A comparative study of lamellar gel phase systems and emzaloids as transdermal drug delivery systems for acyclovir and methotrexate

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ABSTRACT

A Comparative study of Lamellar Gel Phase Systems and Emzaloid® as transdermal drug delivery systems for acyclovir and methotrexate

The skin forms an attractive and accessible route for systemic delivery of drugs as alternative to other methods of administration, such as the oral and parental methods because of the problems associated with last mentioned methods. The lipophilic character of the stratum corneum, coupled with its intrinsic tortuosity, ensures that it almost always provides the principal barrier to the entry of drug molecules into the skin.

Due to the fact that methotrexate (MTX) and acyclovir (ACV) have poor penetration properties through the skin, the aim of this study was to enhance the permeation of methotrexate and acyclovir with the use of two lamellar gel phase systems (LPGS) (Physiogel® NT and Physiogel® Dermaquadrille) and with Emzaloid® as transdermal drug delivery systems.

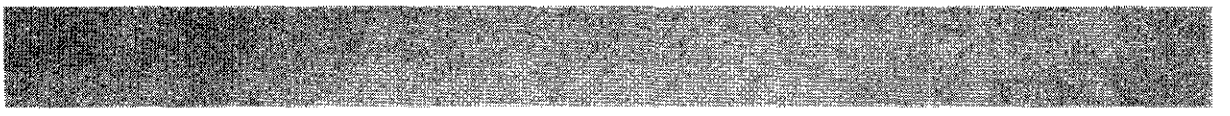
Three different sets of experiments were done in this study: 1) the viscosity of the two Physiogel® creams was measured as an indication of stability and to determine whether the internal structure of the Physiogel® creams were affected by the investigated drugs; 2) the drug release rate from the three drug delivery vehicles was measured with a VanKel® dissolution apparatus; 3) *in vitro* permeation studies were preformed using vertical Franz diffusion cells with human epidermal skin clamped between the donor and receptor compartments. The skin was hydrated with PBS buffer for one hour before 1% mixtures of the drugs in both the Physiogel® creams and Emzaloid® were applied to the donor chamber. Samples were taken at 2, 4, 6, 8, 10, 12 and 24 hours. It was then analysed by HPLC for methotrexate and acyclovir. The fluxes of drug permeation were determined.

The viscosity measurements confirmed that the internal structure of the two Physiogel® creams was not influenced by the drugs. Acyclovir and methotrexate were both released from the delivery vehicles. There was an enhancement of acyclovir through the skin from one of

the Physiogel® creams. The permeability of methotrexate in the presence of the two Physiogel® vehicles was not significantly enhanced. Emzaloid® as delivery vehicle increased the penetration of both drugs through the skin significantly.

The lamellar gel phase system mimics the structure of the stratum corneum, but does not improve the drug permeation through the stratum corneum significantly. The utilisation of Emzaloid® as a drug delivery system could be advocated from these findings. As could be seen from the penetration profiles Emzaloid® was a superior delivery system for methotrexate and acyclovir compared to the lamellar gel phase systems.

Keywords:
acyclovir, methotrexate, transdermal delivery, permeation, drug delivery vehicles, Emzaloid®, Physiogel®



OPSOMMING

'n Vergelykende studie van lamellêre jelfasestelsels en Emzaloid® as transdermale geneesmiddelafleweringstelsels vir asiklovir en metotreksaat

Die vel verskaf 'n aantreklike en toeganklike roete vir die sistemiese aflewering van geneesmiddels as alternatiewe toedieningsroete tot ander metodes soos orale en parenterale roetes vanweë probleme met laasgenoemde roetes. Die lipofiele karakter van die stratum corneum saam met die moeisame en kronkelende deurgang daardeur verseker dat dit feitlik altyd die belangrikste versperring vir die penetrasie van geneesmiddels in die vel is.

Omdat metotreksaat (MTX) en asiklovir (ACV) die vel moeilik penetreer, was die doel van hierdie studie om die penetrasie van metotreksaat en asiklovir te bevorder deur gebruik van twee lamellêre jelfasestelsels (LJFS) (Physiogel® NT en Physiogel® Dermaquadrille) en Emzaloid® as transdermale afleweringstelsels.

Drie verskillende stelle eksperimente is in die studie uitgevoer: 1) die viskositeit van die twee Physiogel®-rome is gemeet as maatstaf vir stabiliteit en om te bepaal of die interne struktuur van die Physiogel®-rome deur die twee geneesmiddels beïnvloed word; 2) die vrystelling van die geneesmiddels uit die drie afleweringstelsels is met behulp van die VanKel® dissolusie-apparaat bepaal; 3) die *in vitro*-diffusie deur menslike epidermis is met behulp van vertikale Franz-diffusieselle bepaal deur die vel tussen die donor- en reseptorkompartemente vas te klem. Die vel is vir een uur met fosfaatbuffer gehidreer voordat mengsels van die geneesmiddel (1% konsentrasie) in sowel Physiogel® en Emzaloid® in die donorkamer aangewend is. Monsters is na 2, 4, 6, 8, 10, 12 en 24 uur getrek en die fluks van die geneesmiddel deur die vel is bepaal.

Metings van die viskositeit het bevestig dat die interne struktuur van die twee Physiogel®-rome nie deur die geneesmiddels beïnvloed word nie. Asiklovir en metotreksaat word albei deur die onderskeie afleweringstelsels vrygestel. Die penetrasie van asiklovir deur die vel word nie noemenswaardig deur enige van die twee Physiogel®-rome bevorder nie.

Die lamellêre jelfasestelsels boots die struktuur van die stratum corneum na, maar het geen noemenswaardige effek op die penetrasie van die geneesmiddels deur die stratum corneum nie. Uit die resultate blyk dit duidelik dat Emzaloid® 'n geskikte afleweringstelsel is. Die diffusieprofiele toon dat Emzaloid® 'n beter afleweringstelsel vir metotreksaat en vir asiklovir as die lamellêre jelfasestelsels is.

Sleutelwoorde:

asiklovir, metotreksaat, transdermale aflewering, permeasie, geneesmiddelafleweringstelsels, Emzaloid®, Physiogel®

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CHAPTER 1

INTRODUCTION AND STATEMENT OF THE PROBLEM

The skin, the largest organ in the body, is a composite of a variety of cell types and organellar bodies, each of which has a particular function. The major function of the skin is protection of the organism from the external environment. Achieving this goal has resulted in the evolution of a complex structure involving several different layers, each with particular properties (Mukhtar, 1991:4).

Although the skin is one of the major sites for non-invasive delivery of therapeutic agents into the body, this task can be relatively challenging owing to the impermeability of the skin, especially the stratum corneum (Foldvari, 2000:417). This layer is the major barrier to penetration of the skin, due to its integral and compact structure comprising of protein-rich cells embedded in a multilamellar lipid domain (Geo *et al.*, 1998:193).

The nature of the topical vehicle is known to play a major role in promoting drug absorption into and through the skin. Conventional topical vehicles, such as ointments, creams or gels, predominantly exert their effect by releasing the drug onto the skin surface and the drug molecules then diffuse through the skin layers. The extent and duration of diffusion depend on the physicochemical properties of the drug absorption kinetics by these vehicles and are the result of their ability to provide increased hydration by occlusion or some other mechanism. If the size and solubility properties of the drug are not amenable, only limited uptake by the skin will occur (Foldvari, 2000:417).

Nevertheless, the transdermal mode offers several distinct advantages: (1) the skin present a relatively large and readily accessible surface-area (1-2 m^2) for absorption; (2) the application of a patch-like device to the skin surface is a non-invasive (and thus patient compliant) product that allows continuous intervention (i.e. system repositioning, removal or

replacement). Further benefits of transdermal drug delivery systems have emerged over the past few years; these include the potential for sustained release (useful for drugs with short biological half-lives requiring frequent oral or parental administration) and the controlled input kinetics, which are particularly indispensable for drugs with narrow therapeutic indices (Naik *et al.*, 2000:319).

Methotrexate is a folic acid antagonist with antineoplastic activity and is used for the treatment of psoriasis and Kaposi's sarcoma. Acyclovir is active in both the treatment and prevention of herpes simplex and varicella zoster viral infections. Due to the complexity of the skin as well as the physicochemical properties of the drugs both these drugs need some kind of enhancement for transdermal delivery. To overcome this problem three drug delivery vehicles (Physiogel® Dermaquadrille, Physiogel® NT and Emzaloid®) were used as delivery vehicles. The formulation of Physiogel® creams is based on the characteristic barrier properties of the skin. Lipids and ceramides found in the Derma-Membrane-Structure (DMS), which are similar to those found in the skin, thus penetrate into deeper layers of the skin and effectively prevent further moisture loss. The Emzaloid® is a submicron type of emulsion that entraps and delivers drugs to specific target sites in the body.

The aims of this study were the following:

- To determine if the investigated drugs will have any influence on the internal structure of the Physiogel® creams;

- To determine the drug release rate from the three delivery vehicles;

- To determine whether the drug delivery vehicles have any influence on the permeation of the investigated drugs through the skin.

CHAPTER 2

TRANSDERMAL DRUG PERMEATION

2.1 STRUCTURE AND THE BARRIER FUNCTIONS OF THE SKIN

The skin functions as the largest organ in the body and is comprised of several layers that protect the underlying tissues. Absorption of chemicals by the skin might have substantial local and systemic consequences. Numerous factors influence the rate and extent of chemical transport through human skin (Gale *et al.*, 2003:976). It is well known that drugs could be applied to the skin for topical treatment of dermatological conditions. The advantages of accessibility and the avoidance of the first-pass metabolism make it attractive for the systemic delivery of drugs. The objective of a transdermal delivery system is to provide a sustained concentration of drug for absorption without breaching the barrier function of the skin and additionally avoids local irritation (Washington & Washington, 1989:182).

The skin is elastic and rugged despite the fact that it is approximately 3 mm thick. An average square centimetre of skin contains 10 hair follicles, 15 sebaceous glands, 12 nerves, 100 sweat glands, 360 cm of nerves and three blood vessels (Michniak, 2000:36). The skin consists of three anatomical layers (Figure 2.1) i.e. the epidermis, dermis and hypodermis or subcutaneous tissue (Washington & Washington, 1989:182).

2.1.1 Epidermis

The epidermis is the thin, dry and tough outer protective layer. It forms a barrier to water, electrolyte and nutrient loss from the body. Simultaneously, it is also responsible for the restriction of the penetration of water and foreign substances from the outside environment into the body (Washington & Washington, 1989:183).

Approximately 95% of the epidermis consists of keratinocytes (of which the lowermost are anchored to the basement membrane *via* hemidesmosomes). The remainder consists of melanocytes, Langerhans cells and Merkel cells (mechanoreceptors). The stratified epidermis, ~100-150 μm thick, is distinguished by four layers: the stratum basale (SB), stratum spinosum (SS), stratum granulosum (SG) and stratum corneum (SC).

The living cells of the epidermis are located directly below the stratum corneum (Lund *et al.*, 1994:136). The basal layer contains the only cells that exhibit cell division and that migrates successively from the spinous, granular and clear layers. The advent of migration induces a gradual loss of cell nuclei as well as a change in cell composition. Ultimately, these cells locate directly above the dermis (Foldvari, 2000:418). The single layer of cells is called the **stratum basale (SB)** and constantly undergoes mytosis to produce keratinocytes (Lund *et al.*, 1994:137).

The **stratum spinosum (SS)** layer appears spiny in histological sections. In addition to typical basal layer cell organelles, the SS presents lipid-enriched lamellar bodies that are known as Orland bodies, keratinosomes and membrane-coating granules. Anatomically, the SS is the first layer to exhibit these bodies in the stratum layers (Menon, 2002:15).

The keratinocytes evolving from the SS migrate to form the stratum granulosum (SG). Migration of the keratinocytes is characterised by continuous cell differentiation. Consequently, keratinocytes produce keratin and evolve to flat-shaped cells. These flattened cells form a three-layer thick zone known as the SG. Enzymes contained in the SG layers eliminate any viable components such as organelles and nuclei.

The lamellar granules are extruded from the cells into the intercellular spaces as the cells approach the upper layer of the **stratum granulosum (SG)** (Williams, 2003:8). Ultimately, due to oxygen and nutrient deprivation, the cells shrine and die to become the cells of the stratum corneum (SC) (Lund *et al.*, 1994:137).

The outermost layer of the epidermis is called the **stratum corneum (SC)** or horny layer. It is a multilayered, wall-like structure into which terminally differentiated keratin-rich epidermal cells (corneocytes) are embedding in an intercellular lipid-rich matrix. This two-

compartment arrangement is usually simplified to a bricks (corneocytes) and mortar (intercellular) domain (Moghimi, 1999:517).

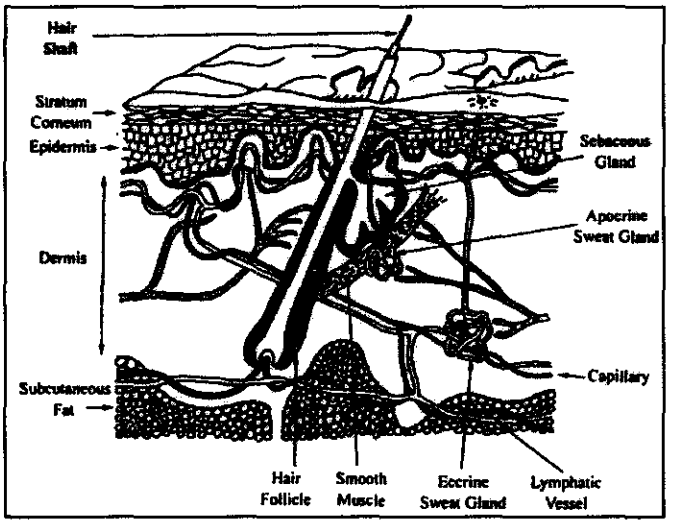


Figure 2.1: Structure of the skin (Washington & Washington, 1989:182).

The human stratum corneum varies in thickness from 10 to 50 μm and is a heterogeneous structure of dead, flattened, interdigitated keratinocytes of 0.5-1.0 μm in thickness (Gale *et al.*, 2003:976). The SC is highly hydrophobic and contains 10-15 layers of interdigitated corneocytes that are continuously shed and renewed. These cells lack phospholipids is, however, enriched in ceramides and neutral lipids (cholesterol, fatty acids and cholesterol esters) that are arranged in a bilayer composition that forms so-called 'lipid channels'.

The barrier function of the skin arises from lamellar granules synthesised in the granular layer. Adequate time lapse will see these granules become organised into the intercellular lipid bilayer domain of the stratum corneum. The barrier lipids are tightly maintained and skin impairment results in the activation of synthetic processes to restore them. The barrier function is apparently dependent on the specific ratio of various lipids. Studies, in which non-polar and relatively polar lipids were selectively extracted with petroleum ether and acetone, indicated that the relatively polar lipids were more crucial to skin barrier integrity. The stratum corneum contains a highly organised structure and is therefore, the major permeability barrier to external materials. In addition, it is regarded as the rate-limiting factor in the penetration of therapeutic agents through the skin. The degree to which absorption is enhanced is dictated by the ability of various agents to interact with the intercellular lipid (Foldvari, 2000:418).

2.1.2 Dermis

The dermis is a fibrous layer that supports and strengthens the epidermis. It ranges from 2 to 3 mm in thickness and in man constitutes 15 - 20% of the total body weight (Washington & Washington, 1989:185). It is largely acellular, rich in blood vessels, lymphatic vessels and nerve endings. An extensive network of dermal capillaries connects to the systemic circulation, with considerable horizontal branching from the arterioles and venules in the papillary dermis to form plexuses. The capillaries are responsible for blood supply to hair follicles and glands. Dermal lymphatic vessels augment the drainage of excess extracellular fluid and clear antigenic materials. The elasticity of the dermis is attributed to a network of protein fibres, including collagen (type I and II) and elastin. These proteins are embedded in an amorphous glycosaminoglycan ground substance. Furthermore, the dermis contains diffuse fibroblasts, microphages and leukocytes. In addition, the dermis contains hair follicles, sebaceous and sweat glands as well as subcutis that might serve as an additional, however, fairly limited pathway for drug absorption (Foldvari, 2000:418).

2.1.3 Hypodermis

The subcutaneous tissue or hypodermis is composed of loosely arranged, elastic fibrous connective tissue as well as fat. The base of the hair follicles is present in this layer, as are the secretory portion of the sweat glands, cutaneous nerves as well as the blood and lymph networks. Generally, it is considered that a drug has entered the systemic circulation if transported to this layer. The fat deposits may, however, serve as a deep compartment or depot for the drug, potentially delaying entry into the blood circulation (Washington & Washington, 1989:184).

2.2 ROUTES OF DRUG PERMEATION ACROSS THE SKIN

The stratum corneum predominates as the rate-limiting barrier to delivery, although some highly lipophilic drugs are primarily stunted by the aqueous epidermal membrane (Williams, 2003:30). Drug diffusion from a transdermal delivery system to the blood could be considered as passage through a series of diffusional barriers (Washington & Washington, 1989:186). There are essentially three pathways facilitating molecule transversion of intact stratum corneum: the appendages (shunt route), the intercellular lipids domains or by a

transcellular route. These pathways are not mutually exclusive and it is likely that combinations thereof allow passage through the stratum corneum. The relative contributions of these pathways to gross flux will depend on the physicochemical properties of the permeant (Williams, 2003:31).

The common routes of drug penetration are depicted below (Figure 2.2).

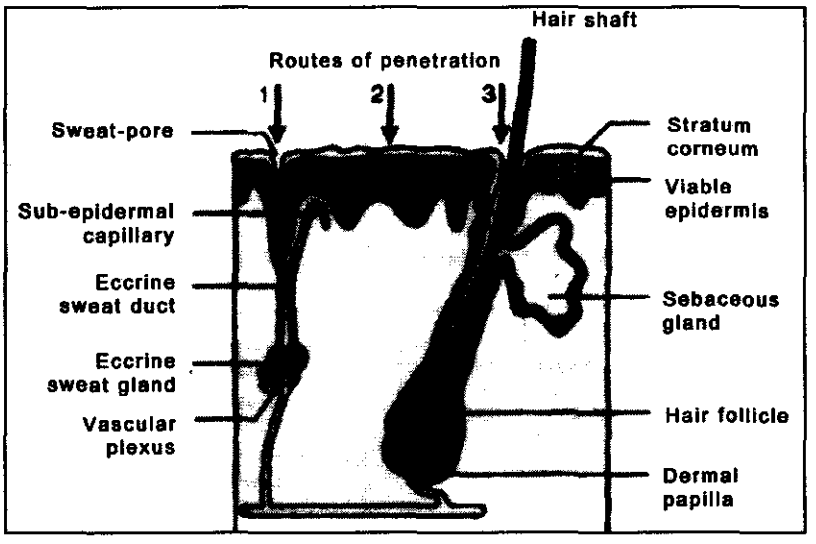


Figure 2.2: Simplified diagram of skin structure and macroroutes of drug penetration: (1) sweat ducts; (2) continuous stratum corneum or (3) hair follicles with their associated sebaceous gland (Barry, 2001:102).

2.2.1 Appendageal pathway

The available diffusional area of the shunt route is approximately 0.1% of the total skin area. Despite their small fractional area, the skin appendages may provide the main portal for ions and larges polar molecules of which permeation is severely impaired by the intact stratum corneum. The shunt route dominants not only the transient phase (non-steady-state) of percutaneous absorption, but makes a negligible contribution to the overall flux in the steady state period (Moghimi, 1999:516).

2.2.2 Transepidermal pathway

There are two routes of passage of drugs through the stratum corneum, i.e. the hydrophilic keratinised cells or the largely organised lipid bilayer channels between cells. The lipoidal nature of lipid channels favours passage of hydrophobic molecules, e.g. several drugs, and provides their major route of entry (Washington & Washington, 1989:86).

A molecule traversing the intact stratum corneum *via* the transcellular route faces numerous challenges. Firstly, there is partitioning into keratinocyte and subsequent diffusion through the hydrated keratin. In order to leave a cell, a molecule should partition into the bilayer lipids prior to diffusion across the lipid bilayer to the adjacent keratinocyte. In traversing the multiple lipid bilayers, the molecule should additionally partition sequentially into the hydrophobic chains and the hydrophilic head groups of the lipids. The characteristics of the permeant will influence the relative importance of the transcellular route in the observed flux. In this regard, for highly hydrophilic molecules, the transcellular route may predominate at a pseudo-steady state. However, the rate-limiting permeation barrier via this route is the multiple bilayered lipids. Molecules are obliged to transverse between the keratinocytes and the application of solvents to extract lipids from the stratum corneum, invariably increases drug flux even observed for highly hydrophilic molecules (Williams, 2003:33).

