Synthesis and Transdermal Permeation of Lamivudine Derivatives

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"Our greatest fear is not that we are inadequate, but that we are powerful beyond measure. It is our light, not our darkness, which frightens us. We ask ourselves, who am I to be brilliant, gorgeous, handsome, talented and fabulous? Actually, who are you not to be?

You are a child of God. Your playing small does not serve the world. There is nothing enlightened about shrinking so that other people won't feel insecure around you. We were born to make manifest the glory of God within us. It is not just in some, it is in everyone. And, as we let our own light shine, we unconsciously give other people permission to do the same. As we are liberated from our fear, our presence automatically liberates others."

-Marianne Williamson-

ABSTRACT

The skin is the organ of the body that comes in contact with the outer environment. This means that it has certain functions like protecting the body from excessive water loss and inhibiting the entry of potentially harmful chemicals. This is called the barrier function of the skin and is enforced by the outermost layer of the skin, the stratum corneum (SC). The transdermal route of delivery has several advantages over the common oral route which include improving patient compliance by decreasing the amount of medication, circumventing the first pass metabolism in the liver, eliminating certain unwanted side-effects which are associated with oral administration (nausea, diarrhoea) and to have better control over the input kinetics of the drug. The SC is a very lipophilic membrane, inhibiting the penetration of hydrophilic molecules. This means that in order for molecules to permeate through the skin, they must have specific physicochemical properties like a specific aqueous solubility, octanol-water partition coefficient (log P), melting point and molecular mass.

Since the epidemic started in 1981, more than 25 million people have died of AIDS worldwide. Statistics at the end of 2007 showed that 33 million people are living with HIV and that 2.7 million people become infected with it each year. There are a total of 16 drugs that have been approved by the U.S. Food and Drug Administration (FDA) for the chemotherapy of AIDS whereof seven are nucleoside reverse transcriptase inhibitors (NRTI), making them the most important class of compounds in the treatment of HIV. Lamivudine (4-amino-1-[2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-1H-pyrimidin-2-one, 3TC) is in this class and is used in combination with zidovudine (AZT) against HIV-1 and HIV-2 because of their synergism. The most common adverse effects of lamivudine are usually associated with the gastro-intestinal system and include vomiting, diarrhoea, abdominal cramps and pain, and nausea.

The objective of this study was to determine the *in vitro* transdermal permeation through the human SC of lamivudine and the synthesised methoxypoly(ethylene glycol) (MPEG) carbonates and carbamates thereof, with and without the use of Pheroid™ as delivery system and to establish a relationship, if any, with selected physicochemical properties.

Three *N4*-methoxypoly(ethylene glycol) carbamates and three 6'-O-metoxypoly(ethylene glycol) carbonates were synthesised by alkoxy carbamylation and carbonylation of lamivudine with acylating agents containing the corresponding short-chain methoxypoly(ethylene glycols). The structures of the products were confirmed by nuclear magnetic resonance spectroscopy (NMR) and liquid chromatography mass spectroscopy (LC-MS).

The aqueous solubility of only lamivudine (188.02 mg/ml) and 2',3'-dideoxy-3'-thiacytidin-N4-yl-methoxy(ethylene glycol) carbamate (8.53mg/ml) could be determined experimentally. The relationship between the aqueous solubility of lamivudine and the other derivatives with their flux could not be determined because these derivatives were oils infinitely miscible with PBS. Theoretical equations using the physicochemical properties like log P were employed to calculate the aqueous solubility. The derivatives became more soluble in aqueous medium as the chain length increased because of the increasing number of intra-chain oxygen atoms forming bonds with surrounding water molecules. However, as the length increased so did the number of ethylene units which ultimately also increased lipid solubility.

The experimental octanol-PBS partition coefficient of lamivudine (-0.83) was lower than that of its derivatives (ranging from -0.16 to -0.63). This meant that the synthesised derivatives were more lipophilic than lamivudine. As the chain length increased the partition coefficient value decreased. This correlates with the increasing aqueous solubility as the chain length increased.

In vitro penetration was measured through excised female human abdominal skin in Franz diffusion cells. The steady-state flux (J_{SS}) of lamivudine in PBS (4.23 μmol/cm^2/h) was higher than that of lamivudine in Pheroid^{TM} (0.20 μmol/cm^2/h). In both PBS and Pheroid^{TM} the median flux of lamivudine was higher than that of the derivatives (0.04 to 2.07 μmol/cm^2/h in PBS and 0.002 to 0.11 μmol/cm^2/h in Pheroid^{TM}) except for the 6'-O-methoxy(ethylene glycol) carbonate (n = 1) which had a higher flux value (0.23 μmol/cm^2/h) in Pheroid^{TM}. Of all the derivatives of lamivudine, the *N4*-methoxy(ethylene glycol) carbamate (n = 1) (2.07 μmol/cm^2/h) and the *N4*-methoxytri(ethylene glycol) carbamate (n = 3) (1.32 μmol/cm^2/h) (in PBS) presented the highest flux.

Two methods for measuring transdermal flux were used: in Method 1 donor solutions were prepared in PBS while Method 2 used the Pheroid^{TM} delivery system for donor solutions. A t-test yielded a *p*-value of 0.0002 indicating that there was a highly statistically significant difference between the mean of the overall flux of all the synthesised derivatives of Method 1 and that of Method 2, showing that the flux in PBS was significantly higher than that in Pheroid^{TM}.

The results of the ANOVA for Method 2 indicated that there were no statistically significant differences between the mean flux values of the different compounds (*p*-value of 0.26). Thus in Pheroid^{TM} there were no statistically significant differences between the mean flux of any compound and the parent drug.

In the case of Method 1, the ANOVA indicated that a significant difference between the means of the flux of all the compounds existed (*p*-value of < 0.0001). A Dunnett test showed that

except for 2',3'-dideoxy-3'-thiacytidin-N4-yl-(methoxy(ethylene glycol)) carbamate, the mean flux values in PBS of all the other compounds were statistically significantly lower than the mean flux of the parent drug.

Although a small data set of only six compounds was used in this study no relationship between the aqueous solubility, partition coefficient (log P) and transdermal flux of any of the compounds was found. Neither derivatisation of lamivudine nor the use of the Pheroid™ delivery system improved the transdermal flux of this drug. This shows that a clinically useful transdermal administration of the derivatives synthesised in this study will not be feasible.

OPSOMMING

Die vel is die orgaan van die liggaam wat met die omgewing in kontak is. Dit beteken dat dit sekere funksies het, soos om die liggaam teen oormatige verlies van water en binnedring van moontlike skadelike chemikalieë te beskerm. Dit word die versperringsfunkie van die vel genoem en dit word deur die buitenste laag, die stratum corneum (SC), bewerkstellig. Die transdermale afleweringsroete het talle voordele bo die algemene orale roete, waaronder beter pasiëntmeewerkendheid deur die hoeveelheid medikasie te verminder, vryspring van eerstedeurgangsmetabolisme in die lewer, uitskakeling van ongewensde newe-effekte wat met orale dosering gepaardgaan (naarheid, diarree) en 'n beter beheer oor die insetkinetika van die geneesmiddel. Die SC is 'n baie lipofiele membraan wat die penetrasie van hidrofiele molekules verhinder. Dit beteken dat as molekules die vel wil deurdring, hulle spesifieke fisies-chemiese eienskappe moet hê, soos 'n spesifieke wateroplosbaarheid, oktanol-water verdelingskoëffisiënt (log P), smeltpunt en molekulêre massa.

Sedert die epidemie in 1981 begin het, het meer as 25 miljoen mense wêreldwyd aan VIGS gesterf. Statistieke aan die einde van 2007 het getoon dat 33 miljoen mense MIV het en dat 2.7 miljoen elke jaar daarmee besmet word. 'n Totaal van 16 middels is tans deur die Amerikaanse Voedsel- en Medisyne-Administrasie (FDA) vir die chemoterapie van VIGS goedgekeur waarvan sewe nukleosied omgekeerdetranskriptaseremmers (NOTR) is wat hulle die belangrikste klas middels vir die behandeling van MIV maak. Lamivudien (4-amino-1-[2-(hidroksimetiel)-1,3-oksatiolan-5-iel]-1H-pirimidien-2-oon, 3TC) is in hierdie klas en word vanweë hulle sinergistiese werking in kombinasie met sidovudien (AZT) teen MIV-1 en MIV-2 gebruik. Die mees algemene newe-effekte van lamivudien is op die gastro-intestinale stelsel en is onder meer braking, diarree, buikkrampe en -pyn en naarheid.

Die doel van hierdie studie was om die *in vitro* transdermale deurgang, met en sonder die gebruik van Pheroid™ as afleweringstelsel, deur menslike SC te bepaal van lamivudine en gesintetiseerde metoksipoli(etileenglikol) (MPEG)-karbonate en -karbamate daarvan en om 'n verwantskap, indien enige, met sekere fisies-chemiese eienskappe te bepaal.

Drie *N4*-metoksipoli(etileenglikol)karbamate en drie 6'-O-metoksipoli(etileenglikol)karbonate is deur alkoksikarbamilering en -karbonilering van lamivudien met die ooreenstemmende kortketting metoksipoli(etileenglikols) gesintetiseer. Die strukture van die produkte is met kernmagnetieseresonansiespektrometrie (KMR) en vloeistofchromatografie-massaspektrometrie (VC-MS) bevestig.

Die wateroplosbaarheid van slegs lamivudien (188.02 mg/ml) en 2',3'-dideoksi-3'-tiasitidien-N4-iel-metoksi(etileenglikol)karbamaat (8.53 mg/ml) kon eksperimenteel bepaal word. Die verband tussen die wateroplosbaarheid van lamivudien en die ander derivate daarvan met hulle vloed deur die vel kon nie bepaal word nie omdat hulle olies en volledig mengbaar met die fosfaatbufferoplossing (FBO) was. Die wateroplosbaarheid is bereken met teoretiese vergelykings wat fisies-chemiese eienskappe soos log P gebruik. Soos wat die kettinglengte langer word, word die derivate meer wateroplosbaar vanweë die toename in die aantal suurstofatome in die ketting wat bindings met die omringende watermolekules vorm. Soos wat die lengte toeneem, neem die aantal etileengroepe egter ook toe wat lipiedoplosbaarheid ook laat toeneem.

Die eksperimentele oktanol-FBO verdelingskoëffisiënt van lamivudien (-0.83) is laer as dié van die derivate (-0.16 tot -0.63). Dit beteken dat die gesintetiseerde derivate meer lipofiel as lamivudien is. Soos wat die kettinglengte toeneem, het die verdelingskoëffisiënt afgeneem. Dit korreleer met die toename in wateroplosbaarheid met toenemende kettinglengte.

In vitro-penetrasie deur vroulike menslike abdominale vel is met Franz-diffusieselle gemeet. Die vloed by gelykvlakke (J_{ss}) van lamivudien in FBO (4.23 µmol/cm^2/h) is hoër as dié van lamivudien in Pheroid^{TM} (0.20 µmol/cm^2/h). In sowel FBO as in Pheroid^{TM} is die mediaanvloed van lamivudien hoër as dié van die derivate (0.04 tot 2.07 µmol/cm^2/h in PBS en 0.002 tot 0.11 µmol/cm^2/h in Pheroid^{TM}) behalwe vir die 6'-O-metoksi(etileenglikol)karbonaat (n = 1) wat 'n hoër vloed het (0.23 µmol/cm^2/h) in Pheroid^{TM}. Van al die derivate van lamivudien, het die *N4*-metoksi(etileenglikol)karbamaat (n = 1) (2.07 µmol/cm^2/h) en die *N4*-metoksitri(etileenglikol)karbamaat (n = 3) (1.32 µmol/cm^2/h) (in PBS) die hoogste vloed.

Twee metodes is gebruik om transdermale vloed te meet: in Metode 1 is donoroplossings in FBO berei terwyl die Pheroid^{TM}-afleweringstelsel in Metode 2 vir donoroplossings gebruik is. 'n *p*-waarde van 0.0002 is met 'n t-toets gekry wat toon dat daar 'n groot statisties beduidende verskil is tussen die gemiddelde van die algehele vloed van al die gesintetiseerde derivate van Metode 1 en dié van Metode 2 en dat die vloed in FBO dus beduidend hoër is as in Pheroid^{TM}.

Die resultate van die ANOVA vir Metode 2 toon dat daar geen statisties beduidende verskil tussen die gemiddelde vloedwaardes van die verskillende verbindings is nie (p-waarde van 0.26). In Pheroid^{TM} is daar dus geen statisties beduidende verskil tussen gemiddelde vloed van enige derivaat en die moederverbinding nie.

In die geval van Metode 1 het die ANOVA 'n beduidende verskil tussen die gemiddeldes van die vloed van al die verbindings aangetoon (*p*-waarde < 0.0001). 'n Dunnetttoets het getoon dat die

gemiddelde vloed in FBO van al die derivate, behalwe vir die eerste karbamaat, statisties beduidende laer is as die van die moederverbinding.

Hoewel 'n klein datastel van net ses verbindings in hierdie studie gebruik is, kon geen verband tussen die wateroplosbaarheid, verdelingskoëffisiënt (log P) en transdermale vloed van enige van die verbindings gekry word nie. Nóg derivatisering van lamivudien nóg die gebruik van die Pheroid™-afleweringstelsel verbeter die transdermale vloed van hierdie geneesmiddel. Dit toon dat klinies bruikbare transdermale toediening van die derivate in hierdie studie gesintetiseer nie haalbaar is nie.

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TABLE OF CONTENTS

TABLE OF FIGURES

TABLE OF TABLES

CHAPTER 1

INTRODUCTION AND PROBLEM STATEMENT

1.1 Introduction

The skin is the largest organ of the body that provides a "multifunctional interface", meaning that it has different functions like protecting the body from the external environment and providing the body with its sensory abilities. Some of these functions include protecting the body from excessive water loss, friction and impact wounds, and external stimuli that can hold a potential threat for the body (Barry, 1983). The skin of the average adult body covers approximately 1-2 m^2 (Naik *et al.*, 2000) and it receives a third of the total blood circulation, thus making it an ideal route by which therapeutic agents can be administered (Chien, 1987). The route by which most drug molecules penetrate the skin is through the stratum corneum (SC), the outermost layer of the skin. It is a compositionally and morphologically unique biomembrane that consists of a layer of compressed keratin-filled corneocytes anchored in a matrix of lipids. This arrangement of the corneocytes and lipids are known as the brick and mortar assembly, which makes the membrane about a thousand times less permeable to water relative to the other membranes in the body (Naik *et al.*, 2000).

Lamivudine (4-amino-1-[2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-1H-pyrimidin-2-one or 2',3'-dideoxy-3'-thiacytidine, 3TC) is a nucleoside reverse transcriptase inhibitor that is used against HIV (1 and 2), Hepatitis B infections and in patients that has been accidentally exposed to HIV (Medicinenet, 2007). According to Gao *et al.* (1993) lamivudine is phosphorylated to its active metabolites, namely 2',3'-dideoxy,3'-thiacytidine-5'-triphosphate, which in turn competes with deoxycytidine triphosphate and stops it being incorporated into the viral DNA *via* reverse transcription. The most common adverse effects of lamivudine are usually associated with the gastric-intestine system and include the following: vomiting, diarrhoea, abdominal cramps and pain, nausea (Gregg, 1999; Sweetman, 2002). Lamivudine is also very hydrophilic with a aqueous solubility of 70 mg/ml and a log P value of -1.4. These properties (aqueous solubility and log P) and adverse effects thus make transdermal delivery of the drug very appealing.

The transdermal drug delivery (TDD) system hosts a few advantages over oral administration that makes it attractive for drug delivery. Some of the advantages include better patient collaboration, control over the input kinetics and less side-effects (Niak *et al.*, 2000). Thus,

incorporating lamivudine in a TDD system will help lower the side-effects that it has on the gastro-intestinal (GI) tract and finally leading to better patient compliance with anti-retroviral (ARV) therapy that leads to less resistance of the HIV-virus. Transdermal application can be advantageous for children or infants that have trouble taking the oral lamivudine formulation, due to its bitter taste (Schiffman *et al.,* 1999). Transdermal drug delivery offers other advantages that include avoiding hepatic first-pass metabolism, improved bioavailability, decreasing the administered dose and it is easy to discontinue in case of toxic effects (Mitragotri, 2000).

As previously mentioned, the skin has a barrier function that inhibits the passive permeation of certain molecules (highly hydrophilic) through the skin. For these molecules to pass through the SC (lipophilic), they need to exhibit certain physicochemical properties that allow it to penetrate the skin's different layers. These properties include the partition coefficient (log P), melting point, aqueous solubility of the drug, molecular mass and pKa of the drug. According to Katz and Poulsen (1971) all of these properties combined, have an influence on the rate at which a drug permeates through the skin.

The log P value is a good indication on how readily a drug will undergo passive diffusion through the SC because it's an indication how lipophilic a drug is. The ideal log P value for a drug to cross over the SC is between 1 and 3 (Guy, 1996). Another important property is the aqueous solubility of a drug. An aqueous solubility of more than 1 mg/ml will be required in order for a drug to permeate through the skin; otherwise it may have some bioavailability problems (Abdou, 1989). Derivatization, by increasing the alkyl chain length of the methoxypoly(ethylene glycol) moiety also has an effect on the rate of the flux over the SC. Usually this type of derivatization increases the lipophilicity of the drug, thus making it easier for the drug molecules to permeate through the lipophilic SC. PEGylation is another form of derivatization. It is the process were methoxypoly(ethylene glycol) (MPEG) is utilised to create derivatives like carbonates and carbamates. MPEG exhibits advantages like increasing stability, enhancing solubility and reducing toxicity (Veronese & Pasut, 2005), thus making it an ideal choice for the use in derivatization.

Pheroid™ is a patented delivery system consisting of plant and essential fatty acids that is stable as a unique submicron emulsion type formulation. It is claimed that it can encapsulate a variety of drugs and deliver them with high efficacy to targeted sites in the body. This system has been manipulated in such a way that it illustrates important advantages, such as high entrapment capabilities, fast rate of transport, delivery and stability, over other lipid based delivery systems like liposomes (Grobler, 2004). These properties make the use of Pheroid™ an ideal choice for drugs that can't penetrate the skin because of their hydrophilic nature. This

makes it possible to administer a wide range of drugs across the skin that could not have been done before.

1.2 Aim and objectives of the study

The primary aim of this study is to synthesise a series of new derivatives of the anti-HIV drug lamivudine, and to evaluate their transdermal penetration, with and without the use of Pheroid™ as delivery system.

In order to achieve this goal, the following objectives were set:

- Synthesise MPEG derivatives of lamivudine and confirm their structures.
- Experimentally determine the physicochemical properties like the aqueous solubility and the partition coefficient for lamivudine and its synthesised derivatives and to compare the experimental aqueous solubility and the partition coefficients of the synthesised lamivudine derivatives to calculated values from commonly used prediction software (ALOGPS 2.1 online prediction software).
- Experimentally determine the transdermal flux of lamivudine and its derivatives in phosphate buffer solution (PBS) at pH 7.4 and in Pheroid™ and to compare the experimental flux data of the synthesised lamivudine derivatives to calculated values from commonly used theoretical equations.
- Determine whether a relationship exists between the physicochemical properties like the aqueous solubility, partition coefficient and the transdermal flux data of lamivudine and its derivatives.
- Contribute to a data base from the data obtained from both this study and other transdermal studies whereby possible correlations between physicochemical properties and the transdermal penetration can be determined.

HIV / AIDS AND LAMIVUDINE AS NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITOR (NRTI)

2.1 Human Immunodeficiency Virus (HIV)

2.1.1 Overview and statistics

The AIDS epidemic has made more impact on public health than even the black plague could. With more than 25 million people vanishing worldwide because of this infection since the early 1980s, and more than 40 million people currently infected with it, HIV is one of the deadliest viruses to ever hit mankind (WHO, 2002). AIDS can be defined as an Acquired Immunodeficiency Syndrome. The primary cause of AIDS is the Human Immunodeficiency Virus (HIV) (Blattner *et al.*, 1988; Fauci, 1993; Weiss, 1993). HIV is a retrovirus that needs a host cell to duplicate itself and thus insuring its survival. HIV infects the CD4+ T-cells that cause the host's immune system to break down and to allow opportunistic infections (OI) to infect the host. Some of these OIs are: tuberculosis (TB), pneumocystis carinii pneumonia (PCP), Kaposi's sarcoma (KS) and Herpes simplex (UNAIDS, 1998). A person can be classified to have AIDS when their CD4+ T-cells count falls below 200 cells per cubic millimetre of blood and/or they have one of the opportunistic infections associated with AIDS (CDC, 1992; WHO, 2006).

Since the epidemic started in 1981, more than 25 million people have died of AIDS. Statistics at the end of 2007 showed that 33 million people are living with HIV and that 2.7 million people become infected with it each year. At the end of 2007 it also showed that 2 million people died of AIDS in that year alone (UNAIDS, 2007). The newly infected people in the age group of under 15 years amounted to 420 000 in 2007. This means that every day about 1180 children became infected with HIV in 2007. Infected women accounted for 50% of all adults living with HIV worldwide. More than three quarters of the people living with AIDS are situated in sub-Saharan Africa. With an estimated 5.5 million people living with HIV (UNAIDS, 2006), South Africa has the largest number of infections in the world. It is estimated that 1.8 million people died of AIDS-related disease in South Africa since the epidemic started (Dorrington *et al.*, 2006). The total annual deaths (from all causes) in South Africa increased by 87% from 1997 to 2005 (Statistics South Africa, 2005 & 2006), with at least 40% of those deaths estimated to

have been AIDS-related (Bradshaw *et al.*, 2004). In developing countries, 9.7 million people are in immediate need of life-saving AIDS drugs; of these, only 2.99 million (31%) are receiving the drugs (UNAIDS, 2007).

2.1.2 Origin and discovery

The AIDS virus HIV had its origin in Africa and is a descendant of a Simian Immunodeficiency Virus (SIV) because it resembles the strains of HIV-1 and HIV-2. In 1999 it was announced that a SIVcpz virus was found that was almost identical to HIV-1. This strain was identified in a frozen sample taken from chimpanzees known as *Pan troglodytes troglodytes*, which were once common in west-central Africa (Gao *et al.*, 1999).

The first cases of AIDS were documented in California in America in the summer of 1981 when cases of Kaposi's sarcoma was reported in young homosexual men. This occurrence was strange because this cancer usually only occurs in older people (Hymes *et al.*, 1981). About the same time, numerous cases of pneumocystis carinii pneumonia (PCP) flared up all over the country. In June, the Centres for Disease Control and Prevention (CDC) published a report, without identifiable cause, of PCP in five men in Los Angeles (CDC, 1982). This signalled the beginning of the general awareness of AIDS.

It was first believed that only homosexuals could get the disease, but in December 1981 it was discovered that the disease also affected other groups of the population. During this time the first case of AIDS was documented in the UK (Dubois *et al.*, 1981). Later, in 1983, the CDC reported that AIDS might be passed on by heterosexual sex after they discovered AIDS among females (CDC, 1983). In May 1983, doctors at the Pasteur Institute in France reported that they had isolated a new virus which may be the cause of AIDS. The virus was called the lymphadenopathy associated virus (LAV) (Barré-Sinoussi *et al.*, 1983). Little notice was taken on this discovery.

In 1998, genetic analysis of a plasma sample from an adult male in the Democratic Republic of Congo suggested that HIV-1 had its origin from a single virus in the period between 1948 and 1952 (Zhu *et al.*, 1998).

The first case of AIDS in South Africa was reported in 1982. Since the 1990s, an increasing amount of attention was being paid to AIDS. It was only in 2002 that the government decided to make the drug nevirapine available to pregnant woman to help prevent mother to child transmission of HIV. In 2003 the government decided to make antiretroviral (ARV) drugs available to the public and in 2005 the first service point for AIDS related care and treatment had been established in all of the districts in the country. Despite this effort by the government, the number of people receiving ARV treatment was well behind the set targets (UNAIDS, 2007).

2.1.3 Transmission of AIDS

The most common ways of HIV infection are:

- unprotected sexual intercourse with an infected person (Nakashima & Flemming, 2003).
- injection with a needle that has been used by an infected person,
- perinatal transmission during the pregnancy, delivery or breastfeeding (Adler, 1993).
- blood or blood products transfusion.

2.1.4 Structure and life cycle of the virus

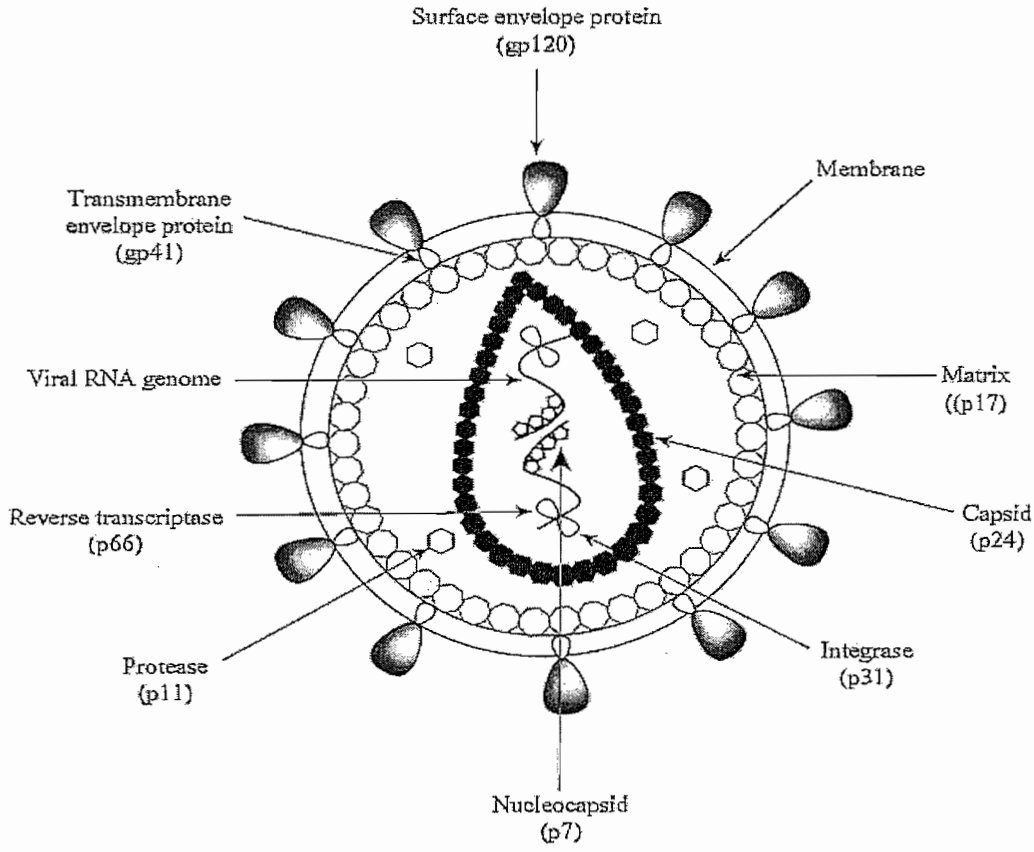


Figure 2.1 HIV's molecular structure (Kirk-Othmer, 2008).

The HIV particle is around 150 nm in diameter (McGovern *et al.*, 2002). Unlike most bacteria, HIV particles are too small to be seen through an ordinary light microscope, but through an electron microscope they can be seen clearly. The HIV particle encloses itself with a membrane consisting of phospholipids creating the viral envelope. From this envelope 72 spikes are formed from the proteins gp120 and gp41 (Freed & Martin, 1995) are projecting. Just below this

envelope is the so-called matrix. The matrix is made from the protein p17. This protein is important to the virus because it is involved in the binding of the viral ribonucleic acid (RNA) (Galley *et al.,* 1995). The protein p24 makes the bullet-shaped viral core which contains three enzymes required for HIV replication and the HIV's genetic material which consists of two identical RNA strands. These RNA strands are (+) single-stranded and they are about 3500-9000 nucleotides long (Kirk-Othmer, 2008). These three enzymes are reverse transcriptase (p66), integrase (p31) and protease (p11) (Doublie *et al.,* 1999; Sarafianos *et al.*, 1999).

The life cycle of HIV can be illustrated in **three** steps as described below.

2.1.4.1 Entry of HIV into the host cells

HIV can only replicate in human cells. The virus bumps against a cell with the surface protein CD4. The spikes on the viral envelope stick to the CD4 and allow the viral envelope to fuse with the cell membrane. The HIV cell's contents are then released into the host cell (Avert, 2008).

2.1.4.2 Reverse transcriptase and integration

Inside the cell, the HIV enzyme's reverse transcriptase converts the viral RNA to DNA. This DNA is compatible with human DNA. The newly formed DNA is transported into the cell's nucleus, where it is incorporated into the human DNA by the HIV enzyme integrase (Avert, 2008; Bouyac-Bertoia *et al.*, 2001). The integrated HIV DNA is known as the provirus. This provirus can lie dormant in a cell for long periods of time, but once activated the cell treats the viral genes in the same manner as human genes. Firstly, the viral genes are converted into messenger RNA (mRNA) by using human enzymes. This mRNA is then transported out of the nucleus and then used as a blueprint to form new HIV proteins and enzymes.

2.1.4.3 Assembly, budding and maturation

The cell produces mRNA that is a complete copy of the HIV genetic material. These gather together with newly made HIV proteins and enzymes to form new viral particles, which are then released by the cell (Avert, 2008). The protease enzyme shortens the protein strands, which then will be used to form new mature viral cores. The mature viruses are then ready to infect other cells and to start the process of replication all over again.

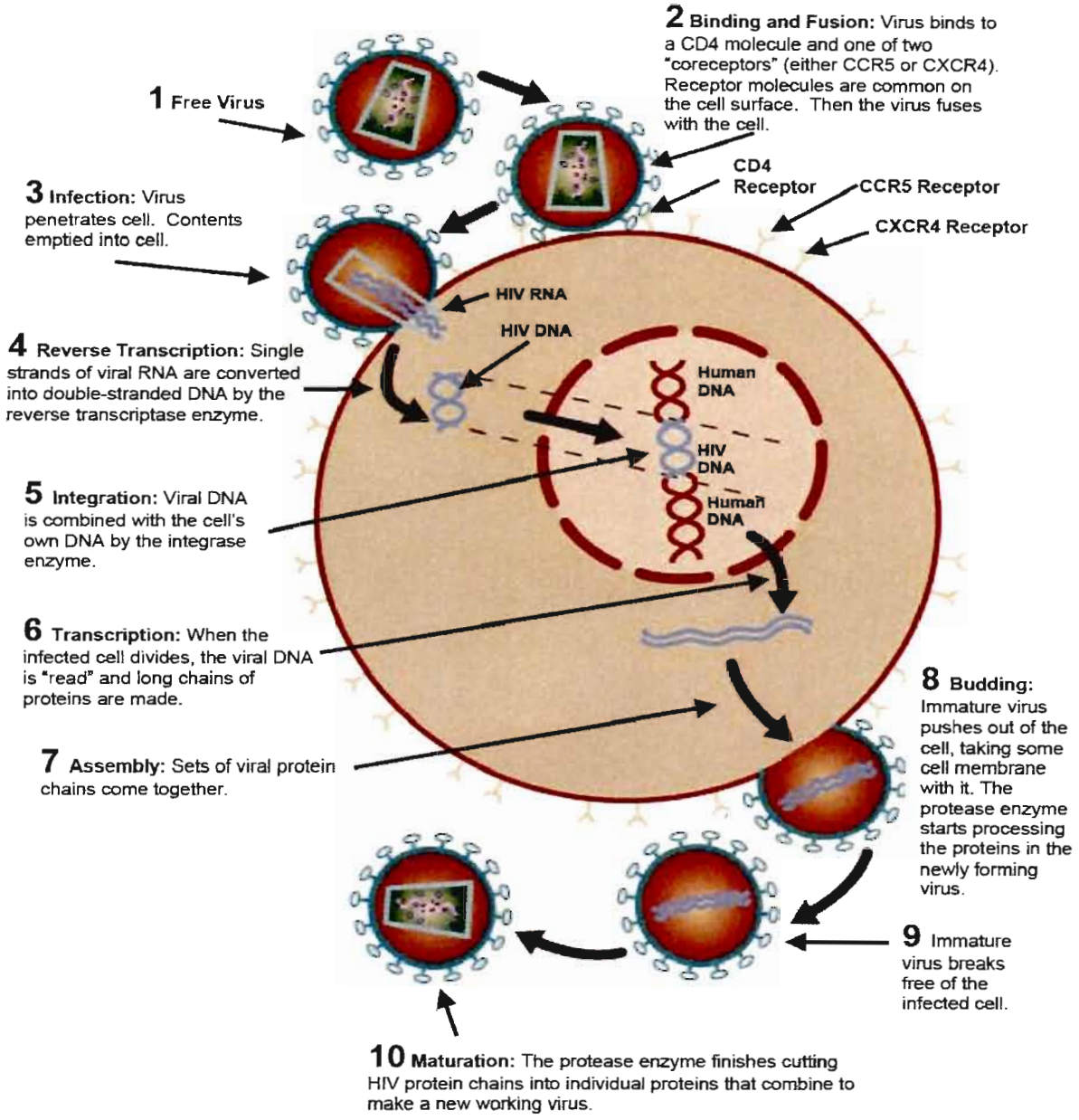


Figure 2.2 Life cycle of HIV (AIDS InfoNet, 2007).

2.1.5 Treatment

The treatment for HIV or AIDS is not a cure, but it can prevent people from becoming sick for many years. These drugs have to be taken for the rest of the person's life.

According to De Clercq (2004) there are five different classes of antiretroviral drugs that can be used as chemotherapeutic agents in the treatment of AIDS:

- Protease inhibitors (PIs),
- Nucleotide reverse transcriptase inhibitors (NtRTIs),
- Nucleoside reverse transcriptase inhibitors (NRTI),
- Non-nucleoside reverse transcriptase inhibitors (NNRTIs) and
- Viral entry inhibitors.

