The Synthesis and Transdermal Delivery of Stavudine Derivatives

Estée-Marie Holmes

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Supervisor: Prof. J.C. Breytenbach

Co-supervisor: Prof. J. du Plessis

Supportive supervisor: M. Gerber

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Abstract

The skin is an amazingly flexible and relatively impermeable barrier that provides protective, perceptive and communication functions to the human body. The interest in transdermal drug delivery may be attributed to the advantages associated with this method of drug delivery. Some of these advantages include more consistent drug levels, the avoidance of hepatic first pass metabolism, and the accommodation of patients who cannot tolerate oral dosage forms. Transdermal therapy, however, is not without limitations. The outermost layer of the skin, the stratum corneum, is the main barrier for penetration of most drugs through the skin and is very selective with respect to the type of molecule that can be transported across this outer covering. This is because the skin serves a protective function, inhibiting compounds from crossing it. Only drugs with the appropriate physicochemical properties are enabled to cross this barrier. Many drugs that possess a hydrophilic structure penetrate too slowly to be of therapeutic benefit. However, drugs with a lipophilic character are better suited for transdermal delivery but compounds with both lipid and water solubilities penetrate much better than substances with either high water or high lipid solubility.

Stavudine (2',3'-didehydro-2',3'-dideoxythymidine, d4T), a synthetic antiretroviral agent, is an inhibitor of nucleoside reverse transcriptase and is used against HIV-1 and HIV-2. It is required to be in combination with other antiretroviral agents and is indicated for the management of mainly HIV-1 infection in adults and paediatric patients. Another important use for stavudine is for post exposure prophylaxis (PEP) that can help decrease the risk of infection after exposure to HIV. Adverse effects, related to stavudine therapy, are mostly due to mitochondrial toxicity resulting from the inhibition of human DNA polymerase gamma. The major adverse effect, peripheral neuropathy, is dependant on the dosage and the duration of the treatment.

Pheroids is a patented system comprising of a unique submicron emulsion type formulation and is capable of encapsulating a variety of drugs and delivering these drugs with high efficacy to target sites in the body. The Pheroid consists primarily of plant and essential fatty acids and is stable within a novel therapeutic system. They are manipulated in a special manner to ensure important advantages over other delivery systems such as high entrapment capabilities, fast rate of transport, delivery and stability.

The primary aim of this study was to synthesise a series of new derivatives of the anti-HIV drug stavudine, and to study the effects of the different substituents on transdermal penetration with and without the use of Pheroids as delivery system. Furthermore, it was to be established if any correlation between the transdermal penetration and selected physicochemical properties of the penetrants existed.

The six derivatives of stavudine were prepared by esterification of stavudine with six different acid chlorides at room temperature. The structures of the products were confirmed by nuclear magnetic resonance spectroscopy (NMR), mass spectroscopy (MS) and infrared spectroscopy (IR). The experimental aqueous solubility and partition coefficients were determined for stavudine and its different derivatives at a pH of 7,0. Interactive analysis (IA) prediction software was used to predict aqueous solubility values while IA, K_{ow}Win and ACD Labs prediction software were used to predict the log P values for each derivative. ACD Labs prediction software gave values relatively close to the experimental partition coefficients that were measured at pH 7,0.

The experimental aqueous solubility, partition coefficient and transdermal flux values were determined for stavudine and each of the derivatives. The experimental aqueous solubility of stavudine (104,75 mg/ml) was much higher than that of the synthesised derivatives, and the partition coefficient of stavudine (-0,846) was lower than that of its derivatives. As could be expected, a direct correlation exists between the aqueous solubility data and the partition coefficients. Stavudine-5'-decanoate had a log P value of approximately 3, but had no flux. This can be ascribed for this specific derivative being insoluble in water. This just proves once again that for a compound to cross the stratum corneum, it should possess both hydrophilic and lipohillic properties.

The aqueous solubility, molecular mass and log D values showed an excellent correlation with the flux values of the compounds in PBS, while no correlation existed between the melting point and the integrity (either before or after) with the flux. The data showed no correlation between the flux values of the compounds in Pheroids for any of the determined physicochemical properties. The experimental transdermal flux of stavudine (1,46 $x10^{-2}$ $\mu g/cm^2.h$) in PBS was much higher than that of its derivatives, while the propionyl (1,86 $x10^{-2}$ $\mu g/cm^2.h$) and the buteryl derivative (2,02 $x10^{2}$ $\mu g/cm^2.h$) were the compounds with the highest transdermal flux in Pheroids. The flux of stavudine improved from 1,46 $x10^{-2}$ $\mu g/cm^2.h$ to 2,02 $x10^{-2}$ $\mu g/cm^2.h$ by synthesising the buteryl

derivative and using Pheroids as delivery system which constitutes a 38% enhancement in flux.

This study has confirmed that transdermal flux is dependent on several factors such as the aqueous solubility of the drug, the partition coefficient, molecular size, melting point and the alkyl chain length, to name a few, and in some instances minor modifications to the drug may be necessary. The best results in this study were achieved by synthesising the propionyl and buteryl derivatives and using Pheroids as delivery system.

Opsomming

Die vel is 'n ongelooflike elastiese en relatief deurlaatbare skans wat beskerming, waarneeming, en kommunikasie aan die menslike liggaam verleen. Die belangstelling in die transdermale aflewering van geneesmiddels is toe te skryf aan die voordele van hierdie metode van geneesmiddelaflewering. Hierdie voordele is onder meer konstante geneesmiddelvlakke, die uitskakeling van eerstedeurgangseffek in die lewer en die akkommodasie van pasiënte wat nie orale doseervorme kan verdra nie. Transdermale terapie is egter nie sonder beperkings nie. Die buitenste laag van die vel, die stratum corneum, is die vernaamste skans vir penetrasie van die meeste geneesmiddels deur die vel en is baie selektief ten opsigte van die tipe molekule wat deur hierdie laag getransporteer kan word. Dit is omdat die vel 'n beskermende funksie vervul en verbindings keer om dit te penetreer. Slegs geneesmiddels met die geskikte fisies-chemiese eienskappe is in staat om hierdie skans te deurdring. Baie geneesmiddels wat hidrofiliese eienskappe besit penetreer te stadig om van terapeutiese waarde te wees, terwyl geneesmiddels met lipofiliese eienskappe beter geskik is vir transdermale aflewering, maar verbindings wat beide lipied- en wateroplosbaar is, penetreer baie beter as verbindings met of 'n hoë water- of 'n hoë lipiedoplosbaarheid.

Stavudien (2',3'-didehidro-2',3'-dideoksitimidien, d4T), 'n sintetiese antiretrovirale middel, is 'n remmer van nukleosiedomgekeerdetranskriptase en word teen MIV-1 en MIV-2 gebruik. Dit word in kombinasie met ander antiretrovirale middels gebruik en is aangedui vir die beheer van hoofsaaklik MIV-1-infeksie in volwasse en pediatriese pasiënte. Nog 'n belangrike gebruik van stavudien is vir profilakse na blootstelling waar dit kan help om die risiko van infeksie te verminder. Newe-effekte van behandeling met stavudien is meestal a.g.v mitokondriële toksisiteit vanweë die inhibisie van menslike gamma-DNA-polimerase. Die belangrikste newe-effek, perifere neuropatie, is afhanklik van die dosis en die duurvan behandeling.

Pheroids is 'n gepatenteerde emulsiestelsel bestaande uit 'n unieke formulering van submikron deeltjies wat in staat is om 'n groot verskeidenheid van geneesmiddels te enkapsuleer en hierdie geneesmiddels met hoë doeltreffendheid na teikens in die liggaam af te lewer. Die Pheroid bestaan hoofsaaklik uit plant- en essensiële vetsure en is stabiel in 'n breë terapeutiese sisteem. Dit word op 'n spesiale manier gemanipuleer

om voordele bo ander afleweringsisteme te verseker, soos hoë mate van enkapsulering, vinnige tempo van transport, aflewering en stabiliteit.

Die hoofdoel van hierdie studie was om 'n reeks nuwe derivate van die antivirale middel stavudien te sintetiseer en om die effekte van die verskillende substituente op transdermale penetrasie te bestudeer, met en sonder die gebruik van Pheroids as afleweringstelsel. 'n Verdere doel was om vas te stel of daar enige korrelasie tussen die transdermale penetrasie en sekere fisies-chemiese eienskappe van die penetrante bestaan.

Die ses verskillende derivate van stavudien is berei deur die verestering van stavudien met ses verskillende suurchloriede by kamertemperatuur. Die strukture van die produkte is met kernmagnetieseresonansspektroskopie (KMR), massaspektroskopie (MS) en infrarooispektroskopie (IR) bevestig. Die eksperimentele wateroplosbaarheid en verdelingskoëffisiënt van stavudien en sy derivate by 'n pH van 7,0 is bepaal. Die rekenaarprogram Interactive Analysis (IA) is gebruik om die wateroplosbaarheid te voorspel, terwyl IA, K_{ow}Win en ACD Labs rekenaarprogramme gebruik is om die verdelingskoëffisiënte te voorspel. ACD Labs rekenaarprogram se voorspelde waardes het relatief goed met die eksperimentele verdelingskoëffisiënt by pH 7,0 gekorreleer.

Die eksperimentele wateroplosbaarheid, verdelingskoëffisiënt en transdermale vloed van stavudien en sy derivate is bepaal. Die eksperimentele wateroplosbaarheid van stavudien (104,75 mg/ml) is baie hoër as dié van die gesintetiseerde derivate, terwyl die verdelingskoëffisiënt van stavudien (-0,846) laer is as dié van die gesintetiseerde derivate. Soos verwag, bestaan daar 'n direkte korrelasie tussen die wateroplosbaarheid en die verdelingskoëffisiënt. Stavudien-5'-dekanoaat het 'n log P-waarde van ongeveer 3, maar geen vloed nie. Dit kan toegeskryf word aan hierdie derivaat se onoplosbaarheid in water. Dit bewys net weereens dat 'n verbinding oor beide lipofiele en hidrofiele eienskappe moet beskik om oor die stratum corneum te beweeg.

Die wateroplosbaarheid, molekulêre massa en log D-waardes het 'n uitstekende korrelasie met die vloed van die verbindings in fosfaatbuffer (PBS) getoon, terwyl daar geen korrelasie was tussen die smeltpunt en die integriteit (voor of na) met die vloed nie. Die data het geen korrelasie tussen die vloed van die verbindings in Pheroids en enige van die bepaalde fisies-chemiese eienskappe getoon nie. Die eksperimentele transdermale vloed van stavudien ($1,46 \times 10^2$ µg/cm^2.h) in PBS was

baie hoër as dié van sy derivate terwyl die propioniel- (1,86 $x10^{-2}$ µg/cm^2.h) en buterielderivate (2,02 $x10^{-2}$ µg/cm^2.h) die verbindings was met die hoogste transdermale vloed in Pheroid. Die vloed van stavudien het dus verbeter van 1,46 $x10^{2}$ µg/cm^2.h tot 2,02 $x10^{-2}$ µg/cm^2.h deur die buterielderivaat te sintetiseer en Pheroids as aflewering sisteem te gebruik wat 'n verbetering van 38% in die vloed is.

Hierdie studie bevestig dat transdermale vloed afhanklik is van 'n aantal faktore soos die wateroplosbaarheid, die verdelingskoëffisient, molekulêre grootte, smeltpunt en die alkiel ketting lengte, om net 'n paar te noem, en dat daar in sommige gevalle klein veranderinge aan die geneesmiddel nodig is. Die beste resultate in die studie is verkry deur die propanoiel- en buterielderivate te sintetiseer en Pheroids as afleweringstelsel te gebruik.

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Chapter 1

INTRODUCTION AND STATEMENT OF THE PROBLEM

1.1 Introduction

The skin is an amazingly flexible and relatively impermeable barrier that provides protective, perceptive and communication functions to the human body (Ramachandran & Fleisher, 2000). It is the largest organ of the body and acts as a protective barrier with sensory and immunological functions (Foldvari, 2000). It protects the body from water loss, friction and impact wounds, and potentially harmful external stimuli (Barry, 1983). In an average adult it covers an area of approximately 1,73 m^2 (Barr, 1962) and receives one third of circulating blood through the body at any given time. Thus the skin is one of the most readily accessible organs of the human body (Chien, 1987).

The main barrier to penetration by most drugs through the skin, is the outermost layer of the skin, the stratum corneum (SC). The SC is very selective with respect to the type of molecule that can be transported across this outer covering, and not all molecules that pass the 'potency' test will have the necessary physicochemical properties (Niak *et al.*, 2000). The primary factors that determine the diffusion rate through human skin are the physicochemical properties of the drug (Idson, 1975), the vehicle and the skin (Katz & Poulsen, 1971). The transdermal penetration is dependent on the aqueous solubility of the drug, the partition coefficient, molecular size, melting point and the alkyl chain length, to name a few. In general, a drug substance should have an aqueous solubility of more than 1 mg/ml or it may represent a potential bioavailability problem (Abdou, 1989). According to Guy (1996), compounds with a log P value between 1 and 3, with relatively low molecular weights and modest melting points, are likely to have acceptable passive skin permeabilities. In addition, the aqueous solubility and the flux decreases as the alkyl chain length and molecular weight increases (Flynn, 1989). The physical and chemical properties of each of these components and their combined interactions all influence the rate at which the drug penetrates the skin (Katz & Poulsen, 1971).

Stavudine (2',3'-didehydro-2',3'-dideoxythymidine, d4T), a synthetic antiretroviral agent, is a nucleoside inhibitor of reverse transcriptase and is used against HIV-1 and HIV-2 (Hayden, 2005). It is required to be in combination with other antiretroviral agents and is indicated for the management of mainly HIV-1 infection in adults and

paediatric patients. Another important use for stavudine is for post exposure prophylaxis (PEP) which can help decrease the risk of infection after exposure to HIV (McEvoy, 2002). Adverse effects related to stavudine therapy, are mostly due to mitochondrial toxicity resulting from the inhibition of human DNA polymerase gamma. The major adverse effect, peripheral neuropathy, is dependant on the dosage and the duration of the treatment (Hurst & Noble, 1999).

Pheroids is a patented system comprising of a unique submicron emulsion type formulation which is capable of encapsulating a variety of drugs and delivering these drugs with high efficacy to target sites within the body. In addition, they are manipulated in a specific manner to ensure important advantages over other delivery systems such as high entrapment capabilities, fast rate of transport, delivery and stability (Grobler, 2004).

Transdermal drug delivery offers a number of significant advantages over more traditional dosage forms. Some of these include more consistent serum drug levels, accommodating patients who cannot tolerate oral dosage forms, thus avoiding direct effects on the stomach and intestine. First pass metabolism can also be avoided with transdermal administration (Wilkosz & Bogner, 2003).

1.2 Aim and objectives of this study

The primary aim of this study was to synthesise a series of new derivatives of the anti-HIV drug stavudine (d4T), and to evaluate effects of the different subtituents on transdermal penetration, with and without the use of Pheroids as delivery system. Furthermore, it was to be established if any correlation between the transdermal penetration and selected physicochemical properties of the penetrant existed.

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

- Synthesise esters of stavudine and confirm their structures.
- Experimentally determine the aqueous solubility and the partition coefficient for stavudine and its synthesised derivatives.
- Compare the experimental aqueous solubility and the partition coefficients of the synthesised stavudine derivatives to calculated values from commonly used prediction software.

- Experimentally determine the transdermal flux of stavudine and its derivatives in PBS and in Pheroids.

- Compare the experimental flux data of the synthesised stavudine derivatives to calculated values from commonly used theoretical equations.

- Determine whether a correlation exists between the physicochemical properties like melting point, aqueous solubility, partition coefficient etc. on the one hand and transdermal flux data of the stavudine and its derivatives.

- Build up 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.

Chapter 2

STAVUDINE AS NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITOR (NRTI)

2.1 Overview of HIV infection

The Acquired Immunodeficiency Syndrome (AIDS) was first recognised in the United States of America, in the summer of 1981, and has since become a major worldwide pandemic (Adler, 1987). The Human Immunodeficiency Virus (HIV) is known as the primary cause of AIDS. According to 2004 statistics an estimated 42 million people worldwide were living with HIV, the greater portion in resource-poor countries. Of those who could benefit from combination antiretroviral therapy, fewer than 5 % were receiving treatment, even though it would have reduced the complications of infection and prolonged life (Hayden, 2005). The Center for Disease Control (CDC) defines AIDS as a disease that is at least moderately predictive of an underlying cellular immune deficiency that results in a cellular defect in an individual, with no known resistance to the disease (Ma & Armstrong, 1984).

The most common route of transmission of HIV worldwide is through sexual intercourse. It is, however, also spread through infected blood, the common use of contaminated needles and from mothers to their babies during pregnancy or birth (Adler, 1987).

HIV is immunosuppressive because it infects cells of the immune system which leads to the destruction or functional impairment of these cells. A diagnosis of AIDS is given to infected HIV-individuals when the CD4+ T-cell count declines below 200 cells/mm^3 of blood. A healthy, uninfected person generally has 800 - 1200 CD4+ T-cells/mm^3 of blood (Caldwell *et al.*, 1994 & Castro *et al.*, 1992).

Two major families of HIV exist, namely HIV-1 and HIV-2 (Hayden, 2005). HIV-1 is the major cause of AIDS in the world today. This virus infects the cells of the immune and central nervous systems. The T helper lymphocyte is the main cell infected with HIV and plays an important role in the immune system, so that a large decrease in the number of T helper cells can dangerously weaken the immune system (Anon, 2006).

The T helper cell is infected with HIV since it has protein CD4 on its surface. HIV uses this protein to attach itself to the cell before entry. The virus then replicates extensively and can infect other healthy cells followed by a severe reduction in the number of T helper cells (Anon, 2006).

After only a few days to a few weeks after exposure to HIV, an acute flu-like illness begins to appear in most infected individuals. The most common symptoms to appear are fever, maculopapular rash, oral ulcers, etc. (Adler, 1987). This stage is known as the primary infection stage (Anon, 2006).

Antiretroviral drugs were introduced with the aim to inhibit the reproduction of retro-viruses. These drugs are virustatic agents, which block the steps in the replication of the virus. This significantly slows the disease progression (Uretsky, 2003).

The purpose of antiretroviral treatment during acute HIV-1 infectivity is to shorten the symptomatic viral illness, decrease the amount of infected cells, defend the HIV-1-specific immune responses and possibly lower the viral set point in the long term (Hurst & Noble, 1999).

In 2004 De Clercq introduced five different classes of anti-HIV chemotherapeutic agents that have been developed in the treatment of AIDS:

- Nucleoside Reverse Transcriptase Inhibitors (NRTI),
- Nucleotide Reverse Transcriptase Inhibitors (NtRTIs),
- Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs),
- Protease Inhibitors (PIs) and
- Viral entry Inhibitors.

Nucleoside Reverse Transcriptase Inhibitors, currently enjoy the greatest field of study.

2.2 Nucleoside Reverse Transcriptase Inhibitors (NRTIs)

The NRTIs form the most important class of compounds active against HIV (Len & Mackenzie, 2006).

To block the replication of HIV in a person's blood, NRTIs should be used in combination with other anti-HIV drugs, usually a total of three drugs, to prevent healthy T-cells in the body from becoming infected with HIV (Staley *et al.*, 2006).

Viral RNA is converted by the HIV-encoded, RNA dependent DNA polymerase, to proviral DNA. The proviral DNA is then incorporated into a host cell gene. For this process to occur, HIV's reverse transcriptase enzyme is required (Hayden, 2005).

The NRTIs contain faulty versions of the building blocks used by reverse transcriptase to convert RNA to DNA. When reverse transcriptase uses these faulty building blocks, the new DNA is thus unable to formulate correctly. Consecutively, HIV's genetic material has difficulty being incorporated into the healthy genetic material of the cell. This then prevents the cell from producing new viruses (Staley *et al.*, 2006).

Although nucleotide analogues are in theory different from nucleoside analogues, they act very similarly. To exert activity, the nucleosides must be triphosphorilated at the 5'-hydroxyl group, while the nucleotide analogues bypass this step, given that they are already chemically activated (Staley *et al.*, 2006).

2.3 Stavudine

2.3.1 History

Stavudine (2',3'-didehydro-2',3'-dideoxythymidine, d4T), a synthetic antiretroviral agent, is an inhibitor of nucleoside reverse transcriptase and is used against HIV-1 and HIV-2 (Hayden, 2005). It was originally synthesised by Dr. Jerome Horowitz of the Michigan Cancer Foundation in 1966, but d4T's capability to treat HIV / AIDS was first discovered by Dr. Tai-Shun Lin and Dr. William Prusoff of Yale University (Love, 2000).

The U.S Food and Drug Administration (FDA) approved d4T on June 24, 1994 for adults and on September 6, 1996 for peadiatric use. It was the fourth antiretroviral drug on the market (Vermund, 2006).

2.3.2 Mechanism of action

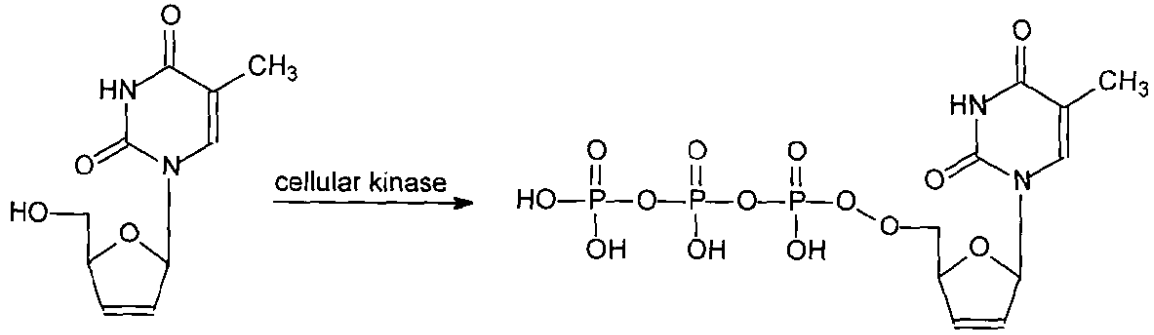


Figure 2.1: Stavudine phosphorylated to the active metabolite stavudine 5'-triphosfate.

Nucleoside analogues are prodrugs, and to exert their activity, they must be metabolised intracellularly (Stein & Moore, 2001). Stavudine, a thymidine nucleoside analogue, penetrates the cells by passive diffusion. Stavudine is more active in stimulated cells (such as T-lympocytes) than in resting cells (such as monocytes and macrophages) because of differing patterns of phosphorylation. The intracellular stavudine is phosphorylated by thymidine kinase to stavudine 5'-monophosphate (d4T-MP), which is then phosphorylated to the diphosphate (d4T-DP) by thymidylate kinase, and lastly phosphorylated to an active metabolite stavudine 5'-triphosphate (d4T-TP) by nucleoside diphosphate (NDP) kinase. The monophosphate and diphosphate metabolites do not accumulate in the cell. HIV replication is inhibited by the 5'-triphosphate metabolite, by competing with the natural substrate deoxythymidine 5'-triphosphate for incorporation into viral DNA by reverse transcriptase, causing early termination of the viral RNA chain if successfully incorporated (Hurst & Noble, 1999). The synthesis of mitochondrial DNA is then markedly reduced because stavudine triphosphate inhibits cellular DNA polymerase beta and gamma (Bristol-Myers Squibb Company, 2006). The rate-limiting step for phosphorylation of stavudine is the conversion to d4T-MP, whereas the conversion to its triphosphate has little effect on the anabolism of thymidine or other nucleosides (Beach, 1998).

2.3.3 Clinical use and adverse effects of stavudine

Stavudine is used for the treatment of HIV infection, which causes AIDS. It is required to be in combination with other antiretroviral agents and is indicated for the

management of HIV-1 infection in adults and paediatric patients. While evaluating the safety and efficacy, stavudine was used in initial studies as monotherapy, but was not effective in the management of HIV infection (McEvoy, 2002). Current guidelines state that three antiretroviral drugs, or 'triple therapy', should be the standard initial treatment for patients with HIV infection or AIDS. The plasma HIV RNA levels, reduced to below the limit of detection when stavudine was used in combination with additional antiretroviral agents. Stavudine should be used with either two NRTIs, an NRTI and a PI, an NRTI and an NNRTI or two PIs (Hurst & Noble 1999).

Although avoiding exposure to HIV is the only reliable way of preventing HIV infection, post exposure prophylaxis (PEP) can decrease the risk of infection after exposure to HIV. This is another important use of stavudine, in conjunction with an additional antiretroviral agent, for health care personnel and other individuals exposed occupationally to blood, tissues, or other body fluids associated with the threat for transmission of the HIV virus (McEvoy, 2002). Only two antiretroviral drugs are provided for post exposure prophylaxis since it is potentially toxic, and does not justify the use in exposures that pose an insignificant risk of transmission. The most common HIV post exposure prophylaxis regimen includes zidovudine (600 mg per day) plus lamivudine (150 mg twice daily). Stavudine is used as an alternate basic HIV post exposure prophylaxis regimen with lamivudine or didanosine (Preboth, 2002).

Adverse effects, related to stavudine therapy, are mostly due to mitochondrial toxicity resulting from the inhibition of human DNA polymerase gamma. The major adverse effect, peripheral neuropathy, is dependant on the dosage and the duration of the treatment. It is more likely to develop in patients who have previously experienced peripheral neuropathy or in patients with underlying peripheral neuropathy. Symptoms include pain, distal sensory loss, numbness in the hands or feet, areflexia and mild muscle weakness may occur. Neurological disorders, diarrhoea, skin rashes, elevation of liver transaminases and/or bilirubin, nausea and abdominal pain are several of the other adverse effects that had been reported during triple therapy, containing stavudine (Hurst & Noble, 1999).

2.4 Transdermal penetration of NRTIs

A number of studies have been performed on the transdermal penetration of NRTIs but was mostly focused on zidovudine, the first antiretroviral drug approved for clinical use.

In 2004 Suwanpidokkul *et al.* investigated the effects of penetration enhancers, vehicles and polymer membranes on zidovudine penetration across cadaver pig skin. Four binary vehicles were tested for zidovudine permeability and solubility across pig skin but ethanol/IPM [isopropyl myristate] (50/50, vol/vol) confirmed the highest zidovudine flux value. When various concentrations of different penetration enhancers were used, the two combinations of ethanol/IPM (20/80 plus 10% *N*-methyl-2-pyrrolidone [NMP]) and ethanol/IPM (30/70 plus 10% NMP) resulted in increased zidovudine solubility and high flux values. When zidovudine penetration across pig skin covered with a microporous polyethylene (PE) membrane was investigated, the flux values decreased to ~50% of that seen with only pig skin.

Seki *et al.* (1990) wanted to improve the skin delivery characteristics of zidovudine and therefore synthesised five aliphatic esters to asses as prodrugs of zidovudine. The aqueous solubilities of the esters were lower than that of zidovudine, while the solubility in IPM and the partition coefficients were higher than that of zidovudine. The acetate and hexanoate esters showed 2,4- and 4,8-fold enhanced penetration in human skin respectively when IPM was used a vehicle relative to application of zidovudine itself.

In a study by Kim & Chien (1995) the possibility of transdermal penetration of the anti-HIV drug zalcitabine were studied in order to maintain blood concentration levels within a therapeutic range for a longer period and to reduce adverse effects associated with this high dose administration. The effects of vehicles and enhancers on the skin permeation rate were investigated to determine the highest penetration rate attainable. Ethanol/tricaprylin or ethanol/water co-solvent systems were studied using excised hairless rat skin and human cadaver skin as skin models. The penetration rate of zalcitabine did not increase when a penetration enhancer such as oleic acid or NMP was added to the ethanol/tricaprylin co-solvent system while oleic acid in ethanol/water co-solvent system noticeably increased the penetration rate of zalcitabine.

Chapter 3

TRANSDERMAL DRUG PENETRATION

3.1 Introduction

The skin is an amazingly flexible and relatively impermeable barrier that provides perceptive, protective and communication functions to the human body (Ramachandran & Fleisher, 2000). As such, in the average course of living, it suffers more physical and chemical abuse than any other organ in the body, but mostly, mechanical abuse. The skin is daily unconsciously exposed to detergents, airborne pollutants, organic solvents, chemical residues in clothing, as well as an extensive variety of contact allergens of diverse origin. It is thus in its healthy state a remarkable fabric, strong and far more complex than any other human-made material (Flynn, 1990).

Over the past few years there has been an increasing interest in percutaneous drug absorption and much research has been done to explain skin structure, physiology, barrier properties, and the mechanisms by which substances enter and cross the skin (Ramachandran & Fleisher, 2000). The interest in transdermal drug delivery may be attributed to the advantages associated with this method of drug delivery. Transdermal therapy, however, does have its limitations. Although the percutaneous delivery of drugs is an efficient way of achieving controlled drug delivery, it is only suitable for a select number of drugs possessing specific physicochemical characteristics (Harrison *et al.*, 1996).

