

Formulation of an emulgel for topical delivery of roxithromycin

V Steenkamp



orcid.org/0000-0002-6449-5570

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Supervisor: Prof J du Plessis

Co-supervisor: Prof M Gerber

Co-supervisor: Dr MA Aucamp

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Student number: 23473142

The dissertation is presented in an article format, which includes one article for publication in a pharmaceutical journal (Chapter 3) and appendices containing experimental results and discussion (Appendix A – F). The article for publication has a specific Guide for Authors (Appendix G) for publishing.

"We've always defined ourselves by the ability to overcome the impossible. And we count these moments. These moments when we dare to aim higher, to break barriers, to reach for the stars, to make the unknown known. We count these moments as our proudest achievements. But we lost all that. Or perhaps we've just forgotten that we are still pioneers. That we've barely begun. That our greatest accomplishment cannot be behind us, because our destiny lies above us."

Cooper, Interstellar

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ABSTRACT

The aim of this study was to establish whether liposomal or niosomal vesicle systems containing roxithromycin could be developed further into semi-solid formulations (emulgels) for topical delivery of the active pharmaceutical ingredient (API). Resistance against the current first-line treatment of inflammatory lesions has increased, urging the development of a novel acne treatment product. It has been found that the acne causing microorganism located in the epidermis-dermis is susceptible to roxithromycin. Previous studies stated that different solid-state forms of roxithromycin could be included into thin-films, rendering it into an amorphous state irrespective of the initial solid-state.

Three solid-state forms of roxithromycin were used for this study, i.e. roxithromycin monohydrate (**MH**), roxithromycin chloroform solvate (**CS**) and roxithromycin anhydrate (**AH**). Fourier-transform infrared spectroscopy (FTIR) and thermogravimetric analysis (TGA) was performed to confirm the properties of these solid-states used; obtained FTIR data correlated with previously reported research.

The formulation strategies made use of liposomes or niosomes as drug delivery vesicles. To ensure the suitability of the chosen solid-states and vesicle systems, the vesicles were characterised by means of morphology, droplet size, pH, zeta-potential and drug entrapment efficiency (EE%). Acceptable results were obtained for both liposomal and niosomal dispersions containing the different encapsulated solid-states of roxithromycin.

As no topical product containing roxithromycin is currently available on the market, it was decided to establish if the encapsulated API could be formulated into a semi-solid formulation. Every vesicle preparation was included in an emulgel separately. All prepared emulgels had a smooth, gel-like consistency. The liposomal emulgels presented with a light yellow colour, while the niosomal emulgels had a white colour, due to the different excipients used during preparation.

The stability of the formulated products was examined over a period of three months, according to the International Conference of Harmonisation (ICH) Tripartite Guideline and the South African Health Products Regulatory Authority (SAHPRA). The stability tests consisted of concentration assay, pH, viscosity, mass loss, zeta-potential, droplet size and physical appearance. The pH and mass variation resulted in satisfactory results over the three months period, whilst the concentration assay, viscosity, zeta-potential and droplet size resulted in unwanted changes. It could thus be concluded that these formulations were not yet suitable for bulk manufacture and improved emulgels ought to be developed.

Membrane release studies were performed on the emulgels, followed by skin diffusion studies and tape stripping. All emulgels resulted in satisfactory API release during the membrane release study. The small amount of API delivery during transdermal diffusion was a favoured outcome, since systemic effects and toxicity would be minimal. All the emulgels permeated the stratum corneum-epidermis. All liposomal emulgels resulted in API delivery to the intended target-site (epidermis-dermis), with the niosomal **CS** resulting in the best target-site delivery.

It was established that quantifiable concentrations of roxithromycin encapsulated in liposomes and niosomes were found at the target-site. It is therefore possible to include encapsulated roxithromycin into an emulgel for topical delivery, but it is necessary to ensure a stable formulation for future use.

Keywords: Emulgel, liposomes, niosomes, roxithromycin, topical drug delivery

UITTREKSEL

Die doel van hierdie studie was om vas te stel of semi-vasteformulerings (emuljelle) ontwikkel kon word vanaf liposoom- of niosoomvesikelsisteme wat roksitromisien bevat vir die topikale aflewering van die aktiewe farmaseutiese bestanddeel (AFB). Weerstandigheid het toegeneem teen die huidige eerste-linie behandeling van inflammatoriese letsels, wat die ontwikkeling van 'n nuwe produk vir akneebehandeling aanmoedig. Daar is gevind dat die mikro-organisme wat aknee veroorsaak in die epidermis-dermis voorkom en dat dit vatbaar is vir roksitromisien. Vorige studies het bewys dat verskillende soliedefasevorme van roksitromisien in dun films ingesluit kon word wat veroorsaak dat roksitromisien oorgaan tot 'n amorfetoestand, ongeag van die aanvanklike soliedefasevorm wat gebruik is.

Drie soliedefasevorme van roksitromisien is tydens die studie gebruik, naamlik roksitromisien-monohidraat (**MH**), roksitromisienchloroformsolvaat (**CS**) en roksitromisienanhidraat (**AH**). Die eienskappe van hierdie soliedefasevorme wat gebruik is, is bevestig deur gebruik te maak van Fourier-transformasie-infrarooispektroskopie (FTIR) en termogravimetriese analise (TGA). Die FTIR resultate wat behaal is, het met voorheen gerapporteerde navorsing verband gehou.

Die formuleringstrategieë het van liposome of niosome gebruik gemaak as vesikelsisteme vir geneesmiddelaflewering. Die vesikels was gekarakteriseer op grond van hul morfologie, druppelgrootte, pH, zeta-potensiaal en geneesmiddelenkapsuleringseffektiwiteit (EE%) om die geskiktheid van die geselekteerde soliedefasevorme en vesikelsisteme te verseker. Aanvaarbare resultate was vir beide die liposomale- en niosomaledispersies verkry wat die verskillende geënkapsuleerde soliedefasevorme van roksitromisien bevat.

Aangesien daar tans geen topikale produk op die mark beskikbaar is wat roksitromisien bevat nie, is daar besluit om vas te stel of 'n semi-soliede formulering vervaardig kon word wat die geënkapsuleerde AFB insluit. Elke vesikelsisteem was afsonderlik in 'n emuljel ingesluit. Alle voorbereide emuljelle het 'n gladde, jelagtige samestelling getoon. As gevolg van die verskillende hulpstowwe wat tydens voorbereiding gebruik was, het die liposoomemuljelle 'n liggeel voorkoms getoon, terwyl die niosoomemuljelle 'n wit kleur gehad het.

Die stabiliteit van die geformuleerde produkte was oor 'n tydperk van drie maande volgens die Internasionale Konferensie vir Harmonisasie (*ICH*) se drieledige riglyne en die Suid-Afrikaanse Gesondheidsprodukte-regulerende owerheid (*SAHPRA*) ondersoek. Die stabiliteitstoetse het konsentrasiebepaling, pH, viskositeit, massaverlies, zeta-potensiaal, druppelgrootte en fisiese voorkoms ingesluit. Die pH en massavariasie het bevredigende resultate oor die drie maande tydperk getoon, terwyl die konsentrasiebepaling, viskositeit, zeta-potensiaal en druppelgrootte ongewenste veranderinge tot gevolg gehad het. Die gevolgtrekking kon dus gemaak word dat

hierdie formulerings nog nie vir grootmaatvervaardiging geskik was nie en dat verbeterde emuljelle ontwikkel moet word.

Membraanvrystellingstudies gevolg deur veldiffusiétudes en kleefbandstropping was op die emuljelle uitgevoer. Alle emuljelle het tydens die membraanvrystellingstudie bevredigende AFB vrystelling getoon. Die klein hoeveelheid AFB wat tydens die transdermalediffusie afgelewer was, was 'n gunstige uitkoms aangesien sistemiese effekte en toksisiteit minimaal sou wees. Al die emuljelle het die stratum korneum-epidermis binnegedring. Al die liposoomemuljelle was suksesvol gewees om die AFB tot in die beplande teikenarea (epidermis-dermis) af te lewer, alhoewel die niosoomemuljel wat **CS** bevat die beste aflewering in die teikenarea getoon het.

Kwantifiseerbare konsentrasies van roksitromisien, wat in die liposome en niosome geënkapsuleer was, was in die teikenarea gevind. Dit is dus moontlik om geënkapsuleerde roksitromisien in 'n emuljel vir topikale geneesmiddelaflewering in te sluit, maar dit is nodig vir toekomstige gebruik om 'n stabiele formulering te verseker.

Sleutelwoorde: Emuljel, liposome, niosome, roksitromisien, topikale geneesmiddelaflewering

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ABBREVIATIONS

%EE	Entrapment efficiency
%RSD	Percentage relative standard deviation
°C	Degrees Celsius
μl	Microliter
μm	Micrometer
AH	Anhydrate form of roxithromycin
ALACC	Analytical laboratory accreditation criteria committee
API	Active pharmaceutical ingredient
ASD	Amorphous solid dispersion
ATL	Analytical Technology Laboratory
C ₄₁ H ₇₆ N ₂ O ₁₅	Roxithromycin monohydrate
cm	centimeters
cP	Centipoises
CS	Chloroform solvate form of roxithromycin
CYP450	Cytochrome P450
Da	Dalton
d.nm	Diameter nanometer
ED	Epidermis-dermis
EE%	Entrapment efficiency
FDA	Food and Drug Administration
FTIR	Fourier-transform infrared spectroscopy
g	grams
h	Hours
H ⁺	Hydrogen
H ₂ O	Water
H ₂ O ₂	Hydrogen peroxide
H ₆ NO ₄ P	Ammonium dihydrogen phosphate
HCl	Hydrochloric acid
HPLC	High-performance liquid chromatography
HREC	Health Research Ethics Committee
HRTEM	High-resolution transmission electron microscopy
ICH	International Conference of Harmonisation

IL-1- α	Interleukin-1 α
IR	Infrared
KBr	Potassium bromide
KH ₂ PO ₄	monopotassium phosphate
kV	Kilovolt
L-AH	Liposomal gel containing anhydrate roxithromycin
L-CD	Liposomal gel containing chloroform solvate roxithromycin
L-MH	Liposomal gel containing monohydrate roxithromycin
LOD	Limit of detection
LOQ	Lower limit of quantification
Log D	Octanol-buffer distribution coefficient
Log P	Octanol-water distribution coefficient
L-PL	Liposomal gel containing no roxithromycin
LUV	Large unilamellar vesicles
MCC	Medicines Control Council
mg	Milligrams
MH	Monohydrate form of roxithromycin
min	Minutes
ml	Milliliters
MLV	Multi-lamellar vesicles
mm	Millimeters
Mol	molar mass
mV	Millivolt
N-AH	Niosomal gel containing anhydrate roxithromycin
NaOH	Sodium hydroxide
NCBI	National Centre for Biotechnology Information
N-CD	Niosomal gel containing chloroform solvate roxithromycin
NH ₃	Ammonia
NH ₄ H ₂ PO ₄	Ammonium di-hydrogen phosphate
nm	Nanometer
N-MH	Niosomal gel containing monohydrate roxithromycin
N-PL	Niosomal gel containing no roxithromycin
NWU	North-West University
OECD	Organisation for Economic Co-operation and Development

OH	Hydroxyl
<i>P. acnes</i>	<i>Propionibacterium acnes</i>
PBS	Phosphate buffer solution
PC	Phosphatidylcholine
PdI	Polydispersity index
PL	Placebo vesicles (without API)
PVDC	Polyvinylidene Chloride
PVDF	Polyvinylidene fluoride
R ₂	Correlation coefficient/ Regression coefficient
RH	Relative humidity
RPM	Revolutions per minute
SAHPRA	South African Health Products Regulatory Authority
SCE	Stratum corneum-epidermis
SD	Standard deviation
sec	seconds
SSCI	Solid-state Chemical Information
SUV	Small unilamellar vesicles
T0	Initial/Month 0
T1	Month 1
T2	Month 2
T3	Month 3
TEM	Transmission electron microscopy
TGA	Thermogravimetric analysis
TNF-α	Tumour necrosis factor-α
USP	United States Pharmacopeia
UV	Ultraviolet
WHO	World Health Organisation

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CHAPTER 3

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CHAPTER 1

INTRODUCTION, AIMS AND OBJECTIVES

1.1 Introduction

The active pharmaceutical ingredient (API), roxithromycin, is classified as a semi-synthetic macrolide antibiotic and is a 14-membered lactone ring derivative of erythromycin (Bryskier, 1998:1). According to Ostrowski *et al.* (2010:83), roxithromycin can be used in the treatment of infections of the respiratory tract, genitourinary tract, skin and soft tissue. The human skin is the largest organ of the integumentary system, providing an ideal surface area for topical drug administration (Williams, 2003:1). Topically applied antibiotics are generally used for the treatment of mild to moderate acne infections due to the bacteriostatic effect of the inflammatory agent, *Propionibacterium acnes* (*P. acnes*) (Oschsendorf, 2006:829).

Previous studies showed that the acne causing microorganism, *P. acnes*, is susceptible to roxithromycin, ensuring effective treatment of mild to moderate inflammatory acne (Glowka *et al.*, 2014:83; Wosicka-Frackowiak *et al.*, 2015:816). In acne, the pilosebaceous units of the skin are affected, resulting in either inflammatory or non-inflammatory lesions (Ayer & Burrows, 2006:500; Bershad, 2001:279). The target-site of roxithromycin is the sebaceous follicles found in the dermis where *P. acnes* accumulates (Gollnick, 2003:1585). First-line treatment for inflammatory lesions currently consists of clindamycin or erythromycin, but in recent years' resistance against *P. acnes* strains has surfaced due to the continuous use of this treatment (Lavers, 2014:334). By applying roxithromycin topically *P. acnes* can be reached directly for the bacteriostatic effect, but up until this point in time this result could not be obtained due to the poor water solubility of roxithromycin. This has led to the interest in screening for other solid-state forms of roxithromycin, as altering the solid-state properties of an API can manipulate the physicochemical factors and even improve the solubility (Threlfall, 1995:2452).

Many different solid-state forms of roxithromycin are known (Aucamp *et al.*, 2012:475, Aucamp *et al.*, 2013:18; Bawa, 2007; Du Plessis, 2004; Milne *et al.*, 2016), as of yet none included in topical applications. It has since been reported that several of these solid-state forms could be included into thin-films, which serve as precursors for drug delivery vesicles (Csongradi, 2015:100; Boshoff, 2018:65; Swart, 2019:86). It was also found that roxithromycin is rendered in an amorphous state when included in thin-films, irrespective of the initial solid-state, due to the preparation of an amorphous solid dispersion. It is most likely that an amorphous solid dispersion occurs when the API is combined with excipients such as cholesterol, phosphatidylcholine and Span[®] 60, which are used when preparing liposome or niosome vesicle systems (Newman *et al.*, 2012:1356). Including roxithromycin into vesicle delivery systems,

such as liposomes and niosomes not only improves bioavailability of the API, it also attributes to targeted topical delivery.

Niosomes are hydrated vesicular systems formed from non-ionic surfactant(s) and are able to encapsulate various compounds with different solubilities (Kumar & Rajeshwarrao, 2011:216). Niosomes and liposomes are very similar vesicle systems, but the former is composed of non-ionic surfactant(s), whereas phospholipids are used to form the latter (Kumar & Rajeshwarrao, 2011:209). Liposomes are very small vesicles that contain one or more concentric hydrophobic bilayer(s) surrounded by a fatty membrane (neutral or charged) that envelopes the internal aqueous volume (Bansal *et al.*, 2012:705). The drugs can be incorporated in the lipid bilayer that contains a hydrocarbon chain core (hydrophobic), the aqueous centre or on the surface of the liposome (Escobar-Chávez *et al.*, 2012:212). They have the ability to increase the solubility of poorly soluble drugs 100 – 1000-fold, as well as delivering hydrophilic and hydrophobic drugs topically (Bansal *et al.*, 2012:709).

Although it is advantageous to include roxithromycin in vesicles such as liposome and niosome, the challenge of providing a user-friendly product still exists. According to Laouini *et al.* (2012), drug delivery vesicles, such as liposomes and niosomes, can easily be formulated in preparations such as suspensions, gels or creams. Considering the target-site for API delivery, including the vesicle systems in a hydrogel base will result in an emulgel, which is a convenient self-administering dosage form (Ning *et al.*, 2005:376). To be able to provide a safe and effective product for the user, it is important to be aware of the stability and all possible degradants whenever a new pharmaceutical product is developed. Accelerated stability tests use exaggerated storage conditions to provide an easy and time effective method to determine the quality, safety and efficacy of a pharmaceutical product (Barnes, 2007:661; Singh & Bakshi, 2000:2). An emulgel containing roxithromycin encapsulated in liposomes or niosomes respectively, proposes to deliver the API topically for the treatment of acne, which can be used as a dual therapy with existing systemic acne treatments.

1.2 Problem statement

Acne, which induces skin scarring, is a common problem that burdens more than 85% of adolescents (Ayer & Burrows, 2006:500). It can be treated topically or systemically with antibiotics, but available treatment options have become problematic due to antimicrobial resistance (Lavers, 2014:334). It has been established that roxithromycin is an effective antibiotic for the use of acne treatment (Glowka *et al.*, 2014:83; Wosicka-Frackowiak *et al.*, 2015:816). In a previous study, Csongradi (2015) managed to improve targeted drug delivery of roxithromycin through topical drug delivery vesicles. This was further investigated by Boshoff (2018) and Swart (2019), where different solid-state forms of roxithromycin were effectively

encapsulated in niosomes and liposomes as vesicle delivery systems. Presently, there is no topical pharmaceutical product on the market that contains roxithromycin.

1.3 Aims and objectives

The aim of this study was to establish whether liposomal or niosomal vesicle systems containing roxithromycin, may be developed further into emulgel formulations for topical application. Considering the formulation of emulgels from a cost point of view, scalability and since it was already established that thin-film preparation renders roxithromycin in the amorphous state, it was decided to only include solid-state forms that could either be purchased or be prepared very easily. The formulation strategies made use of liposomes and niosomes, respectively as drug delivery vesicles, which subsequently was formulated into an emulgel. The stability of the formulated products was examined over a period of three months according to the International Conference of Harmonisation (ICH) Tripartite Guideline and the South African Health Products Regulatory Authority (SAHPRA).

The objectives of this research study were:

- Verification of suitability of the high performance liquid chromatography (HPLC) method for roxithromycin.
- Preparation of anhydrate (**AH**) and chloroform solvate (**CS**) solid-state forms of roxithromycin.
- Solid-state characterisation by investigating the morphology using microscopy techniques, followed by confirming correlation of physicochemical attributes using Fourier-transform infrared spectroscopy (FTIR) with that already reported in literature.
- Performing thermogravimetric analysis (TGA) to determine the hydration or solvation level of the three crystalline forms of roxithromycin.
- Preparation of liposome and niosomes containing the individual solid-state forms of roxithromycin (**AH**, **CS**) and the monohydrate form (**MH**).
- Characterisation of drug delivery vesicles with and without the API in terms of drug entrapment efficiency (EE%), morphology, pH, physical appearance, droplet size and zeta-potential.
- To formulate an emulgel containing these liposomes and niosomes separately as drug delivery vesicles.
- Determination of the stability of the emulgels containing the loaded or unloaded vesicles in accordance with ICH and SAHPRA guidelines. Stability was determined by means of

the following characterisation: pH, viscosity, mass loss, zeta-potential, droplet size and physical appearance. Assay of the API was additionally evaluated for the emulgels containing the API.

- The determination of the concentration of API released from the emulgel by means of membrane release studies.
- The determination of transdermal and topical delivery of the APIs from the emulgel formulation by performing skin diffusion studies followed by tape stripping.

References

- Aucamp, M., Liebenberg, W., Strydom, S.J., van Tonder, E.C. & de Villiers, M.M. 2012. Physicochemical properties of amorphous roxithromycin prepared by quench cooling of the melt or desolvation of a chloroform solvate. *American association of pharmaceutical scientists, PharmSciTech*, 13:467-476.
- Aucamp, M., Stieger, N., Barnard, N. & Liebenberg, W. 2013. Solution-mediated phase transformation of different roxithromycin solid-state forms: Implications on dissolution and solubility. *International journal of pharmaceutics*, 449:18– 27.
- Ayer, J. & Burrows, N. 2006. Acne: more than skin deep. *Postgraduate medical journal*, 82:500-506.
- Bansal, S., Kashyap, C.P., Agarwal, G. & Harikumar, S.L. 2012. A comparative review on vesicular drug delivery systems and stability issues. *International journal of research in pharmacy and chemistry*, 2:704-713.
- Barnes, A.R. 2007. Product stability and stability testing. (*In* Aulton, M.E., ed. *Aulton's pharmaceutics: the design and manufacture of medicines*. 3rd ed. Philadelphia: Elsevier, p.650-665).
- Bawa, Y. 2007. Solvent inclusion properties of Triamterene crystal forms and solubility differences between roxithromycin polymorphic forms. Potchefstroom: NWU. (Dissertation – MSc).
- Bershad, S.V. 2001. The modern age of acne therapy: a review of current treatment options. *The mount Sinia journal of medicine*, 68:279-285.
- Boshoff, S. 2018. Investigation of the stabilising effects of niosomes on the amorphous forms of roxithromycin. Potchefstroom: NWU. (Dissertation – MSc).
- Brittain, H.G. & Grant, D.J.W. 1999. Effects of polymorphism and solid state solvation on solubility and dissolution rate. New York, Basel: Marcel Dekker.
- Bryskier, A. 1998. Roxithromycin: review of its antimicrobial activity. *Journal of antimicrobial chemotherapy*, 41:1–21.
- Csongradi, C. 2015. The effect of solid-state forms on the topical delivery of roxithromycin. Potchefstroom: NWU. (Dissertation – MSc).

Csongradi, C., Du Plessis, J., Aucamp, M.E. & Gerber, M. 2017. Topical delivery of roxithromycin solid-state forms entrapped in vesicles. *European journal of pharmaceutics and biopharmaceutics*, 114:96-107.

Du Plessis, C. 2004. Characterisation of polymorphic, pseudopolymorphic and amorphous Forms of roxithromycin. Potchefstroom: NWU. (Dissertation – MSc).

Escobar-Chávez, J.J., Rodríguez-Cruz, I.M., Domínguez-Delgado, C.L., Díaz-Torres, R., Revilla-Vázquez, A.L. & Aléncaster, N.C. 2012. Nanocarrier systems for transdermal drug delivery. (*In* Sezer, A.D., ed. *Recent advances in novel drug carrier systems*. London: IntechOpen. p.201-240).

Glowka, E., Wisicka--Frakowiak, H., Hyla, K., Stefanowska, J., Jastrzebska, K., Klapiszewski, L., Jesionowski, T. & Cal, K. 2014. Polymeric nanoparticles-embedded organogel for roxithromycin. *European journal of pharmaceutics and biopharmaceutics*, 88:75-84.

Gollnick, H. 2003. Current concepts of the pathogenesis of acne. *Drugs*, 63:1579-1596.

ICH **see** International conference on harmonisation of technical requirements for registration of pharmaceuticals for human use

International conference on harmonisation of technical requirements for registration of pharmaceuticals for human use (ICH). 2003. *Stability testing of new drug substances and products: Q1A(R2)*, p.1-18.

Kumar, G.P. & Rajeshwarrao, P. 2011. Nonionic surfactant vesicular systems for effective drug delivery—an overview. *Acta Pharmaceutica Sinica B*, 1:208-219.

Lavers, I. 2014. Diagnosis and management of acne vulgaris. *Nurse prescribing*, 12:330-336.

Laouini, A., Jaafar-Maalej, C., Limayem-Blouza, I., Sfar, S., Charcosset, C. & Fessi, H. 2012. Preparation, characterization and applications of liposomes: State of the art. *Journal of colloid science and biotechnology*, 1:147-168.

MCC **see** Medicines Control Council

Medicines control council. 2012. *Stability: registration of medicines*. Republic of South Africa, p.1-27. https://www.sahpra.org.za/documents/b22b99e32.05_Stability_Jul12_v7_1.pdf Date of access: 12 Jun. 2019.

- Milne, M., Liebenberg, W. & Aucamp, M.E. 2016. Different amorphous solid-state forms of roxithromycin: a thermodynamic and morphological study. *International journal of pharmaceutics*, 498:304-315.
- Newman, A., Knipp, G. & Zografi, G. 2012. Assessing the performance of amorphous solid dispersion. *Journal of pharmaceutical sciences*, 101:1355-1377.
- Ning, M., Guo, Y., Pan, H., Chen, X. & Gu, Z. 2005. Preparation, in vitro and in vivo evaluation of liposomal/niosomal gel delivery systems for clotrimazole. *Drug development and industrial pharmacy*, 31:375-383.
- Oschsendorf, F. 2006. Systemic antibiotic therapy for acne vulgaris. *Journal der Deutschen dermatologischen gesellschaft*, 4:828-841.
- Ostrowski, M., Wilkowska, E. & Baczek, T. 2010. Impact of pharmaceutical dosage form on stability and dissolution of roxithromycin. *Central European journal of medicine*, 5:83-90.
- Singh, S. & Bakshi, M. 2000. Guidance on conduct of stress test to determine inherent stability of drugs. *Pharmaceutical technology on-line*. <https://semanticsscholar.org/paper/guidance-on-conduct-of-stress-test-to-determine-of-singh-bakshi/fce4347f5ddbc12ac1f74d9f7e7ae6db3be2336b> Date of access: 29 Aug. 2019.
- Swart, M. 2019. Stability of amorphous forms of roxithromycin when encapsulated in liposomes. Potchefstroom: NWU. (Dissertation – MSc).
- Threlfall, T.L. 1995. Analysis of organic polymorphs. *Analyst*, 120:2435-2460.
- Williams, A.C. 2003. *Transdermal and topical drug delivery: from theory to clinical practice*. London: Pharmaceutical press.
- Wosicka-Frackowiak, H., Cal, K., Stefanowska, J., Glowka, E., Nowacka, M., Struck-Lewicka, W., Govedarica, B., Pasikowska, M., Debrowska, R., Jesionowski, T., Srcic, S. & Markuszewski, M.J. 2015. Roxithromycin-loaded lipid nanoparticles for follicular targeting. *International journal of pharmaceutics*, 495:807-815.

CHAPTER 2

FORMULATION AND TOPICAL DELIVERY OF SEMI-SOLID DOSAGE FORMS CONTAINING ROXITHROMYCIN ENCAPSULATED IN VESICLES

2.1 Introduction

Acne is a chronic infection causing prodigious patient discomfort. It is a common problem that burdens more than 85% of adolescents, inducing unprepossessing skin scarring and detrimental psychological damage (Ayer & Burrows, 2006:500; Dunn *et al.*, 2011). The blockage of sebaceous glands, together with the colonisation of *Propionibacterium acnes* is the leading cause of acne (Ayer & Burrows, 2006:500; Bershad, 2001:279). The affected pilosebaceous units of the skin result in either inflammatory or non-inflammatory lesions.

A variety of oral and topical acne treatment options are available and treatment should start as early as possible to minimise skin scarring and psychological damage. The well-known problem with oral therapies is the extensive disadvantages, such as unpredictable absorption due to degradation by stomach acid and enzymes, low solubility, low bioavailability and the mucus barrier preventing drug penetration and subsequent absorption (Ensign *et al.*, 2012:558). The easy non-invasive nature of topical administration, resulting in total patient satisfaction, makes it a very attractive drug delivery option. Topical preparations are also one of the mainstay therapies for acne, which includes antibiotics, however due to the increasing resistance of *P. acnes* towards the available antibiotics, a need for new treatment methods exists (Dessinioti & Katsambas, 2017:164; Feldman *et al.*, 2004:2127; Nakase *et al.*, 2016:160).

The aim of acne treatment should be to reduce non-inflammatory lesions, improving existing inflammation and lowering the *P. acnes* population (Ayer & Burrows, 2006: 501; Feldman *et al.*, 2004:2129). Previous studies showed that *P. acnes* is susceptible to roxithromycin, making it effective in the treatment of mild to moderate inflammatory acne (Csongradi *et al.*, 2017:106; Glowka *et al.*, 2014:83; Wosicka-Frackowiak *et al.*, 2015:816). Currently, there is no topical pharmaceutical product on the market that contains roxithromycin.

The challenge of topical preparations arises when drugs, especially those with a high molecular weight, are poorly lipid soluble resulting in very slow absorption, since the skin is an exceptionally effective barrier (El Maghraby *et al.*, 2008:204; Wang *et al.*, 2003:1612). Altering the solid-state properties of an API can manipulate the physicochemical factors and improve some properties of the API, such as the solubility (Threlfall, 1995:2452), while emulsion-based

gels can also provide an appropriate medium for the delivery of hydrophobic APIs to the skin (Ajazuddin *et al.*, 2013:124).

The skin is composed of various distinct layers, including the lipophilic stratum corneum, which is mainly responsible for the function of being a protective barrier against the loss of physiologically essential substances and to the diffusion of potentially toxic chemicals from the external environment into the body, consequently influencing the topical delivery of drugs (Bouwstra & Ponc, 2006:2081; Foldvari, 2000:417; Naik *et al.*, 2000:318). Overcoming this extraordinary barrier requires the employment of enhancement technologies, such as drug carriers, to contribute to more efficient drug delivery and absorption (Foldvari, 2000:417). In previous studies, liposomes and niosomes have proved to be effective drug carriers (Csongradi *et al.*, 2017:106).

Drug carriers, like liposomes and niosomes, can easily be formulated in preparations such as suspensions, gels or creams (Laouini *et al.*, 2012:160). Compared to other topical drug delivery systems, emulgels show better drug release due to a lack of excess oily bases and insoluble excipients (Ajazuddin *et al.*, 2013:123). Adding vesicle systems to a hydrogel, results in a convenient self-administering dosage form (Ning *et al.*, 2005:376). To guarantee the quality, safety and efficacy of a semi-solid dosage form, stability testing is required (Singh & Bakshi, 2000:2).

The formulation of an emulgel containing liposomes or niosomes as drug carrier systems proposes to deliver roxithromycin topically for the treatment of acne, which can be used as a dual therapy with existing systemic acne treatments.

2.2 Acne

Strauss *et al.* (2007:652) defined acne as a chronic inflammatory dermatosis, which consists of open comedones (blackheads), closed comedones (whiteheads) and inflammatory lesions such as nodules, pustules and papules.

2.2.1 Pathogenesis of acne

Acne is a multifactorial disease, and genetic factors, stress, androgens and excess sweating can all influence the development of the condition (Ayer & Burrows, 2006:500). The four main pathological factors leading to the development of acne are: (1) increased sebum production, (2) hyperproliferation of the follicular epithelium, (3) *P. acnes* proliferation and (4) inflammation of the area (Gollnick *et al.*, 2003:S2).

2.2.1.1 Excess sebum production

The face, chest, upper back and upper arms are known to have a high density of sebaceous glands (Lavers, 2014:330). Increased sebum production and secretion are caused due to androgen hormones, in particular testosterone. The importance of increased sebum production is the direct correlation to the severity and occurrence of acne lesions (Gollnick, 2003:1581).

2.2.1.2 Ductal epidermal hyperproliferation

Follicular hyperkeratinisation leads to a change in the follicular milieu, with consecutive proliferation of bacteria, primarily *P. acnes*. One of the characteristics leading to the development of an acne lesion is hypercornification of the follicle wall (Jeremy *et al.*, 2003:20). The gathering of the abnormal desquamated corneocytes in the sebaceous follicle, along with other lipids and monofilaments, results in comedogenesis (Cunliffe *et al.*, 2003:11). The first step of acne development is the formation of the microcomedone, which forms as a result of abnormal regulation of the cells within the hair follicle. Once the microcomedone forms, dead skin cells and sebum are unable to leave the pore and visible non-inflammatory comedones and inflammatory acne lesions are developed. If sebum continues to accumulate, the closed comedone will continue to expand and may rupture into the surrounding tissue (Gollnick, 2003:1589).

2.2.1.3 *Propionibacterium acnes*

The microflora present in a normal sebaceous follicle includes three coexisting groups of bacteria, namely (1) coagulase-negative staphylococci (*Staphylococcus epidermidis*), (2) anaerobic diphtheroids (*P. acnes* and *Propionibacterium granulosum*) and (3) lipophilic yeasts (*Pityrosporum* species) (Burkhart *et al.*, 1999:328). *P. acnes* colonises in sebaceous follicles and are therefore prevalent in areas of the skin densely packed with sebaceous follicles. These sebaceous follicles produce a rich lipid, anaerobic environment, causing the anaerobic, gram-positive pathogen (*P. acnes*) to flourish (Gollnick, 2003:1585).

Even though all individuals have *P. acnes* present on the surface of the skin, not all individuals will present with acne due to the differences in individual immune response to the pathogen (Webster, 2001:16). According to McInturff and Kim (2005), a lipase enzyme produced by *P. acnes* metabolises the triglycerides of sebum into glycerol and fatty acids, which may in turn assist in the formation of comedones and the inflammation that follows. Being the most plausible organism to cause *Acne vulgaris*, *P. acnes* is the target of oral and topical antibiotic treatments (Burkhart *et al.*, 1999:328).

2.2.1.4 Immune reactions and inflammation

High sebum concentrations and follicular hyperkeratinisation causing proliferation of *P. acnes* leads to increased production of the pro-inflammatory cytokines, interleukin-1 α (IL-1- α) and tumour necrosis factor- α (TNF- α) by T-cells and keratinocytes, this leads to the proliferation of both cell types. Not being able to differentiate by apoptosis, follicular keratinocytes produce hypergranulosis which is similar to the impermeable skin outer layer, resulting in the formation of microcomedones (Gollnick, 2003:1580).

Further inflammatory responses lead to the development of increasing degrees of severity in inflammatory forms of acne (Jeremy *et al.*, 2003:20). The release of chemostatic factors, such as lymphocytes, neutrophils and macrophages, cause follicular damage resorting in the rupture and leakage of bacteria, fatty acids and lipids into the surrounding dermis, giving rise to inflammatory lesions (pustules, nodules, cysts and papules), which are filled with pus and are larger than non-inflammatory lesions (Feldman *et al.*, 2004:2123).

2.3 Novel acne treatment

The treatment of acne aims to reduce sebum production, bacterial proliferation and inflammation, and normalise increased cell turnover, with the aim of preventing lesion development and treating existing lesions (Lavers, 2014:334).

According to Csongradi *et al.* (2017), there is wide variation in existing acne regimens; it can be treated topically or systemically with oral drugs, while other treatment options include the use of natural products or the use of non-drug treatments.

Due to growing resistance in acne treatment an opportunity arises to investigate and develop a novel topical acne treatment (Dessinioti & Katsambas, 2017:164; Feldman *et al.*, 2004:2127; Nakase *et al.*, 2016:160).

2.3.1 Topical antibiotics

Topical antibiotics are commonly prescribed for mild to moderate inflammatory lesions. First-line treatment currently consists of clindamycin or erythromycin, which has activity against *P. acnes* and reduces the stimulus for inflammation of the lesions. In recent years, due to the continuous use of this first-line treatment, resistance against *P. acnes* strains has surfaced (Lavers, 2014:334). To ensure the effective treatment of *P. acnes* a derivate from erythromycin was developed, known as roxithromycin (Glowka *et al.*, 2014:76; Wosicka-Frackowiak *et al.*, 2015:808).

2.4 Roxithromycin as acne treatment

Roxithromycin ((E)-erythromycin-9-[O-[(2-methoxyethoxy)methyl]oxime]) is classified as a second generation macrolide antibiotic, with semi-synthetic properties assembled in a 14-membered lactone ring with a desosamine sugar unit and a cladinose sugar unit (Bryskier, 1998:1). Although the *in vitro* spectrum of antibacterial activity of roxithromycin is similar to erythromycin, it has a greater potency as well as longer action, whilst another advantage to consider is fewer undesired interactions than erythromycin because of roxithromycin's lower cytochrome P450 (CYP450) activity (Glowka *et al.*, 2014:76; Ostrowski *et al.*, 2010:83). The bacteriostatic, as well as bactericidal effects, deems it an ideal candidate for acne treatment.

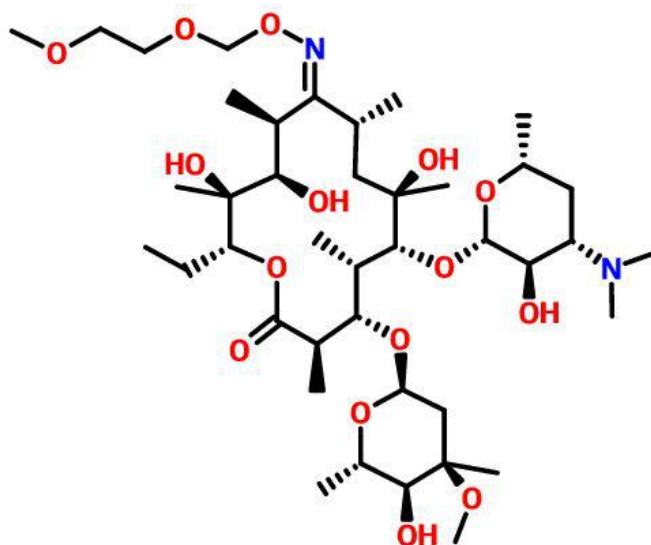


Figure 2.1: Structure of roxithromycin (Drugbank, 2019)

2.4.1 Mechanism of action

Roxithromycin inhibits the production of inflammation causing compounds, such as lipase, by binding to the 50s ribosomal subunit of bacterial micro-organisms; protein synthesis and growth are inhibited in return (Drugbank, 2019; MacDougall & Chambers, 2011; Wosicka-Frackowiak *et al.*, 2015:808). Concentration dependant bacteriostatic and bactericidal effects are possessed by roxithromycin (Medsafe, 2017).

2.4.2 Physicochemical information

Roxithromycin monohydrate ($C_{41}H_{76}N_2O_{15}$) is characterised by a molecular weight of 837.058 g/mol with a octanol-water partition coefficient (log P) of 3.1 and octanol-buffer distribution coefficient (log D) value of 1.52 (Pubchem, 2019; Csongradi, 2015). It can be described as a white crystalline powder, which is a semi-synthetic macrolide antibiotic with similar properties to erythromycin (Pubchem, 2019). It is an anhydrous substance with 96.0 –

102.0% content; meant to be stored in a cool, dry place in a closed container protected from sunlight and moisture (Medsafe, 2017). According to Guidlink (2017:1), it is very slightly soluble in water, only slightly soluble in diluted hydrochloric acid and freely soluble in acetone, alcohol and methylene chloride. It has been found to have a melting point of 116 – 122°C and pKa value of 9.17 (Aucamp *et al.*, 2012:469; Qiang & Adams, 2004:2878).

2.4.3 Clinical uses, adverse effects and contra-indications of roxithromycin

Macrolides, such as the likes of roxithromycin, can be used in the treatment of infections of the respiratory tract, genitourinary tract, skin, soft tissue and orodental infections caused by gram-positive cocci and bacilli, or gram-negative cocci and some bacilli (Bryskier, 1998:1; Ostrowski *et al.*, 2010:83). Roxithromycin is the API of choice for this study, since it has been proven to be effective in the treatment of *P. acnes* (Katz & Mankin, 2009:532-534). The sebaceous follicles in the dermis (where *P. acnes* gather) will be the target-site, as a bacteriostatic effect can be obtained with topical application, while avoiding systemic circulation greatly.

Even with topical dosage forms, possible systemic toxicity should be accounted for as precaution. Gastrointestinal symptoms are common with roxithromycin use; this includes but is not limited to abdominal pain, diarrhoea or nausea and vomiting. Less common side-effects to take note of include hypersensitivity reactions such as headache, pruritus or skin rash (MIMS, 2016:278). Despite its lower affinity to CYP450, it can interact with other drugs and is contra-indicated in patients with known macrolide hypersensitivity or impaired hepatic function, as well as patients using astemizole, cisapride, ergot alkaloid derivatives, pimozone or terfenadine (MIMS, 2016:278).

2.5 Solid-state properties of active pharmaceutical ingredients

The majority of drug products exist at some point as a solid material before formulation or dissolution. A single API can exist in several solid-state forms (Brittain, 2009:1), with these different solid-states of the same drug presenting with diverse properties due to differences in crystal order (Vippagunta *et al.*, 2001:4). Using alternative solid-state forms of a specific drug provide an opportunity to resolve formulation or delivery problems of abandoned APIs, such as the poor water soluble roxithromycin, while cutting the costs of developing an entire new drug (SSCI, 2019; Biradar *et al.*, 2006:22).

2.5.1 Classification, structure and stability of solid-state forms

A solid-state compound can belong to the crystalline or amorphous group (Lee *et al.*, 2008:580), as schematically represented in Figure 2.2.

2.5.1.1 Crystalline form

The most common solid-state that occurs in the industry is the crystalline form, where the structural units are similar and indefinitely recurrent in a three-dimensional space (Vippagunta *et al.* 2001:4). According to Florence and Attwood (2015:8), the highly ordered arrangement of molecules and atoms of crystals are bound by non-covalent interactions. Polymorphs originate from crystalline solids when the material crystallises into different crystal packaging arrangements or conformations and can be subdivided into multicomponent crystals, such as hydrates, solvates or co-crystals (Vippagunta *et al.*, 2001:4). Arora and Zaworotko (2009:282) defines co-crystals as "...a stoichiometric multiple component crystal in which all components are solid under ambient conditions when in their pure form." They further claim that a co-crystal is inclusive of a co-crystal former together with a target ion or molecule coexisting in a single crystal. Hydrates form when a crystal entraps water, while solvates (also known as solvatomorphism) entrap solvents during crystallisation; these solvents occupy voids in the crystal and therefore the crystal lattice is not destroyed by desolvation (Florence & Attwood, 2015:25). Crystalline solids and polymorphs possess identical chemical composition, meaning they will have the same chemical behaviour in solution regardless of the form in which it is presented, although bioavailability, adaptability for manufacture and toxicity may most likely differ (Florence & Attwood, 2015:14).

2.5.1.2 Amorphism

Amorphous solids are less stable than their crystalline counterparts because they exist in a higher energy state. They are more likely therefore to display greater chemical reactivity, causing them to convert back to the more thermodynamically stable crystalline form during handling and storage (Florence & Attwood, 2015:25). Amorphous forms have been investigated since 1969 (Simonelli *et al.*, 1969) to secure higher solubility and faster dissolution of APIs. The two types of amorphous forms known are the neat API and amorphous solid dispersions (ASDs). Even though these forms have vast microscopic variances, they both have the potential to increase solubility and the dissolution rate. A neat API consists only of the pure drug, and the packing of the molecules results in a weakened attractive energy between the drug molecules; thus the drug molecules have a low energy barrier to go into solution. In an ASD, the energy barrier of the drug molecules are disrupted by dispersion in a solid medium (Bellantone, 2014:3).

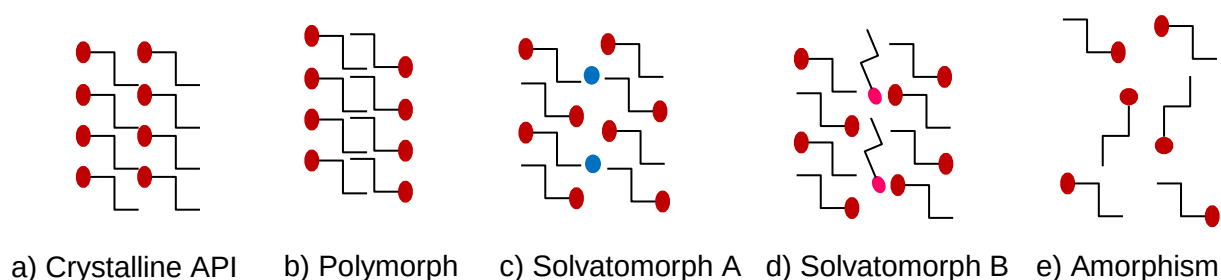


Figure 2.2: Schematic representation of solid-state forms with a) single component API, b) polymorph of single component API, c) hydrate or solvate of API, d) solvate of API and e) amorphous form of API (Adapted from Arora *et al.*, 2009:283)

2.5.2 Thermodynamics of solid-state forms

The dissolved and undissolved form of an API should be taken in consideration when discussing the typical behaviour of material based on energy contents. A dissolved drug can be envisioned as replacing holes in a solvent with drug molecules to form a uniform dispersion in the matrix, where the drug and solvent molecules interact with one another (Bellantone, 2014:7). Although dissolution has been described by numerous models, the Hildebrand-Scott model remains well-known and trusted (Hildebrand & Scott, 1950). Their model embraces the differences in interaction between the pure state, drug *versus* drug or solvent *versus* solvent, and the solution state between drug *versus* solvent. The “hole” model as described by Martin *et al.* (1983) and Hill (1986) can be applied to calculate these previously mentioned differences, where the holes and drug molecules are taken through a sequence of theoretical steps. As the Gibbs free energy is a state function of thermodynamics, these theoretical steps do not have to transpire in reality, but they still offer a way to calculate the changes in energy due to dissolution (Klotz & Rosenberg, 1964). The changes in Gibbs energy only take the initial and final state into account, which corresponds with “Hess’ law” of thermodynamics, as stated by Atkins (1998). Dissolving any solid-state in a fluid solvent is demonstrated by the sequence illustrated in Figure 2.3. The lower activation energy of amorphous systems is due to starting at a higher level than the crystalline API, leading to faster dissolution kinetics, but then again all liquid forms of an API have corresponding Gibbs energy regardless of the initial solid-state (Bellantone, 2014:9). Amorphous forms have advantages over their crystalline equivalent, such as superior solubility and bioavailability, as they are more energetic due to the absence of long-range order (Ilevbare *et al.*, 2010:9).

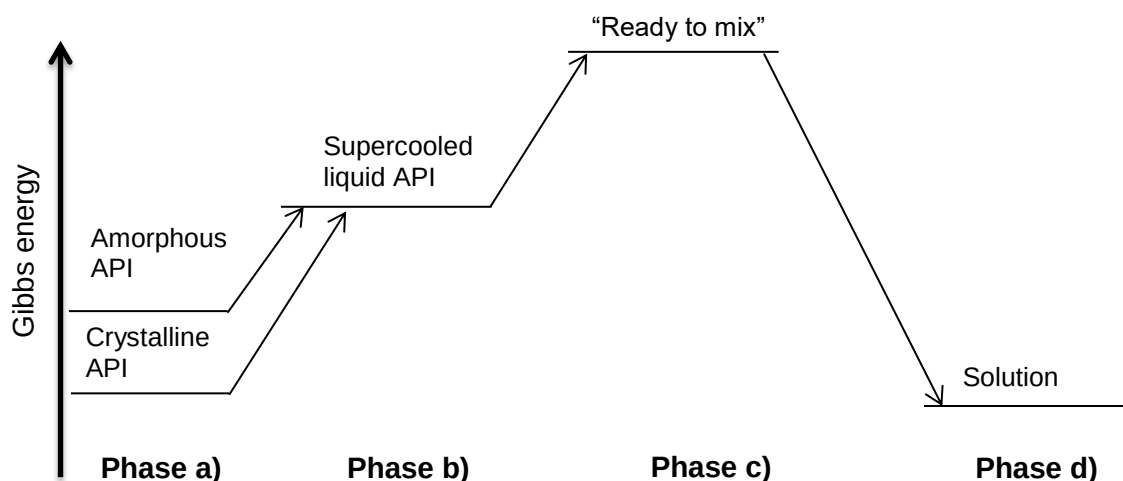


Figure 2.3: Gibbs energy diagram for API dissolution, with phases: a) initial state of undissolved API, b) supercooled liquid API and empty solvent, c) separate drug molecules and holes in the solvent and, d) final solution with dissolved drug in solvent (Adapted from Bellantone, 2014:9)

Results obtained by Aucamp *et al.* (2013:26) underlined the importance of the different solid-state forms during or after product formulation. It was also stated that known metastable amorphous forms are extremely likely to undertake solution-mediated conversion; this statement is also supported by Brittain (2009:34) and Britannica (2009), where it is claimed that it is inevitable for polymorphic and amorphous states not to be subjected to solution-mediated transformation. Roxithromycin is rendered in an amorphous state when thin films are prepared, regardless of whether the crystalline or amorphous forms are used during preparation. An ASD is prepared when the API is combined with excipients such as cholesterol, phosphatidylcholine and Span® 60, resulting in the amorphous state of the API. According to Van den Mooter (2012:e79), ASDs are particularly used to increase the bioavailability of poorly soluble APIs, such as roxithromycin. When an API and polymer is dissolved in a suitable solvent, it results in an ASD (Newman *et al.*, 2012:1356).

2.5.3 Different solid-states of roxithromycin

As mentioned before, an API can exist in more than one form; it is important therefore to be familiar with the properties of the solid-state form used during production as any change in the crystal form can alter the bioavailability of an API. Pappa-Louisi *et al.* (2001:57) stated that roxithromycin gained a lot of interest over the past few years due to its high stability in an acid environment, such as the stomach, increased oral bioavailability and favourable pharmacokinetic performance. Many completed studies focused on identifying possible crystal forms of roxithromycin. Du Plessis (2004) described six different forms of roxithromycin including polymorphic, pseudopolymorphic and amorphous forms. Bawa (2007) described

recrystallised forms of roxithromycin from chloroform, ethyl acetate, dichloromethane and acetonitrile, while Aucamp *et al.* (2012) investigated chloroform solvate and chloroform desolvate solid-state forms of roxithromycin. Aucamp *et al.* (2013) discussed solution-mediated changes of an anhydrate and amorphous form of roxithromycin. It is evident that several solid-state forms of roxithromycin exist, while the challenge of poor oral bioavailability (only 50%) due to low aqueous solubility of the API has not yet been fully resolved (Van Niekerk, 2011:xviii). Taking the latter into consideration, it is worthwhile to investigate alternative methods for the use of solid-state roxithromycin, such as topical treatments, seeing as none are currently available.

2.6 The skin and topical/transdermal drug delivery

Topical and transdermal administration of drugs has gained popularity over the past few years, as they offer excellent patient compatibility, while avoiding first-pass metabolism resulting in fewer side-effects than their oral counterparts. Transport of a drug to viable epidermal or dermal tissue followed by systemic circulation of the administered drug is known as transdermal delivery, while topical delivery aims to retain the administered drug in a certain skin layer identified as the target-site (Roberts *et al.*, 2017:87; Williams, 2013:676). Having a good understanding of the structure and function of the skin layers will contribute to successful transdermal and topical drug delivery.

2.6.1 Advantages and limitations of the topical drug delivery route

When choosing a delivery route, the advantages and disadvantages of all viable options should be considered to determine if the selected route is the most effective and efficient option. Some advantages of topical and transdermal drug delivery to consider over other delivery systems are (Prausnitz & Langer, 2008:1261; Shingade *et al.*, 2012:66):

1. Not exposed to first-pass metabolism;
2. Simple to use with several accessible sites for absorption;
3. Reduced side-effects due to absent or low plasma concentration levels;
4. Easy elimination in case of toxicity;
5. Better patient compliance with reduced inter- and intra-patient variability;
6. Generally inexpensive.

The following challenges should be kept in mind (Ghulaxe & Verma, 2015:3; Shingade *et al.*, 2012:66):

1. A limited number of drugs are pliable to be administered by this route;

2. Molecule size can limit penetration through the stratum corneum if the molecular weight surpasses 500 Da;
3. Hydrophilic drugs result in poor delivery;
4. Extreme partition coefficients can cause drugs not to reach blood circulation;
5. Only very potent drugs with small concentrations can overcome the skin barrier;
6. Skin irritation or similar adverse effects may be experienced with this route.

2.6.2 Structure and function of the skin

Pharmaceutical substances can be conveniently administered to the skin for topical and systemic therapeutic effects (Benson & Watkinson, 2012:3; Naik *et al.*, 2000:319). The skin is the biggest protective barrier against chemical, physical, pathogen and other external factors (Dayan, 2005:67; Hadgraft, 2004:291). Other than the primary function of providing a barrier, the skin also maintains a homeostatic balance to the body, while regulating blood pressure and body temperature (Benson & Watkinson, 2012:3). The skin is also a significant sensory organ and plays a central role in Vitamin D production (Menon, 2002:4), and is composed of various different cells, divided in four anatomical layers, i.e. non-viable stratum corneum, viable epidermis, viable dermis and hypodermis (Pathan & Setty, 2009:174). A basic understanding of every skin layer can contribute to successful drug application to achieve optimal results (Benson & Watkinson, 2013:3; Kute & Saudagar, 2013:272).

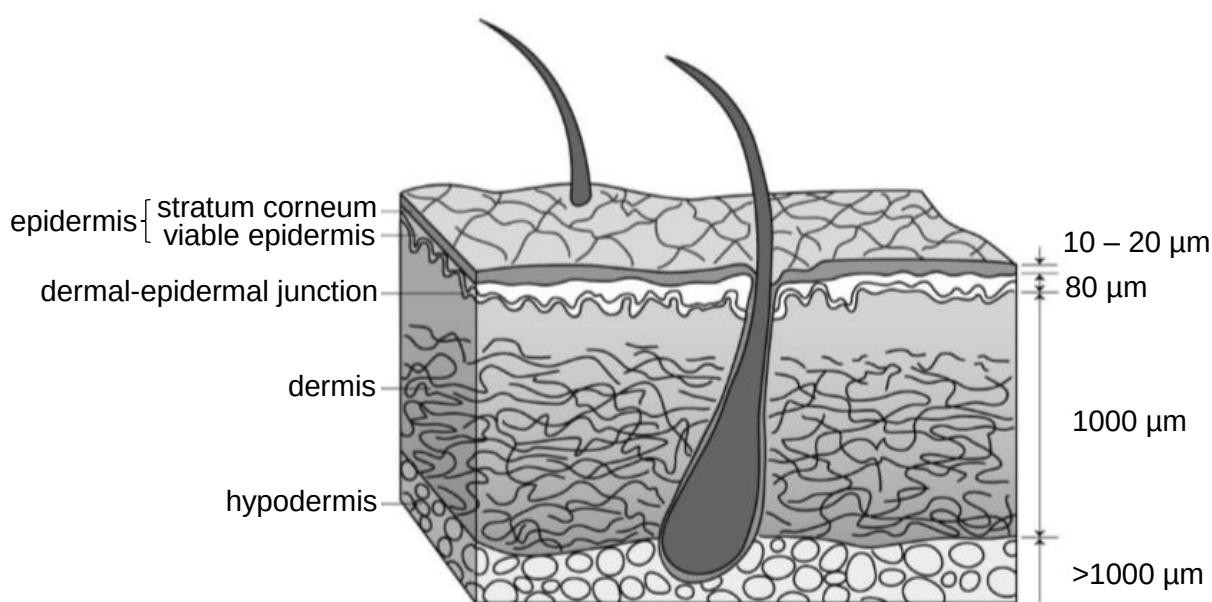


Figure 2.4: Simplified representation of the four major layers of the human skin and their size (adapted from Geerligs, 2010:4)

2.6.1.2.1 The epidermis

The epidermal layer consists of a non-viable and a viable layer, which consist of melanocytes, keratinocytes and Langerhans cells (Williams, 2013:678). The viable layer can be separated in three separate layers: stratum basale, stratum spinosum and stratum granulosum, with the non-viable layer being identified as the stratum corneum (Asbill & Michniak, 2000:36).

2.6.2.1.1 The non-viable epidermis

The outmost layer of the epidermis is compiled of 10 – 15 layers of corneocytes and is known as the stratum corneum, or the non-viable epidermis (Foldvari, 2000:417). According to Barry (2001:101), the stratum corneum function as a rate-limiting hindrance for drug delivery due to numerous layers consisting of proteins and lipids, creating an interlocking resistant complex structure. Washington *et al.* (2001:183) described this complex structure as a “brick and mortar” structure, where keratin-filled corneocytes are identified as bricks and the intercellular matrix, compiled of long chain ceramides, triglycerides, free fatty acids, cholesterol sulphate, sterol esters and cholesterol, which surrounds the cells - the mortar. Due to the intercellular matrix, hydrophilic drugs have a difficult time permeating this layer (Williams, 2013:680). Permeation enhancement is to bypass or disrupt this challenging “brick and mortar” area (Barry, 2006:5).

2.6.2.1.2 The viable epidermis

The first layer of living cells in the outmost layer, consisting of keratinocytes and dermal fibroblasts, are identified as the viable epidermis (Goyal *et al.*, 2016:77). Epidermis cells widely distributed within include melanocytes responsible for producing pigment, Langerhans cells responsible for immunological reactions, Merkel cells attributing sensing sensations and keratinocyte cell types (Kolarsick *et al.*, 2011:203; Ng & Lau, 2015:7; Venus *et al.*, 2010:471; Wickett & Visscher, 2006:S98). The high fraction of water in this layer creates a barrier against lipophilic materials due to their great affinity for a non-polar aqueous environment (Jepps *et al.*, 2013:155). The stratum corneum is maintained by the viable epidermis through proliferation and differentiation of keratinocytes (Brown *et al.*, 2006:175; Williams, 2003:1).

2.6.2.2 The dermis

The largest skin layer, creating a physical barrier for large molecules, is found in the middle of the accumulated layers and is known as the dermis (Sharma *et al.*, 2013:287-288). It is compiled of blood and lymphatic vessels, sensory nerve endings, connective tissue, hair follicles, pilosebaceous and sweat glands, according to Foldvari (2000:418). The layer mostly contains elastin and collagen, which provide elasticity and strength to the skin, surrounded by mucopolysaccharide gel creating a hydrophilic environment (Jepps *et al.*, 2013:677; Williams, 2013:677).

2.6.2.3 The hypodermis

The deepest skin layer is the hypodermis, which consists of fatty tissue composed of lipocytes (Escobar-Chávez *et al.*, 2012:205; Franz & Lehman, 2000:16). The hypodermis serves as a thermal barrier and acts as a shock absorber, while securing underlying muscle (Williams, 2013:677; Yagi & Yonei, 2018:51).

2.6.3 Skin penetration mechanisms

To reach a transdermal or topical destination, a drug molecule has to follow a complex pathway when applied to the skin. The intercellular, transappendageal and transcellular routes, as illustrated in Figure 2.5, are three possible pathways a molecule can follow in combination to reach the destined target-site (Benson & Watkinson, 2012:14).

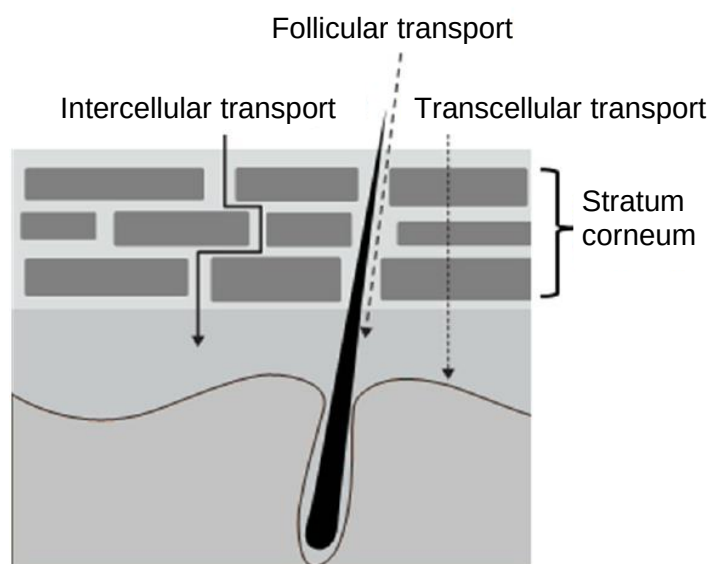


Figure 2.5: Potential transport pathways for transdermal delivery (adapted from Rahimpour & Hamishehkar, 2012:142)

2.6.3.1 Intercellular route

Small and uncharged molecules can follow the intercellular route by diffusing through the lipid matrix with more ease than other molecules (Williams, 2003:34). This is an important pathway for API permeation across the lipid stratum corneum; penetration enhancement techniques are usually the primary focus when considering this pathway (Benson, 2005:24; El Maghraby *et al.*, 2008:205).

2.6.3.2 Transappendageal route

The hair follicles and sweat glands provide direct diffusion for ions and large polar molecules into the epidermis and permeation into the dermis (Ranade & Hollinger, 2004:214). It must however be kept in mind that the small surface area and factors such as sweat and lipophilic sebum in hair follicles can hinder API transport to the target-site (Barry, 2001:101; Morrow *et al.*, 2007:38; Williams, 2003:31-32).

2.6.3.3 Transcellular route

This route is especially fit for highly hydrophilic and polar molecules, as the API moves through the stratum corneum passing both the corneocytes and the lipid matrix (Morrow *et al.*, 2007:38-39). The aqueous compartment surrounded by a lipid compartment can limit the API penetration, as it has to partition and diffuse through the multiple layers before reaching the intended site (Williams, 2013:679).

2.6.4 Mathematical diffusion model

To visualise the permeation process of a molecule through the skin, and to assist in the design and formulation of APIs, mathematical principles and equations are applied (Mitrugotri *et al.*, 2003:71; Williams, 2013:680).

Passive diffusion is the biggest contributor to molecule permeation through the skin, where the molecules spontaneously diffuse from the surface of the stratum corneum to the underlying epidermis and dermis containing a low drug concentration (Surber *et al.*, 1999:396). This transport process follows Fick's first law, which states that the rate of diffusion across an area of skin is proportional to the determined concentration gradient, as seen in Equation 2.1 (Williams, 2003:41-42,47).

$$J = \frac{K \times D}{h} \Delta C \quad \text{Equation 2.1}$$

where:

J = API flux across the stratum corneum ($\mu\text{g}/\text{cm}^2.\text{h}$);

K = partition coefficient of the API;

D = diffusion coefficient of the API (cm^2/h);

ΔC = concentration difference on either side of membrane ($\mu\text{g}/\text{cm}^2$);

h = thickness of the membrane or path length of diffusion (cm).

2.6.5 Determining factors for skin permeation

Several factors can influence API delivery for topical or transdermal effect, with the two main groups to consider being biological and physicochemical factors (Reddy *et al.*, 2014:1097).

2.6.5.1 Biological factors

Biological factors are more difficult to control than physicochemical factors and refer to skin hydration, skin age, blood flow, skin condition, pigmentation, skin metabolism and temperature (Jhawar *et al.*, 2013:49). Skin properties are unique for every human, it should be kept in mind that it can affect API permeation.

2.6.5.2 Physicochemical factors

The transport of vehicles through the skin is largely influenced by physicochemical factors (N'Da, 2014:20785). Optimal physicochemical properties can be compared to API contenders to determine suitability for transdermal delivery (Liu *et al.*, 2016:437).

2.6.5.2.1 Aqueous solubility

The ideal transdermal drug should possess both hydrophilic and lipophilic solubility properties to permeate comfortably through all skin layers. An aqueous solubility close to or equal to 1 mg/ml ensures optimal transdermal delivery and bioavailability of a drug (Naik *et al.*, 2000:319; Williams, 2003:37). Different solid-state forms possess dissimilar solubility due to varying lattice energies. Brittain and Grant (1999:281) reported that the attracting and repulsing forces of a crystal-solvent interface can affect the dissolution rate. Roxithromycin monohydrate has a less than ideal solubility of 0.0335 mg/ml (Aucamp *et al.*, 2013:26). Ponmalar (2017:62) reported a 5-fold improvement in aqueous solubility of the chloroform solvate form of roxithromycin compared to the raw material. Milne *et al.* (2016:313) and Aucamp *et al.* (2013:24) confirmed a 3-fold improved solubility ratio of specific roxithromycin amorphous forms compared to its crystalline counterpart. Aucamp *et al.* (2013:26) also determined the experimental peak solubility of roxithromycin monohydrate as 0.035 mg/ml, and 0.235 mg/ml for roxithromycin anhydrate in distilled water at 25°C; it can thus be concluded that other crystalline solid-state forms of roxithromycin can possibly improve the aqueous solubility. It is evident that using an altered solid-state form of roxithromycin raw material could improve solubility and thus skin penetration.

2.6.5.2.2 Diffusion coefficient

The ease with which a molecule can diffuse through a vehicle and into a membrane is referred to as the diffusion coefficient (Williams, 2003:27). As seen in Equation 2.1, it is a determining

factor for successful drug diffusion. If a constant temperature is maintained, the diffusion coefficient is dependent on the specific properties of the compound and the diffusion medium, as well as the interaction between them (Reddy *et al.*, 2014:1098).

2.6.5.2.3 Drug concentration

The drug concentration is directly proportional to the drug flux; the maximum flux is thus reached when a saturated solution is achieved (Barry, 2002:512). If the drug concentration over the skin surface is higher than the drug concentration in the underlying epidermis and dermis, the concentration gradient will, in turn, also be higher leading to increased diffusion due to passive diffusion (Sharma *et al.*, 2011:75).

2.6.5.2.4 Ionisation, pH and pKa

A pH value lower than 3.0 or higher than 9.0, can cause skin damage and negatively impact permeation of an API (Naik *et al.*, 2000:319). The stratum corneum has a pH of 4.2 – 5.6, whilst the viable epidermis ranges between 7.3 – 7.4 (N'Da, 2014:20787). The pH of an API determines the ionisation, which in turn influences the membrane gradient (Barry, 2002:511; Jhawar *et al.*, 2013:49). Unionised molecules are known for better skin permeation, therefore it is important to determine the state of ionisation of an API (Williams, 2003:38). Equations 2.2 and 2.3 are an adaption of the Henderson-Hasselbalch equation to determine ionisation:

$$\% \text{ionised} = 100 / 1 + \text{antilog} (\text{pKa} - \text{pH}) \quad \text{Equation 2.2}$$

$$\% \text{unionised} = 100 - \% \text{ionised} \quad \text{Equation 2.3}$$

The pKa value of roxithromycin is reported to be 9.08 (Drugbank, 2019). At a pH value of 7.4 the calculated percentage ionisation is 99.17%, indicating difficult skin permeation if the API is used in its raw form.

