

The Transdermal Delivery of Various Anti-emetics

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ABSTRACT

Motion sickness, although viewed as a sickness, is in actuality a psychophysiological response to motion. The most common cause of motion sickness is the mismatch between the vestibular and visual systems. An increase in the activity of the sympathetic nervous system and subsequently a decrease in the parasympathetic nervous system are observed. Symptoms include stomach discomfort, nausea and actual vomiting. Numerous people would rate the severity of their motion sickness on the severity of their nausea. The severity of the symptoms will ultimately be determined by the stimulus and the individual's susceptibility (Muth, 2006:58).

In general, the most frequent treatments include cyclizine, an antihistamine available over the counter, and scopolamine, a muscarinic antagonist, viewed as the only effective drug in combating motion sickness. New strategies to deliver these drugs are thus required to attain the maximum benefit of the drugs with the least possible side-effects.

The skin offers an attractive route to deliver drugs, despite the numerous limitations (Naik *et al.*, 2000:319). Being exposed to chemicals, physical torture and deliberately applied products like cosmetics; the barrier is constantly put to test (Zatz, 1993:11). Plentiful advantages exist when delivering cyclizine and scopolamine transdermally. The oral route might not always be available when treating nausea and vomiting and the first pass metabolism is ruled out (Ball & Smith, 2008:1337-1338).

A new technology, the Pheroid™ technology was incorporated in this study in order to investigate whether it would enhance the permeation of anti-emetics through the skin. The Pheroid™ technology is a vesicular structure that contains neither phospholipids nor cholesterol. The structure is compiled of essential fatty acids and therefore natural to the body (Grobler *et al.*, 2008:283). The aim of this study was to formulate cyclizine and scopolamine in solutions with and without Pheroid™ and subsequently formulated scopolamine in an emulgel with and without Pheroid™.

The octanol-buffer distribution coefficient (log D) and aqueous solubility were determined for both cyclizine and scopolamine at pH 7.4. The aqueous solubility and log D of cyclizine could not accurately be determined due to the insolubility of cyclizine in water and PBS. The literature values for cyclizine of 3.11 (Monene *et al.*, 2005:243) for log D and 1 mg/ml (Drugbank, 2010a) for aqueous solubility was assumed to be correct. The aqueous solubility of scopolamine could not accurately be determined due to the immense amount of scopolamine dissolving in water, resulting in thick, syrup-like solution. The value of 1000 mg/ml was assumed to be correct

(Drugbank, 2010b). A log D of 1.77 indicated that scopolamine would be a favourable drug to consider for transdermal delivery.

A 0.5% cyclizine and a 1% scopolamine solution with and without Pheroid™ was formulated and a 12 h Franz cell diffusion study was conducted with full thickness skin, where after tape-stripping and analysis of the concentration in the dermis was done. The cyclizine solution did not penetrate the skin. This might be due to the low aqueous solubility of cyclizine. The scopolamine solution delivered a result of 14.012 µg.cm² and the scopolamine solution containing Pheroid™ a result of 6.486 µg/cm². The scopolamine solution delivered results of 0.0128 µg/cm² in the stratum corneum (SC)-epidermis and 0.2035 µg/cm² in the epidermis-dermis. For the scopolamine solution containing Pheroid™, the concentrations in the SC-epidermis and epidermis-dermis were 0.0044 µg/cm² and 0.0525 µg/cm² respectively. A 12 h Franz cell diffusion study using only epidermis was performed with the scopolamine emulgel and scopolamine emulgel containing Pheroid™. The emulgel delivered a concentration of 2.649 µg/cm² and the emulgel containing Pheroid™ delivered a concentration of 0.017 µg/cm². When the solutions were compared to the emulgel formulations, the scopolamine solution delivered the highest concentration scopolamine. The Pheroid™ formulations contain a higher oil content, thus decreasing diffusion through the skin (Barry, 2002:513). When previously formulated, scopolamine released only 30 % from its dosage form; the rest of the diffusion was ultimately determined by the patients' skin itself (Barry, 2007:591).

Stability tests were conducted on the emulgel formulations for a period of 3 months. The emulgel formulations were stored at 25 °C/60% relative humidity (RH), 30 °C/60% RH and 40 °C/70% RH. Concentration assays were done on the high performance liquid chromatography (HPLC) to determine the concentration of scopolamine, methyl paraben, propyl paraben, BHT (butylated hydroxytoluene) and tocopherol. Other stability tests included pH, viscosity, visual appearance, mass loss and confocal laser scanning microscopy.

The emulgel formulations were not stable over the 3 months stability test period. A change in colour, viscosity and decreasing active ingredients were observed.

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Alhoewel reisigersnaarheid as 'n siekte beskou word, is dit in werklikheid 'n psigiese-fisiologiese reaksie tot beweging. Die mees algemene oorsaak van reisigersnaarheid is die variasie wat tussen die vestibulêre en visuele sisteme waargeneem word. 'n Verhoging in aktiwiteit van die simpatiese senuweestelsel en 'n verlaging in die parasimpatiese senuweestelsel se aktiwiteit word waargeneem. Simptome van reisigersnaarheid is onder andere naarheid, ongemak van die maag en braking. Mense gradeer hulle reisigersnaarheid aan die mate van die naarheid wat hulle ondervind. Die erns van die simptome word deur die stimulus en die individu se vatbaarheid bepaal (Muth, 2006:58).

Die mees algemene behandeling vir reisigersnaarheid sluit siklisien, 'n antihistamien wat oor die toonbank beskikbaar is en skopolamien, 'n muskariene-antagonis, wat as die mees effektiewe behandeling beskou word, in. Nuwe strategieë word dus benodig om hierdie middels op die uiteinde af te lewer sodat die maksimum voordeel van die middel verkry word; gepaard met die minste moontlike nuwe-effekte.

Ten spyte van die menige beperkings wat die vel toon; beskik dit oor verskeie voordele (Naik *et al.*, 2000:319). Die vel beskerm die liggaam daagliks teen skadelike stowwe onder andere chemikalieë, fisiese skade en kosmetiese stowwe (Zatz, 1993:11). Verskeie voordele kom na vore wanneer siklisien en skopolamien transdermaal afgelewer word. Die orale roete is nie altyd beskikbaar wanneer naarheid en braking behandel word nie en die eerstedeurgangseffek word vermy (Ball & Smith, 2008:1337-1338).

'n Nuwe tegnologie naamlik die Pheroid™ tegnologie was in hierdie studie gebruik om vas te stel of dit die penetrasie van anti-emetika deur die vel kan verhoog. Pheroid™ bevat geen fosfolipiede of cholesterol nie en is verder op 'n blaasagtige struktuur gebaseer. Essensiële vetsure maak die grootste deel van die Pheroid™ uit en word dus deur die liggaam as natuurlik beskou (Grobler *et al.*, 2008:283). Die doel van hierdie studie was om siklisien en skopolamien onderskeidelik in vloeibare doseervorme te vervaardig met en sonder die gebruik van Pheroid™. Die geneesmiddel wat die beste resultate deur die vel gelever het, is in 'n semi-soliede emulgel geformuleer.

Die oktanol-fosfaatbufferoplossing verdelingskoeffisiënt (log D) en wateroplosbaarheid was vir beide siklisien en skopolamien by pH 7.4 vasgestel. Die resultate van siklisien was egter nie akkuraat nie, aangesien dit onoplosbaar in water en die fosfaatbufferoplossing is. Die literatuurwaardes van 3.11 vir log D (Monene *et al.*, 2005:243) en 1 mg/ml (Drugbank, 2010a) vir

wateroplosbaarheid was as korrek aanvaar. Die wateroplosbaarheidresultate van skopolamien was nie akkuraat nie, aangesien skopolamien baie goed oplosbaar in water was en 'n dik, stroopagtige oplossing tot gevolg gehad het, wat nie geanaliseer kon word nie. Die literatuurwaarde van 1000 mg/ml was as korrek aanvaar (Drugbank, 2010b). 'n Log D waarde van 1.77 het aangedui dat skopolamien 'n gunstige middel is om te oorweeg vir transdermale aflewering.

Om vas te stel watter anti-emetikum die beste resultate deur die vel sal lewer, was 'n vloeibare doseervorm met en sonder Pheroid™ met 0.5% siklisien en 'n 1% skopolamien onderskeidelik geformuleer 'n 12 uur Franz-selldiffusiëstudie was met voldikte vel uitgevoer gevolg deur stratum corneum-epidermis en epidermis-dermis analiese. Siklisien het nie die vel gepenetreer nie. Die lae wateroplosbaarheid mag die rede daarvoor wees. Gemiddelde konsentrasies van 14.012 µg/cm² en 6.486 µg/cm² was onderskeidelik vir die skopolamienformulering en die skopolamienformulering met Pheroid™ verkry. Die skopolamienformulering het onderskeidelik konsentrasies van 0.0128 µg/cm² in die stratum korneum-epidermis en 0.2035 µg/cm² in die epidermis-dermis gelewer. Die skopolamienformulering met Pheroid™ het onderskeidelik konsentrasies in die stratum korneum-epidermis en epidermis-dermis van 0.0044 µg/cm² en 0.0525 µg/cm² gelewer. 'n 12 uur Franz-selldiffusiëstudie vir die emulgel beide met en sonder Pheroid™ was met epidermis, alleenlik, uitgevoer. Die emulgel en die emulgel met Pheroid™ het onderskeidelik konsentrasies van 2.649 µg/cm² en 0.017 µg/cm², gelewer. Die emulgel met Pheroid™ beskik oor groter olie druppels wat die penetratsie van die vel moontlik kan benadeel (Barry, 2002:513). Vorige studies het bewys dat slegs 30 % van die skopolamien in die doseervorm vrygestel word. Die res van die diffusie word uiteindelik deur die individu se vel bepaal (Barry, 2007:591).

Stabiliteitstoetse was vir 'n periode van 3 maande vir die emulgelformulerings uitgevoer. Die emulgelformulerings was by 25 °C/60% relatiewe humiditeit (RH), 30 °C/60% RH en 40 °C/70% RH gestoor. 'n Hoë-drukvloeistofkromatografie (HPLC) analiese is gebruik om die konsentrasies van skopolamien in die emulgel te bepaal. Ander stabiliteitstoetse soos pH-variasie, massavariasie, viskositeit, voorkoms en konfokale laserskanderingsmikroskopie is ook uitgevoer.

Die emulgels was nie oor die 3 maande stabiel nie. 'n Verandering in kleur, viskositeit en 'n verlaging in aktiewe bestanddele was waargeneem.

Bronnelys

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FOREWORD

The transdermal delivery of anti-emetic drugs was investigated in this study. Cyclizine and scopolamine was formulated in solutions with and without Pheroid™ technology. The anti-emetic drug that delivered the best results transdermally was subsequently formulated in an emulgel and emulgel containing Pheroid™. Stability tests were conducted for 3 months at 3 different temperatures and humidity.

This dissertation is given in an article format, including introductory chapters, a full length article for publication in a pharmaceutical journal and the attained data in appendices. The article in this thesis will be submitted for publication in Drug Delivery. Appendix E includes a complete guide for authors.

Completing my Masters degree granted me the opportunity to realize the immense role that research plays in the pharmaceutical industry. I have learned a great deal about myself and the importance of endurance, patience and self-discipline. The future holds a lot of opportunities and challenges that I can face head on.

CHAPTER 1

Introduction and Motivation of Study

1.1 Introduction

The transdermal administration of drugs has greatly advanced in the past 30 years. It has become a feasible way to deliver numerous drugs with the potential of delivering many more drugs with the help of penetration enhancers.

The therapeutic efficacy is closely related to the route of administration. Hence, if the drug is easy to administer, the patient compliance and ultimately the bioavailability of the drug will be increased (Farahman *et al.*, 2009:2). Other advantages include that the incompatibility of a drug in the gastrointestinal system is ruled out as well as minimizing the first pass effect (Schulmeister, 2005:48).

Although this route of administration seems ideal, the delivery of drugs is limited due to various factors. The first layer of the skin, the stratum corneum, protects us from harmful substances and poses an excellent barrier to substances. The selected drug for delivery should therefore adhere to a specific criterion. This criterion includes a specified molecular weight of a drug, the log D (octanol-buffer partition coefficient) value and includes the potency thereof (Yano *et al.*, 1986) (as quoted by Brown *et al.*, 2006:177).

Despite of the excellent barrier properties that the skin offers, penetration enhancers have been developed to overcome these properties to ultimately deliver an otherwise impermeable drug systemically. Permeation enhancers are divided into two major groups, namely mechanical and chemical enhancers. The first group includes skin abrasion, microneedles and skin stretching, whereas chemical enhancers compromise the skin's barrier function. An optimum enhancer should elicit no pharmacological action, must act immediately, be compatible to the drug and its actions should be completely reversible. A new technology, namely the Pheroid™ technology has been developed. This technology is unique due to it comprising mainly of essential fatty acids and plant fatty acids. This means that the Pheroid™ is natural to the body and therefore delivers drugs at a remarkable speed (Grobler, 2004:4).

When asking anyone that suffers from motion sickness what they would do to avoid motion sickness, the answer would most likely be “anything that works”. Motion sickness can be defined as a group of nausea syndromes caused due to motion-induced cerebral ischemia, over-stimulation of the vestibular organs of the ear or the stimulation of the abdominal organ

afferents. Nausea and vomiting are the most common symptoms one can perceive when experiencing motion sickness. Other symptoms include sweaty palms, a heaving stomach, headaches and apathy. A variance is perceived in the brain between the visually observed movement and the vestibular system's movement. Therefore the variance in speed at which the eye adjusts relatively to the cochlea is the main cause of this condition. The susceptibility of a person will ultimately determine the severity of motion sickness (Reason & Brand, 1975; Oman, 1990:296) (as quoted by Sherman & Kider, 2007:1).

Many non-pharmacological treatments exist. When considering pharmacological treatments, the first-line medications used in the prevention of motion sickness are antihistamines, including cyclizine and other central anti-cholinergic drugs for example scopolamine (Sherman & Kider, 2007:1).

Cyclizine is easily obtainable due to it being an over-the-counter medicine. It acts as an antihistaminic agent, blocking both the vestibular H_1 -receptor and the chemoreceptor trigger zone (H_2 -receptors). Cyclizine was demonstrated to improve the gastrointestinal symptoms associated with motion sickness (Sherman & Kider, 2007:1).

Scopolamine, a belladonna alkaloid, acts as an anticholinergic agent on the muscarinic receptors. It inhibits the vestibular input into the central nervous system, subsequently inhibiting the vomiting reflex. Identified as one of the most effective medications in combating motion sickness, a prescription is unfortunately required (Wood & Graybiel, 1968:1341-1344).

The route of administration plays a crucial role in the bioavailability of a drug, consequently determining the therapeutic efficacy. The transdermal route supplies a constant rate of drug delivery and provides an easy route for administration. Due to the oral route not being available when treating nausea and vomiting, the transdermal route is a favourable route to consider.

1.2 Aims and Objectives of this study

The aim of this study was to determine the possibility to deliver anti-emetic drugs (i.e. cyclizine and scopolamine) transdermally and thereafter to select the anti-emetic drug that delivered the best results transdermally and prepare different formulations thereof.

The objectives included the following:

- Determining whether cyclizine (0.5 %) and scopolamine (1 %), when formulated in a solution, can be delivered transdermally.

- Formulation of an emulgel, with and without the use of Pheroid™ vesicles, containing one of the aforementioned anti-emetic drugs that delivered the biggest transdermal concentration.
- To determine physicochemical factors like the aqueous solubility and octanol-buffer distribution coefficient (log D).
- To develop and validate an HPLC (high performance liquid chromatography) method for both cyclizine and scopolamine to adequately determine the concentration.
- To determine stability by exposing the emulgel (with and without Pheroid™) to various stability testing.

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CHAPTER 2

Transdermal Drug Penetration of Anti-Emetic drugs

2.1 Introduction

The integument of a human being, the skin, offers an attractive, alternate route for the administration of substances. It protects one from psychological and physical suffering. This multi-layered organ was thought to be impermeable, but over the years, research has shown that not all substances find acquiescence in it (Ball & Smith, 2008:1337).

The skin functions as an interface between the interior of the body and the harsh external environment and is often acknowledged as the principal barrier for skin permeation. Other functions include thermoregulation, mechanical support and immunological effects (Brown *et al.*, 2005:175).

Although transdermal drug delivery offers many advantages, there are equally as many factors that hinder the delivery of a drug, for instance poor permeability, inter-individual variations and skin irritations. To successfully penetrate the skin, the substance should dispose of certain properties. Therefore the physicochemical properties of a drug should be taken into account before incorporating it into a transdermal delivery system (Sloan, 1989:67).

In this study, the transdermal delivery of anti-emetics will be investigated. The emphasis will fall on drugs (scopolamine, cyclizine) indicated for motion sickness.

2.1.1 Anti-Emetics and Anti-Nauseates

2.1.1.1 The Goal of Anti-Emetics and Anti-Nauseates

Nausea and vomiting are common side-effects in various illnesses, which may cause extensive discomfort in patients. Anti-emetics and anti-nauseates were developed to combat this effect completely, consequently improving the quality of a patient's life (Plosker & Milne, 1992:291). The neuropharmacologic basis of vomiting is still not completely understood and offers potential for further investigation.

Selection of an anti-emetic should be according to the cause of nausea. The following steps are required in order to make an appropriate decision:

1. Identify the most likely causes of the symptoms.
2. Identify the pathway by which the cause triggers the vomiting reflex.
3. Identify the neurotransmitter involved in each pathway.
4. Choose the most potent antagonist for the particular receptor.
5. Administer the drug by a route which ensures that it reaches the site of action.
6. Ensure regular drug dosage (Mannix, 2002:18).

The ideal anti-emetic should have no side-effects, have little or no interactions with other substances and should be convenient to use (Borsadia & Patel, 2006:43).

2.1.2 The act of emesis and nausea

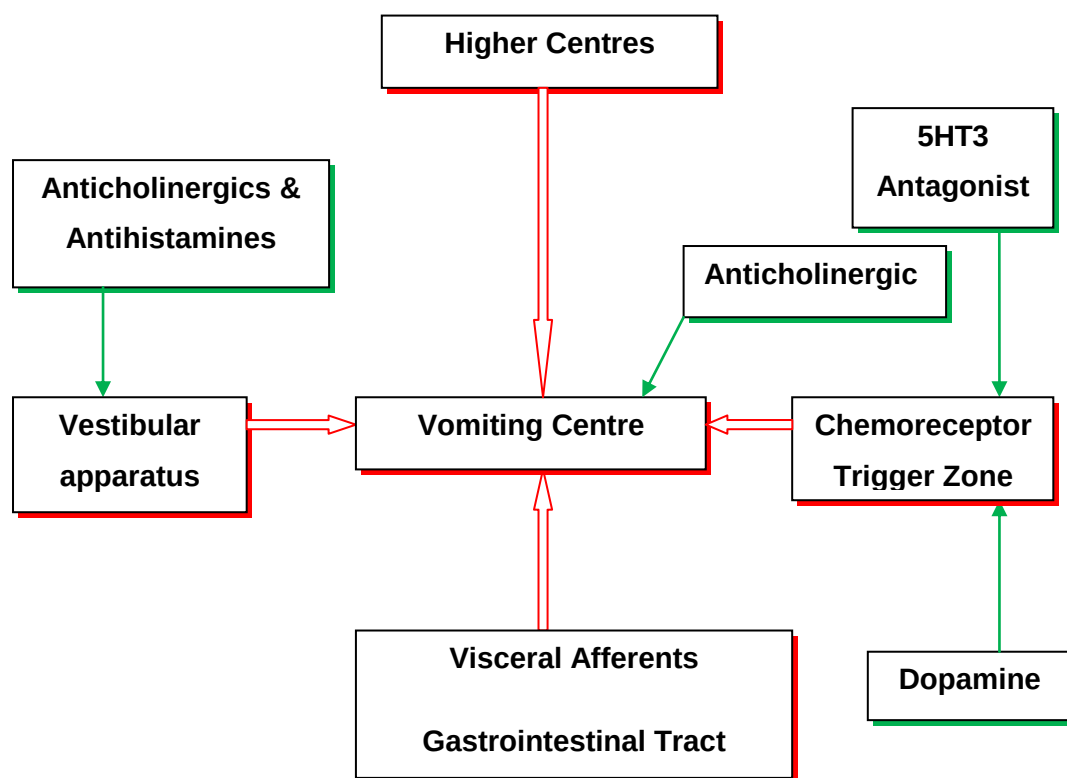


Figure 2.1: Pharmacology of emetic simulation (Medscape, 2010)

Generally the sensation of nausea and the act of emesis are viewed as protective reflexes that prevent the further ingestion of potentially toxic substances. The process is coordinated by the vomiting centre (VC) in the lateral reticular formation of the mid-brainstem adjacent to the fourth

ventricle and solitary tract nucleus (STN) of the vagus nerve at the chemoreceptor trigger zone (CTZ) in the area postrema (Pasricha, 2001:1029). Figure 2.1 illustrates the pharmacological stimulation of emesis.

2.2 Motion Sickness

Motion sickness is a condition that occurs when the brain perceives a variance between the visually observed movement and the vestibular system's movement. Hence, the main cause is the difference in the speed of which the eye adjusts relatively to the cochlea (Reason, 1978:819). Vestibular systems fulfil the most important signal in motion sickness. The labyrinth is situated in the peripheral vestibular system and possesses two types of end organs, namely: the semicircular canals, and the otolith organs. Both organs contain hair cells that function as a receptor to movement. Rotational acceleration is perceived by the three semicircular canals, whereas the two otolith organs, the sacculus and the utricle detect the position of the head in relation to the earth (static position). During angular head movements, a change in the volume in each cylinder is observed in at least two semicircular canals on either side of the head (Souvestre *et al.*, 2008:750). The central nervous system detects the alteration in movement by deciphering the pattern of the discharge by the otolith organs. Various neurotransmitters (histamine, dopamine, serotonin, substance P) play a role in the activity of the vestibular nucleus neurons.

A study conducted on cruise passengers determined that nearly every person would experience motion sickness when exposed to aggressive movements. Roughly 90% of the passengers experienced extensive seasickness in rough sea conditions, whilst only the remaining passengers experienced motion sickness to a moderate extent (Sherman & Kider, 2007:1).

General symptoms of motion sickness include sweaty palms; a heaving stomach and persistent nausea. However the susceptibility of an individual will ultimately determine the severity thereof. The occurrence of motion sickness depends on a number of factors, namely:

- the duration, direction and the rate of recurrence of the stimuli,
- the activity of a person during the motion, and
- personal experience and receptiveness to the stimuli (Sherman & Kider, 2007:1).

As mentioned, the visual observed movements are the main cause for motion sickness as it stimulates the vestibular and visual systems, although it is thought that the auditory and somatosensory systems may also play a role (Oman, 1990:296). A blind person has the same disposition to develop motion sickness as a normal person (Graybiel, 1970:653). Even though

motion sickness is extensively researched, presently we seem to know less about the emetic linkage than we did a few years ago (Oman, 1990:295).

The most important treatment for motion sickness seems to be antihistamines. Cyclizine for instance can be purchased without a prescription, making it accessible to the general public. However, many researchers and patients are sceptic regarding the effectiveness of cyclizine in preventing motion sickness. Generally, scopolamine, a muscarinic antagonist, is viewed as the only successful drug in combating motion sickness. This, however, is a scheduled drug and a prescription is required when purchasing it. Therefore, other alternatives are being researched to effectively prevent motion sickness.

2.3 Classification of Anti-Emetic Drugs

2.3.1 Receptor-specific drugs

2.3.1.1 Dopamine Antagonists

The principal mechanism whereby the dopamine antagonists function is the antagonism of the dopamine D₂-receptor at the CTZ. Haloperidol is the most potent D₂-receptor antagonist at the CTZ and its action is primarily at subcortical levels (Drug bank, 2009). Phenothiazines are weaker D₂-receptor antagonists. Furthermore, it comprises over antihistaminic and anticholinergic activities which are of value in various forms of vomiting (Pasricha, 2001:1029).

2.3.1.2 Histamine antagonists

Antihistamines are generally used as anti-emetics in motion sickness. The inhibition of histamine at the H₁-receptor as well as the indirect inhibition via the vestibular system, reduces vomiting and nausea (Gill & Einarson, 2007) (as quoted by Bottomley *et al.*, 2009:6).

Cyclizine reduces the activity along these pathways. It also has additional anticholinergic effects and is less sedating than other antihistamines.

Hydroxyzine, promethazine and diphenhydramine are other examples in this class.

2.3.1.3 Anticholinergic agents

The muscarinic acetylcholine receptors at the VC are within the blood-brain barrier (Peroutka & Snyders, 1982) (as quoted by Mannix, 2002:18). It is believed that these agents prevent the communication between the nerves of the vestibule and VC; hence anticholinergic agents are primarily used for the treatment of motion sickness. The most commonly used anticholinergic

agent is scopolamine that has a structural similarity to the neurotransmitter acetylcholine (Drug bank, 2009).

2.3.1.4 Serotonin receptor antagonists and agonists

It appears that the serotonin receptors 5-HT₂, 5-HT₃, and 5-HT₄ are mediators of nausea and vomiting. These receptors are located in three sites, namely: the gastrointestinal tract, CTZ and the STN (Sanger & Andrews, 2006:9)).

The highest concentration of 5-HT₃ receptors is found in the CTZ and VC. It is evident that antagonists of these receptors are potent anti-emetics. Examples include granisetron, ondansetron and tropisetron, which are commonly used against chemotherapy nausea (Mannix, 2002:18).

2.3.2 Other Anti-Emetics

2.3.2.1 Corticosteroids

The corticosteroids are commonly underestimated. The working mechanism of these drugs is unclear, but they possess the ability to enhance the activity of other anti-emetics (Mannix, 2002:18).

2.3.2.2 Synthetic cannabinoids and Marijuana

Cannabinoids and Marijuana act as an agonist on the cannabinoid-receptor, consequently exerting its anti-emetic properties (Pasricha, 2001:1031). These anti-emetics are rarely used due to their many side-effects (Mannix, 2002:18).

2.4 Pharmacology of selected anti-emetics suitable for motion sickness

2.4.1 Scopolamine

2.4.1.1 Chemical and physical properties of scopolamine

UIPAC name:	(1 α ,2 β ,4 β ,5 α ,7 β)-9-Methyl-oxa-9-azatricyclo[3.3.1.0]non-7-yl-(α -S)- α -(hydroxymethyl)benzene acetate
Molecular weight:	303.3529 g/mol
Empirical formula:	C ₁₇ H ₂₁ NO ₄

Solubility:	Scopolamine is soluble in water (1:1), alcohol (1:50) and chloroform (1:5), whereas scopolaminehydrobromide is soluble in water (50 mg/ml) at 15°C, freely soluble in hot water, ethanol, chloroform, acetone and ether, not very soluble in petroleum ether or ether
Melting point:	59°C
Log P:	0.8
pKa:	7.6
Acidity:	Comprises over a pH of 7.4
Description:	Colourless to white crystalline powder with a slight efflorescent in dry air

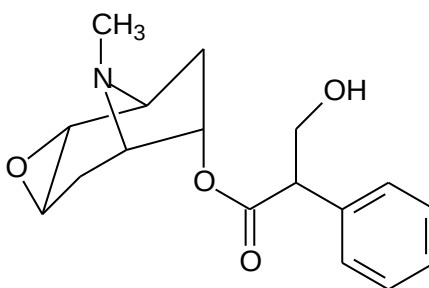


Figure 2.2: Chemical structure of scopolamine

2.4.1.2 Pharmacology and pharmacokinetics of scopolamine

Scopolamine, otherwise known as hyoscine, is an alkaloid obtained from the *Solanaceae* plant family. Scopolamine is a muscarinic antagonist that is similar in structure to acetylcholine; therefore disposes over the ability to block the muscarinic acetylcholine receptors. It causes interference in the transmission of impulses in the parasympathetic nervous system, especially the VC and is generally used in the treatment against motion sickness and extreme salivation. When ingested, scopolamine is greatly metabolised and conjugated; approximately 10% of the drug is excreted unchanged. Depending on the dosage form used, the elimination half life is approximately 2.9 h. The effective dose does not cause any sedation, thus indicating that scopolamine acts specifically in the vestibular nuclei (Yates *et al.*, 1998:400; Drug bank, 2009).

This drug is furthermore branded as the most effective drug treatment against motion sickness although it is renowned for its many side-effects. The most common side effects include blurred vision, dry mouth and dizziness, mainly due to the blocking of the autonomic parasympathetic receptors. CNS (central nervous system) side-effects such as delirium, disorientation, and

somnolence have also been reported (Shutt & Bowes, 1983:478). The following contra-indications exist:

- Hypersensitivity to belladonna alkaloids
- Narrow angle glaucoma
- Alcohol
- Sedatives or tranquilizers

Scopolamine has previously been formulated in a transdermal patch. This dosage form extended the systemic circulation to over 72 h and controlled the absorption process. However, individual variability ultimately determined the efficacy of the product (Barry, 2007:591)). Transdermal scopolamine has many advantages over an oral formulation, but the side-effects due to the blocking of the autonomic parasympathetic receptors still prevailed. In various cases the occurrences of allergic contact dermatitis have been reported (Burton et al., 2010:469)). Hence, the worth of this formulation is still to be proved by well-designed studies (Barry, 2007:591)).

2.4.2 Cyclizine

2.4.2.1 Chemical and physical properties of cyclizine

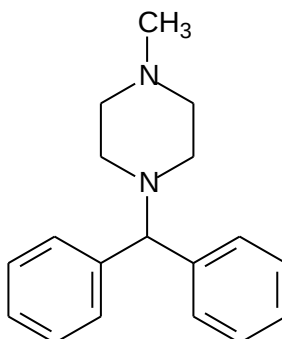


Figure 2.3: Chemical structure of cyclizine

UIPAC name:	1-(diphenylmethyl)-4-methyl piperazine
Molecular weight:	226.40 g/mol
Empirical formula:	C ₁₈ H ₂₂ N ₂
Solubility:	Slight solubility in water (50 mg/ml) and alcohol; soluble in chloroform and insoluble in ether
Melting point:	285°C

Log P:	3.55
pKa:	8.2
Acidity:	Comprises over a pH of 4.5 to 5.5 when made up in a solution of 2% alcohol:water
Description:	Creamy white, relatively odourless, crystalline powder

2.4.2.2 Pharmacology and pharmacokinetics of cyclizine

Cyclizine acts as a first generation antihistaminic agent in the VC, ultimately reducing the activity of the muscarinic pathways. This piperazine derivative is commonly used in preventing nausea and vomiting due to motion sickness. Its activation mechanism in the prevention of motion sickness is not yet completely understood. Cyclizine is presystemically metabolised and reduced to norcyclizine; the N-demethylated derivative which has a slight antihistaminic activity. It is extensively circulated through the tissues, especially the kidneys, lungs, spleen, and liver (Drug bank, 2009).

Although cyclizine causes slight drowsiness, the gastrointestinal symptoms are immensely improved (Muth *et al.*, 1995:1041-1045). Most antihistamines depress the CNS, commonly causing sedation. CNS stimulation, on the other hand, is common in children. The child presents with insomnia, euphoria, irritability, nervousness, and tremors.

Cyclizine also depicts antimuscarinic activity which includes side-effects like: dry mouth, blurred vision and urinary retention (Muth *et al.*, 1995:1041-1045).

Contra-indications of cyclizine are as follows:

- Prostrate hypertrophy
- Glaucoma
- Acute asthma
- Chronic pulmonary disease (Muth *et al.*, 1995:1041-1045).

Patients should monitor their own response to the use of cyclizine (Gibbon, 2005:43). Hypersensitivity reactions may occur when the antihistamines are applied topically (Reynolds, 1993:926).

2.5 The Skin as Barrier to Transdermal Drug Delivery

Covering an area of up to 1.73 m² and weighing approximately 2 kg, the integumentary system of a human being, the skin, is the single heaviest organ of the body.

The skin has a pH ranging between 2.8 and 6.0 and although ranging in thickness from 0.05-2 mm, it is remarkably flexible. Depending on the body site, an average square centimetre of skin contains 12 nerves, 100 sweat glands, 3 blood vessels, 15 sebaceous glands and 10 hair follicles (Barr, 1962:395).

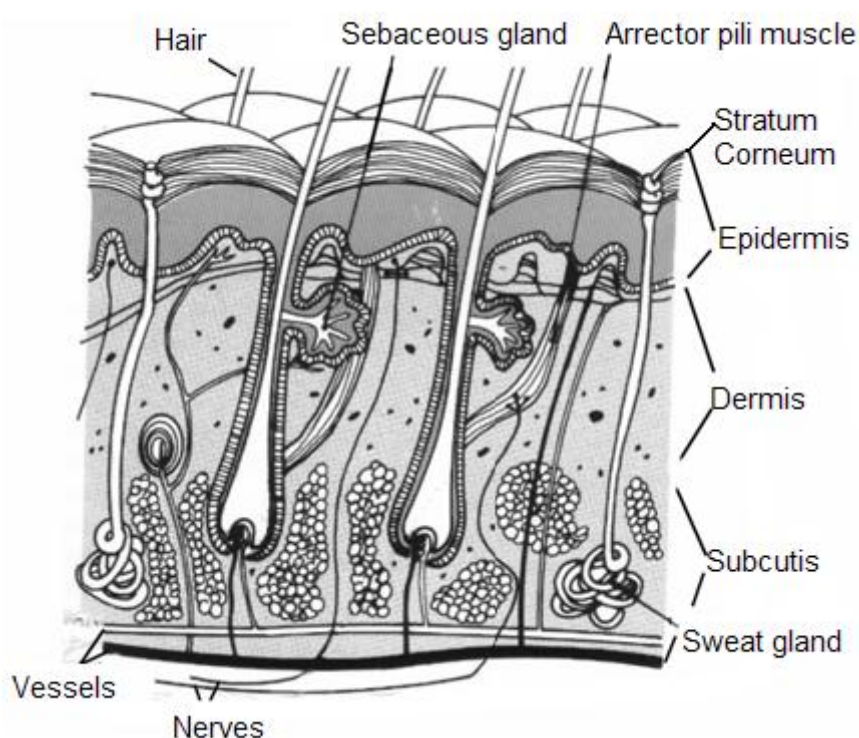


Figure 2.4: The anatomy of the skin (Ylä-Outinen, 2002)

Before reaching the systemic circulation, the administered drug has to cross multiple potential barriers (Rieger, 1993:3436). The four main barriers can be categorized as follows:

1. The stratum corneum (SC)/horny layer – the uppermost, non-viable epidermis
2. The epidermis – the viable, cellular and avascular layer of the skin
3. The dermis – the innermost layer that comprises over acellular connective tissue
4. The hypodermis – the inner subcutaneous fat layer

2.5.1 Stratum Corneum

The uppermost layer of the skin, the SC, functions as the main protective barrier that prevents the loss of essential substances and minimizes the diffusion of harmful chemicals from the environment into the body (Sloan *et al.*, 1984) (as quoted by Ren *et al.*, 2008:43). This horny layer is approximately one hundredth of a millimetre thick and varies in different regions, for example the fingertips, bottom of the toes and palms which is much thicker (Naik *et al.*, 2000:318). The SC consists out of dead skin cells that are the final product of the differentiation process (Elias, 1983) (as quoted by Brown *et al.*, 2005:175). Underlying these dead skin cells are 15-20 layers of keratin filled corneocytes surrounded by a lipid medium. Several cell differentiations exhibit different layers beneath the SC, namely the stratum basale, stratum spinosum, stratum granulosum and stratum lucidum. As the SC cells are sloughed off, the stratum basale is responsible for the replacement of these cells. It takes roughly 28 days for the total turnover from the stratum basale to shedding (Pinkus 1951) (as quoted by Barry, 1983:4).

The SC is often referred to as a brick-and-mortar complex. The corneocytes (brick) are enclosed by the intracellular lipid rich matrix that comprises over ceramides, fatty acids, cholesterol and cholesterol esters (mortar) (Barry & Williams, 1995) (as quoted by Maghraby *et al.*, 2008:203). Known components of the horny layer are intercellular lipids, matured keratinocytes (corneocytes with their protein and lipid shells) and desmosomes that hold the corneocytes together by intercellular connections between the corneocytes (desmosomes and tight junctions).

