Optimised transdermal delivery of pravastatin

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Abstract

Wiechers’ programme “Formulating for Efficacy” initiated a new strategy to optimise the oil phase of topical formulations in order to achieve optimal transdermal drug delivery. This new approach uses the “Delivery Gap Theory” on any active pharmaceutical ingredients (APIs) to test if it could enhance transdermal drug delivery. The aim of the study was to formulate six different semi-solid formulations (three creams and three emulgels) with 2% pravastatin as the API in order to investigate the “Delivery Gap Principle”, by determining which formulation would deliver pravastatin best to the target-site (system circulation). The three cream- and three emulgel formulations had different polarities, i.e. a formulation with polarity equal to that of the stratum corneum (optimised), a non-polar (lipophilic)- and a polar (hydrophilic) formulation. Franz cell diffusion studies were executed over 12 h and the optimised emulgel (2.578 μg/cm²) had the highest median amount per area obtained. Tape stripping followed the diffusion studies and in the stratum corneum-epidermis, the hydrophilic emulgel (1.448 μg/ml) contained the highest median pravastatin concentration and the epidermis-dermis the optimised emulgel (0.849 μg/ml) depicted the highest pravastatin concentration. During this study, it was observed that when both emulgel and cream formulations were compared; the emulgels enhanced the delivery of pravastatin more than the creams.

Keywords: Pravastatin, Wiechers, Transdermal delivery, Formulation, Delivery Gap Theory, Franz cell
Graphical Abstract

Wiechers programme: Formulating for efficacy

Stratum corneum: Main barrier

Delivery Gap Theory to optimise oil phase

Tape-stripping

Cream formulations
- Hydrophilic
- Lipophilic
- Optimised

Emulgel formulations
- Hydrophilic
- Lipophilic
- Optimised

Franz cell diffusion studies
1 Introduction

The human skin is the heaviest organ of the body (Sanders et al., 1999), weighing approximately 5 kg which approximates to 2 m² in surface area (Godin & Touitou, 2007) and consists of heterogeneous layers (Pailler-Mattei et al., 2008). The human skin is composed of three layers, namely the: 1) epidermis, 2) dermis and 3) hypodermis (Pailler-Mattei et al., 2008). The main function of the skin is to protect the human body against outside, hazardous substances and prohibit the loss of endogenous substances (Bouwstra & Honeywell-Nguyen, 2002). The outer-most layer of the skin is the stratum corneum and is known for the barrier it provides against diffusion of active pharmaceutical ingredients (APIs) through the skin to the systemic circulation (Bouwstra & Honeywell-Nguyen, 2002).

Therefore, in order for an API to pass through the lipophilic stratum corneum and hydrophilic epidermal- and dermal layers (Perrie et al., 2012); the API should possess both lipophilic and hydrophilic properties (Perrie et al., 2012).

In this study, pravastatin was selected as a model drug to investigate FFE™ (Formulating for Efficacy). Pravastatin is a HMG-CoA (3-hydroxy-3-methylglutaryl-coenzyme A) reductase inhibitor, which increases the hepatic low-density lipoprotein (LDL)-receptor activity, decreases the level plasma LDL-cholesterol and inhibits the rate limiting step of cholesterol synthesis in the liver (Heath et al., 1999). Adverse effects most commonly associated with statins are poor patient compliance, because of myopathy, hepatitis, rhabdomyolysis, headache, fatigue, gastrointestinal intolerance and general malaise. Patients using statins usually experience myalgia, fatigue, weakness, mild creatine kinase elevations, headaches and increased liver enzymes to 300% in any dosage (Lane, 2005). When taking all the adverse effects into consideration, especially the greatest adverse effect which would be increased liver enzymes, it would be ideal to deliver pravastatin transdermally to exclude the first pass metabolism effect. Sufficient concentrations of pravastatin should permeate through the skin to be delivered to the target-site (blood capillaries) in order for it to exhibit its effect. The therapeutic blood concentration of pravastatin is 64.1 ng/ml (Clarke et al., 2011).
In skin delivery, there are three polarities that must be taken into consideration, namely the polarities of the API, the stratum corneum and the formulation. The API polarity cannot be changed; the stratum corneum polarity can be changed, but not excessively, whereas the formulation polarity can be changed freely. The FFE™ computer program was developed by Wiechers (Wiechers, 2008; Wiechers, 2011) to calculate the polarity of a formulation, in order to ensure that there is a correct balance between the API’s best driving force into the skin from the formulation and to incorporate as much possible API in any formulation chosen (Wiechers, 2008).

To reach the desired polarity (hydrophilic, optimised or lipophilic); the formula has to be manipulated. The FFE™ programme (Wiechers, 2011) is based on the Hansen solubility parameters (HSP) to optimise the skin delivery of APIs from formulations. The solubility parameter is regarded as a route comprised of the following components: the energy from hydrogen bonds between molecules (δₕ), the energy from dipolar intermolecular force between molecules (δₚ) and the energy from dispersion bonds between molecules (δₐ) (Wiechers, 2011). The HSP is the three dimensional (3-D) representation of δₐ, δₚ and δₕ.

According to Hansen solubility, the parameters of the skin are 17, 8 and 8 for δₐ, δₚ and δₕ, respectively. The positions of the HSPs of the skin, the API and the formulation are represented by spheres which are in fact in definite positions. These three parameters in the HSP are provided as co-ordinates for a point (Wiechers, 2011).

When the HSP values of the skin and formulation are similar, the conditions for the API to permeate into the stratum corneum are optimal. Thereafter the API can diffuse to the other layers of the skin whether the physicochemical properties of the API permit this; of which the molecular weight (MW) plays a vital role (Wiechers, 2011).