The treatment plan of AIDS is commonly referred to as HAART (Highly active antiretroviral treatment).

2.1.6 Symptoms and opportunistic infections

According to the CDC (1992) and the WHO (2006) there are four different stages of HIV infection:

Stage 1: Primary HIV infection

This stage is accompanied by flu-like symptoms and only lasts for a few weeks. It is in this stage that diagnosis is often missed because in only about 20 % of individuals the symptoms are serious enough to consult a doctor. In this stage there is a large amount of HIV in the blood and that causes the immune system to react and creating HIV antibodies and cytotoxic lymphocytes. This stage is also known as seroconversion and if an HIV-test is done before the completion of seroconversion, it may have a negative result.

Stage 2: Clinically Asymptomatic Stage

This stage last for about 10 years and patients are often free of al major symptoms of HIV. The only indication is usually swollen lymphatic glands due to the cytotoxic lymphocytes. The HIV antibodies in the blood has dropped to very low amount, but the patient still remains infectious, and thus a HIV test will have a positive result.

The viral load can be tested in this stage by performing a test that measures the HIV RNA that escapes the lymph nodes. This is a very important test because it determines the suitable treatment for the specific individual.

Stage 3: Symptomatic HIV Infection

In this stage the immune system is severely damaged due to:

- over activity of the lymph nodes during stage 2,
- mutation of HIV that becomes more aggressive that leads to more T helper cell destruction, and
- the body can't produce enough T helper cells to make up for the lost cells.

The destruction of the immune system leads to opportunistic infections (Table 2.1).

System	Examples of infection/ Cancer
Respiratory system	Pneumocystis jeroveci Pneumonia (PCP) Tuberculosis (TB) Karposi's sarcoma (KS)
Gastro-intestinal system	Cryptosporodiosis Candida Cytomegolavirus (CMV) Isosporiasis Karposi's sarcoma
Central/ peripheral Nervous system	HIV Toxoplasmosis Cryptococcosis Non-Hodgkin's lymphoma Varicella Zoster Herpes Simplex
Skin	Herpes Simplex Varicella Zoster

Stage 4: Progression from HIV to AIDS

The severity of the opportunistic infections increases as the immune system becomes weaker. This leads to the diagnosis of AIDS. AIDS diagnosis differs from country to country. In the U.S.,

someone is diagnosed with AIDS if their T helper cell count falls beneath a specific number, whereas in the UK someone is diagnosed with AIDS if they develop one or more of the opportunistic infections or cancer.

2.2 Nucleoside Reverse Transcriptase Inhibitors (NRTIs)

There are a total of 16 drugs that have been approved by the U.S. Food and Drug Administration (FDA) for the chemotherapy of AIDS (De Clercq, 2001 & 2002). Seven of the 16 are nucleoside reverse transcriptase inhibitors (NRTI), making them the most important class of compounds in the treatment of HIV.

They are often used in combinations with other NRTIs and NNRTIs because they are not effective on their own (Avert, 2008; Staley *et al.*, 2006) due to viral resistance against these medications. The NRTIs work by producing a faulty blueprint which is then incorporated into the viral nucleic acids. This faulty encoding causes nucleic chain termination in the viral cell (De Clercq, 2001 & 2002; Soriano & De Mendoza, 2002).

2.3 Lamivudine

2.3.1 History

Lamivudine (4-amino-1-[2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-1H-pyrimidin-2-one, 3TC) is a nucleoside reverse transcriptase inhibitor (Medicinenet, 2007). Lamivudine was discovered in 1989 by Bernard Belleau and Nghe Nguyen-Ba at IAF BioChem International, Inc. laboratories in Montreal. They later licensed it to a UK pharmaceutical company GlaxoSmithKline and got 14% of the royalties. The FDA approved it on 17 November 1995 for use in combination with zidovudine (AZT) because of their synergism (Avert, 2008).

2.3.2 Mechanism of action

Lamivudine, like all nucleoside analogues, is a prodrug, meaning that they have to be metabolised intracellularly to wield their activity (Stein & Moore, 2001). According to Gao *et al.* (1993) lamivudine is phosphorylated to its active metabolite, namely 2',3'-dideoxy,3'-thiacytidine-5'-triphosphate, which in turn competes with deoxycytidine triphosphate and stops it from being incorporated into the viral DNA *via* reverse transcription.

Lamivudine enters the cell by means of passive diffusion. Deoxycytidine (dCyd) kinase converts lamivudine to the monophosphate, it is further phosphorylated by deoxycytidine monophosphate (dCMP) kinase and nucleoside 5'-diphosphate (NDP) kinase to yield lamivudine 5'-triphosphate

(lamivudine-TP), which is the active anabolite (Perry & Faulds, 1997). Lamivudine-TP intracellularly competes for the binding to reverse transcriptase (RT) enzyme and is then incorporated into the developing viral nucleic acids. This results in the termination of the nucleic acid chain because of the absence of the important 3'-hydroxy group that is needed by the HIV cell for chain extension (De Clercq, 2001 & 2002; Flexner, 2006; Soriano & De Mendoza, 2002).

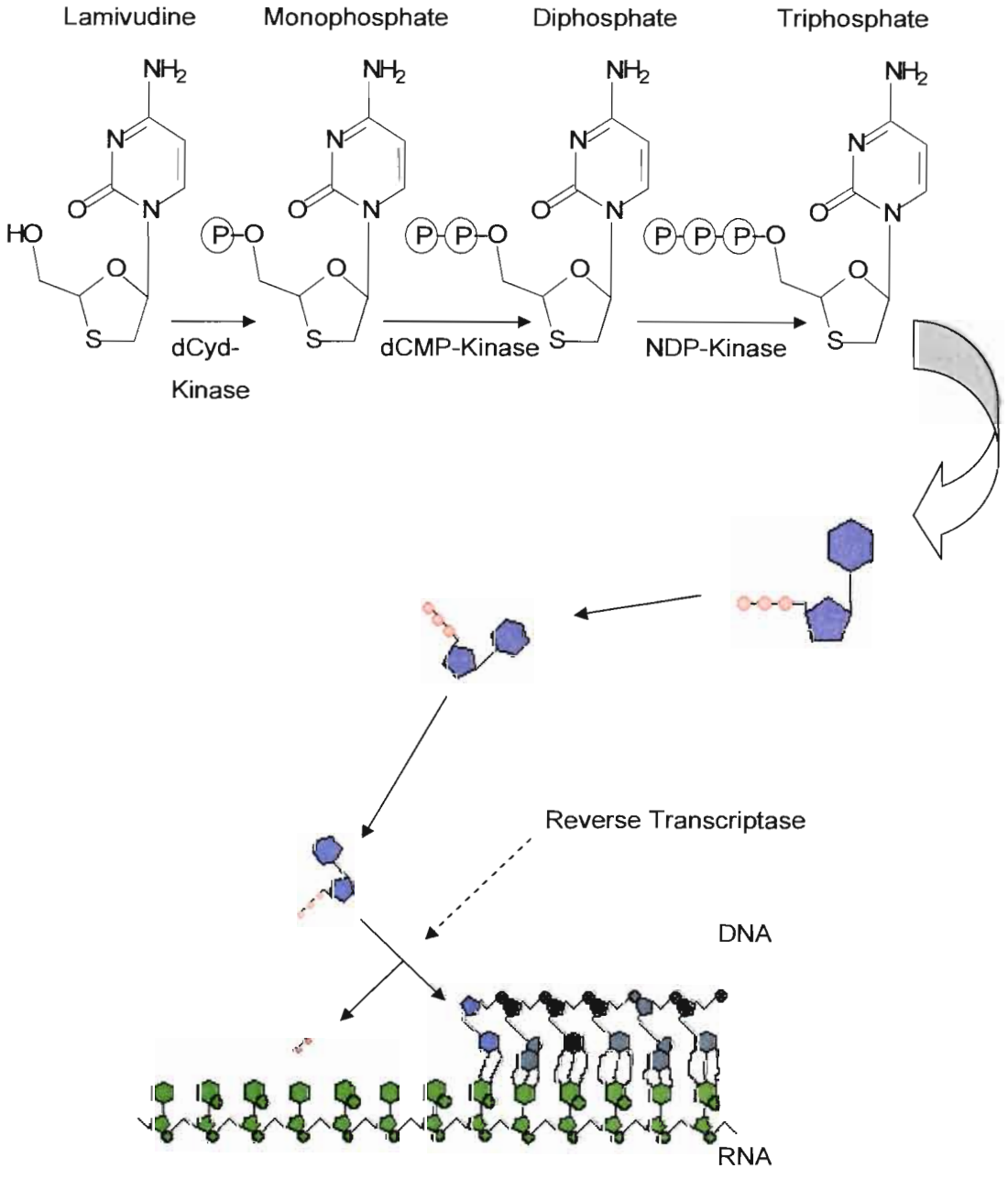


Figure 2.3 Mechanism of action of lamivudine (De Clercq, 2004; Flexner, 2006).

2.3.3 Clinical use and adverse effects of lamivudine

Lamivudine is used against HIV (1 and 2), Hepatitis B infections and in patients that have been accidentally exposed to HIV (Medicinenet, 2007). It is one of the least toxic antiretroviral drugs because it has the lowest affinity for human DNA polymerases (Flexner, 2006).

The most common adverse effects of lamivudine are usually associated with the gastric-intestine system and include the following: vomiting, diarrhoea, abdominal cramps and pain, nausea (Gregg, 1999; Sweetman, 2002).

2.4 Transdermal penetration of NRTIs

One of the first NRTIs on which transdermal diffusion studies had been done was AZT. Kararli *et* a.l (1994) used chemical enhancers like *t*-anethole, carvacrol, thymol and linalool to determine whether they have an enhancing effect on the transdermal penetration of AZT. L-Menthol was used as a reference enhancer. They determined the *in vitro* penetration by using Franz cells and full thickness Sprague-Dawley rat and CD1-1 nude mouse skin. The *in vivo* bioavailability was determined in rats by using a gel formulation containing the enhancers. The *in vitro* studies showed a 24-38 times enhancement of AZT with 5% of the enhancer. This was the optimum concentration for the enhancers. It was shown that the quantity of AZT retained in the skin was dependant of the concentration and type of enhancer being used, indicating that the mechanism of transport enhancement is due to increased diffusion of AZT through the stratum corneum. In this study, the transdermal transport of AZT was higher through the mouse skin as through the rat skin.

Kim & Chien (1995) researched the effect of vehicles and enhancers on the transdermal penetration through hairless rat skin of the dideoxynucleoside-types antiretroviral drugs didanosine (DDI), zalcitabine (DDC) and zidovudine (AZT). It was found that there was an enhancement of permeation rates in each of the drugs as the volume fraction of ethanol (EtOH) was increased. This EtOH/water co-system enhances the skin permeation by means of increasing both the solubility of the drug in the skin and partitioning of the drug into the skin. The enhancement by EtOH/tricaprylin (TCP) co-system is only due to the increase in the partitioning of the drug into the skin. Even the addition of other enhancers like oleic acid (OA) and *N*-methyl-2-pyrrolidone (NMP) to EtOH/TCP could not increase the permeation of the drugs. However, the incorporation of 1% (v/v) OA to the EtOH/water (60:40) co-system significantly enhanced the penetration and shortened the lag time of the drugs. All of the drugs reached their target permeation rate and maintained their required therapeutic systemic levels with the 1% OA in the EtOH/water (60:40) co-system.

In 2002, Thomas & Panchagnula (2002) researched the effect that some vehicles may have on the transdermal penetration of AZT across rat skin. Just like Kim & Chien (1995), they found that the EtOH/water (66.6:33.3) co-system provided the highest flux values across the skin. High flux values were also observed with 33.3% propylene glycol (PG) in PG-water solvents and with 100% EtOH among PG-EtOH combinations. High concentrations of PG in both water and ethanol systems showed a reduction in the steady state flux of AZT. It was also found that PG-water and PG-EtOH systems neither reduce the lag time nor increase the flux of the drug. Thermogravimetric studies revealed that co-systems containing high concentrations of PG dehydrate the epidermis of the skin (Thomas & Panchagnula, 2002).

Gerber *et al.* (2008) synthesised *N*-acyl lamivudine esters and determined the transdermal flux of each one of them with or without the use of Pheroid™. These values were compared to those of lamivudine. It was found that the median flux of lamivudine was still higher than the synthesised compounds, thus concluding that as the alkyl chain length increased and the aqueous solubility decreased, the permeability of the compound decreased. This was ascribed to the fact that the compound penetrated the SC, but could not leave it. The Pheroid™ did not increase the flux of lamivudine and its derivatives and the flux values were even lower than the values in PBS.

Jain *et al.* (2007) used ethosomes, discovered by Touitou (1996) to transport lamivudine across the skin. Ethosomes are lipid vesicular systems that embody EtOH in very high concentrations. These ethosomes contain phospholipids, water and alcohol (EtOH and isopropyl alcohol) in high concentrations. What makes these ethosomes different from classical liposomes is the fact that they could cross the SC of the skin, thus creating higher flux rates of drugs across the skin. The optimised ethosomal formulation showed a 25-fold higher flux than that of lamivudine in PBS. However, a significant reduction in the flux of lamivudine was detected as the concentration of EtOH in the formulation of the ethosomes increased (>45% v/v).

TRANSDERMAL DRUG PERMEATION

3.1 Introduction

The TDD system hosts a few advantages over the common oral route of administration that makes it more applicable for medication delivery.

These advantages include the following (Naik *et al.*, 2000):

- The skin presents a large and readily accessible surface area for administration of drugs.
- Patients tend to comply more with this kind of administration (patch-like device) that allows continuous intervention by the appropriate administrator.
- Sustained release can be acquired with this route of delivery (for agents with short biological half-lives and for drug requiring efficient chrono-pharmacological management).
- The input kinetics can be controlled for drugs that have a narrow therapeutic index.

According to Ranade (1991) the transdermal route of delivery also has the following advantages over the common oral route:

1. It avoids the chemical hostile GI environment that the stomach presents.
2. It does not have the GI distress or other physiological contraindications of the oral route.
3. It provides adequate absorption of drugs with some oral absorption problems.
4. The first-pass effect is avoided.
5. Drugs with short biological half-lives can be used more effectively.
6. The administration of drugs with a narrow therapeutic window is made possible.
7. Controlled plasma levels of potent drugs could be achieved.
8. Prompt interruption of drug input if toxicity occurs.

The transdermal delivery of drugs protects the stomach and intestine from the harmful effect that some drugs may have on it (Wilkosz & Bogner, 2003).

Despite these advantages, the transdermal delivery of drugs has its limitations. Some of the limitations are:

1. The skin acts as a two-way barrier, preventing harmful and unwanted molecules from the external environment from entering the body, while regulating the loss of essential body fluids and electrolytes.
2. Drugs that require high-blood levels can not be administered.
3. Patch adhesives may not adhere to all skin types (allergic responses may be provoked).
4. Drugs or drug formulations may cause skin irritation or sensitisation.
5. The system may not be economical, meaning that it would not be cost effective to formulate, for example, a drug into a transdermal patch if it already has a good oral bioavailability (Ranade, 1991; Naik *et al.*, 2000).

Physico-chemically, it is accepted that molecules with a modest melting point, low molecular weight (<500 daltons) and an oil-water partition coefficient (log P) in the range of 1–2 will have respectable passive skin permeability (Naik *et al.*, 2000).

3.2 Structure of the skin

The human skin consists of two distinct layers which are the stratified avascular cellular epidermis and the underlying dermis of connective tissue (Ranade, 1991). Though many people probably regard the skin as just the outside layer of the body, it is in reality a large and complex organ. In fact, it is the largest in the body, with a total surface area of about 1.72 square meters (Barr, 1962; Naik *et al.*, 2000) and an average mass of between 3 and 4 kg (Schalla & Schaefer, 1982; Stüttgen, 1982). The thickness varies from about 0.5 mm on the eyelids to more than 5 mm on the upper back, with an average of about 2 mm (Foldvari, 2000). It grows constantly from inside, replacing the outer layers as they wear off. Each person sheds on average about 50 kg of skin cells in a lifetime.

Within the skin there are other structures with specialised functions. These include hairs and hair follicles, finger and toe nails, sebaceous (fat-producing) glands, sweat glands, small arteries and veins, and a wide range of nerve types ending in receptors for detecting external stimuli such as heat and cold, pressure, vibration and pain (Hall, 2003).

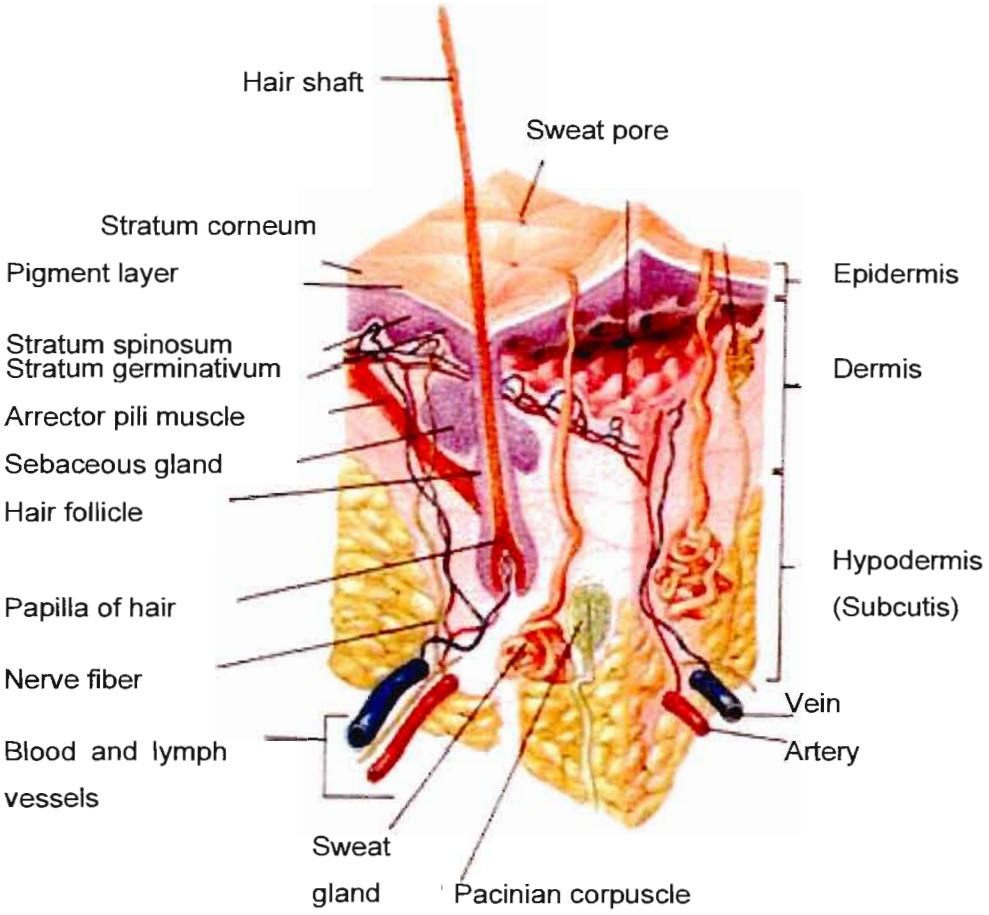


Figure 3.1: A schematic cross-section of human skin (Van de Graaf, 2002).

In order for a drug to have a systemic effect, it has to cross all of the different layers of the skin, namely the epidermis, dermis and hypodermis. These three layers will be discussed in detail and their barrier function will become clear in the following sections.

3.2.1 Stratum corneum

The tissue with the largest effect on drug permeation in this complex membrane is the stratum corneum (SC) or the horny layer. This important layer usually provides the rate-limiting (slowest) step in the percutaneous absorption process (Barry, 1987). This compositionally and morphologically unique biomembrane is extremely thin (about one hundredth of a millimetre) and consists of a shield of dead, anucleate, keratin-filled corneocytes that resembles keratinocytes. These corneocytes are anchored in a lipophilic matrix (Christophers, 1971; Elias, 1981; Elias, 1983).

The stratum corneum consists only of 10-15 corneocyte layers and is only 10 µm thick when dry, but it may swell when it is hydrated (Flynn, 1989). It is the thickest on the foot soles and palms (400 - 600 µm) and the thinnest on the lips. The keratinised cells in the SC are embedded in a mortar of lipid bilayers and are often referred to as the "mortar and brick" model (Figure 3.2) (Michaels *et al.*, 1975; Elias, 1981b). The corneocytes represent the "bricks" while the intracellular lamellar lipids form the "mortar". This wall of cells is kept together by desmosomes that provides the stratum corneum with its stability.

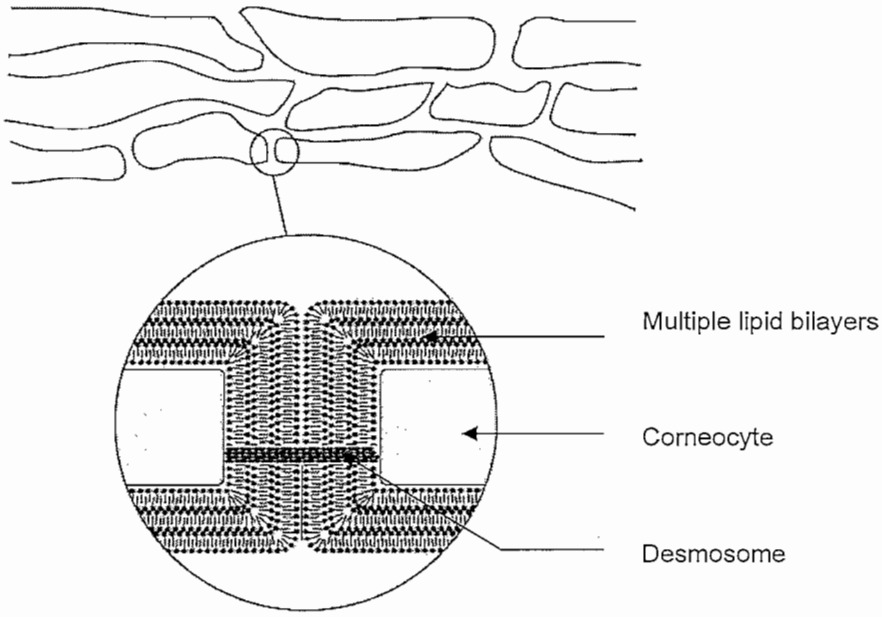


Figure 3.2: A representation of the "brick and mortar" model of the human skin (Michaels *et al.*, 1975; Elias, 1981b).

The major components of the stratum corneum are ceramides, fatty acids, cholesterol, cholesterol sulphate and sterol esters (Elias, 1983; Wertz *et al.*, 1985) which varies between individuals and body site (Lampe *et al.*, 1983). These components provide the stratum corneum with its amphiphilic properties that is needed to form the lipid bilayers. Water plays a vital role in maintaining the integrity of the stratum corneum; it is also involved in the mediation of some hydrolytic enzymes' activity. The keratinocyte water regulates the enzymes that are involved in the manufacturing of natural moisturising factor (NMF). This NMF prevents the skin from cracking and drying up.

3.2.2 Viable epidermis

The epidermis is situated directly beneath the stratum corneum and it is also described as a complex multiple layered membrane. It varies in thickness from 0.06 mm on the eyelids to 0.8 mm on the palms and foot soles that takes a lot of beating from the external environment. The epidermis contains no blood vessels, and thus nutrients and waste products have to travel across this membrane in order for it to maintain its integrity. This also means that a drug must travel across it to reach the systemic circulation in order to have a therapeutic effect.

The epidermis consists of three histologically distinct layers: the stratum basale, stratum spinosum and stratum granulosum (Figure 3.3) (Williams, 2003).

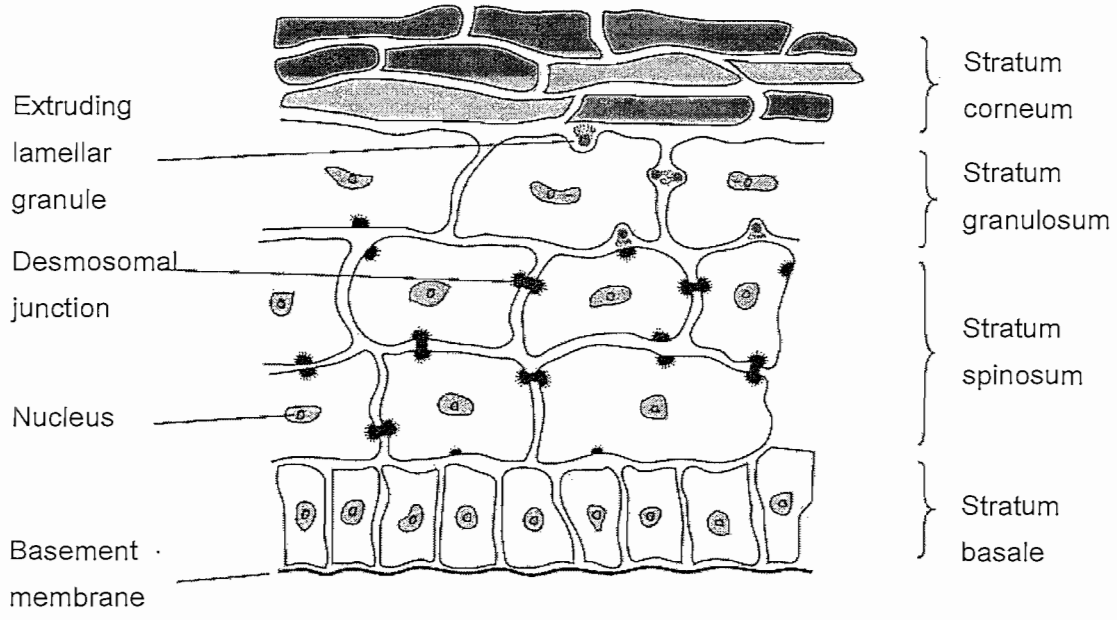


Figure 3.3: A representation of human epidermal cell differentiation (Williams, 2003).

3.2.2.1 The stratum granulosum

This is the first layer of the epidermis, just beneath the stratum corneum. It is usually referred to as the granular layer. It is only one to three cell layers thick and contains enzymes that degrade the cell components such as the nuclei and organelles. In this layer the keratinocytes keep on differentiating, forming keratin and start to flatten. The flattened cells will ultimately form the stratum corneum (horny layer) (Williams, 2003; Hall, 2003).

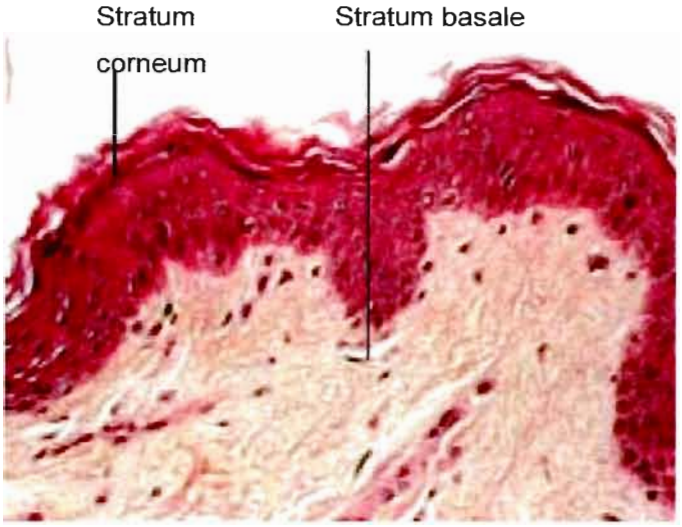
3.2.2.2 The stratum spinosum

This layer is found between the stratum granulosum and stratum basale (Figure 3.3) and is also known as the spinous layer or prickle cell layer. The basale and spinous layers together are called the Malpighian layer. The main component of the spinous layer is two to six layers of keratinocytes that change the morphology from columnar to multilateral cells. The keratinocytes in this layer synthesise keratin that aggregates to form tonofilaments. The adjacent keratinocytes are connected by desmosomes that are formed by condensation of the tonofilaments (Williams, 2003).

3.2.2.3 The stratum basale

This layer is referred to as the stratum germinativum or the basale layer. The cells in this layer are similar to the other cells that are found in the human body. They contain organelles such as mitochondria and ribosomes and they are metabolically active. The keratinocytes in the basale layer are the only cells in the epidermis that undergo mitosis. It takes about 200–400 hours to form new cells, thereafter the newly formed daughter cells stay behind while the older cells start to migrate upwards into the other layers of the epidermis.

The stratum basale contains other specialised cells such as melanocytes that form the pigment melanin from the amino acid tyrosine. Langerhans cells are also found in the stratum basale and are known to be the only antigen-presenting cells in the skin. This is important because the antigens play a role when it comes to allergic skin conditions such as contact dermatitis. Other specialised cells that are found in the basale layer are the Merkel cells. They are very close to the nerve endings in the basale layer, and thus they are located in touch sensitive areas such as the lips and fingertips. These cells are believed to play a role in cutaneous sensation (Williams, 2003).



Figurer 3.4: A cross section of healthy skin illustrating the location of the stratum corneum and stratum basale (Hall, 2003).

3.2.3 Dermis

The dermis (corium) is situated between the epidermis and the underlying subcutaneous fatty layer (hypodermis), with a thickness of 3 - 5 mm. It is the major component of the human skin and predominantly consists of collagen fibrils and elastic tissue that provides support and flexibility to the skin. These cells are embedded in a mucopolysacharide gel (Wilkes *et al.*, 1973). The dermis is very hydrophilic, so that the transdermal transport of hydrophilic drugs will easily take place while lipophilic drugs will struggle in crossing the dermis. Structures like lymphatic and blood vessels, nerve endings hair follicles, sebaceous and sweat glands are found in the dermis (Schaefer & Hensby, 1990).

The dermis is the layers with highest circulation of blood in the skin, regulating the body temperature and removing any toxins and other molecules, thus forming a "sink" condition that allows transdermal permeation to take place due to a concentration gradient that is formed (Cross & Roberts, 1993).

3.2.4 Hypodermis

The hypodermis is also commonly known as the subcutaneous fatty layer and it bridges between the overlying dermis and the underlying body constituents. This layer is very thick, thus insolating the body and protecting it against blows. The fat in this layer can also provide a quick source of energy to the body (Williams, 2003).

3.2.5 Skin appendages

There are three main appendages in the skin, namely the hair follicles, eccrine and apocrine sweat glands (Katz & Poulsen, 1971). The hair follicles are found over the entire body except on the areas on the body that take a lot of strain like the soles of the feet and the palms of the hands. The eccrine and apocrine glands have specific functions.

Eccrine glands are found over most of the body and they secrete sweat, a diluted salt solution with a pH of around 5. Their function is heat regulated. The apocrine glands are situated on specific areas of the body and are usually limited to the axillae, nipples and ano-genital regions. These glands are responsible for the odour of sweat (Williams, 2003). All of these appendages are situated in the dermis (Hunter *et al.*, 1996). In the hair follicles there are sebaceous glands that secrete sebum, a mixture of fatty acids, cholesterol, triglycerides, waxes, cellular debris (Montaga, 1965). This means that more lipophilic drugs will cross this section due to the lipophilic nature of the sebum.

3.3 The process of transdermal permeation of drugs

Over the years there have been many definitions to describe this process like sorption, persorption, permeation and penetration (Ranade, 1991). In Cleary (1984), Rothman described it as percutaneous absorption. But conclusively it can be defined as passively driven mass transfer by means of diffusion through the different layers of the skin until systemic circulation is attained (Ebling, 1977; Schuplein & Blank, 1971).

This process of transdermal absorption can be divided into several steps as shown in Figure 3.5:

1. The first step is the release of the drug from its formulation and the penetration of the first layer of the skin (stratum corneum).
2. Permeation and distribution of the drug through the different layers of the skin.
3. Finally, the absorption from the skin tissue into the systemic circulation (Schaefer *et al.*, 1982).

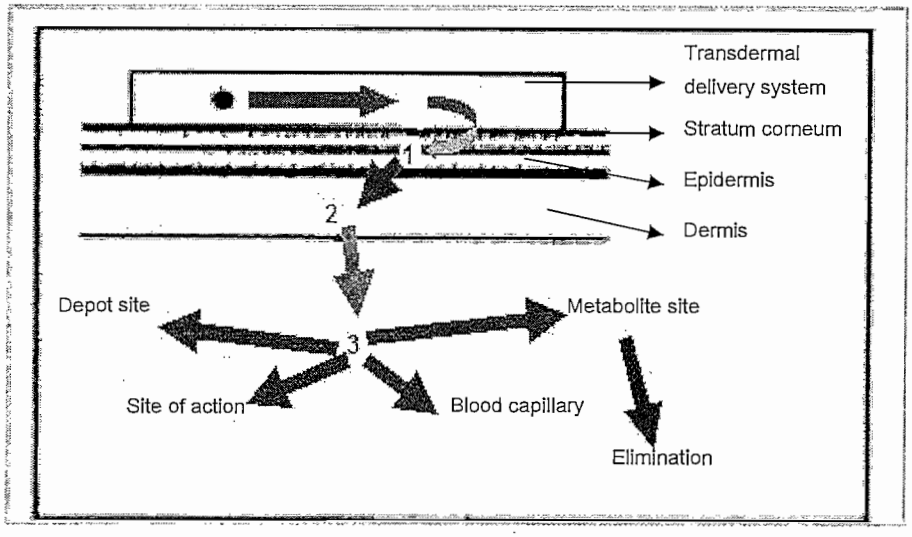


Figure 3.5: The process of transdermal permeation (Higuchi, 1978).

There are many factors that have an influence on the process, both physiological and physicochemical.

The physiological factors that influence this process:

- Skin age,
- Body site,
- Race,
- Anatomy of the skin (different layers),
- Other (gender, temperature, hydration of the skin) (Williams, 2003).

