Emulsion Carrier Formulations for Natural Plant Extracts: Development and Stability Testing

Deveshnee Moodley

Student Number: 11007673

A dissertation submitted in partial fulfilment of the requirements for the degree Master of Chemical Engineering

Department of Chemical Engineering
Faculty of Engineering, Built Environment and Information Technology

University of Pretoria
Pretoria
South Africa

13 May 2019

Supervisor: Me Elizbé du Toit

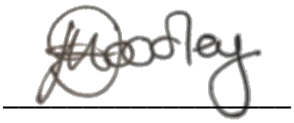
CVD 800

13 May 2019

DECLARATION

I, Deveshnee Moodley, hereby declare that this Master's dissertation is my own original work. I have not copied from any other student's work or from any other sources except where due reference or acknowledgement is made explicitly in the text, nor has any part been written for me by another person.

It is the intention that this Masters dissertation be submitted for the degree of Master of Engineering in Chemical Engineering at the University of Pretoria. Therefore, I further declare that this dissertation has not been submitted to any other educational institution doe any examination, diploma, degree or other form of qualification.



Deveshnee Moodley Date: 13 May 2019

PUBLICATIONS AND PRESENTATIONS

Some of the results presented in this dissertation have been presented at:

Moodley D, Rolfes H, and Lall N (13-14 September 2017). Emulsion Carrier Formulations for Natural Plant Extracts: Developmental and Stability Testing. The Cosmetic Chemistry 40th Annual Scientific Conference (Coschem), Midrand, South Africa.

And a portion of the findings were presented as posters at:

Potgieter L, Ramsawhook L, Moodley D and Rolfes H (14-15 September 2016). Development of Emulsion Formulations for Anti-Cancer Plant Extracts. The Cosmetic Chemistry 39th Annual Scientific Conference (Coschem), Midrand, South Africa.

Yssel J, Abdoola A and Rolfes H (2-3 September 2015). The Development of Emulsion Formulations and Stability Testing for an Anti-Cancer Active Plant Extract. The Cosmetic Chemistry 38th Annual Scientific Conference (Coschem), Midrand, South Africa.

Emulsion Carrier Formulations for Natural Plant Extracts: Development and Stability Testing

SYNOPSIS

South African-grown plants and their possible skin care benefits are increasingly gaining interest. For skin care applications, stable carrier formulations must be developed to incorporate extracts of these plants. Many cosmetic cream and sunscreen formulations are emulsions. The success of these formulations depends on their efficacy, stability and sensory characteristics.

The main objective of this investigation was to develop stable carrier formulations to incorporate four ethanolic plant extracts for topical skin applications. These plant extracts are referred to using the codes HO, BS, PM, and LSSJ. All four plant extracts were ethanol/water extracts and are therefore water soluble. In an emulsion formulation it is expected that the plant extract will report to the water phase. The Department of Plant and Soil Sciences from the University of Pretoria hypothesised and tested the activity of these plants and found strong evidence of anti-acne, anti-cancer and SPF boosting properties.

Development of the carrier formulations involved conducting sensory evaluations and preliminary stability testing on placebo formulations. The best performing formulations were selected and these formulations, in their placebo form and containing 10 % (by mass) of the respective plant extract, underwent long term stability testing. During this testing, formulations were stored under temperature conditions of 4 °C, 25 °C, and 40 °C. The stability of the formulations was tested using three techniques. These were (a)

coalescence analysis which involved determining the rates of coalescence and shelf-lives, (b) microscopy which allowed visual observation of the change in droplet size, and (c) cycle testing which involved exposing samples to two extreme temperatures and evaluating pH, droplet size and response to centrifugation.

Two lotion formulations were developed in this investigation and were referred to as "Formulation A" and "Formulation B". The sunscreen formulation developed was referred to as "Formulation C". Different carrier formulation types were considered including gels, body milks, creams and lotions. Formulation A - a light lotion formulation - showed excellent sensory appeal. It also showed satisfactory long-term stability in its placebo form and with the addition of the HO and LSSJ plant extract where the minimum shelf lives exceeded 1 year under normal storage conditions and 6 months under accelerated conditions.

During the development of Formulation B only locally produced ingredients were used in an attempt to reduce production cost. In the sensory evaluation, formulations were compared to a commercially available lotion. Preliminary stability evaluations of this formulation considered the effect of the plant extract dosage. The best performing formulation was selected by considering its sensory performance as well as its preliminary stability.

Formulation C was chosen based on its long-term stability. It was based on Formulation A and showed good long-term stability and resistance to harsh temperatures during cycle testing. A titanium dioxide dispersion was used as the UV filter in this sunscreen.

Formulation A was suggested for the addition of the HO plant extracts whereas Formulation B showed good stability in the presence of the LSSJ plant extract. The PM plant extract did not perform well in the lotion formulations. It was suggested that a gel

formulation should be considered for this plant extract. Formulation C showed good stability with the addition of the BS plant extract. SPF testing showed slight SPF boosting capability of the BS plant extract. Formulations developed in this study will be sent for efficacy testing. With the efficacy of the formulations containing the plant extracts quantified, these formulations can be introduced into the commercial marked for possible cosmetic and pharmaceutical applications.

Keywords: Ethanolic Plant Extracts, Herbal Cosmetics, Formulation, Stability, Emulsions, Coalescence

ACKNOWLEDGEMENTS

My sincere gratitude to everyone who contributed in any way to the project described in this dissertation.

Firstly, I would like to thank my academic supervisors, Heidi Rolfes for all her guidance, advice and support throughout this project, and Elizbé du Toit for all her patience, support, assistance and useful recommendations. I would also like to thank Danielle Twilley from the Department of Plant and Soil Sciences for her assistance and support, and Christien Gordon from Croda (SA) for her assistance and advice.

A sincere thanks to the final year students, Johan Yssel, Azraa Abdoola, Jessica Lund and Nomalungelo Maphanga who contributed to this project by means of their final year research projects.

I am further grateful to the Microscopy Lab at the University of Pretoria for allowing access to the optical microscope for purposes of this investigation and the National Research Foundation for funding this project.

Finally, a special thanks to my family, friends, and colleagues who continuously supported and encouraged me throughout this process.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

BM	Benchmark formulation
CF	Carrier formulation
CMC	Critical micelle concentration
HLB	Hydrophilic-lipophilic balance
L	Lotion formulation
MED	Minimal erythemal dose
MED_P	Minimal erythemal dose of protected skin
MED_U	Minimal erythemal dose of unprotected skin
O/W	Oil in water
QS	Quantum satis
SOP	Standard operating procedure
SPF	Sun protection factor
SPF_I	Individual sun protection factor
T	Trial formulation
TEWL	Transepidermal water loss
UV	Ultraviolet
UVA	Ultraviolet radiation of wavelength 320 nm – 400 nm
UVAPF	Ultraviolet-A protection factor
UVB	Ultraviolet radiation of wavelength 280 nm – 320 nm
UVC	Ultraviolet radiation of wavelength 100 nm – 280 nm
W/O	Water in oil
Z	Interfacial region mathematical plane

NOMENCLATURE

Roman Symbols

A	Interfacial area	m^2
b	Slope	
d_{32}	Sauter mean diameter	µm
d_{43}	Volume mean droplet diameter	µm
d_{50}	Volume median droplet diameter	µm
G	Surface free energy	J
G_{break}	Breakdown free energy	J
G_{coal}	Free energy of coalescence	J
G^{conf}	Configurational free energy	J
G_{flocc}	Free energy of flocculation	J
G^{form}	Free energy of formation	J
K_C	Apparent rate of coalescence	Day^{-1}
n	Number of moles	mol
N	Number concentration of droplets	m^{-3}
n	Sample size	
n_i	Number of droplets	
r^2	Coefficient of determination	
s	Sensory appeal score	
S	Entropy	J/K
S_{conf}	Configurational entropy	J/K
SE	Standard error	
S_x	Standard deviation of x	

S_y	Standard deviation of *y*	
t	Time	Days
T	Temperature	K

Greek Symbols

μ	Chemical potential	J/mol
α	Bulk phase 1	
β	Bulk phase 2	
γ	Interfacial tension	N/m
δ	Interfacial region thickness	m
ω	Weighting factor of sensory characteristics	

CHAPTER 1: INTRODUCTION

1.1 BACKGROUND

The skin is our largest organ with an average adult carrying 3.6 kg and 2.2 m^2 of it. It does more than just make us look presentable, it acts as a waterproof, insulating shield which guards the body against extremes of temperature, damaging sunlight, and harmful chemicals. Without it we would literally evaporate. Throughout history, great interest has been placed on purifying, beautifying and protecting the skin. Skin diseases account for roughly 34 % of all diseases encountered worldwide. They affect people of all ages and can become a major health burden (Lall & Kishore, 2014). Some of the most common skin diseases include acne and skin cancer.

Currently, there is a growing interest in the skin care benefits of South African-grown plants (Lall & Kishore, 2014). Many individuals still choose to use natural remedies and the global demand for the use of natural ingredients in cosmetic formulations increases continuously (Street & Prinsloo, 2013). Plant extracts may have a number of beneficial properties in skin care products. This may include photoprotection, antiaging, moisturising, antioxidant, astringent, anti-irritant, and antimicrobial activity, to name a few (Chanchal & Swarnlata, 2008).

Despite the known properties of plant extracts, few studies report the development of formulations with them (Ribeiro, Estanqueiro, Oliveira, & Lobo, 2015). Incorporating plant extracts into commercial products requires paying special attention to the extraction method, the interaction of the extract with the formulation and the efficacy of the commercial product.

Emulsions are widely used in a number of industries such as in food, paint, and pharmaceutical and cosmetic applications. Cosmetic cream formulations are emulsions and the success of these products depends on its efficacy, stability and sensory characteristics.

1.2 OBJECTIVES

The medicinal activities of four South African plant extracts were tested by the Department of Plant and Soil Science at the University of Pretoria. There was strong evidence that these plant extracts have anti-acne, anti-cancer or SPF boosting properties (Twilley et al., 2017; Twilley & Lall, 2015). The Department of Chemical Engineering at the University of Pretoria was subsequently approached in order to develop cosmetic products incorporating 10% (on a mass basis) of these plant extracts. The main objective of this investigation is therefore to develop stable carrier formulations for these four ethanolic plant extracts. In order to have potential for commercial application, all formulations must have good sensory appeal and show satisfactory long-term stability. A stable sunscreen formulation containing one of the plant extracts must specifically also be developed. This would allow for testing of the SPF boosting capabilities of one of the plant extracts. Due to IP restrictions, the names of the plant extracts cannot be disclosed at this stage and therefore the code names “HO”, “BS”, “PM” and “LSSJ” are used throughout this document. All four plant extracts were ethanol/water extracts and are therefore water soluble. In an emulsion formulation it is expected that the plant extract will report to the water phase.

1.3 METHOD

A number of placebo formulations (without the plant extracts) were developed and tested. Best case formulations were chosen on the basis of their performance in stability testing

and sensory evaluations. To these, the HO, PM and LSSJ ethanolic plant extracts were added and long-term stability testing was conducted to determine shelf-lives of the developed products.

Sunscreen formulations were developed based on the best-case formulations. Long-term stability testing was conducted on the sunscreen formulations with and without the BS plant extract. The best-case sunscreen formulation was selected. I*n vitro* and *in vivo* SPF testing was then done by the Photobiology Laboratory at Sefako Makgatho Health Sciences University to determine the SPF boosting capabilities of the BS plant extract.

1.4 APPLICATION OF RESEARCH

The formulations suggested in this study will advance the four ethanolic plant extracts into cosmetic applications. The present investigation aims to develop stable formulations with the plant extracts which will be sent for clinical trials to determine its efficacy in the treatment of skin ailments.

1.5 STRUCTURE OF DISSERTATION

This dissertation is structured according to the following chapters:

- Chapter 1: This chapter provides a brief introduction to this research project
- Chapter 2: A literature review which provides background on plant theory; on emulsions and emulsion stability; skin structure, skin diseases and skin care products, and sensory characteristics
- Chapter 3: An overview of the research methodology, experimental procedure and apparatus used in this investigation
- Chapter 4: The development of Formulation A by considering different types of carrier formulations, their sensory characteristics and their stability

- Chapter 5: The development of a cost-efficient lotion formulation by considering the use of various common cosmetic ingredients, sensory characteristics, stability and plant extract dosage sensitivity
- Chapter 6: The formulation of a stable sunscreen formulation by considering the most promising formulations of chapters 4 and 5 and the use of different UV filters
- Chapter 7: Conclusions and recommendations

CHAPTER 2: LITERATURE REVIEW

2.1 INTRODUCTION

A basic understanding of various aspects of the investigation was established. This literature review explores a number of topics including theoretical aspects of emulsions, skin properties, skin care products and sensory characteristics. This review assisted in making informed decisions regarding formulation development and the experimental approach. The choice of formulation ingredients, stability testing methods, and sensory evaluation techniques were chosen accordingly. A structured plan for implementation of the testing procedure was organised.

2.2 EMULSION THEORY

An emulsion is defined as a class of disperse systems consisting of two immiscible liquids (Binks, 1998: 1). The heterogeneous system consists of the first liquid, known as the dispersed phase, which is distributed as droplets of a microscopic or ultramicroscopic size throughout the second liquid referred to as the continuous phase (Tadros, 2013: 1). Since the two liquids are immiscible a third component, known as the emulsifier, is needed to disperse the two phases. The formation and the long term stability of an emulsion crucially depends on the chosen emulsifier (Binks, 1998: 2).

2.2.1 Emulsion Formation

Emulsions are metastable. This implies that once the ingredients are placed in contact with one another, an emulsion is not spontaneously formed. For this reason, intense shear is usually applied to the ingredients during the emulsion preparation process. There are a number of methods that are used in the preparation of emulsions. These techniques can be classified as laminar flow and turbulent flow methods (Leal-Calderon, 2012).

Laminar flow techniques are based on low shear rate application in highly viscous emulsions. Here the applied stress must be large enough to produce droplet elongation and break-up. At low shear rates, droplet fragmentation can occur as long as the viscosity of the emulsion is large enough (Leal-Calderon, 2012).

Turbulent flow techniques are based on mechanical devices which are used to generate turbulence and cavitation phenomena. This induces the disintegration of the dispersed phase and thereby leads to the formation of tiny droplets. Mechanical devices commonly used are high pressure homogenisers, microfluidisers and turbulent blade-type mixers (Leal-Calderon, 2012).

A turbulent blade type mixer was used in the preparation of the formulations developed in this investigation. This mixer is known as the Silverson® L4RT High Shear Mixer and is shown in Figure 2-1.

Figure 2-1: Image of the Silverson® L4RT High Shear Mixer

The Silverson® High Shear Mixer consists of two parts, the high shear rotor and the workhead. The workhead used in this investigation was the emulsor screen attachment which is most suitable for liquid/liquid dispersions and the preparation of all emulsions (Khan et al., 2011).

The mixing principle of the Silverson® comprises of four phases. These phases are illustrated in Figure 2-2. In Phase 1, shown in Figure 2-2(a), the high-speed rotation of the rotor blades exerts a powerful suction which draws both liquid and solid materials upward from the bottom of the vessel into the centre of the workhead which acts as a stator.

Centrifugal forces then drive materials towards the periphery of the workhead where they are subjected to a milling action between the ends to the rotor blades and the inner wall of the stator. Figure 2-2(b) shows Phase 2 which graphically represents this process.

Phase 3 involves inducing the materials to intense hydraulic shear. The materials are forced out through the perforations in the stator at a high velocity and circulated into the main body of the mix which is shown in Figure 2-2(c).

In Phase 4, shown in Figure 2-2(d), the materials expelled from the head are projected radially at high speed towards the sides of the mixing vessel. The mixing cycle is maintained where, at the same time, fresh material is continuously drawn into the workhead through Phase 1. A circulation pattern is set up due to the effect of the suction into the head and the radial expulsion of material, which minimises aeration caused by the disturbance of the liquid's surface (Silverson, 2017).

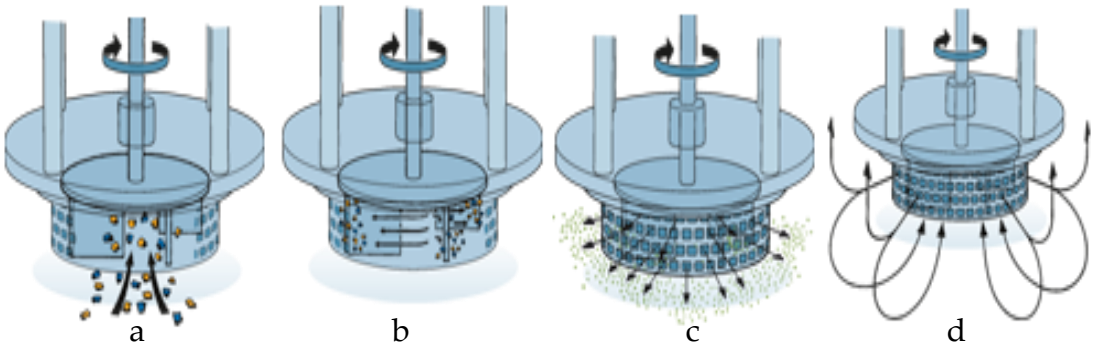


Figure 2-2: Diagram showing the four phase mixing principle used by the Silverson®

2.2.2 Surfactants

The main purpose of a surfactant is to lower the surface tension of a liquid, the interfacial tension between two liquids or the interfacial tension between a liquid and a solid. Surfactants can be used to stabilise emulsions as they act as emulsifiers. Some of the most effective emulsifiers in oil/water (O/W) or water/oil (W/O) emulsions are polymeric surfactants (Tadros, 2013: 1).

Surfactants, also known as surface active agents, are amphipathic molecules consisting of a non-polar hydrophobic, or lipophilic, hydrocarbon section attached to a polar or ionic hydrophilic section. These two sections are joined to form a head and tail structure consisting of both a water-soluble and water-insoluble, or oil-soluble, component as shown in Figure 2-3 below. This structure and the balance between the head and the tail gives systems special properties. The driving force for surfactant adsorption is reducing free energy of the phase boundary (Tadros, 2005: 1). In order to reduce the interfacial free energy, it is favourable for surfactant molecules to adsorb to air-liquid surfaces and liquid-liquid interfaces (Alvarez, 2011).

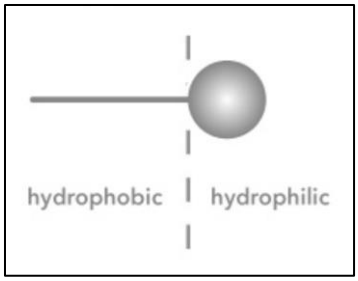


Figure 2-3: Structure of an amphipathic molecule

Classifications of surfactants are based on the nature of the hydrophilic group present in the surfactant. Three main classes are used namely anionic, cationic and amphoteric. Amphoteric surfactants are surfactants which contain both anionic and cationic characteristics. A fourth class of surfactants that is commonly used is polymeric surfactants. Polymeric surfactants are mainly used in the preparation of emulsions and suspensions and play a large role in their stabilisation (Tadros, 2013: 2). Each of the types of surfactants is discussed in greater detail in Section 2.6.8 on emulsifiers.

2.2.2.1 Micelles

An energetically favourable form for surfactant molecules is their association into micelles. Micelles are self-assembled aggregates that have a hydrophobic core and a hydrophilic shell, and are usually spherical (Alvarez, 2011). The driving force for micellisation is the reduction in contact between the hydrocarbon chain and water which consequentially reduces the free energy of the system (Tadros, 2005: 1). A schematic illustration of a spherical micelle is shown in Figure 2-4.

The surfactant concentration at which free monomers transition into micelles is called the c.m.c. or the critical micelle concentration. Each surfactant has a specific c.m.c. at a particular temperature and electrolyte concentration. Above the c.m.c., physical

properties of surfactants change abruptly. Figure 2-5 shows the change of some of these properties as a function of the concentration of the surfactant (Tadros, 2005: 20).

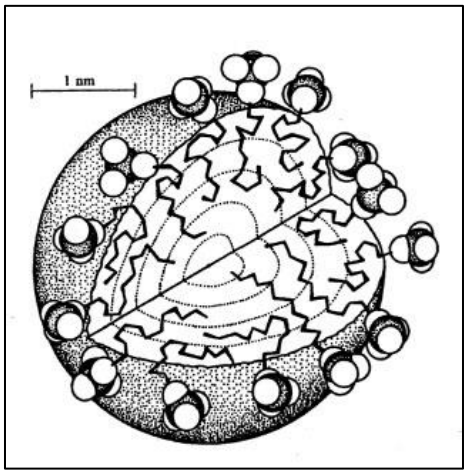


Figure 2-4: Spherical micelle with the hydrophobic tails directed towards the centre of the micelle away from the aqueous solution (Tadros, 2005: 20)

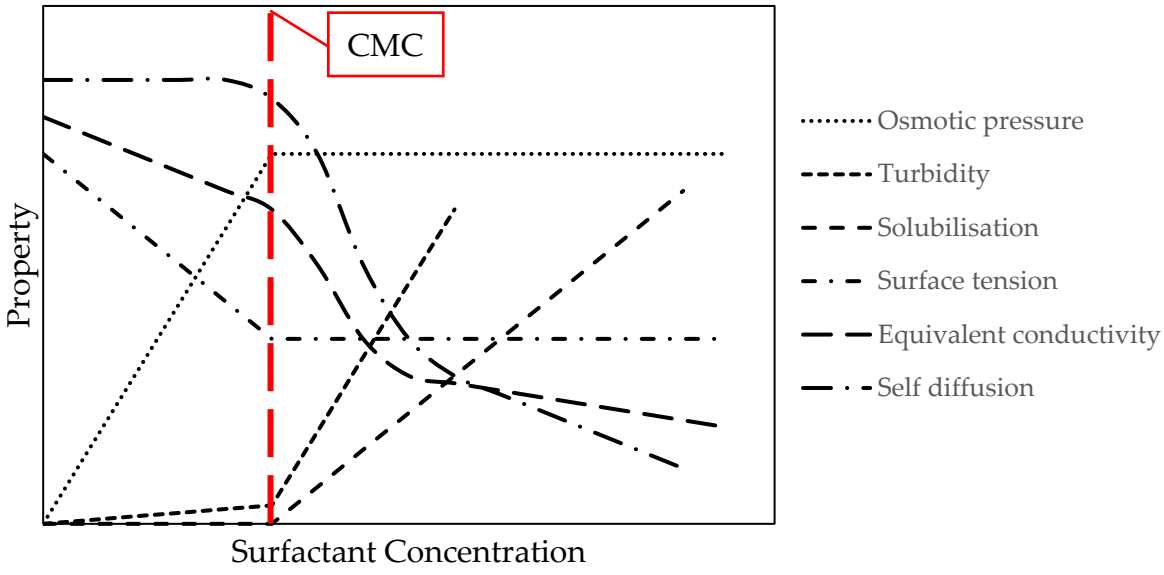


Figure 2-5: Concentration dependence of a number of physical properties of surfactants over the c.m.c. (Tadros, 2005: 19)

2.2.2.2 Hydrophilic-Lipophilic Balance Theory

When preparing O/W or W/O emulsions the selection of emulsifiers, or surfactants, is made on an empirical basis. The Hydrophilic-Lipophilic Balance, or HLB number is a semi-empirical scale used to select surfactants. The HLB scale is based on the relative measure of hydrophilic to lipophilic, or hydrophobic, groups that the surfactant molecule is made up of. There are a number of methods available for calculating the HLB value. One such method was developed purely empirically for non-ionic surfactants (Griffin, 1954). Although this method showed promise its simplicity showed shortfalls when dealing with ionic surfactants (Tadros, 2005: 136).

Another method was devised where the HLB number is calculated using the chemical structure of the surfactant. Here an empirically determined group number is given to individual molecular group taking into account the hydrophobicity of that specific molecular group within the structure of the surfactant (Davies, 1957). The HLB can then be determined using the empirical equation shown in Equation (2-1). The group numbers used to calculate HLB values are shown in Table 2-1.

$$HLB = 7 + \sum(\text{hydrophilic group No.}) + \sum(\text{lipophilic group No.}) \qquad (2\text{-}1)$$

Table 2-1: Group numbers of various hydrophilic, lipophilic and derived groups (Davies, 1957)

Group	Group number
Hydrophilic groups	
$-SO_4Na^+$	38.7
$-COOK^+$	21.1

Group	Group number
-$COONa^+$	19.1
N (tertiary amine)	9.4
Ester (sorbitan ring)	6.8
Ester (free)	2.4
-COOH	2.1
Hydroxyl (free)	1.9
$-O^-$	1.3
Hydroxyl (sorbitan ring)	0.5
Lipophilic groups	
-CH- or $-CH_2-$ or CH_3- or =CH-	-0.475
Derived groups	
$-(CH_2-CH_2-O)-$	+0.33
$-(CH_2-CH_2-CH_2-O)-$	-0.15

HLB values are given in Table 2-2 as a guide for selecting surfactants for specific applications. Insoluble surfactants are obtained when a surfactant's HLB value is lower than 6 due to the fact that it is no longer soluble in water (Alvarez, 2011).

Table 2-2: Classification of emulsifiers according to HLB values (Davies, 1957)

HLB range	Application
3-6	W/O emulsifier
7-9	Wetting agent
8-18	O/W emulsifier
13-15	Detergent

HLB range	Application
15-18	Solubilisation

2.2.3 Emulsion Instability

The stability of emulsions is a kinetic concept whereby a stable emulsion has no distinct change in the number of drops, spatial arrangement of the drops and the droplet size distribution within a chosen time period. This time period or experimental timescale can be anything from a few seconds to a few years which implies that emulsion stability is a relative concept (Binks, 1998: 13).

Long term stability of emulsions is of great interest as this determines the shelf life of any given emulsion product. A number of breakdown processes cause emulsion instability. These may be reversible or irreversible where irreversible separation requires reformulation. The main mechanisms of physical emulsion instability are shown in Figure 2-6. These breakdown processes tend to occur simultaneously rather than consecutively making analysis complicated (Tadros, 2013: 3). The factors affecting these breakdown processes must therefore be well understood in order to produce stable emulsions. Reversible breakdown processes of emulsions include creaming, sedimentation, and flocculation whereas irreversible processes comprise of Ostwald ripening, coalescence, and phase inversion. The principles of these processes are discussed further in the next sections.

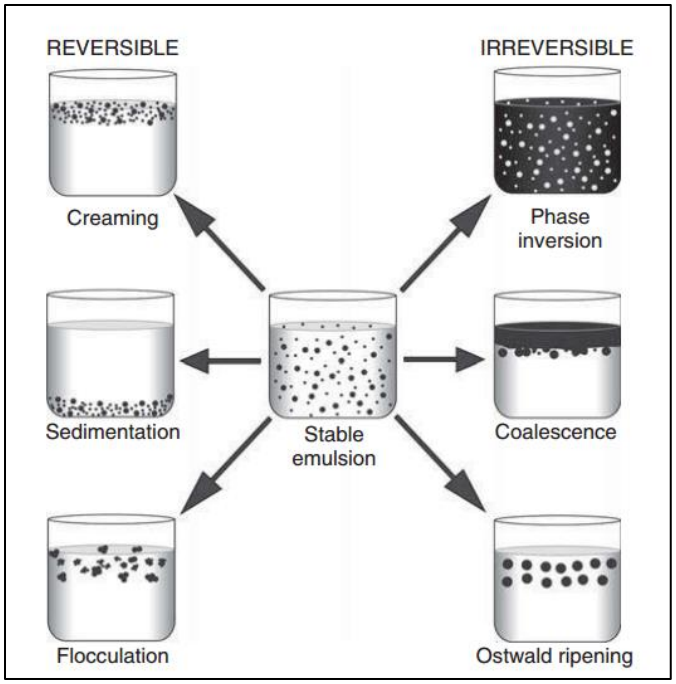


Figure 2-6: Reversible and irreversible emulsion breakdown processes shown schematically (Baki & Alexander, 2015: 178)

2.2.3.1 Creaming and Sedimentation

Creaming and sedimentation occur due to gravity or centrifugal forces, when there is a difference between the densities of the disperse phase and the continuous phase. Creaming occurs when the density of the droplets is lower than that of the continuous medium and sedimentation occurs when the opposite is true, therefore the density of the droplets is higher than that of the continuous medium (Tadros, 2013: 35). During this breakdown process a concentration gradient is built up within the system when the external forces surpass the thermal or Brownian motion of the droplets. This entails that the larger droplets move faster to the top of the container, when creaming occurs, or to the bottom of the container, when sedimentation occurs.

There are a number of procedures which may reduce or eliminate creaming and sedimentation. The first is matching the densities of the oil and aqueous phases. Due to

the fact that density is temperature dependant, matching densities may be possible, if at all, at one specific temperature. This implies that this method is impractical (Tadros, 2013: 37).

The second method is to reduce the droplet size. The required size of the droplets is dependent on the density difference between the oil and aqueous phases. Brownian diffusion within the system may exceed gravity should the droplets be reduced below a specific size. This will prevent creaming or sedimentation from occurring in the emulsion (Tadros, 2013: 37).

Another method of creaming and sedimentation prevention is the use of thickeners. Thickeners are natural or synthetic, high-molecular-weight polymers. They increase the viscosity of emulsions at low stresses or shear rates. Measuring the viscosity of an emulsion at very low stresses, using constant stress or creep measurements, can help predict creaming or sedimentation. At the residual shear rate or zero shear rate, the viscosity can reach very high values which inhibits creaming or sedimentation (Tadros, 2013: 38).

The fourth and fifth methods involves inducing controlled flocculation and depletion flocculation. This deals specifically with weak flocculation but this process has some downsides. The first disadvantage is the temperature dependence. More polymer is required to achieve the same effect at lower temperatures. This is due to the fact that as temperature is increased the hydrodynamic radius of the free polymer decreases due to dehydration. The second disadvantage is once the free polymer concentration is increased above a particular limit, phase separation may occur. Furthermore, the flocculated emulsion droplets may cream or sediment faster than in the absence of the free polymer (Tadros, 2013: 39).

2.2.3.2 Flocculation

Flocculation is a process where the individual emulsion droplets aggregate into larger units. This occurs without breaking the stabilising layer at the interface and without any change in the primary droplet size (Binks, 1998: 17). Flocculation occurs due to van der Waals attraction, which is general in all disperse systems. This van der Waals attraction is inversely proportional to the distance of separation between two droplets. Therefore, flocculation takes place when there is insufficient repulsion between droplets to keep them an appropriate distance apart from each other - thereby ensuring that the van der Waals attraction is weak. Depending on the magnitude of the droplet-droplet attractive energy in the system, weak or strong flocculation is possible (Tadros, 2013: 4).

There are two main methods by which flocculation can be reduced or eliminated. The first is to overcome the van der Waals attraction by charge-stabilising the emulsion. Electrostatic stabilisation is achieved by using ionic surfactants (Tadros, 2013: 43). This forms electrical double layers which introduce repulsive energy that helps droplets overcome the attractive energy. A disadvantage of this method is that the emulsion becomes flocculated at intermediate concentrations of electrolyte. Another more effective method to overcome flocculation is sterical stabilisation of the emulsion. This is done by using non-ionic surfactants or polymers (Tadros, 2013: 40).

2.2.3.3 Ostwald Ripening

Ostwald ripening, also referred to as disproportionation, occurs due to the difference in solubility between small and large droplets. It is believed that small droplets have higher solubility than larger droplets. Over time the molecules of the smaller droplets diffuse into the bulk and are deposited on larger droplets. Theoretically this should lead to the

condensation of all droplets into a single large drop. However, this does not occur in practice due to the fact that the growth rate of the drops decreases as the droplet size increases (Tadros, 2013: 44).

There are many ways to reduce Oswald ripening. Firstly, an extra disperse phase component, which is insoluble in the continuous medium, can be added to the system. In this method a partition between droplets of different sizes is formed. The low solubility component is concentrated in the smaller droplets and further growth of droplets is reduced (Kabalnov & Shchukin, 1992). Another method of reducing Oswald ripening is modifying the interfacial film at the O/W interface. The Oswald ripening rate can be reduced significantly by using surfactants that are strongly adsorbed at the O/W interface and that are insoluble in the continuous phase, which will prevent the surfactant from desorbing during ripening (Kabalnov, 1994).

2.2.3.4 Coalescence

Coalescence is an irreversible process where large drops are formed by two or more emulsion droplets that fuse together. The process occurs due to the thinning and disruption of the liquid film which eventually ruptures when droplets come close enough to each other. Coalescence is driven by surface or film fluctuations which cause droplets to approach each other, thereby increasing their van der Waals forces, preventing separation. It can occur when droplets come in close contact to one another during flocculation, creaming or Brownian diffusion (Tadros, 2013: 45).

The prevention of coalescence can be achieved using two mechanisms. Firstly, by increasing the electrostatic and steric repulsion and secondly, dampening the fluctuation by enhancing the Gibbs elasticity (Tadros, 2013: 46). This is achieved by increasing the

viscosity of the continuous phase. Generally, smaller droplets are less vulnerable to surface fluctuations and therefore coalescence is reduced. These two mechanisms are achieved by two main methods. The first of which is using mixed surfactant films. The second is forming lamellar liquid crystalline phases at the O/W interface. This method produces a multilayer structure that cover the droplets, thereby greatly reducing the van der Waals energy available in the system (Friberg, Jansson, & Cederberg, 1976).

In order to calculate the rate of coalescence in emulsions, a relationship between droplet size and time is considered. The rate of coalescence usually follows first-order kinetics as shown in Equation (2-2)

$$\frac{N_t}{N_0} = e^{-K_c t} \tag{2-2}$$

where N_t is the number concentration of droplets at time t, N_0 is the number concentration of droplets at time zero, and K_C is a rate constant.

The mean droplet diameter, namely the Sauter mean diameter d_{32} and the volume mean diameter d_{43}, are defined by Equation (2-3) (Dickinson, Miller, & Akhtar, 2001).

$$d_{mn} = \frac{\sum_i n_i d_i^m}{\sum_i n_i d_i^n} \tag{2-3}$$

where n_i is the number of droplets of diameter d_i, and d_{mn} represents d_{32} or d_{43}.

The relationship between the N and the mean average droplet diameter, d_{mn} is shown in Equation (2-4). This only holds true if the volume of emulsion droplets remains constant and therefore no oiling-off occurs (Das & Chattoraj, 1982).

$$\frac{4}{3}\pi\left(\frac{d_{mn}}{2}\right)^3 N = \text{constant} \tag{2-4}$$

Using Equation (2-4) at the initial and final time, the relative number of emulsion droplets can be obtained using Equation (2-5).

$$\frac{N_t}{N_0} = \left(\frac{\left(d_{mn}\right)_{t=0}}{\left(d_{mn}\right)_{t=t}}\right)^3 \tag{2-5}$$

By substituting Equation (2-5) into Equation (2-2) and taking the natural logarithm on both sides, Equation (2-6) is obtained. This equation provides a straight-line relationship between the time and the mean droplet diameter. The slope of the graph gives an indication of the apparent rate of coalescence (Ye, Hemar, & Singh, 2004).

$$\ln\left(d_{mn}\right)_{t=t} = \frac{K_c t}{3} + \ln\left(d_{mn}\right)_{t=0} \tag{2-6}$$

2.2.3.5 Phase Inversion

When phase inversion occurs, the disperse phase and the continuous phase interchange. This implies that over time, or with a change in conditions, an O/W emulsion will invert to a W/O emulsion. There are two types of phase inversion namely transitional inversion and catastrophic inversion. Transitional inversion is induced by altering features that affect the HLB of the system. Examples of these features include temperature and

electrolyte concentration. Catastrophic inversion, on the other hand, is induced by increasing the volume fraction of the disperse phase (Tadros, 2013: 48).

2.2.4 Emulsion Thermodynamics

Two components are completely compatible if they do not form an interface upon mixing. The exact opposite is true if two components are incompatible. In this case an interface is formed between the two bulk phases α and β, which may be liquid and air or two immiscible liquids, such as oil and water in the case of emulsions. When the two bulk phases come into contact with each other the interfacial regions of these phases undergo changes resulting in an associated change in internal energy. Deviations in the composition, density, and structure of phases α and β can be observed within this interfacial region. The interfacial region has a thickness δ which depends on the nature of the interface amongst other factors. Gibbs assumed that the two phases α and β have uniform thermodynamic properties up to the interfacial region. To define the interfacial tension γ, he assumed a mathematical plane Z^{σ} in the interfacial region. A schematic representation of the interfacial region as well as the Gibbs mathematical plane is shown in Figure 2-7.

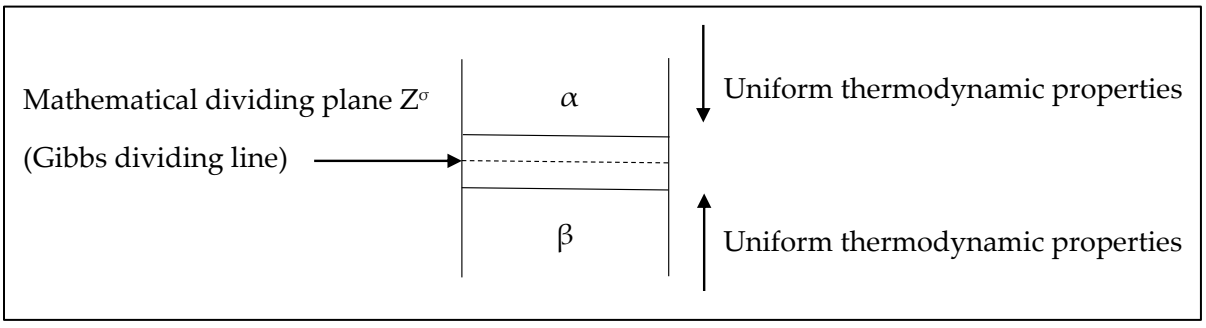


Figure 2-7: Schematic of the Gibbs dividing line (Tadros, 2013)

A definition of the interfacial tension γ is obtained using Gibbs' model. The Gibbs-Deuhem equation is given in Equation (2-7).

$$dG^{\sigma} = -S^{\sigma}dT + Ad\gamma + \sum n_i d\mu_i \tag{2-7}$$

Here dG^{σ} is the surface free energy and is made up of three components namely an entropy term $(S^{\sigma}dT)$ where S is entropy and T is temperature, an interfacial energy term $(A\ d\gamma)$ where A is interfacial area and γ is interfacial tension, and a composition term $(\Sigma n_i d\mu_i)$ where n_i is the number of moles of component i with chemical potential μ_i. At constant temperature and composition Equation (2-7) translates to Equation (2-8) and then Equation (2-9).

$$dG^{\sigma} = Ad\gamma \tag{2-8}$$

$$\gamma = \left(\frac{\partial G^{\sigma}}{\partial A}\right)_{T,n_i} \tag{2-9}$$

In Equation (2-9), γ is positive for a stable interface. This implies that if the interfacial area increases so will the surface free energy G^{σ}.

A schematic representation of emulsion formation and breakdown is shown in Figure 2-8. State I shows a system of an oil (represented as a large drop 2 of area A_1) immersed in liquid 1. The large drop is then segmented into a number of smaller droplets in state II and these droplets have a total area of A_2. Understandably A_2 is much greater than A_1 and the interfacial tension γ_{12} is the same for large and small droplets.

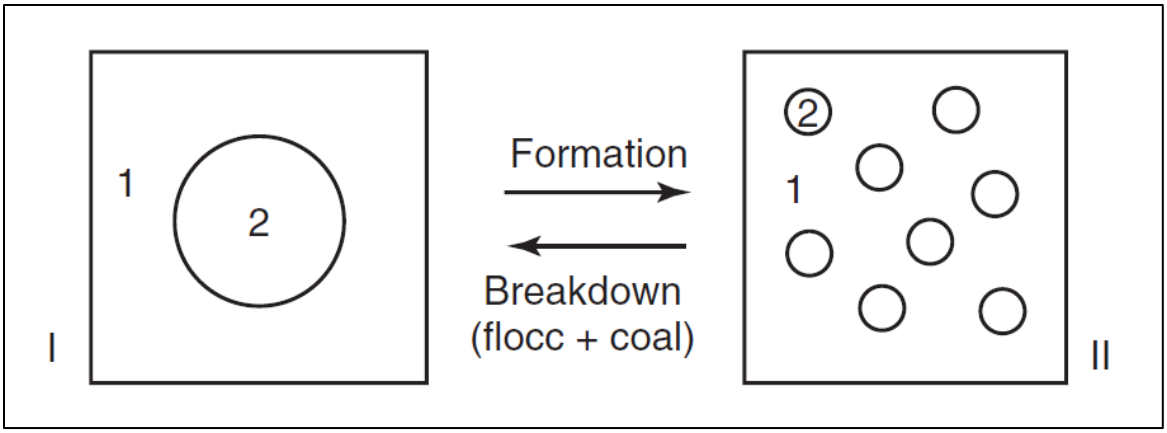


Figure 2-8: Schematic representation of emulsion formation and breakdown (Tadros, 2013)

The second law of thermodynamics is shown in Equation (2-10).

$$\Delta G^{form} = \Delta A\gamma_{12} - T\Delta S^{conf} \tag{2-10}$$

From this, it can be seen that the change in free energy when transitioning from state I to state II is made up of two contributing factors. The first is a positive surface energy term ($\Delta A\gamma_{12}$ where $\Delta A = A_2 - A_1$). Since producing a large number of droplets is accompanied by an increase in configurational entropy, the second factor is a positive entropy of dispersions term ($T\Delta S^{conf}$).

In most cases $\Delta A\gamma_{12}$ is much greater than $T\Delta S^{conf}$ which means that ΔG^{conf} is positive. This implies that the formation of an emulsion is nonspontaneous and the system is thermodynamically unstable. Three free energy paths in emulsion breakdown are shown in Figure 2-9. The emulsion will break by flocculation and coalescence in the absence of any stabilisation mechanism. This is represented by the solid line where there are no free energy barriers for either flocculation or coalescence.

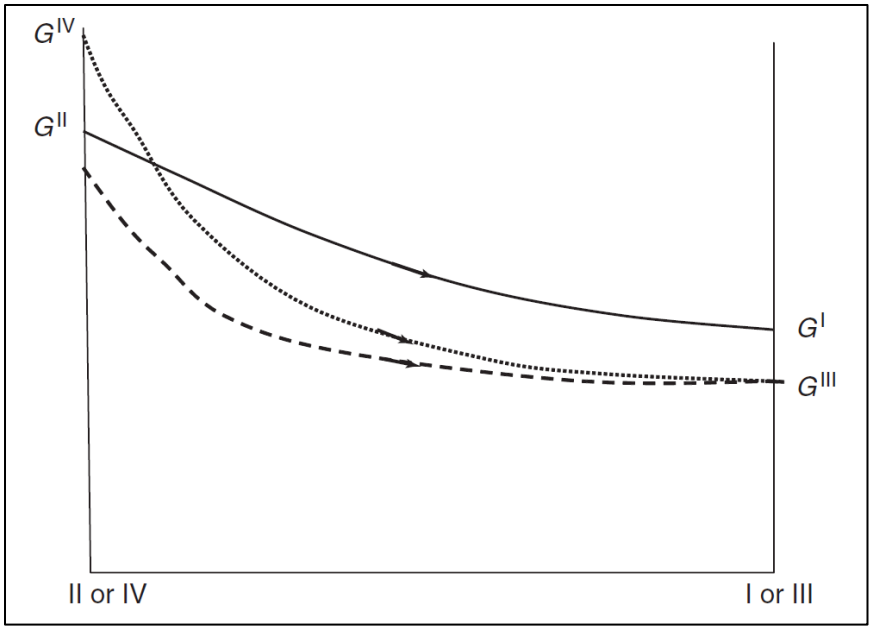


Figure 2-9: Free energy path in emulsion breakdown (Tadros, 2013).

