Development of a topical self-emulsifying drug delivery system for optimised delivery

D van Staden

orcid.org/ 0000-0001-8652-3205

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Supervisor: Prof JM Viljoen

Co-supervisor: Prof J du Plessis

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Student number: 25057146

A bit of science distances one from God, but much science nears one to Him. The more I study science, the more I stand amazed at the work of the Creator.

~Louis Pasteur~

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Abstract

Development of a topical SEDDS for optimised delivery

In order to achieve successful receptor binding affinity at complex pharmacological targets the majority of drugs are of a lipophilic nature. However, optimisation of drug delivery systems for lipophilic drugs presents challenges. Self-emulsifying drug delivery systems (SEDDSs) can provide potential solutions to development problems of lipid-based drug delivery systems. Research reported successful development of SEDDSs designed for oral-, vaginal-, ocular-, rectal- and nasal administration. In contrast, SEDDSs intended for optimised for topical drug delivery has received limited attention.

Clofazimine (log P of 7.66) has been incorporated into multidrug-resistant tuberculosis (MDR-TB) regimens in an attempt to decrease treatment times and enhance activity against resistant bacteria. However, clofazimine poses oral drug delivery challenges as well as adverse effects including gastric accumulation. SEDDSs indicated for topical application can bypass liver metabolism together with gastric accumulation. Nonetheless, the lipophilic outermost skin layer remains a formidable barrier, especially to a drug as lipophilic as clofazimine. Therefore, SEDDSs were chosen as a vehicle since skin penetration enhancers can be included in the form of natural oils, utilised to solubilise clofazimine while establishing *stratum corneum* (SC) lipid disruption to enhance topical delivery. Furthermore, development of a topical SEDDS can provide alternative treatment in terms of extra-pulmonary tuberculosis (EPTB) infections, specifically cutaneous tuberculosis (CTB). This incidence of this otherwise rare EPTB manifestation has increased in recent time due to MDR-TB strains coupled with enhanced tuberculosis (TB) incidence in immunocompromised patients. Currently similar oral anti-tubercular regimens are employed to treat both pulmonary TB and CTB. However, topical SEDDS may have a beneficial outcome in terms of localised effect which will thereby minimise drug interactions if patients are also receiving pulmonary TB treatment.

An approach of quality-by-design and characterisation was followed during development of SEDDS formulations intended for optimised topical delivery of clofazimine. Solubility of clofazimine was determined in water, argan-, avocado-, coconut-, macadamia- and olive oil. Next, water titration experiments were conducted for the purpose of identifying the spontaneous emulsification capacity of different excipients. After establishing self-emulsification regions, check-point formulations were selected within the self-emulsification area itself where favourable drug delivery system properties, in terms of topical application, could be predicted. Hereafter, check-point formulations, that did not depict phase separation

at an ambient temperature after a period of 24 h, were submitted to characterisation experiments. Subsequently, characterisation profiles identified SEDDSs with favourable topical drug delivery properties.

Dermal diffusion studies involving *in vitro* topical delivery of clofazimine were successful. Remarkably, olive oil SEDDS achieved the highest topical clofazimine delivery. This may have been facilitated by increased oleic acid content of olive oil, as oleic acid is known to enhance SC lipid disruption and thereby enhance dermal delivery of drugs. Isothermal microcalometric experiments were conducted in order to confirm compatibility of the SEDDS excipients. Potential interactions were observed between argan oil and clofazimine in addition to the combinations of Span®60 and argan-, macadamia- and olive oil, respectively. Nonetheless, the aim of this study was successfully met through the development of selected topical SEDDS that achieved optimised topical clofazimine delivery.

Keywords: Clofazimine, cutaneous tuberculosis, self-emulsifying drug delivery systems, topical delivery

Preface

This dissertation is introduced in article format comprising sub-chapters, an article submitted to *Tuberculosis Journal* with guidelines for authors included in Annexure G and an article submitted to *AAPS PharmSciTech Journal* with guidelines for authors provided in Annexure H.

Author contributions: Prof JM Viljoen and Prof J du Plessis contributed to the conceptualisation. Prof JM Viljoen and Ms Daniélle van Staden were responsible for the methodology, validation and formal analysis; Ms Daniélle van Staden conducted the investigation and procured the resources; Prof JM Viljoen, Prof J du Plessis and Ms Daniélle van Staden did the data curation; Ms Daniélle van Staden wrote the original draft preparation and contributed in review and editing to generate the final submitted version; Prof JM Viljoen and Prof J du Plessis reviewed theoriginal draft and made suggestions to improve the quality of the final draft; Prof JM Viljoen and Prof J du Plessis were responsible for the supervision of the study; Prof JM Viljoen handled the project administration and Prof JM Viljoen together with Prof J du Plessis the funding acquisition. All authors have read and agreed to the published version of the dissertation.

Chapter 1

Introduction, Problem Statement, Aims and Objectives

1.1 Introduction

The development of novel drug entities is both a prolonged and expensive process (Breckenridge & Jacob, 2019; Pushpakom *et al*., 2018). A possible strategy to avoid these costly procedures is to redirect the use of existing drugs towards treating general as well as orphaned forms of diseases (Marcinkute *et al*., 2019; Pushkaran *et al*., 2019; Muthyala, 2011). Given that existing drugs have been thoroughly characterised in terms of their adverse effect profiles during treatment of a primary disease, these drug entities can be repurposed by taking into account these adverse effect profile (Herma Sree *et al*., 2019). Repurposing of known drugs can be divided into four main approaches, namely: product, concept, action and use (Langedijk *et al*., 2015). For the purpose of this study the known drug clofazimine employed for the treatment of multi-bacillary leprosy (Savla *et al*., 2017; Pai, 2015), will be incorporated into topical self-emulsifying drug delivery systems (SEDDSs) in order to provide a supplementary as well as localised treatment for cutaneous tuberculosis (CTB). Clofazimine has been reintroduced to aid in treatment of TB in an attempt to shorten the current treatment regimen (Lange *et al*., 2019; Mirnejad *et al.*, 2018; Sotgiu *et al*., 2017; van Deun *et al*., 2004). Moreover, synergism between clofazimine and pyrazinamide (a first line anti-tubercular agent) has been established (Ammerman *et al*., 2018), thus, rendering clofazimine as a useful drug (Brennan & Young, 2008).

Redirecting current drugs to provide global relief against anti-microbial resistance, especially observed during the treatment of resistant TB strains, provides an immediate short term solution (Herma Sree *et al*., 2019; Hind *et al*., 2019; Smani *et al*., 2019). The increased anti-tubercular drug resistance is portrayed in the increased incidences of extra-pulmonary TB (EPTB) manifestations (Sharma *et al*., 2017). According to Sharma *et al* (2017) 15% of first time TB cases are of an extra-pulmonary nature. This can be linked to increased anti-microbial resistance and the high prevalence of co-infection with the Human Immunodeficiency Virus (HIV) (Hasan *et al*., 2018; Sharma *et al*., 2017). As CTB is a rare form of EPTB, this daunting disease is frequently misdiagnosed or simply overlooked (De Maio *et al*., 2016; Frankel *et al*., 2009). CTB treatment regimens are torturous considering that similar regimens are prescribed to treat CTB as well as pulmonary TB infection (Chen *et al*., 2019; van Zyl *et al*., 2015). Moreover, anti-tubercular treatment is generally accompanied by unpleasant adverse effects (Erwin *et al*., 2019; Nemati *et al*., 2019). Clofazimine is no exception as this lipophilic drug

tends to accumulate in tissues such as the liver, reticulo-endothelial system, breast, macrophages and intestines as is observed during treatment of leprosy (Swanson *et al*., 2016; Yoon *et al*., 2016). Hence, liver metabolism as well as general adverse effects accompanying treatment by clofazimine treatment will be bypassed by altering the route of clofazimine administration to that involving topical SEDDSs. Bypassing liver metabolism through dermal drug delivery has displayed favourable results in terms of decreasing adverse effects of drugs such as meloxicam and hopefully this study can contribute to achieve similar results for dermal clofazimine delivery (Machado *et al*., 2018).

Clofazimine is considered a highly lipophilic drug with a Log P value of 7.66 (Sangana *et al*., 2018). Lipid based drug delivery systems, including SEDDSs, are extremely versatile platforms employed to optimise delivery of lipophilic drugs (Savla *et al*., 2017). The oily component of SEDDSs can enhance solubility of clofazimine in order to expose the skin to sufficient clofazimine concentrations (Chandrakar *et al*., 2017). Moreover, disruption of the naturally occurring lipids of the *stratum corneum* (SC) can also be effected by the skin penetration enhancing effects of plant based oils (Lin *et al*., 2018; Vaughn *et al*., 2018; Viljoen *et al*., 2015). For the current study, five plant based oils will be individually included as a lipid component of various SEDDS formulations in order to establish which plant based oil facilitates the best dermal drug delivery of clofazimine. Plant based oils present additional topical application benefits; for example, the presence of anti-microbial compounds occurring in the oil may prevent secondary bacterial infections involving CTB disease that leads to open lesions (Lin *et al*., 2018; Vaughn *et al*., 2018; Santos *et al*., 2014). Furthermore, surface active agents are included to facilitate formation of the interfacial film (surfactant) and to ensure flexibility of this interfacial layer (co-surfactant) established between the lipid- and aqueous phase (Rani *et al*., 2019). Moreover, appropriate inclusion of surface active agents can establish formation of self micro-emulsifying drug delivery systems (SMEDDS) or self nano-emulsifying drug delivery systems (SNEDDS) due to its capacity to finely disperse droplets (Rani *et al*., 2019). Additionally, surface active ingredients similarly possess the potential to disrupt lipids present in the SC and render improve dermal drug delivery (Yanase & Hatta, 2018). The to be used in this study are indicated in **Figure 1.1.**

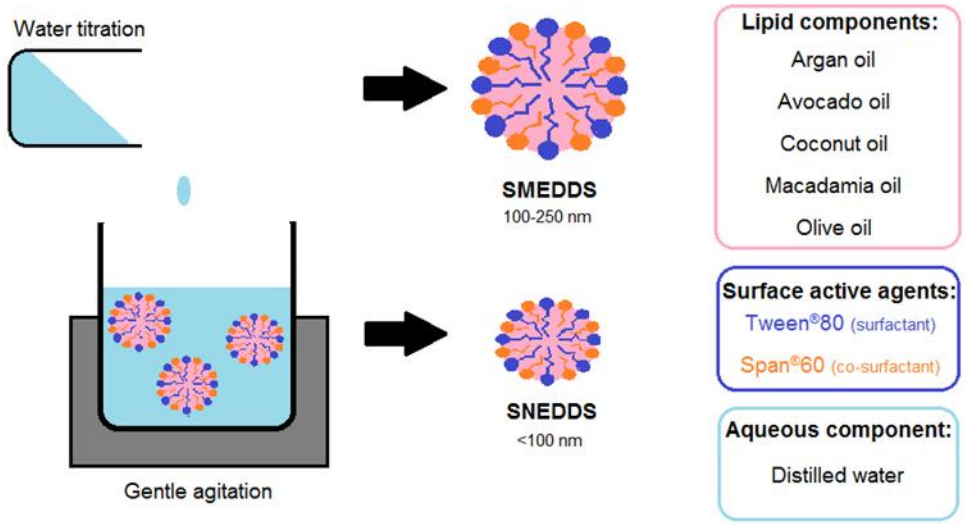

Figure 1.1: Diagrammatic illustration of processes and components employed during formulation of SEDDSs (adapted from Rani *et al*., 2019)

According to **Figure 1.1**, SEDDS formulations may be prepared utilising the water titration method (Prajapat *et al*., 2017). This method is a simplified process when compared to other formulation techniques utilised to formulate general emulsions (Gonçalves *et al*., 2018). The concept of spontaneous emulsification is to establish self-emulsification of oil-in-water emulsions by introducing the already combined oil- and surfactant phase (i.e. surfactant and co-surfactant) to the aqueous phase during gentle agitation at an ambient temperature of 25°C (Rohrer *et al*., 2018; Patel *et al*., 2011). This phenomenon is linked to low interfacial energy that exists between specified combinations as well as concentrations of the incorporated components. This facilitate spontaneous emulsification and thus generate isotropic and thermodynamically stable solutions (Rani *et al*., 2019; Rohrer *et al*., 2018, Solans *et al*., 2016).

In order to determine optimum concentrations of excipients utilised in combination, pseudo-ternary phase diagrams are required (Ujhelyi *et al*., 2018). Pseudo-ternary phase diagrams are employed to illustrate the region of self-emulsification as displayed by different ratios of surfactant phase to oil phase during the dropwise addition of the aqueous phase (Patel *et al*., 2011). Hence, water is added in a dropwise fashion and sufficient time is allowed for gentle agitation of the mixtures between addition of each water drop so as to observe a change in the turbidity of mixtures (Syed & Peh, 2014). The end point marks the concentration of excipients at which the mixture becomes turbid and remains turbid while subjected to moderate agitation (Ke *et al*., 2016; Czajkowska-Kośnik *et al*., 2015; Wang *et al*., 2015). At this point, self-emulsification is established and these concentrations are plotted on pseudo-ternary phase diagrams to indicate the self-emulsification area (Balata, 2018). Once the self-

emulsification regions of the different SEDDSs are identified, the achievability of emulsion formation can be evaluated and the emulsion characteristics may be investigated (Wang *et al*., 2015).

Topical administration of SEDDSs has been used successfully used for treatment of vaginal- and ocular infections (Köllner *et al*., 2017; ElKasabgy, 2014). In contrast, application of SEDDSs to the skin has received limited attention (Khan *et al*., 2018; Pratiwi *et al*., 2017). Therefore, clofazimine is reformulated by using SEDDSs for improving dermal drug delivery in order to present alternative as well as supplementary treatment for CTB.

SEDDSs were originally developed to improve oral delivery of lipophilic drugs (Rohrer *et al*., 2018). Studies aimed at enhancing oral drug delivery by utilising SEDDSs provided promising results (Efiana *et al*., 2018; Bhandari *et al*., 2017; Parakh *et al*., 2016; Chow *et al*., 2015). However, the skin presents a formidable drug delivery barrier due to the lipophilic nature of the outermost layer (Bellefroid *et al*., 2019). SEDDSs can prove to be a useful instrument employed to facilitate dermal drug delivery. Bernkop-Schnürch and Jalil (2018) claimed drug release from SEDDS formulations to be a modest diffusion process that occurs from the lipid phase to the aqueous phase. Therefore, in order to obtain drug release from SEDDSs, the drug has to diffuse to the surface of the oil droplets. Subsequently, the drug molecules will reach the aqueous medium if the interfacial barrier can be crossed (Bernkop-Schnürch & Jalil, 2018). Hence, the lipid component of SEDDSs can contribute towards penetration of the SC, whereas the aqueous component of SEDDSs can possibly facilitate delivery into the underlying, aqueous skin layers (Burger *et al*., 2015; Viljoen *et al*., 2015). As a result, successful topical delivery of the highly lipophilic clofazimine by means of SEDDS may find application in the treatment of CTB.

1.2 Research problem

Currently CTB is treated by anti-tubercular agents via oral or intravenous administration (Chen *et al*., 2019; van Zyl *et al*., 2015). However, as in the case of pulmonary TB, the treatment regimens for CTB are protracted and difficult. As only limited last line anti-tubercular agents are available to treat multi-drug resistant TB (MDR TB) and extensively drug resistant TB (XDR TB) (Roycroft *et al*., 2018; Boru *et al*., 2017), it is vitally important to ensure patient compliance and avoid resistance to first line anti-tubercular agents. Therefore, the development of a formulation utilising a supplementary route for anti-tubercular drug administration should contribute to improve patient compliance, but may also decrease the treatment time (Lange *et al*., 2019). Furthermore, the development of a SEDDSs containing clofazimine may prove vital in improving the solubilisation of this highly lipophilic drug, and consequently the diffusion of clofazimine into the affected skin.

1.3 Aims and objectives

This study is aimed at developing a SEDDS to deliver the highly lipophilic drug clofazimine by means of topical application. Selected plant based oils will be included as critical solubilisation components in the selected SEDDS formulations while providing attractive dermatological benefits (Lin *et al*., 2018). A surfactant (Tween®80) and a co-surfactant (Span®60) will be incorporated into these formulations in a 1:1 ratio to decrease tension at the interface between the oil and water components in order to minimise the input energy required for self-emulsification, as well as to improve the thermodynamic stability of the selected SEDDS formulations (Gurram *et al*., 2015). The chosen plant based oils are moderately accessible, safe for external application and can contribute to improved clofazimine solubilisation (Vaughn *et al*., 2018; Zhang *et al*., 2015). Pseudo-ternary phase diagrams will be employed to provide a supportive method to determine the optimum concentrations of the various ingredients utilised in combination (Prajapat *et al*., 2017; Wang *et al*., 2015). Application of an optimum concentration combination of the ingredients onto the skin may facilitate increased topical delivery of the highly lipophilic clofazimine.

Therefore, the objectives of this study are to;

- Verified that the analytical method, namely high performance liquid chromatography (HPLC) was able to be used for determination of clofazimine concentration.
- Determined the solubility of clofazimine in the selected plant oils, namely argan-, avocado-, coconut-, macadamia- and olive oil.
- Constructed pseudo-ternary phase diagrams to establish the required concentration of each ingredient in order to formulate a SEDDS successfully.
- Evaluated the SEDDSs in terms of droplet size, size distribution, zeta-potential, robustness to dilution, self-emulsification, cloud point determination, thermodynamic stability, viscosity and pH.
- Selected the most optimum topical SEDDS formulations after analysis of the results obtained from the various tests performed.
- Conducted membrane release studies to establish clofazimine release from the selected topical SEDDS formulations.
- Performed skin diffusion as well as tape stripping experiments to determine transdermal and topical delivery of clofazimine, respectively.
- Determined if any potential incompatibilities exist between the included excipients by employing isothermal microcalorimetry.

References

Ammerman, N.C., Swanson, R.V., Bautista, E.M., Almeida, D.V., Saini, V., Omansen, T.F., Guo, H., Chang, Y.S., Li, S.-Y., Tapley, A., Tasneen, R., Tyagi, S., Betoudji, F., Moodley, C., Ngcobo, B., Pillay, L., Bester, L.A., Singh, S.D., Charisson, R.E., Nuermberger, E. & Grosset, J.H. 2018. Impact of clofazimine dosing on treatment-shortening of the first-line regimen in a mouse model of tuberculosis. *Antimicrobial agents and chemotherapy*, 62:Article ID e00636-18. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6021677/. Date of access: 26 Oct. 2019.

Balata, G.F. 2018. Formulation and evaluation of gliclazide in vegetable oil-based self emulsifying delivery system. *Journal of applied pharmaceutical science*, 8:023-033.

Bellefroid, C., Lechanteur, A., Evrard, B. & Piel, G. 2019. Lipid gene nanocarriers for the treatment of skin diseases: current state-of-the-art. *European journal of pharmaceutics and biopharmaceutics*, 137:95-111.

Bernkop-Schnürch, A. & Jalil, A. 2018. Do drug release studies from SEDDS make any sense? *Journal of controlled release*, 271:55–59

Bhandari, S., Rana, V. & Tiwary, A.K. 2017. Antimalarial solid self-emulsifying system for oral use: *in vitro* investigation. *Therapeutic delivery*, 8:201-213.

Boru, C.G., Shimels, T. & Bidal, A.I. 2017. Factors contributing to non-adherence with treatment among TB patients in Sodo Woreda, Gurage Zone, Southern Ethiopia: a qualitative study. *Journal of infection and public health*, 10:527-533.

Breckenridge, A. & Jacob, R. 2019. Overcoming the legal and regulatory barriers to drug repurposing. *Nature reviews drug discovery*, 18:1-2.

Brennan, P.J., & Young, D.B. 2008. Tuberculosis: handbook of anti-tuberculosis agents. Amsterdam: Elsevier, 88(2): 85-170 http://www.tballiance.org/newscenter/research_papers/TB_DB_Final.pdf. Date of access: 22 Jul. 2019.

Burger, C., Gerber, M., Du Preez, J.L. & Du Plessis J. 2015. Optimised transdermal delivery of pravastatin. *International journal of pharmaceutics*, 496:518–525.

Chandrakar, A., Sahu, B., Sahu, H., Dewangan, J., Kumar, N., Kumar, D., Sahu, B., Dewangan, K., Kaushal, R., Agrawal, M. & Tripathi, D.K. 2017. Review on the formulation considerations needed to produce a stable self micro emulsifying drug delivery system (SMEDDS). *Research journal of pharmacy and technology*, 10:1563-1570.

Chen, Q., Chen, W. & Hao, F. 2019. Cutaneous tuberculosis: A great imitator. *Clinics in dermatology*, 37:192-199.

Chow, P.Y., Gue, S.Z., Leow, S.K. & Goh, L.B. 2015. Solid self-microemulsifying system (S-SMECS) for enhanced bioavailability and pigmentation of highly lipophilic bioactive carotenoid. *Powder technology*, 274:199-204.

Czajkowska-Kośnik, A., Szekalska, M., Amelian, A., Szymańska, E. & Winnicka, K. 2015. Development and evaluation of liquid and solid self-emulsifying drug delivery systems for atorvastatin. *Molecules*, 20:21010-21022.

De Maio, F., Trecarichi, E.M., Visconti, E., Sanguinetti, M., Delogu, G. & Sali, M. 2016. Understanding cutaneous tuberculosis: two clinical cases. *JMM case reports*, 3:Article ID e005070. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5343121/. Date of access: 26 Oct. 2019.

Efiana, N.A., Phan, T.N.Q., Wicaksono, A.J. & Bernkop-Schnürch, A. 2018. Mucus permeating self-emulsifying drug delivery systems (SEDDS): About the impact of mucolytic enzymes. *Colloids and surfaces B: biointerfaces*, 161: 28-235.

ElKasabgy, N.A. 2014. Ocular supersaturated self-nanoemulsifying drug delivery systems (S-SNEDS) to enhance econazole nitrate bioavailability. *International journal of pharmaceutics*, 460:33-44.

Erwin, E.R., Addison, A.P., John, S.F., Olaleye, O.A. & Rosell, R.C. 2019. Pharmacokinetics of isoniazid: The good, the bad, and the alternatives. *Tuberculosis*, 116(Suppl.):66-70.

Frankel, A., Penrose, C. & Emer, J. 2009. Cutaneous tuberculosis: a practical case report and review for the dermatologist. *The journal of clinical and aesthetic dermatology*, 2:19-27.

Herma Sree, G.N.S., Saraswathy, G.R., Manikantra, M. & Mamatha, K. 2019. An update on drug repurposing: re-written saga of the drug's fate. *Biomedicine & pharmacotherapy*, 110:700-716.

Gonçalves, A., Nikmaram, N., Roohinejad, S., Estevinho, B.N., Rocha, F., Greiner, R. & McClements, D.J. 2018. Production, properties and applications of solid self-emulsifying delivery systems (S-EDDS) in the food and pharmaceutical industries. *Colloids and surfaces A*, 538:108-126.

Gurram, A.K., Deshpande, P.B., Kar, S.S., Nayak, U.Y., Udupa, N. & Reddy, M.S. 2015. Role of components in the formation of self-microemulsifying drug delivery systems. *Indian journal of pharmaceutical sciences*, 77:249-257.

Hasan, R., Shakoor, S., Hanefeld, J. & Khan, M. 2018. Integrating tuberculosis and antimicrobial resistance control programmes. *Bulletin of the world health organization*, 96:194-200.

Hind, C.K., Dowson, C.G., Sutton, J.M., Jackson, T., Clifford, M., Garner, R.C. & Czaplewski, L. 2019. Evaluation of a library of FDA-approved drugs for their ability to potentiate antibiotics against multidrug resistant Gram-negative pathogens. *Antimicrobial agents and chemotherapy*: 63:Article ID e00769-19. https://aac.asm.org/content/63/8/e00769-19.abstract. Date of access: 26 Oct. 2019.

Ke, Z., Hou, X. & Jia, X.-B. 2016. Design and optimisation of self-nanoemulsifying drug delivery systems for improved bioavailability of cyclovirobuxine D. *Drug design, development and therapy*, 10:2049-2060.

Khan, M., Nadhman, A., Sehgal, S.A., Siraj, S. & Yasinzai, M.M. 2018. Formulation and characterization of a self-emulsifying drug delivery system (SEDDS) of curcumin for the topical application in cutaneous and mucocutaneous leishmaniasis. *Current topics in medicinal chemistry*, 18:1603-1609.

Köllner, S., Nardin, I., Markt, R., Griesser, J., Prüfert, F. & Bernkop-Schnürch, A. 2017. Self-emulsifying drug delivery systems: design of a novel vaginal delivery system for curcumin. *European journal of pharmaceutics and biopharmaceutics*, 115:268-275.

Lange, C., Chesov, D. & Heyckendorf, J. 2019. Clofazimine for the treatment of multidrug-resistant tuberculosis. *Clinical microbiology and infection*, 25:128-130.

Langedijk, J., Mantel-Teeuwisse, A.K., Slijkerman, D.S. & Schutjens, M.H.D. 2015. Drug repositioning and repurposing: terminology and definitions in literature. *Drug discovery today*, 20:1027-1034.

Lin, T.-K., Zhong, L. & Santiago, J.L. 2018. Anti-inflammatory and skin barrier repair effects of topical application of some plant oils. *International journal of molecular sciences*, 19:Article ID 70.

Machado, T.C., Gelain, A.B., Rosa, J., Cardoso, S.G. & Caon, T. 2018. Cocrystallization as a novel approach to enhance the transdermal administration of meloxicam. *European journal of pharmaceutical sciences*, 123:184-190.

Marcinkute, M., Afshinjavid, S., Fatokun, A.A. & Javid, F.A. 2019. Fluoxetine selectively induces p53-independent apoptosis in human colorectal cancer cells. *European journal of pharmacology*, 857:Article ID 172441.

Mirnejad, R., Asadi, A., Khoshnood, S., Mirzaei, H., Heidary, M., Fattorini, L., Ghodousi, A. & Darban-Sarokhalil, D. 2018. Clofazimine: A useful antibiotic for drug-resistant tuberculosis. *Biomedicine & pharmacotherapy*, 105:1353-1359.

Muthyala, R. 2011. Orphan/rare drug discovery through drug repositioning. *Drug discovery today: therapeutic strategies,* 8:71-76.

Nemati, E., Mokhtarzadeh, A., Panahi-Azar, V., Mohammadi, A., Hamishehkar, H., Mesgari-Abbasi, M., Dolatabadi, J.E.N. & de la Guardia, M. 2019. Ethambutol-loaded solid lipid nanoparticles as dry powder inhalable formulation for tuberculosis therapy. *AAPS PharmSciTech*, 20: Article ID 120.

Parakh, D.R., Patil, M.P., Dashputre, N.L. & Kshirsagar, S.J. 2016. Development of self-microemulsifying drug delivery system of mebendazole by spray drying technology: Characterization, *in vitro* and in vivo evaluation. *Drying technology*, 34:1023-1042.

Pai, V.V. 2015. Role of clofazimine in management of reactions in leprosy: A brief overview. *Indian journal of drugs in dermatology*, 1:Article ID 12.

Patel, J., Patel, A., Raval, M. & Sheth, N. 2011. Formulation and development of a self-nanoemulsifying drug delivery system of irbesartan. *Journal of advanced pharmaceutical technology & research*, 2:9-16.

Prajapat, M.D., Patel, N.J., Bariya, A., Patel, S.S. & Butani, S.B. 2017. Formulation and evaluation of self-emulsifying drug delivery system for nimodipine, a BCG class II drug. *Journal of drug delivery science and technology*, 39:59-68.

Pratiwi, L., Fudholi, A., Martien, R. & Pramono, S. 2017. Self-nanoemulsifying drug delivery system (SNEDDS) for topical delivery of mangosteen peels (*Garcinia Mangostana L*.,): formulation design and *In vitro* studies. *Journal of young pharmacists*, 9:341-346.

Pushkaran, A.C., Vinod, V., Vanuopadath, M., Nair, S.S., Nair, S.V., Vasudevan, A.K., Biswas, R. & Mohan, C.G. 2019. Combination of repurposed drug diosmin with amoxicillin-clavulanic acid causes synergistic inhibition of mycobacterial growth. *Scientific reports*, 9:Article ID 6800.

Pushpakom, S., Iorio, F., Eyers, P.A., Escott, K.J., Hopper, S., Wells, A., Doig, A., Guilliams, T., Latimer, J., McNamee, C. & Norris, A. 2018. Drug repurposing: progress, challenges and recommendations. *Nature reviews drug discovery*, 18:1-2.

Rani, S., Rana, R., Saraogi, G.K., Kumar, V. & Gupta, U. 2019. Self-emulsifying oral lipid drug delivery systems: advances and challenges. *AAPS PharmSciTech*, 20:Article ID 129.

Rohrer, J., Zupančič, O., Hetenyi, G., Kurpiers, M. & Bernkop-Schnuerch, A. 2018. Design and evaluation of SEDDS exhibiting high emulsifying properties. *Journal of drug delivery science and technology*, 44:366-372.

Roycroft, E., O'Toole, R.F., Fitzgibbon, M.M., Montgomery, L., O'Meara, M., Downes, P., Jackson, S., O'Donnell, J., Laurenson, I.F., McLaughlin, A.M. & Keane, J. 2018. Molecular epidemiology of multi-and extensively-drug-resistant Mycobacterium tuberculosis in Ireland, 2001–2014. *Journal of infection*, 76:55-67.

Sangana, R., Gu, H., Chun, D.Y. & Einolf, H.J. 2018. Evaluation of clinical drug interaction potential of clofazimine using static and dynamic modelling approaches. *Drug metabolism and disposition*, 46:26-32.

Santos, J.B.D., Figueiredo, A.R., Ferraz, C.E., Oliveira, M.H.D., Silva, P.G.D. & Medeiros, V.L.S.D. 2014. Cutaneous tuberculosis: diagnosis, histopathology and treatment-Part II. *Anais brasileiros de dermatologia*, 89:545-555.

Savla, R., Browne, J., Plassat, V., Wasan, K.M. & Wasan, E.K. 2017. Review and analysis of FDA approved drugs using lipid-based formulations. *Drug development and industrial pharmacy*, 43:1743-1758.

Sharma, S.K., Chaubey, J., Singh, B.K., Sharma, R., Mittal, A. & Sharma, A. 2017. Drug resistance patterns among extra-pulmonary tuberculosis cases in a tertiary care centre in North India. *The international journal of tuberculosis and lung disease*, 21:1112-1117.

Smani, Y., Miró Canturri, A. & Ayerbe Algaba, R. 2019. Drug repurposing for the treatment of bacterial and fungal infections. *Frontiers in microbiology*, 10:Article ID 41.

Solans, C., Morales, D. & Homs, M. 2016. Spontaneous emulsification. *Current opinion in colloid and interface science*, 22:88-93.

Sotgiu, G., Tiberi, S., Centis, R., D'Ambrosio, L., Fuentes, Z., Zumla, A. & Migliori, G.B. 2017. Applicability of the shorter 'Bangladesh regimen'in high multidrug-resistant tuberculosis settings. *International journal of infectious diseases*, 56:190-193.

Syed, H.K. & Peh, K.K. 2014. Identification of phases of various oil, surfactant/co-surfactants and water system by ternary phase diagram. *Acta poloniae pharmaceutica*, 71:301-9.

Swanson, R.V., Ammerman, N.C., Ngcobo, B., Adamson, J., Moodley, C., Dorasamy, A., Moodley, S., Mgaga, Z., Bester, L.A., Singh, S.D. & Almeida, D.V. 2016. Clofazimine contributes sustained antimicrobial activity after treatment cessation in a mouse model of tuberculosis chemotherapy. *Antimicrobial agents and chemotherapy*, 60:2864-2869.

Ujhelyi, Z., Vecsernyés, M., Fehér, P., Kósa, D., Arany, P., Nemes, D., Sinka, D., Vasvári, G., Fenyvesi, F., Váradi, J. & Bácskay, I. 2018. Physico-chemical characterization of self-emulsifying drug delivery systems. *Drug discovery today: technologies*, 27:81-86.

van Deun, A., Salim, H., Kumar Das, A.P., Bastian, I. & Portaels, F. 2004. Results of a standardised regimen for multidrug-resistant tuberculosis in Bangladesh. *The international journal of tuberculosis and lung disease*, 8:560-567.

van Zyl, L., du Plessis, J. & Viljoen, J. 2015. Cutaneous tuberculosis overview and current treatment regimens. *Tuberculosis*, 95:629-638.

Vaughn, A.R., Clark, A.K., Sivamani, R.K. & Shi, V.Y. 2018. Natural oils for skin-barrier repair: Ancient compounds now backed by modern science. *American journal of clinical dermatology*, 19:103-117.

Viljoen, J.M., Cowley, A., du Preez, J., Gerber, M. & du Plessis, J. 2015. Penetration enhancing effects of selected natural oils utilised in topical dosage forms. *Drug development and industrial pharmacy*, 41:2045-2054.

Yanase, K. & Hatta, I. 2018. Disruption of human stratum corneum lipid structure by sodium dodecyl sulphate. *International journal of cosmetic science*, 40:44-49.

Yoon, G.S., Keswani, R.K., Sud, S., Rzeczycki, P.M., Murashov, M.D., Koehn, T.A., Standiford, T.J., Stringer, K.A. & Rosania, G.R. 2016. Clofazimine biocrystal accumulation in

macrophages upregulates interleukin 1 receptor antagonist production to induce a systemic anti-inflammatory state. *Antimicrobial agents and chemotherapy*, 60:3470-3479.

Wang, W., Wei, H., Tai, X. & Wang, G. 2015. Formation and characterization of fully dilutable microemulsion with fatty acid methyl esters as oil phase. ACS *Sustainable chemistry & engineering*, 3:443-450.

Zhang, L., Zhang, L., Zhang, M., Pang, Y., Li, Z., Zhao, A. & Feng, J. 2015. Self-emulsifying drug delivery system and the applications in herbal drugs. *Drug delivery*, 22:475-486.

Chapter 2

Review article submitted for publishing in *Tuberculosis Journal*

This chapter is included as the literature review of this study and is introduced according to the format prescribed by the Author Guidelines of the journal *Tuberculosis*, as attached in Annexure G. This potential review publication was completed for the purpose of complying with the requirements of the NWU that a thorough overview of literature must form part of the final submitted dissertation. Therefore, this review was considered as fulfilment of the literature overview requirements.

Clofazimine: Treatment for Cutaneous Tuberculosis by Employing Self-Emulsifying Drug Delivery Systems

Daniélle van Staden, Jeanetta du Plessis, Joe Viljoen*

Faculty of Health Sciences, Department of Pharmaceutics, Centre of Excellence for Pharmaceutical Sciences (Pharmacen), Building G16, North-West University, 11 Hoffman Street, Potchefstroom, 2520, South Africa

E-mail addresses:

dvanstaden711@gmail.com (D van Staden), Jeanetta.DuPlessis@nwu.ac.za (J du Plessis), Joe.Viljoen@nwu.ac.za (J Viljoen)

Corresponding Author:

Joe Viljoen

Joe.Viljoen@nwu.ac.za

Faculty of Health Sciences, Department of Pharmaceutics, Centre of Excellence for Pharmaceutical Sciences (Pharmacen), Building G16, Room 122, North-West University, 11 Hoffman Street, Potchefstroom, 2520, South Africa

Tel: +27 18 299 2273

Fax: +27 18 299 2248

SUMMARY

Tuberculosis (TB) remains the most notorious infectious disease known to mankind. Despite preventive developments, such as the Bacillus Calmette–Guérin (BCG) vaccination, TB remains a notably intractable disease compared to the success rates achieved with the treatment of Human Immunodeficiency Virus (HIV) and malaria research. Anti-tubercular drug resistance is of increasing concern in both developing countries as well as developed countries, and is especially common in immunocompromised individuals. Moreover, increased incidence of extra-pulmonary TB (EPTB) reflect the increasing antimicrobial drug resistance world wide. Additionally, the increased number of children infected with TB presents an international red flag. For the purpose of establishing global TB elimination, the World Health Organisation (WHO) promulgated the “End-TB” initiative that had as its goal to end this epidemic by 2035. These necessitated interventions to be extended by focusing in part on high impact, integrated and patient-centred approaches, such as individualised therapy. New scientific understanding and innovations are imperative, and the WHO and United Nations declared that the need exists for intensified research and innovation in especially new tools, in addition to investing in the development of new drugs. New tools can include the use of optimised drug delivery systems in order to improve anti-tubercular treatment. While the introduction of new drugs would be ideal, repurposing of known drugs and improving drug delivery should assist in alleviating the global TB burden. This review focusses on the potential of self-emulsifying drug delivery systems (SEDDSs) as alternative drug delivery systems intended for administration by the topical route to aid in treatment of cutaneous TB (CTB) infection. Here we consider the use of clofazimine, a previously under-utilised anti-tubercular agent that recently demonstrated potential in combination with other TB drugs for shortening of anti-tubercular treatment regimens. The concept of formulating clofazimine using self-emulsifying drug delivery systems incorporating plant based oils as emulsifiers and skin penetration enhancers is discussed. Overall, optimised anti-tubercular

drug delivery remains a daunting challenge, and in this review, we present a concept in diversifying drug delivery systems to assist in the treatment of global TB disease.

Keywords:

Antimicrobial resistance, clofazimine, cutaneous tuberculosis, self-emulsifying drug delivery system, topical, tuberculosis

1 Introduction

Control of the global tuberculosis (TB) burden has recently been deemed as of increased concern among both developing as well as developed countries [1,2]. Developing countries burdened with the highest TB incidence are India, Indonesia, China, Nigeria, the Philippines, Pakistan and South Africa [3]. TB might have been wrongly earmarked in the past as a sub-Saharan Africa disease as new studies conducted by developed countries including Germany and Switzerland indicate a definite increase in TB cases in Europe [4–6]. This phenomenon may be linked to African refugees' migration flow to Europe in order to escape their own war tormented countries [6–8]. In contrast, respective research states a decrease of approximately 10% per annum in TB incidences in Europe, since 2010. This same source, though, indicated that Europe has the highest drug-resistant TB burden in the world [9]. For these reasons, it is recommended that the focus of TB treatment must shift to the global antimicrobial resistance crisis, which is clearly visible in the 50% or less cure rate displayed by treatment of drug-resistant TB infections. It is advocated that individualised therapy should rather be implemented [9,10].

However, the search to eliminate TB is not only limited to the aforementioned countries [3]. A recent publication describes the daunting challenge of maintaining control of TB in Australia [11]. Statistics indicate that 90% of reported TB cases in Australia during 2013 were of infected individuals that originated from other countries [12]. This occurrence is linked to the high mobility of modern populations; in this case disease carriers from neighbouring countries such as Papua New Guinea [13]. On the other hand, the United States (US) had 22 years of declining TB incidence until 2015 when the TB frequencies increased minutely to represent the first year in which reported TB cases were higher than that stated in 1983. Multi-drug resistant TB rates in the US are described as stable, but a special interest is taken in the vulnerability of children that develop active TB once infected [14].

TB in children is mostly unrecognised and overlooked. There is nonetheless a vast number of children infected withTB, and more concerning is the fact that this number is increasing [15]. In TB endemic areas, it is also considered one of the ten main causes of death in children younger than 5 years of age [16]. Additionally, TB deaths in children are often misdiagnosed as meningitis, pneumonia, death caused by malnutrition and/or HIV/Acquired Immunodeficiency Syndrome (AIDS), leading to an even higher than noted rate of TB deaths amongst children [15,17]. To worsen the situation, accurate estimates of the true multi-drug resistant TB burden among children are unavailable as TB displays a paucibacillary nature in children, which complicates confirmation of the disease [14]. Therefore, this disease is now receiving close attention in both developing and developed countries in order to maintain control over, and eventually, to expunge TB. [18,19].

Consequently, the WHO implemented the “End-TB” campaign in September 2018 that endeavours to end this epidemic by the year 2035. To achieve this objective, three pillars of the WHO End TB Strategy have been developed, where “Intensified research and innovation” is included as one of the pillars. The two key components of this pillar are “discovery, development and rapid uptake of new tools, interventions and strategies” as well as “research to optimise implementation and impact, and promote innovations”. Moreover, at the first ever United Nations high level meeting on TB, a declaration was undertaken on the necessity for increased research and innovation, with the development of new tools for TB treatment [20–24]. Emphasis has now been placed on finding unique drug delivery systems, while utilising “familiar drugs”, in addition to the more traditional approach of discovering new active compounds.

2 Anti-tubercular drug resistance

TB is one of the principal causes of global morbidity and mortality. This extremely infectious disease is rendered even more intractable by the increasing occurrence of antimicrobial drug resistance. The WHO indicated that 458 000 of the 10 million TB cases reported in 2017 were triggered by *Mycobacterium tuberculosis* (Mtb) with specific

resistance towards rifampicin and isoniazid [25–27]. Mtb is categorised as an acid-fast bacillus with a slow growing rate [1]. Moreover, this organism is able to survive in a quiescent state while harboured in the human body without eliciting any TB symptoms [28]. This quiescent TB state is referred to as latent TB infection (LTBI) [15,28]. Recent research has estimated that at least one in four people worldwide has latent TB, and in almost 10% of the population infected with latent TB, this will develop into active TB. However, numerous individuals, including children, who live with TB patients or in poor, overcrowded conditions, as well as immune-compromised patients are especially vulnerable to LTBI. Subsequently, upon development of an immunocompromised situation such as through infection with HIV, development of diabetes mellitus, through an organ transplant, becoming pregnant, or living in overcrowded and unhygienic living environments coupled with with poor nutritional intake, the inactive TB state might be triggered to become an active TB infection. Persons, for example, infected with HIV, are 20 to 30 times more prone to contract active TB from LTBI [15,29–32].

However, how does drug resistance occur given that TB is both preventable and treatable? Firstly, TB drug resistance imply that Mtb displays resistance to first line anti-tubercular agents including isoniazid, rifampicin, pyrazinamide, streptomycin and ethambutol [33,34]. In order for a TB regimen to be effective, the simultaneous administration of various bactericidal and sterilising anti-tubercular agents must be maintained for a sufficient period of time [1,35] in order to guarantee anti-microbial effectiveness together with the prevention of Mtb mutations that might lead to drug resistance [36]. Resistance generally emerges due to insufficient TB management when anti-tubercular drugs are misused or mismanaged. Examples include patient noncompliance (failure to complete the correct full course of treatment), reinfection with Mtb post treatment, the inappropriate utilisation of medicines such as when health-care providers prescribe the incorrect treatment regime (incorrect employment of antibiotics, prescribing an incorrect dosage, or length of time for treatment), an inconsistent supply, or restricted access to treatment, or when the appropriate drugs are

of poor quality [32,37,38]. It has even been found that clinical and genetic risk factors may also encourage the development of multi-drug resistant TB in non-HIV infected patients [39].

Nonetheless, it has been established that the risk of development of drug-resistant TB is greater among HIV-infected individuals compared to HIV-seronegative sufferers. A study conducted in 1993–1994 in the USA concluded that HIV-infected patients with TB who had not previously been treated for TB, were already infected by bacterial isolates with an 11.3% incidence of isoniazid resistance and an 8.9% incidence of rifampicin resistance. These statistics were almost double compared to those seen in an HIV-negative population [40]. Although the mechanisms involved in the development of rifampicin resistance are not yet clearly understood, malabsorption of antimycobacterial drugs (rifampicin and ethambutol) in HIV-infected patients, associated with acquired drug resistance leading to treatment failure, has been reported [41].

Multi-drug resistant TB (MDR-TB) is defined as the combined resistance to at least two of the front line anti-tubercular agents, isoniazid and rifampicin, and is more prevalent in patients not adhering to anti-TB therapy. However, more concerning though is that multi-resistance is predictive of a poor outcome since these drugs are the two key constituents of anti-TB treatment [25,27,33,42–45]. MDR-TB is generally treated with fluoroquinolones (moxifloxacin and ofloxacin) and injectable agents (amikacin, capreomycin, kanamycin or streptomycin), normally employed as last line anti-tubercular agents, in combination with p-aminosalicylic acid, cycloserine or ethionamide for at least 18 months [45–47]. Again, this regimen is not onlycomplicated, it is furthermore costly and very protracted, and this tends to enhance compliance failure. Moreover, in 8.5% of all patients that contracted MDR-TB, it is projected that Mtb is also resistant against at least one fluoroquinolone and/or one of the second-line injectable aminoglycosides. Resistance against both fluoroquinolones and aminoglycosides is described as extensively drug resistant TB (XDR-TB), whereas pre-XDR refers to either resistance to fluoroquinolones or aminoglycosides, respectively [25,27,45,46,48,49]. The emergence and transmission of these types of TB are again partly driven by the association between TB and HIV (TB/HIV coinfection). Evidence shows that

populations infected with HIV are more prone to contract XDR-TB, which is fundamentally untreatable with normal TB treatment regimens. XDR-TB is a critical health hazard, predominantly in communities with a high incidence of HIV [38]. According to a recent 2018 WHO global TB statement, effective treatment is only accomplished in 55% of individuals that contracted MDR-TB, and is only proficient in 34% of XDR-TB patients [25,27]. The presence of MDR-TB and XDR-TB furthermore enhances the occurrence of extra-pulmonary TB infections [50]. Therefore, treatment is even more complicated as 5% of all new TB cases are considered multi-drug resistant, with the global estimate being that 3.3% of first time TB cases and 20.5% of previously treated TB patients display MDR-TB [43,51].

3 Cutaneous tuberculosis, an extra-pulmonary infection

TB presents normally as a pulmonary infection. However, approximately 20% of TB cases are extrapulmonary (EPTB) and this number is rising; consequently, it cannot be ignored any longer. EPTB includes ocular TB, hepatobiliary and splenic TB, TB spondylodiscitis, pleural TB, TB encephalitis, breast TB, TB pericarditis, TB lymphadenitis, spinal TB, gastrointestinal and peritoneal TB, genitourinary TB, osteoarticular TB, urogenital TB, TB meningitis, and cutaneous TB (CTB). The incidence of EPTB increases with associated HIV infection, an increase in MDR-TB and immunosuppressive treatment, specifically utilising inhibitors of tumor necrosis factor-alpha [1,51–62].

CTB is a relatively uncommon infectious disease where merely 1–2% of all EPTB infections involve cutaneous involvement and of these cases only around 0.1–1% of all cutaneous disorders can be related to CTB [51,55,63]. CTB was first described in 1826 by Théophile Laennec, inventor of the stethoscope, who reported a lesion on his hand that was triggered through access into his skin by means of a causative mycobacterial invasion. This causative organism was however only identified in 1882 when Robert Koch first discovered Mtb [51,52,54,55,63–65].

Skin manifestations due to EPTB are commonly caused by Mtb and are known as true CTB. However, other atypical *Mycobacterium* species may also account for cutaneous

manifestations. These Mycobacteria can be divided into two sub-categories, namely: slow growers (*M. haemophilum*, *M. leprae*, *M. marinum*, *M. ulcerans*, and less regularly *M. avium complex*, *M. kanasii*, *M. malmoense*, *M. scrofulaceum*) and rapid/fast growers (*M. chelonae*, *M. fortuitum*, and infrequently: *M. abscessus*). Rapidly growing organisms have a 7 day or less incubation period, whereas slow growers have a longer incubation time [51,65–69]. *M. bovis* and the Bacille Calmette-Guérin vaccine, a diminished strain of *M. bovis*, have also been reported to cause CTB [70]. Infections by means of atypical mycobacteria appear primarily in immunocompromised populations and there is a tendency for dissemination, whereas CTB infection in immunocompetent hosts follows skin penetration. This type of CTB is normally described as localised [55]. True CTB is more prevalent among women and young adults. The highest affected area on the skin is the face, although it frequently appears on the neck and torso as well [71]. Factors contributing to the diverse manifestations of the different CTB sub-categories include age and sex. For example, scrofuloderma is more common amongst paediatric patients, whereas erythema induratum of Bazin presents more frequently in females and TB verrucosa cutis is predominantly observed in males [72–74]. Individuals exposed to malnutrition or unhygienic conditions, or suffering from diabetes mellitus, end-stage renal disease, malignancies, HIV co-infection, or being submitted to immunosuppressive therapy, and infants and pregnant women, amongst others, are at risk of contracting active CTB [26,63,75,76]. Overall, a diverse collection of individuals is vulnerable, especially due to the global increase in MDR-TB and XDR-TB [77].

Currently three criteria are utilised to classify CTB, i.e. pathogenesis of the causative organism, clinical presentation, and histology. CTB is therefore generally categorised as either an endogenous or exogenous propagation, considering the bacterial basis and the infection means, and as a multibacillary or paucibacillary configuration, depending on the bacterial load in the lesion. The exogenous mechanism refers to direct inoculation into the skin, whereas the endogenous mechanism is secondary to a previously standing infection where transmission arises by means of contiguous, lymphatic or haematogenous dissemination [78,79–82]. This variety of clinical CTB manifestations severely complicates

diagnoses (Figure 1). Moreover, CTB is an elusive dermal condition as it resembles a number of visually similar dermal disorders [51,63,75,78]. In addition, the microbial basis of CTB is vague regardless of the improved detection techniques that include culture smears, polymerase chain reaction examinations and enzyme-linked-immunosorbent serologic assays [63,73,78,82–84].

Figure 1: Classification systems of CTB (adapted from [63,82])

Currently, treatment options for CTB remain the same as for general systemic TB due to the involvement of systemic infections in most cases. Furthermore, surgical interventions in certain indications (surgical excision of lesions and correction of deformities) are sometimes necessary [51,55,85]. CTB treatment comprises two phases. The first phase is the intensive phase and lasts approximately 8 weeks. This phase consists of simultaneous administration of first line anti-tubercular agents; after this regime patient are no longer considered infectious, but do require more treatment in order to eradicate the infection. Subsequently, a maintenance phase is implemented, consisting of simultaneous administration of isoniazid and rifampicin. This phase is maintained for a period of at least 16 weeks for HIV negative patients and 28 weeks for HIV positive patients, but may even last for 9–12 months. For the treatment to be deemed successful, patients need to adhere stringently to this treatment regimen [51,52,84]. However, due to these long-term treatment phases, patient compliance may be problematic, and in order to not only improve patient compliance, but also treatment outcomes, alternative solutions should be found. First, anti-microbial agents must be explored in terms of anti-tubercular agent substitutions to shorten these treatments, fight MDR-TB and XDR-TB, as well as minimise adverse effects accompanied by first line anti-tubercular compounds. Likewise, novel therapeutic combinations of existing antimicrobials and first line TB agents could be more beneficial (such as shortening the TB schedules) as recommended by the WHO and United Nations agreement [2,20–24].

Recently, interest in so-called “repurposed drugs”, i.e. drugs formerly intended for indications other than TB that have been shown to be useful in treating MDR-TB and XDR-

TB, was rekindled [86]. Clofazimine, is one of these compounds that depicted a significant efficacy against resistant TB strains in the Bangladesh regimen [2,25,87]. Individualised anti-tubercular therapy is furthermore increasingly recommended, as it is seen as the most effective and safe manner of targeting the resistance of individual TB patients without inducing further resistance [10,88]. In order to turn the tide against TB and CTB, reintroduction of agents such as clofazimine should not be disregarded as these can assist in both individualised therapy as well as shortening the treatment phases [2,36,89].

4 Clofazimine

Clofazimine, initially known as “B663”, was originally developed in 1954 by Vincent Barry and co-workers to treat TB. It is a highly lipophilic riminophenazine dye, which is significantly active against Mtb. However, despite high bactericidal and sterilising activity *in vitro* as well as in a mouse model of MDR-TB treatment; its good oral absorption in micronised form along with its deposition and persistence in adipose tissue and in cells of the reticuloendothelial system, this compound did not initially progress as an anti-tubercular agent as it was found to be ineffective against TB in humans [15,90–96]. Browne and Hogerzeil [97], however, concluded that due to the accumulation of clofazimine inside macrophages, it could be effective against intracellular diseases. Chang recognised its efficacy against leprosy at the end of 1959, and after clinical trials during the 1960s in Nigeria and elsewhere, the Swiss pharmaceutical company Novartis marketed clofazimine in 1969 under the brand name Lamprene® as an anti-leprosy agent [25,98,99]. In 1982 a WHO Study Group recommended utilising clofazimine as a main constituent in the multidrug triple treatment regimen (i.e. dapsone, rifampicin and clofazimine) of multibacillary leprosy [92,99]. However, Novartis was only granted FDA approval of clofazimine as an orphan drug (a pharmaceutical compound developed to treat medical disorders which, since they are so rare, will not be cost-effective to produce without government assistance) at the end 1986 and its use as anti-tubercular agent lapsed for several decades [98,100,101]. Moreover,

clofazimine was classified as a group 5 drug by the WHO, indicating that it is a drug with unclear efficacy that is not recommended for routine use [102].

Later, interest in clofazimine for the treatment of TB increased flared up following its inclusion in the so-called “Bangladesh regimen” where it was established that approximately 90% of patients that contracted MDR-TB were healed within a 9–11 month period [2,87,103]. This was a substantial improvement from the standard WHO-proposed 24 month regimen that cured less than 50% of MDR-TB victims. Results of a larger clinical trial study in Bangladesh as well as from other clinical trials in Cameroon and Niger confirmed these conclusions [93,104,105–107]. The Bangladesh regimen according to the WHO 2016 guidelines comprises an initial treatment phase consisting of 4–6 months' treatment with kanamycin, moxifloxacin, prothionamide, clofazimine, pyrazinamide, isoniazid (in high dosages) and ethambutol. This initial phase is followed by a 5 month treatment period with moxifloxacin, clofazimine, pyrazinamide and ethambutol [2,105]. The specific bactericidal and treatment-shortening action of clofazimine was furthermore noted in MDR-TB mouse models, and it was likewise displayed in a controlled clinical trial performed in China [108,110]. In contrast, the randomised controlled STREAM trial indicated non-inferiority of the short-course-containing regimen, but not superiority [111]. For these reasons, the full potential of clofazimine as an anti-tubercular agent must still be investigated. Nonetheless, clofazimine is listed in the WHO Model List of Essential Medicines as a leprostatic-, anti-tubercular- and anti-inflammatory agent [112]. It is also categorised by the WHO as a group C agent (i.e. other core second line anti-tubercular agents) [36] and therefore clofazimine may just have the capacity to significantly advance the treatment of MDR-TB and CTB.

Oral absorption of clofazimine is variable, fluctuating from 45–62% when taken on an empty stomach; however, absorption is increased when taken with food. This drug is metabolised by glucuronidation in the liver, incompletely expelled by the biliary route, and only negligibly excreted in urine, sputum, sebum, and sweat. Clofazimine has been reported to cross the placenta and is excreted in breast milk [94,100,113,114].

Second line anti-tubercular drugs are known to be more toxic [115] and clofazimine is no exception as the highly lipophilic nature of this drug leads to accumulation in macrophages and in adipose cells such as in the heart, liver, breasts, adrenal glands, pancreas, spleen, bone marrow and lamina propria of the jejunum thereby extending the half-life to 65–70 days [92–94,100,113,114,116–118]. This accumulation leads to adverse reactions such as reversible skin, hair and corneal dyschromia to brownish-black, dark red, or even to orange pink. Approximately 46% of patients receiving clofazimine treatment display conjunctival deposition, whereas nearly 53% experience crystal deposition in the cornea and 32% of patients present with clofazimine crystals in their tears [119,120]. Deposition of a dark-brown pigmentation is furthermore sometimes visible on the finger nails. Discoloration is more evident in fair-skinned individuals exposed to sunlight and even though it is reversible, it may take years to completely clear up. Breast milk, sweat, urine, and faeces may also discolour. In addition, dry skin and ichthyosis, defined as an increase in skin thickness due to lack of shedding of dead skin cells, often transpires. However, severe gastrointestinal reactions (nausea, vomiting, abdominal pain, diarrhoea) are rather common (reported in >10% of patients) and deemed most concerning as accumulation, as well as precipitation of clofazimine in the wall of the small intestine, ensue after sustained administration [86,92,100,108,113,114,116,121,122]. In 1–10% of patients, weight loss, pruritus, diminished visual perception, and/or eye irritation/dryness occur. Less commonly, more severe adverse effects may be observed including enteropathy possibly complicated by intestinal obstruction, gastrointestinal bleeding, and splenic infarction [113,114,123,124].

Normally, side effects accompanied by clofazimine treatment are dependent on the dose administered and the duration of administration [91]. Clofazimine up to 300 mg daily is often well tolerated, however, 100 mg daily is normally recommended due to gastrointestinal intolerance. Higher dosage regimens given over longer periods, i.e. beyond 4 months, may lead to crystal enteropathy and malabsorption, which is accompanied by effusive vague and/or severe abdominal distress. Coincidentally, discontinuation of treatment normally leads to a reasonably quick clearing of symptoms. Granting the fact that gastrointestinal side

effects are not infrequent and have been observed in roughly one third of patients treated with clofazimine, the severity is usually not substantial enough to evoke termination of the treatment [15,100,108,113,114,118,123–125].

Fortunately, clofazimine is predominantly well-tolerated in children as observed from paediatric leprosy treatment [15]. It is classified as Pregnancy Category C, though little research has been conducted regarding its use to treat MDR-TB during pregnancy and limited data exist for later life teratogenicity. From data obtained from case reports, it was found that only 5 MDR-TB pregnant patients received clofazimine as part of the treatment regimen. All infants born were considered normal. Additionally, no significant adverse reactions due to clofazimine as part of the leprosy treatment regimen during pregnancy have been conveyed [92,113,114,126,127]. Clofazimine is furthermore safe to co-administer with antiretroviral therapy [128] and no known significant drug-drug interactions have been reported [92,126]. Thus, if insight into the physicochemical characteristics of clofazimine are effectively employed to design novel drug delivery systems, the most serious adverse effects of severe gastrointestinal reactions and accumulation of clofazimine in the gastro-intestinal tract might be circumvented. For this reason, the topical administration route might be deemed advantageous as gastrointestinal accumulation can be bypassed [129]. Moreover, an alternative route of administration together with a unique clofazimine dosage form might arm this repurposed drug with a more user-friendly profile.

5 An alternative means to deliver clofazimine more effectively

5.1 Considering topical delivery

As stated, oral delivery of clofazimine is not only variable, but also numerous systemic adverse effects are associated with this route of drug distribution. Topical drug delivery, alternatively, is defined as “the application of a drug onto the surface of the body to a localised area, such as the nose, eyes, rectum, vagina or skin, in order to establish relief of a condition localised at the area of application, without creating any systemic effect” [130,131].

Over the past three decades the trend of drug delivery via the skin has been growing. It is becoming a considerably more popular alternative administration route, especially when aiming to side step complications accompanied when utilising oral drug delivery [129]. Through implementation of this route to treat various topical ailments, patient compliance is increased, the risk of drug and food interactions is decreased, and hepatic metabolism is circumvented amongst others [132,133]; rendering the dermal application route conspicuously beneficial [134]. However, the skin is designed to fulfil a barrier function; and subsequently permeation of various drugs, in concentrations that are of clinical relevance, is limited [135,136]. The barrier function of the skin is linked to the lipophilic nature of the outermost layer of the skin, namely the *stratum corneum* (SC) [136]. Therefore, the main challenge when formulating a topical drug delivery system is to attain an optimum concentration of the included drug at the site of action for a sufficient period of time [130,137].

The skin can be described as a multi-layered organ [138]. Each layer fulfils its own role towards protecting the body, maintaining hydration and allowing sufficient blood perfusion to the skin [139–141]. The skin (Figure 2) consists of three layers, namely the epidermis, dermis and hypodermis [142]. The epidermis is divided into two distinct fragments, i.e. the SC and viable epidermis [139], where the SC forms the outermost layer of the epidermis and is known as the lipophilic, protective layer of the skin that complicates drug delivery of a vast range of substances [141]. This protecting function of the SC is enforced by its lipophilic nature [139]. The SC consists of lifeless, anucleated cells (corneocytes linked by corneodesmosomes) surrounded by a lipid matrix containing a mixture of cholesterol, triglycerides, free fatty acids and ceramides [139]. Epidermis thickness varies according to the cell layer thickness at the different skin regions of the body [140].

Figure 2: Simplified illustration of skin anatomy and drug penetration pathways (adapted from [137,143])

The dermis is known as the second skin layer and can be classified as the most biochemically active layer of the skin [139]. It is responsible for body temperature regulation, cutaneous sensation, skin elasticity, blood circulation in addition to skin nutrition, and the removal of toxic substances [139,140]. Following the dermis, the third skin layer is recognised as the hypodermis [140]. Subcutaneous fat is harboured within the hypodermis [140,143]. Hence, it acts as a supportive membrane for both the dermis and epidermis [140,144]. In order for a drug to be delivered through the skin and into the systemic circulation, it must be able to move through the lipophilic SC and the underlying hydrophilic layers (i.e. the viable epidermis and dermis). Therefore, a drug must have a profile of both hydrophilic and lipophilic properties in order to facilitate successful transdermal drug delivery [139,141].

Furthermore, three pathways for drug diffusion through the skin exists [143]. First, a drug can enter the skin through the intracellular or transcellular pathway [142]. Highly hydrated keratins are part of the corneocytes that are the main building blocks of the epidermis, and therefore, hydrophilic drugs would predominantly pass through the skin via this pathway [139]. Next, intercellular permeation may occur via diffusion of a compound through the lipid matrix of the skin, as mostly observed for lipophilic drugs [139,142]. The third route, the transappendageal pathway, is restricted as a mere 0.1% of the skin surface is occupied by hair follicles and sweat glands that present an opportunity for modest transappendageal permeation of drugs [139,142]. Here, an additional limiting step for permeation of hydrophilic composites is the lipid-rich nature of the sebum that fills the sebaceous glands [145]. In turn, however, delivery of lipophilic drugs may be more beneficial through this route [139]. Sweat, on the other hand, provides a pathway for the delivery of hydrophilic drugs via the appengeal pathway [142]; however, drug delivery in the presence of sweat is limited as diffusion must occur against the diffusion gradient [145]. Hence, as stated previously, the physicochemical properties of a drug are deemed highly important during the development of a drug delivery system to enable topical and/or transdermal drug delivery [146].

5.2 Clofazimine characteristics challenging the topical route

The physicochemical characteristics of clofazimine are portrayed in Table 1. It is stated that molecules smaller than 500 Da, such as those of clofazimine, with a molecular weight of 473.40 Da [147], are capable of easy skin diffusion [146,148]. As skin is a non-homogenous medium, smaller particles will display portray increased skin permeation [149]. Clofazimine furthermore exhibits an exceptionally long half-life as mentioned [99], which is fortunate since an extended half-life may require less frequent dosing. In turn this characteristic might even reduce the adverse skin discoloration normally associated with oral absorption of clofazimine [150].

Table 1: Physicochemical characteristics of clofazimine

Generally, the rate of diffusion through the skin will only occur at a rapid transfer rate if the concentration of clofazimine, present on the skin surface, is saturated [151]. Moser *et al.* [152] found that supersaturation on the skin can increase skin penetration and permeation of lipophilic drugs. The diffusion and partition parameters assumed for lipophilic compounds point to that supersaturation acts entirely by means of increased thermodynamic activity without apparent effect on the barrier function of the skin. Hence, the clofazimine concentration incorporated into formulations, as well as its solubility in these formulations will significantly contribute toward improving the transfer rate of it through the skin [151]. Likewise, a melting point of less than 200ºC is generally recommended to ensure adequate solubility [153]. Clofazimine has a slightly higher melting point (210–212°C) than recommended, and although this difference is only marginally higher, drug solubility might thus possibly present some challenges [99,146]. The solubility parameter of a drug destined for dermal delivery should be similar to that of the skin (approximately 10 cal/cm^3) [154]. An aqueous solubility of more than 1 mg/ml is deemed most favourable for a drug that needs to diffuse through the skin [146].

Clofazimine has a Log P value of 7.66, reflecting the highly lipophilic nature of this drug [155,156]. This negatively affects skin permeation as the ideal Log P range, identified for

topically delivered drugs, is between 1 and 3 [146]. The Log P value is the n-octanol-water partition coefficient, which will reflect the partioning of a drug between the lipophilic SC and the hydrophilic viable epidermis and dermis [157]. Due to clofazimine being highly lipophilic and nearly insoluble in water, these properties will probably add to the expected formulation challenges [99].

Another property to consider is the pH of the skin where the SC is able to tolerate a pH range from 5–9 [146]. For this reason, the pH of a dermal formulation is an important factor in order to avoid skin irritation [158]. Furthermore, the pH of a formulation will determine the fraction of unionised drug molecules available at the skin surface [159]. Drug molecules presented at the skin surface, in the ionised form, will cross the SC by utilising the transappendageal route [160]. Conversely, unionised drug molecules will enter the skin via the intercellular (lipid) route [161]. Pranitha and Lakshmi [162] demonstrated that the acidic or basic properties of a drug can contribute towards which physicochemical properties are deemed more important during topical or transdermal drug delivery. Weakly acidic drugs will follow the order of molecular weight > Log P > solubility, whereas weakly basic drugs, such as clofazimine, will favour the order of molecular weight > solubility > Log P.

As seen, although various challenges exist to effectively deliver a highly hydrophobic compound such as clofazimine topically, it is achievable by means of utilising alternative excipients as well as unconventional drug delivery systems. For example, the inclusion of penetration enhancers such as natural oils, could be deemed useful for the purpose of manipulating topical drug delivery [163]. Natural oils initiate reversible fluidisation and rearrangement of the lipid matrix, harboured by the SC that facilitates penetration enhancement of drugs. In addition, natural oils are frequently employed, during the formulation of dermal drug delivery systems as stabilisers, solubility enhancers and emulsifiers. Furthermore, the application of natural oils onto the skin is considered safe and generally acceptable to the public [164,165].

5.3 Auxiliary natural excipients to enhance topical clofazimine delivery

As stated by the WHO [166], approximately 80% of communities located in developing countries utilise ethno therapy, including topical application of natural oils where skin pathologies are problematic as a more affordable health care and cosmetic alternative. Moreover, present-day research reveals the beneficial capacity of these substances ancient compounds in the world of modern medicine. Insights obtained from current research indicate that the topical application of natural oils is accompanied by mutually beneficial mechanisms influenced by the individual natural oil composition together with the method of extraction. These mechanisms include: enhanced skin barrier homeostasis, anti-oxidative activity, anti-inflammatory action, anti-microbial properties, promotion of wound healing, and anti-carcinogenic characteristics [165,167–171].

Unrefined plant based oils, cold-pressed or cold extracted, are not exposed to heat or chemical processes during manufacturing. Therefore, the components of cold-pressed oils remain unaltered to enrich the skin with improved nutritive properties [167,168,172,173]. Naturally occurring sediments of unrefined plant based oils, such as wax, can further provide assistance as a hydrophobic residue to improve dermal occlusive effects. Occlusive effects establish prevention of skin dehydration while in turn providing prolonged contact time with the skin to improve drug delivery. Moreover, unrefined natural oils retain the highest nutrient concentration together with unaltered fatty acids. Hence these oils present a decreased tendency towards skin irritation [168,174].

The categorisation of natural oils is based on the method of extraction prior to becoming commercially available [167]. First, plant based oils are either categorised as fixed oils (non-volatile at room temperature) or essential oils (aromatic compounds volatile at room temperature) [172]. Fixed oils can be sub-characterised as non-virgin-, virgin- or extra virgin oils [168]. Non-virgin oils are indicative of a compromised oil composition as the oil was refined by means of heat exposure together with chemical processes [167,168]. Virgin oils, contrary, are cold pressed. These oils furthermore display a slightly more acidic nature

compared to extra virgin oils. The waiting time span prior to oil extraction can similarly influence the pH of the oil. Nonetheless, extra virgin oils remain entirely unrefined and are cold pressed by slowly applying pressure to the entire seed or kernel under high pressurised conditions without chemical addition or heat exposure [168,175]. The efficacy of virgin oils compared to extra virgin oils for skin application purposes has however not yet been established [168]. Considering the individual composition of each natural oil, different saturated solubility concentrations of clofazimine could be expected within each selected oil [176]. Therefore, skin penetration could also vary due to the unique composition of each selected plant oil when incorporated into a topical formulation [163].

Oils are mainly composed of glycerides obtained from a condensation reaction between fatty acids and glycerol. Each plant-derived oil possesses a unique composition and provides versatile dermal effects accordingly. The potential dermal benefits accompanied by plant oil application are reflective of the physiological responses due to interactions between the bioactive compounds contained in natural oils and the pathophysiological skin context [163,167,168]. Bioactive compounds present in plant based oils include: triglycerides, essential fatty acids, phospholipids, unsaponifiables, phenolic compounds, triterpenes, tocopherols, carotenoids and flavonoids [177–182]. Considering topical drug delivery, one realises that phospholipids are additionally well-known permeability enhancers [167]. Likewise, it has been proven that various fatty acids present in plant based oils contribute towards increased skin penetration required to improve delivery of topically applied drugs [163,167,168,183–185]. It is their unsaponifiable fractions (such as fatty acids, e.g. oleic acid) that are thought to contribute to their penetration enhancing activities [163]. Fatty acids are classified as being saturated or unsaturated due to the saturation of the carbon backbone with hydrogen [183,186]. Moreover, the character of a free fatty acid is influenced by the carbon backbone as it may vary from 2–22 carbons, as indicated in Figure 3.

Figure 3: Simplified general chemical structure of a fatty acid (adapted from [186])

Free fatty acids are released when natural oils are metabolised within the skin [163]. Research indicates that certain fatty acids are of profound significance during dermal drug delivery. The fatty acids present in plant based oils can either disrupt the lipid formation of the SC and create an entrance for drugs to be delivered into the skin; or the essential fatty acid can contribute towards the barrier repair function of the skin. The essential fatty acid most notorious for skin penetration enhancement by means of disrupting the lipid arrangement of the SC, is oleic acid. Contrary, linoleic acid rather repairs the barrier function of the skin as it is known as an integral component in restoring the lamellar phase of the lipid matrix harboured by the SC [163,167,168,183,187–189]. Vaughn *et al*. [168] observed that either oleic acid or linoleic acid is generally present in a predominant concentration in individual plant based oils. However, no ratio of oleic acid to linoleic acid has yet been standardised as more or less favourable in terms of dermal drug delivery [168,184]. This review focussed in more detail on the following selected plant based oils that can be utilised in various drug delivery systems: argan-, avocado-, coconut-, macadamia- and olive oil. The fatty acid composition of these plant based oils are displayed in Table 2.

Table 2: Fatty acid composition of selected plant based oils [184,185,190]

5.3.1 Argan oil

The first available literature found on argan oil referred to the argan tree as the “tree of life” [191]. Argan oil is traditionally obtained by crushing the fruit kernels harvested from *Argania spinosa* trees [192]. The argan tree is indigenous to the southwestern region of Morocco and possesses a lifespan of up to 200 years [191,193]. Argan oil is utilised for both edible and cosmetic purposes [194] where, when employed for cosmetic purposes, it is procured by grinding unroasted argan fruit kernels. Edible argan oil, alternatively, is prepared by moderately roasting argan fruit kernels prior to grinding [194,195]. In traditional Moroccan medicine argan oil has been reserved for various dermal application purposes (i.e. dry skin, acne, anti-aging, dermal inflammation, psoriasis and eczema) and it is also utilised to prevent dry hair as well as hair loss [194,196,197].

The most abundant unsaturated fatty acid (UFA) components of argan oil are oleic acid (i.e. 45.90 ± 1.84) and linoleic acid (i.e. 34.10% ± 1.08) [185,191] as seen in Table 2. Interestingly, from all of the selected plant based oils consisting predominately of UFAs, that are discussed here, argan oil presents the smallest difference in concentration between oleic acid and linoleic acid. Theoretically, argan oil should thus provide excellent skin barrier repair effects when compared to avocado-, coconut-, macadamia- and olive oil as it has the highest linoleic acid concentration of these plant based oils [167]. Skin barrier repair effects are of significant interest if considered that cutaneous tuberculosis can progress to open lesions [31,63]. Argan oil will probably aid in repairing the skin barrier while solubilising a drug destined for dermal drug delivery [31,167]. Although the oleic acid concentration present in this oil is the lowest comparatively [185], it may still be present in sufficient concentrations to facilitate delivery of the lipophilic drug into the dermis of the skin as studies reported successful dermal drug delivery utilising argan oil [196,198,199].

The saturated analogue of oleic acid, namely stearic acid, is also present in argan oil in a concentration of 6.00% ± 0.05 [185,200]. This is ranked the highest concentration of stearic acid present in the selected plant based oils. Stearic acid has a melting point of 69.3°C [201]. Therefore, it will most probably remain undissolved during dermal application as the skin surface has an approximate temperature of 32°C [202]. Hence, the effects established by stearic acid during dermal drug delivery can be facilitated by the emollient properties of this fatty acid [203]. Gore *et al*. [203] conducted a study on the influence of emollients (i.e. stearic acid and isohexadecane) on the spreadability as well as the film formation achieved by emulsion application. They concluded that stearic acid might mimic the same behaviour as wax on the skin surface by forming a residual film after break down of the emulsion. Thus, occlusion is improved and in turn dermal drug delivery is enhanced through this film formation property [168].

5.3.2 Avocado oil

Avocado oil is extracted from the pulp of *Persia Americana* fruit [167]. This oil is deemed useful for enriching skin that suffer from dryness, damage and cracks [204]. Stimulation of collagen synthesis when avocado oil is applied to skin, has also been described [205]. Moreover, avocado oil has displayed a reduction in inflammation during wound healing in rat models [206]. It is furthermore a rich source of minerals, vitamins A, C, D as well as E, β-sitosterol, β-carotene, and lecithin [167,207].

In terms of fatty acid composition, avocado oil has the highest palmitic acid content (Table 2). Kim *et al*. [200] and Kumar *et al*. [208] deemed palmitic acid a significantly useful penetration enhancer. Kim *et al*. [200] additionally stated that palmitic acid displayed the most potent skin penetration effect of the tested saturated fatty acids (SFAs). Kumar *et al*. [208], on the other hand, reported that palmitic acid did increase drug permeation through the skin, but maximum penetration enhancement was facilitated by the unsaturated oleic acid. Of the fatty acids contained within avocado oil, oleic acid occurs in the highest concentration (59.46% ± 3.88) followed by linoleic acid with a concentration of only 14.66% ± 1.20 [184]. Moreover, avocado oil has the second highest oleic acid content of the selected plant based oils and an approximately similar linoleic acid profile compared to olive oil.

5.3.3 Coconut oil

Coconut oil is extracted from the fruit and fresh juice of *Cocos nucifera* [209]. Southeast Asian countries employ coconut oil for its anti-inflammatory; wound healing and anti-pyretic properties [210]. Interestingly, virgin coconut oil is extracted by wet processing in order to avoid degradation of biologically active ingredients, including vitamins and polyphenols [209,211]. A vast composition of anti-oxidative components is present in coconut oil. These include tocopherols and fatty acids such as palmitic-, stearic-, lauric- and myristic acid [209]. Increased levels of lauric acid are present in coconut oil, i.e. 66.94% ± 4.11 [184] from which monolaurin can be derived [167]. Monolaurin is categorised as a monoglyceride and

demonstrates significant antimicrobial activity by means of lipid disintegration of lipid-coated bacteria, namely *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Propionibacterium acnes* [167,168,212,213]. The open lesions that occur during CTB infection presents an opportunity for development of secondary bacterial infections [31], including the aforementioned bacteria. Hence, the inclusion of coconut oil in a topical formulation can possibly decrease the vulnerability of CTB patients exposed to secondary bacterial infections. Lauric acid additionally renders increased drug permeation through the skin [214] and this capability of lauric acid to cross the SC is facilitated by the formation of complexes between lauric acid and fatty acids naturally present within the skin [215]. The affinity of lauric acid for skin can be linked to its solubility parameter and excellent partitioning coefficient [214].

Myristic acid is a key SFA component of coconut oil [167]. Cotte *et al*. [216] found that myristic acid migrates into the dermis whereas palmitic acid was only detectable in the viable epidermis. The most noted distinction between myristic acid and the saturated palmitic acid is the difference in carbon backbone length. Myristic acid has a total of 14 carbons in its carbon backbone, whereas the structure of palmitic acid is composed of 16 carbons [184,200,216]. Remarkably, SFAs with shorter carbon backbones have furthermore demonstrated deeper skin penetration [200,216]. Comparatively in this review, coconut oil comprises the highest levels of SFAs [184] which may therefore enhance skin penetration. The semi-solid state of coconut oil at room temperature (± 25°C) may in addition be explained by its high SFAs concentration [168].

5.3.4 Macadamia oil

Macadamia oil is produced through cold pressing of the raw nuts of *Macadamia integriolia*, which flourishes in tropical countries, and *Macadamia tetraphilla*, grown in areas with subtropical temperatures [217]. Vitamin E is included in this oil in concentrations of 55 μg/g [218]. The fatty acid composition of macadamia oil is quite peculiar when compared to other plant based oils. The ratio of UFAs to SFAs are 5.86:1, which is the highest relative

ratio between UFAs and SFAs. For nutritional purposes the higher the UFAs to SFAs ratio is, the higher the nutritional value of the oil [184]. Similarly, for topical drug delivery purposes, this high value indicates a vast number of fatty acids available to probably enhance skin permeation.

The palmitic acid profile of macadamia oil is approximately similar to that of coconut oil [184]. Desaturation of palmitic acid delivers palmitoleic acid [219]. Intriguingly, macadamia oil displays the highest palmitoleic acid concentration [184]. Kim *et al*. [200] discovered palmitoleic acid as the second best unsaturated penetration enhancer during transdermal delivery of diclofenac. Additionally, macadamia oil is composed of the highest concentration linolenic acid, a well-known unsaturated penetration enhancer [184,220]. Fox *et al*. [172] stated that the enhanced permeation can be linked to the unsaturated fraction of the fatty acid. Unfortunately, one must bear in mind that an allergic reaction to tree nuts, including macadamia nuts, is a lifelong condition and is hallmarked as the leading cause of anaphylactic shock triggered by food [221].

5.3.5 Olive oil

Olives are harvested from *Olea europaea* trees [167]. These trees were native to the Mediterranean region, but over the past two decades olive tree plantations have been planted world-wide [222]. Lin *et al*. [167] emphasised that more than 200 chemical compounds have been detected in olive oil. For the purpose of dermal drug delivery, olive oil with its high oleic acid content of 72.79 ± 0.21%, is an old favourite [167,168]. Viljoen *et al*. [163] concluded that olive oil is a powerful transdermal drug enhancer; and more specifically oleic acid has been proven an unbeaten skin penetration enhancer in numerous studies [163,168,200,223–226]. This fatty acid is incorporated into the lipids of the SC upon contact with the skin. Subsequently, oleic acid causes disruption of the lipid arrangement and provide hydration changes in the skin that leads to improved skin permeability [163,188].

