Formulation, characterisation and topical application of oil powders from whey protein stabilised emulsions

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TABLE OF CONTENTS

CHAPTER 3: Whey protein- or polysaccharide-stabilised oil powders for topical application: comparison of the release and topical delivery of salicylic acid from oil powders and from their redispersed oil powders

CHAPTER 4: Conclusion

ANNEXURES

List of Tables and Figures

CHAPTER 2: Oil powders as topical delivery systems

CHAPTER 3: Whey protein- or polysaccharide-stabilised oil powders for topical application: comparison of the release and topical delivery of salicylic acid from oil powders and from their redispersed oil powders

APPENDIX B : HPLC Validation

APPENDIX C : Partition Coefficient of Salicylic Acid between Oil and Aqueous Phase

APPENDIX D : Loss on drying

APPENDIX E : Oil leakage and encapsulation efficiency

APPENDIX F : Water dispersibility

APPENDIX G : Droplet size

APPENDIX H : Cumulative release data of salicylic acid

APPENDIX I : Skin absorption data of salicylic acid from redispersed oil powders and oil powders

PREFACE

This dissertation was written in article format, which includes an introductory chapter, a full length article for publication and appendices containing relevant experimental data. The candidate, Magdalena Kotzé was the primary author of the article (chapter 3) and all other chapters included in this thesis. The candidate performed all the experimental work under the supervision and assistance of all promoters.

- Chapter 2 represents a literature overview of the skin and oil powders,
- Chapter 3 includes methods, results and discussion of the investigation of different biopolymers on the release and topical delivery of the oil powders, written in article format and submitted to Skin Pharmacology and Physiology.

The article manuscript was formatted according to a standard format chosen for this dissertation. However, the reference style was maintained according to the guideline for authors of KARGER. The guideline for authors was added in the Appendices.

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ABSTRACT

The available literature indicates that to date, few research has been performed on oil powders for topical delivery. The aim of this project was to investigate the release characteristics of oil powder formulations, as well as their dermal and transdermal delivery properties.

Whey protein-stabilised emulsions were used to develop oil powders. Whey protein was used alone, or in combination with chitosan or carrageenan. Nine oil powders, with salicylic acid as the active ingredient, were formulated by using the layer-by-layer method. Three different pH values (pH 4, 5 and 6) were used to prepare the formulations, because of the different charges that polymeric emulsifiers may have.

The characteristics of the prepared oil powders were determined, including their droplet sizes, particle size distributions, loss on drying, encapsulation efficiencies, oil leakage and water dispersibility.

Release studies (membrane diffusion studies) were conducted by utilising cellulose acetate membranes (0.2 µm pore size) and Franz-type diffusion cells over a period of eight hours. The release of the active ingredient was determined for all nine powders, their respective template emulsions, as well as their respective oil powders redispersed in water. The release of salicylic acid from the respective redispersed oil powders was then further compared to its release from the template emulsions and from the oil powders.

The effect of pH and different polymer types used in preparing the oil powders, their respective redispersed oil powders and the template emulsions were determined with regards to the release of the active ingredient from all these preparations. The effect of pH and different polymers used was furthermore determined on the oil powders and their respective redispersed oil powders, with regards to their dermal and transdermal deliveries.

Transdermal delivery and skin uptake were investigated on specifically selected powders only, based on the outcomes of the oil powder characterisation and release data. The qualifying formulations were chitosan pH 4, 5 and 6, whey and carrageenan pH 6 oil powders, together with their respective redispersed oil powders in water.

Human abdominal skin was dermatomed (thickness 400 µm) for use in the diffusion studies. Franz-type diffusion cells were used over a period of 24 hours.

The results of the membrane release studies indicated that the oil powders had achieved a significantly higher release than their respective redispersed oil powders. The release of salicylic acid from the redispersed oil powders and from their respective emulsions was similar. The transdermal delivery test outcomes showed that the effect of pH could have been

influenced by the degree of ionisation, resulting in a decrease in permeation with increasing ionisation of salicylic acid, in accordance with the pH partition hypothesis. Furthermore, biopolymers, such as chitosan had demonstrated a penetration enhancing effect, which had led to the enhanced dermal and transdermal delivery of salicylic acid. A correlation was also found between the powder particle size and transdermal delivery.

Keywords: oil powders, whey proteins, chitosan, carrageenan, topical delivery, release, salicylic acid.

UITTREKSEL

Die beskikbare literatuur toon aan dat min navorsing tot op hede op olie-poeiers as topikale afleweringsisteem uitgevoer is. Die doel van hierdie studie was om die vrystellingskaraktereienskappe van olie-poeierformulerings, sowel as hul dermale en transdermale afleweringseienskappe te bestudeer.

Wei-proteïen-gestabiliseerde emulsies is gebruik om olie-poeiers te ontwikkel. Wei-proteïene is alleen, of in kombinasie met karrageenan of kitosaan gebruik. Nege olie-poeiers, met salisielsuur as die aktiewe bestanddeel, is geformuleer deur van die laag-op-laag metode gebruik te maak. Drie verskillende pH vlakke (pH 4, 5 en 6) is gebruik waarteen die olie poeiers voorberei is, omrede die verskillende ladings wat polimere mag besit.

Die eienskappe wat op die voorbereide olie-poeiers bepaal is, het hul druppelgroottes, deeltjiegrootte-verspreidings, verlies met droging, enkapsuleringsdoeltreffendhede, olie-lekkasie en waterdeurdringingsvermoë ingesluit.

Vrystellingstudies (membraandiffusiestudies) is uitgevoer deur van sellulose-asetaatmembrane (0.2 µm poriegrootte) en Franz-tipe diffusieselle oor ‘n periode van agt ure gebruik te maak. Die vrystelling van die aktiewe bestanddeel is vir al nege poeiers, hul ooreenkomstige templaat-emulsies, sowel as vir hul ooreenkomstige olie-poeiers wat in water heropgelos is bepaal. Die vrystelling van salisielsuur vanaf die ooreenkomstige heropgeloste olie-poeiers is voorts met die vrystelling vanaf die templaat-emulsies en vanaf die olie-poeiers vergelyk.

Die effek van pH en die verskillende polimere wat in die voorbereiding van die olie-poeiers, hul ooreenkomstige heropgeloste olie-poeiers en templaat-emulsies gebruik is, is met betrekking tot die vrystelling van die aktiewe bestanddeel vanaf al hierdie formulerings bepaal. Die effek van pH en die verskillende polimere wat gebruik is, is voorts op die olie-poeiers en hul ooreenkomstige heropgeloste olie-poeiers bepaal, wat betref hul dermale en transdermale aflewerings.

Transdermale aflewerings- en vel-opname-studies is slegs op spesifiek geselekteerde olie-poeiers uitgevoer, gebaseer op die uitkomstes van die olie-poeier-eienskappe- en die vrystellingsdata. Die kwalifiserende formularings was khitosaan pH 4, 5 en 6, wei en karrageenan pH6 olie-poeiers, tesame met hulle ooreenkomstige heropgeloste olie-poeiers in water.

Menslike abdominale vel is per dermatoom gesny (dikte 400µm) vir gebruik in die diffusiestudies. Franz-tipe diffusieselle is gebruik oor ‘n periode van 24 uur.

Die resultate van die membraan-vrystellings-studies het aangetoon dat die olie-poeiers ‘n aansienlike hoër vrystelling as die heropgeloste olie-poeiers bereik het. Die vrystelling van salisielsuur vanaf die heropgeloste olie-poeiers en hul ooreenkomstige emulsies was soortgelyk. Die transdermale aflewerings-toetsuitkomstes het aangedui dat die effek van pH deur die mate van ionisasie beïnvloed kon gewees het, wat in ‘n afname in deurlating met ‘n toename in ionisasie van salisielsuur gelei het, in ooreenstemming met die pH partisie-hipotese. Voorts het biopolimere, soos kitosaan ‘n penetrasie verbeteringseffek aangetoon, wat tot die verbeterde dermale en transdermale aflewering van salisielsuur aanleiding gegee het. ‘n Korrelasie is ook tussen die poeierdeeltjiegrootte en trandermale aflewering gevind.

Soekwoorde: olie-poeiers, wei-proteïen, kitosaan, karrageenan, topikale aflewering, vrystelling, salisielsuur

CHAPTER 1

Introduction and Statement of the problem

1.1 Introduction

The skin is the largest organ in the human body (Hadgraft, 2004:291) and acts as a barrier against the environment. The outer layer, the stratum corneum, serves as the actual barrier by preventing the ingress of xenobiotics and the loss of endogenous material, such as water (Hadgraft, 2004; Brown *et al*., 2006:175). The human skin can, however, also be used as a route for the administration of drugs (Bernardo *et al*., 2008:3781).

Dermal delivery, also known as topical delivery, targets the pathological sites within the skin. Such dermal delivery results in minimal systemic absorption. Dermatological conditions are treated through dermal delivery systems, due to the cause of the disease being located within the skin. Dermatological conditions that can be treated with topical applications include skin cancer, psoriasis, eczema and microbial infections, for example. Transdermal delivery comprises the transport of a molecule through various layers of the skin, as well as the subsequent uptake into the systemic circulation. Transdermal delivery systems can therefore be used for the treatment of systemic and deeper tissues diseases, e.g. pain, motion sickness and high blood pressure (Brown *et al*., 2006:175). The transfer of drugs from the formulation delivery vehicle into the skin and through the skin into the blood circulation depends on the vehicle properties. The delivery vehicle should be designed to control drug delivery and to achieve the desired therapeutic effects (Bernardo *et al*., 2008:3781).

Topical formulations reach from liquids (e.g. lotions), through semi-solids (e.g. ointments, gels and creams) to solids (e.g. powders and transdermal patches) (Smit *et al*., 1999:781). Among the various types of formulations for topical application, emulsions form an important delivery vehicle type, because they are capable of solubilising hydrophilic and lipophilic ingredients (Förster *et al*., 1997).

It is common practice in the pharmaceutical, cosmetic and food industries to convert an oil-in-water (o/w) emulsion into a solid like powder by evaporating the aqueous continuous phase, usually through spray- or freeze drying. This technique of solidification of an oil-in-water emulsion is used to encapsulate lipophilic active ingredients into the oil droplets, or to prevent the oil from oxidation (Adelmann *et al*., 2012:1694). Different terms are used in the literature for the solid like powder resulting from evaporation of the water phase, for example oil powder (Adelmann *et al*., 2012:1694), dry emulsion (Ghouchi-Eskander *et al*., 2012:384), powdered

redispersible emulsion (Takeuchi *et al*., 1991:1528), or micro-encapsulated oil (Lim *et al*., 2011:1220).

Oil powders offer some advantages, compared to liquid emulsions, such as an increase in physical stabilisation and a reduction in microbial contamination. Moreover, oil powders have proven to enhance the chemical stability of encapsulated substances against light and oxidation and are suitable for controlled release (Hansen *et al*., 2005:204; Jang *et al*., 2006:405).

Two different types of oil powders exist, those that contain solid hydrophilic carriers, and those that don't. Solid hydrophilic carriers, such as maltodextrin, starch, lactose and cellulose (Lim *et al.,* 2011:1220; Adelmann *et al*., 2012:1694; Mezzenga & Ulrich, 2010:16658) are added to the aqueous phase of the oil-in-water emulsion and are required to co-stabilise the oil droplets against coalescence during the evaporation of water, and to prevent oil leakage during storage of the oil powder. However, the amount of carrier that is required for stabilisation, ranges between 30 - 80% of the final oil powder, yielding a very low oil content (Adelmann *et al*., 2012:1694). The second type of oil powders avoids the addition of hydrophilic carriers. In this case, the oil-water interface of the emulsion is physicochemically stabilised to be sufficiently elastic for withstanding the manufacturing of oil powders (Mezzenga & Ulrich, 2010:16658).

With regards to the second type of oil powders, three different methods were found in literature for the stabilisation of the oil-water interface. The first method uses the layer-by-layer technique to assemble a multi-layer of poly-electrolytes (e.g. proteins, polysaccharides, low molecular weight surfactants) at the interface (Adelmann *et al*., 2012:1694). Cheaper and more efficient alternatives to the layer-by-layer stabilisation of interfaces include the thermal, or the enzymatic cross linking of proteins. In a recent study, it was found that the use of thermal, cross-linked *β*-lactoglobulin for the stabilisation of the emulsion had allowed the conversion of the emulsion into the oil powder. No additional hydrocolloids were required and hence the oil content was much higher, compared to oil powders with hydrophilic carriers (> 90 wt %) (Mezzenga & Ulrich, 2010:16659). A third method uses solid particles for the stabilisation of the template emulsion, instead of proteins. Due to the irreversible adsorption of the silica particles to the droplet interface, no further preparation is required prior to spray- or freeze drying, hence simplifying the method (Adelmann *et al*., 2012:1694). It was also noted that the two different techniques, used to evaporate the water, had resulted in different formulations. Oil gels had formed when water was removed slowly (freeze drying), whereas rapid water evaporation through spray drying had yielded oil powders. The oil powders contained nearly 90 wt % of oil and the oil gels comprised nearly 98 wt % of oil (Adelmann *et al*., 2012:1694).

A study by Lim *et al*. (2012) revealed that the choice of wall material to encapsulate red-fleshed pitaya seed oil into the oil powder, had affected the encapsulation efficacy and stability of the oil. They investigated various proteins and polysaccharides as wall materials and found that

sodium caseinate had resulted in the highest micro-encapsulation efficiency and that lactose had been the most effective polysaccharide for slowing down oxidation (Lim *et al*., 2012:1220).

During this study, the layer-by-layer method was used to stabilise the oil-water interface of template emulsions for use in the oil powder preparations. The polysaccharides, carrageenan and chitosan, in combination with whey proteins were employed for the multi-layer assembly at the interface, because these combinations had proven to generate stable emulsions (Li *et al*., 2010; Ru *et al*., 2009). Nano-emulsions, stabilised by whey protein isolate, or *β*-lactoglobulin, the major whey protein used, showed improved stability, compared to nano-emulsions stabilised with traditional emulsifiers (e.g. Tween 80, Poloxamer 188, Cremophor EL). Moreover, *β*-lactoglobulin had exhibited an enhanced emulsifying capacity, which was further increased through denaturation of the protein at 85°C (He *et al*., 2011). Li *et al*. (2010) discovered that the lipid droplets that were surrounded by *β*-lactoglobulin-chitosan coatings had shown a better stability towards droplet coalescence, than those solely surrounded by *β*-lactoglobulin coatings. Ru *et al*. (2009) investigated the combination of *β*-lactoglobulin and *ι*-carrageenan and found that the optimum concentrations to form stable emulsions had been 0.3 wt % - 0.6 wt % at pH 4.0, and 0.4 wt % - 0.7 wt % at pH 3.4.

Chitosan is reported to have also been used in the preparation of spray dried emulsions and had been added to such formulations, because of its anti-oxidative, film forming and emulsifying properties. It was found that a spray dried emulsion of tuna oil, stabilised by chitosan-lecithin, had been more resistant against oxidation than the bulk oil. Spray dried emulsions with tuna oil had also been successfully protected by encapsulated mixtures of chitosan and maltodextrin, or whey protein (Shen *et al.,* 2010:4487).

Different pH values were used during this study to prepare the primary emulsions. A study by Shen *et al*. (2010) indicated the importance of pH on the stability of the fish oil powder, due to changes in the electrostatic interactions between chitosan and emulsifying starch, used in preparing the fish oil powders. By adjusting the pH, the biopolymer's charge and conformation had changed, which in turn had determined the characteristics of the biopolymer mixtures. Shen *et al*. (2010) demonstrated that the oil powders at a higher pH (pH = 6.0) had been more stable against oxidation, than those at pH 4.9. It was postulated that the increased stability of the fish oil powders at higher pH values had been due to increased electrostatic interactions between chitosan and emulsifying starch.

1.2 Research problem and justification

A new type of oil powders, incorporating thermo, cross-linked whey proteins, or solid particles, has been developed recently (Adelmann *et al*., 2012:1694). It is further reported that multiple

layers of polyelectrolytes could be used to manufacture this type of oil powders. To the best of our research team's knowledge, however, neither the release characteristics, nor the dermal and transdermal delivery of this new type of oil powders have yet been investigated. It could therefore be of value to investigate the effects that polysaccharides, adsorbed to the whey proteins at the oil-water interface, would have on the release and topical performance of oil powders. As the wall materials as well as the pH could change the characteristics of the interfacial layer (whey proteins and polysaccharides), the effects of such changes were also investigated during this study. Two methods have been used during this study to manufacture oil powders, i.e. spray- and freeze drying, and their results compared. Furthermore, since no data on dermal and transdermal delivery was found in the literature, oil powder and redispersed oil powders was used to test for the topical performance.

This study formed part of a larger research project, of which the study by Combrinck *et al*. (2014), i.e. 'Formulating and testing whey protein-stabilised emulsions', refers. The emulsions obtained during their study were used to prepare the oil powders during this project. The following objectives were stated for this study:

- Preparation of stable oil powders from oil-in-water emulsions, stabilised solely with whey proteins, whey proteins combined with chitosan, and whey proteins combined with carrageenan. Additionally, three different pH values (pH 4, 5 and 6) for the template emulsions were applied.
- Development and validation of a suitable method for the quantitative determination of the active ingredient, i.e. salicylic acid, in the prepared samples (e.g. oil powder, release samples, skin samples).
- Investigation of the following powder properties:
    - Loss on drying,
    - Encapsulation efficiency,
    - Oil leakage,
    - Particle/Aggregate size and particle size distribution, and
    - Water dispersibility.
- Determination of the release of salicylic acid from the nine prepared oil powders, from their respective template emulsions, and from their respective oil powders redispersed in water. Comparison of the release test outcomes from the redispersed oil powders with those of the template emulsions and the oil powders.
- Determination of the transdermal delivery of salicylic acid and its skin uptake into the various skin layers, including a comparison of the selected oil powders (based on the outcomes of the oil powder characterisation and release data) with their respective redispersed oil powders in water.

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CHAPTER 2

Oil powders as topical delivery systems

2.1 Introduction

This chapter gives a brief overview of the different layers of the skin. The characteristics of the different skin layers emphasise the factors that must be considered when selecting suitable drug delivery vehicles for the optimal transfer and delivery of active ingredients across the skin.

2.2 The skin as barrier

The skin is the largest organ in the human body (Hadgraft, 2004:291) and acts as a barrier against its environment. The human skin consists of multiple histological layers, i.e. the epidermis, the dermis and the hypodermis (De Jager *et al*., 2006:217). The dermis contains the blood vessels, which provide the skin with nutrients and oxygen. The deepest inner layer of the skin is the hypodermis, also known as the subcutaneous fat tissue. The hypodermis supports the dermis and epidermis and it is also responsible for the thermal isolation and mechanical protection of the body (De Jager *et al*., 2006:217). The human skin can also be used as a route for the administration of drugs (Bernardo & Saraiva, 2008:3781).

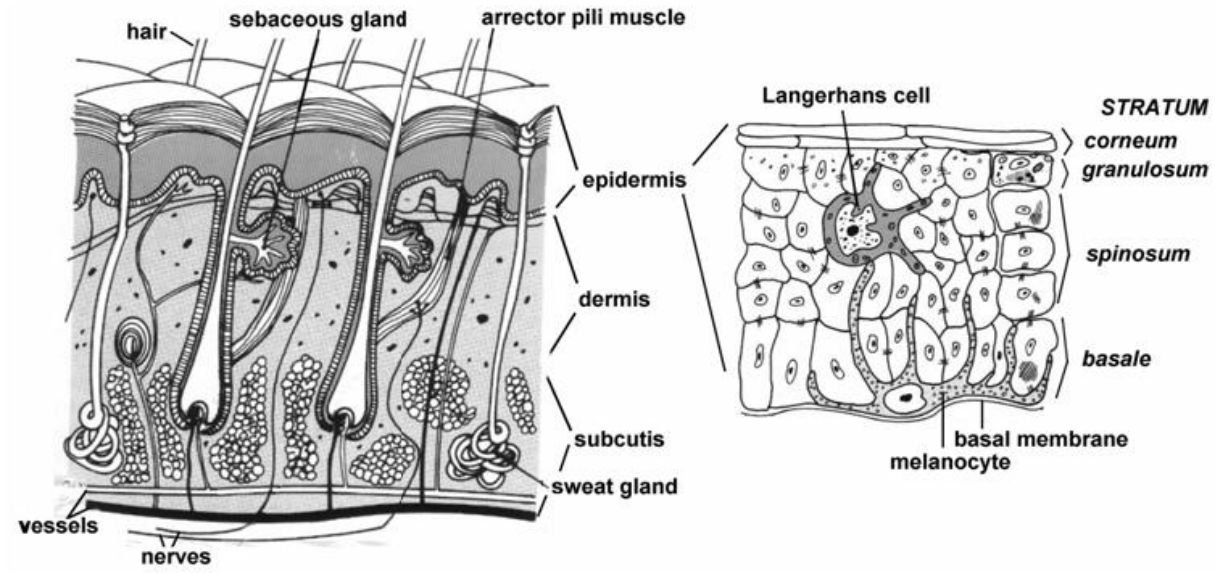

Figure 2.1: The anatomy of the skin (Ylä-Outinen, 2002).

2.3 Structure and function of human skin

2.3.1 Stratum corneum

The uppermost, non-viable layer of the epidermis, i.e. the stratum corneum, serves as the actual barrier against the environment, by preventing the ingress of xenobiotics and the loss of endogenous material, such as water (Brown *et al*., 2006:175; De Jager *et al*., 2006:217; Hadgraft, 2004:291). The stratum corneum consists of flattened, dead skin cells that form ten to fifteen layers of keratin filled corneocytes, surrounded by a lipid medium. This scale-like, protective layer is only 10 µm thick when dry and swells several times this thickness when wet (Barry, 1983:6; Williams, 2003:8).

The stratum corneum is often referred to as a 'brick and mortar' model. The corneocytes are enclosed by the intra-cellular, lipid rich matrix. The human stratum corneum contains a mixture of lipids. The continuous, multiple, bi-layered lipid component of this mixture in the stratum corneum regulates drug flux through the tissue. This model is used to describe the stratum corneum's protein rich corneocytes, which are implanted into an intra-cellular matrix containing ceramides, fatty acids, cholesterol, and cholesterol sulfate and sterol/wax esters (Baran & Maibach, 2010:14; Williams, 2003:9-12). The stratum corneum consists of different components, i.e. the inter-cellular lipids, matured keratinocytes and desmosomes that hold the corneocytes together through inter-cellular connections between the corneocytes (Williams, 2003:9-12).