Diffusion controls the kinetics of both breakdown processes. Flocculation is controlled by the diffusion of the droplets and coalescence is controlled by the diffusion of molecules of liquid 1 out of the thin liquid film formed between two contacting droplets of liquid 2. The dashed line in Figure 2-9 represents the case where sedimentation and creaming are superimposed upon flocculation and coalescence. The final state of the system is shown by state III. The dotted line represents the situation if Ostwald ripening is taken into account an addition to the above effects of flocculation, coalescence, creaming and sedimentation. This occurs if the initial state IV is polydisperse and the liquids have a finite mutual solubility (Tadros, 2013).

An energy barrier is created between the droplets in the presence of a stabiliser (surfactant and/or polymer). The reversal from state II to state I becomes noncontinuous as a result of the presence of these energy barriers. This is shown in Figure 2-10. The system becomes kinetically stable in the presence of the above energy barriers where ΔG_{flocc} and ΔG_{coal} are activation free energies. The intermediate state V is a metastable state

that represents a flocculated emulsion that has undergone no coalescence. It may stay in this state indefinitely if ΔG_{coal} is sufficiently high (Tadros, 2013).

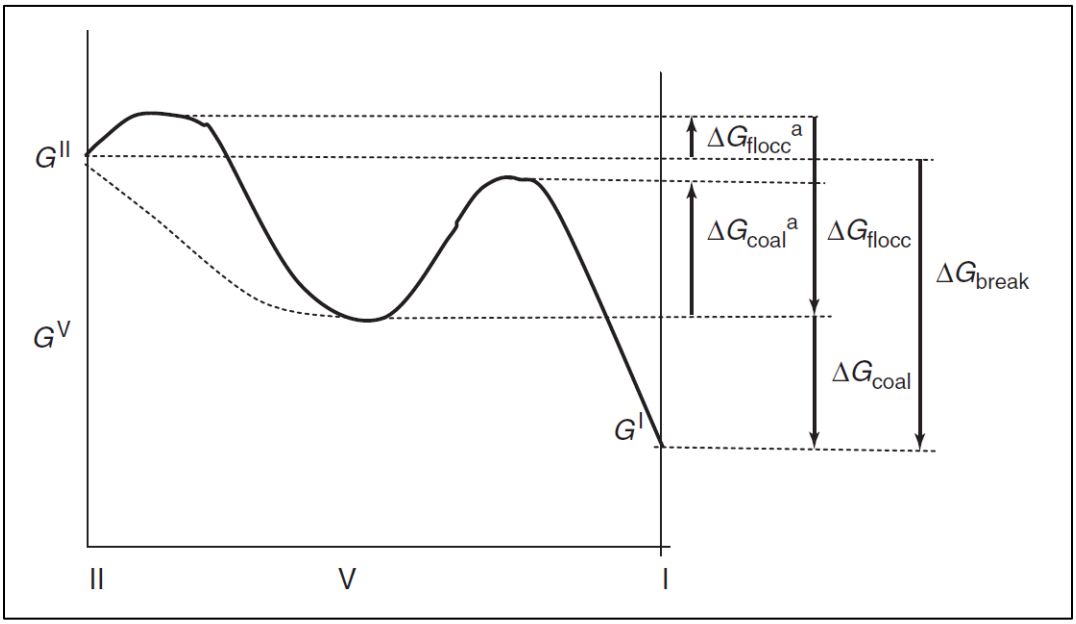


Figure 2-10: Schematic representation of free energy path for breakdown flocculation and coalescence for systems containing an energy barrier (Tadros, 2013)

State II is also an unstable state. If ΔG_{flocc} is sufficiently high, the stable, dispersed state may persist indefinitely. States II and V in these cases represent states of kinetic stability rather than true thermodynamic stability. The dashed curve in Figure 2-10 represents the situation when there is no free energy barrier to flocculation, but there is a large barrier to coalescence. This situation would arise for droplets stabilised by an adsorbed neutral polymer. If ΔG_{flocc} is not too large, the flocculation is reversible and an equilibrium is set up.

It can be seen from Figure 2-10 that,

$$\Delta G_{break} = \Delta G_{flocc} + \Delta G_{coal} \tag{2-11}$$

The excess free energy G^{σ} associated with the presence of an interface is given by Equation (2-12).

$$G^{\sigma} = \Delta A\gamma_2 + \sum_i \mu_i n_i^{\sigma} \tag{2-12}$$

If an interface disappears due to coalescence the change in free energy of coalescence, ΔG_{coal}, is simply given by Equation (2-13). The term $\Sigma_i \mu_i n^{\sigma}{}_i$ disappears since the chemical potential is the same in either bulk phase and in the interface.

$$\Delta G_{coal} = -\Delta\left(\gamma_{12}\Delta A\right) \tag{2-13}$$

Since

$$\Delta\left(\Delta A\gamma_{12}\right) = \gamma_{12}\Delta\Delta A + \Delta A\Delta\gamma_{12} \tag{2-14}$$

it can be concluded that

$$\Delta G_{flocc} = \Delta A\Delta\gamma_{12} - T\Delta S^{conf} \tag{2-15}$$

The free energy of flocculation, ΔG_{flocc}, is made up of two terms. The first is $\Delta A\Delta\gamma_{12}$ and it is associated with the change in interfacial tension in the contact region of two droplets and the second is $T\Delta S^{conf}$ which is associated with the change in configurational entropy. Both these terms are negative. Furthermore, in most cases the $\Delta A\Delta\gamma_{12}$ term dominates so that ΔG_{flocc} is negative which implies that flocculation is thermodynamically spontaneous. If $\Delta A\Delta\gamma_{12}$ is less than $T\Delta S^{conf}$, ΔG_{flocc} is positive and the emulsion is thermodynamically stable against flocculation. This means that flocculation will not occur and the emulsion

has to be concentrated by creaming/sedimentation or centrifugation before coalescence can occur. This may be realised if $\Delta\gamma_{12}$ is small (Tadros, 2013).

2.3 EMULSION STABILITY TESTING

A fundamental component of the developmental process of an emulsion is stability testing. When formulating an emulsion, one of the main considerations is to understand the effects of storage and shipping conditions on the shelf-life of the product. Some of the major concerns involve temperature extremes, sunlight exposure, humidity and vibration. For this reason, the formulations are stored under regulated testing conditions and are vigilantly analysed at fixed intervals (Particle Sciences, 2011). Some general testing conditions that have been used historically and that are still regularly used to evaluate the stability of emulsions are shown in Table 2-3.

Freeze-thaw is regularly used as an accelerated approach to test emulsion stability (Madaan, Chanana, Kataria, & Bilandi, 2014). In this experiment, cycling the temperature influences parameters apart from those that are directly associated with the mechanism of instability. Therefore, it is important to note that this method should not be used exclusively (Particle Sciences, 2011).

Table 2-3: Commonly used testing conditions for emulsion stability testing (Particle Sciences, 2011)

Storage Conditions	**Storage Period**
Ambient temperature	25 °C for 3 years (or projected shelf-life of the product)
Elevated temperature	37 °C for 6 months and 45 °C for 6 months
Refrigerator	Approximately 4 °C for 3 months

Storage Conditions	Storage Period
Freeze/thaw cycles	Approximately -10 °C to ambient for 5 cycles
Cycling chamber	4 °C to 45 °C in 48 hours for 1 month
Light exposure	1 month exposure to north-facing daylight or light cabinet

When evaluating emulsions, sample batches of the formulation are usually stored in glass jars. Glass jars allow for easy observation and frequent measurement of physical characteristics. Ideally the emulsion product should also be assessed in its intended final packaging. This is due to the fact that aspects such as permeation, interfacial wetting behaviour, and leaching of components into or out of the packaging material, may have an influence on the stability of the product (Particle Sciences, 2011).

During stability testing a number of properties are usually monitored. A list of these properties, as well as references to previous studies involving these methods, are shown in Table 2-4. The number of properties is quite extensive and all of the instrumentation required to conduct these tests are not always readily available. For this reason, it is not necessary or practical to conduct all of the tests on all preliminary formulations. The testing done on the initial formulations usually include pH, viscosity, flow behaviour, odour, and physical separation at elevated temperatures (Particle Sciences, 2011). Optimally however, as many of these tests as possible should be conducted on the final formulation.

Droplet size analysis is a reliable and robust technique to determine emulsion stability and was the main technique used in this investigation. The main apparatus used to obtain droplet size distributions was the Malvern Mastersizer 3000™ particle size analyser. The Mastersizer 3000™ uses laser diffraction to measure the size of particles in the range of

10 nm and 3500 µm. This machine consists of a main analysis unit and a range of dispersion units shown in Figure 2-11.

Figure 2-11: Image of the Mastersizer 3000™ analysis and dispersion units

The operating principle of the Mastersizer 3000™ is based on a laser beam that is passed through a dispersed particulate sample. In a single laser diffraction measurement, the angular variation in the intensity of the scattered light is measured (Malvern, 2017). This is achieved using lasers and detectors as can be seen in an illustration of the internal operation of the analysis unit in Figure 2-12.

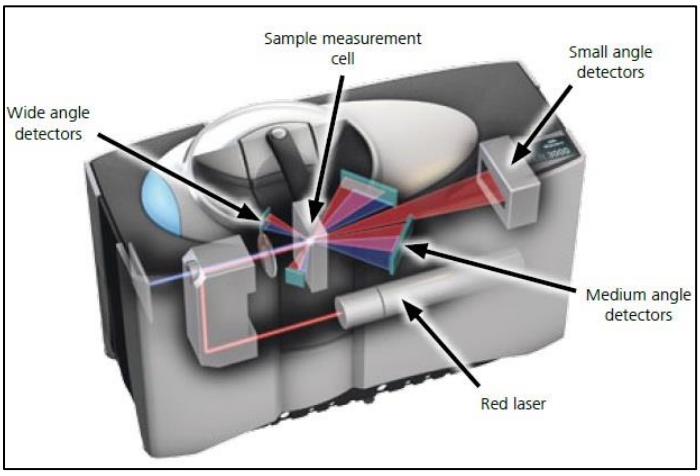


Figure 2-12: Internal operating principle of the Mastersizer 3000™

Small particles scatter light at large angles and large particles scatter light at small angles relative to the laser beam. This is shown graphically in Figure 2-13. Using the Mie theory of light scattering, the angular scattering intensity data is analysed to calculate the size of the particles that created the scattering pattern. The particle size is then reported as a volume equivalent sphere diameter (Malvern, 2017).

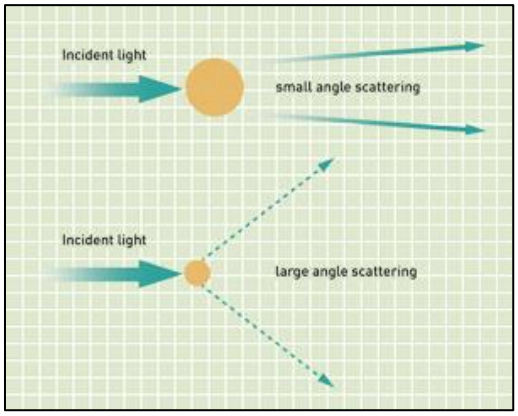


Figure 2-13: Graphic representation of light scattering behaviour of particles

Table 2-4: Properties of emulsions to be monitored during stability testing

Property	**Test Method**	**References**
Colour	Visual or colorimeter	(Particle Sciences, 2011); (Khan et al., 2011); (Khan, Akhtar, Khan, & Braga, 2013)
Conductivity	Conductivity meter	(Alvarado, Wang, & Moradi, 2011); (Henríquez, 2009); (Mahmood & Akhtar, 2013); (Waqas et al., 2016); (Khan et al., 2011); (Khan et al., 2013)
Droplet size distribution	Microscopy and instrumental analysis	(Sobisch & Urbansky, 2014); (Alvarado et al., 2011); (Henríquez, 2009); (André et al., 2003) ; (Bendjaballah, Canselier, & Oumeddour, 2010); (Bjerregaard, Vermehren, Söderberg, & Frokjaer, 2001); (Opedal, Sørland, & Sjöblom, 2009); (Castel, Rubiolo, & Carrara, 2017); (Katepalli, 2014); (Pichot, 2010); (Mahmood & Akhtar, 2013); (Khan et al., 2011)
Flow behaviour	Oscillatory shear viscosity with a rheometer	(Castel et al., 2017); (Katepalli, 2014); (Zografi, 1982); (Mahmood & Akhtar, 2013); (Waqas et al., 2016);
Odour	Organoleptic	(Ragnarsson & Labuza, 1977); (Khan et al., 2013)

Property	Test Method	References
pH	pH meter	(Pichot, 2010); (Mahmood & Akhtar, 2013); (Waqas et al., 2016); (Khan et al., 2013)
Separation	Visual or instrumental creaming value	(Alvarado et al., 2011); (Bjerregaard et al., 2001); (Castel et al., 2017)
Specific gravity	Pycnometer	(Pichot, 2010)
Tack or texture	Extensional and compressional deformation	(Particle Sciences, 2011)
Vibration	Shipping test or shaker table	(Particle Sciences, 2011)
Viscosity	Rotational viscometer	(Henríquez, 2009); (Bendjaballah et al., 2010); (Khan et al., 2011)

2.4 BIOACTIVITY OF PLANT EXTRACTS

For centuries people have been using natural ingredients, such as herbs, roots, flowers and essential oils, for skin care purposes. Today the use of these natural ingredients is especially popular as the search for natural remedies for skin care progresses. It has been discovered that medicinal plants play a major role in the treatment of a number of skin disorders and may have a significant contribution towards the health care of skin (Lall & Kishore, 2014). Phytochemicals from botanicals have many advantageous effects. Botanical ingredients positively influence the biological functions of the skin by providing nutrients for healthy skin. In general, botanical products contain vitamins, antioxidants, essential oils, proteins and many other bioactive molecules (Lall & Kishore, 2014).

In this section the bioactivity of the plant extracts considered in this study are discussed. These extracts are referred to using the code names BS, HO, PM and LSSJ. It is important to note that all work relating to the identification of the plants, bioactivity testing and preparation of the ethanolic plant extract was done by the Department of Plant and Soil Sciences at the University of Pretoria

In the attempt to screen the various plant extracts for their viability in the treatment of a number of skin diseases, various activities were tested. The main applications considered in this study were anti-acne, anti-cancer and SPF boosting capabilities. The current in-vitro activity testing results obtained by the Department of Plant and Soil Science on the plant extracts are shown in Table 2-5. Here the IC_{50} refers to the half maximal inhibitory concentration which represents the concentration required to inhibit a biological process by 50 %. It is measured through dilution assays where the plant extract is added to virus infected host cells. The plant extract concentration obtained is in units of μg/ml which

refers to the mass of dried plant extract powder per volume of medium or assay diluent which in this case is the ethanol/water mixture. The properties usually associated with plant extracts used in the skin care industry include cytotoxicity, antioxidant, anti-tyrosinase, anti-acne and anti-inflammatory activities. Each of these properties have chemical characteristics associated with them. For example, antioxidant bioactivity considers the absorbance of DPPH, and anti-acne considers the activity of the P.acnes bacteria.

Antioxidants are compounds which provide control over free radical formation because they are radical scavengers. They inhibit various oxidising chain reactions thereby protecting the human body against free radicals (Lall & Kishore, 2014). Melanin is a pigment that is responsible for the colour of the skin, eyes, and hair. It is produced and secreted by cells known as melanocytes through a physiological process known as melanogenesis. An essential role of melanin is to protect the skin against UV light. This is achieved by absorbing the UV light and eliminating reactive oxygen species. The key enzyme in the biosynthesis of melanin is tyrosinase. The over-activity of tyrosinase leads to the overproduction of melanin. Tyrosinase inhibitors are used to depigment the skin and treat hyperpigmentation disorders of the skin such as those occurring due to acne and eczema (Vardhan, Khan, & Pandey, 2014). A number of tyrosinase inhibitors are obtained from plants. These plants are traditionally used to treat a variety of skin problems including spot removal and skin lightening (Lall & Kishore, 2014).

Table 2-5: Results of IC_{50} plant extract values obtained through *in vitro* studies of the plant activities (Twilley et al., 2017; Twilley & Lall, 2015)

Property	**BS**	**HO**	**PM**	**LSSJ**
Application	Anti-cancer / SPF boosting	Anti-acne	Anti-acne	Anti-acne
Anti-acne activity (µg/ml)		7.8	7.8	7.8
Cytotoxicity results (µg/ml)				
A375 (non-pigmented melanoma)	66			
A431 (squamous cell carcinoma)	34.4			
B16F10 cells (mouse melanocytes)		>100	>100	>100
HaCat (human keratinocytes)	58.65	>100	>100	>100
HEK-293 (human embryonic kidney)	72.1			
HeLa (cervical cancer)	63.6			
UCT-MEL-1 (pigmented melanoma cells)	31.32			
Antioxidant activity (µg/ml)				
DPPH	18.6	± 50	± 50	± 50
NO		± 200	± 200	± 200
Anti-tyrosinase activity (µg/ml)		± 30	± 30	± 30

2.5 SKIN THEORY

The cosmetic creams considered in this project deals with skin, skin care and skin diseases. It is therefore beneficial to gain a base understanding of the skin and its structure and function.

2.5.1 Skin Anatomy

The skin is the outer covering and protective envelope of the human body. It is the largest sensory and contact organ of the body constituting approximately 16 % of the body's weight and covering a surface area of between 1.5 and 2 m^2 in adults (Derler & Gerhardt, 2012). Human skin comprises of two main layers. The surface layer of epithelium is known as the epidermis and the underlying layer of connective tissue is known as the dermis. A third layer of loose connective tissue exists beneath the dermis known as the hypodermis. This layer is known as the subcutaneous layer and is comprised mainly of fat cells, therefore it is not considered a component of the skin. Its main purpose is to protect vital organs from trauma and against cold. The basic anatomical structure of human skin is shown in Figure 2-14. The skin is a complex organ made up of dead cells, epithelium, connective tissue, muscles, nerves, blood vessels, and appendages. These appendages of the skin include hair, nails, and glands such as sweat glands and sebaceous glands (Krause, 2005: 149).

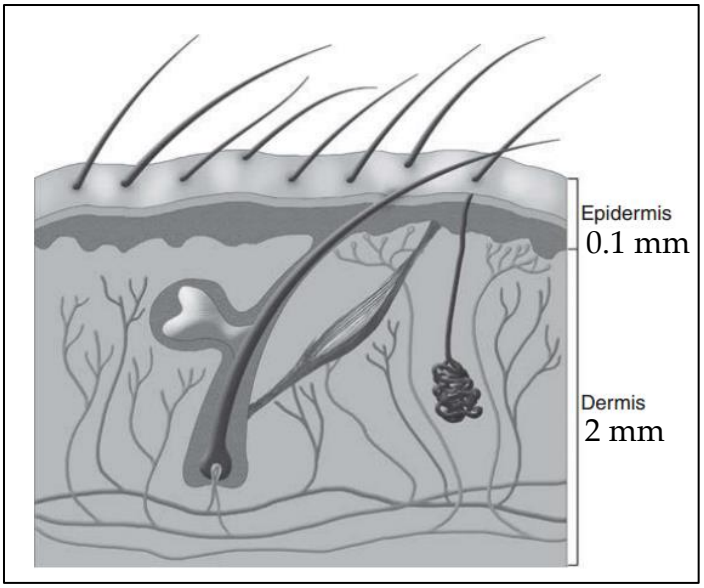


Figure 2-14: Schematic of the basic structure of human skin (Baki & Alexander, 2015: 128)

2.5.1.1 Epidermis

The epidermis is the outer layer of the skin and serves as a protective layer against external influences such as the invasion of micro-organisms and toxic agents (Harding et al., 2003). It is completely dependent on the dermis. This is due to there being no blood vessels present in the epidermis and therefore nutrients are delivered exclusively by the underlying dermis. The strength of the skin is attributable to the abundance of keratin in the epidermis. The basic functions of the epidermis include restricting water loss through the skin and sustaining an optimal water content for the skin, maintaining optimal lipid content, supplying immune protection, functioning as an antioxidant barrier against reactive oxygen species, producing vitamin D, providing photoprotection and skin colour, and allowing stratum corneum cells to desquamate (Baki & Alexander, 2015: 132). The structure of the epidermis is made up of five main layers which is shown in Figure 2-15.

The outermost layer of the epidermis is the stratum corneum which is known as the horny layer. It is made up of fifteen to thirty layers of scale-like dead cells that are continuously shed and replaced by cells in the adjoining layer (Baki & Alexander, 2015: 128). The stratum corneum has a distinctive structure known as the "brick and mortar" structure where dead cells, which are known as corneocytes, represent the bricks and intercellular lamellar lipids represent the mortar (Nemes & Steinert, 1999). This lipid matrix is responsible for limiting the loss of water in the epidermis. It therefore inhibits dehydration and provides the required moisture permeability to the stratum corneum.

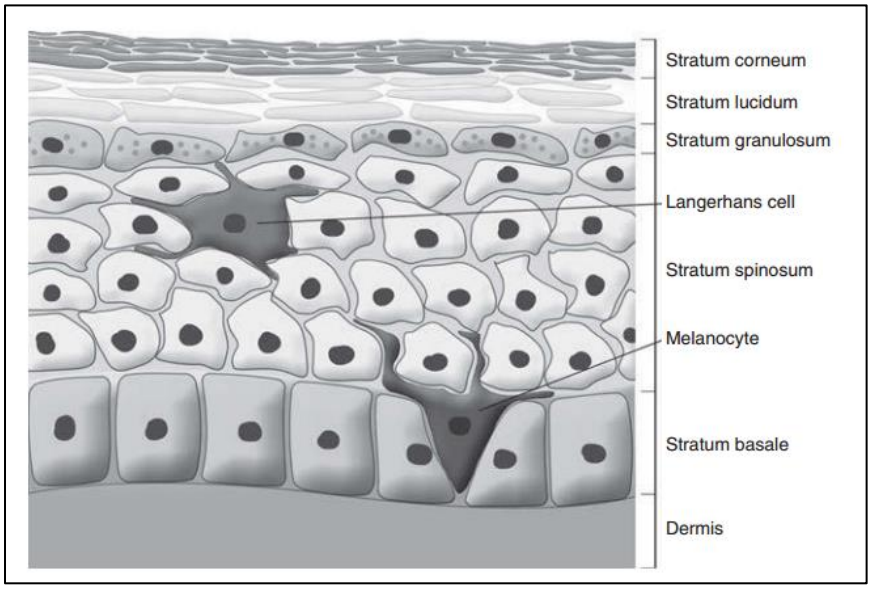


Figure 2-15: Schematic of the layers of the epidermis (Baki & Alexander, 2015: 129)

The thickness of the epidermis differs in thick and thin skin. Thick skin, such as that on the palms of the hands and the soles of the feet, consists of all five layers whereas thin skin, which covers the rest of the body, has a thinner stratum corneum and does not contain a stratum lucidum (Krause, 2005: 150). The stratum corneum is substantially thicker in thick skin than in thin skin. It consists of more than three hundred layers of cells in thick skin whereas it only contains fifteen layers in thin skin.

2.5.1.2 Dermis

The dermis serves as a supporting frame to the epidermis as it is situated beneath the epidermis. It provides nutrients and oxygen to the epidermis by means of blood capillaries. The thickness of the dermis differs between regions of the body and is known to be thicker in men than in women (Krause, 2005: 153). The structure of the dermis is described as an amorphous substance. This substance acts as a mortar for all the components of the dermis. These include elements such as blood vessels, fibroblasts, nerves and sensory organs, sebaceous and sweat glands, hair follicles, and connective tissue containing collagen and elastin fibres.

Sensory signals are transmitted from the skin by means of nerve endings. These sensory signals include touch, pain, pressure, and temperature. Blood vessels and sweat glands play an important role in regulating body temperature. A cooling effect is achieved, and body temperature is reduced, as sweat evaporates from the skin.

2.5.2 Skin Moisture Content

The water content of the skin is of great significance when determining properties of the skin. The skin, dermis and epidermis combined, has an approximate water content of 80 %. The water content of the stratum corneum is much lower, between 10 % and 30 % (Baki & Alexander, 2015: 133). At normal water content, the skin appears soft, smooth, and glowing. When the water content of the skin is below normal, the skin feels dry and tight, lines become more noticeable, and redness and itchiness may be observed.

Water continuously moves from deeper layers of the skin towards the stratum corneum where it eventually evaporates. Transepidermal water loss (TEWL) is a term generally used when considering the hydration state of the skin as it describes the total amount of

water lost through the skin. This loss occurs continuously via passive diffusion through the epidermis (Watson, Fray, Clarke, Yates, & Markwell, 2002).

Skin hydration refers to the water content in the stratum corneum whereas TEWL describes the diffusion of water through the skin (Imhof, De Jesus, Xiao, Ciortea, & Berg, 2009). Cosmetic product claims, such as the mildness of the product, reducing skin irritation reactions, skin hydration, skin repair, and protection against ultraviolet (UV) damage, for instance, are usually based upon and supported by TEWL measurements. Furthermore, TEWL and skin hydration is extensively used as an indicator when assessing skin barrier function (Rogiers, 2001). Should the skin be physically or chemically damaged, the barrier function is compromised. This causes an increase in the TEWL which consequently further decreases the barrier function (Gioia & Celleno, 2002).

Water is essential to the skin in order to conserve its flexibility. If the skin is exceedingly dry it loses its stretch capacity which causes it to crack and peel. The moisture level of the skin will not increase with water alone. A protective lipid layer is required to prevent the evaporation of water from the skin. This is achieved by the use of a moisturising agent. There are a number of different types of moisturising ingredients which act in various ways to increase the moisture content of the skin (Baki & Alexander, 2015: 134).

2.5.3 Types of Skin

There are a number of different criteria by which skin can be classified. Skin can be categorised based on gender, skin colour, UV sensitivity, oiliness, healthiness, and vulnerability, to name a few. The most widely used classification systems are discussed in the following sections.

2.5.3.1 Classification Based on UV Light Sensitivity

The main differences in skin across various ethnic groups is due to hair type, and skin colour, known as skin phototype. A common skin type classification was developed by Fitzpatrick and is based on the skin's reaction to exposure of UV radiation. Although all skin types experience negative effects if overexposed to the sun, some are more vulnerable than others. The Fitzpatrick classification comprises of six skin type categories where skin colour, or melanin content, is correlated with its ability to tan or burn during the exposure of UV light (Fitzpatrick, 1988). These skin types are described in Table 2-6.

Table 2-6: Skin classification according to phototype

Skin Type	Classification
I	Includes people with exceedingly fair skin. They usually have blond or red hair, blue eyes, and freckles. Type I skin never tans and always burns and therefore people with this skin type are extremely sun sensitive.
II	Includes people with fair skin, red or blond hair, and blue, hazel, or green eyes. This skin type is also very sun sensitive, burns easily, and tans minimally.
III	A very common skin type. People with skin that is fair and between cream-white and olive in colour have type III skin. These individuals generally have brown or sandy hair. Although type III skin type is sun sensitive, it can tan gradually to a light brown, and it burns moderately.
IV	Includes people that have light brown skin. They usually have dark brown hair, and green, hazel, or brown eyes. Type IV skin invariably tans

Skin Type	Classification
	to a moderate brown and experiences very little burning. Its sun sensitivity is therefore minimal.
V	Those with moderately brown skin have type V skin. They normally have dark black hair and dark brown eyes. These people tan well to a dark brown, seldom burn, and have sun-insensitive skin.
VI	Includes people with deeply pigmented dark brown or black skin. People with this skin type typically have black hair and dark brown eyes. Skin type VI tans profusely, never burns and is sun-insensitive.

2.5.3.2 Classification Based on Hydration State

In order to prevent water loss, conserve the integrity of the skin barrier, and prevent the penetration of physical and chemical substances, it is vital that the skin is kept hydrated (Zhai & Maibach, 2002). Classification of skin types based on hydration state is usually implemented when choosing cosmetic products such as moisturisers. It has a number of categories each with its own characteristic features. These types include normal skin, dry skin, oily skin, combination skin, and sensitive skin and are described in Table 2-7. When using this classification system, it must be noted that individuals do not have a constant skin type. It may vary over time, depending on a number of external and internal factors. Some if these external factors include the exposure to UV light, wind, humidity, and temperature whereas internal factors involve the hydration state, lipid content, pH, and moisture binding capacity of the skin.

Table 2-7: Skin classification according to hydration state

Skin Type	Classification
Normal skin	Normal skin does not have a strict definition but rather uses other skin types as a point of reference. This skin type is generally described as being not too dry and not too oily. It has a radiant complexion, very few or no imperfections, and no severe sensitivity. On a cosmetological level, normal skin is balanced both structurally and functionally. It has fine pores and is smooth with a good blood supply.
Dry skin	This is a reasonably common skin type as most individuals experience dry skin at some time due to several factors. Dry skin can be described as looking rough, scaly, or dull and feeling taut and itchy. It usually has red patches, a lower elasticity, and a rough complexion. This skin type is inclined toward premature aging and is prone to wrinkles. Dry skin is produced by numerous skin diseases and dietary deficiencies as well as several environmental factors. These include low relative humidity, cold weather, and sunlight. Furthermore, it is also caused by repeated contact with water, surfactants, and solvents.
Oily skin	Oily skin affects most young people and commonly develops with the onset of puberty. During adolescence, people with oily skin tend to suffer from conditions such as acne and dandruff. A characteristic of oily skin is enlarged pores which results from the

Skin Type	Classification
	over-activity of the sebaceous glands making the skin very shiny. Certain parts of the body, such the forehead, nose, and chin, look and feel very oily. There are various factors that may cause or contribute to greasy or oily skin. These include genetic inheritance, diet, hormonal changes, stress, and external agents such as cosmetics, chemicals, and UV light.
Combination skin	Skin that has characteristics of more than one skin type is referred to as combination skin. This occurs commonly as combinations of normal and oily skin, or oily and dry skin. Combination skin tends to be greasy in the central T-zone, which includes the forehead, nose and chin. Furthermore, the skin tends to be normal or dry in other zones such as the cheeks and hairline.
Sensitive skin	Although sensitive skin is considered a skin type, it is better described as a complex dermatological condition. This is because individuals with other skin types may also experience sensitivity to various irritants. Sensitive skin is defined by abnormal sensory symptoms or hyper-reactivity to various environmental factors which causes prickling, tingling, stinging, chafing, or burning and possibly pain or pruritus (Primavera & Berardesca, 2005). The environmental factors include psychological factors such as stress, hormones produced during menstrual cycles, the presence of microorganisms, various chemicals including cosmetics, soaps, water and pollution, and physical factors which include UV light,

Skin Type	Classification
	heat, cold and wind. A common misconception is that sensitive skin is limited to facial skin. This however is not the case as it does concern other areas of the body, mainly, the hands, scalp and feet (Saint-Martory et al., 2008). The pathophysiology of sensitive skin includes altering the skin barrier which allows potential irritants and microorganisms to penetrate the skin and this causes an inflammatory reaction (Luger, 2002).

2.5.3.3 Classification Based on Gender

Although the basic structure, function, and biochemical processes of the skin are fairly constant across the sexes, there are distinct variances between male and female skin. These dissimilarities can be used to formulate gender targeted products in order to better meet the consumers skin needs. The difference between the skin of males and females is discussed in Table 2-8 below.

Table 2-8: Classification according to gender

Characteristic	Description
Hormones	Hormonal differences play a fundamental role in distinction between the sexes. Oestrogen has both advantageous and disadvantageous effects on the skin. It impacts the wound repair rates positively but it negatively influences the distribution of body fat and adversely regulates the growth of body hair. In men, testosterone plays a vital role in the growth of facial and body hair, the production of sebum, and the overall masculine features of

Characteristic	Description
	males (Phillips, Demircay, & Sahu, 2001). Furthermore, an increase in the thickness of the skin is caused by androgen stimulation. This implies that male skin is thicker than female skin.
Age	There are a number of reasons why women appear to age faster than men. This includes the presence of facial hair in men which conceals the fine lines of the skin. Additionally, men have thicker skin and therefore, a higher collagen content (Oblong, 2012). Both genders experience a loss in collagen at the same rate. However, since women have a lower baseline of collagen content, the decrease in collagen results in more visible signs of aging.
Skin thickness	With advancing age, male skin is found to thin progressively while female skin remains reasonably constant until menopause. Once menopause is entered, female skin gradually thins as well. This suggests a hormonal regulation of skin thickness in women as opposed to men (Oblong, 2012).
Sebum content	It has been found that across all age groups, men have a higher sebum content than women at all locations of the body. In men, the sebaceous gland activity remains stable with aging. However, in women it decreases significantly with age, predominantly from the age of 50 to 60 (Luebberding, Krueger, & Kerscher, 2012). The reduction in sebum is accompanied by the depletion of lipids

Characteristic	Description
	within the stratum corneum in women. This could be due to the decline of oestrogen production with advancing age.
TEWL	Besides the biochemical differences between the skin of males and females there are also differences in the functionality of the skin. It has been shown that below the age of 50, men have a significantly lower TEWL than women of the same age (Luebberding, Krueger, & Kerscher, 2013). It is assumed that sebum lipids have a possible occlusive effect on the surface of the skin. For this reason, the higher sebum content in men is a potential explanation for their lower TEWL in comparison to women. With aging however, differences in TEWL due to gender assimilate (Luebberding et al., 2013).
Hydration	The hydration of the stratum corneum is shown to be higher in young men than in women. For women the stratum corneum hydration is stable and even increases with age. However with men, skin hydration begins to decline at the age of 40 and progressively decreases with age (Luebberding et al., 2013).
pH	A further difference between the skin biochemistry of males and females is that males tend to sweat more than females and the sweat of males remains on the skin for a longer period. This produces an environment that is favourable for bacterial growth thereby producing odours. Furthermore, men have more bodily

Characteristic	Description
	hair. This increases the surface area available for bacterial colonisation. The pH of the skin differs between men and women due to the presence of sweat. The skin of females has been found to be significantly more alkaline than that of males except in the underarm area where the pH is similar across both genders. The average pH of the skin of women is 5.6 whereas that of males is found to be 4.3 (Jacobi, Gautier, Sterry, & Lademann, 2005).
UV radiation	The skin of men was found to be more sensitive to UV radiation. This held true for both acute exposure and exposure over a longer time period, known as chronic exposure. It was consequently found that males have a higher risk of developing skin cancer in comparison to females (Oblong, 2012).

2.6 SKIN CARE PRODUCTS

The cosmetic industry is made up of many different product categories. Two major distinctions are colour cosmetics and personal care products. Personal care products are made up of several major categories. The most common are skin care products, hair care products, and dental care products (Baki & Alexander, 2015: 28). The focus of this project is skin care products, specifically moisturisers, products for special skin concerns and sunscreens.

Cosmetics consist of many different ingredients where each is used to fulfil a specific function. A practical classification of the constituents of cosmetics is according to their

function. The constituents used to skin care products are discussed in the following sections.

2.6.1 Antioxidants

The main purpose of antioxidants is to provide protection against oxidative reactions. This implies that they serve two functions in cosmetics. Firstly, antioxidants can contribute to the stability of cosmetic formulations. They can be used to prevent unwanted chemical changes within the formulation which is caused by oxygen in the presence of light, heat or metal ions. These chemical changes include decomposition, rancidity, colour change and odour formation. Antioxidants are generally incorporated in products which contain oxidation sensitive ingredients such as oils, fats, butters, or waxes (Baki & Alexander, 2015: 29).

An additional benefit of antioxidants is its ability to deactivate free radicals in the skin. Free radicals initiate oxidative stress in the skin which accelerates skin aging, and contributes to the formation of wrinkles, pigmentation and malignant processes. Antioxidants fight against various oxidative mechanisms and slow down the skin aging process (Baki & Alexander, 2015: 30).

2.6.2 Chelating Agents

During the preparation process of cosmetics, metallic impurities can contaminate the product from many different sources. These sources can include the raw materials, water sources, metallic equipment, and storage containers. If the metal ions are not deactivated it can cause deterioration of the product by causing rancidity, compromising fragrance intensity, or reducing the clarity and effecting the appearance of the product. Chelating agents are sequestering ingredients which prevent the deterioration of cosmetics and help with stabilisation. They are made up of a particular molecular structure which

enable them to bind and precipitate metals therefore removing trace metals (Baki & Alexander, 2015: 30).

2.6.3 **Moisturisers**

Ingredients that add moisture to the skin and help retain moisture in the skin are referred to as moisturisers. The main purpose of moisturisers is to make the skin feel softer and smoother, and reduce roughness, cracking and irritation (Winter, 2009: 415). They are used in many cosmetic formulations as a main component or as an ingredient that provides additional benefits.

Moisturisers can be subdivided into four categories namely humectants, emollients, occlusives and skin rejuvenators. Each of these are discussed further in following sections. The mechanisms of action of different moisturisers vary from one another and they are therefore used in combination of one another to provide custom benefits in products (Baki & Alexander, 2015: 34).

2.6.3.1 Humectants

Humectants are hygroscopic ingredients which have two main functions in cosmetic products. The first function is to preserve moisture content of products by inhibiting the evaporation of water in the product. The second function is to draw water from the deeper layers of the epidermis and dermis to the outer layer of the skin, the stratum corneum (Baki & Alexander, 2015: 33).

2.6.3.2 Emollients

The main purpose of emollients is to replenish oils and lipids in the skin. This implies that it softens and smoothens the skin. This is done by filling void spaces on the skin surface and replacing lost lipids in the stratum corneum. Furthermore, emollients protect

and lubricate the skin surface, thereby diminishing chafing and improving the skins aesthetic characteristics (Baki & Alexander, 2015: 33).

2.6.3.3 Occlusives

Occlusives are used to prevent water from being lost through the skin. They have a hydrophobic nature which enables them to form a water-repellent layer on the skin. This physically blocks or hinders water loss from the skin (Baki & Alexander, 2015: 34).

2.6.3.4 Skin Rejuvenators

Skin rejuvenators enhance the skin barrier by restoring, protecting, and improving the barrier function of the skin. In addition, skin rejuvenators form a film over the skin surface which smoothens the skin and stretches out fine lines, aesthetically improving the appearance of the skin (Baki & Alexander, 2015: 34).

2.6.4 Neutralising Agents

pH is the hydrogen ion concentration of a solution. It is used to measure acidity and alkalinity and is measured on a scale of 14. Neutral solutions that are not acidic or alkaline have a pH of 7 whereas acids have a pH less than 7 and alkaline solutions have a pH more than 7 (Winter, 2009: 470).

Neutralising agents are used to adjust the pH of formulations. This adjustment may be necessary for a number of reasons. Firstly, since certain ingredients are only stable at a specific pH, neutralising agents can be used to stabilise formulations. In order to achieve an optimum viscosity and thicken a formulation, thickeners are used. Certain thickeners need to be neutralised to achieve optimum results and this can be done with the addition of a neutralising agents. Furthermore, since skin and hair are naturally acidic,

neutralising agents are used to match the pH of the formulation to the surface of application (Baki & Alexander, 2015: 34).

2.6.5 Preservatives

Cosmetics are usually water-based products but water provides the ideal environment for microbial growth. Many types of bacteria, fungi, and yeasts can be found in cosmetic products including pseudomonas, staphylococcus, and streptococcus (Winter, 2009: 502). Moreover, in a number of cases, a product may show no visible indication of microbial contamination but contain actively growing, potentially harmful microorganisms. The presence of microorganisms in products can lead to a number of negative effects such as changes in the general properties of the product as well as the separation of emulsions, discoloration, and odour formation. The main concern however, is the possible infection of consumers.

Preservatives are added to products to prevent unwanted growth of moulds, yeast and bacteria in liquid, semisolid or powder products. The efficacy and mechanisms of action differ amongst types of preservatives. In order to provide protection against a wide variety of microorganisms preservatives are usually used in combination of each other (Baki & Alexander, 2015: 35).

When selecting preservatives some characteristics should be kept in mind. The preservative chosen should be non-toxic for internal and external use, it should not change the character of the product, and it should also be long-lasting and cost effective (Winter, 2009: 502).

2.6.6 Solvents

An important addition to most formulations is a solvent. It is usually in the form of a liquid and is used to dissolve solid ingredients, dispense one or more substances, or combine liquids (Winter, 2009: 567). They can also be used as a formulation medium, or to give the formulation a certain texture.

Solvents have many advantageous effects in formulations. Some of these effects include stabilising formulations, modulating the rate of evaporation, delivering a cooling effect, assisting in the application of products, improving the feel on the skin, adjusting the viscosity of the product, and impacting the film-forming characteristics.

The general rule for solvent interaction is "like dissolves like" which refers to the overall polarity of the solvent and the solute. The solubility of a substance is greatly dependent on the polarity of the solvent. This implies that the solubility of a substance is greatly dependent on the polarity of the solvent which is based on its dielectric constant (Baki & Alexander, 2015: 36).

Solvents can be classified into three main categories namely polar, semi-polar and non-polar and are discussed further below. Although this broad classification is generally used it is not exclusive. Many solvents may have properties of more than one of these categories. Selecting a solvent for a product depends on the type of components present in the formulation, the type of dosage form, and the affinity of the solvent with the surface of application (Baki & Alexander, 2015: 36).

2.6.6.1 Polar Solvents

Polar solvents are made up of strong dipolar molecules, which give them large dipole moments, and they exhibit hydrogen-bonding attributes. They have high dielectric constants and predominantly dissolve polar solutes.

2.6.6.2 Semi-polar Solvents

Semi-polar solvents also consist of strong dipolar molecules but do not form hydrogen bonds. Because they can dissolve both polar and non-polar substances, they can function as a medium for a multicomponent homogeneous system which contains both polar and non-polar solvents.

2.6.6.3 Non-polar Solvents

Non-polar solvents are made up of molecules that have minor dipole characteristics, if any. They have a low dielectric constant and dissolve non-polar substances.

2.6.7 Thickeners

Thickeners have the main function of increasing the viscosity of cosmetic products. Furthermore, they can be used to improve the stability of the formulation, modify the appearance and aesthetics of the product, enhance the applicability, and modify the rheology of the product. Thickeners can act as suspending agents and therefore an additional use of thickeners is to build viscosity in suspensions (Baki & Alexander, 2015: 39).