The AFG (active formulation gap) is known as the ‘distance’ between the API and the formulation and the SFG (skin formulation gap) is known as the ‘distance’ between the skin and the formulation. The API is compatible with the formulation if the AFG increases, meaning the API would rather stay in the formulation. But when the AFG decreases, the API can permeate into the skin because the driving force increases (Wiechers, 2011). The
driving force for diffusion and surface concentration increases when the SFG decreases, which indicates there will be an increase of permeation of API through the skin and therefore the formulation will become more favourable to the skin (Wiechers, 2011).

The SDG (skin delivery gap) is the ratio of the minimum effective concentration (MEC) relative to the concentration reached at the target site ($C_{TS}$) or local tissue. Thus molecules with a SDG less than 1, deliver readily and those above 1 are more complex delivery systems. The local tissue concentration can be predicted by utilising a chain of calculations of molecular modelling of the skin and pharmacokinetic assumptions. By using the SDG both intrinsic activity and deliverability of active molecules can be compared.

The FFE™ programme allows optimal skin delivery of an API from a formula by offering three options:

1) Optimising towards the skin: Depending on the APIs physicochemical properties as well as the skin thickness, the HSPs of the stratum corneum must match those of the formulation. This option suggests large quantities of the API will penetrate the skin.

2) Optimising towards the API: The API must be dissolved adequately so the HSPs of the API could match the HSPs of the formulation.

3) Optimising towards the target concentration: The maximum driving force for the API is reached when the concentration of the API in the chosen formula is close to the maximum solubility limit. The API will then diffuse from the formulation and permeate the skin (Wiechers, 2011). The SFG is minimised when skin delivery is optimised towards the skin. This indicates the spheres are brought as close as possible to each other (which can be presented by the skin and formulation). The normal skin ratio is 17.0:8.0:8.0.

The aim of the study was to assess the 'delivery gap principle' by optimising the oil phase of the six formulations with FFE™ to thereby possibly enhance the penetration of pravastatin. The different formulations will be compared according to its polarity and the permeation efficacy of pravastatin.

2 Materials and Methods

2.1 Materials
Pravastatin sodium was obtained from DB Fine (Johannesburg, RSA). Mineral oil, cetyl alcohol, isopropyl myristate, ethanol analytical grade, analytical grade methanol, stearic acid, polyethylene glycol 400, Span 60 and Tween 80 were obtained from Merck (Midrand, RSA). Dimethyl isosorbide and potassium cetyl phosphate were obtained from Croda (Midrand, RSA). Ultrez 20 was obtained from Lubrizol (Johannesburg, RSA). Glycerine was obtained from Sigma-Aldrich (Johannesburg, RSA). Veegum was obtained from R.T. Vanderbilt Company, Inc. (Kentucky, USA). Sodium hydroxide and orthophosphate used for the preparation of phosphate buffered solution (PBS) were supplied by Merck Laboratory Supplies (Midrand, RSA). Throughout the entire study deionised high performance liquid chromatography (HPLC) grade water (Millipore, Milford, USA) was used.

2.2 Methods

2.2.1 Formulation of semi-solid products

Six formulations containing pravastatin sodium (2%) was developed during this study. The formulations (cream and emulgel) consisted of three different polarities, i.e. a non-polar (lipophilic) formulation, a very polar (hydrophilic) formulation and a formulation where the polarity was equal to that of the stratum corneum (optimised). These formulations were manipulated by means of the programme FFE™ to obtain the HSP values in order to attain the desired polarity.

Table 1:

<table>
<thead>
<tr>
<th>Ingredients used in the formulation for different polarity creams and emulgels</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pravastatin in different polarity formulations were prepared. After the ingredients of the phases were added, each phase was heated between 70 and 75 °C, respectively.</td>
</tr>
<tr>
<td>Pravastatin was added to water and stirred; thereafter the other ingredients of Phase C were added to water and pravastatin mixture. All the ingredients of Phase A were added together; the same was done for Phase B. Subsequently, Phase B was added to Phase C whilst stirring; which was then added to Phase A while stirring continued. The mixture was homogenised at 9500 rpm for 5 min and stirred to cool.</td>
</tr>
</tbody>
</table>
2.2.2 Analysis of pravastatin

An HPLC method was developed and validated at the Analytical Technology Laboratory (ATL), North-West University (NWU), Potchefstroom Campus, RSA. The HPLC (Agilent 1200 Series) was equipped with an Agilent 1200 pump, a diode array detector, an autosampler injection mechanism and Chemstation Rev. A.10.01 software for data analysis (Agilent Technologies, Palo Alto, CA). The UV-detector was set at a wavelength of 238 nm. The mobile phase consisted of 0.1% orthophosphoric acid in HPLC water (600 ml) and acetonitrile (400 ml) and had a 50 µl injection volume and flow rate of 1.0 ml/min. The runtime comprised of 6 min and the retention time was between 2 and 3 min. The limit of detection (LOD) was calculated to be 0.0253 µg/ml and the limit of quantification (LOQ) 0.505 µg/ml. Analytical tests were performed in a laboratory with a controlled environment of 25 ºC.

2.2.3 Standard preparation

Approximately 50 mg of pravastatin was weighed off accurately in a 100 ml volumetric flask, then dissolved in about 10 ml of methanol and filled to volume with PBS (pH 7.4) (BP, 2013a; BP, 2013b) further dilutions were made to obtain standards ranging from 0.5-101.0 µg/ml.