2.3.2 Viable epidermis

The viable epidermis is underlying the stratum corneum and lies outside of the next underlying layer, i.e. the dermis. The viable epidermis is responsible for the generation of the stratum corneum (De Jager *et al*., 2006:217; Rhein & Babajanyan, 2007:4). The viable epidermis (50 - 100 µm thick) consists of three layers. The deepest inner layer is the stratum basale, followed by the stratum spinosum in the middle and the outer stratum granulosum layer (Bouwstra & Ponec, 2006:2081). The epidermis acts as the shielding layer for the entire body against dehydration and damage from foreign substances (Rhein & Babajanyan, 2007:4). The epidermis consists of a matrix of connective tissue, which allows the skin to be elastic and resistant against deformation (De Jager *et al*., 2006:217). New cells are constantly formed in the epidermis, so that shed cells from the surface of the stratum corneum are balanced by new cell growth in the lower epidermis (Bouwstra & Ponec, 2006:2081).

2.3.3 Dermis

The dermis is a major component of the skin and lies just beneath the epidermis. It is 3 - 5 mm thick and fibrous (Williams, 2003:2). Because of the structure that it provides to the membrane, it prevents damage to organs. A network of blood vessels, nerves, lymphatics and skin appendages are found, which lend support to the dermis. The main living cell type in the dermis is the fibroblasts, which generate the fibrous material. The dermis consists of a few connective tissue proteins, such as collagens, elastin and proteoglycans (Barry, 1983:7-8; Rhein *et al*., 2007:5).

2.3.4 Hypodermis

The hypodermis is also known as the subcutaneous fat, or subcutis and occurs across the whole body as a fibro-fatty layer. This hypodermis layer is the deepest layer of the skin and supports the dermis (Barry, 1983:10). The primary purpose of this layer of adipose tissue is to insulate the body and to give mechanical protection against physical shock. This fatty layer carries the main blood vessels and nerves to the skin. It also provides a readily available supply of high-energy molecules (Williams, 2003:2).

2.3.5 Skin appendages

Three main appendages exist on the surface of the human skin. Hair follicles cover the entire body, except for the feet soles, hand palms and lips. Associated with the hair follicles are the sebaceous glands that secrete sebum. Sebum mainly functions as a lubricant and consists of free fatty acids, waxes and triglycerides. The lubricant's function is to maintain a pH value of around 5 on the skin surface. Sweat- and apocrine glands are also found in the dermal tissue. Heat and emotional stress stimulate the sweat glands, which secrete a dilute salt solution with a pH also at around 5. The apocrine glands are limited to specific areas, including the axillae, nipples and ano-genital regions. It's 'milk' protein secretion is stimulated by heat (Williams, 2003:4).

2.4 Dermal and transdermal delivery

Dermal delivery, also known as topical delivery, targets the pathological sites within the skin. Such dermal delivery results in minimal systemic absorption. Dermatological conditions are treated through dermal delivery systems, due to the cause of the disease being located within the skin. Dermatological conditions that can be treated with topical applications include skin cancer, psoriasis, eczema and microbial infections, for example (Brown *et al*., 2006:175). Transdermal delivery comprises the transport of a molecule through various layers of the skin,

as well as the subsequent uptake into the systemic circulation. Transdermal delivery systems can therefore be used for the treatment of systemic and deeper tissues diseases, e.g. pain, motion sickness and high blood pressure (Brown *et al*., 2006:175). The transfer of drugs from the formulation delivery vehicle into the skin and through the skin into the blood circulation depends on the delivery vehicle properties. The vehicle should be designed to control drug delivery and to achieve the desired therapeutic effects (Bernardo *et al*., 2008:3781).

2.4.1 Absorption

Transdermal absorption of a pharmaceutical component consists of various steps. The first comprises penetration from an outside source, where the substance enters the stratum corneum. The second step involves permeation into the viable epidermis, followed by distribution of the drug into the blood capillaries and lymphatic system (Fernandes *et al*., 2005:184).

Multiple steps of transdermal drug delivery include (Kalia & Guy, 2001:160):

1. Dissolution of active ingredient within and release from the formulation.
2. Partitioning of the drug into the stratum corneum.
3. Diffusion through the stratum corneum, mainly *via* the lipidic inter-cellular route.
4. Partitioning from the stratum corneum into the aqueous viable epidermis.
5. Diffusion through the aqueous viable epidermis into the upper dermis.
6. Uptake of the drug into the local capillary network.
7. Reaching the systemic circulation.

2.4.2 Penetration pathways across the skin

Three entry routes exist, i.e. the trans-appendageal, the trans-cellular and the inter-cellular routes. A molecule will follow one, or a combination of these routes from when it is applied on the skin surface, until it appears in the systemic circulation (Hadgraft, 2001:1; Williams, 2003:28).

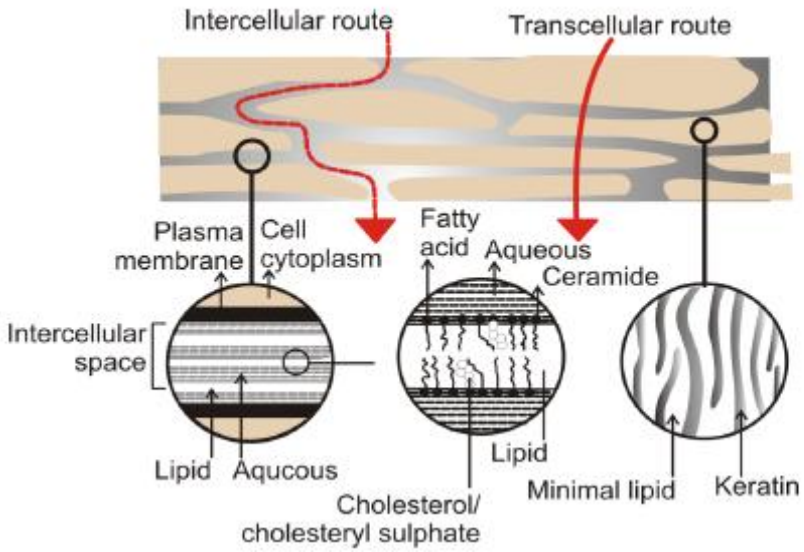

Figure 2.2: Illustration of inter- and trans-cellular drug delivery routes (Barry, 2007:567).

2.4.2.1 Trans-appendageal (shunt route transport)

The appendages, such as the hair follicles and sweat glands offer pores that can bypass the stratum corneum (Williams, 2003:31). The trans-appendageal route is limited to the uptake of substances, due to their low surface area (Hadgraft, 2001:1). Different opinions exist regarding this route, such as that of Lademann *et al.* (2007:159), who believe that the hair follicles play an important part in skin penetration and can influence the penetration process. Higher absorption was found in areas with more hair follicles (Feldmann & Maibach, 1967:181), whereas in scarred, appendage free skin, a lower percutaneous absorption than normal skin is reported (Hueber *et al.*, 1994:245; Tenjarla *et al.,* 1999:147). The fractional area available for trans-appendageal transport is about 0.1%. This route is mainly for molecules that experience high resistance in the stratum corneum, hence it favours larger polar molecules (Barry, 2001:101).

2.4.2.2 Trans-cellular

Hydrophilic substances prefer this route, because of the protein enriched corneocytes (Schnetz & Fartasch, 2001:166). Since the drug molecules move directly across the stratum corneum, polar molecules can cross the stratum corneum *via* this route. The path length is thus equal to

the thickness of the stratum corneum. Highly hydrated keratin offers a route for hydrophilic molecules through the membrane. Although the hydrophilic molecules diffuse rapidly, due to the aqueous environment of this component, they do face a few obstacles when crossing the stratum corneum. The multiple, bi-layered lipids comprise the rate limiting barrier against permeation. Firstly, the molecule partitions into the keratin and then diffuses through the keratin. The molecule should then partition into the bi-layered lipids, diffuse across the bi-layered lipids and only then can it move onto the next keratinocyte. The processes of multiple partitioning and diffusion between hydrophobic and hydrophilic domains are generally unfavourable towards most drugs (Williams, 2003:32-34).

2.4.2.3 Inter-cellular

The inter-cellular route is the most important route of drug delivery. The intra-cellular spaces contain structured lipids. Diffusing molecules need to cross through multiple lipophilic and hydrophilic areas, before they reach the space between the stratum corneum and the epidermis (Hadgraft, 2004:292). It is generally considered that lipophilic substances follow this route, because they are readily dissolved in the lipid bi-layers (Schnetz & Fartasch, 2001:166). The rate limiting barrier to drug flux is still the lipid bi-layers. The path length that a molecule would follow through the inter-cellular route is longer than the thickness of the stratum corneum (Williams, 2003:34-35).

2.5 Delivery vehicles

An active ingredient can be transported into the skin and eventually through the skin in various ways (Abbott, 2012:217). Different delivery vehicles can be used to transport an active ingredient, such as creams, emulsions, foams, gels, lotions, ointments, suspensions, or oil powders (Kurian & Barankin, 2011:4). Three types of delivery vehicles were used during this study for carrying the soluble salicylic acid into and through the skin, i.e. emulsions, oil powders and redispersed oil powders. Vehicles are designed to overcome the barrier function of the stratum corneum (Foldvari, 2000:417). Topical formulations reach from liquids (e.g. lotions), through semi-solids (e.g. ointments, gels and creams) to solids (e.g. powders and transdermal patches) (Smith *et al*., 1999:781). Among the various types of formulations for topical application, emulsions form an important vehicle type, because they are capable of solubilising hydrophilic and lipophilic ingredients (Förster *et al*., 1997). Topical vehicles are important for releasing the drug onto the skin surface and for the drug molecules to diffuse through the skin layers. Delivery vehicles can affect the delivery of an active ingredient, because it can interact with skin and other drugs, and is the scientifically sound choice thereof thus essential.

2.6 Advantages and limitations of transdermal drug delivery

2.6.1 Advantages

- By following the transdermal route, a drug avoids the first pass metabolism. The incompatibility of the gastrointestinal tract is therefore ruled out (Brown *et al*., 2006:177).
- Patients prefer this route, because of its convenience (Davidson *et al.,* 2008:1197).
- The transdermal route is an alternative route of administration, when the oral route is unavailable (Brown *et al*., 2006:177).
- Reduction of side effects, due to optimisation of the blood concentration-time profile (Kydonieus & Wille, 2000:3).
- Reversibility of drug delivery that would allow for the removal of the drug source (Kydonieus & Wille, 2000:3).

2.6.2 Limitations

- Slow drug absorption, which can be incomplete, due to the stratum corneum and outside factors, such as washing, adherence to clothes and shedding of stratum corneum scales (Barry, 2007:571).
- A compound's molecular weight must be less than 500 Da in order to successfully diffuse through the stratum corneum (Bos & Meinardi, 2000:165).
- The Log P (octanol/water) value, required for systemic delivery, should range between 1 - 3 to be soluble in aqueous and lipid areas of the skin (Brown *et al*., 2006:177).

2.7 Physiochemical factors affecting transdermal drug delivery

Specific physicochemical properties are important for a drug to follow the transdermal route (Farahmand & Maibach, 2008:11). The important physicochemical factors that influence permeation through the skin include pH, diffusion coefficient, partition coefficient and the molecular size of the particles.

2.8 Biological factors

Biological factors, such as age, gender, hydration, temperature and diseases influence the skin through the delivery rate of the drug. Any disorders of the skin, for example, will affect the nature of the skin barrier (Williams, 2003:14).

Since ageing of skin does not really change the percutaneous penetration in adults, age itself does not influence drug delivery (Tanner & Marks, 2008:251). Because the skin on the palms, face and genitalia areas are really thin, the drug can penetrate with ease (Tanner & Marks,

2008:251). Variations in permeability, hence variations of drug absorption can thus be observed, because the thickness of the stratum corneum differs from area to area on the body (Williams, 2003:16). Williams (2003:17) states that the keratinocytes are larger in females (37 - 46 µm) than in males (34 - 44 µm), but no differences in transdermal delivery between corresponding locations in the two sexes are reported.

Hydration of the human skin, especially the stratum corneum, can influence its barrier properties. The mechanisms that is known for hydration, may lead to water induced swelling of the corneocytes and water induced expansion of the inter-cellular lipid lamellae (Williams & Barry, 2002:25). As stated by Williams and Barry (2002:25), the water content of the stratum corneum is between 15 - 20% of the tissue's dry weight.

It is known that the average internal temperature of the human body is 37°C and the external temperature 32°C. When the temperature rises, blood flow increases, causing the blood vessels to dilate. This would thus cause the diffusion rate of a drug to increase also. In viscous formulations, with an increase in temperature, the viscosity will decrease, leading to better diffusion through the vehicle (Watkinson & Brian, 2002:85).

Permeation rates can be affected by certain diseases. Corn and warts, for example, thicken the skin, therefore the path length increases and absorption can be delayed. Contrary, psoriatic skin will enhance permeation, as the epidermal structure is different. Other systemic diseases, such as diabetes, are also known to change the epidermal basement membranes and capillary functions (Barry, 2007:575).

2.9 Oil powders

2.9.1 Introduction

Oil powders consist of solid, loose, dry particles of varying degrees of fineness. It is common practice in the pharmaceutical, cosmetic and food industries to convert oil-in-water emulsions into solid like powders by evaporating the aqueous continuous phase, usually through spray- or freeze drying. This technique of solidification of an oil-in-water emulsion is used to encapsulate lipophilic active ingredients into the oil droplets, or to prevent the oil from oxidation (Adelmann *et al*., 2012:1694). Different terms are used in the literature for the solid like powder resulting from evaporation of the water phase, for example oil powder (Adelmann *et al*., 2012:1694), dry emulsion (Ghouchi-Eskander *et al*., 2012:384), powdered redispersible emulsion (Takeuchi *et al*., 1991:1528), or micro-encapsulated oil (Lim *et al*., 2011:1220). The term used in this study is oil powders.

Oil powders offer some advantages, compared to liquid emulsions, such as an increase in physical stabilisation and a reduction in microbial contamination. Moreover, oil powders have proven to enhance the chemical stability of encapsulated substances against light and oxidation and are suitable for the controlled release of active ingredients (Hansen *et al*., 2005:204; Jang *et al*., 2006:405).

Oil powders can be redispersed in water to convert them into their respective emulsions. Another advantage of oil powders is the increased drug concentration, compared to the initial emulsion, due to the evaporation of water. Marefati *et al*. (2013) report that they were able to produce a powder with an oil content of up to 80%. These freeze dried powders were still stable and could be easily redispersed. Romonchuk and Bunge (2006:2526) compared the permeation of powders with saturated aqueous solutions. They found that absorption into homogeneous silicone rubber (polydimethylsiloxane) membranes were similar for powders and liquid solutions. The flux of pure powders, compared to liquid solutions, was, however, smaller for human skin than for the membranes. These researchers were unable to conclude on the reason for the significant differences found between the absorption from powder to membranes and to skin, but proposed the following three possibilities. The membranes are homogeneous, whereas the skin is not. The diffusion of the active ingredient and the solubility in the skin are much higher in fully hydrated skin than in partly hydrated skin. Thirdly, the flux from the powdered chemicals into the skin is lower than the flux from saturated aqueous solutions (Romonchuk and Bunge, 2006:2532).

Since oil powders and redispersed oil powders could potentially be used as topical formulations, it was worth studying the dermal and transdermal deliveries from these formulations. Moreover, to date, few research has been done on oil powders for topical delivery.

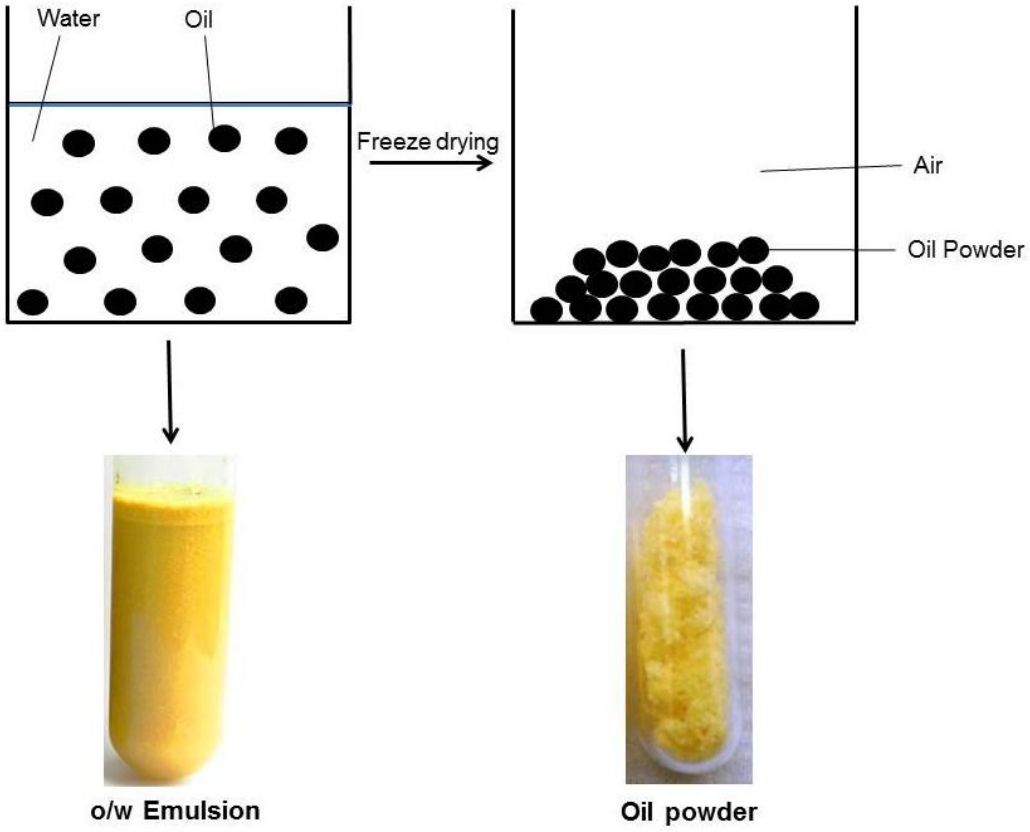

Figure 2.3: Process of oil powder formation.

2.9.2 Different types of oil powders

Two different types of oil powders exist, those that contain solid hydrophilic carriers and those that don't. Solid hydrophilic carriers, such as maltodextrin, starch, lactose and cellulose (Lim *et al.,* 2011:1220; Adelmann *et al*., 2012:1694; Mezzenga & Ulrich, 2010:16658) are added to the aqueous phase of the oil-in-water emulsion and are required to co-stabilise the oil droplets against coalescence during the evaporation of water, and to prevent oil leakage during storage of the oil powder. The amount of carrier that is required for the stabilisation, however, ranges between 30 - 80% of the final oil powder, yielding a very low oil content (Adelmann *et al*., 2012:1694). The second type of oil powders avoids the addition of hydrophilic carriers. In this case, the oil-water interface of the emulsion is physicochemically stabilised to be sufficiently elastic for withstanding the manufacturing of the oil powder (Mezzenga & Ulrich, 2010:16658).

For the second type of oil powders, three different methods were found in literature for the stabilisation of the oil-water interface. The first method uses the layer-by-layer technique to assemble a multi-layer of polyelectrolytes (e.g. proteins, polysaccharides, low molecular weight surfactants) at the interface (Adelmann *et al*., 2012:1694). Cheaper and more efficient alternatives to the layer-by-layer stabilisation of interfaces include thermal, or the enzymatic cross linking of proteins. In a recent study, it was found that the use of thermal, cross-linked *β*-

lactoglobulin for the stabilisation of the emulsion had allowed the conversion of the emulsion into the oil powder. No additional hydrocolloids were required and hence the oil content was much higher, compared to oil powders with hydrophilic carriers (> 90 wt %) (Mezzenga & Ulrich, 2010:16659). A third method includes using solid particles for the stabilisation of the template emulsion, instead of proteins. Due to the irreversible adsorption of the silica particles to the droplet interface, no further preparation is required prior to spray- or freeze drying, hence simplifying the method (Adelmann *et al*., 2012:1694). It was also noted that the two different techniques used to evaporate the water had resulted in different formulations. Oil gels had formed when water was removed slowly (freeze drying), whereas rapid water evaporation through spray drying had yielded oil powders. The oil powders contained nearly 90 wt % oil and the oil gels comprised nearly 98 wt % oil (Adelmann *et al*., 2012:1694).

2.9.3 Encapsulation and stability

Encapsulation is a method during which particles are covered with a thin film of coating, or with wall material (Klinkesorn *et al.,* 2006:449). When an emulsion is converted into a powder, the protein components that remain in the aqueous phase of the emulsion form part of the matrix. The matrix surrounds the encapsulated oil droplets (Augustin *et al*., 2006:30). A study by Lim *et al*. (2012) revealed that the choice of wall material to encapsulate red-fleshed pitaya seed oil in the oil powder had affected the encapsulation efficacy and stability of the oil. They investigated various proteins and polysaccharides as wall materials and found that sodium caseinate had resulted in the highest micro-encapsulation efficiency and that lactose had been the most effective polysaccharide for slowing down oxidation (Lim *et al*., 2012:1220). When the emulsion is dried, water is removed from the emulsion, resulting in oil droplets being surrounded by emulsifier molecules that form the wall material (Klinkesorn *et al*., 2006:450).

To ensure a stable emulsion, optimum encapsulation requires that the emulsion should consist of small oil droplets. Small droplets are also important for rapid absorption. The emulsion must therefore be stable, which requires the selection of the correct emulsifying system. In the food industry, encapsulation is used very efficiently, because important ingredients are merged into food without affecting the taste, aroma, texture, vitamins, or the food itself (Augustin *et al*., 2006:25; Fäldt & Bergnestål, 1996:421). In the cosmetic and pharmaceutical industries, such encapsulation efficiency can also be beneficial, since high concentrations of drugs can be incorporated into oil powders.

A study by Shen *et al*. (2010) indicated the importance of the pH value on the stability of fish oil powder, due to changes in the electrostatic interactions between chitosan and emulsifying starch, used in preparing the fish oil powders. By adjusting the pH, the biopolymer's charge and conformation had changed, which in turn had determined the characteristics of the biopolymer

mixtures. Shen *et al*. (2010) demonstrated that the oil powders at a higher pH (pH = 6.0) had been more stable against oxidation, than those at pH 4.9. It was postulated that the increased stability of the fish oil powders at higher pH values had been due to increased electrostatic interactions between chitosan and emulsifying starch.

2.9.4 Freeze drying

Freeze drying is one of the methods used to obtain oil powders from oil-in-water emulsions. Freeze drying is performed at temperatures lower than ambient temperatures. Because no air is present also, it prevents deterioration (Anwar & Kunz, 2011:368). Freeze drying is based on dehydration through sublimation of the ice fraction of the frozen product. Three main steps exist in the freeze drying method, i.e. freezing, primary drying (sublimation) and secondary drying (desorption). Primary drying begins when the pressure in the chamber is reduced, causing sublimation to start, because of the difference in pressure (Anwar & Kunz, 2011:374). Oxidation and chemical modification can damage the oil powder in this situation. Freeze drying is a suitable method, because it minimises the risk of product damage from changes in structure, texture, appearance and flavour. Freeze drying can also improve the shelf life of food (Anwar & Kunz, 2011:368), and of cosmetic products.