Thickeners are usually classified in two groups. The first group is viscosity-increasing agents for aqueous systems, which increase the viscosity of the aqueous phase or water phase of an O/W emulsion. The second group is viscosity-increasing agents for non-

aqueous systems, which increase the thickness of the oil phase of products or W/O emulsions (Baki & Alexander, 2015: 40).

A number of factors should be considered when selecting thickeners. These include the use of the product, the surface of application, the compatibility of the thickener with the other ingredients of the formulation, the clarity of the products, and the presence of electrolytes in the formulation. Some thickeners are alkali swellable, e.g. carbomers. This means that they cannot be used in an acidic environment and they require an alkaline pH to obtain optimum viscosity. Another factor that should be considered is the processing temperature of the product since some thickeners have to be melted before it can be mixed in the formulation. Lastly, certain thickeners are activated by shear stress, e.g. carbomers, and therefore there has to be shear during processing in order to gain optimum viscosity. On the contrary there are also thickeners that are sensitive to shear stress, e.g. fumed silica, and these thickeners can only be used when processing does not entail shearing (Baki & Alexander, 2015: 40).

2.6.8 Emulsifiers

Nearly all moisturising, nourishing and protective formulations are emulsions and emulsifiers are a vital constituent of emulsions. The selection of an emulsifier is critical to the stability of an emulsion. It also greatly influences the consistency, viscosity, skin feel, colour, odour, and care properties of the final product. In order to customise the characteristics of a product to the needs of the consumer, various types of emulsifiers are used in formulations (Baki & Alexander, 2015: 215).

The most frequently used types of emulsifiers are surfactants. As discussed earlier, surfactants can be categorised into four main classes. These are namely anionic, cationic,

amphoteric and nonionic, or polymeric, surfactants. The applications of each of these classes of surfactants in cosmetic formulations are discussed below.

2.6.8.1 Anionic Surfactants

Anionic surfactants have a negatively charged hydrophilic head and are generally well known for their foaming features and excellent cleaning characteristics. A downfall, however, is that anionic surfactants are potential irritants. This may be of concern to consumers (Baki & Alexander, 2015: 37). Some types and examples of anionic surfactants are shown in Table 2-9.

Table 2-9: Types and examples of anionic surfactants

Type	**Example**
Carboxylic acids	Stearic acid
Soaps	Triethanolamine stearate
	Potassium laurate
Sulfates	Sodium lauryl sulfate
	Ammonium lauryl sulfate
	Sodium laureth sulfate
Sulfonates	Taurates
	Isothionates
	Olefin sulfonates
Sulfosuccinates	Disodium laureth sulfosuccinate

2.6.8.2 Cationic Surfactants

Cationic surfactants have a positively charged hydrophilic head. Skin and hair have an overall negative surface charge. These negative sites attract the cationic surfactant

molecules electrostatically. Consequently, cationic surfactants make up some of the most powerful skin and hair conditioning agents (Baki & Alexander, 2015: 37). Examples of cationic surfactants include:

- amines and their derivatives
- quaternized ammonium compounds, e.g. cetrimonium chloride, stearalkonium chloride and benzalkonium chloride
- quaternium and polyquaternium molecules

2.6.8.3 Amphoteric Surfactants

Amphoteric surfactants contain both a negative and positive charge in their hydrophobic head. These surfactants are able to stabilise and induce foam formation. Furthermore amphoteric surfactants have favourable characteristics such as good cleansing, bacteriostatic, bactericidal, lathering and softening properties (Baki & Alexander, 2015: 38). Examples of amphoteric surfactants include betaines, e.g. coco betaine, lauryl betaine, cocamidopropyl betaine, and hydroxysultaines.

2.6.8.4 Nonionic Surfactants

Nonionic surfactants do not contain any charge in their hydrophobic head and therefore do not dissociate into ions. The presence of alcohol or ethylene oxide groups give them their surface activity. Nonionic surfactants are mainly used for emulsion stabilisation, conditioning and solubilisation applications. They are very versatile because they are not influenced by pH or the presence of electrolytes and are compatible with other types of surfactants. Furthermore, they have a low irritation potential. For these reasons nonionic surfactants are the most extensively used surfactants in cosmetic products (Baki & Alexander, 2015: 38). Types and examples of nonionic surfactants are displayed in Table 2-10.

Table 2-10: Types and examples of nonionic surfactants

Type	Example
Glycol and glycerol esters	Glyceryl monostearate
Sorbitan esters	Sorbitan stearate
	Sorbitan palmitate
Polysorbates (or ethoxylated sorbitan esters)	Polysorbate 20
Fatty alcohols	Cetyl alcohol
	Stearyl alcohol
Polyoxyethylene–polyoxypropylene block copolymers (or poloxamers)	Poloxamer 407
Amine oxides	Cocamine oxide
	Cocamidopropylamine oxide
Alkanolamides	Cocamide monoethanolamine (MEA)
	Diethanolamine (DEA)
Alkylglucosides	Lauryl glucoside

2.7 SUN CARE PRODUCTS

The exposure to the sun can affect the skin in a number of ways depending on the length of exposure to UV radiation. Short term sun exposure can lead to reddening, irritation, and eventually tanning. On the contrary, long-term effects of UV radiation are irreversible. The exposure of skin to UV radiation, acute and chronic, causes an increased risk of skin cancer. Therefore, UV radiation has been found to be an environmental carcinogen (de Gruijl, 1999).

There are three main types of skin cancers. These are namely basal cell carcinoma, squamous cell carcinoma and melanoma. The relationship between UV radiation and each type of skin cancer varies (Rigel, 2008). Studies have shown that acute sun exposure causes the development of basal cell carcinoma and melanoma whereas chronic sun exposure assists in the development of squamous cell carcinoma. Sun protection has become a very important issue because premature wrinkling as well as a number of types of skin cancer are caused by over exposure to the sun (Stechschulte & Kirsner, 2011).

The active ingredients that can protect the skin effectively from UV radiation are known as UV filters. UV filters are not only incorporated into sunscreens but also into cosmetics that are used daily. In order to help skin maintain its integrity and barrier function, one should take appropriate care of their skin after any type of exposure to the sun.

2.7.1 Electromagnetic Spectrum

A constant flow of energy is emitted by the sun in the form of electromagnetic radiation. This radiation ranges over a wide spectrum of wavelengths. The electromagnetic spectrum is divided into a number of categories which can be seen in Figure 2-16. These categories are classified by wavelength, frequency, or energy. Radio waves have the lowest frequency and the lowest energy. They feature long wavelengths and are therefore at the bottom of the energy spectrum. Microwave radiation has a higher energy than radio waves. This is followed by infrared waves, visible waves, UV rays and X-rays as the energy increases. Gamma rays have the highest energy in the spectrum and are therefore at the top of the energy spectrum. They have the shortest wavelength with peaks close to each other.

The Earth's atmosphere prevents most electromagnetic radiation from reaching the surface of the Earth. Rays that are filtered out and do not reach the Earth's surface are

shaded in Figure 2-16. Only a portion of UV light, visible light, near-infrared light and radio waves actually reach the surface of our planet. This implies that the Earth's surface is not exposed to cosmic rays, gamma rays and X-rays, which are all potentially lethal. Of all the wavelengths that reach the surface of the Earth, UV light has the highest energy. For this reason, UV light is of the highest importance when dealing with sun exposure.

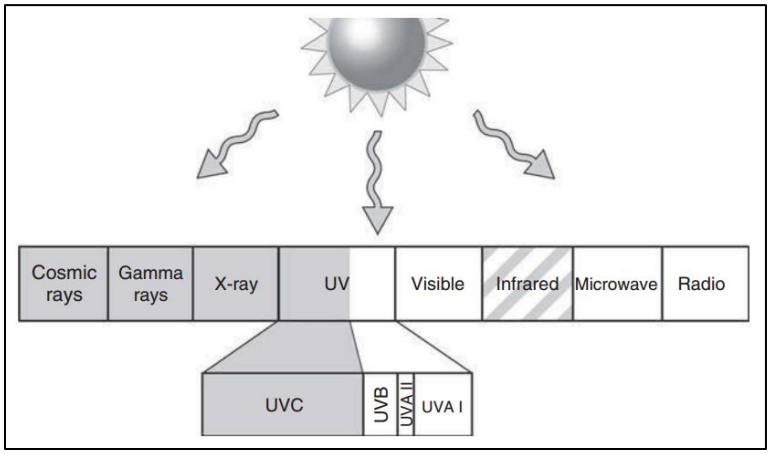


Figure 2-16: Schematic showing the electromagnetic spectrum and their categories (Baki & Alexander, 2015: 276)

Approximately 5% of the total solar radiation that reaches the surface of the Earth is UV radiation (Brugè, Tiano, Astolfi, Emanuelli, & Damiani, 2014). There are three types of UV radiation namely UVA, UVB, and UVC radiation. As UV light passes through the atmosphere, the ozone, oxygen, carbon dioxide, and water vapour in the atmosphere absorb all of the UVC radiation as well as approximately 90 % of the UVB radiation. Therefore, the UV radiation that reaches the surface of the Earth is comprised of about 96 % UVA radiation and 4 % UVB radiation (Brugè et al., 2014).

The three types of UV radiation are based on their difference in wavelength. They also differ in their biological activity and the extent that they penetrate the skin. Although it is more difficult for UV radiation with shorter wavelengths to penetrate the skin, it is

much more harmful to the skin. Figure 2-17 shows the depth of penetration of UVA and UVB radiation.

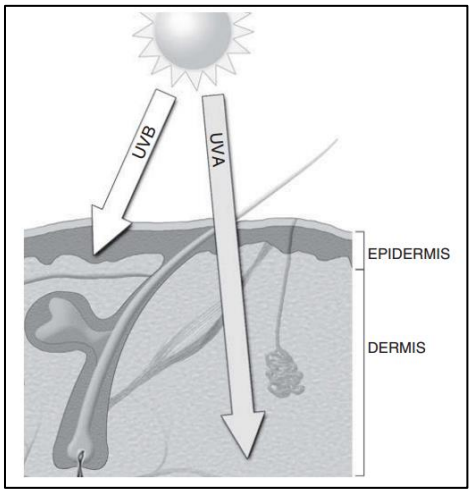


Figure 2-17: Schematic of the skin showing the penetration depth of UVA and UVB light (Baki & Alexander, 2015: 277)

2.7.1.1 UVA Radiation

UVA radiation wavelengths range between 320 nm and 400 nm. It has a longer wavelength than other types of UV radiation and therefore penetrates deeper into the skin, reaching into the dermis. Short term exposure to UVA rays induce tanning of the skin. Over time however, progressive harm is caused due to skin tanning which leads to photoaging (Schulman & Fisher, 2009). It has been found that UVA radiation damages keratinocytes in the basal cell layer of the skin (Garland, Garland, & Gorham, 2003). This is the layer where most skin cancers occur and therefore UVA radiation is known to initiate and contribute to the formation of skin cancer (Coelho & Hearing, 2009). Furthermore, UVA radiation mediates photosensitivity reactions and acts as an immunosuppressant which aids in the development of skin cancer (Frank R. de Gruijl, 2000). Protection against UVA radiation is extremely important due to the fact that it has

the ability to penetrate glass. In addition to this, the energy level of UVA rays remain constant throughout time therefore the damaging effect remains the same regardless of the time of day or the time of year.

2.7.1.2 UVB Radiation

The wavelength of UVB radiation ranges between 280 nm and 320 nm. These rays primarily penetrate the epidermis and is responsible for vitamin D synthesis in the skin. Despite this positive effect, UVB radiation has multiple negative effects on the skin as well. Firstly, UVB radiation is a major cause of sunburn and acute skin damage. In order to minimise the effect of UV radiation on the epidermis, the body has a defence mechanism. This response results in redness of the skin and thickening of the stratum corneum (Gambichler et al., 2005). Even though redness, possible discomfort, and pain subsides reasonably quickly, the latent damage accumulates over time and causes the formation of various types of skin cancer. Furthermore, UVB radiation has been found to have immunosuppressive effects, and promotes photoaging and tanning. UVB radiation can damage the skin throughout the year even though its intensity differs according to location, season and time of day. It is especially influential at high altitudes and on reflective surfaces including ice and snow, but it does not pass through glass (Moehrle, 2008).

2.7.1.3 UVC Radiation

UVC radiation ranges between 100 nm and 280 nm in wavelength. It is blocked out by the ozone layer and therefore has no effect on the skin.

2.7.2 Sun Protection Factor

The efficacy of sunscreens is described by the sun protection factor (SPF). The SPF value increases as sunburn protection increases (Sayre, Agin, LeVee, & Marlowe, 1979).

Erythema refers to the reddening of the skin. The extent of erythema, after the skin is exposed to the sun, is used to measure the efficacy of a given SPF. Minimal erythema dose, MED, is the amount of UV energy required to produce the initial visible signs of skin redness. SPF, by definition, is the UV radiation required to produce 1 MED on sunscreen protected skin after 2 mg/cm^2 of product is applied to it, divided by the UV radiation required to produce 1 MED on unprotected skin (Wilson, Moon, & Armstrong, 2012).

Since the primary cause of erythema is exposure to UVB rays, SPF values only indicate protection against UVB radiation. A typical presumption is that a product with a higher SPF value provides considerably superior sun protection. For example, a product with an SPF of 30 is twice as protective as that with an SPF of 15. This is a common misconception. Sunscreens with an SPF of 15 provide protection against 93 % of UVB radiation whereas those with an SPF of 30 and an SPF of 50 filter 97 % and 98 % of UVB rays respectively. It can be noted that as the SPF value increases, the difference in the amount of UVB protection decreases.

The length of time, in minutes, that skin can be exposed to the sun without burning is theoretically represented by the SPF value multiplied by the time it would normally take for skin to burn. It must be noted that SPF is based on the dosage of solar energy which is affected by a number of factors apart from the length of the period of exposure.

2.7.3 Broad-Spectrum Protection

UVA radiation is accountable for photoaging and the formation of skin cancer. Since SPF is an indication of UVB protection, using a sunscreen with a high SPF value does not provide suitable protection against UVA radiation (Jean-Louis Refrégier, 2004). To prevent the long-term effects of UVA and UVB exposure, protection is required against both types of radiation (Moyal & Fourtanier, 2008). Sunscreens that provide protection against both UVA and UVB rays are said to have broad-spectrum protection. It has been proven that broad-spectrum products with an SPF of 15 or higher can significantly reduce the risk of premature skin aging as well as skin cancer.

2.7.4 UV Filters

UV filters are usually subdivided into two categories based on their mechanism of action. These categories are chemical sunscreens and physical sunscreens. UV filters may be used in combination of one another in a single product formulation in order to achieve optimum photoprotection, a specific SPF value, and broad-spectrum protection. When exposed to sunlight, physical sunscreens remain stable but they may produce free radicals after the exposure to UV rays. This may degrade chemical sunscreens. For this reason, physical sunscreens are usually surface-treated with an aluminium, silica or silicon coating.

2.7.4.1 Chemical Sunscreens

Chemical sunscreens are referred to as organic UV filters and they are typically aromatic compounds. It is the molecular structure of the compound that is responsible for the absorption of UV energy. The working principle of chemical sunscreens is shown in Figure 2-18.

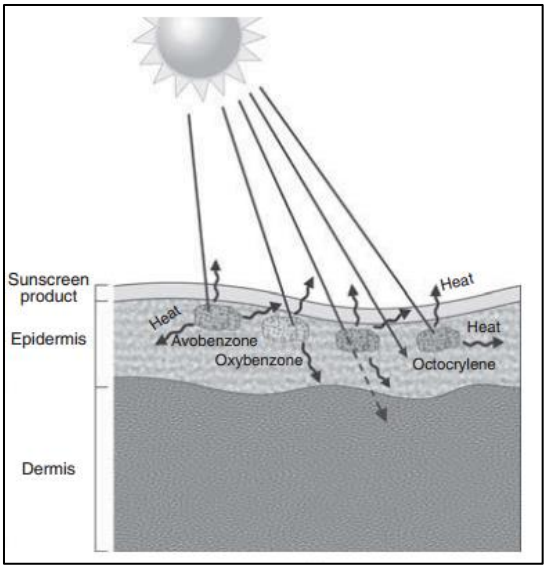


Figure 2-18: Schematic representation of the working principal of chemical sunscreens (Baki & Alexander, 2015: 286)

Once UV radiation is absorbed by the organic UV filter, it induces photoexcitation of the molecules to a higher energy and potentially reactive electronic state. Over time, the system will return to the ground (lowest energy) electronic and vibrational state and the absorbed energy is converted into a lower energy form with longer wavelengths, such as heat and light. This relaxation may occur though a number of photochemical or photophysical processes. For simplicity, a summary of the photophysical processes are shown in Figure 2-19. These include intramolecular vibrational redistribution, internal conversion, intersystem crossing, fluorescence and phosphorescence (Rodrigues, Staniforth, & Stavros, 2016).

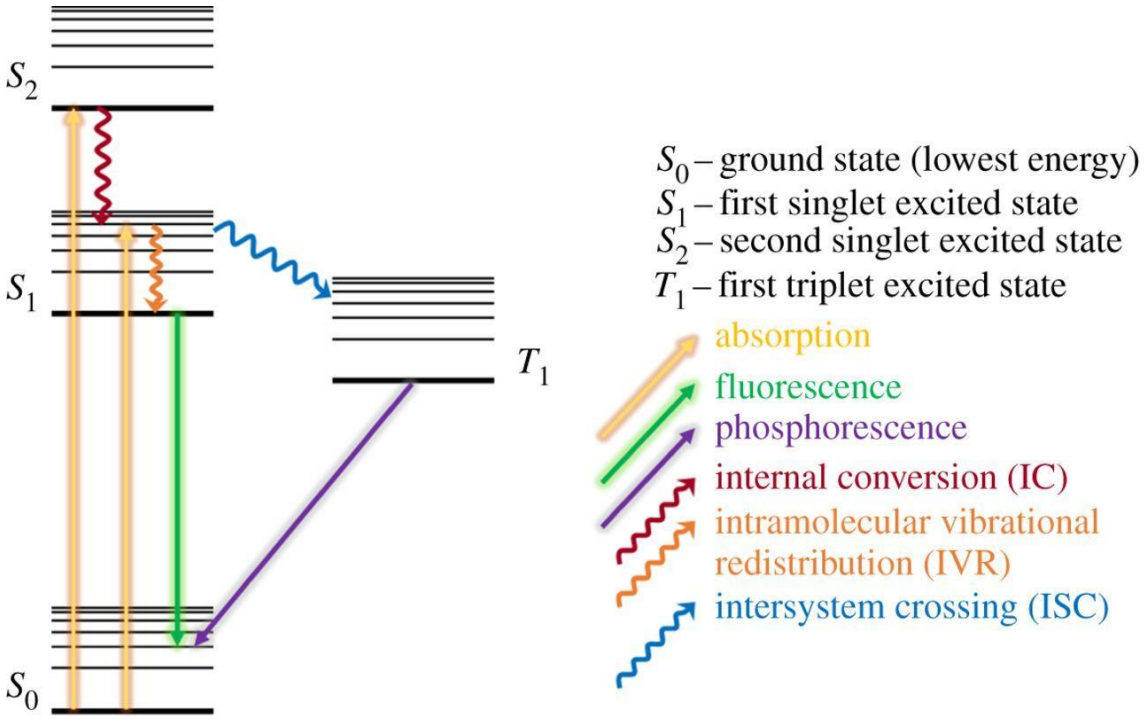


Figure 2-19: Simplified Jablonski diagram showing possible photophysical processes of chemical UV filters (Rodrigues et al., 2016)

Some of these processes may cause damage to the skin by producing free radicals or other harmful products. Ideal chemical UV filters should strongly absorb UVA and UVB rays while being capable of dissipating the excess energy through mechanisms that do not cause chemical change. Subsequently, molecules should be reformed in its original state without producing any potentially harmful species (Rodrigues et al., 2016).

The longer the relaxation mechanism the higher the chance of occurrence of detrimental photochemistry. Therefore, the efficiency of the mechanism by which molecules return to their ground electric state reduces the chance of undesirable photochemistry taking place. Usually once these chemical UV filters are exposed to UV radiation their structure are influenced negatively or even destructed. Therefore, organic UV filters often lose their absorption capacity instead of returning to the ground state. Photostability is a common

issue with many organic sunscreens and therefore photostabilisers are often encompassed in these sunscreen formulations (Baki & Alexander, 2015: 286).

Due to the lipophilic nature of organic sunscreens, they can penetrate the skin thus causing safety concerns (Watkinson, Brain, Walters, & Hadgraft, 1992). Some common UVA organic absorbers include avobenzone, oxybenzone, and meradimate whereas organic UVB filters include octinoxate, ensulizole, octialate and padimate.

Octinoxate has a number of disadvantages on the skin. Since it is absorbed into the skin, it may cause irritation and other adverse reactions. This includes generating free radicals with the exposure to sunlight that causes damage to cells, premature aging, oestrogen and thyroid disruption, and hindrances in brain signalling. Avobenzone in the presence of octinoxate tends to cause instability. The exposure to sunlight tends to break down avobenzone into unknown chemicals. Oxybenzone is very similar to avobenzone but it has been found to be more toxic than avobenzone. It is said to increase the rates of skin cancer, and cause cell damage, hormone disruption and induce allergies. Ensulizole has been found to produce free radicals with the exposure to sunlight. Furthermore, it is said to cause damage to DNA and is a potential cause of cancer (Aliano, 2008).

2.7.4.2 Physical Sunscreens

Physical sunscreens are known as inorganic UV filters. The working principal of physical sunscreens is shown in Figure 2-20. Here it can be seen that inorganic UV filters reflect, scatter and absorb UV radiation (Sayre, Kollias, Roberts, & Baqer, 1990). Furthermore, it can only penetrate the outer layer of the skin which gives these inorganic UV filters an excellent safety profile (Filipe et al., 2009).

Titanium Dioxide and Zinc Oxide are the most commonly used physical filters and they are both white powders. These ingredients are insoluble in base formulations and are therefore suspended in the sunscreen products. Iron oxides provide colour to the skin. It is their reflectance of certain wavelengths that allow us to perceive their colour on the skin. They also have absorption bands throughout the visible and UV region. Titanium Dioxide and Zinc Oxide show strong semiconductor absorption in the ultraviolet region. They scatter, reflect and absorb visible and UV light. The efficacy at which they reflect light is dependent their particle size (Sayre et al., 1990).

Inorganic filters have a number of advantageous effects. Firstly, independent of the base formulation and of other ingredients, inorganic filters are photostable. Secondly, due to the fact that inorganic filters reflect and scatter both UVA and UVB radiation, it provides broad-spectrum protection. Titanium Dioxide provides UVA II, a category of UVA radiation, as well as UVB protection whereas Zinc Oxide offers protection against both UVA I and UVA II, as well as UVB radiation.

The main drawback of inorganic filters is after application, it tends to appear white on the skin, which is less appealing to consumers. This is due to the fact that physical sunscreens reflect and scatter UV radiation into the visible spectrum. By reducing its particle size, the reflection spectrum of the ingredient can be modified thereby altering the white appearance. The reflection in the visible spectrum decreases as the particle size is reduced thus making the sunscreen more aesthetically appealing. Inorganic UV filters with smaller particles have a synergistic effect in sunscreen formulations as it has a lighter feel on the skin. For these reasons micronised and nanonised Titanium Dioxide and Zinc Oxide are commonly used. Nanosized particles do not reflect visible light and therefore instead of appearing white on the skin, they appear transparent.

The reduction in particle size of inorganic UV filters induce a shift in the UV radiation protection profile of the ingredient. It now provides protection in the UVA II and UVB range which causes an imbalance in its UV protection. This situation can be improved firstly, by using nanosized particles in combination with microsized particles or secondly, by using inorganic and organic UV filters in combination with one another (Smijs & Pavel, 2011). This allows for the ideal balance of conservation of aesthetics and delivering broad-spectrum protection.

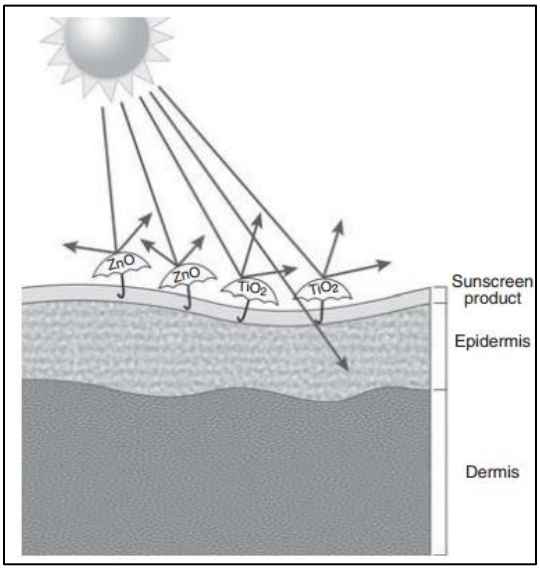


Figure 2-20: Schematic representation of the working principal of physical sunscreens (Baki & Alexander, 2015: 286)

2.8 SENSORY EVALUATION

The success of a cosmetic formulation does not depend solely on the efficacy of its active ingredients but rather on the consumer acceptance as well. One of the objectives in developing a cosmetic product is that it should perfectly satisfy the sensory expectations of the consumer. This is influenced significantly by the sensory properties of the product.

In order to formulate products that provide sufficient efficacy, aesthetic attributes and fulfil the needs of the consumer, many studies have focused on the sensory evaluations of cosmetics (Almeida, Gaio, & Bahia, 2008; Aust, Oddo, Wild, Mills, & Deupree, 1987; Husson, Lê, & Pagès, 2007; Montenegro, Rapisarda, Ministeri, & Puglisi, 2015; Parente, Ares, & Manzoni, 2010). Furthermore, a number of studies have focused on relating instrumental, mechanical, and rheological properties to predict the sensory and textural characteristics of emulsions (Gilbert, Picard, Savary, & Grisel, 2013; Gilbert, Savary, Grisel, & Picard, 2013; Morávková & Stern, 2011; Parente, Gámbaro, & Solana, 2005).

It is not unusual for a clinically effective skin care product to be rejected by consumers due to one or more of its skinfeel properties. Product developers frequently find it difficult to understand information given by the consumer. Terminology such as "rich" and "creamy" often do not offer sufficient insight as to why certain products are interpreted to be less adequate than other products available on the market. Hence, a descriptive sensory evaluation procedure is required.

A good guideline for this procedure is given by the ASTM Standard Practice for Descriptive Skinfeel Analysis of Creams and Lotions. This standard provides a guideline for the selection and training of the panellists, the rating scale range, skin conditioning procedure prior to the tests, definition of each attribute of the product, handling of products, and methods for the manipulation of the product on its own and on the skin. The evaluation process deals with four main sections. These are product delivery, pick-up evaluation, rub-out evaluation and afterfeel characteristics (ASTM E1490-03, 2003). The evaluation criteria for each section are summarised and shown in Figure 2-21.

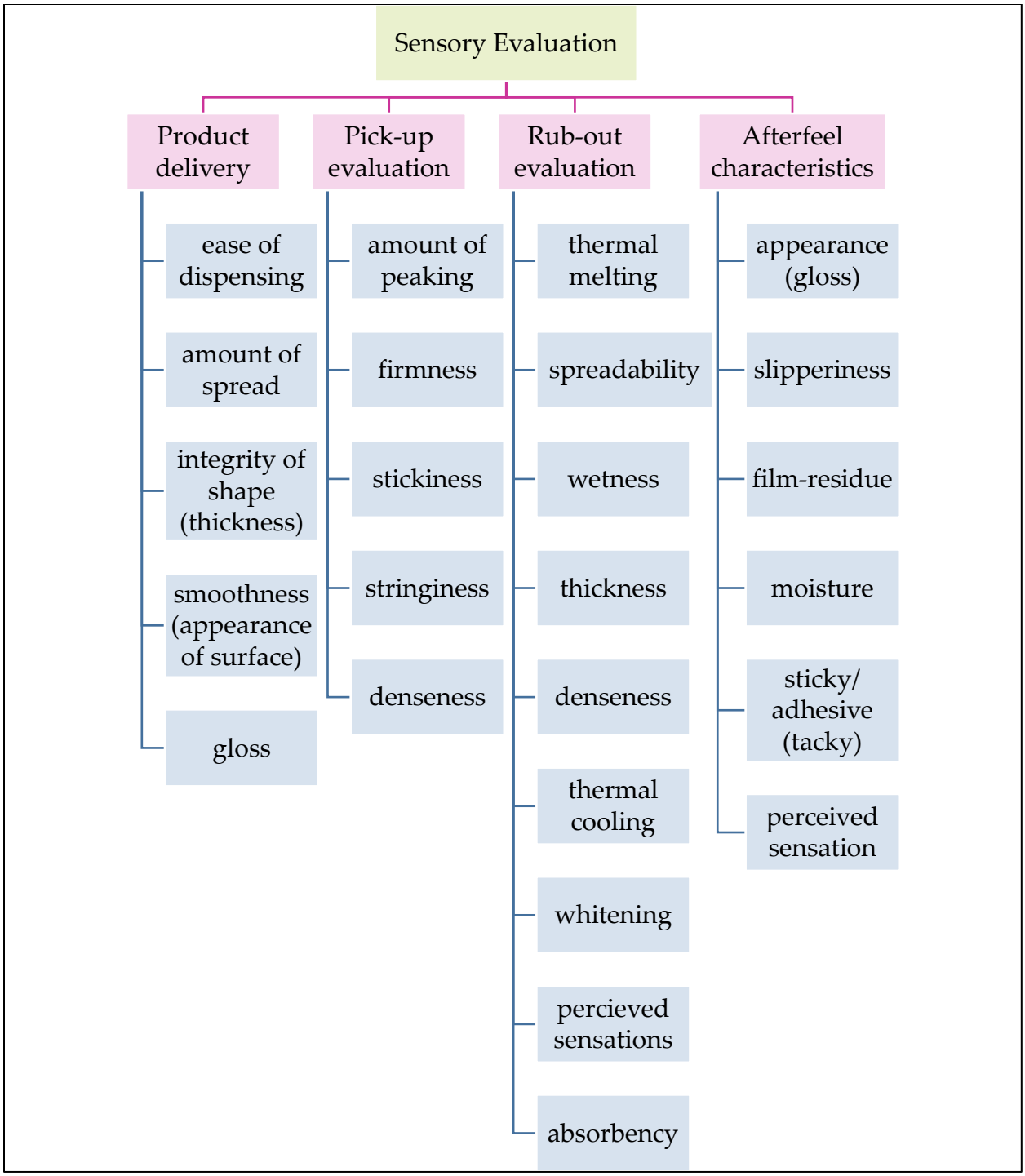


Figure 2-21: Summary of the sensory evaluation criteria covered in ASTM E1490-03

CHAPTER 3: EXPERIMENTAL DESIGN

3.1 INTRODUCTION

Three plant extract containing formulations were developed in this work. Each are discussed in more detail in subsequent chapters as Formulation A, B and C. All four plant extracts were ethanol/water extracts and are therefore water soluble. In an emulsion formulation it is expected that the plant extract will report to the water phase. The HO, PM, and LSSJ plant extracts were included in Formulations A and B whereas BS (hypothesised to have SPF boosting properties) was only included in Formulation C. As requested by the Department of Plant and Soil Sciences at the University of Pretoria, all ethanol/water plant extracts were added to formulations at 10 % by mass. This loading showed good efficacy in past investigations which aimed to incorporate ethanolic plant extracts into skin care formulations (Kucera, Barna, Kálal, Kucera, & Hladíkova, 2005; Kučera1, Barna, Horáček, Kováriková, & Kučera, 2004; Predel, Giannetti, Koll, Bulitta, & Staiger, 2005; Sadaf, Saleem, Ahmed, Iqbal, & Ul-Zafar, 2006; Trakranrungsie, Chatchawanchonteera, & Khunkitti, 2008). The aim of this chapter is to briefly discuss the purpose of the development of each of these three formulations as well as the methodology followed. In addition, the procedure and materials used in the development of the formulations are discussed. Experimental methods as well as equipment and analytical procedures used to analyse the different formulations are also given.

3.2 FORMULATION DEVELOPMENT

Formulation A can be considered as a base case formulation. Here, a number of formulation recipes were sourced from suppliers of raw materials. These formulations were not used as is but rather to guide the choice of ingredients and the concentration at which they were dosed. The formulations included gels, body milks, lotions and creams. Prior to addition of the plant extracts, initial screening tests of formulations based on these recipes were performed. A set

of promising looking formulations resulted from this process. These formulations – still without the plant extracts – were subjected to sensory evaluation as well as preliminary stability testing. Using the results of these procedures, a best-case extract-free formulation, referred to as the placebo formulation, was selected. Formulations using this selected recipe were subsequently prepared that contained 10% by mass of the plant extracts HO, LSSJ and PM and the performance of these three formulations were compared to that of the placebo formulation using a long-term stability testing procedure.

Formulation B aims to be an improvement on Formulation A in terms of cost. Here locally available formulation ingredients were used to replace those used in Formulation A. Once again, placebo formulations were subjected to sensory evaluation and preliminary stability testing in order to identify the most promising formulation recipe using the new ingredients. In addition, the potential effect of the loading of the plant extract on the stability of the formulations were considered by adding a mock extract – consisting of a 40% mass-based ethanol in water mixture – to formulations at two different loadings. Based on the results obtained, the best-case placebo formulation was once again selected. This chosen formulation was then remade in its placebo form as well as in formulations where 10% by mass of plant extracts HO, PM and LSSJ were added. Long term stability testing was used to compare the suitability of these formulations.

Plant extract BS showed promising UV blocking characteristics. Subsequently Formulation C aims to be a sunscreen formulation that contains this plant extract at a 10% (mass basis) loading level together with other traditional UV filters. Formulation C used the best-case formulations from Formulations A and B as a baseline and incorporated different kinds of UV filter additives. Placebo sunscreen formulations with different additives as well as formulations containing extract BS were prepared and subjected to long-term stability testing. A costing analysis of the sunscreen formulations was also performed. These criteria were used to identify the

formulation with the greatest potential to be used as a sunscreen. SPF testing was conducted on Formulation C with and without the BS plant extract. These tests were done by the Photobiology Laboratory at Sefako Makgatho Health Sciences University. A comparison of these results showed the UV blocking capacity of the sunscreen as well as the SPF boosting capability of the BS plant extract.

3.3 MATERIALS

3.3.1 Formulation Ingredients

Each cosmetic ingredient has a specific purpose within a formulation. Many different cosmetic ingredients were considered in this study. The trade names, INCI, or chemical names, and supplier of each of the raw materials used throughout this investigation are grouped according to their functionality in Table 3-1 below.

Table 3-1: List of raw materials and suppliers of the formulation ingredients used in this investigation

Ingredient	INCI/Chemical Name	Supplier
	Chelating Agent	
EDTA	Tetrasodium EDTA	Sigma Aldrich
	Diluent/Solvent	
Water (Distilled)	Aqua	University of Pretoria
	Emollient	
Afrisil 350 cSt Fluid	Dimethicone	Afrisil
Afrisil D5	Cyclopentasiloxane	Afrisil
Crodamol GTCC	Caprylic/Capric Triglyceride	Croda South Africa
Crodamol SFX	PPG-3 Benzyl Ether Ethylhexanoate	Croda South Africa

Ingredient	INCI/Chemical Name	Supplier
Crodamol STS	PPG-3 Benzyl Ether Myristate	Croda South Africa
ERCAREL AB V	C12-15 Alkyl Benzoate	Fourchem
Imex MCT 60/40	Capric/Caprylic Triglyceride	Fourchem
Imex IPM 98	Isopropyl Myristrate	Fourchem
Mineral Oil	Mineral Oil	Fourchem
	Emulsifier	
Crodex M	Cetostearyl Alcohol (and) Potassium Cetyl Phosphate	Croda South Africa
Dermofeel GSC	Glyceryl Steareate Citrate	Evonik
ERCAWAX CS 20 V/FD	Ceteareth 20	Fourchem
Lipowax P	Cetostearyl Alcohol (and) Polysorbate 60	Lipo Chemicals
Palmerol 6830	Cetostearyl Alcohol	Fourchem
	Humectant	
Glycerin	Glycerin	Fourchem
Propylene Glycol	Propylene Glycol	Fourchem
	Neutralising Agent	
Sodium Hydroxide	Sodium Hydroxide	Sigma Aldrich
	Preservative	
Germaben II	Propylene Glycol (and) Diazolidinyl Urea (and) Methylparaben (and) Propylparaben	Fourchem
	Thickening Agent	
Carbopol 940	Carbomer	Fourchem
Sodium Polyacrylate	Sodium Polyacrylate	Sigma Aldrich

Ingredient	INCI/Chemical Name	Supplier
Xanthan Gum	Xanthan Gum	Protea Chemicals
	UV Filter	
SO60MZJ	Zinc Oxide (and) Helianthus Annuus (Sunflower) Seed Oil (and) Jojoba Esters	Cosmetic Ingredients (Pty) Ltd
Solaveil XT-100	Titanium Dioxide, C12-15 Alkyl Benzoate, Polyhydroxystearic Acid, Stearic Acid & Alumina	Croda (SA)
Zinc Oxide BP	Zinc Oxide	Fourchem

3.3.2 Plant Extract Specifications

The plant extracts were prepared by the Department of Plant and Soil Science and was used in the formulations as received. In preparation of the plant extracts, the leaves and stems of each of the plants were identified and collected. The collected material was shade dried and powdered. This powdered plant material was then dissolved in an ethanol/water mixture of concentration mentioned in Table 3-2. It was then shaken for 48 hours. Thereafter, the solution was filtered using a Büchner funnel using Whatman® Grade 1 filter paper. The filtrate was subjected to reduced pressure using a Büchi® Rotavapor® R-200 rotary evaporator. Finally, the prepared extract was stored at 4 °C until further use. The details of the four plant extracts are shown in Table 3-2.

Table 3-2: Details of the plant extracts

Plant Code	Composition of Ethanol/Water Mixture (Mass % Ethanol)	Powdered Plant Extract Loading (g/L)
HO	40 %	6
BS	40 %	6
PM	40 %	6
LSSJ	70 %	7.5

3.4 METHODOLOGY

The experimental methodology is described by firstly explaining the general procedures used to prepare the formulations. Thereafter the sensory evaluation procedures are summarised. Finally, the stability testing methods used in this investigation are explained in detail.

3.4.1 Formulation Preparation

All developed formulations were prepared using standard laboratory equipment. The formulation sheet gave the mass percentage for each ingredient. The ingredients of each phase were weighed in individual glass beakers. Standard formulation preparation procedures were developed and recorded for each formulation. (More detail on composition is provided in the chapters where each formulation is discussed). Temperatures were kept low enough to prevent components from degrading upon heating. A WiseStir® MSH-20D magnetic stirrer hot plate and magnetic stir bar was used to agitate and heat the solutions simultaneously. The temperature of the solutions was continuously monitored using a thermometer.

To avoid clumping of the thickening agent the formulations which included a thickening agent were prepared with an additional step. Here the thickening agent was weighed and dispensed

in a separate beaker. The water phase, usually phase A was placed on a magnetic stirrer hot plate and stirring was commenced until a vortex was formed. The thickening agent was then sprinkled onto the surface of the vortex. Phase A was then mixed at lower speeds until the thickening agent was completely dispersed and the solution was uniform.

The gel formulations consist of a single phase which incorporated a thickening agent. This thickening agent was responsible for the gel like properties of the formulation. In preparation of the gel formulations phase A was prepared where the thickening agent was incorporated as explained above. All the other ingredients were then added and mixed after each addition using the magnetic stirrer. Finally, the complete solution was mixed for 15 minutes using the magnetic stirrer at approximately 1000 rpm to ensure a homogeneous product.

The body milk, cream and lotion formulations were all emulsions. These emulsions comprised of two main phases, phase A – the water phase, and phase B – the oil phase. In preparation of these phases, the ingredients of phases A and B were weighed into separate beakers. If a thickening agent was required it was incorporated using the procedure explained earlier in this section. The phases were then heated using a WiseStir® MSH-20D magnetic stirrer hot plate. This was done to melt the wax ingredients and adjust the individual phases to similar viscosities for ease of mixing. A temperature range of 65 °C and 75 °C was used in the formulations of this study.

Once the individual phases were heated, phase B was added to phase A. Some of the formulations made use of a self-emulsifying wax. In these cases, phase A and B emulsified upon addition and were simply mixed at 1000 rpm for 15 minutes until the product was homogeneous. In other formulations, where phases A and B were not miscible or a UV filter was incorporated more intense mixing was required. In these cases, high shear mixing was used to facilitate emulsification or ensure complete dispersion of the UV filter within the formulation. The Silverson® L4RT High Shear Mixer with the emulsor screen workhead was

used for these purposes. In both these cases the mixture of phases A and B was exposed to high shear mixing at 5000 rpm for 15 minutes.

After the 15 minute mixing period the product was allowed to cool whilst undergoing agitation at 1000 rpm using the magnetic stirrer. Once the temperature of the formulation reached 40 °C the ingredients of phase C were added and mixed after each addition. These ingredients included the preservative, ethanolic plant extract and the neutralising agent. The ethanolic plant extract was added at loading of 10 % by mass as required by the Department of Plant and Soil Science. The neutralising agent was added dropwise while constantly monitoring the pH of product using an EZDO PP-203 portable pH meter. The formulation finally underwent 15 minutes of agitation using the magnetic stirrer at 1000 rpm to ensure that the product was homogeneous.

The preservative and ethanolic plant extract are sensitive to temperature. It was recommended that these ingredients are added to the formulation only after it cooled to 40 °C. Since the boiling point of pure ethanol is 78.5 °C, a conservative operating temperature of 65 °C was chosen for the formulations incorporating the plant extracts. If the plant extract was heated to this temperature it would not degrade and the ethanol within it would not evaporate. This allows room for error should the plant extract be added to one of the heated phases during preparation. In such an event the preservative could be added to the formulation after the final product had cooled completely, even below 40 °C.

3.4.2 Sensory Evaluation Procedure

Two separate sensory evaluations were conducted in this investigation, the first in the development of Formulation A and the second in the development of Formulation B. The ASTM 1490-03 Standard Practice for Descriptive Skinfeel Analysis of Creams and Lotions was used as a guideline for these sensory evaluations. The described procedure spans over a period

of a few weeks where a single group of participants meet repeatedly to go through different phases of the process. Obtaining the commitment of a single group of participants for multiple sessions was impractical in this work. Subsequently the ASTM procedure was adapted and shortened to fit within a single, reasonably short session. In this investigation a time constraint of 90 minutes was placed on each sensory evaluation procedure and multiple groups of participants were used. To ensure validity, the cycle of procedures recommended in the ASTM method was completed within this time frame.