2.2.4 Physicochemical properties

2.2.4.1 Aqueous solubility

The water bath was preheated to 32 ºC (temperature on top of the skin during a diffusion study). PBS (pH 7.4) (BP, 2013a; BP, 2013b) and ethanol:PBS (pH 7.4) (10:90) was separately inserted in polytops with a magnetic stirrers and an excess of pravastatin was added and regularly checked to ensure the solutions were saturated at all times. After 24 h the solutions were removed, filtered and then centrifuged at 5000 rpm for 10 min to ensure complete precipitation. The PBS (pH 7.4) supernatant (1 ml) was diluted to 100 ml with PBS (pH 7.4) (BP, 2013a; BP, 2013b), whereas the ethanol:PBS (pH 7.4) (10:90) supernatant (1 ml) was diluted to 100 ml with ethanol:PBS (pH 7.4) (10:90) and the resultant solutions analysed by HPLC. This experiment was repeated in triplicate.
2.2.4.2 Octanol-buffer distribution coefficient (log D)

*n*-Octanol (100 ml) and equal volumes of PBS (pH 7.4) (BP, 2013a; BP, 2013b) were equilibrated with each other for 24 h in order for co-saturation to take place. Pravastatin sodium (0.4 mg) was dissolved in 3 ml pre-saturated PBS (pH 7.4) (BP, 2013a; BP, 2013b); thereafter 3 ml pre-saturated *n*-octanol was added to the aforementioned pravastatin solution. Subsequently, the solution were placed at 32 °C in the shaker water bath for 3 h and left overnight, then centrifuged to ensure complete separation. The HPLC was used to determine the concentrations of API in the separate phases. The log D was calculated through the logarithmic ratio of the concentration in the PBS (pH 7.4) (BP, 2013a; BP, 2013b) and the concentration in the *n*-octanol phase. This experiment was performed in four fold.

2.3 Characterisation of pravastatin formulations

The characterisation of the six formulations was performed by measuring the following: pH, viscosity, droplet size and zeta-potential.

2.3.1 pH

A Mettler Toledo pH meter (Greifensee, Switzerland) was used to measure the pH of the formulations. The apparatus was calibrated each time before use and the pH of each formulation was measured in triplicate at the same conditions (32 °C).

2.3.2 Viscosity

A Brookfield Viscometer (model DV II, Stoughton, USA) was used to measure the viscosity of the six formulations by determining resistance to a rotating spindle turning at a specific rate (measured in rpm) which was immersed in the formulation medium. Formulations were placed in a water bath to reach room temperature (25 °C), removed and the spindle (Stoughton, USA) was placed in the formulation whilst formulation was positioned in the apparatus. Thereafter the rate was specified and the viscosity reading was measured every 10 sec for approximately 5 min. The average viscosity was determined after the 32 readings were obtained.

2.3.3 Droplet size
The Malvern Mastersizer 2000, equipped with a wet cell Hydro 2000 MU dispersion unit, determined the droplet size (Malvern Instruments, Worcestershire, UK). Measurements were taken from six freshly prepared samples with three readings made per sample.

### 2.3.4 Zeta-potential

The Malvern Zetasizer 2000 (Malvern Instruments, Worcestershire, UK) was used to define the zeta-potential and measurements were taken from six prepared samples in triplicate. Measurements were taken between 3.5 to 65.5 rpm over a period of 300 sec at room temperature. Each sample (0.5 g) was diluted with 3 ml of Milli-Q water. An average of 30 readings were taken of each formulation and calculated. The average percentage torque for the formulations was calculated to be between 47 and 50%.

### 2.4 Diffusion experiments

#### 2.4.1 Membrane release studies

During the release studies, vertical Franz diffusion cells were used. Vacuum grease was applied to both the receptor (with a capacity of approximately 2 ml) and donor (with a capacity of 1 ml and a diffusion area of 1.075 cm$^2$) compartments. A magnetic stirring rod was placed in the receptor compartment. The hydrophilic polyvinylidene fluoride (PVDF) membrane filters (FP Vericel™, 0.45 µm, 25 mm, Pall®) were placed on the receptor compartment and the Franz cell compartments (donor and receptor) were placed together and fastened with a horseshoe clamp. The donor compartment was covered with Parafilm® to avoid any loss of the constituents. The receptor compartment was filled with PBS (pH 7.4) (BP, 2013a; BP, 2013b) and the donor compartment was filled with formulation containing 2% pravastatin sodium at pH 5.0. At a temperature of 37 °C the cell systems were maintained. Every hour for 6 h the receptor compartments were extracted with a syringe and the compartment was refilled with PBS (pH 7.4) (BP, 2013a; BP, 2013b) at 37 °C. The HPLC was used to analyse all the samples. The release rate of the API and the concentration of the API that permeated through the membrane into the receiver fluid were determined for the formulation by means of the HPLC.

#### 2.4.2 Skin preparation
Following abdominoplasty surgery, Caucasian full-thickness abdominal skin (ethical approval reference number: NWU-00114-11-A5) was collected from plastic surgeons. To ensure the skin could easily be separated from the fatty layer; it was kept in a freezer at -20 °C until used. Skin that was dermatomed with a thickness of 400 µm was cut into circles (±15 mm in diameter) and placed on Whatman® filter paper to dry. Thereafter the skin was wrapped in aluminium foil and stored in the freezer at -20 °C. Before diffusion studies commenced, the frozen skin samples were thawed, visually examined for defects and mounted on the diffusion apparatus.