6 The prospect of self-emulsifying drug delivery systems enhancing topical delivery of clofazimine

6.1 Lipid based carrier systems ideal for lipophilic drugs

It is estimated that approximately 30% of currently marketed drugs (including clofazimine) as well as up to 50% of newly discovered drugs are of a highly hydrophobic nature [158,159,227,228]. Due to this difficulty, lipid based carrier systems are yet again receiving special attention in the pharmaceutical industry to provide solutions needed to improve delivery of established drugs instead of developing new drug entities. These systems are a useful platform to improve the delivery of drugs displaying poor aqueous solubility [229,230,232]. They provide an alternative approach by incorporating lipophilic drugs into inert lipid vehicles in order to render repeatable drug concentration profiles [228,233]. Lipid carrier systems include: oils, surfactant dispersions, emulsions, nanoemulsions, liposomes, solid lipid carrier systems, nanostructured lipid carrier systems, and self-emulsifying drug delivery systems (SEDDSs) [228,233,234]. Lipid carrier systems can facilitate targeted drug delivery, implicating improved therapeutic effects and decreased side effects, which are of significance during, for example, anti-tubercular treatment as well as individualised therapy [234].

6.2 Why self-emulsifying drug delivery systems (SEDDSs) are considered more appropriate than other lipid based carrier systems

Liposomes, emulsions and nanoemulsions are frequently employed as drug delivery systems; and these lipid based dosage forms are becoming increasingly more popular to specifically facilitate topical and transdermal drug delivery [234–237]. Liposomes are spherical, bilayered structures of 100–200 nm in diameter. They are generally manufactured from phospholipids that are able to encapsulate a range of diverse drugs, either in their aqueous central or within the lipid bilayer. Their size, number of bilayers and other components included, provide their distinct characteristics. For example, inclusion of a surfactant forms transferosomes that are able to cross the SC; or the inclusion of ethanol

produces more flexible liposomes, termed ethosomes. Liposomes were originally developed for parenteral administration of anticancer and antifungal agents; they are not as widely utilised for topical or transdermal drug delivery and are mainly included in cosmetics and long-acting sunscreen formulations, although their membrane structure is similar to that of natural membranes found in skin. Liposomes are expected to rather remain at the surface of the skin where they fuse with the skin lipids and slowly release their entrapped content for prolonged drug release [235,238–240].

Conversely, emulsions are heterogeneous, thermolabile biphasic liquids consisting of two or more immiscible fluids, which are made miscible through the addition of surfactants or emulsifying agents. They can be further categorised into micro- and nano-emulsions, as well as SEDDSs, depending on the formulation strategies used to prepare them. Conventional emulsions are however sensitive and metastable, which causes difficulty in effective drug delivery [238,241]. Microemulsions are actually not emulsions. They are thermodynamically stable, single-phase systems that form spontaneously once the correct oil to water ratios are achieved and they have a number of different microstructures depending on the nature and concentrations of the components. Then again, nanoemulsions are quite novel thermodynamically unstable dispersions that contain individual small droplets with a mean diameter of less than 200 nm. These dispersions are however considered relatively physically stable as well as possessing reasonably high kinetic stability as the droplets do not collide as frequently as in ordinary emulsions. Their small droplet size furthermore allows for deep penetration into tissues even through fine capillaries. Positively charged (cationic) nanoemulsions have shown to increase skin permeation of poorly soluble drugs due to their interaction with the negatively charged skin epithelia cells. The main advantages of nanoemulsions include increased drug loading, enhanced drug solubility and bioavailability, reduced patient variability, controlled drug release, and protection from enzymatic degradation [234,236,238].

SEDDSs were originally developed from emulsions in order to generate a more physically stable formulation that is easier to manufacture and that was able to facilitate oral drug

delivery of cyclosporine [234,241,242]. Although SEDDSs is not a novel concept and have been utilised in numerous commercial pharmaceutical products destined for oral use, research has lately turned its attention to focussing on more diverse pathways to administer SEDDSs such as the ocular-, vaginal- and sublingual routes [243–246]. However, limited studies concerning the application of SEDDSs on skin in order to aid in dermal pathologies have been published [247,248]. SEDDSs are defined as isotropic and thermodynamically stable solutions containing an oil, surfactant, co-surfactant and drug that spontaneously transform into oil-in-water emulsions upon the addition of aqueous media, under gentle agitation [249]. As the main component of SEDDSs, the incorporated drug should be of a lipophilic nature [250]. The lipophilic drug is solubilised in the oily concentrate and then introduced into the aqueous media where mild agitation occurs [233, 241,242,251]. Interestingly, some studies demonstrated that SEDDSs formulations with lower drug solubility displayed higher oral bioavailability when compared to SEDDSs displaying a higher solubility characteristic [252]. Nonetheless, for the purpose of developing for example, SEDDSs for topical drug delivery of clofazimine, the incorporated clofazimine concentration should not be less than the saturated concentration in order to avoid a decreased clofazimine flux through the skin [253]. Moreover, an improved rate of clofazimine transfer will only be observed if the clofazimine concentration, obtainable at the skin surface, is continuously presented at a concentration nearing saturation [253].

SEDDSs can be formulated in either the nano- or micro droplet size range [254], where nano droplet formation may transpire in the presence of surface active agents that cause spontaneous emulsification of finely dispersed droplets [250,255]. These finely dispersed droplets are evoked from the intrinsic physicochemical properties of the constituents to form nano sized droplets. Moreover, these droplets are stabilised by the ratio of oil to surfactant [256]. Thakare *et al*. [257], however, declared that high surfactant concentrations can cause tissue irritation. Thus, spontaneous emulsification should be able to occur with decreased surfactant concentrations without reducing the stability of the formulations [258].

Table 3 portrays the differences between liposomes, nanoemulsions and SEDDSs in order to demonstrate the topical drug delivery possibilities SEDDSs have to offer. It is clear that the structure of SEDDSs and nanoemulsions are closer in similarity compared to the structure of liposomes. Liposomes are utilised as spherical vesicles that provide the opportunity to incorporate both hydrophilic and lipophilic drugs due to the bilayer formation [259]. Therefore, liposomes are frequently commercially employed to aid in cancer, viral infections, management of pain, photodynamic therapy and fungal infections, though as stated, they are considered less ideal for rapid transdermal drug delivery [234,235,238–240]. Contrary, nanoemulsions portray clear oil-in-water, or water-in-oil dispersions as observed from the arrangement of polar and non-polar structures of surface active agents, whereas SEDDSs normally form clear oil-in-water dispersions [260]. As evident from the structures of nanoemulsions and SEDDSs, notably increased concentrations of surface active agents are present in SEDDSs, rendering these systems more stable than nanoemulsions [237,261]. These systems furthermore differ significantly in their methods of preparation [234,237]. The water titration method employed during the formulation of liquid SEDDSs is considerably easier to master than the methods utilised to produce either liposomes or nanoemulsions. Moreover, upscaling the production process of SEDDS formulations is established with ease [234,262]. As conveyed earlier, individualised therapy still provides the most attractive solution to aid in TB, and more specifically CTB, treatment [9,10]. Therefore, the ease with which SEDDSs are manufactured may probably provide an easy to produce dosage form suitable for individualised therapy.

Table 3: A summary of the most notable differences between liposomes, nanoemulsions and SEDDSs

As stated, to be able to formulate SEDDSs, surfactants are an indispensable inclusion criteria to stabilise these types of formulations. Surfactants included in SEDDSs are mostly non-ionic as they display decreased toxicity profiles, improved agreement with biological systems, and these surfactants are less affected by changes in pH as well as ionic strength

[250,251,255]. What is more, non-ionic surfactants are predominantly utilised during the formulation of topical products as they are known to cause less skin irritation than ionic surfactants [263]. Surfactants can further additionally act as penetration enhancers and improve dermal drug delivery due to their ability to solubilise lipid membranes such as the SC if chosen correctly [264]. During surfactant selection, enhanced emulsification properties are deemed more important than improved drug solubilisation [255]. The efficacy of a surfactant to establish emulsification is displayed by the hydrophilic-lipophilic balance (HLB) value of the said compound. Literature states that a HLB value of ≥12 is deemed adequate. Therefore, surfactants such as Tween®80 (HLB value of 15), Labrasol® (HLB value of 14) and PEG 400 monolaurate (13.1), are regularly used to formulate SEDDSs [228,238,250,255]. Sometimes co-surfactants are also deemed necessary as they improve the flexibility of the interfacial film established by the surfactant, rendering it an even more stable formulation [250,252,255]. The addition of a co-surfactant can facilitate more finely dispersed droplets throughout SEDDS formulations [250]. Hence, the concentration range of the emulsion is improved and solubility of the drug in the SEDDS is additionally enhanced [228].

To summarise, the potential advantages of SEDDSs to name a few, are: 100% drug entrapment capacity; formation of physically more stable formulations, especially when compared to liposomes and emulsions; no dissolution phase is compulsory; formation of submicron droplet sizes is possible, consequently increasing the absorption surface area as well as SEDDSs display increased rates and extent in absorption, and thus increased bioavailability. SEDDSs are able to distribute BCS Class II drugs efficiently. They similarly have potential for effective delivery of BCS class III, BCS class IV and hydrolytically susceptible drugs. SEDDSs furthermore offer consistent progressive profiles with reduced dosing and dosing frequencies. They are easy to manufacture and scale-up; and these dosage form systems are able to direct drug distribution into the lymphatic system [234,252,264,265]. Due to lymphatic absorption of SEDDSs through lymphatic vessels found in the dermis, drug delivery may further proved enhanced [266,267]. Lymphatic absorption of

clofazimine can provide an advantageous future treatment angle as lymphadenitis is considered the most commonly observed manifestation of EPTB in both adults and children [57]. The selected lipid component is extremely important when aiming to achieve lymphatic absorption through the dermis as it was concluded that the utilised oil must be able to solubilise a minimum of 50 mg/ml drug to obtain lymphatic uptake after oral administration. Furthermore, oils containing long chain triglycerides demonstrated increased lymphatic uptake; and the quantity of included oil was established to significantly contribute towards successful lymphatic uptake [252,265,268]. Sun *et al*. [268] established that a concentration of ≥25% oil content provides sufficient lymphatic absorption. Unfortunately, lipids that are highly unsaturated are easily oxidised, and subsequently, the addition of anti-oxidative agents must not be overlooked [265].

Not only is the use of SEDDS enabling the development of alternative drug administration routes, these systems are also being modified to advance muco-adhesion in order to extend contact time at the site of application [269–271]. Moreover, SEDDSs are being reformed to possess zeta-potential changing properties to improve drug delivery across mucus membranes [272]. Menzel and Bernkop-Schnürch [273], for example, published a study describing the success of combining mucolytic enzyme decorated carrier systems with SEDDSs in order to enable local cleavability to mucus membranes, followed by increased drug permeation. Therefore, SEDDSs are able to provide distinct possibilities in enhancing drug delivery [234,250].

The advantages promised by the development of a topical SEDDSs to aid in CTB treatment, is of great value as a reduced drug dose is acquired to establish similar drug absorption as with oral drug delivery [225,257]. Due to the deadly nature of TB, high fixed doses of anti-tubercular drugs are normally recommended as inter-individual variability can lead to sub-therapeutic drug concentrations. Therefore, utilising SEDDSs for dermal clofazimine administration, the use of decreased drug concentrations in these formulations can be considered due to the prolonged elimination half life of clofazimine of approximately 70 days [99]. This will not only provide consistent drug delivery profiles, which will

additionally minimise the risk of Mtb growth and the development of drug resistance [274], it may also possibly reduce the red skin discoloration or even enhance the disappearance of it as this discoloration is dose dependent [91,225,257]. Improved drug loading capacity, displayed during the development of SEDDSs, can further support anti-tubercular treatment by either including higher concentrations of clofazimine if deemed essential, or investigation into the incorporation of fixed-dose drug combinations may prove worthwhile [275,276]. Bhandari *et al*. [275] established successful incorporation of both artemether and lumefantrine in a solid SEDDS double fixed dosage form to assist in the treatment of malaria. Furthermore, a suggested method to delay the development of anti-microbial resistance is the co-administration of two synergistic drugs [277]. For this reason, clofazimine and pyrazinamide, for example, could be incorporated into a SEDDS formulation as a synergistic mechanism between the two drugs has already been established [91,118].

7. Summary

In order enhance treatment of CTB, this altered approach towards drug delivery may provide new means of improving anti-tubercular treatment. Clofazimine is a highly lipophilic drug which presents formulation challenges. However, SEDDSs create a new dimension for the delivery of drugs with poor aqueous solubility. The selected lipid vehicles employed during the formulation of SEDDSs is crucial as it can improve drug solubility, enhance drug loading capacity, facilitate possible lymphatic uptake of clofazimine and decrease the incorporated drug dosage.

Moreover, topical SEDDS administration provides the promise of localised CTB treatment, thus, excluding the possibility of interactions between clofazimine and first-line anti-tubercular agents present in the systemic circulation. Plant based oils, incorporated as the lipid phase during the formulation of SEDDSs, can act as penetration enhancers in order to improve clofazimine delivery into the skin. Additionally, plant based oils can enhance barrier repair of the skin, and in so doing, assist in healing CTB lesions. Some natural oils such as coconut oil contains lauric acid that demonstrates antimicrobial activity against certain

organisms, thus, providing substantial protection against secondary infection of CTB lesions. The optimum potential of lipid-based drug delivery systems must still be reached. This review presents possible approaches towards broadening the horizon of both lipid carrier system applications and assisting in treatment of TB.

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References

[1] Ankrah AO, Glaudemans AWJM, Maes A, van der Wiele C, Dierckx RAJO, Vorster M, Sathekge MM. Tuberculosis. Semin Nucl Med 2018; 48(2):108–130. https://doi.org/(...)mnuclmed.2017.10.005.

[2] Sotgiu G, Tiberi S, Centis R, D'Ambrosio L, Fuentes Z, Zumla A, Migliori GB. Applicability of the shorter 'Bangladesh regimen' in high multidrug-resistant tuberculosis settings. IJID 2017; 56:190–193. https://doi.org/10.1016/j.ijid.2016.10.021.

[3] Grace AG, Mittal A, Jain S, Tripathy JP, Satyanarayana S, Tharyan P, Kirubakaran R. Shortened treatment regimens versus the standard regimen for drug-sensitive pulmonary tuberculosis. Cochrane Database Syst Rev 2018; 1: CD012918. https://doi.org/10.1002/14651858.CD012918.

[4] Nellums LB, Thompson H, Holmes A, Castro-Sánchez E, Otter JA, Norredam M, Friedland JS, Hargreaves S. Antimicrobial resistance among migrants in Europe: a systematic review and meta-analysis. Lancet Infect Dis 2018; 18(7):796–811. https://doi.org/10.1016/S1473-3099(18)30219-6.

[5] Walker TM, Merker M, Knoblauch AM, Helbling P, Schoch OD, van der Werf MJ, Kranzer K, Fiebig L, Kröger S, Haas W, Hoffmann H. A cluster of multidrug-resistant *Mycobacterium tuberculosis* among patients arriving in Europe from the Horn of Africa: a molecular epidemiological study. Lancet Infect Dis 2018; 18(4): 431–440. http://dx.doi.org/10.1016/S1473-3099(18)30004-5.

[6] Dicko A, Faye O, Fofana Y, Soumoutera M, Berthé S, Touré S, Traoré B, Guindo B, Tall K, Keita A, Kéita L. Cutaneous tuberculosis in Bamako, Mali. Pan Afr Med J 2017; 27: 102–102. http://dx.doi.org/10.11604/pamj.2017.27.102.11577.

[7] Nellums LB, Thompson H, Holmes A, Castro-Sánchez E, Otter JA, Norredam M, Friedland JS, Hargreaves S. Antimicrobial resistance among migrants in Europe: a systematic review and meta-analysis. Lancet Infect Dis 2018; 18(7): 796–811. https://doi.org/10.1016/S1473-3099(18)30219-6.

[8] Olaru ID, Albert H, Zallet J, Werner UE, Ahmed N, Rieder HL, Salfinger M, Kranzer K. Impact of quality improvement in tuberculosis laboratories in low-and lower-middle-income countries: a systematic review. Int J Tuberc Lung Dis 2018; 22(3):309–320. https://doi.org/10.5588/ijtld.17.0629.

[9] Monedero-Recuero I. Drug-Resistant Tuberculosis in Europe. What Are We Waiting For? AJRCCM 2018; 198(3):302–304. https://doi.org/10.1164/rccm.201803-0497ED.

[10] Günther G, Van Leth F, Alexandru S, Altet N, Avsar K, Bang D, Barbuta R, Bothamley G, Ciobanu A, Crudu V, Danilovits M. Clinical management of multidrug-resistant tuberculosis in 16 European countries. AJRCCM 2018; 198(3):379–386. https://doi.org/10.1164/rccm.201710-2141OC.

[11] Bainomugisa A, Pande, S, Donnan E, Simpson G, Foster JB, Lavu E, Hiasihri S, McBryde ES, Moke R, Vincent S, Sintchenko V. Cross-border movement of highly drug-resistant mycobacterium tuberculosis from Papua New Guinea to Australia through Torres Strait Protected Zone, 2010–2015. Emerg Infect Dis 2019; 25(3):406. https://doi.org/10.3201/eid2503.181003.

[12] Toms C, Stapledon R, Waring J, Douglas P. Tuberculosis notifications in Australia, 2012 and 2013. Commun Dis Intell Q Rep 2015; 39(2):E217–235. https://www.ncbi.nlm.nih.gov/pubmed/26234258.

[13] Lönnroth K, Mor Z, Erkens C, Bruchfeld J, Nathavitharana RR, van der Werf MJ, Lange C. Tuberculosis in migrants in low-incidence countries: epidemiology and intervention entry points. Int J Tuberc Lung Dis 2017; 21(6):624–636. https://doi.org/10.5588/ijtld.16.0845.

[14] Smith SE, Pratt R, Trieu L, Barry PM, Thai DT, Ahuja SD, Shah S. Epidemiology of pediatric multidrug-resistant tuberculosis in the United States, 1993-2014. Clin Infect Dis 2017; 65(9):437–1443. https://doi.org/10.1093/cid/cix561.

[15] Marais BJ. Improving access to tuberculosis preventive therapy and treatment for children. IJID 2017; 56:122–125. https://doi.org/10.1016/j.ijid.2016.12.015.

[16] Dodd PJ, Yuen CM, Sismanidis C, Seddon JA, Jenkins HE. The global burden of tuberculosis mortality in children: a mathematical modelling study. Lancet Glob Health 2017; 5(9):e898–e906. https://doi.org/10.1016/S2214-109X(17)30289-9.

[17] Graham SM, Sismanidis C, Menzies HJ, Marais BJ, Detjen AK, Black RE. Importance of tuberculosis control to address child survival. Lancet 2014; 383(9928):16051607. https://doi.org/10.1016/S0140-6736(14)60420-7.

[18] Tacconelli E, Pezzani MD. Public health burden of antimicrobial resistance in Europe. Lancet Infect Dis 2019; 19(1):4–6. https://doi.org/10.1016/S1473-3099(18)30648-0.

[19] Baker SJ, Payne DJ, Rappuoli R, De Gregorio E. Technologies to address antimicrobial resistance. PNAS 2018; 115(51):12887–12895. https://doi.org/10.1073/pnas.1717160115.

[20] World Health Organization (WHO). The end TB strategy. http://www.who.int/tb/post2015_TBstrategy.pdf?ua=1; 2016 [accessed 18 May 2019].

[21] World Health Organization (WHO). UN general assembly high-level meeting on the fight against tuberculosis. https://www.who.int/news-room/events/un-general-assembly-high-level-meeting-on-ending-tb 2018 [accessed 10 Apr. 2019].

[22] Stop TB Partnership. http://www.stoptb.org/global/advocacy/unhlm_whatis.asp. 2019 [accessed 20 May 2019].

[23] General Assembly of the United Nations. United Nations high-level meeting on the fight to end tuberculosis 26 September 2018, UNHQ, New York. https://www.un.org/pga/73/event/fight-to-end-tuberculosis/ 2018 [accessed 20 May 2019].

[24] United Nations: Meetings coverage and press releases. World Leaders Reaffirm Commitment to End Tuberculosis by 2030, as General Assembly Adopts Declaration Outlining Actions for Increased Financing, Treatment Access. https://www.un.org/press/en/2018/ga12067.doc.htm 2018 [accessed 20 May 2019].

[25] Lange C, Chesov D, Heyckendorf J. Clofazimine for the treatment of multidrug-resistant tuberculosis. Clin Microbiol Infect 2019; 25:128–130. https://doi.org/10.1016/j.cmi.2018.07.012.

[26] van Zyl L, Viljoen JM, Haynes RK, Aucamp M, Ngwane AH, du Plessis J. Topical delivery of artemisone, clofazimine and decoquinate encapsulated in vesicles and their *in vitro* efficacy against Mycobacterium tuberculosis. AAPS PharmSciTech 2019; 20(1):33–44. https://doi.org/10.1208/s12249-018-1251-5.

[27] World Health Organization (WHO). Global tuberculosis report 2018. Geneva. 2018, http://www.who.int/tb/publications/global_report/en/; 2018 [accessed 19 May 2019].

[28] Osuchukwu O, Nuñez M, Packard S, Ehiri J, Rosales C, Hawkins E, Gerardo Avilés JG, Gonzalez-Salazar F, Oren E. Latent Tuberculosis Infection Screening Acceptability among Migrant Farmworkers. International Migration 2017; 55(5):62–74. https://doi.org/10.1111/imig.12275.

[29] Cardona PJ. Pathogenesis of tuberculosis and other mycobacteriosis. Enferm Infecc Microbiol Clin 2018; 36(1):38–46. https://doi.org/10.1016/j.eimc.2017.10.015.

[30] Centis R, D'Ambrosio L, Zumla A, Migliori GB. Shifting from tuberculosis control to elimination: Where are we? What are the variables and limitations? Is it achievable? IJID 2017; 56:30–33. https://doi.org/10.1016/j.ijid.2016.11.416.

[31] Santos JBD, Figueiredo AR, Ferraz CE, Oliveira MHD, Silva PGD, Medeiros VLSD. Cutaneous tuberculosis: epidemiologic, etiopathogenic and clinical aspects-part I. An Bras Dermatol 2014; 89(2):219–228. http://dx.doi.org/10.1590/abd1806-4841.20142334.

[32] Gavin Churchyard. This kills more South Africans than any other disease. There's a new way to stop it. BHEKISISA Centre for Health Journalism, https://bhekisisa.org/article/2019-03-28-00-3hp-latent-tb-treatment-short-course-guidelines-regimen-isoniazid-rifapentine; 2019 [accessed 19 May 2019].

[33] Yao L, LiangLiang C, JinYue L, WanMei S, Lili S, YiFan L, HuaiChen L.. Ambient air pollution exposures and risk of drug-resistant tuberculosis. Environ Int, 2019; 124:161–169. https://doi.org/10.1016/j.envint.2019.01.013.

[34] Pourakbari B, Sadeghi RH, Mahmoudi S, Parvaneh N, Valian SK, Mamishi S. Evaluation of interleukin-12 receptor β1 and interferon gamma receptor 1 deficiency in patients with disseminated BCG infection. Allergol Immunopathol 2019; 47(1):38–42. https://doi.org/10.1016/j.aller.2018.06.005.

[35] Caminero JA, Cayla JA, García-García JM, García-Pérez FJ, Palacios JJ, Ruiz-Manzano J. Diagnosis and treatment of drug-resistant tuberculosis. Arch Bronconeumol 2017; 53(9):501–509. https://doi.org/10.1016/j.arbr.2017.07.005.

[36] Tiberi S, Muñoz-Torrico M, Duarte R, Dalcolmo M, D'Ambrosio L, Migliori GB. New drugs and perspectives for new anti-tuberculosis regimens. Pulmonology 2018; 24(2):86–98. https://doi.org/10.1016/j.rppnen.2017.10.009.

[37] Tuberculosis (TB). Division of Tuberculosis Elimination. Centers for Disease Control and Prevention. CDC twenty four seven. Saving Lives, Protecting People, https://www.cdc.gov/tb/publications/factsheets/drtb/mdrtb.htm; 2016 [accessed 15 May 2019].

[38] Alexander PE, De P. The emergence of extensively drug-resistant tuberculosis (TB): TB/HIV coinfection, multidrug-resistant TB and the resulting public health threat from extensively drug-resistant TB, globally and in Canada. Can J Infect Dis Med Microbiol 2007; 18:289–291. PubMed PMID: 18923728; PubMed Central PMCID: PMC2533560.

[39] Sharma SK, Turaga KK, Balamurugan A, Saha PK, Pandey RM, Jain NK, Katoch VM, Mehrab NK. Clinical and genetic risk factors for the development of multi-drug resistant tuberculosis in non-HIV infected patients at a tertiary care center in India: a case-control study. Infect Genet Evol 2003; 3:183–188. https://doi.org/10.1016/S1567-1348(03)00086-8.

[40] Munsiff SS, Joseph S, Ebrahimzadeh A, Frieden TR. Rifampin monoresistant tuberculosis in New York City, 1993–1994. Clin Infect Dis 1997; 25: 1465–1467. https://doi.org/10.1086/516146.

[41] Patel KB, Belmonte R, Crowe HM. Drug malabsorption and resistance tuberculosis in HIV-infected patients. N Engl J Med 1995; 332: 336–337. https://doi.org/10.1056/NEJM199502023320518.

[42] Venkatesh U, Srivastava DK, Srivastava AK, Tiwari HC. Epidemiological profile of multidrug-resistant tuberculosis patients in Gorakhpur Division, Uttar Pradesh, India. J Family Med Prim Care. 2018; 7(3):589–595. https://doi.org/10.4103/jfmpc.jfmpc_99_17.

[43] Velayati AA, Farnia P, Hoffner S. Drug-resistant Mycobacterium tuberculosis: Epidemiology and role of morphological alterations. Journal Glob Antimicrob Resist 2018; 12:192–196. https://doi.org/10.1016/j.jgar.2017.10.006.

[44] McBryde ES, Meehan MT, Doan TN, Ragonnet R, Marais BJ, Guernier V, Trauer JM. The risk of global epidemic replacement with drug-resistant Mycobacterium tuberculosis strains. IJID 2017; 56:14–20. https://doi.org/10.1016/j.ijid.2017.01.031.

[45] Aaron L, Saadoun D, Calatroni I, Launay O, Memain N, Vincent V, Marchal G, Dupont B, Bouchaud O, Valeyre D, Lortholary O. Tuberculosis in HIV-infected patients: a comprehensive review. Clin Microbiol Infect 2004; 10:388–398. https://doi.org/10.1111/j.1469-0691.2004.00758.x.

[46] Tiberi S, Scardigli A, Centis R, D'Ambrosio L, Munoz-Torrico M, Salazar-Lezama MA, Spanevello A, Visca D, Zumla A, Migliori GB, Luna JAC. Classifying new anti-tuberculosis drugs: rationale and future perspectives. IJID 2017; 56:181–184. https://doi.org/10.1016/j.ijid.2016.10.026.

[47] Modongo C, Sobota RS, Kesenogile B, Ncube R, Sirugo G, Williams, S.M, Zetola NM. Successful MDR-TB treatment regimens including amikacin are associated with high rates of hearing loss. BMC Infect Dis 2014; 14(1):542–551. https://doi.org/10.1186/1471-2334-14-542.

[48] Peña MJM, García BS, Baquero-Artigao F, Pérez DM, Pérez RP, Echevarría AM, Amador JTR, Durán DGP, Julian AN. Tuberculosis treatment for children: An update. Anales de Pediatría (English Edition) 2018; 88(1):52.e1–52.e12. https://doi.org/10.1016/j.anpede.2017.05.001.

[49] Maitre T, Petitjean G, Chauffour A, Bernard C, El Helali N, Jarlier V, Reibel F, Chavanet P, Aubry A, Veziris N. Are moxifloxacin and levofloxacin equally effective to treat XDR tuberculosis? J Antimicrob Chemother 2017; 72(8):2326–2333. https://doi.org/10.1093/jac/dkx150.

[50] Jiang H, Jin Y, Vissa V, Zhang L, Liu W, Qin L, Wan K, Wu X, Wang H, Liu W, Wang B. Molecular characteristics of mycobacterium tuberculosis strains isolated from cutaneous tuberculosis patients in China. Acta Dermato-venereol 2017; 97(4):472–477. https://doi.org/10.2340/00015555-2577.

[51] van Zyl L, du Plessis J, Viljoen J. Cutaneous tuberculosis overview and current treatment regimens. Tuberculosis 2015; 95(6):629–638. https://doi.org/10.1016/j.tube.2014.12.006.

[52] Chen ST, Cahalane AM, Ryan ET, Foreman RK. Case 2-2019: a 36-year-old man with rash, abdominal pain, and lymphadenopathy. N Engl J Med 2019; 380(3):275–283. https://doi.org/10.1056/NEJMcpc1810390.

[53] Desai U, Joshi JM. Extrapulmonary drug-resistant tuberculosis at a drug-resistant tuberculosis center, Mumbai: Our experience–Hope in the midst of despair! Lung India 2019; 36(1):3–7. http://dx.doi.org/10.4103/lungindia.lungindia_192_18.

[54] Haitz K, Ly A, Smith G. Idiopathic granulomatous mastitis. Cutis 2019; 103(1):38–42. https://www.ncbi.nlm.nih.gov/pubmed/30758334.

[55] Mimesh SA, Memish ZA. Cutaneous Tuberculosis. In: Sener A, Erdem H, editors. Extrapulmonary Tuberculosis, Springer: Cham; 2019, p. 175–180.

[56] de los Santos Moreno A, Gómez AS, Blasco ER, Cuevas MC, Saborido DG. Infecciones bacterianas crónicas (I). Tuberculosis. Medicine-Programa de Formación

Médica Continuada Acreditado 2018; 12(53):3115–3123. https://doi.org/10.1016/j.med.2018.03.020.

[57] Peirse M, Houston A. Extrapulmonary tuberculosis. Medicine 2017; 45(12):747–752. https://doi.org/10.1016/j.mpmed.2017.09.008.

[58] Dusthackeer A, Sekar G, Chidambaram S, Kumar V, Mehta P, Swaminathan S. Drug resistance among extrapulmonary TB patients: Six years' experience from a supranational reference laboratory. Indian J Med Res 2015; 142(5):568-574. https://doi.org/10.4103/0971-5916.171284.

[59] Lee JY. Diagnosis and treatment of extrapulmonary tuberculosis. Tuberc Respir Dis (Seoul) 2015; 78(2):47–55. https://doi.org/10.4046/trd.2015.78.2.47.

[60] Mehta PK, Raj A, Singh N, Khuller GK. Diagnosis of extrapulmonary tuberculosis by PCR. FEMS Immunol Med Mic 2012; 66:20–36.

[61] Joshi JM. Tuberculosis chemotherapy in the 21st century: Back to the basics. Lung India 2011; 28(3):193–200. https://doi.org/10.4103/0970-2113.83977.

[62] Mohan A, Sharma SK. Medical schools and tuberculosis control: bridging the discordance between what is preached and what is practiced. Indian J Chest Dis Allied Sci 2004; 46(1):5–7. https://www.ncbi.nlm.nih.gov/pubmed/14870862.

[63] Frankel A, Penrose C, Emer J. Cutaneous tuberculosis: a practical case report and review for the dermatologist. J Clin Aesthet Dermatol 2009; 2(10):19–27. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2923933/.

[64] Mello RB, Vale ECSD, Baeta IGR. Scrofuloderma: a diagnostic challenge. An Bras Dermatol 2019; 94(1):102–104. https://doi.org/10.1590/abd1806-4841.20188560.

[65] Rullán J, Seijo-Montes RE, Vaillant A, Sánchez NP. Cutaneous manifestations of pulmonary disease. In: Sánchez NP, editor. Atlas of dermatology in internal medicine. 14th ed. San Juan, Puerto Rico: Springer; 2012. p. 17–30.

[66] Ghosh S, Aggarwal K, Jain VK, Chaudhuri S, Ghosh E. Tuberculosis verrucosa cutis presenting as diffuse plantar keratoderma: An unusual sight. Indian J Dermatol 2014; 59(1):80–81. http://dx.doi.org/10.4103/0019-5154.123511.

[67] Walter N, Daley CL. Tuberculosis and nontuberculous mycobacterial infections. In: Spiro SG, Silvestri GA, Agustí A, editors. Clinical respiratory medicine. 4th ed. Philadelphia: Elsevier Ltd.; 2012. p. 383–405.

[68] Jones-Lopez EC, Ellner JJ. Tuberculosis and atypical mycobacterial infections. In: Guerrant RL, Walker DH, Weller PF, editors. Tropical infectious diseases. 3rd ed. London: Elsevier Inc.; 2011. p. 228–247.

[69] Yates VM. Mycobacterial infections. In: Burns T, Breathnach S, Cox N, Griffiths C, editors. Rook's textbook of dermatology. 8th ed., vol. 2. West Sussex, United Kingdom: Blackwell Publishing Ltd; 2010. 31.1–31.41.

[70] Gharabaghi MA. Cutaneous tuberculosis caused by isoniazid-resistant Mycobacterium tuberculosis. BM. J Case Rep 2012: bcr2012006253. http://dx.doi.org/10.1136/bcr-2012-006253.

[71] Hernández Solis A, Herrera González NE, Cazarez F, Mercadillo Pérez P, Olivera Diaz HO, Escobar-Gutierrez A, Cortés Ortíz I, González González H, Reding-Bernal A, Sabido RC. Skin biopsy: a pillar in the identification of cutaneous *Mycobacterium tuberculosis* infection. J Infect Dev Ctries 2012; 6(8):626–631. https://doi.org/10.3855/jidc.2729.

[72] Zhang J, Fan YK, Wang P, Chen QQ, Wang G, Xu AE, Chen LQ, Hu R, Chen W, Song ZQ, Hao F. Cutaneous tuberculosis in China - A multicentre retrospective study of cases diagnosed between 1957 and 2013. J Eur Acad Dermatol Venereol. 2018; 32(4):632–638. https://doi.org/10.1111/jdv.14851.

[73] Ho CK, Ho MH, Chong LY. Cutaneous tuberculosis in Hong Kong: an update. Hong Kong Med J 2006; 12(4):272–277. https://www.ncbi.nlm.nih.gov/pubmed/16912353.

[74] Gopinathan R, Pandit D, Joshi J, Jerajani H, Mathur M. Clinical and morphological variants of cutaneous tuberculosis and its relation to Mycobacterium species. Indian J Med Microbl 2001; 19:193–196. https://www.ncbi.nlm.nih.gov/pubmed/17664831.

[75] De Maio F, Trecarichi EM, Visconti E, Sanguinetti M, Delogu G, Sali M. Understanding cutaneous tuberculosis: two clinical cases. JMM Case Reports 2016; 3(6):e005070–e005076. https://doi.org/10.1099/jmmcr.0.005070.

[76] Teixeira HC, Abramo C, Munk ME. Immunological diagnosis of tuberculosis: problems and strategies for success. J Bras Pneumol 2007; 33:323–334. http://dx.doi.org/10.1590/S1806-37132007000300015.

[77] Sloan DJ, Lewis JM. Management of multidrug-resistant TB: novel treatments and their expansion to low resource settings. Trans R Soc Trop Med Hyg 2016; 110(3):163–172. https://doi.org/10.1093/trstmh/trv107.

[78] Chen Q, Chen W, Hao F. Cutaneous Tuberculosis: A Great Imitator. Clin Dermatol 2019; article in press. https://doi.org/10.1016/j.clindermatol.2019.01.008

[79] Hirt P, Price A, Alwunais K, Schachner L. An interesting case of coincidental epidermolytic hyperkeratosis and erythema annulare centrifugum in the setting of latent tuberculosis in a 12-year-old female. Int J Dermatol 2019; article in print. https://doi.org/10.1111/ijd.14339.

[80] Khan A, Singaraddi R, Shetty D, Rodrigues G, Primary cutaneous 'ulcerative' tuberculosis of the scrotum: a rare occurrence. BMJ Case Rep 2018; 11(1). pii: e227177. https://doi.org/10.1136/bcr-2018-227177.

[81] Yeo PM, Lee SX, Tan YE, Sng LH, Ang CC. Epidemiology, risk factors, and outcomes of adult cutaneous non-tuberculous mycobacterial infection over a 10-year period in Singapore. Int J Dermatol 2018; 58(6):679–687. https://doi.org/10.1111/ijd.14356.

[82] Bravo FG, Gotuzzo, E. Cutaneous tuberculosis. Clin Dermatol 2007; 25(2):173–180. https://doi.org/10.1016/j.clindermatol.2006.05.005.

[83] Khadka P, Koirala S, Thapaliya J. Cutaneous tuberculosis: clinicopathologic arrays and diagnostic challenges. Dermatol Res Pract 2018; 9(2018):7201973. eCollection. https://doi.org/10.1155/2018/7201973.

[84] Mohanty N, Nayak BB. Cutaneous tuberculosis, tuberculosis verrucosa cutis. Med J Dr. D Y Patil Univ 2014; 7(1): 53–55. https://doi.org/10.4103/0975-2870.122776.

[85] Barbagallo J, Tager P, Ingleton R, Hirsch RJ, Weinberg JM. Cutaneous tuberculosis. Am J Clin Dermatol 2002; 3(5):319–328. https://doi.org/10.2165/00128071-200203050-00004.

[86] Dalcolmo M, Gayoso R, Sotgiu G, D'Ambrosio L, Rocha JL, Borga L, Fandinho F, Braga JU, Galesi VMN, Barreira D, Sanchez DA, Dockhorn F, Centis R, Caminero JA, Migliori GB. Effectiveness and safety of clofazimine in multidrug-resistant tuberculosis: a nationwide report from Brazil. Eur Respir J 2017; 49:1602445. https://doi.org/10.1183/13993003.02445-2016.

[87] van Deun A, Salim H, Kumar Das AP, Bastian I, Portaels F. Results of a standardised regimen for multidrug-resistant tuberculosis in Bangladesh. Int J Tuberc Lung Dis 2010; 8(5):560–567. https://www.ncbi.nlm.nih.gov/pubmed/15137531.

[88] Shah MA, Shah I. Increasing Prevalence of Pediatric Drug-resistant Tuberculosis in Mumbai, India, and Its Outcome. Pediatr Infect Dis J 2018; 37(12):1261–1263. https://doi.org/10.1097/INF.0000000000002040.

[89] Cox H, Hughes J, Black J, Nicol MP. Precision medicine for drug-resistant tuberculosis in high-burden countries: is individualised treatment desirable and feasible? Lancet Infect Dis 2018; 18(9):e282–e287. https://doi.org/10.1016/S1473-3099(18)30104-X.

[90] Drugbank. Clofazimine, https://www.drugbank.ca/drugs/DB00845; 2017 [accessed 03 June 2019]

[91] Ammerman NC, Swanson RV, Bautista EM, Almeida DV, Saini V, Omansen TF, Guo H, Chang YS, Li, S-Y, Tapley A, Tasneen R, Tyagi S, Betoudji, F, Moodley C, Ngcobo B, Pillay L, Bester LA, Singh SD, Charisson RE, Nuermberger E, Grosset JH. Impact of clofazimine dosing on treatment-shortening of the first-line regimen in a mouse model of tuberculosis. Antimicrob Agents Chemother 2018; 62(7):e00636–18. https://doi.org/10.1128/AAC.00636-18.

[92] McGuffin SA, Pottinger PS, Harnisch JP. Clofazimine in nontuberculous mycobacterial infections: a growing niche. Open Forum Infect Dis 2017; 4(3):ofx147. https://doi.org/10.1093/ofid/ofx147.

[93] Swanson RV, Adamson J, Moodley C, Ngcobo B, Ammerman NC, Dorasamy A, Moodley S, Mgaga Z, Tapley A, Bester LA, Singh S. Pharmacokinetics and pharmacodynamics of clofazimine in a mouse model of tuberculosis. Antimicrob Agents Chemother 2015; 59(6):3042–3051. https://doi.org/10.1128/AAC.00260-15.

[94] Cholo MC, Steel HC, Fourie PB, Germishuizen WA, Anderson R. Clofazimine: current status and future prospects. J Antimicrob Chemother 2012; 67:290–298. https://doi.org/10.1093/jac/dkr444.

[95] Anonymous. New drug for leprosy. Br Med J 1969; 1(5647):797–798. https://www.ncbi.nlm.nih.gov/pubmed/5774074.

[96] Barry VC, Belton JG, Conalty ML, DEN-STENY JM, Edward DW, O'SULLI-VAN JF, Twomey D, Winder F. A new series of phenazines (rimino-compounds) with high antituberculosis activity. Nature 1957; 179:1013–15. https://doi.org/10.1038/1791013a0.

[97] Browne SG, Hogerzeil LM. “B 663” in the treatment of leprosy. Preliminary report of a pilot trial. Lepr Rev 1962; 33:6–10. http://leprev.ilsl.br/pdfs/1962/v33n1/pdf/v33n1a02.pdf.

[98] Attwood MM, Rask-Andersen M, Schiöth, H.B. Orphan drugs and their impact on pharmaceutical development. Trends Pharmacol Sci 2018; 39(6):525-535. https://doi.org/10.1016/j.tips.2018.03.003.

[99] Brennan PJ, Young DB. Tuberculosis: handbook of anti-tuberculosis agents. Amsterdam: Elsevier 2008; 88(2):85–170. https://doi.org/10.1016/S1472-9792(08)70006-4.

[100] Cholo MC, Mothiba MT, Fourie B, Anderson R. Mechanisms of action and therapeutic efficacies of the lipophilic antimycobacterial agents clofazimine and bedaquiline. J Antimicrob Chemother 2017; 72(20):338–353. https://doi.org/10.1093/jac/dkw426.

[101] Zhang Z, Li T, Qu G, Pang Y, Zhao Y. *In vitro* synergistic activity of clofazimine and other antituberculous drugs against multidrug-resistant Mycobacterium tuberculosis

isolates. Int J Antimicrob Agents 2015; 45(1):71–75. https://doi.org/10.1016/j.ijantimicag.2014.09.012.

[102] Dooley KE, Obuku EA, Durakovic N, Belitsky V, Mitnick C, Nuerm-berger EL, Efficacy Subgroup, RESIST-TB. World Health Organization group 5 drugs for the treatment of drug-resistant tuberculosis: unclear efficacy or untapped potential? J Infect Dis 2013; 207:1352–1358. http://dx.doi.org/10.1093/infdis/jis460.

[103] Mirnejad R, Asadi A, Khoshnood S, Mirzaei H, Heidary M, Fattorini L, Ghodousi A, Darban-Sarokhalil D. Clofazimine: A useful antibiotic for drug-resistant tuberculosis. Biomed Pharmacother 2018; 105:1353–1359. https://doi.org/10.1016/j.biopha.2018.06.023.

[104] Kuaban C, Noeske J, Rieder HL, Ait-Khaled N, Abena Foe JL, Trébucq A. High effectiveness of a 12-month regimen for MDR-TB patients in Cameroon. Int J Tuberc Lung Dis 2015; 19:517–524. http://dx.doi.org/10.5588/ijtld.14.0535.

[105] Aung KJM, Van Deun A, Declercq E, Sarker MR, Das PK, Hossain MA, Rieder, HL. Successful '9-month Bangladesh regimen' for multidrug-resistant tuberculosis among over 500 consecutive patients. Int J Tuberc Lung Dis 2014; 18(10):1180–1187. https://doi.org/10.5588/ijtld.14.0100.

[106] Piubello A, Harouna SH, Souleymane MB, Boukary I, Morou S, Daouda M, Hanki Y, Van Deun A. High cure rate with standardized short-course multidrug-resistant tuberculosis treatment in Niger: no relapses. Int J Tuberc Lung Dis 2014; 18:1188–1194. http://dx.doi.org/10.5588/ijtld.13.0075.

[107] World Health Organization. 2013. Multidrug-resistant tuberculosis (MDR-TB). 2013 update. World Health Organization, Geneva, Switzerland.

[108] Tang S, Yao L, Hao X, Liu Y, Zeng L, Liu G, Li M, Li F, Wu M, Zhu Y, Sun H, Gu J, Wang X, Zhang Z. Clofazimine for the treatment of multidrug-resistant tuberculosis: prospective, multicenter, randomized controlled study in China. Clin Infect Dis 2015; 60:1361–1367. http://dx.doi.org/10.1093/cid/civ027.

[109] Tyagi S, Ammerman NC, Li SY, Adamson J, Converse PJ, Swanson RV, Almeida DV, Grosset JH. Clofazimine shortens the duration of the first-line treatment regimen for experimental chemotherapy of tuberculosis. Proc Natl Acad Sci USA 2015; 112:869–874. http://dx.doi.org/10.1073/pnas.1416951112.

[110] Grosset JH, Tyagi S, Almeida DV, Converse PJ, Li SY, Ammerman NC, Bishai WR, Enarson D, Trébucq A. Assessment of clofazimine activity in a second-line regimen for tuberculosis in mice.AmJ Respir Crit Care Med 2013; 188:608–612. http://dx.doi.org/10.1164/rccm.201304-0753OC.

[111] Seung KJ, Hewison C. Now is the time for shorter all-oral regimens for multidrug-resistant tuberculosis. Lancet Glob Health 2019; 7:e706. https://doi.org/10.1016/S2214-109X(19)30186-X

[112] Durusu İZ, Hüsnügil HH, Ataş H, Biber A, Gerekçi S, Güleç EA, Özen C. Anti-cancer effect of clofazimine as a single agent and in combination with cisplatin on U266 multiple myeloma cell line. Leuk Res 2017; 55:33–40. https://doi.org/10.1016/j.leukres.2017.01.019.

[113] Food and Drug Administration. LAMPRENE® (clofazimine) capsules, Full prescribing information. Reference ID: 3956651. U.S. Food and Drug Administration. Silver Spring, MD. https://www.accessdata.fda.gov/drugsatfda_docs/label/2016/019500s013lbl.pdf. 2016; [Accessed 05 June 2019].

[114] Novartis. Lamprene® Prescribing Information. NDA 19-500/S-010. Novartis. East Hanover, NJ. https://www.accessdata.fda.gov/drugsatfda_docs/label/2003/19500slr010_lamprene_lbl.pdf. 2002; [Accessed 05 June 2019].

[115] Chen Y, Yuan Z, Shen X, Wu J, Wu Z, Xu B. Resistance to second-line antituberculosis drugs and delay in drug susceptibility testing among multidrug-resistant tuberculosis patients in Shanghai. BioMed Res Int, 2016. http://dx.doi.org/10.1155/2016/2628913.

[116] Szeto W, Garcia-Buitrago MT, Abbo L, Rosenblatt JD, Moshiree B, Morris MI. May. Clofazimine enteropathy: a rare and under recognized complication of mycobacterial therapy. Open Forum Infect Dis 2016; 2,3(3):ofw004. https://doi.org/10.1093/ofid/ofw004.

[117] Yoon GS, Keswani RK, Sud S, Rzeczycki PM, Murashov MD, Koehn TA, Standiford TJ, Stringer KA, Rosania GR. Clofazimine biocrystal accumulation in macrophages upregulates interleukin 1 receptor antagonist production to induce a systemic anti-inflammatory state. Antimicrobl Agents Chemother 2016; 60(6):3470–3479. https://doi.org/10.1128/AAC.00265-16.

[118] Gopal M, Padayatchi N, Metcalfe JZ, O'Donnell MR. Systematic review of clofazimine for the treatment of drug-resistant tuberculosis. Int J Tuberc Lung Dis 2013; 17(8):1001–1007. https://doi.org/10.5588/ijtld.12.0144.

[119] Barot RK, Viswanath V, Pattiwar MS, Torsekar RG. Crystalline deposition in the cornea and conjunctiva secondary to long-term clofazimine therapy in a leprosy patient. Indian J Ophthalmol 2011; 59(4): 328–329. https://doi.org/10.4103/0301-4738.82012.

[120] Kaur I, Ram J, Kumar B, Kaur S, Sharma VK. Effect of clofazimine on eye in multibacillary leprosy. Indian J Lepr 1990; 62(1):87–90. https://www.ncbi.nlm.nih.gov/pubmed/2358706.

[121] Zaccagnino A, Managò A, Leanza L, Gontarewitz A, Linder B, Azzolini M, Biasutto L, Zoratti M, Peruzzo R, Legler K, Trauzold, A. Tumor-reducing effect of the clinically used drug clofazimine in a SCID mouse model of pancreatic ductal adenocarcinoma. Oncotarget 2017; 8(24):38276–38293. https://doi.org/10.18632/oncotarget.11299.

[122] Dixit VB, Chaudhary SD, Jain VK. Clofazimine induced nail changes. Indian J Lepr 1989; 61(4):476–478. https://www.ncbi.nlm.nih.gov/pubmed/2621382.

[123] World Health Organization. WHO Model Prescribing Information: Drugs Used in Leprosy. Geneva, Switzerland. World Health Organization,

http://apps.who.int/medicinedocs/pdf/h2988e/h2988e.pdf; 1998 [accessed 5 June 2019].

[124] National Hansen's Disease (Leprosy) Program. Recommended Treatment Regimens. US Department of Health and Human Services. Washington DC. https://www.hrsa.gov/hansensdisease/diagnosis/recommendedtreatment.html. [accessed 5 June 2019].

[125] Hwang TJ, Dotsenko S, Jafarov A, Weyer K, Falzon D, Lunte K, Nunn P, Jaramillo E, Keshavjee S, Wares DF. Safety and availability of clofazimine in the treatment of multidrug and extensively drug-resistant tuberculosis: analysis of published guidance and meta-analysis of cohort studies. BMJ Open 2014; 4:e004143. http://dx.doi.org/10.1136/bmjopen-2013-004143.

[126] Ozturk Z, Tatliparmak A. Leprosy treatment during pregnancy and breastfeeding: a case report and brief review of literature. Dermatol Ther 2017; 30:e12414. http://dx.doi.org/10.1111/dth.12414.

[127] Drobac PC, del Castillo H, Sweetland A, Anca G, Joseph JK, Furin J, Shin S. Treatment of multidrug-resistant tuberculosis during pregnancy: long-term follow-up of 6 children with intrauterine exposure to second-line agents. Clin Infect Dis 2005; 40:1689–1692. https://doi.org/10.1086/430066.

[128] Levis W, Rendini T. Clofazimine Mechanisms of Action in Mycobacteria, HIV, and Cancer. J Infect Dis 2017; 1;215(9):1488. https://doi.org/10.1093/infdis/jix083.

[129] Machado TC, Gelain AB, Rosa J, Cardoso SG, Caon T. Cocrystallization as a novel approach to enhance the transdermal administration of meloxicam. Eur J Pharm Sci 2018; 123:184–190. https://doi.org/10.1016/j.ejps.2018.07.038.

[130] Singh Malik D, Mital N, Kaur G. Topical drug delivery systems: a patent review. Expert Opin Ther Pat 2016; 26(2):213–228. https://doi.org/10.1517/13543776.2016.1131267.

[131] Bhowmik D, Gopinath H, Kumar BP, Duraivel S, Kumar KS. Recent advances in novel topical drug delivery system. Pharma Innovation 2012; 1(9):12–31. http://www.thepharmajournal.com/vol1Issue9/Issue_nov_2012/1.1.pdf.

[132] Gratieri T, Alberti I, Lapteva M, Kalia YN. Next generation intra-and transdermal therapeutic systems: using non-and minimally-invasive technologies to increase drug delivery into and across the skin. Eur J Pharm Sci 2013; 50(5):609–622. https://doi.org/10.1016/j.ejps.2013.03.019.

[133] Prausnitz MR, Mitragotri S, Langer R. Current status and future potential of transdermal drug delivery. Nat Rev Drug Discov 2004; 3(2):115–124. https://doi.org/10.1038/nrd1304.

[134] Zhang Y, Zhang N, Song H, Li H, Wen J, Tan Y, Zheng W. Design, characterization and comparison of transdermal delivery of colchicine via borneol-chemically-modified and borneol-physically-modified ethosome. Drug Deliv 2019; 26(1):70–77. https://doi.org/10.1080/10717544.2018.1559258.

[135] Nan L, Liu C, Li Q, Wan X, Guo J, Quan P, Fang L. Investigation of the enhancement effect of the natural transdermal permeation enhancers from Ledum palustre L. var. angustum N. Busch: Mechanistic insight based on interaction among drug, enhancers and skin. Euro J Pharm Sci 2018; 124(1):105–113. https://doi.org/10.1016/j.ejps.2018.08.025.

[136] Ita K. Transdermal delivery of drugs with microneedles—potential and challenges. Pharmaceutics 2015; 7(3):90–105. https://doi.org/10.3390/pharmaceutics7030090.

[137] Roberts MS, Mohammed Y, Pastore MN, Namjoshi, S, Yousef S, Alinaghi A, Haridass IN, Abd E, Leite-Silva VR, Benson HAE, Grice JE. Topical and cutaneous delivery using nanosystems. J Control Release 2017; 247(1):86–105. https://doi.org/10.1016/j.jconrel.2016.12.022.

[138] Slominski AT, Manna PR, Tuckey RC. On the role of skin in the regulation of local and systemic steroidogenic activities. Steroids 2015; 103(1):72–88. https://doi.org/10.1016/j.steroids.2015.04.006.

[139] Bellefroid C, Lechanteur A, Evrard B, Piel G. Lipid gene nanocarriers for the treatment of skin diseases: current state-of-the-art. Eur J Pharm Biopharm. 2019; 137(1):95–111. https://doi.org/10.1016/j.ejpb.2019.02.012.

[140] Marwah H, Garg T, Goyal AK, Rath G. Permeation enhancer strategies in transdermal drug delivery. Drug Deliv 2016; 23(2):564–578. https://doi.org/10.3109/10717544.2014.935532.

[141] Burger,C, Gerber M, Du Preez JL, Du Plessis J. Optimised transdermal delivery of pravastatin. Int J pharm 2015; 496(2):518–525. http://dx.doi.org/10.1016/j.ijpharm.2015.10.034.

[142] Das A, Ahmed AB. Natural permeation enhancer for transdermal drug delivery system and permeation evaluation: a review. Asian J Pharm Cli Res, 2017; 10(9):5–9. https://doi.org/10.22159/ajpcr.2017.v10i9.19389.

[143] Li J, Xu W, Liang Y, Wang H. The application of skin metabolomics in the context of transdermal drug delivery. Pharmacol Rep 2017; 69(2):252–259. http://dx.doi.org/10.1016/j.pharep.2016.10.011.

[144] Sun R, Celli A, Crumrine D, Hupe M, Adame LC, Pennypacker SD, Park K, Uchida Y, Feingold KR, Elias PM, Ilic D. Lowered humidity produces human epidermal equivalents with enhanced barrier properties. Tissue Eng Part C: Methods 2014; 21(1):15–22. https://doi.org/10.1089/ten.tec.2014.0065.

[145] Ramani R, Pandya S, Motka U, Lakhani D, Ramaney D, Sheth D. Drug penetration enhancement in transdermal drug delivery system by chemical penetration enhancers. Int J Preclin Pharm Res 2013; 4(1):10–17. https://doi.org/10.22159/ajpcr.2017.v10i9.19389.

[146] Naik A, Kalia YN, Guy RH. Transdermal drug delivery: overcoming the skin's barrier function. Pharm Sci Technolo Today 2000; 3(9):318–326. https://doi.org/10.1016/S1461-5347(00)00295-9.

[147] British Pharmacopoeia (BP). Clofazomine, https://www-pharmacopoeia-com.nwulib.nwu.ac.za/bp-2018/monographs/clofazimine.html?date=2018-01-01&page=21; 2018 [Accessed 22 March 2018].

[148] Alkilani A, McCrudden MT, Donnelly R. Transdermal drug delivery: innovative pharmaceutical developments based on disruption of the barrier properties of the

stratum corneum. Pharmaceutics 2015; 7(4):438–470. https://doi.org/10.3390/pharmaceutics7040438.

[149] Su R, Fan W, Yu Q, Dong X, Qi J, Zhu Q, Zhao W, Wu W, Chen Z, Li Y, Lu Y. Size-dependent penetration of nanoemulsions into epidermis and hair follicles: implications for transdermal delivery and immunization. Oncotarget 2017; 8(24):38214. https://doi.org/10.18632/oncotarget.17130.

[150] Gidal BE, Clark AM, Anders B, Gilliam F. The application of half-life in clinical decision making: Comparison of the pharmacokinetics of extended-release topiramate (USL255) and immediate-release topiramate. Epilepsy Res 2017; 129(1):26–32. https://doi.org/10.1016/j.eplepsyres.2016.10.020.

[151] Kathe K, Kathpalia H. Film forming systems for topical and transdermal drug delivery. Asian J Pharm Sci 2017; 12(6):487–497. https://doi.org/10.1016/j.ajps.2017.07.004.

[152] Moser K, Kriwet K, Froehlich C, Kalia YN, Guy RH. Supersaturation: enhancement of skin penetration and permeation of a lipophilic drug. Pharm Res 2001; 18(7):1006–1011. http://dx.doi.org/10.1023/A:1010948630296.

[153] Mao F, Kong Q, Ni W, Xu X, Ling D, Lu Z, Li J. Melting point distribution analysis of globally approved and discontinued drugs: A research for improving the chance of success of drug design and discovery. ChemistryOpen 2016; 5(4):357–368. https://doi.org/10.1002/open.201600015.

[154] Das MK, Bhattacharya A, Ghosal SK. Effect of different terpene-containing essential oils on percutaneous absorption of trazodone hydrochloride through mouse epidermis. Drug Deliv 2006; 13(6):425–431. https://doi.org/10.1080/10717540500395064.

[155] Sangana R, Gu H, Chun DY, Einolf HJ. Evaluation of clinical drug interaction potential of clofazimine using static and dynamic modelling approaches. Drug Metab Dispos 2018; 46(1):26–32. https://doi.org/10.1124/dmd.117.077834.

[156] Zhang Y, Feng J, McManus SA, Lu HD, Ristroph KD, Cho EJ, Dobrijevic EL, Chan HK, Prud'homme RK. Design and solidification of fast-releasing clofazimine nanoparticles

for treatment of cryptosporidiosis. Mol Pharm 2017; 14(10):3480–3488. https://pubs.acs.org/doi/abs/10.1021/acs.molpharmaceut.7b00521.

[157] N'Da D. Prodrug strategies for enhancing the percutaneous absorption of drugs. Molecules 2014; 19(12):20780–20807. https://doi.org/10.3390/molecules191220780.

[158] Schmid-Wendtner MH, Korting HC. The pH of the skin surface and its impact on the barrier function. Skin Pharmacol Physiol 2006; 19(6):296–302. https://doi.org/10.1159/000094670.

[159] Nair A, Jacob S, Al-Dhubiab B, Attimarad M, Harsha S. Basic considerations in the dermatokinetics of topical formulations. Braz J Pharm Sci 2013; 49(3):423–434. http://dx.doi.org/10.1590/S1984-82502013000300004.

[160] Dhote V, Bhatnagar P, Mishra PK, Mahajan SC, Mishra DK. Iontophoresis: A Potential Emergence of a Transdermal Drug Delivery System. Sci Pharm 2012; 80(1):1–28. https://doi.org/10.3797/scipharm.1108-20.

[161] Chaulagain B, Jain A, Tiwari A, Verma A, Jain SK. Passive delivery of protein drugs through transdermal route. Artif Cells Nanomed Biotechnol 2018; 46(sup1):472–487. https://doi.org/10.1080/21691401.2018.1430695

[162] Pranitha A, Lakshmi PK. Effect of pH on weakly acidic and basic model drugs and determination of their ex vivo transdermal permeation routes. Braz J Pharm Sci 2018; 54(2):e00070. http://dx.doi.org/10.1590/s2175-97902018000200070.

[163] Viljoen JM, Cowley A, Du Preez J, Gerber M, du Plessis J. Penetration enhancing effects of selected natural oils utilized in topical dosage forms. Drug Dev Ind Pharm 2015; 41(12):2045–2054. https://doi.org/10.3109/03639045.2015.1047847.

[164] Čižinauskas V, Elie N, Brunelle A, Briedis V. Skin Penetration Enhancement by Natural Oils for Dihydroquercetin Delivery. Molecules 2017; 22(9):1536–1552. https://doi.org/10.3390/molecules22091536.

[165] Zaid AN, Jaradat NA, Eid AM, Al Zabadi H, Alkaiyat A, Darwish SA. Ethnopharmacological survey of home remedies used for treatment of hair and scalp

and their methods of preparation in the West Bank-Palestine. BMC Complement Altern Med 2017; 17(1):355–70. https://doi.org/10.1186/s12906-017-1858-1.

[166] Standard for Olive Oils and Olive Pomace Oils CODEX STAN 33–1981. International Food Standards: World Health Organization Food and Agriculture Organization of the United Nations; 2015.

[167] Lin TK, Zhong L, Santiago J. Anti-inflammatory and skin barrier repair effects of topical application of some plant oils. Int J Mol Sci 2018; 19(1):70–91. https://doi.org/10.3390/ijms19010070.

[168] Vaughn AR, Clark AK, Sivamani RK, Shi VY. Natural oils for skin-barrier repair: Ancient compounds now backed by modern science. Am J Clin Dermatol 2018; 19(1):103–117. https://doi.org/10.1007/s40257-017-0301-1.

[169] Cooke A, Cork M, Danby S. A national survey of UK maternity and neonatal units regarding the use of oil for baby skincare. Br J Midwifery 2011; 19:354–62. https://doi.org/10.12968/bjom.2011.19.6.354.

[170] Kulkarni A, Kaushik JS, Gupta P, Sharma H, Agrawal RK. Massage and touch therapy in neonates: the current evidence. Indian Pediatr 2010; 47(9):771–6. https://doi.org/10.1007/s13312-010-0114-2.

[171] Loden M. Role of topical emollients and moisturizers in the treatment of dry skin barrier disorders. Am J Clin Dermatol 2003; 4(11):771–88. http://dx.doi.org/10.2165/00128071-200304110-00005.

[172] Fox LT, Gerber M, Plessis JD, Hamman JH. Transdermal drug delivery enhancement by compounds of natural origin. Molecules 2011; 16(12):10507–10540. https://doi.org/10.3390/molecules161210507.

[173] Yang JY, Li J, Wang M, Zou XG, Peng B, Yin YL, Deng ZY. A Novel Aqueous Extraction for Camellia Oil by Emulsified Oil: A Frozen/Thawed Method. Eur J Lipid Sci Tech 2019; 121(4):1800431. https://doi.org/10.1002/ejlt.201800431.

[174] Sparr E, Millecamps D, Isoir M, Burnier V, Larsson Å, Cabane B. Controlling the hydration of the skin though the application of occluding barrier creams. J Royal Soc Interface 2013; 10(80):20120788. https://doi.org/10.1098/rsif.2012.0788.

[175] Carranco N, Farrés-Cebriá, M, Saurina J, Núñez O. Authentication and quantitation of fraud in extra virgin olive oils based on HPLC-UV fingerprinting and multivariate calibration. Foods 2018; 7(4):44–59. https://doi.org/10.3390/foods7040044.

[176] Roni, M.A. and Jalil, R.U., 2011. Comparative study of ibuprofen solubility in synthetic and natural lipid vehicles. Dhaka Univ J Pharml Sci 2011; 10(1):65–66. https://doi.org/10.3329/dujps.v10i1.10018.

[177] Sample A, He YY. Mechanisms and prevention of UV-induced melanoma. Photodermatol Photoimmunol Photomed 2018; 34(1):13–24. https://doi.org/10.1111/phpp.12329.

[178] Silva SAME, Michniak-Kohn B, Leonardi GR. An overview about oxidation in clinical practice of skin aging. An Bras Dermatol 2017; 92(3):367–374. http://dx.doi.org/10.1590/abd1806-4841.20175481.

[179] Agra LC, Ferro JNS, Barbosa FT, Barreto E. Triterpenes with healing activity: A systematic review. J Dermatol Treat 2015; 26(5):465–470. https://doi.org/10.3109/09546634.2015.1021663.

[180] Mack Correa MC, Mao G, Saad P, Flach CR, Mendelsohn R, Walters RM. Molecular interactions of plant oil components with stratum corneum lipids correlate with clinical measures of skin barrier function. Exp Dermatol 2014 23:39–44. http://dx.doi.org/10.1111/exd.12296.

[181] Servili M, Esposto S, Fabiani R, Urbani S, Taticchi A, Mariucci F, Selvaggini R, Montedoro GF. Phenolic compounds in olive oil: Antioxidant, health and organoleptic activities according to their chemical structure. Inflammopharmacology 2009; 17(2):76–84. https://doi.org/10.1007/s10787-008-8014-y.

[182] Lercker G, Rodriguez-Estrada MT. Chromatographic analysis of unsaponifiable compounds of olive oils and fat-containing foods. J. Chromatogr. A 2000; 881(1-2):105–129. https://doi.org/10.1016/S0021-9673(00)00455-6.

[183] van Zyl L, du Preez J, Gerber M, du Plessis J, Viljoen J. Essential Fatty Acids as Transdermal Penetration Enhancers. J Pharm Sci 2016; 105(2016):188–193. http://dx.doi.org/10.1016/j.xphs.2015.11.032.

[184] Cicero N, Albergamo A, Salvo A, Bua GD, Bartolomeo G, Mangano V, Rotondo A, Di Stefano V, Di Bella G, Dugo G. Chemical characterization of a variety of cold-pressed gourmet oils available on the Brazilian market. Food Res Int, 2018; 109(1):517–525. https://doi.org/10.1016/j.foodres.2018.04.064.

[185] El Kharrassi Y, Maata N, Mazri MA, El Kamouni S, Talbi M, El Kebbaj R, Moustaid K, Essamadi AK, Andreoletti P, Cherkaoui-Malki M, Nasser B. Chemical and phytochemical characterizations of argan oil (Argania spinosa L. skeels), olive oil (Olea europaea L. cv. Moroccan picholine), cactus pear (Opuntia megacantha salm-dyck) seed oil and cactus cladode essential oil. J Food Meas Charact 2018; 12(2):747–754. https://doi.org/10.1007/s11694-017-9688-x.

[186] Rustan AC, Drevon CA. Fatty acids: structures and properties. Encyclopedia of Life Sciences, John Wiley and Sons, Ltd., Chichester 2001: 1–7. https://doi.org/10.1038/npg.els.0003894.

[187] Viljoen JM, Cowley A, du Preez J, Gerber M, du Plessis J. Penetration enhancing effects of selected natural oils utilized in topical dosage forms. Drug Dev Ind Pharm 2015; 41(12):2045–2054. http://dx.doi.org/10.3109/03639045.2015.1047847.

[188] Aggarwal G, Dhawa, S, L HariKumar S. Natural oils as skin permeation enhancers for transdermal delivery of olanzapine: *in vitro* and in vivo evaluation. Curr Drug Deliv 2012; 9(2):172–181. https://doi.org/10.2174/156720112800234567.

[189] Otto A, du Plessis J, Wiechers JW. Formulation effects of topical emulsions on transdermal and dermal delivery. Int J Cosmetic Sci 2009; 31(1):1–19. https://doi.org/10.1111/j.1468-2494.2008.00467.x.

[190] Rueda A, Seiquer I, Olalla M, Giménez R, Lara L, Cabrera-Vique C. Characterization of fatty acid profile of argan oil and other edible vegetable oils by gas chromatography and discriminant analysis. J Chem 2014: Article ID 843908, 8 pages. http://dx.doi.org/10.1155/2014/843908.

[191] Khallouki F, Eddouks M, Mourad A, Breuer A, Owen R. Ethnobotanic, ethnopharmacologic aspects and new phytochemical insights into moroccan argan fruits. Int J molecular Mol Sci 2017; 18(11):2277–2303. https://doi.org/10.3390/ijms18112277.

[192] Guillaume D, Charrouf Z. Argan oil and other argan products: Use in dermocosmetology. Eur J Lipid Sci Tech 2011; 113(4):403–408. https://doi.org/10.1002/ejlt.201000417.

[193] Badreddine A, Zarrouk A, Karym EM, Debbabi M, Nury T, Meddeb W, Sghaier R, Bezine M, Vejux A, Martine L, Grégoire S. Argan oil-mediated attenuation of organelle dysfunction, oxidative stress and cell death induced by 7-ketocholesterol in murine oligodendrocytes 158N. Int J Mol Sci 2017; 18(10):2220. https://doi.org/10.3390/ijms18102220.

[194] Pagliuca G, Bozzi C, Gallo FR, Multari G, Palazzino G, Porrà R, Panusa A. Triacylglycerol “hand-shape profile” of Argan oil. Rapid and simple UHPLC-PDA-ESI-TOF/MS and HPTLC methods to detect counterfeit Argan oil and Argan-oil-based products. J Pharm Biomed Anal 2018; 150(1):121–131. https://doi.org/10.1016/j.jpba.2017.11.059.

[195] Harhar H, Gharby S, Kartah B, Pioch D, Guillaume D, Charrouf Z. Effect of harvest date of Argania spinosa fruits on Argan oil quality. Ind Crop Prod 2014; 56:156–159. https://doi.org/10.1016/j.indcrop.2014.01.046.

[196] Manca ML, Matricardi P, Cencetti C, Peris JE, Melis V, Carbone C, Escribano E, Zaru M, Fadda AM, Manconi M. Combination of argan oil and phospholipids for the development of an effective liposome-like formulation able to improve skin hydration

and allantoin dermal delivery. Int J Pharm 2016; 505(1-2):204–211. https://doi.org/10.1016/j.ijpharm.2016.04.008.

[197] Charrouf Z, Guillaume D. Ethnoeconomical, ethnomedical, and phytochemical study of Argania spinosa (L.) Skeels. J Ethnopharmacol 1999; 67(1):7–14. https://doi.org/10.1016/S0378-8741(98)00228-1.

[198] Gul R, Ahmed N, Ullah N, Khan MI, Elaissari A. Biodegradable ingredient-based emulgel loaded with ketoprofen nanoparticles. AAPS PharmSciTech 2018; 19(4):1869–1881. https://doi.org/10.1208/s12249-018-0997-0.

[199] Lococo D, Mora-Huertas CE, Fessi H, Zaanoun I, Elaissari A. Argan oil nanoemulsions as new hydrophobic drug-loaded delivery system for transdermal application. J Biomed Nanotechnol 2012; 8(5):843–848. https://doi.org/10.1166/jbn.2012.1445.

[200] Kim MJ, Doh HJ, Choi MK, Chung SJ, Shim CK, Kim DD, Kim JS, Yong CS, Choi HG. Skin permeation enhancement of diclofenac by fatty acids. Drug Deliv 2008; 15(6):373–379. https://doi.org/10.1080/10717540802006898.

[201] Jiménez-Peñalver P, Castillejos M, Koh A, Gross R, Sánchez A, Font X, Gea T. Production and characterization of sophorolipids from stearic acid by solid-state fermentation, a cleaner alternative to chemical surfactants. J Clean Prod 2018; 172(1):2735–2747. https://doi.org/10.1016/j.jclepro.2017.11.138.

[202] Heylings JR, Davies DJ, Burton R. Dermal absorption of testosterone in human and pig skin *in vitro*. Toxicol *in Vitro* 2018; 48(1):71–77. https://doi.org/10.1016/j.tiv.2017.12.014.

[203] Gore E, Picard C, Savary G. Spreading behavior of cosmetic emulsions: Impact of the oil phase. Biotribology 2018; 16:17–24. https://doi.org/10.1016/j.biotri.2018.09.003.

[204] Patzelt A, Lademann J, Richter H, Darvin ME, Schanzer S, Thiede G, Sterry W, Vergou T, Hauser M. In vivo investigations on the penetration of various oils and their influence on the skin barrier. Skin Res Technol 2012; 18(3):364–369. https://doi.org/10.1111/j.1600-0846.2011.00578.x.

[205] Lamaud E, Huc A, Wepierre J. Effects of avocado and soya bean lipidic non-saponifiables on the components of skin connective tissue after topical application in the hairless rat: biophysical and biomechanical determination. Int J Cosmetic Sci, 1982; 4(4):143–152. https://doi.org/10.1111/j.1467-2494.1982.tb00310.x.

[206] Nayak BS, Raju SS, Chalapathi Rao AV. Wound healing activity of Persea americana (avocado) fruit: a preclinical study on rats. J Wound Care 2008; 17(3):123–125. https://doi.org/10.12968/jowc.2008.17.3.28670.

[207] de Oliveira AP, Franco EDS, Rodrigues Barreto R, Cordeiro DP, de Melo RG, de Aquino CMF, de Medeiros PL, da Silva TG, Góes AJDS, Maia MBDS. Effect of semisolid formulation of persea americana mill (Avocado) oil on wound healing in rats. Evid Based Complementary Altern Med 2013; Paper ID 472382, 8 pages. http://dx.doi.org/10.1155/2013/472382.

[208] Kumar A, Aggarwal G, Singh K, Harikumar SL. Comparison of vegetable and volatile oils as skin permeation enhancers for transdermal delivery of losartan potassium. Pharm Lett 2014; 6(1):199–213. https://pdfs.semanticscholar.org/e3e5/60879c5f08aa8a837df2a3344dbcd208efb6.pdf

[209] Kim S, Jang JE, Kim J, Lee YI, Lee DW, Song SY, Lee JH. Enhanced barrier functions and anti-inflammatory effect of cultured coconut extract on human skin. Food Chem Toxicol 2017; 106:367–375. https://doi.org/10.1016/j.fct.2017.05.060.

[210] Zakaria ZA, Rofiee MS, Somchit MN, Zuraini A, Sulaiman MR, Teh LK, Salleh MZ, Long K. Hepatoprotective activity of dried-and fermented-processed virgin coconut oil. Evid Based Complementary Altern Med 2011; Article ID 142739, 8 pages http://dx.doi.org/10.1155/2011/142739.

[211] Naik A, Raghavendra SN, Raghavarao KSMS. Production of coconut protein powder from coconut wet processing waste and its characterization. Biotechnol Appl Biochem 2012; 167(5):1290–1302. https://doi.org/10.1007/s12010-012-9632-9.

[212] Cádiz-Gurrea M, Borrás-Linares I, Lozano-Sánchez J., Joven J, Fernández-Arroyo S, Segura-Carretero A. Cocoa and grape seed by products as a source of antioxidant

and anti-inflammatory proanthocyanidins. Int J Mol Sci 2017; 18(2):376–390. https://doi.org/10.3390/ijms18020376.

[213] Preuss HG, Echard B, Enig M, Brook I. Elliott TB. Minimum inhibitory concentrations of herbal essential oils and monolaurin for gram-positive and gram-negative bacteria. Mol Cell Biochem 2005; 272(1-2):29–34. https://doi.org/10.1007/s11010-005-6604-1.

[214] Kezutyte T, Desbenoit N, Brunelle A, Briedis V. Studying the penetration of fatty acids into human skin by ex vivo TOF-SIMS imaging. Biointerphases 2013; 8(3):1–8. https://doi.org/10.1186/1559-4106-8-3.

[215] Shintani M, Ogiso T. Mechanism for the enhancement effect of fatty acids on the percutaneous absorption of propranolol. J Pharm Sci 1990; 79(12):1065–1071. https://doi.org/10.1002/jps.2600791206.

[216] Cotte M, Dumas P, Besnard M, Tchoreloff P, Walter P. Synchrotron FT-IR microscopic study of chemical enhancers in transdermal drug delivery: example of fatty acids. J Control Release 2004; 97(2): 269–281. https://doi.org/10.1016/j.jconrel.2004.03.014.

[217] Cazolla R, Garziano M, Porta MD, Loreggian L, Cestaro B. First insights of macadamia oil as dietary fat. Potential health benefits. Agro Food Ind Hi Tech 2018; 29(6):18–20. https://www.teknoscienze.com/tks_article/first-insights-of-macadamia-nut-oil-as-dietary-fat-potential-health-benefits/.

[218] Madawala SRP, Kochhar SP, Dutta PC. Lipid components and oxidative status of selected specialty oils. Grasas Aceites 2012; 63(2):143–151. https://doi.org/10.3989/gya.083811.

[219] Parveez GKA, Rasid OA, Masani MYA, Sambanthamurthi R. Biotechnology of oil palm: strategies towards manipulation of lipid content and composition. Plant Cell Rep 2015; 34(4):533–543. https://doi.org/10.1007/s00299-014-1722-4.

[220] Rambharose S, Kalhapure RS, Jadhav M, Govender T. Novel mono, di and tri-fatty acid esters bearing secondary amino acid ester head groups as transdermal permeation enhancers. New J Chem 2018; 42(3):2232–2242. https://doi.org/10.1039/C7NJ04025C.

[221] McWilliam V, Peters R, Tang ML, Dharmage S, Ponsonby AL, Gurrin L, Perrett K, Koplin J, Allen KJ, Dwyer T, Lowe A. Patterns of tree nut sensitization and allergy in the first 6 years of life in a population-based cohort. J Allergy Clin Immunol 2019; 143(2):644–650. https://doi.org/10.1016/j.jaci.2018.07.038.

[222] Rodrigues, F., Pimentel, F.B. and Oliveira, M.B.P., 2015. Olive by-products: Challenge application in cosmetic industry. Ind Crop Prod 2015; 70:116–124. https://doi.org/10.1016/j.indcrop.2015.03.027.

[223] Moreira TSA, de Sousa VP, Pierre MBR. A novel transdermal delivery system for the anti-inflammatory lumiracoxib: influence of oleic acid on *in vitro* percutaneous absorption and in vivo potential cutaneous irritation. AAPS PharmSciTech 2010; 11(2):621–629. https://doi.org/10.1208/s12249-010-9420-1.

[224] Patel RP, Patel G, Patel H, Baria A. Formulation and evaluation of transdermal patch of aceclofenac. RJPDFT 2009; 1(2):108–115. https://pdfs.semanticscholar.org/b1db/5c74b60ded1fba1aa68165ea4523c8862d38.pdf

[225] Singh A., Singh V, Juyal D, Rawat G. 2015. Self emulsifying systems: A review. Asian J Pharm 2015; 9(1):13–18. http://dx.doi.org/10.22377/ajp.v9i1.425.

[226] Güngör S, Bergişadi N. Effect of penetration enhancers on *in vitro* percutaneous penetration of nimesulide through rat skin. Pharmazie 2004; 59(1):39–41. https://www.ncbi.nlm.nih.gov/pubmed/14964420.

[227] Holm R. Bridging the gaps between academic research and industrial product developments of lipid-based formulations. Adv Drug Deliv Rev 2019; article in press. https://doi.org/10.1016/j.addr.2019.01.009.

[228] Rahman MA, Hussain A, Hussain MS, Mirza MA, Iqbal Z. Role of excipients in successful development of self-emulsifying/microemulsifying drug delivery system (SEDDS/SMEDDS). Drug Dev Ind Pharm 2013; 39(1):1–19. https://doi.org/10.3109/03639045.2012.660949.

[229] Bernkop-Schnürch A, Jalil A. Do drug release studies from SEDDS make any sense? J Control Release 2018; 271(1):55–59. https://doi.org/10.1016/j.jconrel.2017.12.027.

[230] Morgen M, Saxena A, Chen X-Q, Miller W, Nkansah R, Goodwin A, Cape J, Haskell R, Su C, Gudmundsson O, Hageman M, Kumar A, Chowan GS, Rao A, Holenarsipur VK. Lipophilic salts of poorly soluble compounds to enable high-dose lipidic SEDDS formulations in drug discovery. Eur J Pharm Biopharm 2017; 117(2017):212–223. https://doi.org/10.1016/j.ejpb.2017.04.021.

