Formulation, *in vitro* release and transdermal diffusion of pravastatin by the implementation of the delivery gap principle

C Burger

21661308

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Supervisor:	Dr M Gerber
Co-Supervisor:	Prof J du Plessis
Assistant Supervisor:	Prof JL du Preez

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ABSTRACT

Active pharmaceutical ingredients (APIs), which are incorporated in different formulations, i.e. creams, gels, foams, etc., are applied to the skin for a therapeutic effect. This therapeutic effect could either be required in the top layer of the skin (topical drug delivery) or deeper layers to reach the blood capillaries (transdermal drug delivery). Transdermal delivery avoids oral administration route limitations, such as first pass metabolism which is the rapid clearance of the drug in the gastrointestinal tract and degradation by enzymes. This delivery targets the drugs to skin sites, where there are significant advantages which include: improved patient compliance, a steady drug delivery state, less frequent dosing, adverse effects are minimal, it is less invasive and issues with the gastrointestinal absorption are avoided by eliminating the first pass metabolism (Perrie *et al.,* 2012:392). This type of delivery is not free from limitations even though the skin can be employed for targeted drug delivery and is a readily available and large accessible surface area for adsorption of drugs. The most upper layer of the human skin, the stratum corneum, which is a watertight barrier, offers defence against hazardous exterior materials such as fungi, allergens, viruses and other molecules. This indicates the stratum corneum controls the drug penetration of most drugs to permeate the skin barrier (Lam & Gambari, 2014:27).

Pravastatin is hydrophilic and is a 3-hydroxy-3-methyl-glutaryl coenzyme A (HMG-CoA) reductase inhibitor which inhibits cholesterol synthesis, a rate-limiting step, in the liver, thus decreasing the level of plasma low density lipoprotein cholesterol (LDL-C) (Heath *et al.,* 1998:42). It can also slow the progression of atherosclerosis and can lower the incident of coronary events (Haria & McTavish, 1997:299).

The first aim of the study is to deliver pravastatin transdermally into the blood circulation. Currently, pravastatin is only administered in oral dosages and can cause highly negative adverse effects such as myopathy and increased liver enzymes. This increase in liver enzymes causes hepatotoxicity and therefore would be ideal if pravastatin could be delivered transdermally, as the first pass metabolic effect would be nullified and adverse effects would decrease drastically (Gadi *et al.*, 2013:648).

Prof JW Wiechers' Delivery Gap Principle was designed in attempt to effectively enhance transdermal drug delivery. This Delivery Gap Principle was incorporated in the computer programme he developed known as "Formulating for Efficacy" (FFE™). The transdermal delivery of suggested APIs, which in this case is pravastatin, when incorporated into a formulation, may be optimised transdermally. The FFE™ computer programme suggests that

the oil phase can be optimised, which in turn leads to better permeation through the skin to the target site (transdermal). The formula can be manipulated to reach desired polarity.

The second aim of this *in vitro* study was to investigate the implementation of Wiechers' Delivery Gap Principle in a semi-solid dosage form, for the transdermal delivery of pravastatin sodium (2%).

Six formulations, of which three were cream and three were emulgel formulations incorporated with pravastatin sodium (2%), were formulated. Each formulation had a different polarity, i.e. hydrophilic cream (HC) and emulgel (HE), lipophilic cream (LC) and emulgel (LE) and optimised cream (OC) and emulgel (OE).

A high performance liquid chromatography (HPLC) method was developed and validated to analyse the concentration of pravastatin. Both the octanol-buffer distribution coefficient (log D) and the aqueous solubility of pravastatin were determined.

For the API to permeate through the skin into the blood circulation, certain physicochemical properties are important and according to Naik *et al* (2000:321), there are specific ideal limits for the API in the formulations which include log D (1 to 3) and a aqueous solubility of >1 mg/ml. The aqueous solubility of 197.5 mg/ml in phosphate buffer solution (PBS) (pH 7.4) at a temperature of 32 °C indicated penetration was favourable (Naik *et al.,* 2000:321), whilst the log D value of -0.703 indicated the API was unfavourable for skin penetration (Naik *et al.,* 2000:321).

Membrane release studies were conducted using a synthetic membrane to determine whether pravastatin was released from the six formulations each containing 2% pravastatin prior to diffusion studies with. The OE yielded the highest median flux value (7.175 µg/cm^2.h), followed the by LE (6.401 µg/cm^2.h), HE (6.355 µg/cm^2.h), HC (5.061 µg/cm^2.h), OC (4.297 µg/cm^2.h) and lastly, LC (3.115 µg/cm^2.h). By looking at the aforementioned data values, it was concluded that the emulgels performed better than the cream formulations when median flux values were compared.

By using dermatomed excised female Caucasian skin, an execution of Franz cell diffusion studies were performed over a period of 12 h, followed by a tape-stripping experiment to determine which semi-solid formulation delivered pravastatin best on the skin and the results of the different polarity formulations were compared.

The median amount per area which permeated through the skin after 12 h was as follows: the OE formulation (2.578 µg/cm^2) depicted the highest median amount per area, followed by OC (1.449 µg/cm^2), HC (0.434 µg/cm^2), LE (0.121 µg/cm^2), HE (0.055 µg/cm^2) and lastly LC

(0.000 µg/cm^2). These results validate Wiechers` theory that when the oil phase is optimised, with regard to the same polarity as the skin, permeation will be enhanced (Wiechers, 2011).

During both the membrane studies and the skin diffusion studies it was evident the emulgel formulations performed better and pravastatin permeated better than the cream formulations. When skin diffusion and membrane median data values were compared, it was evident in both the membrane release studies and the skin diffusion studies that OE yielded the highest median values and LC the lowest median values. It was clear that all six different formulations released pravastatin, but LC displayed no permeation into the systemic circulation (receptor phase).

The data of the different polarity formulations which yielded the best results with regards to median concentrations within the stratum corneum-epidermis and epidermis-dermis, were identified and are: within the stratum corneum-epidermis, HE (1.448 µg/ml) yielded the highest median concentration pravastatin, 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). As emulgels (hydrophilic) contain more water than creams (lipophilic), the penetration enhancement effect can be explained by hydration, since the water hydrated the skin leading the lipids to open and the stratum corneum to swell (Williams & Barry, 2004:606). Therefore more API could permeate into the skin.

Within the epidermis-dermis the highest median concentration median was yielded by 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). Different polarity formulations permeating the viable epidermis could be a result of the solubility characteristics of the formulations. It contained both lipid properties (formulations contained oil content), leading to permeation through the stratum corneum and aqueous properties, which lead to diffusion into the underlying layers of the epidermis (Perrie *et al*., 2012:392).

According to Perrie (2012:392), formulations that need to be delivered transdermally, must permeate through the lipophilic stratum corneum and thereafter the hydrophilic dermal layers to reach the blood circulation, which means formulations must consist of both lipophilic and aqueous solubility properties. When comparing the stratum corneum-epidermis (lipophilic) with the epidermis-dermis (more hydrophilic) and receptor phase (hydrophilic; systemic circulation), it is evident that all formulations had lipophilic and hydrophilic properties, as the API permeated through the stratum corneum and penetrated the deeper layers of the skin (viable epidermis)

When all polarity formulations were compared, i.e. optimised, hydrophilic and lipophilic, it was observed that the optimised formulations depicted the highest median concentration values in the receptor phase (skin diffusion), but lowest median concentration in stratum corneum-epidermis, therefore the optimised formulation permeated best through the stratum corneum-epidermis. The reason for this could be that the optimised formulations had the same polarity

as the skin (17, 8, 8), thus permeating through the skin to the receptor fluid more efficiently (Wiechers, 2011). It was observed that LC penetrated both stratum corneum-epidermis and epidermis-dermis, but did not permeate through the skin to the receptor fluid (systemic circulation), making it a good delivery vehicle for topical delivery.

Overall when the emulgel and cream formulations are compared, according to their ability to deliver pravastatin transdermally, it is evident the pravastatin diffused more from the emulgel formulations than the cream formulations. This could be due to the fact that emulgels are more hydrophilic as they contain more water, resulting in the emulgels diffusing to the deeper layers of the skin (more hydrophilic viable epidermis) (Benson, 2005:28).

All formulations contained not only aqueous (hydrophilic) but also lipid (lipophilic) solubility properties, therefore making it lipophilic enough to permeate the stratum corneum and hydrophilic enough to penetrate to deeper skin layers (viable epidermis) (Perrie *et al*., 2012:392). All formulations could still permeate the viable epidermis despite different polarities being used and all were appropriate candidates, although some were more suitable than others.

The understanding from this study is that:

- pravastatin could be delivered topically by all formulations,
- the best formulation to reach the systemic formulation is the optimised emulgel,
- the best formulation to deliver pravastatin topically is the hydrophilic emulgel.

Keywords: Pravastatin, Wiechers, Franz cell, Diffusion studies, Polarity formulations, Delivery Gap Principle

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Uittreksel

Aktiewe farmaseutiese bestanddele (AFB) wat geïnkorporeer word in verskillende vorme van dosering, onder andere rome, jelle, skuimrome, ensovoorts, word aan die vel vir `n terapeutiese effek aangewend. Hierdie terapeutiese effek word op die boonste laag van die vel (topikale geneesmiddelaflewering) of dieper in die vellae vereis om die bloedsirkulasie te bereik (transdermale geneesmiddelaflewering). Transdermale geneesmiddelaflewering vermy beperkinge wat deur orale administrasie gestel word, onder andere die eerstedeurgangseffek; wat `n vinnige verwydering van die geneesmiddel in die spysverteringskanaal en afbraak deur ensieme is. Hierdie tipe aflewering teiken die geneesmiddel na sekere velareas, wat verskeie voordele inhou, onder andere verbeterde pasiëntmeewerkendheid, houbare geneesmiddelaflewering, minder doserings, newe-effekte is minimaal, dit is minder indringend en kwessies rakende die spysverteringskanaal word geëlimineer (Perrie *et al.,* 2012:392). Hierdie tipe geneesmiddelaflewering is nie vry van beperkinge nie, alhoewel die vel gebruik kan word vir gerigte/geteikende geneesmiddelaflewering; is dit ook geredelik beskikbaar en besit `n groot toeganklike oppervlakarea vir geneesmiddelabsorpsie. Die heel buitenste laag van die menslike vel, die *stratum corneum*, is `n waterdigte skans en bied beskerming teen gevaarlike uiterlike stowwe soos fungi, allergene, virusse en ander molekules. Dit dui aan dat die *stratum corneum* geneesmiddelpenetrasie van meeste stowwe wat die velskans wil deurdring kontroleer (Lam & Gambari, 2014:27).

Pravastatien is hidrofiel en is `n 3-hidroksie-3-metiel-glutariel koënsiem A (HMG-CoA)-reduktase-inhibeerder wat cholesterol sintese inhibeer; `n snelheidsbepalende stap wat in die lewer plaasvind, wat dus die plasmavlak lae-digtheid-lipoproteïen-cholesterol (LDL-C) verlaag (Heath *et al.,* 1998:42). Dit kan ook die progressie van arteriosklerose en die voorkoms van koronêre gevalle verminder (Haria & McTavish, 1997:299).

Die eerste doel van die studie was om pravastatien in die bloedsirkulasie af te lewer. Pravastatien word slegs in orale doseervorme toegedien en kan erge newe-effekte soos miopatie en verhoogde lewerensieme veroorsaak. Verhoging in lewerensieme veroorsaak hepatotoksisiteit en daarom sal dit ideaal wees indien pravastatien transdermaal afgelewer kan word, om sodoende die eerstedeurgangseffek te vermy asook om die newe-effekte drasties te verminder.

Prof J.W. Wiechers se “delivery gap principle” is ontwerp met oogopslag om transdermale geneesmiddelaflewering effektief te verhoog. Die “delivery gap principle” is in 'n rekenaarprogram geïnkorporeer en staan meer bekend as “Formulating for Efficacy” (FFE™).

Die aflewering van `n voorgestelde AFB, wat in die geval pravastatien is, kan sodra dit in ’n formulering geïnkorporeer word, transdermaal geoptimaliseer word. Die FFE™ rekenaarprogram stel voor dat die oliefase van `n formulering geoptimaliseer word om sodoende die penetrasie deur die vel tot in die teikenarea (transdermaal) te verhoog. Formules kan gemanipuleer word om gewenste polariteite te bereik.

Die tweede doel van die studie was om die implementering van Wiechers se “delivery gap principle” te ondersoek, tydens die transdermale aflewering van natriumpravastatien (2%) in semi-soliede doseervorms.

Ses formulerings, drie rome en drie emuljelle, met natriumpravastatien (2%) is geformuleer. Elke formulering het `n ander polariteit gehad onder andere. geoptimaliseerde room (OC) en -emuljel (OE), hidrofiele room (HC) en -emuljel (HE), lipofiele room (LC) en -emuljel (LE).

`n Hoëdrukvloeistofchromatografie (HPLC) metode is ontwikkel en gevalideer om die konsentrasie van pravastatien te analiseer. Beide die oktanol-buffer verdelingskoëffisiënt (log D) en die wateroplosbaarheid van pravastatien is bepaal.

Sekere fisiese-chemiese eienskappe is nodig vir die AFB om deur die vel te penetreer na die bloedsirkulasie en volgens Naik *et al.* (2000:321), is daar spesifieke ideale beperkings vir formulerings en sluit in ’n log D (1 tot 3) en wateroplosbaarheid van >1 mg/ml. Die wateroplosbaarheid van 197.5 mg/ml in die fosfaatbufferoplossing (PBS; pH 7.4) by `n temperatuur van 32 C het gewys dat penetrasie deur die vel voordelig sou wees (Naik *et al.,* 2000:321), terwyl die log D waarde van -0.703 getoon het dat die AFB nie voordelig is vir vel penetrasie nie (Naik *et al.,* 2000:321).

Voor die vel-diffusiestudies plaasgevind het; is membraanvrystellingsstudies uitgevoer deur van `n sintetiese membraan gebruik te maak om vas te stel of pravastatien deur al ses formulerings (elkeen bevat 2% natriumpravastatien) vrygestel is. Die OE het die hoogste mediaan vloedwaarde getoon (7.175 µg/cm^2.h), gevolg deur LE (6.401 µg/cm^2.h), HE (6.355 µg/cm^2.h), HC (5.061 µg/cm^2.h), OC (4.297 µg/cm^2.h) en laastens, LC (3.115 µg/cm^2.h). Deur die voorafgaande data te bestudeer, is daar vasgestel dat emuljelle beter as die rome gevaar het wanneer dit met mekaar vergelyk is.

Deur van gedermatoomde, vroulike, blanke vel gebruik te maak, is `n Franz-sel-diffusiestudie uitgevoer oor `n periode van 12 h, gevolg deur `n kleefbandstropingseksperiment om vas te stel watter semi-solied formulerings pravastatien die beste afgelewer het in die vel en die resultate van die verskillende formulerings met verskillende polariteite is vergelyk.

Die mediaan hoeveelheid per area wat deur die vel gepenetreer het na 12 h was soos volg: die OE formulering (2.578 µg/cm^2) het die hoogste waarde getoon, gevolg deur OC (1.449 µg/cm^2),

HC (0.434 µg/cm^2), LE (0.121 µg/cm^2), HE (0.055 µg/cm^2) en laastens LC (0.000 µg/cm^2). Hierdie waardes valideer Wiechers se teorie dat wanneer die oliefase van `n formulering geoptimaliseer word, met betrekking tot dieselfde polariteit as die vel, penetrasie verbeter sal word (Wiechers, 2011).

Gedurende beide die membraan- en die vel-diffusiestudies, was dit vasgestel dat emuljelle beter presteer en het pravastatien dus beter gepenetreer, vergelyke met rome. Wanneer vel- en membraandiffusie mediaan waardes vergelyk was, is dit vasgestel dat beide die membraan- en die vel-diffusiestudies, die hoogste mediaanwaarde vir OE en die laagste mediaanwaarde vir LC getoon het. Dit is duidelik dat al die formulerings pravastatien vrygestel het, maar die LC formulering het geensins in die reseptorfase gediffundeer nie.

Die data van die verskillende polariteitsformulerings wat die beste resultate getoon het met betrekking tot mediaanwaardes vir die stratum corneum-epidermis en die epidermis-dermis, is soos volg geïdentifiseer: vir die stratum corneum-epidermis het HE (1.448 µg/ml) die hoogste mediaan pravastatien-konsentrasiewaarde getoon, gevolg deur LE (1.301 µg/ml), LC (0.676 µg/ml), HC (0.505 µg/ml), OE (0.505 µg/ml) en laastens OC (0.400 µg/ml). Emuljelle (hidrofiel) bevat baie meer water as rome (lipofiel), en daarom kan die penetrasiebevordering verduidelik word deur die hidrerende effek, omdat water die vel hidreer wat veroorsaak dat die lipiede oopgaan en die stratum corneum swel (Williams & Barry, 2004:606). Daarom kan meer pravastatien in die vel deurgelaat word.

Binne die epidermis-dermis was die hoogste mediaan pravastatien-konsentrasiewaarde verkry deur OE (0.849 µg/ml), gevolg deur LC (0.572 µg/ml), HC (0.524 µg/ml), OC (0.355 µg/ml), HE (0.309 µg/ml) en laastens LE (0.138 µg/ml). Verskillende polariteitsformulerings wat die “viable” epidermis kan deurdring kan as gevolg van die oplosbaarheidskarakteristieke van die formulerings wees. Alle formulerings het lipofiele (formulerings bevat oliebestanddele) eienskappe besit, wat daartoe lei dat penetrasie deur die stratum corneum kan geskied, asook hidrofiele eienskappe besit, wat daartoe lei dat diffusie kan plaasvind na die onderliggende lae van die “viable” epidermis (Perrie *et al*., 2012:392).

Volgens Perrie (2012:392) moet formulerings wat transdermaal afgelewer, eers deur die lipofiele stratum corneum beweeg en daarna deur die hidrofiele dermale lae diffundeer om die bloed sirkulasie te bereik, wat beteken dat formulerings beide lipofiele en hidrofiele oplosbaarheidseienskappe moet bevat. Wanneer die stratum-corneum-epidermis (lipofiel) met die epidermis-dermis (meer hidrofiel) en die reseptorfase (hidrofiele, sistemiese sirkulasie) vergelyk word, is dit duidelik dat al die formulerings beide lipofiele- en hidrofiele eienskappe besit het, omdat die AFB deur die stratum corneum gepenetreer het en daarna na die dieper vellae (“viable” -epidermis).

Wanneer al die verskillende polariteitsformulerings met mekaar vergelyk was onder andere geoptimaliseer, hidrofiel en lipofiel, was dit duidelik dat die geoptimaliseerde formulerings die hoogste mediaan konsentrasiewaardes in die reseptor fase (vel-diffusie) bereik het, maar het die laagste mediaan konsentrasiewaarde in die stratum-corneum-epidermis verkry, daarom het die geoptimaliseerde formulerings die beste deur die stratum corneum-epidermis gepenetreer. Die rede hiervoor kon wees dat die geoptimaliseerde formulerings dieselfde polariteit gehad het as die vel (17; 8; 8), daarom het dit meer effektief deur die vel na die reseptorvloeistof gepenetreer (Wiechers, 2011). Dit is duidelik dat die LC-formulering beide die stratum corneum-epidermis en die epidermis-dermis gepenetreer het, maar nie gediffundeer deur die vel na die reseptorvloeistof (sistemiese sirkulasie) nie, wat dit `n goeie afleweringsdoseervorm maak vir topikale aflewering.

Bowenal, wanneer die emuljelle met die roomformulerings vergelyk word, met betrekking tot die vermoë om pravastatien transdermaal af te lewer, is dit duidelik dat pravastatien meer gediffundeer het vanaf die emuljelformulerings as die roomformulerings. Voorgenoemde kan wees as gevolg van die feit dat emuljelle meer hidrofiel is, want dit bevat meer water; wat veroorsaak dat die emuljelle na die dieper lae van die vel kan diffundeer (meer hidrofiele “viable” epidermis) (Benson, 2005:28).

Alle formulerings bevat nie net hidrofiele oplosbaarheidseienskappe nie, maar ook lipofiele oplosbaarheidseienskappe, daarom is dit lipofiel genoeg om deur die stratum corneum en hidrofiel genoeg om na die dieper lae van die vel te penetreer (Perrie *et al*., 2012:392). Alle formulerings kon nogsteeds die “viable” epidermis bereik ten spyte van die verskillende polariteite wat gebruik is en al die formulerings was geskikte kandidate, alhoewel sekere formulerings meer gepas was as ander.

Die volgende is duidelik uit die studie:

- Pravastatien kon topikaal afgelewer word deur alle formulerings.
- Die beste formulering om die sistemiese sirkulasie te bereik is die geoptimaliseerde emuljel.
- Die beste formulering om pravastatien topikaal af te lewer is die hidrofiele emuljel.

Verwysings

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Chapter 1:

Introduction and problem statement

The skin is known to be 2 m^2 in surface area, occupies approximately 15% total body weight and consists of different tissue layers, making it a multi-layered organ. The top layer of the skin, known as the stratum corneum, has an organised structure which operates as a barrier for drugs to permeate across the skin; a barrier not only applicable to drugs, but to chemical hazards within the environment as well, making the skin a protectant from outside influences (Forslind *et al.,* 1995:117). Transdermal drug delivery offers an alternative to routes such as oral, intramuscular, intravascular, sublingual and subcutaneous delivery (Berty and Lipsky, 1995:581, 582), as it allows the active pharmaceutical ingredient (API) to permeate across the skin and into the blood circulation thus avoiding the hepatic first-pass effect seen in oral administration.

Drug permeation commences by permeating across the stratum corneum either by intercellular, intracellular (better known as transcellular) or follicular (skin appendages) routes (Alexander *et al.,* 2012:27). Compounds which are highly lipophilic and have low molecular weights, result in the greatest permeation through the skin. APIs which need to be delivered to the blood circulation for a therapeutic effect, should not only diffuse through the lipophilic stratum corneum, but also through the remaining underlying layers of the skin (epidermis and dermis), which is the more aqueous region (hydrophilic) of the skin. Lipophilic compounds easily diffuse through the lipid mortar, but thereafter permeation is delayed by the epidermis aqueous layers. Keeping the afore-mentioned in mind, the opposite is true for hydrophilic polar compounds, as these compounds firstly struggle to permeate the outer layer (stratum corneum) which is lipophilic. It is of utmost importance therefore that drugs should maintain affinity for both lipophilic and hydrophilic regions, so absorption and permeation can proceed successfully (Berty and Lipsky, 1995:581, 582).

For many years scientists have tried to modify skin permeability in order to promote transdermal drug permeation. Numerous methods such as chemical, physical or biochemical have been proven to improve drug transportation through the skin layers. The focus was on either increasing drug diffusion properties or reducing the stratum corneum barrier (Reeta *et al.,* 2005:25).

Pravastatin is a HMG-CoA (3-hydroxy-3-methyl-glutaryl coenzyme A) reductase inhibitor, which increases the hepatic low-density lipoprotein (LDL)-receptor activity, decreases the plasma level

LDL-cholesterol and inhibits the rate-limiting step of cholesterol synthesis in the liver (Heath *et al.,* 1999:42). After oral administration, it is rapidly absorbed and peak plasma concentrations are observed at 1 to 1.5 h after dosing (Clarke *et al.,* 2011:1947). Bioavailability, when taken orally, is an estimated 17%, protein binding 50%, with the half-life ranging from 1.3 to 2.6 h. Volume of distribution is 0.46 L/kg at a steady state and the dosage differs from 10 to 40 mg (Clarke *et al.,* 2011:1947). Adverse effects most commonly associated with statins are poor patient compliance because of myopathy, hepatitis, rhabdomyolysis, headache, fatigue, gastro-intestinal 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, it would be ideal to deliver pravastatin transdermally.

Prof JW Wiechers developed the Delivery Gap Principle incorporated in a computer programme called "Formulating for Efficacy" (FFE™), where an API may be chosen for topical formulations to effectively optimise transdermal drug delivery. The FFE™ programme calculates the most favourable composition of the oil phase of a pre-existing formulation. This software will be used to determine the type of formulation such as a cream and emulgel and how to manipulate the formula to reach the desired polarity. The concentration given is near the maximum solubility and the clinical efficacy can be optimised by the programme (Wiechers, 2011). The API chosen for this study will be pravastatin sodium, but will be referred to as only pravastatin for reading purposes. This is applicable for the entire dissertation, except the abstract and Chapter 3.

The first aim of the study was to deliver pravastatin transdermally, as pravastatin's greatest adverse effect is increased liver enzymes to 300% in oral dosage (Lane, 2005), therefore it would be ideal to incorporate pravastatin within a formulation which can be delivered transdermally to exclude the first hepatic metabolism effect. The second aim of the study was to investigate the computer programme FFE™ designed by Prof JW Wiechers, where pravastatin was chosen as API for transdermal formulations to increase transdermal drug delivery.

The objectives of the study were to:

- Use the literature obtained from JW Solutions software (FFE™) to determine the oil phase in the different formulations containing pravastatin as the API.
- Formulate three emulgel formulations and three cream formulations with different polarities, i.e. optimised (polarity is equal to skin) cream and emulgel, lipophilic (non-polar) cream and emulgel as well as hydrophilic (very polar) cream and emulgel.

- Use the high performance liquid chromatography (HPLC) for the development and validation of an analytical method to determine the different concentrations of the pravastatin (with regards to the API) within the six formulations.
- Determine the octanol-buffer distribution coefficient (log D) and aqueous solubility of pravastatin sodium.
- Determine the release of pravastatin from the formulation by means of executing a membrane study.
- Determine the transdermal and topical delivery of pravastatin from the formulation by performing a diffusion study followed by tape stripping, respectively.

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Chapter 2:

The delivery gap principle in transdermal drug delivery

2.1 Introduction

The skin can be classified as the largest organ of the human body which approximates to two square meters in surface area and weighing approximately 5 kg (Godin and Touitou, 2007:1153). The skin is one of the most major sites for non-invasive delivery of drugs into the body Foldvari, 2000:417). The extracellular lipids have a bilayer organisation which creates a chemical barrier to highly polar and non-polar molecules that would have to permeate undesirable environments on penetration (Forslind *et al.,* 1995:117).

The stratum corneum, more commonly known as the upper-most layer of the skin, is responsible for the barrier mechanism which prevents compounds diffusing through the skin (Longsheng *et al*., 2011:53). Significant efforts have been dedicated to the development of different approaches to overcome the permeability barrier (Foldvari, 2000:417). An ideal drug requires appropriate lipophilic properties where it can partition into the stratum corneum, as well as satisfactory hydrophilic properties to allow the second permeation stage into the viable epidermis and thereafter the blood circulation (Kalia and Guy, 2001:160).

There are many advantages of transdermal drug delivery which are not obtained with other administration routes, i.e. patient compliance with no possible infection from injections (Perrie *et al.,* 2012:392), pain-related dosing is avoided (Jepps *et al.,* 2012:7), there is a prolonged delivery of the drug (Alexander *et al.,* 2012:27), transdermal drug delivery is user-friendly (Alexander *et al.,* 2012:27), this type of delivery can be easily terminated (Alexander *et al.,* 2012:27), there is no frequent administration (Alexander *et al.,* 2012:27), drug release can be controlled (Jepps *et al*., 2012:7), the first-pass metabolism is avoided (unlike oral routes) (Perrie *et al.,* 2012:392), there are less side-effects (Perrie *et al.,* 2012:392) and less variability (Perrie *et al.,* 2012:392).

Knowing the advantages of transdermal drug delivery, compared to other conventional dosage forms, is of significant importance to increase the efficacy of these transdermal drugs as well, considering the skin`s barrier makes it more challenging to permeate. This is one of the main reasons why Prof Johan Wiechers designed a computer programme called “Formulating for efficacy” (FFE™). This computer programme suggests oil phase ingredients in which the active

pharmaceutical ingredient (API) will be dissolved, can be chosen to be effective for topical formulations to optimise transdermal drug delivery. This programme will be used to determine the type of formulation. The formula must also be manipulated to reach the polarity that is desired. In this study, pravastatin (which is hydrophilic of nature) was selected as a model drug to investigate FFE™.