The objective of the first sensory evaluation was to determine the carrier formulation type most appealing to the consumer. Here eleven formulations were tested over four criteria. The formulations in this evaluation were of different types including gels, body milks, creams and lotions. The evaluation procedure is discussed in greater detail in Chapter 4.

In the second sensory evaluation, applied during the development of Formulation B, five formulations were compared to a benchmark product. The benchmark product was a commercially available, well performing lotion. In this sensory test, all six formulations were of the same formulation type. The sensory evaluation procedure comprised of four tests and is explained in-depth in Chapter 5.

Both sensory evaluations conducted in this investigation were approved by the EBIT Ethics Committee and the Faculty of Health Sciences Research Ethics Committee of the University of Pretoria. Written approval was granted by both committees (EBIT/98/2017). The evaluation procedures were structured in accordance with the Declaration of Helsinki (last update: October 2013), which deals with recommendations guiding doctors in biomedical research involving human subjects.

3.4.3 Stability Testing Procedure

The stability of the formulations was tested by subjecting them to both preliminary as well as long-term stability testing procedures. The main difference between the two procedures was the length of the testing periods. Preliminary testing extended over 2 to 4 months whereas long term stability testing had a duration of 7 to 12 months. Preliminary stability testing combined with the sensory evaluation data were used to identify the most promising placebo formulation during the initial testing phase of each of the formulation types. Plant extracts were added to the best base case or placebo formulations of each type that were identified in this manner. Freshly prepared formulations with and without the plant extracts were subsequently subjected to long term stability testing.

In evaluating the stability of the formulations, samples were stored under different controlled temperature conditions. Room temperature samples were kept in a temperature regulated room at 25 °C. Accelerated instability conditions were achieved by storing samples in a Labotec Ecotherm oven at 40 °C and a Labotec Labocool refrigerator at 4 °C. During long term stability testing samples were stored under all three temperature conditions.

The stability of the formulations was determined using three methods of analysis. These included coalescence analysis, optical microscopy and cycle testing. The preliminary testing procedure was based mainly on coalescence analysis whereas the long-term stability testing procedure included all three methods of analysis.

3.4.3.1 Coalescence Analysis

Coalescence analysis was the main analysis procedure considered in this investigation. This was due to the fact that majority of the carrier formulations considered were emulsions. Analysis of coalescence is based largely on analysing droplet sizes and the change thereof with time.

3.4.3.1.1 Apparatus

One of the main stability testing apparatus used in this investigation was the Malvern Mastersizer 3000™ particle size analyser. This apparatus uses laser diffraction to produce droplet size distributions. A standard operating procedure (SOP) was set up for analysis of the samples. The properties of this SOP were kept constant amongst all testing to avoid experimental error. These properties are shown in Table 3-3.

Table 3-3: SOP settings for droplet size analysis

Property	**Value**
Particle type	Non-Spherical
Particle refractive index	1.520
Particle absorption index	0.100
Dispersant	Water
Number of measurements	5
Stirrer speed	2600 rpm
Obscuration range	2 % - 20 %
Cleaning	Normal
Analysis model	General Purpose

In this study, an automated wet dispersion unit, the Hydro MV, was used as an attachment to the Mastersizer 3000™. It is a medium volume dispersion unit and is recommended for the analysis of emulsion samples. All formulations developed in this investigation proved miscible in water and was therefore confirmed to be O/W emulsions. For this reason, the dispersant chosen was the continuous phase, water. Although the obscuration range was set between 2 % and 20 %, care was taken to keep the obscuration at approximately 10 % during sample addition.

Droplet size analyses were conducted on the formulations periodically. To make sure that the droplet size distributions obtained were indicative of the entire formulation sample, each sample was mixed thoroughly prior to analysis using a spatula. This ensured that the formulation was completely uniform and not affected by settling. The formulation was then sampled and dispersed into the water within the dispersion unit. At least two samples were tested per formulation and five measurements were obtained for each sample. The data was represented graphically using error bars where the upper and lower boundaries of the error bars were calculated using a 95 % confidence interval.

3.4.3.1.2 Analysis

The results obtained from the Mastersizer 3000™ included the droplet size distribution and other parameters based on the statistical analysis of the distribution. The volume mean diameter, d_{43}, sensitively reflects small changes in the droplet size distributions which mainly occurs as a result of various instability mechanisms (Moschakis, Murray, & Biliaderis, 2010). This implies that analysis of the d_{43} would reveal any signs of coalescence and give conservative results in analysing the change in droplet diameter over time. For this reason, the d_{43} parameter was used to analyse coalescence in this investigation.

A coalescence plot was generated by graphing the natural logarithm of d_{43} versus time. Trendlines were added to the plots and the equations of the lines were determined. Using Equation (2-6) and the slopes of the trendlines, the apparent rates of coalescence, K_C, of each formulation was calculated. This gave a comparative result between formulations and storage temperatures showing how fast the samples were coalescing.

The shelf life of the formulations was determined on the basis of visual appeal. The formulations were assumed to expire once droplets became visible to the naked eye. The smallest size of an object recognisable to the naked human eye is between 55 μm and 75 μm.

A conservative droplet size of 50 μm was considered along with the trendline equations of the coalescence plots. The shelf life was then calculated by determining the time required for the formulation sample to reach a droplet size of 50 μm.

The rate of coalescence and shelf life are both determined through a linear relationship. The interval of uncertainty is therefore obtained through calculating a 95% confidence interval of the slope of the graphs. The estimated slope b tends to miss the true value β by an amount called the standard error of the slope. This is denoted as "*SE of b*" and the formula is shown in Equation (3-1). The 95 % confidence interval for the slope of the regression is estimated by $b \pm 1.96(SE)$ which is shown in Equation (3-2) (Naranjo, 2011).

$$\text{SE of b}=\sqrt{\frac{(1-r^2)S_y^2}{(n-2)S_x^2}} \tag{3-1}$$

$$b \pm 1.96\sqrt{\frac{(1-r^2)S_y^2}{(n-2)S_x^2}} \tag{3-2}$$

In both these equations, r^2 is the coefficient of determination, S_y is the standard deviation of y, S_x is the standard deviation of x, and n is the sample size.

With the confidence interval of the slope now known, the standard error of the rate of coalescence is known. The minimum and maximum slopes were used to calculate an interval of uncertainty for the predicted shelf life. Using the maximum error, the minimum theoretical shelf life was obtained and reported.

3.4.3.2 Optical Microscopy

Optical microscopy was carried out on each of the samples at the beginning and end of the testing period. This was done to qualitatively verify the droplet size analysis results produced by the Mastersizer 3000™. Furthermore, it was used to identify any initial signs of instability and visually compare the change in droplet shape and size with time. The micrographs were produced using the Zeiss Axio Imager M2 microscope with a 100x oil objective. Formulations were mixed thoroughly using a spatula before sampling. This was done to ensure that the sample taken was indicative of the entire formulation and was not affected by external factors such as settling. Microscope slide samples were prepared by dispensing a drop of each formulation onto glass slides. Using a glass coverslip, the droplet was dispersed forming an even layer. The prepared slide was then placed under the microscope and analysed at a magnification of 100x. The analysis was repeated by sampling each formulation three times. Three separate slides were prepared for each formulation and the micrographs with the greatest clarity were included in this dissertation.

3.4.3.3 Cycle Testing

In order to predict the stability of the carrier formulations under extreme conditions cycle testing was conducted. Samples were cycled between the Labotec Ecotherm oven at 40 °C for 24 hours and the Labotec Labocool refrigerator at 4 °C for 24 hours for a period of six or seven cycles. To induce even more stress, the already cycled samples were then alternated between the oven at 40 °C and a Defy™ chest freezer at -18 °C for an additional five cycles.

At the end of each cycle the samples were analysed using three different techniques. Firstly, the pH of the samples was measured using an EZDO PP-203 portable pH meter. Here the samples were mixed thoroughly and five measurements were taken for each sample. Secondly, droplet size analysis of the samples was done using the Mastersizer 3000™. Again, the samples

were mixed thoroughly before sampling using disposable pipettes. Five measurements were taken on each sample and the median droplet size, d_{50}, was analysed over each cycle. The d_{50} is the droplet size where half the sample resides above this size and half below this size. It was therefore used for cycle testing analysis. The rate of coalescence was not considered over each cycle therefore the d_{43} was not analysed. The pH and droplet size data were represented graphically as a function of the number of cycles. In these graphs, error bars were included where the upper and lower bounds were calculated using a 95 % confidence interval. Finally, the samples were subjected to centrifugation at 3000 rpm for 30 minutes using the Eppendorf™ 5810R centrifuge. Visual observations were made before and after centrifugation to identify any signs of separation, creaming or sedimentation.

Since testing and analysis had to take place at room temperature, care was taken to avoid keeping the samples under normal conditions for longer than 10 minutes per cycle. This ensured that the samples were under extreme stress conditions for more than 99 % of the cycle testing period.

CHAPTER 4: FORMULATION CAMPAIGN A

4.1 INTRODUCTION

A number of existing formulation recipes were sourced from raw material suppliers and were used as a guideline for development of formulations in this first cycle. Adapted versions of these recipes were used to prepare gels, body milks, creams and lotions – initially in the placebo form (i.e. without the addition of any plant extracts). As described in Chapter 3, a best-case placebo formulation was identified based on sensory screening and the preliminary stability testing procedure. The formulation selected in this manner is referred to as Formulation A. Subsequently Formulation A was remade in its placebo form and as well as with the addition of the HO, PM and LSSJ plant extracts. The BS plant extract was not included in this formulation. It was said to have SPF boosting characteristics and therefore was only included in the sunscreen formulations of Chapter 6. The samples containing plant extracts underwent long term stability testing according to the procedures described in Chapter 3. Finally, a cost analysis was done on the placebo formulation to determine the raw material cost of a 100 g sample of this formulation.

4.2 PLACEBO FORMULATION DEVELOPMENT

Eighteen initial trial formulations were prepared. Stability of these first trial samples was determined by visual observation over the first 48 hours after preparation. The formulations that proved unstable within this period were modified to try and stabilise them. After final elimination of unsuccessful preparations, eleven potential carrier formulations were identified. These formulations underwent sensory evaluation and preliminary stability testing. The formulations included four gels, one body milk, three creams and three lotions. Summaries and brief descriptions of the eleven formulations

are shown in Table 4-1. The complete formulation recipes and preparation procedures are shown in Appendix A.

Table 4-1: Summarised description of the eleven carrier formulations (CF)

Name	Type	Description
CF 1	Clear Gel	A Carbomer based skin gel. This formulation contained 1.5 % Carbomer thickening agent and formed a stable, clear gel with good spreadability.
CF 2	Clear Gel	A Carbomer based skin gel modified from CF 1. The Carbomer thickening agent loading was increased to 2%. This increased the thickness of CF 1 and formed a thick and stable clear gel with ease of spreading.
CF 3	Clear Gel	A skin gel based on Xanthan Gum as the thickening agent. This formed a thick stable clear gel which spreads easily.
CF 4	White Creamy Gel	A skin gel based on Sodium Polyacrylate as the gelling agent and a combination of emollients including Cyclopentasiloxane and Dimethicone for ease of application and a silky feel.
CF 5	Body Milk	A liquid body milk based largely on the emollient Mineral Oil. It formed a stable spray that spread well and had a silky feel.
CF 6	Light Cream	A light skin cream based on a number of emollients including Mineral Oil, Dimethicone and Cyclopentasiloxane. A stable white cream was formed with good texture and feel. It had properties of fast absorption and ease of spreading.

Name	Type	Description
CF 7	Light Cream	A single phase, light skin cream based largely on the emollient Mineral Oil. A stable white cream was formed which had good texture, feel, absorption and appearance.
CF 8	Light Cream	A light skin cream based on PPG-3 Benzyl Ether emollients and Xanthan Gum as the thickening agent. A thick stable cream was formed which absorbed easily and had a luxurious creamy feel.
CF 9	Light Lotion	A skin lotion based on a number of emollients including Mineral Oil, Dimethicone and Cyclopentasiloxane. This formed a stable light lotion which had easy spreadability, absorbed quickly and had a silky feel.
CF 10	Light Lotion	A single-phase light skin lotion based largely on the emollient Caprylic/Capric Triglyceride. A stable white lotion was formed. It had a good feel, smooth texture and absorbed easily.
CF 11	Light Lotion	A light skin lotion based on a modification of CF 8. In this formulation the thickening agent was excluded to form a lotion that was smooth and stable. It absorbed easily and has a luxurious feel.

4.3 SENSORY EVALUATION

A sensory evaluation was conducted on all eleven placebo carrier formulations. A total of 41 people participated in the sensory evaluation. Participants were asked to give their age, gender, and race to ensure that the results obtained were not biased to any specific skin type. Amongst the 41 participants, there were 33 females and 8 males. Moreover, 18 participants were Caucasian, 14 were Indian, and 9 were African. The age of the

participants ranged from 18 to 66 where 30 participants were between the ages of 18 to 25 and 11 participants were between the ages of 26 and 66. The obtained sensory data was analysed in two ways. Firstly, the population was considered equally and as a whole. Secondly a sensitivity analysis was conducted on the results to observe the consumers preference based to their demographic data.

The sensory evaluation form is shown in Figure 4-1. Four common skin feel properties were considered in this sensory evaluation. The criteria by which each of the carrier formulations were rated was ease of spreading, residue, absorption, and feel. Participants were asked to test each formulation on their skin and rate the samples according to the given criteria. The rating was analysed on a scale of 1 to 5 where a rating of 1 indicated unacceptable performance and 5 indicated exceptional performance. Therefore, the higher the rating given the more appealing the formulation was to the consumer.

4.3.1 Sensory Results

The sensory results were analysed by considering an average of the recorded ratings of each criterion. The results for each carrier formulation are shown in Figure 4-2. Here all ratings above 3 show good characteristics. From the results in Figure 4-2 it was seen that carrier formulations CF 2 and CF 3 have an undesirable skin feel and CF 4, CF 6 and CF 9 leave an undesirable residue on the skin.

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA
Denkleiers • Leading Minds • Dikgopolo tša Dihlalefi

SENSORY EVALUATION FORM

FORMULATION: _____

DEMOGRAPHIC DATA

AGE: _____

GENDER: _____

RACE: _____

QUESTIONS	UNACCEPT-ABLE	ACCEPTABLE	GOOD	VERY GOOD	EXCEPTIO-NAL
DOES THE LOTION SPREAD WITH EASE ON YOUR SKIN?					
DOES THE LOTION LEAVE ANY RESIDUE ON YOUR SKIN?					
ARE YOU SATISFIED WITH THE ABSORPTION ONTO THE SKIN?					
HOW DOES THE LOTION FEEL ON YOUR SKIN?					

Thank you for your time!

Figure 4-1: Sensory evaluation form used in the first sensory evaluation

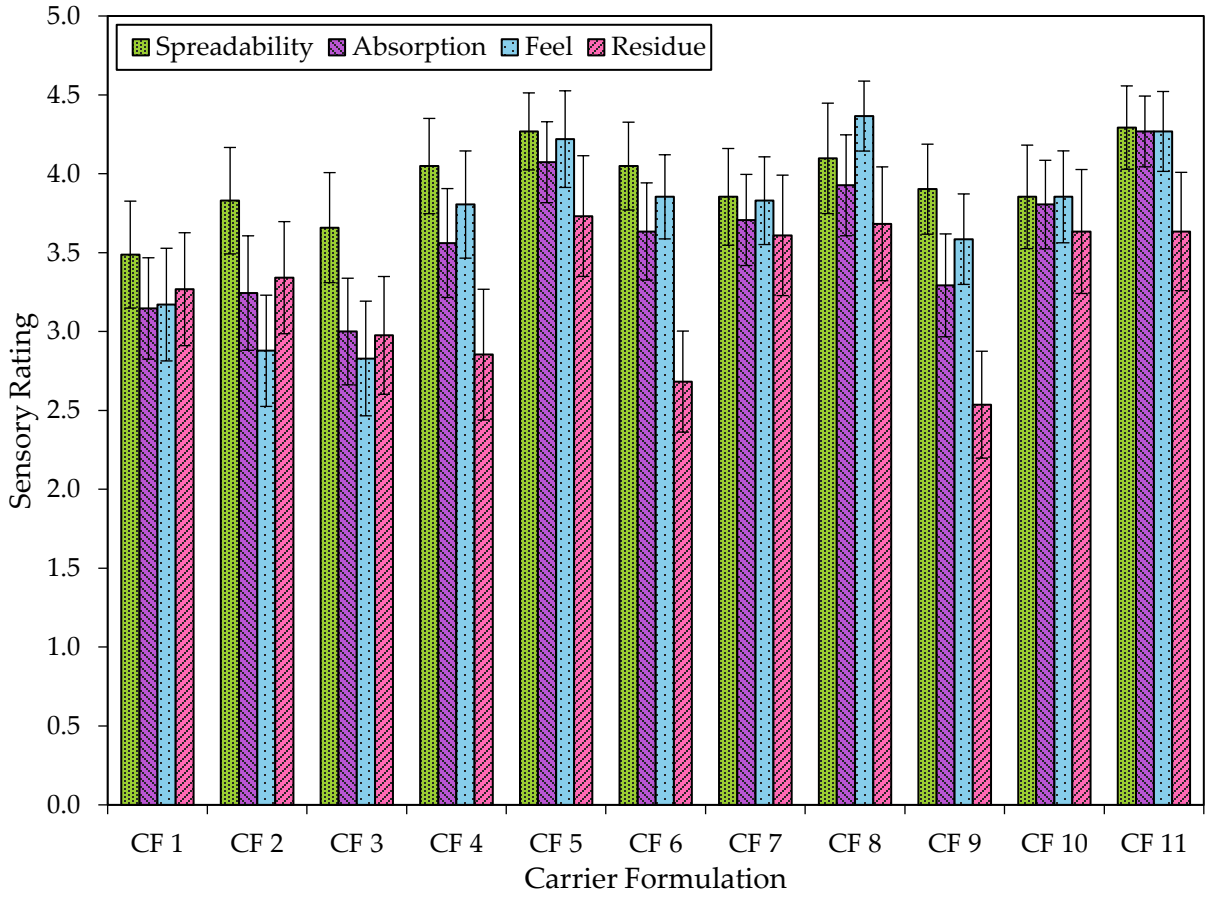


Figure 4-2: Graphical representation of the average sensory ratings of each criterion

The most appealing formulation was decided upon based on an overall rating across all criteria. This overall score was calculated using the following procedure. Firstly, each criterion was assigned a weighting factor. This weighting factor was based on the importance of each characteristic. The weighting factors across the criteria were normalised implying that the summation of the weighting factors would equate to 1. This is shown in Equation (4-1) where the weighting factor of criteria i is ω_i.

$$\sum_{i=1}^{n} \omega_i = 1 \tag{4-1}$$

The overall score for each carrier formulation was calculated using Equation (4-2)

$$Score(j) = \sum_{i=1}^{n} \omega_i s_{ij} \tag{4-2}$$

where ω_i is the weighting factor and s_{ij} is the average score for attribute i of carrier formulation j. The chosen weighted factors for each attribute are shown in Table 4-2 below. These factors were chosen based on the importance of each characteristic in the final product.

Table 4-2: Weighting factor values of each sensory criteria

Sensory Characteristics	Weighting Factor (ω_i)
Spread	0.20
Residue	0.10
Absorption	0.30
Feel	0.40

To avoid bias toward the chosen weighting factors the calculated score was compared to an average score. This average score was calculated by equating each of the weighting factors to 0.25.

The calculated score together with the average score of each formulation is shown graphically in Figure 4-3. From the calculated score results it was concluded that CF 11, a light lotion, had the best sensory characteristics. This was verified by the average score results. It can also be seen that CF 5, a body milk, and CF 8, a light cream, were appealing to the participants. Interestingly, CF 8 was a modification of CF 11 with the addition of a

thickening agent implying that this base formulation has good sensory characteristics in the form of a light lotion or a cream.

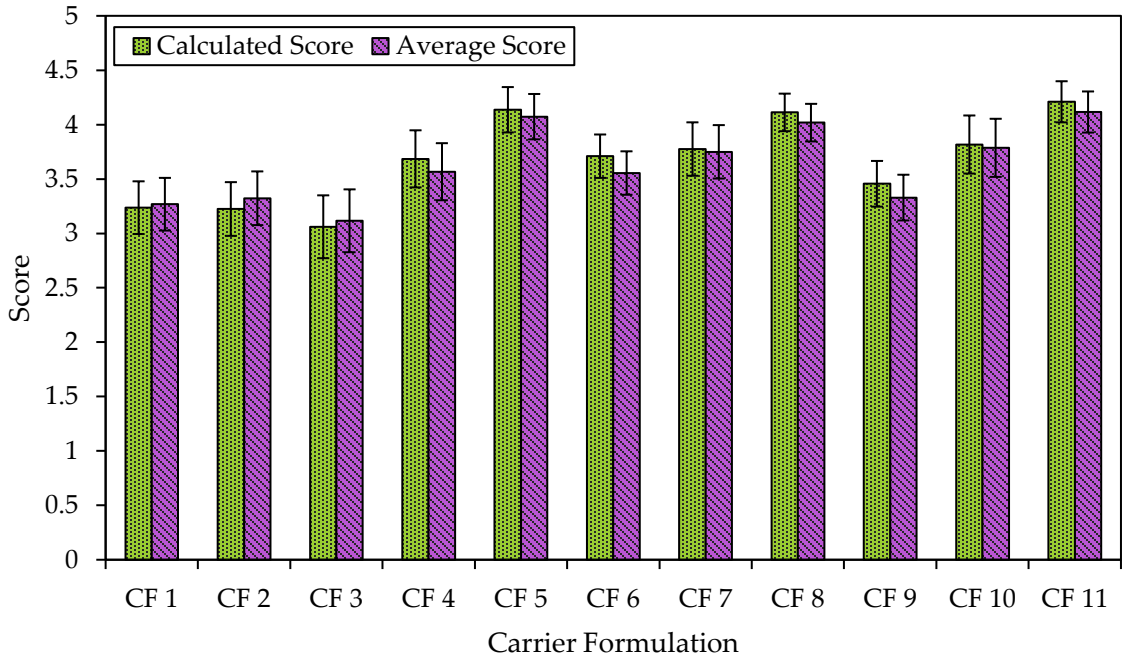


Figure 4-3: Graph of the overall calculated and average sensory scores of each formulation

4.3.2 Sensitivity Analysis

A sensitivity analysis was conducted to investigate the presence of bias based on participants skin type. This was done by analysing the sensory ratings based on the demographic data received from participants. Firstly, the data was analysed based on the gender of the participants. The skin of females and males differ due to the difference in hormones between genders. The results of this analysis are shown in Figure 4-4. From this it was concluded that the most sensory appealing formulation to females was CF 11, a light lotion, and the most appealing to males was CF 5, a body milk.

The second demographic analysis was based on race. The race of the participants effects the skin in terms of its UV sensitivity due to pigmentation, amongst other biological factors. The results based on the race of the participants is shown in Figure 4-5. From this it was seen that Caucasian and African participants showed preference to CF 11 and Indian participants showed preference to CF 5.

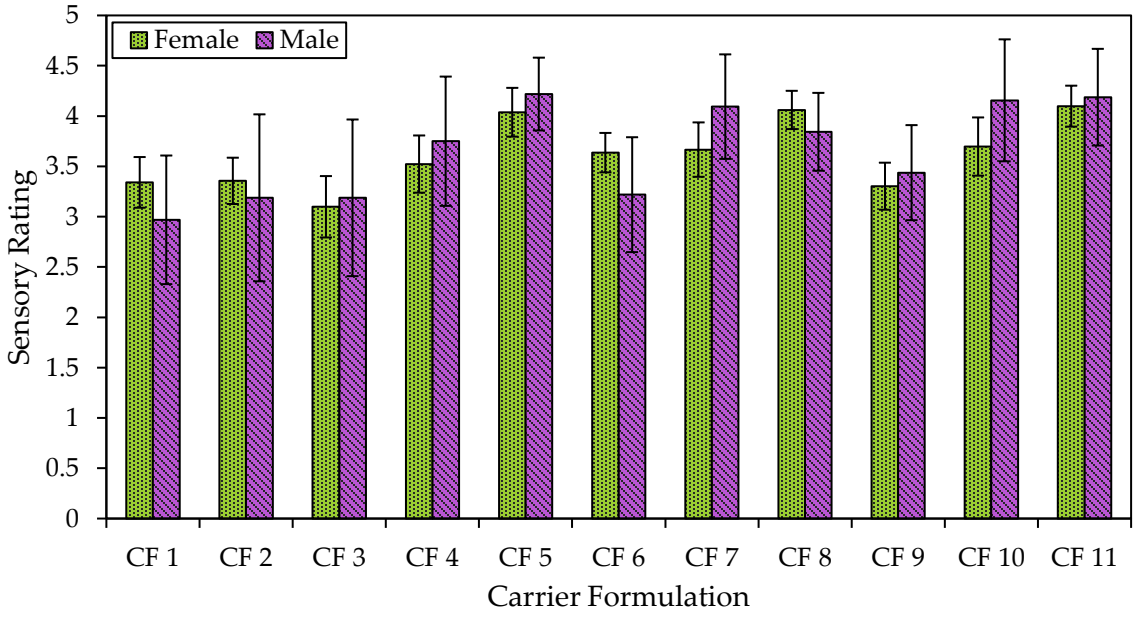


Figure 4-4: Sensory ratings based on the gender of participants

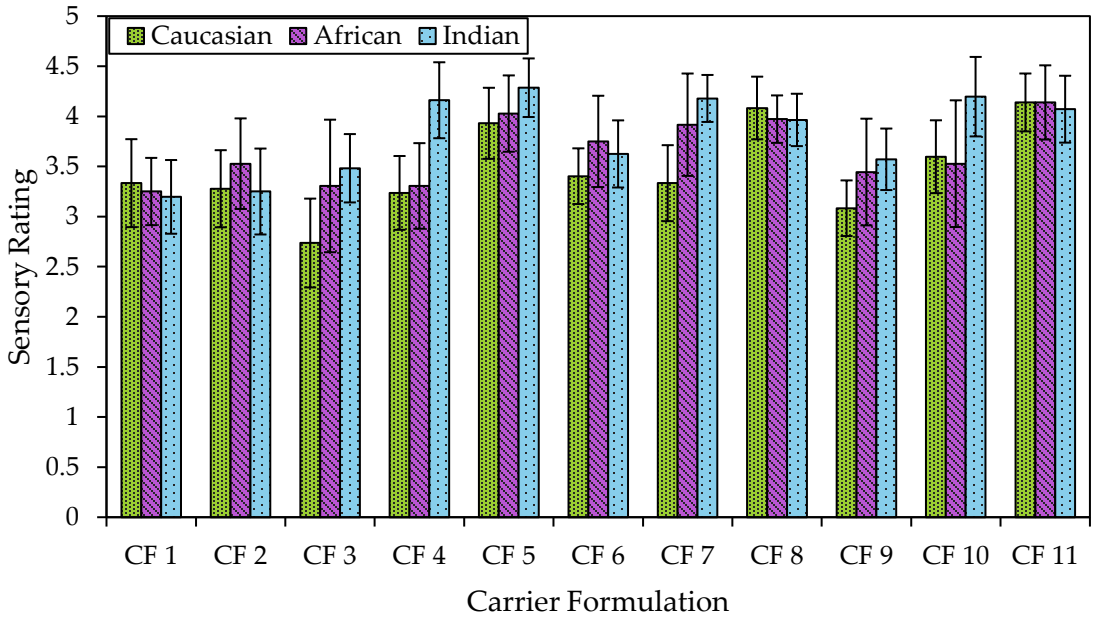


Figure 4-5: Sensory ratings based on the race of participants

The third demographic analysis was based on the age of the participants. The age of the participants effects the thickness of the skin and therefore the collagen content of the skin. This analysis was divided into two categories namely ages within the range of 18 and 25 and ages within the range of 26 and 66. The sensory results based on age is shown in Figure 4-6. From this it can be seen that the preferred formulation for participants of the age of 18 to 25 was CF 5. Furthermore, participants between the ages of 26 and 66 found CF 11 more appealing.

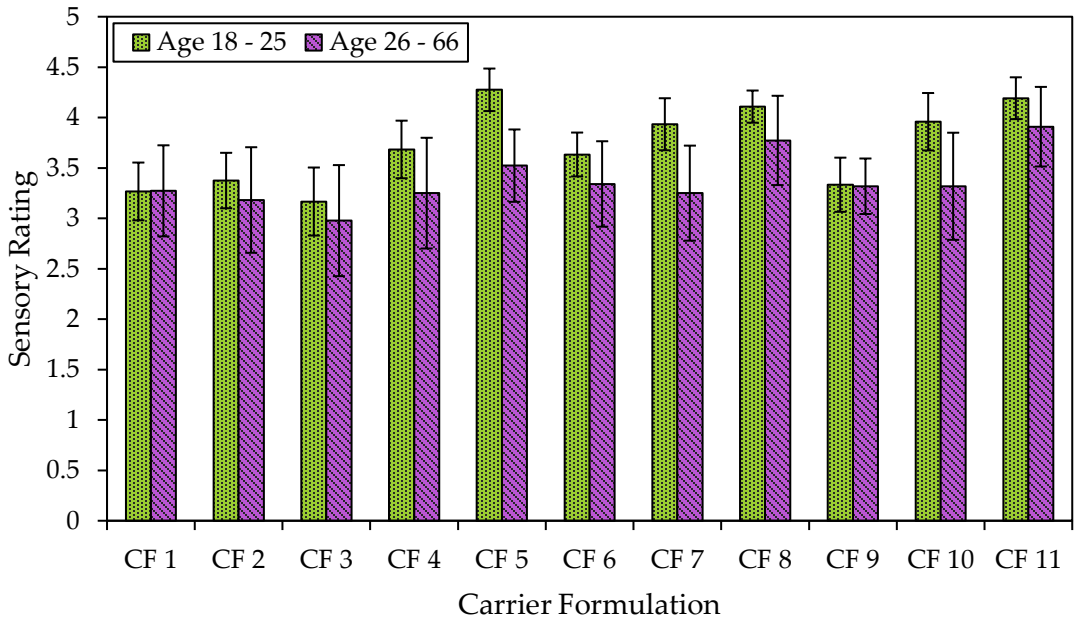


Figure 4-6: Sensory ratings based on the age of participants

The results of the sensitivity analyses showed preference to CF 5, a body milk, in some cases and CF 11, a light lotion, in others. This verified that skin type does in fact affect sensory perception thereby influencing consumer preference. It was concluded that both CF 5 and CF 11 were possible carrier formulations in terms of sensory characteristics. The stability of the formulations was investigated in order to select the best performing carrier formulation.

4.4 PRELIMINARY STABILITY TESTING

As mentioned in Chapter 3, the preliminary stability procedures involved optical microscopy as well as, coalescence analysis where the change in droplet size over time was used to measure the rate of coalescence of each formulation. All carrier formulations were stored in a temperature controlled room at 25 °C for the duration of the testing procedure.

4.4.1 Microscopy

Optical microscopy was conducted on each sample within 24 hours after preparation. Droplets observed in these micrographs were compared to results from the Mastersizer 3000™.

Figure 4-7 shows the micrographs of carrier formulations CF 1, CF 2 and CF 3. These three formulations were gels and the difference in the structure of these gels can be observed from the micrographs. CF 2 shows much larger droplets than CF 1 owing to the fact that CF 2 has a higher loading of Carbomer thickening agent. The thickening agent might not have been fully dispersed throughout the sample thereby forming clumps. This may be the reason why larger droplets were observed in this sample. CF 3 seems to have smaller droplets as it contains less thickening agent than CF 1 and CF 2.

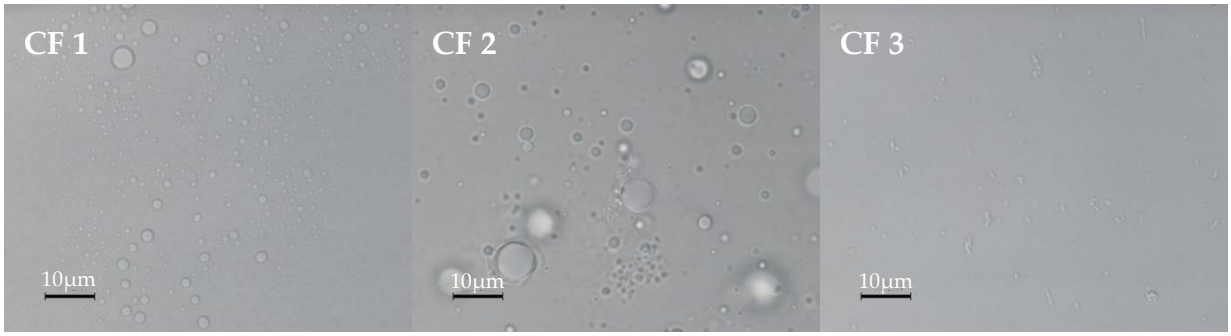


Figure 4-7: Micrographs of the three gel formulations CF 1, CF 2 and CF 3.

The micrographs of the emulsion formulations are shown in Table 4-3. This table gives information of the droplet distribution obtained from the Mastersizer 3000™ including a description of the droplet distribution type, the volume mean droplet diameter, d_{43}, and median droplet diameter, d_{50}.

The micrographs of formulations CF 4, CF 6, CF 7, CF 8, and CF 11 all showed evidence of a range of different droplet sizes. This was verified by the droplet distributions obtained by the Mastersizer 3000™ where bimodal distributions were observed.

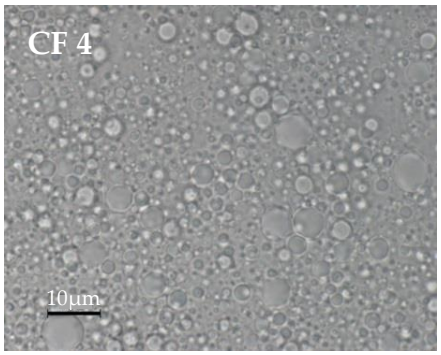
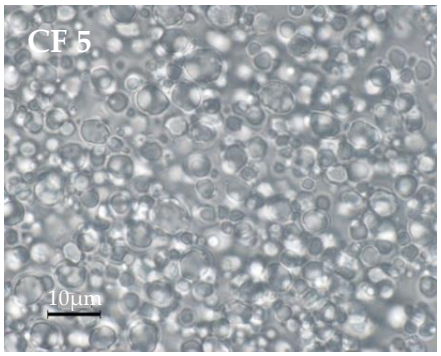
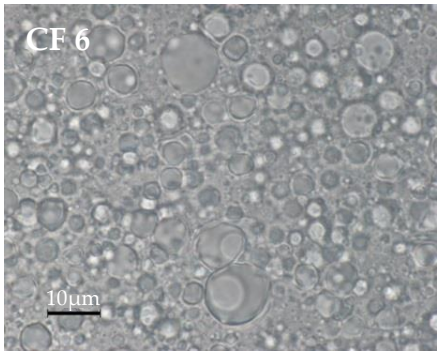
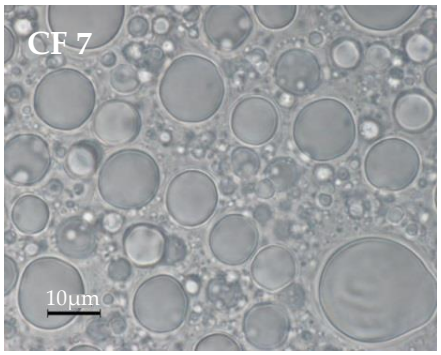
Formulations CF 8 and CF 11 were derived from the same formulation and were therefore compared to each other. The micrographs of these formulations looked similar to each other where the droplets of these formulations ranged over the same diameters. The droplet distributions showed the smaller differences between the samples where the d_{43} was larger in the cream, CF 8, than the lotion, CF 11, possibly due to the presence of the thickening agent in the CF 8.

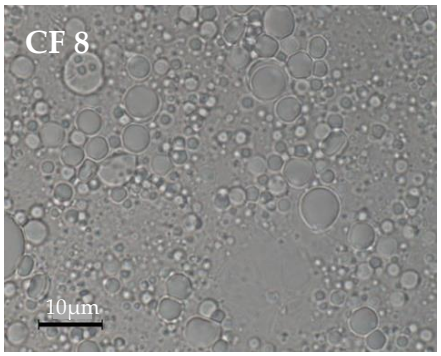
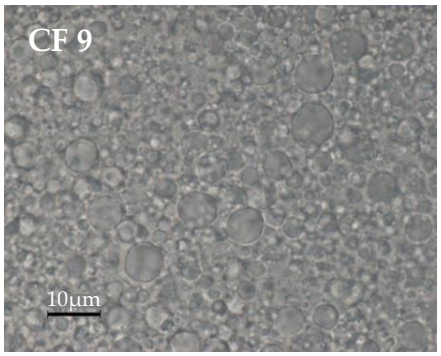
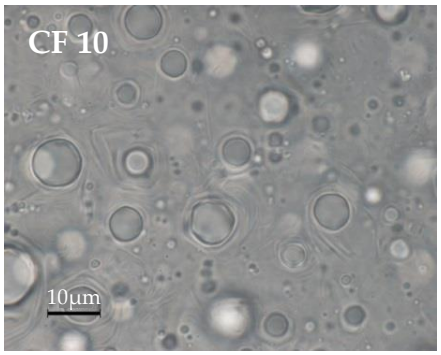
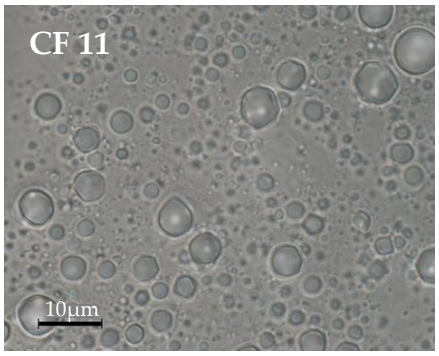
Formulations CF 5, CF 9 and CF 10 showed a large amount of similarly sized droplets. The droplet distributions of these formulations were lognormal. The peak diameter, volume mean diameter and median diameter of these formulations showed similar values. These droplet sizes were confirmed by the micrographs.

There was no evidence of instability in the initial micrographs. This may be due to the fact that the micrographs were obtained less than 24 hours after preparation.

Comparison between the micrographs and the droplet distributions obtained from the Mastersizer 3000™ showed good agreement. The d_{43} parameter represented the average droplet size of the samples appropriately. This supported the analysis of the change in d_{43} with time during coalescence analyses.

Table 4-3: Comparison between micrographs and droplet distribution results

Micrograph	Distribution Description	d_{43} (µm)	d_{50} (µm)
	Bimodal distribution with peaks at 3.12 µm and 6.72 µm	6.03	5.31
	Lognormal distribution with a peak at 3.55 µm	3.99	3.74
	Bimodal distribution with peaks at 0.0597 µm and 6.72 µm	4.61	1.78
	Bimodal distribution with a peak at 14.5 µm and a small peak at 2.75 µm	13.7	13.4

Micrograph	Distribution Description	d_{43} (μm)	d_{50} (μm)
	Bimodal distribution with a peak at 2.75 μm and a bigger peak at 0.0771 μm	5.74	0.153
	Lognormal distribution with a peak at 4.58 μm	4.88	4.42
	Lognormal distribution with a peak at 16.4 μm	14.1	15.8
	Bimodal distribution with peaks at 6.72 μm and 0.0679 μm	2.29	0.166

4.4.2 Coalescence Analysis

Carrier formulations CF 1, CF 2 and CF 3 were gels and not emulsions, therefore droplet size analysis does not indicate its stability as coalescence does not occur. Since these carrier formulations did not perform well in the sensory evaluation, the stability of these formulations was not needed. The stability of the emulsions were determined by comparing the rate of coalescence of each carrier formulation.

Droplet size distributions of the carrier formulations were obtained using the Mastersizer 3000™. These tests were conducted on a weekly basis for a period of eight weeks. The coalescence plots for each carrier formulation were generated by plotting the d_{43} as a function of time in days. Trendlines were added and the equations of these trendlines were used to calculate the apparent rate of coalescence K_C of the formulations using Equation (2-6). The combined coalescence plots for all the formulations are shown in Figure 4-8. The trendlines along with the calculated K_C of each formulation is shown in Table 4-4.

Comparing the rate of coalescence of CF 5, CF 8 and CF 11, it was found that CF 11 was the most stable as it showed the smallest K_C value therefore showing the slowest rate of coalescence. Due to its slow rate of coalescence, the shelf life of CF 11 was suggested to be much longer than the shelf-lives of the other two formulations. Considering the slow rate of coalescence and the long shelf life, CF 11 was selected as the best performing formulation and is referred to as Formulation A. This formulation then underwent long term stability testing in its placebo form and with the addition of the plant extracts.

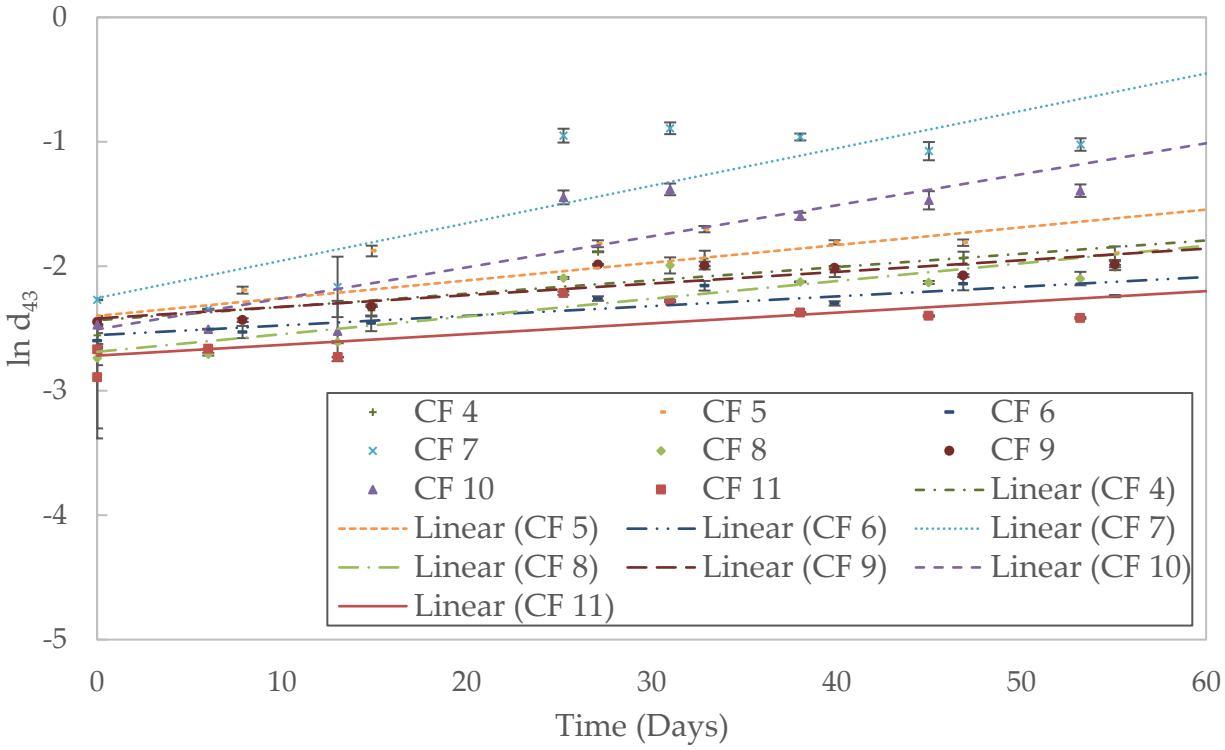


Figure 4-8: Coalescence plot for the emulsion carrier formulations

Table 4-4: Summary of the trendline equations and K_C values

Formulation	**Trendline Equation**	**K_C (Day^{-1})**
CF 4	$y = 0.0107x - 2.4339$	3.20×10^{-2}
CF 5	$y = 0.0142x - 2.3986$	4.27×10^{-2}
CF 6	$y = 0.0078x - 2.5537$	2.33×10^{-2}
CF 7	$y = 0.0301x - 2.2555$	9.02×10^{-2}
CF 8	$y = 0.0142x - 2.6879$	4.26×10^{-2}
CF 9	$y = 0.0094x - 2.4197$	2.81×10^{-2}
CF 10	$y = 0.0249x - 2.5073$	7.48×10^{-2}
CF 11	$y = 0.0086x - 2.7182$	2.59×10^{-2}

4.5 LONG TERM STABILITY TESTING

The selected best performing carrier formulation was CF 11 and is further referred to as Formulation A. Long term stability testing was conducted using three testing procedures namely coalescence analysis, microscopy and cycle testing. These tests were conducted on Formulation A in its placebo form as well as with the addition of the HO, PM and LSSJ plant extracts. The complete Formulation A with its preparation procedure, is shown in Table 4-5. In this formulation the Active Ingredient in Phase C refers to the ethanolic plant extract and its mass percentage.