2.6.5.2.5 Melting point

Organic compounds with a low melting point have the tendency to possess higher aqueous solubility, leading to better skin permeation due to the ability of solvents to easily enter the crystalline structure and dissolve the molecules (N'Da, 2014:19). According to Naik *et al.* (2014:19), a melting point lower than 200°C is favourable for successful transdermal absorption, while Santa Cruz Biotechnology (2007) reported the melting point of roxithromycin as 116 – 122°C, making it a good candidate for skin penetration.

2.6.5.2.6 Molecular size

The size of a molecule is a determining factor during skin permeation, as small molecules can diffuse fast and effortlessly, while skin diffusion proves challenging for large molecules (Williams, 2003:36). A direct correlation is also suggested between molecular weight and transdermal flux, with the ideal molecular mass smaller than 500 g/mol, or 500 Dalton, to achieve optimal diffusion (Barry, 2002:513). With a molecular weight of 837.04 g/mol for roxithromycin, as reported by O'Neil (2001:1486), it may prove more challenging to achieve effective transdermal delivery. Foldvari (2000:417) however stated that the limitation of 500 Da is not trustworthy, as it does not take novel methods into account that are able to improve permeation through the skin. The previous statement was supported by Billich *et al.* (2005:3157), whom achieved successful skin permeation by means of passive diffusion with molecules with a larger molecular weight of 500 Da.

2.6.5.2.7 Partition coefficient

The redistribution of molecules between an oil and water phase is defined as the partition coefficient, which is indicative of aqueous or lipid diffusion ability of a drug in a membrane (Jhawar *et al.*, 2013:49). The log P value is indicative of the hydrophilicity or lipophilicity of an API as a result of the partitioning of an API between the hydrophilic cells of the epidermis and lipophilic stratum corneum. A log P value between 1 and 4 indicates optimal dermal absorption, demonstrating solubility in both aqueous and oil phases (Akhlaq *et al.*, 2014:178). The log P value refers to the octanol-water partition coefficient, while the log D value refers to the octanol-buffer distribution coefficient. Csongradi (2015:178) determined a log D value of 1.52 for roxithromycin, indicating good suitability for skin permeation.

2.7 Approaches to successful topical/transdermal delivery

A limited amounts of drugs are commercially available for transdermal application due to the daunting task of overcoming the barrier function of the skin (Prausnitz *et al.*, 2004:116; Williams, 2013:680). Enhancing drug delivery for transdermal application is essential in current therapy (Barry, 2001:101). An assortment of concepts has been proposed to bridge the transdermal application shortcomings, with special consideration given to lipophilic drugs and drugs with big particle size (Vogt *et al.*, 2016:3). One of the most popular concepts is the use of vesicular drug delivery systems, which is used to overcome limited skin penetration and poor drug bioavailability (Bansal *et al.*, 2012:704). By applying enhancing methods, such as vesicle delivery systems, novel or existing drugs can achieve greater therapeutic effect when applied to the skin (Costa & Santos, 2017:405; Mota *et al.*, 2017:8; Planz *et al.*, 2016:90).

2.7.1 Carrier systems

Lipid-based carrier systems can improve the efficacy of a drug while providing controlled and targeted specific release of an API, especially profiting drugs presenting with poor solubility properties (Planz *et al.*, 2016:90; Zhai & Zhai, 2014:91). Vesicles are the most favoured vesicular system according to Honeywell-Nguyen and Bouwstra (2005:67). Vesicles can be defined as well-ordered gatherings of phospholipid bilayers, which occur when amphiphilic lipid components encounter water molecules (Bansal *et al.*, 2012:704; Kumar & Rajeshwarrao, 2011:209). The amphiphilic constituents attribute to API maintenance, ensuring maximum API availability at the target-site with minimal side-effects, while also enabling both hydrophilic and lipophilic drugs to be incorporated into the carrier systems (Bansal *et al.*, 2012:704-705). The following functions ensure delivery of the API into the skin with the use of vesicle systems (Alexander *et al.*, 2012:33; Honeywell-Nguyen & Bouwstra, 2005:68):

1. Ensuring controlled, steady and sustainable API release by acting as a depot;
2. Limiting systemic absorption by acting as a rate-limiting barrier;
3. Delivering molecules of the API across undamaged skin;
4. Providing prolonged release of API molecules to the target-site;
5. Serving as penetration enhancer by possibly modifying lipids within the stratum corneum.

It is evident that vesicular systems may provide a solution to overcome the challenges that transdermal and topical delivery, as well as unfit APIs present. The physicochemical properties of vesicle systems are mainly decided by the ingredients used and the preparation technique (Kumar & Rajeshwarrao, 2011:209). Non-ionic surfactants and phospholipids usually serve as the main ingredients to compose vesicle systems referred to as liposomes and niosomes (Alexander *et al.*, 2012:33).

2.7.1.1 Interaction of vesicular systems with human skin

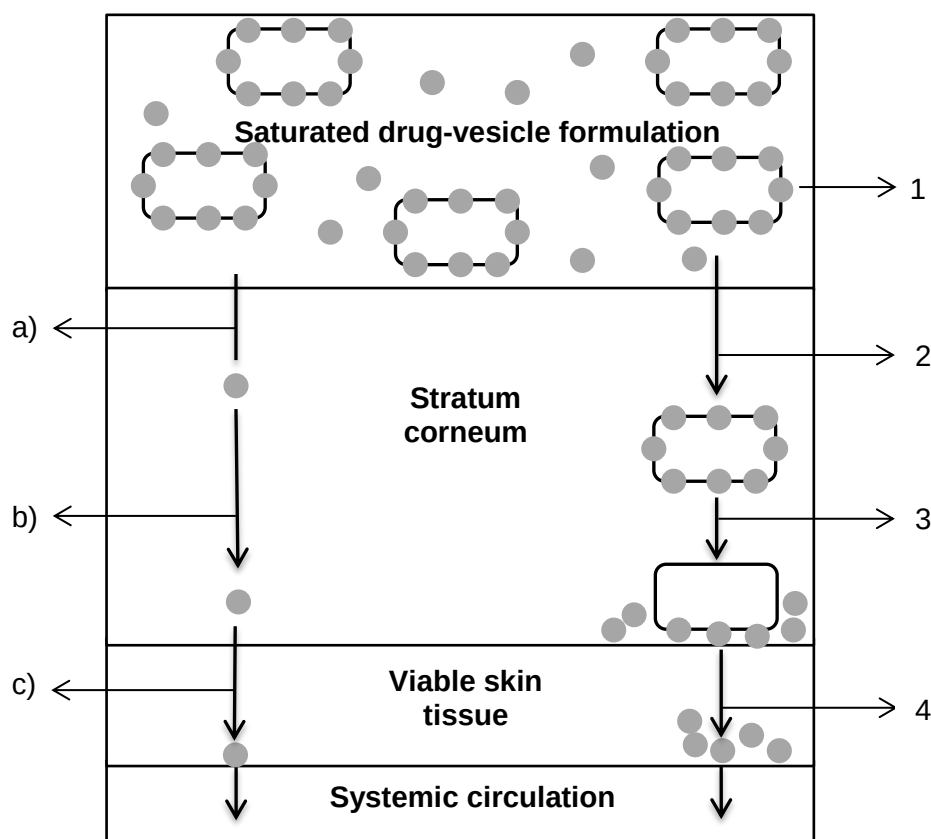


Figure 2.6: Schematic presentation of elastic vesicular API transport within the skin. The API transport from buffer solutions are described as follows: a) partitioning of free API into the stratum corneum; b) diffusion of free API in stratum corneum, and c) partitioning of free API into viable skin tissue (Adapted from Honeywell-Nguyen & Bouwstra, 2008:220)

With three possible pathways as previously discussed, vesicular systems have the upper hand over most common drugs permeating only via the intercellular lipid matrix (Choi & Maibach, 2005:210). To gain a better understanding, Hofland *et al.* (1994:660) and Hofland *et al.* (1995:854) visualised vesicle-skin interaction, where they were able to observe two separate interaction types. The first was between the skin and formulation interface, where new structure formation occurred due to adsorption and fusion of liposomal vesicles into the surface of the stratum corneum layer; the second was the vesicle and skin interaction, which involved modification of the bilayer structures located in the deeper stratum corneum layers. It is possible that the permeation enhancement properties of liposomes and niosomes occur mainly in the intercellular lipid of the stratum corneum, in turn increasing the stratum corneum's fluidity (Choi & Maibach, 2005:210).

The four steps of API permeation enhancement, as observed by elastic vesicles, are illustrated in Figure 2.6 as follows: 1) The API associates with the bilayers of the vesicles, 2) the vesicles

partition into the stratum corneum, 3) the API is released from the vesicles, and 4) the free API is partitioned into the viable tissue.

2.7.1.2 Liposomes and niosomes

The first vesicular systems introduced in the mid-60's were liposomes, with niosomes following in the 70's promising fewer drawbacks than their counterpart (Costa & Santos, 2017:405-406; Kumar & Rajeshwarrao, 2011:208). Liposomes are phospholipid-based vesicles, while niosomes are non-ionic surfactant containing vesicles, sharing the following similarities (Choi & Maibach, 2005:210):

- They are non-toxic;
- They are biodegradable;
- Both have an amphiphilic nature;
- Both modulate drug release properties;
- Their efficiency depends on their physiological properties.

The differences between liposomes and niosomes are presented in Table 2.1 (Rahimpour & Hamishehkar, 2012:145).

Table 2.1: Comparison between liposome and niosome vesicle systems

Liposomes	Niosomes
An aqueous core enclosed in a concentric phospholipid bilayer	An aqueous core enclosed in a non-ionic surfactant bilayer
Naturally occurring vesicles	Synthetic occurring vesicles
Larger vesicles ranging between 10 – 3 000 nm	Smaller vesicles ranging between 10 – 100 nm
Unstable due to phospholipids	More stable due to use of non-ionic surfactants
Expensive to produce	More economical to produce
Special storage conditions required to prevent oxidative degradation	No special storage required

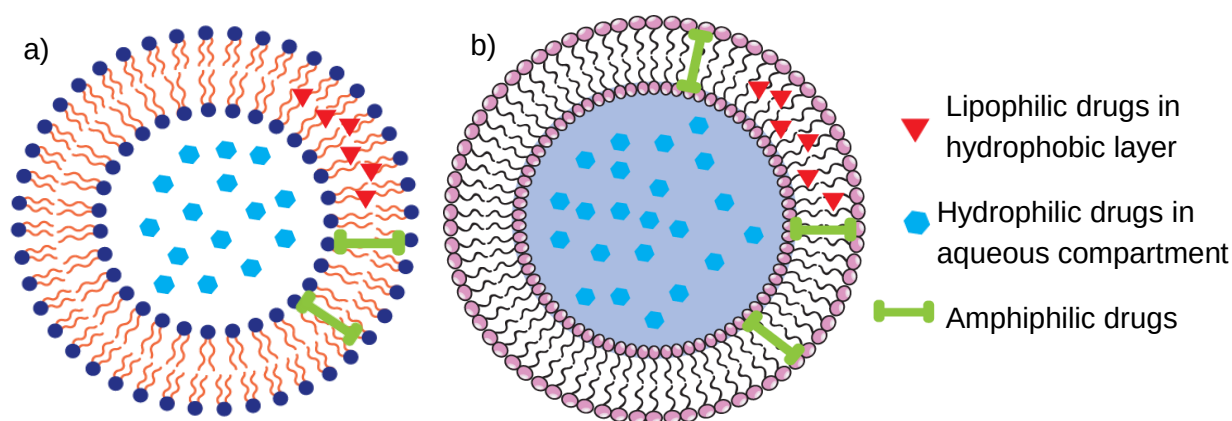


Figure 2.7: Structure of: a) liposomes, and b) niosomes (Adapted from PNG image, 2019 and Saraswathi *et al.*, 2019:7)

2.7.1.2.1 Type of liposomes and niosomes

Vesicles are mainly classified according to their size as follows (Costa & Santos, 2017:405; Moghassemi & Hadjizadeh, 2014:28):

- 10 – 100 nm: Small unilamellar vesicles (SUV);
- 100 – 3 000 nm: Large unilamellar vesicles (LUV);
- 500 – 5 000 nm: Multi-lamellar vesicles (MLV).

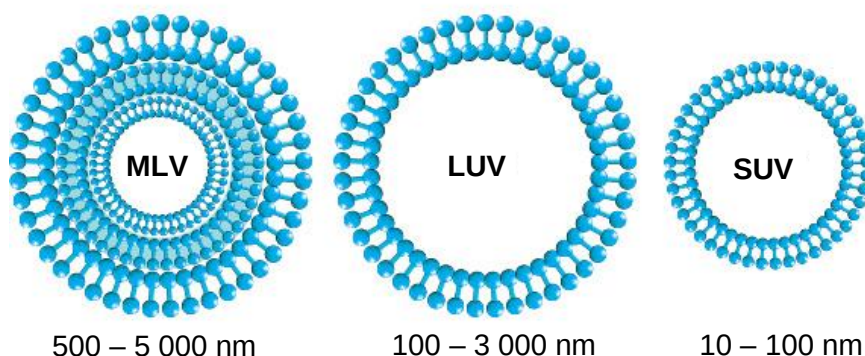


Figure 2.8: Typical vesicle sizes (adapted from Liers, 2017)

2.7.1.2.2 Methods of preparation

The use of vesicles for drug delivery has amplified over the past decade, and the different methods of preparation for obtaining different vesicles. Not only the size, and thus the type of vesicle, but also the distribution, permeability of a membrane and the entrapment efficiency is influenced by the preparation method (Chandu *et al.*, 2012:27). Vesicle formation typically comprises of dispersion of lipid particles in an aqueous phase (Costa & Santos, 2017:406). The

mechanical methods for vesicle preparation include but are not limited to: lipid film hydration, sonification, membrane extrusion, freeze-thaw method, ethanol injection, ether injection, reverse-phase evaporation, dialysis and column chromatography (Kumar & Rajeshwarrao, 2011:210-211; Patil & Jadhav, 2013:10-15). An adapted method of hand shaking/thin-film hydration was used to prepare liposomes and niosomes for this study.

2.7.2 Semi-solid formulations

To improve target-site delivery of transdermal or topical formulations, the delivery systems can be transformed into semi-solid formulations to ensure intact delivery of the dispersion over the skin barrier (Rai *et al.*, 2018:215). These formulations will increase contact time with the skin, with easy application due to increased viscosity (Mahalingam *et al.*, 2008:267; Pund *et al.*, 2015:152; Williams, 2013:689). A diverse range of semi-solid dosage forms exist, such as creams, gels and ointments (Allen *et al.*, 2011:272), which can be greasy and sticky, and also problematic when hydrophilic APIs are concerned (Redkar *et al.*, 2019:588). A gel consists of a liquid phase within a complex polymeric matrix induced by a gelling agent (Bora *et al.*, 2014:3595). The benefit of a gel is the simplicity of the formulation, which provides long-term stability and excellent patient compliance due to its elegance and easy application (Nabi *et al.*, 2014:41). Even with more advantages compared to other semi-solid dosage forms, the challenge of incorporating hydrophobic APIs remains.

2.7.2.1 Emulgel

Emulsion-based strategies have been reported to overcome the challenge of incorporating hydrophobic APIs into a semi-solid dosage form (Redkar *et al.*, 2019:588). With limited available emulgel products on the market, it is not only an evolving but also an exciting and challenging choice to focus on (Sah *et al.*, 2017:26). When a gel and an emulsion are combined, an emulgel is created, providing a two-fold drug release system. Liposomes and niosomes, often referred to as emulsion-based formulations, are dispersed into a gel base to form a vesicle containing emulgel (Rane *et al.*, 2018:818; Wen *et al.*, 2018:263). Combining the great characteristics of a vesicle system with a hydrogel is a promising dosage form, which has gained tremendous attention over the past few years (Rane *et al.*, 2018:818). Including vesicle systems into a gelling agent increases the viscosity and stability, while delivering a convenient self-administering dosage form (Ning *et al.*, 2005:376).

2.7.2.2 Stability testing of semi-solid formulations

To guarantee the quality, safety and efficiency for the duration of the shelf-life of a pharmaceutical product, chemical and physical evaluation occurs (Bajaj *et al.*, 2012:129). Stability is the degree to which a product preserves the same properties as possessed at the initial packaging despite the influence of environmental factors, and to perform standard stability

tests includes intricate procedures, which can be expensive and time consuming (Bajaj *et al.*, 2012:130). Stability testing is essential to predict shelf-life, storage conditions and labelling instructions accurately, and is also a regulatory requirement for approval of any pharmaceutical product (Singh & Bakshi, 2000:2). The following factors should be considered during stability testing: stability of the API, API-exciipient interaction, manufacturing process followed, dosage form type, packaging material, exposure to temperature, light and moisture during shipping and handling (Bajaj *et al.*, 2012:130).

2.7.2.2.1 Methods of stability testing

Depending on the stage of product development, a variety of stability tests are available. In early stages of product development, accelerated testing is used to determine which degradation products are to be expected after long-term storage, while less rigorous conditions are used to determine the shelf-life and expiration date of a product (Bajaj *et al.*, 2012:131). According to Bajaj *et al.* (2012:131-132), stability testing methods can be divided into four main categories:

1. Real-time stability testing;
2. Accelerated stability testing;
3. Retained sample stability testing;
4. Cyclic temperature stress testing.

Accelerated stability testing will be the focus for the purpose of this study. This method is based upon the Arrhenius equation, which stated that the speed of reaction is multiplied or increased three-fold for every 10°C increase in temperature (Bajaj *et al.*, 2012:132). Exaggerated storage conditions are used to increase the rate of degradation and the unstressed product is compared to the stressed counterpart within the same assay (Barnes, 2007:661). The following conditions are used during accelerated stability testing (MCC, 2012:13):

- 40 ± 2°C/75 ± 5% RH (relative humidity) (accelerated storage condition);
- 30 ± 2°C/65 ± 5% RH (intermediate storage condition);
- 25 ± 2°C/60 ± 5% RH (long-term storage condition).

2.7.2.2.2 Factors influenced during storage and acceptance criteria

The following degradation reactions can occur in a pharmaceutical product during storage (Carstensen *et al.*, 2000):

1. Oxidation

2. Reduction
3. Hydrolysis
4. Racemisation

The factors possibly influenced during storage are as follows (Matthews *et al.*, 1999:832):

- Appearance
- Consistency
- Uniformity of contents
- Clarity
- Moisture content
- Particle size
- pH
- Microbiological growth

The accepted criteria, according to the South African Health Products Regulatory Authority (SAHPRA) (MCC, 2012:14) states there should be no significant change during the stability testing period. The following can be seen as a significant change for an API product (ICH, 2003:9):

1. a 5% change in assay from the initial value;
2. any degradant surpassing its specification limit;
3. appearance and physical attributes (ex. colour, phase separation) not meeting its specifications;
4. pH measurements surpassing its specification limits;
5. dissolution for 12 dosage units surpassing its specification limits for capsules and tablets (applicable to oral dosage forms).

Some degradation pathways are intricate and decomposition products may be observed under forced environments; results from such degradation products can attribute to develop and validate suitable analytical methods for future analysis. Some important parameters to keep in mind for the stability of roxithromycin for topical delivery is the assay, which should not surpass

a 5% increase or decrease of the API to ensure efficacy. The appearance during storage is also important as colour or texture change should not occur, as this could negatively influence patient satisfaction; a change in pH is of great significance seeing as a pH lower than 3 or higher than 9 can lead to skin damage and thus poor patient compliance and permeation (Naik *et al.*, 2000:319). The assessment parameters that must be kept in mind during stability testing include appearance, mass loss, odour, pH, particle size, and viscosity (MCC, 2012:24).

2.8 Conclusion

Acne is a widespread disease affecting many of the population. Topical and systemic treatment options available do not provide satisfactory results, which gives rise to the need for novel treatment options. Previous studies revealed that roxithromycin can be an ideal candidate to treat acne topically, but the biggest hindrance is the solubility; the complex structure of the skin also proved challenging for topical delivery of roxithromycin. Using ASDs, where the different solid-states of the API are encapsulated into drug delivery systems, have proven effective in overcoming the challenges posed by solubility and the skin. The main focus of this study was to formulate an emulgel for the topical delivery of roxithromycin. Different solid-state forms of the API were formulated and encapsulated into either liposome or niosome vesicles to improve solubility and bioavailability. Stability testing was performed on the formulated liposomal and niosomal emulgels to determine possible changes that can occur during storage.

References

- Ajazuddin, A.A., Khichariya, A., Gupta, S., Patel, R.J., Giri, T.K. & Tripathi, D.K. 2013. Recent expansions in an emergent novel drug delivery technology: Emulgel. *Journal of controlled release*, 171:122-132.
- Akhlaq, M., Hussain, A., Bakhsh, S., Shakoor, A., Khan, A.M., Iqbal, S. & Kiran, S. 2014. Skin port: a novel route for drug delivery. *Gomal journal of medical sciences*, 12:176-184.
- Alexander, A., Dwivedi, S., Giri, T.K., Saraf, S., Saraf, S. & Tripathi, D.K. 2012. Approaches for breaking the barriers of drug permeation through transdermal drug delivery. *Journal of controlled release*, 164:26-40.
- Allen, L.V., Popovich, N.G. & Ansel, H.C. 2011. Semisolid dosage forms and transdermal systems: ointments, creams, and gels. (*In* Allen, L.V., Popovich, N.G. & Ansel, H.C., eds. *Ansel's pharmaceutical dosage forms and drug delivery systems*. 9th ed. Philadelphia: Lippincott Williams & Wilkins, p.272-293).
- Arora, K.K. & Zawarotko, M.J. 2009. Pharmaceutical co-crystals: A new opportunity in pharmaceutical science for a long-known but little-studied class of compounds. (*In* Brittain, H.G., ed. *Polymorphism in pharmaceutical solids*. 2nd ed. New York: Informa healthcare, p.282-317).
- Asbill, C.S. & Michniak, B.B. 2000. Percutaneous penetration enhancers: local versus transdermal activity. *Pharmaceutical science and technology today*, 3:36-41.
- Atkins, P.W. 1998. *Physical chemistry*. 6th ed. New York: Freeman.
- Aucamp, M., Liebenberg, W., Strydom, S.J., van Tonder, E.C. & de Villiers, M.M. 2012. Physicochemical properties of amorphous roxithromycin prepared by quench cooling of the melt or desolvation of a chloroform solvate. *American association of pharmaceutical scientists, pharmaceutical sciences and technology*, 13:467-476.
- Aucamp, M., Stieger, N., Barnard, N. & Liebenberg, W. 2013. Solution-mediated phase transformation of different roxithromycin solid-state forms: Implications on dissolution and solubility. *International journal of pharmaceutics*, 449:18- 27.
- Ayer, J. & Burrows, N. 2006. Acne: more than skin deep. *Postgraduate medical journal*, 82:500-506.
- Bajaj, S., Singla, D. & Sakhuja, N. 2012. Stability testing of pharmaceutical products. *Journal of applied pharmaceutical science*, 2:129-138.

Bansal, S., Kashyap, C.P., Aggarwal, G. & Harikumar, S.L. 2012. A comparative review on vesicular drug delivery systems and stability issues. *International journal of research in pharmacy and chemistry*, 2:704-713.

Barnes, A.R. 2007. Product stability and stability testing. (*In Aulton, M.E., ed. Aulton's pharmaceuticals: the design and manufacture of medicines. 3rd ed. Philadelphia: Elsevier, p.650-665).*

Barry, B.W. 2001. Novel mechanisms and devices to enable successful transdermal drug delivery. *European journal of pharmaceutical sciences*, 14:101-114.

Barry, B. 2002. Transdermal drug delivery. (*In Aulton, M.E., ed. Pharmaceuticals: the science of dosage form design. 2nd ed. London: Churchill Livingstone, p.499-533).*

Barry, B.W. 2006. Penetration enhancer classification. (*In Smith, E.W. & Maibach, H.I., ed. Percutaneous penetration enhancers. 2nd ed. Boca Raton: CRC/Taylor & Francis, p.3-15).*

Bawa, Y. 2007. Solvent inclusion properties of Triamterene crystal forms and solubility differences between roxithromycin polymorphic forms. Potchefstroom: NWU. (Dissertation-MSc).

Bellantone, R.A. 2014. Fundamentals of amorphous systems: thermodynamic aspects. (*In Shah, N., Sandhu, H., Choi, D.S., Chokshi, H. & Malick, A.W. eds. Amorphous solid dispersions: theory and practice. 1st ed. London: Springer, p.3-34).*

Benson, H.A. 2005. Transdermal drug delivery: penetration enhancement techniques. *Current drug delivery*, 2:23-33.

Benson, H.A.E. & Watkinson, A.C. 2012. Transdermal and topical drug delivery. Hoboken, NJ: Wiley.

Bershad, S.V. 2001. The modern age of acne therapy: a review of current treatment options. *The Mount Sinia journal of medicine*, 68:279-285.

Billich, A., Vyplel, H., Grassberger, M., Schmook, F.P., Steck, A. & Stuetz, A. 2005. Novel cyclosporin derivatives featuring enhanced skin penetration despite increased molecular weight. *Bio-organic & medicinal chemistry*, 13:3157-3167.

Biradar, S.V., Patil, A.R., Sudarsan, G.V. & Pokharkar, V.B. 2006. A comparative study of approaches used to improve solubility of roxithromycin. *Powder technology*, 169:22-32.

Bora, A., Deshmukh, S. & Swain, K. 2014. Recent advances in semisolid dosage form. International journal of pharmaceutical sciences and research, 5:3594-3608.

Bouwstra, J.A. & Ponc, M. 2006. The skin barrier in healthy and diseased state. Biochimica et biophysica acta (BBA) - biomembranes, 1758:2080-2095.

Britannica **see** Encyclopaedia Britannica

Brittain, H.G. 2009. Theory and principles of polymorphic systems. (*In* Brittain, H.G. *ed.* Polymorphism in pharmaceutical solids. 2nd ed. New York: Informa healthcare, p.1-23).

Brittain, H.G. 2009. Application of the phase rule to the characterization of polymorphic and solvatomorphic systems. (*In* Brittain, H.G. *ed.* Polymorphism in pharmaceutical solids. 2nd ed. New York: Informa healthcare, p.24-51).

Brittain, H.G. & Grant, D.J.W. 1999. Effects of polymorphism and solid state solvation on solubility and dissolution rate. New York, Basel: Marcel Dekker.

Brown, M.B., Martin, G.P., Jones, S.A. & Akomeah, F.K. 2006. Dermal and transdermal drug delivery systems: current and future prospects. Drug delivery, 13:175-187.

Bryskier, A. 1998. Roxithromycin: review of its antimicrobial activity. Journal of antimicrobial chemotherapy, 41:1-21.

Burkhart, C.G., Burkhart, C.N & Lehmann, P.F. 1999. Acne: a review of immunologic and microbiologic factors. Postgraduate medical journal, 75:328-331.

Carstensen, J.T. 2000. Drug Stability, Principles and Practices. New York: Marcel Dekker.

Chandu, V.P., Arunashalam, A., Jeganath, S., Yamini, K., Tharangini, K. & Chaitanya, G. 2012. Niosomes: A novel drug delivery system. International journal of novel trends in pharmaceutical sciences, 2:25-31.

Choi, M.J. & Maibach, H.I. 2005. Liposomes and niosomes as topical drug delivery systems. Skin pharmacology and physiology, 18:209-219.

Costa, R. & Santos, L. 2017. Delivery systems for cosmetics - from manufacturing to the skin of natural antioxidants. Powder technology, 322:402-416.

Csongradi, C. 2015. The effect of solid-state forms on the topical delivery of roxithromycin. Potchefstroom: NWU. (Dissertation-MSc).

Csongradi, C., Du Plessis, J., Aucamp, M.E. & Gerber, M. 2017. Topical delivery of roxithromycin solid-state forms entrapped in vesicles. *European journal of pharmaceutics and biopharmaceutics*, 144:96-107.

Cunliffe, W.J., Holland, D.B., Clark, S.M. & Stables, G.I. 2003. Comedogenesis: Some aetiological, clinical and therapeutic strategies. *Dermatology*, 206:11-16.

Dayan, N. 2005. Pathways for skin penetration. *Cosmetics and toiletries® magazine*, 120:67-76.

Dessinioti, C. & Katsambas, A. 2017. *Propionibacterium acnes* and antimicrobia resistance in acne. *Clinics in dermatology*, 35:163-167.

Drugbank. 2019. Roxithromycin. <http://www.drugbank.ca/drugs/DB00778>. Date of access: 26 Jun. 2019.

Dunn, L.K., O'Neill, J. & Feldman, S.R. 2011. Acne in adolescents: Quality of life, self-esteem, mood and psychological disorders. <https://escholarship.org/uc/item/4hp8n68p> Date of access: 25 Mar. 2017.

Du Plessis, C. 2004. Characterisation of polymorphic, pseudopolymorphic and amorphous Forms of roxithromycin. Potchefstroom: NWU. (Dissertation-MSc).

Encyclopaedia Britannica. 2009. Polymorphism. <https://www.britannica.com/science/polymorphism-crystals> Date of access: 20 Jun. 2019.

El Maghraby, G.M., Barry, B.W. & Williams, A.C. 2008. Liposomes and skin: from drug delivery to model membranes. *European journal of pharmaceutical sciences*, 34:203-222.

Ensign, L.M., Cone, R. & Hanes, J. 2012. Oral drug delivery with polymeric nanoparticles: The gastrointestinal mucus barriers. *Advanced drug delivery reviews*, 64:557-570.

Escobar-Chávez, J.J., Rodríguez-Cruz, I.M., Domínguez-Delgado, C.L., Díaz-Torres, R., Revilla-Vázquez, A.L. & Aléncaster, N.C. 2012. Nano-carrier systems for transdermal drug delivery. (In Sezer, A.D., ed. *Recent advances in novel drug carrier systems*. London: Intech, p.201-240).

Feldman, S., Careccia, R.E., Barkham, K.L. & Hancox, J. 2004. Diagnosis and treatment of acne. *American family physician*, 69:2123-2130.

Florence, A.T. & Attwood, D. 2015. *Physicochemical principles of pharmacy in manufacturing, formulation and clinical use*. 6th ed. London: Pharmaceutical Press.

Foldvari, M. 2000. Non-invasive administration of drugs through the skin: challenges in delivery system design. *Pharmaceutical sciences and technology today*, 3:417-425.

Franz, T.J. & Lehman, P.A. 2000. The skin as a barrier: structure and function. (*In* Kydonieus, A.F. & Wille, J.J., eds. *Biochemical modulation of skin reactions. Transdermals, topicals, cosmetics*. Boca Raton: CRC Press, p.15-33).

Geerligs, M. 2010. *Skin layer mechanics*. Netherlands: Universiteitsdrukkerij TU Eindhoven.

Ghulaxe, C. & Verma, R. 2015. A review on transdermal drug delivery system. *The pharma innovation journal*, 4:37-43.

Glowka, E., Wosicka-Frakowiak, H., Hyla, K., Stefanowska, J., Jastrzebska, K., Klapiszewski, L., Jesionowski, T. & Cal, K. 2014. Polymeric nanoparticles-embedded organogel for roxithromycin. *European journal of pharmaceutics and biopharmaceutics*, 88:75-84.

Gollnick, H. 2003. Current concepts of the pathogenesis of acne: implications for drug treatment. *Drugs*, 63:1579-1596.

Gollnick, H., Cunliffe, W., Berson, D., Dreno, B., Finlay, A., Leyden, J.J., Shalita, A.R. & Thiboutot, D. 2003. Management of acne: a report from a global alliance to improve outcomes in acne. *Journal of American academic dermatology*, 49:S1-S37.

Goyal, R., Macri, L.K., Kaplan, H.M. & Kohn, J. 2016. Nanoparticles and nanofibers for topical drug delivery. *Journal of controlled release*, 240:77-92.

Guidlink. 2017. Product information: Rulide® & Rulide® D. <http://www.guidlink.com.au/gc/ws/sw/pi.cfm?product=swprulid10215> Date of access: 29 Aug. 2019.

Hadgraft, J. 2004. Skin deep. *European journal of pharmaceutics and biopharmaceutics*, 58:291-299.

Hildebrand, J. & Scott, R. 1950. *Solubility of non-electrolytes*. 3rd ed. New York: Rheinhold.

Hill, T.L. 1986. *An introduction to statistical thermodynamics*. New York: Dover.

Hofland, H.E.J., van der Geest, R., Bodde, H.E., Junginger, H.E. & Bouwstra, J.A: 1994. Estradiol permeation from nonionic surfactant vesicles through human stratum corneum in vitro. *Pharmaceutical research*; 11:659-664.

Hofland, H.E.J., Bouwstra, J.A., Bodde, H.E., Spies, F. & Junginger, H.E: 1995. Interactions between liposomes and human stratum corneum in vitro: Freeze fracture electron microscopical visualization and small angle X-ray scattering studies. *British journal of dermatology*, 132:853-865.

Honeywell-Nguyen, P.L. & Bouwstra, J.A. 2005. Vesicles as a tool for transdermal and dermal delivery. *Drug discovery today: technologies*, 2:67-74.

Honeywell-Nguyen, P.L. & Bouwstra, J.A. 2008. Vesicles as skin delivery vehicles. (*In* Wiechers, J.W., *ed.* *Science and applications of skin delivery systems*. Carol Stream, Illinois: Allured publishing corporation, p.205-226).

ICH **see** International conference on harmonisation of technical requirements for registration of pharmaceuticals for human use

Ilvabare, G.A., Xu, W., John, C.T., Ormes, J.D., Kuiper, J.L., Templeton, A.C. & Bak, A. 2010. Solubility and dissolution considerations for amorphous solid dispersions. (*In* Gad, S.C., *ed.* *Pharmaceutical sciences encyclopedia: drug discovery, development and manufacturing*. 1st ed. New York: John Wiley & sons inc).

International conference on harmonisation of technical requirements for registration of pharmaceuticals for human use. 2003. ICH harmonised tripartite guideline: Stability testing of new drug substances and products Q1A(R2). <http://www.ich.org/products/guidelines/quality/article/quality-guidelines.html> Date of access: 5 Jun. 2019.

Jepps, O.G., Dancik, Y., Anissimov, Y.G. & Roberts, M.S. 2013. Modelling the human skin barrier - towards a better understanding of dermal absorption. *Advanced drug delivery reviews*, 65:152-168.

Jeremy, A.H., Holland, D.B., Roberts, S.G., Thomson, K.F. & Cuncliffe, W.J. 2003. Inflammatory events are involved in acne lesions initiation. *The Journal of investigative dermatology*, 121:20-27.

Jhawat, V.C., Saini, V., Kamboj, S. & Maggon, N. 2013. Transdermal drug delivery systems: approaches and advancements in drug absorption through skin. *International journal of pharmaceutical sciences review and research*, 20:47-56.

Katz, L. & Mankin, A.S. 2009. Macrolides. (*In* Schaechter, M., *ed.* *Encyclopaedia of microbiology*. 3rd ed. San Diego: Academic press, p.529-589).

Klotz, I.M. & Rosenberg, R.M. 1964. Chemical thermodynamics—basic theory and methods. 3rd ed. New York: Benjamin-Cummings.

- Kolarsick, P.A.J., Kolarsick, M.A. & Goodwin, C. 2011. Anatomy and physiology of the skin. *Journal of the dermatology nurses` association*, 3:203-213.
- Kumar, G.P. & Rajeshwarrao, P. 2011. Non-ionic surfactant vesicular systems for effective drug delivery - an overview. *Acta pharamaceutica Sinica B*, 1:208-219.
- Kute, S.B. & Saudagar, R.B. 2013. Emulsified gel a novel approach for delivery of hydrophobic drugs: an overview. *Journal of advanced pharmacy education & research*, 3:368-376.
- Laouini, A., Jaafar-Maalej, C., Limayem-Blouza, I., Sfar, S., Charcosset, C. & Fessi, H. 2012. Preparation, characterization and applications of liposomes: State of the art. *Journal of colloid science and biotechnology*, 1:147-168.
- Lavers, I. 2014. Diagnosis and management of *Acne vulgaris*. *Nurse prescribing*, 12:330-336.
- Lee, S.L., Raw, A.S. & Yu, L. 2008. Significance of drug substance physicochemical properties in regulatory quality by design. (*In* Adeyeye, M.C. & Brittain, H.G., *Preformulation in solid dosage form development*. New York: Informal healthcare, p.571-583).
- Liers, H. 2017. Recent advances in liposome technology. www.integratedhealthblog.com/tag/liposomes/ Date of access: 29 Aug. 2019.
- Liu, C., Quan, P. & Fang, L. 2016. Effect of drug physicochemical properties on drug release and their relationship with drug skin permeation behaviours in hydroxyl pressure sensitive adhesive. *European journal of pharmaceutical sciences*, 93:437-446.
- MacDougall, C. & Chambers, H.F. 2011. Protein synthesis inhibitors and miscellaneous antibacterial agents. (*In* Brunton, L.L., Chabner, B.A. & Knollmann, B.C., eds. *Goodman & Gilman's The pharmacological basis of therapeutics*. 12th ed. New York: McGraw-Hill). <http://accessmedicine.mhmedical.com/content.aspx?bookid=374&Sectionid=41266266> Date of access: 26 Jun. 2019.
- Mahalingham, R., Li, X. & Jasti, B.R. 2008. Semisolid dosages: ointments, creams, and gels. (*In* Gad, S.C., ed. *Pharmaceutical manufacturing handbook: production and processes*. New Jersey: John Wiley & Sons. p.267-312).
- Martin, A., Swarbrick, J. & Cammarata, A. 1983. *Physical pharmacy*. 3rd ed. Philadelphia: Lea & Febiger.
- Matthews, R.B. 1999. Regulatory aspects of stability testing in Europe. *Drug development and industrial pharmacy*, 25:831-856.

MCC **see** Medicines control council

Mcinturff, J.E & Kim, J. 2005. The role of toll-like receptors in the pathophysiology of acne. *Seminars in Cutaneous Medicine and Surgery*, 24:73-78.

Medicines control council. 2012. Stability: Registration of medicines. https://www.sahpra.org.za/documents/b22b99e32.05_Stability_Jul12_v7_1.pdf Date of access: 5 Jun. 2019.

Medsafe. 2017. New Zealand's medicine and medical devices safety authority. <http://www.medsafe.govt.nz/profs/Datasheet/a/ArrowRoxithromycintab.pdf> Date of access: 26 Jun. 2019.

Menon, G.K. 2002. New insights into skin structure: scratching the surface. *Advanced drug delivery reviews*, 54:3-17.

Milne, M., Liebenberg, W. & Aucamp, M.E. 2016. Different amorphous solid-state forms of roxithromycin: A thermodynamic and morphological study. *International journal of pharmaceutics*, 498:304-315.

MIMS **see** Snyman, J.R.

Mitragotri, S. 2003. Modeling skin permeability to hydrophilic and hydrophobic solutes based on four permeation pathways. *Journal of controlled release*, 86:69-92.

Moghassemi, S. & Hadjizadeh, A. 2014. Nano-niosomes as nanoscale drug delivery systems: an illustrated review. *Journal of controlled release*, 185: 22-36.

Mota, A.H., Rijo, P., Molpeceres, J. & Reis, C.P. 2017. Broad overview of engineers of functional nanosystems for skin delivery. *International journal of pharmaceutics*, 532:710-728.

Morrow, D.I.J., McCarron, P.A., Woolfson, R.F. & Donnelly, R.F. 2007. Innovative strategies for enhancing topical and transdermal drug delivery. *The open drug delivery journal*, 1:36-59.

Nabi, S.A.A., Sheraz, M.A., Ahmed, S., Mustaan, N. & Ahmad, I. 2014. Pharmaceutical gels: a review. *RADS journal of pharmacy and pharmaceutical sciences*, 4:40-48.

Naik, A., Kalia, Y.N. & Guy, R.H. 2000. Transdermal drug delivery: overcoming the skin's barrier function. *Pharmaceutical science and technology today*, 3:318-325.

Nakase, K., Takenaka, Y., Hayashi, N., Kawashima, M. & Noguchi, N. 2016. A novel 23S rRNA mutation in *Propionibacterium acnes* confers resistance to 14-membered macrolides. *Journal of global antimicrobial resistance*, 6:160-161.

National centre for biotechnology information. 2019. Pubchem database: Roxithromycin, CID=444037. <https://pubchem.ncbi.nlm.nih.gov/compound/Roxithromycin> Date of access: 17 Jul. 2019.

Newman, A., Knipp, G. & Zografi, G. 2012. Assessing the performance of amorphous solid dispersion. *Journal of pharmaceutical sciences*, 101:1355-1377.

N'Da, D.D. 2014. Prodrug strategies for enhancing the percutaneous absorption of drugs. *Molecules*, 19:20780-20807.

Ng, K.W. & Lau, W.M. 2015. Skin deep: the basics of human skin structure and drug penetration. (In Dragicevic-Curic, N. & Maibach, H.I., eds. *Percutaneous penetration enhancers: chemical methods in penetration enhancement: drug manipulation strategies and vehicle effects*. Heidelberg: Springer, p.3-12).

Ning, M., Guo, Y., Pan, H., Chen, X. & Gu, Z. 2005. Preparation, in vitro and in vivo evaluation of liposomal/niosomal gel delivery systems for clotrimazole. *Drug development and industrial pharmacy*, 31:375-383.

O'Neil, M.J., ed. 2001. *The Merck index: an encyclopaedia of chemicals, drugs and biologicals*. 13th ed. Whitehouse Station, New Jersey: Merck.

Ostrowski, M., Wilkowska, E. & Baczek, T. 2010. Impact of pharmaceutical dosage form on stability and dissolution of roxithromycin. *Central European journal of medicine*, 5:83-90.

Pappa-louisi, A., Papageorgiou, A., Zitrou, S.S., Georgarakis, E. & Zougrou, F. 2001. Study on the electrochemical detection of the macrolide antibiotics clarithromycin and roxithromycin in reversed phase high performance liquid chromatography. *Journal of chromatography B: biomedical sciences and application*, 755:57-65.

Pathan, I.B. & Setty, C.M. 2009. Chemical penetration enhancers for transdermal drug delivery systems. *Topical journal of pharmaceutical research*, 8:173-179.

Patil, Y.P. & Jadhav, S. 2013. Novel methods for liposomes preparation. *Chemistry and physics of lipids*, 177:8-18.

Planz, V., Lehr, C.M. & Windbergs, M. 2016. In vitro models for evaluating safety and efficacy of novel technologies for skin delivery. *Journal of controlled release*, 242:89-104.

PNG image. 2019. Liposome PNG. <https://Pngimage.net.liposome-png/> Date of access: 29 Aug. 2019.

Ponmalar, P. 2017. A study on solubility and dissolution enhancement of roxithromycin. Chennai: The Tamil Nadu Dr.M.G.R medical university. (Dissertation-M.Pharm).

Prausnitz, M.R., Mitragotri, S. & Langer, R. 2004. Current status and future potential of transdermal drug delivery. *Nature reviews drug discovery*, 3:115-124.

Prausnitz, M.R. & Langer, R. 2008. Transdermal drug delivery. *Nature biotechnology*, 26: 1261-1268.

Pubchem **see** National centre for biotechnology information

Pund, S., Pawar, S., Gangurde, S. & Divate, D. 2015. Transcutaneous delivery of leflunomide nano-emulgel: mechanistic investigation into physicochemical characteristics, in vitro antipsoriatic and anti-melanoma activity. *International journal of pharmaceutics*, 487:148-156.

Qiang, Z. & Adams, C. 2004. Potentiometric determination of acid dissociation constants (pKa) for human and veterinary antibiotics. *Water research*, 38:2874-2890.

Rahimpour, Y. & Hamishehkar, H. 2012. Niosomes as carrier in dermal drug delivery. (*In* Sezer, A.D., ed. *Recent advances in novel drug carrier systems*. London: Intech, p.141-164).

Rai, V.K., Yadav, N.M.K.S. & Yadav, N.P. 2018. Nano-emulsion as pharmaceutical carrier for dermal and transdermal drug delivery: Formulation development, stability issues, basic considerations and applications. *Journal of controlled release*, 270:203-225.

Ranade, V.V. & Hollinger, M.A. 2004. *Drug delivery systems*. 2nd ed. Boca Raton: CPress.

Rane, S., Rane, B. & Jain, A. 2018. Formulation and evaluation of niosomal gel loaded with tinidazole niosomes. *World journal of pharmaceutical research*, 7:817-837.

Reddy, Y.K., Reddy, D.M. & Kumar, M.A. 2014. Transdermal drug delivery system: a review. *Indian journal of research in pharmacy and biotechnology*, 2:1094-1103.

Redkar, M.R., Patil, S.V. & Rukari, T.G. 2019. Emulgel: A modern tool for topical drug delivery. *World journal of pharmaceutical research*, 8:586-597.

Roberts, M.S., Mohammed, Y., Pastore, M.N., Namjoshi, S., Yousef, S., Alinaghi, A., Haridass, I.N., Abd, E., Leite-Silva, V.R., Benson, H.A.E. & Grice, J.E. 2017. Topical and cutaneous delivery using nanosystems. *Journal of controlled release*, 247:86-105.

Sah, S.K., Badola, A. & Nayak, B.K. 2017. Emulgel: Magnifying the application of topical drug delivery. *Indian journal of pharmaceutical and biological research*, 5:25-33.

Santa Cruz Biotechnology. 2007. Product block: Roxithromycin (CAS 80214-83-1). <http://www.scbt.com/datasheet-205845-roxithromycin.html> Date of access: 29 Aug. 2019

Saraswathi, T.S., Mothilal, M. & Jaganathan, M.K. 2019. Niosomes as an emerging formulation tool for drug delivery - a review. *International journal of applied pharmaceutics*, 11:7-15.

Sharma, N., Agarwal, G., Rana, A.C., Bhat, Z.A. & Kumar, D. 2011. A review: transdermal drug delivery system: a tool for novel drug delivery system. *International journal of drug development & research*, 3:70-84.

Sharma, A., Saini, S. & Rana, A.C. 2013. Transdermal drug delivery system: a review. *International journal of research in pharmaceutical and biomedical sciences*, 4:286-292.

SSCI **see** Solid-state chemical information

Shingade, G.M., Aamer, Q., Sabale, P.M., Grampurohit, N.D., Gadhave, M.V., Jadhav, S.L., Gaikwad, D.D. & Patil, K.T. 2012. Review on: recent trend on transdermal drug delivery system. *Journal of drug delivery & therapeutics*, 2:66-75.

Simonelli, A.P., Metha, S.C. & Higuchi, W.I. 1969. Dissolution rates of high energy polyvinyl pyrrolidone (PVP)-sulfathiazole coprecipitates. *Journal of pharmaceutical science*, 58:538-549.

Singh, S. & Bakshi, M. 2000. Guidance on conduct of stress test to determine inherent stability of drugs. *Pharmaceutical technology on-line*. <https://semanticsscholar.org/paper/guidance-on-conduct-of-stress-test-to-determine-of-singh-bakshi/fce4347f5ddbc12ac1f74d9f7e7ae6db3be2336b> Date of access: 29 Aug. 2019.

Snyman, J.R., ed. 2016. *Mims*, 56. Cape Town: Mpasa.

Solid-state chemical information. 2019. What everyone needs to know about polymorphs. <https://www.ssci-inc.com/publications/what-everyone-needs-to-know-about-polymorphs/> Date of access: 21 Jun. 2019.

Strauss, J.S., Krowchuk, D.P., Leyden, J.J., Lucky, A.W., Shalita, A.R., Siegfried, E.C., Thiboutot, D.M., Van Voorhees, A.S., Beutner, K.A., Sieck, C.K. & Bhushan, R. 2007. Guidelines of care for *Acne vulgaris* management. *Journal of the American academy of dermatology*, 56:651-663.

Surber, C., Schwarb, F.P. & Smith, E.W. 1999. Tape-stripping technique. (*In* Bronaugh, R.L. & Maibach, H.I. eds., *Percutaneous absorption: drugs-cosmetics-mechanisms-methodology*. 3rd ed. New York: Marcel Dekker, p.395-409).

- Threlfall, T.L. 1995. Analysis of organic polymorphs. *Analyst*, 120:2435-2460.
- Van den Mooter, G. 2012. The use of amorphous solid dispersions: A formulation strategy to overcome poor solubility and dissolution rate. *Drug discovery today: Technologies*, 9: e79-e85.
- Van Niekerk, E. 2011. Roxithromycin: A solubility and stability study. Potchefstroom: NWU. (Dissertation-MSc).
- Venus, M., Waterman, J. & McNab, I. 2010. Basic physiology of the skin. *Surgery*, 28:469-472.
- Vogt, A., Wischke, C., Neffe, A.T., Ma, N., Alexiey, U. & Lendlein, A. 2016. Nanocarriers for drug delivery into and through the skin - Do existing technologies match clinical challenges? *Journal of controlled release*, 242:3-15.
- Vippagunta, S.R., Brittain, H.G. & Grant, D.J.W. 2001. Crystalline solids. *Advanced drug delivery reviews*, 48:3-26.
- Wang, Y., Fan, Q., Song, Y. & Michniak, B. 2003. Effects of fatty acids and iontophoresis on the delivery of midodrine hydrochloride and the structure of human skin. *Pharmaceutical research*, 20:1612-1618.
- Washington, N., Washington, C. & Wilson, C.G. 2001. *Physiological pharmaceuticals: barriers to drug absorption*. 2nd ed. London: Taylor & Francis.
- Webster, G.F. 2001. *Acne vulgaris* and rosacea: evaluation and management. *Clinical cornerstone*, 4:15-22.
- Wen, J., Gailani, M.A., Yin, N. & Rashidinejad, A. 2018. Liposomes and niosomes. (In Roohinejad, S., Greiner, R., Oey, I. & Wen, J., eds., *Emulsion-based systems for delivery of food active compounds: formation, application, health and safety*. Hoboken: Wiley, p.263-292).
- Wickett, R.R. & Visscher, M.O. 2006. Structure and function of the epidermal barrier. *American journal of infection control*, 34:98-110.
- Williams, A.C. 2003. *Transdermal and topical drug delivery: from theory to clinical practice*. London: Pharmaceutical press.
- Williams, A.C. 2013. Topical and transdermal delivery. (In Aulton, M.E., ed., *Aulton's pharmaceuticals: the design and manufacture of medicines*. 4th ed. London: Churchill Livingstone, p.675-697).

Wosicka-Frackowiak, H., Cal, K., Stefanowska, J., Glowka, E., Nowacka, M., Struck-Lewicka, W., Govedarica, B., Pasikowska, M., Debowska, R., Jesionowski, T., Srcic, S. & Markuszewski, M.J. 2015. Roxithromycin-loaded lipid nanoparticles for follicular targeting. *International journal of pharmaceutics*, 495:807-815.

Yagi, M. & Yonei, Y. 2018. Glycative stress and anti-aging: 7. glycative stress and skin aging. *Glycative stress research*, 5:50-54.

Zhai, Y. & Zhai, G. 2014. Advances in lipid-based colloid systems as drug carrier for topic delivery. *Journal of controlled release*, 193:90-99.

CHAPTER 3

ARTICLE FOR PUBLICATION IN THE INTERNATIONAL JOURNAL OF PHARMACEUTICS

This chapter is written in article format for the purpose of publication in the International Journal of Pharmaceutics. The complete guide for authors is specified in Appendix G. The instructions and formatting, as stipulated by the Guide for Authors, was used during the writing of this article. The text in the manuscript was justified for the ease of reading and UK English was used throughout the manuscript.

Formulation, characterisation and topical delivery of roxithromycin in liposomal and niosomal emulgels

Vernice Steenkamp, Minja Gerber*, Marique E Aucamp and Jeanetta du Plessis

Centre of Excellence for Pharmaceutical Sciences (Pharmacén™), North-West University,

Private Bag X6001, Potchefstroom 2520, South Africa

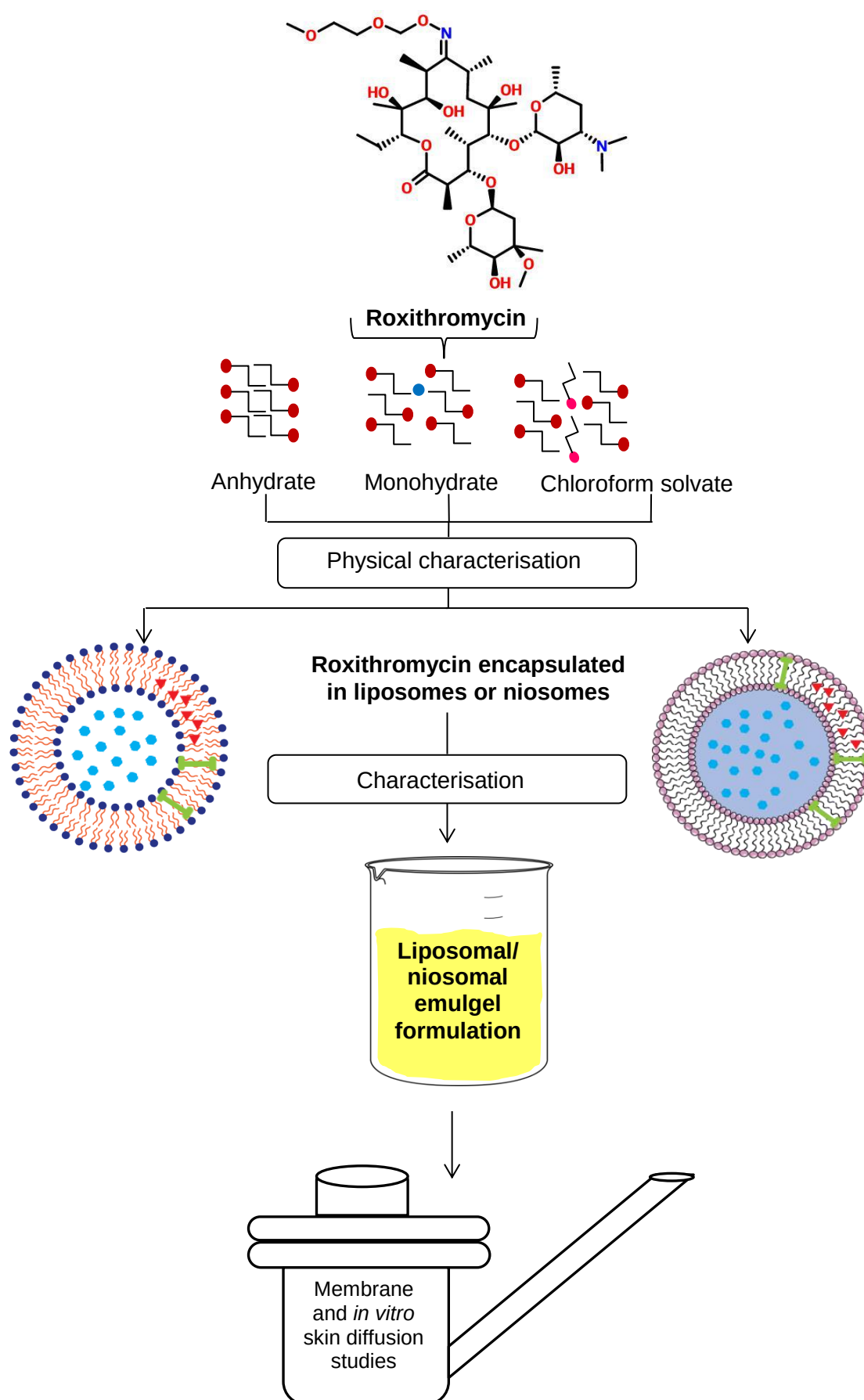
*Corresponding author: Tel.: +2718 299 2328; E-mail address: Minja.Gerber@nwu.ac.za (M Gerber).

Abstract

In recent years, resistance against antibiotic acne treatment has increased, creating the need for a novel topical treatment. Previous research confirmed that roxithromycin could be an ideal contender for topical delivery when alternative solid-states of the active pharmaceutical ingredient (API) are encapsulated into vesicle delivery systems. A product containing the encapsulated roxithromycin has however not yet been developed to date. Easy obtainable solid-states of roxithromycin were included in liposome and niosome vesicle delivery systems. The vesicle dispersions were characterised to confirm stability. The liposomal and niosomal dispersions were included in a proposed emulgel to create a user-friendly application. The topical delivery of these emulgels was determined by performing tape stripping, which revealed successful delivery to the skin layers.

Keywords: Acne, Emulgel, Liposomes, Niosomes, Roxithromycin, Topical delivery

Graphical abstract



1 Introduction

Roxithromycin is a semi-synthetic macrolide antibiotic, which can be used to treat skin infections (Bryskier, 1998). The big surface area of human skin is an ideal route for drug administration, reducing systemic adverse effects and drug interactions, while improving patient compliance due to ease of administration (Prausnitz & Langer, 2008; Shingade et al., 2012). Mild to moderate acne infections are generally treated with topically applied antibiotics because of the bacteriostatic effect it has on *Propionibacterium acnes* (*P. acnes*) (Oschsendorf, 2006). Roxithromycin has proven to be effective in the treatment of mild to moderate inflammatory acne caused by *P. acnes* (Glowka et al., 2014; Wosicka-Frackowiak et al., 2015); the current topical treatment options for inflammatory lesions, including clindamycin and erythromycin, have grown resistant against strains of *P. acnes* due to continuous use (Lavers, 2014). Roxithromycin could be an ideal novel acne topical treatment, as it targets the sebaceous follicles located in the dermis where *P. acnes* accumulates (Gollnick, 2003). The challenge for the topical delivery of roxithromycin however is its poor aqueous solubility, which could be overcome by using methods, such as solid-state modification to improve the physicochemical factors and employing vesicle delivery systems (Threlfall, 1995). Including roxithromycin into thin-films that serve as precursors for drug delivery vesicles renders the API into an amorphous state by creating an amorphous solid dispersion (Aucamp et al., 2013). Different solid-states of roxithromycin have been researched (Du Plessis, 2004; Bawa, 2007; Aucamp et al., 2012; Aucamp et al., 2013), and it has been reported that some of these solid-state forms can be successfully included into drug delivery vesicles such as niosomes and liposomes (Boshoff, 2018; Csongradi, 2015; Swart, 2019). Niosomes are hydrated non-ionic surfactant-based vesicular systems and liposomes are vesicle systems composed of phospholipids (Kumar & Rajeshwarrao, 2011). Liposomes and niosomes are able to encapsulate hydrophilic or lipophilic drugs, which can increase the solubility of poorly soluble drugs 100 to 1000-fold (Bansal et al., 2012). A user-friendly product encapsulating roxithromycin for topical delivery does not yet exist on the market. According to Laouini et al. (2012), drug delivery vesicles

such as liposomes and niosomes can easily be formulated in semi-solid preparations, such
as gels or creams. The best way to ensure a convenient self-administering dosage form is
to include the vesicle systems in a hydrogel base to create an emulgel, which will also be an
ideal application form to reach the intended target-site (Ning et al., 2005). The aim of this
study was to include vesicle-encapsulated roxithromycin in a semi-solid formulation to reach
the target-site, which is the dermis, to serve as a dual therapy with existing systemic acne
treatments.

2 Materials and methods

2.1 Materials

Roxithromycin monohydrate was purchased from DB Fine Chemicals (South Africa). The
excipients used to prepare the liposomes were cholesterol, chloroform, phosphatidylcholine
(PC), tocopherol and Milli-Q[®] water. The excipients used for niosome preparation were
cholesterol, chloroform, methanol, Span[®] 60 and Milli-Q[®] water. Cholesterol from sheep's
wool, tocopherol (DL- α -tocopherol) and egg PC (L- α -PC) was purchased from Sigma-Aldrich
(South Africa). Span[®] 60 were purchased from Merck (South Africa). Chloroform and
methanol were purchased from ACE chemicals (South Africa). Double deionised high
performance liquid chromatography (HPLC) grade water was obtained from a Millipore[®] Milli-
Q[®] water purification system (United States of America). A phosphate buffer solution (PBS)
(pH 7.4) was prepared using UnivAR[®] potassium phosphate (KH_2PO_4) and sodium
hydroxide (NaOH) purchased from Merck (South Africa). HPLC grade LiChrosolv[®] methanol
purchased from Merck Millipore (Germany) and ammonium di-hydrogen phosphate
($\text{NH}_4\text{H}_2\text{PO}_4$) purchased from SAAR Chem (South Africa) served as the mobile phase.

2.2 Methods

2.2.1 High performance liquid chromatography analysis

An HPLC analytical method previously developed and validated by Aucamp et al. (2016)
was verified and applied to quantify the roxithromycin concentration. The analysis was
performed in a controlled laboratory. A Venusil XBP C₁₈ reverse phase 150 x 4.6 mm
column with a 5 μm particle size (Agela Technologies, Germany) was used together with an

Agilent® 1100 Series HPLC system (Agilent Technologies, California). A 0.01 M $\text{NH}_4\text{H}_2\text{PO}_4$ buffer solution mixed with methanol in the ratio 15:85 served as mobile phase (pH 7.0). The mobile phase had a flow rate of 1 ml/min, and an injection volume of 50 μl was used, with a set run time of 9 min and a detection wavelength of 205 nm.

2.2.2 Preparation of different roxithromycin solid-states

The three solid-state forms of roxithromycin used during this study included the purchased crystalline monohydrate form of roxithromycin (**MH**), roxithromycin anhydrate (**AH**) (dehydrated monohydrate) and a polymorphic chloroform solvated (**CS**) form. To prepare the anhydrate, the purchased **MH** was dehydrated using a laboratory drying oven (Binder, Germany). The **MH** was spread out thinly on the surface of a Petri dish, the oven temperature was set at 100°C and the sample was dried for a period of 24 h. To prepare the **CS** form, the method reported by Aucamp et al. (2012) was used. Purchased **MH** was added to chloroform while stirring with a magnetic stirrer and heating the solution to approximately 60±3°C. **MH** was added until an oversaturated solution was obtained, whereafter chloroform was added in a drop-wise manner until the solution was clear. Evaporation occurred in a fume hood at room temperature (25°C) until a dried bulk was obtained.

2.2.3 Preparation of liposomes

Solvent casting, as described by Vasconcelos and Sarmiento (2015) and Vasconcelos et al. (2016), was adapted and combined with the traditional hand-shaking method (New, 1990) to prepare liposome vesicles. A mixture of PC, cholesterol, the API and a drop of α -tocopherol was dissolved in chloroform. The mixture was magnetically stirred in a glass beaker until fully dissolved, whereafter it was poured into Petri dishes and left in a fume hood for 24 h for solvent evaporation to occur and a film to form. The film was scraped off with a stainless steel spatula into a glass beaker. Milli-Q® water was heated to 40±2°C and added to the beaker. The dry lipid film was hydrated using a magnetic stirrer for approximately 30 min until no large droplets were visible and a yellow milky suspension formed. The mixture was

sonicated on ice for 2 min with an ultrasonicator (Model UP200St, Hielscher Ultrasonics, Germany), whereafter it was left for 2 h at room temperature to allow swelling of the vesicles.

2.2.4 Preparation of niosomes

The method described by Agarwal et al. (2001) was adapted for the preparation of niosomes. A lipid mixture of Span[®] 60, cholesterol and the API was dissolved in the chloroform and methanol mixture. The glass beaker was placed on a hot plate at 60±2°C and stirred until all organic solvents had evaporated and a thin-film formed on the bottom. The film was hydrated with 10 ml of Milli-Q[®] water and stirred with a magnetic stirrer for 30 min until no large droplets were visible and a white milky suspension formed. The mixture was sonicated on ice for 2 min with an ultrasonicator (Model UP200St, Hielscher Ultrasonics, Germany), whereafter it was left for 2 h at room temperature to allow swelling of the vesicles.

Table 1

Composition of the vesicle dispersions containing different solid-state forms

2.2.5 Preparation of emulgels

To prepare a hydrogel base, glycerol (10%) was added to water (15 ml). The mixture was stirred with a glass rod until dissolved. Carbopol[®] 934 (1.5%) was sifted into the water and left to hydrate. The impeller of a RZR 2041 homogeniser (Heidolph, Germany) was submerged close to the bottom of the beaker containing the mixture and a vortex of approximately 1000 rpm was used to agitate the liquid until a lump free dispersion was attained. The mixture was neutralised with ammonia (NH₃) (q.s) solution. For the preparation of the final emulgel formulations, the liposomal or niosomal dispersion containing roxithromycin equivalent to 2.0% w/w was incorporated into the gel base. This method was adapted from Ning (2005) and Shirsand et al. (2016), and applied to all vesicle systems.

2.3 Physical characterisation

2.3.1 Fourier-transform infrared spectroscopy (FTIR)

A small quantity of the sample was placed on the IR window of a Bruker Alpha FTIR spectrometer (United States of America), which recorded the IR-spectra over a range of 400-4000 cm^{-1} .

2.3.2 Thermogravimetric analysis (TGA)

TGA was carried out using a TGA 4000 (PerkinElmer Waltham, United States of America). Samples weighing 1 to 3 mg each were placed in a porcelain crucible. A heating programme of 10°C/min from ambient temperature to 250°C was used, which was done under a nitrogen purge of 40 ml/min. The moisture lost during heating of the sample was documented.

2.4 Characterisation of the vesicle systems

2.4.1 Transmission electron microscopy (TEM)

TEM was used to view the morphology and formation of the vesicle systems. An FEI Tecnai G2 20S-Twin 200 kV high-resolution transmission electron microscope (HRTEM) (Czech Republic) fitted with an Oxford INCA X-Sight EDS System was used (Laboratory for Electron Microscopy, NWU, Potchefstroom Campus). A drop of the sample (diluted 10x with distilled water) was stratified onto a 300 mesh copper carbon-coated grid and left for 5 min to dry, any excess sample was removed with a piece of filter paper. The lipid layers of the vesicles were stained using a drop of osmium tetroxide, the sample was left to dry for 1 to 2 min before being inserted and viewed through the microscope. Vesicles containing no API were analysed to avoid damaging the microscope.

