionized molecule is more likely to travel by the intracellular route through the stratum corneum (Malan, Chetty & Du Plessis, 2002:388). According to Menczel and Goldberg (1978) the unionized percentage, P, of a cationic conjugated base or a basic compound is related to the pH of its aqueous solution by the following equation:

$$P = \frac{100}{1+10^{pKa-pH}} \qquad \text{(Equation 2-15)}$$

Where

pK_a = -log K_a of a conjugate base

K_a = the dissociation constant of the conjugation with hydrogen ion.

For a basic compound, the value of pK_a equals the value of

$$[pKw(=14)]-[pK_b] \qquad \text{(Equation 2-16)}$$

Where $pK_b = -\log K_b$ is the dissociation constant of the base. For an anionic (acidic) organic compound this relationship is as follows:

$$P = \frac{100}{1+10^{pH-pK_a}} \qquad \text{(Equation 2-17)}$$

Where: $pK_a = -\log K_a$ is the dissociation constant of the acidic organic compound (Menczel & Touitou, 1989:122).

2.5.7 Melting Point

The permeant melting point was found to be inversely proportional to lipophilicity (log P) (Stott *et al.*, 1998:298). According to Kasting, as quoted by Stott *et al.* (1998), a relationship existed between transdermal permeation and melting point of the permeant, based on ideal solution theory (Stott *et al.*, 1998:298).

Materials with very low melting point will penetrate the skin more readily. Nitroglycerin and nicotine that are delivered transdermally and that diffuse through the skin rapidly, are both liquids at room temperature (Hadgraft & Wolff, 1993:163). Kasting as well as Touitou, as quoted by Stott *et al.* (1998), has proven that a reduction in the melting point of a permeant have a direct effect on its solubility in skin lipids and so increase transdermal permeation. If one can reduce the melting point of a drug without causing unfavorable changes to other physicochemical parameters, then this should enhance transdermal flux (Stott *et al.*, 1998:298).

The melting point can be reduced by a method called eutectic formation. This is a mixture of two components which do not interact to form a new chemical compound but which, at certain ratios, inhibit the crystallization process of one another resulting in a system with a lower melting point than either of the components (Stott *et al.*, 1998:298).

2.6 PHYSICOCHEMICAL PROPERTIES OF ACYCLOVIR

For this study acyclovir, a hydrophilic drug, was chosen as model drug to study its penetration through the stratum corneum. Drugs, which permeate particularly slowly, are those that have high hydrophilicity or are very lipophilic in nature.

The challenge in this study was then to improve the transdermal diffusion of acyclovir with the aim to transport acyclovir in a highly bioavailable drug delivery system. A delivery system with different compositions was examined for the use of the transdermal delivery of this hydrophilic drug.

2.6.1 Chemistry

Acyclovir (Zovirax)
$C_8H_{11}N_5O_3$
9-[(2-Hydroxyethoxy)methyl]guanine

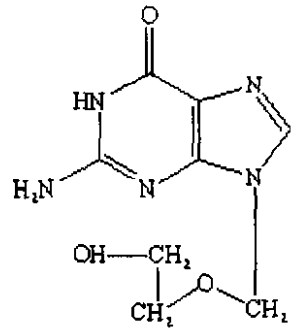

Figure 2-5: Structure of acyclovir (nr. 990909).

Molecular weight	225
pK_a	2.27, 9.25
Solubility in ethanol	< 1 in 5000
in water	1 in 400
Octanol/0.2M phosphate buffer coefficient	0.018 (Dollerry, 1999:A39)
Melting point	230°C (Lund, 1994:711).

Acyclovir is a white powder consisting of elongated rectangular crystals. It is prepared by chemical synthesis. At physiological pH, acyclovir exists as the unionized form with a maximum solubility of 2.5 $g.l^{-1}$ (Dollery, 1999:A39). In an evaluation of oral acyclovir therapy it was reported that oral absorption of acyclovir is slow and variable and that oral bioavalibility is in the range of 15% to 30% (Lund, 1994:713).

2.6.2 Pharmacology

Acyclovir requires three phosphorylation steps for activation. It is converted first to the monophosphate derivative by the virus-specified thymidine kinase and then to the di- and triphosphate compounds by the host's cellular enzymes. Because it requires the viral kinase for the initial phosphorylation, acyclovir is selectively activated and the triphosphate accumulates only in infected cells. Acyclovir triphosphate inhibits viral DNA polymerase, with binding to the DNA template resulting in an irreversible complex and chain termination following incorporation into the viral DNA (Safrin, 2001:824).

2.6.3 Therapeutic Use

Acyclovir has a number of indications:

1. Treatment of herpes simplex keratitis.
2. Treatment and prophylaxis (suppression) of herpes simplex infections of skin and mucous membranes in immunocompetent individuals.
3. Treatment of severe and/or generalized herpes simplex infections in immunocompromised and immunocompetent individuals.
4. Treatment of varicella zoster infections in immunocompromised and immunocompetent individuals.
5. Prophylaxis of herpes simplex, varicella zoster and cytomegalovirus infections in the immunocompromised.
6. Improvement of survival in patients with AIDS (Dollery, 1999:A40).

2.6.4 Summary

Because of the excellent barrier properties of the stratum corneum and the hydrophilicity of acyclovir, it is problematic to obtain sufficient drug levels at the target site to be therapeutic. Drugs that are highly lipophilic of hydrophilic in nature are usually the drugs that permeate particularly slowly. Acyclovir is such a hydrophilic drug resulting in minimal systemic absorption after topical application (Dollery, 1999:A39).

Several approaches to an effective acyclovir topical preparation has been suggested: percutaneous absorption enhancers, using different vehicles and iontophoresis (De Jalón *et al.*, 2001:192). In this study a penetration enhancer was used to improve the limited absorption and secondly, an emulsion was used to incorporate the drug in order to achieve larger percutaneous absorption of acyclovir.

2.7 DRUG DELIVERY VEHICLES

Many of the problems associated with the delivery of a drug could be overcome by the use of specialized delivery systems, such as liposomes, micellular solutions and microemulsions. These three formulations have been proposed as efficient strategies for the administration of drugs with limited solubility in biological fluids. In this respect, micellar solutions and microemulsions offer several potential advantages, such as high

solubilization capacity, stability and ease of preparation (Cortesi & Nastruzzi, 1999:291). These three delivery vehicles can be seen in figure 2-6.

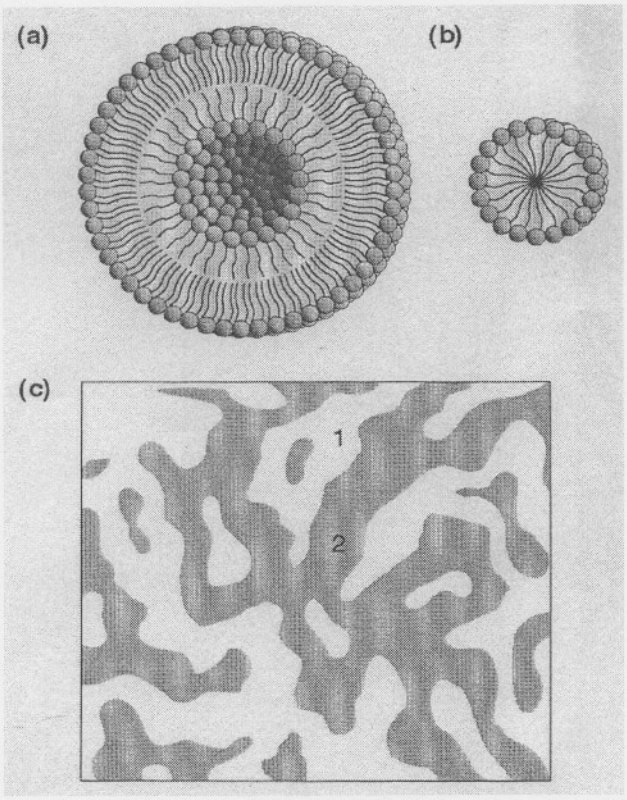

Figure 2-6: Schematic representation of the drug delivery systems. (a) Liposomes; (b) direct micelles; (c) microemulsion, 1 = aqueous phase and 2 = oil phase (Cortesi & Nastruzzi, 1999:291).

Another novel delivery system, to be discussed in this section, is multiple emulsions. This systems consists of droplets which themselves contain smaller droplets within (see § 2.7.4).

2.7.1 Microemulsions

During the recent decades, various studies have suggested that a delivery vehicle described as a 'microemulsion' has the potential of increasing cutaneous drug delivery of both hydrophilic and lipophilic drugs compared to conventional vehicles. As the potential of microemulsion vehicles for cutaneous drug delivery is released, the application of these formulations is becoming increasingly popular. The correlation between components, composition and structure of microemulsions and an increase in drug delivery rate is not yet fully elucidated (Kreilgaard, 2002:S78).

Microemulsions are clear and thermodynamically stable isotropic mixtures of oil and water containing domains of nanometer dimensions stabilized by the interfacial film of surface active agents. Usually microemulsions are produced by homogenization of the

corresponding compounds. Some of them are prepared by titrating to clarity through the addition of a co-surfactant (Valenta & Schultz, 2004:257). The droplet size in the dispersed phase is very small, usually below 140 nm in diameter, which makes the microemulsions transparent liquids (Peltola *et al.* 2003:99) or slightly opalescent systems with low viscosity (Escribano *et al.* 2003:204).

This study investigates the possibility of adjusting viscosity and release characteristics by adding a polymeric gelating agent to microemulsions. For this purpose the polysaccharide carrageenan was chosen. Carrageenan is mostly used as a food additive but have the advantage of good adhesiveness on the skin making it a great component in transdermal delivery vehicles (Valenta & Schultz, 2004:257). In principle, microemulsions can be used to deliver drugs to the patient *via* several routes, but the topical application of microemulsions has gained increasing interest.

The three main factors determining the transdermal permeation of drugs are the mobility of the drug in the vehicle, release of the drug from the vehicle and permeation of the drug into the skin. Microemulsions improve the transdermal delivery of several drugs over the conventional topical preparations such as emulsions and gels. Mobility of drugs in microemulsions is more facile, but the gel former in microemulsions will increase its viscosity and further decrease the permeation in the skin. The superior transdermal flux from microemulsions has been shown to be mainly due to their high solubilization potential for lipophilic and hydrophilic drugs. This generates an increased thermodynamic activity towards the skin. The drug may also be retained in the droplets of the microemulsion formulation, which will decrease its permeation in the skin. For example, the increased concentration of surfactant in dispersed systems may decrease drug release and its permeation in the skin. Several neat compounds used in microemulsions have been reported to improve the transdermal permeation by altering the structure of the stratum corneum (Peltola *et al.*, 2003:99).

2.7.1.1 Microemulsion structure

Within the microemulsion regions, several different internal structures can form from the immiscible water and oil phases and the interfacial surfactant film. The microemulsion structure is influenced by the ratios between the components and the physicochemical properties of these components. Microemulsions are dynamic systems in which the interface is continuously and spontaneously fluctuating. Regular or reverse 'swollen micelle' droplet-like shapes may be approached by microemulsion structures in very dilute systems. However, in between these extremes, the microemulsion components typically form non-spherical aggregates, that may be more or less continuous in the phase with the 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 (Kreilgaard, 2002:S79). These steps in the formation of different microemulsion structures are illustrated in figure 2-7.

Microemulsion systems therefore often do not display emulsion-like behavior with sudden inversion of the 'swollen micelle'. The emulsion terminology of characterizing the systems as oil-in-water (o/w), or water-in-oil (w/o), is thus in many situations not applicable to microemulsions. Typical emulsion-like behavior by some microemulsion systems are being displayed, forming small droplet-like 'swollen micelle' structures with a dispersed and a continuous phase. By continuous addition of the dispersed phase to this system, the droplets may either swell and form other colloidal structures (typically regular macroemulsions) and thus lose the microemulsion structure and characteristics, or they may 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 microemulsions can also be observed for some systems. The exact mechanism behind the structural formations and transitions and the relation to the physicochemical properties of the components is not yet well established. A significant factor is the flexibility of the surfactant film. This film determines the possible structures and ways of structural transitions by changes in component ratios for a given microemulsion system. A very rigid surfactant film will likely result in droplet-like shapes and will not enable existence of bicontinuous structures. This will impede 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 therefore broaden the range of existence, enabling formation of microemulsions with a wide variety of compositions (Kreilgaard, 2002:S80).

2.7.1.2 Formulation

Microemulsion vehicles differ from emulsions in the following way: they are formed spontaneously when mixing the appropriate quantities of the components without requiring additional mechanical energy and they are 'infinitely' physically more stable due to their thermodynamic nature. The characteristics of microemulsions for pharmaceutical formulations and the wide range of oil-water-surfactant compositions, which principally can form microemulsions make them straightforward to prepare (Kreilgaard, 2002:S83).