Transdermal drug delivery offers a number of significant advantages over more traditional dosage forms.

- The transdermal route offers a preferred therapeutical outcome, in that the steady permeation of drugs across the skin allows for more consistent serum drug levels. A constant serum drug level is also achieved with intravenous infusion but is more invasive than transdermal drug delivery. The risk of side effects can be reduced because of the lack of peaks in plasma concentration. Therefore, can drugs that require relatively steady plasma levels, be good candidates for transdermal delivery.

- The transdermal route can be used as an alternative route of administration to accommodate patients who cannot tolerate oral dosage forms. It is therefore of great advantage in patients who are nauseated or unconscious.

- This method avoids direct effects on the stomach and intestine therefore can drugs that usually cause gastrointestinal upset, be good candidates for transdermal delivery. Some drugs that are degraded by the enzymes and acids in the gastrointestinal system may also be good targets because first pass metabolism can be avoided with transdermal administration.

A considerable disadvantage is that the skin's low permeability limits the number of drugs that can be delivered in this manner. The skin serves a protective function, therefore inhibiting compounds from crossing it. Many drugs that possess a hydrophilic structure penetrate too slowly to be of therapeutic benefit. However, drugs with a lipophilic character are better suited for transdermal delivery (Wilkosz & Bogner, 2003), but recent developments have shown that an ideal drug candidate must have sufficient lipophilicity to partition into the stratum corneum, but also sufficient hydophilicity to enable the second partitioning into the viable epidermis (Kalia & Guy, 2001).

3.2 Percutaneous absorption

Percutaneous drug absorption involves the penetration of the substance through the skin, absorption into the blood capillaries of the dermis and distribution into the systemic circulation (Lund, 1994).

The process of transdermal absorption includes several phases:

1. Penetration (the entry of a substance into the particular layer).
2. Permeation (the penetration through one layer into another, which is both structurally and functionally different from the first layer).
3. Absorption (the uptake of a substance into the vascular system of blood vessels, which act as the central compartment) (Schaefer *et al.*, 1982)

Systemic distribution of the compound follows, throughout the body.

Factors that may have an effect on percutaneous absorption are:

- The skin structure (the anatomy and physiology of human skin);

- The physicochemical properties of the penetrant;
- The physicochemical properties of the vehicle in which the penetrant is dosed and
- The dosing conditions (circumstances under which dermal administration of the penetrant occurs) (Wiechers, 1989).

3.2.1 The skin as barrier to transdermal drug delivery

The skin is a membranous tissue forming the external covering or integument of a human being. It is the largest organ of the body and acts as a protective barrier with sensory and immunological functions (Foldvari, 2000).

In an average adult it covers an area of approximately 1,73 m^2 (Barr, 1962) and receives one third of circulating blood through the body at any given time. Thus is the skin one of the most readily accessible organs of the human body (Chien, 1987). The pH of the skin is reported to be between 4,8 and 6,0 (Flynn, 1990). An average square centimeter of skin contains 10 hair follicles, 15 sebaceous glands, 12 nerves, 100 sweat glands, and 3 blood vessels (Asbill & Michniak 2000). The average thickness of the skin is 0,5 mm, ranging from 0,05 mm to 2 mm, in different parts of the body (Foldvari, 2000).

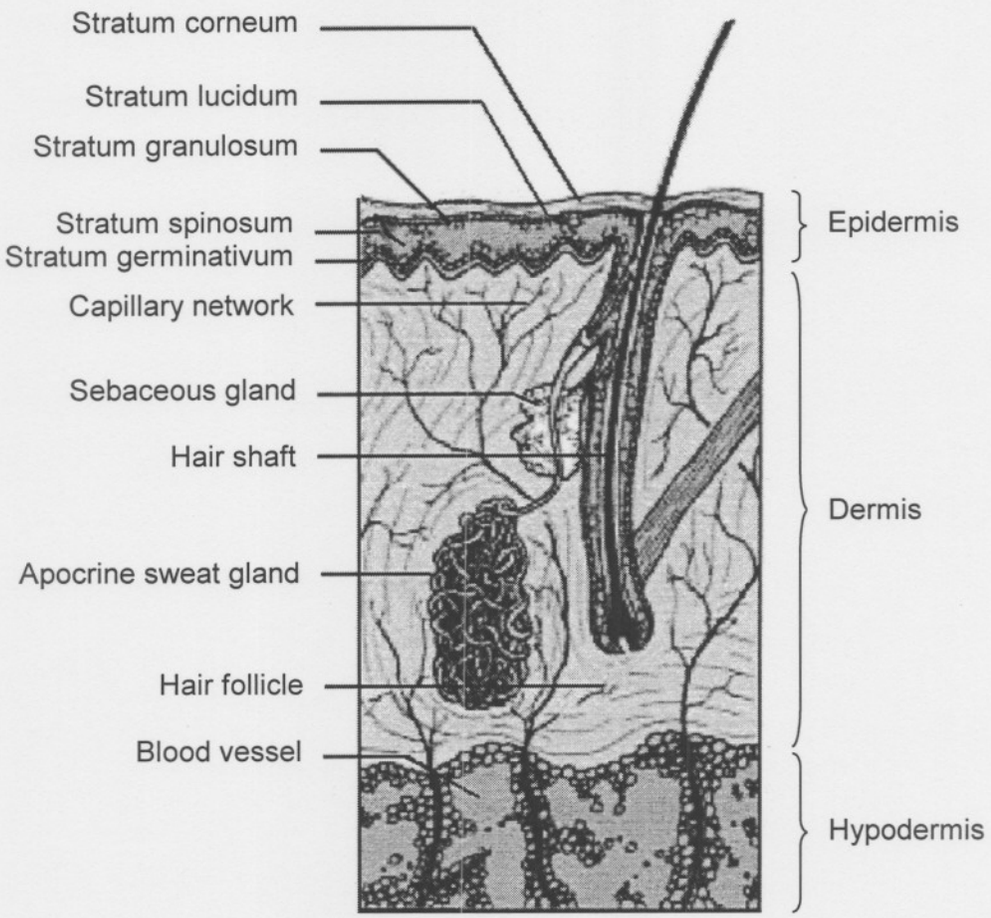


Figure 3.1: A schematic cross-section of the skin with different layers identified (West & Nowakowski,1996).

For a substance to enter the systemic circulation, it has to cross several potential barriers. These include the epidermis (consisting of the stratum corneum and the viable epidermis), the dermis and the hypodermis (Flynn, 1990).

3.2.1.1 Stratum corneum

The main barrier for penetration of most drugs through the skin is the outermost layer of the skin, the stratum corneum (SC). The SC is about 10 μm thick in the non-hydrated state but much thicker on the palms of the hands and foot-soles where it can be as thick as 600 μm. The SC contains 10-25 layers, parallel to the skin surface, consisting of keratin-filled dead cells, the corneocytes, which are entirely surrounded by crystalline lamellar lipid regions. As these dead cells slough off, they are continuously replaced by new cells from the stratum basale (Wiechers, 1989).

The SC can be described as a brick-and-mortar complex. The corneocyte component is the brick, and the intercellular lipid complex, the mortar. The intercellular lipid complex comprises 15 % of the stratum corneum's total weight, with the remainder being protein (70 %) and water (15 %). The combination of the intercellular lipids, matured keratinocytes (corneocytes, with their protein and lipid shells), and intercellular connections between the corneocytes (desmosomes and tight junctions) are the known components of this complete barrier (Fore-Pfliger, 2004).

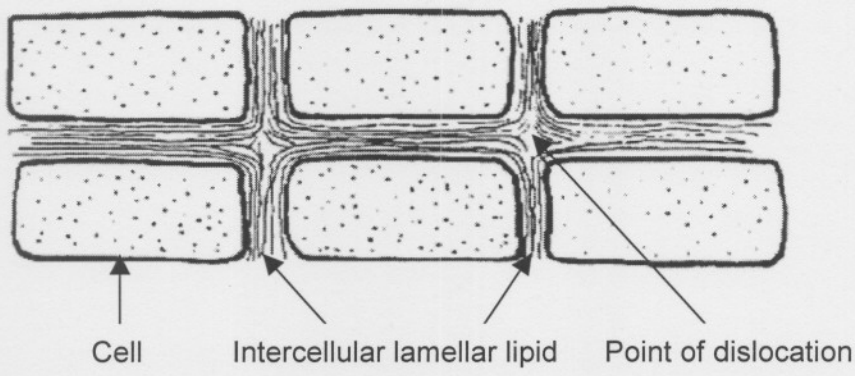


Figure 3.2: The brick and mortar configuration of the SC (Elias, 1983).

The SC is very selective with respect to the type of molecule that can be transported across this outer covering, and not all molecules that pass the 'potency' test will have the necessary physicochemical properties (Niak *et al.*, 2000).

3.2.1.2 Viable epidermis

Situated beneath the SC is the viable epidermis. It ranges in thickness from 75 to 150 µm and consists of three layers: the stratum granulosum, spinosum and basale (Wiechers, 1989). The viable epidermis contains keratinocytes at varying stages of differentiation, as well as melanocytes, Langerhans cells and Merkel cells. The Langerhans cells are important for antigen presentation and immune response where the Merkel cells are involved in sensory perception (Asbill & Michniak, 2000). In the basal layer, mitosis of the cells constantly renews the epidermis and this proliferation compensates for the loss of dead horny cells from the skin surface. As the cells produced by the basal layer move outward, they change morphologically and histochemically, undergoing keratinisation to form the outermost layer, the SC (Barry, 1983).

3.2.1.3 Dermis

The dermis is the innermost layer of the skin and is situated between the viable epidermis and subcutaneous fatty region (Hunter *et al.*, 1996). It consists mainly of a dense network of structural protein fibers, collagen, reticulum, and elastin, embedded in a semi-gel matrix of mucopolysaccharidic "ground substance" (Flynn, 1990) and supports the epidermis structurally and nutritionally (Schaefer & Hensby, 1990). It ranges from 0,1 – 0,5 cm in thickness and is of substantial importance because the microcirculation that subserves the entire skin is located in the epidermis (Flynn, 1990).

3.2.1.4 Hypodermis

The hypodermis or subcutaneous fatty tissue merges with the overlying dermis and is unevenly distributed over the body. It supports the dermis and epidermis and serves as a fat storage area. This layer helps to regulate temperature and provides nutritional support and mechanical protection. It carries the principle blood vessels and nerves to the skin and may contain sensory pressure organs (Barry, 1983).

3.2.1.5 Skin appendages

In addition to the above three layers of skin, the skin has other appendages which affect the percutaneous delivery of drug compounds (Danckwerts, 1991). Some of these include the interspersed hair follicles and associated sebaceous glands, the so-called pilosebaceous glands, and in specific regions two types of sweat glands, the eccrine and apocrine glands (Flynn, 1990). The sebum which is produced by the sebaceous glands lubricates the skin and help to maintain the surface pH at about 5 (Williams, 2003). The sebum consists of a mixture of fatty acids, triglycerides, waxes, cholesterol and cellular debris (Montaga, 1965). Hydrophilic drugs which are incompatible with sebaceous lipids will not be able to utilise this pathway for passive diffusion, while lipophilic drugs that are compatible with sebum will diffuse through the follicles (Ramachandran & Fleisher, 2000).

3.2.2 Functions of the skin

The skin performs a complex role in human physiology. It protects the body from water loss, friction and impact wounds, and other potentially harmful external stimuli. The skin uses its thermoreceptors to perform a very important role in regulating body temperature. Not only does the skin metabolise and synthesise compounds but it

also disposes of chemical waste (Barry, 1983). The skin also produces Vitamin D in the epidermal layer when exposed to the sun's rays (Altruis Biomedical Network, 2000).

3.2.3 The process of transdermal drug penetration

Human skin is an effective, selective barrier to chemical penetration (Barry, 1983). A molecule may use two diffusional routes to penetrate normal intact human skin: the appendageal route and the epidermal route (Williams & Barry, 1992).

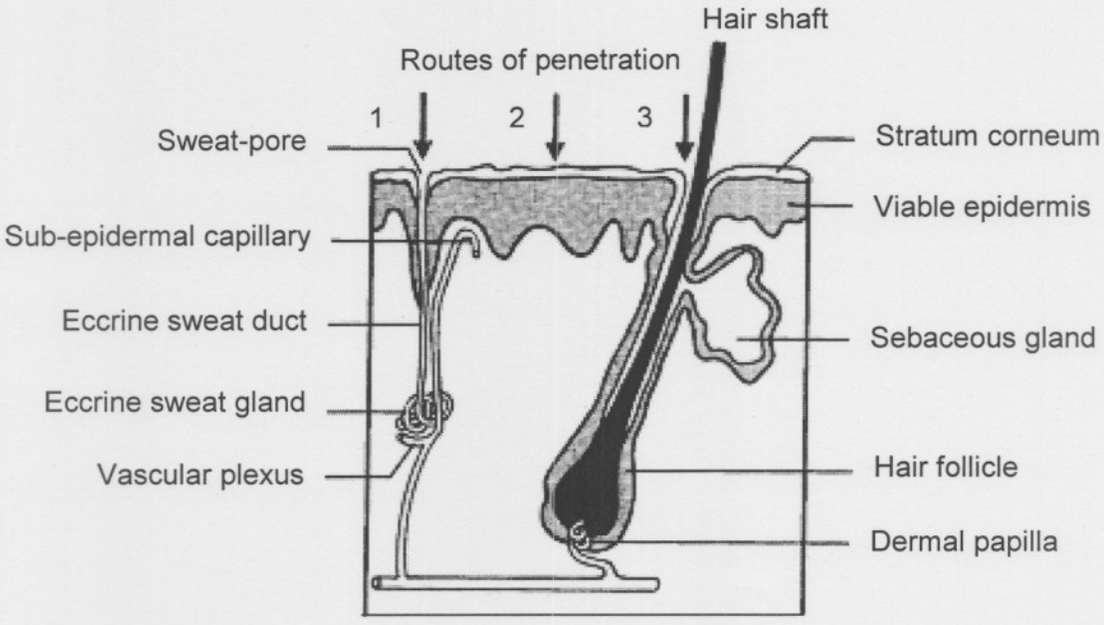


Figure 3.3: Possible macro routes for drug entry through the skin *via* intact horny layer or hair follicles or eccrine sweat glands (appendageal route) (Williams & Barry, 1992).

The appendageal route transports substances *via* the sweat glands and the hair follicles with their associated sebaceous glands (Figure 3.3). These routes are known as shunt routes because they avoid penetration through the SC. However, these routes are considered to be of minor importance because of their relatively small area, approximately 0,1 % of the total skin surface. However, recent studies have shown that follicles may have a greater importance in percutaneous absorption than is commonly understood. The appendageal route may be more important for ions and large polar molecules which hardly penetrate through the SC (Williams & Barry, 1992).

For drugs that mainly cross the intact horny layer, two possible micro routes of entry exists namely the transcellular and intercellular pathways (Figure 3.4). The principle pathway taken by the permeant is primarily decided by the partition coefficient (log K). Hydrophilic permeants partition preferentially into the intracellular domains, whereas lipophilic drugs (octanol/water log K > 2) traverse the stratum corneum *via* the transcellular pathway. Most permeants penetrate the SC by both routes, however the indirect intercellular route is widely considered to provide the principle route and major barrier to the permeation of most drugs (Williams & Barry, 1992).

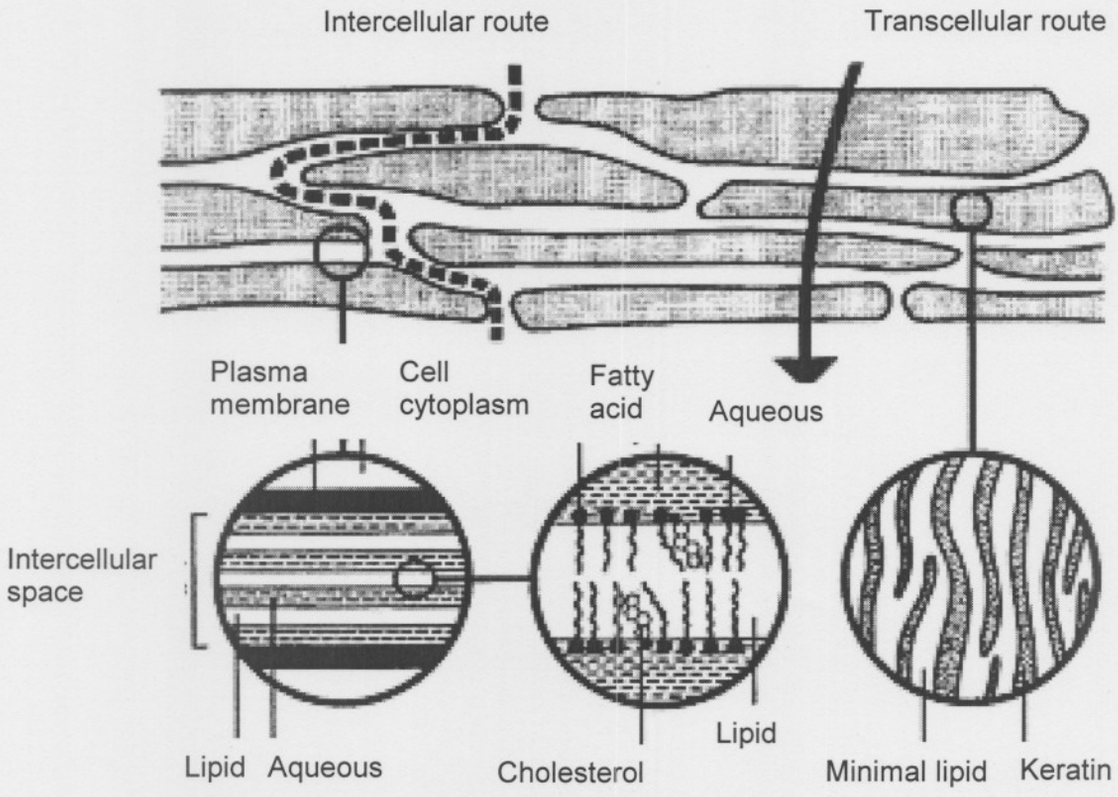


Figure 3.4: Possible micro routes for drug entry across the skin intercellular or transcellular (epidermal route) (Barry, 2001).

Many physiological factors may influence both the rate and extent of penetration into the skin. For example: the mode of application, frequency and duration of application, temperature and condition of the skin, concentration and physicochemical properties of the active ingredient and the influence of the vehicle. If all but the last of the aforementioned factors can be kept constant, it would be possible to determine which physicochemical properties of the compound are most important in determining the absorption through the skin or into the skin (Lien & Tong, 1973).

3.3 Physicochemical factors influencing transdermal delivery

The primary factors that determine the rate of diffusion through human skin are the physicochemical properties of the drug (Idson, 1975), the vehicle and the skin. The physical and chemical properties of each of these components and their combined interactions all influence the rate at which the drug penetrates the skin (Katz & Poulsen, 1971).

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

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

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

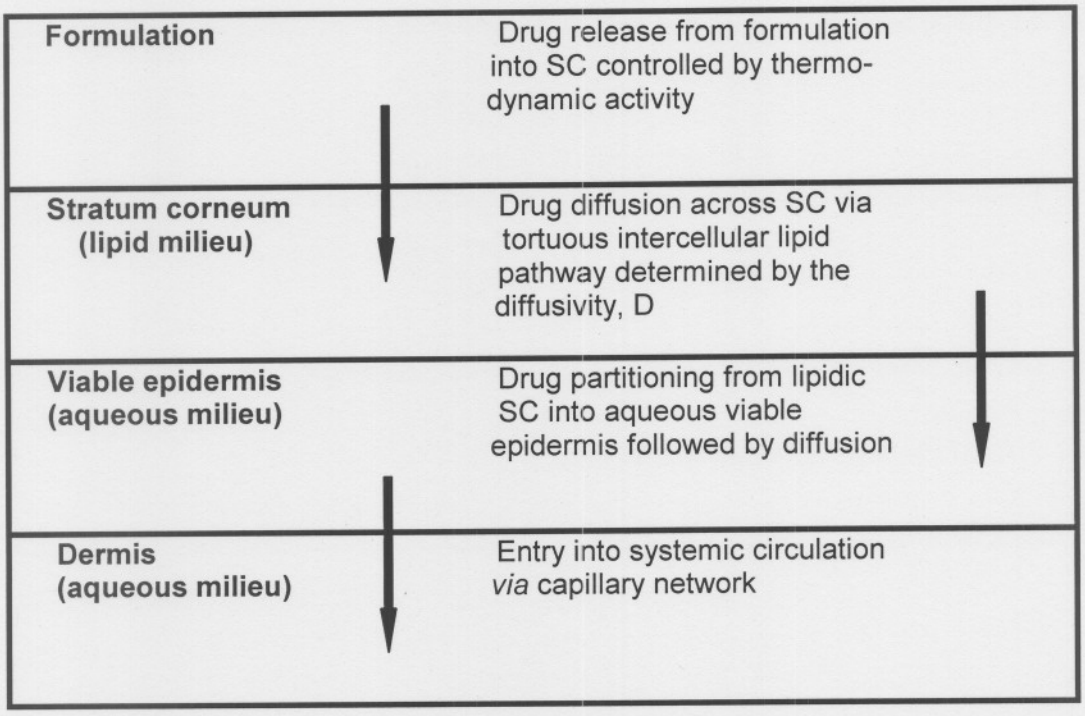


Figure 3.5: The transport process of a therapeutic agent released from formulation to its eventual uptake by dermal capillaries (Kalia & Guy, 2001).

3.3.1 Drug solubility in the SC

The solubility of a penetrant in the various environments of the skin and its surroundings plays an important role in determining the rate of penetration (Smith, 1990). The lipid-water solubility pattern state that, because the epidermal cell membrane consists of a mosaic pattern of lipid and protein molecules, substances soluble in lipids pass through the cell membrane owing to its lipid content. In contrast, water soluble substances pass after the hydration of the protein particles in the cell wall, which leaves the cell permeable to water soluble substances (Idson, 1975).

Solubility is dominant in skin penetration. Its importance was recognised early when it was found that compounds with both water and lipid solubilities penetrate much better than substances with either a high water or high lipid solubility (Liron & Cohen, 1984).

The released drug will partition into the outer layers of the SC. The degree to which this will happen is controlled by the amount supplied and the solubility limit in the SC. In general, the rate of partitioning from the vehicle is greater into the SC than the rate

of diffusion through the SC and the partitioning from the SC into the epidermis (Hadgraft & Wolff, 1993).

3.3.1.1 Solubility parameter

The solubility parameter is one of the indices expressing energetics of molecular interaction. That is, higher miscibility can be released when the two solubility parameters of the components are closer in the binary system. By using the solubility parameter, the solubility of solute in solvent is almost predictable (Otha *et al.*, 1999).

First defined by Hildebrand & Scott (1950), the solubility parameter has been found to be a useful guide for determining solvent miscibility. The solubility parameter of an organic solute in the SC can be estimated from Equation 3.1, 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).

$$\ln X_2 = \frac{-\Delta H_f}{RT}\left(\frac{T_f - T}{T_f}\right) + \frac{\Delta C_p}{R}\left(\frac{T_f - T}{T} - \ln\frac{T_f}{T}\right) - \frac{V_2\Phi_1^2}{RT(\delta_1 - \delta_2)^2} \qquad \text{Equation 3.1}$$

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 supercooled liquid from 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.

The solubility parameter, δ, has been used to explain drug action, structure-activity relationships, drug transport kinetics and *in situ* release of drugs. Therefore, the precise value of the solubility parameter of the drug is of interest (Subrahmanyam & Sarasija, 1997).

The solubility parameter of the skin has been estimated as ~10 and therefore drugs, which possess similar values, would be expected to dissolve readily in the SC (Liron & Cohen, 1984). Thus, penetrants with high solubilities in the SC will tend to exhibit high flux values, or at least will not be limited by solubility considerations.

3.3.1.2 Aqueous solubility

The solubility of the penetrant in the various phases present in the skin and its surrounding areas, play an important role in determining the rate or amount of penetration (Smith, 1990). Lipid solubility is considered to be an essential aspect in transdermal absorption but because of the aqueous nature of the epidermal layers beneath the SC, it can be taken that a drug or molecule should exhibit measurable water solubility to allow it to permeate to the capillary microcirculation (Guy & Hadgraft, 1992). As a common rule, a drug substance should have an aqueous solubility of more than 1 mg/ml or it may represent a potential bioavailability problem. In some instances, minor chemical modifications of the drug chemical, such as salt formation or esterification, are necessary (Abdou, 1989).

Aqueous solubilities of non-polar organic compounds depend on their molecular surface areas, which are essentially hydrophobic in nature. Therefore, the affinity for water decreases exponentially as the molecular hydrophobic surface increases (Barry, 1983). The aqueous solubility of compounds by convention is reported on a molar rather than a mole fraction scale. For inadequately soluble compounds, the molar solubility is simply the mole fraction solubility multiplied by 55,5. The following equation enables the aqueous solubility (S*w*) of either liquid or crystalline organic and crystalline non-electrolytes to be estimated:

$$\log Sw \approx 1{,}00\log PC - 1{,}11\frac{\Delta Sf(mp-25)}{1364} + 0{,}54 \qquad \text{Equation 3.2}$$

Where:

- PC is the octanol/water partition coefficient
- ΔSf is the entropy of fusion and is estimated from the chemical structure

- mp is the melting point

Drugs with low melting points usually have high solubilities and as a result higher dissolution rates.

This equation provides a means of assessing the role crystal structure plays (as reflected by the entropy of fusion and the melting point) and the activity coefficient (as reflected by the octanol/water partition coefficient) in controlling the aqueous solubility of a compound (Yalkowsky & Valvani, 1980).

3.3.2 Diffusion coefficient

The diffusion coefficient or diffusivity, D, may be defined as the measure of ease with which a molecule can move about within a medium, in this case the SC, further influenced by the molecular size of the drug and the viscosity of the surrounding medium (Smith, 1990 & Idson, 1983). The diffusion coefficient of a drug in the skin is also dependent on the properties of the drug, the medium through which it diffuses, and on the degree of interaction between the compound and the SC (Rieger, 1993).

There appears to be an inverse relationship between absorption rate and the molecular weight (Malan *et al.*, 2002). For molecules with similar polarity, those having the higher molecular weight permeate slower. This might be explained by the observed decrease in diffusivity in liquid media with increasing molecular volume according to Equation 3.3.

$$D = A.V_m^{-1/3} \qquad \text{Equation 3.3}$$

Where:

- D is the diffusivity of a spherical penetrant
- A is a constant
- V_m is the molecular volume (Wiechers, 1989)

The drug may bind non-specifically and specifically within the dermis and epidermis, reducing the diffusivity and hence decreasing skin permeability (Barry, 2002 & Wiechers, 1989). Another important factor that may also influence the diffusion coefficient is the drug state, e.g. ionised or unionised, with unionised forms diffusing more freely than the ionised forms (Abdou, 1989).

The affinity of the drug for the vehicle, the temperature of the vehicle and the viscosity are other parameters influencing the diffusion coefficient. The lower the affinity of the drug for the vehicle, the higher the diffusion coefficient (Baber *et al.*, 1990). Diffusivity increases with decreasing viscosity and increasing temperature of the vehicle (Pefile & Smith, 1997 & Gerber, 2003).

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

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

$$J = \frac{dM}{S.dt} \qquad \text{Equation 3.4}$$

Where:

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

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

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

Where:

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

Some of the important factors influencing the penetration of a drug into the skin include:

1. the concentration of the dissolved drug, since penetration rate is proportional to concentration;
2. the partition coefficient between the skin and the vehicle; and

3. the diffusion coefficients, which represent the resistance of the drug molecule movement through the vehicle and the skin barriers (Martin *et al.*, 1983).

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. The diffusing molecule must have some affinity for the SC in order to pass from the solvent to the skin. Once the molecule is in the membrane, it can diffuse in any direction. The permeant tends to move readily from the higher concentration to the lower concentration and therefore the progress is not random. Fick's first law of diffusion often describes the steady-state transport of a compound across a membrane:

$$J = D.A.\frac{K}{h}(C_v - C_r) \qquad \text{Equation 3.6}$$

Where:

- J is the flux
- A is the area of diffusion
- K is the membrane-vehicle partition coefficient
- D is the diffusion coefficient
- h is the diffusional pathway
- C_v is the drug concentration in the vehicle
- C_r is the concentration in the receptor phase

C_r is usually very small, and under sink conditions, (C_v – C_r) is generally approximated to C_v. Fick's laws are more correctly expressed in terms of the chemical potential of the diffusant rather than its concentration. In an ideal system, there should be a linear relationship between the rate of diffusion and the concentration of the diffusant. The maximum flux will occur when the concentration reaches the solubility limit (Barry, 1983).