2.2.3 Intercellular pathway

The intercellular lipid route provides the principal pathway by which most small, intact molecules traverse the stratum corneum. The transport is clearly observed through the lipid domains and the route is highly tortuous, with permeants being transported through the continuous domains between the keratinocytes. The path length followed by the molecule is considerably greater than the stratum corneum thickness (Williams, 2003:35).

2.3 PHYSIOLOGICAL FACTORS AFFECTING TRANSDERMAL DRUG DELIVERY

2.3.1 Skin age

The skin condition and structure varies with age (Washington & Washington, 1989:188). The infant skin ultrastructure is indistinguishable from that of an adult but blood concentrations of

topically applied drugs can be much higher in infants. These differences arise from the fact that the skin is a relatively larger organ in infants than in adults. Additionally, the epidermal enzymes capable of metabolizing applied medicaments may not be completely functional in infants. Furthermore, the skin of prematurely born infants may be even more permeable as the stratum corneum is not completely established until the end of gestation. Old age can also affect elasticity, ultra structure, chemical composition and the barrier properties (Lund *et al.*, 1994:141). Blood flow (dermal clearance of molecules traversing the tissue) tends to decrease with age and this could reduce transdermal flux. However, for the majority of permeants dermal clearance tends not to be the rate-limiting factor in transdermal therapy (Williams, 2003:14).

2.3.2 Body site

The permeability coefficient (k_p) of a penetrant across the stratum corneum is inversely proportional to the diffusion path length (h). It could be expected that the permeability coefficient would be smaller at anatomic sites where thickness of the stratum corneum differs (Mukhtar, 1991:23). In the plantar and palmar areas keratinised layers are thick and absorption rates are consequently slow. The face, particularly behind the ear, presents more rapid absorption.

Factors other than thickness also play a role in the extent of percutaneous absorption at a particular body site. These factors include the size and lipid composition of the cells in the stratum corneum, their number of layers, associated stacking pattern and the depth and distribution of the appendages (Lund *et al.*, 1994:140).

2.3.3 Race

Race appears to influence penetration to a small extent. Negroid stratum corneum has more layers and is generally less permeable, although there is no difference in actual thickness between Negroid and European stratum corneum (Washington & Washington, 1989:189).

2.3.4 Other factors

Several other physiological factors may, to some degree, influence transdermal drug delivery. For example, keratinocytes tend to be slightly larger in females (37-46 μm) than in males

(34-44 μm), however, there are no reports of significant differences in drug delivery between equivalent sites in the two genders. The level of hydration of the stratum corneum may have dramatic effects on the drug permeation through the tissue. High levels of hydration are known to increase transdermal drug delivery of most drugs. Since diffusion through the stratum corneum is a passive process, an increase in temperature results in an increase in the permeant diffusion coefficient. The human body maintains a temperature gradient across the skin of approximately 37 °C inside and around 32 °C at the outer surface. Pronounced elevation of the skin temperature can induce structural alterations within the stratum corneum and these modifications may increase diffusion rate through the tissue (Williams, 2003:17).

2.4 PHYSICOCHEMICAL FACTORS INFLUENCING TRANSDERMAL DELIVERY

Transdermal drug delivery is a viable administration route for potent, low-molecular weight therapeutic agents. Additionally it provides a non-hostile environment to drugs unable to withstand the gastrointestinal tract and/or those subjected to considerable first-pass metabolism by the liver. The choice of therapeutic agent is determined by numerous factors including the physicochemical properties of the drug, membrane interactions and its pharmacokinetic properties.

The release of a therapeutic agent from a formulation applied to the skin surface and its transport to the systemic circulation is a multistep process that involves:

- dissolution within and release from the formulation;
- partitioning into the skin's outermost layer, the stratum corneum (SC);
- diffusion through the SC, principally *via* a lipidic intercellular environment, (i.e. the rate-limiting step for most compounds);
- partitioning from the SC into the aqueous viable epidermis;
- diffusion through the viable epidermis and into the upper dermis and
- uptake into the local capillary network and eventually systemic circulation.

Therefore, an ideal drug candidate would have sufficient lipophilicity to partition into the SC, but also sufficient hydrophilicity to enable the second partitioning step into the viable epidermis and eventually the systemic circulation (Kalia, 2001:160).

2.4.1 Partition coefficient

In order to cross the stratum corneum, a permeant should firstly partition into the membrane. Indeed, partitioning into the skin could be the rate-limiting step in the permeation process (Williams, 2003:35). Partition coefficients are the gate-keepers controlling access of the permeant to the stratum corneum. The passage of a permeant through the stratum corneum cannot initiate unless the permeant has been transferred from the vehicle to one of the stratum corneum components. It is the partition coefficient (K) that controls this process (Rieger, 1993:43). It should be expected that a hydrophilic molecule would preferentially partition into the hydrated keratin-filled keratinocytes, rather than into the lipid bilayers. Additionally lipophilic permeants would preferentially partition into the lipoidal domains. Hydrophilic molecules could be expected to permeate predominantly *via* the intracellular route whereas the intercellular route will dominate for lipophilic molecules (Williams, 2003:35) Partition coefficient is routinely determined by analysis of the concentration of a substance in two immiscible solvents, a solvent and a tissue, or in two tissues at equilibrium. In the case of the stratum corneum, the partition coefficient is defined as:

$$K_1 = \frac{C_{SC}}{C_V}$$

(Equation 2.1)

where C_v is the permeant concentration in the vehicle and C_{sc} the permeant concentration in the stratum corneum.

The partition coefficient is determined by equilibrating the tissue in the stratum corneum with an excess of the permeant in a solvent (Rieger, 1993:43).

Molecules with an intermediate partition coefficient demonstrate moderate solubility in both oil and water phase and the intercellular transport route probably predominates. This would typically encompass most molecules with a log $P_{(octanol/water)}$ of 1 to 3. For more highly

lipophilic molecules (log $P \geq 3$) the intercellular route would be the most exclusive as the pathway of traversing the stratum corneum.

For more hydrophilic molecules (log $P \leq 1$), the transcellular route becomes more relevant, yet there are lipid bilayers to cross between the keratinocytes. In the case of highly hydrophilic (and charged) molecules, the appendageal pathway may also become significant (Williams, 2003:36).

2.4.2 Solubility

The solubility of the penetrant in the various environments of the skin and its surroundings plays an important part in determining the rate of penetration (Smith, 1990:25). It is well known that most organic materials with high melting points and with high enthalpies of melting have relatively low aqueous solubilities at normal temperatures and pressures. A clear relationship is established between melting point and solubility of materials. Lipophilic molecules tend to permeate skin faster than more hydrophilic molecules. Solubility within the intercellular lipids (usually described by the partition coefficient) could be correlated with the permeability coefficient for a homologous series of compounds. Lipophilic permeants may provide a relatively high permeability coefficient: their lipophilicity would usually indicate that the aqueous solubility will be relatively low, with a consequent impact upon drug flux through the tissue (Williams, 2003:37).

The stratum corneum is lipophilic layer with the intercellular lipid lamellae forming a conduit through which drugs should diffuse in order to reach the underlying vascular infrastructure, thereby ultimately gaining access to the systemic circulation. For this reason, lipophilic molecules are better accommodated by the stratum corneum. A molecule should firstly be liberated from the formulation and partition into the uppermost stratum corneum layer, before diffusing through the entire SC. Subsequently, it should repartition into the more aqueous viable epidermis. Ideally, a drug must possess both lipoidal and aqueous solubilities. Extreme hydrophilicity would preclude molecule transfer into the SC. In contradiction, extreme lipophilicity would result in molecules residing in the SC (Naik *et al.*, 2000319).

The released drug molecules will partition into the outer layers of the stratum corneum. The degree of partitioning is controlled by the amount applied and the solubility limit in the

stratum corneum. The rate of partitioning from the vehicle to the skin will be more rapid than the diffusion through the skin and in general could be negated. The solubility constant in the SC σ_{sc} ($\mu g\ cm^{-2}$) could be estimated using either equation 2 or 3:

$$\log \sigma_{sc} = 1.31 \log[oct] - 0.13$$

(Equation 2.2)

$$\log \sigma_{SC} = 1.911(10^3 / mp) - 2.956$$

(Equation 2.3)

where [oct] is the octanol solubility of permeant (g.l-1) and mp is its melting point (K).

The calculation of σ_{sc} and its subsequent use in the prediction of skin penetration assumes that it is not altered by the presence of formulation components (Hadgraft & Wolff, 1993:162).

The solubility parameter of the skin has been estimated as ~10 and therefore, drugs that possess similar values would be expected to dissolve readily in the stratum corneum. Formulation compounds diffusing into the skin e.g. propylene glycol will tend to increase the value of the solubility parameter and would be expected to promote the solubility of polar drugs in the lipids. They will potentially alter the partition coefficient of the drug between the formulation and additionally between the stratum corneum and the viable tissue. The partitioning behaviour of the drugs will be linked with its solubility characteristics and is an important factor that must be taken into account in any assessment of the feasibility of transdermal or topical delivery (Hadgraft & Wolff, 1993:163).

2.4.3 Melting point

Compounds with lower melting points exhibit higher permeability coefficients. Therefore, compounds with the lower melting points exhibited higher solubility in the SC, resulting in their comparatively higher permeation (Roy, 1993:147).

2.4.4 Molecular size

The size and shape of a molecule could also determine its permeation through human skin. The diffusivity of a drug molecule in a medium is dependent on the properties of both the

drug and the medium. The diffusivity (D) in liquid media, in general, tends to decrease with an increase in the molecular volume (MV) and could be expressed as follows:

$$\boxed{D \approx MV^{-1/3}}$$

(Equation 2.4)

The diffusivity in lipids is expected to vary only slightly with increased molecular size. In contrast, in the more structured, semicrystalline lipid regime of the stratum corneum, diffusivities are more sensitive to molecular size (Roy, 1993:143). Molecules of small size in high concentration tend to penetrate more readily than large molecules. However, for a range of chemically equivalent molecules with similar molecular weights, there is little correlation between size and absorption (Lund *et al.*, 1994:141).

2.4.5 Ionisation

If the penetrant is ionisable, both charged and uncharged species are present in quantities that are pH-dependent. Generally, transport of the ionised species occurs much less rapidly than transport of the base or unionised. It is possible that facilitated transport of ion pairs *via* a carrier vehicle may result in enhanced transdermal flux. In general, higher fluxes could be obtained if the pH is such that the penetrant is unionised (Smith, 1990:27). The nonpolar nature of the horny layer suggests that charged compounds should encounter high resistance to permeation. This proposition is best studied by the use of ionigenic compounds for which the ratio of charged species could be manipulated by changing pH of the vehicle (Zatz, 1993:28). In systems where a weakly ionised active substance is present in the aqueous phase of an oil-in water emulsion, adjustment of the pH to values above or below the pK_a value will influence the degree of ionisation with consequent effect on both activity and release (Lund *et al.*, 1994:141).

2.4.6 Hydrogen bonding

Drug binding is a factor that should be considered in the selection of appropriate candidates for drug delivery. Considering the varied nature of skin compounds (lipids, proteins, aqueous regions, enzymes, etc.) and the possible variation within permeants (weak acids/bases, ionised species, neutral molecules, etc.) there is a multitude of potential interactions between drug substances and the tissue. Interactions could vary from hydrogen bonding to weak Van der

Waals forces and the result of drug binding (if any) on flux across the tissue would depend on the permeant. For a poorly water-soluble drug in aqueous donor solution (thus containing relatively few drug molecules), significant binding to the stratum corneum may completely retard drug flux if essentially all the molecules entering the tissue from the donor solution bind to skin components. However, for molecules with moderate water solubility that permeate the skin well, the binding sites within the tissue may be saturated during early periods of transdermal delivery and steady-state flux might be unaffected.

The literature survey revealed that diffusion through human epidermal membranes is not solely dependent on the number of hydrogen bonding groups in a molecule, but also the distribution of these groups with respect to symmetry within the molecule. Consequently, an increase in the number of hydrogen bonding groups on the permeant might inhibit permeation across the stratum corneum (Williams, 2003. 40).

2.5 MATHEMATICS OF SKIN PERMEATION

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

Fick's first law states that the quantity of a diffusing substance (*J*) which migrates in 1 second through 1 cm^2 in the direction X from the skin surface into the horny layer is equal to the diffusion coefficient (*D*) multiplied by the gradient *(-dc/dx)* of the concentration, c

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

(Equation 2.5)

During the diffusion of a drug into the horny layer the concentration gradient in the distribution space is reduced. Fick's second law defines the time-dependent decrease of the gradient:

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

(Equation 2.6)

Transformation of the equation shows that the drug quantity that diffuses from the drug depot up to a given distance is proportional to the square root of the diffusion time. This means that the substance distributes with a decreasing velocity. Over a short distance the diffusion remains constant. Neither the horny layer nor the whole skin is a unique inert membrane. Therefore, the drug concentrations in the formulation are not the same (Wolfgang, 1982:44). At steady-state the amount of drug entering the membrane is equal to the amount leaving the membrane, the flux (J_{ss}), is given in equation:

$$J_{ss} = \left[D \cdot \frac{K_{sc/veh}}{h} \right] \cdot C_{veh} = K_p \cdot C_{veh}$$

(Equation 2.7)

where J_{ss} is the steady-state flux (mg $cm^{-2}hr^{-1}$) across the membrane of thickness h cm; $K_{sc/veh}$ is the drug's SC-vehicle partition coefficient; D is the drug diffusivity (cm^2hr^{-1}) in the SC; C_{veh} is the drug concentration (mg cm^{-3}) in the vehicle and K_p is the formulation-dependent permeability coefficient of the drug.

By increasing the solute diffusivity and partitioning in the stratum corneum as well as the concentration in the applied formulation the fluxes would improve. However, because the skin is a stratified structure wherein the lipoidal stratum corneum is supported by a more aqueous epidermal layer, the physicochemical nature of these underlying structures also contributes to the overall diffusion process. The $K_{sc/veh}$ (governed by drug lipophilicity) must favour both transfer into and out of the stratum corneum; transport across the skin is therefore, not favoured by infinitely increasing $K_{sc/veh}$. By selecting the vehicle with high solubility potential (i.e. affinity) for the permeant, C_{veh} can be increased, but it should be noted that this, in return, is likely to discourage transfer of the permeant into the SC (in other words decrease $K_{sc/veh}$). The vehicle should therefore be carefully selected to optimise the product ($K_{sc/veh} \cdot C_{veh}$) such that it facilitates drug transfer from the formulation into the SC, whilst having a sufficiently 'high holding capacity' for the therapeutic dose (Niak *et al.*, 2000:320). A plot of

the cumulative amount drug passing through a unit area of a membrane (e.g. $\mu g.cm^{-2}$) versus time gives the typical permeation profile (Figure 2.3).

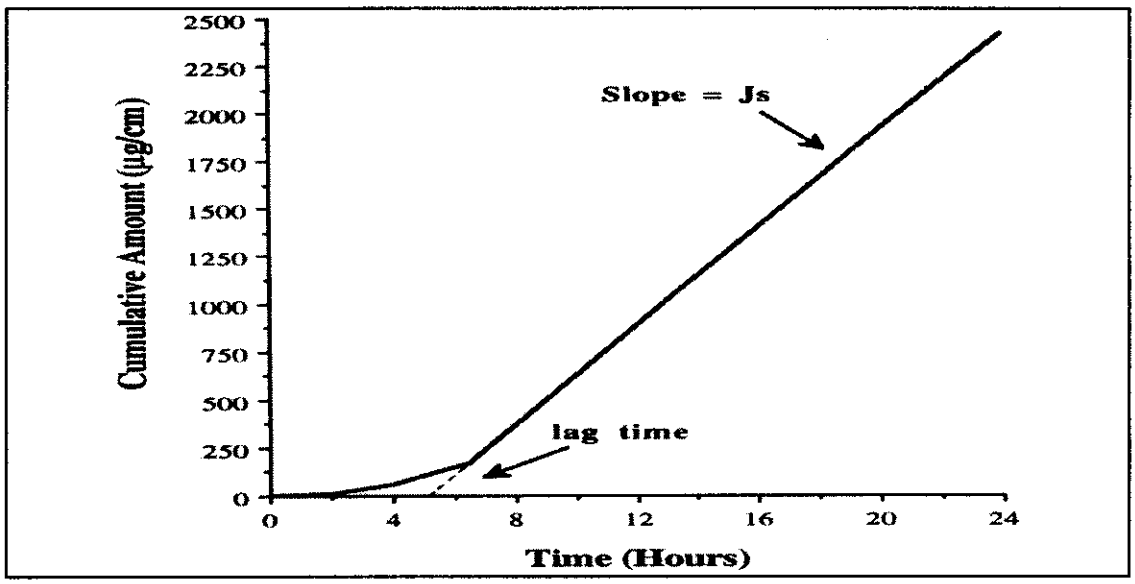


Figure 2.3: Typical Cumulative Amount of Drug Permeated versus Time plot. The slope of the linear portion of the curve provides steady-state skin flux and x-intercept is the lag time (Roy, 1997:145).

The lag time can be obtained from extrapolation of the pseudo-steady-state portion of the permeation profile to the intercept on the time axis. The pseudo-steady-state permeation for most drugs is achieved after approximately 2.7 times the lag time. As stated by Crank (1975) the lag time (L) could be related to the diffusion coefficient by:

$$\boxed{L = \frac{h^2}{6D}}$$

(Equation 2.8)

From the equation it is apparent that the diffusion coefficient of a molecule in the membrane could be obtained by measuring the lag time (Williams, 2003:43). The time lag before steady-state is reached is characteristic of the diffusivity of the penetrant in the membrane (Smith, 1990:31).

2.6 PENETRATION ENHANCERS

Transdermal absorption is relatively slow and has resulted in a large amount of work concerned with finding materials that will increase the penetration rate of drugs through the skin. Such materials are called penetration enhancers and are believed to operate by increasing the permeability of the stratum corneum, either in the lipid or keratinised protein regions (Figure 2.4) (Washington & Washington, 1989:191).

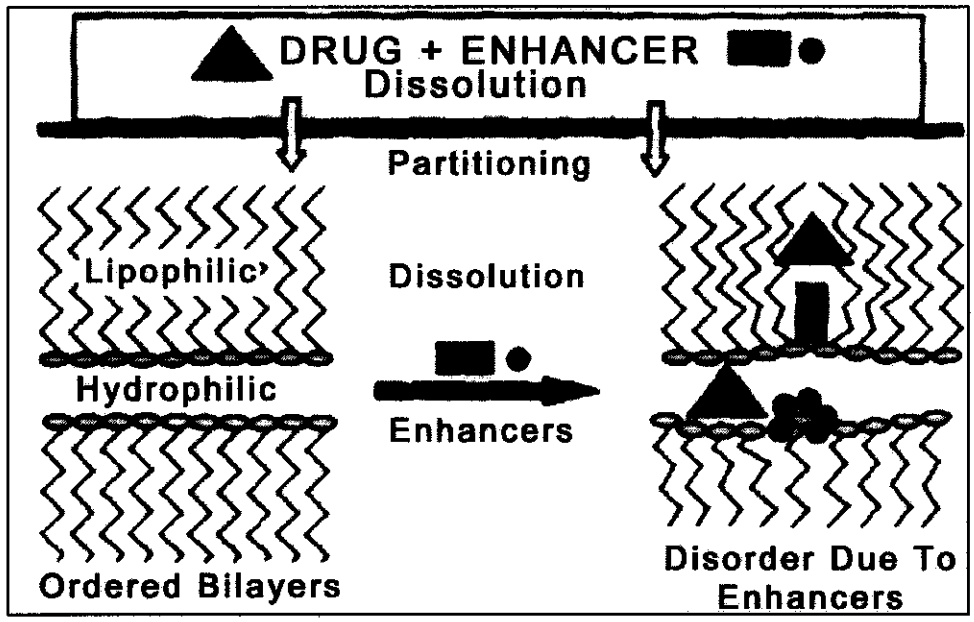


Figure 2.4: Schematic representation of the facilitated drug diffusion channels formed by chemical enhancer disruption of ordered intercellular lipid bilayers (Walker & Smith, 1996).