2.4.2 Droplet size, polydispersity index and zeta-potential

The size, polydispersity index (Pdl) and zeta-potential of the vesicles were determined by using the Malvern Zetasizer Nano ZS (Malvern Instruments Ltd, United Kingdom). Freshly prepared vesicle samples were diluted (approximately 20x) with distilled water and 2 ml was injected into a clear disposable zeta-cell (DTS1070 folded capillary cell). Each sample was measured in triplicate to ensure accurate results.

2.4.3 pH determination

To determine the pH values of the different vesicle systems, a Mettler Toledo[®] pH meter (Mettler Toledo, United States), equipped with a Mettler Toledo[®] InLab[®] 410 electrode (Mettler Toledo, United States) was used. The electrode was inserted into a sufficient volume of each sample after being calibrated. The pH value for each sample was measured in triplicate.

2.4.4 Entrapment efficiency

To determine the amount of API encapsulated in each vesicle system, fresh samples were prepared in triplicate. Approximately 20 ml was transferred into plastic test tubes and centrifuged (Laboratory for Applied Molecular Biology, NWU, Potchefstroom Campus). An OptimaL-100 XP ultracentrifuge (Beckman Coulter, United States of America), with a 50.2 Ti Rotor was used to centrifuge the samples, with a rotation speed of 25000 rpm for 30 min at a temperature of 25±2°C. A pellet containing the entrapped API formed at the bottom of the test tube, with a supernatant solution containing the unentrapped free API. The supernatant was filtered and transferred into HPLC vials for analysis. An equation from Xiang et al. (2009) was adapted to calculate the final entrapment.

$$EE\% = \frac{\text{Drug (total)} - \text{Drug (supernatant)}}{\text{Drug (total)}} \quad \text{Eq. 1}$$

2.5 Diffusion studies

2.5.1 Preparation of the receptor phase

A 0.06 M phosphate buffer was used as the receptor phase, which was prepared by mixing 6.8 g KH₂PO₄ in 250 ml Milli-Q water, with 1.5 g NaOH in 400 ml Milli-Q[®] water, then made up to 1000 ml. Since *in vivo* human blood presents with a pH of 7.4, it consequently determined the pH of the PBS for the *in vitro* skin diffusion studies (OECD, 2011). Before every experiment, the receptor phase was pre-heated in a water bath to represent the human body temperature of 37°C (Williams, 2013).

2.5.2 Preparation of the donor phase

The emulgel containing the liposome or niosome vesicle system encapsulating the different solid-state forms of roxithromycin served as the donor phase. A placebo (vesicles

containing no API) was used as control group during the studies. Ten Franz cells were used, each containing approximately 1 ml of the formulation, while two Franz cells contained 1 ml of the placebo. The prepared samples were placed in a water bath at 32°C, equivalent to human skin surface temperature, before the experiment (Williams, 2013).

2.5.3 Membrane release studies

To accurately represent the temperature of the blood in the human body the PBS, which served as the receptor phase, was placed in a water bath at 37°C before the study. The formulation serving as the donor phase was placed in another water bath at 32°C before the study to represent the temperature of the surface of the skin. A thin layer of Dow Corning® high vacuum grease (Sigma-Aldrich, Germany) was applied to both the donor and receptor compartments. A magnetic stirring rod was placed into the receptor compartments before placing a Polyvinylidene fluoride (PVDF) (Pall® Life Sciences, United States of America) synthetic membrane with a pore size of 0.45 µm and 25 mm diameter between the receptor and the donor compartments. The sides of the Franz cells were sealed with vacuum grease and fastened with a horseshoe clamp to prevent leakage. The receptor compartment was filled with 2 ml of heated PBS (pH 7.4) using a syringe and needle to which a small tube was attached. It was visually inspected to ensure no air bubbles formed when filled and the PBS level was indicated in order to notice any possible leakage or change of PBS level. Each donor compartment was filled with 1 ml of the API vesicle preparation, and then covered with a piece of Parafilm® and a plastic cap to prevent any loss of the formulation during the experiment. The tray with all the assembled Franz cells was placed in a Grant® water bath (Grant Instruments, United Kingdom), at a constant temperature of 37°C on a Variomag® (United States of America) magnetic stirring plate. The entire receptor phase of every cell was extracted through the sampling port and refilled with fresh, preheated PBS (pH 7.4, 37°C) hourly, for 6 h. Any change in the level of the PBS was noted before extraction took place. The extracted contents of the receptor compartments were then placed into corresponding HPLC vials and analysed by means of HPLC.

2.5.4 Skin preparation

The North-West University Health Research Ethics Committee (NWU-HREC) of the Faculty
195 of Health Sciences awarded approval for the study (Ethics number: NWU-00111-17-A1-02).
Skin collected from abdominoplasty of Caucasian females was used for the diffusion studies.
The skin was trimmed of all excess fat and a Zimmer™ electric dermatome (model 8821,
United Kingdom) was used to acquire strips of skin (about 400 µm). The skin strips were
placed onto Whatman® filter paper and allowed to dry, then visually examined for any
200 defects before into cutting circles; the skin circles were wrapped in aluminium foil and stored
in a Ziplock® bag at -20°C until needed. As a result of limited skin per donor, different
donors had to be used for every separate study.

2.5.5 Skin diffusion studies

The same method as discussed in Section 2.5.3 was used, with the following exceptions: 1)
205 Prepared skin samples were placed between the receptor and donor phase of the Franz cell,
with the stratum corneum facing upwards, instead of the PVDF membranes; 2) only one
extraction of the PBS in the receptor phase was made at 12 h, instead of hourly extractions
up to 6 h. Both the skin and PBS were analysed after extraction.

2.5.6 Tape stripping

210 The Franz cells were carefully disassembled after extraction of the receptor phase. The skin
circle was pinned on cling film and Parafilm® covered hardboard. The visible diffusion area
was dabbed with tissue paper to remove any excess donor phase. Sixteen pieces of 3M
Scotch® Magic™ tape were used per skin sample to remove the stratum corneum. The tape
was placed on the diffusion area to remove it; a gleaming appearance of the skin was an
215 indication of complete removal. The first tape was discarded to ensure all superficial donor
phase was removed, and the remaining strips were placed in separate marked polytops
containing 5 ml HPLC grade methanol after dabbing it on the skin to remove the stratum
corneum-epidermis (SCE). Thereafter the diffusion area of the skin (epidermis-dermis (ED))
was cut into smaller pieces and placed in separate marked polytops containing 5 ml HPLC
220 grade methanol. Both the polytops containing the skin and the tape were placed in a

refrigerator (2-5°C) for 8 h to give the API an opportunity to be extracted from the samples. The methanol was removed and filtered into marked HPLC vials for analysis.

2.5.7 Data analysis

The cumulative concentration of each Franz cell was determined by means of HPLC analysis over a period of 6 h for membrane release studies. The concentration of the API in the receptor phase of every Franz cell could be determined using the slope of the linear line (Ng et al., 2010). The analysed results were examined with regard to the average cumulative amount of API per area ($\mu\text{g}/\text{cm}^2$), which had diffused through the membrane, plotted against time (h).

During skin diffusion studies, the average cumulative amount per area ($\mu\text{g}/\text{cm}^2$) of the API diffused through the skin, together with average concentration ($\mu\text{g}/\text{ml}$) of the API in the SCE and ED after 12 h, was examined.

2.6 Statistical data analysis

Statistical analysis was performed on results obtained from membrane release, as well as skin diffusion studies. Statistical methods, such as the analysis of variance (ANOVA), Turkey's honest significance difference (HSD) and t-tests, were performed on the data obtained. The flux values ($\mu\text{g}/\text{cm}^2\cdot\text{h}$) of the liposomal and niosomal emulgels for each roxithromycin solid-state were used to determine median flux values. Median flux values present more accurate results than the average flux, since outliers and extremes are excluded, leaving the median unaffected (Dawson & Trapp, 2004). Box-plots were used to illustrate the summarised data in terms of lower quartile, media, upper quartile, extreme value and mean.

3 Results and discussion

3.1 Preparation and physical characterisation of roxithromycin solid-state forms

MH powder was used to prepare an **AH** and **CS** form of roxithromycin. TGA was used to determine the hydration or solvation level of the three crystalline forms of roxithromycin. The percentage solvent included in crystalline packaging of the solid-states were 2% for **MH**, 0.1% for **AH** and 12.5% for **CS**. The obtained percentage solvent was used to calculate the

molecular weight of each crystalline form. FTIR analysis revealed the most distinctive
variances could be observed at the 4000-3000 cm^{-1} range. **MH** had three distinctive peaks
at 3570.19 cm^{-1} , 3510.21 cm^{-1} and 3455.73 cm^{-1} ; **CS** and **AH** only had one broad peak each
at 3422.05 cm^{-1} and 3457.74 cm^{-1} respectively, which indicated the latter two forms were not
monohydrates. The obtained infrared data correlated with that previously reported by
Aucamp et al. (2012) and Aucamp et al. (2013).

3.2 Characterisation results

The liposomal and niosomal dispersions were characterised in terms of TEM, droplet size,
Pdl, zeta-potential, pH and %EE. The results are summarised in Table 2.

Table 2

Characterisation results for liposome and niosome dispersions

The TEM results of the empty vesicle systems revealed well-formed liposomes and
niosomes, as seen in Fig. 1. To ensure optimal topical delivery, particle sizes between 10-
1000 nm are recommended (Kumar & Rajeshwarrao, 2011). The droplet sizes of all eight
dispersions rendered satisfactory results, with the liposomes between 80-90 nm and the
niosomes ranging between 200-300 nm. The Pdl results for all dispersions were in the lower
range, indicating stable dispersions with uniform droplet dispersion (Marianecchi et al., 2013).
All the samples resulted in highly negative zeta-potential values, which would improve
permeation of the drug into the skin (Duangjit et al., 2011). All vesicle dispersions resulted in
pH values higher than 7 and below 9, making them ideal contenders for skin application, as
it met the requirements for topical formulations (Naik et al., 2000; Paudel et al., 2010). All
the vesicle systems containing the different roxithromycin solid-states resulted in high
entrapment close to 100%, which correlates with previous results reported by Swart (2019).
Better target-site delivery will be obtained as the API was almost fully incorporated within the
vesicles.

Fig. 1: TEM micrographs: a) and b) showing liposome vesicles, and c) and d) showing niosome vesicles

3.3 Preparation of emulgels

To increase the low viscosity of the vesicle dispersions, a 2% emulgel containing the liposome and niosome vesicle systems were prepared. Due to the PC used during liposome preparation, the liposomal emulgels presented with a light yellow colour, while the niosomal emulgels had a white colour due to the use of Span[®] and cholesterol during vesicle preparation. All gels had a smooth, gel-like consistency, with the placebo gels resulting in a lower viscosity than the emulgels containing the different roxithromycin solid-states.

3.4 Diffusion study results

3.4.1 Membrane release

The membrane release studies for the liposome and niosome emulgels indicated that the encapsulated roxithromycin was successfully released from the vesicles. The highest median flux was achieved by the liposomal emulgel containing **CS (L-CS)**, followed by the niosomal emulgel containing **MH (N-MH)**, the liposomal emulgel containing **AH (L-AH)**, the liposomal emulgel containing **MH (L-MH)**, the niosomal emulgel containing **CS (N-CS)** and lastly, the niosomal emulgel containing **AH (N-AH)**.

Fig. 2: Box-plots indicating the flux ($\mu\text{g}/\text{cm}^2\cdot\text{h}$) of the liposomal and niosomal emulgels containing roxithromycin during the membrane release studies over 6 h

3.4.2 Skin diffusion

The receptor phase was analysed after 12 h to determine if the API was delivered transdermally. When the liposomal and niosomal emulgels were compared it was detected that **L-MH** had the highest median amount per area diffused, followed by **L-CS**, and lastly, **L-AH** for the liposomal emulgels. For the niosomal emulgels, **N-CS** had the highest median amount per area diffused, followed by **N-AH**, and lastly, **N-MH**.

When the different solid-state forms of roxithromycin (**MH**, **CS** and **AH**) were compared, it was evident that the type of vesicle used influenced the transdermal delivery (from highest to

lowest median amount per area diffused: **L-MH**; **N-CS**; **L-CS**; **N-AH**; **L-AH** and lastly, **N-MH**).

The liposomal emulgels increased the transdermal delivery of the **MH**, rather than the other two solid-state forms of roxithromycin, while the opposite for the niosomal emulgels was true. Therefore, the poor delivery to the receptor phase can be attributed to the vesicle systems used in the emulgels. The different excipients used, such as PC in liposomes and Span® 60 in niosomes, possibly influenced API delivery. The additional phospholipids could have resulted in an extra barrier on the surface of the skin, which led to slow release of the API from the vesicle systems in the formulations (Kirjavainen et al., 1999; Sakdiset et al., 2018). The poor transdermal delivery was a favoured outcome given that the target-site for the API delivery during this study was the dermis, because unwanted systemic effects and toxicity would be minimal.

Fig. 3: Box-plot indicating the mean and median amount per area diffused ($\mu\text{g}/\text{cm}^2$) for roxithromycin in the liposomal and niosomal emulgels after 12 h

3.4.3 Tape stripping

Tape stripping was performed to determine if the encapsulated roxithromycin was successfully released into the skin from the emulgels. The tape stripping results are divided into delivery to the SCE and the ED.

Fig. 4: Box-plot indicating the mean and median concentration ($\mu\text{g}/\text{ml}$) of the liposomal emulgels for roxithromycin present in the SCE and ED

Fig. 5: Box-plot indicating the mean and median concentration ($\mu\text{g}/\text{ml}$) of the niosomal emulgels for roxithromycin present in the SCE and ED

3.4.3.1 Stratum corneum-epidermis

When the liposomal and niosomal emulgels were compared, it was detected that **L-AH** resulted in an exceptional high stratum corneum delivery, in contrast to **L-MH** and **L-CS**. For the niosomal emulgels, **N-MH** resulted in the highest stratum corneum delivery, followed by

N-AH and lastly, **N-CS**. All the liposomal and niosomal emulgels resulted in median concentrations above the limit of detection (LOD) (0.75 µg/ml) and lower limit of quantification (LOQ) (2.51 µg/ml), with only **N-CS** resulting in a value below the LOQ.

335 When the different solid-state forms of roxithromycin (**MH**, **CS** and **AH**) were compared (from the highest to lowest concentration: **L-AH**; **N-MH**; **N-AH**; **L-MH**; **L-CS** and lastly, and **N-CS**), it was apparent that the **CS** solid-state form of roxithromycin resulted in the lowest SCE delivery. The stratum corneum acts as a rate-limiting hindrance for drug delivery due to the numerous protein and lipid layers (Barry, 2001; Williams, 2013). It was observed that **AH**
340 permeated effortlessly into the SCE from the liposomal vesicles in contrast to niosomal vesicles. PC, which was used in liposome preparation, has the ability to penetrate intercellular lipids of the stratum corneum (Montenegro et al., 2006), which in turn influenced the barrier function and possibly enhanced the penetration of **AH**. **MH** however, permeated with more ease into the SCE from the niosomal vesicles. It is possible that API delivery is
345 more dependent on the excipients used for vesicle preparation than the solid-state form. Highly lipid-soluble excipients used for liposome and niosome preparation, such as cholesterol, would cause the vesicles to have greater difficulty crossing the aqueous region. Since the effect of surfactants, such as PC and Span[®], are extremely complex in biological systems it is possible for the permeation of the APIs to vary extensively (Som et al., 2012).

350 3.4.3.2 Epidermis-dermis

When the liposomal and niosomal emulgels were compared, it was observed that **L-MH** resulted in the highest ED delivery, followed by **L-CS** and lastly, **L-AH**. For the niosomal emulgels, **N-CS** resulted in the highest ED delivery, **N-AH** resulted in the lowest ED delivery and **N-MH** achieved a low median concentration. All liposomal emulgels and **N-CS** resulted
355 in a median concentration above the LOD and LOQ for ED delivery. **N-MH** and **N-AH** achieved values above the LOD, but below the LOQ.

When the different solid-state forms of roxithromycin (**MH**, **CS** and **AH**) were compared, delivery to the epidermis layer ranked from the highest to the lowest concentration as follows: **N-CS**; **L-MH**; **L-CS**; **L-AH**; **N-MH** and **N-AH**. It can be seen that the type of vesicle

360 influenced topical delivery. The rendered amorphous solid dispersions possibly increased the bioavailability of the API leading to a bigger probability for successful ED delivery (Aucamp et al., 2012; Aucamp et al., 2013) and the vesicle composition also contributed to successful ED delivery. **CS** performed much better when encapsulated in niosomes than liposomes, while **MH** and **AH** had much higher ED delivery for liposome vesicles than
365 niosome vesicles. It is probable that the highly lipophilic **N-MH** and **N-AH** had a greater affinity for the various stratum corneum lipids due to the lipophilic excipients, leading to a longer retention time in the SCE layer and resulting in poor diffusion to the highly hydrophilic ED layer. **N-MH** and **N-AH** were not considered successful candidates for ED delivery.

3.5 Statistical analysis results

370 3.5.1 Membrane release studies

The two-way ANOVA performed on the effect of the emulgels (liposomal and niosomal) and effect of the API form (**MH**, **CS** and **AH**) produced a highly significant interaction between the two types of emulgels and three API forms ($F(2, 53)=7.23$ ($p=0.002$)). Due to the significant interaction and no substantial deviation from normality and homogeneity of variances,
375 Tukey's HSD tests were performed to test the significance of pairwise differences between the three means of roxithromycin solid-states, for each of the liposomal and niosomal emulgels. The results of the liposomal formulas reported that all p-values were bigger than the desired level of significance 0.05; thus, they were not of interest. The niosomal formulas resulted in statistical significant p-values (i.e. smaller than 0.05) between **N-MH** and **N-CS**,
380 as well as between **N-MH** and **N-AH** and *vice versa*. T-tests for independent groups, corrected for heterogeneity of variances, were performed on the separate API forms of every emulgel formula. The current effect obtained for the **MH** group could be reported as $t(15.70)=-2.42$ ($p=0.03$), for the **CS** group as $t(11.95)=2.27$ ($p=0.04$) and for the **AH** group as $t(15.68)=1.96$ ($p=0.07$). Thus, a significant difference on a 0.05 level was reported for **MH**
385 and **CS**, with **AH** rendering no significant difference between the means of the two emulgels. Since deviation from normality occurred and could have an effect on the aforementioned results, these latter results were confirmed by performing the Mann-Whitney U

nonparametric test, which reported the p-values for **MH** as 0.05, for **CS** as 0.03 and for **AH** as 0.08.

3.5.2 Skin diffusion studies

3.5.2.1 Transdermal delivery

Logarithmic transformation was done in advance to produce near to normal distribution, since statistical data reflected biased distribution. The two-way ANOVA performed on the transdermal delivery results on the effect of the emulgels (liposomal and niosomal) and the API forms (**MH**, **CS** and **AH**) yielded a highly significant interaction between the two types of formulas ($F(2,53)=41.39$ ($p<0.001$)). Due to the significant interaction and no substantial deviation from normality and homogeneity of variances, Tukey's HSD tests were performed to test the significance of pairwise differences between the three means of the roxithromycin solid-state forms, for each of the liposomal and niosomal emulgels. The results of the liposomal formulas reported p-values for **L-MH** and **L-CS**, as well as **L-MH** and **L-AH**, and *vice versa*, were highly significant (bigger than 0.05). The niosomal formulas also resulted in highly significant p-values for **N-MH** and **N-CS**, as well as **N-MH** and **N-AH**. T-tests for independent groups, corrected for heterogeneity of variances, were performed on the separate API forms of every emulgel formula. The current effect obtained for the **MH** group can be reported as $t(13.14)=9.03$ ($p=0.000001$), for the **CS** group as $t(16.67)=1.72$ ($p=0.10447$) and for the **AH** group as $t(13.85)=0.36$ ($p=0.72687$). Thus, a highly statistically significant p-value was reported for **MH**, while **CS** and **AH** rendered insignificant p-values.

3.5.2.2 Tape stripping

The three-way ANOVA test performed on the effects of the emulgels (liposomal and niosomal), effects of the API form (**MH**, **CS** and **AH**) and effect of different skin layers (SCE and ED), yielded an extremely statistically significant interaction between the liposomal and niosomal emulgels, the three API forms and the two skin layers ($F(2, 106)=52.96$ ($p<0.001$)). Due to the significant interaction and no substantial deviation from normality and homogeneity of variances, Tukey's HSD tests were performed to test the significance of pairwise differences between the three means of roxithromycin solid-state forms, for each of

the liposomal and niosomal emulgels. The results of the niosomal formulas reported that highly significant p-values (bigger than 0.05) for **N-MH** and **N-CS**, as well as **N-CS** and **N-AH** were obtained for the SCE skin layer. For the ED delivery, highly significant p-values were obtained for **N-MH** and **N-CS**, as well as **N-CS** and **N-AH**. T-tests for independent groups, corrected for heterogeneity of variances, were performed on the separate APIs of every emulgel formula, as well as the skin layers. The current effect obtained for the **MH** group for the SCE results can be reported as $t(9.62)=5.61$ ($p=0.0003$) and for the ED as $t(9.10)=-9.17$ ($p=0.000007$). For the **CS** group, the SCE results can be reported as $t(9.57)=-17.01$ ($p=0.000000$) and the ED results as $t(13.93)=4.31$ ($p=0.0007$). For the **AH** group the SCE results can be reported as $t(8.04)=-7.01$ ($p=0.0001$) and the ED results as $t(8.55)=-7.27$ ($p=0.00006$). Thus, a significant difference on a 0.05 level was reported for **MH**, **CS** and **AH** between the means of the two emulgel formulas in both the SCE and ED skin layers. Ranking from the most significant difference to the least significant on a 0.05 level as follows: **SCE-CS > ED-MH > ED-AH > ED-CS > SCE-MH > SCE-AH**. T-tests were also performed on the liposomal and niosomal dispersions with regard to the separate API forms of every emulgel formula. The current effect obtained for the **L-MH** group can be reported as $t(10.79)=-0.23$ ($p=0.082$). For the **L-CS** group, it can be reported as $t(10.19)=-4.55$ ($p=0.001$). For the **L-AH** group the results can be reported as $t(8.16)=7.72$ ($p=0.00005$). The current effect obtained for the **N-MH** group can be reported as $t(9.033)=10.92$ ($p=0.000002$). For the **N-CS** group, it can be reported as $t(9.01)=-9.90$ ($p=0.000004$). For the **N-AH** group the results can be reported as $t(11.66)=28.79$ ($p=0.000000$). Thus, a significant difference on a 0.05 level was reported for **L-CS**, **L-AH**, **N-MH**, **N-CS** and **N-AH** between the means of the two emulgel formulas. Ranking from the most significant difference to the least significant on a 0.05 level was as follows: **N-AH > N-CS > N-MH > L-AH > L-CS > L-MH**.

4 Conclusion

The aim of this study was to establish if roxithromycin encapsulated in liposome and niosome vesicle systems could be developed into emulgels for topical delivery. This aim
445 was reached, proving that it is possible to include encapsulated roxithromycin into an emulgel. It was also determined that quantifiable concentrations of the API were found in the target area of the ED, with the niosomal vesicles containing **CS** resulting in the highest delivery.

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Disclaimer

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

460 Declaration of interest statement

The authors declare no conflict of interest.

References

- Agarwal, R., Katare, O.P. & Vyas, S.P. 2001. Preparation and in vitro evaluation of liposomal/niosomal delivery systems for antipsoriatic drug dithranol. International journal of pharmaceuticals, 228:43-52.
- Aucamp, M., Liebenberg, W., Strydom, S.J., van Tonder, E.C. & de Villiers, M.M. 2012. Physicochemical properties of amorphous roxithromycin prepared by quench cooling of the melt or desolvation of a chloroform solvate. American association of pharmaceutical scientists, pharmaceutical sciences and technology, 13:467-476.
- Aucamp, M., Stieger, N., Barnard, N. & Liebenberg, W. 2013. Solution-mediated phase transformation of different roxithromycin solid-state forms: implications on dissolution and solubility. International journal of pharmaceuticals, 449:18-27.
- Aucamp, M.E., Csongradi, C., Gerber, M. & Du Plessis, J. 2016. A novel RP–HPLC method for the detection and quantification of roxithromycin in topical delivery studies. Pharmazie, 17:175–176.
- Bansal, S., Kashyap, C.P., Agarwal, G. & Harikumar, S.L. 2012. A comparative review on vesicular drug delivery systems and stability issues. International journal of research in pharmacy and chemistry, 2:704-713.
- Barry, B.W. 2001. Novel mechanisms and devices to enable successful transdermal drug delivery. European journal of pharmaceutical sciences, 14:101-114.
- Bawa, Y. 2007. Solvent inclusion properties of Triamterene crystal forms and solubility differences between roxithromycin polymorphic forms. Potchefstroom: NWU. (Dissertation-MSc).
- Boshoff, S. 2018. Investigation of the stabilising effects of niosomes on the amorphous forms of roxithromycin. Potchefstroom: NWU. (Dissertation – MSc).
- Bryskier, A. 1998. Roxithromycin: review of its antimicrobial activity. Journal of antimicrobial chemotherapy, 41:1–21.
- Csongradi, C. 2015. The effect of solid-state forms on the topical delivery of roxithromycin. Potchefstroom: NWU. (Dissertation – MSc).

- Dawson, B. & Trapp, R.G. 2004. Basic & clinical biostatistics. 4th ed. New York: McGraw-Hill.
- Douglas, J.F. 2018. Weak and strong gels and the emergence of the amorphous solid state. *Gels*: 4:1-14.
- 495 Duangjit, S., Opanasopit, P., Rojanarata, T. & Ngawhirunpat, T. 2011. Characterization and in vitro skin permeation of meloxicam-loaded liposomes versus transferosomes. *Journal of drug delivery*, 2011:1-9.
- Du Plessis, C. 2004. Characterisation of polymorphic, pseudopolymorphic and amorphous Forms of roxithromycin. Potchefstroom: NWU. (Dissertation-MSc).
- 500 Glowka, E., Wisicka--Frakowiak, H., Hyla, K., Stefanowska, J., Jastrzebska, K., Klapiszewski, L., Jesionowski, T. & Cal, K. 2014. Polymeric nanoparticles-embedded organogel for roxithromycin. *European journal of pharmaceutics and biopharmaceutics*, 88:75-84.
- Gollnick, H. 2003. Current concepts of the pathogenesis of acne. *Drugs*, 63:1579-1596.
- 505 Honeywell-Nguyen, P.L. & Bouwstra, J.A. 2005. Vesicles as a tool for transdermal and dermal delivery. *Drug discovery today: technologies*, 2:67-74.
- Kumar, G.P. & Rajeshwarao, P. 2011. Nonionic surfactant vesicular systems for effective drug delivery - an overview. *Acta pharmaceutica sinica B*, 1:208-219.
- Lavers, I. 2014. Diagnosis and management of acne vulgaris. *Nurse prescribing*, 12:330-336.
- 510 Laouini, A., Jaafar-Maalej, C., Limayem-Blouza, I., Sfar, S., Charcosset, C. & Fessi, H. 2012. Preparation, characterization and applications of liposomes: State of the art. *Journal of colloid science and biotechnology*, 1:147-168.
- Marianecchi, C., Di Marzio, L., Rinaldi, F., Esposito, S. & Carafa, M. 2013. Niosomes. (*In*
- 515 Uchegbu, I.F., Schätzlein, A.G., Ping Cheng, W. & Lalatsa, A., eds. *Fundamentals of pharmaceutical nanoscience*. New York: Springer, p.65-90).
- Naik, A., Kalia, Y.N. & Guy, R.H. 2000. Transdermal drug delivery: overcoming the skin's barrier function. *Pharmaceutical science and technology today*, 3:318-325.

New, R.R.C. 1990. Liposomes: a practical approach. New York: Oxford University Press.

520 Ng, S.F., Rouse, J., Sanderson, D. & Eccleston, G. 2010. A comparative study of transmembrane diffusion and permeation of ibuprofen across synthetic membranes using Franz diffusion cells. *Pharmaceutics*, 2:209-223.

Ning, M., Guo, Y., Pan, H., Chen, X. & Gu, Z. 2005. Preparation, in vitro and in vivo evaluation of liposomal/niosomal gel delivery systems for clotrimazole. *Drug development and industrial pharmacy*, 31:375-383.

525 OECD **see** Organisation for economic co-operation and development

Organisation for economic co-operation and development. 2004. Guidance document for the conduct of skin absorption studies: OECD series on testing and assessment, No. 28. [http://www.oecd-ilibrary.org/docserver/download/9750281e.pdf?expires=147273](http://www.oecd-ilibrary.org/docserver/download/9750281e.pdf?expires=1472733343&id=id&accname=guest&checksum=C1CE754CE29D5DC604CA843D8951E854)

530 3343&id=id&accname=guest&checksum=C1CE754CE29D5DC604CA843D8951E854 Date of access: 18. Jun. 2019.

Oschsendorf, F. 2006. Systemic antibiotic therapy for acne vulgaris. *Journal der Deutschen dermatologischen gesellschaft*, 4:828-841.

Paudel, K.S., Milewski, M., Swadley, C.L., Brogden, N.K., Ghosh, P. & Stimchcomb, A.L.

535 2010. Challenges and opportunities in dermal/transdermal delivery. *Therapeutic delivery*, 11:109-131.

Prausnitz, M.R. & Langer, R. 2008. Transdermal drug delivery. *Nature biotechnology*, 26: 1261-1268.

Roland, I., Piel, G., Delattre, L. & Evrad, B. 2003. Systematic characterization of oil-in-water emulsions for formulation design. *International journal of pharmaceutics*, 263:85-94.

540 Shingade, G.M., Amer, Q., Sabale, P.M., Grampurohit, N.D., Gadhave, M.V., Jadhav, S.L., Gaikwad, D.D. & Patil, K.T. 2012. Review on: recent trend on transdermal drug delivery system. *Journal of drug delivery & therapeutics*, 2:66-75.

Shirsand, S.B., Para, M.S., Nagendrakumar, D., Kanani, K.M. & Keerthy, D. Formulation

545 and evaluation of Ketoconazole niosomal gel drug delivery system. *International journal of pharmaceutical investigation*, 2:201-207.

- Swart, M. 2019. Stability of amorphous forms of roxithromycin when encapsulated in liposomes. Potchefstroom: NWU. (Dissertation – MSc).
- Threlfall, T.L. 1995. Analysis of organic polymorphs. *Analyst*, 120:2435-2460.
- 550 Vasconcelos, T., Marques, S. & Sarmento, B. 2015. Development of a resveratrol solid dispersion with soluplus®. Controlled release society annual meeting. Edinburgh, Scotland.
- Vasconcelos, T., Marques, S., das Neves, J. & Sarmento, B. 2016. Amorphous solid dispersions: Rational selection of a manufacturing process. *Advanced drug delivery reviews*, 100:85-101.
- 555 Williams, A.C. 2003. Structure and function of human skin. (*In Williams, A.C., ed. Transdermal and topical drug delivery: from theory to clinical practice. London: Pharmaceutical Press*).
- Wosicka-Frackowiak, H., Cal, K., Stefanowska, J., Glowka, E., Nowacka, M., Struck-Lewicka, W., Govedarica, B., Pasikowska, M., Debrowska, R., Jesionowski, T., Srcic, S. &
- 560 Markuszewski, M.J. 2015. Roxithromycin-loaded lipid nanoparticles for follicular targeting. *International journal of pharmaceutics*, 495:807-815.
- Xiang, R., Chen, S., Zhang, F., Ni, J. & Tian, X. 2009. A HPLC method for the determination of entrapment efficiency of a new 5-FPE liposome formulation. *Journal of Chinese pharmaceutical sciences*, 18:186-189.
- 565 Zatz, J.L., Berry, J.J. & Alderman, D.A. 1989. Viscosity-impairing agents in disperse systems. (*In Lieberman, H.A., Rieger, M.M. & Banker, G.S., eds. Pharmaceutical dosage forms: disperse systems. Vol.2. New York: Marcel Dekker, p. 171-203*).

Tables

570 Table 1:

Composition of the vesicle dispersions containing different solid-state forms

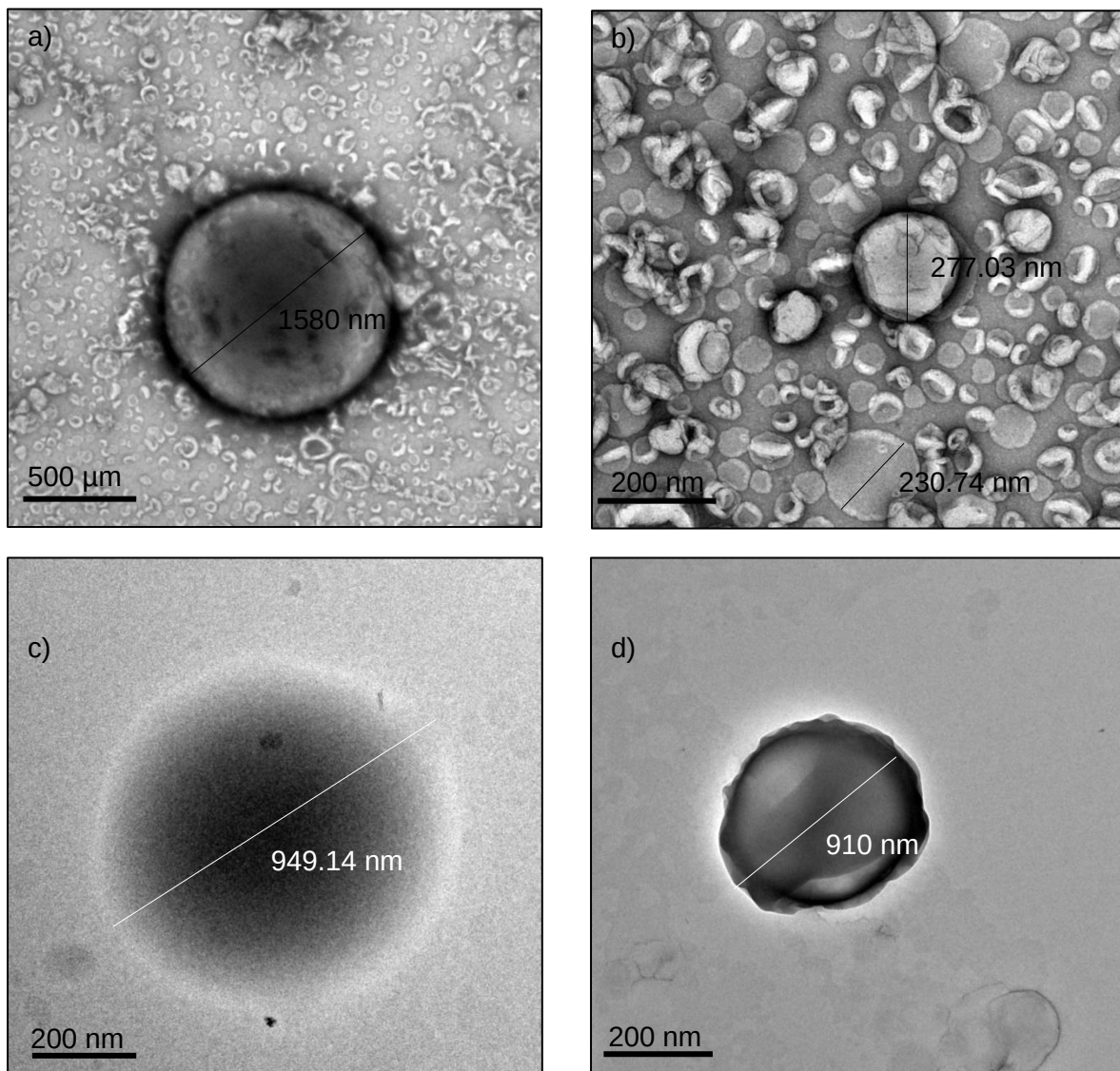
Excipients	MH	CS	AH
Liposomes			
Roxithromycin	200 mg equivalent to 195.76 mg	200 mg equivalent to 174.56 mg	200 mg
Lecithin	560 mg	560 mg	560 mg
Cholesterol	240 mg	240 mg	240 mg
α-Tocopherol	20 μ l	20 μ l	20 μ l
Chloroform	10 ml	10 ml	10 ml
Milli-Q[®] water	10 ml	10 ml	10 ml
Niosomes			
Roxithromycin	200 mg equivalent to 195.76 mg	200 mg equivalent to 174.56 mg	200 mg
Span[®] 60	400 mg	400 mg	400 mg
Cholesterol	400 mg	400 mg	400 mg
Chloroform:methanol (2:1, v/v)	5 ml	5 ml	5 ml
Milli-Q[®] water	10 ml	10 ml	10 ml

Table 2:

575 Characterisation results for liposome and niosome dispersions

Dispersion	Vesicle size (nm)	Pdl	Zeta-potential (mV)	pH	%EE
Liposomes					
PL	90.07±1.44	0.203±0.01	-38.40±0.16	7.87±0.01	NA
MH	90.54±2.83	0.183±0.02	-40.87±1.48	7.07±0.04	99.71±0.01
CS	81.57±2.77	0.167±0.01	-52.05±0.45	7.33±0.03	99.69±0.04
AH	83.91±2.92	0.178±0.01	-48.90±4.10	7.20±0.03	99.73±0.04
Niosomes					
PL	226.27±30.83	0.613±0.01	-33.03±0.53	8.64±0.07	NA
MH	354.17±27.12	0.701±0.07	-61.73±0.83	8.41±0.03	99.88±0.01
CS	330.40±7.06	0.687±0.01	-50.00±0.20	8.41±0.01	99.87±0.01
AH	295.33±37.35	0.615±0.06	-52.73±1.76	8.88±0.01	99.91±0.02

Figures



580 Fig. 1: TEM micrographs: a) and b) showing liposome vesicles, and c) and d) showing niosome vesicles

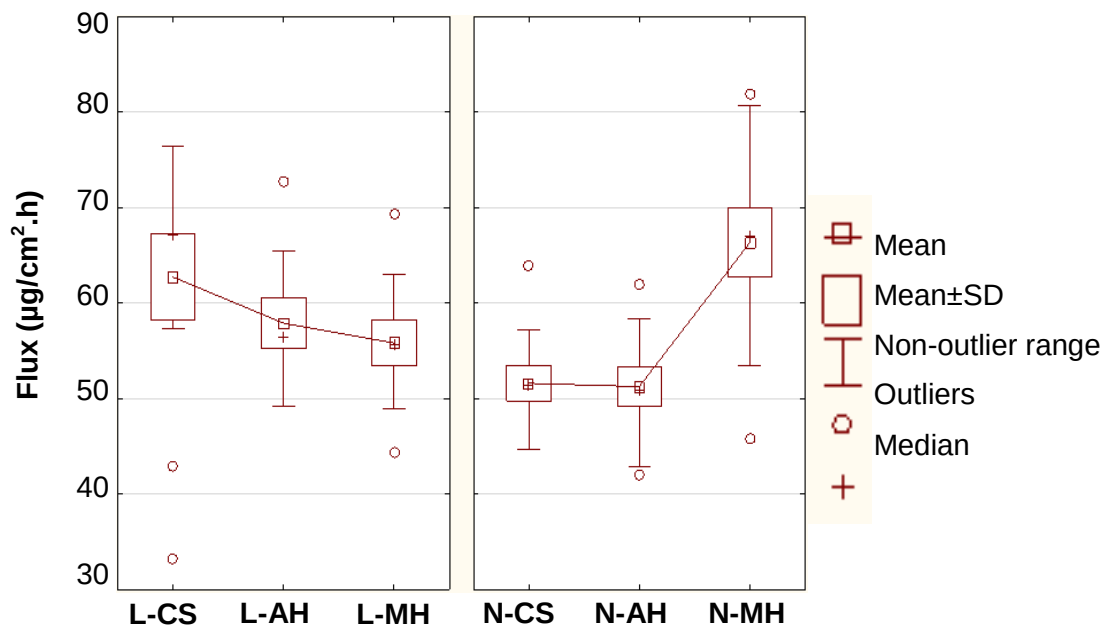


Fig. 2: Box-plots indicating the flux ($\mu\text{g}/\text{cm}^2\cdot\text{h}$) of the liposomal and niosomal emulgels

585 containing roxithromycin during the membrane release studies over 6 h

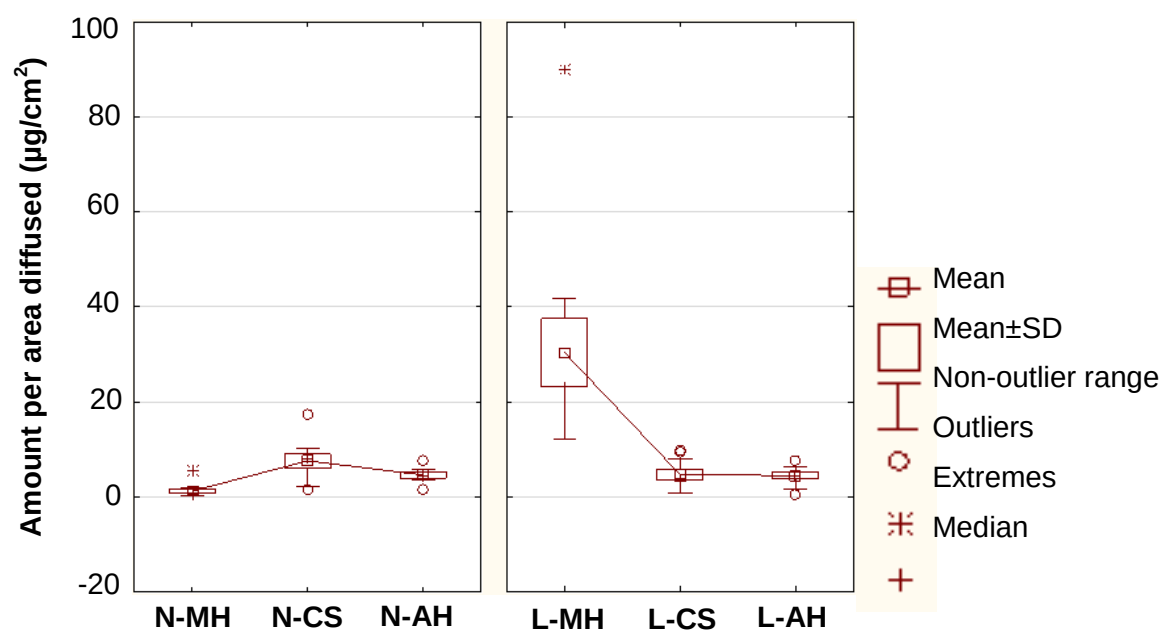


Fig. 3: Box-plot indicating the mean and median amount per area diffused ($\mu\text{g}/\text{cm}^2$) for roxithromycin in the liposomal and niosomal emulgels after 12 h

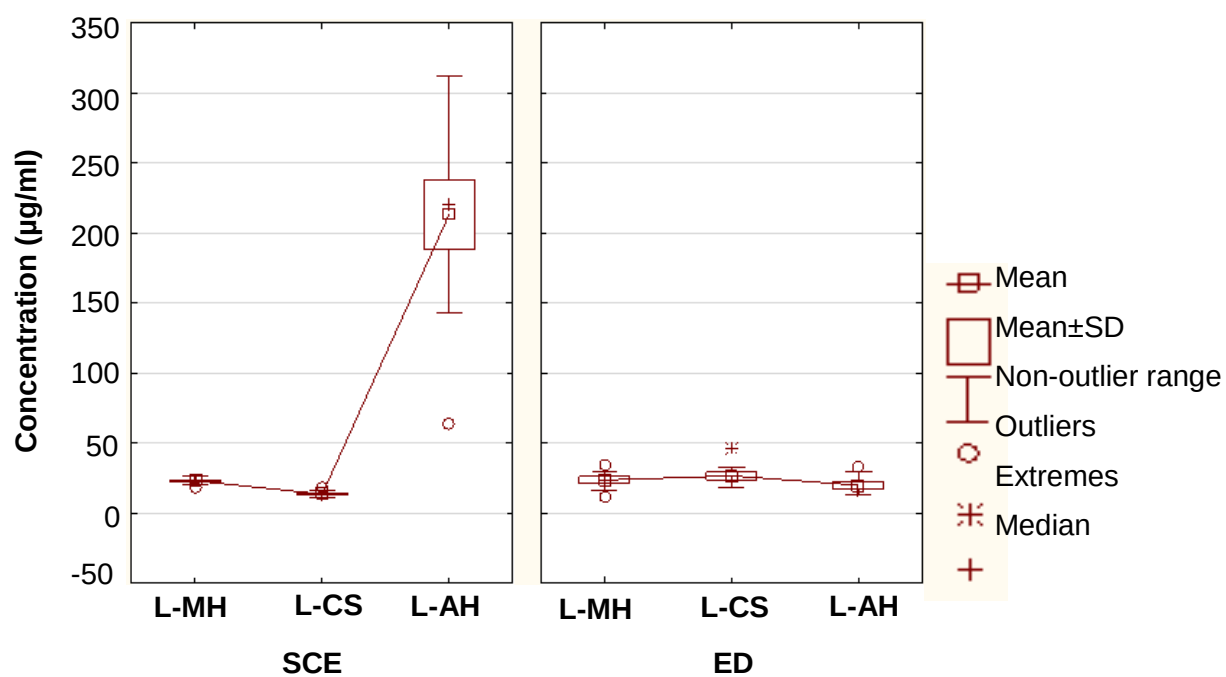


Fig. 4: Box-plot indicating the mean and median concentration ($\mu\text{g/ml}$) of the liposomal emulgels for roxithromycin present in the SCE and ED

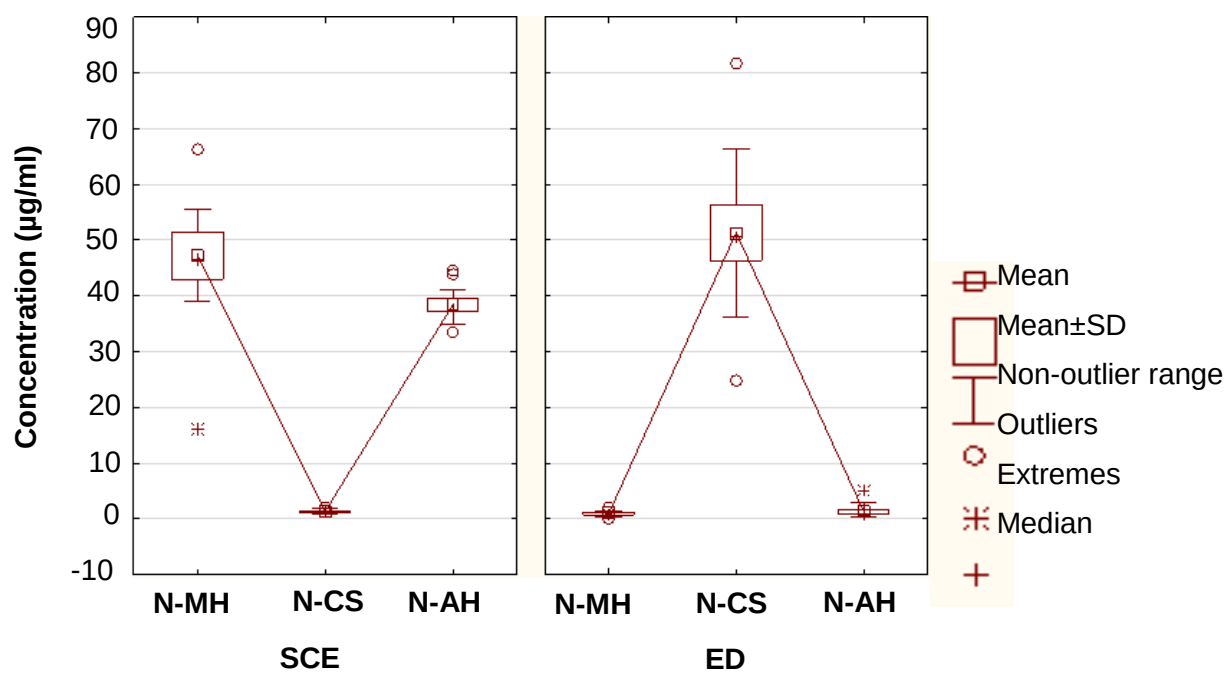


Fig. 5: Box-plot indicating the mean and median concentration (µg/ml) of the niosomal emulgers for roxithromycin present in the SCE and ED

CHAPTER 4

CONCLUSION AND FUTURE RECOMMENDATIONS

Roxithromycin is a semi-synthetic macrolide antibiotic, which can be used to treat skin infections (Bryskier, 1998:1). The big surface area of human skin is an ideal route for drug administration; not only are systemic adverse effects and drug interactions reduced, but patient compliance is also improved due to ease of administration (Prausnitz & Langer, 2008:1261; Shingade *et al.*, 2012:66). Topically applied antibiotics are generally used to treat mild to moderate acne infections due the bacteriostatic effect it has on *P. acnes* (Oschsendorf, 2006:829). The acne causing microorganism, *P. acnes*, is susceptible to roxithromycin and can be used to effectively treat mild to moderate inflammatory acne (Glowka *et al.*, 2014:83; Wosicka-Frackowiak *et al.*, 2015:816).

The target-site of roxithromycin is the sebaceous follicles located in the dermis, where *P. acnes* accumulates, making it an ideal contender for acne treatment (Gollnick, 2003:1585). The current topical treatment options for inflammatory lesions include clindamycin or erythromycin, but with growing resistance against *P. acnes* strains due to continuous use, a need arises for novel treatment options (Lavers, 2014:334). A major challenge for the topical delivery of roxithromycin is the poor aqueous solubility, but altering the solid-state of an API can manipulate the physicochemical factors and improve the solubility (Threlfall, 1995:2452). Different solid-states of roxithromycin have been researched (Aucamp *et al.*, 2012:475; Aucamp *et al.*, 2013:18; Bawa, 2007; Du Plessis, 2004; Milne *et al.*, 2016), and it has been reported that some of these solid-state forms can be included into thin-films, which serve as precursors for drug delivery vesicles (Csongradi, 2015:100; Boshoff, 2018:65; Swart, 2019:86). Including roxithromycin into thin-films renders the API into an amorphous state due to the forming of an ASD. Including roxithromycin into vesicle delivery systems, such as liposomes and niosomes, attributes to targeted topical delivery and improved bioavailability of the API.

Niosomes are hydrated non-ionic surfactant-based vesicular systems, while liposomes are vesicle systems composed of phospholipids (Kumar & Rajeshwarrao, 2011:209). Liposomes and niosomes have the ability to encapsulate hydrophilic or lipophilic drugs, which can increase the solubility of poorly soluble drugs 100 – 1 000-fold (Bansal *et al.*, 2012:709). The aim of this study was to establish whether liposomal or niosomal vesicle systems containing roxithromycin, could be developed further into emulgel formulations for topical application.

In order to achieve the goal of this study, the following objectives were set:

- Verification of suitability of the HPLC method for roxithromycin.

- Preparation of **AH** and **CS** solid-state forms of roxithromycin.
- Solid-state characterisation by investigating the morphology using microscopy techniques, followed by confirming correlation of physicochemical attributes using FTIR, with that which is already reported in literature.
- Performing TGA to determine the hydration or solvation level of the three crystalline forms of roxithromycin.
- Preparation of liposome and niosomes containing the individual solid-state forms of roxithromycin (**AH**, **CS** and the **MH**).
- Characterisation of drug delivery vesicles with and without the API in terms of drug EE%, morphology, pH, physical appearance, droplet size and zeta-potential.
- To formulate an emulgel containing these liposomes and niosomes separately as drug delivery vesicles.
- Determination of the stability of the emulgels containing the loaded or unloaded vesicles in accordance with ICH and SAHPRA guidelines. Stability was determined by means of the following characterisation: pH, viscosity, mass loss, zeta-potential, droplet size and physical appearance. Assay of the API was additionally evaluated for the emulgels containing the API.
- The determination of the concentration of API released from the emulgel by means of membrane release studies.
- Determining the transdermal and topical delivery of the APIs from the emulgel formulation by performing skin diffusion studies, followed by tape stripping.

The previously validated and adapted HPLC method for the quantification of roxithromycin, as reported by Boshoff (2018:68) and Swart (2019:91), was verified. The analysis method proved sensitive, accurate, repeatable and reliable to detect the concentration of roxithromycin. The method was used throughout the study for roxithromycin quantification.

The chloroform solvate solid-state form of roxithromycin was prepared according to the method reported by Aucamp *et al.* (2012). The **AH** of roxithromycin was prepared by dehydrating the purchased roxithromycin **MH** using a laboratory drying oven.

Three solid-states of roxithromycin comprising **MH**, **CS** and **AH** were characterised by means of FTIR. The results obtained correlated with published results of Aucamp *et al.* (2012) and Aucamp *et al.* (2013), and it was established that the **CS** and **AH** solid-state forms were not the monohydrated form of roxithromycin. This was further confirmed using TGA, which was used to

determine the hydration or solvation level of the three crystalline forms of roxithromycin. The obtained percentage solvent that formed part of the crystalline structure was used to calculate the molecular weight of each crystalline form, which was applied when weighing the different APIs throughout the study.

The **MH**, **CS** and **AH** solid-state forms were encapsulated into liposome and niosome vesicles, respectively. Liposomes were prepared using an adapted method of solvent casting (Vasconcelos & Sarmento, 2015; Vasconcelos *et al.*, 2016:89), which was combined with the thin-film/hand shaking method, as originally reported by New (1990:36-38). Niosomes were prepared using an adapted method described by Agarwal *et al.* (2001:44). A placebo vesicle system for both the liposome and niosome systems was also prepared. The three solid-states were encapsulated into the vesicle systems separately, which resulted in a total of eight vesicle dispersions.

The prepared vesicle dispersions were characterised in terms of droplet size, pH and zeta-potential. Transmission electron microscopy (TEM) was performed in addition to the other characterisation methods for the niosome and liposome dispersions containing no API, while the EE% was determined for the vesicle dispersions containing API. The TEM results of the empty vesicle systems revealed well-formed liposomes and niosomes, with sizes smaller than 3 µm, making it ideal for topical drug delivery (Barry, 2001:101; Bolzinger *et al.*, 2012:163). To ensure optimal topical delivery, particle sizes between 10 – 1 000 nm are recommended (Kumar & Rajeshwarrao, 2011:209). The droplet sizes of all eight dispersions rendered satisfactory results, with the liposome dispersions ranging between 80 – 90 nm and the niosome dispersions ranging between 200 – 300 nm. All the samples resulted in highly negative zeta-potential values, which would improve permeation of the drug into the skin (Duangjit *et al.*, 2011:6). All vesicle dispersions resulted in pH values higher than 7 and below 9, making them ideal contenders for skin application, since they met the requirements for topical formulations (pH should be between 3 – 9) (Naik *et al.*, 2000:319; Paudel *et al.*, 2010:118). All the vesicle systems containing the different roxithromycin solid-states resulted in high entrapment near 100%. Better target-site delivery would be obtained as the API was almost fully incorporated within the vesicles. Overall, satisfactory results were obtained during characterisation, confirming the roxithromycin **MH**, **CS** and **AH** could be successfully included in liposomes and niosomes for topical delivery.

To increase the low viscosity of the vesicle formulations, a topical application containing the liposome and niosome vesicle systems was prepared. An emulgel was the chosen application, which consisted of mixing the vesicle dispersions with a hydrogel base. This method was adapted from Shirsand *et al.* (2016:203) and Ning (2005:378), and was applied to both vesicle systems, with and without API. Theoretically, all the emulgels contained 2% of the different

solid-state forms of roxithromycin encapsulated in vesicle delivery systems, separately. Due to the phosphatidylcholine (PC) used during liposome preparation, the liposomal emulgels presented with a light yellow colour. The niosomal emulgels had a white colour due to the use of Span[®] and cholesterol during vesicle preparation. All gels had a smooth gel-like consistency, with the placebo gels resulting with a lower viscosity than the emulgels containing the different roxithromycin solid-states.

Adequate quantities of the vesicle emulgels were prepared and used to perform stability testing at different storage conditions, as stipulated by ICH and SAHPRA. The stability tests consisted of the following characterisation: pH, viscosity, mass loss, zeta-potential, droplet size and physical appearance. The assay of the API was additionally evaluated for the emulgels containing the API. All liposomal and niosomal emulgels containing the API remained within the acceptable pH range for transdermal delivery, with the lowest pH value measured at 3.82 and the highest measured at 6.46 over the three-month testing period. The ideal pH range for skin delivery is between 3 and 9 (Naik *et al.*, 2000:319). There was a significant change in viscosity observed over the three months, with fluctuating values for both the liposomal and niosomal emulgels. The macroscopic results also confirmed that temperature and humidity influenced the viscosity of the emulgels. There was an insignificant change in mass during the three-month period, confirming that the storage containers used were suitable. The ideal zeta-potential value should be higher than + 30 mV or lower than - 30 mV, which indicates a stable formulation (Malvern Instruments, 2015:3). Significant changes occurred during the three-month period, and many of the liposomal and niosomal zeta-potential values did not comply with this limit. The droplet sizes varied significantly during the three-month testing period, as well as the polydispersity index (Pdl), the products varied from monodispersed to polydispersed, resulting in unsatisfactory results due to large droplets or aggregation. The physical assessment revealed that high temperatures and humidity influenced the colour, the viscosity and skin feel of emulgels significantly. The most significant colour change was observed with the liposomal emulgels due to the absence of PC. The liposomal emulgel containing **MH** revealed micro-bacterial contamination during storage. The appearance of all emulgels containing API went from smooth to uneven, with detectable changes in the viscosity of the products, but the “skin feel” remained smooth whenever applied. The niosomal emulgels physically revealed a less significant change than the liposomal emulgels during storage. According to ICH (2003:9), there should be no variation larger than 5% in the assayed concentration from the initial value. The concentration in both the liposomal and niosomal emulgels fluctuated during the three months testing period, and therefore it can be stated that none of the loaded emulgels complied with this limit over the three months period. With only the pH and mass loss resulting in satisfactory results during the accelerated stability testing, the

observation was that an improved emulgel formulation with better stability needed to be developed.

Membrane release studies were performed to determine if the encapsulated solid-states of roxithromycin were being released from the encapsulated vesicles. The results revealed that all six vesicle dispersions successfully released the API from the vesicles formulated in an emulgel. The highest median flux was obtained by the niosomal emulgel containing **MH** ($67.08 \mu\text{g}/\text{cm}^2\cdot\text{h}$), while the lowest median flux was observed by the niosomal emulgel containing **AH** ($50.51 \mu\text{g}/\text{cm}^2\cdot\text{h}$). The excipients used to prepare the emulgels were identical for the separate vesicle systems, therefore it could be confirmed that the initial solid-state influenced the delivery.

Skin diffusion studies followed the membrane release studies. By applying the tape stripping method, it was possible to determine if the API was delivered topically. Small amounts of the API were delivered transdermally, with the highest median amount per area diffused achieved by the liposomal emulgel containing **MH** ($23.20 \mu\text{g}/\text{cm}^2$); all the other emulgels resulted in minimal delivery with median amount per area diffused values below $7.5 \mu\text{g}/\text{cm}^2$. The lowest amount per area diffused was observed by the niosomal emulgel containing **MH** ($0.58 \mu\text{g}/\text{cm}^2$), confirming that the type of vesicle used can be a deciding factor for the diffusion rate. Limited systemic delivery is desirable, to avoid possible adverse systemic effects.

Delivery to the stratum corneum-epidermis (SCE) is ranked from the highest to the lowest concentration as follows: the liposomal emulgel containing **AH**, the niosomal emulgel containing **MH**, the niosomal emulgel containing **AH**, the liposomal emulgel containing **MH**, the liposomal emulgel containing **CS** and lastly the niosomal emulgel containing **CS**. All emulgels resulted in values above the limit of detection (LOD) ($0.75 \mu\text{g}/\text{ml}$) and lower limit of quantification (LOQ) ($2.51 \mu\text{g}/\text{ml}$), except for the niosomal emulgel containing **CS** which resulted in a value below the LOQ with a median concentration of $1.30 \mu\text{g}/\text{ml}$. All emulgels were successful in reaching the target-site, which was the epidermis-dermis (ED), with the exception of the niosomal emulgels containing **MH** and **AH** with average concentration values below the LOQ. It is possible that the highly lipophilic **MH** and **AH** niosomal emulgels had a higher affinity for the various stratum corneum lipids, leading to a longer retention time in the SCE layer, resulting in poor ED delivery. The highest ED concentration was achieved by the niosomal emulgel containing **CS** ($50.54 \mu\text{g}/\text{ml}$), indicating that even though the initial solid-state was rendered to ASDs, physicochemical differences occurred, influencing topical delivery. The liposomal emulgel with the highest ED concentration was **CS** ($22.92 \mu\text{g}/\text{ml}$). The ED delivery can be ranked from highest to lowest concentration as follows: the niosomal emulgel containing **CS**, the liposomal emulgel containing **MH**, the liposomal emulgel containing **CS**, the liposomal emulgel containing **AH**, the niosomal emulgel containing **MH** and the niosomal emulgel containing **AH**. **CS**

revealed the lowest SCE delivery and the highest ED delivery for both liposomal and niosomal emulgels, suggesting that this solid-state form of roxithromycin had the most favourable physicochemical properties to reach the target-site when included in an ASD. The aim of this study was to establish if roxithromycin encapsulated in liposome and niosome vesicle systems could be developed into emulgel formulations for topical delivery. This aim was reached, proving that it was possible to include encapsulated roxithromycin into an emulgel. It was however observed that it is necessary to refine the emulgel formulation and to include preservatives. It was also determined that quantifiable concentrations of the API were found in the target area of the ED, with the niosomal vesicles containing **CS** resulting in the highest delivery.

Future recommendations include:

- Incorporate a higher concentration of roxithromycin into the vesicle systems to improve skin permeation results.
- Since a jelly instead of a gel is prone to form, due to the development of an overly strong matrix between Carbopol® and roxithromycin, it is recommended to use excipients with a weaker affinity to the API for emulgel formulation.
- If Carbopol® is used for emulgel formulation it must be given adequate hydration time and thoroughly mixed thereafter to avoid phase separation during storage.
- Add preservatives in emulgel formulations.
- Use amber storage jars for liposomal formulations due to possible degradation during storage.

References

- Agarwal, R., Katare, O.P. & Vyas, S.P. 2001. Preparation and in vitro evaluation of liposomal/niosomal delivery systems for antipsoriatic drug dithranol. *International journal of pharmaceutics*, 228:43-52.
- Aucamp, M., Liebenberg, W., Strydom, S.J., van Tonder, E.C. & de Villiers, M.M. 2012. Physicochemical properties of amorphous roxithromycin prepared by quench cooling of the melt or desolvation of a chloroform solvate. *American association of pharmaceutical scientists, PharmSciTech*, 13:467-476.
- Aucamp, M., Stieger, N., Barnard, N. & Liebenberg, W. 2013. Solution-mediated phase transformation of different roxithromycin solid-state forms: Implications on dissolution and solubility. *International journal of pharmaceutics*, 449:18– 27.
- Bansal, S., Kashyap, C.P., Agarwal, G. & Harikumar, S.L. 2012. A comparative review on vesicular drug delivery systems and stability issues. *International journal of research in pharmacy and chemistry*, 2:704-713.
- Barry, B.W. 2001. Novel mechanisms and devices to enable successful transdermal drug delivery. *European journal of pharmaceutical sciences*, 14:101-114.
- Bawa, Y. 2007. Solvent inclusion properties of Triamterene crystal forms and solubility differences between roxithromycin polymorphic forms. Potchefstroom: NWU. (Dissertation-MSc).
- Bolzinger, M., Briancon, S., Pelletier, J. & Chevalier, Y. 2012. Penetration of drugs through skin, a complex rate-controlling membrane. *Current opinion in colloid & interface science*, 17:156-165.
- Boshoff, S. 2018. Investigation of the stabilising effects of niosomes on the amorphous forms of roxithromycin. Potchefstroom: NWU. (Dissertation – MSc).
- Bryskier, A. 1998. Roxithromycin: review of its antimicrobial activity. *Journal of antimicrobial chemotherapy*, 41:1–21.
- Csongradi, C. 2015. The effect of solid-state forms on the topical delivery of roxithromycin. Potchefstroom: NWU. (Dissertation – MSc).
- Duangjit, S., Opanasopit, P., Rojanarata, T. & Ngawhirunpat, T. 2011. Characterization and in vitro skin permeation of meloxicam-loaded liposomes versus transferosomes. *Journal of drug delivery*, 2011:1-9.

Du Plessis, C. 2004. Characterisation of polymorphic, pseudopolymorphic and amorphous Forms of roxithromycin. Potchefstroom: NWU. (Dissertation-MSc).

Glowka, E., Wisicka--Frakowiak, H., Hyla, K., Stefanowska, J., Jastrzebska, K., Klapiszewski, L., Jesionowski, T. & Cal, K. 2014. Polymeric nanoparticles-embedded organogel for roxithromycin. *European journal of pharmaceutics and biopharmaceutics*, 88:75-84.

Gollnick, H. 2003. Current concepts of the pathogenesis of acne. *Drugs*, 63:1579-1596.

ICH **see** International conference on harmonisation of technical requirements for registration of pharmaceuticals for human use

International conference on harmonisation of technical requirements for registration of pharmaceuticals for human use. 2003. ICH harmonised tripartite guideline: Stability testing of new drug substances and products Q1A(R2). <http://www.ich.org/products/guidelines/quality/article/quality-guidelines.html> Date of access: 5 Jun. 2019.

Kumar, G.P. & Rajeshwarrao, P. 2011. Nonionic surfactant vesicular systems for effective drug delivery—an overview. *Acta Pharmaceutica Sinica B*, 1:208-219.

Lavers, I. 2014. Diagnosis and management of acne vulgaris. *Nurse prescribing*, 12:330-336.

Malvern Instruments LTD. 2015. Zetasizer nano series technical note. <http://www3.nd.edu/~rroeder/ame60647/slides/zeta.pdf> Date of access: 5 Jun. 2019.