[231] Koul A, Arnoult E, Lounis N, Guillemont J, Andries K. The challenge of new drug discovery for tuberculosis. Nature 2011; 469(7331):483–490. http://dx.doi.org/10.1038/nature09657.

[232] Balganesh TS, Alzari PM, Cole ST. Rising standards for tuberculosis drug development. Trends Pharmacol Sci 2008; 29(11):576–581. https://doi.org/10.1016/j.tips.2008.08.001.

[233] Chatterjee B, Hamed Almurisi S, Ahmed Mahdi Dukhan A, Mandal UK, Sengupta P. Controversies with self-emulsifying drug delivery system from pharmacokinetic point of view. Drug Deliv 2016; 23(9):3639–3652. https://doi.org/10.1080/10717544.2016.1214990.

[234] Gonçalves A, Nikmaram N, Roohinejad S, Estevinho BN, Rocha F, Greiner R, McClements DJ. Production, properties, and applications of solid self-emulsifying delivery systems (S-SEDS) in the food and pharmaceutical industries. Colloids Surf A Physicochem Eng Asp 2018; 538(1):108–126. http://dx.doi.org/10.1016/j.colsurfa.2017.10.076.

[235] Watanabe A, Murayama S, Karasawa K, Yamamoto E, Morikawa S, Takita R, Murata S, Kato M. A simple and easy method of monitoring doxorubicin release from a liposomal drug formulation in the serum using fluorescence spectroscopy. Chem Pharm Bull 2019; 67:367–371.

[236] Gupta PK, Bhandari N, Shah HN, Khanchandani V, Keerthana R, Nagarajan V, Hiremath L. An update on nanoemulsions using nanosized liquid in liquid colloidal systems. In: Nanoemulsions – Properties, fabrications and applications. [Online First], IntechOpen; 2019. https://doi.org/10.5772/intechopen.84442.

[237] Chime SA, Kenechukwu FC, Attama AA. Nanoemulsions—advances in formulation, characterization and applications in drug delivery. In Application of nanotechnology in drug delivery. IntechOpen; 2014. http://dx.doi.org/10.5772/15371.

[238] Aulton ME, Taylor KMG. Aulton's Pharmaceutics: The design and manufacture of medicines. 5th ed. Edinburgh: Churchill Livingstone; 2018.

[239] Barry BW. Novel mechanisms and devices to enable successful transdermal drug delivery. Eur J Pharm Sci 2001; 14(2):101–114. https://doi.org/10.1016/S0928-0987(01)00167-1.

[240] El Maghraby GMM, Williams AC, Barry BW. Oestradiol skin delivery from ultradeformable liposomes: refinement of surfactant concentration. Int J Pharm 2000; 196:63–74. https://doi.org/10.1016/S0378-5173(99)00441-X.

[241] Patil JM, Yadava SK, Mokale VJ, Naik J. Development of surfactant free nanoparticles by a single emulsion high pressure homogenization technique and effect of formulation parameters on the drug entrapment and release. Int J Pharm 2013; 3:843–852.

[242] Rohrer J, Zupančič O, Hetenyi G, Kurpiers M, Bernkop-Schnuerch A. Design and evaluation of SEDDS exhibiting high emulsifying properties. Journal of Drug Delivery Science and Technology 2018; 44(1):366–372. https://doi.org/10.1016/j.jddst.2018.01.013.

[243] Köllner S, Nardin I, Markt R, Griesser J, Prüfert F, Bernkop-Schnürch A. Self-emulsifying drug delivery systems: design of a novel vaginal delivery system for curcumin. Eur J Pharm Biopharm 2017; 115(1):268–275. https://doi.org/10.1016/j.ejpb.2017.03.012.

[244] Pattewar SV, Kasture SB, Pande VV, Sharma SK. A New Self Microemulsifying Mouth Dissolving Film. Indian J Pharm Educ Res 2016; 50(3):S191–199. https://doi.org/10.5530/ijper.50.3.29.

[245] ElKasabgy NA. Ocular supersaturated self-nanoemulsifying drug delivery systems (S-SNEDDS) to enhance econazole nitrate bioavailability. Int J Pharm 2014; 460(1-2):33–44. https://doi.org/10.1016/j.ijpharm.2013.10.044.

[246] Xiao L, Yi T, Liu Y. A new self-microemulsifying mouth dissolving film to improve the oral bioavailability of poorly water soluble drugs. Drug Dev Ind Pharm 2013; 39(9):1284–1290. https://doi.org/10.3109/03639045.2012.723716.

[247] Khan M, Nadhman A, Sehgal SA, Siraj S, Yasinzai MM. Formulation and Characterization of a Self-Emulsifying Drug Delivery System (SEDDS) of Curcumin for the Topical Application in Cutaneous and Mucocutaneous Leishmaniasis. Curr Top Med Chem 2018; 18(18):1603–1609. https://doi.org/10.2174/1568026618666181025104818.

[248] Pratiwi L, Fudholi A, Martien R, Pramono S. Self-nanoemulsifying Drug Delivery System (Snedds) for Topical Delivery of Mangosteen Peels (Garcinia Mangostana L.,): Formulation Design and *In vitro* Studies. JYP 2017; 9(3):341–346. https://doi.org/10.5530/jyp.2017.9.68.

[249] Vasconcelos T, Marques S, Sarmento B. Measuring the emulsification dynamics and stability of self-emulsifying drug delivery systems. Eur J Pharm Biopharm 2018; 123:1–8. https://doi.org/10.1016/j.ejpb.2017.11.003.

[250] Rani S, Rana R, Saraogi GK, Kumar V, Gupta U. Self-Emulsifying Oral Lipid Drug Delivery Systems: Advances and Challenges. AAPS PharmSciTech 2019; 20(3):129. https://doi.org/10.1208/s12249-019-1335-x.

[251] Nikolakakis I, Partheniadis I. Self-Emulsifying Granules and Pellets: Composition and Formation Mechanisms for Instant or Controlled Release. Pharmaceutics 2017; 9(4):50–77. https://doi.org/10.3390/pharmaceutics9040050.

[252] Gurram AK, Deshpande PB, Kar SS, Nayak UY, Udupa N, Reddy M.S. Role of components in the formation of self-microemulsifying drug delivery systems. Indian J Pharm Sci 2015; 77(3):249–257. https://doi.org/10.4103/0250-474X.159596.

[253] Smith, K.L. Penetrant characteristics influencing skin absorption. In: Kemppainen BW, Reifenrath WG, editors. Methods for skin absorption, Boca Raton: CRC; 1990, p. 23–34.

[254] Rajeshwar V, Shrivastava B. Self-emulsifying drug delivery system (SEDDS): a conventional alternative and approach to improve oral bioavailability. IJPSR 2018; 9(8):3114–3127. https://doi.org/10.13040/IJPSR.0975-8232.9(8).3114-27.

[255] Zhang L, Zhang I, Zhang M, Pang Y, Li Z, Zhao A, Feng J. Self-emulsifying drug delivery system and the applications in herbal drugs. Drug Deliv 2015; 22(4):475–486. https://doi.org/10.3109/10717544.2013.861659.

[256] Anton N, Vandamme TF. Nano-emulsions and micro-emulsions: clarifications of the critical differences. Pharm Res 2011; 28(5):978–985. https://doi.org/10.1007/s11095-010-0309-1.

[257] Thakare P, Mogal V, Borase P, Dusane J, Kshirsagar S. A review on self-emulsified drug delivery system. Pharmaceutical and Biological Evaluations 2016; 3(2):140–153. http://www.onlinepbe.com/index.php/PBE/article/view/88.

[258] Senapati PC, Sahoo SK, Sahu AN. Mixed surfactant based (SNEDDS) self-nanoemulsifying drug delivery system presenting efavirenz for enhancement of oral bioavailability. Biomed Pharmacother 2016; 80:42–51. https://doi.org/10.1016/j.biopha.2016.02.039.

[259] Akbarzadeh A, Rezaei-Sadabady R, Davaran S, Joo SW, Zarghami N, Hanifehpour Y, Samiei M, Kouhi M, Nejati-Koshki K. Liposome: classification, preparation, and applications. Nanoscale Res. Lett. 2013; 8(1):102. https://doi.org/10.1186/1556-276X-8-102.

[260] Hegde RR, Verma A, Ghosh A. Microemulsion: new insights into the ocular drug delivery. ISRN pharmaceutics 2013; Article ID 826798, 11 pages. http://dx.doi.org/10.1155/2013/826798.

[261] Niu Z, Conejos-Sanchez I, Griffin BT, O'Driscoll CM, Alonso MJ. Lipid-based nanocarriers for oral peptide delivery. Adv Drug Deliv Rev 2016; 106:337–354. https://doi.org/10.1016/j.addr.2016.04.001.

[262] Mojeiko G, de Brito M, Salata GC, Lopes LB. Combination of microneedles and microemulsions to increase celecoxib topical delivery for potential application in

chemoprevention of breast cancer. Int J Pharm 2019; 560:365–376. https://doi.org/10.1016/j.ijpharm.2019.02.011.

[263] Effendy I, Maibach HI. Surfactants and experimental irritant contact dermatitis. Contact dermatitis 1995; 33(4):217–225. https://doi.org/10.1111/j.1600-0536.1995.tb00470.x.

[264] Gupta S, Kesarla R,Omri A. Formulation strategies to improve the bioavailability of poorly absorbed drugs with special emphasis on self-emulsifying systems. ISRN Pharm 2013; 2013:848043. https://doi.org/10.1155/2013/848043.

[265] Akula S, Gurram AK, Devireddy SR. Self-microemulsifying drug delivery systems: an attractive strategy for enhanced therapeutic profile. Int Sch Res Notices 2014; Article ID 964051, 11 pages. http://dx.doi.org/10.1155/2014/964051.

[266] Jepps OG, Dancik Y, Anissimov YG, Roberts MS. Modelling the human skin barrier—Towards a better understanding of dermal absorption. Adv Drug Deliv Rev 2013; 65(2):152–168. https://doi.org/10.1016/j.addr.2012.04.003.

[267] King MJ, Michel D, Foldvari M. Evidence for lymphatic transport of insulin by topically applied biphasic vesicles. J Pharm Pharmacol 2003; 55(10):1339–1344. https://doi.org/10.1211/0022357021918.

[268] Sun M, Zhai X, Xue K, Hu L, Yang X, Li G, Si L. Intestinal absorption and intestinal lymphatic transport of sirolimus from self-microemulsifying drug delivery systems assessed using the single-pass intestinal perfusion (SPIP) technique and a chylomicron flow blocking approach: linear correlation with oral bioavailabilities in rats. Eur J Pharm Sc 2011; 43(3):132–140. https://doi.org/10.1016/j.ejps.2011.04.011.

[269] Elbahwy IA, Lupo N, Ibrahim HM, Ismael HR, Kasem AA, Caliskan C, Matuszczak B, Bernkop-Schnuerch A. Mucoadhesive self-emulsifying delivery systems for ocular administration of econazole. Int J Pharm 2018; 541(1-2):72–80. https://doi.org/10.1016/j.ijpharm.2018.02.019.

[270] Efiana NA, Mahmood A, Lam HT, Zupančič O, Leonaviciute G, Bernkop-Schnürch A. Improved mucoadhesive properties of self-nanoemulsifying drug delivery systems

(SNEDDS) by introducing acyl chitosan. Int J Pharm 2018; 519(1-2):206–212. https://doi.org/10.1016/j.colsurfb.2017.10.032.

[271] Leonaviciute G, Adamovic NT, Lam HT, Rohrer J, Partenhauser A., Bernkop-Schnürch A. Self-emulsifying drug delivery systems (SEDDS): proof-of-concept how to make them mucoadhesive. Eur J Pharm Biopharm 2017; 112:51–57. https://doi.org/10.1016/j.ejpb.2016.11.019.

[272] Salimi E, Le-Vinh B, Zahir-Jouzdani F, Matuszczak B, Ghaee A, Bernkop-Schnürch A. Self-emulsifying drug delivery systems changing their zeta-potential via a flip-flop mechanism. Int J Pharm 2018; 550(1-2):200–206. https://doi.org/10.1016/j.ijpharm.2018.08.046.

[273] Menzel C, Bernkop-Schnuerch A. Enzyme decorated drug carriers: targeted swords to cleave and overcome the mucus barrier. Adv Drug Deliv Rev 2018; 124(1):164–174. https://doi.org/10.1016/j.addr.2017.10.004.

[274] Xu Y, Wu J, Liao S, Sun Z. Treating tuberculosis with high doses of anti-TB drugs: mechanisms and outcomes. Ann Clin Microbiol Antimicrob 2017; 16(1):67–80. https://doi.org/10.1186/s12941-017-0239-4.

[275] Bhandari S, Rana V, Tiwary AK. Antimalarial solid self-emulsifying system for oral use: *in vitro* investigation. Ther Deliv 2017; 8(4):201–213. https://doi.org/10.4155/tde-2016-0092.

[276] Nigade PM, Patil SL, Tiwari SS. Self-emulsifying drug delivery system (SEDDS): A review. IJPBS 2012; 2(2):42–52. https://pdfs.semanticscholar.org/246a/ef881fe67b31419e799eec4a486d980eca49.pdf.

[277] Gaur RK. Antibiotic resistance: alternative approaches. Indian J Pharmacol 2017; 49(2):208–210. https://doi.org/10.4103/ijp.IJP_574_16.

[278] Jaiswal M, Dudhe R, Sharma PK. Nanoemulsion: an advanced mode of drug delivery system. 3 Biotech 2015; 5(2):123–127. https://doi.org/10.1007/s13205-014-0214-0.

[279] Bhosale RR, Osmani RA, Ghodake PP, Shaikh SM, Chavan SR. Nanoemulsion: A review on novel profusion in advanced drug delivery. IJPBR 2014; 2(1):122-127. https://doi.org/10.30750/ijpbr.2.1.19.

Table 1:
Physicochemical characteristics of clofazimine [99,147,158]

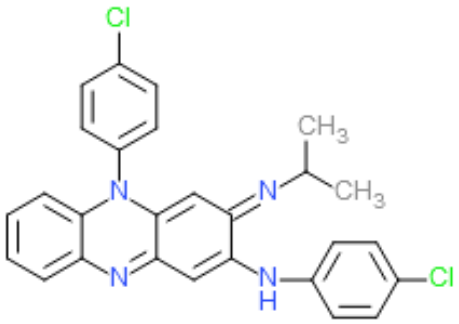
Physicochemical characteristic	
Chemical formula	$C_{27}H_{22}Cl_2N_4$
Molecular weight	473.40 D_a
Chemical structure	
Melting point	210–212°C
Appearance	Reddish-brown, fine powder.
Aqueous solubility	Practically insoluble in water.
Log P (octanol-water partition coefficient)	7.66
Detection on spectrophotometer	254 nm
Elimination half life	70 days
pK_a value	8.51
Solubility	Soluble in methylene chloride, very slightly soluble in ethanol (96%).

Table 2:
Fatty acid composition of selected plant based oils [184,185,190]

Fatty acid (% ± SD)	**Argan oil**	**Avocado oil**	**Coconut oil**	**Macadamia oil**	**Olive oil**
Lauric acid	Not detected	Not detected	66.94 ± 4.11	Not detected	Not detected
Myristic acid	0.12 ± 0.01	0.01 ± 0.008	18.81 ± 0.98	0.91 ± 0.17	Not detected
Palmitic acid	11.80 ± 0.53	14.21 ± 1.02	6.54 ± 1.20	6.84 ± 1.87	0.73 ± 0.05
Palmitoleic acid	0.18 ± 0.02	7.06 ± 0.79	Not detected	9.68 ± 0.90	2.04 ± 0.03
Stearic acid	6.00 ± 0.05	2.15 ± 0.07	2.01 ± 0.12	3.39 ± 0.96	2.83 ± 0.01
Oleic acid	45.90 ± 1.84	59.46 ± 3.88	4.75 ± 0.1	50.65 ± 8.05	72.79 ± 0.21
Linoleic acid	34.10 ± 1.08	14.66 ± 1.20	0.82 ± 0.32	8.63 ± 1.77	13.20 ± 0.03
Linolenic acid	0.13 ± 0.01	1.3 ± 0.15	Not detected	14.59 ± 1.20	0.92 ± 0.06
SFAs	17.58 ± 0.63	16.93 ± 1.09	94.40 ± 5.08	14.56 ± 2.38	11.60 ± 0.33
MUFAs	46.08 ± 1.82	67.11 ± 1.77	4.78 ± 0.17	62.22 ± 9.19	73.52 ± 0.21
PUFAs	33.63 ± 1.25	15.96 ± 1.15	0.082 ± 0.32	23.22 ± 1.62	14.13 ± 0.10
UFA/SFA	3.85	4.90	0.059	5.86	4.97

Table 3:

A summary of the most notable differences between liposomes, nanoemulsions and SEDDSs [222,234,250,254, 257,259,261,279,280]

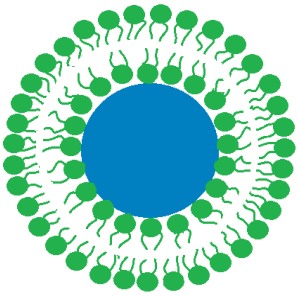
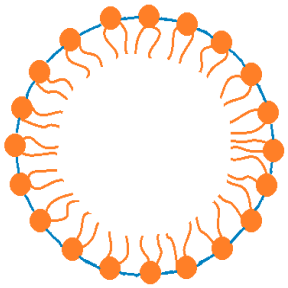
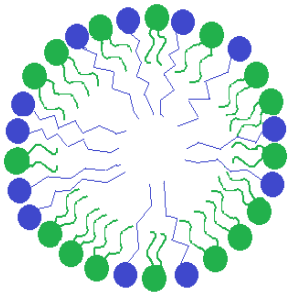
	Liposomes	Nanoemulsions	SEDDSs
Structure			
Size	0.025-2.5µm	20-200 nm	Nano or micro range
Constituents	• Natural or synthetic phospholipids • Sometimes cholesterol or other sterols	• Natural or synthetic oil phase • Surfactant and co-surfactant • Water phase	• Natural or synthetic oil phase • Surfactant and co-surfactant • Water phase
Production methods	• The Bangham method • Ethanol/ether injection • Sonication • Reverse phase evaporation	• High-pressure valve homogenisation • Microfluidisation • Ultrasonification • Phase inversion temperature (PIT) • Phase inversion composition (PIC) • Solvent diffusion	• Water titration method (liquid SEDDSs) **Solidification techniques:** • Spray-drying • Adsorption to solid carriers • Melt granulation • Melt extrusion
Advantages	• Increased efficacy and therapeutic index • Enhanced stability by encapsulation • Decrease toxicity of encapsulated drug • Effect of site avoidance • Decreased exposure of sensitive tissue to irritating drugs	• Substitute for liposomes • Non-toxic and decreased tissue irritation • Enhanced physical stability • Increased absorption • Various formulation possibilities • Enhanced solubility of lipophilic drugs	• Decreased dosage of drug needed • Increased drug loading capacity • Manufacture and upscale with ease • Consistent drug absorption profiles • Lymphatic uptake • Enhances solubility of lipophilic drugs
Limitations	• Decreased solubility • Short half-life • Oxidation and hydrolysis reactions of phospholipids • Fusion and leakage of drugs encapsulated • Increased production cost	• High surfactant concentrations to ensure stability of formulations • Stability is influenced by changes in temperature and pH • Ostwald ripening effect can lead to instability	• High surfactant concentrations can cause tissue irritation • Poor stability without surface active agent • Development challenges of validation methods

Figure captions

Figure 1: Classification systems of CTB (adapted from [63,82])

Figure 2: Simplified illustration of skin anatomy and drug penetration pathways (adapted from [137,143])

Figure 3: Simplified chemical structure of l fatty acid (adapted from [186])

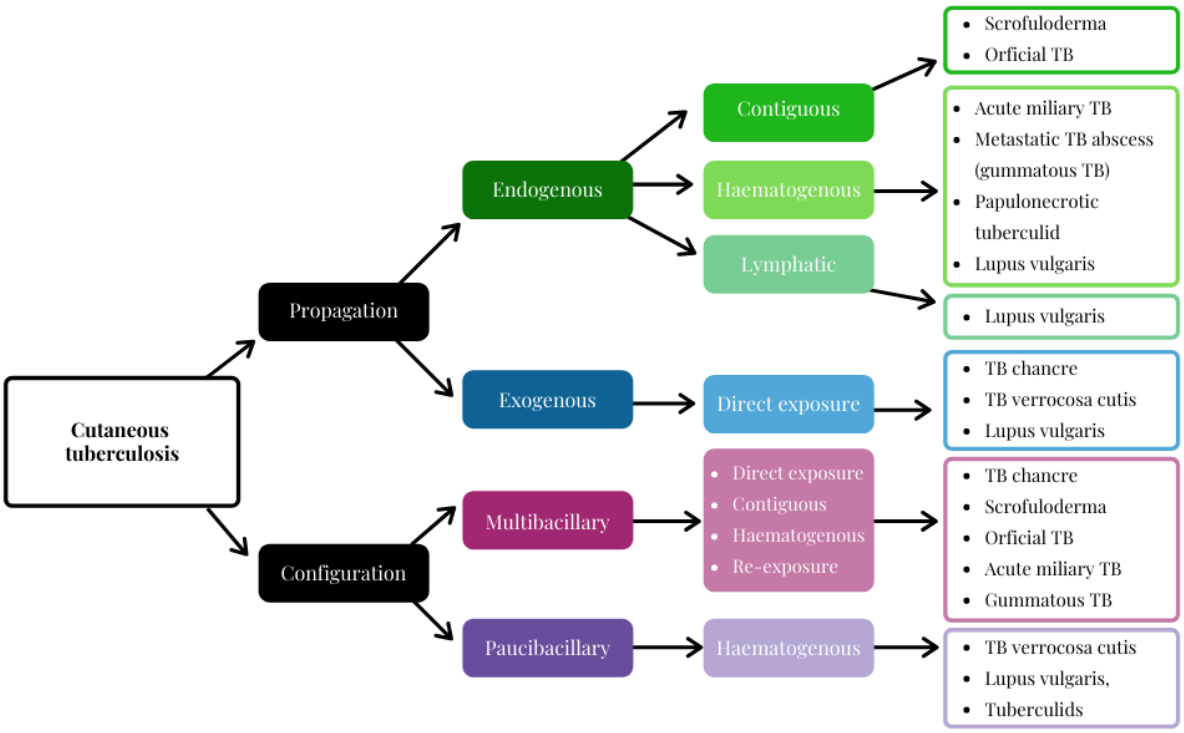


Figure 1: Classification systems of CTB (adapted from [63,82])

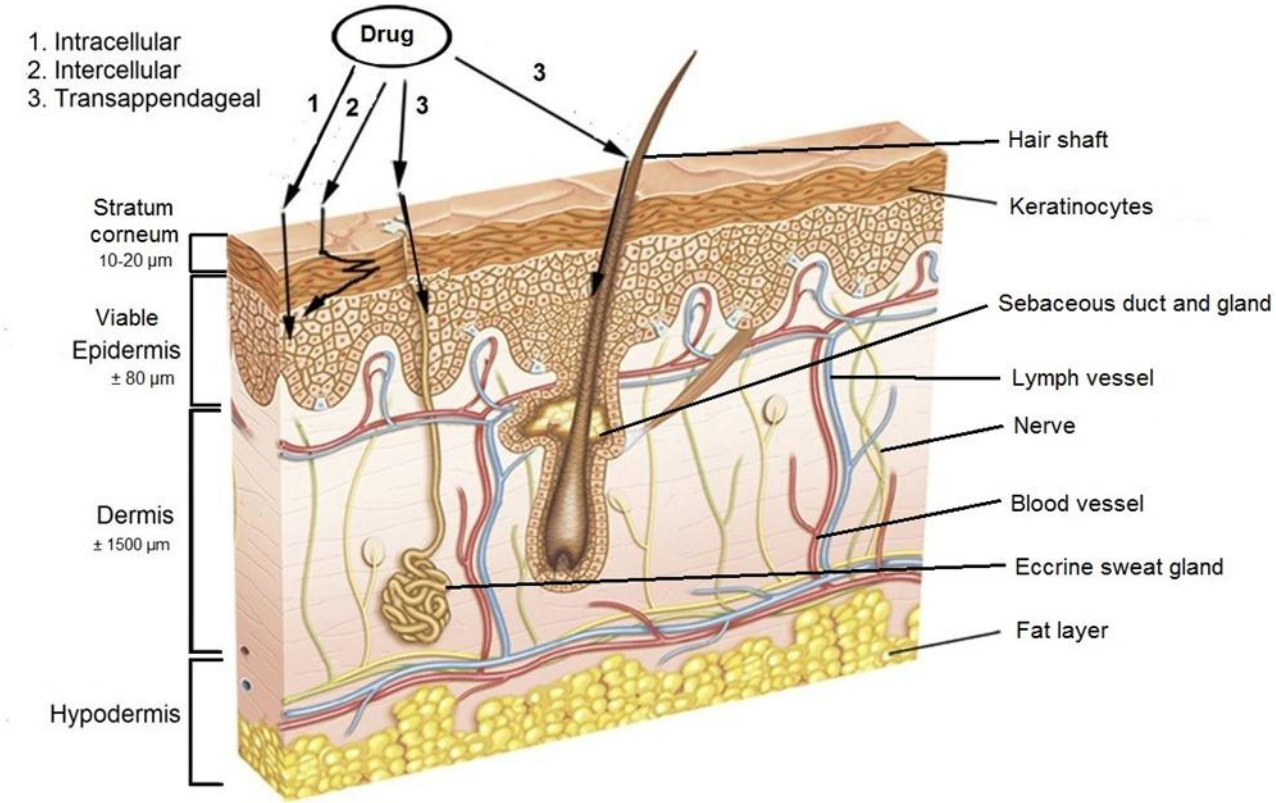


Figure 2: Simplified illustration of skin anatomy and drug penetration pathways (adapted from [137,143])

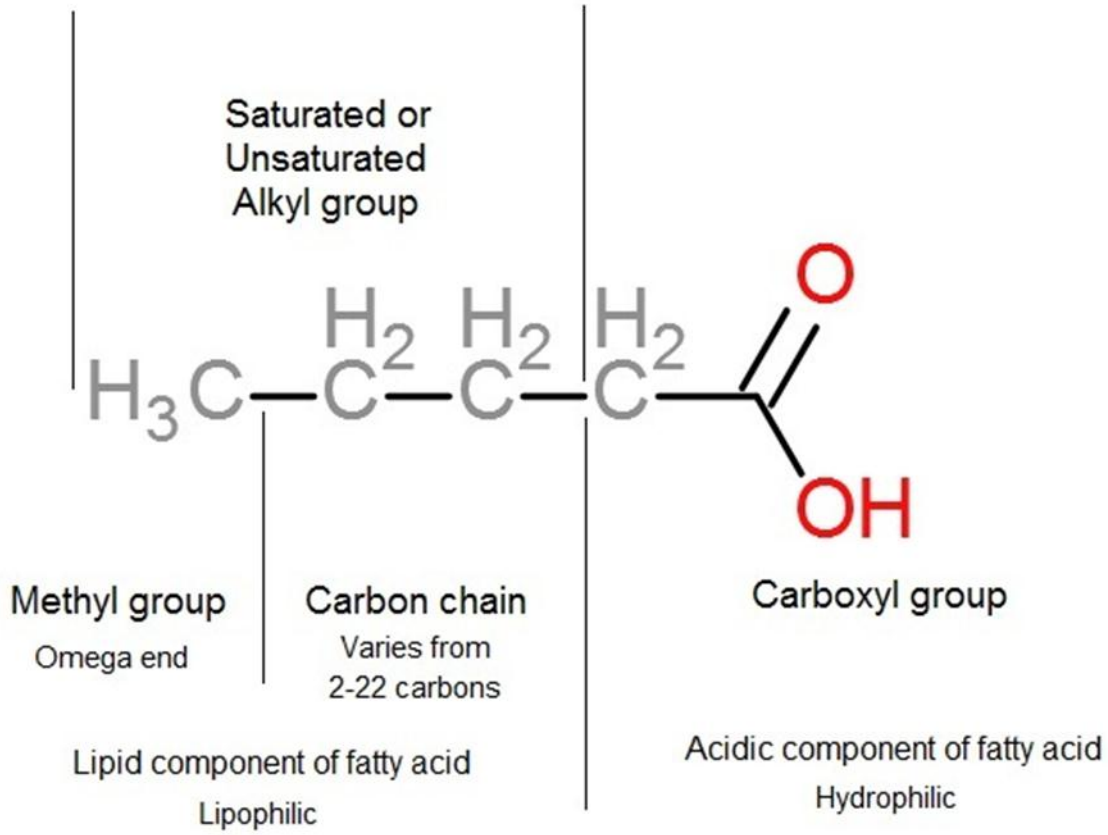


Figure 3: Simplified chemical structure of a fatty acid (adapted from [186])

Chapter 3

Manuscript submitted to *AAPS PharmSciTech Journal*

Chapter 3 is presented in article format according to the Author Guidelines of *AAPS PharmSciTech Journal*, as provided by Annexure H.

Development of a Self-emulsifying Drug Delivery System for Optimised Topical Delivery of Clofazimine

Daniélle van Staden, Jeanetta du Plessis, Joe Viljoen*

Faculty of Health Sciences, Department of Pharmaceutics, Centre of Excellence for Pharmaceutical Sciences (Pharmacen), Building G16, North-West University, 11 Hoffman Street, Potchefstroom, 2520, South Africa

E-mail addresses:

dvanstaden711@gmail.com (D van Staden), Jeanetta.DuPlessis@nwu.ac.za (J du Plessis), Joe.Viljoen@nwu.ac.za (JM Viljoen)

Corresponding Author:

Joe Viljoen

Joe.Viljoen@nwu.ac.za

Faculty of Health Sciences, Department of Pharmaceutics, Centre of Excellence for Pharmaceutical Sciences (Pharmacen), Building G16, Room 122, North-West University, 11 Hoffman Street, Potchefstroom, 2520, South Africa

Tel: +27 18 299 2273

Fax: +27 18 299 2248

Abstract

A quality-by-design and characterisation approach was followed to ensure development of a self-emulsifying drug delivery system (SEDDS) destined for optimised topical delivery of the highly lipophilic clofazimine. Solubility and water titration experiments were employed to identify the spontaneous emulsification capacity of divergent excipients and clofazimine utilised in combination. After identifying self-emulsification regions, check-point formulations were selected within a specific area of the region of self-emulsification by considering characteristics required to achieve optimised topical drug delivery. Next, check-point formulations that did not exhibit phase separation at an ambient temperature after 24 h, were subjected to characterisation experiments. These experiments involved evaluation of droplet size; size distribution; zeta-potential; robustness towards dilution; efficacy of self-emulsification; self-emulsification time; viscosity and pH measurement; cloud point assessment; and thermodynamic stability studies. Characterisation profiles were studied to identify SEDDSs with favourable properties since criteria must still be established for SEDDSs intended for topical drug delivery. Dermal diffusion studies displayed successful *in vitro* topical clofazimine delivery. Remarkably, the olive oil SEDDS facilitated the highest topical delivery of clofazimine probably due to the increased oleic acid content of olive oil that induced increased *stratum corneum* (SC) lipid disruption followed by enhanced dermal clofazimine delivery. Finally, isothermal microcalometric studies were conducted to confirm compatibility of excipients. Potential interactions were depicted between argan oil and clofazimine as well as between Span®60 and argan-, macadamia- and olive oil, respectively. However, despite some incompatibilities between certain excipients, topical SEDDSs that achieved enhanced topical clofazimine delivery were successfully developed.

Keywords: Clofazimine, penetration enhancers, self-emulsifying drug delivery system (SEDDS), topical delivery

INTRODUCTION

Resistance against anti-tubercular (A-TB) treatment regimens are considered a global health threat of the modern-age (1). An estimated 500 000 individuals are newly infected with multi-drug resistant tuberculosis (MDR-TB) each year (2). Additional to increased incidence of MDR-TB, rare extra-pulmonary manifestations of tuberculosis (TB), such as cutaneous tuberculosis (CTB), are escalating due to A-TB drug resistance (3). A-TB treatment is notably protracted as well as costly (1). Moreover, presently no topical treatment is available to aid CTB treatment (3). Therefore, CTB patients are subjected to general A-TB regimens (4). A-TB treatment regimens rely on drugs with both reasonable and serious adverse effect profiles (1). However, the development of a topically administered dosage form that assists in CTB therapy, can eliminate gastro-intestinal adverse effects by circumventing liver metabolism (5). Moreover, topical A-TB drug delivery will enable treatment at the affected site without interference with oral A-TB management since most patients infected with CTB also suffer from pulmonary TB disease (4,6).

Clofazimine is currently listed by the World Health Organisation (WHO) as a group 5 drug to for treatment of MDR-TB (7). This riminophenazine antibiotic agent is furthermore administered as part of the triple regimen employed during treatment of multibacillary leprosy (7). Increased lipophilicity as well as the redox potential of clofazimine establish anti-microbial effectiveness by means of oxidation of reduced clofazimine followed by formation of reactive oxygen species (8). Additionally, possible synergism between clofazimine and A-TB drugs including capreomycin and moxifloxacin have been reported (8). The focus of A-TB research has shifted towards repurposing known drug entities, with relatively safe adverse effect profiles to form part of new A-TB regimens for the resolution of decreasing treatment time and improving efficacy against TB disease (9). Clofazimine is a favourable candidate as this drug has exhibited *in vivo* as well as *in vitro* efficacy against strains of MDR-TB with limited toxicity (10,11). However, the physicochemical properties of clofazimine, that has an aqueous solubility of 10 mg/l and Log P value of 7.66, are not particularly suitable for dermal drug delivery (3,12). Nonetheless, development of an efficacious topical drug delivery system could potentially enable optimised topical delivery of clofazimine.

The concept of self-emulsifying drug delivery systems (SEDDSs) was pioneered during the 1960s when components of poor aqueous solubility were incorporated into mixtures of lipophilic and hydrophilic excipients to achieve enhanced solubility of lipophilic substances (13). However, Pouton (13) suggested the first concrete solution towards improved lipophilic drug delivery by utilising SEDDSs in 1985. Despite several decades devoted to development of SEDDS for various routes of administration, namely: oral-, rectal-, vaginal-, ocular- and nasal administration, the dermal route has remained relatively untouched compared to other routes of administration (14-20). The formidable barrier provided by the outermost skin layer can possibly be conquered by SEDDSs as these isotropic, thermodynamically stable mixtures, comprising oil, water and surface active agents, have the potential capacity to facilitate entry of drugs into underlying skin layers

(21,22). Alteration of *stratum corneum* (SC) lipid arrangement can be achieved by natural oils, included as the oil phase of topical SEDDSs (23-26). Additionally, natural oils can assist in solubilising the highly lipophilic clofazimine as only solubilised drug particles can partition into the SC (27,28). The inclusion of surface active agents can maintain formulation stability while facilitating increased disruption of SC lipids (26). Finally, water is the most established and safest skin penetration enhancer due to lipid disruption being achieved by skin hydration that loosens the strict lipid structure of the SC (28). Though various choices of vehicles are available to investigate topical delivery of clofazimine, this research is aimed at exploring potential development of a topical clofazimine SEDDS to aid in CTB since SEDDSs provide simplified techniques suitable for industrial upscaling together with improved solubility of the highly lipophilic clofazimine (21,22).

MATERIALS AND METHODS

Materials

Clofazimine was generously donated by Cipla Pty Ltd. (Mumbai, India). Argan-, avocado-, coconut-, macadamia- and olive oil were purchased from Scatters Oils (Johannesburg, South Africa). Tween®80 and Span®60 were obtained from Associated Chemical Enterprises (Pty) Ltd (Johannesburg, South Africa) and Sigma Aldrich Chemistry GmbH (Darmstadt, Germany). Distilled water was attained by a Rephile Bioscience Ltd system (Boston, United States of America).

Pre-formulation studies

Solubility studies

Excess clofazimine was added to 5 ml of each natural oil tested, and vortexed for 2 min. Samples were placed in a shaking water bath (32 ± 0.5°C) for 48 h. Thereafter, the samples were centrifuged at 3 000 rpm for 15 min at 22°C. Next, 1 ml supernatant was removed from each sample and diluted to 20 ml with methanol. All samples were analysed utilising an Agilent® 1100 Series HPLC system (25 ± 0.5°C) equipped with an Agilent® 1100 pump, UV detector, auto-sampler injection mechanism. Chemstation Rev. A10.02 software were employed for data acquisition and analysis at a UV wavelength of 284 nm. All samples were analysed in triplicate (Table I) (29-32).

Construction of pseudo-ternary phase diagrams

The water titration method was employed during construction of pseudo-ternary phase diagrams. The three components utilised to construct the triplot consisted of a natural oil, water and surfactant phase, i.e. Tween®80 and Span®60. The surfactant phase was fixed at a 1:1 ratio as literature concluded that this ratio forms more stable SEDDSs, whereas higher concentration ratios enlarge the emulsion range, but in turn facilitate decreased stability that could possibly

cause precipitation of an incorporated drug (33,34). The clofazimine concentration included varied according to its solubility in each natural oil (29). Specifically, 0.6% clofazimine was incorporated into avocado oil preparations, whereas 0.2% clofazimine was added to preparations containing argan- or coconut oil, respectively. Likewise, 0.1% clofazimine was included in preparations comprising macadamia- or olive oil. The surfactant phase and individual natural oils were prepared in fixed ratios (9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8 and 1:9) while water, the varying component, was added in a dropwise fashion at an ambient temperature of 25°C (29). The point at which the preparations turned turbid is considered the end-point. The end-point of different preparations was plotted implementing Triplot software version 4.1.2 to construct pseudo-ternary phase diagrams that identified the area of spontaneous emulsification.

Preparation of topical SEDDSs

In brief, the surfactant phase was prepared by continuous heating and stirring for 25 min. Next, clofazimine was dissolved in each of the oil phases while subjected to sonication for 2 min employing a UP400St (400W, 24kHz) Hielscher's digital ultrasonic device (Teltow, Germany). Subsequently, each oil phase was added to the surfactant phase while continuously stirred for an additional 25 min. Small increments of water were added with waiting periods between water additions, until the full predetermined quantity of water was added (Table III). The SEDDSs were left to cool before it was stored at room temperature (25 ± 0.5ºC) for 24 h followed by visual analysation (35-37). Heating SEDDSs during production is not traditional since low inert energy of excipients normally facilitate spontaneous emulsification once exposed to gentle agitation (35). However, heating during water titration followed by temperature stabilising at 25 ºC has been reported to render self-emulsification, especially in systems that should conquer high kinetic barriers to achieve spontaneous emulsification (35).

Characterisation of topical SEDDS formulations

Droplet size, zeta-potential and size distribution

Droplet size, zeta-potential and size distribution of topical SEDDSs were assessed by means of dynamic light scattering performed by a Zetasizer Nano® ZS (Malvern® Instruments Ltd., Worcestershire, United Kingdom) at a maintained temperature of 25°C. Drug delivery facilitated by SEDDSs are fundamentally influenced by droplet size, droplet size distribution and zeta-potential (30,38,39). Size characterisation is considered one of the most profound tests performed during development of SEDDSs as size influences drug release from SEDDS as well as stability of the formulations (38,40). Moreover, an increasingly negative or positive zeta-potential value is indicative of elevated electrostatic repulsion between droplets, and thus, improved stability of SEDDSs. Additionally, uniform size distribution within dispersions confirms stability of the said SEDDS formulations, and is evaluated utilising the polydispersity index (PDI) (41).

Robustness to dilution

In context of dermal drug delivery, SEDDS formulations were diluted with distilled water as well as phosphate buffer solution (PBS) comprising different pH values. The pH of PBS was adjusted to a value of 5, similar to skin surface pH; and 7.4 to resemble the change in pH just before partitioning into the epidermis (42,43). Dilutions of 100 fold were prepared with the aforementioned dilution fluids. Next, diluted SEDDSs were visually inspected for phase separation after storage at 25°C for 24 h (32).

Efficacy of self-emulsification and self-emulsification time

Efficacy of self-emulsification is also referred to as dispersability assessment (44-46). Swift emulsification is considered highly favourable, if exhibited in SEDDSs intended for oral administration as spontaneous emulsification is identified as the rate limiting step that must occur before successful absorption can ensue (46). Contrary, during topical drug delivery, diffusion through the lipophilic SC marks the rate limiting step for most drugs (26,47). Thus, prolonged contact time between SEDDSs and skin can determine if sufficient diffusion of the drug across the SC can be achieved (16,48). The efficiency of spontaneous emulsification was evaluated using a type II Distek 2500 dissolution system apparatus (North Brunswick, United States of America). This characterisation investigation consisted of the addition of 1 ml of each SEDDSs to 100 ml distilled water. The temperature was sustained at 32 ± 0.5°C, (skin surface temperature) while SEDDDSs were exposed to mild agitation with a set paddle speed of 50 rpm. Following, by means of visual observation, the time required by individual SEDDSs to transpire into homogenous dispersions was noted. Furthermore, the efficacy of spontaneous emulsification was graded according to the grading system (Table II) (32,49).

Viscosity and pH

A Brookfield® Viscometer model DV-II+ (Stoughton, USA), attached to a circulating water bath equipped with a Brookfield® temperature controller was employed to obtain viscosity readings of individual SEDDSs. The temperature of the water jacket was sustained at 25 ± 0.5°C (50). Spindles utilised are: SC4-34 LV, SC4-25 LV, T-bar F LV and T-bar E LV. These spindles were employed at 20 rpms, where torque values of approximately 20% were maintained.

The pH of the selected SEDDS formulations was assessed using a Mettler® Toledo pH meter (Mettler® Toledo International Inc., USA) with a Mettler® Toledo Inlab® 410 NTC electrode 9823. Calibration of the Mettler Toledo® InLab® 410 NTC electrode 9823 was executed at a pH of 4, 7 and 10 prior to measurement (51).

Cloud point

Dilutions of individual SEDDSs were prepared with the addition of distilled water (1:100). Subsequently, samples were positioned in a water bath at a starting temperature of 25°C. The

initial temperature was slowly increased at 2°C/min until a turbid appearance of the formulations was visually observed upon dehydration of excipients (52).

Thermodynamic stability studies

Thermodynamic stability included exposure of selected SEDDSs to differing temperatures during six cycles. This experiment included subjection of these SEDDSs to temperatures of approximately 4°C or 45°C, respectively. Thus, the formulations were heated and cooled to these temperatures for six sequences for a period not exceeding 48 h. Each tested SEDDS formulation was visually inspected for any possible phase separation or drug precipitation during the test (32). Hereafter, the SEDDSs were centrifuged at 3 500 rpm for 30 min (53). Succeeding centrifugation, the formulations were again visually assessed for any indications of instability, e.g. phase separation, cracking and/or creaming (53).

Topical delivery

Skin preparation

Caucasian female skin donated by anonymous patients that underwent abdominoplastic surgery, was employed during dermal diffusion experiments. Full-thickness tummy-tuck skin was stored at -20°C upon arrival at the bio-safety laboratory for no longer than 6 months. Ethics approval, concerning procurement and preparation of skin, was granted (ethics number: NWU-00111-17-A1-07) by the Ethics Committee of the North-West University (South Africa). Skin was examined for abnormalities that might affect dermal diffusion, for example the presence of *striae distensae*. Next, skin was dermatomed utilising a Zimmer™ electric dermatome, model 8821 (Warsaw, United States of America) to enable harvesting of skin pieces with continues thickness (i.e. 400 µm) to ensure that harvested skin pieces included SC, epidermis-dermis and dermis. Whatman® filter paper was used to position harvested pieces of skin. Hereafter, Whatman® filter paper with harvested skin was wrapped in foil and maintained at -20°C until conduction of dermal diffusion experiments (3).

Skin diffusion studies

Franz diffusion cells (n = 10) were employed to conduct *in vitro* dermal diffusion experiments. Harvested skin pieces were cut into skin circles and mounted between the receptor- and donor compartment of each Franz cell. The receptor phase consisted of a mixture of PBS (pH 7.4) and analytical grade ethanol (9:1); it was continuously stirred at 720 rpm with a magnetic stirrer. The donor compartment contained 1 ml of a selected SEDDS formulation. Franz cells were positioned in a water bath at a sustained temperature of 37°C and the receptor phase completely extracted after 12 h. All samples were analysed in triplicate utilising the validated HPLC method (3).

Tape stripping

After 12 h dermal diffusion experiments, skin circles were removed from Franz cells and positioned onto a board covered with Whatman® filter paper. The skin surface was carefully dapped with paper towels to remove excess SEDDSs prior to tape stripping. Scotch® magic tape was applied to strip only the region of each skin circle submitted to dermal diffusion. The first tape strip was discarded and the following 15 tape strips that removed the SC-epidermis fraction, were deposited into a polytop filled with 5 ml analytical grade ethanol and stored in the refrigerator (2–8°C) for a period of approximately 8 h. The remaining skin should demonstrate a gleaming appearance to ensure complete SC removal and prevalence of the epidermis-dermis skin layer. The epidermis-dermis fractions were cut into small pieces, for the purpose of creating an enlarged surface; and were placed into individual polytops filled with 5 ml analytical grade ethanol prior to approximately 8 h storage in the refrigerator (2-8°C). Next, samples were transferred into HPLC vials by means of filtration through a 45 µm filter (3) and subsequently analysed.

Isothermal microcalometric studies

Compatibility of excipients were determined by means of a 2277 Thermal Activity Monitor, TAMIII, (TA Instruments, New Castle, United States of America) equipped with an oil bath with a stability of ±100 µK over 24 h. The temperature was maintained at 40°C throughout 48 h experiments. During these trials both individual samples and samples containing combined excipients (100 mg) were analysed to establish compatibility. The heat flow displayed by individual samples are added to obtain a theoretical response (i.e. baseline). Next, comparison of theoretical response to the measured calometric output is done to determine if any interactions exist between components. If the theoretical response drastically differs from the measured calometric output, interactions between excipients can be suspected. Generally, a change in heat flow that exceeds 10 µW/g, with additional slopes observed on the heat flow graph itself, signify potential incompatibility (3).

RESULTS AND DISCUSSION

Pre-formulation and characterisation

Solubility studies

Natural oils are incorporated into SEDDSs for enhancing and maintaining solubility of highly lipophilic drugs, including clofazimine, as only solubilised drug particles are able to partition into the outermost skin layer (27,28). Moreover, natural oils provide reversible alteration of SC lipids that facilitate dermal penetration enhancement (23-26). The aqueous solubility of clofazimine is 10.0 mg/l (3) Therefore, clofazimine solubility is significantly improved when incorporated into the selected natural oils (Table I) as an increased fraction of solubilised clofazimine will be available at the skin surface.

Construction of pseudo-ternary phase diagrams

Pseudo-ternary phase diagrams provided assistance in identifying the self-dispersability potential of topical SEDDSs (27). Moreover, these diagrams schematically represent the concentration range of the utilised excipients that can facilitate self-emulsification, when incorporated in combination (54). It is evident from observing the pseudo-ternary phase diagrams for argan-, avocado-, macadamia- and olive oil SEDDSs (Fig. 1) that a vast region can possibly facilitate spontaneous emulsification. Thus, a specific area within the self-emulsification region was identified to ensure evaluation of SEDDSs predicted suitable for topical application.

This zone was indicated by eliminating areas within self-emulsification regions that are known for unfavourable properties in terms of topical drug delivery. Therefore, only regions comprising a surfactant phase ratio of less than 5 were considered for further investigation as an increased surfactant concentration is known to cause skin irritation (55,56). Likewise, micelles are unwanted structures due to the tendency of the structures to establish poor dermal drug delivery as facilitated by their rigidity and reduced deformability (57,58). Micelles are found in water rich areas (59). Hence, formulations considered suitable for further investigation should not exceed a ratio of 7 in terms of water content. Additionally, areas of high oil content tend to produce reverse micelles (59). The focus of this study remains the development of SEDDSs and not reversed micelles destined for topical clofazimine delivery. Therefore, self-emulsification regions exceeding an oil ratio of 7 were furthermore excluded from additional investigational studies. Thus, a possible 5 formulations were selected from the self-emulsification region of each pseudo-ternary phase diagram. This was done by choosing two check-points at the top, two check-points at the bottom, and one check-point in the centre of the identified region of self-emulsification for each oil (Fig. 2). The self-emulsification area of the pseudo-ternary phase diagram representing coconut oil rendered a notably smaller area of self-emulsification compared to the other natural oils. Nonetheless, 5 possible check-point formulations were selected from this self-emulsification region as similarly as possible to the selection method utilised for the other natural oils (Fig. 2).

Preparation of topical SEDDSs

Visual inspection of topical SEDDSs, retained at ambient temperature for 24 h, portrayed phase separation in formulations ARG 2, ARG 4, AVO 1, MAC 4, OLV 4 as well as all of the prepared coconut oil SEDDSs. Hence, the remaining 15 topical SEDDSs that did not exhibit phase separation were considered suitable for further investigation. Characterisation experiments were performed to generate profiles of topical SEDDSs to establish which SEDDS formulations are most suitable for dermal drug delivery, prior to conducting dermal diffusion studies.

Droplet size, zeta-potential and size distribution

It has been reported that reduced droplet size can contribute towards rapid, and significantly increased drug permeation during dermal drug delivery (41). In addition, smaller droplets portray

a decreased tendency towards emulsion instability such as cohesion. Most topical SEDDS formulations tested (Table IV) fell within the micro-sized range, i.e. 100–250 nm (27). Favourably, formulations ARG 1, AVO 2 and AVO 5 could be classified as nano SEDDSs (<100 nm) (27). However, formulations ARG 5, AVO 3, MAC 5, OLV 1 and OLV 3 demonstrated droplet sizes >250 nm and are therefore not deliberated ideal for dermal diffusion studies (41).

Uniform size distribution of droplets within dispersions is considered indicative of formulation stability and is scrutinised through the polydispersity index (PDI) (41). However, no fixed PDI criteria has been established for dermal drug delivery, except for lipid based carrier systems (PDI ≤ 0.3) and polymer-based nanoparticles (PDI ≤ 0.2) developed specifically for transdermal drug delivery (60). Moreover, a PDI exceeding the generally accepted pharmaceutical range of 0.05–0.7, can designate that microscopic techniques must be employed for size characterisation rather than dynamic light scattering as it could possibly mistakenly identify many small particles clustered together as single large particles (60). Thus, in this study only SEDDS formulations that obtained a PDI of 1 (ARG 5, AVO 4, AVO 5, MAC 5, OLV 1, OLV 2 and OLV 3) were excluded from further analysis (Table IV).

It is known that increasingly negative or positive zeta-potential values (i.e. >30 mV or <-30 mV) signify increased electrostatic repulsion between droplets and are therefore considered favourable as coagulation is circumvented (61). Nevertheless, a minute deviation is allowed as research recognised that emulsions stabilised by both steric and combined electrostatic forces, as enabled by Tween®80, with a minimum zeta-potential value of -20 mV, can be contemplated acceptable (62,63). The negatively charged zeta-potential values obtained during this study (Table IV) are initiated by the presence of free fatty acids within the oil phase (3). However, as the net charge of the skin is negative, a positively charged formulation should theoretically facilitate increased affinity between the applied formulation and skin (64,65). Contrary, free fatty acids are skin penetration enhancers that function by disruption and fluidisation of SC lipids to enhance dermal drug delivery (23-26). Therefore, negatively charged SEDDSs can still potentially facilitate dermal drug delivery, but just in a slower fashion compared to positively charged formulations (64). Accordingly, all SEDDSs tested, complied with the criteria set for zeta-potential, where overall, the SEDDS formulations comprising avocado oil are regarded most stable (average zeta-potential: -37.35 mV). Interestingly, a correlation probably exists between the pH of SEDDS and zeta-potential measurements as avocado oil SEDDS displayed decreased pH measurements with increasingly negative zeta-potential values.

Robustness to dilution

It was established upon visual observation of diluted topical SEDDSs that formulations ARG 5, AVO 5, OLV 2 and OLV 3 demonstrated complete phase separation upon dilution with distilled water and PBS comprising different pH values. Thus, these formulations were considered

unsuitable for dermal drug delivery. Although formulations ARG 3, AVO 2, MAC 1 and OLV 1 displayed robustness towards exposure to different pH environments, but failed to withstand phase separation upon dilution with distilled water, they were still deliberated suitable for further analysis. This decision was based on the assumption that stability of these formulations will not be influenced while diffusing through different skin layers, as these layers have similar pH environments to the different PBS utilised during this experiment. Exposure to large volumes of water on the skin surface will probably not transpire as sweat is the most noteworthy fluid that can influence stability of topically applied SEDDSs. The sweat rate of healthy individuals is between 500–700 ml daily over the entire body surface (66). Thus, the possibility of exposing topically applied SEDDSs to a similar fluid volume of 99 ml, utilised to prepare dilutions, on a single region of the body, is considered highly unlikely.

Efficacy and time of self-emulsification

Considering the grading system, SEDDSs that obtained a C or D grading (Table II) could be deemed favourable for dermal clofazimine delivery. Contrary, SEDDSs that demonstrated poor emulsification properties, with consequent E-grading, were considered unsuitable as slow emulsification is favourable, but complete inability to self-emulsify is undesired. Moreover, SEDDSs that rendered rapid emulsification (A or B emulsions) were also reasoned unsuitable as these SEDDSs can be easily washed away once exposed to sweat or external water. Therefore, rapid spontaneous emulsification is suggestive of decreased occlusivity that sequentially reduces topical clofazimine delivery (48,67). Hence, formulation MAC 5 was discarded in terms of dermal drug delivery due to a received B-grading (Table IV).

Self-emulsification time of individual SEDDSs illustrates the free energy needed to enable self-emulsification that can be influenced by the energy decreasing capabilities of surface active agents, which dictate entropy of formulations (68). Literature confirmed that spontaneous emulsification can either occur swiftly or be prolonged, depending on the presence of kinetic barriers between excipients included in SEDDSs. What is more, kinetic barriers can be overcome through applying heat or mild agitation (35). Thus, definite kinetic barriers are present as clofazimine SEDDSs required exposure to heightened temperatures, followed by cooling afterwards, to enable spontaneous emulsification (69). Argan oil SEDDS required longer intervals to achieve self-emulsification that possibly indicates increased kinetic barriers between argan oil SEDDSs excipients.

Viscosity and pH

Viscosity refers to internal friction of a fluid that can elicit an impact on flow resistance and spontaneous emulsification (69,70). The incorporated natural oils have a definite influence on the viscosity of SEDDSs considering that formulations ARG 3, AVO 3, MAC 3 and OLV 3 comprises exactly similar excipient ratios, thus indicating the role of natural oils on formulation viscosity. The

influence on viscosity can be ordered from highest to lowest as: olive oil > argan oil > macadamia oil > avocado oil (Table IV). Prolonged self-emulsification times were depicted by formulations of increased viscosity (i.e. ARG 3 and OLV 3) compared to self-emulsification times exhibited by SEDDSs of decreased viscosity (i.e. MAC 3 and AVO 3). Thus, signifying a potential correlation between the ease of spontaneous emulsification and viscosity.

For dermal drug delivery, the pH range deemed suitable for SEDDSs is 5.0–9.0 (71). However, an optimal pH would closely resemble the natural pH of skin and will thus range between 4.5–5.0 (42). Most formulations depicted values between 5.0–9.0, except formulation AVO 3 that exhibited a pH measurement of 3.82 (Table IV). Remarkably, avocado oil SEDDS portrayed pH measurements closely related to the natural pH of skin.

Cloud point

Cloud point specifies the temperature where SEDDSs are incapable of retaining their spontaneous emulsification properties (72). This leads to erratic release of the incorporated drug and can further cause irreversible phase separation (52), which is linked to dehydration of excipients once exposed to heightened temperatures (38). Formulations ARG 1, MAC 1 and MAC 3 portrayed excipient dehydration below skin surface temperature (32°C) (49).

Thermodynamic stability

Inclusion of surface active agents in SEDDSs cannot promise physical stability due to the complexation of emulsified systems sustained by surfactants (73). These excipients create interfacial tension gradients and provide modification to breakup dynamics of droplets (73). Hence, the physical stability of SEDDSs was investigated through thermodynamic stability experiments (32,53). It is evident that formulations ARG 5, MAC 1, OLV 1 and OLV 2 revealed incapability to withstand environments of thermodynamic- and kinetic stress. Contrary, formulation OLV 3 exclusively displayed instability once subjected to differing temperatures; whereas formulation AVO 5 was unable to remain stable upon centrifugation. Thus, all SEDDSs that illustrated any instability once subjected to thermodynamic and/or kinetic stress conditions were deemed inapt for topical drug delivery.

Skin diffusion

Post characterisation, only formulations ARG 3, AVO 2, MAC 2 and OLV 5 were reasoned suitable candidates for topical clofazimine delivery. To improve clofazimine detection and incorporate a therapeutic concentration, 2% w/w clofazimine was included in each of these formulations. Moreover, delivery of supersaturated clofazimine concentrations at skin surface will probably enhance dermal flux (47,74) as delayed nucleation and crystal growth are enabled by SEDDSs due to enhanced kinetic- and thermodynamic inhibition of clofazimine precipitation (27).

No clofazimine was detected in the receptor phase post dermal diffusion regardless the SEDDS, thus favouring topical delivery and not distribution of clofazimine into the systemic circulation (5). Patients diagnosed with CTB generally also suffer from pulmonary TB and will already be on systemic treatment (75). Accordingly, topical therapy should not interfere with systemic regimes as it may cause additional side effects, therefore, the outcome of this experiment is deemed ideal. Furthermore, clofazimine could only be detected in the epidermis-dermis layer after application of formulations ARG 3, AVO 2 and OLV 5; whereas it could be quantified in both the SC-epidermis and epidermis-dermis layers once formulation MAC 2 was tested. Clofazimine concentrations were statistically significantly ($p < 0.001$) increased in the SC-epidermis relatively to the epidermis-dermis (Fig. 4). A previous study specified that macadamia oil comprises a high palmitoleic acid content that has preferred affinity for the epidermis (76). This increased affinity is due to a higher molecular weight owing to a longer carbon backbone (> C14) that deduces mobility of fatty acids throughout different skin layers (77).

Formulation OLV 5 rendered the highest dermal cumulative clofazimine concentration, followed by ARG 3 (Fig. 3). This outcome may be attributed to the increased oleic acid content known for its penetration enhancement properties (78,79) by means of SC disruption. The natural lipid structure is disordered and fluidisation of these lipids occur, which in turn facilitates enhanced dermal drug delivery (26;79,80). Interestingly, speculation indicates that only the *cis* form of oleic acid is responsible for skin penetration enhancement as established by the unsaturated structure of the molecule itself (26). On the other hand, clofazimine delivery enabled by SEDDS containing argan oil, is probably due to an increased stearic acid concentration. Stearic acid is a saturated fatty acid with a melting point of 69.3°C; which is significantly higher than skin surface temperature (49,81). Consequently, stearic acid in its undissolved state, possibly provides a residual layer on the skin surface that enables improved occlusivity (23,82). Occlusion decreases transepidermal water loss and thus enhances swelling of the SC, which sequentially disrupts the strictly packed lipid structure of the SC rendering improved drug diffusion (83,84).

The AVO 2 SEDDS displayed significantly ($p < 0.018$) lower permeation of the epidermis-dermis layer, probably due to the increased palmitic acid (saturated fatty acid) levels present within this oil (76,85). Saturated fatty acids are deliberated less powerful skin penetration enhancers due to the decreased potential to insert themselves into the lipid barrier (24,86,87).

Isothermal microcalometric studies

The argan oil and clofazimine combination provided an average heat flow of 2.261 µW/g and an interaction error of 12.296 µW/g, signifying a potential incompatibility. Potential hydrogen peroxide formation due to redox reactions facilitated by clofazimine might be responsible for these results (88). Remarkably, potential incompatibility between argan oil and clofazimine can confirm the presence of enhanced kinetic barriers within argan oil SEDDS as heightened self-

emulsification times were observed (Table IV). Moreover, possible incompatibilities were depicted between Span®60 and argan-, macadamia- and olive oil, respectively; with observed changes in heat flow exceeding 10 µW/g; which were probably due to physical interactions as Span®60 is a solid substance at room temperature and were heated to dissolve Span®60 in Tween®80 during surfactant phase preparation.

CONCLUSIONS

Detection of interactions should not discourage further investigation as incompatibilities can either be of a physical or chemical nature (89). Physical interactions between excipients are not exclusively unfavourable as it does not necessarily influence formulation stability (89). Although not all intrinsic properties of clofazimine are considered suitable for dermal drug delivery (12, 67), and despite potential incompatibilities, SEDDSs comprising argan-, avocado-, macadamia- and olive oil induced efficient dermal clofazimine transport. Thus, this work indicates that the transition of oral SEDDSs to novel topical vehicles should be successful. Furthermore, clofazimine was detected in the epidermis-dermis skin layer during analysis; thus, the possibility of delivering "unsuitable" drugs into the lymphatic circulation provides exciting prospects (90). These prospects include targeting conditions worsened by lymphatic dissemination such as Human Immunodeficiency Virus (HIV), metastatic cancers and endogenous extra-pulmonary TB (90).

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

References

1. Churchyard GJ. A Short Regimen for Rifampin-Resistant Tuberculosis. NE J Me. 2019; 380:1279-80.

2. Nunn AJ, Phillips PP, Meredith SK, Chiang CY, Conradie F, Dalai D, Van Deun A, Dat PT, Lan N, Master I, Mebrahtu T. A trial of a shorter regimen for rifampin-resistant tuberculosis. NE J Me. 2019; 380:1201-13.

3. Van Zyl L, Viljoen JM, Haynes RK, Aucamp M, Ngwane AH, du Plessis J. Topical delivery of artemisone, clofazimine and decoquinate encapsulated in vesicles and their *in vitro* efficacy against Mycobacterium tuberculosis. AAPS PharmSciTech. 2019; 20:33.

4. van Zyl L, Du Plessis J, Viljoen J. Cutaneous tuberculosis overview and current treatment regimens. Tuberculosis. 2015; 95:629-38.

5. Bellefroid C, Lechanteur A, Evrard B, Piel G. Lipid gene nanocarriers for the treatment of skin diseases: current state-of-the-art. Eur J Pharm Biopharm. 2019; 137:95-111.

6. Chen Q, Chen W, Hao F. Cutaneous tuberculosis: A great imitator. Clinics in dermatology. 2019; 1:192-99.

7. Li G, Xu Z, Jiang Y, Liu H, Zhao LL, Li M, Xu D, Zhao X, Liu Z, Wang R, Wan K. Synergistic activities of clofazimine with moxifloxacin or capreomycin against Mycobacterium tuberculosis in China. Intl J of antimicrob agents. 2019; 11: article in press.

8. Mirnejad R, Asadi A, Khoshnood S, Mirzaei H, Heidary M, Fattorini L, Ghodousi A, Darban-Sarokhalil D. Clofazimine: A useful antibiotic for drug-resistant tuberculosis. Biomed Pharmacother. 2018; 1:1353-59.

9. Rao M, Valentini D, Zumla A, Maeurer M. Evaluation of the efficacy of valproic acid and suberoylanilide hydroxamic acid (vorinostat) in enhancing the effects of first-line tuberculosis drugs against intracellular Mycobacterium tuberculosis. IJID. 2018; 1:78-84.

10. Jagannath C, Reddy MV, Kailasam S, O'Sullivan JF, Gangadharam PR. Chemotherapeutic activity of clofazimine and its analogues against Mycobacterium tuberculosis. *In vitro*, intracellular, and in vivo studies. Am J Respir Crit Care Med. 1995; 151:1083-6.

11. Dey T, Brigden G, Cox H, Shubber Z, Cooke G, Ford N. Outcomes of clofazimine for the treatment of drug-resistant tuberculosis: a systematic review and meta-analysis. J Antimicrob Chemother. 2012; 68:284-93.

12. Sangana R, Gu H, Chun DY, Einolf HJ. Evaluation of clinical drug interaction potential of clofazimine using static and dynamic modeling approaches. Drug Metab Dispos. 2018; 46:26-32.

13. Singh B, Beg S, Khurana RK, Sandhu PS, Kaur R, Katare OP. Recent advances in self-emulsifying drug delivery systems (SEDDS). Crit Rev Ther Drug. 2014; 3:121-85.

14. Leichner C, Baus RA, Jelkmann M, Plautz M, Barthelmes J, Dünnhaupt S, Bernkop-Schnürch A. *In vitro* evaluation of a self-emulsifying drug delivery system (SEDDS) for nasal administration of dimenhydrinate. Drug Deliv Transl Re. 2019; 14:945-55.

15. Kauss T, Gaubert A, Tabaran L, Tonelli G, Phoeung T, Langlois MH, White N, Cartwright A, Gomes M, Gaudin K. Development of rectal self-emulsifying suspension of a moisture-labile water-soluble drug. Int J Pharm. 2018; 30:283-91.

16. Rohrer J, Zupančič O, Hetényi G, Kurpiers M., Bernkop-Schnürch A. Design and evaluation of SEDDS exhibiting high emulsifying properties. J Drug Deliv Sci Technol. 2018; 44:366-72.

17. Köllner S, Nardin I, Markt R, Griesser J, Prüfert F, Bernkop-Schnürch A. Self-emulsifying drug delivery systems: design of a novel vaginal delivery system for curcumin Eur J Pharm Biopharm. 2017; 115:268-75.

18. Pattewar SV, Kasture SB, Pande VV, Sharma SK. A New Self Microemulsifying Mouth Dissolving Film. Indian J Pharm Educ Res. 2016; 50 Supple 3:191-99.

19. ElKasabgy NA. Ocular supersaturated self-nanoemulsifying drug delivery systems (S-SNEDDS) to enhance econazole nitrate bioavailability. Int J Pharm. 2014; 460:33-44.

20. Xiao L, Yi T, Liu Y. A new self-microemulsifying mouth dissolving film to improve the oral bioavailability of poorly water soluble drugs. Drug Dev Ind Pharm. 2013; 39:1284-90.

21. Abdulkarim M, Sharma PK, Gumbleton M. Self-emulsifying drug delivery system: Mucus permeation and innovative quantification technologies. Adv Drug Deliv Rev. 2019; 1:62-74.

22. El Khayat NW, Donia AA, Mady OY, El Maghraby GM. Optimization of eugenol microemulsion for transdermal delivery of indomethacin. J Drug Deliv Sci Technol. 2018; 48:311-8.

23. Vaughn AR, Clark AK, Sivamani RK, Shi VY. Natural oils for skin-barrier repair: Ancient compounds now backed by modern science. Am J Clin Dermato. 2018; 19:103-17.

24. Van Zyl L, Du Preez J, Gerber M, Du Plessis J, Viljoen J. Essential fatty acids as transdermal penetration enhancers. J Pharm Sci. 2016; 105:188-93.

25. Viljoen JM, Cowley A, Du Preez J, Gerber M, Du Plessis J. Penetration enhancing effects of selected natural oils utilized in topical dosage forms. Drug Dev Ind Pharm. 2015; 41:2045-54.

26. Lane ME. Skin penetration enhancers. Int J Pharm. 2013; 447:12-21.

27. Rani S, Rana R, Saraogi GK, Kumar V, Gupta U. Self-Emulsifying Oral Lipid Drug Delivery Systems: Advances and Challenges. AAPS PharmSciTech. 2019; 20:129.

28. Haque T, Talukder MM. Chemical enhancer: a simplistic way to modulate barrier function of the stratum corneum. Adv Pharm Bull. 2018; 8:169.

29. Prajapat MD, Patel NJ, Bariya A, Patel SS, Butani SB. Formulation and evaluation of self-emulsifying drug delivery system for nimodipine, a BCG class II drug. JDDST. 2017; 39:59-68.

30. Balata GF, Essa EA, Shamardl HA, Zaidan SH, Abourehab MA. Self-emulsifying drug delivery systems as a tool to improve solubility and bioavailability of resveratrol. Drug Des Devel Ther. 2016; 10:117-28.

31. Ke Z, Hou X, Jia XB. Design and optimization of self-nanoemulsifying drug delivery systems for improved bioavailability of cyclovirobuxine D. Drug Des Devel Ther. 2016; 10:2049-60.

32. Czajkowska-Kośnik A, Szekalska M, Amelian A, Szymańska E, Winnicka K. Development and evaluation of liquid and solid self-emulsifying drug delivery systems for atorvastatin. Molecules. 2015; 20:21010-22.

33. Syed HK, Peh KK. Identification of phases of various oil, surfactant/co-surfactants and water system by ternary phase diagram. Acta Pol Pharm. 2014; 71:301-9.

34. Kang BK, Lee JS, Chon SK, Jeong SY, Yuk SH, Khang G, Lee HB, Cho SH. Development of self-microemulsifying drug delivery systems (SMEDDS) for oral bioavailability enhancement of simvastatin in beagle dogs. Int J Pharm. 2004; 274:65-73.

35. Solans C, Morales D, Homs M. Spontaneous emulsification. Curr Opin Colloid Interface Sci. 2016; 22:88-93.

36. Sanka K, Suda D, Bakshi V. Optimization of solid-self nanoemulsifying drug delivery system for solubility and release profile of clonazepam using simplex lattice design. JDDST. 2016; 33:114-24.

37. Parmar K, Patel J, Sheth N. Self nano-emulsifying drug delivery system for Embelin: Design, characterization and in-vitro studies. Asian J Pharm Sci. 2015; 10:396-404.

38. Ujilestari T, Dono ND, Ariyadi B, Martien R, Zuprizal Z. Formulation and characterization of self-nano emulsifying drug delivery systems of lemongrass (cymbopogon citratus) essential oil. Mal J Fund Appl Sci. 2018; 14:360-3.

39. Mahapatra AK, Murthy PN, Swadeep B, Swain RP. Self-emulsifying drug delivery systems (SEDDS): An update from formulation development to therapeutic strategies. Int. J. PharmTech Res. 2014; 6:546-68.

40. Zaichik S, Steinbring C, Menzel C, Knabl L, Orth-Höller D, Ellemunter H, Niedermayr K, Bernkop-Schnürch A. Development of self-emulsifying drug delivery systems (SEDDS) for ciprofloxacin with improved mucus permeating properties. Int J Pharm. 2018; 547:282-90.

41. Kaur R, Ajitha M. Transdermal delivery of fluvastatin loaded nanoemulsion gel: Preparation, characterization and in vivo anti-osteoporosis activity. Eur J Pharm Sci. 2019; 136:104956.

42. Sheshala R, Anuar NK, Samah NH, Wong TW. *In vitro* drug dissolution/permeation testing of nanocarriers for skin application: a comprehensive review. AAPS PharmSciTech. 2019; 20:164.

43. Margetts L, Sawyer R. Transdermal drug delivery: principles and opioid therapy. BJA Education. 2007; 7:171-6.

44. Chaudhari KS, Akamanchi KG. Novel bicephalous heterolipid based self-microemulsifying drug delivery system for solubility and bioavailability enhancement of efavirenz. Int J Pharm. 2019; 560:205-18.

45. Vasconcelos T, Araújo F, Lopes C, Loureiro A., das Neves J., Marques, S, Sarmento B. Multicomponent self nano emulsifying delivery systems of resveratrol with enhanced pharmacokinetics profile. Eur J Pharm. 2019; 137:105011.

46. Nasr A, Gardouh A, Ghorab M. Novel solid self-nanoemulsifying drug delivery system (S-SNEDDS) for oral delivery of olmesartan medoxomil: Design, formulation, pharmacokinetic and bioavailability evaluation. Pharmaceutics. 2016; 8:20.

47. Parhi R, Swain S. Transdermal Evaporation Drug Delivery System: Concept to Commercial Products. Advanced pharmaceutical bulletin. 2018; 8:535-50.

48. Kathe K, Kathpalia H. Film forming systems for topical and transdermal drug delivery. Asian J Pharm Sci. 2017; 12:487-97.

49. Heylings JR, Davies DJ, Burton R. Dermal absorption of testosterone in human and pig skin *in vitro*. Toxicol *in Vitro*. 2018; 48:71-7.

50. Bharadwaj R, Haloi J, Medhi S. Topical delivery of methanolic root extract of Annona reticulata against skin cancer. S Afr J Bot. 2019; 124:484-93.

51. Clausen ML, Slotved HC, Krogfelt KA, Agner T. Tape stripping technique for stratum corneum protein analysis. Sci Rep. 2016; 6:19918.

52. Zupančič O, Rohrer J, Thanh Lam H, Grießinger JA, Bernkop-Schnürch A. Development and *in vitro* characterization of self-emulsifying drug delivery system (SEDDS) for oral opioid peptide delivery. Drug Dev Ind Pharm. 2017; 43:1694-702.

53. Suvarna V. Development and characterization of solid self-emulsifying drug delivery system containing nateglinide. AJP. 2017; 11:27-36.

54. Ibrahim TM, Abdallah MH, El-Megrab NA, El-Nahas HM. Upgrading of dissolution and anti-hypertensive effect of Carvedilol via two combined approaches: self-emulsification and liquisolid techniques. Drug Dev Ind Pharm. 2018; 44:873-85.

55. Mojeiko G, de Brito M, Salata GC, Lopes LB. Combination of microneedles and microemulsions to increase celecoxib topical delivery for potential application in chemoprevention of breast cancer. Int J Pharm. 2019; 560:365-76.

56. Kiselmann C, Dobler D, Schmidts T, Eicher AC, Möbs C, Pfützner W, Runkel F. Development of a skin-friendly microemulsion for dermal allergen-specific immunotherapy. Int J Pharm. 2018; 550:463-9.

57. Abdel-Messih HA, Ishak RA, Geneidi AS, Mansour S. Tailoring novel soft nano-vesicles 'Flexosomes' for enhanced transdermal drug delivery: Optimization, characterization and comprehensive ex vivo–in vivo evaluation. Int J Pharm. 2019; 560:101-15.

58. El Zaafarany GM, Awad GA, Holayel SM, Mortada ND. Role of edge activators and surface charge in developing ultradeformable vesicles with enhanced skin delivery.Int J Pharm. 2010; 397:164-72.

59. Hegde RR, Verma A, Ghosh A. Microemulsion: new insights into the ocular drug delivery. ISRN Pharm. 2013; 27:826798.

60. Danaei M, Dehghankhold M, Ataei S, Hasanzadeh Davarani F, Javanmard R, Dokhani A, Khorasani S, Mozafari MR. Impact of particle size and polydispersity index on the clinical applications of lipidic nanocarrier systems. Pharmaceutics. 2018;10:57.

61. Sharma S, Shukla P, Misra A, Mishra PR. Interfacial and colloidal properties of emulsified systems: pharmaceutical and biological perspective. In: Oshima H, Makino K, editors. Colloid and interface science in pharmaceutical research and development. Kidlington: Elsevier; 2014. p. 149-172.

62. Gupta V, Trivedi P. *In vitro* and in vivo characterisation of pharmaceutical topical nanocarriers containing anti-cancer drugs for skin cancer treatment. In: Grumezescu AM, editors. Lipd nano carriers for drug targeted therapy. Kidlington: William Andrew applied science publishers; 2018. p. 563-627.

63. Zafeiri I, Norton JE, Smith P, Norton IT, Spyropoulos F. The role of surface active species in the fabrication and functionality of edible solid lipid particles. J Colloid Interface Sci. 2017; 500:228-40.

64. Carter P, Narasimhan B, Wang Q. Biocompatible nanoparticles and vesicular systems in transdermal drug delivery for various skin diseases. Int J Pharm. 2019; 555:49-62.

65. Gumustas M, Sengel-Turk CT, Gumustas A, Ozkan SA, Uslu B. Effect of polymer-based nanoparticles on the assay of antimicrobial drug delivery systems. In: Grumezescu AM, editors. Multifunctional systems for combined delivery, biosensing and diagnostics. Amsterdam: Elsevier; 2017. p. 67-108.

66. Salvo P, Pingitore A, Barbini A, Di Francesco F. A wearable sweat rate sensor to monitor the athletes' performance during training. Sci Sport. 2018. doi.org/10.1016/j.scispo.2017.03.009

67. Keurentjes AJ. Maibach HI. Percutaneous penetration of drugs applied in transdermal delivery systems: an in vivo based approach for evaluating computer generated penetration models. Regul Toxicol Pharmacol. 2019; 18:104428.

68. Shahnaz G, Hartl M, Barthelmes J, Leithner K, Sarti F, Hintzen F, Rahmat D, Salvenmoser W, Bernkop-Schnürch A. Uptake of phenothiazines by the harvested chylomicrons ex vivo model: Influence of self-nanoemulsifying formulation design. Eur J Pharm Biopharm. 2011; 79:171-80.

69. Eid AM, El-Enshasy HA, Aziz R, Elmarzugi NA. The preparation and evaluation of self-nanoemulsifying systems containing Swietenia oil and an examination of its anti-inflammatory effects. Int J Nanomed. 2014; 9:4685.

70. Perazzo A, Preziosi V, Guido S. Phase inversion emulsification: Current understanding and applications. Adv Colloid Interface Sci. 2015; 222:581-99.

71. Rastogi V, Yadav P. Transdermal drug delivery system: An overview. AJP. 2014; 6:161-70.

72. Akula S, Gurram AK, Devireddy SR. Self-microemulsifying drug delivery systems: an attractive strategy for enhanced therapeutic profile. Int Sch Res Notices. 2014; 2014:964051.

73. Roumpea E, Kovalchuk NM, Chinaud M, Nowak E, Simmons MJ, Angeli P. Experimental studies on droplet formation in a flow-focusing microchannel in the presence of surfactants. Chem Eng Sci. 2019; 195:507-18.

74. Ganti, S.S., Nguyen, H.X., Murnane, K.S., Blough, B.E. & Banga, A.K. 2018. Transdermal formulation of 4-benzylpiperidine for cocaine-use disorder. J Drug Deliv Sci Technol. 2018; 47:299-308.

75. Tshisevhe V, Mbelle N, Peters RP. Cutaneous tuberculosis in HIV-infected individuals: Lessons learnt from a case series. South Af. J HIV Med. 2019; 20:1-3.

76. Čižinauskas V, Elie N, Brunelle A, Briedis V. Skin Penetration Enhancement by Natural Oils for Dihydroquercetin Delivery. Molecules. 2017; 22:1536.

77. Balázs B, Vizserálek G, Berkó S, Budai-Szűcs M, Kelemen A, Sinkó B, Takács-Novák K, Szabó-Révész P, Csányi E. Investigation of the efficacy of transdermal penetration enhancers through the use of human skin and a skin mimic artificial membrane. J Pharm Sci. 2016;105:134-40.

78. Cicero N, Albergamo A, Salvo A, Bua GD, Bartolomeo G, Mangano V, Rotondo A, Di Stefano V, Di Bella G, Dugo G. Chemical characterization of a variety of cold-pressed gourmet oils available on the Brazilian market. Food Res Int. 2018;109:517-25.