Statins such as pravastatin, which are widely prescribed in cholesterol-lowering therapy, can be synthetic or natural in source. Statins exhibit biological pleiotropism due to the rate-limiting step enzyme which converts 3-hydroxy-3-methyl-glutaryl coenzyme A (HMG-CoA) to mevalonate. Not only do statins prevent biosynthesis of cholesterol, but by inhibiting the mevalonate pathway, they block the synthesis of other biological products which are important for several cellular functions (Mariucci *et al.,* 2011:381). Statins have a neuroprotective action which is due to the selective up-regulation of endothelial NO (nitrogen oxide) synthase, which leads to an increased bioavailability in vascular NO as well as recovery of endothelial functions. Other functions of statins include inhibiting leukocyte and platelet adhesion, protecting neurons from glutamate-mediated excitotoxicity, decreasing inflammation and oxidative stress, inhibiting the thrombogenic response and also significantly reducing serum cholesterol (Mariucci *et al*., 2011:381, 382).

2.2 Transdermal drug delivery

Different layers of the skin ranging from 0.05 to 2.00 mm in thickness, the skin is on average about 5 mm thick (Foldvari, 2000:417). Weighing about 5 kg (Godin and Touitou, 2007:1153), this makes the skin the heaviest organ of the body, while avoiding the first-pass effect (Perrie *et al*., 2012:392) and is responsible for 16% of weight (Wickett and Visscher, 2006:99). The human skin is one of the most readily accessible organs of the human body, receiving approximately one third of blood circulation (Chien, 1987:2). The skin can be classified as a multi-layered organ consisting of various histological layers (Alexander *et al.,* 2012:27). The skin`s primary function can be viewed as the prevention of any water loss or dehydration, thus making the skin act as a protective barrier from any hazardous or hostile environment (physical, chemical or biological) (Cevc *et al.,* 1996:351), while providing the body with thermal regulation.

Not only does the skin protect the body against all these factors, but it also protects the body against free radicals and ultraviolet (UV) radiation (Venus *et al.,* 2010:469). The stratum corneum, with its barrier function, prevents molecules with a molecular weight of > 500 Da to penetrate the intact skin (Andrews *et al.,* 2012). The human skin can be regarded as water-tight, but despite this, the human skin loses approximately 250 to 300 ml of water daily. This water loss is compensated for with the intake of water and food (Forslind *et al*., 1995:117).

Transdermal drug delivery consists of passing through the lipophilic stratum corneum, followed by hydrophilic epidermal and dermal layers to reach the capillaries of the human body (Perrie *et al*., 2012: 392). The average human skin consists of three main layers, namely the epidermis, dermis and the subcutaneous fatty layer called the hypodermis (Potts *et al.,* 1992:14). Transdermal drugs should have both aqueous and lipophilic solubilities, therefore they must diffuse through the thick stratum corneum and repartition in the aqueous epidermis reaching the vascular infrastructure (Perrie *et al*., 2012: 393).

2.2.1 The skin structure

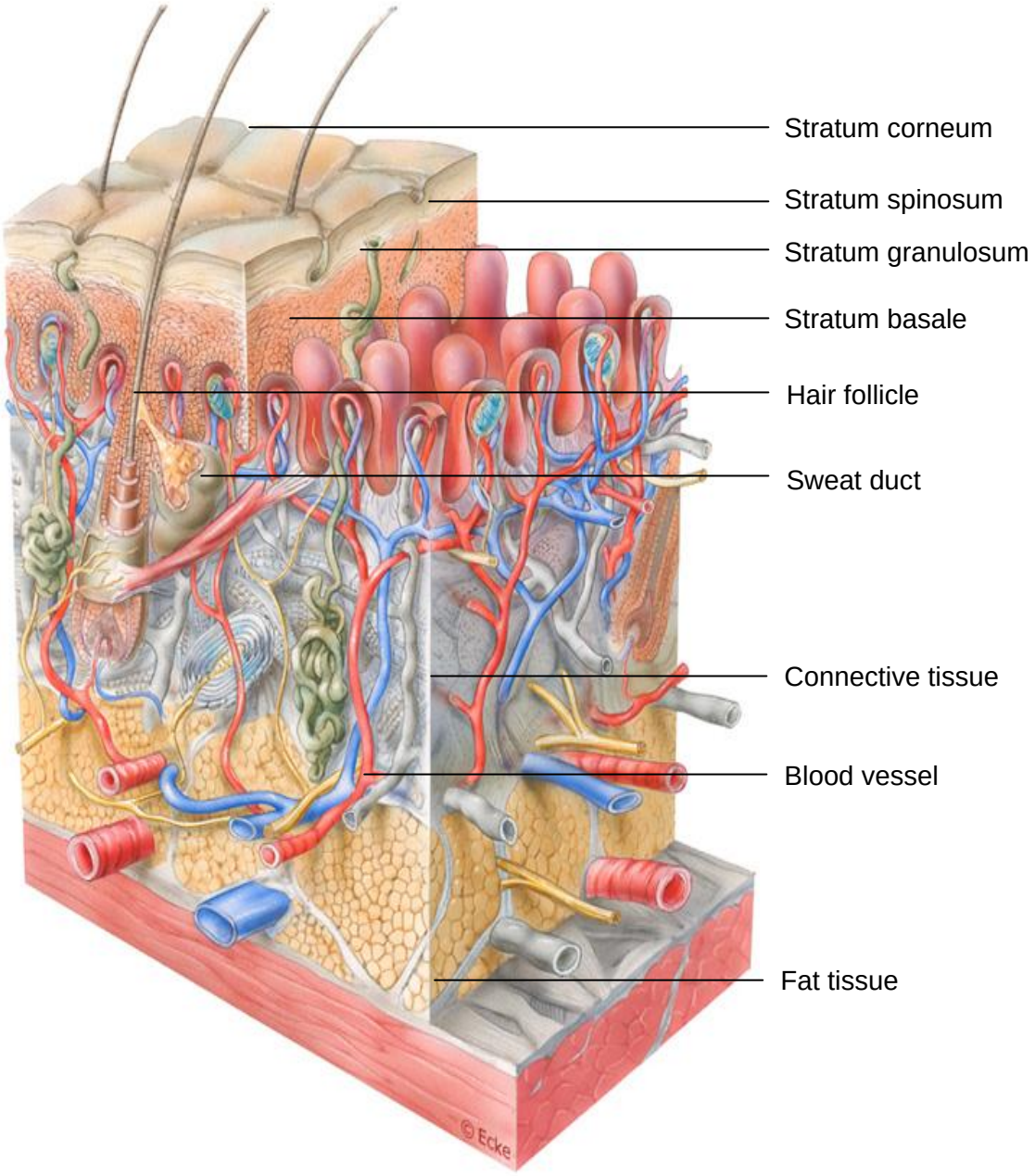

Figure 2.1: Schematic representation of skin layer (Leonardt, 1990:343).

2.2.1.1 Epidermis

This major layer can be sub-dived into two layers, namely the stratum corneum and the viable epidermis (Potts *et al.*, 1992:14) and when these are put together it is known as the full epidermis (Andrews *et al.,* 2012).

Primarily, the stratum corneum helps with homeostasis (Wickett and Visscher, 2006:98) and is the most outer layer of the human skin, differentiating on different parts of the body where it takes up approximately 5% of full thickness skin (Perrie *et al*., 2012:392). The epidermis consists of keratinocytes which form the head cells of the 'brick and mortar' structure, which includes marker cells, Langerhans cells and melanocytes (Alexander *et al.,* 2012:27). It is highly hydrophobic, filled with a non-living layer of corneocytes, where 90% is the intercellular proteins and ~ 10% the extracellular lipids (Foldvari, 2000:417, 418). This tissue is supposedly homogenous (Elias *et al.,* 2002:79). The bricks are keratin-rich corneocytes which are embedded in the intercellular lipid-rich matrix known as the mortar (Maghraby *et al.,* 2008:204).

This intercellular lipid matrix is composed of fatty acids and a mixture of cholesterol, triglycerides and ceramides (Jepps *et al.,* 2012:7). The main barrier to penetration is the outer lipids of the stratum corneum. When epidermal differentiation proceeds, the composition of the lipid shifts from a polar to a neutral mixture. The bricks represent tightly-packed corneocytes, hexagonal, flattened and highly proteinaceous cells, which are the endpoint of keratinocytes that differentiates and interconnected by corneodesmosomes (Moss *et al.,* 2012:167). Desquamation is the process where the cells migrate from the dermal-epidermal junction over a period of two weeks at the base of the epidermis to the stratum corneum (Jepps *et al.,* 2012:7). When the stratum corneum is hydrated, the thickness range changes to 40 µm; this is also known as the rate limiting barrier in transdermal permeation (Maghraby *et al.,* 2008:204).

The viable epidermis can be defined as the epidermis without the stratum corneum (Maghraby *et al.,* 2008:204) and can therefore be divided into the stratum basale, stratum spinosum and stratum granulosum. It is composed of 15 to 20% lipids, 40% water and about 40% keratinocytes and is also an avascular environment (Jepps *et al.,* 2012:4). The diffusion mechanism through the viable epidermis works on the principle that the drug diffuses through the aqueous medium. This medium is hindered by proteins which means this barrier is more effective against lipophilic permeants because it has a greater affinity with a non-polar environment (Jepps *et al.,* 2012:4). There might be evidence that the viable epidermis consists of tight junctions (Andrews *et al.,* 2012). The viable epidermis consists of layers of keratinocytes which differentiate at various stages. Presence of keratin can affect diffusion from the stratum corneum into the viable epidermis (Jepps *et al.*, 2012:4).

2.2.1.2 Dermis

The dermis is connective tissue which contains cells, ground substances (polysaccharides and proteins) and fibres. The hygroscopic proteoglycan macromolecules are produced by ground substances (Venus *et al.*, 469).

This part of the skin is responsible for the support of the epidermis and divides the epidermis from the fatty layer. The dermis thickness is between 3 and 5 mm in depth, with the upper layer being 100 to 200 µm thick and consists of fibrous proteins (also known as fibroblasts) such as elastin and collagen which promotes flexibility and strength, thus proving to be a barrier to infection (Venus *et al.,* 2010:469).

The dermis also consists of gel containing water, salts and glycosaminoglycans which means it also functions as a water storage organ (Perrie *et al.,* 2012:392). Embedded within the dermis are nerve endings, blood and lymphatic vessels, hair follicles, sebaceous glands and sweat glands and is where the appendageal route of skin permeation takes place (the pilosebaceous units open directly into the environment of the skin surface) (Maghraby *et al.,* 2008:204).

The dermis is acellular, which means the vascularisation of the dermis helps with distribution and drug transport as well as the lymphatic system (Jepps *et al.,* 2012:4). This is the primary site where drugs or molecules are taken up in the systemic circulation (Andrews *et al.,* 2012).

2.2.1.3 Hypodermis

Composed out of lipocytes, this layer consists of the fatty layer of tissue (Venus *et al.,* 2010). This part of the skin serves as a thermal barrier and a mechanical cushion (Perrie *et al.,* 2012:392).

2.2.1.4 Skin appendages

Skin appendages can be classified as the hair follicles and sebaceous glands that differ in specific regions of the human body (Franz and Lehman, 2000:24) and include the apocrine and eccrine sweat glands. These apocrine and sebaceous glands moisten the skin with fluid secretions, whilst the eccrine secretions regulate the body temperature (Perrie *et al.*, 2012:392).

The hair follicle entrance to the skin is 500 µm in depth to the sebaceous duct. These sebaceous glands are found mainly on the face and produce sebum (consisting of squalene, cholesterol, esters and triglycerides) which takes an estimated eight hours to pass to the skin surface from the sebaceous gland (Jepps *et al.,* 2012:5). This sebum repels water and is a bacteriostatic and fungistatic mixture which serves as a lubricant for the hair and skin (Wosicka and Cal, 2010:84).

Hair follicles are perceived to be connected with the blood capillaries and below the hair canal there is no mature stratum corneum, therefore it can be said that molecules can penetrate these follicles and move to the surrounding tissue of the follicle and reach the network of blood capillaries thus reaching the blood circulation (Wosicka and Cal, 2010:83).

Hair follicles can be an alternative route for skin permeation because of their points of major entry, but they can also serve as reservoir for other substances that are dermally applied (Wosicka and Cal, 2010:84). The pilosebaceous unit can describe the structure of the hair follicle, the hair shaft and the sebaceous glands.

2.2.2 Transdermal routes

Any drug applied to the skin either enters by appendages such as hair follicles and ducts (transappendageal) leading to eccrine sweat glands, or through the stratum corneum (transepidermal) where the drug needs to pass through another series of skin layers such as the viable dermis, dermis and hypodermis (Finnin and Morgan, 1999:955).

The appendageal glands and hair follicles have a vascular nature which means drug distribution can be systematic. It is important to note that the stratum corneum is the main barrier for penetration but the other layers of skin also play a role in penetration and distribution for instance when lipophilic drugs want to be administered, the lower layers are important and need to be considered (Jepps *et al*., 2012:5).

2.2.2.1 Transappendageal

This route, better known as the shunt route (Maghraby, 2008:204), goes directly through the stratum corneum across the appendages of the skin such as hair follicles, sweat ducts and glands. This type of penetration only works when the hair follicles are “active” meaning there is sebum production or hair growth involved (Wosicka and Cal, 2010:86). This pathway favours the highly hydrophilic molecules or substances but in the presence of the lipophilic sebum, it can favour lipophilic molecules as well. The penetrants lipophilicity influences the follicle that is targeted whilst in the presence of propylene glycol or surfactants.

This type of penetration can be explained by the “geared pump” hypothesis where the particles penetrating are size depended, therefore molecules that have a similar size to the follicles, are pushed in by movement of the hair and depending by different physicochemical properties of the molecule, can reach the network of blood capillaries thus reaching the blood circulation (Wosicka and Cal, 2010:88).

2.2.2.2 Transepidermal

This route is where molecules can permeate across the unbroken, intact stratum corneum (Maghraby, 2008:205) and can be divided into two other routes, namely transcellular and intercellular which are discussed below.

2.2.2.2.1 Transcellular

This route consists of the path travelled across the stratum corneum, passing through other regions such as the corneocytes and then the lipid layers (Morrow *et al*., 2007:38). This means diffusion through and partitioning into the keratin bricks and also across and into the intercellular lipids (Maghraby, 2008:205).

2.2.2.2.2 Intercellular

This route is the main route of molecules despite the surface area being so small (Maghraby, 2008:204). This process consists of the API moving through the lipid layer. This continuous lipid layer surrounds the cells and the API must diffuse through the lipid and aqueous components in the matrix (Potts *et al*, 1992:22).

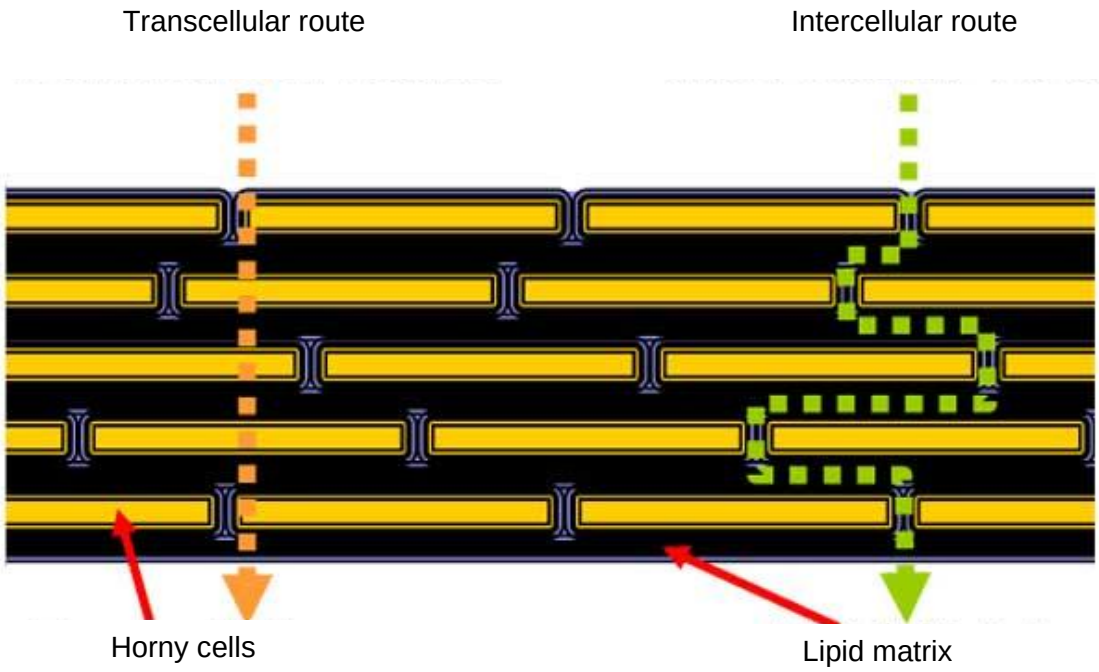

Figure 2.2: Routes of permeation (Fartasch *et al.,* 1993:128).

2.2.3 Mathematics of skin absorption

There are diverse types of chemical, physical or biological interactions that influence skin permeation. Fick's law has been accepted for skin transport of the permeant, because the main barrier is the nonviable stratum corneum. Diffusion is the course of the mass transferred of the

individual solutes, where it is driven by molecular movement and the rate of transport can be calculated in the following equation:

$$dC/dt = K \times D \times Co/h \qquad \textbf{Equation 2.1}$$

Where K is the partition coefficient, D is the diffusion coefficient, Co is the donor concentration and h is the thickness of the barrier. When highly hydrophilic drugs are used, the mathematical relationship changes due to heterogeneity of the skin structure. Where two patterns (polar and non-polar) of diffusion are established, is where, in the former layer, the skin permeation proceeds (Godin and Touitou, 2007:1158). Flux of an active ingredient can be increased by increasing D and C.

These parameters can be increased individually as well. By increasing the absolute concentration, C can be increased in the formulation. By reducing the API in the formulation, which is relative to that of the stratum corneum, K can also be increased. Driving force of the diffusion can also be increased when the API is more soluble in the stratum corneum than the formulation (Wiechers, 2008:94).

Fick's first law states that rate of transfer of diffusing molecule through a unit area of a specific section is equivalent to the concentration gradient that is measured normal to this section. Flux is the amount of material passing through a unit area per unit time.

$$J = -D\,\partial C / \partial x \qquad \textbf{Equation 2.2}$$

Where C is the concentration of the diffusing molecule, D is the diffusion coefficient, x is the space that coordinates the measured normal to the skin and J is the flux of the permeant. A drug`s steady state flux (J) can be calculated by Fick`s second law of diffusion:

$$\partial c / \partial t = D\,(\partial^2 C) / (\partial^2 x) \qquad \textbf{Equation 2.3}$$

Where x is the surface of the skin, D is the diffusion coefficient in the stratum corneum, C is the concentration and t is time (Wiiliams, 2003:41, 42).

2.2.4 Physicochemical factors that influences human skin permeation

This is where Fick's law comes into consideration. These factors can be altered to improve skin permeation such as drug concentration, solubility, skin hydration, diffusion coefficient, molecular shape, temperature and pH.

2.2.4.1 Drug concentration

According to Fick's law, the concentration of a drug applied to the skin, is directly equivalent to the flux (J). This means a high drug concentration results in a bigger diffuse gradient where

molecule diffusion is enhanced. There are some factors that can alter this diffusion such as complex formation or pH (Barry, 2007:576).

2.2.4.2 Skin hydration

Hydration of the skin can be seen when water diffuses from epidermal layers. When the skin is hydrated, the percutaneous absorption is improved (Jackson, 1993:177).

2.2.4.3 Aqueous solubility

Pravastatin`s aqueous solubility is 197.5 mg/ml (Squibb, 2013). An aqueous solubility larger than 1 mg/ml is required for maximum permeability, which indicates that pravastatin would permeate well, but oil solubility is also needed to permeate the skin (Hadgraft and Wolff, 1993:162), because most topically applied products have an aqueous formulation (Williams, 2003:37).

2.2.4.4 Partition coefficient

An increased flux (J) is the result of a high partition coefficient (K) according to Fick's first law. The reason why the partition coefficient is so important is because the initial concentration of the diffusion is determined in the membranes first layer. Compounds that have high lipid solubility also have high partition coefficient, which means partition in the stratum corneum is easy but does not often pass into the aqueous tissue (Barry, 2007:578).

Hydrophilic molecules (octanol-water partition coefficient (log P < 1)), such as pravastatin with a log P of - 0.7 (Squibb, 2013), uses the transcellular route; where highly charged hydrophobic molecules use the appendageal pathway that is shown by localisation. Intermediate molecules which are soluble in water and oil would use the intercellular route. This is usually molecules with a log P ranging from 1 to 3. Highly lipophilic molecules with a log P > 3 exclusive pathway would be the intercellular route as well traversing the stratum corneum (Williams, 2003:36). An ideal limit for log D (octanol-buffer distribution coefficient) should be between 1 to 3, showing that pravastatin (- 0.7) would not permeate very well (Naik *et al.,* 2000:319), therefore extra measurements must be taken, such as optimal formulation, in order for this API to be delivered.

2.2.4.5 Molecular shape and size

The skin allows molecular absorption for molecules with low molecular weights (smaller than 500 Da) as a result of the non-equivalent relationship between the flux and the molecular weight. This result shows that smaller molecules are absorbed faster (Barry, 2007:579).

Bos and Meinardi (2000:166) explained there is an upper molecular weight limit which enables drugs and compounds to be absorbed through the skin barrier, but if the molecular weight

exceeds 500 Da the absorption decreases rapidly. When a molecule cannot penetrate the human skin barrier in effective quantities, it is not a sensitising agent (Bos and Meinardi, 2000:166).

Investigators believe that when a molecule is larger and penetrates the skin it can be metabolised quickly making them ineffective, but it is believed this mechanism is the same for smaller molecules (Bos and Meinardi, 2000:168).

2.2.4.6 Diffusion coefficient

As an API is administered, molecules from this API move and diffuse through the stratum corneum and concentrate in the membrane where a gradient of concentration is the result. The API primary function should be able to move from the vehicle and diffuse through the skin`s natural barrier therefore, the simplicity that a molecule diffuses through one medium to the next is the diffusion coefficient (D) (Hadgraft and Wolf, 1993:163).

2.2.4.7 Ionisation and pH

Permeation through the skin happens in several ways where molecules traverse the human skin. Ionised drugs can cross the membrane via the shunt route but the amount of permeant can be less than if the species were unionised (Williams, 2003:38). The pH influences the dissociation however ionised molecules, passes the stratum corneum to an extent (Potts *et al.,* 1992:24).

Table 2.1: Formulation consideration for ideal limits (Naik *et al.,* 2000:319).

Physicochemical factors	Ideal limits	Pravastatin
Log D	1 < 3	-0.703*
Molecular weight	< 500 Da	424.53 Da
Melting point	< 200 °C	171.2-173.0 °C
Aqueous solubility	> 1 mg/ml	197.5 mg/ml
pH of saturated aqueous solution	pH 5 – 9	pH 5.0
Dose deliverable	< 10 $mg.day^{-1}$	-

* Properties which are not ideal for permeation

According to Table 2.1, pravastatin is a good candidate for transdermal drug delivery; the molecular weight is below 500 Da and the aqueous solubility more than 1 mg/ml. A compound's existence in ionised or unionised form depends on the pH. With respect to unionised form, ionised compounds have higher aqueous solubility, but lower permeability (Admescope, 2012). Ionisation of an API is a function of its pKa (acid dissosation constant) value, where pH of the skin surface is a big factor. The unionised form of an API is more permeable through membranes, whereas ionised molecules do not voluntarily penetrate the

skin surface (Williams, 2003:38). Pravastatin (pKa of 4.70) is 33.39% unionised at a pH 5 where it can permeate through the skin.

2.3 Cholesterol

Cholesterol is a lipid sterol which is found in the body`s cell membranes. Sterol is better known as a combination of an alcohol and a steroid. The function of cholesterol is to maintain and build cell membranes, thus it increases the membranes fluidity and also helps to absorb fat soluble vitamins (A, D, E and K) better. Cholesterol is a regulator in homeostasis, which functions as a precursor to sex hormones and corticosteroids. It is a very important factor in the maintenance of the cell wall integrity. The total cholesterol levels should be less than 200 mg/dL, as high levels of the low density lipoprotein can lead to atherosclerosis. This is a predisposing factor where there is an accumulation of these blood lipids, lipoproteins, in the macrophage and cholesterol esters found in the arteries which lead to plaque formation and vessel occlusion (Tiwari and Pathak, 2011:983).

Table 2.2: Cholesterol levels (Wells *et al.*, 2009:101).

Level mg/dL	Level mmol/L	Interpretation
< 200	< 5.2	Desirable level
200 – 239	5.2 – 6.2	Borderline high risk
> 240	> 6.2	High risk

2.3.1 High-density lipoprotein function

Dysfunctional high-density lipoprotein (HDL) is predisposed in patients with cardiovascular heart diseases (CHD) and high-density lipoprotein cholesterol (HDL-C) greater than 85 mg/dL. Large HDL particles increase the risk of CHD by suggesting dysfunctional reverse cholesterol transport, where there is an accumulation of lipid-loaded HDL in plasma and also high HDL-C levels. Emphasis is placed on plasma HDL-C seeing that RCT (reverse cholesterol transport) which removes cellular cholesterol derives from the tissue of accumulation. This accumulation causes atheroma formation where cholesterol should be activated to exit this site. Not only the atherogenic process is influenced by the HDL but also through the anti-oxidant and anti-inflammatory properties (Gadi *et al.,* 2013:647).

2.3.2 Hypercholesterolemia

Hypercholesterolemia is an inheritable disorder characterised by the accumulation of low-density lipoprotein (LDL) particles in the plasma itself, which then leads to premature cardiovascular diseases (Farnier and Bruckert, 2012:656). One of the most common inherited disorders is the autosomal dominant familial hypercholesterolemia. This disorder is due to an

increase of the low-density lipoprotein cholesterol (LDL-C) characterised by elevated plasma LDL, due to synthesis by the liver and can be characterised by tendon xanthomas or premature coronary heart disease (Heath *et al.,* 1999:42). This type of disorder can increase the risk of atherosclerotic illnesses which is secondary to a lifetime of elevations in the LDL-C. The main gene involved with familial hypercholesterolemia is the LDL receptor gene. There can also be mutations in other genes, for example Apo-lipoprotein B (Apo B) and proprotein convertase subtilisin/kexin type 9 (PCSK9) (Farnier and Bruckert, 2012:657). Factors leading to an increase of LDL are increased alcohol consumption, smoking, Cushing's syndrome, hypothyroidism, hypertension, age, gender, Diabetes Mellitus, body mass and physical inactivity (Iversen *et al.,* 2009:140). A high saturated fat and cholesterol diet can also increase the blood cholesterol levels which have serious complications that result in stroke, heart attack or angina (Wells *et al.*, 2009:100).

A blood test measures the levels of the total cholesterol which consist of the LDLs, triglycerides (TG) and HDLs. Other risk factors can also be examined such as lipoprotein-a, Apo B, C-reactive protein and homocysteine. A coronary artery calcium scan may also be done where electron beams are computed and take up to 40 pictures of the heart between beats (Wells *et al.*, 2009:100).

2.3.3 Treatment of hypercholesterolemia

Non drug treatment that can be followed consists of a diet of low saturated fat and cholesterol as well as being rich in water-soluble fibre which can help prevent high cholesterol levels. Alcohol should be consumed moderately (no more than 2 units a day) which can be beneficial and raise HDL cholesterol (Heath *et al.,* 1999:42).

The primary treatment for hypercholesterolemia is the HMG-CoA reductase inhibitors which increases the hepatic LDL-receptor activity, decreases the level plasma LDL-C and inhibits the rate limiting step of cholesterol synthesis in the liver (Heath *et al.,* 1999:42). These drugs are better known as statins, which are known to reduce the endogenous synthesis of cholesterol by preventing the development of the predisposing factor atherosclerosis. Statins also have vasculoprotective actions. Their pleiotropic effects can be described as enhancing the ischemic vasodilatory response, improving the function of the endothelial, increasing the nitric oxide bioavailability, ischemia-perfusion protection, having antioxidant properties, inhibiting the inflammatory response, regulating the progenitor cells and stabilising the atherosclerotic plaques (Tiwari and Pathak, 2011:984).

Statins can be combined with losartan, which is an angiotensin II type 1 receptor antagonist which prevents apoptosis. Statins used in the treatment of hypercholesterolemia are: pravastatin, simvastatin, fluvastatin, atorvastatin, rosuvastatin and cerivastatin. Pravastatin, simvastatin and lovastatin are derived from fungi, while rosuvastatin, cerivastatin and fluvastatin

are synthetic. Bisphosphonates contain nitrogen (used to treat osteoporosis), which can act as an inhibitor of farnesyl pyrophosphate synthase and may enhance the effect of statins. Statins such as simvastatin, cerivastatin, fluvastatin and atorvastatin have higher levels in non-hepatic tissue (Tiwari and Pathak, 2011:984). Adverse effects such as rhabdomyolysis (when myoglobin is released into the circulation) and myopathy have been seen in some statins. Weakness, renal failure and muscle pain have also been noted (Wells *et al*, 2009:106). In this study, pravastatin was examined and will be discussed further.

