Synthesis and Transdermal Penetration of Stavudine-5'-esters

Estée-Marie Holmes[a], Jaco C. Breytenbach[a], *Minja Gerber[b] and Jeanetta du Plessis[b]

[a] Pharmaceutical Chemistry, [b] Unit for Drug Research and Development, North-West University, Private Bag X6001, Potchefstroom 2520, South Africa

* Corresponding author. Tel.: +2718 299 2328; fax: +2718 293 5219. E-mail address: Minja.Gerber@nwu.ac.za (M Gerber)

Abstract

The aim of this study was to investigate the effects of different ester groups in position 5' of stavudine on the transdermal penetration with and without the use of Pheroid™ as the delivery system. Six esters were prepared by reaction of stavudine with six different acid chlorides at room temperature. Female human abdominal skin was used for *in vitro* penetration in Franz diffusion cells. The experimental aqueous solubility of stavudine (104.75 mg/mL) was much higher than that of the synthesized derivatives (ranging from 0.08 to 5.17 mg/mL), while the log D (octanol-buffer partition coefficient) of stavudine (-0.85) was lower than that of its derivatives (ranging from -0.41 to 3.06). The experimental transdermal flux of stavudine (6.52 μmol/cm^2.h) in PBS (phosphate buffer solution) was much higher than that of any of its derivatives (0.06 – 0.23 μmol/cm^2.h), while the propionyl (6.64 μmol/cm^2.h) and the butyryl esters (6.87 μmol/cm^2.h) had the highest transdermal flux using the Pheroid™ (0.75 – 6.87 μmol/cm^2.h) system.

Keywords: Delivery system; Pheroid™; Skin penetration; Stavudine, Stavudine esters, Transdermal delivery.

1 Introduction

The skin is an amazingly flexible and relatively impermeable barrier that provides protective, perceptive and communication functions to the human body [1]. It is the largest organ of the body and acts as a protective barrier with sensory and immunological functions [2]. It protects the body from water loss, friction and impact wounds, and potentially harmful external stimuli [3]. In an average adult it covers an area of approximately 1.73 m^2 [4] and receives one third of circulating blood through the body at any given time. The skin thus is one of the most readily accessible organs of the human body [5].

The main barrier to penetration by most drugs through the skin is the outermost layer of the skin, the stratum corneum. The stratum corneum 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 [6]. The primary factors that determine the diffusion rate through human skin are the physicochemical properties of the drug [7], the vehicle and the skin [8]. 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 [6, 16].

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 and, thus avoiding direct effects on the stomach and intestine. First pass metabolism can also be avoided with transdermal administration [9].

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 infection [10]. 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 pediatric patients. Another important use of stavudine is to help decrease the risk of infection after exposure to HIV [11]. 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 dependent on the dosage and the duration of the treatment [12].

Pheroid™ 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. The basic Pheroid™ has a vesicular structure which ranges in size from 200 - 440 nm [13]. The main essential fatty acids used in Pheroid™ are linolenic and linoleic acid as well as oleic acid. These essential fatty acids are emulsified in water and saturated with nitrous oxide [14; 13]. 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 [15].

The aim of this study was to investigate the effects of different ester groups in position 5’ of stavudine on the transdermal penetration with and without the use of Pheroid™ as delivery system and to determine a correlation, if any, between transdermal penetration and selected physicochemical properties of the penetrants.

2 Materials and methods

2.1 Materials

Stavudine was a gift from Aspen Pharmacare, Port Elizabeth, South Africa. Pheroid™, as utilized in this study, is a patented system of the Unit for Drug Research and Development, North-West University, Potchefstroom, South Africa. A specific Pheroid™ format, *viz*. 'micro-sponges', was used. Other reagents and chemicals used in this study were of analytical grade.

2.2 General procedures

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. An analytical VG 7070E mass spectrometer using fast atom bombardment (FAB) at 70 eV as ionization technique was used to record the MS spectra. KBr pellets were used to record the IR spectra on a Nicolet Magna 550 IR spectrometer. Differential scanning calorimetry (DSC) was used to determine melting point.