79. ElMasry .R, Hathout RM, Abdel-Halim M, Mansour S. *In Vitro* transdermal delivery of sesamol using oleic acid chemically-modified gelatin nanoparticles as a potential breast cancer medication. J Drug Deliv Sci Technol, 2018; 48:30-39.

80. Hadgraft J, Lane ME. Advanced topical formulations (ATF). Int J Pharm. 2016; 514:52-7.

81. Jiménez-Peñalver P, Castillejos M, Koh A, Gross R, Sánchez A, Font X. Gea T. Production and characterization of sophorolipids from stearic acid by solid-state fermentation, a cleaner alternative to chemical surfactants. J Clean Prod. 2018; 172:2735-47.

82. Gore E, Picard C, Savary G. Spreading behavior of cosmetic emulsions: Impact of the oil phase. Biotribology. 2018;16:17-24.

83. Vaz S, Silva R, Amaral MH, Martins E, Lobo JS, Silva AC. Evaluation of the biocompatibility and skin hydration potential of vitamin E-loaded lipid nanosystems formulations: *In vitro* and human in vivo studies. Colloids Surf B Biointerfaces. 2019; 179:242-9.

84. Warner RR, Stone KJ, Boissy YL. Hydration disrupts human stratum corneum ultrastructure J Investig Dermatol. 2003; 120:275-84.

85. Mansour RS, Sallam AA, Hamdan II, Khalil EA, Yousef I. Elucidation of penetration enhancement mechanism of Emu oil using FTIR microspectroscopy at EMIRA laboratory of SESAME synchrotron. Spectrochim. Acta A. 2017; 185:1-0.

86. Lundborg M, Wennberg CL, Narangifard A, Lindahl E, Norlén L. Predicting drug permeability through skin using molecular dynamics simulation. JCR. 2018; 283:269-79.

87. Kim MJ, Doh HJ, Choi MK, Chung SJ, Shim CK, Kim DD, Kim JS, Yong CS, Choi HG. Skin permeation enhancement of diclofenac by fatty acids. Drug Deliv. 2008; 15:373-9.

88. Murashov M, Diaz-Espinosa J, LaLone V, Tan J, Laza R, Wang X, Stringer K, Rosania G. Synthesis and characterization of a biomimetic formulation of Clofazimine hydrochloride microcrystals for parenteral administration. Pharmaceutics. 2018; 10:238.

89. Patel P, Ahir K, Patel V, Manani L, Patel C. Drug-Excipient compatibility studies: First step for dosage form development. J Pharm Innov. 2015; 4:14.

90. Khadke S, Roces CB, Cameron A, Devitt A, Perrie Y. Formulation and manufacturing of lymphatic targeting liposomes using microfluidics. JCR. 2019; 307:211-20.

Table I. Solubility of clofazimine in natural oils

Natural oil	Solubility of clofazimine (μg/ml)
Argan oil	2.23 ± 0.54
Avocado oil	6.29 ± 0.44
Coconut oil	1.78 ± 0.67
Macadamia oil	1.25 ± 0.46
Olive oil	1.09 ± 0.65

Table II. Grading system for SEDDSs

Grading	Description
Grade A	Swift emulsion formation, demonstrating a clear/bluish appearance (60 s)
Grade B	Swift formation of emulsion, which appears bluish (60 s)
Grade C	Emulsion displays fine milky appearance (120 s)
Grade D	Dull, greyish white appearance with an additional oily appearance together with slow emulsification, (>120 s)
Grade E	Poor or minimal emulsification noted with large oil droplets noticed on the surface

Table III. Check-point SEDDS formulations

SEDDS	Ratio of excipients (oil: surfactant phase: water)
ARG 1	7: 5: 7
ARG 2	**7.4: 5: 5**
ARG 3	6: 4: 6
ARG 4	**7: 3: 3**
ARG 5	4.4: 3: 7
AVO 1	**7: 5: 5**
AVO 2	6.6: 5: 5
AVO 3	6: 4: 6
AVO 4	3.4: 3: 7
AVO 5	7: 3: 7
CCT 1	**9: 3: 7**
CCT 2	**9: 2: 8**
CCT 3	**8: 2: 8**
CCT 4	**9: 1: 9**
CCT 5	**6.3: 1: 9**
MAC 1	7: 5: 5
MAC 2	6.6: 5: 5
MAC 3	6: 4: 6
MAC 4	**7: 3: 7**
MAC 5	4.4: 3: 7
OLV 1	7: 5: 5
OLV 2	6.7: 5: 5
OLV 3	6: 4: 6
OLV 4	**4.4: 3: 7**
OLV 5	4.4: 3: 7

Table IV. Characterisation profiles of topical SEDDSs

SEDDS	Droplet size (nm)	PDI	Zeta-potential (mV)	Self-emulsification grading	Self-emulsification time (s)	Viscosity (mPa.s)	pH	Cloud point (°C)
ARG 1	66.24	0.39	-30.60	D	499	230.53	6.95	**27.00**
ARG 3	107.32	0.62	-29.90	D	482	9436.27	6.00	34.00
ARG 5	**440.78**	**1.00**	-23.40	D	423	1060.14	6.60	46.90
AVO 2	64.11	0.34	-32.80	D	423	4103.20	5.01	40.00
AVO 3	**345.75**	**1.00**	-40.90	D	131	710.40	**3.82**	45.90
AVO 4	221.95	**1.00**	-37.50	D	130	6971.10	5.02	44.00
AVO 5	59.013	0.55	-38.20	D	430	1178.40	5.05	50.60
MAC 1	108.01	0.80	-25.00	D	374	23071.00	7.40	**31.90**
MAC 2	108.71	0.76	-29.20	D	346	17476.53	6.61	32.50
MAC 3	116.68	0.73	-27.70	D	181	5748.37	7.01	**31.40**
MAC 5	**462.32**	**1.00**	-26.00	**B**	59	993.60	7.02	36.00
OLV 1	**360.25**	**1.00**	-21.30	D	393	2511.03	7.37	34.80
OLV 2	222.87	**1.00**	-30.00	D	385	6006.17	7.13	34.80
OLV 3	**502.08**	**1.00**	-31.00	D	274	11740.00	7.13	33.80
OLV 5	154.80	0.80	-23.60	C	90	7220.00	6.97	35.90

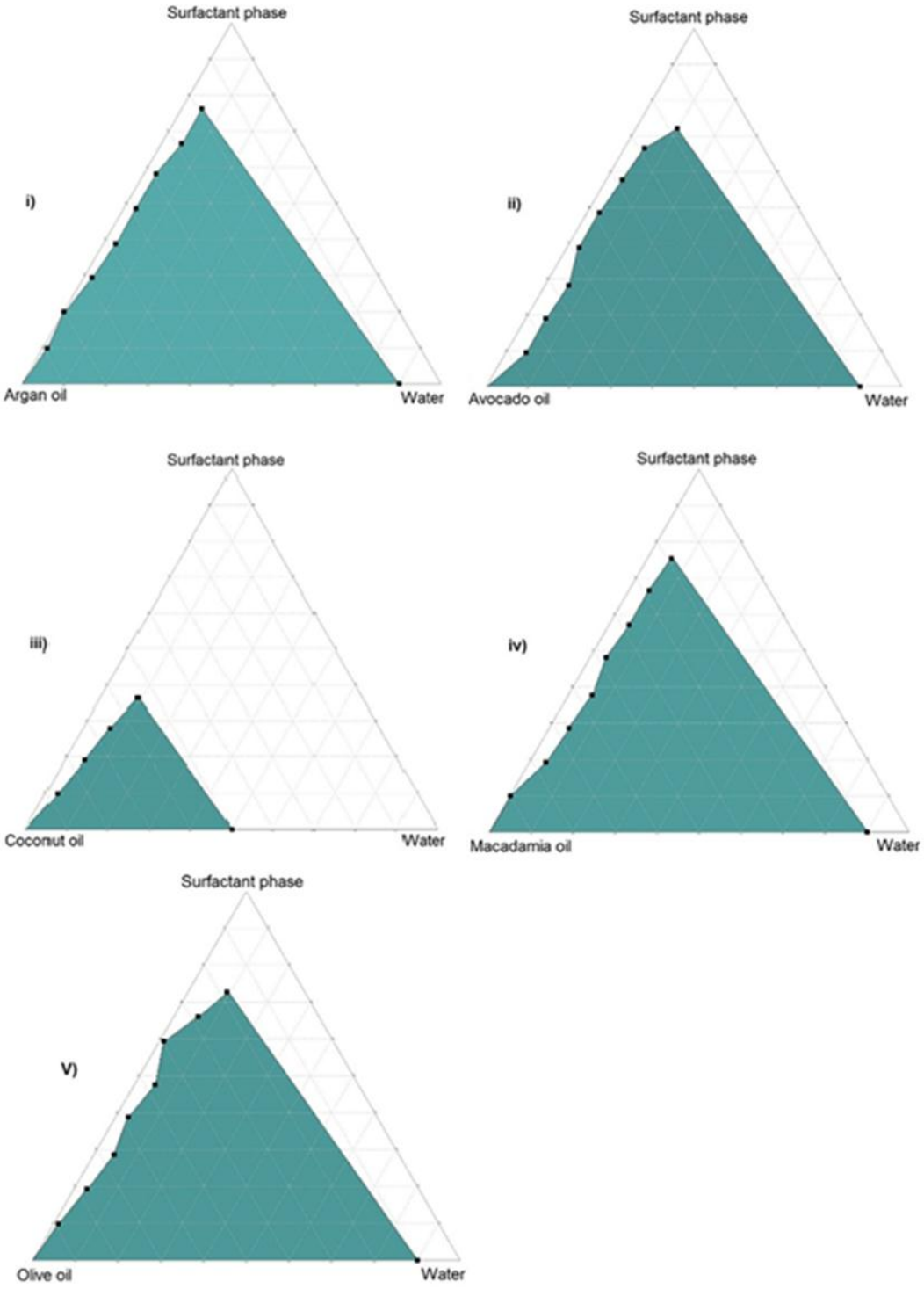

Fig.1: Pseudo-ternary phase diagram of **(i)** argan oil, clofazimine, surfactant phase and water **(ii)** avocado oil, clofazimine, surfactant phase and water **(iii)** coconut oil, clofazimine, surfactant phase and water **(iv)** macadamia oil, clofazimine, surfactant phase and water and **(v)** olive oil, clofazimine, surfactant phase and water

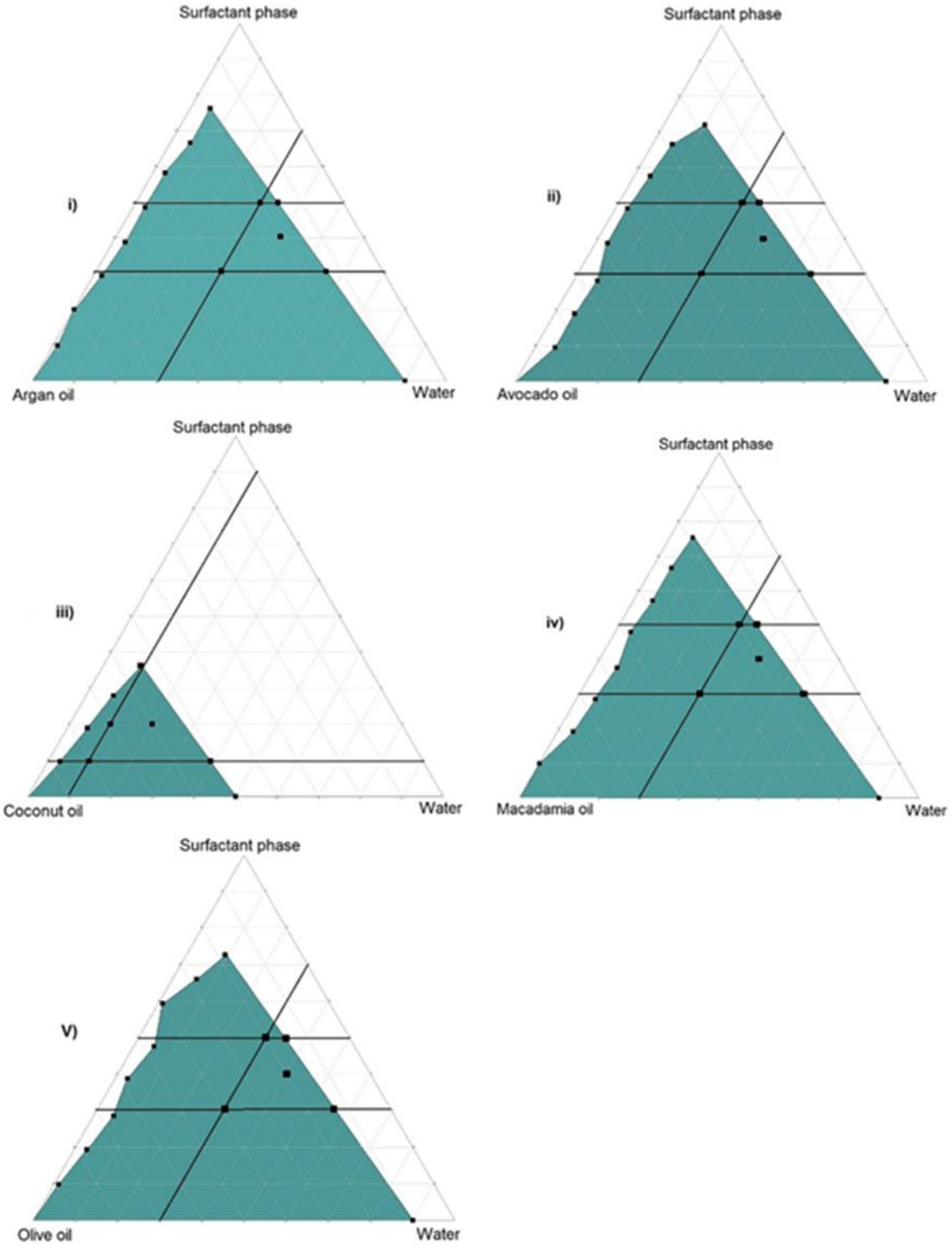

Fig. 2. Pseudo-ternary phase diagram indicating check-point formulations for **(i)** argan oil, clofazimine, surfactant phase and water **(ii)** avocado oil, clofazimine, surfactant phase and water **(iii)** coconut oil, clofazimine, surfactant phase and water **(iv)** macadamia oil, clofazimine, surfactant phase and water and **(v)** olive oil, clofazimine, surfactant phase and water

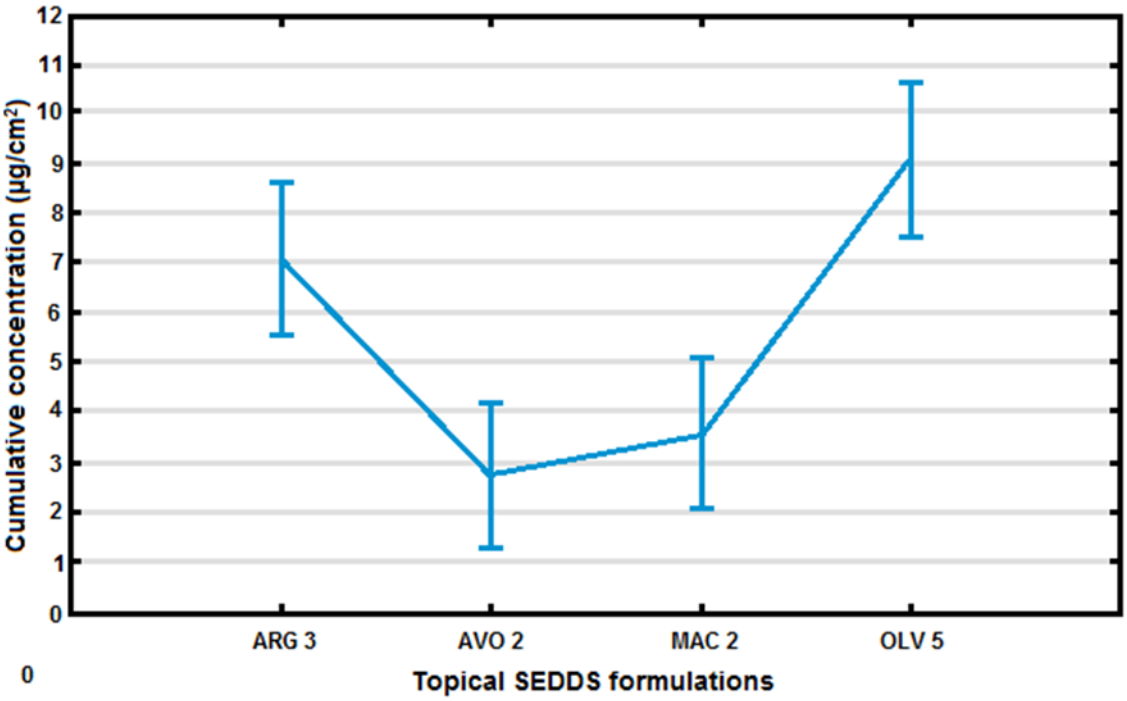

Fig. 3. Cumulative clofazimine concentrations observed in epidermis-dermis

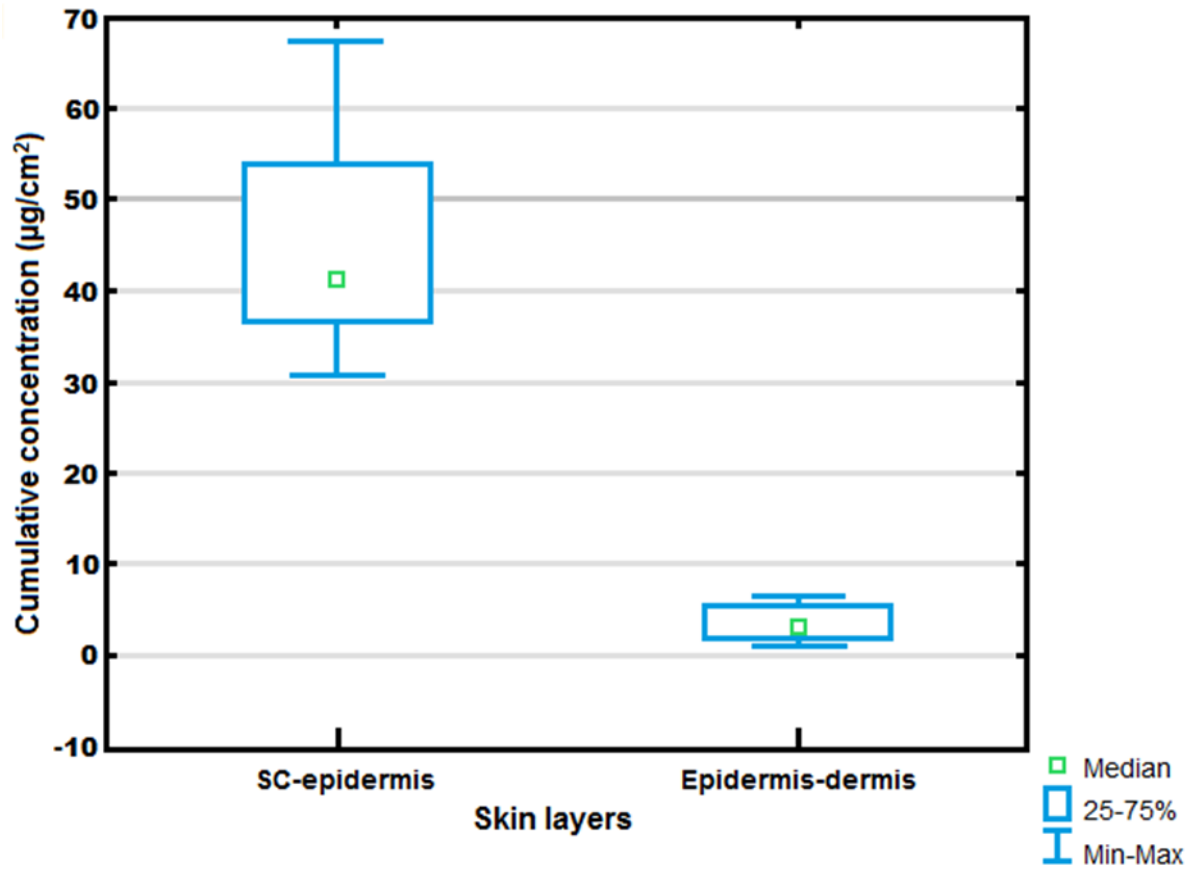

Fig. 4. Cumulative concentration of clofazimine delivered in different skin layers as achieved by MAC 2 over a duration of 12 h

Chapter 4

Conclusion and Future Prospects

4.1 Conclusion

The increased incidence of resistant TB strains adds to the global burden of anti-microbial resistance (Dougan *et al*., 2019; Tacconelli *et al*., 2018). CTB is a rare dermatological disease that has become more common due to growing drug resistance against first line anti-tubercular agents as well as heightened numbers of immunocompromised patients (Chen *et al*., 2019). Additionally, modern-day migration contributes to a worldwide increase of CTB (Chen *et al*., 2019). However, the development of new drug entities to aid in treatment of resistant TB disease is a complicated, expensive as well as a prolonged process (Breckenridge & Jacob, 2019; Pushpakom *et al*., 2018). Hence, repurposing of drugs primarily employed to treat a disease other than TB can TB burden by providing current relief whilst the quest continues for ong-term solutions to end TB (Herma Sree, 2019; Savla *et al*., 2017; Langedijk *et al*., 2015). The World Health Organisation (WHO) initiated the "End-TB" strategy aimed at complete elimination of TB by 2035 (John, 2019). Interventions are focused on high impact approaches such as individualised therapy (Akkerman *et al*., 2018; Lange *et al*., 2018). Moreover, new scientific insights are essential for the purpose of developing new tools in addition to investing in the development of novel drug entities (Floyd *et al*., 2018). This study is in line with the "End-TB" strategy as it provides the possibility of a solution to adjunct treatment of an ancient disease by incorporating a repurposed drug into topical SEDDSs.

Topical delivery of a drug as lipophilic as clofazimine presented unique challenges (Czajkowska-Kośnik *et al*., 2018; Jones *et al*., 2016; Ita, 2014). However, these challenges were managed by adding excipients known for their skin penetration enhancement properties. A skin penetration enhancer, considered ideal for topical drug delivery, should possess properties, including as follows: it should depict pharmacologically inert behaviour, not display any toxic effects, have no tendency towards skin irritation, it should exclude any possibility of allergic reactions, and facilitate reversible structural changes of the SC (Lane, 2013). Therefore, plant based oils can be considered almost ideal skin penetration enhancers as they possess all the previously mentioned characteristics (Lin *et al*., 2018; Vaughn *et al*., 2018; van Zyl *et al*., 2016; Viljoen *et al*., 2015; Lane, 2013). Moreover, plant based oils are affordable alternatives to certain chemical skin penetration enhancers (Haque & Talukder, 2018). Additionally, SEDDSs provide topical drug delivery advantages,

for example, increased thermodynamic stability, simplified preparation techniques, improved drug loading capacity, reduced droplet sizes, decreased surface tension, as well as skin penetration enhancement potential (El Khayat *et al*., 2018).

4.2 Objectives

The objectives of this study were to;

- Verify the analytical method, namely high performance liquid chromatography (HPLC) to enable determination of clofazimine concentrations.
- Establish the solubility of clofazimine in the selected plant based oils (i.e. argan-, avocado-, coconut-, macadamia- and olive oil).
- Construct pseudo-ternary phase diagrams to determine the required concentration of each ingredient for the purpose of formulating a SEDDS successfully.
- Evaluate SEDDSs in terms of droplet size, size distribution, zeta potential, robustness to dilution, self-emulsification, cloud point assessment, thermodynamic stability, viscosity and pH.
- Select the most optimum topical SEDDS formulations after interpretation of the results obtained from the various evaluation tests performed.
- Conduct membrane release studies to determine clofazimine release from finally selected topical SEDDS formulations.
- Perform dermal diffusion as well as tape stripping experiments to investigate transdermal and topical delivery of clofazimine, respectively.
- Investigate if any potential incompatibilities are depicted between the included excipients by utilising isothermal microcalorimetry.

Verification of the HPLC analytical method was conducted in conjunction with the expertise of Prof Jan du Preez at the analytical laboratory of the Potchefstroom Campus. This analytical method, previously developed for the purpose of clofazimine detection, rendered accurate, specific and repeatable results that enabled reliable calculation of the cumulative clofazimine concentrations released during membrane release studies and the amount diffused during dermal diffusion studies, respectively (Aucamp *et al*., 2016; Indrayanto *et al*., 2012).

Pre-formulation studies were conducted to contribute toward development of topical SEDDSs for optimised delivery (Honmane *et al*., 2017). Firstly, solubility of clofazimine in individual plant based oils was successfully determined by utilising the shake flask method (Ghanbari *et al*., 2016; Czaikowska-Kósnik *et al*., 2015). The lipophilic nature of clofazimine was confirmed by observing increased solubility of clofazimine in plant based oils compared to the aqueous solubility exhibited by clofazimine itself (Sangana *et al*., 2018;

Zhang *et al*., 2017). Next, pseudo-ternary phase diagrams were employed to determine optimal quantities of excipients employed in combination (Ibrahim *et al*., 2018). Moreover, by constructing pseudo-ternary phase diagrams the self-emulsification area of SEDDSs comprising divergent plant based oils, were illustrated by adding distilled water in a dropwise fashion (i.e. water titration method) to mixtures containing predetermined ratios of other excipients and clofazimine (Rani *et al*., 2019; Ke *et al*., 2016; Czaikowska-Kósnik *et al*., 2015). Hereafter, the area of interest was identified, within the displayed self-emulsification region, for the purpose of investigating SEDDS formulations that might be most suitable for topical clofazimine delivery. From these regions of interest, 25 topical SEDDS formulations were identified for further investigation.

The 25 identified topical SEDDS formulations were formulated according to the water titration method while subjected to stirring and heating, followed by cooling and subsequent spontaneous emulsification (Prajapat *et al*., 2017; Hong *et al*., 2016; Solans *et al*., 2016; Aparna *et al*., 2015). Next, the 25 formulated topical SEDDSs were visually inspected for phase separation after being stored at an ambient temperature of 25°C for a period of 24 h (Sanka *et al*., 2016; Parmar *et al*., 2015). Argan oil SEDDSs displayed phase separation of formulations ARG 2 and ARG 4. Additionally, avocado oil SEDDSs rendered phase separation of formulation AVO 1. Coconut oil SEDDSs depicted phase separation of all formulated coconut oil SEDDSs and therefore confined coconut oil SEDDSs unsuitable for further investigation. In contrast, macadamia oil and olive oil SEDDSs depicted phase separation of formulations MAC 4 and OLV 4. Therefore, a total of only 15 topical SEDDS formulations did not display any phase separation after the 24 h period and were thus further subjected to characterisation experiments.

Characterisation studies included: measurement of droplet size; size-distribution; obtaining zeta potential readings; robustness to dilution displayed by SEDDSs; efficacy of self-emulsification experiments; viscosity readings; assessment of cloud point; evaluation of thermodynamic stability; and pH measurement. The results obtained during characterisation were aimed at eliminating SEDDS formulations that displayed undesirable properties in terms of topical clofazimine delivery. Hence, the final SEDDS formulations destined for membrane release and dermal diffusion studies were identified as formulations ARG 3, AVO, 2, MAC 2 and OLV 5. After studying the characterisation profiles of the finally selected topical SEDDS formulations, formulation AVO 2 was considered the SEDDSs most suitable for topical clofazimine delivery as this formulation fell within the nano-sized range and decreased droplet size tend to facilitate enhanced dermal permeation (Kaur & Ajitha, 2019). Moreover, formulation AVO 2 exhibited the most negative zeta potential value (i.e.

-32.8 mV). The increasingly negative zeta potential of formulation AVO 2 signified increased stability of the topical SEDDS formulation (Liang *et al*., 2017). Additionally, formulation AVO 2 depicted the highest cloud point temperature (40°C) between finally selected SEDDS formulations, indicating dehydration of excipients at temperatures exceeding 40°C (Ujilestari *et al*., 2018; Akula *et al*., 2014). Moreover, this formulation received D-grading in terms of efficacy of self-emulsification that predicts enhanced occlusive effects (Keurentjes & Maibach, 2019; Kathe & Kathpalia, 2017). Lastly, the pH value obtained for formulation AVO 2 demonstrated a value of 5.009 that is considered compatible with natural skin pH (Sheshala *et al*., 2019).

Membrane release studies confirmed release of clofazimine from all of the selected topical SEDDS formulations. The flux values obtained during membrane release and dermal diffusion studies signified formulation OLV 5 as the SEDDS that rendered the highest release as well as topical delivery of clofazimine. This increased permeation of clofazimine from formulation OLV 5 is linked to the high oleic acid content of olive oil. Oleic acid is known for significant skin penetration enhancement properties as facilitated by lipid disruption of the SC, fluidisation of lipids naturally present within the SC and formation of reversible, permeable changes to the SC structure (Hadgraft & Lane, 2016; Lane, 2013). In contrast, formulation OLV 5 has the highest water content comparatively to the other finally selected topical SEDDS formulations. This increased presence of water is suspected to have an influence on partitioning of the highly lipophilic clofazimine into the lipophilic SC (Chauhan & Sharma, 2019; Fantini *et al*., 2019).

Finally, compatibility of clofazimine with other excipients, as well as compatibility between these excipients were investigated by employing isothermal microcalorimetry (van Zyl *et al*., 2019; Patel *et al*., 2015). This highly sensitive test detected possible interactions between the combinations of Span®60 and argan oil, macadamia oil or olive oil, respectively. Moreover, a potential incompatibility was observed between argan oil and clofazimine. Nonetheless, isothermal microcalometric experiments only indicate the potential existence of incompatibilities between substances (Patel *et al*., 2015; Fathima *et al*., 2011). Hence, the type of interaction must be further investigated. However, despite possible incompatibilities, topical application of SEDDS rendered successful dermal permeation of the highly lipophilic clofazimine and therefore signifies the potential of SEDDSs as a topical and/or transdermal drug delivery vehicle.

4.3 Future recommendations

Recommendations are to:

- Consider the beneficial capacity of other co-surfactants to replace Span®60 in SEDDS formulations. Examples include Span®80 and propylene glycol (Sekrani & Poncet, 2018; Mahdi *et al*., 2011). Both of the aforementioned alternative co-surfactants have displayed successful topical drug delivery in combination with Tween®80 (Sambhakar *et al*., 2017). As possible incompatibilities exist between Span®60 and certain plant based oils, the investigation of alternative co-surfactants for the purpose of possibly substituting Span®60 is of utmost importance.
- Investigate the nature of possible incompatibilities depicted between Span®60 and argan-, macadamia, and olive oil, respectively. This can be conducted utilising alternative techniques such as isothermal stress testing followed by HPLC analysis (Patel *et al*., 2015).
- Determine the solubility of clofazimine in alternative plant based oils as enhanced solubility of clofazimine in the oil phase can potentially establish an increased concentration gradient at the skin surface that intensifies dermal drug diffusion (Ruela *et al*., 2016).
- Stability testing of topical SEDDS formulations should be conducted. The presence of UFAs may influence the stability of SEDDSs as these components tend to fall victim to peroxidation upon exposure to light or air (Almeida *et al*., 2018). This tendency towards peroxidation is facilitated by double bonds present within UFAs (Yun & Surh, 2012). Additionally, PUFAs are notoriously known for causing decreased stability of formulations (Hu *et al*., 2019). Hence, the influence of MUFAs and PUFAs can also be distinguished during stability experiments.
- In the food industry the potential of blending plant based oils in certain ratios in order to provide optimum nutritional value, together with decreased health risks, are discussed (Torri *et al*., 2019). However, by combining plant based oils and including more than one plant based oil in the oil phase of topical SEDDSs, the possibility of achieving optimum skin penetration enhancement together with improved solubility of clofazimine can be established (Arslan *et al*., 2017). This proposed idea is specifically aimed at reducing possible skin irritation reactions induced by powerful, natural skin penetration enhancers such as oleic acid in high concentrations (Karagounis *et al*., 2019). This concept may also contribute towards creating topical SEDDSs less sensitive to peroxidation by decreasing the quantity of undesired components, including elevated levels of PUFAs (Hu *et al*., 2019). Thus, by designing an oil phase of natural origin the inexpensiveness of the oil phase is not lost during formulation of SEDDSs, but the possibility of producing an oil phase for optimised topical delivery of clofazimine is a definite possibility to be confirmed.

- Limited studies have been conducted on employing a flip-flop mechanism to change the zeta-potential of SEDDSs for the purpose of enhancing drug delivery across mucus membranes (Salimi *et al*., 2018). The principal of this mechanism is to include an excipient that is able to alter the zeta-potential of a formulation from an initially negatively charged to a positively charged formulation in order to improve mucus permeation (Salimi *et al*., 2018). Thus, the flip-flop mechanism is established by loss of the added excipient during crossing of mucus and this facilitates a positively charged formulation that is desired for increased diffusivity across cell membranes within the body (Salimi *et al*., 2018). The same principal can be investigated to further enhance dermal permeation of drugs from SEDDSs as the formulations in this study were negatively charged due to the presence of fatty acids in the plant based oils (Umerska *et al*., 2016). In theory, the flip-flop mechanism can be investigated to improve dermal drug delivery by utilising the net charge of the skin surface and its negatively charged capacity to improve affinity between the skin and formulation by applying a positively charged SEDDS that facilitates enhanced dermal permeation (Carter *et al*., 2019; Gumustas *et al*., 2017).
- Development of fixed dose drug combination SEDDSs destined for topical or transdermal drug delivery should be considered. This suggestion is of specific importance during the treatment of TB infections of a pulmonary and/or extra-pulmonary nature as anti-tubercular treatment regimens include simultaneous administration of numerous drugs for prolonged time periods (Wu *et al*., 2019; Tiberi *et al*., 2018; Sotgiu *et al*., 2017). The inclusion of more than one drug within the same dosage form can contribute towards enhanced efficacy of anti-tubercular treatment while improving patient adherence (Wu *et al*., 2019; Sotgiu *et al*., 2017). Potential examples of drugs than may be utilised in combination with clofazimine, are drugs included in the shortened Bangladesh regimen (Tiberi *et al*., 2018; Sotgiu *et al*., 2017). This regimen displayed regimen shortening potential by decreasing treatment time from 24 months to 9-12 months of MDR-TB (Tiberi *et al*., 2018; Sotgiu *et al*., 2017). The initial phase of the shortened Bangladesh regimen refers to administration of kanamycin, prothionamide, clofazimine, moxifloxacin together with high doses of isoniazid and ethambutol for a period of 4-6 months (Sotgiu *et al*., 2017). This phase is followed by a maintenance phase consisting of clofazimine, moxifloxacin, ethambutol and pyrazinamide for the duration of 5 months (Sotgiu *et al*., 2017). Hence, numerous drugs may possibly also be incorporated into an individual topical SEDDS to aid in CTB.

References

Akkerman, O.W., Grasmeijer, F., de Lange, W.C., Kerstjens, H.A., de Vries, G., Bolhuis, M.S., Alffenaar, J.W., Frijlink, H.W., Smith, G., Gajraj, R. & de Zwaan, R. 2018. Cross border, highly individualised treatment of a patient with challenging extensively drug-resistant tuberculosis. *European respiratory journal*, 51:Article ID 1702490.

Akula, S., Gurram, A.K. & Devireddy, S.R. 2014. Self-microemulsifying drug delivery systems: an attractive strategy for enhanced therapeutic profile. *International scholarly research notices*, Article ID 964051.

Almeida, D.T.D., Viana, T.V., Costa, M.M., Silva, C.D.S. & Feitosa, S. 2018. Effects of different storage conditions on the oxidative stability of crude and refined palm oil, olein and stearin (Elaeis guineensis). *Food science and technology*, 39:211-217.

Aparna, C., Pathima, S. & Rao Patnaik, K.S.K. 2015. Formulation and evaluation of oral self-emulsifying drug delivery system of lornoxicam. *International journal of pharma and bio sciences*, 6:381-395.

Arslan, F.N., Şapçı, A.N., Duru, F. & Kara, H. 2017. A study on monitoring of frying performance and oxidative stability of cottonseed and palm oil blends in comparison with original oils. *International journal of food properties*, 20:704-717.

Aucamp, M.E., Csongradi, C., Gerber, M. & Plessis, J.D. 2016. A novel RP-HPLC method for the detection and quantification of roxithromycin in topical delivery studies. *Die Pharmazie*, 71:175-176.

Breckenridge, A. & Jacob, R. 2019. Overcoming the legal and regulatory barriers to drug repurposing. *Nature reviews drug discovery*, 18:1-2.

Carter, P., Narasimhan, B. & Wang, Q. 2019. Biocompatible nanoparticles and vesicular systems in transdermal drug delivery for various skin diseases. *International journal of pharmaceutics*, 555:49-62.

Chauhan, M.K. & Sharma, P.K. 2019. Optimization and characterization of rivastigmine nanolipid carrier loaded transdermal patches for the treatment of dementia. *Chemistry and physics of lipids*, 224:Article ID 104794.

Chen, Q., Chen, W. & Hao, F. 2019. Cutaneous tuberculosis: a great imitator. *Clinics in dermatology*, 37:192-199.

Czajkowska-Kośnik, A., Szekalska, M., Amelian, A., Szymańska, E. & Winnicka, K. 2015. Development and evaluation of liquid and solid self-emulsifying drug delivery systems for atorvastatin. *Molecules*, 20:21010-21022.

Czajkowska-Kośnik, A., Szekalska, M. & Winnicka, K. 2018. Nanostructured lipid carriers: A potential use for skin drug delivery systems. *Pharmacological reports*, 71:156-166.

Dougan, G., Dowson, C. & Overington, J. 2019. Meeting the discovery challenge of drug-resistant infections: progress and focusing resources. *Drug discovery today*, 24:452-461.

El Khayat, N.W., Donia, A.A., Mady, O.Y. & El Maghraby, G.M. 2018. Optimization of eugenol microemulsion for transdermal delivery of indomethacin. *Journal of drug delivery science and technology*, 48:311-318.

Fantini, A., Padula, C., Nicoli, S., Pescina, S. & Santi, P. 2019. The role of vehicle metamorphosis on triamcinolone acetonide delivery to the skin from microemulsions. *International journal of pharmaceutics*, 565:33-40.

Fathima, N., Mamatha, T., Qureshi, H.K., Anitha, N. & Rao, J.V. 2011. Drug-excipient interaction and its importance in dosage form development. *Journal of applied pharmaceutical science*, 1:66-71.

Floyd, K., Glaziou, P., Houben, R.M.G.J., Sumner, T., White, R.G. & Raviglione, M. 2018. Global tuberculosis targets and milestones set for 2016–2035: definition and rationale. *The international journal of tuberculosis and lung disease*, 22:723-730.

Ghanbari, A., Sarbaz, Y., Jouyban-Gharamaleki, V., Jouyban-Gharamaleki, K., Soleymani, J. & Jouyban, A. 2016. An improved automated setup for solubility determination of drugs. *Pharmaceutical sciences*, 22:210- 214.

. Herma Sree, G.N.S., Saraswathy, G.R., Manikantra, M. & Mamatha, K. 2019. An update on drug repurposing: re-written saga of the drug's fate. *Biomedicine & pharmacotherapy*, 110:700-716.

Gumustas, M., Sengel-Turk, C.T., Gumustas, A., Ozkan, S.A. & Uslu, B. 2017. Effect of polymer-based nanoparticles on the assay of antimicrobial drug delivery systems. (*In* Grumezescu, A.M., *ed.* Multifunctional systems for combined delivery, biosensing and diagnostics. Amsterdam: Elsevier. p. 67-108).

Hadgraft, J. & Lane, M.E. 2016. Advanced topical formulations (ATF). *International journal of pharmaceutics*, 514:52-57.

Haque, T. & Talukder, M.M.U. 2018. Chemical enhancer: a simplistic way to modulate barrier function of the stratum corneum. *Advanced pharmaceutical bulletin*, 8:169-179.

Hong, E.-P., Kim, J.-Y., Kim, S.-H., Hwang, K.-M., Park, C.-W., Lee, H.-J., Kim, D.-W., Weon, K.-Y., Jeong, S.Y. & Park, E.-S. 2016. Formulation and evaluation of a self-microemulsifying drug delivery system containing bortezomib. *Chemical and pharmaceutical bulletin*, 64:1108-1117.

Honmane, S.M., Dange, Y.D., Osmani, R.A.M. & Jadge, D.R. 2017. General considerations of design and development of dosage forms: pre-formulation review. *Asian journal of pharmaceutics*, 11(Suppl.):479-488.

Hu, W., Fitzgerald, M., Topp, B., Alam, M. & O'Hare, T.J. 2019. A review of biological functions, health benefits, and possible de novo biosynthetic pathway of palmitoleic acid in macadamia nuts. *Journal of functional foods*, 62:Article ID 103520.

Ibrahim, T.M., Abdallah, M.H., El-Megrab, N.A. & El-Nahas, H.M. 2018. Upgrading of dissolution and anti-hypertensive effects of carvedilol via two combined approaches: self-emulsification and liquisolid techniques. *Drug development and industrial pharmacy*, 44:873-885.

Indrayanto, G. 2012. Validation of analytical methods- update 2011. *Profiles of drug substances, excipients, and related methodology*, 37:439-463.

Ita, K.B. 2014. Transdermal drug delivery: progress and challenges. *Journal of drug delivery science and technology*, 24:245-250.

John, C.A. 2019. Realizing the World Health Organization's end TB strategy (2016–2035): How can social approaches to tuberculosis elimination contribute to progress in Asia and the Pacific?. *Tropical medicine and infectious disease*, 4:Article ID 28.

Jones, J.G., White, K.A.J. & Delgado-Charro, M.B. 2016. A mechanistic approach to modelling the formation of a drug reservoir in the skin. *Mathematical biosciences*, 281:36-45.

Karagounis, T.K., Gittler, J.K., Rotemberg, V. & Morel, K.D. 2019. Use of "natural" oils for moisturization: Review of olive, coconut, and sunflower seed oil. *Pediatric dermatology*, 36:9-15.

Kathe, K. & Kathpalia, H. 2017. Film forming systems for topical and transdermal drug delivery. *Asian journal of pharmaceutical sciences*, 12:487-497.

Kaur, R. & Ajitha, M. 2019. Formulation of transdermal nanoemulsion gel drug delivery system of lovastatin and its in vivo characterization in glucocorticoid induced osteoporosis rat model. *Journal of drug delivery science and technology*, 52:968-978.

Ke, Z., Hou, X. & Jia, X.-B. 2016. Design and optimisation of self-nanoemulsifying drug delivery systems for improved bioavailability of cyclovirobuxine D. *Drug design, development and therapy*, 10:2049-2060.

Keurentjes, A.J. & Maibach, H.I. 2019. Percutaneous penetration of drugs applied in transdermal delivery systems: an in vivo based approach for evaluating computer generated penetration models. *Regulatory toxicology and pharmacology*, 18:Article ID 104428.

Lane, M.E. 2013. Skin penetration enhancers. *International journal of pharmaceutics*, 447:12-21.

Lange, C., Alghamdi, W.A., Al-Shaer, M.H., Brighenti, S., Diacon, A.H., DiNardo, A.R., Grobbel, H.P., Gröschel, M.I., von Groote-Bidlingmaier, F., Hauptmann, M. & Heyckendorf, J. 2018. Perspectives for personalized therapy for patients with multidrug-resistant tuberculosis. *Journal of internal medicine*, 284:163-188.

Langedijk, J., Mantel-Teeuwisse, A.K., Slijkerman, D.S. & Schutjens, M.H.D. 2015. Drug repositioning and repurposing: terminology and definitions in literature. *Drug discovery today*, 20:1027-1034.

Liang, L., Wang, L., Nguyen, A.V. & Xie, G. 2017. Heterocoagulation of alumina and quartz studied by zeta potential distribution and particle size distribution measurements. *Powder technology*, 309:1-12.

Lin, T.K., Zhong, L. & Santiago, J. 2018. Anti-inflammatory and skin barrier repair effects of topical application of some plant oils. *International journal of molecular sciences*, 19:Article ID 70.

Mahdi, E.S., Sakeena, M.H., Abdulkarim, M.F., Abdullah, G.Z., Sattar, M.A. & Noor, A.M. 2011. Effect of surfactant and surfactant blends on pseudoternary phase diagram behavior of newly synthesized palm kernel oil esters. *Drug design, development and therapy*, 5:311-323.

Parmar, K., Patel, J. & Sheth, N. 2015. Selfnano-emulsifying drug delivery system for embelin: design, characterisation and in-vitro studies. *Asian journal of pharmaceutical sciences*, 10:396-404.

Patel, P., Ahir, K., Patel, V., Manani, L. & Patel, C. 2015. Drug-excipient compatibility studies: first step for dosage form development. *The pharma innovation*, 4:14-20.

Prajapat, M.D., Patel, N.J., Bariya, A., Patel, S.S. & Butani, S.B. 2017. Formulation and evaluation of self-emulsifying drug delivery system for nimodipine, a BCG class II drug. *Journal of drug delivery science and technology*, 39:59-68.

Pushpakom, S., Iorio, F., Eyers, P.A., Escott, K.J., Hopper, S., Wells, A., Doig, A., Guilliams, T., Latimer, J., McNamee, C. & Norris, A. 2018. Drug repurposing: progress, challenges and recommendations. *Nature reviews drug discovery*, 18:1-2.

Rani, S., Rana, R., Saraogi, G.K., Kumar, V. & Gupta, U. 2019. Self-emulsifying oral lipid drug delivery systems: advances and challenges. *AAPS PharmSciTech*, 20:Article ID 129.

Ruela, A.L.M., Perissinato, A.G., Lino, M.E.D.S., Mudrik, P.S. & Pereira, G.R. 2016. Evaluation of skin absorption of drugs from topical and transdermal formulations. *Brazilian journal of pharmaceutical sciences,* 52:527-544.

Salimi, E., Le-Vinh, B., Zahir-Jouzdani, F., Matuszczak, B., Ghaee, A. & Bernkop-Schnürch, A. 2018. Self-emulsifying drug delivery systems changing their zeta potential via a flip-flop mechanism. *International journal of pharmaceutics*, 550:200-206.

Sambhakar, S., Paliwal, S., Sharma, S. & Singh, B. 2017. Formulation of risperidone loaded proniosomes for effective transdermal delivery: an in-vitro and in-vivo study. *Bulletin of faculty of pharmacy, Cairo University*, 55:239-247.

Sangana, R., Gu, H., Chan, D.Y. & Einolf, H.J. 2018. Evaluation of clinical drug interaction potential of clofazimine using static and dynamic modeling approaches. *ASPET journal*, 46:1-34.

Sanka, K., Suda, D. & Bakshi, V. 2016. Optimisation of solid-selfnanoemulsifying drug delivery system for solubility and release profile of clonazepam using simple lattice design. *Journal of drug delivery science and technology*, 33:114-124.

Savla, R., Browne, J., Plassat, V., Wasan, K.M. & Wasan, E.K. 2017. Review and analysis of FDA approved drugs using lipid-based formulations. *Drug development and industrial pharmacy*, 43:1743-1758.

Sekrani, G. & Poncet, S. 2018. Ethylene-and propylene-glycol based nanofluids: a litterature review on their thermophysical properties and thermal performances. *Applied sciences*, 8:Article ID 2311.

Sheshala, R., Anuar, N.K., Samah, N.H.A. & Wong, T.W. 2019. *In vitro* drug dissolution/permeation testing of nanocarriers for skin application: a comprehensive review. *AAPS PharmSciTech*, 20:Article ID 164.

Solans, C., Morales, D. & Homs, M. 2016. Spontaneous emulsification. *Current opinion in colloid and interface science*, 22:88-93.

Sotgiu, G., Tiberi, S., Centis, R., D'Ambrosio, L., Fuentes, Z., Zumla, A. & Migliori, G.B. 2017. Applicability of the shorter 'Bangladesh regimen'in high multidrug-resistant tuberculosis settings. *International journal of infectious diseases*, 56:190-193.

Tacconelli, E., Carrara, E., Savoldi, A., Harbarth, S., Mendelson, M., Monnet, D.L., Pulcini, C., Kahlmeter, G., Kluytmans, J., Carmeli, Y. & Ouellette, M. 2018. Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. *The lancet infectious diseases*, 18:318-327.

Torri, L., Bondioli, P., Folegatti, L., Rovellini, P., Piochi, M. & Morini, G. 2019. Development of Perilla seed oil and extra virgin olive oil blends for nutritional, oxidative stability and consumer acceptance improvements. *Food chemistry*, 286:584-591.

Tiberi, S., Muñoz-Torrico, M., Duarte, R., Dalcolmo, M., D'Ambrosio, L. & Migliori, G.B. 2018. New drugs and perspectives for new anti-tuberculosis regimens. *Pulmonology*, 24:86-98.

Ujilestari, T., Dono, N.D., Ariyadi, B., Martien, R. & Zuprizal, Z. 2018. Formulation and characterization of self-nano emulsifying drug delivery systems of lemongrass (cymbopogon citratus) essential oil. *Malaysian journal of fundamental and applied sciences*, 14:360-363.

Umerska, A., Cassisa, V., Matougui, N., Joly-Guillou, M.L., Eveillard, M. & Saulnier, P. 2016. Antibacterial action of lipid nanocapsules containing fatty acids or monoglycerides as co-surfactants. *European journal of pharmaceutics and biopharmaceutics*, 108:100-110.

van Zyl, L., du Preez, J., Gerber, M., du Plessis, J. & Viljoen, J. 2016. Essential fatty acids as transdermal penetration enhancers. *Journal of pharmaceutical sciences*, 105:188-193.

van Zyl, L., Viljoen, J.M., Haynes, R.K., Aucamp, M., Ngwane, A.H. & du Plessis, J. 2019. Topical delivery of artemisone, clofazimine and decoquinate encapsulated in vesicles and their *in vitro* efficacy against Mycobacterium tuberculosis. *AAPS PharmSciTech*, 20:33.

Vaughn, A.R., Clark, A.K., Sivamani, R.K. & Shi, V.Y. 2018. Natural oils for skin-barrier repair: Ancient compounds now backed by modern science. *American journal of clinical dermatology*, 19:103-117.

Viljoen, J.M., Cowley, A., Du Preez, J., Gerber, M. & Du Plessis, J. 2015. Penetration enhancing effects of selected natural oils utilized in topical dosage forms. *Drug development and industrial pharmacy*, 41:2045-2054.

Wu, S., Lan, L., Jiang, J., Ding, X., Ho, C.M., Lou, Y. & Fan, G. 2019. Simultaneous determination of the potent anti-tuberculosis regimen—pyrazinamide, ethambutol, protionamide, clofazimine in beagle dog plasma using LC–MS/MS method coupled with 96-well format plate. *Journal of pharmaceutical and biomedical analysis*, 168:44-54.

Yun, J.M. & Surh, J. 2012. Fatty acid composition as a predictor for the oxidation stability of Korean vegetable oils with or without induced oxidative stress. *Preventive nutrition and food science*, 17:158-165.

Zhang, Y., Feng, J., McManus, S.A., Lu, H.D., Ristroph, K.D., Cho, E.J., Dobrijevic, E.L., Chan, H.K., Prud'homme, R.K. 2017 Design and solidification of fast-releasing clofazimine nanoparticles for treatment of cryptosporidosis. *Molecular oharmaceutics*, 14:3480-3488.

Annexure A

Analytical method verification for the HPLC determination of clofazimine

A.1 Introduction

Analytical method validation is defined as the process by which laboratory experiments are conducted in order to obtain evidence that the performance characteristics of a drug correlate with the requirements established for the analytical application (Belouafa *et al*., 2017, Indrayanto, 2012). For the purpose of this study a chromatographic method was verified in order to confirm the reliability as well as the sensitivity of this analytical technique. Moreover, method verification is crucial to enable determination of the clofazimine concentration observed after conducting membrane release and dermal diffusion studies (Aucamp *et al*., 2016).

A.2 High performance liquid chromatography verification method

The analytical method employed during this study was developed by Prof Jan du Preez at the Analytical Technology Laboratory (ATL) of the North-West University (NWU) Potchefstroom Campus (Janse van Rensburg, 2016; Joubert, 2016). Therefore, analytical method verification was completed by reemployment of the HPLC analytical technique formerly created for clofazimine.

A.2.1 Chromatographic conditions

The following chromatographic instruments and conditions were employed:

Analytical instrument:	The analysis of clofazimine was enabled by utilising an Agilent® 1100 Series HPLC system at a temperature of 25°C in order to maintain a controlled environment. The instrument is equipped with an Agilent® 1100 pump, UV detector, auto-sampler injection mechanism and Chemstation Rev. A10.02 software employed for data acquisition and analysis.
Column:	A Venusil C_{18} column, 150 x 4.6 mm (Agilent Technologies, Newark, Delaware) was utilised.
Mobile phase:	The mobile phase consisted of acetonitrile together with a 0.005 M octane sulphonic acid (pH 3.5)/octane sulfonic acid mixture (70:30).

Solvent:	Methanol (100%)
Run time:	6 min
Retention time:	± 3.94 min
Flow rate:	1.0 ml/min
Injection volume:	5 µl
Wavelength of detection:	UV at 284 nm

A.2.2 Standard and sample preparation

Approximately 10 mg of clofazimine was accurately weighed and diluted to 100 ml in a volumetric flask, with the addition of methanol, in order to obtain a solution with a concentration of 100 µg/ml. The HPLC chromatograph of the standard clofazimine solution is displayed in **Figure A.1**.

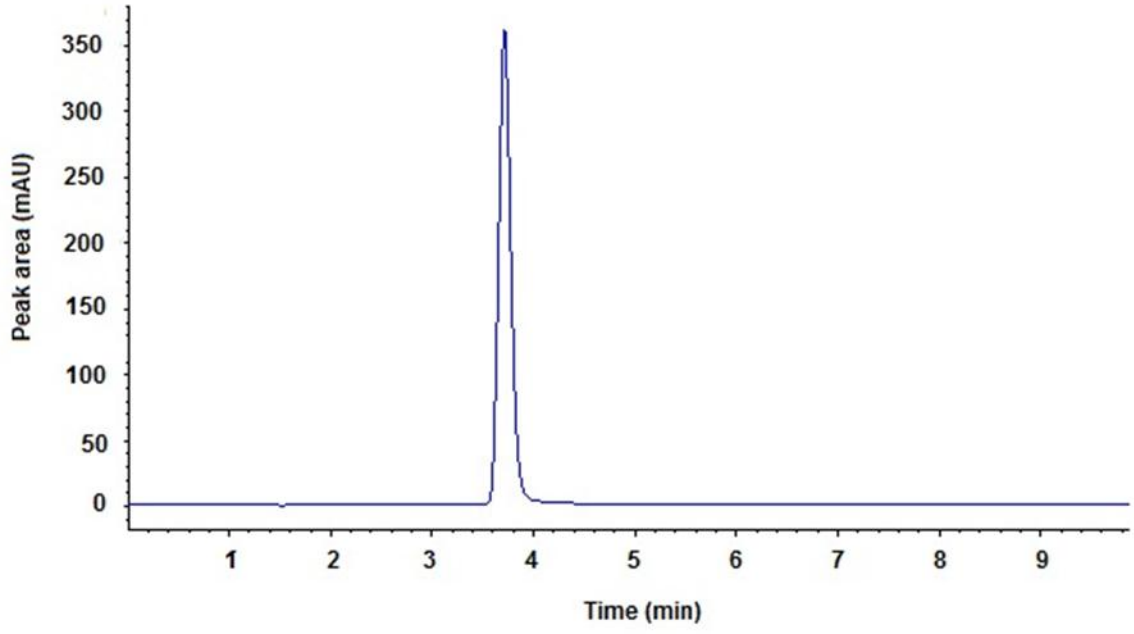

Figure A.1: HPLC chromatograph of clofazimine standard solution (i.e. 100 µg/ml)

A.2.3 Analytical verification of test procedure and acceptance criteria

A.2.3.1 Linearity

The ability displayed by an analytical method to render responses directly proportional to the concentration of an analyte included in a sample, within a given range, is defined as linearity (Alquadeib, 2018; Aparicio-Ruiz *et al*., 2018; Shabir, 2003). Therefore, linearity can be measured by how well data is able to fit to the portrayed linear equation **(Equation A:1)** (Araujo, 2009):

$$y = mx + c \qquad \textbf{Equation A.1}$$

Where:

- y - represents the peak area
- m - indicates the slope
- x - is the concentration
- c - displays the y-intercept

An acceptable correlation coefficient (R^2) value > 0.998 is generally accepted as evidence of adequate relation between the data and the regression line (Singh, 2013; Shabir, 2003). This criterion is based on the principal that the closer the correlation coefficient is to a value of one, the stronger the positive linear relationship can be described as (Ratner, 2009). Linearity is normally attained by plotting peak areas of calibration standards on the Y-axis against concentration on the X-axis. A series of three to six injections of a minimum of five or more standards is employed to determine linearity (Singh, 2013; ICH, 2005). For assays, a minimum specified range of the target concentration is 80–120% (Shabir, 2003).

Figure A.2 and **Table A.1** indicate a co-relation coefficient for clofazimine as 0.9998 (~1) which is suggestive of a perfect co-relation between the obtained data and the clofazimine concentration according to the ICH (2005) guidelines.

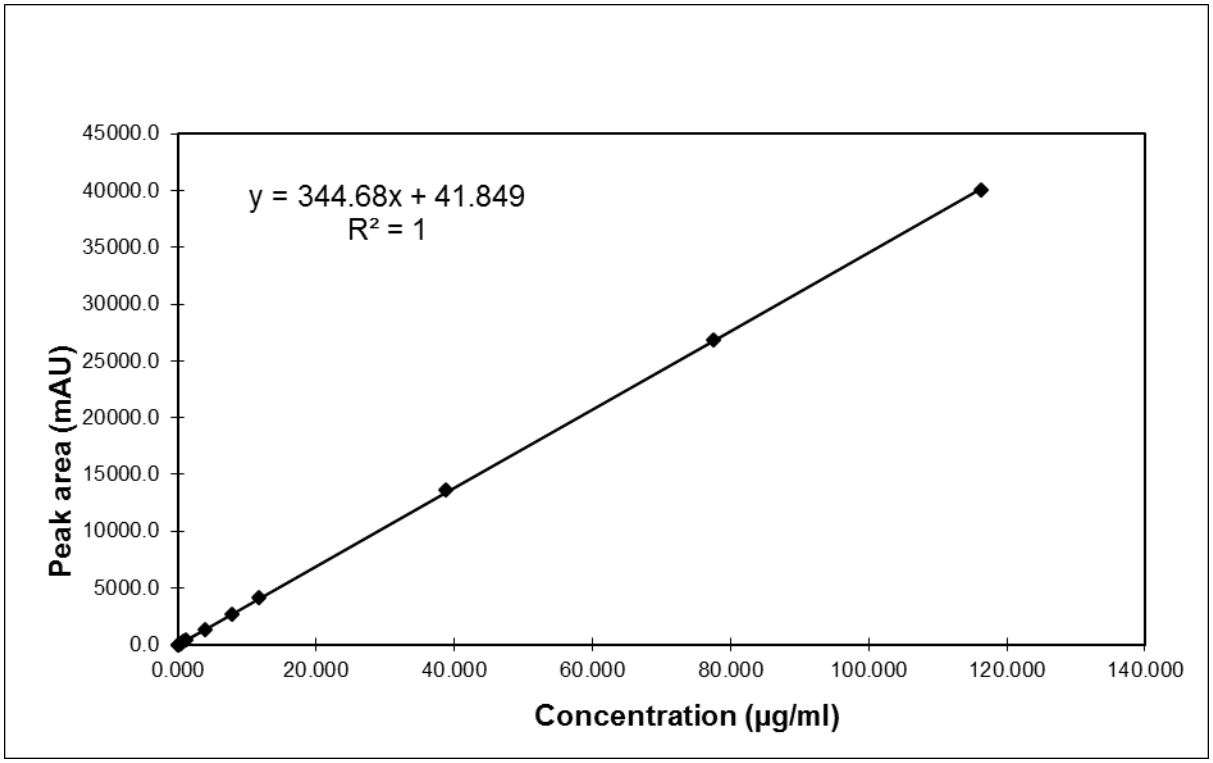

Figure A.2: Linear regression curve of clofazimine

Table A.1: Linearity data for clofazimine

Concentration (µg/ml)	Peak area (mAU)		Mean (mAU)
0.078	14.90	14.2	14.6
0.194	34.90	35.2	35.1
0.388	70.40	70.3	70.4
0.776	142.80	139.9	141.4
1.163	211.70	213.80	212.80
1.551	285.20	287.20	286.20
1.939	361.80	359.20	360.50
3.878	711.20	695.30	703.30
7.756	1424.40	1418.90	1421.70
11.634	2133.10	2136.60	2134.90
15.512	2825.30	2883.70	2854.50
19.390	3584.10	3656.50	3620.30
38.780	7182.10	7133.80	7158.00
77.560	14047.70	14484.30	14266.00
116.340	21338.80	21061.30	21200.10
155.120	28001.60	28229.00	28115.30
193.900	35284.50	35116.50	35200.50
R^2	**0.9998**	**Lower 95%**	**Upper 95%**
Intercept	**0**	**0**	**0**
Slope	**34.66**	**34.28**	**35.05**

A.2.3.2 Limit of detection and quantitation

The limit of detection (LOD) can be described as the lowest concentration of a drug in a sample that can be depicted, but is not necessarily possible to quantify adequately (ICH, 2005). Conversely, the limit of quantitation (LOQ) can be determined with acceptable precision and accuracy under the conditions specified by the analytical method (ICH, 2005).

In order to determine the LOD and LOQ of clofazimine, multiple standard solutions of different concentrations were prepared. These standard solutions were prepared in low concentrations. Each sample was injected seven consecutive times to be verified by HPLC analysis. The acceptance parameter for the percentage relative standard deviation (%RSD) of LOD is established as ≤ 15 % and the acceptance parameter for the %RSD of LOQ is instructed to be ≤ 20% (De Vito *et al*., 2016; Shabir, 2003). The LOD obtained for this method was 0.04 µg/ml, whereas the %RSD was calculated as 5.29%. LOQ was determined as 0.30 µg/ml, with a %RSD of 1.60%. Therefore, both the LOD and LOQ values obtained complied with the acceptance parameters as portrayed in **Table A.2**.

Table A.2: Summary of the limit of detection and lower limit of quantification as obtained for clofazimine

Injection volume (µl)	**1**	**2.5**	**5**	**7.5**	**10**	**12**
Concentration (µg/ml)	0.04	0.10	0.20	0.30	0.41	0.49
Peak area (mAU)	1.24	3.18	5.80	8.90	11.30	8.40
	1.42	3.07	5.80	8.90	11.80	9.00
	1.31	3.03	6.00	8.80	11.60	9.00
	1.45	3.11	6.10	8.60	11.80	8.80
	1.24	2.95	5.80	8.60	12.00	8.80
	1.36	3.16	6.00	8.80	12.40	8.90
	1.32	3.15	5.90	8.70	11.30	9.00
	1.38	3.25	5.80	8.50	11.70	9.00
Mean peak area (mAU)	**1.34**	**3.11**	**5.90**	**8.73**	**11.74**	**8.86**
SD	**0.07**	**0.09**	**0.11**	**0.14**	**0.34**	**0.19**
%RSD	**5.29**	**2.84**	**1.89**	**1.60**	**2.89**	**2.18**

1. Standard deviation (SD)
2. Relative standard deviation (%RSD)

A.2.3.3 Accuracy

The accuracy of an analytical method is established by comparing the closeness of the measured values to the true values (Singh, 2013; Indrayanto, 2012; Shabir, 2003). Additionally, accuracy can only be confirmed if the true and measured values are ascertained across a fixed concentration range (Ermer, 2005). For the purpose of this study a specific clofazimine concentration range, i.e. 80 µg/ml, 100 µg/ml and 120 µg/ml, was employed to verify the accuracy of the HPLC analytical method. Three samples of each clofazimine concentrate were prepared and each individual sample was injected in duplicate. The analytical value obtained for accuracy analysis should deliver a mean recovery value between the range of 98% - 102%, in order to be deemed successful (ICH, 2005; Shabir, 2003). Moreover, a %RSD equal or less than 2% is recommended (Ermer, 2005). As demonstrated in **Table A.3** the mean recovery value for clofazimine was obtained at 99.36% and the %RSD established at 1.9%. Therefore, this verification method complies with the established criteria.

Table A.3: Accuracy results for clofazimine

Concentration spiked (µg/ml)	Peak area 1 (mAU)	Peak area 2 (mAU)	Mean peak area (mAU)	Recovery	
				µg/ml	%
79.6	5974.20	6054.90	6014.55	82.59	103.76
80.4	5796.90	5811.40	5804.15	79.66	99.07
79.8	5701.00	5702.80	5701.90	78.23	97.98
99.5	7354.70	7285.10	7319.90	100.81	101.32
100.5	7234.30	7236.90	7235.60	99.64	99.14
99.8	7093.10	7087.80	7090.45	97.61	97.81
119.4	8566.60	8463.60	8515.10	117.50	98.41
120.6	8639.90	8649.70	8644.80	119.31	98.93
119.8	8493.10	8484.00	8488.55	117.13	97.80
				Mean	**99.36**
				SD	**1.97**
				%RSD	**1.98**

1. Standard deviation (SD)
2. Relative standard deviation (%RSD)

A.2.3.4 Precision

Precision considers the degree of repeatability displayed by an analytical method under prescribed operating conditions by conducting a series of sampling from the same sample (ICH, 2005; BPCC, 2001). Moreover, precision is specified as the %RSD obtained for a number of samples (McPolin, 2009). This number of samples should include a minimum of five to nine measurements, obtained at a minimum of three concentrations, in a specified range (Singh, 2013; McPolin, 2009). Generally, precision is performed at three levels, namely repeatability (intra-day precision), intermediate precision (inter-day precision) and reproducibility (Shabir, 2003). The specifications for an assay method employed for drug analysis is a %RSD ≤ 2% (Huber, 2015; Shabir, 2003).

A.2.3.4.1 Intra-day precision

Intra-day precision is performed in order to determine if the system is able to render similar responses under corresponding conditions on the same day (Araujo, 2009). Nine clofazimine samples were prepared on the same day and each sample was injected in duplicate. As observed in **Table A.4** the %RSD did not exceed 2%, hence the rendered %RSD of 0.8% complies with the prescribed limit (Huber, 2015; Shabir, 2003).

Table A.4: Intra-day precision results for clofazimine

Concentration spiked (µg/ml)	Peak area 1 (mAU)	Peak area 2 (mAU)	Mean peak area (mAU)	Recovery	
				µg/ml	%
32.09	3577.40	3561.50	3569.45	41.71	129.98
32.09	3594.10	3589.50	3591.80	41.97	130.78
32.30	3584.20	3585.60	3584.90	41.89	129.67
40.38	4476.10	4432.90	4454.50	52.11	129.40
40.47	4551.80	4536.80	4544.30	53.10	131.35
40.47	4454.00	4441.90	4447.95	52.03	128.57
48.07	5226.30	5285.30	5255.80	61.52	127.97
48.10	5301.20	5294.70	5297.95	62.02	128.93
48.23	5363.10	5344.00	5353.55	62.67	129.94
				Mean	**129.62**
				SD	**1.06**
				%RSD	**0.82**

1. Standard deviation (SD)
2. Relative standard deviation (%RSD)

A.2.3.4.2 Inter-day precision

Inter-day precision demonstrates the possibility of variable responses that could occur during sample preparation on two or more separate days (Singh, 2013; McPollin, 2009; ICH, 2005). For this particular analytical method verification analysis was performed by preparing samples of 52 µg/ml in triplicate over a period of three consecutive days. As displayed in **Table A.5** a %RSD value of less than 2% was obtained (i.e. 1.24%). Hence, inter-day precision verification was considered acceptable (Shabir, 2003).

Table A.5: Inter-day precision results for clofazimine

Parameters (%Recovery)	Day 1	Day 2	Day 3	Between days
	128.57	128.10	127.69	
	128.93	127.15	123.27	
	127.97	123.11	126.17	
Mean	128.49	127.14	126.62	**127.42**
SD	0.48	0.97	0.90	**0.97**
%RSD	0.62	0.76	0.71	**1.24**

1. Standard deviation (SD)
2. Relative standard deviation (%RSD)

A.2.3.4.3 System repeatability

System repeatability is described as the precision displayed by an analytical method as observed over a short period of time (Ermer, 2001). In order to determine the system repeatability of clofazimine, a standard clofazimine solution (i.e. 100 µg/ml) was prepared and injected six at consecutive times, as demonstrated in **Table A.6**.

Table A.6: System repeatability results obtained for clofazimine

Injection number	Peak area (mAU)	Retention time (min)
1	11914.60	3.93
2	11911.10	3.93
3	11990.00	3.94
4	11920.00	3.94
5	11992.30	3.94
6	11992.50	3.94
Mean	**11953.42**	**3.94**
SD	**41.93**	**0.01**
%RSD	**0.35**	**0.18**

1. Standard deviation (SD)
2. Relative standard deviation (%RSD)

As seen in **Table A.6** the %RSD obtained for peak area was 0.35% and 0.18% for retention time. Hence, the system repeatability for clofazimine is considered suitable as the calculated %RSD values did not exceed a value of 2% (Shabir, 2003).

A.2.3.5 Stability

Stability tests are conducted in order to determine the chemical stability of a drug when exposed to specified conditions, in a distinct environment over a fixed period of time (Ravisankar *et al*., 2015). A standard sample of clofazimine was prepared, as noted in Section A.2.2. The stock solution was repeatedly injected over a period of 24h at hourly intervals. Moreover, the peak area obtained at a time of 0 h was employed as a reference value for each peak area subsequently obtained. The derived peak areas together with the percentage recovery for each injection are displayed in **Table A.7.**

Table A.7: Stability results portrayed by clofazimine

Time (h)	Peak area (mAU)	% Recovery
0	11908.60	100.00
1	11869.10	99.70
2	11813.70	99.20
3	11970.40	100.50
4	11988.70	100.60
5	11995.40	100.70
6	11837.50	99.40
7	11999.10	100.70
8	11957.20	100.40
9	11841.30	99.40
10	12037.80	101.10
11	12037.30	101.10
12	11821.50	99.20
13	12021.80	100.90
14	11811.80	99.20
15	11756.50	98.70
16	11796.40	99.10
17	11759.70	98.70
18	11821.60	99.30
19	12017.30	100.90
20	11868.50	99.70
21	11918.60	100.10
22	12056.80	101.20
23	12077.10	101.40
24	11800.10	99.10
Mean	**11911.35**	**100.01**
SD	**102.56**	**0.85**
%RSD	**0.86**	**0.85**

1. Standard deviation (SD)
2. Relative standard deviation (%RSD)

For the purpose of ensuring optimum sample stability, clofazimine samples should not be utilised or retained for periods that exceed the degrading of clofazimine with a value of 2% (Huber, 2015). Favourably, the collected stability results of clofazimine demonstrated virtually no degradation, with a %RSD value of 0.86 and 0.85, obtained for % recovery and peak area, respectively. As this analytical method verification test was conducted over a period of 24 h and virtually no degradation of clofazimine was observed, it can be assumed that clofazimine should remain stable for the 12 h period required to conduct dermal diffusion studies.

A.2.3.6 Specificity

The specificity of an analytical method should not be confused with the selectivity (Indrayanto, 2012). In analytical method validation, selectivity is graded as low or high (Indrayanto, 2012; Araujo, 2009); whereas, specificity can be described as 100% selectivity or 0% interference of excipients included in a formulation (Indrayanto, 2012). Therefore, specificity is deemed an essential analytical method validation test to indicate if differentiation between compounds of similar HPLC chromatographic structure is possible.

In order to establish the specificity, a standard clofazimine solution of 25 μg/ml was prepared. Next, 2 ml of the standard clofazimine solution was extracted and vortexed together with 200 μl of hydrochloric acid, sodium hydroxide or hydrogen peroxide and methanol in a test tube. **Figure A.3** displays the HPLC chromatographs obtained for hydrochloric acid, sodium hydroxide and hydrogen peroxide together with the HPLC chromatograph of a standard clofazimine solution.

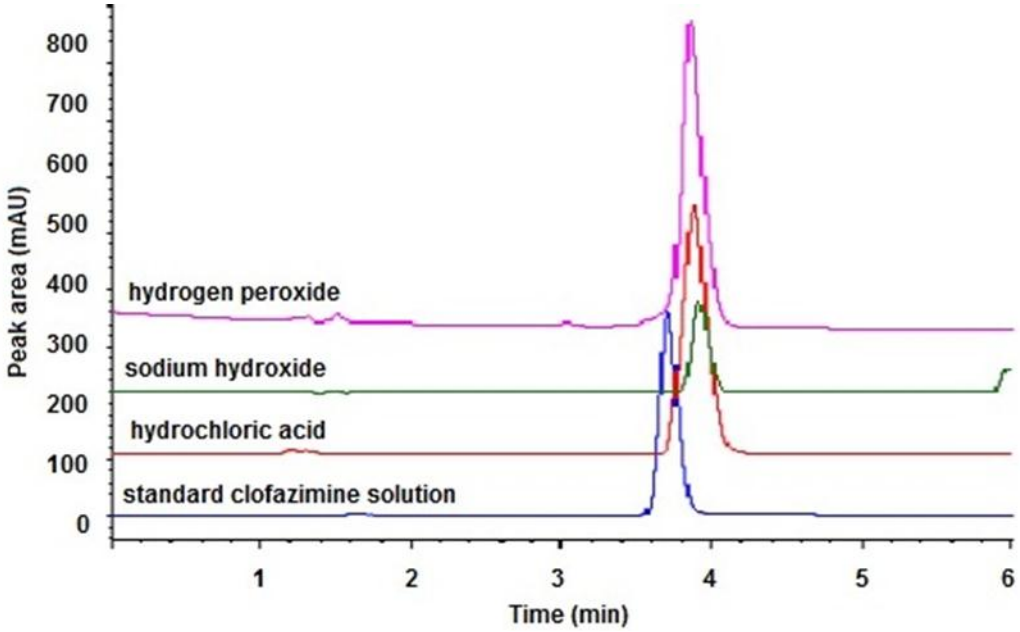

Figure A.3: HPLC chromatographs of hydrochloric acid, sodium hydroxide, hydrogen peroxide solutions of clofazimine and standard clofazimine solution

As observed in **Figure A.3** virtually no decomposition of clofazimine can be detected in the presence of hydrochloric acid, sodium hydroxide or hydrogen peroxide. Conversely, it should be noted that the retention time of clofazimine differs minimally as influenced by the presence of hydrochloric acid, sodium hydroxide or hydrogen peroxide.

For the purpose of obtaining HPLC chromatographs for excipients included in formulations, 100 µl of plant oil (i.e. argan-, avocado-, coconut-, macadamia- and olive oil) were vortexed with approximately 5 ml methanol prior to being analysed by means of HPLC. In order to exhibit an HPLC chromatographic structure for the surface active ingredient, 100 mg of Span®60 were weighed, heated in a test tube and approximately 5 ml methanol was added before subjected to vortexing. The Span®60 sample was filtered prior to HLPC analysis. **Figure A.4** depicts the HPLC chromatographs of the various excipients tested.

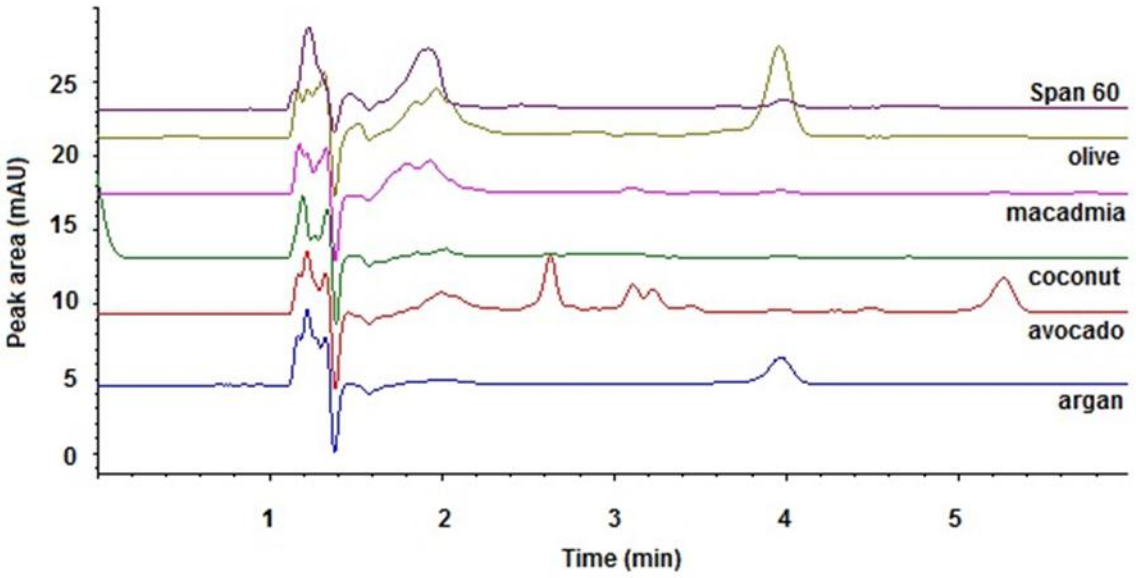

Figure A.4: HPLC chromatograph of excipients included in SEDDSs formulations

From **Figure A.4** it is clear that argan oil, olive oil and Span®60 are the only excipients that might be able to interfere with clofazimine detection as the HPLC chromatograph shows peaks between the retention time of 3.8 min and 4.2 min. These peak retention times could complicate accurate detectability of clofazimine as the average retention time displayed by clofazimine is established at 3.94 min. However, when the HPLC chromatographs of the excipients and the standard clofazimine solution are placed into context (**Figure A.5**), the interference with clofazimine detection is deemed insignificant and specificity of the analytical method was thus proven.