Some physicochemical factors that influence this process:

- Aqueous solubility,
- Lipophilicity,
- Molecular weight,
- Melting point,
- pH of the saturated solution (Naik *et al.*, 2000).

The passing of the molecule through the skin is mediated by passive diffusion that takes place due to a diffusion gradient that has been created. Due to the lipophilicity of the SC, the gradient is much steeper than in the underlying epidermis (Flynn, 1989; Naik *et al.*, 2000). This passive process of diffusion may be influenced by various factors, for example, steroid molecules may bind with the keratin in the SC, forming a "reservoir" effect. This means that the concentration gradient is shifting, slowing down the process and thus creating a depot site for the drug in the skin. The advantage of this phenomenon is that a sustained release of the drug can be obtained (Williams, 2003).

3.4 Routes of transdermal delivery

For molecules to have an effect in the body, they must cross the different layers of the skin. In order for them to achieve this crossing of the skin they can take different routes.

The three routes which a molecule can take to reach the systemic circulation are:

1. Transepidermal route (intra- and intercellular),
2. Through the sweat glands,
3. Through the hair follicles (Barry, 2001; Williams, 2003).

3.4.1 Transepidermal route

This route is always divided into two:

- The intercellular route (crossing of the molecule between the cells),
- The intracellular route (crossing of the molecule through the cells) (Williams, 2003).

3.4.1.1 Intracellular

This route is also known as the transcellular route by which a molecule permeates through the stratum corneum. According to Barry (2001), this is the route where the drug molecules move across and through the corneocytes that is located in SC. Travelling across and through the cells brings forth its own obstacles.

The stratum corneum's keratinocytes are very hydrophilic, thus the natural way of thinking would be that hydrophilic drugs would cross the SC with more ease. But it must be taken into account that these hydrophilic keratinocytes are enclosed in a multi-layered lipophilic membrane and surrounded by a lipophilic matrix between these cells. This lipophilic environment thus prevents hydrophilic drugs from crossing the SC (Williams, 2003). The lipophilic matrix is made up of 4-20 lamellae (cells with a hydrophilic head and lipophilic tail) between each keratinocyte, making it very difficult for hydrophilic drugs to pass through it (Wertz & Downing, 1989). It also makes it difficult for lipophilic drugs to pass through the hydrophilic head space in lamellae as the drug diffuses perpendicular to the SC.

3.4.1.2 Intercellular

The intercellular route accommodates the molecules that pass between the cells of the stratum corneum. According to Williams (2003), the lipid bilayers make up about 1% of the SC but are the only continuous phase in the SC. The importance of these lipids is that they hinder water loss through the skin (Elias, 1981). This means that only lipophilic drugs can transverse by this route. It is believed that this route is predominantly suitable for most small, uncharged molecules (Guy & Hadgraft, 1989).

3.4.2 Transappendageal (Shunt route)

The sweat glands and hair follicles also offer pathways by which a molecule can travel through the SC. According to Scheuplein (1971), these appendages only make up about 0.1% of the total skin surface area and can be seen as rather insignificant (Scheuplein & Blank, 1971). The drugs that use this route are normally the high molecular weight and charged molecules that struggle to cross the bulk of the SC (Williams, 2003).

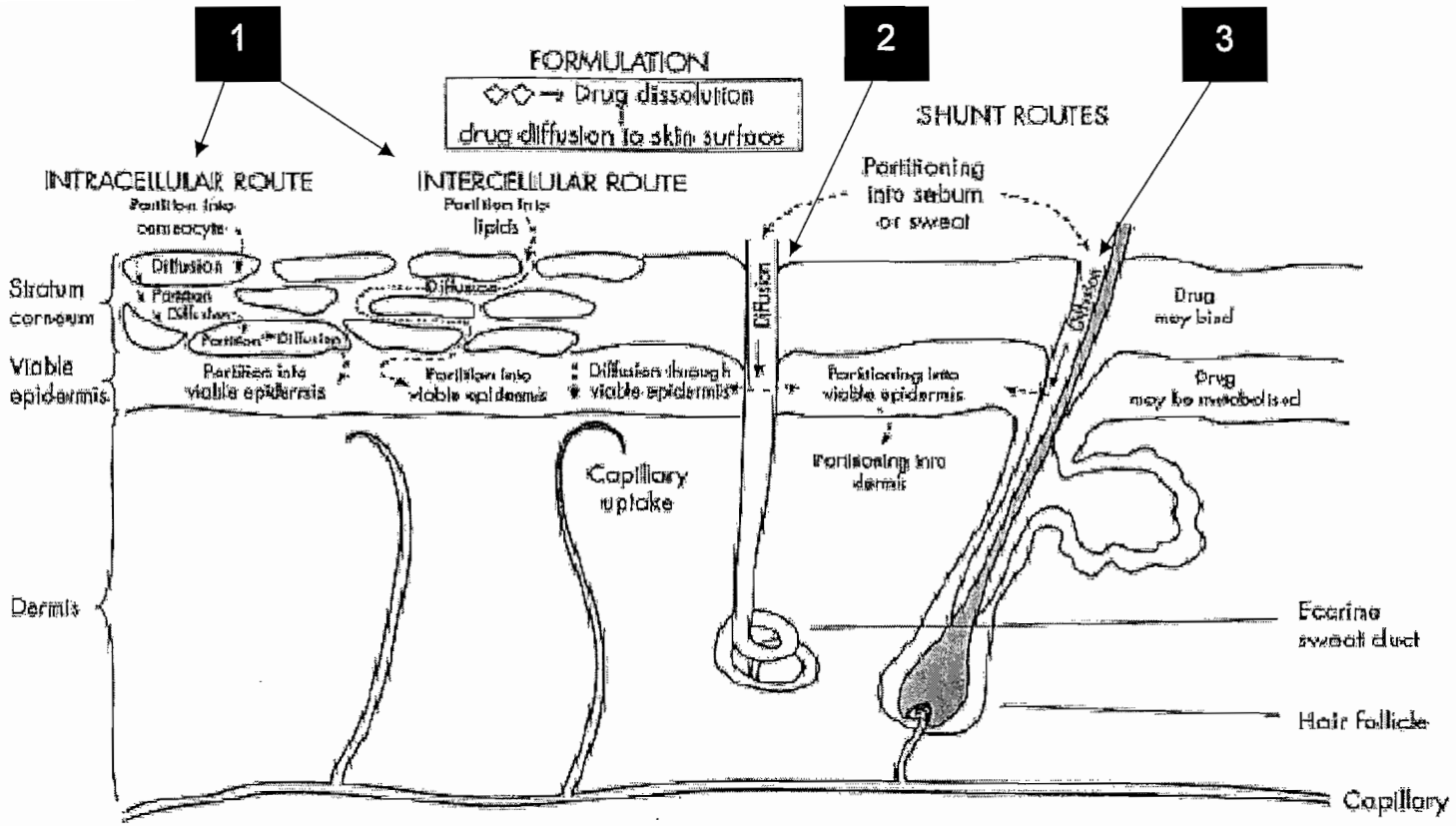


Figure 3.6: A representation of the pathways by which a molecule must travel through the skin: (1) transepidermal route (intra- and intercellular); (2) down the sweat glands; or (3) *via* the hair follicle (Williams, 2003).

3.5 Factors influencing transdermal absorption

When it comes to the transport of molecules across the skin, there are two types of factors that have an influence on the transdermal transport:

- Physiological factors and
- Physicochemical factors (Williams, 2003; Mukhtar, 1992).

3.5.1 Physiological factors

It is generally accepted that if the barrier function of the skin is affected, the permeation of molecules through the skin will also be affected. There are even factors that influence the absorption of molecules through the skin when it is healthy.

Some of the factors include the skin age, body (anatomical) site, race, gender and temperature (Williams, 2003).

3.5.1.1 Skin age

This physiological factor has received much attention over the years. As the membrane ages, some functional and structural changes take place that can have an effect on transdermal absorption (Fenske & Lober, 1986). The hydration of the skin plays a big role in transdermal absorption and the skin looses its moisture content as it ages (Potts *et al.*, 1984). Thus proving that aging of the skin is a telling factor. The enzymatic activity in the skin also differs with age. Children also have a higher surface area to body weight ratio. These two factors implicate that risk assessments should be done when using transdermal preparations for use in children (Plunkett *et al.*, 1992). Another change that takes place with age is that the blood flow to the skin in older people is lower than that of younger people, meaning that the clearance of the drug in the stratum corneum will be slower and that will in turn influence the flux gradient (Williams, 2003).

3.5.1.2 Anatomical site

The skin differs in appearance all over the body and the stratum corneum is thicker in the soles and palms than, for example, on the lips. The thickness of the SC is not the determining factor when it comes to variations in absorption. Wester and Maibach (1989) found that even sites with the same SC thickness have different absorption rates and that sites with different SC thickness have the same absorption. This brings researchers to the conclusion that the regional absorption is different all over the body. According to Williams (2003), the generalised rank of order when it comes to absorption is:

genitals > head and neck > trunk > arm > leg

This shows that the site where a transdermal patch is applied has an effect on the absorption. These regional variations may be different for each individual (Southwell *et al.*, 1984).

3.5.1.3 Race

According to Berardesca *et al.* (1991) the water content of the skin differs from race to race. This will theoretically have an effect on the absorption, but very little research has thus far been done on this.

3.5.1.4 Gender

The skin of males and females has a couple of anatomical differences that may have an influence on permeation of drugs through the skin. For example, the keratinocytes in females (37–46 µm) are larger than those of males (34–44 µm), but this has shown a very small effect (Williams, 2003).

3.5.1.5 Temperature

The permeation of molecules across the skin is a passive process and increasing the temperature (energy) will result in an increase in kinetic energy of the molecules. This means that molecules will move faster through the stratum corneum. Increasing the temperature will also cause structural alterations in the stratum corneum and underlying tissue. The result is a faster movement of the drug through the different skin layers (Sun, 1997; Williams, 2003).

3.5.1.6 Skin hydration

The water content of the skin plays a vital role in transdermal permeation. Jackson (1993) found that if the skin gets hydrated by means of soaking, moisturising, humidity, etc. the corneocytes in the stratum corneum swells, letting molecules through much easier. In studies done it was found that using a co-solvent like EtOH/water (very hydrophilic) increases the permeation of anti-HIV drugs through the skin (Li & Chan, 1999).

3.5.1.7 Skin conditions (diseases)

When the skin is affected by disease the natural barrier function is compromised. This will have an effect on the transdermal absorption. According to Williams (2003) the rate of transdermal absorption will increase when disease is ravaging the skin, but will start to decrease as the skin starts to heal itself. The skin can be affected by numerous diseases like:

- psoriasis,
- dermatitis (eczema),
- acne,
- infections (*Candida, Staphylococcus aureus, Streptococcus pyogenes*),
- tumours and
- ichthyosis (disorder of keratinisation) (Hall, 2003; Williams, 2003).

3.5.1.8 Skin metabolism

Enzymes in the skin are responsible for metabolism and elimination of drugs. For prodrugs that have to undergo biotransformation, this will be the rate-limiting step in the process of transdermal absorption (Gupta *et al.*, 1997). According to Tojo (1997) the place where enzymatic activity is the highest is in the viable epidermis of the skin.

3.5.2 Physicochemical factors

The physicochemical factors are the primary factors that influence transdermal absorption. For molecules to pass through the SC, they need to have certain physicochemical properties (Niak *et al.*, 2000). These properties are the partition coefficient, melting point, aqueous solubility, lipid solubility and molecular size (Barry, 1983).

In Katz & Poulsen (1971) it is stated that the physical and chemical nature of each compound and its interactions is different, thus influencing the rate of transdermal penetration in a different

way. According to Naik *et al.* (2000) the formulation considerations for passive transdermal delivery must comply with those listed in Table 3.1.

Tabel 3.1: Considerations for passive transdermal delivery (Naik *et al.*, 2000).

Aqueous solubility	> 1 mg ml^{-1}
Lipophilicity (log P)	1-2
Molecular weight	< 500 Da
Melting Point	< 200 °C
pH of saturated aqueous solution	pH 5-9
Dose deliverable	10 mg day^{-1}

3.5.2.1 Solubility of the drug in the stratum corneum

The stratum corneum is the most important barrier in the skin and therefore the rate limiting factor of the crossing of any drug through the skin. This can be attributed to the lipohilicity of this important membrane (Flynn, 1989). The amount of drug that accumulates in the stratum corneum bears some relationship to the solubility that drugs may have in some organic solvents like hexane which is highly lipophilic. It has been shown that saturated solutions have better permeation through the stratum corneum. This is because it represents maximum thermodynamic activity (Higuchi, 1960).

It is very important to take the solubility requirements in account when selecting to prepare compounds for transdermal delivery (Idson, 1975). The importance of a drug's solubility was noticed when it was found that drugs with both water and lipid solubility had better permeation than those with only high water or lipid solubility (Sloan, 1989; Sloan *et al.*, 1984). Niak *et al.* (2000) also showed that if a drug is too hydrophilic, the molecule will be unable to penetrate the skin; if it is to lipophilic, the drug will have a propensity to remain in the layers of the stratum corneum.

3.5.2.1.1 Aqueous solubility

The lipid solubility is seen as one of the most important properties that are needed for sufficient transdermal permeation, but sometimes another important factor is overlooked, namely the aqueous solubility of the drug. This property is important because of the aqueous nature of the layers beneath the stratum corneum, thus the drug should embody some hydrophilic properties (Sloan, 1989). Sometimes a small change in the molecular structure, like salt formation or etherification, could either enhance the aqueous or lipid solubility (Abdou, 1989). Solomons (1996) stated that MPEG derivatives showed an increase in aqueous solubility due to the increasing number of oxyethylenic units in the added chain. This allows the derivative to form multiple hydrogen bonds with water molecules in the near proximity thus increasing the molecules' hydrophilicity.

The following equation enables the estimation of the aqueous solubility, S*w*, of compounds (Yalkowsky & Valvani, 1980):

$$\log Sw \approx 1.00 \log PC - 1.11 \frac{\Delta Sf\,(mp-25)}{1364} + 0.54 \qquad \textbf{Equation 3.1}$$

where PC is the octanol-water partition coefficient, ΔSf is the entropy of fusion and is estimated from the chemical structure and mp is the melting point.

The aqueous solubility (S*w*) can also be theoretically calculated by using equations (shown below) extensively used by other research groups (Yalkowsky & Valvani, 1980; Osborne & Lambert, 1992; Bonina *et al.*, 2001; Puglia *et al.*, 2006). Equation 3.2 is exclusive to crystalline compounds while Equation 3.3 is dedicated to oils.

$$\log Sw = -\log P - 0.01mp + 1.05 \qquad \textbf{Equation 3.2}$$

$$\log Sw = -1.072 \log P + 0.672 \qquad \textbf{Equation 3.3}$$

where S*w* is the calculated water solubility of a compound, mp is its melting point and log *P* is the *n*-octanol-water partition coefficient.

3.5.2.1.2 Solubility parameter

The solubility parameter, δ, may sometimes be used to give an indication of how soluble a compound is in the stratum corneum (Guy & Hadgraft, 1992). This parameter can scientifically be defined as the square root of the cohesive energy density. This cohesive energy keeps the molecules in the compound together and is thus the total effect of all the intermolecular interactions together (Gerber, 2006).

This parameter was defined by Hildebrand & Scott (1950) for the first time and has been found to be a useful guide for solvent miscibility. The solubility parameter of an organic solute in the SC can be estimated from Equation 3.4, if the solubility of the solute in a non-polar organic solvent (like hexane) is known, as well as the heat of fusion and the melting point, and the solubility parameter of the solvent (hexane) (Hildebrand *et al.*, 1970).

$$\ln X_2 = \frac{-\Delta H_f}{RT}\left(\frac{T_f - T}{T_f}\right) + \frac{\Delta C_p}{R}\left(\frac{T_f - T}{T} - \ln\frac{T_f}{T}\right) - \frac{V_2\Phi_1^2}{RT(\delta_1 - \delta_2)^2} \qquad \textbf{Equation 3.4}$$

- X_2 is the solute's mole fraction solubility in hexane
- ΔH_f is the heat if fusion of a solid
- R is the gas constant
- T_f is the melting point of the solid in Kelvin
- T is any experimental temperature lower than T_f
- ΔC_p is the difference in heat capacity between the solid form and the hypothetical super cooled liquid form of the compound, both at the same temperature
- V_2 is the molar volume of the liquid solute
- Φ_1 is the volume fraction of the solvent
- δ_1 is the solubility parameter or square-root of the cohesive energy density of the solvent (hexane)
- δ_2 is the solubility parameter or square-root of the cohesive energy density of the solute.

This parameter is useful in the prediction of the solubility of a compound in a specific solvent, thus making it easier to determine if a compound would be suitable for transdermal delivery (Otha *et al.*, 1999). Liron and Cohen (1984) estimated that the solubility parameter of the skin is in the region of 10 and therefore came to the conclusion that compounds with more or less the same value would be soluble in the SC.

3.5.2.2 Diffusion coefficient

Rieger's (1993) described diffusion as the transfer of matter that resulted from the intermolecular movement of the molecules in the substrate. Therefore the diffusion coefficient can be defined as the quantity of mol of a compound that diffuses across a membrane per given unit area per time unit (Idson, 1983). The value of the diffusion coefficient, D, measures the penetration rate of a molecule under specified conditions and is therefore useful information (Barry, 2002).

Important factors influencing the penetration of a drug into the skin according to Martin *et al.* (1983) are:

1. the concentration of the dissolved drug in the donor solution, since penetration rate is relative to concentration (not the case if a saturated solution is used);
2. the partition coefficient (K) between the skin and the vehicle; and
3. the diffusion coefficients (D), which represent the difficulty a drug molecule encounters when moving through the vehicle and the different skin barriers.

The flux (J) is known as the amount of drug flowing through a unit cross section of a membrane (in this case the stratum corneum) in a unit time (Martin *et al.*, 1983):

$$J = \frac{\Delta M}{S.\Delta t}$$ **Equation 3.5**

Where:

- J is the flux ($mg/cm^2/h$)
- S is the area (cm^2)
- t is the time (sec)
- M is the amount/weight of the drug (mg)

The flux is proportional to the concentration gradient (dC/dh) in the membrane and, inversely proportional to the thickness of the membrane (Martin *et al.*, 1983):

$$J = -D\frac{dC}{dh}$$ **Equation 3.5**

Where:

- D is the diffusion coefficient of the drug (cm/sec)
- h is the thickness of the membrane (cm)
- C is the concentration

Fick's laws are generally viewed as the mathematical description of the diffusion process through the membranes. Fick's laws are applicable whenever the chemical or physical nature of the membrane controls the rate of diffusion (Rieger, 1993). The diffusing molecule must have some affinity for the SC in order to pass from the donor solution to the skin and once the drug molecule is in the SC, it can distribute in any possible direction. The molecule has a tendency to diffuse readily from the higher concentration to the lower concentration and therefore showing that this is not random occurrence. Fick's first law has to do with steady-state transport of a compound across a membrane and can be mathematically defined by Equation 3.7:

$$J = D.A.\frac{K}{h}\Delta C \qquad \textbf{Equation 3.7}$$

where

- J ($\mu g/cm^2/h$) is the flux
- A (cm^2) is the area of the membrane where diffusion takes place
- K (cm/h) is the partition coefficient between the vehicle and the skin
- D (cm^2/h) is the diffusion coefficient
- h (cm) is the thickness of the diffusion pathway
- ΔC ($\mu g/cm^3$) = (C_v- C_r) where C_v is the drug concentration in the vehicle and C_r is the concentration in the receptor phase

C_r is usually insignificantly small, and under sink conditions (when there is no drug molecules present in the membrane or underlying tissue), (C_v – C_r) is generally approximated to C_v. In an ideal system, there should be a linear relationship between the rate of diffusion and the concentration of the diffusing molecules. The maximum flux will occur when the concentration reaches the solubility limit (Barry, 1983).

3.5.2.3 Partition coefficient

In order for a molecule to penetrate the SC it has to partition into the membrane. This partitioning into the membrane is also known as the rate-limiting step of the transdermal penetration process (Hadgraft & Wolff, 1993). According to Williams (2003), partitioning can be defined as a process of molecules distributing in two domains. In transdermal work it can be simplified as the molecular movement from a one domain to another, like from an aqueous to a lipophilic domain. The partition coefficient is usually the telling factor in determining which pathway a drug molecule will follow when passing through the SC (Barry, 1987). Thus, it would be expected that aqueous molecules will pass through the keratin-filled keratinocytes (intracellular route) whereas more lipophilic molecules will pass through the lipoidal areas (intercellular route). This permeation process is a very slow process due to all the different barriers that is found within the skin. Both lipophilic and hydrophilic permeants must pass through the hydrophilic and lipophilic phases to give transdermal delivery.

After the molecule has passed through the entire layer of the SC, it reaches the more aqueous viable epidermis beneath. This means that the drug should exhibit some hydrophilic properties in order for the molecule to pass through this layer otherwise if the molecules are too lipophilic; they will remain in the SC layer and will be unable to reach the systemic circulation for the drug to have an effect (Niak *et al.*, 2000). It is important though that the molecule possesses more lipophilic (oil-like) properties in order for it to leave the vehicle, enter the SC and finally have a pharmacological effect (Guy & Hadgraft, 1989).

In order to study and determine the partition coefficient of a drug, organic solvents can be used to mimic the different phases/domains which a molecule must pass through. The octanol-water partition coefficient is a good representation of the partitioning of a drug between the lipophilic SC and the underlying hydrophilic living cells of the epidermis (Tenjarla *et al.*, 1996). The log P value (the log P value at a specific pH) of a compound is usually a good way of telling whether a molecule would be a good candidate for transdermal permeation or not. The lower the log P value is, the more hydrophilic the compound. Some researchers (Guy & Hadgraft, 1989) views are that compounds which have a log P lower than -1 will have difficulty in distributing from the vehicle into the SC and as a result only compounds with log P >-1 may be considered as possible candidates for transdermal delivery. Another problem arises when compounds have a log P value that is larger than 2. This is due to the drug being delayed in the stratum corneum where a reservoir can be established, thus creating problems in achieving steady plasma concentrations in a reasonable time span (Guy & Hadgraft, 1989).

Molecules with a log P value ranging between 1 and 3 is believed by Williams (2003) to exhibit both aqueous and lipophilic properties sufficient enough to obtain proper transdermal

permeation, because they could cross the lipophilic (SC) and hydrophilic layers (epidermis) of the skin. It is recognised that in a homologous series, by increasing the non-polar portion of a molecule through extending the length of the (aliphatic) chain produces certain characteristic features, such as elevating boiling point, decreasing aqueous solubility and increasing the partition coefficient (Abdou, 1989). As seen by Flynn & Yalkowsky (1972) relationships can be drawn for the influence of chain length on the partition coefficient and solubility. Partition coefficients of membranes of a homologous series between the immiscible polar and non-polar phases, increase by a constant factor as the series ascend. The representative diffutional curves are shown in figure 3.7

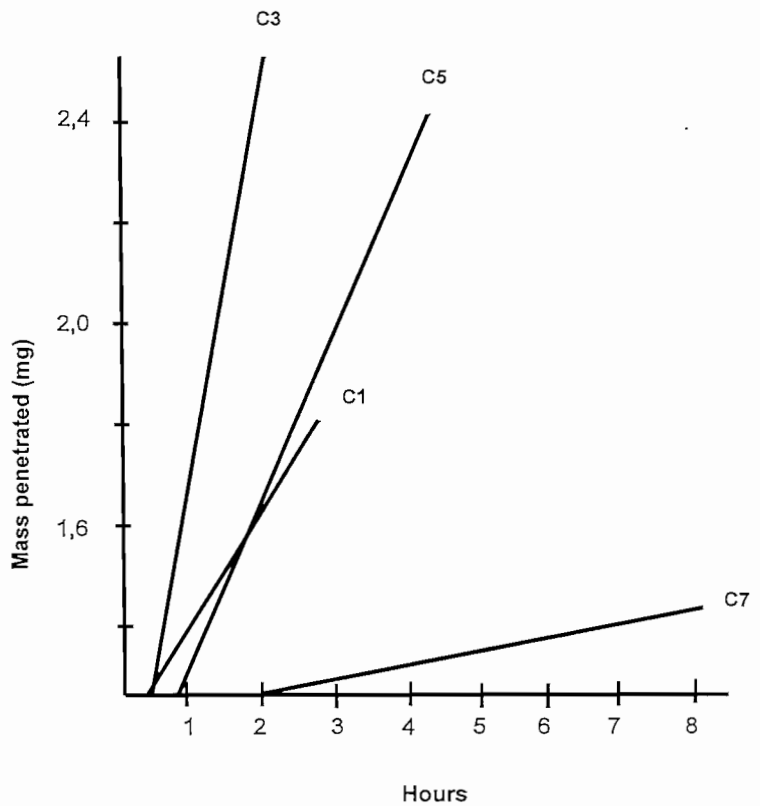


Figure 3.7: Cumulative mass penetrated through a membrane for the odd chain length ester series at 37 °C as a function of time. C1, C3, C5, and C7 designate for methyl, propyl, pentyl and heptyl derivatives of *p*-aminobenzoic acid, respectively (Flynn & Yalkowski, 1972).

The curves indicate that (Flynn & Yalkowski, 1972):

- there is initially an increase in the steady state flux as the homologous series is ascended,
- the flux drops off markedly at longer chain lengths. Additionally, lag times, which appear to be approximately constant initially, increase sharply for the longest chain esters.

3.5.2.4 Molecular mass

The skin has a compact membrane that makes is difficult for anything to penetrate the body. This means that smaller molecules will have a more rapid permeation rate through the skin than bulkier molecules. This just shows the importance that the molecular size has in transdermal absorption. Idson (1975) suggested that an inverse relationship exists between the molecular size and transdermal permeation. It was also found that drugs with a molecular mass between 100 and 500 daltons would be suitable for transdermal transport (Niak *et al.*, 2000; Williams 2003).

Potts & Guy (1992) determined that the permeability through human skin could be predicted by Equation 3.8:

$$\log K_p = -2.7 + 0.71 \log K_{oct} - 0.0061\ MW \qquad \textbf{Equation 3.8}$$

where

- K_p is the permeability coefficient (cm/sec)
- K_{oct} is the octanol/water partition coefficient
- MW is the molecular weight

By using this equation it is clear that as the molecule becomes more lipophilic its permeability increases due to better partitioning into the skin (Gerber, 2006). It is still debatable whether the upper range could not be higher than 500 daltons because studies have been done with molecules that had a molecular weight higher than 500, which showed an increase in transdermal flux of the compound's derivatives compared to the parent compound (N'Da & Breytenbach, 2009).

3.5.2.5 Ionisation

The stratum corneum is very lipophilic, consisting of lipid bilayer domains. This led to the belief that ionisable drugs would make a poor choice for transdermal delivery which was founded by the partition hypothesis developed by Shore in 1957. This hypothesis was developed to explain the absorption of drugs from the gastro-intestinal (GI) tract. It stated that only the unionised form of the drug could cross the lipid membranes in high enough amounts to have an effect. However, with the complex structure of the skin, this model could not be applied as for drug absorption from the GI tract. As described in the previous section, drugs can cross the skin *via*

various pathways. The transcellular route can be seen as having intermediate properties, whereas the intracellular route is mainly for molecules that are lipophilic. This means that ionised drugs would prefer the shunt route when crossing the skin. However, the amount of molecules that pass through this route is significantly less than unionised molecules that pass through the intracellular route (Williams, 2003).

According to Pardo *et al.* (1992), the pH range of the viable epidermis is 7.3–7.4 and that of the stratum corneum is 4.2 – 5.6. The drug concentration that exists in the unionised form is a function of both the dissociation constant of the drug and the pH at the absorption site (Abdou, 1989). The pKa of the diffusant also plays a role. Ansel (1981) determined the pKa of compounds by deriving it from the Henderson-Hasselbalch equation:

For an acid:

$$pH = pKa + \log\frac{(salt)\,(ionised)}{(acid)\,(unionised)} \qquad \textbf{Equation 3.9}$$

For a base:

$$pH = pKb + \log\frac{(base)\,(unionised)}{(salt)\,(ionised)} \qquad \textbf{Equation 3.10}$$

Thus, the fraction of the unionised drug is a function of the pH (Barry, 1983).

Even though unionised molecules are preferred above ionised ones, it does not mean that weakly ionised drugs can not be used for transdermal transport. Flynn (1989) highlighted the fact that these ionised molecules can form ion pairs and that even the salt form of a molecule could be soluble in the lipophilic domains of the SC, thus creating the possibility that aqueous soluble drugs can permeate through the skin.

3.5.2.6 Hydrogen bonding

The drug binding factor should be taken into consideration when selecting possible candidates for transdermal delivery. This practically means that the number of groups that could have interactions, like forming hydrogen bonds or weak Van der Waals forces, should be taken into account. When 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.) are considered, there is a multitude of potential interactions between drug substances and the tissue (Williams, 2003). One important factor that is influenced by a

drug binding to the tissue is the lag time. If drugs bind to the tissue of the SC, a prolonged lag time will be observed which in effect means that the drug will take longer to have its effect. Pugh *et al.* (1996) showed that the SC tissue is mainly a hydrogen bond acceptor. Therefore, an increase in the number of hydrogen bonding groups on the drug might inhibit permeation across the layers of the SC (Williams, 2003).

3.5.2.7 Melting point

It is a well known fact that organic substances with a high melting points and high enthalpies of melting have lower aqueous solubility properties because the solvent can not enter the crystalline structure of the molecule and the compound can not dissolve (Lipinski *et al.*, 1997). Thus a direct relationship can be drawn between the melting point and solubility of a drug. There are a few models that use the melting point to determine the solubility of a drug. One of these models was developed by Hadgraft *et al.* (1990) that accurately estimated the solubility parameter in the SC, δ_{SC}, by using the melting point of the drug.

This is given by Equation 3.11:

$$\log\delta_{SC} = 1.911\frac{10^3}{mp} - 2.956 \qquad \textbf{Equation 3.11}$$

where

- δ_{SC} is the solubility parameter in the SC and
- mp is the melting point (Kelvin).

Stott *et al.* (1998) concluded that lowering the melting point of the permeant will cause an increase in the permeation across the skin.

3.6 The influence of derivatization on transdermal permeation

A drug molecule's physicochemical properties like lipophilicity can be modified by derivatization. This also highlights the nature of the drug development process, in that a drug has never been developed from the start to be a suitable transdermal candidate, i.e. the physicochemical makeup has been specifically modified for skin permeation. This is due to the fact that most drugs are only designed for oral administration. Thus derivatives of a drug may enhance its

ability to permeate through the skin. This strategy (derivatization) is the basis of the pro-drug approach in which the drug molecule is physically modified to facilitate its permeation, although being designed to later yield the parent (active) drug after enzymatic or chemical release (Sloan, 1992).

Some of the derivatization strategies that can be used are:

- Increasing the lipophilicity by alkyl-chain lengthening (Waranis & Sloan, 1987), and
- Using MPEG (PEGylation) to synthesise carbonates and carbamates with different physicochemical properties than the parent compound that could more readily cross the skin (Veronese & Pasut, 2005).

These strategies could increase the transdermal delivery of drugs, thus increasing their pharmacological action in the body.

3.7 Pheroid™ delivery system

3.7.1 Introduction to Pheroid™ technology

Pheroid™ technology, previously known as Emzaloid™ technology, concerns a therapeutic delivery system which comprises of a distinctive submicron oil-water emulsion type formulation. It consists of a stable, spherical or vesicular structure within this system that can be manipulated with regard to its morphology, structure, size and function (Schlebusch, 2002). The novelty of Pheroid™ technology is underlined by patents registered in Europe, the USA, South Africa and China. The Pheroid™ technology is described by these patents as a delivery system that increases the absorption and efficacy of dermatological, biological and oral medicines. Throughout this thesis this trademarked delivery system will be referred to as Pheroid (or Pheroid™).

Pheroid closely resembles other lipid-based delivery systems, such as liposomes. What makes the Pheroid unique amongst its other counterparts is that its components are altered in a very specific manner to ensure its properties such as:

- high entrapment capabilities,
- very fast rate of transport,
- fast delivery, and
- high stability (Schlebusch, 2002).

Grobler *et* al. (2008) found that Pheroid™ is able to enhance the absorption and/or efficacy of several active ingredients and compounds. This was obtained by major improvements in the control of size, charge and hydrophilic-lipophilic characteristics of therapies, when compared to other systems.

Some of the key advantages claimed for the Pheroid™ delivery system are the following (Grobler, 2004; Schlebusch, 2002):

- Increased delivery of active compounds.
- Decreased time to onset of action.
- Reduction of minimal effective concentration.
- Increased therapeutic efficacy.
- Reduction in cytotoxicity.
- Penetration of most known barriers in the body and in cells.
- Ability to target treatment areas.
- Lack of immunological response.
- Ability to transfer genes to cell nuclei.
- Reduction of drug resistance.

Drugs are entrapped in the Pheroid vesicles with high efficiency. The Pheroid penetrates the keratinised tissue, skin, intestinal lining, vascular system, fungi, bacteria, and parasites (Grobler, 2004). All components used in the manufacturing of Pheroid are pharmaceutically safe and the system is based on the naturally occurring molecules of the body, thus making the Pheroid biodegradable. The stability of the Pheroid delivery system has been proven for Pheroid-based commercialised products (Grobler *et al.,* 2008). Gerber *et al.* (2008) found no significant improvement in the transdermal delivery of lamivudine when using Pheroid. This might suggest that Pheroid might be responsible for a sustained release when delivering drugs over the skin.

3.7.2 Structure, composition and mechanism of Pheroid™

Pheroid™ consists mainly of plant and essential fatty acids, amongst which is oleic acid. There are various types of Pheroid and each type has a particular composition. The different types of Pheroid are the following (Schlebusch, 2002):

- Lipid bilayer vesicles with nano- and micrometer diameters.
- Micro-sponges.