2.3 High performance liquid chromatography

Table **1**: HPLC conditions.

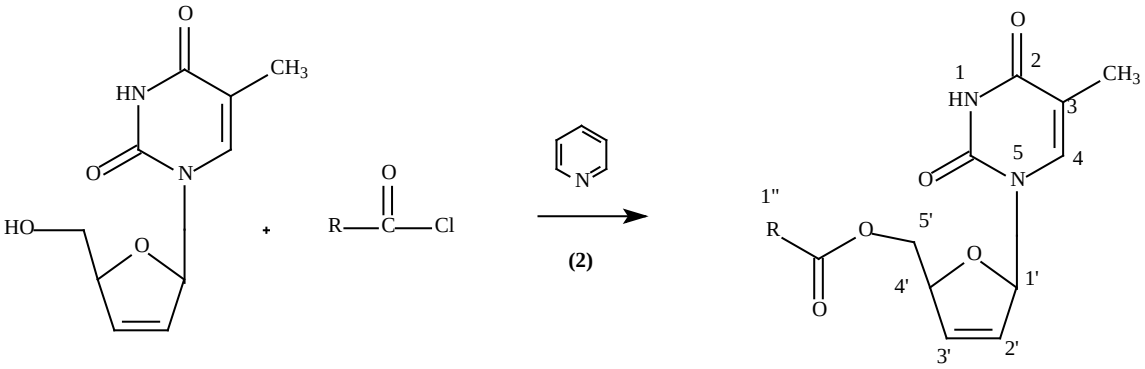
Compound	Flow rate (mL/min)	Wavelength (nm)	Mobile phase H_2O:acetonitrile	Retention time (min)
(1)	1.0	270	90:10	3.96
(4)	1.0	270	80:20	2.46
(5)	1.0	270	70:30	3.53
(6)	1.0	270	60:40	3.18
(7)	1.0	270	60:40	7.03
(8)	1.0	270	50:50	8.13
(9)	1.0	270	40:60	9.27

The HPLC system consisted of an Agilent 1100 auto sampler, UV detector and HP1100 series HPLC pump (Agilent, Palo Alto, CA). A Phenomenex (Luna C-18, 150 x 4.60mm, 5µm) column protected with a Securityguard pre-column (C-18, 4 x 3mm) (Phenomenex, Terronce, CA) was used. Chemstation for LC Systems software package was used for data analysis. The flow rate, UV wavelengths, mobile phase compositions and retention times are presented in Table **1**. The mobile phase consisted of acetonitrile and water containing 0.2% triethylamine with orthophosphoric acid to adjust the pH 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.

2.4 Esterification of stavudine

The esters were synthesized using standard organic chemical procedures. The esterification of stavudine (3 g; 13.38 mmol) **(1)** was done in dry pyridine **(2)** with 1.2 equivalent of the corresponding acid chloride **(3)** at room temperature. Stirring was continued for 72 h for the highest percentage yield. The mixture was poured into excess dichloromethane (± 50 mL) and washed several times with distilled water (± 300 mL) to remove the pyridine. The organic phase was dried over anhydrous $MgSO_4$ and the dichloromethane removed under vacuum. The resulting product was collected and purified by re-crystallization from methanol.



(1) **(3)**

R = CH_3 **(4)**
= CH_2CH_3 **(5)**
= $(CH_2)_2CH_3$ **(6)**
= $(CH_2)_4CH_3$ **(7)**
= $(CH_2)_6CH_3$ **(8)**
= $(CH_2)_8CH_3$ **(9)**

Scheme **1**: Synthesis of stavudine derivatives

*2.4.1 Stavudine-5'-acetate **(4)***

A yield of 3.04 g (85.3%) white crystalline compound was obtained; mp 187.94°C ; R_f 0.71 (dichloromethane:methanol) (9:1); $C_{12}H_{14}N_2O_5$; m/z: 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} (cm^{-1}): 1120; 1260; 1480; 1680; 2790; 3480. δ_H (ppm, $CDCl_3$) 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). δ_C (ppm, $CDCl_3$) 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").