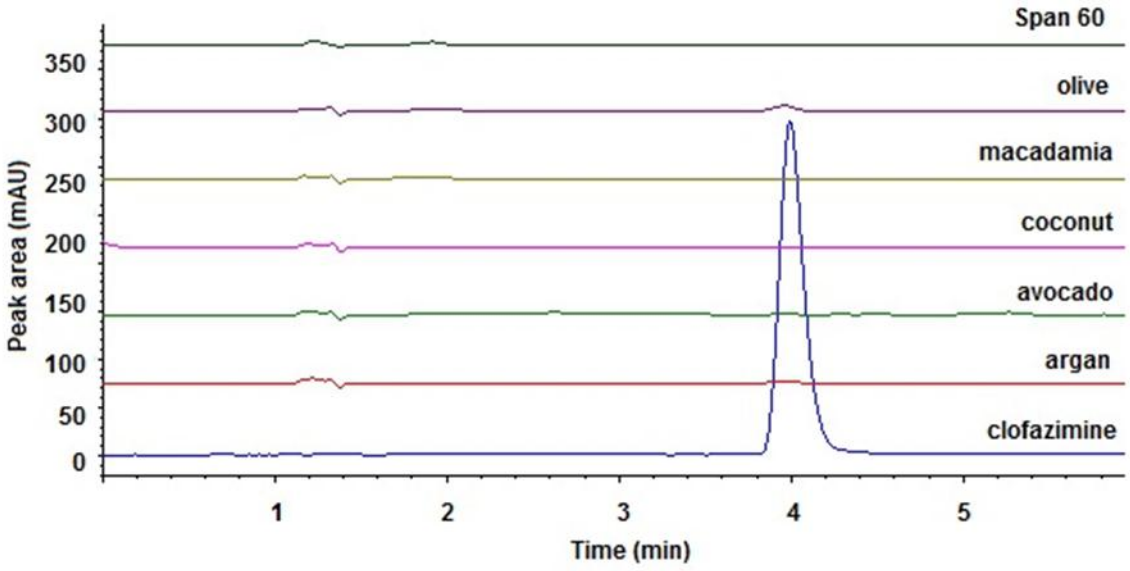

Figure A.5: HPLC chromatograph of clofazimine standard solution and excipients included in SEDDSs formulations

A.2.3.7 Robustness

Robustness evaluates the ability of an analytical method to remain unaffected if certain parameters are slightly altered (Shabir, 2003). For the purpose of demonstrating analytical method robustness the initial chromatographic conditions, as summarised in Section A.2.1, were altered while injecting a standard clofazimine solution. Two robustness experiments were performed while four chromatographic conditions were adjusted simultaneously. During the first robustness test the initial injection volume was changed from 1 µg/l to 1.2 µg/l, the initial flow rate was altered from 1.0 ml/min to 2.5 ml/min, the mobile phase ratio of 70:30 (acetonitrile to octane sulfonic acid) was modified to a mobile phase ratio of 65:35 (acetonitrile to octane sulfonic acid), and the wavelength was adjusted from the initial 284 nm to 280 nm. For the second robustness assessment the initial injection volume was altered from 1 µg/l to 0.8 µg/l, the initial flow rate was changed from 1.0 ml/min to 1.5 ml/min, the mobile phase ratio of 70:30 (acetonitrile to octane sulfonic acid) was modified to a mobile phase ratio of 75:25 (acetonitrile to octane sulfonic acid) and the wavelength was amended from an initial 284 nm to 288 nm. Results obtained for both robustness tests, together with the HPLC chromatograph of clofazimine under initial chromatographic conditions, are presented in **Figure A.6**.

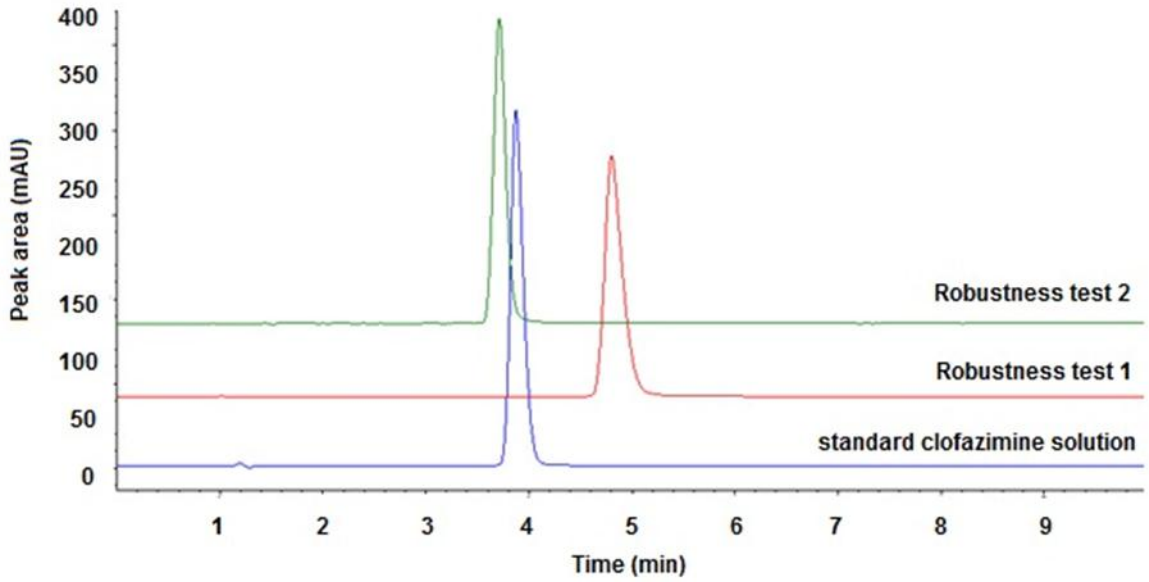

Figure A.6: HPLC chromatographs of clofazimine standard solutions subjected to different injection volumes, flow rates, mobile phase ratios and wavelengths

As illustrated (**Figure A.6**) the results obtained during the robustness analysis displayed approximately similar HPLC chromatographic peak times despite the altered chromatographic conditions. Hence, the outcomes obtained are deemed acceptable.

A.3. Conclusion

The HPLC method verified for clofazimine was found reliable as well as adequately sensitive in determining clofazimine concentrations. **Tables A.1** – **A.7** indicate that the set criteria were met.

References

Alquadeib, B.T. 2018. Development and validation of a new HPLC analytical method for the determination of diclofenac tablets. *Saudi pharmaceutical journal*, 27:66-70.

Aparicio-Ruiz, R., García-González, D.L., Morales, M.T., Lobo-Prieto, A. & Romero, I. 2018. Comparison of two analytical methods validated for the determination of volatile compounds in virgin olive oil: GC-FID vs GC-MS. *Talanta*, 187:133-141.

Araujo, P. 2009. Key aspects of analytical method validation and linearity evaluation. *Journal of chromatography B*, 877:2224-2234.

Aucamp, M.E., Csongradi, C., Gerber, M. & Plessis, J.D. 2016. A novel RP-HPLC method for the detection and quantification of roxithromycin in topical delivery studies. *Die Pharmazie*, 71:175-176.

Belouafa, S., Habti, F., Benhar, S., Belafkih, B., Tayane, S., Hamdouch, S., Bennamara, A. & Abourriche, A. 2017. Statistical tools and approaches to validate analytical methods: methodology and practical examples. *International journal of metrology and quality engineering*, 8:Article ID 9.

BPCC (Biopharmaceutics Coordinating Committee). 2001. Guidance for industry. Bioanalytical method validation. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/bioanalytical-method-validation-guidance-industry. Date of access: 26 Oct. 2019.

De Vito, V., Saba, A., Lee, H., Owen, H., Poapolathep, A. & Giorgi, M. 2016. Detection and quantification of the selective EP4 receptor antagonist CJ-023423 (grapiprant) in canine plasma by HPLC with spectrofluorometric detection. *Journal of pharmaceutical and biomedical analysis*, 118:251-258.

Ermer, J. 2001. Validation in pharmaceutical analysis part I: an integrated approach. *Journal of pharmaceutical and biomedical analysis*, 24:755-767.

Ermer, J. Accuracy. 2005. (*In* Ermer, J. & Miller, J.H., *ed.* Method validation in pharmaceutical analysis: a guide to best practice. Weinheim: Wiley-VCH Verlag GmbH & Co. p.55-63).

Huber, L. 2015. Validation of analytical methods. Agilent Technologies. https://www.fws.gov/aah/PDF/QI-Terms%20and%20Defs.pdf Date of access: 12 Nov. 2018.

ICH ***see*** International Conference Harmonisation.

Indrayanto, G. 2012. Validation of analytical methods- update 2011. *Profiles of drug substances, excipients, and related methodology*, 37:439-463.

International conference on harmonisation of technical requirements for registration of pharmaceuticals for human use. 2005. Validation of analytical procedures: text and methodology Q2 (R1). http://www.gmp-compliance.org/guidemgr/files/Q2(R1).PDF. Date of access: 26 Oct. 2019.

Janse van Rensburg, E. 2016. Formulation and topical delivery of proliposomes containing clofazimine. Potchefstroom: NWU. (Dissertation: MSc).

Joubert, H. 2016. Topical delivery of clofazimine from an avocado oil nano-emulsion. Potchefstroom: NWU. (Dissertation: MSc).

McPolin, O. 2009. Precision. (*In* McPolin, O., *ed*. Analytical methods for pharmaceutical analysis. Newry: Moure Training Services. p. 42-49).

Ratner, B. 2009. The correlation coefficient: Its values range between+ 1/− 1, or do they?. *Journal of targeting, measurement and analysis for marketing*, 17: 139-142.

Ravisankar, P., Navya, C.N., Pravallika, D. & Sri, D.N. 2015. A review on step-by-step analytical method validation. *IOSR journal of pharmacy*, 5:7-19.

Shabir, G.A. 2003. Validation of high-performance liquid chromatography methods for pharmaceutical analysis understanding the differences and similarities between validation requirements of the US Food and Drug Administration, the US Pharmacopeia and International Conference Harmonization. *Journal of chromatography A*, 987:57-66.

Singh, R. 2013. HPLC method development and validation- an overview. *Journal of pharmaceutical education and research*, 4:26-33.

Annexure B

Pre-formulation studies of self-emulsifying drug delivery systems

B.1 Introduction

Pre-formulation studies contribute toward the rational development of an optimised drug delivery system by collecting information utilised to develop a safe, stable and administration route appropriate dosage form (Honmane *et al*., 2017). During pre-formulation studies of topical self-emulsifying drug delivery systems (SEDDSs), solubility tests were conducted (Kazi *et al*., 2017; Prajapat *et al*., 2017; Ghanbari *et al*., 2016; Czaikowska-Kósnik *et al*., 2015). Sufficient solubility of a drug in the oil phase is a crucial factor to consider as decreased solubility can lead to precipitation of the incorporated drug during the shelf-life of the product (Czajkowska-Kósnik *et al*., 2015; Miryala & Kurakula, 2013). Moreover, insufficient solubility can usher precipitation of the drug during the addition of water while formulating SEDDSs (Czaikowska-Kósnik *et al*., 2015). Next, pseudo-ternary phase diagrams were constructed by implementing the water titration method (Prajapat *et al*., 2017). The art of formulating SEDDSs lies in combining excipients in optimum concentrations in order to facilitate self-emulsification (Rani *et al*., 2019). The combination of excipients as well as the concentration of these excipients are considered by pseudo-ternary phase diagrams (Wang *et al*., 2015). Thus, pseudo-ternary phase diagrams were considered an irreplaceable instrument during the pre-formulation stage of this study.

B.2 Solubility studies

Glass centrifuge tubes were employed to solubilise a supersaturated concentration of clofazimine in 5 ml of a plant based oil or water. An excess quantity of clofazimine should be added in order to ensure reliable solubility study results (Ghanbari *et al*., 2016; Czaikowska-Kósnik *et al*., 2015) and the shake flask method was utilised (Kazi *et al*., 2017; Ghanbari *et al*., 2016). The excess quantity of clofazimine needed to establish the supersaturated concentration varied for each of the individual vehicles. Argan-, avocado- and coconut oils necessitated 500 mg clofazimine per 5 ml; water required 300 mg per 5 ml; and the macadamia- and olive oils needed 400 mg clofazimine per 5 ml to obtain a supersaturated solution. Subsequently, each glass centrifuge tube, containing a mixture of clofazimine with one of the various vehicles, was subjected to vortexing for a period of 2 min to create a uniform dispersion (Prajapat *et al*., 2017; Czaikowska-Kósnik *et al*., 2015).

Next, all vortexed samples were placed in a shaking water bath for the duration of 48 h in order to obtain equilibration of the samples (Prajapat *et al*., 2017; Balata *et al*., 2016;

Ke *et al*., 2016). The temperature of the shaking water bath was fixed at 32°C, similar to the temperature at the surface of the skin (Heylings *et al*., 2018). Samples of each vehicle were prepared in triplicate to attain an average solubility concentration value (Prajapat *et al*., 2017; Balakumar *et al*., 2013). After the 48 h period was completed, the samples were removed and centrifuged at 3 000 rpm for 15 min at a temperature of 22°C to remove the excess solid from the equilibrated solutions (Suvarna *et al*., 2017; Ghanbari *et al*., 2016; Czaikowska-Kósnik *et al*., 2015). Only 1 ml of the supernatant was removed from each of the plant based oil samples and diluted with methanol to a volume of 20 ml, prior to HPLC analysis (Prajapat *et al*., 2017). The 1 ml supernatant collected from the samples containing water and clofazimine, was diluted to 20 ml with HPLC grade water; and these samples had to be filtered with a Millipore membrane filter (45µm) prior to HPLC analysis (Annexure A) as clear lumps of clofazimine were visible (Prajapat *et al*., 2017). Thereafter, an average of the obtained clofazimine concentrations were determined as presented in **Table B.1**.

Table B.1: Solubility of clofazimine in the various vehicles

Vehicles	Solubility of clofazimine (µg/ml)
Argan oil	2.230 ± 0.54
Avocado oil	6.292 ± 0.44
Coconut oil	1.781 ± 0.67
Macadamia oil	1.247 ± 0.46
Olive oil	1.090 ± 0.65
Water	0.220 ± 0.88

* Data is presented as mean ± standard deviation

As observed from **Table B.1**, avocado oil displayed the highest clofazimine solubility with an average value of 6.292 mg/ml, whereas clofazimine proofed to be least soluble in water (0.220 mg/ml). The solubility of clofazimine in the selected vehicles can be ranked from highest to lowest in the following order: avocado oil >>>> argan oil > coconut oil > macadamia oil > olive oil >> water. These experiments confirmed the necessity of solubilising clofazimine in a plant based oil as all the oils were able to increase this active ingredient's solubility; even the macadamia- and olive oils that were considered lowest in the ranking order were able to portray solubility values of

more than double that of the aqueous solubility. Results confirmed that clofazimine is a highly lipophilic compound with low aqueous solubility (Sangana *et al*., 2018; Zhang *et al*., 2017).

B.3 Construction of pseudo-ternary phase diagrams

Pseudo-ternary phase diagrams are a valuable instrument when aiming to illustrate the self-dispersability potential of a lipid based formulation, such as SEDDSs (Rani *et al*., 2019). Additionally, pseudo-ternary phase diagrams demonstrate the concentration range of the excipients needed to enable self-emulsification when utilised in combination (Ibrahim *et al*., 2018). In order to construct pseudo-ternary phase diagrams the water-titration method was employed (Rani *et al*., 2019; Czaikowska-Kósnik *et al*., 2015).

The water-titration method can be described as adding distilled water, in a dropwise fashion, to the homogenous mixture of clofazimine, plant based oil, surfactant and co-surfactant while visually observing changes in the clarity of the mixture (Ke *et al*., 2016). During distilled water addition the mixture is gently stirred while positioned on a magnetic stirring plate. The ease at which water penetrates the mixture is indicative of the ease at which emulsification will transpire (Midha *et al*., 2017). Subsequently, the end point of the mixture is achieved when the mixture changes from a clear appearance to a turbid appearance (Rani *et al*., 2019; Patil *et al*., 2013). This indicates the end-point at which the mixture will turn to spontaneous self-emulsification (Rani *et al*., 2019). The point at which permanent turbidity of the mixture is observed is then plotted on the pseudo-ternary phase diagram to indicate the concentration of excipients needed to enable self-emulsification (Nazari-Vanani *et al*., 2018; Patil *et al*., 2013).

To find an appropriate concentration range for all of the components (active ingredient, oil, surfactant, co-surfactant and water) at room temperature (approximately 25°C) in which they spontaneously form emulsions, the surfactant and co-surfactant (i.e. Tween®80: Span®60) were first mixed together. This mixture will be referred to as the “surfactant phase” from hereafter and was fixed at a ratio of 1:1 (Syed & Peh, 2014). Kang *et al* (2004) concluded that a 1:1 ratio forms more stable SEDDSs, whereas higher concentration ratios improved the emulsion range, but a decrease in stability was depicted that could lead to precipitation of the incorporated active ingredient. Following, the surfactant and co-surfactant were stirred and slightly heated on a magnetic stirring plate to ensure homogenous distribution of the co-surfactant in the surfactant. Next, mixtures were prepared for each plant based oil and surfactant phase in ratios of 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2 and 9:1 to enable construction of the pseudo-ternary phase diagrams after the addition of distilled water (Ke *et al*., 2016; Aparna *et al*., 2015; Patil *et al*., 2013). The water phase was titrated into the various mixtures in a dropwise fashion until the first sign of turbidity was noted so as to identify the end-point of

the emulsion range. Once equilibrium of the mixture was achieved the mixtures were visually inspected, by means of polarised lenses, for transparency and for optical isotropicity; and the pseudo-ternary phase diagrams constructed. Each apex of the pseudo-ternary phase diagrams illustrates the concentration of distilled water, oil phase and surfactant phase, respectively (Dash *et al*., 2015). The pseudo-ternary phase diagrams obtained for SEDDSs, formulated with individual plant based oils, are displayed in **Figure B1**.

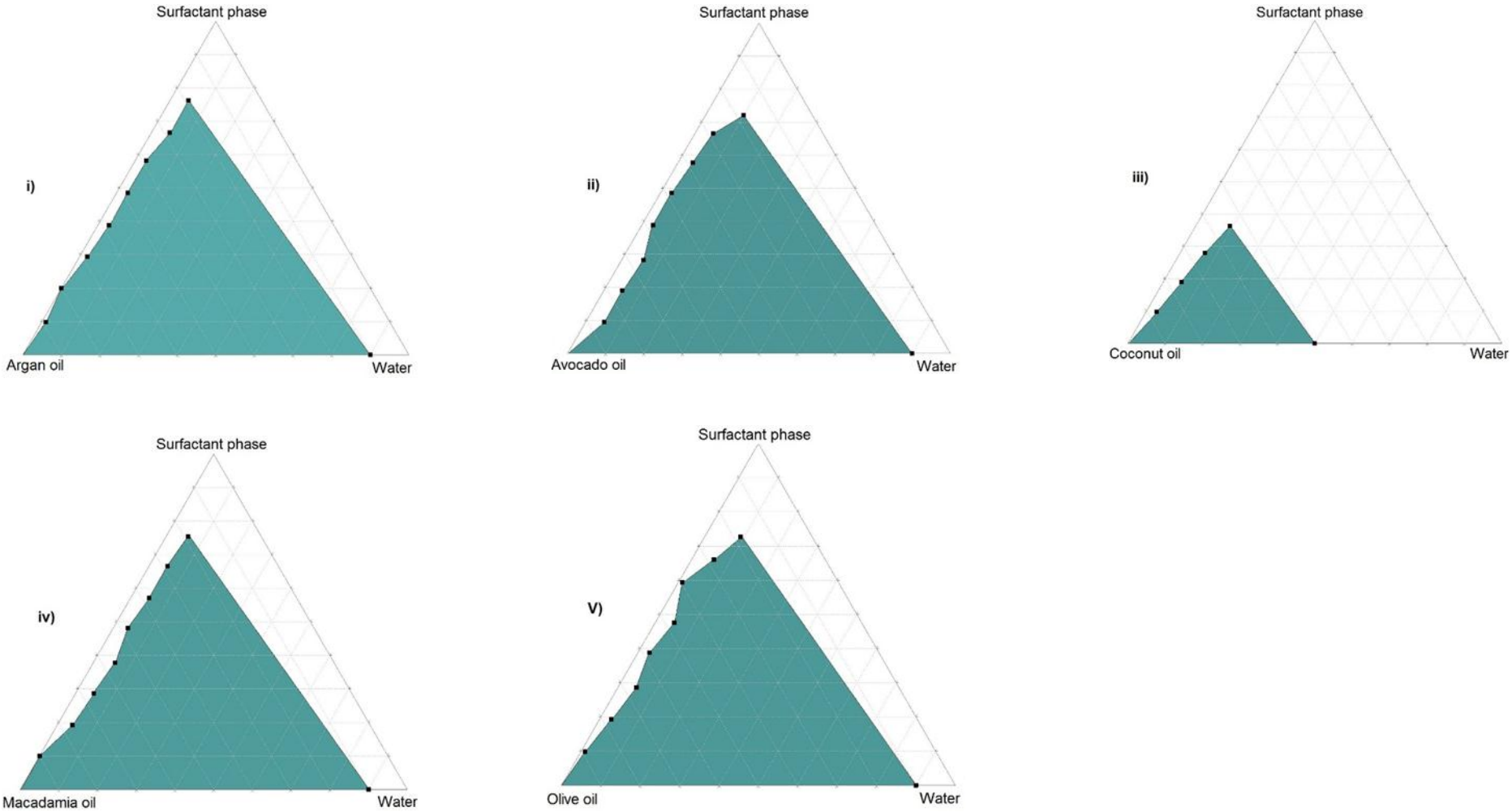

Figure B.1: Pseudo-ternary phase diagram of **(i)** argan oil, clofazimine, surfactant phase and water **(ii)** avocado oil, clofazimine, surfactant phase and water **(iii)** coconut oil, clofazimine, surfactant phase and water **(iv)** macadamia oil, clofazimine, surfactant phase and water and ***(v)*** olive oil, clofazimine, surfactant phase and water

As seen in **Figure B.1**, the plant based oil ranked with the highest self-emulsification potential, is avocado oil. In contrast, coconut oil displayed the smallest self-emulsification area; and the argan-, macadamia-, and olive oils rendered approximately similar self-emulsification areas. Coconut oil has the highest saturated fatty acid composition of the selected plant based oils (Cicero *et al*., 2018). Thus, the elevated saturated fatty acid composition of coconut oil can provide a possible explanation for the unique self-emulsification behaviour of this plant based oil compared to the other plant based oils utilised (Cicero *et al*., 2018; Vaughn *et al*., 2018). Average virgin coconut oil contains 75% medium chain triglycerides and 90% of these triglycerides are saturated (Santos *et al.*, 2014). Due to the high saturated fatty acid composition of coconut oil it will return to a semi-solid state after being left at room temperature (Vaughn *et al*., 2018). Hence, coconut oil might present formulation challenges especially in terms of maintaining a state of self-emulsification.

A possible solution to improve the self-emulsification potential of the SEDDSs containing coconut oil would be to substitute the surfactant or co-surfactant for one with increased phase penetration characteristics in order to increase the distribution of water droplets throughout the SEDDS formulation which may increase the self-emulsification potential of the SEDDSs formulated with coconut oil (Rajeshwar & Shirvastava, 2018; Zhang *et al*., 2015). The role of surfactants and co-surfactants is to adsorb at the interphase of SEDDSs in order to decrease the interfacial energy while in turn reducing the free energy needed to enable the formation of emulsions with increased thermodynamic stability (Czaikowska-Kósnik *et al*., 2015). Moreover, another possible solution to enlarge the self-emulsification area of SEDDSs created with coconut oil is to increase the concentration of surfactant included (Czaikowska-Kósnik *et al*., 2015).

Interestingly, the obtained pseudo-ternary phase diagrams delivered larger self-emulsification areas than anticipated. It is declared that self-emulsification arises if the entropy change in favour of dispersion formation is greater than the energy needed to enhance the surface area of the dispersion (Balata *et al*., 2016). Subsequently, a method of selecting the most acceptable SEDDSs for formulation and further investigation had to be chosen. Authentication of predicting phase behaviour is provided by constructing pseudo-ternary phase diagrams according to different ratios of excipient concentrations when working in different areas of a pseudo-ternary phase diagram (Rani *et al*., 2019; Hegde *et al*., 2013). The predicted phase behaviour exhibited by pseudo-ternary phase diagrams are illustrated in **Figure B.2**.

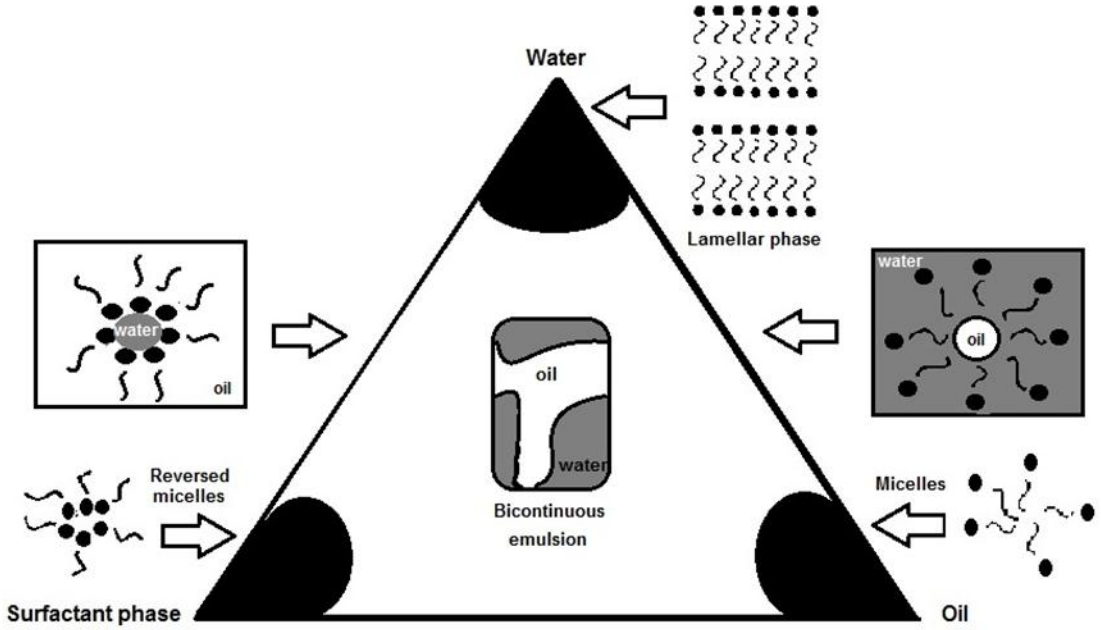

Figure B.2: Hypothetical pseudo-ternary phase diagram utilised to predict phase behaviour of SEDDSs (adapted from Rani *et al.*, 2019; Hegde *et al*., 2013*;* Lawrence & Rees, 2000)

Each section of the pseudo-ternary phase diagram illustrated in **Figure B.2** predicts characteristics of the dispersion according to the concentrations of excipients included in SEDDS formulations (Rani *et al*., 2019). Hence, for the purpose of this study one must bear in mind that the developed SEDDSs are destined for topical delivery and therefore the regions of interest were chosen according to the properties desired to enable successful drug delivery into the epidermis-dermis of the skin.

An increased surfactant concentration can cause skin irritation (Mojeiko *et al*., 2019; Kiselmann *et al*., 2018). Skin irritation induced by surfactants are linked to the ability of these compounds to solubilise lipid membranes (Effendy & Maibach, 1995). In contrast, the disruption of lipids in the *stratum corneum* will enhance drug penetration and in turn improve topical drug delivery (Viljoen *et al*., 2015). Therefore, only SEDDSs containing a surfactant ratio of lower than 5 were considered for further investigation. Likewise, during the formulation of SEDDSs, micelles are unwanted structures as micelles tend to lead to poor dermal drug delivery due to their rigidity and decreased deformability (Abdel-Messih *et al*., 2019; El Zaafanay *et al*., 2010). Hence, areas on the pseudo-ternary phase diagram that illustrate the presence of micelles were avoided during further formulation. Areas known to render micelles are areas of high water content (Hegde *et al*., 2013), whereas oil rich regions on the pseudo-ternary phase diagram will deliver reverse micelles (Hegde *et al*., 2013;). Therefore, it was decided that the ratio of water included in formulations may not exceed a value of 7

during this study. Reverse micelles are an area of interest in drug delivery as reverse micelles have been used as simplified systems to obtain insight into the structure of entrapped water (Lépori *et al*., 2019; Silva *et al*., 2012; Barauch *et al*., 2006). Reverse micelles possess the ability to solubilise both polar and non-polar substances and therefore have numerous applications in chemical as well as enzymatic reactions, the synthesis of nanomaterials and drug delivery systems (Lépori *et al*., 2019; Kogan & Garti, 2006). The focus of this study remains to develop SEDDSs consisting of an oil- in-water emulsion and/or water-in-oil emulsion to enable comparison between SEDDSs as an alternative emulsion formulation method. For this reason, areas exceeding a value of 7 in oil content will be ignored during further formulation of SEDDSs. Thus, in order to eliminate an elevated surfactant concentration, areas that contain micelles or reverse micelles, additional pseudo-ternary phase diagrams were constructed and are displayed in **Figure B.3** for the purpose of illustrating the self-emulsifying regions suitable for further investigation.

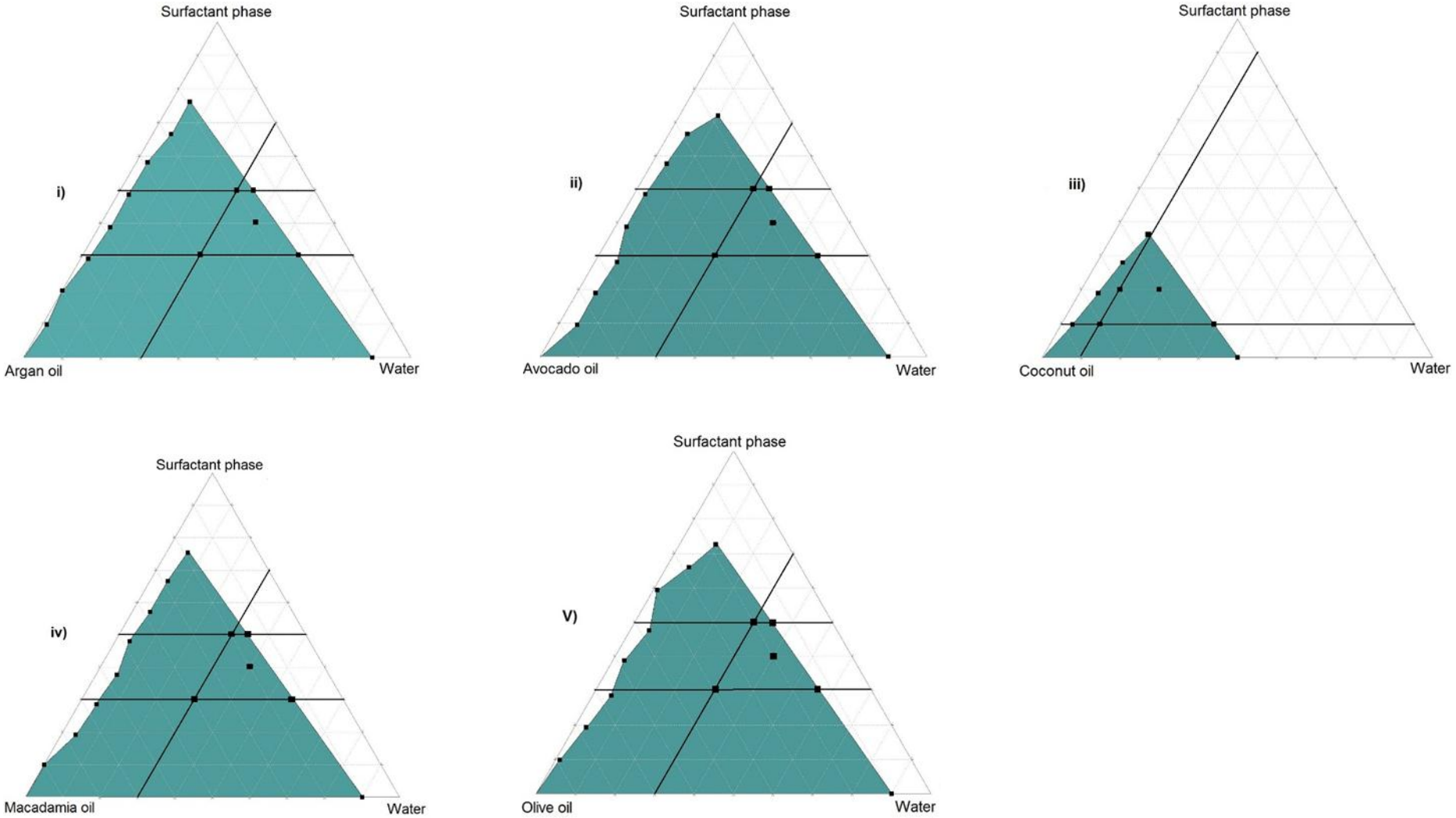

Figure B.3: Pseudo-ternary phase diagram illustrating self-emulsifying region of interest for **(i)** argan oil, clofazimine, surfactant phase and water **(ii)** avocado oil, clofazimine, surfactant phase and water **(iii)** coconut oil, clofazimine, surfactant phase and water **(iv)** macadamia oil, clofazimine, surfactant phase and water and ***(v)*** olive oil, clofazimine, surfactant phase and water

The pseudo-ternary phase diagrams (**Figures B.3**) illustrate the points within the sufficient self-emulsifying area that were chosen for further investigation. Furthermore, five points were selected in the suitable self-emulsification region. Four points at the edges of this region were selected as well as an extra point, located in the centre of the remaining self-emulsification region. These selected points on the pseudo-ternary phase diagrams were formulated and characterised in order to determine which SEDDS formulations were considered most acceptable to perform membrane release studies with. Coconut oil is the only exception to the selection method as the displayed self-emulsification region is too small to eliminate areas in this region in exactly the same manner utilised for the other plant based oils Therefore, coconut oil was handled individually, but as similar as possible to the selection method employed for the previously mentioned plant based oils. The five points (**Figure B.3 iii**) selected on the pseudo-ternary phase diagram of coconut oil was chosen as four points on the edges of the self-emulsification region and one point at the centre of this region.

B.4 Conclusion

The main objective of pre-formulation studies remains to obtain information that will assist during further dosage form development. As the first step during dosage form development, pre-formulation studies provide a framework to consider the safety of the final product. Additionally, the choices made during pre-formulation of topical SEDDSs must correlate with the properties desired to develop a product for optimised topical drug delivery (Honmane *et al*., 2017; Chaurasia, 2016).

References

Abdel-Messih, H.A., Ishak, R.H.H., Geneidi, A.S. & Mansour, S. 2019. Tailoring novel of nano-vesicles 'flexomes' for enhanced transdermal drug delivery: optimisation, characterisation and comprehensive ex vivo-in vivo evaluation. *International journal of pharmaceutics*, 6:101-115.

Aparna, C., Pathima, S. & Rao Patnaik, K.S.K. 2015. Formulation and evaluation of oral self-emulsifying drug delivery system of lornoxicam*. International journal of pharma and bio sciences*, 6:381-395.

Balakumar, K., Raghavan, C.V., Selvan, N.T., Prasad, R.H. & Abdu, P.S. 2013. Self-nano-emulsifying drug delivery system (SNEDDS) of rosuvastatin calsium: design, formulation, bioavailability and pharmacokinetic evaluation. *Colloids and surfaces B: biointerfaces*, 1112:337-343.

Balata, G.F., Essa, E.A., Sharmardl, H.A., Zaidan, S.H. & Abourehab, M.A.S. 2016. Self-emulsifying drug delivery system as a tool to improve solubility and bioavailability of resveratrol. *Drug design, development and therapy*, 10:117-128.

Barauch, B., Roden, J.M., Sedgnick, M., Correa, N.M., Levinger, N.E. & Crans, D.S. 2006. When is water not water? Exploring water confined in large reverse micelles using a highly charged inorganic molecular probe. *Journal of American Chemical Society*, 128:12758-12765.

Chaurasia, G. 2016. A review on pharmaceutical preformulation studies in formulation and development of new drug molecules. *International journal of pharmaceutical sciences and research*, 7: Article ID 2313.

Cicero, N., Albergamo, A., Salvo, A., Bua, G.D., Bartolomeo, G., Mangano, V., Rotondo, A., Di Stefano, V., Di Bella, G. & Dugo, G. 2018. Chemical characterisation of a variety of cold pressed gourmet oils on the Brazilian market. *Food reseach international*, 109:517-525.

Czajkowska-Kośnik, A., Szekalska, M., Amelian, A., Szymańska, E. & Winnicka, K. 2015. Development and evaluation of liquid and solid self-emulsifying drug delivery systems for atorvastatin. *Molecules*, 20:21010-21022.

Dash, P.N., Habibuddin, M., Humaira, T. & Ramesh, D. 2015. Design, optimisation and elevation of glipizide solid self-nanoemulsifying drug delivery for enhanced solubility and dissolution. *Saudi pharmaceutical journal*, 23:528-540.

Effendy, I. & Maibach, H.I. 1995. Surfactants and experimental irritant contact dermatitis. *Contact dermatitis*, 33:217-225.

El Zaafarany, G.M., Awad, G.A., Holayel, S.M. & Mortda, N.D. 2010. Role of edge activators and surface charge in developing ultra-deformable vesicles with enhanced skin delivery. *International journal of pharmaceutics*, 397:164-172.

Ghanbari, A., Sarbaz, Y., Jouyban-Gharamaleki, V., Jouyban-Gharamaleki, K., Soleymani, J. & Jouyban, A. 2016. An improved automated setup for solubility determination of drugs. *Pharmaceutical sciences*, 22:210- 214.

Hegde, R.R., Verma, A. & Ghosh, A. 2013. Microemulsion: new insights into the ocular drug delivery. *ISRN pharmaceutics*, 27:1-11.

Honmane, S.M., Dange, Y.D., Osmani, R.A.M. & Jadge, D.R. 2017. General considerations of design and development of dosage forms: pre-formulation review. *Asian journal of pharmaceutics*, 11(Suppl.):479-488.

Heylings, J.R., Davies, D.J. & Burton, R. 2018. Dermal absorption of testosterone in human and pig skin *in vitro*. *Toxicology in vitro*, 48:71-77.

Ibrahim, T.M., Abdallah, M.H., El-Megrab, N.A. & El-Nahas, H.M. 2018. Upgrading of dissolution and anti-hypertensive effects of carvedilol via two combined approaches: self-emulsification and liquisolid techniques. *Drug development and industrial pharmacy*, 44:873-885.

Kang, B.K., Lee, J.S., Chon, S.K., Jeong, S.Y., Yuk, S.H., Khang, G., Lee, H.B. & Cho, S.H. 2004. Development of self-microemulsifying drug delivery systems (SMEDDS) for oral bioavailibilty enhancement of simvastatin in beagle dogs. *International journal of pharmaceutic*s, 274:65-73.

Kazi, M., Al-Amri, K.A. & Alanazi, F.K. 2017. The role of lipid-based drug delivery systems for enhancing solubility of highly selective antiviral agent acyclovir. *Pharmaceutical development and technology*, 22:312-321.

Ke, Z., Hou, X. & Jia, X.-B. 2016. Design and optimisation of self-nanoemulsifying drug delivery systems for improved bioavailability of cyclovirobuxine D. *Drug design, development and therapy*, 10:2049-2060.

Kiselmann, C., Dobler, D., Schmidts, T., Eicher, A.C., Möbs, C., Pfüntzner, W. & Runkel, F. 2018. Development of a skin-friendly microemulsion for dermal allergen specific immunotherapy. *International journal of pharmaceutics*, 550:463-469.

Kogan, A. & Garti, N. 2006. Microemulsions as transdermal drug delivery vehicles. *Advances in colloid and interface science*, 16:123-126.

Lawrence, M.J. & Rees, G.D. 2000. Micro emulsion-based media as novel drug delivery sytems. *Advanced drug delivery*, 48:89-121.

Lépori, C.M.O., Correa, N.M., Silber, J.J., Chávez, F.V. & Falcone, R.D. 2019. Interfacial properties modulated by the water confinement in reverse micelles created by the ionic liquid-like surfactant bmim-AOT. *Soft matter*, 15:947-955.

Midha, H., Nagpal, M., Singh, G. & Aggarwal, G. 2017. Prospects of solid self-micro-emulsifying systems in novel drug delivery. *Current drug delivery*, 14:1078-1096.

Miryala, V. & Kurakula, M. 2013. Self-nano-emulsifying drug delivery system (SEDDS) for oral delivery of atorvastatin- formulation and bioavailability studies. *Journal of drug delivery and therapeutics*, 3:131-140.

Mojeiko, G., de Brito, M., Salata, G.C. & Lopex, L.B. 2019. Combination of microneedles and micro emulsions to increase celecoxib topical delivery for potential application in chemoprevention of breast cancer. *International journal of pharmaceutics*, 60:365-376.

Nazari-Vanani, R., Azarpira, N. & Henli, H. 2018. Development of self-nanoemulsifying drug delivery systems for oil extracts of Citrus aurantiun. L. blossoms and Rose demascena and evaluation of anticancer properties. *Journal of drug delivery science and technology*, 47:330-336.

Patil, S., Suryavanshi, S., Pathak, S., Sharma, S. & Patravale, V. 2013. Evaluation of novel lipid based formulation of Beta-artemether and lumefantrine in murine malaria model. *International journal of pharmaceutics*, 455:229-234.

Prajapat, M.D., Patel, N.J., Bariya, A., Patel, S.S. & Butani, S.B. 2017. Formulation and evaluation of self-emulsifying drug delivery system for nimodipine, a BCG class II drug. *Journal of drug delivery science and technology*, 39:59-68.

Rajeshwar, V. & Shirvastava, B. 2018. Self-emulsifying drug delivery system (SEDDS): a conventional and alternative approach to improve oral bioavailability. *International journal of pharmaceutical sciences and research*, 9:3114-3127.

Rani, S., Rana, R., Saraogi, G.K., Kumar, V. & Gupta, U. 2019. Self-emulsifying oral lipid drug delivery systems: advances and challenges. *AAPS PharmSciTech*, 20:Article ID 129.

Sangana, R., Gu, H., Chan, D.Y. & Einolf, H.J. 2018. Evaluation of clinical drug interaction potential of clofazimine using static and dynamic modeling approaches. *ASPET journal*, 46:1-34.

Santos, S.S., Lorenzoni, A., Pegoraro, N.S., Denardi, L.B., Alves, S.H., Schaffazick, S.R. & Cruz, C. 2014. Formulation and *in vitro*-evaluation of coconut oil-core cationic nano-capsules intended for vaginal delivery of clotrimazole. *Colloids and surfaces B: biointerfaces*, 116:270-276.

Silva, O.F., Fernández, M.A., Silber, J.J., De Rossi, R.H. & Correa, N.M. 2012. Inhibited phenol ionisation in reverse micelles: confinement effect at the nanometre scale. *ChemPhysChem*, 3:124-130.

Suvarna, V., Pagdhare, U., Kadu, A. & Oza, M. 2017. Development and characterisation of solid self-emulsifying drug delivery system containing nateglinide. *Asian journal of pharmaceutics*, 11:27-36.

Syed, H.K. and Peh, K.K. 2014. Identification of phases of various oil, surfactant/co-surfactants and water system by ternary phase diagram. *Acta poloniae pharmaceutica*, 71:301-309.

Vaughn, A.R., Clark, A.K., Sivamani, R.K. & Shi, V.Y. 2018. Natural oils for skin-barrier repair: Ancient compounds now backed by modern science. *American journal of clinical dermatology*, 19:103-117.

Viljoen, J.M., Cowley, A., du Preez, J., Gerber, M. & du Plessis, J. 2015. Penetration enhancing effects of selected natural oils utilised in topical dosage forms. *Drug development and industrial pharmacy*, 41:2045-2054.

Wang, W., Wei, H., Tai, X. & Wang, G. 2015. Formation and characterization of fully dilutable microemulsion with fatty acid methyl esters as oil phase. *ACS Sustainable chemistry & engineering,* 3:443-450.

Zhang, L., Zhang, I., Zhang, M., Pang, Y., Li, Z., Zhao, A. & Feng, J. 2015. Self-emulsifying drug delivery system and the applications in herbal drugs. *Drug delivery*, 22:475-486.

Zhang, Y., Feng, J., McManus, S.A., Lu, H.D., Ristroph, K.D., Cho, E.J., Dobrijevic, E.L., Chan, H.K., Prud'homme, R.K. 2017 Design and solidification of fast-releasing clofazimine nanoparticles for treatment of cryptosporidosis. *Molecular pharmaceutics*, 14:3480-3488.

Annexure C

Formulation of self-emulsifying drug delivery systems

C.1 Introduction

It has been established that the vehicle utilised to present a drug at the skin surface plays a significant part in the efficacy of dermal drug delivery (Otto *et al*., 2009). Another factor that complicates drug delivery is sufficient solubility of lipophilic drugs in the vehicle utilised to present the drug at the skin surface (Pratiwi *et al*., 2017). SEDDSs provide a useful alternative dosage form as the lipid is inert and the plant based oil, utilised during SEDDSs formulation, can contribute towards enhanced skin penetration by altering lipids, naturally present in the *stratum corneum* (SC), in order to facilitate dermal drug delivery (Viljoen *et al*., 2015). Decreased energy input is required for formulation of SEDDSs when compared to other emulsion formulation techniques (Gonçalves *et al*., 2018).

The decreased energy input needed to deliver SEDDSs can be linked to the mechanism of spontaneous emulsification (Rani *et al*., 2019; Gonçalves *et al*., 2018). Solans *et al* (2016) states that the chemical potential between the aqueous and oil phase might render gradients that precipitate spontaneous emulsification under specified conditions. These specified conditions include regulation of external energy input such as heat application and stirring of formulations (Solans *et al*., 2016; López-Montilla *et al*., 2002). The choice of lipid component contributes toward the phase behaviour of SEDDSs as oils with increased molecular volumes depict a reduced extent of solubilisation when compared to oils of decreased molecular volumes (Zhang *et al*., 2015; Warisnoicharoen *et al*., 2000). Additionally, research indicate that self-emulsification is influenced by the ratio of oil phase to surfactant phase (Eid *et al*., 2014). Hence, during the manufacturing of SEDDSs spontaneous droplet formation is influenced by the inherent properties of the included excipients that can either promote or restrict self-emulsification (Anton & Vandamme, 2011).

C.2 Mechanism of self-emulsification

Droplet formation is achieved during spontaneous emulsification by exposing two immiscible liquids, which are not in equilibrium, without applying any external energy (Solans *et al*., 2016). The mechanism of self-emulsification was discovered by Johannes Gad in 1879; and yet the mechanism is still not completely understood (Rani *et al*., 2019; Gad, 1879). Research conducted with and without the presence of surfactants rendered explanations for various aspects of spontaneous emulsification (Solans *et al*., 2016). Firstly, turbulent flow is known to enhance the rate at which emulsification occurs. However, turbulent

flow can be supressed by including surfactants in formulations, yet self-emulsification definitely ensues within the presence of a surfactant. Secondly, it has been stated that low, or in other words negative interfacial energy, is required to establish spontaneous emulsification of a system. Nonetheless, by experimenting with the inclusion and exclusion of surfactants it was observed that self-emulsification can occur far from the set critical point of low energy, even in surfactant-free systems. Lastly, self-emulsification can be defined as a diffusion driven process as both large droplets, such as micro sized droplets, and small droplets, for instance nano sized droplets, can be created during spontaneous emulsification (Solans *et al*., 2016; López-Montilla *et al*., 2002).

Emulsification can transpire by applying either internal energy (**Figure C.1**), external energy (**Figure C.2**) or both internal and external energy (**Figure C.3**) where a kinetic barrier is formed and stabilised according to the energy source (Solans *et al*., 2016).

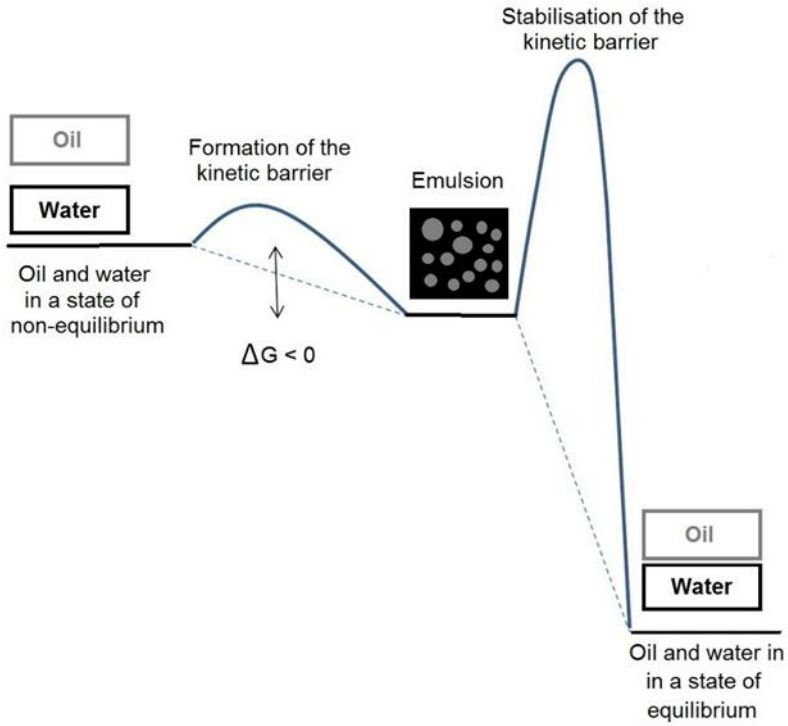

Figure C.1: Emulsification achieved through the application of internal energy (adapted from Solans *et al*., 2016)

Figure C.1 portrays the nature of self-emulsification of two immiscible phases in non-equilibrium. Negative values of free energy are created by variances in the gradient of the chemical potential between the two liquids. This phenomenon is demonstrated by **Equation C.1.**

$\Delta G < 0$ **Equation C.1**

Where ΔG indicates the total energy of emulsion formation.

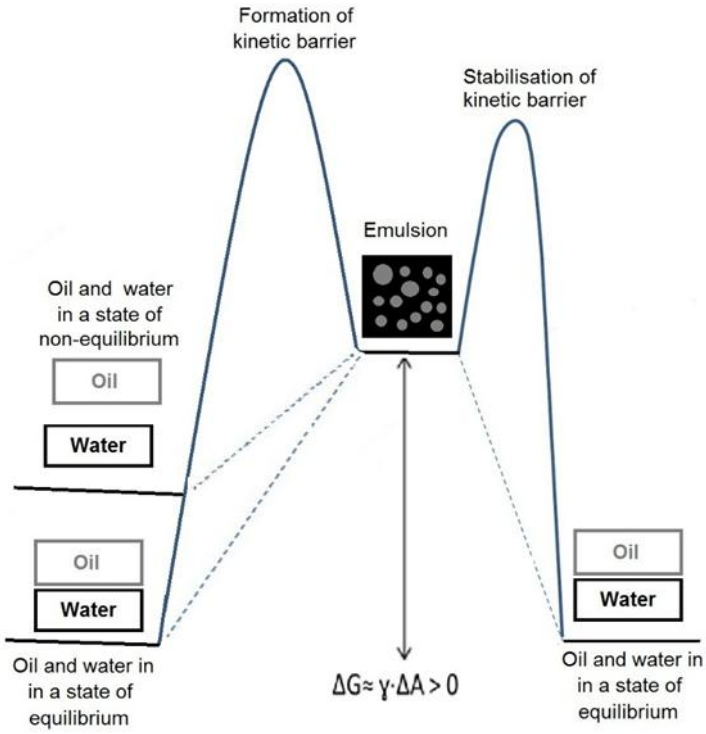

Figure C.2: Emulsification achieved through the application of external energy (adapted from Solans *et al*., 2016)

Figure C.2 demonstrates the need for external energy (i.e. mixing or heating) to establish emulsification. Here the two immiscible phases are in thermodynamic equilibrium and the energy required to enlarge the interface is positive as well as large; and no compensation can be made for the positive together with small entropy of the phases. Hence, more energy is needed to overcome the formation of the kinetic barrier. This observation is displayed by **Equation C.2** and simplified by **Equation C.3**.

$\Delta G = \gamma \Delta A - T \Delta S$ **Equation C.2**

Where ΔGspecifies the total free energy of emulsion formation; γ indicates the interfacial tension; ΔA denotes an increase in the interfacial area; T is the absolute temperature; and ΔS designates an increase in entropy.

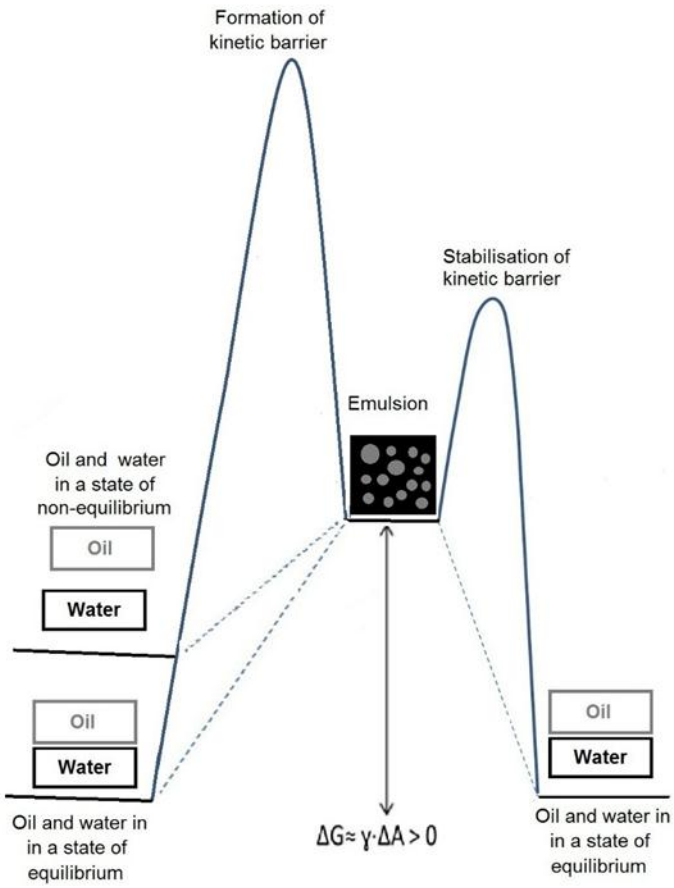

Figure C.3: Emulsification achieved through the application of both internal and external energy (adapted from Solans *et al*., 2016)

$$\Delta G > 0$$ **Equation C.3**

When applying both external and internal energy, a significantly lower external energy input is required to establish emulsification when compared to the energy needed to render emulsification established by only an external source of energy (**Figure C.3**). This is the process most frequently utilised in practice to obtain SEDDSs (Solans *et al*., 2016) as internal energy as well as external energy input are required by some systems that are temperature sensitive. During the formulation of these systems heating is needed to provide phase inversion and spontaneous emulsification occurs during cooling of the formulation. Heating of SEDDS formulations during this study was necessary as it was the only procedure that enabled complete phase inversion of clofazimine SEDDSs. Clear self-emulsification was displayed after removing the formulations from heat.

C.3 Materials

The materials needed to prepare the individual topical SEDDS formulations are portrayed in **Tables C.1–C.5**.

Table C.1: Abbreviations, ratio of excipients and quantity of each excipient needed to formulate 40 ml SEDDSs utilising argan oil

Name of SEDDS	**Ratio of excipients** *(oil: surfactant phase: water)*	**Quantity of excipient**	
ARG 1	**7 : 5 : 5**	Argan oil	16.50 ml
		Tween®80	11.80 ml
		Span®60	11.80 mg
		Water	11.80 ml
ARG 2	**7.4 : 5 : 5**	Argan oil	17.00 ml
		Tween®80	11.50 ml
		Span®60	11.50 mg
		Water	11.50 ml
ARG 3	**6 : 4 : 6**	Argan oil	15.00 ml
		Tween®80	10.00 ml
		Span®60	10.00 mg
		Water	15.00 ml
ARG 4	**7 : 3 : 7**	Argan oil	16.50 ml
		Tween®80	7.10 ml
		Span®60	7.10 mg
		Water	16.50 ml
ARG 5	**4.4 : 3 : 7**	Argan oil	12,20 ml
		Tween®80	8.30 ml
		Span®60	8.30 mg
		Water	19.40 ml

Table C.2: Abbreviations, ratio of excipients and quantity of each excipient needed to formulate 40 ml SEDDSs employing avocado oil

Name of SEDDS	Ratio of excipients *(oil: surfactant phase: water)*	Quantity of excipient	
AVO 1	**7 : 5 : 5**	Avocado oil	16.50 ml
		Tween®80	11.80 ml
		Span®60	11.80 mg
		Water	11.80 ml
AVO 2	**5.6 : 5 : 5**	Avocado oil	14.40 ml
		Tween®80	12.80 ml
		Span®60	12.80 mg
		Water	12.80 ml
AVO 3	**6 : 4 : 6**	Avocado oil	15.00 ml
		Tween®80	10.00 ml
		Span®60	10.00 mg
		Water	15.00 ml
AVO 4	**3.4 : 3 : 7**	Avocado oil	10.10 ml
		Tween®80	9.00 ml
		Span®60	9.00 mg
		Water	20.90 ml
AVO 5	**7 : 3 : 7**	Avocado oil	16.50 ml
		Tween®80	7.10 ml
		Span®60	7.10 mg
		Water	16.50 ml

Table C.3: Abbreviations, ratio of excipients and quantity of each excipient needed to formulate 40 ml SEDDSs including coconut oil

Name of SEDDS	Ratio of excipients *(oil: surfactant phase: water)*	Quantity of excipient	
CCT 1	**9 : 3 : 7**	Coconut oil	18.90 ml
		Tween®80	6.30 ml
		Span®60	6.30 mg
		Water	14.70 ml
CCT 2	**9 : 2 : 8**	Coconut oil	18.90 ml
		Tween®80	4.20 ml
		Span®60	4.20 mg
		Water	16.80 ml
CCT 3	**8 : 2 : 8**	Coconut oil	17.80 ml
		Tween®80	4.40 ml
		Span®60	4.40 mg
		Water	17.80 ml
CCT 4	**9 : 1 : 9**	Coconut oil	18.90 ml
		Tween®80	2.10 ml
		Span®60	2.10 mg
		Water	18.90 ml
CCT 5	**6.3 : 1 : 9**	Coconut oil	15.50 ml
		Tween®80	2.50 ml
		Span®60	2.50 mg
		Water	22.10 ml

Table C.4: Abbreviations, ratio of excipients and quantity of each excipient needed to formulate 40 ml SEDDSs comprising macadamia oil

Name of SEDDS	Ratio of excipients *(oil: surfactant phase: water)*	Quantity of excipient	
MAC 1	**7 : 5 : 5**	Macadamia oil	16.50 ml
		Tween®80	11.80 ml
		Span®60	11.80 mg
		Water	11.80 ml
MAC 2	**6.6 : 5 : 5**	Macadamia oil	15.90 ml
		Tween®80	12.00 ml
		Span®60	12.00 mg
		Water	12.00 ml
MAC 3	**6 : 4 : 6**	Macadamia oil	15.00 ml
		Tween®80	10.00 ml
		Span®60	10.00 mg
		Water	15.00 ml
MAC 4	**7 : 3 : 7**	Macadamia oil	16.50 ml
		Tween®80	7.10 ml
		Span®60	7.10 mg
		Water	16.50 mg
MAC 5	**4.4 : 3 : 7**	Macadamia oil	12.20 ml
		Tween®80	8.30 ml
		Span®60	8.30 mg
		Water	19.40 ml

Table C.5: Abbreviations, ratio of excipients and quantity of each excipient needed to formulate 40 ml SEDDSs containing olive oil

Name of SEDDS	Ratio of excipients *(oil: surfactant phase: water)*	Quantity of excipient	
OLV 1	**7 : 5 : 5**	Olive oil	16.50 ml
		Tween®80	11.80 ml
		Span®60	11.80 mg
		Water	11.80 ml
OLV 2	**6.7 : 5 : 5**	Olive oil	16.00 ml
		Tween®80	12.00 ml
		Span®60	12.00 mg
		Water	12.00 ml
OLV 3	**6 : 4 : 6**	Olive oil	15.00 ml
		Tween®80	10.00 ml
		Span®60	10.00 mg
		Water	15.00 ml
OLV 4	**7 : 3 : 7**	Olive oil	16.50 ml
		Tween®80	7.10 ml
		Span®60	7.10 mg
		Water	16.50 ml
OLV 5	**4.4 : 3 : 7**	Olive oil	12.20 ml
		Tween®80	8.30 ml
		Span®60	8.30 mg
		Water	19.40 ml

C.4 Preparation of topical SEDDSs

First, Tween®80 and Span®60 were magnetically stirred and slightly heated to ensure uniform distribution of surfactant and co-surfactant (Hong *et al*., 2016). Clofazimine was consecutively weighed to formulate argan oil and coconut oil SEDDSs with a concentration of 0.2%; avocado oil SEDDSs with a concentration of 0.6%; as well as macadamia oil and olive oil SEDDSs containing 0.1% clofazimine. Next, the oil phase of each topical SEDDS formulation was accurately measured in established quantities, as illustrated in **Tables C.1-C.5**. Clofazimine

was added to each of the measured oil phases and subjected to sonication for a period of 2 min in order to properly dissolve the active ingredient in the oil phase prior to the addition of the surfactant phase in the established ratios (Aparna *et al*., 2015; Prajapat *et al*., 2017). Subsequently, these mixtures were continuously magnetically stirred for a period of 25 min to ensure uniform clofazimine distribution (Parmar *et al*., 2015). The water titration method was employed by adding small increments of distilled water to the mixture (Aparna *et al*., 2015). Water was slowly incorporated, with waiting periods in between water additions, until the fixed amount of water required by each formulation was completely added. The SEDDSs were left to cool down prior to closing the lids of the glass containers. Thereafter, the topical SEDDS formulations were stored at room temperature (25°C ± 0.5ºC) for the duration of 24 h prior to visual examination (Sanka *et al*., 2016; Parmar *et al*., 2015).

C.5 The day succeeding topical SEDDS formulation

All of the formulated SEDDSs were visually inspected for phase separation or any change in appearance that portrays an unstable emulsion (Sanka *et al*., 2016; Parmar *et al*., 2015). SEDDSs formulated with argan oil delivered two formulations with clear phase separation (i.e. ARG 2 and ARG 4) as visible in **Figure C.4**, whereas only AVO 1 SEDDS that included avocado oil was considered instable. Interestingly, SEDDSs manufactured with avocado oil delivered nearly black formulations. For this reason, it proofed difficult to clearly illustrate phase separation of AVO 1 in **Figure C.5**.

Figure C.4: SEDDSs containing argan oil as the oil phase, where ARG 1 to ARG 5 are from right to left. ARG 2 and ARG 4 are undoubtedly considered instable as phase separation is distinctly visible

Figure C.5: AVO 1 SEDDS comprising avocado oil illustrating phase separation

All coconut oil containing SEDDSs displayed undisputable phase separation as demonstrated in **Figure C.6**. Macadamia oil SEDDSs formulations, on the other hand, only produced one SEDDS that was considered instable due to phase separation (MAC 4) as seen in **Figure C.7**. Lastly, SEDDSs formulated with olive oil rendered one formulation with visible phase separation (i.e. OLV 4) as observed in **Figure C.8**.

Figure C.6: Coconut oil SEDDSs (CCT 1–5) displaying instability due to phase separation

Figure C.7: Macadamia oil SEDDSs (MAC 5–1) where phase separation is clearly observed with only MAC 4 SEDDS, indicating instability. The other MAC SEDDSs are considered stable

Figure C.8: SEDDSs containing olive oil as the oil phase, where OLV 1 to OLV 5 are from right to left. Only OLV 4 SEDDS depicted phase separation

Due to phase separation obtained with the ARG 4, CCT 4, MAC 4 and OLV 4 SEDDSs, these formulations were not considered for any further characterisation and evaluation experiments. All four of these SEDDS formulations were manufactured in a 7: 3: 7 ratio (i.e. oil phase: surfactant phase: water) where both the oil and water phases were included in equal quantities. Thus, a possible explanation may be provided for the instability of these SEDDSs.

Stable emulsions normally consist of two immiscible phases that are in a state of equilibrium (Gupta *et al*., 1999). Coalescence of droplets in emulsions are driven by interfacial tension. Hence, surfactants must be included to establish stable emulsion formation by decreasing interfacial tension between the two immiscible phases (Gupta *et al*., 1999). Two mechanisms are utilised to establish decreased interfacial tension, namely: electrical double layer formation (stabilisation is achieved by charge on droplets) and the formation of protective layers (steric stabilisation on droplets) (Gupta *et al*., 1999). An ionic surfactant achieves stabilisation by inducing a charge by means of adsorption of ions or polyelectrolytes at the surface of droplets. This mechanism of emulsion stabilisation facilitates flocculation prevention of similarly charged droplets. Additionally, ionic surfactants also retard drainage of the liquid film due to the electro viscous effect (Bhamla *et al*., 2017; Gupta *et al*., 1999). The electro viscous effect refers to the electrostatic attraction between flowing films as well as liquid interfaces (Gupta *et al*., 1999). In contrast, non-ionic surfactants employ steric stabilisation of droplets by preventing mass transport from crossing interfaces by creating a protective layer on the surface of droplets. Therefore, non-ionic surfactants, which were utilised in this study, are known to be less efficacious when compared to ionic surfactants (Amiri-Rigi & Abbasi, 2016; Gupta *et al*., 1999). Substitution of a non-ionic surfactant such as Tween®80 for an ionic surfactant can consequently improve emulsion stabilisation (Gupta *et al*., 1999). However, one must bear in mind that ionic surfactants are more prone to dermal irritation (Kiselmann *et al*., 2018; Kaur & Mehta, 2017; Otto *et al*., 2009). For this reason, it was opted in the study not to investigate varying the type of surfactants, but rather to further explore the

viable SEDDSs procured during formulation as the non-ionic surfactants will circumvent possible skin discomfort.

The role fulfilled by the co-surfactant is to increase flexibility of the interfacial film to further promote penetration of the dispersed phase into the continuous phase (Guram *et al.*, 2015). Thus, the addition of co-surfactants further improves the emulsification capacity of surfactants by reducing interfacial tension (Kamal & Nazzal, 2018). Tubtimsri *et al*., (2014) investigated the most favourable hydrophilic-lipophilic balance (HLB) value for modified coconut oil. Various ratios of surface active agents, Span®80 and Tween®80, rendered HLB values ranging from 4.3 to 15. A HLB value of 12 was deemed most stable in terms of reduced size distribution as well as decreased droplet size. The combination of Tween®80 and Span®80 was utilised to obtain an HLB value of 12. Hence, Span®80 can be substituted for Span®60 in order to possibly improve the stability of coconut oil SEDDSs.

As stated beforehand, ARG 2 and AVO 1 topical SEDDS formulations also demonstrated instability. The SEDDSs component ratios of these SEDDSs are approximately similar; i.e. the ratios utilised to formulate ARG 2 and AVO 1 are: 7.4: 5: 5 and 7: 5: 5 (oil:surfactant:water phases), respectively. In these cases, the oil phase is included in the highest concentration ratio, whereas one can reason that the surfactant phase concentration is equal to the amount of water included. Thus, a notably high surfactant content was included into these SEDDS formulations. Moreover, the possibility of bicontinuous emulsion formation is enhanced by an increased surfactant concentration (Marquez *et al*., 2019). If one pays attention to the hypothetical pseudo-ternary phase diagram (**Figure B.2**), displayed in Annexure B, the bicontinuous emulsion region is located at approximately the same position where formulations ARG 2 and AVO 1 were chosen to be formulated at (Rani *et al*., 2019; Hegde *et al*., 2013).

Bicontinuous emulsions can be defined as particle-stabilised emulsions consisting of two continuous phases with sustained inter-penetration between the phases (Cai *et al*. 2017). According to Amiri and Abbasi (2016) non-ionic surfactants are almost without exception too weak to sustain decreased interfacial flexibility by themselves. Hence, the choice of co-surfactant is vital when utilising a non-ionic surfactant such as Tween®80 (Amiri & Abbasi, 2016). Marquez *et al*., (2019) mentioned the possibility of having all the surfactant trapped in the middle-phase of the bicontinuous emulsion, which in turn could explain the instability of these emulsions. The consequence of having surfactant trapped in the middle-phase of a bicontinuous emulsion is that the emulsion is behaving as if no surfactant is available to stabilise either oil or water droplets. Therefore, phase separation occurs as no surfactant is available to decrease interfacial tension (Marquez *et al*., 2019).

C.6 Conclusion

It was indeed possible to formulate at least 15 different topical SEDDS formulations comprising different oil: surfactant: water phases. These formulations did not display any visual phase separation after a period of 24 h. Thus, these 15 topical SEDDS formulations will be subjected to characterisation and evaluation experiments in order to establish which SEDDS formulation(S) is/are deemed most suitable for topical application.

References

Amiri-Rigi, A. & Abbasi, S. 2016. Microemulsion-based lycopene extraction: effects of surfactants, co-surfactants and pretreatments. *Food chemistry*, 197:1002-1007.

Anton, N. & Vandamme, T.F. 2011. Nano-emulsions and micro-emulsions: clarifications of the critical differences. *Pharmaceutical research*, 28:978-985.

Aparna, C., Pathima, S. & Rao Patnaik, K.S.K. 2015. Formulation and evaluation of oral self-emulsifying drug delivery system of lornoxicam*. International journal of pharma and bio sciences*, 6:381-395.

Bhamla, M.S., Chai, C., Álvarez-Vaenzuela, A., Tajuelo, J. & Fuller, G.G. 2017. Interfacial mechanis,s for stability of surfactant-laden films. *PLoS ONE*, 12:1-14.

Cai, D., Clegg, P.S., Li, T., Rumble, K.A. & Tavacoli, J.W. 2017. Bijels formed by direct mixing. *Soft matter*, 13:4824-4829.

Eid, A.M., El-Enshasy, H.A., Aziz, R. & Elmarzugi, N.A. 2014. The preparation and evaluation of self-nanoemulsifying systems containing Swietenia oil and an examination of its anti-inflammatory effects. *International journal of nanomedicine*, *9*:Article ID 4685.

Gad, J. 1879. Zur Lehre von der Blutrache*. Archives of anatomy and physiology*, 1:181-192.

Gupta, P., Mauri, R. & Shinnar, R. 1999. Phase separation of liquid mixtures in the presence of surfactants. *Industrial and engineering chemistry research*, 38:2418-2424.

Guram, A.K., Deshpande, P.B., Kar, S.S., Nayak, U.Y., Udupa, N. & Reddy, M.S. 2015. Role of components in formulation of self-microemulsifying drug delivery systems. *Indian journal of pharmaceutical sciences*, 77:249-257.

Gonçalves, A., Nikmaram, N., Roohinejad, S., Estevinho, B.N., Rocha, F., Greiner, R. & McClements, D.J. 2018. Production, properties and applications of solid self-emulsifying delivery systems (S-EDDS) in the food and pharmaceutical industries. *Colloids and surfaces A*, 538:108-126.

Hegde, R.R., Verma, A. & Ghosh, A. 2013. Microemulsion: new insights into the ocular drug delivery. *ISRN pharmaceutics*, 27:1-11.

Hong, E.-P., Kim, J.-Y., Kim, S.-H., Hwang, K.-M., Park, C.-W., Lee, H.-J., Kim, D.-W., Weon, K.-Y., Jeong, S.Y. & Park, E.-S. 2016. Formulation and evaluation of a self-microemulsifying drug delivery system containing bortezomib. *Chemical and pharmaceutical bulletin*, 64:1108-1117.

Kamal, M.M. & Nazzal, S. 2018. Development of a new class of sulforaphane-enabled self-emulsifying drug delivery system (SFN-SEDDS) by high throughput screening: a case study with curcumin. *International journal of pharmaceutics*, 539:147-156.

Kaur, G. & Mehta, S.K. 2017. Development of polysorbate (tween) based microemulsions: preclinical drug delivery, toxicity and anti-microbial applications. *International journal of pharmaceutics*, 29:134-160.

Kiselmann, C., Dobler, D., Schmidts, T., Eicher, A.C., Möbs, C., Pfüntzner, W. & Runkel, F. 2018. Development of a skin-friendly microemulsion for dermal allergen specific immunotherapy. International journal of pharmaceutics, 550:463-469.

López-Montilla, J.C., Herrera-Morales, P.E., Pandey, S. & Shah, D.O. 2002. Spontaneous emulsification: mechanisms, physicochemical aspects, modeling, and applications. *Journal of dispersion science and technology*, 23:219-268.

Marquez, R., Antón, R., Vejar, F., Salager, J.-L. & Forgiarini, A.M. 2019. New interfacial rheology characteristics measured using a spinning drop rheometer at the optimum formulation. Part 2. Surfactant-oil-water systems with a high volume of middle-phase microemulsion. *Jounal of surfactants and detergents*, 22:177-188.

Otto, A., du Plessis, J. & Wiechers, J.W. 2009. Formulation effects of topical emulsions on transdermal and dermal delivery. *International journal of cosmetic science*, 31:1-19.

Parmar, K., Patel, J. & Sheth, N. 2015. Selfnano-emulsifying drug delivery system for embelin: design, characterisation and in-vitro studies. *Asian journal of pharmaceutical sciences*, 10:396-404.

Prajapat, M.D., Patel, N.J., Bariya, A., Patel, S.S. & Butani, S.B. 2017. Formulation and evaluation of self-emulsifying drug delivery system for nimodipine, a BCG class II drug. *Journal of drug delivery science and technology*, 39:59-68.

Pratiwi, L., Fudholi, A., Martien, R. & Pramono, S. 2017. Self-nanoemulsifying drug delivery system (snedds) for topical delivery of mangosteen peels (Garcinia Mangostana L.,): formulation design and in-vitro studies. *Journal of young pharmacists*, 9:341-346.

Rani, S., Rana, R., Saraogi, G.K., Kumar, V. & Gupta, U. 2019. Self-emulsifying oral lipid drug delivery systems: advances and challenges. *AAPS PharmSciTech*, 20:Article ID 129.

Sanka, K., Suda, D. & Bakshi, V. 2016. Optimisation of solid-selfnanoemulsifying drug delivery system for solubility and release profile of clonazepam using simple lattice design. *Journal of drug delivery science and technology*, 33:114-124.

Solans, C., Morales, D. & Homs, M. 2016. Spontaneous emulsification. *Current opinion in colloid and interface science*, 22:88-93.

Tubtimsri, S., Limmatvapirat, C., Sriamornsak, P. & Limmatvapirat, S. 2014. Determination of required hydrophile-lipophile balance value of modified coconut oil. *Advanced materials research*, 1060:172-175.

Viljoen, J.M., Cowley, A., du Preez, J., Gerber, M. & du Plessis, J. 2015. Penetration enhancing effects of selected natural oils utilised in topical dosage forms. *Drug development and industrial pharmacy*, 41:2045-2054.

Warisnoicharoen, W., Lansley, A.B. & Lawrence, M.J. 2000. Nonionic oil-in-water microemulsions: the effect of oil type on phase behaviour. *International journal of pharmaceutics*, 198:7-27.

Zhang, L., Zhang, I., Zhang, M., Pang, Y., Li, Z., Zhao, A. & Feng, J. 2015. Self-emulsifying drug delivery system and the applications in herbal drugs. *Drug delivery*, 22:475-486.

Annexure D

Characterisation of topical self-emulsifying drug delivery systems

D.1 Introduction

Factors that significantly contribute toward optimised topical drug delivery are: physicochemical properties of the incorporated drug, physiology of the site of application as well as the chosen drug delivery system (Roohnikan *et al*., 2019; Shu *et al*., 2018; Kathe & Kathpalia, 2017). Due to the physicochemical properties of clofazimine rendering topical drug delivery challenges (Sangana *et al*., 2018; Marwah *et al*., 2016; Bolla & Nangia, 2012; Naik *et al*., 2000; Peters *et al*., 2000), one should also consider the unique physiological characteristics of the skin and the importance of the type of the drug delivery system in order to formulate an optimum dosage form. This can be achieved through examining which properties or behaviour exhibited by this system will contribute toward optimised topical drug delivery of the highly lipophilic clofazimine (Riaz *et al*., 2019; Roohnikan *et al*., 2019; Sangana *et al*., 2018; Shu *et al*., 2018; Kathe & Kathpalia, 2017). Hence, characterisation of the 15 different topical SEDDS formulations containing different oil: surfactant: water phases, that did not exhibit phase separation after 24 h when visually inspected, was conducted. Characterisation experiments included determination of the droplet size; measurement of zeta-potential; size distribution; robustness of SEDDSs to dilution; evaluation of self-emulsification capacity; time required to enable self-emulsification; viscosity measurement; cloud point assessment; thermodynamic stability studies; and measurement of pH values. The obtained results are displayed and discussed in Annexure D in order to eliminate topical SEDDS formulations that displayed unfavourable physical properties prior to conducting membrane release and dermal diffusion studies.

D.2 Characterisation of topical SEDDS formulations

D.2.1 Droplet size, size distribution and zeta-potential

Performance of SEDDSs are substantially influenced by droplet size, droplet size distribution and zeta-potential (Ujilestari *et al*., 2018; Balata et al., 2016; Mahapatra *et al*., 2014). Ujilestari *et al* (2018) described size characterisation as one of the most significant assessments performed during the development of SEDDSs as droplet size predicts not only drug release from the SEDDSs, but also the stability of the formulations (Zaichik *et al*., 2018). Moreover, multiple studies have concluded that a decreased droplet size notably enhanced dermal drug delivery (Hardiningtyas *et al*., 2019; Kaur & Ajitha, 2019; Zhou *et al*., 2018; Su *et al*., 2017; Lovelyn & Attama, 2011). Kaur and Ajitha (2019) concluded that a decreased

droplet size facilitates faster, as well as more significant drug permeation. However, one must bear in mind that an increased surfactant concentration together with a decreased droplet size are associated due to reduced interfacial tension, facilitated by the surface active agents, that enable finer droplet formation (Ali & Hussein, 2017; Akula *et al*., 2014; Otto *et al*., 2009). Additionally, surface active agents are capable of SC lipid disruption; and increased concentrations of these compounds will render decreased droplet sizes as well as penetration enhancement, followed by elevated dermal drug delivery (Abdel-Messih *et al*., 2019; Otto *et al*., 2009).