There are three pathways suggested for drug penetration through the skin: polar, non-polar, and polar/nonpolar routes. The enhancers act by alteration of these pathways. The key to altering the polar pathway is to produce protein conformational changes or solvent swelling. The key to altering the nonpolar pathway is to alter the rigidity of the lipid structure and fluidise the crystalline pathway (this substantially increases diffusion). The fatty acid enhancers increase the fluidity of the lipid portion of the stratum corneum. Some enhancers (binary vehicles) act on both polar and nonpolar pathways and alteration of the multilaminate pathway for penetrants. Enhancers can increase the drug diffusivity in the stratum corneum by dissolving the skin lipids or by denaturing skin proteins.

The type of enhancer employed has a significant impact on design and development of the product (Shah, 1994:20). Properties of an ideal penetration enhancer are the following:

- It should be pharmacologically inert.
- It should be non-toxic, non irritating and non-allergenic.
- It should be have a rapid onset of action; predictable and suitable duration of action for the drug used.
- Following removal of the enhancer, the stratum corneum should immediately and fully recover its normal barrier property.
- The barrier function of the skin should decrease in one direction only and efflux of endogenous materials should not occur.
- It should be chemically and physically compatible with the delivery system.
- It should be readily incorporated into the delivery system.
- It should be inexpensive and cosmetically acceptable.

(Finnin & Morgan, 1999:956).

2.6.1 Physical enhancers

The iontophoresis and ultrasound (also known as phonophoresis or sonophoresis) techniques are examples of physical means of enhancement that have been used for enhancing percutaneous penetration (and absorption) of various therapeutic agents. One of the major concerns in the application of iontophoresis is that the device may cause painful destruction of the skin with high current settings.

It is essential to use high quality electrodes with adequate skin adhesion, uniform current distribution, and well-controlled ionic properties. The mechanism of transdermal penetration by this technology is still not clear (Shah, 1994:21).

2.6.2 Supersaturation

The thermodynamic activity of a drug could be increased by employing supersaturated systems that give rise to unusually high thermodynamic potentials; this effect was first shown in a volatile-nonvolatile vehicle. However, topical vehicles relying on supersaturation have the major limitation of formulation instability, both prior to and during application to the skin, unless the formulation could be stabilised with antinucleant and anticrystal-growth agents (Finnin & Morgan, 1999:956).

2.6.3 Chemical enhancers

Chemicals that promote the penetration of topically applied drugs are commonly referred to as accelerants, absorption promoters, or penetration enhancers. A prime research objective is to identify chemicals that significantly enhance drug penetration through the epidermis but do not severely irritate or damage the skin. The enhancers have the following effects:

- Increase drug permeability through the skin by producing reversible damage to the stratum corneum.
- Increase (and optimise) thermodynamic activity of the drug when functioning as co-solvent.
- Increase the partition coefficient of the drug to promote its release from the vehicle into the skin.
- Operate by conditioning the stratum corneum to promote drug diffusion.
- Promote penetration and establish drug reservoir in the stratum corneum.

Many of the vehicles, in spite of being effective enhancers, are limited in their functions as vehicles because of their deleterious effects on the skin e.g. dimethyl sulphoxide (DMSO). DMSO is a powerful solvent and it increases drug penetration, however, it simultaneously alters the biochemical and structural integrity of the skin and effects this by the direct insult to the stratum corneum (Shah, 1994:21). Substances reported to render the stratum corneum more permeable include alcohols, polyalcohols, pyrrolidones, amines, amides, fatty acids, sulphoxides, esters, terpenes, alkanes, surfactants and phospholipids (Naik, 2000:321).

2.6.4 Metabolic or biochemical enhancers

Chemicals that provoke biochemical and metabolic events within the skin could potentially be used to alter skin permeability. These types of enhancers could reduce the barrier properties of the skin either by inhibition of enzymes responsible for the synthesis of specific stratum corneum lipids during stratum corneum repair or by promotion of the metabolism of existing skin lipids that are responsible for skin barrier function. It should be noted that chemical penetration enhancers could provoke unwanted events that could alter skin permeability (Finnin & Morgan, 1999:956).

2.7 DRUG DELIVERY VEHICLES

2.7.1 Liposomes and lipid vesicles

Liposomes, or lipid vesicles, are spherical, self-closed composed of concentric lipid bilayers that entrap part of the vehicle or active in the centre core. They may consist of one or several membranes (i.e. unilamellar or multilamellar). The size of liposomes ranges from 20 nm to 100 μm, of which the thickness of each membrane is approximately 4 nm. Liposomes are made predominantly from amphiphiles that may be natural lipids (e.g. phospholipids) or synthetic surfactants (Figure 2.5).

Liposomes are ideal vehicles for cosmetic and dermatological applications. It aids the dissolution and formulation of water-insoluble or hydrophobic ingredients. They can encapsulate water-soluble or hydrophilic drugs and moisturises and enhance water retention in the skin *via* their bilayer structure. They can increase water retention in the stratum corneum resulting in an improved skin elasticity and barrier function. Liposomes offer several attributes in topical delivery. Their bilayer structure and lipid components enable the active to have prolonged retention in the skin, be released in a sustained fashion, and show less irritation, as seen with retinoic acid (Liu & Wisniewski, 1997:594).

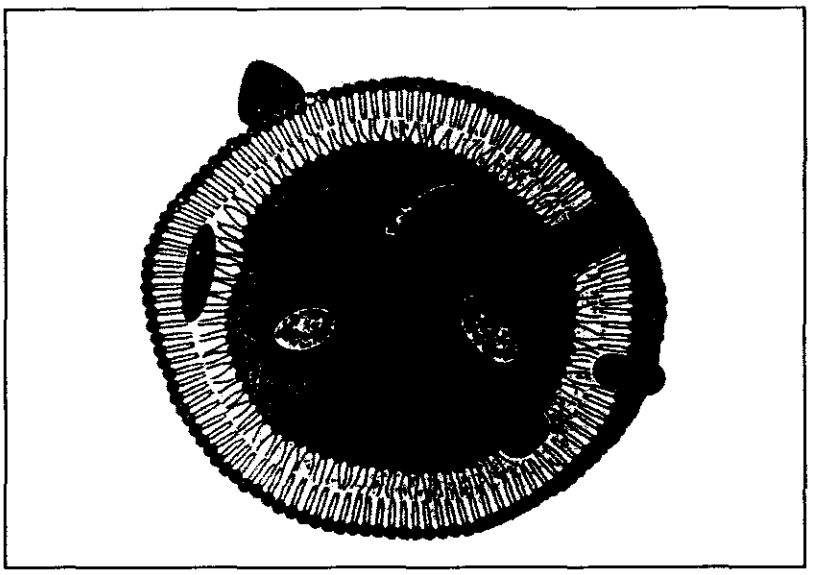


Figure 2.5: Diagram of a liposome interacting with hydrophilic (in centre core or surface) and hydrophobic (dissolve in bilayer) molecules (Liu & Wisniewski, 1997:594).

2.7.2 Lamellar gel phase systems

Lamellar gel phase systems consist of DMS (Derma Membrane Structure). The special features of DMS originate from the fact that its structure is similar to the skin's own lipid barrier (Figure 2.6), with lipids and ceramides that penetrate into deeper layer of the skin (Schöffling, 2002:9).

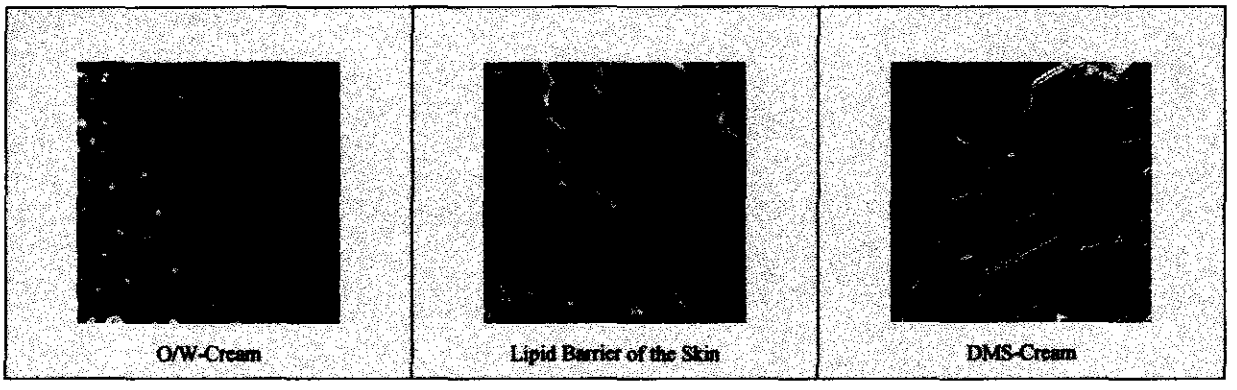


Figure 2.6: Comparison of different structures.

The DMS is part of the membrane family. In contrast to liposomes and nanoparticles which consist of native phosphatidylcholine (PC) (its fatty acid population is mainly linoleic acid), the DMS contains a hydrogenated phosphatidylcholine (PC) (with a fatty acid population of stearic acid and palmitic acid) with ceramide-like properties. Hence, hydrogenated PC has a high affinity to lipid bilayers of the skin barrier, stabilizes the transepidermal water loss in a physiologically useful balance and protects the skin against the penetration of foreign substances (Lautenschlaeger, 2002:167).

The production of this lamellar structure is based on special high–pressure technology (more than 1400 bar). The components are forced into a microcrystalline, lamellar structure and thereby a high viscosity is achieved. Conventional emulsifiers were avoided because they accumulate in the skin and allow barrier lipids to be washed out (Schöffling, 2002:9). The major components of DMS-based creams are:

- Water
- Hydrogenated phosphatidylcholine
- Oil-based substances
- Phytosterols (e.g. extracted from shea butter)
- Moisturising agents (glycerine, etc.)

The lamellar gel phase systems consist of two creams: 1) Physiogel® Dermaquadrille, 2) Physiogel® NT. The lamellar gel phase systems were used as sponsored by Kush Kosmetiek, Germany.

2.7.3 Emzaloid®

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

There are various types of emzaloids: 1) a lipid bilayer vesicle in both the nano- and micrometer size ranges, 2) micro-sponges and 3) depots or reservoirs that contain pro-

emzaloids. Each type of Emzaloid® has a specific composition. In this study Emzaloid® in a cream formulation with lipid bilayers were used (Figure 2.7).

Although there are many lipid based delivery systems, the Emzaloid® is unique among these in that its composition, the essential fatty acids are manipulated in a very specific manner to ensure its high entrapment capabilities, very fast rate of transport, delivery and stability (MeyerZall laboratories, 2002:8).

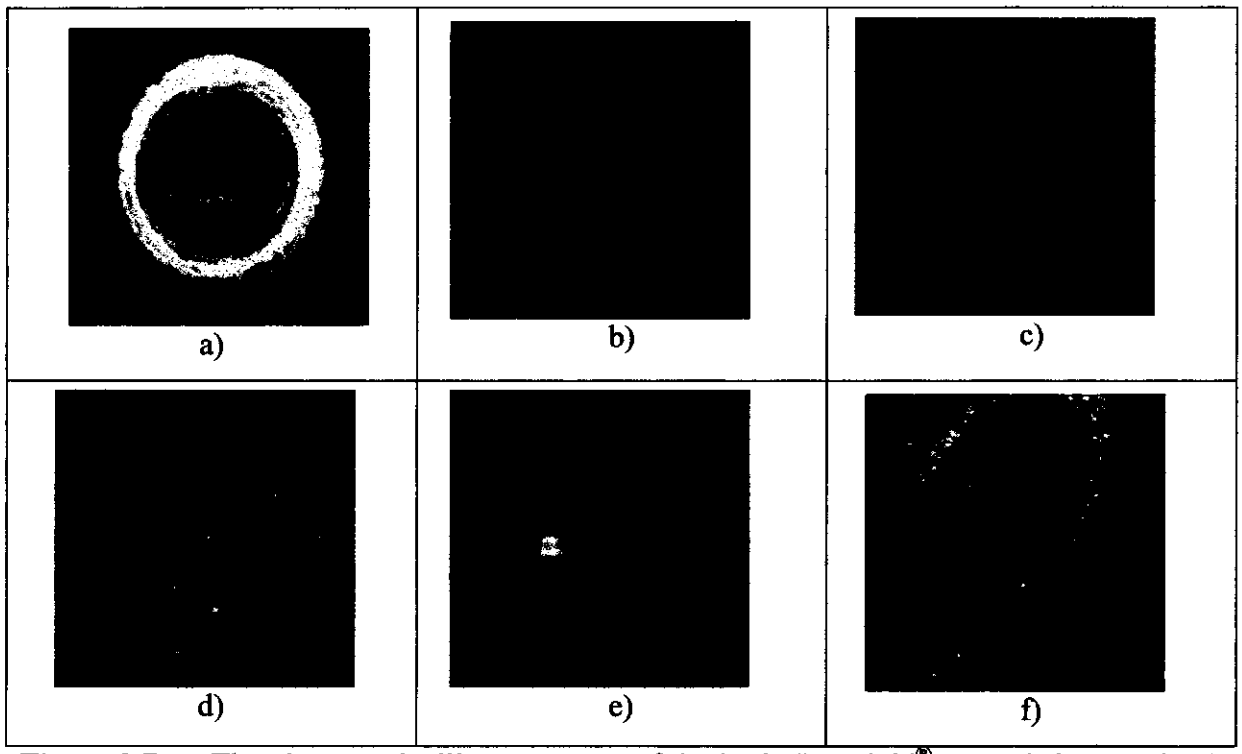


Figure 2.7: The photographs illustrate some of the basic Emzaloid® types. Micrograph (a) shows a bilayer membrane vesicle with a diameter of 100 nm containing rifampicin; (b) a highly elastic or fluid bilayer vesicle with loose lipid packing, containing rifampicin; (c) a small pro- Emzaloid®, (d) a reservoir with multiple particles of coal tar; (e) the Emzaloid® in the process of entrapping fluorescently labelled water-soluble diclofenac; it is very small (diameter less than 30 nm) and the membrane packing is sponge-like; (f) a depot with a hydrophobic core containing pro- Emzaloid® formulation, a surrounding hydrophilic zone and an outer vesicle containing zone.

2.8 PHYSICOCHEMICAL PROPERIES OF THE INVESTIGATED DRUGS

In order to assess the feasibility of delivering a drug either onto or through the skin, it is important to consider both its physicochemical and pharmacokinetic properties. The physicochemical ones will determine the rate at which it can penetrate. These should be correlated to the pharmacokinetic factors that control its clearance to ensure that concentrations in the lower regions of the skin or the plasma may be estimated (Hadgraft & Wolff, 1993:161).

The ideal limits for passive transdermal delivery (Table 2.1) for any given formulation should be the following:

Table 2.1: Ideal limits of physicochemical parameters of transdermally delivered drugs.

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

2.8.1 Acyclovir

Acyclovir (ACV) or 9-(2-hydroxyethoxy)methylguanine is a synthetic purinic nuleosidic analogue derived form guanine. This drug is structurally differentiated from guanine due to the presence of an acylic chain (Fernández *et al.*, 2003:357). Acyclovir was the first specific antiviral drug to become widely used against herpes viruses, particularly herpes simplex viruses types I and II and varicella zoster virus (Dollery, 1999:39)

2.8.1.1 Identification

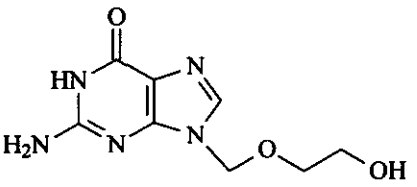


Figure 2.8: Molecular structure of acyclovir or 9-(2-hydroxyethoxy)methylguanine (Dollery, 1999:39).

Molecular weight:	225
Formula:	$C_8H_{11}N_5O_3$
Colour, odour and appearance:	A white almost white crystalline powder (Dollery, 1999:39).

2.8.1.2 Physicochemical properties

2.8.1.2.1 Melting point

Acyclovir is stated (BP) to melt with decomposition at approximately 230 C. Since lower melting points will promote permeation of the compounds through the skin (see § 2.4.3) acyclovir's high melting point will limit its permeation.

2.8.1.2.2 Dissociation constants

The drug has pK_as of 2.27 and 9.25. Acyclovir is in an unionised form at a physiological pH of 7.4 (Dollery, 1999:39).

2.8.1.2.3 Partition coefficient

The log P octanol/0.2 M phosphate buffer partition coefficient for acyclovir was determined as 0.018 (Dollery, 1999:39). For transdermal drug delivery a log P ± 3 is desired and as the log P decreases, diffusion through the intercellular lipids is hindered.

2.8.1.2.4 Solubility

Acyclovir is slightly soluble in water with maximum solubility of 2.5 $g.l^{-1}$(Dollery, 1999:39), insoluble in ethanol, practically insoluble in most organic solvents, soluble in dilute aqueous solutions of alkali hydroxides and mineral acids (Lund *et al.*, 1994:712).

2.8.1.3 Stability

Refrigeration of reconstituted solutions of acyclovir can result in the formation of a precipitate. The precipitate redissolves at room temperature. Acyclovir exhibited greater stability in an alkaline solution than in an acidic solution. When acyclovir was boiled for 10 minutes in 1 N sulphuric acid or in 1 N sodium hydroxide loss of 'potency' was about 12 % and 5 %, respectively (Lund *et al.*, 1994:712).

2.8.1.4 Pharmacology

2.8.1.4.1 Mechanism of action

Acyclovir is converted to acyclovir monophosphate principally *via* virus-coded thymidine kinase; the monophosphate is phosphorylated to the diphosphate *via* cellular guanylate kinase and then to the triphosphate *via* other enzymes (e.g., phosphoglycerate kinase, pyruvate kinase, phosphoenolpyruvate carboxykinase) (BP, 1993:1983).

2.8.1.4.2 Therapeutic use

- Treatment of herpes simplex keratitis;
- Treatment and prophylaxis (suppression) of herpes simplex infections of the skin and mucous membranes in immunocompetent individuals;
- Treatment of varicella zoster infections in immunocompromised and immuno-competent individuals;
- Prophylaxis of herpes simplex, varicella zoster and cytomegalovirus infections in the immunocompromised and
- Improvement of survival in patients with AIDS (Dollery, 1999:40).

2.8.2 Methotrexate

Methotrexate (MTX), amethopterine or N-[4-[[(2,4-diamino-6-pteridinyl)methyl]-methylamino]benzoyl]-L-glutamic acid is an analogue of folic acid (4-NH_2, *N*10-methylfolic acid) and is a widely used antimetabolite anticancer drug, with a role in the treatment of both haematological malignancies and solid tumours. It is also used commonly as immunosuppressive agent in the treatment of a number of autoimmune and inflammatory disorders (e.g., severe psoriasis and rheumatoid arthritis) (Dollery, 1999:90).

2.8.2.1 Identification

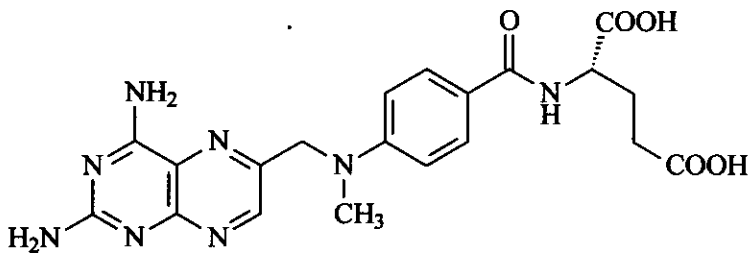


Figure 2.9: Molecular structure of methotrexate (Dollery, 1999:90).

Molecular weight:	454.5
Formula:	$C_{20}H_{22}N_8O_5$
Colour, odour and appearance:	MTX is a yellow to orange-brown crystalline powder (Dollery, 1999:90).

2.8.2.2 Physicochemical properties

2.8.2.2.1 Melting point

As discussed in § 2.4.3 lower melting points will promote permeation of the compounds through the skin. Methotrexate's relative high melting point will limited its permeation through the skin, because it is stated to melt in the range of 182 C to 189 C (Lund *et al.*, 1994:949).

2.8.2.2.2 Dissociation constants

The drug has pK_as of 3.8, 4.8, 5.6, (Lund *et al.*, 1994:949).

2.8.2.2.3 Partition coefficient

For transdermal drug delivery a log P of ±3 is desired; as the log P decreases, diffusion through the intercellular lipids is hindered. Wallace *et al* (1978:67) determined partition coefficient values for MTX between octanol and water to be 0.034 at pH 3.5, 0.21 at pH 5.3 (buffered suspensions) and 0.005 at pH 8.2 (solution in 0.5% bicarbonate) (Lund *et al.*, 1994:949).