2.4.3 Skin diffusion

The same method as described under Section 2.4.1 was used during the skin diffusion studies, except dermatomed skin instead of PVDF membrane filters (FP Vericel, 0.45 µm, 25 mm, Pall®) was used with the stratum corneum facing the upper donor compartment. The receptor compartment was filled with a mixture of PBS (pH 7.4) (BP, 2013a; BP, 2013b) and ethanol (90:10) and the entire receptor volume was withdrawn after 12 h. Ethanol was added since there was no diffusion of pravastatin into the receptor phase and therefore no median fluxes were observed by using only PBS (pH 7.4). It should also be kept in mind that only a 2% formulation was used and therefore a higher concentration gradient will act as a driving force in increasing drug permeation (Barry, 2002). The API concentration that permeated through the skin, into the receiver fluid, was determined and samples were analysed by means of the HPLC (Baert et al., 2011).

2.4.4 Tape stripping

To determine the API concentration present in the stratum corneum-epidermis (SCE) and epidermis-dermis (ED) the tape stripping technique was used after the 12 h diffusion studies were completed. The Franz cells were dismantled and the skin was pinned to a solid surface covered with Parafilm®; any remaining formulation on the skin was dabbed dry with a paper towel. 3M Scotch® Magic™ tape was cut into the same size as the diffusion area. The first tape strip was disposed of due to possible contamination with the formulation still left on the skin; the following 15 tape strips removed SCE and API until the skin glistened.
(Pellet et al., 1997); this was placed in a polytop, filled with 5 ml PBS (pH 7.4) (BP, 2013a; BP, 2013b) and ethanol (90:10). The ED that was left after the procedure was cut into smaller pieces to enhance the surface area (Pellet et al., 1997) and placed in another polytop containing 5 ml PBS (pH 7.4) (BP, 2013a; BP, 2013b) and ethanol (90:10). These solutions containing the tape strips (SCE) and skin pieces (ED) were stored overnight at 4 °C, in order for the API to dissolve. SCE and ED samples were analysed by means of HPLC.

2.5 Data analysis
The linear portion of the graph of pravastatin represented the flux during membrane studies. Mean flux values were obtained by using the slope of the straight line where cumulative concentrations are compared over time. The percentage released during the membrane studies was determined after 6 h. The mean amount per area of pravastatin which permeated the skin after 12 h for each Franz cell was plotted during the diffusion studies for the different formulations. By using the yield of each cell a percentage of the applied amount per area was expressed, where the percentage diffused was also determined after 12 h for the diffusion studies.

2.6 Statistical analysis
Descriptive statistics involve calculations of the median (middle score in distribution), mean (with standard deviation (SD)) of the flux values during membrane studies and the concentration values of diffusion studies (Sheskin, 2000). Box-plots were used to illustrate data by using first and third quartiles of distribution as well as the median values (Dawson & Trapp, 2004). To determine the significance of the effects of two formulations (such as emulgel and cream), of the different polarity formulations and of the interaction between the different polarity formulations of cream and emulgel, a two-way analysis of variance (ANOVA) was used in the membrane study. By using the ANOVA, p-values were determined. A statistical significant effect is indicated is a p-value of 0.05 or less (Steyn et al., 1994). In the membranes studies to determine if there was any significant differences between the mean data values in the different polarity formulations, i.e. hydrophilic emulgel
Tukey HSD (honestly significant difference) test was used for unplanned comparisons, when in a set of data possible comparisons were made (Sheskin, 2000). A two-way ANOVA was used in the skin diffusion study where the different polarity formulations (H, L and O) were compared, the formulations (cream and emulgel) were compared, as well as the formulation (cream and emulgel) and different polarity formulation interaction was determined. By applying three-way ANOVA the mean concentration and tape stripping were compared, where the different polarity formulations were compared within emulgel and cream formulations. By means of Statistica (Statsoft, 2008) and SAS (SAS Institute Inc., 2005) the abovementioned statistical analyses were performed. When there is a significant variation between the mean and median values; the median is a more exact method to determine, i.e. flux and concentration (Dawson & Trapp, 2004). Both mean and median values will be presented throughout this study, but median will be used to describe the data.

3 Results and Discussion

3.1 Formulation and semi-solid products

All six formulations, three of which were creams and three emulgels, contained 2% pravastatin and were not too oily, applied easily and had a homogeneous white texture with a soft feel.

Table 2:

<table>
<thead>
<tr>
<th>Hansen solubility parameters, molecular weight, active formulation gap and skin formulation gap of the formulations with different polarities</th>
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</thead>
</table>

Table 2 consists of the HSP-, MW-, AFG- and SFG values of the three different polarity formulations that were calculated by the FFE™ computer programme. It is evident that the δ values are more or less the same when comparing the hydrophilic (HC and HE), lipophilic (LC and LE) and optimised (OC and OE) formulations. Although, when the lipophilic
formulations and the hydrophilic formulations are compared to the optimised formulations, the lipophilic formulations have lower $\delta_p$ and $\delta_h$ values and the hydrophilic formulations have higher $\delta_p$ and $\delta_h$ values than the the optimised $\delta_p$ and $\delta_h$ values, respectively. It is noticeable that the optimised formulations’ HSP values are much closer to the HSP values of the skin, than the HSP values of the hydrophilic- or lipophilic formulations. Hence, the optimised formulations should therefore increase permeation through the skin (Wiechers, 2011).

When comparing the AFG values of the different polarity formulations from lowest to highest value, the hydrophilic formulations (HC and HE) had the lowest AFG value, followed by the optimised formulations (OC and OE) and then lastly the lipophilic formulations (LC and LE). Therefore the hydrophilic formulations are expected to increase the driving force and in turn enhance the permeation of the API into the skin (Wiechers, 2011).

From the lowest to the highest value, the optimised formulations (OC and OE) had the lowest SFG value, followed by the lipophilic formulations (LC and LE) and then lastly the hydrophilic formulations (HC and HE). Consequently, the optimised formulations, when compared to the hydrophilic- and lipophilic formulations, are projected to improve the permeation through the skin (Wiechers, 2011).