2.9.5 Biopolymers for transdermal delivery systems

During this study, the layer-by-layer method was used to stabilise the oil-water interface of template emulsions for use in the oil powder preparations. The polysaccharides, carrageenan and chitosan, in combination with whey proteins, were employed for the multi-layer assembly at the interface, because these combinations had proven to generate stable emulsions (Li *et al*., 2010; Ru *et al*., 2009). Shen *et al.* (2010:4487) report that biopolymer compounds are important for stabilising unsaturated oils and for formulation of the micro-encapsulation of oils.

A wide variety of biopolymers, e.g. proteins and polysaccharides, exists. The three biopolymers, used during this study, are discussed below. Bouyer *et al.* (2012:359) state that in the food industry, biopolymers are used to stabilise emulsions. Apart from some biopolymers adsorbing at a globule surface to therefore decrease the interfacial tension, they can also improve the interfacial elasticity (Bouyer *et al*., 2012:359). They conclude that most polysaccharides stabilise emulsions by improving the viscosity of the continuous phase of the emulsions.

2.9.5.1 Whey proteins

Milk is the primary source of whey proteins (whey). Whey proteins consist of two major proteins, i.e. *β*-lactoglobulin and *α*-lactalbumin (He *et al*., 2011:522; Livney, 2010:74). Fäldt and Bergenståhl (1996:421) emphasise the ability of whey proteins to encapsulate fat and that whey protein is one of many proteins used to stabilise emulsions.

Nano-emulsions, stabilised by whey protein isolate, or *β*-lactoglobulin, the major whey protein, showed improved stability, compared to nano-emulsions stabilised with traditional emulsifiers (e.g. Tween 80, Poloxamer 188, Cremophor EL). Moreover, *β*-lactoglobulin had exhibited an enhanced emulsifying capacity, which was further increased through denaturation of the protein at 85°C (He *et al*., 2011).

2.9.5.2 Chitosan

Chitosan is a cationic polymer and is produced through the deacetylation of chitin. Chitin can be found in exoskeletal material of invertebrates (Kumar *et al*., 2004:6019; Lima *et al*., 2012:322). It can be used in combination with an anionic component to form an electrostatic complex (Shen *et al*., 2010:4487). Li *et al*. (2010) discovered that the lipid droplets, surrounded by *β*-lactoglobulin-chitosan coatings, had shown a better stability to droplet coalescence, than those solely surrounded by *β*-lactoglobulin coatings. Chitosan is also reported to have been used for the preparation of spray dried emulsions and was it hence added to the formulations during this study, because of its anti-oxidative, film forming and emulsifying properties. It was found that a spray dried emulsion of tuna oil, stabilised by chitosan-lecithin, had been more resistant against oxidation, than the bulk oil. Spray dried emulsion with tuna oil were also found to be protected by encapsulated mixtures of chitosan and maltodextrin, or whey protein (Shen *et al.,* 2010:4487). Klinkesorn *et al.* (2006:449) also investigated a tuna oil-in-water emulsion, which had been stabilised by an electrostatic layer-by-layer deposition process, using lecithin-chitosan. They found that the resultant powder had showed good physicochemical properties and dispersibility. This method could thus be used more commonly as a food preservative (Klinkesorn *et al.*, 2006:449).

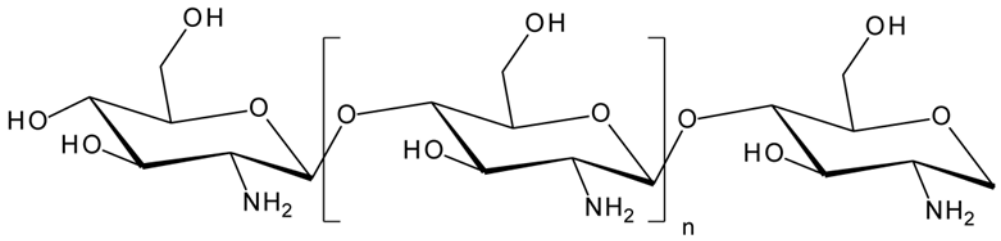

Figure 2.4: Molecular structure of chitosan.

2.9.5.3 Carrageenan

Carrageenan is one of three unique carbohydrate residues that are found in marine organisms. Commercial carrageenan can be divided into three major groups (*κ*-, *ι*-, and *λ*-carrageenan), with the difference among these groups being the amount of sulphate groups present, and hence their water solubility and gelling properties. Carrageenan, together with pectin, is a gelling polysaccharide, found in plants and seaweed. It is also the generic name of natural, water-soluble, sulphated galactans, found in red seaweed (De Ruiter & Rudolph, 1997:389). Carrageenan is used as a high quality ingredient in food and in cosmetics.

Ru *et al*. (2009) investigated the combination of *β*-lactoglobulin and *ι*-carrageenan and found that the optimum concentrations to form stable emulsions had been 0.3 wt % - 0.6 wt % at pH 4.0, and 0.4 wt % - 0.7 wt % at pH 3.4.

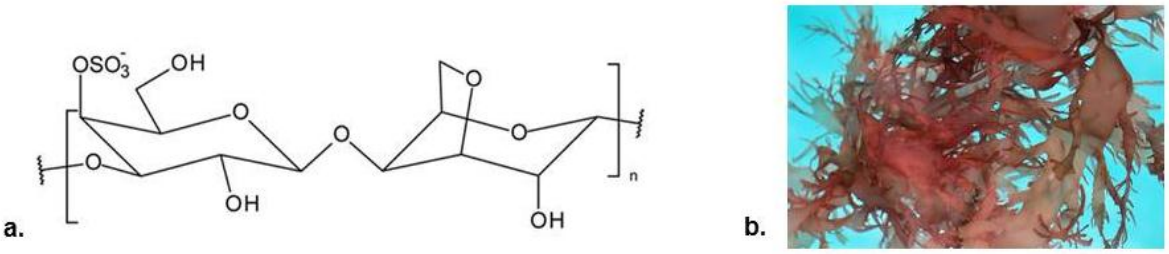

Figure 2.5: **a.** Molecular structure of *κ*-carrageenan.
b. Image of red seaweed.

2.10 Conclusion

During this literature study, it was found that oil powders could serve as suitable delivery vehicles for active ingredients. Oil powders offer some advantages, compared to liquid emulsions, such as an increase in physical and chemical stabilisation, as well as a reduction in microbial contamination. Recent studies have indicated that the wall material and the pH are important factors that could affect the stability of oils and thus of active ingredients, as well as the encapsulation efficacy of wall materials. Both the shelf life and encapsulation efficacy can be improved through careful selection of the formulation ingredients and parameters. A recent study indicated that the powder of active ingredients could be absorbed into skin in the absence of liquids, suggesting that oil powders could serve as potential delivery vehicles for topical applications.

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CHAPTER 3

Whey protein- or polysaccharide-stabilised oil powders for topical application: comparison of the release and topical delivery of salicylic acid from oil powders and from their redispersed oil powders

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Running head: How oil powders as topical delivery systems compare to their counterpart redispersed oil powders

Keywords: oil powders, whey proteins (whey), chitosan (Chi), carrageenan (Car), topical delivery, release, salicylic acid

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Abstract

During this study, the release, the dermal and transdermal delivery of salicylic acid from oil powders, as well as how these parameters would be affected by the type of polymer and different pH used to prepare the various oil powders, were investigated. Physical characterisation of the oil powders was performed, such as loss on drying, oil leakage, encapsulation efficiency, water dispersibility and particle size analysis. The release and topical delivery of salicylic acid containing oil powders were assessed and compared with those from their respective template emulsions and redispersed powders in water. Dermal and transdermal delivery studies were performed on the five most stable oil powders and on their counterpart redispersed oil powders, by using Franz type diffusion cells and by utilising human, abdominal skin membranes. From the outcomes of this study it was concluded that the redispersed oil powders had compared well with their respective template emulsions, because of the similar results obtained during the release and particle size determinations. The oil powders furthermore showed a significantly higher release of the active, than from their corresponding redispersed oil powders. The release of salicylic acid from the oil powders and from their counterpart redispersed oil powders showed the same trend, whereas the transdermal delivery of the active ingredient among the related formulations differed. In this study, it was found that the type of polymer used as emulsifier during preparation, as well as the pH of the formulation, had influenced both the release and topical delivery of salicylic acid.

Keywords

Oil powders, whey proteins (whey), chitosan (Chi), carrageenan (Car), topical delivery, release, salicylic acid

3.1 Introduction

It is common practice in the pharmaceutical, cosmetic and food industries to convert an oil-in-water emulsion into a solid like powder by evaporating the aqueous continuous phase, usually through spray- or freeze drying. This technique of solidification of an oil-in-water emulsion is used to encapsulate lipophilic active ingredients in the oil droplets, or to prevent the oil from oxidation (Adelmann *et al*., 2012:1694). Different terms are used in the literature for the resultant solid like powder, following evaporation of the water phase, such as oil powder (Adelmann *et al*., 2012:1694), dry emulsion (Ghouchi-Eskander *et al*., 2012:384), powdered redispersible emulsion (Takeuchi *et al*., 1991:1528), and microencapsulated oil (Lim *et al*., 2011:1220).

Generally, hydrophilic carriers, such as maltodextrin, starch, lactose and cellulose (Adelmann *et al.*, 2012:1694; Lim *et al*., 2012:1220; Mezzenga & Ulrich, 2010:16659) are added to the aqueous phase of the oil-in-water emulsion, in order to co-stabilise the oil droplets against coalescence during evaporation of the water, and to prevent oil leakage during storage of the oil powder. More recent studies have, however, shown that stable oil powders could also be obtained without the addition of hydrophilic carriers. In such cases, the oil-water interface of the emulsion was physicochemically stabilised for it to be sufficiently elastic to survive the manufacturing of the oil powder (Mezzenga & Ulrich, 2010:16658).

Three different methods were found in literature for the stabilisation of the oil-water interface. The first method uses the layer-by-layer technique to assemble a multilayer of polyelectrolytes (e.g. proteins, polysaccharides, low-molecular-weight surfactants) at the interface (Adelmann *et al*., 2012:1694). Cheaper and more efficient alternatives to the layer-by-layer stabilisation of interfaces are the thermal, or the enzymatic cross linking of proteins. In a recent study, it was found that the use of thermal, cross-linked *β*-lactoglobulin for the stabilisation of the emulsion had allowed the conversion of the emulsion into the oil powder. No additional hydrocolloids were required and was the oil content hence much higher, compared to oil powders with hydrophilic carriers (> 90 wt %) (Mezzenga & Ulrich, 2010:16659). A third method uses solid particles for the stabilisation of the template emulsion, instead of proteins. Due to the irreversible adsorption of the silica particles to the droplet interface, no further preparation is required prior to spray- or freeze drying, hence simplifying the method (Adelmann *et al.*, 2012:1694).

A study by Lim *et al*. (2012:1220) revealed that the choice of wall material to encapsulate red-fleshed pitaya seed oil into the oil powder had affected the encapsulation efficacy and stability of the oil. They investigated various proteins and polysaccharides as wall materials and found that sodium caseinate had resulted in the highest microencapsulation efficiency and that lactose had been the most effective polysaccharide for slowing down oxidation (Lim *et al*., 2012:1220).

During this study, the layer-by-layer method was used to stabilise the oil-water interface of template emulsions during preparation of the oil powders. The polysaccharides, carrageenan and chitosan, in combination with whey proteins were employed for the multilayer assembly at the interface, because these combinations had proven to generate stable emulsions (Combrinck *et al*., 2014; Li *et al*., 2010; Ru *et al*., 2009). Furthermore, different pH values (pH 4, 5 and 6) were utilised in preparing the emulsions, with the aim of introducing different charges to the emulsion droplets. A study by Shen *et al*. (2010:4487) indicated the importance of the pH value on the stability of fish oil powders, due to changes in the electrostatic interactions between chitosan and emulsifying starch, used in preparing these fish oil powders. By adjusting the pH, the biopolymer's charge and conformation had changed, which in turn had determined the

characteristics of the biopolymer mixtures. Shen *et al*. (2010:4487) demonstrated that those oil powders at a higher pH (pH = 6.0) had been more stable against oxidation, than those prepared at pH 4.9. It was postulated that the increased stability of the fish oil powders at higher pH values had been due to increased electrostatic interactions between chitosan and emulsifying starch. In addition, chitosan was also added to the formulation, because of its anti-oxidative, film-forming and emulsifying properties. It was found that a spray dried emulsion of tuna oil, stabilised by chitosan-lecithin, had been more resistant against oxidation, than the bulk oil. Spray dried emulsion with tuna oil was also found to be protected by encapsulated mixtures of chitosan and maltodextrin, or whey protein (Shen *et al.,* 2010:4487).

During this study, the release and topical delivery of salicylic acid containing oil powders were assessed and compared with the release of the active ingredient from their respective template emulsions and redispersed powders. Characterisation of the various oil powders was also performed, i.e. particle size, oil leakage, encapsulation efficiency and redispersibility, to further investigate the effects of the various polymers and different pH values used during preparation, on the release of salicylic acid and also on its dermal and transdermal delivery.

3.2 Materials and methods

3.2.1 Materials

Whey protein isolate (BiPro®) was kindly donated by Davisco Foods International (Le Sueur, MN, USA). As per the manufacturer, the whey protein isolate powder comprised of at least 97% of dry basis protein, with the main proteins being *β*-lactoglobulin and *α*-lactalbumin. Miglyol 812 N® was kindly donated by Cremer (Hamburg, Germany). Carrageenan (commercial grade, type I, predominantly *κ*-carrageenan, lesser amounts of *λ*-carrageenan) and chitosan glutamate were purchased from Sigma Aldrich (Johannesburg, RSA) and from CarboMer (San Diego, CA, USA), respectively. Salicylic acid (99+%) was acquired from SAFC (St. Louis, MO, USA). Potassium chloride was purchased from Sigma Aldrich (Johannesburg, RSA). Sodium dihydrogen orthophosphate, di-sodium hydrogen orthophosphate anhydrous, propylene glycol, 1 N hydrochloric acid (HCl), 1 N sodium hydroxide (NaOH) and methanol were purchased from ACE Chemicals (Johannesburg, RSA). Acetonitrile (LiChrosolv®, gradient grade for liquid chromatography) and acetic acid (glacial) were acquired from Merck Chemicals (Johannesburg, RSA). Sicomet Yellow F 11920 oil colorant was acquired from BASF (Ludwigshafen, Germany).

3.2.2 Aqueous and oil phase preparations

The aqueous phase was prepared by dispersing 3.5% *wt*. of whey protein powder into deionised water, adjusted with 1 N HCl or 1 N NaOH to pH 4, 5 and 6 on a mixing plate at room temperature for approximately two hours to ensure complete hydration (three solutions). 1% *wt*. of chitosan glutamate and 0.5% *wt*. of carrageenan were separately dissolved in deionised water, each adjusted with 1 N HCl or 1 N NaOH to pH 4, 5 and 6 at 60°C and the resultant six solutions allowed to cool to room temperature.

The oil phase consisted of 2% *wt*. salicylic acid in Miglyol 812 N®. Salicylic acid was left to dissolve in Miglyol 812 N® overnight at 37°C.

3.2.3 Emulsion preparation

The template emulsions were prepared in two phases. The primary emulsions were prepared by ultrasonication for 30 seconds (Model UP200St, Hielscher Ultrasonics, Teltow, Germany) at room temperature to obtain 30% *wt*. oil-in-water emulsions. The aqueous phase of the primary emulsions consisted of 3.5% *wt*. whey solution at pH 4, 5 and 6 each. The primary emulsions were then placed in a water bath at 80°C for fifteen minutes and subsequently allowed to cool to room temperature. The secondary emulsions were prepared by diluting the primary emulsions 1:1 (*v/v*) with either deionised water, 1% chitosan solution, or 0.5% carrageenan solution, followed by ultrasonication for 30 seconds at room temperature. After the preparation of the secondary emulsions, their pH values were adjusted to pH 4, 5 and 6 each, using 1 N HCl or 1 N NaOH. These emulsions were then sonicated for a further 30 seconds at room temperature.

3.2.4 Oil powder preparation

The prepared emulsions were first allowed to cool at 4°C for three hours and subsequently placed and left in the freezer overnight at -80°C, prior to placing them in the freeze dryer. Freeze drying was carried out using a VirTis freeze dryer (SP Scientific, Gardiner, NY, USA). The freeze drying conditions were set according to those used by Adelmann *et al*. (2012). Briefly, the drying chamber was set at room temperature, the cooling unit at -50°C at a vacuum of 10^{-2} mbar. The final composition of the oil powders are presented in Table 3.1.

Table 3.1: Composition of oil powders

Ingredient % *wt.*	Whey protein (Whey)	Whey protein / carrageenan (Car)	Whey protein / chitosan (Chi)
Salicylic acid	1.8	1.8	1.8
Miglyol 812 N®	90.0	90.0	90.0
Whey protein	8.2	6.7	5.2
Chitosan glutamate	–	–	3.0
Carrageenan	–	1.5	–

3.2.5 Oil leakage

All nine powders were tested for oil leakage. For this purpose, the same oil powders, as described above, were prepared and a known amount of Sicomet Yellow F 11920 oil colourant added into the oil phase. After preparation of the oil powders, a weighted amount of oil powder (100 mg) was dispersed in 1.5 ml of Miglyol 812 N® and vortex mixed for one minute. The Miglyol 812 N®, containing the leaked coloured oil, was then separated from the powder by centrifugation, then filtered and diluted with Miglyol 812 N®, and analysed using a UV-VIS spectrophotometer (Specord 200 Plus, Analytic Jena, Germany) at 387 nm. Samples per oil powder were prepared in duplicate and two measurements taken per sample. The extent of oil leakage was determined over a period of three months as an estimation of the stability of the powders.

3.2.6 Water dispersibility

The dispersity value *D(t)* was determined using the equation by Takeuchi *et al*. (1991).

$$D(t)=\frac{\textit{Total amount of SA dispersed in the test solution at time } t}{\textit{Total amount of SA in the added oil powder}} \times 100\% \qquad \text{Eq. 1}$$

For the water dispersibility testing, a weighted amount of each oil powder (200 mg) was each dispersed in 2 ml of deionised water in a test tube at room temperature. Each test tube was hand shaken ten times and then allowed to stand for 30 minutes. Two aliquots of 100 µl each of the test solution was withdrawn from the centre of the tube after 5 and 30 minutes. Two ml of methanol was added to each sample in order to extract the salicylic acid. The samples were then filtered, using hydrophilic PVDF pre-filters with a pore size of 0.45 µm (Agela Technologies Inc., Wilmington, DE, USA), and subjected to HPLC-UV analysis to determine the amount of dispersed salicylic acid. Measurements were taken from three samples per oil powder.

3.2.7 Particle size analysis

Droplet sizes of the template emulsions (before freeze drying) and of the redispersed powder (after freeze drying) were determined by using the Malvern Mastersizer 2000, equipped with a wet cell Hydro 2000 SM dispersion unit (Malvern Instruments, Worcestershire, UK). The template emulsions were diluted with deionised water on the day of preparation to yield obscuration values between 10% - 20%. The oil powders were redispersed in deionised water at the day of removal from the freeze dryer to also yield obscuration values between 10% - 20%. Measurements were taken from two freshly prepared samples each per emulsion and redispersed powder, and readings taken in duplicate.

The particle sizes were confirmed on the day of preparation of the template emulsions and redispersed oil powders by means of microscopy. A Motic microscope (Motic, Hong Kong) was used, equipped with a Moticam 3 camera and Motic Images Plus 2.0 software.

Additionally, grains of oil powder were embedded in resin. 0.5 µm thick sections were cut with a Reichert Jung Ultracut E microtome (Newark, DE, USA) and stained with 0.5% of aqueous toluidine blue and 0.05% of neofuchsin. Sections were also viewed with the Motic microscope (Motic, Hong Kong), equipped with the Moticam 3 camera and Motic Images Plus 2.0 software.

3.2.8 Release of active from formulations

The release rates of the active ingredient from all nine emulsions, oil powders and the redispersed oil powders were tested in triplicate, using Franz type diffusion cells, having a diffusion area of 1.13 cm^2 and a receptor capacity of approximately 2 ml. These release tests were performed at 37 ± 1°C in a heated water bath for eight hours. Cellulose acetate membranes (0.2 µm pore size, Whatman, Dassel, Germany) were used and the receptor phase consisted of phosphate buffer pH 7.4 and propylene glycol (PG) (1:1, *v/v*). 50% of PG was included into the receptor phase to increase the solubility of salicylic acid, thereby ensuring sink conditions of the active ingredient throughout the duration of the study. The cellulose acetate membranes were soaked overnight in the receptor phase. The receptor compartment was filled with 2 ml of preheated and degassed receptor phase and left to equilibrate before adding the donor phase. The donor compartment was filled with 1 ml of emulsion, 200 mg of powder, or 200 mg of oil powder redispersed in 1 ml of deionised water. The entire volume of the receptor phase was withdrawn at 1, 2, 3, 4, 6 and 8 hours each and replaced with fresh pre-heated receptor media. The samples were analysed by HPLC-UV.

3.2.9 Skin preparation

The utilisation of human skin for research purposes had been approved by the Ethics Committee of the North-West University, Potchefstroom, South Africa (Ethics number: NWU-00114-11-A5). Caucasian female skin was collected from cosmetic surgeries, following abdominoplastic surgery, with the written informed consent of each patient for use of their skin in research. The skin was thawed at room temperature and skin membranes with a thickness of 400 µm were removed, using an electric dermatome (Zimmer Inc. Warsaw, Indiana, USA). Each dermatomed skin membrane was placed on Whatman® filter paper, with the stratum corneum facing upwards. The skin samples were stored in aluminium foil and frozen at -20°C until use. Prior to the diffusion experiments, the skin membranes had been thawed at room temperature, cut into the required size (circular pieces of approximately 1.5 cm in diameter) and mounted onto the Franz diffusion cells.

3.2.10 *In vitro* skin absorption study

A randomised, complete block design was used to assess the effect of skin donor variability, as well as the effect of formulation on dermal and transdermal delivery. Three different skin donors were used per formulation and each formulation was applied in duplicate on each donor skin, giving a total of six replicates per formulation.