There has been established that there is a direct connection between the SC and the absorption of substances (Langer, 2004:557)). Therefore a molecule should dispose over certain properties in order to cross the barrier. The SC might be comprised out of dead cells, but the response certainly is not.

2.5.2 Viable Epidermis

The skin layer situated between the SC and the dermis, the viable epidermis (VE), can be considered as aqueous gel that doesn't post a remarkable barrier to penetration, therefore to obtain percutaneous absorption it is important to enhance penetration of a substance through the SC (Scheuplein, 1967:79-88).

The VE is more densely packed than the SC and keratinocytes and dendritic cells are the main substances present. This layer ranges from 40-50 up to 400 μm and in comparison to the SC; is ten times thicker (Rieger, 1993:36).

The cells in the VE differentiate and renew itself constantly to eventually after enduring keratinisation, structural and histochemically changes, deliver the SC. Also present in the VE are melanocytes that are pigment producing cells, Langerhans cells that contribute to the immune effects of the skin and Merkel cells which are responsible for observing sensory stimuli (Barry, 1983:3-7).

2.5.3 Dermis

The dermis (0.1-0.5 cm thick) is the lowest layer of the skin that offers no resistance to passing molecules (Walters & Roberts, 2002:11). Made up of collagen and elastin together with glycosaminoglycans, salts and water, the dermis provides an important component of the body as it provides nutritive, immune and other support systems (Walters & Roberts, 2002:11). A network of lymphatics, blood vessels and nerve endings is revealed in the dermis which lends support to the epidermis. Hair follicles and sweat ducts derive from the dermis and ultimately provide the appendageal route (Barry, 1983:10-12; Bisett, 1987:32).

2.5.4 Hypodermis

The hypodermis, otherwise known as the subcutis, is the deepest layer of the skin that supports the dermis (Barry 1983:10:). This fatty layer consists out of fat cells which are linked to the dermis by collagen and elastin fibres. It also contains other cells, mainly fibroblasts and macrophages. The main functions of the hypodermis are to attach the skin to the core muscles and to provide the skin with neural and vascular systems. It is estimated that 50% of the body's fat comprises out of the subcutis.

2.5.5 Skin appendages

Four main appendages exist in the skin namely hair follicles with sebaceous glands, eccrine sweat glands, apocrine sweat glands and nails. Each of these appendages is associated with a different function (Poet & McDougal, 2002:22). Hair follicles are distributed over almost the entire body, except the lips, soles of the feet and the palms. The follicles have an association with a sebaceous gland (200-2000 μm in diameter) that is responsible for the secretion of sebum that functions mainly as a lubricant. Eccrine glands are uncomplicated epidermal structures that secrete a saline solution with pH 5, when stimulated by heat or the cholinergic system. The previously mentioned secretion aids as a cooling mechanism. Apocrine glands are approximately 10 times bigger than the eccrine glands. It is limited to specific areas of the body namely the axillae, nipples and anogenital regions and its milky protein secretion is stimulated by heat. Apocrine sweat glands make up one thirds of the total glands on the body, whereas eccrine sweat glands gives account for 2/3 of the total glands.

The fourth appendage, the nails, is made up of layers of keratinized cells that are flattened and welded together to form a rather elastic bundle. It is limited to the ends of the toes and fingers and serves as a protective plate.

2.6 Advantages and Limitations of Transdermal Drug Delivery

Continuous improvements of transdermal drug delivery offer a number of advantages over the traditional methods of drug administration, but intertwined with this advances are various limitations.

2.6.1 Advantages

- Transdermal delivery minimizes the effect of first-pass metabolism on the drug, therefore improving drug availability
- The incompatibility of the gastrointestinal tract is ruled out
- Transdermal administration of a drug is accepted more by patients and therefore increases their compliance (Payne *et al.*, 1998:1588-1593).
- Painful administration can be avoided
- When the oral route is not available it can provide a possible route for administration (Ball, 2008:1337-1338)

2.6.2 Limitations

- Penetration is limited to the SC (Scheuplein, 1965:334).
- Molecular weight is limited to 500 Da in order to successfully diffuse through the SC (Bos & Meinardi, 2000:165).
- The permeation coefficient ($\log P_{\text{oct/water}}$) should range between 1 and 3 to be soluble in the aqueous and lipid regions of the skin (Yano *et al.*, 1986) (as quoted by Brown *et al.*, 2006:177).
- Bacteria found on the skin can break down drugs before penetration.
- Presystemic metabolism can influence the penetration and absorption of the drug.
- Drugs must be potent, so that the small amounts delivered will be sufficient (Gill & Prausnitz, 2007) (as quoted by Davidson *et al.*, 2008:1197).

2.7 Transdermal absorption and routes of penetration

2.7.1 Transdermal absorption

Conventional methods of applying a drug to the skin include ointments, creams, gels and patches. Ideally for a drug to be successfully absorbed into the skin, the applicable drug should be lipophilic enough to penetrate the SC, but should also dispose over sufficient hydrophilicity to penetrate the VE. If the drug is too lipophilic, it will subside in the SC. On the other hand, if a drug is too polar, it won't be able to partition from the vehicle (Hadgraft, 1996:168). The nature of the solvent also affects the interaction between the SC lipids and the drug (Wang *et al.*, 2004:207-216) (as quoted by Drakulić *et al.*, 2008:40).

Transdermal drug delivery involves multiple steps as follows (Kalia & Guy, 2001:160):

1. Dissolution within and release from the formulation to the skin surface.
2. Partitioning of the drug into the outermost layer of the skin.
3. Diffusion through the SC via the lipidic intercellular way, which is also the rate-limiting pathway
4. Partitioning from the SC into the hydrophilic VE.
5. Diffusion through CE into the upper dermis.
6. Uptake of the drug into the capillary network in order to reach systemic circulation.

Transdermal absorption can be divided into different steps, the first of which is the penetration from an outside source, where a substance enters the layer. The second step is the permeation from the SC to the VE and finally, the distribution into the blood capillaries and lymphatic system (Fernandes *et al.*, 2005:184).

2.7.2 Routes of penetration

Three possible routes have been identified for the penetration of substances through the skin namely the transcellular route, intercellular route and the transappendageal route (Barry, 2001:101);. Figure 2.5 illustrates these various routes.

The transappendageal route, also known as the shunt route, occupies less than one percent of the total area of the human skin. Molecules making use of this route, permeates mainly via the hair follicles with their associated sebaceous glands and sweat glands. The number, opening diameter and volume of a follicle should be taken into account when using this route (Otberg *et al.*, 2004:14).

Within the SC, two routes of penetration can be identified. The first route, the intercellular route, involves the passage through the intercellular lipid domain, whilst the second route crosses cells and intercellular spaces (transcellular route) (Flynn, 1989:27). The intercellular pathway is the predominant route of permeation, but the penetrants' physicochemical characteristics will ultimately determine the route through which it will permeate. Even though this route transports substances continuously via a very tortuous route (much longer than the thickness of the SC), absorption of the penetrant was found to be much faster than the transcellular pathway due to the high diffusion coefficient.

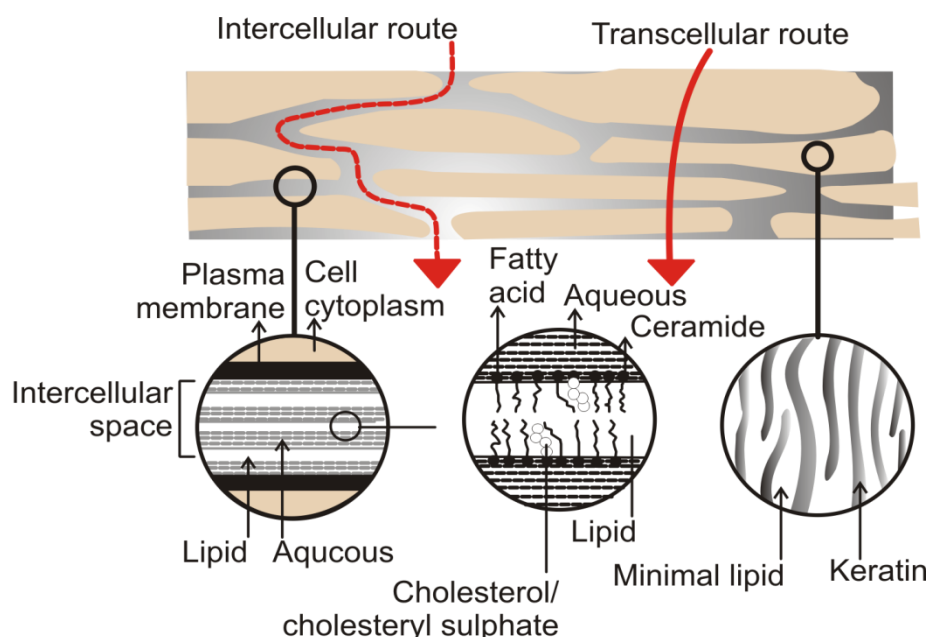


Figure 2.5: Illustration of intercellular and transcellular route (Barry, 2007:567).

Water, ions and uncharged hydrophilic molecules are primarily accommodated by the transcellular route. Although much shorter, this route is not the preferred route, as it has low permeability. In contrast to the polar route, the pores are non-discriminating and as long as the molecule size isn't the limiting factor, will accommodate any molecules. The "lipid" route handles everything else (Zats, 1993:16).

2.8 Physiological factors affecting transdermal drug delivery

Besides the variation between individuals in regards to genetic aspects (i.e. metabolism, gender and body weight); the two main factors that influence transdermal drug delivery are biological and physicochemical properties (Farahmand & Maibach, 2008:11). As these factors vary, changes in the delivery of drugs can be observed. Predictions of the quantity and rate of drug delivery are therefore complicated by these factors.

2.8.1 Biological factors

2.8.1.1 Age

Resistance to diffusion gradually increases with gestational and postnatal age. In comparison to adults, an infant's skin is a relatively larger organ, therefore a higher blood concentration of a topically applied drug can be found in infants (Williams, 2003:242). Transdermal flux can decrease as the blood flow to the skin decreases with age. Elasticity and changes in the ultra-structure together with barrier properties and chemical composition of the skin can affect permeation in the elderly.

2.8.1.2 Gender

Significant differences are observed in the overall appearance and the distribution of hair follicles between males and females. Females generally have a thicker layer of subcutaneous fat. Transdermal drug delivery can therefore be affected (Schwartz, 2003:106).

2.8.1.3 Metabolism

Growing evidence has proved that the barrier function of the skin may not only be a passive, but also an active one. The skin is capable of metabolizing endogenous as well as exogenous substances. Biotransformation may therefore be an alternative mechanism to protect the body against substances which may have crossed the SC. Metabolism can thus become a rate-limiting step in transdermal drug delivery.

2.8.1.4 Temperature

The human body has an average internal temperature of 37°C and an external temperature of approximately 32°C. An increase of temperature will result in an increase of blood flow due to blood vessels that dilate. The rate of removal of an applied drug, as well as the rate of diffusion, will therefore increase. In viscous formulations, the viscosity will decrease with the increase in temperature, thus aiding the diffusion through the vehicle (Watkinson & Brian, 2002:85)).

2.8.1.5 Hydration

We can modify the SC by hydration in order to promote better delivery of most, but not all, substances (Menon *et al.*, 1994) (as quoted by Barry 2001:101). Under normal circumstances, the SC holds between 15-20% water, and following excessive soaking, it can be increased up to 400% (Vavasour *et al.*, 1998:101-104). The flux of almost all drugs is increased with the hydration of the SC. This can be a result of the cornified tissue opening up (Barry, 2001:969). The permeation profile ultimately changes shape.

2.8.1.6 Disease

The barrier function of the skin is hindered with certain diseases. Permeation rates can therefore be affected. Corns and warts thicken the skin, resulting in an increase in path length and delaying the absorption. In contrast psoriatic skin will enhance permeation as the epidermal structure is altered. Systemic diseases e.g. diabetes are also known to change the epidermal basement membranes and capillary functions (Barry, 2007:575).

2.8.2 Physicochemical Properties

To accurately determine the permeation of a drug through the skin, it is important to take the physicochemical properties into consideration. The correct physicochemical properties will ensure the translocation of the molecules across the barrier at an acceptable rate (Barry 2001:102).

2.8.2.1 Solubility/mobility in SC

The environment with which an applied drug may interact is described by the morphological features of the skin. To ultimately reach the systemic circulation, the substance must penetrate a number of layers. After the drug partitioned into the membrane, it must diffuse over the SC. The SC is lipophilic to a great extent, with intercellular lipid lamellae that forms a conduit through which substances diffuse. Lipophilic drugs are generally better accepted by the SC, but ideally the drug should possess both lipoidal and aqueous solubility (Naik *et al.*, 2000:319).

An important factor to take into account in skin permeation is the solubility of a substance. It can be described as the energetics of a molecular interaction, needed to convert the substance from the solid solute to a molecular form, the energy of dissolution in the SC and the energy of dissolution in a vehicle. Solutes used in topical delivery are usually solids; hence it is necessary to express solubilities and partition coefficients in terms of its ideal solubility (X_i^0).

This ideal solubility varies with the nature of the solute crystal. It is also related to the energy associated with the formation of the pure liquid form by melting of the crystals at a specified melting point (Roberts, *et al.*, 2002:104)).

$$\ln X_i^0 = -\frac{\Delta H_f}{RT} \left[\frac{1}{T} - \frac{1}{T_m} \right] + \frac{\Delta C_p}{R} \left[\frac{T_m - T}{T} - \ln \frac{T_m}{T} \right] \quad \text{Equation 2.1}$$

Where:

- X_i^0 is the ideal solubility
- $-\Delta H_f$ is a function of the molar heat of fusion
- R is the gas constant

- T is the room temperature
- T_m is the melting point
- C_p is the difference in heat capacity of the crystalline and molten states

2.8.2.2 Diffusion coefficient (D)

The diffusion coefficient can be defined as a measure of the speed at which a substance crosses an applicable barrier (in this case the SC) and the effect of the viscosity of the surroundings, binding and the sinuosity of the path (*Smith, 1990:219*). The diffusion coefficient is furthermore dependent on the properties of the drug. Molecular weight exhibits an inverse relationship to absorption rate. Molecules with similar polarity and a higher molecular weight therefore permeate slower (*Malan et al., 2002:386*). Ideally, a molecule would be round and spherical in shape (*Dennis, 1990:27*). Diffusivity decreases in the liquid media with an increase in molecular volume according to Equation 2.2.

$$D = A \cdot V_m^{-1/3}$$

Equation 2.2

Where:

- D indicates the diffusivity of a spherical penetrant
- A is a constant
- $V_m^{-1/3}$ indicates the molecular volume (*Wiechers, 1989:185-198*)

In order to pass from the solvent to the skin, the diffusing molecule must comprise over some affinity for the SC. When the molecule has entered the membrane, it can diffuse in any direction. Progress, however is uniform because the molecule tends to move from a high concentration to a lower concentration of the substance (*Rieger, 1993:39*), following the path with least resistance (*Dennis, 1990:24*). The state of a molecule will also influence the diffusion rate e.g. an ionised molecule will diffuse slower than a unionised molecule.

The flux (diffusive flow) through a membrane should be inversely proportional to the thickness of the membrane and proportional to the concentration differences between the two sides of the membrane (*Rieger, 1993:39*).

$$J = \frac{dC}{dl}$$

Equation 2.3

Where:

- J is the flux (mole/cm²sec)
- dC is the concentration differences between the two sides of the membrane
- dl is the thickness of the membrane

Fickian's relationship signifies the passive permeation of a solute across a membrane. Fick's laws can only be applied if the membrane controls the rate of diffusion, in this case the SC. The quantity of drug entering the membrane is equivalent to the amount of drug leaving the membrane at steady state. The first law of Fick describes the steady-state diffusion of a substance through a membrane, as derived from Equation 2.2 (Davidson *et al.*, 2008:1199; Farahmand & Maibach, 2008:3).

$$J = -D \frac{dC}{dx} \quad \text{Equation 2.4}$$

Where:

- J is the flux of the drug through the skin
- D is the diffusion coefficient in the SC
- dC/dx is the concentration gradient
- C is the initial concentration of the applied drug

The flux can then be represented by Equation 2.5

$$J = D.A \frac{k}{h} (C_v - C_r) \quad \text{Equation 2.5}$$

Where:

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

Important factors that affect the penetration of a drug include:

1. The partition coefficient between the vehicle and the skin.
2. Diffusion coefficients, that proposes the resistance of a molecule movement through the vehicle and the skin barriers.
3. Concentration of a dissolved drug, as it is proportional to penetration rate (Martin *et al.*, 1983:664).

2.8.2.3 Molecular size

The size and shape of a diffusing molecule could play a role in its permeation rate through the skin (Idson, 1975:914). Considering that the diffusing drug molecules follow a sinuous path through the complex SC, it might be self-evident that the molecule size would be inversely related to the diffusion coefficient.

$$D_m = D_m^0 \cdot MW^{-1}$$

Equation 2.6

Where:

- D_m is the diffusion coefficient for each compound
- D_m^0 is a constant that depends on the membrane
- n is a constant that depends on the membrane
- MW is the molecular weight

A molecular weight of not more than 500 Da seems adequate to optimally penetrate the skin. Small molecules penetrate the aqueous routes more readily than the larger molecules, which will prefer the lipoidal route (Naik *et al.*, 2000:319).

Farahmand & Maibach (2008:14) suggests that an increase in inter-individual variability is exhibited when molecular weight is decrease (MW ranging from between 200–400 g/mo).

A non-linear relationship was established between the apparent sigmoidal dependence of $\log k_p$ on $\log k_{oct}$. The relationship between the molecular weight and the absorption was found to be related (Barry, 2002:513).).

According to Potts and Guy (1992:663), as described by Farahmand & Maibach (2008:1), the combination of octanol-water partition coefficients and solute size are determinants in transdermal transport. Large molecules will be prone to diffuse slowly. Permeability can therefore be predicted by Equation 2.7.

$$\text{Log}K_p = -2.7 + 0.71 \text{ Log}K_{oct} - 0.0061 MW$$

Equation 2.7

Where:

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

The substitution of molecular weight with molecular volume represents an equally fit model.

The two chosen substances for this study (cyclizine: 277.38 g/mol; scopolamine: 303.35 g/mol) comprises over molecular weights that seem optimum for transdermal delivery. If transdermal delivery only depended on the size of the molecule, these three substances would be good candidates for optimal delivery.

2.8.2.4 Ionisation

A compound in its unionised form will penetrate the skin more readily than an ionised compound, therefore the absorption will decrease. This is due to the non-polar nature of the SC favouring unionised molecules (Zats, 1993:28). Lower concentrations of unionised drugs are available for transdermal absorption if the drug is highly ionised (Abdou, 1989:554). The pH of the dermal tissues varies from 4.2-5.6 in the SC and from 7.3-7.4 in the VE (Pardo *et al.*, 1992:990-995). By changing the pH of the vehicle, the ratio of the uncharged to the charged species can be altered (Zats, 1993:28). Depending on the vehicle, generally free acids and free bases may exist in an ionised or unionised form according to the pH partition theory (Cordero *et al.*, 1997:503).

The pKa and pH of a permeant, indicates the fraction of ionised and unionised species (Zats, 1993:28)).

The pKa can be deduced from the Henderson-Hasselbach equation and also plays a role in the permeation:

For an acid:

$$\text{pH} = \text{pK}_a + \log \frac{(\text{salt})(\text{ionised})}{(\text{acid})(\text{unionised})} \quad \text{Equation 2.8}$$

For a base:

$$\text{pH} = \text{pK}_a + \log \frac{(\text{base})(\text{unionised})}{(\text{salt})(\text{ionised})} \quad \text{Equation 2.9}$$

This does not mean that an ionic species cannot penetrate the skin, for ion pairing is possible. Adding an oppositely charged species forms a lipophilic ion pair that can readily partition into the lipids of the SC when the temporary charge neutralizes. The created ion pair diffuses to the VE (aqueous of nature) thereafter dissociating into its charged species that can partition into the epidermis onward (Megwa *et al.*, 2000a:919; Megwa *et al.*, 2000b:929; Valenta *et al.*, 2000:84; Stott *et al.*, 2001:171).

2.8.2.5 Partition Coefficient

The partition coefficient can be defined as a measure of the affinity that a penetrant has for its two media (i.e. the vehicle and the membrane) (Watkinson & Brain, 2002:71).

For decades the SC was thought to be a lipoidal barrier to skin penetration. The partition coefficient is a critical factor in predicting the extent and rate of a penetrant through the skin. According to Lien & Tong (1973:371-384), as mentioned in Mourgues *et al.* (2004:297), the partition coefficients are related to the penetration of a drug. It is also the product of partition into and the diffusion across the SC (Pugh *et al.*, 1996:149). Kai *et al.* (1992: 2490-2504), also found a linear coefficient between the partition coefficient and lipophilicity of the drug which suggests that the permeation of a drug is essentially governed by the process of partitioning to the membrane.

The most widely used parameters for predicting skin penetration are molecular weight and logarithmically transformed octanol-water partition coefficient (log K_{oct}) (Farahmand & Maibach, 2008:2). In the octanol/water system the molecules in a neutral state are favoured towards the ionised form.

$$K_p = \frac{k_m \cdot D_m}{h} \quad \text{Equation 2.10}$$

Where:

- K_p is the permeability
- h is the thickness of the SC
- D_m is the permeant diffusivity in the membrane
- K_m is the partition coefficient between the SC and the vehicle

K_m is regularly substituted by K_{ow} (the octanol-water partition coefficient) (Fitzpatrick *et al.*, 2004:1309).

Research has proven that a drug with a log P in the range of 1-2, has an optimal permeation into the skin (Hadgraft, 1996:165-173; Roberts & Sloan, 2000:1415, 1416). Potential problems can be experienced in achieving steady state plasma concentrations, should the drug possess a log P of greater than 2 (Watkinson & Brain, 2002:71). A reservoir is formed and therefore the drug is delayed in the SC. In contrast, if a drug has a log P of less than -1, it will have difficulty partitioning from the vehicle into the SC. Scopolamine comprises over a log P of 0.8 (Drug bank, 2009) making it rather suitable for the transdermal delivery. It might have some difficulty partitioning from the vehicle into the SC mentioned above. It might have a tendency to form a reservoir in the SC. Cyclizine has a log P value of 3.55 (Drug bank, 2009), making it relatively

unfavourable to penetrate the skin optimally. In contrast, with the new Pheroid™ technology, the characteristics of a substance used in transdermal delivery, do not have to be optimised.

2.8.2.6 Aqueous solubility

The solubility of a penetrant in the different components and surrounding areas of the skin plays a considerable role in determining the rate of penetration (Smith, 1990:219). The skin comprises over both hydrophilic and lipophilic components. Hence, to adequately permeate the skin, the penetrant should be both soluble in water and in oil (Hadgraft & Somers, 1956:628). Ideally a drug should have an aqueous solubility not exceeding 1 mg/ml; otherwise it might present a potential bioavailability problem (Sloan, 1989:67-101).

It has been demonstrated that the aqueous solubility of a substance is partially dependent on other relevant physicochemical properties for example the partition coefficient (Malan *et al.*, 2002:385,391).

2.8.2.7 Hydrogen bonding

The hydrogen bond formed by a compound is an important factor to consider (Abraham *et al.*, 1999:78-88) (as quoted by Fitzpatrick *et al.*, 2004:1310). Consequently, the diffusivity of a solute in the SC is a function of the hydrogen bonding of a solute as well as the size of the solute. The SC is to a great extent a hydrogen bond donor rather than a hydrogen bond acceptor (Pugh *et al.*, 1996:149-165).

Both the number and the distribution (in regards to symmetry) of these hydrogen bonds affect the diffusion through the epidermal membranes. Thus an increase in the hydrogen bonds might hinder the permeation across the SC, as it is the main determinant of permeability (Roberts *et al.*, 1996:24).

2.8.2.8 Melting point

In order to select the best candidate for transdermal drug delivery, an attempt should be made to keep the melting point as low as possible (Benson, 2005:26)). A substance with a melting point of less than 200°C proves to be suitable for transdermal delivery because of the higher aqueous solubilities and dissolution rates (Vecchia & Bunge, 2003:25-57). The melting point is often used as an indicator of maximum flux through the skin (Guy & Hadgraft, 1989:324; Stott *et al.*, 1998:298). Hence, if one can lower the melting point of a drug without bringing forth unwanted changes to the physicochemical parameters, the transdermal flux will increase (Stott *et al.*, 1998:298). It was established that lipophilicity (log K_{oc}) is inversely proportional to the melting point of the permeant and therefore consequently transdermal flux (Guy & Hadgraft,

1989:324; Stott *et al.*, 1998:298). Scopolamine is the only one of the two selected substances that exhibits over an optimum melting point (59°C), whereas cyclizine (285°C) might present a potential problem as the aqueous solubility decreases with an increase of melting point (Drug bank, 2009).

2.9 The influence of permeation enhancers on transdermal delivery

The SC is such a good barrier; therefore it is necessary to improve bioavailability (Guy & Hadgraft, 1989:324). Various methods of manipulation have been developed to optimize the permeation of a substance through the skin.

Some functions of enhancers are to:

- reversibly decrease the barrier function of the SC,
- promote diffusivity of a substance into the skin and
- increase the release of a drug into the skin by affecting the partition coefficient (Shah, 1994:20).

Enhancers have diverse chemical structures. It is therefore likely to act by more than one mechanism. The activity will ultimately depend on the physicochemical properties of both the enhancer and the penetrant (Walters & Hadgraft, 1993:iii; Yu *et al.*, 2003:448-455).

Chemical enhancers are the most often used. It compromises the skin's barrier functions, consequently allowing molecules that under normal circumstances poorly penetrate the skin, through the membrane and into the systemic circulation (Walters & Hadgraft, 1993:iii).

In order to be characterised as an optimum enhancer, it should elicit some of the following criteria:

1. It should be specific in its action.
2. It should show no pharmacological action of its own.
3. It must be reversible.
4. It should act immediately.
5. It should be physically and chemically stable.
6. It must be compatible with the drug.
7. It should be colourless, odourless and tasteless.
8. It should be non-toxic and non-allergenic.

It is highly unlikely that an enhancer will comprise all of the criteria, but compromises will have to be accepted (Katz& Poulsen, 1971:160).

Hydration of the SC is an alternate and safer method for enhancing permeability, but unfortunately not all substances are affected. By using water for the hydration, the compact structure of the SC opens up (Barry, 2001:105), allowing substances to permeate up to ten times better than in the dry SC (Menon *et al.*, 1994) (as quoted by Dennis, 1990:27).

Other enhancers include electrically assisted delivery (iontophoresis and electroporation), mechanical methods (microneedles, skin abrasion and skin stretching) and other miscellaneous methods (ultrasound, radiofrequency and magnetophoresis) (Brown *et al.*, 2006:177).

2.10 Pheroid™

Pheroid™, previously known as Emzaloid™, surpasses the barrier properties of the skin. This distinctive submicron emulsion type formulation entraps a drug and delivers it at a considerable speed. It was first developed by the MeyerZall (Pty) Ltd Laboratories as a biomaterial active ingredient delivery system. The founder, Piet Meyer, initially developed this technology for his own treatment of psoriasis. In 2003, the North West University acquired the property on which Pheroid™ technology was based (Grobler *et al.*, 2007:284).

Many delivery systems exist, but the Pheroid™ delivery system is unique in that it is comprised mainly of essential fatty acids and plant fatty acids, therefore natural to the body. Drugs are enclosed in the vesicles with high efficacy and deliver the drug to the target sites at an extraordinary speed. This patent has shown the potential in increasing the absorption of various dermatological, oral and biological products. Various successes using the Pheroid™ technology include the entrapment of bacteria, viruses, and anti-malaria drugs. The main reasons for using Pheroid™ technology in this study is the effectiveness and the versatility it offers. Additionally, it is cost effective and inexpensive, and shows promise for application in the commercial market (Grobler, 2004:4).

The process by which Pheroid™ are absorbed, has not yet been clarified. However, the hypothesis exists that transport is facilitated through the process of protein mediated transfer. It readily penetrates the skin, fungi, keratinized tissue, bacteria and parasites. Pheroid™ can be morphologically manipulated and altered in size and structure.

Research has proven that the Pheroid™ offers many advantages and are as follows:

- Minimising the cytotoxicity
- Penetrates the most known barriers in the body and in the cells

- Reducing the drug resistance
- Increase the delivery of active substances
- Increase the therapeutic effects of a substance
- Reducing the time to onset of action
- Target treatment areas
- Reduces the minimal effective concentration
- Transfers genes to the cell nuclei (Grobler, 2004:3-4)

Table 2.1: Comparison between Pheroid™ and other delivery systems (Grobler, 2004:5)

Pheroid™	Other delivery systems
Consists mainly of essential fatty acids, natural to the body	Delivery systems mostly contain substances foreign to the body
Entrapment efficiency in all the tested compounds range between 85% and 100%	Entrapment may be problematic due to steric limitations and charge of the delivery system
The Pheroid™ type used determines the loading capacity thereof	The loading capacity is mostly dependent on the intra- or inter-membrane volume, thus having limitations
Due to the composition of the Pheroid™, it is sterically stable without the disadvantages of decreased elasticity or increase in size	In general, these systems need to be stabilized; usually resulting in rigidity and an increase in size of the carrier
Cytokine studies showed that no immune response was elicited in man when using the Pheroid™	Some liposomal formulations elicit immune responses in man
Depending on the method of compounding or composition various Pheroid™ can be formulated	Various liposomes have been described namely multi-lamellar vesicles, nanosomes, multivesicular vesicles and single lamellar vesicles

Pheroid™ is formed through a self-amass method in unison with micro-emulsions and low-energy emulsions. In comparison with its peers (liposomes), it comprises of a lipid bilayer, but

without the presence of cholesterol or phospholipids. It is generally viewed as a lipid-base colloidal system containing spherical structures (micron- and submicron size) which is consistently distributed in a formulation (Grobler *et al.*, 2007:284). What makes this colloidal system unique is that it can contain particles that are between 200 nm and 2 µm in size, whereas normal colloidal systems only contain particles between the sizes of 1-100 nm (Grobler *et al.*, 2007:285). In comparison to emulsions that only contain two liquid phases, the Pheroid™ system possesses an additional dispersed gas phase. This gas phase is related to the fatty acid dispersed phase. Essentially Pheroid™ comprises mainly over cis-formatted pegylated and ethylated polyunsaturated fatty acids (with the exception of arachidonic acid) (Grobler *et al.*, 2007:285).

Various types of Pheroid™ exist, namely:

- micro-sponges
- lipid bilayer vesicles with nano- and micrometer diameters, and
- reservoirs or depots which contain pro-Pheroid™ (Grobler 2004:5).

Other than fatty acids, Pheroid™ also contains nitrous oxide. In the dispersal medium, this dispersed gas assists in the miscibility of fatty acids. It also contributes to the self-amass and stability of the Pheroid™. The interactions between the nitrous oxide and the fatty acids present an unwavering vesicular Pheroid™ structure, thus providing a matrix able to effectively transport hydrophilic and hydrophobic drugs (Grobler *et al.*, 2007:289).

The fatty acid component of the Pheroid™ assures that the water loss by the epidermis is inhibited. The barrier properties of the skin are also maintained by the internal lipid layers. For this reason, it is ideal to incorporate the Pheroid™ in the transdermal delivery of drugs.

2.11 Summary

Nausea and vomiting caused by motion sickness are amongst the most unpleasant symptoms one can experience. The occurrence of motion sickness depends on the activity of a person exposed to the motion, the direction and duration of the motion and ultimately the individual's susceptibility. Visual observance is however the main factor. The biggest contributing aspect in motion sickness is the variance perceived by the brain between the speeds at which the eye adjusts relative to the cochlea. A heaving stomach, sweaty palms and extensive nausea are the most common symptoms experienced in motion sickness.

Anti-histamines (including cyclizine) are viewed as the first regimen treatment for motion sickness and therefore easily to obtain as an over the counter product. Other treatments

available include dopamine-antagonists, anticholinergic agents, serotonin receptor antagonists and agonists. These treatments are administered orally and thus not the most preferable route when treating nausea and vomiting. The skin consequently offers an attractive alternate route for administration. Three routes exist for transdermal penetration across the skin namely the transcellular diffusion route, the intercellular route and the appendageal diffusion route. Due to the excellent barrier properties the skin proposes, various limitations exist when considering the transdermal delivery route. Penetration enhancers can be utilized to optimize the transdermal delivery of a drug. Pheroid™ technology is a new technology used to enhance the permeation of a drug through the skin. Pheroid™ mainly consists out of essential fatty acids and is therefore natural to the body.

The development of suitable HPLC methods to determine the concentrations of the active ingredients quantitatively will be carried out in this study. Other objectives and aims are the formulation of scopolamine emulgels with Pheroid™, to determine the aqueous solubility and the LogD value of the selected drugs.

Transdermal delivery has become increasingly popular and offers a lot of potential in decreasing adverse effects and eliminating the first pass metabolism.

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CHAPTER 3

Article for publication in Drug Delivery

Chapter 3 is given in an article format for publication in Drug Delivery. The guide for authors is provided in Appendix E.

The formulation and transdermal diffusion of an anti-emetic for motion sickness

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Abstract

Context: Motion sickness is among the most unpleasant conditions one can experience, causing extensive discomfort. Symptoms of motion sickness include sweaty palms, a heaving stomach and persistent nausea. Scopolamine is generally viewed as one of the most successful drugs in combating motion sickness. **Objective:** The aim of this study was to establish whether scopolamine, once formulated in emulgel formulations, can be delivered transdermally. **Materials and methods:** Franz cell permeation studies for both the solutions and emulgel formulations with and without Pheroid™ were conducted over a 12 h period to determine the transdermal delivery. Pheroid™ technology was used to enhance transdermal delivery. This technology contains neither cholesterol nor phospholipids. **Results & Discussion:** Scopolamine solution penetrated full thickness skin successfully and resulted in the highest % diffused when compared to the solution with Pheroid™, emulgel and emulgel containing Pheroid™. Stratum corneum-epidermis and epidermis-dermis concentrations for the solution were higher than the solution with Pheroid™, respectively. Flux values for the scopolamine solution were slightly higher than the solution containing Pheroid™. The octanol-buffer partition coefficient (log D) value indicated that scopolamine would be favorable to consider for transdermal delivery. **Conclusion:** The highest concentration of scopolamine was delivered by the solution. All of the formulations delivered adequate therapeutic concentrations of scopolamine to combat motion sickness.

1 Introduction

Motion sickness is a common condition perceived by travelers, should it be by air, car or sea. This condition is characterized by sweaty palms, a heaving stomach and persistent nausea (Oman, 1990). A recent study conducted by Sherman & Kider (2007) determined that roughly 90% of the passengers on a cruise ship experienced motion sickness whilst only 25-30% of the passengers experienced motion sickness to a moderate extent. The susceptibility of an individual will ultimately determine the severity of this condition (Sherman & Kider, 2007).

Nausea and emesis are synchronized by the vomiting centre, the lateral reticular formation of the mid-brainstem adjacent to the fourth ventricle and the solitary tract nucleus of the vagus nerve at the chemoreceptor trigger zone in the area postrema (Pasricha, 2001). The vestibular system fulfils a crucial role in generating the signal triggering motion sickness. Two end organs are present in the vestibular system namely the semicircular canals and the otolith organs. Both organs contain hair cells that detect movement. Hence, motion sickness is elicited when the brain perceives a difference between the visually observed movement and the vestibular systems' movement (Souvestre *et al.*, 2008). Auditory and somatosensory systems are also thought to contribute to this condition (Oman, 1990). The occurrence and the severity of motion sickness depend on a number of factors including the duration, direction and rate of recurrence of the stimuli, the activity of a person during the motion, as well as an individuals' receptiveness to the stimuli (Sherman & Kider, 2007). The ideal anti-emetic agent would combat the effect of nausea and emesis completely and would elicit no side effects or other drug interactions (Borsadia & Patel, 2006), hence, improving the quality of a patients' life. Scopolamine has been considered to be the only truly effective drug in combating motion sickness. This anti-cholinergic agent acts as a muscarinic antagonist. It is structurally similar to the neurotransmitter acetylcholine, hence, preventing the communication between the vomiting centre and the nerves of the vestibule (Drugbank, 2009). In the recent years, the skin has been researched extensively and has become an attractive, alternate route to deliver various drugs. Psychological and physical suffering can be ruled out when making use of this delivery route, also known as the transdermal route (Ball & Smith, 2008). Together with many advantages,

there are equally as many limitations that prevent a drug from being delivered transdermally. A drug should dispose over certain properties for example optimum molecular weight and should be potent in small amounts(Gill & Prausnitz, 2007) (as quoted by Davidson *et al.*, 2008).