3.2 Physicochemical properties

3.2.1 Aqueous solubility

The solubility of pravastatin was determined to be 197.5 mg/ml in PBS (pH 7.4) (BP, 2013a; BP, 2013b) and 205.14 mg/ml in ethanol:PBS (pH 7.4) at a temperature of 32 °C. Molecules ideally permeate through the skin when it has an aqueous solubility of more than 1 mg/ml (Naik et al., 2000). Hence, taking the aforementioned into account, pravastatin is an ideal candidate to permeate through the skin.

3.2.2 Log D

Log D can be described as the ability of a drug to dissolve both in water and oil. This value should be between 1 and 3 (Naik et al., 2000), which indicates the compound would permeate the skin exceptionally fast (Brain et al., 1998). The log D of pravastatin was determined to be -0.703, therefore expecting penetration would not be optimal.
3.2.3 Characterisation of semi-solid formulations

The data obtained from the pH, viscosity, droplet size and zeta-potential analysis are summarised in Table 3.

Table 3:
Characterisation results of pravastatin formulations

The pH influences the degree of ionisation of an API. The unionised form has a higher permeability whereas ionised compounds have higher aqueous solubility, but lower permeability. Pravastatin dissociates at a pH of 3. This is not suitable for transdermal delivery since a non-physiological pH alters skin permeation and may affect the solubility (Barry, 2007); therefore the lowest pH that can be used is pH 5. Pravastatin molecules (pKa = 4.7) are 33.39% unionised at pH 5 where it can diffuse through the skin (Admescope, 2012).

The viscosity readings indicated that all the formulations had very high viscosity.

Emulsion droplet sizes ranged from 10 nm to 10 µm (Wiechers, 2008). When the size of the droplet changes a change in total area of the dispersed phase occurs (Wiechers, 2008).

Literature reports that when the droplet size decreases, it does not suggest that penetration increases, seeing as emulsions differ in system components or composition (Wiechers, 2008), thus, accumulation did take place in the stratum corneum even though droplet sizes were not in range.

Stable zeta-potential values ranged from below -30 mV or above 30 mV (Malvern instruments, 2014). It was observed that the zeta-potential values were in the acceptable range (between 40 mV to 78 mV), which indicates the formulas were stable.

3.3 Membrane diffusion experiments

An oversaturated solution was also used to determine whether there would be any diffusion as possible control. No flux data was obtained during this experiment. The highest median %pravastatin diffused after 6 h was OE (0.115%), followed by HE and LE (0.103%), HC (0.082%), OC (0.068%) and LC (0.050%). The highest median flux values were as follow:
firstly the OE (7.175 μg/cm².h), followed by LE (6.401 μg/cm².h), HE (6.355 μg/cm².h), HC (5.061 μg/cm².h), OC (4.297 μg/cm².h) and lastly, the LC (3.115 μg/cm².h). It is clear the emulgel formulations released pravastatin better than the comparing cream formulations.

3.4 Diffusion experiment

3.4.1 Diffusion study

During the diffusion studies the amount of pravastatin diffused per area (μg/cm²) after two hourly extractions were too low; therefore no flux values (μg/cm².h) could be obtained. The extractions were then only taken after 12 h for each Franz cell in order to see if any pravastatin diffused through the skin. Illustrated in the box-plot in Fig. 1 is the mean and median amount per area (μg/cm²) of pravastatin that diffused through the skin in different semi-solid formulations.

Fig. 1: Box-plot representing the amount per area (μg/cm²) of pravastatin that diffused through the skin after 12 h for the six different formulations. The mean and median flux values are indicated by the diamond shapes and lines, respectively.

The median amount per area of pravastatin that permeated through the skin after 12 h were compared with each other and the following was observed: the OE formulation (2.578 μg/cm²) had the highest median amount per area obtained, followed by OC (1.449 μg/cm²), HC (0.434 μg/cm²), LE (0.121 μg/cm²), HE (0.055 μg/cm²) and lastly LC (0.000 μg/cm²).

When comparing the mean and median concentration values of the different formulations (in Fig. 1), it is evident that the most significant differences between the mean and median concentration values were for the OC- and OE formulation. Inaccurate estimation of true concentration values could be given by the mean values as they are influenced by skewed distributions around the central location. Therefore, median amount per area would represent the true concentration for pravastatin better than the mean concentration values, as the outliers do not affect the data (Dawson & Trapp, 2004).

When the OE and OC formulations were compared to the other hydrophilic and lipophilic formulations (HE, HC, LE and LC), the optimised formulations permeated better. The oil
phase was optimised from both the OE and OC formulations and according to Wiechers (2008), when the formulation and the skin polarity is the same, permeation would be optimal. When HE and HC (hydrophilic) were compared to LE and LC (lipophilic) it can be observed that both hydrophilic formulations increased the permeation of pravastatin through the skin, whereas only LE showed enhanced permeation. The LC formulation did not allow any permeation of pravastatin. The barrier properties of the stratum corneum is very good (Hadgraft, 2004), which could explain why LC did not permeate the stratum corneum; since, LC is the most lipophilic formulation. It is possible the LC formulation concentrated pravastatin in the lipophilic stratum corneum (Wiechers, 2008).

When the OE formulation was compared to the OC formulation, it was observed the OE formulation permeated better. The emulgel formulations overall permeated pravastatin better through the skin than the cream formulations, when the different polarity emulgels were compared to the different polarity creams. Emulgel formulations are more hydrophilic (formulations contain more water) and creams are more lipophilic (contains more oil content). The assumption can therefore be made that the emulgels, which are more hydrophilic, will permeate to the more aqueous regions, which in this case is the receptor fluid (Wiechers, 2008), whilst the cream formulations (more lipophilic) would rather remain in the lipophilic stratum corneum as it has the same affinity (Wiechers, 2008).