- Depots or reservoirs that contain pro-Pheroid (see Figure 3.8).

The Pheroid™ delivery system is a colloidal system with the lipid-based submicron- and micron sized spherical structures (the Pheroid), uniformly dispersed in the formulation. Pheroid is specifically formulated to have a diameter of between 200 nm and 2 pm (Grobler *et al.,* 2008).

Pheroid generally contain a lipid bilayer, as does a liposome, but it contains no phospholipids or cholesterol. In contrast to liposomes, Pheroid are formed by a self-assembly process similar to that of low-energy emulsions and micro-emulsions and no lyophilization or hydrations of the lipid components is necessary. The Pheroid are, like emulsions, dispersed within a dispersion medium, but it contains not only two liquid phases, but also a dispersed gas phase (N_2O) which is associated with the fatty acid dispersed phase (Grobler *et al.,* 2008). The primary components of Pheroid are ethylated and pegylated polyunsaturated fatty acids, including the omega-3 and -6 fatty acids but excluding arachidonic acid. The fatty acids are in the cis-formation and therefore compatible with the orientation of the fatty acids in man (Grobler *et al.,* 2008).

Apart from the fatty acids, the Pheroid also contains nitrous oxide (N_2O), which is found distributed in close association with the dispersed phase throughout the continuous phase.

Some interaction between the fatty acids and the nitrous oxide has been indicated by means of molecular modelling. This interaction gives stable vesicular Pheroid structures as a result. The nitrous oxide essential fatty acid (NOEFA) matrix thus provides a functional model for the transport of hydrophobic and hydrophilic drugs. Controlled experiments were performed on various formulations and it was determined that if either the N_2O or the EFAs (essential fatty acids) were absent from the formulations, a dramatic decline in efficacy and stability of the formulations would be observed (Grobler *et al.,* 2008).

The Pheroid delivery system is ideal in a transdermal situation due to the fact that the composition support the binding of the EFAs to the fatty acid binding proteins of the keratinocyte, the chief cell of the stratum corneum (Grobler *et al.,* 2008). It is also believed that Pheroid may enhance transdermal permeation by entrapping drug molecules with unfavourable properties (for transdermal permeation) and transporting them through the different layers of the skin, thus allowing molecules that could not previously penetrate the skin, to do so.

3.7.3 Types of Pheroid™

Due to the flexibility in the manufacturing process, gives the possibility several different types of Pheroid.

These different Pheroid™ types are:

a) A bilayer membrane vesicle containing rifampicin (100 nm in diameter).

b) A highly elastic or fluid bilayered vesicle with loose lipid packing containing rifampicin.

c) The formation of small pro-Pheroid™ that are used in oral delivery.

d) A reservoir (size: 1 – 10 μm) that contains multiple particles of coal tar. Reservoirs have large loading capacity to surface area ratios and are good entrappers of insoluble compounds.

e) This Pheroid™ is in the process of entrapping fluorescently labelled water-soluble diclofenac. It is very small (approximately 30 nm) and the membrane packing is sponge-like.

f) A depot (size: 5 – 100 μm) with a hydrophobic core containing pro-Pheroid™ formulation, a surrounding hydrophilic zone and an outer vesicle-containing zone. Selective addition of fluid results in the release of vesicles from a release zone. The depots are used for sustained release according to a concentration gradient. The sizes of Pheroid™ reflected above are not all to scale.

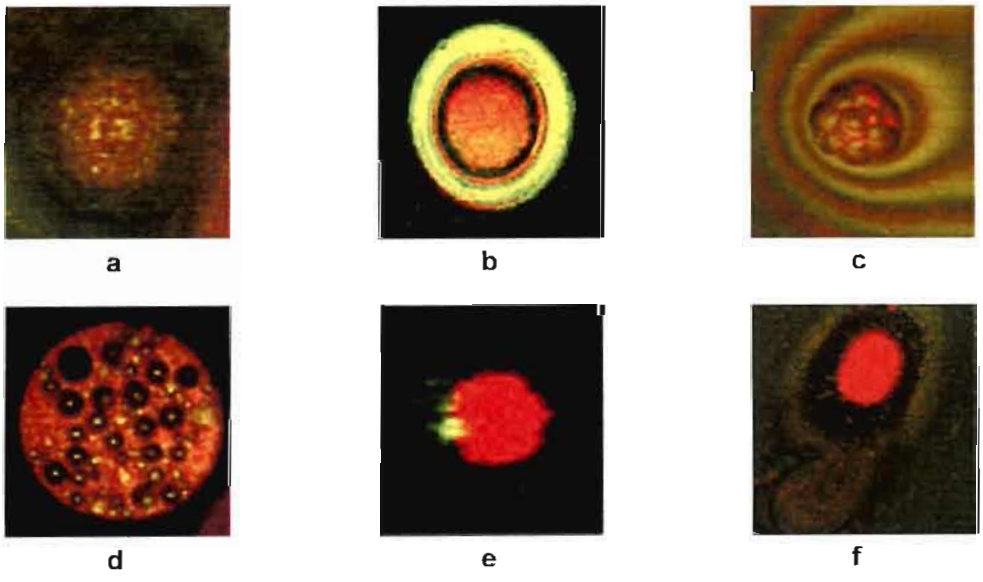


Figure 3.8: Confocal laser scanning microscopy (CLSM) micrographs of several of the basic Pheroid™ types.

3.7.4 The rational behind selecting Pheroid™ as delivery system for transdermal delivery

Pheroid™ was selected for use in this study because of its entrapment properties and ability to transport hydrophilic molecules over lipophilic membranes (Grobler, 2004). Lamivudine is a very hydrophilic molecule, thus it will struggle to permeate through the skin. In theory the Pheroid™ will entrap the drug and enable it to cross the highly lipophilic stratum corneum and the rest of the skin's layers to finally have a pharmacological effect.

ARTICLE FOR SUBMISSION

Chapter 4 contains the manuscript of an article to be submitted to the European Journal of Pharmaceutical Sciences. The article contains the background, aims, all the experimental details and results of this study, including the physicochemical properties and transdermal flux results (in PBS and in Pheroid™) of lamivudine and its derivatives. The article is prepared according to the Guide for Authors that can be found on the website of this journal (http://www.elsevier.com/wps/find/journaldescription.cws_home/523997/description#description), except that for easy reading figures, schemes and tables are inserted at their logical places as they would appear in the printed version.

Synthesis and *in vitro* transdermal penetration of methoxypoly(ethylene glycol) carbonate and carbamate derivatives of lamivudine (3TC)

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Abstract

The objective of this study was to determine the *in vitro* transdermal permeation through the human stratum corneum (SC) of lamivudine (3TC) (**6**) and the synthesised methoxypoly(ethylene glycol) (MPEG) carbamates and carbonates thereof, with and without the use of Pheroid™ as delivery system and to establish a relationship, if any, with selected physicochemical properties. The synthesis and *in vitro* human skin permeation flux of three *N4*-methoxypoly(ethylene glycol) carbamates (**9-11**) and three 6'-O-methoxypoly(ethylene glycol) carbonates (**12-14**) of the anti-HIV drug lamivudine are reported. The derivatives were synthesised in a two-step process by coupling activated MPEG oligomers of various chain lengths (n = 1, 2, 3) to either the 4-amino or 6'-hydroxy group of (**6**). The aqueous solubility and lipophilic properties (log P values) showed all derivatives to be increasingly water soluble while the lipid solubility also increased slightly as the methoxypoly(ethylene glycol) chain lengthened. This finding can be ascribed to the ratio of aqueous to lipid solubility being so big that the derivatives showed increasing aqueous solubility. The *N4*-methoxypoly(ethylene glycol) carbamates are more hydrophilic than the 6'-methoxypoly(ethylene glycol) carbonates on the basis of their log P values. The use of the Pheroid™ delivery system retarded skin permeation rate significantly. *In vitro* transdermal diffusion through excised human skin showed all derivatives except (**9**) to possess a statistically significant lower mean flux than lamivudine itself.

Keywords: Transdermal permeation; methoxypoly(ethylene glycol) (MPEG); derivatives; lamivudine (3TC); Pheroid™; aqueous solubility; log P

1. Introduction

With 18.3 % (about 5.5 million) of the population's adults infected with HIV/AIDS, South Africa has the highest prevalence of HIV in the world (UNAIDS, 2006). This has led to the urge for researching new types of drugs and new ways of delivering those drugs. A promising route of delivery which has been receiving increased attention is transdermal administration. This route has advantages over the oral route and could lead to more effective treatment of HIV/AIDS because of better patient compliance that has a decrease in resistance of HIV as the result. It will also be an alternative route when it comes to increasing patient compliance in paediatric patients due to the bad palatability of the anti-retroviral (ARV) medication. Despite all the advantages of this pathway, the skin's barrier effect limits this route merely to drugs that possess specific physicochemical properties (Niak et al., 2000). These limitations forced the scientific community to develop alternative strategies to enhance the transdermal permeation of drugs through the skin. Some of these strategies included developing derivatives as prodrugs, using penetration enhancers and incorporating transport systems (ethosomes and liposomes) to enhance the transdermal permeation. The inclusion of an enhancer within a formulation may however also increase the absorption of other components, which can lead to skin damage and irritancy. Developers of these strategies, like Kim & Chien (1995), Thomas & Panchagnula (2003) and Jain et al. (2007) all contributed to better delivery but not without limitations. A promising alternative to the use of penetration enhancers is the prodrug approach (Sloan, 1989; Sloan, 1992). This involves modifying the chemical structure of a drug with concomitant changes in both its physicochemical and pharmacokinetic characteristics in order to enhance its delivery. In this study, MPEG was used to synthesise derivatives of lamivudine (**6**) to determine if an increase in the transdermal flux of the drug (**6**) can be obtained and because MPEG had certain properties like increasing the stability, reducing toxicity, enhancing solubility and reducing proteolytic activity of a drug (Veronese & Pasut, 2005). The amphiphilic property of MPEG gives the derivatives the ability to be soluble in both hydrophilic and lipophilic

environments. This would theoretically mean that the derivative would pass through the lipophilic SC and then through the hydrophilic underlying dermis. Gerber et al. (2008) synthesised N-acyl esters of lamivudine and found that transdermal flux increased with increase in water solubility (decrease in log P values). The water solubility of all these N-acyl lamivudine esters was lower than that of lamivudine itself as were their fluxes. It was also found that the Pheroid™ vesicle transport system had a retarding effect on the permeation of these compounds through the SC. These results prompted the current study, *viz.* to synthesise derivatives of lamivudine with higher water solubility than the parent compound and to determine their steady-state flux across the skin thus creating the possibility to compare the results of these studies and drawing certain conclusions that could have a significant contribution in the field of transdermal permeation of anti-retrovirals.

Lamivudine (4-amino-1-[2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-1H-pyrimidin-2-one, 3TC) (**6**) is a nucleoside reverse transcriptase inhibitor (Medicinenet, 2007). The FDA approved it on 17 November 1995 for use in combination with zidovudine (AZT) because of their synergism (Avert, 2008). It is used against HIV (1 and 2), hepatitis B infections and in patients that has been accidentally exposed to HIV (Medicinenet, 2007). The most common adverse effects of lamivudine are usually associated with the gastric-intestine system and include vomiting, diarrhoea, abdominal cramps and pain and nausea (Gregg, 1999; Sweetman, 2002) which might be avoided by transdermal administration. More-over, transdermal application can be advantageous for children or infants who have trouble taking the oral lamivudine formulation due to its unpleasant taste (Schiffman et al., 1999). Lamivudine itself is also not a suitable candidate for transdermal delivery, as was shown in a previous study (Gerber et al. 2008) and confirmed here. Therefore, the transdermal strategy using the prodrug approach is an alternative to the common oral route to be explored for controlled delivery of lamivudine, and minimizing adverse effects while achieving better therapeutic results. Numerous systems, like reservoir devices, matrix diffusion-controlled devices, multiple polymer devices, and multilayer matrix systems, are available that enhance

the delivery of drugs across the skin (Hadgraft and Lane, 2006) and some attention has also been given to the use of Pheroid™ to improve transdermal permeation of drugs (Grobler et al., 2008).

The Pheroid™ delivery system is a colloidal system with the lipid-based submicron- and micron sized spherical structures (the pheroids), uniformly dispersed in the formulation. It comprises mostly essential and plant fatty acids, i.e. ethyl esters of the essential fatty acids (EFAs) oleic, linolenic and linoleic acids, which are emulsified in water and saturated with nitrous oxide. The Pheroid™ delivery system is claimed to be ideal in a transdermal situation due to the fact that the composition support the binding of the EFAs to the fatty acid binding proteins of the keratinocyte, the main cell of the stratum corneum (Grobler *et al.,* 2008).

The aim of this study was to determine the transdermal permeation of lamivudine (**6**) and the synthesised derivatives, with and without the use of Pheroid™ as delivery system and to establish a relationship, if any, with selected physicochemical properties.

2. Materials and methods

2.1. Materials

2-Methoxyethanol, 2-(2-methoxyethoxy)ethanol and 2-[2-(2-methoxyethoxy)ethoxy]ethanol were all purchased from Fluka South Africa, Ltd. Lamivudine (3TC) was kindly donated by Aspen Pharmacare South Africa. *para*-Nitrophenyl chloroformate (*p-NPCF*) was purchased from Sigma-Aldrich South Africa, Ltd. HPLC grade methanol was obtained from Labchem South Africa Ltd. All the reagents and chemicals were of analytical grade.

2.2. General procedures

The ^{1}H and ^{13}C NMR spectra were recorded on a Varian Gemini 300 spectrometer (at a frequency of 300.075 and 75.462 MHz, respectively) and on a Bruker Avance III 600 spectrometer (at a frequency of 600.17 and 150.913 MHz, respectively) in dimethyl sulfoxide (DMSO) or chloroform ($CDCl_3$). Chemical shifts are reported in parts per million δ (ppm) using tetramethylsilane (TMS) as internal standard. The splitting pattern abbreviations are as follows: s (singlet), d (doublet), t (triplet), q (quartet), bs (broad singlet) and m (multiplet).

Mass spectra (MS) were recorded on a LC-MS Applied Biosystems API 2000 triple quadrupole mass spectrometer and Analyst 1.4 data acquisition and analysis software by using direct infusion with a Harvard syringe pump at a flow rate of 10 µl/min. The mass spectrometer featured the following: atmospheric pressure electron ionisation (Turbo ion spray source), positive ion mode. The full scan from 100-1200 amu was obtained in 1 s. The declustering, focusing and entrance potentials were 120, 400 and 10 V, respectively while the ion spray voltage was 4500 V. The ion source gas 1 and 2 were used at a flow rate of 20 l h^{-1}, and the temperature was 350 °C. Additional MS spectra were recorded on an analytical VG 7070E mass spectrometer using electron impact (EI) at 70 eV as ionisation technique.

Melting points were determined by differential scanning calorimetry (DSC) with a Shimadzu DSC-50 instrument. Thin-layer chromatography was performed using silica gel plates ($60F_{254}$ Merck). Preparative flash column chromatography was carried out on silica gel (230-240 mesh, G60 Merck). Analytical quantities of samples were weighed on a Sartorius/BP211D balance (capacity, resolution: 210 g, 0.0001; and 80 g, 0.00001).

2.3. High performance liquid chromatography (HPLC)

The HPLC system consisted of an Agilent 1100 series auto sampler, Agilent 1100 series variable wave detector (VWD) and Agilent 1100 series isocratic pump. A Zorbax Eclipse XDB

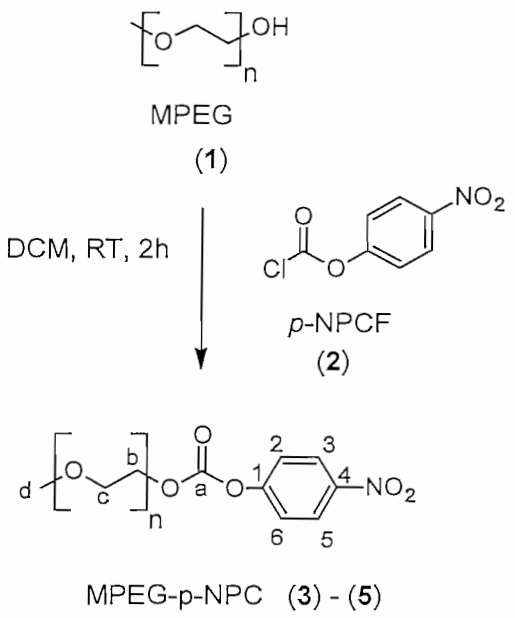
C18, 5 µm (150 x 4.60 mm) column was used and the Agilent Chemstation rev A08.03 for LC systems software package for data analysis. The compounds were quantified using a gradient method (A = 0.2 % triethylamine in H_2O, pH 7.0, B = methanol) at a flow rate of 1 ml/min with 5 µl standard sample injections. The gradient consisted of 10 % of solvent B until 1 min, then increased linearly to 40 % of B after 6 min, and held until 10 min. A calibration plot of peak area versus drug concentration for each compound showed excellent linearity ($0.999 < r^2 \leq 1$) over the concentration range (0.24–600 µg/ml) employed for the assays.

The absorption maximum for 3TC and all its derivatives was at 240 nm; this wavelength was consequently used for the HPLC detection. New mobile phase was prepared for each sample batch that was analyzed by HPLC. The peak retention times (t_R) were 2.45 min for (**6**) (3TC), 5.81 min for (**9**), 6.02 min for (**10**), 6.24 min for (**11**), 6.43 min for (**12**), 6.59 min for (**13**) and 6.75 min for (**14**).

*2.3.1. Synthesis of methoxypoly(ethylene glycol) p-nitrophenyl carbonates (**3**-**5**) (Scheme 1)*

The synthesis of methoxypoly(ethylene glycol) *p*-nitrophenyl carbonates required the activation of the terminal hydroxyl group of methoxypoly(ethylene glycol) using *para*-nitrophenyl chloroformate (*p*-NPCF) (Scheme 1). This was achieved by adapting with slight modifications the method reported by Bodansky (1955). Thus, for example to a solution of methoxyethanol, (**1**) (18.8 mmol) dissolved in 50 ml of dry dichloromethane (DCM) and stirried at room temperature, was added triethylamine (TEA) (20.7 mmol, 1.1 equiv., relative to MPEG). After 10 min *p*-nitrophenyl chloroformate (**2**) (4.00 g, 19.8 mmol) was added portion wise, and the stirring continued for 2 h. The evaporation of the solvent *in vacuo* resulted in the appearance of triethylammonium chloride as white precipitate, which was washed several times with diethyl ether and filtered off. The filtrate was concentrated to an oily residue and further purified by flash chromatography. With the exception of methoxypoly(ethylene glycol) *p*-nitrophenyl carbonate (**3**, n = 1), isolated in crystalline form,

all other activated compounds were oils, and failed to crystallize. ^{1}H and ^{13}C NMR chemical shifts as well as HRMS data of compounds (**3**-**5**) (Scheme 1) are reported.



n	compound
1	(**3**)
2	(**4**)
3	(**5**)

Scheme 1. Activation of MPEG using *p*-nitrophenyl chloroformate

*2.3.1.1. Methoxyethylene glycol p-nitrophenyl carbonate (**3**) (n = 1)*

Carbonate (**3**) was purified by flash silica gel column chromatography eluting with DCM:EtOAc (10:1) to give white crystals: 4.35 g (91%) yield after crystallisation in hexane:EtOAc (5:1). m.p. 47.8 °C, $C_{10}H_{11}NO_6$. ^{1}H NMR δ (ppm) 3.40 (s, 3H, H-d), 3.66-3.69 (m, 2H, H-c), 4.39-4.3 (m, 2H, H-b), 7.37 (d, 2H, H-3, H-5, J = 9.26 Hz), 8.25 (d, 2H, H-2, H-6, J = 9.26 Hz). ^{13}C NMR δ (ppm): 59.02 (C-d), 68.10 (C-c), 69.83 (C-b), 115.53 (C-4), 121.68 (C-5), 125.47 (C-3), 126.09 (C-2), 145.37 (C-6), 152.45 (C-1), 155.47 (C-a). MS FAB 242 ($(M+H^+)$ 70%), *289* (10%), 210 (20%), 165 (12%), 154 (90%), 137 (100%), 123 (32%), 119 (20%), 107 (44%).

2.3.1.2. Methoxydi(ethylene glycol) p-nitrophenyl carbonate (***4***) (n = 2)

Compound (**4**) was purified by flash silica gel column chromatography eluting with DCM:EtOAc (10:1) to give a yellowish oil: 4.95 g, 87% yield. $C_{12}H_{15}NO_7$. ^{1}H NMR δ (ppm): 3.36 (s, 3H, H-f), 3.50-3.54 (m, 2H, H-e), 3.55-3.60 (m, 2H, H-d), 3.80-3.85 (m, 2H, H-c), 4.40-4.50 (m, 2H, H-b), 7.36 (d, 2H, H-3, H-5, J = 9.19 Hz), 8.25 (d, 2H, H-2, H-6, J = 9.19 Hz). ^{13}C NMR δ (ppm): 57.60 (C-f), 68.25 (C-e), 68.37 (C-d), 69.35 (C-c), 71.50 (C-b), 121.57 (C-4), 124.85 (C-5), 126.09 (C-3), 144.95 (C-6), 152.25 (C-2), 154.97 (C-a). MS FAB 286 ((M+H$^+$) 40%), 210 (72%), 165 (12%), 154 (22%), 137 (42%), 123 (22%), 119 (16%), 107 (26%), 103 (100%).

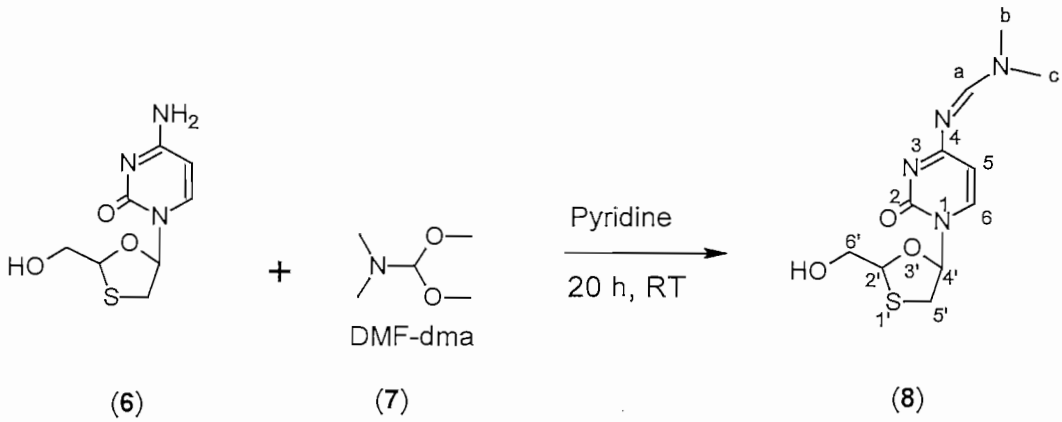
2.3.1.3. Methoxytri(ethylene glycol) p-nitrophenyl carbonate (***5***) *(n = 3)*

Purification by flash silica column chromatography eluting with DCM:EtOAc (10:1) afforded 3.73 g (57%) of carbonate (**5**) as a yellowish oil. $C_{14}H_{19}NO_8$. ^{1}H NMR δ (ppm):3.28 (s, 3H H-h), 3.44-3.47 (m, 2H, end chain H-g), 3.55-3.63 (m, 6H, H-d, H-e, H-f), 3.71-3.74 (m, 2H, H-c), 4.33-4.36 (m, 2H, H-b), 7.37 (d, 2H, H-3, H-5, J = 9.27 Hz), 8.25 (d, 2H, H-2, H-6, J = 9.27 Hz). ^{13}C NMR δ (ppm): 53.40 (C-h), 58.69 (C-g), 67.50 (C-f), 68.07 (C-e), 68.37 (C-d), 70.32 (C-c), 71.69 (C-b), 121.55 (C-4), 125.04 (C-5), 126.09 (C-3), 145.15 (C-6), 152.20 (C-2), 155.33 (C-a). MS FAB 330 ((M+H$^+$) 30%), 392 (30%), 210 (66%), 167 (18%), 154 (34%), 149 (100%), 137 (42%), 103 (50%).

2.3.2. Synthesis of N4-dimethylimidoformamide lamivudine (***8***) *(protected lamivudine) (Scheme 2)*

N-dimethylimidoformamide lamivudine was synthesised by adapting with slight modifications the method used by Anastasi et al. (2004) depicted in Scheme 2 and, described as follows to protect the amine group of lamivudine (protected lamivudine) (**8**). To a solution of (**6**) (20.00 g, 87.20 mmol) in dry pyridine (200 ml) and stirring at room

temperature was added dimethylformamide dimethyl acetal (DMF-dma) (**7**) (96 mmol, 1.1 equiv). After 24 h of stirring at room temperature, the solvent was evaporated in *vacuo* and the residue was purified by silica gel flash chromatography followed by crystallisation to afford the product (**8**) in crystalline form. ^{1}H and ^{13}C NMR chemical shifts as well as LC-MS data for compounds (**8**) (Scheme 2) are reported below.



(**6**) (**7**) (**8**)

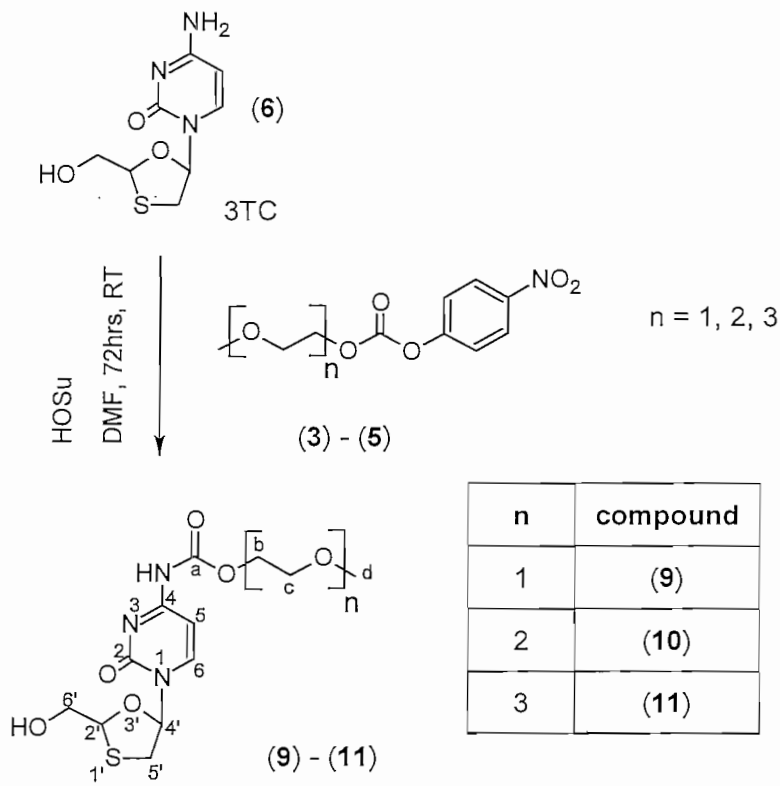
Scheme 2. Synthesis of N4-dimethylimidoformamide lamivudine (**8**)

2.3.2.1. N4-dimethylimidoformamide lamivudine (protected lamivudine, 8)

A yield of 24.07 g (95%) of white crystalline compound was obtained after flash chromatography eluting with DCM:Methanol (10:1). m.p. 285.0 °C. $C_{11}H_{16}N_4O_3S$. ^{1}H NMR δ (ppm) (DMSO): 2.99 (s, 3H, H-c), 3.06 (dd, 1H, H-5'b, J = 11.86, 4.92 Hz), 3.13 (s, 3H, H-b), 3.41 (dd, 1H, H-5'a, J = 11.86, 4.92 Hz), 3.68 (m, 2H, H-6'), 5.14 (m, 1H, H-2'), 5.28 (m, 1H, OH), 5.85 (d, 1H, H-5, J = 7.28 Hz), 6.13 (m, 1H, H-4'), 7.90 (d, 1H, H-6, J = 7.28 Hz), 8.49 (s, 1H, H-a). ^{13}C NMR δ (ppm): 34.75 (C-5'), 36.84 (C-c), 40.81 (C-b), 62.48 (C-6'), 86.61 (C-2'), 86.88 (C-4'), 101.37 (C-5), 142.09 (C-6), 154.67 (C-4), 157.86 (C-a), 171.25 (C-2). *m/z* (ESI+,%): *($M+H^+$)* 285 (100), 167 (94), 128 (23), 101 (41).

*2.3.3. Synthesis of MPEG carbamates of lamivudine (**9-11**) (Scheme 3)*

MPEG carbamates of lamivudine were synthesised following a general method depicted in Scheme 3 and, described as follows to exemplify the preparation of carbamate (**9**) (n =1). To a solution of (**3**) (3.00 g, 12.4 mmol) in dry DMF (40 ml) and stirring at room temperature was added hydroxysuccinimide (HOSu) (1.2 mmol, 0.1 equiv). Lamivudine, (**6**) (13.7 mmol, 3.1 g, 1.1 equiv) previously dissolved in DMF (5 ml) was added drop wise over a 5-10 min period to solution of (**3**). After 72 h of stirring at room temperature, the solvent was evaporated in *vacuo* and the residue was purified by silica gel flash chromatography. ^{1}H and ^{13}C NMR chemical shifts as well as LC-MS data for compounds (**9-11**) (Scheme 3) are reported below.



n	compound
1	(**9**)
2	(**10**)
3	(**11**)

Scheme 3. Synthesis of N4-carbamates (**9-11**)

*2.3.3.1. 2',3'-dideoxy-3'-thiacytidin-N4-yl-(methoxy(ethylene glycol)) carbamate (n = 1, **9**)*

A yield of 1.20 g (40%) of white crystalline (**9**) was obtained after flash chromatography eluting with DCM:MeOH (10:1). m.p. 125 °C. $C_{12}H_{17}N_3O_6S$. ^{1}H NMR δ (ppm) (DMSO): 3.08 (dd, 1H, H-5'b, J = 12.23, 3.44 Hz), 3.22 (s, 3H, H-d), 3.47 (m, overlapping, 3H, H-c, H-5'a), 3.72-3.93 (s, 2H, H-b), 4.17-4.37 (m, 2H, H-6'a, H-6'b), 5.2 (t, 1H, H-4', J = 4.12 Hz), 5.30 (s, 1H, OH), 6.05-6.33 (m, 1H, H-2'), 6.87 (d, 1H, H-6), 8.22 (d, 1H, H-5). ^{13}C NMR δ (ppm): 37.49 (C-5'), 58.05 (C-d), 61.98 (C-b), 64.34 (C-6'), 69.72 (C-c), 87.07 (C-2'), 87.83 (C-4'), 94.06 (C-5), 144.89 (C-6), 153.19 (C-a), 154.02 (C-2), 162.97 (C-4). *m/z* (ESI+, %): *($M+H^+$)* 332.1 (100), 280.1 (63), 236 (91), 230.1 (15), 214.1 (74), 137.8 (17), 134.8 (24), 111.9 (16), 60.1 (16).

*2.3.3.2. 2',3'-dideoxy-3'-thiacytidin-N4-yl-(methoxydi(ethylene glycol)) carbamate (n = 2, **10**)*

Compound (**4**) (4.00g, 14.0 mmol) afforded 2.50 g (63%) of (**10**) as a yellowish oil after purified by flash silica gel column chromatography eluting with DCM:MeOH (10:1). $C_{14}H_{21}N_3O_7S$. ^{1}H NMR δ (ppm) ($CDCl_3$): 3.17 (dd, 1H, H-5'b, J = 12.4, 3.4 Hz), 3.34 (d, 3H, H-f, J = 8.8 Hz), 3.49-3.54 (m, 2H, H-e), 3.55-5.64 (m, overlapping, 3H, H-5'a, H-d), 3.65-3.73 (m, overlapping, 3H, H-2', H-c), 3.93 (dd, 1H, H-6'b, J = 12.4, 3.4 Hz), 4.07 (dd, 1H, H-6'a, J = 12.4, 3.4 Hz), 4.28-4.34 (m, 2H, H-b), 5.31 (t, 1H, H-4', J = 3.4 Hz), 6.30 (t, 1H, H-5, J = 4.1 Hz), 7.16 (s, 1H, NH_2), 8.28 (d, 1H, H-6, J = 7.2 Hz). ^{13}C NMR δ (ppm): 38.94 (C-5'), 59.05 (C-f), 62.77 (C-e), 65.30 (C-d), 68.86 (C-c), 70.51 (C-b), 71.81 (C-6'), 88.02 (C-4', C-2'), 94.63 (C-5), 144.86 (C-6), 152.22 (C-a), 154.94 (C-2), 162.49 (C-4). *m/z* (ESI+, %): *($M+H^+$)* 376 (59), 281 (23), 280 (100), 258.1 (48), 160.2 (19), 143.1 (17), 138.0 (49), 112.4 (12).