Another limitation is the excellent barrier properties that the first layer of the skin, the stratum corneum, elicits. Despite these outstanding barrier properties, it is still possible to deliver some drugs transdermally (Sloan, 1989). Three possible routes have been identified through which a drug can be delivered transdermally. The first of these routes is the transcellular route, where a drug diffuses through the cells and intercellular spaces, the second route, the intercellular route, involves the diffusion of a drug through the intercellular lipid domain and the third route involves the diffusion through hair follicles and sweat ducts (Flynn, 1989). The physical condition of the skin can also influence the penetration of a drug through the skin. The age, metabolism, gender and hydration are some of these contributing factors (Dayan, 2007).

Penetration enhancers can be utilized to overcome some of these barrier properties of the skin. Physical as well as chemical enhancers exist. Recently new technologies have been developed such as Pheroid™. This patented technology, mainly comprises over natural fatty acids that are natural to the body. Pheroid™ is produced through a self-amass method in unison with micro-emulsions and low-energy emulsions. It comprises over a lipid bilayer, without the presence of phospholipids and cholesterol. It is generally viewed as a lipid-base colloidal system containing spherical structures (micron- and submicron size) which is consistently distributed in a formulation (Grobler *et al.*, 2008). In comparison to colloidal systems that comprise over particles that are 1-100 nm in size, the Pheroid™ particle size varies from 200 nm to 2 µm, thus making this technology unique (Grobler *et al.*, 2008). In comparison to emulsions that only contain two liquid phases, the Pheroid™ system possesses an additional dispersed gas phase. This gas phase is related to the fatty acid dispersed phase. Essentially Pheroid™ comprises mainly over *cis*-formatted pegylated and ethylated polyunsaturated fatty acids (with the exception of arachidonic acid) (Grobler *et al.* 2008). Other than fatty acids, Pheroid™ also contains nitrous oxide. In the dispersal medium, this dispersed gas assists in the miscibility of fatty acids. It also contributes to the self-amass and stability of the Pheroid™. The interactions

between the nitrous oxide and the fatty acids present an unwavering vesicular Pheroid™ structure, thus providing a matrix able to effectively transport hydrophilic and hydrophobic drugs. The fatty acid component of the Pheroid™ assures that the water loss by the epidermis is inhibited. The barrier properties of the skin are also maintained by the internal lipid layers. For this reason, it is ideal to incorporate the Pheroid™ in the transdermal delivery of drugs (Grobler *et al.*, 2008).

The main objective of this study was to determine whether scopolamine could be successfully delivered transdermally when formulated in an emulgel and to determine the effect of Pheroid™, a delivery system, on the permeation of scopolamine.

2 Methods

2.1 Materials

Scopolamine was obtained from BASF (South Africa). Other ingredients include deionized HPLC (high performance liquid chromatography) grade water prepared by the Milli-Q water purification system (Millipore, Milford, USA). Pheroid™ ingredients, xanthan gum (Warren Chem Specialities Johannesburg, South Africa) and tocopherol (obtained from Chempure, Pretoria, South Africa). Span 60 (obtained from Fluka Analytical, Germany), tween 80, liquid paraffin, methyl paraben and propyl paraben (Merck Laboratory Supplies, Midrand, South Africa) were also used in the formulations. Nile red was obtained from Sigma-Aldrich Corporation (Johannesburg, South Africa). Acetonitrile, octane sulphonic acid and methanol (Merck Laboratory Supplies, Midrand, South Africa) acted as the HPLC mobile phases. The phosphate buffer solution (PBS) consisted out of potassium dihydrogen orthophosphate (KH_2PO_4) and sodium hydroxide (NaOH).

2.2 Preparation of PBS (pH 7.4)

Potassium orthophosphate crystals (13.62 g) was dissolved in 500 mL fresh Milli-Q water and added to a solution prepared by dissolving sodium hydroxide pearls (3.1472 g) in 786.8 mL Milli-Q water. The pH was adjusted to 7.4 with phosphoric acid.

2.3 Scopolamine solutions

2.3.1 Preparation of scopolamine solutions

The active ingredient (1% scopolamine) was accurately weighed and added to the freshly prepared PBS at pH 7.4 (Phase A) and subsequently heated to 70°C. Phase B consisted of the anti-oxidant (0.20% butylated hydroxytoluene (BHT) and preservatives (0.20% methyl paraben and 0.04% propyl paraben)) which were accurately weighed and heated to 70°C. Phase B was added to Phase A whilst being homogenized at a speed of 13 500 rpm until the solution cooled down to approximately 40°C. The solution was swirled until cooled to room temperature ($\pm$ 25°C) resulting in a homogenous solution.

2.3.2 Preparation of scopolamine solution containing Pheroid™

The same procedure was followed as described in Section 2.3.1 with the exception of adding α -dl-tocopherol and the other Pheroid™ ingredients.

2.3.3 Preparation of placebo scopolamine solutions

The same procedure was followed as described in Sections 2.3.1 and 2.3.2 to manufacture a solution with or without Pheroid™, with the exception that the active ingredient, scopolamine, was left out of the solution.

2.4 Scopolamine formulation

2.4.1 Formulation of scopolamine emulgel

Phase A of the formulation consisted of scopolamine (1%), the gelling agent (1.5% xanthan gum) and HPLC grade water. Scopolamine and water were accurately weighed and heated together to a temperature of 40°C. The xanthan gum was slowly added to the scopolamine mixture whilst homogenizing at 777 rpm. Phase A was subsequently heated to 80°C. Phase B consisted out of the carrier of the emulsion (20% liquid paraffin), the emulsifying agent (0.5% Span 60), the surfactant (4.5% Tween 80), preservatives (0.4% methyl paraben, 0.08% propyl paraben) and the anti-oxidant (0.2% BHT). Phase B was accurately weighed and heated to 80°C. The heated Phase B was added to the heated Phase A whilst being homogenized at 13 500 rpm until it cooled down to 40°C. The mixture was stirred until cooled down to 25°C.

2.4.2 Formulation of an emulgel containing Pheroid™

The same procedure was followed as in Section 2.4.1 with the exception of adding Pheroid™ ingredients together with α -dl-tocopherol to Phase B.

2.4.3 Formulation of placebo emulgel formulations

An emulgel and emulgel with Pheroid™ were prepared as described in Sections 2.4.1 and 2.4.2, respectively with the exception of not adding the active ingredient (scopolamine).

2.5 Octanol-buffer distribution coefficient (log D)

In order to determine the log D of a substance, saturated solutions of both *n*-octanol and PBS are required. Equivalent amounts of PBS (pH 7.4) and *n*-octanol were combined. The mixture was shaken vigorously and left for 24 h to attain saturation. The two substances were separated and stored in different containers. Scopolamine (2 mg) was dissolve in 20 mL of the saturated *n*-octanol and 5 mL of this solution was transferred to a test tube, thereafter 5 mL of the saturated PBS was added. The test tube was agitated for 24 h where after it was

centrifuged for 30 min. The experiment was done in triplicate. The PBS phase of each test tube was analyzed on the HPLC in duplicate.

2.6 HPLC analysis of scopolamine

2.6.1 Preparation of standard solution

The instigation of a calibration curve is important in order to make sure that the equipment and method is accurate. Scopolamine (25 mg) was accurately weighed in a 100 mL volumetric flask. The volumetric flask was filled to volume with deionized HPLC grade water and sonicated to ensure a homogenous solution. A sample vial was filled with the solution and analyzed on the HPLC in duplicate.

2.6.2 HPLC method

The HPLC method was previously validated in the Analytical Technology Laboratory at the North West University (South Africa). The Agilent® 1100 series HPLC was used in the analysis. This instrument, fitted with an Agilent® 1100 pump, makes use of an auto sampler injection mechanism and diode array detector. Chemstation Rev. A.10.02 was used for data analysis and acquirement. All reagents were of HPLC standard and the analyses were done in a controlled laboratory environment at 25°C. The Verusil XBP C18 (2), 5 µm, 100 Å, 4.6 x 150 mm analytical column was used. Detection took place at 210 nm. The mobile phase flow rate was 1.0 mL/min with an injection volume of 10 µL. Two mobile phases were used. Mobile phase I was prepared by adding 1 g of octanesulphonic acid sodium to 1 L of fresh Milli-Q water. The pH was adjusted to 3.5 with phosphoric acid (10%) and ammoniumhydroxide. Mobile phase II consisted out of 100% acetonitrile. The retention time for scopolamine was 5 min.

2.7 Membrane permeation studies

Ten vertical Franz cells were utilized in this study. The Franz cells comprise over a volume of 2 mL. A small amount of Dow Corning vacuum grease was applied to the top of the receptor compartment and to the bottom of the donor compartment. Polytetrafluoroethylene (PTFE) membranes, resembling the skin, were used instead of real human skin. The release of the scopolamine from the formulation was subsequently determined. A layer of vacuum grease was applied right around the Franz cells in order to make the compartments waterproof. Horse shoe clamps were subsequently used to clamp the cells together. Small magnetic stirrer bars were inserted into every Franz cell. The solutions were placed in a water bath set to 32°C and the PBS solution was placed in a separate water bath set to 37°C. The heated emulgel (1 mL at 32°C) was placed in the donor compartment. A piece of Parafilm was placed on the top of the donor compartment together with a cap. This was done to prevent the emulgel from evaporating. Heated PBS (2 mL at 37°C) was injected into the receptor compartments. The Franz cells were placed in a Grant water bath on a stand on a Variomag magnetic stirrer plate. The temperature was maintained at 37°C throughout the study. The PBS was withdrawn at 2, 4, 6, 8, 10 and 12 h and immediately analyzed on the HPLC.

2.8 Skin permeation studies

2.8.1 Skin preparation for solutions

Female Caucasian skin was obtained from various medical institutions performing abdominal plastic surgeries. Approval was attained from the Research Ethics Committee of the North-West University (reference number 04D08) as well as the doctor and the patient herself. After collection, the skin was immediately frozen at -20°C. Adipose tissue was removed from the skin and the skin was blotted to ensure that all the fatty tissue was removed. Circles with a diameter of 2 cm was punched out of the skin and placed on a filter paper. The circles were inspected for blemishes and stretch marks and refrozen at -20°C until utilized.

2.8.2 Skin preparation for emulgel formulations

Female Caucasian skin was obtained from various medical institutions performing abdominal plastic surgeries as described in Section 2.8.1. After attaining the circles from the skin, it was submerged in a water bath set to 60°C for 1 min. Tweezers and a scalpel were used to separate the epidermis from the dermis. The epidermis circles were placed on a filter paper and left to dry. The circles were inspected for any defects, stretch marks or blemishes and frozen at -20°C until utilized.

2.8.3 Skin permeation studies for solutions utilizing full thickness skin

The same technique was used as described in Section 2.7 with the exception of twelve vertical Franz cells being utilized in this study. Two Franz cells functioned as controls and the remaining ten were used for the actual experiment. The prepared human skin circles (as described in Section 2.8.1) were placed on top of the receptor compartments of the Franz cells and the donor compartments were placed on top. The heated scopolamine solution (1 mL at 32°C) was placed in the donor compartment. The control Franz cells donor compartments were filled with 1 mL PBS (32°C) and 1 mL placebo solutions (32°C). The Franz cells were placed in a Grant water bath on a stand on a Variomag magnetic stirrer plate. The temperature was maintained at 37°C throughout. The PBS was withdrawn at 2, 4, 6, 8, 10 and 12 h and immediately analyzed on the HPLC.

2.8.4 Skin permeation studies for emulgel utilizing epidermal skin

The same procedure was followed as described in Section 2.8.3. Epidermal skin circles (as described in Section 2.8.2) were utilized. The emulgel formulations were heated to 32°C and placed in the donor compartments. The membrane study revealed that the emulgel did not release a high concentration of scopolamine and thus there was decided that the only extraction of PBS from the receptor compartment would be done at 12 h.

2.9 Stratum corneum-epidermis and epidermis-dermis studies

This simple and proficient procedure is carried out to analyze the amount of drug in the stratum corneum and epidermis (Lademann *et al.* 2009). After the duration of the skin permeation study, the Franz cells were dismantled and the drug preparation was removed from the skin instantaneously. The skin was blotted dry with a piece of paper towel. 16 scotch tape strips were cut per piece of skin. The aim of this is to successfully remove the stratum corneum-epidermis from the skin to establish whether any concentration of the drug penetrated it. The first strip of scotch tape was discarded as it is only viewed as part of the cleaning process (Pellet *et al.*, 1997). When the stratum corneum is removed the viable epidermis glistens. The remaining strips were placed in a politop filled with 5 mL PBS. After being left overnight in the PBS, it was withdrawn and filtered before assaying it on the HPLC. To analyze the amount of drug in the epidermis-dermis the diffusion area on the skin was cut out and cut into small pieces where after it was placed in 5 mL PBS. It was left overnight and centrifuged before assaying (Pellet *et al.*, 1997).

2.10 Data analysis

Before analyzing the samples of the diffusion studies, a standard solution was prepared and linearity was determined. The cumulative amount per area was plotted against time. The flux of scopolamine is represented by the linear portion of the graph. The slope of the straight line was determined and the average flux values were obtained.

2.11 Statistical analysis

The statistical analysis of the data included determining the median (statistically calculated centre of the data points). The Spearman's Rho correlation coefficient was determined for the formulations in order to determine the relationship between the various measurements i.e. flux, stratum corneum-epidermis and epidermis-dermis concentrations. The data was subsequently plotted on scatter-plots and box-plots. Inferential statistical analyses were performed together with T-tests and the non-parametric Mann-Whitney. Should a big variation in the data be

observed, it is more accurate to use the median value (Gerber *et al.*, 2008), due to the fact that median is not affected by outliers. The two-way analysis of variance (ANOVA) was performed to investigate the effect of the formulations and the scopolamine solution and scopolamine solution containing Pheroid™ together with the interaction effect on the concentration measurement at 12 h. All statistical inference was performed at the 5% level of significance. Statistical analysis was performed utilizing the SPSS and R statistical software.

2.12 Previous studies conducted

The importance of aiming to deliver anti-emetics transdermally can be derived from previous studies. Various other anti-emetics have been investigated for transdermal delivery, including tetrahydrocannabinol in rats, resulting in a delivery of 50 ng/mL concentration (Touitou *et al.*, 1988). It was noted by Roberge (2006) that the transdermal scopolamine patch contains a concentration of 1.5 mg and releases an initial dose of 140 µg followed by the continuous absorption of 5 µg for a duration of 72 h. Hadgraft *et al.* (1995) found that theoretically, several anti-emetics including scopolamine could be likely candidates for successful dermal activity. Solutions (5 mg/mL) were prepared and tested on hairless mice. A permeation of 0.27 µg/mL was obtained and they estimated a log k_p value of - 3 to - 3.95 in humans. Another study conducted established that post operative nausea and vomiting treated with odansetron with the addition of a transdermal scopolamine patch improved the symptoms immensely (Sah *et al.*, 2009).

3 Results & Discussion

3.1 Scopolamine solutions

3.1.1 Franz cell diffusion study results

The scopolamine solution and solution containing Pheroid™ delivered concentrations of 14.012 µg/cm² (0.075% diffused) and 6.486 µg/cm² (0.035% diffused), respectively. After the duration of 12 h, the scopolamine solution delivered a concentration of more than twice the amount of

the scopolamine solution containing Pheroid™. Average and median flux values for the scopolamine solution (1.488 and 1.416 $\mu\text{g}\cdot\text{cm}^2\cdot\text{h}$) were higher than the solution containing Pheroid™ (0.636 and 0.584 $\mu\text{g}\cdot\text{cm}^2\cdot\text{h}$) and can clearly be observed by Figure 1a & 2b. The average and median flux values illustrated minor differences, thus either one would be accurate to use to determine flux.

The lower concentration of scopolamine delivered by the solution containing Pheroid™ might be attributed to the higher oil content in the solution containing Pheroid™. With an increase in oil content of a formulation, the diffusion decreases (Barry, 2002). Hydration might also be a contributing factor to the Pheroid™ solution permeating the skin less than the solution. The Pheroid™ solution contains a higher oil content than the solution and the drive force due to the hydration gradient might thus be smaller (Bouwstra *et al.*, 2003).

3.1.2 Stratum corneum-epidermis and epidermis-dermis studies

The average (0.0128 $\mu\text{g}/\text{mL}$) and median (0.0132 $\mu\text{g}/\text{mL}$) concentration values of the stratum corneum-epidermis are higher in the scopolamine solution when compared to that of the scopolamine solutions' containing Pheroid™ (average: 0.0044 $\mu\text{g}/\text{mL}$ and median: 0.0035 $\mu\text{g}/\text{mL}$). When studying the epidermis-dermis; the scopolamine solution delivered higher average (0.2035 $\mu\text{g}/\text{mL}$) and median (0.1662 $\mu\text{g}/\text{mL}$) concentration values than that of the solution with Pheroid™ (average: 0.0525 $\mu\text{g}/\text{mL}$ and median 0.0504 $\mu\text{g}/\text{mL}$).

The average and median concentration values are comparable, with minor differences, thus both the median and average values would be accurate to use. These results demonstrate that scopolamine is a favorable drug to be delivered transdermally, as the rate limiting barrier, the stratum corneum, is overcome. It can subsequently be determined that the Pheroid™ vesicles did not assist scopolamine in penetrating neither the stratum corneum-epidermis nor the epidermis-dermis.

A study conducted by Bouwstra *et al.* (2003) revealed that vesicles might not penetrate lower than the uppermost layer than the skin. The lower concentration in the stratum corneum-

epidermis and epidermis-dermis might therefore be due to the lack of penetration of the skin by the Pheroid™ in the solution. The scopolamine in PBS penetrated the skin deeper than the scopolamine with Pheroid™ solution. In order to combat motion sickness, scopolamine has to be delivered systemically. The average cumulative concentration attained through the skin is much higher than the concentrations remaining in the epidermis and dermis, making scopolamine a favorable drug for transdermal delivery.

3.2 Emulgel diffusion studies utilizing the epidermis

3.2.1 Membrane release study

An average concentration of 0.116% (21.579 µg/cm²) was released by the emulgel and 0.079% (14.769 µg/cm²) by the emulgel containing Pheroid™. The emulgel released more of the active ingredient than the emulgel with Pheroid™. The Pheroid™ vesicles subsequently did not enhance the delivery of the drug. Due to the poor release of scopolamine by the emulgel formulations, the only extraction when performing the Franz cell diffusion study with emulgel formulations were done at 12 h.

3.2.2 Franz-cell diffusion studies

The influence of the Pheroid™ on the delivery of scopolamine was investigated and the permeation results were compared to the delivery of scopolamine without Pheroid™. The transdermal delivery of scopolamine was higher in the emulgel in comparison with the emulgel containing Pheroid™ after the 12 h extraction. An average concentration of 2.649 µg/cm² and 0.017 µg/cm² was delivered transdermally after 12 h by the emulgel and emulgel containing Pheroid™, respectively. The emulgel delivered a concentration of approximately 155 times bigger than the emulgel containing Pheroid™.

From the membrane release studies it can be derived that the emulgel released almost 32% more of the active ingredient than the emulgel with Pheroid™, thus releasing less active ingredient to diffuse across the skin. These results might be due to the Pheroid™ formulation

being more complex and thus not adequately releasing the active ingredient. Bouwstra *et al.* (2003) stated that vesicles in a gel state can inhibit permeation of the skin. The vesicles in a gel can act as an extra barrier due to the gel only acting on the outermost layer of the skin (Bouwstra *et al.*, 2003). Subsequently, the Pheroid™ consisting of vesicles might decrease the permeation of the skin when formulated in an emulgel. Barry (2007) mentioned that when considering the scopolamine patch, 70% of the scopolamine resided in the patch and the release of the remainder of the scopolamine was determined by the patients' skin itself. Thus scopolamine might not be adequately released when formulated in a transdermal dosage form. When comparing the solutions to the emulgel formulations (Figure 2), the scopolamine delivered the highest concentration, followed by the solution containing Pheroid™, the emulgel and lastly the emulgel containing Pheroid™. The poor release of scopolamine from a formulation can subsequently be noted again.

In a previous study conducted by Roberge (2006), it was determined that a concentration of 50 pg/mL is required to effectively combat motion sickness. Both emulgel with and without Pheroid™ will thus deliver a concentration of more than the required effective therapeutic dosage to combat motion sickness.

3.3 Statistical analysis

The correlation coefficients were calculated in order to determine whether there was a significant difference or a significant relationship between the solutions. Except for the significant relationship between stratum corneum-epidermis and epidermis-dermis for the scopolamine solution, no noteworthy relationship could be determined. A larger sample site might however contain more information regarding to this research question. In order to determine whether there was a significant relationship between the formulations, the T-test and Man-Whitney tests were performed. The flux, stratum corneum-epidermis and epidermis-dermis values of the scopolamine solution were compared to the flux of the scopolamine solution containing Pheroid™. It can clearly be observed when studying Figure 2 that the scopolamine solution resulted in the highest average value, followed by the solution with

Pheroid™, the emulgel and lastly the emulgel containing Pheroid™. A two way ANOVA was conducted to investigate the interaction between these formulas and the addition of Pheroid™. It was conducted that a statistical significant interaction was observed, hence the difference between Pheroid™ and non-Pheroid™ should be investigated separately for each formulation. It was concluded from the T-test and Mann-Whitney tests that there was a statistical significant difference between the concentrations measured for the Pheroid™ and non-Pheroid™ groups of both the solutions and emulgel formulations. Subsequently, a bigger difference is observed for the solutions than the emulgel formulations (Figure 2).

4 Conclusion

In this study solutions containing scopolamine and scopolamine together with Pheroid™ was investigated for transdermal delivery. The average concentration of scopolamine solution when compared to that of the solution containing Pheroid™ delivered more than twice the amount of scopolamine. The scopolamine solution demonstrated a slightly higher flux than the scopolamine solution containing Pheroid™ in full-thickness skin. Stratum corneum-epidermis and epidermis-dermis analysis revealed that scopolamine penetrated both the epidermis and dermis.

Scopolamine was formulated in an emulgel and an emulgel containing Pheroid™. Membrane studies revealed that the emulgel released the highest concentration of scopolamine when compared to that of the emulgel containing Pheroid™. During skin diffusion studies utilizing epidermis, the emulgel delivered an average scopolamine concentration after 12 h of approximately 155 times higher than that of the emulgel containing Pheroid™. The higher oil content in the Pheroid™ emulgel and the poor entrapment of scopolamine might influence the diffusion of a drug (Barry, 2002).

All the solutions and formulations containing scopolamine will transdermally deliver therapeutic concentrations (50 pg/mL) for scopolamine to combat motion sickness Roberge (2006).

Statistical analysis determined that there was a strong relationship between the stratum corneum-epidermis and epidermis-dermis for the scopolamine solution. A significant relationship was indicated between the formulations with and without Pheroid™, with a bigger difference for the solutions.

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Declaration of interest

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Figure Legends

Figure 1: Cumulative amount per area ($\mu\text{g}/\text{cm}^2$) representing each individual Franz cell for **(A)** scopolamine solution and **(B)** scopolamine solution containing Pheroid™ over a period of 12 h. The dotted and solid lines represent the average and median values, respectively.

Figure 2: Box-plot representation of concentration values ($\mu\text{g}.\text{cm}^2$) at 12 h for the solutions and emulgel formulations. The dotted line represents the average concentration values.

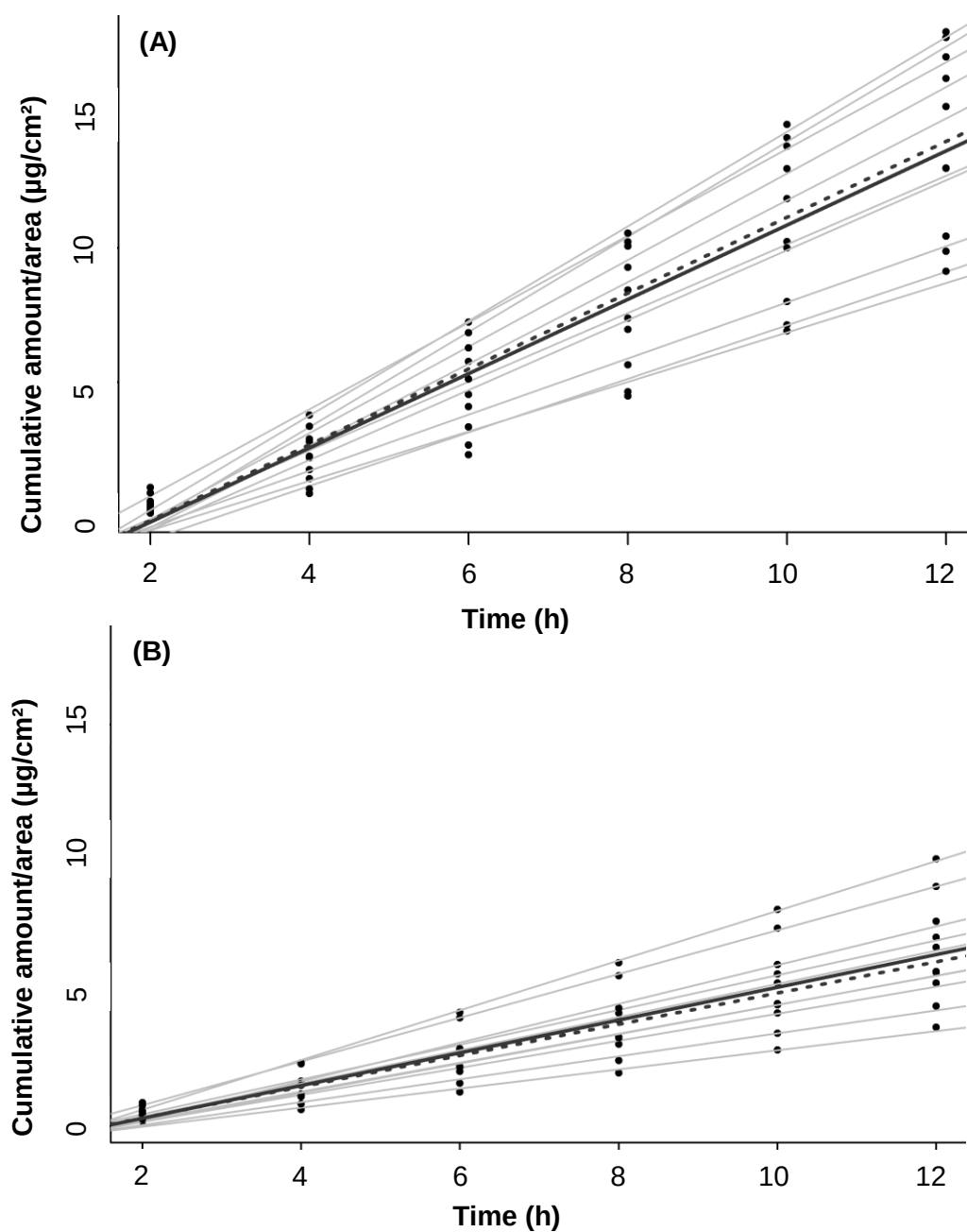


Figure 1: Cumulative amount per area ($\mu\text{g}/\text{cm}^2$) representing each individual Franz cell for **(A)** scopolamine solution and **(B)** scopolamine solution containing Pheroid™ over a period of 12 h. The dotted and solid lines represent the average and median values, respectively.

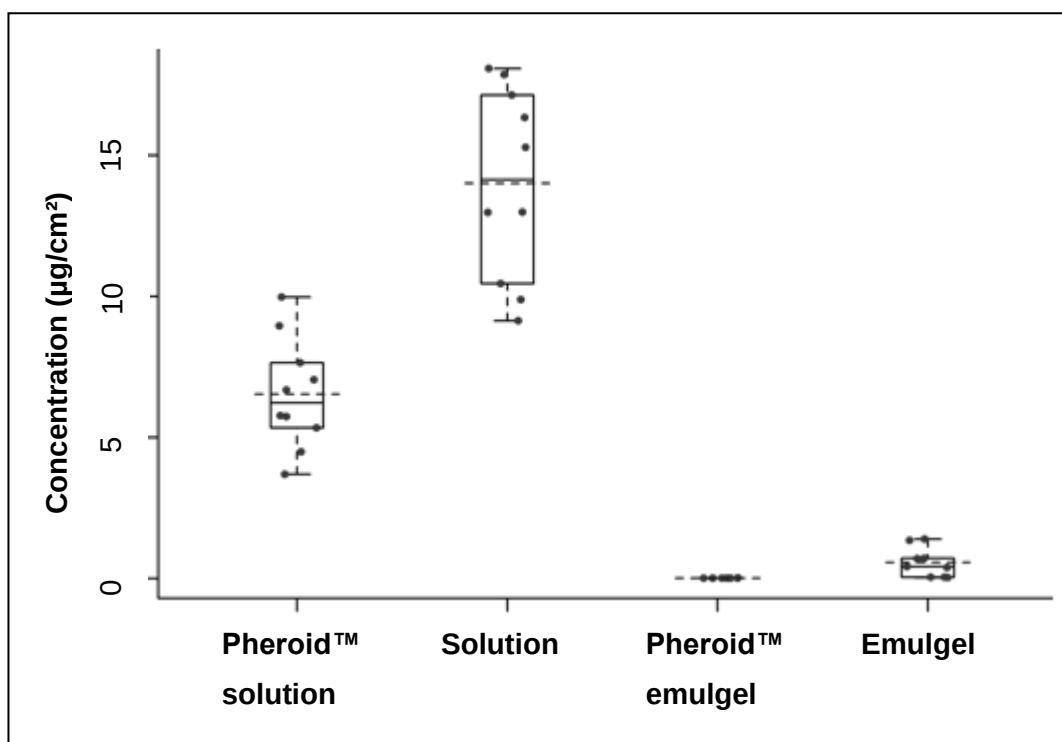


Figure 2: Box-plot representation of concentration values ($\mu\text{g}.\text{cm}^2$) at 12 h for the solutions and emulgel formulations. The dotted line represents the average concentration values.

CHAPTER 4

Conclusion and Future Prospects

Nausea and vomiting associated with motion sickness can cause extensive discomfort in patients. Motion sickness occurs when the brain perceives a variance between the speeds at which the eye's visually observed movement adjusts relatively to the vestibular system's movement. The most common symptoms include sweaty palms, a heaving stomach and persistent nausea (Sherman & Kider, 2007:1). Antihistamines (including cyclizine) and scopolamine are viewed as the most successful treatments to combat motion sickness (Shutt & Bowes, 1983:478). New potential strategies are subsequently being developed to deliver these drugs.

The aims set in this study were to:

- Establish whether cyclizine (0.5%) and scopolamine (1.0%) solution can be delivered transdermally.
- Formulate an emulgel, with and without the use of Pheroid™ vesicles, containing the anti-emetic (cyclizine or scopolamine) that delivered the biggest transdermal concentration.
- Determine the aqueous solubility and log D of the two drugs.
- Develop and validate a HPLC method for both cyclizine and scopolamine to accurately determine the concentration of the drugs.
- Determine the stability of the emulgel formulations when exposed to different temperatures and humidity.

Solutions containing either cyclizine or scopolamine were investigated for transdermal penetration using full thickness skin. Franz-cell diffusion studies were done for 12 h. The results obtained demonstrated that cyclizine did not penetrate the skin and therefore would not be a suitable drug to formulate as a transdermal dosage form. Scopolamine penetrated the full thickness skin and delivered an average cumulative concentration of 14.012 $\mu\text{g}/\text{cm}^2$ without Pheroid™ and 6.486 $\mu\text{g}/\text{cm}^2$ with Pheroid™, respectively. Evaluation of the sc-epidermis and epidermis-dermis followed the Franz-cell diffusion studies to determine the amount of drug present in the dermis and epidermis. Cyclizine did not penetrate either of the layers. Scopolamine delivered average concentration values of 0.0128 $\mu\text{g}/\text{ml}$ for the stratum corneum (sc)-epidermis and 0.2035 $\mu\text{g}/\text{ml}$ for the dermis-epidermis. Scopolamine with Pheroid™ delivered average concentration values of 0.0044 $\mu\text{g}/\text{ml}$ for the sc-epidermis and 0.0525 $\mu\text{g}/\text{ml}$

for the epidermis-dermis. A higher oil content in the Pheroid™ solution and thus an increase in particle size; decreases the diffusion through the skin. As noted by Nachum *et al.* (2000:121) a higher loading dose is required to attain an effective concentration when delivering scopolamine transdermally. Hydration is one of the key drive forces to transport vesicles across the skin. The solution with Pheroid™ contains a bigger oil phase and therefore the drive force is smaller to permeate the skin (Bouwstra *et al.*, 2003:31).

Scopolamine emulgel formulations were formulated with and without Pheroid™. Membrane release studies were done on both formulations to determine whether scopolamine would be released from the emulgel. The average cumulative concentrations of 0.116% for the emulgel and 0.079% for the emulgel containing Pheroid™ confirmed the release from the dosage form. Epidermal skin diffusion studies were subsequently done over 12 h to determine the transdermal permeation of scopolamine. The obtained result for the scopolamine emulgel was 2.649 µg/cm² and 0.017 µg/cm² for the scopolamine emulgel with Pheroid™. The reason for the lower concentration delivered by the emulgel containing Pheroid™ might be attributed to the smaller amount of scopolamine released by the emulgel formulation. Barry (2002:513) stated that a bigger particle size decreases the transdermal permeation. The bigger particle size of the emulgel containing Pheroid™ due to the larger oil concentration in the formulation might therefore attribute to the lower diffusion of scopolamine.. A smaller concentration of scopolamine is released by the emulgel with Pheroid™ and thus a smaller concentration is available for transdermal diffusion. Bouwstra *et al.*, (2003:23) previously stated that vesicles in a gel state can inhibit the permeation of the skin. The gel might even act as an extra barrier due to it only acting on the outer layer (SC) of the skin (Bouwstra *et al.*, 2003:26-31).

Statistical analyses of the skin permeation data obtained for the solutions revealed a significant difference between the formulations. When comparing the concentration of the emulgel formulations after 12 h, a statistical significant difference was observed between the formulations. When the concentrations of the solutions and emulgel formulations were compared after 12 h, the scopolamine solution delivered the highest concentration transdermally, followed by the solution with Pheroid™, the emulgel and lastly the emulgel with Pheroid™.

The log D value of scopolamine was determined in order to establish whether it would be a favourable drug to deliver transdermally. An optimum log D value of 1.77 was obtained and demonstrated that scopolamine would be a great candidate to consider for transdermal formulations (Yano *et al.*, 1986) (as quoted by Brown *et al.*, 2006:177).

The formulated emulgel formulations were placed in stability chambers for 3 months. The emulgel formulations were stored at 25 °C/60% RH, 30 °C/60% RH and 40 °C/70% RH,

respectively. The scopolamine concentration remained in the acceptable range after the 3 month stability test period. The pH remained relatively constant and no noteworthy change in the visual appearance was observed. The increase in viscosity and CLSM micrographs revealed instability in the emulgel formulation. Instability in the scopolamine emulgel containing Pheroid™ was exposed in the formulation. The scopolamine concentration was no longer in the accepted range and the pH, viscosity, CLSM micrographs and visual appearance greatly varied over the stability test period.