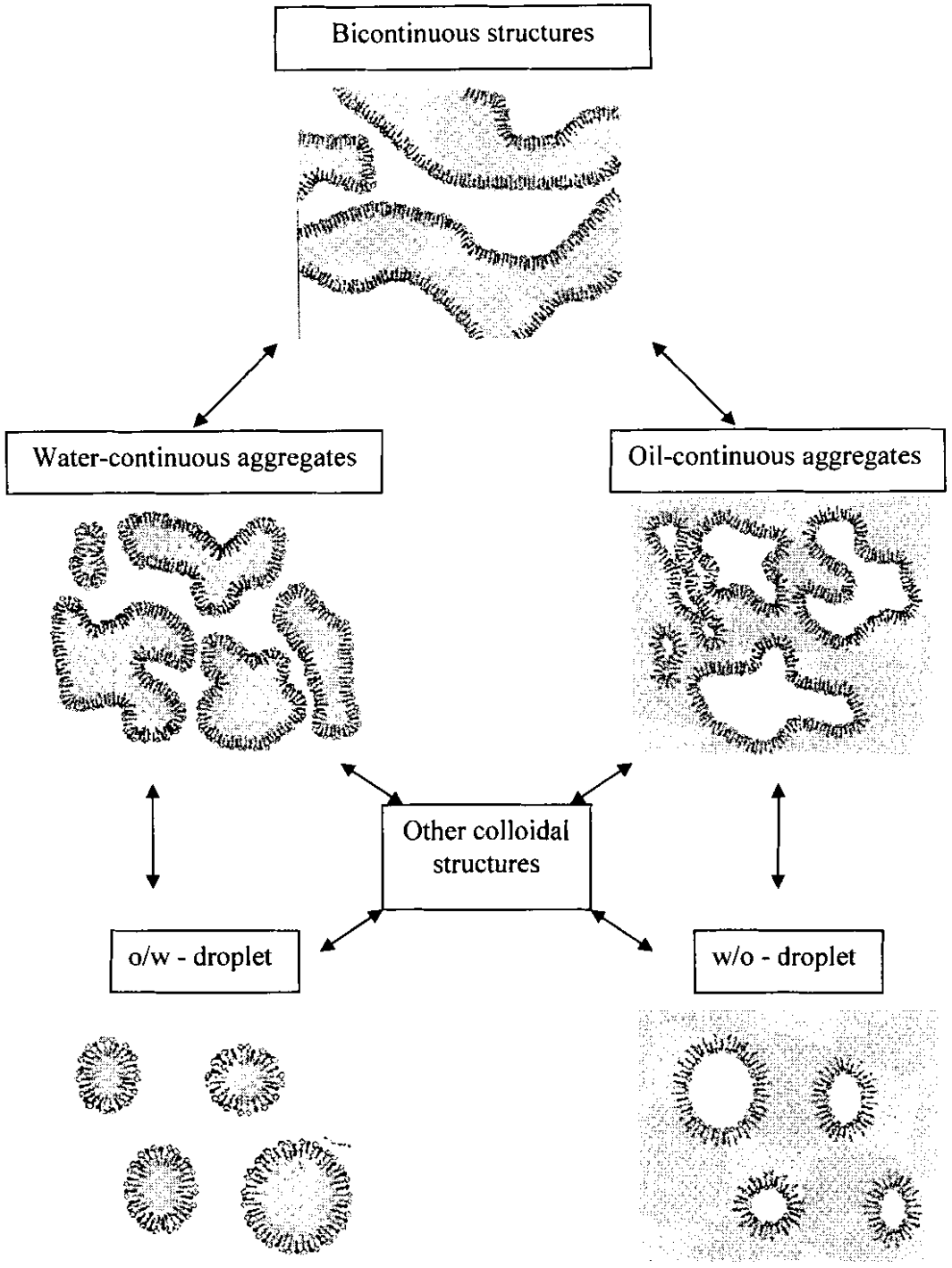

Figure 2-7: Basic dynamic microemulsion structures formed by oil phase (grey), aqueous phase (white) and surfactant/co-surfactant interfacial film, and plausible transition between the structures (indicated by arrows) by increase of oil fraction (clockwise from left to right) and water fraction (anti-clockwise from right to left), respectively. (Kreilgaard, 2002:S81).

2.7.1.3 *In vitro* investigations

The following three microemulsions were investigated in previous studies:

1. a basic microemulsion
2. the basic microemulsion with 2 % cholesterol
3. the basic microemulsion with 5 % oleic acid.

The *in vitro* investigations showed that incorporation of cholesterol into the microemulsion significantly enhanced the dermal delivery of the drug into all skin layers, and particularly into the stratum corneum layer by increasing the hydrophilic domains in the stratum corneum, thereby facilitating the passage of hydrophilic substances. Interestingly, the addition of oleic acid, which is generally acknowledged as a penetration enhancer, did not increase dermal delivery of the drug in any layers of the skin. As for oleic acid, it is believed to alter the viscosity of the skin lipids, facilitating diffusion of lipophylic substances (Kreilgaard, 2002:S89).

Skin permeation tests that were done by Valenta & Schultz (2004) with microemulsions contained carrageenan as a viscosity enhancer. Carrageenan needed to be present in the microemulsion to apply the emulsion transdermally without difficulty. In their study they concluded that carrageenan had a positive influence on their hydrophilic model drug permeation in all preparations. All their preparations that consisted of carrageenan, had a decrease in lag time and they ascribed it to the good adhesiveness of carrageenan on the skin (Valenta & Schultz, 2004: 264).

2.7.1.4 Means of increasing cutaneous drug delivery

Because of the ability of dissolving large amounts of both lipophilic and hydrophilic drugs in microemulsions, without a concurrent increase in vehicle affinity, the overall transdermal flux is increased due to the larger concentration gradients (Kreilgaard, 2002:S94)

The combined effect of hydrophilic and lipophilic components of the microemulsion enhances the activity of the whole system. Microemulsions are able to reduce the interfacial tension between skin and vehicle because of its close contact to skin lipids. This results in a faster penetration and permeation into deeper skin layers (Schmalfuß *et al.*, 1997:283).

Increasing surfactant and oil content of the microemulsion formulations showed a general trend of decrease in cutaneous drug delivery. The potential enhancer effect of microemulsions is typically attributable to the individual constituents, i.e. the oil of surfactant phase, rather than the specific microemulsion structure. An enhancer effect of the frequently applied oil phase for microemulsion, IPP, has been indicated by several studies (Kreilgaard, 2002:S94).

Two recent publications (Kreilgaard *et al.*, 2000 and Kreilgaard *et al.*, 2001) demonstrated that the intradermal permeation rates of a lipophilic drug were significantly

increased by microemulsions as compared to commercial macroemulsions. In macroemulsions, the free mobility of the active material between the internal (continuous) phases within the structure of the formulated system is limited due to the strong interaction between the surfactants that form the interfacial membrane film. In microemulsions, the co-surfactant lowers the interfacial tension of the surfactant film, resulting in a more flexible and dynamic layer as suggested by previous reports. The drug in this energy-rich system can diffuse across the flexible interfacial surfactant film between the phases; a thermodynamic process that increase partitioning and diffusion into the stratum corneum (Sintov & Shapiro, 2004:174).

The enhancer effect of the constituents is, however, also dependent on the favour of hydrophilic or lipophilic pathways for the drug through the stratum corneum. There is a significant influence of the composition and internal structure of the microemulsion on the drug delivery potential of individual microemulsion vehicles, and thus on the mobility of the drug in the vehicle. While a microemulsion vehicle may exhibit excellent solubility of a drug and contain penetration enhancers, the full potential of the vehicle for high cutaneous drug delivery rates can be hampered by either diffusional hindrances of the drug (i.e. encapsulation of the solvent phase or adsorption to the surfactant film), unfavorable partitioning of the drug from the internal to the external phase, or an unfavorable overall partition coefficient between the microemulsion and the skin (Kreilgaard, 2002:S94).

2.7.1.5 Tolerability studies of topically applied microemulsions

The potential risk of toxicological skin reactions from application of microemulsions is closely related to interaction properties of the constituents with the skin. The use of penetration enhancers should therefore be minimized to ensure the therapeutic relevance of the vehicle. However, as microemulsions typically are composed of large amounts of surfactants and oil, it is particularly important to consider the potential skin irritation and toxicological reactions by topical application of these formulations (Kreilgaard, 2002:S95). Saito and Shinoda showed that clear isotropic systems could be achieved by using non-ionic surfactants without the addition of a co-surfactant. Based on these investigations various co-surfactant free microemulsions were established. This is interesting for dermal application because many of the co-surfactants have been shown to exhibit irritative effects (Valenta & Schultz, 2004:1). The favorable cutaneous drug delivery and solvent properties, together with the ease of formulation and the 'infinite' physical stability of these unique oil-water-surfactant mixture, makes microemulsions promising vehicles for topical formulations (Kreilgaard, 2002:S96).

Microemulsions can increase the transdermal delivery of a compound by different mechanisms:

- a large amount of drug can be included in the formulation due to the high solubilization power;
- an increase in the transdermal flux can be expected in that the thermodynamic activity of the drug in the microemulsion can be modified to favor partitioning into the stratum corneum, or

- the surfactants in the microemulsion may reduce the diffusional barrier of the stratum corneum (Delgado-Charro *et al.*, 1997:37).

2.7.2 Liposomes

Interest in the use of liposomes as topical drug vehicles has been spurred, in part, by the commercial success and popularity of liposome-based cosmetics. This has provided a firm basis for further investigation of their utility as topical delivery systems. Indeed, it has given a clear indication of the general safety, lack of toxicity or irritation and cosmetic acceptability of topically applied lipids. Liposomes are thus attractive candidates for dermatological formulations (Redelmeier & Kitson, 1999:283).

2.7.2.1 Structure and formulation

Liposomes are microscopic spheres consisting of lipid bilayer(s) enclosing an inner aqueous core. Formulation strategies which focus on achieving "liposomal" delivery of a bioactive compound to viable skin tissue generally involve encapsulating the compound in the inner aqueous core. High drug/lipid ratios are generally required for topical delivery and that is why a poor trapping efficiency for a liposome formulation may restrict the suitability of the formulation. Because of multilamellar vesicles, comparatively higher entrapment volumes (5-20 l/mol lipid) may be more suitable for topical application. An additional consideration when formulating liposomes for use in dermatological preparations relates to the solubility of hydrophobic compounds in the liposomes. Though it has been argued that liposomes offer ideal vehicles for solubilizing hydrophobic compounds, many non-amphipathic molecules do not readily formulate into liposome bilayers (Redelmeier & Kitson, 1999:284).

Release kinetics from topical formulations have been shown to be influenced by encapsulation of compounds into liposomes, though the extent of this depends upon the physicochemical properties of the drug. The permeability of liposome carrier systems depends upon the lipid composition - increasing the acyl chain length or decreasing the degree of saturation of the lipid hydrocarbon reduces the rate of release of an encapsulated compound. *In vivo*, the kinetics of drug release from the liposome is determined by the thermodynamic activity of the compound, the flux across the liposome membrane, as well as the stability of the liposome itself. Conditions which result in fusion or breakdown of topically applied vesicles are likely to result in the release of the encapsulated contents. If a drug's "affinity" for the liposome is greater than for the stratum corneum it will be retained on the skin surface (Redelmeier & Kitson, 1999:284).

2.7.2.2. Lipid penetration of the stratum corneum

Though physical evidence that liposomes penetrate the stratum corneum is lacking, there is substantial evidence that lipid monomers can and do penetrate the intercellular lipid. Topical application of lipids either as liposomes, or from other structures, will undoubtedly lead to diffusion of lipid monomers into the intercellular lipid domain. In turn, it is possible that these lipids may influence the properties of the intercellular lipid,

as seen in figure 2-8. Therefore, lipids, perhaps even in the form of liposomes, are good candidates to modulate skin barrier properties. However, the relative advantage of these formulations should be evaluated on a case by case basis in comparison with other enhancers or retarders of percutaneous absorption and under clinically appropriate conditions (Redelmeier & Kitson, 1999:291).

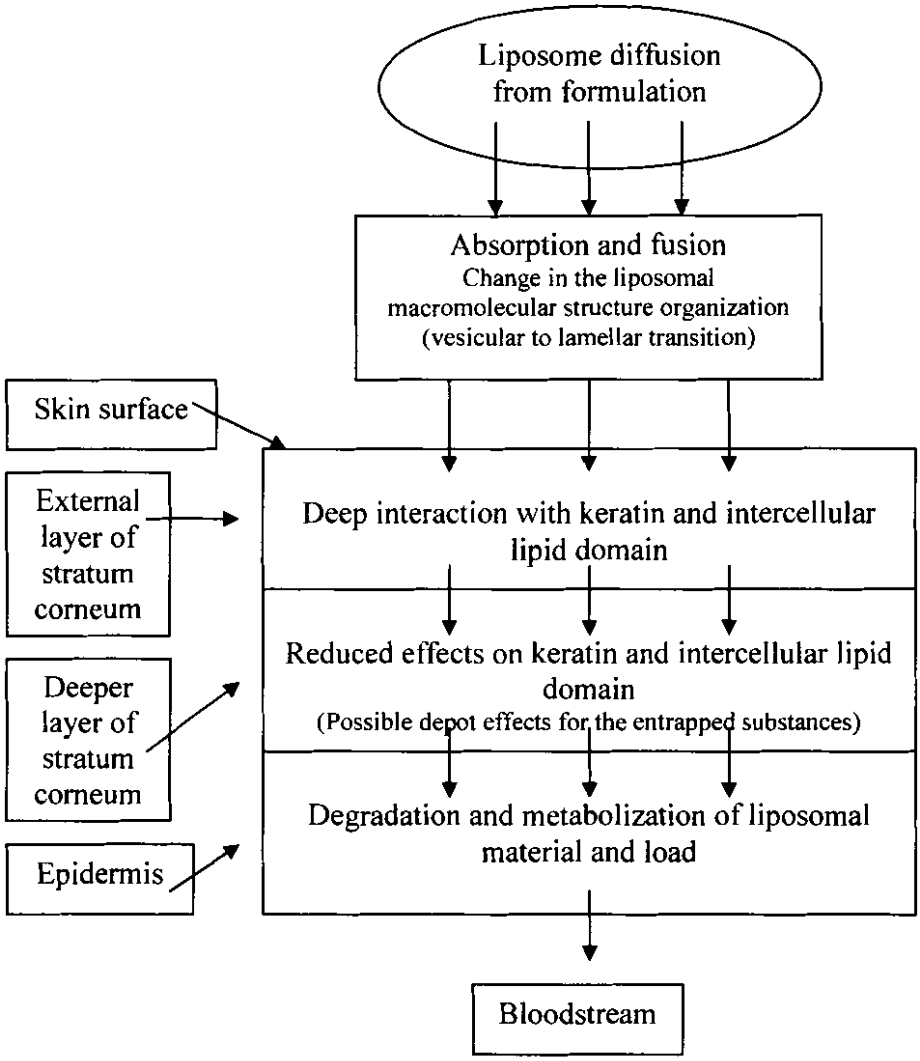

Figure 2-8: Schematic representation of the proposed skin interactions and disruption-degradation mechanisms of liposome (Cortesi & Nastruzzi, 1999:297).