3.3.3 Partition coefficient

Since it is experimentally problematical to obtain the appropriate lipid/water partition coefficient which is relevant for drug transport across the SC, many investigators have chosen to use the octanol/water partition coefficient (log K_{oct}) as the index of lipophilicity (Potts *et al.*, 1992). In the octanol/water system, the partition of drug

molecules in a neutral state is relatively favoured towards the ionised form (Avdeef *et al.*, 1998). Compounds which are either highly insoluble in water and/or have very low lipid solubility will have low rates of diffusion through the SC (Parikh *et al.*, 1984).

Partition coefficients are determined by dissolving drugs in an aqueous solution with an organic solvent, and assaying both phases for drug content. The partition coefficient is the organic solvent to water drug concentration ratio (Ansel, 1981). It is generally believed that the octanol/water partition coefficient is a good representation of the partitioning of a drug between the lipophilic SC and the underlying hydrophilic epidermis (Tenjarla *et al.*, 1996). Compounds with high partition coefficients are most likely to be the best penetrants of the skin (Takahashi *et al.*, 1993). It is likely that compounds which have a log K_{oct} of less than -1 will have difficulty in distributing from the vehicle into the SC and as a result only compounds with log K_{oct} > -1 may be considered as possible candidates for transdermal delivery. Once the compound has diffused into the SC, it will partition reasonably well into the underlying tissue. Compounds with this particular type of lipophilic properties are well suited candidates for transdermal delivery. For compounds with log K_{oct} > 2, there could be problems in achieving steady plasma concentrations in a reasonable time span. This is due to the drug being delayed in the stratum corneum where a reservoir can be established (Guy & Hadgraft, 1989).

According to Guy (1996) compounds with a log P value between 1 and 3, with relatively low molecular weights and modest melting points, are likely to have acceptable passive skin permeabilities.

3.3.4 Ionisation

Generally drugs permeate through the skin better in their unionised form, because of their greater lipid solubility (Adbou, 1989). The non-polar nature of the horny layer suggests that charged compounds should encounter high resistance to permeation through it. This proposition is best studied by the use of ionigenic compounds for which the ratio of charged species could be manipulated by changing the pH of the vehicle (Zatz, 1993).

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 pH range of the viable epidermis is 7,3 – 7,4 and of the SC 4,2 – 5,6 (Pardo *et al.*, 1992). Therefore, drugs that are weak acids or bases, according to the pH-

partition theory, may exist in an ionised or unionised form, depending on the pH of the vehicle (Barr *et al.*, 1962). The pKa or pKb of the diffusant also plays a role. The concept of pKa is derived from the Henderson-Hasselbach equation (Ansel, 1981):

For an acid:

$$pH = pKa + \log \frac{(salt)(ionised)}{(acid)(unionised)} \qquad \text{Equation 3.7}$$

For a base:

$$pH = pKa + \log \frac{(base)(unionised)}{(salt)(ionised)} \qquad \text{Equation 3.8}$$

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

This does not indicate that ionic species cannot pass through the skin, for ion pairing is possible and, in the form of ion pairs, a salt can be soluble to some extent, within a lipid continuum, whereby diffusion can take place (Flynn, 1989).

3.3.5 Melting point

Another factor which has been considered in skin permeability studies, and one which can be modified by comparatively simple synthetic changes, is the melting point of a drug (Higuchi, 1977).

The permeant melting point was established to be inversely proportional to lipophilicity (log K_{oct}) and consequently, transdermal flux. The melting point of a substance is often considered to be an indicator of the maximum flux possible through the skin. It was attempted to find a correlation between flux and the reciprocal of the melting point, given that the entropy of fusion of the permeant (ΔS_f) slowly varies with melting point. As the melting point decreases, the ideal solubility properties increase exponentially for any given molecular mass. It is assumed that there should be an exponential increase in transdermal flux with a decrease in melting point (Guy & Hadgraft, 1989 & Stott *et al.*, 1998).

Drugs with lower melting points are to be preferred due to their higher aqueous solubilities and higher dissolution rates (Yalkowski,1990), and according to Hadgraft *et al.*, (1990) it is clear that the solubility parameter in the SC, δ_{SC}, can be estimated

more accurately if the melting point of the drug is also taken into account using Equation 3.6.

$$\log\delta_{sc} = 1{,}911\frac{10^3}{mp} - 2{,}956 \qquad \text{Equation 3.9}$$

Where:

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

A study carried out by Kommuru and co-workers (1998) showed that enantiomers with a lower melting point may exhibit higher solubility than the racemate, and as a result have higher skin permeation profiles. For example, the flux of a pure enantiomer of nivaldipine, a calcium channel blocker, across human cadaver skin was about 7 fold higher than that of the racemate. In this particular case, there was a melting point difference of about 34 °C.

It can therefore be concluded that a reduction in melting point of a permeant will have a direct effect on its solubility in skin lipids and as a result increase transdermal permeation (Stott *et al.*, 1998).

3.3.6 Hydrogen bonding

An important factor to consider when selecting appropriate candidates for drug delivery, is the drug binding factor. 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. Interactions might vary from hydrogen bonding to weak Van der Waals forces and the effect of drug binding (if any) on flux across the tissue would depend on the permeant. Significant binding to the SC may completely slow drug flux down for a poorly water soluble drug in aqueous donor solution (thus containing relatively few drug molecules), if essentially all the molecules entering the tissue from the donor solution bind to skin components. However, for molecules with moderate aqueous solubility that permeate the skin well, the binding sites within the tissue may become saturated during early periods of transdermal delivery and thus the steady-state flux will possibly not be affected (Williams, 2003).

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

3.3.7 Molecular size

The size and shape of a molecule could also determine its permeation rate through the human skin. Small molecules penetrate more rapidly than larger molecules, within a narrow range of molecular size. There is little correlation between size and penetration rate. Diffusion constants through the hydrated SC for many low molecular mass compounds are more or less similar. The specific effect on the penetration rate of size and shape of the penetrating molecules can only be determined if the effect of size and shape can be separated from the influence of solubility characteristics (Idson, 1975).

There seems to be an inverse relationship between the permeant diffusivity and the permeant size. For the SC and the other lipid membranes, it has been suggested that the functional dependence of permeant diffusivity on molecular size is exponential. A model for compounds ranging in molecular weight from 18 to > 750 and log K_{oct} from -3 to +6 was introduced by Potts & Guy in 1992. They observed that the permeability through human skin could be predicted by Equation 3.7:

$$\log k_p = -2,7 + 0,71 \log K_{oct} - 0,0061\ MW \qquad \text{Equation 3.10}$$

Where:

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

They also found that the substitution of molecular weight for molecular volume provides an equivalent fit in the model. In conclusion they found that the apparently sigmoidal dependence of log k_p upon log K_{oct} suggests a non-linear relationship between these parameters. However, when molecular volume is taken into account, the data lies on a three-dimensional surface defined by log K_p, log K_{oct} and the molecular volume (Potts & Guy, 1992).

The direct relationship between log K_p and log K_{oct} was confirmed by Pugh *et al.* (2000) but they found that the relationship between log K_p and MW is also direct, and not inversely as was found by Potts & Guy.

A matter which still raises discussion, however, is the upper limit of molecular weight for permeation. Although some authors mention weights of not more than 3000, there seems to be a limit of about 5000. Nonetheless, influx of compounds into the skin does decrease with increasing molecular weight due a respective decrease in the diffusion coefficient in water (Idson, 1975 & Schalla & Schaeffer, 1982).

3.3.8 Lipophilicity

Basically, the SC barrier is lipophilic with the intercellular lipid lamellae forming a conduit through which drugs must diffuse in order to reach the underlying vascular infrastructure and ultimately to access the systemic circulation. Therefore, lipophilic molecules are better accepted by the SC. A molecule must first be liberated from the formulation and partition into the uppermost SC layer, before diffusing through the entire thickness, and must then repartition into the more aqueous viable epidermis beneath. Ideally, a drug must possess both lipodial and aqueous solubilities; if it is too hydrophobic, the molecule will be unable to transfer into the SC; if it is too lipophilic, the drug will tend to remain in the SC layers (Niak *et al.*, 2000). It appears that the permeability of a molecule is directly related to its lipophilicity (which is often the key parameter) (Potts *et al.,* 1992).

3.3.9 Hydrophilicity

Progesterone, a lipophilic steroid, progressively increased hydrophilicity when one or more hydroxyl substituents were incorporated at different positions on the steroidal skeleton. Effects of these hydrophilic substituents on the permeation of progesterone across both the intact and stripped skin of hairless mice, were studied. The steady state rate of permeation across the intact and stripped skin was found to be approximately proportional to the solubility of drugs in the SC or the viable skin, respectively. As the hydrophilicity of the penetrant increased, it was noted that the solubility of progesterone and its hydroxyl derivatives in the SC decreased gradually. However, the solubility of these progestins in the viable skin was observed to be dependent not only on the penetrant hydrophilicity, but also on the position of the OH-group on the penetrant molecule. The diffusivity of progesterone and its hydroxyl

derivatives across the SC was almost independent on the hydrophilicity of the drugs (Tojo *et al.*, 1987).

3.4 The influence of alkyl chain length on transdermal delivery

It is a well recognised fact that in a homologous series, increasing the non-polar portion of a molecule by extending the length of the chain produces certain characteristic features, such as lowering of the melting point, leading to a decrease in aqueous solubility and an increase in 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 diffusional curves are shown in Figure 3.6:

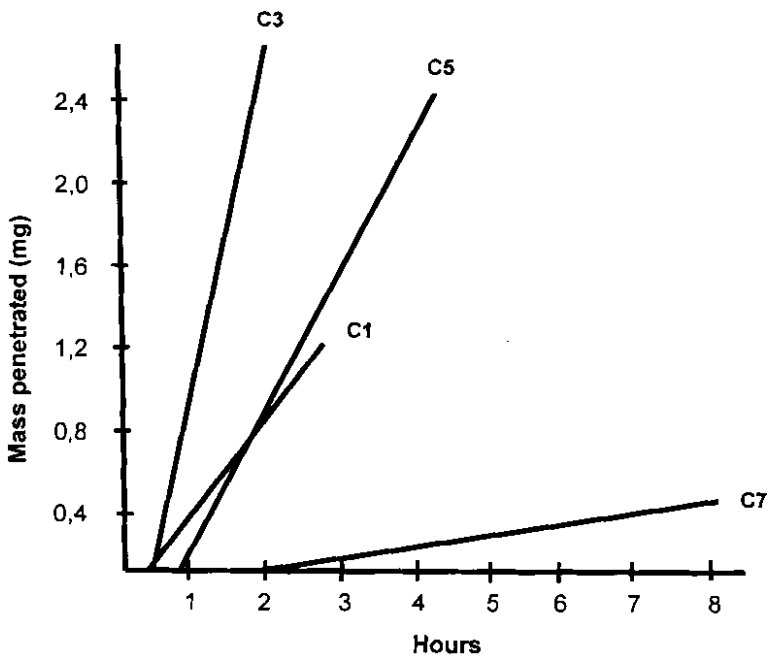


Figure 3.6: Cumulative milligrams penetrated through a membrane for the odd chain length ester studied at 37 °C as a function of time. C1; C3; C5; and C7 designate for methyl, propyl, pentyl and heptyl derivatives, respectively (Flynn & Yalkowski, 1972).

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

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

The aqueous solubility and the flux decreased as the alkyl chain length and molecular weight increased. At the lower end of the range it takes an addition of three methylene units to produce a 10 fold increase in the partition coefficient. While partition coefficients grew exponentially as the homologous series ascended, there appears to be little effect on diffusion coefficients (Flynn, 1989).

3.5 Skin Integrity

The increased use of *in vitro* dermal tests as a replacement to *in vivo* testing has produced a need to ensure that the skin membranes selected are strong yet conservative exposure models. It was therefore necessary to distinguish between undamaged and damaged skin preparations. As a result, a validated electrical resistance (ER) method was developed that offered an attractive alternative approach for qualifying skin preparations appropriate for use on *in vitro* dermal tests (Fasano *et al.*, 2002).

3.6 Pheroids

MeyerZall Laboratories developed Pheroids, previously known as Emzaloids™, as a biomaterial active ingredient delivery system. Pheroids is a patented system comprising of a unique submicron emulsion type formulation that is capable of encapsulating a variety of drugs and delivering these drugs with high efficacy to target sites in the body. The Pheroid consists primarily of plant and essential fatty acids and is stable within a novel therapeutic system. They can entrap, transport and deliver pharmacologically active compounds and other valuable molecules (Grobler, 2004).

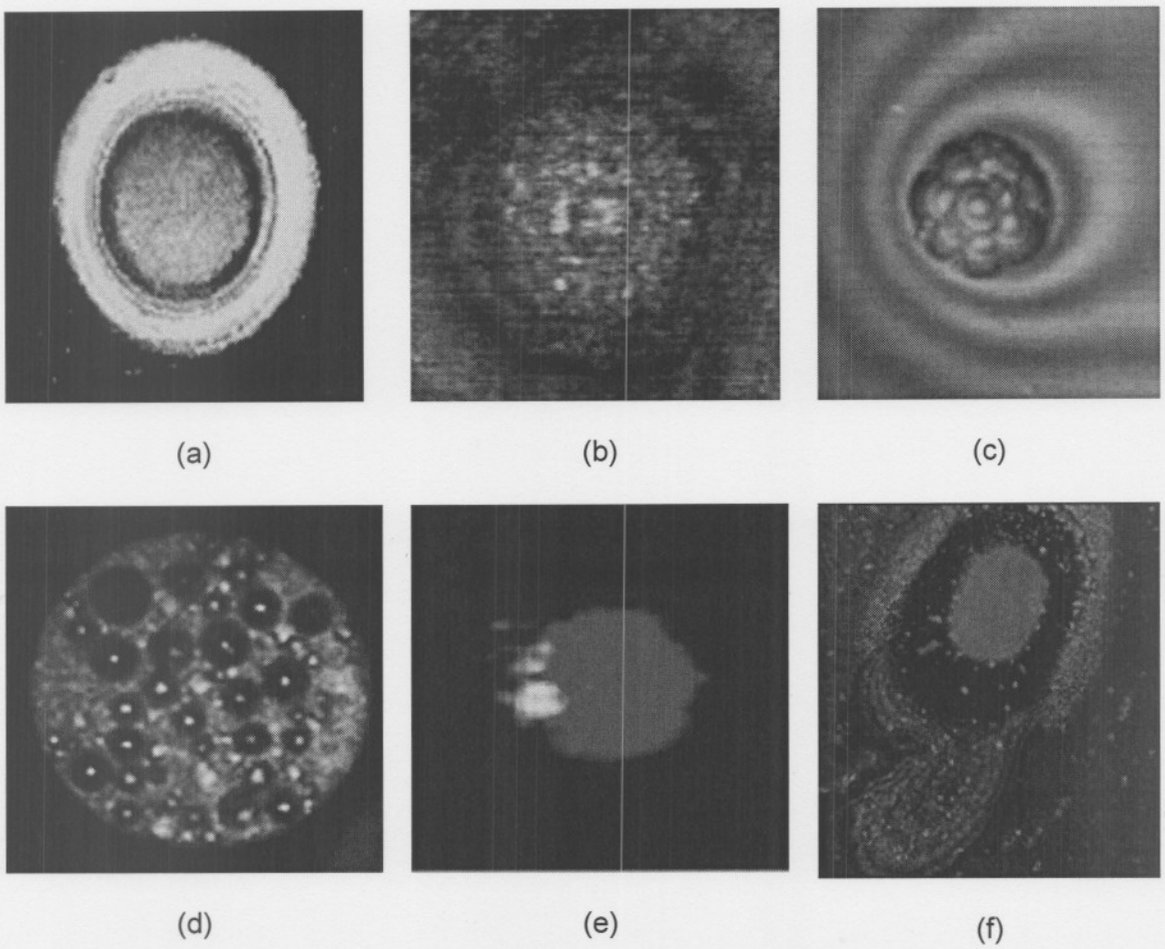


Figure 3.7: The micrographs illustrate some of the basic Pheroid types (Grobler, 2004). Micrograph (a) shows a bilayer membrane vesicle containing rifampicin with a diameter of 100 nm. (b) shows a highly elastic or fluid bilayered vesicle with loose lipid packing, also containing rifampicin. Micrograph (c) illustrates a small pro-Pheroid. (d) shows a reservoir that contain multiple particles of coal tar. The reservoir is a good entrapper of insoluble compounds and has a large loading capacity to surface area. The general size is 1 µm. The Pheroid in (e) shows the process of entrapping fluorescently labelled water soluble diclofenac. It has a diameter smaller than 30 nm. Micrograph (f) depicts a depot with a hydrophobic core containing pro-Pheroid formulation. Also seen is a surrounding hydrophilic zone and an outer vesicle containing zone (Grobler, 2004).

Although there are many existing delivery system technologies, Pheroids are unique among these, given that they are comprised of essential fatty acids which are natural ingredients of the human body. These substances are manipulated in a specific

manner, ensuring important advantages over other delivery systems, such as high entrapment capabilities, fast rate of transport, delivery and stability (Grobler, 2004).

EXPERIMENTAL

4.1 General experimental methods

4.1.1 Instrumentation

4.1.1.1 Nuclear magnetic resonance spectroscopy (NMR)

A Varian Gemini 300 spectrometer was used to record the ^{1}H and ^{13}C NMR spectra at a frequency of 300,075 MHz and 75,462 MHz respectively. All the chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane (δ =0). Abbreviations used to describe the multiplicity of the ^{1}H signals are: s = singlet, d = doublet, dd = doublet of doublets, dt = doublet of triplets, dq = doublet of quartets, t = triplet, q = quartet and m = multiplet.

4.1.1.2 Mass spectroscopy (MS)

An analytical VG 7070E mass spectrometer using fast atom bombardment (FAB) at 70 eV as ionisation technique was used to record the MS spectra.

4.1.1.3 Infrared spectroscopy (IR)

KBr pellets were used to record the IR spectra on a Nicolet Magna – IR 550 spectrometer.

4.1.1.4 Melting points

Differential scanning calorimetry (DSC) was used to determine melting points. The DSC thermograms were recorded with a Mettler Toledo DSC822e700 instrument (Mettler Switzerland). A sample weight of approximately 2 mg, an aluminium crimp cell sample holder, nitrogen gas flow at 30 ml/min and a heating rate of 10 °C/min were necessary conditions for measurement.

4.1.1.5 Integrity

A Model 6401 LCR Databridge (H. Tinsley, Inc., Croydon, Surrey, UK) was used to determine the integrity of the skin before and after the transdermal procedure. Resistance readings were taken by immersing stainless-steel test probe lead tips into the PBS filled donor and receptor compartments.

4.1.2 Chromatographic techniques

4.1.2.1 Thin-layer chromatography (TLC)

Silica gel aluminium backed sheets (Merck® 5554 DC – Alufolien 60 F_{254}), 0,20 mm thick were used for analytical TLC. Dichloromethane (DCM) and Methanol (9:1) were used as mobile phase. For the detection of the compounds the chromatograms were examined under UV-light (245 nm).

4.1.2.2 High pressure liquid chromatography (HPLC)

The HPLC system consisted of an Agilent 1100 auto sampler, UV detector and HP1100 series HPLC pump (Agilent, Polo Alto, CA). A Phenomex (Luna C-18 (2), 150 x 4,60 mm, 5 µm) column was used and in order to prolong column life, a Securityguard pre-column (C-18, 4 x 3 mm) (Phenomex, Terronce, CA) was used. Chemstation for LC Systems software package was used for data analysis. For stavudine and each of the derivatives the flow rate, UV wavelengths, mobile phase compositions and retention times are presented in Table 4.1. The mobile phase consisted of acetonitrile and water with 0,2 % triethylamine and orthophosphoric acid used to adjust the pH of the mobile phase to 7. A different volume was injected for each of the compounds in order to compensate for the difference in concentration for each of the various derivatives.

Table 4.1: Data of the HPLC method

Compound	Flow rate (ml/min)	Wavelength (nm)	Mobile phase H_2O:acetonitrile	Retention time (min)
Stavudine	1	270	90:10	3,959
Stavudine-5'-acetate	1	270	80:20	2,459
Stavudine-5'-propionate	1	270	70:30	3,530
Stavudine-5'-buterate	1	270	60:40	3,177
Stavudine-5'-hexanoate	1	270	60:40	7,033
Stavudine-5'-octanoate	1	270	50:50	8,132
Stavudine-5'-decanoate	1	270	40:60	9,271

4.1.3 Theoretical water solubility

The aqueous solubility of stavudine and each of the derivatives was predicted by Interactive Analysis (http://www.logp.com/) prediction software. These values were compared to the experimental values (§ 5.2.1).

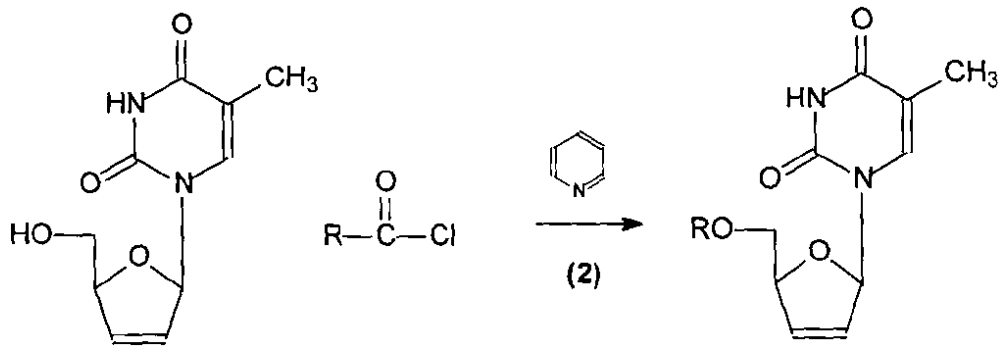
4.1.4 Theoretical partition coefficients

The partition coefficients of stavudine and its derivatives were predicted by ACD Labs, K_{ow}Win (http://www.syrres.com/esc/est_kowdemo.htm) and Interactive Analysis (http://www.logp.com/). These values were compared to the experimental values (§ 5.2.3).

4.2 Synthesis and physical data of compounds

4.2.1 Esterification

The compounds studied were synthesised using standard organic chemical procedures to make ester derivatives. The esterification of d4T (3g; 13,38 mmol) **(1)** was carried out in dry pyridine **(2)** with 1,2 equivalent of a corresponding acid chloride **(4)** – **(9)** at room temperature. Stirring was continued for 72 hours for the highest percentage yield. The mixture was poured into excess DCM and washed several times with distilled water to remove the pyridine. The organic phase was dried over anhydrous magnesium sulphate ($MgSO_4$) and the DCM removed under vacuum. The resulting product was collected and purified by recrystallisation out of methanol.



(1) **(3)**

R = $COCH_3$ **(4)**
= $COCH_2CH_3$ **(5)**
= $CO(CH_2)_2CH_3$ **(6)**
= $CO(CH_2)_4CH_3$ **(7)**
= $CO(CH_2)_6CH_3$ **(8)**
= $CO(CH_2)_8CH_3$ **(9)**

Scheme 4.1: Synthesis of stavudine derivatives

4.2.1.1 Stavudine-5'-acetate (4)

A yield of 3,037 g (85,25%) white crystalline compound was obtained; mp 187,94°C (thermogram 1); R_f 0,71 (dichloromethane : methanol) (9:1); $C_{12}H_{14}N_2O_5$; m/e (FAB, spectrum 1): 267 ($(M+H^+)$ 28,7%), 207 (18,3%), 193 (6,3%), 180 (8,4%), 162 (17,6%), 154 (100,0%), 150 (8,4%), 136 (98,2%), 127 (100,0%), 120 (18,3%), 115 (10,9%), 107 (43,4%); ν_{max} (spectrum 7, KBr, cm^{-1}): 1120; 1260; 1480; 1680; 2790; 3480; δ_H (spectrum 13, 300,075MHz, $CDCl_3$): 1,82 (s; 3H; CH_3), 2,10 (s; 3H; H-2''), 4,20 (dd; 1H; J = 12,45; 3,07 Hz; H-5'b), 4,36 (dd; 1H; J = 12,42; 4,07 Hz H-5'a), 4,98 – 5,01 (m; 1H; H-4'), 5,86 (dq; 1H; J = 2,35; 1,47 Hz; H-2'), 6,23 (dt; 1H; J = 6.00; 1,74 Hz; H-3'), 6,96 (dq; 1H; J = 1,93; 1,37 Hz; H-1'), 7,17 (dd; 1H; J = 2,46; 1,19 Hz; H-6), 9,35 (s; 1H; H-3). δc (spectrum 19, 75,462MHz, $CDCl_3$): 12,52 (CH_3), 20,76 (C-2''), 64,65 (C-5'), 84,13 (C-1'), 89,74 (C-4'), 111,00 (C-5), 127,32 (C-2' / 3'), 133,10 (C-2' / 3'), 135,345 (C-6), 150,83 (C-2), 163,79 (C-4), 170,30 (C-1'').

4.2.1.2 Stavudine-5'-propionate (5)

A yield of 2,360 g (94,40%) white crystalline compound was obtained; mp 191,12°C (thermogram 2); R_f 0,72 (dichloromethane : methanol) (9:1); $C_{13}H_{16}N_2O_5$; m/e (FAB, spectrum 2): 281 ($(M+H^+)$ 24,8%), 207 (19,9%), 180 (11,3%), 165 (13,6%), 154 (100,0%), 150 (12,5%), 136 (100,0%), 127 (100,0%), 120 (31,1%), 115 (16,1%), 107 (65,8%); ν_{max} (spectrum 8, KBr, cm^{-1}): 1130; 1265; 1490; 1700; 2800; 3450; δ_H (spectrum 14, 300,075MHz, $CDCl_3$): 1,08 (t; 1H; J = 7,55 Hz; H-3''), 1,87 (s; 3H; CH_3), 2,32 (dd; 2H; J = 7,56; 15,11 Hz; H-2''), 4,17 (dd; 1H; J = 12,36; 3,03 Hz; H-5'b), 4,39 (dd; 1H; J = 12,40; 4,05 Hz; H-5'a), 4,98-5,01 (m; 1H; H-4'), 5,86 (dq; 1H; J = 2,37; 1,42Hz H-2'), 6,24 (dt; 1H; J = 6,04; 1,72 Hz; H-3'), 6,96 (dq; 1H; J = 1,40; 1,93 Hz; H-1'), 7,18 (dd; 1H; J = 2,45; 1,19 Hz; H-6'), 9,42 (s; 1H; H-3). δc (spectrum 20, 75,462MHz, $CDCl_3$): 8,95 (C-3''), 12,46 (CH_3), 27,32 (C-2''), 64,50 (C-5'), 84,22 (C-1'), 89,74 (C-4'), 111,00 (C-5), 127,28 (C-2' / 3'), 133,10 (C-2' / 3'), 135,34 (C-6), 150,84 (C-2), 163,84 (C-4), 173,85 (C-1'').

4.2.1.3 Stavudine-5'-buterate (6)

A yield of 2,580 g (65,53%) white crystalline compound was obtained; mp 174,26°C (thermogram 3); R_f 0,73 (dichloromethane : methanol) (9:1); $C_{14}H_{18}N_2O_5$; m/e (FAB, spectrum 3): 295 ($(M+H^+)$ 46,7%), 207 (16,9%), 168 (8,1%), 162 (12,2%), 154 (20,1%), 147 (11,1%), 136 (24,6%), 127 (100,0%), 120 (5,5%), 111 (5,0%), 107 (10,0%); ν_{max} (spectrum 9, KBr, cm^{-1}): 1125; 1270; 1480; 1690; 2810; 3470; δ_H

(spectrum 15, 300,075MHz, $CDCl_3$): 0,091 (t; 3H; J = 7,42 Hz; H-4''), 1,62 (m; 2H; J = 14,86; 7,44 Hz; H-3''), 1,88 (s; 3H; CH_3), 2,26 (t; 2H; J = 7,42 Hz; H-2''), 4,18 (dd; 1H; J = 12,36; 3,02 Hz; H-5'b), 4,40 (dd; 1H; J = 12,38; 4,11 Hz; H-5'a), 4,99-5,01 (m; 1H; H-4'), 5,87 (dq; 1H; J = 2,27; 1,37 Hz; H-2'), 6,23 (dt; 1H; J = 6,02; 1,72 Hz; H-3'), 6,96 (dq: 1H; J = 1,95; 1,46 Hz; H-1'), 7,19 (t; 1H; J = 1,22 Hz; H-6), 9,41 (s; 1H; H-3). δc (spectrum 21, 75,462MHz, $CDCl_3$): 12,47 (C-4''), 13,51 (CH_3), 19,24 (C-2''), 35,00 (C-3''), 64,39 (C-5'), 84,24 (C-1'), 89,76 (C-4'), 110,98 (C-5), 127,29 (C-2' / 3'), 133,10 (C-2' / 3'), 135,36 (C-6), 150,83 (C-2), 163,84 (C-4), 173,01 (C-1'').