Based on the results of the release and oil powder characterisation data, ten formulations were selected for the skin diffusion study. Chi oil powders at pH 4, 5, and 6, whey oil powder at pH 6 and Car oil powder at pH 6 were chosen and applied as powders, and also as powders redispersed in water. The setting up of the skin absorption study was the same as for the release study. Prior to the skin absorption testing, the skin integrity was assessed by measuring the electric resistance across the skin. For skin resistance, the donor and the receptor compartments were filled with a 0.9% potassium chloride solution and placed in a heated water bath for an equilibration period of 30 minutes. Electrical resistance was measured by using a Tinsley LCR Databridge Model 6401 (Tinsley Precision Instruments, Croydon, UK). The reading was taken at 1 kHz with a maximum voltage of 300 mV root-mean-square in the parallel equivalent circuit mode, using an alternating current (Fasano *et al*., 2002:731). Skin samples with resistance values below 10 kΩ were excluded from the study. The donor compartment was filled with 200 mg of oil powder, or 200 mg of oil powder redispersed in 1 ml of deionised water. Receptor samples were collected after 24 hours and analysed by HPLC-UV.

3.2.11 Skin sample preparation

After completion of the 24 hour transdermal diffusion studies, the formulations were removed from the donor compartments and the skin membranes pinned onto Whatman® filter paper, with the stratum corneum facing upwards. The skin samples were gently dabbed with paper towel to remove most of the formulation from the skin surface. 3M Scotch® Magic™ tape was used to remove the stratum corneum, using sixteen strips in total per sample. The first strip of each sample was discarded due to possible traces of formulation still left on the skin. The remainder of the skin after tape stripping was cut into pieces to increase the surface area for extraction. The tape stripped samples and the remainder of the skin per skin sample were placed into two separate vials, containing 5 ml of methanol and stored for a period of at least twelve hours at 4°C for subsequent extraction. The samples were then vortex mixed, filtered through hydrophilic PVDF pre-filters with a pore size of 0.45 µm (Agela Technologies Inc., Wilmington, DE, USA) and analysed by HPLC-UV.

3.2.12 Statistical analysis

Data from the release study was analysed through one-way analysis of variance (ANOVA), using STATISTICA® (StatSoft Inc., Tulsa, OK, USA). Data from the skin absorption study was log transformed and analysed through two-way ANOVA, employing the formulation as fixed effect and the skin donor as random effect. Subsequently, Tukey's Honestly Significant Difference (HSD) test was performed on both data sets to compare the various formulations with each other. The release data of each emulsion and its corresponding redispersed powder, as well as the skin absorption data of each oil powder and its respective redispersed powder was analysed, using the Student's *t*-test in Excel® (Microsoft Corp., Seattle, WA, USA). Data with $p < 0.05$ was considered statistically significant.

3.2.13 HPLC-UV method

HPLC analysis of salicylic acid was performed using an Agilent® 1100 Series HPLC system (Agilent Technologies, Palo Alto, CA, USA), equipped with a high performance, silica based, reversed phase C_{18}-2 column (150 x 4.60 mm) with 5 µm particle size (Venusil XBP Agela Technologies, Wilmington, DE, USA), in a controlled laboratory environment at 25°C. This instrument was equipped with a G1311A quaternary pump, a G1313A autosampler and a G1315A multi-wavelength, diode array detector. The mobile phase consisted of 1% acetic acid, 45% acetonitrile and 54% Milli-Q® water that had been degassed after preparation. The flow rate was set at 1 ml/min, with a run time of approximately six minutes per sample. The UV-detector was set at 236 nm. All samples were injected in duplicate. Chromatograms were analysed, using ChemStation Rev. A.10.03 software.

3.3 Results

3.3.1 Characterisation of oil powders and their respective redispersed powders in water

Upon freeze drying of thermal, cross-linked whey protein or polysaccharide-stabilised emulsions, white fluffy oil powders, consisting of aggregated oil droplets, were obtained. Most of these powders felt dry on touch, except for the Car oil powder (pH 4) and whey oil powder (pH 6) that felt slightly sticky, while the whey oil powders (pH 4 and 5) appeared very sticky. Light microscopic images of whey protein oil powders are illustrated in Figure 3.1. The whey proteins were stained with toluidine blue or neofuchsin for improved vision. Figure 3.1 clearly illustrates the shells that the whey proteins had formed around the oil droplets. The sizes of the oil droplets at all pH values were determined as being below 10 µm, which were similar to the results reported by Mezzenga and Ulrich (2010).

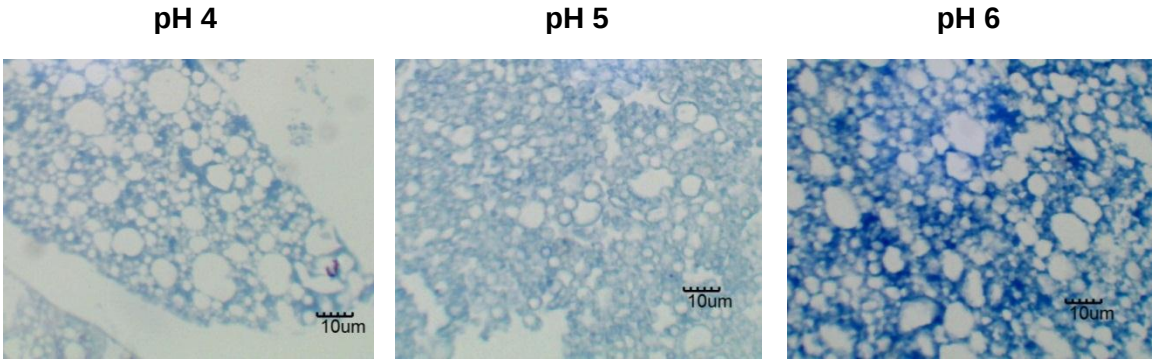

Figure 3.1: Light microscopic images of toluidine blue or neofuchsin stained whey oil powders at pH 4, 5 and 6. The scale bar in each image represents 10 µm.

The outcomes of the droplet size analyses of each template emulsion (whey, chitosan and carrageenan) and their respective redispersed oil powders in water are summarised in Table 3.2 and are presented as volume weighted means (D [4,3]) and surface weighted means (D [3,2]). The light microscopic images of the template emulsions and redispersed oil powders in water are furthermore represented in Figures 3.2 and 3.3, respectively. Overall, the laser light scattering data revealed that the droplet sizes of the redispersed powders were much larger than those of the emulsions. However, the droplet size data was contradicted by the microscopic images (Figures 3.2 and 3.3), in which the redispersed particles were much smaller. The light microscopic images in Figures 3.2 and 3.3 show that the droplet sizes of the various template emulsions and their respective redispersed oil powders were similar, with a slight tendency of larger oil droplets shown by the redispersed oil powders. All of the various template emulsions and redispersed oil powders consisted of oil droplets in the lower

micrometer range (≤ 5 µm). Moreover, agglomeration of oil droplets was present in most of the formulations tested.

Table 3.2: Droplet size data of emulsions and redispersed oil powders

		Emulsion		Redispersed oil powder	
		D [4,3] [µm]	**D [3,2] [µm]**	**D [4,3] [µm]**	**D [3,2] [µm]**
Whey	**pH 4**	7.64 ± 4.95	2.47 ± 0.10	192.57 ± 14.33	19.59 ± 5.30
	pH 5	12.15 ± 13.60	2.25 ± 0.25	238.37 ± 25.12	35.41 ± 6.23
	pH 6	2.69 ± 0.06	1.99 ± 0.02	227.40 ± 09.16	36.78 ± 1.37
Car	**pH 4**	61.07 ± 08.42	4.13 ± 0.12	246.82 ± 08.79	64.74 ± 14.59
	pH 5	6.80 ± 3.10	2.94 ± 0.07	305.87 ± 24.72	60.46 ± 9.61
	pH 6	2.23 ± 0.04	1.74 ± 0.04	289.31 ± 44.77	55.58 ± 9.93
Chi	**pH 4**	14.49 ± 04.62	4.07 ± 0.48	126.67 ± 05.47	9.05 ± 0.38
	pH 5	4.29 ± 0.39	2.68 ± 0.11	62.72 ± 10.42	7.62 ± 0.91
	pH 6	3.61 ± 0.06	2.38 ± 0.03	519.10 ± 52.94	98.51 ± 19.47

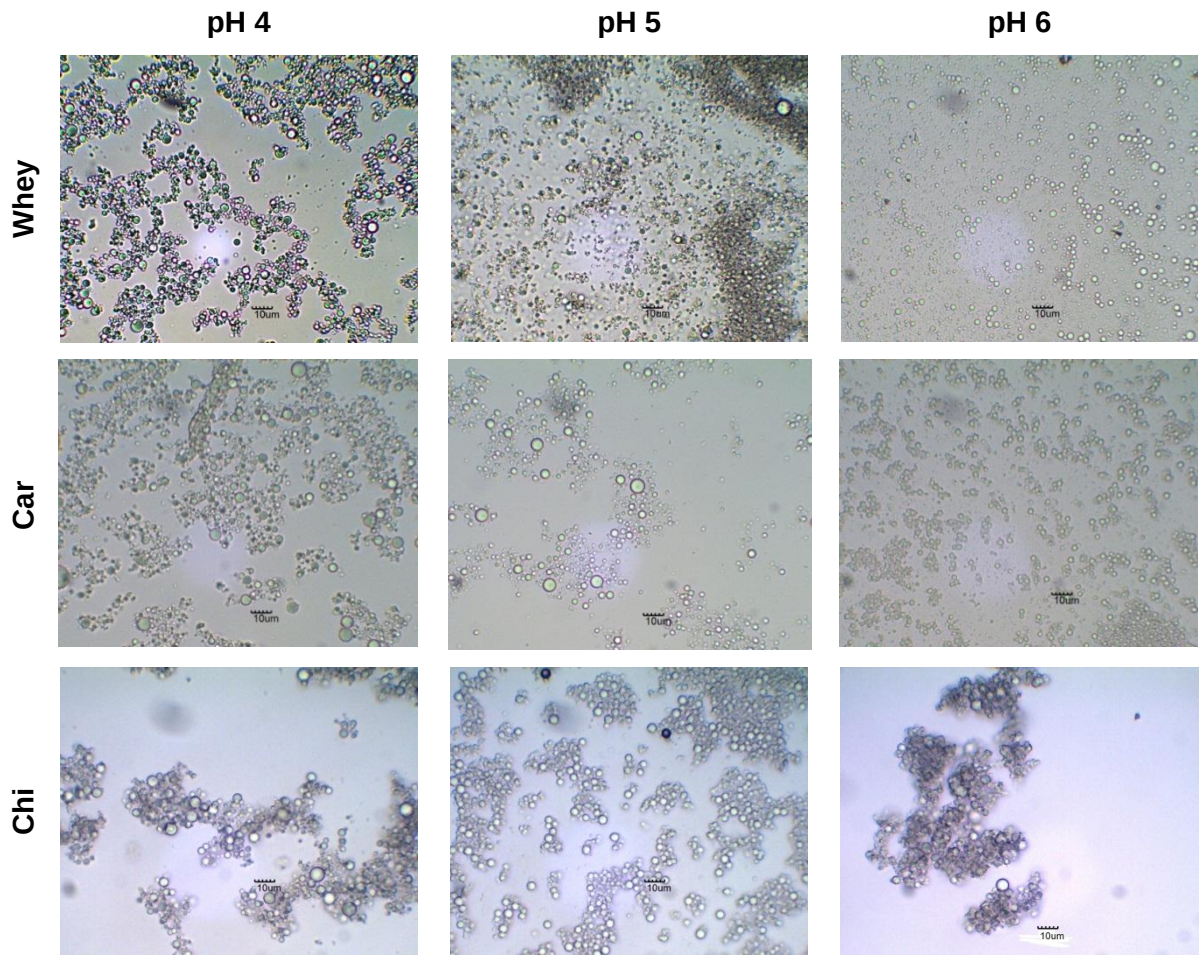

Figure 3.2: Light microscopic images of whey, carrageenan and chitosan template emulsions (before being subjected to freeze drying) at pH 4, 5 and 6. The scale bar in each image represents 10 µm.

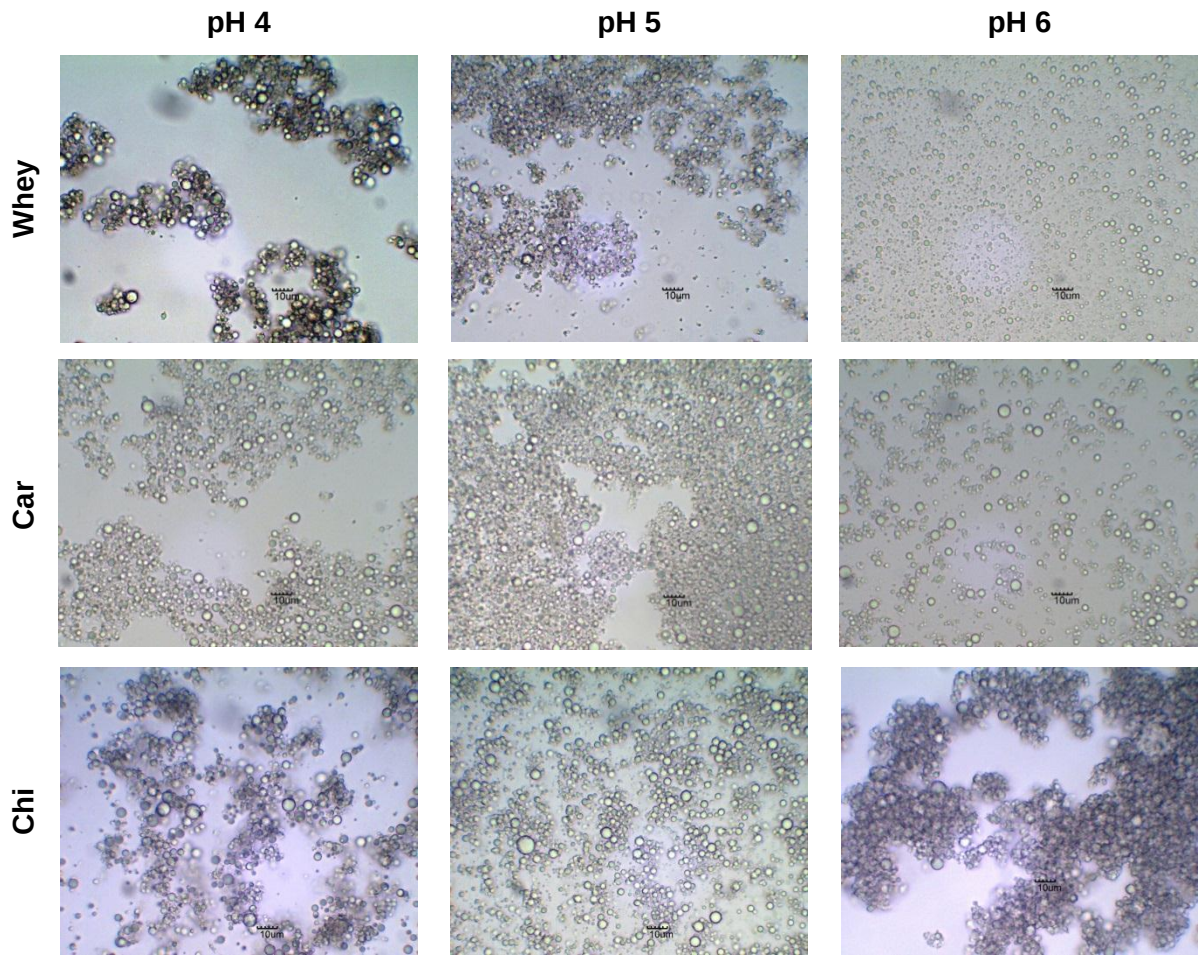

Figure 3.3: Light microscopic images of whey, carrageenan and chitosan redispersed oil powders at pH 4, 5 and 6 in water. The scale bar in each image represents 10 µm.

The oil leakage results are summarised in Table 3.3. The data shows that oil leakage had not increased over the three months of testing. Furthermore, no significant differences were found amongst samples taken at different locations (e.g. bottom, middle and top) within one powder sample, with the exception of the whey protein oil powder at pH 4. In general, oil leakage was similar for the various oil powders, except for the whey protein oil powders at pH 4 and 5, which yielded higher oil leakage values.

The determined dispersion values are listed in Table 3.4. Overall, the dispersion values after 5 and 30 minutes were similar and no significant reduction of water dispersibility occurred after 30 minutes, compared to 5 minutes. It was also observed that the dispersibility was increased with increasing pH for the three oil powder types (whey, chitosan and carrageenan).

Table 3.3: Data generated during the oil leakage studies

		Centre of powder 1 week	Top of powder 2 months	Side of powder (glass wall) 3 months	Bottom of powder 3 months
		Oil leakage [%]	**Oil leakage [%]**	**Oil leakage [%]**	**Oil leakage [%]**
Whey	**pH 4**	39.5 ± 0.53	21.9 ± 0.24	16.8 ± 1.01	17.5 ± 0.59
	pH 5	41.0 ± 0.07	34.0 ± 1.26	33.2 ± 0.22	30.9 ± 0.85
	pH 6	20.8 ± 0.16	13.8 ± 1.29	14.7 ± 0.93	12.1 ± 1.19
Car	**pH 4**	25.7 ± 2.25	19.7 ± 0.19	22.6 ± 0.88	20.5 ± 0.73
	pH 5	21.0 ± 0.01	20.1 ± 1.70	23.0 ± 1.32	20.7 ± 0.43
	pH 6	13.1 ± 0.02	9.9 ± 0.78	9.9 ± 0.03	13.7 ± 0.11
Chi	**pH 4**	24.1 ± 1.11	25.9 ± 0.71	27.7 ± 0.59	24.4 ± 0.03
	pH 5	21.5 ± 0.55	18.1 ± 0.42	21.1 ± 0.85	18.7 ± 0.89
	pH 6	20.5 ± 1.38	21.4 ± 0.59	21.9 ± 1.19	21.7 ± 0.36

Table 3.4: Water dispersibility data

		5 min [%]	**30 min [%]**
Whey	**pH 4**	42.10 ± 0.03	46.79 ± 0.01
	pH 5	67.78 ± 0.10	71.08 ± 0.04
	pH 6	89.33 ± 0.07	90.84 ± 0.11
Car	**pH 4**	64.88 ± 0.01	64.54 ± 0.04
	pH 5	80.01 ± 0.20	82.07 ± 0.09
	pH 6	101.28 ± 0.03	103.49 ± 0.13
Chi	**pH 4**	147.49 ± 0.23	145.65 ± 0.18
	pH 5	86.16 ± 0.09	83.35 ± 0.12
	pH 6	102.89 ± 0.15	114.14 ± 0.10

3.3.2 Release of active from formulations

The release study was divided into two groups, i.e. the effect of pH (Figure 3.4) and the effect of polymer (Figure 3.5) on release. The release of salicylic acid from the oil powders was furthermore compared to the release from the respective redispersed oil powders in water, as well as to the release of salicylic acid from the corresponding template emulsions (before being subjected to freeze drying) (Table 3.5).

Figures 3.4 and 3.5 represent the cumulative amounts of salicylic acid released per surface area, plotted against the square root of time, according to the Higuchi equation (Eq. 2).

$$f_t = Q = K_H t^{1/2} \qquad \text{Eq. 2}$$

where f_t is the cumulative amount of salicylic acid released per surface area, K_H is the Higuchi dissolution constant, while $t^{1/2}$ represents the square root of time. The release rate K_H was calculated from the linear portion ($t_{1h} – t_{8h}$) of the correlation between the cumulative amount of released salicylic acid per surface area and the square root of time. Calculated release rates and regression coefficients are presented in Table 3.5. As illustrated by Figures 3.4 and 3.5, and confirmed by the regression coefficients as summarised in Table 5, the amount of salicylic acid being released per surface area showed a linear relationship with the square root of time ($R^2 \geq 0.973$) for all of the formulations tested, in accordance with the Higuchi model.

The first set of data regarding the effect of pH on the release of salicylic acid from the emulsions, oil powders and redispersed oil powders, generally showed an increase in the release of salicylic acid with increasing pH. No significant differences were observed among the release (neither release rate, nor cumulative amount of salicylic acid released after eight hours) from the emulsions and from the redispersed powders ($p > 0.05$). However, the oil powders had yielded higher release rates (release rate and cumulative amount released) of salicylic acid, with the trend being similar to those of the emulsions and redispersed powders. It was further observed that the lag time was considerably longer for the oil powders, than for the respective emulsions and their redispersed powders.

The second set of test outcomes, representing the effect of polymer on release, revealed no significant trend in the performance of the three polymers with regards to the release of salicylic acid. However, a tendency for a lower release of salicylic acid from the chitosan containing formulations was observed.

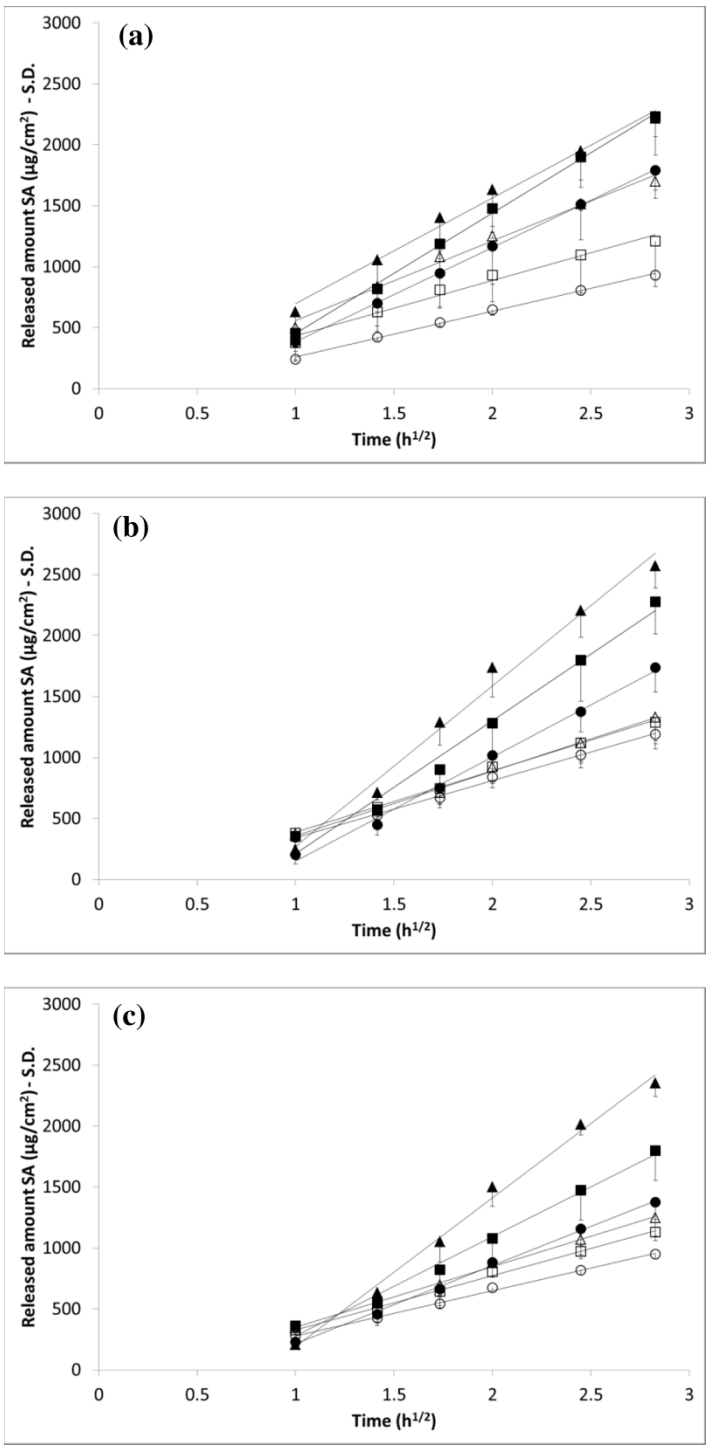

Figure 3.4: Salicylic acid release through cellulose acetate membranes from (a) whey, (b) Car and (c) Chi oil powders (closed symbols) and redispersed oil powders (open symbols). Effect of pH: (●) pH 4, (■) pH 5 and (▲) pH 6. The data is represented as linear fit against square root of time (according to the simplified Higuchi equation). Average S.D. (n = 3).