2.7.3 Micellar Systems

Solubility and stability of lipophilic compounds in water is known to be influenced by micellar solutions. For example, lipophilic drugs can be solubilized by the hydrophobic environment within the micelles (direct micelles), allowing for improvements in the level of bioavailability (Cortesi & Nastruzzi, 1999:295).

2.7.4 Multiple Emulsions

Multiple emulsions are of interest because of their potential for protecting encapsulated substances and their ability to provide sustained release (Piemi *et al.*, 1998:208). They are complex systems, where droplets of the dispersed phase themselves contain even smaller droplets, identical to or different from the continuous phase, as shown in figure 2-9. Water-in-oil-in-water (w/o/w) and oil-in-water-in-oil systems (o/w/o) are the two basic types of multiple emulsions (Nielsen & Gohla, 1998:76).

2.7.4.1 History

Boys first reported multiple emulsions in 1890. According to Seifritz, key parameters for a multiple droplet formation, as an unstable intermediate state of the manufacturing process, are intensity of stirring, stirring speed, chemical composition and the ratio of the lipophilic and hydrophilic ingredients. The first reproducible manufacturing method for multiple emulsions was developed by Herbert, an endocrinologist of Edinburgh Medical School. This manufacturing process is a two step technique (Nielsen & Gohla, 1998:67).

2.7.4.2 Formulation

The literature reports on several approaches to formulate multiple emulsions and focus mainly on the formulation of the w/o/w type (Nielsen & Gohla, 1998:69).

2.7.4.3 Stability control

Stability assessment is basically the same as for simple emulsions. Appropriate methods are macroscopical evaluation, micrographical evaluation with polarized and non-polarized light, particle size distribution and viscosity change check as well as storage behavior under different conditions. While macroscopical stability of said multiple systems may remain unaltered, multiple systems can convert into simple o/w or w/o emulsions. Therefore, assessment of macro- and microcoalescence are of particular importance (Nielsen & Gohla, 1998:75).

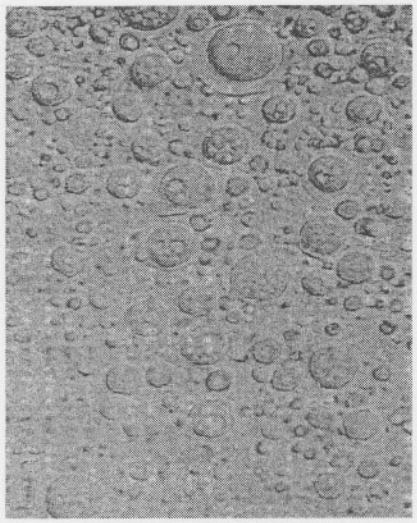

Figure 2-9: Micrograph of a multiple emulsion (Nielsen & Gohla, 1998:76).

2.7.4.4 Summary

Multiple emulsions have great promise. Firstly, because of the potential of these systems to protect the drug in the internal phase of the emulsion and, secondly because of the possibility to control the release rate of the drug (Piemi *et al.* 1998:214). They are interesting for several technological and industrial uses, such as separation techniques for waste-water regeneration, pharmaceutics (antigen inoculation, topical treatment, sustained release in topical treatment, etc.) and metal separation techniques. In cosmetics, sensorial features as well as moisturising and release patterns are of particular interest. Their release patterns give rise to the assumption that these emulsions can be used as product base for sustained topical release products (Nielsen & Gohla, 1998:77).

2.8 CONCLUSION

A very crucial function of the skin, most of the time overlooked, is to prevent the body from loosing water into the environment and to block the entry of exogenous agents. An effective barrier to drug permeation is therefore formed by the skin and in particular by the stratum corneum (Asbill & Michniak, 2000:37).

An advantage of percutaneous absorption is that the "first-pass" effect is avoided. The major problem accociated with treating dermal conditions is the excellent barrier function of the skin. This is a result of the tortuous route the permeant has to take through the structured lipids of the intercellular channels.

Delivery vehicles are used to increase transdermal delivery of a wide range of drugs. It is often necessary to incorporate a drug in such a vehicle to improve the percutaneous absorption. The motivation for this investigative study of emulsions is to determine

whether the absorption of acyclovir can be improved transdermally with the use of a delivery vehicle.

In this study, formulations with different percentages of surfactant was used for the transdermal delivery of the model drug, acyclovir. This formulation is based on that of microemulsions. Microemulsions are of pharmaceutical interest because of their considerable potential to act as drug delivery systems by incorporating drug molecules. The viscosity was adjusted by adding a gelating agent, carrageenan, which was responsible for better application transdermally. In the following chapter the permeability of the stratum corneum was investigated by using Franz diffusion cells *in vitro*.

CHAPTER 3

The Effect of a Surfactant, Brij 97, and Viscosity Enhancer, Carrageenan, on the Transdermal Delivery of Acyclovir

3.1 INTRODUCTION

The layer that provides the most significant barrier from the outside, the stratum corneum, prevents most external solutes from permeating the body. A few approaches to overcome this excellent barrier function, to improve the impermeability of the skin, to lesson the biological variablility and increase the amount of drugs for transdermal drug delivery, has been investigated. One of the approaches includes the use of penetration enhancers (Suhonen *et al.* 1999:149).

κ-Carrageenan is widely used as a thickening, gelling and stabilizing agent in the food industry (Uruakpa & Arntfield, 2004:420). Valenta & Schultz (2004) concluded that microemulsions in combination with suitable polymers such as carrageenan offers better application on large skin areas and that high permeation rates for incorporated drugs can be predicted. In this study the objective was to confirm the formulation of a microemulsion and to determine the influence of carrageenan on the transdermal delivery of acyclovir.

3.2 MATERIALS

Acyclovir and Brij 97 (poly-oxy-ethylene-10-oleyl-ether) was obtained from Sigma-Aldrich Co. (Johannesburg, SA). Soya oil from *Glycine max* was purchased from Fluka (Steinheim, Switzerland). HPLC analytical grade methanol (BDH Laboratory Supplies, Poole, England) were used in the experiments. Sodium chloride (NaCl), sodium dihydrogen orthophosphate dihydrate ($NaH_2PO_4.2H_2O$), disodium hydrogen orthophosphate dihydrate ($Na_2HPO_4.2H_2O$), dipotassium hydrogen orthophosphate (K_2HPO_4), lead citrate ($C_{12}H_{10}O_{14}Pb_3$) and uranyl acetate ($(CH_3COO)_2U$) were obtained from Merck Laboratory Supplies (Midrand, South-Africa). Orthophosphoric acid 85% AR from Associated Chemical Enterprises (ACE) (Pty) Ltd (Southdale, England) was used. Nile red was used, obtained from Molecular probes (California). Double distilled deionised water was prepared by a Milli-Q 50 water purification system (Millipore, Milford, USA). Throughout the study HPLC grade water was used.

3.3 ANALYTICAL METHODS

3.3.1 High-Pressure Liquid Chromatography (HPLC) Method for the Analysis of Acyclovir

3.3.1.1 Apparatus

The HPLC system used for the analysis of acyclovir was an Agilent® 1100 Series equipped with a variable wavelength UV detector, pump, injection device, and Chemstation Rev. A.06.02 data acquisition and analysis software or equivalent. A Luna 5 µ C18 (2) 100A column (Phenomenex, Germany) was used, together with a HPLC Security Guard Cartridge System™ to prolong column life.

3.3.1.2 Chromatographic conditions

All analyses were performed at a flow rate of 1 ml/min. The mobile phase consisted of a mixture of 10% methanol, 90% dipotassium hydrogen orthophosphate and water; the pH (3.5) was adjusted with orthophosphoric acid. The column temperature was held constant at 25°C and the effluent was monitored at a wavelength of 254 nm for acyclovir. The retention time for acyclovir was 6.0 – 7.5 minutes. The injection volume for all the samples was 100 µg/ml.

3.3.1.3 Column maintenance

After each analysis, HPLC water was passed through the column for about 20 minutes at a flow rate of 1 ml/min. The column was then rinsed with HPLC water/methanol, 70/30, for 20 minutes at a flow rate of 1 ml/min in which solution it was also stored.

3.3.1.4 Preparation of standard solutions

Ten milligrams of acyclovir was transferred into a 100 ml volumetric flask and dissolved in water to produce a 100 µg/ml stock solution. Standard solutions with concentrations of 5; 30; 50; 300; 500; 2000; 5000 ng/ml acyclovir were prepared from the stock solution. These dilutions were used for the validation procedure.

3.3.2 Validation of HPLC Procedure

3.3.2.1 Linearity

The assay of linearity for acyclovir was determined by performing linear regression analysis on the plot of the peak area versus concentration. The standard solutions were prepared as described in § 3.3.1.4, to obtain concentrations ranging from 5 to 5000 ng/ml. The regression (as shown in figure 3-1) value was 1.000, the slope was 0.3223 and the Y-intercept was 0.1281.

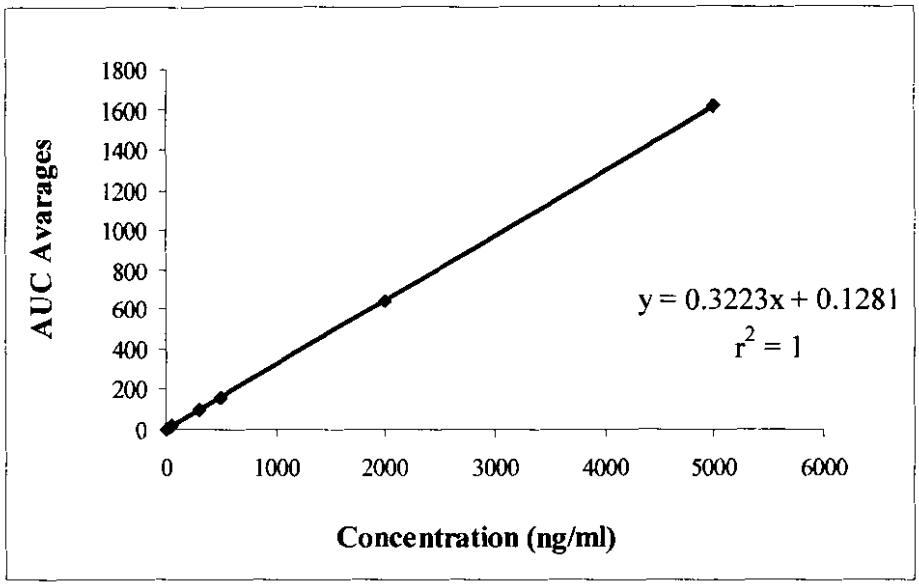

Figure 3-1: Linear regression curve of acyclovir standards.

3.3.2.2 Precision

The method's precision was investigated in terms of interday (reproducibility) and intraday (repeatability) variations.

- **Interday precision**

The interday precision was determined by performing HPLC analysis of seven known concentrations on three consecutive days. Results are shown in table 3-1.

Table 3-1: Interday precision parameters of acyclovir standards.

Standards (ng/ml)	Day 1	Day 2	Day 3	AUC Mean	SD	%RSD
5	7.7	2.3	2.2	4.1	3.2	78.6
30	11.2	10.1	10.2	10.5	0.6	5.7
50	16.6	16.6	16.5	16.6	0.1	0.5
300	97.4	96.5	96.9	96.9	0.5	0.5
500	154.6	159.8	160.1	158.2	3.1	2.0
2000	627.5	639.0	643.2	636.6	8.1	1.3
5000	1609.0	1568.5	1629.7	1602.4	31.1	1.9

- **Intraday precision**

The intraday precision was determined by performing HPLC analysis of seven known concentrations at three times during the same day. Results can be seen in table 3-2.

Table 3-2: Intraday precision parameters of acyclovir standards.

Concentration (ng/ml)	AUC 1	AUC 2	AUC 3	AUC Mean	SD	%RSD
5	2.2	2.0	2.0	2.1	0.1	7.0
30	10.2	10.1	9.6	9.9	0.3	3.0
50	16.4	16.3	16.6	16.4	0.2	1.0
300	96.1	96.0	96.1	96.1	0.04	0.04
500	163.6	164.9	163.8	164.1	0.7	0.4
2000	658.4	661.2	659.6	659.7	1.4	0.2
5000	1665.4	1681.1	1695.2	1680.6	14.9	0.9

3.3.2.3 Selectivity

Selectivity is the ability of the analytical method to detect and analyze a component in the presence of other components. The compounds, mobile phase and phosphate buffer solution (PBS) pH 7.4 were separately analyzed by HPLC. This method was selective since there was no interfering peaks at the same retention time (6 - 7.5 minutes) as acyclovir.

3.3.2.4 System repeatability

In an evaluation of the repeatability of the peak area, samples with known concentrations (5000 ng/ml) of acyclovir were injected six times on the same day and under the same conditions. The variation in the response (%RSD) of the repeatability of the peak area was found to be 1.35%. This was well within acceptable criteria.

