

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

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Dissertation submitted in fulfilment of the requirements for the degree *Magister Scientiae* in *Pharmaceutics* at the Potchefstroom Campus of the North-West University

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November 2014

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Acknowledgements

I give praise to the Lord for without His grace, support and strength the completion of this study would not have been possible. I thank Him for the people He blessed me with to support me throughout this study.

I realise that the completion of this study would not have been possible without the wisdom, help and support from the following people:

- ◆ Ivan, thank you for your unfailing support, love and motivation. Thank you for always believing in me and encouraging me to do my best. You have made this journey so much easier. I love you with my whole heart.
- ◆ Mom and Dad thank you for all your prayers and support. Thank you for all the opportunities you gave me and for supporting me in everything I do. Thank you for always having faith in me and encouraging me to do more. I love you very much. My sisters, Linmarie and Elmien, thank you for your love, friendship and support. I love you and I am truly blessed to have you as my family
- ◆ My colleagues and friends thank you for your support and your friendship. Anina thank you for all the chats, laugh and support. You are a true friend. Johann and Lizelle thank you for always being friendly and willing to help. Candice thank you for your friendliness and help during my study.
- ◆ Prof Jan du Preez my supervisor, thank you for your wisdom, guidance and support. Thank you for all your help during my study and for your friendliness. Prof made a huge contribution to the success of my study.
- ◆ Prof Jeanetta du Plessis thank you for your help and guidance and the opportunity to undertake the study.
- ◆ Dr Minja Gerber thank you for all your help during my study, especially with the formatting.
- ◆ Prof Faans Steyn thank you for the statistical analysis and helping me to interpret my results.
- ◆ Prof Jan Steenekamp thank you for your help with the Mastersizer and your support.
- ◆ Mark Chandler, Prof Steven Abbott and Dr Charles Hansen for your correspondence and help to understand the software and HSP.
- ◆ Thank you to the National Research Foundation (NRF) of South Africa and the Centre of Excellence for Pharmaceutical Sciences (Pharmacoen), North-West University, Potchefstroom Campus, South Africa, for funding this study

Abstract

The transdermal delivery route has become a popular alternative to more conventional routes, such as oral administration, but has not yet reached its full potential (Prausnitz & Langer, 2008:1261). Although the transdermal route proves to have several advantages over the conventional route, the greatest challenge is to overcome the effective barrier of the skin (Jepps *et al.*, 2012:153). The permeation of the active pharmaceutical ingredient (API) through the skin is a complex, multi-step process and therefore predicting the permeability of the API is difficult (Jepps *et al.*, 2012:153; Williams, 2003:30). Various approaches have been developed to overcome the skin barrier and it is recognised that the nature of the vehicle in which the API is applied plays a significant role in promoting transdermal delivery (Foldvari, 2000:417). It is important to consider the fate of the formulation ingredients and the API after application and how this changes the composition of the formulation on the skin when developing a vehicle for transdermal delivery (Lane *et al.*, 2012:496; Otto *et al.*, 2009:2).

Wiechers (2012) proposed the Skin Delivery Gap (SDG) as an indicator for the permeability of an API. An API with a SDG < 1 will readily permeate the skin, whilst an SDG > 1 indicates a more complex delivery system is required. The partitioning of the API between the skin and the formulation is influenced by the formulation and by altering the formulation properties it is possible to manipulate the transdermal delivery of the API. The relative polarity index (RPI), based on the octanol-water partition coefficient ($\log P$) of the stratum corneum, formulation and the API, was initially developed by Wiechers as a tool for developing formulations with an optimal polarity, to ensure the transdermal delivery of at least 50% of the API (Lane *et al.*, 2012:498; Wiechers, 2008:94; Wiechers *et al.*, 2004:174). The use of $\log P$ as an indicator of polarity was considered impractical by Hansen (2013) and acknowledged by both Wiechers and Abbott, who consequently developed the Formulating for Efficacy™ (FFE™) software which uses Hansen solubility parameters (HSP) instead of $\log P$ to indicate polarity (Hansen, 2013). The FFE™ calculates HSP distances, known as gaps, between the skin, API and the formulation to indicate the solubility of the different components in each other. A smaller HSP gap indicates a high solubility. The FFE™ enables the formulator to develop a formulation with a good balance between the active-formulation gap (AFG) and the skin-formulation gap (SFG) to ensure sufficient diffusion of the API into the skin.

The FFE™ software was used to develop formulations containing 1.5% atropine as a model drug. Formulations of different polarity (optimised towards the stratum corneum, more hydrophilic and more lipophilic) were developed to determine the effect of the polarity of the formulation and the relevant HSP gaps on the transdermal delivery of the API. The same

formulations were utilised for atropine sulphate to determine the effect the salt form has on the transdermal delivery of the API compared to the base compound.

The log P and octanol-buffer partition coefficient (log D) of both atropine and atropine sulphate were determined. Log D is a more reliable indicator of distribution compared to log P, since, it considers the degree of ionisation of the API (Ashford, 2007:294). The log P and log D of atropine (0.22 and -1.26) and atropine sulphate (-1.32 and -1.23) both predicted poor skin penetration (Brown *et al.*, 2005:177). The aqueous solubility of atropine (0.9 mg/ml) also predicted limited transdermal delivery, while the solubility of atropine in phosphate buffer solution (PBS pH 7.4) (5.8 mg/ml) indicated favourable permeation (Naik *et al.*, 2000:321). The high degree of ionisation of the API (99.68 %), at pH 7.4, predicts only a small amount will penetrate the skin (Barry, 2007:576).

The membrane release study confirmed the API was released from the different formulations and subsequently skin diffusion studies were conducted, followed by tape stripping after 12 h, to determine which formulation resulted in the highest transdermal delivery of the API. The atropine hydrophilic formulation released the highest percentage of API after 6 h (13.930%). This was explained by the low affinity the lipophilic atropine has towards the hydrophilic formulation (Otto *et al.*, 2009:9). The highest percentage transdermal delivery (0.065%) was observed with the lipophilic formulation containing atropine. The higher SFG compared to the AFG of the lipophilic formulation initially predicted poor transdermal delivery, but when considering the HSP profile and molar volume of the different ingredients, it was observed the dimethyl isosorbide (DMI) penetrated and provided a desirable environment for the API in the skin. The residual formulation (containing less DMI and more polyethylene glycol 400 (PEG 8) and liquid paraffin) was less desirable for the API and was therefore forced out of the formulation (Abbott, 2012:219). Both these factors contributed to the high transdermal delivery of atropine from the lipophilic formulation. The atropine sulphate hydrophilic formulation had the highest percentage in the stratum corneum-epidermis (0.29 µg/ml) and the hydrophilic formulation of both atropine and atropine sulphate had the highest concentration in the epidermis-dermis (both 0.55 µg/ml). The hydrophilic formulations had the lowest driving force provided by the AFG and the only driving force for the API to leave the formulation was the concentration gradient. These formulations had the lowest transdermal delivery which indicates the API had not fully traversed through the skin after 12 h.

According to Wiechers, a minimised SFG would indicate the formulation is optimised towards the stratum corneum and should essentially deliver the highest percentage of API through the skin. The results obtained are contrary to this belief and it is concluded that the total HSP profile and the molar volume of the formulation and the API should be considered when developing a formulation with optimal transdermal delivery rather than just the SFG.

Keywords: Transdermal delivery, Formulation, Hansen Solubility Parameters

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Uittreksel

Die transdermale roete het 'n populêre alternatief geword vir konvensionele roetes soos orale toediening, maar het nog nie die volle potensiaal bereik nie (Prausnitz & Langer, 2008:1261). Alhoewel die transdermale roete verskeie voordele bo die konvensionele roetes inhou, is die grootste uitdaging om die effektiewe skans van die vel te oorkom (Jepps *et al.*, 2012:153). Die penetrasie van die aktiewe farmaseutiese bestanddeel (AFB) deur die vel is 'n komplekse, multi-stapproses en dus is dit moeilik om die penetrasie van die AFB te voorspel (Jepps *et al.*, 2012:153; Williams, 2003:30). Verskeie benaderings is al ontwikkel om die velskans te oorkom en dit is erken dat die aard van die medium waarin die AFB aangewend word 'n betekenisvolle bydra maak in die bevordering van die AFB se transdermale aflewering (Foldvari, 2000:417). Tydens die ontwikkeling van 'n medium vir transdermale aflewering is dit belangrik om die lot van die verskillende formuleringsbestanddele, die AFB na aanwending en hoe dit die samestelling van die formulering op die vel verander, in ag te neem (Lane *et al.*, 2012:496; Otto *et al.*, 2009:2).

Wiechers (2012) het die velafleweringsgaping (VAG) voorgestel om die penetrasie vermoë van 'n AFB aan te dui. 'n AFB met 'n VAG < 1 sal maklik die vel penetreer, terwyl 'n VAG > 1 aandui dat 'n meer komplekse afleweringstelsel benodig word om die AFB effektief af te lewer. Die verdeling van die AFB tussen die vel en die formulering word beïnvloed deur die formulering en deur die eienskappe van die formulering te verander is dit moontlik om die transdermale aflewering van die AFB te manipuleer. Die relatiewe polariteit indeks (RPI), gebaseer op die oktanol-water verdelingskoëffisiënt ($\log P$) van die stratum corneum, die formulering en die AFB, was aanvanklik ontwikkel deur Wiechers as 'n hulpmiddel om formulerings te ontwikkel met 'n optimale polariteit wat die transdermale aflewering van ten minste 50% van die AFB sal verseker (Lane *et al.*, 2012:498; Wiechers, 2008:94; Wiechers *et al.*, 2004:174). Die gebruik van $\log P$ om polariteit aan te dui is as onprakties geag deur Hansen (2013). Hierdie feit was erken deur beide Wiechers en Abbott en hul het die "Formulating for Efficacy™ (FFE™)" sagteware ontwikkel wat gebruik maak van Hansen oplosbaarheid parameters (HOP) in plaas van $\log P$ om polariteit aan te dui (Hansen, 2013). Die FFE™ bereken die HOP afstand, bekend as gapings, tussen die vel, AFB en die formulering; om die oplosbaarheid van die verskillende komponente in mekaar aan te dui. 'n Kleiner HOP afstand dui goeie oplosbaarheid aan. Die FFE™ stel die formuleerder in staat om 'n formulering te ontwikkel met 'n goeie balans tussen die aktief-formuleringsgaping (AFG) en die vel-formuleringsgaping (VFG) om voldoende diffusie van die AFB in die vel in te verseker.

Die FFE™ sagteware is gebruik om formulerings wat 1.5% atropien as 'n modelgeneesmiddel bevat te ontwikkel. Formulerings met verskillende polariteite (geoptimaliseer tot die stratum corneum, meer hidrofiel en meer lipofiel as die stratum corneum), is ontwikkel om die effek van die polariteit van die formulering en die relevante HOP gapings op die transdermale aflewering van die AFB te bepaal. Dieselfde formulering is gebruik vir atropiensulfaat om die effek van die sout vorm op die transdermale aflewering van die AFB te vergelyk met die basisverbinding.

Die log P en oktanol-buffer verdelingskoëffisiënt (log D) van beide atropien en atropiensulfaat was bepaal. Log D is 'n meer betroubare aanduiding van verdeling in plaas van log P, aangesien dit die graad van ionisasie van die AFB in ag neem (Ashford, 2007:294). Die log P en log D van beide atropien (0.22 en -1.26) en atropiensulfaat (-1.32 en -1.23) voorspel swak velpenetrasie (Brown *et al.*, 2005:177). Die wateroplosbaarheid van atropien (0.9 mg/ml) het ook beperkte transdermale aflewering voorspel, terwyl die oplosbaarheid van atropien in 'n fosfaatbuffer-oplossing (FBO pH 7.4) (5.8 mg/ml) gunstige penetrasie aandui (Naik *et al.*, 2000:321). Die hoë mate van ionisasie van die AFB (99.68 %) by pH 7.4 voorspel dat slegs 'n klein hoeveelheid die vel sal penetreer (Barry, 2007:576).

Die membraanvrystellingsstudie het bevestig dat die AFB vrygestel word vanuit die verskillende formulerings waarna veldiffusiestudies uitgevoer is gevolg deur die kleefbandafstropingsstudie na 12 h om te bepaal watter formulering die hoogste transdermale aflewering van die AFB tot gevolg gehad het. Die atropien hidrofiele formule het die hoogste persentasie van die AFB vrygestel na 6 h (13.93%). Die verduideliking hiervoor was die lae affiniteit wat die lipofiele atropien het vir die hidrofiele formulering (Otto *et al.*, 2009:9). Die hoogste persentasie transdermale aflewering (0.065%) is waargeneem met die lipofiele formulering wat atropien bevat. Die hoër VFG in vergelyking met die AFG van die lipofiele formulering het aanvanklik swak transdermale aflewering voorspel, maar nadat die HOP profiel en die molêre volume van die verskillende bestanddele in ag geneem is, is daar bevind dat die dimetielisosorbied (DMI) die vel gepenetreer het en 'n gunstige omgewing vir die AFB in die vel veroorsaak het. Die oorblywende formulering (wat minder DMI en meer poliëtileenglikool 400 (PEG 8) en vloeibare paraffien bevat) was minder gunstig vir die AFB en daarom was dit uit die formulering geforseer (Abbott, 2012:219). Beide hierdie twee faktore het bygedra tot die hoë transdermale aflewering van atropien uit die lipofiele formulering. Die atropiensulfaat hidrofiele formulering het die hoogste konsentrasie in die stratum corneum-epidermis (0.29 µg/ml) gehad en die hidrofiele formulering van beide atropien en atropiensulfaat het die hoogste konsentrasie in die epidermis-dermis (beide 0.55 µg/ml) gehad. Die hidrofiele formulering het die laagste dryfkrag as gevolg van die AFG gehad en die enigste dryfkrag vir die AFB om die formulering te verlaat was die konsentrasie gradiënt. Hierdie formulerings het die laagste transdermale aflewering getoon wat aandui dat die AFB nog nie die vel ten volle gekruis het na 12 h nie.

Volgens Wiechers sal 'n verkleinde VFG aandui dat 'n formulering geoptimaliseer is tot die stratum corneum en moet daarom die hoogste persentasie van die AFB deur die vel aflewer. Die resultate verkry is in teenstelling hiermee en die gevolgtrekking is gemaak dat die totale HOP profiel en die molêre volume van die formulering en die AFB in ag geneem moet word wanneer 'n formulering met optimale transdermale aflewering ontwikkel word in plaas van net die VFG.

Sleutel woorde: Transdermale aflewering, Formulering, Hansen oplosbaarheid parameters

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Chapter 1

Introduction and problem statement

1.1 Introduction

The transdermal route of administration is an attractive alternative to the standard oral route and possibly to hypodermic injection (Prausnitz & Langer, 2008:1261). Compared to the oral route, transdermal delivery has several advantages, such as eliminating the first-pass metabolism of drugs and the effects of the gastrointestinal-tract on the active pharmaceutical ingredient (API) (Kornick *et al.*, 2003:953; Walters & Roberts, 2002:4). Due to the reduction of metabolism and loss of API via the transdermal route, lower doses may be administered which may reduce the occurrence of adverse effects (Kornick *et al.*, 2003:953). In the event of an adverse effect, the transdermal therapy can be terminated immediately by removing the formulation (Delgado-Charro & Guy, 2001:216). Since transdermal delivery avoids possible infection and pain from injections, the patient's acceptance and compliance are higher (Delgado-Charro & Guy, 2001:216; Jepps *et al.*, 2012:153).

The human skin is the largest organ in the human body with multiple possible application sites for transdermal delivery. Although the skin is easily accessible, it has a highly efficient barrier function preventing the entry and loss of molecules through the skin (Jepps *et al.*, 2012:153; Williams, 2003:1). The barrier function is primarily caused by the 10 to 15 μm thick stratum corneum and needs to be overcome when delivering an API transdermally (Prausnitz, 1999:62). APIs follow a complex process consisting of multiple steps when permeating the skin (Williams, 2003:30); essentially via three different pathways known as the transappendageal, the transcellular and intercellular route (Williams, 2003:31). Predicting the permeability of an API is difficult because of the complexity of the mechanism and structure of these pathways (Jepps *et al.*, 2012:153). Most APIs will penetrate the skin via a combination of the different pathways depending on the physicochemical properties of the API (Williams, 2003:31), with only a few being compliant for delivery via the transdermal route (Prausnitz & Langer, 2008:1261). According to Yano (cited by Brown *et al.*, 2005:177), a molecule should have a log P of 1 to 3 to ensure sufficient aqueous and lipid solubility for skin diffusion. The transdermal route is limited to molecules having a molecular weight less than 500 Da (Bos & Meinardi, 2000:169).

Although the transdermal delivery of APIs have made a substantial contribution to medical practice, it has not yet achieved its full potential as an alternative for oral or hypodermic delivery (Prausnitz & Langer, 2008:1261). In the transdermal delivery of an API, the vehicle in which the API is applied has a unique effect on its delivery (Otto *et al.*, 2009:2). It is important to

understand the fate of the different formulation components and the API after application on the skin (Lane *et al.*, 2012:496). After application onto the skin, the composition of the formulation will change as some ingredients permeate the skin, some evaporate and some components are extracted from the skin (Otto *et al.*, 2009:2). When developing an optimised formulation for transdermal delivery it is important to follow an integrated approach considering five principles. These principles include the fact that all APIs have a maximum ideal solubility in a solvent that cannot be exceeded and that the API and the different ingredients will partition into the skin based on the partition coefficient. The diffusion of the API is determined by the concentration gradient and the diffusion coefficient which are influenced by the molecular shape and size and the concentration of the solvent in the skin. It is important to consider the fact that most formulations contain multiple ingredients and the formulation will be delivered as a finite dose (Abbott, 2012:217).

According to Wiechers (2012), the Skin Delivery Gap (SDG) can be used to compare different molecules based on their intrinsic activity and deliverability. A SDG < 1 indicates that an API will permeate the skin, whilst an API with an SDG > 1 may need a more complex delivery system. For transdermal delivery to be possible, the API needs to partition from the formulation into the skin. The formulation influences the stratum corneum/formulation partition coefficient of an API and by altering the properties of the formulation, it is possible to manipulate the transdermal delivery of the API. Wiechers proposed the Relative Polarity Index (RPI) as a tool to obtain the optimal polarity of the formulation to ensure that at least 50 % of the API would be delivered to the skin (Lane *et al.*, 2012:498; Wiechers, 2008:94; Wiechers *et al.*, 2004:174). The RPI uses the polarities (octanol-water partition coefficient (log P)) of the stratum corneum, the formulation and the API to measure the differences in behaviour between the different entities. A small RPI will indicate a small difference and thus better compatibility. The optimal polarity of the formulation is calculated using the following equations and is illustrated in Figure 1.1 (Wiechers *et al.*, 2004:176, 177):

Polarity of formulation > polarity of penetrant + penetrant polarity gap **Equation 1.1**

Polarity of formulation < polarity of penetrant – penetrant polarity gap **Equation 1.2**

The penetrant polarity gap (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 1.3**

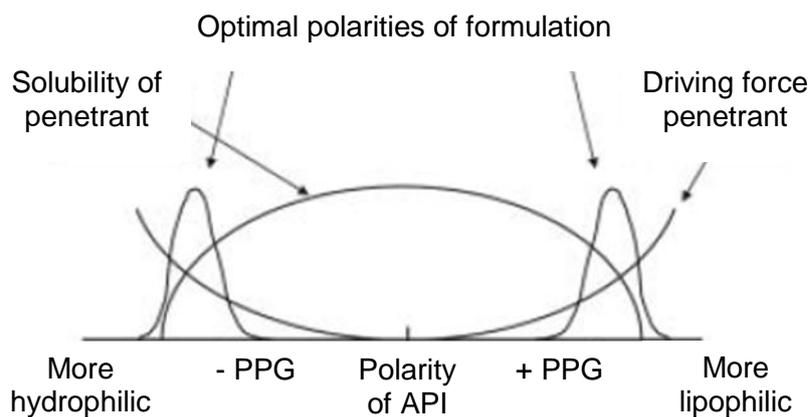


Figure 1.1: A schematic representation of the optimal polarity of the formulation (adapted from Wiechers *et al.*, 2004:177).

The RPI scale has some limitations regarding the use of the log P values of the different entities to describe the polarities. Hansen (2013) states the log P is an impractical indication of polarities, since it is a ratio of the solubility of a compound in something extraordinary (water) and something tedious (octanol). Since it is a ratio, a molecule having a 5:1 ratio and one with a 0.005:0.001 ratio, will have the same log P values and therefore the log P does not fully represent the polarity of the compound. Wiechers and Abbott acknowledged this fact and developed the Formulating for Efficacy™ (FFE™) software using Hansen Solubility Parameters (HSP) as an indicator of polarity (Hansen, 2013). HSP includes general dispersion interaction (E_D), polar cohesion energy (E_P) and hydrogen bonding (E_H) (Hansen, 2007a:4). The combination of these three parameters provides a numerical way to describe the polarity of a molecule (Abbott, 2012:218). The human skin is assumed to be a polymeric barrier with HSP values of $[\delta D, \delta P, \delta H; 17, 8, 8]$ (Abbott, 2012:219). By calculating the HSP distance between the skin, API and formulation using Equation 1.4, it is possible to determine the solubility of the different components in each other.

$$\text{Distance} = \sqrt{4(\delta D_1 - \delta D_2)^2 + (\delta P_1 - \delta P_2)^2 + (\delta H_1 - \delta H_2)^2} \quad \text{Equation 1.4}$$

The smaller the HSP distance, the more soluble the different compounds are in each other. A small distance between the API and the formulation (API-formulation gap (AFG)) indicates that a high concentration of the API can be dissolved in the formulation to provide a high concentration gradient. The smaller the HSP distance is between the formulation and the skin (skin-formulation gap (SFG)), the more likely the ingredients are to penetrate the skin. The penetration of the formulation into the skin will cause swelling of the skin and a more welcome environment for the API is created within the skin. A good balance between the AFG and SFG

will ensure the diffusion of the API into the skin by providing a substantial driving force and additional solubility of the API in the skin caused by the formulation (Abbott, 2012:218).

1.2 Aims and objectives

This study forms part of a larger research project on the optimisation of transdermal API delivery. The transdermal delivery of atropine and atropine sulphate will be investigated by using the FFE™ software and implementing the Delivery Gap principle. The aim of the study is to obtain significant insight on the optimisation of transdermal API delivery by using current science and the understanding of percutaneous absorption, the mechanisms thereof and the most recent developments in strategies for transdermal formulation.

Formulations containing atropine as a model drug for transdermal delivery will be optimised and the *in vitro* skin permeation of the different formulations will be compared. The same formulations will be used for atropine sulphate in order to determine the effect of the salt form on the transdermal delivery.

The objectives of the study are to:

- Develop and validate a high performance liquid chromatography (HPLC) method for atropine.
- Determine the aqueous solubility of atropine.
- Determine the log P and octanol-buffer distribution coefficient (log D) of atropine and atropine sulphate.
- Develop a gel optimised towards the stratum corneum, a more hydrophilic gel and a more lipophilic emulgel containing atropine using the FFE™ software.
- Compound the atropine formulations.
- Use the formulations developed for atropine to compound the atropine sulphate formulations.
- Perform membrane diffusion studies to determine API release from the formulation.
- Perform transdermal diffusion studies followed by tape-stripping to determine and compare the transdermal and topical delivery respectively, of the API from the formulations.

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Chapter 2

Transdermal delivery of atropine by implementing the Delivery Gap principle and the Formulating for Efficacy software

2.1 Introduction

Prior to the 1980's, only a small amount of compounds formulated in relatively simple gels and ointments was delivered via the transdermal route (Wiedersberg & Guy, 2014:150). Although the transdermal delivery of APIs has made a significant contribution to the practice of medicine, it has not yet reached its full potential as an alternative delivery route. Transdermal drug delivery, although having its own limitations, has several advantages over conventional routes of delivery. The greatest challenge for transdermal delivery is that only a limited number of APIs can be administered via this route (Prausnitz & Langer, 2008:1261). Significant efforts have been made to develop various approaches to overcome the skin barrier. The nature of the transdermal delivery vehicle plays a significant role in the promotion of API delivery over the skin (Foldvari, 2000:417).

Prof. J.W. Wiechers established the RPI as a basis to obtain the optimised polarity of the formulation to ensure that at least 50% of the API is delivered (Wiechers *et al.*, 2004:176). This initial theory was further developed by J.W. Wiechers and S. Abbott to provide an integrated approach for the optimisation of the transdermal delivery of cosmetic and pharmaceutical actives using (HSP) (Abbott, 2012:217). This approach focuses on the use of HSP as an indication for solubility rather than the log P.

This chapter focuses on the transdermal delivery of APIs for a systemic effect, factors influencing the delivery and the optimisation of the transdermal formulations.

2.2 Transdermal drug delivery

The topical application of medicaments to the skin dates back over thousands of years when the ancient Greeks made a moisturising balm consisting of water, olive oil and lead oxide which was applied to the skin. The skin was considered as an impermeable barrier until Bourgat and his co-workers proved that topical salicylic acid could be used for the treatment of acute rheumatoid arthritis in 1893 (as cited by Morrow *et al.*, 2007:36). Since their discovery, topical preparations were only prescribed for the treatment of skin diseases. After World War II, nitroglycerine ointment was produced to manage angina attacks after employees working with this ingredient showed less frequent angina attacks. Since then many other topical preparations

were developed to yield a systemic effect (Morrow *et al.*, 2007:36). Most topically applied preparations are relatively simple semi-solids including gels, creams and ointments (Förster *et al.*, 2009:309) or a more complex transdermal patch (Thomas & Finn, 2004:697). According to Barry (2002:500) the aim in dermatological pharmaceuticals is ultimately to design active drugs/pro-drugs and to incorporate them into vehicles or devices for delivery to the active site in the bio-phase at a controlled rate. The transdermal delivery of an API does however have some advantages and disadvantages.

2.2.1 Advantages and disadvantages

As with many alternative routes of administration, transdermal delivery has some advantages and disadvantages in comparison to the oral route.

2.2.1.1 Advantages

The following are some of the advantages of the transdermal drug delivery (TDD):

- TDD eliminates first-pass metabolism and gastrointestinal absorption (Kornick *et al.*, 2003:953).
- Lower dosages may be administered in comparison to oral administration, which may lead to a reduction in adverse effects (Kornick *et al.*, 2003:953).
- TDD provides improved patient acceptance and compliance (Delgado-Charro & Guy, 2001:216).
- Drug therapy can be terminated in the event of adverse effects by removing the formulation from the skin (Delgado-Charro & Guy, 2001:216).
- TDD avoids pain and possible infections associated with injections (Jepps *et al.*, 2012:153).
- TDD provides an alternative route in patients who are unable to take oral dosage forms (Kornick *et al.*, 2003:967).

2.2.1.2 Disadvantages

The following are some of the disadvantages of the TDD:

- The skin is an effective barrier that limits drug delivery (Jepps *et al.*, 2012:153).
- It is difficult to predict the permeability of a compound because of the complex structure and mechanisms of the delivery pathway (Jepps *et al.*, 2012:153).

- The size of a molecule intended for transdermal delivery should be restricted to a molecular weight (MW) of less than 500 Da to ensure easy diffusion (Bos & Meinardi, 2000:169).
- According to Yano (cited by Brown *et al.*, 2005:177), the permeant has to be sufficiently soluble in both aqueous and lipid material (log P of 1-3) in order for it to diffuse through the lipophilic stratum corneum and the underlying aqueous layers to deliver it systemically.
- The enzymes present in the skin can lead to pre-systemic metabolism of the permeant (Steinsträsser & Merkle, 1995:3-25).

2.2.2 Skin permeation

The permeation of an API through the skin is a complex process. After application, the API needs to partition from the formulation into the stratum corneum; only the API molecules adjacent to the skin surface partition into the stratum corneum. This initial step in skin permeation is dependable on the physicochemical properties of both the API and the formulation. The API present in the outer layers of the stratum corneum diffuses through the stratum corneum then partitions into the viable epidermis. The API will then diffuse through the viable epidermis, partition into the dermal-epidermal junction, partition into and diffuse through the dermis to eventually partition into the capillaries and lymphatic vessels for removal into the systemic circulation (Williams, 2003:28).

As seen in Figure 2.1 an API can cross the stratum corneum in three ways: diffusion through the appendages (shunt route), diffusion through the intercellular lipid lamellae and transcellular diffusion through the corneocytes and the lipid lamellae (Lane, 2013:13; Morrow *et al.*, 2007:38; Yamashita & Hashida, 2003:1187).

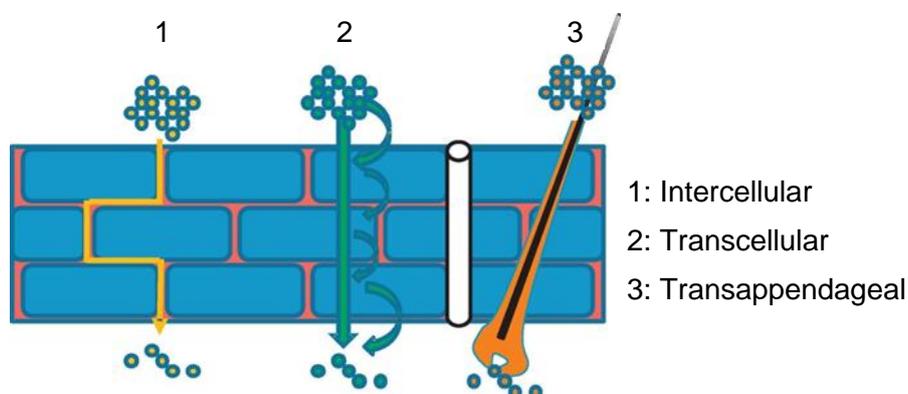


Figure 2.1: Permeation pathways across the skin (adapted from Morrow *et al.*, 2007:38).