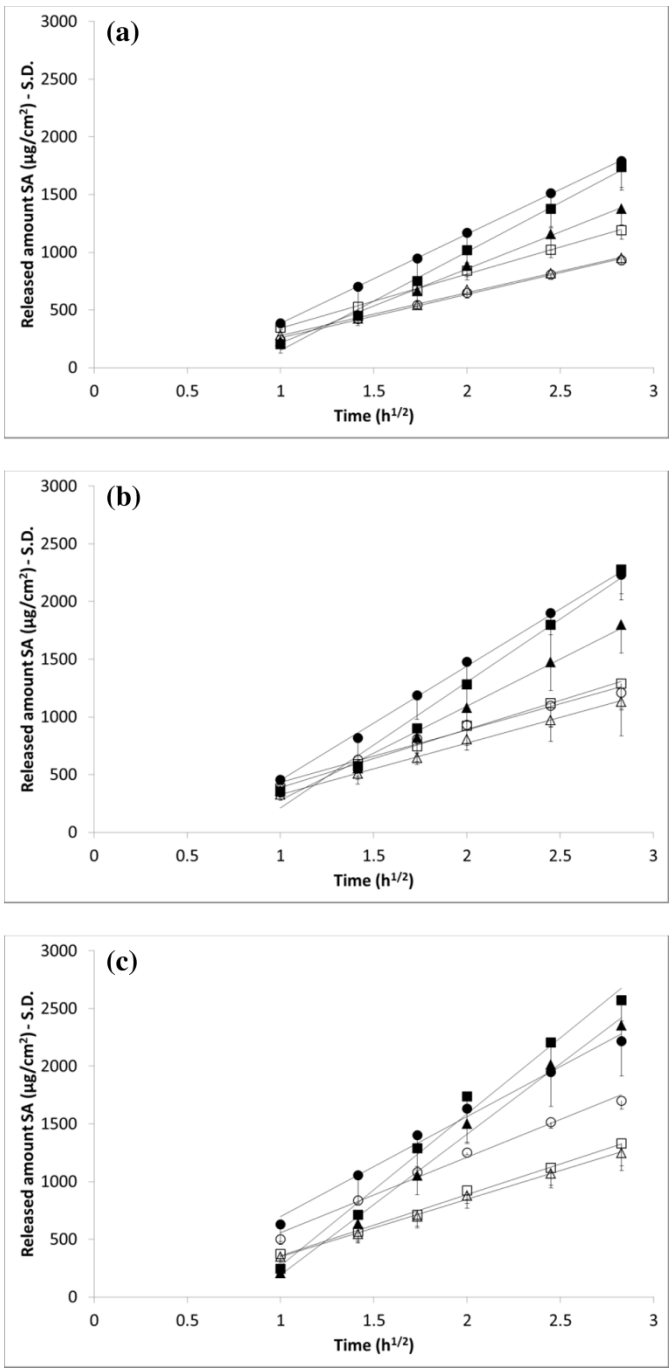

Figure 3.5: Release of salicylic acid through cellulose acetate membranes from oil powders (closed symbols) and redispersed oil powders (open symbols) at (a) pH 4, (b) pH 5 and (c) pH 6. Effect of polymer: (●) whey, (■) Car and (▲) Chi. The data is represented as linear fit against square root of time (according to simplified Higuchi equation). Average S.D. (n = 3).

Table 3.5: Cumulative release data of salicylic acid

			Release rate [µg/cm^2/h$^{1/2}$]	R^2	Cumulative release in 8 hr [µg/cm^2]	Lag time [min]
Emulsion	Whey	pH 4	370.6 ± 56.3	0.998	976.1 ± 059.8	4.1 ± 6.2
		pH 5	501.7 ± 06.5	0.999	1274.3 ± 032.9	5.4 ± 2.3
		pH 6	463.7 ± 36.0	0.999	1360.3 ± 113.8	0
	Car	pH 4	365.3 ± 59.4	0.998	1004.4 ± 113.3	1.3 ± 1.1
		pH 5	435.4 ± 40.6	0.999	1144.0 ± 055.1	6.7 ± 11.2
		pH 6	515.9 ± 94.8	0.997	1262.2 ± 178.6	8.6 ± 7.3
	Chi	pH 4	415.5 ± 56.4	0.998	1075.4 ± 098.5	4.8 ± 6.1
		pH 5	422.5 ± 13.1	0.998	1137.0 ± 057.6	1.7 ± 1.8
		pH 6	460.9 ± 48.0	0.999	1181.0 ± 112.5	4.4 ± 1.0
Redispersed oil powder	Whey	pH 4	376.3 ± 14.1	0.999	931.7 ± 41.5	6.0 ± 1.0
		pH 5	453.7 ± 195.8	0.973	1210.5 ± 373.8	15.6 ± 20.3
		pH 6	653.8 ± 034.2	0.995	1700.0 ± 071.3	1.4 ± 0.9
	Car	pH 4	469.4 ± 34.1	0.999	1192.3 ± 79.4	4.4 ± 0.6
		pH 5	502.6 ± 65.4	0.999	1290.2 ± 220.3	4.1 ± 4.2
		pH 6	517.8 ± 55.3	0.995	1329.7 ± 191.1	5.4 ± 1.1
	Chi	pH 4	371.3 ± 09.9	0.999	950.50 ± 41.95	4.0 ± 2.2
		pH 5	444.1 ± 30.4	0.999	1130.70 ± 72.71	4.1 ± 1.1
		pH 6	498.0 ± 55.5	0.999	1248.93 ± 153.07	5.8 ± 2.9
Oil powder	Whey	pH 4	771.5 ± 72.8	0.996	1789.7 ± 229.1	18.1 ± 12.3
		pH 5	993.8 ± 83.0	0.998	2230.6 ± 166.0	19.6 ± 10.1
		pH 6	866.5 ± 106.0	0.993	2218.2 ± 302.6	4.8 ± 4.9
	Car	pH 4	862.0 ± 151.6	0.997	1736.9 ± 199.4	41.6 ± 15.2
		pH 5	1089.5 ± 209.6	0.986	2278.8 ± 264.5	38.3 ± 5.8
		pH 6	1346.3 ± 124.2	0.992	2572.1 ± 182.9	41.6 ± 1.6
	Chi	pH 4	645.2 ± 75.1	0.996	1375.8 ± 138.4	27.8 ± 8.5
		pH 5	815.8 ± 126.8	0.991	1798.0 ± 246.7	26.5 ± 6.4
		pH 6	1239.5 ± 43.8	0.994	2354.2 ± 112.1	45.7 ± 7.9

3.3.3 *In vitro* skin absorption

The skin absorption test results are shown in Figures 3.6 and 3.7, where the data is represented as the total amount of active delivered over 24 hours of permeation as least square means (formulation effect) ± 95% confidence interval (C.I.), obtained after two-way ANOVA. The results from the skin absorption study were also divided into two sets, i.e. the effect of pH on chitosan oil powders and their respective redispersed oil powders in water, as well as the effect of various polymers used in preparing the formulations at pH 6.

The investigation of the effect of pH (Figure 3.6) demonstrated no differences in dermal delivery of salicylic acid among the various pH values, neither from the oil powders, nor from their corresponding redispersed powders in water. However, significant differences were obtained in transdermal delivery, with the trend being different for the oil powders and the redispersed powders. After 24 hours, significantly more salicylic had permeated the skin from chitosan oil powder at pH 5, compared to pH 4 (p = 0.0268) and pH 6 (p = 0.0002). The redispersed oil powder of chitosan at pH 4, however, showed statistically higher salicylic acid permeation, than at pH 5 (p = 0.0002) and pH 6 (p = 0.0063).

The second set of results also showed no significant differences in dermal delivery between the various polymers, whereas statistically significant differences occurred in transdermal delivery, with the trend again being different for the oil powders and the redispersed powders. The Chi formulation statistically significantly showed the lowest permeation of salicylic acid from the oil powder (p = 0.0015 against whey, and p = 0.0040 against Car), whereas permeation had been the highest from the redispersed oil powder (p = 0.0654 against whey, and p = 0.0314 against Car).

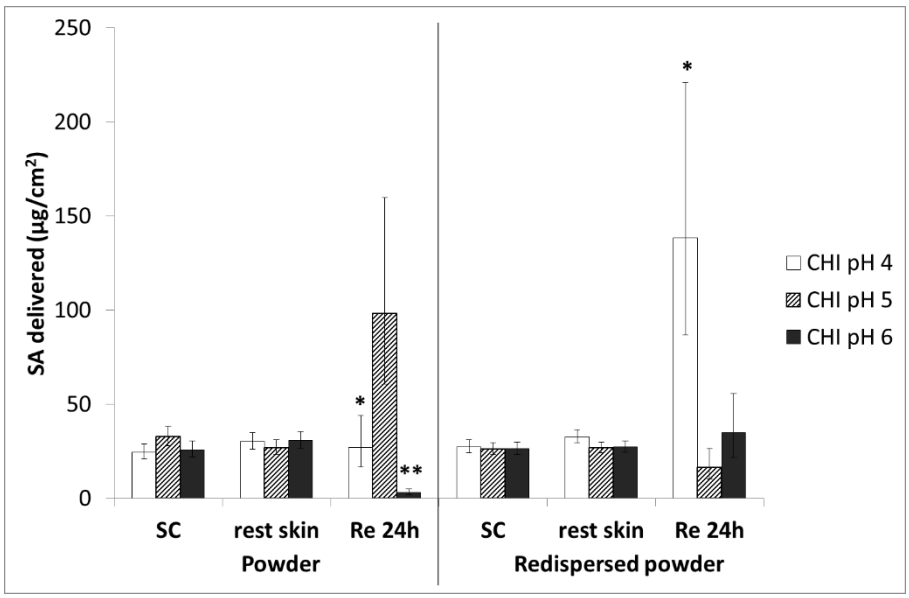

Figure 3.6: Salicylic acid skin absorption from Chi oil powders and redispersed oil powders through human abdominal skin, expressed as total amount delivered over 24 hours as least square means (formulation effect) ± 95% confidence interval (C.I.), obtained after two-way ANOVA (n = 6). Effect of pH: * $p < 0.05$ at pH 5 and 6, ** $p < 0.05$ at pH 4 and 5.

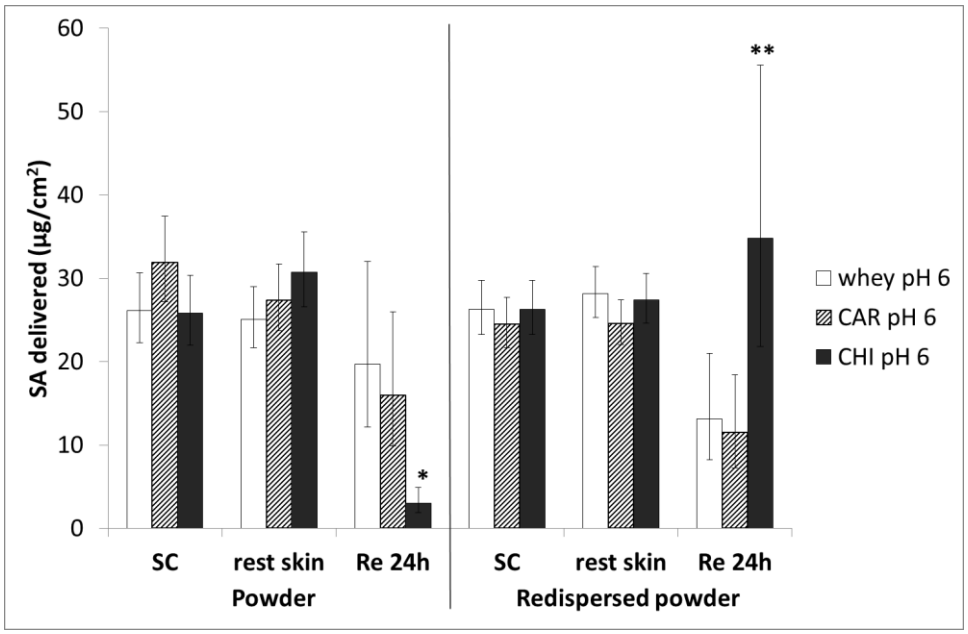

Figure 3.7: Salicylic acid skin absorption from whey, Car and Chi oil powders and redispersed oil powders at pH 6 through human abdominal skin, expressed as total amount delivered over 24 hours as least square means (formulation effect) ± 95% confidence interval (C.I.), obtained after two-way ANOVA (n = 6). Effect of polymer: * $p < 0.05$ against whey and Car, ** $p < 0.05$ against Car.

3.4 Discussion

3.4.1 Characterisation of oil powders

The layer-by-layer technique, as described by Shchukina and Shchukin (2012), was used to prepare the template emulsions during this study. The whey proteins at the oil-water interface were first thermally cross linked and then coated with a layer of polysaccharides, since thermal cross linking provides elasticity to the interface, which protects the proteins during the stresses caused by the manufacturing process (Adelmann *et al*., 2012; Mezzenga & Ulrich, 2010). Thermal cross linking was performed by placing the emulsions in a water bath at 80°C for fifteen minutes (Adelmann *et al.,* 2012). Mezzenga and Ulrich (2010) emphasise that cross linking of the protein shell is the most important step in stabilising the oil powder.

The increase in particle sizes from the emulsions to their corresponding redispersed oil powders, according to the particle size study outcomes, could have been caused by coalescence, or flocculation of the oil droplets. Contrary, the light microscopic images revealed no increase in droplet size between the emulsions and their respective redispersed oil powders, indicating that the increase in particle size, as evidenced in Table 3.2, had instead been caused by agglomeration of the particles during the preparation of the oil powders, rather than by coalescence. Not all of the light microscopic images of the redispersed oil powders, e.g. whey (pH 6) and Car (pH 6) showed extensive agglomeration, as indicated by the laser light scattering data. This may be explained by the fact that only a small part of the redispersed oil powder was visible, which may not necessarily have represented aggregates, due to creaming of the larger particles/aggregates at the top of the redispersed oil powder samples, whereas the samples taken for the preparation of the microscopic slides, were taken from around the middle of the samples.

The dispersity value is an indication for the stability of the water dispersion of the oil powders. However, one has to consider that the method used to determine the dispersity values did not distinguish between the salicylic acid encapsulated in the redispersed oil powder particles and emulsion droplets, and the dissolved salicylic acid in the water. The increase in dispersity values with increasing pH therefore could not have been solely related to a better water dispersibility, but also to an enhanced solubility of salicylic acid in water at higher pH values. In general, the addition of chitosan and carrageenan had improved the water dispersibility, which was congruent with the oil leakage data. Oil powders with higher oil leakage had resulted in more sticky oil powders, which were more difficult to redisperse, thus yielding lower dispersity values.

3.4.2 Release of active from the formulations

The release data obtained for the emulsions and their respective redispersed powders was similar to that obtained by Combrinck *et al.* (2014). It could therefore be concluded that electrostatic interactions between the salicylic acid and the emulsifier of the emulsion droplets had affected the release of salicylic acid from these formulations. The same trend was also observed for the release of salicylic acid from the oil powders, although initially no water had been present in these formulations. During the release studies, however, water had diffused into the donor phase, which may have resulted in electrostatic interactions between salicylic acid and the emulsifiers. Figure 3.8 illustrates that more water appeared in the donor phase over time when cellulose acetate membranes were used, compared to skin membranes. As the water had to first diffuse from the receptor into the donor phase, the onset of release had been delayed, as indicated by the longer lag times of the oil powders, compared to their corresponding emulsions and redispersed powders. The higher cumulative release concentrations from the oil powders could be explained by the lower amount of water present in the donor phase, thus yielding higher salicylic acid concentrations and therefore causing higher driving forces for diffusion.

Figure 3.8: Images of Franz type diffusion cells containing whey oil powder at pH 6.

3.4.3 *In vitro* skin absorption

The transdermal delivery results of salicylic acid from the redispersed oil powders were also similar to those obtained by Combrinck *et al*. (2014). With regards to the effects of pH, the degree of ionisation of salicylic acid could have affected the transdermal delivery thereof, with a

decrease in permeation with increasing ionisation of salicylic acid, according to the pH partition hypothesis. With regards to the effects of polymer, the enhanced permeation of salicylic acid from the redispersed chitosan oil powder could have been caused by the interaction between the positively charged amino groups of chitosan and the negatively charged skin surface. Additionally, the enhancing effect could also have been related to the permeation enhancing effects of chitosan. Lima *et al*. (2012) state that chitosan is known to lower the diffusion resistance of the stratum corneum and hence to enhance permeation across skin.

The oil powders showed a different trend in skin absorption. With regards to the effects of pH, the ionisation degree of salicylic acid could not have played a role, because no water was present in the donor phase. However, a correlation between the powder particle size and transdermal delivery was observed, because with an increase in particle size (pH 5 < pH 4 << pH 6, with reference to the Mastersizer results of the redispersed powders in Table 3.2), the transdermal delivery decreased (pH 5 > pH 4 > pH 6). It was hypothesised that smaller powder particles and agglomerates may have caused better contact with skin (achieving a better coverage of the skin diffusion area), hence resulting in higher percutaneous absorption. The same explanation could have applied with regards to the different polymers, where with increasing particle size, according to the Mastersizer results of the redispersed powders (Whey < Car << Chi), transdermal delivery decreased (whey > Car >> Chi). The differences in transdermal performance between the Chi oil powder at pH 6 (the lowest transdermal delivery) and the Chi redispersed oil powder at pH 6 (the highest transdermal delivery), may have been due to the fact that chitosan was undissolved in the oil powder and therefore it could not have acted as a permeation enhancer. Contrary, with regards to the redispersed oil powder containing water, the chitosan could have dissolved and acted as permeation enhancer, hence resulting in a higher transdermal delivery of salicylic acid from the redispersed powder (Figure 3.7).

It was also observed that in general, the redispersed powders exhibited better transdermal delivery than their respective oil powder formulations. It was hypothesised that the oil powders had resulted in a longer lag time for salicylic acid to permeate the skin, whereas the presence of water in the donor phase of redispersed powders may have accelerated the transport of salicylic acid across skin.

3.5 Conclusion

The outcomes of this study demonstrated that redispersed oil powders could be compared to their respective template emulsions, because of the similar release and particle size results obtained. Furthermore, the oil powders showed a significantly higher release of salicylic acid than from their counterpart redispersed oil powders. Release data showed the same trend

among all of the oil powder formulations and their corresponding redispersed oil powders, whereas transdermal delivery among the related formulations differed. During this study it was found that the type of polymer used as emulsifier, as well as the pH of the formulation had influenced both the release and topical delivery of salicylic acid. With regards to the release study outcomes, it was concluded that electrostatic interactions between salicylic acid and the emulsifier of the emulsion droplets had affected the release of salicylic acid from these formulations. The observed higher cumulative release from the oil powders could be explained by the lower amount of water present in the donor phase, thus yielding higher salicylic acid concentrations and therefore higher driving forces for diffusion. With regards to transdermal delivery, the effect of pH could have been affected by the degree of ionisation, resulting in a decrease in permeation with increasing ionisation of salicylic acid, in accordance with the pH partition hypothesis. Regarding the effect of polymer type, the enhanced permeation of salicylic acid from the redispersed chitosan oil powder could have been due to the permeation enhancing effects of chitosan. The oil powders showed a different trend in skin absorption. With regards to the effect of pH, the ionisation degree of salicylic acid could not have played a role, because no water had been present in the donor phase. A correlation between the powder particle size and the transdermal delivery was observed. It was also observed that in general, the redispersed powders had exhibited a better transdermal delivery than their respective oil powder formulations.

Acknowledgements

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(Please note that the Referencing Style applied here is for the purposes of submission of this article and deviates from the rest of the dissertation).

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CHAPTER 4

CONCLUSION

A literature study on oil powders was performed, with a focus on oil powders as possible topical delivery vehicles. It became clear from the literature that oil powders could very well be employed as appropriate delivery vehicles for pharmaceutical active ingredients. Oil powders have some advantages compared to liquid emulsions, such as an increase in physical and chemical stabilisation, and a reduction in microbial contamination. Recent studies indicated that both the wall material and pH are important factors that could affect the stability of the oil and thus the active ingredient, as well as the encapsulation efficacy. Both parameters (shelf life and encapsulation efficacy) could be improved by careful selection of the formulation ingredients and parameters. A recent study indicated that the powder of active ingredients could be absorbed into skin in the absence of liquids, suggesting that oil powders are potential delivery vehicles for topical application.

Three different biopolymers were used in this study, i.e. whey protein, chitosan and carrageenan. The oil powders were formulated from emulsions that had been prepared at three different pH values (pH 4, 5 and 6). The different pH values introduced different charges to the polymers, which had shown to have affected the properties of the emulsions and of the oil powders, as well as the delivery of the active ingredient. Salicylic acid was used as active ingredient during this study.

Nine oil powders were formulated and characterised through droplet size, particle size distribution, loss on drying, encapsulation efficiency, oil leakage and water dispersibility determinations. Particle sizes were determined by using a Mastersizer. The particle sizes of the redispersed powders were much larger than those of the emulsions and was it confirmed by microscopy that the increase in particle sizes was due to agglomeration of the particles of the redispersed oil powders. There were no significant differences in oil leakage among the different oil powders, except for the whey protein oil powders at pH 4 and 5, which had yielded higher oil leakage values. Oil leakage had not increased over the duration of the three months of testing, indicating that the oil powders were stable for at least three months. The encapsulation efficiency of salicylic acid was between 55.0% and 79.6% and did not differ significantly among the various oil powders. The water dispersibility test results showed that there had been no significant reduction in water dispersibility after 30 minutes. Furthermore, the dispersibility properties increased with an increase in pH value, due to the increasing solubility of salicylic acid at higher pH. In general, the addition of chitosan and carrageenan had improved the water dispersibility, which was congruent with the oil leakage data. Oil powders

with higher oil leakage had resulted in stickier oil powders, which were more difficult to redisperse, hence yielding lower dispersity values. The loss on drying was ≤ 0.20% after a period of two days. The loss on drying was insignificant and therefore it was concluded that the oil powders did not consist of much water.