When comparing the hydrophilic and lipophilic formulations (HE, LE, HC and LC) to the optimised formulations (OE and OC), it was observed that the optimised formulations permeated better. The API of the optimised formulation can permeate freely into the stratum corneum; since, the HSP values of the optimised formulations (17.1, 7.6, 8.5) are the nearest to the HSP values of the skin (17.0, 8.0, 8.0). Transdermal delivery should improve as pravastatin is hydrophilic and would therefore permeate into the aqueous layers of the skin (Barry, 2007). The HSP values of the lipophilic (17.0, 6.5, 7.5) and hydrophilic (17.0, 8.5, 12.4) formulations do not allow adequate permeation because the HSP values of these formulations differ too much from the HSP values of the skin.
It is evident that when the AFG values (Table 2) of the hydrophilic formulations (1.3) and lipophilic formulations (5.7) were compared, the hydrophilic formulations had lower AFG values than that of the lipophilic formulations. This may explain why the hydrophilic formulations (HE and HC) permeated more efficiently through the skin than the lipophilic formulations (LE and LC); hence, due to the driving force effect (Wiechers, 2008).

Although, when the SFG values (Table 2) were compared, the hydrophilic formulations (7.9) and lipophilic formulations (4.2) had much higher SFG values than that of the optimised formulations (1.5), indicating that the API can diffuse easier through the skin as this formulation is more favourable for the skin.

Table 4:
The concentration (µg/ml) of pravastatin that diffused through the skin after 12 h for all the different polarity formulations

When comparing the therapeutic blood concentration of pravastatin to the different polarity formulations, it is observed that all the formulations had higher concentration values than the therapeutic blood concentration, except for LC and HE, which had lower concentrations. Therefore the aforementioned formulations (OE, OC, HC and LE) will achieve therapeutic concentrations after transdermal application.

3.5 Tape stripping

3.5.1 Stratum corneum-epidermis

Fig. 2: Box-plot indicating the concentration (µg/ml) pravastatin present in the SCE after tape stripping for the different formulations. The mean and median concentration values are indicated by the lines and squares, respectively. The tape stripping results for the SCE are depicted in Fig. 2. The results for SCE pravastatin concentrations were as follow: firstly HE (1.448 µg/ml) contained the highest median pravastatin concentration, followed by LE (1.301 µg/ml), LC (0.676 µg/ml), HC (0.505 µg/ml), OE (0.505 µg/ml) and lastly OC (0.400 µg/ml). It is observed that the hydrophilic and
lipophilic formulations accumulated more in the SCE than the optimised formulations, however all the formulations did accumulate in the SCE. Fig. 2 suggested the emulgel formulations (HE, LE and OE) improved the permeation of pravastatin into the SCE more than the cream formulations (HC, LC and OC). This could be explained by the hydrating effect, because emulgels contain more water than creams (Williams & Barry, 2004). During the experiments the skin was exposed to water vapour for 12 h, which led the skin to be hydrated. Hence, this can cause the lipids to open and the stratum corneum to swell (Williams & Barry, 2004), explaining the increased permeation of the API into the skin.

3.5.2 Epidermis-dermis

Fig. 3: Box-plot indicating the concentration (µg/ml) pravastatin present in the ED after tape stripping for the different formulations. The mean and median concentration values are indicated by the lines and squares, respectively.

In Fig. 3 it is evident that the results for ED pravastatin concentrations were as follow: firstly OE (0.849 µg/ml), followed by LC (0.572 µg/ml), HC (0.524 µg/ml), OC (0.355 µg/ml), HE (0.309 µg/ml) and lastly LE (0.138 µg/ml).

It is observed after comparing the optimised formulations (OE and OC) with each other that OE increased diffusion of pravastatin more into the ED than OC. It is detected that the cream formulations permeated better than the emulgel formulations when the hydrophilic (HE and HC) and lipophilic (LE and LC) were compared. This could be due to the aqueous regions within the skin lipids in the stratum corneum, which indicates that permeation into the viable epidermis decreased as well. It was evident when the formulations contained both lipid and aqueous solubility characteristics, the lipophilic part contributed to the capabilities of the API to diffuse into the stratum corneum and the hydrophilic part promoted permeation to the other layers of the skin (Perrie et al., 2012).

3.6 Statistical analysis

3.6.1 Membrane release studies
A two-way ANOVA was applied on the flux values. There was a statistical significance between the cream compared to emulgel (p < 0.001), as well as between the different polarity of the emulgel and cream formulations, i.e. optimised (OE, OC), hydrophilic (HE, HC) and lipophilic (LE, LC) (p < 0.001). There was a statistical significant interaction between formulations and different polarity formulations (p = 0.0168). One-way ANOVA’s were applied separately on the flux values of emulgel and cream since the above-mentioned interaction was encountered. The results were as follow: there was no statistical significance between different polarity formulations of emulgel (p = 0.0645), however there was statistical significance between the different polarity formulations of cream (p < 0.001). Thereafter Tukey HSD test for the unplanned comparisons were performed, which resulted in the means of the different polarity formulations of creams O, H and L were mutually significantly different on a 0.05 level of significance. There were no significant differences between any means of emulgel of the different polarity formulations.