2.2.2.1 Diffusion through the appendages (shunt route)

The transappendageal route bypasses the barrier of the stratum corneum by providing a direct channel across the stratum corneum (Morrow *et al.*, 2007:38; Lane, 2013:13). Sweat glands and hair follicles only occupy approximately 1% of the total surface area of the skin and therefore a limited surface area is available for contact with the formulation. Although the transappendageal route provides a small surface area, it is considered as the dominant pathway in the initial phase of skin transport and plays an important role in the delivery of ions and polar compounds as well as compounds such as nanoparticles which have a very high molecular weight (Morrow *et al.*, 2007:38; Lane, 2013:13; Yamashita & Hashida, 2003:1187). The sweat ducts provide an aqueous pathway for drugs across the skin, which can be desirable for many drugs, but in an active secreting sweat duct the aqueous salt solution is moving against the permeant's diffusion pathway which may limit permeation. The sebaceous glands contain sebum which is rich in lipids and this lipophilic sebum can cause a barrier for the permeation of hydrophilic drugs (Morrow *et al.*, 2007:38).

2.2.2.2 Diffusion through the intercellular lipid lamellae

In intercellular diffusion, the permeants follow a tortuous route through the lipid matrix surrounding the corneocytes. The intercellular pathway can be an obstacle for the permeation of substances since the permeants repeatedly diffuse through and partition into aqueous and lipid material (Morrow *et al.*, 2007:38). The path length of the intercellular route is greater than the thickness of the stratum corneum and can range from 150 to 500 μm (Williams, 2003:35). This route is the predominant permeation pathway for small uncharged molecules (Morrow *et al.*, 2007:38).

2.2.2.3 Transcellular diffusion through the corneocytes and the lipid lamellae

Drugs that permeate the skin via the transcellular route diffuse through the keratin containing corneocytes. The highly hydrated keratin provides hydrophilic drugs with an aqueous pathway through which it can diffuse. A lipid envelope surrounds the corneocytes and connects it to the interstitial lipids. Keratinised skin cells are separated by multiple lipid bilayers. The API following the transcellular route will therefore follow a series of diffusion and partitioning steps. The API will partition into and diffuse through the corneocytes after which it will partition into the lipid envelope and finally into the multiple lipid bilayers. During steady-state flux the transcellular route is the major permeation pathway for hydrophilic APIs (Morrow *et al.*, 2007:38).

2.2.3 Physicochemical factors influencing permeation

Some of the physicochemical factors which can influence the permeation of an API through the skin are skin hydration, temperature, pH, pKa and unionised and ionised forms, diffusion coefficient, drug concentration and molecular size (Barry, 2007:576).

2.2.3.1 Skin hydration

The permeability of skin is significantly increased when skin is saturated with water because the tissue swells, softens and wrinkles. Skin hydration can be a result of water diffusion from the underlying epidermal layers or the accumulation of perspiration after the application of a dressing or occlusive vehicle. According to Barry (2002:511), stratum corneum hydration is an important factor that can increase the penetration rate of most substances that permeate the skin.

2.2.3.2 Temperature

Temperature variations can cause changes in the penetration of an API through human skin. A decrease in temperature leads to a decreased diffusion coefficient. Fluctuations in temperature and penetration are usually prevented in humans by adequate clothing on the majority of the body (Barry, 2002:511).

2.2.3.3 pH, pKa and unionised/ionised forms

Only unionised molecules can readily cross the lipid membranes according to the simple form of the pH-partition hypothesis (Aulton, 2007:37; Barry, 2007:576). The degree of dissociation of weak acids and bases is determined by the pH and their pK_a and pK_b values. The ratio of unionised/ionised forms of the API can be calculated using the Henderson-Hasselbalch equation. For a weak base the equation is as follows (Aulton, 2007:37):

$$\text{pH} = \text{pK}_a + \log \frac{[\text{B}]}{[\text{BH}^+]} \quad \text{Equation 2.1}$$

Where:

$$\begin{aligned} \text{pK}_a &= \text{pK}_a \text{ of the API} \\ \frac{[\text{B}]}{[\text{BH}^+]} &= \text{partition coefficient} \end{aligned}$$

The effective membrane gradient is determined by the fraction of unionised API in the applied formula. A limited amount of the ionised form of the API does however penetrate the skin (Barry, 2007:576). These molecules may make a substantial contribution to the total flux since their aqueous solubility is usually higher than that of the unionised species in saturated or near-

saturated solutions. The stratum corneum is remarkably resistant to alterations in the pH and can tolerate a pH range of 3-9 (Barry, 2002:511). The pK_a value for atropine is 9.9 (Moffat *et al.*, 2011:933). At pH 7.4, atropine and atropine sulphate will be almost completely ionised (99 %), which means atropine is highly soluble in the solution but will pose problems in the transdermal delivery.

Aqueous solubility is directly linked to the degree of ionisation (Aulton, 2007:37). An aqueous solubility of > 1 mg/ml is necessary for transdermal delivery (Naik *et al.*, 2000:319). Atropine has an aqueous solubility of 2.19 mg/ml which is sufficient for transdermal delivery (Moffat *et al.*, 2011:933).

2.2.3.4 Diffusion coefficient (D)

The diffusion coefficient (D) is the penetration rate of a molecule under specified conditions. A molecule's diffusional speed is dependable on the state of matter of the medium through which it is diffusing. The diffusion coefficient of a molecule in air and gases is large because the void space available to the molecules is great in comparison to its size and the mean free path is large between molecular collisions. The diffusion coefficient in liquids is decreased because of a decreased free volume and a decreased mean free path. The diffusivities in skin progressively decreases and the lowest values are reached in the compacted stratum corneum matrix. If a constant temperature is maintained, the diffusion coefficient of a drug in the skin or in a topical vehicle is determined by the properties of both the drug and the medium and the interaction between them (Barry, 2002:512). Relative diffusion coefficients can be estimated using the molar volume; the diffusion coefficient depends on (molar volume)^x. Skin has a dependence on molar volume with $x \sim 2$ (Abbott, 2012:219). Atropine has a molar volume of 240.2 cm³/mol (Vafai *et al.*, 1993:126). In solvent blends molecules with smaller molar volumes will enter the skin faster than those with higher molar volumes. If the resulting mixture of solvents produces a less favourable environment for the API, a resulting driving force will be provided to force the API into the skin. If this blend is too unfavourable for the API, the API may precipitate out of the solution and will not be delivered into the skin (Abbott, 2012:219).

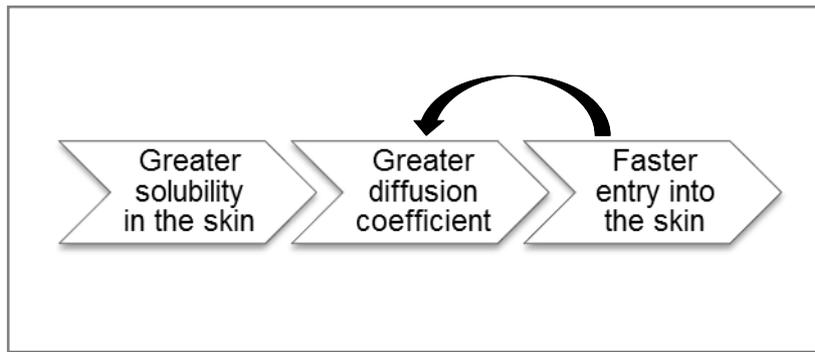


Figure 2.2: The feedback system seen with a solvent that swells the skin (adapted from Abbott, 2012:219).

The diffusion coefficient is strongly dependant on the concentration of additional components in the skin. The diffusion coefficient of both the solvent and the API will be increased if a solvent swells the skin. The effect of this can be seen in Figure 2.2.

2.2.3.5 Molecular shape and size

Drug properties that determine the diffusion coefficient includes molecular shape and size (Abbott, 2012:219). Molecular size and absorption presents an inverse relationship where smaller molecules penetrate the skin faster than larger molecules. The specific effect the size of a molecule has on flux can, however, only be determined if the effect of the size and the resultant change in solubility characteristics can be separated (Barry, 2002:513). Most therapeutic agents selected for transdermal therapy lie within a molecular weight range of 100-500 Da (Williams, 2003:37). Atropine has a molecular weight of 289.4 Da which falls within this range (Moffat *et al.*, 2011:933), therefore based on molecular size, atropine should penetrate the skin.

2.2.3.6 Drug concentration

The permeation of a drug usually follows Fick's law of diffusion. Fick's first law can be written as follows (Rieger, 1993:39):

$$J = \frac{DK}{l} \Delta C$$

Equation 2.2

Where:

- J = flux ($\mu\text{g}/\text{cm}^2 \cdot \text{h}$)
- D = diffusion coefficient (cm^2/h)
- K = partition coefficient
- l = membrane thickness (cm)
- ΔC = concentration gradient ($\mu\text{g}/\text{cm}^2$)

According to Fick's law, the flux of a drug across the skin is proportional to the concentration gradient across the entire barrier phase (Barry, 2002:512). For fast diffusion, a high concentration of API is needed in the outer nanometre of the stratum corneum (Abbott, 2012:219). The solubility of a drug can be optimised by altering the composition of the solvent in the vehicle (Barry, 2002:512). This means the HSP distance should be as small as possible between the HSP of the skin and the permeating species (Abbott, 2012:219).

2.2.3.7 Partition coefficient ($\log P_{\text{octanol/water}}$)

The partition coefficient of an API is an indication of how the compound will distribute between two phases. A partition coefficient between octanol and water is often used in transdermal studies and is an indication for how well the API will distribute between the lipids and water in the stratum corneum. The partition coefficient of an API is usually the principal factor that determines the pathway it will follow through the skin. Atropine has a log P of 1.8 (Moffat *et al.*, 2011:933). It is expected that the dominant route of permeation for hydrophilic compounds will be the intracellular pathway and for lipophilic compounds the intercellular pathway. According to Flyn and Yalkowski (as cited by Williams, 2003:35) an increase in the lipophilicity of an API will increase flux. The bilayered lipids are rate limiting in the flux of lipophilic permeants, indicating that permeation via the intercellular route is allowed by the partition coefficient. The lipid bilayers contain polar areas and hydrophilic compounds may partition into these polar areas and therefore it can cross the skin via the intercellular route as well as the intracellular route. According to Roberts *et al.* (as cited by Williams, 2003:36), this has led to the mixed permeation model proposal which indicates that most drugs permeate the skin via the continuous intercellular domains. The micro-routes of permeation are provided by both the lipid and polar regions in the lipid bilayer and are dependable on the partition coefficient. Molecules such as atropine, with an intermediate partition coefficient ($\log P$ 1-3), will predominately follow the intercellular route. More hydrophilic compounds ($\log P < 1$) will permeate via the transcellular route, but there are still lipid bilayers between keratinocytes which need to be crossed. The transappendageal route may become significant for highly hydrophilic and polar molecules. Highly lipophilic compounds ($\log P > 3$) will almost exclusively traverse the stratum corneum via the intercellular pathway, but the permeant has to partition into the essentially aqueous viable epidermis which can cause restrictions for transdermal delivery (Williams, 2003:36).

2.3 Optimisation of transdermal delivery systems

2.3.1 Theoretical considerations

The first step in the transdermal delivery of an API is the partitioning from the formulation into the stratum corneum. This partitioning is indicated by the stratum corneum/formulation partition coefficient ($K_{sc/formulation}$) of the penetrating molecule and can be defined as:

$$K_{sc/formulation} = \frac{C^{\text{penetrant}} \text{ in stratum corneum}}{C^{\text{penetrant}} \text{ in formulation}} \quad \text{Equation 2.3}$$

Where:

$K_{sc/formulation}$ = Stratum corneum/formulation partition coefficient

$C^{\text{penetrant}}$ = Solubility of the API in the stratum corneum relative to the formulation

To increase the partitioning of an API from the formulation into the skin, the solubility of the API in the formulation can be decreased, or the solubility in the stratum corneum increased. Conversely, the $C^{\text{penetrant}}$ needs to be large in the formulation in order to increase the flux of the API over the stratum corneum, according to Equation 2.3 (Wiechers *et al.*, 2004:174). Changing the parameters of Equation 2.3 can influence the penetration of an API into the skin. The only parameter that can easily be altered by the formulator without having to repeat efficacy studies is the stratum corneum/formulation partition coefficient ($K_{SC/formulation}$), since it depends on the formulation. The formulation determines how much API is dissolved in it and is available for penetration into the skin. A higher concentration of the API in the formulation will mean more of the API will penetrate before saturation is reached. To achieve this, it is required that the API should be highly soluble in the formulation. Another parameter influenced by the formulation is the polarity of the formulation relative to the polarity of the stratum corneum. If an API is better dissolved in the stratum corneum than in the formulation, the API will prefer to penetrate the skin rather than to stay in the formulation. It is therefore required that the API be more soluble in the stratum corneum relative to the formulation. These two requirements cannot be fully adhered to at the same time, but the optimal polarity for the formulation can be obtained by using the novel concept RPI (Lane *et al.*, 2012:498; Wiechers, 2008:94; Wiechers *et al.*, 2004:174) described below. By using the RPI, a formulation can be developed with the best balance between having the highest possible concentration of API in the formulation and ensuring the best driving force for the partitioning of the API into the skin. The optimal polarity of a formulation determined by the RPI will allow the penetration of 50% of the API into the skin (Wiechers, 2008:94).

2.3.2 Skin delivery gap

The SDG, as described by Wiechers (2012), is calculated as the ratio between the minimum effective concentration (MEC) and the local tissue concentration (LTC). An API with a SDG < 1 will be readily delivered to the skin, whilst a higher SDG (> 1) will require a more complex system to deliver an effective concentration of the API at the action site. The LTC can be predicted by utilising a chain of calculations based on the molecular modelling of the skin and pharmacokinetic assumptions. By using the SDG, active molecules can be compared on both their intrinsic activity and their deliverability (Wiechers, 2012).

2.3.3 Relative polarity index

The RPI is a new method in which the polarity of an API can be compared to that of the stratum corneum and the emollients found in the formulations. This unique method can be visualised as a vertical line with a logarithmic scale. The highest polarity is at the top and the highest lipophilicity at the bottom and the log P of the different components is used to express the polarity. The use of this concept requires the following three polarities (on log₁₀ scale): the polarity of the stratum corneum, the API and the formulation. These three polarities are placed on the RPI scale (Wiechers *et al.*, 2004:176). The RPI measures the difference in behaviour between two molecules; a small RPI will indicate a small difference and a large RPI will indicate a large difference (Wiechers. 2008: 95).

To obtain a higher concentration of the API in the stratum corneum than in the formulation, the following equations can be used to determine the required polarity of the formulation (Wiechers *et al.*, 2004:176):

$$\text{Polarity of formulation} > \text{polarity of penetrant} + \text{penetrant polarity gap} \quad \text{Equation 2.4}$$

$$\text{Polarity of formulation} < \text{polarity of penetrant} - \text{penetrant polarity gap} \quad \text{Equation 2.5}$$

The PPG is the difference in polarity between the API and the stratum corneum and can be calculated as follows:

$$\text{Penetrant polarity gap} = |\text{polarity API} - \text{polarity stratum corneum}| \quad \text{Equation 2.6}$$

Figure 2.3 illustrates the optimal polarity of a formulation which will provide the penetration of at least 50% of the API into the stratum corneum.

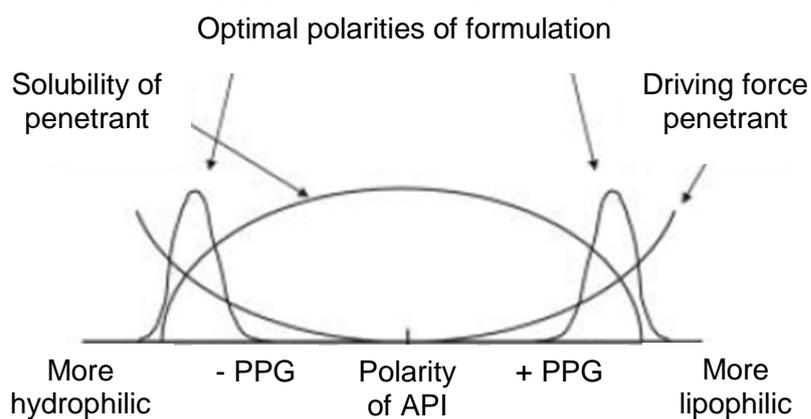


Figure 2.3: A schematic representation of the optimal polarity of the formulation (adapted from Wiechers *et al.*, 2004:177).

Three scenarios can be defined using the RPI scale:

- 1) the polarity of the API is equal to that of the stratum corneum,
- 2) the polarity of the API is larger than that of the stratum corneum,
- 3) the polarity of the API is smaller than that of the stratum corneum (Wiechers *et al.*, 2004:176).

2.3.3.1 Polarity of API equal to the polarity of stratum corneum

When using an API with a polarity equal to that of the stratum corneum in a formulation with the same polarity, the solubility of the API will be the same in both the stratum corneum and the formulation. The only driving force for the API to leave the formulation and enter the stratum corneum is the initial concentration difference upon application. The API will leave the formulation until equilibrium is reached and the concentration of the API in the formulation is equal to the concentration in the stratum corneum. The absolute amount of the API in the two layers will depend on the volumes. The penetration of the API into the stratum corneum will still be significant although the polarity difference is absent. In reality this situation is highly unlikely since most API's have a polarity different to that of the stratum corneum (Wiechers *et al.*, 2004:176).

2.3.3.2 Polarity of API larger than the polarity of stratum corneum

In a situation where the polarity of the API is higher (more hydrophilic) than the polarity of the stratum corneum, the PPG needs to be calculated using Equation 2.6. The PPG is always positive since an absolute difference is used. The polarity of the phase in which the API is dissolved should be either greater than the polarity of the API plus the PPG or less than the

polarity of the API minus the PPG. The larger the polarity difference between the API and the formulation, the greater the driving force will be for the partitioning of the API into the stratum corneum. The negative impact of a large polarity difference between the formulation and the API is a reduction in the solubility of the API in the formulation. The optimal polarity of the formulation can be determined with Equations 2.4 and 2.5, to ensure there will be penetration of at least 50% of the API into the stratum corneum (Wiechers *et al.*, 2004:176).

2.3.3.3 Polarity of API smaller than the polarity of stratum corneum

For an API that is more lipophilic than the stratum corneum, the PPG also needs to be calculated. Again the polarity of the formulation should be more than the polarity of the API plus the PPG, or less than the polarity of the API minus the PPG. In a formulation that is more hydrophilic than the stratum corneum, the API will prefer to penetrate the stratum corneum since its solubility is higher in the stratum corneum than in the formulation. As stated above, a more extreme difference in polarities between the API and the formulation will provide a bigger driving force for the API to penetrate the stratum corneum, but the solubility of the API in the formulation will decrease with an increase of the polarity difference. Equations 2.4 & 2.5 are used to determine the optimal polarity of the formulation (Wiechers *et al.*, 2004:176).

2.3.4 Application of the RPI

To obtain the optimal polarity of the formulation the solubility of the API in the formulation should be optimised (step 1) as well as the driving force (step 2). After determining the log P of the API, the PPG should be calculated. With the PPG known, the two polarities of the formulation can be calculated using Equations 2.4 and 2.5, which will give an indication of whether the API will be dissolved in a hydrophilic or lipophilic phase. A primary emollient or water-miscible solvent should be identified in which the API is dissolved. The RPI-value of this primary emollient or solvent should be identical or close to that of the API (Wiechers *et al.*, 2004:178-179).

The driving force for the penetration of the API into the skin is increased by reducing its solubility in the primary emollient or solvent. To achieve this, a secondary emollient or solvent is incorporated into the formulation. The API should be less soluble in the secondary emollient or solvent, but this secondary emollient or solvent should still be miscible with the primary emollient or solvent. The addition of increasing amounts of the secondary emollient or solvent will decrease the solubility of the API and consequently the amount of dissolved API relative to the amount that could be dissolved increases. When a value of 90% is reached for the fraction of maximum solubility in the solvent mixture, sufficient secondary emollient has been added. This value will allow for temperature changes during transport or storage and will avoid the crystallisation of the API in decreased temperatures (Wiechers *et al.*, 2004:179).

The optimal polarity of the formulation can alternatively be obtained by selecting a single emollient with the correct RPI-value however, this does not allow the combination of different emollients in order to obtain the preferred skin sensory characteristics (Wiechers *et al.*, 2004:179). More than one emollient can be used in fixed ratios as well. An emulsifier should be selected for this to obtain optimised delivery of the API (Wiechers. 2008:98).

2.3.5 Limitations of the RPI scale

The term 'polar' is generally misused in literature since it is possible for a molecule to be mainly polar but insoluble in water (Hansen, 2007c:5). According to Hansen (2013), log P is impractical for many applications including the prediction of skin permeation. Hansen's first objection is the fact that log P is the solubility ratio of a compound in something extraordinary (water) and something tedious (octanol). Secondly, since log P is a ratio, a molecule with a 5:1 ratio will have the same log P as a compound with a ratio of 0.005:0.001. From this it is clear that log P does not fully represent the polarity of the compound. The last objection was the fact that log P primarily depends on the molar volume of a compound. Both Wiechers and Abbott, the creators of the FFE™ software, acknowledged the fact that log P is not a rational indicator for skin permeation and that HSP should rather be used (Hansen, 2013).

2.4 Optimising skin delivery using an integrated approach

To overcome the limitations stated above, Abbott (2012:217) suggests an integrated approach when optimising the skin delivery of pharmaceutical and cosmetic actives. This approach takes the following five key principles into account:

- 1) All actives have a maximum 'ideal solubility'.
- 2) The activity coefficients of actives and solvents will determine their partitioning into the skin.
- 3) Transdermal diffusion can be modelled on a concentration gradients and diffusion coefficients basis and depends on the shape/size of the molecule and the solvent concentration at each point in the skin.
- 4) All ingredients have an effect on the system behaviour.
- 5) Many cosmetic/pharmaceutical formulations are delivered as a finite dose rather than an infinite dose.

2.4.1 Ideal solubility

Thermodynamically, a perfect solvent will have an activity coefficient of one. A high activity coefficient indicates a bad solvent and the API will therefore be insoluble in it (Abbott,

2012:217). Several factors determine the ideal solubility of any compound. These factors include the physicochemical properties of the compound as well as its physical state of matter and environmental factors such as temperature and pressure (Yalkowsky & Wu, 2010:1100). The solubility of an active in an ideal solute can be determined by the crystal-liquid fugacity ratio (CLFR) also known as the ideal mole fractional solubility (X^{ideal}). The CLFR depends on the melting point of the solute, its entropy of melting and differential heat capacity of melting of the solute. According to Yalkowsky & Wu (2012:1105) the estimated ideal solubility can be determined using only the melting point, unless full data are available, using the following equation:

$$\log(\text{CLFR}) = -0.01(\text{MP} - 25) \quad \text{Equation 2.7}$$

This equation is only used when full data about the above mentioned factors are unavailable. From the equation, it is clear a higher melting point (MP) indicates lower solubility (Yalkowsky & Wu, 2010:1105). This estimated solubility can be used to determine if the solvent will sufficiently dissolve at least the minimum effective dose of the API (Abbott, 2012:218).

2.4.2 Solubility and partitioning

Human skin is assumed to be a polymeric barrier (Hansen, 2007a:250). Some of the aspects of human skin and formulations can be characterised using HSP (Hansen, 2007b:316). HSP can be used to predict the solubility of formulation components and how it will partition into the skin. If the HSP is similar for materials, they will have a high affinity for each other. HSP includes three parameters general dispersion interactions (E_D), polar cohesion energy (E_P) and hydrogen bonding (E_H). According to Hansen (2007a:4), the total cohesion energy, E , is the sum of the three individual energies (Equation 2.6).

$$E = E_D + E_P + E_H \quad \text{Equation 2.8}$$

All three components combined provides a numerical way in which a molecule can be described and is more informative than using the term 'polar' (Abbott, 2012:218).

2.4.2.1 General dispersion interactions

Nonpolar interactions are the most general type of interactions in common organic materials. All molecules contain dispersion interactions that are derived from atomic forces since all molecules contain atoms. For saturated aliphatic hydrocarbons the only cohesion interaction is dispersion interactions and therefore the energy of vaporisation is assumed to be equal to the dispersion cohesion energy. The first step for calculating the HSP of a molecule is to find the dispersion cohesion energy, as the homomorph or hydrocarbon counterpart's cohesion energy (Hansen, 2007c:5). Since the dispersion interaction parameter is based on atomic forces, the

atom size plays an important role. For atoms larger than carbon a correction factor is needed when calculating the dispersion energy (Hansen, 2007c:15).

2.4.2.2 Polar cohesion energy

Polar cohesion energy is molecular interactions that most molecules contain. The polar cohesion energy is caused by the permanent dipole-permanent dipole interactions of a molecule. The polar cohesion energy is primarily calculated from the dipole moment (Hansen, 2007c:5).

2.4.2.3 Hydrogen bonding

Hydrogen bonding is also known as the electron exchange parameter and resembles the polar interactions of molecules. This parameter is based on the attraction of molecules caused by hydrogen bonds. The E_H are used to collect the energies not included in the E_D and E_P (Hansen, 2007c:5).

2.4.2.4 Hansen solubility parameter and skin delivery

The oldest solubility rule is that like dissolves like. HSP is used as a measure of how alike components are. The HSP distance between two compounds can be calculated with the following equation:

$$\text{Distance} = \sqrt{4(\delta D_1 - \delta D_2)^2 + (\delta P_1 - \delta P_2)^2 + (\delta H_1 - \delta H_2)^2} \quad \text{Equation 2.8}$$

A smaller HSP distance indicates the molecules are more alike and will therefore have a higher solubility. The HSP values of the skin are assumed to be close to $[\delta D, \delta P, \delta H; 17, 8, 8]$ (Abbott, 2012:219). With the HSP of the skin known, a formulation can be developed with either a good match for the API or for the skin. A small HSP distance between the API and the formulation will indicate high solubility and a high concentration of the API can be incorporated in the formulation increasing the concentration gradient driving force. If there is a small HSP distance between the formulation and the skin, the solvent will easily enter the skin and swell it. This will create a desirable environment for the API in the skin. As more of the solvent enters the skin, less solvent will be available on the skin and the API will thus be encouraged to enter the skin. It is important to obtain a good balance between the AFG and the SFG to ensure there is a substantial driving force and that the API will be encouraged to diffuse into the skin by the additional solubility provided by the solvent in the skin (Abbott, 2012:218).

2.4.3 Transdermal diffusion

The permeation of a topically applied API through the skin is usually facilitated by unidirectional diffusion due to the concentration gradient (Williams, 2003:41). Passive diffusion is the movement of matter from one region to another following random molecular movement. According to Barry (2002:506), Fick's first law of diffusion states that the rate of transfer of diffusing substances per unit area of a section is proportional to the concentration gradient measured normal to the section.

2.4.4 Multi-ingredient formulations

Formulations usually contain several ingredients fulfilling different functions. Most ingredients used in skin formulations have poor solubility profiles. In a solvent mixture, the volume-weighted average of each solvent is used to determine the HSP. Since there are few good solvents for skin delivery, this enables the formulator to create a solvent mixture from poor solvents to obtain a formulation with the correct HSP for skin delivery (Abbott, 2012:219).

2.4.5 Finite dose delivery

Formulations are delivered as a finite dose in practice and therefore it is important to conduct transdermal experiments using a finite dose (Abbott, 2012:220). In contrast with infinite-dosing, the exact starting concentration is known when using a finite dose. As absorption takes place, the drug concentration on the skin surface decreases and the flux falls during the experiment. Using the finite-dose technique mimics *in vivo* situations in a realistic manner (Surber & Davis, 2002:451).

2.6 Summary

Conventional routes of drug delivery have several limitations for many APIs and the transdermal route of administration provides a possible alternative delivery route. Although TDD has many advantages over conventional routes, it also has several limitations, the biggest being the barrier function of the skin. The stratum corneum provides an effective barrier for the skin, which prevents the penetration of many unwanted substances. It is possible however for some compounds to penetrate the skin via the intercellular, transcellular or the transappendageal route. Many different approaches have been developed to overcome the barrier function of the skin. Wiechers developed the RPI to determine the optimised polarity to deliver at least 50% of the API. This theory was further developed by Wiechers and Abbott to an integrated approach that takes five key principles into account when optimising transdermal formulations. These principles include the fact that each API has a maximum ideal solubility, that an API will diffuse and partition into different parts of a system based on the activity coefficient, that most

transdermal formulations consist of multiple ingredients and that a finite dose delivery regime will be followed. This approach uses HSP distance as an indication of how alike different compounds are to indicate solubility and to determine the optimised polarity, rather than the log P scale. The main HSP distances used in this theory are the AFG and the SFG. According to the theory, the AFG should be bigger than the SFG to ensure the optimal delivery of the API. It is important therefore to obtain a suitable balance between the AFG and AFG to ensure optimal delivery and stability.

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Chapter 3

**Article for publication in:
International Journal of Pharmaceutics**

Chapter 3 is written in article format for publication in the International Journal of Pharmaceutics. The complete author guidelines for this journal are in Appendix D. The alignment of the paragraph is justified to ease reading.