Milne, M., Liebenberg, W. & Aucamp, M.E. 2016. Different amorphous solid-state forms of roxithromycin: a thermodynamic and morphological study. *International journal of pharmaceutics*, 498:304-315.

Naik, A., Kalia, Y.N. & Guy, R.H. 2000. Transdermal drug delivery: overcoming the skin's barrier function. *Pharmaceutical science and technology today*, 3:318-325.

New, R.R.C. 1990. *Liposomes: a practical approach*. New York: Oxford University Press.

Ning, M., Guo, Y., Pan, H., Chen, X. & Gu, Z. 2005. Preparation, in vitro and in vivo evaluation of liposomal/niosomal gel delivery systems for clotrimazole. *Drug development and industrial pharmacy*, 31:375-383.

Oschsendorf, F. 2006. Systemic antibiotic therapy for acne vulgaris. *Journal der Deutschen dermatologischen gesellschaft*, 4:828-841.

Paudel, K.S., Milewski, M., Swadley, C.L., Brogden, N.K., Ghosh, P. & Stimchcomb, A.L. 2010. Challenges and opportunities in dermal/transdermal delivery. *Therapeutic delivery*, 11:109-131.

Prausnitz, M.R. & Langer, R. 2008. Transdermal drug delivery. *Nature biotechnology*, 26: 1261-1268.

Shingade, G.M., Aamer, Q., Sabale, P.M., Grampurohit, N.D., Gadhave, M.V., Jadhav, S.L., Gaikwad, D.D. & Patil, K.T. 2012. Review on: recent trend on transdermal drug delivery system. *Journal of drug delivery & therapeutics*, 2:66-75.

Shirsand, S.B., Para, M.S., Nagendrakumar, D., Kanani, K.M. & Keerthy, D. Formulation and evaluation of Ketoconazole niosomal gel drug delivery system. *International journal of pharmaceutical investigation*, 2:201-207.

Swart, M. 2019. Stability of amorphous forms of roxithromycin when encapsulated in liposomes. Potchefstroom: NWU. (Dissertation – MSc).

Threlfall, T.L. 1995. Analysis of organic polymorphs. *Analyst*, 120:2435-2460.

Vasconcelos, T., Marques, S. & Sarmiento, B. 2015. Development of a resveratrol solid dispersion with soluplus®. Controlled release society annual meeting. Edinburgh, Scotland.

Vasconcelos, T., Marques, S., das Neves, J. & Sarmiento, B. 2016. Amorphous solid dispersions: Rational selection of a manufacturing process. *Advanced drug delivery reviews*, 100:85-101.

Wosicka-Frackowiak, H., Cal, K., Stefanowska, J., Glowka, E., Nowacka, M., Struck-Lewicka, W., Govedarica, B., Pasikowska, M., Debrowska, R., Jesionowski, T., Srcic, S. & Markuszewski, M.J. 2015. Roxithromycin-loaded lipid nanoparticles for follicular targeting. *International journal of pharmaceutics*, 495:807-815.

APPENDIX A

ANALYTICAL METHOD VERIFICATION FOR HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC ANALYSIS OF ROXITHROMYCIN

A.1 Introduction

Validated methods that are justified and suitable to test the quality parameters of a particular pharmaceutical ensure accurate and dependable results. According to the USP (2013:1), the validation of a method is the demonstration that an analytical method used for a specific test is suitable, precise and true for its intended purpose. It is redundant to fully re-validate a method that has been validated; the existing validated method needs only to be verified by partially validating the method (FDA, 2014:19; Moffat, 2011:362; Etse, 2007:736; WHO, 2010:16, WHO, 2018:6). Method verification is performed to demonstrate and confirm the laboratory can perform the validated method as intended in the hands of the user (FDA, 2014:17). This HPLC verification was used to confirm the validated method, which will subsequently be used to detect the concentration of roxithromycin, as published by Aucamp *et al.* (2016:175) and adapted by Boshoff (2018:68) and Swart (2019:91).

A.2 Chromatographic conditions

All research was performed at the Analytical Technology Laboratory (ATL) at the North-West University (NWU), Potchefstroom Campus, in a controlled research facility at room temperature (25°C). The parameters used are noted below:

Analytical instrument:	An Agilent® 1100 Series HPLC system, equipped with an Agilent® 1100 isocratic pump, auto sampler and ultraviolet (UV) detector (Agilent Technologies, California), was used.
Column:	A Venusil XBP C ₁₈ (2) reversed phase column, consisting of pure silica (150 x 4.6 mm) with a particle size of 5 µm was used (Agela Technologies, Germany).
Mobile phase:	The mobile phase consisted of 0.01 M ammonium dihydrogen phosphate (H ₆ NO ₄ P) buffer solution, with pH adjusted to 7.0 with diluted ammonia solution (7% w/v). The buffer solution was mixed with HPLC grade methanol in an 85:15 (methanol:buffer) ratio. The mobile phase was filtered through a 0.22 µm nylon membrane filter (Membrane Solutions®, United States of America) before use.
Flow rate:	1.0 ml/min

Injection volume:	50 µl
Detection:	UV at 205 nm
Retention time:	± 5 min
Stop time:	9 min
Solvent:	Methanol

A.3 Standard preparation

The standard solution was prepared by weighing 20 mg of roxithromycin reference standard powder and dissolving it in methanol in a 100 ml volumetric flask. The solution was agitated in an ultrasonic bath for 1 min before being made up to volume. The solution was filtered into a HPLC vial and injected in duplicate using different injection volumes.

A.4 Verification parameters

The parameters to verify this validated analytical method included accuracy (recovery), linearity, limit of detection (LOD) together with lower limit of quantification (LOQ), precision (repeatability) and specificity (ALACC, 2007:6; USP, 2013:1; WHO, 2018:10).

A.4.1 Accuracy

Accuracy, also known as recovery, is the closeness of agreement between the accepted reference value and the measured value (USP, 2011:2). It is recommended to cover the specified range over a minimum of nine standards with three concentrations (ICH, 2005:10). An acceptable percentage recovery ranges between 95 – 105% (FDA, 2014:19; Lobrutto & Patel, 2007:461). To evaluate the accuracy of the analytical method a reference standard solution, with a known concentration of 205 µg/ml, was prepared. Three different sample solutions at three concentration levels (n = 9) were prepared and analysed in duplicate. From Table A.1, the mean percentage recovery was calculated to be 98.61% (%RSD = 4.9%) and deemed acceptable.

Table A.1: Results for accuracy of roxithromycin

Concentration (µg/ml)	Peak area			Recovery	
	1	2	Average	µg/ml	%
23.30	328.10	344.32	336.21	23.09	99.08
22.50	330.36	313.19	321.77	22.10	98.20
21.00	281.39	251.11	266.25	18.28	87.06
58.25	888.86	897.25	892.97	61.32	105.27
56.25	841.73	809.15	825.44	56.68	100.77
52.50	710.43	742.43	726.43	49.88	95.01
233.00	3463.18	3443.64	3453.41	237.14	101.78
223.00	3280.96	3270.55	3275.76	224.94	99.97
210.00	3060.15	3077.79	3068.97	210.74	100.35
				Mean	98.61
				SD	4.8
				%RSD	4.9

A.4.2 Linearity

The linearity is a validation parameter to measure the ability to acquire results directly proportional to the concentration of the API present in the sample within a specified range (ICH, 2005:5). The interval between the upper and lower concentrations refers to the range of analytical procedure. Linear regression is used to analyse the results. The acceptance criteria specify a regression coefficient of ≥ 0.997 (Lobrutto & Patel, 2007:461). To evaluate the linearity of the analytical method, standard solutions with different concentrations were prepared and injected in duplicate. A linear equation was used to interpret the data yielded by the regression and adapted by Graham (2011:352).

$$y = mx + c$$

Equation A.1

Where:

y = peak area

m = slope

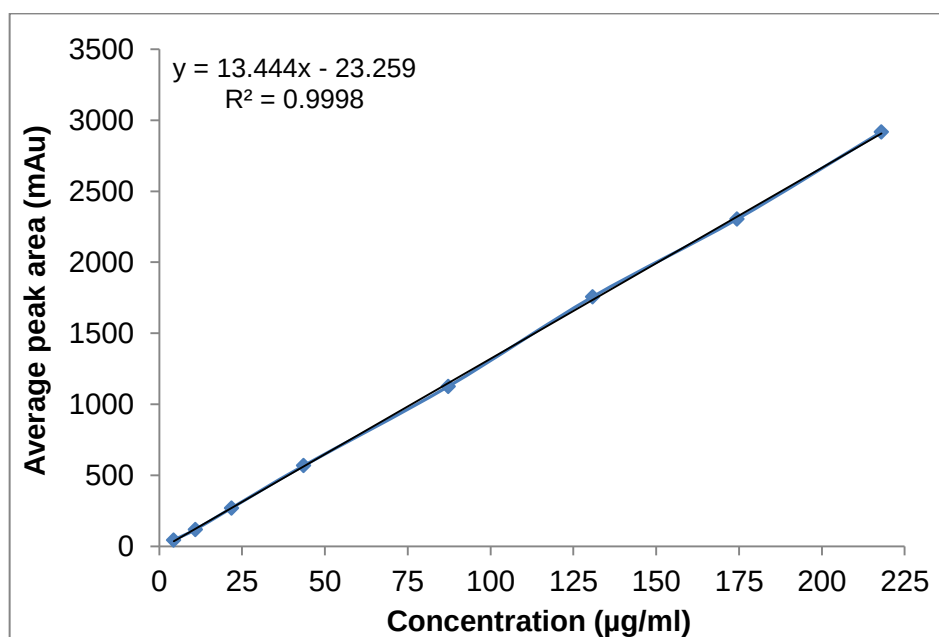
x = concentration of roxithromycin (µg/ml)

c = y-intercept

It is evident that the concentration range of roxithromycin (4.40 – 436.00 µg/ml) *versus* the mean peak area provides a linear plot. From Table A.2 and Figure A.1, it can be observed the regression coefficient (R^2) obtained for roxithromycin for the analytical method was 0.9998, which is indicative that the analytical method is acceptable and within the desired specifications.

Table A.2: Linearity results of roxithromycin standard solution

Standard concentration($\mu\text{g/ml}$)	Peak area		
	1	2	Average
4.4	39.5	50.4	44.9
10.9	121.3	116.2	118.7
21.8	273.9	265.5	269.7
43.6	566.5	569.8	568.2
87.2	1126.0	1124.9	1125.4
130.8	1780.0	1732.9	1756.5
174.4	2279.8	2327.1	2303.4
218.0	2972.3	2862.7	2917.5
R^2			0.9998
y-intercept (c)			-23.259
Slope (m)			13.44

**Figure A.1:** Average peak area plotted against the concentration of roxithromycin to prove the linearity of the analytical method

A.4.3 Limit of detection and lower limit of quantification

The lowest amount of analyte detected in the sample, which is not necessarily quantifiable into an exact numerical value, is defined as the detection limit (LOD). The lowest amount of analyte that can be determined with acceptable accuracy and precision is defined as the quantitation limit (LOQ) (ICH, 2005:5). According to Swartz and Krull (2011:46), the acceptance criteria for LOD is that the signal noise level should be equal to or surpass 3, while the LOQ signal noise level should target 10. To evaluate the LOD and LOQ, a standard with low concentration was

prepared and injected six consecutive times at different injection volumes. To calculate the results, the slope of a calibration curve and standard deviation of the response using the following formulas were applied (Swartz & Krull, 2011:46):

$$\text{LOD} = K (\text{SD}/S) \quad \text{Equation A.2}$$

$$\text{LOQ} = K (\text{SD}/S) \quad \text{Equation A.3}$$

Where:

K = constant (3 for LOD, and 10 for LOQ),

SD = standard deviation of y-intercepts, and

S = slope

Table A.3: Results of limit of detection (LOD) and quantitation (LOQ) of roxithromycin

Concentration (µg/ml)		0.63	1.25	1.88	2.5	5	7.5
Peak area	1	30.79	31.17	38.94	35.60	85.90	125.39
	2	30.86	33.62	41.70	44.01	79.09	129.04
	3	30.18	32.48	39.99	37.63	82.37	132.70
	4	0.00	32.04	36.94	42.59	82.30	129.14
	5	30.16	32.61	41.60	39.86	83.28	130.37
	6	30.52	33.34	36.20	45.22	87.90	129.58
Area mean		25.42	32.54	39.23	40.82	83.47	129.37
SD		11.37	0.81	2.11	3.44	2.81	2.16
%RSD		44.73	2.48	5.39	8.43	3.36	1.67
LOD (µg/ml)		0.752		LOQ (µg/ml)		2.506	

A.4.4 Precision

The agreement between replicate measurements of a sample is defined as precision, this validation parameter can be divided into three levels: repeatability, intermediate precision and reproducibility (USP, 2011:3; Graham, 2011:352). For the verification of a validated method it is only necessary to determine the repeatability, also known as intra-day precision (ALACC, 2007:6; FDA, 2014:19).

A.4.4.1 Repeatability

Repeatability or intra-day precision is used to determine the preciseness of a method over a short interval under the same conditions (ICH, 2005:5; Lavanya *et al.*, 2013:1285). A system is believed repeatable if the %RSD is 2% or less (Lobrutto & Patel, 2007:461). To evaluate the system repeatability, a standard solution with a known concentration was injected six consecutive times.

The results, as seen in Table A.4, confirm the system performance proved repeatable with %RSD values of 1.68% for peak area and 0.25% for the retention time.

Table A.4: Results for repeatability of roxithromycin

Injection number	Peak area	Retention time (min)
1	565.42	5.276
2	566.51	5.298
3	558.79	5.302
4	541.85	5.302
5	549.66	5.305
6	547.01	5.320
Mean	555.87	5.30
SD	9.31	0.01
%RSD	1.68	0.25

A.4.5 Specificity

When an analytical method can selectively distinguish between the analyte of interest in the presence of other components, it may be considered specific (ICH, 2005:4). If no peaks interfere with the peak of the specific analyte it confirms specificity as acceptable. Specificity was evaluated by preparing a standard as previously mentioned in Section A.3. A pipette was used to transfer 2 ml of the prepared standard solution into seven different test tubes, each containing 500 µl of either distilled water (H₂O), sodium hydroxide (NaOH), hydrogen peroxide (H₂O₂), Span[®] 60, phosphatidylcholine (PC) or cholesterol. The test tubes were left to stand for 30 min, where after a volume from each test tube was placed into an HPLC vial and analysed. All samples were injected in duplicate.

Figure A.2 illustrates the standard containing roxithromycin, and serves as a reference standard when comparing the different samples. Figures A.3 to A.5 display the chromatograms of results obtained after analysis of the samples. The roxithromycin peak was easily identifiable in the presence of the degradants. In Figure A.6, the effect of the added components of niosome and

liposome formulations on the roxithromycin peak can be seen. It is discernible that no peaks interfered with the roxithromycin peak.

The specificity experiment proved that the degraded samples, obtained by adding different reagents to roxithromycin solutions, did not interfere with the determination and quantification of roxithromycin.

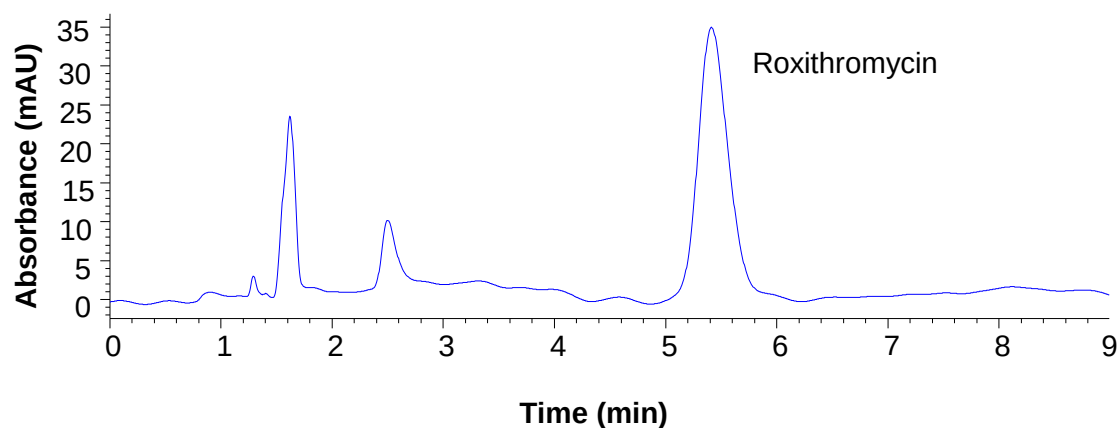


Figure A.2: Roxithromycin standard for specificity analysis

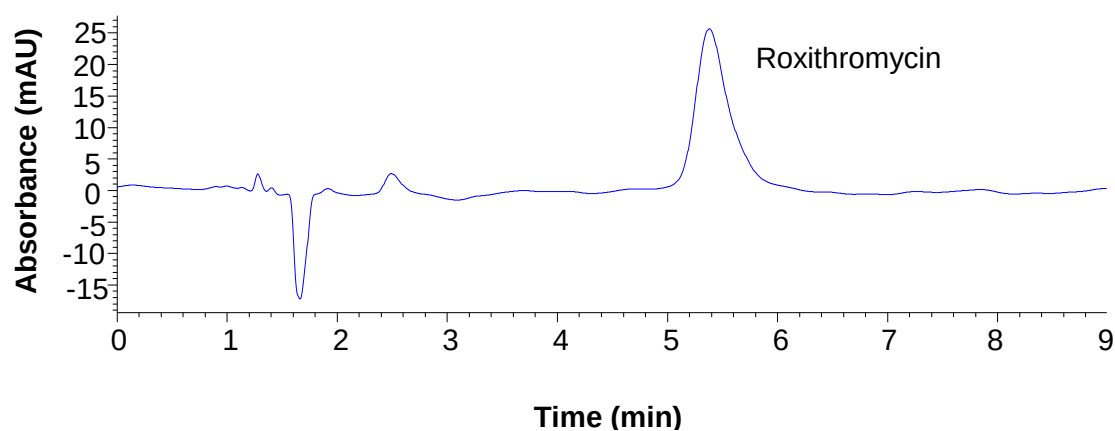


Figure A.3: Specificity analysis results using distilled water (H₂O) as reagent

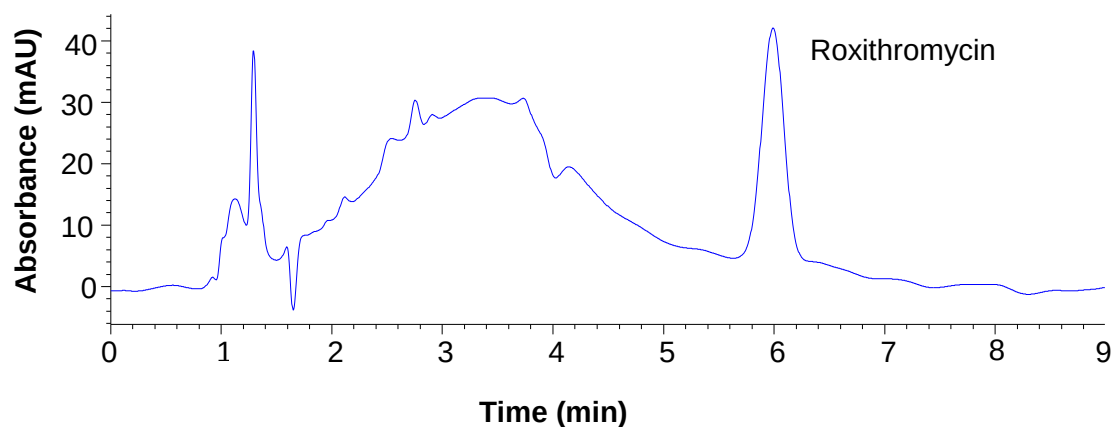


Figure A.4: Specificity analysis results using sodium hydroxide (NaOH) as reagent

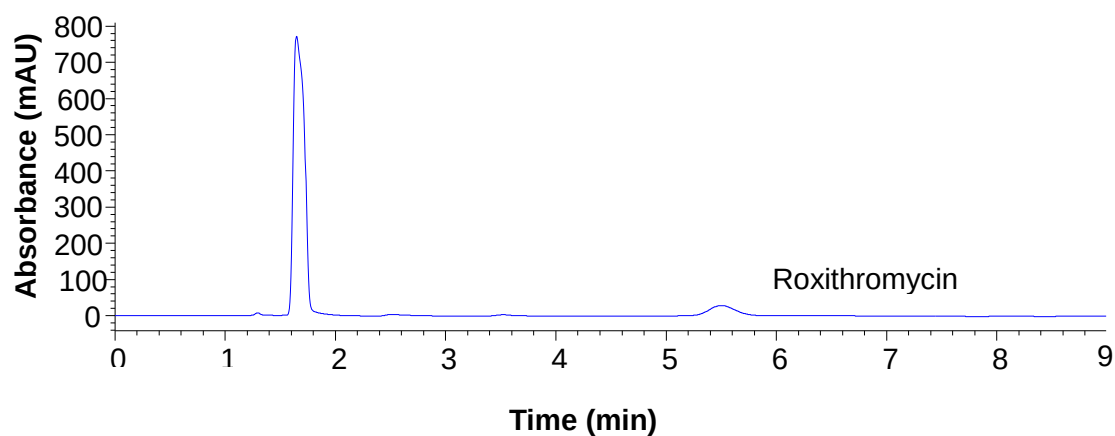


Figure A.5: Specificity analysis results using hydrogen peroxide (H₂O₂) as reagent

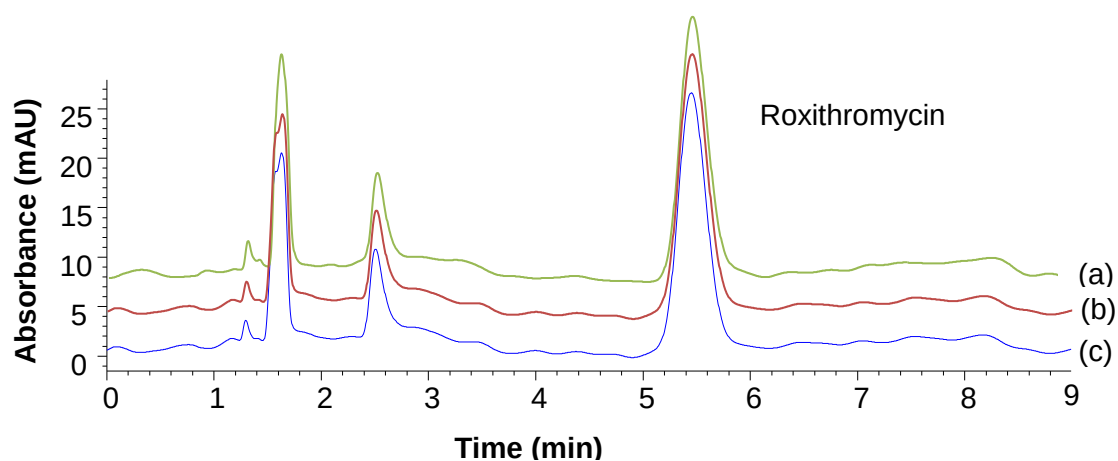


Figure A.6: Specificity results for (a) Phosphatidylcholine (b) Span® 60 and (c) Cholesterol

A.6 Conclusion

Method verification is essential to ensure established methods perform as intended under alternate conditions or in the hands of another user. The parameters that were verified included accuracy (recovery), linearity, LOD together with LOQ, precision (repeatability) and specificity. The criteria for method verification were met for all instances, which confirmed the successful transfer of these methods to the user in the laboratory. This confirms the method is dependable, reproducible and suitable for its intended use of identifying the concentration of roxithromycin encapsulated in the liposome and niosome vesicle systems.

References

ALACC **see** Analytical laboratory accreditation criteria committee

Analytical laboratory accreditation criteria committee. 2007. How to meet ISO 17025 requirements for method verification. http://www.aoac.org/aoac_prod_imis/aoac_docs/lptp/alacc_guide_2008.pdf Date of access: 21 Apr. 2019.

Aucamp, M.E., Csongradi, C., Gerber, M. & Du Plessis, J. 2016. A novel RP–HPLC method for the detection and quantification of roxithromycin in topical delivery studies. *Pharmazie*, 17:175–176.

Boshoff, S. 2018. Investigation of the stabilising effects of niosomes on the amorphous forms of roxithromycin. Potchefstroom: NWU. (Dissertation – MSc).

FDA **see** Food and drug administration

Food and drug administration. 2014. Appendix 1 – ORA validation and verification guidance for human drug analytical methods. <https://www.fda.gov/downloads/ScienceResearch/FieldScience/UCM173090.pdf> Date of access: 21 Apr. 2019.

Graham, P. 2011. Quality control in the pharmaceutical industry. (*In* Moffat, A.C., Osselton, M.D. & Widdop, B., eds. *Clarke's analysis of drugs and poisons*. London: Pharmaceutical press, p.350–360).

ICH **see** International conference of harmonisation

International conference of harmonisation. 2005. Validation of analytical procedures: text and methodology Q2 (R1). https://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q2_R1/Step4/Q2_R1__Guideline.pdf Date of access: 21 Apr. 2019.

Lavanya, G., Sunil, M., Eswarudu, M.M., Chinna–Eswaraiah, M., Harisudha, K. & Naga Spandana, B. 2013. Analytical method validation: an updated review. *International journal of pharmaceutical sciences and research*, 4:1280–1286.

LoBrutto, R. & Patel, T. 2007. Method validation. (*In* Kazakevich, Y. & LoBrutto, R., eds. *HPLC for pharmaceutical scientists*. Hoboken, New Jersey: Wiley Interscience, p.455–502).

Etse, J. 2007. The role of HPLC in technical transfer and manufacturing. (*In* Kazakevich, Y. & LoBrutto, R., eds. *HPLC for pharmaceutical scientists*. Hoboken, New Jersey: Wiley Interscience, p.735–762).

Moffat, A.C. 2011. Quality Control and accreditation in the toxicology laboratory. (*In* Moffat, A.C, Osselton, M.D. and Widdop, B., ed. Clarke's analysis of drugs and poisons. London: Pharmaceutical press, p.361–370).

Swart, M. 2019. Stability of amorphous forms of roxithromycin when encapsulated in liposomes. Potchefstroom: NWU. (Dissertation – MSc).

Swartz, M. & Krull, I.S. 2011. Analytical method validation: back to basics, part II. LCGC North America, 29:44–52.

USP **see** United States pharmacopoeia

United States pharmacopoeia. 2011. Validation of compendial procedures. https://hmc.usp.org/sites/default/files/documents/HMC/GCs-Pdfs/c1225_1SUSP40.pdf Date of access: 21 Apr. 2019.

United States pharmacopoeia. 2013. Verification of compendial procedures. <https://hmc.usp.org/sites/default/files/documents/HMC/GCs-Pdfs/c1226.pdf> Date of access: 21 Apr. 2019.

WHO **see** World health organisation

World health organisation. 2010. WHO guiding principles in transfer of technology. https://www.who.int/immunization/sage/9_TOT_QAS08_259Rev2_27072010.pdf?ua=1 Date of access: 21 Apr. 2019.

World health organisation. 2018. Guidelines on validation – appendix 4 analytical method validation. https://www.who.int/medicines/areas/quality_safety/quality_assurance/28092018_Guideline_Validation_AnalyticalMethodValidation-Appendix4_QAS16-671.pdf Date of access: 21 May 2019.

APPENDIX B

PREPARATION AND PHYSICAL CHARACTERISATION OF ROXITHROMYCIN CRYSTALLINE SOLID-STATE FORMS

B.1 Introduction

The two main classes of solid-state of a pharmaceutical active identified by researchers are crystalline or amorphous. The crystalline class can also occur as a desolvate, hydrate or solvate form (Lee *et al.*, 2008:580). To identify a specific solid form is an indispensable task during any pharmaceutical product development. An awareness of the solid-state forms of an API can expose various challenges or successes prior to or during product formulation.

Vippagunta *et al.* (2001:4) define polymorphism as crystalline material with identical chemical composition, but dissimilar molecular packing. According to Byrn *et al.* (1999:14), amorphism and polymorphism are vital in the formulation of pharmaceuticals as they give rise to a multiplicity of contemporary drug molecules. Du Plessis (2004:72) screened for amorphous and polymorphic forms of roxithromycin, and the development of amorphous forms was further explored by Aucamp *et al.* (2012:467), whom also proved solution-mediated phase transformation of metastable solid-state forms of roxithromycin. Furthermore, it was proven that roxithromycin could exist as either a monohydrate or an anhydrate (Aucamp *et al.*, 2013:26). The amorphous solid-states of roxithromycin were also explored by Milne (2016:305), and it has since been reported that some of these different solid-state forms of roxithromycin can be successfully included into thin-films, which are the precursors to the preparation of drug delivery vesicles, such as liposomes and niosomes (Csongradi, 2015:100; Boshoff, 2018:65; Swart, 2019:86). During these precursor studies, it was found that irrespective of whether crystalline roxithromycin monohydrate or either of the two different amorphous forms of roxithromycin were used in the preparation of thin-films, roxithromycin was rendered in an amorphous state. This can be ascribed to the fact that an amorphous solid dispersion is prepared when the API is combined with excipients such as cholesterol, phosphatidylcholine and Span[®] 60.

A dispersion of a hydrophobic API in a hydrophilic polymer matrix, to produce a single-phase amorphous mixture, is known as an amorphous solid dispersion (Kalia & Poddar, 2011:10). A simple preparation method of an amorphous solid dispersion is to dissolve the chosen API and polymer in a suitable solvent (Newman *et al.*, 2012:1356). The most important use of an amorphous solid dispersion is to increase the bioavailability of poorly soluble APIs, which is achieved by refining the speed and degree of dissolution (Van den Mooter, 2012:e79).

The rationale of this study was to establish whether liposomal or niosomal thin-films of easily obtainable roxithromycin solid-state forms may be developed further into a semi-solid formulation such as an emulgel. Considering the formulation of emulgels from a cost point of view and scalability, and since it was already established that thin-film preparation renders roxithromycin in the amorphous state, it was decided to include only solid-state forms that could be purchased or prepared easily.

This annexure discusses the methods of preparing thin-films containing either roxithromycin monohydrate, anhydrate or the chloroform solvated form. The characterisation was performed by investigating the morphology using microscopy techniques followed by confirming correlation of physicochemical attributes using FTIR and TGA, with that already reported in literature (Aucamp *et al.* 2012:467; Aucamp *et al.*, 2013:26).

B.2 Preparation of roxithromycin solid-state forms

The three solid-state forms of roxithromycin used during this study included the purchased crystalline monohydrate form of roxithromycin (**MH**) (see Section C.2.1), roxithromycin anhydrate (**AH**) (dehydrated monohydrate) and a polymorphic chloroform solvated (**CS**) form.

B.2.1 Preparation of roxithromycin anhydrate

The purchased roxithromycin monohydrate was dehydrated using a laboratory-drying oven (Binder, Germany). The roxithromycin monohydrate was spread out thinly on the surface of a Petri dish, the oven temperature was set at 100°C and the sample was dried for a period of 24 h.

B.2.2 Preparation of the chloroform solvated polymorphic form

To prepare the chloroform solvate form, the method reported by Aucamp *et al.* (2012) was used. Purchased roxithromycin monohydrate was added to chloroform while stirring with a magnetic stirrer and the solution heated to approximately $60 \pm 3^\circ\text{C}$. Roxithromycin monohydrate was added until a milky solution was obtained, which indicated an oversaturated solution. Chloroform was added in a drop-wise manner until the solution was clear. Evaporation occurred in a fume hood at room temperature (25°C) until a dried bulk was obtained.

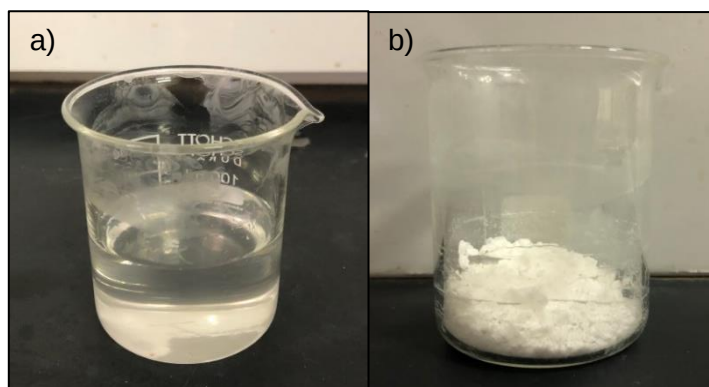


Figure B.1: Preparation of chloroform solvated form of roxithromycin: a) roxithromycin monohydrate dissolved in chloroform and b) prepared granules of chloroform solvated form

B.3 Preparation of the lipid films encapsulating the different solid-state forms

The three solid-state forms of roxithromycin used during this study were encapsulated into liposomes and niosomes respectively by making use of an adapted thin-film technique. Lipid films serve as precursors to vesicle systems such as liposomes and niosomes.

B.3.1 Preparation of the lipid films for liposome formulation

Solvent evaporation defined as solvent casting by Vasconcelos *et al.* (2015) and Vasconcelos *et al.* (2016:89) were used to prepare thin-films for liposome vesicle preparation for this study. A lipid mixture of phosphatidylcholine and cholesterol, a drop of α -tocopherol and the API was dissolved in chloroform by stirring the mixture in a glass beaker on a magnetic stirrer until fully dissolved. The mixture was poured into Petri dishes and left in a fume hood for 24 h to ensure the solvent evaporated and any residual was completely removed. This resulted in a thin-film on the bottom of the Petri dish.

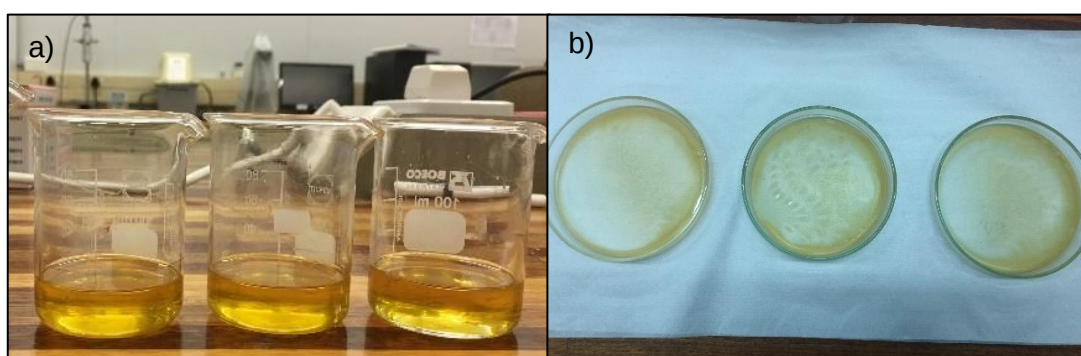


Figure B.2: Preparation of thin-films for liposomes: a) dissolved excipients and b) film formed after evaporation

B.3.2 Preparation of the lipid films for niosome formulation

A lipid mixture of Span® 60, cholesterol and the API was dissolved in a chloroform and methanol mixture. The glass beaker was placed on a hot plate at $60 \pm 2^\circ\text{C}$ and stirred until a thin-film formed on the bottom and all organic solvents had evaporated.



Figure B.3: Preparation of thin-films for niosomes: a) undissolved excipients before heating and b) film formed after evaporation

B.4 Analytical techniques used for physical characterisation

B.4.1 Fourier-transform infrared spectroscopy

A small quantity of sample was placed on the IR window of a Bruker Alpha FTIR spectrometer (United States of America), which recorded the IR-spectra over a range of $400 - 4000\text{ cm}^{-1}$.



Figure B.4: Bruker Alpha FTIR spectrometer

B.4.2 Thermogravimetric analysis

TGA was carried out using a TGA 4000 (PerkinElmer Waltham, United States of America). The samples, weighing $1 - 3\text{ mg}$ each were placed in a porcelain crucible. Subsequently, a heating programme of $10^\circ\text{C}/\text{min}$ from ambient temperature to 250°C was used, this was done under a nitrogen purge of $40\text{ ml}/\text{min}$. The moisture lost during heating of the sample was documented.

B.5 Results and discussion

B.5.1 Fourier-transform infrared spectroscopy results

IR spectroscopy is known as a green fingerprint technique that provides rapid and reliable information about functional groups present in compounds, making it a useful tool for quality control testing of pharmaceuticals (Rohman, 2012:1; Stuart, 2004:71). The results obtained for FTIR analysis are seen in Table B.1. The most distinctive variances can be observed at the 4 000 – 3 000 cm^{-1} range, where roxithromycin **MH** had three distinctive peaks at 3 570.19 cm^{-1} , 3 510.21 cm^{-1} and at 3 455.73 cm^{-1} . **CS** only had one broad peak at 3 422.05 cm^{-1} and **AH** also had a single peak at 3 457.74 cm^{-1} indicating the latter two forms were not monohydrates. The broad peaks observed can be attributed to the fact that no water molecules were bound (Hollenbeck, 2007:2378). The obtained infrared data correlates with that previously reported by Aucamp *et al.* (2012) and Aucamp *et al.* (2013).

Table B.1: Wavenumbers of roxithromycin solid-state forms

Wavenumbers of roxithromycin solid-state forms				
Functional groups	Regions (cm^{-1})	MH	CS	AH
O-H (strong & broad) N-H (strong & broad)	4000 – 3200 3400 – 3150	3570.19 3510.21 3455.73	3422.05	3457.74
O-H (weak & broad) C-H (strong & broad)	3000 – 2700 3000 – 2500	2980.71 2969.47 2932.57 2878.37 2829.02	2971.83 2936.79 2875.95 2829.59	2969.29 2932.51 2876.15
C≡N (weak) C≡C (weak)	2400 – 2100 2300 – 2100	2285.92		

B.5.2 Thermogravimetric analysis results

TGA was used to determine the hydration or solvation level of the three crystalline forms of roxithromycin. The obtained percentage solvent, which forms part of the crystalline structure, was used to calculate the molecular weight of each crystalline form, as presented in Table B.2.

Table B.2: Percentage solvent included in crystalline packing of roxithromycin solid-state forms and the resulting molecular weight (g/mol)

Solid-state form	Percentage solvent (%) obtained from analysis	Calculated molecular weight (g/mol)
MH	2.0	837.05
AH	0.1	819.30
CS	12.5	938.68

B.6 Conclusion

It is important to understand the properties of solid-state forms used for formulation during pharmaceutical development. It is guaranteed that structural differences occur between crystalline forms in which drugs may exist, but according to Brittain (2009:34), it must be kept in mind that these differences occur solely in solid-state form. Irrespective of the initial solid-state form, roxithromycin is rendered to the amorphous state when encapsulated in liposome or niosome thin-films. The results obtained for FTIR analysis confirmed that the **CS** and **AH** solid-state forms were not monohydrates and the data corresponded with the published results of Aucamp *et al.* (2012) and Aucamp *et al.* (2013). The opportunity to resolve formulation problems of abandoned APIs may be addressed by employing different solid-state forms of an API (SSCI, 2019). As this is exactly the case for roxithromycin, the solid-state forms previously discussed could be the ideal resolution to novel product development. The main objective of this study is to develop an emulgel containing vesicle systems encapsulating roxithromycin for successful topical delivery. The characterisation of the vesicle systems encapsulating these three different solid-states of roxithromycin will be discussed in Annexure C, while the formulation of an emulgel will be discussed in Annexure D; the possibility of topical delivery of these solid-state forms of roxithromycin will be discussed in Annexure F.

References

- Aucamp, M., Liebenberg, W., Strydom, S.J., Van Tonder, E.C. & De Villiers, M.M. 2012. Physicochemical properties of amorphous roxithromycin prepared by quench cooling of the melt or desolvation of a chloroform solvate. *American association of pharmaceutical scientist, PharmSciTech*, 13:467-476.
- Aucamp, M., Stieger, N., Barnard, N. & Liebenberg, W. 2013. Solution-mediated phase transformation of different roxithromycin solid-state forms: implications on dissolution and solubility. *International journal of pharmaceutics*, 449:18-27.
- Boshoff, S. 2018. Investigation of the stabilising effects of niosomes on the amorphous forms of roxithromycin. Potchefstroom: NWU. (Dissertation – MSc).
- Brittain, H.G. 2009. Application of phase rule to the characterization of polymorphic and solvatomorphic systems. (*In* Brittain, H.G. ed. *Polymorphism in pharmaceutical solids*. 2nd ed. New York: Informa healthcare, p.24-51).
- Byrn, S.R., Pfeiffer, R.R. & Stowell, J.G. 1999. *Solid state chemistry of drugs*. 2nd ed. West Lafayette, Indiana: SSCI Inc.
- Csongradi, C. 2015. The effect of solid-state forms on the topical delivery of roxithromycin. Potchefstroom: NWU. (Dissertation – MSc).
- Du Plessis, C. 2004. Characterisation of polymorphic, pseudopolymorphic and amorphous forms of roxithromycin. Potchefstroom: NWU. (Dissertation – MSc).
- Hollenbeck, R.G. 2007. Moisture in pharmaceutical products. (*In* Swarbrick, J. ed. *Encyclopedia of Pharmaceutical Technology*. 3rd ed. New York: Informa healthcare, p.2368-2383) <http://www.dawsonera.com.nwulib.nwu.ac.za/depp/reader/protected/external/EBookView/S9780849393983/S2430>. Date of access: 21 Jun. 2019.
- Kalia, A. & Poddar, M. 2011. Solid dispersions: an approach towards enhancing dissolution rate. *International journal of pharmacy and pharmaceutical sciences*, 3:9-19.
- Lee, S.L., Raw, A.S. & Yu, L. 2008. Significance of drug substance physicochemical properties in regulatory quality by design. (*In* Adeyeye, M.C. & Brittain, H.G. eds. *Preformulation in solid dosage form development*. New York: Informa Healthcare, p.571-583).
- Milne, M., Liebenberg, W. & Aucamp, M.E. 2016. Different amorphous solid-state forms of roxithromycin: a thermodynamic and morphological study. *International journal of pharmaceutics*, 498:304-315.

Newman, A., Knipp, G. & Zografi, G. 2012. Assessing the performance of amorphous solid dispersion. *Journal of pharmaceutical sciences*, 101:1355-1377.

Rohman, A. Application of fourier transform infrared spectroscopy for quality control of pharmaceutical products: a review. 2012. *Indonesian journal of pharmacy*, 23:1-8.

SSCI **see** Solid-state chemical information

Solid-state chemical information. 2019. What everyone needs to know about polymorphs. <https://www.ssci-inc.com/publications/what-everyone-needs-to-know-about-polymorphs/> Date of access: 21 Jun.2019.

Stuart, B.H. 2004. *Infrared spectroscopy: fundamentals and applications*. 1st ed. Hoboken, New Jersey: Wiley.

Swart, M. 2019. Stability of amorphous forms of roxithromycin when encapsulated in liposomes. Potchefstroom: NWU. (Dissertation – MSc).

Van den Mooter, G. 2012. The use of amorphous solid dispersions: A formulation strategy to overcome poor solubility and dissolution rate. *Drug discovery today: Technologies*, 9:e79-e85.

Vasconcelos, T., Marques, S. & Sarmento, B. 2015. Development of a resveratrol solid dispersion with soluplus®. Controlled release society annual meeting. Edinburgh, Scotland. https://www.researchgate.net/publication/281862903_Development_of_a_Resveratrol_Solid_Dispersion_with_SoluplusR Date of access: 18 Jun. 2019.

Vasconcelos, T., Marques, S., das Neves, J. & Sarmento, B. 2016. Amorphous solid dispersions: Rational selection of a manufacturing process. *Advanced drug delivery reviews*, 100:85-101.

Vippagunta, S.R., Brittain, H.G. & Grant, D.J.W. 2001. Crystalline solids. *Advanced drug delivery reviews*, 48:3-26.

APPENDIX C

FORMULATION AND CHARACTERISATION OF VESICULAR SYSTEMS ENTRAPPING ROXITHROMYCIN IN LIPOSOMES AND NIOSOMES

C.1 Introduction

Vesicle delivery systems have gained attention for possible drug delivery in various routes of administration (Carafa *et al.*, 1998:51). The ingredients used when preparing vesicle systems, as well as the method followed, ultimately determines the structure and properties of that specific carrier (Abdelkader *et al.*, 2014:93, Naeem *et al.*, 2015:579). It has been reported that various solid-state forms of roxithromycin can be successfully included into thin films, which are the precursors to the preparation of drug delivery vesicles, such as liposomes and niosomes (Boshoff, 2018:109; Milne, 2015:305; Swart, 2019:140). This appendix discusses the methods of the encapsulation of the roxithromycin into two different vesicle systems, and the physicochemical properties, such as pH, droplet size and zeta-potential of the prepared liposomal and niosomal vesicles. Once the formulation methods were finalised, it remained unchanged throughout the remainder of the study.

C.2 Ingredients used to formulate vesicular systems

C.2.1 Roxithromycin

Roxithromycin was the active ingredient encapsulated into the niosome and liposome vesicles. With a poor aqueous solubility of 0.0335 mg/ml at 25°C for the monohydrate form (Aucamp *et al.*, 2013:26), other solid-state forms of the API were also explored. Roxithromycin raw material, also known as the monohydrate form, was purchased from DB Fine Chemicals (South Africa).

C.2.2 Cholesterol

Cholesterol was added to the formula of the dispersions due to its ability to improve the rigidity and fluidity of vesicles, which in turn leads to less leakage of the vesicles (Karim *et al.*, 2010:378; Kumar & Rajeshwarrao, 2011:211; Varun *et al.*, 2012:633). Cholesterol forms part of the lipophilic phase of the vesicles and will regulate cohesion and water permeability of the membrane (Marianecchi *et al.*, 2013:67). Due to the insolubility of cholesterol in water, a synergistic interaction between the hydrophobic alkyl chain of the surfactant and cholesterol forms bilayer structures in an aqueous medium (Ghosh *et al.*, 2015:2311). Cholesterol (from sheep wool) was purchased from Sigma-Aldrich (South Africa).

C.2.3 Non-ionic surfactants

Non-ionic surfactants are chosen due to their ability to enhance solubility and ultimately increase the bioavailability of poorly soluble drugs (Mahale *et al.*, 2012:47). Their compatibility, stability and non-toxic nature is of importance for topical formulations. The choice of non-ionic surfactant to form part of the vesicle's lipid layer for this study was sorbitan monostearate (Span[®] 60) purchased from Merck Millipore (South Africa), as it produces the highest entrapment efficiency (EE%) amongst all the available grades of Tween and Span (Kumar & Rajeshwarrao, 2011:210; Mali *et al.*, 2013:587; Ruckmani & Sankar, 2010:1124). Together with cholesterol, it forms part of the lipophilic phase of the vesicles.

C.2.4 Phosphatidylcholine

Phosphatidylcholine (PC) was the phospholipid of choice for the preparation of liposomes due to delivering stable vesicles (Narenji *et al.*, 2016:88). L- α -phosphatidylcholine (from egg yolk) was purchased from Sigma-Aldrich (South Africa).

C.2.5 α -Tocopherol

α -Tocopherol (vitamin E), purchased from Sigma-Aldrich (South Africa), is added to liposomes to contribute to stabilising the lipid membrane by decreasing the oxidation of lipids (Quinn, 2012:158).

C.2.6 Organic solvents

Chloroform is a non-irritating, non-flammable organic solvent (NCBI; 2019) and according to New (1990:33), is a popular solvent commonly used in the preparation of various types of vesicle systems. Methanol is a volatile, flammable organic solvent (NCBI; 2019) and again according to New (1990:33), it is mostly used as a mixture with chloroform, with the volume ratio of chloroform:methanol customarily 2:1. Chloroform and methanol, both purchased from ACE Chemicals (South Africa), are used as the organic solvent for the cholesterol, Span[®] 60 and PC in this study.

C.2.7 Purified water

Purified water serves as the aqueous phase to hydrate the lipid film (Patil & Jadhav, 2013:10). All experiments during the course of the study were done with Milli-Q[®] HPLC grade water, available in-house.

C.3 Preparation of vesicular systems

Two carrier systems were prepared during this study. Both liposomes and niosomes were prepared using the thin film method, with minor adaptations to existing methods to yield multilamellar vesicles.

C.3.1 Method for preparation of liposome vesicles

Table C.1 presents the formulas used to formulate drug containing or empty (without API) multilamellar liposomes.

Table C.1: Composition of the liposome dispersions containing different solid-state forms

Excipients	PL	MH	CS	AH
Roxithromycin	NA	200 mg equivalent to 195.76 mg	200 mg equivalent to 174.56 mg	200 mg
Lecithin	700 mg	560 mg	560 mg	560 mg
Cholesterol	300 mg	240 mg	240 mg	240 mg
α-Tocopherol	20 μ l	20 μ l	20 μ l	20 μ l
Chloroform	10 ml	10 ml	10 ml	10 ml
Milli-Q[®] water	10 ml	10 ml	10 ml	10 ml

A method for solvent evaporation, described as solvent casting by Vasconcelos and Sarmento (2015) and Vasconcelos *et al.* (2016:89), was adapted and combined with the traditional hand shaking method as originally elucidated by New (1990:36-38) to prepare liposome vesicles for this study. A lipid mixture of phosphatidylcholine and cholesterol, a drop of α -tocopherol and the API (where applicable), were dissolved in chloroform by stirring the mixture in a glass beaker on a magnetic stirrer until fully dissolved. The mixture was poured into Petri dishes and left in a fume hood for 24 h to ensure the solvent evaporated; a film formed and the residual was completely removed. The film was scraped off with a stainless steel lab spatula into a glass beaker. Milli-Q[®] water was heated to $40 \pm 2^\circ\text{C}$ and added to the beaker. The dry lipid film was hydrated using a magnetic stirrer for approximately 30 min until no large droplets were visible and a yellow milky suspension formed. The mixture was sonicated on ice for 2 min with an ultrasonicator (Model UP200St, Hielscher Ultrasonics, Germany), thereafter it was left for 2 h at room temperature to allow swelling of the vesicles.

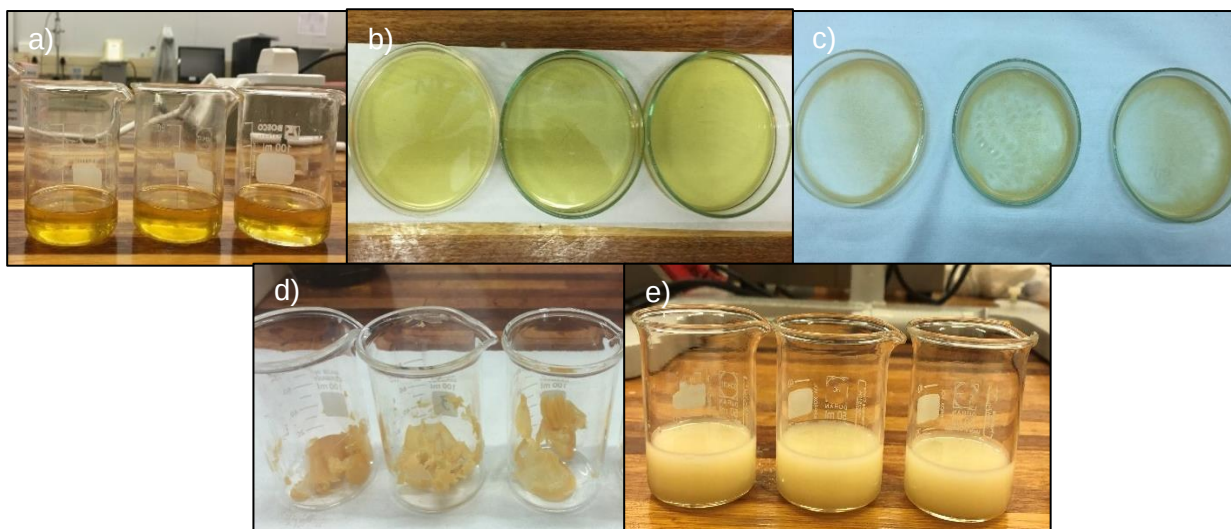


Figure C.1: Preparation process of liposomes: a) dissolved lipid mixture, b) dissolved lipid mixture in Petri dishes before evaporation in fume hood, c) thin films that formed after evaporation of the organic solvents, d) dry lipid film in beaker, and e) milky solution after hydration of thin films

C.3.2 Method for preparation of niosome vesicles

Table C.2 presents the formulas of drug containing or empty (without API) multilamellar niosomes.

Table C.2: Composition of the niosome dispersions containing different solid-state forms

Excipients	PL	MH	CS	AH
Roxithromycin	NA	200 mg equivalent to 195.76 mg	200 mg equivalent to 174.56 mg	200 mg
Span® 60	500 mg	400 mg	400 mg	400 mg
Cholesterol	500 mg	400 mg	400 mg	400 mg
Chloroform:methanol (2:1, v/v)	5 ml	5 ml	5 ml	5 ml
Milli-Q® water	10 ml	10 ml	10 ml	10 ml

The method described by Agarwal *et al.* (2001:44) was adapted for the preparation of niosomes. A lipid mixture of Span® 60 and cholesterol, and the API (where applicable), was dissolved in the chloroform and methanol mixture. The glass beaker was placed on a hot plate at $60 \pm 2^\circ\text{C}$ and stirred until a thin film formed on the bottom and all organic solvents had evaporated. The film was hydrated with 10 ml of Milli-Q® water and stirred with a magnetic stirrer for 30 min until no large droplets were visible and a white milky suspension formed. The mixture was sonicated on ice for 2 min with an ultrasonicator (Model UP200St, Hielscher

Ultrasonics, Germany) thereafter it was left for 2 h at room temperature to allow for swelling of the vesicles.

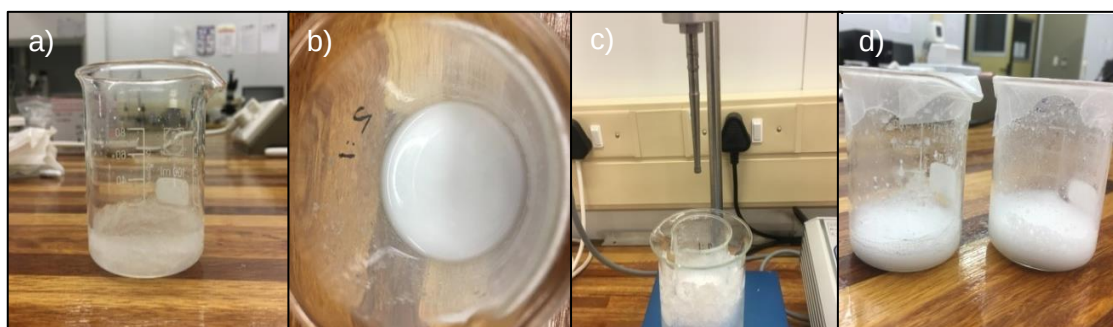


Figure C.2: Preparation process of niosomes: a) thin films that formed after evaporation of the organic solvents, b) hydrated solution, c) sonication of the dispersion with a sonicator probe and d) final niosome dispersions

C.4 Methods used to characterise the vesicles formulated

C.4.1 Transmission electron microscopy

TEM was used to view the morphology and formation of the vesicle systems. Due to the high resolution and magnification, this technique has proven to be superior to other imaging methods, such as conventional light microscopy (Laouini *et al.*, 2012:156; Wibroe *et al.*, 2016:3). An FEI Tecnai G2 20S-Twin 200 kV high-resolution transmission electron microscope (HRTEM) (Czech Republic), as seen in Figure C.3, fitted with an Oxford INCA X-Sight EDS System was operated by Dr A. Jordaan (Laboratory for Electron Microscopy, NWU, Potchefstroom Campus). A drop of sample (diluted 10x with distilled water) was stratified onto a 300 mesh copper carbon-coated grid and left for 5 min to dry, any excess sample was removed with a piece of filter paper. The lipid layers of the vesicles were stained using a drop of osmium tetroxide, and the sample was left to dry for 1 to 2 min before being viewed through the microscope.



Figure C.3: Transmission electron microscope

C.4.2 Droplet size and distribution

The size of the vesicles was determined using the Malvern Zetasizer Nano ZS (Malvern Instruments Ltd, United Kingdom). The instrument uses dynamic light scattering to determine particle sizes. The freshly prepared vesicle samples were diluted approximately 20 times with distilled water and 2 ml was injected into a clear disposable zeta-cell (DTS1070 folded capillary cell). Each sample was measured in triplicate to ensure accurate results.

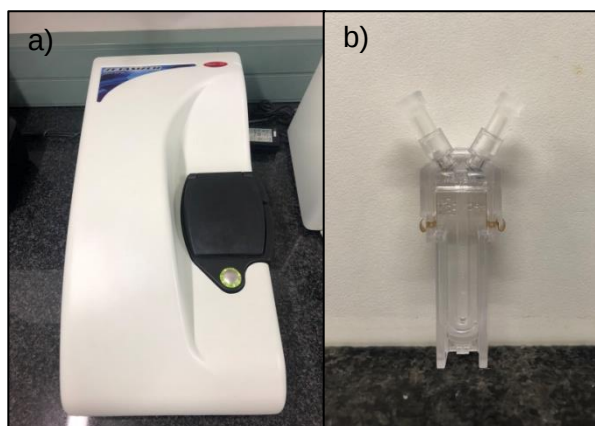


Figure C.4: a) Malvern Zetasizer Nano ZS, and b) disposable zeta-cell

C.4.3 Zeta-potential

The zeta-potential of the vesicles was determined using the Malvern Zetasizer Nano ZS, as seen in Figure C.4.a (Malvern Instruments Ltd, United Kingdom). The prepared vesicle samples were diluted roughly 20 times with distilled water, and 2 ml was injected into a clear disposable zeta-cell (Figure C.4.b), taking measurements in triplicate.

C.4.4 pH determination

To determine the pH values of the different vesicle systems, a Mettler Toledo® pH meter (Mettler Toledo, United States), equipped with a Mettler Toledo® InLab® 410 electrode (Mettler Toledo, United States), was used as seen in Figure C.5.



Figure C.5: Mettler Toledo® pH meter

The electrode was inserted into a sufficient volume of each sample after being calibrated. The measured pH value for each sample is reported in Tables C.7 and C.8, after taking measurements in triplicate.

C.4.5 Entrapment efficiency

To determine the amount of API encapsulated in each vesicle system, fresh samples were prepared in triplicate. Approximately 20 ml was transferred into plastic test tubes and centrifuged with the assistance of Miss S. Lowe (Laboratory for Applied Molecular Biology, NWU, Potchefstroom Campus). An OptimaL-100 XP ultracentrifuge (Beckman Coulter, United States of America), with a 50.2 Ti Rotor, was used to centrifuge the samples, with a rotation speed of 25 000 rpm for 30 min at a temperature of $25 \pm 2^\circ\text{C}$. A pellet containing the entrapped API formed at the bottom of the test tube, with a supernatant solution containing the unentrapped free API. The supernatant was filtered and transferred into HPLC vials for analysis.

C.5 Results and discussion

C.5.1 Transmission electron microscopy

TEM can only be performed on vesicles containing no API, as precipitation of the API can cause damage to the microscope. As reported by Varun *et al.* (2012:636), the vesicles are presumed spherical, with sizes reported by previous studies ranging from 20 nm to 50 000 nm (Moghassemi & Hadjizadeh, 2014:30). The ideal size for topical delivery is smaller than $3\ \mu\text{m}$, as it will permeate through the stratum corneum (Allec *et al.*, 1997:119; Barry, 2001:101; Bolzinger *et al.*, 2012:163).

C.5.1.1 TEM results for liposome vesicles

Figure C.6 represents the micrograph of the liposomes with no API. It is evident that the liposomes presented with strong intact lipid membranes, as seen by the darker osmium tetroxide coloured membranes. Densely packed liposomes were observed in various sizes, which correlated with the observed liposomes of Chen *et al.* (2016:78) having many diameters in the lesser nanometre range and a few in the larger nanometre range. The formed liposomal vesicles are deemed fit for topical drug delivery with all sizes smaller than $3\ \mu\text{m}$.

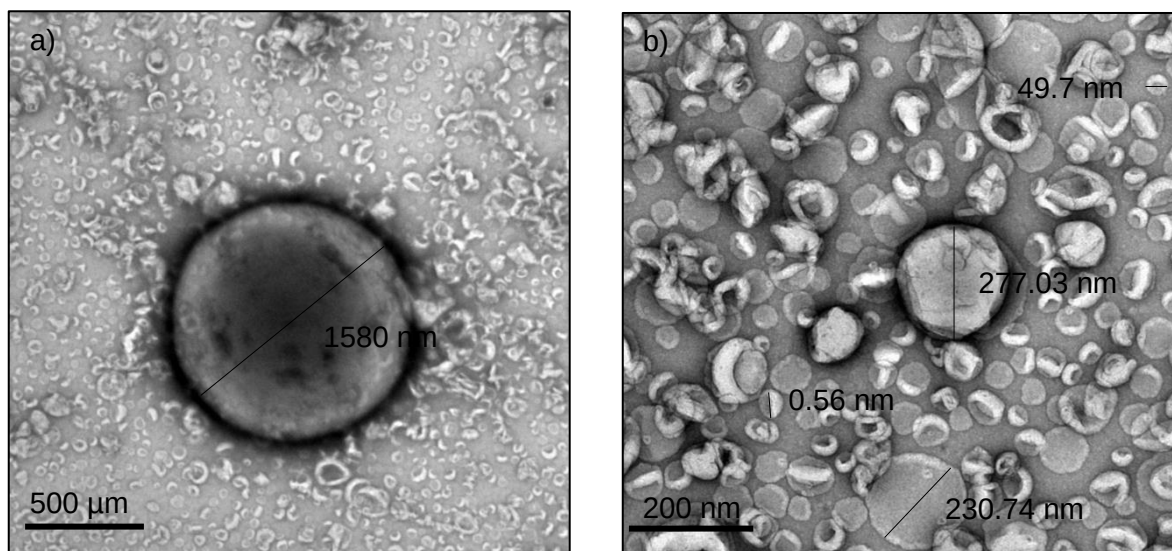


Figure C.6: TEM micrographs: a) and b) showing liposome vesicles

C.5.1.2 TEM results for niosome vesicles

Figure C.7 illustrates the micrograph of the niosomes with no API. The osmium tetroxide fixed to the unsaturated fatty acid of the phospholipids illustrates the vesicle membrane as a dark circular region of the vesicle. Just as these TEM micrographs confirmed niosome vesicles in different size ranges, they correlated with the TEM images of Asthana *et al.* (2016:4), which also displayed a variety of diameter ranges. All the vesicles resulted in sizes smaller than 3 μm, confirming the suitability for topical drug delivery.

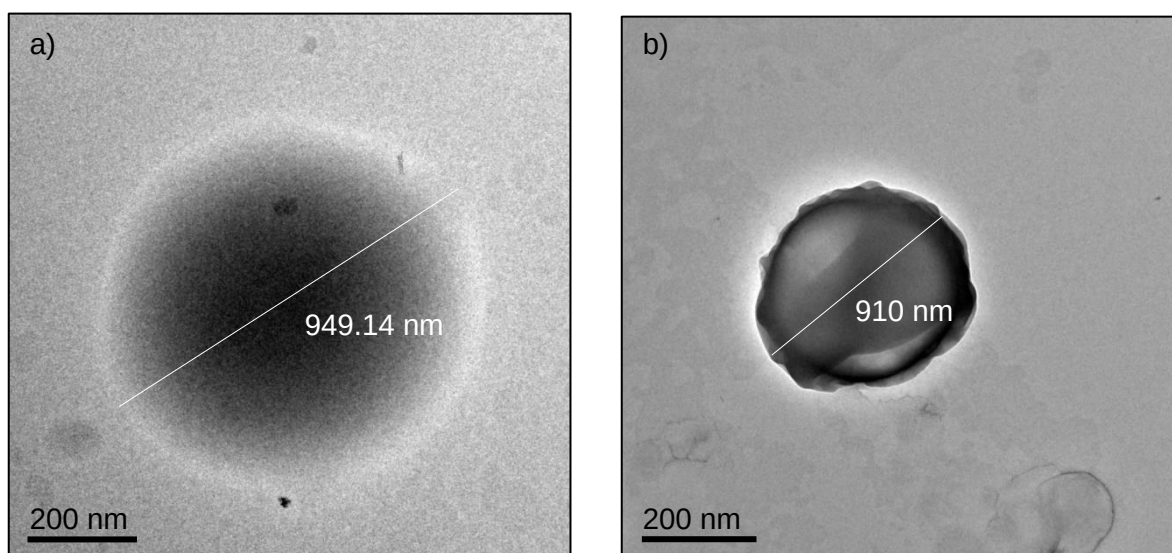


Figure C.7: TEM micrographs: a) and b) showing niosome vesicles

C.5.2 Droplet size and distribution

The TEM micrographs in Section C.5.1 established that the liposomal and niosomal vesicles had a large variety of different sized droplets. To ensure optimal delivery of the API through the stratum corneum, particle sizes in the nanometre range 10 – 1000 nm are required (Kumar & Rajeshwarrao, 2011:209). The polydispersity index (Pdl) indicates the distribution of droplets throughout the dispersion (Shah *et al.*, 2014:65; Shakeel *et al.*, 2007:6). A measured Pdl close to 0 indicates the vesicles are monodispersed, whilst a Pdl value approaching 1 indicates the droplets are polydispersed (Gaumet *et al.*, 2008:3; Gaur *et al.*, 2014:36).

C.5.2.1 Droplet size results for liposome vesicles

According to the results of the droplet size distribution, it can be established that the majority of particles were around the 80 – 90 nm size range, which resulted in a good size distribution curve, and all the sizes measured for the liposomes were well within the desired ranges for topical drug delivery. This affirms all the samples would have no problem permeating through the skin despite the fact they presented with size variation within the dispersion. Similar to the results of Ammar *et al.* (2011:148), a variation was visible on the curves (Figures C.8.a to d), such as a smaller tail at the end of the curve, which can be attributed to droplet aggregation or large sediments in the sample. The Pdl results for all dispersions were in the lower range. Dispersions presenting with a Pdl value of less than 0.400, is usually considered as stable with uniform droplet dispersion (Marianecchi *et al.*, 2013:74).