Future prospects and recommendations include the following:

- The stability of the formulations can be improved by using different anti-oxidants and preservatives, making sure that scopolamine is kept in the accepted range
- Incorporating other delivery systems to optimally deliver scopolamine

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APPENDIX A

Validation of the HPLC Analytical Method of Analysis

Introduction

Validation is a crucial step in establishing whether the substances used satisfies a certain criterion (Karnes *et al.*, 1991:421). This establishes if the method developed for a substance is reliable and sensitive enough to determine the amount and recovery of a drug when formulated. Active ingredients include scopolamine and cyclizine. This validation process was undertaken in a regulated laboratory environment at a temperature of 25°C. The validation process usually consists of the following steps:

1. System qualifications: This step determines if an instrument is reliable and suitable for the anticipated analysis.
2. Sampling: This ensures that the sample selected represents the material as a whole.
3. Sample preparation: Representing a crucial step in validation and operational costs in a laboratory.
4. Analysis: In close relation with the instrument to deliver quantitative and qualitative information.
5. Data evaluation: Gaining insight and summarizing the data collected (Karnes *et al.*, 1991:421).

In Section A.1 the HPLC method of analysis will be discussed for the solutions containing cyclizine and scopolamine, respectively. Due to the positive results obtained in the transdermal permeation of scopolamine (Appendix D), an emulgel with and without the use of Pheroid™ will subsequently be formulated. Section A.2 discusses the HPLC method of analysis for the emulgel containing scopolamine.

A.1 Validation of active ingredients

A.1.1 Chromatography

Equipment:

The Agilent 1100 series HPLC was used in the analysis. This instrument, fitted with an Agilent® 1100 pump, makes use of an autosampler injection mechanism and diode array detector. Chemstation Rev. A.10.02 was used for data analysis and acquirement.

Chromatographic conditions:

Milli-Q water (HPLC grade, double deionised) was used throughout this study. The acetonitrile and octanesulphonic acid (sodium salt) used were both of HPLC grade. Detection took place at 210 nm at a flow rate of 1.0 ml/min. An injection volume of 5 µl and 10 µl of cyclizine and scopolamine, respectively, was injected into the HPLC. Retention time of cyclizine was 5 min and 2.8 min for scopolamine.

Column:

Verusil XBP C18 (2), 5 µm, 100 Å, 4.6 x 150 mm.

Mobile phase I:

A solution was prepared by adding 1 g of octanesulphonic acid sodium to 1000 ml of fresh Milli-Q water. The pH was subsequently adjusted to 3.5 with phosphoric acid (10%).

Mobile phase II:

This phase consisted of 100% acetonitrile HPLC grade.

Gradient:

Table A.1.1: Mobile phases and retention times

Substance	Retention time (min)	%Mobile Phase I	%Mobile Phase II
Cyclizine	11	55	45
Scopolamine	10	50	50

A.1.2 Preparation of standard solution

The establishment of a calibration curve was done by preparing cyclizine hydrochloride and scopolamine solutions daily. Five different concentrations within the accepted range must be utilized (Karnes *et al.*, 1991:423).

Standards solutions were prepared that contained 25 mg of dry powder dissolved in HPLC water and sonicated for approximately 5 min to assist in the dissolution process of the solute. The volumetric flasks were filled to 100 ml. The aforementioned solution (5 ml) was diluted with HPLC water and filled to 50 ml in a volumetric flask. Another 5 ml of this solution was diluted with HPLC water and filled to 50 ml. The obtained concentrations were 2.5 µl, 25 µl and 250 µl. A range of volumes (2.5 µl, 5 µl, 10 µl, 25 µl, 50 µl) were analyzed on the HPLC from each flask

in order to obtain a broader range of concentrations. It is crucial to have a wide range as it is not certain how much of a substance will be delivered transdermally.

A.1.3 Linearity

The importance of determining the linearity is to establish the fit of the regression line on the plot of the peak area versus concentration. This can be obtained by constructing a calibration curve. Three standard solutions were prepared daily as mentioned above in Section A.1.2. For both cyclizine and scopolamine a range of 0.006-250 µg/ml was used. The data is described by the linear equation $y = mx + c$, where:

- y: peak area
- m: slope
- x: concentration
- c: intercept on the y-axis

A straight line correlation between the input (x) and output (y) should be obtained. In common practice, the correlation coefficient (r) must be inspected. The closer the value is to 1, the more efficient the method is considered to be (Araujo, 2009:2225).

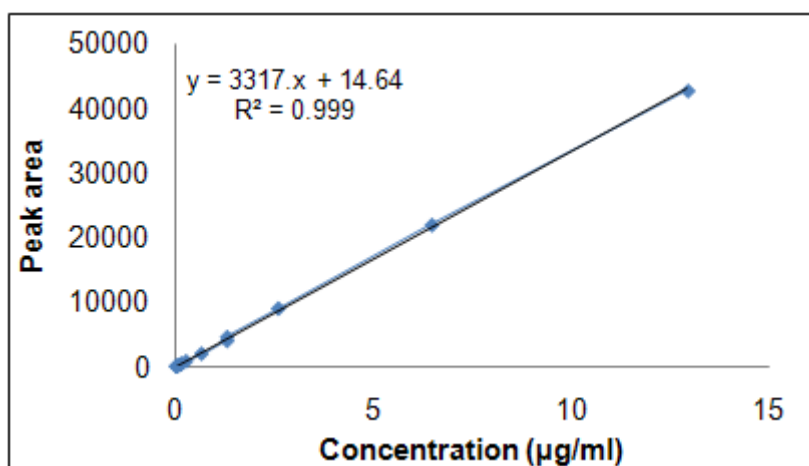


Figure A.1.1: Linearity with peak area vs. concentration (µg/ml)

A.1.2: Linearity of cyclizine

Standard (µg/ml)	Peak area ratio
0.0065	21.306
0.0130	42.584
0.0259	78.354
0.0658	191.273
0.1295	384.868
0.2590	799.278
0.6475	1994.634
1.2950	4553.162
2.5900	8984.971
6.4750	21921.700
12.9500	42710.800
Slope	3317.0
y-intercept	14.640
r²	0.999

Table A.1.3: Linearity of scopolamine

Standard (µg/ml)	Peak area ratio
0.01	11.064
0.03	22.293
0.07	55.359
0.13	111.611
0.27	220.635
0.66	548.633
1.33	1092.170
2.65	2287.505
6.63	5684.883
13.25	11312.000
Slope	854.9
y-intercept	- 2.230
r²	1.000

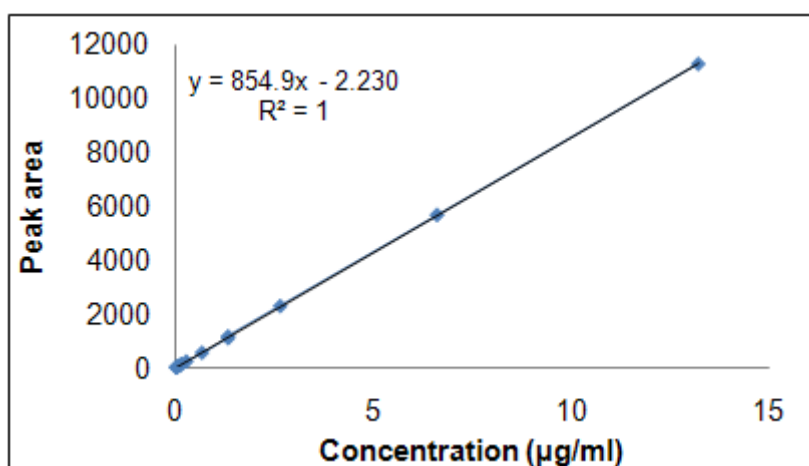


Figure A.1.2: Linearity with peak area vs. concentration (µg/ml)

A.1.4 Precision

This determines the degree of closeness and proximity of a value to the definite value by various testing and sampling under the same prescribed conditions. Without precision, the reproducibility and repeatability of the method can not be determined (Araujo, 2009:2227). Precision consists out of intra-day and inter-day variability (Karnes *et al.*, 1991:424).

A.1.4.1 Intra-day

This term describes the assessment of the batch within the same day (Karnes *et al.*, 1991:424). It will determine the repeatability of the method.

Table A.1.4: Intra-day precision of cyclizine

Concentration spiked (µg/ml)	Peak area 1	Peak area 2	Mean	Concentration recovered (µg/ml)	%
0.710	2199.230	2123.670	2161.450	0.704	99.11
0.730	2204.817	2214.661	2209.739	0.719	98.55
0.773	2255.718	2245.534	2250.626	0.733	94.86
0.750	2288.350	2345.540	2316.905	0.754	100.59
0.587	1798.570	1799.625	1799.010	0.585	99.50
0.558	1734.849	1715.320	1725.085	0.561	100.50
Mean	98.91				
SD*	1.96				
%RSD**	1.98				

*SD refers to standard deviation

**%RSD refers to relative standard deviation

Three replicate standard samples of different concentrations were prepared on the same day. Each of the three samples was spiked on the HPLC in duplicate and a mean peak area was used to obtain the relative standard deviation (RSD) and the standard deviation (SD). As previously mentioned, values should not exceed 2%.

According to the USP, the %RSD must be 2% or less. The calculated %RSD of 1.98 falls within the acceptable range.

Table A.1.5: Intra-day precision of scopolamine

Concentration spiked (µg/ml)	Peak area 1	Peak area 2	Mean	Concentration recovered (µg/ml)	%
0.570	475.70	475.30	476.40	0.576	101.00
0.580	477.50	476.59	476.10	0.576	99.22
0.640	534.10	533.09	533.10	0.645	100.70
0.650	526.90	527.06	527.06	0.635	98.06
0.812	684.02	671.56	671.50	0.810	100.08
0.810	669.49	672.52	672.50	0.810	100.53
Mean	99.95				
SD*	1.02				
%RSD**	1.02				

*SD refers to standard deviation

**%RSD refers to relative standard deviation

According to the USP, the %RSD must be 2% or less. The calculated %RSD of 1.02 falls within the acceptable range.

A.1.4.2 Inter-day

The reproducibility of a method is determined through inter-day precision and also determines how accurate the method of analysis is. The reproducibility of a method is determined by performing inter-day. This test was carried out over three consecutive days. Three standard solutions with known concentrations were prepared. Samples were spiked in duplicate on the HPLC and the mean peak area was used to determine the RSD and SD. This value should not exceed 5%.

Table A.1.6: Inter-day precision of cyclizine

Day	Mass (g)	Peak area	Concentration recovered (µg/ml)	%	SD*	%RSD*	Mean
1	0.019	1534.5	20.3	101.8	1.78	1.75	99.29
	0.025	1831.9	24.2	97.9			
	0.030	2230.6	29.4	98.2			
2	0.020	1513.0	20.0	100.4	1.10	1.10	99.64
	0.025	1828.0	24.1	98.1			
	0.030	2283.0	30.1	100.5			
3	0.020	1514.0	20.9	100.6	0.64	0.64	100.04
	0.025	1897.0	25.0	99.4			
	0.030	2262.0	29.9	99.8			
Between days					0.3	0.3	99.66

*SD refers to standard deviation

**%RSD refers to relative standard deviation

The USP stipulates that the %RSD for inter-day variation must not exceed 5%. Hence, the obtained value of 0.3 falls within the acceptable range.

Table A.1.7: Inter-day precision of scopolamine

Day	Mass (g)	Peak area	Concentration recovered (µg/ml)	%	SD*	%RSD*	Mean
1	0.020	572.0	20.1	100.7	0.27	0.27	100.31
	0.025	629.5	24.8	100.0			
	0.030	763.5	30.1	100.3			
2	0.023	512.0	20.2	99.4	0.64	0.64	100.10
	0.025	629.0	24.7	100.0			
	0.030	769.0	30.1	100.9			
3	0.020	504.0	19.9	99.3	0.27	0.27	99.57
	0.025	632.0	24.9	99.5			
	0.030	764.0	30.1	99.9			
Between days					0.31	0.31	99.99

*SD refers to standard deviation

**%RSD refers to relative standard deviation

The USP stipulates that the %RSD for inter-day variation must not exceed 5%. Hence, the obtained value of 0.31 falls within the acceptable range.

A.1.5 Accuracy

Table A.1.8: Accuracy of cyclizine

Concentration spiked (µg/ml)	Peak area 1	Peak area 2	Mean	Recovery (µg/ml)	%
0.49	1534.8	1534.3	1534.6	0.49	100.3
0.49	1536.2	1534.9	1535.5	0.49	100.4
0.49	1535.4	1536.4	1535.9	0.50	100.4
0.62	1871.6	1879.3	1875.5	0.61	99.2
0.62	1871.1	1872.7	1871.9	0.61	99.1
0.62	1871.8	1870.7	1871.2	0.61	99.0
0.75	2278.1	2278.8	2278.5	0.74	98.9
0.75	2282.0	2280.1	2281.1	0.74	99.0
0.75	2282.0	2342.6	2312.3	0.75	100.4
Mean	99.64				
SD*	0.66				
%RSD**	0.66				

*SD refers to standard deviation

**%RSD refers to relative standard deviation

Table A.1.9: Accuracy of scopolamine

Concentration spiked (µg/ml)	Peak area 1	Peak area 2	Mean	Recovery (µg/ml)	%
0.55	462.2	451.7	456.9	0.75	100.8
0.59	484.1	482.1	483.1	0.74	99.8
0.59	495.7	486.6	491.2	0.61	99.4
0.61	496.9	507.1	502.1	0.74	99.1
0.62	512.9	518.7	515.8	0.61	99.4
0.65	543.2	537.5	540.3	0.50	99.8
0.77	646.4	627.0	636.7	0.49	100.4
0.77	637.2	637.3	637.3	0.49	100.2
0.81	672.6	674.8	673.7	0.61	100.4
Mean	99.64				
SD*	0.66				
%RSD**	0.66				

*SD refers to standard deviation

**%RSD refers to relative standard deviation

Accuracy is the most crucial step in any analytical method. It can be defined as the degree of agreement between the reference value and true experimental value. This parameter estimates the influence of systematic errors on an analytical method (Araujo, 2009:2226).

Three replicate standard solutions were prepared. Three samples of each of the three solutions were prepared and spiked in duplicate for repeatability testing. Together with precision this determines the error of the analytical measurement.

A.1.6 Sensitivity

The lowest amount and limit of a sample was determined in order to assess the sensitivity of the analytical method. This is the lowest concentration of a substance that can be determined with adequate precision. The lowest detectable limit for both cyclizine and scopolamine was $< 0.0006 \mu\text{g}$.

A.1.7 Ruggedness

A.1.7.1 Influence of pH

Four replicate standard solutions were prepared. Sulphonic acid, water, hydrogen peroxide and hydrochloric acid was added to each standard. Each of the three samples was subsequently investigated at extremes of pH. Most drugs have their maximum stability at pH 4 and 8 (Wells *et al.*, 2007:353).

A.1.7.2 System repeatability

This assesses the repeatability of a result on the HPLC.

Table A.1.10: System repeatability of cyclizine

Peak area	Retention time (min)
2098.26	5.99
2099.50	5.99
2098.42	5.99
2097.18	5.99
2096.48	6.01
2091.97	6.01
2094.41	6.01
2089.18	6.01
Mean	2095.67
SD*	3.34
%RSD**	00.16

*SD refers to standard deviation

**%RSD refers to relative standard deviation

Table A.1.11: System repeatability of scopolamine

Peak area	Retention time (min)
567.04	2.53
566.12	2.53
566.67	2.53
566.59	2.53
566.26	2.52
566.05	2.53
565.41	2.54
565.84	2.54
Mean	566.25
SD*	0.48
%RSD**	0.08

*SD refers to standard deviation

**%RSD refers to relative standard deviation

A.1.7.3 Sample stability

This assesses the repeatability of a method under various conditions. Three standard replicate solutions were prepared and left for 24 h to subsequently determine both the formation of deterioration products and sample stability in new conditions.

Table A.1.12: Sample stability of cyclizine

Time (h)	Peak area	%
0	4231.197	100.0
1	4243.916	100.301
2	4273.957	101.011
3	4255.595	100.577
4	4264.495	100.787
5	4238.586	100.175
6	4236.077	100.115
7	4275.567	101.049
8	4234.602	100.081
9	4215.087	99.619
10	4183.362	98.869
11	4237.404	100.147
12	4181.141	98.817
13	4211.812	99.542
14	4193.313	99.105
15	4181.216	98.819
16	4160.916	98.339
17	4172.543	98.614
18	4181.211	98.819
19	4169.973	98.553
20	4202.956	99.333
21	4188.696	98.996
22	4183.252	98.867
23	4203.369	99.342
24	4200.206	99.268
Mean	4212.819	99.566
SD*	33.500	0.791
%RSD**	0.794	0.794

*SD refers to standard deviation

**%RSD refers to relative standard deviation

Table A.1.13: Sample stability of scopolamine

Time (h)	Peak area	%
0	1249.569	100.000
1	1243.999	99.600
2	1252.967	100.300
3	1244.423	99.600
4	1247.007	99.800
5	1238.218	99.100
6	1255.815	100.500
7	1244.527	99.600
8	1261.765	100.900
9	1240.874	99.300
10	1248.237	99.900
11	1246.540	99.800
12	1249.784	100.020
13	1238.297	99.100
14	1254.140	100.400
15	1258.516	100.700
16	1258.517	100.700
17	1241.552	99.400
18	1242.267	99.400
19	1253.120	100.600
20	1245.485	99.700
21	1243.559	99.500
22	1237.322	99.000
23	1239.935	99.200
24	1237.876	99.100
Mean	1247.010	99.800
SD*	6.980	0.560
%RSD**	0.560	0.560

*SD refers to standard deviation

**%RSD refers to relative standard deviation

A.2 HPLC method of scopolamine emulgel

A.2.1 Chromatography

Equipment:

The same equipment and analytical column was utilized as described in Section A.1.1 with the exception of the following:

Chromatographic conditions:

All chromatographic conditions were the same (as in Section A.1.1) except the retention time for scopolamine was 7.2 min.

Mobile phase I:

The same solution and pH were used for mobile phase I as in Section A.1.1.

Mobile phase II:

This phase consisted of 100% methanol HPLC grade.

Gradients:

Table A.2.1: Percentages of mobile phases used at specific time intervals

Run time (min)	%Mobile phase I	%Mobile phase II
0.0	0	10
8.0	0	100
20.0	0	100
20.1	60	40

A.2.2 Preparation of standard solutions for the emulgel

Methanol and water was used as solvents for the preparation of standard solutions. Before conducting any test, a standard solution was prepared. The solution consisted of all the ingredients present in the formulated gel, see Table A.2.2. A placebo gel was also prepared, functioning as a control, see Table A.2.3. The ratios remained the same as in the final gel formulations.

A standard solution was prepared by accurately weighing each of the ingredients in a 50 ml volumetric flask and then diluting it by adding methanol. The amount of propyl paraben could not be accurately weighed. Hence, 10 mg of propyl paraben was weighed in a 100 ml volumetric flask and diluted by adding methanol. The solution (8 ml) was extracted and added to the standard solution, subsequently delivering the desired concentration. A calibration curve was instituted by injecting different volumes of the standard solution.

Table A.2.2: Standard solution formulation as for 100%

Substance	Amount weighed (mg)	Concentration (µg/ml)
α-dl-tocopherol*	2	40
Methyl paraben	4	80
BHT (butylated hydroxytoluene)	2	40
Scopolamine	10	200
Propyl paraben	10	16

* Hereafter referred to tocopherol

Table A.2.3: Placebo standard as for 100%

Substance	Amount weighed (mg)
Vitamin F	2.8
Cremophor RH	1
Liquid paraffin	20
Span 60	0.5
Tween 80	4.5
Xanthan gum	1.5
Water	67.12

A.2.3 Linearity

As describe in Section A.1.3, the basic steps in determining linearity are the input, conversion and output and can be expressed mathematically as the equation: $y = mx + c$.

A standard 125% solution was prepared before assessing the linearity. One gram of both placebo and Pheroid™ gel were weighed in separate 50 ml volumetric flasks and diluted by adding water and methanol. Various dilutions were made from the 125% delivering concentrations from 155-310 µg/ml (See Table A.2.4).

Table A.2.4: Linear regression of scopolamine standard

Standard (µg/ml)	Peak area ratio
155	1107.4
186	1320.0
217	1576.0
248	1786.2
279	2022.2
310	2227.0
Slope	7.295
y-intercept	-22.910
r²	0.999

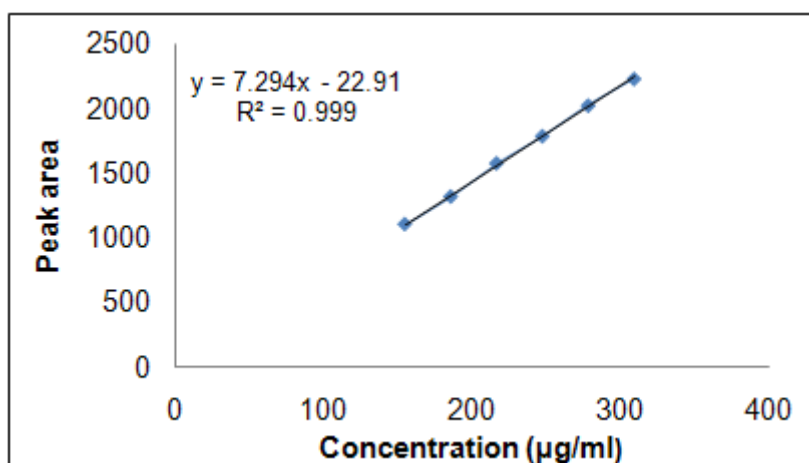


Figure A.2.1: Linear regression with peak area vs. concentration (µg/ml)

The attained regression value (r^2) indicates an optimum degree of linearity, therefore demonstrating a favourable stability of the analysis system.

Table A.2.5: Linear regression of methyl paraben standard

Standard (µg/ml)	Peak area ratio
62.5	4007.6
75.0	4850.0
87.5	5678.5
100.0	6472.7
112.5	7249.9
125.0	8027.9
Slope	64.320
y-intercept	27.310
r²	0.999

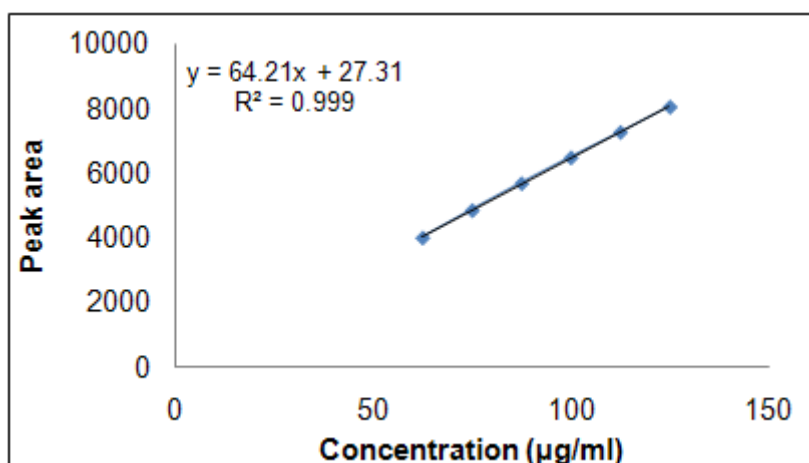


Figure A.2.2: Linear regression with peak area vs. concentration (µg/ml)

The attained regression value (r^2) indicates an optimum degree of linearity, therefore demonstrating a favourable stability of the analysis system.

Table A.2.6: Linear regression of propyl paraben standard

Standard (µg/ml)	Peak area ratio
160	435.0
192	525.0
224	601.0
256	700.5
288	770.0
320	849.5
Slope	2.595
y-intercept	23.900
r²	0.998

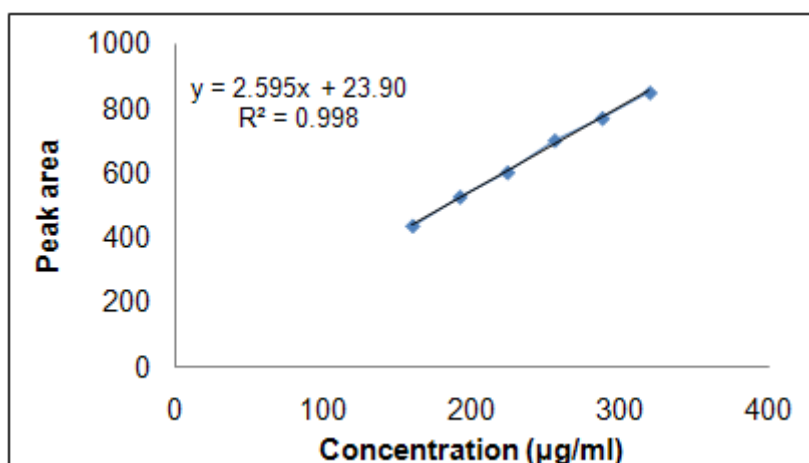


Figure A.2.3: Linear regression with peak area vs. concentration (µg/ml)

The attained regression value (r^2) indicates an optimum degree of linearity, therefore demonstrating a favourable stability of the analysis system.

Table A.2.7: Linear regression of BHT standard

Standard (µg/ml)	Peak area ratio
33.8	771.0
40.5	932.3
47.3	1089.0
54.0	1244.0
60.8	1404.0
67.5	1544.0
Slope	2.595
y-intercept	23.900
r²	0.998

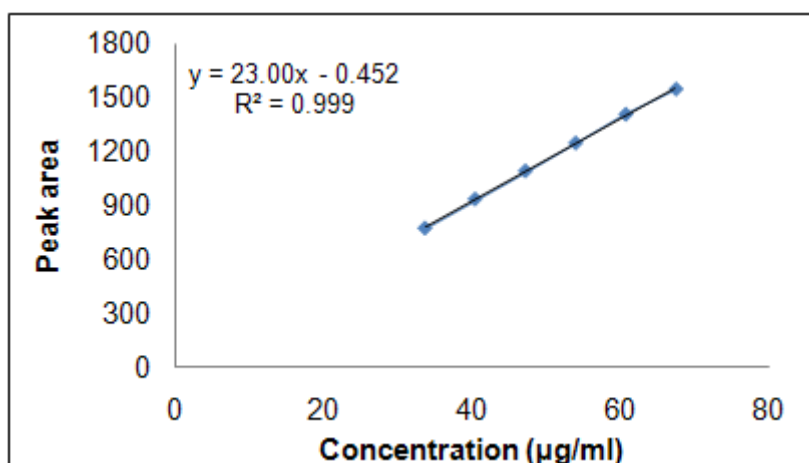


Figure A.2.4: Linear regression with peak area vs. concentration (µg/ml)

The attained regression value (r^2) indicates an optimum degree of linearity, therefore demonstrating a favourable stability of the analysis system.

Table A.2.8: Linear regression of tocopherol

Standard (µg/ml)	Peak area ratio
31.3	585.0
37.5	715.0
43.8	838.0
50.0	960.0
56.3	1113.5
62.5	1291.0
Slope	22.160
y-intercept	121.600
r²	0.994

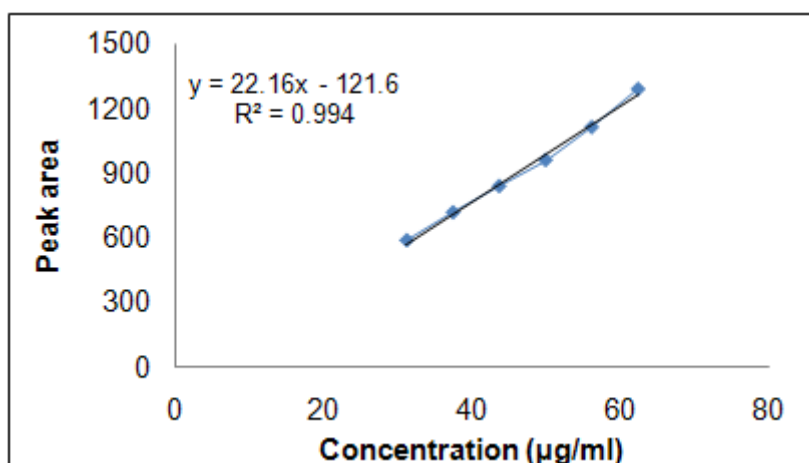


Figure A.2.5: Linear regression with peak area vs. concentration (µg/ml)

The attained regression value (r^2) indicates an optimum degree of linearity, therefore demonstrating a favourable stability of the analysis system.

A.2.4 Precision

The relevance and importance of precision is described in Section A.1.4 and consists of intra-day and inter-day.

A.2.4.1 Intra-day

A standard solution was prepared as described in Section A.2.2 and analysed on the HPLC. The Pheroid™ gel was accurately weighed in triplicate in 100 ml volumetric flasks. The first three contained 0.8 g Pheroid™ gel (80%), the next three contained 1.0 g (100%) and the last three contained 1.2 g (120%). The nine flasks were filled to volume with water and methanol.

After being sonicated, the samples were injected in the HPLC in duplicate. A %RSD and SD value less than 2% should be obtained to have an accurate method.

Table A.2.9: Scopolamine intra-day precision

Mass (g)	Peak area 1	Peak area 2	Mean	Concentration recovered (µg/ml)	%
0.819	1520	1500	1510	209.2	100.6
0.809	1464	1478	1471	206.3	99.2
0.814	1473	1475	1474	205.4	98.8
1.003	1910	1918	1914	216.5	104.1
1.000	1901	1909	1905	216.1	103.9
1.000	1906	1916	1911	216.5	104.2
1.260	2279	2285	2282	205.5	98.8
1.190	2274	2280	2277	217.1	104.4
1.280	2278	2270	2274	211.5	101.1
Mean	101.7				
SD*	2.3				
%RSD**	2.3				

*SD refers to the standard deviation

**%RSD refers to the relative standard deviation

According to the USP, the %RSD for intra-day repeatability must be 2% or less. A value of 2.3 is somewhat higher than the required value, but is still expectable. This result can be attributed to the emulgel not being homogenous.

Table A.2.10: Methyl paraben intra-day precision

Mass (g)	Peak area 1	Peak area 2	Mean	Concentration recovered (µg/ml)	%
0.819	3314	3288	3301.0	80.4	100.5
0.809	3297	3177	3237.0	79.8	99.8
0.814	3300	3125	3212.5	78.7	98.4
1.003	4217	4019	4117.0	81.9	102.4
1.000	4205	4015	4110.0	82.6	102.5
1.000	4107	4214	4160.0	83.1	103.8
1.260	4998	4957	4977.5	78.8	98.5
1.190	4996	4793	4894.5	82.1	102.6
1.280	4954	4973	4963.5	81.2	101.5
Mean	99.2				
SD*	0.5				
%RSD**	0.5				

*SD refers to the standard deviation

**%RSD refers to the relative standard deviation

According to the USP, the acceptable %RSD for intra-day repeatability must be 2% or less. The obtained %RSD of 0.5 is within acceptable range.

Table A.2.11: Propyl paraben intra-day precision

Mass (g)	Peak area 1	Peak area 2	Mean	Concentration recovered (µg/ml)	%
0.819	530.0	529	529.5	154.6	96.6
0.809	527.0	528	527.5	155.9	97.5
0.814	531.0	531	531.0	156.0	97.5
1.003	669.1	676	683.0	162.9	101.8
1.000	680.0	678	679.0	162.4	101.5
1.000	678.0	685	681.5	163.0	101.9
1.260	832.0	837	835.5	158.0	99.0
1.190	809.0	828	818.5	164.5	102.8
1.280	829.0	831	830.0	162.7	101.7
Mean	100.0				
SD*	2.2				
%RSD**	2.2				

*SD refers to the standard deviation

**%RSD refers to the relative standard deviation

According to the USP, the acceptable %RSD for intra-day repeatability must be 2% or less. The obtained value of 2.2 is slightly higher than the specified value of the USP, but is still acceptable. This value may be due to the emulgel not being homogenous.

Table A.2.12: BHT intra-day precision

Mass (g)	Peak area 1	Peak area 2	Mean	Concentration recovered (µg/ml)	%
0.819	914	921	917.5	41.4	98.5
0.809	920	911	915.5	41.8	99.5
0.814	919	913	916.0	41.6	99.0
1.003	1132	1160	1146.0	42.2	100.5
1.000	1156	1160	1158.0	42.8	101.8
1.000	1188	1107	1147.0	42.4	100.9
1.260	1369	1363	1366.0	40.0	95.3
1.190	1354	1376	1465.0	42.4	100.9
1.280	1362	1365	1363.5	41.3	99.3
Mean	99.4				
SD*	1.8				
%RSD**	1.8				

*SD refers to the standard deviation

**%RSD refers to the relative standard deviation

According to the USP, the acceptable %RSD for intra-day repeatability must be 2% or less. The obtained %RSD of 1.8 falls within the accepted range.

Table A.2.13: Tocopherol intra-day precision

Mass (g)	Peak area 1	Peak area 2	Mean	Concentration recovered (µg/ml)	%
0.819	820	813	816.5	39.9	99.7
0.809	807	808	807.5	39.9	99.8
0.814	815	808	811.5	39.9	99.7
1.003	1002	1004	1003.0	40.0	100.0
1.000	1014	1021	1017.5	40.7	101.8
1.000	1025	1035	1030.0	41.2	103.0
1.260	1263	1263	1263.0	40.1	100.2
1.190	1159	1200	1159.5	39.6	99.1
1.280	1169	1262	1215.5	39.9	99.6
Mean	100.3				
SD*	1.2				
%RSD**	1.2				

*SD refers to the standard deviation

**%RSD refers to the relative standard deviation

According to the USP, the acceptable %RSD for intra-day repeatability must be 2% or less. The obtained %RSD of 1.2 falls within the accepted range.

A.2.4.2 Inter-day (inter laboratory variation)

Inter-day was carried out over three separate days, in order to access the influence of different conditions (i.e. temperature, technique etc.). A standard solution was prepared (Section A.2.1) and injected in the HPLC. Pheroid™ gel (1 g) was accurately weighed in 50 ml volumetric flasks in triplicate and filled to volume with water and methanol. After being sonicated, the samples were analysed on the HPLC. The %RSD and SD value should not exceed 5% to prove to be accurate and reproducible.

Table A.2.14: Inter-day precision of scopolamine

Day	Mass (g)	Peak area	Concentration recovered (µg/ml)	%	SD*	%RSD*	Mean
1	1.03	1914.0	210.8	101.4	0.9	0.9	100.5
	1.03	1905.0	209.8	100.9			
	1.05	1911.0	206.5	99.3			
2	1.017	1833.0	202.5	101.2	1.3	1.2	101.3
	1.005	1867.0	208.7	104.3			
	1.020	1865.0	205.4	102.7			
3	1.060	1865.5	203.3	100.6	2.9	2.8	102.3
	1.060	1856.0	201.9	100.0			
	1.020	1899.5	214.8	106.3			
Between days					1.70	1.63	101.86

*SD refers to the standard deviation

**0%RSD refers to the relative standard deviation

According to the USP, an acceptable range of %RSD for inter-day variability must be 5% or below. The obtained value of 1.63 complies with this requirement.

Table A.2.15: Inter-day precision of methyl paraben

Day	Mass (g)	Peak area	Concentration recovered (µg/ml)	%	SD*	%RSD*	Mean
1	1.030	4117.0	79.8	99.7	0.4	0.4	99.3
	1.030	4110.0	79.6	99.5			
	1.050	4160.0	79.1	98.8			
2	1.017	4090.0	81.6	99.5	0.9	0.9	98.6
	1.005	4020.0	81.1	99.0			
	1.020	4018.5	79.9	97.4			
3	1.060	4373.0	79.4	99.3	1.0	1.0	99.9
	1.060	4360.5	79.3	99.1			
	1.020	4301.5	81.1	101.4			
Between days					0.76	0.76	99.26

*SD refers to the standard deviation

**0%RSD refers to the relative standard deviation

According to the USP, an acceptable range of %RSD for inter-day variability must be 5% or below. The obtained value of 0.76 falls within the acceptable range.

Table A.2.16: Inter-day precision of propyl paraben

Day	Mass (g)	Peak area	Concentration recovered (µg/ml)	%	SD*	%RSD*	Mean
1	1.030	672.0	156.2	97.6	1.8	1.8	99.3
	1.030	679.0	157.7	98.5			
	1.050	681.5	163.0	101.9			
2	1.017	673.0	157.6	98.5	0.3	0.3	98.9
	1.005	668.3	158.3	99.0			
	1.020	680.0	158.7	99.2			
3	1.060	720.0	157.6	98.5	0.1	0.1	98.6
	1.060	720.5	157.7	98.6			
	1.020	694.5	158.0	98.8			
Between days					0.73	0.73	98.93

*SD refers to the standard deviation

**%RSD refers to the relative standard deviation

According to the USP, an acceptable range of %RSD for inter-day variability must be 5% or below. The obtained %RSD value of 0.73 complies with the required range.