2.8.2.2.4 Solubility

Methotrexate is practically insoluble in water, in ethanol, in chloroform, in 1,2-dichloro-ethane and in ether. It dissolves in solutions of mineral acids and in dilute solutions of alkali hydroxides and carbonates. It is slightly soluble in 6 M hydrochloric acid (Lund *et al.*, 1994:950).

2.8.2.3 Stability

Methotrexate, in solution, is subject to photolytic and thermal degradation. When methotrexate solutions (pH 8.3) were kept under laboratory fluorescent light at room temperature the major degradation products were identified by ultraviolet spectrophotometry and HPLC as 2,4-diaminio-6-pteridine carbaldehyde, 2-4-diamino-6-pteridine carboxylic acid and *p*-aminobenzoylglutamic acid. Degradation was subjected to general acid-base catalysis by various buffers. The temperature dependence of methotrexate degradation was examined in isotonic buffer-free solution (initially pH 8.5) over a range of 65 C to 95 C and an activation energy of 96.8 kJ/mol was established. The $t_{10\%}$ of this solution (initial pH 8.5) at 25 C and at 4 C was predicted to be 4.5 years and 88.7 years, respectively.

Methotrexate solutions (1.25 to 12.5 mg/ml in sodium chloride 0.9% for injection) were physically and chemically stable for up to 15 weeks of refrigerated storage followed by an additional week at room temperature in polyvinyl chloride (Viaflex) or glass containers, protected from light (Lund *et al.*, 1994:950).

2.8.2.4 Pharmacology

2.8.2.4.1 Mechanism of action

Methotrexate, an antimetabolite-type cytotoxic agent that acts by inhibiting dihydrofolate reductase and interfering with folic acid synthesis, has long been used for the suppression of various malignant diseases. In low dosage it has proved valuable in some patients with rheumatoid arthritis and shares pride of place with sulfasalazine as a relatively rapid-acting disease modifying agent (Gibbon, 2000:332).

2.8.2.4.2 Therapeutic use

- It has antitumour activity against a wide variety of tumours and is used most often in combination chemotherapy regimens;
- In treatment of severe psoriasis and with increasing frequency in other immunologically mediated disease states (Dollery, 1999:93b).

2.9 SUMMARY

The skin forms an attractive and accessible route of delivery for systemic drugs because of the problems associated with other methods of administration, i.e. oral and parental. However, few drugs are able to passively diffuse across the uppermost layer of the skin, the stratum corneum, as a result if its effective barrier properties. The stratum corneum is lipophilic, with intercellular lipid lamellae forming a conduit through which drugs must diffuse to access the systemic circulation. Effective drug therapies must overcome the challenge of finding a technology to administer and deliver the required quantity of drug into or through the skin.

Through interactions with the skin, delivery vehicles provide a complementary action by which the skin can be 'prepared' to allow passage of the drug. An ideal drug must possess both lipoidal and aqueous solubilities to partition through the various layers of the skin. Although there are many physiological and physiochemical properties that influence transdermal drug delivery it can be promoted by several techniques, including supersaturation, drug modification and chemical enhancers. Thus, in this study, methods to circumvent the barrier function of skin by utilising lamellar gel phase systems and Emzaloid® will be investigated.

CHAPTER 3

THE PERMEATION OF TRANSDERMAL DRUG DELIVERY VEHICLES

3.1 INTRODUCTION

The stratum corneum forms the outermost layer of the skin and is essentially a multilamellar lipid milieu punctuated by protein-filled corneocytes. These corneocytes augment membrane integrity and significantly increase membrane tortuosity. The lipophilic character of the stratum corneum in conjunction with its intrinsic tortuosity ensures that it almost always poses the principal barrier to the entry of drug molecules into the skin (Kalia *et al.*, 2001:159).

Numerous challenges are posed to transdermal drug delivery, arising from skin impermeability and biological variation in any given population. Various approaches to reversibly remove the barrier resistance have been investigated to increase the number of drug candidates for transdermal drug delivery. These approaches include the utilisation of penetration enhancers, chemical modification of drugs (prodrugs), electrical methods such as iontophoresis, ultrasound and specialised drug delivery vehicles such as microemulsions and vesicles (Suhonen *et al.*, 1999:149).

In this study, the influence of Physiogel® and Emzaloid® was investigated as potential transdermal drug delivery vehicles for the selected drugs.

3.2 MATERIALS

Acyclovir and methotrexate (selected model drugs) were acquired from Sigma-Aldrich Co. (Johannesburg, SA). Anhydrous dipotassium hydrogen orthophosphate (K_2HPO_4), HPLC-grade methanol, phosphoric acid as well as disodium hydrogen ortophosphate dihydrate ($Na_2HPO_4.2H_2O$), sodium chloride (NaCl) and sodium dihydrogen orthophosphate dihydrate ($NaH_2PO_4.2H_2O$) were purchased from Merck Laboratory Supplies (Midrand, South Africa).

Physiogel Dermaquadrille® and Physiogel NT®, the lamellar gel phase systems were supplied by Kush-Kosmetiek in Germany. The Emzaloid® was formulated by Ms Anne Grobler (Department of Pharmaceutics, School of Pharmacy, North-West University, Potchefstroom, South Africa). HPLC-grade water (double deionised) produced with a Milli Q 50 water purification system (Millipore, Milford, USA) was employed in all studies.

3.3 ANALYTICAL METHODS

3.3.1 Analysis of acyclovir

3.3.1.1 The HPLC system

An Agilent® 1100 Series HPLC system was used for all analyses. The system comprised of an Agilent® 1100 pump, UV detector and autosampler. The HPLC system was interfaced with data acquisition and analysis software (Chemostation Rev A06.02) as configured for a Hewlett Packard® computer.

A Luna (5μ, 250 x 4.60mm) C18 column was employed for sample separation and was fitted to the appropriate Phenomemex® Security Guard precolumn.

Analyses were preformed with the mobile phase comprising of 90% aqueous buffer (6.69 g K_2HPO_4 per litre) and 10% methanol. The mobile phase was degassed by filtration (0.45 μm Millipore® filter) and the pH was adjusted to pH 7.00 (phosphoric acid).

The samples were eluted at a flow rate of 1.0 ml/min with retention times ranging 5-6 minutes. A stop time of 15 minutes was set. Analyses were preformed at a detection wavelength of 254 nm at room temperature (25 ± 1 °C).

3.3.1.2 Preparation of standard solution

Ten milligrams of acyclovir was weighed and quantitatively dissolved in a 100 ml volumetric flask filled with HPLC grade water to obtain a concentration of 100 μg/ml (stock solution). Standard solutions with concentrations of 0.05, 0.30, 0.50, 2.00 and 5.00 μg/ml were prepared on a daily basis from the stock solution.

3.3.1.3 Validation of the HPLC method

The objective of the HPLC validation was to ensure that the analytical methods were sensitive and reliable in the determination of the amount of drug that permeates the skin.

3.3.1.3.1 Linearity

The linearity of an analytical method is described as its ability (within a given range) to obtain the results that are directly proportional to the concentration (amount) of analyte in the sample. The linearity of acyclovir was determined by plotting the peak area to concentration (Table 3.1). The standard concentrations were prepared as described (§ 3.3.1.2).

Table 3.1: Average AUC values of acyclovir standards.

Standard (µg/ml)	AUC
0.03	12.70
0.05	18.30
0.30	118.2
0.50	165.1
2.00	654.7
5.00	1647.6
Slope	328.01
Y-intercept	5.32
r^2	0.99

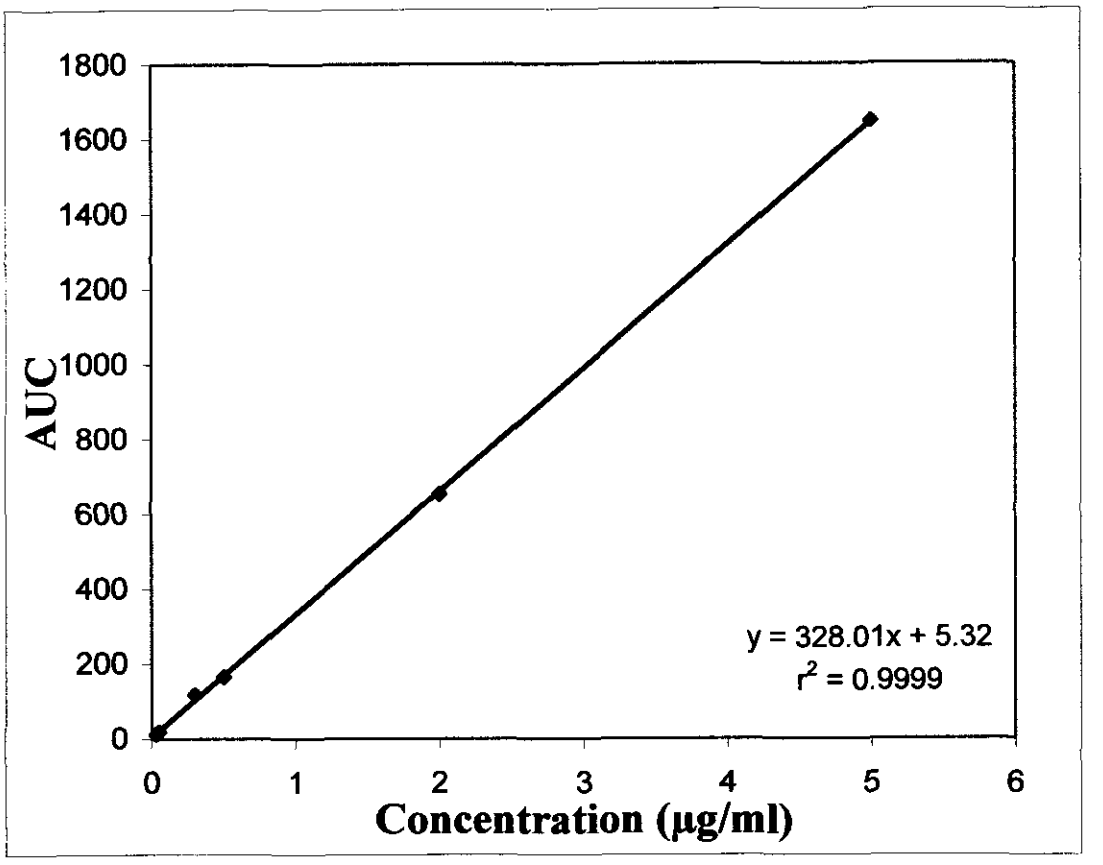


Figure 3.1: Linear regression curve of acyclovir standards.

3.3.1.3.2 Precision

The precision of an analytical procedure expresses the proximity of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision was investigated in terms of interday (reproducibility) and intraday (repeatability) variation.

- **Interday precision**

The interday precision was determined by performing HPLC-analysis of five different standards of acyclovir on three consecutive days. The results can be seen in Table 3.2 with all results within acceptable limits.

Table 3.2: Interday precision parameters of acyclovir standards.

Standards (µg/ml)	Day 1	Day 2	Day 3	AUC Mean	SD	%RSD
0.03	12.70	12.40	56.71	27.27	25.5	93.5
0.05	18.30	22.54	22.12	20.99	2.34	11.1
0.30	118.2	104.9	108.9	110.7	6.82	6.16
0.50	165.1	164.4	164.4	164.7	0.39	0.24
2.00	654.7	667.9	652.7	658.4	8.28	1.26
5.00	1647.6	1674.2	1640.8	1654.2	17.7	1.07

- **Intraday precision**

The intraday precision was determined via analysis of five known standards at three times during the same day. The results can be seen in Table 3.3.

Table 3.3: Intraday precision parameters of acyclovir standards.

Concentration (µg/ml)	AUC 1	AUC 2	AUC 3	AUC Mean	SD	%RSD
0.03	12.70	12.60	12.60	12.63	0.06	0.46
0.05	18.30	18.20	18.20	18.23	0.06	0.32
0.30	118.2	118.2	118.2	118.2	0.00	0.00
0.50	165.1	165.2	165.2	165.1	0.06	0.03
2.00	654.7	654.4	654.4	654.5	0.17	0.03
5.00	1647.6	1648.3	1648.9	1648.2	0.65	0.04

3.3.1.3.3 Limit of detection

Limit of detection (LOD) is the lowest concentration of the analyte that can be detected, but not necessarily quantified. On a signal-to-noise ratio of three-to-one, the LOD for acyclovir was 0.5 ng/ml.

3.3.1.3.4 Limit of quantification

The limit of quantification (LOQ) is the lowest concentration of the analyte that can be determined with acceptable precision and accuracy. The LOQ of acyclovir was expressed as a concentration at a signal-to-noise ratio of ten-to-one and was determined as 3.5 ng/ml.

3.3.1.3.5 Selectivity

Selectivity is the ability of the method to selectively detect the analyte in the presence of other compounds that may interfere with analyte detection. Methanol as well as the receptor phase (PBS) was separately analysed and illustrated no interference.

3.3.1.3.6 System repeatability

In an attempt to evaluate the repeatability of the peak area calculations, samples with known concentrations 5 µg/ml and 30 ng/ml were injected 6 times. The variation in response (%RSD) of the repeatability of the peak area for the concentration 5 µg/ml was found to be 0.13% and that of the retention times was 0.135%, for the concentration 30 ng/ml the peak area was found to be 1.06% and that of the retention time was 0.101%.

3.3.2 Analysis of methotrexate

3.3.2.1 The HPLC system

An Agilent® 1100 Series HPLC system was used for all analyses. The system comprised of an Agilent® 1100 pump, UV detector, autosampler. The HPLC system was interfaced with data acquisition and analysis software (Chemostation Rev A06.02) as configured for a Hewlett Packard® computer

A Luna (5µ, 250 x 4.60mm) C18 column was employed for sample separation and was fitted to the appropriate Phenomemex® Security Guard precolumn

Analyses were preformed with a mobile phase comprising of 70% aqueous buffer (6.69 g K_2HPO_4 per litre) and 30% methanol. The mobile phase was degassed by filtration (0.45 µm Millipore® filter) and pH was adjusted to pH 7.00 (phosphoric acid).

The samples were eluted at a flow rate of 0.5 ml/min with retention times ranging 8-10 minutes. A stop time of 15 minutes was set. Analyses were preformed at a detection wavelength of 302 nm at room temperature (25 ± 1 °C).

3.3.2.2 Preparation of standard solutions

Ten milligrams of methotrexate was weighed and dissolved in a 100 ml volumetric flask filled with HPLC grade water to obtain a concentration of 100 µg/ml (stock solution). Standard solutions with concentrations of 0.05, 0.25, 0.50, 1.00, 2.00 and 5.00 µg/ml were prepared on a daily basis from the stock solution.

3.3.2.3 Validation of the HPLC method

3.3.2.3.1 Linearity

The linearity of an analytical method is its ability (within a given range) to obtain the results that are directly proportional to the concentration (amount) of analyte in the sample. The linearity of methotrexate was determined by plotting the peak area to concentration (Table 3.4). The standard concentrations were prepared as describe in § 3.3.2.2.

Table 3.4: Average AUC values of methotrexate standards.

Standards (µg/ml)	AUC
0.05	33.95
0.10	61.85
0.25	141.3
0.50	311.9
1.00	572.2
2.00	1168.6
5.00	3045.4
Slope	607.7
Y-intercept	10.49
r^2	0.99

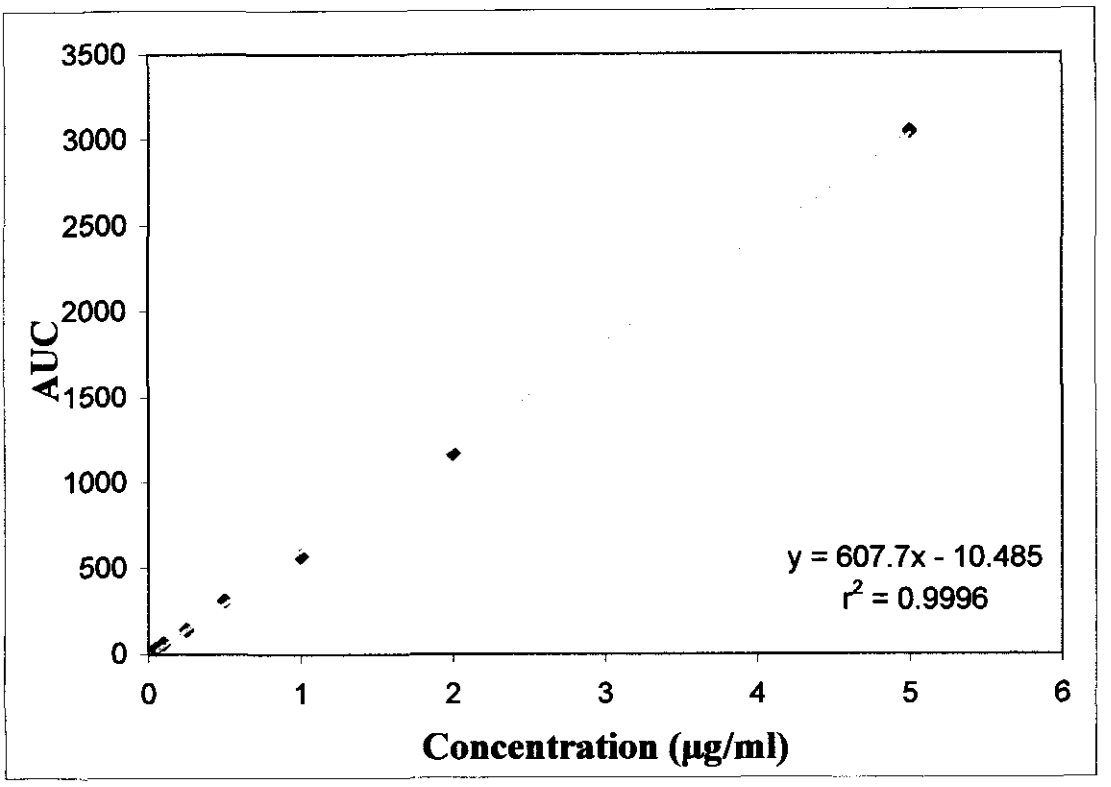


Figure 3.2: Linear regression curve of methotrexate standards.

3.3.2.3.2 Precision

The precision of an analytical procedure expresses the proximity of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision was investigated in terms of interday (reproducibility) and intraday (repeatability) variation.

- **Interday precision**

The interday precision was determined by performing HPLC-analysis of five different standards methotrexate on three consecutive days. The results can be seen in Table 3.5 and were within acceptable limits.

Table 3.5: Interday precision parameters of methotrexate standards.

Standards (µg/ml)	Day 1	Day 2	Day 3	AUC Mean	SD	% RSD
0.05	33.95	50.06	35.09	39.71	8.99	22.6
0.10	61.85	62.50	60.31	61.55	1.12	1.83
0.25	141.3	158.0	148.9	149.4	8.35	5.59
0.50	311.9	314.2	298.2	308.1	8.66	2.81
1.00	572.2	593.4	574.5	580.0	11.6	2.01
2.00	1168.6	1138.5	1164.4	1157.1	16.3	1.41
5.00	3045.4	3075.4	2993.8	3038.2	41.3	1.36

- **Intraday precision**

The intraday precision was determined via analysis of five known standards at three times during the same day. The results can be seen in Table 3.6.

Table 3.6: Intraday precision parameters of methotrexate standards.

Standards (µg/ml)	AUC 1	AUC 2	AUC 3	AUC Mean	SD	%RSD
0.05	30.93	29.32	35.09	31.78	2.98	9.38
0.10	57.79	59.31	60.31	59.14	1.27	2.15
0.25	156.6	140.1	148.9	148.6	8.24	5.55
0.50	293.2	282.0	298.2	291.2	8.28	2.84
1.00	594.9	561.0	574.5	576.8	17.1	2.96
2.00	1183.9	1141.5	1164.4	1163.3	21.2	1.83
5.00	2970.2	2938.2	2993.8	2967.4	27.9	0.94

3.3.2.3.3 Limit of detection

The limit of detection (LOD) is the lowest concentration of the analyte that can be determined but not necessarily quantified. The LOD of methotrexate was expressed as a concentration at a signal-to-noise ratio of three-one and was determined as 0.01 µg/ml.

3.3.2.3.4 Limit of quantification

Limit of quantification (LOQ) is the lowest concentration of the analyte that can be determined with acceptable precision and accuracy. On a signal-to-noise ratio of ten-to-one, the LOQ for methotrexate was 0.02 μg/ml.

3.3.2.3.5 Selectivity

Selectivity is the ability of the method to selectively detect the analyte in the presence of other compounds that may interfere. Methanol as well as the receptor phase (PBS) was separately analysed and illustrated no interference.