2.4 Pravastatin

2.4.1 Mechanism of action

Pravastatin is a selective and potent inhibitor of HMG-CoA reductase and is known to decrease concentration of LDL and increase HDL. There are two different mechanisms that reduce the serum total cholesterol (TC). The first mechanism is known to inhibit HMG-CoA reductase in the liver that reduces the cholesterol amount in this specific tissue, which leads to increasing LDL receptor mRNA and protein levels. An increase in the catabolic rate decreases the concentration of LDL-cholesterol (Miyazaki and Koga, 2002:299). The second mechanism decreases the very low density lipoprotein (VLDL) which is secreted from the liver, taking into consideration that hepatic cholesteryl ester (CE) is a significant component for the formation of VLDL. By reducing the CE amount caused by the inhibition of cholesterol biosynthesis in the liver, it limits secretion and production of VLDL (Miyazaki and Koga, 2002:299,300).

Gender, age, dispensed statin dose and a combination of drugs are known influencing risk factors when taking statin medication (Settergren *et al.,* 2013:1). Pravastatin does not have significant effect on the basis of human cytochrome P450 3A4 (CYP 3A4) inhibition and is mainly excreted unchanged through kidneys and via bile into the faeces (Settergren *et al.,* 2013:3).

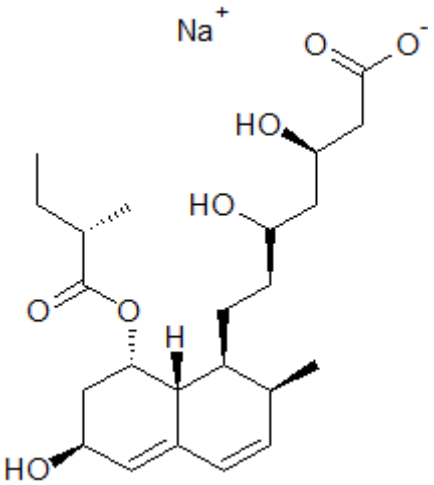

Figure 2.3: Structure of pravastatin (Squibb, 2013).

2.5 Penetration enhancers

The human skin causes difficulties for transdermal delivery of a variety of therapeutic agents because of its barrier. Strategies, whether physical or chemical methods, have been developed to enhance penetration, by providing a driving force that acts on the drug or where the skin`s permeability is increased (Alexander *et al.,* 2012:27).

Thus drugs can now be delivered effectively by using penetration enhancers that promote the drug flux (Williams and Barry, 2012:129). There are certain properties that the penetration enhancers must have, namely:

- They must be cosmetically acceptable.
- Barrier properties should return to normal when removed from the skin.
- They should be compatible with both drugs and excipients.
- They should be non-toxic, non-allergenic and non-irritating.
- They should have no pharmacological activity within the body – no binding to receptors.
- They should work rapidly and the effect of activity and duration should be reproducible and predictable.
- They should work unidirectionally, thus preventing the loss of endogenous material from the body whilst allowing therapeutic agents into the body

No material possesses the ideal properties, but only several of the properties have been named and they are usually used to improve the flux of the drug through the diverse membranes. The passive membrane (stratum corneum) controls the diffusion through the skin. Penetration enhancers are formulated in eutectic systems or slow release delivery systems. The penetration enhancing activity of dimethyl isosorbide (DMI) used in this study will be discussed below (Williams and Barry, 2012:129).

2.5.1 Influence of penetration enhancers on the structure of the stratum corneum

There are three main mechanisms of penetration enhancement on the stratum corneum: 1) interactions with the intercellular keratin, 2) interactions with the intercellular lipids and 3) improved dissolving capacity into the stratum corneum (Alexander *et al.,* 2012:30). There are numerous penetration enhancers available commercially, but only two will be discussed, namely water and DMI as they were used during this study. Water is included as a solute and has a hydrating effect on the skin (Williams and Barry, 2012:129); DMI is included as it is a solvent for poor hydrophobic and hydrophilic actives such as pravastatin, it is stable for hydrolysis, it improves the spreading of very viscous materials such as glycerine, it reduces gelatine cross-

linking and cellular irritation, enhances API and formulation stability as well as epidermal penetration (Arlasolve-DMI, 2014).

2.5.1.1 Water

The human stratum corneum content is usually 15 to 20% of dry tissue weight, but can vary depending on the humidity of the external environment. One can expose the skin to higher humidity by soaking the skin in water or occluding the tissue to prevent transepidermal water loss so the stratum corneum can reach the water equilibrium with underlying epidermal skin cells. The outer membrane can reach 400% of dry weight with occlusion.

Transdermal delivery can be increased by increasing tissue hydration for both lipophilic and hydrophilic permeants. Percutaneous absorption may not be necessarily increased by occlusion such as hydrophilic compounds.

Occlusion may cause some local skin irritation. Water within the tissue is available and 'free' and can act as a solvent for polar permeants within the membrane. Hygroscopic humectants mixture of salts, amino acid derivatives and amino acids causes the natural moistening factor (NMF) in human skin, which helps with tissue pliability and retains water within the stratum corneum.

The corneocytes contains functional groups such as -COOH and -OH binds water molecules within the human tissue. When the stratum corneum is fully hydrated it shows there are water pools with vesicle like-structures in the intercellular lipid bilayers (Williams and Barry, 2012:129).

2.5.1.2 Sulphoxides

Dimethyl isosorbide (DMI) is one of the most frequent used penetration enhancers. This is an aprotic solvent that would rather bind with hydrogen than with water itself as seen in Figure 2.4. It is also hygroscopic, colourless and odourless and is known as a universal solvent, but more commonly used as a co-solvent within a vehicle. This penetration enhancer is effective for both hydrophilic and lipophilic permeants.

One adverse effect of DMI is that is can cause erythema as the dimethyl sulphoxide (DMSO) can denature some proteins in the stratum corneum. Dimethylacetamide (DMAC) and dimethylformamide (DMF) can also be used as penetration enhancers, which is more effective for hydrophilic permeants than lipophilic permeants. The mechanism of these sulphoxides is known to denature proteins on the human skin where the intercellular keratin confirmation is changed from α helical to a β sheet (Williams and Barry, 2012:130).

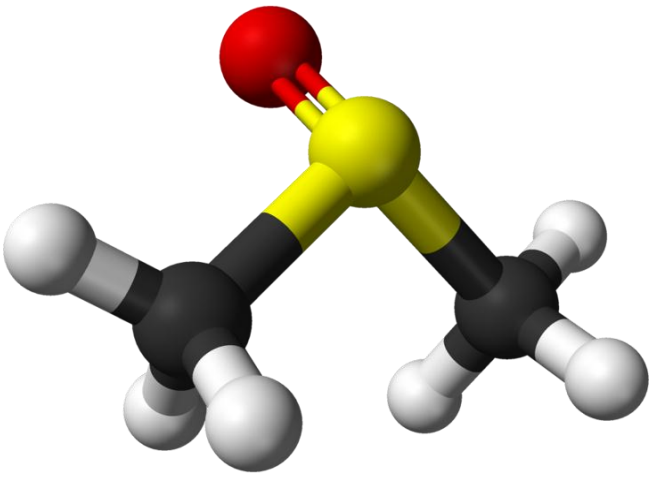

Figure 2.4: Aprotic solvents, which act as potent penetration enhancers (Williams and Barry, 2012:131).

2.5.2 General principles of penetration enhancers

The general principles of penetration enhancers are as follows:

- They work well with polyethylene glycol or ethanol (co-solvents) (Williams and Barry, 2012:135).
- They are concentration dependant (Williams and Barry, 2012:135).

2.6 Delivery gap theory/Wiechers theory

Pravastatin has ideal aqueous solubility properties, but not adequate Log D properties, which make it more difficult to permeate through the skin and into the bloodstream.

It is important to note that in skin delivery, there are three polarities which must be taken into consideration; firstly, the API which is pravastatin, secondly the stratum corneum and lastly 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 programme was therefore ideally developed to calculate the polarity of a formulation, so there is a correct balance between the active`s best driving force into the skin from the formulation and to incorporate as much possible API in any formulation chosen.

To create the right polarity for pravastatin, the polarity indexes (PI), penetrant polarity gap (PPG) and relative polarity indexes (RPI) are used to see which emollient makes a better solvent. These PI values are calculated by the FFE™ programme. In order to receive these PI values, a molecular structure of the API is inserted, which calculates the PI. This PI value on its own is meaningless, but in combination with the right emollients, gives meaningful RPI values. The RPI value is how different the levels are between two different molecules, where one

molecule is the emollient and the other molecule is the API. Therefore, if the RPI value is very small, it means the structures are very alike, as well as the PI values being small and vice versa. A few emollients should always be chosen to get a better RPI value (Wiechers, 2008:95). However, a primary emollient should be chosen with a low RPI (where solubility of the API in emollient is very high) value which is combined with a secondary emollient with a high RPI value. These two emollients, when mixed in the right proportion, will give optimal polarity of the formulation.

The process of how the skin is penetrated is as follows: the API firstly has to diffuse from the formulation to the skin surface, where it partitions into the skin. It must then diffuse through the stratum corneum (outermost layer) and partition into the viable epidermis. Lastly it diffuses through and reaches the dermis where it will also partition. All these layers of skin play a significant role in skin penetration (Wiechers *et al*., 2004:174). As the API diffuses through all these layers of skin, it can partition into the fat deposits or be redistributed via the blood capillaries (Wiechers *et al*., 2004:174). Thus diffusion and partition are extremely important parameters for skin penetration, which can be combined in the following formula:

$$Kp = (K_{oct} / D_{water}) / L \qquad \textbf{Equation 2.4}$$

Where Koct/water is the partition coefficient, L, is the length of pathway of diffusion of the molecule penetrant, Kp is the permeability coefficient and D is the diffusion coefficient (Wiechers *et al*., 2004:174). Aqueous solution permeation of a chemical demonstrated by Potts *et al* (1992:663) through the stratum corneum could be explained by only two parameters which are the molecular weight (MW) and the octanol/water partition coefficient in the following formula:

$$\text{Log Kp } (cm.s^{-1}) = - 6.3 + 0.71 \text{ Log Koct / water} - 0.0061 \text{ MW} \qquad \textbf{Equation 2.5}$$

It is said that when the lipophilicity increases of a penetrating molecule, the permeability Kp increases. The Kp decreases when MW increases.

The stratum corneum/formulation partition coefficient ($K_{SC/formulation}$) indicates the rate which the API partitioned from the formulation into the skin and is defined as:

$$K_{SC/formulation} = \frac{\text{C in stratum corneum}}{\text{C in formulation}} \qquad \textbf{Equation 2.6}$$

C is the solubility of the API. Penetration of the stratum corneum can be increased by decreasing the solubility of the formulation or increasing the solubility of the molecules in the stratum corneum. The amount of penetration per unit or time can be increased (flux) by

increasing the formulation molecule solubility (Wiechers *et al*., 2004:174). If the difference increases between the last layers of the stratum corneum and the formulation, the more molecules will penetrate through the stratum corneum (Wiechers *et al*., 2004:175).

$$J = k_p = \Delta C \frac{KD}{L} \Delta D$$ **Equation 2.7**

ΔC represents the difference in concentrations between the formulation, and the inner most layers of the stratum corneum, K being the partition coefficient and D is the diffusion coefficient. With an increase in ΔC, the flux through the stratum corneum will also increase (Wiechers *et al.*, 2004:175).

Table 2.3: Effect of parameter extent of skin delivery of ingredients (Wiechers *et al.*, 2004:175).

Parameter	Change	Effect	How
Permeation coefficient	Greater	Speeding up permeation rate	Increasing K, D Reducing MW, L
	Smaller	Slowing down permeation rate	Reducing K, D Increasing MW, L
Diffusion	Greater	Speeding up diffusion	Use skin penetration enhancers
	Smaller	Reducing diffusion	Use skin binding to retard penetration
Octanol-water partition coefficient	Greater	Increased levels in stratum corneum	Make penetrant more lipophilic
	Smaller	Reduced level in stratum corneum	Make penetrant more hydrophilic
Stratum corneum / formulation partition coefficient	Greater	Depending on the polarity of the formulation	Change the polarity of the formulation
	Smaller	Depending on the polarity of the formulation	Change the polarity of the formulation
Solubility	Greater	Increasing flux without affecting stratum corneum/ formulation partition coefficient	Formulate as closely as possible maximum solubility of API in formulation
	Smaller	Decreasing flux without affecting stratum corneum/ formulation partition coefficient	Formulate far below the maximum solubility of API in formulation

The log P can be changed as well as the diffusivity of the penetrating molecule, but by changing the physicochemical properties of the API formulated, the delivery can be enhanced much more effortlessly (Wiechers *et al*., 2004:175).

Where the polarity of the penetrants is equal to the stratum corneum, the log P that is equal to that of the stratum corneum (0.8), meaning the solubility of the penetrant in the formulation as well as the stratum corneum, is equal. Well after equilibrium, the concentration of the API will be the same in both phases (stratum corneum and formulation) although the absolute amounts will depend on their respective volumes (Wiechers *et al*., 2004:176).

In the case where the penetrants are more polar than the stratum corneum, the polarity can be calculated by subtracting the polarity of the penetrant from the stratum corneum. The PPG is the difference in polarity between the API and the stratum corneum and can be calculated as follows:

Penetrant polarity gap = |polarity API – polarity stratum corneum| **Equation 2.8**

Hence the two absolute signs (|....|) in Equation 2.8, the PPG should always be positive. The formulation`s polarity is calculated in the second step. To ensure that at least 50% of the API can penetrate into the stratum corneum, the phase polarity in which the API is dissolved should be less or greater than the API itself (Wiechers *et al*., 2004:177).

The following equation is used to obtain API concentration that is higher in the stratum corneum (Wiechers *et al*., 2004:176):

Polarity of formulation > Polarity of penetrant + penetrant polarity gap **Equation 2.9**

Polarity of formulation < Polarity of penetrant – penetrant polarity gap **Equation 2.10**

The equation above can be defined as: ‘The difference in polarity between the API and stratum corneum’ (Wiechers, 2008:95). The driving force for partitioning into the stratum corneum depends on the extreme difference in polarity between the API and the formulation. Whether the polarity between the API and the formulation is enlarged, the solubility of the formulation penetrant will reduce (Wiechers *et al*., 2004:178).

The formulation determines the total amount of the API is dissolved and ready for skin penetration and the polarity of the formulation relative to the stratum corneum. Penetration will elevate as the amount of API rises in the formulation so that high solubility can be obtained. The stratum corneum desires a low solubility in the formulation in order for the API to leave the formulation thus the API must be more soluble in the stratum corneum than in the formulation and permeate the stratum corneum (Wiechers *et al*., 2004:175). The polarity of the formulation must differ greatly from the polarity of the API, but also be close enough so that high

concentrations can be reached at the site of action, consequently the stratum corneum (Wiechers *et al*., 2004:178).

Both conditions cannot be applied at once, but by making use of the RPI, this difficulty may be dealt with (Wiechers *et al*., 2004:175). By using the RPI the polarity of the formulation, the API and the stratum corneum can be compared. This method is applied by visualising a vertical line with high lipophilicity at the bottom and high polarity at the top where the partition expresses the polarity. This all depends on the values on the log 10 scale, stratum corneum polarities penetrating the formulation and the molecule (Wiechers *et al*., 2004:176).

The RPI scale holds these polarity values by marking their position on the vertical line. There are three different scenarios: i.e. 1) when the polarity of the API phase is greater than that of the stratum corneum; 2) the polarity of the API phase is equal to that of the stratum corneum; 3) the polarity of the API phase is smaller than the polarity of the stratum corneum (Wiechers *et al*., 2004:176). When the right primary emollient/solvent is chosen, it can optimise the solubility of the API. The phase in which the API is present, will either be more hydrophilic or lipophilic. The RPI value of the emollient or solvent should be near to that of the API (Wiechers *et al*., 2004:179). By selecting the right secondary emollient/solvent, the driving force can be optimised. This happens when the solubility of the primary solvent is reduced and as a result, the total amount of API dissolved relative to what could be dissolved increases (Wiechers *et al*., 2004:179).

The total quantity of the API absorbed into the skin can be greatly influenced by the choice of emollients used. The distribution of the API in the skin is influenced by different emulsifiers whether it is O/W or W/O emollients (Wiechers *et al*., 2004:181).

The ratio of the minimum effective concentration (MEC) relative to the concentration reached at the target site (C_{TS}) or local tissue is known as the skin delivery gap (SDG) and can be calculated by JW Solutions (2012). Thus molecules with an SDG above 1 are more complex delivery systems and those less than 1, deliver readily. Molecular modelling of the skin and pharmacokinetic assumptions by means of utilising a chain of calculations, can predict the local tissue concentration. Active molecules can be compared on both their intrinsic activity and their deliverability by using the SDG.

2.7 Conclusion

Whether lipophilic or hydrophilic penetrant is used, selecting the right emollient by using the RPI concept, thus optimising the oil phase, the efficacy of formulations can be improved without increasing the API. The API must be dissolved in the highest possible concentration in the primary emollient and the adding of the secondary emollient to reduce the solubility to an acceptable level. Emollients influence the extent of skin delivery whereas the emulsifiers

influence the rate of skin delivery. Increasing the solubility of the API by making corrections in the formula; FFE™ works on the principle that it optimises the driving force of the API where it penetrates into the stratum corneum, without affecting the stratum corneum itself.

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Chapter 3:

Article for publication in International Journal of Pharmaceutics

Chapter 3 is written in article format in for publication in The International Journal of Pharmaceutics. The complete author's guide is given in Appendix D. For ease of reading the paragraphs of this chapter have been justified.

Optimised transdermal delivery of pravastatin

Cornel Burger, Minja Gerber*, Jan L du Preez and Jeanetta du Plessis

Centre of Excellence for Pharmaceutical Sciences, North-West University, Private Bag X6001, Potchefstroom 2520, South Africa

* Corresponding author. Tel.: +2718 299 2328; Fax: +2787 231 5432. E-mail address: Minja.Gerber@nwu.ac.za (M Gerber).

Abstract

The skin`s primary function is to protect the body from environmental factors. The outermost layer of the skin, known as the stratum corneum, is the main barrier of the skin. The stratum corneum is lipophilic which consists of lipids and when topical formulations are applied onto the skin for systemic effect, it has to pass firstly the main barrier, then the other layers of the skin. Wiechers programme “Formulating for Efficacy” initiated a new strategy to optimise the oil phase of formulations. 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 emulgel formulations) with 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 different polarity formulations consisted of a formulation with a polarity equal to the stratum corneum, a lipophilic and a hydrophilic formulation. Franz cell diffusion studies were executed over a period of 12 h, which was followed by tape-stripping.

Keywords: Pravastatin, Wiechers, Transdermal delivery, Formulation, Delivery Gap theory, Franz cell

1 Introduction

The skin consists of heterogeneous layers (Pailler-Mattei *et al.*, 2008) and is the heaviest organ of the body (Sanders *et al.*, 1999), weighing approximately 5 kg which approximates to two square meters in surface area (Godin & Touitou, 2007). 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 endogenous substances loss (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 through the skin to the systemic circulation (Bouwstra & Honeywell-Nguyen, 2002).

Literature suggests the skin serves as a potential anatomical site for the application of various dosage forms for topical and transdermal delivery. The target site of action differs from the stratum corneum, epidermis, dermis, appendages or even the blood vessels (Ponec, 2002). Good bioavailability is needed since the stratum corneum's barrier properties are exceptional (Hadgraft, 2004). Any transdermal drug needs to pass through the stratum corneum which is lipophilic; thereafter the hydrophilic epidermal and dermal layers have to be permeated before the active pharmaceutical ingredient (API) reaches the blood stream (Perrie *et al*., 2012). This said, it is highly important for transdermal drugs to consist of both lipophilic and hydrophilic properties, so these drugs can partition through the stratum corneum as well as the epidermis, the dermis and into the blood capillaries (Perrie *et al*., 2012).

The physicochemical properties are very important for the permeation of the API (pravastatin), therefore the penetration mechanism must be understood so the API can permeate across the stratum corneum (Hadgraft, 2004). To design transdermal dosage forms, physicochemical and mechanistic properties should be taken into consideration when a formulation is made with a specific API (Hadgraft, 2004). There are many advantages to

transdermal drug delivery which cannot be obtained by other administration routes, which include: avoidance of pain, patient compliance improves (no needles, etc.), drug release can be controlled over time and no large amounts of drugs are lost since the first pass metabolism is avoided. However, there are also challenges in transdermal drug delivery of which the most important will be variability in penetration between patients on different sites of action (Jepps, 2012).

Pravastatin, which is the API in this study, is unique because this drug contains a hydroxyl salt, it is liver specific and is taken up by the multi-specific anion transporter (Yamazaki *et al*., 1997). Sufficient concentrations of pravastatin should permeate through the skin to be delivered to the target site (blood capillaries) in order for it to exhibit it`s effect. *In vitro* permeation studies and tape-stripping play an important role in skin penetration to optimise the formulation design in transdermal and dermal delivery (Leveque *et al.*, 2004). The vertical Franz diffusion cells are reproducible, which is why they are widely used and it also helps with drug release from semi-solid dosage forms.

Prof Johan Wiechers designed a computer programme called “Formulating for Efficacy” (FFE™) which optimises oil phase ingredients in which the API will be dissolved, thus transdermal drug delivery might be optimised. The type of formulation will be determined by the computer programme. To reach the desired polarity (hydrophilic, optimised or lipophilic), the formula will be manipulated. In this study, pravastatin (hydrophilic) was selected as a model drug to investigate FFE™. The aim of this study was to determine the success of Prof Wiechers Delivery Gap Principle by optimising the oil phase of the six formulations to penetrate the skin more adequately and according to polarity and penetration, compare the different formulations. Membrane studies were performed with these six different formulations prior to the *in vitro* diffusion studies across human skin. 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 (δ_h), the energy from dipolar intermolecular force between molecules (δ_p) and the energy from dispersion bonds between molecules (δ_d) (Wiechers, 2011). The HSP is the three dimensional (3-D) representation of δ_d, δ_p and δ_h. According to Hansen solubility, the parameters of the skin are 17, 8 and 8, 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). The HSP values of the skin, pravastatin and the three formulations used for this study are shown in Table 1.

Table 1:

The Hansen solubility parameters of pravastatin, the formulations and the skin

The FFE™ programme allows optimal API skin delivery in a certain formula and offers three options:

1) Optimising towards the skin: Depending on the API`s 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 (TC): 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, which can leave the formulation and permeate the skin (Wiechers, 2011). The skin formulation gap (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.

2 Materials and Methods

2.1 Materials

Pravastatin was obtained from DB Fine (Johannesburg, South-Africa). 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, South Africa). Dimethyl isosorbide and potassium cetyl phosphate were obtained from Croda (Midrand, South-Africa). Ultrez 20 was obtained from Lubrizol (Johannesburg, South-Africa). Glycerine was obtained from Sigma-Aldrich (Johannesburg, South-Africa). Veegum was obtained from R.T Vanderbilt Company, Inc. (Kentucky). Sodium hydroxide and orthophosphate used for the preparation of phosphate buffered solution (PBS) were supplied by Merck Laboratory Supplies (Midrand, South Africa). Throughout the entire study deionised high pressure 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 then be manipulated by means of the programme FFE™ to obtain the desired polarity.

2.2.2 Preparation of PBS (pH 7.4)

Sodium hydroxide (3.15 g) was weighed and dissolved in 786.8 ml HPLC water and 13.62 g potassium dihydrogen orthophosphate was weighed and dissolved in 500.0 ml HPLC water, according to the British Pharmacopoeia (BP) standards (BP, 2013a; BP, 2013b). The two above-mentioned solutions were mixed and the pH was set to 7.4 with 10% orthophosphoric acid. The solution was filtered and degassed.

2.2.3 Analysis of pravastatin

A HPLC method had been developed and validated in conjunction with Prof Jan du Preez at the Analytical Technology Laboratory (ATL), North-West University (NWU), Potchefstroom Campus, South Africa. The HPLC (Agilent 1200 Series) was equipped with an Agilent 1200 pump, a diode array detector, an auto sampler 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. This wavelength was set in order for pravastatin to be detected. 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. Analytical tests were performed in a laboratory with a controlled environment of 25 °C.

2.2.4 Standard preparation

The standard preparation was used in the HPLC. 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) (Standard 1); 5 ml of this solution was diluted to 50 ml with PBS (pH 7.4) (Standard 2); a further 5 ml of this solution was diluted to 50 ml with PBS (pH 7.4) (Standard 3); 5 ml of this solution was diluted to 50 ml with PBS (pH 7.4) (Standard 4). The standards were transferred into auto sampler vials and analysed. The concentration range of the standard was between 0.5 and 101.0 µg/ml.

2.2.5 Preparation of different formulations

Table 2:

Ingredients used in the formulation for different polarity cream and emulgel

The following procedure was used to prepare the pravastatin in different polarity formulations: pravastatin was added to water and stirred; the other ingredients of Phase C were added to water and heated to 70 to 75 °C; Phase A was added together and heated to

70 to 75 °C; Phase B was added together and heated to 70 to 75 °C; Phase B was added to C whilst stirring; the water phase was added to Phase A while it was stirred; the mixture was homogenised at 9500 rpm for 5 min and stirred to cool.

2.2.6 Physicochemical properties

2.2.6.1 Aqueous solubility

The water bath was preheated to 32 °C which is the same temperature on top of the skin during a diffusion study. PBS (pH 7.4) was inserted in the polytop with a magnetic stirrer and an excess of pravastatin was added and regularly checked to ensure the solution was saturated at all times. After 24 h the solution was removed and filtrated then centrifuged at 5000 rpm for 10 min to ensure complete precipitation. The supernatant (1 ml) was diluted to 100 ml with PBS (pH 7.4) and the resultant solution analysed by HPLC. This experiment was repeated in triplicate.

2.2.6.2 Octanol-buffer distribution coefficient (log D)

n-Octanol (100 ml) and equal volumes of PBS (pH 7.4) 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); thereafter 3 ml pre-saturated *n*-octanol was added to each of the aforementioned pravastatin solutions. Subsequently, the solutions were placed at 32 °C in the shaker water bath for 3 h and left overnight, then centrifuged thereafter to ensure complete precipitation. The HPLC was used to determine the concentrations of API in the separated phases. The log D was calculated through the logarithmic ratio of the concentration in the PBS (pH 7.4) and the concentration in the *n*-octanol phase. This experiment was performed in quaternary.

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

2.3.2 Viscosity

A Brookfield Viscometer (model DV II, Stoughton, Massachusetts, 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), then removed and the spindle (Stoughton, MA) 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 also used to define the zeta-potential. Suitable pH zeta-potential 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. 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 to the lower half of the Franz cell 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) 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) 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.

2.4.2 Skin preparation

Following abdominoplastic surgery, Caucasian full-thickness abdominal skin (ethical approval reference number: NWU-00114-11-A5) was collected from plastic surgeons. To ensure the skin could be easily separated from the fatty layer it was kept in a refrigerator at -20 °C for no more than 24 h. 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 and the differences will be discussed in this section. Dermatomed skin instead of membrane was used; the stratum corneum faced the upper donor compartment; the receptor compartment was filled with a mixture of PBS (pH 7.4) and ethanol (90:10); after 12 h the entire receptor volume was withdrawn. 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) with 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) with ethanol (90:10). These solutions containing the tape strips and skin pieces 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 concentrative 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 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 (HE), lipophilic emulgel (LE), optimised emulgel (OE), hydrophilic cream (HC), lipophilic cream (LC) and optimised cream (OC) (Sheskin, 2000), a two one-way ANOVA's was used. 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 above statistical analyses were performed. Flux or concentration determined by a more exact method is the median; this is used when there is a significant variation between the mean and median values (Dawson & Trapp, 2004). Both mean and median values will be presented throughout this study and we will make use of median 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 summarises the ingredients.

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) 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: Results of physicochemical properties 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 are dissociated 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 dissociated 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 ranges 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 range from below -30 mV or above 30 mV (Malvern instruments, 2014). It is observed that the zeta-potential values are in an acceptable range (between 40 mV to 78 mV), which indicates the formulas were stable.