Table C.3: Droplet size (d.nm) and Pdl results of different liposome dispersions

Liposomes	Measurement (d.nm)			Average droplet size (d.nm)	Average Pdl
	1	2	3		
PL	91.57	88.12	90.51	90.07 ± 1.443	0.203 ± 0.007
MH	94.51	88.07	89.05	90.54 ± 2.833	0.183 ± 0.018
CS	85.73	83.97	79.17	81.57 ± 2.772	0.167 ± 0.007
AH	81.61	88.03	82.1	83.91 ± 2.918	0.178 ± 0.011

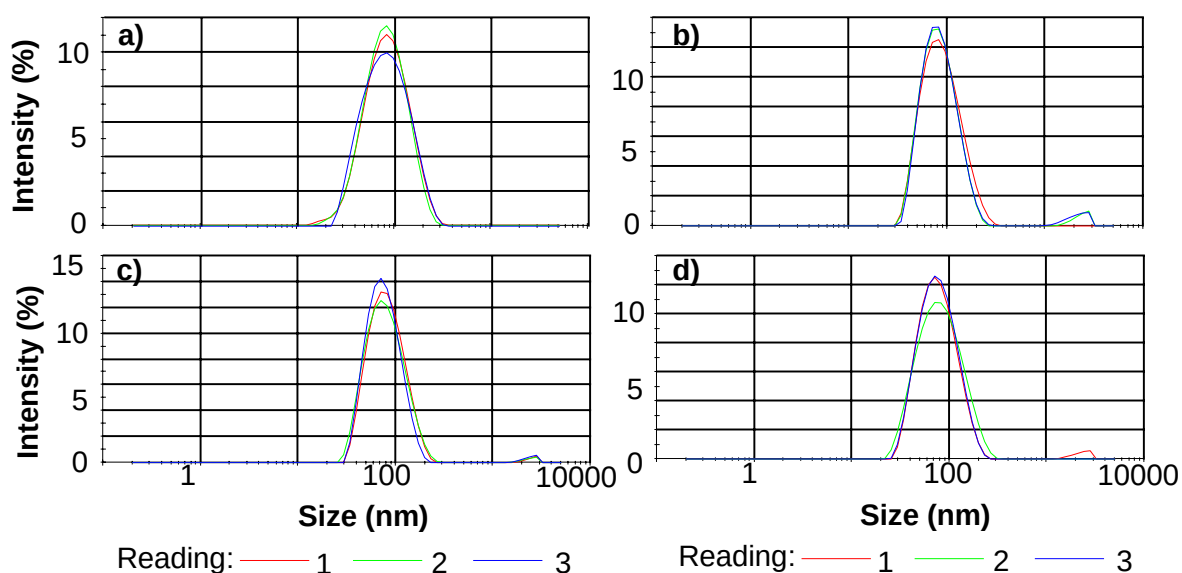


Figure C.8: Size distribution results of liposome dispersions: a) **PL**, b) **MH**, c) **CS** and, d) **AH**

C.5.2.2 Droplet size results for niosome vesicles

The droplet sizes for the niosome vesicles were much larger than the liposomes, ranging between 200 – 300 nm. All the samples could permeate through the skin easily even though they present with a variation in sizes within the dispersion. A study by Ammar *et al.* (2011:148) showed the size distribution of their niosomes was between 50 nm – 980 nm; this demonstrated that many particles of different sizes in a formulation could still result in a good size distribution curve. Variants, such as a smaller tail at the end of the curve (Figures C.9.a to d), can be attributed to droplet aggregation or large sediments in the sample (Ammar *et al.*, 2011:148). The Pdl values of the resulting dispersions also measured much higher than the liposomes, indicating that the dispersions were more polydispersed.

Table C.4: Droplet size (d.nm) and Pdl results of different niosome dispersions

Niosomes	Measurement (d.nm)			Average droplet size (d.nm)	Average Pdl
	1	2	3		
PL	217.3	193.8	267.7	226.27 ± 30.829	0.613 ± 0.009
MH	342.2	328.6	391.7	354.17 ± 27.115	0.701 ± 0.074
CS	343.3	326.0	345.2	330.40 ± 7.063	0.687 ± 0.010
AH	285.5	255.3	345.2	295.33 ± 37.354	0.615 ± 0.055

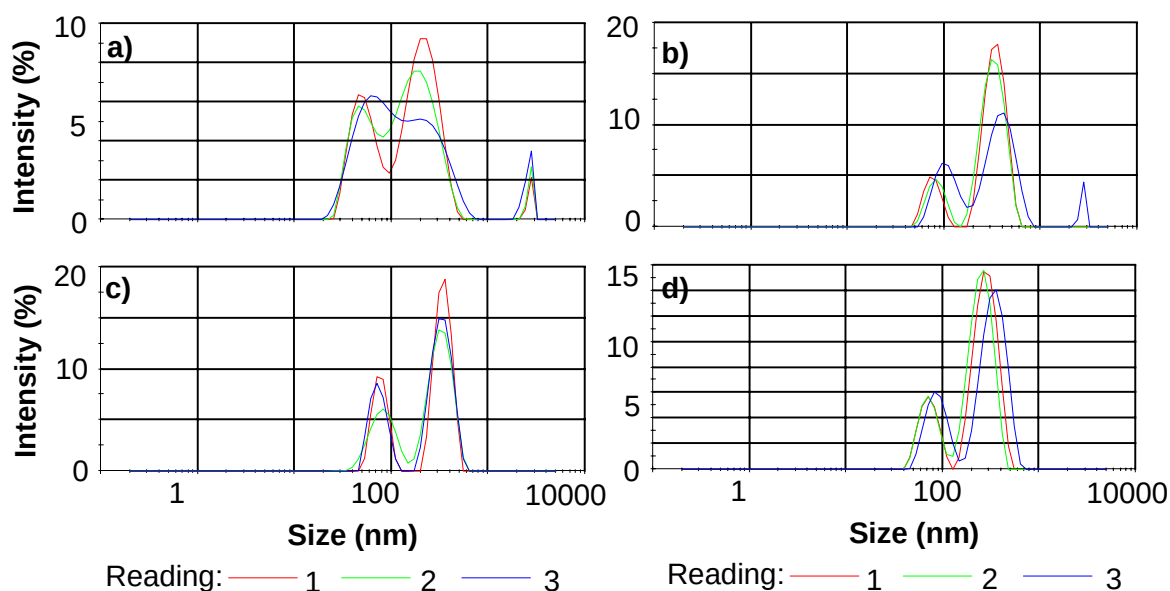


Figure C.9: Size distribution results niosome dispersions: a) **PL**, b) **MH**, c) **CS** and, d) **AH**

C.5.3 Zeta-potential

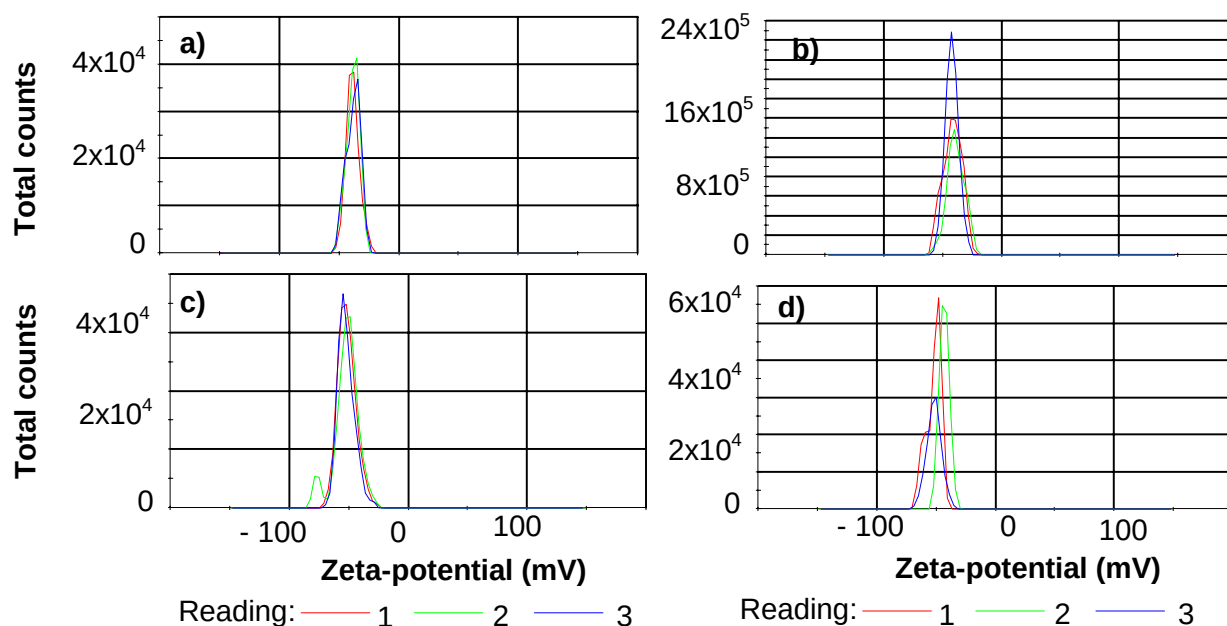
Zeta-potential results of dispersions can be attributed to the determination of the stability, entrapment efficiency and interaction with the skin (Bhatt & Madhav, 2011:2296; Klang *et al.*, 2015:262; Silva *et al.*, 2012:860). Great stability over time can be observed in dispersions with a highly negative zeta-potential (Eid *et al.*, 2014:2; Silva *et al.*, 2012:860), the reason being repelling particles lead to minimal fusion of vesicles (Bayindir & Yuksel, 2010:2055). According to Chibowksi and Szcześ (2016:760), a choline group located below the phosphate group at a low ionic strength results in a negative zeta-potential. It has been found that negative zeta-potential values result in improved skin permeation of a drug during transdermal delivery, as stated by Duangjit *et al.* (2011:6).

C.5.3.1 Zeta-potential results for liposome vesicles

Satisfactory results were obtained of highly negative zeta-potential values. The empty liposomes exhibited slightly higher values than the vesicles containing API; the highly negative results could be attributed to the adsorption of counter ions, or the preferential adsorption of hydroxyl ions at the vesicle surface (Junyaprasert *et al.*, 2008:854).

Table C.5: Zeta-potential (mV) results of different liposome dispersions

Liposomes	Measurement (mV)			Average zeta-potential (mV)
	1	2	3	
PL	- 38.6	- 38.4	- 38.2	- 38.40 ± 0.163
MH	- 41.6	- 38.8	- 42.2	- 40.87 ± 1.482
CS	- 51.6	- 51.9	- 52.2	- 52.05 ± 0.245
AH	- 51.9	- 43.1	- 51.7	- 48.90 ± 4.102

**Figure C.10:** Zeta-potential results for liposome dispersions: a) PL, b) MH, c) CS and, d) AH

C.5.3.2 Zeta-potential results for niosome vesicles

Desirable results of highly negative zeta-potential values were obtained, possibly ensuring improved skin permeation during diffusion studies. The niosomes containing the API resulted in lower zeta-potential values than the blank niosomes, proposing more stable vesicles over time with less aggregation and coagulation (Bayindir & Yuksel, 2010:2055).

Table C.6: Zeta potential (mV) results of different niosome dispersions

Niosomes	Measurement (mV)			Average zeta-potential (mV)
	1	2	3	
PL	- 33.0	- 32.4	- 33.7	- 33.03 ± 0.531
MH	- 61.0	- 62.9	- 61.3	- 61.73 ± 0.834
CS	- 49.6	- 50.2	- 49.8	- 50.00 ± 0.200
AH	- 54.9	- 52.7	- 50.6	- 52.73 ± 1.756

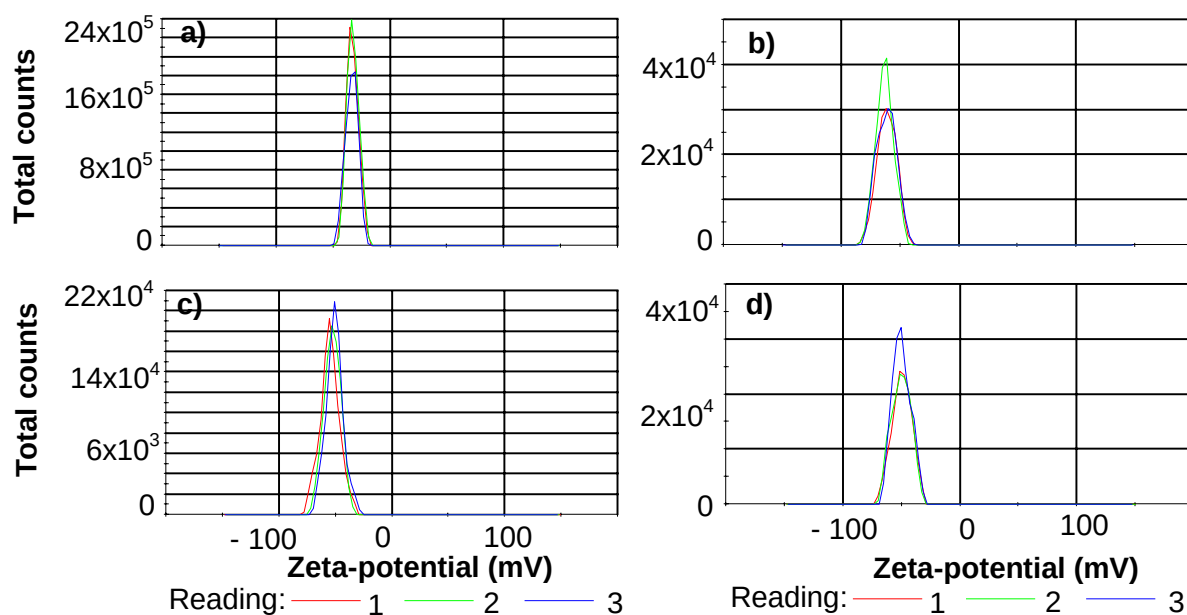


Figure C.11: Zeta-potential results for niosome dispersions: a) **PL**, b) **MH**, c) **AH** and, d) **CS**

C.5.4 pH determination

A wide pH range from 3 to 9 can be viable when dealing with the stratum corneum (Barry, 2002:512). The intended pH of a topical formulation is to range between 3 and 9, to avoid complications such as skin irritation (Naik et al., 2000:319; Paudel *et al.*, 2010:118).

C.5.4.1 pH results for liposome vesicles

The results obtained for the different liposome dispersions endorse that the integrity of the skin will not be compromised due to the pH values.

Table C.7: pH reading of different liposome dispersions

Liposomes	Measurement			Average pH
	1	2	3	
PL	7.87	7.89	7.86	7.87 ± 0.013
MH	7.03	7.07	7.12	7.07 ± 0.037
CS	7.37	7.30	7.32	7.33 ± 0.029
AH	7.20	7.16	7.23	7.20 ± 0.029

C.5.4.2 pH results for niosome vesicles

The measured pH values determined that all niosome vesicle dispersions corresponded with the ideal range for the skin, ensuring there are no unwanted effects on the skin. The niosome pH values were slightly higher than the liposome vesicles possibly because of the different excipients used.

Table C.8: pH reading of different niosome dispersions

Niosomes	Measurement			Average pH
	1	2	3	
PL	8.73	8.56	8.62	8.64 ± 0.070
MH	8.39	8.45	8.40	8.41 ± 0.026
CS	8.41	8.40	8.42	8.41 ± 0.008
AH	8.89	8.87	8.87	8.88 ± 0.009

C.5.5 Entrapment Efficiency

The equation from Xiang *et al.* (2009:186) was adapted to calculate the final entrapment.

$$EE\% = \frac{\text{Drug (total)} - \text{Drug (supernatant)}}{\text{Drug (total)}} \quad \text{Equation C.1}$$

C.5.5.1 Entrapment efficiency results for liposome vesicles

All the liposomal vesicles resulted in high entrapment, with results near 100%. This suggests the roxithromycin was almost fully incorporated within the vesicles, resulting in better target-site delivery due to the high entrapment, leading to improved bioavailability of the API (Loureiro *et al.*, 2015:96). The high entrapment can be awarded to the long chain fatty acid of phosphatidylcholine, which is capable of accommodating more of the API molecules (Alvarez-Trabado *et al.*, 2017:212).

Table C.9: Entrapment efficiency (%) results of different liposome dispersions

Liposomes	Experiment			Average EE%
	1	2	3	
MH	99.71	99.71	99.72	99.71 ± 0.006
CS	99.69	99.70	99.77	99.69 ± 0.040
AH	99.76	99.75	99.66	99.73 ± 0.041

C.5.5.2 Entrapment efficiency results for niosome vesicles

Excellent entrapment was attained with the niosomal dispersions. Good site delivery of roxithromycin will be possible with all entrapment results nearing 100%. The high transition temperature of Span[®] 60, the inclusion of the lipophilic nature of cholesterol and the API all contributed to a high entrapment result (Bhushan *et al.*, 2014:414; Chandu *et al.*, 2012:28-29).

Table C.10: Entrapment efficiency (%) results of different niosome dispersions

Niosomes	Experiment			Average EE%
	1	2	3	
MH	99.87	99.88	99.87	99.88 ± 0.008
CS	99.86	99.89	99.88	99.87 ± 0.013
AH	99.88	99.92	99.92	99.91 ± 0.017

C.6 Conclusion

It is important to accurately predict and determine the properties, behaviour and results of vesicle systems. The characterisation of the vesicles thus provides assurance when witnessing effects on topical delivery to the skin and any further findings. The liposomal and niosomal vesicles for use in this study were characterised according to their morphology, size distribution, zeta-potential, pH and entrapment efficiency.

Table C.11: Final characterisation results for liposome and niosome vesicle dispersions

Dispersion	Vesicle size (nm)	Pdl	Zeta-potential (mV)	pH	%EE
Liposomes					
PL	90.07	0.203	- 38.40	7.87	NA
MH	90.54	0.183	- 40.87	7.07	99.71
CS	81.57	0.167	- 52.05	7.33	99.69
AH	83.91	0.178	- 48.90	7.20	99.73
Niosomes					
PL	226.27	0.613	- 33.03	8.64	NA
MH	354.17	0.701	- 61.73	8.41	99.88
CS	330.40	0.687	- 50.00	8.41	99.87
AH	295.33	0.615	- 52.73	8.88	99.91

All the prepared vesicles entrapping the different solid-state forms of roxithromycin produced satisfactory results with regard to characterisation, encouraging further formulation of an emulgel to produce a more manageable topical application that will be discussed in Appendix D.

References

- Abdelkader, H., Alani, A.W.G. & Alany, R.G. 2014. Recent advances in non-ionic surfactant vesicles (niosomes): self-assembly, fabrication, characterization, drug delivery applications and limitations. *Drug delivery*, 21:87-100.
- Agarwal, R., Katare, O.P. & Vyas, S.P. 2001. Preparation and in vitro evaluation of liposomal/niosomal delivery systems for antipsoriatic drug dithranol. *International journal of pharmaceutics*, 228:43-52.
- Allec, J., Chatelus, A. & Wagner, N. 1997. Skin distribution and pharmaceutical aspect of adapalene gel. *Journal of the American academy of dermatology*, 36:119-125.
- Alvarez-Trabado, J., Diebold, Y. & Sanchez, A. Designing lipid nanoparticles for topical ocular drug delivery. *International journal of pharmaceutics*, 532:204-217.
- Ammar, H.O., Ghorab, M., El-Nahhas, S.A. & Higazy, I.M. 2011. Proniosomes as a carrier system for transdermal delivery of tenoxicam. *International journal of pharmaceutics*, 405:142-152.
- Asthana, G.S., Asthana, A., Singh, D. & Sharma, P.K. 2016. Etodolac containing topical niosomal gel: formulation development and evaluation. *Journal of drug delivery*, 2016:1-8.
- Aucamp, M., Stieger, N., Barnard, N. & Liebenberg, W. 2013. Solution-mediated phase transformation of different roxithromycin solid state forms: implications on dissolution and solubility. *International journal of pharmaceutics*, 449:18-27.
- Barry, B.W. 2001. Novel mechanisms and devices to enable successful transdermal drug delivery. *European journal of pharmaceutical sciences*, 14:101-114.
- Barry, B. 2002. Transdermal drug delivery. (*In* Aulton, M.E., ed. *Pharmaceutics: the science of dosage form design*. 2nd ed. London: Churchill livingstone, p.499-533).
- Bayindir, Z.S. & Yuksel, N. 2010. Characterization of niosomes prepared with various nonionic surfactants for paclitaxel oral delivery. *Journal of pharmaceutical sciences*, 99:2049-2060.
- Bhatt, P. & Madhav, S. 2011. A detailed review on nanoemulsion drug delivery system. *International journal of pharmaceutical science and research*, 2:2482-2489.
- Bolzinger, M., Briancon, S., Pelletier, J. & Chevalier, Y. 2012. Penetration of drugs through skin, a complex rate-controlling membrane. *Current opinion in colloid & interface science*, 17:156-165.

Boshoff, S. 2018. Investigation of the stabilising effects of niosomes on the amorphous forms of roxithromycin. Potchefstroom: NWU. (Dissertation - MSc).

Bhushan, B., Luo, D., Schricker, S.R., Sigmund, W. & Zauscher, S. 2014. Handbook of nanomaterials and properties. Springer sciences & business media.

Carafa, M., Santucci, E., Alhaique, F., Coviello, T., Murtas, E., Riccieri, F.M., Luciana, G. & Torrisi, M.R. 1998. Preparation and properties of new unilamellar non-ionic/ionic surfactant vesicles. International journal of pharmaceutics, 160:51-59.

Chandu, V.P., Arunashalam, A., Jeganath, S., Yamini, K., Tharangini, K. & Chaitanya, G. 2012. Niosomes: a novel drug delivery system. International journal of novel trends in pharmaceutical sciences, 2:25-31.

Chen, Y., van Minh, L., Liu, J., Angelov, B., Drechsler, M., Garamus, V.M., Willumeit-Romer, R. & Zou, A. 2016. Baicalin loaded in folate-PEG modified liposomes for enhanced stability and tumor targeting. Colloids and surfaces B: biointerfaces, 140:74-82.

Chibowski, E. & Szcześ, A. 2016. Zeta potential and surface charge of DPPC and DOPC liposomes in the presence of PLC enzyme. Adsorption, 22:755-765.

Duangjit, S., Opanasopit, P., Rojanarata, T. & Ngawhirunpat, T. 2011. Characterization and in vitro skin permeation of meloxicam-loaded liposomes versus transferosomes. Journal of drug delivery, 2011:1-9.

Eid, A.M., El-Enshasy, H.A., Aziz, R. & Elmarzughi, N.A. 2014. Preparation, characterization and anti-inflammatory activity of Swietenia macrophylla nanoemulgel. Journal of nanomedicine and nanotechnology, 5:1-10

Gaumet, M., Vargas, A., Gurny, R. & Delie, F. 2008. Nanoparticles for drug delivery: the need for precision in reporting particle size parameters. European journal of pharmaceutics and biopharmaceutics, 69:1-9.

Ghosh, S., Roy, A., Banik, D., Kundu, N., Kuchlyan, J., Dhir, A. & Sarkar, N. 2015. How does the surface charge of ionic surfactant and cholesterol forming vesicles control rotational and translational motion of rhodamine 6G perchlorate (R6G ClO₄)? American chemical society: Langmuir, 31:2310-2320.

Guar, S., Garg, A., Yadav, D., Beg, M. & Gaur, K. 2014. Nano-emulsion gel as novel oil based colloidal nanocarrier for topical delivery of bifonazole. Indian research journal of pharmacy and science, 1:36-54.

Junyaprasert, V.B., Teeranachaideekul, V. & Supaperm, T. 2008. Effect of charged and non-ionic membrane additives on physicochemical properties and stability of niosomes. *American association of pharmaceutical scientists: Pharmaceutical sciences and technology*, 3:851-859.

Karim, K.M., Mandal, A.S., Biswas, N., Guha, A., Chatterjee, S., Behera, M. & Kuotsu, K. 2010. Niosome: a future of targeted drug delivery systems. *Journal of advanced pharmaceutical technology & research*, 1:374-380.

Klang, V., Schwarz, J.C. & Valenta, C. 2015. Nano-emulsions in dermal drug delivery. (*In* Dragicevic-Curic, N. & Maibach, H.I., eds. *Percutaneous penetration enhancers: chemical methods in penetration enhancement: drug manipulation strategies and vehicle effects*. Heidelberg: Springer, p.255-266).

Kumar, G.P. & Rajeshwarrao, P. 2011. Nonionic surfactant vesicular systems for effective drug delivery - an overview. *Acta pharmaceutica sinica B*, 1:208-219.

Laouini, A., Jaafar-Maalej, C., Limayem-Blouza, I., Sfar, S., Charcosset, C. & Fessi, H. 2012. Preparation, characterization and application of liposomes: state of the art. *Journal of colloid science and biotechnology*, 1:147-168.

Loureiro, A., Nogueira, E., Azoia, N.G., Sárria, M.P., Abreu, A.S., Shimanovich, U., Rollett, A., Härmark, J., Hebert, H., Guebitz, G., Bernardes, G.J.L., Preto, A., Gomes, A.C. & CavacoPaulo, A. 2015. Size controlled protein nano-emulsions for active targeting of folate receptor positive cells. *Colloids and surfaces B: biointerfaces*, 135:90-98.

Mahale, N.B., Thakkar, P.D., Mali, R.G., Walunj, D.R. & Chaudhari, S.R. 2012. Niosomes: novel sustained release nonionic stable vesicular systems - an overview. *Advances in colloid and interface science*, 183-184:46-54.

Mali, N., Darandale, S & Vavia, P. 2013. Niosomes as a vesicular carrier for topical administration of minoxidil: formulation and in vitro assessment. *Drug delivery and translational research*, 3:587-592.

Marianecchi, C., Di Marzio, L., Rinaldi, F., Esposito, S. & Carafa, M. 2013. Niosomes. (*In* Uchegbu, I.F., Schätzlein, A.G., Ping Cheng, W. & Lalatsa, A., eds. *Fundamentals of pharmaceutical nanoscience*. New York: Springer, p.65-90).

Milne, M. 2015. The physico-chemical properties and recrystallisation kinetics of selected amorphous active ingredients. Potchefstroom: NWU. (Dissertation - MSc).

Moghassemi, S. & Hadjizadeh, A. 2014. Nano-niosomes as nanoscale drug delivery systems: an illustrated review. *Journal of controlled release*, 185:22-36.

Naeem, S., Kiew, L.V., Chung, L.Y. & Fui, K.S. 2015. A comparative approach for the preparation and physicochemical characterization of lecithin liposomes using chloroform and non-halogenated solvents. *Journal of surfactant detergents*, 18:579-587.

Naik, A., Kalia, Y.N. & Guy, R.H. 2000. Transdermal drug delivery: overcoming the skin's barrier function. *Pharmaceutical science and technology today*, 3:318-325.

Narenji, M., Talaei, M.R. & Moghimi, H.R. 2016. Investigating the effects of size, charge, viscosity and bilayer flexibility on liposomal delivery under convective flow. *International journal of pharmaceutics*, 513:88-96.

NCBI **see** National center for biotechnology information

National center for biotechnology information. 2019. PubChem database. Chloroform, CID=6212. <https://pubchem.ncbi.nlm.nih.gov/compound/Chloroform> Date of access: 24 Jun. 2019.

National center for biotechnology information. 2019. PubChem database. Methanol, CID=887. <https://pubchem.ncbi.nlm.nih.gov/compound/Methanol> Date of access: 24 Jun. 2019.

New, R.R.C. 1990. *Liposomes: a practical approach*. New York: Oxford University Press.

Patil, Y.P. & Jadhav, S. 2013. Novel methods for liposome preparation. *Chemistry and physics of lipids*, 177:8-18.

Paudel, K.S., Milewski, M., Swadley, C.L., Brogden, N.K., Ghosh, P. & Stimchcomb, A.L. 2010. Challenges and opportunities in dermal/transdermal delivery. *Therapeutic delivery*, 11:109-131.

Quinn, P.J. 2012. The effect of tocopherol on the structure and permeability of phosphatidylcholine liposomes. *Journal of controlled release*, 160:158-163.

Ruckmani, K. & Sankar, V. 2010. Formulation and optimization of Zidovudine niosomes. *Pharmacy science and technology*, 11:1119-1127.

Shah, R., Eldridge, D., Palombo, E. & Harding, I. 2014. Optimisation and stability assessment of solid lipid nanoparticles using particle size and zeta potential. *Journal of physical sciences*, 25:59-75.

Shakeel, F., Baboota, S., Ahuja, A., Ali, J., Aqil, M. & Shafiq, S. 2007. Nano-emulsions as vehicle for transdermal delivery of aceclofenac. *American association of pharmaceutical scientists: Pharmaceutical sciences and technology*, 8:1-9

Silva, H.D., Cerqueira, M.A. & Vicente, A.A. 2012. Nano-emulsions for food applications: development and characterization. *Food and bioprocess technology*, 5:854-867.

Swart, M. 2019. Stability of amorphous forms of roxithromycin when encapsulated in liposomes. Potchefstroom: NWU. (Dissertation - MSc).

Varun, T., Sonia, A., Bharat, P. & Patil, V. 2012. Niosomes and liposomes - vesicular approach towards transdermal drug delivery. *International journal of pharmaceutical and chemistry sciences*, 1:632-644.

Vasconcelos, T., Marques, S. & Sarmiento, B. 2015. Development of a resveratrol solid dispersion with soluplus®. Controlled release society annual meeting. Edinburgh, Scotland.

Vasconcelos, T., Marques, S., das Neves, J. & Sarmiento, B. 2016. Amorphous solid dispersions: Rational selection of a manufacturing process. *Advanced drug delivery reviews*, 100:85-101.

Wibroe, P.P., Ahmadvand, D., Oghabian, M.A., Yaghmur, A. & Moghimi, S.M. 2016. An integrated assessment of morphology, size, and complement activation of the PEGylated liposomal doxorubicin products Doxil®, Caelyx®, DOXOrubicin, and SinaDoxome. *Journal of Controlled Release*, 221:1-8.

Xiang, R., Chen, S., Zhang, F., Ni, J. & Tian, X. 2009. A HPLC method for the determination of entrapment efficiency of a new 5-FPE liposome formulation. *Journal of Chinese pharmaceutical sciences*, 18:186-189.

APPENDIX D

FORMULATION OF AN EMULGEL CONTAINING ROXITHROMYCIN ENCAPSULATED IN LIPOSOMES AND NIOSOMES

D.1 Introduction

To treat topical conditions, a semi-solid dosage form is preferred ensuring prolonged skin contact of the API and better patient compliance, which is one of the most important deciding factors for treating a condition (Mahalingham *et al.*, 2008:267; Kurian & Barankin, 2011:4). Semi-solid dosage forms do not flow freely and are divided into gels, creams, ointment, poultices or pastes (Stahl, 2015:209-218). A gel is the ideal choice to reach faster release of the API, but the delivery of hydrophobic APIs can prove challenging. Changing an emulsion into a semi-solid formulation to form an emulgel, for instance, can overcome this limitation (Redkar *et al.*, 2019:586). To further improve topical delivery, vesicles are used as a delivery system in emulgel formulations; providing increased skin penetration with less systemic absorption due to better retention of the API in skin layers (Mali *et al.*, 2013:587; Nasir *et al.*, 2012:484). Previous studies confirmed that prepared amorphous solid-dispersions containing different encapsulated solid-states of roxithromycin are able to successfully deliver the API for topical treatment (Csongradi, 2015; Boshoff, 2018; Swart, 2019).

Currently, there is no topical treatment using roxithromycin available for acne. The purpose of this Appendix was to increase the low viscosity of the vesicle formulations, as discussed in Appendix C, to achieve a patient-friendly and easy to use topical application for acne using roxithromycin.

D.2 Intended purpose of the formulation and dosage form selection

The main purpose was to increase the viscosity of the dispersions (vesicles), for ease of use, by formulating a semi-solid dosage form. Normally, a semi-solid dosage form consists of two phases, where a larger portion identified as the external phase is combined with a dispersed phase, while the API is dissolved in either the oil or water phase (Valentine, 2014:153). Other topical dosage forms, such as creams and ointments, are greasy and sticky with low spreading coefficient, therefore gel formulations are more advantageous in this instance (Redkar *et al.*, 2019:588). For this study, a gel and versions thereof were investigated for the topical delivery of roxithromycin.

D.2.1 Semi-solid dosage form: gel

A gel is a significantly dilute cross-linked system, immobilised by surface rigidity of network fibres, presenting with both liquid and solid properties (Allen *et al.*, 2011:278; Kute & Saudagar, 2013:368; Kaur & Guleri, 2013:1). The challenge a gel poses is to incorporate lipophilic APIs, which could possibly be addressed by using emulsion based strategies as described by Pant *et al.* (2015:1729).

D.2.2.1 Emulgel

A conventional gel could not suffice to incorporate the vesicle dispersions, therefore an emulgel would be the perfect solution to incorporate lipophilic actives within a semi-solid dosage form (Gaur *et al.*, 2014:38; Kute & Saudagar, 2013:368). The combination of an emulsion with a gel base defines an emulgel. Properties such as easily spreadable, emollient, greaseless, non-staining and thixotropic can be used to describe an emulgel, with another advantage being enhanced skin penetration due to the emulgels hydrophilic and hydrophobic nature (Redkar *et al.*, 2019:589).

D.3 Excipients used to formulate a liposomal or niosomal emulgel

D.3.1 Gelling agent

Carbopol® 934 (Lubrizol, South Africa) consist of polymers of acrylic acid cross-linked with polyalkenyl ethers or divinyl glycol. When dispersed in water, the polymers swell and produce a viscous dispersion, and according to Islam *et al.* (2004:1198), they are suitable in the use of topical formulations. Carbopol polymers are well-known for their safety and low toxicity profiles, thus making them effective gelling agents (Tang *et al.* 2007:2785).

D.3.2 Glycerine

Glycerine, also known as glycerol, was purchased from Merck Millipore (South Africa). Glycerine served as an emollient, enhancing the feeling on use, by contributing to smoothness while avoiding skin irritation.

D.3.3 Purified water

Purified water served as the aqueous phase to form the gel. All experiments throughout the study were done with Milli-Q® HPLC grade water available in-house.

D.4 Formulation of an emulgel containing vesicle systems

D.4.1 Pre-formulation of a topical emulgel containing vesicles

Pre-formulation ensures the development of a stable and safe dosage form. After a comprehensive literature study, a trial-and-error approach was used to eliminate failed gel formulations. Different methods were used and the formulations were examined visually, the final formulation was chosen due to favourable viscosity and colour. The end goal for viscosity was to achieve a smooth gel, with high consistency that would be easy to apply, while the colour should preferably be neutral or light to ensure patient acceptability. The method described by Lundie (2016:130) and Rane *et al.* (2018:821) was adapted and employed for the vesicle gel formulations. This method used propylene glycol, methylparaben and propylparaben as preservatives, together with a carbomer and water mixture with the vesicle dispersion added. At first it seemed to render satisfactory results, a light and smooth emulgel that was not sticky. After storing the gel for 6 h, the formulations changed from being smooth to irregular, with visible pieces in the formulation. The visual examination of the samples revealed texture change and possible phase separation, thereafter other methods were tested. An adapted method of Shirsand *et al.* (2016:203) and Ning (2005:378) delivered the most favourable results and was used for all further emulgel preparation.

D.4.2 Method of preparation for emulgels containing encapsulated roxithromycin

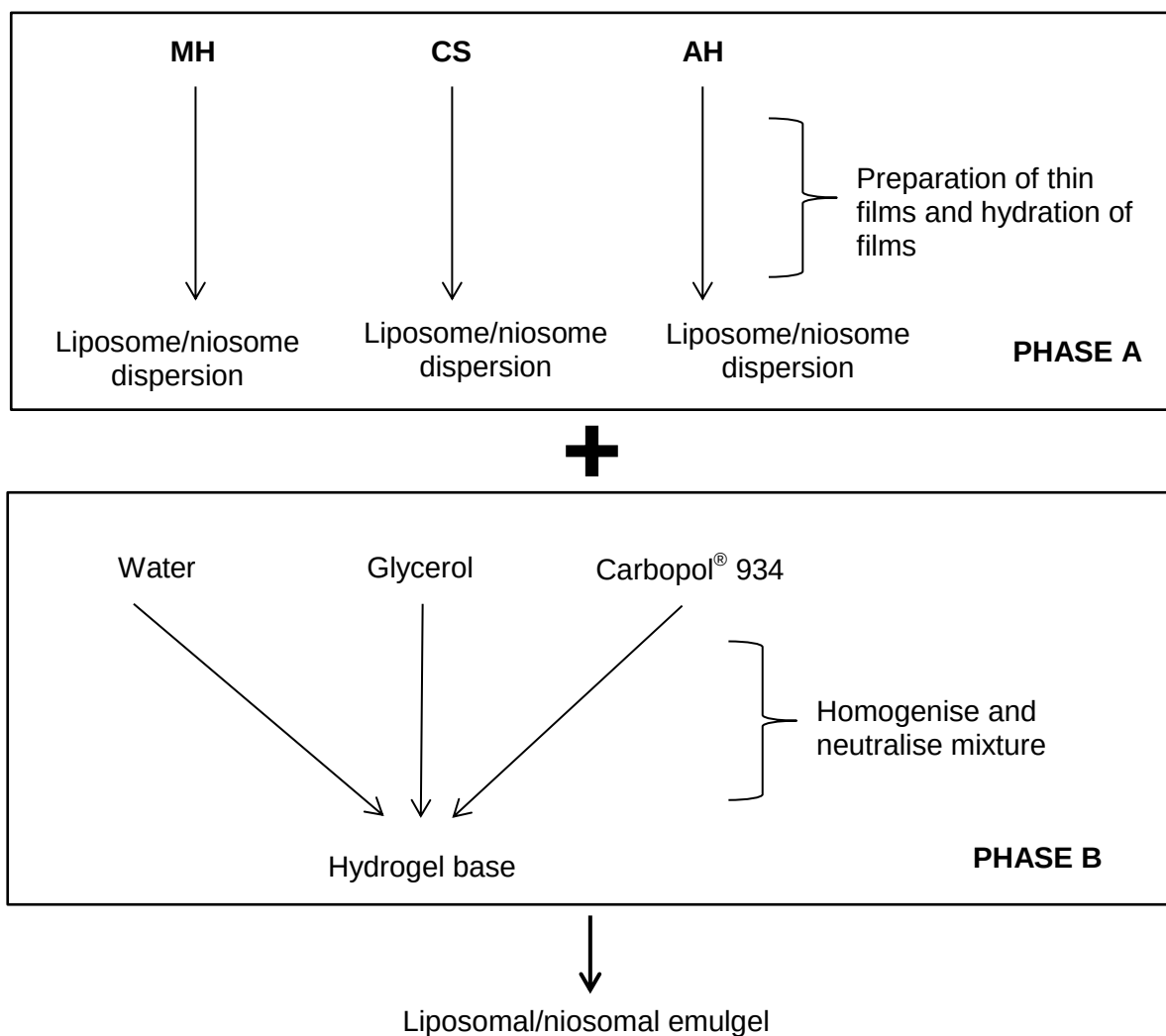


Figure D.1: Diagrammatic representation of the formulation of a liposomal or niosomal emulgel

Table D.1: The final formula of the vesicle emulgel containing roxithromycin

Excipients	Amount
Phase A	
Vesicles (containing 2% roxithromycin)	10 ml
Phase B	
Glycerol	10.0%
Carbopol® 934	1.5%
Milli-Q® water	Add to 15 ml

D.4.2.1 Preparation of the liposomal and niosomal dispersions

When an API is encapsulated in a vesicle it can promote the bioavailability and reduce the side-effects of the chosen API. Surfactants used during formulation of vesicles also act as penetration enhancers by eliminating the mucous layer (Kaur *et al.*, 2018:72). The method for preparation of the liposomal dispersion is described in Appendix C.3.1, and for the niosomal dispersion the method is fully discussed in Appendix C.3.2.

D.4.2.2 Preparation of the hydrogel base

The glycerol was accurately weighed, added to the water and stirred with a glass rod until dissolved. The Carbopol® 934 was sifted into the water through a metal sieve to break any agglomerates due to the static charge of the particles. The Carbopol® 934 was left to hydrate for at least 30 min. The impeller of a RZR 2041 homogeniser (Heidolph, Germany) was submerged close to the bottom of the beaker of the hydrated Carbopol® 934 and water mixture. A vortex of approximately 1 000 rpm was used to agitate the liquid until a lump-free dispersion was attained. The mixture was neutralised with ammonia solution (NH₃). Water, glycerol and Carbopol® 934 form strong OH-bonds, which in turn deliver a clear and smooth gel.

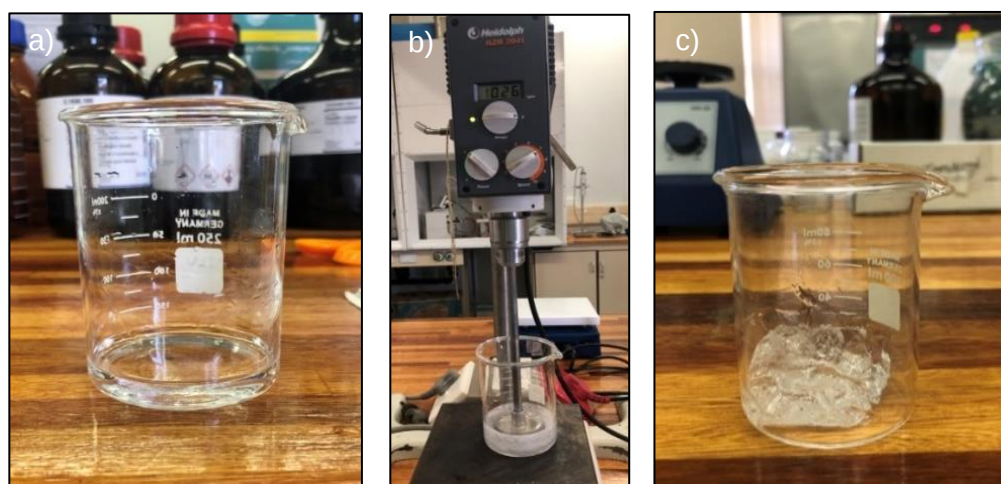


Figure D.2: Method of formulation of the hydrogel base: a) water containing dissolved glycerol, b) homogenising of Carbopol® 934 and water mixture and c) prepared clear hydrogel base

D.4.2.3 Preparation of the emulgels containing the vesicles

For the preparation of the final emulgel formulations, the selected vesicle dispersion was added to a calculated amount of hydrogel base and mixed using a glass rod until the gel was smooth and thoroughly mixed. Both the liposomal and niosomal emulgels, containing the different solid-states of roxithromycin, presented a homogenous and smooth gel-like substance, it had a light and pleasant feel when applied to the skin with no oily residue.



Figure D.3: A niosomal and liposomal emulgel

D.5 Characterisation of emulgels

Evaluating the properties of the formulated liposomal and niosomal emulgels would help to determine which emulgel could be regarded as optimal. The following characteristics of both liposomal and niosomal emulgels were determined, and are fully discussed in Appendix E:

- concentration assay of active ingredient;
- pH;
- viscosity;
- zeta-potential;
- mass loss;
- droplet size;
- visual appearance.

D.6 Conclusion

Emulgels are a front-runner when compared to other delivery systems (Kumar *et al.*, 2015:1182). The formulated liposomal emulgel presented with a light yellow colour due to PC used during preparation of the liposome vesicle systems, while the niosomal gel had a white colour. Both liposomal and niosomal emulgels had a gel-like consistency, with favourable smoothness when applied to the skin. The emulgels theoretically contained 2% of the different solid-state forms of roxithromycin encapsulated in vesicle delivery systems, separately. These emulgel formulations succeeded in improving the viscosity of the vesicle dispersions. Adequate quantities of the vesicle emulgels were prepared and used to perform stability testing at different storage conditions, as discussed in Appendix E.

References

- Allen, L.V., Popovich, N.G. & Ansel, H.C. 2011. Ointments, creams, and gels. (*In* Allen, L.V., Popovich, N.G. & Ansel, H.C., eds. *Ansel's pharmaceutical dosage forms and drug delivery systems*. 9th ed. Philadelphia: Lippincott Williams & Wilkins, p.272-293).
- Gaur, S., Garg, A., Yadav, D., Beg, M. & Gaur, K. 2014. Nano-emulsion gel as novel oil based colloidal nano-carrier for topical delivery of bifonazole. *Indian research journal of pharmacy and science*, 1:36-54.
- Islam, M.T, Rodriguez-Hornedo,N., Ciotti, S. & Ackermann, C. 2004. Rheological characterization of topical carbomer gels neutralized to different pH. *Pharmaceutical research*, 21:1192-1199.
- Kuar, L.P. & Guleri, T.K. 2013. Topical gel: A recent approach for novel drug delivery. *Asian journal of biomedical and pharmaceutical sciences*, 3:1-5.
- Kuar, P., Dua, J.S. & Prasad, D.N. 2018. Formulation and evaluation of ketoconazole niosomal gel. *Asain journal of pharmaceutical research and development*, 6:71-75.
- Kumar, S., Singh, N. & Arora, S. 2015. Emulgel: an insight. *European journal of pharmaceutical and medical research*, 2:1168-1186.
- Kurian, A. & Barankin, B. 2011. Delivery vehicle advances in dermatology. *Skin therapy letter*, 7:4-5.
- Kute, S.B. & Saudagar, R.B. 2013. Emulsified gel: a novel approach for delivery of hydrophobic drugs: an overview. *Journal of advanced pharmacy education and research*, 3:368-376.
- Lundie, M.M. 2016. Formulation and topical delivery of niosomes and proniosomes containing carnosine. Potchefstroom: NWU. (Dissertation - MSc).
- Mahalingam, R., Li, X. & Jasti, B.R. 2008. Semisolid dosages: ointments, creams and gels. (*In* Gad, S.C., ed. *Pharmaceutical manufacturing handbook: production and processes*. New Jersey: John Wiley & Sons, p.267-309).
- Mali, N., Darandale, S. & Vavia, P. 2013. Niosomes as a vesicular carrier for topical administration of minoxidil: formulation and in vitro assessment. *Drug delivery and translational research*, 3:587-592.
- Nasir, A., SL, H. & Amanpreet, K. 2012. Niosomes: an excellent tool for drug delivery. *International journal of research in pharmacy and chemistry*, 2:479-487.

Ning, M., Guo, Y., Pan, H., Chen, X. & Gu, Z. 2005. Preparation, in vitro and in vivo evaluation of liposomal/niosomal gel delivery systems for clotrimazole. *Drug development and industrial pharmacy*, 31:375-383.

Pant, S., Badola, A., Baluni, S. & Pant, W. 2015. A Review on emulgel novel approach for topical drug delivery system. *World journal of pharmacy and pharmaceutical sciences*, 4:1728-1743.

Rane, S., Rane, B. & Jain, A. 2018. Formulation and evaluation of niosomal gel loaded with tinidazole niosomes. *World journal of pharmaceutical research*, 7:817-837.

Redkar, M.R., Patil, S.V. & Rukari, T.G. 2019. Emulgel: A modern tool for topical drug delivery. *World journal of pharmaceutical research*, 8:586-597.

Shirsand, S.B., Para, M.S., Nagendrakumar, D., Kanani, K.M. & Keerthy, D. Formulation and evaluation of Ketoconazole niosomal gel drug delivery system. *International journal of pharmaceutical investigation*, 2:201-207.

Stahl, J. 2015. Dermal and transdermal formulations: how they can affect the active compound. (*In* Dragicevic-Curic, N. & Maibach, H.I., eds. *Percutaneous penetration enhancers, chemical methods in penetration enhancers*. Berlin: Springer, p.209-220).

Tang, C., Yin, L., Yu, J., Yin, C. & Pei, Y. 2007. Swelling behavior and biocompatibility of carbopol-containing superporous hydrogel composites. *Journal of applied polymer science*, 104:275-2791.

Valentine, N. 2014. Semi solid dosage forms manufacturing: tools, critical process parameters, strategies, optimization and validation. *Scholars academic journal of pharmacy*, 3:153-161.

APPENDIX E

STABILITY TESTING OF LIPOSOMAL AND NIOSOMAL EMULGELS

E.1 Introduction

It is important for a pharmaceutical product to remain potent, non-toxic and uniform, and to retain chemical integrity during storage. To store a product under normal conditions for a long period is uneconomical and time consuming. Exposing an API product to a variety of environmental factors, such as temperature and humidity, can provide evidence on the variability of the quality of the product, while also establishing recommended storage conditions and shelf-life at an accelerated rate (Bajaj *et al.*, 2012:131). Thorough stability testing consists of both chemical and physical characterisation during storage over a recommended period, with a report on physical appearance and assay of all APIs included (ICH, 2003:1). Arrhenius stated for every 10°C increase in temperature, the speed of reaction is multiplied, or increased threefold (Bajaj *et al.*, 2012:132). Accelerated testing use exaggerated storage conditions in order to increase the rate of chemical degradation and physical alteration of a product, therefore using the Arrhenius equation is the most efficient method to shorten the stability test period of pre-formulation studies, according to Barnes (2007:661). The accepted criteria, according to the South African Health Products Regulatory Authority (SAHPRA) (MCC, 2012:14), indicates there should be no significant change during the stability testing period. The following can be seen as a significant change for an API product (ICH, 2003:9):

6. a 5% change in assay from the initial value;
7. any degradant surpassing its specification limit,
8. appearance and physical attributes (e.g. colour, phase separation) not meeting its specifications;
9. pH measurements surpassing its specification limits;
10. dissolution for 12 dosage units surpassing specification limits for capsules and tablets.

E.2 Methods

E.2.1 Sample preparation

The emulgels containing liposome or niosome vesicle systems encapsulating roxithromycin, as described in Appendix D, were prepared in bulk for use during stability testing. A test sample of every formulation was analysed directly after the preparation of the initial test results. A placebo

(vesicles containing no API) was used as the control group during the studies. All formulations were stored upright in 50 ml glass jars with polyvinylidene chloride (PVDC) liners.

E.2.2 Accelerated stability testing method

Stability tests were performed using the guidelines provided by SAHPRA for accelerated stability testing. The formulations were stored in three different airtight Labcon® humidity chambers (Maraisburg, South Africa) for a period of three months, the temperature and humidity was noted every day for the duration of the study to ensure stable conditions throughout the assessment period.

The chambers were set to the following conditions to increase the speed of reaction, as predicted by Arrhenius (Bajaj *et al.*, 2012:132; MCC, 2012:13):

- $40 \pm 2^{\circ}\text{C}/75 \pm 5\% \text{ RH}$ (Stability chamber 1)
- $30 \pm 2^{\circ}\text{C}/65 \pm 5\% \text{ RH}$ (Stability chamber 2)
- $25 \pm 2^{\circ}\text{C}/60 \pm 5\% \text{ RH}$ (Stability chamber 3)



Figure E.1: Labcon® humidity chambers used to perform accelerated stability tests

A sample of each formulation was removed from storage at months 1, 2 and 3 and allowed to reach room temperature before assessment. Each sample was analysed in triplicate. The following assessments were performed on all formulations at monthly intervals:

- Concentration assay of active ingredient;
- pH;
- viscosity;
- zeta-potential;

- mass loss;
- droplet size;
- visual appearance.

E.2.2.1 Concentration assay

Approximately 1 ml sample representative of each storage condition was accurately weighed and dissolved in methanol in a 100 ml volumetric flask. The previously verified HPLC method, as fully described in Appendix A, was used to evaluate the concentration assay of the API in the emulgels. A Venusil XBP C18 (2) reverse phase 150 x 4.6 mm column with a 5 µm particle size (Agela Technologies, Germany) was used together with an Agilent® 1100 Series HPLC system (Agilent Technologies, California). The mobile phase (pH 7) consisted of a 0.01 M $\text{NH}_4\text{H}_2\text{PO}_4$ buffer solution mixed with methanol in the ratio 15:85. A flow rate of 1 ml/min and injection volume of 50 µl, set with a run time of 9 min and 205 nm detection wavelengths, was utilised. The concentrations were quantified, the mean percentages relative to the initial assay results were evaluated and a standard sample was prepared, as stated in Section A.3, to serve as a control for the analysis. The experiment was done in triplicate to ensure reliable results.



Figure E.2: An Agilent® 1100 Series HPLC system used to perform assay assessments

E.2.2.2 pH

The pH of samples representative of every storage condition was measured in triplicate using a Mettler Toledo pH meter (Mettler Toledo, Switzerland) after calibration.



Figure E.3: Mettler Toledo pH meter used to perform pH measurements

E.2.2.3 Viscosity

A Brookfield Viscometer DV2T Ultra (United States of America) was used to measure the viscosity. The viscosity was measured in centipoises (cP) at 25°C using a LV-4 spindle. Multipoint data was collected on Rheocalc T 1.2.19 software; it was recorded at intervals of 10 sec for 2 min with a speed of 5 rpm, after which an average was calculated.

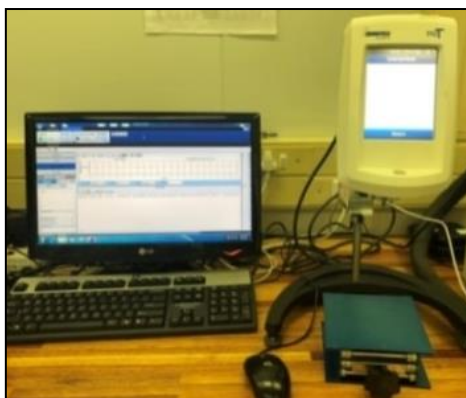


Figure E.4: A Brookfield Viscometer DV2T Ultra used to measure viscosity

E.2.2.4 Zeta-potential

Approximately 1 ml of each of the samples representing every storage condition was diluted with 20 ml purified water. Approximately 2 ml of a dilution was injected into a clear disposable zeta-cell (DTS1070 folded capillary cell) and the zeta-potential was measured in triplicate using a Malvern zeta-sizer Nano ZS (Malvern Instruments, United Kingdom).



Figure E.5: Malvern zeta-sizer Nano ZS used to measure zeta-potential

E.2.2.5 Mass loss

The same container for every sample, for every storage condition was weighed in triplicate every month for three months using a calibrated Mettler Toledo (Switzerland) balance.



Figure E.6: Mettler Toledo balance used to determine mass loss

E.2.2.6 Droplet size

The average droplet size and Pdl were measured in triplicate using a Malvern zeta-sizer Nano ZS (Malvern Instruments, United Kingdom). To reduce interaction between droplets approximately 1 ml of each of the samples representing every storage condition was diluted with 20 ml purified water. About 2 ml of a dilution was injected into a clear disposable zeta-cell (DTS1070 folded capillary cell) for analysis.

E.2.2.7 Visual appearance

A sample from each storage condition was inspected monthly to report any macroscopic changes. Colour, smell and feel were reported, and photos of every sample at the different storage conditions were taken at every monthly assessment to be compared to the initial

photographs. Visual and sensory assessments were performed at the same time of the day at each time interval.

E.3 Results and discussion

E.3.1 Concentration assay

The concentration assay results obtained over the three-month period were summarised in Tables E.1 to E.2. The mean percentage concentration change of the API in the samples representing each different storage condition, relative to the initial values, was calculated. Some formulations resulted in an initial concentration higher than 100%, which can be seen as acceptable, since Barnes (2007:650) applied this method to increase the shelf life of a product. The only liposome formulations to comply with the acceptable limit (5.0% variation) were **L-CS** in month 2, with a 2.8% variation (40°C/75% RH) and **L-AH** in month 2 (4.3% variation) and month 3 (1.9% variation) at 30°C/65% RH and 25°C/60% RH, respectively. Values for **N-CS** were within the variation limit on month 1 (4.1% variation) and month 2 (4.8% variation) at 30°C/65% RH and 25°C/60% RH, respectively. **N-AH** also had two values within the limit on month 2 (3.8% variation) and month 3 (1.2% variation) at 40°C/75% RH and 30°C/65% RH, respectively. The concentration assay in liposomal and niosomal emulgels fluctuated during the three month testing period. This could be attributed to poor mixing of the semi-solids during bulk formulation. It is also possible that poor mixing resulted in uneven distribution of the API in the separate glass containers delivering fluctuating results. The degradation could also be due to the absence of preservatives leading to insufficient protection of the API in the emulgel. It can be concluded that none of the emulgels delivered satisfactory results with regard to the assay of API.

Table E.1: The average concentration assay (%) results of an emulgel containing roxithromycin encapsulated in liposomes

Emulgel	Storage condition	Initial (T0)	Month 1 (T1)	Month 2 (T2)	Month 3 (T3)
L-MH	40°C/75% RH	98.6 ± 1.30	61.9 ± 1.79	51.3 ± 2.81	37.8 ± 3.26
	30°C/65% RH	98.6 ± 1.30	64.6 ± 7.84	69.2 ± 2.44	30.9 ± 2.52
	25°C/60% RH	98.6 ± 1.30	61.5 ± 2.75	97.4 ± 9.49	33.4 ± 1.15
L-CS	40°C/75% RH	77.5 ± 0.33	73.6 ± 1.99	75.3 ± 4.90	16.1 ± 0.51
	30°C/65% RH	77.5 ± 0.33	63.8 ± 2.39	115.2 ± 4.50	49.9 ± 1.84
	25°C/60% RH	77.5 ± 0.33	89.3 ± 1.39	72.8 ± 0.60	45.2 ± 1.54
L-AH	40°C/75% RH	104.8 ± 1.30	86.1 ± 2.16	70.96 ± 2.12	33.2 ± 1.62
	30°C/65% RH	104.8 ± 1.30	47.7 ± 1.64	109.3 ± 1.32	61.7 ± 1.02
	25°C/60% RH	104.8 ± 1.30	91.7 ± 2.90	102.8 ± 7.94	72.0 ± 2.09

Table E.2: The average concentration assay (%) results of an emulgel containing roxithromycin encapsulated in niosomes

Emulgel	Storage condition	Initial (T0)	Month 1 (T1)	Month 2 (T2)	Month 3 (T3)
N-MH	40°C/75% RH	102.4 ± 4.16	83.4 ± 0.43	62.8 ± 2.28	45.9 ± 0.95
	30°C/65% RH	102.4 ± 4.16	85.7 ± 1.52	95.4 ± 12.70	59.3 ± 1.37
	25°C/60% RH	102.4 ± 4.16	77.2 ± 12.31	111.8 ± 9.73	79.3 ± 1.53
N-CS	40°C/75% RH	77.5 ± 0.76	101.9 ± 1.23	89.2 ± 1.57	43.0 ± 0.49
	30°C/65% RH	77.5 ± 0.76	80.7 ± 1.15	86.1 ± 7.88	49.6 ± 23.44
	25°C/60% RH	77.5 ± 0.76	61.8 ± 6.22	81.2 ± 3.26	71.1 ± 2.45
N-AH	40°C/75% RH	112.9 ± 0.16	145.5 ± 1.57	108.6 ± 3.69	51.1 ± 1.80
	30°C/65% RH	112.9 ± 0.16	79.1 ± 1.34	130.3 ± 9.17	111.6 ± 0.69
	25°C/60% RH	112.9 ± 0.16	92.4 ± 0.75	146.0 ± 0.91	111.6 ± 0.17

E.3.2 pH

The pH values of the liposomal and niosomal emulgel samples over a three months period, representing each storage condition, are indicated in Tables E.3 and E.4. A pH value indicates the ratio between hydroxide ions [OH⁻] and hydrogen ions [H⁺] in a solution, which should remain constant in a stable solution (Hach company, 2018:6). It can thus be accepted that a disturbance of the [H⁺] balance occurred if a change in pH was noted. It is known that roxithromycin contains OH-groups, which could also influence the pH level of the formulations. To prevent possible skin damage, the ideal pH range for skin delivery is between 3 and 9 (Naik *et al.*, 2000:319). Taking the results as seen in Tables E3 and E4 into consideration, it is evident that both [H⁺] and [OH⁻] concentration variations occurred during storage. As no trend in the pH values was observed over the three months testing period, a possibility of chemical instability could be considered. To ensure satisfactory permeability of the API through the skin layers, a good pH balance must be maintained throughout storage. Both the liposomal and niosomal emulgels for all storage conditions resulted in increased acidity at month 3 in comparison to month 2. The increased pH levels indicated an increase in the [OH⁻] concentration and a decrease of the [H⁺] concentration in the formulations during storage. It is known that the pH level effects the ionisation of a drug, influencing the permeation in turn (Williams, 2003:38). The unionised form of a weak acidic product will permeate the lipophilic stratum corneum bilayers more easily, while the opposite is true for the ionised form. The increased hydroxyl groups can be attributed to degradation of the emulgels, confirming possible chemical instability. All liposomal and niosomal emulgels containing the API did however remain in the acceptable pH range for transdermal delivery, with the lowest pH value measured at 3.82 and the highest at 6.46.

Table E.3: The average pH results of an emulgel containing roxithromycin encapsulated in liposomes

Emulgel	Storage condition	Initial (T0)	Month 1 (T1)	Month 2 (T2)	Month 3 (T3)
L-PL	40°C/75% RH	9.65 ± 0.01	8.50 ± 0.02	8.22 ± 0.19	8.67 ± 0.02
	30°C/65% RH	9.65 ± 0.01	8.19 ± 0.08	8.41 ± 0.20	9.03 ± 0.05
	25°C/60% RH	9.65 ± 0.01	8.30 ± 0.11	8.65 ± 0.03	9.38 ± 0.10
L-MH	40°C/75% RH	6.20 ± 0.02	5.59 ± 0.13	4.65 ± 0.04	5.72 ± 0.10
	30°C/65% RH	6.20 ± 0.02	5.59 ± 0.30	4.88 ± 0.11	5.74 ± 0.10
	25°C/60% RH	6.20 ± 0.02	5.49 ± 0.08	4.85 ± 0.04	6.17 ± 0.03
L-CS	40°C/75% RH	6.46 ± 0.11	5.32 ± 0.03	4.61 ± 0.03	5.67 ± 0.12
	30°C/65% RH	6.46 ± 0.11	5.28 ± 0.05	4.61 ± 0.03	5.48 ± 0.08
	25°C/60% RH	6.46 ± 0.11	5.19 ± 0.04	4.71 ± 0.01	6.36 ± 0.19
L-AH	40°C/75% RH	6.15 ± 0.03	5.06 ± 0.05	4.34 ± 0.01	5.25 ± 0.10
	30°C/65% RH	6.15 ± 0.03	5.25 ± 0.14	4.42 ± 0.01	5.53 ± 0.07
	25°C/60% RH	6.15 ± 0.03	5.01 ± 0.03	4.46 ± 0.00	5.72 ± 0.07

Table E.4: The average pH results of an emulgel containing roxithromycin encapsulated in niosomes

Emulgel	Storage condition	Initial (T0)	Month 1 (T1)	Month 2 (T2)	Month 3 (T3)
N-PL	40°C/75% RH	7.46 ± 0.12	5.22 ± 0.01	4.64 ± 0.04	5.57 ± 0.08
	30°C/65% RH	7.46 ± 0.12	4.97 ± 0.01	4.51 ± 0.00	5.18 ± 0.04
	25°C/60% RH	7.46 ± 0.12	5.37 ± 0.13	5.88 ± 0.00	5.48 ± 0.01
N-MH	40°C/75% RH	5.37 ± 0.04	4.52 ± 0.02	4.02 ± 0.01	5.15 ± 0.01
	30°C/65% RH	5.37 ± 0.04	4.47 ± 0.01	3.82 ± 0.01	4.69 ± 0.05
	25°C/60% RH	5.37 ± 0.04	4.79 ± 0.14	3.93 ± 0.01	4.78 ± 0.01
N-CS	40°C/75% RH	5.68 ± 0.01	4.94 ± 0.01	4.42 ± 0.02	5.05 ± 0.01
	30°C/65% RH	5.68 ± 0.01	4.94 ± 0.04	4.45 ± 0.02	5.12 ± 0.05
	25°C/60% RH	5.68 ± 0.01	5.19 ± 0.03	4.45 ± 0.02	5.18 ± 0.04
N-AH	40°C/75% RH	5.76 ± 0.01	5.00 ± 0.01	4.46 ± 0.01	5.16 ± 0.02
	30°C/65% RH	5.76 ± 0.01	4.95 ± 0.01	4.42 ± 0.01	5.17 ± 0.03
	25°C/60% RH	5.76 ± 0.01	5.20 ± 0.01	4.51 ± 0.01	5.20 ± 0.01

E.3.3 Viscosity

Tables E.5 and E.6 indicated the average viscosity readings at monthly intervals over a period of three months. A significant change in viscosity was observed over the three months, with fluctuating values observed for both the liposomal and niosomal emulgels. It was observed there was no certain trend visible in the viscosity results over the three-month period. Chemical

changes in the products due to chemical instability could have attributed to the fluctuation in viscosity results (Zatz *et al.*, 1989:174). The viscoelastic response of a product can be attributed to constrictions in the material, such as chemical cross-linking, which assists in confining the constituent molecules to their average positions in space (Douglas, 2018:1). The fair amount of OH-groups between roxithromycin and Carbopol® (as stated in Section D.4.2.3) can thus be seen as the chemical constraints, which results in the firm gel matrix. Factors such as high temperature influence the gel matrix by leading to broken Van der Waals forces between the molecules due to higher kinetic energy, which is also a probable reason for the fluctuation in viscosity results. This can be confirmed by mass loss due to evaporation, which will be discussed in Section E.3.5. The macroscopic results (discussed in Section E.3.7) also revealed that temperature and humidity influenced the viscosity of the emulgels.