Droplet size, droplet size distribution and zeta-potential of topical SEDDSs were thus investigated through employing dynamic light scattering performed by a Zetasizer Nano® ZS (Malvern® Instruments Ltd., Worcestershire, United Kingdom) at a maintained temperature of 25°C. The results obtained for droplet size are illustrated in **Figure D.1.**

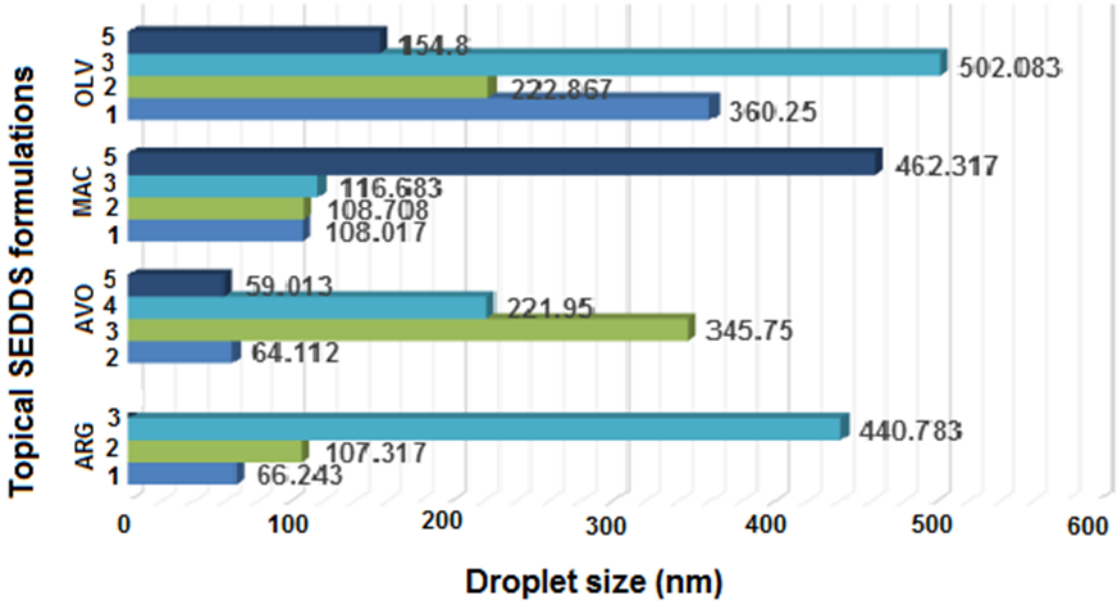

Figure D.1: Droplet size displayed by topical SEDDS formulations

As exhibited in **Figure D.1**, most of the topical SEDDSs are classified within the micro-sized range, i.e., 100–250 nm (Rani *et al*., 2019). However, some SEDDS formulations displayed larger droplet sizes that exceed the micro-sized range (Rani *et al*., 2019). Hence, formulations ARG 5, AVO 3, MAC 5, OLV 1 and OLV 3 were not considered for membrane release, and dermal diffusion studies as these SEDDS formulations neither belong to the nano- nor micro-sized range (Rani *et al*., 2019). Contradictory, formulations ARG 1, AVO 2 and AVO 5 exclusively fall within the nano-sized range (i.e. <100 nm). These nano-sized SEDDS formulations are favoured as decreased droplet size renders an increased surface area available for absorption; and it furthermore contributes towards lymphatic uptake

(Syukri *et al*., 2018; Dokania & Joshi, 2015). Dokania and Joshi (2015) identified the range for subcutaneously injected particles suitable for lymphatic uptake as between 80–100 nm. Interestingly, AVO 5 is classified as a nano-sized formulation even though this formulation did not contain an increased surfactant concentration as exhibited in Annexure C, **Table C.2**. Smaller droplets are known to contribute toward increased emulsion stability because smaller droplets are less prone to droplet cohesion and subsequent phase separation (Ishii & Nill, 2014).

In order to confirm the predicted stability of the SEDDS formulations, measuring the zeta-potential is frequently employed (Liang *et al*., 2017). Zeta-potential identifies the charge observed on droplet surfaces within the dispersion medium (Kaur & Ajitha, 2019). The measured value is indicative of the extent of electrostatic repulsion that exists between droplets within the dispersion (Gupta & Trivedi, 2018). These repulsion forces contemplate the ease with which small droplets tend to coagulate in order to form larger droplets (Gupta & Trivedi, 2018). For these reasons, increased zeta-potential values portray elevated electrostatic repulsion instead of attraction between droplets, and therefore, increased stability of SEDDS formulations (Ding *et al*., 2018). Literature has established that the ideal zeta-potential value is >30 mV or <-30 mV for stable emulsions (Sharma *et al*., 2014). However, a small deviation is allowed as research concluded that emulsions subjected to both steric and combined electrostatic stabilisation, as facilitated by Tween®80, with a minimum zeta-potential value of -20 mV, can be considered acceptable (Gupta & Trivedi, 2018; Zafeiri *et al*., 2017). Therefore, the software of the Zetasizer Nano ZS Malvern Instruments (Worcestershire, United Kingdom) specify that all SEDDS formulations with a zeta-potential of <-20 mV could be considered stable formulations. These zeta-potential results achieved for the selected SEDDS formulations are depicted in **Figure D.2**.

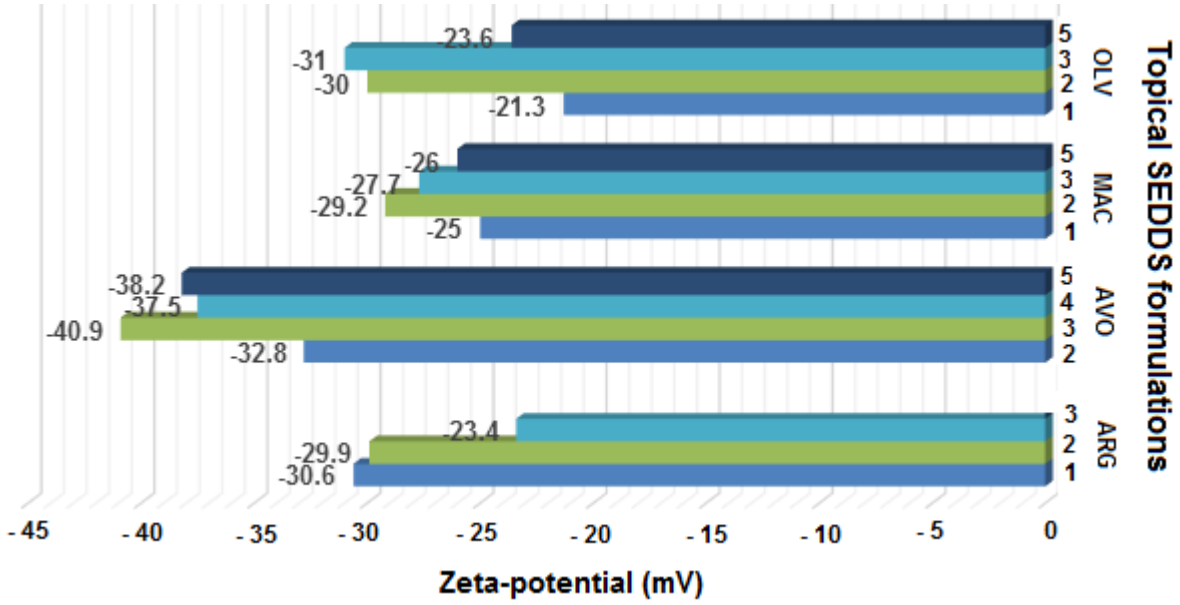

Figure D.2: Zeta-potential measured for SEDDS formulations

As seen in **Figure D.2** all SEDDSs formulations complied with the criteria set for zeta-potential. However, avocado oil SEDDSs displayed notably higher zeta-potential values compared to SEDDS formulations containing argan-, macadamia- and olive oil. As stated, zeta-potential values lower than -30 mV are known to render highly stable formulations (Krstić *et al*., 2018). Therefore, SEDDS formulations including avocado oil could be considered the most stable formulations in this study.

What is more, Gumustas *et al* (2017) stated that pH has a definite influence on zeta-potential. As seen in **Section 2.8** of Annexure D avocado oil SEDDSs showed remarkably decreased pH values compared to other SEDDS formulations. Moreover, zeta-potential is not only indicative of formulation stability, but it also portrays the possible extent of drug permeation (Carter *et al*., 2019; Gumustas *et al*., 2017). Considering the fact that skin surface has a net negative charge, it is conceivable that positively charged formulations might exhibit increased diffusivity (Carter *et al*., 2019). This increased diffusivity can be linked to increased affinity between the skin surface and the applied formulation (Gumustas *et al*., 2017). However, without exception, all SEDDS formulations produced in this study are negatively charged due to the presence of free fatty acids as provided by the inclusion of plant based oils (Umerska *et al*., 2016). Nonetheless, plant based oils are included to enhance dermal drug delivery due to skin penetration enhancement effects (Lin *et al*., 2018; Vaughn *et al*., 2018; Viljoen *et al*., 2015). The penetration enhancement effects established by plant based oils are due to mechanisms such as lipid disruption of the SC and lipid fluidisation, that facilitate reversible changes to the SC and in turn increase drug permeation into the skin (Lane, 2013).

Therefore, the negatively charged SEDDSs can still possibly establish topical drug delivery; probably just in a slower fashion than a positively charged formulation (Carter *et al.*, 2019).

A uniform size distribution of droplets within dispersions is likewise an indication of the stability of the said formulations and is determined by utilising the polydispersity index (PDI) (Kaur & Ajitha, 2019). A PDI value of 0.0 is deliberated a perfectly homogenous sample, whereas a PDI value of 1.0 is deemed a sample of high polydispersity (Danaei *et al*., 2018). However, PDI values ranging from 0.05–0.7 are considered acceptable in the pharmaceutical industry (Danaei *et al*., 2018). However, no fixed PDI criteria are recognised for dermal drug delivery with the exceptions of lipid based carrier systems (PDI ≤ 0.3) and polymer-based nanoparticles (PDI ≤ 0.2) destined for transdermal drug delivery (Danaei *et al*., 2018). Moreover, a PDI value exceeding 0.7 does not necessarily portray unpredictable drug release (Danaei *et al*., 2018). Additionally, a PDI value of 0.7 can indicate that the dynamic light scattering technique is not suitable for analysis of this sample and employment of microscopic observations can prove beneficial as this technique does not take morphology and individual shapes of particles into consideration (Danaei *et al*., 2018). Therefore, a highly polydispersed sample can be considered unsuitable although small particles are present in oval or cylindrical shapes; these particles are only significantly closely packed together, and can thus be perceived as one large particle (Danaei *et al*., 2018). The only clear fact remains that a PDI value nearing 1.0 indicates unpredictable dermal drug delivery as divergent droplet size populations are present within the applied formulation and controlled drug delivery cannot be guaranteed (Danaei *et al*., 2018). Thus, it was decided to consider all SEDDS formulations, except those that obtained a PDI value of 1.0. The PDI values acquired for the various SEDDS formulations are exhibited in **Figure D.3**.

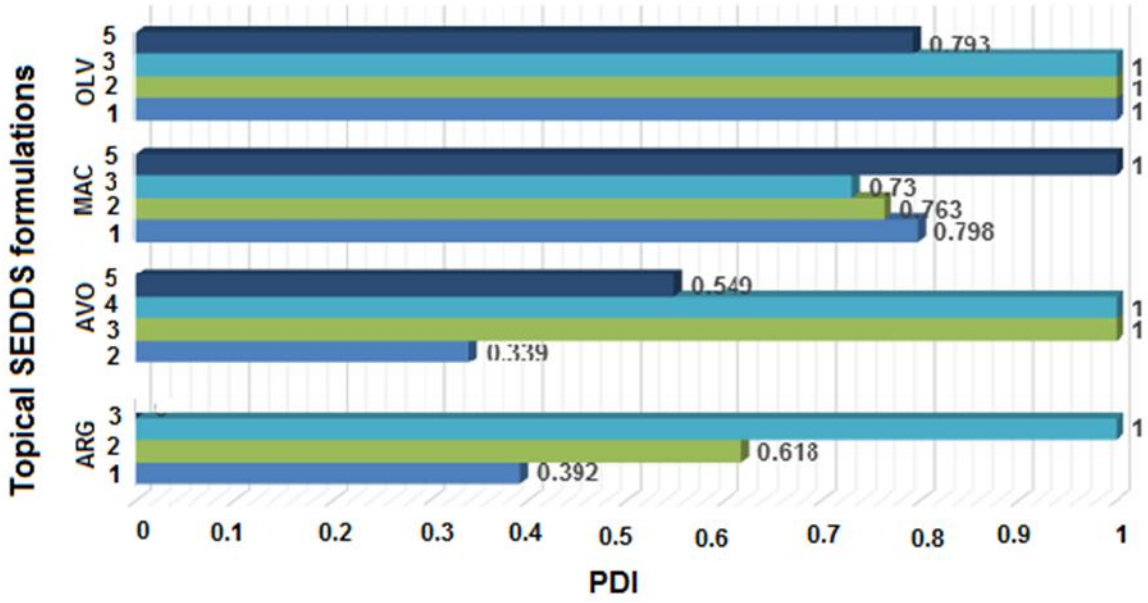

Figure D.3: Polydispersity index values obtained for SEDDS formulations

As observed in **Figure D.3** formulations ARG 5, AVO 4, AVO 5, MAC 5, OLV 1, OLV 2 and OLV 3 rendered a PDI value of 1.0 and were consequently deemed unsuitable for further investigation.

D.2.2 Robustness to dilution

Robustness to dilution displays the ability of SEDDSs to withstand drug precipitation and phase separation upon dilution (Nasr *et al*., 2016). If SEDDSs are considered in terms of oral drug delivery, this test is of importance as these formulations will be exposed to gastric fluids and therefore dilution (Dokania & Joshi, 2015). However, during dermal drug delivery the focus will shift towards robustness to pH upon mild dilution as the SEDDS formulations will not be exposed to similar amounts of fluids during dermal drug delivery; even more so, if the aim is to achieve topical drug delivery instead of utilising the transdermal route to achieve systemic drug delivery. Nonetheless, topically delivered formulations might be exposed to sweat and pH changes throughout skin layers (Sheshala *et al*., 2019; Marghetts & Sawyer, 2007). The pH at the surface of the SC ranges from 4.5 to 5.0, but approaches a neutral pH value in the final fractions of the SC, just before reaching the underlying epidermis (Sheshala *et al*., 2019). Therefore, it was decided to determine the robustness of the topical SEDDSs upon dilution in fluids of various pH values. The dilution fluids of choice were: distilled water, phosphate buffer solution (PBS) with an adjusted pH of 5, to resemble the pH of the skin surface, and PBS with an altered pH value of 7.4 as to mimic the pH value just before entering the epidermis. The SEDDSs were diluted 100 fold within the aforementioned fluids and these diluted SEDDS formulations were retained at an ambient

temperature of 25°C for a period of 24h prior to visual examination (Aparna *et al*., 2015; Czajkowska-Kośnik *et al*., 2015). The visual observations are displayed in **Table D.1**.

Table D.1: Phase separation displayed by SEDDSs after a period of 24 h at a temperature of 25°C

SEDDS formulation	Dilution with distilled water (pH of 5.8)	Dilution with PBS (pH of 5)	Dilution with PBS (pH of 7.4)
ARG 1	✓	✓	✓
ARG 3	×	✓	✓
ARG 5	×	×	×
AVO 2	×	✓	✓
AVO 3	✓	✓	✓
AVO 4	✓	×	✓
AVO 5	×	×	×
MAC 1	×	✓	✓
MAC 2	✓	✓	✓
MAC 3	✓	×	×
MAC 5	✓	×	×
OLV 1	×	✓	✓
OLV 2	×	×	×
OLV 3	×	×	×
OLV 5	✓	✓	✓

Data is presented as passed (✓) or failed (×)

As demonstrated by **Table D.1**, formulations ARG 5, AVO 5, OLV 2 and OLV 3 displayed complete phase separation after the 24 h storage period. Therefore, these topical SEDDS formulations could not be considered for membrane release and dermal diffusion studies as they are clearly sensitive towards dilution within different pH environments. In contrast, formulations ARG 1, AVO 3, MAC 2 and OLV 5 exhibited perfect robustness toward both dilution and change in pH. Additionally, formulations such as ARG 3, AVO 2, MAC 1 and OLV 1 showed robustness toward variable pH environments, but failed to withstand phase separation when diluted with distilled water.

The most noteworthy fluid these topical SEDDS formulations will have contact with is sweat (Margetts & Sawyer, 2007). Promphet *et al* (2019) described sweat content as water containing decreased quantities of electrolytes, metabolites, small molecules and proteins with a pH range of 4.0–6.5. The fact that formulations ARG 3, AVO 2, MAC 1 and OLV 1 did not display any phase separation after dilution with PBS having a lower pH of 5; or PBS with a pH of 7.4, they could still be regarded for membrane release and dermal diffusion studies. This decision is based on the understanding that these formulations revealed robustness toward pH ranges that comply with the set pH range for sweat; and that the human sweat rate is not evenly distributed over the whole skin surface (Promphet *et al*., 2019; Patterson *et al*., 2000). Salvo *et al* (2018) reported that the average sweat rate of a healthy individual is approximately 500–700 ml daily as established over the entire surface of the body. If the daily average sweat rate value is compared to the sweat rate over a period of one hour, the average sweat rate is in the region of 20.333–29.167 ml/h. Moreover, an excessive sweat rate, as brought about by intensive exercise, is quantified at 58.333 ml/h, which still does not render a similar volume of 99 ml as was performed during the dilution test (Salvo *et al*., 2018). Hence, robustness towards pH values similar to skin surface and pH values resembling physiological pH values were deemed sufficient for this characterisation test.

D.2.3 Assessment of efficiency of self-emulsification

Assessment of self-emulsification is often referred to as the dispersibility test (Chaudhari & Akamanchi, 2019; Vasconcelos *et al*., 2019; Nasr *et al*., 2016). For the purpose of characterising SEDDSs intended for oral drug delivery, this experiment implies the possible *in vitro* performance that can be expected from the tested SEDDSs (Nasr *et al*., 2016). Rapid emulsification is a desirable property, if exhibited by SEDDSs destined for oral drug delivery as spontaneous emulsification is the rate limiting step that must be completed before successful absorption can occur (Nasr *et al*., 2016). However, in terms of topical drug delivery, the rate limiting step for most drugs is diffusion through the lipophilic, outermost skin layer (Parhi & Swain, 2018; Lane, 2013). Contact time is highly important during dermal drug delivery as sufficient exposure of the drug to the skin must be facilitated in order to establish dermal drug diffusion (Rohrer *et al*., 2018; Kathe & Kathpalia, 2017). Therefore, this assessment was conducted to determine the ability of SEDDSs to withstand exposure to moist environments. This characteristic is essential as the occlusive effect of topically applied products can be interrupted if SEDDS formulations are influenced by the presence of external water addition such as sweat secretion or exposure of the skin to water while topical SEDDSs are applied (Margetts & Sawyer, 2007). The following grading system was utilised to categorise the SEDDS formulations (Chaudhari & Akamanchi, 2019):

Grade A	Swift emulsion formation, demonstrating a clear/bluish appearance (60 s)
Grade B	Swift formation of emulsion, which appears bluish (60 s)
Grade C	Emulsion displays fine milky appearance (120 s)
Grade D	Dull, greyish white appearance with an additional oily appearance together with slow emulsification, (>120 s)
Grade E	Poor or minimal emulsification noted with large oil droplets noticed on the surface

According to the grading system, SEDDS formulations that received a C or D grading could be deemed suitable for dermal drug delivery. On the other hand, SEDDS formulations that displayed poor or minimal emulsification, with consequent E-grading, were discarded in terms of further investigation as slow emulsification is desired and a complete inability to self-emulsify should be avoided. Furthermore, SEDDS formulations that reveal rapid emulsification and received an A or B grading were also considered unsuitable as these topical SEDDS formulations can easily be washed away by sweat or external water exposure and in turn decrease occlusive effects that enhance dermal drug delivery (Keurentjes & Maibach, 2019; Kathe & Kathpalia, 2017).

The efficiency of spontaneous emulsification, once exposed to large volumes of aqueous media, was assessed by employment of a type II Distek 2500 dissolution system apparatus (North Brunswick, United States of America). This experiment was performed by adding a 1 ml sample of each SEDDS formulation to 100 ml distilled water. A temperature of 32°C ± 0.5°C, similar to the surface temperature of skin, was maintained while subjected to moderate agitation that was facilitated by a set paddle speed of 50 rpm. Subsequently, upon visual observation, the self-emulsification time of each SEDDS formulation was noted as the time required to form a distinct homogenous dispersion after dilution (Heylings *et al*., 2018; Czajkowska-Kośnik *et al*., 2015). The time exhibited by individual SEDDS formulations are presented in **Table D.2**, together with the grading received by these topical SEDDS formulations.

Table D.2: Self-emulsification time and emulsion grading exhibited by topical SEDDS formulations

Formulations	Grade of emulsion	Time (s)
ARG1	**D**	499
ARG3	**D**	482
ARG5	**D**	423
AVO2	**D**	423
AVO3	**D**	131
AVO4	**D**	130
AVO5	**D**	430
MAC1	**D**	374
MAC2	**D**	346
MAC3	**D**	181
MAC5	**B**	59
OLV1	**D**	393
OLV2	**D**	385
OLV3	**D**	247
OLV5	**C**	90

As seen in **Table D.2**, the majority of the tested SEDDS formulations received a D-grading that is indicative of improved occlusive effects as slow emulsification occurred. Interestingly, the OLV 5 SEDDS formulation displayed swift emulsification compared to the other SEDDS formulations with a self-emulsification time of 90 s, rendering formulation OLV 5 with a C-grading. Formulations such as OLV 5 might exhibit decreased occlusive effects as these types of formulations are more easily washed away from the skin surface. Only one SEDDS formulation, MAC 5, failed the set criteria due to receiving a B-grading. This SEDDS

formulation also failed the robustness to dilution test. Thus, the rapid emulsification time of MAC 5 can be considered unstable behaviour if deliberated for dermal drug delivery.

D.2.4 Self-emulsification time

It is frequently stated that self-emulsification is facilitated by an inert low interfacial energy that exists between components of emulsions (Dizdarević *et al*., 2019; Gonçalves *et al*., 2018; Solans *et al*., 2016; Zhang *et al*., 2015; Akula *et al*., 2014). However, the free energy required to enable spontaneous emulsification can be influenced by the extent at which the surfactant decreases the tension present between the aqueous- and oil phases; and in turn dictates change in entropy (Shahnaz *et al*., 2011). However, analyses of the self-emulsification time experiments displayed contradicting results as increased surfactant concentrations rendered shortened emulsification times. Soalns *et al* (2016) stated that spontaneous emulsification can occur instantaneously or be prolonged, depending on kinetic barriers present between the various SEDDS components. According to the same authors, kinetic barriers can be reduced by application of heat or moderate agitation (Solans *et al*., 2016). Therefore, definite kinetic barriers are present as SEDDS formulations prepared during this study required an increase in temperature, followed by cooling afterwards to achieve self-emulsification. Eid *et al* (2014) however reported an increased viscosity upon enhanced surfactant concentrations; and subsequent difficulty to self-emulsify.

D.2.5 Viscosity

Viscosity refers to internal friction present within fluids that can provide resistance against flow and spontaneous emulsification (Perazzo *et al*., 2015; Eid *et al*., 2014). Thus, increased viscosity is indicative of strong attraction forces that exist within the fluid itself (Li *et al*., 2019); whereas, decreased viscosity portrays the presence of reduced attraction forces within a fluid (Li *et al*., 2019). These attraction forces are sensitive toward changes in temperature (Akilu *et al*., 2019). Therefore, reduced temperatures will render increased viscosity and elevated temperatures will facilitate enhanced viscosity (Akilu *et al*., 2019). Structural changes of reversible as well as irreversible nature can be induced by the flow of dispersions (Mewis & Wagner, 2009). Therefore, the measurement of viscosity is considered important for the purpose of predicting flow-facilitated structural changes that can influence formulation stability as well as topical drug delivery (Mendonsa *et al*., 2019; Mewis & Wagner, 2009).

Flow behaviour of fluids can be classified as Newtonian or non-Newtonian (Kazemian *et al*., 2012). Newtonian fluids exhibit unchanged viscosity values despite being exposed to different shear rates (Kazemian *et al*., 2012); whereas the viscosity of non-Newtonian fluids is influenced by shear rate (Kondratiev & Ilyushechkin, 2018). Moreover, non-Newtonian fluid behaviour can be categorised as either time dependant (i.e. thixotropic-

and rheopexy fluids) or time independent (i.e. pseudo-plastic-, dilatant- and visco-elastic fluids) (Pietrzyk *et al*., 2019; Kondratiev & Ilyushechkin, 2018, Ding *et al*., 2013; Kazemian *et al*., 2012). In this study viscosity experiments were conducted once off where the formulations were exposed to altered spindle speeds in order to generate a viscosity profile. Hence, no conclusion could be drawn for time dependant behaviour of the selected topical SEDDS formulations.

Viscosity readings were obtained utilising a Brookfield® Viscometer model DV-II+ (Stoughton, United States of America). Samples of approximately 10 ml of each SEDDS formulation were placed in a water bath at a maintained temperature of 25 ± 0.5°C, prior to viscosity experiments so as to exclude the influence of temperature on the rheology of the SEDDSs. The temperature of the water bath was regulated by a Brookfield® temperature controller (Bharadwaj *et al*., 2019). Viscosity of the selected SEDDS formulations was measured employing different spindles. Each spindle was tested for the purpose of finding a spindle that rendered sufficient torque values as influenced by the consistency of topical SEDDS formulations. The spindles employed during this study are listed in **Table D.3**.

Table D.3: Spindles employed to determine viscosity of the various SEDDS formulations

Type of spindle	**Topical SEDDS formulations**
SC4-34 LV	ARG 5
SC4-25 LV	ARG 1, ARG 3, AVO 2, AVO 3, AVO 5, MAC 5, OLV 5
T-bar F LV	MAC 1, MAC 2, OLV 1, OLV 2
T-bar E LV	AVO 4, MAC 3, OLV 3

As displayed in **Table D.3** all measurements occurred within the LV torque range. During this test, the Helipath stand D20733 raised and lowered the viscometer in a slow fashion at a rate of 7/8-inches/min. The spindle rotated within the SEDDS formulations while the viscometer was slowly lowered and raised. Spindles were carefully immersed in order to avoid fluid disturbance while paying close attention that the spindle did not come into contact with the bottom of the container (Bharadwaj *et al*., 2019). Readings were noted every 10 sec for a period of 5 min at various shear rates (5; 10; 20; 30; 60; 100 rpm). An average was calculated from the approximate 30 readings obtained at each shear rate. The viscosity profiles of the tested topical SEDDS formulations are illustrated according to the calculated average values at the set spindle speed as displayed in **Figures D.4–D.8**.

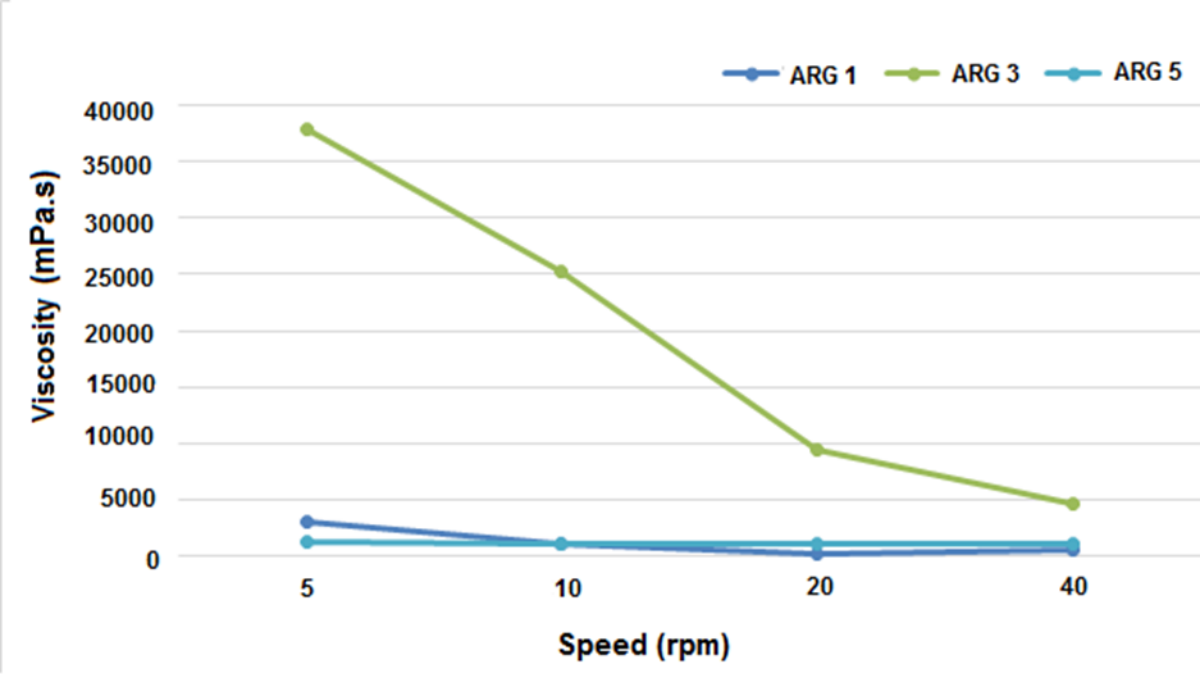

Figure D.4: Viscosity readings observed for SEDDSs comprising argan oil

As seen in **Figure D.4** the viscosity profile of argan oil SEDDSs only exhibited viscosity readings observed at spindle speeds of 5, 10, 20 and 40 rpm. The ARG 5 SEDDS did not render satisfactory torque values or viscosity measurements above a speed of 40 rpm. Hence, the viscosity profile was only constructed by utilising viscosity readings obtained at the aforementioned spindle speeds. The inability to obtain sufficient torque values for spindle speeds of 60 and 100 rpm for the ARG 5 SEDDS can be linked to a phenomenon known as dilatancy or a shear thickening fluid behaviour (Ding *et al*., 2013; Kazemian *et al*., 2012). A dilatant fluid refers to increased viscosity of a liquid as facilitated by enhanced stirring rates (Pietrzyk *et al*., 2019; Ding *et al*., 2013). Consequently, upon visual observation, during the viscosity experiments, apparent solidification of the ARG 5 SEDDS occurred that established a clay-like appearance. This observation is suggestive of volume expansion and frictional interactions that occur between particles (Chen *et al*., 2017); and this phenomenon transpires due to disruption of particle arrangement within the formulation (Chen *et al*., 2017; Ding *et al*., 2013). Kazemian *et al* (2012) stated that void spaces are readily present within dilatant fluids, but these void spaces do not cause any disturbance during rest conditions as the liquid present within the fluid itself sufficiently furnish voids. In contrast, once exposed to increased shear rates volume expansion of the fluid will transpire due to insufficient amounts of liquid present to fill the void spaces (Ding *et al*., 2013; Kazemian *et al*., 2012). As the formed

void spaces remain present within the formulation, resistance towards stirring remains problematic (Kazemian *et al*., 2012). The critical shear rate is inversely related to droplet size observed within the fluid (Fall *et al*., 2012). Therefore, increased droplet size can attribute towards dilatancy at decreased shear rates (Fall *et al*., 2012). Moreover, the ARG 5 SEDDS depicted the third highest droplet size during characterisation of the selected topical SEDDS and therefore provides a possible explanation for the flow behaviour displayed by this formulation.

Dilatancy mimics characteristics that correlate with flocculation during storage of SEDDS formulations (Dickinson, 2019; Rognon *et al*., 2011; Starov & Zhdanov, 2003; Bergström, 1998). Subsequently, dilatancy is an unwanted characteristic and the ARG 5 SEDDS could, for these reasons, not be considered for membrane release and dermal diffusion experiments. As depicted in **Figure D.4** the ARG 3 SEDDS displayed drastically decreased viscosity upon increased stirring speeds. On the other hand, the ARG 1 SEDDS displayed only modestly reduced viscosity values when compared to the ARG 3 SEDDS. Both these formulations could nonetheless be categorised as non-Newtonian fluids of a pseudo-plastic nature as their viscosity profiles displayed decreased viscosity upon increased stirring rates (Ding *et al*., 2013).

As visible in **Figure D.5** the AVO 3 and AVO 5 SEDDS formulations demonstrated viscosity readings that fluctuated minutely when compared to viscosity readings obtained for the AVO 2 and AVO 4 SEDDS formulations. A possible explanation can be found in the ratio excipients included in the AVO 2 and AVO 3 SEDDS formulations. The aqueous and oil phases were included in equal quantities in both formulations, as exhibited in **Table C.2** of Annexure C. Contrary, the ratios of oil to surfactant are 7: 3 and 6: 4 for the AVO 5 and AVO 3 SEDDS formulations, respectively. The AVO 5 SEDDS displayed increased viscosity despite a slightly reduced surfactant concentration. Hence, the influence of the oil phase on the viscosity of SEDDSs cannot be overlooked (Nowak *et al*., 2016). Both the AVO 2 and AVO 4 SEDDS formulations depicted pseudo-plastic behaviour as a decreased viscosity was observed when the SEDDSs were exposed to increased stirring speeds (Ding *et al*., 2013).

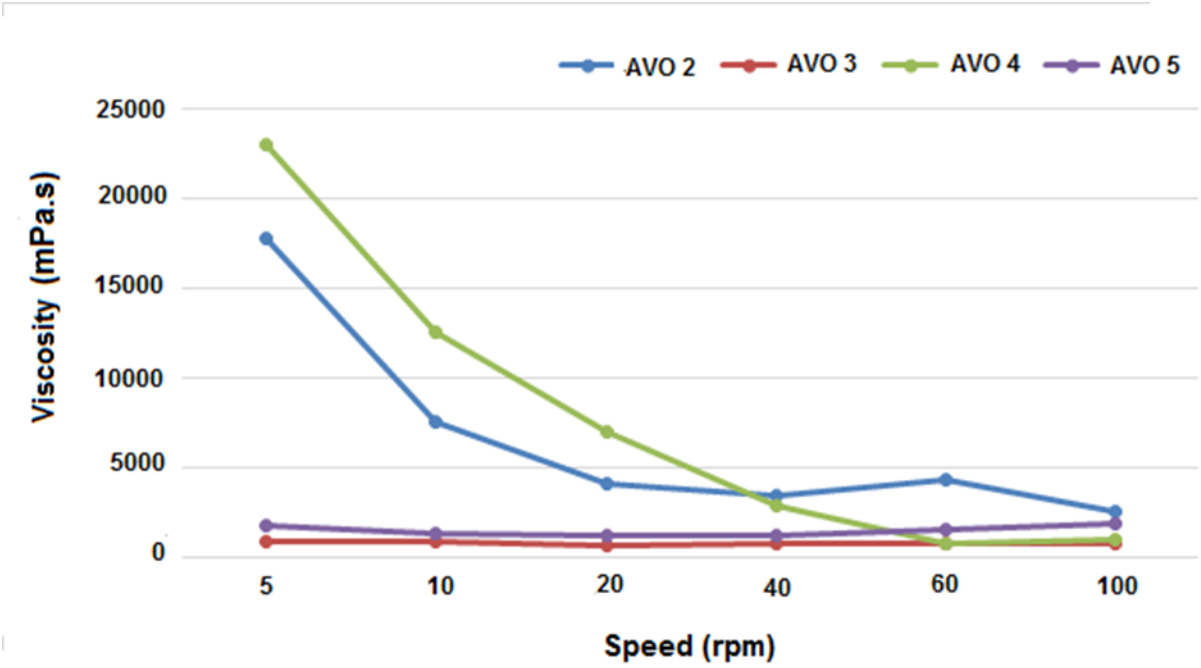

Figure D.5: Viscosity readings observed for SEDDSs comprising avocado oil

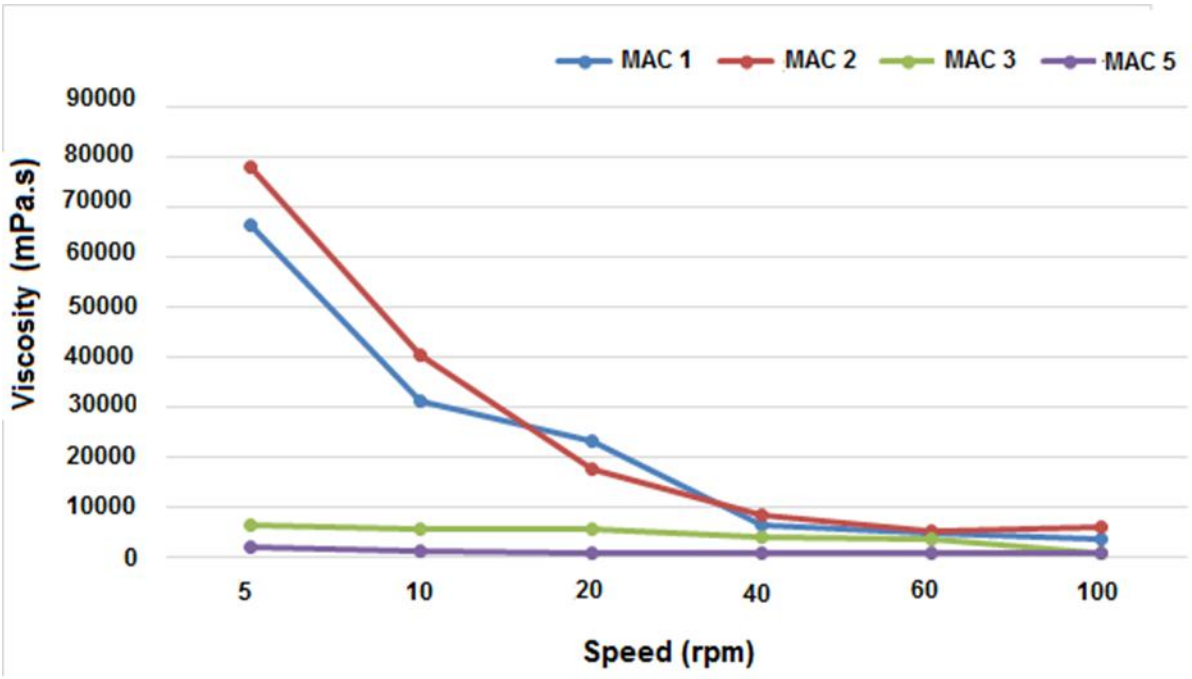

Figure D.6: Viscosity readings observed for SEDDSs comprising macadamia oil

Figure D.6 illustrates increased viscosity readings for both SEDDS formulations MAC 1 and MAC 2 as probably facilitated by increased surfactant concentrations (Eid *et al*., 2014). Moreover, the MAC 3 and MAC 5 SEDDS formulations demonstrated decreased viscosity values for all the shear rates tested, possibly due to the lower surfactant phase concentrations

(Eid *et al*., 2014). Therefore, indicating that macadamia oil SEDDSs rendered pseudo-plastic flow behaviour (Ding *et al*., 2013).

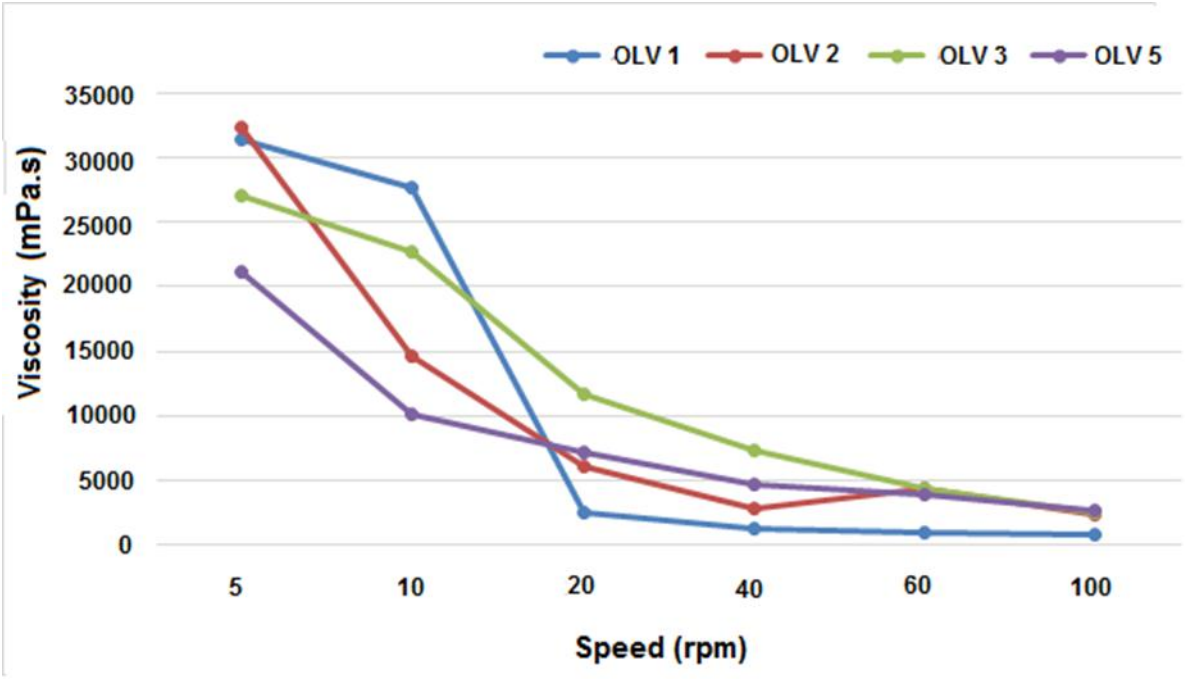

Figure D.7: Viscosity readings observed for SEDDSs comprising olive oil

If **Figure D.7** is compared to **Figures D.4–D.6**, the olive oil SEDDSs exhibited clear flow behaviour that correlate with the surfactant concentration included in these formulations. As seen in **Table C.5** of Annexure C olive oil SEDDSs can be ranked from highest to lowest surfactant content as follow: OLV 1>OLV 2>OLV 3>OLV 5. Thus, the viscosity results of the olive oil SEDDSs can definitely be linked to the extent of surfactant phase inclusion. Moreover, Eid *et al* (2014) established similar findings upon increased and decreased surfactant concentrations. However, the influence of the selected plant based oil, incorporated in different SEDDS formulations cannot be ignored as indicated by **Figure D.8**. This figure demonstrates the influence of the oil phase on the observed viscosity for the ARG 3, AVO 3, MAC 3 and OLV 3 SEDDS formulations with similar ratios of oil: surfactant phase: water (6: 4: 6).

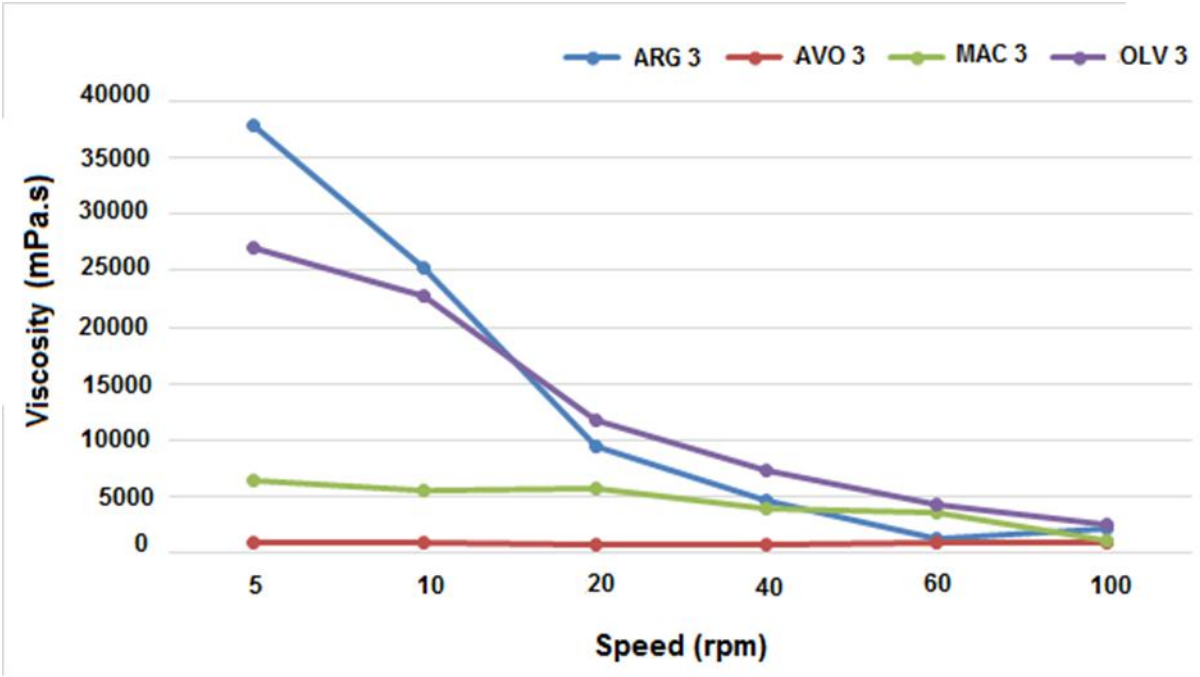

Figure D.8: Influence of various plant based oils on viscosity of SEDDSs

From this figure it is evident that the type of oil phase significantly influenced the flow behaviour of these SEDDS formulations. The impact of plant based oils on viscosity can be ranked from highest to lowest at a speed of 20 rpm as: olive oil > argan oil > macadamia oil > avocado oil. Interestingly, argan oil SEDDSs depicted increased self-emulsification time as displayed in **Table D.2** as well as the highest viscosity of at speeds of 5 rpm and 10 rpm, respectively. Therefore, increased viscosity probably influence the time required to facilitate spontaneous emulsification (Eid *et al*., 2014). All SEDDS formulations depicted a non-Newtonian behaviour of pseudo-plastic nature that can also be referred to as shear-thinning (Nguyen *et al*., 2017). Shear thinning is a favourable property if displayed by topical formulations as it avoids the occurrence of instantaneous skin injury during application of a formulation by means of rubbing (Nguyen *et al*., 2017). Rubbing is assumed to enhance topical drug delivery due to lipid disruption of the outermost skin layer (Nguyen *et al*., 2017). Moreover, as decreased viscosity is established by increased shear rates, topical SEDDS formulations will depict similar behaviour once rubbed onto the skin (Nguyen *et al*., 2017). Therefore, decreased viscosity can facilitate improved spreadability and in turn establish treatment of a larger area of affected skin (Nguyen *et al*., 2017). Additionally, as viscosity is sensitive towards changes in temperature, cloud point experiments were conducted to investigate the temperature at which each formulation exhibits dehydration of the included excipients.

D.2.6 Cloud point determination

Cloud point refers to the temperature at which a SEDDS formulation is no longer capable of preserving its self-emulsification properties (Akula *et al*., 2014). The loss of spontaneous emulsification risks erratic drug release and irreversible phase separation (Zupančič *et al*., 2017). This irreversible phase separation is caused by the dehydration of ingredients as facilitated by increased temperatures (Ujilestari *et al*., 2018). Cloud point can be visually observed as a sudden change in appearance from transparent to turbid (AboulFotouh *et al*., 2018). Cloud point is especially significant when non-ionic, hydrophilic surfactants are included in SEDDS formulations as these surfactants are no longer soluble in the aqueous media after the cloud point is reached (Pouten, 1997). However, the presence of oils and drugs can have an impact on the cloud point displayed by a surfactant (Pouten, 1997). Consequently, the cloud point must be determined for each individual SEDDS formulation.

Topical SEDDS formulations were diluted (1:100) with distilled water. Hereafter, the samples were placed in a water bath at a starting temperature of 25°C. The temperature was moderately increased at 2°C/min until a cloudy appearance of the topical SEDDS formulations was observed (Zupančič *et al*., 2017). The observed cloud point temperatures are noted in **Table D.4**.

Table D.4: Cloud point temperatures observed for topical SEDDSs

SEDDS formulation	Cloud point temperature (°C)
ARG1	27.00
ARG3	34.00
ARG5	46.90
AVO2	40.00
AVO3	45.9
AVO4	44.00
AVO5	50.60
MAC1	31.90
MAC2	32.50
MAC3	31.40
MAC5	36.00
OLV1	34.80
OLV2	34.80
OLV3	33.80
OLV5	35.90

As revealed in **Table D.4**, the various cloud point temperatures varied from 27.00°C to 50.60°C. In order to avoid dehydration of the SEDDS components at the skin surface, the temperature at which cloud point is observed must exceed a temperature of 32°C in order to be deemed suitable for topical drug delivery (Heylings *et al*., 2018). Therefore, SEDDS formulations ARG 1, MAC 1 and MAC 3 were considered unsuitable for dermal application as cloud point temperatures of only 27.00°C, 31.90°C and 31.40°C were acquired, respectively. Concern might be raised about SEDDS formulations nearing 32.00°C, for example the MAC 2 SEDDS with a cloud point temperature of 32.50°C; the reason being that the cloud point temperature is quite similar to skin surface temperature of 32.00°C (Heylings *et al*., 2018).

However, Al-Sabagh *et al* (2011) stated that surfactants generally demonstrate optimal effectiveness if utilised near to their cloud point. Nonetheless, the stability of SEDDS upon exposure to different environments during handling, storage and transport should be investigated. For this reason, thermodynamic stability experiments were conducted.

D.2.7 Thermodynamic stability studies

Emulsified systems can exhibit instabilities such as creaming, aggregation, flocculation, Ostwald ripening and/or cracking during storage periods that lead to macroscopic phase separation of these formulations (Liu *et al*., 2019). These unstable mechanisms are linked to decreased contact between the lipid- and aqueous phases (Liu *et al*., 2019; McClements & Li, 2010). Therefore, surface active agents are included to enforce the stability of the dispersed droplets (Liu *et al*., 2019; Roumpea *et al*., 2019; Priorkowski & McClements, 2014). The stability of emulsified systems is dependent on the opacity and charge properties of the interfacial membrane (Liu *et al*., 2019). However, surfactant inclusion cannot guarantee physical stability of the emulsified system as surfactants add complexation to emulsified systems by facilitating gradients of interfacial tension and alteration of droplet breakup dynamics (Roumpea *et al*., 2019). Therefore, the physical stability of topical SEDDS formulations was determined by exposing these formulations to thermodynamic stress environments, for example, cycles of heating and cooling, as well as centrifugation (Suvarna *et al*., 2017; Aparna *et al*., 2015; Czajkowska-Kośnik *et al*., 2015).

First, heating and cooling were varied during six cycles (Aparna *et al*., 2015). This test was performed through the exposure of the SEDDS formulations to temperatures of approximately 4°C and 45°C, respectively. SEDDS formulations were not exposed to one of these varied temperatures for more than 48 h at a time. After removal from each environment, the SEDDS formulations were visually inspected for phase separation or drug precipitation (Czajkowska-Kośnik *et al*., 2015). Next, the SEDDS formulations were subjected to centrifugation at 3 500 rpm for 30 min (Suvarna *et al*., 2017; Aparna *et al*., 2015). Succeeding centrifugation, the SEDDS formulations were visually examined for indications of phase separation, cracking and/or creaming (Suvarna *et al*., 2017). The thermodynamic stability results acquired for these topical SEDDS formulations are presented in **Table D.5**.

Table D.5: Thermodynamic stability results acquired for SEDDS formulations

SEDDS formulation	Heat-cool cycles	Centrifugation test
ARG 1	✓	✓
ARG 3	✓	✓
ARG 5	×	×
AVO 2	✓	✓
AVO 3	✓	✓
AVO 4	✓	✓
AVO 5	✓	×
MAC 1	×	×
MAC 2	✓	✓
MAC 3	✓	✓
MAC 5	✓	✓
OLV 1	×	×
OLV 2	×	×
OLV 3	×	✓
OLV 5	✓	✓

Data is presented as passed (✓) or failed (×)

From **Table D.5** it is clear that the ARG 5, MAC 1, OLV 1 and OLV 2 SEDDS formulations displayed instability after being exposed to thermodynamic stress environments as well as kinetic stress experiments and were thus considered unsuitable for membrane release and dermal diffusion studies. In contrast, the OLV 3 SEDDS only exhibited instability upon exposure to thermodynamic stress conditions and the AVO 5 SEDDS revealed instability after kinetic stress experiments. However, both SEDDS formulations, OLV 3 and AVO 5, were deemed unsuitable for further investigation as physical instability was observed. An additional factor that can contribute towards successful dermal drug delivery of SEDDSs is the pH of SEDDS formulations.

D.2.8 pH

The pH of the selected SEDDS formulations was measured by employing a Mettler® Toledo pH meter (Mettler® Toledo International Inc., United States of America) with a Mettler® Toledo

Inlab® 410 NTC electrode 9823. Calibration of the Mettler Toledo® InLab® 410 electrode NTC electrode 9823 was executed at a pH of 4, 7 and 10 prior to measurement (Ritchie *et al*., 2017; Clausen *et al*., 2016). The acceptable pH range established for formulations destined for dermal drug delivery, is 5.0–9.0 (Rastogi & Yadav, 2014), though the optimal pH would be a value that resembles natural skin pH; and will thus vary between 4.5–5.0 (Sheshala *et al*., 2019). The obtained pH measurements for the SEDDS formulations are shown in **Table D.6**.

Table D.6: Measured pH values obtained for topical SEDDS formulations

Formulation	pH
ARG1	6.949
ARG3	5.998
ARG5	6.600
AVO2	5.009
AVO3	3.820
AVO4	5.024
AVO5	5.045
MAC1	7.404
MAC2	6.605
MAC3	7.007
MAC5	7.021
OLV1	7.365
OLV2	7.125
OLV3	7.133
OLV5	6.963

All of the SEDDSs formulations could be considered suitable for topical application as all measured pH values (**Table D.6**) fell within the 5–9 range, except for the AVO 3 SEDDS that

depicted a pH value of 3.82. If the optimal pH range is considered, the avocado oil SEDDSs would be the most compatible with the natural pH of the skin surface, with values of 5.009, 5.024 and 5.045 as obtained by the AVO 2, AVO 4 and AVO 5 SEDDS formulations, correspondingly.

D.3 Conclusion

After numerous characterisation experiments, the behaviour and properties of the tested topical SEDDS formulations were considered in order to establish which of the topical SEDDS formulations could be utilised to conduct membrane release and dermal diffusion studies. Considering that the main focus of this study is directed at the development of a topical SEDDS formulation for optimised drug delivery of an extremely lipophilic drug, all SEDDS formulations that exhibited unfavourable behaviour or properties, in terms of topical drug delivery and general SEDDS characterisation guidelines, were eliminated due to the fact that characterisation results rendered them unsuitable for further investigation. Topical SEDDS formulations that were considered unsuitable for membrane release and dermal diffusion studies are shown in **Figure D.9**.

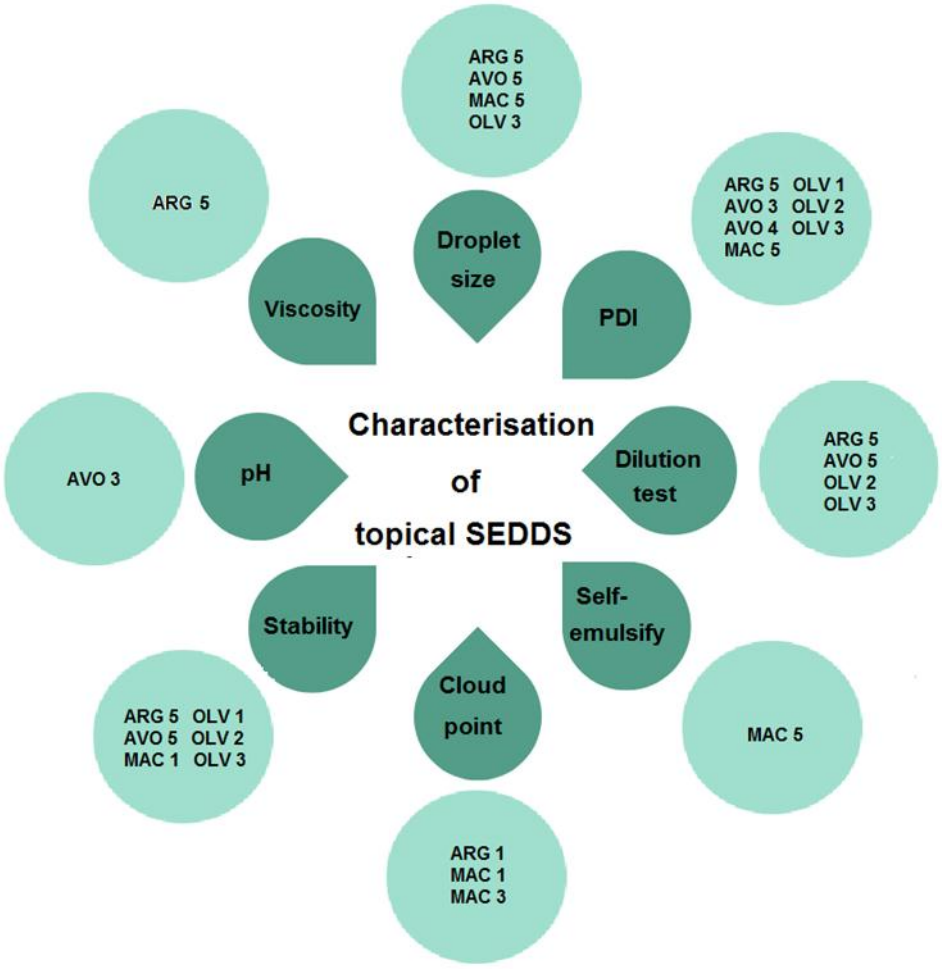

Figure D.9: Topical SEDDS formulations considered unsuitable for topical drug delivery

As demonstrated by **Figure D.9** a modest quantity of formulations was deemed suitable for topical drug delivery, namely: SEDDS formulations ARG 3, AVO 2, MAC 2 and OLV 5. These formulations met all of the criteria set for SEDDSs destined for topical drug delivery. The properties exhibited by these selected SEDDS formulations are summarised in **Table D.7**.

Table D.7: Summary of properties exhibited by SEDDSs suitable for topical delivery

	ARG3	AVO2	MAC2	OLV5
Droplet size (nm)	107.317	64.112	108.708	154.800
Polydispersity Index (PDI)	0.6175	0.339	0.763	0.793
Zeta-potential (mV)	-29.900	-32.800	-29.200	-23.600
Grading of self-emulsification	D	D	D	C
Self-emulsification time (s)	482.000	423.000	346.000	90.000
Cloud point (°C)	34.000	40.000	32.500	35.900
pH	5.998	5.009	6.605	6.963

From the above table, it is evident that the AVO 2 SEDDS formulation is the only acceptable formulation residing in the nano-sized range. Moreover, this formulation rendered the smallest deviation in size distribution and it depicted the highest zeta-potential comparatively to the SEDDS formulations considered for further investigation.

For these reasons, it was predicted that the AVO 2 SEDDS formulation will probably be the most stable formulation of the remaining SEDDS formulations. The temperature at which cloud point was observed for the AVO 2 SEDDS is also the highest temperature obtained by the finally selected SEDDS formulations. In addition, the AVO 2 SEDDS received a D-grading for efficacy of self-emulsification and therefore predicts improved occlusive effects. Finally, this formulation depicted a pH value nearing an optimum skin compatibility pH value. Therefore, the AVO 2 SEDDS formulation can be considered the topical SEDDS formulation that displayed the most suitable properties if destined for topical drug delivery. However, the correlation between paper and practice is established in Annexure E.

References

Abdel-Messih, H.A., Ishak, R.A., Geneidi, A.S. & Mansour, S. 2019. Tailoring novel soft nano-vesicles 'Flexosomes' for enhanced transdermal drug delivery: Optimization, characterization and comprehensive ex vivo–in vivo evaluation. *International journal of pharmaceutics*, 560:101-115.

AboulFotouh, K., Allam, A.A., El-Badry, M. & El-Sayed, A.M. 2018. Role of self-emulsifying drug delivery systems in optimizing the oral delivery of hydrophilic macromolecules and reducing interindividual variability. *Colloids and surfaces B: biointerfaces*, 167:82-92.

Agarwal, V., Bell, G.W., Nam, J.W. & Bartel, D.P. 2015. Predicting effective microRNA target sites in mammalian mRNAs. *elife*, 4: Article ID e05005. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4532895/. Date of access: 26 Oct. 2019.

Akilu, S., Baheta, A.T., Kadirgama, K., Padmanabhan, E. & Sharma, K.V. 2019. Viscosity, electrical and thermal conductivities of ethylene and propylene glycol-based β-SiC nanofluids. *Journal of molecular liquids*, 284:780-792.

Akula, S., Gurram, A.K. & Devireddy, S.R. 2014. Self-microemulsifying drug delivery systems: an attractive strategy for enhanced therapeutic profile. *International scholarly research notices*, Article ID 964051. https://www.hindawi.com/journals/isrn/2014/964051/. Date of access: 26 Oct. 2019.

Ali, H.H. & Hussein, A.A. 2017. Oral solid self-nanoemulsifying drug delivery systems of candesartan citexetil: formulation, characterization and *in vitro* drug release studies. *AAPS open*, 3:Article ID 6.

Al-Sabagh, A.M., Nasser, N.M., Migahed, M.A. & Kandil, N.G. 2011. Effect of chemical structure on the cloud point of some new non-ionic surfactants based on bisphenol in relation to their surface active properties. *Egyptian journal of petroleum*, 20:59-66.

Aparna, C., Pathima, S. & Rao Patnaik, K.S.K. 2015. Formulation and evaluation of oral self-emulsifying drug delivery system of lornoxicam. *International journal of pharma and bio sciences*, 6:381-395.

Balata, G.F., Essa, E.A., Shamardl, H.A., Zaidan, S.H. & Abourehab, M.A. 2016. Self-emulsifying drug delivery systems as a tool to improve solubility and bioavailability of resveratrol. *Drug design, development and therapy*, 10:117-128.

Bergström, L. 1998. Shear thinning and shear thickening of concentrated ceramic suspensions. *Colloids and surfaces A: physicochemical and engineering aspects*, 133:151-155.

Bharadwaj, R., Haloi, J. & Medhi, S. 2019. Topical delivery of methanolic root extract of Annona reticulata against skin cancer. *South African journal of botany*, 124:484-493.

Bolla, G. & Nangia, A. 2012. Clofazimine mesylate: a high solubility stable salt. *Crystal growth & design*, 12:6250-6259.

Carter, P., Narasimhan, B. & Wang, Q. 2019. Biocompatible nanoparticles and vesicular systems in transdermal drug delivery for various skin diseases. *International journal of pharmaceutics*, 555:49-62.

Chaudhari, K.S. & Akamanchi, K.G. 2019. Novel bicephalous heterolipid based self-microemulsifying drug delivery system for solubility and bioavailability enhancement of efavirenz. *International journal of pharmaceutics*, 560:205-218.

Chen, Q., Liu, M., Xuan, S., Jiang, W., Cao, S. & Gong, X. 2017. Shear dependent electrical property of conductive shear thickening fluid. *Materials & design*, 121:92-100.

Clausen, M.-L., Slotved, H.-C., Krogfelt, K.A. & Agner, T. 2016. Tape stripping technique for stratum corneum protein analysis. *Scientific reports*, 6:Article ID 19918.

Czajkowska-Kośnik, A., Szekalska, M., Amelian, A., Szymańska, E. & Winnicka, K. 2015. Development and evaluation of liquid and solid self-emulsifying drug delivery systems for atorvastatin. *Molecules*, 20:21010-21022.

Danaei, M., Dehghankhold, M., Ataei, S., Hasanzadeh Davarani, F., Javanmard, R., Dokhani, A., Khorasani, S. & Mozafari, M. 2018. Impact of particle size and polydispersity index on the clinical applications of lipidic nanocarrier systems. *Pharmaceutics*, 10:Article ID 57.

Dickinson, E. 2019. Strategies to control and inhibit the flocculation of protein-stabilized oil-in-water emulsions. *Food hydrocolloids*, 96:209-223.

Ding, J., Tracey, P., Li, W., Peng, G., Whitten, P. G. & Wallace, G. G. 2013. Review on shear thickening fluids and applications. *Textiles and light industrial science and technology*, 2:161-173.

Ding, Z., Jiang, Y. & Liu, X. 2018. Nanoemulsions-based drug delivery for brain tumors. (*In* Kreharwani, P. & Gupta, U., *ed*. Nanotechnology-based targeted drug delivery system for brain tumors. London: Academic Press. p. 327-358).

Dizdarević, A., Efiana, N.A., Phan, T.N.Q., Matuszczak, B. & Schnürch, A.B. 2019. Imine bond formation: a novel concept to incorporate peptide drugs in self-emulsifying drug delivery systems (SEDDS). *European journal of pharmaceutics and biopharmaceutics*, 142:92-100.

Dokania, S. & Joshi, A.K. 2015. Self-microemulsifying drug delivery system (SMEDDS)–challenges and road ahead. *Drug delivery*, 22:675-690.

Eid, A.M., El-Enshasy, H.A., Aziz, R. & Elmarzugi, N.A. 2014. The preparation and evaluation of self-nanoemulsifying systems containing Swietenia oil and an examination of its anti-inflammatory effects. *International journal of nanomedicine*, 9:Article ID 4685.

Fall, A., Bertrand, F., Ovarlez, G. & Bonn, D. 2012. Shear thickening of cornstarch suspensions. *Journal of rheology*, 56:575-591.

Gonçalves, A., Nikmaram, N., Roohinejad, S., Estevinho, B.N., Rocha, F., Greiner, R. & McClements, D.J. 2018. Production, properties and applications of solid self-emulsifying delivery systems (S-EDDS) in the food and pharmaceutical industries. *Colloids and surfaces A*, 538:108-126.

Gumustas, M., Sengel-Turk, C.T., Gumustas, A., Ozkan, S.A. & Uslu, B. 2017. Effect of polymer-based nanoparticles on the assay of antimicrobial drug delivery systems. (*In* Grumezescu, A.M., *ed.* Multifunctional systems for combined delivery, biosensing and diagnostics. Amsterdam: Elsevier. p. 67-108).

Gupta, V. & Trivedi, P. 2018. *In vitro* and in vivo characterisation of pharmaceutical topical nanocarriers containing anti-cancer drugs for skin cancer treatment. (*In* Grumezescu, A.M., *ed.* Lipd nano carriers for drug targeted therapy. Kidlington:William Andrew applied science publishers. p. 563-627).

Hardiningtyas, S.D., Nagao, S., Yamamoto, E., Shirakigawa, N., Wakabayashi, R., Goto, M., Ijima, H. & Kamiya, N. 2019. A non-sized gel in-in-oil suspension for transcutaneous protein delivery. *International journal of pharmaceutics*, 567:Article ID 118495.

Heylings, J.R., Davies, D.J. & Burton, R. 2018. Dermal absorption of testosterone in human and pig skin *in vitro*. *Toxicology in vitro*, 48:71-77.

Ishii, F. & Nill, T. 2014. Lipid emulsions and lipid vesicles prepared from various phospholipids as drug carriers. (*In* Oshima, H. & Makino, K., *ed*. Colloid and interface science in pharmaceutical research and development. Kidlington: Elsevier. p. 469-501).

Kazemian, S., Arun, P. & Haut, B. B. 2012. Review of Newtonian and non-Newtonian fluids behaviour in the context of grouts. *Geotechnical aspects of underground construction in soft ground*, 321-326.

Kathe, K. & Kathpalia, H. 2017. Film forming systems for topical and transdermal drug delivery. *Asian journal of pharmaceutical sciences*, 12:487-497.

Kaur, R. & Ajitha, M. 2019. Formulation of transdermal nanoemulsion gel drug delivery system of lovastatin and its in vivo characterization in glucocorticoid induced osteoporosis rat model. *Journal of drug delivery science and technology*, 52:968-978.

Keurentjes, A.J. & Maibach, H.I. 2019. Percutaneous penetration of drugs applied in transdermal delivery systems: an in vivo based approach for evaluating computer generated penetration models. *Regulatory toxicology and pharmacology*, 18:Article ID 104428.

Kondratiev, A. & Ilyushechkin, A. 2018. Flow behaviour of crystallising coal ash slags: shear viscosity, non-newtonian flow and temperature of critical viscosity. *Fuel*, 224:783-800.

Krstić, M., Medarević, D., Đuriš, J. & Ibrić, S. 2018. Self-nano-emulsifying drug delivery systems (SNEDDS) and self-microemulsifying drug delivery systems (SMEDDS) as lipid nanocarriers for improving dissolution rate and bioavailability of poorly soluble drugs. (*In* Oshima, H. & Makino, K., *ed*. Colloid and interface science in pharmaceutical research and development. Kidlington: Elsevier. p. 473-508).

Lane, M.E. 2013. Skin penetration enhancers. *International journal of pharmaceutics*, 447: 12-21.

Liang, L., Wang, L., Nguyen, A.V. & Xie, G. 2017. Heterocoagulation of alumina and quartz studied by zeta-potential distribution and particle size distribution measurements. *Powder technology*, 309:1-12.

Li, J., Cheng, Y., Chen, X. & Zheng, S. 2019. Impact of electroviscous effect on viscosity in developing highly concentrated protein formulations: lessons from non-protein charged colloids. *International journal of pharmaceutics,* 1:Article ID 100002.

Lin, T.K., Zhong, L. & Santiago, J. 2018. Anti-inflammatory and skin barrier repair effects of topical application of some plant oils. *International journal of molecular sciences*, 19:Article ID 70.

Liu, H., Han, G., Zhang, H., Liu, Q. & Kong, B. 2019. Improving the physical and oxidative stability of emulsions based on the interfacial electrostatic effects between porcine bone protein hydrolysates and porcine bone protein hydrolysate-rutin conjugates. *Food hydrocolloids*, 94:418-427.

Lovelyn, C. & Attama, A.A. 2011. Current state of nanoemulsions in drug delivery. *Journal of biomaterials and nanobiotechnology*, 2:Article ID 626.

Mahapatra, A.K., Murthy, P.N., Swadeep, B. & Swain, R.P. 2014. Self-emulsifying drug delivery systems (SEDDS): An update from formulation development to therapeutic strategies. *International journal of pharmtech research*, 6:546-568.

Margetts, L. & Sawyer, R. 2007. Transdermal drug delivery: principles and opioid therapy. *Continuing education in anaesthesia, critical care & pain*, 7:171-176.

Marwah, H., Garg, T., Goyal, A.K. & Rath, G. 2016. Permeation enhancer strategies in transdermal drug delivery. *Drug delivery*, 23:564-578.

McClements, D.J. & Li, Y. 2010. Structured emulsion-based delivery systems: controlling the digestion and release of lipophilic food components. *Advances in colloid and interface science*, 159:213-228.

Mendonsa, N.S., Pradhan, A., Sharma, P., Prado, R.M., Murthy, S.N., Kundu, S. & Repka, M.A. 2019. A quality by design approach to develop topical creams via hot-melt extrusion technology. *European journal of pharmaceutical sciences*, 136:Article ID 104948.

Mewis, J. & Wagner, N.J. 2009. Thixotropy. *Advances in colloid and interface science*, 147:214-227.

Naik, A., Kalia, Y.N. & Guy, R.H. 2000. Transdermal drug delivery: overcoming the skin's barrier function. *Pharmaceutical science & technology today*, 3:318-326.

Nasr, A., Gardouh, A. & Ghorab, M. 2016. Novel solid self-nanoemulsifying drug delivery system (S-SNEDDS) for oral delivery of olmesartan medoxomil: design, formulation, pharmacokinetic and bioavailability evaluation. *Pharmaceutics*, *8*:Article ID 20.

Nguyen, H.X., Puri, A. & Banga, A.K. 2017. Methods to simulate rubbing of topical formulation for *in vitro* skin permeation studies. *International journal of pharmaceutics*, 519*:*22-33.

Nowak, E., Kovalchuk, N.M., Che, Z. & Simmons, M.J. 2016. Effect of surfactant concentration and viscosity of outer phase during the coalescence of a surfactant-laden drop with a surfactant-free drop. *Colloids and surfaces A: physicochemical and engineering aspects*, 505:124-131.

Otto, A., Du Plessis, J. & Wiechers, J.W. 2009. Formulation effects of topical emulsions on transdermal and dermal delivery. *International journal of cosmetic science*, 31:1-19.

Parhi, R. & Swain, S. 2018. Transdermal evaporation drug delivery system: concept to commercial products. *Advanced pharmaceutical bulletin*, 8:Article ID 535.

Patterson, M.J., Galloway, S.D. & Nimmo, M.A. 2000. Variations in regional sweat composition in normal human males. *Experimental physiology*, 85:869-875.

Perazzo, A., Preziosi, V. & Guido, S. 2015. Phase inversion emulsification: current understanding and applications. *Advances in colloid and interface science*, 222:581-599.

Peters, K., Leitzke, S., Diederich, J.E., Borner, K., Hahn, H., Müller, R.H. & Ehlers, S. 2000. Preparation of a clofazimine nanosuspension for intravenous use and evaluation of its therapeutic efficacy in murine *Mycobacterium avium* infection. *Journal of antimicrobacterial chemotherapy*, 45:77-83.

Pietrzyk, K., Nganguia, H., Datt, C., Zhu, L., Elfring, G.J. & Pak, O.S. 2019. Flow around a squirmer in a shear-thinning fluid. *Journal of non-newtonian fluid mechanics*, 268:101-110.

Piorkowski, D.T. & McClements, D.J. 2014. Beverage emulsions: Recent developments in formulation, production, and applications. *Food hydrocolloids*, 42:5-41.

Pouton, C.W. 1997. Formulation of self-emulsifying drug delivery systems. *Advanced drug delivery reviews*, 25:47-58.

Promphet, N., Rattanawaleedirojn, P., Siralertmukul, K., Soatthiyanon, N., Potiyaraj, P., Thanawattano, C., Hinestroza, J.P. & Rodthongkum, N. 2019. Non-invasive textile based colorimetric sensor for the simultaneous detection of sweat pH and lactate. *Talanta*, 192:424-430.

Rani, S., Rana, R., Saraogi, G.K., Kumar, V. & Gupta, U. 2019. Self-Emulsifying Oral Lipid Drug Delivery Systems: Advances and Challenges. *AAPS PharmSciTech*, 20:Article ID 129.

Rastogi, V. and Yadav, P. 2014. Transdermal drug delivery system: an overview. *Asian journal of pharmaceutics*, 6:161-170.

Riaz, A., Ahmed, N., Khan, M.I., Haq, I.-U., Rehman, A.U. & Khan, G.M. 2019. Formulation of topical NLCs to target macrophages for cutaneous Leishmaniasis. *Journal of drug delivery science and technology,* 54:Article ID 101232.

Ritchie, E.K., Martin, E.B., Racher, A. & Jaques, C. 2017. Extraction of indirectly captured information for use in comparison of offline pH measurement technologies. *Journal of biotechnology*, 257:160-165.

Rognon, P.G., Einav, I. & Gay, C. 2011. Flowing resistance and dilatancy of dense suspensions: lubrication and repulsion. *Journal of fluid mechanics*, 689:75-96.

Rohrer, J., Zupančič, O., Hetenyi, G., Kurpiers, M. & Bernkop-Schnuerch, A. 2018. Design and evaluation of SEDDS exhibiting high emulsifying properties. *Journal of drug delivery science and technology*, 44:366-372.

Roohnikan, M., Laszlo, E., Babity, S. & Brambilla, D. 2019. A snapshot of transdermal and topical drug delivery research in Canada. *Pharmaceutics*, 11:Article ID 256.

Roumpea, E., Kovalchuk, N.M., Chinaud, M., Nowak, E., Simmons, M.J. & Angeli, P. 2019. Experimental studies on droplet formation in a flow-focusing microchannel in the presence of surfactants. *Chemical engineering science*, 195:507-518.

Salvo, P., Pingitore, A., Barbini, A. & Di Francesco, F. 2018. A wearable sweat rate sensor to monitor the athletes' performance during training. *Science & sports*, 33:Article ID e51-e58. https://www.sciencedirect.com/science/article/abs/pii/S076515971730182X Date of access: 26 Oct. 2019.

Sangana, R., Gu, H., Chan, D.Y. & Einolf, H.J. 2018. Evaluation of clinical drug interaction potential of clofazimine using static and dynamic modeling approaches. *ASPET journal*, 46:1-34.

Shahnaz, G., Hartl, M., Barthelmes, J., Leithner, K., Sarti, F., Hintzen, F., Rahmat, D., Salvenmoser, W. & Bernkop-Schnürch, A. 2011. Uptake of phenothiazines by the harvested chylomicrons ex vivo model: Influence of self-nanoemulsifying formulation design. *European journal of pharmaceutics and biopharmaceutics*, 79:171-180.

Sharma, S., Shukla, P, Misra, A. & Mishra, P.R. 2014. Interfacial and colloidal properties of emulsified systems: pharmaceutical and biological perspective. (*In* Oshima, H. & Makino, K., *ed*. Colloid and interface science in pharmaceutical research and development. Kidlington: Elsevier. p. 149-172).

Sheshala, R., Anuar, N.K., Samah, N.H.A. & Wong, T.W. 2019. *In vitro* drug dissolution/permeation testing of nanocarriers for skin application: a comprehensive review. *AAPS PharmSciTech*, 20:Article ID 164.

Shu, J., Zhao, J. & Guo, F. 2018. Systematically optimized topical delivery system for Loperamide hydrochloride: Formulation design, *in vitro* and in vivo biopharmaceutical evaluation. *Asian journal of pharmaceutical sciences*, 13:255-264.

Solans, C., Morales, D. & Homs, M. 2016. Spontaneous emulsification. *Current opinion in colloid and interface science*, 22:88-93.

Starov, V.M. & Zhdanov, V.G. 2003. Viscosity of emulsions: influence of flocculation. *Journal of colloid and interface science*, 258:404-414.

Su, R., Fan, W., Yu, Q., Dong, X., Qi, J., Zhu, Q., Zhao, W., Wu, W., Chen, Z., Li, Y. & Lu, Y. 2017. Size-dependent penetration of nanoemulsions into epidermis and hair follicles: implications for transdermal delivery and immunization. *Oncotarget*, 8:Article ID 38214.

Suvarna, V., Pagdhare, U., Kadu, A. & Oza, M. 2017. Development and characterization of solid self-emulsifying drug delivery system containing nateglinide. *Asian journal of pharmaceutics*, 11:27-36.

Syukri, Y., Martien, R., Lukitaningsih, E. & Nugroho, A.E. 2018. Novel self-nano emulsifying drug delivery system (SNEDDS) of andrographolide isolated from Andrographis paniculata Nees: characterization, in-vitro and in-vivo assessment. *Journal of drug delivery science and technology*, 47:514-520.

Ujilestari, T., Dono, N.D., Ariyadi, B., Martien, R. & Zuprizal, Z. 2018. Formulation and characterization of self-nano emulsifying drug delivery systems of lemongrass (cymbopogon citratus) essential oil. *Malaysian journal of fundamental and applied sciences*, 14:360-363.

Umerska, A., Cassisa, V., Matougui, N., Joly-Guillou, M.L., Eveillard, M. & Saulnier, P. 2016. Antibacterial action of lipid nanocapsules containing fatty acids or monoglycerides as co-surfactants. *European journal of pharmaceutics and biopharmaceutics*, 108:100-110.

Vasconcelos, T., Araújo, F., Lopes, C., Loureiro, A., das Neves, J., Marques, S. & Sarmento, B. 2019. Multicomponent self nano emulsifying delivery systems of resveratrol with enhanced pharmacokinetics profile. *European journal of pharmaceutics*, 137:Article ID 105011.

Vaughn, A.R., Clark, A.K., Sivamani, R.K. & Shi, V.Y. 2018. Natural oils for skin-barrier repair: Ancient compounds now backed by modern science. *American journal of clinical dermatology*, 19:103-117.

Viljoen, J.M., Cowley, A., Du Preez, J., Gerber, M. & Du Plessis, J. 2015. Penetration enhancing effects of selected natural oils utilized in topical dosage forms. *Drug development and industrial pharmacy*, 41:2045-2054.

Zafeiri, I., Norton, J.E., Smith, P., Norton, I.T. & Spyropoulos, F. 2017. The role of surface active species in the fabrication and functionality of edible solid lipid particles. *Journal of colloid and interface science*, 500:228-240.