3.3 Membrane diffusion experiments

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 follows: firstly the OE (7.175 µg/cm^2.h), followed by LE (6.401 µg/cm^2.h), HE (6.355 µg/cm^2.h), HC (5.061 µg/cm^2.h), OC (4.297 µg/cm^2.h) and lastly, the CL (3.115 µg/cm^2.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 there were no flux values (µg/cm².h) obtained, as the flux values were too low when two hourly withdrawals were extracted, therefore only one extraction was taken for each individual Franz cell after 12 h which resulted in only concentration (µg/cm^2) values being obtained.

Illustrated in the box-plot in Fig. 1 is the mean and median amount per area (µg/cm^2) of

pravastatin that diffused through the skin in different semi-solid formulations.

Fig. 1: Box-plot representing the amount of pravastatin per area (µg/cm²) diffused through the skin between 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 highest median amount per area obtained was the OE formulation (2.578 µg/cm^2), followed by OC (1.449 µg/cm^2), HC (0.434 µg/cm^2), LE (0.121 µg/cm^2), HE (0.055 µg/cm^2) and lastly LC (0.000 µg/cm^2).

In Fig. 1, the most significant differences between the mean and median concentration values from all the formulations 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 permeation of pravastatin through the skin increased, 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 had rather 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).

Table 4, calculated by the FFE™ computer programme, consists of the HSP values, AFG and SFG values of three different polarity formulations. These values are important to explain why median amounts of the formulations differ.

Table 4:

Hansen solubility parameters of three different semi-solid formulations, active formulation gap and skin formulation gap

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 plays a vital role (Wiechers, 2011).

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 and therefore show more improved median amount per area diffused. 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.

The AFG is known as the 'distance' between the API and the formulation and the SFG 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. 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).

It is evident that when the AFG values of the hydrophilic formulations (1.3) and lipophilic formulations (5.7) were compared, the hydrophilic values were smaller than those of the lipophilic values. Prof Wiechers Delivery Gap Principle suggests that when the AFG decreases, the driving force will increase and therefore the API can permeate into the skin (Wiechers, 2011). Due to this driving force effect, as the concentration increases as the API accumulates in the skin, it is more prone to diffuse through the skin (Wiechers, 2008). This resulted in the hydrophilic formulations (HE and HC) permeating more efficiently through the skin than the lipophilic formulations (LE and LC).

When the SFG values were compared of the optimised formulations (1.5), hydrophilic (7.9) and lipophilic formulations (4.2), it can be seen that the optimised formulations SFG value was very low, indicating API can diffuse easier through the skin as this formulation is more favourable for the skin.

The same amount of API, as well as penetration enhancer, was incorporated in each formulation so the use of penetration enhancer could be ruled out. The effects of penetration enhancers (DMI which was used in this study) are nullified when used to

improve the solubility of lipophilic APIs; however this it can be corrected by using more secondary emollients (Wiechers 2008). Many factors play an important role as to whether APIs should penetrate the skin; the difficulty transpires when these factors are taken into consideration and need to be manipulated and controlled (Wiechers, 2008).

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 concentrations were as follows: firstly HE (1.448 µg/ml) contained the highest median concentration pravastatin, 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 stratum corneum than the optimised formulations, however all the formulations did accumulate in the stratum corneum. Fig. 2 suggested the emulgel formulations (HE, LE and OE) improved the permeation of pravastatin into the SCE 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 why the API into the skin increased.

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 was depicted that the highest median concentration in ED was 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 was 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 was observed 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 viable epidermis permeation 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 ($p < 0001$), 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 also 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 follows: 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.

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Any opinions, findings, conclusions or recommendations expressed in this material are those of the authors and therefore the NRF does not accept any liability with regard thereto.

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Tables

Table 1:

The Hansen solubility parameters of pravastatin, the formulations and the skin

	δ_d	δ_p	δ_h
Skin	17.0	8.0	8.0
Pravastatin	16.7	8.6	11.5
Optimised cream / emulgel	17.1	7.6	8.5
Hydrophilic cream / emulgel	17.0	8.5	12.4
Lipophilic cream / emulgel	17.0	6.5	7.5

Table 2:

Ingredients used in the formulation for different polarity cream and emulgel

	Cream ingredients	%m/m	Emulgel ingredients	%m/m
A	Stearic acid	4.0%	Cetyl alcohol	1.5%
	Cetyl alcohol	0.5%	MCK	1.7%
	MCK	1.7%	Isopropyl myristate	1.0%
	Span 60	0.4%		
B **Optimised**	Dimethyl isosorbide	13.0%	Dimethyl isosorbide	13.0%
	Polyethylene glycol 400	5.0%	Polyethylene glycol 400	5.0%
Hydrophilic	Dimethyl isosorbide	13.0%	Dimethyl isosorbide	13.0%
	Water	2.4%	Water	2.4%
	Ethanol	2.6%	Ethanol	2.6%
Lipophilic	Dimethyl isosorbide	13.0%	Dimethyl isosorbide	13.0%
	Mineral oil	5.0%	Mineral oil	5.0%
C	Glycerine	10.0%		
	Tween 80	3.2%	Ultrez 20	0.8%
	Veegum	0.5%	Pravastatin	2.0%
	Pravastatin	2.0%	Water (dH_2O)	75.0%
	dH_20	60.0%		

Table 3:

Results of physicochemical properties of pravastatin formulations

	pH	Viscosity (cP)	Droplet size (µm)	Zeta-potential (mV)
Optimised cream	5.09	4818.65	44.375	-46.67
Hydrophilic cream	5.05	3546.26	29.705	-42.90
Lipophilic cream	5.01	3856.35	31.777	-56.03
Optimised emulgel	5.09	4064.10	55.295	-75.00
Hydrophilic emulgel	5.00	3843.54	32.214	-63.53
Lipophilic emulgel	5.07	4157.79	78.428	-77.37

Table 4:

Hansen solubility parameters of three different semi-solid formulations, active formulation gap and skin formulation gap

Formulations	**δ_d**	**δ_p**	**δ_h**	**MW**	**AFG**	**SFG**
Hydrophilic	17.0	8.5	12.4	179	1.3	7.9
Lipophilic	17.0	6.5	7.5	272	5.7	4.2
Optimised	**17.1**	**7.6**	**8.5**	230	4.1	1.5
Skin	17.0	8.0	8.0	-	-	-

Figures:

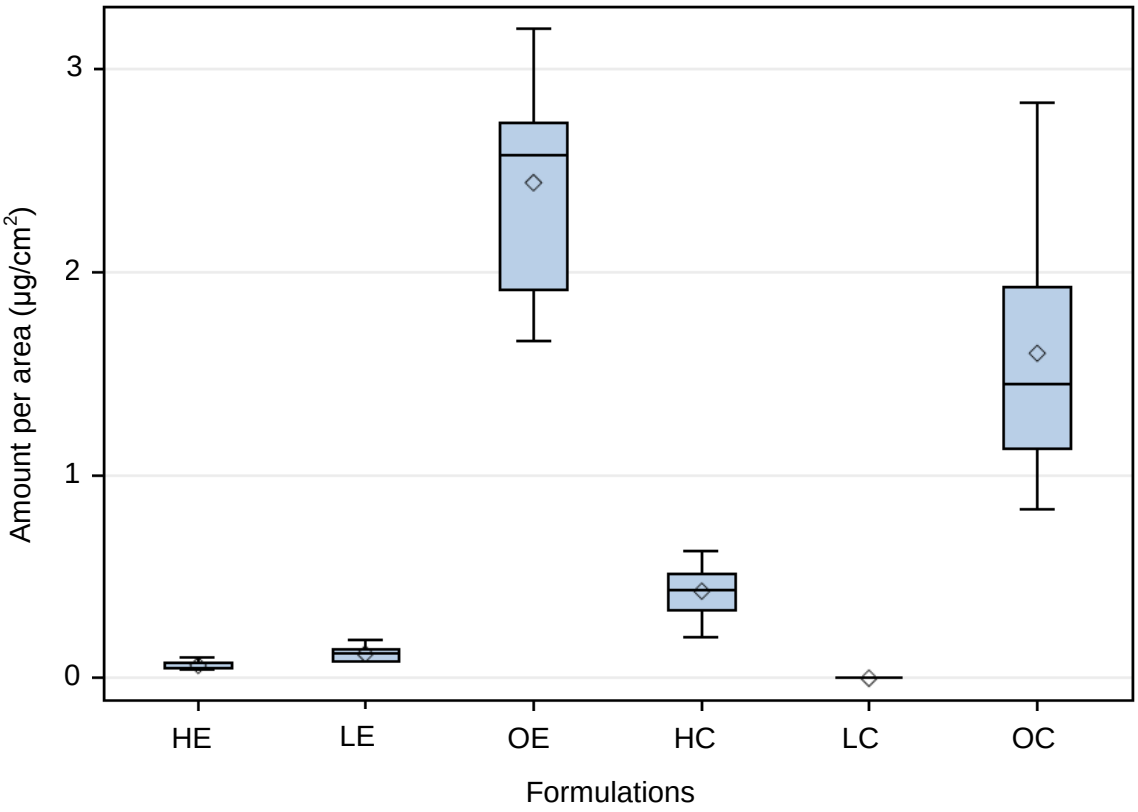

Fig. 1: Box-plot representing the amount of pravastatin per area (µg/cm²) diffused through the skin between the six different formulations. The mean and median flux values are indicated by the diamond shapes and lines, respectively.

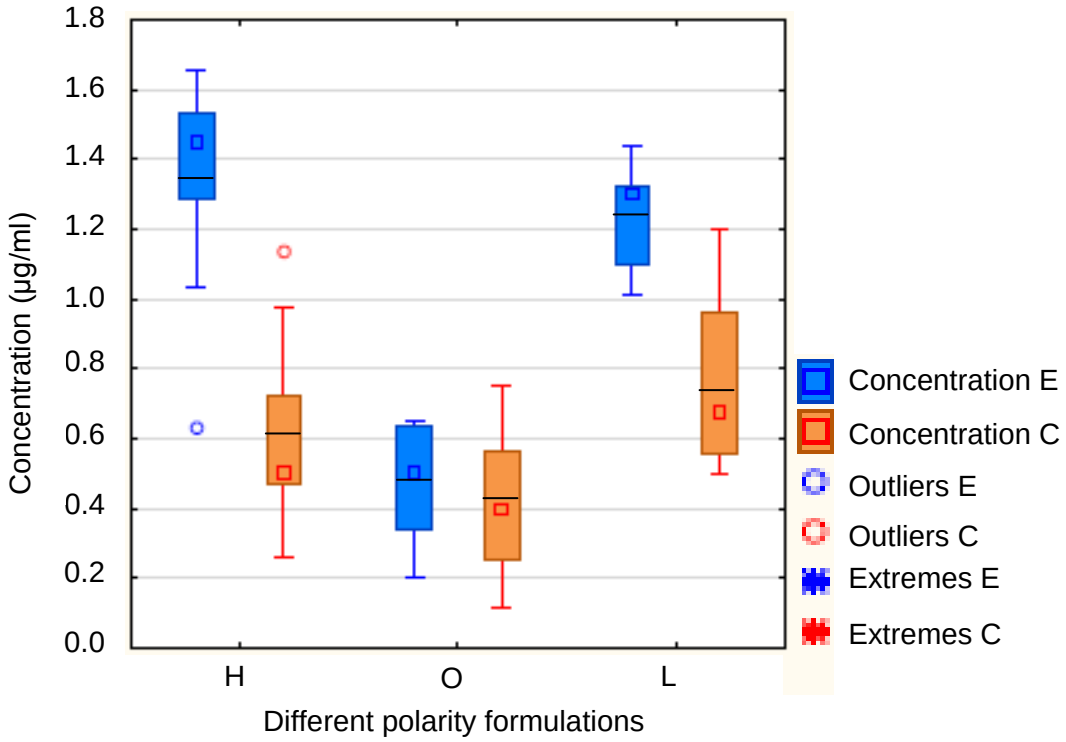

Fig. 2: Box-plot indicating the concentration (µg/ml) pravastatin present in the SCE after tape stripping for the different formulations. The mean and median concentration values are indicated by the lines and squares, respectively.

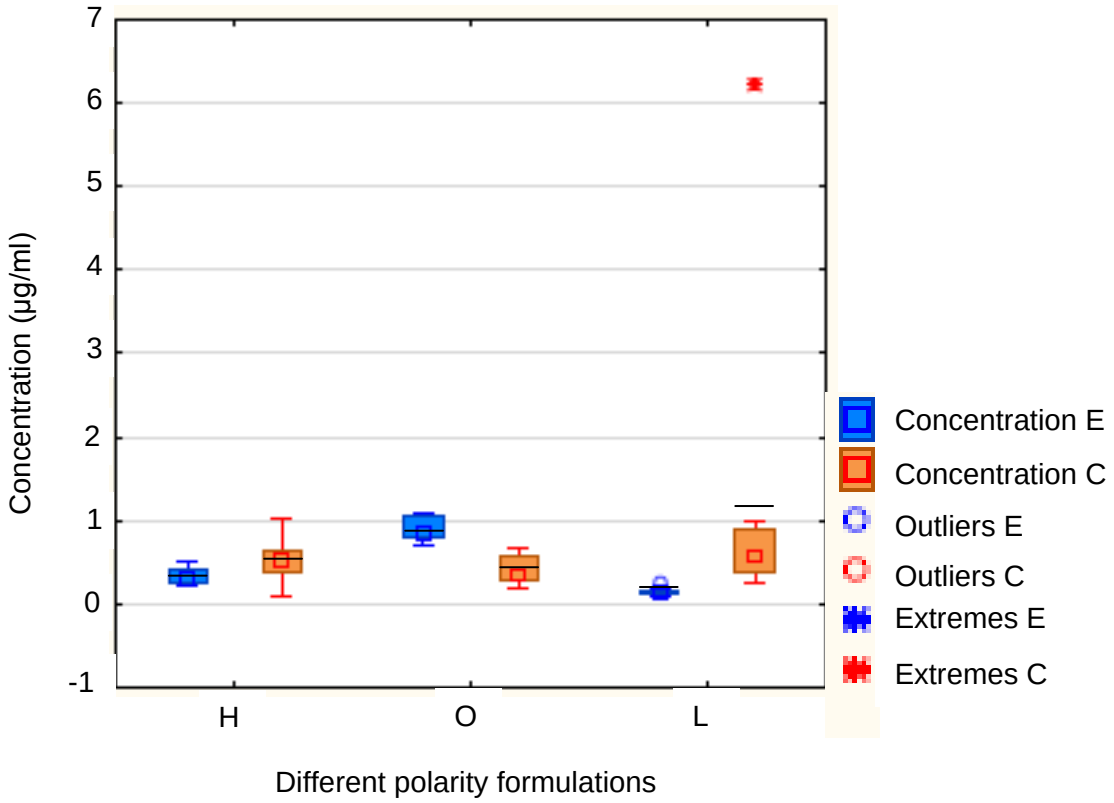

Fig. 3: Box-plot indicating the concentration (µg/ml) pravastatin present in the ED after tape stripping for the different formulations. The mean and median concentration values are indicated by the lines and squares, respectively.

Chapter 4:

Conclusion and future prospects

Transdermal drugs are topically administered formulations intended to deliver continuous and controlled delivery of APIs into the blood circulation, via the stratum corneum and finally into the systemic circulation, for a therapeutic effect (Latheeshjlal *et al.,* 2011:2140). However, transdermal drug delivery is limited by a variety of biological (skin age, blood flow, skin site, skin metabolism, etc.) and physiological (skin hydration, pH, temperature, ionisation, drug concentration, etc.) factors (Latheeshjlal *et al.,* 2011:2140).

The first aim of the study was to deliver pravastatin transdermally as pravastatin's greatest adverse effect is increased liver enzymes of up to 300% in oral dosage (Lane, 2005), therefore it would be ideal to incorporate pravastatin within a formulation that can be delivered transdermally to exclude the first hepatic metabolism effect. The second aim of the study was to use the computer programme FFE™ designed by Prof JW Wiechers, where pravastatin was chosen as API for transdermal formulations to increase transdermal drug delivery.

The objectives of the study were to:

- Determine the optimal oil phase in the different formulations containing pravastatin through the literature from the JW Solutions software (FFE™)
- Formulate three emulgel formulations as well as three cream formulations with different polarities, i.e. optimised (polarity is equal to skin) cream and emulgel, lipophilic (non-polar) cream and emulgel, as well as hydrophilic (very polar) cream and emulgel.
- Develop and validate a HPLC analytical method to determine the different concentrations of the pravastatin within the six formulations.
- Determine the log D and aqueous solubility of pravastatin sodium.
- Determine the release of pravastatin from the formulation by means of executing a membrane study.
- Determine the transdermal and topical delivery of pravastatin from the formulation by performing a diffusion study followed by tape stripping, respectively.

During this study, semi-solid products were formulated using Wiechers Delivery Gap Principle. Pravastatin sodium (2%) was selected as API. Six formulations (cream and emulgel) containing

pravastatin sodium (2%) were developed and manufactured and consisted of three different polarities, i.e. a non-polar (lipophilic) formulation, a very polar (hydrophilic) formulation and a formulation where the polarity is equal to that of the stratum corneum (optimised). These formulations were manipulated by means of the programme FFE™ to obtain the desired polarity as mentioned above. The six formulations were manufactured successfully. Formulations had a homogeneous white texture, applied very easily and were not too oily.

The HPLC method used in this study to determine concentration was successfully developed and validated for the six formulas in conjunction with Prof Jan du Preez from the Analytical Technology Laboratory (ATL), North-West University (NWU), Potchefstroom Campus, South Africa.

Transdermal drug delivery through the skin was investigated. The stratum corneum, which is the most important consideration or limitation in dermal delivery because it acts as a protective barrier prohibiting external elements penetrating the skin, needs to be permeated. An ideal property of the skin penetrant is having a log P of 1 to 3 (Naik *et al.,* 2000:319) and an aqueous solubility of > 1 mg/ml to permeate adequately through the skin. The log D value obtained for pravastatin was -0.703 indicating that transdermal drug delivery would not be optimal compared to the aqueous solubility of pravastatin (197.5 mg/ml) which indicated it would permeate through the skin.

After membrane release study experiments were conducted with the six formulations (three of which were emulgels and three creams, each containing 2% pravastatin), the optimised emulgel (OE) had the highest median flux value (7.175 µg/cm^2.h), followed by the lipophilic emulgel (LE) (6.401 µg/cm^2.h), the hydrophilic emulgel (HE) (6.355 µg/cm^2.h), the hydrophilic cream (HC) (5.061 µg/cm^2.h), the optimised cream (OC) (4.297 µg/cm^2.h) and lastly, the lipophilic cream (LC) (3.115 µg/cm^2.h). It was determined that the emulgels had higher median flux values when compared to the cream formulations; hence pravastatin was released better from the emulgels than from the creams.

The skin diffusion was conducted after the membrane studies with the different polarity formulations and results of the median amount per area which permeated through the skin after 12 h was compared. The OE formulation (2.578 µg/cm^2) presented the highest median amount per area, followed by OC (1.449 µg/cm^2), HC (0.434 µg/cm^2), LE (0.121 µg/cm^2), HE (0.055 µg/cm^2) and lastly LC (0.000 µg/cm^2).

The median stratum corneum-epidermis (SCE) concentrations were also compared to each other and the results were as follows: the HE (1.448 µg/ml) portrayed the highest median concentration pravastatin, 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).

Median concentrations in epidermis-dermis (ED) were compared and the results were as follow: OE (0.849 µg/ml) yielded the highest median concentration, 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).

During membrane studies, it was noticeable that the emulgel formulations performed better than the cream formulations when compared to each other. In the skin diffusion studies, the emulgel formulations also permeated pravastatin better than the cream formulations. When membrane and diffusion data was compared, OE depicted the highest median values and the LC showed the lowest median values in both cases. Despite the fact all six formulations released pravastatin the observation, when looking at LC, was that no pravastatin diffused into the systemic circulation (receptor phase).

When comparing the lipophilic SCE with the more hydrophilic ED and systemic circulation (receptor phase), the formulations had both lipophilic and hydrophilic characteristics seeing that the API penetrated the stratum corneum and thereafter diffused into the deeper layers of the skin (Potts, 1992:22). When all the formulations were compared, i.e. optimised, hydrophilic and lipophilic, it was evident the optimised formulations had the lowest median concentration in SCE, but the highest median concentration in the receptor phase, thus the optimised formulation diffused the best through the SCE. As afore-mentioned this could be because the formulations had the same polarity as the skin and therefore penetrated the skin to the receptor fluid more adequately (Wiechers, 2011). It is also noteworthy that when examining LC, pravastatin penetrated the SCE as well as the ED, but did not permeate through to the systemic circulation, which makes it a good candidate for topical delivery.

Generally, when comparing the emulgel and cream formulations the observation is that the emulgel formulations permeated more pravastatin than the cream formulations. This can be explained by the hydrating effect of emulgel, as emulgels contain more amounts of water which lead it to be more hydrophilic. Consequently this results in more hydrophilic emulgels permeating to the more aqueous regions, in this case, the receptor fluid (Benson, 2005:28). Hence, all formulations contain both aqueous (hydrophilic) and lipid (lipophilic) solubility properties, which means it was sufficiently lipophilic to penetrate the stratum corneum and hydrophilic enough to permeate to other deeper skin layers such as the aqueous regions and even though different formulations were used, it could still permeate the viable epidermis (Perrie *et al*., 2012:392).

Overall, it was evident that between the different polarity formulations (optimised, hydrophilic and lipophilic), the lipophilic and hydrophilic formulations increased the diffusion of pravastatin into the SCE, whereas the optimised formulations improved the diffusion of pravastatin into the systemic circulation (receptor fluid). The optimised formulations had the same polarity as the skin, therefore increasing penetration the most (Wiechers, 2011). Consequently, the FFE™

programme is effective to increase skin penetration by using Prof Wiechers` Delivery Gap Principle to optimise the oil phase (Wiechers, 2011); the delivery of the API (pravastatin) was transdermally enhanced by the optimised formulation.

Future prospects include:

- Other statins may be used as an API to examine if it is possible to deliver them transdermally, in order to bypass first-pass metabolism.
- Other statins may also be investigated to determine if the FFE™ programme is efficient to optimise drug delivery.
- More polar, i.e. very hydrophilic or less lipophilic, formulations could be studied to see if the results could increase transdermal and/or topical drug delivery.
- Other formulations (other than cream or emulgel) can be explored to see if they would have any influence on the optimal drug delivery using the FFE™ programme.

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Appendix A:

Validation of the HPLC analytical method for assay analysis and diffusion

A.1 Purpose of validation

The purpose of the validation is to ensure that the method of analysis is suitable, sensitive and reliable for the determination of the amount of active ingredients in different semi-solid formulations (ICH, 1995:8; ICH 1996:8).

A.2 Chromatographic conditions

The analytical HPLC method was created and developed by Professor Jan du Preez, of the North-West University (NWU), Potchefstroom and the procedures were carried out in the Analytical Technology Laboratory (ATL).

Certain factors of the chromatographic conditions had been taken into account when this method of analysis was developed and these were:

Analytical instrument:	HP1100 series HPLC equipped with a pump, autosampler, UV detector and Chemstation Rev. A.10.03 data acquisition and analysis software or equivalent
Column:	Column L1, USP 24, 2000, p 1925 (Luna C18-2 column, 150 x 4.6 mm, 5 µm, 100 Å pores, 17.8% carbon load, endcapped, Phenomenex, Torrance, CA and Venusil XBP C18(2), 150 x 4.6 mm, 5 µm, Agela Technologies, Newark, DE was used)
Mobile phase:	Acetonitrile/0.1% orthophosphoric acid in water, 40:60
Flow rate:	1.0 ml/min
Injection volume:	50 µl
Detection:	UV at 238 nm
Retention time:	± 4 min
Stop time:	6 min
Solvent:	Phosphate buffer solution (PBS; pH 7.4)

A.3 Preparation of standard and samples

A.3.1 Standard preparation

The standard preparation of pravastatin is as follows:

1. Weigh approximately 50 mg of pravastatin accurately in a 100 ml volumetric flask.
2. Dissolve in approximately 10 ml of methanol; fill to volume with PBS (pH 7.4) (Standard 1).
3. Dilute 5 ml of the solution to 50 ml with PBS (pH 7.4) (Standard 2).
4. Dilute 5 ml of the solution to 50 ml with PBS (pH 7.4) (Standard 3).
5. Further dilute 5 ml of the solution to 50 ml with PBS (pH 7.4) (Standard 4).
6. Transfer the standards into auto sampler vials and analyse.

A.3.2 Preparation of samples for the analysis of formulations

The following ingredients and quantities were weighed off as shown in Table A.1. A, B and C were heated separately and added together and stirred. After the cream was obtained, 1 g was transferred to a 100 ml volumetric flask and diluted with methanol.

Table A.1: Cream standard formula

Ingredients		%m/m
A	Stearic acid	4.0%
	Cetyl alcohol	0.5%
	Potassium cetyl phosphate (MCK)	1.7%
	Span 60	0.4%
B	Dimethyl isosorbide	13.0%
	Polyethelene glycol 400	5.0%
C	Glycerin	10.0%
	Tween 80	3.2%
	Veegum	0.5%
	Pravastatin	2.0%
	dH_20	60.0%

Table A.2 shows the different ingredients and quantities that were measured to formulate the emulgel. A, B and C were heated separately and added together and stirred. After the emulgel was obtained, 1 g was transferred to a 100 ml volumetric flask and diluted with methanol.

Table A.2: Emulgel standard formula

Ingredient		%m/m
A	Cetyl alcohol	1.5%
	MCK	1.7%
	Isopropyl myristate	1.0%
B	Dimethyl isosorbide	13.0%
	Polyethelene glycol 400	5.0%
C	Ultrez 20	0.8%
	Pravastatin	2.0%
	Water	75.0%

A.3.3 Placebo preparation

The placebo was prepared as mentioned above; only the API was left out of the formula.

A.3.4 Sample preparation for diffusion studies

Samples were collected from Franz diffusion cells which were transferred into auto sampler vials and analysed without any further processing.

A.4 Validation parameters

A.4.1 Linearity

Linearity expresses differences in precision at different points of a given range. The linearity of an analytical procedure is its ability (within a given range) to obtain test results, which are directly proportional to the concentration (amount) of analyte in the sample.

A.4.1.1 Linear regression analysis

Linear regression analysis determined the linearity of the API on the plot of the peak area versus concentration (µg/ml). The data is described by a linear equation, namely:

$$y = mx + c$$ **Equation A.1**

Where:

y = Peak area ratios of the different API

m = Slope

x = Concentration of the different API in µg/ml

c = y-intercept

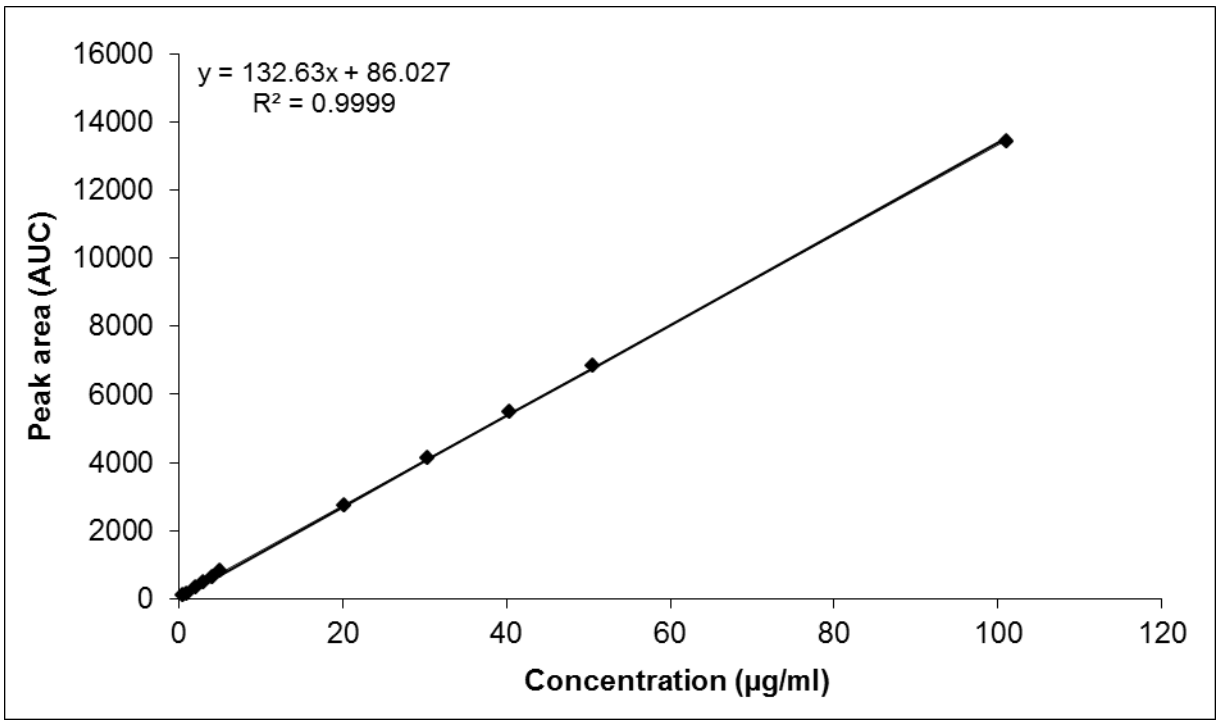

Figure A.1: Linear regression curve of pravastatin standards

Linear regression analysis should yield a regression coefficient (R squared) of ≥ 0.99. The regression value (R^2) obtained, was within the acceptance criteria of 0.99 as seen in Figure A.1, which indicates a high degree of linearity and therefore demonstrates good stability of the analysis system.