*2.4.2 Stavudine-5'-propionate **(5)***

A yield of 2.36 g (94.4%) white crystalline compound was obtained; mp 191.12°C; R_f 0.72 (dichloromethane:methanol) (9:1); $C_{13}H_{16}N_2O_5$; m/z: 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} (KBr, cm^{-1}) 1130, 1265, 1490, 1700, 2800, 3450. δ_H (ppm, $CDCl_3$) 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). δ_C (ppm, $CDCl_3$) 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").

*2.4.3 Stavudine-5'-butyrate **(6)***

A yield of 2.58 g (65.5%) white crystalline compound was obtained; mp 174.26°C; R_f 0.73 (dichloromethane:methanol) (9:1); $C_{14}H_{18}N_2O_5$; m/z: 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} (KBr, cm^{-1}) 1125, 1270, 1480, 1690, 2810, 3470. δ_H (ppm, $CDCl_3$) 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 (ppm, $CDCl_3$) 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").

*2.4.4 Stavudine-5'-hexanoate **(7)***

A yield of 4.15 g (96.2%) white crystalline compound was obtained; mp 159.62°C; R_f 0.75 (dichloromethane:methanol) (9:1); $C_{16}H_{22}N_2O_5$; m/z: 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} (KBr, cm^{-1}) 1090, 1260, 1470, 1680, 2820, 3480. δ_H (ppm, $CDCl_3$) 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). δ_C (ppm, $CDCl_3$) 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'').

*2.4.5 Stavudine-5'-octanoate **(8)***

A yield of 4.53 g (96.2%) white crystalline compound was obtained; mp 154.36°C; R_f 0.79 (dichloromethane:methanol) (9:1); $C_{18}H_{26}N_2O_5$; m/z: 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} (KBr, cm^{-1}) 1100, 1250, 1470, 1660, 2880, 3460. δ_H (ppm, $CDCl_3$) 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).). δ_C (ppm, $CDCl_3$) 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'').

*2.4.6 Stavudine-5'-decanoate **(9)***

A yield of 4.08 g (80.6%) white crystalline compound was obtained; mp 151.05°C; R_f 0.83 (dichloromethane:methanol) (9:1); $C_{20}H_{30}N_2O_5$; m/z: 379 (($M+H^+$) 15.6%), 207 (14.3%), 162 (10.0%), 154 (14.6%), 136 (15.4%), 127 (100%), 107 (9.1%). ν_{max} (KBr, cm^{-1}) 1120, 1270, 1480, 1700, 2890, 3460. δ_H (ppm, $CDCl_3$) 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). δ_C (ppm, $CDCl_3$) 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'').

2.5 Physicochemical properties

2.5.1 Aqueous solubility

Saturated solutions of stavudine and its derivatives were used to determine the aqueous solubility in phosphate buffer solution (PBS) at pH 7. In a water bath at 32°C the slurries were stirred with magnetic bars for 24h. The

solutions were saturated at all times with an excess of the solute present. The solutions were filtered after 24h and diluted prior to being analyzed by HPLC to determine the concentration of the solute dissolved in the solvent. The experiment was performed in triplicate.

2.5.2 Octanol-PBS partition coefficient (log D)

Equal volumes of *n*-octanol and PBS pH 7 were saturated with one another by stirring for 24h and then separated. An excess of stavudine and of each derivative was suspended in pre-saturated *n*-octanol (1 mL) and pre-saturated PBS (1 mL). The solutions were saturated at all times with an excess of the solute present. The solutions were stoppered and agitated for 90 min where after 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 analyzed by HPLC. The partition coefficients were calculated as logarithmic ratios of the stavudine derivative concentrations in the *n*-octanol phase to the concentrations in the PBS. The experiment was performed in triplicate.

2.6 Preparation of Pheroid™

Pheroid™, as utilized in this study, is a patented system manufactured by the Unit for Drug Research and Development, North-West University, Potchefstroom, South Africa. The 'micro-sponges' Pheroid™ formulation was used.