Table A.2.17: Inter-day precision of BHT

Day	Mass (g)	Peak area	Concentration recovered (µg/ml)	%	SD*	%RSD*	Mean
1	1.030	1146.0	44.2	100.5	0.8	0.8	100.0
	1.030	1158.0	42.5	98.9			
	1.050	1147.0	44.2	100.5			
2	1.017	1114.5	42.1	100.3	0.0	0.0	100.2
	1.005	1100.0	42.1	100.2			
	1.020	1116.5	42.1	100.2			
3	1.060	1421.0	39.9	99.9	0.5	0.5	100.2
	1.060	1437.0	40.4	101.0			
	1.020	1367.0	39.9	101.0			
Between days					0.43	0.42	100.13

*SD refers to the standard deviation

**%RSD refers to the relative standard deviation

According to the USP, an acceptable range of %RSD for inter-day variability must be 5% or below. The %RSD value obtained (0.42) falls within the acceptable range.

Table A.2.18: Inter-day precision of tocopherol

Day	Mass (g)	Peak area	Concentration recovered (µg/ml)	%	SD*	%RSD*	Mean
1	1.030	1003.0	40.0	99.9	1.2	1.2	101.5
	1.030	1017.5	40.7	101.6			
	1.050	1030.0	41.2	102.9			
2	1.017	1119.0	39.3	98.3	1.1	1.2	98.8
	1.005	1179.0	40.2	100.4			
	1.020	1115.5	39.1	97.7			
3	1.060	1134.0	41.6	99.1	1.1	1.1	99.6
	1.060	1157.5	42.5	101.2			
	1.020	1085.5	41.4	98.6			
Between days					1.13	1.17	99.97

*SD refers to the standard deviation

**%RSD refers to the relative standard deviation

According to the USP, an acceptable range of %RSD for inter-day variability must be 5% or below. The %RSD value of 1.17 complies with the specified standards.

A.2.5 Accuracy

Accuracy (described in Section A.1.5) plays a crucial role when validating a HPLC method. A placebo gel was prepared as described in Section A.2.2. It was subsequently divided into nine 50 ml volumetric flasks. The first three contained 0.8 g placebo gel (80%), the second three flasks contained 1.0 g placebo gel (100%) and the last three flasks 1.2 g placebo gel (120%). A ten times stronger standard solution was also prepared (described in Section A.2.2.).

The placebo gels were spiked with different concentrations of the ten times stronger standard. The following preparations were done:

- 4 ml standard solution added to the 80% placebo gel;
- 5 ml standard solution added to the 100% placebo gel; and
- 6 ml standard solution added to the 120% placebo gel.

The different concentrations were filled to volume with methanol and water. The solutions were spiked in duplicate and analysed on the HPLC.

Table A.2.19: Accuracy of scopolamine

Concentration spiked (µg/ml)	Peak area 1	Peak area 2	Mean	Recovery (µg/ml)	%
84.8	1375	1395	1385.0	86.9	102.5
84.8	1368	1385	1377.0	86.4	101.9
84.8	1365	1382	1373.0	86.2	101.3
106.0	1700	1700	1700.0	106.7	100.6
106.0	1708	1709	1708.5	106.2	100.1
106.0	1715	1719	1717.0	107.8	101.7
127.2	2063	2064	2063.5	129.5	101.8
127.2	2067	2064	2065.5	129.6	101.9
127.2	2067	2067	2067.0	129.7	100.0
Mean	101.68				
SD*	0.49				
%RSD**	0.48				

*SD refers to the standard deviation

**%RSD refers to the relative standard deviation

The range of recovered scopolamine ranged between 100% and 102.5%; with an average of 101.68%. These values might indicate that scopolamine was detained in the column, resulting in the values exceeding 100%.

Table A.2.20: Accuracy of methyl paraben

Concentration spiked (µg/ml)	Peak area 1	Peak area 2	Mean	Recovery (µg/ml)	%
130.7	3291	3289	3289	131.6	100.6
130.7	3301	3309	3305	132.2	101.1
130.7	3309	3306	3308	132.3	101.2
163.4	3837	4300	4068	162.7	99.6
163.4	4100	4015	4057	162.3	99.3
163.4	3837	4439	4138	165.5	101.3
196.1	4835	4841	4838	193.5	98.7
196.1	4841	4831	4836	193.4	98.7
196.1	4801	4875	4838	193.5	98.7
Mean	99.92				
SD*	1.09				
%RSD**	1.09				

*SD refers to the standard deviation

**%RSD refers to the relative standard deviation

The recovered methyl paraben ranged between 98.7% and 101.3%. The average methyl paraben recovered was 99.92%.

Table A.2.21: Accuracy of propyl paraben

Concentration spiked (µg/ml)	Peak area 1	Peak area 2	Mean	Recovery (µg/ml)	%
129.6	541	542	541.5	132.7	102.4
129.6	538	536	537.0	131.6	101.5
129.6	532	535	535.0	130.7	100.9
162.0	649	651	650.0	159.3	98.3
162.0	656	656	656.0	160.7	99.2
162.0	655	652	653.5	160.1	98.8
194.4	798	798	798.0	195.5	100.6
194.4	799	803	801.0	196.3	101.0
194.4	796	800	800.0	196.0	100.8
Mean	100.39				
SD*	1.25				
%RSD**	1.25				

*SD refers to the standard deviation

**%RSD refers to the relative standard deviation

The recovered propyl paraben ranged between 98.3% and 102.4%. The average propyl paraben recovered was 100.39%.

Table A.2.22: Accuracy of BHT

Concentration spiked (µg/ml)	Peak area 1	Peak area 2	Mean	Recovery (µg/ml)	%
32.8	809	801	805.0	33.0	100.5
32.8	802	798	800.0	32.8	99.9
32.8	814	791	802.5	32.9	100.2
41.0	990	987	988.5	40.5	98.7
41.0	978	984	981.0	40.2	98.7
41.0	978	990	984.0	40.3	98.3
49.2	1182	1175	1178.5	48.2	98.1
49.2	1194	1203	1198.5	40.1	99.7
49.2	1187	1181	1184.0	48.5	99.5
Mean	99.09				
SD*	0.92				
%RSD**	0.93				

*SD refers to the standard deviation

**%RSD refers to the relative standard deviation

The recovered BHT ranged between 98.1% and 100.5%. The average BHT recovered was 99.09%.

Table A.2.23: Accuracy of tocopherol

Concentration spiked (µg/ml)	Peak area 1	Peak area 2	Mean	Recovery (µg/ml)	%
34.4	628	631	629.5	34.2	99.5
34.4	634	621	627.5	34.1	99.1
34.4	619	628	623.5	33.9	98.5
42.0	730	827	728.5	39.6	94.3
42.0	732	740	736.0	40.0	95.2
42.0	728	725	726.5	39.5	94.0
50.4	788	765	776.5	42.2	83.7
50.4	787	798	792.5	43.1	85.5
50.4	787	785	780.0	42.7	84.8
Mean	92.75				
SD*	6.03				
%RSD**	6.05				

*SD refers to the standard deviation

**%RSD refers to the relative standard deviation

The recovered tocopherol ranged between 83.7% and 99.5%. The average tocopherol recovered was 92.75%.

A.2.6 Ruggedness

Ruggedness can be divided into two different categories, namely the stability of a sample solution and system repeatability.

A.2.6.1 Stability of a sample solution

In order to determine the stability of a substance, ruggedness should be assessed. A standard solution was prepared (Section A.2.2). Pheroid™ gel (1.0 g (100%)) was weighed in a 50 ml volumetric flask and filled with water and methanol. The solution was sonicated and analysed on the HPLC at various time-intervals for the duration of 24 h. This will determine the stability and degradation of a product.

Table A.2.24: Sample stability of scopolamine

Time (h)	Peak area	%
0	1926	100
1	1895	101.6
2	1892	100.2
3	1900	99.6
4	1892	100.4
5	1901	99.5
6	1898	100.2
24	1899	99.99
Mean	1900.4	100.2
SD*	10.2	0.6
%RSD**	0.5	0.6

*SD refers to the standard deviation

**%RSD refers to the relative standard deviation

The scopolamine sample is stable for 24 h.

Table A.2.25: Sample stability of methyl paraben

Time (h)	Peak area	%
0	4324	100.0
1	4344	99.5
2	4368	99.5
3	4319	101.1
4	4319	100.0
5	4306	100.3
6	4325	99.6
24	4326	100.0
Mean	4328.9	100.0
SD*	17.8	0.5
%RSD**	0.4	0.5

*SD refers to the standard deviation

**%RSD refers to the relative standard deviation

Methyl paraben is stable for 24 h.

Table A.2.26: Sample stability of propyl paraben

Time (h)	Peak area	%
0	678	100.0
1	668	101.5
2	660	101.2
3	665	99.2
4	668	99.6
5	662	100.9
6	668	99.1
24	667	100.1
Mean	667.0	100.2
SD*	5.0	0.9
%RSD**	0.8	0.8

*SD refers to the standard deviation

**%RSD refers to the relative standard deviation

The propyl paraben sample is stable for 24 h.

Table A.2.27: Sample stability of BHT

Time (h)	Peak area	%
0	1256	100.0
1	1224	102.6
2	1235	99.1
3	1236	99.9
4	1241	99.6
5	1237	100.3
6	1232	100.4
24	1228	100.3
Mean	1236.1	100.3
SD*	9.0	1.0
%RSD**	0.7	1.0

*SD refers to the standard deviation

**%RSD refers to the relative standard deviation

BHT is stable for 24 h.

Table A.2.28: Sample stability of tocopherol

Time (h)	Peak area	%
0	1425	100.0
1	1409	101.1
2	1370	102.8
3	1342	101.6
4	1142	118.0
5	1368	83.5
6	1349	101.4
24	1299	103.8
Mean	1339.1	101.5
SD*	82.4	8.7
%RSD**	6.2	8.6

*SD refers to the standard deviation

**%RSD refers to the relative standard deviation

The results obtained showed that tocopherol starts degrading over a period of 24 h.

A.2.6.2 System repeatability

A standard solution was prepared (Section A.2.1) and injected in the HPLC six times. This is to assess the repeatability of analysis. The % RSD is assessed and should be 2% or less to be acceptable.

Table A.2.29: System repeatability of scopolamine

Time (h)	Peak area
1	1755
2	1758
3	1751
4	1751
5	1748
6	1751
Mean	1752.3
SD*	3.2
%RSD**	0.2

*SD refers to the standard deviation

**%RSD refers to the relative standard deviation

The difference in the values obtained proved to be acceptable with a %RSD of 0.2.

Table A.2.30: System repeatability of methyl paraben

Time (h)	Peak area
1	4159
2	4166
3	4164
4	4157
5	4158
6	4156
Mean	4160
SD*	3.7
%RSD**	0.1

*SD refers to the standard deviation

**%RSD refers to the relative standard deviation

The variation in the all of the sample responses proved to be acceptable, with a %RSD of 0.1.

Table A.2.31: System repeatability of propyl paraben

Time (h)	Peak area
1	686
2	691
3	688
4	680
5	684
6	690
Mean	686.5
SD*	3.7
%RSD**	0.5

*SD refers to the standard deviation

**%RSD refers to the relative standard deviation

The obtained value %RSD of 0.5 proved to be acceptable.

Table A.2.32: System repeatability of BHT

Time (h)	Peak area
1	1366
2	1364
3	1359
4	1368
5	1368
6	1363
Mean	1364.7
SD*	3.1
%RSD**	0.2

*SD refers to the standard deviation

**%RSD refers to the relative standard deviation

The %RSD value obtained (0.2) proved to be acceptable.

Table A.2.33: System repeatability of tocopherol

Time (h)	Peak area
1	907
2	911
3	920
4	905
5	913
6	915
Mean	911.8
SD*	5.0
%RSD**	0.5

*SD refers to the standard deviation

**%RSD refers to the relative standard deviation

The obtained %RDS proved to be acceptable with a value of 0.5.

A.2.7 Specificity

The standard solution was prepared (Section A.2.2) as follows: 1 ml of the standard solution was transferred in four separate test tubes. Hydrogen peroxide (H₂O₂) (1 ml) was added to the first of the four test tubes. In the three remaining test tubes 1 ml of hydrogen chloride (HCl), sodium hydroxide (NaOH) and water (H₂O) was added, respectively. The test tubes were left over night. The following day a fresh standard solution was prepared and analysed on the HPLC. A sample of each of the four test tubes were analysed on the HPLC to determine the degradation of a product as well as the products reaction when exposed to different conditions.

All active ingredients degraded when exposed to the various conditions, being slightly more stable in an acid environment.

A.3 Conclusion

The HPLC methods for the determination of scopolamine, methyl paraben, propyl paraben, BHT and tocopherol were found to be sensitive and reliable enough to determine the concentration thereof in an emulgel.

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APPENDIX B

The Formulation of the Donor Phase Solutions and an Emulgel

Introduction

The latter half of the twentieth century brought forth many advances in formulation techniques, providing us with a vast amount of new information that changed our way of living and thinking. There is subsequently an ongoing search for new cosmetic products with advanced drug delivery capacity and its resulted combined biological effects. The formulation of cosmetic products most often includes an active ingredient together with an assortment of other products, hence, giving the product its physical form and controlling the release of an active ingredient. The water-oil emulsion is currently the most widespread formulation, including various creams and gels that not only appears more attractive, but propose an easier application and drug release (Magdassi *et al.*, 1999:1).

Dr. Albert Kligman has identified the differences and relationships between cosmetics and pharmaceutical drugs, hence the term cosmeceuticals. Products that lay on the spectrum between cosmetics and drugs still exert a pharmaceutical therapeutic effect, but not necessarily a systemic therapeutic effect. Despite these former mentioned effects, the difference of a drug to a cosmeceutical is that cosmeceuticals are not regulated by the U.S. Food and Drug Administration (FDA) and are therefore not subjected to all the requirements regarding the proof of safety and efficacy (Choi & Berson, 2006:163).

It remains of utmost importance to consider the choice of drug for transdermal delivery. The irritancy, sensitization and cutaneous metabolism must also be considered (Barry, 2007:591).

Scopolamine was previously formulated as a transdermal patch. This dosage form was used in the treatment of motion sickness and was also utilized by American astronauts. Various side-effects are reduced when delivering scopolamine transdermally i.e. hallucinations and confusion. Some of the side-effects were however turned into a positive. Woodwind instrumentalists suppress their salivation by administering a scopolamine patch (Barry, 2007:590). Although this administering of scopolamine seems favourable, the nature of a patient's skin will ultimately determine the effectiveness.

B.1 Donor phase solutions containing cyclizine or scopolamine

B.1.1 Preparation of donor phase solutions

A donor solution was prepared to subsequently determine whether a drug can be delivered transdermally. Equipment suitable for mixing and cooling must be available.

B.1.1.1 Formulation in phosphate buffer solution

A donor solution formula was obtained and a 0.5 % cyclizine and 1 % scopolamine was subsequently prepared. A phosphate buffer solution (PBS) at pH 7.4 functioned as the basis of the donor solution. It is important that the pH is maintained throughout the experiments. The ratio between ionised versus unionised drug was calculated at various pH solutions. Hence determining that pH 7.4 is adequate. The percentage of unionised drug was 81.7% and 72% for cyclizine and scopolamine respectively. When delivering a drug transdermally it is crucial that this ratio is satisfactory (described in Section 2.8.2.4). A homogenous solution was prepared. The ingredients used in the two formulations are listed below in Table B.1.1 and Table B.1.2.

Table B.1.1: Ingredients used in the cyclizine formulation

Ingredients	%m/m	Activity
A: Methyl paraben	0.20%	Preservative
Propyl paraben	0.04%	Preservative
BHT	0.20%	Anti-oxidant
B: Cyclizine	0.50%	Active ingredient
PBS (pH 7.4)	To 100%	Solvent/Buffer

Table B.1.2: Ingredients used in scopolamine formulation

Ingredients	%m/m	Activity
A: Methyl paraben	0.20%	Preservative
Propyl paraben	0.04%	Preservative
BHT	0.20%	Anti-oxidant
B: Scopolamine	1.00%	Active ingredient
PBS (pH 7.4)	To 100%	Solvent/Buffer

The procedure for both formulations was done using the same principles.

- Weigh the active ingredient and add it to the PBS
- Heat Phase A to 70°C
- Weigh Phase B and heat to 70°C
- Add both heated Phases A and B together
- Homogenize the solution at 13500 rpm until it has cooled down to approximately 40°C
- Swirl the solution to room temperature (approximately 25°C)

B.1.1.2 Formulation in Pheroid™

The same ingredients and procedure was followed, with the exception of adding the Pheroid™ ingredients and tocopherol. Tocopherol aids in the formation of vesicles where the drug is incorporated into.

B.2 Formulation of an emulgel containing scopolamine

B.2.1 Design of a drug formulation

B.2.1.1 Preformulation

The term preformulation literally means the step before formulation. It is crucial that the various chemical and physical properties are determined before a dosage form is developed. The feasibility, instability and bioavailability of a drug will usually be exposed during this period. Important pointers in terms of suitability of various excipients can also be obtained.

There is an emergent belief that many cosmetics will one day be replaced with products with advanced technology and superior delivery systems (Wells & Aulton, 2007:337). The Pheroid™ technology being one of the first steps in accomplishing this goal.

B.2.1.2 Formulation of a gel

By definition, a gel is a two-compartment formulation containing a large amount of organic molecules interpenetrated by water and a small amount of emulsified lipids (Radulescu *et al.*, 2010). A three dimensional matrix is built by a synthetic or natural polymer in a hydrophilic liquid (Barry, 2007:593).

B.2.1.2.1 Function and purpose of a gel

Gels are vastly accepted as a pleasant dosage form. It consists of a two-component semi-solid system that is affluent in liquid. Solid-like properties are provided by the presence of a continuous structure, thus one of the main characteristics. A gel is usually soothing and easy to

apply and has a moist and cooling feel. Gels can also act as moisturizers, make-up removers and cleanser (Barry, 2007:593).

The main criteria for any dermatological formulations can be divided into various factors including the following:

- The stability of both the active ingredient and the adjuvants
- The rheological properties
- Evident pH
- Phase changes
- Particulate contamination (Barry, 2007:595).

B.2.1.2.2 Main ingredients of a gel

The main ingredients of a gel include preservatives, humectants, surfactants and perfumes (Mitsui 1997:351). It consists of a polymer, soluble in water for example methyl cellulose, providing the gel with its structure. Gels can vary from transparent to a cloudy colour, depending on the formulation. When preparing an oily gel, it is of utmost importance to ensure the compatibility of the oils. The liquid crystal structure and gelling ability of a surfactant will ultimately determine the appearance of an oily gel. A vast knowledge of preservation, safety and the ingredients is required before preparing a gel.

B.2.1.2.3 Rheology

Gels and emulsions normally exert non-Newtonian flow.

B.2.2 Method for fabricating an emulgel and Pheroid™ emulgel

Lopez-Cervantez *et al.* (2009:1) defined an emulgel as an oil-in-water (O/W) emulsion integrated in a gel formulation.

The droplet size of a formulation is directly proportionate to the viscosity and the rate of creaming in an emulsion. A homogenizer is used to attain a uniform product and smaller globules of the dispersed phase. Typically the oil phase is added to the water phase. Any solid or semi-solid ingredients are melted before compounding. The water phase should subsequently be heated to the same temperature as the oil phase in order to prevent premature solidification. When combining the two phases, it is important to keep on stirring until the emulgel is cooled. This ensures that demulsification doesn't occur (Billany, 2007:404). Equipment suitable for filtering, transporting and cooling should be available whilst

manufacturing an emulgel. One of the main focus points of a gel is the visual appearance, the transparency should also be noted, and the distribution of ingredients should be consistent.

B.2.2.1 Formulation of Scopolamine containing emulgel and Pheroid™ emulgel

A basis gel formulation was obtained and the active ingredient was added. The amounts were later altered and some ingredients were substituted with other similar ingredients; in order to produce an emulgel with a homogeneity, stability and attractive appearance.

The final ingredients used in the formulation are listed below in Table B.2.1.

Table B.2.1: Ingredients used in emulgel formulation

Ingredients	%m/m	Activity
A: Liquid paraffin	20.00%	Oil phase of emulsion
Span 60	0.50%	Emulsifier
Tween 80	4.50%	Surfactant
Methyl paraben	0.40%	Preservative
Propyl paraben	0.08%	Preservative
BHT	0.20%	Anti-oxidant
B: Xanthan gum	1.50%	Gelling agent
Scopolamine	1.00%	Active ingredient
dH ₂ O	To 100.00%	Solvent

The procedure in preparing the emulgel is as follows:

- Weigh the active ingredient and add it to the water
- Heat until 40°C
- Add the xanthan gum slowly to the heated mixture
- Homogenize at 777 rpm
- Heat the mixture to 80°C
- Weigh the rest of the ingredients of Phase A and heat to 80°C
- Add the heated Phase A to the heated Phase B whilst homogenizing at 13500 rpm until 40°C
- Stir occasionally and let it set over night at approximately 25°C

B.2.3 Pheroid™ emulgel

The exact same procedure was followed as described except for the Pheroid™ ingredients added to the formula. Tocopherol was also incorporated into this formulation to aid the formation of vesicles that encapsulate the drug.

B.3 Outcome

Both emulgel formulations were the same in terms of feel and appearance. The emulgel formulations were manufactured in sufficient quantities in order to perform stability studies (discussed in Appendix C).

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APPENDIX C

Stability Testing of an Emulgel

C.1 Introduction

Stability can be defined as the process of ensuring the acceptability of a products key attributes. It is crucial to assess the change of these characteristics and the degree of change thereof. Consequently, it must be decided as to what constitutes acceptable degradation. Before this step is possible, one should have a common knowledge about the use and the chemical composition of the product (Masmoudi *et al.*, 2005:118).

Stability testing acts as an early warning system that can make one aware of potential formulation problems. This forms part of the trial and error in the formulation process. Looking from a profitability point of view, it is of utmost importance to market a new product as swiftly as possible. However, before a product can be launched, it must demonstrate stability over at least seven months at various temperatures and be able to endure many external influences (Masmoudi *et al.*, 2005:118). Cosmeceutical products should be aesthetically pleasing and instabilities like discolouration and odour are sure to be noticed by the consumer (Romanowski & Schueller, 1999:115).

One has to decide what the most important factors are (for example appearance or active ingredient characteristics) to be able to determine the required tests (Romanowski & Schueller, 1999:115).

Stability tests can be divided into two main criterions, namely:

- Physical stability:
This consists of the production of an emulsion of small droplet size, breaking, cracking, separation and change in viscosity.
- Chemical instability:
This consists of microbiological contamination, odour, staining and discolouration.

If there are no noticeable instabilities found, the formulations are stored at different temperatures and humidities, respectively. This accelerates the degradation process, and thus speeds up the stability testing of a formulation (Masmoudi *et al.*, 2005:118).

In this study, the two formulations (described in Appendix B) were stored at 25°C/60% RH (relative humidity), 30°C/60% RH and 40°C/75% RH for 3 months to be analysed at month 0, 1, 2 and 3.

The formulations were inspected for noteworthy changes. These changes can be defined as the following:

1. A 5% change in the ingredients from its original value.
2. Degradation exceeding the acknowledged criterion.
3. Not meeting the expected criteria in terms of physical appearance, colour, cracking etc.
4. Failing to meet the criteria for pH (ICH, 2003:9).

C.2 Emulgel stability tests

Emulsions are some of the most common delivery systems used in cosmetic products. Many ingredients can easily be incorporated into an emulsion. Unfortunately, this dosage form is known to be a rather unstable formulation, thus the emphasis on the stability tests being carried out (Masmoudi *et al.*, 2005:118).

Stability tests carried out in this study were as follows:

- Assay of concentration of the active ingredient
- Viscosity
- pH
- Assessment of the mass variation
- Confocal laser scanning microscopy (CLSM)
- Particle size
- Zeta-potential

C.2.1 Assay of concentration

The concentration of methyl paraben, propyl paraben, tocopherol, scopolamine and BHT were determined in the formulations by means of HPLC analysis. The validation of the HPLC method is described in Appendix A. The reduction of the drug concentration down to 90% is still deemed acceptable (Barnes, 2007:650).

C.2.1.1 Chromatography

The same equipment, analytical column, chromatographic conditions, mobile phases and gradients were used as discussed in Section A.2.1. The retention time of the ingredients are given in Table C.1.

Table C.1: Retention times of the ingredients

Ingredient	Time (min)
Methyl paraben	6.01
Propyl paraben	7.17
Scopolamine	7.20
BHT	11.14
Tocopherol	20.09

C.2.1.2 Standard solutions

The standard solution consisted of the following ingredients:

- Methyl paraben 4 mg
- Propyl paraben 10 mg
- Scopolamine 10 mg
- BHT 2 mg
- Tocopherol 2 mg

All the ingredients, except propyl paraben, were accurately weighed together in a 50 ml volumetric flask and filled to volume with methanol. Propyl paraben was weighed and diluted with methanol in a 100 ml volumetric flask. Thereafter this solution (8 ml) was extracted with a pipet and added to the other ingredients to obtain the accurate concentration relation identical to that of the formulation.

The standard solution was analysed on the HPLC in triplicate.

C.2.1.3 Sample preparations

One gram of each of the emulgel and Pheroid™ emulgel were accurately weighed in a 50 ml volumetric flask and filled to volume with methanol/HPLC water. This action was repeated in duplicate. The samples were injected in the HPLC in duplicate in order to determine the ingredients accurately.

C.2.2 pH

When considering the pH of a product, it is important to take the ratio of ionised versus unionised molecules of the active ingredients into account. Unionised molecules penetrate the stratum corneum readily (Section 2.6.2.2). The stratum corneum is amazingly resistant to the variation in pH and can tolerate a range of 3-9 (Barry, 2007:565). The pH of the emulgel formulations were maintained at 7.4. This pH range ensures that more than half of the molecules are unionised in the emulgel formulations (28.0112 % ionised molecules versus 71.988 % unionised). The Mettler Toledo Inlab® 410 electrode pH-meter was utilized and was tested in duplicate.

C.2.3 Viscosity

Rheology describes the flow of a material in its constituent phase. The rheology is one of the most prominent features of an emulsion, taking into account its high viscosity in comparison with its constituent phases. Emulsions are known to exhibit non-Newtonian fluid characteristics. This means that when placed under stress, a solid-like behaviour is exposed.

Viscosity, defined as the resistance to flow due to internal friction, is the beginning of the assessment of a formulations rheology. The Brookfield viscometer (Model DV – II+, Stoughton, Massachusetts, USA) was utilized when measuring the formulations' viscosity. The most accurate spindle is selected to ensure that an optimum torque exists. The spindle then turns at a selected rate, measured in rotations per minute (rpm). A constant temperature of 25°C was maintained by placing the formulation in a water bath set to temperature. As the spindle turns at the selected speed, a reading is taken every ten seconds until 32 readings are obtained. The viscosity is subsequently calculated. A high viscosity is required initially to maintain the emulsions physical stability. With the decrease in viscosity, creaming often occurs especially when the formulation comprises over low oil content. It is crucial to remember that the consumers' or patients' acceptability of a product is of utmost importance in a competitive market. The formulation should therefore be easily retained from its container (Billany, 2007:391).

C.2.4 Confocal laser scanning microscopy

CLSM samples were prepared by weighing 0.1 g of each of the emulgel and Pheroid™ emulgel samples in an Eppendorf vial. Nile red dye (2 µl) was added with water (500 µl). The samples (25 µl) were placed on a microscope plate and closely looked at under the CLSM. A photograph was taken of the samples during the stability tests and the CLSM micrographs were inspected for variations.

C.2.5 Visual appearance

Initially the appearances of both emulgel formulations were homogenous, white in colour and non-greasy. The appearance of each emulgel was inspected for variance during stability testing. The formulations' colour was matched to a graded white colour chart obtainable from paint stores. Photos were taken and matched up to the previous months.

C.2.6 Mass variation

The mass of each formulation was initially determined on the Shimadzu scale. The scale was regularly calibrated to ensure accuracy when determining the variation in mass. The same container was again used at month 1, 2 and 3 and compared to the previous values. The loss of mass usually indicates water loss from the formulation.

C.2.7 DT-1200 used for particle size and zeta-potential determination

C.2.7.1 Background

The DT-1200 offers a great advantage over other methods due to it being able to record readings on undiluted, concentrated emulsion samples (Hsu & Nacu, 2002:375). Both the zeta-potential and the distribution of an emulsion drop size were calculated using the DT-1200. The sound speed and the attenuation were measured making use of acoustic spectroscopy. This acoustic sensor can also be utilized when measuring the viscosity of both non-Newtonian and Newtonian liquids. Colloidal vibration on the other hand is measured by electro-acoustic spectroscopy. When determining the zeta-potential of the emulsion, the colloidal vibration is taken into account. The DT-1200 is able to generate ultrasound attenuation frequency spectra, ranging from 1-100 MHz. This is utilized when determining the distribution of a drop size. Electro-acoustic spectroscopy and acoustic spectroscopy can not be viewed as total separates, as the accurate calculation of the zeta-potential requires information about the drop size (Takeda *et al.*, 1998:273). The DT-1200 software automatically calculates the droplet size distributions. The thermal losses and intrinsic losses are both taken into account. Droplet size is independent on the intrinsic losses, but is thought to be thermal sensitive (Dukhin & Goetz, 2002:282).

.2.7.1.1 Acoustic theory

The ideal acoustic theory would incorporate the following factors:

1. Reflect colloids using the various mechanisms of ultrasound interactions, for example viscous, structural and electro-kinetic interactions;
2. The theory must apply for a big range of particles (10 nm-1 mm);
3. It may be utilized in concentrated suspensions, therefore taking particle interactions into consideration.

One of the most common acoustic theories is the ECAH (Epstein, Carhart, Allegra and Hawley) theory. Although it is widely used, it fails to comply with numerous of these factors, making it far from ideal (Epstein & Carhart, 1953:553-565).

C.2.7.1.2 Electro-acoustic theory

O'Brien proposed an alternative method when determining the electro-acoustic phenomena, namely Colloid Vibration Current /Potential (CVI). This method can be utilized when determining the electro-acoustic properties in both concentrated and diluted suspensions (O'Brien, 1988:71-86).

C.2.7.1.3 Model theory

As with any theory, a dispersed particle should be modelled in terms of chemical and physical properties. Poli-dispersity for example the variation of one particle to another should also be taken into account. Despite the diverse properties of a particle and its medium, the acoustic and the electro-acoustics may still be determined

Due to the heterogeneous particles, it is crucial to use the relative density of a sample when calculating its properties (Dukhin & Goetz, 2002:28).

C.2.7.1.4 Particle size

In colloid systems, the particle size in sample varies, thus making it a poli-dispersed system. Both the particle distribution and particle size has to ensure that particles will neither aggregate nor form a sediment, causing the suspension to be non-uniform (Leschonski, 1984:89-95).

C.2.7.1.5 Zeta-potential

Scientifically, the zeta-potential can be defined as the electro-kinetic potential in a colloidal system. When considering it theoretically however, there is a necessity to consider the electro-chemical aspects of the interfacial layer and the double layer. Figure C.1 represents a schematic portrayal of the zeta-potential.

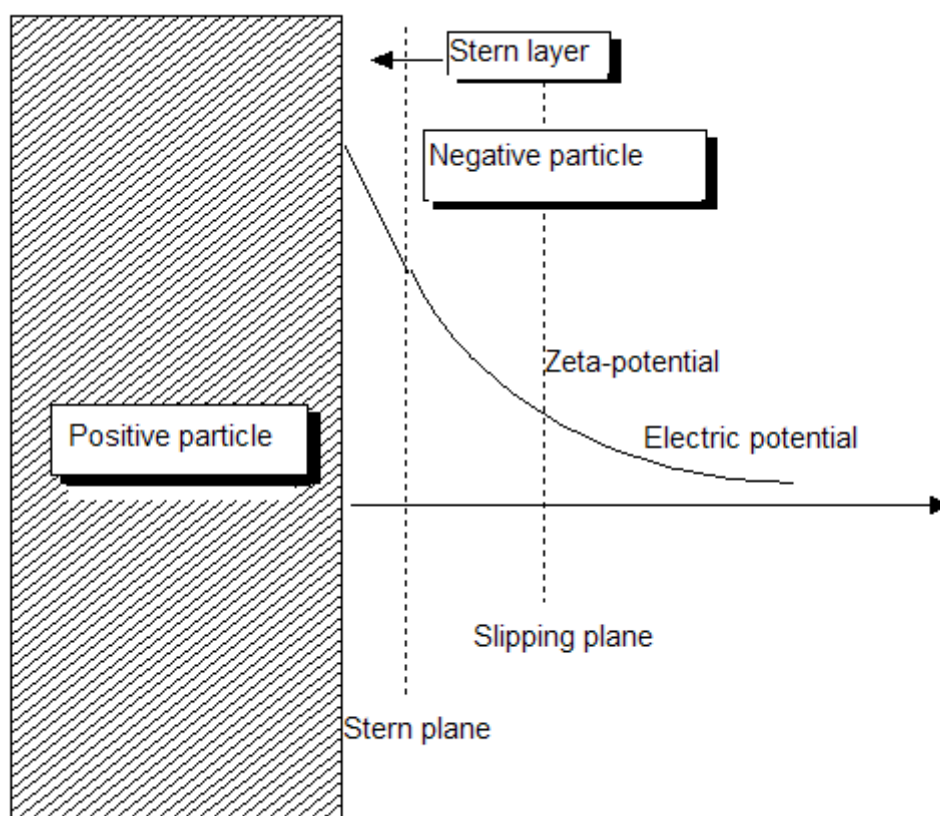


Figure C.1: Schematic representation of zeta-potential (Dispersion Technology Inc. 2010).)

When a solid, exhibiting an electric charge, comes into contact with an aqueous solution, comprising over both positively and negatively charged ions, the distribution of ions will be affected. The electrical double layer is subsequently formed, indicating the distribution of ions and the magnitude thereof. This layer is divided into two parts via the Stern plane. The Stern plane possesses the opposite charge to that of the particle. A balanced layer of unbound ions gather at the Stern plane. This layer together with the Stern plane is termed the double layer. A variance in potential is delivered as a result of the double layer generating a potential difference between the particles and solvent, termed the slipping plane. The potential at this plane is termed the zeta-potential (Attwood, 2007:77).

Zeta-potential is a key factor when determining the stability of a colloidal dispersion, as it indicates the degree of repulsion between the adjoining, similarly charged particles. The magnitude of the zeta-potential is therefore a crucial factor. A force more negative than -30 mV and more positive than +30 mV is usually considered to be stable. Should the zeta-potential be too low, there will be no force exerted to prevent the particles from separating, resulting in dispersion instabilities.

pH is one of the most important factors that affect the zeta-potential. The more alkali the emulsion is, the more negative the zeta-potential becomes and the more acid an emulsion is, the more positive the zeta-potential becomes.

Previously, the zeta-potential was measured with micro-electrophoresis or other electro-kinetic phenomenon. With the advance in technology, the zeta-potential may now be determined with the Dispersion Technology 1200 (DT-1200) (Hsu & Nacu, 2002:375).

C.2.7.2 Determining the droplet size of an emulsion

The acoustic sensor of the DT-1200 enabled us to determine the particle size determination in an emulsion without dilution. The density of the emulgel was firstly determined by weighing 10 ml of the emulgel in a volumetric flask. The particle size was determined at 120% wt (weight). 120 ml of the emulgel was subsequently emptied into the sample chamber. This chamber incorporates a built-in magnetic stir bar. Continuous mixing prevents the emulgel from settling. The DT-1200 software calculates the droplet size distribution automatically. Attenuation is interpreted as a combination of thermal losses and intrinsic losses. Droplet size is independent on the intrinsic losses. Thermal losses, however, is droplet size sensitive (Hsu & Nacu, 2002:375-376). Three consecutive measurements were taken and an average particle size was determined. A variation coefficient of under 1-2% is usually viewed as acceptable (Wells & Aulton, 2007:355). The measurement time for a sample was 10 min. There is still an ongoing dispute about the influence of droplet size on the penetration of an emulsion through the skin. Various studies still indicate that penetration is dependant on the droplet size (Schwarz *et al.*, 1995:687-692).