3.3.2.3.6 System repeatability

In an attempt to evaluate the repeatability of the peak area calculations, samples with known concentrations 5 μg/ml and 0.02 μg/ml were injected 6 times. The variation in response (%RSD) of the repeatability of the peak area for the concentration 5 μg/ml was found to be 0.92% and that of the retention times was 0.53%, for the concentration 0.02 μg/ml the peak area was found to be 0.54% and that of the retention time was 0.25%.

3.4 METHOD FOR PREPARATION OF THE TWO DELIVERY VEHICLES

3.4.1 The lamellar gel phase systems

The lamellar gel phase systems consisted of two types, Physiogel® Dermaquadrille and Physiogel® NT. The preparation in this study, Physiogel®, complies with German regulations for cosmetics with regards to its production and composition. Both the Physiogel® creams have a Derma-Membrane Structure (DMS) and contain the components as given in Table 3.7.

Table 3.7: The Physiogel® ingredients.

Physiogel® Dermaquadrille	***Physiogel® NT***
Aqua	Aqua
Caprylic/ Capric triglyceride	Olea europaea
Glycerin	Glycerin
Pentylene glycol	Pentylene glycol
Cocos nucifera	Palm glycerides
Hydrogenated lecithin	Olus
Butyrospermum parkii	Hydrogenated lecithin
Squalane	Squalane
Hydroxyethylenecellulose	Bataine
Sodium carbomer	Palmitamide MEA
Xanthan gum	Acetamide MEA
Carbomer	Sarcosine
Ceramide 3	Hydroxyethylcellulose
	Sodium carbomer
	Carbomer
	Xanthan gum

The specific quantity of each ingredient and the formulation method could not be specified by Kush Kosmetiek. For the experiments each Physiogel® was formulated to contain 1% of the

active ingredient in 40 g Physiogel® and was left for one week at room temperature to allow diffusion of all active particles.

3.4.2 Emzaloid®

The Emzaloid® was used in a cream formulation with lipid bilayer formulation. The Emzaloid® was formulated by Ms Anne Grobler (Department of Pharmaceutics, School of Pharmacy, North-West University, Potchefstroom, South Africa).

3.5 EXPERIMENTAL METHODS

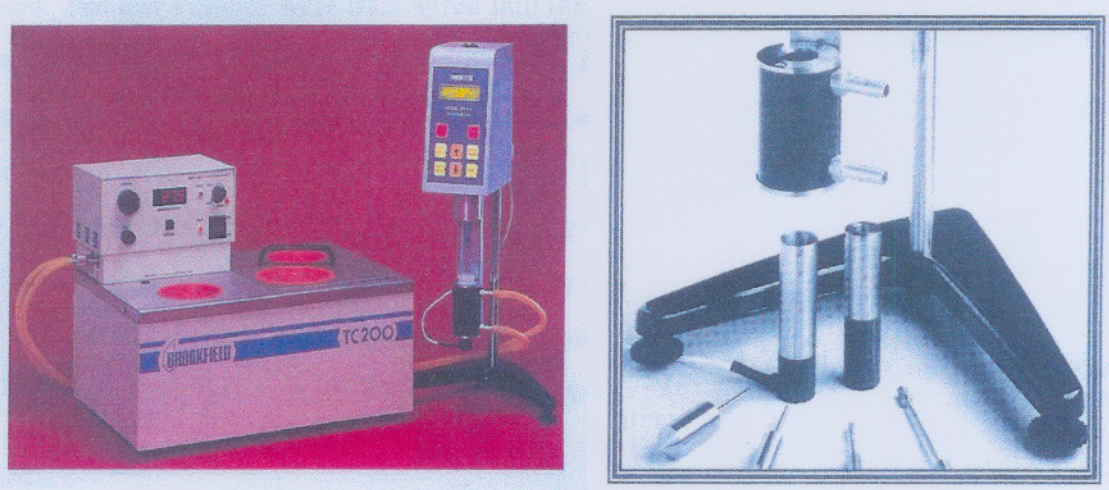
3.5.1 Confocal laser scanning microscopy

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

3.5.2 Viscosity

Rheology is the science of the flow behaviour of matter. The initial investigation of rheology is characterisation of the viscosity (the resistance of flow that arise from interparticle friction) properties of the fluid.

Material rheological characteristics are paramount in the prediction of its purity, performance in dipping of coating operations and processing properties. Viscosity data provides an accurate benchmark of batch to batch consistency (Brookfield, 1998:2).



a) **b)**

Figure 3.3: a) The Brookfield® Model DV-II+ viscometer with a b) Brookfield® small sample adapter.

Viscosity was determined on a calibrated Brookfield® Model DV-II+ viscometer (Figure 3.3a) with a Brookfield® small sample adapter and RV spindles (number 25) (Figure 3.3b). The Brookfield® small sample adapter was used for its rheological evaluation of materials where sample volume is limited. The sample chamber could be easily exchanged and the flow jacket controls the temperature at 25 °C for each sample measurement (Brookfield, 1998:22).

The test samples were transferred into the sample chamber that fitted into the flow jacket so that precise temperature control could be achieved. The sample was left to stabilise for 1 hour to assure that no air bubbles were present. The stirring action of the spindle and the small sample volume ensured a stable temperature gradient across the sample. Direct readout of sample temperature was provided using sample chambers with embedded RTD sensors connected to the Brookfield® Digital Temperature Indicator (DTI). The samples were measured at different rpm's to generate viscosity, shear stress and shear rate values.

3.5.3 Measurement of drug release from two delivery vehicles (dissolution)

The release rates of the drugs from the two delivery vehicles (lamellar gel phase systems and Emzaloid®) were measured using a release unit that fits directly onto the VanKel® VK 700 six station apparatus. Six stations (containing 200 ml dissolution vessels) were employed in the experiments (Figure 3.4a). The enhancer cell consisted of a cap, a washer, a membrane, a ring and the body of that is the reservoir for the sample (Figure 3.4b). The reservoir of the dissolution cell was filled in each experiment with the delivery vehicles and covered with a cellulose acetate membrane (pore size 0.45 μm), ensuring that no air bubbles were present in the reservoir between the sample and the membrane. The cell was capped and placed in the dissolution vessel that contained the receptor medium (190 ml PBS). The experiments were performed at 32 °C and the rotation speed of the paddles was set at 150 rpm. Samples of 200 μl were extracted, using a micropipette, at time intervals of 30, 60, 120, 180, 240, 300, 360 minutes. The samples were then analysed by HPLC methods in order to determine the content of the active in the samples.

a) b)

Figure 3.4: a) The dissolution vessel and b) the enhancer cell for the VanKel® dissolution apparatus.

3.5.4 Transdermal diffusion studies

3.5.4.1 Skin preparation

The abdominal skin used in the permeation studies was obtained from white female donors. The full thickness skin was frozen at –20 °C not exceeding 24 hours after excision. Before separation, the skin was thawed at room temperature. The excess fat was removed and the epidermal layers were separated by immersing the skin for 1 minute at 60 °C. The epidermis was carefully removed, placed on a Whatman® filter and left to dry. When dry it was wrapped in aluminium foil and frozen at –20 °C for a maximum of two months. The frozen skin was examined for defects and cut into circles of approximately 10 mm diameter preceding mounting onto the diffusion apparatus.

3.5.4.2 Diffusion studies

Vertical Franz diffusion cells (Figure 3.2) with a maximum capacity 2.3 ml and a 1.075 cm^2 diffusion area were used. The epidermal layer was mounted between the donor and receptor compartments with the stratum corneum facing upwards. The receptor compartment was filled with PBS and the Franz cells were placed in the water bath 1 hour prior to the experiment at 37 °C on a magnetic stirrer to equilibrate.

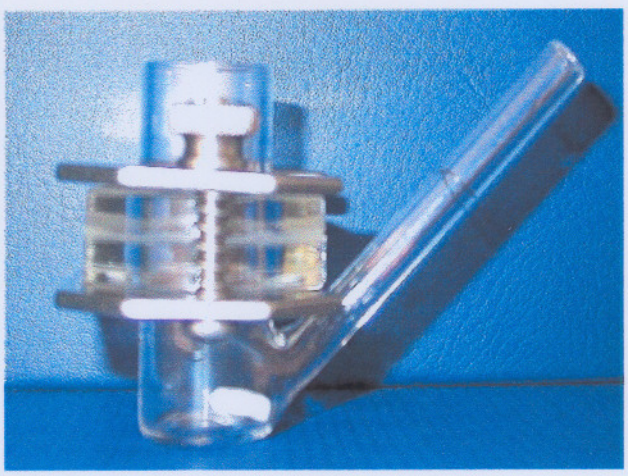


Figure 3.5: Vertical Franz diffusion cell.

The donor compartment contained 1 ml of a 1.0 % aqueous solution of acyclovir, filtered with a 0.22 nm nylon filter and methotrexate in water:propylene glycol (50:50) with pH of 4.00-

5.00. To ensure that the correct quantity of the delivery vehicle was applied to the donor compartment, the delivery vehicle was placed in a syringe, weighed before and after applying to the donor compartment. The amount applied (difference in weight) to the donor compartment for both delivery vehicles were ± 0.2 g and evenly spread over the whole donor compartment area. This compartment was covered with Parafilm® in order to prevent evaporation.

The receptor compartment contained phosphate buffered saline (PBS) at physiological pH 7.00, 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 to1000 ml. At predetermined intervals at 2, 4, 6, 8, 10, 12, 24 hours the entire content of the receptor compartment was withdrawn and replaced with equivalent amount of fresh PBS at 37 °C. Each sample was directly assayed by HPLC to determine the concentration of the active in the receptor medium.

3.6 DATA ANALYSIS

3.6.1 Measurement of the drug released rate

The amount of the drug released from the drug delivery vehicles (μg/cm^2) as a function of the square root time (minutes) was calculated by multiplying the concentration of the drug (μg/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

Diez-Sales *et al.*, (1991:3) developed an equation (3.1) to describe the amount of the drug crossing a membrane at a given time.

$$Q(t) = AKhC\left[D\frac{t}{h^2} - \frac{1}{6} - \frac{2}{\pi^2}\sum_{n-1}^{\infty}\frac{(-1)^n}{n^2}\exp\left(\frac{-Dn^2\pi^2 t}{h^2}\right)\right]$$

(Equation 3.1)

where:

- Q(t) is the quantity passed through the membrane at a given time (t);
- A is the actual surface area;
- K is the partition coefficient between the membrane and the donor vehicle;
- h is the membrane thickness;
- D is the diffusion coefficient of the permeant in the membrane and
- C is the concentration in the donor solution.

As *t* approaches infinity the exponential terms become negligible and the linear steady-state expression is given by the following equation (3.2):

$$\boxed{Q(t) = AKhQ\left[D\frac{t}{h^2} - \frac{1}{6}\right]}$$

(Equation 3.2)

The cumulative amount of the drug that penetrated through the epidermis (n = 6) per unit area (μg/cm^2) was calculated by multiplying the concentration (μg/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 corrected amount of drug, Q, was then plotted as a function of time *t* (h). The flux (μg/cm^2/h) was obtained from the slope of the linear part of the curve and was calculated by the following equation (3.3):

$$\boxed{J_s = \frac{D_m \Delta C_m}{L}}$$

(Equation 3.3)

where:

- J_s is the steady-state flux;
- D_m is the membrane diffusivity;
- ΔC_m is the concentration gradient across the membrane;
- L is the length of the membrane.

The enhancing effect of the drug delivery vehicles on the permeation of the drugs were calculated using the enhancement ratios of flux that is the ratio of the flux with the enhancer and that without the enhancer (Equation 3.4) (El-Katton, *et al.*, 2000).

$$E.R. = \frac{FLUX_{\ WITH\ (ENHANCER\ )}}{FLUX_{\ WITHOUT\ (ENHANCHER\ )}}$$

(Equation 3.4)

3.7 RESULTS

Three sets of experiments were conducted on each drug for the three different delivery vehicles:

- The viscosity was measured with a Brookfield® viscometer
- Measurement of the drug released form the delivery vehicles with the VanKel® dissolution apparatus
- Transdermal diffusion studies with vertical Franz diffusion cells

3.7.1 Acyclovir

3.7.1.1 Viscosity

The viscosity of the two Physiogel® creams was measured. This measurement was made to determine if the investigated drugs had any influence on the internal cream structure; therefore, viscosity of the creams. Two sets of viscosity measurements had been done for each Physiogel® cream, first the blank cream and then the Physiogel® with acyclovir. In the following graphs the viscosity (cP) were plotted against the rpm (agitation rate) were plotted for both Physiogel® NT and Physiogel® Dermaquadrille.

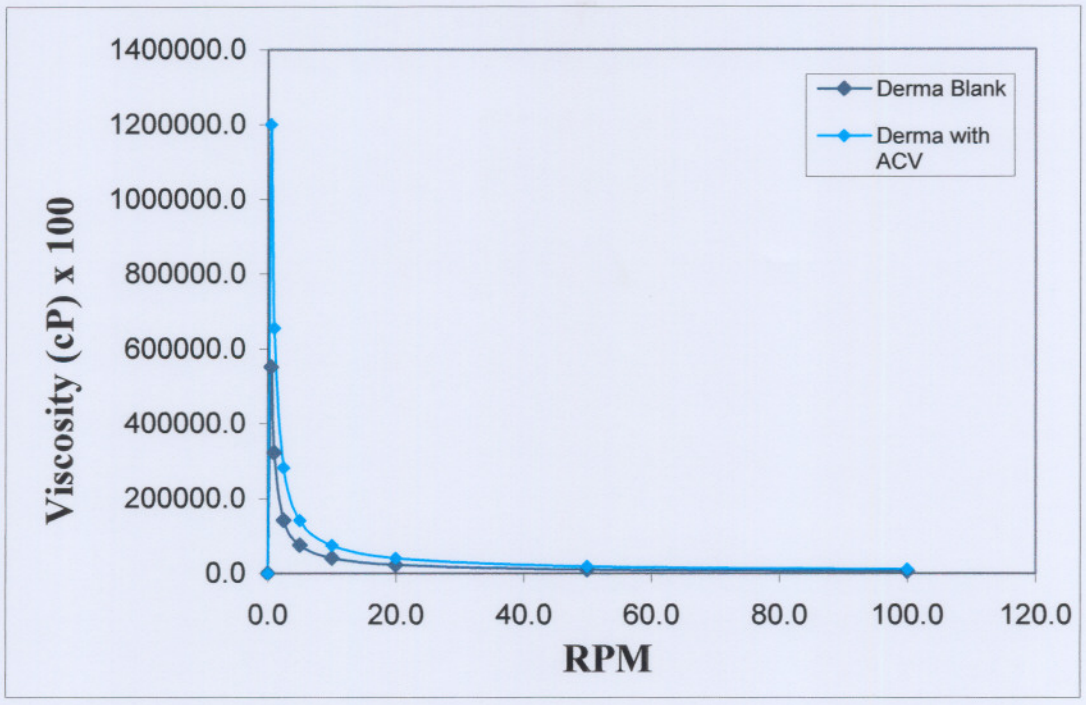


Figure 3.6: The viscosity profile of Physiogel® Dermaquadrille (Derma).

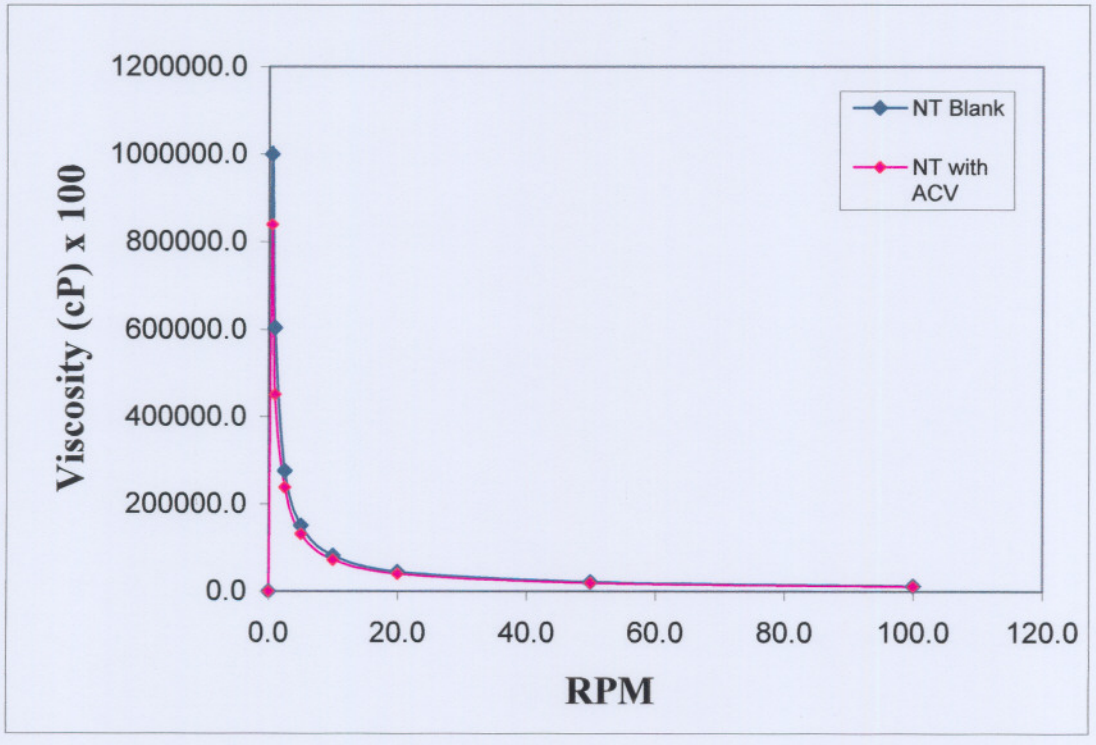


Figure 3.7: The viscosity profile of Physiogel® NT (NT).

3.7.1.2 Measurement of the drug released rate for the delivery vehicles

The released rate of acyclovir from the three delivery vehicles, Physiogel® NT, Physiogel® Dermaquadrille and Emzaloid® were measured over a six hour period using membrane diffusion. The amount of the drug released from each delivery vehicle (μg/cm^2) as a function of square root time (√h) was calculated by multiplying the concentration of the drug (μg/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). The results are given in figure 3.8.

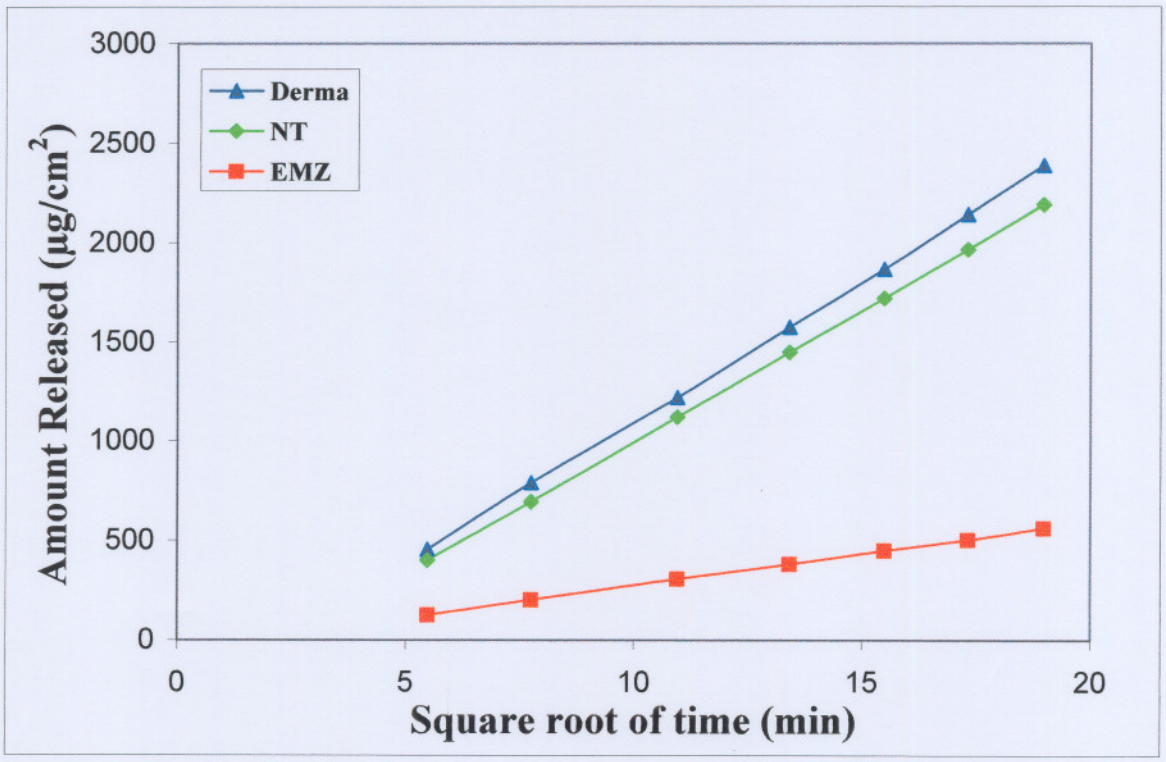


Figure 3.8: The amount of acyclovir released (μg/cm2) from the three delivery vehicles (n = 6); Physiogel® Dermaquadrille (Derma), Physiogel® NT (NT) and Emzaloid® (EMZ) as a function of the square root of time (min).