Table A.3: Linearity results of pravastatin

Standard (µg/ml)	Peak area
0.51	108.0
1.01	161.8
2.02	323.5
3.03	485.0
4.04	647.0
5.05	807.6
20.20	2756.8
30.31	4128.5
40.41	5488.8
50.51	6838.8
101.00	13432.5
Slope	132.63
y-intercept	86.027
R^2	0.9999

A.4.1.2 Lower limit of detection and quantification

The limit of detection (LOD), is defined as the lowest concentration of an analyte in a sample that can be detected, not quantified. It is expressed as a concentration at a specified signal: noise ratio (S/N), usually between 3:1 and 2:1.

The limit of quantification (LOQ), is defined as the lowest concentration of an analyte in a sample that can be determined with acceptable precision and accuracy under the stated operational conditions of the method. SD refers to the standard deviation and %RSD refers to the relative standard deviation.

Table A.4: The lower limit of detection and quantification (LLOD and LLOQ) of pravastatin

	Concentration (µg/ml)	
	0.0253	**0.5050**
Area	4.38	97.62
	6.12	99.54
	5.54	101.23
	4.75	100.51
	5.39	97.77
	4.26	101.78
Mean	5.07	99.74
SD*	0.67	1.60
%RSD**	13.12	1.61

LLOD must yield a percentage more than 10% and LLOQ more than 1%. Both LLOD and LLOQ produced acceptable %RSD values of 13.2% and 1.61%, respectively.

A.4.2 Accuracy

The accuracy of an analytical method expresses the closeness of agreement between the value which is accepted either as a conventional true value, or an accepted reference value and the value found. Since this process does not include any sample preparation, accuracy and precision can only be completed by preparing a set of standards and comparing it against another set of standard solutions.

A.4.2.1 Accuracy analysis

1. Weigh approximately 8 mg of pravastatin into a 100 ml volumetric flask and dissolve in PBS (pH 7.4).
2. Transfer 10 ml of this solution into a 20 ml volumetric flask and fill up to volume with PBS (pH 7.4).

3. Transfer 10 ml of the latter solution into a 20 ml volumetric flask and fill to volume with PBS (pH 7.4).
4. Transfer 5 ml of the latter solution into a 20 ml volumetric flask and fill to volume with PBS (pH 7.4).

This will yield solutions containing approximately 5, 20, 40 and 80 µg/ml of pravastatin. These solutions should then be transferred into auto sampler vials and analysed for accuracy experiment against a standard solution (see Section A.3.1). This experiment will be done in quantenar.

Table A.5: Accuracy results of pravastatin

Concentration spiked	**Peak area**			**Recovery**	
µg/ml	**Area 1**	**Area 2**	**Mean**	**µg/ml**	**%**
5.26	715.1	714.9	715.0	5.36	101.92
5.25	710.7	711.6	711.2	5.33	101.50
5.16	672.9	674.6	673.8	5.05	97.91
21.03	2858.9	2868.6	2863.8	21.46	102.06
21.00	2841.7	2846.8	2844.2	21.31	101.48
20.63	2755.2	2757.5	2756.3	20.65	100.13
42.05	5669.7	5667.6	5668.7	42.47	101.01
42.00	5623.7	5611.6	5617.7	42.09	100.22
41.25	5455.3	5464.4	5459.9	40.91	99.17
84.10	11272.0	11273.0	11272.5	84.46	100.43
84.00	11158.0	11162.0	11160.0	83.62	99.55
82.50	10964.0	10961.0	10962.5	82.14	99.56

Table A.6: Statistical analysis results of pravastatin

Statistical analysis	
Mean	100.41
SD	1.19
%RSD	1.19
95% confidence intervals	
Lower limit	100.83
Upper limit	98.61
Estimated median	99.48
Confidence level (95.0%)	0.55

Recovery must be between 98 to 102%. Over the range of 5 to 85 µg/ml the method yielded a mean recovery of 100.41%.

A.4.3 Precision

The precision (variability) of an analytical technique is usually expressed as the standard deviation (S), variance (S2), or coefficient of variation (%RSD) of a sequence of measurements. It expresses the proximity of agreement between a series of measures obtained from multiple sampling of the same homogeneous substance under the prescribed conditions. Precision was investigated by the terms repeatability (intra-day) and reproducibility (inter-day).

A.4.3.1 Intra-day precision (repeatability)

Repeatability states the precision (spread of the data, variability) under similar operating conditions over a short interlude of time. Repeatability more known as intra-assay precision and is prepared as follows:

1. Prepare three samples each of low, medium and high concentration (n = 9).
2. Prepare a set of standards as described under sample preparation.
3. Inject into the HPLC in duplicate.

Table A.7: Repeatability results of pravastatin

Concentration spiked	**Peak area**			**Recovery**	
µg/ml	**Area 1**	**Area 2**	**µg/ml**	**µg/ml**	**%**
30.90	4189.4	4139.9	4164.6	31.20	100.99
29.40	3993.9	3894.5	3944.2	29.55	100.52
27.32	3773.8	3658.1	3716.0	27.84	101.91
54.08	7355.2	7382.5	7368.8	55.21	102.10
51.45	6985.3	6910.2	6947.7	52.06	101.18
47.78	6541.5	6567.3	6554.4	49.11	102.78
77.25	10323.2	10625.8	10474.5	78.48	101.60
73.50	9928.1	9820.1	9874.1	73.98	100.66
68.30	9284.3	9202.5	9243.4	69.26	101.40
				Mean	101.46
				SD	0.69
				%RSD	0.68

Precision should yield a percentage up to 2%. Precision was satisfactory with a %RSD of 0.68%.

A.4.3.2 Inter-day precision (reproducibility)

Inter-day precision must be 5% or less. The inter-day and the intra-day %RSD total values of 0.64% and 0.68% means reproducibility was within acceptable limits as shown in Table A.7 and Table A.8.

Table A.8: Reproducibility results of pravastatin

	Day 1	Day 2	Day 3	Between days
	102.10	102.37	100.14	
	101.18	100.78	100.11	
	102.78	100.78	99.97	
Mean	102.02	101.31	100.08	101.14
SD	0.66	0.75	0.07	0.99
%RSD	0.64	0.74	0.07	0.98

A.4.4 Ruggedness

A.4.4.1 Sample stability

A sample was left on the auto sampler tray and re-analysed over several time intervals to determine stability.

Sample solutions should not be used for a period extensive than it takes to degrade by 2% and in this case, distinctive provisions should be followed to compensate for the degradation. The pravastatin sample solution was stable over a period of 24 h with only a 0.85% variation in concentration over this period.

Table A.9: Results of sample stability of pravastatin

Time (h)	Peak area	%Remaining
0	2080.07	100.0
1	2092.50	100.6
2	2064.45	99.2
3	2051.82	98.6
4	2053.02	98.7
5	2051.43	98.6
6	2039.45	98.0
7	2040.79	98.1
8	2053.41	98.7
9	2009.65	96.6
10	2035.69	97.9
11	2042.49	98.2
12	2044.11	98.3
13	2035.59	97.9
14	2039.45	98.0
15	2036.64	97.9
16	2030.15	97.6
17	2026.27	97.4
18	2026.57	97.4
19	2033.57	97.8
20	2040.50	98.1
21	2025.89	97.4
22	2029.77	97.6
23	2026.96	97.4
24	2034.12	97.8
Mean	2041.80	98.20
SD	17.33	0.83
%RSD	0.85	0.85

A.4.4.2 System repeatability

A sample was injected six times in order to test the repeatability of the peak area as well as the retention time.

Table A.10: Results of system repeatability of pravastatin

	Peak area	Retention time (min)
	5720.4	4.009
	5725.3	4.003
	5722.1	4.012
	5716.8	4.013
	5710.7	4.003
	5741.8	4.012
Mean	5722.9	4.009
SD	9.63	0.004
%RSD	0.17	0.105

The peak area and retention times should have a %RSD of 2% or less. System performance proved to be well within the acceptable range, with %RSD values of 0.17% for peak area and 0.11% for retention time, respectively.

A.4.5 Specificity

Specificity is the capability to assess a clear analyte in the presence of components, which may be anticipated to be present. Typically these might include impurities and excipients. The specificity method is prepared as follows:

1. Prepare a placebo by filling a vial with PBS (pH 7.4) buffer.
2. Inject in duplicate.
3. Dilute a standard solution by adding 100 µl of water, 2.0 M hydrochloric acid, 2.0 M sodium hydroxide and 10% hydrogen peroxide to 1 ml of standard and mix by vortexing.
4. Store these solutions overnight in closed test tubes at room temperature to degrade.
5. Inject the samples into the HPLC with a run time of 10 min.
6. Examine the chromatograms to determine whether any additional peaks were formed.

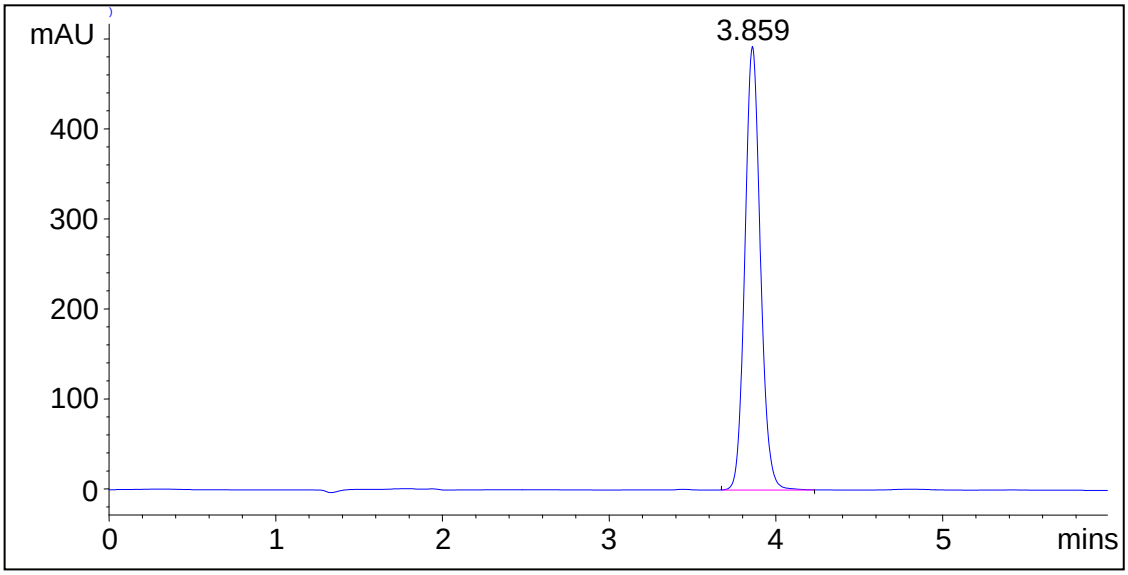

Figure A.2: HPLC chromatogram of the standard solution of pravastatin

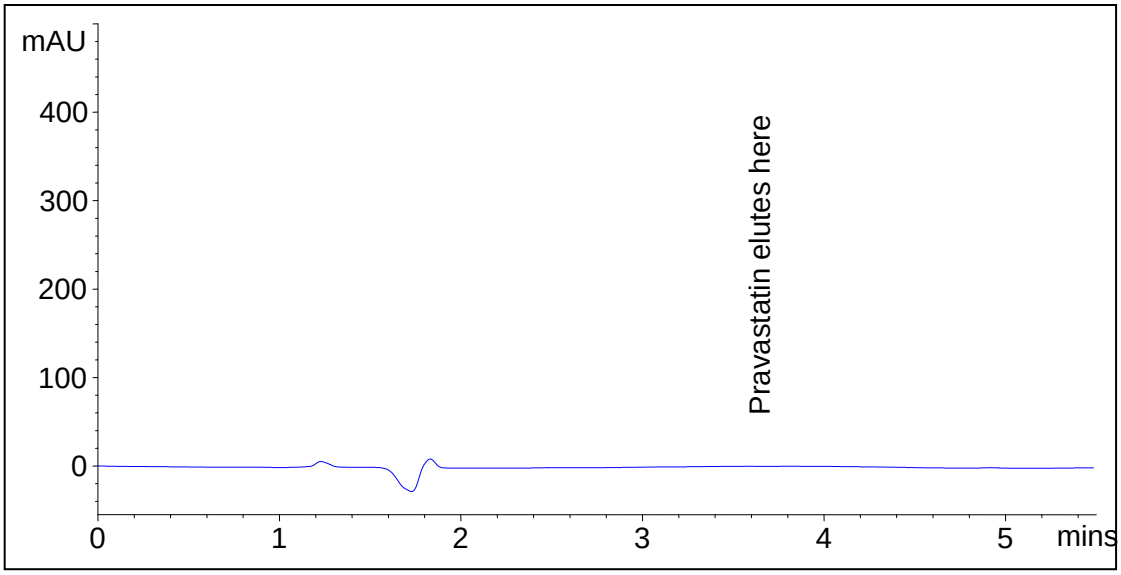

Figure A.3: HPLC chromatogram of the placebo

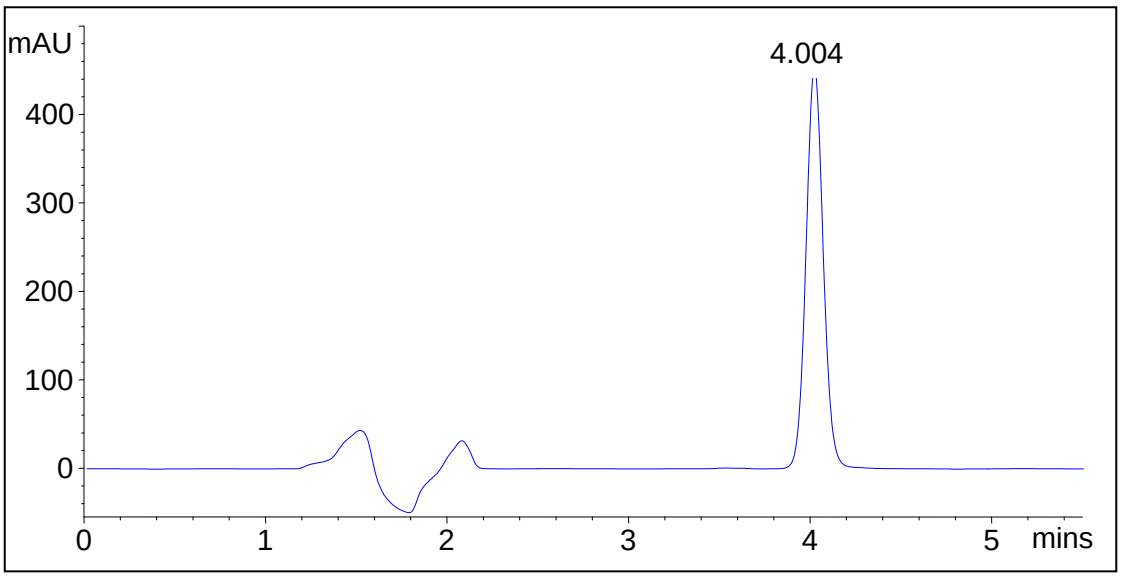

Figure A.4: HPLC chromatogram of the sample solution stressed in water at 40 °C for 24 h

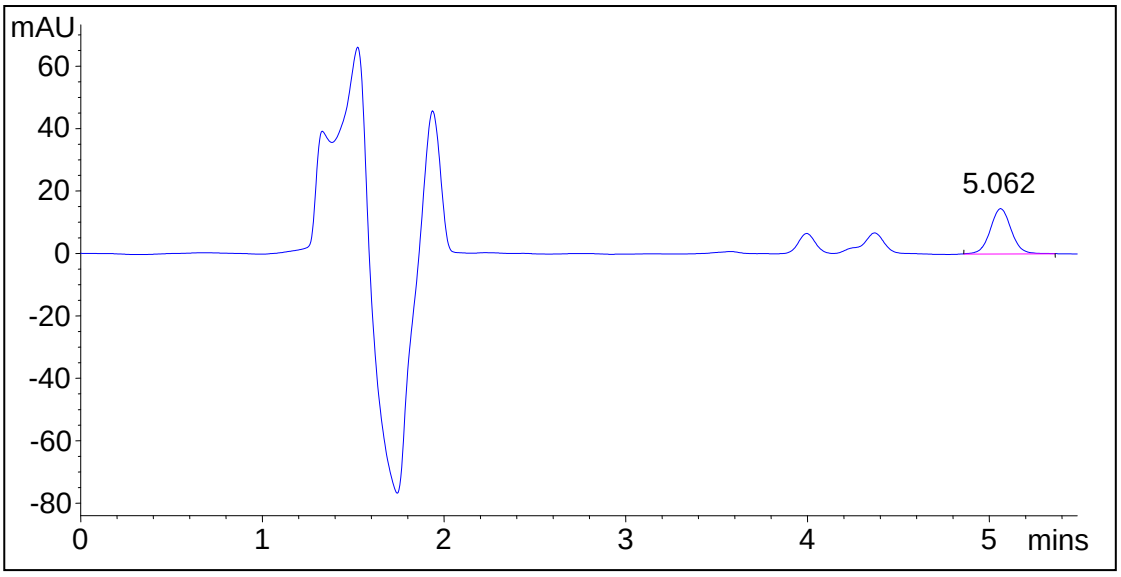

Figure A.5: HPLC chromatogram of the sample solution stressed in 0.1 M hydrochloric acid at 40 °C for 24 h

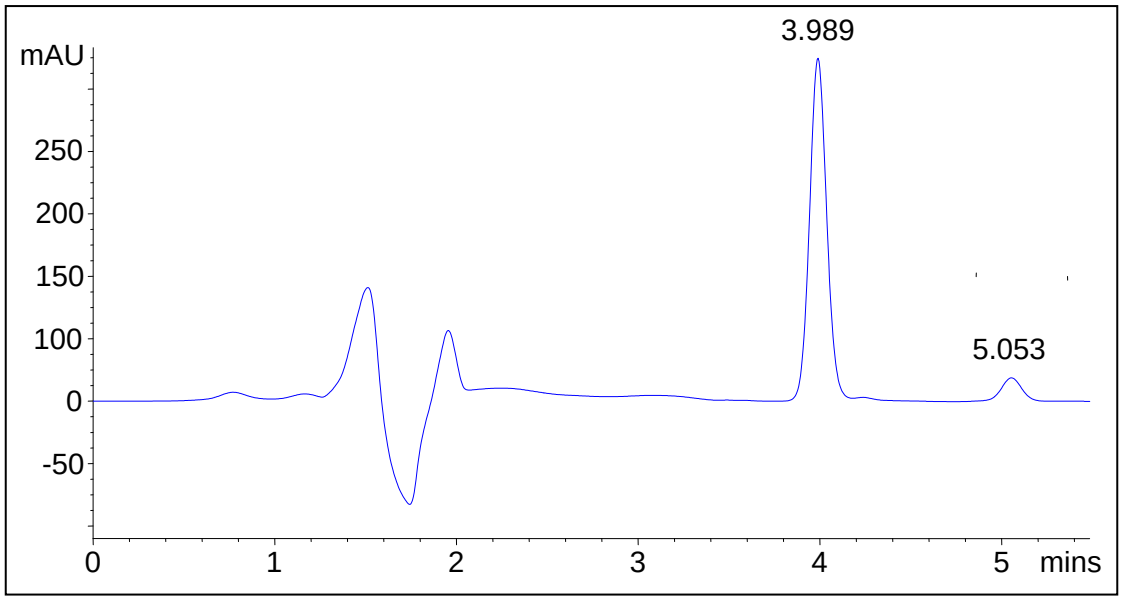

Figure A.6: HPLC chromatogram of the sample solution stressed in 0.1 M sodium hydroxide at 40 °C for 24 h

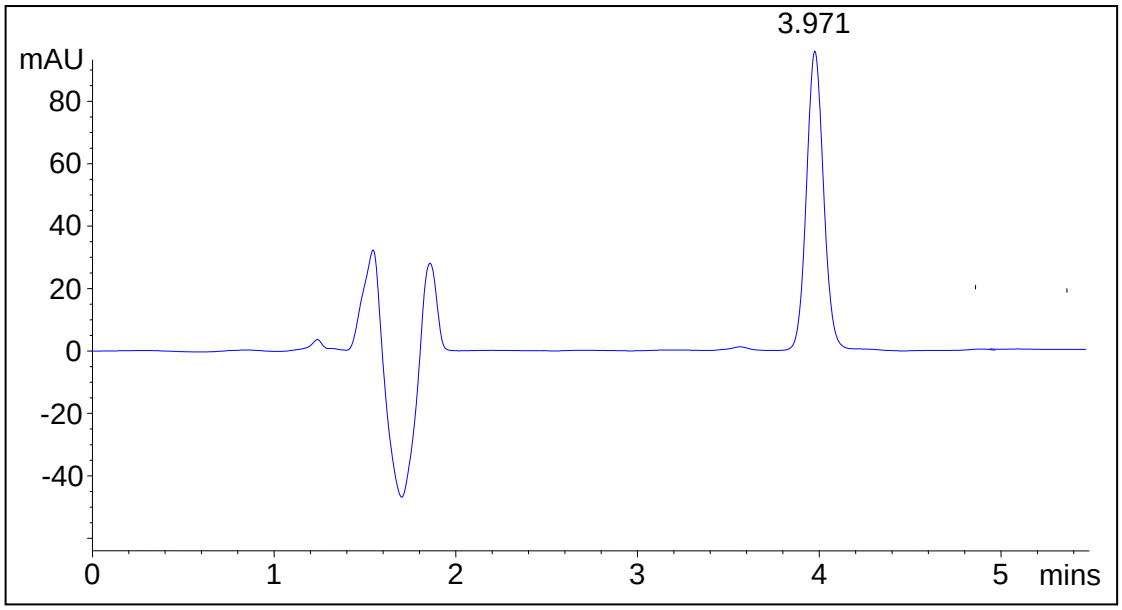

Figure A.7: HPLC chromatogram of the sample solution stressed in 10% hydrogen peroxide at 40 °C for 24 h

The degraded samples should not enclose any peaks that will hinder the determination of pravastatin. The placebo should not affect the pravastatin and none of the ingredients in the placebo (Figure A.3) affected the analyte peak. Additional peaks formed throughout forced degradation (shown in Figures A.5, A.6 and A.7) did not obstruct the remainder of the pravastatin peak. Peak purity testing of the remaining peaks after forced degradation in water

was tested. Figure A.4 showed the peak was still pure, thus proving the method is stability-indicating.

A.5 Conclusion

The method performed well and should be appropriate to analyse pravastatin in diffusion samples in addition to stability testing, quality control and batch release purposes. No interference came across from stressed samples or known related substances, thus the method can be considered stability-indicating.

References

ICH ***see*** INTERNATIONAL CONFERENCE ON HARMONISATION.

International Conference on Harmonisation of technical requirements for registration of pharmaceuticals for human use (ICH). 1995. Guideline for industry: text on validation of analytical procedures, ICH-Q2A. 8p. Mar.

International Conference on Harmonisation of technical requirements for registration of pharmaceuticals for human use (ICH). 1996. Validation of analytical procedures: methodology, ICH-Q2B. 8p. Nov.

Appendix B:

Formulation of a semisolid cream and emulgel with pravastatin as an active ingredient and the implementation of the delivery gap theory

B.1 Introduction

The objective of this study was to use Professor Johann Wiechers' computer programme, FFE™, to formulate six preparations with different polarities, of which three were semisolid creams and three were emulgels, containing pravastatin as the API. One semisolid cream and emulgel formula was optimised towards the stratum corneum, one semisolid cream and emulgel formula had increased polarities and one semisolid cream and emulgel formula had decreased polarities, when compared to the optimised preparation. These formulations were then used to determine the transdermal delivery and release of the API.

The FFE™ programme calculated the optimal composition of the oil phase of both the cream and emulgel formula containing 2% pravastatin. The concentration is near the maximum solubility, calculated by using the unionised species, which optimises the efficacy of the API (Wiechers, 2011).

Prof J Wiechers developed a theory which can be described as the ratio between the necessary concentration of an API and the definite concentration which can be delivered from the particular formulation. The SDG was consequently incorporated into the FFE™ database as a new feature to estimate the ratio.

B.2 The function of FFE™ computer programme

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 (δ_h)
- the energy from dipolar intermolecular force between molecules (δ_p),
- the energy from dispersion bonds between molecules (δ_d) (Wiechers, 2011).

The three dimensional (3-D) representation of δ_d, δ_p and δ_h is described as the HSP. According to Hansen, the parameters of the skin's solubility are 17, 8 and 8, respectively. The positions of the HSPs of the API, formulation and skin are represented by spheres in their certain positions. In the Hansen space, these three parameters serve as co-ordinates for a point (Wiechers, 2011). The HSP values of the skin, pravastatin and the three formulations used for this study are shown in Table B.1.

Table B.1: The Hansen solubility parameters of pravastatin, the formulations and the skin

	δ_d	δ_p	δ_h
Skin	17.0	8.0	8.0
Pravastatin	16.7	8.6	11.5
Optimised cream/emulgel	17.1	7.6	8.5
Hydrophilic cream emulgel	17.0	8.5	12.4
Lipophilic cream/emulgel	17.0	6.5	7.5

The FFE™ programme allows the user to choose one of three options by which the skin delivery of the API can be optimised in a certain formula (Wiechers, 2011):

- Optimise towards the skin - the HSPs of the formulation must match those of the stratum corneum, depending on the physicochemical properties of the API and skin thickness. This option declares skin permeation of large quantities of the API.
- Optimise towards the API - the HSPs of the formulation must match those of the API, therefore the API must be dissolved as much as possible.
- Optimise towards the target concentration (TC) - the concentration of the API in the chosen formula is close to the maximum solubility limit, so that the maximum driving force for the API is reached to enter the skin and leave the formulation (Wiechers, 2011).

When optimising skin delivery towards the skin, the skin formulation gap (SFG) is minimised, therefore the spheres representing the skin and the formulation are brought as close together as possible. Smaller HSP values are associated with lipophilic ingredients, the ratio for example being 17.0:5.4:3.6 and higher HSP values are associated with more hydrophilic ingredients, ratio being 17.0:10.5:16.8 when compared to the skin ratio 17.0:8.0:8.0.

B.3 Development programme for the formulation of products

Research of product development includes developing products and the acquired packaging such as containers. By extensive investigation of quality characteristics such as stability, safety, efficacy and usability is required for the specifications for ingredients, packaging and a manufacturing method (Mitsui, 1997:7).

B.3.1 Formulation of products

Defining the product requirements before the development stage minimises the cost and the time to market (Radd, 1994:50). Defining areas that of importance include: product form, product vision, critical ingredients, essential ingredients, product performance, product cost and regulatory requirements. An idea must exist for the forthcoming product or line of product which is relative to the other products in the category. Product development will depend on the type of product which will be formulated. Each form requires different processing needs as well as different key ingredients. Essential ingredients indicate the technologies around which the product will be developed, whereas critical ingredients are those that are necessary to create the products such as penetration enhancers or thickeners. Ingredients which should be avoided should also be identified. A project timetable should be established and drafted to identify cost and timing milestones so reformulation can be minimised and turnaround time for testing and purchasing can be optimised. Typical product use conditions should be specified as well as efficacy and sensory product attribute. The regulatory requirements convey the minimum stability, expiry date, minimum/maximum active levels, ingredients restrictions and claim requirements (Radd, 1994:51).