Table E.5: The average viscosity (cP) results of an emulgel containing roxithromycin encapsulated in liposomes

Emulgel	Storage condition	Initial (T0)	Month 1 (T1)	Month 2 (T2)	Month 3 (T3)
L-PL	40°C/75% RH	57030 ± 2337.06	24520 ± 3205.81	40690 ± 7214.07	24920 ± 320.91
	30°C/65% RH	57030 ± 2337.06	32780 ± 3898.14	60110 ± 968.77	27030 ± 1288.51
	25°C/60% RH	57030 ± 2337.06	26380 ± 4578.57	66270 ± 472.09	21200 ± 494.33
L-MH	40°C/75% RH	32060 ± 6331.81	29990 ± 4517.44	30200 ± 6703.10	35050 ± 1027.78
	30°C/65% RH	32060 ± 6331.81	43710 ± 3756.29	46660 ± 5890.31	15260 ± 1278.01
	25°C/60% RH	32060 ± 6331.81	35490 ± 5907.24	67170 ± 7180.54	18840 ± 2768.29
L-CS	40°C/75% RH	94890 ± 3466.29	96677 ± 6551.25	97238 ± 15744.39	84810 ± 2094.85
	30°C/65% RH	94890 ± 3466.29	77000 ± 6151.12	115383 ± 4865.81	70470 ± 2837.01
	25°C/60% RH	94890 ± 3466.29	67033 ± 9158.60	99085 ± 10226.40	90520 ± 4515.67
L-AH	40°C/75% RH	50230 ± 1691.23	88018 ± 7164.57	63320 ± 8461.85	85030 ± 1878.27
	30°C/65% RH	50230 ± 1691.23	75990 ± 6277.47	110992 ± 6084.92	73170 ± 3169.59
	25°C/60% RH	50230 ± 1691.23	83422 ± 15789.9	99803 ± 7466.45	107358 ± 3674.10

Table E.6: The average viscosity (cP) results of an emulgel containing roxithromycin encapsulated in niosomes

Emulgel	Storage condition	Initial (T0)	Month 1 (T1)	Month 2 (T2)	Month 3 (T3)
N-PL	40°C/75% RH	7810 ± 263.20	24520 ± 5474.99	25730 ± 6173.24	34080 ± 954.53
	30°C/65% RH	7810 ± 263.20	15310 ± 1429.47	37640 ± 1906.54	19440 ± 1361.50
	25°C/60% RH	7810 ± 263.20	19910 ± 1930.51	23610 ± 2111.04	27040 ± 993.95
N-MH	40°C/75% RH	55450 ± 1612.78	28737 ± 7021.04	35890 ± 11456.12	28220 ± 2647.84
	30°C/65% RH	55450 ± 1612.78	41100 ± 4380.64	106583 ± 7409.31	29480 ± 2918.98
	25°C/60% RH	55450 ± 1612.78	42670 ± 7817.09	76480 ± 3581.53	18970 ± 2440.81
N-CS	40°C/75% RH	35180 ± 1652.37	44820 ± 6105.65	58270 ± 7153.39	28860 ± 1444.09
	30°C/65% RH	35180 ± 1652.37	42690 ± 5690.75	86310 ± 8098.50	15800 ± 3006.47
	25°C/60% RH	35180 ± 1652.37	42270 ± 4458.13	54240 ± 7064.91	41020 ± 3885.69
N-AH	40°C/75% RH	41620 ± 2176.20	52580 ± 7607.01	90280 ± 4776.31	35720 ± 4120.97
	30°C/65% RH	41620 ± 2176.20	45440 ± 5951.66	49640 ± 5290.73	26100 ± 3586.70
	25°C/60% RH	41620 ± 2176.20	36630 ± 4306.68	58110 ± 4027.73	35790 ± 3533.24

E.3.4 Zeta-potential

The zeta-potential of a pharmaceutical product points to the determination of the stability, EE% and interaction with the skin (Bhatt & Madhav, 2011:2296; Klang *et al.*, 2015:262; Silva *et al.*, 2012:860). Zeta-potential values for the emulgel formulation fluctuated significantly over the three months testing period, as seen in Tables E.7 and E.8. Subsequently the emulgels were viewed as unstable over the three months. A highly positive or negative zeta-potential value indicates electrical stabilisation, while low values indicate possible coagulation or flocculation (Marianecchi *et al.*, 2013:75). A zeta-potential value higher than + 30 mV or lower than - 30 mV indicates a stable formulation (Malvern Instruments, 2015:3). Some emulgels had repulsive forces exceeding the attractive forces resulting in zeta-potential values lower than - 30 mV, indicating dispersed particles and deflocculated systems after the three months testing period (Roland *et al.*, 2003:8). The liposomal emulgels that did not meet the zeta-potential limits after the testing period were **L-MH** at 40°C/75% RH and 30°C/65% RH, and **L-CS** at all three storage

conditions. The niosomal emulgels that did not meet the criteria were **N-MH** at 30°C/65% RH, **N-CS** at 40°C/75 RH and 25°C/60% RH, together with **N-AH** at 40°C/75% RH.

Table E.7: The average zeta-potential (mV) results of an emulgel containing roxithromycin encapsulated in liposomes

Emulgel	Storage condition	Initial (T0)	Month 1 (T1)	Month 2 (T2)	Month 3 (T3)
L-PL	40°C/75% RH	-80.2 ± 2.39	-99.2 ± 3.07	-59.8 ± 0.87	-68.6 ± 1.27
	30°C/65% RH	-80.2 ± 2.39	-68.6 ± 2.54	-70.4 ± 1.73	-64.8 ± 0.30
	25°C/60% RH	-80.2 ± 2.39	-71.1 ± 3.91	-71.2 ± 0.91	-68.1 ± 1.32
L-MH	40°C/75% RH	-63.4 ± 5.75	-64.1 ± 2.85	-18.4 ± 1.94	-7.1 ± 1.74
	30°C/65% RH	-63.4 ± 5.75	-33.6 ± 0.85	-31.2 ± 4.82	-9.8 ± 0.95
	25°C/60% RH	-63.4 ± 5.75	-48.5 ± 1.15	-23.7 ± 1.66	-42.0 ± 4.97
L-CS	40°C/75% RH	-59.9 ± 3.74	-19.0 ± 2.50	-12.2 ± 0.81	-12.4 ± 1.68
	30°C/65% RH	-59.9 ± 3.74	-34.4 ± 8.76	-62.5 ± 2.17	2.5 ± 7.67
	25°C/60% RH	-59.9 ± 3.74	-42.4 ± 0.58	-12.8 ± 0.60	-18.0 ± 0.81
L-AH	40°C/75% RH	-58.7 ± 2.71	-33.7 ± 3.61	-15.9 ± 4.96	-49.3 ± 0.15
	30°C/65% RH	-58.7 ± 2.71	-49.6 ± 0.70	-50.4 ± 0.53	-42.1 ± 16.66
	25°C/60% RH	-58.7 ± 2.71	-46.0 ± 1.73	-21.1 ± 2.29	-13.9 ± 2.80

Table E.8: The average zeta-potential (mV) results of an emulgel containing roxithromycin encapsulated in niosomes

Emulgel	Storage condition	Initial (T0)	Month 1 (T1)	Month 2 (T2)	Month 3 (T3)
N-PL	40°C/75% RH	-104.0 ± 2.00	-92.2 ± 1.78	-59.5 ± 2.57	-46.1 ± 5.12
	30°C/65% RH	-104.0 ± 2.00	-102.9 ± 2.87	-51.4 ± 3.67	-88.2 ± 0.26
	25°C/60% RH	-104.0 ± 2.00	-94.2 ± 0.75	-54.7 ± 7.21	-68.2 ± 2.37
N-MH	40°C/75% RH	-74.5 ± 1.53	-42.4 ± 3.00	-22.5 ± 0.62	-32.4 ± 0.87
	30°C/65% RH	-74.5 ± 1.53	-39.5 ± 0.67	-37.1 ± 3.05	-17.1 ± 3.22
	25°C/60% RH	-74.5 ± 1.53	-38.7 ± 1.18	-46.8 ± 0.75	-36.9 ± 10.23
N-CS	40°C/75% RH	-78.9 ± 14.95	-39.1 ± 3.01	-32.6 ± 2.70	-21.5 ± 1.54
	30°C/65% RH	-78.9 ± 14.95	-31.9 ± 2.06	-41.8 ± 1.40	-41.4 ± 0.81
	25°C/60% RH	-78.9 ± 14.95	-38.3 ± 1.85	-44.0 ± 2.30	-28.4 ± 1.06
N-AH	40°C/75% RH	-54.1 ± 10.77	-42.6 ± 0.60	-35.3 ± 0.52	-20.8 ± 1.12
	30°C/65% RH	-54.1 ± 10.77	-36.1 ± 2.40	-41.8 ± 9.56	-31.3 ± 5.94
	25°C/60% RH	-54.1 ± 10.77	-36.2 ± 0.95	-45.7 ± 3.59	-30.9 ± 1.42

E.3.5 Mass loss

Tables E.9 and E.10 indicated the mass variation at monthly intervals over a period of three months. A slight decrease in mass occurred for the different liposomal and niosomal emulgels, which could be attributed to evaporation during storage. If evaporation did occur, it could also be a reason for the variation observed in pH and viscosity of the formulations. All emulgels resulted in negligible mass loss, with **L-AH** at 40°C/75% RH resulting in the highest percentage loss for the liposomal emulgels with a 1.31% variation, and **N-AH** at 40°C/75% RH resulting the highest niosomal emulgel variation of 0.96%; the smallest mass loss was observed by **L-CS** at 30°C/65% RH with a 0.05% variation from the initial value. These results confirmed the storage containers used were suitable, resulting in minimal condensation.

Table E.9: The average mass (g) of an emulgel containing roxithromycin encapsulated in liposomes

Emulgel	Storage condition	Initial (T0)	Month 1 (T1)	Month 2 (T2)	Month 3 (T3)
L-PL	40°C/75% RH	80.11 ± 0.0003	80.07 ± 0.0111	80.04 ± 0.0002	80.00 ± 0.0003
	30°C/65% RH	81.68 ± 0.0002	81.64 ± 0.0002	81.61 ± 0.0002	81.59 ± 0.0007
	25°C/60% RH	84.47 ± 0.0002	84.41 ± 0.0002	84.40 ± 0.0005	84.39 ± 0.0002
L-MH	40°C/75% RH	83.14 ± 0.0005	83.08 ± 0.0002	83.05 ± 0.0001	83.01 ± 0.0003
	30°C/65% RH	85.36 ± 0.0004	85.33 ± 0.0002	85.30 ± 0.0002	85.28 ± 0.0009
	25°C/60% RH	87.94 ± 0.0004	87.91 ± 0.0005	87.89 ± 0.0002	87.88 ± 0.0003
L-CS	40°C/75% RH	89.50 ± 0.0002	89.45 ± 0.0130	89.41 ± 0.0002	89.37 ± 0.0007
	30°C/65% RH	84.40 ± 0.0004	84.39 ± 0.0003	84.37 ± 0.0001	84.36 ± 0.0005
	25°C/60% RH	83.74 ± 0.0001	83.79 ± 0.1560	83.68 ± 0.0001	83.55 ± 0.1911
L-AH	40°C/75% RH	84.23 ± 0.0004	83.77 ± 0.0002	83.53 ± 0.0001	83.13 ± 0.0003
	30°C/65% RH	93.79 ± 0.0009	93.71 ± 0.0001	93.68 ± 0.0002	93.65 ± 0.0006
	25°C/60% RH	96.75 ± 0.0007	96.71 ± 0.0005	96.69 ± 0.0001	96.68 ± 0.0002

Table E.10: The average mass (g) of an emulgel containing roxithromycin encapsulated in niosomes

Emulgel	Storage condition	Initial (T0)	Month 1 (T1)	Month 2 (T2)	Month 3 (T3)
N-PL	40°C/75% RH	86.52 ± 0.0002	86.50 ± 0.0004	86.48 ± 0.0003	86.45 ± 0.0084
	30°C/65% RH	86.09 ± 0.0001	86.06 ± 0.0002	86.03 ± 0.0004	86.00 ± 0.0014
	25°C/60% RH	86.47 ± 0.0002	86.43 ± 0.0002	86.41 ± 0.0002	86.39 ± 0.0012
N-MH	40°C/75% RH	82.54 ± 0.0002	82.47 ± 0.0028	82.45 ± 0.0004	82.41 ± 0.0001
	30°C/65% RH	83.77 ± 0.0035	83.56 ± 0.0002	83.42 ± 0.0002	83.28 ± 0.0003
	25°C/60% RH	85.75 ± 0.0001	85.64 ± 0.0004	85.61 ± 0.0001	85.58 ± 0.0002
N-CS	40°C/75% RH	77.48 ± 0.0001	77.37 ± 0.0019	77.28 ± 0.0001	77.20 ± 0.0008
	30°C/65% RH	79.32 ± 0.0002	79.17 ± 0.0004	79.07 ± 0.0002	79.01 ± 0.0006
	25°C/60% RH	80.45 ± 0.0001	80.32 ± 0.0004	80.28 ± 0.0001	80.25 ± 0.0005
N-AH	40°C/75% RH	82.18 ± 0.0002	81.79 ± 0.0012	81.54 ± 0.0001	81.39 ± 0.0006
	30°C/65% RH	84.41 ± 0.0003	84.10 ± 0.0001	83.86 ± 0.0002	83.65 ± 0.0003
	25°C/60% RH	86.12 ± 0.0011	85.72 ± 0.0003	85.66 ± 0.0002	85.61 ± 0.0001

E.3.6 Droplet size and distribution

Tables E.11 to E.14 indicated the average droplet size and Pdl at monthly intervals over a period of three months. The Pdl is an indication of droplet distribution (Shah *et al.*, 2014:65); whereas a value nearing 1 indicates polydispersed droplets (Gaur *et al.*, 2014:36). From Tables E.12 and E.14, it is evident the samples analysed fluctuated significantly every month, with droplets in the emulgels ranging from monodispersed to very polydispersed. Therefore, the droplet sizes also varied vastly each month. It is possible that droplet aggregation occurred, or large sediments were present in the sample, which resulted in fluctuating results.

Table E.11: The average droplet size (d.nm) of liposomal emulgels

Emulgel	Storage condition	Initial (T0)	Month 1 (T1)	Month 2 (T2)	Month 3 (T3)
L-PL	40°C/75% RH	4418.73 ± 1124.66	3055.03 ± 2392.01	5444.60 ± 62.21	1754.57 ± 2773.74
	30°C/65% RH	4418.73 ± 1124.66	4293.33 ± 5336.10	158.51 ± 15.65	138.49 ± 58.54
	25°C/60% RH	4418.73 ± 1124.66	4802.53 ± 627.71	1823.30 ± 2889.67	1523.40 ± 2429.91
L-MH	40°C/75% RH	4962.23 ± 64.28	4079.11 ± 461.30	2791.79 ± 1900.21	1399.20 ± 1219.09
	30°C/65% RH	4962.23 ± 64.28	4958.07 ± 234.74	358.60 ± 164.73	3161.49 ± 930.22
	25°C/60% RH	4962.23 ± 64.28	4668.27 ± 110.15	3315.41 ± 1214.12	578.19 ± 226.74
L-CS	40°C/75% RH	186.20 ± 12.33	2620.70 ± 2975.06	719.55 ± 285.59	1232.53 ± 1675.34
	30°C/65% RH	186.20 ± 12.33	497.39 ± 314.08	3539.00 ± 2619.56	4896.80 ± 633.02
	25°C/60% RH	186.20 ± 12.33	887.29 ± 157.16	3263.33 ± 488.08	787.49 ± 2092.53
L-AH	40°C/75% RH	4855.53 ± 207.85	4581.81 ± 696.43	714.67 ± 208.21	224.57 ± 18.07
	30°C/65% RH	4855.53 ± 207.85	5911.13 ± 454.15	5112.83 ± 254.39	2935.59 ± 416.07
	25°C/60% RH	4855.53 ± 207.85	1867.46 ± 562.26	294.73 ± 39.00	145.39 ± 39.64

Table E.12: The average Pdl of liposomal emulgels

Emulgel	Storage condition	Initial (T0)	Month 1 (T1)	Month 2 (T2)	Month 3 (T3)
L-PL	40°C/75% RH	0.258 ± 0.01	0.285 ± 0.03	0.444 ± 0.06	0.191 ± 0.01
	30°C/65% RH	0.258 ± 0.01	0.345 ± 0.00	0.157 ± 0.01	0.143 ± 0.02
	25°C/60% RH	0.258 ± 0.01	0.374 ± 0.07	0.173 ± 0.01	0.278 ± 0.01
L-MH	40°C/75% RH	0.222 ± 0.01	1.000 ± 0.00	0.339 ± 0.02	0.362 ± 0.04
	30°C/65% RH	0.222 ± 0.01	0.686 ± 0.07	0.460 ± 0.01	0.343 ± 0.01
	25°C/60% RH	0.222 ± 0.01	0.694 ± 0.01	0.283 ± 0.03	0.648 ± 0.30
L-CS	40°C/75% RH	0.208 ± 0.01	0.847 ± 0.13	0.439 ± 0.02	0.469 ± 0.02
	30°C/65% RH	0.208 ± 0.01	0.797 ± 0.18	0.458 ± 0.10	0.301 ± 0.16
	25°C/60% RH	0.208 ± 0.01	0.432 ± 0.03	0.334 ± 0.14	0.570 ± 0.13
L-AH	40°C/75% RH	0.267 ± 0.02	0.778 ± 0.04	0.593 ± 0.07	0.210 ± 0.02
	30°C/65% RH	0.267 ± 0.02	0.648 ± 0.09	0.266 ± 0.03	0.365 ± 0.02
	25°C/60% RH	0.267 ± 0.02	0.818 ± 0.06	0.281 ± 0.06	0.415 ± 0.03

Table E.13: The average droplet size (d.nm) of niosomal emulgels

Emulgel	Storage condition	Initial (T0)	Month 1 (T1)	Month 2 (T2)	Month 3 (T3)
N-PL	40°C/75% RH	783.11 ± 35.09	4418.99 ± 2204.26	2818.70 ± 1850.86	3094.99 ± 2382.05
	30°C/65% RH	783.11 ± 35.09	1246.63 ± 459.38	3220.73 ± 1470.17	1676.71 ± 961.40
	25°C/60% RH	783.11 ± 35.09	995.33 ± 59.36	5703.95 ± 601.33	1507.37 ± 673.67
N-MH	40°C/75% RH	588.46 ± 183.15	3156.16 ± 1897.56	2835.43 ± 2357.16	1511.67 ± 2391.21
	30°C/65% RH	588.46 ± 183.15	4453.82 ± 2897.01	1628.83 ± 2539.84	2917.55 ± 2466.58
	25°C/60% RH	588.46 ± 183.15	4188.73 ± 904.85	1633.33 ± 2535.00	3382.53 ± 2817.73
N-CS	40°C/75% RH	1291.75 ± 758.48	5344.84 ± 718.54	3052.26 ± 2546.46	4158.18 ± 2393.32
	30°C/65% RH	1291.75 ± 758.48	5271.43 ± 558.68	1528.20 ± 2441.17	114.10 ± 1.80
	25°C/60% RH	1291.75 ± 758.48	5408.38 ± 345.04	1635.63 ± 2667.36	1593.23 ± 2524.44
N-AH	40°C/75% RH	235.01 ± 171.41	488.10 ± 269.48	1345.91 ± 2182.92	3426.33 ± 1426.33
	30°C/65% RH	235.01 ± 171.41	5442.88 ± 750.50	2670.45 ± 2222.22	82.60 ± 4.52
	25°C/60% RH	235.01 ± 171.41	2441.24 ± 2387.58	1555.51 ± 2520.84	82.92 ± 3.09

Table E.14: The average Pdl of niosomal emulgels

Emulgel	Storage condition	Initial (T0)	Month 1 (T1)	Month 2 (T2)	Month 3 (T3)
N-PL	40°C/75% RH	0.968 ± 0.03	0.914 ± 0.05	0.372 ± 0.14	0.326 ± 0.02
	30°C/65% RH	0.968 ± 0.03	0.798 ± 0.05	0.336 ± 0.01	0.749 ± 0.19
	25°C/60% RH	0.968 ± 0.03	0.882 ± 0.04	0.576 ± 0.16	0.599 ± 0.17
N-MH	40°C/75% RH	0.765 ± 0.04	0.601 ± 0.08	0.253 ± 0.01	0.254 ± 0.01
	30°C/65% RH	0.765 ± 0.04	0.650 ± 0.11	0.236 ± 0.03	0.209 ± 0.03
	25°C/60% RH	0.765 ± 0.04	0.346 ± 0.05	0.255 ± 0.04	0.347 ± 0.08
N-CS	40°C/75% RH	0.804 ± 0.07	0.428 ± 0.03	0.263 ± 0.02	0.227 ± 0.01
	30°C/65% RH	0.804 ± 0.07	0.747 ± 0.03	0.255 ± 0.01	0.120 ± 0.01
	25°C/60% RH	0.804 ± 0.07	0.498 ± 0.04	0.207 ± 0.02	0.259 ± 0.01
N-AH	40°C/75% RH	0.916 ± 0.15	0.805 ± 0.09	0.212 ± 0.03	0.240 ± 0.01
	30°C/65% RH	0.916 ± 0.15	0.689 ± 0.06	0.272 ± 0.01	0.134 ± 0.02
	25°C/60% RH	0.916 ± 0.15	0.398 ± 0.05	0.201 ± 0.02	0.156 ± 0.01

E.3.7 Physical assessment of samples

The physical and visual observation results, including colour change, smell and feel, were reported in Tables E.15 to E.22 for the different liposomal emulgels and Tables E.23 to E.30 for the different niosomal emulgels. The high temperature and humidity influenced the emulgels, affecting the colour and skin feel over the three months period. The viscosity of the emulgels was also influenced by these accelerated test conditions, due to possible condensation or changes in chemical structures.

The liposomal emulgels revealed the biggest physical change, which could be attributed to the PC included in the formulations. High temperatures resulted in a colour change due to possible oxidation, and the distinctive PC odour also diminished over the 30-day period. The **L-MH** emulgel was prone to signs of micro-bacterial contamination over the three-month testing period, especially for the samples stored at 25°C/60% RH and 30°C/65% RH. The skin feel of most liposomal and niosomal emulgels containing API went from a thick jelly-like gel to a more spreadable emulgel, possibly due to disrupted Van der Waals forces. Despite an uneven appearance of some liposomal and niosomal emulgels, the skin feel remained smooth and wet corresponding with the hydrogel base. The niosomal emulgels did not result in any major colour or odour change, which could be attributed to the more stable ingredients used for the niosome preparation. With regards to physical observation, the niosomal emulgels appeared more stable than their liposomal counterparts.

Table E.15: Physical assessment results of the placebo liposomal emulgel

		T0 (Initial)	T1	T2	T3
40°C/75% RH	Colour	Light yellow	Slightly darker yellow	No change from T1	No change from T2
	Skin feel	Smooth but sticky	Smooth but less sticky	Smooth and thin	No change from T2
	Smell	Distinctive smell	Distinctive smell less notable	No distinctive smell	No change from T2
30°C/65% RH	Colour	Light yellow	No change from T0	No change from T1	Slightly darker yellow
	Skin feel	Smooth but sticky	Smooth and soft	No change from T1	Smooth and thin
	Smell	Distinctive smell	Distinctive smell less notable	No distinctive smell	No change from T2
25°C/60% RH	Colour	Light yellow	No change from T0	No change from T1	Slightly darker yellow
	Skin feel	Smooth but sticky	Smooth and thin	No change from T1	No change from T2
	Smell	Distinctive smell	Distinctive smell less notable	No distinctive smell	No change from T2

Table E.16: Visual results of the placebo liposomal emulgel

	T0	T1	T2	T3
40°C/75% RH				
30°C/65% RH				
25°C/60% RH				

Table E.17: Physical assessment results of the liposomal emulgel containing **MH**

		T0 (Initial)	T1	T2	T3
40°C/75% RH	Colour	Light yellow	Slightly darker yellow	Slightly darker yellow	No change from T2
	Skin feel	Smooth but sticky	No change from T0	No change from T1	No change from T2
	Smell	Distinctive smell	Distinctive smell less notable	No change from T1	No distinctive smell
30°C/65% RH	Colour	Light yellow	Slightly lighter yellow	No change from T1	Slightly lighter yellow
	Skin feel	Smooth but sticky	Thick and uneven	No change from T1	No change from T2
	Smell	Distinctive smell	Distinctive smell less notable	No change from T1	No distinctive smell
25°C/60% RH	Colour	Light yellow	Slightly lighter yellow	Slightly lighter yellow	No change from T2
	Skin feel	Smooth but sticky	No change from T0	Thick and uneven	No change from T2
	Smell	Distinctive smell	Distinctive smell less notable	No change from T1	No distinctive smell

Table E.18: Visual results of the liposomal emulgel containing **MH**

	T0	T1	T2	T3
40°C/75% RH				
30°C/65% RH				
25°C/60% RH				

Table E.19: Physical assessment results of the liposomal emulgel containing **CS**

		T0 (Initial)	T1	T2	T3
40°C/75% RH	Colour	Light yellow	Darker yellow	No change from T1	Slightly darker yellow
	Skin feel	Smooth but sticky	Thick and sticky	No change from T1	Thick and uneven
	Smell	Distinctive smell	Distinctive smell less notable	No change from T1	No distinctive smell
30°C/65% RH	Colour	Light yellow	No change from T0	No change from T1	No change from T2
	Skin feel	Smooth but sticky	Thick and sticky	No change from T1	Thick and uneven
	Smell	Distinctive smell	Distinctive smell less notable	No change from T1	No distinctive smell
25°C/60% RH	Colour	Light yellow	No change from T0	No change from T1	No change from T2
	Skin feel	Smooth but sticky	Thick and sticky	No change from T1	Thick and uneven
	Smell	Distinctive smell	Distinctive smell less notable	No change from T1	No distinctive smell

Table E.20: Visual results of the liposomal emulgel containing **CS**

	T0	T1	T2	T3
40°C/75% RH				
30°C/65% RH				
25°C/60% RH				

Table E.21: Physical assessment results of the liposomal emulgel containing **AH**

		T0 (Initial)	T1	T2	T3
40°C/75% RH	Colour	Light yellow	Darker yellow	No change from T1	No change from T2
	Skin feel	Smooth but sticky	No change from T0	No change from T1	No change from T2
	Smell	Distinctive smell	Distinctive smell less notable	No change from T1	No distinctive smell
30°C/65% RH	Colour	Light yellow	Slightly darker yellow	No change from T1	No change from T2
	Skin feel	Smooth but sticky	No change from T0	No change from T1	No change from T2
	Smell	Distinctive smell	Distinctive smell less notable	No change from T1	No distinctive smell
25°C/60% RH	Colour	Light yellow	No change from T0	No change from T1	No change from T2
	Skin feel	Smooth but sticky	No change from T0	No change from T1	Sticky and uneven
	Smell	Distinctive smell	Distinctive smell less notable	No change from T1	No distinctive smell

Table E.22: Visual results of the liposomal emulgel containing **AH**

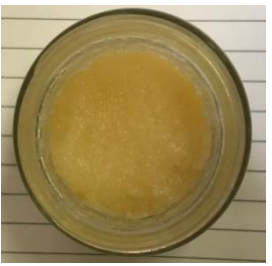
	T0	T1	T2	T3
40°C/75% RH				
30°C/65% RH				
25°C/60% RH				

Table E.23: Physical assessment results of the placebo niosomal emulgel

		T0 (Initial)	T1	T2	T3
40°C/75% RH	Colour	White	No change from T0	No change from T1	No change from T2
	Skin feel	Smooth and thin	No change from T0	No change from T1	No change from T2
	Smell	No distinctive smell	No change from T0	No change from T1	No change from T2
30°C/65% RH	Colour	White	No change from T0	No change from T1	No change from T2
	Skin feel	Smooth and thin	No change from T0	No change from T1	No change from T2
	Smell	No distinctive smell	No change from T0	No change from T1	No change from T2
25°C/60% RH	Colour	White	No change from T0	No change from T1	No change from T2
	Skin feel	Smooth and thin	No change from T0	No change from T1	No change from T2
	Smell	No distinctive smell	No change from T0	No change from T1	No change from T2

Table E.24: Visual results of the placebo niosomal emulgel

	T0	T1	T2	T3
40°C/75% RH				
30°C/65% RH				
25°C/60% RH				

Table E.25: Physical assessment results of the niosomal emulgel containing **MH**

		T0 (Initial)	T1	T2	T3
40°C/75% RH	Colour	White	No change from T0	No change from T1	No change from T2
	Skin feel	Smooth and soft	No change from T0	No change from T1	Soft but uneven
	Smell	No distinctive smell	No change from T0	No change from T1	No change from T2
30°C/65% RH	Colour	White	No change from T0	No change from T1	No change from T2
	Skin feel	Smooth and soft	No change from T0	No change from T1	No change from T2
	Smell	No distinctive smell	No change from T0	No change from T1	No change from T2
25°C/60% RH	Colour	White	No change from T0	No change from T1	No change from T2
	Skin feel	Smooth and soft	No change from T0	No change from T1	No change from T2
	Smell	No distinctive smell	No change from T0	No change from T1	No change from T2

Table E.26: Visual results of the niosomal emulgel containing **MH**

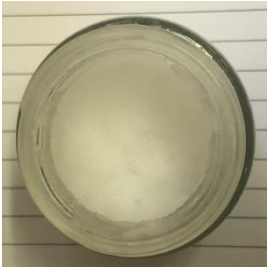
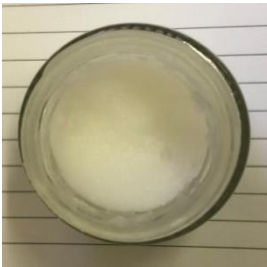
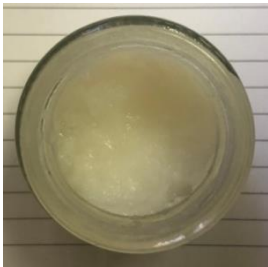
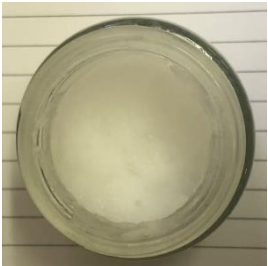
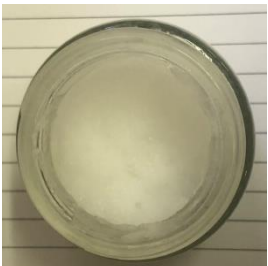
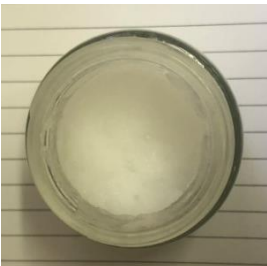
	T0	T1	T2	T3
40°C/75% RH				
30°C/65% RH				
25°C/60% RH				

Table E.27: Physical assessment results of the niosomal emulgel containing **CS**

		T0 (Initial)	T1	T2	T3
40°C/75% RH	Colour	White	No change from T0	No change from T1	Slightly darker
	Skin feel	Smooth and soft	No change from T0	No change from T1	Soft but uneven
	Smell	No distinctive smell	No change from T0	No change from T1	No change from T2
30°C/65% RH	Colour	White	No change from T0	No change from T1	No change from T2
	Skin feel	Smooth and soft	No change from T0	No change from T1	No change from T2
	Smell	No distinctive smell	No change from T0	No change from T1	No change from T2
25°C/60% RH	Colour	White	No change from T0	No change from T1	No change from T2
	Skin feel	Smooth and soft	No change from T0	No change from T1	No change from T2
	Smell	No distinctive smell	No change from T0	No change from T1	No change from T2

Table E.28: Visual results of the niosomal emulgel containing **CS**

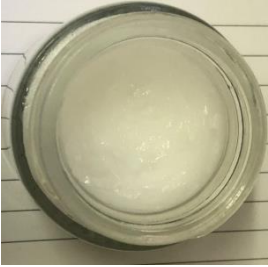
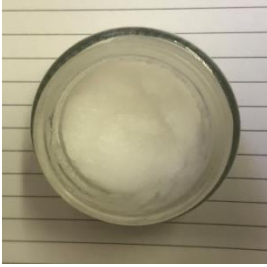
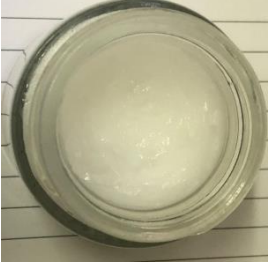
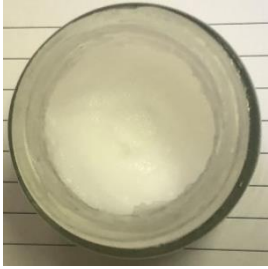
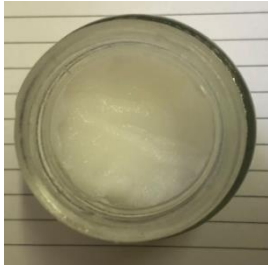
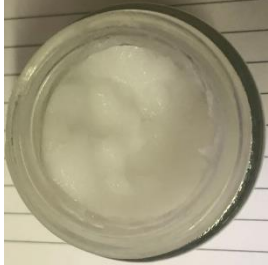
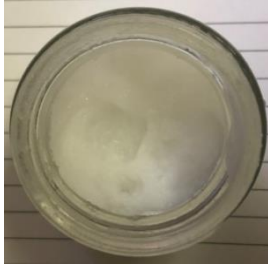
	T0	T1	T2	T3
40°C/75% RH				
30°C/65% RH				
25°C/60% RH				

Table E.29: Physical assessment results of the niosomal emulgel containing **AH**

		T0 (Initial)	T1	T2	T3
40°C/75% RH	Colour	White	No change from T0	No change from T1	Slightly darker
	Skin feel	Smooth and soft	No change from T0	No change from T1	Soft but uneven
	Smell	No distinctive smell	No change from T0	No change from T1	No change from T2
30°C/65% RH	Colour	White	No change from T0	No change from T1	No change from T2
	Skin feel	Smooth and soft	No change from T0	No change from T1	No change from T2
	Smell	No distinctive smell	No change from T0	No change from T1	No change from T2
25°C/60% RH	Colour	White	No change from T0	No change from T1	No change from T2
	Skin feel	Smooth and soft	No change from T0	No change from T1	No change from T2
	Smell	No distinctive smell	No change from T0	No change from T1	No change from T2

Table E.30: Visual results of the niosomal emulgel containing **AH**

	T0	T1	T2	T3
40°C/75% RH				
30°C/65% RH				
25°C/60% RH				

E.4 Conclusion

The formulated liposomal and niosomal emulgels containing different solid-state forms of roxithromycin underwent a three months accelerated stability test at different storage conditions, as endorsed by the ICH and SAHPRA, to determine product stability. Some degradation pathways can be very complex and pharmaceutical products exposed to forced conditions may lead to the observation of unlikely decomposition products, which would not occur under normal circumstances. However, information on these unlikely decomposition products can be very useful to predict real-time degradation, ensuring the development and validation of suitable analytical methods.

The following assessments were performed monthly, concentration assay of the API, pH, viscosity, zeta-potential, mass loss, droplet size and visual appearance. As seen from the results discussed, the emulgels did not result in satisfactory stability. All assessments resulted in significant changes and possible degradation, which proved unsatisfactory. The only exception for both formulations was the assessment of mass loss that revealed excellent stability. The latter indicated that the choice of container was successful in limiting moisture absorption and evaporation. The only improvement to be considered with regard to the storage

container was to store the liposomal emulgels in amber jars, due to possible oxidation leading to colour change.

When considering all the stability parameters, the two vesicle emulgels did not meet the acceptance criteria and is therefore not suitable for manufacturing without improvement. Possible improvements to consider is to include preservatives in the emulgel formulations or to reconsider the gelling agent, since the OH-groups of the hydrogel base together with the OH-groups of the API have an immense effect on viscosity and stability of the final products.

References

- Bajaj, S., Singla, D. & Sakhuja, N. 2012. Stability Testing of Pharmaceutical Products. *Journal of applied pharmaceutical science*, 2:129-138.
- Barnes, A.R. 2007. Product stability and stability testing. (*In* Aulton, M.E., ed. *Aulton's pharmaceuticals: the design and manufacture of medicines*. 3rd ed. Philadelphia: Elsevier, p.650-665).
- Bhatt, P. & Madhav, S. 2011. A detailed review on nanoemulsion drug delivery system. *International journal of pharmaceutical science and research*, 2:2482–2489.
- Douglas, J.F. 2018. Weak and strong gels and the emergence of the amorphous solid state. *Gels*: 4:1-14.
- Guar, S., Garg, A., Yadav, D., Beg, M. & Gaur, K. 2014. Nano–emulsion gel as novel oil based colloidal nanocarrier for topical delivery of bifonazole. *Indian research journal of pharmacy and science*, 1:36–54.
- Hach Company. 2018. What is pH and how is it measured? www.hach.com/asset-get.download.jsa?id=7639984488 Date of access: 5 Jun. 2019.
- ICH **see** International conference on harmonisation of technical requirements for registration of pharmaceuticals for human use
- International conference on harmonisation of technical requirements for registration of pharmaceuticals for human use. 2003. ICH harmonised tripartite guideline: Stability testing of new drug substances and products Q1A(R2). <http://www.ich.org/products/guidelines/quality/article/quality-guidelines.html> Date of access: 5 Jun. 2019.
- Klang, V., Schwarz, J.C. & Valenta, C. 2015. Nano–emulsions in dermal drug delivery. (*In* Dragicevic–Curic, N. & Maibach, H.I., eds. *Percutaneous penetration enhancers: chemical methods in penetration enhancement: drug manipulation strategies and vehicle effects*. Heidelberg: Springer, p.255–266).
- Malvern Instruments LTD. 2015. Zetasizer nano series technical note. <http://www3.nd.edu/~rroeder/ame60647/slides/zeta.pdf> Date of access: 5 Jun. 2019.
- Marianecchi, C., Di Marzio, L., Rinaldi, F., Esposito, S. & Carafa, M. 2013. Niosomes. (*In* Uchegbu, I.F., Schätzlein, A.G., Ping Cheng, W. & Lalatsa, A., eds. *Fundamentals of pharmaceutical nanoscience*. New York: Springer. p. 65-90).

MCC **see** Medicines control council

Medicines control council. 2012. Stability: Registration of medicines. https://www.sahpra.org.za/documents/b22b99e32.05_Stability_Jul12_v7_1 Date of access: 5 Jun. 2019.

Naik, A., Kalia, Y.N. & Guy, R.H. 2000. Transdermal drug delivery: overcoming the skin's barrier function. *Pharmaceutical science and technology today*, 3:318-325.

Roland, I., Piel, G., Delattre, L. & Evrad, B. 2003. Systematic characterization of oil-in-water emulsions for formulation design. *International journal of pharmaceutics*, 263:85-94.

Shah, R., Eldridge, D., Palombo, E. & Harding, I. 2014. Optimisation and stability assessment of solid lipid nanoparticles using particle size and zeta potential. *Journal of physical sciences*, 25:59–75.

Silva, H.D., Cerqueira, M.A. & Vicente, A.A. 2012. Nano-emulsions for food applications: development and characterization. *Food and bioprocess technology*, 5:854–867.

Williams, A.C. 2003. Structure and function of human skin. (*In Williams, A.C., ed. Transdermal and topical drug delivery: from theory to clinical practice. London: Pharmaceutical Press*).

Zatz, J.L., Berry, J.J. & Alderman, D.A. 1989. Viscosity-impairing agents in disperse systems. (*In Lieberman, H.A., Rieger, M.M. & Banker, G.S., eds. Pharmaceutical dosage forms: disperse systems. Vol.2. New York: Marcel Dekker, p. 171-203*).

APPENDIX F

DIFFUSION STUDIES OF ROXITHROMYCIN ENCAPSULATED IN VESICLES AND FORMULATED IN AN EMULGEL FOR TOPICAL DELIVERY

F.1 Introduction

The use of *in vitro* skin diffusion studies to assess skin permeability has evolved into an unparalleled research methodology, since transdermal drug delivery is advantageous in many ways when compared to other routes of drug administration. Not only can diffusion studies provide in depth comprehension into the relationship between drug, formulation and skin interaction; but it is also valuable for quality control purposes of novel formulations (Ng *et al.*, 2010:210). A topically applied antibiotic, such as roxithromycin, can decrease inflammation caused by bacteria accumulating in the pilosebaceous glands, located in the dermis, which causes to acne (Williams *et al.*, 2012:164). Franz-type diffusion studies are used to measure the success of transdermal delivery of an API. Synthetic membranes or biological skin are usually employed to perform Franz-type diffusion studies; for this study membrane studies were performed using a synthetic polyvinylidene fluoride (PVDF) membrane to determine if the encapsulated roxithromycin was being released from the vesicles formulated into an emulgel. This was followed by biological skin diffusion studies and tape stripping to determine the success of API delivery through and into the skin, respectively.

F.2 Methods

F.2.1 HPLC analysis of the roxithromycin concentration

To determine the amount of API diffused, the verified HPLC method as discussed in Appendix A was applied. The receptor phase, skin and tape strip samples were all analysed with the use of the validated and verified HPLC method. A Venusil XBP C₁₈ reverse phase 150 x 4.6 mm column with a 5 µm particle size (Agela Technologies, Germany) was used together with an Agilent® 1100 Series HPLC system (Agilent Technologies, California). A 0.01 M NH₄H₂PO₄ buffer solution mixed with methanol in the ratio 15:85 served as mobile phase (pH 7). The mobile phase had a flow rate of 1 ml/min, and used an injection volume of 50 µl, with a set run time of 9 min and a detection wavelength of 205 nm. A standard curve was determined before sample analysis from a standard solution that was prepared by dissolving 20 mg of API in a 100 ml volumetric flask, which contained methanol. For the skin studies, the standard solution was diluted by transferring 10 ml of the solution into a 100 ml volumetric flask and making it up to volume with methanol.

F.2.2 *In vitro* diffusion studies: vertical Franz cell method

Any *in vitro* study should possess similar or identical conditions to *in vivo* study conditions, with closely monitored temperatures to ensure uninterrupted diffusion and subsequent results (Modi & Shah, 2015:1; Shahzad *et al.*, 2015:2). The vertical Franz cell method (Wiechers, 2008:24) was employed to perform *in vitro* release and diffusion studies to determine the release (membrane) or transdermal diffusion (skin) of roxithromycin solid-state forms from the vesicle transport systems formulated into an emulgel.

F.2.2.1 Vertical Franz cell components

The vertical Franz cell method is an effective *in vitro* study method to determine skin penetration; this method requires a synthetic membrane or skin sample to be positioned between a donor and receptor phase (Williams, 2013:683). The vertical Franz cell diffusion method was used for membrane release studies, as well as skin diffusion studies as described in Sections F.2.2.4 and F.2.2.5. The components of a vertical Franz cell are illustrated in Figure F.1.

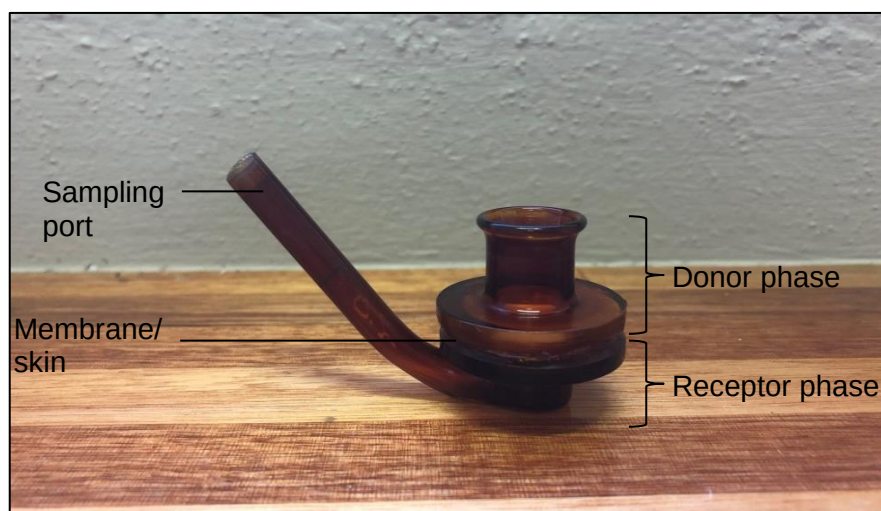


Figure F.1: Different components of a vertical Franz diffusion cell

F.2.2.2 Preparation of the receptor phase

A 0.06 M phosphate buffer solution (PBS) was used as the receptor phase. The mixture was prepared by mixing 6.8 g monopotassium phosphate (KH_2PO_4) in 250 ml Milli-Q[®] water with 1.5 g sodium hydroxide (NaOH) in 400 ml Milli-Q[®] water, made up to 1000 ml. Since human blood presents with a pH of 7.4 *in vivo*, it consequently determined the pH of the PBS for the *in vitro* skin diffusion studies (OECD, 2011:29). Before every experiment the receptor phase was pre-heated to $37 \pm 3^\circ\text{C}$ in a water bath, equivalent to human body temperature (Williams, 2013:68).

F.2.2.3 Preparation of the donor phase

The emulgel containing the liposome or niosome vesicle system encapsulating the different solid-state forms of roxithromycin, as described in Appendix D, was used as the donor phase. A placebo (vesicles containing no API) was used as the control group during the studies. Ten Franz cells were used, each containing approximately 1 ml of the formulation, while two Franz cells contained 1 ml of the placebo. The prepared samples were placed in a water bath at $32 \pm 3^\circ\text{C}$, equivalent to human skin surface temperature, 1 h before the experiment (Williams, 2013:685).

F.2.2.4 Membrane release studies method

A membrane release study took place as follows:

- The PBS, which served as the receptor phase, was placed in a water bath at $37 \pm 3^\circ\text{C}$, 1 h before the study to accurately represent the temperature of the blood in the human body.
- The formulation, serving as the donor phase, was placed in another water bath at $32 \pm 3^\circ\text{C}$, 1 h before the study to accurately represent the temperature on the surface of the skin.
- A thin layer of Dow Corning® high vacuum grease (Sigma-Aldrich, Germany) was applied to both the donor and receptor compartments.
- A small magnetic stirring rod was placed into the receptor compartments before placing a PVDF (Pall® Life Sciences, United States of America) synthetic membrane with a pore size of $0.45 \mu\text{m}$ and 25 mm diameter between the receptor and the donor compartments (Figure F.2.a).
- The sides of the Franz cells were sealed with vacuum grease to prevent leakage, and fastened with a horseshoe clamp (Figure F.2.b).
- The receptor compartment was filled with 2 ml of heated PBS (pH 7.4) using a syringe and needle with a small tube attached to it.
- When filled, it was visually inspected to ensure no air bubbles formed, and the PBS level was marked to notice any possible leakage or change of PBS level.
- Each donor compartment was filled with 1 ml of the API vesicle preparation, and then covered with a piece of Parafilm® and a plastic cap to prevent any loss of the formulation during the experiment.

- The Franz cell stand with all the assembled Franz cells were placed in a Grant® water bath (Grant Instruments, United Kingdom) at a constant temperature of $37 \pm 3^\circ\text{C}$ on a Variomag® (United States of America) magnetic stirring plate (Figure F.2.c).
- The entire receptor phase of every cell was extracted through the sampling port and refilled with fresh, preheated PBS (pH 7.4, 37°C) hourly, for 6 h. Before the extraction took place, any change in the level of the PBS was noted.
- Each Franz cell had its own marked syringe with a needle and tube to extract the PBS. The extracted contents of the receptor compartments were then placed into corresponding HPLC vials (Figure F.2.d) and analysed by means of HPLC, as discussed in Section F.2.1.

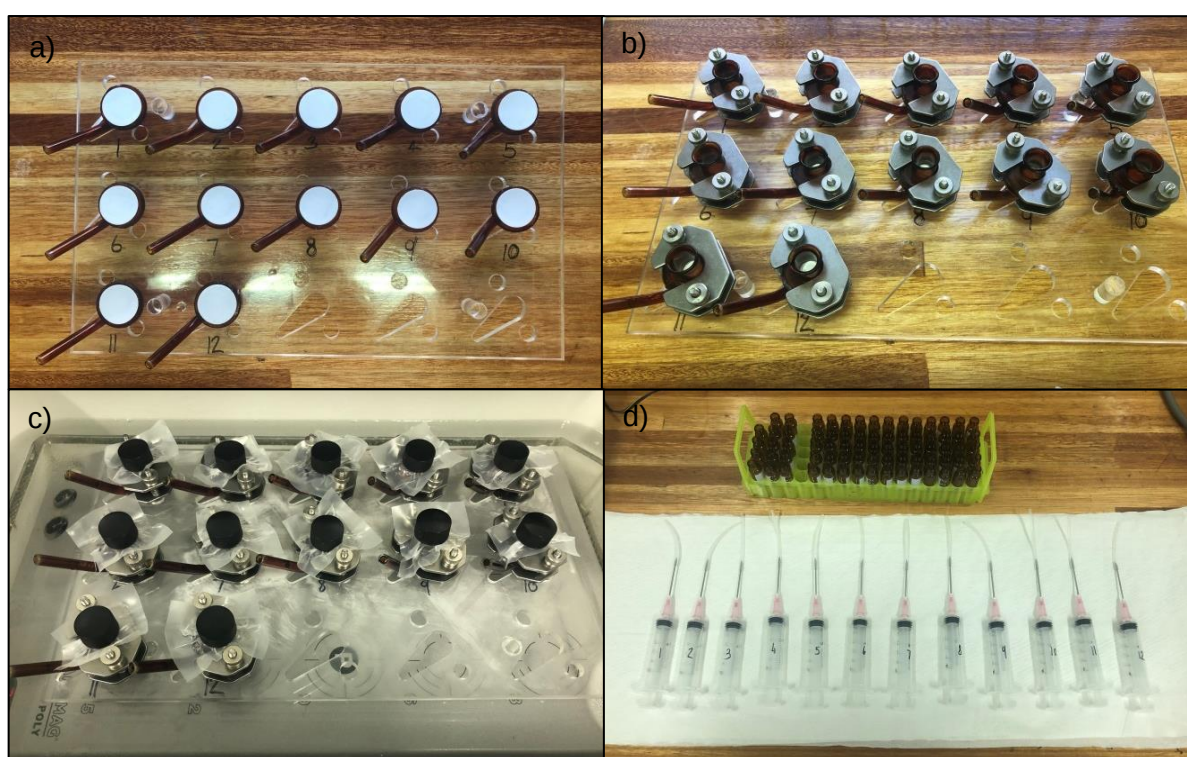


Figure F.2: Diffusion study method: a) lightly greased receptor compartments of Franz cells with PVDF membranes; b) horseshoe clamp used to fasten Franz cell compartments; c) vertical Franz cells fully assembled and placed on a magnetic stirrer plate in the water bath and d) syringes with needles and tubes attached used for extraction of the receptor phase and marked HPLC vials

F.2.2.5 *In vitro* skin diffusion studies methods

F.2.2.5.1 Skin ethics and collection

Skin obtained from abdominoplasty of Caucasian females was used for the diffusion studies. Willing patients completed an informed consent form with a corresponding code assigned to the skin to ensure confidentiality. The skin was collected from donor clinics and hospitals, and transported in a cooled icebox, whereafter it was frozen in a designated freezer at - 20°C in the Transdermal laboratory, North-West University, Potchefstroom Campus. Approval for the study was awarded by the North-West University Health Research Ethics Committee (NWU-HREC) of the Faculty of Health Sciences (ethics number: NWU-00111-17-A1-02).

F.2.2.5.2 Preparation of the skin

After defrosting the skin, it was trimmed of all excess fat. The skin was stretched and secured over a polystyrene block with the stratum corneum facing upwards. The prepared skin was wiped clean with a paper towel, next a Zimmer™ electric dermatome (model 8821, United Kingdom) (Figure F.3.a) was used to acquire strips of dermatomed skin (about 400 µm). The strips were placed onto Whatman® filter paper and allowed to dry (Figure F.3.b). The skin was examined visually for any defects before cutting circles (Figure F.3.c). The skin circles were wrapped in aluminium foil and stored in a Ziploc® bag at - 20°C until needed. Due to the limited amount of skin per donor, different donors had to be used for every separate study. Skin samples were disposed of after six months. All waste was disposed of according to the approved standard operating procedures (SOPs) of the laboratory (Ethics number: NWU-00369-16-A1), which included the following SOPs: biological waste disposal (Pharmacn_SOP001_v02) and chemical, toxic and pharmaceutical waste disposal (Pharmacn_SOP002_v02).

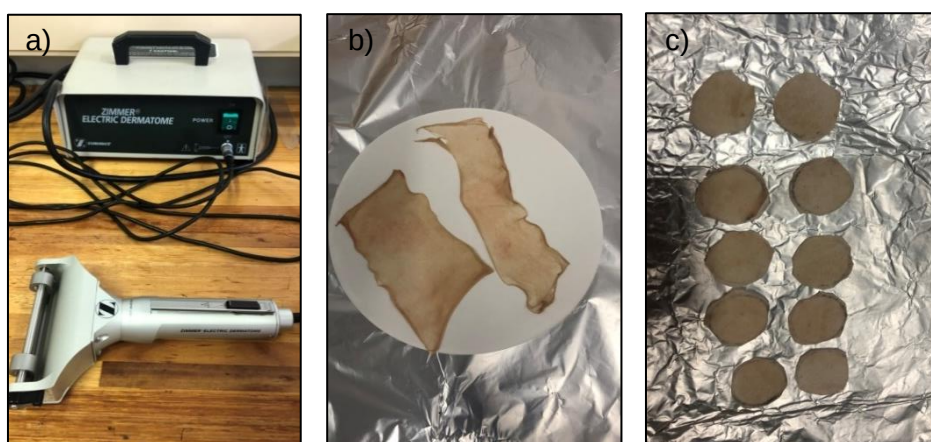


Figure F.3: Method of skin preparation: a) The Zimmer™ electric dermatome, b) dermatomed skin and c) prepared skin circles

F.2.2.5.3 Transdermal diffusion studies method

The same method as discussed in Section F.2.2.4 was used, with the following exceptions:

- Prepared skin samples were placed between the receptor and donor phase of the Franz cell, with the stratum corneum facing upwards, instead of the PVDF membranes. The skin samples were analysed after tape-stripping (as discussed in Section F.2.2.5.4).
- Only one extraction of the PBS in the receptor phase was made at 12 h, instead of 6 hourly. The PBS was analysed after extraction.

F.2.2.5.4 Tape stripping method

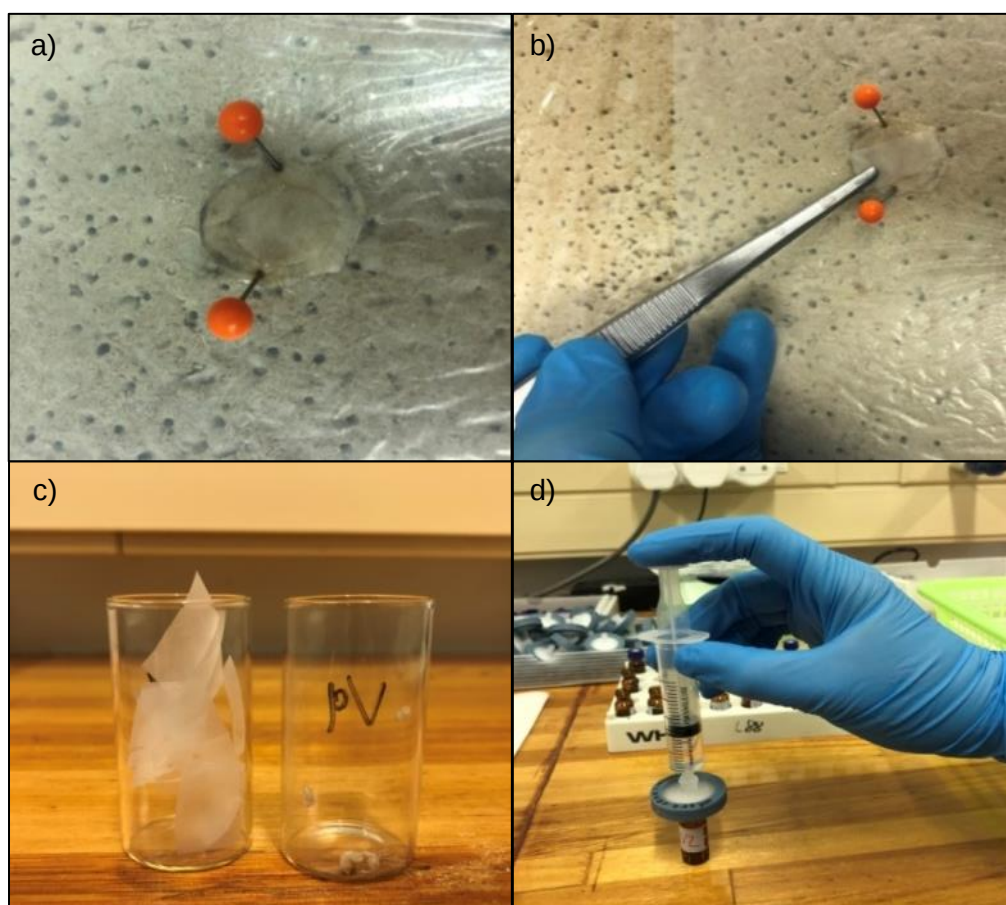


Figure F.4: Skin diffusion study: a) skin sample with the diffusion area visible, b) tape stripping, c) polytops containing tape strips (SCE) and skin pieces (ED) of a single Franz cell before adding methanol and, d) extracted methanol being filtered into a HPLC vial after 8 h

Tape stripping took place as follows:

- After the extraction of the receptor phase, the Franz cells were carefully disassembled. The skin circles were pinned on hardboard covered with glad wrap and Parafilm®.

- The visible diffusion area was dabbed with tissue paper to remove any excess donor phase (Figure F.4.a).
- Sixteen pieces of 3 M Scotch® Magic™ tape were used per skin sample to remove the stratum corneum-epidermis (SCE). This was done by placing the tape on the diffusion area and removing it and a gleaming appearance of the skin was an indication of complete removal. The first tape was discarded to ensure all superficial donor phase was removed (Figure F.4.b).
- The remaining 15 strips were placed in separate marked polytops containing 5 ml HPLC grade methanol after dabbing it on the skin to remove the SCE.
- Next the diffusion area of the remaining skin (epidermis-dermis (ED)) was cut into smaller pieces and placed in separate marked polytops (Figure F.4.c); 5 ml HPLC grade methanol was added to every polytop.
- The polytops containing both the skin (ED) and tapes (SCE) were placed in a refrigerator (2 – 5°C) for at least 8 h to give the API an opportunity to be extracted from the samples.
- The methanol was removed and filtered into marked HPLC vials for analysis (Figure F.4.d).

F.3 Physicochemical properties of roxithromycin solid-state forms

F.3.1 Aqueous solubility

Csongradi (2015:177-178) experimentally determined the solubility of **MH** in PBS (pH 7.4) as 1.98 mg/ml at 32°C, since the receptor phase for diffusion studies consists of PBS. Aucamp *et al.* (2013:26) determined the experimental peak solubility of **MH** as 0.035 mg/ml and 0.235 mg/ml for **AH** in distilled water at 25°C, it can thus be concluded that other crystalline solid-state forms of roxithromycin can possibly improve the aqueous solubility.

F.3.2 *n*-Octanol-buffer partition coefficient

N'Da (2014:20786) and Williams (2013:677) described the importance of the log P value for successful transdermal delivery of an API, indicating the drug distribution between the lipophilic stratum corneum and the more hydrophilic regions of the epidermis and dermis. Mixtures with a log P value of 1 to 3 are expected to easily diffuse across the skin (Khalid *et al.*, 2016:129). The predicted log P value of 2.9 (Drugbank, 2019), exceeded the experimental value reported as 1.7 for roxithromycin (Sakdiset *et al.*, 2017). The log P value at a predetermined pH is referred to as the log D value; this value specifies whether an API can be delivered transdermally (N'Da,

2014:20786). Roxithromycin proved to be lipophilic in nature, with a determined log D value of 1.52 by Csongradi (2015:178) for roxithromycin **MH**.

F.4 Results and discussion

F.4.1 Membrane release studies results

Membrane release studies were completed before performing skin diffusion studies, as it indicates whether the API is released from the encapsulated vesicles. A total of six membrane release studies were performed, each containing a different emulgel sample serving as the donor phase.

Figures F.5 to F.16 visualise the results obtained. One Franz cell was discarded for the **L-AH** study due to signs of leakage. Two figures per study are represented, with the first showing the average cumulative amount API release per area ($\mu\text{g}/\text{cm}^2$) over a period of 6 h, whilst the second figure depicts the cumulative amount per area ($\mu\text{g}/\text{cm}^2$) released for every individual Franz cell (FC).