An HPLC method was developed and validated for the quantitative determination of the concentration of salicylic acid in the oil powders, release samples, skin samples and transdermal samples.

The release of salicylic acid was determined for all nine template emulsions and for their respective oil powders and redispersed oil powders. Each sample had the same concentration of salicylic acid. The effect of pH on the release of salicylic acid from the corresponding emulsions, oil powders and redispersed oil powders generally showed an increase in the release of this active with an increase in pH. The oil powders had yielded higher release rates (release rate and cumulative amount released) of salicylic acid, with the trend being similar to those of their related emulsions and redispersed powders. The higher cumulative release of the active from the oil powders could be explained by the lower amount of water present in the donor phase, thus yielding higher salicylic acid concentrations and therefore higher driving forces for diffusion. The release of salicylic acid from the redispersed oil powders and their corresponding emulsions were similar.

Six oil powders and six redispersed oil powders were selected for use in the skin diffusion studies. The results from the skin absorption study were also divided into the effect of pH on chitosan and into the effect of various polymers used during the preparation of the formulations at pH 6. No differences were found in dermal delivery with changing pH, whereas significant differences were obtained with regards to transdermal delivery. The second set of tests outcomes also showed no differences in dermal delivery among various polymers used, whereas statistically significant differences occurred in respect of transdermal delivery. With regards to the effects of pH, the degree of ionisation of salicylic acid could have affected the transdermal delivery thereof, with a decrease in permeation with increasing ionisation of salicylic acid, in accordance with the pH partition hypothesis. With regards to the effects of polymer used, the enhanced permeation of salicylic acid from the redispersed chitosan oil powder may have been caused by the interaction between the positively charged amino groups of chitosan and the negatively charged skin surface. The oil powders resulted in a longer lag time for salicylic acid to permeate the skin, whereas the presence of water in the donor phase of the redispersed oil powders could have accelerated the transport of salicylic acid across the skin. A positive correlation was found between powder particle size and transdermal delivery. In general, the redispersed oil powders had demonstrated better transdermal delivery than the respective oil powder formulations.

Future perspectives

To date, the available literature indicates that very little research has been performed on oil powders for possible use in topical delivery. A suggestion for future projects could be to investigate oil powders containing different active ingredients, oils as well as wall materials, for possible application in the cosmetic and pharmaceutical industries. Another suggestion could be to formulate an oil powder with increased oil content that would decrease oil leakage.

APPENDIX A

INSTRUCTIONS FOR AUTHORS—KARGER

Contents

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Using and Understanding Medical Statistics, ed 3, revised. Basel, Karger, 1996.

(d) Edited books: Parren PWHI, Burton DR:

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APPENDIX B

Validation of HPLC-UV analytical method for salicylic acid determinations

An HPLC method was developed and validated for the quantitative determination of the concentration of salicylic acid in the oil powders, release samples, skin samples and transdermal samples at the Analytical Technology Laboratory, North-West University, Potchefstroom Campus, South Africa under the supervision of Prof. Jan L. du Preez.

B.1 Linearity

HPLC-UV calibration curves were generated by dissolving approximately 100 mg of salicylic acid in 100 ml of methanol, to yield a stock solution having a concentration of 1 mg/ml. The standard solutions were prepared by further diluting samples from the stock solution with methanol to obtain six solutions with salicylic acid concentrations of 1.25 µg ml^{-1}, 2.5 µg ml^{-1}, 25 µg ml^{-1}, 62.5 µg ml^{-1}, 125 µg ml^{-1} and 250 µg ml^{-1}. Five calibration curves were generated for these standard solutions on five consecutive days. The equation below describes the linear regression between the concentration of the active ingredient and the peak area that also represented the average of the five calibration curves.

$$y = 73.384\,x - 6.839 \qquad \text{Equation 1}$$

Regression coefficients of all calibration curves were determined at $R^2 \geq 0.999$, indicative of the strong linearity between the salicylic acid concentrations and the peak areas over the range of 1.25 µg ml^{-1} – 250 µg ml^{-1}. A calibration curve was also prepared for the receptor phase of the release and skin absorption studies. The receptor phase contained phosphate buffer pH 7.4/propylene glycol (1:1) and the following regression line was generated, with a regression coefficient higher than 0.999.

$$y = 72.13\,x + 0.405 \qquad \text{Equation 2}$$

Since the regression lines for methanol and the receptor phase were similar, further calibration curves were prepared in methanol.

B.2 Accuracy and Precision

Accuracy describes the closeness of the mean concentration value obtained for the method to the true concentration, whereas precision is the relative standard deviation obtained by repeatedly analysing a sample. Accuracy and precision were calculated from five

determinations per salicylic acid concentration. Three concentration levels (low, medium and high) of the calibration curve were used and the results are presented in Table B.1:

Table B.1: Accuracy and precision test outcomes

Actual conc. [µg ml^{-1}]	Analysed conc. [µg ml^{-1}]	Accuracy [%]	Precision [%]
1.25	1.16 ± 0.14	92.8	12.1
62.5	64.4 ± 2.2	103.0	3.4
250	249.8 ± 9.2	99.9	3.7

According to the Food and Drug Administration's (FDAs) validation guideline for industry (FDA, United States, 2001), the mean value should be within 15% of the actual value (acceptance criteria for accuracy) and the precision should not exceed 15%. The data in Table B.1 demonstrates that the acceptance criteria were met at all of the three concentration levels and was the method therefore found to be accurate and precise for the analysis of salicylic acid on HPLC within the concentration range of 1.25 µg ml^{-1} – 250 µg ml^{-1}.

B.3 Lower limit of quantification

Lower limit of quantification is the lowest concentration of the standard curve that can be analysed with acceptable accuracy and precision. Acceptance criteria for both parameters is set below 20% according to the FDA validation guideline for industry (United States, 2001). According to Table B.1, the analysis of the lowest concentration of 1.25 µg ml^{-1} resulted in an accuracy value within 20% of the actual concentration and in a precision value below 20% and was this concentration hence established as the lower limit of quantification of salicylic acid on HPLC.

B.4 Repeatability

Repeatability was determined through six consecutive injections of the same sample having a concentration of 25 µg ml^{-1}. The analysis of six injections resulted in a measured concentration of 26.26 ± 0.03 µg ml^{-1}. As the resulting relative standard deviation (0.11%) was found to be below 2%, repeatability was favourably proven.

B.5 Selectivity

Blank emulsions (without salicylic acid), as well as methanol solutions with skin from two different skin donors and tape strips were injected and compared to a standard sample of

salicylic acid in methanol. No interferences were indicated at the retention time for salicylic acid at 4.4 minutes (Figure B.1 – B.6).

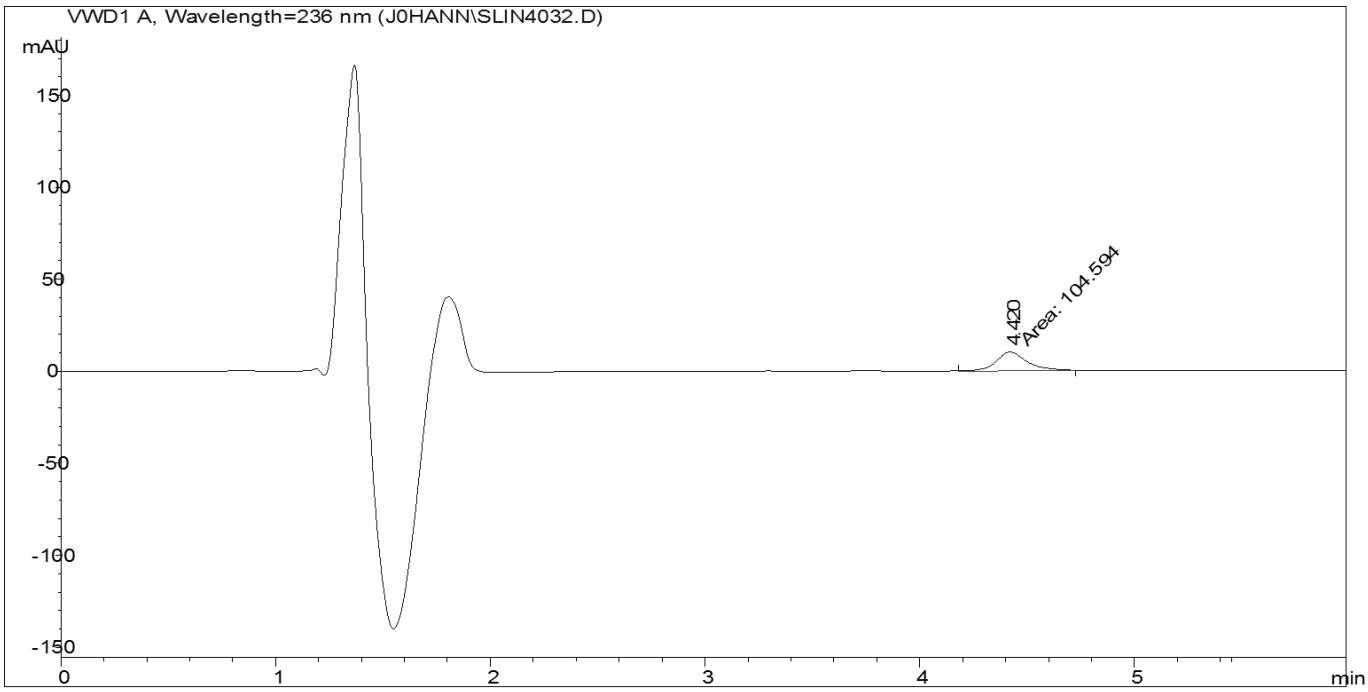

Figure B.1. : HPLC chromatograph of salicylic acid (1.25 µg ml^{-1}).

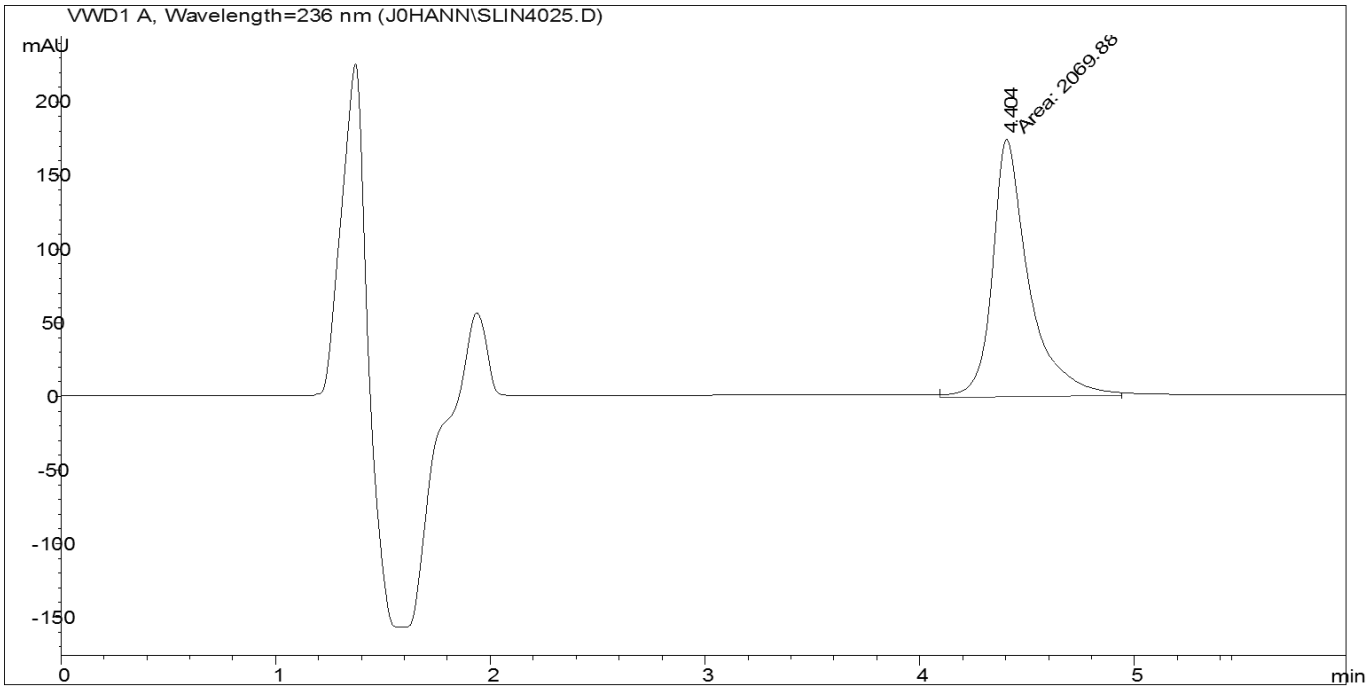

Figure B.2. : HPLC chromatograph of salicylic acid (25 µg ml^{-1}).

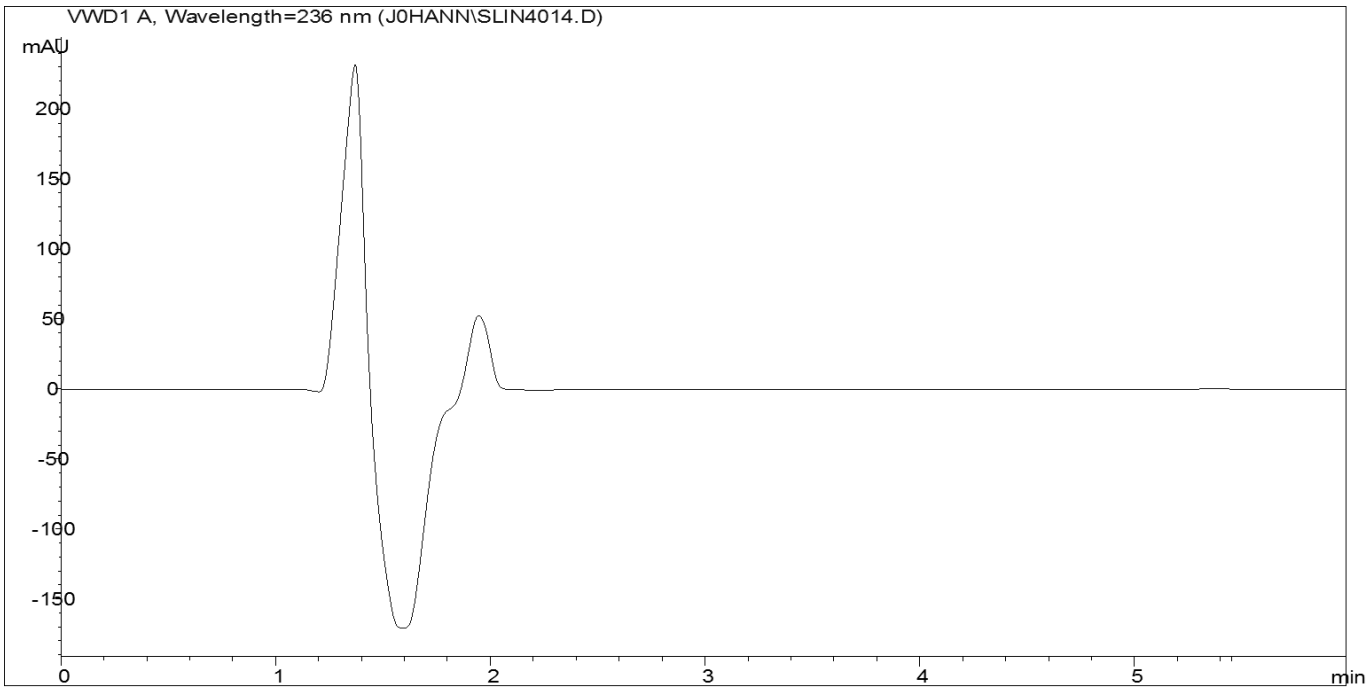

Figure B.3. : HPLC chromatograph of PBS:PG (1:1, v/v).

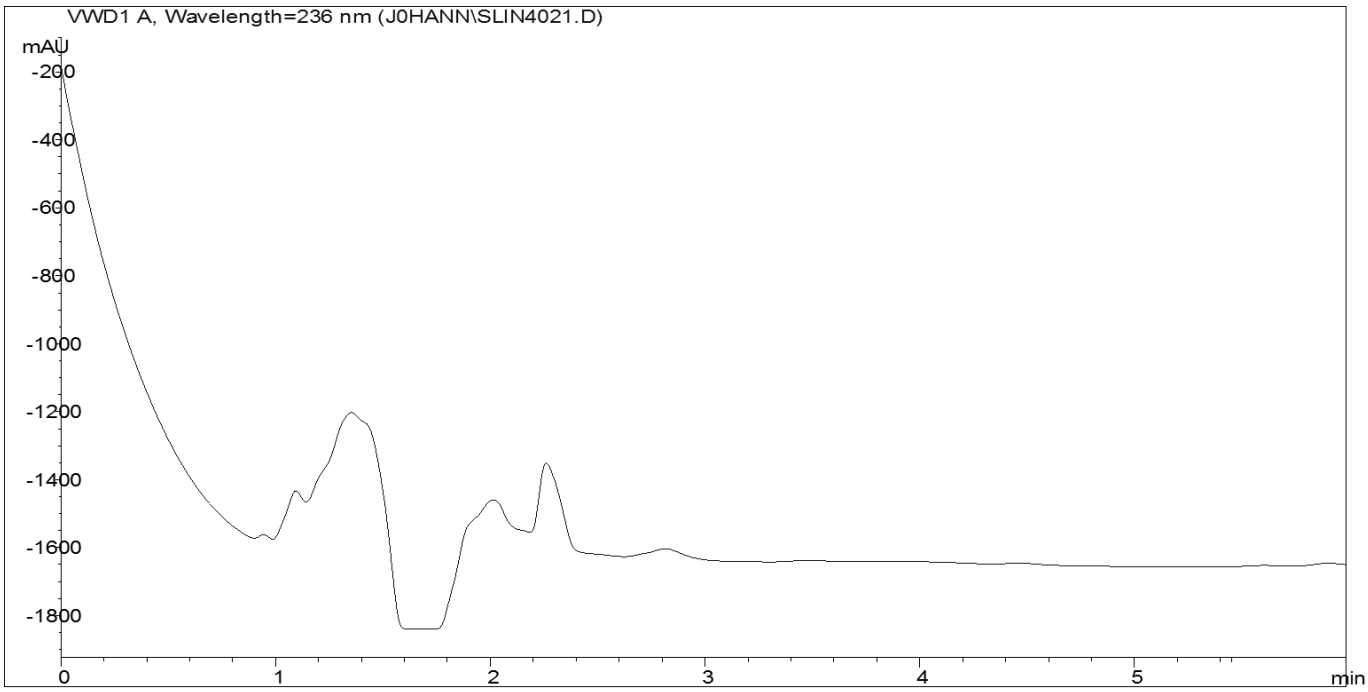

Figure B.4. : HPLC chromatograph of blank emulsion.

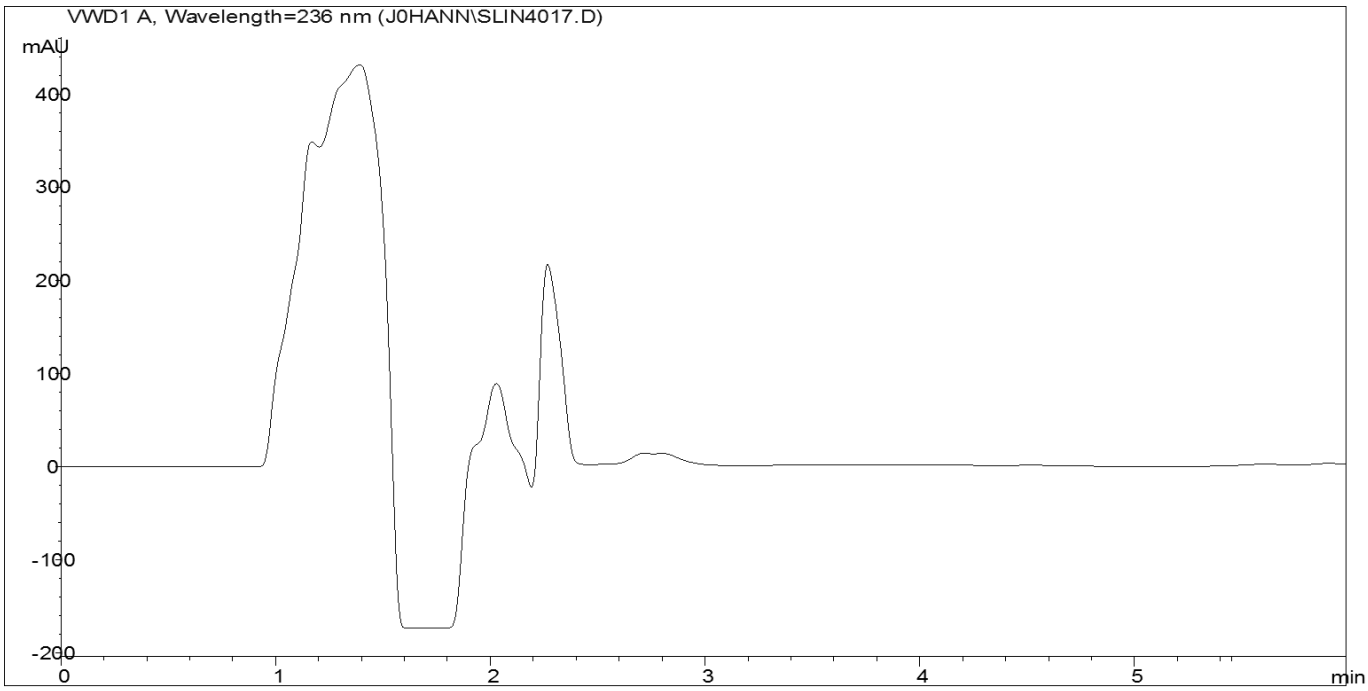

Figure B.5. : HPLC chromatograph of blank skin sample.