4.2.1.4 Stavudine-5'-hexanoate (7)

A yield of 4,150 g (96,22%) white crystalline compound was obtained; mp 159,62°C (thermogram 4); R_f 0,75 (dichloromethane : methanol) (9:1); $C_{16}H_{22}N_2O_5$; m/e (FAB, spectrum 4): 323 ($(M+H^+)$ 19,4%), 207 (13,8%), 162 (10,1%), 154 (8,9%), 136 (10,0%), 127 (100,0%), 107 (7,1%); ν_{max} (spectrum 10, KBr, cm^{-1}): 1090; 1260; 1470; 1680; 2820; 3480; δ_H (spectrum 16, 300,075MHz, $CDCl_3$): 0,85 (t; 3H; J = 6,94 Hz; H-6''), 1,23-1,29 (m; 2H; H-5''), 1,23-1,29 (m; 2H; H-4''), 1,60 (m; 2H; J = 7,56 Hz; H-3''), 1,87 (s; 3H; CH_3), 2,23 (t; 2H; J = 7,56 Hz; H-2''), 4,18 (dd; 1H; J = 12,36; 3,16 Hz; H-5'b), 4,34 (dd; 1H; J = 12,36; 4,12 Hz; H-5'a), 4,98-5,02 (m; 1H; H-4'), 5,86 (dq; 1H; J = 2,37; 1,41 Hz; H-2'), 6,22 (dt; 1H; J = 5,99; 1,74 Hz; H-3'), 6,97 (dq; 1H; J = 1,94; 1,39 Hz; H-1'), 7,20 (dd; 1H; J = 2,47; 1,21 Hz; H-6), 9,32 (s; 1H; H-3). δc (spectrum 22, 75,462MHz, $CDCl_3$): 12,50 (C-6''), 13,77 (CH_3), 22,20 (C-2''), 24,43 (C-3''), 31,17 (C-4''), 34,02 (C-5''), 64,43 (C-5'), 84,27 (C-1'), 89,78 (C-4'), 110,99 (C-5), 127,31 (C-2' / 3'), 133,10 (C-2' / 3'), 135,36 (C-6), 150,82 (C-2), 163,80 (C-4), 173,21 (C-1'').

4.2.1.5 Stavudine-5'-octanoate (8)

A yield of 4,530 g (96,23%) white crystalline compound was obtained; mp 154,36°C (thermogram 5); R_f 0,79 (dichloromethane : methanol) (9:1); $C_{18}H_{26}N_2O_5$; m/e (FAB, spectrum 5): 351 ($(M+H^+)$ 24,2%), 225 (6,7%), 207 (14,0%), 162 (9,4%), 154 (38,1%), 136 (36,7%), 127 (100,0%), 120 (8,6%), 107 (17,8%); ν_{max} (spectrum 11, KBr, cm^{-1}): 1100; 1250; 1470; 1660; 2880; 3460; δ_H (spectrum 17, 300,075MHz, $CDCl_3$): 0,83 (t; 3H; J = 6,8 Hz; H-8''), 1,21-1,28 (m; 2H; H-4''), 1,21-1,28 (m; 2H; H-5''), 1,21-1,28 (m; 2H; H-6''), 1,21-1,28 (m; 2H; H-7''), 1,58 (t; 2H; J = 7,48 Hz; H-3''), 1,88 (s; 3H; CH_3), 2,28 (t; 2H; J = 7,54 Hz; H-2''), 4,15 (dd; 1H; J = 12,42; 3,08 Hz; H-5'b), 4,40 (dd; 1H; J = 12,36; 4,12 Hz; H-5'a), 4,99-5,01 (m; 1H; H-4'), 5,86 (dq; 1H; J = 2,34; 1,38 Hz; H-2'), 6,24 (dt; 1H; J = 6,03; 1,73 Hz; H-3'), 6,96 (m; 1H; J = 6,61;

1,69 Hz; H-1'), 7,12 (dd; 1H; J = 2,42; 1,12 Hz; H-6), 9,31 (s; 1H; H-3).). δc (spectrum 23, 75,462MHz, $CDCl_3$): 12,51 (C-8''), 13,94 (CH_3), 22,48 (C-2''), 24,75 (C-3''), 28,80 (C-4''), 28,98 (C-5''), 31,53 (C-6''), 34,06 (C-7''), 64,24 (C-5'), 84,27 (C-1'), 89,77 (C-4'), 111,00 (C-5), 127,32 (C-2' / 3'), 133,10 (C-2' / 3'), 135,36 (C-6), 150,81 (C-2), 163,79 (C-4), 173,21 (C-1'').

4.2.1.6 Stavudine-5'-decanoate (9)

A yield of 4,082 g (80,61%) white crystalline compound was obtained; mp 151,05°C (thermogram 6); R_f 0,83 (dichloromethane : methanol) (9:1); $C_{20}H_{30}N_2O_5$; m/e (FAB, spectrum 6): 379 ($(M+H^+)$ 15,6%), 207 (14,3%), 162 (10,0%), 154 (14,6%), 136 (15,4%), 127 (100%), 107 (9,1%); ν_{max} (spectrum 12, KBr, cm^{-1}): 1120; 1270; 1480; 1700; 2890; 3460; δ_H (spectrum 18, 300,075MHz, $CDCl_3$): 0,84 (t; 3H; J = 6,73 Hz; H-10''), 1,25 (s; 2H; H-4''), 1,25 (s; 2H; H-5''), 1,25 (s; 2H; H-6''), 1,25 (s; 2H; H-7''), 1,25 (s; 2H; H-8''), 1,25 (s; 2H; H-9''), 1,6 (t; 2H; J = 7,31 Hz; H-3''), 1,89 (s; 3H; CH_3), 2,28 (t; 2H; J = 7,56 Hz; H-2''), 4,16 (dd; 1H; J = 12,43; 3,09 Hz; H-5'b), 4,44 (dd; 1H; J = 12,40; 4,02 Hz; H-5'a), 4,99-5,01 (m; 1H; H-4'), 5,87 (dq; 1H; J = 2,34; 1,37 Hz; H-2'), 6,24 (dt; 1H; J = 6,04; 1,72 Hz; H-3'), 6,98 (dq; 1H; J = 2,17; 1,65 Hz; H-1'), 7,18 (dd; 1H; J = 2,41; 1,16 Hz; H-6), 9,22 (s; 1H; H-3). δc (spectrum 24, 75,462MHz, $CDCl_3$): 12,54 (C-10''), 14,01 (CH_3), 22,57 (C-2''), 24,77 (C-3''), 29,05 (C-4''), 29,15 (C-5''), 29,17 (C-6''), 29,32 (C-7''), 31,76 (C-8''), 34,08 (C-9''), 64,43 (C-5'), 84,29 (C-1'), 89,78 (C-4'), 111,01 (C-5), 127,32 (C-2' / 3'), 133,12 (C-2' / 3'), 135,36 (C-6), 150,79 (C-2), 163,76 (C-4), 173,22 (C-1'').

4.3 Physicochemical properties and solubility

4.3.1 Aqueous solubility

Saturated solutions of stavudine and its derivatives were used to determine the aqueous solubility in a phosphate buffer solution (PBS) at pH 7. In a water bath at 32°C the slurries were stirred with magnetic bars for 24 hours. The solutions were saturated at all times with an excess of the solute present. The solutions were filtered after 24 hours and diluted prior to being analysed by HPLC to determine the concentration of the solute dissolved in the solvent. The experiment was performed in triplicate.

4.3.2 Partition coefficient

Equal volumes of *n*-octanol and PBS pH 7 were saturated with one another by stirring for 24 hours and then separated. An excess of stavudine and of each derivative was dissolved in 1 ml pre-saturated *n*-octanol and 1 ml pre-saturated PBS buffer. The solutions were saturated at all times with an excess of the solute present. The solutions were stoppered and agitated for 90 min. thereafter they were filtered and centrifuged at 4000 rpm for 20 min at 25 °C. The aqueous phase was diluted with PBS and the *n*-octanol phase with methanol prior to being analysed by HPLC. The partition coefficients (K_{oct}) were calculated as logarithmic ratios of the stavudine derivative concentrations in the *n*-octanol phase to the concentrations in the PBS buffer. The experiment was performed in triplicate.

4.4 Preparation of Pheroids

Pheroids, as utilised in this study, is a patented system and manufactured by Dale Elgar, Department of Pharmaceutics, School of Pharmacy, North-West University, Potchefstroom. The specific Pheroid format, 'micro-sponges', was used.

4.5 Transdermal permeation

4.5.1 Preparation of skin

For the permeation studies female human abdominal skin, obtained subsequent to cosmetic procedures from the Sandton Surgical Centre, Johannesburg, was used. The skin was separated from the fat layer using a scalpel after which the epidermis was removed by placing the skin in HPLC water at 60 °C for 60 seconds. Teasing gently, the epidermis was removed from the skin taking special care that the integrity of the skin was not ruptured as this would compromise the results. In a bath filled with HPLC water, the epidermis was cautiously placed on Whatman® filter paper and left to dry before being wrapped in foil and stored in a freezer at - 20 °C. The epidermis was used within 6 months after being prepared. After examining the epidermis for any defects it was mounted on the Franz diffusion cells.

4.5.2 Donor solutions preparation

In a water bath, saturated solutions of stavudine and its derivatives were prepared by stirring the slurries with magnetic bars for 24 hours at 32°C. These slurries were used to fill the donor compartments of vertical Franz diffusion cells. An excess amount of

solute was present at all times in a phosphate buffer solution (PBS) at pH 7. The same method was used for the Pheroids. The compound was saturated in the Pheroids solution and the receptor compartments were filled with isotonic PBS buffer at pH 7,4 as before.

4.5.3 Skin penetration method

Vertical Franz diffusion cells were used for the skin penetration studies. Each diffusion cell had a 1,0751 cm^2 effective diffusion area and a 2,0 ml receptor compartment. Vacuum grease was applied on the surface of each cell to keep it from leaking. Magnetic bars were inserted in the receptor compartments and the epidermal skin layer cautiously positioned on the lower half of the diffusion cell. The donor and receptor compartments were separated by the epidermal skin layer and clamped together. The receptor compartments were filled with 2,0 ml isotonic PBS with a pH of 7,4 and the donor compartments filled with 1,0 ml buffer solution before being equilibrated for half an hour in a water bath at 37 °C. Exceptional care was taken to ensure that no air bubbles came between the epidermis and the buffer solution. The integrity of the skin was measured before 1 ml of the newly prepared saturated solution was added to the donor compartments and covered with Parafilm®. The latter prevents any evaporation of the constituents within the saturated solution for the duration of the experiment. The donor compartments were saturated at all times with an excess amount of solute present.

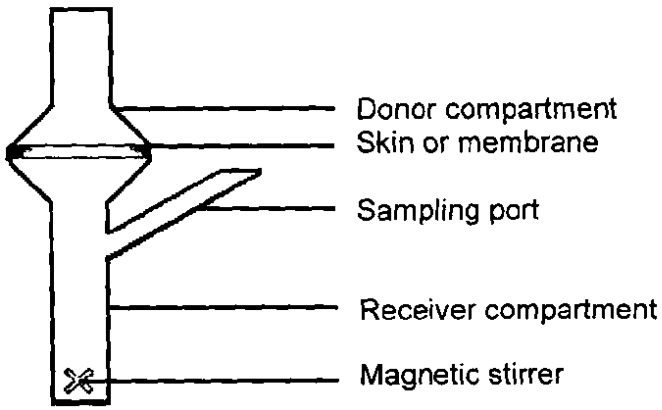


Figure 4.1: Schematic representation of the standard, original diffusion cell developed by Franz (Bronaugh & Collier, 1993).

The entire receptor volumes were withdrawn after 2, 4, 6, 8, 10, 12 and 24 hours and replaced with 37 °C fresh buffer solution at pH 7,4 to mimic sink conditions as they occur in the human body. The experiments were conducted over 24 hour periods.

These samples were assayed directly by HPLC to determine the drug concentration of each of the compounds that had permeated through the epidermis.

Data collected for all cells was used, except for those which leaked.

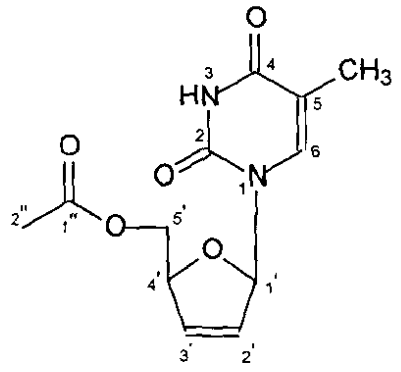
RESULTS AND DISCUSSION

5.1 Stavudine esterification

The stavudine derivatives were synthesised, isolated and analysed by NMR, MS, IR and DSC. It was obvious from TLC that esterfication had occurred between stavudine **(1)** and the six different acid chlorides to give products **(4)** – **(9)**. NMR analysis indicated that the samples were all pure compounds, after purification by recrystallisation was successful. The ^{1}H and ^{13}C NMR data of all the stavudine derivatives were similar to that of stavudine, therefore only differences will be discussed.

5.1.1 Structures of synthesised compounds

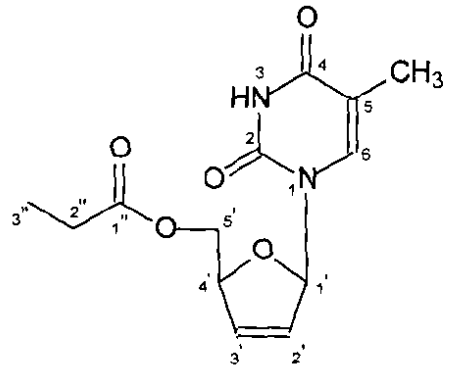
5.1.1.1 Stavudine-5'-acetate (4)



(4)

The ^{13}C NMR data of **(4)** were similar to that of stavudine, except that the signal of the carbonyl carbon atom (C-1") appeared at δ 170,30 in the ^{13}C NMR spectrum, while the presence of the methyl group was indicated by the signal at δ 20,78 representing the carbon atom C-2". In the ^{1}H NMR spectrum the singlet at δ 2,10 represents H-2". The MS data confirmed the presence of the molecular ion of **(4)** at *m/z 267*, corresponding to a molecular formula of $C_{12}H_{14}N_2O_5$. In the IR spectrum the carbonyl group stretching vibration was at 1700 cm^{-1}, NH stretching vibration at 1480 cm^{-1} and the stretching vibration of the nitrogen at 1120 cm^{-1}.

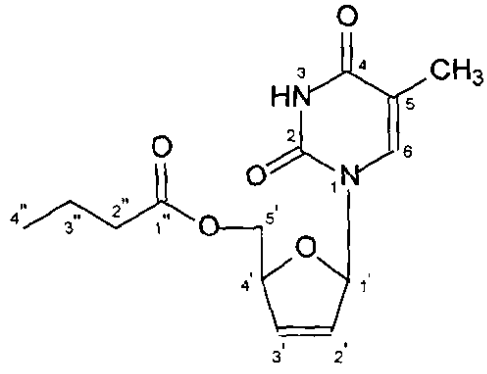
5.1.1.2 Stavudine-5'-propionate (5)



(5)

The ^{13}C NMR data of **(5)** were similar to that of stavudine, except that the signal of the carbonyl carbon atom (C-1") appeared at δ 173,85 in the ^{13}C NMR spectrum, while the presence of the methyl and methylene groups was indicated by signals at δ 8,95 and 27,32 representing the carbon atoms C-3" and C-2" respectively. In the ^{1}H NMR spectrum the triplet at δ 1,08 and the doublet of doublets at 2,32 represents H-3" and H-2" respectively. The MS data confirmed the presence of the molecular ion of **(5)** at m/z 281, corresponding to a molecular formula of $C_{13}H_{16}N_2O_5$. The IR spectrum stretching vibrations for the propionate derivative was relatively the same as for the acetate derivative.

5.1.1.3 Stavudine-5'-buterate (6)

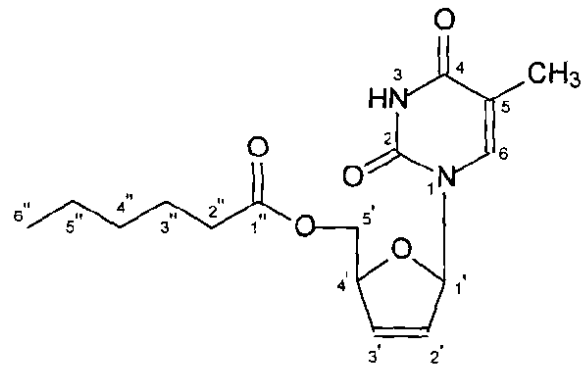


(6)

The ^{13}C NMR data of **(6)** were similar to that of stavudine, except that the signal of the carbonyl carbon atom (C-1") appeared at δ 173,01 in the ^{13}C NMR spectrum, while the presence of the methylene groups were indicated by signals at δ 19,24 and 35,00 representing the carbon atoms C-2" and C-3" respectively, with the methyl

group, C-4" represented by the peak at δ 12,47. In the ^{1}H NMR spectrum the triplet at δ 0,091, the multiplet at 1,62 and the triplet at 2,26 represents H-4", H-3" and H-2" respectively. The MS data confirmed the presence of the molecular ion of **(6)** at m/z 295, corresponding to a molecular formula of $C_{14}H_{18}N_2O_5$. The IR spectrum stretching vibrations for the buterate derivative was relatively the same as for the acetate derivative.

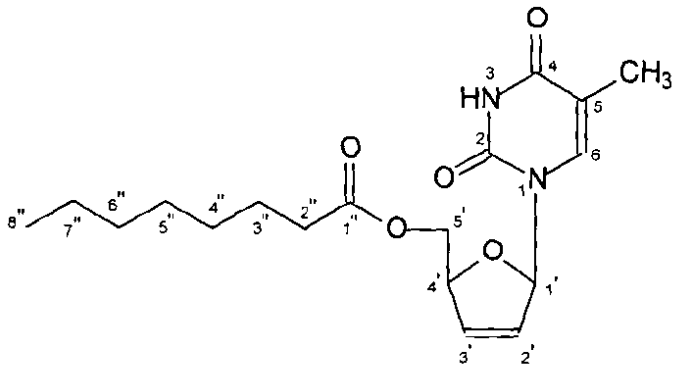
5.1.1.4 Stavudine-5'-hexanoate (7)



(7)

The ^{13}C NMR data of **(7)** were similar to that of stavudine, except that the signal of the carbonyl carbon atom (C-1") appeared at δ 173,21 in the ^{13}C NMR spectrum, while the presence of the methylene groups were indicated by signals at δ 22,20; 24,43; 31,17 and 34,02 representing the carbon atoms C-2", C-3", C-4" and C-5" respectively, with the methyl group, C-6" represented by the peak at δ 12,50. In the ^{1}H NMR spectrum the triplets at δ 0,85 and 2,23 represents H-6" and H-2" respectively and the multiplets at 1,23-1,29 and 1,60 represents H-5", H-4" and H-3" respectively. The MS data confirmed the presence of the molecular ion of **(7)** at m/z 323, corresponding to a molecular formula of $C_{16}H_{22}N_2O_5$. The IR spectrum stretching vibrations for the hexanoate derivative was relatively the same as for the acetate derivative.

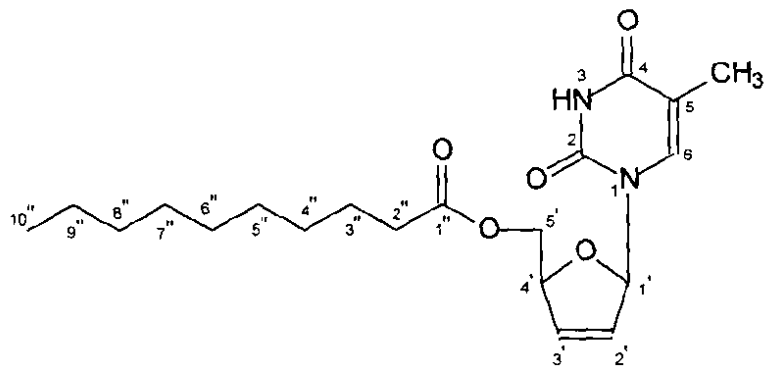
5.1.1.5 Stavudine-5'-octanoate (8)



(8)

The ^{13}C NMR data of **(8)** were similar to that of stavudine, except that the signal of the carbonyl carbon atom (C-1") appeared at δ 173,21 in the ^{13}C NMR spectrum, while the presence of the methylene groups were indicated by signals at δ 22,48 24,75; 28,80; 28,98; 31,53 and 34,06 representing the carbon atoms C-2"; C-3"; C-4"; C-5"; C-6" and C-7" respectively, with the methyl group, C-8" represented by the peak at δ 12,51. In the ^{1}H NMR spectrum the triplet at δ 0,83 represents H-8", the multiplet at 1,21-1,28 represents H-4", H-5", H-6" and H-7" and the triplets at 1,58 and 2,28 represents H-3" and H-2" respectively. The MS data confirmed the presence of the molecular ion of **(8)** at m/z 351, corresponding to a molecular formula of $C_{18}H_{26}N_2O_5$. The IR spectrum stretching vibrations for the octanoate derivative was relatively the same as for the acetate derivative.

5.1.1.6 Stavudine-5'-decanoate (9)



(9)

The ^{13}C NMR data of **(9)** were similar to that of stavudine, except that the signal of the carbonyl carbon atom (C-1") appeared at δ 173,22 in the ^{13}C NMR spectrum,

while the presence of the methylene groups were indicated by signals at δ 22,57, 24,77; 29,05; 29,15; 29,17; 29,32; 31,76 and 34,08 representing the carbon atoms C-2", C-3", C-4", C-5", C-6", C-7", C-8" and C-9" respectively, with the methyl group, C-10" represented by the peak at δ 12,54. In the ^{1}H NMR spectrum the triplet at δ 0,84 represents H-10", the singlet at 1,25 represents H-4", H-5", H-6", H-7",H-8" and H-9" and the triplets at 1,60 and 2,28 represents H-3" and H-2" respectively. The MS data confirmed the presence of the molecular ion of **(9)** at m/z 379, corresponding to a molecular formula of $C_{20}H_{30}N_2O_5$. The IR spectrum stretching vibrations for the decanoate derivative was relatively the same as for the acetate derivative.

5.1.2 Conclusion

The ^{1}H and ^{13}C NMR, IR and MS spectroscopy data confirmed that the stavudine derivatives were successfully synthesised.

5.2 Physicochemical properties

5.2.1 Aqueous solubility

Presented in Table 5.1 below, are the predicted aqueous solubility values, obtained from interactive analysis (IA) prediction software together with the experimentally determined aqueous solubility values.

Table 5.1: The aqueous solubility values of stavudine and its synthesised derivatives

	Aqueous solubility (mg/ml)	
Compound	**Predicted[a] (IA)**	**Experimental[b]**
Stavudine	14,578	104,75
Stavudine-5'-acetate	0,1713	5,17
Stavudine-5'-propionate	0,0733	1,73
Stavudine-5'-buterate	0,0961	1,05
Stavudine-5'-hexanoate	0,0339	0,11
Stavudine-5'-octanoate	0,0142	0,08
Stavudine-5'-decanoate	0,4414	nd*

[a] calculated using IA (interactive analysis)

[b] experiments conducted at pH 7 in PBS buffer at 32°C

*not dectectable

5.2.2 Discussion

The solubilities of the derivatives in water are lower than that of stavudine, which inherently has a higher water solubility (104,75 mg/ml). By referring to the above results, the solubility in general decreased with an increase in chain length in accordance with data in the literature (§ 3.3.1.2) (Abdou, 1989).

The experimentally determined aqueous solubility was higher than predicted values. This difference in data may be attributed to the method of calculation, the fact that the software does not specify temperature, nor the buffer used or the pH at which they were calculated. The solubility values are likely to be influenced by a different temperature, pH values and buffers.

5.2.3 Partition coefficient

Presented in Table 5.2 below, are the predicted partition coefficient values, obtained from interactive analysis (IA), ACD Labs and K_{ow}Win prediction software, together with the experimentally determined partition coefficients (log P) values.

Table 5.2: The partition coefficients of stavudine and its derivatives (log P)

Compound	Predicted[a] (IA)	Predicted[b] (ACD)	Predicted[c] (K_{ow}Win)	Experimental[d]
Stavudine	-0,53	-0,90	-0,79	-0,8462
Stavudine-5'-acetate	0,40	-0,52	0,21	-0,4085
Stavudine-5'-propionate	1,07	0,01	0,71	0,2666
Stavudine-5'-buterate	0,58	0,54	1,20	0,8883
Stavudine-5'-hexanoate	1,34	1,60	2,18	18,605
Stavudine-5'-octanoate	2,25	2,67	3,16	26,580
Stavudine-5'-decanoate	3,23	3,73	4,14	30,600

[a] calculated using (IA) interactive analysis

[b] calculated using ACD software

[c] calculated using K_{ow}Win

[d] experiments conducted at pH 7 in PBS buffer

5.2.4 Discussion

As expected from the above data, all the esters are more lipophillic than the parent compound in terms of partition coefficients between *n*-octanol and pH 7,0 buffer, due to an increase in the alkyl chain length. This leads to an increase in partition coefficients in accordance with data in the literature (§ 3.3.3) (Guy & Hadgraft, 1989).

ACD Labs prediction software gave values relatively close to the experimental partition coefficients that were performed at pH 7,0. Thus, for ACD programs to correlate with the experimental values it might predict values at pH 7,0. The difference between data for the other programs may be attributed to the method of calculation, the fact that the software does not specify the pH at which they were calculated, or the buffers used. Partition coefficient values are likely to be influenced by different pH values and buffers.

The results of the experimental partition coefficients specify that esterification of stavudine results in a higher partition coefficient. The aqueous solubility data validate these results and show that compounds with higher partition coefficients present an increased lipophilicity and therefore have lower aqueous solubilities.

5.3 Transdermal penetration of stavudine and its derivatives

5.3.1 Transdermal penetration

In Appendix 1, the flux was calculated by using the *average* cumulative amount of compound that had penetrated through the skin as a function of time.

However, to account for the possibility of outlier values, the following research methodology was followed:

The flux for every individual cell was determined by the slope of a straight line of time (independent variable) vs. average cumulative amount per area (dependent variable) graph. Consider the following graph as example:

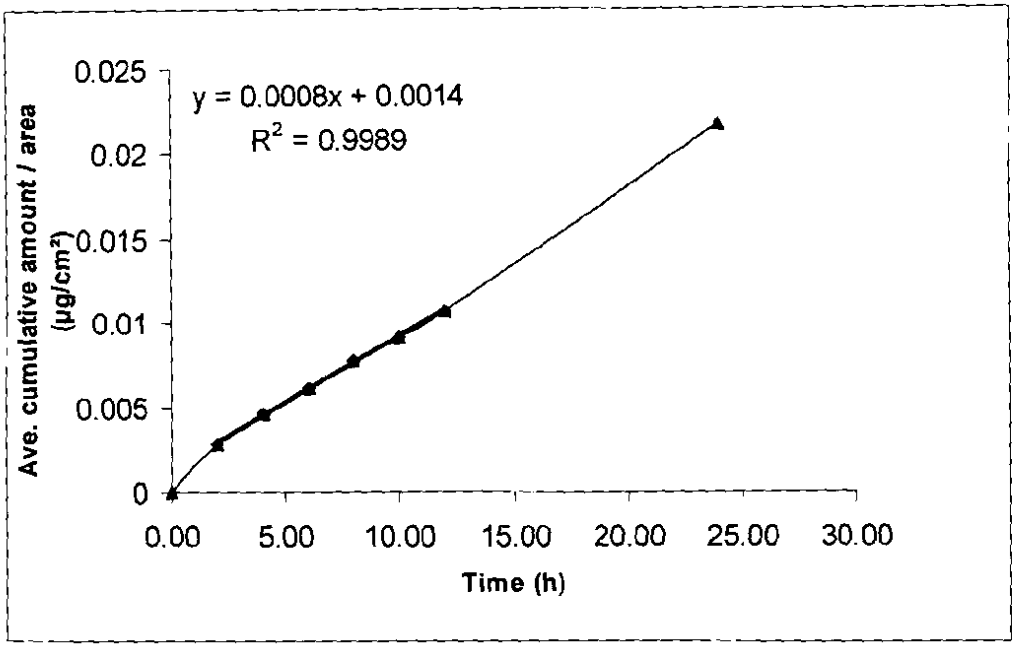


Figure 5.1: Average cumulative amount per area vs. time.