3.4 METHOD FOR PREPARATION OF THE DELIVERY VEHICLE

Emulsions were prepared by individually mixing the required weights of oil with surfactant and heating up to 70-80°C. This mixture was poured into the hot water (70-80°C) and stirred. Acyclovir was dissolved in the applicable amount of water of the formulation (see table 3-3 for different compositions of the emulsions). Initially a saturated solution was made, but crystals formed. It was then decided to decrease the amount of acyclovir to 1 mg/ml in order to prevent the formation of crystals. Carrageenan was mixed into the above mixture at room temperature (Valenta and Schultz, 2004:258).

Table 3-3: Composition of the various emulsions.

	4%	8%	15%	25%
Brij 97	0.4 g	0.78 g	1.5 g	2.5 g
Water	8.72 g	8.34 g	7.62 g	6.62 g
Soybean oil	0.78 g	0.78 g	0.78 g	0.78 g

3.5 EXPERIMENTAL METHODS

3.5.1 pH Measurement

The pH of the controls (emulsions without acyclovir) and emulsions (with acyclovir) with and without carrageenan was measured with a Metrohm Autotitrator 785 DMP Titrino pH meter. Three pH readings were taken for each sample and the average was used.

3.5.2 Zeta Potential

The zeta potential was determined with the Malvern zetasizer (Malvern Instruments). Diluted samples of 20 µl/ 10 ml were prepared. The zetasizer was rinsed with 5 ml ethanol and then 20 ml of Milli Q water before the diluted sample (± 8 ml) was injected. Ten readings were taken for each sample and the average was used.

3.5.3 Particle Size

Particle size was measured with a Mastersizer (Malvern Instruments). For each sample determined two measurements were taken with a 20 second delay in between. The setup presentation was: 4NHD = 1.4564, 0.1000 in 1.3300; with a refraction index of olive oil in water. The Mastersizer was rinsed with Milli Q water for 30 seconds and set for alignment of the laser. The background was first measured and then the sample.

3.5.4 Confocal Laser Scanning Microscopy

- **Nile Red labelling**

2 µl Nile Red (diluted in DMSO to a concentration of 10 µg/ml) was added to 100 µl of the sample. Five minutes was allowed for optimal labelling of the sample after which 20 µl of the labelled sample was applied to a microscope slide. A cover slip was placed on the microscope slide and positive pressure applied to remove any excess sample. The microscope slide was then inverted (cover slip facing the objective) and placed on the viewing stage of an inverted microscope (see below).

- **Confocal microscopy**

Digital images of the Nile red labelled samples were captured by a Nikon PCM 2000 confocal microscope. The PCM 2000 was connected to a Nikon (TE300) inverted

microscope equipped with a 60x/1.40 Apo Planar oil objective. A 10% neutral density filter was used for viewing of the sample with the least required excitation light. The system's helium-neon ion laser was used to excite Nile Red at 505 nm and emission waves were obtained at 565 nm. A pinhole size of 1/4 airy units was used to minimize photobleaching.

3.5.5 Electron Microscopy

After diffusion studies were completed a few pieces of skin were collected and fixated in Todd's solution (Todd, 1986:87). Acetone was used to dehydrate the skin, which was then imbedded in Spurr's resin. After 24 hours, sections of 100 nm thickness were made by using an Ultra microtome (Reichert-Jung, Ultracut E) and the sample was stained with 2% uranyl acetate and lead citrate. The sample was then examined with the Philips CM10 transmission electron microscope.

3.5.6 Membrane Diffusion Release Studies

The *in vitro* release from the emulsion was measured with the release unit illustrated below.

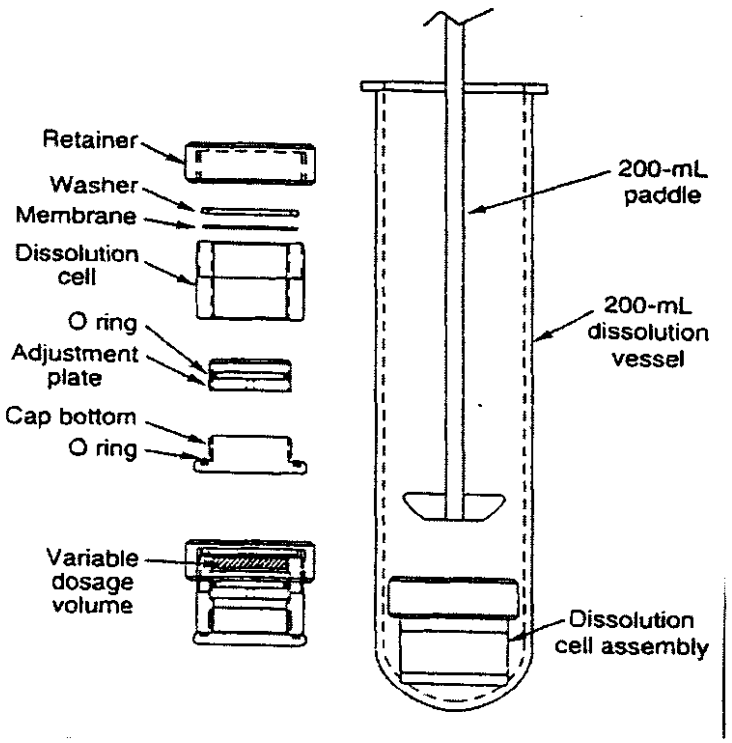

Figure 3-2: Release unit in the dissolusion studies (Fares & Zats, 1995:54).

The release unit fits directly onto the Vankel VK 700 six station dissolution apparatus. The release experiment was carried out in six fold for each preparation. The reservoir of

the dissolution cell (enhancer cell) was filled with the emulsion and covered with the membrane (cellulose acetate; 0.45 μm pore size), taking care to exclude air bubbles between the emulsion and the membrane. The cell was capped and placed in the dissolution vessel containing the receptor medium phosphate buffer solution (PBS); 190 ml). The paddle speed was 150 rpm and the temperature 32°C. Samples of 250 μl were withdrawn from the receptor at 30, 60, 120, 180, 240, 300 and 360 minutes. The samples were analysed for acyclovir content by HPLC (Fares and Zatz, 1995:54) (See § 3.3.1).

3.5.7 *In Vitro* Transdermal Diffusion Studies

3.5.7.1 Skin preparation

In order to minimize variability only white, female abdominal skin was used for the permeation studies (obtained after cosmetic procedures). A scalpel was used to separate the skin from the fat layer; subsequently the stratum corneum was removed by means of immersion in 60°C HPLC water for 60 seconds. The stratum corneum was gently teased away from the skin with forceps. Special care was taken that the integrity of the stratum corneum was not ruptured, as this would compromise the validity of the results. The stratum corneum was placed in a bath filled with HPLC water and carefully set on Whatman® filter paper, left to air dry and was wrapped in aluminium foil. The foil containing the stratum corneum was stored in a freezer at -20°C and was used within two months after being prepared. Prior to use, the stratum corneum was thawed and examined for any defects, before mounting them in the Franz diffusion cells (Myburgh, 2003:39).

3.5.7.2 Skin permeation studies

Vertical Franz diffusion cells (see Figure 3-3) were used in the permeation studies. The skin layer (cut into circles with a diameter of ± 10 mm) was placed on the lower half of the Franz cell with the stratum corneum facing upwards. The maximum capacity of the receptor compartment is 2.3 ml with a diffusion area of 1.075 cm^2.

The receptor and donor compartment was filled with phosphate buffered solution (PBS) at physiological pH 7.4, consisting of 6.69 g disodium orthophosphate dihydrate ($Na_2PO_4.2H_2O$), 4.4 g sodium chloride (NaCl) and 2.1 g sodium dihydrogen orthophosphate dihydrate ($NaH_2PO_4.2H_2O$) in water to a final volume of 1000 ml. Franz cells containing the buffer were equilibrated for one hour in a water bath at 37°C. After half an hour the integrity of each cell was measured, the PBS in the donor compartment were discarded before the saturated solution was added to the donor compartments and allowed to equilibrate for an hour. Only the receptor compartment was submerged in the water and was continuously stirred with a small magnetic bar at 500 rpm. After one hour, 1 ml of freshly prepared emulsion was added to each donor compartment, which was immediately covered with Parafilm® in order to prevent evaporation. An excess amount of solute was always present in the donor compartment. The entire receptor volumes were withdrawn every 2, 6, 8, 10, 12, 14, 16, 18, 20 and 24 hours and replaced with 37°C fresh PBS buffer. Withdrawn samples were assayed by HPLC to determine the drug

concentration. The experiment was carried out in six fold for each preparation (Myburgh, 2003:39).

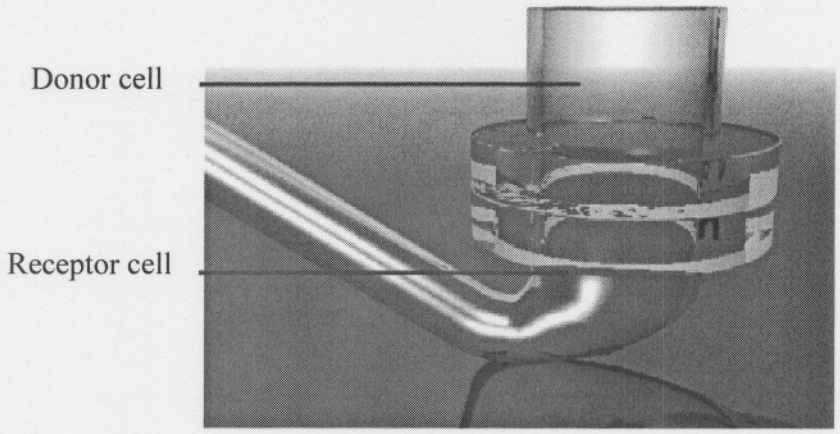

Figure 3-3: Vertical Franz diffusion cell.

3.5.8 Aqueous Solubility Determination

The aqueous solubility of acyclovir was determined by equilibrating an excess amount of acyclovir in HPLC water at 37°C. A water bath (Grant Instruments, UK) was used to keep the temperature constant. The solution was vigorously stirred using magnetic stirring bars. Care was taken that there was always an excess amount of drug to keep the concentration at a constant level. The solution was left stirring for 24 h after which the saturated solubility of acyclovir was determined by filtering the solution through a 0,45 µm filter (Glassfibre Prefilter, Sartorius AG) and assaying by HPLC. The experiment was carried out in three fold (Van der Westhuizen, 2002:39).

3.6 DATA ANALYSIS

3.6.1 Measurement of the Drug Release Rate

The amount of acyclovir released from the drug delivery vehicle (ng/cm^2) as a function of the square root time (minutes) was calculated by multiplying the concentration of the drug (ng/ml) in the receptor phase with the amount of the receptor phase used (190 ml) and dividing it by the membrane area (3.98 cm^2).

3.6.2 Drug Permeation

The steady state flux (J) of a diffusant across the stratum corneum is given by Fick's 1st law of diffusion:

$$J = A \cdot k_p \cdot c_v \qquad \text{(Equation 3-1)}$$

Where

- A is the cross sectional area
- k_p is the permeability coefficient
- c_v is the concentration of the drug in the vehicle (Hadgraft, 1996:166).

"The flux is the amount of drug that permeates a given area of the skin in a defined time-period. Flux is typically expressed as µg per cm^2 per h. Q is the cumulative amount of drug per unit area of skin that has passed through the skin after 24 hours, and is expressed as µg per cm^2." (Asbill & Michniak, 2000:37.)

The cumulative amount of acyclovir that penetrated through the epidermis (n = 6) per unit area (ng/cm^2) was calculated by multiplying the concentration (ng/ml) in the receptor phase with the amount of receptor phase used (1.9 - 2.4 ml) and dividing it by the area of epidermis (1.075 cm^2).

The cumulative amount of drug, Q, was then plotted as a function of time t (h). The flux (J_{ss}, ng/cm^2/h) was obtained from the slope of the linear part of the curve and was calculated by the following equation (Myburgh, 2003:42):

$$J_{ss} = \frac{\Delta Q}{\Delta t} \qquad \text{(Equation 3-2)}$$

"A simple method of expressing the final data collected from experiments on enhancers is to use enhancement ratios (ER). The enhancement ratio for a given enhancer is calculated by dividing the experimental parameter, such as skin-drug content obtained, with the enhancer by the same parameter from the control experiment." (Asbill & Michniak, 2000:37.)

$$ER = \frac{Flux\ of\ ACV\ with\ enhancer}{Flux\ of\ ACV\ without\ enhancer\ (control)} \qquad \text{(Equation 3-3)}$$

3.7 RESULTS

3.7.1 pH Measurement

The pH has a definite influence on transdermal delivery because only the unionized form of the drug is able to cross the lipoidal membranes in significant amounts (Martin, 1993:540). The dissociation coefficients of acyclovir are 2.27 and 9.25 (Dollery, 1999:A39). pH values can be seen in table 3-4.

Table 3-4: The average pH values of the emulsions before and after the addition of acyclovir (ACV). Mean ± SD, (n = 3).

	Concentration Brij 97			
	4%	**8%**	**15%**	**25%**
Without Carrageenan				
Emulsion without ACV	6.22 ± 0.02	5.59 ± 0.03	5.05 ± 0.02	5.17 ± 0.03
Emulsion with ACV	5.8 ± 0.05	5.55 ± 0.04	5.20 ± 0.05	5.20 ± 0.03
With Carrageenan				
Emulsion without ACV	6.64 ± 0.02	6.45 ± 0.03	6.55 ± 0.006	5.93 ± 0.05
Emulsion with ACV	7.35 ± 0.03	6.85 ± 0.03	6.40 ± 0.05	6.08 ± 0.05

3.7.2 Zeta Potential

The zeta potential can be defined as the difference in potential between the surface of the tightly bound layer (shear plane) and the electroneutral region of the solution (Martin, 1993:387). The zeta potential was measured and the results are shown in table 3-5.

Table 3-5: The zeta potential of emulsions without and with carrageenan (n = 10).