Table 4-5: Formulation CF 11 with the addition of the plant extract

Ingredient	%	Application	INCI/Chemical Name
		Phase A	
Water	77.2	Diluent/solvent	Aqua
EDTA	0.10	Chelating agent	Tetrasodium EDTA
Glycerin	1.80	Humectant	Glycerin
		Phase B	
Crodex M	6.30	Emulsifier	Cetostearyl Alcohol (and) Potassium Cetyl Phosphate
Crodamol STS	1.80	Emollient	PPG-3 Benzyl Ether Myristate
Crodamol SFX	1.80	Emollient	PPG-3 Benzyl Ether Ethylhexanoate
		Phase C	
Active ingredient	10.0	Active ingredient	Ethanolic Plant Extract
Germaben II	1.00	Preservative	Propylene Glycol (and) Diazolidinyl Urea (and) Methylparaben (and) Propylparaben

Method

1. Mix ingredients of Phase A together in a clean beaker and heat to 65 °C.
2. Mix ingredients of Phase B together in a clean beaker and heat to 65 °C.
3. Slowly add Phase B to Phase A while stirring using a Silverson® high shear mixer at 5000 rpm for 15 minutes.
4. Allow to cool to 40 °C with stirring using a magnetic stirrer.
5. Add the active ingredient and preservative, stirring after each addition.
6. Mix for 15 minutes until homogeneous.

4.5.1 Coalescence Analysis

Droplet size analyses were conducted on the samples for a period of 12 months. The coalescence plot for placebo Formulation A at storage conditions of 25 °C, 40 °C and 4 °C is shown in Figure 4-9. The coalescence plots for Formulation A with the addition of the plant extracts are also shown below, where the formulation with the addition of the HO plant extract is shown in Figure 4-10, PM plant extract is shown in Figure 4-11 and LSSJ plant extract is shown in Figure 4-12. A summary of the results obtained from the plots are shown in Table 4-6. In this table, the equations of the trendlines, calculated rates of coalescence, projected shelf lives and the minimum theoretical shelf lives of each formulation are given. Finally, Figure 4-13 shows the rate of coalescence as a function of temperature for each of the formulations.

In Figure 4-9 the slopes of all three trendlines for placebo Formulation A seem fairly close to each other. The placebo sample stored at room temperature had a slightly larger slope than the sample stored in the oven. The difference between these two slopes was 2×10^{-4} which is exceptionally small. It can therefore be concluded that storage temperature has very little effect on the placebo formulation. The droplet size distribution of the placebo formulation showed little variation over the testing period. For this reason, the slopes of

the trendlines were quite small. This indicated slow rates of coalescence and suggested a stable emulsion with a good shelf life.

Formulation A samples without any plant extract and inclusive of the HO and LSSJ, stored at 25 °C, showed minimum theoretical shelf-lives greater than 1 year as displayed in Table 4-6. The formulation containing the PM plant extract showed the shortest shelf-lives across all three storage conditions. This suggested a possible incompatibility of the PM plant extract with Formulation A. The formulation containing the LSSJ plant extract which was stored at 25 °C showed a slightly longer shelf life than the placebo formulation suggesting a synergistic effect between the extract and the formulation under normal conditions. The formulation containing the HO plant extract showed a much longer shelf life in the sample that was stored at 4 °C than under the other two storage conditions suggesting a sensitivity to the storage temperature. In general, the samples stored at 4 °C showed much longer shelf-lives implying that these products could be preserved at lower temperatures to extend the intended shelf life. Since the minimum theoretical shelf-lives at normal storage conditions exceed 1 year and at accelerated conditions, all exceed 6 months, it was concluded that Formulation A with the addition of HO and LSSJ plant extracts had acceptable shelf-lives.

The effect of the addition of the plant extracts on the stability of the emulsions is highlighted in Figure 4-13 where the rates of coalescence are plotted as a function of storage temperature. Here, the formulations with the addition of the plant extracts show a definite increase in rates of coalescence. This suggests that the ethanolic plant extracts have a destabilising effect on the emulsion formulation. Furthermore, the rate of coalescence showed a directly proportional relationship to the storage temperatures. This was expected as an increase in storage temperature accelerates coalescence and thereby accelerates instability.

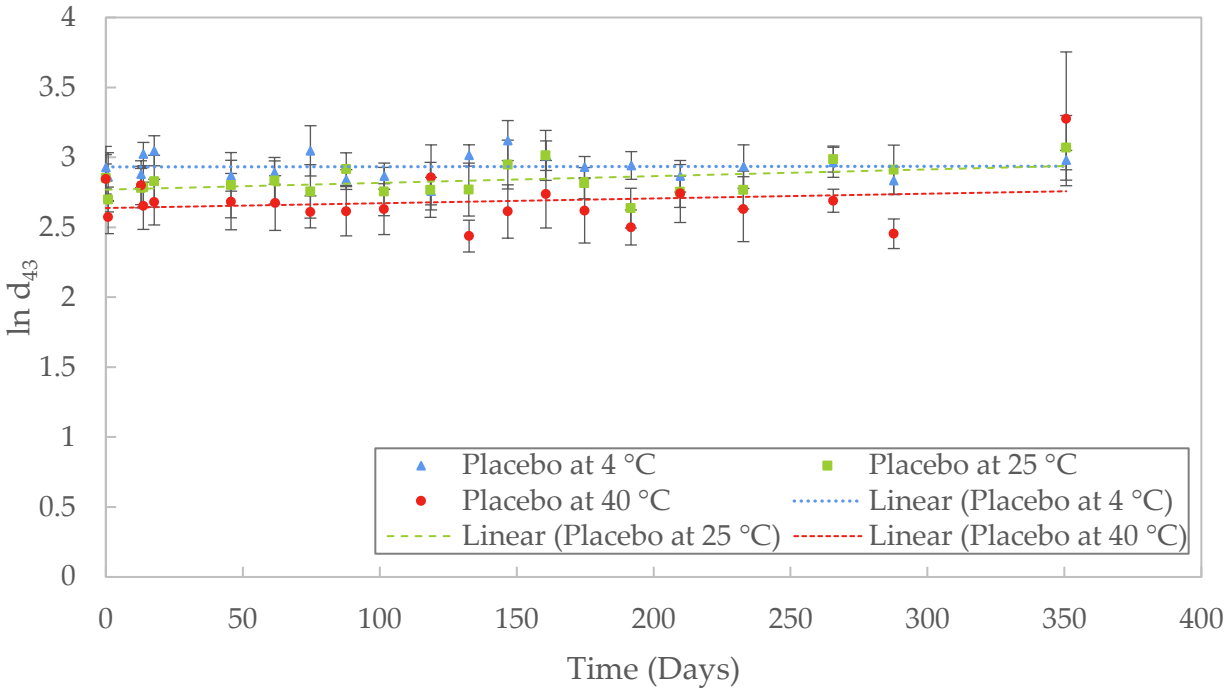


Figure 4-9: Coalescence plot of placebo Formulation A

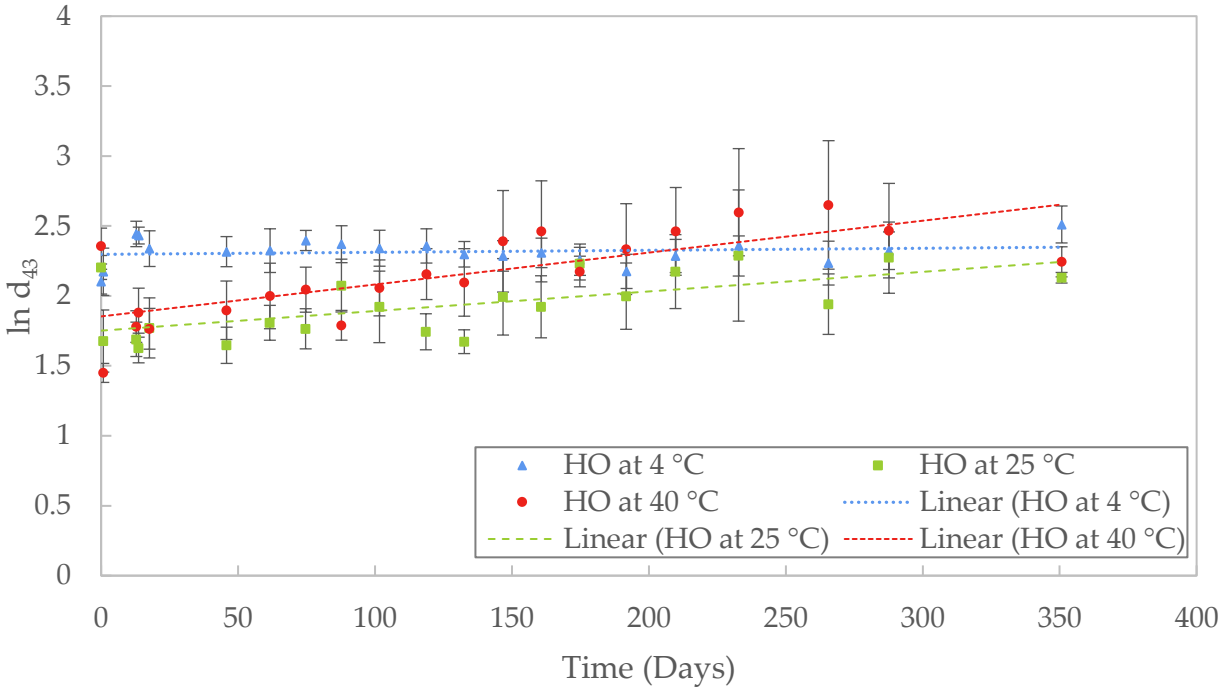


Figure 4-10: Coalescence plot of Formulation A with the HO plant extract

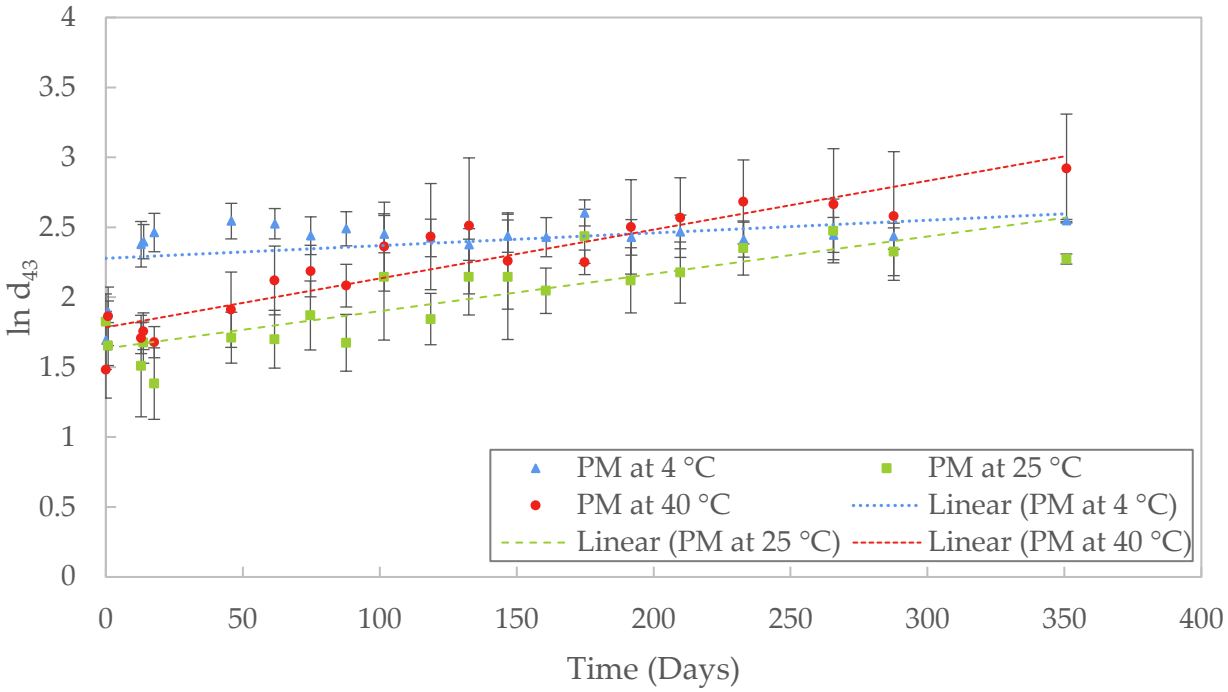


Figure 4-11: Coalescence plot of Formulation A with the PM plant extract

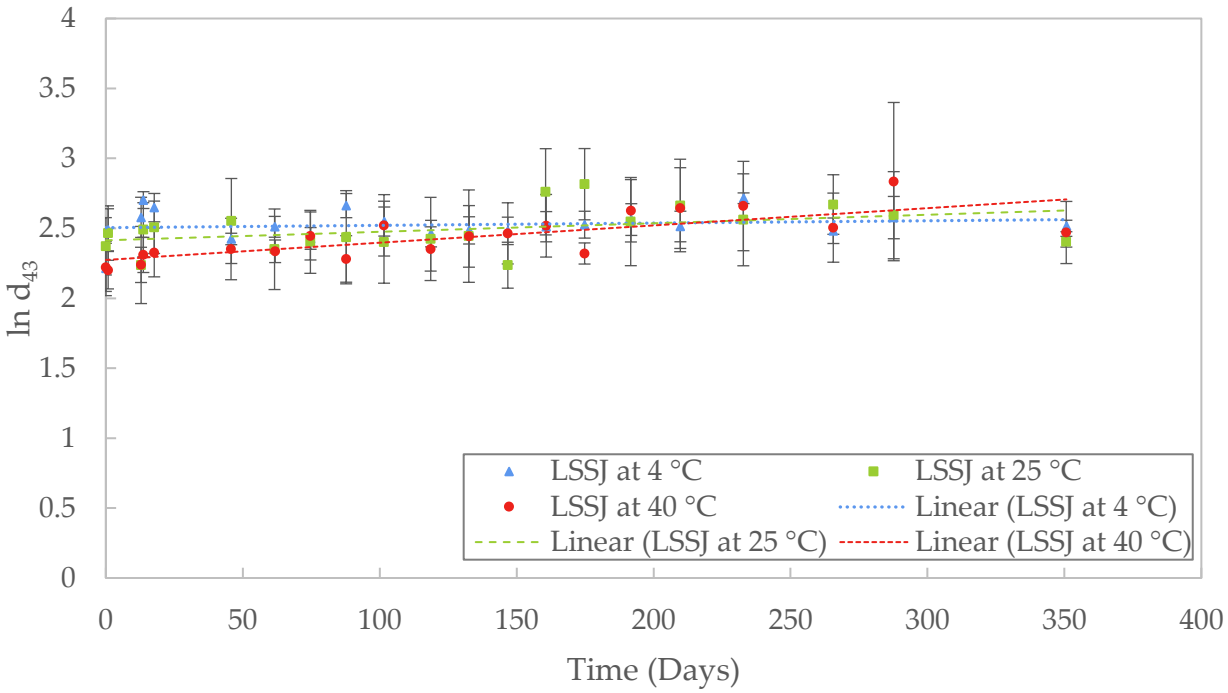


Figure 4-12: Coalescence plot of Formulation A with the LSSJ plant extract

Table 4-6: Summary of the trendline equations, calculated K_C, projected shelf life and minimum theoretical shelf life for Formulation A incorporated with the plant extracts

Temperature (°C)	Trendline Equation	K_C (Day^{-1})	Shelf Life (Years)	Minimum Shelf Life (Years)
Placebo Formulation A				
4	$y = 0.00001x + 2.9318$	0.03×10^{-3}	269 ± 7	262
25	$y = 0.0005x + 2.7694$	1.5×10^{-3}	6.26 ± 3	2.98
40	$y = 0.0003x + 2.6379$	0.9×10^{-3}	11.6 ± 2	8.57
HO in Formulation A				
4	$y = 0.0001x + 2.2971$	0.3×10^{-3}	44.2 ± 9	35.7
25	$y = 0.0014x + 1.7517$	4.2×10^{-3}	4.23 ± 3	1.48
40	$y = 0.0023x + 1.8534$	6.9×10^{-3}	2.45 ± 2	0.726
PM in Formulation A				
4	$y = 0.0009x + 2.2783$	2.7×10^{-3}	4.97 ± 2	2.42
25	$y = 0.0027x + 1.6336$	8.1×10^{-3}	2.31 ± 2	0.510
40	$y = 0.0035x + 1.7848$	10.5×10^{-3}	1.67 ± 1	0.291
LSSJ in Formulation A				
4	$y = 0.0002x + 2.5045$	0.6×10^{-3}	19.3 ± 6	13.6
25	$y = 0.0006x + 2.4143$	1.8×10^{-3}	6.84 ± 3	3.74
40	$y = 0.0012x + 2.2737$	3.6×10^{-3}	3.74 ± 2	1.14

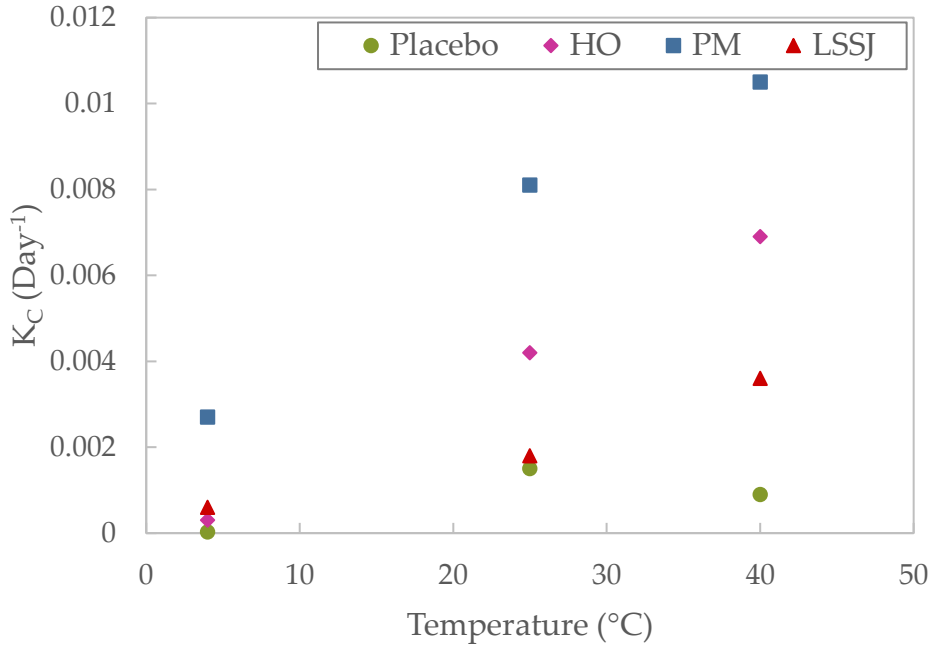


Figure 4-13: Graphic representation of the apparent rate of coalescence of each formulation as a function of temperature

4.5.2 Microscopy

Optical micrographs were obtained at the beginning and end of the 12 month testing period in order to compare droplet sizes.

Figure 4-14 shows the four micrographs of placebo Formulation A. A slight change in droplet size was observed between the initial micrograph and the final micrograph of the sample stored at 4 °C. This was verified by the droplet size distribution and coalescence plot shown in the previous section where a low coalescence rate was observed. A definite increase in the droplet size was observed between the initial micrograph and final micrographs of the samples stored at 25 °C and 40 °C. These two samples showed a faster rate of coalescence than the sample stored at 4 °C.

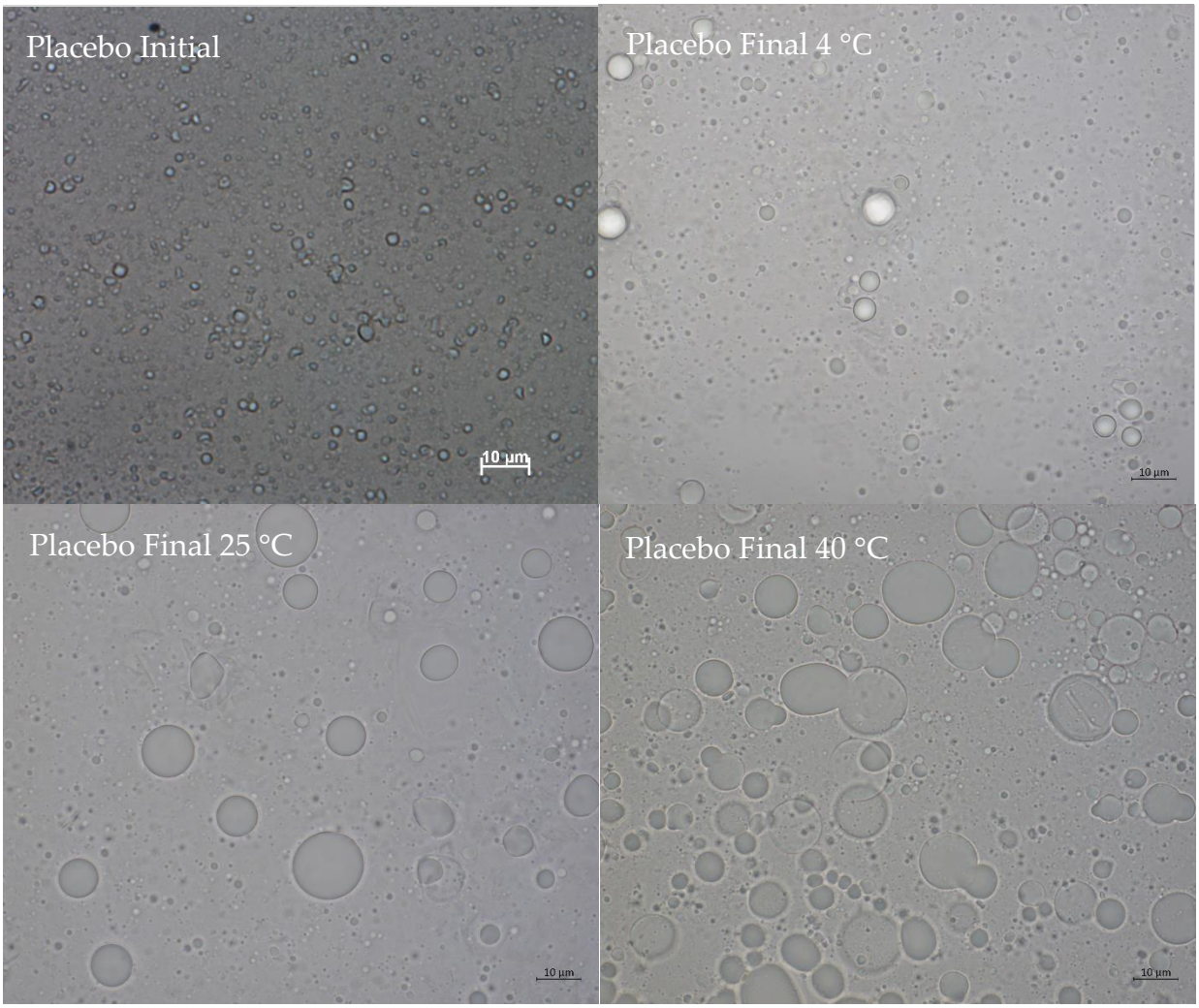


Figure 4-14: Micrographs of placebo Formulation A

The micrographs of Formulation A with the addition of the HO plant extracts is shown in Figure 4-15. Although the sample stored at 4 °C showed the largest increase in droplet size over the testing period the coalescence rate for this samples was found to be the smallest. This was due to the fact that an initial increase in droplet size was observed over the first two weeks after which the droplet size remained fairly constant.

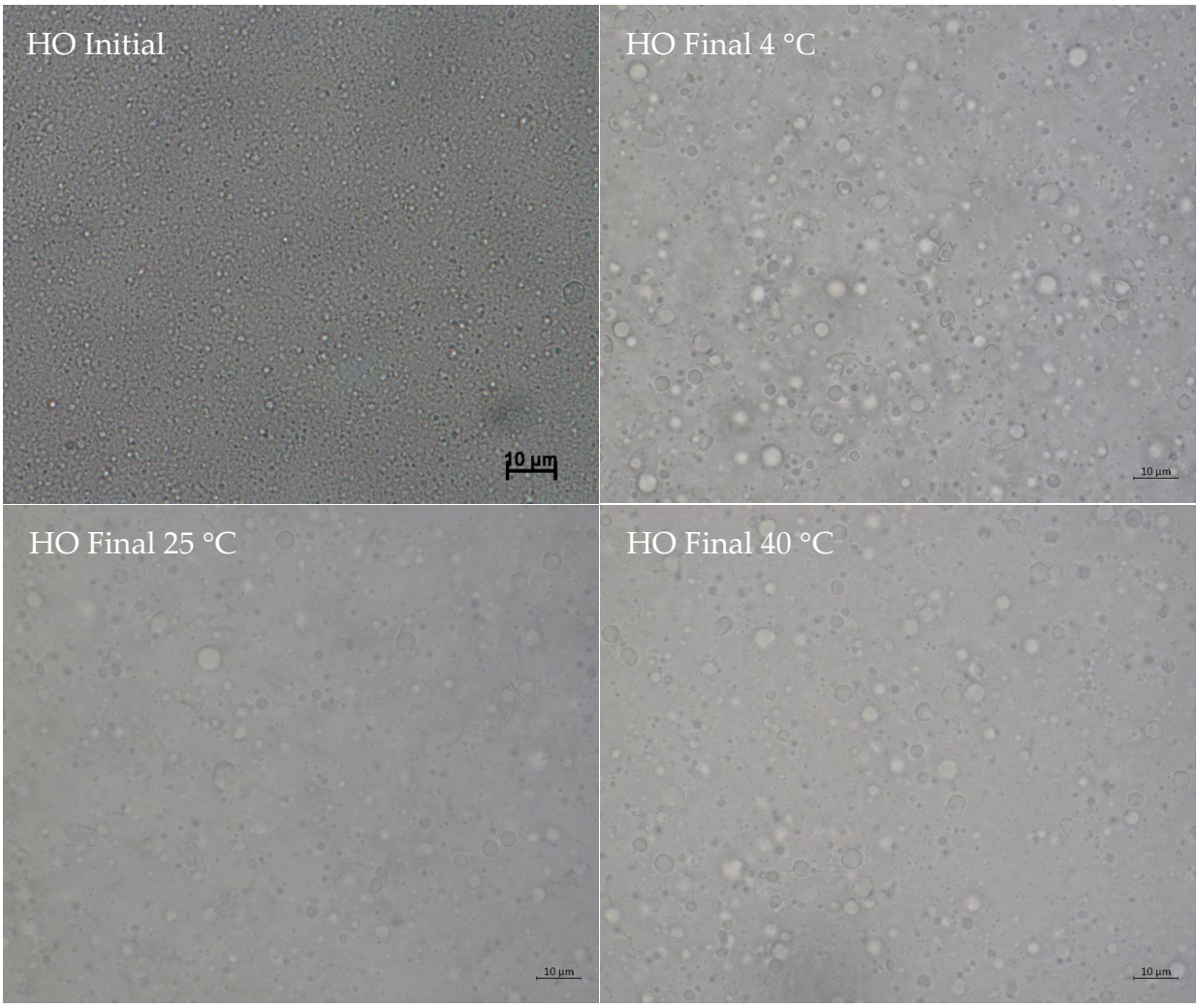


Figure 4-15: Micrographs of Formulation A with the HO plant extract

Figure 4-16 shows the micrographs for Formulation A with the addition of the PM plant extract at the start and end of the testing period. The droplet sizes of the 25 °C and 40 °C micrographs look fairly similar to each other. This is confirmed by their similar rates of coalescence. A slight increase in droplet size was noticed between the initial and final micrographs which is confirmed by the relatively slow rates of coalescence.

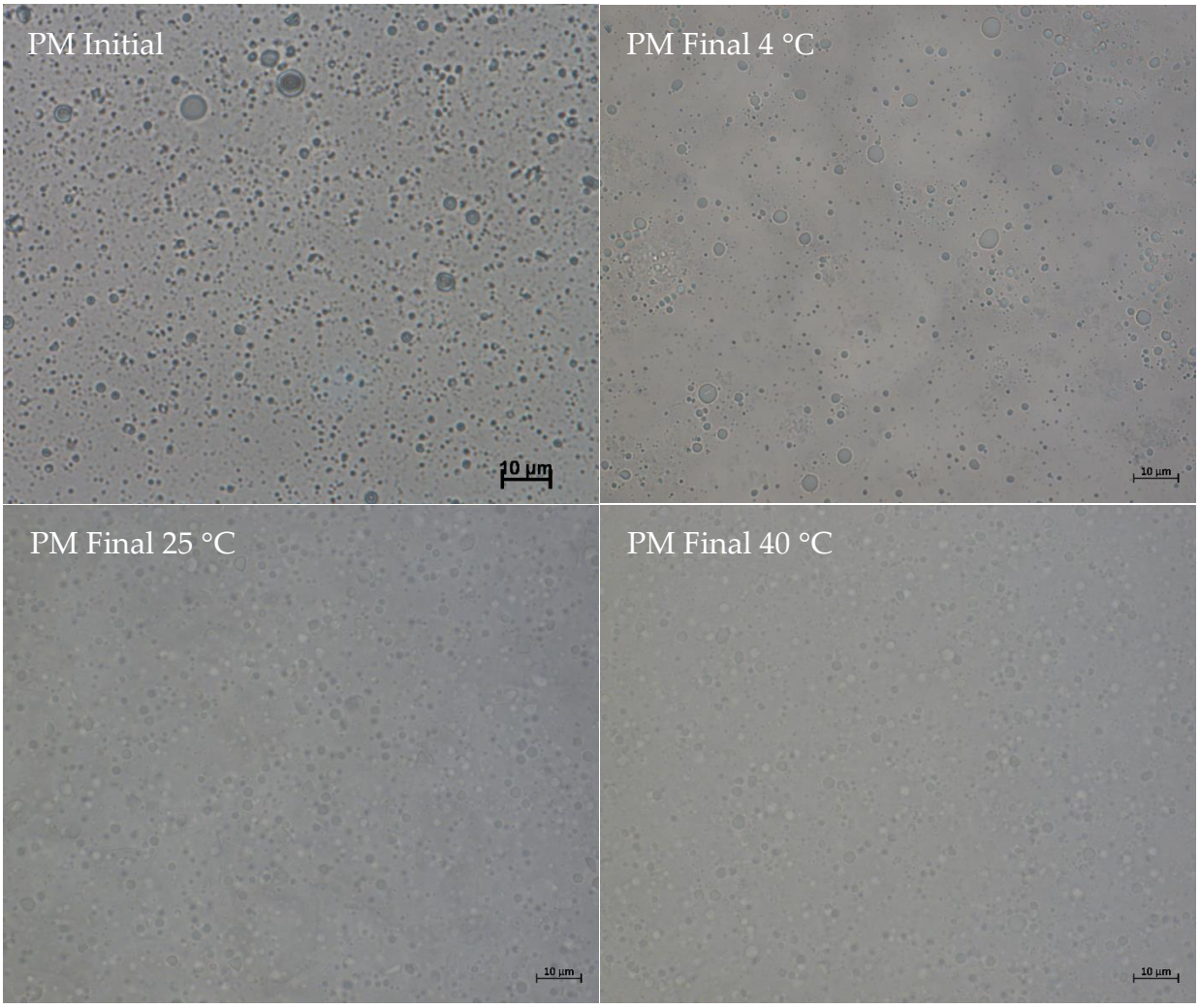


Figure 4-16: Micrographs of Formulation A with the PM plant extract

The micrographs of Formulation A with the addition of the LSSJ plant extracts is shown in Figure 4-17. Although a small increase in droplet size was observed over the testing period a trend was noticed where the change droplet size is directly proportional to the storage temperature. This was confirmed by the coalescence results where the rate of coalescence showed a direct proportionality to the storage temperature of the formulation.

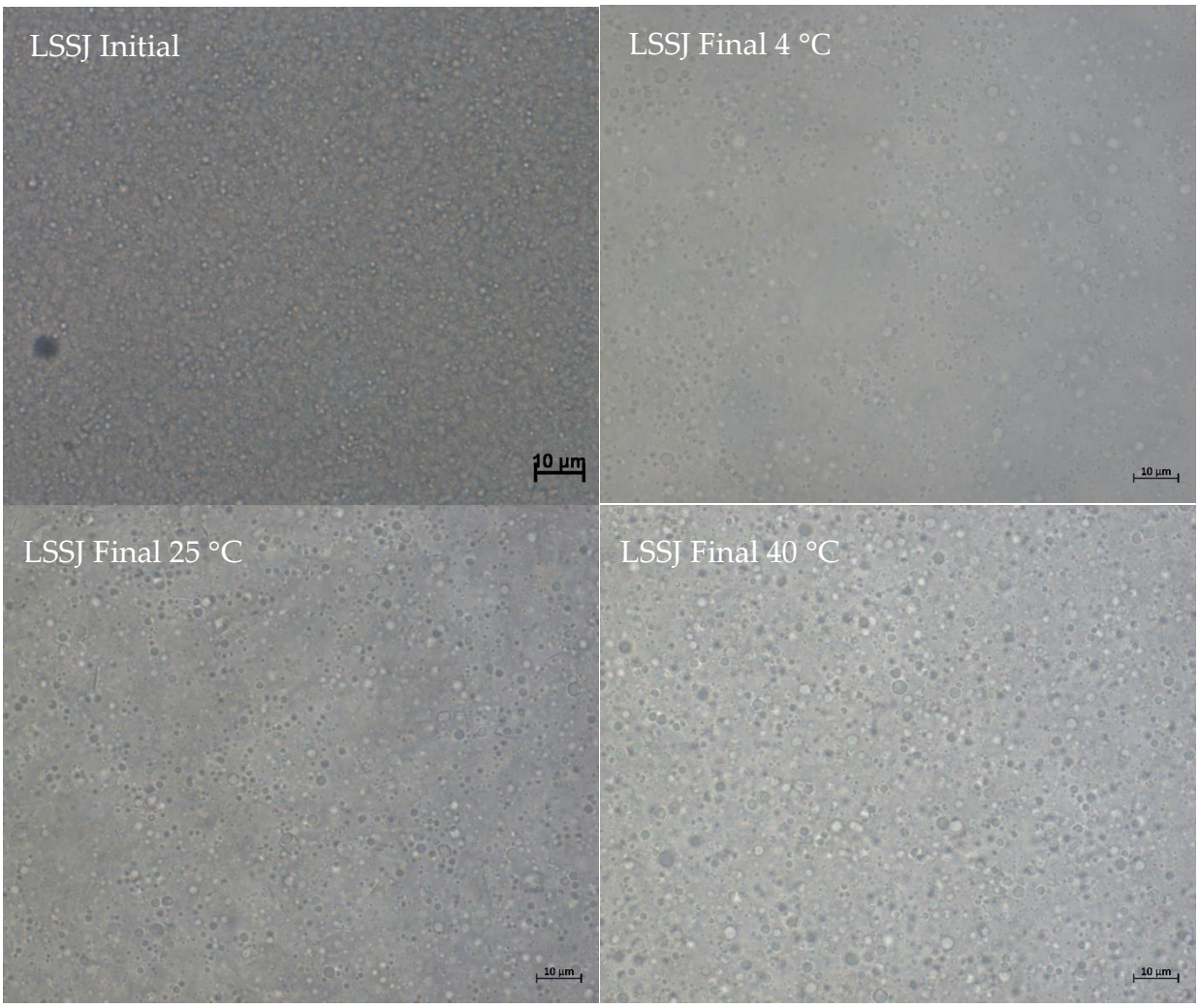


Figure 4-17: Micrographs of Formulation A with the LSSJ plant extract

4.5.3 Cycle testing

Cycle testing was conducted on Formulation A in its placebo form and with the addition of the plant extracts. These samples underwent six cycles between 4 °C and 40 °C followed by five cycles between -18 °C and 40 °C. After each cycle the pH of the samples were measured, droplet size analysis was done and the samples were observed visually after undergoing centrifugation.

4.5.3.1 pH Analysis

The pH of the samples was measured at the end of each cycle and is shown graphically in Figure 4-18. All four formulations showed a larger variation in pH over the last five cycles which were the freezer-oven cycles. This variation is owed to the increase in stress due to the freezing and thawing of the emulsions. In all four graphs the pH over the cycles show variation of less than 1 pH unit over the cycling period. This implies that the formulations are all stable. The overall pH of placebo Formulation A was 4.06 ± 0.03. The pH of Formulation A with the addition of HO, PM, and LSSJ was 4.03 ± 0.03, 4.13 ± 0.03, and 4.02 ± 0.03 respectively. Since the pH of all four formulations stayed within the range of 4 and 6 across stressed conditions, all four formulations are suitable for skin care applications.

4.5.3.2 Droplet Size Analysis

Droplet size analysis was conducted at the end of each 48 hour cycle. The median droplet diameter, d_{50}, at the end of each cycle is shown graphically in Figure 4-19. Formulation A in its placebo form and the formulations containing the HO and PM plant extracts showed a drastic increase in droplet size at the end of cycle 7. This was expected as cycle 7 was the first freezer-oven cycle where the samples were exposed to increased stress conditions during freezing and thawing. These samples all showed evidence of freezer burn after cycle 7 where the samples became thicker and drier. In the cycles thereafter, the samples returned back to its creamy state with a slightly lower moisture content than the initial sample. This is also evident in Figure 4-19 where the median droplet size decreased with every cycle over the five freezer-oven cycles.

Formulation A containing LSSJ performed differently to the other samples. The initial median droplet size of this sample was much larger than the other three samples. This

sample showed bigger fluctuations in the droplet sizes which can be seen by the larger error bars. The first freezer-oven cycle, cycle 7, showed a smaller increase in the droplet size of this sample. This was however, followed by a decrease in droplet size over the last five cycles similar to that observed in the graphs of the other three samples.