3.7.1.3 Transdermal diffusion studies

The permeation of acyclovir was investigated in the presence of three drug delivery vehicles to determine if it had any effect on the penetration of acyclovir through the skin. The *in vitro* percutaneous absorption profiles were calculated and are given in Table 3.8.

Table 3.8: The effect of the three delivery vehicles on the percutaneous diffusional parameters of acyclovir.

Acyclovir	**Flux (μg/cm^2/h), n = 6**	**ER_{Flux}**
Control	0.25 ± 0.10	-
Physiogel® Dermaquadrille	0.33 ± 0.11	1.32
Physiogel® NT	0.88 ± 0.03	0.32
Emzaloid®	0.73 ± 0.23	2.92

The flux (J_s ± standard deviation) was determined from the slope of the linear portion of the cumulative amount *versus* time plot. The enhancement ratios of acyclovir were calculated from the flux of the delivery vehicles divided by the flux of the control.

The mean flux and standard deviation of acyclovir in the three drug delivery vehicles as well as the control were plotted and are presented in Figure 3.9. The transdermal flux values of the control experiments were lower than that of the delivery vehicles. The flux value of Physiogel® NT was lower than that of the control experiment. Emzaloid® affected a two–fold higher acyclovir permeation compared to the control values. Emzaloid® additionally superseded the flux illustrated by the Physiogel®. The statistical analysis of the flux values showed that all three delivery vehicles had a significant effect on acyclovir permeation ($p < 0.1$).

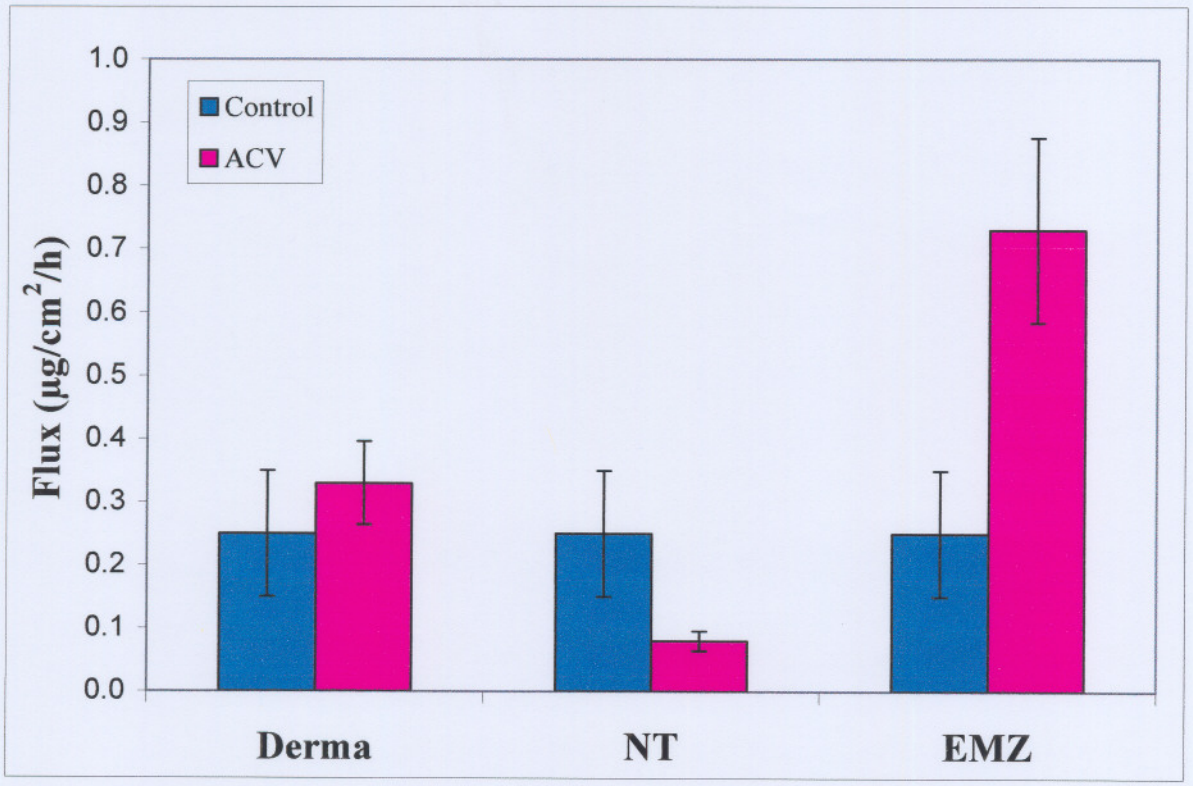


Figure 3.9: The mean transdermal flux ± SD of acyclovir in the three drug delivery vehicles; Physiogel® Dermaquadrille (Derma), Physiogel® NT (NT) and Emzaloid® (EMZ).

The E.R. value of Emzaloid® (2.92) is two times higher than of the E.R. values of the Physiogel® Dermaqaudrille (1.32). A lower enhancement was observed with the Physiogel® NT (0.32) compared to Physiogel® Dermaquadrille.

3.7.2 Methotrexate

3.7.2.1 Viscosity

This measurement of the viscosity was an indication of internal structure stability of the creams in the presence of the investigated drugs. Only the viscosity of the two Physiogel® creams was measured. For each Physiogel® two sets of measurement were done, the blank cream was first measured, subsequently the Physiogel® with methotrexate. In the following graphs the viscosity (cP) against the rpm were plotted for both Physiogel® NT and Physiogel® Dermaquadrille.

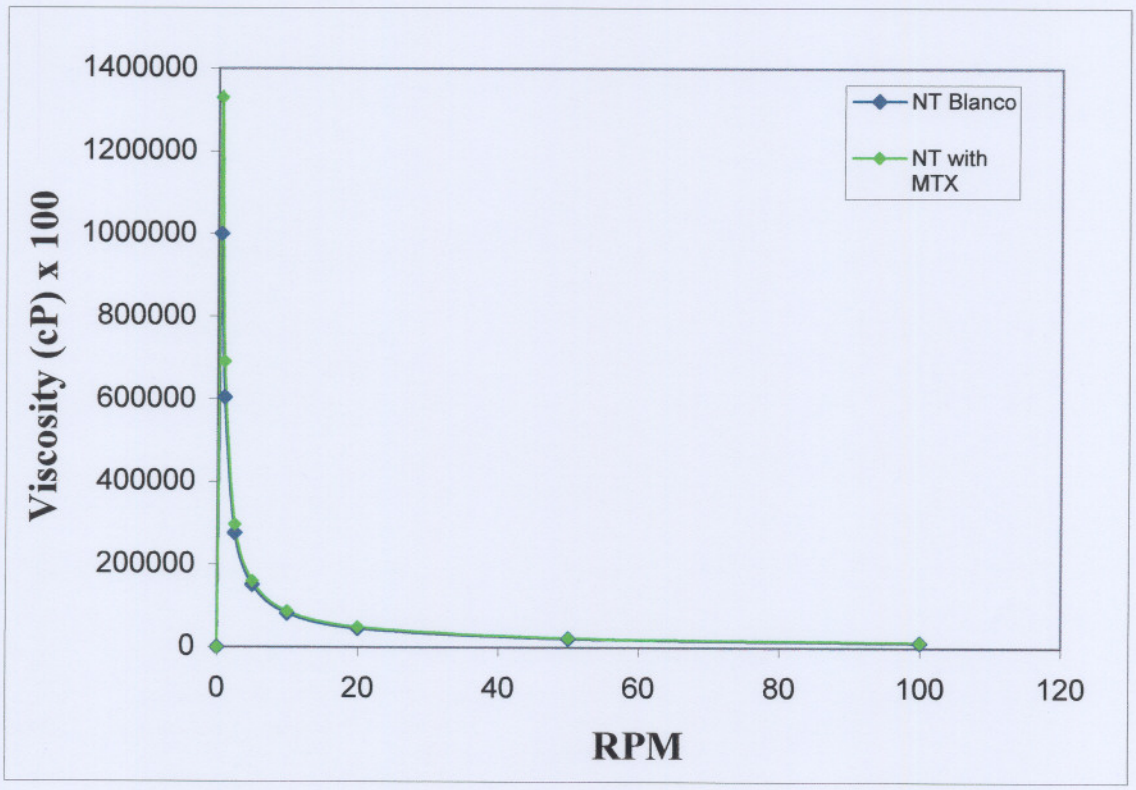


Figure 3.10: The viscosity profile of Physiogel® NT (NT).

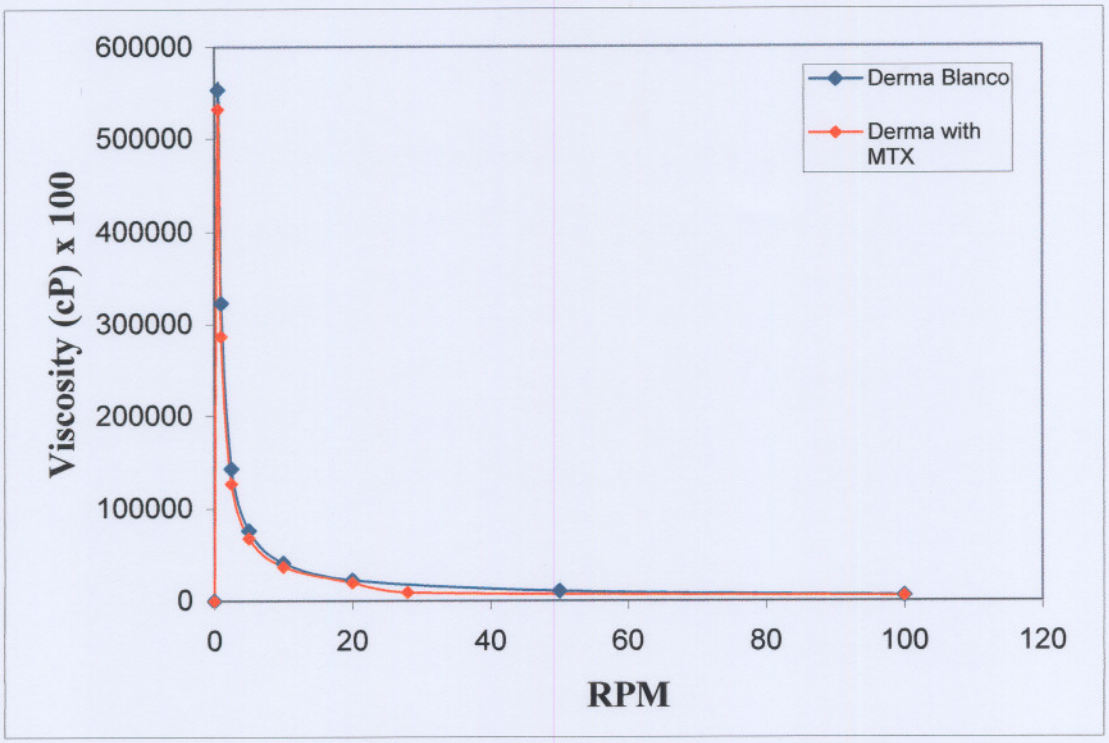


Figure 3.11: The viscosity profile of Physiogel® Dermaquadrille (Derma).

3.7.2.2 Measurement of the drug released rate for the delivery vehicles

The release rate of methotrexate from the three delivery vehicles, Physiogel® NT, Physiogel® Dermaquadrille and Emzaloid® was measured over a six hour period using membrane diffusion. The amount of the drug released from each delivery vehicle ($\mu g/cm^2$) as a function of square root time ($\sqrt{h}$) was calculated by multiplying the concentration of the drug ($\mu g/ml$) 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). The results are given in Figure 3.12.

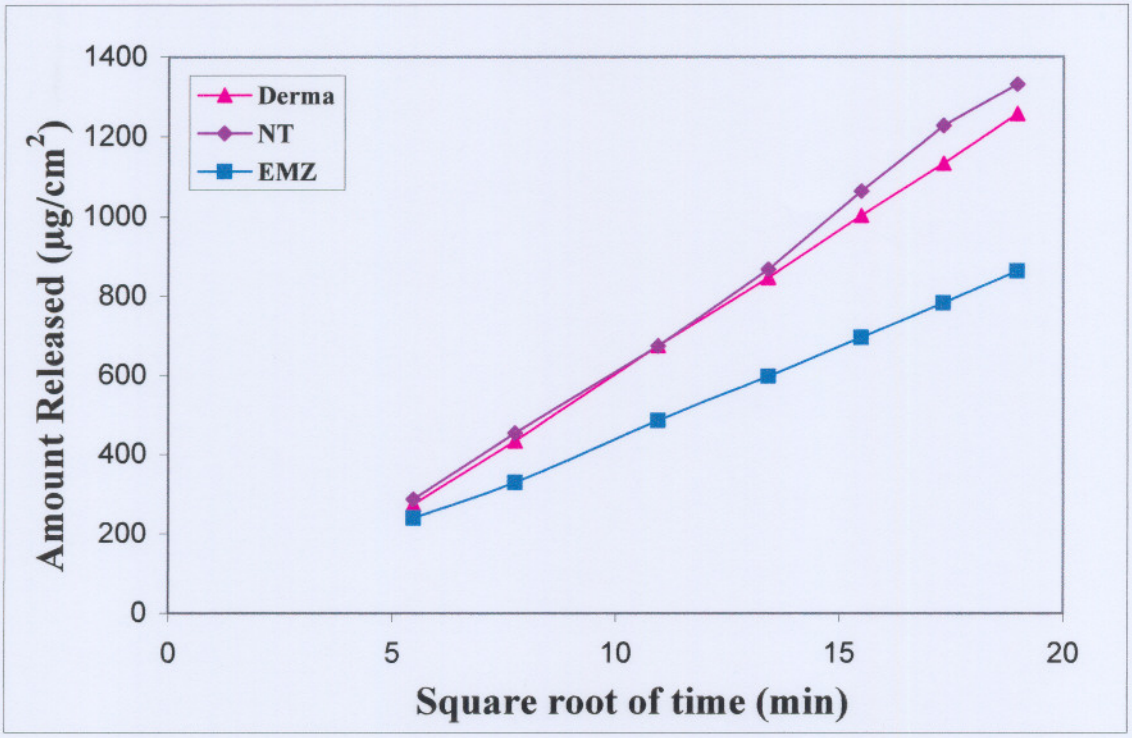


Figure 3.12: The amount of methotrexate released (µg/cm2) from the three delivery vehicles (n = 6); Physiogel® Dermaquadrille (Derma), Physiogel® NT (NT) and Emzaloid® (EMZ) as a function of the square root of time (min).

3.7.2.3 Transdermal diffusion studies

The permeation of methotrexate was investigated in the presence of three drug delivery vehicles to determine if it had any effect on the penetration of methotrexate through the skin. The *in vitro* percutaneous absorption profiles were calculated and are given in Table 3.9.

Table 3.9: The effect of the three delivery vehicles on the percutaneous diffusional parameters of methotrexate.

Methotrexate	**Flux ($\mu g/cm^2/h$), $n = 6$**	**ER_{Flux}**
Control	0.06 ± 0.01	-
Physiogel® Dermaquadrille	0.06 ± 0.02	1.00
Physiogel® NT	0.07 ± 0.02	1.22
Emzaloid®	0.09 ± 0.04	1.56

The flux (J_s ± standard deviation) was determined from the slope of the linear portion of the cumulative amount *versus* time plot. The enhancement ratios of methotrexate were calculated from the flux of the delivery vehicles divided by the flux of the control.

The mean flux and standard deviation of methotrexate in the three drug delivery vehicles as well as the control were plotted and are presented in Figure 3.13. No difference in flux value was found between the MTX fluxes of the control and Physiogel® Dermaquadrille. A higher flux value was obtained from both Physiogel® NT and Emzaloid® compared to that of the control experiments. Physiogel® Dermaquadrille flux value was equal to the control experiment. Emzaloid® has the highest permeation of methotrexate through the skin if compared to Physiogels® and control experiments. The statistical analysis of the flux values showed that all three delivery vehicles had a significant effect on methotrexate permeation ($p < 0.05$).

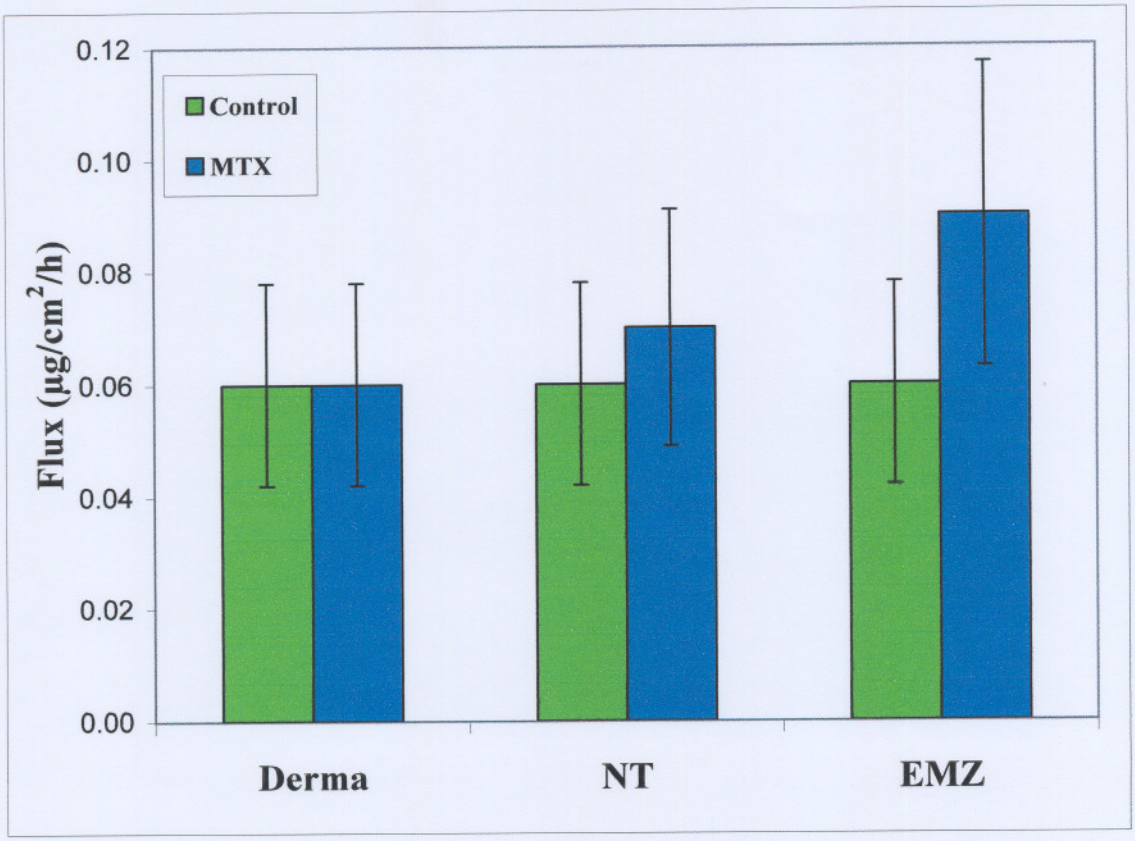


Figure 3.13: The mean transdermal flux ± SD of methotrexate in the three drug delivery vehicles; Physiogel® Dermaquadrille (Derma), Physiogel® NT (NT) and Emzaloid® (EMZ).

The E.R. ratio of Emzaloid® (1.56) is higher than the E.R. ratio of the Physiogel® vehicles. Physiogel® NT and Emzaloid® have enhancing effects with E.R. ratios of 1.22 and 1.55. There was no enhancement for methotrexate with Physiogel® Dermaquadrille, with an E.R. ratio of 1.0.