% Brij 97	Zeta potential (mV)	Standard error
Without Carrageenan		
4%	-34.1	2.1
8%	-33.1	0.6
15%	-20.1	0.9
25%	-18	7
With Carrageenan		
4%	-46.7	2.5
8%	-25.6	0.7
15%	-22.2	2.7
25%	-24.9	7.7

3.7.3 Particle Size

Any collection of particles is usually polydisperse. Therefore it is necessary to know not only the size of a certain particle, but also how many particles of the same size exist in the sample. Thus, we need an estimate of the size range present and the number or weight fraction of each particle size. This is the particle size distribution, and from it maybe calculated an average particle size for the sample (Martin, 1993:425). Figure 3-4 to 3-7 reflects the mean volume and the median volume distribution with and without acyclovir.

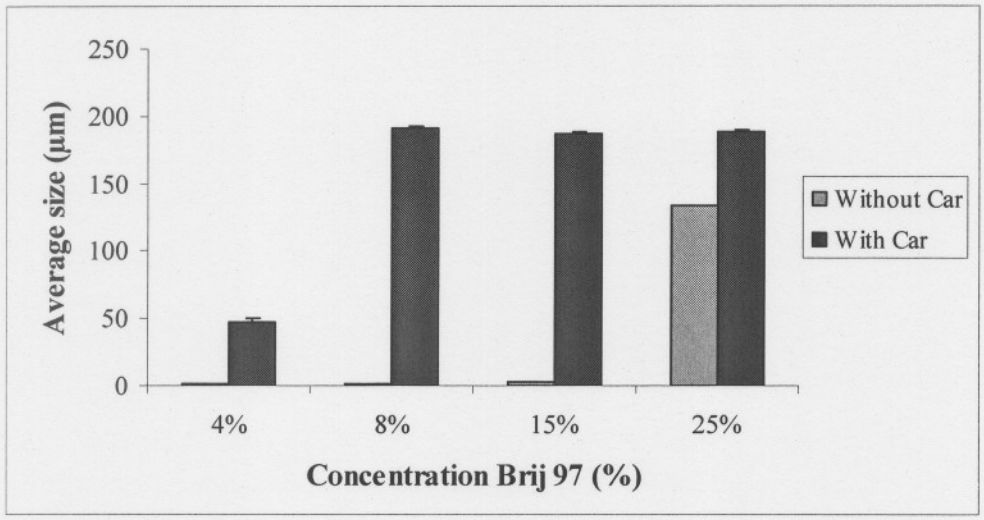

Figure 3-4: The average of the mean volume distribution (D (4,3)) of the emulsions without acyclovir, with and without carrageenan (Car). Mean ± SD, n = 2.

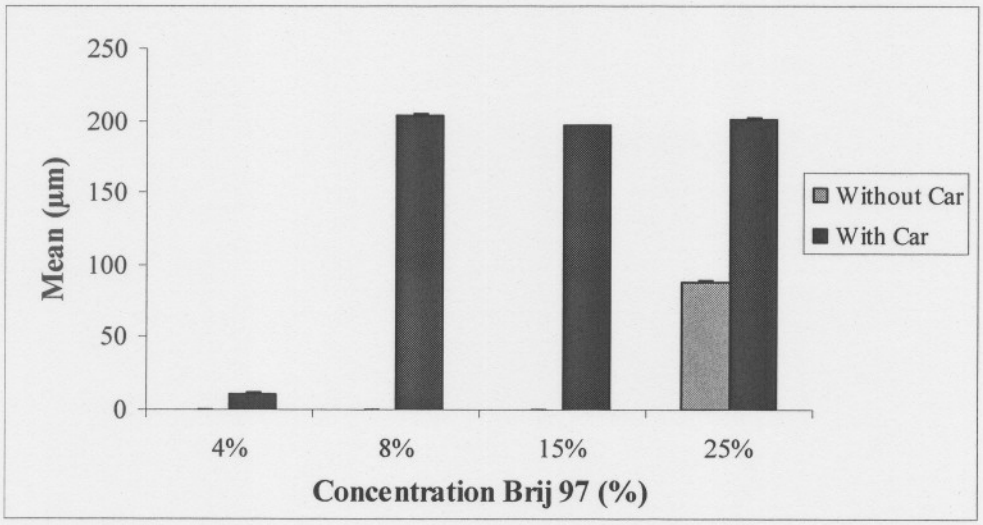

Figure 3-5: The average of the median volume distribution (D (V 0,5)) of the emulsions without acyclovir, with and without carrageenan. Mean ± SD, n = 2.

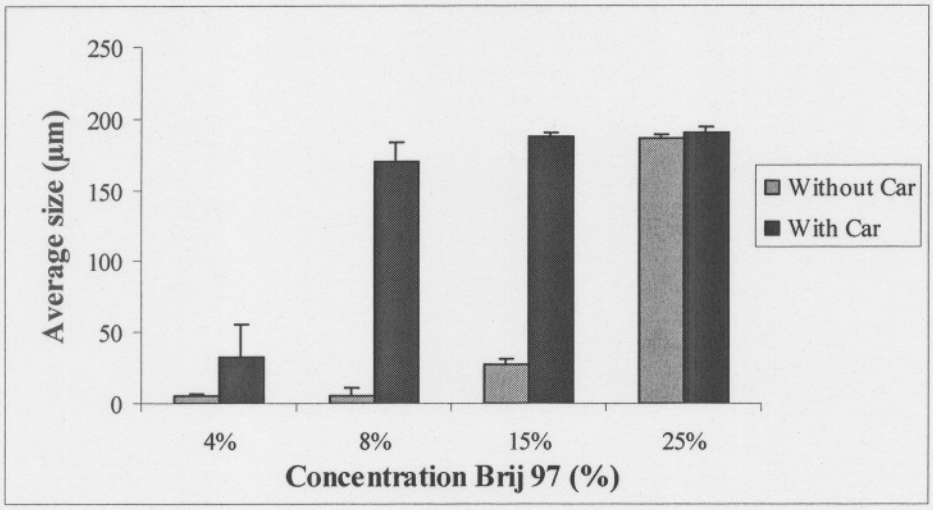

Figure 3-6: The average of the mean volume distribution (D (4,3)) of the emulsions with acyclovir, with and without carrageenan. Mean ± SD, n = 2.

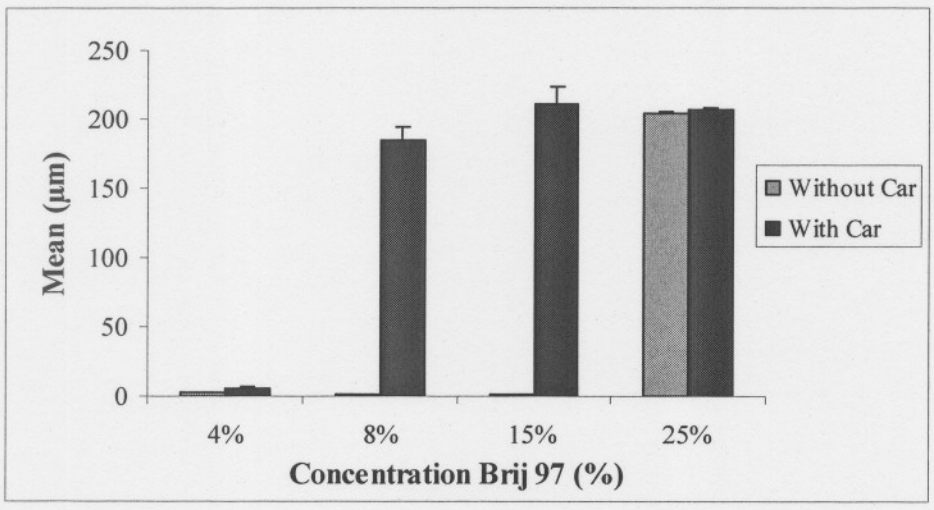

Figure 3-7: The average of the median volume distribution (D (V 0,5)) of the emulsions with acyclovir, with and without carrageenan. Mean ± SD, (n = 2).

3.7.4 Confocal Laser Scanning Microscopy (CLSM)

The confocal microscope was used to verify the presence of vesicles in the different emulsions. The following results (as shown in figure 3-8) show that only concentrations 4% and 8% had vesicles whereas the 15% and 25% showed only the background with no vesicles. In the 4% and 8% with carrageenan the agglomeration caused by carrageenan can clearly be seen. The 15% e) and f) have some resemblance of particulate matter, probably indicating a matrix-like internal structure in the emulsion. This may relate to the various microemulsion structures described in figure 2-7.

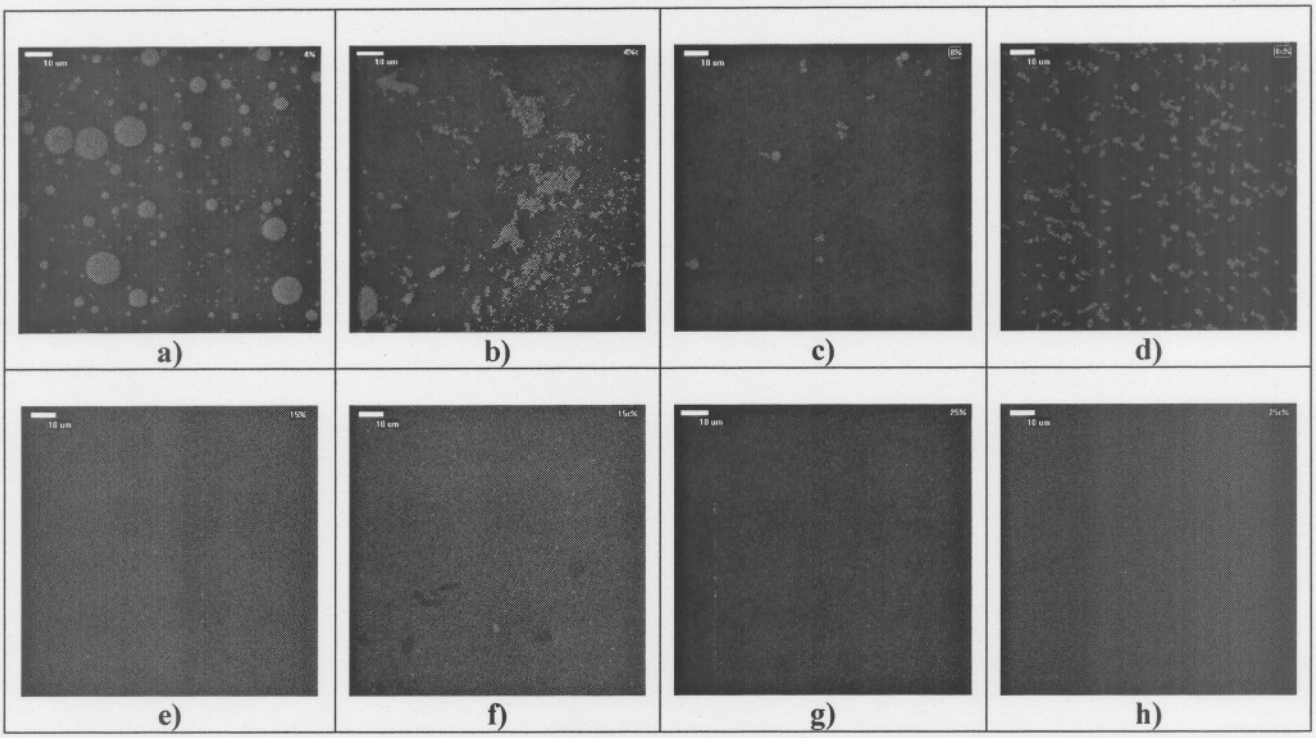

Figure 3-8: Confocal micrograph of the emulsions with different percentages Brij 97 a) 4% b) 4% with carrageenan c) 8% d) 8% with carrageenan e) 15% f) 15% with carrageenan g) 25% and h) 25% with carrageenan.

3.7.5 Electron Microscopy

Electron microscopy micrographs of the stratum corneum were inspected for changes in the stratum corneum after the application of the different concentrations of surfactant. A representative micrograph can be seen in figure 3-9. The application of emulsions with concentrations of 4-25% Brij 97 did not effect the histology of the stratum corneum.

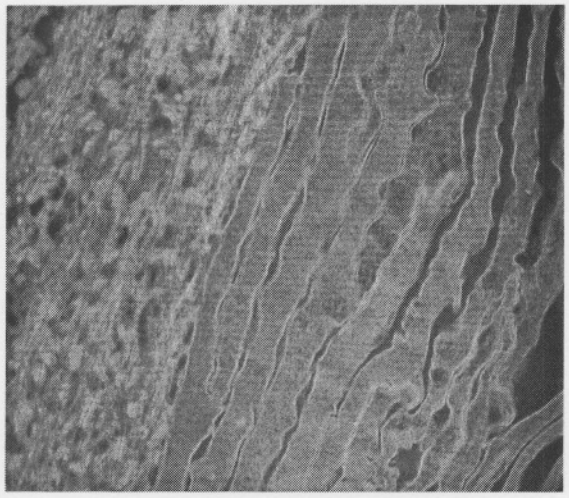

Figure 3-9: Electron microscopy photo of the stratum corneum after the application of the emulsion, 4% Brij 97 with carrageenan. Epidermis. The strata spinosum, granulosum and corneum from left to right.

3.7.6 Membrane Diffusion Release Studies

The amount of acyclovir released were determined as described in § 3.6.1. Replicate membrane diffusion release studies were done and the average release was calculated for each analysis point. The results can be seen in figures 3-10 and 3-11. Acyclovir released in micrograms per cm² membrane versus time was plotted and can be seen in figure 3-10. The release rate decreases with time which is to be expected due to depletion.