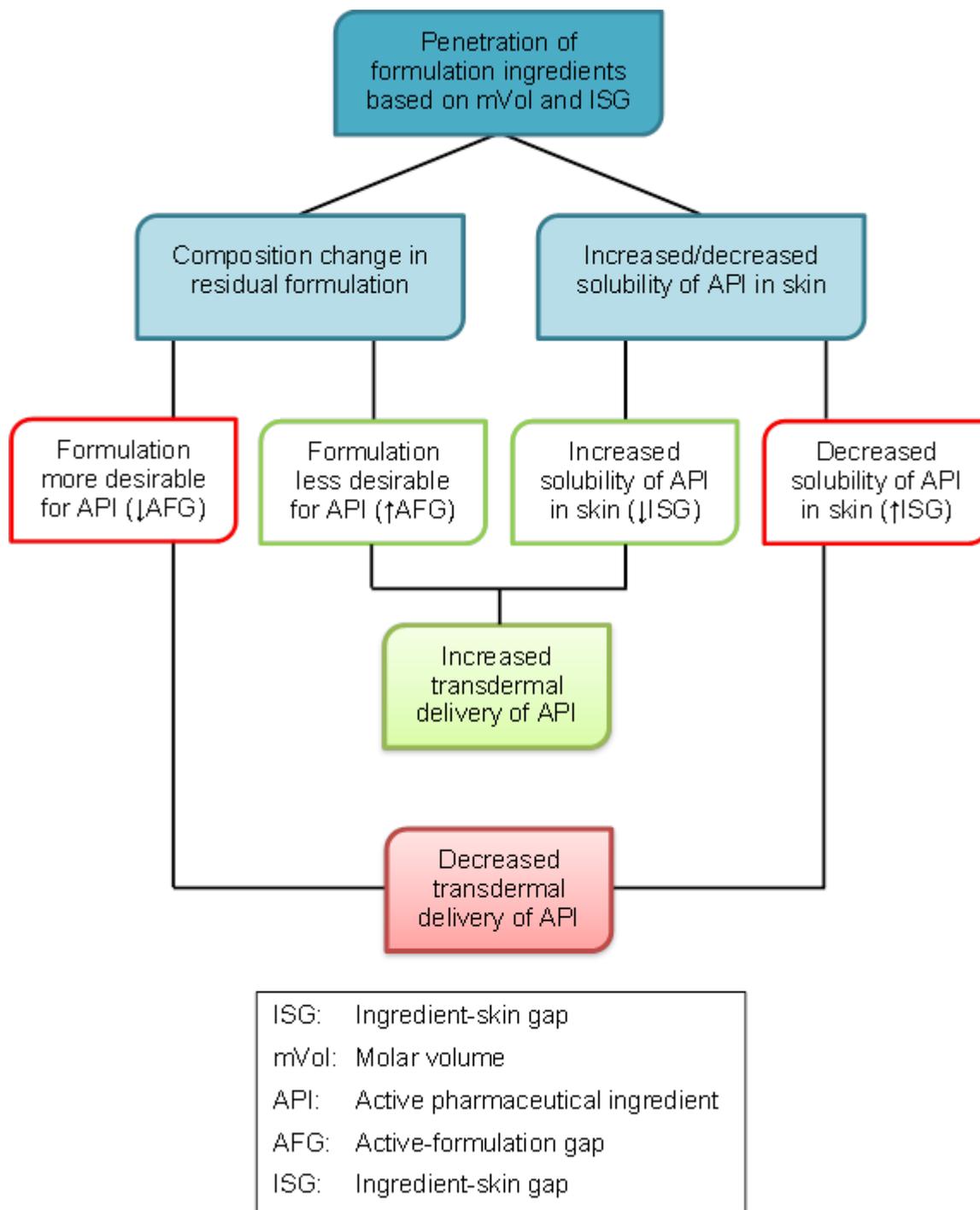
Influence of formulation polarity on the transdermal delivery of atropine by implementation of the delivery gap principle

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Graphical abstract



Abstract

The study was conducted to determine the effect of formulation polarity on the transdermal delivery of atropine and how the sulphate salt form of atropine influences its delivery. The Formulating for Efficacy™ (FFE™) software was employed to develop a gel optimised towards the stratum corneum, a more hydrophilic and a more lipophilic formulation for atropine. The same formulations were used for atropine sulphate. The Skin Delivery Gap (SDG) and the Hansen solubility parameter (HSP) profile of the active pharmaceutical ingredient (API), ingredients and the formulations were determined using the FFE™ software. Membrane release studies were performed over a period of 6 h to confirm the release of the API from the formulations and subsequently transdermal diffusion studies were performed, each over a period of 12 h, followed by tape-stripping. The results indicated the transdermal delivery of an API is influenced by the polarity and the HSP profile of the formulation. The atropine formulations had a higher transdermal diffusion compared to atropine sulphate. The highest transdermal diffusion was obtained from the more lipophilic formulations.

Keywords: Formulation polarity, Hansen solubility parameter, Transdermal delivery

1 Introduction

The transdermal delivery route is an attractive alternative to the conventional oral route (Prausnitz & Langer, 2008). In comparison to the oral route, transdermal delivery has the advantage of eliminating the first-pass metabolism of the active pharmaceutical ingredient (API) as well as the gastrointestinal-tract effects on the API and because of this, lower doses are needed and the occurrence of adverse effects are reduced (Kornick *et al.*, 2003; Walters & Roberts, 2002). If such adverse reactions occur, the treatment can be terminated immediately by removing the formulation (Delgado-Charro & Guy, 2001). The patient acceptance and compliance is higher with transdermal delivery, since it avoids pain and possible infections associated with injections (Delgado-Charro & Guy, 2001; Jepps *et al.*, 2012).

The skin is the largest organ in the human body with many possible application sites for transdermal delivery, but its highly efficient barrier function prevents the penetration of molecules through the skin (Jepps *et al.*, 2012; Williams, 2003). The stratum corneum is primarily responsible for the barrier and needs to be overcome for transdermal delivery to be possible (Prausnitz, 1999). The permeation of an API through the skin is a complex, multi-step process following three possible pathways known as the transappendageal, the transcellular and the intercellular route (Williams, 2003). The permeability of an API is not easily predicted due to the complexity of the mechanisms and the structure of these pathways and an API usually follows a combination of the different pathways, determined primarily by the physicochemical properties of the API (Jepps *et al.*, 2012; Williams, 2003). Only a few APIs are compliant for transdermal delivery, since they should have a log P between 1 and 3 and the molecular weight should not exceed 500 Da (Bos & Meinardi, 2000; Brown *et al.*, 2005; Prausnitz & Langer, 2008).

The Skin Delivery Gap (SDG) was proposed by Wiechers (2012) as an indicator for the permeability of an API. An SDG < 1 indicates sufficient permeability, while an API with an SDG > 1 requires a more complex delivery system. The transdermal delivery of an API is influenced by the properties of the formulation in which it is applied and by altering the properties of the formulation it is possible to manipulate the delivery. Wiechers initially developed the Relative Polarity Index (RPI) as a tool for developing a formulation with an optimal polarity to ensure the delivery of at least 50% of the API. The RPI uses the log P of the stratum corneum, the formulation and the API as an indication of polarity. Hansen (2013) was opposed to the use of log P, regarding it as an impractical indication of polarity and developed the Hansen solubility parameters (HSP) to predict the solubility of two components in each other. Wiechers and Abbott both acknowledged this fact and developed the Formulating for Efficacy™ (FFE™) software which utilises HSP to indicate polarity (Hansen, 2013). HSP uses a combination of

three parameters (general dispersion interaction (E_D), polar cohesion energy (E_P) and hydrogen bonding (E_H)) to provide a numerical way to describe the polarity of a compound (Hansen, 2007a; Abbott, 2012). The HSP profile of the human skin is assumed to be $[\delta D:17, \delta P:8, \delta H:8]$ (Abbott, 2012). To indicate the solubility of one component in another, the HSP distance is calculated using Equation 1.

$$\text{Distance} = \sqrt{4(\delta D_1 - \delta D_2)^2 + (\delta P_1 - \delta P_2)^2 + (\delta H_1 - \delta H_2)^2} \quad \text{Equation 1}$$

A smaller HSP distance will indicate a higher solubility. It is generally accepted that a desirable HSP gap, in terms of solubility and compatibility, is < 4 and that a HSP gap > 8 indicates insolubility and incompatibility (Abbott, 2012). The relevant distances used in the FFE™ software is the ingredient-skin gap (ISG), ingredient-API gap (IAG), API-formulation gap (AFG) and the skin-formulation gap (SFG). HSP is used to characterise many biological materials (Hansen & Poulsen, 2007). Once the HSP profile of the human skin $[17.0, 8.0, 8.0]$, the API $[18.1, 4.7, 8.5]$ and the formulations are identified, the HSP distance/gaps can be used to predict the solubility of the API in the formulation and the skin diffusion (HSP, 2013). The IAG indicates how close the ingredient and the API are in terms of HSP distance and the ISG indicates the HSP distance between the ingredient and the skin. The AFG is an indication of how soluble the API is in the formulation. A smaller AFG will indicate better solubility. The SFG is an indication of how alike the formulation is to the skin. A smaller AFG shows the formulation and the skin are mutually soluble in terms of HSP distance. Wiechers stated that a formulation optimised to the stratum corneum, should have a small SFG to ensure the transdermal delivery of at least 50% of the API, which essentially means two similar layers are applied on each other and the API will be evenly distributed between the formulation and the skin.

In this study, three formulations of different polarities were developed for a model drug (atropine) using the FFE™ software, one optimised towards the stratum corneum, one more hydrophilic than the stratum corneum and one more lipophilic than the stratum corneum. The transdermal delivery of the API from the different formulations was compared to determine the effect of the relevant delivery gaps. To evaluate the effect of the salt form on the delivery the studies were repeated with atropine sulphate instead of atropine in the same formulations.

2 Materials and Methods

2.1 Materials

Atropine and atropine sulphate were obtained from Sigma-Aldrich (Kempton Park, South Africa). Other ingredients used in the formulations include: Carbopol[®] Ultrez 10 polymer (Lubrizol Advanced Materials, Brussels, Belgium), dimethyl isosorbide (DMI) (Sigma-Aldrich, Kempton Park, South Africa), polyethylene glycol 400 (PEG-8), Tween[®] 80 (PEG-20 sorbitan monooleate), Span[®] 60 (sorbitan monostearate) and paraffin liquid (all from Merck Chemicals, Halfway House, South Africa) and ethanol (ACE Chemicals, Johannesburg, South Africa). For the phosphate buffer solution (PBS) potassium dihydrogen orthophosphate and sodium hydroxide (both obtained from Merck Chemicals, Halfway House, South Africa) was used. Merck Chemicals (Halfway House, South Africa) also supplied the 1-octane sulphonic acid sodium salt and HPLC (high performance liquid chromatography) grade methanol. Deionised water was prepared with a Milli-Q[®] water purification system (Millipore, Milford, USA).

2.2 HPLC analysis

The HPLC method for atropine was developed and validated in the Analytical Technology Laboratory at the North-West University, South Africa. An Agilent HP1100 series HPLC with a pump, autosampler, UV detector and Chemstation Rev. A.10.02 data acquisition and analysis software was used (Agilent Technologies, Palo Alto, CA). A Luna C18-2 column (150 x 4.6 mm, 5 µm, Phenomenex, Torrance, CA) was used. The mobile phase consisted of methanol and 0.005 M 1-octane sulphonic acid-Na in water (pH adjusted to 3.5 with diluted phosphoric acid) in a ratio of 58:42. The flow rate was 1.0 ml/min with a default injection volume of 50 µl. The UV detector was set at 210 nm for atropine. The retention time of atropine was ± 5.1 min and the stop time was set at 8.0 min.

2.3 Phosphate buffer solution (pH 7.4) preparation

Potassium dihydrogen phosphate (6.805 g/250.0 ml water) and sodium hydroxide (1.574 g/393.4 ml water) were mixed and the pH adjusted to 7.4 with sodium hydroxide and phosphoric acid (BP, 2014).

2.4 Formulation of gels

All the formulations were prepared following the same method. The Carbopol[®] was sprinkled over the water and left for ± 2 min to ensure wetting, after which it was heated to 40 °C, followed by homogenisation at 800 rpm. All the ingredients of the oil phase were mixed and atropine was added. Both phases were separately heated to 50 °C, after which the oil phase was slowly

added to the water phase during homogenisation at 1800 rpm. The mixture was homogenised until a temperature of 40 °C was reached, after which it was stirred with a glass rod until it cooled down to 25 °C. The pH was adjusted to 7.4 using sodium hydroxide.

For the preparation of the atropine sulphate formulations, the atropine was substituted with the atropine sulphate and this was added to the water phase instead of the oil phase due to its hydrophilicity.

2.5 Viscosity

The viscosity of the formulations was measured using a Brookfield DV2T Viscometer (Stoughton, Massachusetts, USA). After heating the formulation to 25 °C in a water bath it was placed in a small sample adapter. Viscosity measurements were made every 10 sec for 2 min using a SC4-25 spindle turning at a speed of 0.70 rpm.

2.6 Physicochemical properties

2.6.1 Solubility of atropine

Triplicate saturated solutions of atropine in PBS (pH 7.4), water and *n*-octanol were prepared and shaken in a water bath for 24 h at 32 °C. Excessive amounts of atropine were used to ensure the solution remained saturated. After 24 h the solutions were centrifuged, diluted and analysed using HPLC.

2.6.2 *n*-Octanol/PBS distribution coefficient

Equal amounts of PBS (pH 7.4) and *n*-octanol were mixed and left to separate to produce *n*-octanol pre-saturated with PBS and vice versa. Atropine (10.84 mg) was dissolved in the pre-saturated *n*-octanol and equal volumes (3 ml) of this and pre-saturated PBS was inserted into a test tube. The test tube was shaken in a water bath at 32 °C for 24 h after which it was centrifuged at 4500 rpm for 10 min. The *n*-octanol phase (2 ml) was diluted to 10 ml using methanol and both solutions were analysed using HPLC. The logarithmic ratio of the atropine concentration in the *n*-octanol and the PBS (pH 7.4) were used to calculate the log D (*n*-octanol/PBS). The experiment was performed in triplicate and the aforementioned method was used to determine the log D for atropine sulphate as well.

2.7 Skin preparation

During this study, Caucasian abdominal skin obtained after abdominoplastic surgery was used. The donors gave informed consent and ethical approval was obtained from the Ethics Committee of the North-West University, Potchefstroom (Ethics number: NWU-00114-11-A5).

Split thickness skin (400 μm) containing stratum corneum, viable epidermis and upper dermis was removed using an electric dermatome (Zimmer Inc.) and placed on Whatman[®] filter paper with the stratum corneum facing upwards, wrapped in aluminium foil and frozen at $-20\text{ }^{\circ}\text{C}$ until use. The skin samples were thawed and cut into circles with a diameter of $\pm 2\text{ mm}$ prior to the skin diffusion study.

2.8 Diffusion studies

Vertical Franz type diffusion cells with a 1.075 cm^2 diffusion area and a $\pm 2\text{ ml}$ receptor capacity were used during this study. Ten cells, with a magnetic stirring bar inserted in the receptor compartment of each to ensure stirring during the experiment, were used. The Franz cells were assembled with the membrane/skin samples (stratum corneum facing upwards) mounted between the donor and receptor compartment and sealed and secured using Dow Corning[®] high vacuum grease and horseshoe clamps. The receptor phase (2 ml), pre-heated to $37\text{ }^{\circ}\text{C}$, was injected into the receptor compartment whilst preventing air bubble formation and 1 ml of the semi-solid formulation (pre-heated to $32\text{ }^{\circ}\text{C}$, temperature of the skin when diffusion is performed at $37\text{ }^{\circ}\text{C}$) was inserted in the donor compartment and covered with Parafilm[®]. The entire study was performed in a water bath ($37 \pm 1\text{ }^{\circ}\text{C}$ to compare with the human body temperature) with a magnetic stirrer. The entire receptor phase content was extracted and replaced with fresh receptor phase on predetermined time after which the extracted receptor phase was injected into HPLC vials for analysis.

2.8.1 Membrane release

Membrane release studies were performed to determine release of atropine and atropine sulphate from the formulations following the method discussed in Section 2.8. Hydrophilic polyvinylidene fluoride (PVDF) membrane filters (Pall[®] Life Sciences, Michigan, USA) were used and the receptor phase (PBS pH 7.4) content was extracted and replaced hourly for 6 h.

2.8.2 Skin diffusion

Skin diffusion studies were performed following the method discussed in Section 2.8. During initial studies the receptor phase (PBS pH 7.4 and methanol (1:1, v/v)) were extracted and replaced hourly, but the concentration atropine in the receptor phase was below the limit of detection. It was then decided to change the sampling times to one single sampling time, in order to be able to measure the total amount diffused during that time period. The receptor phase was therefore only extracted after 12 h and tape-stripping commenced immediately afterwards.

2.8.3 Tape-stripping

The Franz cells were disassembled after 12 h and the skin samples were pinned to a solid surface followed by removal of the semi-solid formulation by light dabbing with tissue paper. Sixteen strips of 3M Scotch® Magic™ tape (discarding the first strip) were used to remove the stratum corneum-epidermis (SCE). The remainder of the skin (epidermis-dermis (ED)) was cut into small pieces; thereafter the strips (SCE) and the skin (ED) were placed in separate polytop glass vials filled with 5 ml receptor phase, capped and kept overnight at 4 °C.

2.9 Data analysis

The cumulative concentration of the API that permeated the membrane was plotted against time for the membrane release studies. The average flux was obtained by the slope of the straight line between 2 and 6 h. For the diffusion studies the percentage yield after 12 h was determined.

2.10 Statistical analysis

Statistica (StatSoft, 2014) was utilised for the statistical analysis using both descriptive and inferential statistics. Both parametric and non-parametric statistical analyses were performed since the data was not distributed normally. A two-way analysis of variance (ANOVA) followed by Tukey's HSD (honestly significant difference) were performed on the membrane data, whilst a univariate test of significance was performed for the skin diffusion studies. For the tape-stripping data, a three-way ANOVA and *t*-test was performed.

Non-parametric statistical analyses of the membrane and skin diffusion studies were performed using the Kruskal-Wallis test and the Mann-Whitney U test for the tape-stripping data. A *p*-value < 5 indicated statistical significance.

3 Results and discussion

3.1 Formulation of gels

The FFE™ software was used to develop a formulation optimised towards the stratum corneum (A-O), a more hydrophilic (A-H) and a more lipophilic (A-L) formulation containing 1.5% atropine. The formulations developed for atropine were used for atropine sulphate as well (AS-O, AS-H and AS-L). The formulations of different polarities were obtained by adapting the optimised formulation. For the more hydrophilic formulation, 10% ethanol was added to the water phase and for the more lipophilic formulation, 10% liquid paraffin was added to the oil phase.

In total six formulations were prepared, all of which applied easily. All the formulations had a uniform appearance; the optimised and lipophilic formulations had an acceptable skin feel, whilst the hydrophilic formulations were a bit tacky. The optimised and hydrophilic formulations were opaque, whilst the lipophilic formulations were white.

The FFE™ software was used to determine a hypothetical SDG (0.001) for atropine based on a plasma concentration of 2 ng/ml obtained from literature (Kradjan *et al.*, 1985). According to the Delivery Gap principle, atropine should readily penetrate the skin (Wiechers, 2012).

3.2 Formulation characteristics

3.2.1 HSP profile

The smallest IAG was observed with DMI (3.09) indicating the best solubility, whilst the IAG of ethanol (13.14) and liquid paraffin (47.62) indicated insolubility. The IAG of PEG-8 (4.81) indicates solubility but not within the preferred range. DMI will readily permeate the skin based on the small ISG (1.96). The ISG values of PEG-8 (7.16) and ethanol (7.1) indicates the ingredient is partly soluble in the skin but the solubility is undesirable. Liquid paraffin is highly unlikely to penetrate the skin based on its ISG (47.62).

For the optimised formulation the AFG (3.5) was bigger than the SFG (1.9), indicating sufficient solubility of the API in the formulation and good penetration of the formulation into the skin. As the formulation penetrates, the skin swells and a more welcome environment for the API is created (Abbott, 2012). The penetration of the formulation results in less solvent left on the skin for the API and the composition of the residual formulation is different. Based on the mVol (158.9 mol/ml and 320.0 mol/ml, respectively) and ISG of DMI it will penetrate the skin faster than PEG-8 indicating that more PEG-8 is left in the residual formulation and the SFG will therefore be higher. The more favourable environment for the API (caused by a decreased

AFG) indicates a decrease in the permeation of the API.

For the hydrophilic formulation, the AFG (5.3) and SFG (5.7) are almost the same and the AFG/SFG close to one (0.9). Both distances are > 4 indicating the formulation may have solubility and compatibility problems. The low solubility of the API in the hydrophilic formulation indicates the API may precipitate out of the formulation.

The API is sufficiently soluble and compatible in the more lipophilic formulation based on the AFG (3.8). The high SFG (9.5) indicates the formulation is insoluble and incompatible with the skin which predicts poor delivery, but individual ingredients with desirable ISG's, such as DMI, might still penetrate the skin leaving a formulation with a less desirable composition on the skin. This will provide a high driving force for the API to leave the formulation and enter the skin.

3.2.2 Viscosity

A much higher viscosity was observed with the atropine formulations compared to the atropine sulphate formulations. The highest viscosity was measured with A-H (288.80 ± 0.97 P, pH 7.67), followed by the A-L (264.91 ± 0.99 P, pH 7.57) and lastly, the A-O (242.46 ± 1.99 P, pH 7.47). AS-L (92.32 ± 0.45 P, pH 7.49) had the highest viscosity, followed by AS-H (71.30 ± 0.00 P, pH 7.5) and AS-O (67.30 ± 0.40 P, pH 7.48). A higher viscosity resists the diffusion of the API through the formulation and therefore a high viscosity may reduce the permeation of the API (Cross *et al.*, 2001). The higher viscosity of the atropine formulations compared to the atropine sulphate formulations predicts that the API will reach the skin surface faster from the sulphate formulations and penetration can commence faster.

3.3 Physicochemical properties

3.3.1 Solubility

Naik *et al.* (2000) state an aqueous solubility > 1 mg/ml is required to ensure effective transdermal delivery of an API. The aqueous solubility of atropine (0.9 mg/ml) therefore predicted limited transdermal delivery. Atropine had a much higher solubility in PBS (pH 7.4) (5.8 mg/ml) because of the high degree of ionisation and the formation of ion-pairs with the phosphate salt.

3.3.2 *n*-octanol/PBS distribution coefficient

Log D is a more reliable indication of distribution compared to the octanol/water partition coefficient (log P), since it takes the degree of ionisation of the API into account. For both atropine and atropine sulphate, the log D (-1.26 and -1.23, respectively) predicted that

transdermal delivery may be suboptimal (Brown *et al.*, 2005).

3.4 Diffusion studies

3.4.1 Membrane release studies

During the membrane release studies, formulations containing 0.5% API were used and the results confirmed the release of API from all formulations. The data contained outliers and therefore the median values are used to describe the data. Compared to the average, the median is a more reliable representation of data as it is more resistant to outliers (Smith, 2012). The highest median flux for the atropine formulations were observed with A-H (155.06 $\mu\text{g}/\text{cm}^2\cdot\text{h}$), followed by A-O (136.74 $\mu\text{g}/\text{cm}^2\cdot\text{h}$) and A-L (129.91 $\mu\text{g}/\text{cm}^2\cdot\text{h}$). For atropine sulphate the highest median flux was observed with AS-O (150.29 $\mu\text{g}/\text{cm}^2\cdot\text{h}$), followed by AS-H (117.76 $\mu\text{g}/\text{cm}^2\cdot\text{h}$) and AS-L (115.99 $\mu\text{g}/\text{cm}^2\cdot\text{h}$) (see Figure 1).

Figure 1: Flux ($\mu\text{g}/\text{cm}^2\cdot\text{h}$) of atropine and atropine sulphate from the different formulations in the membrane release studies after 6 h. The average and median concentration values are indicated by the lines and squares, respectively (AS-O: n = 10; A-O, A-L, AS-L: n = 9; A-H, AS-H n = 8).

The highest median percentage released was observed with A-H (13.93%), whilst AS-H resulted in the lowest median percentage released (11.05%). The low affinity of the lipophilic atropine for the hydrophilic formulation causes a driving force for the API to leave the undesirable formulation, resulting in the high release, while the more hydrophilic atropine sulphate has a high solubility in the hydrophilic formulation and therefore low release is observed (Otto *et al.*, 2009). The atropine formulations only had slight differences in the percentage released after 6 h (A-H: 13.93%, A-O: 13.16% and A-L: 12.58%). Atropine has a high affinity towards the lipophilic formulation which resulted in low release; the atropine sulphate formulations indicated more variation in the percentage released compared to the atropine formulations. The highest median percentage released was obtained with the AS-O (13.12%), followed by AS-L (11.07%) and AS-H (11.05%).

From the results it was observed that atropine resulted in a higher release compared to atropine sulphate from formulations with the same polarity. This indicates that the salt form of the API reduces its release from the formulations due to the higher HSP of the salt and therefore a high affinity for the water content of the formulations (Hansen, 2007b).

3.4.2 Skin diffusion studies

The diffusion studies were initially performed with formulations containing 0.5% API, but either very low concentrations or no API at all was delivered transdermally. The explanation for this was because of an insignificant concentration gradient which existed between the donor and the receptor and therefore it was decided that the concentration of the API in the formulation had to be increased to 1.5%.

Figure 2: Amount per area ($\mu\text{g}/\text{cm}^2$) of atropine and atropine sulphate that diffused through the skin from the different formulations. The average and median concentration values are indicated by the lines and squares, respectively (AS-O: n = 10; A-O, A-L, AS-L: n = 9; A-H, AS-H n = 8).

For both A and AS the highest median amount/area was observed from the L-formulations followed by the O- and H-formulations. A-O ($10.40 \mu\text{g}/\text{cm}^2\cdot\text{h}$) and AS-O ($6.66 \mu\text{g}/\text{cm}^2\cdot\text{h}$) resulted in nearly double the median amount/area diffused of A-H ($4.50 \mu\text{g}/\text{cm}^2\cdot\text{h}$) and AS-H ($2.78 \mu\text{g}/\text{cm}^2\cdot\text{h}$), respectively. For A-L ($18.04 \mu\text{g}/\text{cm}^2\cdot\text{h}$) the median amount/area diffused was almost twice that of A-O, whilst the median amount/area diffused for AS-L ($17.6 \mu\text{g}/\text{cm}^2\cdot\text{h}$) was almost three times that of AS-O.

When comparing the diffusion from the formulations of different polarities the highest median percentage diffused was observed with the L-formulation for both A and AS (0.065% and 0.063%) followed by the O-formulation (0.037% and 0.024%) and the H-formulation (0.015% and 0.010%). For AS-L the median percentage diffused was almost three times that of AS-O, whilst A-O was almost half that of A-L. A-O and AS-O resulted in nearly double the median percentage diffused of A-H and AS-H, respectively.

The differences in the diffusion can be explained by considering the IAG, ISG, AFG and SFG of the different formulations. Although the SFG of the lipophilic formulation indicates poor penetration into the skin, it resulted in the highest transdermal delivery of the API. It is hypothesised that the DMI penetrated the skin to create a more welcome environment for the API in the skin while the PEG-8 and liquid paraffin was left on the skin. The residual formulation was an undesirable environment for the API based on the IAG values of PEG-8 and liquid paraffin. The combination of the more welcome environment in the skin and the less desirable residual formulation provided a driving force for the API to leave the formulation and enter the skin (Abbott, 2012). For the optimised formulation both the AFG and SFG indicated desirable solubility and the formulation should easily penetrate the skin (Abbott, 2012). A more desirable

environment for the API in the skin was created by the penetrating formulation and the API distributed almost evenly between the formulation and the skin because of their similarity (Abbott, 2000). The only driving force for the API to leave the formulation was the concentration gradient and therefore lower transdermal delivery was observed with the optimised formulation compared to the more lipophilic formulation. The lowest transdermal delivery was observed from the hydrophilic formulations, since both the AFG (5.3) and SFG (5.7) indicate undesirable solubility and thus low skin penetration (Abbott, 2012).

A comparison between the diffusion of A and AS from formulations of similar polarity indicated that A resulted in higher transdermal delivery than AS. A-H (0.015%) and A-O (0.370%) resulted in almost twice the median amount/area diffused of AS-H (0.100%) and AS-O (0.240%). No significant difference was observed between A-L (0.065%) and AS-L (0.063%). These results can be explained by considering the log P values of both A and AS. A gel typically is a polar formulation (H and O) and the more hydrophilic AS would therefore prefer to reside in the formulation (Barry, 2007). The gel formulations are an undesirable environment for the lipophilic atropine and it would prefer to leave the formulation. The higher transdermal delivery of A, compared to AS, can be explained by the higher release from the formulations observed with A, which indicates more of A is on the skin surface and available for penetration.

3.3.3 Tape-stripping

Figure 3: Concentration ($\mu\text{g/ml}$) of atropine and atropine sulphate in the stratum corneum-epidermis for the different formulations after tape stripping. The average and median concentration values are indicated by the lines and squares, respectively (AS-0: n = 10; A-O, A-L, AS-L: n = 9; A-H, AS-H n = 8).

Figure 4: Concentration ($\mu\text{g/ml}$) of atropine and atropine sulphate in the epidermis-dermis for the different formulations after tape-stripping. The average and median concentration values are indicated by the lines and squares, respectively (AS-0: n = 10; A-O, A-L, AS-L: n = 9; A-H, AS-H n = 8).

A higher median concentration of the API was observed in the ED compared to the SCE, except with A-O and AS-O which had no API in the SCE or ED and A-L and AS-L for which no concentration of API was found in the SCE. The highest concentration in both the SCE and the ED was observed with the A-H (0.21 $\mu\text{g/ml}$ and 0.55 $\mu\text{g/ml}$) and AS-H (0.29 $\mu\text{g/ml}$ and 0.55 $\mu\text{g/ml}$), followed by the lipophilic formulations in the ED (A-L: 0.48 $\mu\text{g/ml}$ and AS-L: 0.52 $\mu\text{g/ml}$)

(see Figure 3 & 4). Due to the absence of a driving force for the API to leave the formulation and enter the skin, based on the HSP profile of the H-formulation, the penetration of the API was only driven by the concentration gradient. The API penetrated the skin much slower compared to the O- and L-formulation and after 12 h the API had not yet fully traversed the skin resulting in high concentrations in the skin and poor transdermal delivery. This indicates the added driving force provided by the HSP profile (as seen with the O- and L-formulations) increases the penetration of the API through the skin. A slightly higher median concentration in the SCE was observed with AS-H compared to A-H, while being the same in the ED. The higher concentration of the API in the ED than in the SCE is opposite to what was expected, since the lipophilic atropine has a higher affinity towards the more lipophilic stratum corneum.