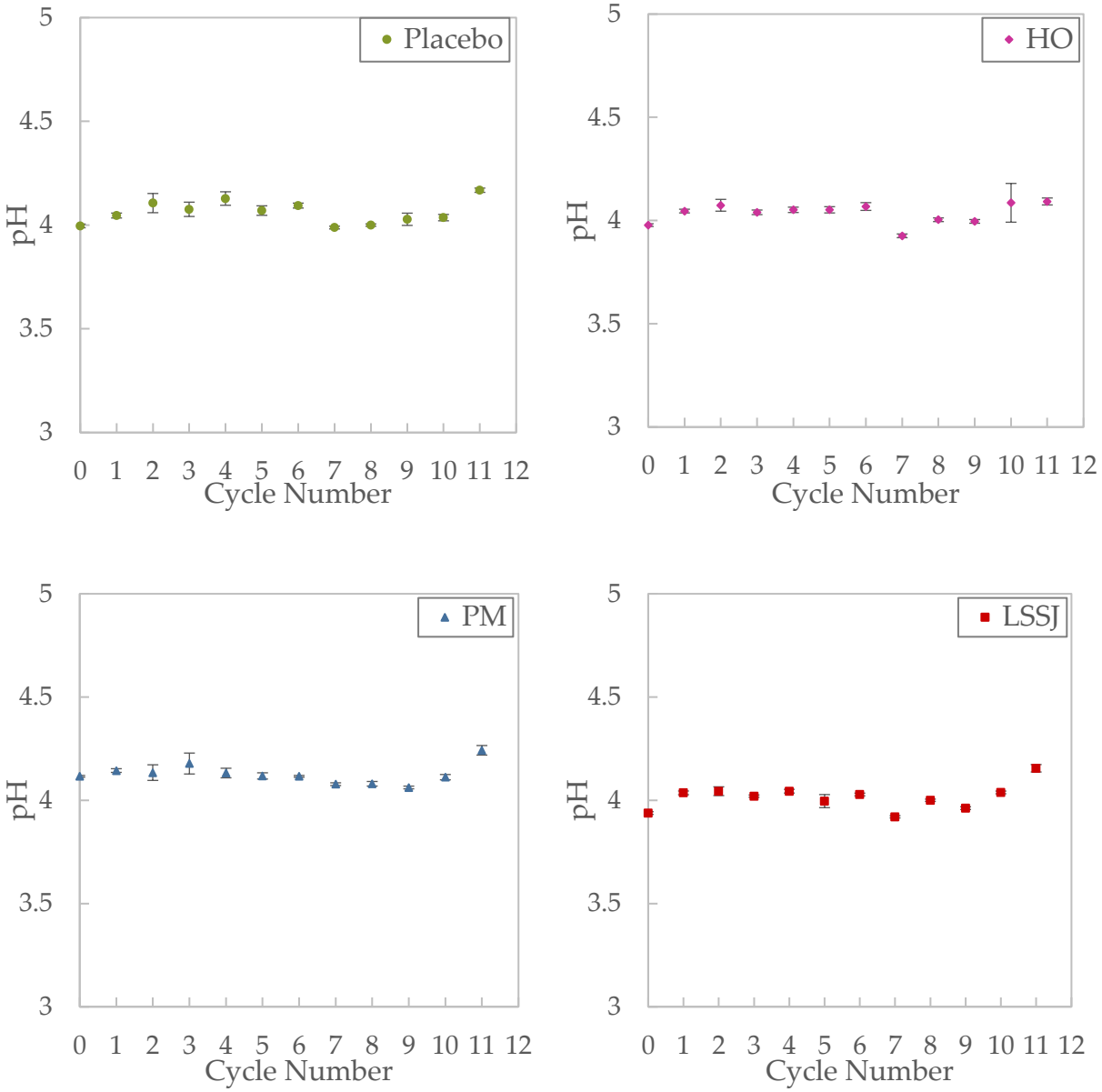


Figure 4-18: pH results over six fridge-oven cycles followed by five freezer-oven cycles

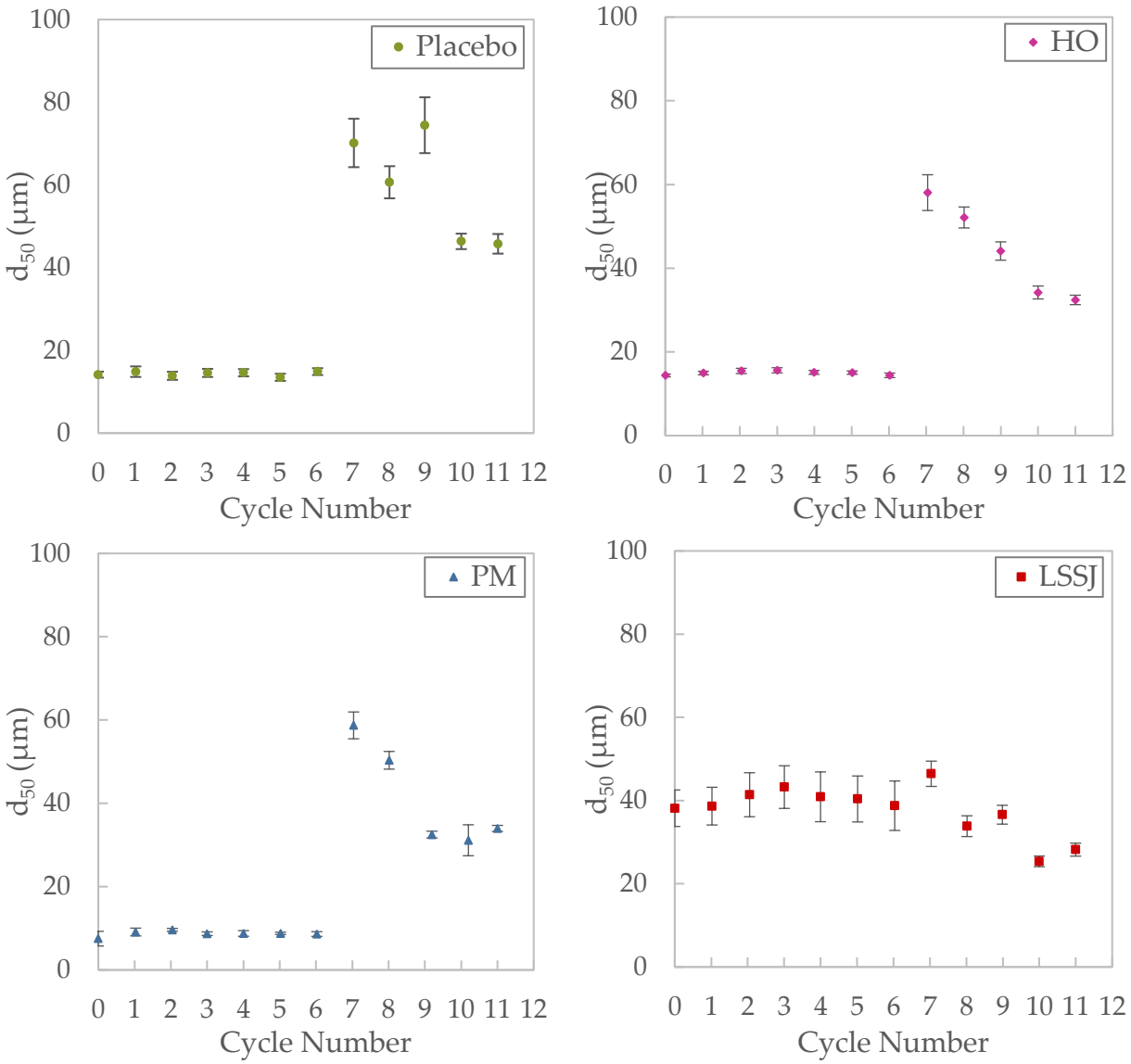


Figure 4-19: Median droplet size results over six fridge-oven cycles followed by five freezer-oven cycles

4.5.3.3 Centrifugation

Visual observations were made and recorded after samples underwent centrifugation of 3000 rpm for 30 minutes. Over all eleven cycles none of the formulations showed any signs of separation. All four formulations performed exceptionally well since no sign of creaming, sedimentation or phase separation was evident as samples withstood extreme

temperature conditions. This showed that Formulation A in its placebo form and with the addition of the plant extracts have good stability even under extreme conditions.

4.6 COSTING ANALYSIS

A costing analysis was done on placebo Formulation A and the cost breakdown is shown in Table 4-7. The cost of each raw material was obtained from bulk suppliers. Using the mass percentage of each component the cost of 100 g of the formulation was calculated and found to be R 2.16.

Table 4-7: Cost breakdown of placebo Formulation A

Ingredient	**INCI/Chemical Name**	**%**	**Cost (R/kg)**
Water	Aqua	85.9	0.00714
EDTA	Tetrasodium EDTA	0.10	34.40
Glycerin	Glycerin	2.00	14.45
Crodex M	Cetostearyl Alcohol (and) Potassium Cetyl Phosphate	7.00	67.00
Crodamol STS	PPG-3 Benzyl Ether Myristate	2.00	387.00
Crodamol SFX	PPG-3 Benzyl Ether Ethylhexanoate	2.00	368.00
Germaben II	Propylene Glycol (and) Diazolidinyl Urea (and) Methylparaben (and) Propylparaben	1.00	143.00

Croda (SA) is the main supplier of Crodex M, Crodamol STS and Crodamol SFX. The minimum order quantity of each of these ingredients is 25 kg. These three ingredients make up 92% of the total cost of this formulation. For this reason, identifying an alternate supplier or similar generic materials for these ingredients may prove beneficial in reducing the overall cost of the formulation.

4.7 CONCLUSION

In developing Formulation A, existing formulations were considered which were sourced from raw material suppliers. These formulations were used as a guideline to formulate samples of different carrier formulations types including gels, body milks, creams and lotions. From the sensory evaluation the most appealing carrier formulations types were identified. This was a body milk (CF 5), light cream (CF 8), and light lotion (CF 11).

The preliminary stability of the placebo carrier formulations was tested by means of microscopy and coalescence analysis. Comparison between the initial micrographs and the droplet size distribution results obtained from the Mastersizer 3000™ showed good agreement with little variation in the results. This supported the use of the Mastersizer 3000™ in analysing coalescence in this study. Coalescence analysis showed slow rates of coalescence for formulations CF 5, CF 8 and CF 11, with CF 11 showing the slowest coalescence rate and therefore the longest shelf life. Considering the sensory appeal and preliminary stability, CF 11 was selected as the best performing formulation and was referred to as Formulation A.

Long term stability testing was conducted on Formulation A in its placebo form and with the addition of the HO, PM and LSSJ plant extracts. The testing procedure involved coalescence analysis, microscopy and cycle testing. Through coalescence analysis it was found that the formulation with the plant extract showed an increase in the rate of coalescence suggesting a possible destabilizing effect by the ethanolic plant extract. The storage temperature showed little effect on the rate of coalescence of the placebo formulation suggesting temperature resistance. The formulations with the addition of the plant extracts showed a direct proportionality between coalescence rate and storage temperature. The minimum theoretical shelf life of the placebo formulation and the formulations containing the HO and LSSJ plant extracts were more than 1 year making

Formulation A viable for long term applications with these plant extracts. The sample containing the PM plant extract showed a minimum theoretical shelf lives of less than 1 year at a storage temperature of 25 °C. This makes Formulation A unfavourable for the addition of the PM plant extract.

The microscopy results verified the droplet size analysis and showed the change in the droplet size and shape due to time and the various storage conditions. During cycle testing over extreme stress conditions the samples performed well. There were signs of degradation through observation of droplet size analysis over the freezer-oven cycles. This was expected due to extreme freeze-thaw conditions and was not indicative of an unstable formulation since this was a harsh test. The stability of Formulation A in its placebo form and with the addition of the plant extracts was substantiated by the pH and centrifugation results which remained constant and did not show signs of instability. The pH of all four samples remained between the values of 4 and 6 making it viable for skin care usage.

The costing analysis of placebo Formulation A showed a raw material cost of R 2.16 for 100 g of sample. Crodex M, Crodamol STS and Crodamol SFX made up 92% of the cost of the sample. Therefore, the cost of Formulation A could be reduced by finding an alternate supplier for Crodex M, Crodamol STS and Crodamol SFX or finding generic versions or similar, more cost-effective ingredients to replace these ingredients. This is the subject of the next chapter.

CHAPTER 5: FORMULATION CAMPAIGN B

5.1 INTRODUCTION

A main objective of Formulation B was to develop a stable formulation to provide a more cost-effective option than Formulation A. For this reason, locally produced and less expensive chemicals were used as alternatives to the emulsifier and emollients used in Formulation A.

Since the findings of Chapter 4 showed that the best performing carrier formulation type was a lotion, all formulations developed in this chapter were lotions. A more in-depth sensory evaluation was conducted on the placebo formulations developed in this chapter. This sensory evaluation involved four separate tests. The placebo formulations used in the sensory evaluation underwent preliminary stability testing which considered coalescence analysis. In addition, a mock-extract consisting of a 40 % (mass based) ethanol solution in water without the plant powder, was added to formulations at two levels, 10 % and 20 % by mass, in order to investigate the potential effect of extract loading on formulation stability. Considering the preliminary stability results and the sensory evaluation results, the best performing formulation was selected as Formulation B.

Formulation B was remade in its placebo form and with the addition of the HO, PM and LSSJ plant extract at a 10% mass-based loading level. These formulations underwent long term stability testing which involved coalescence analysis, microscopy and cycle testing. The BS plant extract was not included in Formulation B as the core interest of BS was its incorporation in a sunscreen formulation which is the subject of Chapter 6. A costing analysis was finally conducted on Formulation B in its placebo form and compared to that of the Formulation A.

5.2 PLACEBO FORMULATION DEVELOPMENT

In formulating, a number of ingredients were selected based on their cost. A core constraint was using a local producer. A local agent, Fourchem, was identified that offers commonly used cosmetic ingredients manufactured by local producers. The ingredients selected included two humectants, two emulsifiers, and two emollients. All the ingredients used, together with its function and INCI or chemical names, are shown in Table 5-1. The chosen thickening agent was Carbopol 940. This thickening agent requires pH adjustment to activate its thickening ability. For this reason, sodium hydroxide was used to neutralise the formulations.

Table 5-1: A list of the ingredients used to formulate Formulation B

Trade Name	**INCI/ Chemical Name**	**Function**
Water	Aqua	Diluent/solvent
EDTA	Tetrasodium EDTA	Chelating Agent
Carbopol 940	Carbomer	Thickener
Sodium Hydroxide	Sodium Hydroxide	Neutralising Agent
Glycerin	Glycerin	Humectant
Propylene Glycol	Propylene Glycol	Humectant
Palmerol 6830	Cetostearyl Alcohol	Emulsifier
ERCAWAX CS 20 V/FD	Ceteareth 20	Emulsifier
ERCAREL AB V	C12-15 Alkyl Benzoate	Emollient
Imex MCT 60/40	Capric/Caprylic Triglyceride	Emollient
Germaben II	Propylene Glycol (and) Diazolidinyl Urea (and) Methylparaben (and) Propylparaben	Preservative

A number of different lotion formulations were made up by using formulating guidelines, varying the ingredients and varying the quantities of the ingredients. A list of the trial formulations is shown in Table 5-2. The amount of sodium hydroxide added to certain formulations is described as "Q.S." This is an abbreviation for the Latin term "Quantum satis" meaning the amount which is enough. This simply implies that the sodium hydroxide was added to the respective formulations until a pH of 5.5, which is the average pH of skin, was obtained. In formulation of T12 the pH was increased to a value of 7 and therefore the amount of sodium hydroxide added is described as "Q.S. (7)".

Using a small-scale sensory evaluation, with ten participants, the formulations were eliminated based on ease of absorption and immediate afterfeel. Using these criteria, the five best formulations were T14, T6, T7, T13, and T4. These formulations were renamed as L1, L2, L3, L4 and L5 and were subjected to a more rigorous sensory evaluation procedure. The complete formulation recipes and preparation methods for the five chosen formulations are shown in Table 5-3.

The emulsifiers used were in the form of waxes. These emulsifiers are known as self-emulsifying waxes therefore high shear was not required to combine phases A and B or induce emulsification. A magnetic stirrer at 1000 rpm was used to combine the phases and an operating temperature of 65 °C was chosen. This temperature was sufficiently high to melt the waxes and low enough to avoid degradation of the ingredients.

Formulations L1 and L2 included a Carbomer. For this reason, sodium hydroxide was added to the formulations to neutralise the pH. A pH of 5.5 was selected as this pH sufficiently increased the viscosity of the samples. In addition to this, the average pH of skin is 5.5 therefore the formulations would be viable for skin care applications at this pH. During pH adjustment the samples were continuously agitated and sodium hydroxide was added dropwise while monitoring the pH until a reading of 5.5 was obtained.

Table 5-2: Trial formulations made up using common cosmetic ingredients

Trial Formulation	Ingredient (%)										
	Water	EDTA	Glycerin	Propylene Glycol	Carbopol 940	Palmerol 6830	ERCAWAX CS 20 V/FD	ERCAREL AB V	Imex MCT 60/40	Germaben II	Sodium Hydroxide
T1	86.9	0.1	2			3	3	4		1	
T2	86.9	0.1	2			2	2	6		1	
T3	76.9	0.1	2			3	5	12		1	
T4	81.9	0.1	2			6	4	5		1	
T5	81.9	0.1	2			8	4	3		1	
T6	85.8	0.1	2		0.1	3.5	3.5	4		1	Q.S.
T7	86.9	0.1	2			3	3		4	1	
T8	85.8	0.1	2		0.1	3.5	3.5		4	1	Q.S.
T9	81.9	0.1	2			7	5	3		1	
T10	81.9	0.1	2			8	4		3	1	
T11	81.3	0.1	2		0.1	7.5	5	3		1	Q.S.
T12	85.8	0.1	2		0.1	3.5	3.5	4		1	Q.S. (7)
T13	86.9	0.1		2		3	3	4		1	
T14	85.8	0.1		2	0.1	3.5	3.5	4		1	Q.S.
T15	86.9	0.1		2		3	3		4	1	
T16	85.8	0.1		2	0.1	3.5	3.5		4	1	Q.S.

Table 5-3: The complete formulations of the top five trial lotions

			Mass Percentage (%)				
Ingredient	**Function**	**INCI/Chemical Name**	**L1**	**L2**	**L3**	**L4**	**L5**
		Phase A					
Water	Diluent/solvent	Aqua	85.8	85.8	86.9	86.9	81.9
EDTA	Chelating Agent	Tetrasodium EDTA	0.10	0.10	0.10	0.10	0.10
Glycerin	Humectant	Glycerin		2.00	2.00		2.00
Propylene Glycol	Humectant	Propylene Glycol	2.00			2.00	
Carbopol 940	Thickener	Carbomer	0.10	0.10			
		Phase B					
Palmerol 6830	Emulsifier	Cetostearyl Alcohol	3.50	3.50	3.00	3.00	6.00
ERCAWAX CS 20 V/FD	Emulsifier	Ceteareth 20	3.50	3.50	3.00	3.00	4.00
ERCAREL AB V	Emollient	C12-15 Alkyl Benzoate	4.00	4.00		4.00	5.00
Imex MCT 60/40	Emollient	Capric/Caprylic Triglyceride			4.00		
		Phase C					
Germaben II	Preservative	Propylene Glycol (and) Diazolidinyl Urea (and) Methylparaben (and) Propylparaben	1.00	1.00	1.00	1.00	1.00

			Mass Percentage (%)				
Ingredient	**Function**	**INCI/Chemical Name**	**L1**	**L2**	**L3**	**L4**	**L5**
Sodium Hydroxide	Neutralising Agent	Sodium Hydroxide	QS	QS			

Method

1. Mix ingredients of Phase A together in a clean beaker and heat to 65 °C.
2. Mix ingredients of Phase B together in a clean beaker and heat to 65 °C.
3. Slowly add Phase B to Phase A while stirring using a magnetic stirrer at 1000 rpm for 15 minutes until homogeneous.
4. Allow to cool to 40 °C with gentle stirring using the magnetic stirrer.
5. Add the preservative, stirring after addition.
6. Add the sodium hydroxide dropwise while measuring the pH until a pH of 5.5 is reached.
7. Mix for 15 minutes until homogeneous.

5.3 SENSORY EVALUATION

For the more rigorous sensory evaluation that formulations L1, L2, L3, L4 and L5 were subjected to, ASTM E 1490-03 - the standard practice for descriptive skinfeel analysis of creams and lotions - was considered. The original procedure comprises of many short sessions which span over a period of 1 month. The methodology goes through a number of phases which take panellists through screening, training and finally product evaluation. This study was conducted on campus where volunteers had between 1 and 2 hours to partake and little commitment to attend multiple sessions. For this reason, the E 1490 standard was adapted to span over a maximum period of 90 minutes. To ensure validity, all phases were retained within the single session.

5.3.1 Participant Demographics

The testing was conducted on campus at the University of Pretoria, therefore, the majority of participants were between the ages of 18 and 25. Care was taken to include participants above the age of 25 but minors under the age of 18 were not included. To ensure safety, participants were asked if they had ever had allergic or adverse reactions to creams, lotions or fragrances, if they had any skin or health conditions, or if they were taking any medication. Participants who answered affirmatively to any of these questions were advised to refrain from further participation in the sensory evaluation.

Since skin type plays a role in sensory perception, participants were asked to give information describing their skin type. This included giving their age and gender. Furthermore, volunteers were asked to select criteria that best described their skin type based on UV sensitivity as well as their skin hydration state. The form given to participants to gain this insight is shown in Figure 5-1.

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Welcome to our Sensory Testing

Age:		Gender:	Male	Female
Date:		Time:		

1. Please select your skin type based on its UV sensitivity:

Type I	Type II	Type III	Type IV	Type V	Type VI
-Never tans. -Always burns. -Extremely sun sensitive.	-Tans minimally. -Burns easily. -Very sun sensitive.	-Tans to a light brown. -Burns moderately. -Sun sensitive.	-Tans to a moderate brown. -Very little burning. -Minimal sun sensitivity.	-Tans well to a dark brown. -Seldom burns. -Sun-insensitive.	-Tans profusely. -Never burns. -Sun-insensitive.

2. Please select your skin type based on it hydration state:

Normal skin	Dry skin	Oily skin	Combination skin (Please specify, i.e. normal and oily) _____	Sensitive skin

3. Have you ever had an allergic or adverse reaction to any lotion, fragrance or cream? If yes, describe

_____
_____
_____
_____
_____

4. Do you have any of the following?

	Yes	No
Psoriasis		
Eczema		
Central nervous system disorder		
Unusually cold or warm hands		
Skin rashes		
Calluses on hands/fingers		
Hypersensitive skin		
Tingling in the fingers		

5. Are you currently taking any medication? Please list.

_____
_____
_____
_____
_____
_____
_____

Ballot number:	
Room Temperature:	
Room Humidity:	
Skin Temperature:	

Figure 5-1: Personal details form used to gain insight into the participants skin type

Forty-four participants partook in the sensory evaluation. These participants were between the ages of 18 and 30. The distribution of the participants in terms of gender is shown in Figure 5-2. From this it was noticed that there were a few more male participants than female participants. The skin type of participants is shown in Figure 5-3 and Figure 5-4 which is based on UV sensitivity and skin hydration state respectively. Figure 5-3 shows a fairly equal distribution amongst all the skin types but a majority of the participants had skin type VI. This may be due to the fact that skin type VI is common amongst the African and Indian race groups. Typical properties of this skin type include profuse tanning, never burning and insensitivity to the sun. Finally, the distribution based on skin hydration state showed the presence of all skin types. As expected, a large majority of participants had a normal skin hydration state. This was ideal for this study as the formulations developed in this investigation were not designed for any specific skin hydration state which may require more or less oily properties.

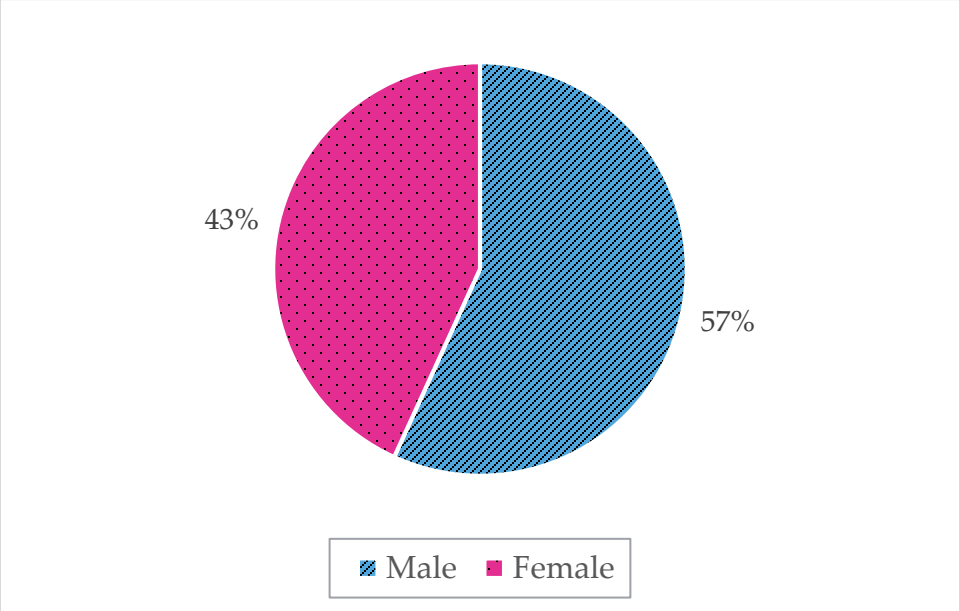


Figure 5-2: Participant distribution amongst genders

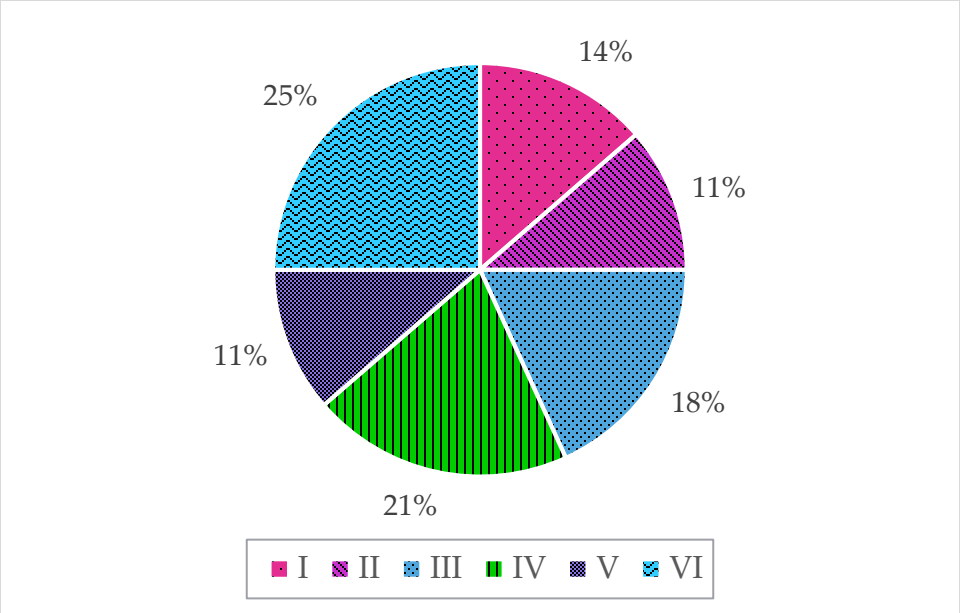


Figure 5-3: Participant distribution amongst skin types based on UV sensitivity

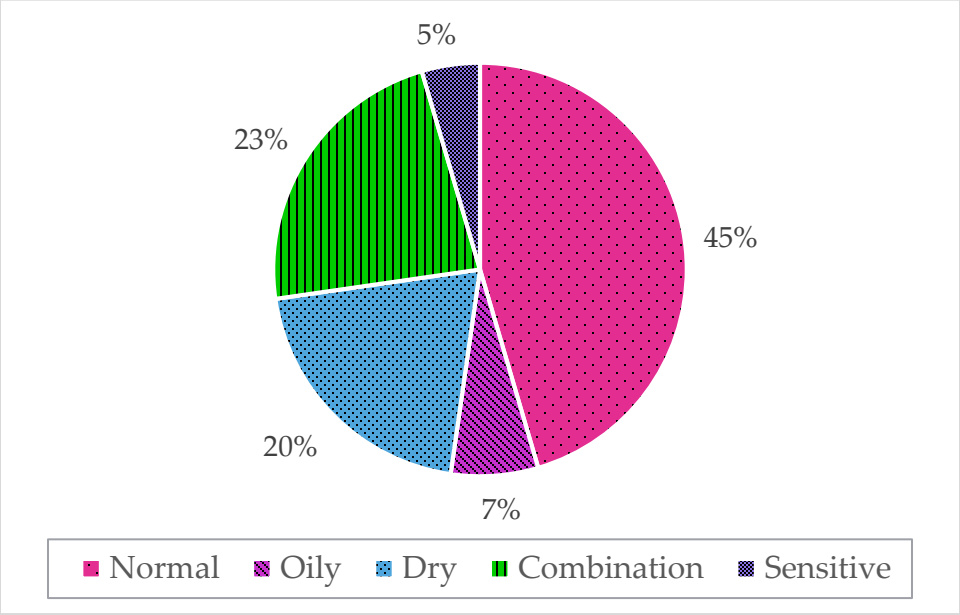


Figure 5-4: Participant distribution amongst skin types based on skin hydration state

5.3.2 Testing Conditions

The environmental conditions of the testing room were controlled as far as possible. The venue wherein the evaluation took place was temperature and humidity controlled. To avoid bias participants were taken through a short orientation while they acclimatised to the conditions of the room. The conditions of the room were monitored by measuring the humidity of the room using a hygrometer, the room temperature using a thermometer, and the participant's skin temperature using a skin thermometer. The average humidity of the room was measured as 29.3 ± 0.4 %. The average temperature of the room was 24.3 ± 0.2 °C and the average skin temperature of the participants was 31.6 ± 0.5 °C. The error of these measurements was quite low showing little bias toward the environmental conditions of the testing room.

The room had no natural lighting. The venue had no windows since it was on a basement level. Fluorescent tube lights were the core lighting of the room ensuring a consistent light source and viewing conditions for the evaluation of shine across all testing sessions.

The samples were all kept in the same packaging to avoid bias based on the colour of the samples. The sample packaging was chosen to be white, opaque bottles with a lotion pump attachment. This ensured that a uniform quantity was dispensed to each participant with every pump. Each bottle was labelled numerically between 1 and 6 and this number corresponded to the column wherein participants recorded their ratings. The labels of the bottles were changed over the test period to ensure no bias exists.

5.3.3 Testing procedure

The sensory evaluation consisted of four tests namely rub out, product delivery, pick up and afterfeel evaluations. The results of the tests are discussed separately in the next section.

During the orientation process participants were informed on various definitions used throughout the evaluation. A touch quiz was done to familiarise participants with general tactile properties and descriptive words they may use to describe characteristics. A scaling exercise was also done to acquaint participants with the rating scale.

In each test reference materials were used to represent the extremes of the rating scale. A scale of 0 to 10 was used where participants were asked to rate specific criterion by comparing the product to the reference materials. A simple example is explained as follows. Participants were asked to rate the wetness of the product where the wetness is described as the amount of water perceived whilst rubbing. The reference materials were talc powder, which represented a rating of 0, and water, which represented a rating of 10. After touching the talc powder, the water and the product, participants could decide on and record an appropriate rating which best described the product. The ballot sheet for each test showed the criteria, its definition and the two reference materials for a rating of 0 and 10.

After the reference materials of the rating extremes were presented and participants were allowed to feel the different materials, they were instructed to wash their hands and forearms for at least 10 seconds with a provided aqueous surfactant solution of 5 % TEA-Lauryl Sulfate. Approximately 5 minutes after drying the forearms six tests sites, three on each arm, were marked. This was done using a template circle, which had a diameter of 51 mm, and a skin pencil. It was important that a template circle was used as this ensured that consistently measured areas were allocated for each product application and evaluation.

In the study, participants compared each of the five lotion formulations to set criteria. A sixth product was also added to the sensory evaluation. This product was a well

performing, non-fragranced, commercially available lotion. Participants were not informed of the different formulations or the presence of the commercial formulation and therefore the commercial formulation was used as a blind benchmark. The sensory characteristics of each of the formulations were compared to the benchmark product which is labelled "BM" in the results that follow.

5.3.4 Sensory Results

5.3.4.1 Test 1: Rub Out Evaluation

The first test of the sensory evaluation was a rub out evaluation. In this test, participants were instructed to dispense a single pump of a product into the middle of a marked circle on the forearm. The droplet of product was then spread over the inscribed surface using gentle circular motions with a clean index or middle finger. A metronome was used to guide and regulate strokes at 2 strokes per second. Sensory characteristics were evaluated after 3 rubs, 9 rubs and 15 rubs. After 3 rubs the thermal melting, spreadability, wetness, thickness, denseness and thermal cooling was rated. The thermal melting, spreadability and whitening were rated after 9 and 15 rubs. The ballot sheet criteria of this test, as given to participants, is shown in Figure 5-6.

The results of the first test are shown in Figure 5-7. Participants were asked to rate the thermal melting, spreadability and whitening three times. Since the ratings over the number of rubs did not show large differences, an average rating over the three instances was calculated and plotted.

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Instructions:

You will be conducting 4 tests today. The procedure and evaluation criteria is listed in each test. A rating scale of 0 to 10 will be used to evaluate each criteria. Reference materials are given for the extremes of each scale. These materials correspond to a rating of 0 and 10 for the specific criterion.

Test 1: Rub Out Evaluation

Circles will be drawn on your arms using a skin pencil. Dispense a single pump of the product you are evaluating into a circle. Spread the product over the inscribed surface with the index or middle finger, using gentle circular motions, at the rate of one to two strokes per second. Use the metronome as a guide to regulate your strokes.

	0	10	Carrier 1	Carrier 2	Carrier 3	Carrier 4	Carrier 5	Carrier 6
After 3 rubs								
Thermal Melting: Amount of change in viscosity due to body heat and friction.	Lanolin (No change)	Coco butter (High amount of melting)						
Spreadability: Ease of moving product over the skin.	Lanolin (Difficult to spread or drag)	Mineral oil (Easy to spread or slip)						
Wetness: Amount of water perceived whilst rubbing.	Talc powder (None)	Water (High amount)						
Thickness: Amount of product perceived between finger and skin.	Ethanol (No amount of product or thin)	Petrolatum (High amount of product or thick)						
Denseness: Amount of compactness.	Whipped egg white (No amount)	Petrolatum (High amount)						
Thermal cooling: Amount of cool sensation felt at the point of contact due to rate of product evaporation.	Mineral oil (None)	Ethanol (High amount)						

Figure 5-5: First part of the ballot sheet criteria for test 1 – rub out evaluation

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

After 9 Rubs								
Thermal Melting: Amount of change from a solid to a semi-solid to liquid state due to body heat and friction.	Lanolin (No change)	Coco butter (High amount of melting)						
Spreadability: Ease of moving product over the skin.	Lanolin (Difficult to spread or drag)	Mineral oil (Easy to spread or slip)						
Whitening: Degree product turns white when rubbed (soapiness).	Mineral oil (None)	Teals solution (High amount)						
After 15 Rubs								
Thermal Melting: Amount of change from a solid to a semi-solid to liquid state due to body heat and friction.	Lanolin (No change)	Coco butter (High amount of melting)						
Spreadability: Ease of moving product over the skin.	Lanolin (Difficult to spread or drag)	Mineral oil (Easy to spread or slip)						
Whitening: Degree product turns white when rubbed (soapiness).	Mineral oil (None)	Teals solution (High amount)						
Provide the following code for the sensation you experienced:	Warming: W Cooling: C Tingling: T None: N							
Absorbency : Product loses the wet moist feeling and resistance to continued pressure is perceived	Record the number of rubs necessary to achieve total rub-in							

Figure 5-6: Second part of the ballot sheet criteria for test 1 – rub out evaluation

All six products had similar ratings for thermal melting, spreadability, thermal cooling and whitening where the margin of error of each criterion was less than 0.4. Formulation L1 performed closest to the benchmark over the spreadability, wetness, thickness, and denseness criteria. Surprisingly the benchmark product showed a higher rating for whitening. In this case whitening is the degree that the product turns white when rubbed. This could also be described as soapiness and is an unwanted characteristic. The product with the least whitening was L2. Thermal melting and thermal cooling were not of major concern but products L3 and L2 performed closest to the benchmark for these criteria respectively. In the rub out evaluation L1 performed the closest to the benchmark product. Formulations L4 and L3 also rated close to the benchmark product.

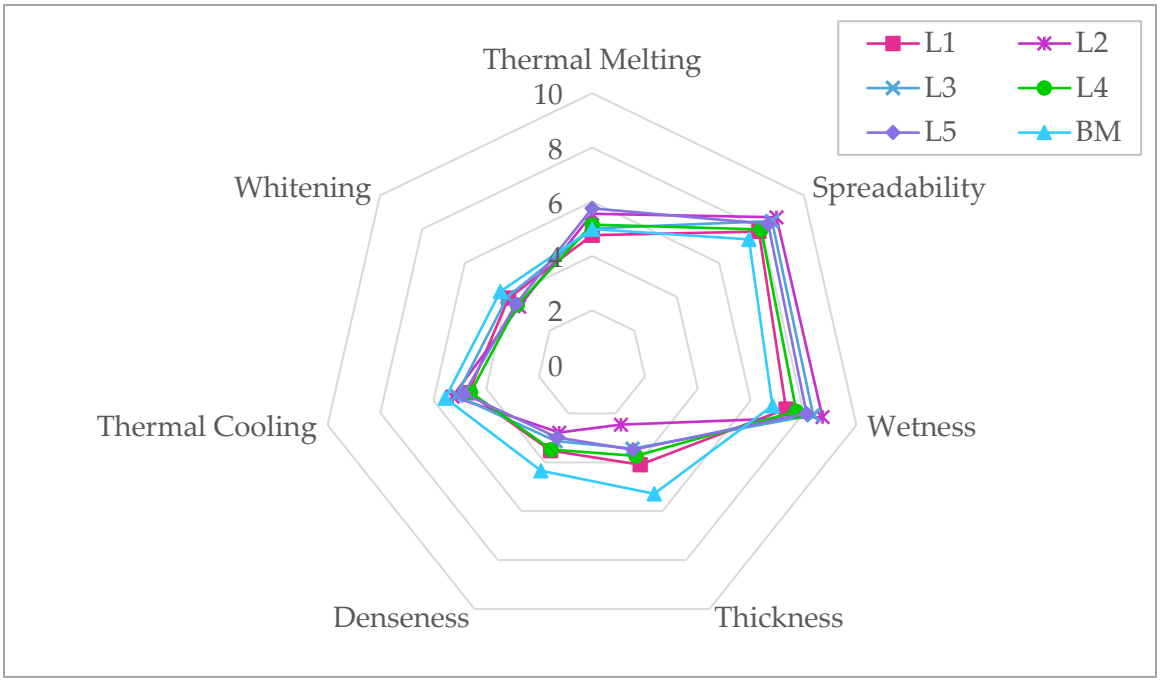


Figure 5-7: Sensory results of test 1 – rub out evaluation

5.3.4.2 Test 2: Product Delivery

Test 2 was the product delivery test. For this test each participant was given a petri dish. They were then instructed to dispense a single pump of the product into the petri dish keeping it on a flat, level surface. In evaluating the gloss, participants were asked to tilt the petri dish at a 45 ° angle to observe the refractive properties. The appearance of the products was then evaluated based on the criteria shown in the ballot sheet in Figure 5-8.

Figure 5-9 shows the results of the product delivery test. The amount of spread and integrity of shape was observed twice. The first was immediately after dispensing into the petri dish and the second was after 10 seconds. Again, the ratings over the two observations did not show great variation therefore an average rating over the two instances was calculated and plotted.

The results of this test showed little variation in smoothness and gloss amongst the six products. The margin of error for both these characteristics was less than 0.2. Formulation L2 performed closest to the benchmark product for ease of dispensing and gloss whereas formulation L4 performed the best for the amount of spread. Formulation L1 showed the closest rating for the integrity of shape and smoothness. This formulation also showed the closest overall rating for this test where L4 performed closely as well.

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Test 2: Product Delivery

Dispense a single pump of the product into the petri dish. Be sure to keep the petri dish on a flat, level surface. Evaluate the appearance.

	0	10	Carrier 1	Carrier 2	Carrier 3	Carrier 4	Carrier 5	Carrier 6
Immediate observation								
Ease of dispensing: Ease of squeezing the product from the container.	Petrolatum (Not at all easy)	Water (Very easy)						
Amount of spread: Distance the product spreads in petri dish.	Petrolatum (None)	Mineral oil (High amount)						
Integrity of shape (thickness): Degree product holds shape, peaks.	Mineral oil (Flattens)	Petrolatum (Retains shape)						
Observation after 10 seconds								
Integrity of shape (thickness): Degree product holds shape, peaks.	Mineral oil (Flattens)	Petrolatum (Retains shape)						
Amount of spread: Distance the product spreads in petri dish.	Petrolatum (None)	Mineral oil (High amount)						
Smoothness: Degree of even surface, not marked by roughness.	Cooked porridge (Rough, lumpy)	Mineral oil (Very smooth)						
Tilt petri dish to an approximate angle of 45° to observe the reflective properties								
Gloss: Amount or degree of light reflected from product.	Talc powder (Dull or flat)	Mineral oil (Shiny or glossy)						

Figure 5-8: Ballot sheet criteria for test 2 – product delivery test

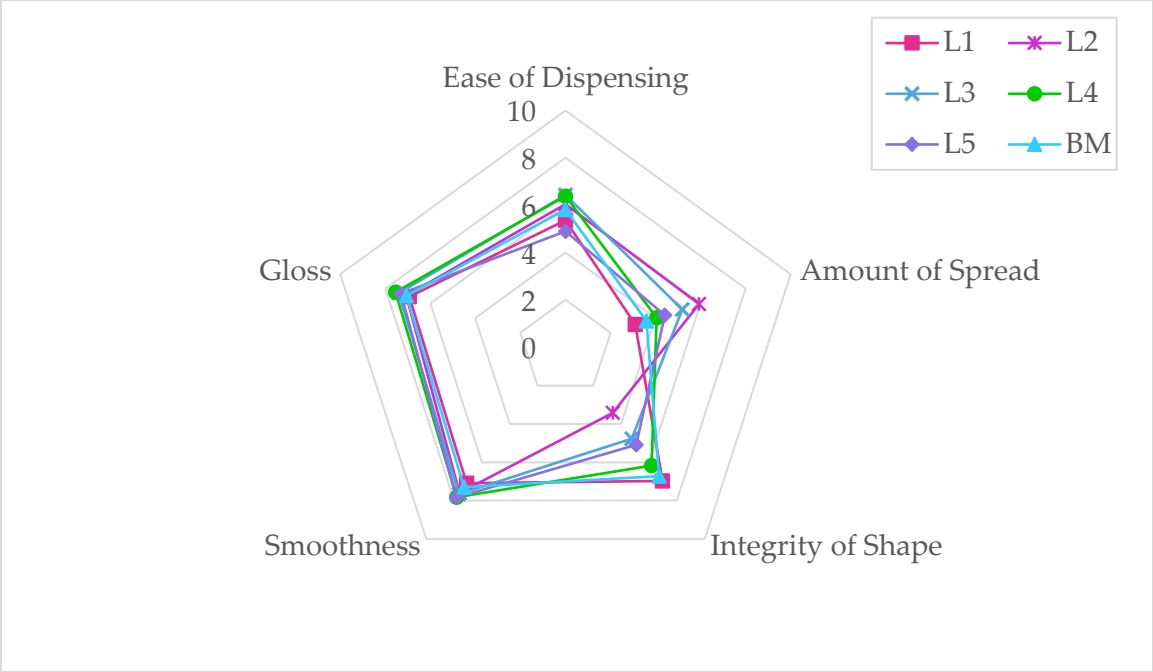


Figure 5-9: Sensory results of test 2 – product delivery test

5.3.4.3 Test 3: Pick-Up Evaluation

In the third test the initial perception of each product was evaluated. Participants were instructed to evaluate one product at a time cleansing their fingers between each sample evaluation. The pre-dispensed product from test 2 could be used in this test. Participants were instructed to lightly tap the product in the petri dish using the middle finger and evaluate the amount of peaking, compress the product between the index finger and thumb and evaluate the firmness, separate the fingers slightly and evaluate the stickiness and stringiness, and finally rotate the fingers and evaluate the denseness. The criteria of this test are shown in Figure 5-10.

Test 3: Pick-up Evaluation

Evaluate one product at a time cleaning fingers between samples. Dispense a single pump of the product into the petri dish or use the pre-dispensed amount in Test 2.

	0	10	Carrier 1	Carrier 2	Carrier 3	Carrier 4	Carrier 5	Carrier 6
Lightly tap product in petri dish using middle finger								
Amount of peaking: Degree to which product stands up straight when tapped.	Mineral oil (No peak)	Petrolatum (High straight peak)						
Compress product slowly between index and thumb three times								
Firmness: Force required to fully compress product between thumb and forefinger.	Mineral oil (No force)	Bees wax (High force)						
Separate fingers slightly								
Stickiness: Force required to separate fingers.	Mineral oil (No force)	Lanolin (High force)						
Stringiness: Amount sample deforms or strains rather than breaks when fingers are separated.	Mineral oil (Low string or cohesion)	Lanolin (high string or cohesion)						
Rotate fingers								
Denseness: Compactness of the product.	Whipped egg white (Airy/ light)	Petrolatum (Compact or heavy)						

Figure 5-10: Ballot sheet criteria for test 3 – pick up evaluation

The pick-up evaluation results are shown in Figure 5-11. Stickiness and stinginess were unwanted properties. The stickiness of the products performed closely to each other with a margin of error of less than 0.3. In this test product L4 performed closest to the benchmark product with the amount of peaking, stickiness, and stringiness. L1 showed the closest rating to the benchmark for firmness and denseness. Formulation L4 performed the closest to the benchmark in this test with L1 performing close as well.