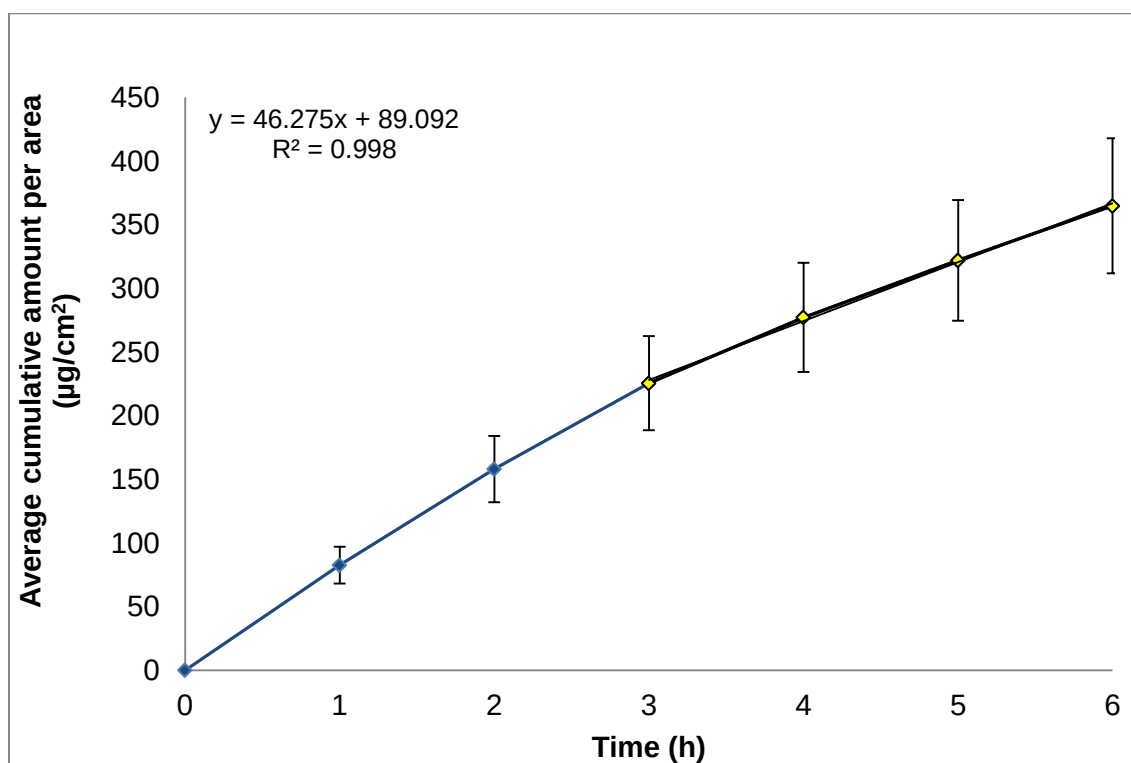


Figure F.5: Average cumulative amount per area ($\mu\text{g}/\text{cm}^2$) of roxithromycin from L-MH that was released through the membranes to indicate the average flux from 3 – 6 h ($n = 10$)

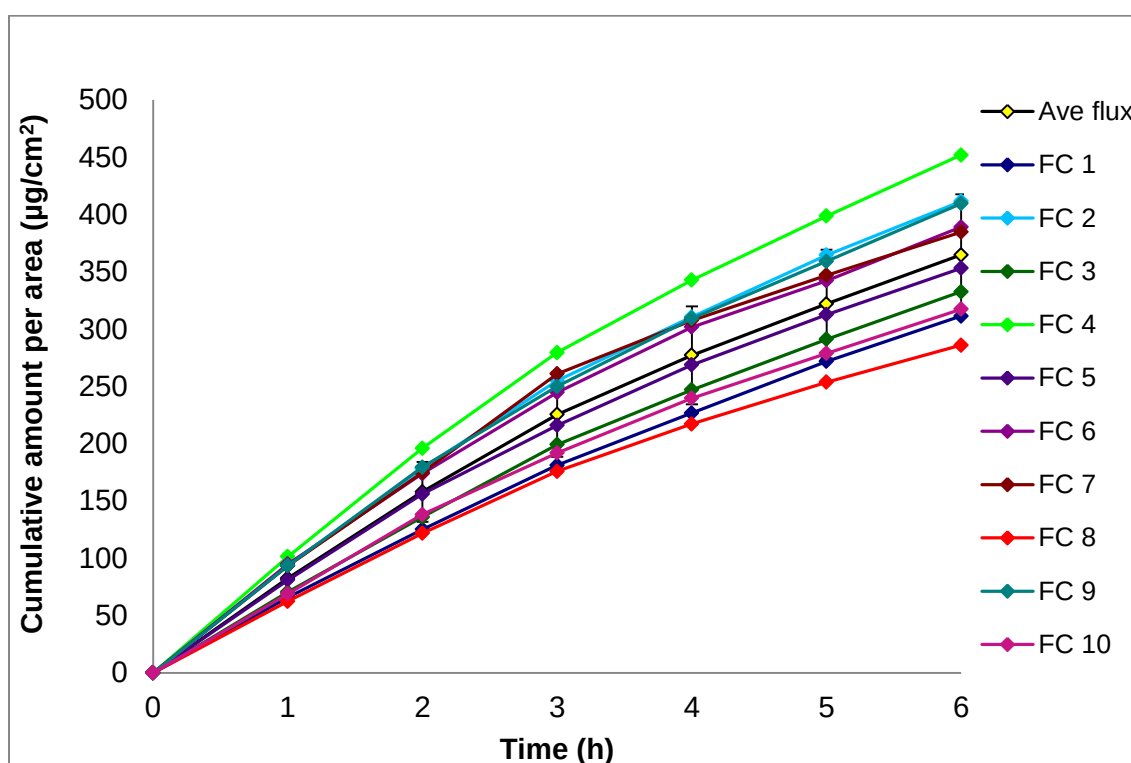


Figure F.6: Cumulative amount per area ($\mu\text{g}/\text{cm}^2$) of roxithromycin from L-MH that was released through the membranes of each individual Franz cell over 6 h ($n = 10$)

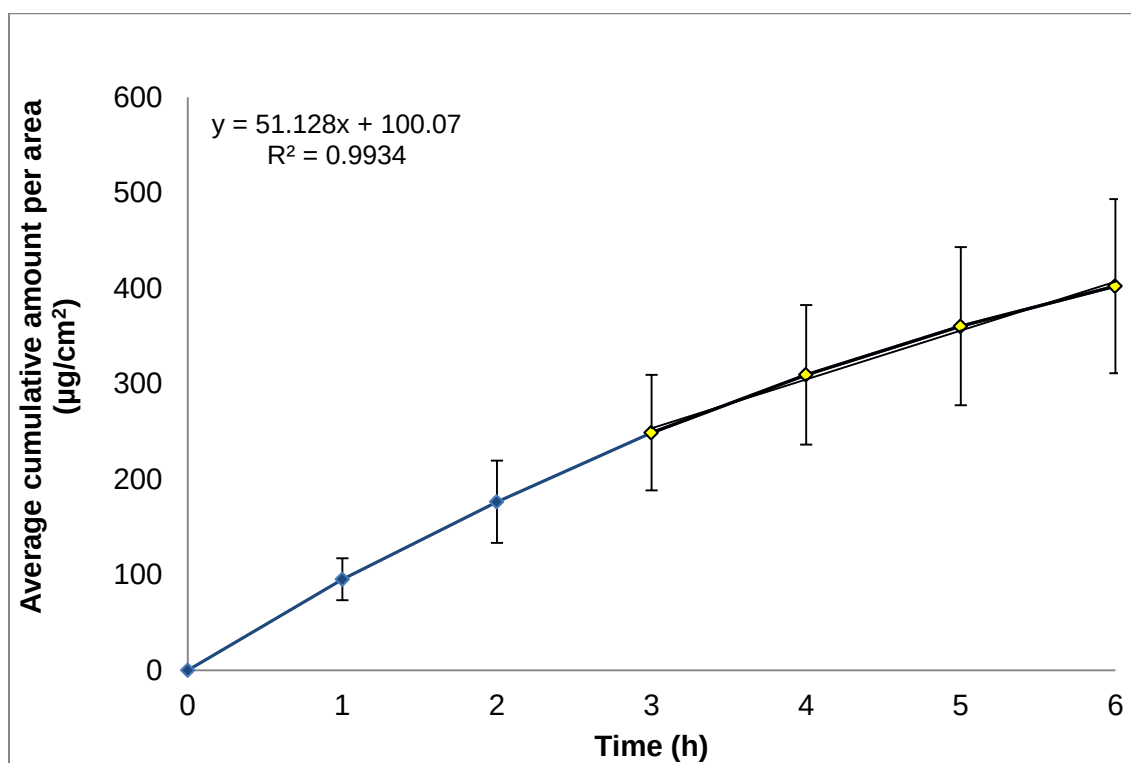


Figure F.7: Average cumulative amount per area ($\mu\text{g}/\text{cm}^2$) of roxithromycin from L-CS that was released through the membranes to indicate the average flux from 3 – 6 h ($n = 10$)

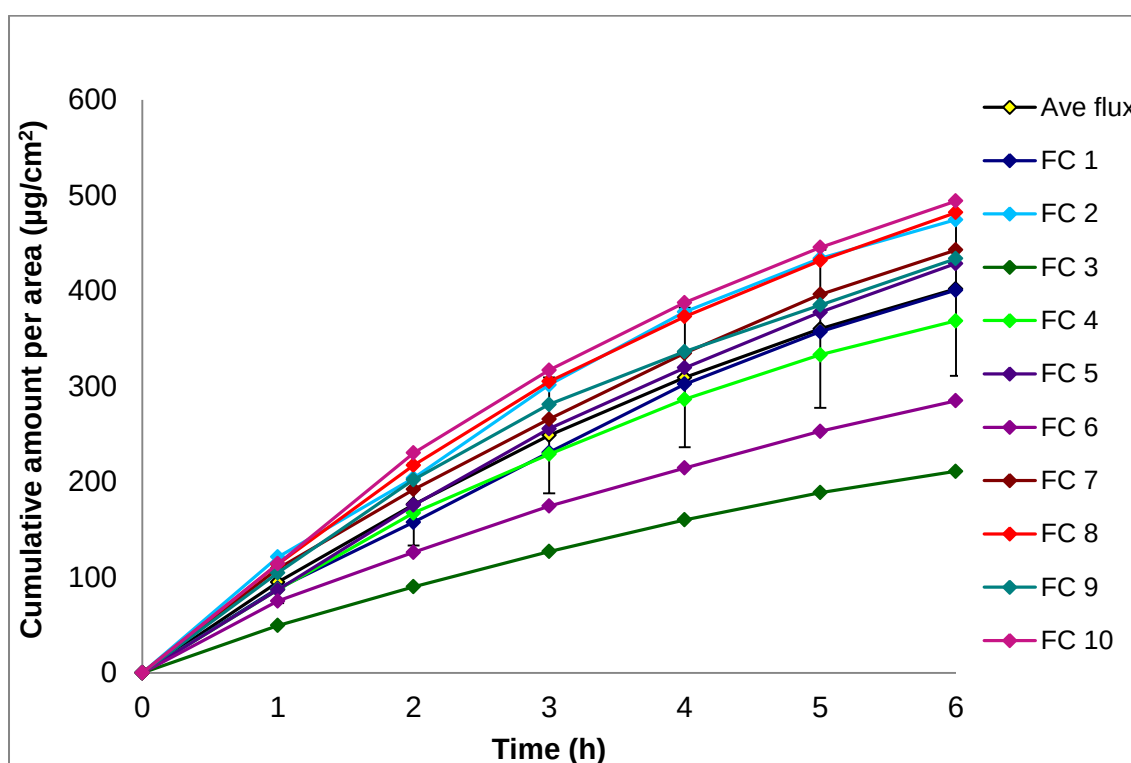


Figure F.8: Cumulative amount per area ($\mu\text{g}/\text{cm}^2$) of roxithromycin from L-CS that was released through the membranes of each individual Franz cell over 6 h ($n = 10$)

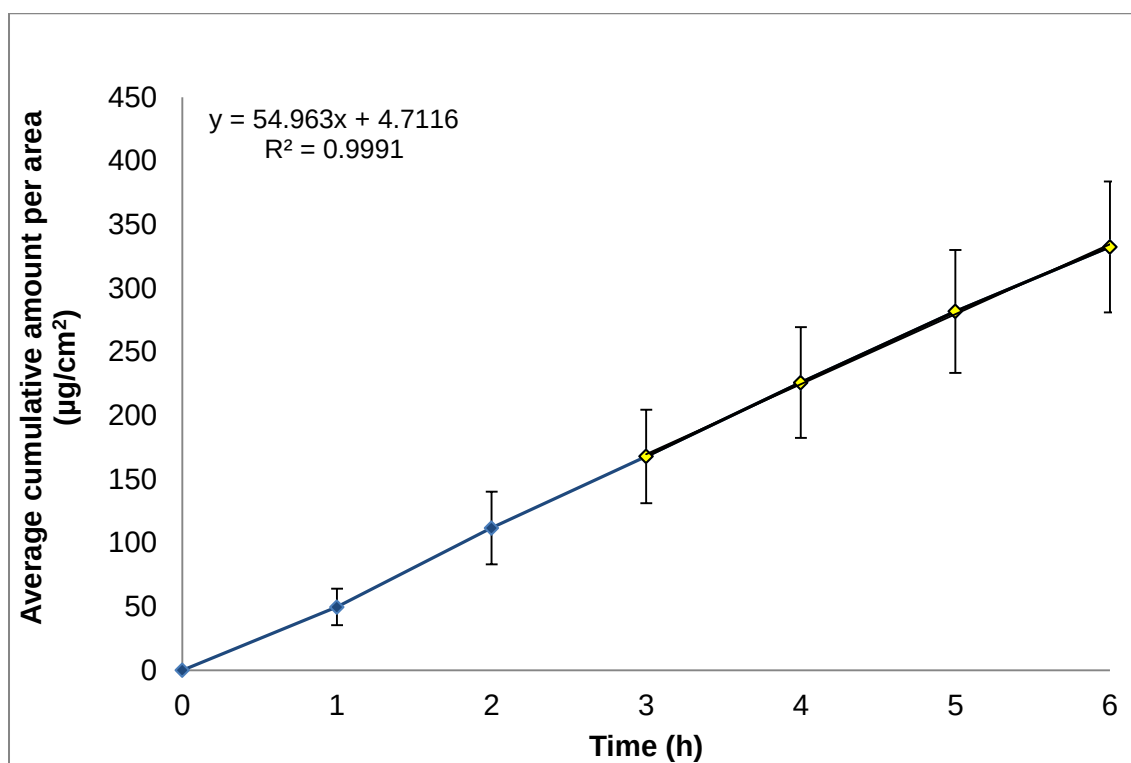


Figure F.9: Average cumulative amount per area ($\mu\text{g}/\text{cm}^2$) of roxithromycin from L-AH that was released through the membranes to indicate the average flux from 3 – 6 h ($n = 9$)

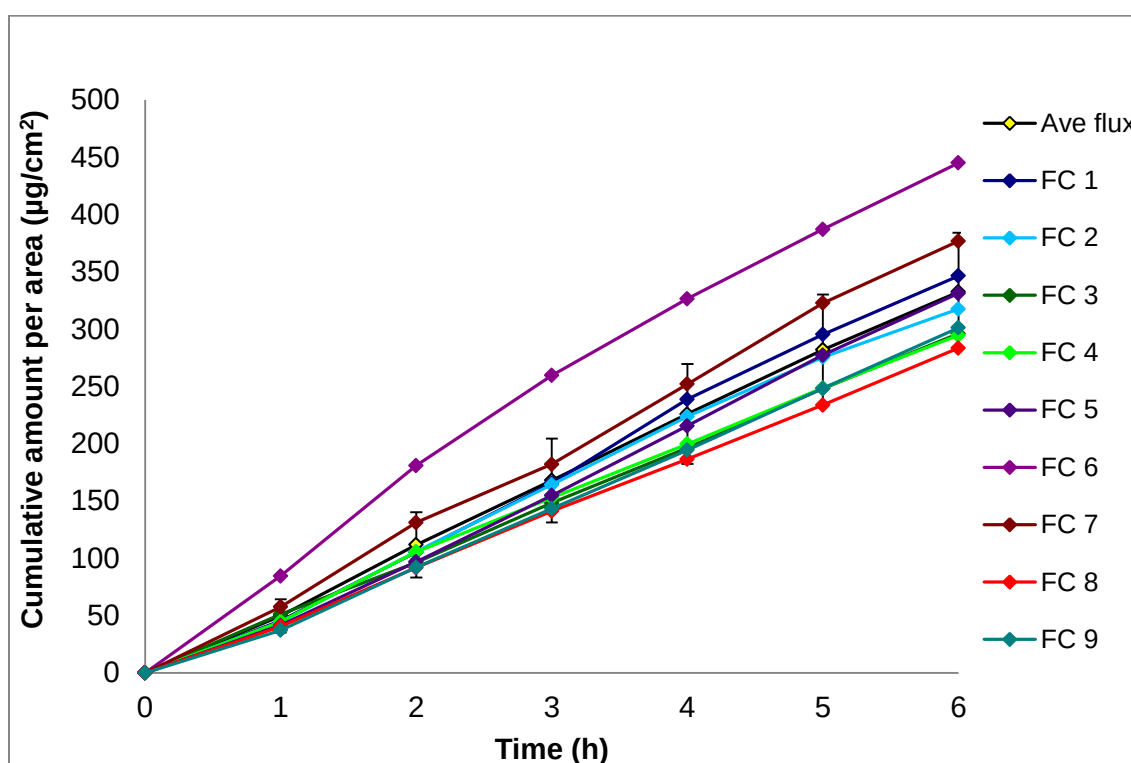


Figure F.10: Cumulative amount per area ($\mu\text{g}/\text{cm}^2$) of roxithromycin from L-AH that was released through the membranes of each individual Franz cell over 6 h ($n = 9$)

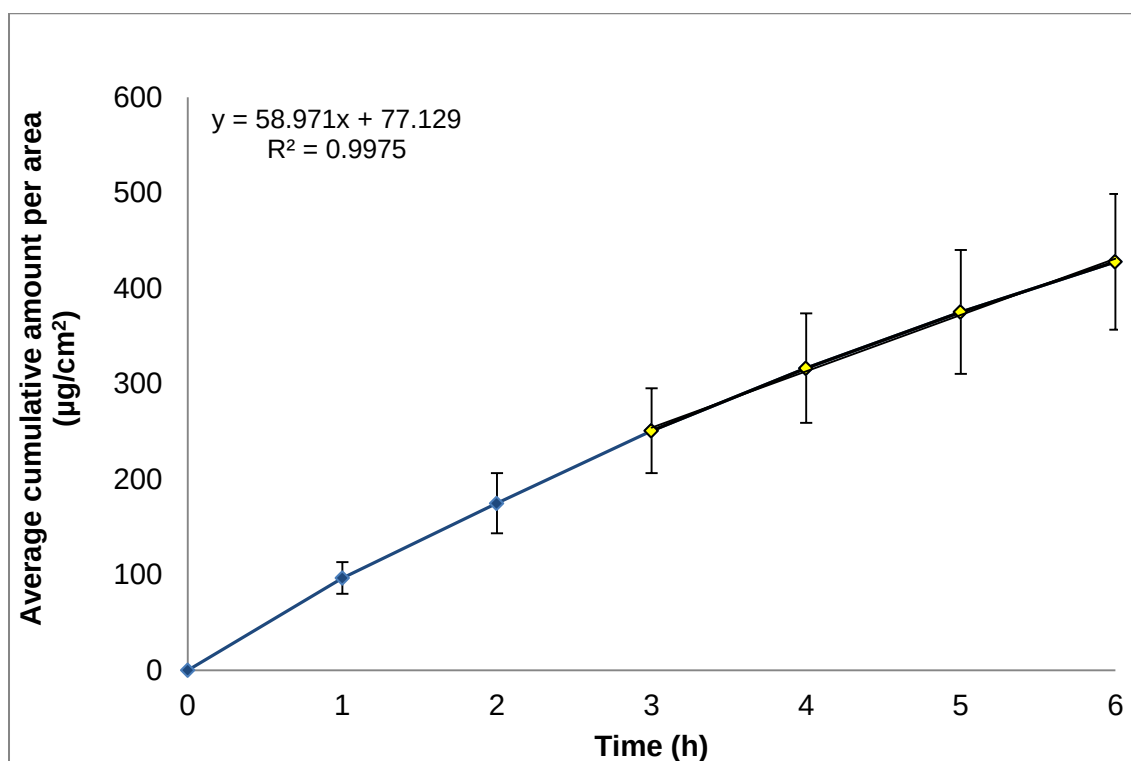


Figure F.11: Average cumulative amount per area ($\mu\text{g}/\text{cm}^2$) of roxithromycin from N-MH that was released through the membranes to indicate the average flux from 3 – 6 h ($n = 10$)

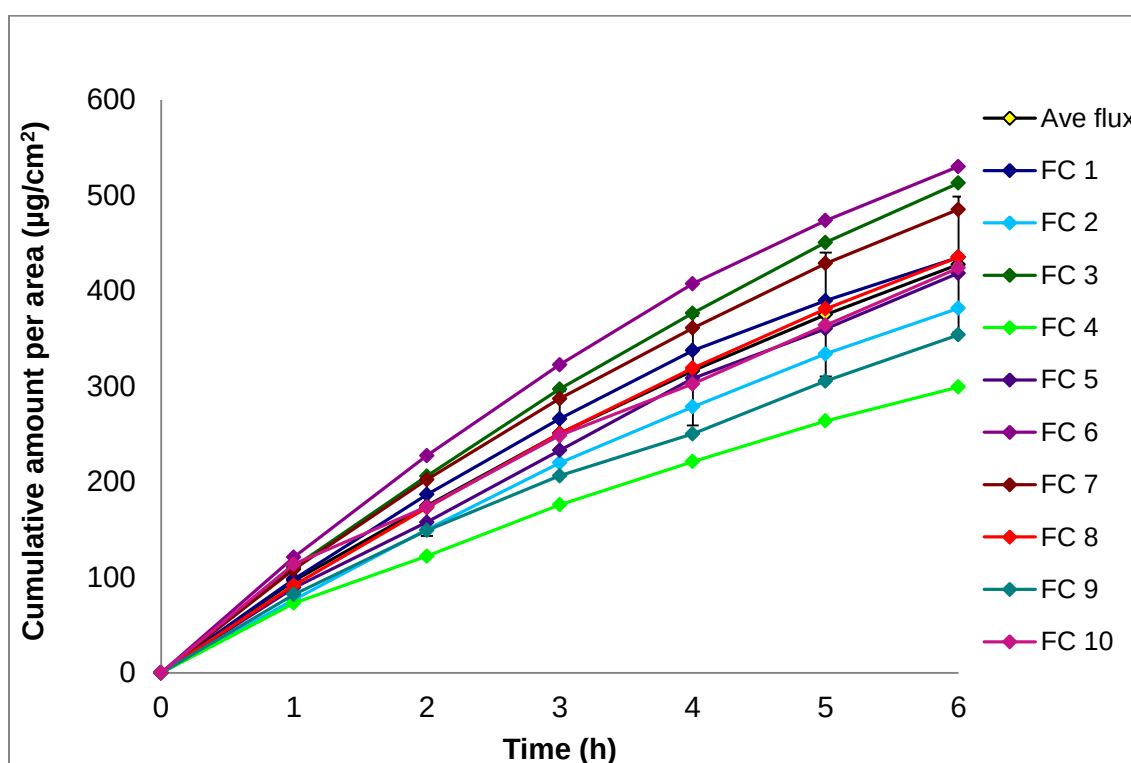


Figure F.12: Cumulative amount per area ($\mu\text{g}/\text{cm}^2$) of roxithromycin from N-MH that was released through the membranes of each individual Franz cell over 6 h ($n = 10$)

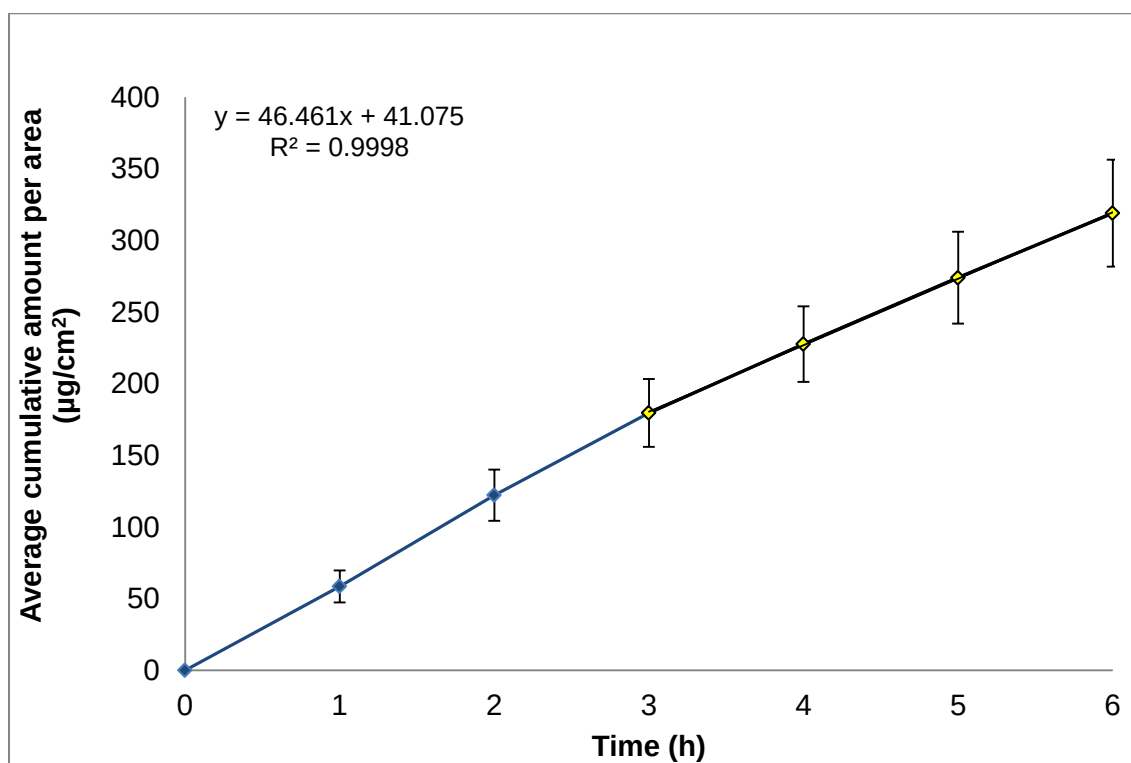


Figure F.13: Average cumulative amount per area ($\mu\text{g}/\text{cm}^2$) of roxithromycin from N-CS that was released through the membranes to indicate the average flux from 3 – 6 h ($n = 10$)

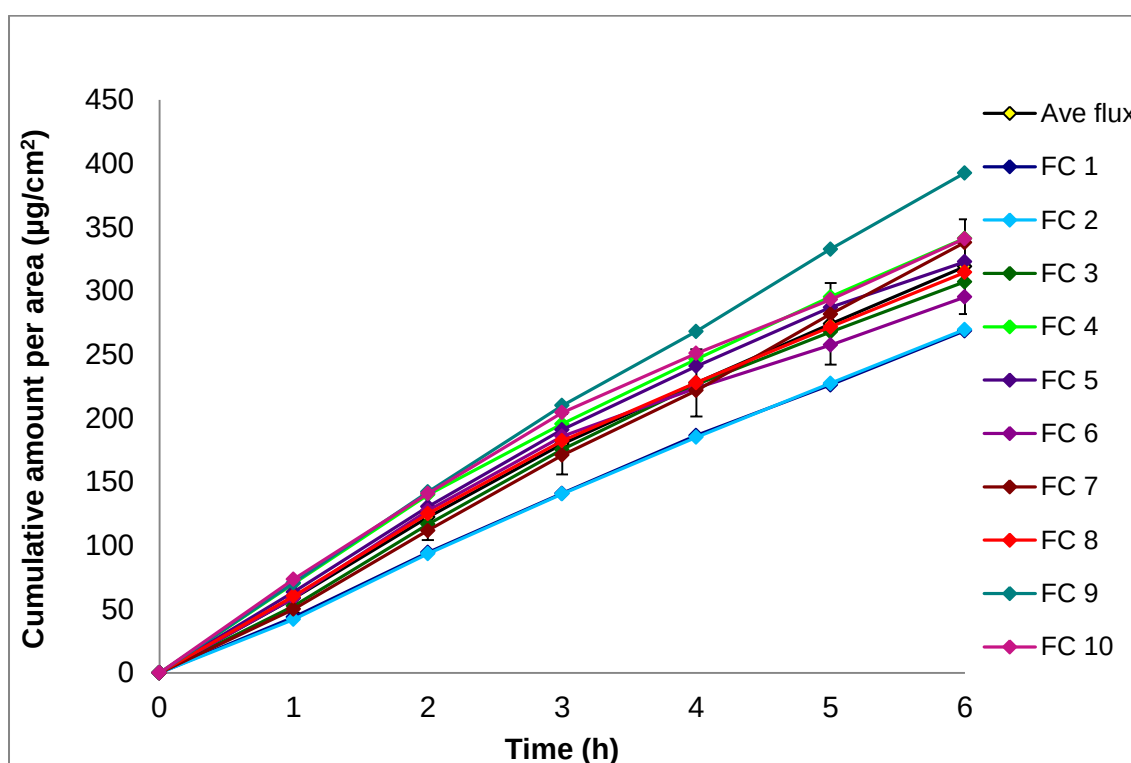


Figure F.14: Cumulative amount per area ($\mu\text{g}/\text{cm}^2$) of roxithromycin from N-CS that was released through the membranes of each individual Franz cell over 6 h ($n = 10$)

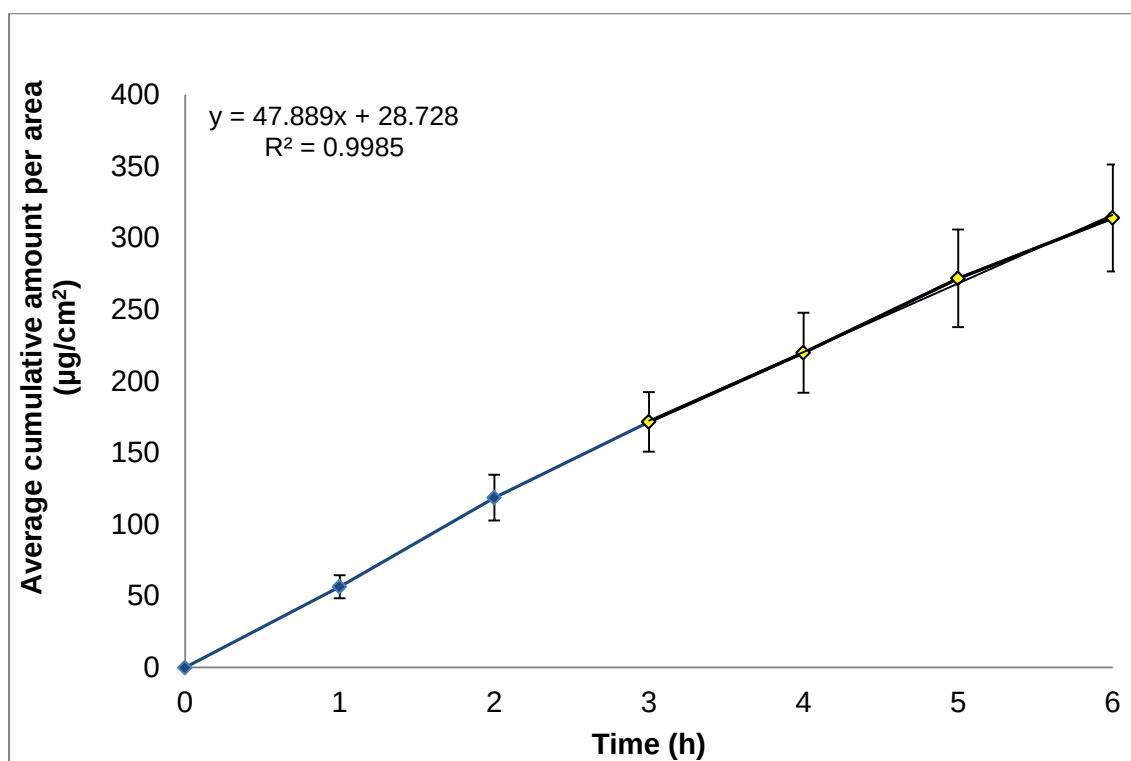


Figure F.15: Average cumulative amount per area ($\mu\text{g}/\text{cm}^2$) of roxithromycin from **N-AH** that was released through the membranes to indicate the average flux from 3 – 6 h ($n = 10$)

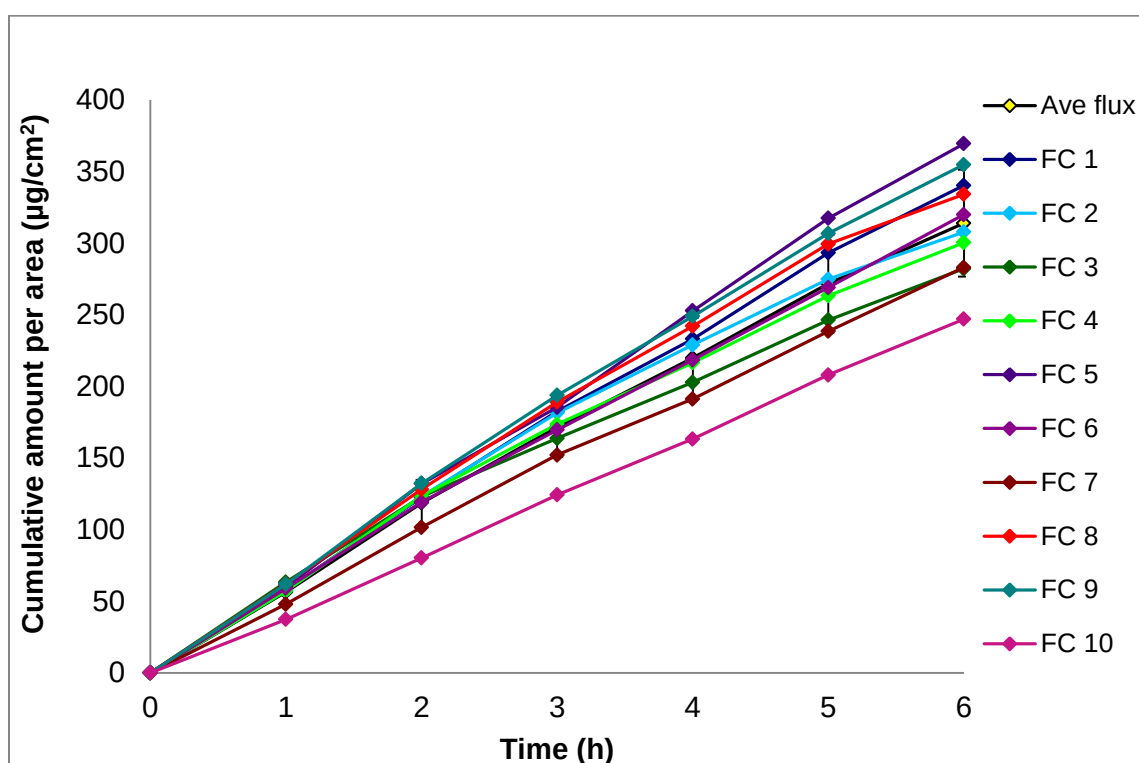


Figure F.16: Cumulative amount per area ($\mu\text{g}/\text{cm}^2$) of roxithromycin from **N-AH** that was released through the membranes of each individual Franz cell over 6 h ($n = 10$)

Table F.1: The average flux ($\mu\text{g}/\text{cm}^2\cdot\text{h}$), average percentage released (%) and median flux of roxithromycin released through the membranes for the liposomal and niosomal emulgels after 6 h (n = amount of Franz cells used)

Emulgel	n	Average flux ($\mu\text{g}/\text{cm}^2\cdot\text{h}$)	Average percentage released (%)	Median flux ($\mu\text{g}/\text{cm}^2\cdot\text{h}$)
L-MH	10	46.28 ± 6.44	2.49 ± 0.36	55.75
L-CS	10	51.13 ± 9.38	3.08 ± 0.70	67.17
L-AH	9	54.96 ± 4.81	2.19 ± 0.34	56.50
N-MH	10	58.97 ± 8.11	2.87 ± 0.48	67.08
N-CS	10	46.46 ± 7.78	2.45 ± 0.29	51.35
N-AH	10	47.89 ± 7.11	1.76 ± 0.21	50.51

The flux values ($\mu\text{g}/\text{cm}^2\cdot\text{h}$) of the liposomal and niosomal emulgels for each roxithromycin solid-state were used to determine median flux values. Median flux values present more accurate results than the average flux, since outliers and extremes are excluded, leaving the median unaffected (Dawson & Trapp, 2004). Therefore, only median values will be discussed.

It is evident from Table F.1 that the liposome and niosome formulations containing the different solid-state forms of roxithromycin had competing results during membrane release studies. The highest median flux was achieved by **L-CS** ($67.17 \mu\text{g}/\text{cm}^2\cdot\text{h}$), and the lowest achieved by **N-AH** ($50.51 \mu\text{g}/\text{cm}^2\cdot\text{h}$). The **N-MH** demonstrated a higher release than its liposomal counterpart, while **L-CS** and **L-AH** revealed increased release when compared to its niosomal counterpart. The reason why the liposomal formulations had a slightly better release rate can be attributed to the excipients used during formulation. Various factors can influence transdermal and topical delivery, such as excipients used for vesicle formulations, as well as the solid-state properties. Higher flux values for membrane release studies were obtained during this study compared to previous studies, where niosome and liposome vesicles encapsulated the amorphous solid-states of roxithromycin, as reported by Boshoff (2018) and Swart (2019). A possible reason for better delivery is the type of formulation used; an emulgel would most likely possess better API delivery because of a longer contact time with the skin (Sah *et al.*, 20:26). The membrane release studies for the liposome, as well as niosome emulgels achieved their purpose of confirming that the encapsulated roxithromycin was being released from the vesicles. It is notable to mention that the membrane study results will not necessarily correlate with results from the skin diffusion study.

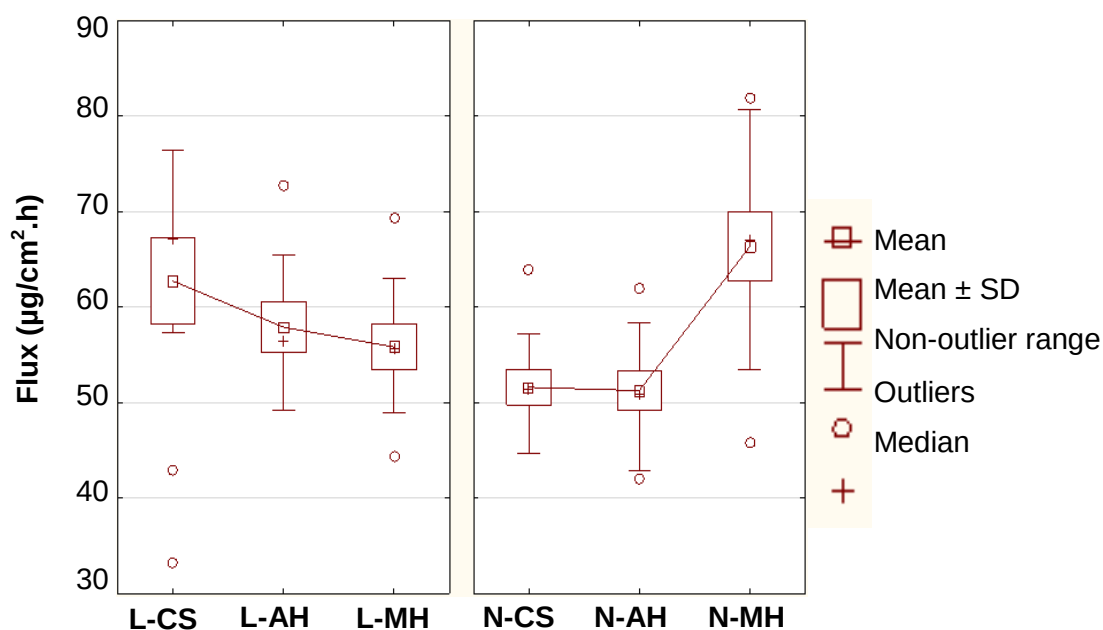


Figure F.17: Box-plots indicating the flux ($\mu\text{g}/\text{cm}^2\cdot\text{h}$) of the liposomal and niosomal emulgels containing roxithromycin during the membrane release studies over 6 h

F.4.2 *In vitro* skin diffusion studies results

Skin diffusion studies can evaluate the correlation between the skin, skin permeation and API delivery system used (Ng *et al.*, 2010:210). Skin diffusion studies were conducted to determine the transdermal delivery of three solid-state forms of roxithromycin. Ten cells containing the API formulation as donor phase were used for each study, with two additional cells containing the placebo to serve as the control group. In total, six skin diffusion studies were performed, with each experiment having a different vesicle and form of roxithromycin encapsulated. One Franz cell was discarded from the **L-AH** study due to signs of leakage.

The amount per area diffused ($\mu\text{g}/\text{cm}^2$) of the liposomal and niosomal emulgels for each roxithromycin solid-state were used to determine the median values. To avoid outliers and extremes, only median flux values will be discussed. The average flux does not provide for outliers and extremes, while the median flux is unaffected by them (Dawson & Trapp, 2004).

The amount per area ($\mu\text{g}/\text{cm}^2$) of roxithromycin that diffused through the skin of each Franz cells from the liposomal and niosomal emulgels during *in vitro* skin diffusion studies after 12 h is presented in Figures F.18 to F.23.

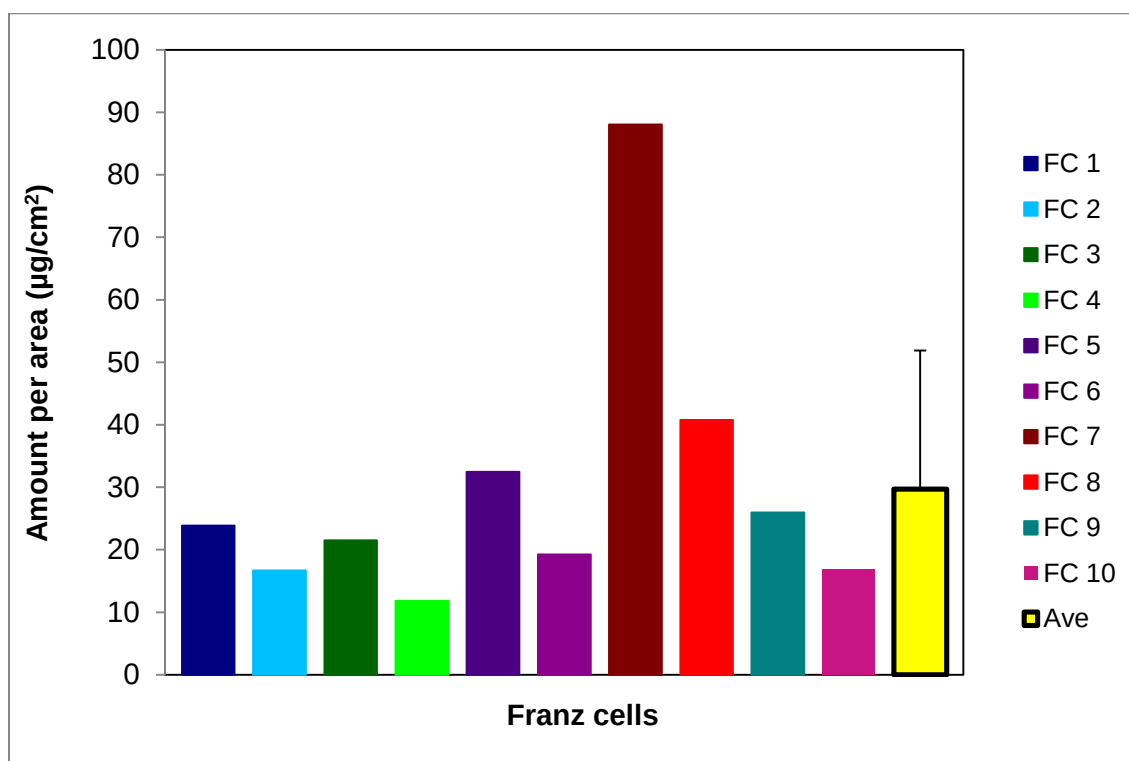


Figure F.18: The amount per area diffused ($\mu\text{g}/\text{cm}^2$) of roxithromycin from **L-MH** after the 12 h diffusion study ($n = 10$)

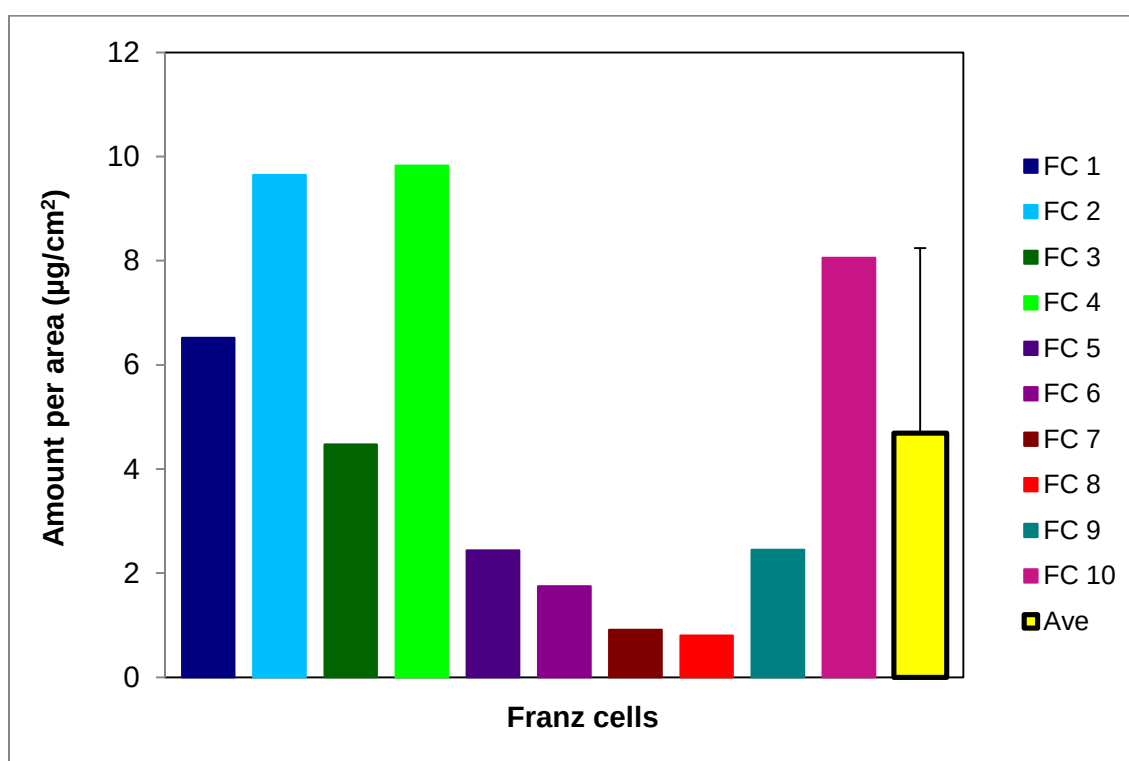


Figure F.19: The amount per area diffused ($\mu\text{g}/\text{cm}^2$) of roxithromycin from **L-CS** after the 12 h diffusion study ($n = 10$)

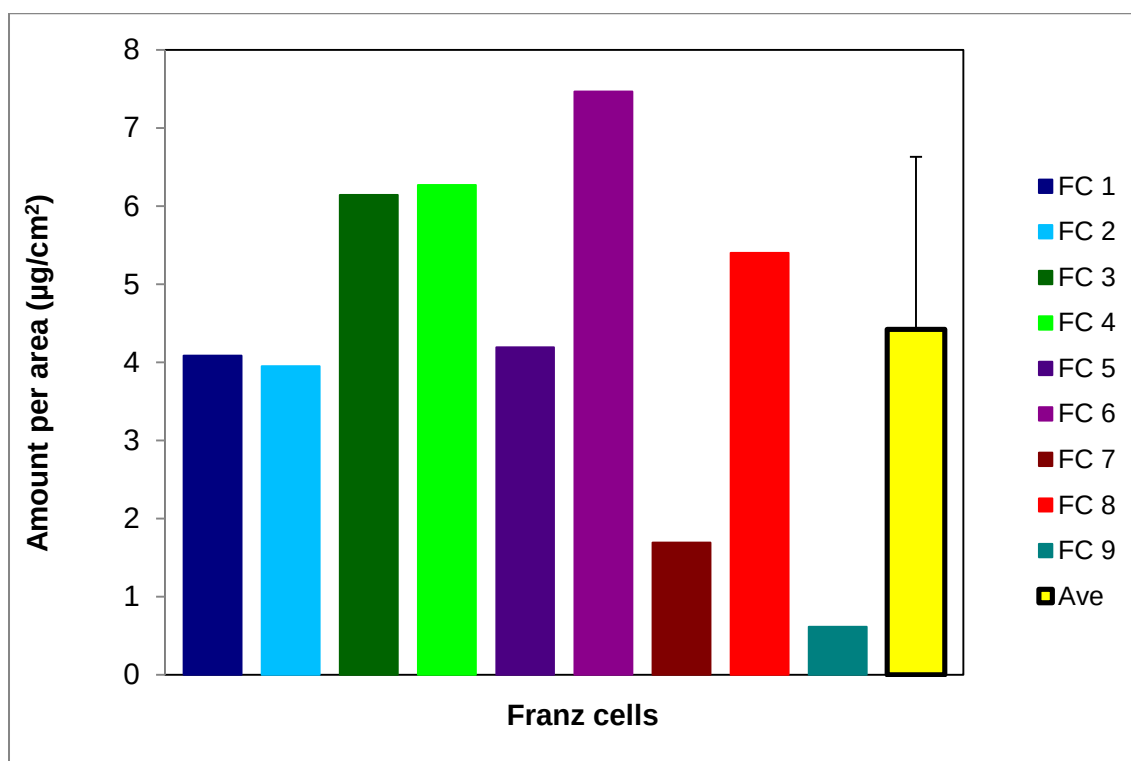


Figure F.20: The amount per area diffused ($\mu\text{g}/\text{cm}^2$) of roxithromycin from **L-AH** after the 12 h diffusion study ($n = 9$)

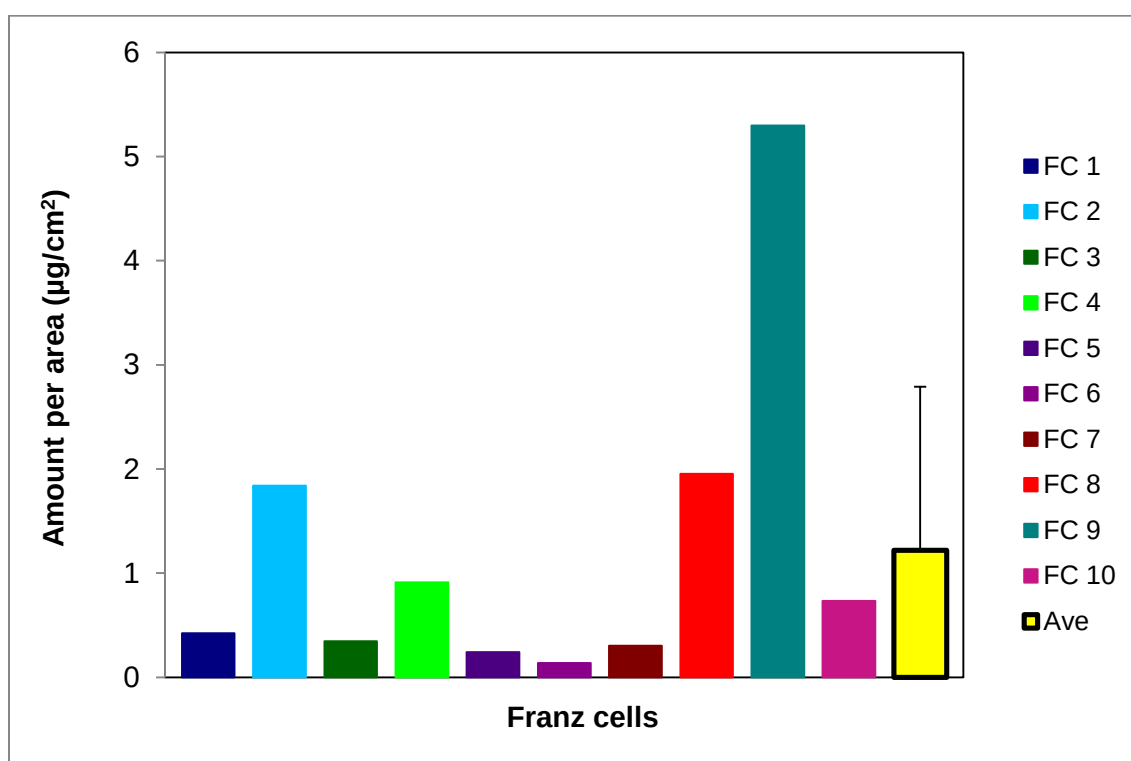


Figure F.21: The amount per area diffused ($\mu\text{g}/\text{cm}^2$) of roxithromycin from **N-MH** after the 12 h diffusion study ($n = 10$)

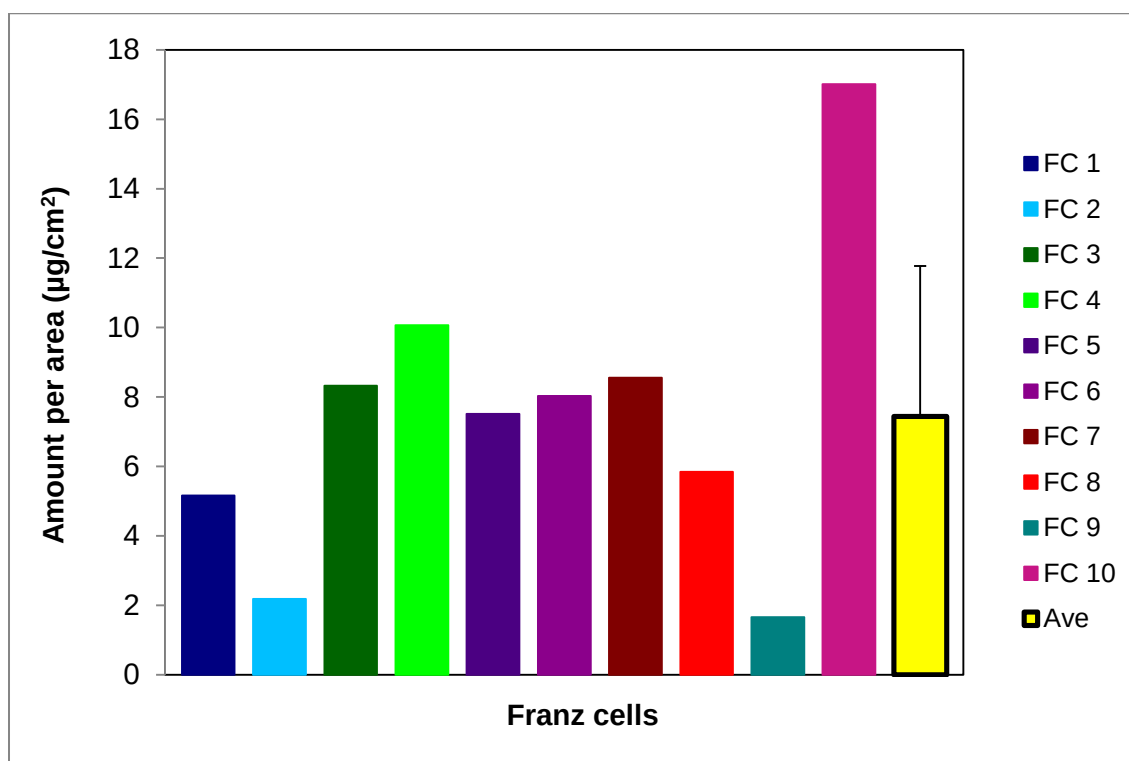


Figure F.22: The amount per area diffused ($\mu\text{g}/\text{cm}^2$) of roxithromycin from **N-CS** after the 12 h diffusion study ($n = 10$)

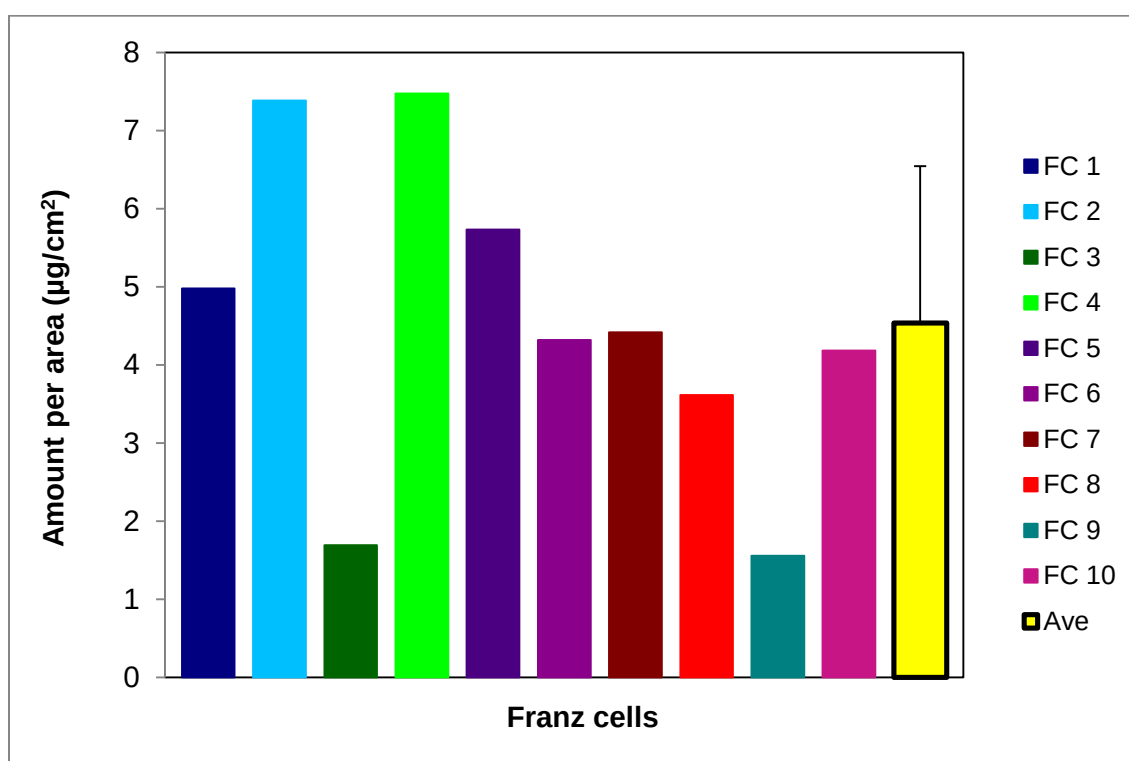


Figure F.23: The amount per area diffused ($\mu\text{g}/\text{cm}^2$) of roxithromycin from **N-AH** after the 12 h diffusion study ($n = 10$)

Table F.2: The average percentage diffused (%), average amount per area diffused ($\mu\text{g}/\text{cm}^2$) and median amount per area diffused of roxithromycin for the liposomal and niosomal emulgels found in the receptor phase (n = amount of Franz cells used)

Emulgel	n	Average percentage diffused (%)	Average amount per area diffused ($\mu\text{g}/\text{cm}^2$)	Median amount per area diffused ($\mu\text{g}/\text{cm}^2$)
L-MH	10	0.20 ± 0.15	29.72 ± 22.15	23.20
L-CS	10	0.04 ± 0.03	4.57 ± 3.48	3.46
L-AH	9	0.03 ± 0.01	4.42 ± 2.21	4.27
N-MH	10	0.01 ± 0.01	1.22 ± 1.57	0.58
N-CS	10	0.06 ± 0.03	7.44 ± 4.34	7.94
N-AH	10	0.03 ± 0.01	4.54 ± 2.01	4.46

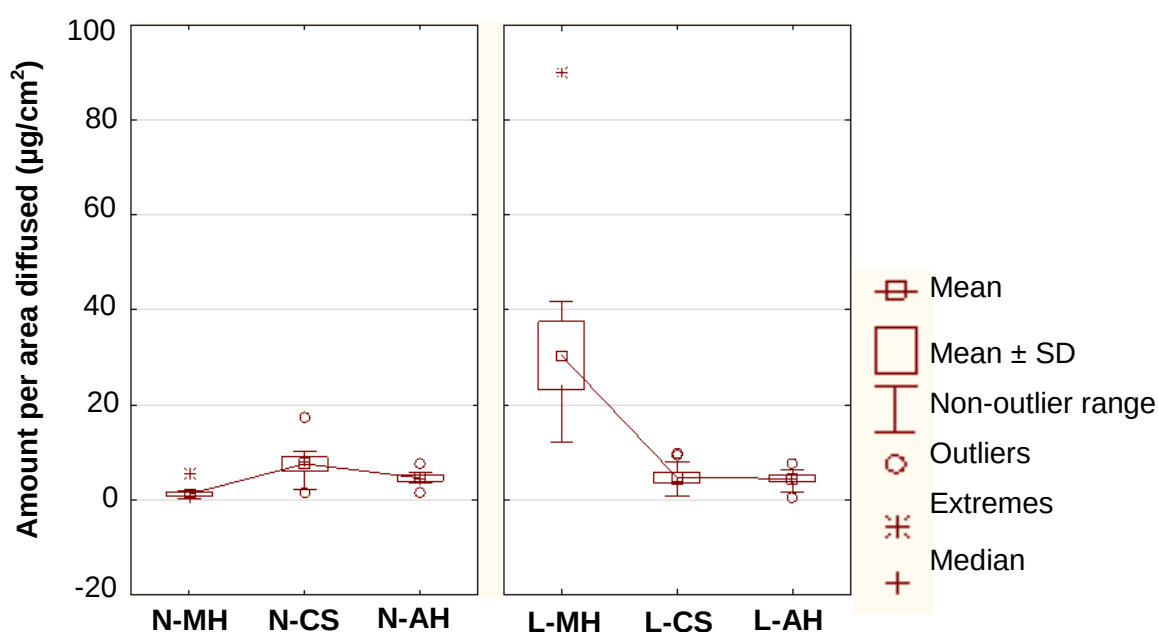


Figure F.24: Box-plot indicating the mean and median amount per area diffused ($\mu\text{g}/\text{cm}^2$) for roxithromycin in the liposomal and niosomal emulgels after 12 h

It can be seen from the results that very small amounts of API were delivered to the receptor phase, representative of transdermal diffusion. When the vesicle emulgels (liposomal and niosomal emulgels) were compared, it was detected that **L-MH** had the highest median amount per area diffused, followed by **L-CS**, and lastly, **L-AH** for the liposomal emulgels, while for the niosomal emulgels, **N-CS** had the highest median amount per area diffused, followed by **N-AH**, and lastly, **N-MH**.

When the different solid-state forms of roxithromycin (**MH**, **CS** and **AH**) were compared, it was evident that the type of vesicle used influenced the transdermal delivery, rather than the API used (from highest to lowest median amount per area diffused: **L-MH**; **N-CS**; **L-CS**; **N-AH**; **L-AH**

and lastly, **N-MH**). Hence, the liposomal emulgels increased the transdermal delivery of the **MH**, rather than the other two solid-state forms of roxithromycin, while the opposite for the niosomal emulgels was true. The different physicochemical characteristics of the different types of vesicle delivery systems, as well as the excipients used for vesicle composition influenced API delivery (Honeywell-Nguyen & Bouwstra, 2005:68). Therefore, the poor delivery to the receptor phase can be attributed to the vesicle systems used in the emulgel formulations. The different excipients used, such as PC in liposomes and Span[®] 60 in niosomes, possibly influenced API delivery, seeing as the additional phospholipids could have resulted in an extra barrier on the surface of the skin, leading to slow release of the API from the vesicles (Kirjavainen *et al.*, 1999:285; Sakdiset *et al.*, 2018:63). Given that the target-site for the API delivery during this study was the dermis, the poor transdermal delivery was a favoured outcome, because systemic effects and toxicity would be minimal.

F.4.3 Tape stripping results

Tape stripping provides a good indication of possible topical delivery of an API. By removing the stratum corneum from the skin, the amount of API present in the skin layers can be determined (Ademola, 1997:532; Surber *et al.*, 1999:395). To determine if the encapsulated roxithromycin was successfully released into the skin from the emulgel formulations, tape-stripping was performed. If the API resided in the dermis, the target-site would be reached, resulting in successful topical delivery. The tape stripping results are divided firstly into delivery to the SCE and secondly, delivery to the ED. The concentration ($\mu\text{g/ml}$) of the roxithromycin that was delivered topically (SCE and ED) from the emulgels is presented in Figures F.25 to F.36.