High concentrations of the API were expected from the formulations optimised towards the stratum corneum. An explanation for the low concentration of API in the skin from the optimised formulations may be a fast initial penetration and a decrease in the concentration gradient over time reduced the driving force and the amount of API in the skin was below the limit of detection of the analytical method. The highest driving force for the API to enter the skin was observed with the lipophilic formulations and the high percentage diffused compared to the skin concentration indicates that the API had fully crossed the skin.

The lipophilic formulations had the highest driving force to leave the formulation and permeate the skin based on the HSP profile. The high driving force pushed the API into the SC where it diffused to the ED. The high transdermal delivery from the L-formulations indicates most of the API fully traversed through the SC into and through the ED.

3.4 Statistical analysis

3.4.1 Membrane release studies

A one-way ANOVA followed by Tukey's studentised range HSD (honestly significant difference) tests were used to analyse the data of H, L and O for both A and AS. Due to the non-normality of the data, non-parametric analysis was performed using the Kruskal-Wallis test followed by multiple comparisons between H, L and O. Significant differences were indicated between A-H and A-L ($p = 0.039$), AS-O and AS-H ($p = 0.018$), as well as between AS-O and AS-L ($p < 0.001$).

3.4.2 Skin diffusion studies

Both the amount/area diffused and the log amount/area diffused was analysed using the univariate ANOVA test. Both indicated non-normality of the data and subsequently non-parametric statistical analyses was performed using the Kruskal-Wallis test, followed by multiple

comparisons. Significant differences were indicated between A-O and A-H ($p = 0.032$) and AS-H and AS-L ($p = 0.005$).

3.4.3 Tape- stripping

The tape-stripping data was analysed using the Mann-Whitney U test which indicated a significant difference between A-O and AS-O in the SCE ($p = 0.0256$), but the significance was disregarded due to the absence of the API in the skin. The test indicated no significant difference between A-H and AS-H in both the SCE ($p = 0.1035$) and ED ($p = 0.9581$) and for A-L and AS-L in the ED ($p = 0.2703$).

4 Conclusion

The highest transdermal delivery of both atropine and atropine sulphate was obtained from the more lipophilic formulations when compared to the optimised and more hydrophilic formulation. The results of the study indicated that atropine provides better transdermal delivery compared to the sulphate salt.

The results obtained in the study supported the Delivery Gap Principle developed by Wiechers (2012), which indicates that an API with an SDG < 1 will be readily delivered to the skin. It is concluded that the HSP profile and the polarity of a formulation plays a significant role in the transdermal delivery of an API. It is believed that a formulation with a small SFG is optimised towards the stratum corneum and should result in the highest transdermal delivery. According to Abbott (2012), a good balance between the SFG and AFG will provide a driving force for the API to leave the formulation and generate a more desirable environment in the skin resulting in sufficient penetration of the API. The results indicated higher transdermal delivery is observed from a formulation with an SFG $>$ AFG. From the results it can be concluded that not only the HSP profile of the formulation, but also the HSP profile of the different ingredients and their molar volumes should be taken into account when developing a formulation optimised for transdermal delivery. Different ingredients in the formulation will penetrate at different rates and this will change the composition of the residual formulation. If the residual formulation is less desirable for the API it will be forced out of the formulation, increasing transdermal delivery. If the penetrating ingredients have a small IAG it will create a welcome environment for the API in the skin which will also contribute to a higher transdermal delivery.

The study proves the FFE™ software, developed by Wiechers, is valuable when developing formulations optimised for the transdermal delivery of an API. It can be concluded that the base API (atropine) will result in a higher transdermal delivery compared to the sulphate salt form (atropine sulphate) due to the higher HSP of the salt, which indicates a higher aqueous solubility and low solubility in polymers such as the human skin (Hansen, 2007b)

Acknowledgements

The authors would like to thank the National Research Foundation (NRF) of South Africa and the Centre of Excellence for Pharmaceutical Sciences (Pharmacén), North-West University, Potchefstroom Campus, South Africa, for funding this study.

We would like to thank Prof F Steyn for the statistical analysis.

Any opinions, findings, conclusions, or recommendations expressed in this material are those of the author(s) and are not necessarily attributed to the NRF.

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Figure legends:

Figure 1: Flux ($\mu\text{g}/\text{cm}^2\cdot\text{h}$) of atropine and atropine sulphate from the different formulations in the membrane release studies after 6 h. The average and median concentration values are indicated by the lines and squares, respectively (AS-0: n = 10; A-O, A-L, AS-L: n = 9; A-H, AS-H n = 8).

Figure 2: Amount per area ($\mu\text{g}/\text{cm}^2$) of atropine and atropine sulphate which diffused through the skin from the different formulations. The average and median concentration values are indicated by the lines and squares, respectively (AS-0: n = 10; A-O, A-L, AS-L: n = 9; A-H, AS-H n = 8).

Figure 3: Concentration ($\mu\text{g}/\text{ml}$) of atropine and atropine sulphate in the stratum corneum-epidermis for the different formulations after tape-stripping. The average and median concentration values are indicated by the lines and squares, respectively (AS-0: n = 10; A-O, A-L, AS-L: n = 9; A-H, AS-H n = 8).

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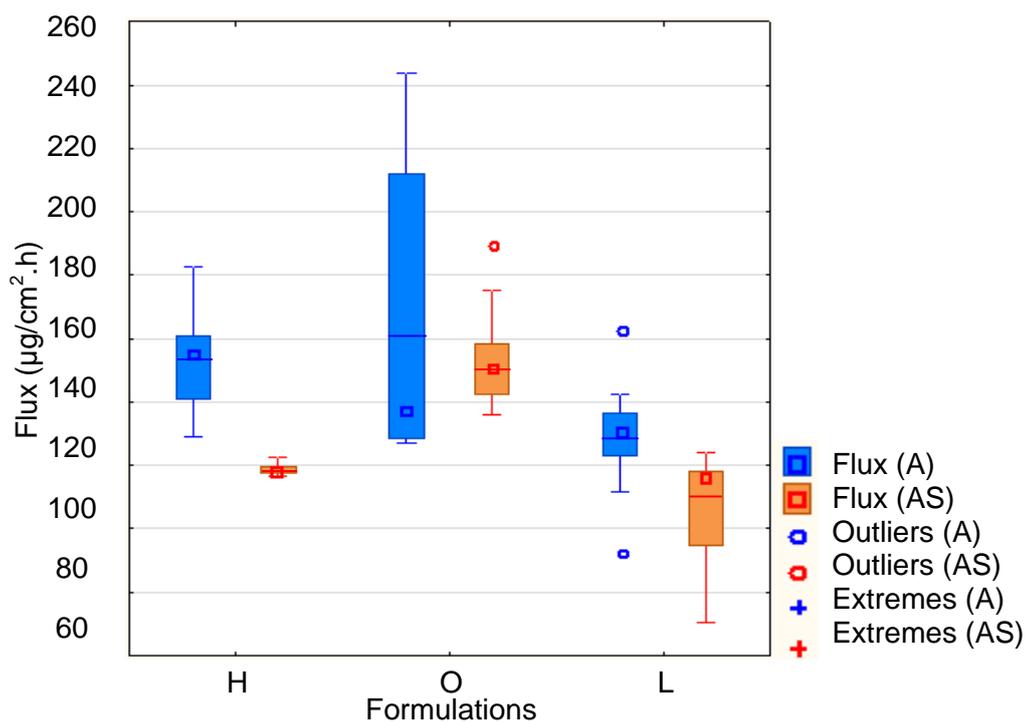


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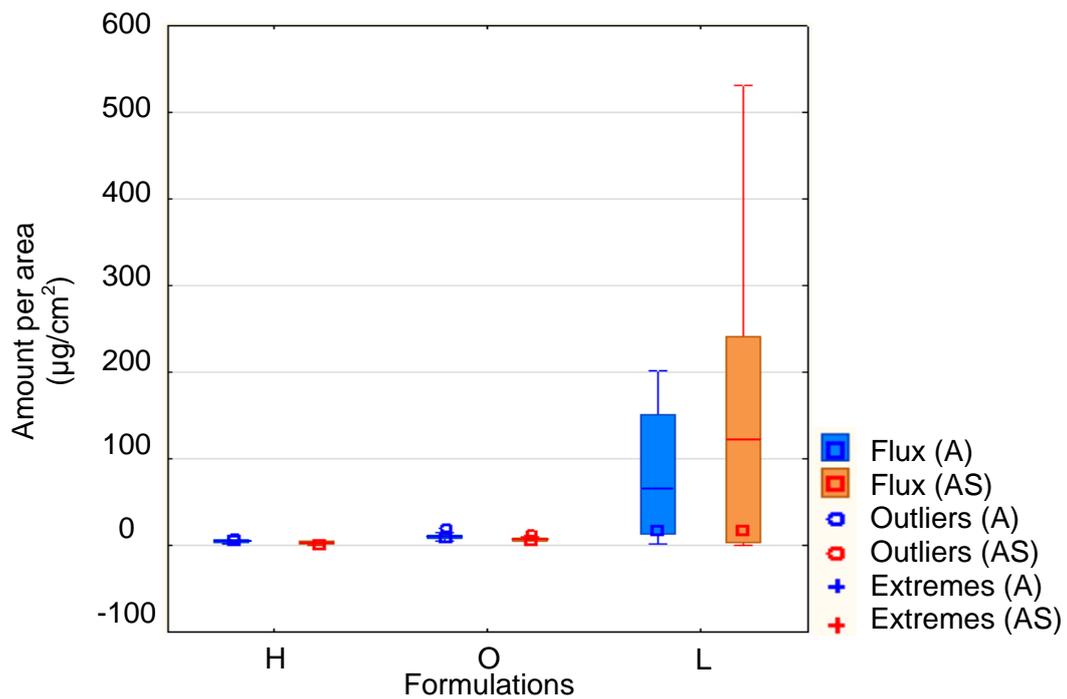


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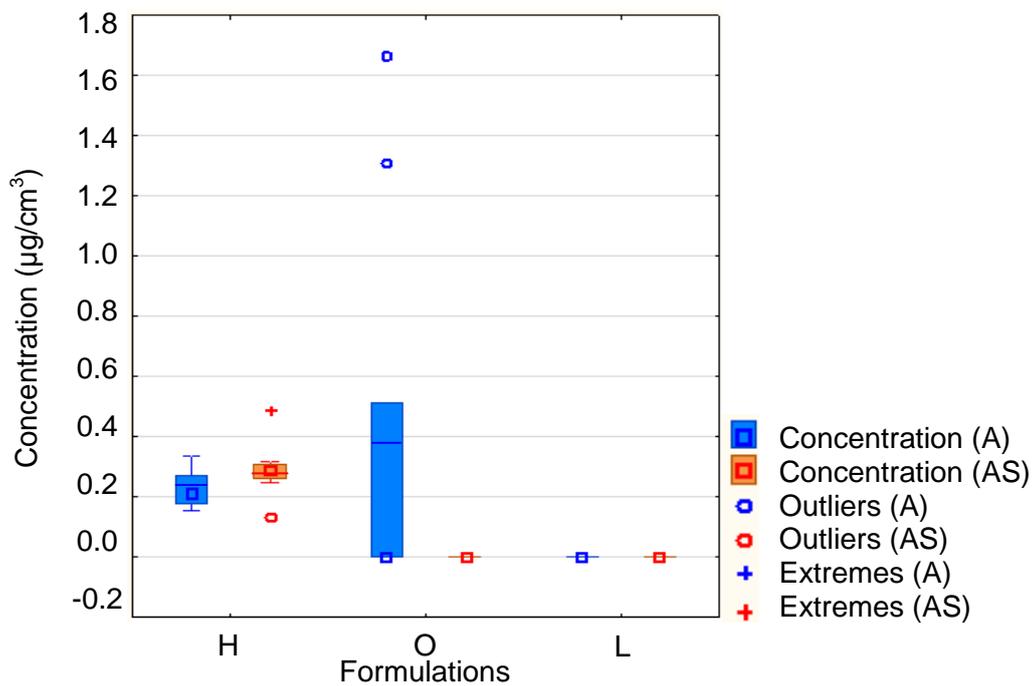


Figure 3: Concentration ($\mu\text{g}/\text{ml}$) of atropine and atropine sulphate in the stratum corneum-epidermis for the different formulations after tape-stripping. The average and median concentration values are indicated by the lines and squares, respectively (AS-O: $n = 10$; A-O, A-L, AS-L: $n = 9$; A-H, AS-H $n = 8$).

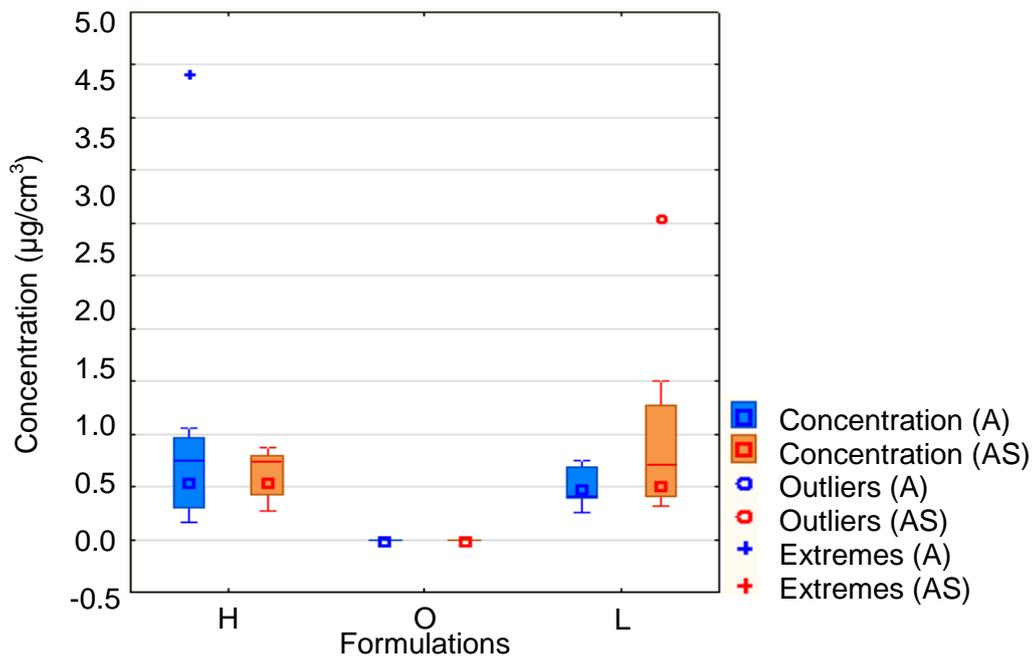


Figure 4: Concentration ($\mu\text{g}/\text{ml}$) of atropine and atropine sulphate in the epidermis-dermis for the different formulations after tape-stripping. The average and median concentration values are indicated by the lines and squares, respectively (AS-0: $n = 10$; A-O, A-L, AS-L: $n = 9$; A-H, AS-H $n = 8$).

Chapter 4

Final conclusion and future prospects

4.1 Final conclusion

The FFE™ software was designed by Wiechers and Abbott to assist the development of formulations optimised for transdermal delivery. The software utilises HSP as an indication of solubility and can also determine the SDG. According to Wiechers (2012), an SDG < 1 will indicate sufficient permeability of the API, whilst an API with a SDG > 1 will need a more complex delivery system. It is believed that a formulation with a small SFG would optimally deliver the API, since you effectively apply two similar layers on each other and the API will distribute evenly between the formulation and the skin.

The aim of this study was to determine the effect of formulation polarity on the transdermal delivery of a model drug, atropine and how the sulphate salt influences the transdermal delivery of atropine. The objectives of this study were as follows:

- Develop and validate an HPLC method for atropine.
- Determine the aqueous solubility of atropine.
- Determine the log P and log D of atropine and atropine sulphate.
- Develop a gel optimised towards the stratum corneum, a more hydrophilic gel and a more lipophilic emulgel containing atropine using the FFE™ software.
- Compound the atropine formulations.
- Use the formulations developed for atropine to compound the atropine sulphate formulations.
- Perform membrane diffusion studies to determine API release from the formulation.
- Perform transdermal diffusion studies followed by tape-stripping to determine and compare the transdermal and topical delivery, respectively of the API from the formulations.

The HPLC method was developed and validated and performed well and was used for the analysis of experimental data.

The solubility of atropine in water, PBS (pH 7.4) and *n*-octanol were determined to be

0.9 mg/ml, 5.8 mg/ml and 3.2 mg/ml, respectively. According to Naik *et al.* (2000:319), a compound should have an aqueous solubility > 1 mg/ml to be transdermally delivered. The aqueous solubility of atropine indicated transdermal delivery may be limited. The high solubility of atropine in the PBS (pH 7.4) was explained by the high degree of ionisation (99.68%) and the formation of ion-pairs with the phosphate salt. The log D of both atropine and atropine sulphate predicted suboptimal transdermal delivery (Brown *et al.*, 2005:177).

Atropine and atropine sulphate were both formulated in a gel with a polarity optimised towards the stratum corneum, a more hydrophilic gel and a lipophilic emulgel. The formulations for atropine were developed using the FFE™ software and those formulations were used for atropine sulphate. The atropine formulations had a higher viscosity compared to the atropine sulphate formulations. The optimised and lipophilic formulations applied easily and had an acceptable skin feel, whilst the hydrophilic formulation was a little tacky. The optimised and hydrophilic formulations had a uniform and opaque appearance, whilst the lipophilic formulations were white.

The membrane release studies confirmed the API was released from all formulations. The highest percentage released after 6 h was observed with the more hydrophilic formulation containing atropine. The lipophilic atropine has a low affinity for the hydrophilic formulation and this caused a driving force for the atropine to leave the formulation, resulting in the high release (Otto *et al.*, 2009:9).

Transdermal diffusion studies were performed over a period of 12 h to determine the transdermal delivery of the API. The lipophilic formulations for both atropine and atropine sulphate resulted in the highest percentage transdermal delivery (0.65% and 0.63%, respectively). The lipophilic formulations had a higher SFG compared to the AFG, which initially predicted poor transdermal delivery. The individual ingredients might however still penetrate the skin resulting in a change in the residual formulation on the skin. The dimethyl isosorbide (DMI) penetrated the skin faster than the other ingredients and resulted in a less desirable residual formulation, which resulted in a driving force for the atropine and the atropine sulphate to leave the formulation. The penetrated DMI provided a more welcome environment for the API in the skin which also contributed to the high delivery (Abbott, 2012:219).

Tape-stripping was employed after the transdermal diffusion experiments to determine the distribution of the API between the stratum corneum-epidermis (SCE) and the epidermis-dermis (ED). The hydrophilic formulations of both atropine and atropine sulphate resulted in the highest concentration in the SCE and ED. This was explained by the lack of driving force caused by the HSP profile and the only driving force for the API to leave the hydrophilic formulation was the concentration gradient. After 12 h, the API had not fully traversed the skin

and therefore low transdermal results were obtained compared to the highest concentration in the skin.

The results obtained in this study support the Delivery Gap Principle of Wiechers, since effective transdermal delivery was obtained with an API with a SDG < 1 (Wiechers, 2012). The results also confirmed that the polarity of the formulation has a definite effect on the transdermal delivery of an API. Wiechers stated that a formulation with a small SFG (optimised towards the stratum corneum) should result in a transdermal delivery of at least 50% of the API. The results from this study contradict this, since the more lipophilic formulation had the highest transdermal delivery. According to Abbott (2012:218), a good balance between the AFG and SFG is needed to ensure sufficient penetration of the API through the skin by generating a driving force and by increasing the solubility of the API in the skin as the formulation penetrates. From the results it was observed that the lipophilic formulation with a higher SFG, compared to AFG, resulted in the highest transdermal delivery. The AFG and SFG initially predicted poor delivery but the composition change of the formulation, as some ingredients penetrated, resulted in a less desirable environment for the API causing a driving force for it to leave the formulation. The penetrated ingredients generated a more welcome environment for the API in the skin and also contributed to the higher delivery. It can be concluded that the polarity of the formulation affects the transdermal delivery of an API, but it is important to consider the total HSP profile and molar volume of the API and the ingredients to predict the transdermal delivery of an API rather than just the SFG or AFG. The results indicated that the sulphate salt of the API reduced the transdermal delivery of it and it is therefore better to use the API base instead of the salt form due to. The HSP of a salt is generally higher compared to the base compound and therefore a higher affinity towards water and a low solubility in the skin is observed (Hansen, 2007:337)

4.2 Future prospects

Future prospects for further investigation of this study include:

- 1) Determine the stability of the atropine lipophilic formulation.
- 2) Formulate atropine in different semi-solid formulations like a cream or ointment.
- 3) Evaluate a different API in a gel formulation.
- 4) Extend the investigation of the FFE™ theory by employing other APIs and formulation types.

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Appendix A

Method validation for the high performance liquid chromatography assay of atropine

A.1 Introduction

The purpose of the validation process is to confirm that the analytical method used to determine the amount of API in the samples is both sensitive and reliable. The APIs used during this study were atropine and atropine sulphate.

Table A.1: A summary of the results obtained from the validation tests for atropine

Test	Results
Specificity	Complies
Range	0.03-300.00 µg/ml
Linearity	$r^2 = 0.99917$
Accuracy	102.6%
Precision	RSD* = 1.62%

*Relative standard deviation

This method was developed and validated primarily for use in transdermal and membrane diffusion studies as well as for the determination of aqueous solubility, log P and log D.

A.2 Chromatographic conditions

The chromatographic conditions were as follows:

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 (Agilent Technologies, Palo Alto, CA)

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)

Mobile phase: Methanol/0.005 M 1-octane sulphonic acid sodium in water, pH adjusted to 3.5 with dilute phosphoric acid 58:42

Flow rate:	1.0 ml/min
Injection volume:	50 µl
Detection:	UV at 210 nm
Retention time:	± 5.1 min
Stop time:	8 min
Solvent:	PBS pH 7.4

A.3 Sample preparation

Samples are prepared/collected and transferred into autosampler vials without any further processing and analysed.

A.4 Standard preparation

The standard solution was prepared using the following method:

1. Weigh approximately 30 mg of atropine accurately in a 100 ml volumetric flask.
2. Dissolve in about 50 ml of methanol; fill to volume with PBS (pH 7.4) (Standard 1).
3. Dilute 5 ml of this solution to 50 ml with PBS (pH 7.4) (Standard 2).
4. Further dilute 5 ml of this solution to 50 ml with PBS (pH 7.4) (Standard 3).
5. Further dilute 5 ml of this solution to 50 ml with PBS (pH 7.4) (Standard 4).
6. Transfer the standards into autosampler vials and analyse.

A.5 Calculations

The concentrations of standard solutions are entered into an Excel worksheet with the peak areas of the standards and samples. A standard curve is calculated by means of linear regression. The slope and y-intercept are used to calculate the concentration of the samples from the peak areas.

A.6 Validation test procedures and acceptance criteria

A.6.1 Specificity

The specificity of the method was validated using the following method:

1. Prepare a placebo by filling a vial with PBS (pH 7.4).
2. Inject in duplicate.

3. Dilute a standard solution by adding 100 µl of water, 2.0 M hydrochloric acid (HCl), 2.0 M sodium hydroxide (NaOH) and 10% hydrogen peroxide (H₂O₂) 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 in the chromatograph with a run time of 10 min.
6. Examine the chromatograms to determine whether any additional peaks were formed.

A.6.1.1 Acceptance criteria

The degraded samples should not contain any peaks that will interfere with the determination of atropine. The placebo should not interfere with the atropine.

A.6.2 Linearity

The linearity of the method was validated by the following method:

1. Prepare a standard as described in Section A.4.
2. Inject 5, 10, 20, 30, 40 and 50 µl of each standard solution (Standards 1-4) in duplicate into the HPLC.

A.6.2.1 Acceptance criteria

Linear regression analysis should yield a regression coefficient (r^2) of ≥ 0.99 . The range is determined as the lowest and highest concentrations between which the response remains linear and/or where acceptable precision is obtained.

A.6.3 Accuracy

The following method was used for the validation of the accuracy:

1. Since the method does not involve any sample preparation, accuracy and precision can only be done by preparing a set of standards and analysing them against another set of standard solutions.
2. Weigh approximately 25 mg of atropine in a 100 ml volumetric flask. Dissolve in approximately 50 ml of methanol and fill to volume with PBS (pH 7.4). Transfer 10 ml of this solution into a 20 ml volumetric flask and fill up to volume with PBS (pH 7.4). Transfer 5 ml of the latter solution into a 50 ml volumetric flask and fill to volume with PBS (pH 7.4). This will yield solutions containing approximately 12.5, 125.0 and 250.0 µg/ml of atropine. Transfer these solutions into autosampler vials and analyse for accuracy experiment against a standard solution prepared as described in Section A.4.

A.6.3.1 Acceptance criteria

According to the Food and Drug Association (FDA, 2001), the mean value determined for accuracy should be within 15% of the true value. For the purpose of our experiments, we set a limit of between 95 to 105%.

A.6.4 Precision

A.6.4.1 Intra-day precision (repeatability)

The intra-day precision was validated using the following method:

1. Prepare three samples each of low, medium and high concentration (n = 9).
2. Prepare a set of standards as described in Section A.4.
3. Inject into the chromatograph in duplicate.

A.6.4.2 Inter-day precision

Analyse three samples of the middle concentration as described under intra-day precision (Section A.6.4.1) on two more days to determine the between-day variability of the method. If possible, a different analyst should perform the analysis, preferably using different equipment.

A.6.4.3 Acceptance criteria

Limits set for precision of bioanalytical methods are 15% of the coefficient of variation, except for the lower limit of quantification (LLOQ), where it should not exceed 20% (FDA, 2001).

For the purposes of our study, we set the limits as follows:

- Intra-day repeatability must be better than 5% (n = 9).
- Inter- day precision must be better than 10% (n = 9).

A.6.5 Limit of detection and lower limit of quantification

The limit of detection (LOD) is defined as the lowest amount of analyte which can be detected (discerned from baseline noise), but not quantified. The LLOQ is the lowest amount of an analyte that can be determined with suitable accuracy and precision (ICH, 1995). The LLOQ should be chosen to suit the purpose for which the method is to be used.

A.6.5.1 Acceptance criteria

For the purpose of this study, the LOD will be the lowest concentration that yields an RSD of approximately 15%, whereas the LLOQ will be the lowest concentration that yields an RSD of less than 5% (n = 6).

A.6.6 Ruggedness

A.6.6.1 Stability of sample solutions

The stability of the samples was validated using the following method:

1. Prepare a standard solution as described in Section A.4.
2. Inject the sample into the chromatograph.
3. Leave the sample in the autosampler tray and reanalyse at hourly intervals up to 24 h in order to determine the stability of the sample.
4. Programme the pump to reduce the flow rate to 0.1 ml/min after elution of the peak and reset the flow rate to 1 ml/min 5 min before injecting the next sample.

A.6.6.1.1 Acceptance criteria

Sample solutions should not be used for a period longer than it takes to degrade by 2% and in this case, special precautions should be followed to compensate for the degradation.

A.6.6.2 System repeatability

Inject a sample or standard six times consecutively in order to test the repeatability of the peak area as well as the retention time.

A.6.6.2.1 Acceptance criteria

The peak area and retention times should have an RSD of 2% or less.

A.6.7 Robustness

Make deliberate changes to the flow rate, injection volume, wavelength and mobile phase composition. Determine the influence of these changes on the chromatographic results.

A.6.8 System and method performance characteristics (system suitability)

Generate an extended performance report on the standard solution, taking care that only the relevant peaks are integrated.

A.6.8.1 Acceptance criteria

Examine the performance results obtained. Set realistic performance characteristics that must be complied to, in order to do the analysis successfully.

A.6.9 Uncertainty of measurement

The uncertainty of measurement was determined empirically as well as from validation data. The calculated uncertainty of measurement was obtained by combining the uncertainties of each step in the analysis process and is expressed as a contribution factor. Set a value for uncertainty of measurement to include on reports done with this method.