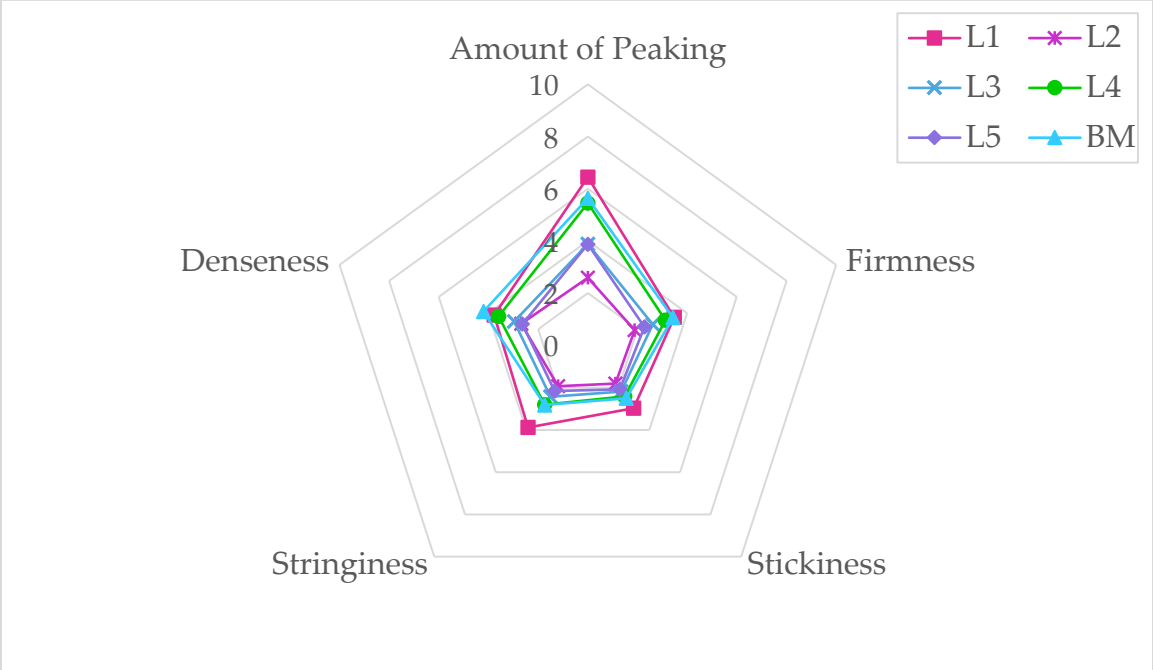


Figure 5-11: Sensory results of test 3 – pick up evaluation

5.3.4.4 Test 4: Afterfeel Characteristics

In the final test the afterfeel characteristics of the products were evaluated by analysing each testing site where each of the lotions had been rubbed in during test 1. By visually analysing, stroking and tapping fingers on the testing site the criteria shown in Figure 5-12 was evaluated.

The results of test 4 is shown in Figure 5-13. The slipperiness is described as ease of moving fingers across the skin. All six products performed close to each other within a margin of error of 0.2. The average rating was 5.26 which is in the middle of the two extremes. This is a desired score as it implies that the products didn't show drag or slip. Film-residue and stickiness or tackiness are unwanted properties. Surprisingly, the benchmark formulation showed high ratings for both these qualities. Product L1 showed the lowest rating for film residue and L3 showed the lowest rating for stickiness. Formulation L4 performed closest to the benchmark product over all criteria.

Over all four tests formulations L1 and L4 performed closest to the benchmark formulation and showed good characteristics. These two formulations are variations of each other where L1 has the addition of a thickening agent. The stability of these two formulations were considered in determining the best performing formulation.

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Test 4: Afterfeel Characteristics

	0	10	Carrier 1	Carrier 2	Carrier 3	Carrier 4	Carrier 5	Carrier 6
Visually analyse forearm								
Appearance-gloss: Amount/ degree of light reflected off the skin.	Talc powder (Dull)	Eye shadow (Very shiny)						
Stroke cleansed fingers lightly across forearm								
Slipperiness Ease of moving fingers across skin.	Lanolin (Difficult to move or drag)	Mineral oil (Easy to move/slip)						
Film-residue: Indicate type and amount of product left on skin. ***Select one:*** *Waxy/Greasy/Oily*	Untreated skin (None)	Lip ice (Waxy) Petrolatum (Greasy) Mineral oil (Oily)						
Moisture: Amount of moisture perceived when moving fingers across skin.	None (Dry)	Water (High amount)						
Tap fingers lightly over application site								
Sticky/adhesive (tacky): Degree to which fingers adhere to residue product.	Untreated skin (Not sticky)	Lanolin (Very sticky)						
Provide the following code for the sensation you experienced:	Warming: W Cooling: C Burning: B Tingling: T None: N							
Please rate the creams according to your overall preference using each number (1 to 6) only once	1: most preferred	6: least preferred						

Comments:

_____

_____

_____

Thank you for your contribution!

Figure 5-12: Ballot sheet criteria for test 4 – after feel test

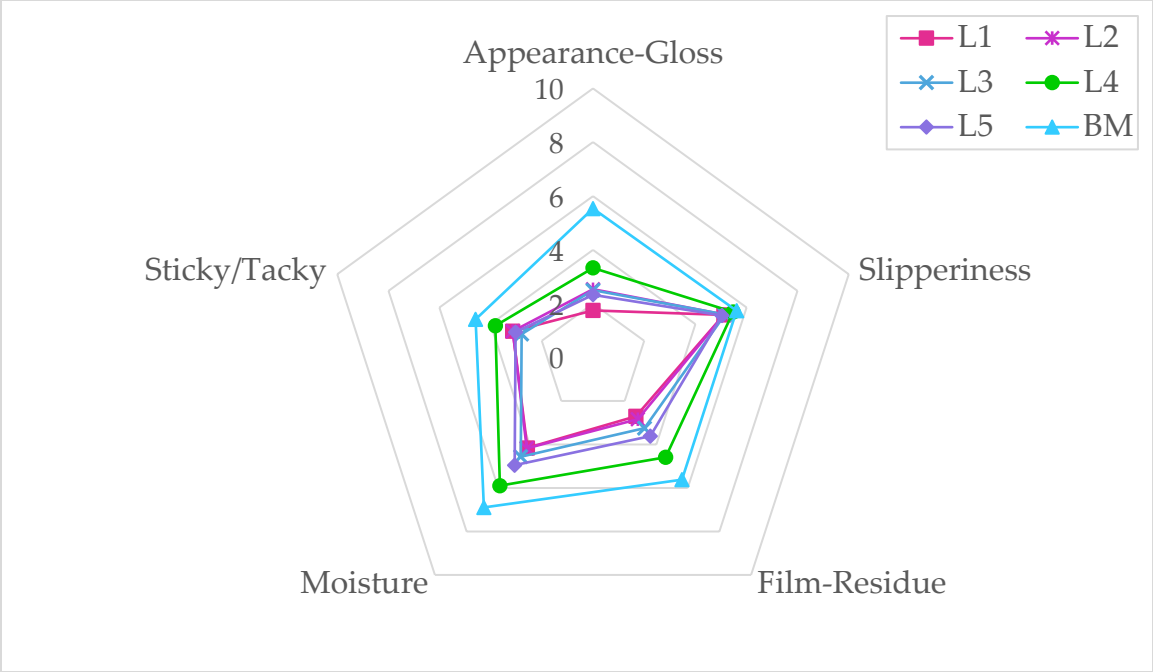


Figure 5-13: Sensory results of test 4 – afterfeel test

5.4 PRELIMINARY STABILITY TESTING

The preliminary stability of the five placebo formulations was determined using coalescence analysis. Droplet size analysis was conducted periodically to determine the change in droplet size over time and from this, the rates of coalescence were determined. In the previous chapter it was noticed that the addition of the plant extract had a large effect on the stability of the formulations. The effect that the dosage of plant extract has on the stability of each of the five formulations was therefore investigated. All carrier formulations were stored at ambient room temperature of 25 °C and in an oven at 40 °C for the duration of analysis which spanned over 4 months.

5.4.1 Coalescence Analysis

The coalescence plots for formulations L1, L2, L3, L4 and L5 are shown in Figure 5-14, Figure 5-15, Figure 5-16, Figure 5-17, Figure 5-18 respectively. Table 5-4 shows a summary

of the trendline equations, the calculated K_C, the projected shelf life and the minimum theoretical shelf life of each sample.

All five coalescence plots showed very little variation between the samples stored at room temperature and the samples stored in the oven. This may suggest resistance to high temperatures. The minimum theoretical shelf-lives of placebo formulations L2, L4 and L5 exceeded 1 year making all of them viable formulations. Formulations L3 and L4 showed a direct proportionality between the shelf life and the storage temperature whereas the other three formulations showed an inverse proportionality.

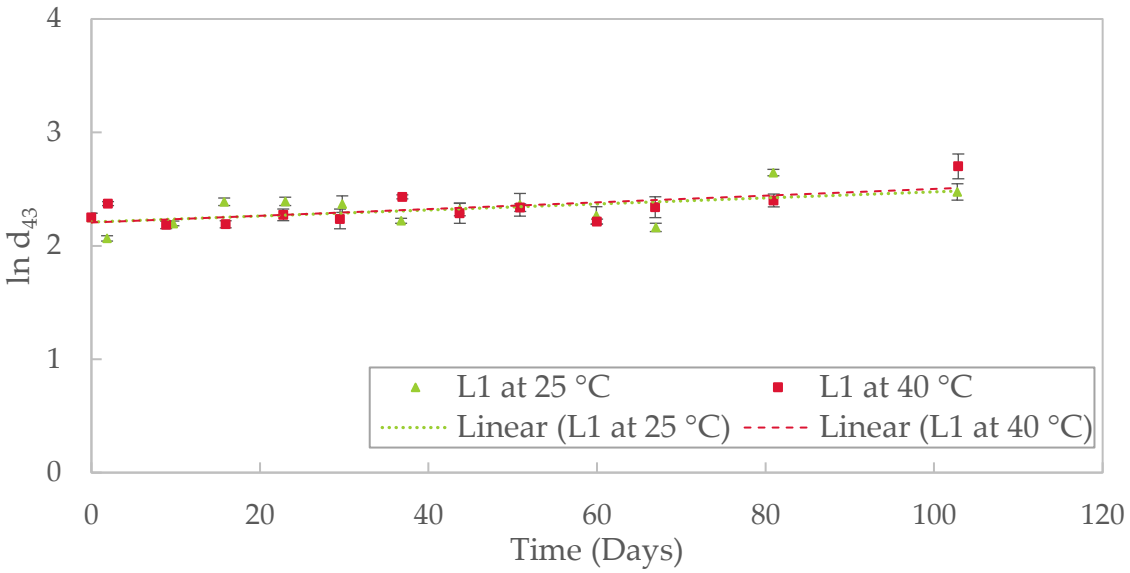


Figure 5-14: Coalescence plot of formulation L1

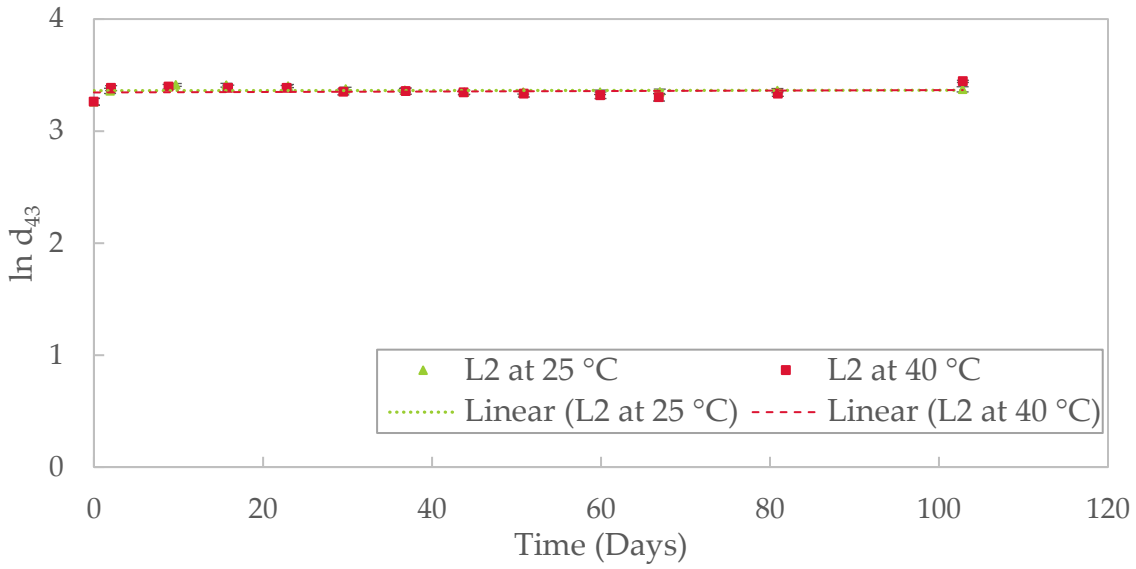


Figure 5-15: Coalescence plot of formulation L2

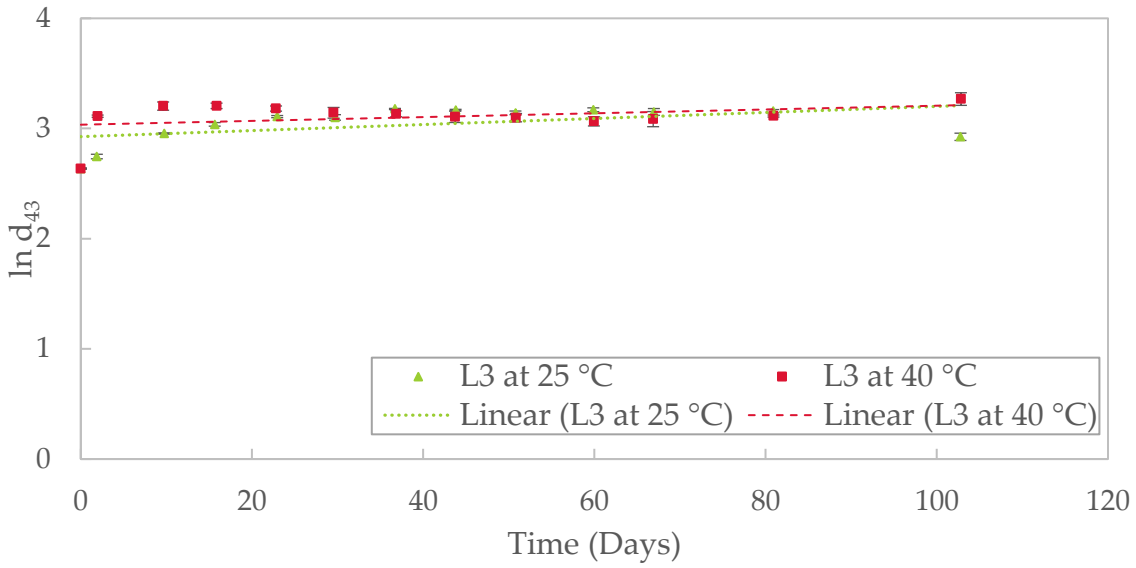


Figure 5-16: Coalescence plot of formulation L3

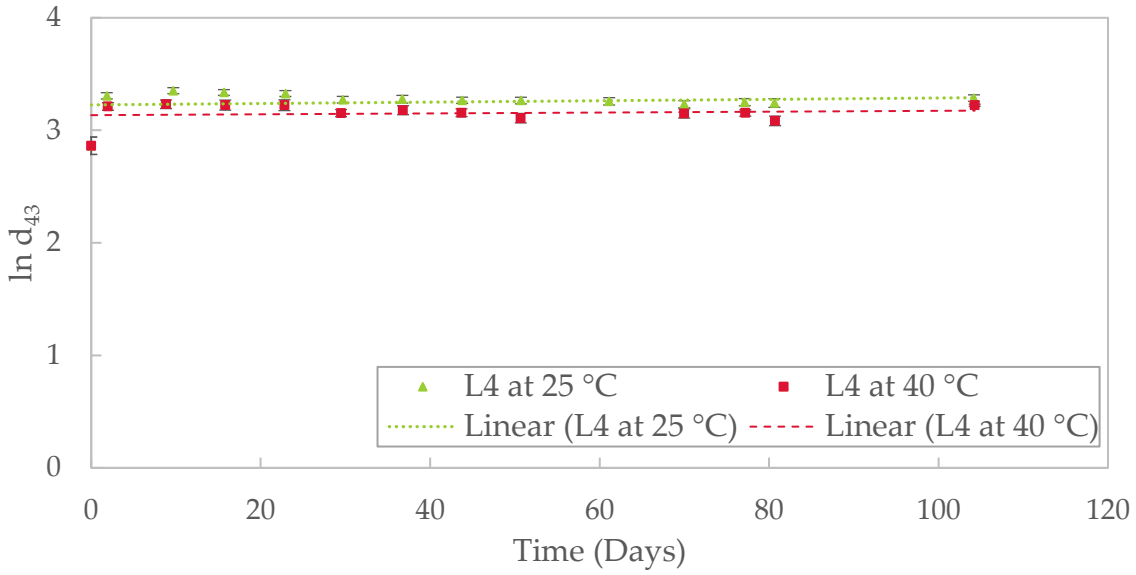


Figure 5-17: Coalescence plot of formulation L4

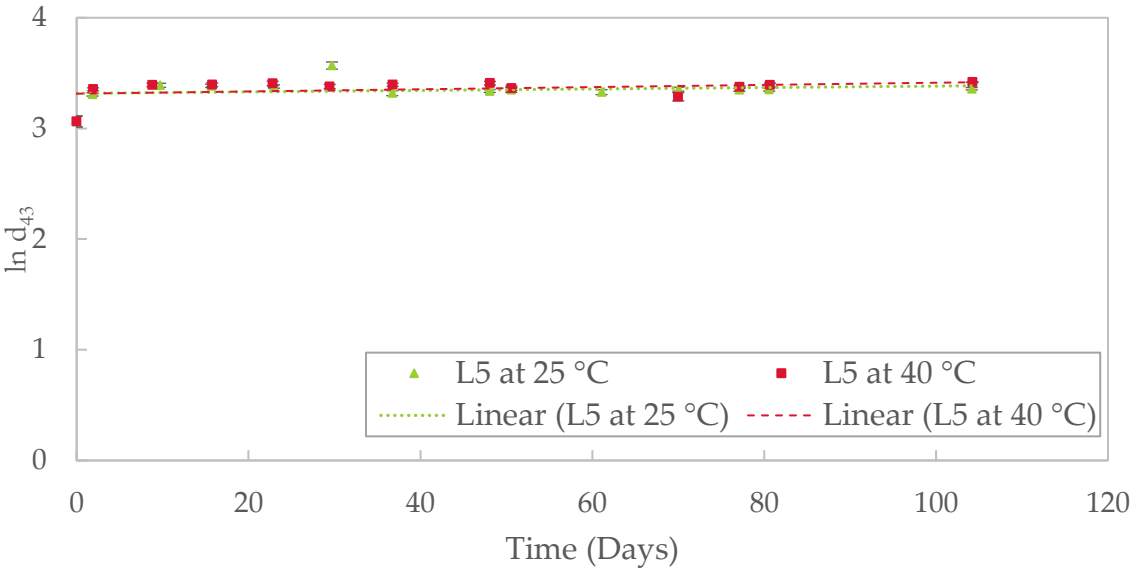


Figure 5-18: Coalescence plot of formulation L5

Table 5-4: Summary of the trendline equations, K_C values, projected shelf life and minimum theoretical shelf life for the five placebo lotion formulations

Temperature (°C)	Trendline Equation	K_C (Day^{-1})	Shelf Life (Years)	Minimum Shelf Life (Years)
		Formulation L1		
25	$y = 0.0027x + 2.2094$	8.10×10^{-3}	1.73 ± 1	0.710
40	$y = 0.003x + 2.2054$	9.00×10^{-3}	1.56 ± 1	0.606
		Formulation L2		
25	$y = 4\times10^{-5}x + 3.3605$	0.12×10^{-3}	37.78 ± 2	35.8
40	$y = 0.0002x + 3.3445$	0.60×10^{-3}	7.77 ± 2	6.31
		Formulation L3		
25	$y = 0.0027x + 2.9263$	8.1×10^{-3}	1.00 ± 0.5	0.515
40	$y = 0.0017x + 3.0345$	5.10×10^{-3}	1.41 ± 0.5	0.863
		Formulation L4		
25	$y = 0.0006x + 3.2258$	1.8×10^{-3}	3.13 ± 1	2.42
40	$y = 0.0004x + 3.1345$	1.2×10^{-3}	5.32 ± 1	4.33
		Formulation L5		
25	$y = 0.0007x + 3.3171$	2.10×10^{-3}	2.32 ± 0.6	1.67
40	$y = 0.001x + 3.3142$	3.00×10^{-3}	1.64 ± 0.6	1.00

5.4.2 Dosage Sensitivity

In this section the sensitivity of the formulations to the loading of the plant extract was investigated. Since large amounts of plant extract were not available, a mock extract was used. All the plant extracts are ethanolic which comprise largely of ethanol and water. Of the four plant extracts, three comprised of 40 % ethanol, therefore the mock extract used was simply an ethanol/water mixture of the ratio 40:60. The LSSJ plant extract was

made up of 70 % ethanol and was not considered in this study. Two dosages were tested, namely, the prescribed 10 % loading and an extreme loading of 20 %. The results of both cases were compared to the stability results of the placebo formulation.

The coalescence plots for the five formulations with the addition of 10 % and 20 % mock extracts are shown below. Figure 5-19, Figure 5-20, Figure 5-21, Figure 5-22, and Figure 5-23 shows the coalescence plots of formulations L1, L2, L3, L4, and L5 respectively. A summary of the trendline equations for each sample, the calculated coalescence rate, the projected shelf life and the minimum theoretical shelf life is shown in Table 5-5.

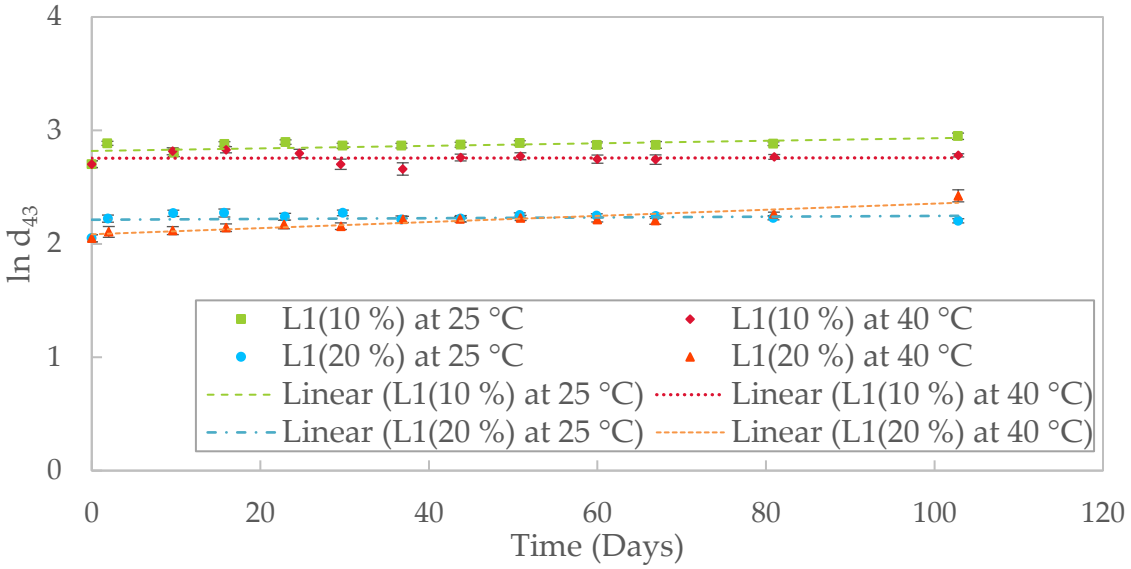


Figure 5-19: Coalescence plot for formulation L1 with 10 % and 20 % extract loading

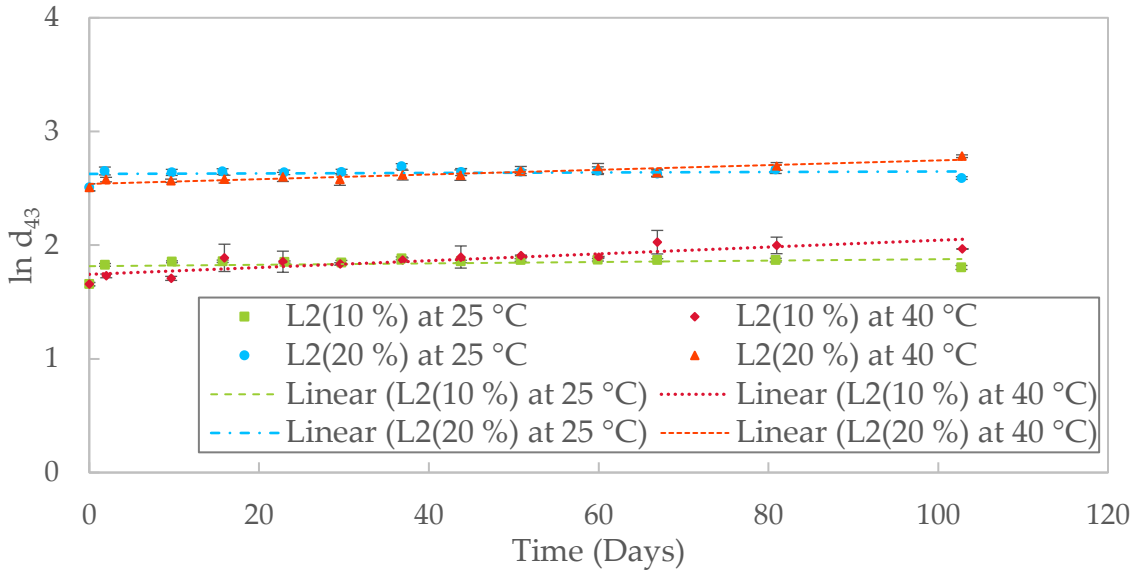


Figure 5-20: Coalescence plot for formulation L2 with 10 % and 20 % extract loading

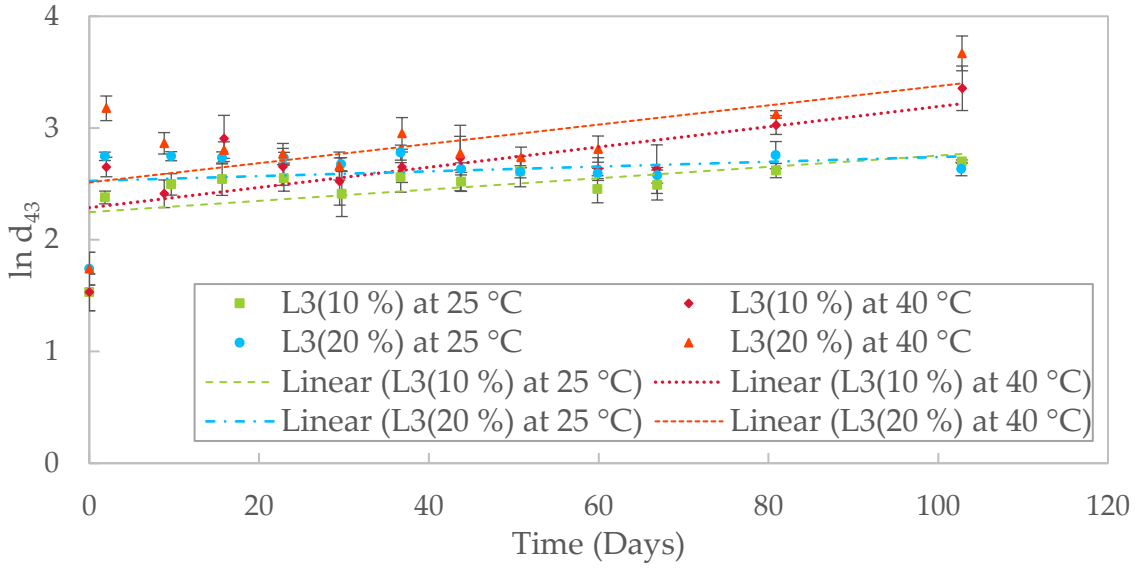


Figure 5-21: Coalescence plot for formulation L3 with 10 % and 20 % extract loading

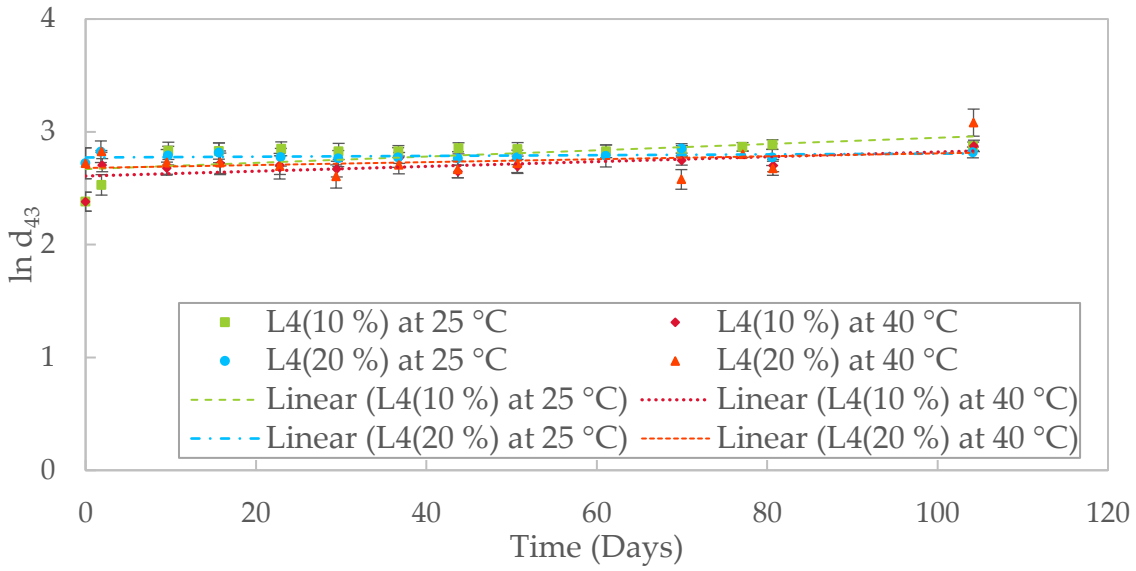


Figure 5-22: Coalescence plot for formulation L4 with 10 % and 20 % extract loading

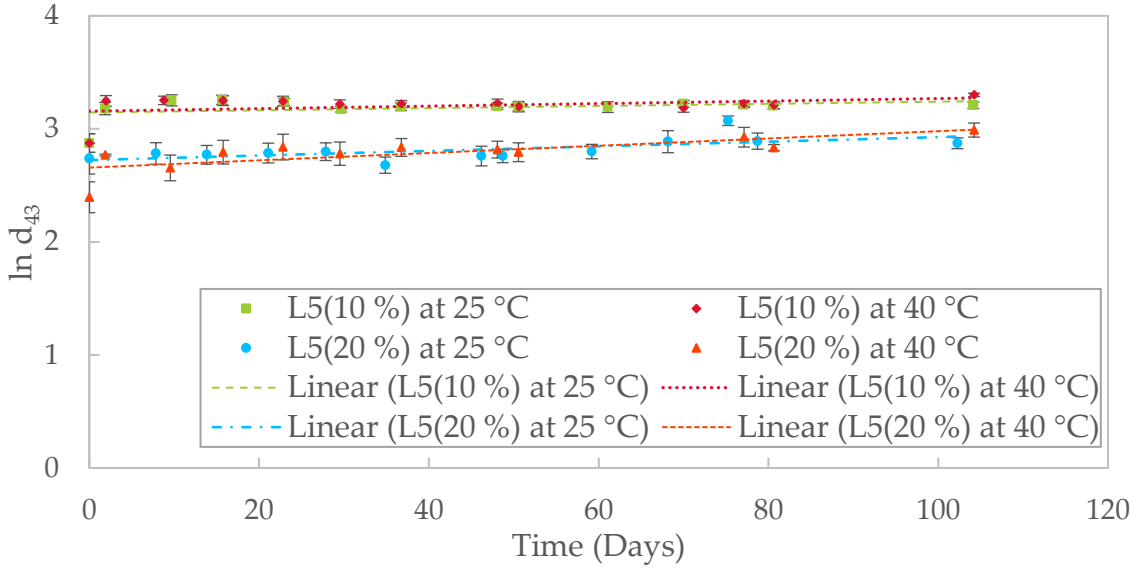


Figure 5-23: Coalescence plot for formulation L5 with 10 % and 20 % extract loading

Table 5-5: Summary of the trendline equations, K_C values, projected shelf life and minimum theoretical shelf life for the five lotion formulations with 10 % and 20 % mock extract loading

Dosage (%)	Temperature (°C)	Trendline Equation	K_C (Day^{-1})	Shelf Life (Years)	Minimum Shelf Life (Years)
		Formulation L1			
10	25	$y = 0.0011x + 2.8188$	3.30×10^{-3}	2.72 ± 1	1.20
	40	$y = 4\times10^{-5}x + 2.7545$	0.12×10^{-3}	79.3 ± 3	76.3
20	25	$y = 0.0003x + 2.212$	0.9×10^{-3}	15.5 ± 3	12.1
	40	$y = 0.0027x + 2.084$	8.10×10^{-3}	1.86 ± 1	0.351
		Formulation L2			
10	25	$y = 0.0006x + 1.8152$	1.80×10^{-3}	9.58 ± 3	6.08
	40	$y = 0.003x + 1.7435$	9.00×10^{-3}	1.98 ± 1	0.522
20	25	$y = 0.0002x + 2.6266$	0.6×10^{-3}	17.6 ± 3	14.2
	40	$y = 0.0021x + 2.5387$	6.3×10^{-3}	1.79 ± 1	0.299
		Formulation L3			
10	25	$y = 0.0051x + 2.2469$	15.3×10^{-3}	0.895 ± 0.4	0.422
	40	$y = 0.0091x + 2.2868$	27.3×10^{-3}	0.489 ± 0.3	0.193
20	25	$y = 0.0021x + 2.5265$	6.30×10^{-3}	1.81 ± 1	1.27
	40	$y = 0.0086x + 2.5132$	25.8×10^{-3}	0.446 ± 0.2	0.196
		Formulation L4			
10	25	$y = 0.0028x + 2.6695$	8.40×10^{-3}	1.22 ± 1	0.511
	40	$y = 0.0021x + 2.6082$	6.30×10^{-3}	1.70 ± 1	0.764
20	25	$y = 0.0003x + 2.7728$	0.9×10^{-3}	10.4 ± 3	6.89

Dosage (%)	Temperature (°C)	Trendline Equation	K_C (Day^{-1})	Shelf Life (Years)	Minimum Shelf Life (Years)
	40	$y = 0.0013x + 2.6798$	3.9×10^{-3}	2.60 ± 1	1.60
Formulation L5					
10	25	$y = 0.001x + 3.1448$	3.00×10^{-3}	2.10 ± 1	1.28
	40	$y = 0.0011x + 3.1566$	3.30×10^{-3}	1.88 ± 1	1.15
20	25	$y = 0.002x + 2.7235$	6.00×10^{-3}	1.63 ± 1	0.682
	40	$y = 0.0032x + 2.6573$	9.60×10^{-3}	1.07 ± 1	0.397

The combined coalescence rate constants for the samples stored at room temperature is shown in Figure 5-24 (a). Here L2, L3 and L4 showed an increase in coalescence rate between 0 % and 10 % dosage and then a decrease in coalescence rate between 10 % and 20 % dosage. L1 showed an inversely proportional relationship between coalescence rate and dosage whereas L5 showed a direct proportionality between coalescence rate and dosage.

The rate of coalescence varied amongst formulations, therefore there was no direct trend between the stability of the formulation and the dosage of the mock extract. The overall dosage sensitivity was inconclusive however the effect of the mock extract was considered in each individual formulation separately.

The combined coalescence rate constants for the samples stored at oven temperature are shown in Figure 5-24 (b). Here L2 and L4 showed an increase in coalescence rate between 0 % and 10 % dosage and then a decrease in coalescence rate between 10 % and 20 %. L3 showed the same general trend however this formulation had a large increase in rate of coalescence between 0 % and 10 % and then only slight decrease between 10 % and 20 % indicating sensitivity to the presence of plant extract but not to the dosage of the plant

extract. Again, L1 showed an inversely proportional relationship and L5 showed a direct proportionality between coalescence rate and dosage.

Considering the shelf-lives of the placebo formulations and of the formulations with the addition of the mock extracts at different dosages the following trends were noticed. The placebo formulation L2 had the longest shelf-lives at both storage conditions followed by formulation L4 at both storage conditions. Amongst the formulations with a 10 % mock extract dosage, L1 and L2 showed the longest shelf-lives suggesting a stabilising effect with the addition of the thickening agent Carbopol 940. At higher dosages of 20 % formulations L1, L2 and L4 showed the longest shelf-lives.

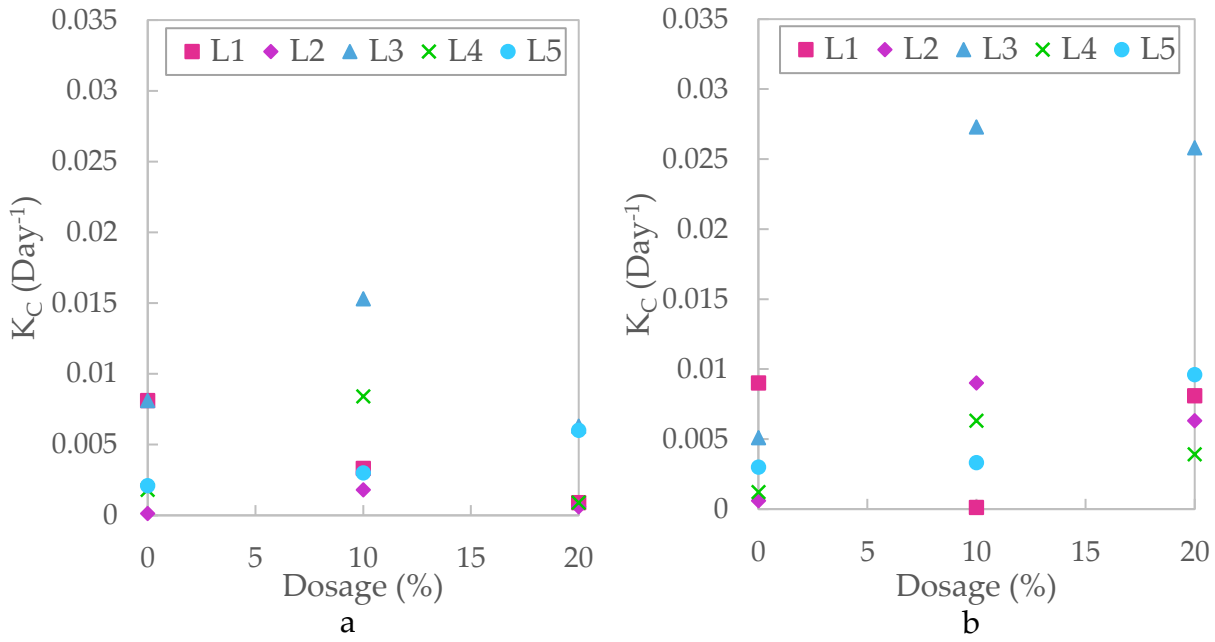


Figure 5-24: The rate of coalescence as a function of the plant extract dosage for the formulations stored at a) room temperature and b) in an oven at 40 °C

Considering the sensory analysis results and the rates of coalescence of formulations L1 and L4 at 10 % dosage it was found that formulation L1 has a slower rate of coalescence than L4. This held true under both storage conditions. Since the plant extract is dosed at

10 %, formulation L1 was selected as the best performing formulation. Formulation L1 is further referred to as Formulation B.

5.5 LONG TERM STABILITY TESTING

Long term stability testing was conducted using coalescence analysis, microscopy and cycle testing. These tests were conducted on Formulation B in its placebo form and inclusive of the HO, PM and LSSJ plant extracts. The BS plant extract was not included in this formulation as it did not contain a UV filter. The complete formulation of Formulation B with the addition of the plant extract is shown in Table 5-6, where the active ingredient in Phase C refers to the ethanolic plant extract and its mass percentage.

Table 5-6: The complete formulation of Formulation B with the addition of the plant extract

Ingredient	%	Function	INCI/Chemical Name
		Phase A	
Water	77.0	Diluent/solvent	Aqua
EDTA	0.10	Chelating Agent	Tetrasodium EDTA
Propylene Glycol	1.80	Humectant	Propylene Glycol
Carbopol 940	0.10	Thickener	Carbomer
		Phase B	
Palmerol 6830	3.18	Emulsifier	Cetostearyl Alcohol
ERCAWAX CS 20 V/FD	3.18	Emulsifier	Ceteareth 20
ERCAREL AB V	3.64	Emollient	C12-15 Alkyl Benzoate
		Phase C	

Ingredient	%	Function	INCI/Chemical Name
Active ingredient	10.0	Active ingredient	Ethanolic Plant Extract
Germaben II	1.00	Preservative	Propylene Glycol (and) Diazolidinyl Urea (and) Methylparaben (and) Propylparaben
Sodium Hydroxide	QS	Neutralising Agent	Sodium Hydroxide

Method

1. Mix ingredients of Phase A together in a clean beaker and heat to 65 °C.
2. Mix ingredients of Phase B together in a clean beaker and heat to 65 °C.
3. Slowly add Phase B to Phase A while stirring using a magnetic stirrer at 1000 rpm for 15 minutes until homogeneous.
4. Allow to cool to 40 °C with gentle stirring using the magnetic stirrer.
5. Add the active ingredient and preservative, stirring after each addition.
6. Add the sodium hydroxide drop wise while measuring the pH until a pH of 5.5.
7. Mix for 15 minutes until homogeneous.

5.5.1 Coalescence Analysis

In this case, droplet size analyses were conducted on the samples for a period of 7 months. The coalescence plots for placebo Formulation B at storage conditions of 25 °C, 40 °C and 4 °C are shown in Figure 5-25. The coalescence plots for Formulation B with the addition of the plant extracts follow where the formulation with the addition of the HO plant extract is shown in Figure 5-26, PM plant extract is shown in Figure 5-27 and LSSJ plant extract is shown in Figure 5-28. A summary of the results obtained from the plots are shown in Table 5-7. In this table the equations of the trendlines, calculated rates of coalescence, projected shelf life and minimum theoretical shelf life of each formulation

are given. Finally, Figure 5-29 shows the rate of coalescence as a function of temperature for each of the formulations.