2.7 Transdermal permeation

2.7.1 Preparation of skin

The project "*In vitro* transdermal delivery of drugs through human skin" was approved by the Ethics Committee (reference number 04D08) of the North-West University (Potchefstroom, South Africa). For the permeation studies female human abdominal skin, obtained with informed consent subsequent to cosmetic procedures from the Sandton Surgical Centre, Johannesburg, South Africa, 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 skin was not ruptured. The epidermis was cautiously placed on Whatman® filter paper and then 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 visual examination for any defects, the epidermis was mounted on the Franz diffusion cells with the stratum corneum facing upwards.

2.7.2 Donor solutions preparation

Saturated solutions of stavudine and its derivatives were prepared by stirring the slurries with magnetic bars in a water bath for 24 h at 32°C. These slurries were used to fill the donor compartments of vertical Franz diffusion cells. An excess amount of solute in PBS (pH 7) was present at all times. The same method was used for the Pheroid™. The compound was encapsulated in the Pheroid™ solution and the receptor compartments were filled with isotonic PBS at pH 7.4 as before.

2.7.3 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 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 (stratum corneum side up) 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 mL isotonic PBS with a pH of 7.4 and the donor compartments filled with 1 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. Newly prepared PBS (1 mL) or Pheroid™ suspension (1 mL) was added to the donor compartments and it was covered with Parafilm® to prevent any evaporation of the constituents for the duration of the experiment. The donor compartments were saturated at all times with an excess amount (as visually observed) of solute present. The entire receptor volumes were withdrawn after 2, 4, 6, 8, 10, 12 and 24 h 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 24h periods and were performed in triplicate.

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

Data, as collected for all cells was used, except for those cells, which by observation, had leaked (as seen through the rise of receptor buffer past the 2 mL mark or indicated by the milky receptor phase with the use of Pheroid™). Average flux was calculated from the gradient of the cumulative concentration versus time graph.

3 Results and discussion

3.1 Esterification of stavudine

The ^{13}C NMR data of **(4)** were similar to that of stavudine, except for the signal of the carbonyl carbon atom (C-1'') at δ 170.30 and that of the methyl group (C-2'') at δ 20.78. In the ^{1}H NMR spectrum the singlet at δ 2.10 represents H-2''. Confirmation that the acylation took place on the hydroxyl group on C-5' (as opposed to N-1) is rendered by the downfield shift of the ^{1}H NMR signals of H-5'a and H-5'b by *ca*. 1 ppm each relative to that of stavudine indicating conversion to an ester. The MS data confirmed the presence of the molecular ion of **(4)** at m/z 267, corresponding to a molecular formula of $C_{12}H_{14}N_2O_5$. Within 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}. The spectral properties of the other derivatives were similar to that of **(4)** and the structures of all the derivatives were determined in the same way.

3.2 Physicochemical properties

Table **2**: Flux, aqueous solubility and log D of compounds **(1)**, **(4)** – **(9)**.

Compound	Flux (μmol/cm^2.h)				Aqueous solubility (mg/mL)	Log D
	PBS	SD [a]	Pheroid™	SD [a]		
(1)	6.52	1.402	6.12	2.857	104.75	-0.85
(4)	0.23	0.015	0.75	0.038	5.17	-0.41
(5)	0.21	0.018	6.64	8.429	1.73	0.27
(6)	0.17	0.068	6.87	3.776	1.05	0.89
(7)	0.06	0.000	1.55	0.310	0.11	1.86
(8)	nd*	nd*	0.86	0.000	0.08	2.66
(9)	nd*	nd*	nd*	nd*	nd*	3.06

* not detectable, [a] Standard deviation

The solubility values of the derivatives in PBS are lower than that of stavudine, which inherently has a higher aqueous solubility (104.75 mg/mL). **(4)** has the highest aqueous solubility value of all the derivatives. By referring to the results in Table **2**, the solubility in general decreased with an increase in chain length in accordance with data in the literature [16].