A.7 Validation results

A.7.1 Specificity

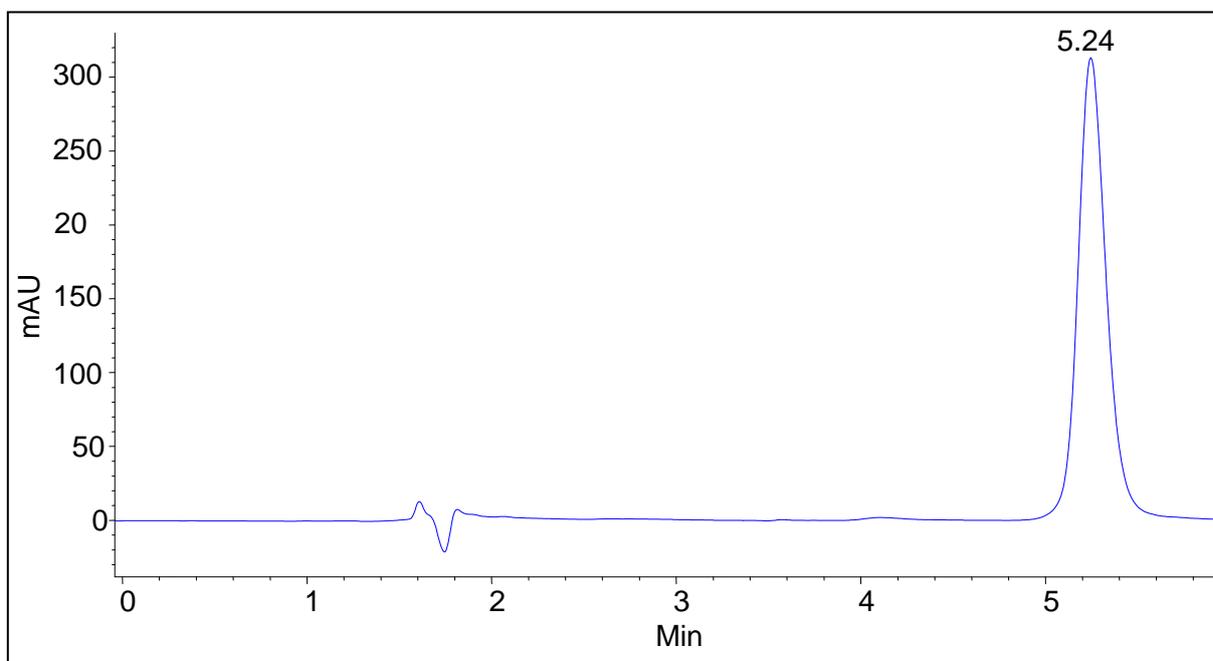


Figure A.1: HPLC chromatogram of a standard solution of atropine

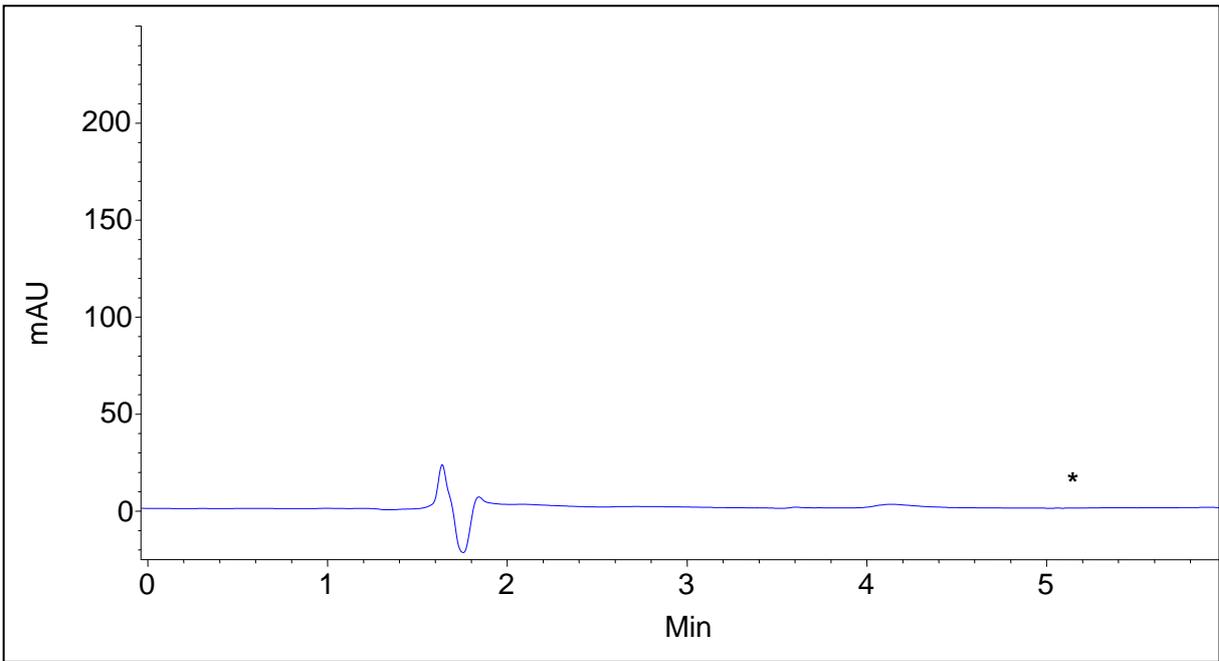


Figure A.2: HPLC chromatogram of a placebo (*Atropine elutes here)

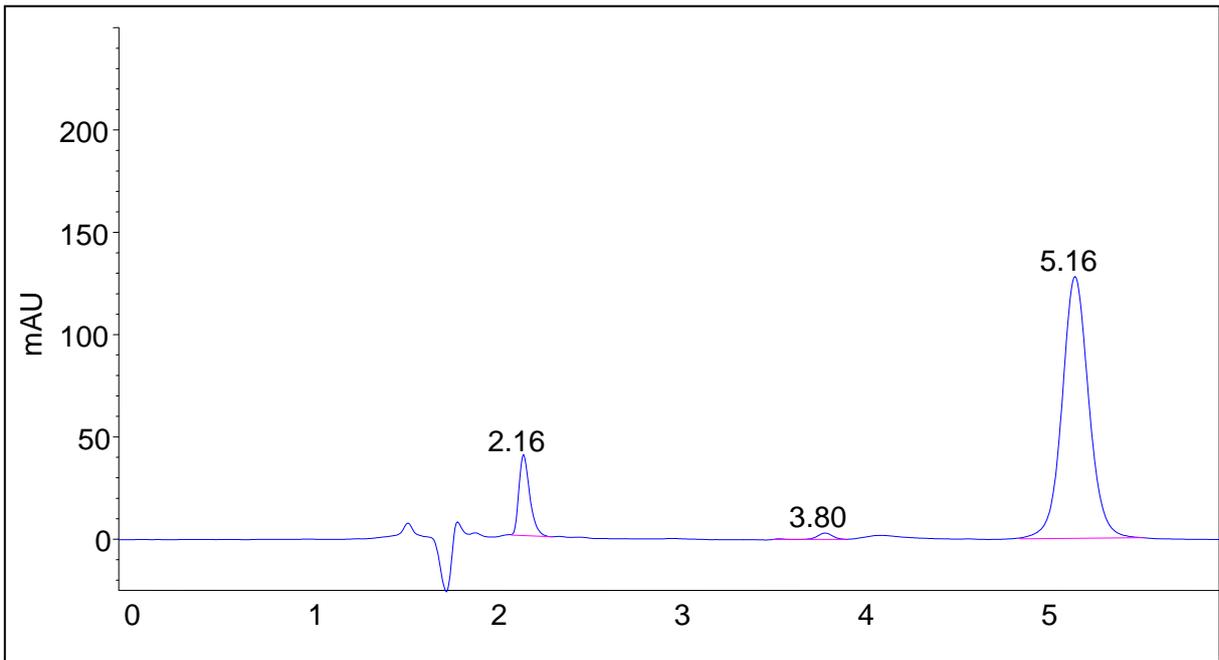


Figure A.3: HPLC chromatogram of a sample solution stressed in water

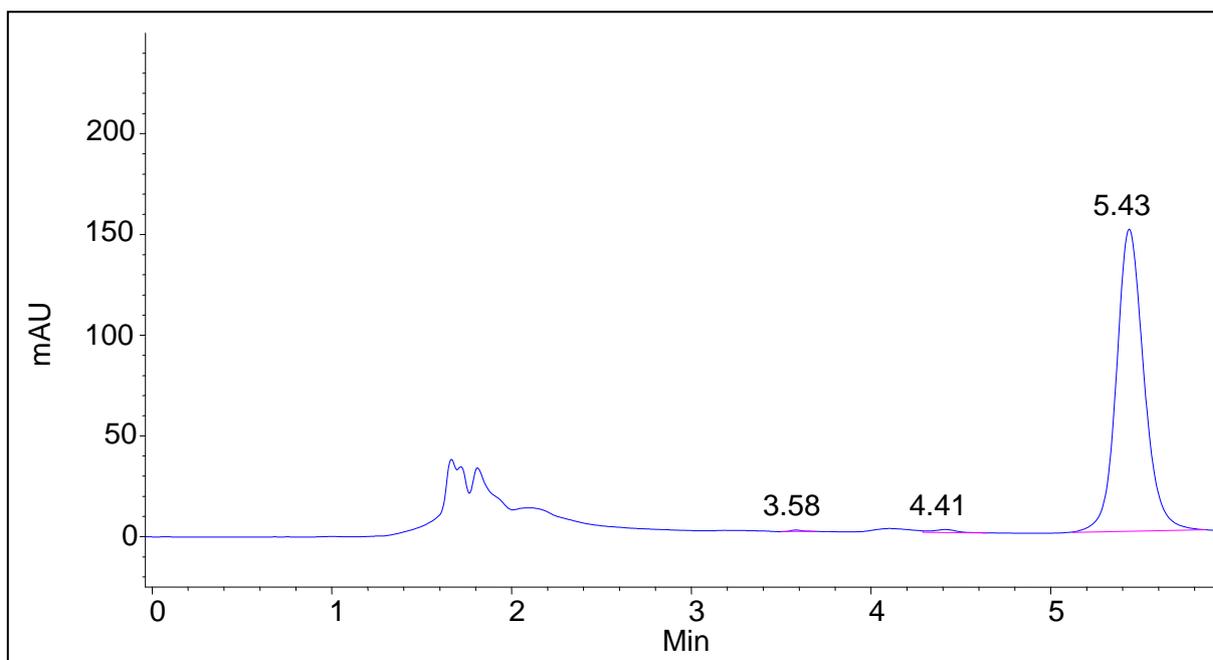


Figure A.4: HPLC chromatogram of a sample solution stressed in 0.1 M HCl

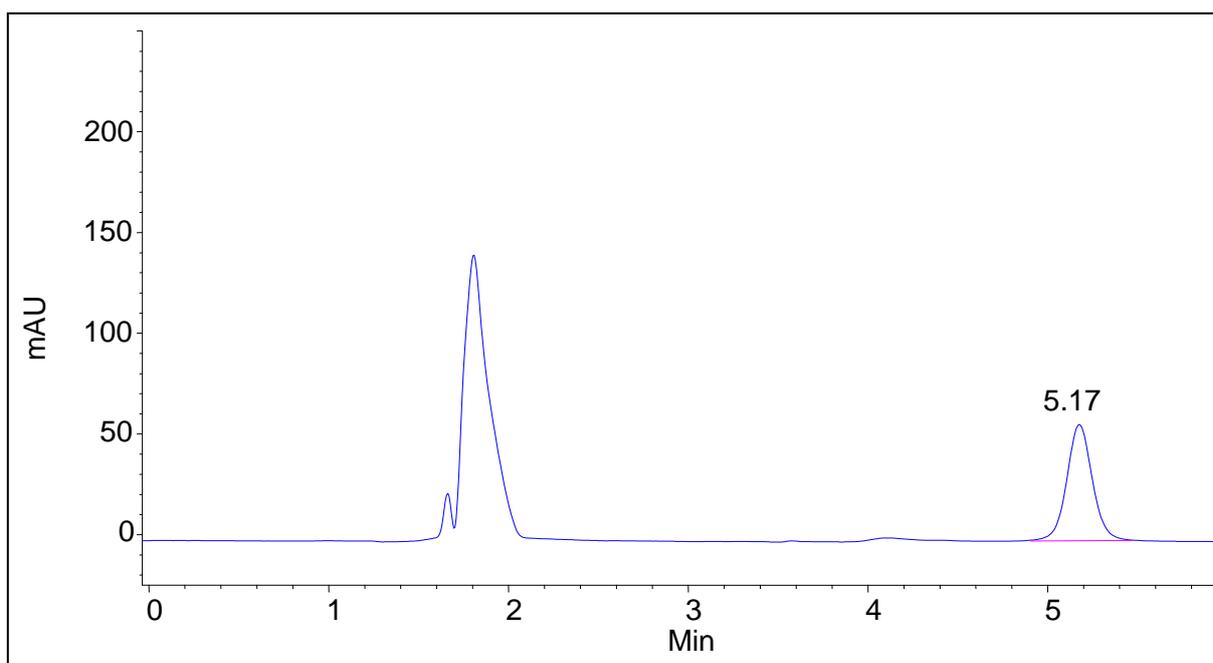


Figure A.5: Chromatogram of a sample solution stressed in 0.1 M NaOH

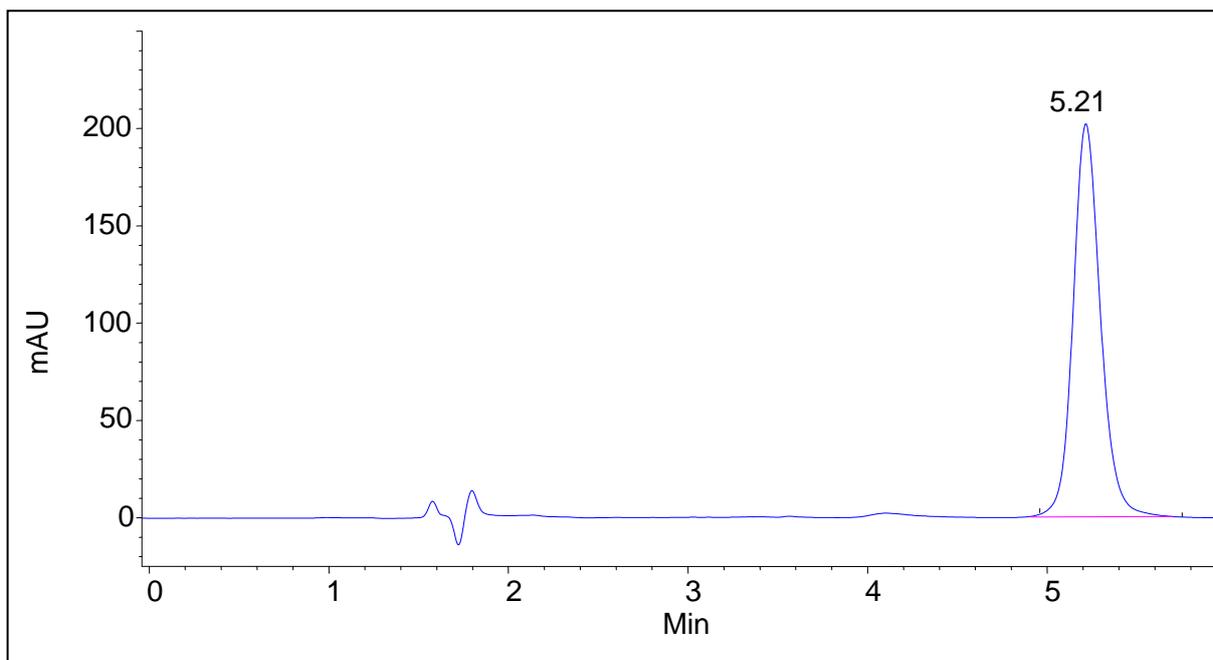


Figure A.6: HPLC chromatogram of a sample solution stressed in 10% H₂O₂

A.7.1.1 Peak purity

The remainder of the atropine peaks in the above stressed samples were examined by means of diode array peak purity analysis to ascertain whether any interference from degradation were present and co-eluted with the atropine peak.

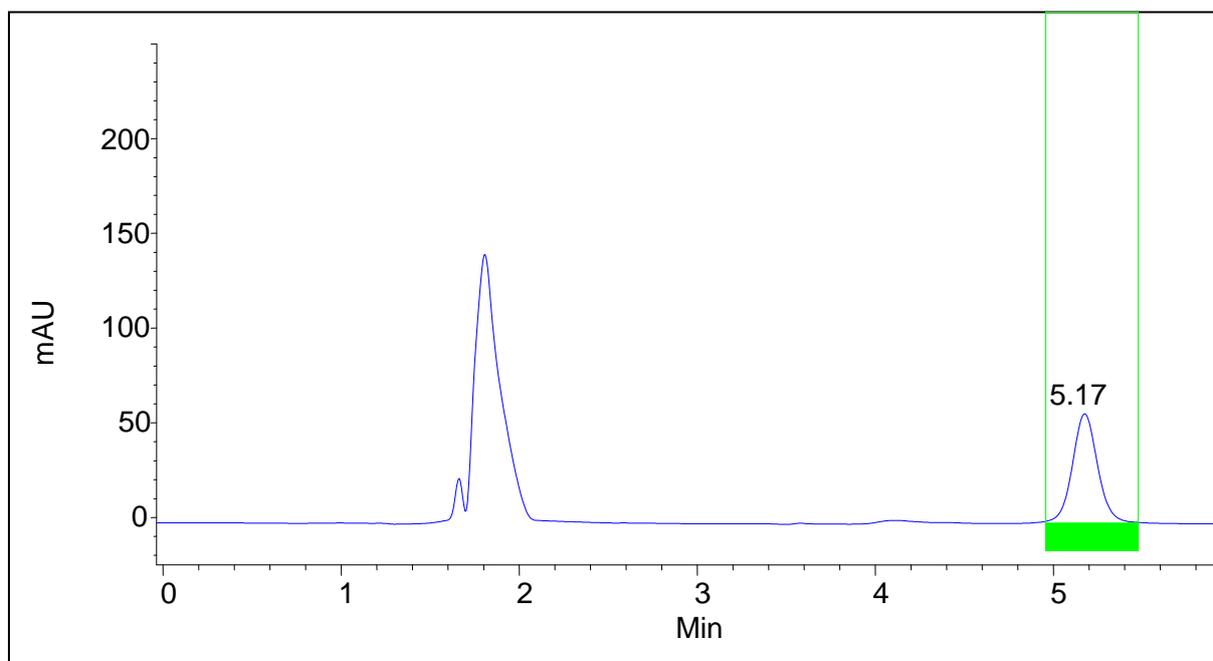


Figure A.7: Purity testing of chromatogram of a sample solution stressed in 0.1 M NaOH

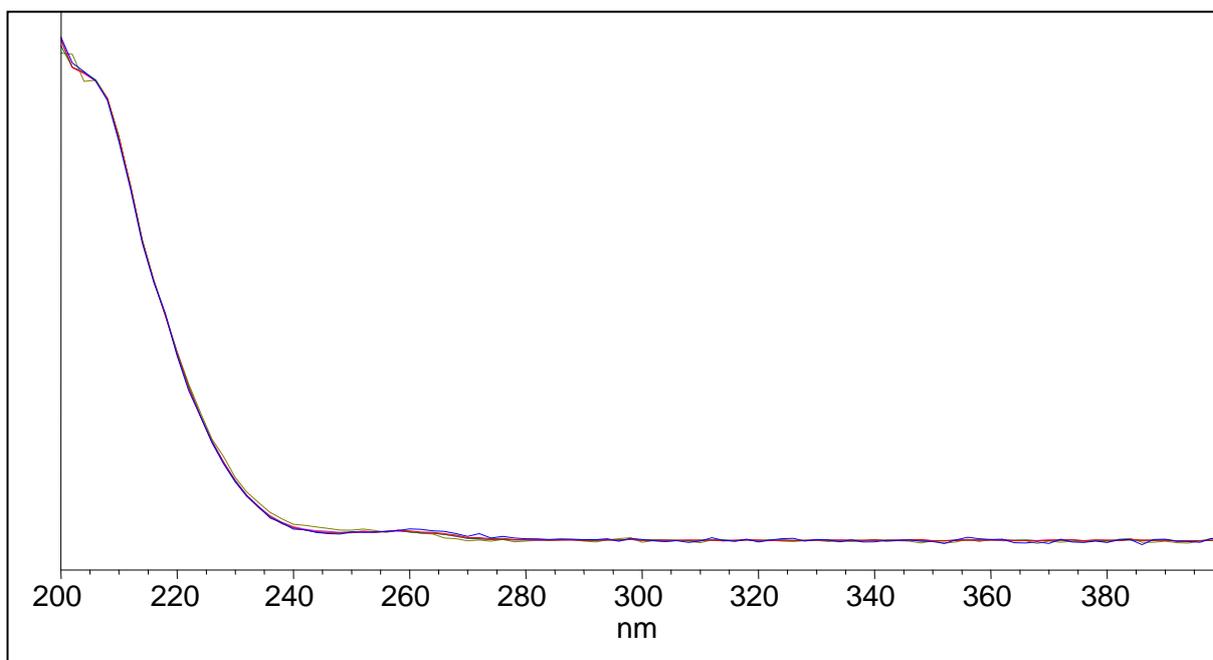


Figure A.8: Overlaid UV spectra of atropine peak

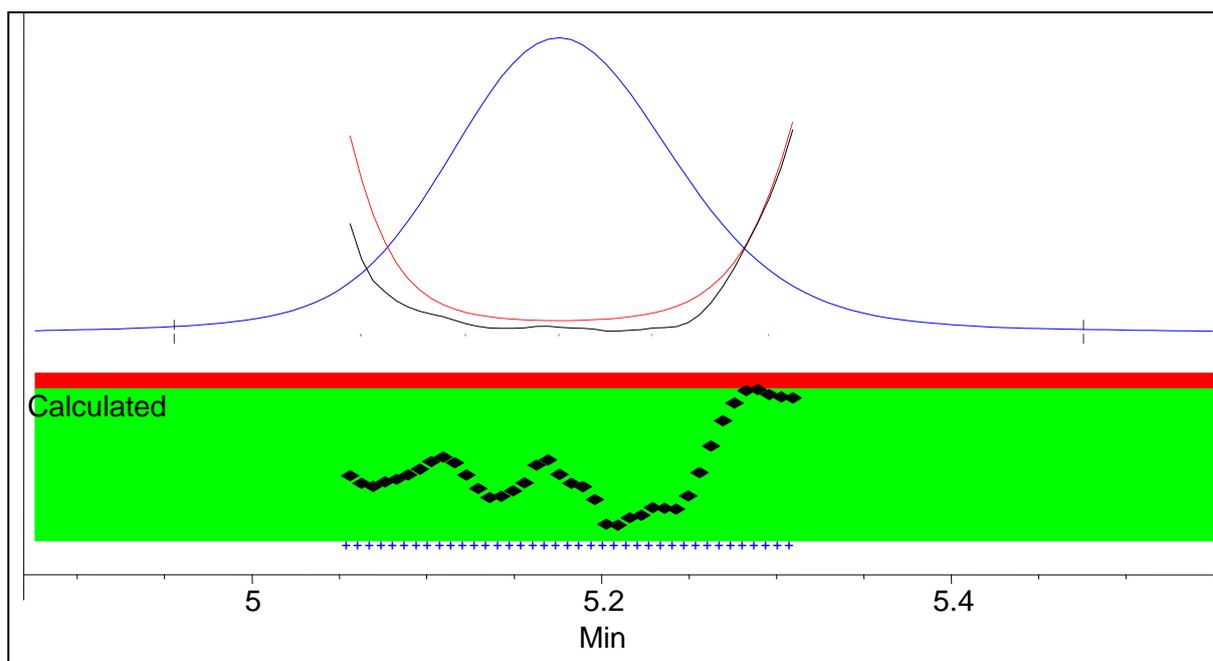


Figure A.9: Graph of purity profile of atropine peak

None of the ingredients in the placebo interfered with the analyte peak. Extra peaks formed during forced degradation did not interfere with the remainder of the atropine peak. Peak purity testing of the remaining peaks, after forced degradation in all forced degradation samples, indicated the peak was still pure (> 99.98%), thus proving the method is stability-indicating.

A.7.2 Linearity and range

Table A.2: Linearity results for atropine

Standard concentration ($\mu\text{g/ml}$)	Peak area
0.030	3.2
0.060	6.8
0.121	11.4
0.181	25.0
0.241	34.5
0.302	36.3
0.603	52.3
1.206	101.5
1.810	164.9
2.413	227.8
3.016	273.6
6.032	430.5
12.064	873.5
18.096	1342.9
24.128	1868.0
30.160	2318.1
60.320	4428.9
120.640	8718.4
180.960	12961.8
241.280	16957.1
301.600	20451.1

Table A.3: Range for atropine

R Squared	0.999	Lower 95%	Upper 95%
Intercept	79.667		
Slope	69.250	69.294	70.206

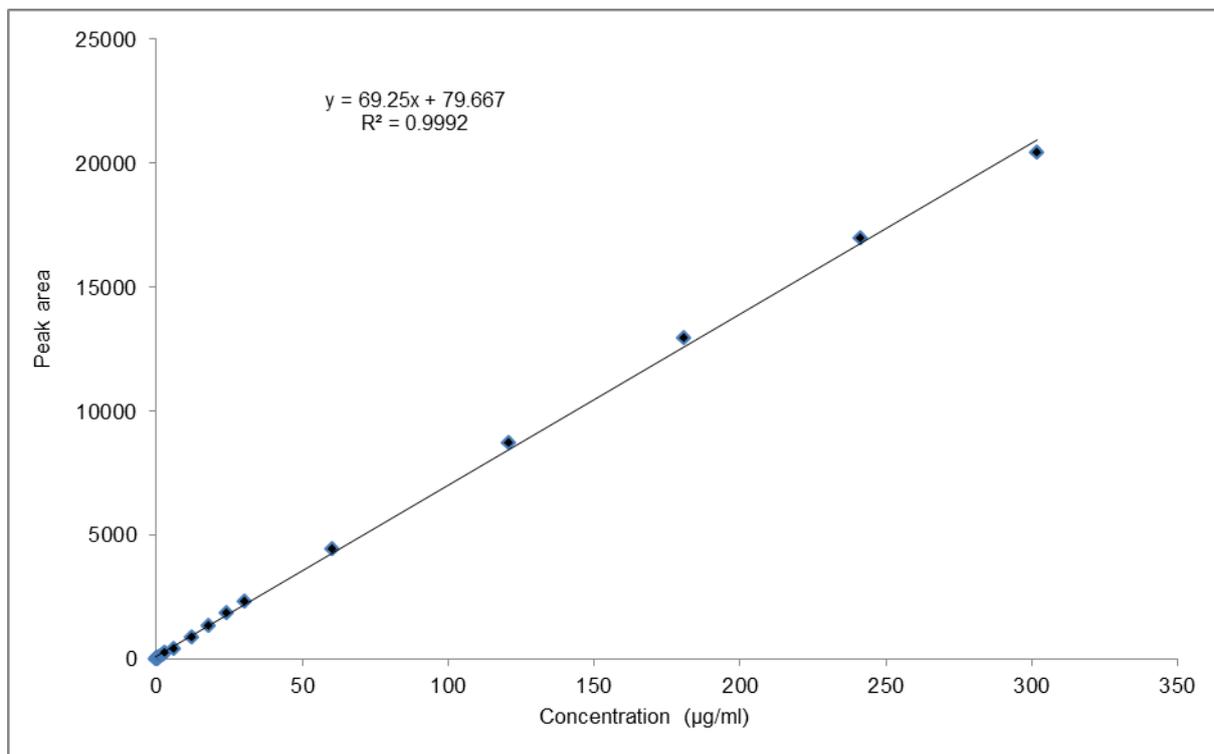


Figure A.10: Linear regression graph for atropine

The method is linear over the concentration range 0.03-301.60 µg/ml.

A.7.3 Accuracy

Table A.4: Accuracy parameters of atropine

Concentration spiked ($\mu\text{g/ml}$)	Peak area 1	Peak area 2	Mean peak area	Recovery ($\mu\text{g/ml}$)	Recovery (%)
9.95	742.6	692.7	717.7	9.89	99.40
10.08	721.1	729.5	725.3	10.00	99.20
9.88	745.7	730.3	738.0	10.18	103.03
99.52	7551.9	7362.9	7457.4	103.50	104.00
100.80	7598.4	7618.0	7608.2	105.60	104.76
98.76	7346.4	7388.0	7367.2	102.25	103.54
248.80	18456.9	18223.2	18340.1	254.66	102.36
252.00	19020.3	18720.7	18870.5	262.03	103.98
246.90	18380.7	18293.6	18337.2	254.62	103.13
				Mean	102.60
				SD	1.88
				%RSD	1.83

Over the range of 10 to 250 $\mu\text{g/ml}$, the method yielded a mean recovery of 102.60%. Precision was satisfactory with an RSD of 1.83% (see Section A.6.3).

A.7.4 Precision

A.7.4.1 Intra-day precision (repeatability) and inter-day precision (reproducibility)

Table A.5: Intra- and Inter-day precision parameters of atropine

	Day 1	Day 2	Day 3	Between days
	104.03	99.21	102.78	
	104.79	100.62	99.02	
	103.57	98.91	101.25	
Mean	104.13	99.58	101.02	101.58
SD	0.50	0.75	1.54	2.61
%RSD	0.48	0.75	1.53	2.13

The repeatability is within acceptable limits (intra-day variance of 0.48%, 0.75% and 1.53%, for Days 1, 2 and 3, respectively and inter-day variance of 2.13%) and the assay should perform well, even when executed by other personnel in a different laboratory (see Section A.6.4).

A.7.5 Limit of detection and lower limit of quantification

Table A.6: Limit of detection and lower limit of quantification of atropine

	LOD ($\mu\text{g/ml}$)	LLOQ ($\mu\text{g/ml}$)
	0.040	0.120
	Peak area	Peak area
	3.575	9.737
	2.750	9.908
	2.539	9.853
	2.768	9.236
	3.822	9.253
	2.810	10.165
Mean	3.04	9.69
SD	0.48	0.34
%RSD	15.64	3.52

The LOD of this method is 0.040 $\mu\text{g/ml}$ and the LLOQ is 0.120 $\mu\text{g/ml}$.

A.7.6 Ruggedness

A.7.6.1 Stability of sample solutions

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

Table A.7: Sample stability parameters of atropine

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	2041.8	98.2
SD	17.33	0.83
%RSD	0.85	0.85

The atropine sample solution is stable over a period of 24 h with only a 0.85% variation in concentration over this period (see Section A.6.6.1).

A.7.6.2 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.8: System repeatability for the peak area and retention time of atropine

	Peak area	Retention time (min)
	8009.0	5.143
	8236.2	5.188
	8150.8	5.202
	8158.0	5.163
	8074.7	5.174
	8004.5	5.093
Mean	8105.5	5.161
SD	84.02	0.035
%RSD	1.04	0.686

System performance proved well within the acceptable range with RSD values of 1.04% for peak area and 0.686% for retention time, respectively (see Section A.6.6.2).

A.7.7 Robustness

The following changes in the chromatographic operating parameters were found to be acceptable:

Column: Luna C18-2 column, 150 x 4.6 mm, 5 µm particle size, 100 Å pores, 17.8% carbon load, endcapped, Phenomenex, Torrance, CA, and a Venusil XBP C18(2) column, 150 x 4.6 mm, 5 µm particle size, 100 Å pores, 19% carbon load, endcapped, were found to be suitable.

Mobile phase: Concentrations of 56 to 62% methanol was still suitable in spite of differences in retention time. Atropine was found to be very sensitive to organic content and this must be carefully controlled to obtain reproducible retention times.