*2.3.3.3. 2',3'-dideoxy-3'-thiacytidin-N4-yl-(methoxytri(ethylene glycol)) carbamate (n = 3, **11**)*

Compound (**5**) (4.00g, 12.15 mmol) afforded 1.60 g (40%) of compound (**11**) as yellowish oil after purification by flash silica gel column chromatography eluting with a mixture of DCM:MeOH (10:1). $C_{16}H_{25}N_3O_8S$. ^{1}H NMR δ (ppm) ($CDCl_3$): 3.14 (dd, 1H, H-5'b, J = 12.4, 3.8 Hz), 3.34 (s, 1H, H-h), 3.51 (dd, 2H, H-g, J = 5.6, 4.0 Hz), 3.55-5.65 (m, overlapping, 7H, H-5'a, H-d, H-e, H-f), 3.66-3.74 (m, 2H, H-c), 3.92 (dd, 1H, H-6'b, J = 12.4, 3.8 Hz), 4.07 (dd, 1H, H-6'a, J = 12.4, 3.8 Hz), 4.26-4.34 (m, 2H, H-b), 5.31 (t, 1H, H-4', J = 3.4 Hz), 6.25-6.35 (m, 1H, H-5), 7.16 (s, 1H, NH_2), 8.28 (d, 1H, H-6, J = 7.0 Hz). ^{13}C NMR δ (ppm): 38.93 (C-5'), 59.01 (C-h), 62.77 (C-g), 65.26 (C-f), 68.82 (C-e), 70.48 (C-d, C-c), 70.60 (C-b), 71.86 (C-6'), 87.97 (C-4'), 88.03 (C-2'), 94.67 (C-5), 144.80 (C-6), 152.29 (C-a), 154.96 (C-2), 162.55 (C-4). *m/z* (ESI+, %): ($M+H^+$) 420 (100), 413 (5), 339.9 (8), 324.2 (11), 302.1 (11), 256.2 (5), 186.6 (11), 138 (20).

*2.3.4. Synthesis of MPEG carbonates of lamivudine (**12-14**) (Scheme 4)*

MPEG carbonates of lamivudine were synthesised following a general method depicted in Scheme 4 and, described as follows to exemplify the preparation of carbonate (**12**) (n =1). To a solution of (**3**) (3.00 g, 12.4 mmol) in dry DMF (40 ml) stirred at room temperature was added hydroxysuccinmide (HOSu) (1.24 mmol, 0.12 equiv). Protected lamivudine (**8**) (13.7 mmol, 3.9 g, 1.1 equiv) previously dissolved in DMF (5 ml) was added drop wise over 5-10 min period to solution of (**3**). After 72 h of stirring at room temperature, the solvent was evaporated in *vacuo* and the residue was purified by silica gel flash chromatography to afford the prodrug (**12**) as a yellowish oil. ^{1}H and ^{13}C NMR chemical shifts as well as LC-MS data for compounds (**12-14**) (Scheme 4) are reported below.

*2.3.4.1. 2',3'-dideoxy-3'-thiacytidin-6'-yl-O-(methoxy(ethylene glycol)) carbonate (n = 1, **12**)*

A yield of 0.66 g (22%) of compound (**12**) as a yellowish oil was obtained after purification by flash silica gel column chromatography eluting with a mixture of DCM:MeOH (10:1). $C_{12}H_{17}N_3O_6S$. ^{1}H NMR δ (ppm) (DMSO): 2.98 (dd, 1H, H-5'b, J = 11.63, 5.64 Hz), 3.22 (s, 3H, H-d), 3.38 (dd, 1H, H-5'a, J = 11.63, 5.64 Hz), 3.48-3.63 (m, 2H, H-c), 4.14-4.28 (m, 2H, H-b), 4.36-4.49 (m, 2H, H-6'a, H-6'b), 5.26 (t, 1H, H-2', J = 4.77 Hz), 5.62 (t, 1H, H-4', J = 7.41 Hz), 6.12 (t, 1H, H-5, J = 5.5 Hz), 7.04 (d, 2H, NH_2), 7.56 (d, 1H, H-6). ^{13}C NMR δ (ppm): 35.68 (C-5'), 58.03 (C-d), 66.95 (C-b), 67.84 (C-c), 69.51 (C-6'), 80.83 (C-2'), 86.84 (C-4'), 94.40 (C-5), 140.53 (C-6), 154.25 (C-2), 154.63 (C-a), 165.62 (C-4). *m/z* (ESI+, %): *($M+H^+$)* 331.9 (100), 324.1 (5), 211.8 (5), 187.3 (3), 162 (3), 112 (87), 101.1 (5), 59.4 (5).

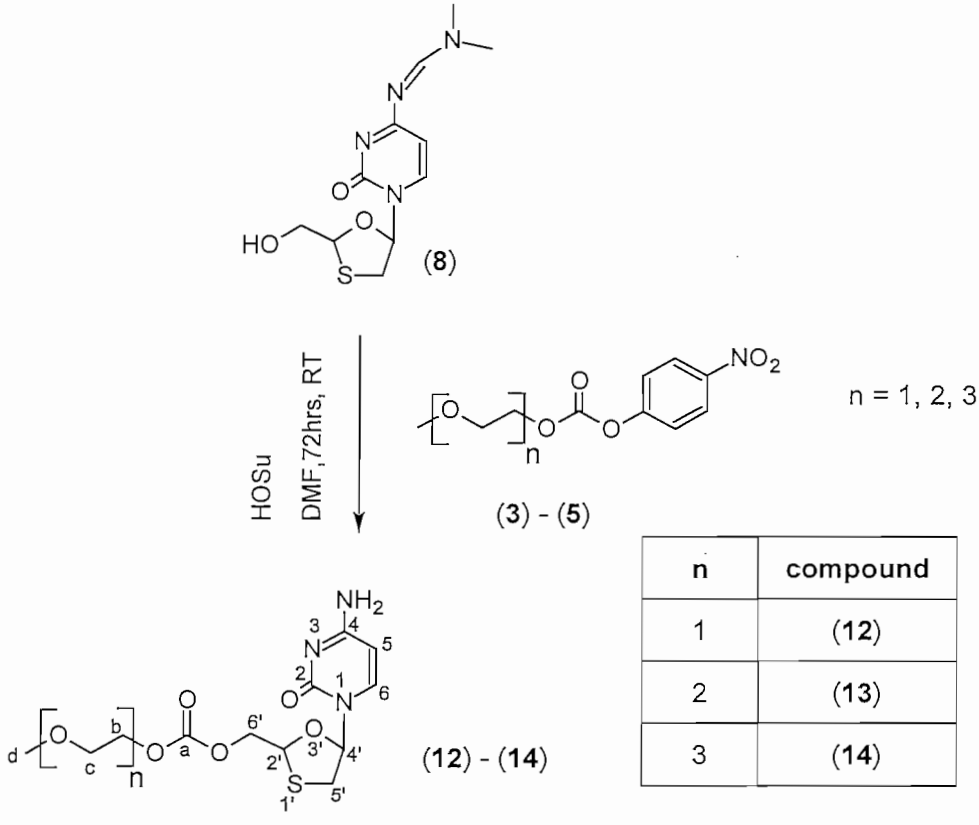
*2.3.4.2. 2',3'-dideoxy-3'-thiacytidin-6'-yl-O-(methoxydi(ethylene glycol)) carbonate (n = 2, **13**)*

Compound (**4**) (1.20 g, 4.2 mmol) afforded 0.5 g (42%) of compound (**13**) as a yellowish oil after purified by flash silica gel column chromatography eluting with DCM:MeOH (10:1). $C_{14}H_{21}N_3O_7S$. ^{1}H NMR δ (ppm) ($CDCl_3$): 3.23 (dd, 1H, H-5'b, J = 12.5, 3.7 Hz), 3.25-3.29 (m, 3H, H-f), 3.42-3.47 (m, 2H, H-e), 3.49-3.51 (m, 1H, H-5'a), 3.54-3.61 (m, 2H, H-d), 3.62-3.64 (m, 2H, H-c), 4.18-4.28 (m, 2H, H-b), 4.36-4.52 (m, 2H, H-6'), 5.24-5.39 (m, 1H, H-2'), 6.17-6.26 (m, 1H, H-4'), 6.45 (d, 1H, H-5, J = 7.9 Hz), 7.89-7.98 (m, 1H, H-6). ^{13}C NMR δ (ppm): 38.62 (C-5'), 58.80 (C-f), 66.71 (C-e), 67.65 (C-d), 68.92 (C-c), 70.32 (C-b), 71.84 (C-6'), 83.52 (C-2'), 87.43 (C-4'), 94.38 (C-5), 144.78 (C-6), 154.65 (C-2), 154.74 (C-a), 165.86 (C-4). *m/z* (ESI+, %): *($M+H^+$)* 376.1 (70), 224.2 (32), 166.9 (41), 128.2 (52), 119.1 (51), 101.2 (100), 78.1 (10), 73.3 (10).

*2.3.4.3. 2',3'-dideoxy-3'-thiacytidin-6'-yl-O-(methoxytri(ethylene glycol)) carbonate (n = 3, **14**)*

Compound (**5**) (3.5g, 10.63 mmol) afforded 1.68 g (48%) of compound (**14**) as yellowish oil

after purification by flash silica gel column chromatography eluting with a mixture of DCM:MeOH (10:1). $C_{16}H_{25}N_3O_8S$. ^1H NMR δ (ppm) ($CDCl_3$): 3.11 (dd, 1H, H-5'b, J = 12.2, 3.8 Hz), 3.39 (d, 3H, H-h, J = 69.1 Hz), 3.46-3.70 (m, overlapping, 11H, H-5'a, H-c, H-d, H-e, H-f, H-g), 4.30 (s, 2H, H-b), 4.51 (dt, 2H, H-6', J = 12.4, 7.2 Hz), 5.37 (d, 1H, H-2', J = 50.9 Hz), 5.88 (d, 1H, H-5, J = 7.51 Hz), 6.35 (t, 1H, H-4', J = 4.4 Hz), 7.79 (d, 1H, H-6, J = 7.5 Hz). ^{13}C NMR δ (ppm): 38.47 (C-5'), 58.87 (C-h), 66.84 (C-g), 67.61 (C-f), 68.90 (C-e), 70.36 (C-d), 70.45 (C-c), 70.53 (C-b), 71.74 (C-6'), 83.27 (C-2'), 87.48 (C-4'), 94.43 (C-5), 140.92 (C-6), 154.78 (C-2), 155.61 (C-a), 165.95 (C-4). MS *m/z* (ESI+, %): ($M+H^+$) 420.1 (100), 231.2 (20), 184.9 (8), 134.8 (4), 119 (28), 112.1 (5), 101 (8), 87.4 (4).



n	compound
1	(12)
2	(13)
3	(14)

Scheme 4. Synthesis of 6'-O-carbonates (**12-14**)

2.4. Physicochemical properties

2.4.1. Solubility

The aqueous solubility values of compounds (**6**) and (**9**) were obtained by preparing saturated solutions in phosphate buffer at pH 7.4. The slurries were stirred with magnetic bars in a water bath at 32 °C for 24 h. An excess of solute was present at all times to provide saturated solutions. After 24 h, the solutions were filtered and analyzed directly by HPLC to determine the concentration of solute dissolved in the solvent. The experiment was performed in triplicate (Table 2). It was not possible to determine the aqueous solubility of compounds (**10**) – (**14**) due to the fact that they were infinitely miscible.

2.4.2. Experimental log P

Equal volumes of *n*-octanol and phosphate buffer solution of pH 7.4 were saturated with each other under vigorous stirring for at least 24 h. Accurately weighed 30 mg of (**6**) (10 mg/ml) as well as each derivative was dissolved in 3 ml of pre-saturated *n*-octanol, stoppered and agitated for 10 min in a 10 ml graduated tubes (0.5 ml division). Subsequently 3 ml of pre-saturated buffer was transferred to the tubes containing the before-mentioned solutions. The tubes were stoppered and agitated for 45 min then centrifuged at 4000 rpm for 30 min. The *n*-octanol and aqueous phases were allowed to separate at room temperature for 5 min and thereafter the volume ratio (v/v; *n*-octanol:buffer) was established. In all cases, the ratio was found to be 1. The *n*-octanol phases were diluted with methanol (MeOH), HPLC analyzed and the concentrations determined. Consequently the concentrations in aqueous phases were deduced. This method had previously been used by Taylor and Sloan (1998). The log P values (log (octanol: pH 7.4 buffer partition coefficient)) were calculated as logarithmic ratios of the concentrations in the *n*-octanol phase to the concentrations in the buffer. Five micro litre of each tested *n*-octanol phase was injected

onto the column in triplicate. The experiment was performed in triplicate and the results expressed as means are listed in Table 2.

2.5. Skin permeation

2.5.1. Donor solutions

Donor solutions (0.2 M) of (**6**) and its derivatives (**9**-**14**) were obtained by dissolving with stirring the exact amount of compound in 5 ml of phosphate buffer solution of pH 7.4 and Pheroid™ (formulated by the Department of Pharmaceutics, School of Pharmacy, North-West University, Potchefstroom, South Africa). The process was carried out in stoppered flasks in a water bath at 32 °C over a period of 24 h in order for saturation to occur.

2.5.2. Skin preparation

The project "*In vitro* transdermal delivery of drugs through human skin" was approved (human ethics approval reference number 04D08) by the Ethics Committee of the North-West University (Potchefstroom campus, South Africa) and skin was obtained with informed consent of the donors. Caucasian female human abdominal skin was obtained after cosmetic procedures. A scalpel was used to separate the skin from the fat; subsequently, the stratum corneum-epidermis (SCE) layer was removed by means of immersion in 60 °C HPLC water for 60 s. SCE layer was then peeled away from the dermis using forceps (Harrison et al., 1984). Special care was taken that the integrity of the layer was not ruptured, as this would compromise the validity of the results. The layer was placed in a bath filled with HPLC water and carefully set on Whatman® filter paper, left to air dry, wrapped in foil and stored in a freezer at -20 °C for ultimate use within a period not exceeding 6 months after preparation. Prior to use, it was thawed and visually examined before being mounted on the Franz diffusion cells.

2.5.3. Skin permeation

Vertical Franz diffusion cells with 2.0 ml receptor compartment and 1.08 cm^2 effective diffusion area was used for the permeation studies. The SCE skin layer was carefully mounted on the lower half of the Franz cell with the stratum corneum facing upwards. A clamp was used to fasten the upper and lower parts of the Franz cell together. The receptor compartment was filled with sodium phosphate buffer (pH 7.4). Special care was taken that no air bubbles came between the buffer solution and skin layer. The Franz cell, containing the buffer solution was equilibrated for 1 h in the water bath at 32 °C, prior to the addition of the 0.2 M solution to the donor compartment. Only the receptor compartment which was submerged in the water was equipped with a stirring magnet. After a period of 1 h, 1.0 ml of freshly prepared 0.2 M solution was added to each donor compartment, and immediately covered with Parafilm® to prevent the evaporation of any constituents from the solution throughout the duration of the experiment. The data were collected by using human skin from a single donor with five cells in each diffusion experiment.

The entire receptor volumes were withdrawn and replaced with 32°C fresh buffer solution (pH 7.4) after 2, 4, 6, 8, 10 and 24 h. The entire receptor volume was withdrawn to mimic sink conditions as they occur in the human body. The experiment was conducted over 24 h periods.

The withdrawn samples were assayed immediately by HPLC. Five micro litre of each sample was injected onto the column, and the results expressed as means allow the determination of concentration of (**6**) or the derivatives (**9–14**) that had permeated the epidermis. The cumulative amount of drug or prodrug collected in the receptor compartment was plotted as a function of the time. The flux value for a given experiment was obtained from the slope of the steady-state portion of the cumulative amount of drug permeated versus time. Two methods were used, *viz.* Method 1 with the PBS donor solutions and Method 2 with the Pheroid™ donor solutions.

2.6. Statistical methods (Table 1)

Two methods were used during the transdermal study (see section 2.5.3).

With both methods the experiment was repeated on 5 different diffusion cells for each compound. The fluxes of six compounds, i.e. the six derivatives (**9**-**14**), were compared with that of the parent compound lamivudine (**6**). As a result of this experimental design parametric statistics could be performed for each method. In both methods the following statistical procedures were used.

A one-way analysis of variance (ANOVA) was done to determine if there were statistical significant differences between the means of the flux values of all the tested compounds. A Dunnetts test was done to determine which of the mean flux values of the derivatives differed statistically significantly from that of lamivudine. As a result of the fact that the standard deviations of the flux of all the compounds were large a transformation on the flux values was made by the square root of the flux values, when the one-way analysis of variance was done (Field, 2005). By performing this transformation homogeneity of variances of the different compounds were obtained and verified by Levene's test. Interpretation of results, however, was done on the mean of the flux values.

To determine which method (in PBS or in Pheroid^{TM}) was the most efficient, a t-test for independent groups were done on the overall mean flux values of each method using the statistical computer package SAS (SAS Institute inc., 2005) and SPSS (SPSS Inc., 2007). The steady-state flux (J_{ss}), standard deviation (STD) and *p*-values for the derivatives by both methods are reported in Table 1 and Table 2.

Table 1. Dunnett tests' p-values of the derivatives (Method 1 and 2)

Compound	*p*-value (Method 1)	*p*-value (Method 2)
(**9**)	0.1956	0.4247
(**10**)	0.0028*	0.9714
(**11**)	0.0102*	0.1505
(**12**)	< 0.0001*	0.9875
(**13**)	0.0009*	nd [a]
(**14**)	< 0.0001*	0.3458

* statistical significant. [a] not determined.

3. Results and Discussion

3.1. Chemistry (Schemes 1, 2, 3 and 4)

Carbamates (**9-11**) and carbonates (**12-14**) were obtained in 22-63% yields by alkoxy carbamylation and carbonylation of lamivudine (**6**) with corresponding short-chain methoxypoly(ethylene glycol)(s) followed by chromatographic purification. Methoxypoly(ethylene glycol) (MPEG) were chosen because of the useful properties like a wide range of solubility in both organic and aqueous solvents, biocompatibility meaning less toxicity, no antigenicity and immunogenicity, no interference with enzymatic activities and conformations of polypeptides, and easy excretion (glomular filtration in the kidneys) from living organisms, making MPEG an ideal choice for making derivatives (Zalipsky & Lee, 1992). Bonora et al. (1997) found that PEG may cause an increase in the stability of the conjugated molecules. PEG may also extend circulation life of drugs in the body, reduce toxicity and enhance protection from proteolytic degradation (Veronese & Pasut, 2005). It is one of only a few synthetic polymers that are approved by the US Food and Drug Administration (FDA) to be used internally in food, cosmetics and pharmaceutical products (Moore & Roberts, 1982).

Prior to carbamylation and carbonylation, each MPEG was activated at its terminal OH using *p*-nitrophenyl chloroformate, as outlined in Scheme 1. This first synthetic step resulted in unsymmetrical alkoxy aryl carbonates (**3**-**5**) which possessed the highly nucleofuge moiety (*p*-nitrophenoxide). Shaik and Sivaram (1996) showed that the electron-withdrawing nature of the *p*-nitro group increases the vulnerability of such carbonates towards carbonylation exchange reactions. Subsequently, the reaction between the activated MPEG and 3TC led to the title compounds as result of the nucleophilic groups (*N4 and 6'-OH*) of (**6**) displacing the nucleofuge as delineated in Scheme 2.

Before the 6' carbonates could be synthesised, the highly reactive $-NH_2$ group of 3TC had to be protected which was done by using dimethylformamide dimethyl acetal (DMF-dma) (**7**). This forced the activated MPEG to undergo carbonylation at the 6'-OH group and thus forming the corresponding carbonate of (**6**). During the reaction the protecting group was hydrolysed, leaving only the carbonates (**12**-**14**) as the final products.

The chemical structures of the title compounds (**9**-**14**) (Schemes 3 and 4) were confirmed by NMR and LC-MS data. The presence of the linker was confirmed by the resonance of carbon C-a at 153-154 ppm in ^{13}C NMR. ^{1}H NMR spectra of all the derivatives exhibited resonances in the 3.48-4.34 ppm region characteristic of methylene protons $-OCH_2\text{-}CH_2O-$ belonging to MPEG chain.

The LC-MS data for the compounds confirmed the presence of molecular ions (*m/z*) at 332 (**9** and **12**), 376.1 (**10** and **13**) and 420.1 (**11** and **14**), corresponding to the molecular formulae $C_{12}H_{17}N_3O_6S$ (**9** and **12**), $C_{14}H_{21}N_3O_7S$ (**10** and **13**) and $C_{16}H_{25}N_3O_8S$ (**11** and **14**). These formulae in turn indicate the number of oxyethylene units, **n**, to be 1 for (**9** and **12**), 2 for (**10** and **13**) and 3 for (**11** and **14**), respectively.

3.2. Aqueous solubility and lipophilicity (Table 2)

Aqueous solubility and lipophilicity influence the way a drug molecule passes through the skin. They determine drugs' lipophilicity, which in turn is important because of the lipophilic nature of the SC. The lipophilic SC prevents hydrophilic molecules from entering the skin, thus preventing drugs with a high aqueous solubility from penetrating the skin and entering the systemic circulation. The aqueous solubility of a drug is also important because of the complex membrane structure of the skin. This is seen when looking at the pathway a drug must undertake when passing through the skin. As mentioned above, a drug must be lipophilic in order to penetrate and cross the SC, but once it has crossed the SC it reaches the more aqueous viable epidermis. If the drug molecule is too lipophilic, it will remain in the SC layer and be unable to reach the systemic circulation in order for the drug to have an effect (Niak *et al.*, 2000). Thus, a drug molecule should possess both hydrophilic and lipophilic properties to readily diffuse across the skin (Rautio et al., 1999), but a more lipophilic nature is needed in order for the drug molecule to penetrate the SC (Guy & Hadgraft, 1989). The two characteristics expressed as logarithm of the partition coefficient (log P) between *n*-octanol and phosphate buffer (pH 7.4) are listed in Table 2 for compounds (**9-14**). Methoxypoly(ethylene glycol) exhibits the ability to increase molecules' hydrophilicity by the increasing number of oxyethylenic units in the methoxypoly(ethylene glycol) moiety. Solomons & Fryhle (2007) states that the aqueous solubility of MPEGs (polyether) arises from their capability to form number of hydrogen bonds with water molecules in the surrounding environment. These bonds are formed by the oxygen atoms in the chain of the MPEGs. Thus, increasing the chain length automatically increases the number of intra-chain oxygen atoms that can interact with the water molecules, leading to an increase in aqueous solubility. However, as the length increased so did the number of ethylene units which ultimately increases lipid solubility. The derivatives (**9-14**) showed higher log P values than (**6**) (Table 2). In Table 2 are also compiled calculated aqueous solubility S_w values for all compounds (**6**) and (**9-14**) using previously mentioned log P values. They were obtained

theoretically from equations (shown below) extensively used by other research groups (Yalkowsky & Valvani, 1980; Yalkowsky et al., 1983; Osborne and Lambert, 1992; Bonina et al., 2001; Puglia et al., 2006) to compare the aqueous solubility of a parent drug with that of a homologous series of its derivatives as well as the influence of this parameter on the *in vitro* skin permeation of investigated compounds.

Equation (1) is exclusive for crystalline compounds while Equation (2) applies to oils.

$$\text{Log } S_w = -\log P - 0.01\ MP + 1.05 \quad (1)$$

$$\text{Log } S_w = -1.072 \log P + 0.672 \quad (2)$$

where S_w is the calculated water solubility of a compound, MP is its melting point and log *P* is the *n*-octanol-water partition coefficient.

The S_w values for (**9-14**), as expected, followed the decreasing trend of the log P values irrespective of the series, as the chain length increased thus firmly validating both structure-aqueous solubility and structure-lipophilicity relationships within the series.

3.3 In vitro *skin permeation* (Tables 2 and 3)

The permeation of lamivudine (**6**) and derivatives (**9-14**) through excised human skin was evaluated from isotonic phosphate buffer solution (0.01 M, pH 7.4). Solutions of standard concentration of 0.2 M were prepared for each compound and used as donor in the diffusion study. The cumulative permeated amount displayed in portion a linear relationship with time. Steady-state fluxes (J_{ss}) were obtained from slopes of the linear portions of these plots. The experimental flux values were compared to flux values (Table 3) that were calculated by using the Potts and Guy equation (Equation 3). Theoretical values obtained for aqueous solubility, log P and the molecular weight (MW) were used to estimate the flux values (J_{max}) for (**6**) and its derivatives (Table 3).

Table 2. Physicochemical properties and transdermal flux of lamivudine (**6**) and its derivatives (**9**) - (**14**)

Compound	n	M_w (g/mol)	Mp (°C)	S_w (mg/ml)	STD	S_w (µmol/ml)	STD	Log P [d]	STD	Log P [e]	J_{ss} [g] (µmol/cm²/h)	STD	J_{ss} [h] (µmol/cm²/h)	STD
(**6**)		229.3	162	1.82 [a] (188.02 [b,d])	2.24	7.94 (819.97)	9.77	-0.83	0.061	-1.16	4.23 [I]	2.98	0.20 [J]	0.22
(**9**)	1	331.34	125	0.93 [a] (8.53 [b,d])	0.22	2.79 (25.66)	0.66	-0.17	0.008	-0.69	2.07	0.62	0.04	0.06
(**10**)	2	375.40	oil	8.29 [c]	-	22.02	-	-0.23	0.016	-0.74	0.88	0.45	0.11	0.17
(**11**)	3	419.46	oil	22.25 [c]	-	52.92	-	-0.63	0.043	-0.81	1.32	0.86	0.002	0.01
(**12**)	1	331.34	oil	6.97 [c]	-	20.96	-	-0.16	0.006	-0.53	0.04	0.03	0.23	0.47
(**13**)	2	375.40	oil	8.29 [c]	-	22.02	-	-0.23	0.017	-0.60	0.66	0.36	nd [f]	nd [f]
(**14**)	3	419.46	oil	10.09 [c]	-	23.99	-	-0.31	0.026	-0.67	0.22	0.32	0.02	0.03

Methoxypoly(ethylene glycol) index (n), molecular weight (M_w), melting point (Mp), standard deviation (STD), aqueous solubility (S_w), partition coefficient, log P (*n*-octanol-PBS, pH 7.4), steady-state flux (J_{ss}).

[a] calculated using Eq. (1). [b] determined experimentally. [c] calculated using Eq. (2). [d] data represent the mean and STD of 3 measurements. [e] calculated using ALOGPS 2.1 online software (http://www.vcclabs.org/lab/alogps). [f] not detected. [g] Method 1: each experiment was run on five different cells and data represent the mean and STD in PBS (pH=7.4). [h] Method 2: each experiment was run on five different cells and data represent the mean and STD in Pheroid™ (pH=7.4). [I] the flux of lamivudine in PBS obtained in Gerber's study was 4.289 µmol/cm²/h. [J] the flux of lamivudine in Pheroid™ obtained in Gerber et al. (2008) was 0.011 µmol/cm²/h.

The Potts and Guy equation (Equation 3) were used to calculate the log K_p, from where the permeability coefficient (K_p) was obtained (Hadgraft et al., 2000). The estimated flux ($\mu mol.cm^{-2}.h^{-1}$) was obtained from the product of the permeability coefficient and the aqueous solubility at the same pH (Equation 4).

$$\log K_p = -2.7 + 0.71 \log P - 0.0061 MW \quad (3)$$

$$J_{max} = K_p \times \text{aqueous solubility} \quad (4)$$

The predicted flux values were all lower than the experimental flux values (Table 3). In both the prediction and experiment, the parent drug (**6**) displayed the highest flux value. However, the experimental value was at least 2000-fold higher than the predicted one.

Table 3. Transdermal permeation: Experimental flux vs. predicted flux

	Flux ($\mu mol/cm^2/h$)	
Compound	**Predicted [a]**	**Experimental (PBS)**
(**6**)	9.81×10^{-3}	4.23
(**9**)	1.56×10^{-4}	2.07
(**10**)	6.63×10^{-5}	0.88
(**11**)	7.65×10^{-5}	1.32
(**12**)	1.65×10^{-4}	0.04
(**13**)	8.33×10^{-5}	0.66
(**14**)	4.36×10^{-5}	0.22

[a] calculated using ALOGPS 2.1 predicted log P and predicted aqueous solubility values of al the compounds (except for compound (**6**) and (**9**) where the experimental aqueous solubility values were used) in the Potts and Guy equation (Equation 3 & 4).

All of the derivatives (**9**-**14**) showed lower steady-state fluxes than 3TC. The N4-carbamates' steady-state fluxes were higher than those of the 6'-O carbonates.

Furthermore, the carbamates showed a decrease in the transdermal flux as the chain length increased. On the other hand the carbonates showed a different pattern, starting very low with (**12**) at 0.04 μmol/cm^2/h, reaching a maximum flux of 0.66 μmol/cm^2/h for (**13**) and then decreasing again to 0.22 μmol/cm^2/h for (**14**). However, a realistic conclusion can only be drawn by extending each series.

When comparing flux in PBS with that in Pheroid^{TM} it is observed that all the compounds except (**12**) have a lower flux in Pheroid^{TM}. Hence, Pheroid^{TM} does not improve transdermal flux of this series of compounds. This could be due to the fact that Pheroid^{TM} might have a sustained release effect on the drug molecules, thus prolonging the time it takes for the molecules to cross the SC. The flux of (**13**) could not be determined because it does not seem to penetrated through the SC. Gerber et al. (2008) suggested that drugs with a higher aqueous solubility will have better flux values in Pheroid^{TM}.

When the two methods (PBS and Pheroid^{TM}) were compared a *p*-value of 0.0002 indicated that there was a highly statistically significant difference between the mean of the overall flux of Method 1 and that of Method 2. The mean flux value of Method 1 was 1.17 μmol/cm^2/h with a STD of 1.50, while the mean value of Method 2 was 0.10 μmol/cm^2/h with a STD of 0.22, showing that the flux of Method 1 was significantly higher than that of Method 2.

The results of the ANOVA for Method 2 indicated that there were no statistically significant differences between the mean flux values of the different compounds (*p*-value of 0.26). Thus there were no statistically significant differences between the mean flux of any compound and the parent drug.

In the case of Method 1, the ANOVA indicated that a significant difference between the means of the flux of all the compounds existed (*p*-value of < 0.0001). A Dunnett test showed

that except for (**9**), all the mean flux values of the other compounds were statistically lower than the mean flux of the parent drug (Table 1).

Gerber et al. (2008) found the experimental flux value of lamivudine to be 4.289 μmol/cm^2/h and it correlated well with the experimental flux value of 4.23 μmol/cm^2/h in this study. This confirms the repeatability of the experiment and that the values can be compared. All of the derivatives in this study had better flux values than those in Gerber's study. This might be attributed to the amphiphilic properties of MPEG that enables the derivatives to permeate more readily through the skin than the more lipophilic derivatives.

N'Da & Breytenbach (2009) synthesised carbonates of zidovudine (AZT) by using MPEG and found that the derivatives with 1–3 or 8 oxyethylene units in the methoxypoly(ethylene glycol) moiety permeated through the skin, whereas those with 12 or 17 units did not. This correlates well with the results of the carbonates synthesised in this study, since carbonates (**12** – **14**), containing 1-3 oxyethylene units, permeated through the skin.

4. Conclusion

In conclusion, we have synthesised a series of N-4 methoxypoly(ethylene glycol) carbamates (**9**-**11**) and 6'-O methoxypoly(ethylene glycol) carbonates (**12**-**14**) as derivatives of lamivudine through derivatisation at its –NH_2 or –OH group respectively.

The structures were verified by ^{1}H and ^{13}C NMR and LC-MS spectroscopy. None of the derivatives showed better flux than that of lamivudine. It was also found that the flux in Pheroid™ (Method 2) was much lower than in PBS (Method 1). Thus, neither derivatisation nor the use of Pheroid™ improved the flux of this series of compounds. This shows that a clinically useful transdermal administration of the derivatives synthesised in this study will not be feasible.

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CHAPTER 5

SUMMARY AND FINAL CONCLUSIONS

This section gives a summary and final conclusion of the whole study, including results from Chapter 4 (article) plus additional results from Appendix 1 (not included in the article). It therefore endeavours to unify the outcomes of the whole study and draws overall conclusions from all data, points out relevance and specific scientific advances made by the study and finally points out remaining scientific questions and proposes appropriate prospective studies.

The transdermal route of drug administration has advantages over the common oral route which include improving patient compliance by decreasing the amount of medication, circumventing the first pass metabolism in the liver, eliminating certain unwanted side-effects which are associated with oral administration (nausea, diarrhoea) and better control over the input kinetics of the drug. The skin's barrier function, however, limits penetration through it and has inspired researchers to look at ways to enhance delivery through the skin in order for the drug to reach the circulation in sufficient quantities to have a pharmacological effect. One of the ways to address this problem is to synthesise derivatives that have more suitable physicochemical properties for transdermal penetration than the parent drug.

Lamivudine is a nucleoside reverse transcriptase inhibitor (NRTI) that is commonly used in combination with zidovudine (AZT) in the treatment of HIV-1 and HIV-2. It is also used as a treatment against Hepatitis B and prophylactically for patients that have accidentally been exposed to HIV. The most common adverse effects of lamivudine are associated with the gastro-intestinal system and include vomiting, diarrhoea, abdominal cramps and pain, and nausea (Gregg, 1999) and nucleoside medications are perceived as having a bitter taste (Sweetman, 2002).

The adverse effects encouraged a study into the possible transdermal delivery of lamivudine with the aim of avoiding hepatic first pass metabolism, reducing side effects, improving patient compliance and bioavailability and decreasing the administered dose. Transdermal delivery can also improve patient compliance in younger children by avoiding the bitter taste of these suspensions when using a transdermal patch (Schiffman *et al.,* 1999).

Much attention has been given to the use of penetration enhancers and delivery vehicles to improve transdermal permeation. A promising alternative to the use of penetration enhancers is the prodrug approach (Sloan, 1989; Sloan, 1992). The approach involves modifying the chemical structure of a drug in order to achieve a change in both its pharmaceutical and pharmacokinetic characteristics and thus enhancing its transdermal delivery. Molecules with a log P value between 1 and 3 are believed by Williams (2003) to exhibit sufficient aqueous and lipophilic properties to obtain proper transdermal permeation.

The primary aim of this study was to synthesise a series of new derivatives of the anti-HIV drug 3TC, and to evaluate the effects of the different subtituents on transdermal penetration, with and without the use of Pheroid™ as delivery system. Furthermore, it was to be established if any relationship exists between the transdermal permeation and selected physicochemical properties like aqueous solubility and partition coefficient (log P) of the penetrant.