The procedure was repeated for a number of cells for each compound and the expected flux calculated by using the *median* value. This can be motivated by looking at the following box-plot as example:

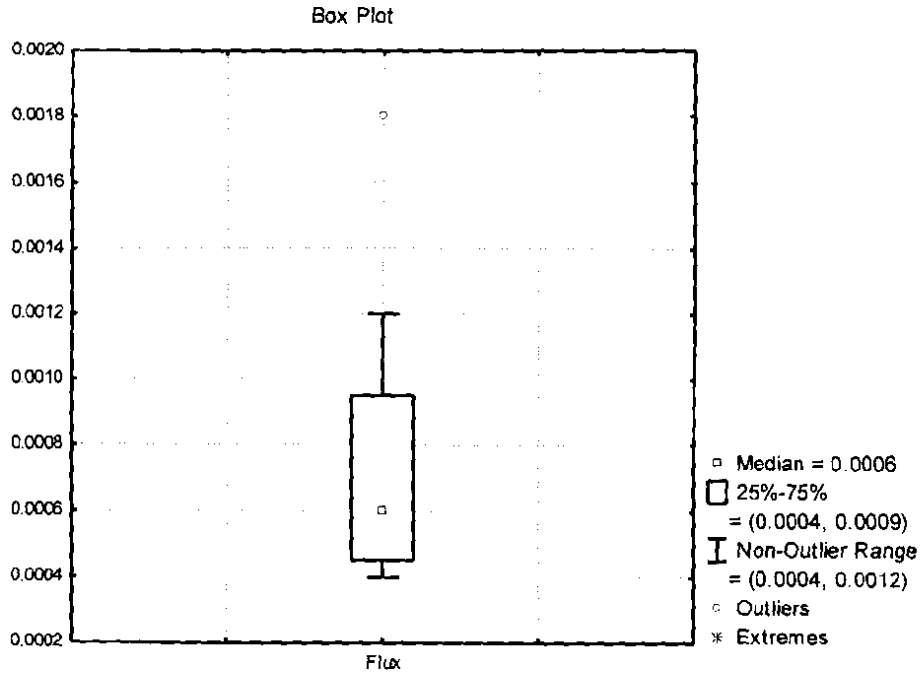


Figure 5.2: Box-plot to show the skewedness of data when using the mean flux value

The mean is influenced by skewed distributions. Hence if some, or all of the flux distributions are skewed, with the possibility of outliers, the median will be used instead of the mean. This is due to the median being more robust should the underlying data be skewed as was found with the flux values in this study.

Flux values (J_{max}) can be estimated for stavudine and its derivatives when aqueous solubility, log P and molecular mass (MW) values are obtained. The Potts and Guy equation (Equation 5.1) can be used to calculate the log k_p, from where the permeability coefficient (k_p) may be obtained (Hadgraft *et al.*, 2000). At the same pH, the estimated flux ($\mu g/cm^2.h$) can be obtained from the product of the permeability coefficient and the aqueous solubility using equation 5.2.

$$\text{Log } k_p = -2,7 + 0,71 \log P - 0,0061 \text{ MW} \qquad \text{Equation 5.1}$$

$$J_{max} = k_p \text{ x aqueous solubility} \qquad \text{Equation 5.2}$$

Presented in Table 5.3 is the experimentally determined median flux data of the different stavudine derivatives in PBS and in Pheroids, together with the predicted flux values, using predicted partition coefficient values as well as experimentally determined partition coefficient values.

Table 5.3: Experimental and predicted flux data (J).

	Experimental ($\mu g/cm^2.h$)		**Predicted($\mu g/cm^2.h$)**	
Compound	**PBS**	**Pheroid**	**a**	**b**
Stavudine	$1,46x10^{-2}$ ± 0,0314	$1,37x10^{-2}$ ± 0.0064	$2,21x10^{-3}$	$2,15x10^{-3}$
Stavudine-5'-acetate	$0,06x10^{-2}$ ± 0,0004	$0,02x10^{-2}$ ± 0.0001	$0,12x10^{-3}$	$0,11x10^{-3}$
Stavudine-5'-propionate	$0,06x10^{-2}$ ± 0,0005	$1,86x10^{-2}$ ± 0.0236	$0,11x10^{-3}$	$0,07x10^{-3}$
Stavudine-5'-buterate	$0,05x10^{-2}$ ± 0,0002	$2,02x10^{-2}$ ± 0.0111	$0,14x10^{-3}$	$0,08x10^{-3}$
Stavudine-5'-hexanoate	$0,02x10^{-2}$ ± 0,0000	$0,05x10^{-2}$ ± 0.0010	$0,05x10^{-3}$	$0,03x10^{-3}$
Stavudine-5'-octanoate	nd*	$0,03x10^{-2}$ ± 0.0000	$0,09x10^{-3}$	$0,09x10^{-3}$
Stavudine-5'-decanoate	nd*	nd*	nd*	nd*

a calculated using experimental log P values in the Potts and Guy equation (Equation 5.1 & 5.2)

b calculated using ACD predicted log P values in the Potts & Guy equation (Equation 5.1 & 5.2)

*not detectable

The following graph (Figure 5.3) shows the medians of the experimental transdermal flux data for stavudine and all the stavudine derivatives in PBS and in Pheroids.

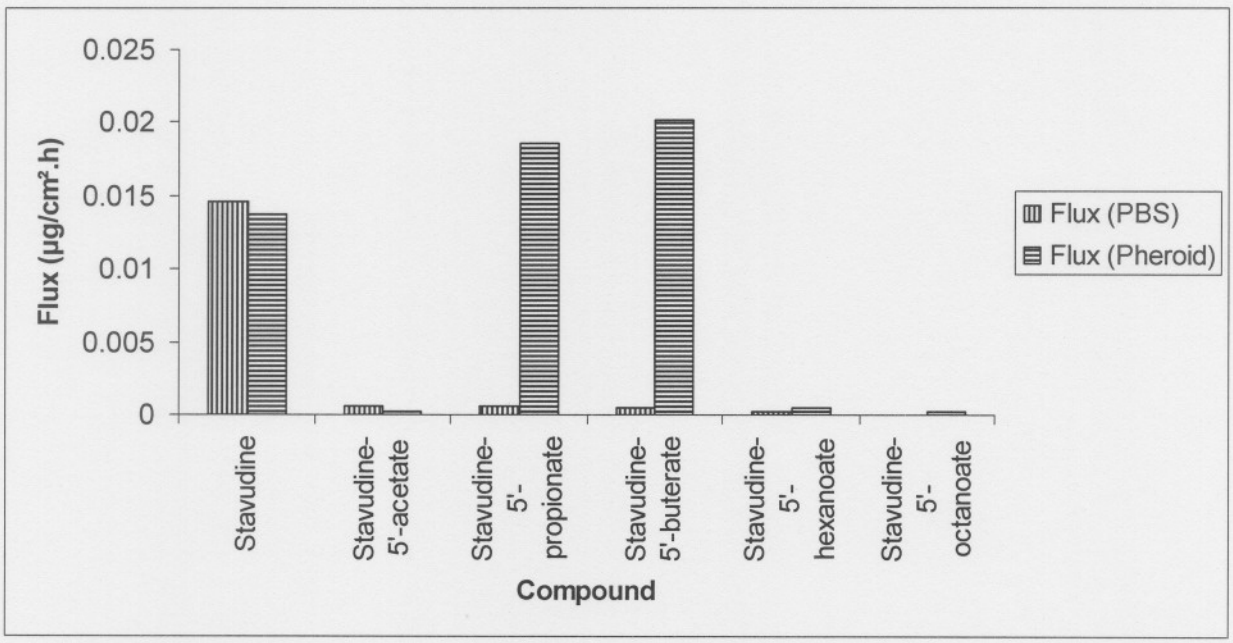


Figure 5.3: Experimental stavudine and stavudine derivative flux data in PBS and in Pheroids.

5.3.2 Discussion

The experimental transdermal flux of stavudine ($1{,}46 \times 10^{-2}$ µg/cm^2.h) in PBS was much higher than that of its derivatives. Stavudine was the compound with the highest aqueous solubility and the lowest partition coefficient, thus being more hydrophilic than the other compounds. According to the mosaic theory, water-soluble substances may penetrate membranes since the proteins in skin may take up water, resulting in swollen membranes (Rothman, 1954). The higher flux is most probably the result of stavudine penetrating through the rich protein spaces between the SC and the corneosytes (Foldvari, 2000). The SC is hydrated in a polar solution for 24 hours making it possible for hydrophilic compounds to penetrate (Hull, 2002).

The propionyl ($1,86x10^{-2}$ $\mu g/cm^2.h$) and the buteryl derivatives ($2,02x10^{-2}$ $\mu g/cm^2.h$) were the compounds with the highest transdermal flux in Pheroids, because of the Pheroid enhancing the penetration for this specific compounds through the skin. The mechanism by which this happen, is unknown at this stage. Also seen in Figure 5.1 is that the flux of stavudine was higher in PBS than in Pheroids.

Stavudine-5'-decanoate had a log P value of approximately 3, but had no flux. This can be ascribed for this specific derivative being insoluble in water. This just proves once again that to cross the SC, a drug should possess both hydrophilic and lipohillic properties.

The predicted flux values were obtained by using a) experimentally predicted partition coefficient data and b) predicted partition coefficient values obtained from ACD software in the Potts and Guy equation (Equation 5.1 & 5.2). No correlation existed between the experimental and predicted flux data. This could be due to the model used to predict transdermal flux values as it does not regard the state of ionisation. It therefore makes it hard to predict flux and compare it with experimental flux values.

From Figure 5.1 can be seen that no correlation exists between the flux values of PBS and the flux values of Pheroids of the different compounds.

5.3.3 Flux and physicochemical properties of stavudine and its derivatives

The so-called Spearman rank correlation was used to calculate the correlation between the expected median flux and the determined physicochemical properties of stavudine and the synthesised derivatives.

5.3.3.1 Median flux vs. aqueous solubility

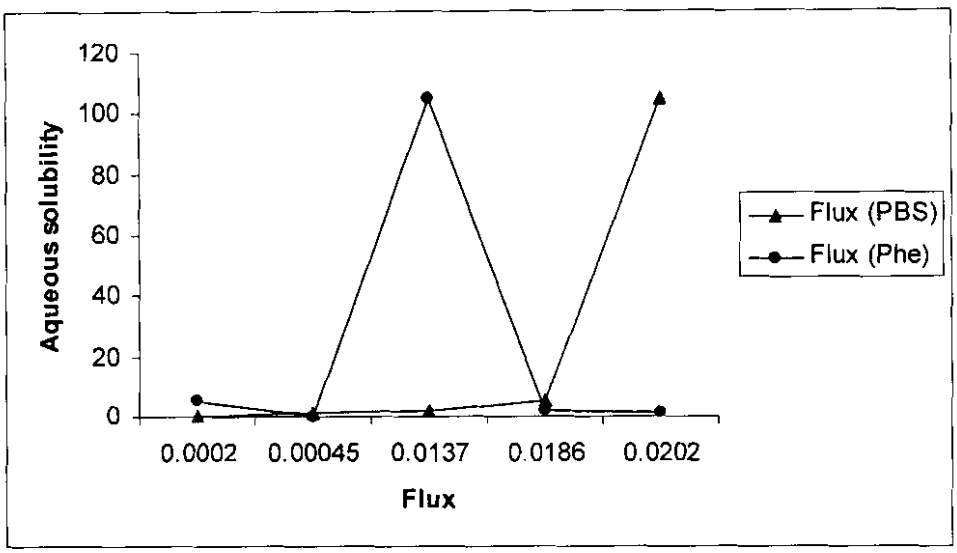


Figure 5.4: Aqueous solubility vs. time for stavudine and its derivatives in PBS and in Pheroids.

As the aqueous solubility of the compounds increased the flux also increased when the experiment was performed in PBS. Therefore a correlation exists between flux and the water solubility of the drug. No correlation exists between the flux values of the derivatives in Pheroids and the aqueous solubility of stavudine or its derivatives.

5.3.3.2 Median flux vs. molecular mass

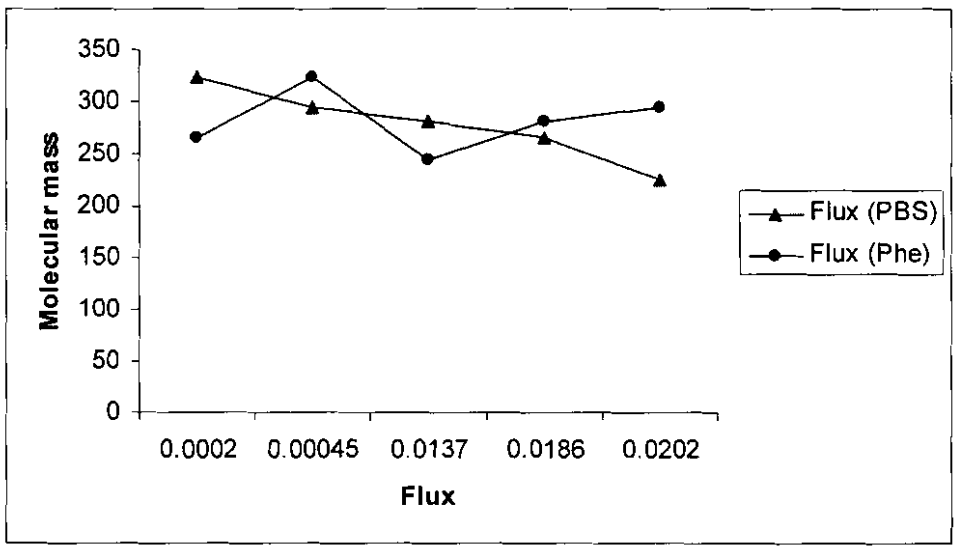


Figure 5.5: Molecular mass vs. time for stavudine and its derivatives in PBS and in Pheroids.

The flux value increased with a decrease in molecular mass when the experiment was performed in PBS. Therefore a negative correlation exists between flux and the molecular mass of the drug. No correlation exists between the flux values of the derivatives in Pheroids and the molecular mass of stavudine or its derivatives.

5.3.3.3 Median flux vs. log D

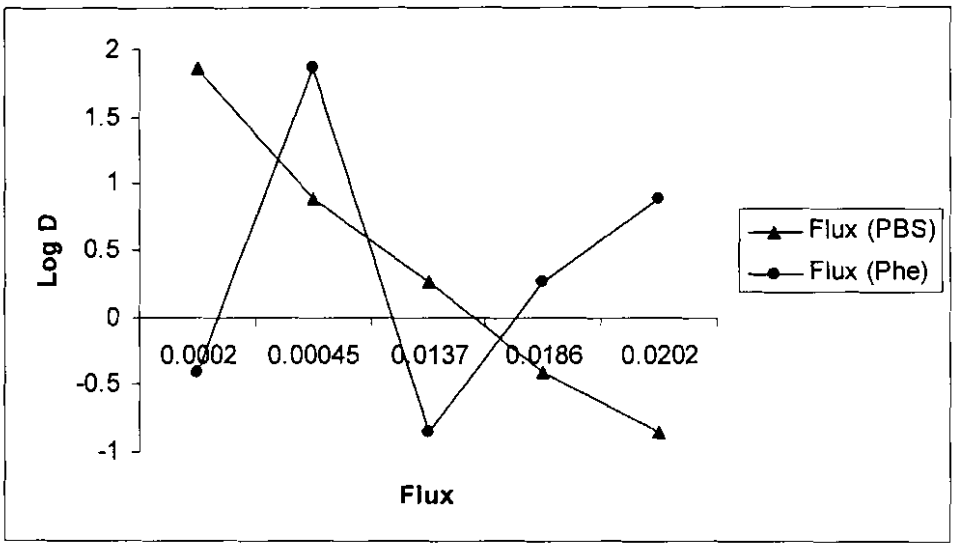


Figure 5.6: Log D vs. time for stavudine and its derivatives in PBS and in Pheroids.

The flux value increased with a decrease in log D when the experiment was performed in PBS. Therefore a negative correlation exists between flux and the log D value of the drug. No correlation exists between the flux values of the derivatives in Pheroids and the log D value of stavudine or its derivatives.

5.3.3.4 Median flux vs. melting point

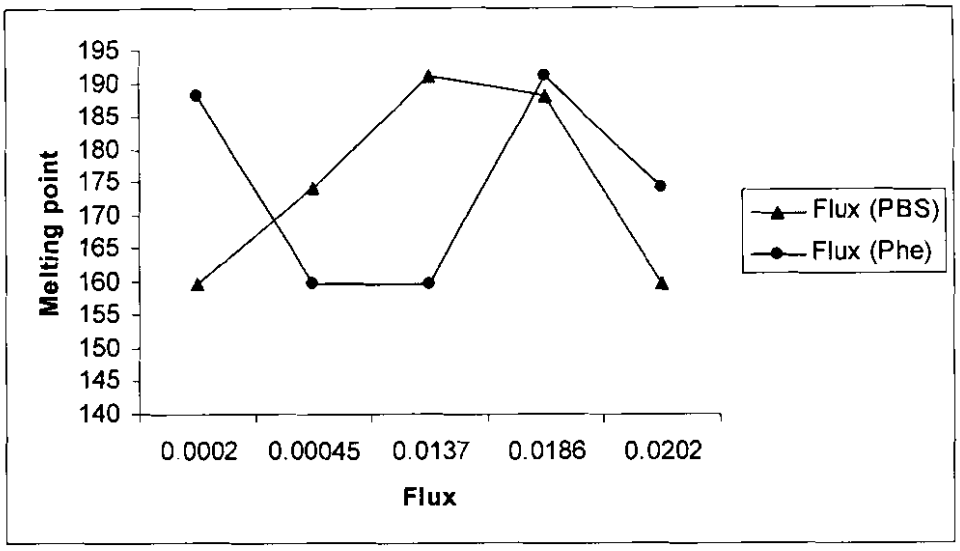


Figure 5.6: Melting point vs. time for stavudine and its derivatives in PBS and in Pheroids.

No correlation exists between the flux and the melting point whether the experiment was performed in PBS or in Pheroids.

5.3.3.5 Median flux vs. integrity (before)

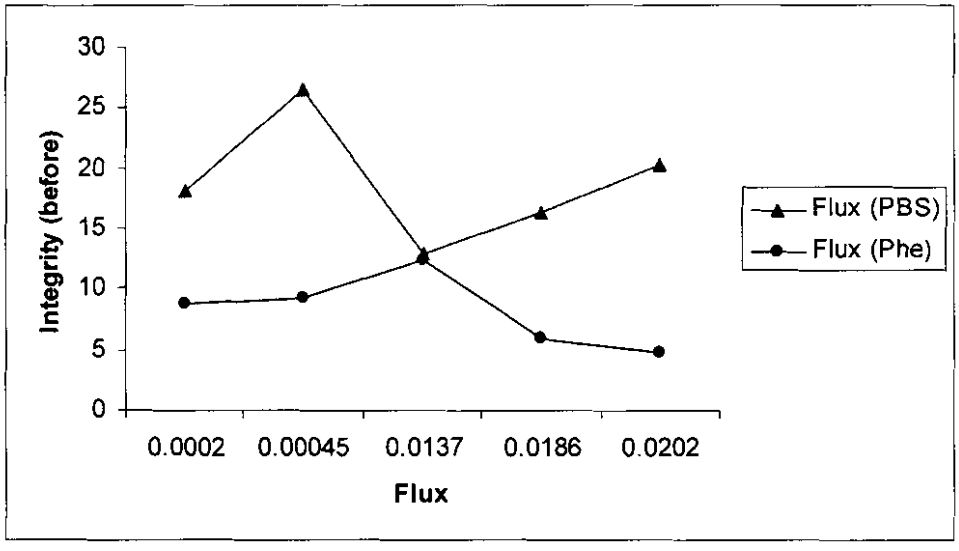


Figure 5.7: Integrity (before) vs. time for stavudine and its derivatives in PBS and in Pheroids.

No correlation exists between the flux and the integrity (before) whether the experiment was performed in PBS or in Pheroids.

5.3.3.6 Median flux vs. integrity (after)

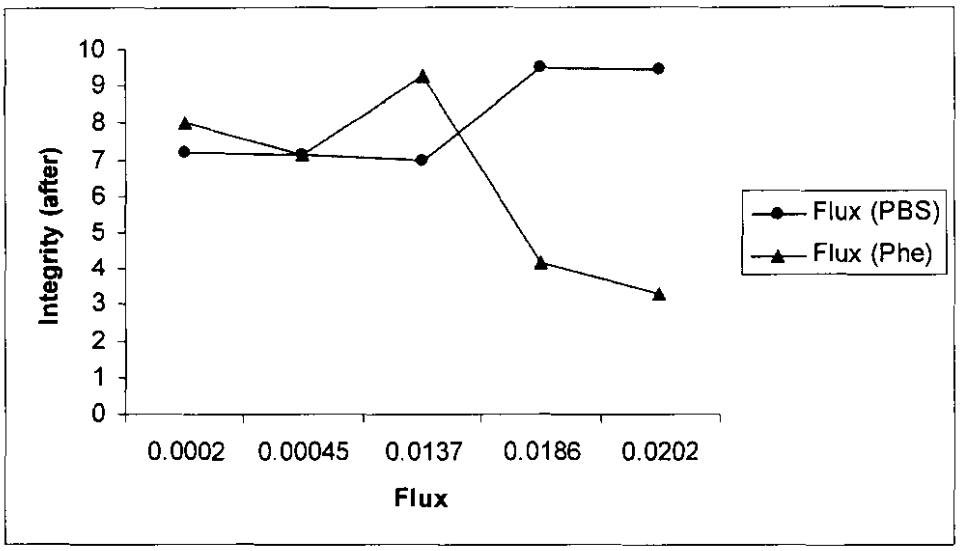


Figure 5.8: Integrity (after) vs. time for stavudine and its derivatives in PBS and in Pheroids.

No correlation exists between the flux and the integrity (after) whether the experiment was performed in PBS or in Pheroids.

Chapter 6

SUMMARY AND CONCLUSION

The vast interest in transdermal drug delivery may be attributed to the advantages associated with this method of drug delivery. Transdermal therapy, however, is not without limitations. The skin provides a protective function, inhibiting compounds from crossing it, and only drugs with the appropriate physicochemical properties are enabled to cross this protective barrier.

Stavudine is one agent of the most important class of compounds, the nucleoside reverse transcriptase inhibitors, and is active against HIV. Stavudine must be metabolised intracellulary to form the active compound stavudine 5'-triphosphate, in order to exert activity. Adverse effects related to stavudine containing therapy include skin rashes, nausea, abdominal pain, etc. but most importantly, peripheral neuropathy due to mitochondrial toxicity.

The toxicity of this drug encouraged further development of a transdermal delivery system. The primary aim was not only to eliminate some of the known adverse effects, but include the advantages associated with transdermal therapy. Of these advantages include more consistent drug levels, the avoidance of hepatic first pass metabolism, and the accommodation of patients who cannot tolerate oral dosage forms.

Not all drugs possess the correct physicochemical properties to cross the skin, therefore in some instances minor chemical modifications to the drug, such as salt formation or esterification, may be necessary (Abdou, 1989).

During this study the primary aim was to synthesise new derivatives of stavudine and to evaluate the effects of the different substituents on transdermal penetration, with and without the use of Pheroids as delivery system. Furthermore, it was to be established if any correlation between the transdermal penetration and selected physicochemical properties of the penetrant existed.

The ^{1}H and ^{13}C NMR, IR and MS spectroscopy data confirmed that the stavudine derivatives were successfully synthesised.

As expected, the aqueous solubility of stavudine (104,75 mg/ml) was much higher than that of the synthesised derivatives. This was in accordance with data in the literature (§ 3.4) which proved that the solubility decreased as the alkyl chain length and molecular weight increased.

The partition coefficient of stavudine (-0,846) was lower than that of its derivatives. This was in accordance with data in the literature (§ 3.3.3) which proved that the partition coefficient increased with an increase in chain length. The experimental partition coefficients were also compared to values obtained from IA, ACD Labs and KowWin prediction software, but ACD Labs was the only software that gave values relatively close to the experimental partition coefficients. As could be expected a direct correlation was found to exist between the aqueous solubility data and the partition coefficients.

The experimental flux was determined for every compound using the median value for every individual cell. The experimental transdermal flux of stavudine (1,46 $x10^{2}$ $\mu g/cm^{2}.h$) in PBS was much higher than that of its derivatives. Stavudine was more hydrophilic than the other compounds and most probably penetrated through the protein rich spaces between the SC and the corneosytes, resulting in higher fluxes. The propionyl derivative (1,86 $x10^{-2}$ $\mu g/cm^{2}.h$) and the buteryl derivative (2,02 $x10^{2}$ $\mu g/cm^{2}.h$) were the compounds with the highest transdermal flux in Pheroids. The flux of stavudine therefore improved from 1,46 $x10^{-2}$ $\mu g/cm^{2}.h$ to 2,02 $x10^{-2}$ $\mu g/cm^{2}.h$ by synthesising the buteryl derivative and using Pheroids as delivery system which constitutes a 38% enhancement in flux.

The predicted flux values didn't correlate with the experimental flux values. This could be due to the model used to predict transdermal flux values as it does not regard the state of ionisation. Thus is it hard to compare predicted flux with experimental flux values when using the available model.

No correlation existed between the flux values of the derivatives in PBS and the flux values of the derivatives in Pheroids but the transdermal flux values in the Pheroids were much higher than that in PBS. The aqueous solubility, molecular mass and log D values showed an excellent correlation between these mentioned physicochemical properties and the flux values of the compounds in PBS, while no correlation existed between the melting point and the integrity (either before or after). The data showed no correlation between flux and Pheroids for any of the determined physicochemical properties.

This study has confirmed that transdermal flux is dependent on several factors such as aqueous solubility, log P etc. and in some instances minor modifications to the drug may be necessary. The best results in this study were achieved by synthesising the propionyl and buteryl derivatives and using Pheroids as delivery system.