Flow rate: 0.8-1.2 ml/min

Wavelength: The wavelength can be altered by ± 3 nm without any ill effect.

Environment: The analysis was performed by two different analysts on Days 2 and 3 of the inter-day precision experiment, on two different instruments (Agilent 1100 series with diode array detection and Agilent 1200 series with variable wavelength UV detection). The intra-day variation was only 2.13% (see Section A.6.4).

The method was able to tolerate small changes in the chromatographic conditions and should perform well under normal use.

A.8 Chromatographic performance parameters

Reference	(USP, 2005)
Retention time (min):	5.206
Number of theoretical plates (N) plates/column (tangent method):	5950
USP tailing factor (T):	1.138
Capacity factor (k')	2.34

A.9 System suitability parameters

The system suitability parameters were determined using the following method:

- Inject a standard solution in triplicate.
- Calculate the relative standard deviation of the peak areas obtained.
- Calculate the number of theoretical plates for the atropine peak.
- Use the tangent method to calculate the parameters.

A.9.1 System suitability criteria

The system is suitable to perform the analysis if the following criteria are met:

- RSD < 2% for 3 injections
- The column must have more than 4460 theoretical plates for atropine (75% of validation value).

A.10 Uncertainty measurements

Empirical calculation:

Weighing of standard: $0.01 \text{ mg}/25.00 \text{ mg} \times 100$	=	0.04%
100 ml flask: $0.08 \text{ ml}/100.00 \text{ ml} \times 100$	=	0.08%
5 ml pipette: $0.015 \text{ ml}/5.000 \text{ ml} \times 100$ (3 x diluted)	=	0.90%
50 ml volumetric flask: $0.05 \text{ ml}/50.00 \text{ ml} \times 100 \times 3$	=	0.30%
Injection inaccuracy (repeatability)	=	<u>1.04%</u>
Total uncertainty:		<u>2.36%</u>

From validation data:

Recovery: 102.6%, thus $100.00 - 102.6$	=	2.6%
Intra-day and inter-day precision $(1.83 + 1.62)/2$	=	<u>1.72%</u>
Total uncertainty: (2.6 + 1.72)	=	<u>4.33%</u>

A.11 Conclusion

The method performed well and should be suitable to analyse atropine in membrane and diffusion study samples and for the determination of aqueous solubility, log P and log D. Measurement uncertainty is well within the limits for assays in biological matrices. No interference was encountered from stressed samples or known related substances, thus the method can be regarded as being stability-indicating.

References

FDA see Food and drug administration

Food and Drug Administration. 2001. Guidance for Industry: Bio analytical method Validation. <http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm070107.pdf> Date of access: 21 Oct. 2014.

ICH see International Conference on Harmonisation of technical requirements for registration of pharmaceuticals for human use.

International Conference on Harmonisation of technical requirements for registration of pharmaceuticals for human use. 2005. ICH harmonised tripartite guidelines. Validation of analytical procedures: text and methodology Q2(R1). <http://www.ich.org/products/guidelines/quality/quality-single/article/validation-of-analytical-procedures-text-and-methodology.html> Date of access: 25 Jun. 2013

USP (United States Pharmacopoeia). 2005. Chromatography. <https://mc.usp.org/sites/default/files/documents/GeneralChapterPDFs/621Chromatography.pdf> Date of access: 10 Nov. 2014

Appendix B

Formulation of a gel containing atropine using the Formulating for Efficacy™ software

B.1 Introduction

The objective of the study was to develop formulations of different polarities for both atropine and atropine sulphate using the FFE™ software. Atropine was chosen as a model drug in this study and atropine sulphate was used to determine the effect of the salt form on the delivery of the API from the same formulations. The FFE™ software allows the formulator to optimise the formulation to the API, the stratum corneum or the target concentration (JW solutions, 2014). In this study, the formulation was optimised towards the skin to ensure optimal transdermal delivery of the API. Two additional formulations were developed which were more hydrophilic and more lipophilic than the optimised formulation. The six formulations were prepared and membrane release and skin diffusion studies were conducted to determine the release and transdermal delivery of the API, respectively.

B.2 Preformulation and formulation

Preformulation studies are done before the initiation of formulation development to ensure the rational development of formulations that are stable, safe and efficient. Preformulation is mainly concerned with the physicochemical properties of the API (Walters & Brian, 2002:321). There are eight chronological phases in preformulation studies and include the general description of the API, calorimetry, polymorphism, hygroscopicity, analytical development, intrinsic stability, solubility and partitioning and drug delivery characteristics (Walters & Brian, 2002:322).

During this study the FFE™ software was used for formulation development as it uses the simplified molecular-input line-entry system (SMILES) string of the API to determine certain physicochemical properties of the API. This simplifies formulation development and eliminates the preformulation phase. The aqueous solubility, log P and the log D of the API was determined and described in Appendix C.

B.3 Developing a product using the “Formulating for Efficacy” software

The general method for developing a formulation using the FFE™ software is illustrated in Figure B.1.

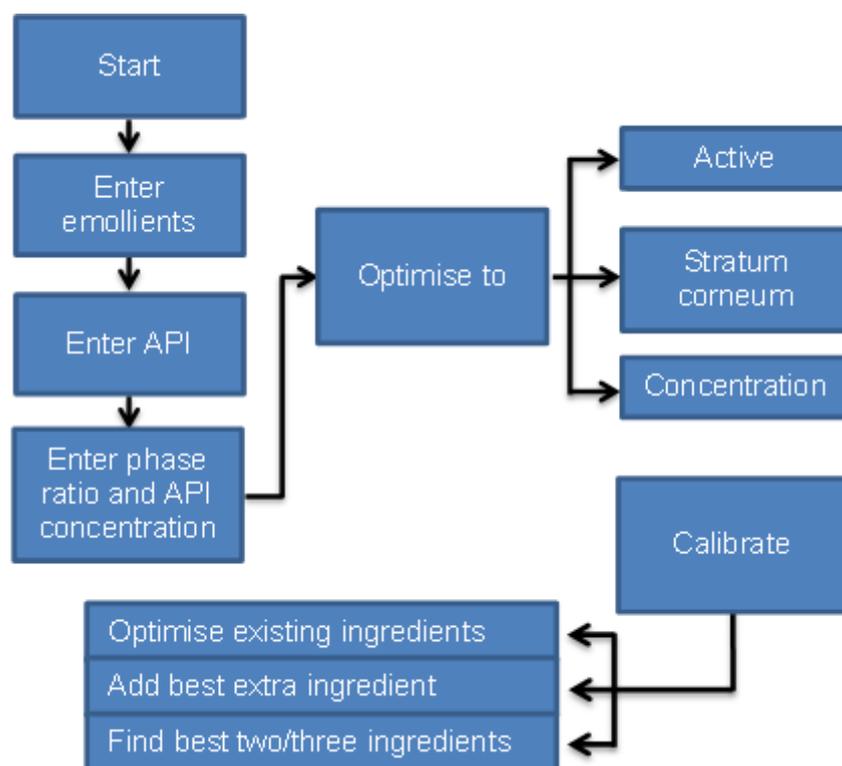


Figure B.1: General method for developing a formulation using FFE™ software (Adapted from JW Solutions, 2014).

The first step in developing a formulation is to identify and to select the emollients in which the API is soluble. The API is then added to the active list using the SMILES string. The software uses the SMILES string to calculate the HSP, molar volume and melting point. After the intended API is selected, the percentage oil phase in the formulation and the overall percentage required API is entered.

The formulation can then be either optimised towards the API, the stratum corneum or the target concentration. To optimise the formulation towards the API, the amount of API must reach a maximum. The aforementioned is used especially for APIs that have a very low solubility in most ingredients. This ensures that an adequate concentration gradient is obtained to ensure clinical efficacy. Optimising the formulation towards the stratum corneum ensures that the API penetrates the stratum corneum in sufficient amounts, thus penetrating deeper into the skin. When the formulation is optimised towards the target concentration, the selected API concentration is close to the maximum limit of solubility to ensure a maximum driving force for the API to leave the formulation and penetrate the skin (JW Solutions, 2014).

The selected ingredient list can be optimised using the programme and the optimal ratio of these will be calculated. The programme can also be used to find the best extra ingredient to create the optimised formulation. If no emollients are selected, the programme can be used to

find the best two or three ingredients and will generate the best combination of all the ingredients listed to obtain the optimised formulation (JW Solutions, 2014).

The formulation obtained using the FFE™ software should in theory provide a good transdermal delivery of the API. The formulation can be finalised by adding different additives to provide the needed viscosity, preservation, skin feel, etc. (JW Solutions, 2014).

B.4 Semi-solid formulations: gel and emulgel

A gel is a liquid rich, semi-solid formulation. The gel consists of an external solvent phase that is immobilised in a three-dimensional matrix. A gel usually contains a gelling agent such as a carbomer or natural gum that is dispersed in water. The gelling agent swells to form the three-dimensional polymer network (Barry, 2007:593; Rehman & Zulfakar, 2014:433).

An emulgel is a combination between an emulsion and a gel. A gelling agent is incorporated into the water phase of the emulsion. The emulgel can be a water-in-oil (w/o) or an oil-in-water (o/w) emulsion based gel (Rehman & Zulfakar, 2014:433).

B.5 Skin delivery gap

Atropine was used as a model drug during this study and therefore the minimum effective concentration (MEC) is not of interest. A hypothetical SDG was however determined from the serum levels obtained from literature. The serum concentration of atropine was 2 ng/ml after an intravenous injection of 0.32 mg atropine (Kradjan *et al.*, 1985). This serum level was used as the MEC to determine the SDG using the FFE™ software. The SDG of atropine was calculated to be 0.001 (SDG < 1) and it should therefore readily penetrate the skin. An SDG > 1 would have indicated that a more complex delivery system was needed to ensure effective transdermal delivery of the API (Wiechers, 2012).

B.6 Formulation of an optimised gel, hydrophilic gel and lipophilic emulgel for both atropine and atropine sulphate

The FFE™ software was used to develop an optimised formulation of atropine. DMI was chosen as the primary emollient and the software was used to find the best extra ingredient. The API concentration of 1.5% was used and an oil phase percentage of 25.0% was chosen; the formulation was then optimised towards the skin.

Table B1 provides a list of the ingredients used to make the different formulations as well as the suppliers and batch numbers.

Table B.1: Ingredients used in the formulations together with the suppliers and batch numbers

Ingredient	Supplier	Batch number
Atropine	Sigma-Aldrich	070M1206V
Atropine sulphate	Sigma-Aldrich	BCBH 8339V
Carbopol® Ultrez 10 polymer	Lubrizol Advanced Materials	0100922762
DMI	Sigma-Aldrich	STBD7240V
Polyethylene glycol 400 (PEG-8)	Merck Chemicals	1040534
Tween® 80 (PEG-20 sorbitan monooleate)	Merck Chemicals	1042689
Span® 60 (sorbitan monostearate)	Merck Chemicals	S5361721 034
Liquid paraffin	Merck Chemicals	1034378
Ethanol	ACE Chemicals	6676

The concentration ratio of the DMI:PEG-8 was kept the same for all the formulations to eliminate the possible penetration enhancement effect of the DMI. For the more hydrophilic gel, 10% ethanol was added and for the more lipophilic emulgel, 10% liquid paraffin was added. The formulations were kept the same for the atropine sulphate formulations.

B.6.1 Formulation of an optimised gel containing atropine/atropine sulphate

An optimised formulation of atropine was developed using the FFE™ software. The same formulation was used for atropine sulphate.

Table B.2: Formula of atropine/atropine sulphate optimised gel

Phase	Ingredients	%m/m	Activity
A	Water	To 100.0	Solvent
	Carbopol®	0.6	Thickening/gelling agent
B	DMI	15.3	Primary emollient
	PEG-8	8.2	Secondary emollient
	Tween® 80	4.5	Surface active agent
	Span® 60	0.5	Emulsifier
	Atropine*	1.5	API

* The atropine sulphate was added to Phase A

B.6.1.1 Preparation of the atropine optimised gel

Carbopol® was sprinkled over the water and left for ± 2 min for wetting to occur. Phase A was then heated to 40 °C after which it was homogenised at 800 rpm. All the ingredients of Phase B were mixed and both Phases A and B were heated to 50 °C. Phase B was slowly added to Phase A whilst homogenising at 1800 rpm, until a temperature of 40 °C was reached. The final

mixture was stirred whilst cooling to 25 °C and then the pH was adjusted to pH 7.4 with NaOH.

B.6.1.2 Preparation of the atropine sulphate optimised gel

The method described in Section B.6.1.1 was followed to prepare the optimised atropine sulphate gel. The only difference was that the atropine sulphate was dissolved in the water (Phase A) prior to the addition of the Carbopol®.

B.6.1.3 Outcome

The formulations prepared had an acceptable skin feel and applied easily. The appearances of the formulations were uniform and opaque.

B.6.2 Formulation of a hydrophilic gel containing atropine/atropine sulphate

For the more hydrophilic gel 10% ethanol was added to the water in Phase A.

Table B.3: Formula of atropine/atropine sulphate hydrophilic gel

Phase	Ingredients	%m/m	Activity
A	Water	To 100.0	Solvent
	Ethanol	10.0	Co-solvent
	Carbopol®	0.6	Thickening/gelling agent
B	DMI	15.3	Primary emollient
	PEG-8	8.2	Secondary emollient
	Tween® 80	4.5	Surface active agent
	Span® 60	0.5	Emulsifier
	Atropine*	1.5	API

* The atropine sulphate was added to Phase A

B.6.2.1 Preparation of the atropine hydrophilic gel

The method described in Section B.6.1.1 was followed to prepare the hydrophilic atropine gel. The only difference was that the water and ethanol was mixed prior to the addition of the Carbopol®.

B.6.2.2 Preparation of the atropine sulphate hydrophilic gel

The method described in Section B.6.1.1 was followed to prepare the hydrophilic atropine sulphate gel. The only difference was the atropine sulphate was dissolved in the water (Phase A) and then mixed with the ethanol prior to the addition of the Carbopol®.

B.6.2.3 Outcome

The formulations had a uniform, opaque appearance with a good consistency. The formulation applied easily, but was a bit tacky.

B.6.3 Formulation of a lipophilic emulgel containing atropine/atropine sulphate

For the more lipophilic emulgel 10% liquid paraffin was added. The oil phase percentage was increased to 35%.

Table B.4: Formula of atropine/atropine sulphate lipophilic emulgel

Phase	Ingredients	%m/m	Activity
A	Water	To 100.0	Solvent
	Carbopol®	0.6	Thickening/gelling agent
B	DMI	15.3	Primary emollient
	PEG-8	8.2	Secondary emollient
	Tween® 80	4.5	Surface active agent
	Span® 60	0.5	Emulsifier
	Liquid paraffin	10.0	Lipophilic solvent
	Atropine*	1.5	API

* The atropine sulphate was added to Phase A

B.6.3.1 Preparation of the atropine lipophilic emulgel

The method described in Section B.6.1.1 was followed to prepare the lipophilic atropine emulgel. The only difference was the addition of the liquid paraffin to Phase B.

B.6.3.2 Preparation of the atropine sulphate lipophilic emulgel

The method described in Section B.6.1.1 was followed to prepare the lipophilic atropine sulphate emulgel. The only difference was the addition of the liquid paraffin to Phase B and the atropine sulphate was dissolved in the water (Phase A) prior to the addition of the Carbopol®.

B.6.3.3 Outcome

The appearance of the formulations was uniform white. The formulations had an acceptable skin feel and were easily applied.

B.7 Formulation characteristics

B.7.1 HSP values

Many biological materials are characterised by HSP (Hansen, 2007:270). With the HSP values of the human skin [17.0, 8.0, 8.0], the API [18.1, 4.7, 8.5] and the formulations (see Table B.6) known, it is possible to predict adequate solubility in the formulation and skin diffusion using the HSP distance/gap (Hansen, 2013). Table B.5 summarises the HSP characteristics of the different ingredients and the API. The ingredient-active-gap (IAG) is an indication of how close the ingredient and the API are in terms of HSP distance and the ingredient-skin-gap (ISG) indicates the HSP distance between the ingredient and the skin. Tables B.5 and B.6 respectively, give a summary of the HSP characteristics of the different ingredients and the HSP characteristics of the formulations containing atropine as provided by the FFE™ software. The AFG is an indication of how soluble the API is in the formulation. A smaller AFG will indicate better solubility. The SFG is an indication of how alike the formulation is to the skin. A smaller AFG shows that the formulation and the skin are mutually soluble in terms of HSP distance. It is generally accepted that a HSP gap > 8 indicates insolubility. A desirable HSP gap in terms of solubility and compatibility is < 4 (Abbott, 2012:221).

Table B.5: HSP characteristics of atropine and the ingredients in the formulations

Ingredients	δD	δP	δH	Mvol (mol/ml)	IAG	ISG
Atropine	18.1	4.7	5.8	253.2		
DMI	17.5	7.4	7.6	158.9	3.09	1.96
PEG-8	16.0	17.0	8.0	320.0	4.81	7.16
Ethanol	15.4	9.2	19.6	58.7	13.14	7.10
Liquid paraffin	15.7	1.3	3.0	543.9	8.05	47.62

Ingredients with lower molar volumes (mVol) will penetrate the skin faster than those with higher molar volumes (Abbott, 2012:219). When excluding the API; ethanol has the lowest mVol followed by DMI, PEG-8 and liquid paraffin. This indicates, studying only mVol, that ethanol will penetrate the skin the fastest and liquid paraffin the slowest.

The smaller the IAG, the more soluble the API is in the different ingredients. From Table B.5 it is observed that the API is most soluble in the DMI with an IAG of 3.09. The IAG of PEG-8 indicates the API is soluble to some extent in the solvent, but it is not in the preferable HSP distance range for solubility and compatibility. The IAG of ethanol and liquid paraffin indicates that the API is insoluble (HSP gap > 8) in both solvents.

DMI has the smallest ISG indicating that the DMI will readily permeate the skin. PEG-8 and ethanol have an ISG of 7.16 and 7.1, respectively. Although these values are below eight and indicate the ingredient is partly soluble in the skin, it does not provide a desirable solubility. Liquid paraffin has an ISG of 47.62 which indicates it is highly unlikely to penetrate the skin.

From Table B.5 it is clear the single ingredients, except DMI, will not provide sufficient solubility of the API and will not penetrate the skin. For this reason multiple ingredients are combined to obtain the desired solubility.

Table B.6 gives a summary of the HSP characteristics of the different formulations. Figures D.2, D.3 and D.4 are visual 3D representations of the HSP space. This indicates the Hansen distance between the API, formulation, skin and the different ingredients. The further the distance, the less soluble the different components are in each other (Abbott, 2012:218).

Table B.6: HSP characteristics of the different atropine formulations

	δD	δP	δH	Mvol (mol/ml)	AFG	SFG	AFG/ SFG
Atropine optimised gel							
Ingredients	17.0	7.3	7.7	215			
Ingredients + API	17.0	7.1	7.8	208	3.5	1.9	1.8
Atropine hydrophilic gel							
Ingredients	16.5	7.8	11.3	169			
Ingredients + API	16.6	7.7	11.2	172	5.3	5.7	0.9
Atropine lipophilic emulgel							
Ingredients	16.6	5.5	6.3	314			
Ingredients + API	16.7	5.5	6.4	311	3.8	9.5	0.4

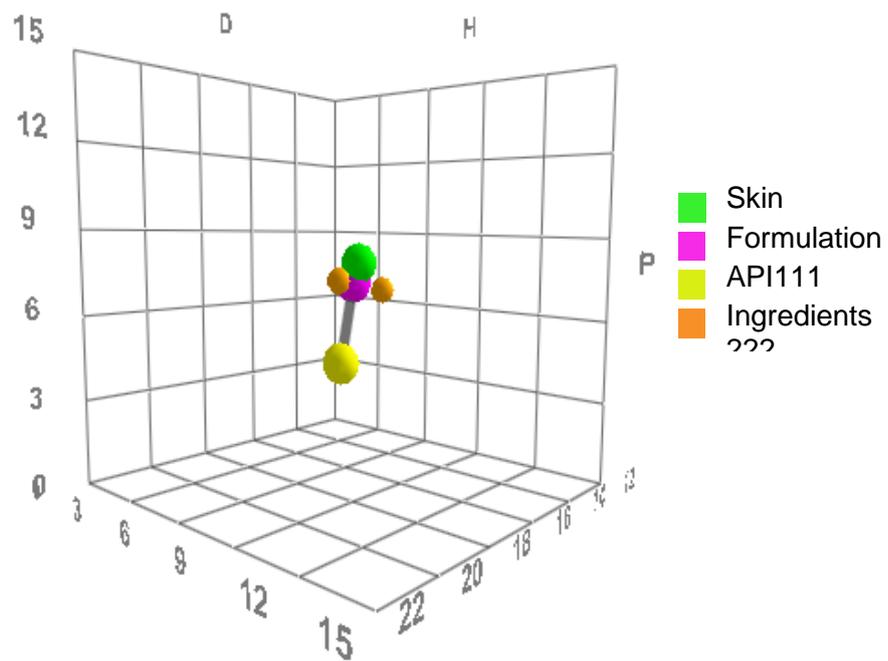


Figure B.2: 3D HSP of atropine optimised gel (D = general dispersion interactions; P = polar cohesion energy and H = hydrogen bonding)

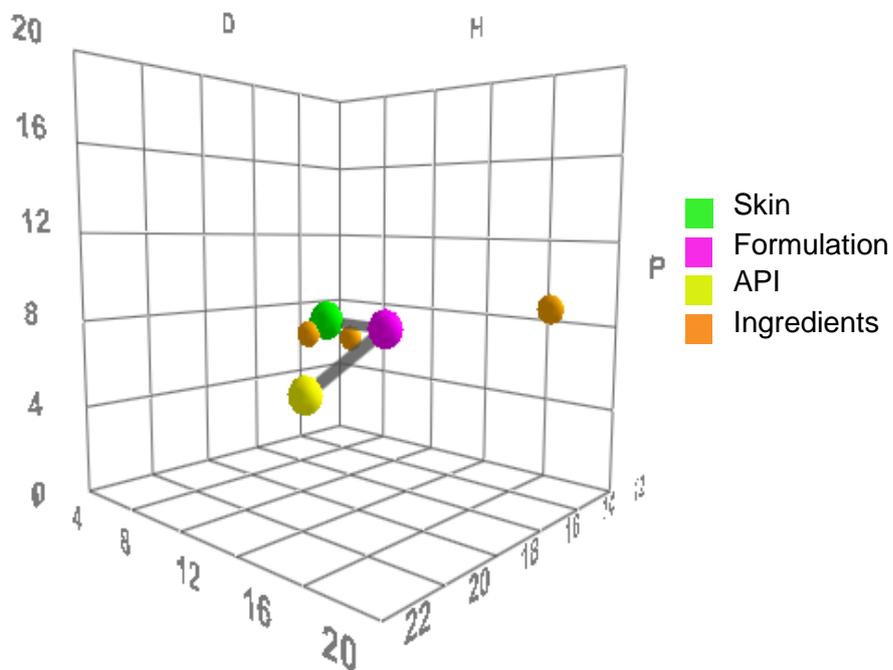


Figure B.3: 3D HSP of atropine hydrophilic gel (D = general dispersion interactions; P = polar cohesion energy and H = hydrogen bonding)

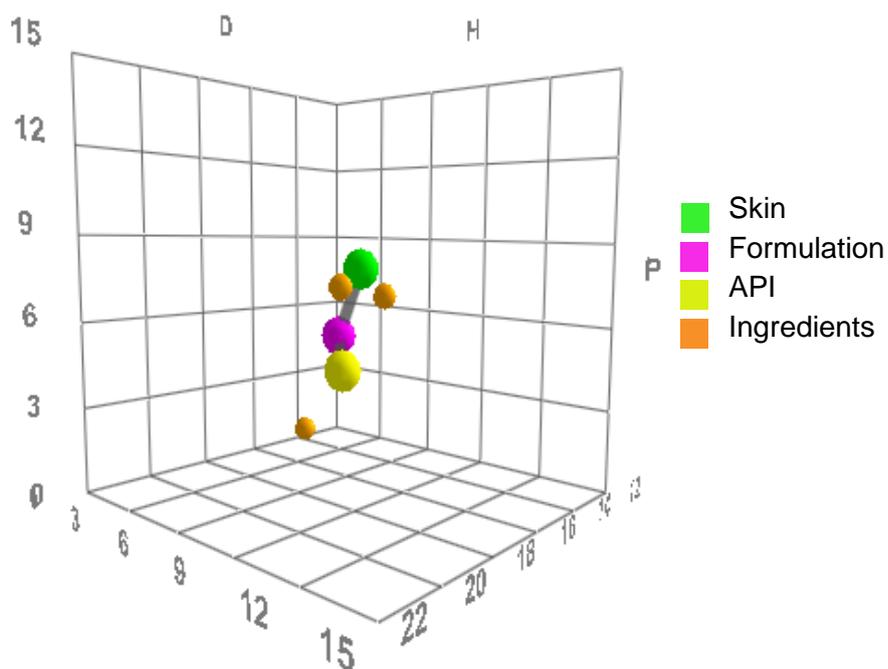


Figure B.4: 3D HSP of atropine lipophilic emulgel (D = general dispersion interactions; P = polar cohesion energy and H = hydrogen bonding)

Table B.5 and Table B.6 illustrate the ingredient combinations give a different HSP profile than the single ingredients. This indicates that a combination of two or more single ingredients may result in a formulation with a more or less favourable HSP profile. Table B.6 and Figure B.2 illustrates the AFG is bigger than the SFG for the atropine optimised gel, indicating that the API is less soluble in the formulation than the formulation is in the skin. The SFG predicts a good penetration of the formulation into the skin (< 4). The penetration of the formulation into the skin causes the skin to swell and generates a more welcome environment for the API (Abbott, 2012:218). As the formulation enters the skin, less formulation is left on the skin surface meaning less solvent for the API. The composition of the formulation on the skin also changes which results in a change in the SFG and AFG. DMI will penetrate the skin faster than the PEG-8 based on mVol and ISG. This indicates that more PEG-8 will be left in the formulation relative to the DMI and the resulting SFG will be higher. The residual formulation on the skin is more favourable for the API (decreased AFG), indicating that the permeation of the API will decrease over time.

The AFG and SFG for the atropine hydrophilic gel is almost the same with an AFG/SFG close to one as seen in Table B.6 and Figure B.3. Both these distances are above the desired distance. The formulation may have solubility and compatibility difficulties and will not penetrate the skin as easy as the optimised formulation. The API is also less soluble in the hydrophilic formulation than in the optimised formulation, which could result in precipitation of the API out of the

hydrophilic formulation.

The more lipophilic formulation has an AFG of 3.8 and a SFG of 9.5 (see Table B.6 and Figure B.4). The AFG is below four, which indicates sufficient solubility and compatibility of the API in the formulation. The SFG (9.5) however, is above eight which indicates insolubility and incompatibility of the formulation in/with the skin. The individual ingredients with desirable ISG's might still penetrate the skin. In this case DMI may penetrate the skin, leaving an undesirable formulation on the skin with respect to API solubility. The IAG of atropine in liquid paraffin (8.05) and PEG-8 (4.81) are above four, indicating undesirable solubility.

B.7.2 Viscosity and pH

The viscosity of each formulation was measured using a Brookfield DV2T Viscometer (Stoughton, Massachusetts, USA). The formulation was heated to 25 °C in a water bath, then placed in the small sample adapter and the spindle (SC4-25) was connected and inserted. The spindle turned at a speed of 0.70 rpm and a measurement was taken every 10 sec for 2 min. The average viscosities of the formulations are given in Table B.7.

Table B.7: Average viscosities and pH values of the different formulations of atropine and atropine sulphate

	Atropine		Atropine sulphate	
	Average viscosities (P)*	pH	Average viscosities (P)*	pH
Optimised	242.46 ± 1.99	7.47	67.30 ± 0.40	7.48
Hydrophilic	288.80 ± 0.97	7.67	71.30 ± 0.00	7.50
Lipophilic	264.91 ± 0.99	7.57	92.32 ± 0.45	7.49

*P = Poise

The pH of the formulations was adjusted to ±7.4 to eliminate the effect of possible salt formation. This pH falls within the acceptable pH ranges for transdermal preparations (3-9) (Barry, 2007:576). At this pH the atropine and atropine sulphate is however mostly ionised (99 %) and will only penetrate the skin to a limited extent.

The atropine formulations had a much higher viscosity than the atropine sulphate formulations. The viscosity of the atropine hydrophilic formulation was highest, followed by the lipophilic and lastly, the optimised formulation. For the atropine sulphate formulations the highest viscosity was measured with the lipophilic formulation. The optimised formulation of atropine sulphate had the lowest viscosity. A higher viscosity may lead to a decrease in permeability. This is because a higher viscosity causes resistance to the diffusion of the API through the formulation (Cross *et al.*, 2001:149). This may predict the API will diffuse faster through the atropine

sulphate formulations than through the atropine formulations, which means the API will reach the skin surface faster from the atropine sulphate formulations and skin permeation can take place sooner.

B.7.3 Particle size

A gel consists of a continuous structure and therefore no particle size was determined for the optimised and hydrophilic gels (Barry, 2007:593). The particle size of the two lipophilic emulgels was determined using the Malvern Mastersizer 2000 equipped with Hydro 2000SM wet cell dispersion unit (Malvern Instruments, Worcestershire, UK). The emulgel was dispersed in deionised water and added to the dispersion unit until an obscuration value between 5 to 10% was obtained. Two freshly prepared samples per formulation were used and three measurements were taken from each sample.