Zaichik, S., Steinbring, C., Menzel, C., Knabl, L., Orth-Höller, D., Ellemunter, H., Niedermayr, K. & Bernkop-Schnürch, A. 2018. Development of self-emulsifying drug delivery systems (SEDDS) for ciprofloxacin with improved mucus permeating properties. *International journal of pharmaceutics*, 547:282-290.

Zhang, L., Zhang, L., Zhang, M., Pang, Y., Li, Z., Zhao, A. & Feng, J. 2015. Self-emulsifying drug delivery system and the applications in herbal drugs. *Drug delivery*, 22:475-486.

Zhou, X., Hao, Y., Yuan, L., Pradhan, S., Shrestha, K., Pradhan, O., Liu, H. & Li, W. 2018. Nano-formulations for transdermal drug delivery: A review. *Chinese chemical letters*, 29:1713-1724.

Zupančič, O., Rohrer, J., Thanh Lam, H., Grießinger, J.A. & Bernkop-Schnürch, A. 2017. Development and *in vitro* characterization of self-emulsifying drug delivery system (SEDDS) for oral opioid peptide delivery. *Drug development and industrial pharmacy*, 43:1694-1702.

Annexure E

Clofazimine release and dermal diffusion studies of finally selected topical SEDDS formulations

E.1 Introduction

Senapati *et al* (2018) stated that not only a potent drug is required to aid in disease treatment regimens, but also an efficacious drug delivery system should be employed. During the development of topical drug delivery systems, the vehicle utilised to present a drug at the skin surface is of profound significance (Fantini *et al*., 2019). In fact, the vehicle can influence a drug partitioning into the SC, diffusion of the said drug across the SC as well as drug retention within the different skin layers (Fantini *et al*., 2019; Zhang *et al*., 2017). Moreover, one must bear in mind the impact of vehicle metamorphosis on the efficacy of topically delivered drugs (Fantini *et al*., 2019; Surber & Kottner, 2017). Vehicle metamorphosis refers to compositional changes of the drug delivery system as brought about by evaporation of excipients, such as water; and extraction of SC lipids upon prolonged contact with the skin (Fantini *et al*., 2019). Evaporation of water from applied formulations can contribute towards exposing the skin surface to supersaturated clofazimine concentrations that render enhanced dermal drug delivery (Parhi & Swain, 2018). Moreover, extraction of SC lipids can be utilised as a drug delivery technique in order to facilitate permeation of clofazimine into the epidermis and dermis of the skin (Haque & Talukder, 2018). Additionally, plant based oils together with the included surface active agents that form part of the vehicle, can cause disruption of SC lipid arrangement and in turn establish drug penetration enhancement (Lane, 2013). Therefore, *in vitro* studies were performed by employment of Franz cell diffusion studies for the purpose of identifying the specific plant based oil, incorporated in topical SEDDS formulations, which demonstrated the most successful topical delivery of clofazimine (Čuříková *et al*., 2017).

Franz cell diffusion studies are useful for investigating dermal drug delivery (Čuříková *et al*., 2017; Simon *et al*., 2016; Liu *et al*., 2015). Despite drawbacks, such as difficult data reproducibility and no consideration of metabolic processes that occur within different skin layers, these experiments provide a controlled environment where only the tested formulation and skin can be considered as variables (Čuříková *et al*., 2017; Li *et al*., 2017). During these experiments the *in vivo* situation can be predicted my means of *in vitro* observations (Levintova *et al*., 2011). Ganti *et al* (2018) reported that maximum flux of a drug presented at the skin surface will be achieved if a supersaturated drug concentration is maintained by the applied formulation. However, the argument can be made that not only steady-state flux, as established by exposure to supersaturated drug concentrations, but also

exposure to components included in topical formulations that can render reversible changes to barrier properties of the skin, such as plant based oils, can facilitate increased drug delivery (Ganti *et al*., 2018; Lin *et al*., 2018; Vaughn *et al*., 2018). Furthermore, highly lipophilic drugs, including clofazimine, present unique challenges during topical drug delivery (Czajkowska-Kośnik *et al*., 2018; Jones *et al*., 2016; Ita, 2014; Frelichowska *et al*., 2009). Therefore, drug release and dermal diffusion studies were conducted in order to evaluate not only the release of clofazimine from topical SEDDS formulations, but also to establish the possibility of successful dermal delivery of clofazimine.

E.2 HPLC preparation

The verified HPLC method, described in Annexure A, was utilised during drug release and dermal diffusion studies to determine topically delivered clofazimine concentrations. All HPLC experiments were conducted in a controlled laboratory environment at 25°C. A 0.005 M solution of octane sulphonic acid was prepared in HPLC grade water. Thereafter, the pH of the mobile phase solution was adjusted to a pH of 3.5 by means of orthophosphoric acid (H_3PO_4) addition. The mobile phase was injected at a ratio of 70:30 (acetonitrile: octane sulphonic acid) with an injection volume of 5 µl and a flow rate of 1 ml/min. An optimal wavelength of 284 nm was used to enable detection of clofazimine. The runtime of each injection was 6 min and the approximate retention time of clofazimine was ± 4.0 min.

A standard solution of clofazimine was prepared by weighing 10 mg of clofazimine and adding methanol to a volume of 100 ml. Subsequently, 5 ml of the mother solution was removed with a glass pipet and transferred into a 20 ml volumetric flask where methanol was added again. This standard solution was injected in volumes of 2.5 µl, 5 µl, 10 µl, 15 µl and 20 µl to obtain a standard curve utilised to calculate the concentration clofazimine detected in the receptor phase.

E.3 Preparation of the donor- and receptor phase

The donor phase was prepared by means of the water titration method; and subsequent cooling of the SEDDS formulations, as described in Annexure C. During Franz cell studies placebo SEDDS formulations were prepared as well as SEDDSs containing clofazimine. The clofazimine SEDDS formulations varied in clofazimine concentration during drug release studies: ARG 3 contained 0.2% clofazimine; 0.6% clofazimine was incorporated in AVO 2; and 0.1% clofazimine was incorporated into both the MAC 2 and OLV 5 formulations. The concentration of clofazimine included in each of the finally selected topical SEDDS formulations is similar to the solubility of clofazimine depicted in individual plant based oils, as demonstrated in Annexure B. However, during dermal diffusion studies the clofazimine concentration was increased to 2% for all SEDDS formulations in order to enable improved

detection during HPLC quantification. Moreover, by exposing the skin to supersaturated clofazimine concentrations maximal flux of clofazimine can possibly be achieved (Ganti *et al*., 2018; Parhi & Swain, 2018). In addition, Rani *et al* (2019) stated that SEDDSs displayed detained nucleation as well as crystal growth due to increased kinetic- and thermodynamic inhibition of drug precipitation if a saturated drug concentration was included in SEDDS formulations. The excipients needed to prepare the final SEDDS formulations are presented in **Table E.1**.

Table E.1: Quantity of excipients needed to prepare final SEDDS formulations

	ARG 3	**AVO 2**	**MAC 2**	**OLV 5**
Oil	7.5 ml	7.2 ml	8 ml	6.1 ml
Tween®80	5 ml	6.4 ml	6 ml	4.2 ml
Span®60	5 mg	6.4 mg	6 mg	4.2 mg
Water	7.5ml	6.4 ml	6 ml	9.7 ml

The receptor phase, consisting of phosphate buffer solution (PBS) and HPLC grade ethanol (9:1), was prepared as follows:

- A mass of 6.5 g KH_2PO_4 was weighed and dissolved in 250 ml HPLC grade water.
- Next, 1.5 g NaOH was weighed and dissolved in 400 ml HPLC grade water.
- The two solutions were added, mixed and HPLC grade water was added to obtain a final solution of 1000 ml.
- Thereafter, the pH of the PBS solution was adjusted to a pH of 7.4 when necessary (Čuříková *et al*., 2017).

The phosphate buffer solution was kept in a refrigerator (for not more than 6 months) at a temperature of ± 4°C until utilised. It is stated that drugs with an aqueous solubility ≤ 0.1 mg/ml acquire the inclusion additional solubilisers in the receptor compartment for the purpose of providing a solvent that solubilises the lipophilic drug as a drug must be in solution in order to be detected by means of HPLC analysis (Trombino *et al*., 2019; Esposito *et al*., 2018). For this reason, 10% analytical grade ethanol was included in the receptor phase for the purpose of improving the solubility of clofazimine during the conduction of dermal diffusion studies (Trombino *et al*., 2019; Esposito *et al*., 2018).

E.4 Conditions of Franz cell experiments

During both drug release and dermal diffusion studies 12 Franz cells were prepared for each experiment. Every experiment consisted of 10 Franz cells containing a specific clofazimine SEDDS formulation and 2 Franz cells with placebo SEDDS formulations as donor phases. Franz cell preparation was done as follows:

- First, a small stirring magnet was placed in each receptor compartment that was set at a stirring rate of 750 rpm throughout the duration of the experiments (Buhecha *et al*., 2019).
- Dow Corning® vacuum grease (Sigma-Aldrich, Germany) was thinly spread across the connecting parts of the donor- and receptor phases before placing an artificial membrane with a pore size of 0.45 µm (i.e. drug release studies) or a cut skin circle (i.e. dermal diffusion studies) between the uniting parts (Simon *et al*., 2016).
- Next, the donor- and receptor phases were sealed by applying copious amounts of vacuum grease to seal the donor-and receptor compartments.
- The SEDDS formulations were transferred into donor compartments (i.e. 1 ml in each compartment) and each receptor compartment was filled with 2 ml PBS: analytical grade ethanol (9:1) solution (Liu *et al*., 2015).
- Thereafter, Parafilm® together with a lid were placed over each donor compartment before positioning the Franz cells in a pre-heated water bath.

During this study two Grant® water baths (Grant Instruments, United Kingdom), equipped with Variomag® magnetic hot plates (Variomag Instruments, United States of America), were utilised at different temperatures:

Water bath (± 32°C)

- The SEDDS formulations (placebo formulations and SEDDSs containing clofazimine) were placed in this pre-heated water bath, in order to mimic the skin surface temperature of 32°C, prior to transfer into the donor phase of the individual Franz cells (Čuříková *et al*., 2017).

Water bath (± 37°C)

- The PBS: analytical grade ethanol (9:1) solution was kept in this pre-heated water bath to enable heat transfer to the receptor phase. This facilitates similar heat profiles of the receptor phase to the temperature of blood beneath the skin.
- The 12 prepared Franz cells were placed into this water bath during both drug release and dermal diffusion studies.

- Drug release studies involved an experiment of 6 h with hourly extractions for the purpose of maintaining sink conditions (Buhecha *et al*., 2019).
- Diffusion studies, on the other hand, consisted of one extraction from the respective receptor phases after a 12 h period, as topical delivery is desired.

E.5 Skin preparation

Skin utilised during this study was obtained from anonymous Caucasian female donors that underwent abdominoplastic surgery, performed by qualified surgeons. Full-thickness tummy-tuck skin was frozen, at a temperature of -20°C upon arrival at the bio-safety laboratory at the Potchefstroom campus of the North West University. Ethics approval was granted (ethics number: NWU-00111-17-A1-07) by the Research Ethics Committee of the North-West University for the purpose of collecting biological material as well as utilising human skin during dermal diffusion experiments. The full-thickness skin was inspected for abnormalities that might influence dermal diffusion such as the presence of *striae distensae* (Mitts *et al*., 2010). Thereafter, the skin was dermatomed using a Zimmer™ electric dermatome, model 8821, to harvest skin pieces of a continues thickness (i.e. 400 µm) for the purpose of obtaining harvested skin pieces comprising SC, epidermis-dermis and dermis. The harvested skin was placed on Whatman® filter paper, wrapped in foil and stored at a temperature of -20°C for no longer than a period of 6 months. Prior to dermal diffusion studies the harvested skin were cut into skin circles with a diameter of 15 mm.

E.6 Tape stripping

After performing dermal diffusion studies, as described in Sections E.3 and E.4, the skin circles were removed from the Franz cells and dapped clean with paper towels. Scotch® magic tape was employed to remove only the area of each skin circle that was subjected to dermal diffusion. The first tape strip utilised on each skin circle was discarded; and the following 15 tape strips were placed into a polytop. Skin that was tape stripped is the SC-epidermis layer of the skin and the remaining skin must have a gleaming appearance to indicate complete removal of the SC. This layer where the SC was removed from, is the epidermis-dermis of the skin. The epidermis-dermis parts were cut into small pieces, within the polytop, to enlarge the surface area, and placed into individual polytops. Each polytop was filled with 5 ml analytical grade ethanol and transferred into the refrigerator to remain overnight at a temperature of 4°C. After removing the polytops from the refrigerator, ethanol was extracted from each polytop and filtered through a 45 µm filter prior to HPLC analysis.

E.7 Data analysis

E.7.1 Analysis of drug concentration after topical application

After completion of dermal diffusion studies, the cumulative clofazimine concentration (µg/cm^2) was plotted against time. Likewise, flux values (µg/cm^2.h) were established by constructing the steady-state slope of the average cumulative clofazimine concentrations against time. The total clofazimine percentage yielded after a period of 12 h, as observed for dermal diffusion studies, and after a time span of 6 h, as depicted by drug release studies, were determined.

HPLC chromatographs of the placebo SEDDS formulations indicated whether the obtained results indeed represented clofazimine concentrations or other excipients present within SEDDS formulations. However, outlier data obtained during dermal diffusion studies were omitted as certain Franz cells fell victim to leakage or early depletion of topical SEDDS formulations. Hence, the relevant number of Franz cells utilised during each experiment is indicated in Section E.9.

E.7.2 Statistical data analysis

Data obtained during drug release and dermal diffusion experiments were processed by both descriptive and interferential statistical methods. Descriptive statistics refer to the employment of data such as the median (statistical centre point) and mean together with the standard deviation (Brownstein *et al*., 2019). The aim of descriptive statistics is to describe and also compare groups within the same population or sample (Ali & Bhaskar, 2016). These descriptive statistical values were graphically illustrated by utilising box-plot representations which enabled inclusion of error bars (measuring of data distribution). One must bear in mind that the median value can be preferred during statistical data analysis as the median is less affected by data of an outlier nature that can facilitate skew data distribution. On the other hand, the mean is disproportionately influenced by outlier data (Rodriguez & Valdora, 2019). Contrary, inferential statistical analysis includes analysis of variance (ANOVA) as well as testing of parametric assumptions (Ali & Bhaskar, 2016; Marshall & Jonker, 2011).

For the purpose of identifying significant flux differences between topical SEDDS formulations, subjected to drug release and dermal diffusion studies, one-way ANOVA was utilised to determine the significance of the effect of groups. This parametric method can only be used if the data did not deviate from normality and the variances (or standard deviations) of groups are equal. When these assumptions are not met, the non-parametric Kruskal-Wallis test was employed to compare groups analogously to one-way ANOVA (McDonald, 2009). In addition, the post-hoc Tukey honestly significant difference (HSD) test was employed after one-way

ANOVA analyses of flux values obtained during drug release studies. This parametric test is preferred when pairwise comparisons are conducted (Kim, 2015), but if the parametric assumption does not hold, pairwise multiple comparisons between groups can be performed after applying the Kruskal-Wallis test. The statistical methods employed during this study are illustrated in **Figure E.1**.

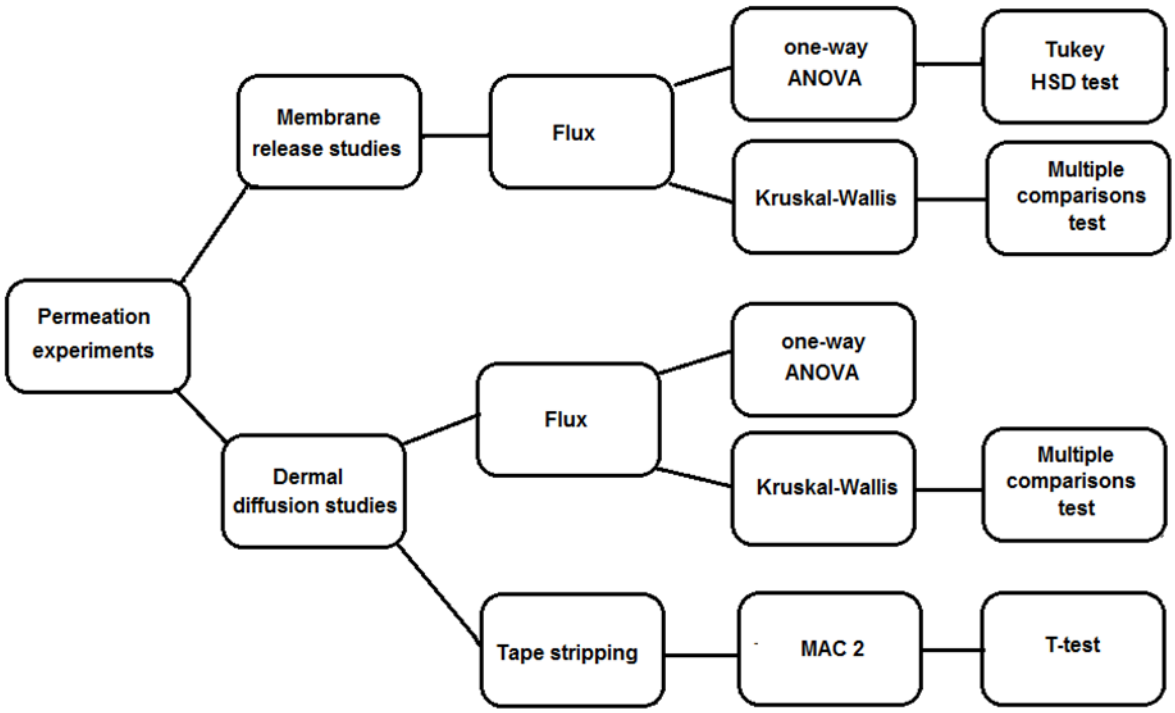

Figure E.1: Flow diagram of statistical methods employed during analyses of topical SEDDS formulations

As seen in **Figure E.1** the first parametric assumption of normal data distribution can result in discarding the results of a one-way ANOVA and a Tukey HSD test; and rather use the non-parametric results of the Kruskal-Wallis and multiple comparison tests. These data analyses could be done for the purpose of identifying the differences observed between cumulative clofazimine concentrations as displayed by divergent topical SEDDS formulations. These results entailed the actual p-values for pairwise comparisons, group means and medians, and test statistics in order to illustrate differences between topical SEDDS formulations (McDonald, 2009).

After conducting dermal diffusion studies, it was found that clofazimine was only detected in the SC-epidermis skin layer, as obtained by tape stripping, for macadamia oil SEDDS. Hence, the T-test by means of the group pairing method was utilised to compare differences between cumulative clofazimine concentrations observed within the SC-epidermis and epidermis-dermis, respectively. T-test analyses involve comparison of mean values of two groups also

assuming the normal distribution of data (Marshall & Jonker, 2011). Therefore, SEDDSs comprising, argan-, avocado- and olive oil were exclusively analysed by one-way ANOVA and the non-parametric Kruskal-Wallis test. Here the one-way ANOVA results were discarded due to the fact that normality of the data did not hold, and the non-parametric Kruskal-Wallis test was employed, followed by the multiple comparisons in order to signify differences between cumulative clofazimine concentrations as obtained by SEDDS formulations comprising argan-, avocado-, macadamia- and olive oil. Statistical analyses of topical SEDDS formulations were performed using Statistica (version 10) by the Statistical Consultation Services, North-West University, Potchefstroom Campus.

E.8 Analysis of clofazimine release from the finally selected SEDDS formulations

Drug release studies utilising synthetic membranes were conducted for the purpose of confirming release of clofazimine from the dosage form prior to performance of dermal diffusion studies. Favourably, clofazimine was depicted in the receptor phase during these experiments of topical SEDDS formulations comprising argan-, avocado-, macadamia- and olive oil. Data obtained after drug release studies are expressed as a percentage of the initial quantity of clofazimine included in the finally selected SEDDS formulations as seen in **Table E.2**.

Table E.2: Data obtained for clofazimine release studies after 6 h

Topical SEDDS formulation	Average % released	Average cumulative concentration (µg/cm^2)	Median of cumulative concentration (µg/cm^2)
ARG 3	0.5 ± 0.002	0.729 ± 0.030	0.726
AVO 2	2.5 ± 0.007	1.685 ± 0.161	1.707
MAC 2	2 ± 0.002	0.586 ± 0.038	0.571
OLV 5	7 ± 0.002	0.459 ± 0.350	0.445

Average cumulative concentration is presented as mean ± standard error, whereas average percentage released is displayed as mean ± standard deviation

As seen (**Table E.2**) the average percentage clofazimine released can be ranked as follows: OLV 5 >>> AVO 2 > MAC 2 > ARG 3. However, it should be highlighted that topical SEDDS formulations utilised to conduct drug release studies contained different concentrations of clofazimine. These concentrations were determined according to the solubility of clofazimine in each plant based oil. Nonetheless, after completion of the drug release studies it was decided to include 2% clofazimine into each finally selected topical SEDDS formulation in

order to improve detectability of clofazimine by means of HPLC analysis; as well as to reduce the variables between the different SEDDS formulations. Moreover, as stated previously, SEDDSs formulated in a state of supersaturation have exhibited kinetic as well as thermodynamic inhibition of drug precipitation by detaining crystal growth and nucleation (Rani *et al*., 2019). Also, the flux of clofazimine can possibly be enhanced if the clofazimine is presented in a state of saturation at the skin surface (Ganti *et al*., 2018; Parhi & Swain, 2018). One-way ANOVA depicted significant differences between cumulative clofazimine concentrations, i.e., $F(3)=40.755$, $p < 0.001$. Note that while a deviation from normality was observed, a highly significant difference between topical SEDDS formulations justified the use this parametric analysis (the non-parametric analysis was also applied and resulted in the same conclusion). As evident from the p-value, clear differences can be observed between the mean values of the tested/selected SEDDS formulations. Hence, the post-hoc Tukey HSD test was employed to illustrate the observed differences and these results are displayed in **Table E.3**.

Table E.3: Data differences as indicated by post-hoc Tukey HSD test

Cell number	SEDDS formulations	Flux mean (µg/cm^2)
4	**OLV 5**	0.459^a
3	**MAC 2**	0.586^a
1	**ARG 3**	0.729^a
2	**AVO 2**	1.685^b

a, b indicates formulations not statistically significant on 5% level where only AVO 2 statistically differs from OLV 5, MAC 2 and ARG 3

E.9 Dermal diffusion

The dermis marks the final barrier to be crossed before a drug can be systemically delivered (Bellefroid *et al*., 2019). As patients suffering from CTB can also be infected with pulmonary TB, topically applied clofazimine should not diffuse through the skin and into the systemic circulation as this can interfere with systemic TB regimens utilised to treat pulmonary TB (Tshisevhe *et al*., 2019). Therefore, no clofazimine should be detected in the receptor phase after a 12 h period of Franz cell diffusion studies. Fortunately, clofazimine was not detected in any receptor phase after completion of the 12 h period. However, clofazimine was only detected in the epidermis-dermis skin layer and not in the SC-epidermis skin layer for formulations ARG 3, AVO 2 and OLV 5. Therefore, indicating clear topical drug delivery of clofazimine. The permeation results obtained by the finally selected topical SEDDS formulations are depicted in **Table E.4**.

Table E.4: Permeation of clofazimine into epidermis-dermis after 12 h

Topical SEDDS formulation	Number of Franz cells	Average % diffused	Average cumulative concentration (µg/cm^2)	Median of cumulative concentration (µg/cm^2)
ARG 3	8	0.95 ± 4.166	7.099 ± 1.473	6.471
AVO 2	9	0.25 ± 0.557	2.755 ± 0.220	2.616
MAC 2	8	0.45 ± 2.013	3.573 ± 0.712	3.132
OLV 5	8	1.2 ± 7.576	9.105 ± 2.679	7.602

Average cumulative concentration is presented as mean ± standard error, whereas average percentage released is displayed as mean ± standard deviation

From **Table E.4** it is clear that the OLV 5 SEDDS formulation facilitated the highest average cumulative clofazimine concentration as well as the highest average clofazimine percentage diffused. Furthermore, the difference between the average cumulative concentration and median depicted by the OLV 5 SEDDS is considered small; thus, illustrating a decreased quantity of outlier data. In contrast, the SEDDS formulation, AVO 2, that exhibited the second highest clofazimine amount released during drug release experiments, displayed the lowest clofazimine permeation during dermal diffusion studies. Since the data were not normally distributed, statistical analysis by means of non-parametric Kruskal-Wallis testing was done and rendered statistically significant differences. These clear differences, indicated in red as assisted by divergent flux values, are presented in **Table E.5**.

Table E.5: Multiple comparisons of p-values depicted for topical SEDDS formulations

ARG 3				
AVO 2	0.018			
MAC 2	0.217	1.000		
OLV 5	1.000	0.010	0.142	
	ARG 3	**AVO 2**	**MAC 2**	**OLV 5**

The signified statistical differences displayed in **Table E.5** can be linked to the unique composition of each plant based oil (Kala & Juyal, 2018; Viljoen *et al*., 2015; Kumar *et al*., 2014). The presence of components such as certain fatty acids can enhance permeation of clofazimine into the skin layers (Kala & Juyal, 2018; Lin *et al*., 2018; Vaughn *et al*., 2018). Different mechanisms of increased permeation can be established by

fatty acids, such as disruption or fluidisation of SC lipids (Lane, 2013). However, the extent of skin penetration enhancement is influenced by the type of fatty acid as well as the concentration of specific fatty acids naturally present within individual plant based oils (Lin *et al*., 2018; Vaughn *et al*., 2018). Average cumulative clofazimine concentrations rendered by the SEDDS AVO 2 formulation differs from both the ARG 3 and OLV 5 SEDDS formulations, respectively. Whereas, the AVO 2 and MAC 2 SEDDS formulations do not display any statistically significant differences in terms of dermal clofazimine delivery. The cumulative clofazimine concentrations obtained by topical SEDDS formulations are illustrated by **Figure E.2**.

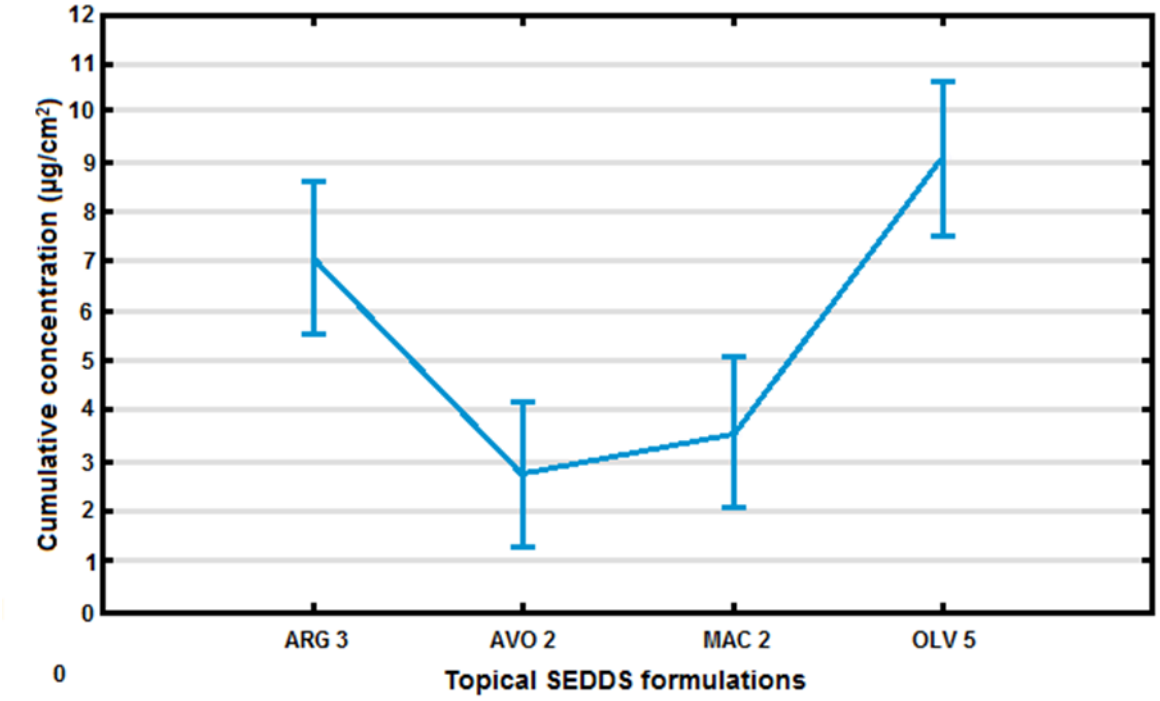

Figure E.2: Cumulative clofazimine concentrations as depicted by the selected topical SEDDS formulations

Olive oil displayed the highest permeation of clofazimine during both drug release and dermal diffusion studies. This increased permeation can be enabled by the naturally incorporated high concentration of oleic acid present within olive oil (Cicero *et al*., 2018; ElMasry *et al*., 2018). Lane (2013) described oleic acid as a penetration enhancer which applies disordering effects on the SC lipids. Additionally, oleic acid has displayed increased fluidising properties of SC lipids that contribute toward increased drug permeation and flux values (ElMasry *et al*., 2018; Hadgraft & Lane, 2016). However, it has been speculated that the *cis* form of oleic acid is the only form that can facilitate skin penetration enhancement due to the unsaturated structure present within the molecule itself (Lane, 2013). In addition, this theory is confirmed by

observation of oleic acid as separate pools within lipid areas of the SC that assist enhanced permeation within fluidised domains (Hadgraft & Lane, 2016). This non-homogenous distribution of oleic acid within the SC lipids can be indicative of the *cis* formation that predominantly condenses with itself instead of evenly distributing throughout the SC lipids (Lane, 2013). Besides, non-homogenous oleic acid distribution can implicate reversible as well as permeable defects formed within the SC lipids which facilitate enhanced dermal drug delivery (Hadgraft & Lane, 2016). One must bear in mind that argan-, avocado-, macadamia- and olive oil all contain oleic acid (Cicero *et al*., 2018; Rueda *et al*., 2014). However, each plant based oil consists of a different oleic acid concentration and can be ranked from highest to lowest oleic acid concentrations as follow: olive oil >>> avocado oil >> macadamia oil > argan oil. Contrary, dermal diffusion studies rendered permeation results that displayed penetration enhancement effects as follow: olive oil >argan oil >> macadamia oil > avocado oil.

Argan oil SEDDS formulations depicted the second highest permeation enhancement of clofazimine during dermal diffusion studies. This plant based oil contains the highest stearic- and linoleic acid concentrations between the selected plant based oils (Cicero *et al*., 2018; Rueda *et al*., 2014). Linoleic acid is associated with intensified skin repair properties due to increased hydration of the SC (Hu *et al*., 2019). Interestingly, stearic acid is also known as the saturated form of oleic acid (Almeida *et al*., 2018; El Kharrassi *et al*., 2018). The melting point of this unsaturated fatty acid is reported as 69.3°C (Jiménez-Peñalver *et al*., 2018). Hence, stearic acid will most probably be presented at the skin surface in an undissolved form as the melting point of this free fatty acid far exceeds skin surface temperature of approximately 32°C (Heylings *et al*., 2018). Therefore, undissolved stearic acid can possibly act as an occlusive agent by forming a residual layer on the skin surface after emulsion depletion (Gore *et al*., 2018; Vaughn *et al*., 2018). Thus, providing skin hydration that is known to enhance dermal drug delivery by means of a swelling process within the SC (Vaz *et a*l., 2019; Pham *et al*., 2016; Warner *et al*., 2003). This hydrating effect, possibly established by argan oil, can loosen the strictly packed structure of the SC and in turn achieve increased permeation of clofazimine (Vaz *et al*., 2019; Warner *et al*., 2003).

The AVO 2 SEDDS formulation displayed the lowest cumulative clofazimine concentration in the epidermis-dermis skin layer. A possible reason for this decreased permeation can be linked to the high palmitic acid concentration naturally present within avocado oil (Tan, 2019; Čižinauskas *et al*., 2017). Palmitic acid is classified as a saturated fatty acid (SFA) (Čižinauskas *et al*., 2017; Mansour *et al*., 2017). Despite the increased drug permeation facilitated by palmitic acid, SFAs are deliberated less powerful skin penetration enhancers

when compared to unsaturated fatty acids (UFAs) (Lundborg *et al*., 2018; Čižinauskas *et al*., 2017; van Zyl *et al*., 2016; Viljoen *et al*., 2015). **Table 2** in Chapter 2 showed that avocado oil comprises the second highest SFA concentration compared to argan-, avocado-, macadamia- and olive oil. Argan oil exceeds the SFA content of avocado oil (Cicero *et al*., 2018). However, argan oil contains a higher stearic acid level when related to avocado oil, which has been mentioned for its possibly improved occlusive effects (Gore *et al*., 2018; Vaughn *et al*., 2018). The less effective skin permeation facilitated by SFAs can be clarified by the decreased lipid solubility of SFAs within the SC lipids as established by their increased melting points (Knothe & Dunn, 2009; Chi *et al*., 1995; Aungst, 1989). For this reason, SFAs portray a decreased potential to insert themselves into the SC that is accompanied by reduced disruption of the SC lipid arrangement (Lundborg *et al*., 2018; van Zyl *et al*., 2016; Kim *et al*., 2008). Additionally, SFAs vary from UFAs in terms of chemical structure that can also possibly contribute towards decreased dermal permeation (Rodríguez *et al*., 2019; Kanikkannan *et al*., 2006).

UFAs can be divided into two sub-categories, namely: polyunsaturated fatty acids (PUFAs) and monounsaturated fatty acids (MUFAs) (Rodríguez *et al*., 2019; Cicero *et al*., 2018). Remarkably, Hu *et al.* (2019) as well as Kochhar & Henry (2009) claimed that the presence of PUFAs can decrease shelf-life of topical SEDDS formulations as these fatty acids are more prone toward oxidative instability. The PUFA content of the utilised plant based oils can be ranked as: argan oil >> macadamia oil >> avocado oil >olive oil. Whereas, the inverse relationship is obtained when the MUFA content of these plant based oils is categorised: olive oil > avocado oil > macadamia oil >>> argan oil. MUFAs, such as oleic acid and linolenic acid, indicated increased permeation of clofazimine when compared to the PUFA content (van Zyl *et al*., 2016). This finding is confirmed as the oils comprising higher oleic acid amounts seem to most successfully enhance topical drug penetration in this study.

If the ratio of components included in the finally selected SEDDS formulations is considered (**Table E.1**), the OLV 5 SEDDS formulation clearly has the highest water content between these formulations. This formulation rendered the highest dermal permeation of clofazimine despite having the lowest concentrations plant based oil as well as surfactant present. Overall, the individual plant based oil together with the surfactant are capable of enhanced dermal drug delivery; and theoretically increased concentrations of these components should render elevated dermal permeation of clofazimine. However, the effectiveness of SEDDS formulations as a topical dosage form can possibly be linked to increased water content that facilitates enhanced flux of lipophilic drugs (Chauhan & Sharma, 2019; Fantini *et al*., 2019; Leite-Silva *et al*., 2012; Barry, 1991). This increased flux is accomplished by the hydrophilic

nature of the vehicle compared to lipophilic nature of both the SC and clofazimine that forces clofazimine to permeate into the SC instead of remaining in the hydrophilic SEDDS formulation (Chauhan & Sharma, 2019; Sawant *et al*., 2010; Barry, 1991). Interestingly, the MAC 2 SEDDS formulation comprises the highest oil content of the finally selected SEDDS formulations and was also the only SEDDS formulation that rendered clofazimine delivery in both the SC-epidermis and epidermis-dermis skin layers. Hence, the possibility that the lipophilic clofazimine depicted slower release as well as permeation from the SEDDS containing macadamia oil can possibly be explained by clofazimine favouring the lipophilic environment of the formulation and SC instead of the hydrophilic epidermis-dermis skin layer (Chauhan & Sharma, 2019; Leite-Silva *et al*., 2012; Sawant *et al*., 2010; Barry, 1991).

E.10 Tape stripping

The lipophilic nature of the outermost skin layer shields the body from entry of dangerous substances (Bellefroid *et al*., 2019). However, during topical drug delivery the protective mechanisms of the outermost skin layer must be manipulated in order to allow permeation of drugs (Kala & Juyal, 2018). Plant based oils were included as skin penetration enhancers to observe which plant based oil included in the formulated SEDDSs possessed the most desirable penetration enhancement profile to deliver the highly lipophilic clofazimine. Fortunately, all topical SEDDS formulations tested reached the underlying skin layers. Though, as displayed in **Table E.6**, the MAC 2 SEDDS formulation is the only topical SEDDS in this study that yielded cumulative clofazimine concentrations in both the SC-epidermis and epidermis-dermis skin layers, respectively.

Table E.6: Permeation of clofazimine into SC-epidermis after 12 h

Topical SEDDS formulation	Number of Franz cells	Average % diffused	Average cumulative concentration (µg/cm^2)	Median of cumulative concentration (µg/cm^2)
ARG 3	8	0	0	0
AVO 2	9	0	0	0
MAC 2	8	1.95 ± 0.011	45.142 ± 4.707	41.069
OLV 5	8	0	0	0

Average cumulative concentration is presented as mean ± standard error, whereas average percentage released is displayed as mean ± standard deviation

Clofazimine was not detected in the individual tape stripping samples of the SEDDS formulations, ARG 3, AVO 2 or OLV 5. Thus, it is probable that the fatty acid composition of macadamia oil can provide prolonged permeation of clofazimine. The variance in

concentration observed by the MAC 2 SEDDS formulation in the SC-epidermis and epidermis-dermis are shown in **Table E.7**.

Table E.7: Permeation of formulation MAC 2 after 12 h

Skin layers	Average % diffused	Average cumulative concentration (µg/cm^2)	Median of cumulative concentration (µg/cm^2)
SC-epidermis	1.95 ± 0.011	45.142 ± 4.707	41.069
Epidermis-dermis	0.45 ± 2.013	3.573 ± 0.712	3.132

Average cumulative concentration is presented as mean ± standard error, whereas average percentage released is displayed as mean ± standard deviation

It is evident that an increased cumulative concentration of clofazimine was depicted in the outermost SC-epidermis skin layer. Moreover, the closely related average cumulative concentration and median value indicate the absence of outlier data. Significant statistical differences were observed by employment of both the T-test and the grouping method. The statistical observations can be described as $t(14)=8.732$ ($p < 0.001$). Distribution of permeation results obtained for the MAC 2 SEDDS formulation is illustrated in the box-plot representation of the aforementioned results (**Figure E.3**).

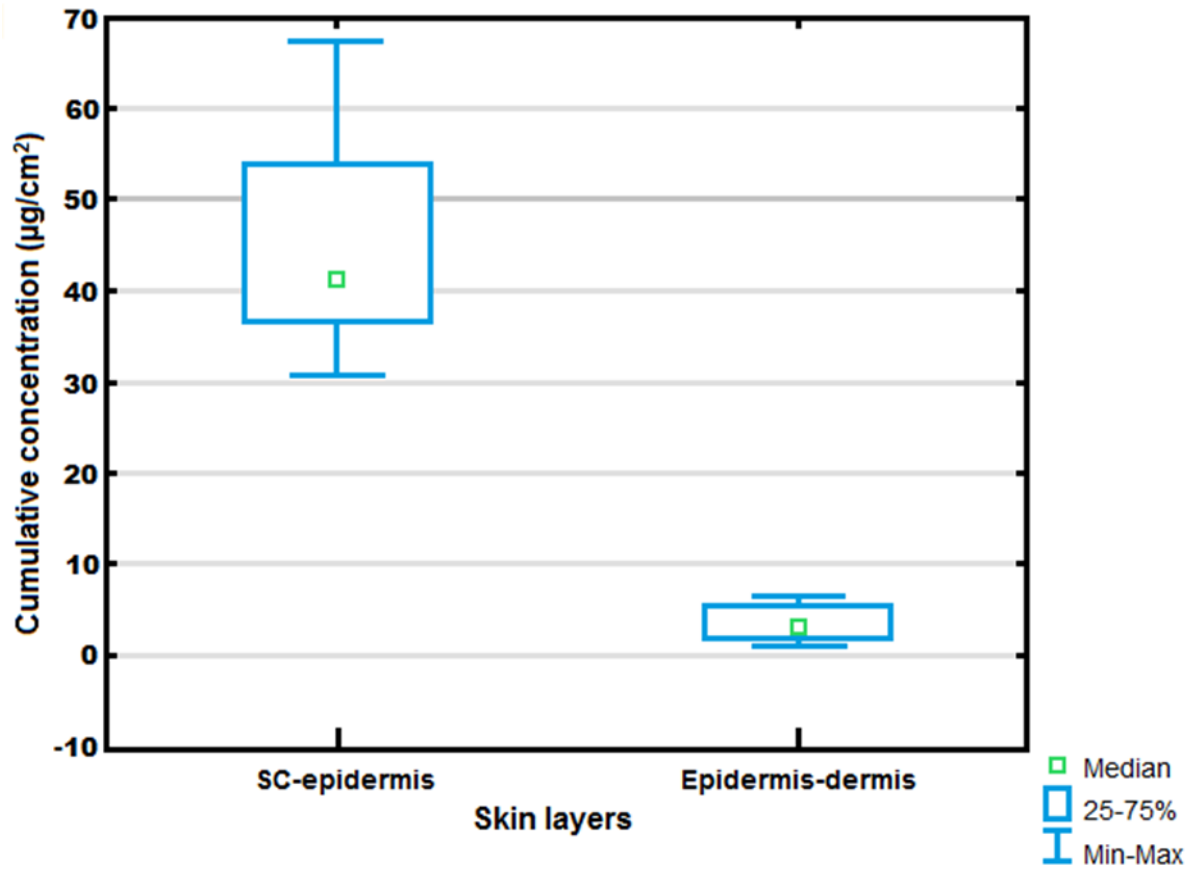

Figure E.3: Cumulative clofazimine concentrations depicted in different skin layers as facilitated by the MAC 2 SEDDS formulation after 12 h

The box-plot representation clearly indicates that the cumulative clofazimine concentrations found in the epidermis-dermis skin layer are closely distributed compared to the increased data distribution displayed in the SC-epidermis skin layer. This is a favourable observation as topical clofazimine delivery facilitated by macadamia oil can possibly yield delivery of clofazimine in predictable concentrations. However, one must bear in mind that this prediction can only be made if skin contact can be maintained for sufficient periods of time as this study was conducted over a period of 12 h.

Macadamia oil is known for its high levels of palmitoleic acid (Cicero *et al*., 2018). Sea-buckthorn pulp oil is also considered a rich source of palmitoleic acid (Olas, 2018). Remarkably, Čižinauskas *et al* (2017) utilised sea-buckthorn pulp oil during their studies concerning the influence of skin penetration enhancement by natural oils and found that palmitoleic acid depicted significantly higher concentrations within the epidermis when compared to palmitoleic concentrations observed in the dermis. Therefore, it can be speculated that palmitoleic acid has a preferred affinity for the epidermis of skin instead of the dermis that can conceivably explain the increased cumulative concentration of clofazimine in

the SC-epidermis skin layer. On the other hand, it has been reported that increased carbons (> C14) present in the carbon backbone of free fatty acids will probably contribute toward increased affinity for the SC and in turn render delayed partitioning of clofazimine into underlying skin layers (Sinha & Kaur, 2000). This increased affinity can be linked to increased molecular weight as facilitated by longer carbon backbone structures that complicate mobility of fatty acids within different skin layers (Balázs *et al*., 2016). Considering that the carbon backbone of palmitoleic acid (C16:1) exceeds 14 carbons, this probably can provide an explanation for the slow permeation into the epidermis-dermis skin layer of the MAC 2 SEDDS formulation compared to the other finally selected SEDDS formulations.

The possibility of vehicle metamorphosis should be considered as this phenomenon can conceivably provide a clarification for only depicting clofazimine in the epidermis-dermis, as exhibited by argan-, avocado- and olive oil SEDDS formulations. Water evaporation can be excluded as the donor compartments of the Franz cells were covered by Parafilm® and a lid for the purpose of avoiding loss of volatile excipients (Fantini *et al*., 2019). However, vehicle metamorphosis also refers to changes that occur in terms of emulsion stability while applied to the skin (Fantini *et al*., 2019). These changes include phase transitions, flocculation and coalescence as facilitated by interactions between a formulation and the skin surface (Fantini *et al*., 2019; Kreilgaard, 2002). During the cleaning process following dermal diffusion studies, it was observed that the MAC 2 SEDDS formulation was the only formulation that displayed clear phase separation in the donor compartment of the Franz cells after the 12 h experiments. Phase separation did not transpire during drug release studies when the formulation was exposed to the same environment for a period of 6 h without exposure to human skin. What is more, the MAC 2 SEDDS formulation provided satisfactory results during thermodynamic stability testing as well as excellent robustness to dilution; and was thus considered a stable topical SEDDS formulation. Nevertheless, this formulation delivered a cloud point temperature of 32.5°C and might therefore be more sensitive for changes established by vehicle metamorphosis due to easier dehydration of excipients within the formulation itself (Ujilestari *et al*., 2018). For this reason, the MAC 2 SEDDS formulation might have struggled to deliver clofazimine as effectively as the ARG 3 and OLV 5 SEDDS formulations.

E.11 Conclusion

Topical drug delivery of the highly lipophilic clofazimine presented challenges due to the fact that drugs as hydrophobic as clofazimine tend to partition into and accumulate in the SC after being released from the applied dosage form (Jones *et al*., 2016; Ita, 2014; Frelichowska *et al*., 2009). The lipophilic nature of the SC creates an ideal environment for lipophilic drugs to establish a reservoir effect while these substances avoid full partitioning into the hydrophilic epidermis (Czajkowska-Kośnik *et al*., 2018; Jones *et al*., 2016; Frelichowska *et al*., 2009). Hence, for most drugs delivered via topical application, partitioning into the lipophilic SC is considered the rate limiting step (Qi & Mitragotri, 2019). Contrary, the rate limiting step for drugs as lipophilic as clofazimine is the partitioning into the hydrophilic epidermis (Lane, 2013). The process of drug delivery from a topically applied SEDDS is illustrated by **Figure E.4**

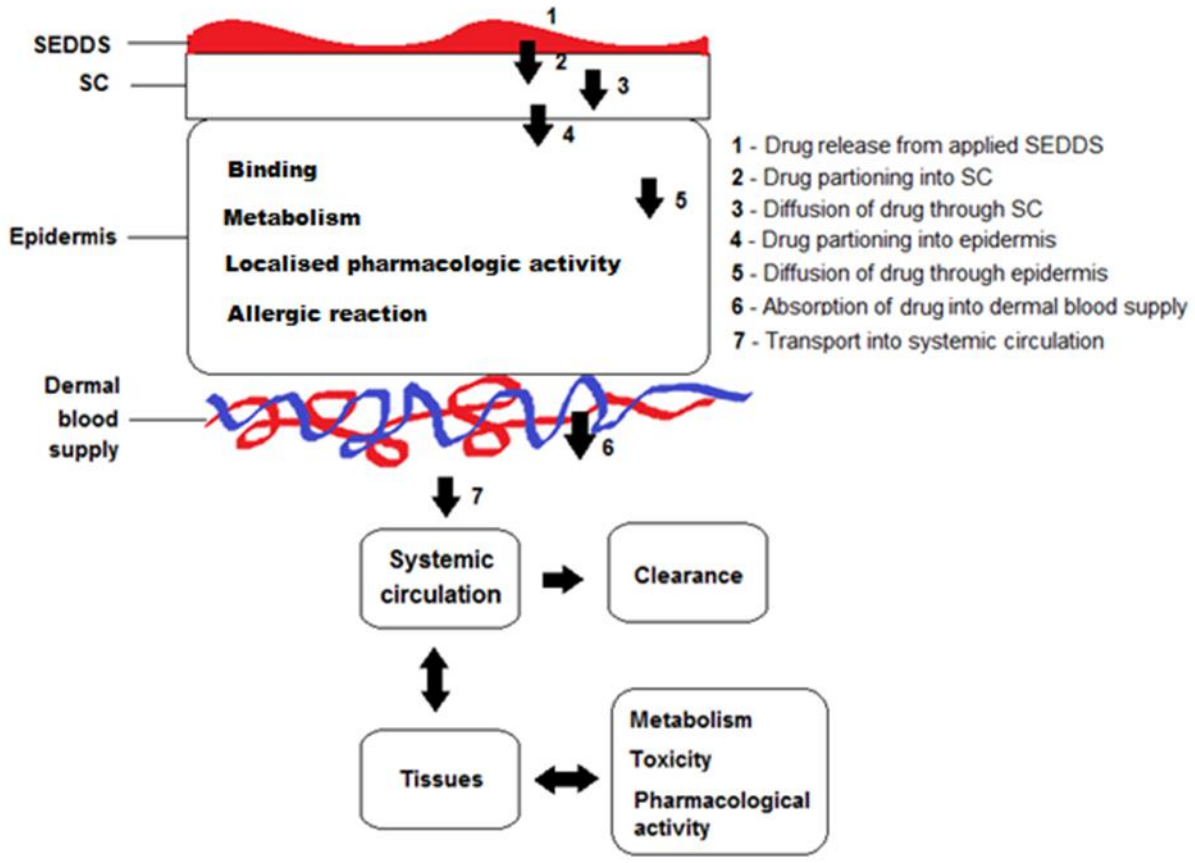

Figure E.4: Schematic illustration of a drug transported from a SEDDS formulation across different skin layers as adapted from Lane, 2013

It is evident from **Figure E.4** that topically applied SEDDS formulations must reach the epidermis skin layer in order to establish a localised pharmacological effect (Lane, 2013). Therefore, the inclusion of penetration enhancers in topical SEDDS formulations are

considered successful as all finally selected SEDDS formulations rendered delivery of clofazimine into the epidermis-dermis skin layer. However, variances observed between different SEDDS formulations and conclusive clofazimine delivery can be linked to the unique composition of each plant based oil (Vaughn *et al*., 2018). Free fatty acids are naturally present within plant based oils and are known skin penetration enhancers (Lin *et al*., 2018; Vaughn *et al*., 2018; van Zyl *et al*., 2016; Viljoen *et al*., 2015; Lane, 2013). Moreover, lipid disruption of the SC is established in many different ways as assisted by individual free fatty acids (Lane; 2013). However, MUFAs are considered more efficient skin penetration enhancers compared to SFAs as also displayed by the notably increased permeation of clofazimine (van Zyl *et al*., 2016). The finally selected olive oil SEDDS formulation comprising the highest oleic acid content rendered the most effective skin penetration enhancement properties during this study. In contrast, argan oil can be considered a useful skin penetration enhancer, despite decreased oleic acid content, as the possible occlusive effects of this plant based oil established the second highest permeation characteristics of clofazimine during this research. To conclude, topical SEDDS formulations can be noted as a definite possibility in terms of facilitating dermal drug delivery of highly lipophilic drugs.

References

Ali, Z. & Bhaskar, S.B. 2016. Basic statistical tools in research and data analysis. *Indian journal of anaesthesia*, 60:662-669.

Almeida, E.D.P., Dipieri, L.V., Rossetti, F.C., Marchetti, J.M., Bentley, M.V.L., Nunes, R.D.S., Sarmento, V.H.V., Valerio, M.E.G., Júnior, J.J.R., Montalvão, M.M. & Correa, C.B. 2018. Skin permeation, biocompatibility and antitumor effect of chloroaluminum phthalocyanine associated to oleic acid in lipid nanoparticles. *Photodiagnosis and photodynamic therapy*, 24:262-273.

Aungst, B.J. 1989. Structure/effect studies of fatty acids isomers as skin penetration enhancers and skin irritants. *Pharmaceutical research*, 3:244-247.

Balázs, B., Vizserálek, G., Berkó, S., Budai-Szűcs, M., Kelemen, A., Sinkó, B., Takács-Novák, K., Szabó-Révész, P. & Csányi, E. 2016. Investigation of the efficacy of transdermal penetration enhancers through the use of human skin and a skin mimic artificial membrane. *Journal of pharmaceutical sciences*, 105:1134-1140.

Barry, B.W. 1991. Lipid-protein-partitioning theory of skin penetration enhancement. *Journal of controlled release,* 15:237-248.

Bellefroid, C., Lechanteur, A., Evrard, B. & Piel, G. 2019. Lipid gene nanocarriers for the treatment of skin diseases: current state-of-the-art. *European journal of pharmaceutics and biopharmaceutics*, 137:95-111.

Brownstein, N.C., Adolfsson, A. & Ackerman, M. 2019. Descriptive statistics and visualization of data from the R datasets package with implications for clusterability. *Data in brief*, 25:Article ID 104004.

Buhecha, M.D., Lansley, A.B., Somavarapu, S. & Pannala, A.S. 2019. Development and characterization of PLA nanoparticles for pulmonary drug delivery: Co-encapsulation of theophylline and budesonide, a hydrophilic and lipophilic drug. *Journal of drug delivery science and technology*, 53:Article ID 101128.

Chauhan, M.K. & Sharma, P.K. 2019. Optimization and characterization of rivastigmine nanolipid carrier loaded transdermal patches for the treatment of dementia. *Chemistry and physics of lipids*, 224:Article ID 104794.

Chi, S.-C., Park, E.S. & Kim, H. 1995. Effect of penetration enhancers on flurbiprofen permeation through rat skin. *International journal of pharmaceutics*, 126:267-274.

Cicero, N., Albergamo, A., Salvo, A., Bua, G.D., Bartolomeo, G., Mangano, V., Rotondo, A., Di Stefano, V., Di Bella, G. & Dugo, G. 2018. Chemical characterization of a variety of cold-pressed gourmet oils available on the Brazilian market. *Food research international*, 109:517-525.

Čižinauskas, V., Elie, N., Brunelle, A. & Briedis, V. 2017. Skin penetration enhancement by natural oils for dihydroquercetin delivery. *Molecules*, 22:Article ID 1536.

Čuříková, B.A., Procházková, K., Filková, B., Diblíková, P., Svoboda, J., Kováčik, A., Vávrová, K. & Zbytovská, J. 2017. Simplified stratum corneum model membranes for studying the effects of permeation enhancers. *International journal of pharmaceutics*, 534:287-296.

Czajkowska-Kośnik, A., Szekalska, M. & Winnicka, K. 2018. Nanostructured lipid carriers: A potential use for skin drug delivery systems. *Pharmacological reports*, 71:156-166.

El Kharrassi, Y., Maata, N., Mazri, M.A., El Kamouni, S., Talbi, M., El Kebbaj, R., Moustaid, K., Essamadi, A.K., Andreoletti, P., Cherkaoui-Malki, M. & Nasser, B. 2018. Chemical and phytochemical characterizations of argan oil (Argania spinosa L. skeels), olive oil (Olea europaea L. cv. Moroccan picholine), cactus pear (Opuntia megacantha salm-dyck) seed oil and cactus cladode essential oil. *Journal of food measurement and characterization*, 12:747-754.

ElMasry, S.R., Hathout, R.M., Abdel-Halim, M. & Mansour, S. 2018. *In Vitro* transdermal delivery of sesamol using oleic acid chemically-modified gelatin nanoparticles as a potential breast cancer medication. *Journal of drug delivery science and technology*, 48:30-39.

Esposito, E., Carducci, F., Mariani, P., Huang, N., Simelière, F., Cortesi, R., Romeo, G. & Puglia, C. 2018. Monoolein liquid crystalline phases for topical delivery of crocetin. *Colloids and surfaces B: biointerfaces*, 171:67-74.

Fantini, A., Padula, C., Nicoli, S., Pescina, S. & Santi, P. 2019. The role of vehicle metamorphosis on triamcinolone acetonide delivery to the skin from microemulsions. *International journal of pharmaceutics*, 565:33-40.

Frelichowska, J., Bolzinger, M.A., Pelletier, J., Valour, J.P. & Chevalier, Y. 2009. Topical delivery of lipophilic drugs from o/w Pickering emulsions. *International journal of pharmaceutics*, 371:56-63.

Ganti, S.S., Nguyen, H.X., Murnane, K.S., Blough, B.E. & Banga, A.K. 2018. Transdermal formulation of 4-benzylpiperidine for cocaine-use disorder. *Journal of drug delivery science and technology*, 47:299-308.

Gore, E., Picard, C.& Savary, G. 2018. Spreading behavior of cosmetic emulsions: Impact of the oil phase. *Biotribology*, 16:17-24.

Hadgraft, J. & Lane, M.E. 2016. Advanced topical formulations (ATF). *International journal of pharmaceutics*, 514:52-57.

Haque, T. & Talukder, M.M.U. 2018. Chemical enhancer: a simplistic way to modulate barrier function of the stratum corneum. *Advanced pharmaceutical bulletin*, 8:169-179.

Heylings, J.R., Davies, D.J. & Burton, R. 2018. Dermal absorption of testosterone in human and pig skin *in vitro*. *Toxicology in Vitro*, 48:71-77.

Hu, W., Fitzgerald, M., Topp, B., Alam, M. & O'Hare, T.J. 2019. A review of biological functions, health benefits, and possible de novo biosynthetic pathway of palmitoleic acid in macadamia nuts. *Journal of functional foods*, 62:Article ID 103520.

Ita, K.B. 2014. Transdermal drug delivery: progress and challenges. *Journal of drug delivery science and technology*, 24:245-250.

Jiménez-Peñalver, P., Castillejos, M., Koh, A., Gross, R., Sánchez, A., Font, X. & Gea, T. 2018. Production and characterization of sophorolipids from stearic acid by solid-state fermentation, a cleaner alternative to chemical surfactants. *Journal of cleaner production*, 172:2735-2747.

Jones, J.G., White, K.A.J. & Delgado-Charro, M.B. 2016. A mechanistic approach to modelling the formation of a drug reservoir in the skin. *Mathematical biosciences*, 281:36-45.

Kala, S. & Juyal, D. 2018. Recent developments on natural transdermal penetration enhancers. *International journal of pharmaceutical sciences and research*, 9:2190-2196.

Kanikkannan, N., Babu, R,J. & Singh, M. 2006. Structure-activity relationship of chemical penetration enhancers. (*In* Smith, E.W. & Maibach, H.I., *eds*. Percutaneous penetration enhancers. 2nd ed. Boca Raton: CRC/Taylor & Francis. p. 17-33.)

Kim, H.Y. 2015. Statistical notes for clinical researchers: post-hoc multiple comparisons. *Restorative dentistry & endodontics*, 40:172-176.

Kim, M.J., Doh, H.J., Choi, M.K., Chung, S.J., Shim, C.K., Kim, D.D., Kim, J.S., Yong, C.S. & Choi, H.G. 2008. Skin permeation enhancement of diclofenac by fatty acids. *Drug delivery,* 15:373-379.

Knothe, G. & Dunn, R.O. 2009. A comprehensive evaluation of the melting points of fatty acids and esters determined by differential scanning calorimetry. *Journal of the American oil chemists' society*, 86:843-856.

Kochhar, S.P. & Henry, C.J.K. 2009. Oxidative stability and shelf-life evaluation of selected culinary oils. *International journal of food sciences and nutrition*, 60:289-296.

Kreilgaard, M. 2002. Influence of microemulsions on cutaneous drug delivery. *Advanced drug delivery reviews*, 54(Suppl.):77-98.

Kumar, A., Aggarwal, G., Singh, K. & Harikumar, S.L. 2014. Comparison of vegetable and volatile oils as skin permeation enhancers for transdermal delivery of losartan potassium. *Der Pharmacia Lettre*, 6:199-213.

Lane, M.E. 2013. Skin penetration enhancers. *International journal of pharmaceutics*, 447:12-21.

Leite-Silva, V.R., de Almeida, M.M., Fradin, A., Grice, J.E. & Roberts, M.S. 2012. Delivery of drugs applied topically to the skin. *Expert review of dermatology*, 7:383-397.

Levintova, Y., Plakogiannis, F.M. & Bellantone, R.A. 2011. An improved *in vitro* method for measuring skin permeability that controls excess hydration of skin using modified Franz diffusion cells. *International journal of pharmaceutics*, 419:96-106.

Li, J., Xu, W., Liang, Y. & Wang, H. 2017. The application of skin metabolomics in the context of transdermal drug delivery. *Pharmacological reports*, 69:252-259.

Lin, T.K., Zhong, L. & Santiago, J. 2018. Anti-inflammatory and skin barrier repair effects of topical application of some plant oils. *International journal of molecular sciences*, 19:Article ID 70.

Liu, D., Zhang, C., Zhang, X., Zhen, Z., Wang, P., Li, J., Yi, D., Jin, Y. & Yang, D. 2015. Permeation measurement of gestodene for some biodegradable materials using Franz diffusion cells. *Saudi pharmaceutical journal*, 23:413-420.

Lundborg, M., Wennberg, C.L., Narangifard, A., Lindahl, E. & Norlén, L. 2018. Predicting drug permeability through skin using molecular dynamics simulation. *Journal of controlled release*, 283:269-279.

Mansour, R.S., Sallam, A.A., Hamdan, I.I., Khalil, E.A. & Yousef, I. 2017. Elucidation of penetration enhancement mechanism of emu oil using FTIR microspectroscopy at EMIRA laboratory of SESAME synchrotron. *Spectrochimica acta part A: molecular and biomolecular spectroscopy*, 185:1-10.

Marshall, G. & Jonker, L. 2011. An introduction to inferential statistics: A review and practical guide. *Radiography*, 17:Article ID e1-e6. https://www-sciencedirect-com.nwulib.nwu.ac.za/science/article/pii/S1078817409001436. Date of access: 26 Oct. 2019.

McDonald, J.H. 2009. Normality. (*In* McDonald, J.H., *ed.* Handbook of biological statistics. Baltimore: Sparky House Publishing. p. 141-144)

Mitts, T.F., Bunda, S., Wang, Y. & Hinek, A. 2010. Aldosterone and mineralocorticoid receptor antagonists modulate elastin and collagen deposition in human skin. *Journal of investigative dermatology*, 130:2396-2406.

Olas, B. 2018. The beneficial health aspects of sea buckthorn (Elaeagnus rhamnoides (L.) A. Nelson) oil. *Journal of ethnopharmacology*, 213:183-190.

Parhi, R. & Swain, S. 2018. Transdermal evaporation drug delivery system: concept to commercial products. *Advanced pharmaceutical bulletin*, 8:535-550.

Pham, Q.D., Björklund, S., Engblom, J., Topgaard, D. & Sparr, E. 2016. Chemical penetration enhancers in stratum corneum—Relation between molecular effects and barrier function. *Journal of controlled release*, 232:175-187.

Qi, Q.M. & Mitragotri, S. 2019. Mechanistic study of transdermal delivery of macromolecules assisted by ionic liquids. *Journal of controlled release*, 311-312:162-169.

Rani, S., Rana, R., Saraogi, G.K., Kumar, V. & Gupta, U. 2019. Self-emulsifying oral lipid drug delivery systems: advances and challenges. *AAPS PharmSciTech*, 20:Article ID 129.

Rodriguez, D. & Valdora, M. 2019. The breakdown point of the median of means tournament. *Statistics & probability letters*, 153:108-112.

Rodríguez, J.F., Dynarowicz-Latka, P. & Conde, J.M. 2019. Interactions of cholesterol and 7-ketocholesterol with unsaturated fatty acids of different unsaturation degree–The monolayer study. *Biochimica et Biophysica Acta (BBA)-Biomembranes*, 1861:1428-1436.

Rueda, A., Seiquer, I., Olalla, M., Giménez, R., Lara, L. & Cabrera-Vique, C. 2014. Characterization of fatty acid profile of argan oil and other edible vegetable oils by gas chromatography and discriminant analysis. *Journal of chemistry*, Article ID 843908. https://www.hindawi.com/journals/jchem/2014/843908/. Date of access: 26 Oct. 2019.

Sawant, P.D., Luu, D., Ye, R. & Buchta, R. 2010. Drug release from hydroethanolic gels. Effect of drug's lipophilicity (log P), polymer–drug interactions and solvent lipophilicity. *International journal of pharmaceutics*, 396:45-52.

Senapati, S., Mahanta, A.K., Kumar, S. & Maiti, P. 2018. Controlled drug delivery vehicles for cancer treatment and their performance. *Signal transduction and targeted therapy*, 3:Article ID 7.

Simon, A., Amaro, M.I., Healy, A.M., Cabral, L.M. & de Sousa, V.P. 2016. Comparative evaluation of rivastigmine permeation from a transdermal system in the Franz cell using synthetic membranes and pig ear skin with *in vivo-in vitro* correlation. *International journal of pharmaceutics*, 512:234-241.

Sinha, V.R. & Kaur, M.P. 2000. Permeation enhancers for transdermal drug delivery. *Drug development and industrial pharmacy*, 26:1131-1140.

Surber, C. & Kottner, J. 2017. Skin care products: What do they promise, what do they deliver. *Journal of tissue viability*, 26:29-36.

Tan, C.X. 2019. Virgin avocado oil: An emerging source of functional fruit oil. *Journal of functional foods*, 54: 381-392.

Trombino, S., Russo, R., Mellace, S., Varano, G.P., Laganà, A.S., Marcucci, F. & Cassano, R. 2019. Solid lipid nanoparticles made of trehalose monooleate for cyclosporin-a topic release. *Journal of drug delivery science and technology*, 49:563-569.

Tshisevhe, V., Mbelle, N. & Peters, R.P. 2019. Cutaneous tuberculosis in HIV-infected individuals: lessons learnt from a case series. *Southern African journal of HIV medicine*, 20:1-3.

Ujilestari, T., Dono, N.D., Ariyadi, B., Martien, R. & Zuprizal, Z. 2018. Formulation and characterization of self-nano emulsifying drug delivery systems of lemongrass (cymbopogon citratus) essential oil. *Malaysian journal of fundamental and applied sciences*, 14:360-363.

Van Zyl, L., Du Preez, J., Gerber, M., Du Plessis, J. & Viljoen, J. 2016. Essential fatty acids as transdermal penetration enhancers. *Journal of pharmaceutical sciences*, 105:188-193.

Vaughn, A.R., Clark, A.K., Sivamani, R.K. & Shi, V.Y. 2018. Natural oils for skin-barrier repair: Ancient compounds now backed by modern science. *American journal of clinical dermatology*, 19:103-117.

Vaz, S., Silva, R., Amaral, M.H., Martins, E., Lobo, J.S. & Silva, A.C. 2019. Evaluation of the biocompatibility and skin hydration potential of vitamin E-loaded lipid nanosystems formulations: *In vitro* and human in vivo studies. *Colloids and surfaces B: biointerfaces*, 179:242-249.

Viljoen, J.M., Cowley, A., Du Preez, J., Gerber, M. & Du Plessis, J. 2015. Penetration enhancing effects of selected natural oils utilized in topical dosage forms. *Drug development and industrial pharmacy*, 41:2045-2054.

Warner, R.R., Stone, K.J. & Boissy, Y.L. 2003. Hydration disrupts human stratum corneum ultrastructure. *Journal of investigative dermatology*, 120:275-284.

Zhang, A., Jung, E.C., Zhu, H., Zou, Y., Hui, X. & Maibach, H. 2017. Vehicle effects on human stratum corneum absorption and skin penetration. *Toxicology and industrial health*, 33: 416-425.

Annexure F

Compatibility studies of excipients included in topical SEDDS formulations

F.1 Introduction

Development of topical SEDDSs for the purpose of optimised delivery involved confirmation of compatibility of the included excipients (Kaur *et al*., 2017; Patel *et al*., 2015; Chadha & Bhandari, 2014). Each excipient fulfils its own purpose towards establishing optimised topical drug delivery (Rani *et al*., 2019; Chatterjee *et al*., 2016). For example, plant based oils are included to enhance, as well as maintain solubility of the highly lipophilic clofazimine; considering that only solubilised drug particles can partition into the SC (Rani *et al*., 2019; Haque & Talukder, 2018). Additionally, plant based oils are employed during development of topical SEDDSs for their skin penetration enhancement properties (Lin *et al*., 2018; Vaughn *et al*., 2018; van Zyl *et al*., 2016; Viljoen *et al*., 2015). Without the inclusion of these penetration enhancers a drug as lipophilic as clofazimine will not be able to disrupt the SC lipid arrangement in order to partition into this barrier and diffuse across different skin layers (Czajkowska-Kośnik *et al*., 2018; Jones *et al*., 2016; Ita, 2014; Frelichowska *et al*., 2009). Moreover, if plant based oils are compared to synthetic skin penetration enhancers, the natural origin of plant based oils provides a more affordable production alternative together with decreased skin irritancy (Shehadeh *et al*., 2019; Chen *et al*., 2016).

The synthetic Tween®80 and Span®60, which are considered surface active ingredients or emulsifiers, are said to also be able to assist with skin penetration enhancement (Lane, 2013). Nevertheless, these excipients are included to reduce surface tension and in turn establish interfacial stabilisation of the oil- and water phases incorporated into topical SEDDS formulations (Rani *et al*., 2019; Chatterjee *et al*., 2016). On the other hand, the co-surfactant, Span®60, also allows flexibility of the interfacial film established by Tween®80 for the purpose of ensuring fluidity between the dispersed and continuous phases (Rani *et al*., 2019; Chatterjee *et al*., 2016). This increased fluidity facilitates a reduced risk of surfactant induced skin irritation by decreasing the quantity of surfactant required to stabilise dispersed droplets when a co-surfactant is not included (Chatterjee *et al*., 2016). Water is incorporated as the polar component of the two immiscible phases. Haque and Talukder (2018) concluded that water is the most generally used, as well as safest skin penetration enhancer due to the fact that hydration of the SC drastically contributes towards swelling of this skin layer that in turn facilitates disruption of the strict SC structure, allowing increased permeation of topically

applied drugs. Therefore, confirming compatibility of excipients utilised in combination during development of topical SEDDSs is of profound significance for the purpose of ensuring optimal stability in addition to efficacious drug delivery (Kaur *et al*., 2017; Patel *et al*., 2015; Chadha & Bhandari, 2014).

F.2 Utilising isothermal microcalorimetry in order to establish excipient compatibility

Isothermal microcalorimetry studies are based on the understanding that both physical and chemical processes are followed by heat exchange between surrounding components (Viljoen *et al*., 2018; Braissant *et al*., 2010; Phipps & Mackin, 2000). This is a rapid, highly sensitive test that can even detect thermal changes by minute intervals (Chadha & Bhandari, 2014). Through exposing a sample to isothermal conditions within a microcalorimeter, an interaction between components can be detected as a temperature gradient will exist between the sample and its surroundings (Phipps & Mackin, 2000). The heat flow observed during these experiments is measured as a function of time (Phipps & Mackin, 2000). Isothermal microcalorimetry techniques are non-specific and can be employed to confirm stability of formulations, including compatibility studies and characterisation of the physical form (Simončič *et al*., 2007). However, for the purpose of this study isothermal microcalorimetry experiments were conducted to determine if the employed excipients demonstrated compatibility within the selected SEDDS formulations.

In order to establish compatibility of excipients, a 2277 Thermal Activity Monitor, TAMIII, (TA Instruments, United States of America) supplied with an oil bath with a stability of ±100 µK over 24 h, was employed. The temperature was maintained at 40°C throughout 48 h experiments. During these experiments both individual samples and samples containing combined excipients were analysed to establish compatibility. The results displayed by individual samples are added to render a theoretical response. Thereafter, the theoretical response is compared to the measured response in order to compare expected results to obtained results for the purpose of confirming excipient compatibility. If the theoretical response significantly differs from the measured result, incompatibility can be suspected (TA Instruments, 2017).

Samples of individual components were prepared by weighing approximately 100 mg of individual excipients into Thermal Activity Monitor (TAM) vials. Following, the TAM vials were sealed and subjected to isothermal conditions. Samples containing combinations of excipients were prepared by weighing excipients according to established ratios of excipients present within the finally selected SEDDS formulations in order to obtain samples of approximately

100 mg. These established ratios were fixed according to the SEDDS formulations that were deemed suitable for further investigation, namely: ARG 3, AVO 2, MAC 2 and OLV 5. All samples were prepared and measured in duplicate for the purpose of confirming isothermal microcalometric results.

F.2.1 Combination of clofazimine with argan oil, Span®60, Tween®80 and distilled water

The compatibility of excipients included in formulation ARG 3 were evaluated by comparing the heat flow measurement of clofazimine with argan oil, Span®60, Tween®80 and distilled water to ascertain whether any interactions coexist between these various components. **Figure F.1** depicts the observed normalised heat flow versus the hypothetical response.

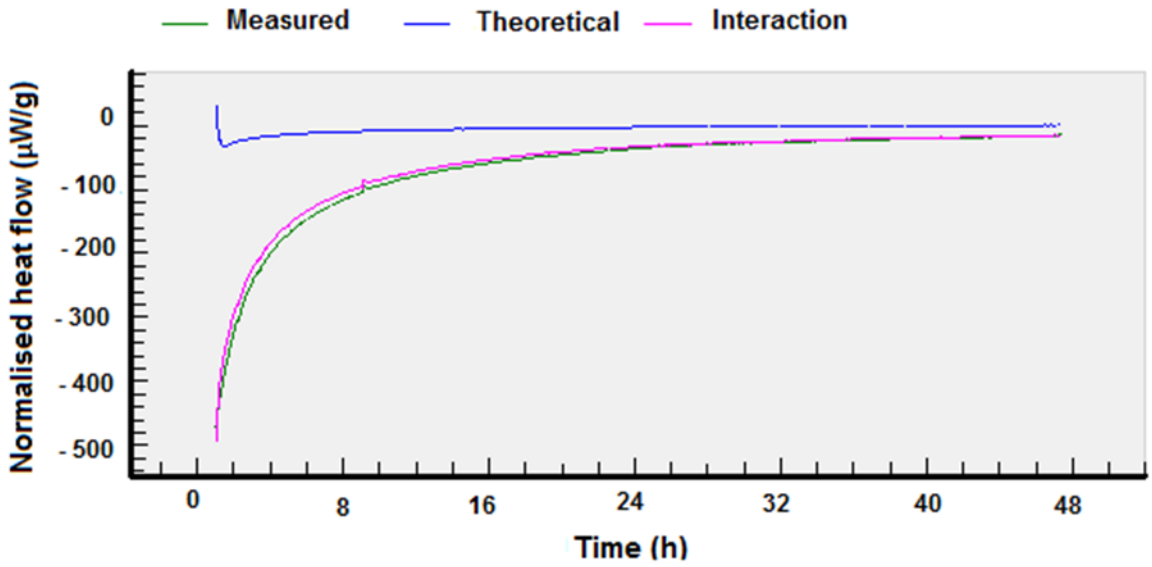

Figure F.1: Heat flow graph depicted for the combination of clofazimine with argan oil, Span®60, Tween®80 and distilled water

Average interaction heat flow measurements of -61.04 µW/g and -44.32 µW/g were depicted for sample 1 and sample 2, respectively. The interaction error of these two samples were measured as 91.49 µW/g for the first sample and a value of 63.93 µW/g was obtained for the second sample. These interaction heat flow values for the two samples are, according to compatibility studies, considered marginally high due the differences observed between the average interaction heat flow and interaction error that exceeds 10 µW/g (Viljoen *et al.*, 2018). Therefore, it may be concluded that an incompatibility is present within the argan oil SEDDS.

F.2.2 Combination of clofazimine with avocado oil, Span®60, Tween®80 and distilled water

The interaction heat flow results for the combination of clofazimine with avocado oil, Span®60, Tween®80 and distilled water are illustrated in **Figure F.2**.

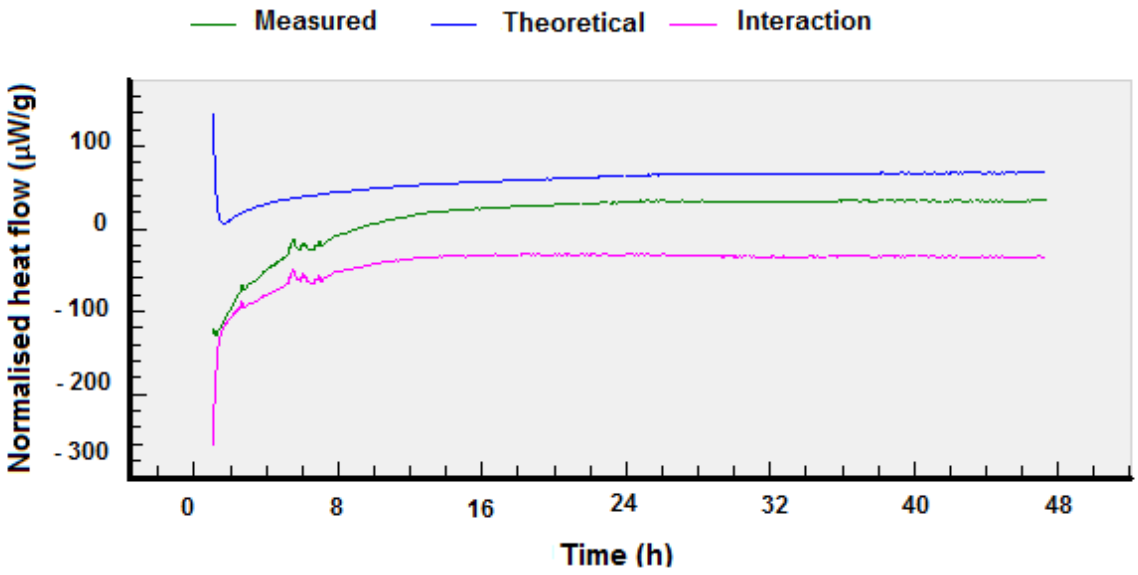

Figure F.2: Heat flow graph depicted for the combination of clofazimine with avocado oil, Span®60, Tween®80 and distilled water

Average interaction heat flow values for samples 1 and 2 of this combination excipients were calculated as -41.24 µW/g and 68.04 µW/g, respectively; whereas the interaction errors of these sample were determined as 46.35 µW/g and 64.40 µW/g, individually. Average interaction heat flow values and interaction error responses do not differ by more than 10 µW/g, and thus, it may be stated that excipients included in avocado oil SEDDS can be considered compatible and suitable for dermal delivery of clofazimine.

F.2.3 Combination of clofazimine with macadamia oil, Span®60, Tween®80 and distilled water

Figure F.3 demonstrates the interaction heat flow results for the combination of clofazimine, macadamia oil, Span®60, Tween®80 and distilled water. The average interaction heat flow value attained for sample 1 is -1.64 mW/g with an interaction error of 1.99 mW/g. On the other hand, an average interaction heat flow of -1.37 mW/g was depicted for sample 2 together with an interaction error of 1.57 mW/g. These results obtained specify that some degree of incompatibility between the incorporated excipients probably exist.