F.4.3.1 Stratum corneum-epidermis

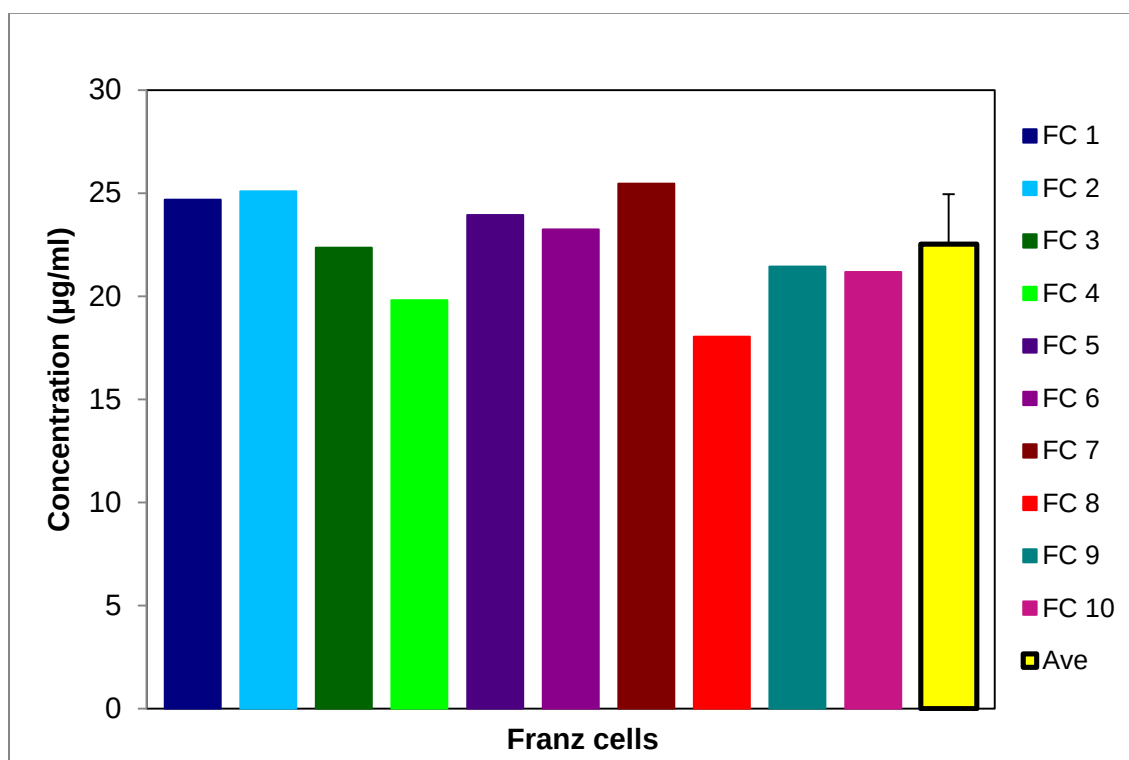


Figure F.25: Roxithromycin concentration (µg/ml) from the L-MH present in the SCE ($n = 10$)

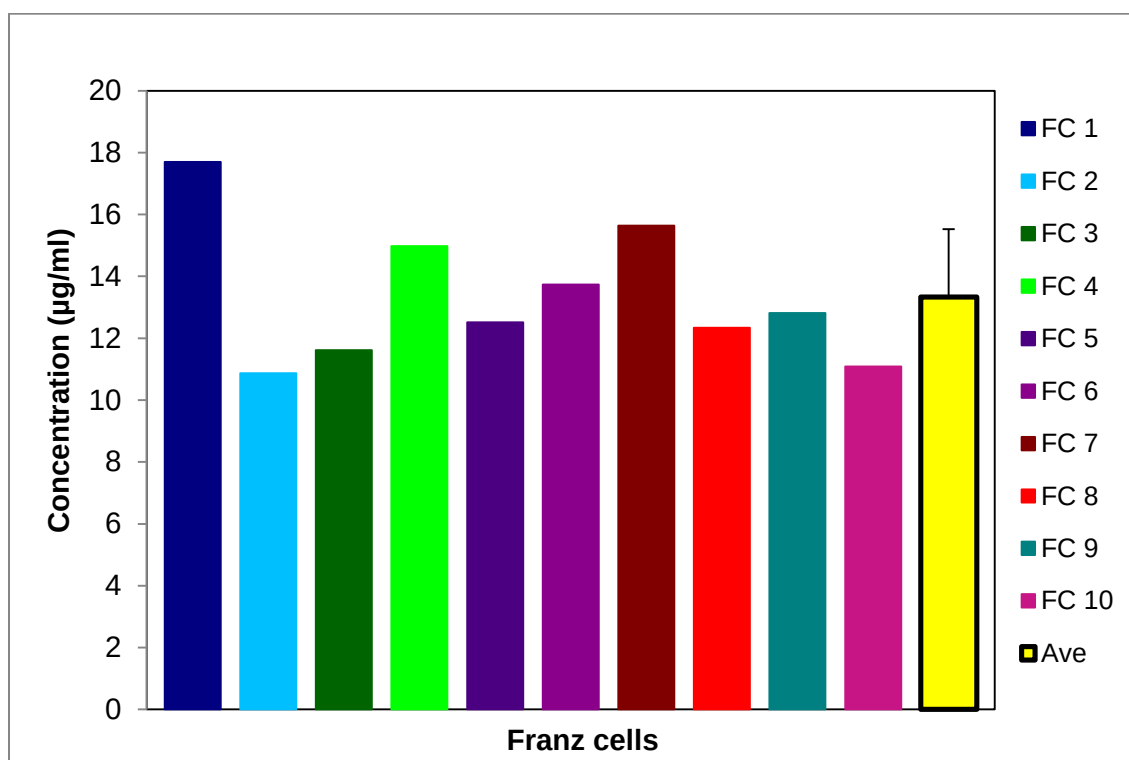


Figure F.26: Roxithromycin concentration (µg/ml) from the L-CS present in the SCE ($n = 10$)

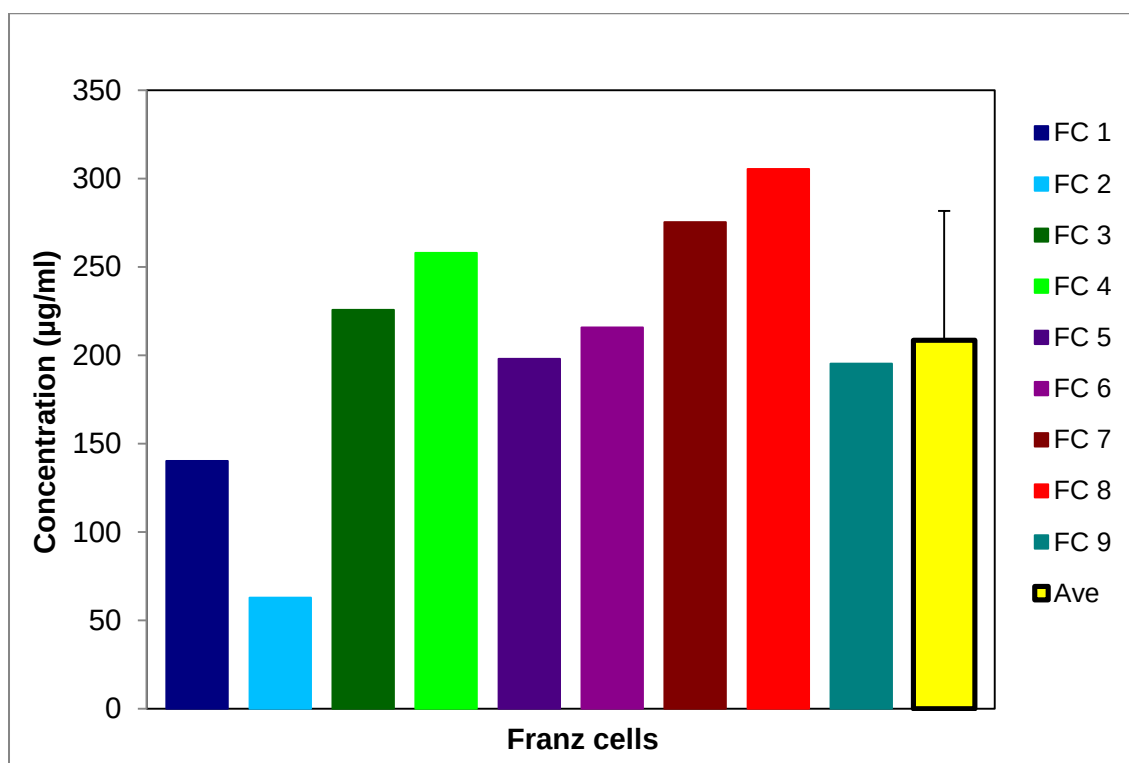


Figure F.27: Roxithromycin concentration (µg/ml) from the L-AH present in the SCE ($n = 9$)

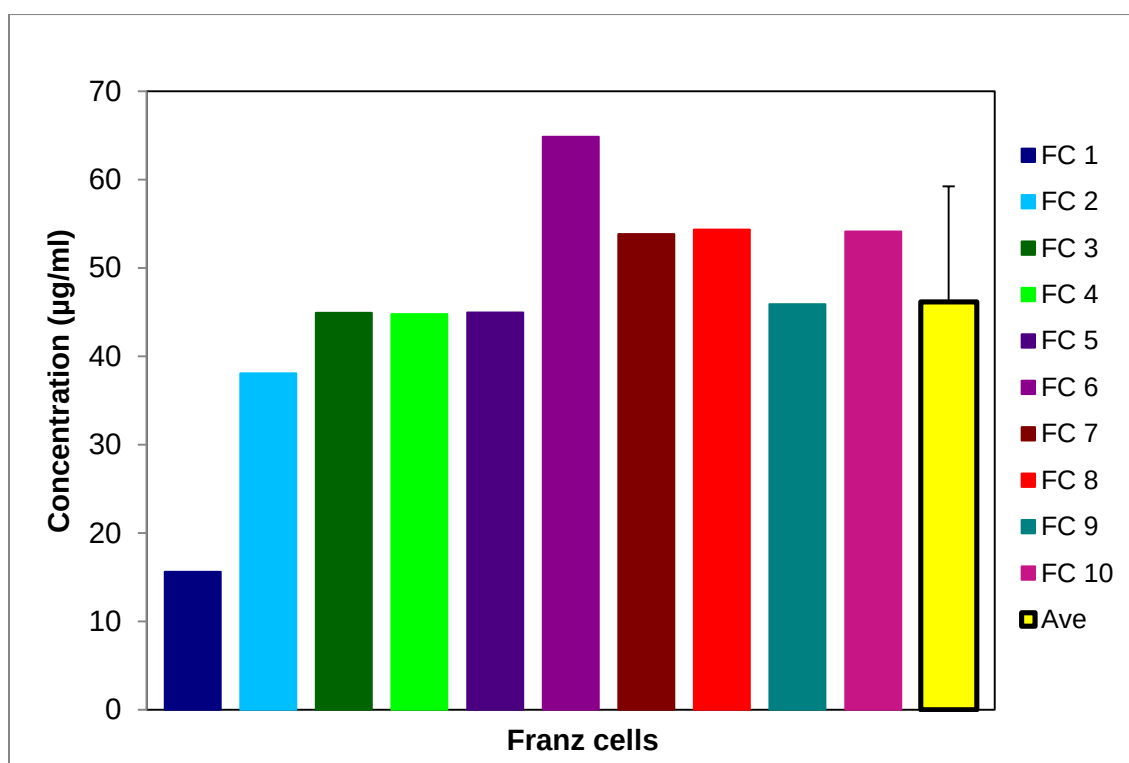


Figure F.28: Roxithromycin concentration (µg/ml) from the N-MH present in the SCE ($n = 10$)

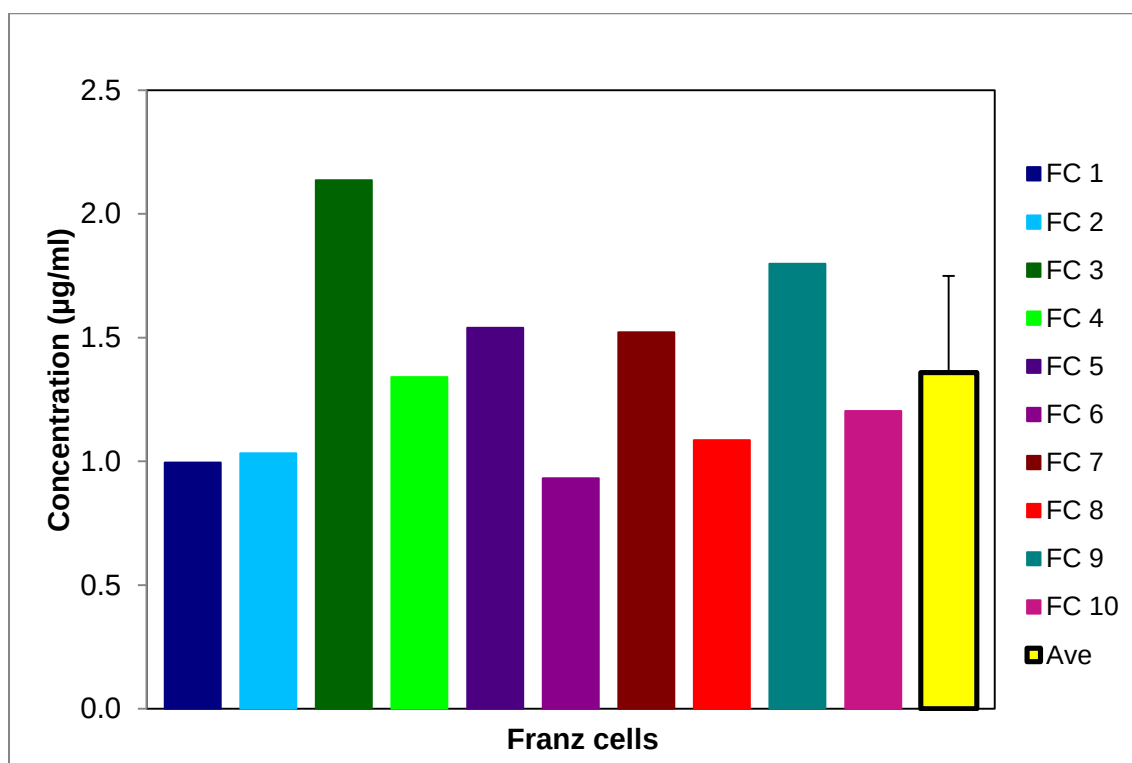


Figure F.29: Roxithromycin concentration (µg/ml) from the **N-CS** present in the SCE ($n = 10$)

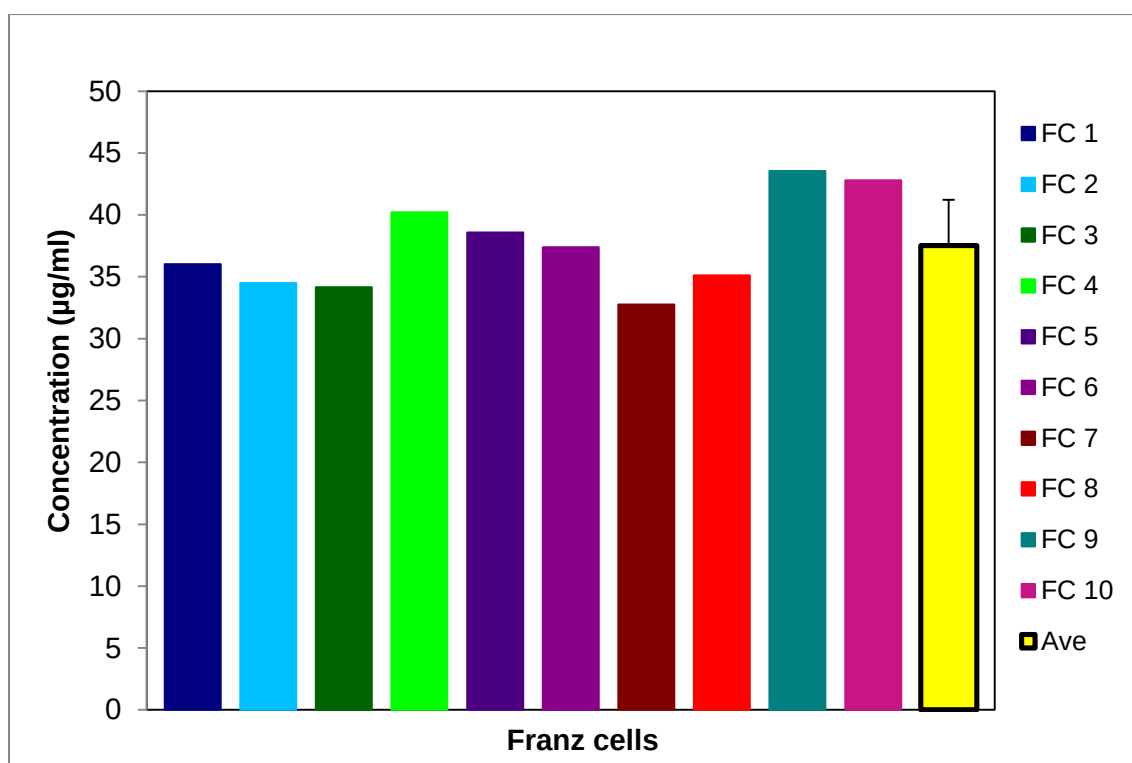


Figure F.30: Roxithromycin concentration (µg/ml) from the **N-AH** present in the SCE ($n = 10$)

Table F.3: The average and median concentration ($\mu\text{g/ml}$) of roxithromycin present in the SCE after tape stripping (n = amount of Franz cells used)

Emulgel	n	Average concentration in the SCE ($\mu\text{g/ml}$)	Median concentration in the SCE ($\mu\text{g/ml}$)
L-MH	10	22.52 ± 2.42	23.30
L-CS	10	13.33 ± 2.19	12.96
L-AH	9	208.52 ± 73.14	220.58
N-MH	10	46.14 ± 13.08	46.43
N-CS	10	1.36 ± 0.39	1.30
N-AH	10	37.51 ± 3.71	37.52

When the vesicle emulgels (liposomal and niosomal emulgels) were compared, it was observed that **L-AH** resulted in an exceptional high stratum corneum delivery ($220.58 \mu\text{g/ml}$), in contrast to **L-MH** and **L-CS** with a median concentration of $23.30 \mu\text{g/ml}$ and $12.96 \mu\text{g/ml}$, respectively. For the niosomal emulgels, **N-MH** resulted in the highest stratum corneum delivery ($46.43 \mu\text{g/ml}$), followed by **N-AH** ($37.52 \mu\text{g/ml}$) and lastly, **N-CS** ($1.30 \mu\text{g/ml}$). All the liposomal and niosomal emulgels resulted in median concentrations above the LOD ($0.75 \mu\text{g/ml}$) and LOQ ($2.51 \mu\text{g/ml}$), with only **N-CS** resulting in a value below the LOQ.

When the different solid-state forms of roxithromycin (**MH**, **CS** and **AH**) were compared (from the highest to lowest concentration: **L-AH**; **N-MH**; **N-AH**; **L-MH**; **L-CS** and lastly and **N-CS**), it was evident that the **CS** solid-state form of roxithromycin resulted in the lowest stratum corneum delivery. It is known that the stratum corneum acts as a rate-limiting hindrance for drug delivery due to the numerous protein and lipid layers (Barry, 2001:101; Williams, 2013:680). It was observed that **AH** permeated effortlessly in the SCE from the liposomal vesicles in contrast to niosomal vesicles. PC, which is used in liposome preparation, has the ability to penetrate intercellular lipids of the stratum corneum (Montenegro *et al.*, 2006:137), influencing the barrier function and possibly enhancing the penetration of **AH**. **MH** however, permeated with more ease into the SCE from the niosomal vesicles rather than the liposomal vesicles. It is possible that API delivery is more dependent on the excipients used for vesicle preparation than the solid-state form. Highly lipid-soluble excipients used for liposome and niosome preparation, such as cholesterol, would cause the vesicles to have more difficulty crossing the aqueous region. As the effect of surfactants, such as PC and Span[®], are extremely complex in biological systems, it is possible for the permeation of the APIs to vary extensively (Som *et al.*, 2012:3).

F.4.3.2 Concentration in epidermis-dermis

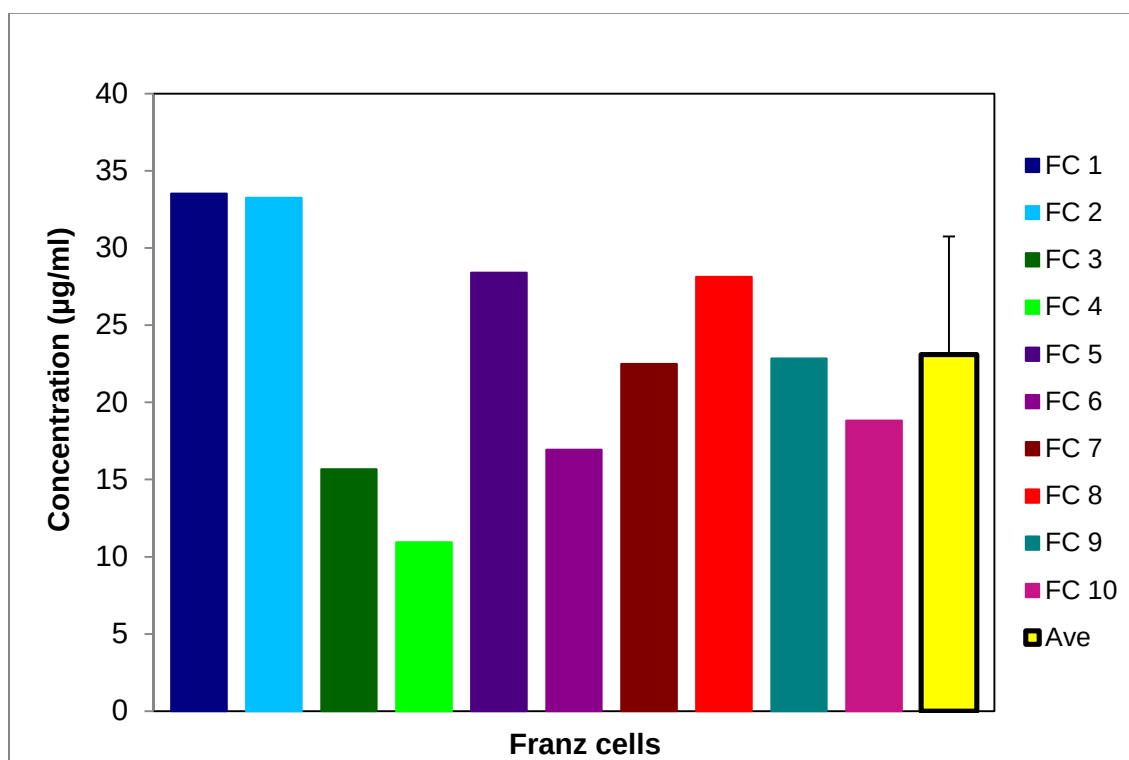


Figure F.31: Roxithromycin concentration (µg/ml) from the L-MH present in the ED ($n = 10$)

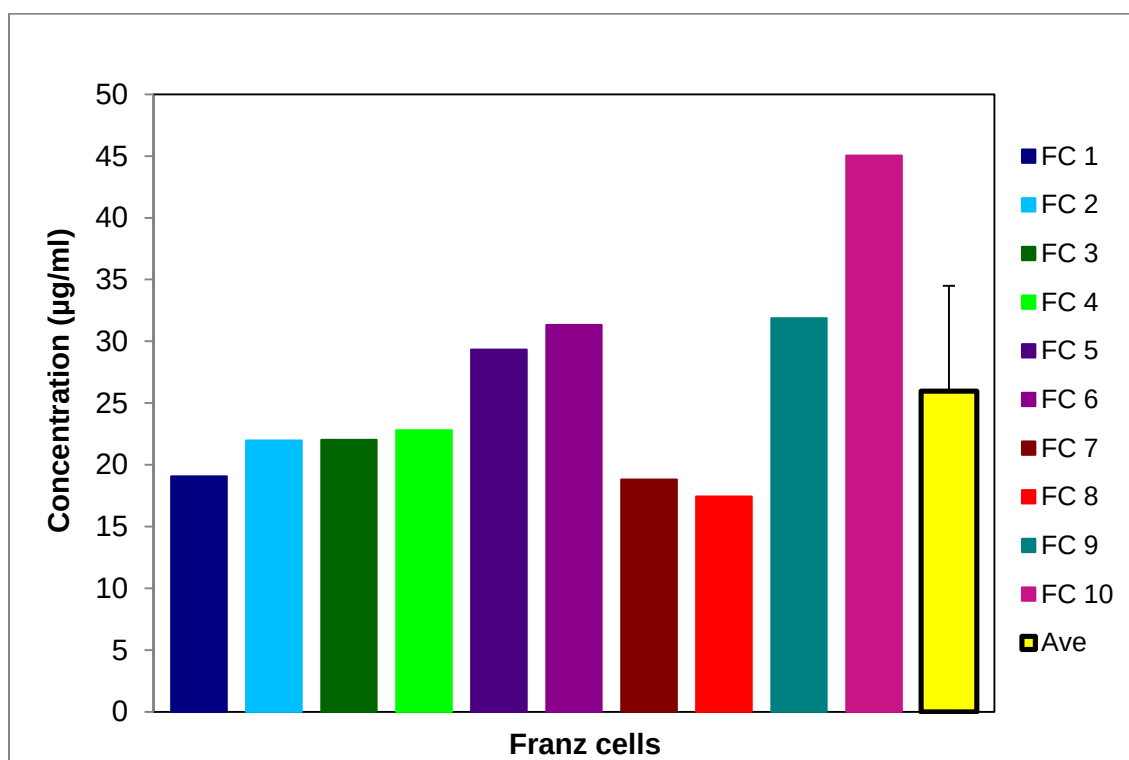


Figure F.32: Roxithromycin concentration (µg/ml) from the L-CS present in the ED ($n = 10$)

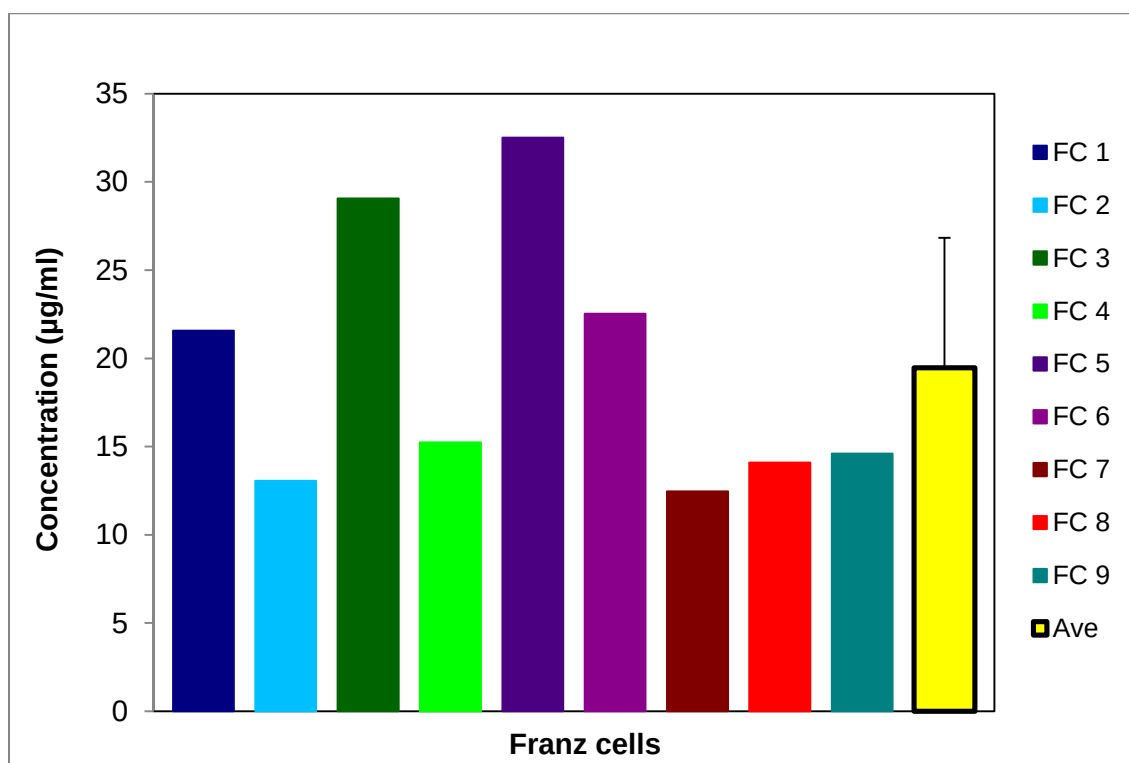


Figure F.33: Roxithromycin concentration (µg/ml) from the **L-AH** present in the ED ($n = 9$)

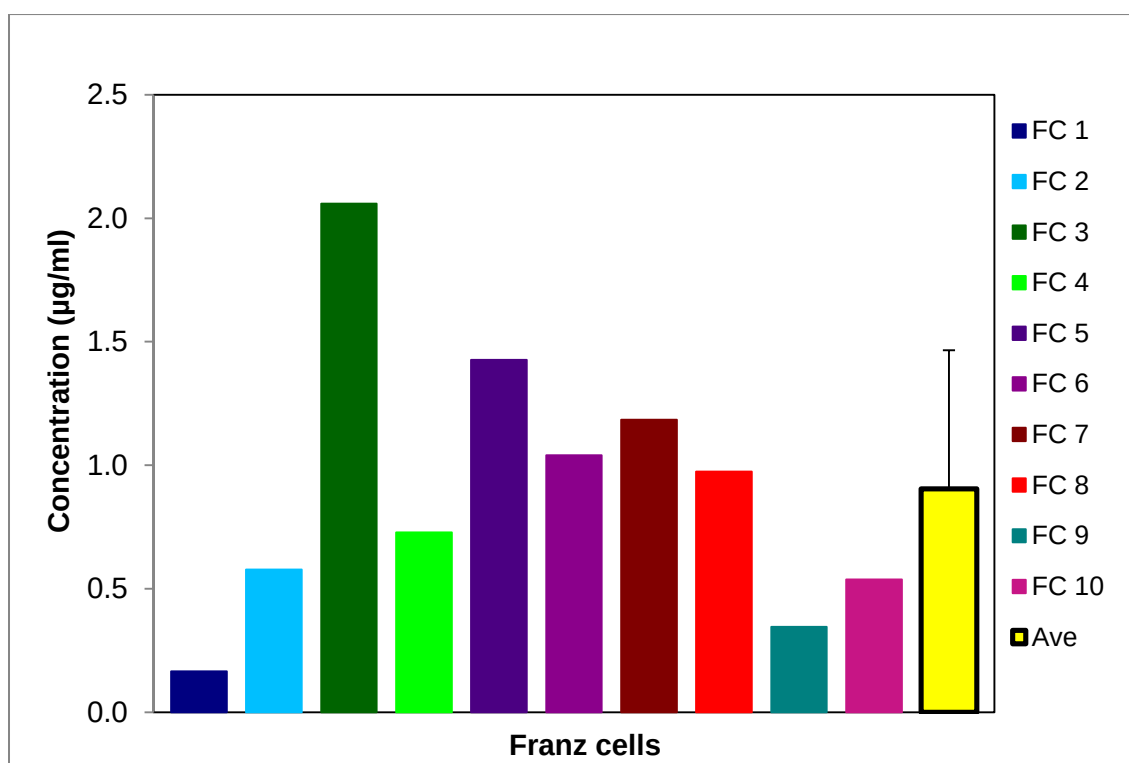


Figure F.34: Roxithromycin concentration (µg/ml) from the **N-MH** present in the ED ($n = 10$)

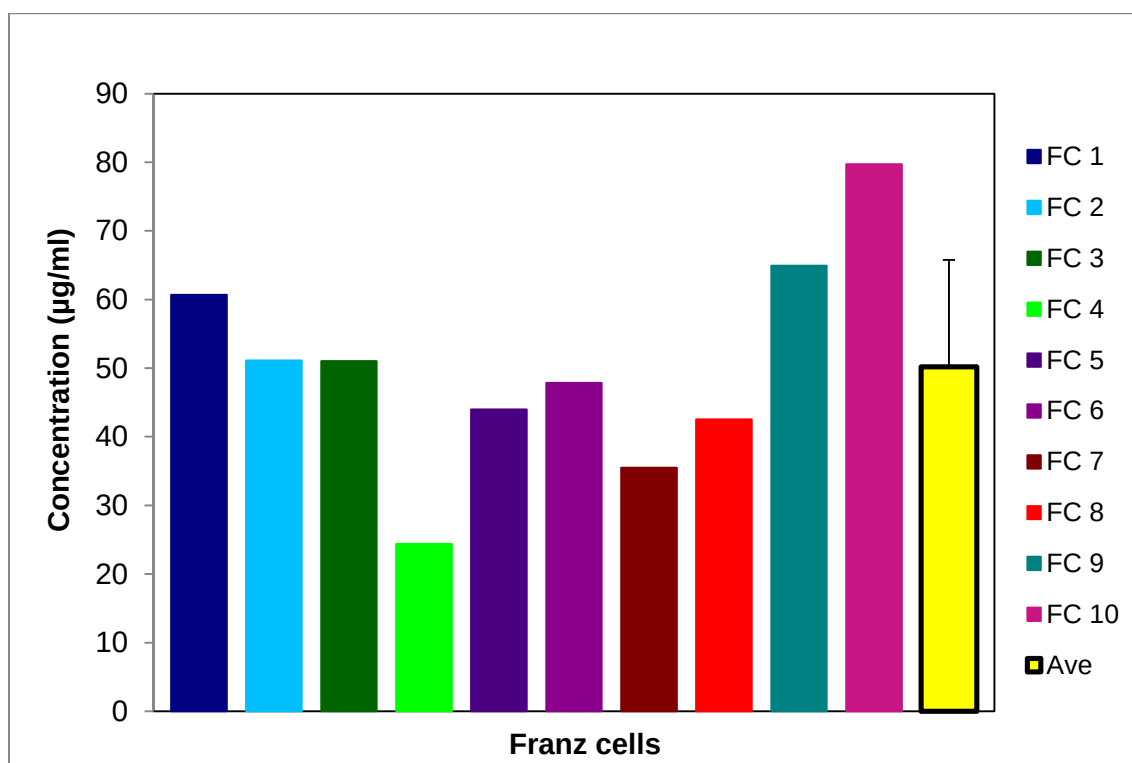


Figure F.35: Roxithromycin concentration (µg/ml) from the **N-CS** present in the ED ($n = 10$)

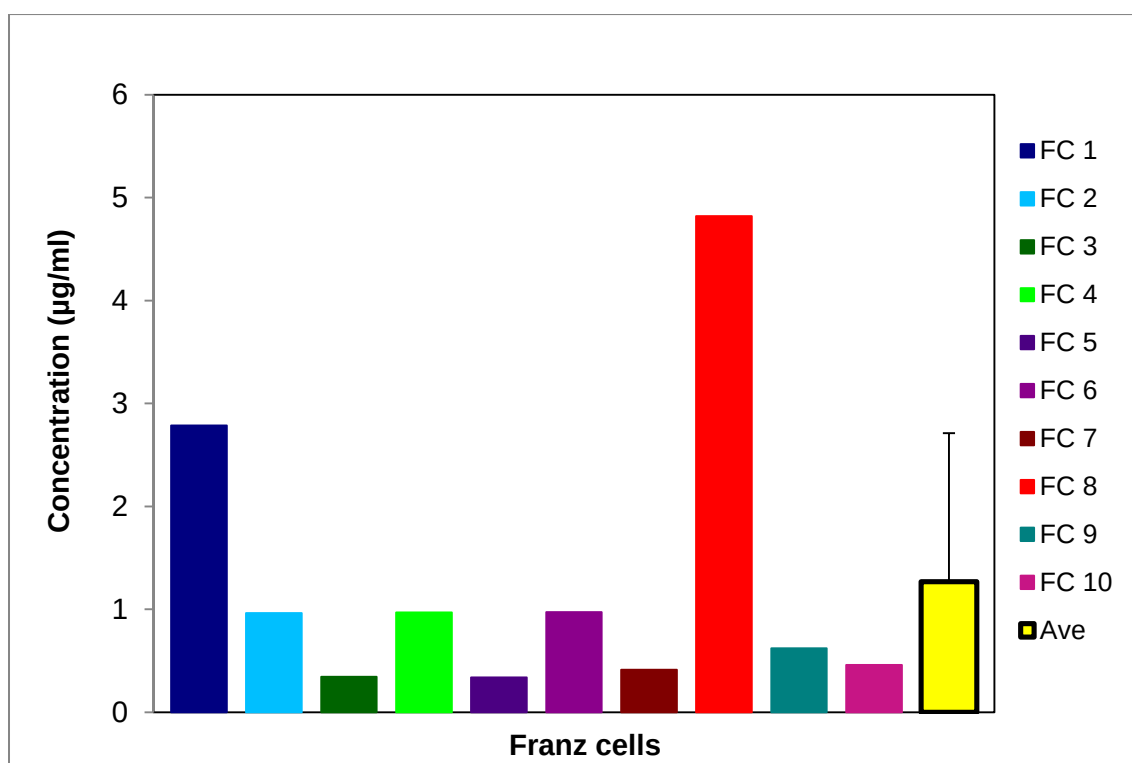


Figure F.36: Roxithromycin concentration (µg/ml) from the **N-AH** present in the ED ($n = 10$)

Table F.4: The average and median concentration ($\mu\text{g/ml}$) of roxithromycin present in the ED after tape stripping (n = amount of Franz cells used)

Emulgel	n	Average concentration in the ED ($\mu\text{g/ml}$)	Median concentration in the ED ($\mu\text{g/ml}$)
L-MH	10	23.10 ± 7.64	23.18
L-CS	10	25.97 ± 8.51	22.92
L-AH	9	19.46 ± 7.38	15.58
N-MH	10	0.90 ± 0.56	0.86
N-CS	10	50.18 ± 15.59	50.54
N-AH	10	1.27 ± 1.44	0.81

When the vesicle emulgels (liposomal and niosomal emulgels) were compared, it was seen that **L-MH** resulted in the highest ED delivery ($23.18 \mu\text{g/ml}$), followed by **L-CS** ($22.92 \mu\text{g/ml}$) and lastly, **L-AH** ($15.58 \mu\text{g/ml}$). For the niosomal emulgels, **N-CS** resulted in the highest ED delivery with a median concentration of $50.54 \mu\text{g/ml}$. **N-AH** resulted in the lowest ED delivery ($0.81 \mu\text{g/ml}$), and **N-MH** also achieved a low median concentration ($0.86 \mu\text{g/ml}$). All liposomal emulgels and **N-CS** resulted in a median concentration above the LOD ($0.75 \mu\text{g/ml}$) and LOQ ($2.51 \mu\text{g/ml}$) for ED delivery. **N-MH** and **N-AH** achieved a value above the LOD, but below the LOQ.

When the different solid-state forms of roxithromycin (**MH**, **CS** and **AH**) were compared, delivery to the epidermis layer ranked from the highest to the lowest concentration as follows: **N-CS**; **L-MH**; **L-CS**; **L-AH**; **N-MH** and **N-AH**. It can be observed that the type of vesicle influenced topical delivery. The rendered amorphous forms conceivably increased the bioavailability of the API (Aucamp *et al.*, 2012:467; Aucamp *et al.*, 2013:18) and the vesicle composition contributed to successful ED delivery. **CS** performed much better when encapsulated in niosomes than liposomes, while **MH** and **AH** had much higher ED delivery for liposome vesicles than niosome vesicles. It is possible that the highly lipophilic **N-MH** and **N-AH** had a greater affinity for the various stratum corneum lipids due to the lipophilic excipients, cholesterol and Span[®], leading to a longer retention time in the SCE layer resulting in poor diffusion to the highly hydrophilic ED layer. **N-MH** and **N-AH** therefore cannot be considered as successful candidates for ED delivery.

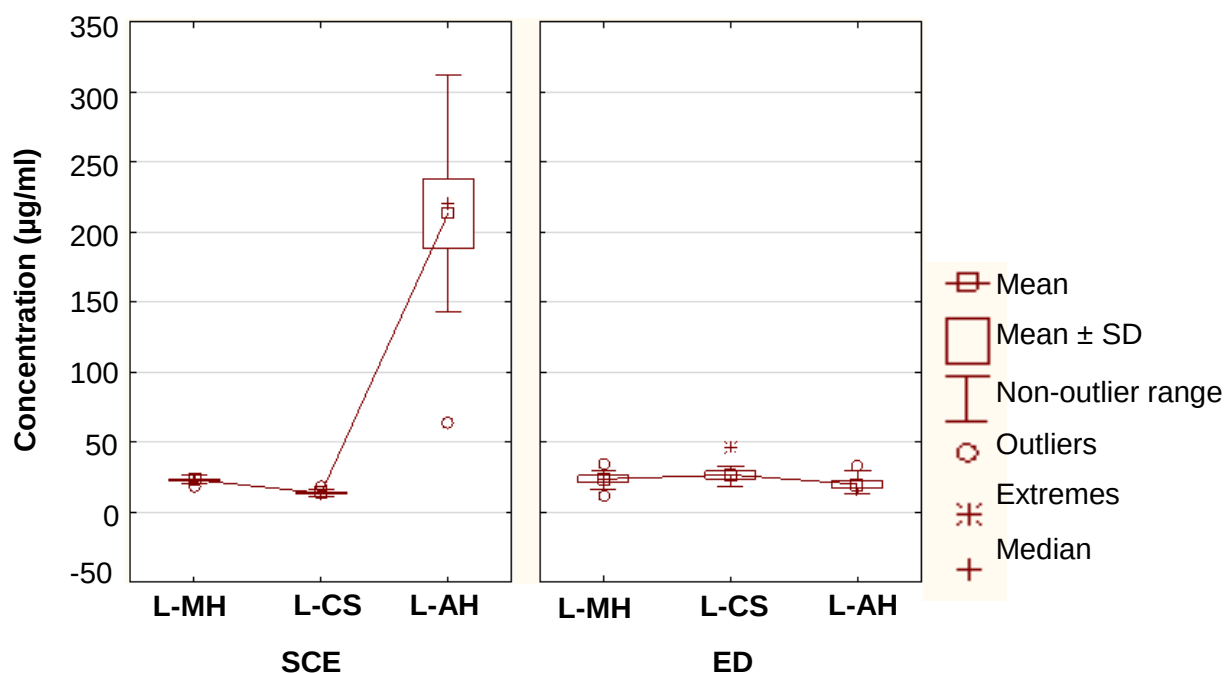


Figure F.37: Box-plot indicating the mean and median concentration (µg/ml) of the liposomal emulgels for roxithromycin present in the SCE and ED

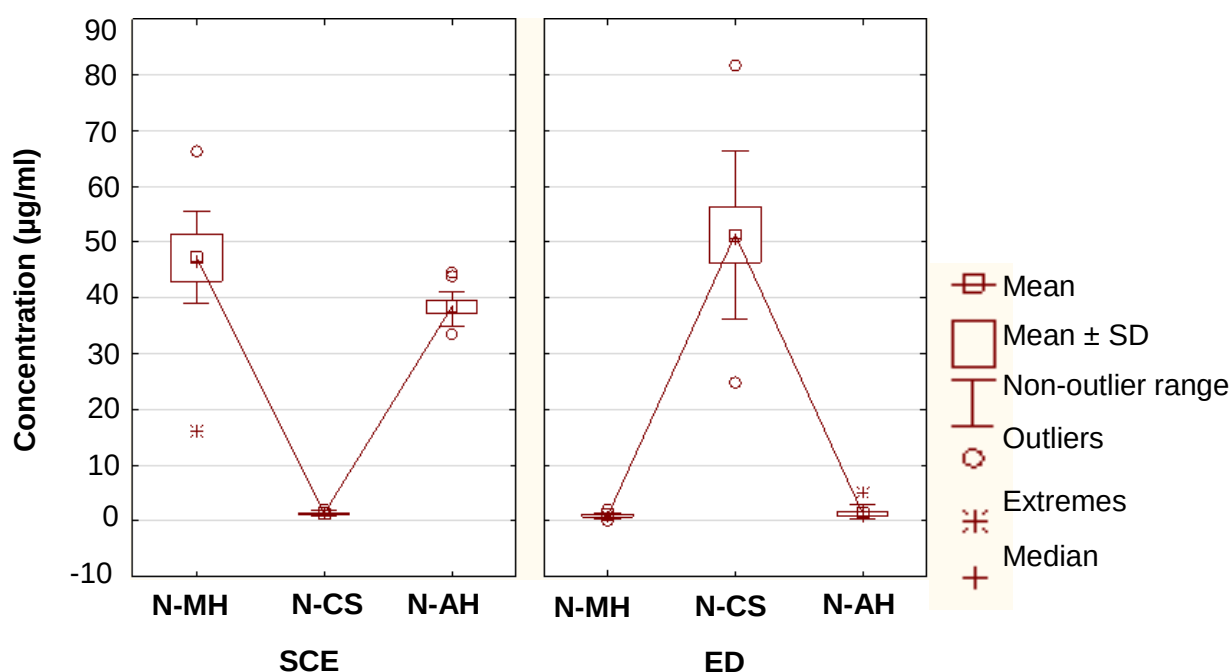


Figure F.38: Box-plot indicating the mean and median concentration (µg/ml) of the niosomal emulgels for roxithromycin present in the SCE and ED

F.5 Statistical data analysis

Statistical methods, such as the analysis of variance (ANOVA), Turkey's honest significance difference (HSD) and t-tests, were performed on the data obtained from diffusion studies.

Results of statistical tests were evaluated with p-values (Consonni & Bertazzi, 2017:328). p-Values obtained for each effect smaller than 0.05, indicated a statistical significant effect according to Concato and Hartigan (2016:1166). Logarithm transformation can be performed in advance on data reflecting non-normal distribution (Hamada, 2018:16). A null hypothesis should be suggested before conducting any statistical analysis (McCluskey & Lalkhen, 2007:209). Under the null hypothesis, the equality of the group means obtained can be equal or superior to what was truly observed, which is defined as the p-value.

The six roxithromycin emulgels were compared during membrane diffusion studies in terms of the respective emulgel formula (liposome or niosome dispersions) and the respective solid-states of roxithromycin. For the skin diffusion studies, data was also compared with regard to the emulgel formulas and the respective solid-states, with the exception of tape stripping data, which was compared in terms of the SCE and ED, in combination with the separate emulgel formulas and solid-states.

F.5.1 Statistical analysis of membrane release studies

The two-way ANOVA performed on the effect of the emulgels (liposomal and niosomal) and effect of the API form (**MH**, **CS** and **AH**) yielded a highly significant interaction between the two types of emulgels and three API forms ($F(2, 53) = 7.23$ ($p = 0.002$)).

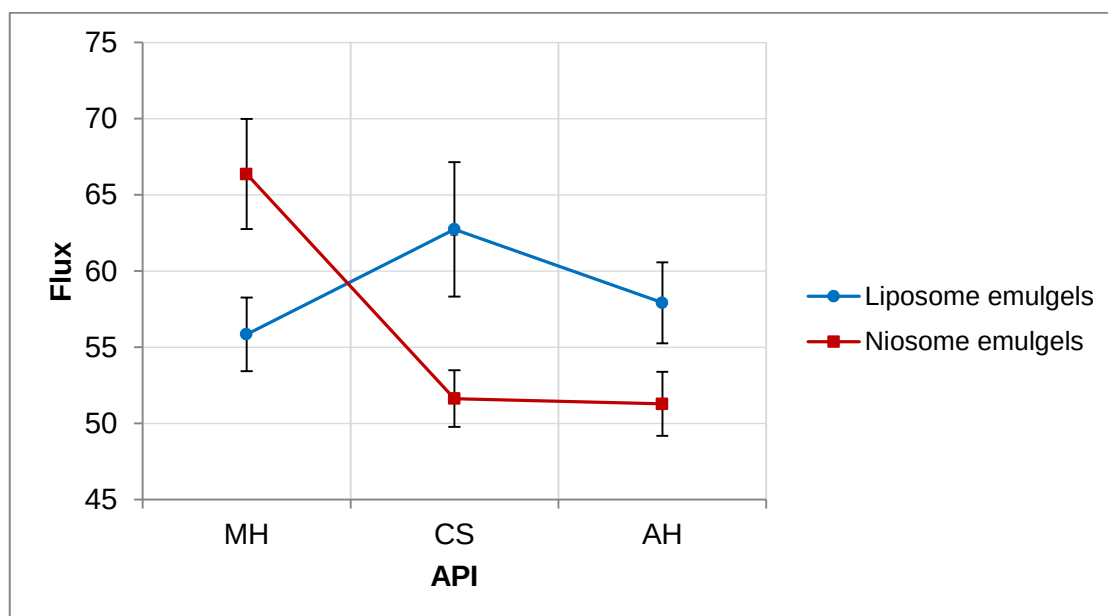


Figure F.39: Means of emulgels containing liposomal and niosomal vesicles capturing the API forms: **MH**, **CS** and **AH**, with standard errors

Due to the significant interaction and no substantial deviation from normality and homogeneity of variances, Tukey's HSD tests were performed to test the significance of pairwise differences between the three means of roxithromycin solid-states, for each of the liposomal and niosomal

emulgels. The results of the liposomal formulas (Table F.5) reported that all p-values were bigger than the desired level of significance 0.05; thus, they were not of interest. The niosomal formulas (Table F.6) resulted in statistical significant p-values (i.e. smaller than 0.05) between **N-MH** and **N-CS**, as well as between **N-MH** and **N-AH** and vice versa.

Table F.5: Tukey's HSD post-hoc test p-values for the pairwise differences in the three solid-states liposomal emulgel formulas

API	MH	CS	AH
MH		0.324	0.909
CS	0.324		0.600
AH	0.909	0.600	

Table F.6: Tukey's HSD post-hoc test p-values for the pairwise differences in the three solid-states niosomal emulgel formulas

API	MH	CS	AH
MH		0.002	0.001
CS	0.002		0.996
AH	0.001	0.996	

T-tests for independent groups, corrected for heterogeneity of variances, were performed on the separate API forms of every emulgel formula. The current effect obtained for the **MH** group could be reported as $t(15.70) = -2.42$ ($p = 0.03$), for the **CS** group as $t(11.95) = 2.27$ ($p = 0.04$) and for the **AH** group as $t(15.68) = 1.96$ ($p = 0.07$). Thus, a significant difference on a 0.05 level was reported for **MH** and **CS**, with **AH** rendering no significant difference between the means of the two emulgels. Since deviation from normality occurred and could have an effect on the aforementioned results, these latter results were confirmed by performing the Mann-Whitney U nonparametric test, which reported the p-values for **MH** as 0.05, for **CS** as 0.03 and for **AH** as 0.08.

F.5.2 Statistical analysis of transdermal delivery

The *in vitro* skin diffusion study results were statistically analysed by means of an ANOVA test, t-tests and Tukey's HSD post-hoc test.

Logarithmic transformation was done in advance to produce near to normal distribution, since statistical data reflected biased distribution. The two-way ANOVA performed on the transdermal delivery results on the effect of the emulgels (liposomal and niosomal) and the API forms (**MH**, **CS** and **AH**) yielded a highly significant interaction between the two types of formulas ($F(2.53) = 41.39$ ($p < 0.001$)).

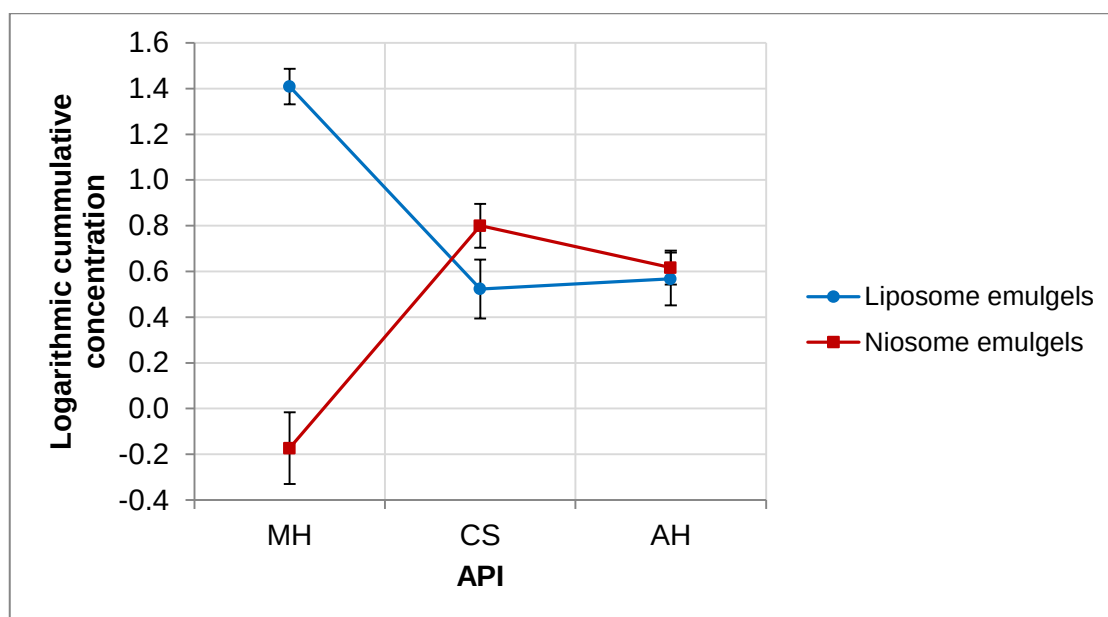


Figure F.40: ANOVA statistical results of transdermal delivery for liposomal and niosomal emulgels including standard errors

Due to the significant interaction and no substantial deviation from normality and homogeneity of variances, Tukey's HSD tests were performed to test the significance of pairwise differences between the three means of the roxithromycin solid-state forms, for each of the liposomal and niosomal emulgels. The results of the liposomal formulas (Table F.7) reported p-values for **L-MH** and **L-CS**, as well as **L-MH** and **L-AH**, and *vice versa*, were highly significant (bigger than 0.05). The niosomal formulas (Table F.8) also resulted in highly significant p-values for **N-MH** and **N-CS**, as well as **N-MH** and **N-AH**.

Table F.7: Tukey's HSD post-hoc test p-values for the pairwise differences in the three solid-states liposomal emulgel formulas

API	MH	CS	AH
MH		0.0001	0.0002
CS	0.0001		0.5048
AH	0.0002	0.5048	

Table F.8: Tukey's HSD post-hoc test p-values for the pairwise differences in the three solid-states niosomal emulgel formulas

API	MH	CS	AH
MH		0.0001	0.0002
CS	0.0001		0.9591
AH	0.0002	0.9591	

T-tests for independent groups, corrected for heterogeneity of variances, were performed on the separate API forms of every emulgel formula. The current effect obtained for the **MH** group can be reported as $t(13.14) = -9.03$ ($p = 0.000001$), for the **CS** group as $t(16.67) = 1.72$ ($p = 0.10447$) and for the **AH** group as $t(13.85) = 0.36$ ($p = 0.72687$). Thus, a highly statistically significant p-value was reported for **MH**, while **CS** and **AH** rendered insignificant p-values.

F.5.3 Statistical analysis of topical delivery

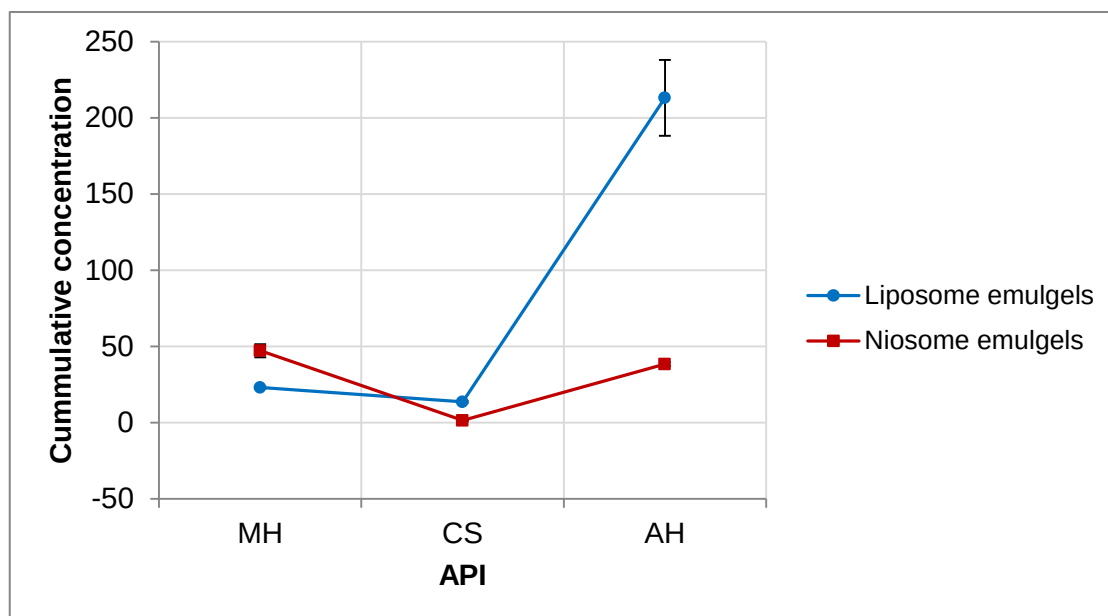


Figure F.41: ANOVA statistical results of SCE delivery for liposomal and niosomal emulgels including standard errors

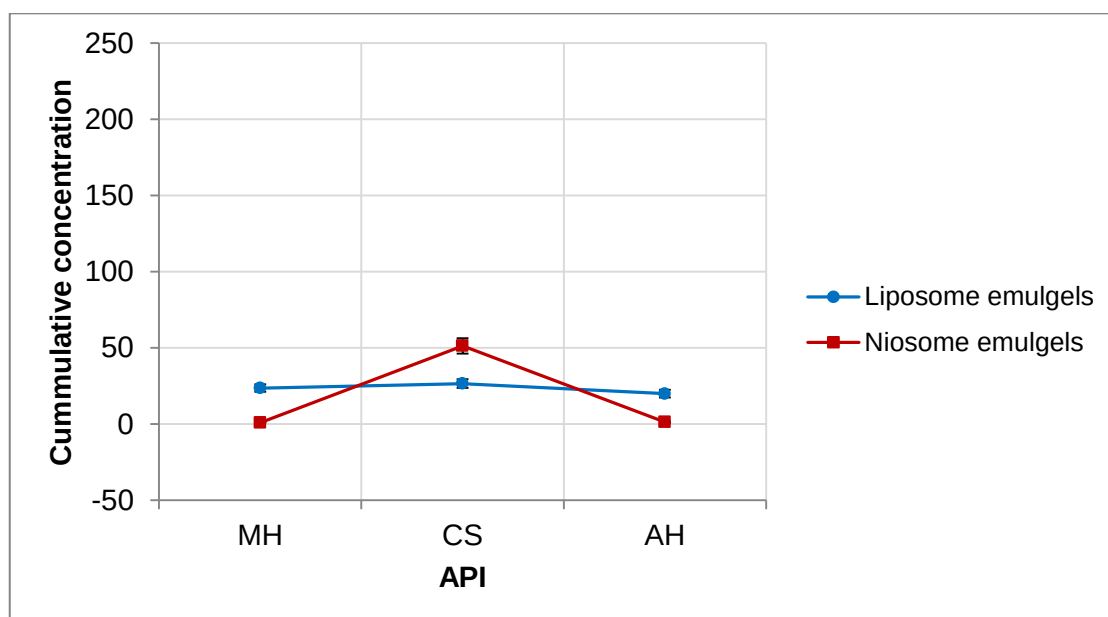


Figure F.42: ANOVA statistical results of ED delivery for liposomal and niosomal emulgels including standard errors

The three-way ANOVA test performed on the effects of the emulgels (liposomal and niosomal), effects of the API form (**MH**, **CS** and **AH**) and effect of different skin layers (SCE and ED), yielded an extremely statistically significant interaction between the liposomal and niosomal emulgels, the three API forms and the two skin layers ($F(2, 106) = 52.96$ ($p < 0.001$)).

Due to the significant interaction and no substantial deviation from normality and homogeneity of variances, Tukey's HSD tests were performed to test the significance of pairwise differences between the three means of roxithromycin solid-state forms, for each of the liposomal and niosomal emulgels.

Table F.9: Tukey's HSD post-hoc test p-values for the pairwise differences in the three solid-states liposomal emulgel formulas for the SCE skin layer

API	MH	CS	AH
MH		0.8692	0.0001
CS	0.8692		0.0001
AH	0.0001	0.0001	

Table F.10: Tukey's HSD post-hoc test p-values for the pairwise differences in the three solid-states liposomal emulgel formulas for the ED skin layer

API	MH	CS	AH
MH		0.6975	0.5935
CS	0.6975		0.2041
AH	0.5935	0.2041	

The results of the liposomal formulas (Table F.9) reported that highly significant p-values (bigger than 0.05) for **L-MH** and **L-AH**, as well as **L-CS** and **L-AH** were obtained for the SCE skin layer. For the ED delivery, no significant p-values were obtained (Table F.10).

Table F.11: Tukey's HSD post-hoc test p-values for the pairwise differences in the three solid-states niosomal emulgel formulas for the SCE skin layer

API	MH	CS	AH
MH		0.0001	0.0526
CS	0.0001		0.0001
AH	0.0526	0.0001	

Table F.12: Tukey's HSD post-hoc test p-values for the pairwise differences in the three solid-states niosomal emulgel formulas for the ED skin layer

API	MH	CS	AH
MH		0.0001	0.9956
CS	0.0001		0.0001
AH	0.9956	0.0001	

The results of the niosomal formulas (Table F.11) reported that highly significant p-values (bigger than 0.05) for **N-MH** and **N-CS**, as well as **N-CS** and **N-AH** were obtained for the SCE skin layer. For the ED delivery, highly significant p-values were obtained for **N-MH** and **N-CS**, as well as **N-CS** and **N-AH** (Table F.12).

T-tests for independent groups, corrected for heterogeneity of variances, were performed on the separate APIs of every emulgel formula, as well as the skin layers. The current effect obtained for the **MH** group for the SCE results can be reported as $t(9.62) = 5.61$ ($p = 0.0003$) and for the ED as $t(9.10) = -9.17$ ($p = 0.000007$). For the **CS** group, the SCE results can be reported as $t(9.57) = -17.01$ ($p = 0.000000$) and the ED results as $t(13.93) = 4.31$ ($p = 0.0007$). For the **AH** group the SCE results can be reported as $t(8.04) = -7.01$ ($p = 0.0001$) and the ED results as $t(8.55) = -7.27$ ($p = 0.00006$). Thus, a significant difference on a 0.05 level was reported for **MH**, **CS** and **AH** between the means of the two emulgel formulas in both the SCE and ED skin layers. Ranking from the most significant difference to the least significant on a 0.05 level as follows: **SCE-CS > ED-MH > ED-AH > ED-CS > SCE-MH > SCE-AH**.

T-tests were also performed on the liposomal and niosomal dispersions with regard to the separate API forms of every emulgel formula. The current effect obtained for the **L-MH** group can be reported as $t(10.79) = -0.23$ ($p = 0.082$). For the **L-CS** group, it can be reported as $t(10.19) = -4.55$ ($p = 0.001$). For the **L-AH** group the results can be reported as $t(8.16) = 7.72$ ($p = 0.00005$). The current effect obtained for the **N-MH** group can be reported as $t(9.033) = 10.92$ ($p = 0.000002$). For the **N-CS** group, it can be reported as $t(9.01) = -9.90$ ($p = 0.000004$). For the **N-AH** group the results can be reported as $t(11.66) = 28.79$ ($p = 0.000000$). Thus, a significant difference on a 0.05 level was reported for **L-CS**, **L-AH**, **N-MH**, **N-CS** and **N-AH** between the means of the two emulgel formulas. Ranking from the most significant difference to the least significant on a 0.05 level was as follows: **N-AH > N-CS > N-MH > L-AH > L-CS > L-MH**.

F.6 Conclusion

The target-site for API delivery was the epidermis-dermis skin layer, as this is where *P. acnes* collects in sebaceous glands leading to acne (Gollnick, 2003:1581, 1585). It was observed

during membrane release studies that the API encapsulated in vesicle systems and formulated into an emulgel successfully released into the receptor compartment, with the liposomal and niosomal vesicle systems achieving corresponding results. The conclusion could thus be made, since the APIs were released, to continue with skin diffusion studies and tape stripping to study if the API would diffuse through the skin or into the different skin layers.

The targeted topical delivery proved successful during skin diffusion studies, as there was little transdermal API delivered (as seen in Table F.2), with bigger traces of roxithromycin found in the skin layers. The SCE results, as seen in Table F.3, indicated that roxithromycin permeated the outer skin layer from the liposomal and niosomal emulgels. The six emulgels containing the vesicle systems were able to deliver the API into deeper skin layers (ED); **N-MH** and **N-AH** resulted in values lower than the LOQ, making them less ideal for the intended use of the emulgel. It was evident that **AH** had the lowest ED delivery for both the liposomal and niosomal emulgels, whilst **CS** revealed the lowest SCE delivery and the highest ED delivery for both the liposomal and niosomal emulgels, suggesting that this solid-state form of roxithromycin had the most favourable physicochemical properties to reach the target-site when included in an ASD. It is important to remember that the selected excipients during formulation play a significant role in the outcome of topical delivery. Considering the target-site, **N-CS** was the best contender for the topical treatment of acne resulting in the highest ED delivery. It can be concluded that an emulgel containing roxithromycin entrapped in either the liposomal or niosomal emulgel can successfully serve as a novel acne treatment.

References

- Ademola, J. 1997. Drug delivery from topical formulations. (*In* Ghosh, T.K. & Pfister, W.R., eds. Transdermal and topical delivery systems. Illinois: Interpharms Press Inc, p.511-538).
- Aucamp, M., Liebenberg, W., Strydom, S.J., Van Tonder, E.C. & De Villiers, M.M. 2012. Physicochemical properties of amorphous roxithromycin prepared by quench cooling of the melt or desolvation of a chloroform solvate. American association of pharmaceutical scientist, PharmSciTech, 13:467-476.
- Aucamp, M., Stieger, N., Barnard, N. & Liebenberg, W. 2013. Solution-mediated phase transformation of different roxithromycin solid state forms: implications on dissolution and solubility. International journal of pharmaceutics, 449:18-27.
- Barry, B.W. 2001. Novel mechanisms and devices to enable successful transdermal drug delivery. European journal of pharmaceutical sciences, 14:101-114.
- Boshoff, S. 2018. Investigation of the stabilising effects of niosomes on the amorphous forms of roxithromycin. Potchefstroom: NWU. (Dissertation - MSc).
- Censi, R. & Martino, P.D. 2015. Polymorph impact on the bioavailability and stability of poorly soluble drugs. Molecules, 20:18759-18776.
- Concato, J. & Hartigan, J.A. 2016. P-values: from suggestion to superstition. Journal of investigative medicine, 64:1166-1171.
- Consonni, D. & Bertazzi, P.A. 2017. Health significance and statistical uncertainty: The value of P-value. Medicina del lavoro, 108: 327-331.
- Csongradi, C. 2015. The effect of solid-state forms on the topical delivery of roxithromycin. Potchefstroom: NWU. (Dissertation - MSc).
- Dawson, B. & Trapp, R.G. 2004. Basic & clinical biostatistics. 4th ed. New York: McGraw-Hill.
- Drugbank. 2019. Roxithromycin. <http://www.drugbank.ca/drugs/DB00778> Date of access: 17 Jun. 2019.
- Gollnick, H. 2003. Current concepts of the pathogenesis of acne: Implications for Drug Treatment. Drugs, 63:1579-1596.
- Hamada, C. 2018. Statistical analysis for toxicity studies. Journal of toxicological pathology, 31:15-22.

- Honeywell-Nguyen, P.L. & Bouwstra, J.A. 2005. Vesicles as a tool for transdermal and dermal delivery. *Drug discovery today: technologies*, 2:67-74.
- Khalid, F., Goroudi, F. & Maibach, H.I. 2016. Anti-aging topical peptides and proteins. (In Sivamani, R.K., Jagdeo, J.R., Elsner, P. & Maibach, H.I., eds. *Cosmeceuticals and active cosmetics*. New York: CRC Press, p.127-131).
- Kirjavainen, M., Urtti, A., Valjakka-Koskela, R., Kiesvaara, J. & Mönkkönen, J. 1999. Liposome-skin interactions and their effects on the skin permeation of drugs. *European journal of pharmaceutical sciences*, 7:279-286.
- McCluskey, A. & Lalkhen, A.G. 2007. Statistics IV: Interpreting the results of statistical tests. *Continuing education in anaesthesia, critical care & pain*, 7:209-212.
- Modi, P.B. & Shah, N.J. 2015. Optimization of an *in vitro* release test for topical formulations containing eberconazole nitrate and mometasonefuroate. *Der pharma chemica*, 7:1-9.
- Montenegro, L., Paolino, D., Drago, R., Pignatello, R., Fresta, M., Puglisi, G. 2006. Influence of liposome composition on *in vitro* permeation of diosmin through human stratum corneum and epidermis. *Journal of drug delivery and scientific technology*, 6:133-140.
- Naik, A., Kalia, Y.N. & Guy, R.H. 2000. Transdermal drug delivery: overcoming the skin's barrier function. *Pharmaceutical science and technology today*, 3:318-326.
- N'Da, D.D. 2014. Prodrug strategies for enhancing the percutaneous absorption of drugs. *Molecules*, 19:20780-20807.
- Ng, S.F., Rouse, J., Sanderson, D. & Eccleston, G. 2010. A comparative study of transmembrane diffusion and permeation of ibuprofen across synthetic membranes using Franz diffusion cells. *Pharmaceutics*, 2:209-223.
- OECD **see** Organisation for economic co-operation and development
- Organisation for economic co-operation and development. 2004. Guidance document for the conduct of skin absorption studies: OECD series on testing and assessment, No. 28. <http://www.oecd-ilibrary.org/docserver/download/9750281e.pdf?expires=1472733343&id=id&accname=guest&checksum=C1CE754CE29D5DC604CA843D8951E854> Date of access: 18. Jun. 2019.
- Purohit, R. & Venugopalan, P. 2009. Polymorphism: an overview. *Resonance*, 14:882-893.

Sakdiset, P., Okada, A., Todo, H. & Sugibayashi, K. 2018. Selection of phospholipids to design liposome preparations with high skin penetration - enhancing effects. *Journal of drug delivery science and technology*, 44:58-64.

Sah, S.K., Badola, A. & Nayak, B.K. 2017. Emulgel: magnifying the application of topical drug delivery. *Indian journal of pharmaceutical and biological research*, 5:25-33.

Shahzad, Y., Louw, R., Gerber, M. & Du Plessis, J. 2015. Breaching the skin barrier through temperature modulations. *Journal of controlled release*, 202:1-13.

Som, I., Bhatia, K., & Yasir, M. 2012. Status of surfactants as penetration enhancers in transdermal drug delivery. *Journal of pharmacy and bioallied sciences*, 4:2-9.

Surber, C., Schwarb, F.P. & Smith, E.W. 1999. Tape-stripping technique. (*In* Bronaugh, R.L. & Maibach, H.I. eds. *Percutaneous absorption: drug--cosmetics-mechanisms-methodology*. 3rd ed. New York: Marcel Dekker, p.395-409).

Swart, M. 2019. Stability of amorphous forms of roxithromycin when encapsulated in liposomes. Potchefstroom: NWU. (Dissertation - MSc).

Wiechers, J.W. 2008. Measuring skin delivery. (*In* Wiechers, J.W., ed. *Science and applications of skin delivery systems*. Carol Stream, IL: Allured publishing corporation, p.23-28).

Williams, A.C. 2013. Topical and transdermal drug delivery. (*In* Aulton, M.E., ed. *Aulton's pharmaceuticals: the design and manufacture of medicines*. 4th ed. London: Churchill Livingstone, p.675-697).

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