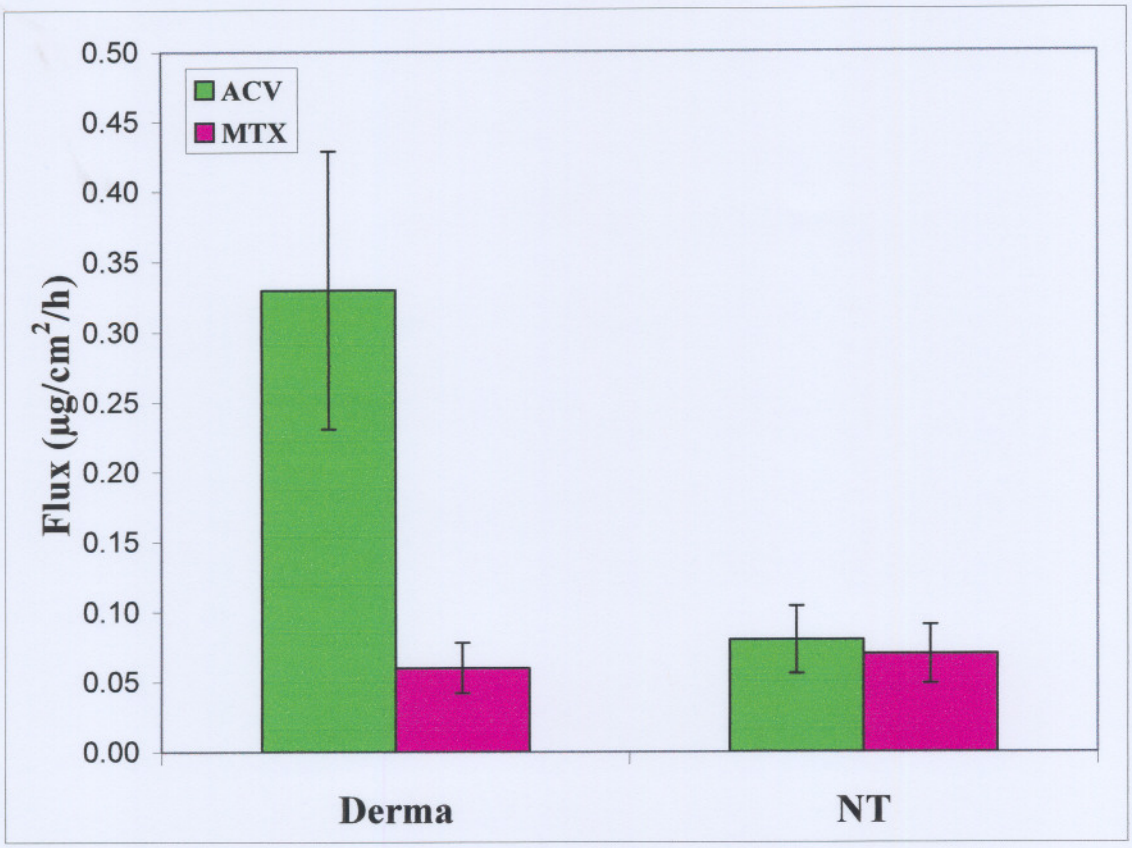


Figure 3.14: The transdermal flux (mean ± SD) of the two drugs in the presence of the Physiogel® formulations.

If the flux values of the two drugs in the Physiogel® formulations were compared, the highest flux was observed in Physiogel® Dermaquadrille with acyclovir. For both the drugs a higher flux were obtained in the Physiogel® NT compared to the Physiogel® Dermaquadrille with methotrexate (Figure 3.14).

The flux of Emzaloid® (0.73 µg/cm^2/h) with acyclovir shows six fold higher value than that of the Emzaloid® with methotrexate (0.09 µg/cm^2/h).

3.7.3 Statistical analysis

An analysis of variance (ANOVA) was done to determine if there were statistically significant differences between the mean of the flux values ($\mu g/cm^2/h$) of acyclovir and methotrexate, the amount of drug released from the drug delivery vehicles and if there were any significant differences in the flow properties of the delivery vehicles. The values were considered significant at $p < 0.1$ for acyclovir and $p < 0.05$ for methotrexate. There were no significant similarities between the three delivery vehicles and the release rate of Emzaloid® for both acyclovir and methotrexate were the lowest.

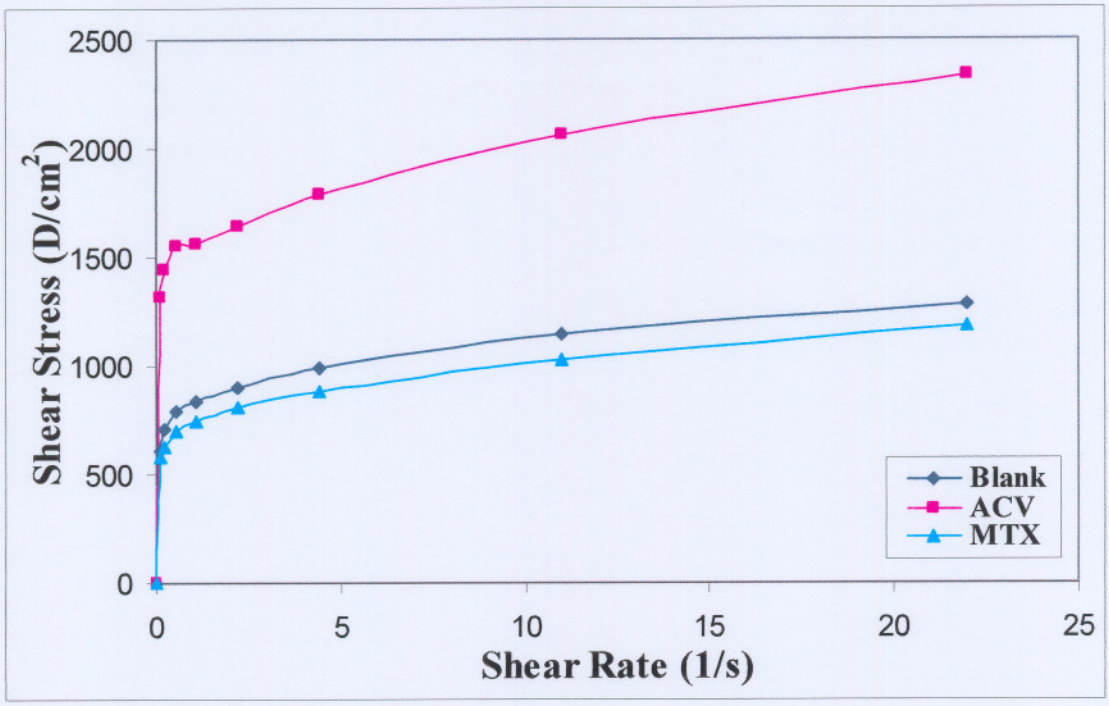
3.8 DISCUSSION

The application of medicines to the skin surface serves the purpose of delivering the active ingredient into the skin (dermal delivery) or into the systemic circulation (transdermal delivery). The permeation of acyclovir and methotrexate in the presence and absence of three delivery vehicles were investigated.

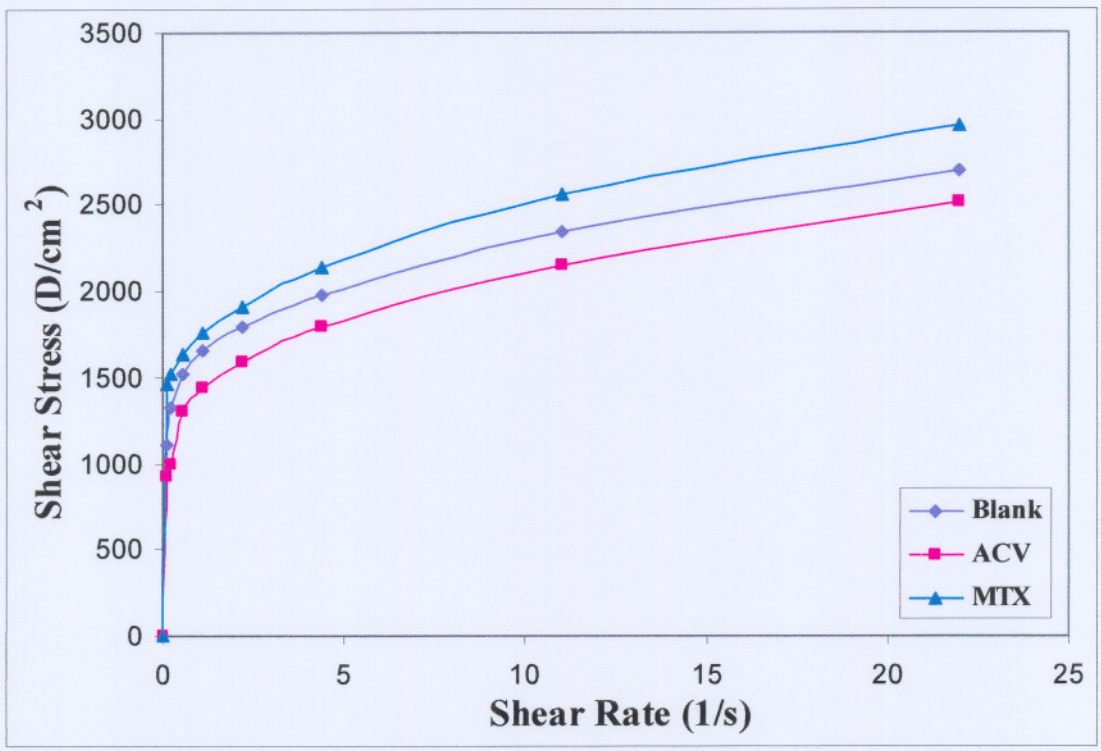
3.8.1 Viscosity

As one of the measurements for stability, viscosity was use to determine if the investigated drugs would affect the internal structure of the creams. In both Physiogel® creams, the viscosity decreased if the rpm were to increase. No significant changes were found in the viscosity if the blank cream was compared to the one with drug and thus the internal structure of both creams was still intact.

Typical pseudoplastic flow rheograms are constructed from the values of shear stress versus shear rate. The characteristic threshold value of shear stress is clearly observed. This type of flow is very common in pharmaceutical preparations, occurring in aqueous dispersions of many suspending agents and with emulsions and creams (Lund *et al.*, 1994:263).



a)



b)

Figure 3.15: Pseudoplastic flow profiles of the different Physiogel® formulations; a) Physiogel® Dermaquadrille and b) Physiogel® NT.

If the shear stress values of the two Physiogel® formulations were compared (Figure 3.15), the Physiogel® Dermaquadrille viscosity did increase with acyclovir as active ingredient. The

reason for this may be due to the formulation differences of the two Physiogel® formulations. In the Physiogel® Dermaquadrille formulation acyclovir interact on the surface of the molecules and was not entrap in the molecules itself causing interparticle friction and the viscosity to increase.

3.8.2 Measurement of the drug release from the delivery vehicle

The release rate of a drug from its vehicle is an indication of the ability of the vehicle to introduce the drug into the receptor medium and the availability of the drug from the dosage form for transdermal absorption. Due to the permeability differences between the synthetic cellulose acetate membrane and the skin, the release rate gives no indication of the drug amount that will penetrate through the skin (Shah *et al.*, 1991: 58).

The cumulative amount of drug released was linear and directly proportional to the square root of time as shown in Figures 3.8 and 3.12. In a study conducted by Fares *et al.*, (1995) the release rate of the active from the delivery vehicles is higher in the dissolution cells due to the differences in the mixing and the temperature within the cream. The amount of acyclovir released from Physiogel® NT (2388.4 $\mu g/cm^2$) was lower than that of the Physiogel® Dermaquadrille (5433.7 $\mu g/cm^2$), due to the formulation differences of the two Physiogel® creams. A study done by Coetzee (2002) shown that carbomer impaired or sustained the release of an active from a delivery vehicle and may thus be the reason for the lower amount of acyclovir released from Physiogel® NT.

There was a considerable lower amount of acyclovir released from the Emzaloid® formulation. The explanation for this can be that acyclovir forms big depots that restrict the exposure of drug to the dissolution medium in the Emzaloid® formulation. Consequently, there was a smaller amount of acyclovir released from the Emzaloid® but over a longer period of time as can be seen in Figure 3.8.

There was an equal amount of methotrexate released from the two Physiogel® creams (1250-1330 $\mu g/cm^2$). The permeation of acyclovir in the presence of two delivery vehicles (Physiogel® Dermaquadrille and Emzaloid®) were higher if compared to methotrexate. Von Plessing *et al.*, (2000) found that the dissolution rate of acyclovir in a inclusion compound or

vehicle was evidently higher than the drug alone and if the crystallinity of the inclusion complex is decreased the solubility will be improved. Unfavourable crystallisation of acyclovir in the Physiogel® vehicles could be the explanation of the poor release profiles.

3.8.3 Transdermal diffusion studies

3.8.3.1 Acyclovir

Acyclovir (ACV) is an antiviral agent used in the treatment of herpes simplex infections that occurs at the lower epidermis. There is a low efficacy of dermatological formulations due to the inadequate drug percutaneous penetration and to heterogeneous distribution of drug in skin layers (Volpato *et al.*, 1998). Improved antiviral results have been achieved using a barrier-altering solvent (dimethyl sulphoxide) or a modified aqueous cream, as compared to ACV formulated in polyethylene glycol. The primary aim of this study was to determine the permeation of acyclovir through the skin in the presence of three drug delivery vehicles.

The stratum corneum barrier properties (i.e. porosity and tortuosity) that are associated with the polar regions of the stratum corneum intercellular lipids may be viewed as the transport along the polar/aqueous regions between the bilayers of the intercellular lipids. This route is highly tortuous and the physicochemical properties of acyclovir (§ 2.7.1) precludes its diffusion to a large extent through the stratum corneum, compared to a more lipophilic compound.

Three flux values were obtained from the three drug delivery vehicles with an applied concentration of 1% for acyclovir. The thermodynamic activity of a drug in a particular vehicle indicates the potential of the active substance to become available for therapeutic purposes. A saturated solution is, therefore, preferable for topical drug delivery as it represents maximum thermodynamic activity (leaving potential) (Kemken *et al.*, 1992). There is a need to keep the solubility of a drug in a vehicle as near to the saturation point as possible and this may be one explanation of the low flux of the Physiogel® NT compared to the control experiment. It could be suggested that the leaving potential of the drug was too low in the NT formulation to effect pronounced flux.

Smaller molecules penetrate the skin more rapidly than larger molecules, but within a narrow range of molecular mass (of 200-500) there is little correlation between size and penetration rate (Liron and Cohen, 1984; Guy and Hadgraft, 1985). The molecular mass of acyclovir is 225.2 and falls in a range of ideal molecular mass, however, the effect on acyclovir permeation remains uncertain in this study.

Drugs should preferably have a balanced lipophilic/hydrophilic character and drugs with log *P* values of 1-3 are considered to be potential candidates for transdermal delivery (Guy *et al.*, 1989). The extent of interaction between drug and vehicles plays a significant role in determining the partition coefficient of a drug. The nature of the vehicle controls drug activity, the rate of diffusion in the vehicle and the partition coefficient between the vehicle and the drug (Dancwerts, 1991). The log *P* value of acyclovir (0.018) is outside the range of acceptability and is detrimental to drug penetration.

Acyclovir was used at a pH of 7.00 in unionised form. In a study preformed by Volpato (1998) the amount of acyclovir in the basal epidermis was to increase by iontophoresis. After iontophoresis the concentration of acyclovir in the skin was generally higher than after passive transport in particular at a pH 3.00. However, at a pH 7.40, the amount recovered in the SC and epidermis after iontophoresis was similar to that obtained after passive diffusion (0.3 $\mu g/cm^2/h$). Thus the flux value of acyclovir in the Physiogel® Dermaquadrille in this study was equal to that of the passive diffusion at a pH 7.00 in the unionised form.

The flux value of acyclovir in Physiogel® NT was considerably lower than that of the acyclovir in Physiogel® Dermaquadrille and the control experiments. The carbomer in the formulation may be the potential cause of release inhibition of an active from a delivery vehicle as well as a prolonged release of a drug Coetzee (2002).

Fatty acids are inherent part of chemical constitution of the stratum corneum, by virtue of which this group of compounds have a capability to intercalate within the lipid bilayer and modulate the barrier property of the stratum corneum (Nair *et al.*, 2003). Fatty acids are one of the essential compounds in the Emzaloid® formulation. The high flux value of acyclovir in Emzaloid® may be attributed to the fact that fatty acids are one of the essential compounds in the Emzaloid® formulation. A study done by Nair *et al.*, (2003) indicate that unsaturated fatty acids make the stratum corneum more permeable by interacting with lipid arrays of the skin

leading to an increase in motional freedom or excitability of their hydrocarbon chain (disordering effect).

The permeation of acyclovir from the Emzaloid® cream was two times higher than that from the Physiogel® creams. A study done by de Jalón *et al.,* (2001) suggested that the most of the acyclovir is located at the Poly($_{D,L}$-lactic-co-glycolic acid) microparticle surface, although a part of acyclovir is entrapped within the particles. For topical application both features are of interest, since the burst release effect can be useful to improve the initial penetration of the drug and sustained release becomes important to supply the skin with acyclovir over a prolonged period of time. Confocal micrographs of acyclovir in the Emzaloid® formulation showed the big depots with the entrapped acyclovir as well as the crystals that have formed (Figure 3.16).

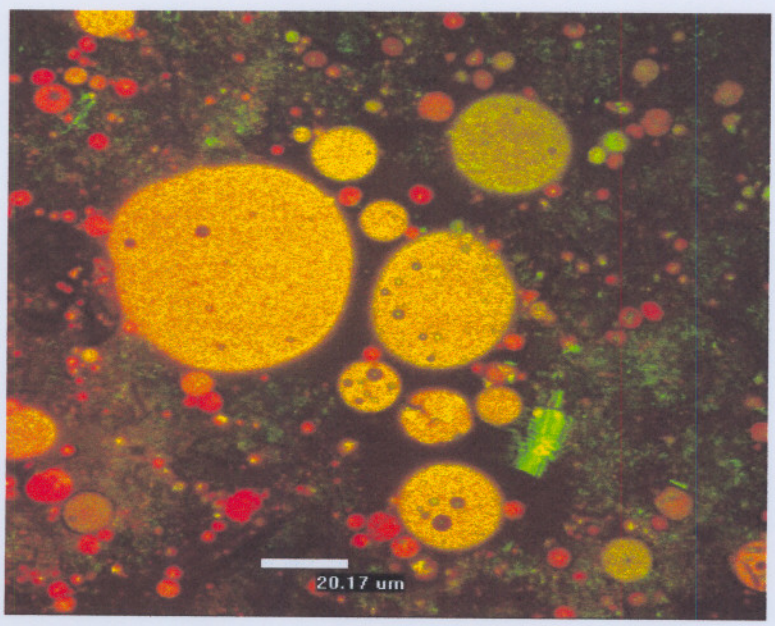


Figure 3.16: Confocal micrograph of acyclovir in the Emzaloid® formulation.

The crystals that have formed in the interfaces may provoke the permeation of acyclovir through the skin. A sustained release of acyclovir from the depots may be the result of a prolonged enhancement effect of acyclovir from the Emzaloid®. The crystallisation difficulty could be solved by an increase in the interphase volume and changing the pH of the cream formulation of the Emzaloid® prior to drug inclusion.

3.8.3.2 Methotrexate

The low dose of methotrexate (MTX) is approved for the treatment of psoriasis and rheumatoid arthritis (RA). The objective of this study was to determine the effect of three drug delivery vehicles on the percutaneous absorption of methotrexate for transdermal drug delivery.

A major challenge posed to transdermal methotrexate delivery is its hydrophilic properties and a high molecular weight 454.56 g/mol (Alvarez-Figueroa *et al.,* 2001). The diffusion rate of molecules through liquids is inversely proportional to the square root of the molecular weight. This proportionality is also applicable to a membrane without major modification of parameters. Therefore; one might expect large molecules to diffuse more slowly because they require more space to be created in the medium and this in turn leads to diminished permeability. Small molecules penetrate more rapidly than large ones, but in a narrow range of molecular size (Liron *et al.,* 1984). Since the diffusion of molecules through liquids is inversely proportional to the square root of the molecular weight and the dependency is generally not much higher for the diffusion through a membrane, one might expect higher permeability coefficients to be associated with drugs of low molecular weight (Roy *et al.,* 1989).