Table B.8: Particle size (μm) of the lipophilic emulgels for both atropine and atropine sulphate

Formulations	d(0.1) (μm)	d(0.5) (μm)	d(0.9) (μm)
Atropine lipophilic	0.51 ± 0.19	1.18 ± 0.07	11.90 ± 1.05
Atropine sulphate lipophilic	3.51 ± 0.00	7.26 ± 0.37	24.38 ± 4.32

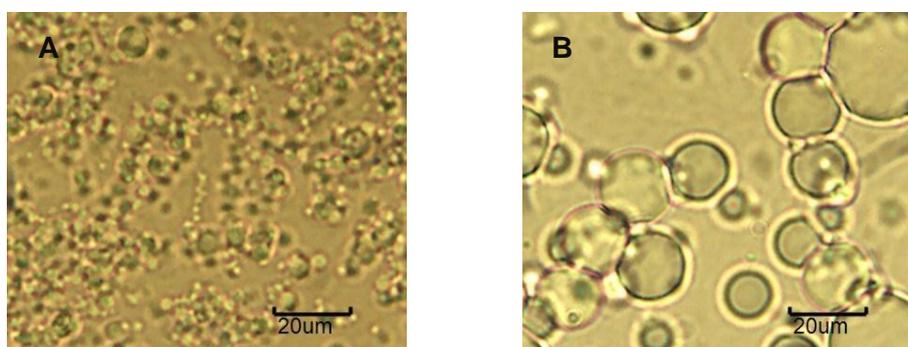


Figure B.5: Micrographs of (A) atropine lipophilic emulgel and (B) atropine sulphate lipophilic emulgel using a Nikon Optiphot light microscope equipped with a Motic Images Advanced 3.2 camera system

As seen in Table B.8 and Figure B.5 the atropine sulphate emulgel had larger particles than the atropine emulgel. For the atropine emulgel, 90% of the particles were smaller than $11.90 \pm 1.05 \mu\text{m}$ and 50% of the particles were smaller than $1.18 \pm 0.07 \mu\text{m}$ compared to $24.38 \pm 4.32 \mu\text{m}$ and $7.26 \pm 0.37 \mu\text{m}$, respectively for the atropine sulphate formulation. Although the atropine sulphate is more hydrophilic than the atropine base, the IAG indicates that both the sulphate salt and the base will be dissolved in the gel matrix rather than the

droplets (consisting mainly of liquid paraffin). In a study performed by Izquierdo *et al.* (as cited by Otto *et al.*, 2009:15) no correlation between the droplet size and dermal/transdermal delivery was observed.

B.8 Summary

Atropine has a theoretical SDG of 0.001 which indicates it should easily penetrate the skin (Wiechers, 2012). Three formulations of different polarities were developed using the FFE™ software. A gel optimised towards the skin was developed for atropine. For the more hydrophilic formulation 10% ethanol and for the more lipophilic formulation 10% liquid paraffin was added to the optimised formulation. The same formulations for atropine were used to prepare the atropine sulphate formulations and all the formulations had an acceptable appearance, viscosity and applied easily.

The formulation characteristics determined were the HSP values and -distances, pH, viscosity, particle size and zeta-potential. The IAG and ISG of the different ingredients indicated DMI was the most compatible single ingredient for API solubility and skin permeation and liquid paraffin was determined to be the least compatible ingredient for skin permeation. Transdermal formulations however, always consist of multiple ingredients (Abbott, 2012:217). The combination of different ingredients may yield a formulation with more desirable HSP values and -distances than the single ingredients (Abbott, 2012:218). The AFG and SFG of the different formulations indicated the optimised formulations should provide the best transdermal delivery of the API. The more lipophilic formulation had the highest SFG which indicates the formulation will permeate the skin to a limited extent. The AFG of the lipophilic formulation indicates the API will prefer to stay in the formulation rather than penetrate the skin. Both the AFG and the SFG of the hydrophilic formulation indicated there may be solubility and compatibility problems for the API in the formulation and the permeation of the formulation into the skin.

To eliminate the salt formation possibility of atropine, the formulation pH was adjusted to ± 7.4 . The atropine formulations had a much higher viscosity than the atropine sulphate formulations. The higher viscosity may lead to a decrease in the permeation of the API into the skin since there is a higher resistance to the diffusion of the API through the formulation (Cross *et al.*, 2001:149). The lower viscosity of the atropine sulphate formulations provides less resistance to diffusion of the API through the formulation and therefore the permeation from the formulation may be higher than for the atropine formulation.

The particle size of the atropine lipophilic emulgel was significantly smaller than that of atropine sulphate. There is however no correlation between the droplet size of an emulgel and the dermal/transdermal delivery of an API (Otto *et al.*, 2009:15). According to the formulation

characteristics, the optimised formulations should provide the best transdermal delivery.

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Appendix C

Franz cell diffusion studies

C.1 Introduction

The use of Franz diffusion cells is the most common technique for the assessment of skin permeability (Bartosova & Bajgar, 2012:4673; Ng *et al.*, 2010:210). The skin sample is mounted between the two compartments of the cell, known as the donor and receptor compartment. The formulation containing the API is applied in the donor compartment onto the skin surface (Bartosova & Bajgar, 2012:4673). The receptor phase should have a sufficient solubilising capacity for the API and should remain in contact with the skin at all times (Bartosova & Bajgar, 2012:4673). The pH of the receptor phase requires adjustment to 7.4 ± 0.1 , which is close to the pH of human skin and the temperature should be kept at 37.0 ± 1 °C, throughout the study (Shah *et al.*, 2013:29).

During this study, three formulations of different polarities were prepared for both atropine and atropine sulphate. Membrane release studies were performed to determine the release of the API from these formulations. Subsequent skin diffusion studies were to determine the transdermal delivery of the API, followed by tape stripping to determine the topical delivery of the API. The results obtained were compared in order to determine the effect of the formulation polarity and HSP profile on the transdermal delivery of the API. According to Wiechers' theory the optimised formulation should result in the highest transdermal delivery since the SFG is the smallest.

C.2 Methods

C.2.1 Formulations preparation

Three formulations of different polarities containing 1.5% API were prepared for both atropine (A) and atropine sulphate (AS). The FFE™ software was utilised to develop a formulation for atropine optimised towards the stratum corneum (A-O), a more hydrophilic gel (A-H) and a more lipophilic emulgel (A-L). The same formulations were used for atropine sulphate (AS-O, AS-H and AS-L). The optimised formulation was adapted to develop formulations of different polarities. The more hydrophilic formulation was obtained by adding 10% ethanol to the water phase of the optimised formulation, whilst 10% liquid paraffin was added to the oil phase to produce the more lipophilic formulation.

Six formulations were prepared (see Appendix B) and all applied easily and had an acceptable skin feel, except for the hydrophilic formulations which were a little tacky. The optimised and hydrophilic gels had a uniform and opaque appearance, whilst the lipophilic emulgels were uniformly white.

A hypothetical SDG was determined using the FFE™ software for atropine, using a serum concentration of 2 ng/ml obtained from literature (Kradjan *et al.*, 1985). The SDG (0.001) indicated the API should readily penetrate the skin (Wiechers, 2012).

C.2.2 Phosphate buffer solution (pH 7.4) preparation

Potassium dihydrogen phosphate (6.805 g) and sodium hydroxide (1.5736 g) were weighed and dissolved in 250 ml and 393.4 ml water, respectively. The solutions were mixed and the pH was adjusted to 7.4 using NaOH and phosphoric acid (H₃PO₄) (BP, 2014).

C.2.3 High performance liquid chromatography analysis

The HPLC method for atropine was developed and validated in conjunction with Prof JL du Preez, from the Analytical Technology Laboratory at the North-West University, Potchefstroom Campus (see Appendix A). An Agilent HP1100 series HPLC with a pump, autosampler, UV detector and Chemstation Rev. A.10.03 data acquisition and analysis software were used (Agilent Technologies, Palo Alto, CA), as well as a Luna C18-2 column (150 x 4.6 mm, 5 µm, 100 Å pores, 17.8% carbon load, end-capped from Phenomenex, Torrance, CA). The mobile phase consisted of methanol and 0.005 M 1-octane sulphonic acid sodium salt in water (pH was adjusted to 3.5 with diluted phosphoric acid) in a 58:42 ratio. The flow rate was 1.0 ml/min with a default injection volume of 50 µl. The UV detector was set at 210 nm for atropine. The retention time of atropine was ± 5.1 min and the stop time was set at 8.0 min.

C.2.4 Solubility of atropine

Saturated solutions of atropine in PBS (pH 7.4), water and *n*-octanol, respectively, were prepared in triplicate by shaking them in a water bath at 32 °C for 24 h. An excessive amount of atropine was added to ensure the solution remained saturated at all times. The solutions were then centrifuged, diluted and analysed using HPLC.

C.2.5 *n*-Octanol/PBS distribution coefficient and *n*-octanol/water partition coefficient

The log P does not take the degree of ionisation of the API into account, therefore log D is a more reliable indication of distribution compared to log P (Ashford, 2007:294). PBS (pH 7.4) and *n*-octanol were saturated by shaking equal amounts of each and leaving to separate.

Atropine (10.84 mg) was dissolved in pre-saturated *n*-octanol. Equal volumes (3 ml) of both the atropine/pre-saturated *n*-octanol and the pre-saturated PBS (pH 7.4) were inserted into a test-tube and shaken in a water bath for 24 h at 32 °C. The solutions were centrifuged for 10 min at 4500 rpm. The *n*-octanol phase (2 ml) was diluted with methanol to 10 ml and both solutions were analysed using HPLC. The log D was calculated using the logarithmic ratio of the atropine concentration in the *n*-octanol and the PBS (pH 7.4). The experiment was performed in triplicate.

The above method was followed to determine the log D for atropine sulphate and also the log P of both atropine and atropine sulphate (using water instead of PBS (pH 7.4)).

C.2.6 Skin preparation

Caucasian abdominal skin was obtained after abdominoplastic surgery, with informed consent of the donors. Ethical approval was obtained from the Ethics Committee of the North-West University, Potchefstroom (Ethics number: NWU-00114-11-A5). An electric dermatome (Zimmer Inc.) was used to remove split thickness skin (400 µm) containing stratum corneum, viable epidermis and upper dermis. The dermatomed skin sample was placed on Whatman® filter paper with the stratum corneum facing upwards and wrapped in aluminium foil. The skin samples were frozen at -20 °C until used. Prior to the skin diffusion study, the skin samples were thawed and cut into circles with a diameter of approximately 12 mm.

C.2.7 Diffusion studies

Ten vertical Franz type diffusion cells, with a receptor capacity of ± 2 ml and a diffusion area of 1.075 cm² were used for each study. A small magnetic stirring bar was placed in the receptor compartment of the cell to maintain stirring throughout the experiment. The membrane/skin samples (stratum corneum facing upwards) were mounted between the donor and receptor compartments. The cells were sealed using Dow Corning® high vacuum grease and secured with a horseshoe clamp. The receptor compartment was filled with 2 ml of the receptor phase (PBS (pH 7.4) and methanol (1:1, v/v)) pre-heated to 37 °C, whilst ensuring no air bubbles were formed. The donor compartment was filled with 1 ml of the semi-solid formulation (pre-heated to 32 °C) and covered with Parafilm® to prevent evaporation. The study was performed on a magnetic stirrer in a water bath kept at 37 ± 1 °C. The entire receptor phase content was extracted on predetermined time intervals and replaced with 2 ml fresh receptor phase pre-heated to 37 °C. The extracted receptor phase was then injected in the HPLC vials and placed in the HPLC for analysis.

C.2.7.1 Membrane release

The aim of the membrane studies is to determine the release of the atropine and atropine sulphate from the semi-solid formulations. The method discussed in Section C.2.7 was used for the membrane diffusion studies. Hydrophilic polyvinylidene fluoride (PVDF) membrane filters were used during this study (FP Vericel, 0.45 μm , 25 mm, Pall[®] Life Sciences, Michigan, USA). The receptor phase content (PBS pH 7.4) was extracted and replaced hourly for six hours.

C.2.7.2 Skin diffusion

The method discussed in Section C.2.7 was used for the skin diffusion studies. The receptor phase content was initially extracted hourly for 12 h, but no data was obtained and therefore only one extraction was done after 12 h. Tape stripping commenced immediately after this (see Section C.2.7.3).

C.2.7.3 Tape stripping

After 12 h, the Franz cells were disassembled and the skin samples were pinned to a solid surface. The semi-solid formulation was removed from the skin sample by dabbing lightly with tissue paper. Small strips of 3M Scotch[®] Magic[™] tape were used; the first strip was discarded and fifteen more strips were used to remove the stratum corneum-epidermis (SCE). The tape strips were placed in a polytop glass vial, whilst the remainder of the skin (epidermis-dermis (ED)) was cut into small pieces and placed in a separate polytop. The polytops were filled with 5 ml of the receptor phase, capped and kept overnight at 4 °C after which the contents were filtered and injected into the HPLC for analysis.

C.2.7 Release and diffusion data analysis

For the membrane release studies, the cumulative amount of the API released from the formulation was plotted against time. The average flux was determined from the slope of the linear regression fit between 2 to 6 h. The release of the API from the formulations was expressed as a percentage of the applied concentration in the donor compartment after 6 h.

For the transdermal diffusion studies, the amount/area diffused after 12 h was calculated. The yield of each cell was expressed as a percentage of the concentration in the donor phase.

The statistical analysis was performed using Statistica (StatSoft, 2014). Both descriptive and inferential statistics were utilised for the analysis of the data obtained from the membrane release, skin diffusion and the tape stripping studies. Descriptive statistics are used to summarise data using the measures of central tendency (mode, median and mean) and variability (standard deviation and variance) (Sheskin, 2000:1, 3, 5). For the purpose of this

study the mean, median and standard deviation were calculated for the flux and concentration values. Box-plots were utilised to illustrate the data. The box connects the 25th and 75th percentile and the length of the box is the interquartile range. The median of the data is represented by the square and the average by the line inside the box. The minimum and maximum values are denoted by the whiskers (straight lines) extending from the box. A data point is considered an outlier if its distance from the box exceeds 1.5 times the interquartile range and is represented by a circle (Smith, 2011:78). In order to draw conclusions from the data, inferential statistics were utilised using the analysis of variance (ANOVA) and non-parametric statistical analysis.

A two-way ANOVA was performed on the membrane release study data to determine if the different APIs and polarities of the formulations had an interaction with the flux values. To evaluate the difference between the mean values obtained from different polarity formulations a one-way ANOVA was employed. To determine exactly where the differences between the mean values were, a Tukey's HSD (honestly significant difference) test was performed. For the skin diffusion studies, a univariate test of significance was performed for both the concentration and the log transformed concentration values. A three-way ANOVA and *t*-test was performed on the data obtained from the tape stripping study to determine the differences between the mean values in the different skin layers obtained from the different formulations.

The data did not have a normal distribution and therefore non-parametric statistical analysis was performed. The Kruskal-Wallis test was utilised to evaluate the data from the membrane release and skin diffusion studies. For the tape stripping data the Mann-Whitney U test was employed. Statistical tests were performed at a 5% significance level.

C.3 Results and discussion

C.3.1 Physicochemical properties

C.3.1.1 Aqueous solubility

Table C.1: Solubility results of atropine

	Solvent	Solubility at 32 °C (mg/ml)
Atropine	PBS (pH 7.4)	5.8
	Water	0.9
	<i>n</i> -octanol	3.2
Atropine sulphate	Water	2500.0*

*literature value

An aqueous solubility of >1 mg/ml is necessary for transdermal delivery (Naik *et al.*, 2000:319). The aqueous solubility of atropine sulphate was obtained from literature (Moffat *et al.*, 2011:933). Due to the high aqueous solubility and the limited amount of atropine sulphate on hand, the aqueous solubility was not determined and the literature value was used. Atropine sulphate should easily be delivered transdermally based on the aqueous solubility. The solubility of atropine in water was determined to be 0.9 mg/ml, which may limit transdermal delivery. The solubility of atropine in PBS (pH 7.4) was determined to be 5.8 mg/ml. The high solubility of atropine (pKa 9.9) in the PBS (pH 7.4) is due to the high degree of ionisation (99.68%) and the formation of ion-pairs with the phosphate salt. The solubility of atropine in *n*-octanol was determined to be 3.2 mg/ml. Atropine has the highest solubility in PBS (pH 7.4) followed by *n*-octanol and water.

C.3.1.2 *n*-Octanol/PBS distribution coefficient and *n*-octanol/water partition coefficient

Table C.2: Log D and log P of atropine and atropine sulphate

Atropine	
Log D	-1.26
Log P	0.22
Atropine sulphate	
Log D	-1.23
Log P	-1.32

According to Yano (cited by Brown *et al.*, 2005:177), a log P of 1 to 3 indicates sufficient solubility in aqueous and lipid material to ensure diffusion through the stratum corneum and the aqueous layers of the epidermis and dermis. The log P of atropine and atropine sulphate was experimentally determined to be 0.22 and -1.32, respectively. These values do not fall in the desired range set by Yano (cited by Brown *et al.*, 2005:177) and thus may predict poor skin penetration of the API. Molecules with a log P < 1, such as atropine and atropine sulphate, may however penetrate the skin primarily via the transcellular route (Williams, 2003:36). The log D of both atropine (-1.26) and atropine sulphate (-1.23) predicted the transdermal delivery might be suboptimal.

C.3.2 Membrane release studies

Formulations containing 0.5% API were used during the membrane release studies, with the results illustrated in Table C.3.

Table C.3: The average and median flux ($\mu\text{g}/\text{cm}^2\cdot\text{h}$), as well as average and median percentage atropine and atropine sulphate released from the formulations with different polarities through membranes after 6 h

Formulations	Average flux ($\mu\text{g}/\text{cm}^2\cdot\text{h}$)	Median flux ($\mu\text{g}/\text{cm}^2\cdot\text{h}$)	Average percentage released (%)	Median percentage released (%)
A-H	153.60 ± 16.77	155.06	13.77 ± 1.33	13.93
A-O	160.94 ± 45.00	136.74	13.58 ± 1.84	13.16
A-L	128.87 ± 18.85	129.91	12.31 ± 1.25	12.58
AS-H	118.76 ± 2.21	117.76	9.48 ± 0.47	11.05
AS-O	154.63 ± 17.88	150.29	13.06 ± 0.64	13.12
AS-L	106.90 ± 17.38	115.99	10.56 ± 1.40	11.07

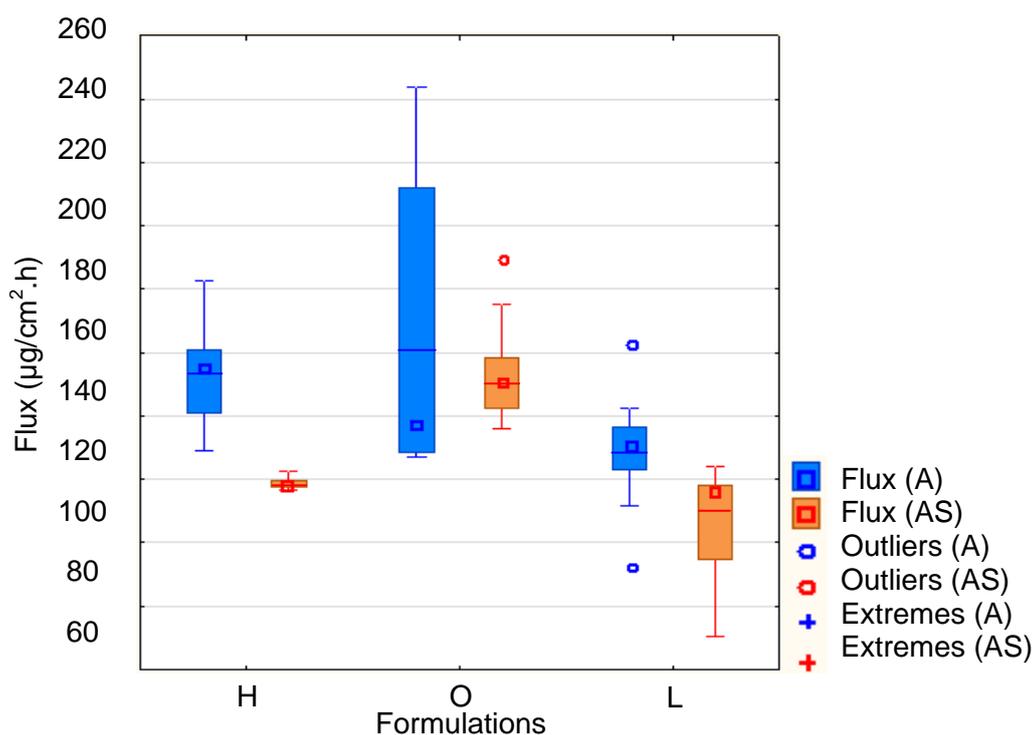


Figure C.1: Flux ($\mu\text{g}/\text{cm}^2\cdot\text{h}$) of atropine and atropine sulphate from the different formulations in the membrane release studies after 6 h. The average and median concentration values are indicated by the lines and squares, respectively (AS-O: $n = 10$; A-O, A-L, AS-L: $n = 9$; A-H, AS-H: $n = 8$).

The results confirmed the API was released from the semi-solid formulations sufficiently enough to be available for transdermal delivery. A comparison of the release of the API from the formulation between the different formulations of A and AS, showed no pattern. The mean or average is calculated by adding all the data values and dividing it by the number of observations (Smith, 2012:73). The median is the middle value of a set of data arranged from highest to lowest or *vice versa*. The median indicates that half of the observations are bigger or smaller than that specific value (Smith, 2012:75). The median is more resistant to outliers than the average and thus a more reliable representation of data (Smith, 2012:76). Outliers were observed in the data and therefore the median is used to describe the data. The biggest differences between the average and median flux values were observed with A-O and AS-L. For the atropine formulations, A-H had the highest median flux (155.06 $\mu\text{g}/\text{cm}^2\cdot\text{h}$) followed by A-O (136.74 $\mu\text{g}/\text{cm}^2\cdot\text{h}$) and A-L (129.91 $\mu\text{g}/\text{cm}^2\cdot\text{h}$). The highest median flux for the atropine sulphate formulations was observed with AS-O (150.29 $\mu\text{g}/\text{cm}^2\cdot\text{h}$) followed by AS-H (117.76 $\mu\text{g}/\text{cm}^2\cdot\text{h}$) and AS-L (115.99 $\mu\text{g}/\text{cm}^2\cdot\text{h}$).

A-H had the highest (13.93%) and AS-H the lowest (11.05%) median percentage released of all six formulations. The reduced solubility of the lipophilic atropine in the hydrophilic formulation provides a driving force for atropine to leave the formulation, whilst being a desirable environment for the hydrophilic atropine sulphate (Otto *et al.*, 2009:9). The three formulations containing atropine only had minor differences in the median percentage released after 6 h with A-H having the highest median percentage (13.93%) followed by A-O (13.16%). A-L had the lowest median percentage released (12.58%) after 6 h. The lipophilic atropine has a high affinity for the lipophilic emulgel and would therefore prefer to stay in the formulation, resulting in poor release. For the atropine sulphate formulations there were more variation than for the atropine formulations. The highest median percentage released was obtained with the AS-O (13.12%), followed by AS-L (11.07%) and AS-H (11.05%). The median percentage release for AS-H and AS-L did not differ significantly. AS-L is an emulgel consisting of lipophilic droplets dispersed in a hydrophilic continuous phase. The hydrophilic atropine sulphate has a high affinity for the continuous phase and it will therefore prefer to stay in the formulation.

When comparing the atropine and the atropine sulphate formulations of the same polarity, a higher median percentage release was observed from the atropine formulations than from the atropine sulphate formulations. This indicates the API base has a higher release than the API sulphate salt form.

C.3.3 Skin diffusion studies

Diffusion studies were done with formulations containing 0.5% API. The transdermal delivery was very poor and in some cases no data was obtained. The explanation for the results was an

insignificant concentration gradient to provide a driving force. The API concentration was increased to 1.5% and the diffusion studies repeated. Table C.4 presents the results obtained from the diffusion study.

Table C.4: Data obtained from skin diffusion studies

Formulations	Average amount per area diffused ($\mu\text{g}/\text{cm}^2$)	Median amount per area diffused ($\mu\text{g}/\text{cm}^2$)	Average percentage diffused (%)	Median percentage diffused (%)
A-H	4.500 ± 2.450	4.50	0.018 ± 0.010	0.015
A-O	10.886 ± 4.436	10.40	0.039 ± 0.020	0.037
A-L	65.695 ± 81.723	18.04	0.236 ± 0.290	0.065
AS-H	2.982 ± 1.906	2.78	0.011 ± 0.010	0.010
AS-O	7.722 ± 2.972	6.66	0.028 ± 0.010	0.024
AS-L	122.800 ± 181.698	17.60	0.440 ± 0.650	0.063

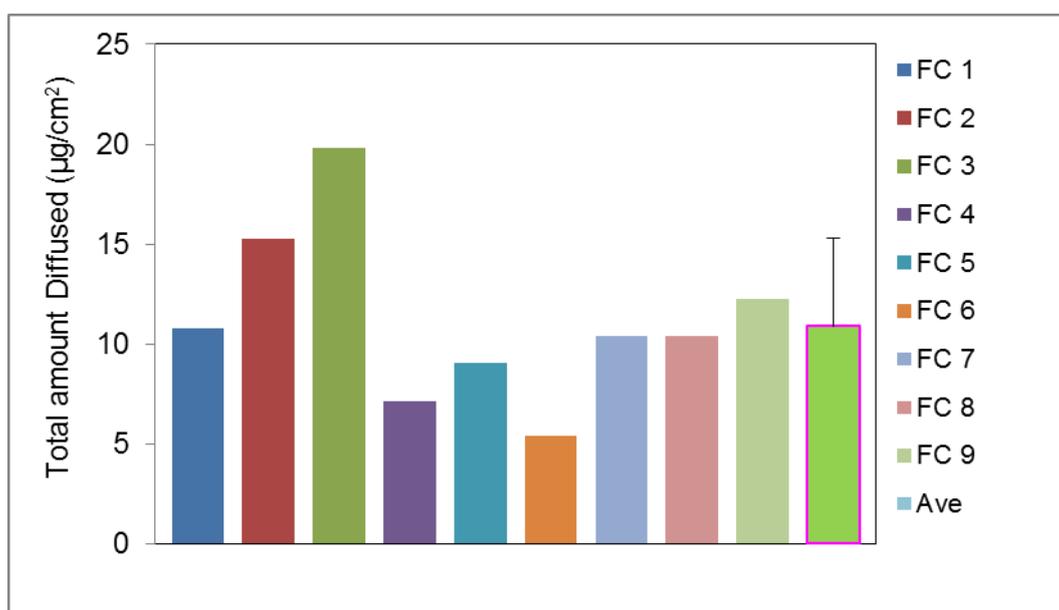


Figure C.2: The amount of atropine per area ($\mu\text{g}/\text{cm}^2$) for A-O gel which diffused through the skin after 12 h (n = 9)

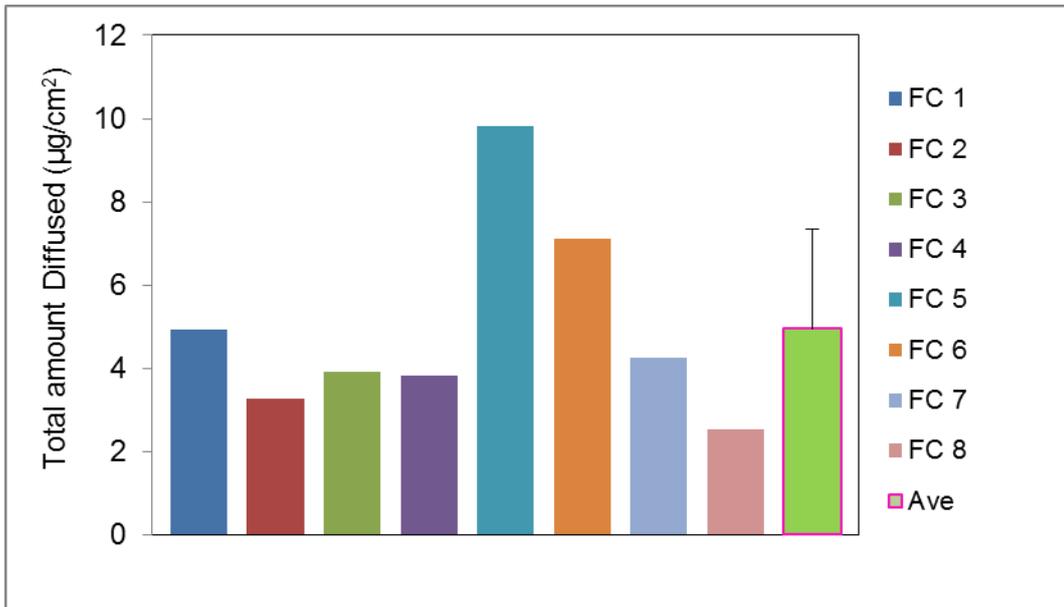


Figure C.3: The amount of atropine per area ($\mu\text{g}/\text{cm}^2$) for A-H gel which diffused through the skin after 12 h (n = 8)

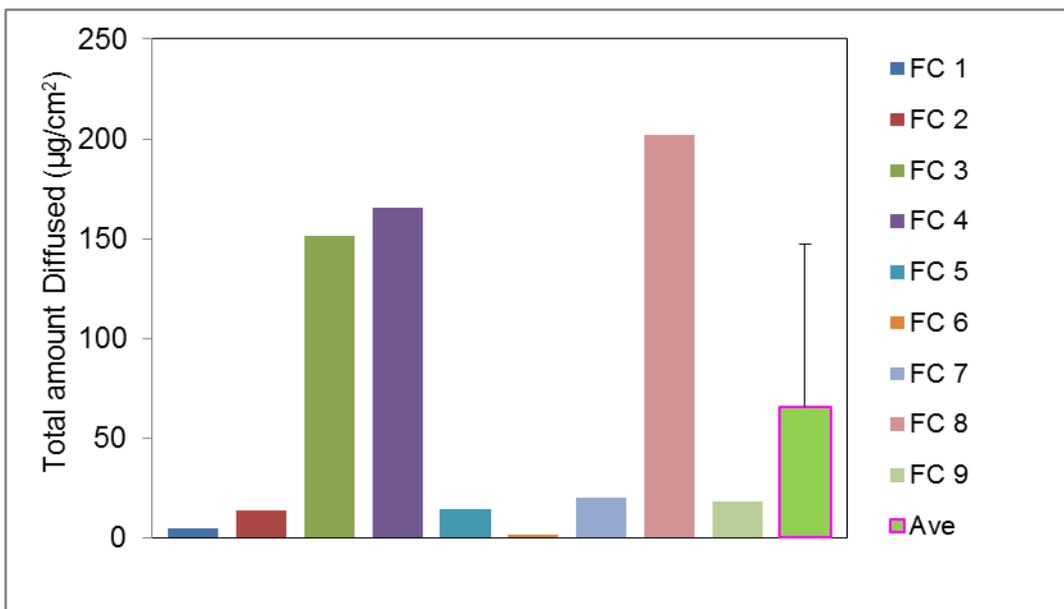


Figure C.4: The amount of atropine per area ($\mu\text{g}/\text{cm}^2$) for A-L gel which diffused through the skin after 12 h (n = 9)