C.2.7.3 Determining the zeta-potential of an emulsion

Zeta-potential and particle size are key factors in the stability of emulsions. These factors are affected by the manufacturing technique, oil/water ratio and concentration to a great extent. The colloidal vibration current supplies the zeta-potential. In order to accurately determine the zeta-potential, information about the drop size is required (Hsu & Nacu, 2002:374-375) and was therefore determined before hand (see Section 2.7.2). Three consecutive readings were taken

and the average zeta-potential was calculated. Tables C.10 and C.11 demonstrate the zeta-potentials of the emulgel formulations.

C.3 Results

C.3.1 Assay concentration

Table C.2: The percentages of active ingredients present in emulgel

Ingredient	Temperature	Month			
		0	1	2	3
Scopolamine	25	97.0	100.0	96.8	90.9
	30	-	96.6	95.7	90.5
	40	-	98.4	95.7	89.4
Methyl paraben	25	95.8	99.2	97.6	69.9
	30	-	95.5	95.7	70.3
	40	-	97.4	96.2	68.0
Propyl paraben	25	98.6	100.4	90.7	94.9
	30	-	97.5	90.4	92.2
	40	-	99.1	92.7	91.1
BHT	25	98.8	96.5	33.5	41.8
	30	-	95.4	58.4	37.2
	40	-	78.4	37.0	36.0

Scopolamine remained in the accepted range of 90-110% when exposed to 25 °C/60% °RH, 30 °C/60% RH and 40 °C/75% RH for the stability test period of 3 months. Methyl paraben and BHT did not remain in the accepted range after the duration of 3 months. Propyl paraben remained in the accepted range after the 3 months stability test period. The decrease in the preservative, methyl paraben, and the anti-oxidant, BHT, may be due to the protection of scopolamine. The biggest variation of actives was observed in the emulgel exposed to 40 °C/75% RH.

Table C.3: The percentage of active ingredients present in emulgel with Pheroid™

Ingredient	Temperature	Month			
		0	1	2	3
Scopolamine	25	97.3	98.5	96.6	87.9
	30	-	95.0	94.8	94.9
	40	-	96.9	71.7	68.7
Methyl paraben	25	98.0	91.3	87.3	62.6
	30	-	88.1	85.6	65.9
	40	-	88.6	71.7	66.6
Propyl paraben	25	97.6	92.2	89.5	82.6
	30	-	89.1	85.5	88.2
	40	-	90.7	73.0	71.6
BHT	25	98.3	93.1	59.6	37.4
	30	-	78.3	31.6	46.9
	40	-	43.6	27.9	26.8
Tocopherol	25	97.3	73.9	19.9	0
	30	-	53.6	20.1	0
	40	-	41.3	17.8	0

The concentration of scopolamine in the emulgel with Pheroid™ decreased in the 3 months stability test period. The scopolamine concentration is no longer in the acceptable concentration range (90-110%). The concentration of methyl paraben, propyl paraben, BHT and tocopherol decreased significantly over the 3 month period. The decrease in the actives exposes instability in the formulation. The biggest decrease of active ingredients can be observed in the emulgel with Pheroid™ exposed to 40°C/75% RH.

When compared to the emulgel, the active ingredients of the emulgel containing Pheroid™ decrease more significantly. The emulgel containing Pheroid™ exposes more instability in the formulation.

C.3.2 pH

C.3.2.1 pH for emulgel

Table C.4: pH values for emulgel formulation over 3 months

Storage temperature	Initial pH	Month 1	Month 2	Month 3	Mean	*SD	**RSD
25 °C/60% RH	5.16	5.21	5.14	5.06	5.14	0.06	1.19
30 °C/60% RH	5.16	5.14	5.00	4.87	5.00	0.11	2.20
40 °C/75% RH	5.16	4.98	4.68	4.55	4.79	0.18	3.78

*SD refers to the standard deviation

**RSD refers to the relative standard deviation

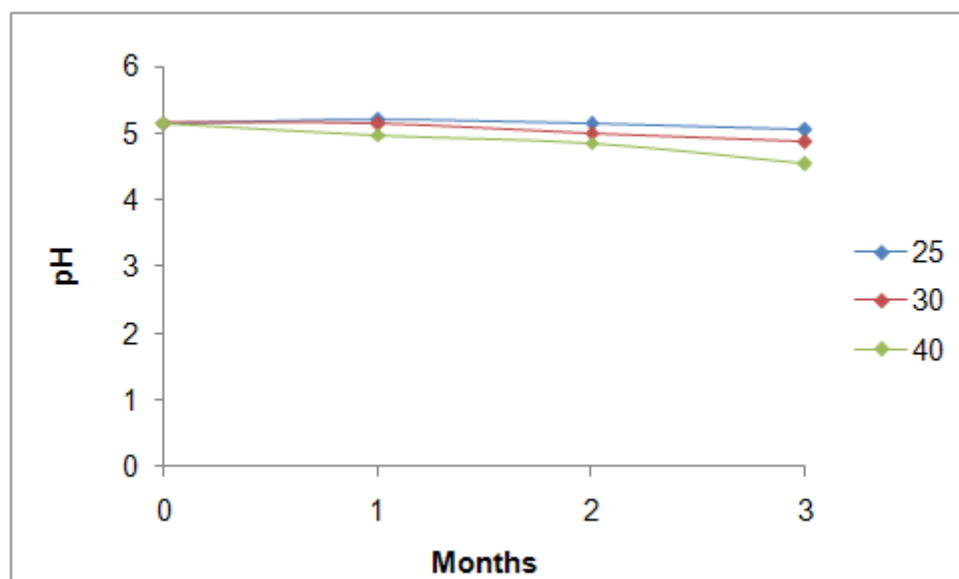


Figure C.2: pH of emulgel over 3 months

During the first month of stability testing, the pH of the emulgel exposed to 25 °C/60% RH increased from the initial value. This might be explained by the formulation still having to set. The pH of the emulgel formulations decreased in months 2 and 3 with a value of ± 0.7 . The emulgel formulations exposed to the higher temperatures, 30 °C and 40 °C, revealed a greater decrease in pH over the 3 months. The emulgel exposed to 40 °C proved to be the least unstable. Even though the pH decreased over the stability test period, the pH remained in the accepted pH range of 3-9 and would still be viewed satisfactory to use as a topical preparation. The active ingredients may however be influenced and become unstable when exposed to a varying pH.

C.3.2.2 pH for emulgel containing Pheroid™

Table C.5: pH values for emulgel formulations containing Pheroid™ over 3 month's stability test period

Storage temperature	Initial pH	Month 1	Month 2	Month 3	Mean	*SD	**RSD
25 °C/60% RH	5.34	5.23	5.21	4.97	5.14	0.12	2.34
30 °C/60% RH	5.34	5.22	5.06	4.84	5.01	0.19	3.73
40 °C/75% RH	5.34	4.97	4.74	4.48	4.76	0.21	4.36

*SD refers to the standard deviation

**RSD refers to the relative standard deviation

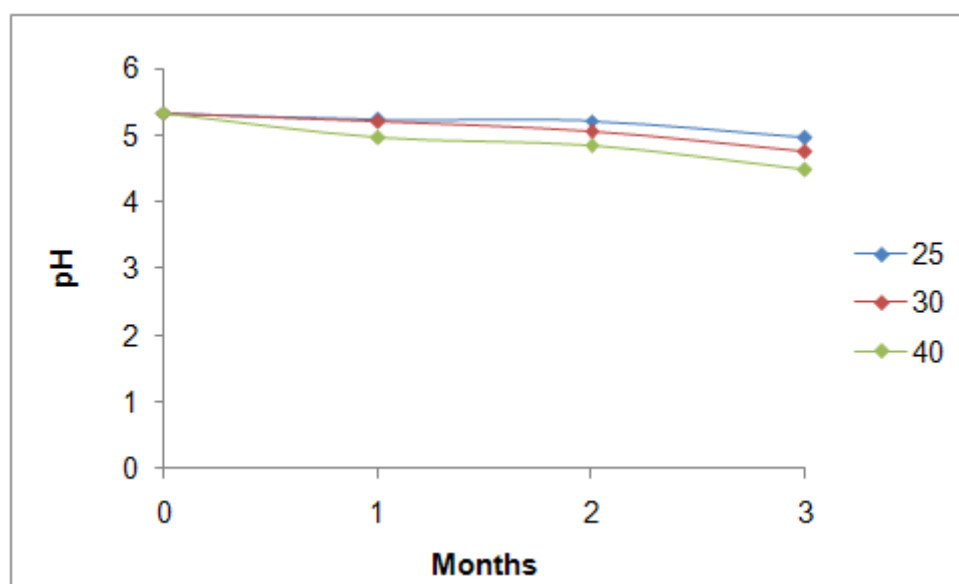


Figure C.3: pH of emulgel containing Pheroid™ over 3 months

The emulgel containing Pheroid™ exposed to 25 °C/60% RH revealed the least significant decrease in pH (± 25). The emulgel with Pheroid™ exposed to 40 °C revealed the most significant decrease in pH over the 3 months stability test period. In order to be topically accepted, the pH of a formulation should be within the pH range of 3-9. The emulgel formulations are thus still in the accepted criteria. A decrease in pH might reveal instabilities in the formulation.

C.3.3 Viscosity

C.3.3.1 Viscosity of Emulgel

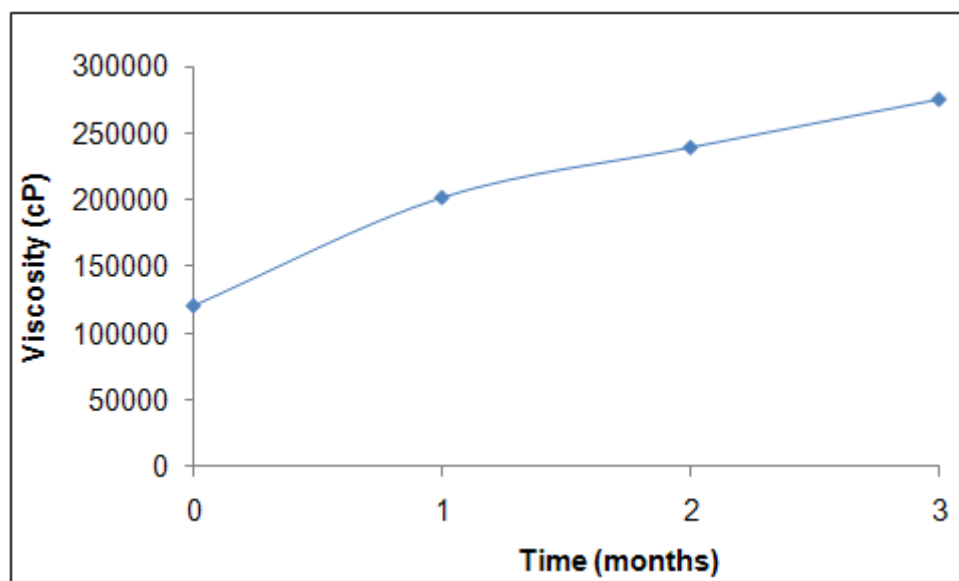


Figure C.4: Viscosity (cP) of emulgel after 3 months

The emulgel formulations exposed to 25°C/60% RH for 3 months was analysed for any change in viscosity. An increase of approximately 150000 cP was observed, indicating instability. This might be attributed to the emulgel still having to reach its settling stage. The emulgel revealed rheopetic behaviour (viscosity increasing with time). The change might be attributed to evaporation of water during the of the stability testing. The time of recovery of an emulgel is dependant on the Brownian motion. The structure of an emulgel might not recover at all after being exposed to stress, consequently increasing the viscosity of a system (Mariott, 2007:51).

C.3.3.2 Viscosity of emulgel containing Pheroid™

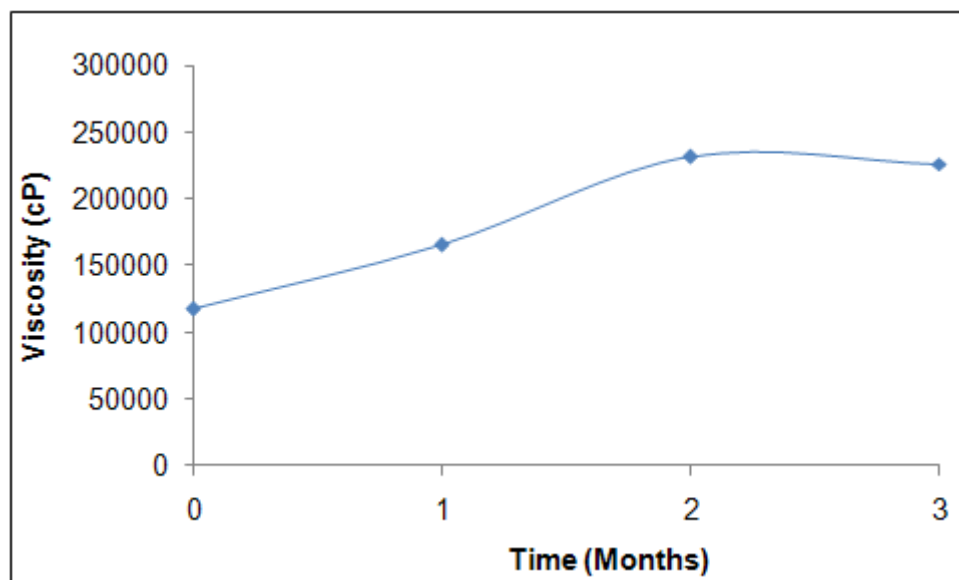


Figure C.5: Viscosity (cP) of emulgel containing Pheroid™

The viscosity of the emulgel with Pheroid™ exposed to 25°C/60% RH increased at month 1 and 2. The viscosity started to decrease at month 3. When compared to the initial viscosity of the formulation, the value of month 3 differs with approximately 100000 cP. This might be attributed to the product still having to reach its settling stage. The formulation reveals instability.

C.3.4 Confocal laser scanning microscopy (CLSM)

Figure C.6 illustrates the CLSM micrographs of the emulgel after the duration of 3 months stability test period. The particle size of the scopolamine gel changed significantly over the 3 months stability period. The emulgel exposed to 40 °C/60% RH displayed the biggest change in particle size. The emulgel formulations were no longer homogenous, proposing a problem with varying concentration of the active ingredient when the emulgel is applied.

3.5.1 Confocal laser scanning micrographs for scopolamine gel

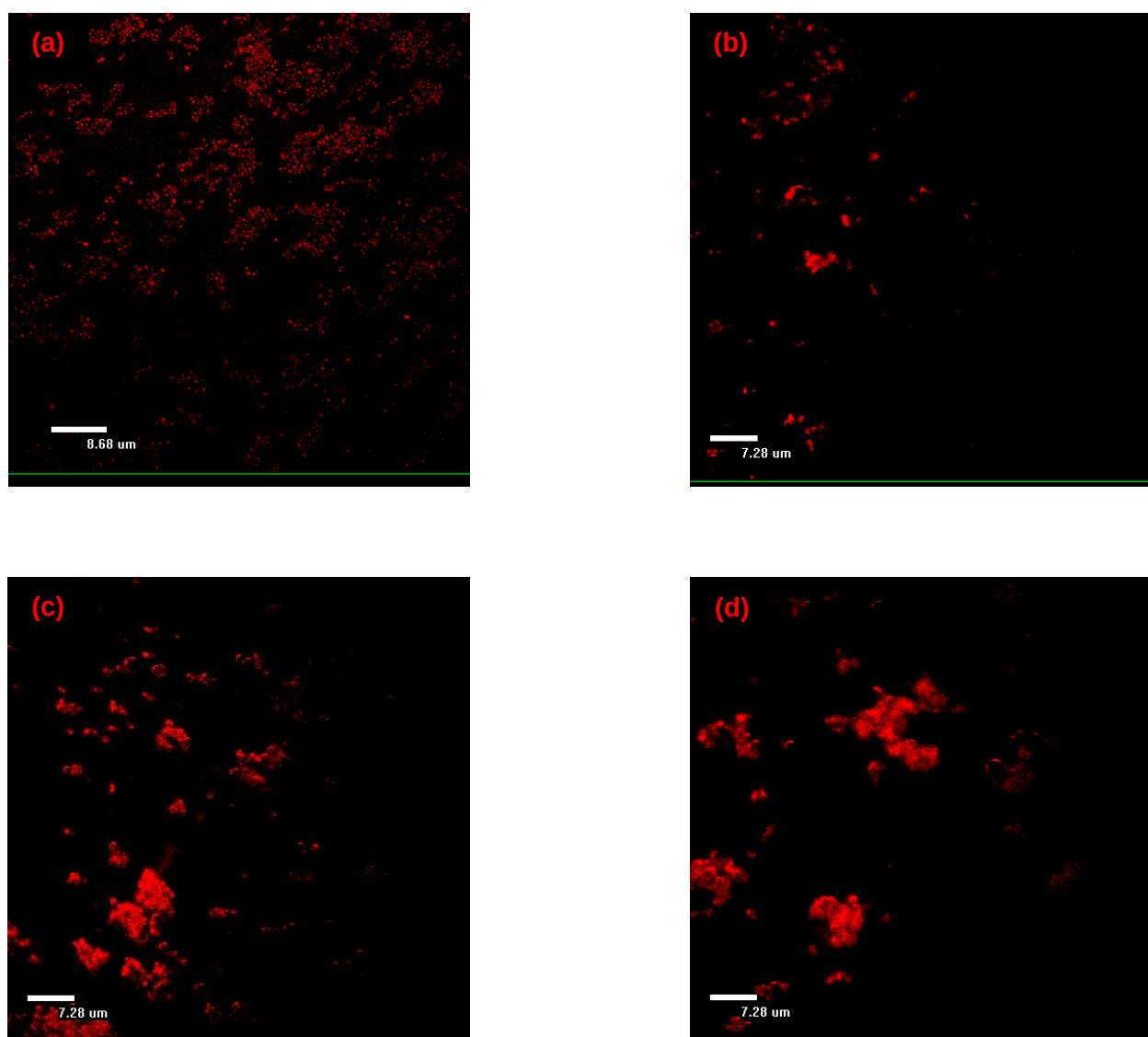


Figure C.6: Representation of confocal laser scanning micrographs of scopolamine gel after 3 months with **(a)** being the initial appearance, **(b)** at 25 °C/60% RH, **(c)** at 30 °C/60% RH and **(d)** at 40 °C/75% RH

3.5.2 Confocal laser scanning micrographs for scopolamine gel containing Pheroid™

After the 3 month stability test period, the emulgel formulations containing Pheroid™ exposed a significant change in particle size. The emulgel containing Pheroid™ was no longer homogenous and could propose a problem with varying concentrations of the active ingredients.

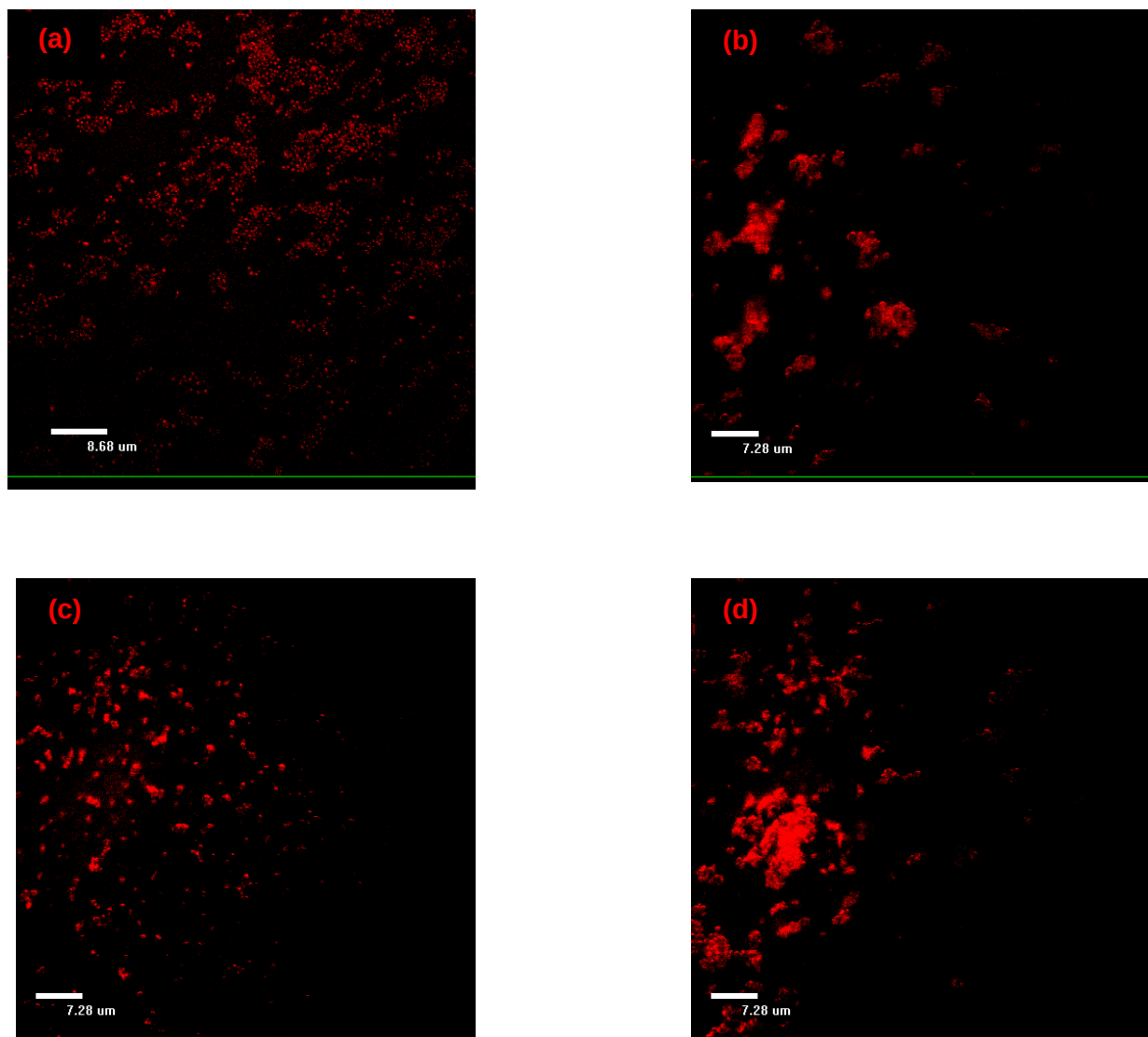


Figure C.7: Representation of confocal laser scanning micrographs of scopolamine gel containing Pheroid™ after 3 months. (a) illustrates the initial appearance, (b) at 25 °C/60% RH, (c) at 30 °C/60% RH and (d) at 40 °C/75% RH

C.3.6 Visual appearance

No significant change was observed in the emulgel exposed to the various temperatures. When compared to the colour chart, the emulgel formulations maintained a brilliant white colour (Figure C.8). The emulgel formulations with Pheroid™ exposed to temperatures 25°C/60% RH and 30°C/60% RH maintained a brilliant white colour (Figure C.9). The colour of the emulgel with Pheroid™ exposed to temperature 40°C/75% RH changed from a brilliant white colour to luxurious silk timeless at month 2 to a vanilla mist 4 colour at month 3. The emulgel with Pheroid™ consequently exhibits instability in the formulation and could propose a problem with the acceptability from the consumers.

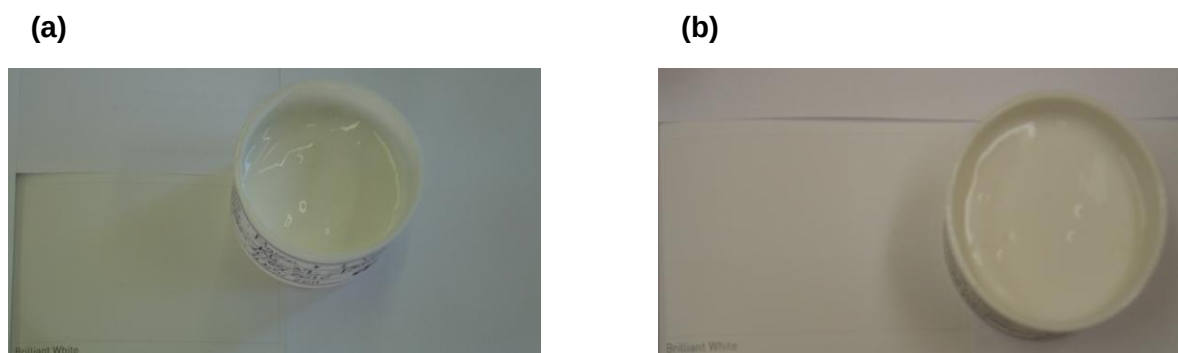


Figure C.8: Illustration of visual appearance of emulgel. **(a)** represents the initial visual appearance of emulgel formulations exposed to 25°C/60% RH, 30°C/60% RH and 40°C/75% RH and **(b)** represents the visual appearance of emulgel formulations exposed to 25°C/60% RH, 30°C/60% RH and 40°C/75% RH after 3 months

(a)



(b)



(c)



Figure C.9: Illustration of visual appearance of emulgel containing Pheroid™. **(a)** represents the initial visual appearance of emulgel formulations exposed to 25°C/60% RH, 30°C/60% RH and 40°C/75% RH and **(b)** represents the visual appearance of emulgel formulations exposed to 25°C/60% RH, 30°C/60% RH after 2 months and **(c)** represents the visual appearance of emulgel with Pheroid™ after 3 months exposed to 40 °C/75% RH

C.3.7 Mass variation

Table C.6: Mass variation of emulgel over 3 months

Emulgel	Initial (g)	Month 1 (g)	Month 2 (g)	Month 3 (g)	Average (g)
25°C/60% RH	50.32	53.28	53.02	52.38	52.89
30°C/60% RH	53.65	49.95	49.70	49.63	50.63
40°C/75% RH	49.62	53.28	49.70	49.03	48.84

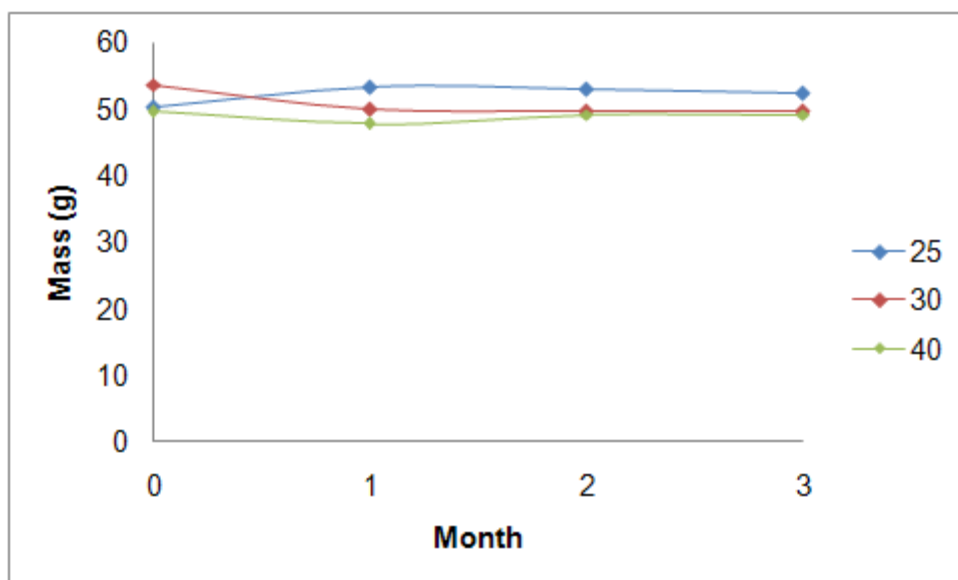


Figure C.10: Illustration of pH of emulgel over 3 months

No significant change was observed over the 3 month stability test period. The loss in mass is mostly attributed to the water loss due to the temperatures. The emulgel exposed to 40 °C revealed the biggest variation in mass due to the higher temperature.

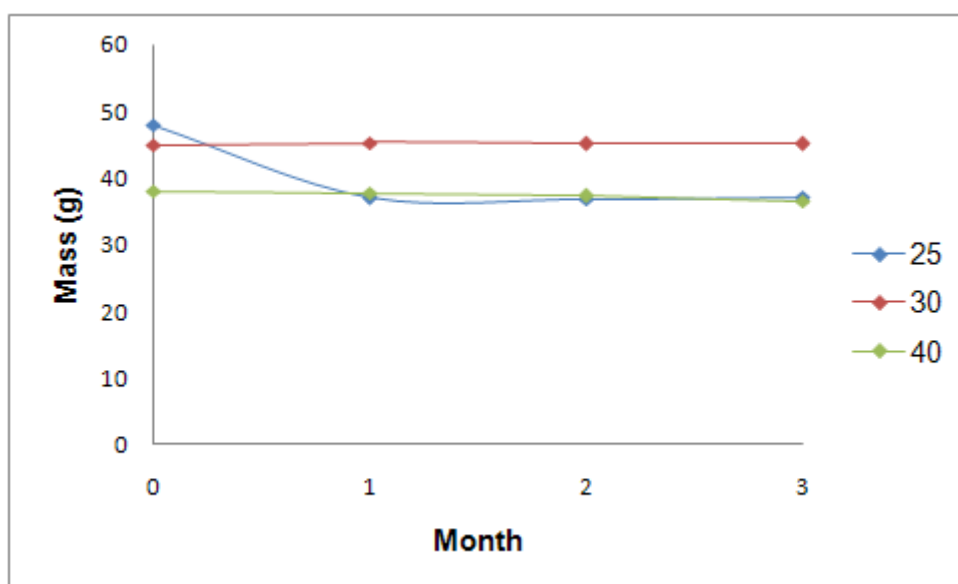


Figure C.11: Illustration of pH of emulgel containing Pheroid™ over 3 months

Table C.7: Mass variation of emulgel with Pheroid™ over 3 months

Emulgel	Initial (g)	Month 1 (g)	Month 2 (g)	Month 3 (g)	Average (g)
25°C/60% RH	47.92	47.80	47.27	47.21	47.55
30°C/60% RH	45.06	45.14	45.14	45.10	45.14
40°C/75% RH	38.02	37.10	36.88	36.60	39.63

No significant change in mass was observed over the 3 month stability test period. The biggest variation was noted in the emulgel with Pheroid™ exposed to temperature 40°C/75% RH. The loss of mass is attributed to the exposure of the emulgel to the higher temperature.

C.3.8 Particle size

Table C.8: Emulgel particle size for 3 months

Month	Storage Condition	Size	Standard Deviation	Fitting Error
1	25°C/60% RH	89.974	0.05	32.17
	30°C/60% RH	90.5	0.05	26.03
	40°C/75% RH	90.3	0.05	32.5
2	25°C/60% RH	89.4	0.05	38.8
	30°C/60% RH	89.0	0.05	42.26
	40°C/75% RH	89.6	0.05	36.8
3	25°C/60% RH	89.148	0.05	34.06
	30°C/60% RH	89.148	0.05	36.83
	40°C/75% RH	89.296	0.05	42.50

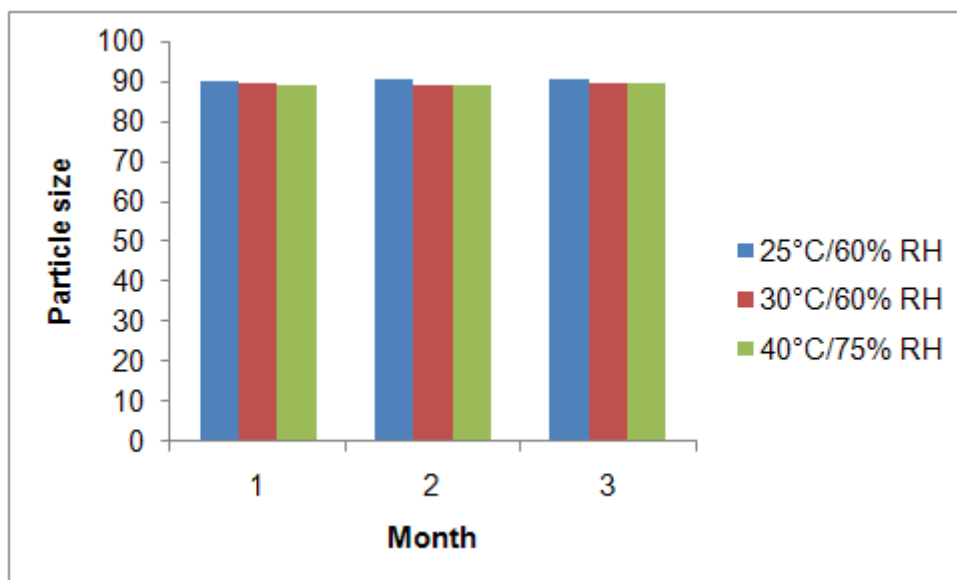


Figure C.12: Illustration of particle size over 3 months stability period.

The particle size of the emulgel formulations exposed to the different temperatures and RH, remained approximately the same for the duration of 3 months. The standard deviation was consistent over the 3 months. The fitting errors varied slightly. This can be attributed to a small fraction of large droplets that cause the levelling of the attenuation curve at low frequencies. Particle size is particularly important when formulating a transdermal dosage form. It is crucial that high potency, low-dose drugs be blended adequately and homogenous in a formulation. The acceptable variation coefficient ranges from 1-2%.

Table C.9: Particle size of emulgel containing Pheroid™ over 3 months

Month	Storage Condition	Size	Standard Deviation	Fitting Error
1	25°C/60% RH	90.2	0.05	26.97
	30°C/60% RH	90.2	0.05	26.97
	40°C/75% RH	89.8	0.053	24.4
2	25°C/60% RH	93.5	0.05	32.0
	30°C/60% RH	93.4	0.05	19.9
	40°C/75% RH	35.2	0.05	26.5
3	25°C/60% RH	72.7	0.05	22.3
	30°C/60% RH	38.8	0.057	22.6
	40°C/75% RH	46.74	0.053	23.5

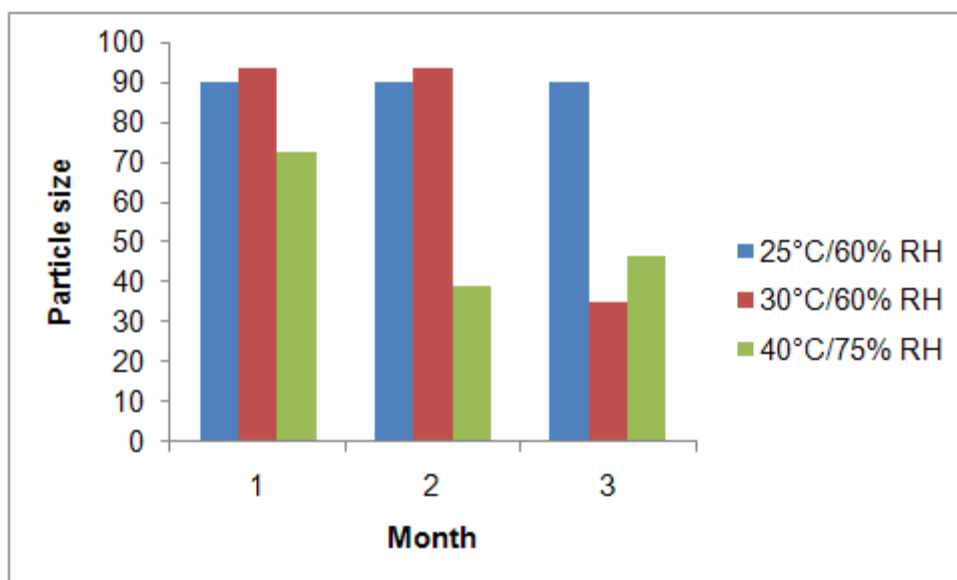


Figure C.13: Illustration of particle size of emulgel containing Pheroid™ over 3 months

The particle size of the emulgel formulations containing Pheroid™ exposed to the different temperatures and RH varied over the 3 months. This proposes a problem when formulating a transdermal preparation. The formulation is no longer homogenous and for low-dose, high potent drugs become potentially dangerous. The size of the particles does not adhere to the acceptable variation coefficient limit of 1-2%. The standard deviations showed no significant change over the 3 months. The fitting errors varied slightly over the 3 months, but showed no significant change. Due to a small fraction of large droplets, the attenuation curve is levelled at a low frequency, exhibiting a large fitting error.