As expected from the data seen in Table **2**, all the esters are more lipophilic than the parent compound (-0.85) in terms of partition coefficients between *n*-octanol and pH 7.0 buffer, due to an increase in the alkyl chain length.

(9) has the highest log D value (3.06) of all the compounds. This leads to an increase in partition coefficients in accordance with data in the literature [17].

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 solubility values.

3.3 Transdermal diffusion of the compounds and the effect of encapsulation in Pheroid™

The experimental transdermal flux of stavudine in PBS was much higher than that of its derivatives and slightly higher in PBS (6.52 µmol/cm^2.h) than in Pheroid™ (6.12 µmol/cm^2.h). 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 [18]. The higher flux is most probably the result of stavudine penetrating through the rich protein spaces between the stratum corneum and the corneocytes [2]. The stratum corneum is hydrated in a polar solution for 24h making it possible for hydrophilic compounds to penetrate [19].

The esters **(4)**, **(5)**, **(6)** and **(7)** diffused better through the skin in the Pheroid™ medium than in the PBS and **(8)**; in PBS did not diffuse through the skin, but in Pheroid™ it did. **(5)** and **(6)** were the compounds with the highest transdermal flux in Pheroid™ with a flux value of 6.64 and 6.87 µmol/cm^2.h, respectively. It seems that the more lipophilic compounds with an aqueous solubility higher than 1 mg/mL are better encapsulated in the Pheroid™ formulation that consists of natural oils, which may assist in the penetration through the skin.

(9) had a log D value of approximately 3.00, but did not diffuse through the skin. This can be ascribed for this specific derivative being insoluble in water. This just proves once again that to cross the stratum corneum, a drug should possess both hydrophilic and lipophilic properties.

4 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. Stavudine is one agent of the most important class of compounds, the nucleoside reverse transcriptase inhibitors, and is active against HIV. 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 also to include the advantages associated with transdermal therapy.

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 [16].

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

As expected, the aqueous solubility of stavudine (104.75 mg/mL) was much higher and the partition coefficient of stavudine (-0.85) was lower than that of the synthesized derivatives. This was in accordance with data in the literature which proved that the solubility decreases and partition coefficient increases as the alkyl chain length and molecular weight increased.

Stavudine in PBS gave much higher experimental flux values when compared to its derivatives. Stavudine was more hydrophilic than the other compounds and most probably penetrated through the protein rich spaces between the stratum corneum and the coenocytes, resulting in higher fluxes.

The esters had better penetration with Pheroid™ than with PBS. Compounds **(5)** and **(6)** had aqueous solubility values higher than 1 mg/mL and log D values of less than 1, but also gave the best penetration of all the esters in Pheroid™. Compounds **(7)** and **(8)** had aqueous solubility values lower than 1 mg/mL and log D values of more than 1, but it did not penetrate better than **(5)** and **(6)**. In Pheroid™, compounds **(7)** and **(8)** diffused slightly better than **(4)**. Pheroid™ consists of essential oils that may improve penetration through the skin, which explains why the esters in Pheroid™ gave better diffusion than in PBS. In this study it is seen that diffusion was better with both lipophilic and hydrophilic properties with an aqueous solubility of at least more than 1 mg/mL.

This differs from the results we obtained in a previous study with lamivudine where we could not obtain any enhancement in transdermal penetration by either preparing derivatives or using the Pheroid™ delivery system, although entrapment in Pheroid™ had taken place and was seen in the micrographs taken with confocal laser scanning microscopy [20]. No single delivery system can claim that it will improve the transdermal delivery of all compounds. This study has confirmed that transdermal flux is dependent on several factors such as aqueous

solubility, log D and in some instances minor modifications to the drug may be necessary. The best results in this study were achieved by synthesizing **(5)** and **(6)** and using Pheroid™ as delivery system.

Acknowledgements

We thank Aspen Pharmacare, Port Elizabeth, South Africa, for a generous gift of stavudine, Anne Grobler and Dale Elgar for assistance with Pheroid™, Dr Gerhard Koekemoer for help with statistical analysis and Prof JL du Preez for HPLC analysis. The financial support of the National Research Foundation and Medical Research Council of South Africa is highly appreciated. Special thanks to Proff Kenneth Sloan, University of Florida, USA and Jonathan Hadgraft, University of London, for valuable advice.