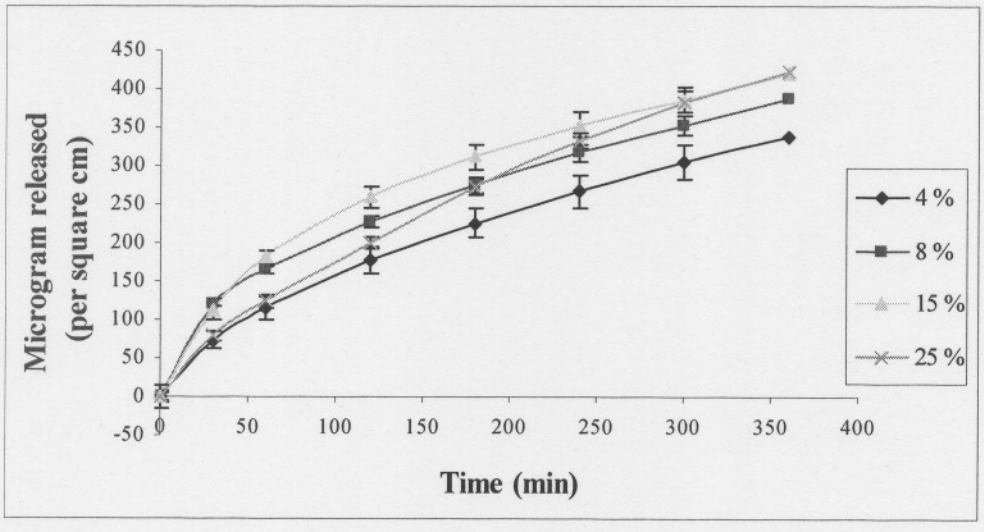

Figure 3-10: The average amount of acyclovir released (µg/cm²) from the four emulsions containing different percentages Brij 97, with carrageenan, as the function of time (min). Mean ± SD (n = 6).

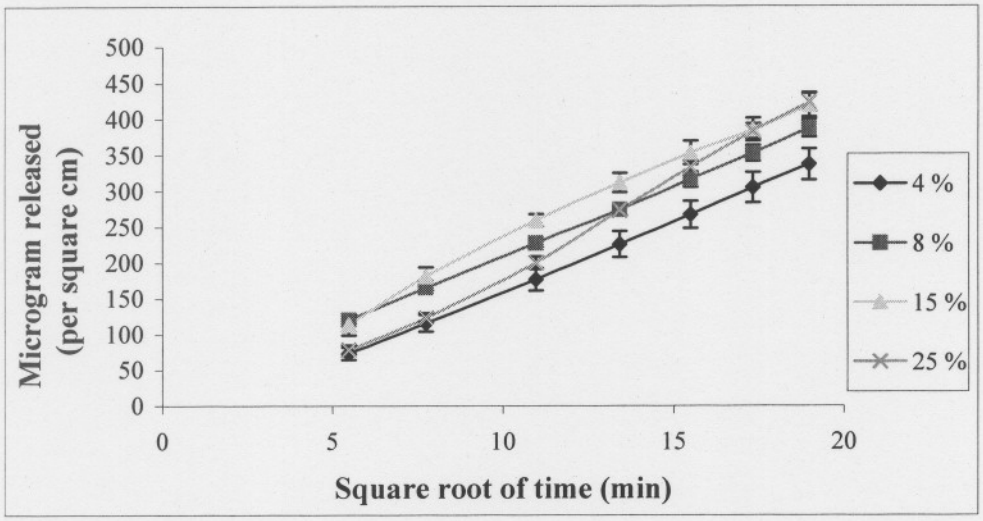

Figure 3-11: The amount of acyclovir released (μg/cm²) from the four emulsions containing different percentages Brij 97, with carrageenan, as a function of the square root of time (min). Mean ± SD (n = 6).

A straight line would be expected when a plot of micrograms acyclovir released per cm² membrane versus the square root of time (minutes). This is indeed the case in figure 3-11 with correlation coefficient as presented in table 3-6 for each emulsion. The slope of each line represents per definition the release rate (or flux) (table 3-6).

Table 3-6: The correlation coefficient and release rate of the emulsions with carrageenan.

Concentration Brij 97	Correlation coefficient	Release Rate
4%	0.9998	19.577
8%	0.9998	19.674
15%	0.9938	22.283
25%	0.9971	26.209

3.7.7 *In Vitro* Transdermal Diffusion Studies

In vitro transdermal diffusion studies were carried out as described in § 3.5.7.2. The drug containing delivery vehicle with its ingredients namely Brij 97 and carrageenan was investigated to determine if it had any effect on the permeation of acyclovir through the skin. The *in vitro* transdermal absorption profiles are shown in table 3-7.

Table 3-7: The effect of Brij 97 and carrageenan on the diffusion of acyclovir.

Acyclovir	Flux (ng/cm²/h), n = 6	ER (Flux)
Control	8.65 ± 2.04	1.00
4% Brij 97	11.14 ± 7.28	1.29
4% Brij 97 + Carrageenan	1.15 ± 0.76	0.13
8% Brij 97	4.34 ± 3.28	0.50
8% Brij 97 + Carrageenan	0.74 ± 0.15	0.09
15% Brij 97	382.06 ± 79.18	44.12
15% Brij 97 + Carrageenan	4.97 ± 2.69	0.57
25% Brij 97	556.34 ± 190.84	64.24
25% Brij 97 + Carrageenan	1.98 ± 0.49	0.23

In figure 3-12 the mean transdermal flux of the control and four emulsions with acyclovir are shown. The 0% represents the control which consisted of acyclovir in water.

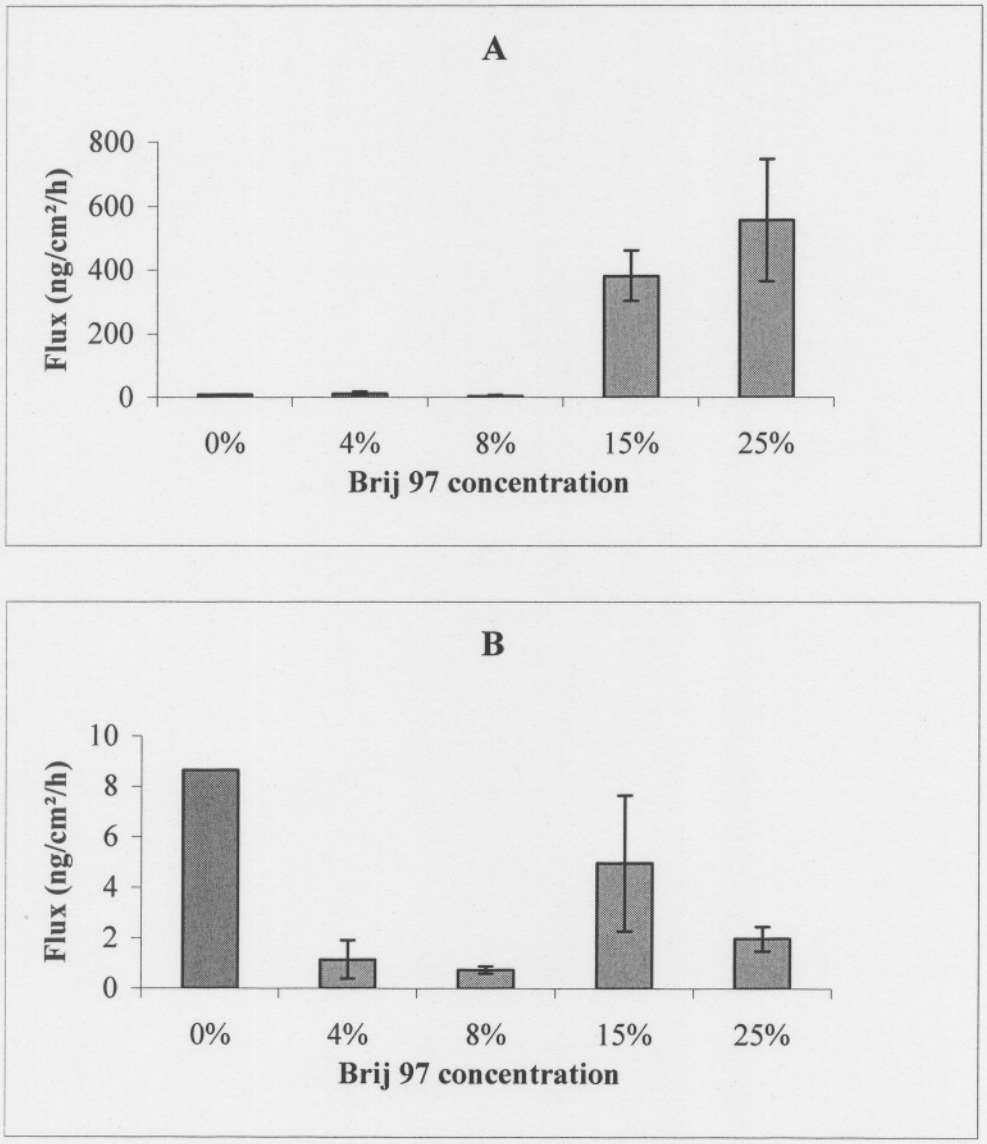

Figure 3-12: The mean transdermal flux and ± SD of the control (0%) and four emulsions A) without carrageenan and B) with carrageenan (n = 6).

3.8 STATISTICAL ANALYSIS

One-way analysis of variance (ANOVA) was performed to compare the mean of the flux values (ng/cm²/h) of the control to the different emulsions. A significant group effect was

detected ($p < 0,0001$). A Dunnett multiple comparison test was done which resulted in a significant difference between 15% and the control and 25% and the control ($p < 0,0001$).

This was followed by the Tukey multiple comparison test where variances and sample sizes are assumed to be unequal. Significant differences ($p < 0,05$) were found between all possible pairs of the four emulsion means except that of 4% and 8%. The Tukey test was also performed to compare all the possible pairs of four emulsion means with carrageenan resulting in significant differences ($p < 0,05$) for 4% vs. 15%, 8% vs. 15% and 15% vs. 25%.

Each of the emulsions' mean of the flux values without carrageenan was compared to that with carrageenan by using a Student t-test adjusted for unequal variances. All these comparisons were significant on a 5% level of significance and had the following p-values:

Table 3-8: Results of a Student t-test.

Concentration of emulsion	t value(adjusted df)	p values (2 sided)
4% 4% + Carrageenan	t (5.095) = 3,348	p = 0,0198
8% 8% + Carrageenan	t (5.020) = 2,684	p = 0,043
15% 15% + Carrageenan	t (5.012) = 11,658	p = 0,0001
25% 25% + Carrageenan	t (5.001) = 7,115	p = 0,0008

3.9 DISCUSSION

3.9.1 pH Measurement

The pH values of emulsions without carrageenan were lower when compared to the emulsions with carrageenan (see figure 3-13 and 3-14).

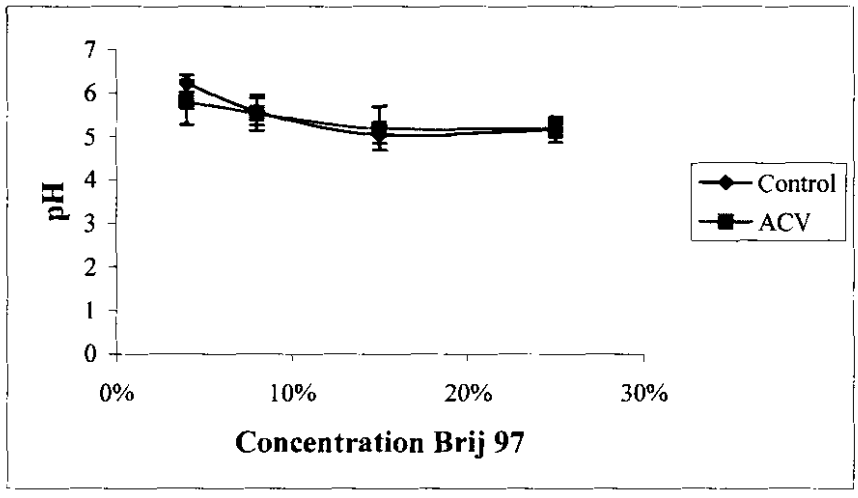

Figure 3-13: The pH values of the control (emulsion without acyclovir) and emulsions without carrageenan in correlation with the concentration of the surfactant. Mean ± SD (n = 3).

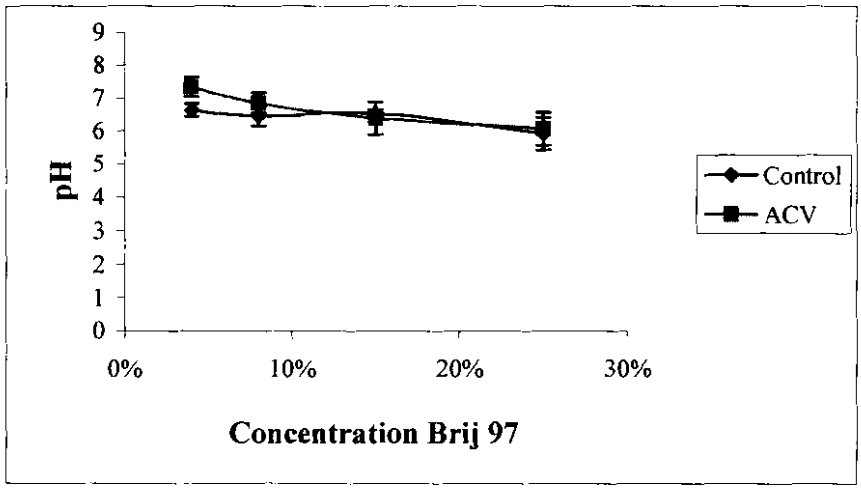

Figure 3-14: The pH values of the control (emulsion without acyclovir) and emulsions with carrageenan in correlation with the concentration of the surfactant. Mean ± SD (n = 3).