A study completed by Alvarez-Figueroa *et al.* (2001) of iontophoresis at a pH 4.00-5.00, demonstrated that the amine group of methotrexate was predominantly ionised ($\geq$ 80%), positively charged, zwitterionic and must be delivered from the anode. Anodic iontophoresis should offer the advantage of adding an electroosmotic component to the iontophoretic flux. However, methotrexate was not detected either in the receptor solution or in the skin after anodic iontophoresis at either pH. This is probably due to the low solubility of methotrexate in the donor solution. Methotrexate at a medium pH (4.0) with a log P value of -2.52 and -1.85 was used in this study. Vaidyananthan *et al.,* (1985) suggested that the pH value of 4.00 – 5.00 would provide the most favourable environment for passive diffusion since the concentration of unionised methotrexate would be optimal in this pH range.

The flux values obtained from the Physiogel® (0.06 $\mu g/cm^2/h$ and 0.07 $\mu g/cm^2/h$) was higher than the flux values of a study done by Brain *et al.* (1991) where Azone® was used as an enhancer for percutaneous absorption of methotrexate. The higher flux values could be

explained by the specific composition of the two Physiogel® creams, which are similar to the structure of the skin and promote drug penetration through the polar and lipid routes. Vaidyananthan *et al.* (1985) found that a saturated solution of methotrexate in propylene glycol:water (50:50) vehicle provided the best system for passive diffusion. Methotrexate in this study was used in a concentration of 1% that was not close to the saturation point and this explain the lower flux value of methotrexate in the two Physiogel® creams.

The amount of methotrexate released from Emzaloid® as drug delivery vehicle was higher than the amount released from the two Physiogel® creams. Several studies have compared o/w and w/o microemulsions with other vehicles for the transdermal administration of medicines, such as lotions, suspensions, gels or emulsions (Linn *et al.*, 1990; Gasco *et al.*, 1991; Trotta *et al.*, 1997).

Nair *et al.*, (2003) found that fatty acids form 'grain boundaries' through which large polar molecules may be transported and they lower the diffusional resistance of the stratum corneum to polar and relatively polar solutes by lowering thermal phase properties of lipid components without affecting that of keratin. The high flux value that was obtained from methotrexate in Emzaloid® cream may be due to the fact that one of the essential compounds in the Emzaloid® formulation is the fatty acids and the molecular weight (§ 2.8.2.1) of methotrexate that favours the transport with fatty acids across the stratum corneum.

The structure of the Emzaloid® is analogous to microemulsions and consists of one or more water and oil phases. In a study conducted by Alvarez-Figueroa (2001) it had been observed that microemulsions had a large capacity to release drugs to the skin. This may be due to the fact that drugs contained in microemulsions are in dissolved or suspended form, so that their absorption is faster and more effective.

The flux value of methotrexate in Emzaloid® was not as pronounced as that of acyclovir, due to the fact that there was only a small amount of methotrexate entrapped in the Emzaloid® vesicle itself (Figure 3.17). The higher concentration was in the interphase and is represented in Figure 3.18.

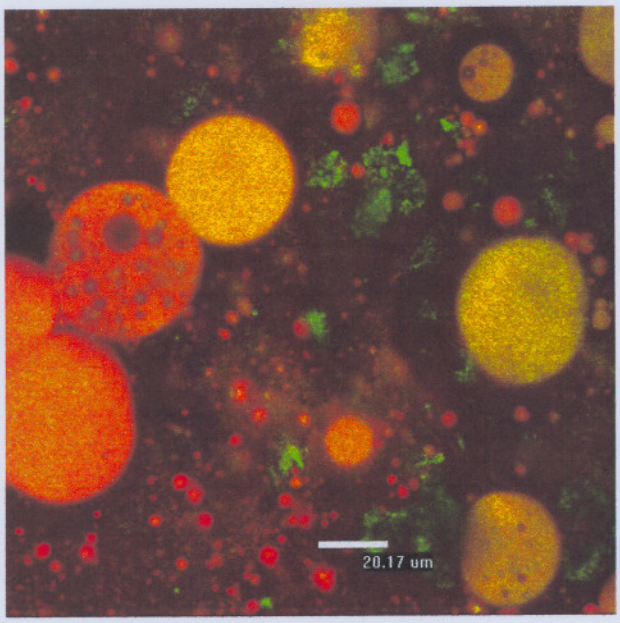


Figure 3.17: A confocal micrograph of the small amount of methotrexate entrapped in the Emzaloid® vesicles.

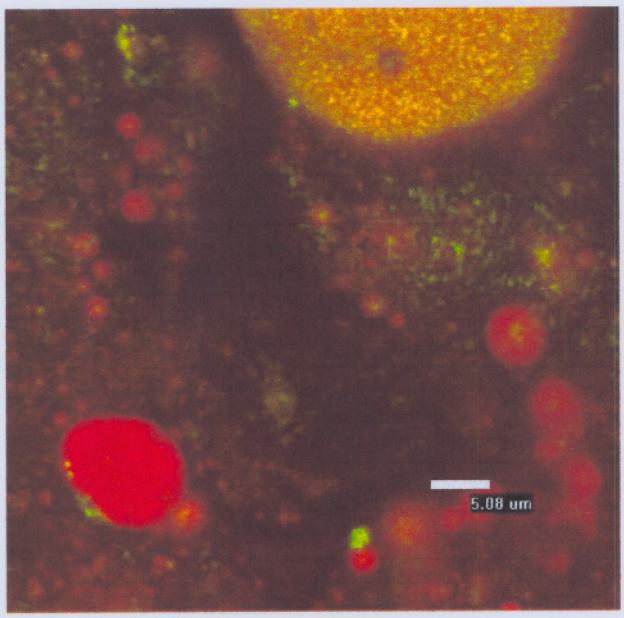


Figure 3.18: The confocal micrograph of methotrexate in the interphases of the Emzaloid® formulation.

Alvarez-Figueroa (2001) found that the solubility of methotrexate in the microemulsions (pH 7.40) was higher in the system I (ethyl oleate as oil phase) than in system II (myristic isopropyl ester as oil phase). This may be attributable to the fact that system I is o/w

(oil/water), while system Π is w/o (water/oil). Ionised methotrexate is hydrosoluble and thus has a higher affinity for the aqueous phase: since this was the external phase in the system I microemulsion, a greater amount of drug is solubilised. Through the manipulation of the pH, the ionisation state of methotrexate will determine which phase methotrexate would have an affinity for, either the oil, water or intern phases. Thus, both the micro-sponges and a lipid bilayer vesicle formulation for the Emzaloid® would be appropriate due to the low solubility as well as the hydrosolubility properties of the drug.

3.9 CONCLUSIONS

In this chapter HPLC methods and different drug delivery vehicles have been outlined. The HPLC methods for the analysis of methotrexate and acyclovir have been validated and proven in both instances to be simple, rapid, specific and sensitive. Acceptable levels of sensitivity, reproducibility and repeatability were obtained.

The transdermal permeation of acyclovir and methotrexate in the presence of three drug delivery vehicles were investigated. Three sets of experiments were done for each active ingredient and the following observations were made:

- Through the viscosity measurements it was determined that the internal structure of the Physiogel® vehicles were still intact.
- A consistent amount of methotrexate and acyclovir were released from all three drug delivery vehicles over a period of time.
- Transdermal diffusion studies were done on all three drug delivery vehicle.

Significant enhancement of transdermal flux of acyclovir and methotrexate were observed with Emzaloid® as drug delivery vehicle. This was due to the fact that Emzaloid® entraps and delivery drug with higher efficiency than the ordinary drug delivery vehicles.

CHAPTER 4

SUMMARY AND FINAL CONCLUSIONS

The major limitation to transdermal drug delivery is the inherent barrier function of the skin. Being the outermost layer of the human organism, separating the internal from the external environment, the skin acts as a two-way barrier, i.e. preventing the ingress of foreign molecules and the egress of endogenous substances (Suhonen, 1999:149).

The main objective of a drug delivery vehicle is to improve the permeation of drug molecules by controlled reduction of the barrier properties of the stratum corneum. A primary requisite is that this effect should be reversible, so that there is no permanent damage to the skin (Ghosh *et al.*, 1993:88). Through interactions with the skin, delivery vehicles provide a complementary action by which the skin can be prepared to allow passage of the drug. The thermodynamic activity of the substance and its potential for absorption by the skin are more important factors than the ability of the vehicle to penetrate through the skin (Lund *et al.*, 2000:141). The drug's physicochemical properties (solubility, molecular weight, structure, size, ionisation state) have an effect on the permeability of the drug through the skin.

The study was conducted using Physiogel® Dermaquadrille, Physiogel® NT and Emzaloid® as transdermal drug delivery systems. Methotrexate, an antineoplastic agent and acyclovir, an antiviral agent, were used in the presence of the three delivery vehicles. The objectives of the study were:

- to determine by measurement of the viscosity whether the internal structure of the Physiogel® creams were still intact following incorporation of drugs;
- to measure the extent and rate drug released from the delivery vehicles and
- finally, to determine the influence of the delivery vehicles on the permeation of the drugs through the skin.

Through the viscosity measurements it was determined that the internal structure of both the Physiogel® creams was not influenced by the investigated drugs. Methotrexate and acyclovir

were both released from all three the delivery vehicles. The diffusion experiments were conducted from a 1% concentration of the drug in the delivery vehicles. The flux values of acyclovir from Physiogel® Dermaquadrille (enhancement ratio 1.32) and Emzaloid® (enhancement ratio 2.92) showed an enhancement. The flux values reported for methotrexate from Physiogel® NT (enhancement ratio 1.22) and Emzaloid® (enhancement ratio 1.56) have also shown enhancement.

In conclusion the following should be considered:

There was significant enhancement for both methotrexate (enhancement ratio 1.56) and acyclovir (enhancement ratio 2.92) from Emzaloid® as drug delivery vehicle.

More stability tests (time-dependent viscosity measurements, crystallography and solubility) should be performed on the Emzaloid®.

An optimal cream formulation for various Emzaloid® systems can be formulated.

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ANNEXURE I

Viscosity

Viscosity of Physiogel NT - Blank

Model:
RV **Spindle: SC4-25**

No	Speed	Torque	Viscosity	Shear Stress	Shear Rate	Temp
	RPM	%	cP	D/cm^2	1/sec	°C
1	0.0	0.0	0.0	0.0	0.0	25
2	0.5	9.8	1000000	1104	0.11	25
3	1	11.8	604000	1329	0.22	24.9
4	2.5	13.5	276000	1521	0.55	24.9
5	5	14.7	151000	1656	1.10	25
6	10	15.9	81408	1791	2.20	25
7	20	17.6	45056	1982	4.40	25
8	50	20.8	21299	2343	11.0	25
9	100	24	12288	2703	22.0	25

Viscosity of Physiogel Dermaquadrille- Blank

Model: RV **Spindle: SC4-25**

No	Speed	Torque	Viscosity	Shear Stress	Shear Rate	Temp
	RPM	%	cP	D/cm^2	1/sec	°C
1	0.0	0.0	0.0	0.0	0.0	25
2	0.5	5.4	553000	608.3	0.11	25
3	1	6.3	323000	709.6	0.22	25
4	2.5	7	143000	788.5	0.55	25
5	5	7.4	75776	833.5	1.10	25
6	10	8	40960	901.1	2.20	25
7	20	8.8	22528	991.2	4.40	25
8	50	10.2	10445	1149	11.0	25
9	100	11.4	5837	1284	22.0	25

Viscosity of methotrexate

Viscosity of Physiogel NT - METHOTREXATE

Model: RV **Spindle: SC4-25**

No	Speed	Torque	Viscosity	Shear Stress	Shear Rate	Temp
	RPM	%	cP	D/cm^2	1/sec	°C
1	0.0	0.0	0.0	0.0	0.0	24.9
2	0.5	13	1330000	1464	0.11	24.9
3	1	13.5	691000	1521	0.22	25
4	2.5	14.5	297000	1633	0.55	25
5	5	15.6	160000	1757	1.10	24.9
6	10	17	87040	1915	2.20	24.9
7	20	19	48640	2140	4.40	24.9
8	50	22.7	23245	2557	11.0	25
9	100	26.3	13466	2962	22.0	25

Viscosity of Physiogel Dermaquadrille- METHOTREXATE

Model: RV **Spindle: SC4-25**

No	Speed	Torque	Viscosity	Shear Stress	Shear Rate	Temp
	RPM	%	cP	D/cm^2	1/sec	°C
1	0.0	0.0	0.0	0.0	0.0	24.9
2	0.5	5.2	532000	585.7	0.11	25
3	1	5.6	287000	630.8	0.22	24.9
4	2.5	6.2	127000	698.4	0.55	24.9
5	5	6.6	67584	743.4	1.10	24.9
6	10	7.2	36864	811	2.20	24.9
7	20	7.8	19968	878.6	4.40	24.9
8	28	9.1	9318	1025	11.0	24.8
9	100	10.5	5376	1183	22.0	24.9

ANNEXURE 2

Summary acyclovir Vankel dissolution

ACV DERMAQUADRILLE

sqrt time	TIME	[ ] 1	[ ] 2	[ ] 3	[ ] 4	[ ] 5	[ ] 6	AVE	amt (µg/sqcm)	stdev
5.47723	30	10.21	8.772	8.845	10.3	9.18	9.94	9.536	455.5061583	0.686
7.74597	60	16.8	16.22	16.69	17.1	15.7	16.7	16.55	790.3432055	0.503
10.9545	120	26.37	24.34	24.42	26.8	25.2	26.3	25.57	1221.206531	1.0724
13.4164	180	33.9	31.33	31.98	34.5	32.5	33.7	32.98	1575.38891	1.2178
15.4919	240	40.14	37.13	38.18	41	38.5	39.8	39.11	1868.342543	1.4247
17.3205	300	46.13	42.6	44.16	46.5	44.3	45.5	44.86	2142.984922	1.456
18.9737	360	51.41	47.76	49.33	51.7	49.6	50.2	50	2388.43307	1.4546

ACV NT

sqrt time	TIME	[ ] 1	[ ] 2	[ ] 3	[ ] 4	[ ] 5	[ ] 6	AVE	amt (µg/sqcm)	stdev
5.47723	30	8.358	8.622	8.563	8.6	7.94	8.44	8.421	402.2536178	0.2549
7.74597	60	14.74	14.97	14.95	14.7	13.5	14.4	14.56	695.5471681	0.54
10.9545	120	23.85	24.52	23.98	23.5	22.1	23	23.47	1121.301465	0.8636
13.4164	180	30.85	31.65	31	30.2	28.6	29.8	30.33	1448.944347	1.0805
15.4919	240	36.5	37.72	36.75	35.9	34.3	35.1	36.03	1721.037576	1.2418
17.3205	300	42.05	43.08	41.8	40.9	39.2	40	41.18	1966.883773	1.4286
18.9737	360	46.62	47.87	46.63	45.6	44.2	44.4	113.8	5433.690353	1.426

ACV IN EMZALOID®

sqrt time	TIME	[ ] 1	[ ] 2	[ ] 3	[ ] 4	[ ] 5	[ ] 6	AVE	amt (µg/sqcm)	stdev
5.47723	30	2.132	3.139	2.757	2	2.73	3.26	2.669	127.5082148	0.5146
7.74597	60	3.631	4.877	4.434	3.14	4.42	5.07	4.262	203.5949035	0.7395
10.9545	120	5.704	7.38	6.749	4.85	6.56	7.54	6.464	308.7785426	1.0262
13.4164	180	6.892	9.221	8.387	6.33	8.09	9.24	8.027	383.4087489	1.1988
15.4919	240	8.287	10.75	9.894	7.51	9.4	10.7	9.427	450.2718549	1.3156
17.3205	300	9.459	12	10.61	8.59	10.8	11.9	10.56	504.5478242	1.3453
18.9737	360	10.6	13.35	11.89	9.75	11.8	13.3	11.79	563.0052415	1.4339

Summary methotrexate Vankel dissolution

MTX DERMAQUADRILLE

sqrt time	TIME	[ ] 1	[ ] 2	[ ] 3	[ ] 4	[ ] 5	[ ] 6	AVE	amt (µg/sqcm)	stdev
5.477226	30	5.206	6.021	5.67	5.606	6.379	5.62	5.7501	274.662862	0.4024
7.745967	60	8.52	9.63	9.024	8.867	9.844	8.6232	9.0848	433.95089	0.5399
10.95445	120	14.3	14.78	13.53	13.76	14.9	13.365	14.106	673.813794	0.6516
13.41641	180	17.24	18.49	17.1	17.57	18.68	17.145	17.703	845.626811	0.7047
15.49193	240	20.65	21.76	20.13	20.99	21.94	20.258	20.954	1000.91622	0.7597
17.32051	300	23.37	24.78	22.84	23.75	24.66	22.833	23.706	1132.36132	0.8595
18.97367	360	25.89	27.36	25.5	26.21	27.31	25.448	26.287	1255.65914	0.8573

MTX NT

sqrt time	TIME	[ ] 1	[ ] 2	[ ] 3	[ ] 4	[ ] 5	[ ] 6	AVE	amt (µg/sqcm)	stdev
5.477226	30	5.96	5.506	5.696	5.856	6.908	6.1773	6.0171	287.417918	0.4924
7.745967	60	9.655	8.723	9.184	9.174	10.75	9.5102	9.4994	453.75557	0.692
10.95445	120	14.69	13.36	13.87	13.05	15.13	14.489	14.099	673.454464	0.8113
13.41641	180	18.27	17.58	18.43	16.32	18.91	19.299	18.135	866.264831	1.0628
15.49193	240	22.84	21.54	22.42	20.09	23.92	22.708	22.252	1062.92366	1.3068
17.32051	300	25.95	24.5	25.77	23.4	27.01	27.459	25.682	1226.75714	1.5227
18.97367	360	29.31	26.67	28	28.27	29	25.799	27.84	1329.79932	1.3585

MTX IN EMZALOID®

sqrt time	TIME	[ ] 1	[ ] 2	[ ] 3	[ ] 4	[ ] 5	[ ] 6	AVE	amt (µg/sqcm)	stdev
5.477226	30	3.163	4.728	4.932	6.19	5.567	5.65	5.0384	240.666687	1.0589
7.745967	60	4.394	6.613	6.723	8.356	7.664	7.6652	6.9025	329.709272	1.3918
10.95445	120	6.681	9.917	9.811	11.91	11.48	11.057	10.143	484.518817	1.8922
13.41641	180	8.405	12.3	11.96	14.51	14.25	13.595	12.503	597.206335	2.2546
15.49193	240	9.914	14.37	13.86	16.68	16.62	15.783	14.54	694.505272	2.5434
17.32051	300	11.27	16.16	15.62	18.58	18.74	17.772	16.358	781.367711	2.7961
18.97367	360	12.54	17.83	17.15	20.31	20.71	19.638	18.03	861.224314	3.0314

ANNEXURE 3

Summary of acyclovir flux values

Control cells

Cell	*Flux (µg/cm²/h)*
1	0.140
2	0.202
3	0.152
4	0.322
5	0.274
6	0.403

Average Control	0.25
SD Control	0.10
% RSD Control	41.44

ACV in Derma

Cell	*Flux (µg/cm²/h)*
1	0.149
2	0.419
3	0.292
4	0.284
5	0.402
6	0.452

Average Derma	0.33
SD Derma	0.11
% RSD Derma	34.04

ACV in NT

Cell	*Flux (µg/cm²/h)*
1	0.063
2	0.041
3	0.077
4	0.072
5	0.122
6	0.109

Average NT	0.08
SD NT	0.03
% RSD NT	37.13

ACV in Emzaloid®

Cell	*Flux (µg/cm²/h)*
1	0.902
2	0.455
3	0.970
4	0.952
5	0.500
6	0.752

Average EMZ	0.73
SD EMZ	0.23
% RSD EMZ	31.14

Summary of methotrexate flux values

Control cells

Cell	*Flux (μg/cm²/h)*
1	0.053
2	0.058
3	0.077
4	0.050
5	0.059
6	0.057

Average Control	0.06
SD Control	0.01
% RSD Control	16.01

MTX in Derma

Cell	*Flux (μg/cm²/h)*
1	0.048
2	0.068
3	0.058
4	0.086
5	0.047
6	0.051

Average Derma	0.06
SD Derma	0.02
% RSD Derma	25.28

MTX in NT

Cell	*Flux (μg/cm²/h)*
1	0.083
2	0.104
3	0.067
4	0.081
5	0.040
6	0.061

Average NT	0.073
SD NT	0.022
% RSD NT	30.11

MTX in Emzaloid®

Cell	*Flux (μg/cm²/h)*
1	0.063
2	0.118
3	0.060
4	0.066
5	0.149
6	0.101

Average EMZ	0.093
SD EMZ	0.036
% RSD EMZ	38.97