References

[1] Ramachandran, C.; Fleisher, D. Transdermal delivery of drugs for the treatment of bone diseases. Adv. Drug Delivery Rev., **2000**, 42, 197 - 223.

[2] Foldvari, M. Non-invasive administration of drugs through the skin: Challenges in delivery system design. Pharm. Sci. Technol. To., **2000**, 3, 417 - 425.

[3] Barry, B.W. In: Dermatological formulations; Bronaugh, P.L.; Maibach, H.I., Eds.; Marcel Dekker: New York, **1983**; 664 p.

[4] Barr, M. Percutaneous absorption. J. Pharm. Sci., **2008**, 97, 395 - 409.

[5] Chien, Y.W. In: Advances in transdermal systemic medication; Chien, Y.W., Ed.; Marcel Dekker: New York, **1987**; pp. 1 - 22.

[6] Naik, A.; Kalia, Y.N.; Guy, R.H. Transdermal drug delivery: overcoming the skin's barrier function. Pharm. Sci. Technol. To., **2000**, 3, 318 - 326.

[7] Idson, B. Percutaneous absorption. J. Pharm. Sci., **1975**, 64, 901 - 924.

[8] Katz, M.; Poulson, R.T. In: Absorption of drugs through the skin; Brodie, B.B.; Gilette, J.R., Eds.; Springer Verlag: New York, **1971**; pp. 103 - 162.

[9] Wilkosz, M.F.; Bogner, R.H. Transdermal drug delivery. Part 1: current status. U.S. Pharmacist, **2003**, 28, 04.

[10] Hayden, F.G. In: Antiretroviral agents and treatment of HIV infection; Hardman, J.G.; Limbird, L.E.; Molinoff, P.B.; Ruddon, W.R.; Gillman, A.G., Eds.; McGraw-Hill: New York, **2005**; pp. 1273 - 1314.

[11] McEvoy, G.K. AHFS Drug information. United States of America, **2002**.

[12] Hurst, M.; Noble, S. Stavudine: An update of its use in the treatment of HIV infection. Drugs, **1999**, 58, 919 - 949.

[13] Grobler, A.; Kotze, A.; Du Plessis, J. In: The design of a skin-friendly carrier for cosmetic compounds using Pheroid™ technology; Wiechers, J., Ed.; Science and applications of skin delivery systems. Allured Publishing: Wheaton, IL., **2008**; pp. 283 - 311.

[14] Saunders, J.C.J.; Davis, H.J.; Coetzee, L.; Botha, S.; Kruger, A.E.; Grobler, A. A novel skin penetration enhancer: Elevation by membrane diffusion and confocal microscopy. J. Pharm. Pharm. Sci., **1999**, 2, 99 - 107.

[15] Grobler, A. Emzaloid™ Technology. Ref Type: Report, North-West University: Potchefstroom, **2004**; 20 p.

[16] Abdou, H.M. Dissolution, bioavailability and bioequivalence; Mack Publishing Company: Easton, Pennsylvania, **1989**.

[17] Guy, R.H.; Hadgraft, J. In: Selection of drug candidates for transdermal drug delivery; Guy, R.H.; Hadgraft, J., Eds.; Marcel Dekker: New York, **1989**; 324 p.

[18] Rothman, S. Physiology and biochemistry of the skin. The University of Chicago Press, **1954**, 27 - 53.

[19] Hull, W. Heat-enhanced transdermal drug delivery: a survey paper. The journal of applied research, 2(1). http://www.jrnlappliedresearch.com/articles/Vol2Iss1/Hull.htm (Accessed May 27, **2010**).

[20] Gerber, M.; Breytenbach, J.C.; Du Plessis, J. Transdermal penetration of zalcitabine, lamivudine and synthesised N-acyl lamivudine esters. Int. J. Pharm., **2008**, 351, 186 - 193.