The following objectives were set:

- Synthesise MPEG derivatives of lamivudine and confirm their structures.
- Experimentally determine the physicochemical properties like the aqueous solubility and the partition coefficient for lamivudine and its synthesised derivatives and to compare the experimental aqueous solubilities and the partition coefficients of the synthesised lamivudine derivatives to calculated values from commonly used prediction software (ALOGPS 2.1 online prediction software).
- Experimentally determine the transdermal flux of lamivudine and its derivatives in PBS at pH 7.4 and in Pheroid™ and to compare the experimental flux data of the synthesised lamivudine derivatives to calculated values from commonly used theoretical equation.
- Determine whether a relationship exists between the physicochemical properties like the aqueous solubility, partition coefficient and the transdermal flux data of lamivudine and its derivatives.
- Contribute to a data base with results from both this and other transdermal studies whereby possible correlations between physicochemical properties and the transdermal penetration can be determined.

The lamivudine derivatives were successfully synthesised and the structures were verified by ^{1}H and ^{13}C NMR, LC-MS spectroscopy.

It was not possible to determine the aqueous solubility of compounds (**10**) – (**14**) due to the fact that they were infinitely soluble in water. This led to the use of theoretical equations to determine the aqueous solubility. The derivatives showed an increase in aqueous solubility (ranging from 8.29 to 10.09 mg/ml). This could be due to the fact that as the intra-chain oxygen atoms increased, more bonds were formed with surrounding water molecules and thus increased the aqueous solubility.

The experimental partition coefficient (-0.83) of lamivudine was lower than that of its derivatives (ranging from -0.16 to -0.63). As the chain length increased the partition coefficient value decreased. This correlates with the aqueous solubility values which showed that the aqueous solubility increased as the chain length increased due to an increase in the intra-chain oxygen atoms. However, as the length increased so did the number of ethylene units which ultimately increases lipid solubility, thus making the derivatives more lipophilic than lamivudine.

The transdermal flux (4.23 μmol/cm^2/h) of lamivudine in PBS was much higher than that of its derivatives (ranging from 0.04 to 2.07 μmol/cm^2/h). With the *N4*-methoxy(ethylene glycol) carbamate (n = 1) (2.07 μmol/cm^2/h) and the *N4*-methoxytri(ethylene glycol) carbamate (n = 3) (1.32 μmol/cm^2/h) being the only derivatives with appreciable flux. In the Pheroid^{TM}, the flux values of lamivudine (0.20 μmol/cm^2/h) and its derivatives (ranging from 0.002 to 0.23 μmol/cm^2/h) were much lower than those in PBS. This showed that Pheroid^{TM} did not enhance the transdermal permeation of lamivudine and its derivatives in this study. This could be due the fact that Pheroid^{TM} may have a sustained release effect, thus decreasing the rate of diffusion over the stratum corneum.

Lamivudine (**6**) and 2',3'-dideoxy-3'-thiacytidin-N4-yl-methoxy(ethylene glycol) carbamate (n = 1, **9**) are both crystalline compounds, while the rest of the lamivudine derivatives (**10** – **14**) are oils. Therefore in the supersaturated buffer solutions the crystals were at the bottom of the donor phase, keeping the resultant solution homogenous which may explain the higher flux observed for these compounds. With the oils it was hard to determine if the solution was saturated or not, thus 0.2 M donor solutions of each derivative were prepared. This inability to determine the saturation may have caused the lower flux values due to the fact that there may not have been enough of the compound in the donor to give sufficient flux over the stratum corneum.

In this study it has been shown that lamivudine has a better transdermal flux than the derivatives that was synthesised, both in PBS and Pheroid^{TM}. This correlated with results obtained in a previous study (Gerber *et al.* 2008). It also confirmed that transdermal flux is dependant on several factors including optimum solubility, partitioning, diffusion and the degree of ionisation in the stratum corneum in addition to a suitable partition coefficient and high aqueous solubility. It has also shown that some penetration enhancers may have a possible

sustained release effect. The possible sustained release effect of Pheroid™ and the effect that substitution on the different functional groups of lamivudine may have on the transdermal flux, could be investigated in future studies.

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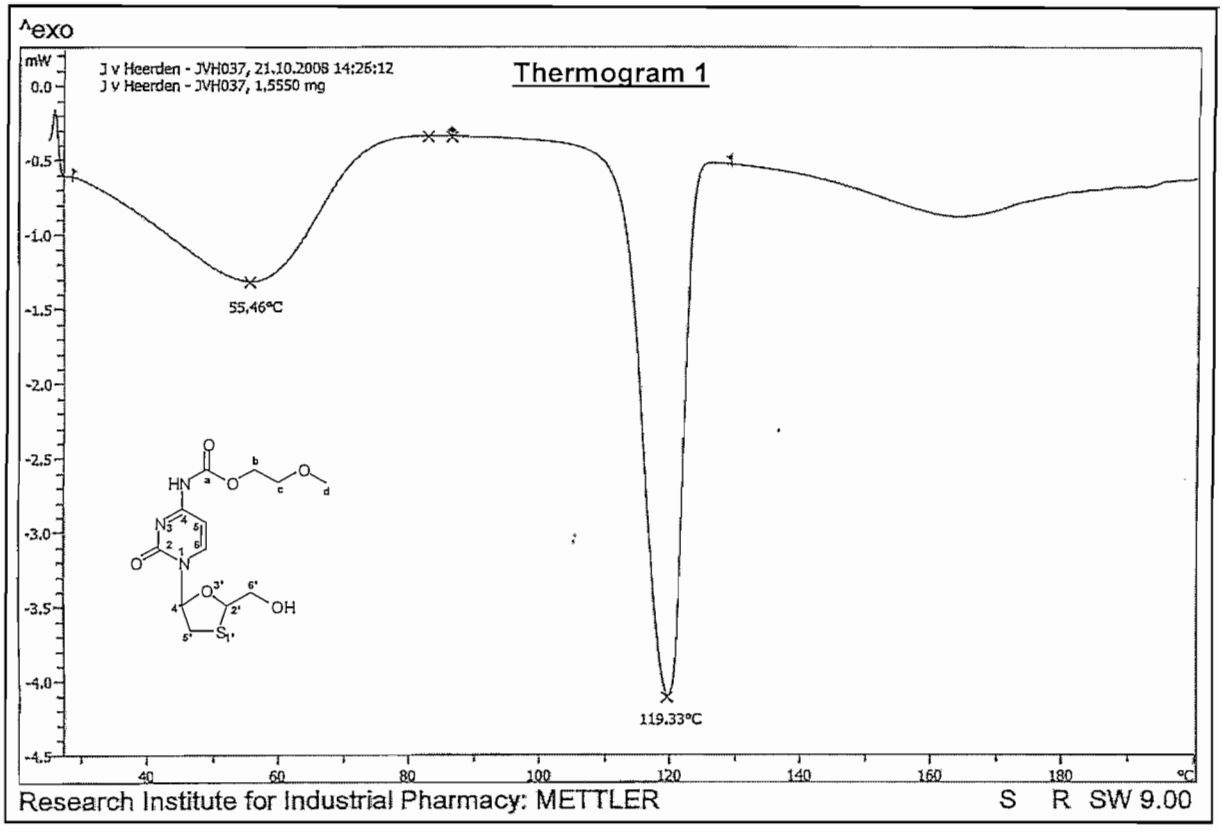
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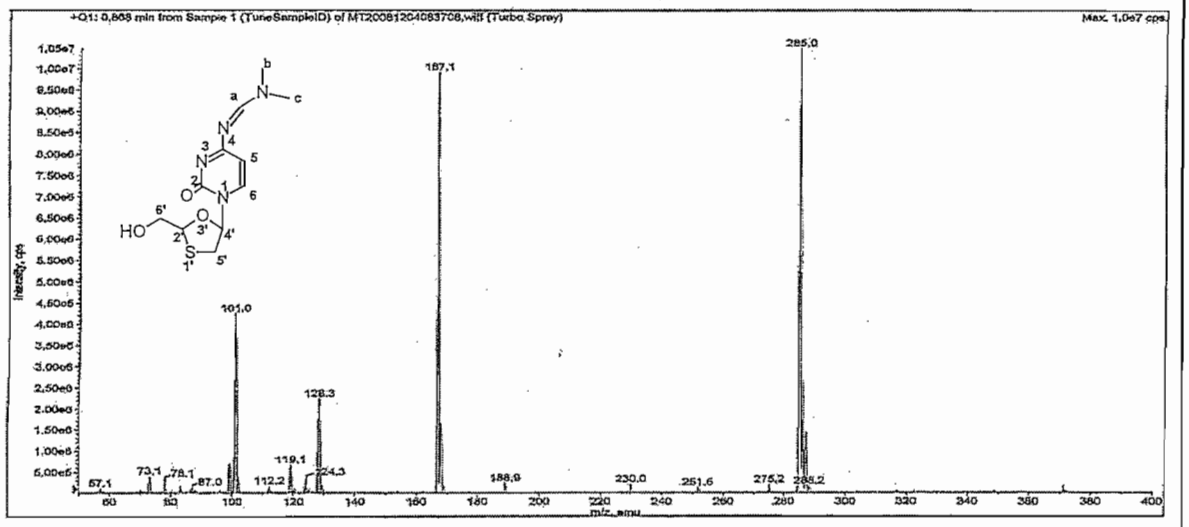

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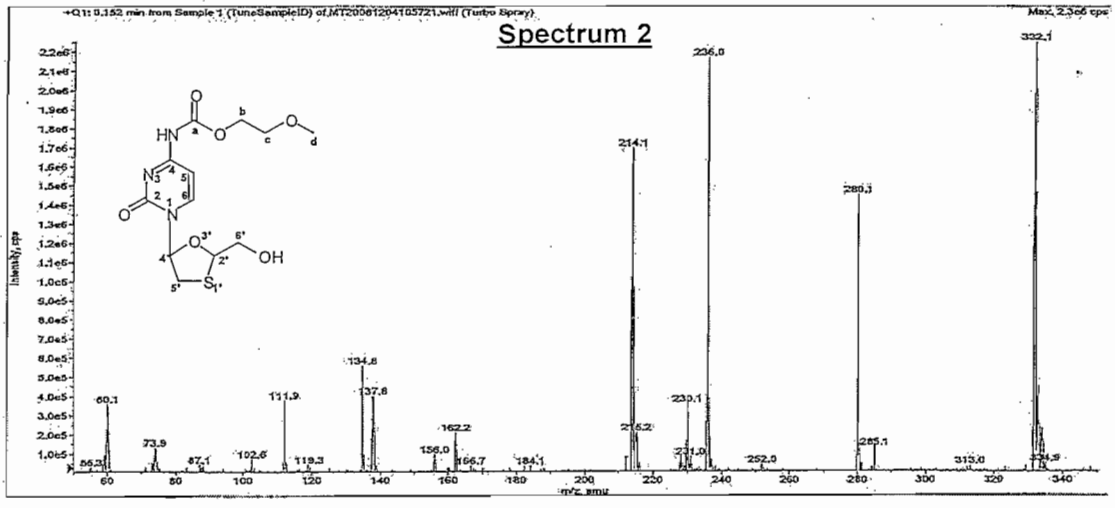

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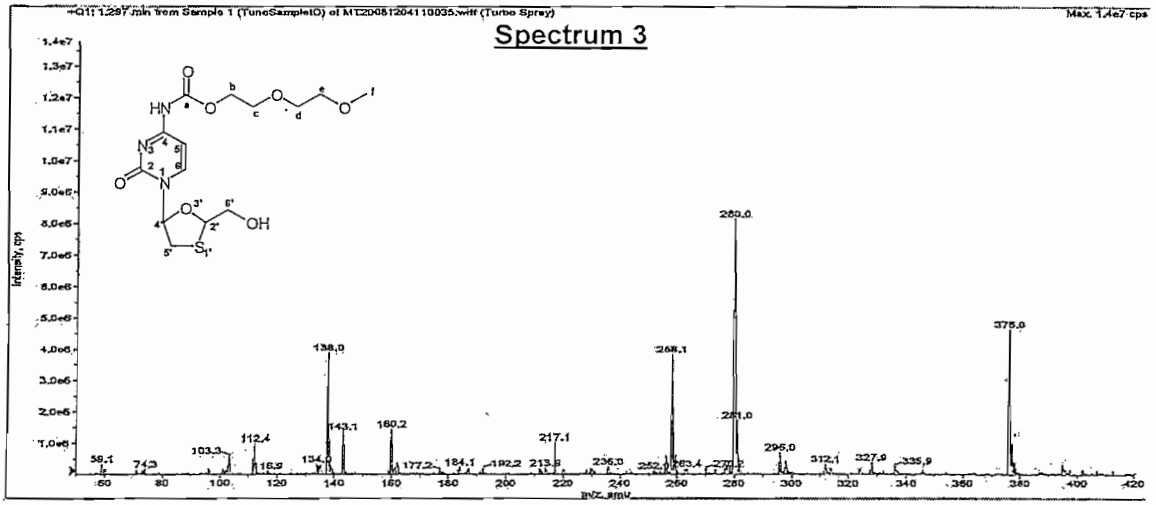

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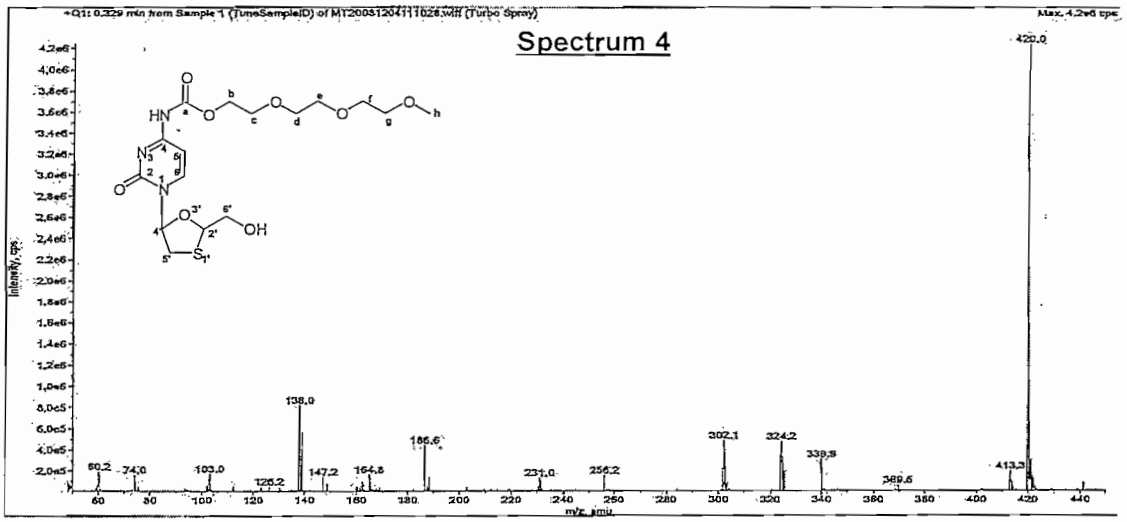

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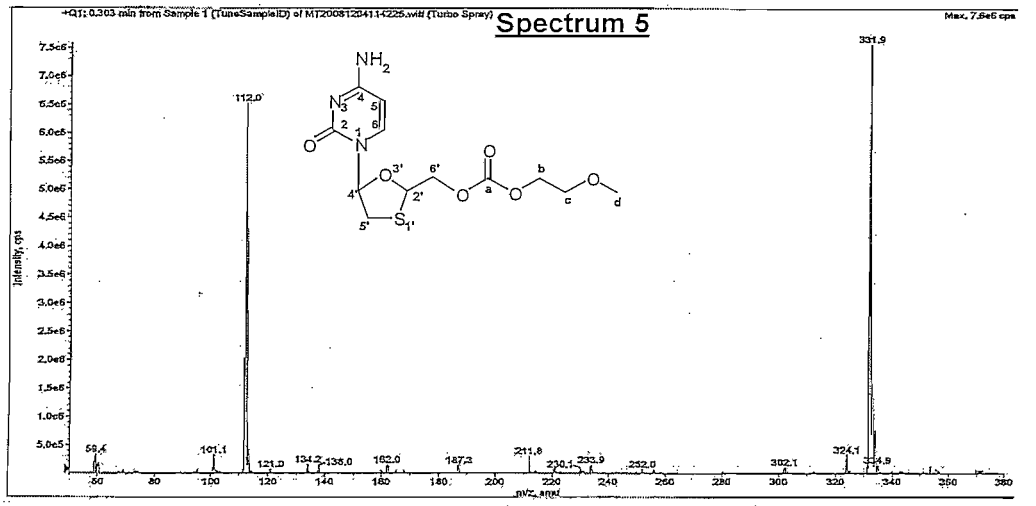

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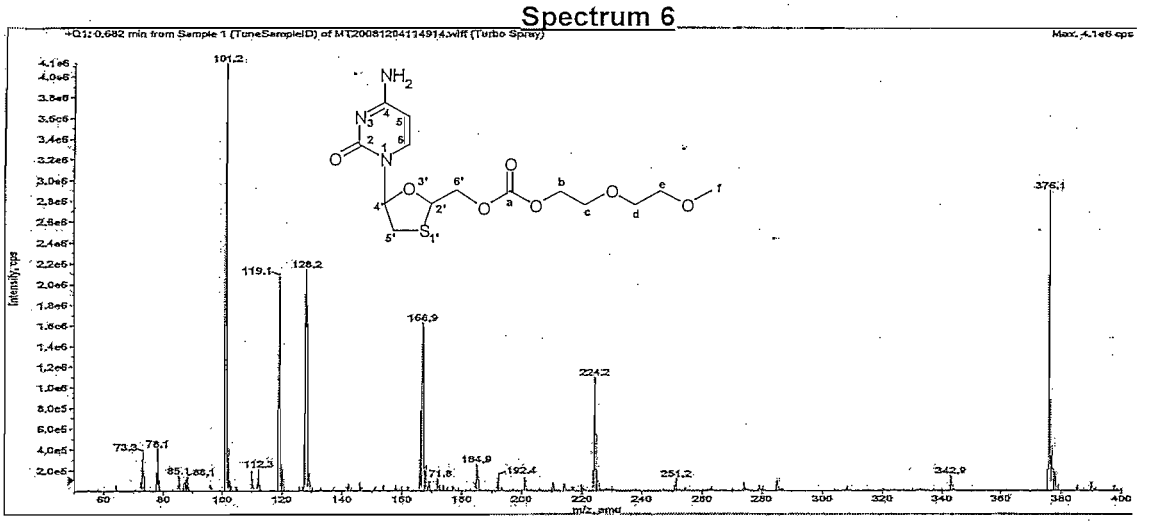

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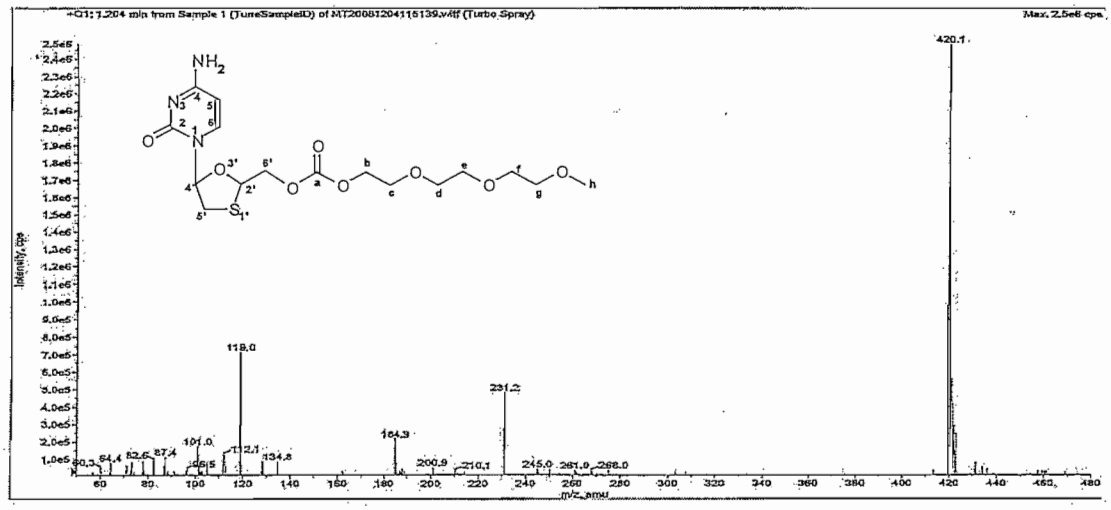
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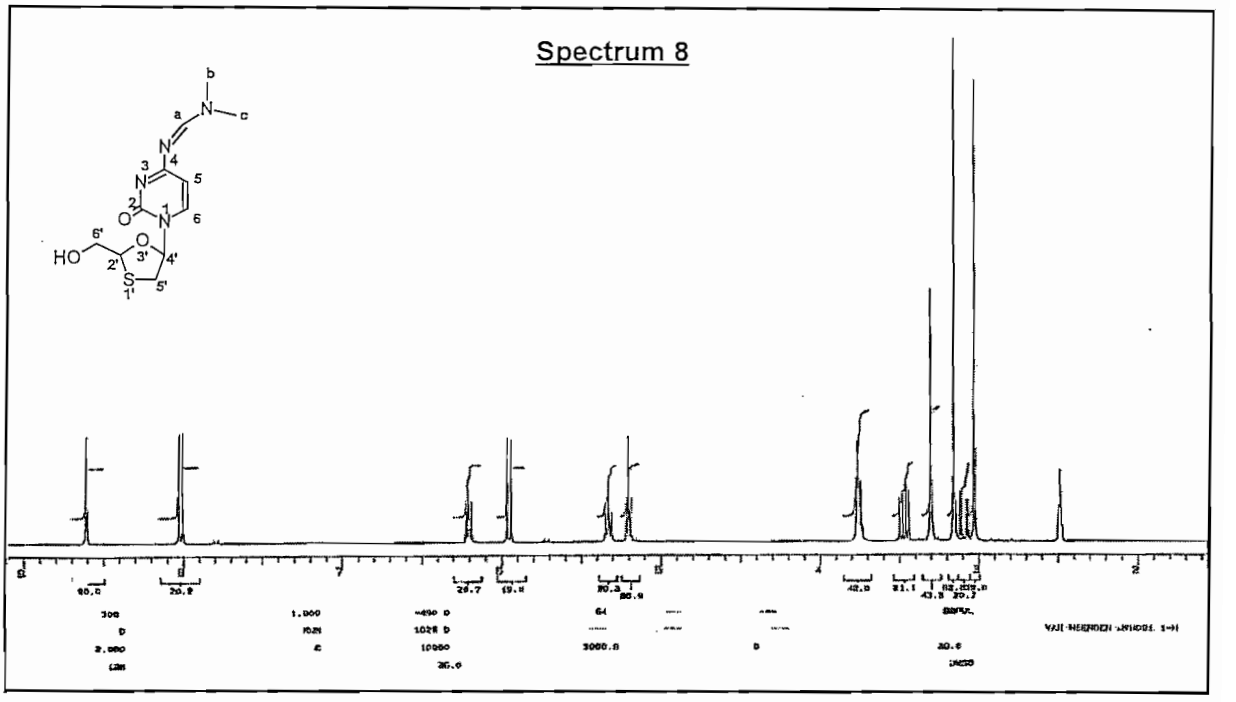
Spectrum 8



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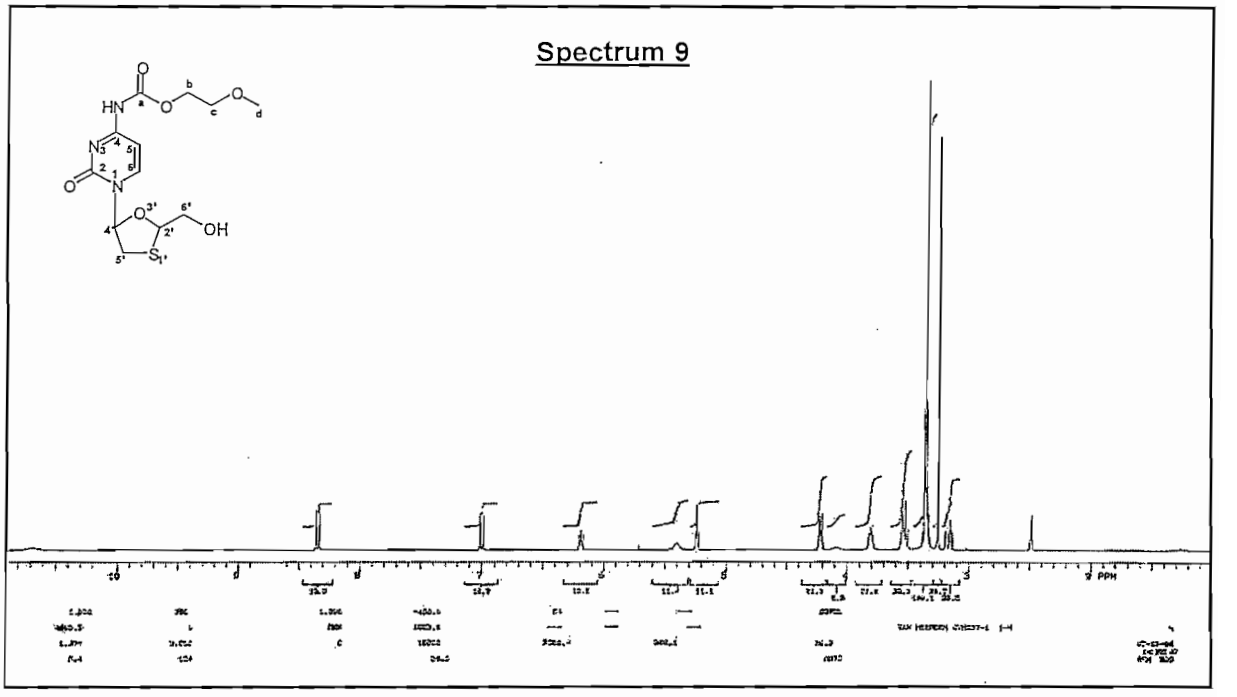

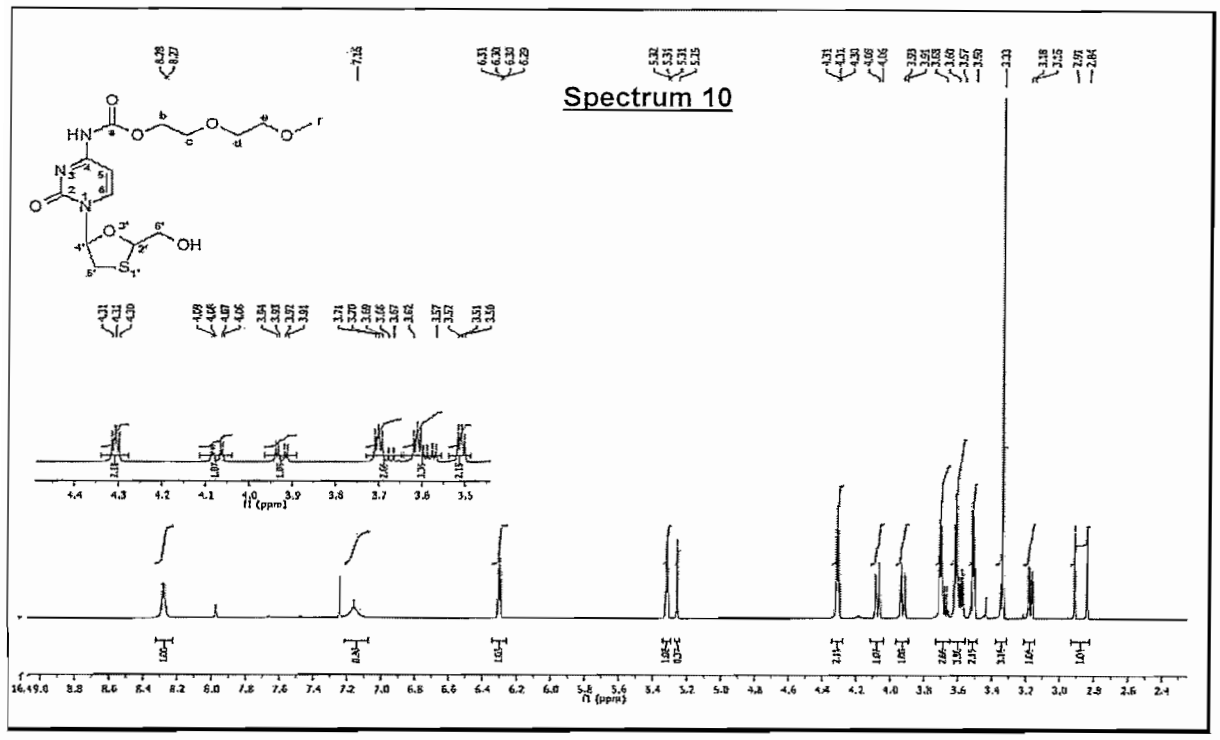
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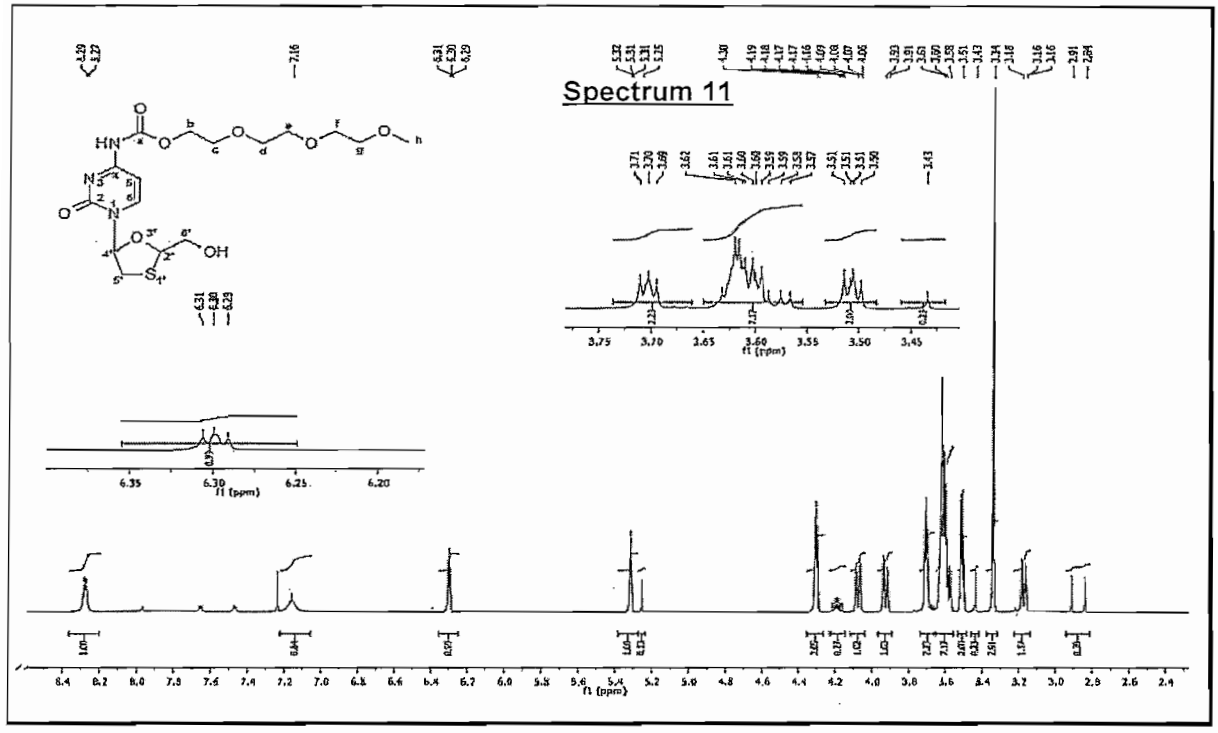
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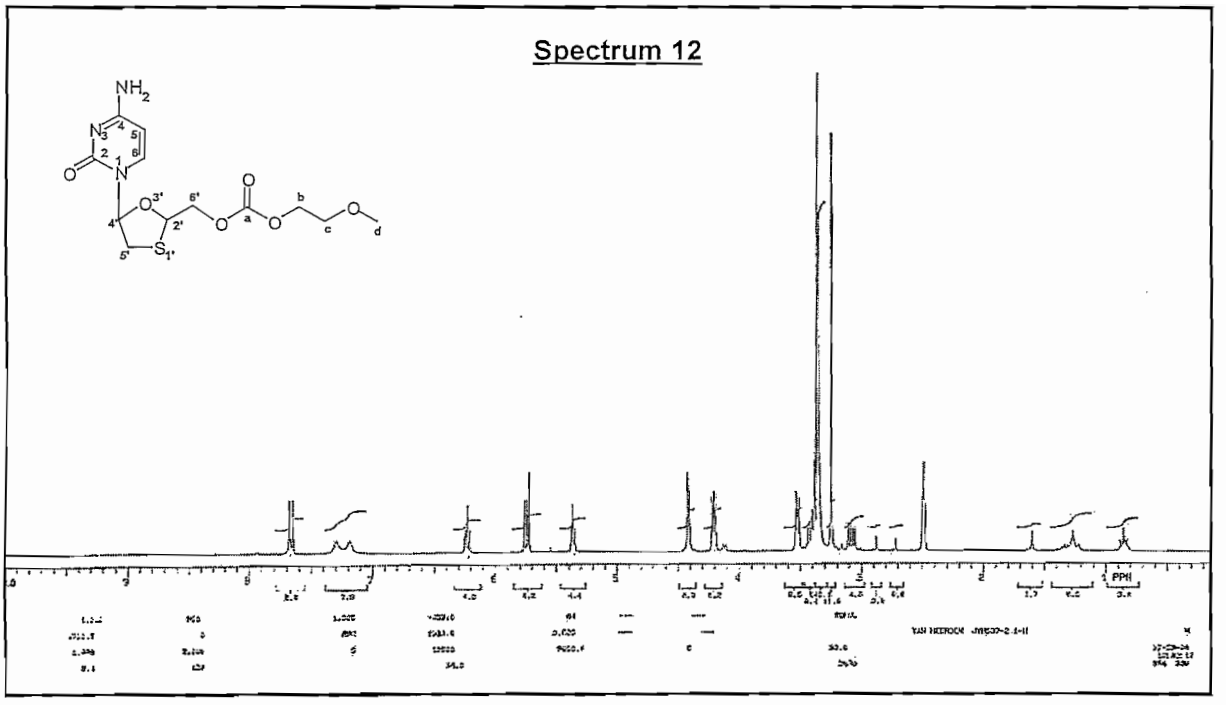