3.6.2 Skin diffusion studies

Two one-way ANOVA’s were performed on the formulations (emulgel and cream) since statistical significant interaction between the formulations (emulgel and cream) and different polarity formulations exist, which was followed by the Tukey HSD tests. The following results were observed for the cream formulation: the mean values of the different polarity formulations OC, HC and LC were mutually significant different (p < 0.001). The following was observed for the emulgel formulations: there were significant differences between the means of LE/HE (p < 0.001), between the means of OE/LE (p < 0.001) as well as between OE/HE (p < 0.001).

3.6.3 Tape stripping

A three-way ANOVA was applied on the SCE and ED to indicate if there were any significant difference between the effects on the formulations as well as the different polarity formulations and the different interactions between them. It was observed that there was a statistical significance between SCE and ED (p = 0.0262), the interactions with the SCE/ED
of both formulations (emulgel and cream) \((p = 0.0015)\) and between the different polarity formulations (H, L and O) \((p = 0.0007)\).

4 Conclusion

The formulation of these six preparations which had different polarities containing pravastatin as the API was compiled by the FFE™ computer programme. The optimal oil phase was calculated by the programme, thereafter the formulations were made according to these specifications (Wiechers, 2011).

During release studies it was observed that the emulgel formulations released pravastatin better than the cream formulations when compared to each other. The same phenomenon was noticed with the skin diffusion studies, where the emulgel formulations permeated pravastatin better than the cream formulations. After release and diffusion data was evaluated, LC presented with the lowest median values and OE showed the highest median values in both cases. Even though all six formulations released pravastatin, it was noted that LC did not permeate pravastatin into the receptor phase (target-site).

While comparing the SCE (lipophilic) with the ED (hydrophilic), as well as the receptor phase (hydrophilic), it is depicted that the formulations had both hydrophilic and lipophilic properties, since the API penetrated the stratum corneum and thereafter diffused into the deeper layers of the skin (Potts, 1992). When all the formulations, with regard to their polarity, were compared, it was observed that the optimised formulations had the highest median concentration in the receptor phase, but the lowest median concentration in SCE, consequently the optimised formulation diffused the best into the target-site and through the SCE. This could be due to the fact that the oil phase for these formulations was optimised and had the same polarity as the skin and subsequently pravastatin permeated through the skin into the systemic circulation more effectively (Wiechers, 2011). It is noticeable that LC might be a good candidate for topical drug delivery since, pravastatin penetrated the skin layers (SCE and ED), but did not diffuse into the receptor phase.

After the emulgel and cream formulations in this study were compared, it was evident that the emulgels enhanced the delivery of pravastatin more than the creams. The
aforementioned can be justified by the hydrating effect of emulgels since, emulgels when
compared to creams, contain more water and less oil. Hence, emulgels are more
hydrophilic and would rather diffuse to the more hydrophilic (aqueous) regions, i.e. the
receptor phase (Benson, 2005). It should be noted that all the formulations had both
lipophilic and hydrophilic characteristics and therefore could permeate into the lipophilic
stratum corneum and diffuse to the deeper hydrophilic skin layers (Perrie et al., 2012).
When comparing the formulations with regard to polarity, it is observed, in general, that the
more hydrophilic and more lipophilic formulations enhanced the delivery of pravastatin into
the SCE, but the optimised formulations increased the delivery of pravastatin transdermally
(receptor fluid). Hence, using the FFE™ programme was successful in optimising the oil
phase in order to improve transdermal delivery of pravastatin.
Lastly, four of the six formulations (OE, OC, HC and LE) were successful after transdermal
application in reaching the same (LE) or even higher pravastatin concentration levels than
the therapeutic blood concentration after oral dosing.

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abdominoplasty surgery, which was used during the transdermal diffusion studies.

Conflict of Interest
The authors declare no conflict of interest.
References

Admescope. 2012. pKa-Dissociation constant, pH partition.

http://www.admescope.com/learnadme/physicochemical-properties/pka-dissociation

costant-ph-partition.html Date of access: 10 Jan 2015.


http://www.pharmacopoeia.co.uk.nwulib.nwu.ac.za/bp2013/ixbin/bp.cgi?&a=query&title=%22Phosphate%20Buffer%20Solution%20pH%207.4%22&tab=a-z%20index&l=P&xh=1 Date of access: 23 Jan 2014.


http://www.pharmacopoeia.co.uk.nwulib.nwu.ac.za/bp2013/ixbin/bp.cgi?&a=query&title=%22Phosphate%20Buffer%20Solution%20pH%206.5%2c%200.1%3Csmallcaps%3Em%3C%2fsmallcaps%3E%22&tab=az%20index&l=P&xh=1 Date of access: 23 Jan 2014.