B.3.2 Pre-formulation

Pre-formulation consist of studies that should be carried out before formulation development is commenced. The primary goal of this process is to allow rational development of safe, stable, efficacious dosage forms and is mainly concerned with characterisation of the physicochemical properties of the API. Protocols must cover all required aspects at the pre-formulation stage (Waters and Brain, 2002:321). The pre-formulation stage has distinct phases and these tasks are as follows: general description of the compound, calorimetry, polymorphism, hygroscopicity, analytical development, intrinsic development, solubility and partitioning characteristics and drug delivery characteristics (Walter and Brain, 2002:322).

B.3.3 Early formulation

Existing formulas were taken during early formulation and changed as necessary by using a trial-and-error approach.

B.3.4 Final formulation

The final formulation was chosen after the oil phase was optimised by the FFE™ computer programme and the correct formula made. The products were then formulated in bulk for membrane studies and diffusion studies.

B.3.5 Preservation of pharmaceutical products

Substances such as glycerine and sorbitol which provide a source of carbon for micro-organisms and substances such as amino acid derivatives and proteins, which can be a source of nitrogen, are found in pharmaceuticals. Pharmaceutical products can be contaminated by fungi, bacteria and other micro-organisms by the above mentioned substances. Bacterial contamination and product deterioration can be prevented by adding preservatives to cosmeceuticals. It is mainly bacteria which contaminate cosmeceuticals, but fungi and yeasts are also known to do so (Mitsui, 1997:199). Microbiostasis is known as the suppression of the proliferation of micro-organisms. Typical preservatives such as parabens are used in cosmeceuticals (Mitsui, 1997:201).

The characteristics of preservatives are as follow:

- efficacy against many species of micro-organisms;
- water solubility or easy dissolution in commonly used cosmetic ingredients;
- neutral with no effect on product pH;
- no reduction of product ingredient effectiveness;
- no adverse effect on product appearance;
- stability over wide temperature and pH range;
- readily available and stable supply;
- low in price and economical to use;
- high safety;
- no irritation (Mitsui, 1997:202).

B.4 Formulation of semisolids

B.4.1 Formulation of a cream

Creams are like lotions and have been generally used throughout history. There is a great variety of creams made today due to technology advancements in surface chemistry, development of pharmaceutical products and higher level production techniques (Mitsui, 1997:341).

B.4.1.1 Purpose and function of a cream

Creams are a type of emulsion where two liquids such as water and oil, which do not mix together, are made into a stable dispersion. This is accomplished by making a liquid into a

dispersion phase and dispersing it through the other liquid which will be the dispersion medium. The main function of creams is to keep skin moist and supple through the supply of water, oil and humectants as well as maintain the moisture balance of the human skin (Mitsui, 1997:342).

B.4.1.2 Main ingredient of a cream

Creams mainly consist of aqueous ingredients, oily ingredients, preservatives, surfactants, chelating agents, perfumes and a pharmaceutical agent. Creams can either be oil in water (O/W) or water in oil (W/O) emulsions depending on the oily ingredients and surfactants. When the cream is O/W, hydrophilic surfactants are used, whereas W/O emulsions consist of a bigger oil phase (Mitsui, 1997:343). The main components of creams are shown in Table B.2.

Table B.2: Main ingredients of a cream (Mitsui, 1997:344).

Components	Typical raw material
Oil phase components	Hydrocarbons – liquid paraffin, etc. Fats and oils – olive oil, almond oil, etc. Waxes – beeswax, lanolin, etc. Fatty acids – stearic acid, oleic acid, etc. Higher alcohols – cetanol, stearyl alcohol, etc. Synthetic esters – isopropyl myristate, glycerine trimester, etc. Others – silicone oil, polysiloxane, etc.
Water phase components	Humectants – glycerine, propylene glycol etc. Thickening agents – quince seeds, pectin etc. Alcohols – ethanol, isopropyl alcohol Purified water – ion exchange water
Surfactants	Non-ionic – glycerine monostearate, sorbitan fatty acid esters, etc. Anionic – fatty acid soaps, sodium alkyl sulphate, etc.
Others	Alkalis – potassium hydroxide, sodium hydroxide, etc. Perfumes Colourants – pigments Chelating agent – ethylenediaminetetraacetic acid (EDTA) Preservatives – parabens, sorbic acid, etc. Antioxidants – vitamin E, etc. Buffers – citric acid, sodium citrate, etc. Pharmaceutical agents – vitamins, UV absorbents, etc.

B.4.1.3 General method for manufacturing a cream

To make the water phase, humectants and other water phase ingredients are added to purified, distilled water and subsequently heated to 70 °C. A solution of the solid oils, liquid oils, semi-solid oils, antioxidants and preservatives is prepared by heating and stirring this at 70 °C. Perfume can also be stirred into the oil phase before emulsification takes place. The oil phase is then added to the water phase then mixed with a homogeniser to make the emulsion uniform. This method is used for an O/W emulsion type cream (Mitsui, 1997:343). A W/O type cream is manufactured by adding the water phase gradually to the oil phase, where the preliminary emulsification is carried out, the process is then the same as that for the O/W (Mitsui, 1997:344).

When a cream is manufactured, quality characteristic values can be affected by processes such as:

- The cooling process
- The process for emulsion particle size adjusting

In the cooling process, a surface heat exchanger is used which is important to obtain a stable quality cream, so care must be given to the setting of the cylinder rotation speed as well as the final temperature (Mitsui, 1997:345).

B.4.2 Formulation of a gel

A gel can be defined as a two-compartment system. It has a semi-solid nature, which is high in liquid, with a continuous structure that provides a solid-like property. A natural or synthetic polymer in a typical polar gel, at low concentration, builds a three-dimensional matrix throughout a hydrophilic liquid. The gel can either be clear or turbid, depending on the gelling agent that does not fully dissolve and disperses the light when it forms aggregates (Barry, 1983:300).

B.4.2.1 Purpose and function of a gel

Gels and jellies consist of a base which produces a uniform external appearance, which can range from semi-transparent to transparent and give a moist feeling. Aqueous gels are mostly used in summer because of the light, cool and moist feeling. Oily gels can be applied together with lotion to supply oil to the skin and are used as a winter product because of their moisturising properties, for dry skin (Mitsui, 1997:351).

B.4.2.2 Main ingredients of a gel

Aqueous gels usually contain water soluble polymer substances with gelling abilities such as methyl cellulose or carboxyvinyl polymer. The gel consists of a gel base to which humectants,

preservatives, surfactants, colouring agents, pharmaceutical agents and perfumes are added. Oily gels have a liquid crystal structure as well as gelling ability. When a gel formula is made, careful decisions must be made considering ingredients for preservation, stability and ease of use (Mitsui, 1997:352).

B.4.2.3 General method for manufacturing a gel

It is necessary to carefully select equipment for making gels because of the high viscosities. The equipment must be capable of removing air bubbles, uniform mixing as well as suited to filtering, transportation and cooling of high viscosity substances. The raw materials must consist of good dissolution properties (Mitsui, 1997:353).

B.5 Formulation of a pravastatin containing cream and emulgel during this study

Table B.3: Ingredients, suppliers and batch numbers

Ingredient	Supplier	Batch number
Pravastatin	DB Fine	0651001
Ultrez 20	Lubrizol	8x2z7211
Cetyl alcohol	Merck	5513404018
MCK	Croda	498133
Isopropyl myristate	Merck	5499302826
Stearic acid	Saarchem	5872120
Veegum	R.T Vanderbilt Company, Inc.	12199370
Mineral oil (light)	Merck	1034378
Ethanol	Merck	1042281
Polyethylene glycol 400	Saarchem	5040100
Span 60	Merck	5361721034
Tween 80	Merck	1042689
Glycerine	Sigma	2002895
Dimethyl isosorbide	Croda	0000744889

The purpose of this study was to formulate pravastatin in an optimised cream, lipophilic cream and hydrophilic cream, as well as an optimised emulgel, lipophilic emulgel and hydrophilic emulgel. Different formulations were prepared and the best were selected on grounds of stability, appearance and homogeneity.

The ingredients chosen are listed in Table B.3, as well as the supplier and batch number.

B.5.1 Formulation of a pravastatin containing cream during this study

B.5.1.1 Formula of pravastatin cream (optimised)

The formula of the optimised pravastatin cream is given in Table B.4.

Table B.4: Pravastatin optimised cream

Ingredients		%m/m	Activity
A	Stearic acid	4.0%	Thickening agent
	Cetyl alcohol	0.5%	Thickening agent
	MCK	1.7%	Emulsifier
	Span 60	0.4%	Emulsifier
B	Dimethyl isosorbide	13.0%	Penetration enhancer
	Polyethelene glycol 400	5.0%	Oil phase of emulsion
C	Glycerine	10.0%	Humectant
	Tween 80	3.2%	Surface active agent
	Veegum	0.5%	Thickening agent
	Pravastatin	2.0%	API
	dH_20	60.0%	Solvent

The procedure to prepare the pravastatin optimised cream was as follows:

- Add pravastatin to water and stir
- Add other ingredients of phase C to water and heat to 70 to 75 °C
- Add phase A together and heat to 70 to 75 °C
- Add phase B together and heat to 70 to 75 °C
- Add phase B to C while stirring
- Add water phase to phase A while stirring
- Homogenise mixture at 9500 rpm for 5 mins
- Stir to cool

B.5.1.2 Formula of pravastatin cream (lipophilic)

The formula of the lipophilic pravastatin cream is given in Table B.5.

Table B.5: Pravastatin lipophilic cream

Ingredients		%m/m	Activity
A	Stearic acid	4.0%	Thickening agent
	Cetyl alcohol	0.5%	Thickening agent
	MCK	1.7%	Emulsifier
	Span 60	0.4%	Emulsifier
B	Dimethyl isosorbide	13.0%	Penetration enhancer
	Mineral oil	5.0%	Oil phase of emulsion
C	Glycerine	10.0%	Humectant
	Tween 80	3.2%	Surface active agent
	Veegum	0.5%	Thickening agent
	Pravastatin	2.0%	API
	dH_20	60.0%	Solvent

The procedure to prepare pravastatin lipophilic cream was as follows:

- Add pravastatin to water and stir
- Add other ingredients of phase C to water and heat to 70 to 75 °C
- Add phase A together and heat to 70 to 75 °C
- Add phase B together and heat to 70 to 75 °C
- Add phase B to C while stirring
- Add water phase to phase A while stirring
- Homogenise mixture at 9500 rpm for 5 mins
- Stir to cool

B.5.1.3 Formula of pravastatin cream (hydrophilic)

The formula of the hydrophilic pravastatin cream is given in Table B.6.

Table B.6: Pravastatin hydrophilic cream

Ingredients		%m/m	Activity
A	Stearic acid	4.0%	Thickening agent
	Cetyl alcohol	0.5%	Thickening agent
	MCK	1.7%	Emulsifier
	Span 60	0.4%	Emulsifier
B	Dimethyl isosorbide	13.0%	Penetration enhancer
	Water	2.4%	Solvent
	Ethanol	2.6%	Penetration enhancer
C	Glycerine	10.0%	Humectant
	Tween 80	3.2%	Surface active agent
	Veegum	0.5%	Thickening agent
	Pravastatin	2.0%	API
	dH_20	60.0%	Solvent

The procedure to prepare pravastatin hydrophilic cream was as follows:

- Add pravastatin to water and stir
- Add other ingredients of phase C to water and heat to 70 to 75 °C
- Add phase A together and heat to 70 to 75 °C
- Add phase B together and heat to 70 to 75 °C
- Add phase B to C while stirring
- Add water phase to phase A while stirring
- Homogenise mixture at 9500 rpm for 5 min
- Stir to cool

B.5.1.4 Outcome

All three cream formulations were not too oily and applied easily and also had a homogeneous white texture with a soft feel.

B.5.2 Formulation of pravastatin containing emulgel during this study

B.5.2.1 Formula of pravastatin emulgel (optimised)

The formula of the optimised pravastatin emulgel is given in Table B.7.

Table B.7: Pravastatin optimised emulgel

Ingredient		%m/m	Activity
A	Cetyl alcohol	1.5%	Thickening agent
	MCK	1.7%	Emulsifier
	Isopropyl myristate	1.0%	Emulsifier
B	Dimethyl isosorbide	13.0%	Penetration enhancer
	Polyethelene glycol 400	5.0%	Oil phase of emulsion
C	Ultrez 20	0.8%	Gelling agent
	Pravastatin	2.0%	API
	Water (dH_2O)	75.0%	Solvent

The procedure to prepare pravastatin optimised emulgel was as follows:

- Add phase C together, stir and heat to 70 °C
- Add phase A together and heat to 70 °C
- Add phase B together and heat to 70 °C
- Add phase B to C while stirring
- Add water phase to oil phase while stirring
- Homogenise at 9500 rpm for 1 min
- Stir to cool

B.5.2.2 Formula of pravastatin emulgel (lipophilic)

The formula of the lipophilic pravastatin emulgel is given in Table B.8.

Table B.8: Pravastatin lipophilic emulgel

Ingredient		%m/m	Activity
A	Cetyl alcohol	1.5%	Thickening agent
	MCK	1.7%	Emulsifier
	Isopropyl myristate	1.0%	Emulsifier
B	Dimethyl isosorbide	13.0%	Penetration enhancer
	Mineral oil	5.0%	Oil phase of emulsion
C	Ultrez 20	0.8%	Gelling agent
	Pravastatin	2.0%	API
	Water (dH_2O)	75.0%	Solvent

The procedure to prepare pravastatin lipophilic emulgel was as follows:

- Add phase C together, stir and heat to 70 °C
- Add phase A together and heat to 70 °C
- Add phase B together and heat to 70 °C
- Add phase B to C while stirring
- Add water phase to oil phase while stirring
- Homogenise at 9500 rpm for 1 min
- Stir to cool

B.5.2.3 Formula of pravastatin emulgel (hydrophilic)

The formula of the hydrophilic pravastatin emulgel is given in Table B.9.

Table B.9: Pravastatin hydrophilic emulgel

Ingredient		%m/m	Activity
A	Cetyl alcohol	1.5%	Thickening agent
	MCK	1.7%	Emulsifier
	Isopropyl myristate	1.0%	Emulsifier
B	Dimethyl isosorbide	13.0%	Penetration enhancer
	Water	2.4%	Solvent
	Ethanol	2.6%	Penetration enhancer
C	Ultrez 20	0.8%	Gelling agent
	Pravastatin	2.0%	API
	Water (dH_2O)	75.0%	Solvent

The procedure to prepare pravastatin hydrophilic emulgel was as follows:

- Add phase C together, stir and heat to 70 °C
- Add phase A together and heat to 70 °C
- Add phase B together and heat to 70 °C
- Add phase B to C while stirring
- Add water phase to oil phase while stirring
- Homogenise at 9500 rpm for 1 min
- Stir to cool

B.5.2.4 Outcome

All three emulgel formulations were not oily, applied easily and had a homogeneous white texture.

B.6 Physicochemical properties of pravastatin formulations

The following physicochemical properties from the six formulations were also tested and are as follows:

- pH
- Viscosity
- Droplet size
- Zeta-potential

B.6.1 pH

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

B.6.2 Viscosity

A Brookfield Viscometer (model DV II, Stoughton, Massachusetts, USA) was used to measure the viscosity of the six formulations by determining resistance to a rotating spindle that turns at a specific rate (measured in rpm), which is immersed in the formulation medium.

Formulations were placed in a water bath to reach room temperature (25 °C). The formulations were then removed and the spindle (Stoughton, MA) was placed in the formulation which in turn was put into the apparatus. Thereafter the rate was specified and the viscosity reading was measured every 10 sec for approximately 5 mins. The average viscosity was determined after 32 readings were obtained.

B.6.3 Droplet size

The Malvern Mastersizer 2000 was used to determine droplet size and is equipped with a wet cell Hydro 2000 MU dispersion unit (Malvern Instruments, Worcestershire, UK). Measurements were taken from six freshly prepared samples with three readings per sample.

B.6.4 Zeta-potential

The Malvern Zetasizer 2000 (Malvern Instruments, Worcestershire, UK) was used to define the

zeta-potential. Suitable pH zeta-potential measurements were taken from six prepared samples in triplicate.

Table B.10: Results of physicochemical properties of pravastatin formulations

	pH	Viscosity (cP)	Droplet size (µm)	Zeta-potential (mV)
Optimised cream	5.09	4818.65	44.375	-46.67
Hydrophilic cream	5.05	3546.26	29.705	-42.90
Lipophilic cream	5.01	3856.35	31.777	-56.03
Optimised emulgel	5.09	4064.10	55.295	-75.00
Hydrophilic emulgel	5.00	3843.54	32.214	-63.53
Lipophilic emulgel	5.07	4157.79	78.428	-77.37

B.6.5 Discussion of results

Droplet size of these emulsions, range from 10 nm to 10 µm (Wiechers, 2008:173). In this case, for topical delivery, accumulation of the molecule within the skin is preferred (Wiechers, 2008:174). It has also been reported that there is a relationship between the droplet size of the formulation and the permeability coefficient of a drug, where there is a change in total area of the dispersed phase which occurs when the size of the droplet changes (Wiechers, 2008:175). In most of the reports found in literature, it is not always possible that when the droplet size decreases, penetration increases, since emulsions differ not only in droplet size, but in system components or composition (Wiechers, 2008:177). Consequently, droplet sizes were not in range, but accumulation did take place in the stratum corneum. A compound ionised or unionised form depends on the pH. With respect to unionised form, ionised compounds have higher aqueous solubility, but lower permeability. Pravastatin molecules are dissociated at 33.39% at pH 5, where it can permeate through the skin (Admescope, 2012).

Stable zeta-potential values range from above 30 mV or below -30 mV (Malvern instruments, 2014). Results differ from -40 mV to -78 mV, which is in the acceptable range above 30 mv or below -30, indicating that formulas were stable.

B.7 Conclusion

Pravastatin was formulated into the different semi-solid formulations:

- 2% Optimised cream
- 2% Optimised emulgel
- 2% Lipophilic cream
- 2% Lipophilic emulgel

- 2% Hydrophilic cream
- 2% Hydrophilic emulgel

The FFE™ computer programme was used to formulate these six preparations with different polarities containing pravastatin as the API, respectively. The optimal composition of the oil phase was calculated by the programme and the formulations were made accordingly. The efficacy of the API was optimised by using the unionised species, where the concentration is near the maximum solubility (pH 5). All semisolids were stable as seen by the zeta-potential values, although droplet sizes were not in range.

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Appendix C:

Franz cell diffusion studies

C.1 Introduction

The skin, accountable for more or less 16% of body weight, is the heaviest most versatile organ of the body (Sanders *et al.*, 1999:168). The first layer of the skin, the stratum corneum, is a heterogeneous membrane, which controls absorption. The stratum corneum, only 15 to 20 mm thick, provides an adequate barrier which cannot be penetrated very easily (Hadgraft, 2001:1). Since the stratum corneum's barrier properties are excellent, it is of utmost importance to improve the bioavailability (Hadgraft, 2004:294). Permeation of the API (pravastatin) is a function of the physicochemical properties and its task of penetration mechanism must be understood to cross the stratum corneum (Hadgraft, 2004:294). Thus with knowledge of physicochemical and mechanistic properties, it makes it possible to design transdermal dosage forms when taking the formulation and the API into consideration (Hadgraft, 2004:294). Transdermal delivery, or more descriptively, transcutaneous delivery, includes topically administered drug dosage forms which are intended to let the API into the blood circulation, whilst the lipid dermatics only aim to penetrate the skin (Cevc *et al*., 1996:350).

A few advantages of transdermal drug delivery include, no frequent administration, prolonged delivery of drug, user friendly, delivery is easily terminated (Alexander *et al.,* 2012:27) and less variability (Perrie *et al.,* 2012:392). As mentioned, bioavailability needs to be improved and there are various ways to enhance permeation through the skin (Hadgraft, 2004:294) and into the circulation. Main mechanisms of enhancing skin penetration are: 1) interactions with the intercellular lipids, 2) interactions with the intercellular keratin and 3) improved dissolving capacity into the stratum corneum (Alexander *et al.,* 2012:30).

The aim of this study was to determine whether Prof Wiechers' Delivery Gap Principle was successful by enhancing formulations to better penetrate the skin by optimising the oil phase of the six formulations and to compare the different formulations according to polarity and penetration. Prior to the *in vitro* diffusion studies across human skin, membrane studies were performed with these six different formulations which were made by using the FFE™ programme.

C.1.1 Hansen solubility parameters

Charles Hansen developed HSP, which is a way to predict whether a material can dissolve in another to form a solution. Three Hansen parameters are awarded to each molecule which is measured in $MPa^{1/2}$ and they are: 1) δ_d – energy from dispersion forces between molecules 2) δ_p – energy from dipolar intermolecular force between molecules and 3) δ_h – energy from hydrogen bonds between molecules. The Hansen space is known as these three parameters, which are coordinates for a specific point in three dimensions. The closer these molecules are in Hansen's space, the more likely they are to dissolve (Hansen, 2007:4, 5).

C.2 Methods

C.2.1 Preparation of formulations

During this study six formulations were manufactured which consisted of an emulgel and cream formulation with different polarities, i.e. hydrophilic emulgel (HE), lipophilic emulgel (LE), optimised emulgel (OE), hydrophilic cream (HC), lipophilic cream (LC) and optimised cream (OC) (see Appendix B).

C.2.2 Preparation of PBS (pH 7.4)

Potassium dihydrogen orthophosphate (13.62 g) was weighed and dissolved in 500.0 ml HPLC water. Sodium hydroxide (3.15 g) was weighed and dissolved in 786.8 ml HPLC water according to the British Pharmacopoeia standards (BP, 2013a:1; BP, 2013b:1). The two aforementioned solutions were mixed and the pH was set to 7.4 with 10% orthophosphoric acid; the solution was filtered and degassed.

C.2.3 HPLC analysis of pravastatin

In conjunction with Prof Jan du Preez at the ATL, NWU, Potchefstroom Campus, South Africa, a HPLC method had been developed and validated. The HPLC (Agilent 1200 Series) was equipped with an Agilent 1200 pump and auto sampler injection mechanism, a diode array detector and ChemstationRev. A.10.01 software for data analysis (Agilent Technologies, Palo, Alto, CA). The UV-detector was set at a wavelength of 238 nm in order for pravastatin to be detected. The mobile phase consisted of acetonitrile (400 ml) and 0.1% orthophosphoric acid in HPLC water (600 ml), with a 50 µl as injection volume and flow rate of 1.0 ml/min. The runtime consisted of 6 min and the retention time was between 2 and 3 min. These analytical tests were performed in a laboratory with a controlled environment of 25 °C.

C.2.4 Physicochemical properties of pravastatin

C.2.4.1 Aqueous solubility

The water bath was preheated to 32°C (temperature on top of the skin during a diffusion study). A polytop was used and a magnetic stirrer was included; PBS (pH 7.4) was inserted into the polytop and additional pravastatin was added to ensure the solution was saturated at all times. This solution was mixed for 24h and regularly checked to see if it stayed saturated. After 24h the solution was removed and filtrated. If the solution concentration was too high, it was diluted. The solution was centrifuged at 5000 rpm for 10 min to ensure complete precipitation. The supernatant (1 ml) was diluted to 100 ml with PBS (pH 7.4) and the resultant solution analysed by HPLC. This experiment was repeated in triplicate.

C.2.4.2 Octanol-buffer distribution coefficient (log D)

PBS at pH 7.4 (100 ml) and equal volumes of *n*-octanol were equilibrated with each other for 24h in order for the two phases to co-saturate. The pre-saturated PBS phase was used as solvent for pravastatin sodium. Added to each of these pravastatin solutions was an equal volume of pre-saturated *n*-octanol (3 ml). Solutions was placed in the shaker water bath for 3h at 32°C and left overnight where they were centrifuged thereafter to ensure complete precipitation. Using HPLC, the concentrations of API in the separated phases was determined. The partition coefficient was calculated as the ratio of API concentration in the *n*-octanol phase to the buffer phase. This experiment was performed in quaternary. The logarithmic ratio of the concentration in the *n*-octanol phase to the concentration in the PBS (pH 7.4) was used to calculate the log D.

C.2.5 Diffusion experiments

C.2.5.1 Membrane release studies

Vertical Franz diffusion cells were used for release studies. Vacuum grease was applied to the donor (1 ml) and receptor (capacity of approximately 2 ml) compartments with a diffusion area of 1.075 cm^2 and a magnetic stirring rod placed into the receptor compartment. The hydrophilic polyvinylidene fluoride (PVDF) membrane filters (FP Vericel, 0.45 µm, 25 mm, Pall® was then placed on to the lower half of the Franz cell. Franz cell compartments (donor and receptor) were placed together and fastened with a horseshoe clamp. To avoid any loss of the constituents, the donor compartment was covered with Parafilm®. PBS (pH 7.4) was used to fill the receptor compartment and in the donor compartment the API formulation (HE, LE, OE, HC, LC and OC) at pH 5.0 was added. The cell systems were maintained at a temperature of 37 °C. The receptor compartments were extracted with a syringe every hour for 6h and the compartment refilled with PBS (pH 7.4) at 37 °C. The HPLC was used to analyse all the

samples. The concentration of the API which permeated through the membrane into the receiver fluid and the release rate of the API were determined for the formulation.

C.2.5.2 Skin preparation

Caucasian full-thickness abdominal skin (ethical approval reference number: NWU-00114-11-A5) was collected from plastic surgeons following abdominoplastic surgery. The skin was kept in a refrigerator at -20°C for no more than 24h to ensure it could be easily separated from the fatty layer. 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, then wrapped in aluminium foil and stored in the freezer at -20°C. Prior to diffusion studies, the frozen skin samples were thawed, visually examined for defects and mounted on the diffusion apparatus.

C.2.5.3 Skin diffusion

Dermatomed skin was positioned on the outer half of the diffusion cells with the stratum corneum facing the upper donor compartment. The receptor compartment was filled with a mixture of PBS (pH 7.4) and ethanol (90:10) at a temperature of 37°C. The formulation containing 2% pravastatin sodium was added to the donor compartment. The entire receptor volume was withdrawn after 12h and samples were analysed by means of the HPLC and the API concentration, which permeated through the skin into the receiver fluid, was determined (Baert *et al.,* 2011:472, 473).

C.2.5.4 Tape stripping

The tape stripping technique was used to determine the API concentration present in the stratum corneum-epidermis (SCE) and epidermis-dermis (ED) after the 12h diffusion studies were completed. 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, thereafter, the following 15 tape strips removed SCE and API until the skin glistened (Pellet *et al*., 1997:94). The strips were placed in a polytop, filled with 5 ml PBS (pH 7.4) with ethanol (90:10). The ED that was left after the procedure, was cut into smaller pieces to enhance the surface area (Pellet *et al.,* 1997:94) and placed in another polytop containing 5 ml PBS (pH 7.4) with ethanol (90:10). These solutions, containing the tape strips and skin pieces, were stored overnight at 4 °C, in order for the API to dissolve. SCE and ED samples were analysed by means of HPLC.

C.2.6 Data analysis

The flux, during membrane studies of pravastatin, was represented by the linear portion of the graph. By using the slope of the straight line where cumulative concentrative is compared over time, mean flux values were obtained. Membrane studies percentage released have been determined after 6h.

Each individual Franz cell, together with its mean amount per area of pravastatin, which permeated the skin after 12h was plotted for the different formulations during the diffusion studies. A percentage of the applied amount was expressed by using the yield of each cell, where the percentage diffused was also determined after 12h for diffusion studies.

C.2.7 Statistical analysis

Descriptive statistics consist of procedures, which involve the calculation of the mean (with standard deviation (SD) and median (middle score in distribution) of the flux values (membrane studies) as well as the concentration values (diffusion studies) (Sheskin, 2000:1, 4). Box-plots were used to illustrate data by using first and third quartiles of distribution, as well as the median values (Dawson and Trapp, 2004:38). These were drawn with the bottom as first quartile and top as third quartile, where the vertical straight lines (whiskers) which extended 1.5 times range above 25^{th} and below 75^{th} percentiles. The height of this box-plot illustrates the middle of data (50%). Values below or above the whiskers (outliers) are illustrated as circles (Dawson and Trapp, 2004:39). A two-way analysis of variance (ANOVA) was used in the membrane study 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.