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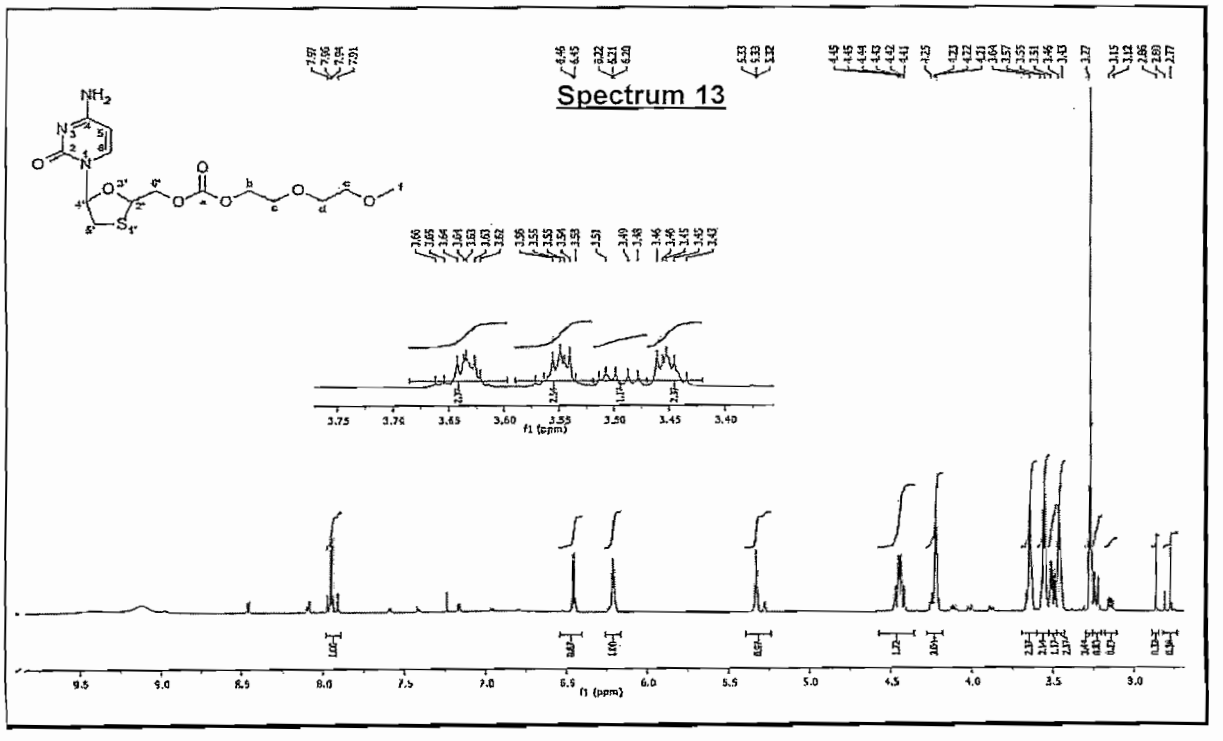
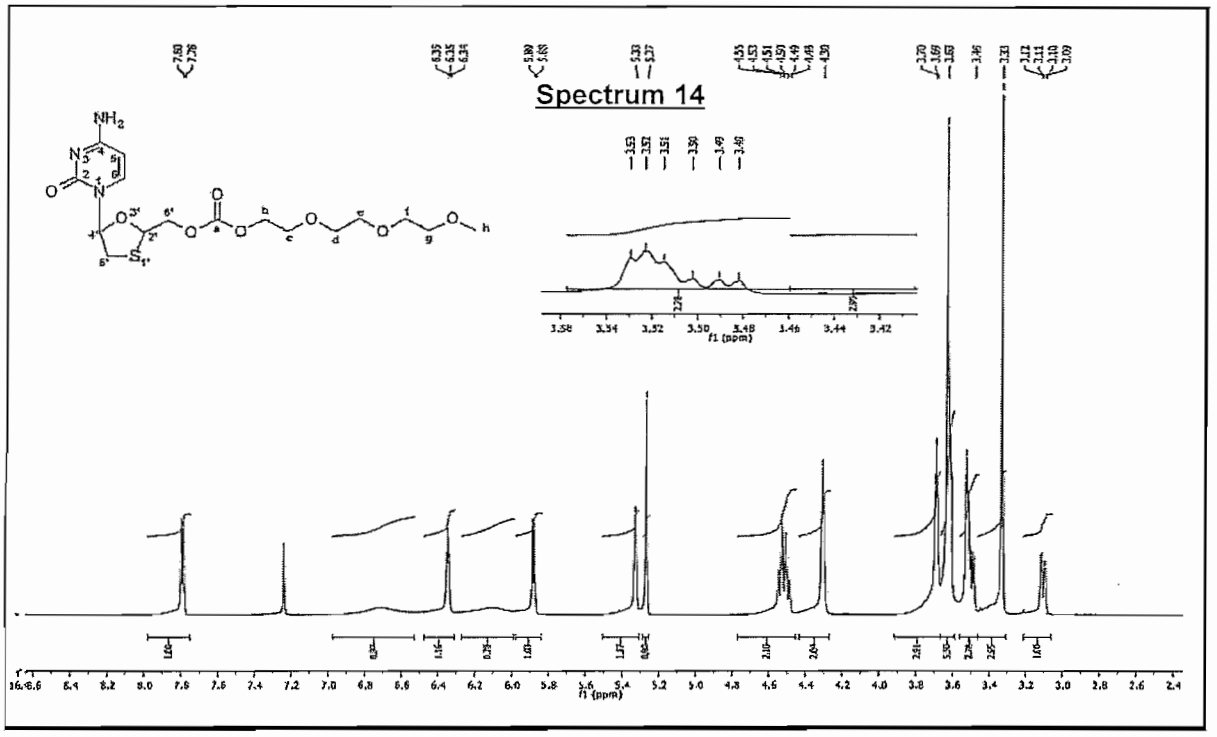
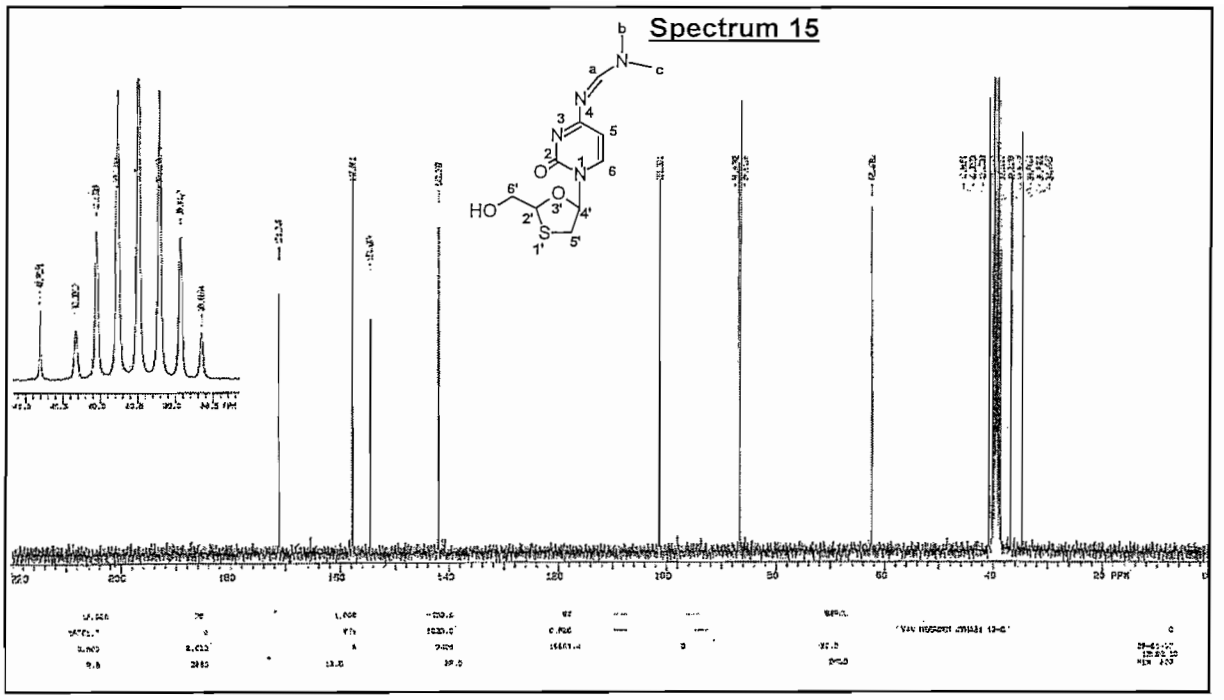



Spectrum 14
f1 (ppm)

Spectrum 15



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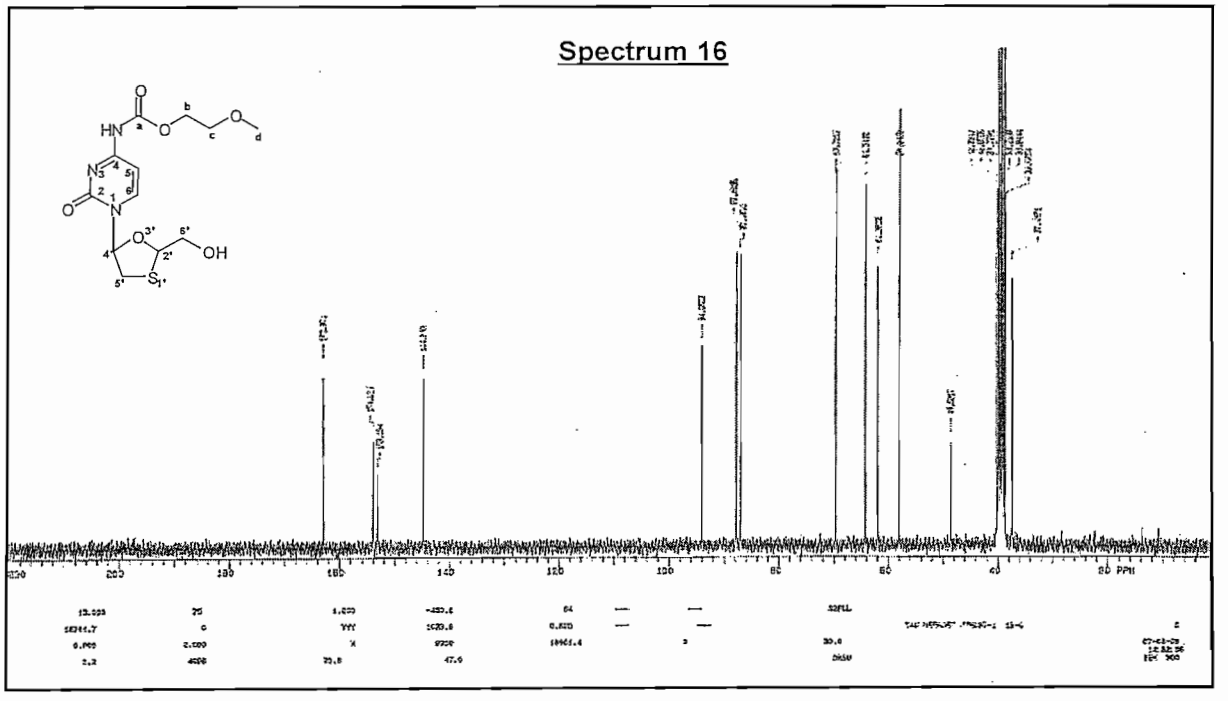

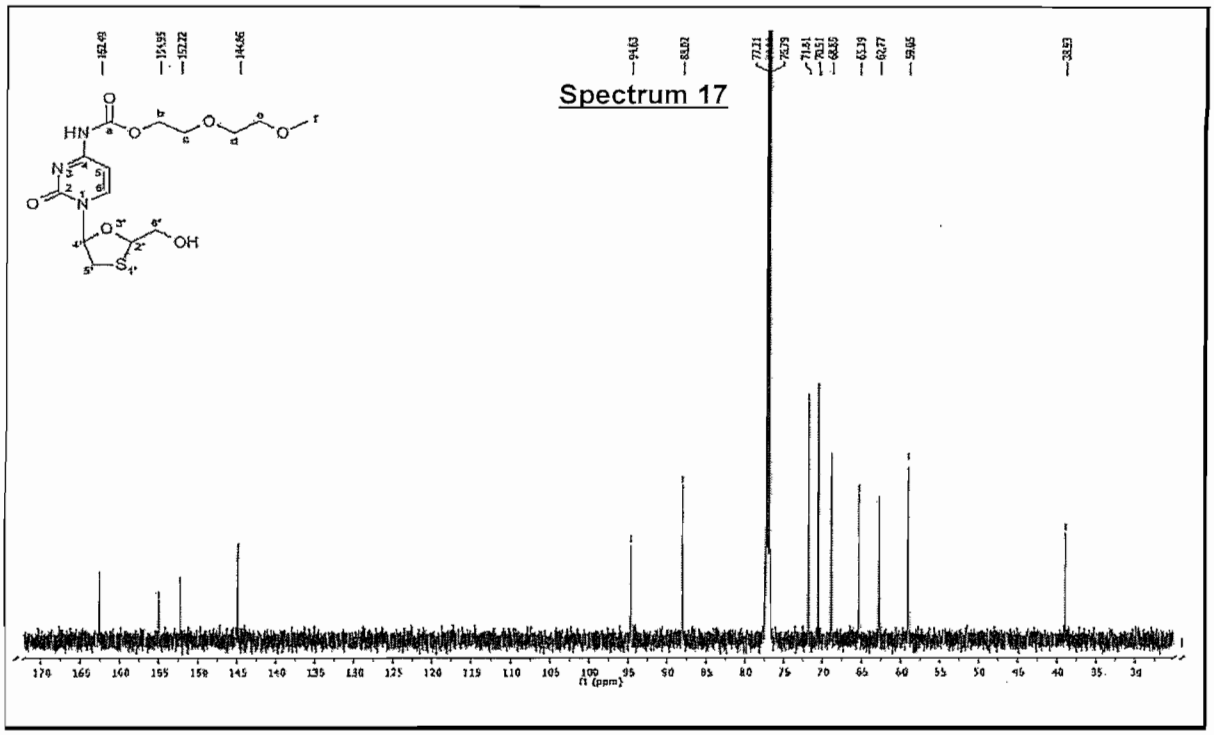
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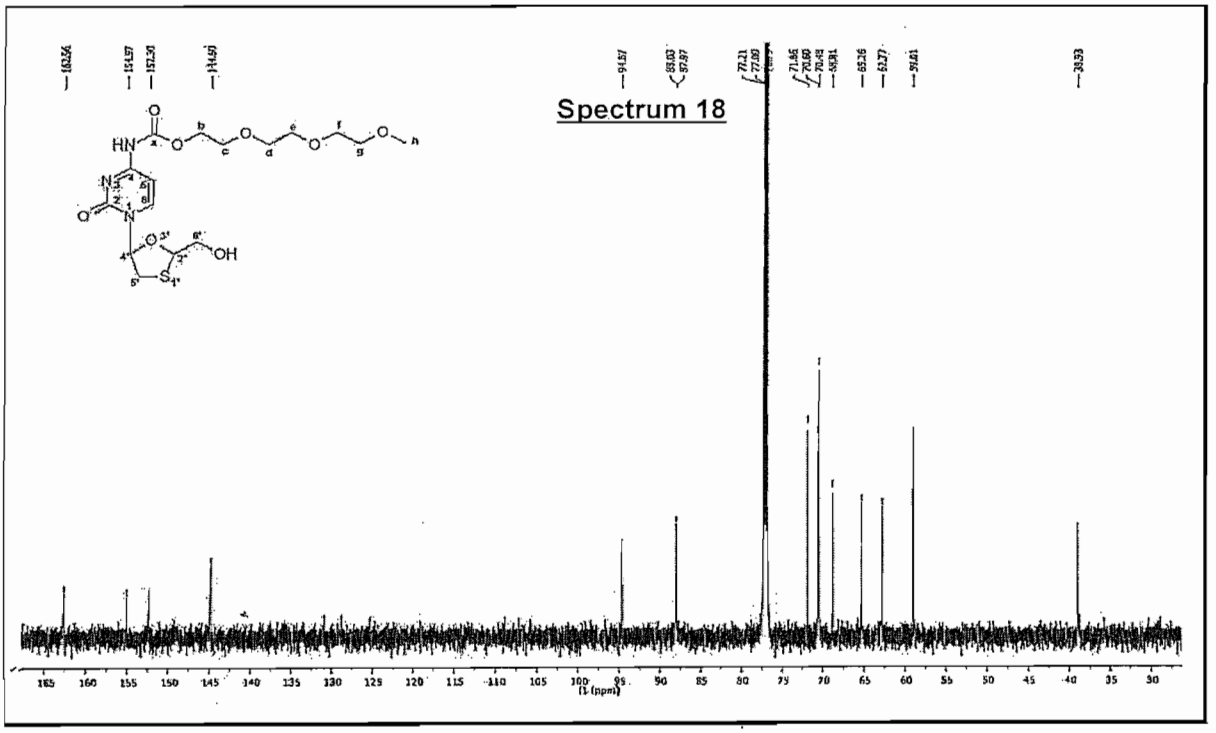
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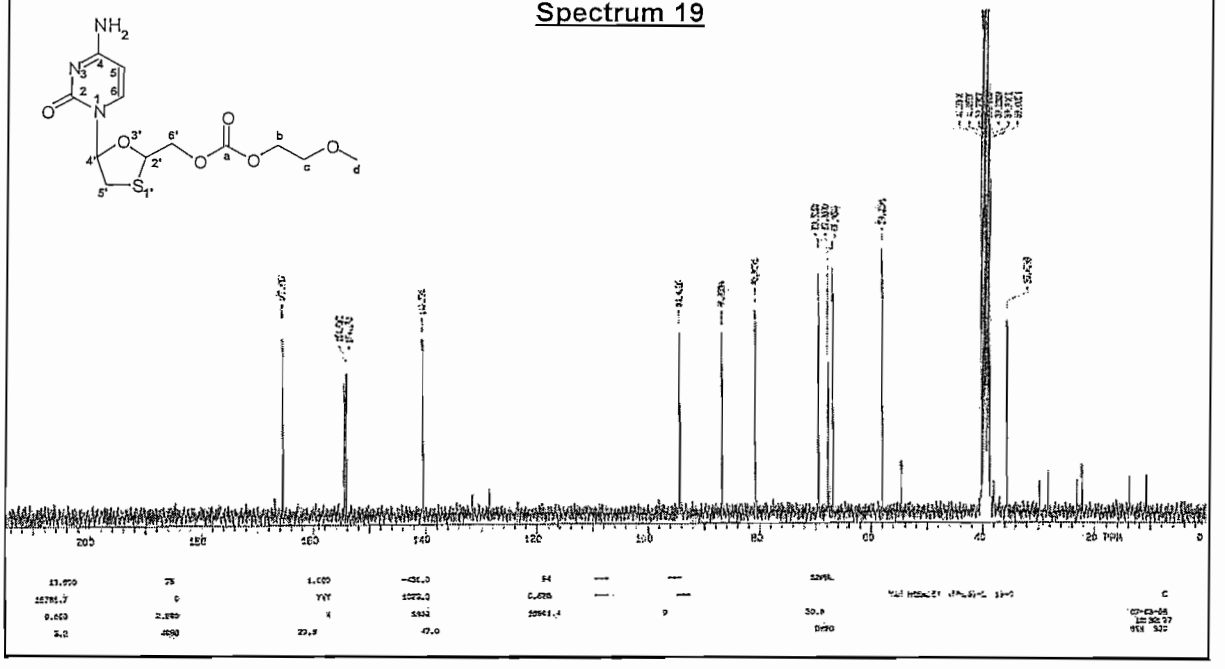

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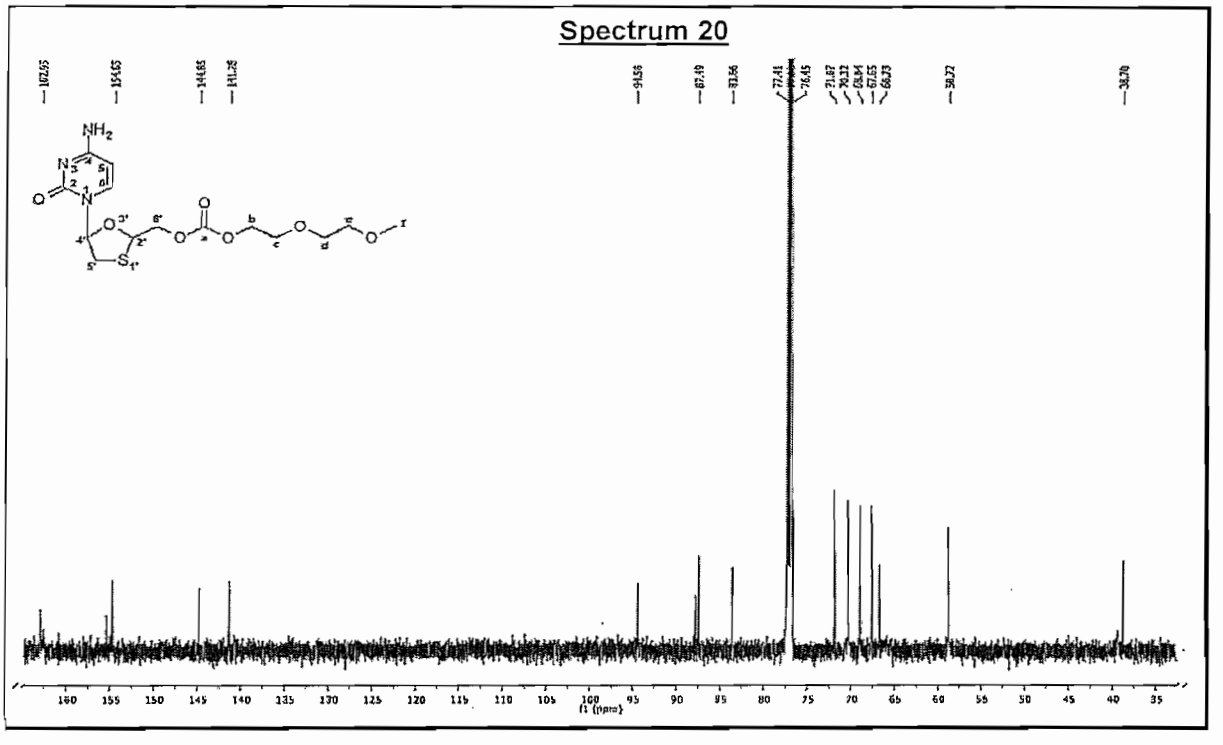

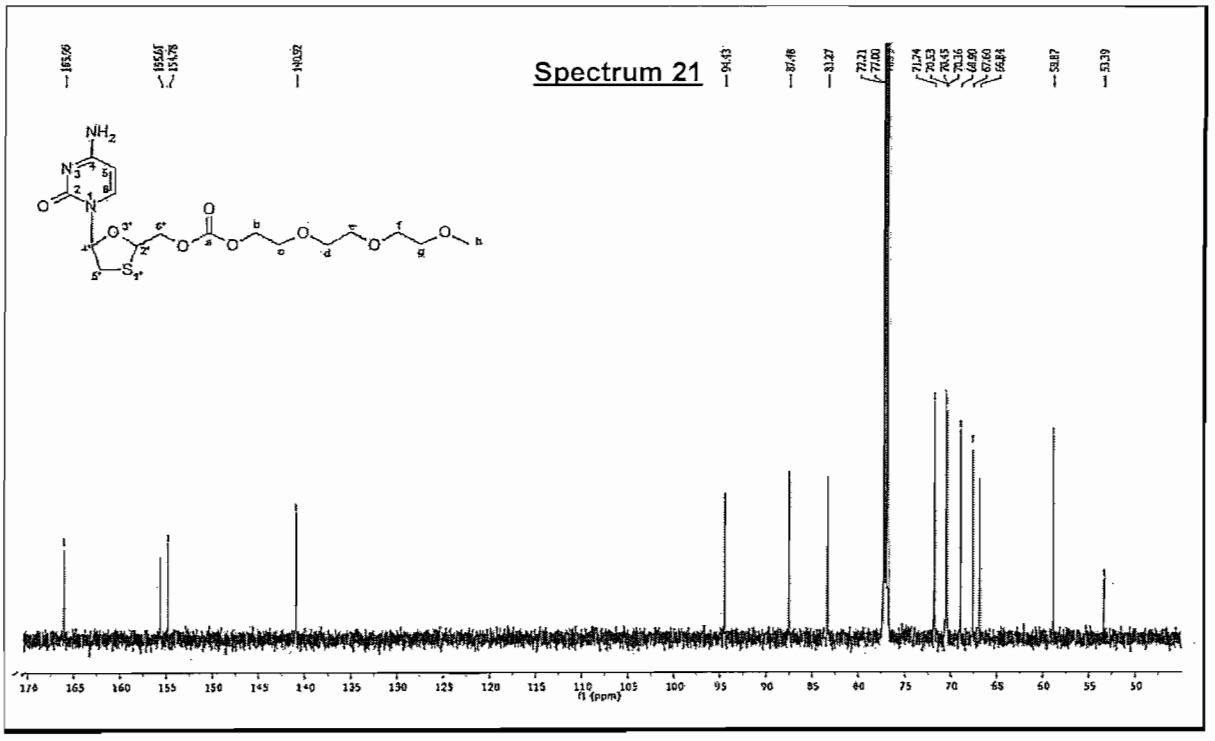
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O
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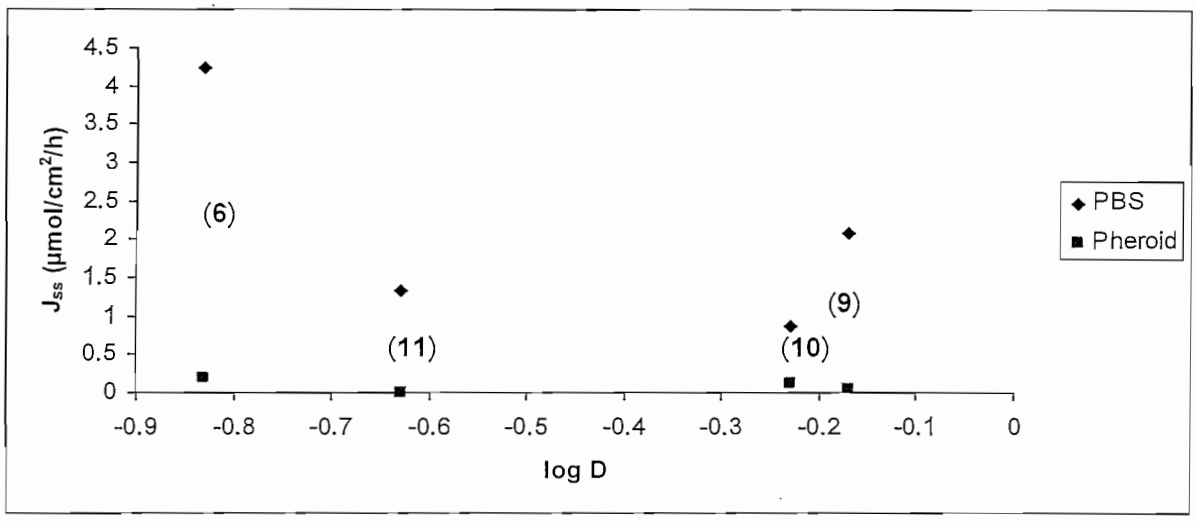

Figure A.1: Steady-state flux (J_{SS}) vs. log P of lamivudine (**6**) and carbamates (**9 – 11**) in PBS and Pheroid™.

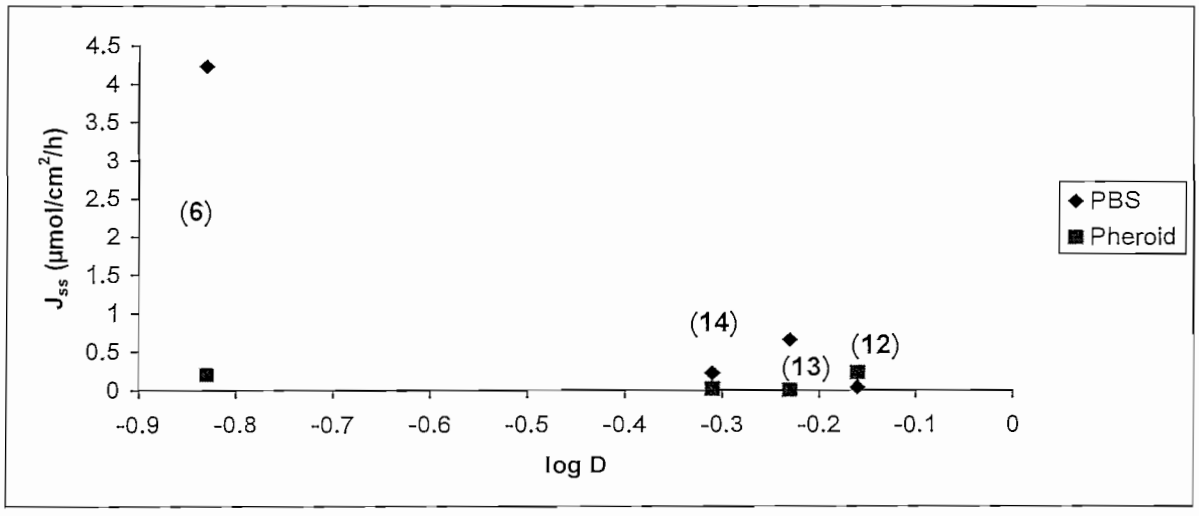

Figure A.2: Steady-state flux (J_{SS}) vs. log P of lamivudine (**6**) and carbonates (**12 – 14**) in PBS and Pheroid™.

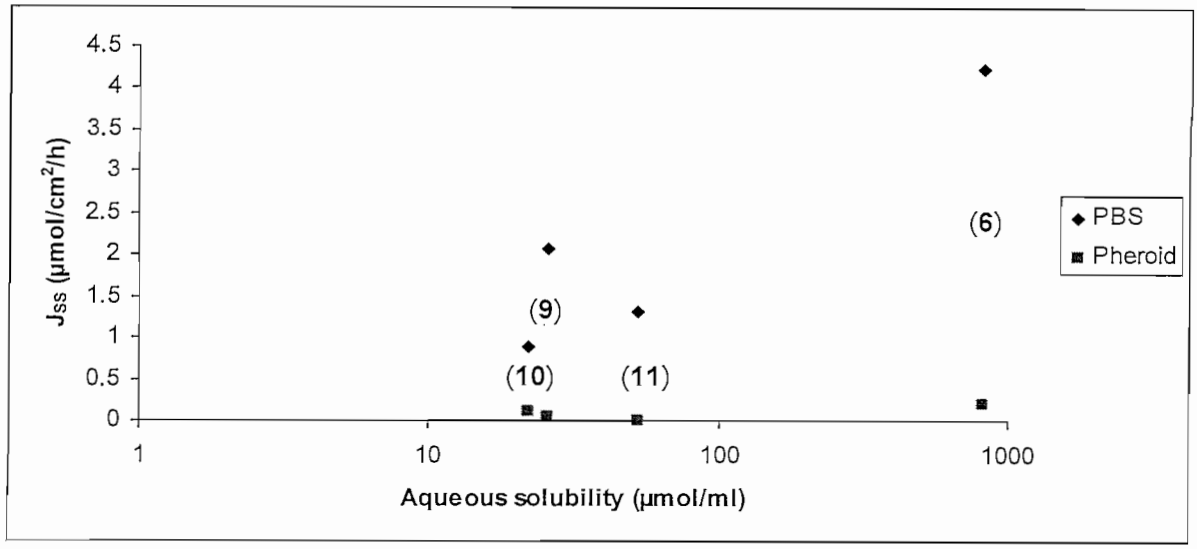

Figure A.3: Steady-state flux (J_{ss}) vs. aqueous solubility of lamivudine (**6**) and carbamates (**9 – 11**) in PBS and Pheroid™.

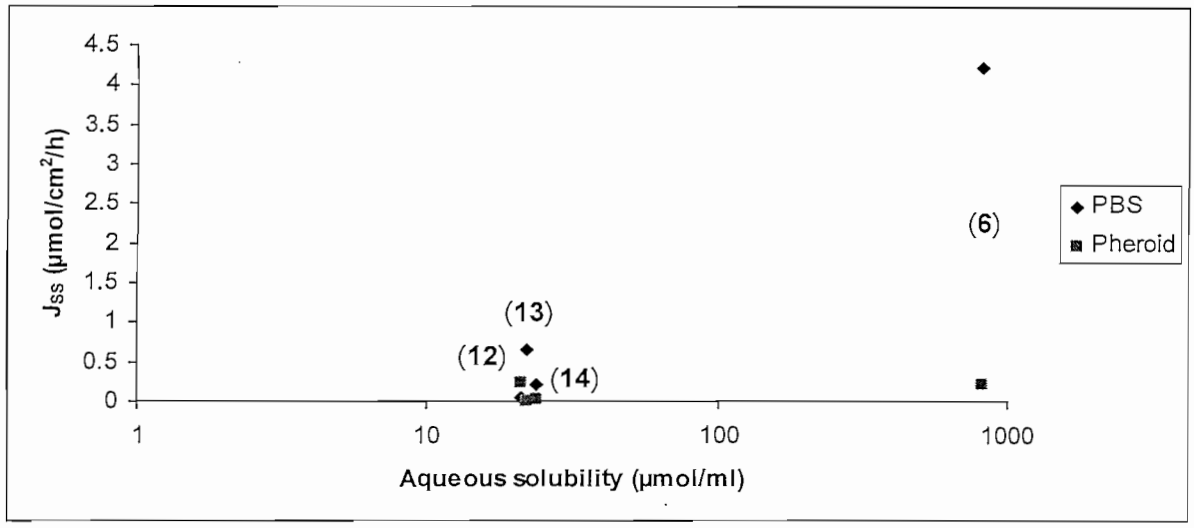

Figure A.4: Steady-state flux (J_{ss}) vs. aqueous solubility of lamivudine (**6**) and carbonates (**12 – 14**) in PBS and Pheroid™.