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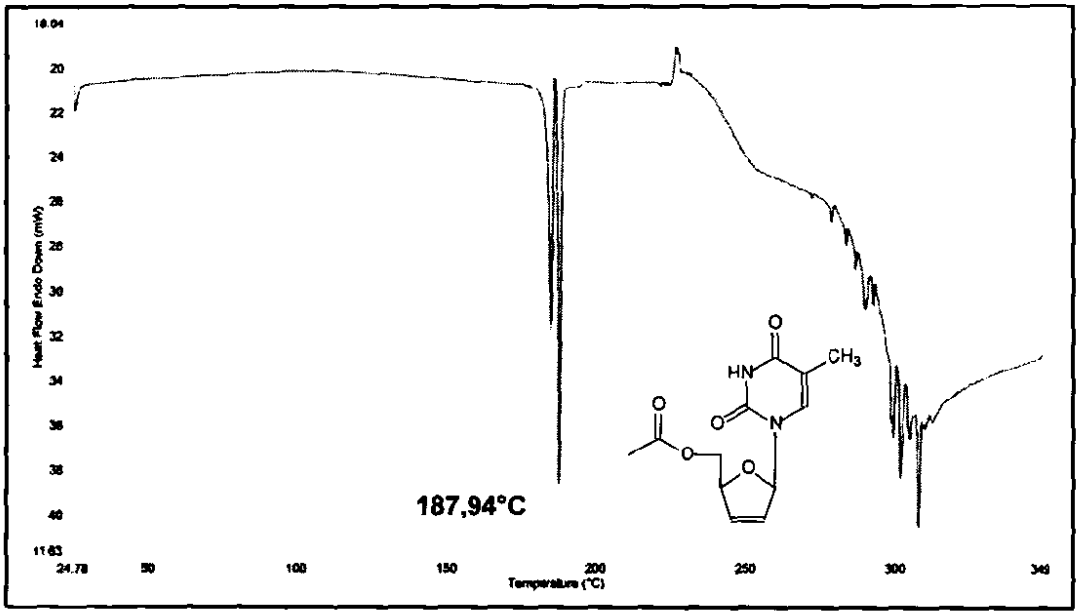
Thermogram 1



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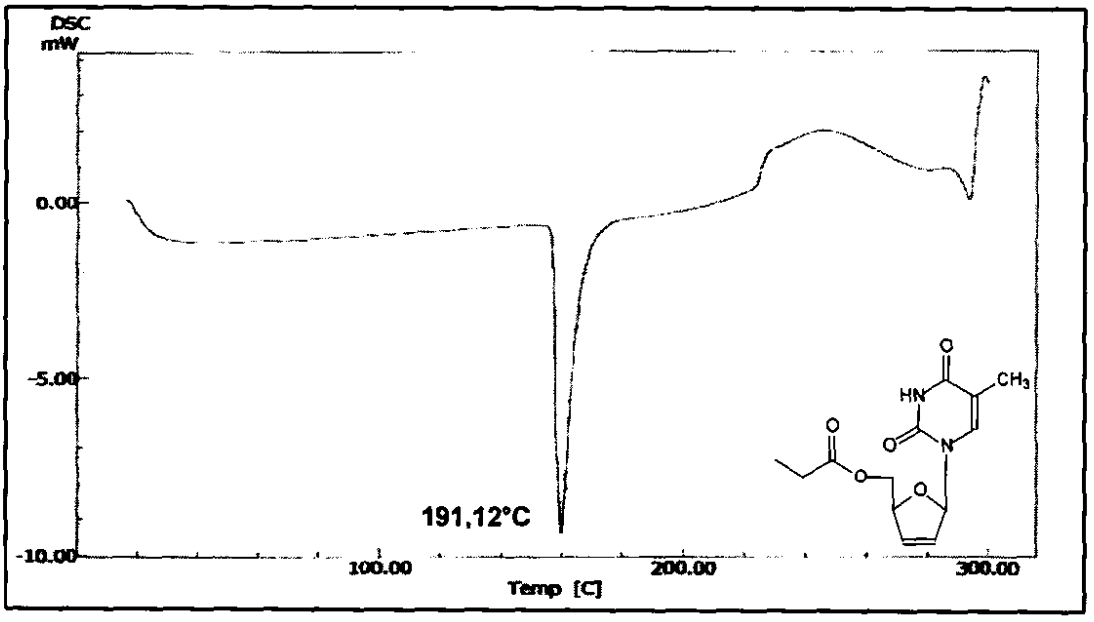

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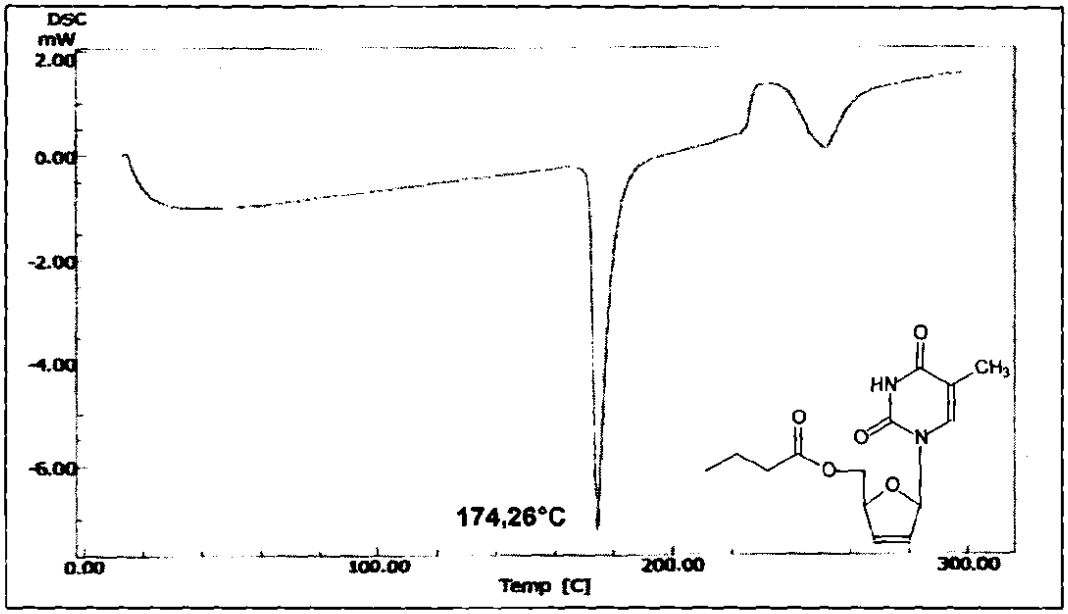

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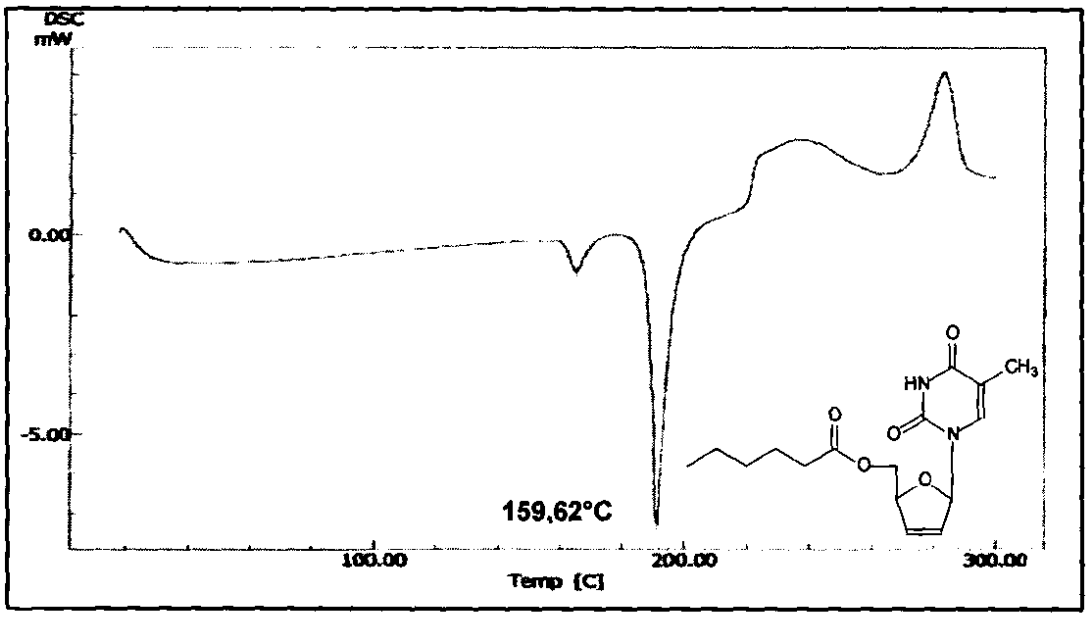

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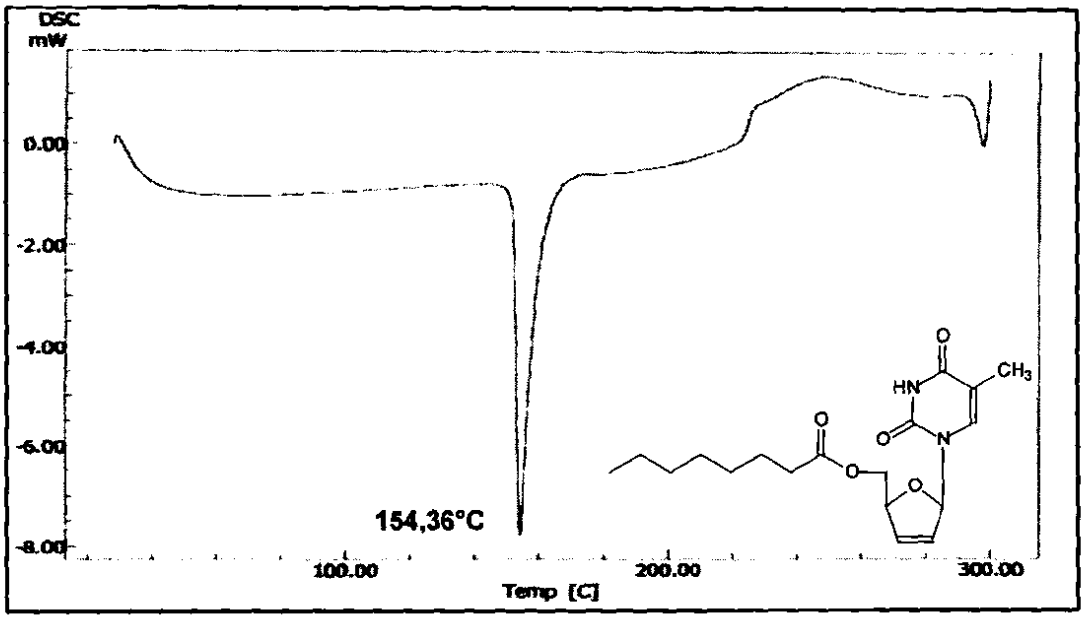

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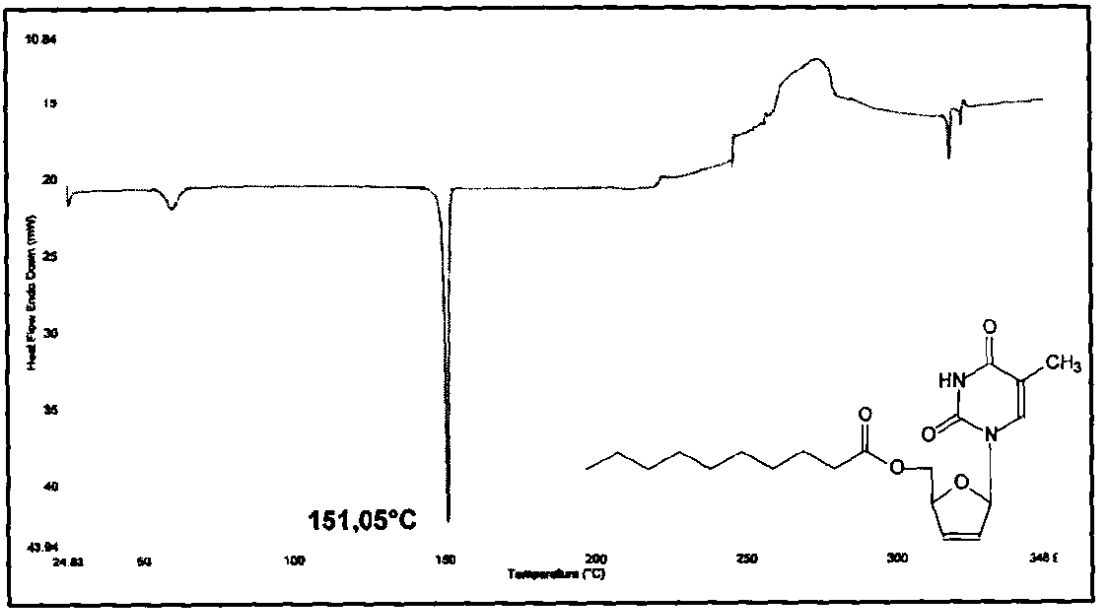

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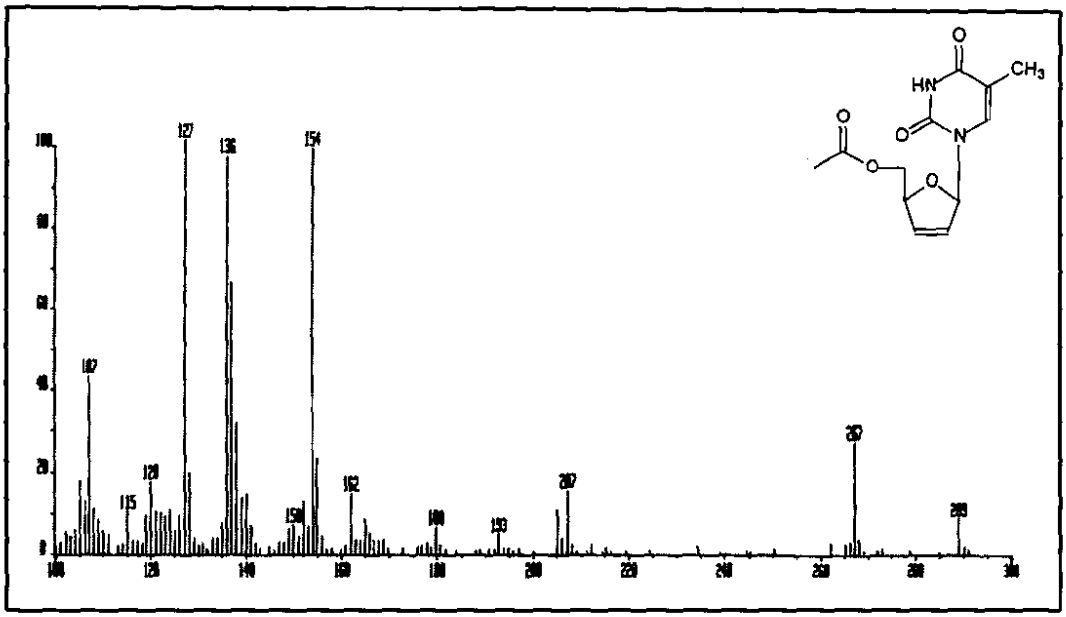

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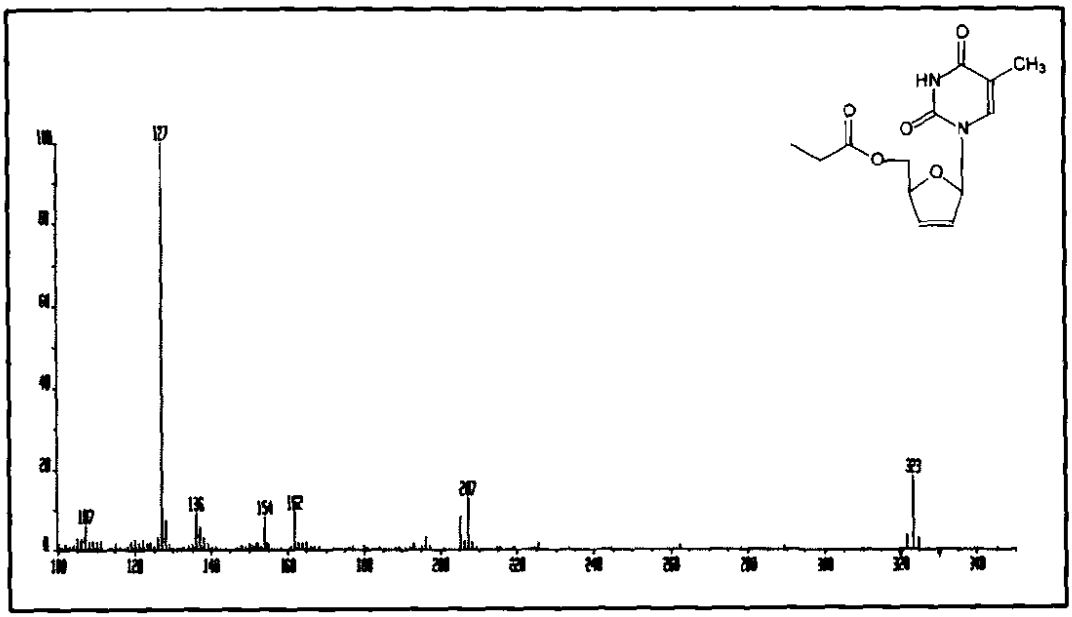

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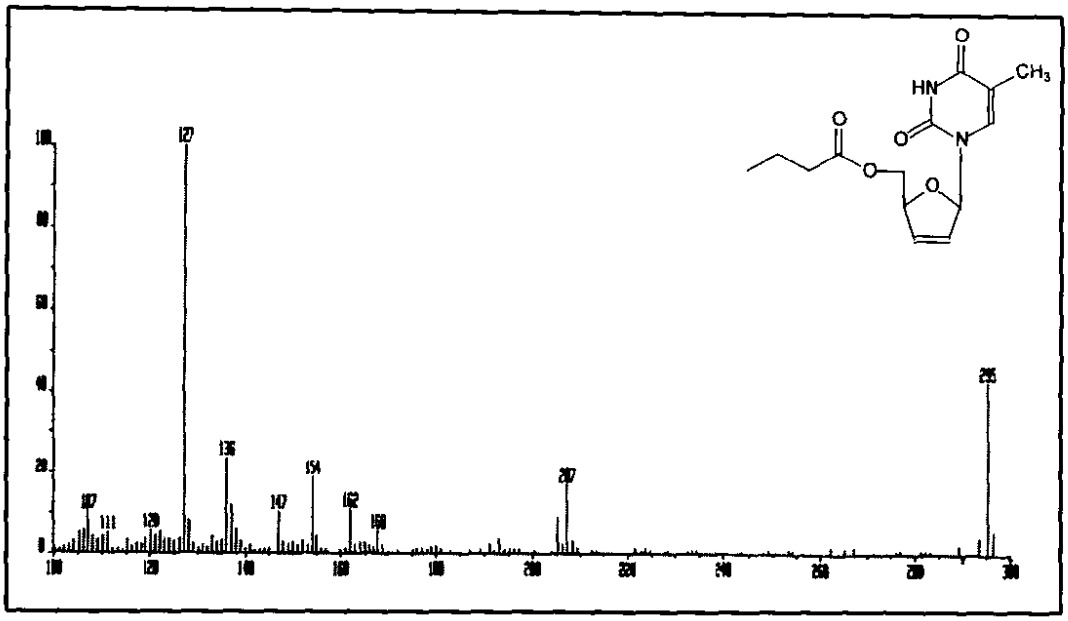

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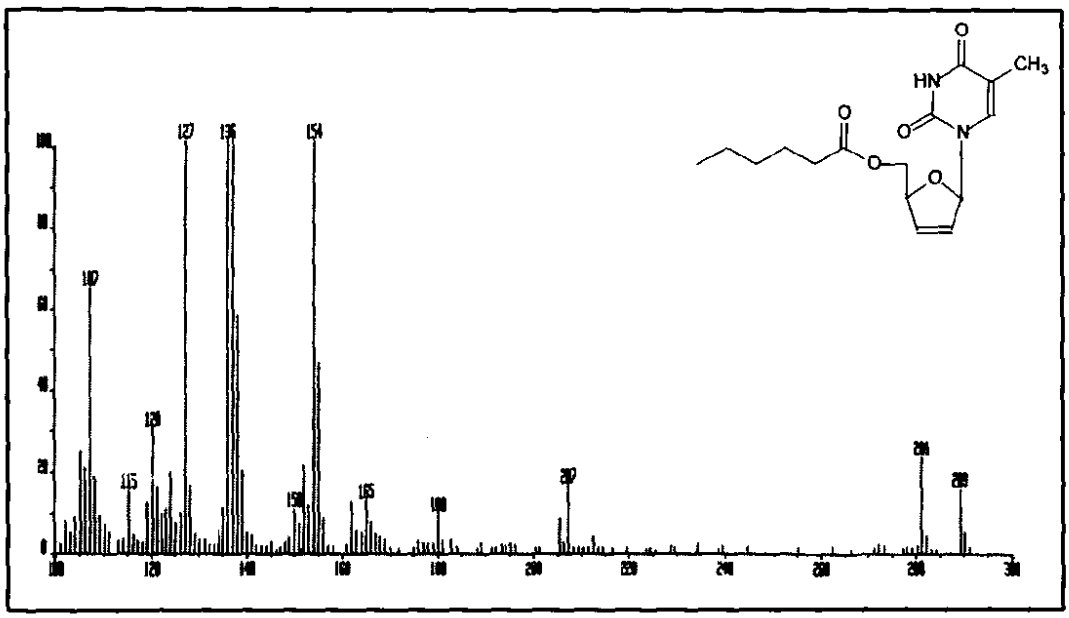

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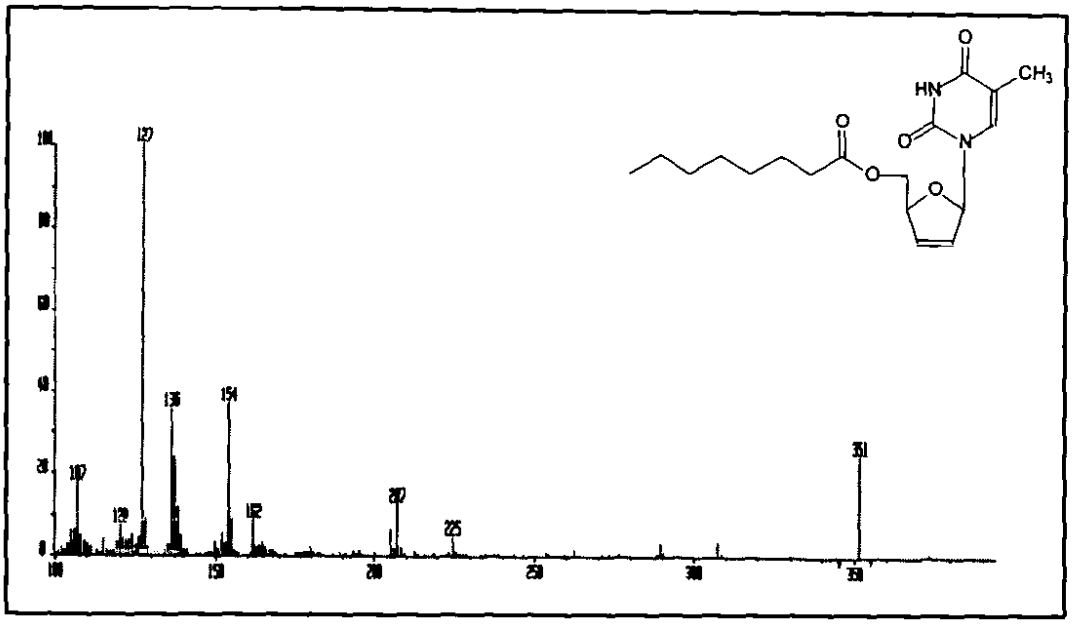

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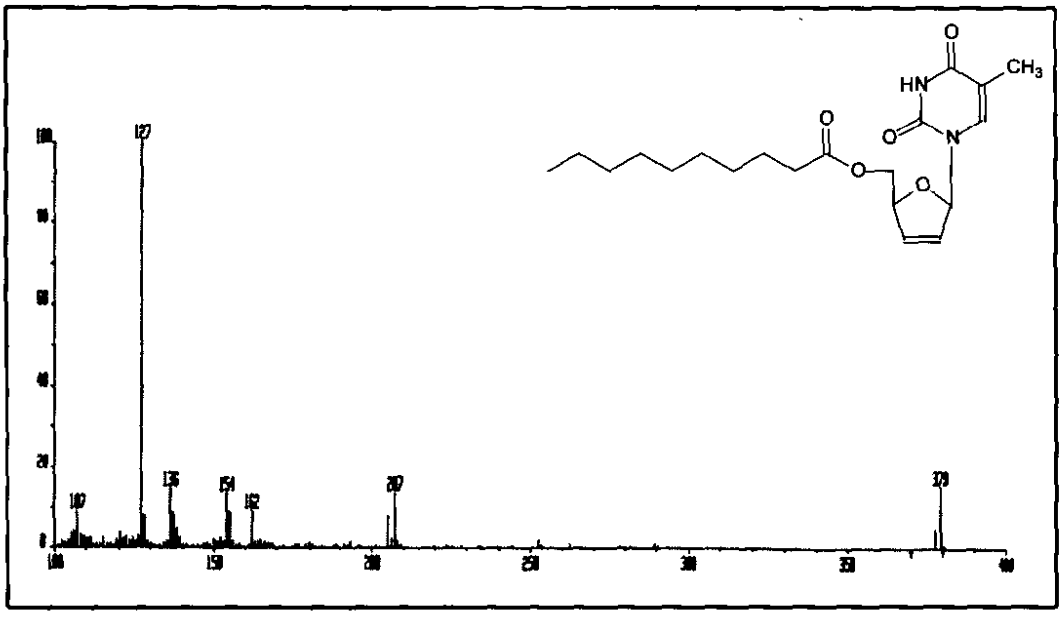

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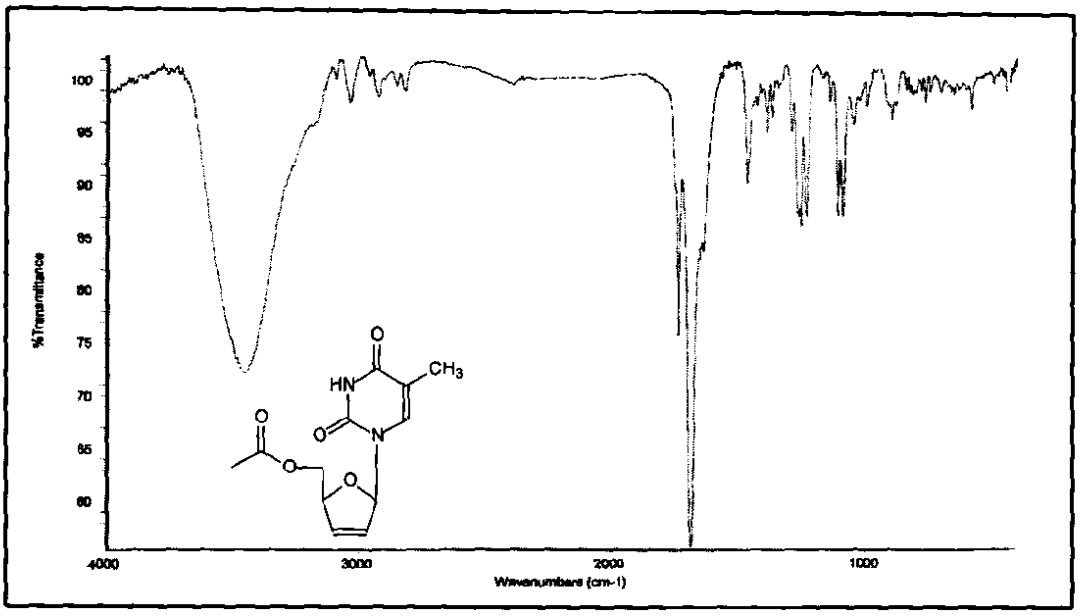
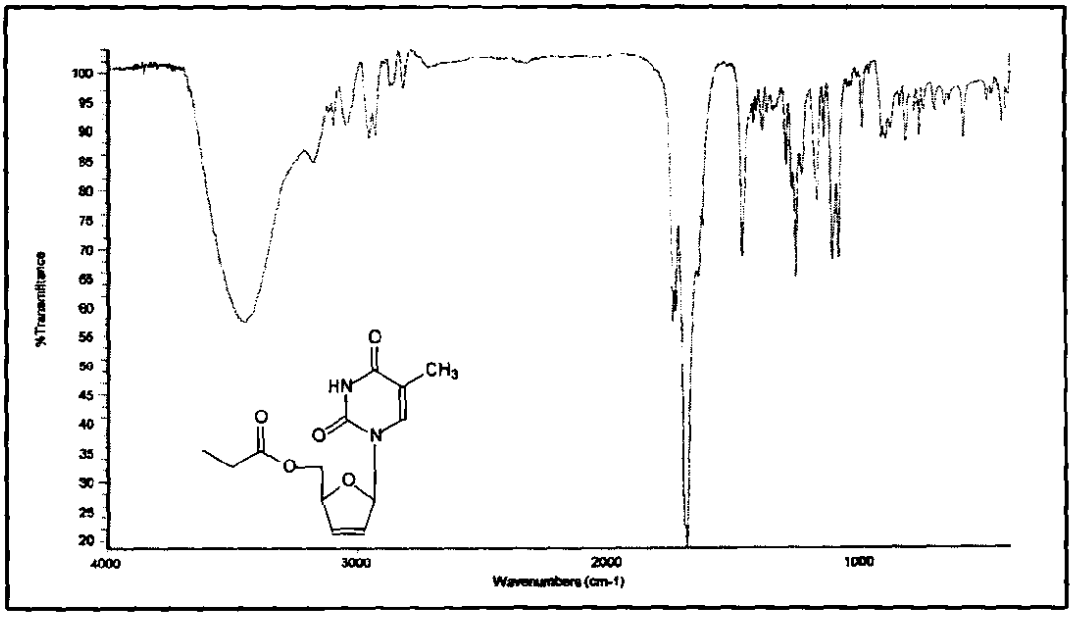
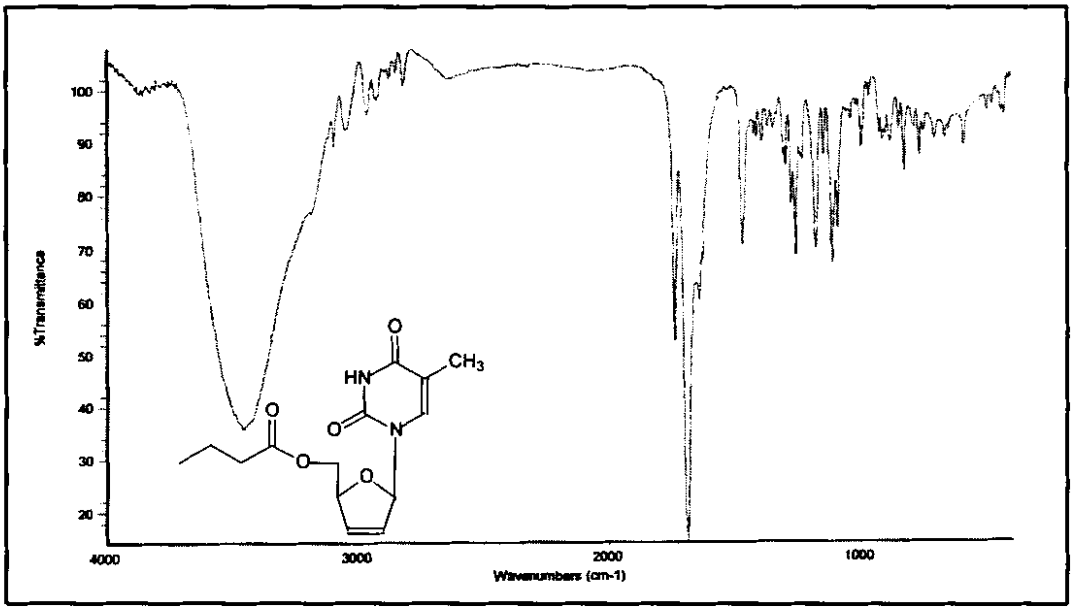