P-values were determined by using the ANOVA. A p-value of 0.05 or less would indicate a statistical significant effect (Steyn *et al.,* 1994:604-606). Two one-way ANOVA's were used in the membranes studies to determine if there was any significant differences between the mean data values in the different polarity formulations, i.e. HE, LE, OE, HC, LC and OC (Sheskin, 2000:511). For unplanned comparisons the Tukey HSD (honestly significant difference) test was used, when in a set of data, possible comparisons were made (Sheskin, 2000:534). In the skin diffusion study, a two-way ANOVA was used where the formulations (cream and emulgel) were compared, the different polarity formulations (H, L and O) were compared, as well as the formulation (cream and emulgel) and different polarity formulation interaction were determined. Following this, mean concentration and tape stripping were compared by applying three-way ANOVA, where the different polarity formulations were compared within emulgel and cream formulations. The above statistical analyses were performed by means of Statistica (Statsoft, 2008) and SAS (SAS Institute Inc., 2005). A more exact method to determine flux or

concentration is the median; this is used when there is a significant variation between the mean and median values (Dawson and Trapp, 2004:30). Consequently, throughout this study we will make use of median to describe the data, although both mean and median values will be presented.

C.3 Results and discussion

C.3.1 Formulation of semi-solid products

Six formulations were made with a 2% pravastatin as API. These formulations consisted of three creams and three emulgels with different polarities such as hydrophilic, lipophilic and optimised formulations. All the formulations were not too oily, applied easily and had a homogeneous white texture.

C.3.2 Physicochemical properties

C.3.2.1 Aqueous solubility

Pravastatin's solubility was determined to be 197.5 mg/ml in PBS (pH 7.4) at a temperature of 32 °C. For a molecule to ideally permeate through the skin, it must have an aqueous solubility of more than 1 mg/ml (Naik *et al.,* 2000:319). Taking the aforementioned into account, pravastatin is expected to permeate well through the skin.

C.3.2.2 Octanol-buffer distribution coefficient (log D)

The log D should be between 1 and 3 (Naik *et al.,* 2000:319), which indicates the ability of a drug to dissolve both in oil and water, ensuring a compound would permeate the skin exceedingly quick (Brain *et al.*, 1998). Pravastatin's log D was determined to be -0.703, thus predicting permeation would not be optimal.

C.3.3 Diffusion experiment

C.3.3.1 Membrane release study

Six membrane studies were conducted and the mean and median flux, as well as the mean and median percentage diffused, was determined from the receptor compartments to establish whether pravastatin was released from all six formulations, after optimising the oil phase according to the FFE™ computer programme. Summary results obtained from the membrane release studies are shown in Table C.1 and comparative box-plots are represented in Figure C.1.

Table C.1: Mean and median flux (μg/cm^2.h) as well as mean and median percentage pravastatin released from six different formulations through membranes after 6h.

Formulations	Mean flux (μg/cm^2.h)	Median flux (μg/cm^2.h)	Mean %diffused (%)	Median %diffused (%)
OE	6.993 ± 1.254	7.175	0.113 ± 0.020	0.115
LE	5.850 ± 1.165	6.401	0.094 ± 0.019	0.103
HE	6.308 ± 0.584	6.355	0.102 ± 0.010	0.103
OC	4.230 ± 0.803	4.297	0.067 ± 0.012	0.068
LC	3.209 ± 0.518	3.115	0.051 ± 0.008	0.050
HC	4.981 ± 0.358	5.061	0.080 ± 0.006	0.082

In Table C.1 it is evident that the highest median flux values were obtained from OE (7.175 μg/cm^2.h), followed by LE (6.401 μg/cm^2.h), HE (6.355 μg/cm^2.h), HC (5.061 μg/cm^2.h), OC (4.297 μg/cm^2.h) and lastly, the CL (3.115 μg/cm^2.h).

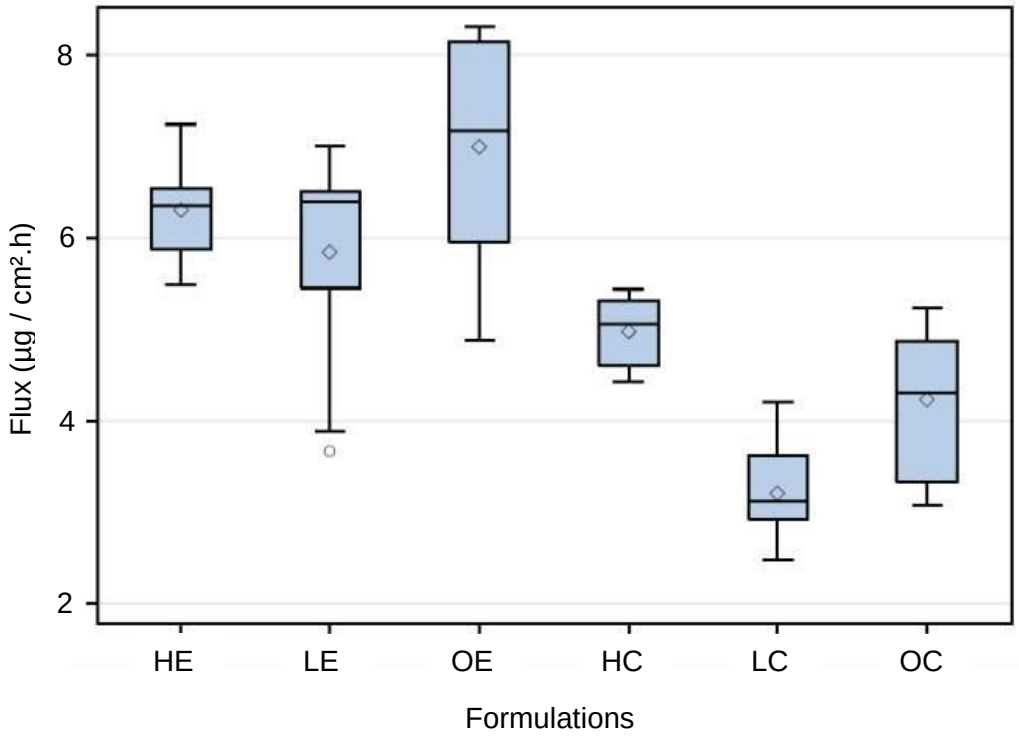

Figure C.1: Box-plots representing the flux values (μg/cm^2.h) of pravastatin of the different formulations in the membrane diffusion studies. The mean and median flux values are indicated by the diamond shape and line, respectively.

It is clear that all the emulgel formulations released pravastatin better than the cream formulations. When examining the median flux values, it was also observed that the LE formulation did not differ much from the HE formulation. For all the formulations the mean and median flux values did not differ much, except for LE. The mean flux values could give an inaccurate estimation of true flux values as they are influenced by distorted distributions around the central location, therefore median flux would better represent the true flux of pravastatin

from the different formulations, as the outliers do not affect the data (Dawson and Trapp, 2004:30).

C.3.3.2 Diffusion study

The mean and median amount per area diffused as well as the mean and median percentage diffused of pravastatin after 12h is depicted in Table C.2. The amount of pravastatin per area (µg/cm²) which permeated the skin was plotted for each Franz cell together with the mean of that formulation after 12h and can be seen in Figures C.2, C.3, C.4, C.5 and C.6. There was no flux values (µg/cm².h) obtained for the diffusion studies, since the flux values were too low when two hourly withdrawals were extracted. Only one extraction after 12h was taken for each individual Franz cell, which resulted in no flux values being obtained, only concentration (µg/cm^2) values. The mean and median amount per area (µg/cm^2) of pravastatin which diffused through the skin in different semi-solid formulations is illustrated in the box-plot in Figure C.7. In Table C.3, the HSP is included to illustrate the difference in the solubility parameters between the six formulations.

Table C.2: The mean and median amount per area (µg/cm²) as well as mean and median percentage diffused of pravastatin which permeated the skin after 12h.

Formulation	Mean amount per area diffused (µg/cm^2)	Median amount per area diffused (µg/cm^2)	Mean %diffused (%)	Median %diffused (%)
OE	2.443 ± 0.533	2.578	0.007 ± 0.001	0.007
LE	0.120 ± 0.036	0.121	3×10^{-4} ± 0.0001	3×10^{-4}
HE	0.064 ± 0.021	0.055	2×10^{-4} ± 0.0001	1×10^{-4}
OC	1.600 ± 0.632	1.449	0.004 ± 0.002	0.004
LC	0.000 ± 0.000	0.000	0.000 ± 0.000	0.000
HC	0.429 ± 0.132	0.434	0.001 ± 0.000	0.001

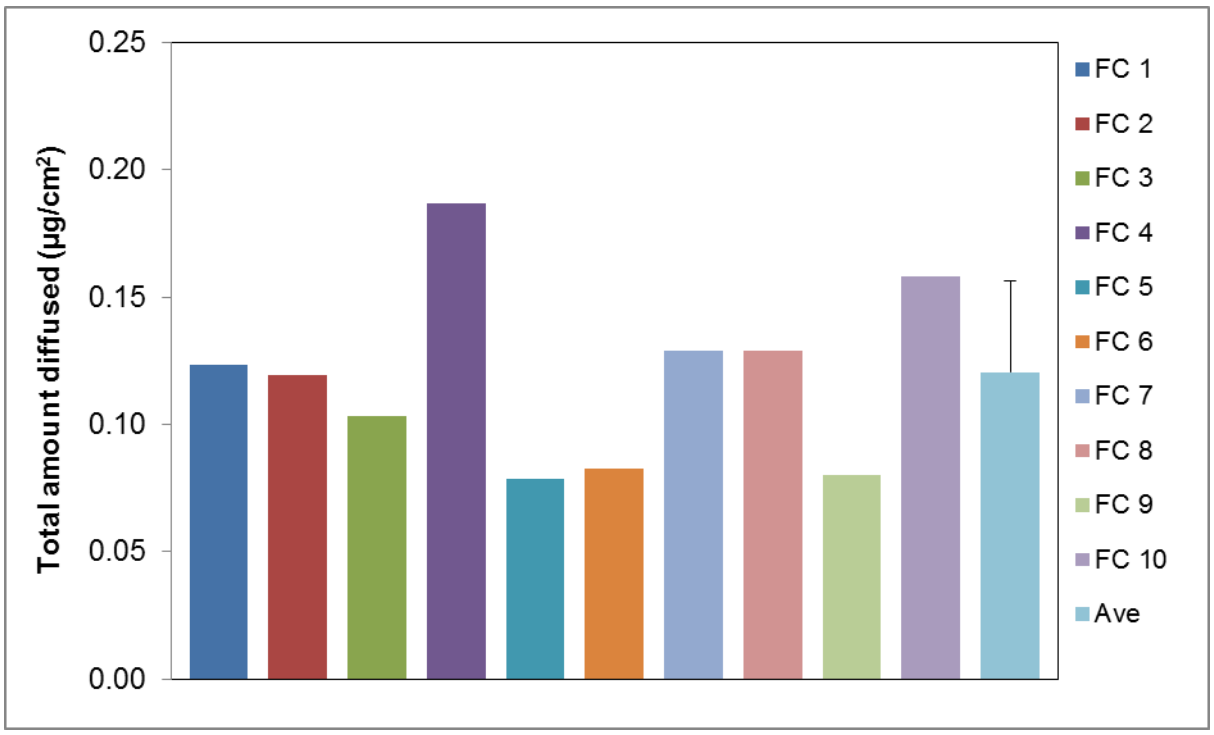

Figure C.2: Total amount of pravastatin diffused per area (µg/cm^2) from the lipophilic emulgel after 12h (n = 10)

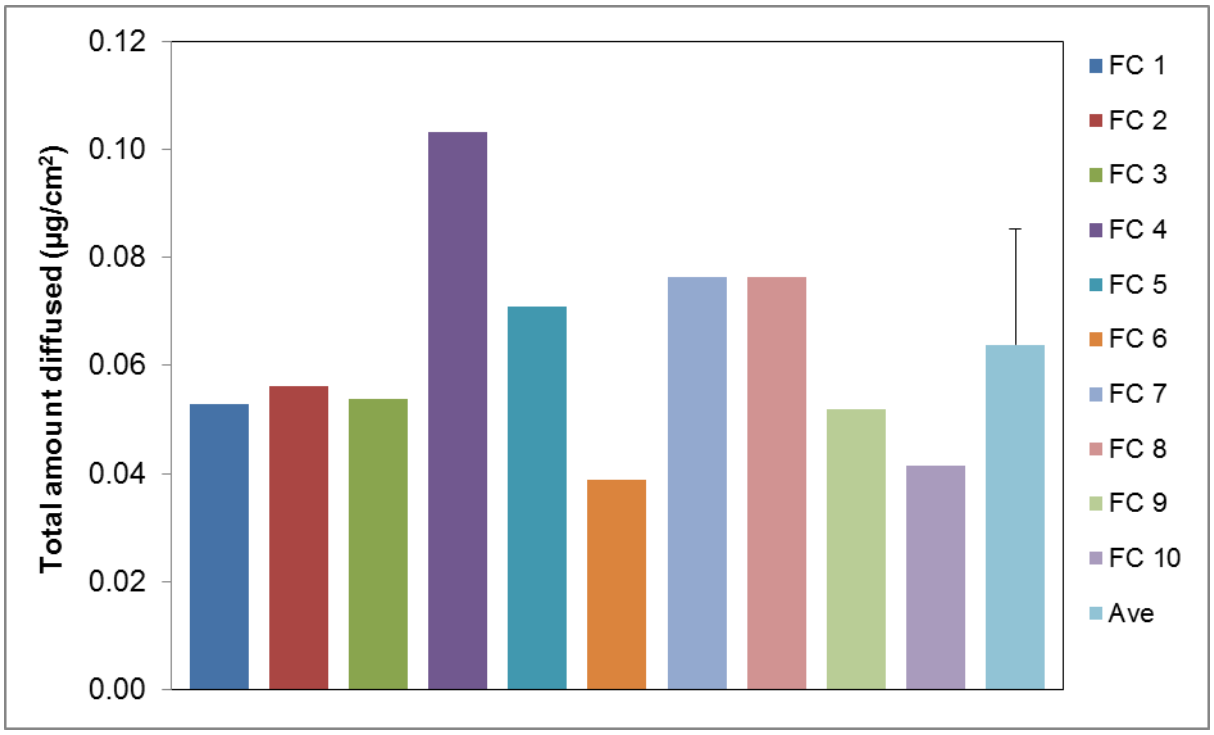

Figure C.3: Total amount of pravastatin diffused per area (µg/cm^2) from the hydrophilic emulgel after 12h (n = 10)

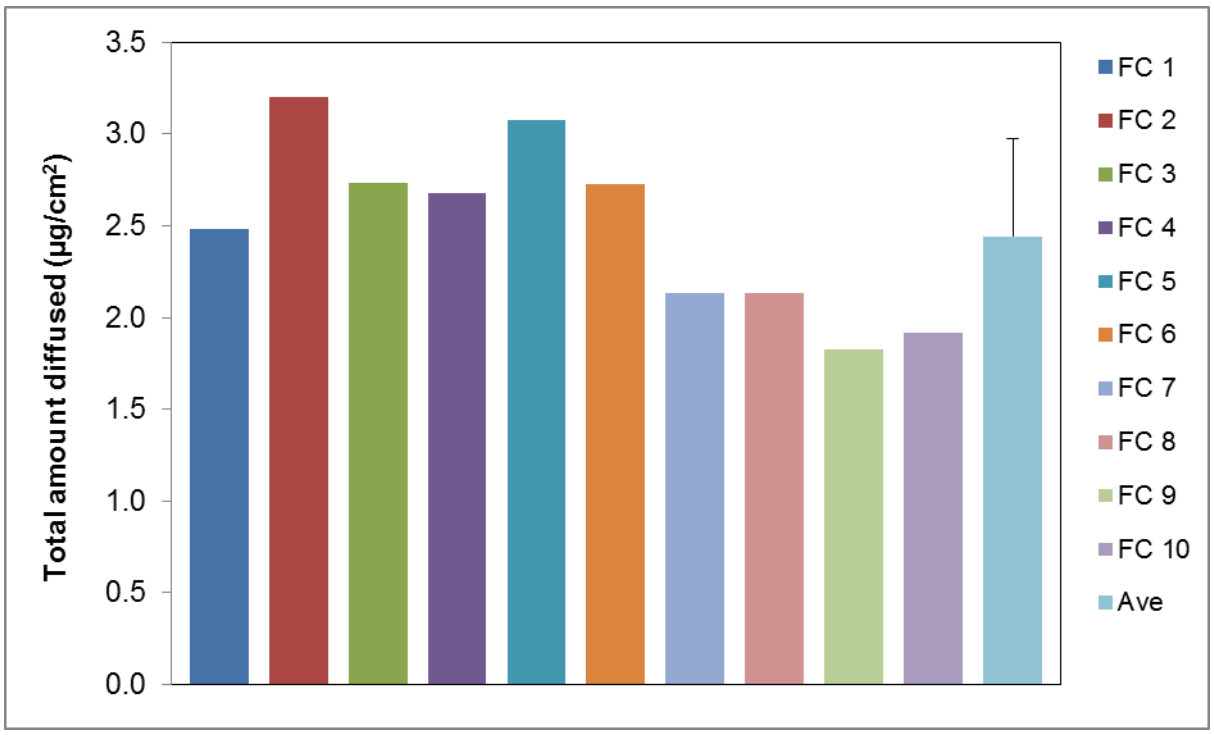

Figure C.4: Total amount of pravastatin diffused per area (µg/cm^2) from the optimised emulgel after 12h (n = 10)

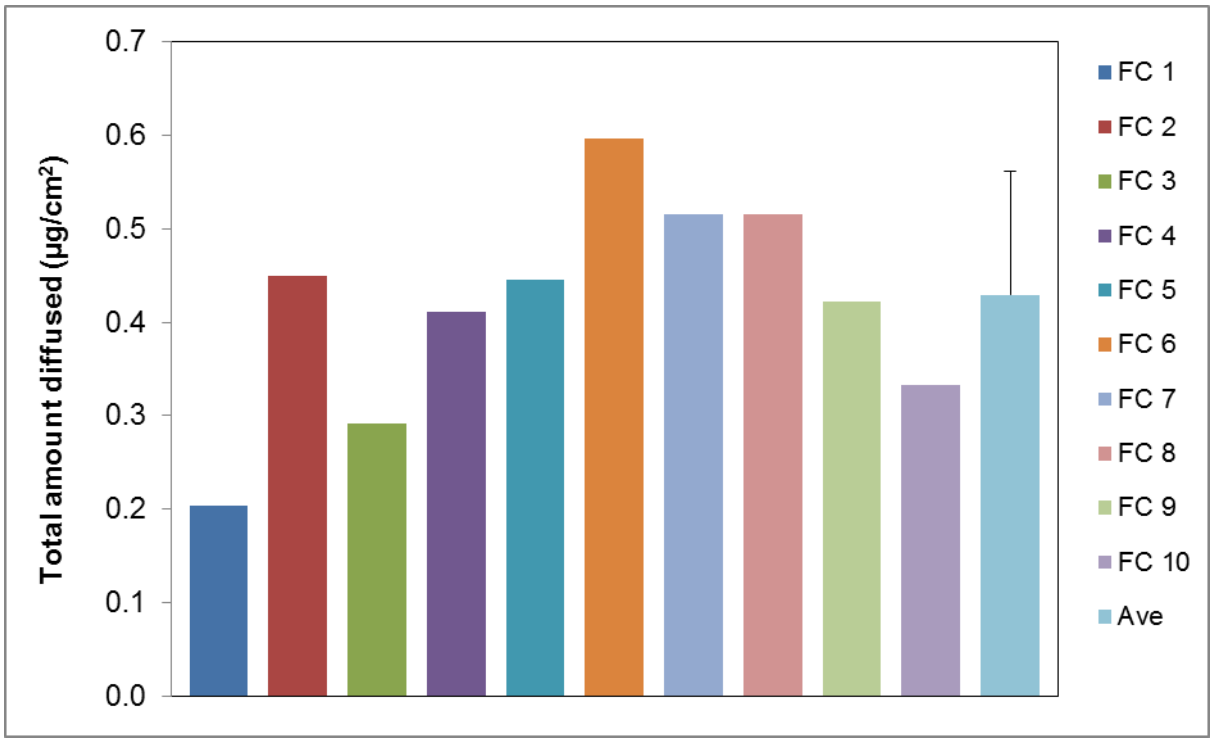

Figure C.5: Total amount of pravastatin diffused per area (µg/cm^2) from the hydrophilic cream after 12h (n = 10)

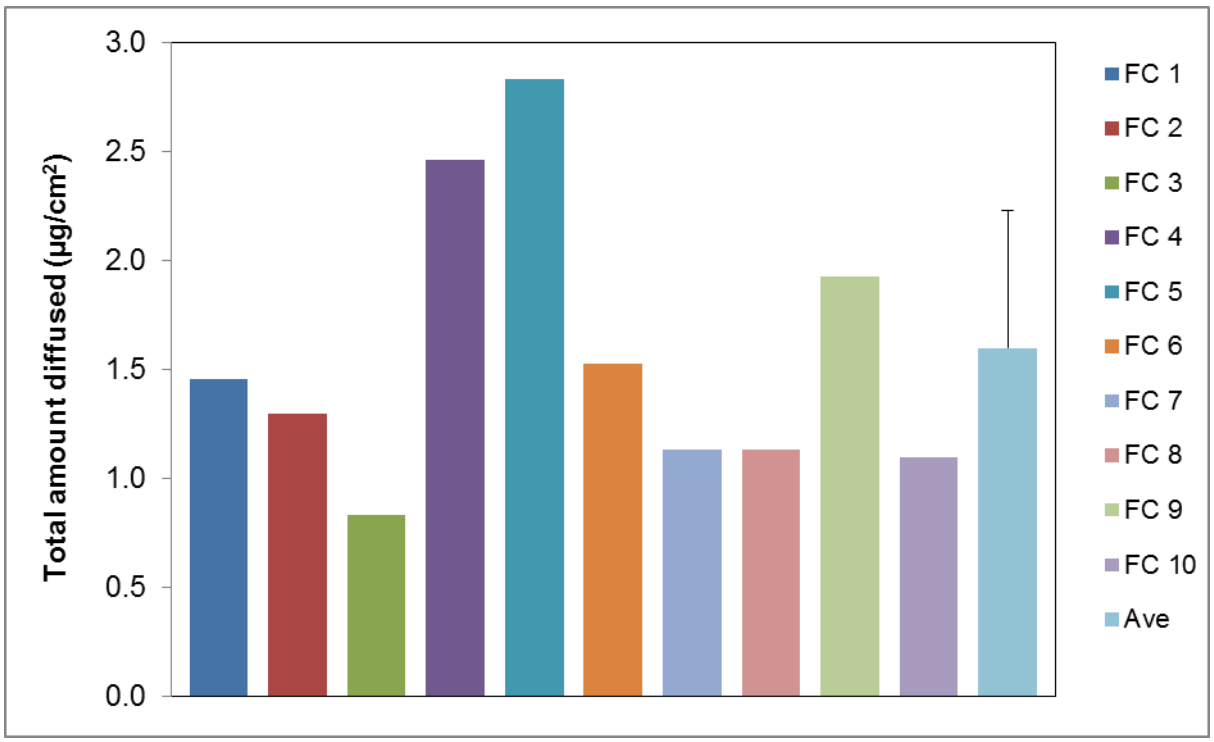

Figure C.6: Total amount of pravastatin diffused per area (µg/cm^2) from the optimised cream after 12h (n = 10)

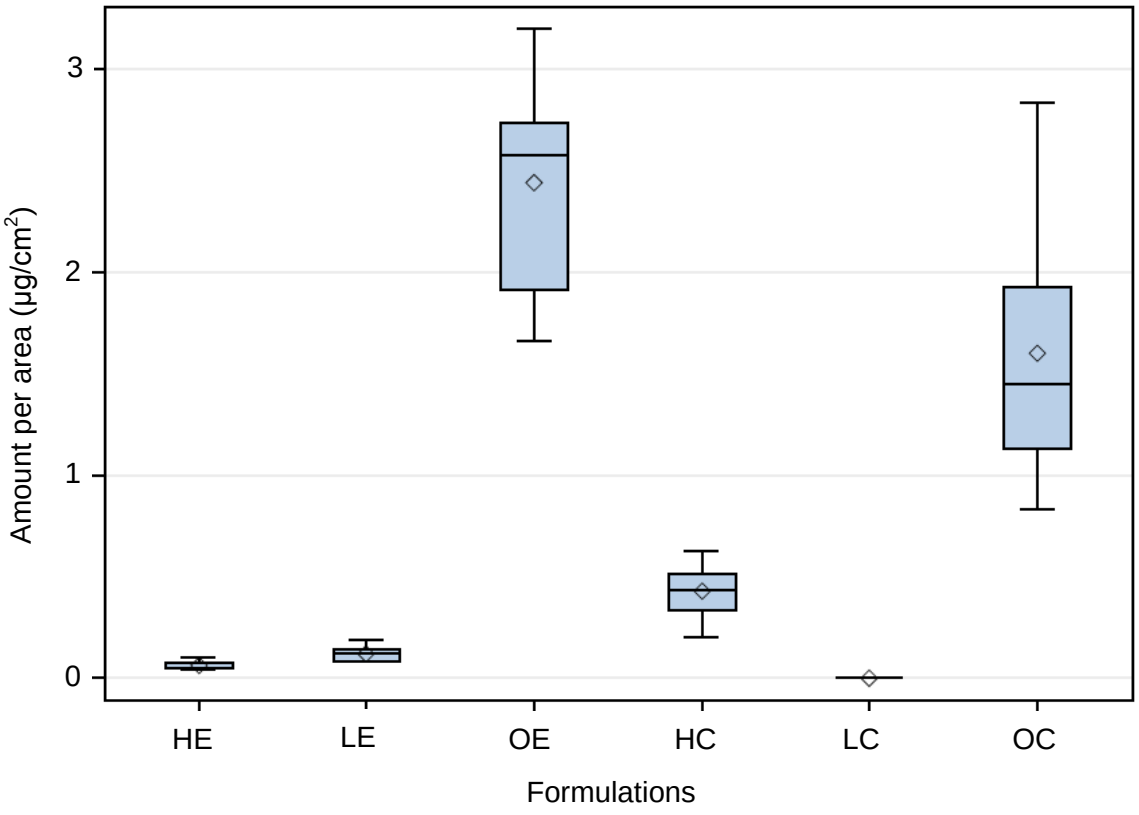

Figure C.7: Box-plot representing the total amount of pravastatin diffused per area (µg/cm²) for the different formulations. The mean and median flux values are indicated by the diamond shapes and lines, respectively.

After comparing the median amount per area which permeated through the skin after 12h with each other, the following was observed: the OE formulation (2.578 µg/cm^2) obtained the highest median amount per area, followed by OC (1.449 µg/cm^2), HC (0.434 µg/cm^2), LE (0.121 µg/cm^2), HE (0.055 µg/cm^2) and lastly LC (0.000 µg/cm^2).

The OC and OE formulations had the most significant difference between the mean and median concentration values from all the formulations. The median amount per area, which takes all the data into account and is not affected by outliers of the data, provides more accurate results of the true value (Dawson and Trapp, 2004:30). Consequently, the median amount per area is described instead of the mean amount per area.

When comparing the emulgel formulations with different polarities (HE, LE and OE) with the cream formulations with different polarities (HC, LC and OC), the emulgels increased the permeation of pravastatin through the skin more than the creams. Emulgels contain more water than creams which makes it more hydrophilic, whereas creams (with more oil content) can be seen as more lipophilic therefore, the more hydrophilic emulgel formulations will tend to diffuse to the more hydrophilic aqueous regions, i.e. the receptor phase (Wiechers, 2008:11). In contrast, the more lipophilic cream formulations would rather remain in the lipophilic stratum corneum (Wiechers, 2008:11). However, the OE and OC formulations both enhanced the penetration of pravastatin through the skin more when compared to HE, LE, HC and LC. It can also be observed that the OE formulation was superior to the OC formulation. The OE formulation had higher water content, which makes it more hydrophilic (Benson, 2005:28) and will therefore penetrate the skin via the hydrophilic transappendageal route, leaving the stratum corneum to penetrate the hydrophilic epidermal layers (Dayan, 2007:32). The OC formulation is more lipophilic, which means the API will permeate the stratum corneum and accumulate (Wiechers, 2008:11). The transappendageal route favours the highly hydrophilic molecules or substances but in the presence of the lipophilic sebum, it can favour lipophilic molecules as well (Wosicka & Cal., 2010:88) which means the OC formulation could also permeate through the skin. According to Wiechers (2011), since the oil phase was optimised, it explains why both the optimised formulations (formulations that had the same polarity as the skin) improved permeation the most.

When the hydrophilic formulations (HE and HC) were compared to the lipophilic formulations (LE and LC), the hydrophilic formulations improved the permeation of pravastatin more, i.e. both HE and HC increased the permeation of pravastatin through the skin whereas only LE enhanced permeation for the lipophilic formulations.