C.3.9 Zeta-potential

Zeta-potential indicates the stability of a system. Particles comprise over either a positive or negative zeta-potential. The particles that are similarly charged repel each other. In an aqueous system, a charge of more than 30 mV or more negative than – 30 mV are generally viewed as stable. If all the particles have a high positive or negative charge, the particles repel each other, resulting in a stable dispersion system (Silver-colloids,2010).

Table C.10: Emulgel zeta-potential

Storage Temperature	Zeta-potential (mV)		
	Month 1	Month 2	Month 3
25°C/60% RH	-245676	-114128	-109393
30°C/60% RH	-254384	-129939	-107328
40°C/75% RH	-242847	-118420	-123508

The emulgel exhibited no significant change in zeta-potential over the 3 month stability test period. The large negative charge of the particles indicates that the particles greatly repel each other and result in a stable dispersion. The emulgel formulations are stable over a 3 month period.

Table C.11: Pheroid™ emulgel zeta-potential

Storage Temperature	Zeta-potential (mV)		
	Month 1	Month 2	Month 3
25°C/60% RH	18699	23900	846
30°C/60% RH	16122	23635	851
40°C/75% RH	15991	22163	883

The zeta-potential of the emulgel with Pheroid™ varied significantly over the 3 months. However, the zeta-potential of the particles maintained a positive charge large enough to repel the similarly charged particles successfully. The dispersion system is subsequently viewed as stable.

C.3.10 Conclusion

The formulations underwent a stability test period of 3 months. Significant changes were prominent in the formulations. The emulgel proved to be more stable than the emulgel containing Pheroid™. Changes in the emulgel containing Pheroid™ including colour change, viscosity increase and particle size variation were noted. Throughout the stability tests, it was apparent that neither of the formulations was completely stable and that the necessary criteria were not met.

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APPENDIX D

The Transdermal Delivery of Drugs Utilizing Franz cells

D.1 Introduction

Transdermal delivery of various drugs has become increasingly popular due to numerous advantages over other traditional delivery methods. Although this offers an attractive route, there are many factors limiting diffusion such as the size and aqueous solubility of a drug.

The likelihood of transdermal delivery of anti-emetics was highlighted by Hadgraft *et al.* (1995:286) and will be discussed later in this Appendix. The aforementioned included the prediction of scopolamine; hence many permeability studies have been conducted.

Emulsions are by far the most conventional method used to deliver many active ingredients. Some of the more sophisticated forms of the traditional emulsions include emulgel formulations. Emulgel formulations were developed to improve the stability of an active ingredient and enhance its appearance. It is cosmetically and pharmaceutically perceived as a pleasant formulation to apply topically (Magdassi & Touitou, 1999:1).

As previously stated, the transdermal delivery route has recently been recognized as one exhibiting great potential for drug delivery. It is crucial to develop an understanding of how the transdermal delivery of an active ingredient is influenced when incorporated in various formulations. The aim of this study was subsequently to determine whether cyclizine and scopolamine could be delivered transdermally with or without the use of Pheroid™.

D.2 Materials

Both cyclizine and scopolamine were identified according to the described methodology (see Appendix A.1). Scopolamine was formulated into an emulgel (see Appendix B) in order to determine the effect of an emulsion on an active ingredient and the delivery thereof. Actives were obtained from BASF (South Africa).

D.2.1 Phosphate buffer solution (PBS)

Potassium orthophosphate crystals (13.62 g) was dissolved in 500 ml fresh Milli-Q water and added to a solution prepared by dissolving sodium hydroxide pearls (3.1472 g) in 786.8 ml Milli-Q water. The pH was adjusted to 7.4 with phosphoric acid.

D.3 Methods

D.3.1 Physicochemical properties

D.3.1.1 Aqueous solubility

Three test tubes were filled with 5 ml Milli-Q water each. Undetermined amounts of drug were added to the water to compose an over-saturated solution. The test tubes were then submerged in a water bath set to a temperature of 32 °C and vigorously shaken to accelerate the equilibrium state. It was closely monitored over a period of 24 h to ensure that a saturated solution was maintained over this period where after it was analysed on the HPLC.

D.3.1.2 Octanol-buffer distribution coefficient (log D)

Equivalent amounts of PBS (pH 7.4) and *n*-octanol were combined. The mixture was shaken vigorously and left for 24 h to attain saturation. The two substances were separated and stored in different containers. A solution of cyclizine was prepared in the buffer phase due to its lipophilicity. Scopolamine was subsequently prepared in the saturated *n*-octanol because of its hydrophilic characteristics. These solutions (5 ml) were deposited in three test tubes and equal volumes of saturated-PBS or saturated-octanol were added. The test tubes were agitated for 24 h to blend the solutions, where after it was centrifuged for 30 min to separate the *n*-octanol and PBS solutions. Samples of both solutions were appropriately diluted, 1 ml of sample to 100 ml of PBS and analyzed on the HPLC. The ratio of drug concentration in both phases were determined and calculated.

D.3.2 Experimental manufacturing of solutions

D.3.2.1 Cyclizine

D.3.2.1.1 Cyclizine in PBS

Table D.1 demonstrates the ingredients used in the cyclizine solution.

Table D.1: Ingredients used in the 0.5 % cyclizine solution

Raw Material	Quantity (g)
PBS	99.06
BHT	0.20
Methyl paraben	0.20
Propyl paraben	0.04
Cyclizine	0.50
Total	100.00

D.3.2.1.2 Cyclizine with Pheroid™

Table D.2 illustrates the ingredients used in cyclizine solution containing Pheroid™.

Table D.2: Ingredients used in the 0.5 % cyclizine in Pheroid™ solution

Raw Material	Quantity (g)
Pheroid™ ingredients	3.80
dl- α -tocopherol	0.20
PBS	95.06
BHT	0.20
Methyl paraben	0.20
Propyl paraben	0.04
Cyclizine	0.50
Total	100.00

D.3.2.1.3 Placebo solutions for cyclizine

Ingredients used in placebo cyclizine solution are given in Table D.3.

Table D.3: Ingredients used in the placebo solution for cyclizine

Raw Material	Quantity (g)
PBS	99.56
BHT	0.20
Methyl paraben	0.20
Propyl paraben	0.04
Total	100.00

D.3.2.1.4 Placebo solutions for cyclizine with Pheroid™

Table D.4 illustrates the ingredients used in the manufacturing of placebo cyclizine solution containing Pheroid™.

Table D.4: Ingredients used in the placebo Pheroid™ solution for cyclizine

Raw Material	Quantity (g)
Pheroid™	3.80
dl- α -tocopherol	0.20
PBS	95.56
BHT	0.20
Methyl paraben	0.20
Propyl paraben	0.04
Total	100.00

D.3.2.2 Scopolamine

D.3.2.2.1 Scopolamine in PBS

Table D.5 illustrates the ingredients used to manufacture scopolamine solution.

Table D.5: Ingredients utilized in the 1 % scopolamine solution

Raw Material	Quantity (g)
PBS	98.56
BHT	0.20
Methyl paraben	0.20
Propyl paraben	0.04
Scopolamine	1.00
Total	100.00

D.3.2.2.2 Scopolamine with Pheroid™

Ingredients utilized in manufacturing scopolamine solution with Pheroid™ are displayed in Table D.6.

Table D.6: Ingredients utilized in 1 % scopolamine in Pheroid™ solution

Raw Material	Quantity (g)
Pheroid™	3.80
dl- α -tocopherol	0.20
PBS	95.56
BHT	0.20
Methyl paraben	0.20
Propyl paraben	0.04
Scopolamine	1.00
Total	100.00

D.3.2.2.3 Placebo solutions for scopolamine

Table D.7 demonstrates the ingredients used in the manufacturing of placebo scopolamine solution.

Table D.7: Ingredients utilized in the placebo solutions for scopolamine

Raw Material	Quantity (g)
PBS	99.56
BHT	0.20
Methyl paraben	0.20
Propyl paraben	0.04
Total	100.00

D.3.2.2.4 Placebo solution for scopolamine with Pheroid™

Ingredients supplied in Table D.8 are used in the manufacturing of placebo scopolamine solution containing Pheroid™.

Table D.8: Ingredients utilized in Pheroid™ placebo solutions for scopolamine

Raw Material	Quantity (g)
Pheroid™	3.80
dl- α -tocopherol	0.20
PBS	95.56
BHT	0.20
Methyl paraben	0.20
Propyl paraben	0.04
Total	100.00

D.3.3 Experimental batch manufacturing of emulgel formulations

Both a scopolamine emulgel with and without Pheroid™ were formulated as described in Appendix B.2.

D.3.4 Skin preparation

D.3.4.1 Skin preparation for solutions

Female Caucasian skin was obtained from various medical institutions performing abdominal plastic surgeries. Approval was attained from the Research Ethics Committee of the North-West University (reference number 04D08) as well as the patient herself. The skin was immediately frozen at -20°C after collection. Adipose tissue was removed with a blunt scalpel until the dermis was visible. Circles with a diameter of approximately 2 cm were punched out of the skin and inspected for any defects, for example stretch marks, blemishes or scars. It was then wrapped in foil and refrozen until utilized.

D.3.4.2 Skin preparation for emulgel formulations

The same procedure was followed in obtaining the female Caucasian skin (described in Appendix D.3.4.1). Not only the adipose tissue, but also the dermis and hypodermis, were removed from the skin with a blunt scalpel. The skin was submerged in a Grant water bath set to 60°C for 1 min. Making use of tweezers and a scalpel, the SC-epidermis was carefully removed from the skin. The SC-epidermis was placed on a filter paper and left to dry. Circles with a diameter of approximately 2 cm were cut out and inspected for defects and puncture holes. The circles were subsequently placed on a clean filter paper and frozen at -20°C until utilized.

D.3.5 Skin permeation studies

D.3.5.1 Skin permeation studies for solutions

Vertical Franz diffusion cells, with a receptor compartment (2 ml) and donor compartment (1 ml) were used (Figure D.1). Twelve Franz cells were utilized in this study. Ten were investigated for skin permeation and two functioned as the controls. A small amount of Dow Corning vacuum grease was applied on both the receptor and donor compartments before positioning the prepared skin circles with the SC facing upwards on the receptor compartment. The donor compartment was then replaced and a layer of vacuum grease was applied in order to make the Franz cells waterproof. Drug preparations were submerged in a water bath set to 32 °C and the PBS was placed in a water bath set to 37 °C until the preparations reached their temperatures, respectively. The donor compartments were filled with 1 ml of the drug preparation. A piece of Parafilm™ was placed on top of the compartments together with a cap in order to prevent evaporation of the preparations. For the two control-Franz cells, one was filled with 1 ml placebo preparation (see Appendix D.3.2) and the other with basic (containing no actives or preservatives) 1 ml PBS or Pheroid™, depending on the experiment done. The receptor

compartments were filled with 37 °C PBS and a magnetic stirrer bar was added. All the cells were clamped thereafter and placed on a Variomag magnetic stirrer plate (Figure D.2). The temperature of the Franz cells were constantly monitored and maintained at 37 °C to resemble the human body. It was determined that that when the Franz cells were placed in a water bath set to 37 °C, the skin maintains a temperature of 32 °C. At predetermined times (2, 4, 6, 8, 10, and 12 h) the PBS was withdrawn from the receptor compartment and put in a vial. Fresh PBS (37 °C) was immediately replaced after withdrawal. The vials were stored in the fridge and analyzed on the HPLC as soon as possible.



Figure D.1: Franz cell and horseshoe clamp



Figure D.2: Grant water bath fitted with a Variomag magnetic stirrer plate and Franz cell stand

D.3.5.2 Skin permeation studies for emulgel formulations

The same procedure was used as described in Section D.3.5.1 with the exception that the only extraction done was at 12 h. The objective of delivering scopolamine transdermally is to have a sustained release over a long period of time (longer than 12 h). The amount of scopolamine delivered over shorter times was not sufficient to analyse. Hence, the predetermined time for the withdrawal of the receptor PBS was 12 h. The PBS was then analyzed on the HPLC and the amount of drug delivered was calculated.

It was previously determined that the scopolamine patch withheld 70% of its active ingredient and that the formative factor ultimately remained the patients' skin itself (Barry, 2007:591).

D.3.6 Membrane studies for emulgel formulations

The exact same procedure was followed as described in Section D.3.5.1 with the exception of using preferred membranes (Pall) that resembles the human skin instead of real human skin. This determines whether the drug is released from its dosage form before performing the actual skin permeation study. The receptor PBS was withdrawn every 2 h for up to 12 h.

D.3.7 SC-epidermis and epidermis-dermis evaluation*

This simple and proficient procedure is carried out to analyse the quality of cosmetic formulations as well as the amount of drug in the SC-epidermis and epidermis-dermis (Lademann *et al.* 2009:317). After the duration of the skin permeation study, the Franz cells were dismantled and the drug preparation was removed from the skin instantaneously. The skin was blotted dry with a piece of paper towel. 16 Scotch Magic™ tape strips were cut per piece of skin. The aim of this is to successfully remove the SC-epidermis from the skin to establish whether any drug penetrated the skin layers. The first strip of Scotch Magic™ tape was discarded as it is only viewed as part of the cleaning process (Pellet *et al.*, 1997:94). When the SC-epidermis is removed the viable epidermis glistens. The remaining strips were placed in a politop filled with 5 ml PBS. After being left overnight in the PBS, it was withdrawn and filtered before assaying it on the HPLC. To analyze the amount of drug in the epidermis-dermis the diffusion area on the skin was cut out and cut into small pieces where after it was placed in 5 ml PBS. It was left overnight and centrifuged before assaying it with HPLC (Pellet *et al.*, 1997:94).

D.3.8 HPLC

Active ingredients, cyclizine and scopolamine were analysed by means of HPLC using an Agilent 1100 series, fitted with an Agilent® 1100 pump, an autosampler injection mechanism and diode array detector. Data analysis and calculations were performed by the Chemstation Rev. A 10.02 software. All analyses were done in a controlled laboratory environment at 25 °C. Quantitation of the active ingredient was done based on the ratio of active ingredient to the peak area of the standard solution (see Appendix A.1).

*Hereafter referred to tape stripping and dermis

D.3.8.1 HPLC of cyclizine

Each of the samples (5 µl) were analysed by HPLC. A flow rate of 1 ml/min was maintained throughout the analyses. The Verusil XBP C18 (2), 5 µm, 100 Å, 4.6 x 150 mm was utilized with a mobile phase consisting of the following:

A: Octanesulphonic acid sodium (1 g) added to 1000 ml of Milli-Q water. Phosphoric acid (1 ml) was added to the solution and stirred on a magnetic stirrer plate. The pH was subsequently adjusted to 3.5 with ammoniumhydroxide.

B: HPLC grade acetonitrile.

The mobile phase was used mixed at a 50:50 concentration of A and B, respectively. The runtime was 10 min in total and the retention time of cyclizine was 5 min.

D.3.8.2 HPLC of scopolamine

Each of the scopolamine samples (10 µl) were injected into the HPLC. The Verusil XBP C18 (2), 5 µm, 100 Å, 4.6 x 150 mm was utilized for all analyses. The mobile phase as described in Section A.1.1 was used and maintained at a constant flow rate of 1 ml/min. Detection took place at 210 nm. The retention time of scopolamine was 2.8 min and the total runtime was 10 min.

D.3.8.3 HPLC of scopolamine emulgel formulations

The same equipment and HPLC conditions were used as described in Section D.3.8.2.

D.3.9 Data analysis

Before analysing the samples of the diffusion studies, a standard solution was prepared and linearity was determined as described in Appendix A. The cumulative concentration was plotted against time. The flux of scopolamine is represented by the linear portion of the graph. The slope of the straight line was determined and the average flux values were obtained.

D.3.10 Statistical analysis

The statistical analysis includes the following descriptive statistics:

- Median (the statistical centre point of the data) and standard deviation calculations.
- Representation of the data by means of scatter-plots and box-plots with data point superimposed.
- Calculation of the Spearman's Rho correlation coefficient in order to determine the relationship between the various measurement variables i.e. flux, tape stripping and dermis of the formulations.

In addition to the previous mentioned statistics analysis, inferential statistical analyses were performed. The T-test and the non-parametric Mann-Whitney investigated the effect of the formulations for the various measurement variables (flux, tape stripping and dermis). Should a big variation in the data be observed, it is more accurate to use the median value (Gerber *et al.*, 2008:190). The two-way analysis of variance (ANOVA) was performed to investigate the effect of the formulations and the scopolamine solution and scopolamine solution containing Pheroid™ together to test the interaction effect on the concentration measurement at 12 h. All statistical inference was performed at the 5 % level of significance. Statistical analyses were performed utilizing the SPSS and R statistical software.

D.4 Results and discussion

D.4.1 Physicochemical properties

D.4.1.1 Aqueous solubility

Naik *et al.* (2000:319) noted that an ideal aqueous solubility of a drug considered for transdermal delivery should be more than 1 mg/ml.

D.4.1.1.1 Aqueous solubility of cyclizine

Cyclizine exhibits a low aqueous solubility. When adding the cyclizine to water a milky solution was obtained. After the duration of 24 h, the sample was filtered, using a pre-filter/PVDF 0.45 µm filter and centrifuged before it was analysed on the HPLC. The results obtained were not accurate as the solution was oversaturated and it was not clear whether as to the cyclizine residue was removed completely from the sample before analysis. According to Drugbank (2010) the theoretical value for cyclizine is 1 mg/ml and was assumed to be correct.

D.4.1.1.2 Aqueous solubility of scopolamine

Scopolamine is very soluble in water. Whilst monitoring the samples (described in Section D.3.1.1) for the 24 h period, scopolamine was added several times to maintain equilibrium state. This resulted in a thick, syrup-like solution. The solution was centrifuged and diluted 1:100. The results obtained varied greatly and thus the theoretical value, according to Drugbank (2010) of 1000 mg/ml for aqueous solubility of scopolamine was assumed to be correct and used in further calculations.

D.4.1.2 *n*-Octanol-buffer distribution coefficient (log D)

D.4.1.2.1 Log D of cyclizine

Cyclizine samples were prepared as described in Section D.3.1.2. The samples were adequately diluted and analysed on the HPLC. All of the cyclizine was found to be in the *n*-octanol phase. The log D value of 3.11 assumed to be correct and used in further calculations (Monene *et al.*, 2007:243).

D.4.1.2.2 Log D of scopolamine

Samples were prepared as described in Section D.3.1.2. The samples were analysed on the HPLC. The log D of scopolamine was calculated and determined to be 1.77. This value indicates that scopolamine would be a good candidate to consider for transdermal delivery.

D.4.2 Skin permeation studies

D.4.2.1 Skin permeation for cyclizine solutions using full thickness skin

No data were obtained with the cyclizine solutions with or without the use of Pheroid™. Cyclizine did not penetrate through the full thickness skin. Physicochemical parameters including lipophilicity and aqueous solubility were proven to be contributing factors for transdermal delivery (Goosen *et al.*, 1998:207). The low aqueous solubility and high melting point of cyclizine might thus be a reason for it not penetrating the skin successfully.

Monene *et al.* (2003:243) conducted a Franz cell diffusion study by making use of a saturated cyclizine solution and utilized epidermal skin layers. The extraction of PBS was done at times 2, 4, 6, 8, 10, 12 and 24 h and they obtained an average flux of 0.1318 µg/cm².h. Various other alkyl analogues were also synthesised to produce cyclizine analogues with higher aqueous solubility values, subsequently improving the transdermal delivery. Monene *et al.* (2003:243) attained flux values for cyclizine which might be due to the use of saturated solutions and

therefore provides a bigger drive force for diffusion than the prepared 0.5 % solution used in our study.

D.4.2.2 Skin permeation for scopolamine solutions using full thickness skin

Figure D.3 represents the comparison of the scopolamine solution and solution containing Pheroid™ in terms of the average cumulative concentrations ($\mu\text{g}/\text{cm}^2$) released over 12 h. It can be observed that the scopolamine solution (0.075 % diffused) delivered more than twice the concentration than the solution containing Pheroid™ (0.035 % diffused). It can be observed that the Pheroid™ technology did not enhance the release or the diffusion of scopolamine. The higher oil content of the Pheroid™ solution might be the contributing factor to the aforementioned. With an increase in oil content of a formulation, the diffusion decreases (Barry, 2002:513).

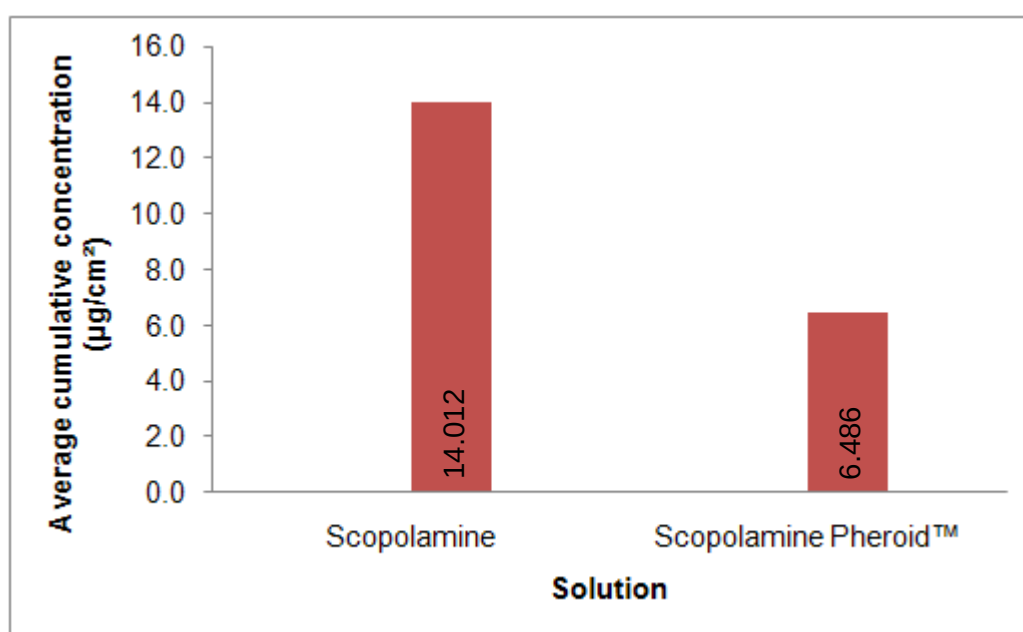


Figure D.3: Average cumulative concentration ($\mu\text{g}/\text{cm}^2$) after 12 h for the 1% scopolamine solutions with or without the use of Pheroid™

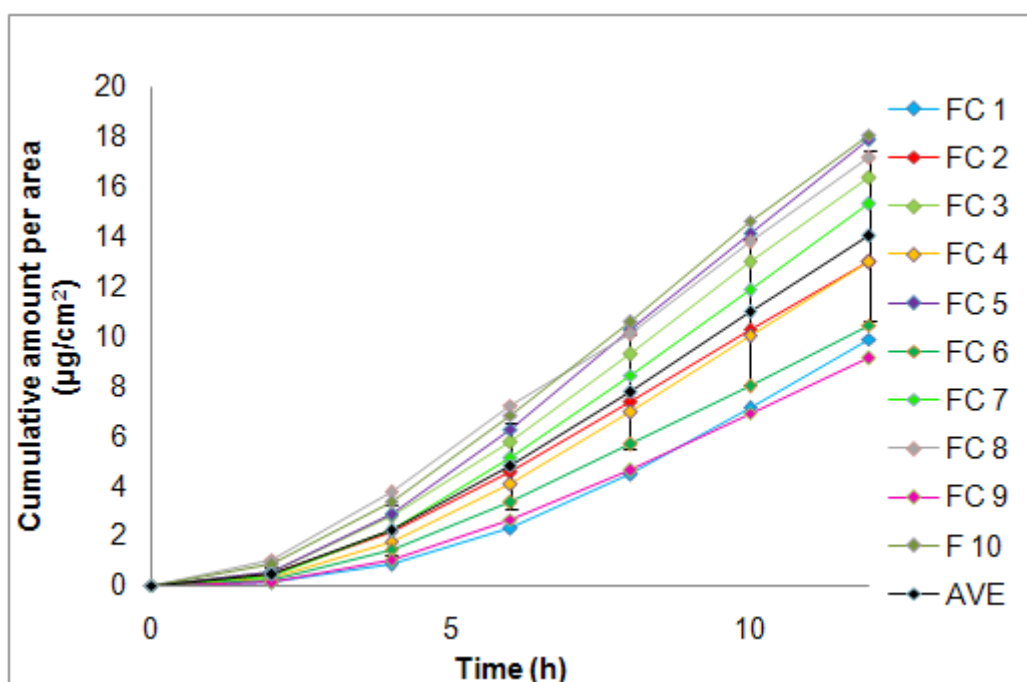


Figure D.4: Cumulative amount/area ($\mu\text{g}/\text{cm}^2$) of each individual Franz cell for scopolamine solution

Figures D.4 and D.6 illustrate the cumulative amount/area for all the individual Franz cells over a period of 12 h for the scopolamine solution and the scopolamine solution containing Pheroid™, respectively. Figures D.5 and D.7 represent the average cumulative amount/area of all the Franz cells combined over a period of 12 h for the scopolamine solution and the scopolamine solution containing Pheroid™, respectively, to illustrate average flux.

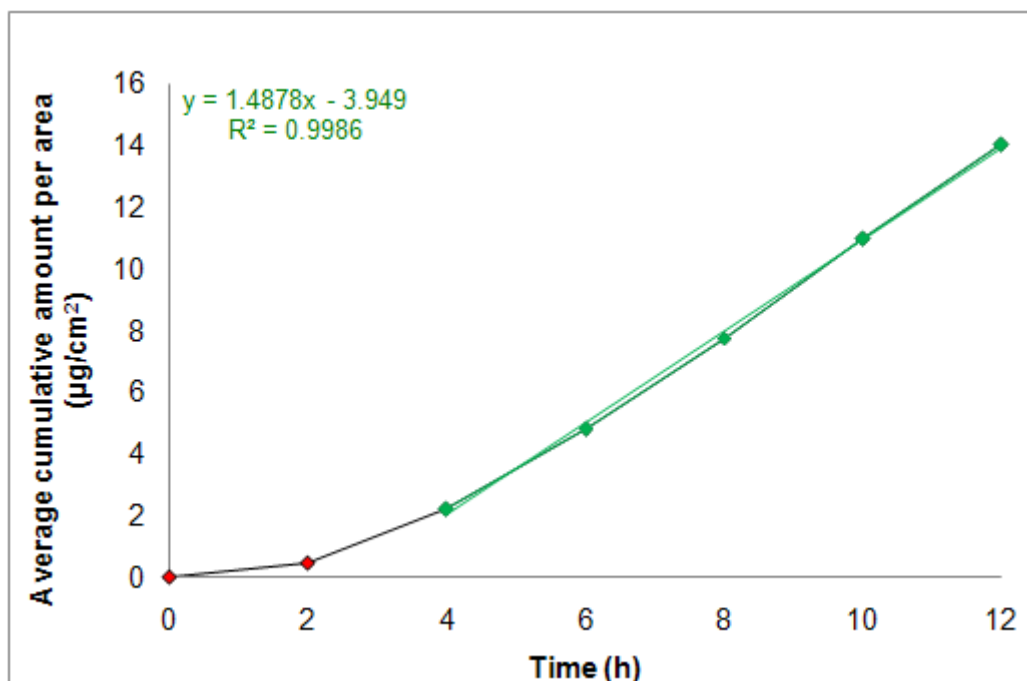


Figure D.5: Average cumulative amount/area ($\mu\text{g}/\text{cm}^2$) of scopolamine solution that penetrated through the skin as a function of time

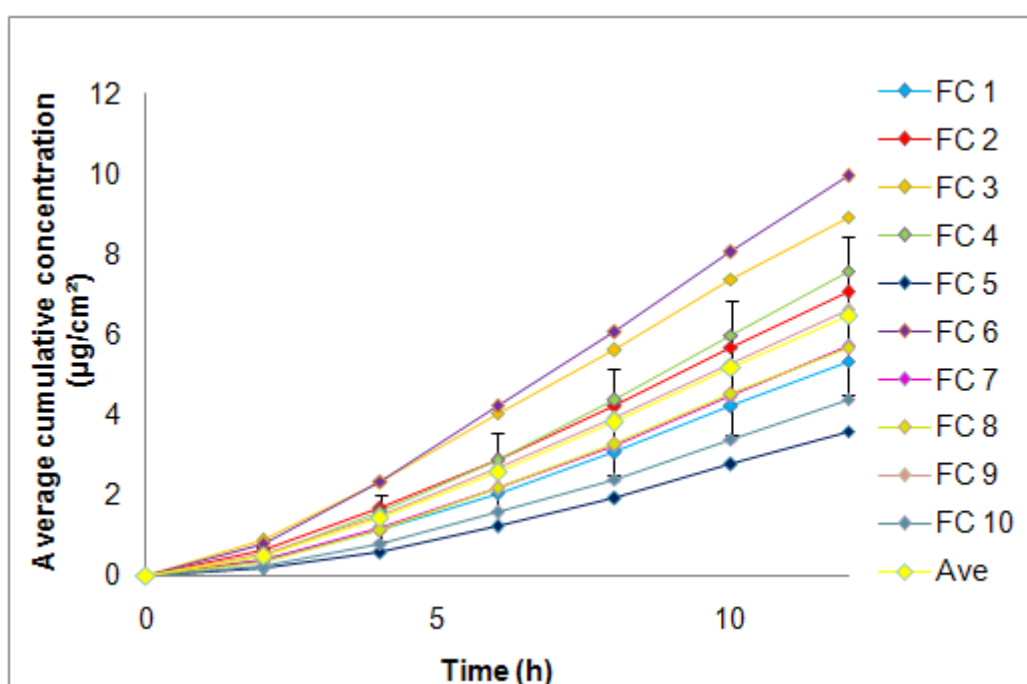


Figure D.6: Cumulative amount/area ($\mu\text{g}/\text{cm}^2$) of each individual Franz cell for scopolamine solution containing Pheroid™

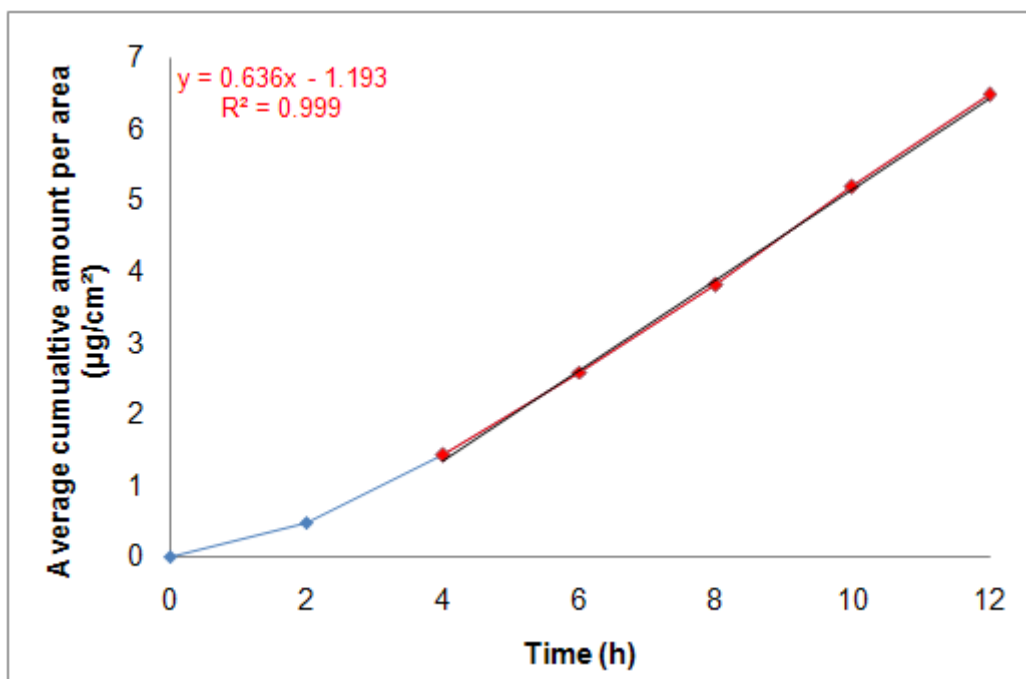


Figure D.7: Average cumulative amount/area ($\mu\text{g}/\text{cm}^2$) of scopolamine solution containing Pheroid™ that penetrated through the skin as a function of time

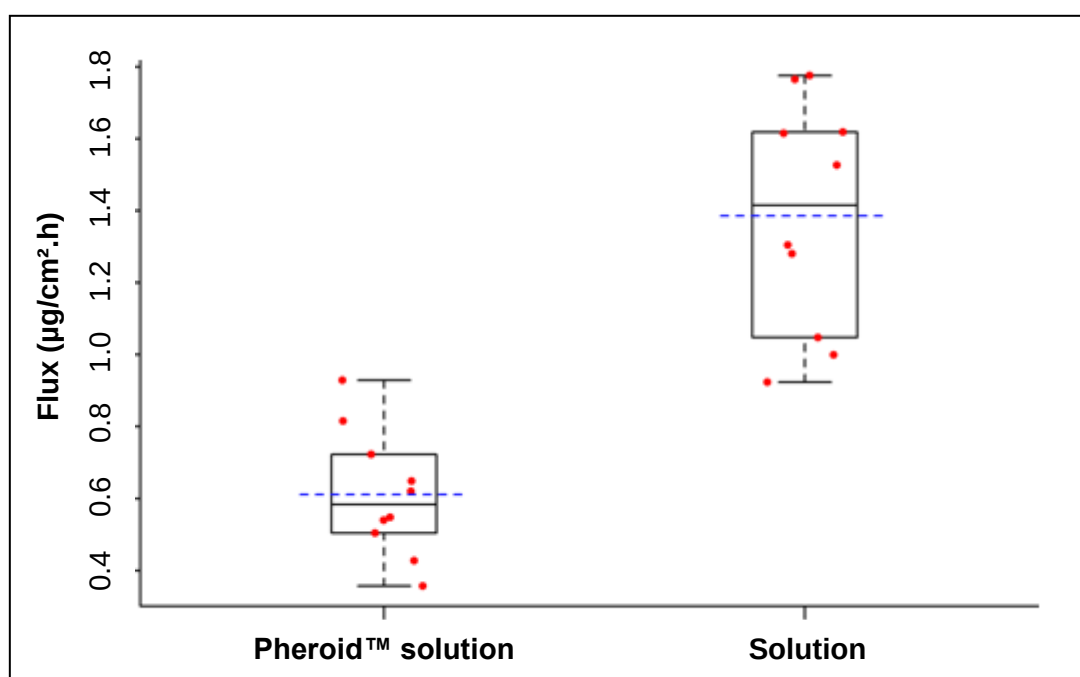


Figure D.8: Box-plot representation of the flux values ($\mu\text{g}.\text{cm}^2.\text{h}$) for the solutions. The blue and black lines represent the average and median flux values, respectively.

A box-plot is presented in Figure D.8, to illustrate the difference between average (0.636 and 1.488 $\mu\text{g}.\text{cm}^2.\text{h}$) and median (0.584 and 1.416 $\mu\text{g}.\text{cm}^2.\text{h}$) flux values for scopolamine containing Pheroid™ and scopolamine solution, respectively. When studying the aforementioned figure it can clearly be observed that the scopolamine solution delivered a greater flux than the solution containing Pheroid™. Again it might be contributed to the higher oil content of the solution (Barry, 2002:513). Pheroid™ subsequently did not enhance the penetration of scopolamine. It can also be observed that there are minor differences between the average and the median values. The small difference in data indicates that there weren't many outliers in the data and therefore both average and median values can be used to determine flux.