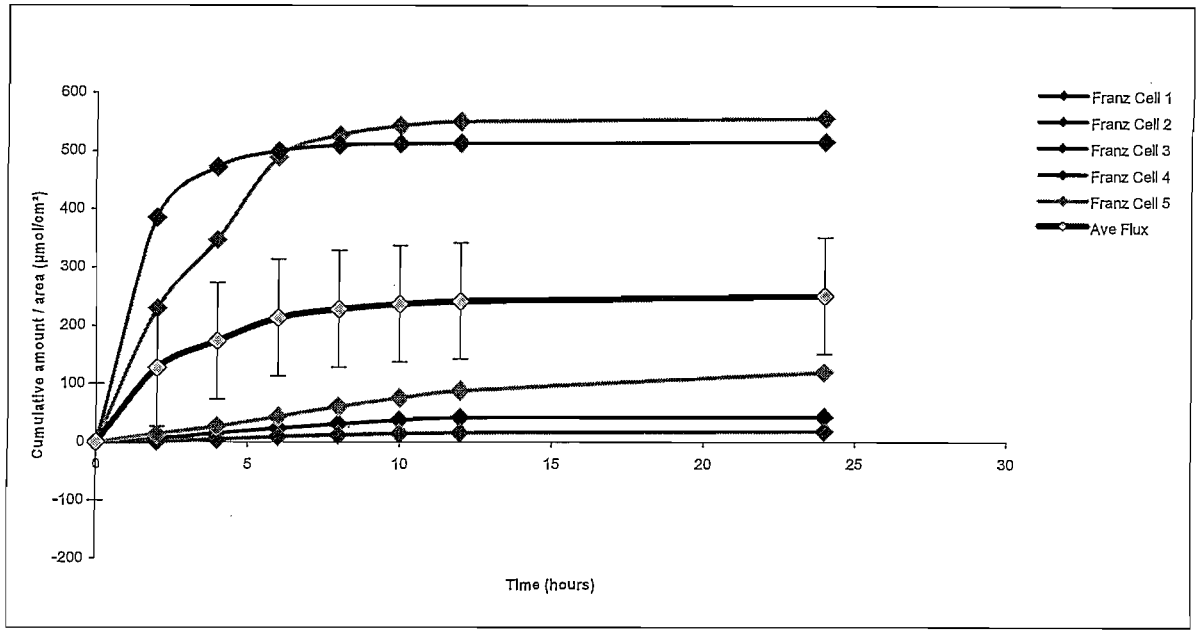

Figure A.5: Experimental cumulative amount of (**6**) per area and average cumulative amount of (**6**) that had penetrated the skin as a function of time (in PBS).

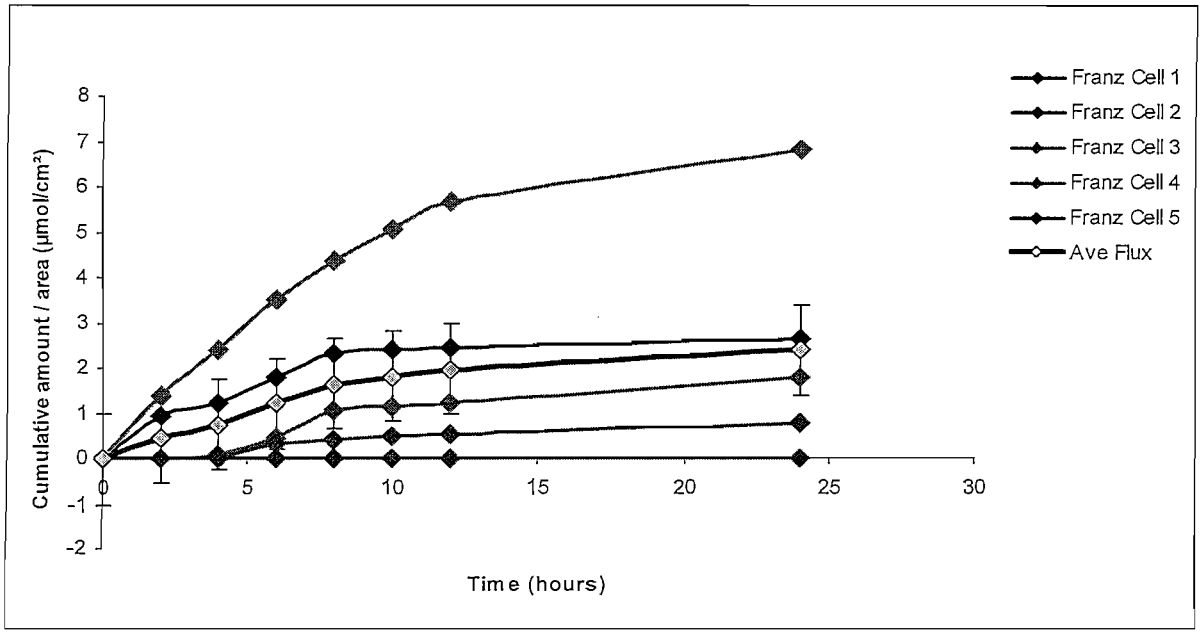

Figure A.6: Experimental cumulative amount of (**6**) per area and average cumulative amount of (**6**) that had penetrated the skin as a function of time (in Pheroid^{TM}).

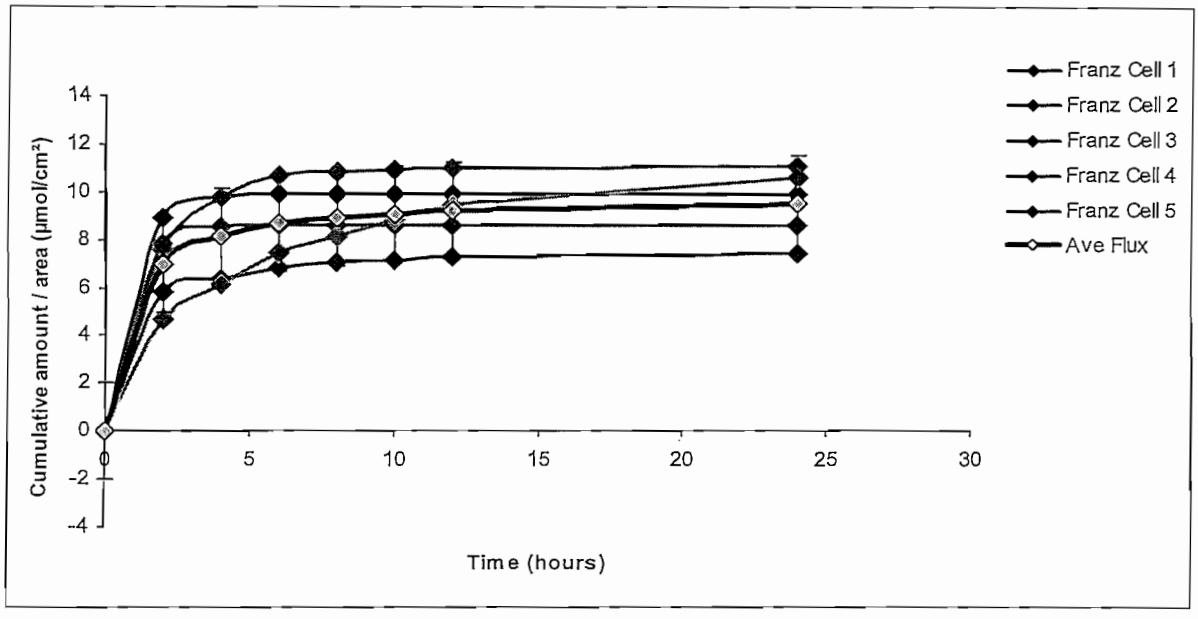

Figure A.7: Experimental cumulative amount of (**9**) per area and average cumulative amount of (**9**) that had penetrated the skin as a function of time (in PBS).

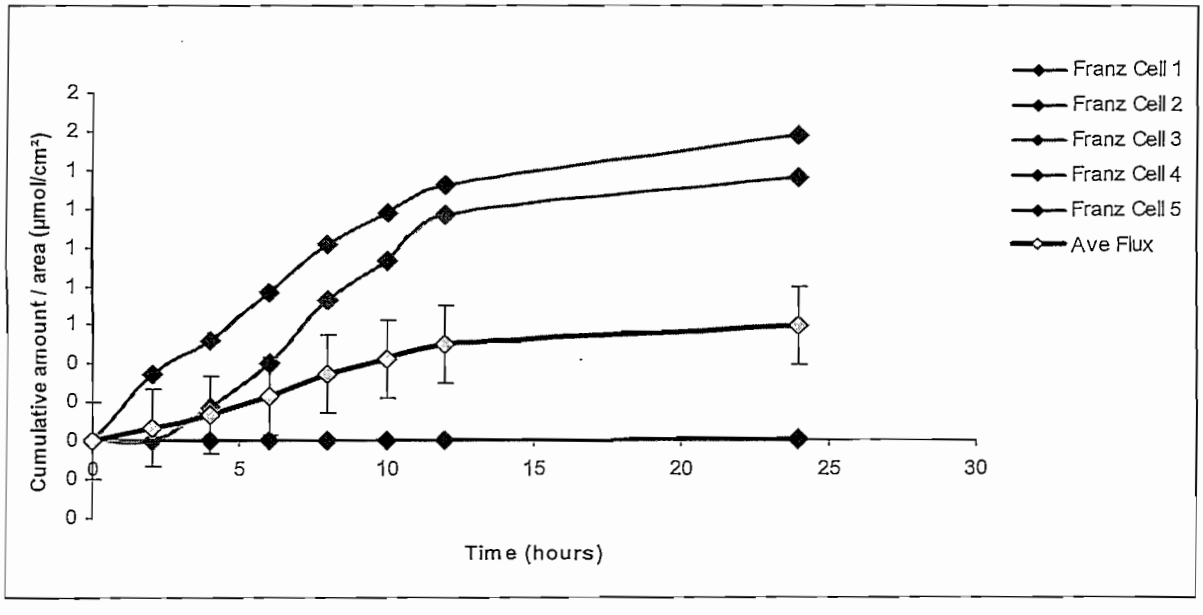

Figure A.8: Experimental cumulative amount of (**9**) per area and average cumulative amount of (**9**) that had penetrated the skin as a function of time (in Pheroid^{TM}).

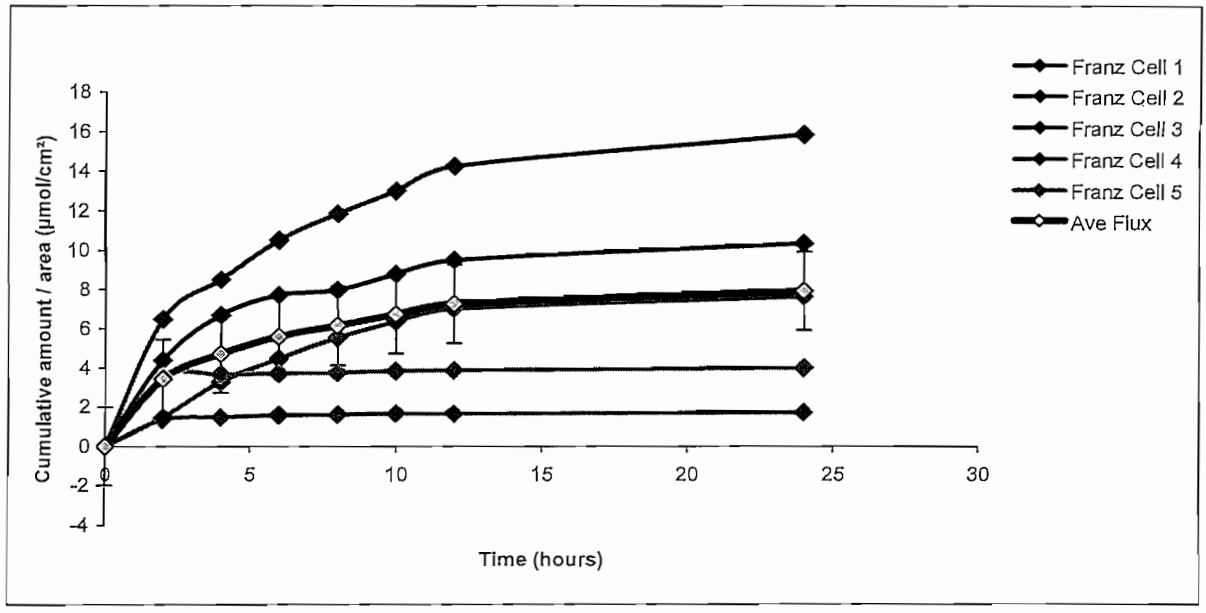

Figure A.9: Experimental cumulative amount of (**10**) per area and average cumulative amount of (**10**) that had penetrated the skin as a function of time (in PBS).

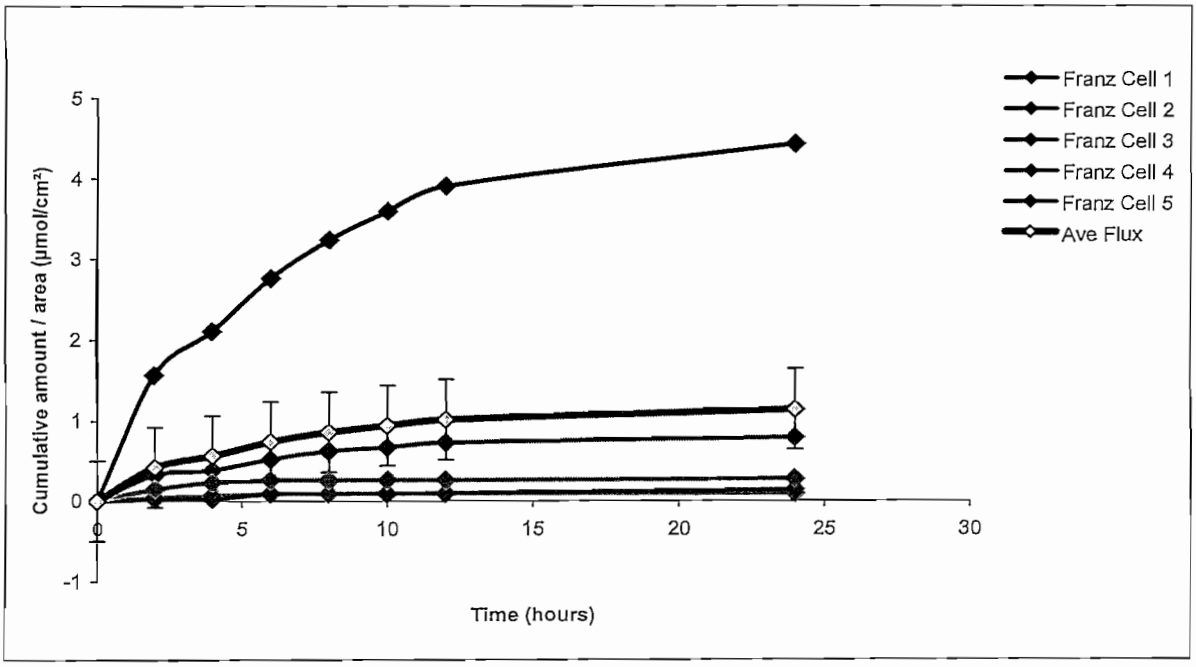

Figure A.10: Experimental cumulative amount of (**10**) per area and average cumulative amount of (**10**) that had penetrated the skin as a function of time (in Pheroid^{TM}).

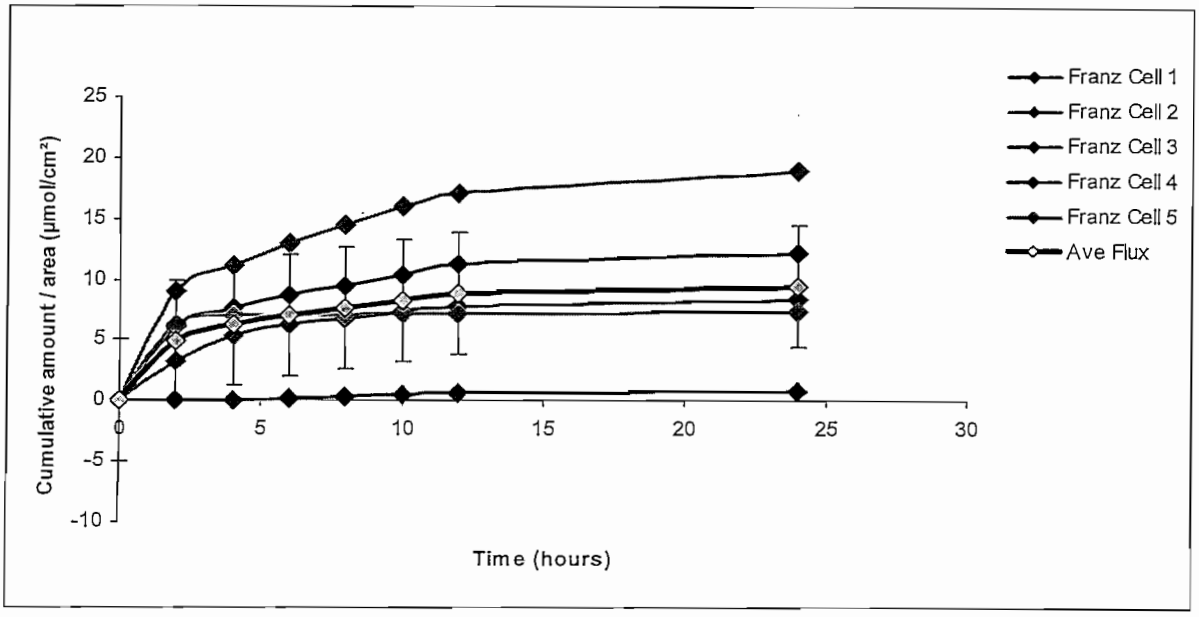

Figure A.11: Experimental cumulative amount of (**11**) per area and average cumulative amount of (**11**) that had penetrated the skin as a function of time (in PBS).

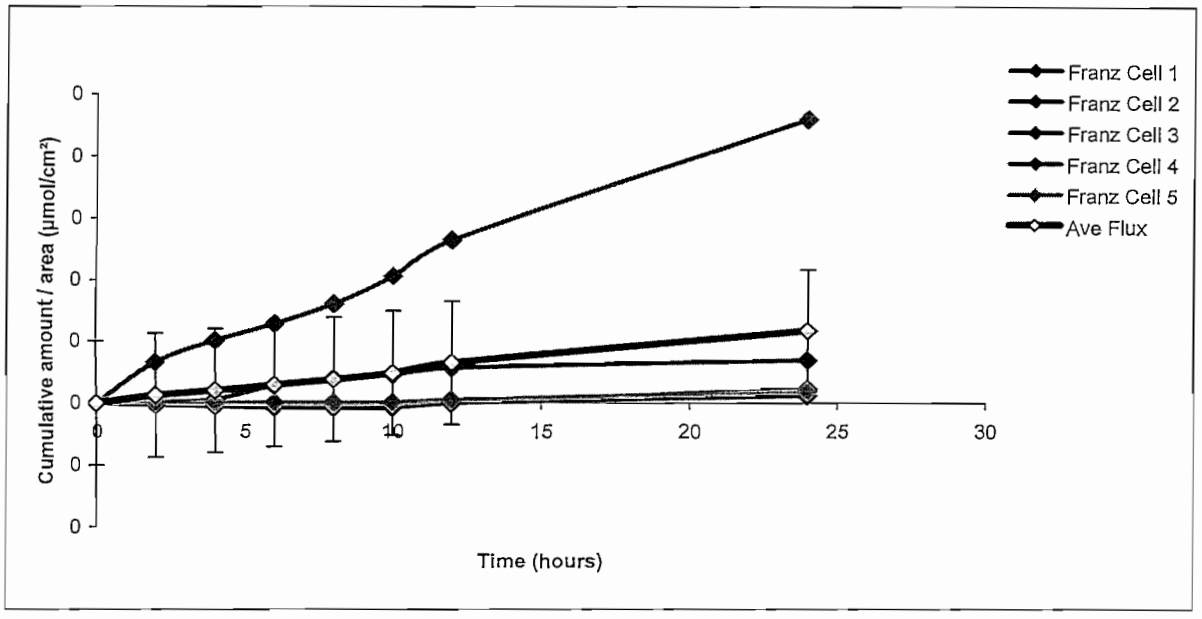

Figure A.12: Experimental cumulative amount of (**11**) per area and average cumulative amount of (**11**) that had penetrated the skin as a function of time (in Pheroid^{TM}).

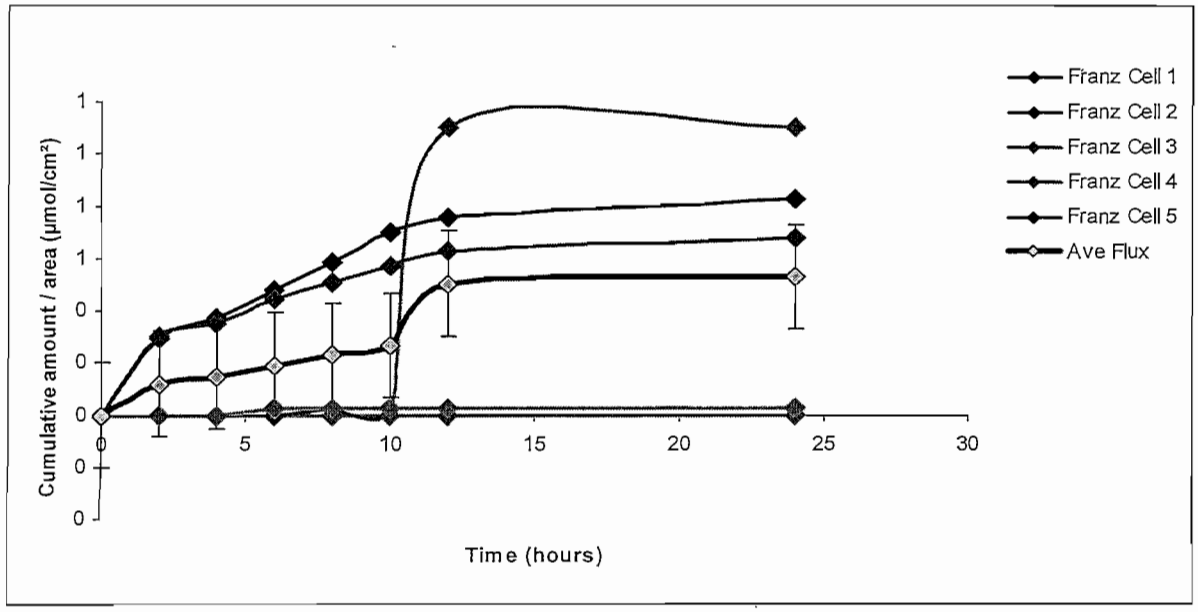

Figure A.13: Experimental cumulative amount of (**12**) per area and average cumulative amount of (**12**) that had penetrated the skin as a function of time (in PBS).

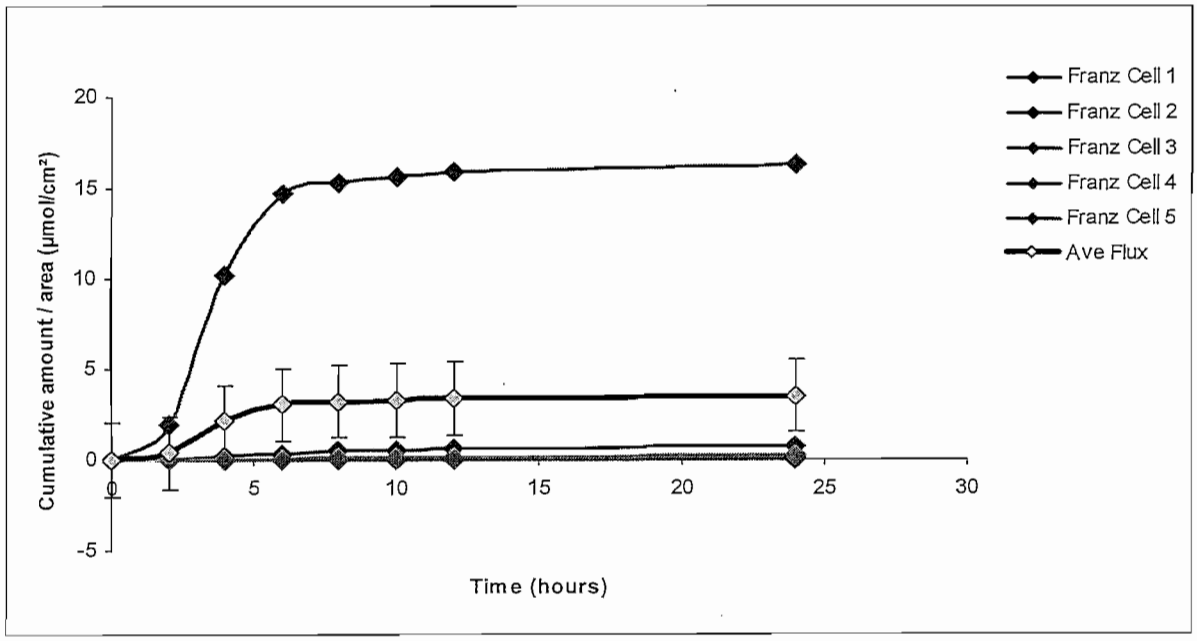

Figure A.14: Experimental cumulative amount of (**12**) per area and average cumulative amount of (**12**) that had penetrated the skin as a function of time (in Pheroid^{TM}).

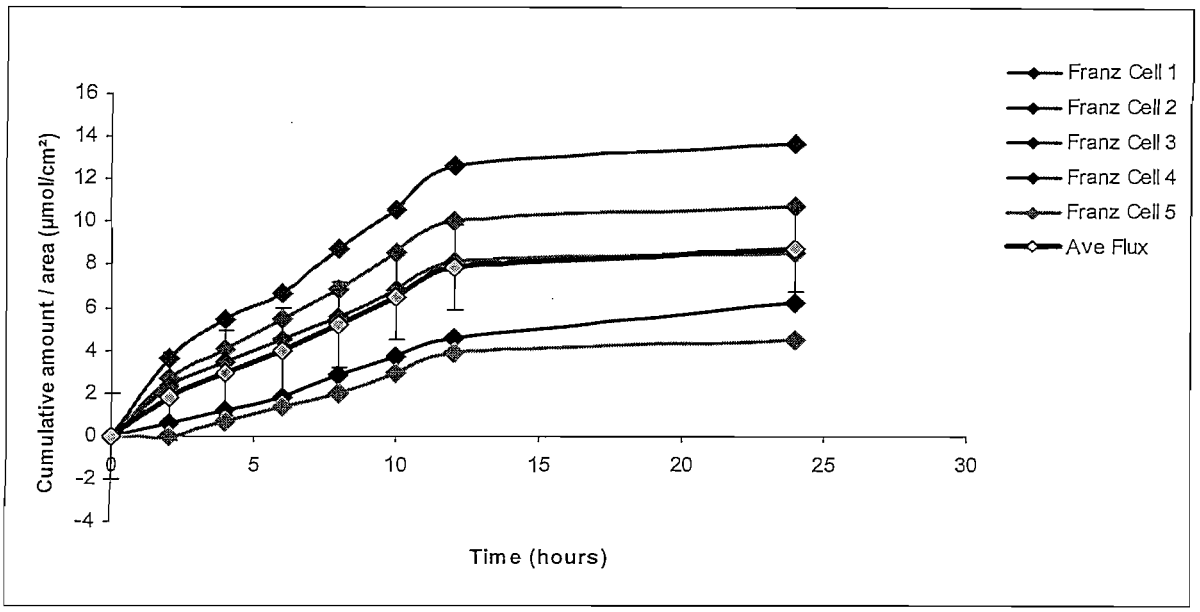

Figure A.15: Experimental cumulative amount of (**13**) per area and average cumulative amount of (**13**) that had penetrated the skin as a function of time (in PBS).

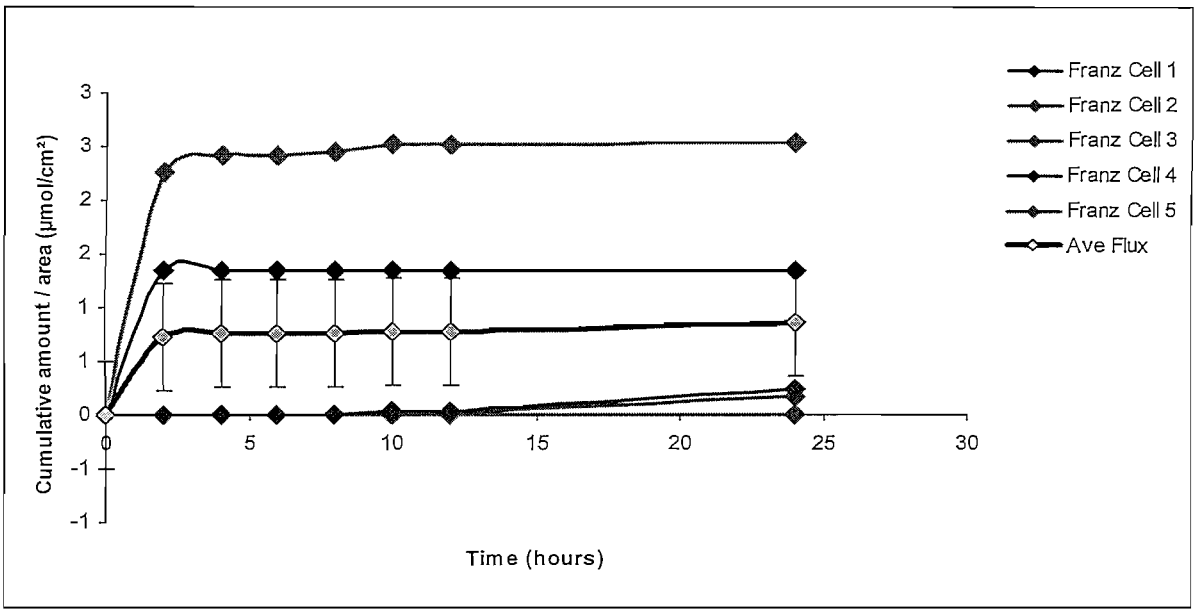

Figure A.16: Experimental cumulative amount of (**14**) per area and average cumulative amount of (**14**) that had penetrated the skin as a function of time (in PBS).

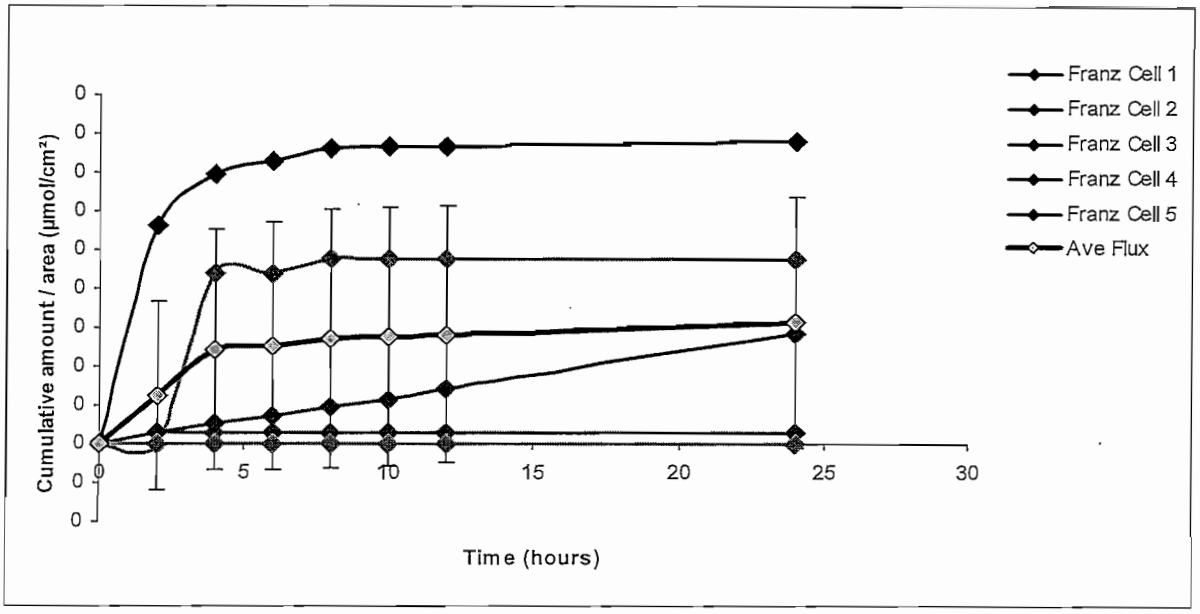

Figure A.17: Experimental cumulative amount of (**14**) per area and average cumulative amount of (**14**) that had penetrated the skin as a function of time (in Pheroid^{TM}).