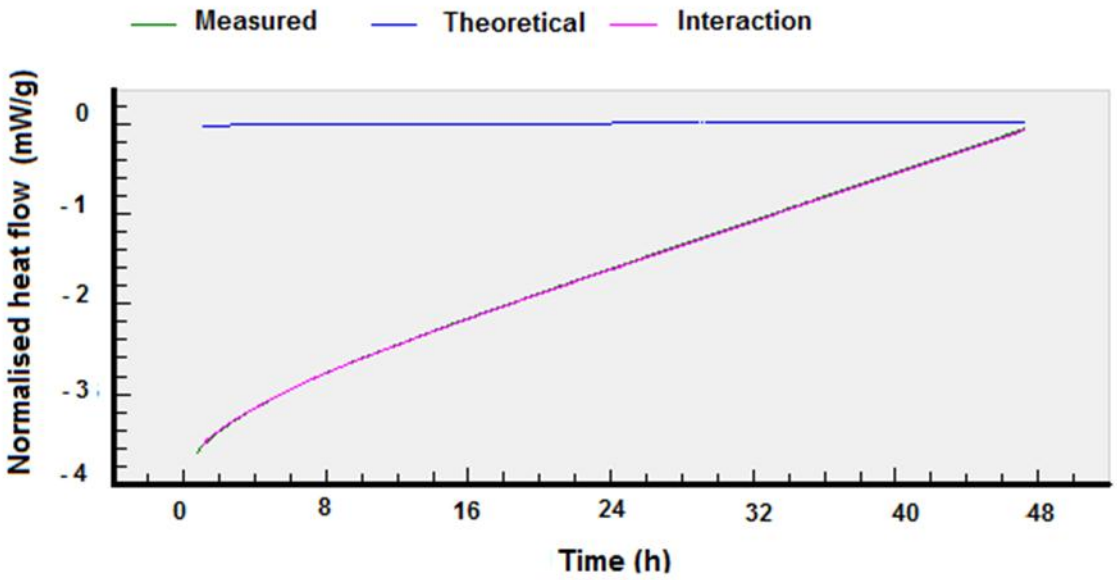

Figure F.3: Heat flow graph depicted for the combination of clofazimine with macadamia oil, Span®60, Tween®80 and distilled water

F.2.4 Combination of clofazimine with olive oil, Span®60, Tween®80 and distilled water

Data obtained for the combination components comprised within the olive oil SEDDS indicated that some degree of incompatibility exists between the various components as the average interaction heat flow measured for each sample tested was 1.09 mW/g and -1.28 mW/g, individually. The interaction error obtained for sample 1 and sample 2 were determined as 1.25 mW/g and 1.47 mW/g, respectively. As seen in **Figure F.4** the heat flow diagram obtained for formulation OLV 5, during microcalometric experiments, clearly designate some incompatibility.

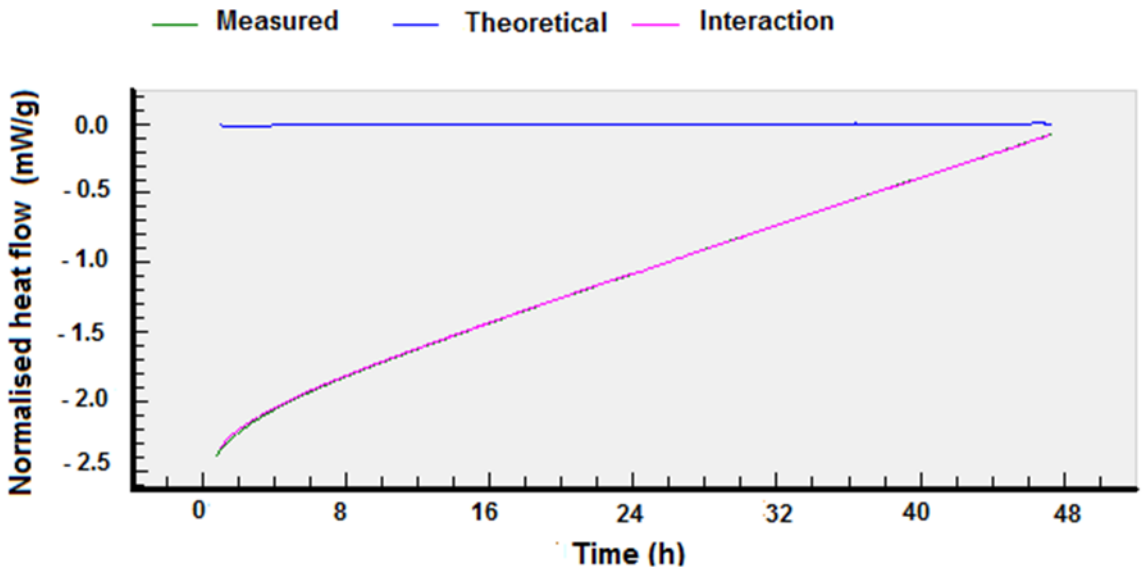

Figure F.4: Heat flow graph depicted for the combination of clofazimine with olive oil, Span®60, Tween®80 and distilled water

F.3 Isothermal microcalometric analysis of paired excipients

From the results obtained in section F.2 (**Figures F.1-F.4**) numerous incompatibilities can possibly exist between components of SEDDS formulations containing either argan-, macadamia- or olive oil. In order to determine which excipients are incompatible with one another, various samples of approximately 100 mg were prepared that contained a combination of only two excipients in each TAM vial. For example: argan oil and clofazimine; argan oil and Span®60; etcetera, were combined. Hereafter, these samples were again subjected to the set isothermal conditions in order to identify specific excipients that might have potential interactions. It was concluded that a possible incompatibility was detected for the combination of argan oil and clofazimine as observed in **Figure F.5**.

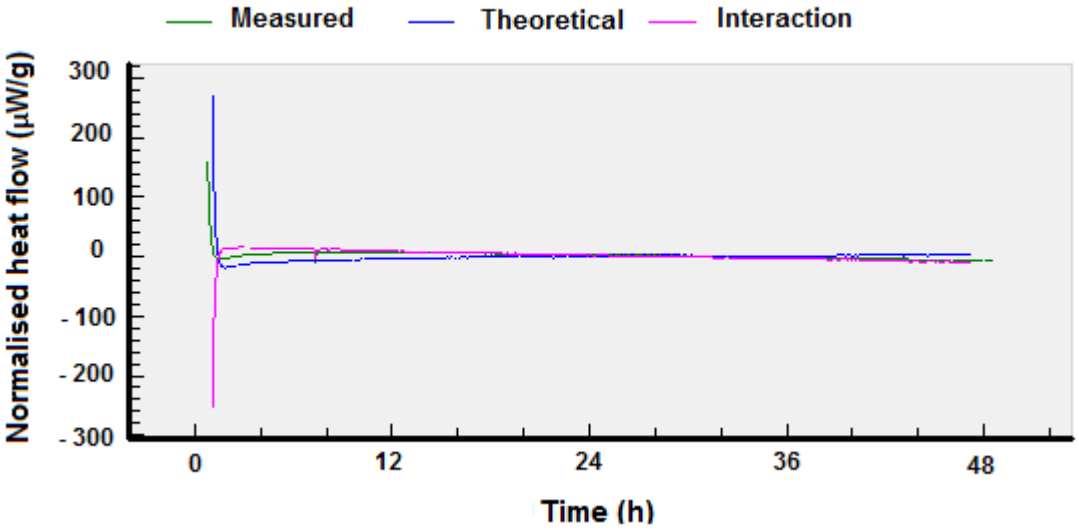

Figure F.5: Heat flow graph obtained for the combination of argan oil and clofazimine

The argan oil and clofazimine combination rendered an average heat flow of 2.261 µW/g together with an interaction error of 12.296 µW/g. This difference observed between these two values signify a possible interaction between the two components. The incompatibility observed between argan oil and clofazimine is suspected to be of a chemical nature due to the fact that clofazimine is a drug known to participate in redox reactions (Murashov *et al*., 2018; Cholo *et al*., 2012; Yano *et al*., 2011). Reported redox activity of clofazimine indicated the formation of hydrogen peroxide, a known oxidising agent (Cholo *et al*., 2016; Tyagi *et al*., 2015; Yano *et al*., 2011). Elevated concentrations of hydrogen peroxide have been detected in the skin of patients suffering from inflammatory skin conditions, such as vitiligo (Eskandari *et al*., 2019). Interestingly, application of polyphenols has proven significant anti-oxidative capability in terms of reducing inflammation caused by oxidative agents such as hydrogen peroxide (Eskandari *et al*., 2019; Kouka *et al*., 2019). Plant based oils are rich sources of polyphenols (Eskandari *et al*., 2019; Lin *et al*., 2018). Therefore, questions can be raised about the polyphenol content of plant based oils employed as solubilisers and skin penetration enhancers during this study due to the possibility that the polyphenols present are able to stabilise the redox reactions as brought about by clofazimine.

Seiquer *et al* (2015) noted that only a certain fraction of polyphenols was considered bio-accessible after subjected to *in vitro* metabolism. Hence, the probability can be considered that the initial polyphenols present within topical SEDDS formulations are not as important as the remaining bio-accessible fraction after 12 h diffusion studies in order to best interpret the influence of polyphenols on stability of SEDDSs. This statement is made while bearing in mind that formulation ARG 3 rendered the second highest cumulative clofazimine concentration

during dermal diffusion studies, despite the depicted interaction between argan oil and clofazimine. Therefore, the likelihood of argan oil not possessing sufficient polyphenols, compared to other plant based oils employed to withstand interactions upon exposure to increased temperatures after 48 h isothermal microcalorimetric experiments, cannot be over ruled. In contrast, hydrogen peroxide has displayed pore enlargement properties, similar to micro channel formation within the skin that can enhance topical drug delivery of clofazimine (Shimizu & Krištof, 2017; Kalluri *et al*., 2011). However, hydrogen peroxide is a causative agent of corrosive damage, formation of oxygen gas as well as lipid peroxidation (Watt *et al*., 2004). Therefore, prolonged exposure to hydrogen peroxide can lead to tissue damage (Watt *et al*., 2004). Consequently, this possible chemical incompatibility between clofazimine and argan oil can provide an explanation for the increased clofazimine permeation. Nonetheless, hydrogen peroxide is a dangerous substance upon prolonged skin contact and is therefore not considered favourable for optimal topical drug delivery of clofazimine.

Mechanisms of incompatibility between drugs and excipients can be categorised as either of a physical or chemical nature (Patel *et al*., 2015; Fathima *et al*., 2011). Chemical incompatibilities are almost without exception viewed as unfavourable interactions (Patel *et al*., 2015; Fathima *et al*., 2011). These types of interactions can lead to degradation of components within formulations that decreases efficacious functionality as well as safety of the final product (Patel *et al*., 2015; Fathima *et al*., 2011). Contrary, physical interactions should not be considered deleterious until confirmed by further investigation as these interactions may sometimes contribute toward increased drug delivery. An example of favourable physical interactions observed between a drug and excipient is complexation. This interaction can be beneficial if a lipophilic drug, such as clofazimine, forms complexes with an excipient for the purpose of obtaining increased bioavailability (Patel *et al*., 2015). A well-known example of favourable complexation is the employment of cyclodextrin to enhance the rate and extent of dissolution for orally administered drugs (Patel *et al*., 2015; Loftsson & Brewster, 2012). However, cyclodextrin is not generally included in topical or transdermal formulations (Haque & Talukder, 2018; Patel *et al*., 2015). Even so, Haque and Talukder (2018) stated that the inclusion of cyclodextrin and free fatty acids in the same formulation can complement each other as cyclodextrin improves aqueous solubility of lipophilic drugs, while free fatty acids facilitate skin permeation of the solubilised drug. Therefore, isothermal microcalometric results are employed to indicate potential incompatibilities that exist between components, but are not used to identify the specific nature of the interaction itself (Patel *et al*., 2015). Potential interactions were observed for the combination of Span®60 with argan oil, macadamia oil and olive oil, respectively. These potential interactions are represented in **Figures F.6–F.8**.

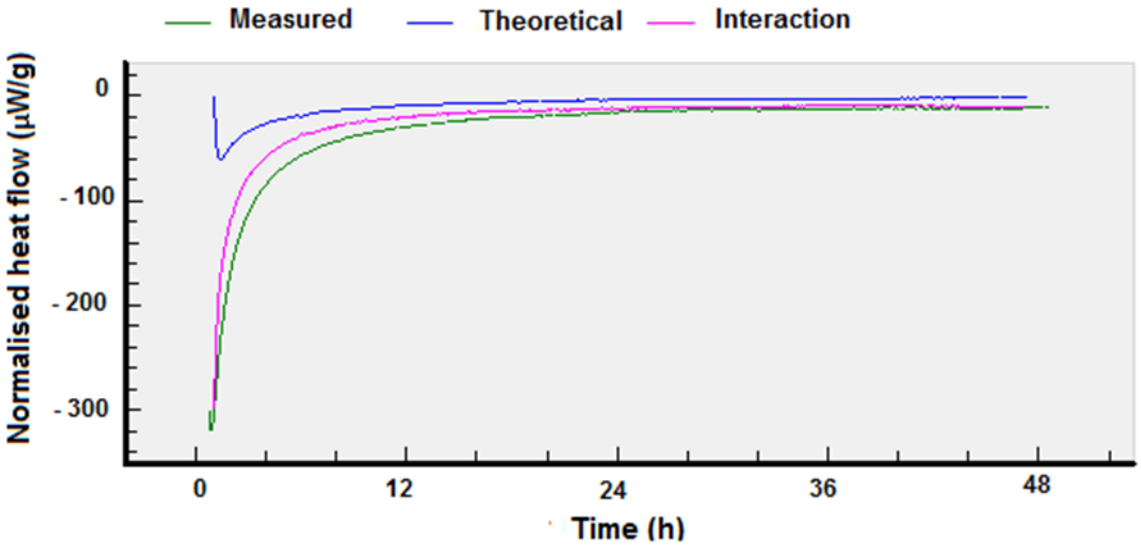

Figure F.6: Heat flow graph obtained for the combination of argan oil and Span®60

An average heat flow of -21.674 µW/g was depicted for the combination of argan oil and Span®60, whereas an interaction error for this combination was established at 35.245 µW/g. The difference between the measured average heat flow and calculated interaction error was found to be higher than 10 µW/g; and therefore, it is possible that an incompatibility might exist between the two compounds.

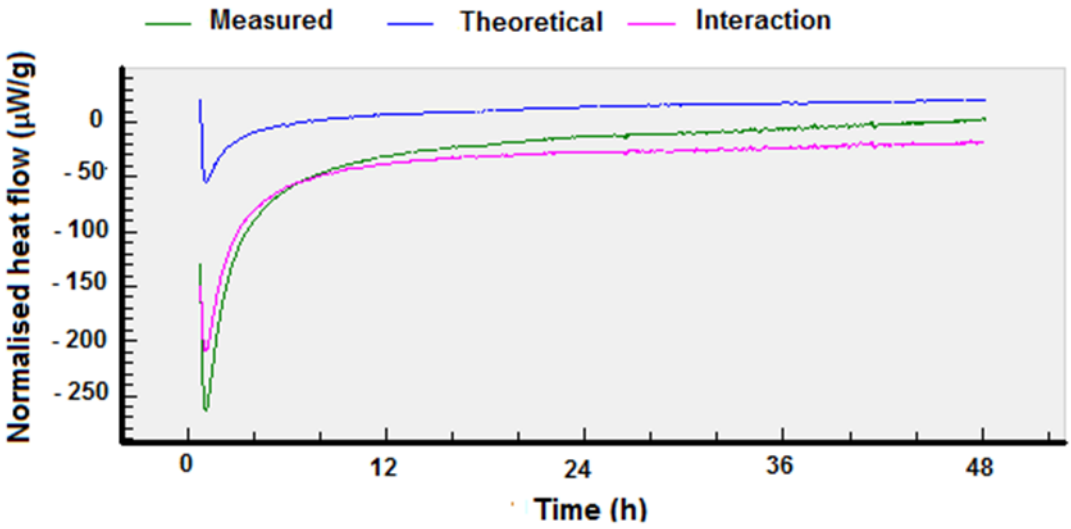

Figure F.7: Heat flow graph obtained for the combination of macadamia oil and Span®60

An average heat flow of -37.516 µW/g and an interaction error of 48.270 µW/g was obtained for the combination, macadamia oil and Span®60. The difference between the measured

average heat flow and determined interaction error is again indicative of an incompatibility between macadamia oil and Span®60.

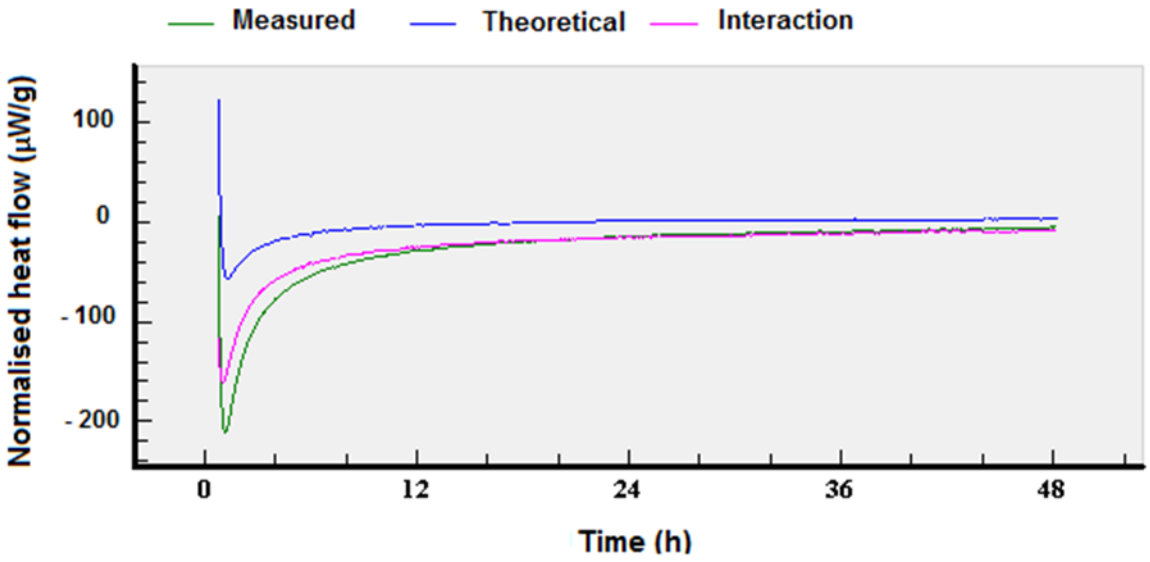

Figure F.8: Heat flow graph obtained for the combination of olive oil and Span®60

For the olive oil and Span®60 combination, an average heat flow of -23.858 µW/g and an interaction error of 33.930 µW/g were measured. The difference between the measured average heat flow and calculated interaction error barely exceeds 10 µW/g, and hence, this slight potential of an incompatibility between olive oil and Span®60 must be subjected to further investigation.

Potential interactions observed between argan-, macadamia- or olive oil and Span®60, are suspected to be of a physical origin. This speculation is based on the finding that Span®60 is frequently included as a co-surfactant within topical formulations as this non-ionic co-surfactant does not easily cause skin irritation (Lémery *et al*., 2015). Additionally, Span®60 is generally used in combination with Tween®80 during development of topical preparations as this combination has depicted improved drug entrapment properties (Sambhakar *et al*., 2017). What is more, no incompatibilities between Span®60 and any natural oil has been previously reported. However, it might be useful to investigate other co-surfactants that can possibly establish increased stability of the formulated SEDDS formulations and which do not depict potential incompatibilities.

Span®60 is a solid substance at room temperature and difficulties arose during formulation when Span®60, due to increased phase transition temperature, had to be dissolved in Tween®80 while exposed to prolonged heating and stirring (Sambhakar *et al*., 2017). Therefore, a co-surfactant with a decreased melting point (i.e. a co-surfactant that is a liquid

state at room temperature) can possibly be investigated to substitute Span®60 in these formulated topical SEDDS formulations. Examples of liquid state non-ionic co-surfactants include: Span®80 and propylene glycol (Sekrani & Poncet, 2018; Mahdi *et al*., 2011). The combination of Span®80 and Tween®80 is known to provide improved incorporation of the aqueous phase as these two excipients are prone to form fibre-resembling structures that physically interact with one-another while providing a networked structure ideal to maintain droplets dispersed in the continuous phase (Sagiri *et al*., 2012). Interestingly, Span®80 differs from Span®60 in structure as well as droplet stabilising mechanism (Sambhakar *et al*., 2017). Span®60 possesses a longer alkyl chain length compared to Span®80, which provides a possible explanation for the improved drug entrapment capacity of Span®60 (Sambhakar *et al*., 2017). Span®80, in contrast, comprises an unsaturated alkyl chain ideal for improved flexibility and rotation of the alkyl chain (Sambhakar *et al*., 2017). This improved flexibility as well as rotational properties improve stearic hindrance facilitation by Span®80 (Sambhakar *etal*., 2017).

Abd-Allah *et al* (2010) reported the combination of Tween®80 and propylene glycol, with oleic acid as the oil phase, the most successful vehicle utilised to topically deliver peroxicam. Moreover, propylene glycol can be employed as an emollient, modulator of viscosity as well as a skin penetration enhancer (Pushpalatha *et al*., 2019). Additionally, propylene glycol has been reported a low risk substance in terms of causing skin irritation in a study that employed data obtained over a period of 10 years (Lessmann *et al*., 2005). As cutaneous TB patients are subjected to extended treatment periods, this decreased risk of skin irritancy as well as low risk of skin sensitisation reactions are deemed highly favourable and predict possible beneficial contributions toward safe and efficacious topical drug delivery (Gunawan *et al*., 2018).

F.4 Conclusion

Despite the depiction of potential incompatibilities between components utilised during this study, the finally selected SEDDS formulations facilitated successful topical delivery of clofazimine. Therefore, incompatibilities can be subjected to further investigation while also exploring alternative excipient combinations. Further studies can include exposing samples to isothermal stress conditions followed by HPLC analysis in order to establish if physical or chemical interactions occurred between excipients (Patel *et al*., 2015). Research involving development of topical SEDDSs are considered highly favourable in terms of improved thermodynamic stability, simplified formulation techniques, enhanced capacity for drug loading, decreased droplet sizes, reduced interfacial tension together with intensified skin penetration possibilities (Rani *et al*., 2019; El Khayat *et al*., 2018). Thus, this drug delivery

system, originally developed to enhance oral drug delivery of lipophilic drugs, can be considered a prospective topical and/or transdermal vehicle in terms of optimised dermal drug delivery, especially for drugs as lipophilic as clofazimine.

References

Abd-Allah, F.I., Dawaba, H.M. & Ahmed, A.M. 2010. Development of a microemulsion-based formulation to improve the availability of poorly water-soluble drug. *Drug discoveries and therapeutics*, 4:257-66.

Braissant, O., Wirz, D., Göpfert, B. & Daniels, A.U. 2010. Biomedical use of isothermal microcalorimeters. *Sensors*, 10:Article ID 9369-9383.

Chadha, R. & Bhandari, S. 2014. Drug–excipient compatibility screening—role of thermoanalytical and spectroscopic techniques. *Journal of pharmaceutical and biomedical analysis*, 87:82-97.

Chatterjee, B., Hamed Almurisi, S., Ahmed Mahdi Dukhan, A., Mandal, U.K. & Sengupta, P. 2016. Controversies with self-emulsifying drug delivery system from pharmacokinetic point of view. *Drug delivery*, 23:3639-3652.

Chen, J., Jiang, Q.D., Chai, Y.P., Zhang, H., Peng, P. & Yang, X.X. 2016. Natural terpenes as penetration enhancers for transdermal drug delivery. *Molecules*, 21:Article ID 1709.

Cholo, M.C., Mothiba, M.T., Fourie, B. & Anderson, R. 2016. Mechanisms of action and therapeutic efficacies of the lipophilic antimycobacterial agents clofazimine and bedaquiline. *Journal of antimicrobial chemotherapy*, 72:338-353.

Cholo, M.C., Steel, H.C., Fourie, P.B., Germishuizen, W.A. & Anderson, R. 2012. Clofazimine: current status and future prospects. *Journal of antimicrobial chemotherapy*, 67:290-298.

Czajkowska-Kośnik, A., Szekalska, M. & Winnicka, K. 2018. Nanostructured lipid carriers: A potential use for skin drug delivery systems. *Pharmacological reports*, 71:156-166.

El Khayat, N.W., Donia, A.A., Mady, O.Y. & El Maghraby, G.M. 2018. Optimization of eugenol microemulsion for transdermal delivery of indomethacin. *Journal of drug delivery science and technology*, 48:311-318.

Eskandari, M., Rembiesa, J., Startaitė, L., Holefors, A., Valančiūtė, A., Faridbod, F., Ganjali, M.R., Engblom, J. & Ruzgas, T. 2019. Polyphenol-hydrogen peroxide reactions in skin: *In vitro* model relevant to study ROS reactions at inflammation. *Analytica chimica acta*, 1075:91-97.

Fathima, N., Mamatha, T., Qureshi, H.K., Anitha, N. & Rao, J.V. 2011. Drug-excipient interaction and its importance in dosage form development. *Journal of applied pharmaceutical science*, 1:66-71.

Frelichowska, J., Bolzinger, M.A., Pelletier, J., Valour, J.P. & Chevalier, Y. 2009. Topical delivery of lipophilic drugs from o/w Pickering emulsions. *International journal of pharmaceutics*, 371:56-63.

Gunawan, H., Achdiat, P.A., Hindritiani, R., Essary, E.D., Ningtias, L.D., Siregar, E.P., Sori, P.R. & Febrina, D. 2018. Various cutaneous tuberculosis with rare clinical manifestations: a case series. *International journal of mycobacteriology*, 7:288-291.

Haque, T. & Talukder, M.M.U. 2018. Chemical enhancer: a simplistic way to modulate barrier function of the stratum corneum. *Advanced pharmaceutical bulletin*, 8:169-179.

Ita, K.B. 2014. Transdermal drug delivery: progress and challenges. *Journal of drug delivery science and technology*, 24:245-250.

Jones, J.G., White, K.A.J. & Delgado-Charro, M.B. 2016. A mechanistic approach to modelling the formation of a drug reservoir in the skin. *Mathematical biosciences*, 281:36-45.

Kalluri, H., Kolli, C.S. & Banga, A.K. 2011. Characterization of microchannels created by metal microneedles: formation and closure. *The AAPS journal*, 13:473-481.

Kaur, M., Sharma, S. & Sinha, V.R. 2017. Polymer based microspheres of aceclofenac as sustained release parenterals for prolonged anti-inflammatory effect. *Materials science and engineering: C*, 72:492-500.

Kouka, P., Tsakiri, G., Tzortzi, D., Dimopoulou, S., Sarikaki, G., Stathopoulos, P., Veskoukis, A.S., Halabalaki, M., Skaltsounis, A.L. & Kouretas, D. 2019. The polyphenolic composition of extracts derived from different Greek extra virgin olive oils is correlated with their antioxidant potency. *Oxidative medicine and cellular longevity*: Article ID 1870965. https://www.hindawi.com/journals/omcl/2019/1870965/. Date of access: 26 Oct. 2019.

Lane, M.E. 2013. Skin penetration enhancers. *International journal of pharmaceutics*, 447:12-21.

Lémery, E., Briançon, S., Chevalier, Y., Bordes, C., Oddos, T., Gohier, A. & Bolzinger, M.A. 2015. Skin toxicity of surfactants: Structure/toxicity relationships. *Colloids and surfaces A: physicochemical and engineering aspects*, 469:166-179.

Lessmann, H., Schnuch, A., Geier, J. & Uter, W. 2005. Skin-sensitizing and irritant properties of propylene glycol: Data analysis of a multicentre surveillance network (IVDK*) and review of the literature. *Contact dermatitis*, 53:247-259.

Lin, T.K., Zhong, L. & Santiago, J. 2018. Anti-inflammatory and skin barrier repair effects of topical application of some plant oils. *International journal of molecular sciences*, 19:Article ID 70.

Loftsson, T. & Brewster, M.E. 2012. Cyclodextrins as functional excipients: methods to enhance complexation efficiency. *Journal of pharmaceutical sciences*, 101:3019-3032.

Mahdi, E.S., Sakeena, M.H., Abdulkarim, M.F., Abdullah, G.Z., Sattar, M.A. & Noor, A.M. 2011. Effect of surfactant and surfactant blends on pseudoternary phase diagram behavior of newly synthesized palm kernel oil esters. *Drug design, development and therapy*, 5:311-323.

Murashov, M., Diaz-Espinosa, J., LaLone, V., Tan, J., Laza, R., Wang, X., Stringer, K. & Rosania, G. 2018. Synthesis and characterization of a biomimetic formulation of clofazimine hydrochloride microcrystals for parenteral administration. *Pharmaceutics*, 10:Article ID 238.

Patel, P., Ahir, K., Patel, V., Manani, L. & Patel, C. 2015. Drug-excipient compatibility studies: first step for dosage form development. *The pharma innovation*, 4:14-20.

Phipps, M.A. & Mackin, L.A. 2000. Application of isothermal microcalorimetry in solid state drug development. *Pharmaceutical science & technology today*, 3:9-17.

Pushpalatha, R., Selvamuthukumar, S. & Kilimozhi, D. 2019. Cyclodextrin nanosponge based hydrogel for the transdermal co-delivery of curcumin and resveratrol: Development, optimization, *in vitro* and ex vivo evaluation. *Journal of drug delivery science and technology*, 52:55-64.

Rani, S., Rana, R., Saraogi, G.K., Kumar, V. & Gupta, U. 2019. Self-emulsifying oral lipid drug delivery systems: advances and challenges. *AAPS PharmSciTech*, 20:Article ID 129.

Sagiri, S.S., Behera, B., Sudheep, T. & Pal, K. 2012. Effect of composition on the properties of tween-80–span-80-based organogels. *Designed monomers and polymers*, 15:253-273.

Sambhakar, S., Paliwal, S., Sharma, S. & Singh, B. 2017. Formulation of risperidone loaded proniosomes for effective transdermal delivery: an in-vitro and in-vivo study. *Bulletin of faculty of pharmacy, Cairo University*, 55:239-247.

Sekrani, G. & Poncet, S. 2018. Ethylene-and propylene-glycol based nanofluids: a litterature review on their thermophysical properties and thermal performances. *Applied sciences*, 8:Article ID 2311.

Shehadeh, M., Jaradat, N., Al-Masri, M., Zaid, A.N., Hussein, F., Khasati, A., Suaifan, G. & Darwish, R. 2019. Rapid, cost-effective and organic solvent-free production of biologically active essential oil from Mediterranean wild Origanum syriacum. *Saudi pharmaceutical journal*, 27:612-618.

Shimizu, K. & Krištof, J. 2017. Enhancement of percutaneous absorption on skin by plasma drug delivery method. *Advanced technology for delivering therapeutics*, 11:Article ID 111.

Simončič, Z., Zupančič, P., Roškar, R., Gartner, A., Kogej, K. & Kmetec, V. 2007. Use of microcalorimetry in determination of stability of enalapril maleate and enalapril maleate tablet formulations. International journal of pharmaceutics, 342:145-151.

Seiquer, I., Rueda, A., Olalla, M. & Cabrera-Vique, C. 2015. Assessing the bioavailability of polyphenols and antioxidant properties of extra virgin argan oil by simulated digestion and caco-2 cell assays. Comparative study with extra virgin olive oil. *Food chemistry*, 188:496-503.

TA instruments. 2017. Microcalorimetry. http://www.tainstruments.com/pdf/brochure/MicrocalorimetryBrochure.pdf. Date of access: 6 October 2019.

Tyagi, P., Dharmaraja, A.T., Bhaskar, A., Chakrapani, H. & Singh, A. 2015. Mycobacterium tuberculosis has diminished capacity to counteract redox stress induced by elevated levels of endogenous superoxide. *Free radical biology and medicine*, 84:344-354.

Van Zyl, L., Du Preez, J., Gerber, M., Du Plessis, J. & Viljoen, J. 2016. Essential fatty acids as transdermal penetration enhancers. *Journal of pharmaceutical sciences*, 105:188-193.

Vaughn, A.R., Clark, A.K., Sivamani, R.K. & Shi, V.Y. 2018. Natural oils for skin-barrier repair: Ancient compounds now backed by modern science. *American journal of clinical dermatology*, 19:103-117.

Viljoen, J.M., Botes, D. & Steenekamp, J.H. 2018. Formulation and evaluation of selected transmucosal dosage forms containing a double fixed-dose of acyclovir and ketoconazole. *European journal of pharmaceutical sciences*, 111:503-513.

Viljoen, J.M., Cowley, A., Du Preez, J., Gerber, M. & Du Plessis, J. 2015. Penetration enhancing effects of selected natural oils utilized in topical dosage forms. *Drug development and industrial pharmacy*, 41:2045-2054.

Watt, B.E., Proudfoot, A.T. & Vale, J.A. 2004. Hydrogen peroxide poisoning. *Toxicological reviews*, 23:51-57.

Yano, T., Kassovska-Bratinova, S., Teh, J.S., Winkler, J., Sullivan, K., Isaacs, A., Schechter, N.M. & Rubin, H. 2011. Reduction of clofazimine by mycobacterial type 2 NADH: quinone oxidoreductase a pathway for the generation of bactericidal levels of reactive oxygen species. *Journal of biological chemistry*, 286:10276-10287.

Annexure G

Author guidelines for *Tuberculosis Journal*

G.1 Description

Tuberculosis is a speciality journal focusing on basic experimental research on tuberculosis, notably on bacteriological, immunological and pathogenesis aspects of the disease. The journal publishes original research and reviews on the host response and immunology of tuberculosis and the molecular biology, genetics and physiology of the organism, however discourages submissions with a metaanalytical focus (for example, articles based on searches of published articles in public electronic databases, especially where there is lack of evidence of the personal involvement of authors in the generation of such material).

Areas on which submissions are welcomed include:

- Immunology
- Immunogenetics
- Pathogenetics
- Microbiology
- Microbial physiology
- Pathogenesis
- Pathology
- Molecular epidemiology
- Diagnostics
- Vaccine development
- Drug resistance

The resurgence of interest in tuberculosis has accelerated the pace of relevant research and *Tuberculosis* has grown with it, as the only journal dedicated to experimental biomedical research in tuberculosis.

G.2 Impact factor

2018: 2.790 © Clarivate Analytics Journal Citation Reports 2019

G.3 Abstracting and indexing

- Scopus
- ADONIS
- Cambridge Scientific Abstracts
- Embase
- Biological Abstracts
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- PubMed/Medline
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G.5 Guide for authors

G.5.1 Article types

The following types of manuscripts are routinely accepted for consideration by Editors (please note that word count is from abstract to references but excluding references):

Original Articles: The form of these articles is discussed fully below under manuscript preparation; an abstract is required.

Letters: Headings should not be used in a letter; no abstract or keywords are required. The text should be no more than 800 words; there should be a maximum of 5 references and one table or figure may be included.

Reviews: An abstract and keywords are required. The text should be divided into sections by suitable headings. Tables and figures may be used as appropriate for the text.

Comments: Editorial comments are generally invited by the Tuberculosis Editorial team. They are 1,500 words in length with no abstract or keywords.

Short Communications: These should be no more than 2,500 words, with up to 15 references and a maximum of 3 figures or tables. It is strongly advised that Authors provide a list of 4 or 5 potential reviewers (e-mail, phone and fax numbers) who are knowledgeable in the subject matter, have no conflict of interest, and are likely to agree to review the manuscript. When submitting your manuscript please specify under which of the following categories your

paper should be included if accepted for publication. This will be of great assistance to the Editors with the review process:

- Reviews
- Comments
- Molecular Aspects
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- Mechanisms of Pathogenesis
- Model Systems
- Bovine Tuberculosis
- Diagnostics
- Drug Discovery and Resistance
- Host Genetics of Susceptibility
- Non-tuberculous Mycobacteria

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Books for review may be sent to **either Editor** at the addresses given below.

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Centenary Institute, and Disciplines of Medicine,
Immunology and Infectious Diseases,
Sydney Medical School, The University of Sydney, NSW 2006
Australia.
Tel: +61 2 9515 5210 (work)
Email: wbritton@med.usyd.edu.au

Brian D. Robertson, PhD

Imperial College London
Department of Microbiology
Centre for Molecular Microbiology and Infection
Flowers Building
London SW7 2AZ
UK
Tel: +44 (0)20 7594 3198
Fax: +44 (0)20 7594 3095
E-mail: b.robertson@imperial.ac.uk

Reviews' Editor **Patrick J. Brennan** Department of Microbiology, Immunology and PathologyCollege of Veterinary Medicine and Biomedical SciencesColorado State UniversityFort CollinsCO 80523-1682USA

G.5.2 Submission checklist

You can use this list to carry out a final check of your submission before you send it to the journal for review. Please check the relevant section in this Guide for Authors for more details.

G.5.3 Ensure that the following items are present:

- One author has been designated as the **corresponding author with contact details:**
    - E-mail address
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- **All necessary files have been uploaded**:
- **Manuscript:**
    - Include keywords
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    - All references mentioned in the Reference List are cited in the text, and vice versa
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    - A competing interests statement is provided, even if the authors have no competing interests to declare
    - Journal policies detailed in this guide have been reviewed
    - Referee suggestions and contact details provided, based on journal requirements
- For further information, visit our **Support Center**.

G.6 Before you begin

G.6.1 Ethics in publishing

Please see our information pages on Ethics in publishing and Ethical guidelines for journal publication.

G.6.2 Ethics

Work on human beings that is submitted to Tuberculosis should comply with the principles laid down in the Declaration of Helsinki; Recommendations guiding physicians in biomedical research involving human subjects. Adopted by the 18th World Medical Assembly, Helsinki, Finland, June 1964, amended by the 29th World Medical Assembly, Tokyo, Japan, October 1975, the 35th World Medical Assembly, Venice, Italy, October 1983, and the 41st World Medical Assembly, Hong Kong, September 1989. The manuscript should contain a statement that the work has been approved by the appropriate ethical committees related to the institution(s) in which it was performed and that subjects gave informed consent to the work. Studies involving experiments with animals must state that their care was in accordance with institution guidelines. Studies on patients or volunteers require ethics committee approval and informed consent which should be documented in your paper. Patients have a right to privacy. Therefore, identifying information, including patients, images, names, initials, or hospital numbers, should not be included in videos, recordings, written descriptions, photographs, and pedigrees unless the information is essential for scientific purposes and you have obtained written informed consent for publication in print and electronic form from the patient (or parent, guardian or next of kin where applicable). If such consent is made subject to any conditions, Elsevier must be made aware of all such conditions. Written consents must be provided to Elsevier on request. Even where consent has been given, identifying details should be omitted if they are not essential. If identifying characteristics are altered to protect anonymity, such as in genetic pedigrees, authors should provide assurance that alterations do not distort scientific meaning and editors should so note. If such consent has not been obtained, personal details of patients included in any part of the paper and in any supplementary materials (including all illustrations and videos) must be removed before submission.

G.6.3 Randomised controlled trials

All randomised controlled trials submitted for publication in Tuberculosis should include a completed Consolidated Standards of Reporting Trials (CONSORT) flow chart. Please refer to the CONSORT statement website at http://www.consort-statement.org for more information. Tuberculosis has adopted the proposal from the International Committee of Medical Journal Editors (ICMJE) which require, as a condition of consideration for publication of clinical trials, registration in a public trials registry. Trials must register at or before the onset

of patient enrolment. The clinical trial registration number should be included at the end of the abstract of the article. For this purpose, a clinical trial is defined as any research project that prospectively assigns human subjects to intervention or comparison groups to study the cause-and-effect relationship between a medical intervention and a health outcome. Studies designed for other purposes, such as to study pharmacokinetics or major toxicity (e.g. phase I trials) would be exempt. Further information can be found at www.icmje.org.

G.6.4 Declaration of interest

All authors must disclose any financial and personal relationships with other people or organizations that could inappropriately influence (bias) their work. Examples of potential competing interests include employment, consultancies, stock ownership, honoraria, paid expert testimony, patent applications/registrations, and grants or other funding. Authors must disclose any interests in two places:

1. A summary declaration of interest statement in the title page file (if double-blind) or the manuscript file (if single-blind). If there are no interests to declare then please state this: 'Declarations of interest: none'. This summary statement will be ultimately published if the article is accepted.
2. Detailed disclosures as part of a separate Declaration of Interest form, which forms part of the journal's official records. It is important for potential interests to be declared in both places and that the information matches.

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Submission of an article implies that the work described has not been published previously (except in the form of an abstract, a published lecture or academic thesis, see 'Multiple, redundant or concurrent publication' for more information), that it is not under consideration for publication elsewhere, that its publication is approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out, and that, if accepted, it will not be published elsewhere in the same form, in English or in any other language, including electronically without the written consent of the copyright holder. To verify originality, your article may be checked by the originality detection service Crossref Similarity Check.

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Inclusive language acknowledges diversity, conveys respect to all people, is sensitive to differences, and promotes equal opportunities. Articles should make no assumptions about the beliefs or commitments of any reader, should contain nothing which might imply that one individual is superior to another on the grounds of race, sex, culture or any other characteristic, and should use inclusive language throughout. Authors should ensure that writing is free from bias, for instance by using 'he or she', 'his/her' instead of 'he' or 'his', and by making use of job titles that are free of stereotyping (e.g. 'chairperson' instead of 'chairman' and 'flight attendant' instead of 'stewardess').

G.6.8 Authorship

All authors should have made substantial contributions to all of the following:

1. the conception and design of the study, or acquisition of data, or analysis and interpretation of data,
2. drafting the article or revising it critically for important intellectual content, (3) final approval of the version to be submitted.

G.6.9 Changes to authorship

Authors are expected to consider carefully the list and order of authors **before** submitting their manuscript and provide the definitive list of authors at the time of the original submission. Any addition, deletion or rearrangement of author names in the authorship list should be made only **before** the manuscript has been accepted and only if approved by the journal Editor. To request such a change, the Editor must receive the following from the **corresponding author**: (a) the reason for the change in author list and (b) written confirmation (e-mail, letter) from all authors that they agree with the addition, removal or rearrangement. In the case of addition or removal of authors, this includes confirmation from the author being added or removed. Only in exceptional circumstances will the Editor consider the addition, deletion or rearrangement of authors **after** the manuscript has been accepted. While the Editor considers the request, publication of the manuscript will be suspended. If the manuscript has already been published in an online issue, any requests approved by the Editor will result in a corrigendum.

G.6.10 Reporting clinical trials

Randomized controlled trials should be presented according to the CONSORT guidelines. At manuscript submission, authors must provide the CONSORT checklist accompanied by a flow diagram that illustrates the progress of patients through the trial, including recruitment, enrollment, randomization, withdrawal and completion, and a detailed description of the

randomization procedure. The CONSORT checklist and template flow diagram are available online.

G.6.11 Registration of clinical trials

Registration in a public trials registry is a condition for publication of clinical trials in this journal in accordance with International Committee of Medical Journal Editors recommendations. Trials must register at or before the onset of patient enrolment. The clinical trial registration number should be included at the end of the abstract of the article. A clinical trial is defined as any research study that prospectively assigns human participants or groups of humans to one or more health-related interventions to evaluate the effects of health outcomes. Health-related interventions include any intervention used to modify a biomedical or health-related outcome (for example drugs, surgical procedures, devices, behavioural treatments, dietary interventions, and process-of-care changes). Health outcomes include any biomedical or health-related measures obtained in patients or participants, including pharmacokinetic measures and adverse events. Purely observational studies (those in which the assignment of the medical intervention is not at the discretion of the investigator) will not require registration.

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G.6.18 Elsevier Researcher Academy

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Please write your text in good English (American or British usage is accepted, but not a mixture of these). Authors who feel their English language manuscript may require editing to eliminate possible grammatical or spelling errors and to conform to correct scientific English may wish to use the English Language Editing service available from Elsevier's Author Services. Original articles and suggestions for review articles should be submitted by online submission at: https://www.evise.com/profile/api/navigate/TUBE. During the submission process, the author should submit the manuscript file, figures, tables, acknowledgements, supplements and any other files, which need to be reviewed, in one file. The cover letter and any additional documents should be submitted as a separate file.

G.6.20 Referees

Please submit the names and institutional e-mail addresses of several potential referees. For more details, visit our Support site. Note that the editor retains the sole right to decide whether or not the suggested reviewers are used.

G.7 Preparation

Papers should be submitted in journal style and must be written in good English. Failure to do so may lead to significant delays in publication. Spelling may be British or American, but this must be consistent throughout the paper. It is highly recommended that authors use the "spell-checking" facility on their word processing software. Format the entire manuscript using 1.5 double spacing and 2 cm margins. Your paper should include page numbers and consecutive line numbering. Ensure that each new paragraph is clearly indicated. Present tables and figure legends on separate pages at the end of the manuscript. If possible, consult a recent issue of the journal to become familiar with layout and conventions. Number all pages consecutively. Authors should note that upon submission of their manuscripts to the Editor, they must ensure that they have adhered to the following checklist:

One author is designated as the **corresponding author** and provides the following information:

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Papers should be set out as follows, with each section beginning on a separate sheet: title page, summary, keywords, text, acknowledgements, references, tables, captions to illustrations.

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- Title. Concise and informative. Titles are often used in information-retrieval systems. Avoid abbreviations and formulae where possible.
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general, those for experimental papers should follow the usual conventions. Other papers can be subdivided as the author desires; the use of headings enhances readability. Normally only two categories of headings should be used: major ones should be typed in capital letters; minor ones should be typed in lower case (with an initial capital letter) at the left hand margin and underlined. Do not use "he", "his", etc where the sex of the person is unknown; say "the patient", etc. Avoid inelegant alternatives such as "he/she". Patients should not be automatically designated as "she", and doctors as "he".

G.8 Short communications

Editors/Section Editors may recommend conversion of longer manuscripts to shorter versions, or short communications may be submitted as such. We recommend the format described by ASM publications (asm.journals.org).

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This journal operates a single blind review process. All contributions will be initially assessed by the editor for suitability for the journal. Papers deemed suitable are then typically sent to a minimum of two independent expert reviewers to assess the scientific quality of the paper. The Editor is responsible for the final decision regarding acceptance or rejection of articles. The Editor's decision is final. More information on types of peer review.

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G.8.3 Subdivision - numbered sections

Divide your article into clearly defined and numbered sections. Subsections should be numbered 1.1 (then 1.1.1, 1.1.2, ...), 1.2, etc. (the abstract is not included in section numbering). Use this numbering also for internal cross-referencing: do not just refer to 'the

text'. Any subsection may be given a brief heading. Each heading should appear on its own separate line.

G.8.4 Conclusions

The main conclusions of the study may be presented in a short Conclusions section, which may stand alone or form a subsection of a Discussion or Results and Discussion section.

G.8.5 Essential title page information

- ***Title.*** Concise and informative. Titles are often used in information-retrieval systems. Avoid abbreviations and formulae where possible.
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Highlights are optional yet highly encouraged for this journal, as they increase the discoverability of your article via search engines. They consist of a short collection of bullet points that capture the novel results of your research as well as new methods that were used during the study (if any). Please have a look at the examples here: example Highlights. Highlights should be submitted in a separate editable file in the online submission system. Please use 'Highlights' in the file name and include 3 to 5 bullet points (maximum 85 characters, including spaces, per bullet point).

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Define abbreviations that are not standard in this field in a footnote to be placed on the first page of the article. Such abbreviations that are unavoidable in the abstract must be defined at their first mention there, as well as in the footnote. Ensure consistency of abbreviations throughout the article.

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Collate acknowledgements in a separate section at the end of the article before the references and do not, therefore, include them on the title page, as a footnote to the title or otherwise. List here those individuals who provided help during the research (e.g., providing language help, writing assistance or proof reading the article, etc.).

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List funding sources in this standard way to facilitate compliance to funder's requirements: Funding: This work was supported by the National Institutes of Health [grant numbers xxxx, yyyy]; the Bill & Melinda Gates Foundation, Seattle, WA [grant number zzzz]; and the United States Institutes of Peace [grant number aaaa]. It is not necessary to include detailed descriptions on the program or type of grants and awards. When funding is from a block grant or other resources available to a university, college, or other research institution, submit the name of the institute or organization that provided the funding. If no funding has been provided for the research, please include the following sentence: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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G.8.11 Footnotes

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Ensure that each illustration has a caption. Supply captions separately, not attached to the figure. A caption should comprise a brief title (**not** on the figure itself) and a description of the illustration. Keep text in the illustrations themselves to a minimum but explain all symbols and abbreviations used.

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Please ensure that every reference cited in the text is also present in the reference list (and vice versa). Any references cited in the abstract must be given in full. Unpublished results and personal communications are not recommended in the reference list, but may be mentioned in the text. If these references are included in the reference list, they should follow the standard reference style of the journal and should include a substitution of the publication date with either 'Unpublished results' or 'Personal communication'. Citation of a reference as 'in press' implies that the item has been accepted for publication.

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All sources of funding should be declared as an acknowledgement at the end of the text. Authors should declare the role of study sponsors, if any, in the study design, in the collection, analysis and interpretation of data; in the writing of the manuscript; and in the decision to submit the manuscript for publication. If the study sponsors had no such involvement, the authors should so state.

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This journal encourages you to cite underlying or relevant datasets in your manuscript by citing them in your text and including a data reference in your Reference List. Data references should include the following elements: author name(s), dataset title, data repository, version (where available), year, and global persistent identifier. Add [dataset] immediately before the reference so we can properly identify it as a data reference. The [dataset] identifier will not appear in your published article.

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Examples:

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[1] Van der Geer J, Hanraads JAJ, Lupton RA. The art of writing a scientific article. J Sci Commun 2010;163:51–9. https://doi.org/10.1016/j.Sc.2010.00372.

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[2] Van der Geer J, Hanraads JAJ, Lupton RA. The art of writing a scientific article. Heliyon. 2018;19 e00205. https://doi.org/10.1016/j.heliyon.2018.e00205

Reference to a book:

[3] Strunk Jr W, White EB. The elements of style. 4th ed. New York: Longman; 2000.

Reference to a chapter in an edited book:

[4] Mettam GR, Adams LB. How to prepare an electronic version of your article. In: Jones BS, Smith RZ, editors. Introduction to the electronic age, New York: E-Publishing Inc; 2009, p. 281–304.

Reference to a website:

[5] Cancer Research UK. Cancer statistics reports for the UK, http://www.cancerresearchuk.org/aboutcancer/statistics/cancerstatsreport/; 2003 [accessed 13 March 2003].

Reference to a dataset:

[dataset] [6] Oguro M, Imahiro S, Saito S, Nakashizuka T. Mortality data for Japanese oak wilt disease and surrounding forest compositions, Mendeley Data, v1; 2015. https://doi.org/10.17632/xwj98nb39r.1.

Note shortened form for last page number. e.g., 51–9, and that for more than 6 authors the first 6 should be listed followed by 'et al.' For further details you are referred to 'Uniform Requirements for Manuscripts submitted to Biomedical Journals' (J Am Med Assoc 1997;277:927–34) (see also Samples of Formatted References).

Journal abbreviations source:

Journal names should be abbreviated according to the List of Title Word Abbreviations.

G.8.23 Data visualisation

Include interactive data visualizations in your publication and let your readers interact and engage more closely with your research. Follow the instructions here to find out about available data visualization options and how to include them with your article.

G.8.24 Supplementary and multimedia data

Elsevier accepts electronic supplementary and multimedia data to support and enhance your scientific research. Supplementary files offer the author additional possibilities to publish supporting applications, movies, animation sequences, high-resolution images, background datasets, sound clips and more. Supplementary files supplied will be published online alongside the electronic version of your article in Elsevier Web products, including

ScienceDirect: http://www.sciencedirect.com. In order to ensure that your submitted material is directly usable, please ensure that data are provided in one of our recommended file formats. At this time the following file types are not supported in Evise and would need to be sent outside of Evise to the JM to process after acceptance EPS, CSV, Video, KML/ KMZ, mol, Newick or NeXML, MATLAB, PDB, PSE or MOL/MOL2, or NlfTl).

- EES: For supported file types in EES, please visit our Support site for EES.
- Evise: For supported file types in Evise, please visit our Support site for Evise.

Note: Both lists will be expanded as more file types are supported.

G.8.25 Research data

This journal requires and enables you to share data that supports your research publication where appropriate, and enables you to interlink the data with your published articles. Research data refers to the results of observations or experimentation that validate research findings. To facilitate reproducibility and data reuse, this journal also encourages you to share your software, code, models, algorithms, protocols, methods and other useful materials related to the project. Below are a number of ways in which you can associate data with your article or make a statement about the availability of your data when submitting your manuscript. When sharing data in one of these ways, you are expected to cite the data in your manuscript and reference list. Please refer to the "References" section for more information about data citation. For more information on depositing, sharing and using research data and other relevant research materials, visit the research data page.

G.8.26 Data linking

If you have made your research data available in a data repository, you can link your article directly to the dataset. Elsevier collaborates with a number of repositories to link articles on ScienceDirect with relevant repositories, giving readers access to underlying data that gives them a better understanding of the research described. There are different ways to link your datasets to your article. When available, you can directly link your dataset to your article by providing the relevant information in the submission system. For more information, visit the database linking page. For supported data repositories a repository banner will automatically appear next to your published article on ScienceDirect. In addition, you can link to relevant data or entities through identifiers within the text of your manuscript, using the following format: Database: xxxx (e.g., TAIR: AT1G01020; CCDC: 734053; PDB: 1XFN).

G.8.27 Mendeley Data

This journal supports Mendeley Data, enabling you to deposit any research data (including raw and processed data, video, code, software, algorithms, protocols, and methods) associated with your manuscript in a free-to-use, open access repository. During the submission process, after uploading your manuscript, you will have the opportunity to upload your relevant datasets directly to *Mendeley Data*. The datasets will be listed and directly accessible to readers next to your published article online. For more information, visit the Mendeley Data for journals page.

G.8.28 Data statement

To foster transparency, we require you to state the availability of your data in your submission if your data is unavailable to access or unsuitable to post. This may also be a requirement of your funding body or institution. You will have the opportunity to provide a data statement during the submission process. The statement will appear with your published article on ScienceDirect. For more information, visit the Data Statement page.

G.9 After acceptance

G.9.1 Online proof correction

Corresponding authors will receive an e-mail with a link to our online proofing system, allowing annotation and correction of proofs online. The environment is similar to MS Word: in addition to editing text, you can also comment on figures/tables and answer questions from the Copy Editor. Web-based proofing provides a faster and less error-prone process by allowing you to directly type your corrections, eliminating the potential introduction of errors. If preferred, you can still choose to annotate and upload your edits on the PDF version. All instructions for proofing will be given in the e-mail we send to authors, including alternative methods to the online version and PDF. We will do everything possible to get your article published quickly and accurately. Please use this proof only for checking the typesetting, editing, completeness and correctness of the text, tables and figures. Significant changes to the article as accepted for publication will only be considered at this stage with permission from the Editor. It is important to ensure that all corrections are sent back to us in one communication. Please check carefully before replying, as inclusion of any subsequent corrections cannot be guaranteed. Proofreading is solely your responsibility.

G.9.2 Offprints

The corresponding author will, at no cost, receive a customized Share Link providing 50 days' free access to the final published version of the article on ScienceDirect. The Share Link can be used for sharing the article via any communication channel, including email and social

media. For an extra charge, paper offprints can be ordered via the offprint order form which is sent once the article is accepted for publication. Both corresponding and co-authors may order offprints at any time via Elsevier's Author Services. Corresponding authors who have published their article gold open access do not receive a Share Link as their final published version of the article is available open access on ScienceDirect and can be shared through the article DOI link.

G.9.3 Proprietary names

Proprietary names of drugs, instruments, etc. should be indicated by the use of initial capital letters.

G.10 Author enquiries

Visit the Elsevier Support Center to find the answers you need. Here you will find everything from or find out when your accepted article will be published. Frequently Asked Questions to ways to get in touch. You can also check the status of your submitted article.

Annexure H

Author guidelines for *AAPS PharmSciTech Journal*

H.1 Introduction

AAPS PharmSciTech (ISSN 1530-9932) is a peer-reviewed online-only journal owned by the American Association of Pharmaceutical Scientists (AAPS). The journal's mission is to disseminate scientific and technical information on drug product design, development, evaluation, and processing to the global pharmaceutical research community. The journal is indexed by PubMed/Medline, Index Medicus, Institute of Scientific Information's Science Citation Index Expanded, and Chem Abstracts.

Editor-in-Chief **Robert O. (Bill) Williams III**, Ph.D., oversees an international editorial board of leading researchers in the pharmaceutical sciences. Williams is a professor of pharmacy at the University of Texas at Austin, College of Pharmacy.

H.2 Types of manuscripts

AAPS PharmSciTech publishes the following article types. For examples of published articles, please visit www.PharmaGateway.net.

Reviews, usually by invitation and sometimes organized into theme issues, report on recent advances in pharmaceutical research. Unsolicited reviews are considered only if they are authored by investigators who have demonstrated expertise in the relevant areas.

Mini-reviews discuss a more narrowly focused topic of recent research. Unsolicited mini-reviews are considered only if they are authored by investigators who have demonstrated expertise in the relevant areas.

Original research papers contain innovative, hypothesis-driven research that is supported by sound experimental design, methodology, and data interpretation.

Brief technical notes, normally more limited in scope than original research papers, must be of high quality, general interest, and sufficient importance to warrant publication.

Rapid communications provide a venue for fast-breaking research updates or other news items. The justification for rapid communication should be stated in the cover letter during submission.

Regulatory notes provide a summary of regulatory decisions made and rationale for the regulatory decision made on a product. These notes are typically submitted by invitation only, but authors may propose notes to the editors.

Editorials, commentaries, or summaries are usually published by invitation only. These articles contain topical issues of public and scientific interest.

Meeting reports on AAPS or AAPS-affiliated meetings provide readers with summaries of such meetings, including consensus views. When a meeting report purports to be a consensus report, it may, at the editor-in-chief's discretion, adhere to the AAPS Guideline for Review of Opinions and Summary Reports, available at www.aaps.org/guidelineopinions. The corresponding author must 2 complete and submit the Corresponding Author's Consent to Publish form (www.aaps.org/consentcorr) and all contributing authors must complete and submit the Contributing Author's Consent to Publish form (www.aaps.org/consentcont).

Meeting notices provide readers with information on upcoming AAPS or AAPS-affiliated meetings. They should include the title of the meeting, date, time, location, an outline, description of meeting topics, and a list of invited speakers. If possible, meeting notices should include contact information for the organizers and a URL to the meeting's webpage. Meeting notices should be submitted to the AAPS Editorial Office 2–3 months prior to the meeting.

Letters to the editor may be submitted by readers commenting on articles already published by the journal.

White Papers describe a current problem or issue and propose a solution. White papers written on behalf of AAPS groups are subject to the AAPS Guideline for Review of Opinions and Summary Reports (www.aaps.org/guidelineopinions) at the discretion of the editor-in-chief.

All articles published in the journal will follow the Springer Online First production workflow, enabling publication on the SpringerLink website after receipt of author corrections to page proofs.

H.3 Manuscript submission

AAPS uses Editorial Manager as its peer review tracking system. Manuscripts must be submitted online by the corresponding author at www.editorialmanager.com/aapspt. You may be required to register as a new user with Editorial Manager upon your first visit. Straightforward sign in and registration procedures can be found on the website. Editorial Manager allows authors to track the progress of manuscript review in real time. Detailed, step-

by-step instructions for submitting manuscripts can be found on the website. All correspondence regarding your manuscript must go through Editorial Manager.

H.3.1 Special features, Appendices, and supplementary material

These materials can be accommodated and may contain highly interactive features or large databases. All authors are encouraged to take full advantage of the Web-only capabilities of online publishing, including 3-D, video, and interactive graphics. All special features must be created by the author.

Authors who wish to publish electronic supplementary material with their article (Excel files, images, audio/video files, etc.) must submit the supplementary files/materials with their manuscript submission via our online peer review tracking system, Editorial Manager. These files may be submitted as one file or multiple files as desired by the authors. Accepted formats for these files include DOC, DOCX, XLS, XLSX, JPG, PDF, and for videos, MPEG-3 format. If a desired technical feature is in a different format or not covered in the author instructions, please contact the AAPS Editorial Office (AAPSPT@aaps.org) for assistance. Note that supplementary files are not automatically sent to production if the article is accepted. Please therefore note in a cover letter if these materials should be evaluated by reviewers only (not published), or if the supplemental files should be included for review and be published with the article should the manuscript be accepted for publication. If the supplemental files are intended for publication, references using “supplemental 1,” “supplemental 2,” etc. should be placed in the appropriate location within the text of the manuscript body. Supplemental files are published as-is, with no copyediting or formatting by Production.

H.3.2 Hypertext links

Authors may identify URLs for websites that provide the reader with additional information on the topic addressed in the manuscript. Although URLs are an important feature of electronic publishing, authors are encouraged to be selective in their choice of sites to include. Do not include URLs for webpages with newspaper or journal articles that will be removed or archived to another web page. Links to pharmaceutical manufacturers or other sources of product information are acceptable; however, providing a URL to the reader should not be substituted for adequate discussion within the manuscript itself. Do not include links to sites that are not accessible without a password.

www.editorialmanager.com/aapspt

H.4 Terms of manuscript consideration

H.4.1 AAPS Journals' ethics policy

The editors-in-chief of the three AAPS journals, *Pharmaceutical Research*, *The AAPS Journal*, and *AAPS PharmSciTech*, along with the AAPS Publications Committee, developed an integrated ethics policy to guide decision-making across the three journals. The document is based on the recommendations on publication ethics policies for medical journals published by the World Association of Medical Editors (WAME), posted at www.wame.org/about/recommendations-on-publication-ethics-policie .

Authors are required to review and adhere to the AAPS Journals Ethics Policy (www.aaps.org/journalsethics) in full prior to submitting manuscripts to *AAPS PharmSciTech*. Excerpts from the policy are outlined below.

H.4.2 Full disclosure

During the manuscript submission process, all authors will be required to confirm that the manuscript has not been previously published in any language anywhere and that it is not under simultaneous consideration by another journal. The journal policy regarding compound disclosure centers on the data presented. If specific data relating to the study compound are reported, then the journal requires identification so that reviewers and readers can judge, based on general principles, whether the data are plausible and internally consistent, and to potentially allow future examination and/or validation of the results and conclusions reported. If however a library of compounds is used to generate correlations, such as in QSAR when the scope and diversity of the compounds are described, then it is possible that the specific chemical compositions of the compounds need not be identified, subject to the discretion of the reviewers and the editor.

H.4.3 Conflicts of interest

Authors will be required to declare all conflicts of interest (or their absence) during the submission of a manuscript. This conflict declaration includes conflicts or potential conflicts of all listed authors. If any conflicts are declared, AAPS will publish them with the paper. In cases of doubt, the circumstance should be disclosed so that the editors may assess its significance.

Conflicts may be financial, academic, commercial, political, or personal. Financial interests may include employment, research funding (received or pending), stock or share ownership, patents, payment for lectures or travel, consultancies, nonfinancial support, or any fiduciary interest in a company.

www.aaps.org/journalsethics

H.4.4 Copyright transfer

The corresponding author, on behalf of all authors, must transfer copyright of the work to the publisher (the American Association of Pharmaceutical Scientists) in order to provide for the widest possible dissemination of professional and scientific literature. A signed Transfer of Copyright form must be submitted online with the manuscript. The Transfer of Copyright form for an accepted manuscript must be on file with the AAPS Editorial Office prior to production for publication. Corresponding authors may print and sign or electronically sign the form on behalf of all authors. The Transfer of Copyright form can be found at www.aaps.org/copyright.

H.4.5 Research data policy

The journal encourages authors, where possible and applicable, to deposit data that support the findings of their research in a public repository. Authors and editors who do not have a preferred repository should consult Springer Nature's list of repositories and research data policy. General repositories – for all types of research data – such as figshare and Dryad may also be used. Datasets that are assigned digital object identifiers (DOIs) by a data repository may be cited in the reference list. Data citations should include the minimum information recommended by DataCite: authors, title, publisher (repository name), identifier. Springer Nature provides a research data policy support service for authors and editors, which can be contacted at researchdata@springernature.com. This service provides advice on research data policy compliance and on finding research data repositories. It is independent of journal, book and conference proceedings editorial offices and does not advise on specific manuscripts.

H.5 Ethics in animals and clinical investigations

H.5.1 Human subjects and clinical trials

AAPS journals require author(s) at the time of manuscript submission to make a statement in the cover letter indicating documented review and approval from a formally constituted review board (Institutional Review Board or ethics committee) for all studies involving people, medical records, and human tissues, per the uniform guidelines from the World Medical Association (www.wma.net/en/30publications/10policies/index.html). Studies and research using human subjects, medical records, and human tissues (including educational research) must also state this compliance within the Methods section of the paper.

AAPS journals also require that controlled clinical trials must be registered in a publicly available database or the journals will not publish the results of these trials. Manuscripts

submitted to the journals must include trial registration information in the cover letter. To register a clinical trial, authors should go to the NIH registry (www.clinicaltrials.gov) or the International Standard Randomized Controlled Trials database (http://isrctn.org). Further information can be obtained from the International Committee of Medical Journal Editors (ICMJE) at www.icmje.org/about-icmje/faqs/clinical-trials-registration.

H.5.2 Animal use and assurances

AAPS journals require author(s) at the time of manuscript submission to make a statement in the cover letter indicating that animal experiments are conducted in full compliance with local, national, ethical, and regulatory principles and local licensing regulations, per the spirit of Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International's expectations for animal care and use/ethics committees (www.aaalac.org/education/module_1.cfm). Investigations using experimental animals (including educational research) must also state this compliance within the Methods section of the paper.

H.6 Originality of manuscripts

Authors of manuscripts submitted to AAPS are obliged to present accurate representation of the research performed along with an objective discussion of the significance of their findings. The author's submission should be original work that reflects research undertaken with integrity and honesty, and that conforms to ethical practices outlined in the AAPS Journals Ethics Policy. Authors should be willing to reply to any reasonable request from editors, referees, and scientists for materials, methods, or data necessary for verification of the conclusions reported in the paper.

H.6.1 Use of copyrighted tables and figures

It is the authors' responsibility to obtain permission of any tables/figures in compliance with the AAPS Journals Transfer of Copyright Agreement and AAPS Electronic Journals Ethics Policy for any copyrighted material used in an AAPS journal submission. A copy of the granted permission to use copyrighted figures, tables, or other material must be included with the submitted manuscript.

H.6.2 Peer review

All submissions will be reviewed anonymously by at least 2 independent reviewers. Authors are encouraged to submit names and email addresses of expert reviewers, but selection remains the prerogative of the editors. Authors should not recommend work colleagues or co-authors/collaborators as expert reviewers for their own papers. Authors may include supplementary notes to facilitate the review process. If an accepted paper is cited that has not

yet appeared in print and is required for evaluation of the submitted manuscript, authors should provide an electronic version for use by the reviewers. Authors are responsible for all statements in their work, including changes made by the copyeditor after a manuscript is accepted.

www.icmje.org/recommendations/browse/manuscript-preparation/preparing-for-submission.html

H.7 Points on novelty and significance

Novelty: Your results must be new and not resemble something formerly known or used. You must clearly make the case that your paper presents novel results.

Significance: Your paper must emphasize why anyone would care about your work and why someone would want to read your work. You must make a compelling case in the Introduction and Discussion sections of the significance of your work.

H.8 Manuscript organisation

Several components of the manuscript must be submitted as individual files within Editorial Manager: cover letter, title page, manuscript body (including references list), individual figure/table files, and the Transfer of Copyright.

H.8.1 Cover letter

A cover letter is recommended, but not required. Please note, a cover letter is required for a rapid communication submission. Authors who wish to submit names and email addresses of recommended reviewers for the peer review process may indicate those in the cover letter or during the submission process.

H.8.2 Title page

The title page must be submitted as a separate file and should include:

1. the title of the article,
2. author names with full first name (no degrees),
3. each author's affiliation, and
4. a suggested running head (of less than 50 characters, including spaces).

The affiliation should comprise the department, institution (usually university or company), city, and state (or nation) and should be typed as a footnote to the author's name. For the corresponding author designated to correspond with the AAPS Editorial Office and review

proofs, indicate his/her complete mailing address, office/cellular telephone number, fax number, and email address.

H.8.3 Transfer of copyright

A signed copy of the Transfer of Copyright must be submitted online as part of the manuscript submission process. The Transfer of Copyright is available at:

www.aaps.org/copyright

H.8.4 Abstract

The abstract is limited to 250 words or less. For original research articles, the abstract should include a brief (2 to 3 sentence) statement for each of the following sections: Introduction, Materials and Methods, Results, Discussion, and Conclusion, written in paragraph form. All abstracts must be written in one paragraph, with no subheadings, equations, tables, reference citations, or graphics.

The abstract must state the significance of the paper and the problem or question being addressed in the first sentence; what you found (include key data numbers); and what you conclude. Tenses should not be mixed in the paper: use the past tense.

Only reviews, mini-reviews, original research articles, rapid communications, and meeting reports require an abstract.

AAPS journals do not publish graphical abstracts.

H.8.5 Keywords

Provide a list of no more than 5 key words.

H.8.6 Manuscript body

For original research articles, brief technical notes, and rapid communications, organize the main text as follows:

1. Introduction
2. Materials and Methods
3. Results
4. Discussion
5. Conclusion

The use of subheadings to divide the text is encouraged. Primary, secondary, and tertiary level headings should be clearly defined; do not use numbers or letters in headings**.**

Introduction: The introduction must define the scope of your paper; articulate the question or hypothesis; summarize relevant work to the study being reported; and inform the reader of the rationale and significance of the study. Required for reviews, mini-reviews, original research articles, and meeting reports only.

Materials and Methods: Clearly define in detail how you conducted the study. Colleagues reading your paper must be able to replicate your experiments and determine whether your conclusions are warranted.

Results: Report what you found. Supplement the text with tables and figures.

Discussion: In this section, preferably separate from the Results section, explain what your findings mean, using the literature in great detail to compare and contrast your data with literature; use the literature as evidence to support your assertion. Explain the significance of your results and their practical implications.

Recommended maximum word counts are as follows:

- Reviews, original research articles, meeting reports, and white papers: 5,000
- Mini-reviews: 2,500
- Brief technical notes and rapid communications: 1,200

Conclusion: Did your findings support your hypothesis? Explain.

Citations: 45–50 relevant citations

H.8.7 General Manuscript Body Guidelines

- The submission must include continuous line numbers in the manuscript body file.
- Use abbreviations sparingly and define them at the first insertion in the text. Define all abbreviations used in tables within the table footnotes.
- Use the metric system for all measurements. Express metric abbreviations in lowercase letters without periods (cm, mL, sec).
- Define all symbols used in equations and formulas. When symbols are used extensively, the authors may include a list of all symbols in a table.
- Numbers should be reported to reflect the precision of the instrumentation utilized. Calculated numbers, such as means and standard deviations, should be expressed to no more than one significant digit beyond the precision of the instrument. Normally, data reported to more than 3 significant figures should be justified. The precision of the variability (e.g., standard deviation) should not exceed that of the reported mean value.

H.8.8 Acknowledgements

May be included in the manuscript body. Include funding source(s) and other contributions. If the work has been funded by NIH, please provide name(s) of funding institute(s) and grant number(s). This information is required for automatic deposit into PUBMED Central by the publisher.

H.9 References

References should conform to Vancouver style and be numbered consecutively in the order in which they are cited in the text. Cite in the text by the appropriate Arabic numeral enclosed in parentheses, e.g., (1), (2-5), etc. You should typically use 45–50 relevant references, including references from this journal, but you may use more if warranted.

Maximum reference limits are as follows:

- Reviews, original research articles, meeting reports, and white papers: 100
- Mini-reviews: 40
- Brief technical notes and rapid communications: 20

References to unpublished, peer-reviewed, personal communications, including conference abstracts and papers in preparation or in review, cannot be listed but can be notated parenthetically in the text.

Abbreviations for journal names should conform to those of Vancouver style. The style and punctuation of the references should conform to the following examples:

H.9.1 Examples by type

1. **Journal article** Smith JJ. The world of science. Am J Sci. 1999;36:234–5.
2. **Journal article with DOI (and with page numbers)** O'Mahony S, Rose SL, Chilvers AJ, Ballinger JR, Solanki CK, Barber RW, et al. Finding an optimal method for imaging lymphatic vessels of the upper limb. Eur J Nucl Med Mol Imaging. 2004;31:555–63. doi:10.1007/s00259-003-1399-3.
3. **Journal article by DOI (before issue publication with page numbers)** O'Mahony S, Rose SL, Chilvers AJ, Ballinger JR, Solanki CK, Barber RW, et al. Finding an optimal method for imaging lymphatic vessels of the upper limb. Eur J Nucl Med Mol Imaging. 2004. doi:10.1007/s00259-003-1399-3.
4. **Article in electronic journal by DOI (no paginated version)** Slifka MK, Whitton JL. Clinical implications of dysregulated cytokine production. Dig J Mol Med. 2000. doi:10.1007/s801090000086.

5. **Journal article in a supplement** Frumin AM, Nussbaum J. Esposito M. Functional asplenia: demonstration of splenic activity by bone marrow scan. Blood 1979; 59 Supple 1:26-32.
6. **Book chapter** Wyllie AH, Kerr JFR, Currie AR. Cell death: the significance of apoptosis. In: Bourne GH, Danielli JF, Jeon KW, editors. International review of cytology. London: Academic; 1980. p. 251–306.
7. **OnlineFirst chapter in a series (without a volume designation but with a DOI)** Saito Y, Hyuga H. Rate equation approaches to amplification of enantiomeric excess and chiral symmetry breaking. Top Curr Chem. 2007. doi:10.1007/128_2006_108.
8. **Book, authored** Blenkinsopp A, Paxton P. Symptoms in the pharmacy: a guide to the management of common illness. 3rd ed. Oxford: Blackwell Science; 1998.
9. **Online document** Doe J. Title of subordinate document. In: The dictionary of substances and their effects. Royal Society of Chemistry. 1999. http://www.rsc.org/dose/title of subordinate document. Accessed 15 Jan 1999.
10. **Online database** Healthwise Knowledgebase. US Pharmacopeia, Rockville. 1998. http://www.healthwise.org. Accessed 21 Sept 1998.
11. **Supplementary material/private homepage** Doe J. Title of supplementary material. 2000. http://www.privatehomepage.com. Accessed 22 Feb 2000.
12. **University site** Doe, J.: Title of preprint. http://www.uni-heidelberg.de/mydata.html (1999).
13. **FTP site** Doe, J.: Trivial HTTP, RFC2169. ftp://ftp.isi.edu/in-notes/rfc2169.txt (1999). Accessed 12 Nov 1999. Accessed 25 Dec 1999.
14. **Organization site** ISSN International Centre: The ISSN register. http://www.issn.org (2006). Accessed 20 Feb 2007.

For a full description of the Vancouver reference style, including numerous examples, please access:

www.nlm.nih.gov/bsd/uniform_requirements.html

H.10 Tables

Tables must be created in Microsoft Word table format. Tables should be numbered (with Roman numerals) and referred to by number in the text. Center the title above the table and type explanatory footnotes (indicated by superscript lowercase letters) below the table. Data must be placed in separate cells of the table to prevent text and numbers from shifting when the table is converted for publication on the Internet. Empty cells may be inserted to create spacing. Tables should not duplicate information provided in the text. Instead, tables should

be used to provide additional information that illustrates or expands on a specific point the author wishes to make. Each table should be self-explanatory.

H.11 Figures

AAPS PharmSciTech offers authors the use of color figures in online published manuscripts, free of charge. Figures (as well as photographs, drawings, diagrams, and charts) are to be numbered in sequential Arabic numerals. The description of each figure should be separated from the manuscript body text and collated in a separate file called “Legend to Figures.” All electronic artwork must be submitted online via our online peer review tracking system, Editorial Manager. Figure files should be submitted in TIFF or EPS format (1200 dpi for line drawings and 300 dpi for half-tones and gray-scale art); however, JPG, GIF, BMP, and PDF files may also be submitted as long as the dpi specifications above are met. Use of a professional graphics program such as Adobe Photoshop to edit and/or save photographs and graphics is highly recommended. Because of quality issues, it is not recommended that images be submitted in DOC, DOCX, PPT, or PPTX format.

Maximum combined count for tables and figures are as follows:

- Reviews, original research articles, meeting reports, and white papers: 10 (suggested)
- Mini-reviews: 6
- Brief technical notes and rapid communications: 6

H.12 Footnotes

Footnotes in the manuscript body should be avoided. When their use is absolutely necessary, footnotes should be numbered consecutively using Arabic numerals and should be typed at the bottom of the page to which they refer. Place a line above the footnote, so that it is set off from the text. Use the appropriate superscript numeral for citation in the text.

H.13 Does Springer provide English language support?

For editors and reviewers to accurately assess the work presented in your manuscript you need to ensure the English language is of sufficient quality to be understood. If you need help with writing in English, you should consider:

- Asking a colleague who is a native English speaker to review your manuscript for clarity.
- Visiting the English language tutorial which covers the common mistakes when writing in English.

- Using a professional language editing service where editors will improve the English to ensure that your meaning is clear and identify problems that require your review. Two such services are provided by our affiliates Nature Research Editing Service and American Journal Experts.
- English language tutorial
- Nature Research Editing Service
- American Journal Experts

Please note that the use of a language editing service is not a requirement for publication in this journal and does not imply or guarantee that the article will be selected for peer review or accepted. If your manuscript is accepted it will be checked by our copyeditors for spelling and formal style before publication.

Annexure I

Language editing certificate

Gill Smithies

Proofreading & Language Editing Services

59, Lewis Drive, Amanzimtoti, 4126, Kwazulu Natal

Cell: 071 352 5410 E-mail: moramist@vodamail.co.za

Work Certificate

To	Ms D. van Staden
Address	Centre of Excellence for Pharmaceutical Sciences, Faculty of Health Sciences, North West University, Private Bag X6001, Potchefstroom.
Date	22/10/2019
Subject	Development of a topical self-emulsifying drug development system for optimised delivery
Ref	GS/DvS/01

I, Gill Smithies, certify that I have proofed the following for language, grammar and style:

Chapters 1 and 4: Development of a topical self-emulsifying drug development system for optimised delivery,

to the standard as required by NWU, Potchefstroom Campus.

Gill Smithies

Annexure J

Ethics training proof of Attendance and Assessment

Private Bag X6001, Potchefstroom
South Africa 2520

Tel: +2718 299-1111/2222
Web: http://www.nwu.ac.za

Health Sciences Ethics Office for Research, Training and Support
Tel: +2718 299 2089
Email: minrie.greeff@nwu.ac.za

7 March 2018

Dear Ms Danielle Van Staden

PROOF OF ATTENDANCE AND ASSESSMENT

This letter certifies that you have attended the 2 day ethics training and successfully completed the associated assessment.

The Basics of Health Research Ethics

(Accreditation number: PSB002/037/01/2018 from University of Free State CPD accreditation department accredited by the HPCSA)

Presenter: Prof Minrie Greeff (Head of the Health Sciences Ethics Office for Research, Training and Support) **on 22 and 23 January 2018.**

This letter of attendance, as proof of ethics training and assessment, is valid for 3 years and expires on 31 Janaury 2021 (Where applicable, Ethics CEUs awarded: 14 CEUs).

Yours sincerely,

Prof Minrie Greeff
Head of Health Sciences Ethics Office for Research, Training and Support

Prof Awie Kotzé
Executive Dean: Faculty of Health Sciences

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