As observed, pravastatin did not permeate through the skin from the LC formulation, because the stratum corneum provides a barrier that does not allow penetration very easily (Hadgraft, 2001:1). As the stratum corneum's barrier properties are very good (Hadgraft, 2004:294), it

could explain why LC (the most lipophilic formulation of all the formulations studied) did not permit the diffusion of pravastatin through the stratum corneum and instead may have concentrated pravastatin in the lipophilic stratum corneum (Wiechers and Watkinson, 2008:77).

Table C.3 consists of the HSP values, AFG and SFG values of three different polarity formulations calculated by the FFE™ computer programme. These values are important to explain why median amounts of the formulations differ.

Table C.3: Hansen solubility parameters of three different semi-solid formulations, active formulation gap and skin formulation gap

Formulations	δD	δP	δH	MW	AFG	SFG
Hydrophilic	17.0	8.5	12.4	179	1.3	7.9
Lipophilic	17.0	6.5	7.5	272	5.7	4.2
Optimised	17.1	7.6	8.5	230	4.1	1.5
Skin	17.0	8.0	8.0	-	-	-

If the HSP values of the formulation and the HSP values of the skin are similar, the API can permeate freely into the stratum corneum; thereafter it will diffuse to the deeper layers of the skin depending on the physicochemical properties of the API. The aforementioned, is subject to its molecular weight (Wiechers, 2011). Using this information it is clear the optimised formulations (OE and OC) would permeate better and therefore show improved median amount per area diffused when compared to the other formulations (HE, LE, HC and LC). 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), indicating that the API can permeate adequately into the stratum corneum. Since pravastatin is hydrophilic, it would permeate into the aqueous layer of the skin (Barry, 2007:578) and therefore should improve transdermal delivery. The HSP values of the lipophilic (17.0, 6.5, 7.5) and hydrophilic (17.0, 8.5, 12.4) formulations differ too much from the HSP values of the skin for adequate permeation into and through the skin.

The AFG is better known as the ‘distance’ between the formulation and the API, whereas the SFG is known as the ‘distance’ between the formulation and the skin. If the AFG increases, the API would be more compatible with the formulation, thus it would not want to leave the formulation, but if the AFG decreases rapidly, the driving force increases allowing the API to permeate into the skin. When SFG decreases, the driving force for diffusion and surface concentration increases, which in turn leads to an increased diffusion of API through the skin, meaning the formulation also becomes more favourable to the skin. If we compare the AFG value of the hydrophilic formulations with the lipophilic formulations, it is evident that the AFG value for the hydrophilic formulations (1.3) is smaller compared to the lipophilic formulations (5.7). According to the delivery gap principle of Prof Wiechers if the AFG decreases, the driving

force increases in order for the API to permeate into the skin (Wiechers, 2011). If an API accumulates in the skin and as the concentration increases, it is more prone to diffuse through the skin due to this driving force effect (Wiechers, 2008:11); as a result the hydrophilic formulations (HE and HC) diffused better through the skin than the lipophilic formulations (LE and LC). Furthermore, the SFG of the optimised formulations (1.5) is very low when compared to the hydrophilic- (7.9) and lipophilic formulations (4.2), indicating that the optimised formulation is more favourable for the skin and therefore the API can diffuse more freely through the skin.

Each formulation contained the same amount of API as well as penetration enhancer thus ruling out the possibility that by using a penetration enhancer the optimised formulations would perform better in permeating the skin. Wiechers (2008:122) concluded that with lipophilic APIs, the effects of penetration enhancers (DMI) improve the solubility of the APIs and although the function of DMI is nullified, it can be corrected by using more secondary emollient if necessary. Remember that multiple factors play a role in APIs which should penetrate the skin and difficulty arises when all these factors need to be manipulated and controlled (Wiechers, 2008:122).

C.3.3.3 Tape-stripping

Mean and median concentrations of pravastatin in the SCE and ED are shown in Table C.4. The median concentrations of the SCE and ED are graphically represented in the box-plots in Figures C.8, and C.9 for the different formulations.

Table C.4: The mean and median concentration (µg/ml) pravastatin accumulated in the SCE and ED over a 12 h period in different formulations

Formulations	Mean concentration in SCE (µg/ml)	Median concentration in SCE (µg/ml)	Mean concentration in ED (µg/ml)	Median concentration in ED (µg/ml)
HE	1.343 ± 0.303	1.448	0.336 ± 0.103	0.309
LE	1.243 ± 0.150	1.301	0.150 ± 0.047	0.138
OE	0.467 ± 0.181	0.505	0.888 ± 0.132	0.849
HC	0.607 ± 0.264	0.505	0.521 ± 0.260	0.524
LC	0.741 ± 0.244	0.676	1.134 ± 1.805	0.572
OC	0.418 ± 0.216	0.400	0.418 ± 0.164	0.355

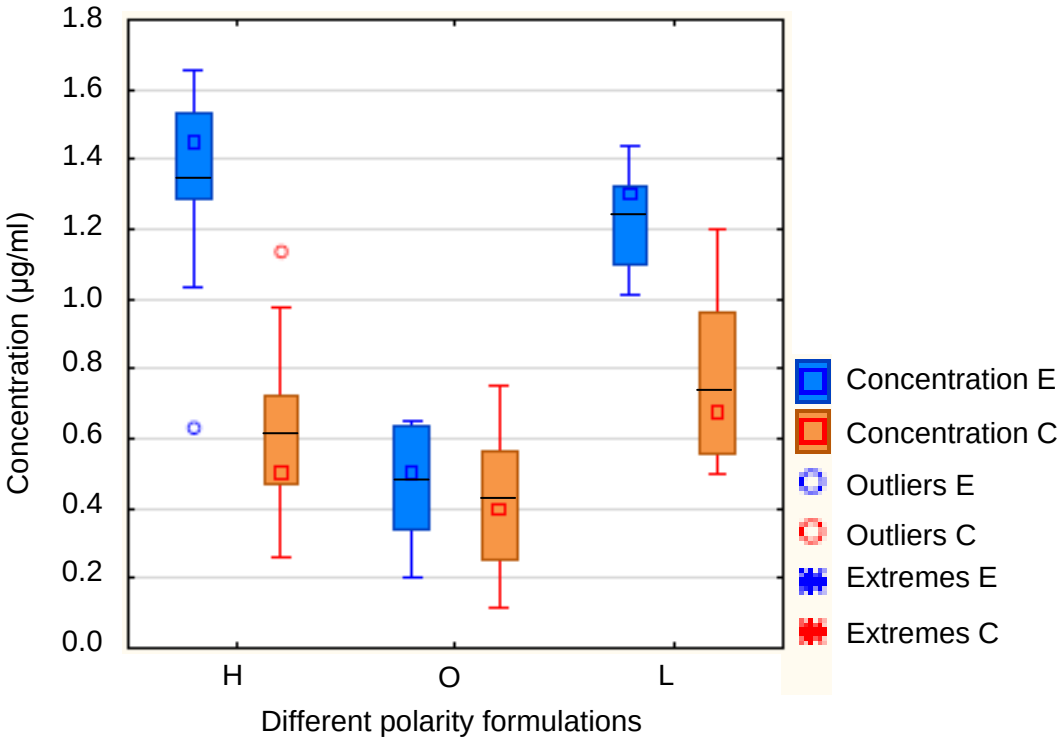

Figure C.8: Box-plot indicating the concentration (µg/ml) pravastatin present in the SCE after tape-stripping for the different formulations. The mean and median concentration values are indicated by the lines and squares, respectively.

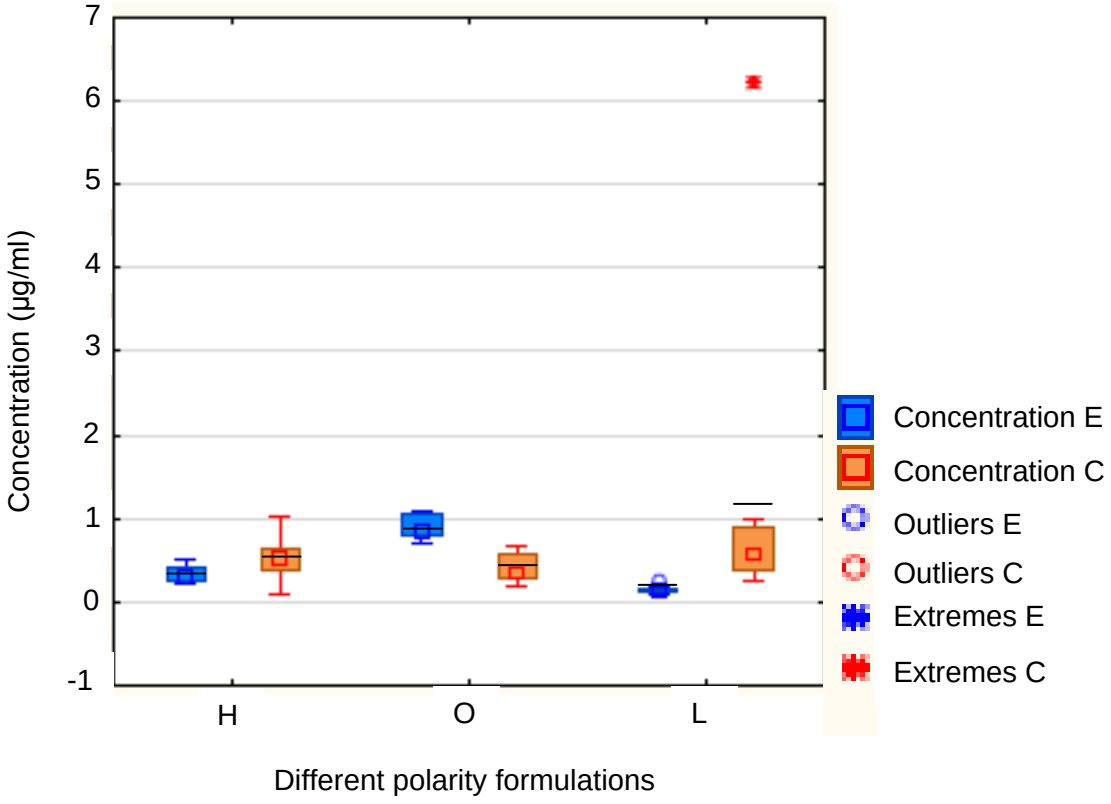

Figure C.9: Box-plot indicating the concentration (µg/ml) pravastatin present in the ED after tape-stripping for the different formulations. The mean and median concentration values are indicated by the lines and squares, respectively.

C.3.3.3.1 Stratum corneum-epidermis

When median SCE concentrations were compared, the HE (1.448 µg/ml) yielded the highest median concentration pravastatin, 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).

All the formulations accumulated in the stratum corneum, but the hydrophilic and lipophilic formulations accumulated more than the optimised formulations. By looking at the data in Table C.4 and Figure C.8, it is evident that the emulgel formulations (HE, LE and OE) improved the permeation of pravastatin more into the SCE than the cream formulations (HC, LC and OC), i.e. HE permeated better than HC, etc. Emulgels are more hydrophilic because they contain more water than creams. This explains the hydrating effect (Williams and Barry, 2004:606). The skin was exposed to water vapour for 12h during the experiments, consequently the water hydrated the skin and caused the stratum corneum to swell, which in turn forced the lipids to open (Williams and Barry, 2004:606). Consequently, the aforementioned can result in an increase of API into the skin.

C.3.3.3.2 Epidermis-dermis

The highest median concentration in ED was 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). When comparing mean and median concentration values it was observed that the greatest difference between these concentration values was for LC.

After comparing the optimised formulations (OE and OC) with each other, it was observed that OE increased permeation of pravastatin more than double into the ED than that of OC. When the hydrophilic (HE and HC) and lipophilic formulations (LE and LC) are compared it is evident that the cream formulations enhanced the permeation of pravastatin more than the emulgel formulations. Due to aqueous regions in the skin lipids the stratum corneum delays partitioning, which decreases permeation into the viable epidermis. In this instance, where the formulations contain both aqueous (hydrophilic) and lipid (lipophilic) solubility characteristics, it will be lipophilic enough to permeate through the stratum corneum and hydrophilic enough to diffuse into the deeper skin layers. This could be the reason, even when different formulations have been used, it still permeated the viable epidermis (Perrie *et al*., 2012:392).

C.3.4 Statistical analysis

C.3.4.1 Membrane release studies

A two-way ANOVA was applied on the flux values with 60 readings that shows statistical significance between the formulations of cream and emulgel ($p < 0001$), also between the

different polarity formulations of optimised (OE, OC), hydrophilic (HE, HC) and lipophilic (LE, LC) ($p < 0.001$), but there was also statistical significance interaction between formulations and different polarity formulations ($p = 0.0168$). Since this interaction was encountered, one-way ANOVA's were applied separately on the flux values of emulgel and cream, which show statistical significance between the different polarity formulations of cream ($p < 0.001$), but no real statistical significance between different polarity formulations of emulgel ($p = 0.0645$). Tukey HSD tests were performed for unplanned comparisons and the results were as follows: for cream the means of the different polarity formulations O, H and L were mutually significantly different on a 0.05 level of significance, while no significant differences existed between any of the different polarity formulations' means of emulgel.

C.3.4.2 Skin diffusion studies

Table C.5: Two-way ANOVA values of the different formulations

Formulations	Two-way ANOVA p-value
Formulation of emulgel and cream	**0.028**
Different polarity formulations H, L and O	**< 0.001**
Interaction of formulation (E and C) and different polarity formulations (HE, LE, OE, HC, LC and OC)	**< 0.001**

Since a statistical significant interaction between the formulations (E and C) and different polarity formulations exists, two one-way ANOVA's were performed on the formulations of each of emulgel and cream, followed by Tukey HSD tests and the results were as follows: for cream the means of the different polarity formulations O, H and L were mutually significant different on a 0.05 level of significance (< 0.001), whilst significant differences existed between the means of L and H, between the means of O and L (< 0.001) and also between O and H (< 0.001) for emulgel.

C.3.4.3 Tape stripping

A three-way ANOVA was applied on the SCE and ED with 120 readings to determine the significance of effects on the formulations and the different polarity formulations, as well as all the different interactions between them. Table C.6 consists of the p-values of all the different formulation combinations used in the tape-stripping studies.

Table C.6: P-values obtained from the three-way ANOVA of all the formulations

Formulations	P-value
Formulation E and C	**0.3355**
Different polarity formulations H, L and O	**0.0985**
Interaction: Formulation and different polarity formulations (EH, EL, EO, CH, CL and CO)	**0.0656**
SCE and ED	**0.0262**
Interaction: Emulgel SCE and ED with cream SCE and ED	**0.0015**
Different polarity formulations H (SCE and ED), L (SCE and ED) and O (SCE and ED)	**0.0085**
Formulation and different polarity formulations: SCE and ED (EH: SCE and ED; EL: SCE and ED; EO: SCE and ED; CH: SCE and ED; CL: SCE and ED; CO: SCE and ED)	**0.0007**

There is only a statistical significance between SCE and ED (0.0262) and all the interactions with SCE/ED with the emulgel and cream formulations (0.0015), as well as with the different polarity formulations (0.0085 and 0.0007).

C.4 Conclusion

The aqueous solubility of pravastatin (197.5 mg/ml) indicated it would permeate very well through the skin compared to the -0.703 log D value which indicated decreasing permeation (Naik *et al.,* 2000:319).

The membrane release studies determined that pravastatin (2%) was released from all six different formulations; although the emulgel formulations had the highest percentage pravastatin release (OE (0.113%), LE (0.102%) and HE (0.094%)) through the membrane when compared to the cream formulations. It was also observed when the membrane and diffusion data were compared, the OE formulation in both cases depicted the highest median value, as did LC depict the lowest median value. It was also observed that LC did not diffuse any pravastatin into the systemic circulation (receptor phase).

In general, it was observed that when the emulgel formulations were compared to the cream formulations, the former increased the diffusion of pravastatin more than the latter. It should also be noted that during the release studies, the emulgel formulations released pravastatin better than the cream formulations. Another reason may be that due to the more hydrophilic nature of the emulgel formulations it will tend to diffuse to the more aqueous regions, i.e. the systemic circulation (Jepps *et al.,* 2012:4). During the skin diffusion studies it was observed that the optimised formulations permeated the best and presented the highest median amount per area diffused when compared to the hydrophilic- and lipophilic formulations. Therefore, the use of the FFE™ programme is successful in order to optimise the oil phase of the formulations.

Hence, Prof Wiechers' Delivery Gap Principle which claims that by optimising the oil phase of formulations it is possible to enhance skin penetration is valid, as the API permeated adequately through the stratum corneum into the systemic circulation (Wiechers, 2011).

All six formulations resulted in pravastatin diffusing into the SCE (lipophilic) as well as the ED (hydrophilic), which implies that the formulations had sufficient hydrophilic and lipophilic properties to cause the API to permeate through the stratum corneum and diffuse into the layers of the skin (Potts, 1992:22).

It was observed that the optimised formulations had the least diffusion of pravastatin into the SCE when compared to the hydrophilic and lipophilic formulations. When comparing the SCE data with the skin diffusion data it is evident that the optimised formulations increased the diffusion of pravastatin the most into the systemic circulation, hence, the lower concentrations of pravastatin in the SCE when compared to the hydrophilic and lipophilic formulations. Keep in mind that the LC did in fact penetrate both the SCE and ED and is therefore a great candidate for topical delivery.

The following conclusions can be made:

- It is possible to deliver pravastatin topically.
- The best formulation for penetration through the skin (transdermal) is the optimised emulgel.
- The best formulation to penetrate the top layer of the skin (topical) is the hydrophilic emulgel.
- The best formulation for pravastatin to reach the dermis is the optimised emulgel.

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Appendix D:

International Journal of Pharmaceutics: Guide for Authors

D.1 Scope of the journal

The International Journal of Pharmaceutics publishes innovative papers, reviews, mini-reviews, rapid communications and notes dealing with physical, chemical, biological, microbiological and engineering studies related to the conception, design, production, characterisation and evaluation of drug delivery systems *in vitro* and *in vivo*. "Drug" is defined as any therapeutic or diagnostic entity, including oligonucleotides, gene constructs and radiopharmaceuticals.

Areas of particular interest include: pharmaceutical nanotechnology; physical pharmacy; polymer chemistry and physical chemistry as applied to pharmaceutics; excipient function and characterisation; biopharmaceutics; absorption mechanisms; membrane function and transport; novel routes and modes of delivery; responsive delivery systems, feedback and control mechanisms including biosensors; applications of cell and molecular biology to drug delivery; prodrug design; bioadhesion (carrier-ligand interactions); and biotechnology (protein and peptide formulation and delivery).

Note: In 2004, a new section was started on pharmaceutical nanotechnology. For more details, see Editorials in 279/1-2, 281/1, and 288/1.

D.2 Editorial Policy

The over-riding criteria for publication are originality, high scientific quality and interest to a multidisciplinary audience. Papers not sufficiently substantiated by experimental detail will not be published. Any technical queries will be referred back to the author, although the Editors reserve the right to make alterations in the text without altering the technical content. Manuscripts submitted under multiple authorship, are reviewed on the assumption that all listed authors concur with the submission and that a copy of the final manuscript has been approved by all authors and tacitly or explicitly by the responsible authorities in the laboratories where the work was carried out. If accepted, the manuscript shall not be published elsewhere in the same form, in either the same or another language, without the consent of the Editors and Publisher.

Authors must state in a covering letter when submitting papers for publication the novelty embodied in their work or in the approach taken in their research. Routine bioequivalence studies are unlikely to find favour. No paper will be published which does not disclose fully the nature of the formulation used or details of materials which are key to the performance of a product, drug or excipient. Work which is predictable in outcome, for example the inclusion of another drug in a cyclodextrin to yield enhanced dissolution, will not be published unless it provides new insight into fundamental principles.

D.3 Submission of Manuscripts

Authors are strongly encouraged to submit their manuscript electronically by using the Elsevier submission site at (http://www.elsevier.com/iournals).

After registration, authors will be asked to upload their manuscript and associated artwork. Full instructions on how to use the online submission tool are available at the web address listed above.

If an author cannot submit their manuscript electronically, then for the initial submission of manuscripts for consideration, hardcopies are sufficient. The original plus two copies, complete with two sets of figures (including originals or duplicates of sufficient quality for clarity of reproduction) and tables, must be submitted in English. All data that would help referees to evaluate the paper should also be supplied. Manuscripts should be typewritten with double spacing and adequate margins on one side of the sheet only (not more than 26 lines per page). All pages should be numbered sequentially. Manuscripts should be sent to one of the following Editors-in-Chief according to the geographical origin of the author. Please include full contact information - corresponding author name, e-mail address, telephone and fax numbers, and full postal address.

After final acceptance for publication, your revised manuscript on disk together with two printed hard copies should be submitted to the accepting editor. It is important that the file on disk and the printout are identical. Both will then be forwarded by the editor to Elsevier. In-depth guidelines for submitting artwork/illustrations can be found at: http://www.elsevier.com/artworkinstructions.

When the paper is to be published as a Rapid Communication, this should be clearly indicated to the Editor-in-Chief.

D.3.1 Europe, Africa, Near East

Prof AT. Florence, The School of Pharmacy, University of London, 29-39 Brunswick Square, London WCIN 1AX, U.K., Fax: +44 20 7837 5092; E-mail: jip(5)pharmacv.ac.uk

D.3.2 The Americas, Australia and New Zealand

Prof J.H. Rytting, Pharmaceutical Chemistry Dept, University of Kansas, 2095 Constant Avenue, Lawrence, KS 66047, U.S.A., Fax: +1 785 864 5736; E-mail: iip@ku.edu

D.3.3 Japan and far East

Prof. T. Sonobe, Department of Pharmaceutical Engineering, School of Pharmaceutical Sciences, University of Shizuoka, 52-1 Yada, Shizuoka-shi 422-8526, Japan. Fax: +81-54-264-5614; E-mail: sonobe@gakushikai.jp

D.4 Manuscript Types

D.4.1 Full length manuscripts

The arrangement of full length papers should accord with the following:

D.4.1.1 Title

The full title should not exceed 85 characters including spaces between words.

D.4.1.2 List of authors

Initial(s) (one given name may be used) followed by the surname of author(s) together with their affiliations. When the work has been carried out at more than one address, the affiliation of each author should be clearly indicated using superscript, lower-case letters. The author to whom correspondence should be directed must be indicated with an asterisk.

D.4.1.3 Affiliation(s) Name(s) and address (es) of the establishment(s) where the work was done designated by superscript, lower-case letters where appropriate.

D.4.1.4 Abstract

An Abstract not exceeding 200 words (a single paragraph) should be provided typed on a separate sheet.

D.4.1.5 Keywords

A maximum of 6 keywords or short phrases suitable for indexing should be supplied. If possible keywords should be selected from Index Medicus or Excerpta Medica Index. Authors may also wish to refer to the Subject Index published in International Journal of Pharmaceutics, for example, Vol. 287/1-2, pp. 205-219.

D.4.1.6 Corresponding author

The author to whom correspondence should be directed should be designated with an asterisk (do not include the address unless different from that indicated by the author's affiliation). Telephone, fax and e-mail address of the corresponding author must be provided.

D.4.1.7 Text

The text should be divided into main sections, such as the following: 1. Introduction; 2. Materials and methods; 3. Results; 4. Discussion; Acknowledgements; References; Figure legends; Tables and Figures. These sections must be numbered consecutively as indicated. Subdivisions of a section should also be numbered within that section, for example, 2.1. Materials, 2.2. Relative humidity measurement, 2.3. Sample preparation, etc.

D.4.1.8 Nomeclature

Standard nomenclature should be used throughout; unfamiliar or new terms and arbitrary abbreviations should be defined when first used. Unnecessary or ambiguous abbreviations and symbols are to be avoided. Data should be expressed in SI units.

D.4.1.9 Figure legends, table legends, footnotes

Figure legends, tables and footnotes should be typed on separate sheets, lines double spaced. Footnotes, to be numbered consecutively in superscript throughout the text, should be used as little as possible.

D.4.1.10 References

See below for full details.

D.4.2 Rapid communications

- These articles should not exceed 1500 words or equivalent space.
- Figures should not be included otherwise delay in publication will be incurred.
- Do not subdivide the text into sections. An Abstract should be included as well as a full reference list.
- No proofs will be sent to the author(s).

D.4.3 Notes

Should be prepared as described for full length manuscripts, except for the following:

- The maximum length should be 1500 words, including figures and tables.
- Do not subdivide the text into sections. An Abstract and reference list should be included.

D.4.4 Reviews and mini reviews

Suggestions for review articles will be considered by the Editors-in-Chief. "Mini-reviews" of a topic are especially welcome.

D.5 References

D.5.1 Text citation

The Harvard system of citation must be used. References should be cited in the text within parentheses: where several citations are given within a single set of parentheses, they should be arranged in ascending order of year of publication; where more than one reference with the same year of publication is cited, they should be arranged in alphabetical order of the first authors' names. When referring to a work of more than two authors, the name of the first author should be given, followed by et al.

Examples of text citations:

(Gesztes et al., 1988; Chestnut et al., 1989; Legros et al., 1990; Mhando and Li Wan Po, 1990; Korsten et al., 1991; Langerman et al., 1991, 1992a,b; Masters et al., 1991; Bonhomme et al., 1992; Kollietal., 1992).

(Shaw et al., 1978; Nakano and Arita 1990b; Nakano et al., 1990a,b; Bone et al., 1992)

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All references cited in the text should be listed at the end of the paper (typed with double spacing) and assembled alphabetically. More than one paper from the same author(s) in the same year must be identified by the letters a b c, etc. placed after the year of publication.

References must consist of names and initials of all authors, year, title of paper, abbreviated title of periodical, and volume and first and last page numbers. 'Personal communication' and 'unpublished data' should be cited in the text only. Papers referred to as 'submitted for publication' must include the name of the journal to which submission has been made. Journal titles should be abbreviated according to the 'List of Serial Title Word Abbreviations' (available from International Serials Data System, 20, rue Bachaumont, 75002 Paris, France. ISBN 2-904939-02-8).

Example of arrangement in the reference list:

Crowe, J.H., Crowe, L.M., Chapman, D., 1984a. Infrared spectroscopic studies on interactions of water and carbohydrates with a biological membrane. Arch Biochem. Biophys., 232, 400-407.

Crowe, J.H., Crowe, L.M., Hoekstra, F.A., 1989. Phase transitions and permeability changes in dry membranes during rehydration. J. Bioenerg. Biomembr., 21, 77-92.

Crowe, J.H., Crowe, L.M., Carpenter, J.F., Aurell Wistrom, C, 1987. Stabilization of dry phospholipid bilayers and proteins by sugars. Biochem. J., 242, 1-10.

Crowe, J.H., Crowe, L.M., Carpenter, J.F., Rudolph, A.S., Wistrom, C.A., Spargo, B.J., Anchordoguy, T.J., 1988. Interactions of sugars with membranes. Biochim. Biophys. Acta, 947, 367-384.

Crowe, L.M., Crowe, J.H., Womersley, C, Reid, D., Appel, L, Rudolph, A., 1986. Prevention of fusion and leakage in freeze-dried liposomes by carbohydrates. Biochim. Biophys. Acta, 861, 131-140.

Crowe, L.M., Mouradian, R., Crowe, J.H., Jackson, S.A., Womersley, C, 1984b. Effects of carbohydrates on membrane stability at low water activities. Biochim. Biophys. Acta, 769, 141-150.

Examples of presentation for various types of publication

Langerman, L., Chaimsky, G., Golomb, E., Tverskoy, M., Kook, A.I., Benita, S., 1990. A rabbit model for evaluation of spinal anaesthesia: chronic cannulation of the subarachnoid space. Anesth. Analg., 71, 529-535.

Timsina, M.P., Martin, G.P., Marriott, C, Ganderton, D., Yianneskis, M., 1994. Drug delivery to the respiratory tract using dry powder inhalers. Int. J. Pharm., 101, 1-13.

Gibaldi, M. and Perrier, D., 1982. Pharmacokinetics, 2nd Ed., Dekker, New York. 171

Deppeler, H.P., 1981. Hydrochlorothiazide. In: Florey, K. (Ed.), Analytical Profiles of Drug Substances, Vol. 10, Academic Press, New York, pp. 405-441.

US Pharmacopeia XXII, 1990. US Pharmacopeial Convention, Rockville, MD, pp. 1434-1435.

Mueller, L.G., 1988. Novel anti-inflammatory esters, pharmaceutical compositions and methods for reducing inflammation. UK Patent GB 2 204 869 A, 23 Nov.

Du Plessis, J., 1992. Topical liposomal delivery of biologically active peptides. Ph.D Thesis, Potchefstroom University for CHE, South Africa.

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