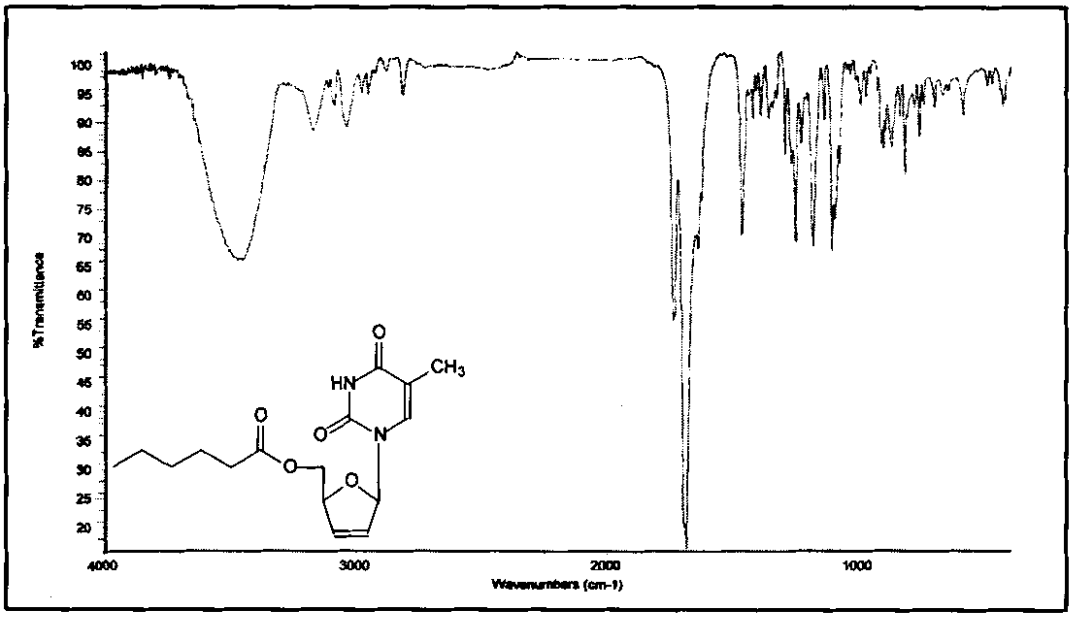
Spectrum 8



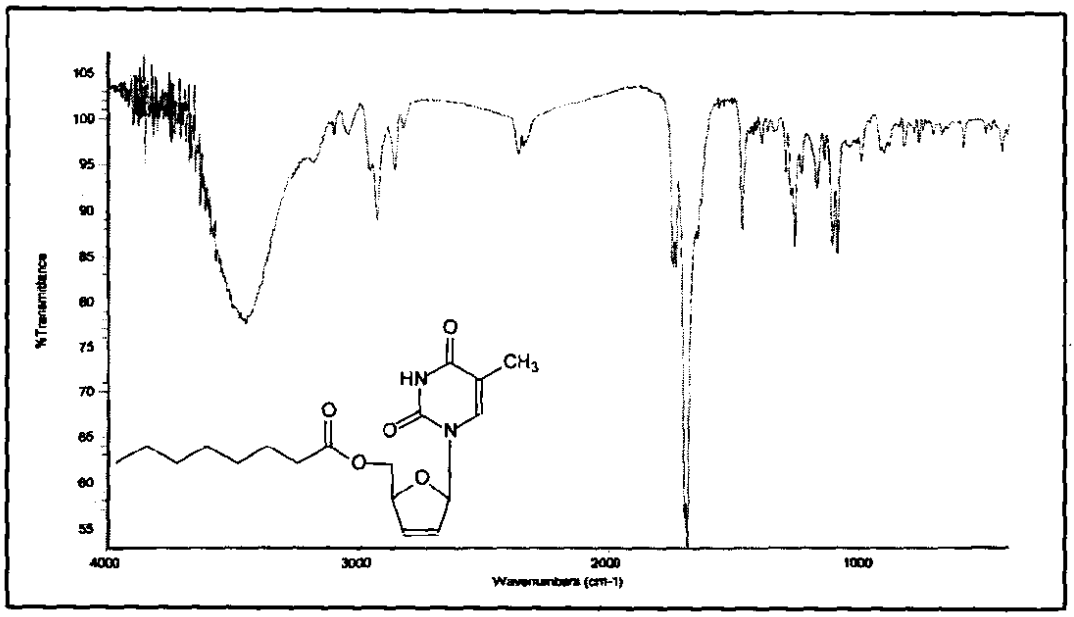
Spectrum 9



Spectrum 10



Spectrum 11



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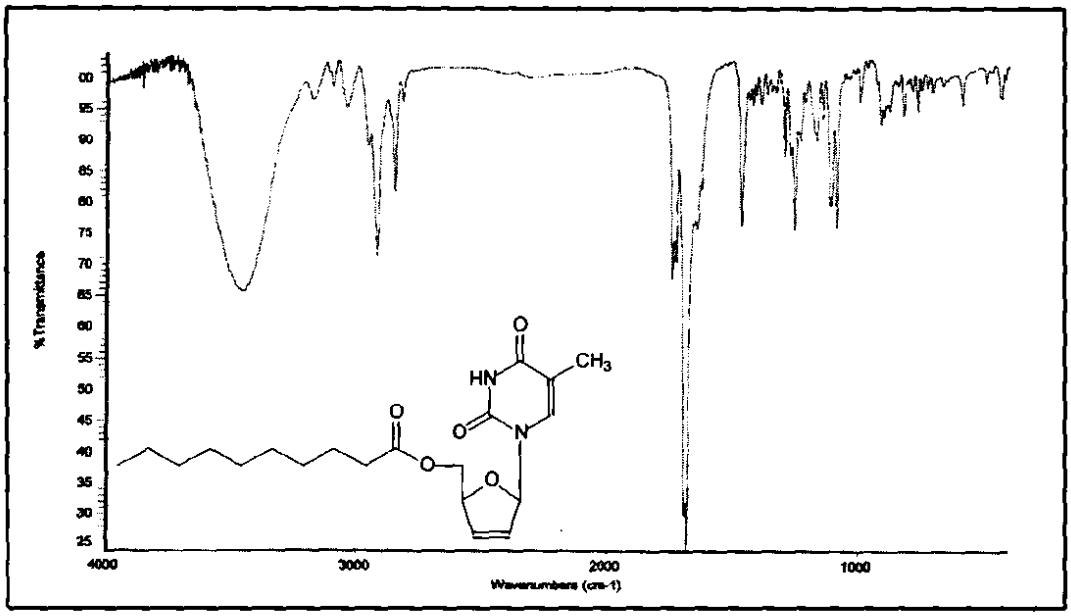

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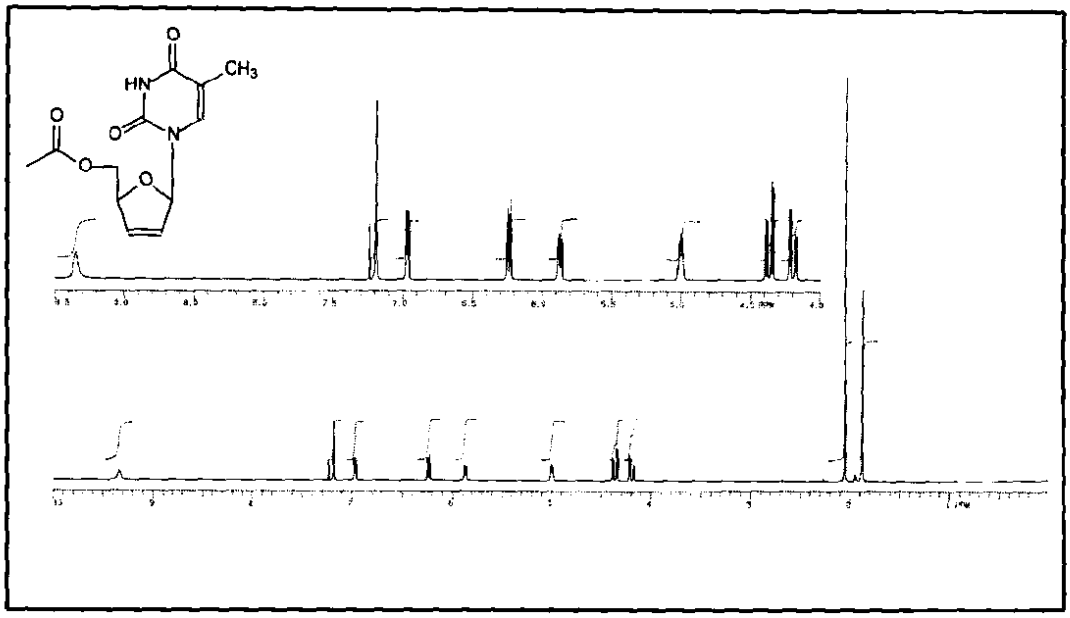

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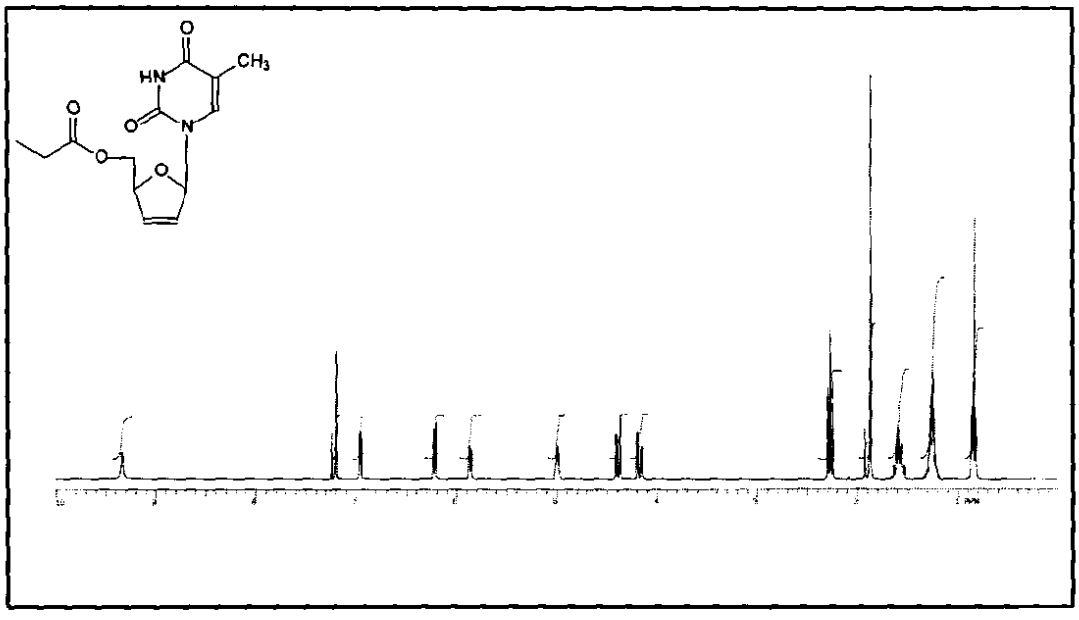

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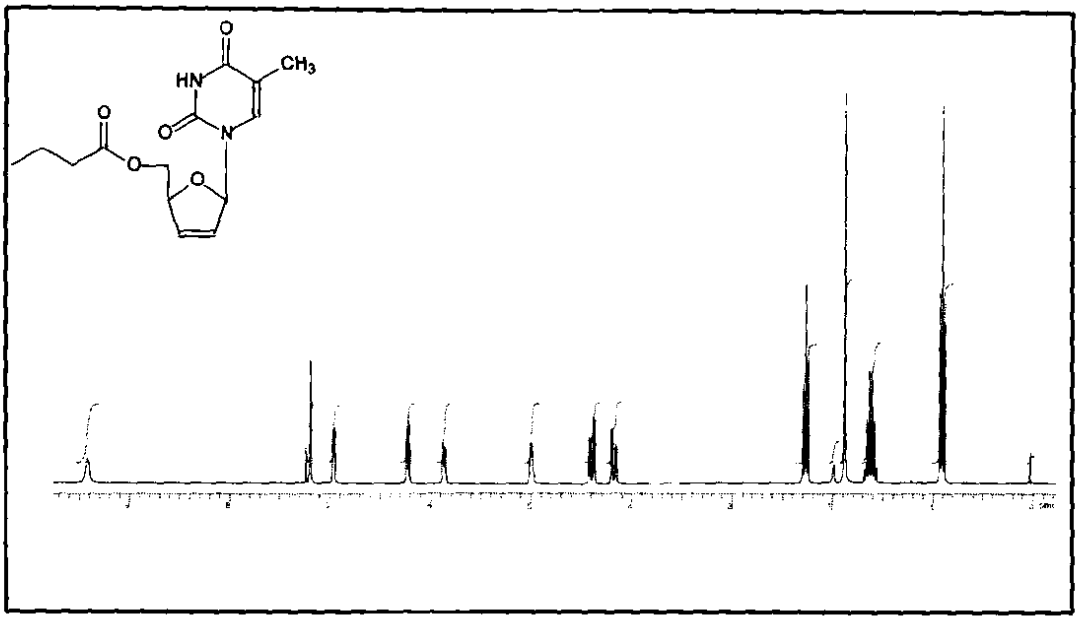

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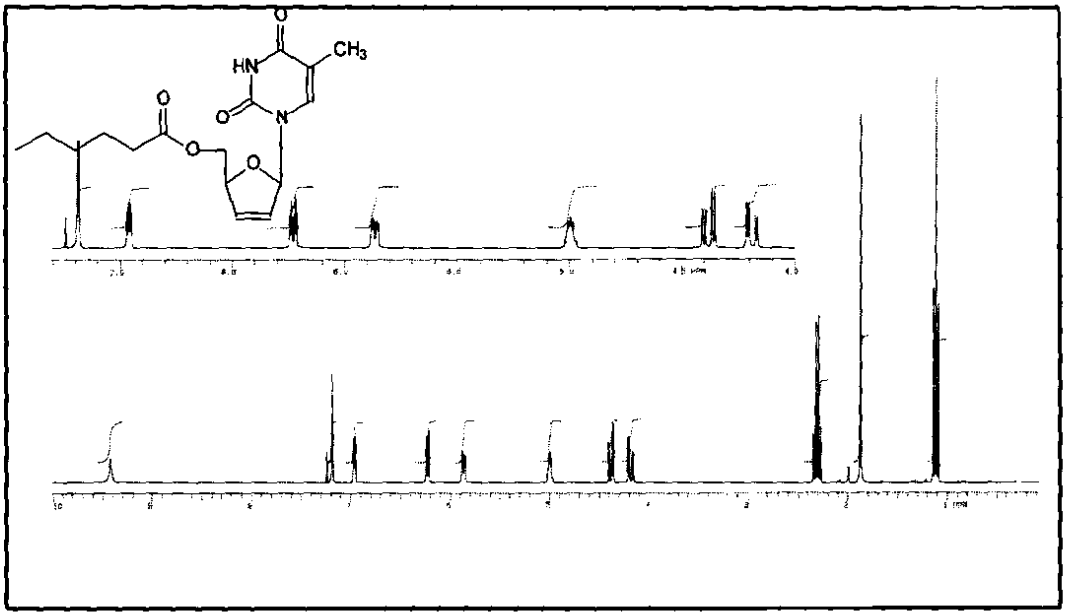

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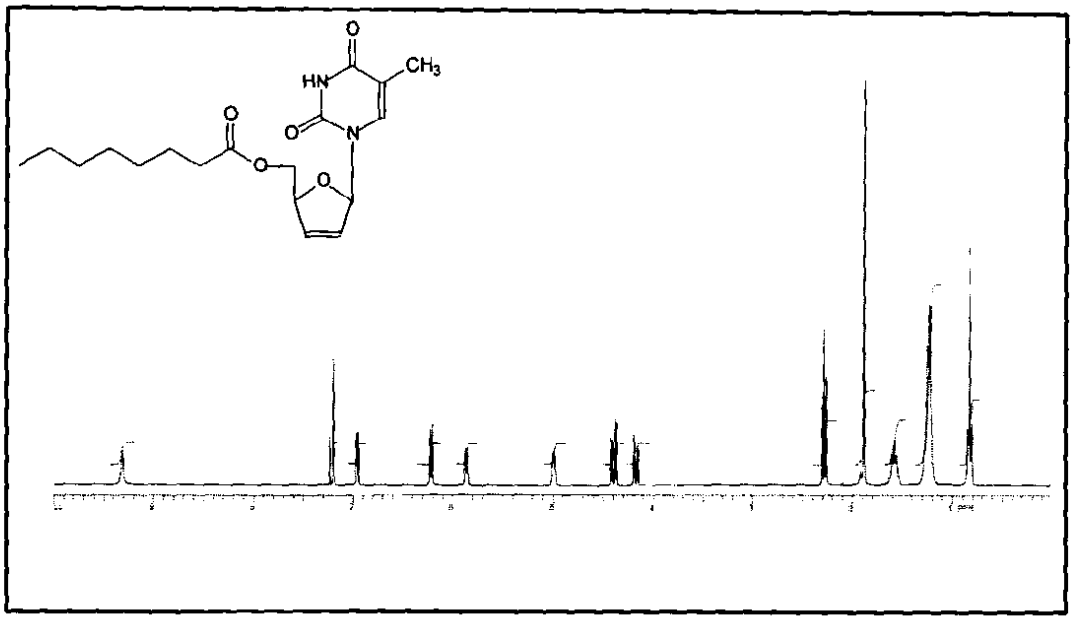

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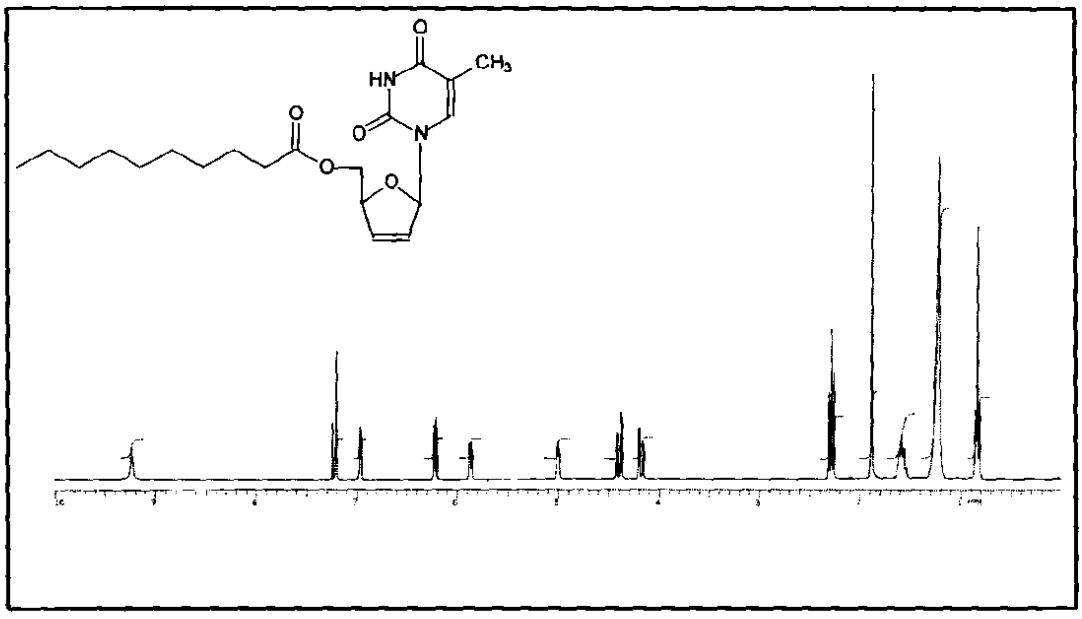

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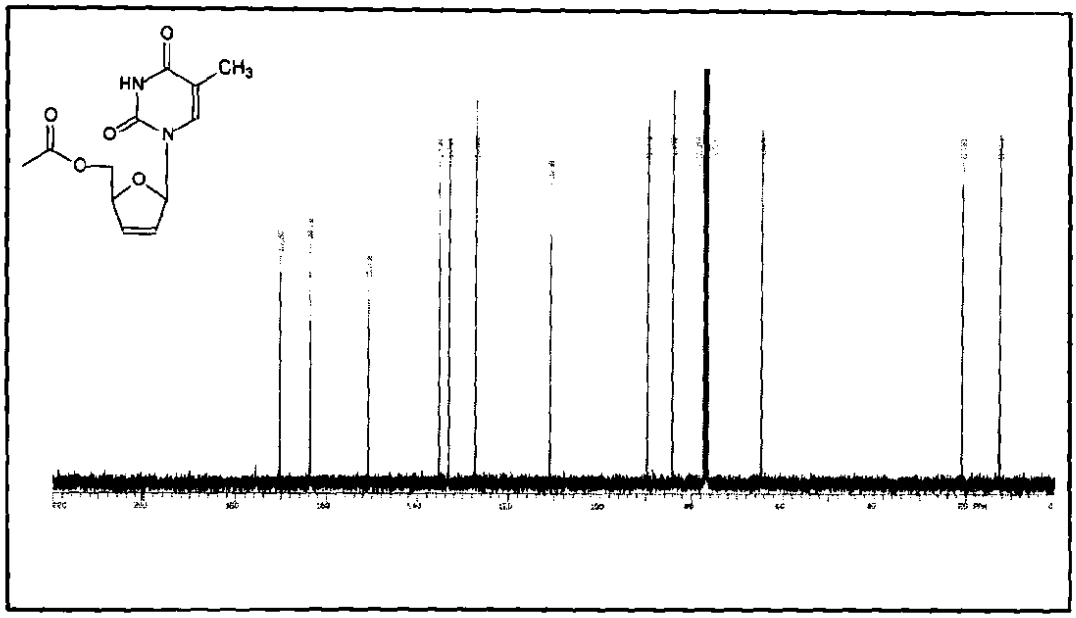

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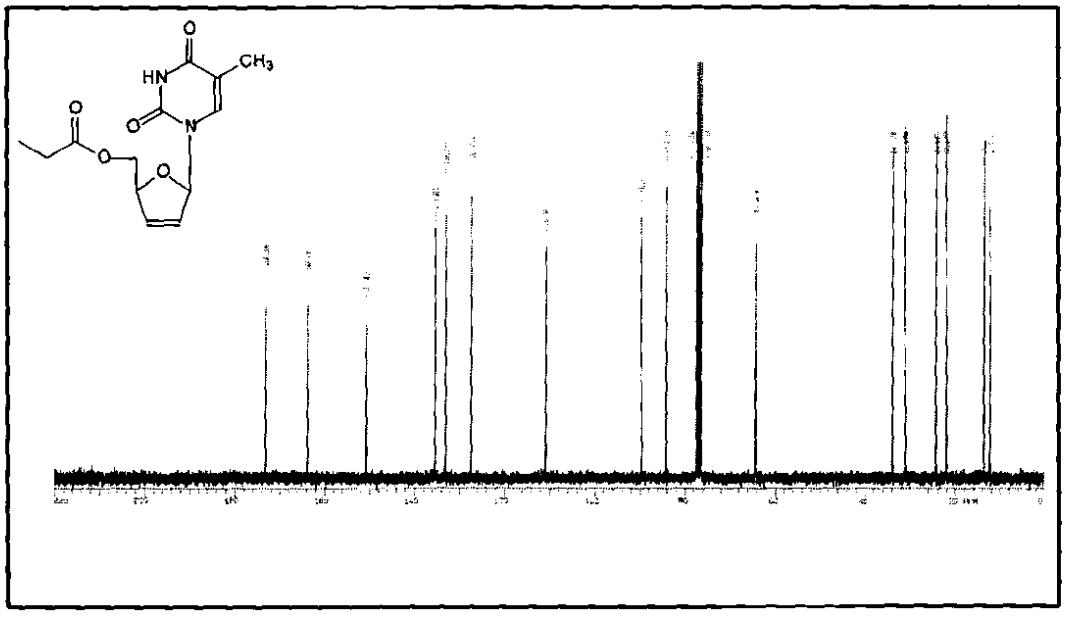

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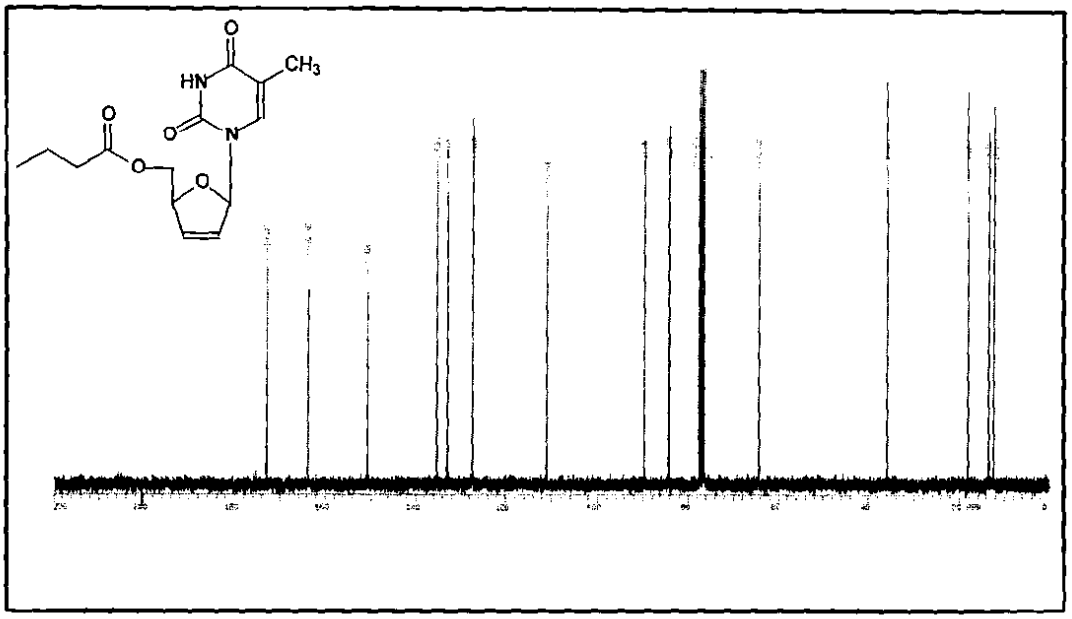

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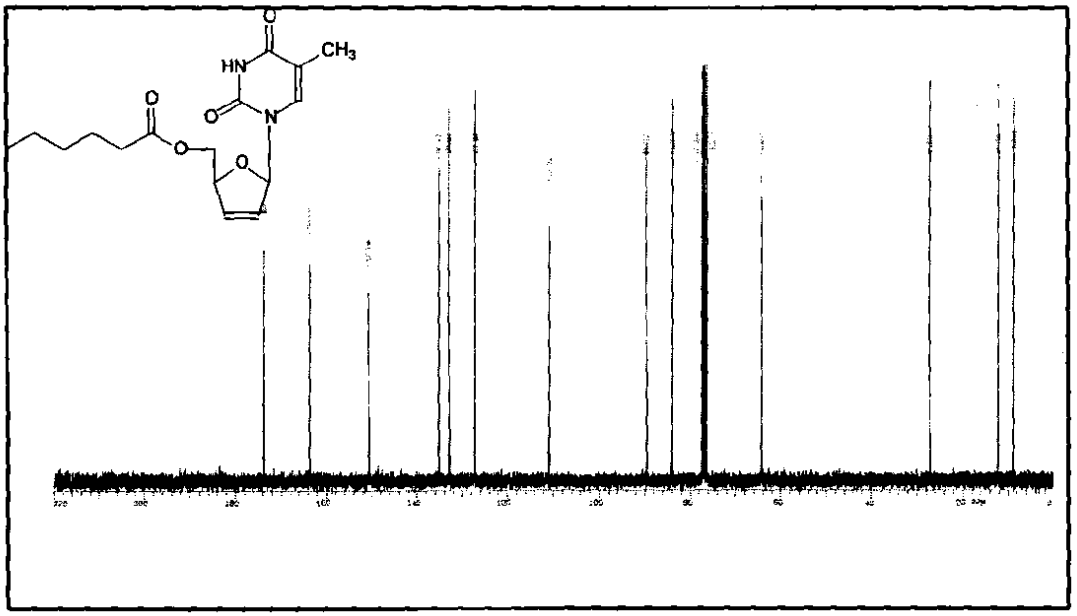