The pH was stable for all emulsions. As the surfactant concentration increased the pH progressively lowers. The stratum corneum's non-polar nature has a high resistance to diffusion through it (Zats, 1993:28). The more unionized the drug is the more lipophilic and the greater the permeation through the stratum corneum.

3.9.2 Zeta Potential

Zeta potential has practical application in the stability of dispersions since this potential governs the degree of repulsion between adjacent, similarly charged, dispersed particles. If the zeta potential is reduced below a certain value (which depends on the particular system being used), the attractive forces exceed the repulsive forces and the particles fuse (Martin, 1993:387).

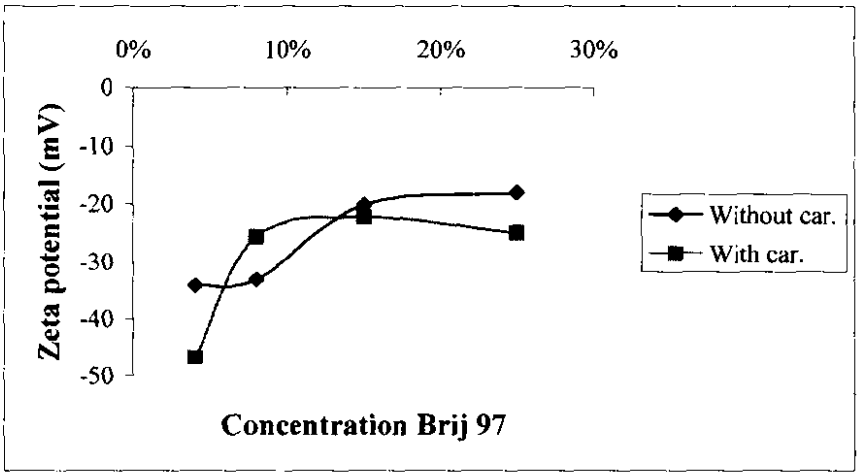

Figure 3-15: The relationship between zeta potential and the concentration of the surfactant.

The stability of an emulsion has long been known to require zeta potentials, of either sign (+ or -), to be greater than about 30 mV (Kitchener & Mussellwhite, 1968:106). In all emulsions, with and without carrageenan, the increasing of the surfactant concentration had a simultaneously decreasing effect on the zeta potential. This decreasing effect proves that the higher the concentration of the surfactant the lower the stability becomes. At high surfactant levels, carrageenan had a more stabilizing effect than the emulsions without carrageenan (see figure 3-15).

3.9.3 Particle Size

The rate of creaming in an emulsion will slow down as the globules of the dispersed phase get smaller. Creaming in an emulsion is the separation of the emulsion into two regions. The viscosity can also be affected by the size of these globules. It has been found that the best emulsions with respect to physical stability and texture exhibit a mean globule diameter of between 0.5 and 2.5 μm (Billany, 2002:353).

Peltola *et al.* (2003) stated that a gel former in a microemulsion will increase its viscosity and further decrease the permeation in the skin. This was confirmed by the addition of carrageenan to the emulsions in this study, where particle size increased and resulted in less surface contact which also had a decreasing effect on transdermal diffusion. The reason for this was believed to be the agglomeration of particles with the addition of

carrageenan as seen in figures 3-4 to 3-7. The increase in particle size was evident at the lowest concentration of Brij 97. The growth in size of the particles stabilized at 15% Brij 97.

This was in contrast to the findings of Valenta & Schultz (2004) who experimentally determined that their formulations, microemulsions with carrageenan, had an increasing effect on the permeation through the skin.

Dennis (1990) confirmed that if the molecular size of the penetrant molecule and viscosity of the membrane increase the diffusion will be reduced.

In the emulsions without the addition of carrageenan the particle size was directly proportional to the concentration of the surfactant.

D (4,3), the average size and D (V 0,5), the mean showed the same tendancy.
A moderately coarse dispersion of uniform-sized particles should have the best stability (Martin, 1993:491). From data collected the following results were obtained which was representative for each preparation with and without acyclovir.

90% particles are more or less smaller than 200 µm,
10% particles are more or less smaller than 1,5 µm and
50% particles are more or less smaller than 11 µm.

Particle size distribution of all the samples was similar to the one presented in figure 3-16. This is an indication that there's not a uniform size distribution in the emulsions and this has a further lowering influence on the stability (as previously mentioned) of the emulsion.

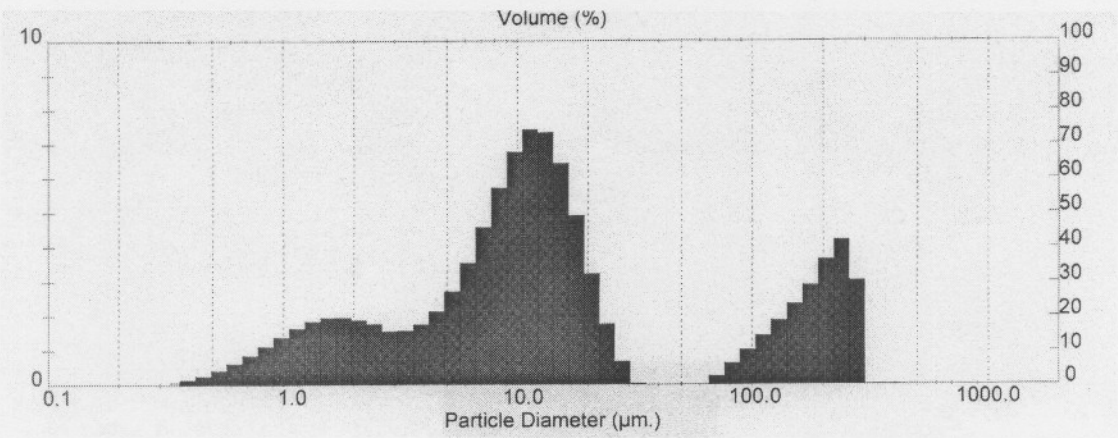

Figure 3-16: The particle size distribution of the 4% Brij 97 with carrageenan emulsion.

3.9.4 Confocal Laser Scanning Microscopy (CLSM)

The different percentages Brij 97 emulsions with and without carrageenan were viewed with the use of confocal laser scanning microscopy.

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 by using the Hamamadzu CCD camera. Dynamic changes could be visualised in real time. The confocal microscope system used was a Nikon PCM 2000 equiped with argon and helium/neon lasers. A 60 × Plan Achromat oil immersion objective with a numerical aperture of 1.4 was used to confirm that the emulsions consisted of vesicles (Saunders *et al.*, 1999:2).

As mentioned in § 3.5.4, Nile red was chosen as the colorant as it associates with lipids and therefore readily stains the soya oil component of the formulations.

In figure 3-8 the micrographs of each emulsion can be seen. In figure 3-8a) and b), without and with carrageenan respectively, the vesicles are clearly visible. In figure a) the vesicles are round and separated from each other. In the figure with carrageenan (b) the clotting of those vesicles can clearly be seen. Similar results obtained for the 8% Brij 97 emulsion in figure 3-8 c) and d). In c) and d) less vesicles are present than in a) and b). For the rest of the figures ((e) to h)) no vesicles are visible.

According to Peltola *et al.* (2003) a microemulsion's droplet size is about 140 nm which make the emulsion a transparent liquid. On the micrographs the particle size wasn't determined precisely but from the scale on the micrograph of the 4% Brij 97 emulsion the largest vesicle size was approximately 10 μm, which is much bigger than 140 nm.

Rhee *et al.* (2001) define a microemulsion as an oil-in-water or water-in-oil emulsion producing a transparent product that has a droplet size smaller than 0.15 μm and does not have the tendency to coalesce. The fact that the formulation with carrageenan clotted together like it did in this study is also a reason for not being a microemulsion.

Valenta and Schultz (2004) concluded in their study that the 25% Brij 97 emulsion, which formulation is the same as in this study, is a microemulsion. In the micrographs in figure 3-8, as discussed previously, it can clearly be seen that figure e) to h) has no vesicles, despite of digital zooming, where it is possible to resolve signs of less than 100 nm vesicles. Figure a) and c), 4% and 8% Brij 97 emulsion respectively, are the closest formulation to a microemulsion because of their vesicles. The 4%, 8% and 15% Brij 97 emulsions are white and milky liquids where as the 25% Brij 97 emulsion is a transparent liquid as described by Valenta and Schultz (2004).

It can thus be said that none of the formulations we prepared were microemulsions and can only be seen as oil-in-water emulsions.

3.9.5 Electron Microscopy

In electron microscopy studies that were carried out the impact of the formulation on the stratum corneum was investigated. Micrographs of the stratum corneum after the application of all the preparations, including a control which consisted of skin lying in PBS for 24 hours, were taken. These micrographs were investigated and found to be similar to each other and to the control.

Because there was no visible damage to the skin (or in this case the stratum corneum), it can thus be said that the surfactant had no degenerating effect on the stratum corneum but rather that the solubility of the drug in the skin may have been increased by the surfactant as stated by Kreilgaard (2002).

3.9.6 Membrane Diffusion Release Studies

Emulsions are systems that contain, in addition to oil components, different amounts of water and perhaps surfactants. In such a complex system different kinds of interactions may occur between the active ingredients and components of the vehicle, which might influence the release and availability of the drug (Refai & Müller-Goymann, 1999:756).

Membrane diffusion release studies were performed on the emulsions with carrageenan to see what the effect of the different components on the release of acyclovir was. The results plotted against the square root of time can be seen in figure 3-11 and this resulted in a straight line as described in § 3.7.6. There was a slow, smooth release of acyclovir through the membrane from the emulsions which was an indication that acyclovir was definitely released from all four systems and that acyclovir was available for absorption when diffusion studies were carried out.

It is interesting to note that the concentration of the surfactant didn't have any notable effect on the release rate for the first three hours. Thereafter the two emulsions with the highest concentration surfactant, namely 15% and 25%, had an increasing effect on the release rate, especially for the 25%. An explanation for this can be that in the vesicles in the 4% and 8% Brij 97 emulsion the drug, acyclovir, is held back a little and in the 15% and 25% Brij 97 emulsion, where no vesicles are seen, the drug is free to be released and is not trapped in a vesicle.

The drug solubility, initial drug concentration, diffusion constant of the drug in the vehicle and other formulation adjuvants play a major role in affecting the drug release rate (Shah *et al.*, 1992:105).

3.9.7 *In Vitro* Transdermal Diffusion Studies

The specific structure of the skin, especially the barrier properties and the physiology, present one of the most important difficulties to be overcome in order to improve the penetration of molecules through this biological membrane (Piemi *et al.*, 1998:207). Because of these barrier properties of the skin, low efficacy of dermatological formulations of acyclovir for recurrent infections in immunocompetent subjects (Volpato *et al.*, 1998: 291) have been a problem for many years.

In a study done by Yamashita *et al.*, (1993) the excellent barrier properties of the stratum corneum was yet again realized. The penetration of acyclovir through the intact skin was very slow due to its high hydrophilicity but after the removal of the stratum corneum the skin permeation of acyclovir was markedly enhanced and the lag time was decreased.

This is just an indication that enhancement of skin permeation of acyclovir may be required for more effective therapy in humans and that penetration enhancers, such as surfactants, may be used (Yamashita *et al.*, 1993:204).

The lipophilic character of the stratum corneum through which drugs must diffuse to ultimately access the systemic circulation is the reason why lipophilic molecules are better accepted by the stratum corneum. This is why the use of a delivery system with components such as surfactants are needed to help carry acyclovir, a hydrophilic drug, through the stratum corneum. 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 surfactant used in this study was Brij 97. Surfactants mainly penetrate the skin in their monomer form according to Kreilgaard (2002). Monomer surfactants act as enhancers either by disrupting the lipid structure of the stratum corneum, facilitating diffusion through the barrier phase, or by increasing the solubility of the drug in the skin (Kreilgaard, 2002:S94). The solubility of the drug in the skin as mentioned in § 3.9.5 was rather true than the lipid structure being disrupted. This can be said because no structural change to the stratum corneum was visible when studying the micrographs given in figure 3-9.

The results obtained after diffusion studies were done (see figure 3-12a) with emulsions with different concentrations of surfactant, Brij 97, without carrageenan were analysed. The increasing concentration of Brij 97 had a simultaneously increasing effect on the permeation of the skin and confirmed Kreilgaards' (2002) results regarding the enhancing effect of surfactants.

The high standard deviation in the 25% Brij 97 emulsion without carrageenan might be because of skin damage. According to the study of Coceani *et al.* (2003) the dermis-epidermis membrane of the older animals are less permeable to acyclovir than that of younger animals. They say that this may probably be due to the ageing. In this study not all ages of the women used were known, which might also have had an influence on the high standard deviation.

The results also relate to Valenta & Schultz's (2004) article where they stated that the 25% Brij 97 emulsion, also used in this study, had the highest amount of the hydrophilic model compound sodium fluorescein permeated compared to their other formulations. They concluded that it was due to the phospholipids from the soybean oil, which also had a positive influence on other drugs (Valenta & Schultz, 2004:264).

The explanation for the low permeation of the 4% and 8% Brij 97 emulsions cannot be due to the absence of soybean oil because they also had soybean oil. The greater success of the permeation of 15% and 25% Brij 97 emulsions may be due to the fact that they did not possess any vesicles (see § 3.1.8.4) and that the drug was freely available for diffusion. The 4% and 8% Brij 97 emulsions which actually had vesicles, held on tightly

to them and had a decreasing effect on the penetration of the skin. Some of the particles were rather large (see § 3.9.3) and lowered the contact surface which also played an important role on diffusion.