D.4.2.3 Statistical correlations

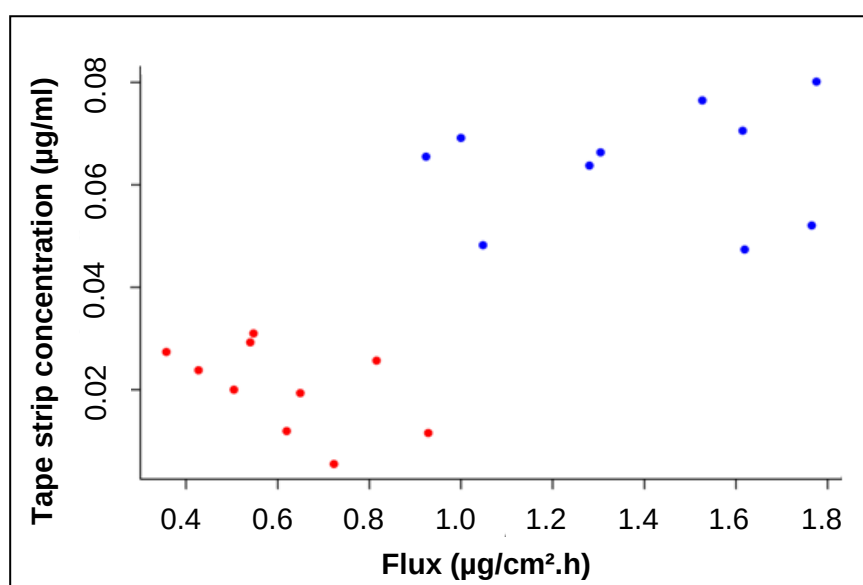


Figure D.9: Scatter-plot representation of tape stripping concentration ($\mu\text{g}/\text{ml}$) and flux ($\mu\text{g}/\text{cm}^2.\text{h}$) utilizing the Spearman's Rho correlation coefficient. (Red represents the scopolamine solution with Pheroid™ and blue the scopolamine solution).

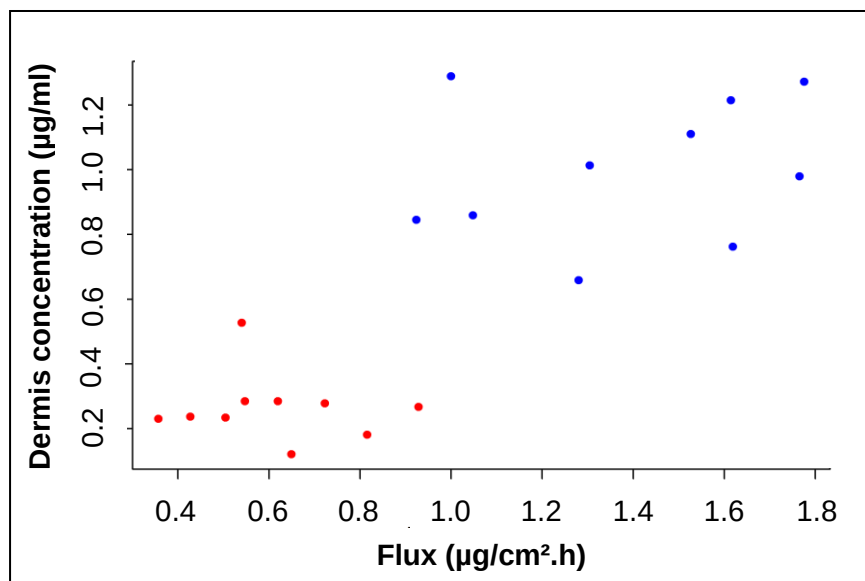


Figure D.10: Scatter-plot representation of dermis concentrations (µg/ml) and flux (µg/cm².h) utilizing the Spearman's Rho correlation coefficient. (Red represents the scopolamine solution with Pheroid™ and blue the scopolamine solution).

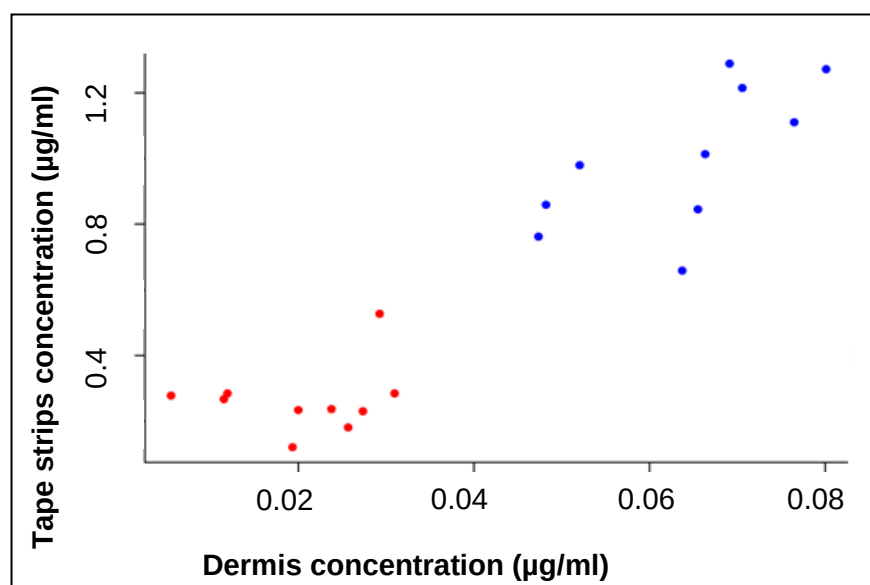


Figure D.11: Scatter-plot representation of tape stripping and dermis concentrations (µg/ml) utilizing the Spearman's Rho correlation coefficient. (Red represents the scopolamine solution with Pheroid™ and blue the scopolamine solution).

The relationships, once quantified utilizing the Spearman's Rho correlation coefficient, are given in Table D.9.

Table D.9: Spearman's Rho correlation coefficients (significant correlations indicated in bold).

		Flux	Tape Stripping	Dermis
Scopolamine Solution	Flux	1.000	0.164	0.188
	Tape Stripping	0.164	1.000	0.782
	Dermis	0.188	0.782	1.000
Scopolamine Solution with Pheroid™	Flux	1.000	-0.539	-0.036
	Tape Stripping	-0.539	1.000	0.109
	Dermis	-0.036	0.109	1.000

Except for the significant relationship between tape stripping and dermis for the scopolamine solution, no noteworthy relationship (positive or negative correlation (slope)) could be determined, as illustrated by Figures D.9 to D.11. A larger sample site might however contain more information regarding to this research question.

In order to determine whether there was a significant relationship between the formulations, the T-test and Man-Whitney tests were performed. The flux, tape stripping and dermis values of the scopolamine solution were compared to the flux of the scopolamine solution containing Pheroid™ are represented in Figure D.8 using a box-plot representation. The T-test and Mann-Whitney test confirmed that this observed differences were statistically significant at the 5 % level.

D.4.3 Tape stripping and dermis concentrations

D.4.3.1 Concentration of cyclizine present in the SC-epidermis and epidermis-dermis

No tape stripping or dermis data were obtained with the cyclizine solutions. Cyclizine did neither penetrate the SC-epidermis, nor the epidermis-dermis. Cyclizine comprises over a low aqueous solubility and a high melting point. These physicochemical properties make cyclizine not a suitable drug for transdermal penetration (Goosen *et al.*, 1998:207). The importance of the penetration of the SC for transdermal delivery was described by Jantunen *et al.*, (2008:103) and it was found that cyclizine was not capable of penetrating the SC and therefore the epidermis-dermis layer can subsequently not be reached.

D.4.3.2 Concentration of scopolamine present in the SC-epidermis and epidermis-dermis

The median values of the tape stripping and dermis is higher in the scopolamine solution when compared to the scopolamine solution containing Pheroid™. There were no outliers in the data

(Gerber *et al.*, 2008:190) hence; there were no significant differences between the average and median concentration values. Both median and average can be used to determine concentration. The relationship between the various measures grouped of the scopolamine solution and scopolamine solution containing Pheroid™ were investigated and are represented in the box-plots, illustrated by Figures D.13 and D.14.

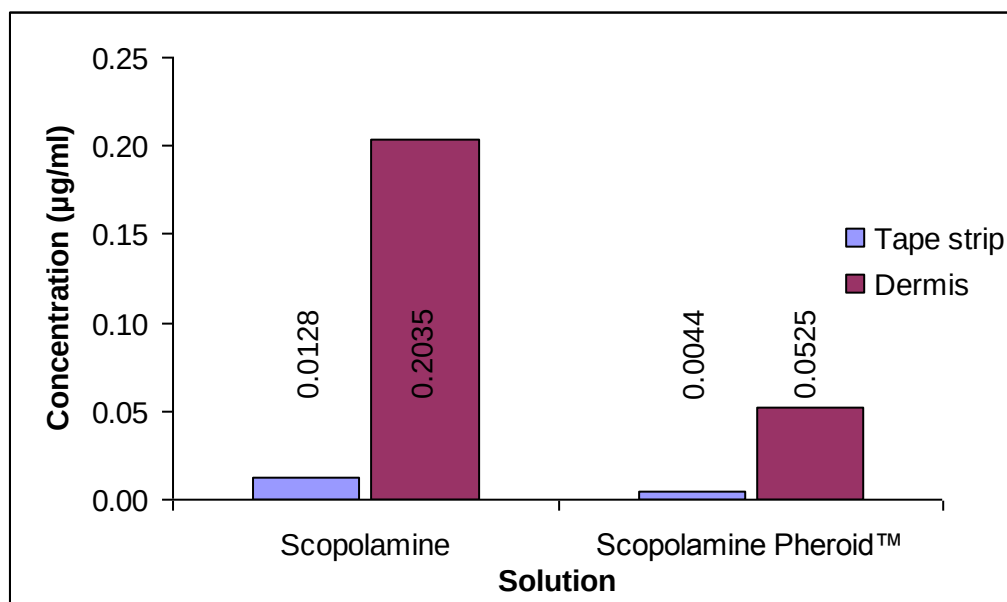


Figure D.12: Average concentration (µg/ml) of scopolamine present in tape strips and dermis

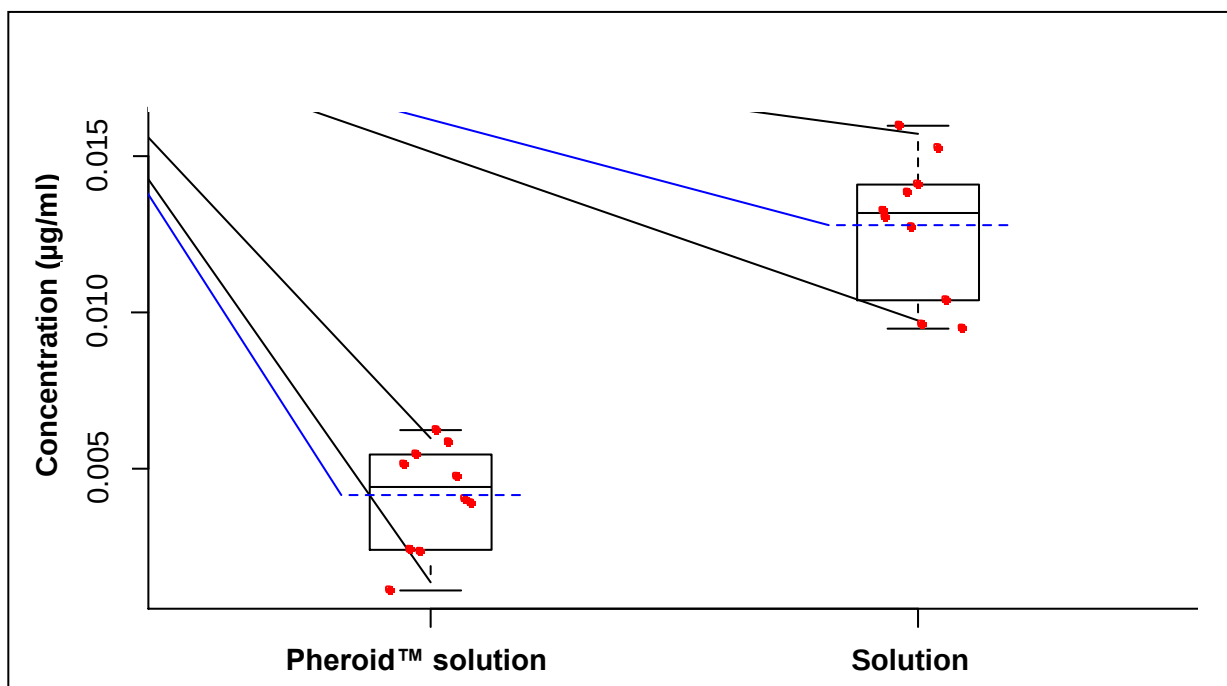


Figure D.13: Box-plot representation of the tape stripping concentrations ($\mu\text{g/ml}$) for the solutions. The blue and black lines represent the average and median values, respectively.

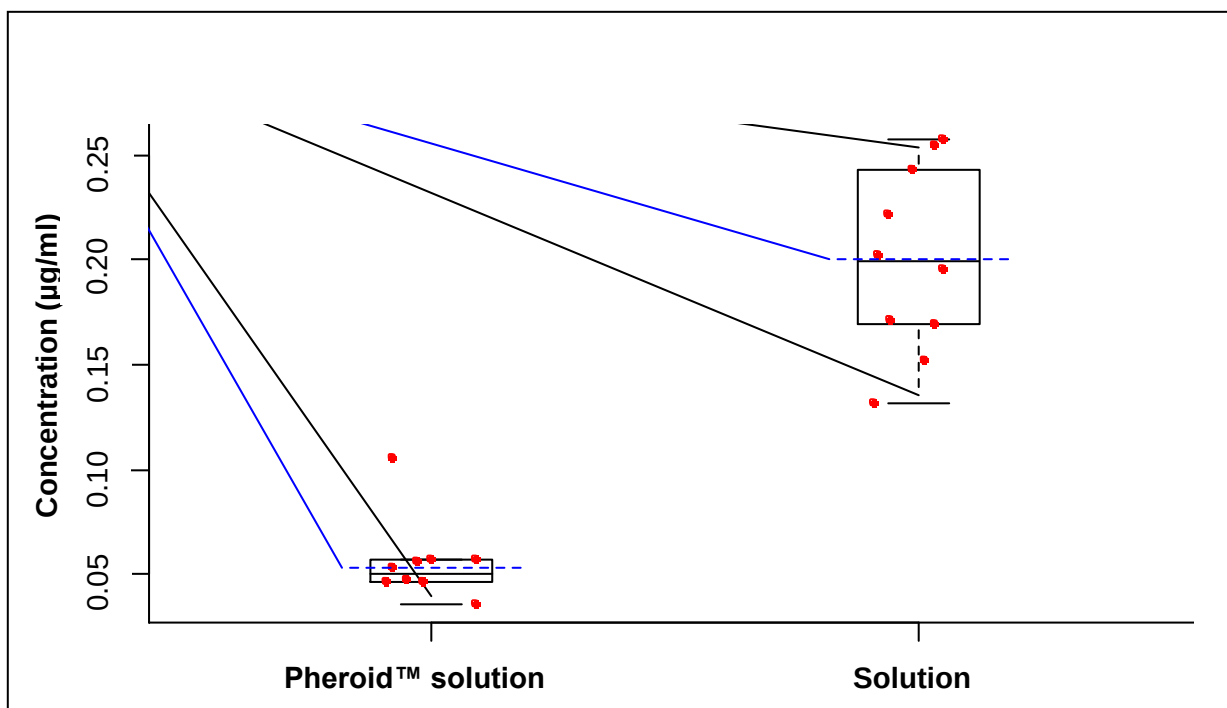


Figure D.14: Box-plot representation of the dermis concentrations ($\mu\text{g/ml}$) for the solutions. The blue and the black lines represent and median and average values, respectively.

The scopolamine in PBS revealed the greatest average and median concentration values in the epidermis (0.0128 µg/ml; 0.0132 µg/ml) and the dermis (0.2035 µg/ml; 0.1662 µg/ml) when compared to that of the scopolamine in Pheroid™ solution in the epidermis (0.0044 µg/ml; 0.0035 µg/ml) and the dermis (0.0525 µg/ml; 0.0504 µg/ml), respectively. It can subsequently be determined that the Pheroid™ vesicles did not assist scopolamine in penetrating neither the epidermis nor the dermis. The scopolamine in PBS penetrated the skin deeper than the scopolamine with Pheroid™ solution. Hence the higher oil content of the Pheroid™ solution might be the attributing factor (Barry, 2002:513).

D.4.4 Franz cell diffusion study using the scopolamine emulgel formulations

D.4.4.1 Membrane diffusion studies of emulgel formulations

The average cumulative concentrations (µg/cm²) are given in Figure D.15. The membrane release study was performed to determine the release of the active ingredient from the formulation. The emulgel released 0.116% (21.579 µg/cm²) of its active ingredient, thus almost 32 % more than the Pheroid™ emulgel (0.079%; 14.769 µg/cm²). When taking a previous study into account that was performed on the scopolamine patch, only 30 % of the active ingredient was released and the diffusion of the remainder of scopolamine was ultimately determined by the patient's skin itself (Barry, 2007:591). Scopolamine might thus not be adequately released when formulated. The Pheroid™ vesicles subsequently did not enhance the release of the drug. This might also be attributed to the emulgel with Pheroid™ having a higher oil content. The diffusion decreases with the increase of oil content (Barry, 2002:513). Due to the emulgel formulations not releasing an adequate percentage of scopolamine, there was decided that the only extraction for the skin diffusion studies would be done at 12 h.

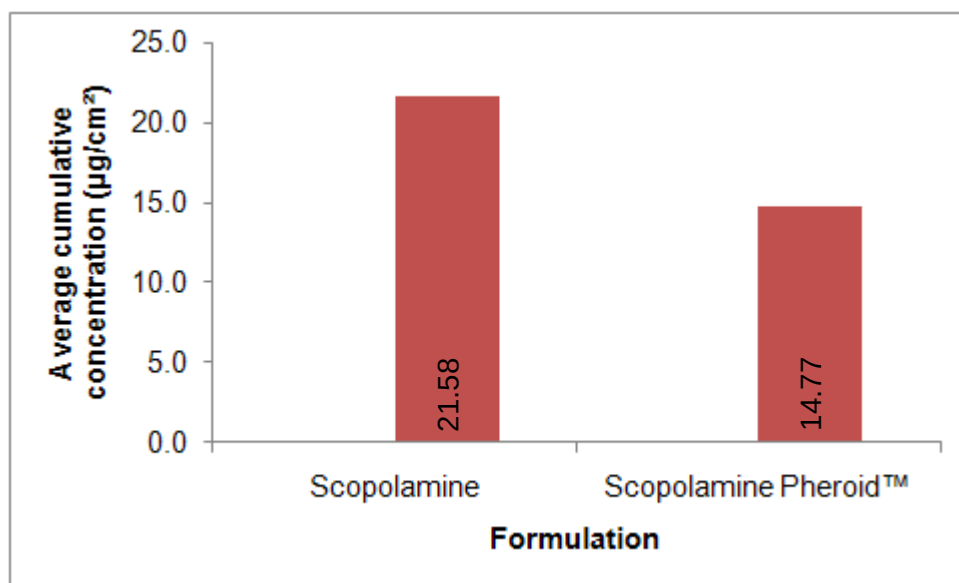


Figure D.15: Average cumulative concentration ($\mu\text{g}/\text{cm}^2$) after 12 h for the scopolamine emulgel formulations with or without the use of Pheroid™ after membrane diffusion

D.4.4.2 Skin diffusion studies of emulgel formulations

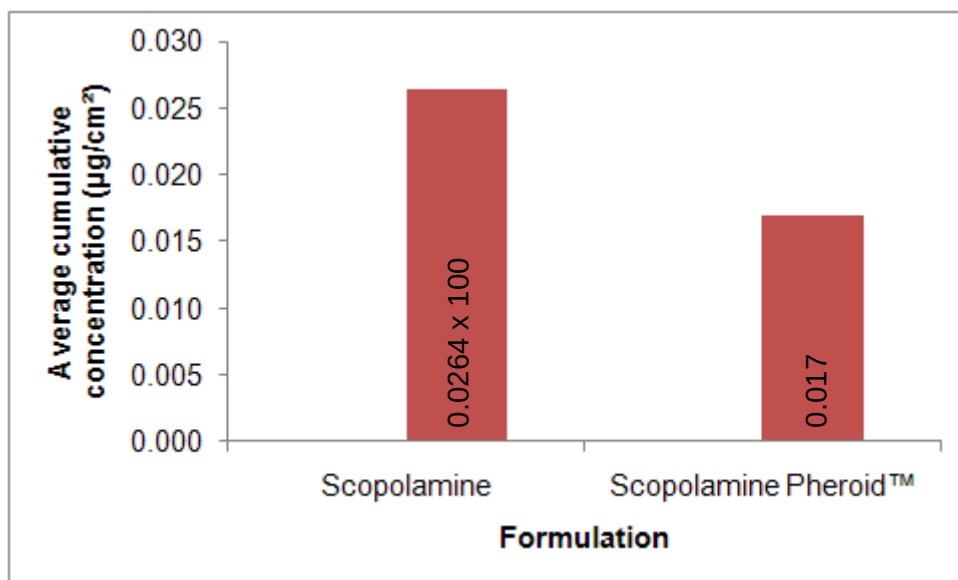


Figure D.16: Average cumulative concentration after 12 h for the scopolamine emulgel formulations with or without the use of Pheroid™ after epidermal skin diffusion

A higher concentration of scopolamine was delivered transdermally after using the emulgel ($2.649 \mu\text{g}/\text{cm}^2$). The Pheroid™ vesicles ($0.017 \mu\text{g}/\text{cm}^2$) did not enhance the transdermal

permeation when compared to the emulgel without Pheroid™. The emulgel delivered a concentration of approximately 155 times bigger than the emulgel containing Pheroid™. These results might be due to a more complex formulation and thus not adequately releasing the scopolamine. From the membrane release studies it can be derived that the emulgel released almost 32 % more of the active ingredient than the emulgel with Pheroid™. The higher oil content of the Pheroid™ emulgel might also be an attributing factor to it not diffusing properly across the skin (Barry, 2002:513). Bouwstra *et al.*, (2003:23) also revealed that vesicles in a gel state can inhibit permeation of the skin and act as an extra barrier. The Pheroid™ vesicles might thus restrain the permeation of scopolamine when using the Pheroid™ emulgel.

The emulgel will deliver an adequate concentration in order to combat motion sickness. A concentration of 50 pg/ml is required to be effective (Roberge, 2006:299-300). Both emulgel with or without Pheroid™ will thus deliver a concentration of more than the required effective therapeutic dosage to combat motion sickness.

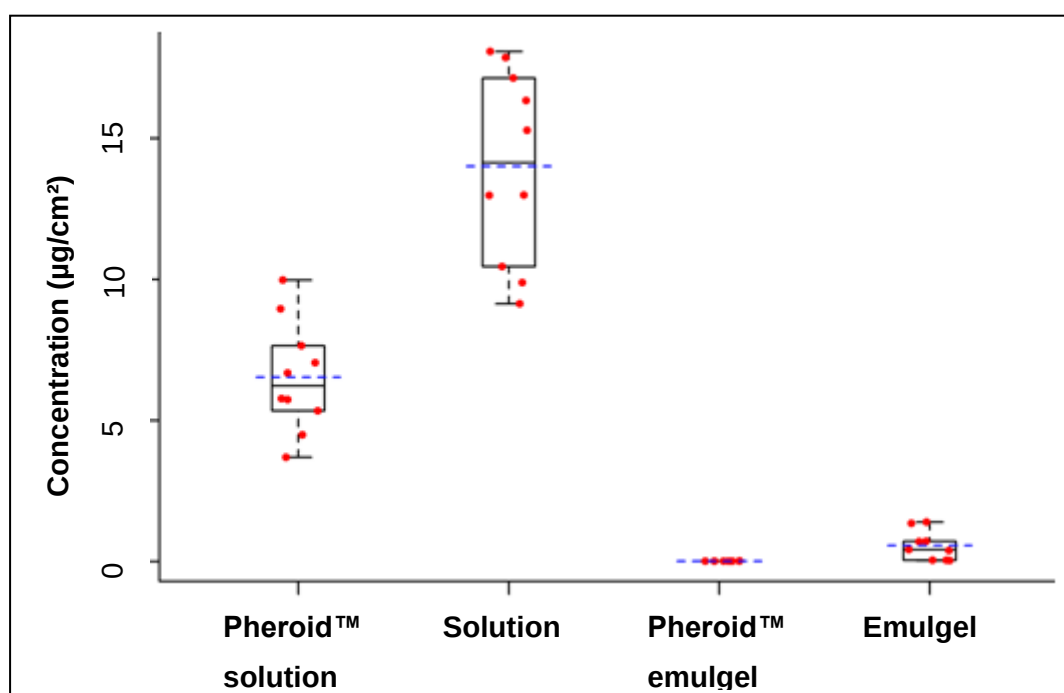


Figure D.17: Box-plot representation with and without the use of Pheroid™ for the solutions when compared to the emulgel formulations

When comparing the solutions to the formulations it is observed that the scopolamine solutions resulted in the highest transdermal concentration being delivered and that the solutions and formulations without Pheroid™ gave the lowest concentrations. These results might be attributed to the scopolamine not being adequately released from the formulation and thus not being able to penetrate the skin. Another reason for the Pheroid™ formulation not penetrating the skin more sufficiently, might be the unsuccessful entrapment of scopolamine and therefore

just contributes to the increases in oil content in the Pheroid™ formulations, subsequently resulting in a decrease in diffusion (Barry, 2002:513)

When compared to the scopolamine solution and scopolamine solution containing Pheroid™, the scopolamine solution delivered the highest average cumulative concentration ($\mu\text{g}/\text{cm}^2/\text{h}$) followed by the solution containing Pheroid™, the emulgel and lastly the emulgel containing Pheroid™. The concentrations after the duration of 12 h were measured for both the solutions and emulgel formulations. The box-plots of these comparisons are displayed in Figure D.17.

A two way ANOVA was conducted to investigate the interaction between these formulae and the addition of Pheroid™. It was conducted that a statistical significant interaction was observed, hence the difference between Pheroid™ and non-Pheroid™ should be investigated separately for each formulation. It was concluded from the T-test and Mann-Whitney tests that there was a statistical significant difference between the concentrations measured for the Pheroid™ and non-Pheroid™ groups of both the solutions and emulgel formulations. Subsequently, a bigger difference is observed for the solutions than the emulgel formulations. It can therefore be concluded that the release of scopolamine is not as sufficient when formulated in an emulgel as when scopolamine is formulated in solutions. The Pheroid™ technology did not assist the transdermal permeation of scopolamine.

D.5 Previous studies conducted

Hadgraft *et al.* (1995:285-289) found that theoretically, several anti-emetics including scopolamine could be likely candidates for successful dermal activity. Solutions (5 mg/ml) were prepared and tested on hairless mice. A permeation of 0.27 $\mu\text{g}/\text{ml}$ was obtained and they estimated a $\log k_p$ value of - 3 to - 3.95 in humans. It was noted by Roberge (2006:299-300) that the transdermal scopolamine patch contains a concentration of 1.5 mg and releases an initial dose of 140 μg followed by the continuous absorption of 5 μg for a duration of 72 h. Another study conducted established that post operative nausea and vomiting treated with ondansetron with the addition of a transdermal scopolamine patch improved the symptoms immensely (Sah *et al.*, 2009:251). Various other anti-emetics have been tested for transdermal delivery, including tetrahydrocannabinol in rats, resulting in a delivery of 50 ng/ml concentration (Touitou *et al.*, 1988:9).

D.6 Conclusion

In order to develop a suitable pharmaceutical formulation, various factors should be taken into account. Identifying the condition that is to be treated is the most important factor to consider followed by the site of action, identifying the rate-limiting step and ultimately to deliver the drugs to the active sites (Barry, 2007:596). The aim of this study was to deliver cyclizine and

scopolamine, both very effective anti-emetics, transdermally and thus to deliver these active ingredients systemically where the therapeutic action is required.

The low aqueous solubility and high log D value (3.11) indicated that cyclizine might not permeate the skin adequately. Scopolamine, comprising over a log D value of 1.77 and an aqueous solubility of 1 g/ml predicted optimum transdermal permeation. Both the cyclizine solution and the cyclizine solution containing Pheroid™ exhibited no results for transdermally. As stated by Monene *et al.*, (2007:243) the transdermal permeation of cyclizine increased with the increase of aqueous solubility. The concentration of cyclizine was possibly too low to create a driving force for cyclizine through full thickness skin.

Scopolamine solution (14.012 µg/cm²) delivered an average cumulative concentration of more than a 50 % higher than that of the scopolamine solution containing Pheroid™ (6.486 µg/cm²). This result might be attributed to the higher oil content in the scopolamine solution containing Pheroid™. With the increase in oil content, the permeation decreases (Barry, 2002:513).

The cyclizine solution and the cyclizine solution containing Pheroid™ did not penetrate the SC, consequently delivering no results for the SC-epidermis or the epidermis-dermis. These results might again be due to the cyclizine not comprising over adequate aqueous solubility or the concentration of the drug in both solutions being too low (Naik *et al.*, 2000:319).

The concentration in the epidermis for the scopolamine solution was much higher than the scopolamine solution containing Pheroid™. When statistically analysed, by means of the Mann-Whitney and T-tests, a significant difference between the solutions were obtained. Higher oil content in the Pheroid™ solution might decrease the permeation of the skin. Another reason might be that the Pheroid™ did not entrap scopolamine successfully.

The concentration of scopolamine present in the dermis was considerably higher for the scopolamine solution, revealing a concentration of 4 times higher, when compared to the scopolamine solution containing Pheroid™. A deeper penetration of scopolamine is consequently exhibited with the scopolamine solution.

Membrane release studies were conducted with the scopolamine formulations. The scopolamine emulgel released 0.116 % of scopolamine after the duration of 12 h. The scopolamine emulgel containing Pheroid™ released 0.079 % of scopolamine, exhibiting very poor release of the active ingredient. The Pheroid™ vesicles subsequently did not enhance the release of scopolamine.

When conducting the skin diffusion studies, the emulgel delivered an average cumulative concentration of 2.649 µg/ml over the 12 h period. The emulgel containing Pheroid™ delivered

a concentration of 0.017 µg/ml over 12 h. The higher oil content of the Pheroid™ emulgel and the poor entrapment of scopolamine might influence the diffusion of a drug (Barry, 2002:513).

Both emulgel formulations delivered scopolamine transdermally, hence it has reached its site of action. Previous studies indicated that a concentration of 50 pg/ml would be therapeutically effective in combatting motion sickness (Roberge, 2006:299-300). Subsequently, the emulgel formulations delivered an adequate therapeutic concentration that will in turn successfully combat against motion sickness.

In conclusion, the scopolamine solution delivered the greatest amount of drug when compared to the Pheroid™ solution. When formulated into emulgel formulations, the emulgel containing Pheroid™ released less of its active ingredient. The emulgel delivered the greatest concentration of scopolamine and therefore in this study be the best formulation to consider when treating nausea and vomiting. There exists a great opportunity to formulate scopolamine in various other dosage forms in order to deliver the most effective treatment for motion sickness.

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APPENDIX E

Drug Delivery: Instructions for Authors

Manuscript Submission

E.1 About the Journal

E.1.1 Aims and Scope

Drug Delivery serves the academic and industrial communities with peer reviewed coverage of basic research, development, and application principles of drug delivery and targeting at molecular, cellular, and higher levels. Topics covered include all delivery systems and modes of entry, such as controlled release systems; microcapsules, liposomes, vesicles, and macromolecular conjugates; antibody targeting; protein/peptide delivery. Papers on drug dosage forms and their optimization will not be considered unless they directly relate to the original drug delivery issues. Published articles present original research and critical reviews.

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Manuscripts should be compiled in the following order: title page; abstract; main text; acknowledgments; Declaration of Interest statement; appendices (as appropriate); references; tables with captions (on separate pages); figures; figure captions (as a list).

Drug Delivery publishes the following manuscript types:

Original papers Reviews Book reviews

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A title page should be provided comprising the manuscript title plus the full names and affiliations of all authors involved in the preparation of the manuscript. One author should be clearly designated as the corresponding author and full contact information, including phone number and email address, provided for this person. Five key terms that are not in the title should also be included on the title page. The keywords will assist indexers in cross indexing your article. The title page should be uploaded separately to the main manuscript and designated as “title page – not for review” on ScholarOne Manuscripts.

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All original articles and reviews should start with an abstract of 250 or fewer words, summarising the central core of knowledge that is the focus of the paper. The recommended format is as a structured abstract, with the following headings for an original article: context, objective, materials and methods, results, discussion and conclusion. For a review article, it should be structured as follows: context, objective, methods (including data sources, study selection and data extraction), results and conclusion. It should be written in an informative style permitting its use, without revision, by abstracting services, give essential details of research findings without further reference to the text, and avoid generalisations and nonessential information.

E.3.4 Main Text

E.3.4.1 Original articles

The body of the article should include the following sections: introduction; methods; results; discussion; conclusions.

Introduction: This section should state the relevance and background to the study, and its rationale and purpose.

Methods: This section should include only information that was available at the time the plan or protocol for the study was being written. You should describe your selection of the observational or experimental participants, identify the methods, apparatus and procedures in sufficient detail to allow others to reproduce the results, and describe statistical methods with enough detail to enable a knowledgeable reader with access to the original data to verify the reported results. Drug Delivery requires that studies involving humans, both volunteers and patients, or animals be approved by an institutional review board, in accordance with approved published guidelines, prior to actually performing the research and publishing the data. Details including clinical trial registration number must be provided in the methods section if research includes studies conducted on human volunteers.

Results: Present your results in logical sequence in the text, tables, and illustrations.

Discussion: This should include implications of the findings and their limitations, with reference to all other relevant studies and the possibilities these suggest for future research.

Conclusions: This must summarize the main paper. Ensure that extrapolations are reasonable and that conclusions are justified by the data presented, and indicate if the study design can be generalized to a broader study population.

E.3.4.2 Reviews

The body of a review article should be a comprehensive, scholarly evidence-based review of the literature, accompanied by critical analysis and leading to reasonable conclusions. Wherever appropriate details of the literature search methodology should be provided, i.e. the databases searched (normally Medline and at least one or two other databases), the search terms and inclusive dates, and any selectivity criteria imposed.

Wherever possible, use primary resources, avoiding “Data on File”, “Poster” or other unpublished references.

E.3.5 Acknowledgments and Declaration of Interest sections

Acknowledgments and Declaration of interest sections are different, and each has a specific purpose. The Acknowledgments section details special thanks, personal assistance, and dedications. Contributions from individuals who do not qualify for authorship should also be acknowledged here.

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Journal: Iyengar BS, Dorr RT, Remers WA. (2004). Chemical basis for the biological activity of Imexon and related Cyanaziridines. J Med Chem, 47, 218-23.

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Contribution to a Book: Chandrasekaran SK, Benson H, Urquhart J. (1978). Methods to achieve controlled drug delivery: The biomedical engineering approach. In: Robinson JR, ed. Sustained and Controlled Release Drug Delivery Systems. New York: Marcel Dekker, 557-93.

Electronic Resources: Lin A-S, Shibano M, Nakagawa-Goto K, Tokuda H, Itokawa H, Morris-Natschke, SL, Lee K-H, (2007). Cancer Preventive Agents. 7. Antitumor-Promoting Effects of Seven Active Flavonolignans from Milk Thistle (*Silybum marianum*) on Epstein-Barr Virus Activation. Pharm Biol [Online] Available at: <http://www.informapharmascience.com/doi/abs/10.1080/13880200701585592>. Accessed on 12 April 2009.

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Tables should be used only when they can present information more efficiently than running text. Care should be taken to avoid any arrangement that unduly increases the depth of a table, and the column heads should be made as brief as possible, using abbreviations liberally. Lines of data should not be numbered nor run numbers given unless those numbers are needed for reference in the text. Columns should not contain only one or two entries, nor should the same entry be repeated numerous times consecutively. Tables should be grouped at the end of the manuscript on separate pages.

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3. Use standard convention for human and animal genes and proteins: italics for genes and regular font for proteins, and upper case for human products and lower case for animal products.
4. "US" is preferred to "American", "USA" to "United States", and "UK" to "United Kingdom".
5. Double quotation marks rather than single are used unless the "quotation is "within another".
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8. Apostrophes should be used sparingly. Thus, decades should be referred to as follows: “The 1980s [not the 1980.s] saw...”. Possessives associated with acronyms (e.g. APU), should be written as follows: “The APU’s findings that...” but note that the plural is “APUs”.

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