## Tables

### Table 1:

Ingredients used in the formulation for different polarity creams and emulgels

<table>
<thead>
<tr>
<th></th>
<th>Cream ingredients</th>
<th>Emulgel ingredients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% m/m</td>
<td>% m/m</td>
</tr>
<tr>
<td><strong>A</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Stearic acid</td>
<td>Cetyl alcohol</td>
</tr>
<tr>
<td></td>
<td>4.0%</td>
<td>1.5%</td>
</tr>
<tr>
<td></td>
<td>Cetyl alcohol</td>
<td>MCK</td>
</tr>
<tr>
<td></td>
<td>0.5%</td>
<td>1.7%</td>
</tr>
<tr>
<td></td>
<td>Potassium cetyl phosphate</td>
<td>1.7%</td>
</tr>
<tr>
<td></td>
<td>(MCK)Span 60</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.4%</td>
<td></td>
</tr>
<tr>
<td><strong>B</strong></td>
<td>Dimethyl isosorbide</td>
<td>13.0%</td>
</tr>
<tr>
<td>Optimised</td>
<td>Polyethylene glycol 400</td>
<td>5.0%</td>
</tr>
<tr>
<td></td>
<td>13.0%</td>
<td>5.0%</td>
</tr>
<tr>
<td><strong>Hydrophilic</strong></td>
<td>Dimethyl isosorbide</td>
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</tr>
<tr>
<td></td>
<td>Water</td>
<td>Water</td>
</tr>
<tr>
<td></td>
<td>2.4%</td>
<td>2.4%</td>
</tr>
<tr>
<td></td>
<td>Ethanol</td>
<td>Ethanol</td>
</tr>
<tr>
<td></td>
<td>2.6%</td>
<td>2.6%</td>
</tr>
<tr>
<td><strong>Lipophilic</strong></td>
<td>Dimethyl isosorbide</td>
<td>13.0%</td>
</tr>
<tr>
<td></td>
<td>Mineral oil</td>
<td>Mineral oil</td>
</tr>
<tr>
<td></td>
<td>5.0%</td>
<td>5.0%</td>
</tr>
<tr>
<td></td>
<td>Glycerine</td>
<td>Ultrez 20</td>
</tr>
<tr>
<td></td>
<td>10.0%</td>
<td>0.8%</td>
</tr>
<tr>
<td></td>
<td>Tween 80</td>
<td>Pravastatin</td>
</tr>
<tr>
<td></td>
<td>3.2%</td>
<td>2.0%</td>
</tr>
<tr>
<td><strong>C</strong></td>
<td>Veegum</td>
<td>Water (dH$_2$O)</td>
</tr>
<tr>
<td></td>
<td>0.5%</td>
<td>75.0%</td>
</tr>
<tr>
<td></td>
<td>Pravastatin</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.0%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>dH$_2$O</td>
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<tr>
<td></td>
<td>60.0%</td>
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Table 2:
Hansen solubility parameters, molecular weight, active formulation gap and skin formulation gap of the formulations with different polarities

<table>
<thead>
<tr>
<th>Formulations</th>
<th>δ_d</th>
<th>δ_p</th>
<th>δ_h</th>
<th>MW</th>
<th>AFG</th>
<th>SFG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrophilic</td>
<td>17.0</td>
<td>8.5</td>
<td>12.4</td>
<td>179</td>
<td>1.3</td>
<td>7.9</td>
</tr>
<tr>
<td>Lipophilic</td>
<td>17.0</td>
<td>6.5</td>
<td>7.5</td>
<td>272</td>
<td>5.7</td>
<td>4.2</td>
</tr>
<tr>
<td>Optimised</td>
<td>17.1</td>
<td>7.6</td>
<td>8.5</td>
<td>230</td>
<td>4.1</td>
<td>1.5</td>
</tr>
<tr>
<td>Skin</td>
<td>17.0</td>
<td>8.0</td>
<td>8.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Pravastatin</td>
<td>16.7</td>
<td>8.6</td>
<td>11.5</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Table 3:
Characterisation results of pravastatin formulations

<table>
<thead>
<tr>
<th></th>
<th>pH</th>
<th>Viscosity (mPa s)</th>
<th>Droplet size (µm)</th>
<th>Zeta-potential (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Optimised cream (OC)</td>
<td>5.09</td>
<td>4818.65</td>
<td>44.375</td>
<td>-46.67</td>
</tr>
<tr>
<td>Hydrophilic cream (HC)</td>
<td>5.05</td>
<td>3546.26</td>
<td>29.705</td>
<td>-42.90</td>
</tr>
<tr>
<td>Lipophilic cream (LC)</td>
<td>5.01</td>
<td>3856.35</td>
<td>31.777</td>
<td>-56.03</td>
</tr>
<tr>
<td>Optimised emulgel (OE)</td>
<td>5.09</td>
<td>4064.10</td>
<td>55.295</td>
<td>-75.00</td>
</tr>
<tr>
<td>Hydrophilic emulgel (HE)</td>
<td>5.00</td>
<td>3843.54</td>
<td>32.214</td>
<td>-63.53</td>
</tr>
<tr>
<td>Lipophilic emulgel (LE)</td>
<td>5.07</td>
<td>4157.79</td>
<td>78.428</td>
<td>-77.37</td>
</tr>
</tbody>
</table>
Table 4:
The concentration (µg/ml) of pravastatin that diffused through the skin after 12 h for all the different polarity formulations

<table>
<thead>
<tr>
<th>Formula</th>
<th>Concentration (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Therapeutic blood concentration (oral dosage)</strong></td>
<td>0.0641 *</td>
</tr>
<tr>
<td>Optimised cream (OC)</td>
<td>0.8600</td>
</tr>
<tr>
<td>Hydrophilic cream (HC)</td>
<td>0.2310</td>
</tr>
<tr>
<td>Lipophilic cream (LC)</td>
<td>0.0000</td>
</tr>
<tr>
<td>Optimised emulgel (OE)</td>
<td>1.3140</td>
</tr>
<tr>
<td>Hydrophilic emulgel (HE)</td>
<td>0.0341</td>
</tr>
<tr>
<td>Lipophilic emulgel (LE)</td>
<td>0.0646</td>
</tr>
</tbody>
</table>

* Clarke et al., 2011
Figures:

Fig. 1: Box-plot representing the amount per area (μg/cm²) of pravastatin that diffused through the skin after 12 h for the six different formulations. The mean and median flux values are indicated by the diamond shapes and lines, respectively.
Fig. 2: Box-plot indicating the concentration (µg/ml) pravastatin present in the SCE after tape stripping for the different formulations. The mean and median concentration values are indicated by the lines and squares, respectively.
Fig. 3: Box-plot indicating the concentration (µg/ml) pravastatin present in the ED after tape stripping for the different formulations. The mean and median concentration values are indicated by the lines and squares, respectively.