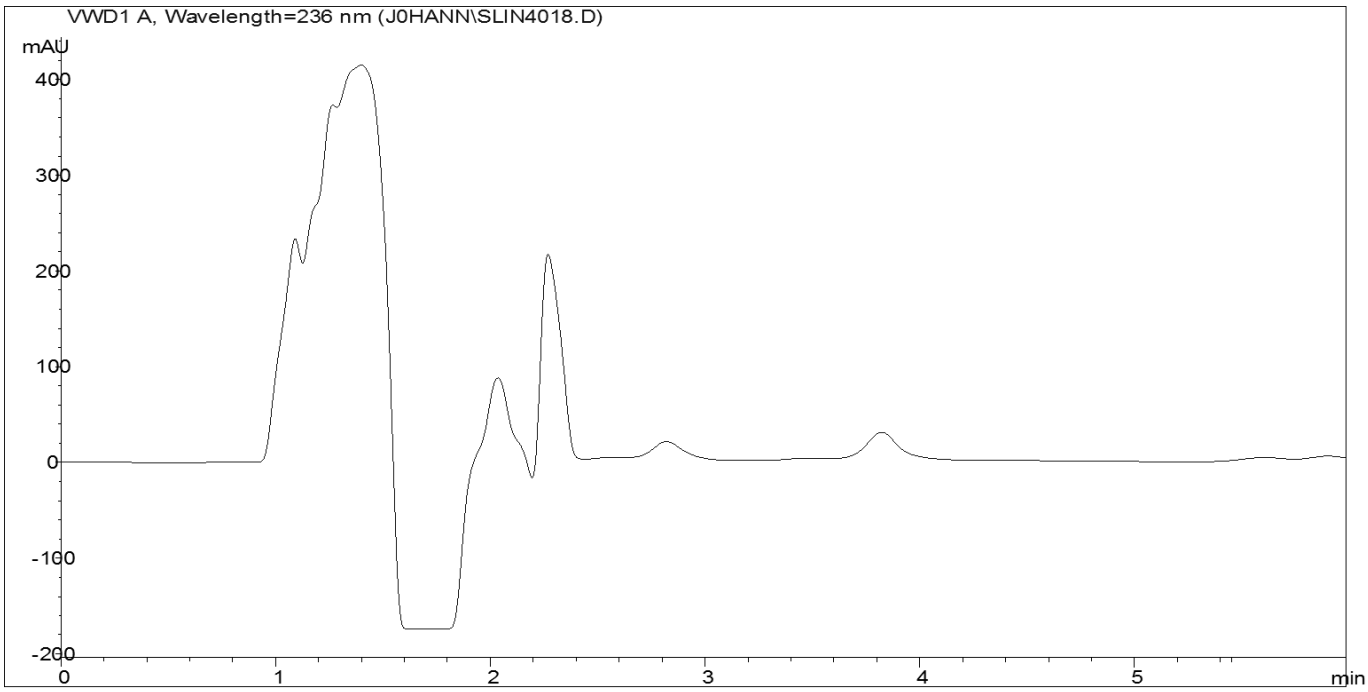

Figure B.6. : HPLC chromatograph of blank tape strip sample.

B.6 Stability

The stability of salicylic acid (250 µg ml^{-1}) in methanol and in phosphate buffer pH 7.4: propylene glycol (PG) (1:1) was determined every two hours for up to 24 hours and at 36 hours. These samples were kept at room temperature. The results are summarised in Table B.2.

Table B.2: Outcomes from stability determinations of salicylic acid in methanol and in the receptor phase

	Area	Percentage of initial value [%]	Analysed conc. [µg ml^{-1}]
Methanol	18703.8 ± 177.1	100.0 ± 1.0	254.97 ± 2.4
PBS:PG	18489.8 ± 73.0	100.6 ± 0.4	252.1 ± 1.0

The average of the area of methanol and PBS:PG was calculated at time 0 and at each two hour interval until 24 hours, as well as at 36 hours.

The data in Table B.2 illustrates that no change in area had occurred over time. It could therefore be concluded that salicylic acid had remained stable in methanol and in the receptor phase for at least 36 hours.

Reference

United States. Department of Health and Human Services. Food and Drug Administration. 2001. Guidance for industry-bioanalytical method validation. http://www.fda.gov/downloads/Drugs/Guidances/ucm070107.pdf.

APPENDIX C

Partition coefficient determinations of salicylic acid between oil and aqueous phases

C.1 Method

The partition coefficient of salicylic acid was determined by preparing deionised water at pH 4, 5 and 6. The Miglyol 812 N® was prepared to contain 2% of salicylic acid. Subsequently, 5 ml of the deionised water at each pH value was added to 5 ml of Miglyol 812 N® containing 2% of salicylic acid, and then magnetically stirred for 24 hours. After 24 hours of stirring, the two phases were separated, filtered and then subjected to HPLC-UV analysis for determination of the total salicylic acid content.

C.2 Results

The partition coefficient was utilised to determine possible oil leakage from the salicylic acid oil powders and to calculate the encapsulation efficiency. It was found that the solubility increased with a decrease in the partition coefficient.

Table C.1: Outcomes from water-Miglyol 812 N® partition coefficient of salicylic acid testing and from the determination of the percentage of ionised salicylic acid at different pH values of the water phase

	Log P	% ionised SA
pH 4	1.76	91.46
	1.54	
	1.57	
pH 5	1.70	99.08
	1.48	
	1.50	
pH 6	1.64	99.90
	1.47	
	1.46	

APPENDIX D

Loss on drying determinations

D.1 Method

Loss on drying was determined by using the oven drying method. Approximately 500 mg of oil powder was placed on a suitable dish and dried at 80°C in an oven. The weight of the oil powder was determined at time zero (before placing it into the oven) and at pre-defined time intervals. Drying was continued until two consecutive weighing measurements did not differ by more than 0.2%.

D.2 Results

The loss on drying percentage values are listed in Table D.1 below. The loss on drying determinations revealed that after a period of two days, the loss on drying of the oil powders was ≤ 0.20%. The whey, Car and Chi oil powders at pH 6 had experienced no loss on drying between days 1 and 2.

D.3 Discussion

The measured loss on drying values was insignificant and therefore it was concluded that the oil powders did not contain much water. The slightly higher values for the Car and Chi powders may have been due to swelling of the polysaccharides and hence would it have been more difficult to remove the water during freeze drying.

Table D.1: Loss on drying test outcomes

		Day 1 [%]	Day 2 [%]
Whey	**pH 4**	0.20	0.20
	pH 5	0.20	0.19
	pH 6	0.20	0.00
Car	**pH 4**	0.80	0.20
	pH 5	0.59	0.00
	pH 6	0.80	0.00
Chi	**pH 4**	0.60	0.20
	pH 5	0.59	0.00
	pH 6	0.20	0.00

APPENDIX E

Oil leakage and encapsulation efficiency determinations

E.1 Method

The following equation was used to calculate the encapsulation efficiency (EE) (Klinkersorn *et al.,* 2005).

$$\mathrm{EE\%} = [(\text{total SA} - \text{extractable SA}) \times 100]/\text{total SA} \qquad \text{Equation 1}$$

The extractable salicylic acid (SA) content is the amount of salicylic acid that has not been encapsulated into the oil powder, i.e. the amount of salicylic acid that has leaked during the manufacturing of the oil powders. The extractable amount of salicylic acid was calculated by using the oil leakage and partition coefficient values of salicylic acid between the oil- and the aqueous phases. First, the amount of salicylic acid that had been lost in the oil phase of the emulsion, due to partitioning into the aqueous phase, was determined by applying the partition coefficient. Subsequently, the amount of salicylic acid that had been lost due to oil leakage during freeze drying of the emulsions, was added. This value was determined from the known amount of salicylic acid in the oil phase and the amount of oil that had leaked during preparation of the oil powders.

For the determination of the total salicylic acid content, a known amount of oil powder was redispersed in water to dissolve the shell of the oil powders consisting of whey proteins and polysaccharides. The resulting emulsion was subsequently de-emulsified with methanol, during which the salicylic acid was simultaneously dissolved also. The resulting solution was filtered and subjected to HPLC-UV analysis for the determination of the total salicylic acid content. Duplicate measurements were taken for each of the duplicate samples per oil powder.

E.2 Results

The results for oil leakage and encapsulation efficiency are summarised in Table E.1. The data shows that oil leakage did not increase over the period of three months of testing. Furthermore, no significant differences were observed among samples taken from different locations (e.g. bottom, middle and top) within one powder sample. In general, the oil leakage was similar for the various oil powders, except for the whey protein oil powders at pH 4 and 5, which had yielded higher oil leakage values. The encapsulation efficiency of salicylic acid was determined to be between 55.0% - 79.6% and did not differ significantly among the various powders. However, slightly higher values were observed for the carrageenan and chitosan oil powders, compared to those of the whey protein oil powders.

Table E.1: Oil leakage and encapsulation efficiency study test outcomes

		Centre of powder 1 week		Top of powder 2 months		Side of powder (glass wall) 3 months		Bottom of powder 3 months	
		%	% SA EE	%	% SA EE	%	% SA EE	%	% SA EE
Whey	pH 4	39.90	54.19	21.71	71.09	17.52	77.03	17.91	74.91
		39.15	55.81	22.05	71.10	16.10	74.37	17.08	75.10
	pH 5	41.09	53.41	33.10	59.03	33.00	60.97	30.30	61.64
		41.00	52.59	34.88	59.57	33.31	59.23	31.50	62.55
	pH 6	20.67	69.83	12.90	76.61	14.09	75.81	12.91	77.01
		20.90	70.16	14.72	75.78	15.40	74.78	11.22	78.38
Car	pH 4	27.30	65.51	19.82	73.97	21.94	71.04	20.01	72.60
		24.12	68.09	19.55	72.23	23.18	69.96	21.04	72.00
	pH 5	21.02	71.04	21.27	72.02	22.03	69.97	20.96	69.97
		21.00	70.95	18.87	71.78	23.90	68.63	20.35	72.64
	pH 6	13.07	75.99	9.39	79.15	9.91	79.15	13.58	75.79
		13.10	77.60	10.50	80.05	9.95	80.04	13.74	76.81
Chi	pH 4	23.33	68.91	25.41	67.14	27.58	65.91	24.41	68.03
		24.90	69.29	26.42	67.66	27.80	65.68	24.37	69.57
	pH 5	21.91	71.03	18.36	73.01	21.92	71.54	18.08	73.21
		21.14	70.17	17.76	74.39	20.21	70.46	19.34	72.99
	pH 6	21.50	70.91	20.97	68.92	20.87	68.46	21.92	69.94
		19.55	69.49	21.80	70.08	22.89	69.54	21.41	68.46

E.4 Oil leakage

This experiment was performed to confirm the oil leakage data obtained during HPLC-UV analysis. A known amount of oil powder was placed on filter paper. A microscope slide was then used to cover the powder and exert gravitational pressure on the oil powder over a period of 24 hours. Pictures were taken at zero minutes, at 60 minutes and after 24 hours. The pictures confirmed the oil leakage data, i.e. in general the oil leakage was similar for the various oil powders, except for the whey protein oil powders at pH 4 and 5, which had yielded higher oil leakage values.

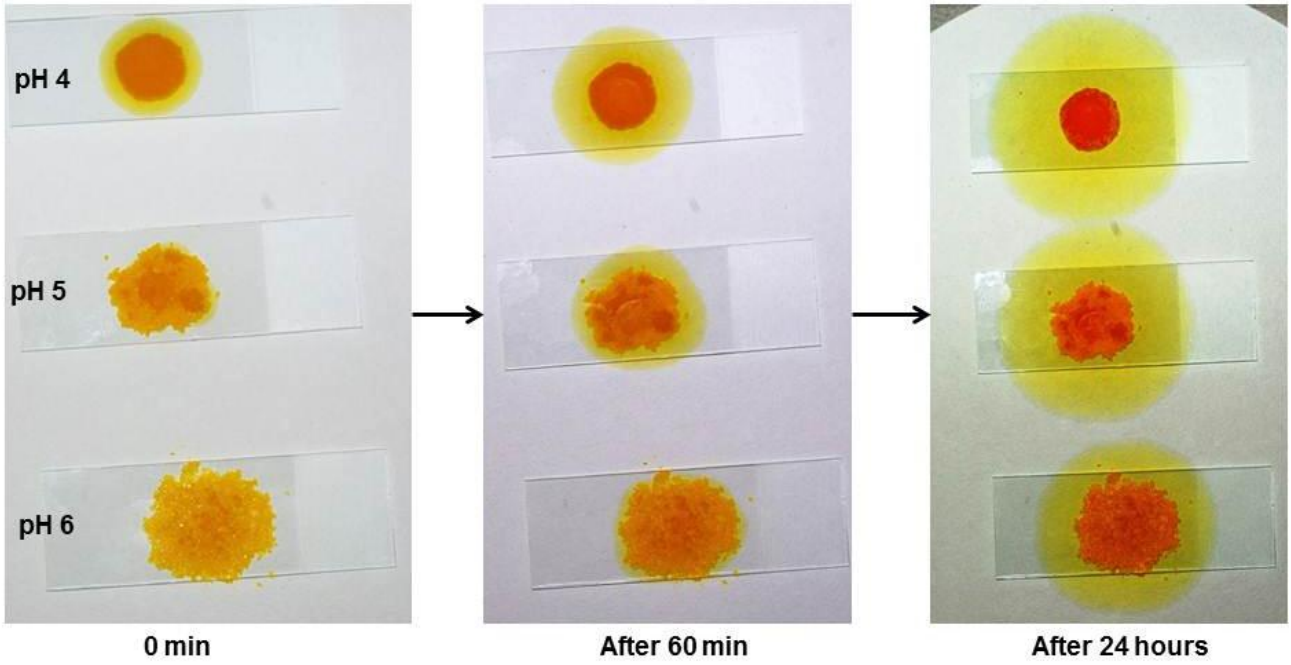

Figure E.1: Illustrations of oil leakage of whey protein oil powder over 24 hours.

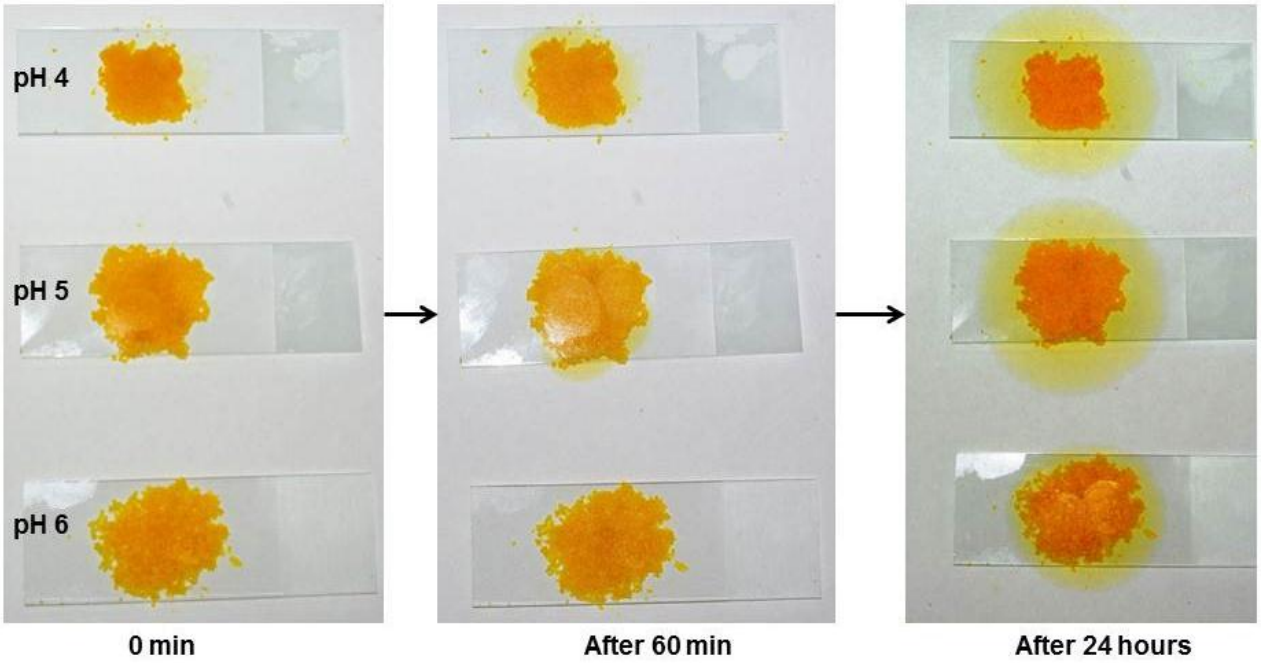

Figure E.2: Illustrations of oil leakage of carrageenan oil powder over 24 hours.

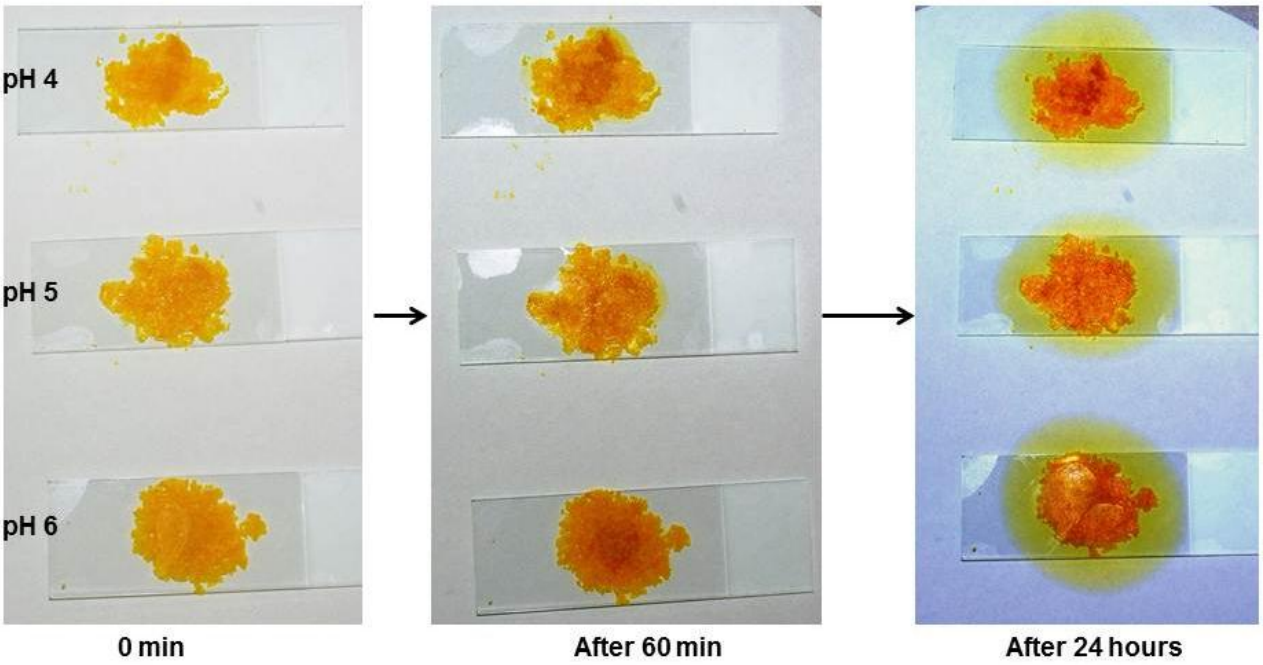

Figure E.3: Illustrations of oil leakage of chitosan oil powder over 24 hours.

Reference

Klinkesorn, U., Sorphanodora, P., Chinachoti, P., McClements, D.J. & Decker, E.A. 2005. Stability of spray-dried tuna oil emulsions encapsulated with two-layered interfacial membranes. *Journal of Agricultural Food Chemistry*, 53:8365-8371.

APPENDIX F

Water dispersibility determinations

F.1 Method

The dispersity value *D(t)* was determined using the equation by Takeuchi *et al*. (1991).

$$\mathrm{D(t)} = \frac{\text{Total amount of SA dispersed in the test solution at time t}}{\text{Total amount of SA in the added oil powder}} \times 100\% \qquad \text{Equation 1}$$

For the water dispersibility testing, a weighted amount of each oil powder (200 mg) was each dispersed in 2 ml of deionised water in a test tube at room temperature. Each test tube was hand shaken ten times and allowed to stand for 30 minutes. Two aliquots of 100 µl each of the test solution was withdrawn from the centre of the tube after 5 and 30 minutes. 2 ml of methanol was added to each sample in order to extract the salicylic acid. The samples were filtered using hydrophilic PVDF pre-filters with a pore size of 0.45 µm (Agela Technologies Inc., Wilmington, DE, USA) and subjected to HPLC-UV analysis to determine the amount of dispersed salicylic acid. Duplicate measurements were taken from each of the three samples per oil powder.

F.2 Results

The determined dispersity values are listed in Table F.1. Overall, the dispersity values after 5 and 30 minutes were similar and no significant reduction in water dispersibility had occurred after 30 minutes, compared to 5 minutes. It was also observed that the dispersibility had increased with increasing pH for the three oil powder types (whey, chitosan and carrageenan).

F.3 Discussion

The dispersity value is an indication of the stability of the water dispersion of oil powders. However, one has to consider that the method used to determine the dispersity values did not distinguish between the salicylic acid being encapsulated in the redispersed oil powder particles and in the emulsion droplets, and the dissolved salicylic acid in water. The increase in dispersity values with increasing pH could therefore not have solely related to a better water dispersibility, but also to an enhanced solubility of salicylic acid in water at higher pH values. In general, the addition of chitosan and carrageenan had improved water dispersibility, which was congruent with the oil leakage data. Oil powders with higher oil leakage had resulted in more sticky oil powders, which were more difficult to redisperse, hence yielding lower dispersity values.

Table F.1: Water dispersibility test outcomes

		5 min [%]	30 min [%]
Whey	**pH 4**	41.09	46.98
		43.24	46.93
		41.97	46.46
	pH 5	67.21	71.28
		68.37	71.04
		67.75	70.92
	pH 6	89.09	90.53
		89.55	90.97
		89.35	91.03
Car	**pH 4**	64.80	64.39
		64.84	64.75
		64.99	64.50
	pH 5	79.87	82.17
		80.13	82.01
		80.02	82.03
	pH 6	101.43	103.63
		101.07	103.04
		101.34	103.80
Chi	**pH 4**	147.63	145.78
		147.50	145.73
		147.34	145.40
	pH 5	86.07	82.40
		86.23	83.71
		86.17	83.95
	pH 6	102.36	114.16
		102.90	114.59
		103.40	113.66

Reference

Takeuchi, H., Sasaki, H., Niwa, T., Hino, T., Kawashima, Y., Uesugi, K., Kayano, M., Miyake, Y. 1991. Preparation of powdered redispersible vitamin E acetate emulsion by spray-drying technique. *Chemical Pharmaceutical Bulletin*, 39:1528-1531.

APPENDIX G

Droplet size determinations

G.1 Method

Droplet sizes of the template emulsions (before freeze drying) and of the redispersed powders (after freeze drying) were determined by using the Malvern Mastersizer 2000, equipped with a wet cell Hydro 2000 SM dispersion unit (Malvern Instruments, Worcestershire, UK). The template emulsions were diluted with deionised water on the day of preparation, to yield obscuration values between 10% - 20%. The oil powders were redispersed in deionised water on the day of removal from the freeze dryer to also yield obscuration values between 10% - 20%. Measurements were taken from two freshly prepared samples each per emulsion and from the redispersed powder, and readings per sample taken in duplicate.