Peltola *et al.* (2003) found that an increase of viscosity further decreased the penetration of the skin. In figure 3-12b this can clearly be seen. The carrageenan increases the viscosity which makes the vesicles clot together. This may be the reason why the diffusion of 15% and 25% Brij 97 emulsion were higher than that of 4% and 8% Brij 97 emulsions, as they had no vesicles according to the micrograph images.

Špiclin *et al.* (2003) also added thickening agents to their microemulsions to increase the viscosity. According to them the thickening agent changes the appearance of the system, usually improves stability and influences the drug release. In this study figure 3-12b clearly shows the negative influence that carrageenan, the thickening agent, had on the release of acyclovir.

The pH of a vehicle is a vital factor in the lipophylicity and hydrophilicity of a drug. In a study done by Volpato *et al.* (1998) they concluded that at a pH of 7.4, 98% of acyclovir is present in the unionized form and thus is more lipophilic. This just confirms why the diffusion of the emulsions with carrageenan is as low as can be seen in figure 3-12b. The emulsions' pH can be seen in table 3-4. All four concentrations with carrageenan is near to a pH of 7.4, which is an indication that acyclovir is more lipophilic in the vehicle. The result of this is that the drug will be situated in the vesicles, which consist of soybean oil. Because of the added carrageenan the vesicles are bigger and flow together This results in a smaller contact surface between the skin and the vesicles, which leads to a decrease of diffusion of the acyclovir (mentioned earlier in § 3.9.3). The drug, acyclovir, does get released from the vehicle as shown in § 3.1.6.6. Membrane diffusion release studies should, however, be done in future on emulsions without carrageenan to see the difference between them and the emulsions with carrageenan and to have an explanation to the influence that carrageenan has on the release of acyclovir. This will conclude if it is skin specific.

3.10 CONCLUSION

The stratum corneum, top layer of the skin, is the most significant barrier layer of the skin. This study was conducted using a delivery vehicle to try yet again to find a way past this excellent barrier, the stratum corneum, to succeed in the transdermal delivery of acyclovir.

Validation of the HPLC method for the analysis of acyclovir has for this study proven to be simple, rapid, specific and sensitive. The levels of reproducibility, repeatability and sensitivity were obtained and found to be acceptable.

In this study the transdermal permeation of acyclovir in a delivery vehicle in the presence and absence of a gelating agent, carrageenan, was investigated.

The following observations were made:

- The specific formulation of the delivery vehicle had a large influence on the transdermal flux value. As the concentration of the surfactant increased the transdermal flux value of acyclovir also increased. This may be due to the surfactant that increases the solubility of the drug in the skin (Kreilgaard, 2002:S94).

- The gelating agent, carrageenan, surprisingly had a decreasing effect on the transdermal permeation of acyclovir. These results are in contrast to results obtained by Valenta & Schultz (2004). The mechanism of influence of carrageenan on the permeation of acyclovir is unknown. An explanation may be found in the observed increase in particle size.

CHAPTER 4

Summary and Final Conclusion

The skin, a multilayered complex organ that is regarded as a nearly impermeable membrane, serves to provide several significant functions that include:

- Maintaining physical protection (barrier function) against external agents and dessication;
- Receiving sensory stimuli from the environment;
- Regulating body temperature and water balance;
- Excreting a variety of substances;
- Participating in metabolic pathways and
- Serving as a compartmentalized component of the immune system to provide protection against certain pathogens, toxins and neoplasia (Lynch & Roberts, 1990:87).

The outermost layer of the skin, the stratum corneum, has an essential role as a barrier against the transport of water and of chemical and biological agents. Investigations into the individual structure and overall organization of the stratum corneum lipids have been extensive (Friberg *et al.*, 1990:29).

The penetration of acyclovir, an antiviral drug, was shown to be very slow due to its high hydrophilicity (Yamashita *et al.*, 1993:204). A transdermal delivery system or penetration enhancer was needed to obtain sufficient drug concentrations through the skin.

The objectives of this study were to determine:

- How the transdermal delivery of acyclovir will be affected by a drug delivery vehicle;
- The specific formulation of a microemulsion;
- What influence a gelating agent will have on the transdermal delivery of acyclovir.

At first a microemulsion was formulated according to the formulation of Valenta & Schultz (2004). Different percentages Brij 97 was used to determine at what concentration of the surfactant the microemulsion formed.

The 25% Brij 97 formulation without carrageenan in this study was identical to formulation C of Valenta & Schultz (2004). The formulation was observed to form a microemulsion. After investigation by using a confocal laser scanning micrograph it was

demonstrated that none of the 8 formulations had the vesicles which are typical of a microemulsion. The vesicles that could be seen in the 4% and 8% Brij 97 were too large to be microemulsions. It was concluded that none of the formulations formed a microemulsion, but that they were normal oil-in-water emulsions.

The formulation of microemulsion still needs a lot of attention. If the concentration of the surfactant is maybe further increased a clear microemulsion may be formed.

Carrageenan, a gelating agent, was incorporated in the emulsion. This was done according to Valenta & Schultz (2004) to adjust the viscosity of the microemulsion. Valenta & Schultz concluded that in combination with carrageenan, the microemulsions resulted in an additional permeation enhancement. This was in contrast to the findings of this study. Carrageenan had a decreasing effect on the skin permeation of all four the emulsions, compared to the four emulsions without carrageenan. As mentioned in § 3.9.3, our results were comparable with those of Peltola *et al.* (2003) who stated that a gelating agent will decrease the permeation of a microemulsion while increasing its viscosity.

A reason for this decrease in skin permeation in the present study might have been that the carrageenan made the vesicles clot together and increased the particle size which then decreased the contact surface and resulted in a lower flux value.

In conclusion, according to the present study, carrageenan did not prove to be a promising gelating agent in an emulsion for transdermal delivery of acyclovir. It is suggested that other gelating agents be tested in future to determine if they have a more favourable enhancing effect on the transdermal delivery of acyclovir.

Brij 97, a surfactant, was incorporated in the emulsion at increasing percentages. As the concentration of the surfactant increased the flux simultaneously increased. This was in agreement with Kreilgaard's (2002) theory that surfactants are believed to increase skin permeation on various ways as discussed in § 3.9.7.

The mechanism of action of the surfactant increasing transdermal permeation is unkown. In electron micrographs no damage could be observed to the stratum corneum. It is believed that the surfactant increased the solubility of the drug in the skin and through that increased the flux.

The following are suggested for future studies:

- After permeation studies have been done the skin should be studied to reveal and explain the mechanism of action of surfactants on permeation enhancement.

- Several drugs should be used in the emulsions used in this study to determine the influence of different physicochemical properties on the permeation of the emulsions.

- More studies on particle size and a way of decreasing the size of the vesicles that form should be done.

- Studies correlating membrane diffusion and transdermal delivery in the absence and presence of various surfactants and gelating agents should be done.

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Annexure 1

Summary acyclovir Vankel dissolution

4% Brij 97 Emulsion with carrageenan

Area samples at time (min)						
30	**60**	**120**	**180**	**240**	**300**	**360**
418	673	1050	1358	1624	1862	2086
484	777	1187	1508	1785	2039	2237
515	820	1269	1624	1930	2200	2455
601	911	1352	1698	1956	2238	2430
484	755	1155	1474	1754	1997	2211
513	820	1268	1620	1913	2175	2421

	Release rate (µg/square cm) in time (min)						
	30	**60**	**120**	**180**	**240**	**300**	**360**
	61.05	98.30	153.37	198.35	237.21	271.97	304.69
	70.69	113.49	173.38	220.26	260.72	297.82	326.74
	75.22	119.77	185.35	237.21	281.90	321.34	358.59
	87.78	133.06	197.48	248.02	285.70	326.89	354.93
	70.69	110.28	168.70	215.30	256.20	291.69	322.95
	74.93	119.77	185.21	236.62	279.42	317.69	353.62
Ave %	73.40	115.78	177.25	225.96	266.86	304.57	336.92
%RSD	9.45	9.27	8.98	8.47	7.91	7.51	7.26

8% Brij 97 Emulsion with carrageenan

Area samples at time (min)						
30	**60**	**120**	**180**	**240**	**300**	**360**
817	1125	1593	1917	2223	2482	2731
806	1120	1534	1875	2172	2440	2686
884	1181	1578	1890	2162	2409	2648
798	1111	1532	1852	2136	2390	2628
811	1131	1512	1785	2046	2279	2503
834	1129	1599	1944	2252	2480	2728

	Release rate (µg/square cm) in time (min)						
	30	**60**	**120**	**180**	**240**	**300**	**360**
	119.33	164.32	232.68	280.00	324.70	362.53	398.90
	117.73	163.59	224.06	273.87	317.25	356.39	392.33
	129.12	172.50	230.49	276.06	315.79	351.87	386.78
	116.56	162.28	223.77	270.51	311.99	349.09	383.85
	118.46	165.20	220.85	260.72	298.85	332.88	365.60
	121.82	164.91	233.56	283.95	328.93	362.24	398.46
Ave %	120.50	165.47	227.57	274.19	316.25	352.50	387.65
%RSD	1.03	0.18	0.19	0.72	0.67	0.04	0.06

15% Brij 97 Emulsion with carrageenan

Area samples at time (min)						
30	**60**	**120**	**180**	**240**	**300**	**360**
685	1173	1776	2167	2503	2694	2908
734	1220	1824	2262	2550	2810	3030
691	1145	1680	2028	2291	2524	2745
777	1264	1829	2193	2506	2682	2952
947	1391	1809	2111	2363	2593	2857
830	1265	1734	2041	2299	2539	2754

	Release rate (µg/square cm) in time (min)						
	30	60	120	180	240	300	360
	100.05	171.33	259.41	316.52	365.60	393.49	424.75
	107.21	178.20	266.42	330.40	372.46	410.44	442.57
	100.93	167.24	245.39	296.22	334.63	368.66	400.94
	113.49	184.62	267.15	320.32	366.03	391.74	431.18
	138.32	203.17	264.23	308.34	345.15	378.74	417.30
	121.23	184.77	253.27	298.12	335.80	370.85	402.26
Ave %	113.54	181.56	259.31	311.65	353.28	385.66	419.83
%RSD	9.33	3.70	1.18	2.95	4.22	2.94	2.68

25% Brij 97 Emulsion with carrageenan

	Release rate (µg/square cm) in time (min)						
	30	60	120	180	240	300	360
	100.05	171.33	259.41	316.52	365.60	393.49	424.75
	107.21	178.20	266.42	330.40	372.46	410.44	442.57
	100.93	167.24	245.39	296.22	334.63	368.66	400.94
	113.49	184.62	267.15	320.32	366.03	391.74	431.18
	138.32	203.17	264.23	308.34	345.15	378.74	417.30
	121.23	184.77	253.27	298.12	335.80	370.85	402.26
Ave %	113.54	181.56	259.31	311.65	353.28	385.66	419.83
%RSD	9.33	3.70	1.18	2.95	4.22	2.94	2.68

	Release rate (µg/square cm) in time (min)						
	30	60	120	180	240	300	360
	75.95	119.48	200.54	267.15	325.87	374.07	413.65
	81.80	123.28	201.57	266.57	331.71	379.91	419.79
	73.91	118.02	196.75	268.76	326.89	378.16	424.02
	89.68	134.38	211.35	286.87	349.38	402.84	448.71
	79.31	127.66	207.85	276.94	335.80	385.31	422.71
	72.16	119.77	185.21	272.85	328.50	380.79	412.92
Ave %	78.80	123.76	200.54	273.19	333.02	383.51	423.63
%RSD	2.41	0.12	3.82	1.04	0.39	0.88	0.09

Annexure 2

Summary of acyclovir flux values

Control cells:

Cell	Flux ($ng/cm^2/h$)
1	6.31
2	11.64
3	10.24
4	8.93
5	7.93
6	6.83

Average control	8.65
SD control	2.04
%RSD control	23.60

4% Brij 97 emulsion without carrageenan

Cell	Flux ($ng/cm^2/h$)
1	14.43
2	5.11
3	2.14
4	7.40
5	17.73
6	20.03

Average control	11.14
SD control	7.28
%RSD control	65.32

4% Brij 97 emulsion with carrageenan

Cell	Flux ($ng/cm^2/h$)
1	1.46
2	0.68
3	0.74
4	0.70
5	1.12
6	2.74
7	0.61

Average control	1.15
SD control	0.76
%RSD control	66.56

8% Brij 97 emulsion without carrageenan

Cell	Flux ($ng/cm^2/h$)
1	2.03
2	7.56
3	2.25
4	2.50
5	9.43
6	2.25

Average control	4.34
SD control	3.28
%RSD control	75.57

8% Brij 97 emulsion with carrageenan

Cell	Flux ($ng/cm^2/h$)
1	0.85
2	0.94
3	0.73
4	0.72
5	0.71
6	0.51

Average control	0.74
SD control	0.15
%RSD control	19.86

15% Brij 97 emulsion without carrageenan

Cell	Flux ($ng/cm^2/h$)
1	248.42
2	438.64
3	429.15
4	458.57
5	334.97
6	382.60

Average control	382.06
SD control	79.18
%RSD control	20.73

15% Brij 97 emulsion with carrageenan

Cell	Flux (ng/cm²/h)
1	2.05
2	9.21
3	3.70
4	6.10
5	6.11
6	2.63

Average control	4.97
SD control	2.69
%RSD control	54.14

25% Brij 97 emulsion without carrageenan

Cell	Flux (ng/cm²/h)
1	665.84
2	395.31
3	334.61
4	633.86
5	467.35
6	841.10

Average control	556.34
SD control	190.84
%RSD control	34.30

25% Brij 97 emulsion with carrageenan

Cell	Flux ($ng/cm^2/h$)
1	2.44
2	2.09
3	1.66
4	2.50
5	1.98
6	1.20

Average control	1.98
SD control	0.49
%RSD control	24.88