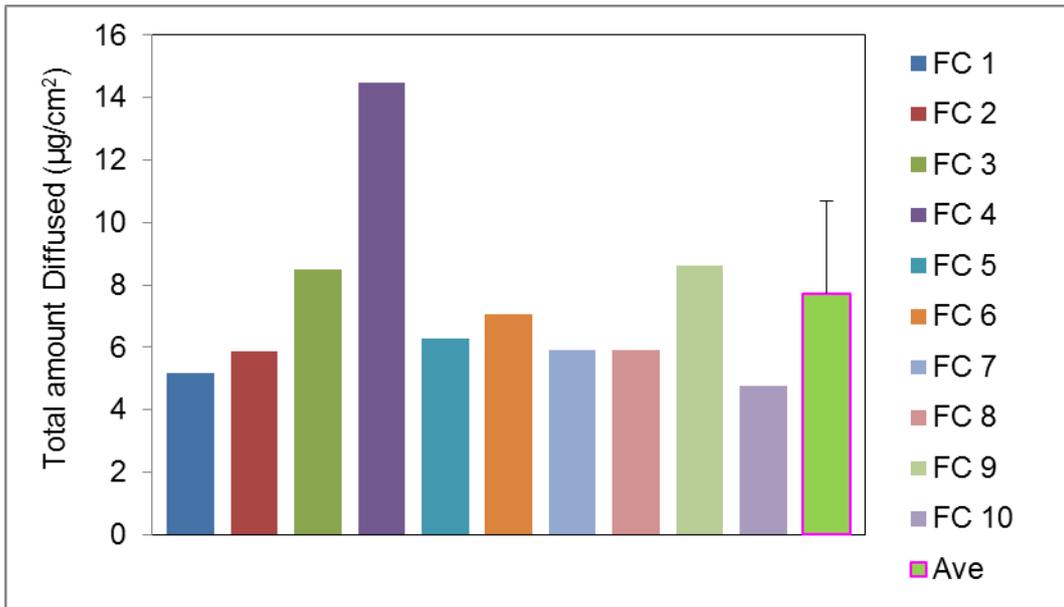


Figure C.5: The amount of atropine per area ($\mu\text{g}/\text{cm}^2$) for AS-O gel which diffused through the skin after 12 h ($n = 10$)

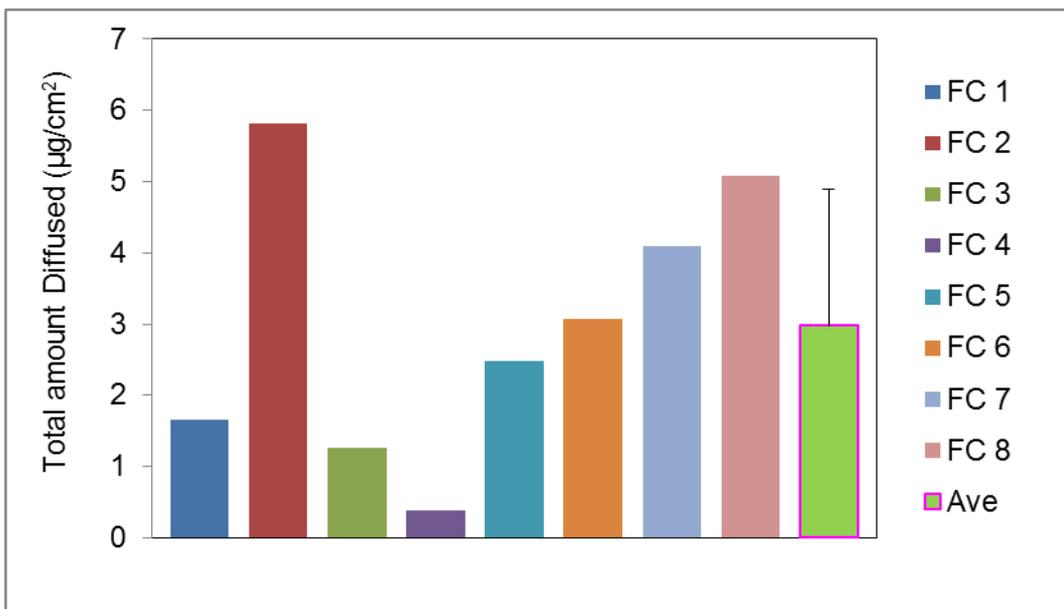


Figure C.6: The amount of atropine per area ($\mu\text{g}/\text{cm}^2$) for AS-H gel which diffused through the skin after 12 h ($n = 8$)

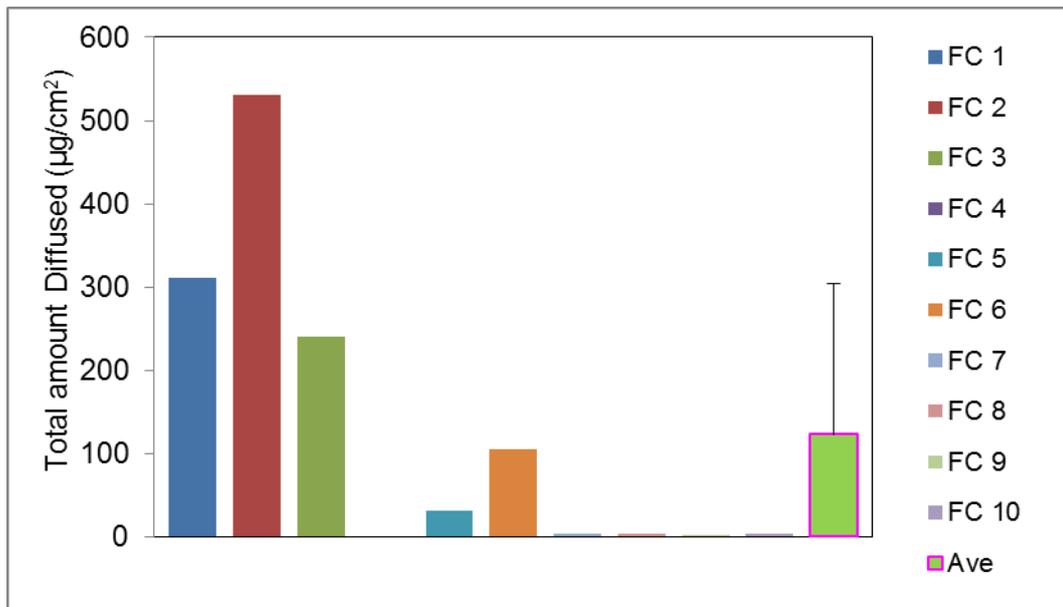


Figure C.7: The amount of atropine per area ($\mu\text{g}/\text{cm}^2$) for AS-L gel which diffused through the skin after 12 h ($n = 9$)

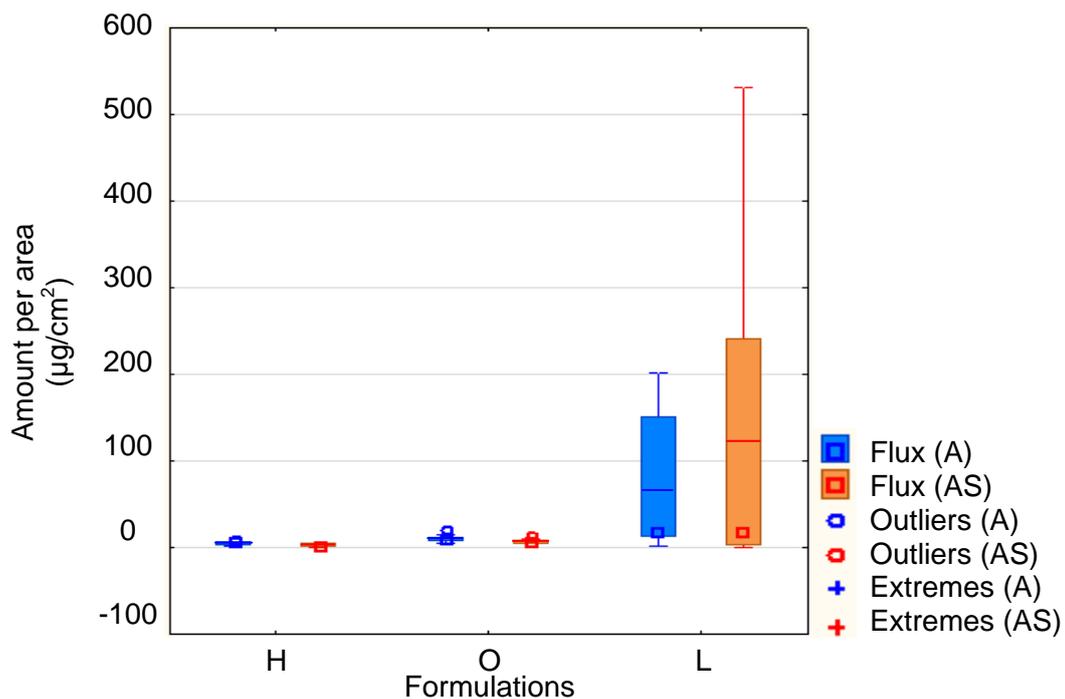


Figure C.8: Amount per area ($\mu\text{g}/\text{cm}^2$) of atropine and atropine sulphate which diffused through the skin from the different formulations. The average and median concentration values are indicated by the lines and squares, respectively (AS-O: $n = 10$; A-O, A-L, AS-L: $n = 9$; A-H, AS-H: $n = 8$).

The average and median amount/area diffused for the A-O, A-H, AS-O and AS-H did not have a significant difference (see Section C.4 for statistical analysis). The average and median amount/area diffused differed significantly for A-L and AS-L, indicating the data contains outliers and therefore only the median values are used to describe the data. The highest median amount/area diffused was observed from the lipophilic formulation followed by the optimised formulation and the more hydrophilic formulation for both A and AS. A-H (4.50 $\mu\text{g}/\text{cm}^2\cdot\text{h}$) and AS-H (2.78 $\mu\text{g}/\text{cm}^2\cdot\text{h}$) yielded nearly half the median amount/area diffused of A-O (10.40 $\mu\text{g}/\text{cm}^2\cdot\text{h}$) and AS-O (6.66 $\mu\text{g}/\text{cm}^2\cdot\text{h}$), respectively. The median amount/area diffused for A-L (18.04 $\mu\text{g}/\text{cm}^2\cdot\text{h}$) was double the median amount/area diffused for A-O, whilst AS-L (17.6 $\mu\text{g}/\text{cm}^2\cdot\text{h}$) resulted in almost three times the median amount/area diffused of AS-O.

A comparison of the different formulations indicated a significant difference between the median percentages diffused (see Section C.4 for statistical analysis). For both A and AS the L-formulation yielded the highest median percentage diffused, followed by the O-formulation and the H-formulation. The median percentage diffused for AS-L (0.063%) was almost triple the median percentages diffused of AS-O (0.024%), whilst A-L (0.065%) was almost double that of A-O (0.037%). A-H (0.015%) and AS-H (0.010%) resulted in nearly half the median percentage diffused of A-O (0.370%) and AS-O (0.024%), respectively, which could be explained by considering the IAG, ISG, AFG and SFG of the different formulations (see Tables B.5 and B.6). For the lipophilic formulation, the SFG (9.5) is greater than the AFG (3.8), meaning that although the API is sufficiently soluble in the formulation, the formulation is not likely to penetrate the skin. The individual ingredients might however still penetrate the skin. It is hypothesised that the penetration of DMI (IAG:3.09) into the skin resulted in a change in the composition of the residual formulation on the skin (Otto *et al.*, 2009:2). Both PEG 8 and liquid paraffin have undesirable IAG values (4.81 and 8.05, respectively), which indicates the residual formulation is an unwelcome environment for the API. This undesirable residual formulation, together with the increased solubility of the API in the skin created by the penetrated DMI, provided a driving force for the API to leave the formulation and enter the skin resulting in the higher transdermal delivery (Abbott, 2012:218). For the optimised formulations, both the AFG (3.5) and SFG (1.9) indicate desirable solubility, but the AFG is greater than the SFG. The SFG suggests the formulation and the skin are close together in terms of HSPs and therefore will easily penetrate the skin (Abbott, 2012:218). The penetration of the formulation into the skin caused the skin to swell and a more welcome environment in the skin was created for the API. Due to the similarity of the formulation and the skin, the API was distributed between the formulation and the skin almost evenly (Abbott, 2012:218). The optimised formulations however did not have the additional driving force caused by the undesirable residual formulation on the skin, as seen with the lipophilic formulations. The hydrophilic formulations resulted in the lowest transdermal delivery because the AFG (5.3) and SFG (5.7) both indicate undesirable solubility

(AFG > 4 and SFG > 4) and consequently low skin permeation (Abbott, 2012:221).

When comparing the formulations of A and AS with each other, A yielded a higher median amount/area diffused and median percentage diffused than AS for all three formulations. The biggest difference in the median amount/area diffused between A and AS was observed with the H and O formulations. A-H (0.015%) and A-O (0.370%) yielded almost double the median amount/area diffused of AS-H (0.100%) and AS-O (0.240%), respectively, whilst the values for A-L (0.065%) and AS-L (0.063%) were practically the same. This observation can be explained by the log P values of A and AS. A gel is typically a polar formulation (H and O) and therefore the more hydrophilic AS (log P = -1.31) would prefer to stay in the formulation, whilst the more lipophilic A (log P = 0.22) would prefer to leave the formulation (Barry, 2007:593).

C.3.4 Tape stripping

Table C5: Data obtained from tape stripping

Formulations	Average concentration in SCE (µg/ml)	Median concentration in SCE (µg/ml)	Average concentration in ED (µg/ml)	Median concentration in ED (µg/ml)
A-H	0.23 ± 0.06	0.21	1.03 ± 1.40	0.55
A-O	0.39 ± 0.63	0.00	0.00 ± 0.00	0.00
A-L	0.00 ± 0.00	0.00	0.51 ± 0.17	0.48
AS-H	0.29 ± 0.10	0.29	0.59 ± 0.22	0.55
AS-O	0.00 ± 0.00	0.00	0.00 ± 0.00	0.00
AS-L	0.00 ± 0.00	0.00	0.97 ± 0.84	0.52

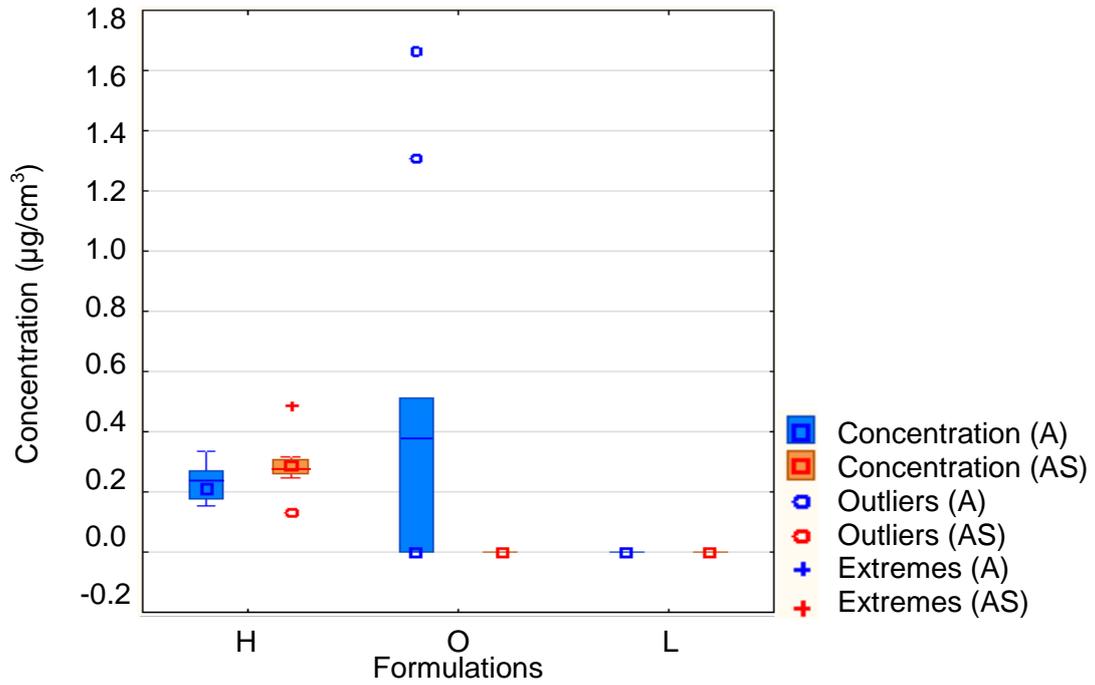


Figure C.9: Concentration ($\mu\text{g/ml}$) of atropine and atropine sulphate in the SCE for the different formulations after tape stripping. The average and median concentration values are indicated by the lines and squares, respectively (AS-O: $n = 10$; A-O, A-L, AS-L: $n = 9$; A-H, AS-H: $n = 8$).

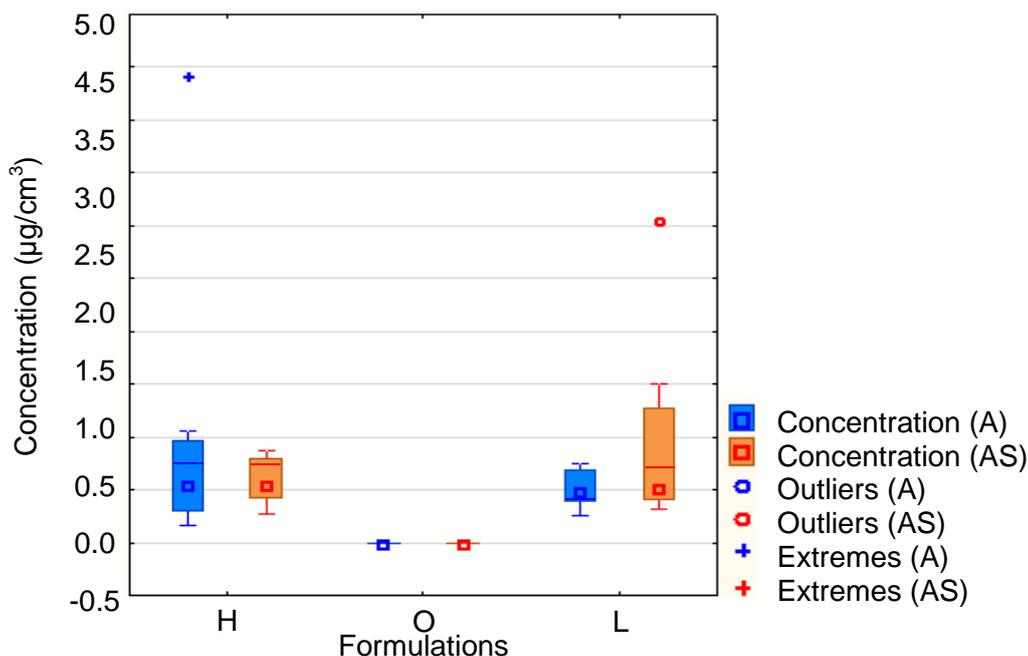


Figure C.10: Boxplots illustrating the concentration ($\mu\text{g}/\text{ml}$) of atropine and atropine sulphate in the ED for the different formulations after tape stripping. The average and median concentration values are indicated by the lines and squares, respectively (AS-O: $n = 10$; A-O, A-L, AS-L: $n = 9$; A-H, AS-H: $n = 8$).

The differences in the average and median values indicated there were outliers in the data and therefore only the median values will be discussed. The median concentration of the API was higher in the ED than in the SCE, except for A-O and AS-O, for which no API was observed in the SCE or ED. No amount of API was observed in the SCE from the lipophilic formulations. The hydrophilic formulations had the highest concentration in both the SCE (A-H: $0.21 \mu\text{g}/\text{ml}$ and AS-H: $0.29 \mu\text{g}/\text{ml}$) and the ED (A-H: $0.55 \mu\text{g}/\text{ml}$ and AS-H: $0.55 \mu\text{g}/\text{ml}$), followed by the lipophilic formulations in the ED (A-L: $0.48 \mu\text{g}/\text{ml}$ and AS-L: $0.52 \mu\text{g}/\text{ml}$). AS-H yielded a slightly higher median concentration in the SCE compared to A-H. The highest median concentration in the ED was obtained from A-H and AS-H, followed by AS-L and A-L. More API is obtained in the ED compared to the SCE, which is contrary to what was expected since the ED is essentially aqueous and atropine would therefore rather stay in the more lipophilic SC.

C.4 Statistical analysis

C.4.1 Membrane release studies

The data for A and AS were separately analysed by means of one-way analyses of variance (ANOVA) to compare H, L and O, followed by Tukey's studentised range HSD (honestly

significant difference) tests. Significant differences were observed, but due to the non-normality of the data subsequently non-parametric analyses were done using Kruskal-Wallis tests, followed by multiple comparisons between H, L and O. The test indicated there was a significant difference between A-H and A-L ($p = 0.039$), between AS-O and AS-H ($p = 0.018$), as well as between AS-O and AS-L ($p < 0.001$).

C.4.2 Skin diffusion studies

The univariate ANOVA test was used to analyse the amount/area diffused and the log amount/area diffused. Since, non-normality was encountered for both, it was decided to resort to non-parametric statistics using the Kruskal-Wallis test, followed by multiple comparisons. These analyses indicated significant differences between A-O and A-H ($p = 0.032$), as well as between AS-H and AS-L ($p = 0.005$).

C.4.3 Tape stripping

The Mann-Whitney U test indicated a significant difference ($p = 0.0256$) between A-O and AS-O in the SCE, but since there was no value for AS-O in the SCE it is regarded as insignificant. No significant difference was observed between A-H and AS-H in both the SCE ($p = 0.1035$) and the ED ($p = 0.9581$), or between A-L and AS-L in the ED ($p = 0.2703$). The p-value for A-O and AS-O in the ED and A-L and AS-L in the SCE could not be calculated since no concentration of the API in respective skin layers was observed.

C.5 Conclusion

The aqueous solubility of atropine (0.9 mg/ml) and the log D of atropine and atropine sulphate (-1.26 and -1.23, respectively) indicated transdermal delivery may be suboptimal (Brown *et al.*, 2005:177; Naik *et al.*, 2000:319). The high degree of ionisation (99.68%) of the API also predicted poor delivery.

The release of the API from the formulation was confirmed during the membrane release studies. The highest median percentage released was observed from A-H, while AS-H had the lowest median percentage released after 6 h. The H-formulation provides a desirable environment for the AS and it would therefore prefer to stay in the formulation. The more lipophilic A has a low affinity for the H-formulation resulting in a higher driving force and consequently a high release (Otto *et al.*, 2009:9). When comparing the median percentage released from the atropine formulations (A-H (13.93%), A-O (13.16%) and A-L (12.58%)) to the atropine sulphate formulations (AS-H (11.05%), AS-O (13.12%) and AS-L (11.07%)), the indication was that the API base provides a better release than the sulphate salt.

The skin diffusion studies indicated that, for both atropine and atropine sulphate respectively, the lipophilic formulations (0.063% and 0.065%) had the highest percentage transdermal delivery followed by the optimised (0.037% and 0.023%) and the hydrophilic (0.015% and 0.010%) formulations. The SFG of the lipophilic formulation is higher than the AFG, which indicates the API is sufficiently soluble in the formulation, but the formulation is not likely to penetrate the skin (Abbott, 2012:218). Some of the ingredients in the lipophilic formulation penetrated the skin faster than others (like DMI) and resulted in a change in the composition of the formulation over time (Abbott, 2012:221; Otto *et al.*, 2009:2). The undesirable residual formulation provided a driving force for the API to enter the skin and a more desirable environment in the skin, created by the penetrating DMI, resulted in a high transdermal delivery.

The atropine formulations resulted in a higher median percentage diffused compared to the atropine sulphate formulations of the same polarity. This indicated a higher release from the formulations resulted in a higher transdermal delivery, since more API is on the skin surface and available for penetration.

The highest concentration in the skin was observed with A-H and AS-H. A-H and AS-H had the lowest driving force for the API to leave the formulation based on the HSP profile and the penetration of the API was only driven by the concentration gradient over the skin, indicating the API penetrated the skin slower from the H-formulations compared to the O- and L-formulations. After 12 h, the API had not fully traversed through the skin and high concentrations were observed in the skin compared to the poor transdermal delivery, indicating the added driving force provided by the HSP profile (as seen with the O- and L-formulations) increases the penetration of the API through the skin.

The O-formulations were optimised towards the skin and it was expected that high concentrations of the API would be obtained in the skin from these formulations, but no API was observed. The reason for this may be due to fast initial penetration of the API and as the concentration gradient decreases so does the skin penetration. After 12 h the concentration gradient may be too low to provide a driving force for penetration and the amount of API in the skin may be below the LOD of the analytical method. The lipophilic formulations had the highest driving force to leave the formulation and permeate the skin based on the HSP profile. The high driving force forced the API into the SC where it diffused to the ED. The high transdermal delivery from the L-formulations indicates that most of the API fully traversed through the SC, into and through the ED.

For both A-H and AS-H, the concentration in the ED (0.55 µg/ml for both) was higher than in the SCE (0.21 µg/ml and 0.29 µg/ml, respectively). A-L and AS-L also had a higher concentration in the ED (0.48 µg/ml and 0.52 µg/ml) than in the SCE (no concentration for both). This

indicates the API penetrated the skin deeper than the superficial layers of the skin and will be available for systemic absorption. The amount of API which diffused through the skin was considerably higher than the concentrations in the skin for A-O, AS-O, A-L and AS-L, which indicates deeper penetration and essentially systemic delivery.

In conclusion, the HSP profile of a formulation has a definite effect on the delivery of the API. Wiechers states that a formulation optimised towards the SC with a small SFG should result in the highest transdermal delivery of an API. According to Abbott (2012:218), a good balance is needed between the AFG and SFG to ensure sufficient penetration of the API through the skin by generating a driving force and by increasing the solubility of the API in the skin as the formulation penetrates. It was observed that a higher SFG, compared to AFG, resulted in a higher transdermal delivery of the API. It is important to note that the HSP profile of the formulation changes as some ingredients penetrate the skin faster than others and therefore one cannot only use the HSP profile of the formulation when predicting the transdermal delivery of an API. The HSP profile of individual ingredients and the API should also be considered when developing a formulation optimised for transdermal delivery; smaller ingredients with a small ISG will penetrate faster than ingredients with a larger ISG and mVol, and it is therefore important to consider the composition change and resulting change in the HSP profile when developing a formulation. When the smaller ingredient also has a small IAG (such as DMI), the penetration of the ingredient into the skin will generate a welcome environment for the API and it will be encouraged to enter the skin. If the remaining ingredients have larger IAGs, there will be an additional driving force for the API to leave the formulation caused by the insolubility of the API in the residual formulation. The lipophilic formulation resulted in the highest transdermal delivery compared to the optimised and more hydrophilic formulations. When comparing the transdermal delivery of the atropine with the atropine sulphate, it can be concluded that the base API provides better transdermal delivery than the salt form.

It can therefore be concluded that the polarity of the formulation affects the transdermal delivery of an API, but it is important to consider the total HSP profile and molar volume of the API and the ingredients to predict the transdermal delivery of an API, rather than just the SFG or AFG. The results indicated the sulphate salt of API reduced the transdermal delivery of the API, therefore it is better to use the API base instead of the salt form. The salt form of an organic compound has a higher HSP indicating it is more soluble in water and less soluble in polymers like the human skin and therefore poor transdermal results are obtained from the atropine sulphate formulations. The results obtained in this study supported the Delivery Gap Principle of Wiechers, since effective transdermal delivery was obtained with an API with a SDG < 1 (Wiechers, 2012).

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Appendix D

International Journal of Pharmaceutics: Author Guidelines

D.1 Introduction

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, characterization 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 characterization; 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).

D.2 Types of paper

D.2.1 Full length manuscripts

D.2.2 Rapid communications

- a) These articles should not exceed 1500 words or equivalent space.
- b) Figures should not be included otherwise delay in publication will be incurred.
- c) Do not subdivide the text into sections. An Abstract should be included as well as a full reference list.

D.2.3 Notes

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

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

D.2.4 Reviews and mini-reviews

Suggestions for review articles will be considered by the Review-Editor. "Mini-reviews" of a topic are specially welcome.

D.3 Before you begin

D.3.1 Ethics in publishing

For information on Ethics in publishing and Ethical guidelines for journal publication see <http://www.elsevier.com/publishingethics> and <http://www.elsevier.com/journal-authors/ethics>.

D.3.2 Human and animal rights

If the work involves the use of animal or human subjects, the author should ensure that the work described has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans <http://www.wma.net/en/30publications/10policies/b3/index.html>; EU Directive 2010/63/EU for animal experiments http://ec.europa.eu/environment/chemicals/lab_animals/legislation_en.htm; Uniform Requirements for manuscripts submitted to Biomedical journals <http://www.icmje.org>. Authors should include a statement in the manuscript that informed consent was obtained for experimentation with human subjects. The privacy rights of human subjects must always be observed.

D.3.3 Conflict of interest

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