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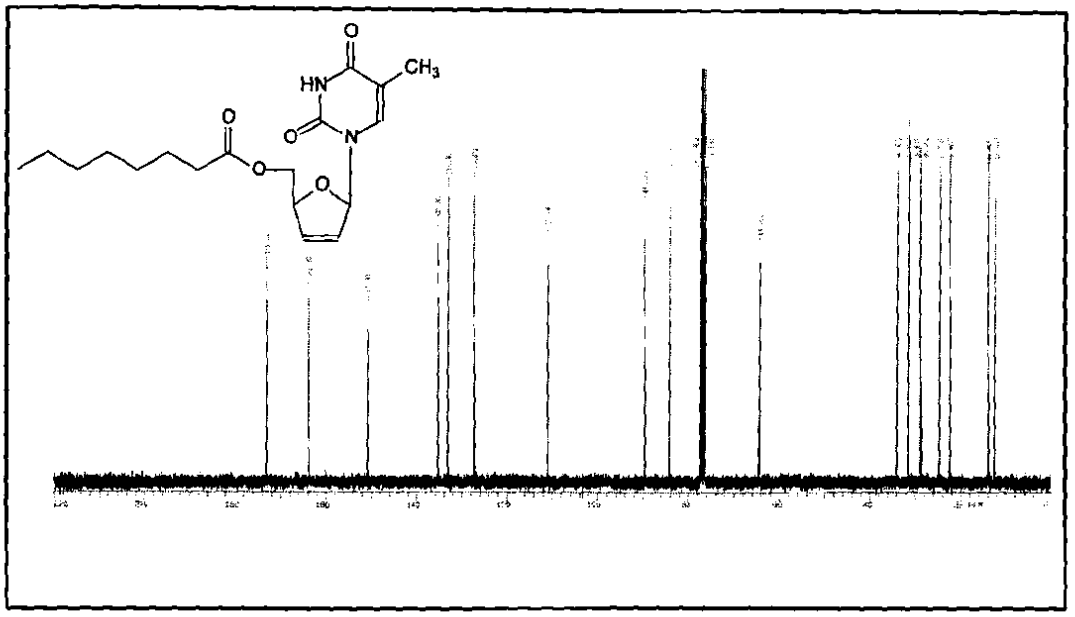

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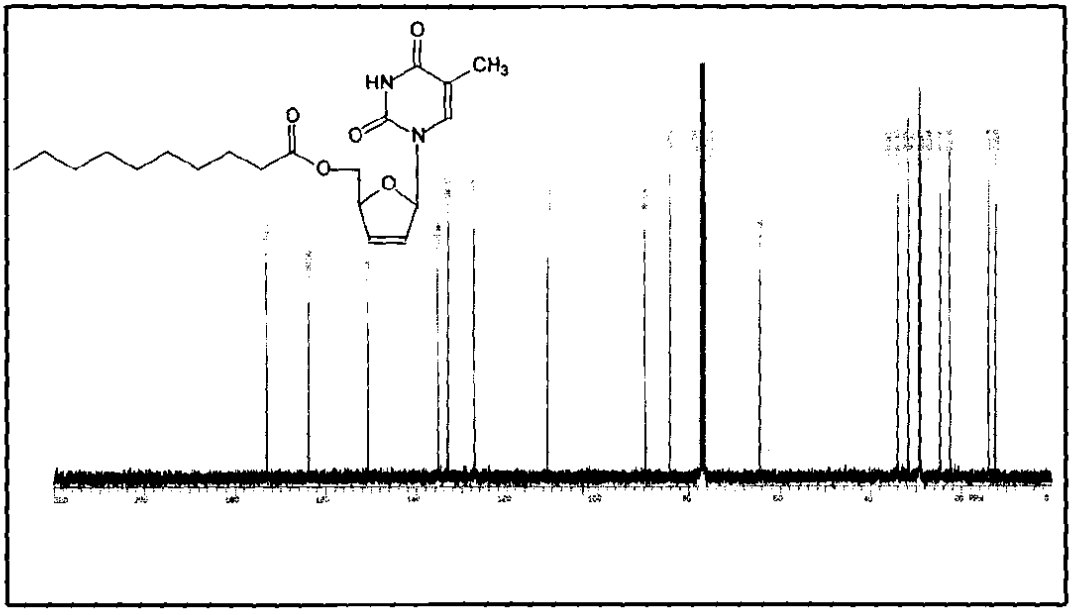
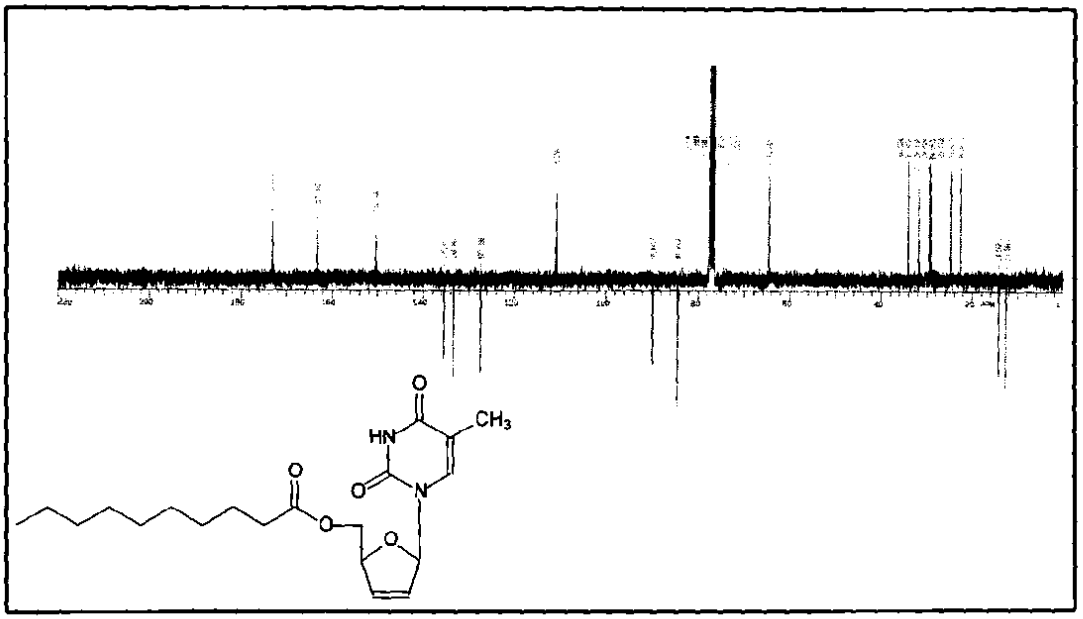


Spectrum 25



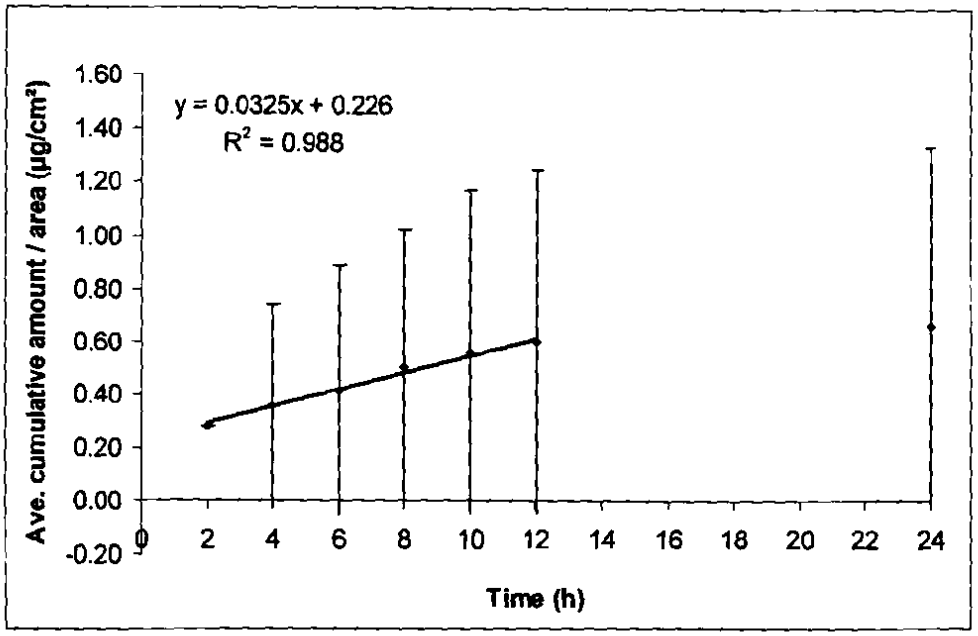


Figure A.1: Average cumulative amount of stavudine penetrated through the skin as a function of time (PBS).

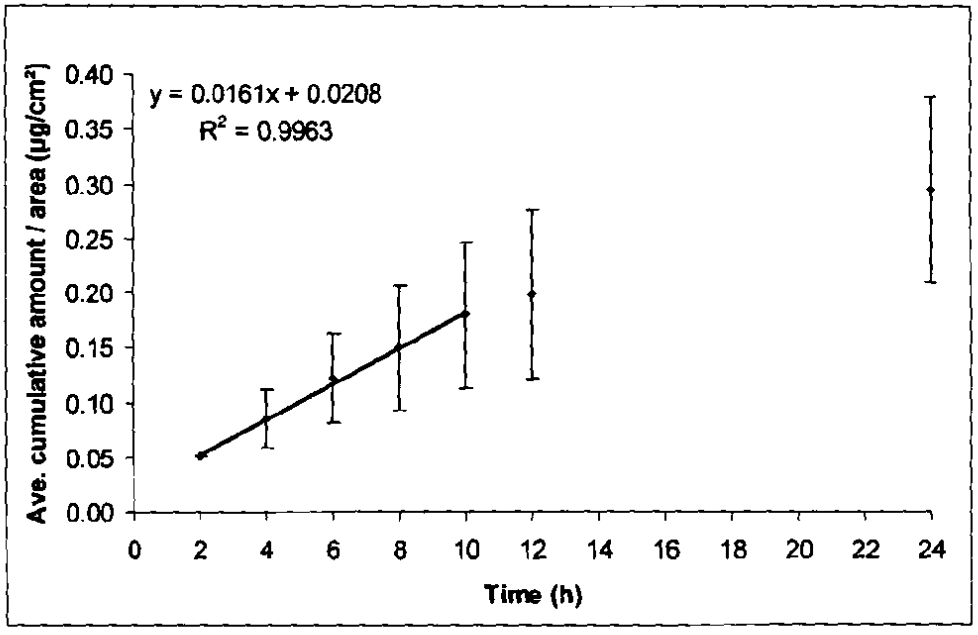


Figure A.2: Average cumulative amount of stavudine penetrated through the skin as a function of time (Pheroids).

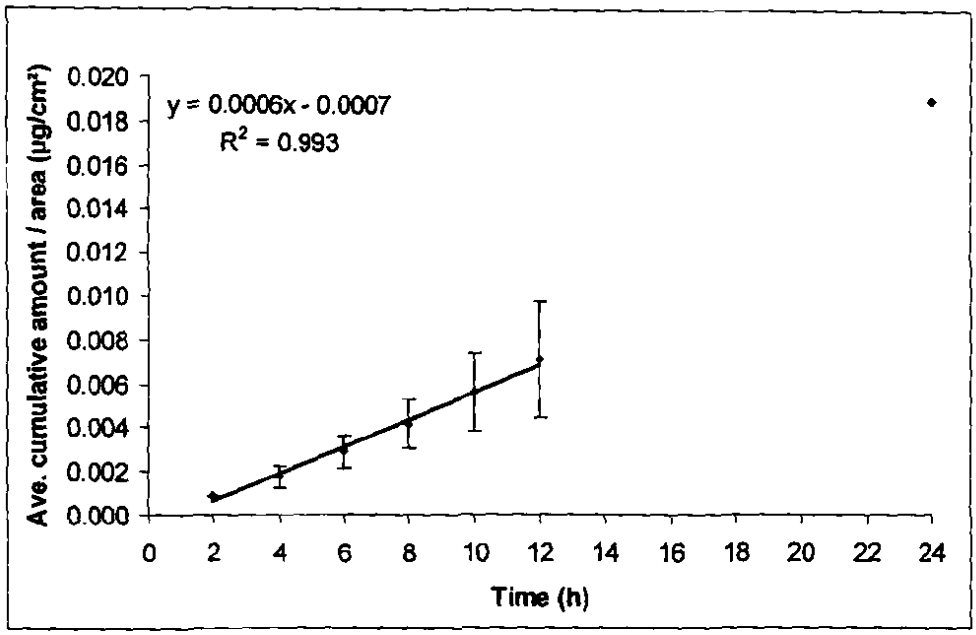


Figure A.3: Average cumulative amount of stavudine-5'-acetate penetrated through the skin as a function of time (PBS).

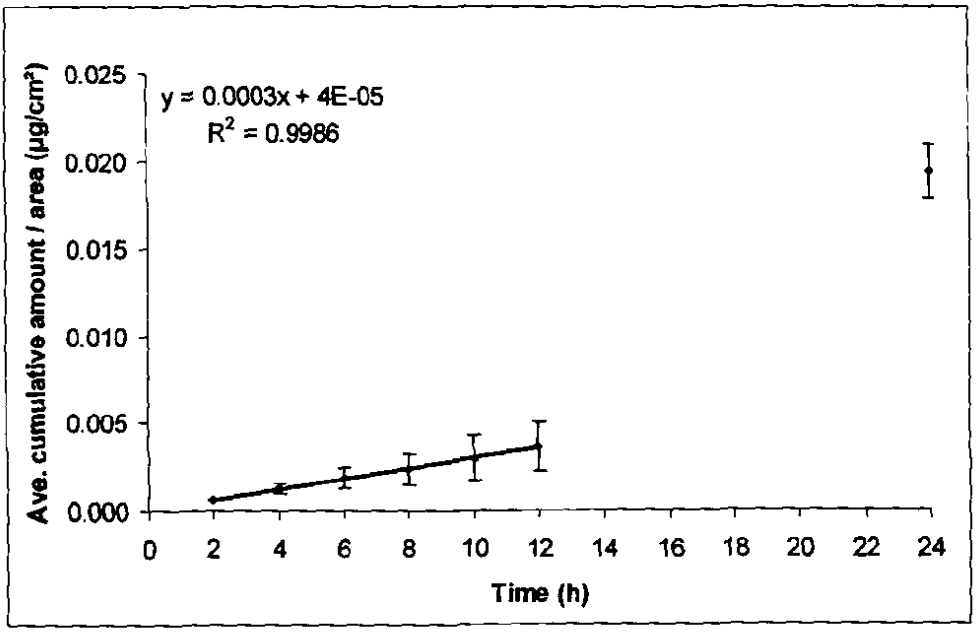


Figure A.4: Average cumulative amount of stavudine-5'-acetate penetrated through the skin as a function of time (Pheroids).

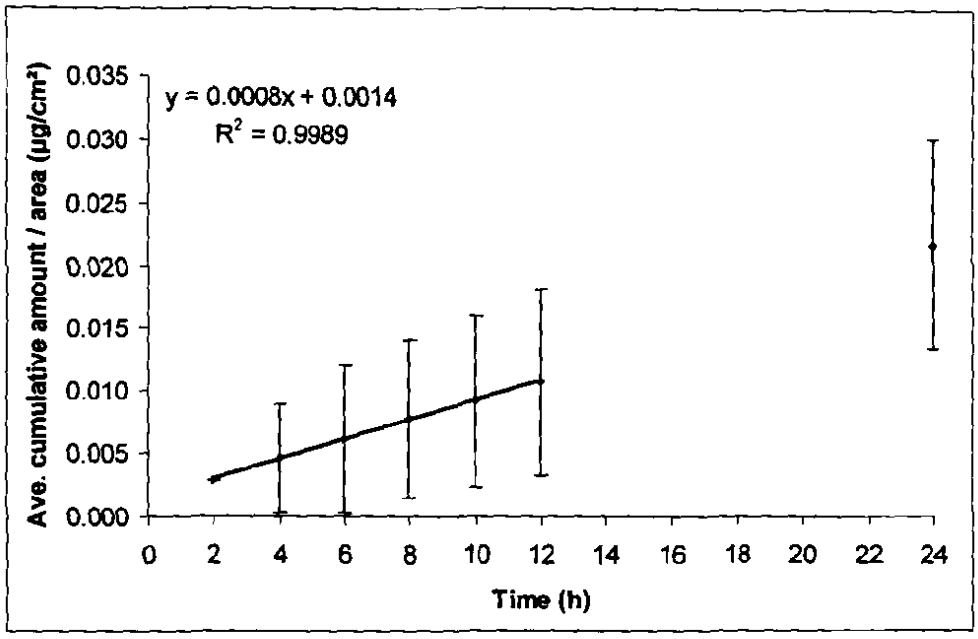


Figure A.5: Average cumulative amount of stavudine-5'-propionate penetrated through the skin as a function of time (PBS).

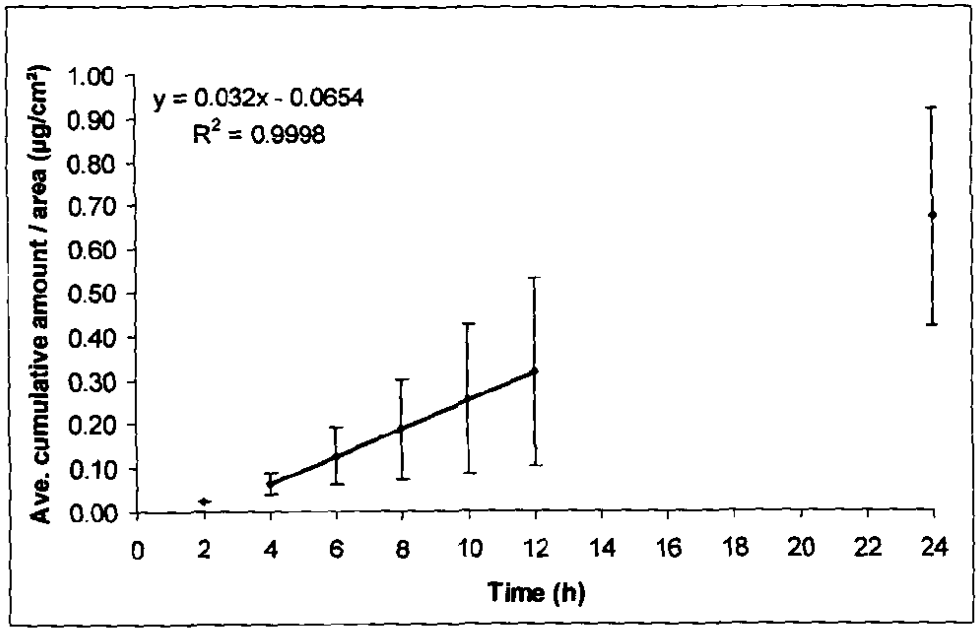


Figure A.6: Average cumulative amount of stavudine-5'-propionate penetrated through the skin as a function of time (Pheroids).

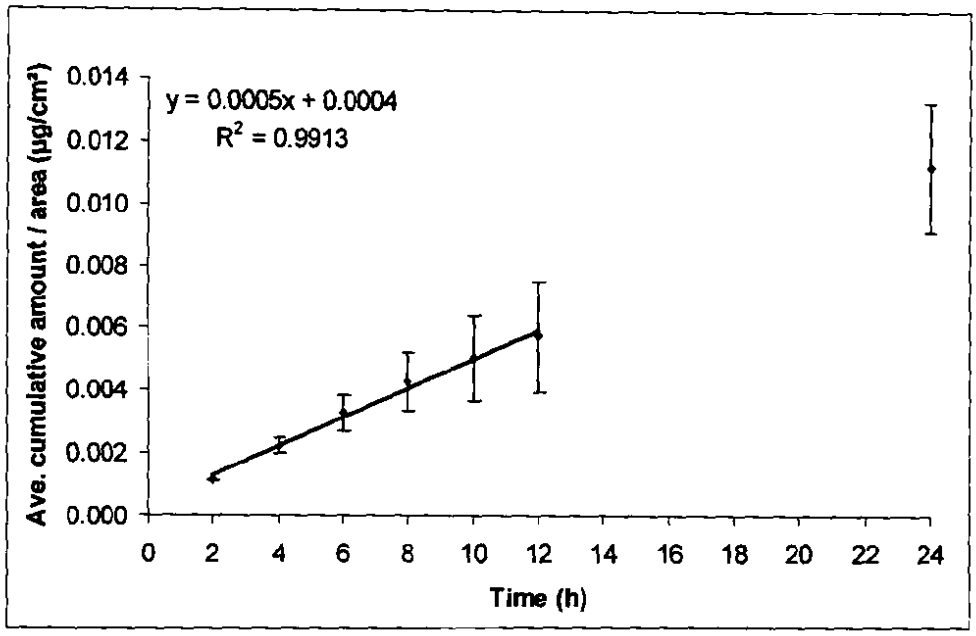


Figure A.7: Average cumulative amount of stavudine-5'-buterate penetrated through the skin as a function of time (PBS).

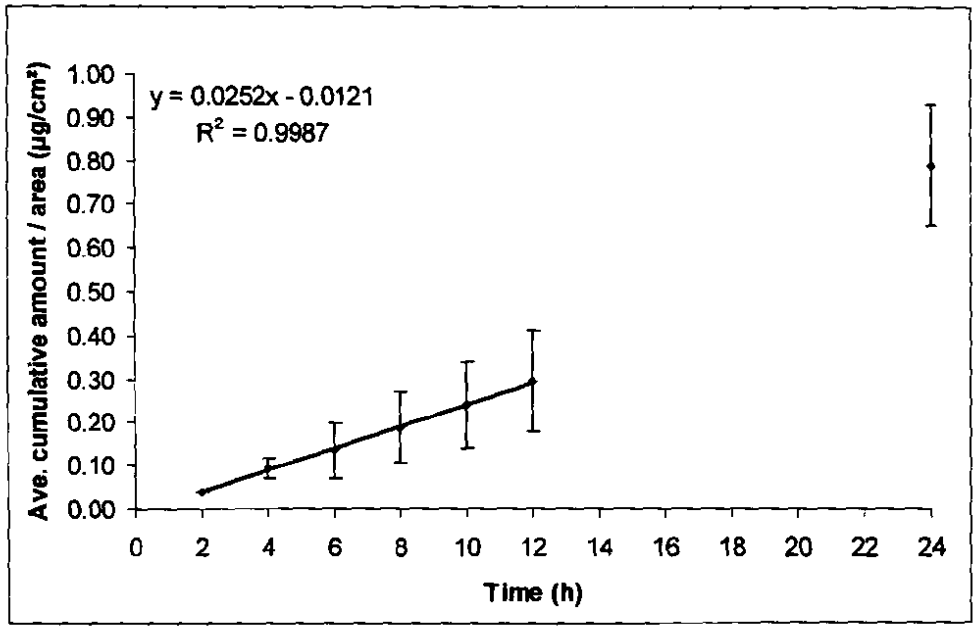


Figure A.8: Average cumulative amount of stavudine-5'-buterate penetrated through the skin as a function of time (Pheroids).

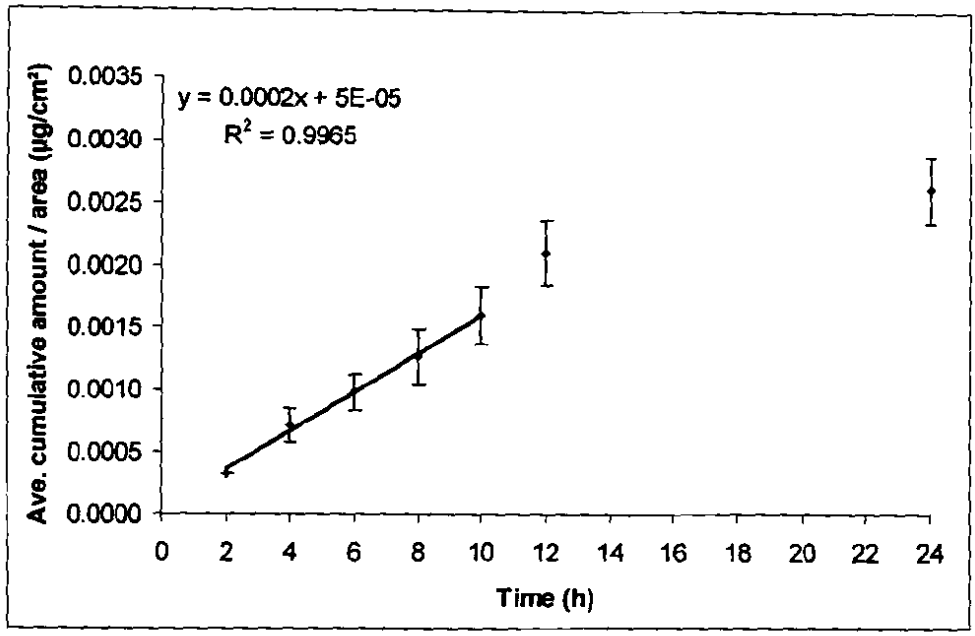


Figure A.9: Average cumulative amount of stavudine-5'-hexanoate penetrated through the skin as a function of time (PBS).

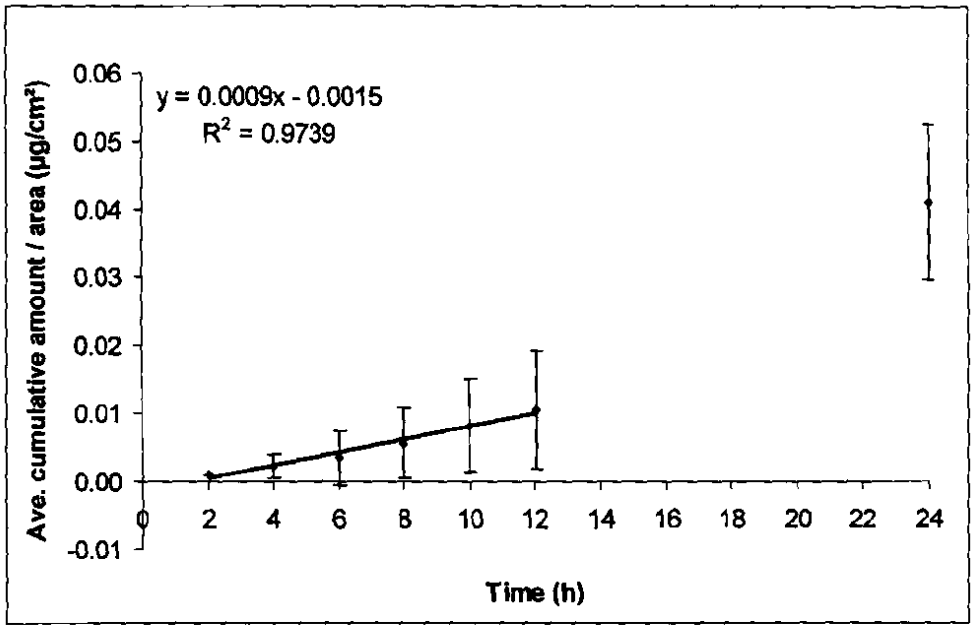


Figure A.10: Average cumulative amount of stavudine-5'-hexanoate penetrated through the skin as a function of time (Pheroids).

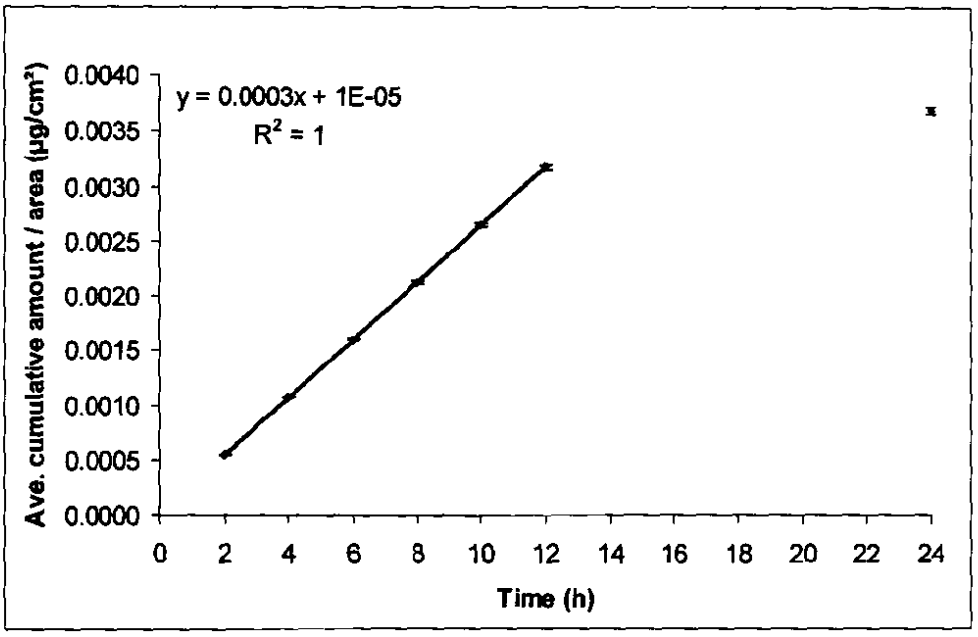


Figure A.11: Average cumulative amount of stavudine-5'-octanoate penetrated through the skin as a function of time (Pheroids).