G.2 Results

The data obtained from the Malvern Mastersizer 2000 for the particle sizes of each emulsion (whey, chitosan and carrageenan) and for each corresponding redispersed oil powder in water, is summarised in Tables G.1 and G.2. The results of the particle size analyses were presented as volume weighted means (D [4,3]) and surface weighted means (D [3,2]). Overall, the data showed that the particle sizes of the redispersed powders were much larger than those of their respective emulsions. However, the particle size data from the Mastersizer was contradicted by the microscopic images, which showed that the redispersed particles were much smaller.

G.3 Discussion

The increase in particle size from the emulsion to the redispersed oil powders could have been due to coalescence, or flocculation of the oil droplets. The light microscopic images, however, revealed no significant increase in droplet size among the emulsions and redispersed oil powders, indicating that the increase in particle sizes, as observed from the data in Table G.2, had rather been caused by agglomeration of the particles during preparation of the oil powders, than by coalescence. Whey pH 4 and 5 and chitosan pH 4 and 6 images were indicative that agglomeration had occurred.

Table G.1. : Droplet size data of emulsions

		d (0.1) [µm]	d (0.5) [µm]	d (0.9) [µm]	Span	D [4,3] [µm]	D [3,2] [µm]	Obscuration [%]
Whey	**pH 4**	1.36	3.01	8.18	2.27	12.32	2.57	14.01
		1.29	2.90	6.08	1.66	3.36	2.37	14.30
		1.35	2.98	7.47	2.05	11.52	2.54	12.91
		1.29	2.90	6.10	1.66	3.37	2.38	13.08
	pH 5	1.35	2.94	16.75	1.83	10.74	2.50	13.60
		1.30	2.92	6.10	1.64	3.38	2.39	13.77
		1.16	2.47	5.82	1.89	31.81	2.14	18.21
		1.07	2.34	4.81	1.60	2.69	1.95	18.43
	pH 6	1.08	2.37	4.88	1.60	2.73	1.98	14.78
		1.13	2.35	4.58	1.47	2.65	2.00	14.97
		1.04	2.26	4.56	1.56	2.75	1.92	12.47
		1.04	2.25	4.53	1.56	2.61	1.95	12.44
Car	**pH 4**	1.70	7.21	257.83	35.54	70.51	4.26	13.19
		1.68	6.91	198.14	28.43	62.29	4.16	13.30
		1.67	6.70	212.79	31.50	61.48	4.11	13.17
		1.64	6.38	166.30	25.80	50.03	4.00	13.41
	pH 5	1.35	4.32	11.69	2.39	6.19	2.91	11.78
		1.46	4.01	9.39	1.98	4.84	2.92	11.85
		1.38	4.58	14.55	2.88	11.35	3.04	13.37
		1.45	3.98	9.35	1.99	4.81	2.90	13.89

Droplet size data of emulsions *(continued)*

Car	**pH 6**	1.03	2.04	3.76	1.34	2.25	1.77	13.31
		0.99	1.98	3.69	1.37	2.19	1.70	14.26
		1.05	2.07	3.80	1.33	2.28	1.80	16.79
		0.99	1.98	3.70	1.36	2.20	1.71	16.43
Chi	**pH 4**	1.52	8.45	50.78	5.82	17.18	4.11	15.82
		1.41	6.81	21.56	2.96	10.28	3.55	16.77
		1.90	9.05	60.88	6.52	19.60	4.69	16.37
		1.64	7.20	20.42	2.61	10.91	3.95	17.51
	pH 5	1.39	4.00	9.01	1.93	4.82	2.82	14.01
		1.35	3.44	7.45	1.78	4.01	2.60	14.03
		1.37	3.70	8.27	1.87	4.36	2.71	13.72
		1.34	3.40	7.44	1.79	3.99	2.58	13.72
	pH 6	1.24	2.97	6.65	1.83	3.56	2.36	12.59
		1.23	2.95	6.63	1.83	3.56	2.35	12.62
		1.26	3.02	6.72	1.81	3.63	2.40	11.06
		1.26	3.04	6.85	1.84	3.69	2.41	11.03

Table G.2. : Droplet size data of redispersed oil powder

		d (0.1) [µm]	d (0.5) [µm]	d (0.9) [µm]	Span	D [4,3] [µm]	D [3,2] [µm]	Obscuration [%]
Whey	**pH 4**	13.09	180.68	436.24	2.34	208.07	24.49	12.95
		12.26	117.87	413.15	2.25	199.96	23.83	13.04
		6.18	147.98	429.75	2.86	186.55	15.37	12.71
		5.74	136.32	409.37	2.96	175.70	14.65	12.79
	pH 5	36.05	228.08	504.39	2.05	254.03	38.91	11.45
		36.42	226.04	499.11	2.05	251.68	39.09	11.64
		18.20	177.56	440.55	2.38	209.40	28.22	10.87
		18.57	175.75	452.66	2.47	212.12	28.46	11.01
	pH 6	35.48	205.30	442.58	1.98	227.94	37.53	11.43
		36.89	211.66	470.72	2.05	239.63	38.33	11.57
		32.31	193.80	428.06	2.04	217.82	35.70	11.78
		31.83	197.26	442.24	2.08	224.23	35.56	11.84
Car	**pH 4**	93.98	221.30	447.23	1.60	249.28	83.12	11.51
		89.34	217.12	424.44	1.54	238.90	61.76	14.24
		89.76	220.91	467.63	1.71	258.15	66.36	10.21
		76.46	217.76	436.77	1.66	240.94	47.73	13.03
	pH 5	70.36	228.79	731.92	2.89	326.08	71.52	11.90
		53.06	210.96	735.12	3.23	315.86	54.76	13.81
		64.19	210.29	715.95	3.10	311.61	65.13	14.09
		47.50	194.62	610.54	2.89	269.93	50.42	16.17

Droplet size data of redispersed oil powder *(continued)*

Car	**pH 6**	80.32	261.30	757.54	2.59	351.25	69.28	11.78
		54.10	195.89	511.85	2.34	247.11	49.47	13.09
		66.76	228.43	603.24	2.35	289.49	56.37	11.77
		50.20	193.84	592.47	2.80	269.40	47.21	12.98
Chi	**pH 4**	3.20	100.62	293.74	2.89	123.94	9.43	11.18
		3.15	99.17	291.20	2.91	122.45	9.29	11.29
		2.99	88.13	348.81	3.92	134.64	8.86	10.78
		2.91	82.39	317.61	3.82	125.65	8.61	10.86
	pH 5	2.56	23.86	153.46	6.32	68.18	7.35	13.29
		2.36	19.40	145.79	7.39	47.14	6.45	13.42
		3.14	32.74	181.12	5.44	66.81	8.34	11.40
		3.14	32.74	188.57	5.66	68.75	8.34	11.39
	pH 6	78.23	509.406	1106.30	2.02	558.53	115.15	9.66
		78.39	519.14	1130.73	2.03	569.69	115.57	9.77
		63.44	398.66	975.05	2.29	463.10	80.83	9.96
		64.71	418.89	1022.83	2.29	485.08	82.49	9.99

APPENDIX H

Table H.1. : Cumulative release data of salicylic acid from emulsions *(Raw data of Chapter 3)*

		1h [µg cm^{-2}]	2h [µg cm^{-2}]	3h [µg cm^{-2}]	4h [µg cm^{-2}]	6h [µg cm^{-2}]	8h [µg cm^{-2}]	Rate [µg cm^{-2} $h^{-1/2}$]	R^2	Lag time[min]	% salicylic acid released after 8h
Whey		309.3	434.9	511.8	616.8	776.0	920.4	334.8	0.997	0.969	34.668
	pH 4	344.3	483.4	560.4	670.7	829.2	968.6	341.5	0.998	0.028	36.484
		231.9	448.5	559.2	694.8	874.4	1039.3	435.4	0.999	11.153	39.146
		376.9	601.6	725.1	890.4	1091.9	1312.1	504.3	0.999	3.821	27.924
	pH 5	355.5	531.8	662.4	835.1	1060.5	1252.4	506.5	0.999	7.987	26.652
		370.9	556.4	700.3	887.2	1083.8	1258.3	494.3	0.998	4.261	26.778
		472.9	675.8	786.9	931.5	1120.9	1280.6	440.7	0.999	0.470	48.236
	pH 6	568.7	791.9	922.6	1089.6	1308.9	1490.6	505.2	0.999	1.047	56.146
		498.4	685.5	793.1	943.5	1141.3	1309.7	445.3	0.999	0.681	49.333
Car		332.8	453.8	529.5	616.4	756.1	893.6	304.0	0.998	0.216	33.657
	pH 4	356.7	514.1	615.2	759.3	956.3	1120.0	422.6	0.998	2.387	42.194
		322.2	477.1	564.7	675.9	849.8	999.7	369.4	0.999	1.337	37.657
		405.5	584.9	678.8	807.2	1000.5	1194.1	425.8	0.998	0.345	44.979
	pH 5	215.2	403.3	533.4	696.6	903.6	1084.9	479.9	0.999	19.585	40.865
		419.5	588.6	685.6	812.0	993.6	1152.7	400.4	0.999	0.064	43.420
		331.2	528.1	624.3	746.7	926.4	1086.3	407.3	0.999	1.694	40.918
	pH 6	275.8	465.6	663.0	872.3	1103.0	1257.1	558.7	0.996	16.275	47.351
		409.9	600.6	738.9	946.2	1236.9	1443.3	581.8	0.996	7.950	54.366

Cumulative release data (continued)

		1h [µg cm^{-2}]	2h [µg cm^{-2}]	3h [µg cm^{-2}]	4h [µg cm^{-2}]	6h [µg cm^{-2}]	8h [µg cm^{-2}]	Rate [µg cm^{-2} $h^{-1/2}$]	R^2	Lag time[min]	% salicylic acid released after 8h
Chi		295.7	410.1	550.6	710.9	916.9	1097.6	454.0	0.995	11.745	41.341
	pH 4	331.4	476.8	560.0	682.9	839.5	967.7	350.8	0.999	0.302	36.449
		356.0	540.6	662.9	803.7	995.6	1161.0	441.6	1.000	2.359	43.732
		325.9	500.5	610.2	758.6	943.1	1102.9	427.9	0.999	3.815	41.545
	pH 5	363.4	541.9	646.5	777.9	964.0	1104.5	407.6	0.999	0.670	41.602
		408.2	579.5	671.9	809.8	1007.2	1203.5	432.0	0.997	0.582	45.330
		337.7	519.7	632.8	801.5	1009.8	1169.5	462.7	0.998	5.169	44.050
	pH 6	378.3	577.1	707.1	885.1	1105.1	1298.7	508.0	0.999	4.690	48.916
		324.9	490.3	591.9	727.8	914.2	1074.5	412.0	0.999	3.333	40.474

Table H.2. : Cumulative release data of salicylic acid from redispersed oil powder *(Raw data of Chapter 3)*

		1h [µg cm^{-2}]	2h [µg cm^{-2}]	3h [µg cm^{-2}]	4h [µg cm^{-2}]	6h [µg cm^{-2}]	8h [µg cm^{-2}]	Rate [µg cm^{-2} $h^{-1/2}$]	R^2	Lag time [min]	% salicylic acid released after 8h
Whey		259.8	449.5	577.5	695.2	855.3	977.7	392.6	0.998	4.859	27.165
	pH 4	221.9	402.4	531.4	626.9	780.7	897.2	367.6	0.998	6.731	23.807
		242.8	408.9	520.9	616.0	785.9	920.2	368.8	1.000	6.311	25.173
		442.6	737.5	981.0	1178.7	1452.6	1630.0	659.3	0.997	4.722	52.906
	pH 5	300.4	507.6	689.9	801.8	966.5	1089.0	432.4	0.994	3.047	35.347
		382.1	644.2	762.7	805.8	874.4	912.6	269.5	0.928	38.977	29.769
		501.7	825.8	1074.1	1234.4	1464.4	1638.2	617.8	0.992	0.446	48.837
	pH 6	518.5	855.9	1097.2	1270.8	1563.4	1778.0	685.8	0.998	1.996	53.006
		482.6	821.0	1070.9	1249.6	1512.3	1683.8	657.8	0.994	1.860	50.1982
Car		352.2	549.0	702.9	882.2	1073.3	1250.0	497.0	0.999	4.090	33.162
	pH 4	359.4	541.2	687.4	862.5	1048.5	1225.1	480.0	0.999	4.170	31.039
		325.2	486.7	616.2	778.7	943.6	1101.7	431.3	0.998	4.078	28.777
		462.5	700.9	865.9	1061.1	1289.8	1484.9	563.9	0.999	1.746	44.268
	pH 5	411.1	630.8	800.1	981.2	1168.0	1334.6	510.1	0.998	1.614	39.787
		265.3	442.5	568.5	730.3	894.3	1051.0	433.8	0.999	8.920	31.489
		334.8	515.5	659.5	832.5	1019.9	1194.6	476.8	0.999	5.810	34.520
	pH 6	431.7	653.3	827.4	1054.0	1288.3	1464.8	580.7	0.997	4.132	42.328
		351.5	529.0	649.7	885.8	1045.3	n.d.	496.0*	0.990	6.284	30.205

Cumulative release data (continued)

		1h [µg cm^{-2}]	2h [µg cm^{-2}]	3h [µg cm^{-2}]	4h [µg cm^{-2}]	6h [µg cm^{-2}]	8h [µg cm^{-2}]	Rate [µg cm^{-2} $h^{-1/2}$]	R^2	Lag time [min]	% salicylic acid released after 8h
Chi		274.0	422.0	540.8	678.9	824.3	962.0	381.1	0.999	4.867	23.120
	pH 4	315.9	458.7	572.6	704.8	852.0	985.5	371.3	0.999	1.496	22.618
		249.5	395.9	506.6	633.4	774.7	904.0	361.4	0.999	5.631	21.390
		352.8	528.4	671.5	842.9	1027.7	1196.1	468.8	0.999	4.112	34.655
	pH 5	319.1	504.2	638.4	804.6	971.8	1143.6	453.4	0.999	5.161	33.136
		307.5	485.9	615.9	767.3	912.1	1052.4	410.2	0.998	2.940	30.646
		386.2	607.6	781.2	974.1	1190.6	1400.7	559.0	0.999	5.831	40.794
	pH 6	286.4	456.1	589.7	759.2	938.1	1094.6	450.5	0.998	8.582	31.880
		377.4	571.7	720.6	896.3	1084.7	1251.5	484.6	0.999	2.856	36.448

Table H.3. : Cumulative release data of salicylic acid from oil powders *(Raw data of Chapter 3)*

		1h [µg cm^{-2}]	2h [µg cm^{-2}]	3h [µg cm^{-2}]	4h [µg cm^{-2}]	6h [µg cm^{-2}]	8h [µg cm^{-2}]	Rate [µg cm^{-2} h$^{-1/2}$]	R^2	Lag time [min]	% salicylic acid released after 8h
Whey	**pH 4**	598.6	970.7	1306.1	1534.5	1803.1	2010.4	782.8	0.992	1.475	55.8575
		365.7	824.1	1019.7	1308.2	1720.6	1964.0	870.0	0.997	16.241	52.1137
		280.5	491.4	707.4	875.3	1248.5	1578.2	714.4	0.995	28.955	43.1706
		290.4	521.4	750.5	947.2	1272.0	1606.2	718.7	0.998	25.532	42.6188
	pH 5	620.2	1097.1	1467.5	1732.0	2113.7	2385.3	965.9	0.996	4.658	77.4208
		415.4	678.3	998.3	1291.7	1734.4	2113.9	948.7	0.998	23.852	68.6993
		387.0	796.1	1220.0	1530.8	2007.6	2360.5	1117.5	0.998	27.270	76.998
		392.2	704.1	1067.9	1358.1	1746.2	2062.7	943.1	0.998	22.469	68.3161
	pH 6	504.2	901.7	1269.5	1435.9	1688.5	1888.2	756.0	0.988	2.717	56.2925
		480.6	805.2	1104.4	1321.2	1694.9	2036.6	847.9	1.000	11.533	60.716
		852.1	1384.1	1714.4	1949.7	2233.2	2442.8	850.9	0.986	1.725	72.8262
		671.5	1123.0	1511.6	1826.9	2183.5	2505.1	1011.0	0.997	4.936	74.6838
Car	**pH 4**	170.4	448.8	826.5	1170.4	1619.6	1996.1	1045.9	0.997	50.466	50.8288
		118.7	332.3	656.2	921.7	1326.8	1775.7	926.7	0.995	58.144	43.6798
		224.7	481.1	754.5	985.3	1254.6	1532.3	735.7	0.998	31.775	37.8759
		300.2	531.5	766.2	984.7	1300.9	1643.5	739.7	0.998	26.104	41.6401
	pH 5	419.8	498.9	707.0	1006.5	1546.1	2146.0	956.6	0.969	37.928	62.4178
		331.0	554.1	821.1	1267.3	1773.6	2201.3	1075.0	0.991	41.120	64.339
		316.5	612.5	1210.3	1731.4	2279.4	2670.4	1390.2	0.993	43.744	78.4338
		342.7	622.5	858.9	1112.6	1594.5	2097.4	936.3	0.991	30.377	61.9074

Cumulative release data of salicylic acid from oil powders (continued)

		1h [µg cm^{-2}]	2h [µg cm^{-2}]	3h [µg cm^{-2}]	4h [µg cm^{-2}]	6h [µg cm^{-2}]	8h [µg cm^{-2}]	Rate [µg cm^{-2} h$^{-1/2}$]	R^2	Lag time [min]	% salicylic acid released after 8h
Car	**pH 6**	230.2	755.2	1475.1	1914.4	2326.3	2652.6	1418.5	0.984	40.840	74.4193
		249.8	713.0	1239.0	1712.3	2243.0	2647.3	1383.1	0.996	43.772	75.7419
		275.2	754.7	1397.6	1914.4	2372.8	2689.1	1421.7	0.989	40.082	76.5607
		225.8	626.5	1048.9	1404.7	1886.8	2299.2	1161.9	0.999	41.564	66.1091
Chi	**pH 4**	196.8	332.3	474.0	714.1	1016.8	1265.4	605.5	0.991	38.337	28.7563
		235.3	442.9	666.1	896.0	1103.1	1300.8	609.0	0.996	24.760	29.7066
		212.5	529.3	779.4	1010.1	1342.3	1574.5	757.8	0.999	30.003	36.5033
		272.8	522.8	726.8	911.5	1165.5	1362.3	608.3	0.999	18.124	31.5835
	pH 5	455.2	659.3	818.1	986.7	1493.3	1853.9	768.1	0.985	17.334	55.094
		370.8	555.2	734.7	1063.4	1491.4	1790.5	813.9	0.991	27.079	52.4039
		274.7	362.8	724.1	915.6	1156.3	1475.2	690.9	0.990	31.748	42.302
		344.9	635.6	1004.5	1345.2	1759.6	2072.3	990.1	0.997	29.622	60.3463
	pH 6	211.0	531.0	967.9	1477.4	1951.5	2374.5	1254.8	0.995	50.046	69.5016
		175.4	475.4	856.2	1288.6	1926.1	2250.3	1213.4	0.995	54.014	66.5361
		223.9	722.9	1204.3	1656.9	2059.0	2288.7	1195.9	0.989	36.364	68.3647
		212.2	805.2	1173.9	1572.8	2110.6	2503.3	1293.8	0.997	42.344	72.9046

APPENDIX I

Table I.1. : Skin absorption data of salicylic acid from redispersed oil powder *(Raw data of Chapter 3)*

		24h [µg/cm^2]	Rest skin [µg/cm^2]	SC [µg/cm^2]	El. Res. [kΩ]
Whey	**pH 6**	11.181	9.479	6.614	24.47
		8.860	5.489	7.655	22.76
		22.410	5.915	6.635	16.13
		12.746	5.126	5.553	19.57
		22.415	5.563	5.723	18.55
		8.177	5.035	6.235	31.42
Carr	**pH 6**	8.916	6.131	6.473	25.70
		8.010	4.391	6.583	25.64
		27.084	5.823	6.194	22.88
		15.484	5.890	5.210	20.74
		8.294	5.861	5.529	33.13
		9.558	5.368	3.870	34.10
Chi	**pH 4**	119.976	5.718	6.981	30.71
		167.259	6.799	7.063	25.27
		207.043	6.089	6.865	22.37
		194.177	6.397	8.241	17.64
		78.922	5.561	7.689	24.91
		110.609	6.872	7.801	25.89
	pH 5	14.773	6.699	5.953	41.03
		16.346	7.183	5.797	31.97
		23.403	5.529	6.143	26.33
		28.211	4.961	6.840	16.43
		12.023	5.524	7.123	15.39
		10.964	5.876	4.868	17.23
	pH 6	21.807	8.393	8.131	30.37
		130.466	6.164	7.220	19.11
		17.361	5.420	6.993	20.71
		32.392	6.023	6.517	15.89
		93.565	4.753	6.472	31.53
		11.927	4.951	6.465	29.17

Table I.2. : Skin absorption data of salicylic acid from oil powder *(Raw data of Chapter 3)*

		24h [µg/cm^2]	Rest skin [µg/cm^2]	SC [µg/cm^2]	El. Res. [kΩ]
Whey	**pH 6**	8.787	12.422	11.194	27.51
		13.681	12.718	7.949	21.40
		27.367	11.464	9.218	17.87
		41.161	10.350	9.965	21.22
		49.774	8.088	10.295	17.21
		54.142	8.584	12.112	29.05
Car	**pH 6**	5.989	12.167	10.504	25.45
		10.179	14.239	10.884	24.29
		22.113	10.249	10.733	20.41
		33.211	11.088	11.066	20.28
		38.780	9.682	10.292	31.78
		44.447	22.584	12.445	34.61
Chi	**pH 4**	6.411	9.404	12.556	31.14
		16.954	9.747	12.496	24.97
		33.667	9.893	12.608	23.69
		41.682	10.533	10.990	17.26
		46.825	10.380	13.276	26.58
		62.847	9.235	10.725	22.47
	pH 5	21.656	12.952	11.849	39.14
		50.489	13.386	11.832	32.01
		92.546	14.066	13.271	21.94
		132.890	11.118	5.537	17.57
		151.409	13.012	11.558	19.90
		176.649	14.378	13.067	18.41
	pH 6	1.968	14.854	14.392	28.50
		8.379	10.909	12.778	27.65
		9.790	9.593	12.377	26.00
		18.747	10.661	11.535	20.13
		20.801	8.412	11.454	19.37
		23.189	8.763	11.443	29.43