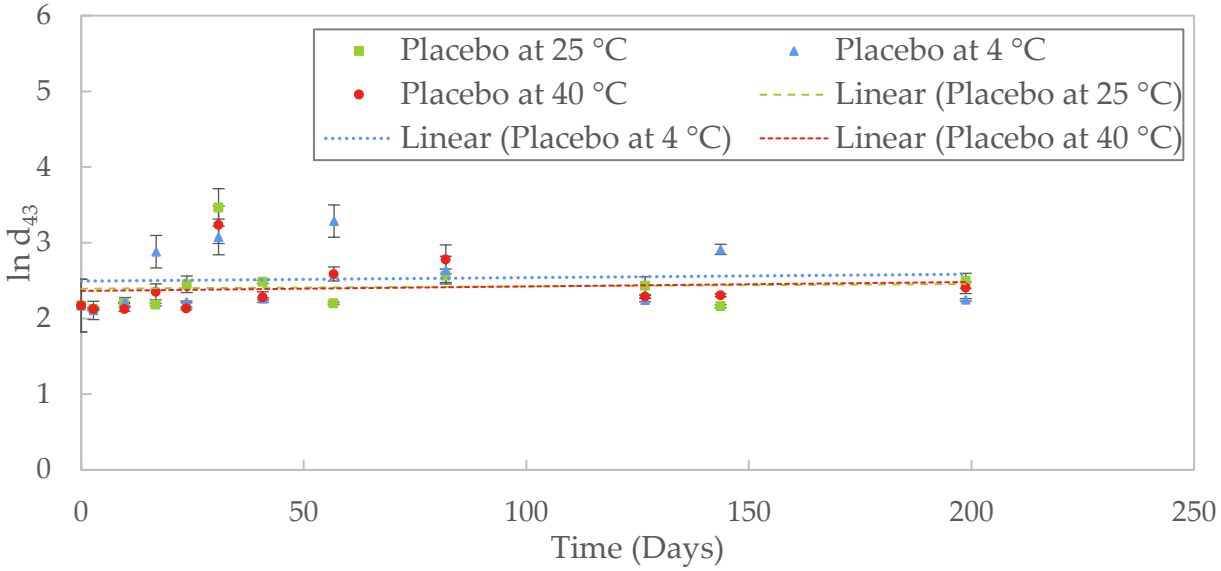


Figure 5-25: Coalescence plot of placebo Formulation B

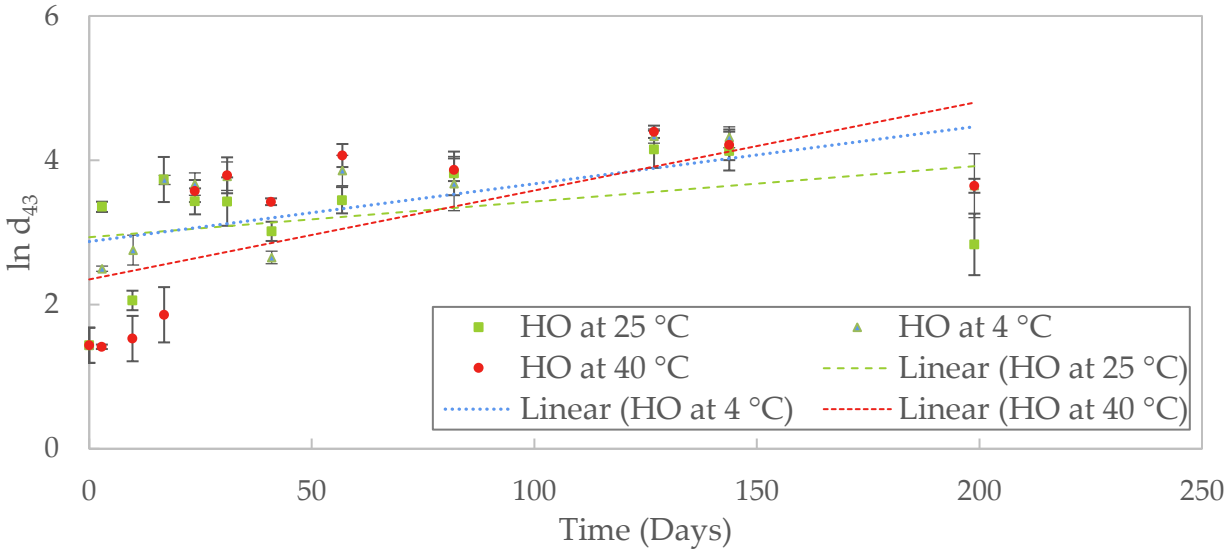


Figure 5-26: Coalescence plot of Formulation B with the HO plant extract

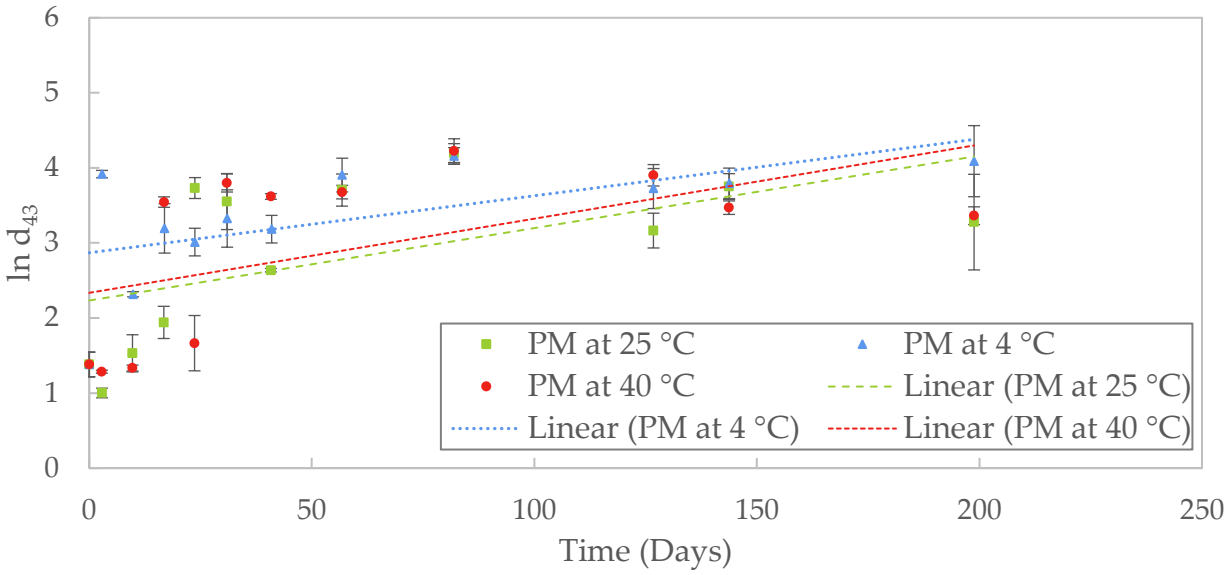


Figure 5-27: Coalescence plot of Formulation B with the PM plant extract

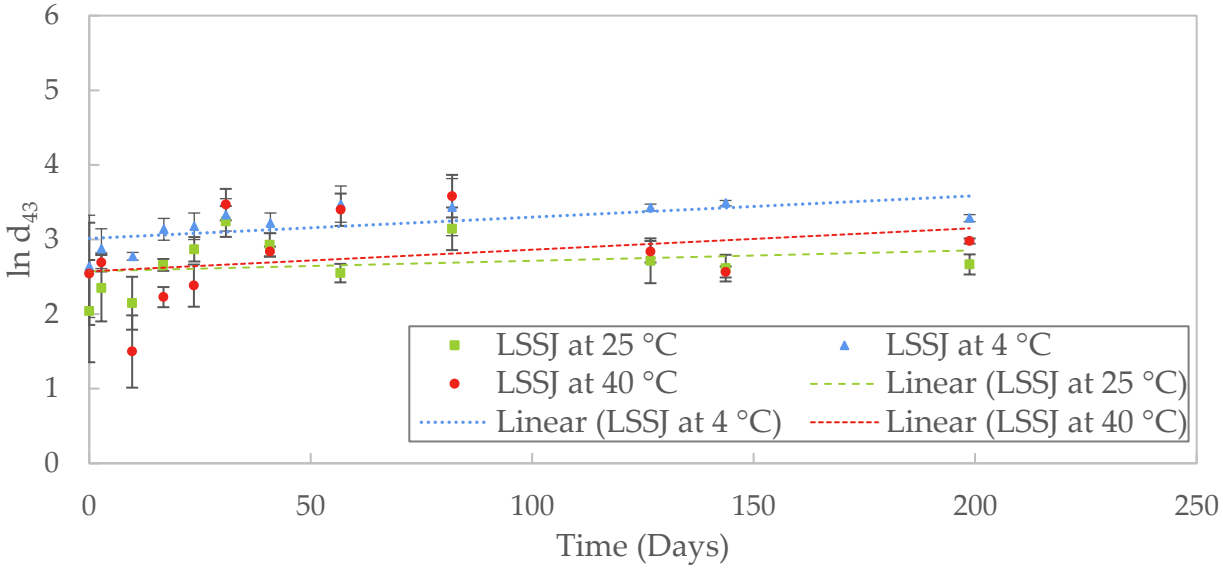


Figure 5-28: Coalescence plot of Formulation B with the LSSJ plant extract

Table 5-7: Summary of the trendline equations, apparent rate of coalescence (K_C), projected shelf life and minimum theoretical shelf life of Formulation B with the plant extracts

Temperature (°C)	Trendline Equation	K_C (Day^{-1})	Shelf Life (Years)	Minimum Shelf Life (Years)
Placebo Formulation B				
4	$y = 0.0005x + 2.4939$	1.5×10^{-3}	7.77 ± 1	6.91
25	$y = 0.0003x + 2.3929$	0.9×10^{-3}	13.9 ± 1	12.8
40	$y = 0.0006x + 2.3641$	1.8×10^{-3}	7.07 ± 1	5.94
HO in Formulation B				
4	$y = 0.008x + 2.8729$	24×10^{-3}	0.356 ± 0.2	0.164
25	$y = 0.005x + 2.9329$	15×10^{-3}	0.537 ± 0.2	0.327
40	$y = 0.0125x + 2.3224$	38×10^{-3}	0.348 ± 0.2	0.148
PM in Formulation B				
4	$y = 0.0076x + 2.8693$	23×10^{-3}	0.376 ± 0.2	0.197
25	$y = 0.0097x + 2.2329$	29×10^{-3}	0.474 ± 0.2	0.244
40	$y = 0.0099x + 2.3357$	30×10^{-3}	0.436 ± 0.2	0.225
LSSJ in Formulation B				
4	$y = 0.0029x + 3.0137$	8.7×10^{-3}	0.849 ± 0.4	0.371
25	$y = 0.0014x + 2.5743$	4.2×10^{-3}	2.35 ± 1	1.90
40	$y = 0.0029x + 2.5737$	8.7×10^{-3}	1.34 ± 0.4	0.825

From Figure 5-29 it was noticed that the placebo, HO and LSSJ samples stored at 4 °C showed a faster rate of coalescence than the samples at 25 °C and 40 °C. This implies that Formulation B is sensitive to lower temperatures. Since this formulation was developed

for use mainly in South Africa where low climate temperatures are not experienced for long periods of time, the sensitivity to low temperatures was not of major concern.

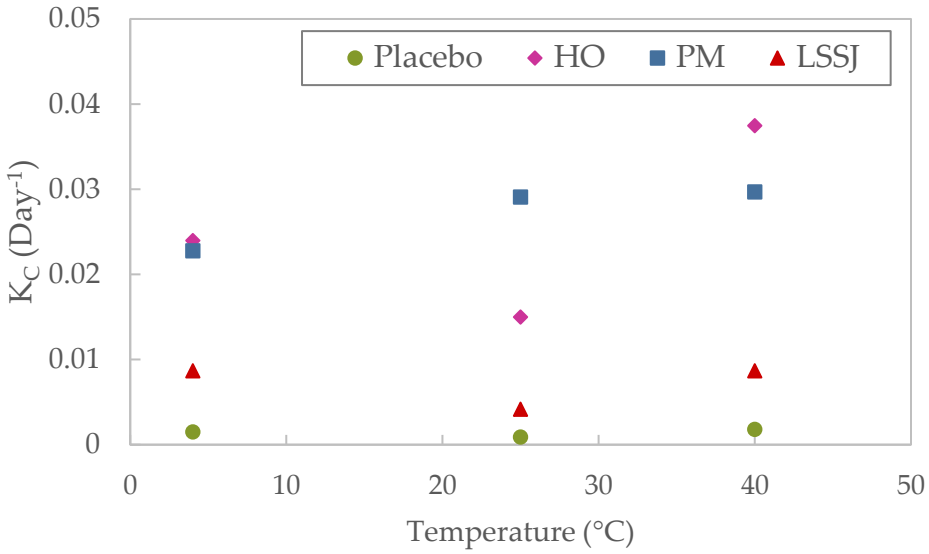


Figure 5-29: Graphic representation of the rate of coalescence as a function of temperature for each form of Formulation B

Formulation B showed less stability with the addition of the HO and PM plant extracts. Both these samples also showed unsteady droplet size results and measurements had to be repeated a number of times to obtain reliable results. The PM sample followed the general direct proportionality trend between coalescence rate and temperature but showed much faster rates of coalescence than the placebo formulation. The HO sample showed the most sensitivity to temperature due to the large difference in coalescence rate with reference to storage temperature.

The minimum theoretical shelf-lives of placebo Formulation B under the three storage conditions exceeded 1 year showing good long-term stability. Formulation B with the addition of the LSSJ plant extract showed a shelf life of more than 1 year for samples stored at 25 °C and more than 6 months when stored at 40 °C making this formulation

appropriate for the addition of LSSJ. The PM and HO plant extracts showed a shelf life of less than 6 months across all storage temperatures therefore this formulation is not suitable for the addition of these plant extracts.

5.5.2 Microscopy

Micrographs were taken at the start and end of the 7 month testing period. Figure 5-30 shows the micrographs of placebo Formulation B where the increase in droplet size between the initial micrographs and the three final micrographs are clearly noticed.

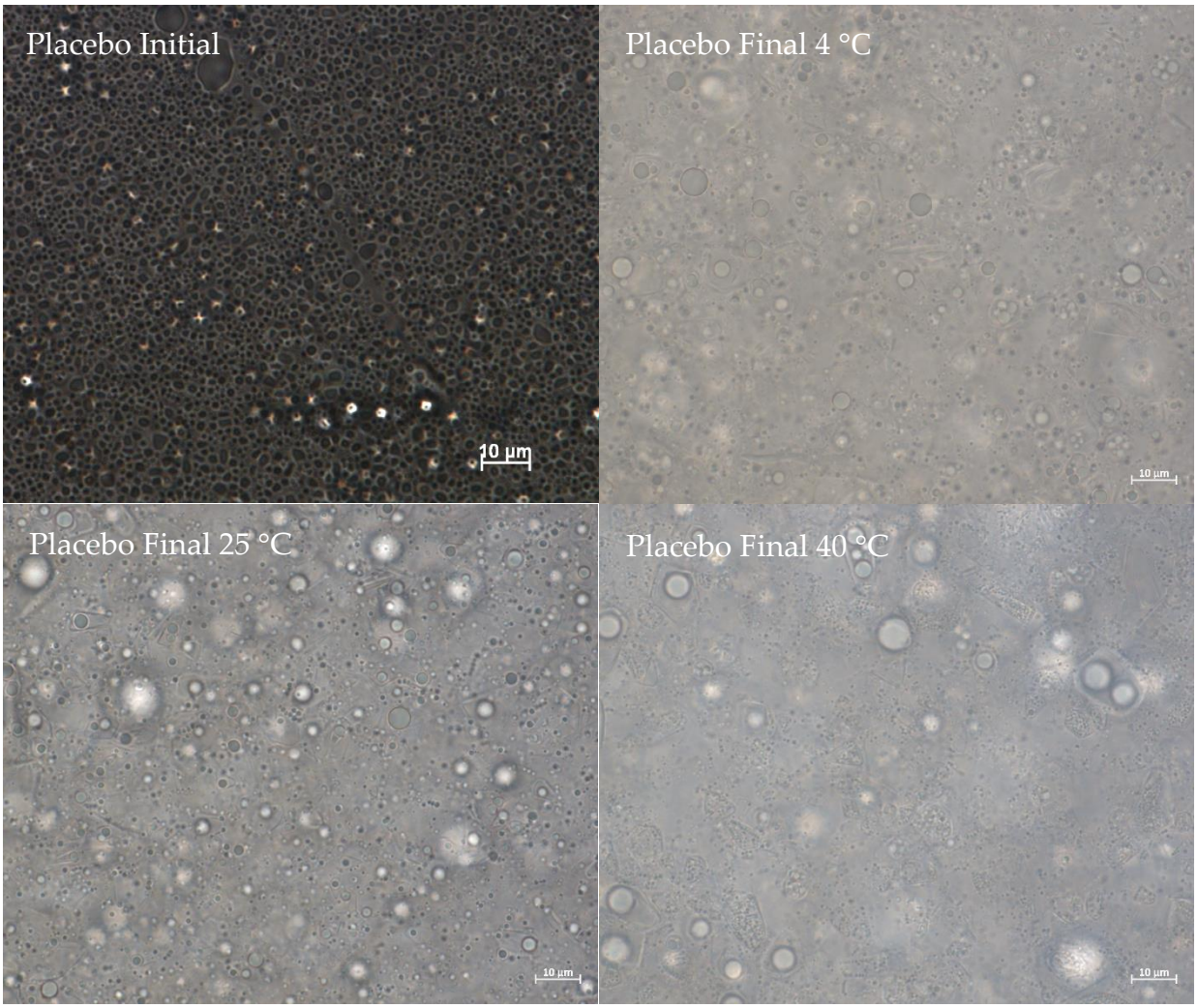


Figure 5-30 Micrographs of placebo Formulation B

The micrograph of Formulation B with the addition of the HO plant extract is shown in Figure 5-31. Here individual droplets were difficult to distinguish from the bulk. This may be the reason why difficulty was experienced when trying to gain consistent droplet sizes using laser diffraction.

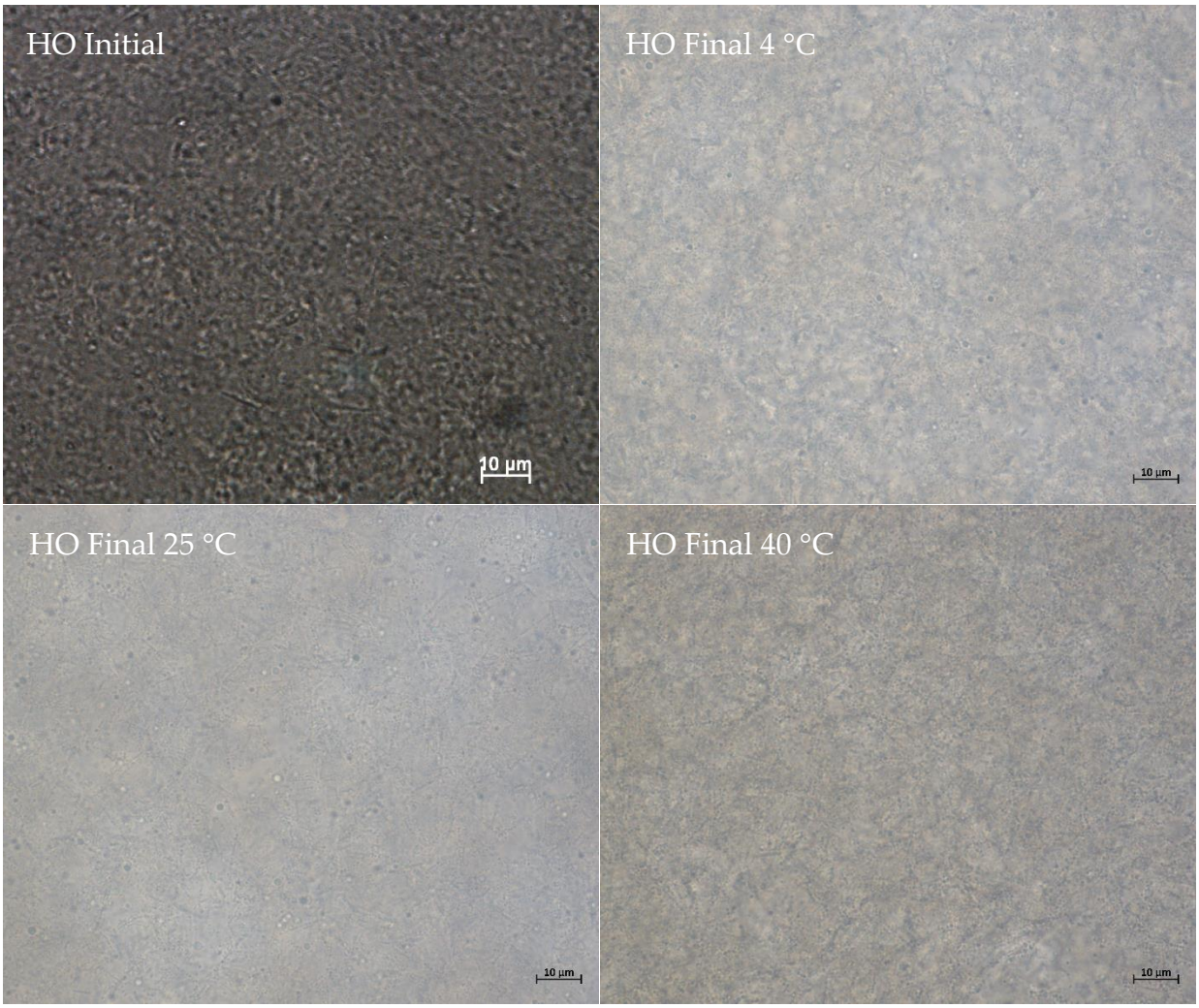


Figure 5-31: Micrographs of Formulation B with the HO plant extract

Figure 5-32 shows the micrographs of Formulation B with the PM extract. The initial micrograph shows distinct droplets but individual droplets are almost indistinguishable in all three final micrographs. Again, this may be the reason why droplet size measurements for these samples proved difficult using laser diffraction.

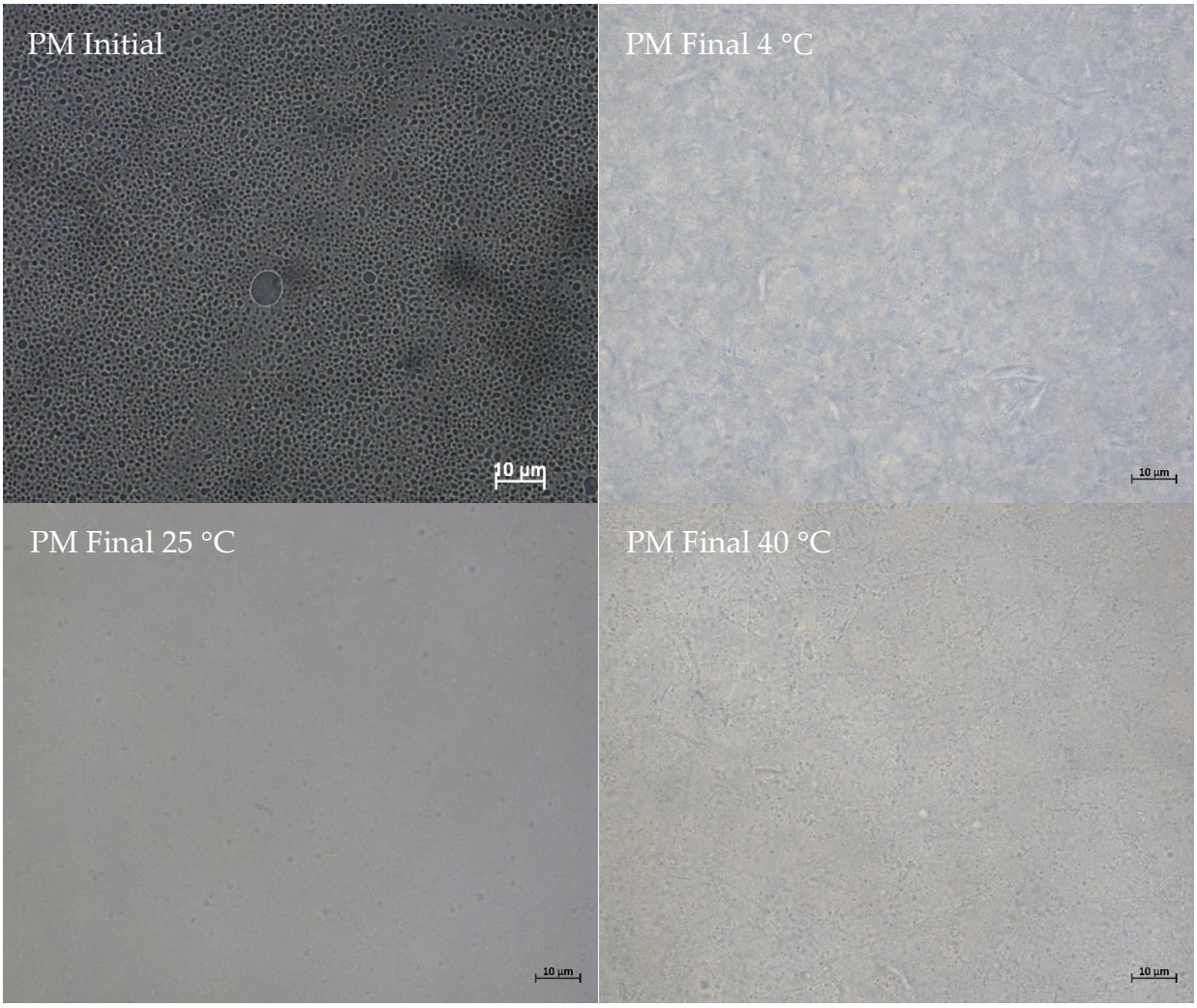


Figure 5-32: Micrographs of Formulation B with the PM plant extract

Figure 5-33 shows the micrographs of Formulation B with the addition of the LSSJ plant extract. Here individual droplets are distinguishable in all four micrographs. There is a clear increase in droplet size between the initial and final micrographs but the three final micrographs showed similar droplet sizes. This verifies the similar rates of coalescence of this sample stored under the three different storage conditions.

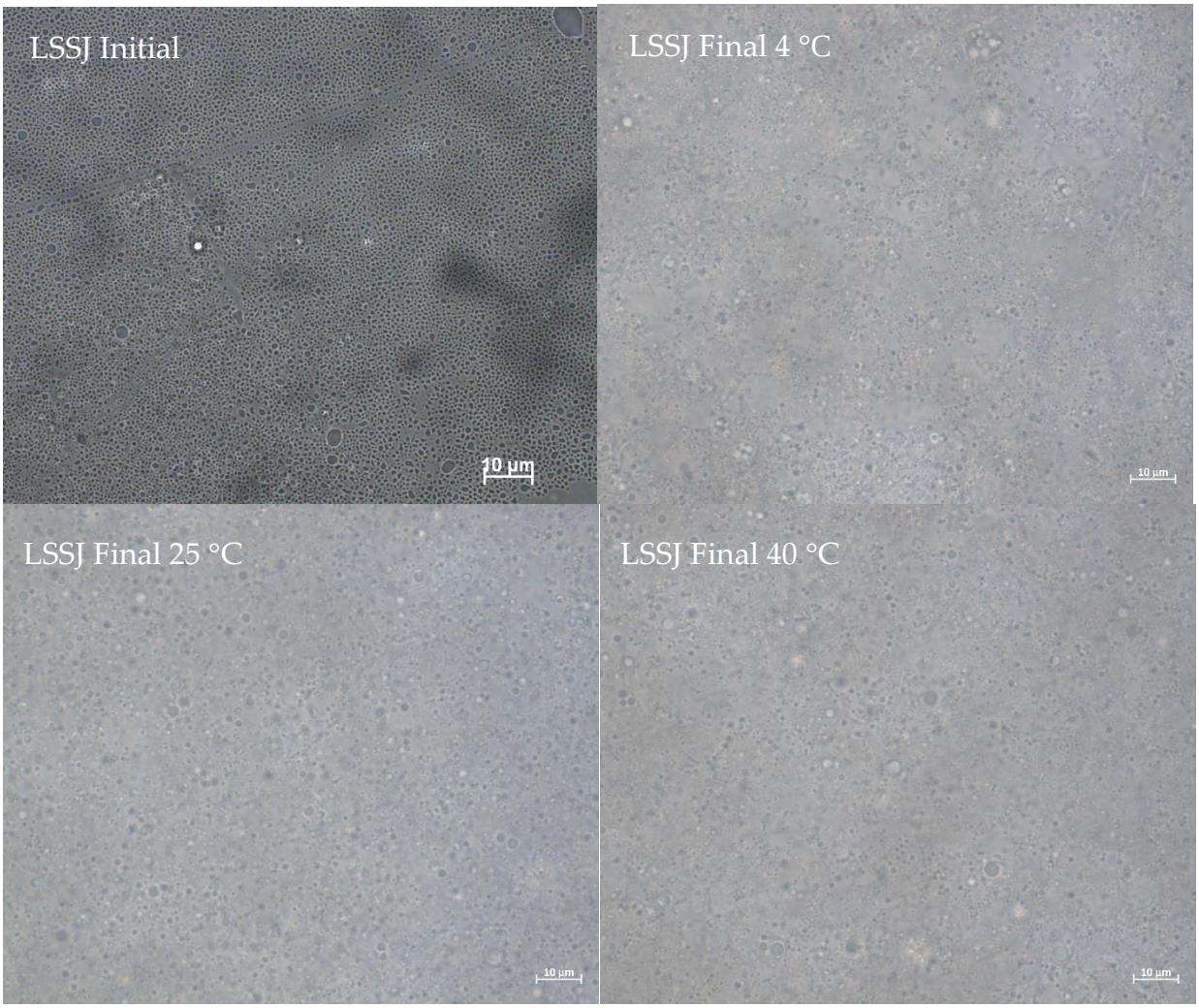


Figure 5-33: Micrographs of Formulation B with the LSSJ plant extract

5.5.3 Cycle Testing

Cycle testing involved 7 fridge-oven cycles followed by five freezer-oven cycles. At the end of each 48-hour cycle, measurements of pH, droplet size analysis and centrifugation were conducted. The results thereof are discussed in this section.

5.5.3.1 pH Analysis

The pH results for each formulation is shown graphically in Figure 5-34. The pH of placebo Formulation B and Formulation B with the addition of the HO, PM and LSSJ

plant extracts showed variation of less than 1 pH unit. This suggested a stable formulation under high stress conditions. The pH of all four formulations remained close to a pH of 5.5 which is the pH of skin. This implies that these formulations can be used safely in skin care applications. The placebo formulation showed an average pH of 5.46 ± 0.05. Furthermore, the formulations containing HO, PM and LSSJ showed an average pH of 5.43 ± 0.06, 5.40 ± 0.06, 5.39 ± 0.06 respectively.

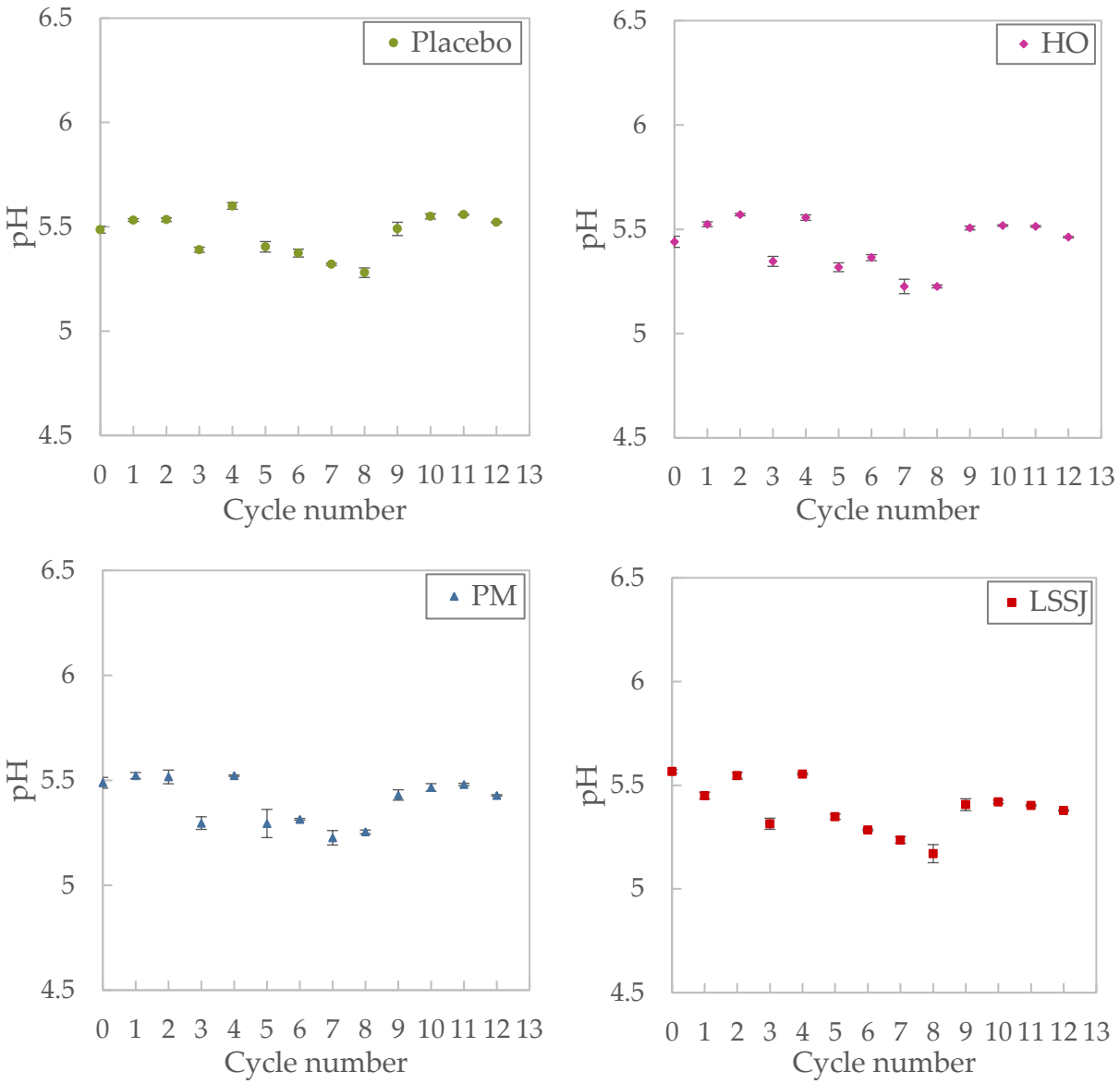


Figure 5-34: pH results of Formulation B at the end of each cycle

5.5.3.2 Droplet Size Analysis

The results of the median droplet size at the end of each cycle is shown in Figure 5-35. Here the placebo formulation showed little variation in the droplet size over the fridge-oven cycles but showed a constant increase in droplet size over the five freezer-oven cycles where the sample experienced high stress conditions.

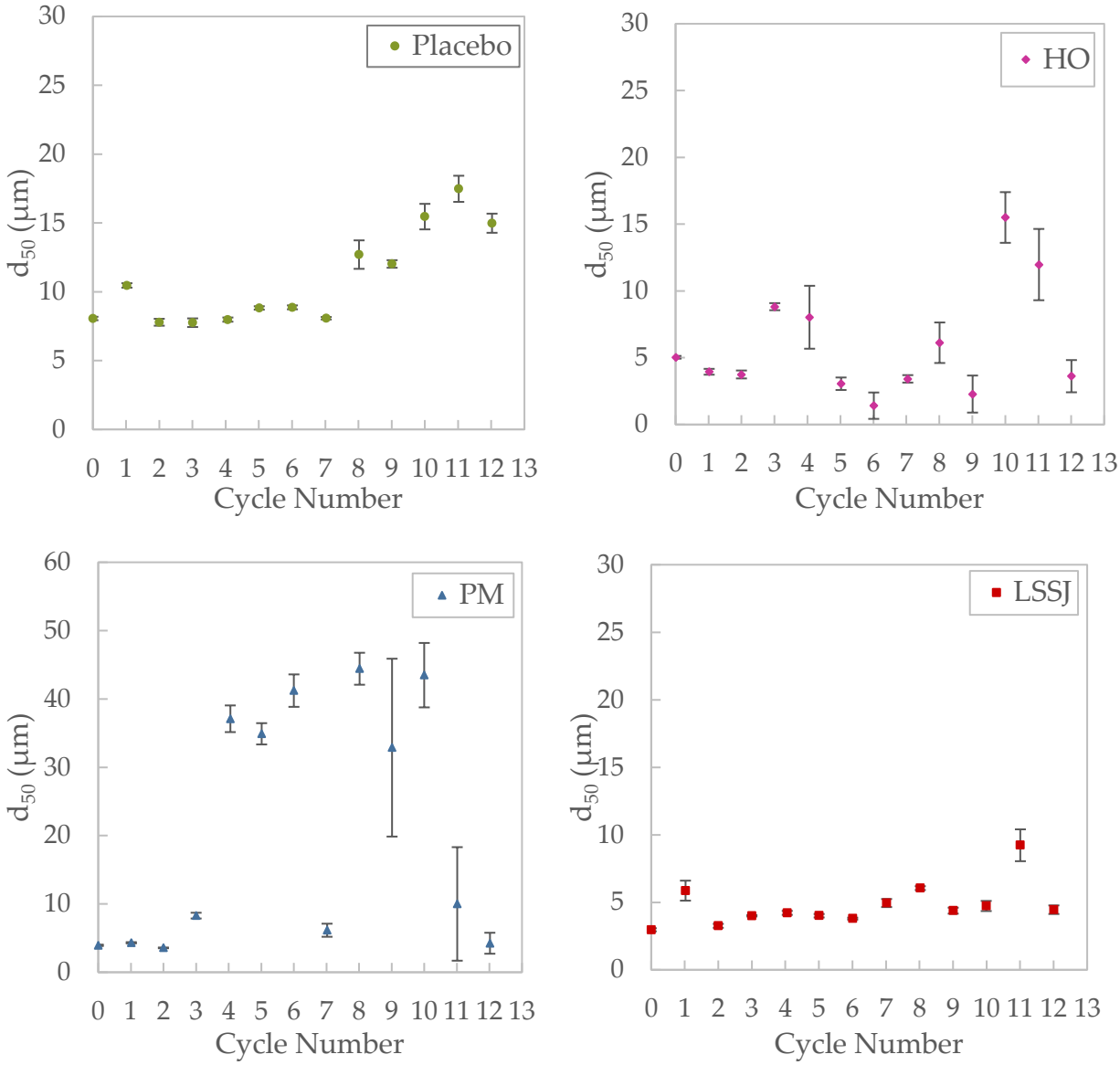


Figure 5-35: Median droplet size results of Formulation B

The formulation containing the HO and PM plant extracts showed variation in the median droplet size at the end of the third fridge-oven cycle. This implied that these formulations are sensitive to extreme conditions and may degrade under high stress conditions. The formulation containing the LSSJ plant extract showed little variation over all twelve cycles. An increase in droplet size was observed at the end of the first fridge-oven cycle and the fourth freezer-oven cycle.

5.5.3.3 Centrifugation

Formulation B in its placebo form and inclusive of the plant extracts underwent centrifugation at the end of each cycle for 30 minutes at 3000 rpm. After centrifugation the samples were visually observed and no signs of separation was evident over any of the cycles. This implied no creaming, sedimentation or phase separation occurred under extreme conditions indicating good stability.

5.6 COSTING ANALYSIS

A cost analysis was done on the basis of 100 g of placebo Formulation B. The ingredients, loadings and raw material cost is shown in Table 5-8. All of the ingredients were locally produced and most of the ingredients were sourced from a local supplier, Fourchem. In this formulation, sodium hydroxide was added on a Q.S. basis until the pH of the batch was 5.5. There was no set loading for this ingredient therefore a conservative weight percentage of 0.1 % was considered in the costing analysis. The cost of 100 g of placebo Formulation B was found to be R 0.99. This was a significant reduction in cost in comparison to Formulation A, which was R 2.16 for 100 g of sample.

Table 5-8: Cost break down of placebo Formulation B

Ingredient	INCI/Chemical Name	%	Cost (R/kg)
Water	Aqua	85.8	0.00714
EDTA	Tetrasodium EDTA	0.10	34.40
Propylene Glycol	Propylene Glycol	2.00	23.40
Carbopol 940	Carbomer	0.10	82.58
Palmerol 6830	Cetostearyl Alcohol	3.50	26.15
ERCAWAX CS 20 V/FD	Ceteareth 20	3.50	41.29
ERCAREL AB V	C12-15 Alkyl Benzoate	4.00	137.63
Germaben II	Propylene Glycol (and) Diazolidinyl Urea (and) Methylparaben (and) Propylparaben	1.00	143.00
Sodium Hydroxide	Sodium Hydroxide	0.10	5.51

5.7 CONCLUSION

In this chapter Formulation B was developed based on locally produced, cost-efficient raw materials. Five of the best performing trial formulations were used in a sensory evaluation and compared to a commercially available lotion which served as a benchmark. The sensory evaluation showed that formulations L1 and L4 performed closest to the benchmark formulation. These formulations were modifications of each other with the only difference being the inclusion of a thickening agent.

Preliminary stability testing, which involved coalescence analysis, was done on the five formulations used in the sensory evaluation. The sensitivity of these formulations to the dosage of the plant extract was tested using a mock extract. This mock extract was an ethanol/water mixture of the ratio 40:60. A dosage of 10 % and 20 % was tested and

compared to the stability of the placebo formulations. Between L1 and L4, formulation L1 showed the longest shelf life with the addition of the mock extract at 10 % and 20 %. For this reason, formulation L1 was selected as the best performing formulation for the addition of the HO, PM and LSSJ plant extracts. This formulation is further referred to as Formulation B.

Long term stability testing was conducted on Formulation B in its placebo form and with the addition of the HO, PM and LSSJ plant extracts. This testing procedure involved coalescence analysis, microscopy and cycle testing. Through coalescence analysis the stability of the formulations with the addition of the HO and PM extracts showed short shelf-lives. The placebo formulation and the formulation inclusive of the LSSJ plant extract showed shelf-lives greater than 1 year at room temperature and greater than 6 months at accelerated conditions. In addition to this, the rates of coalescence of these formulations showed little variation due to storage temperature. Cycle testing showed that all four formulations showed pH readings close to 5.5 which is the pH of skin. Droplet size analysis over the cycle testing period showed more variation in the HO and PM formulations suggesting instability in these formulations. All four samples showed no separation after undergoing centrifugation. Considering the long-term stability of the formulations, Formulation B was suggested as a viable option for the addition of the LSSJ plant extract. Due to the short shelf-lives and variation in droplet size throughout the testing period Formulation B is not recommended for the addition of the HO and PM plant extracts. The cost analysis of the placebo cost-efficient formulation showed a cost of R 0.99 for 100 g of placebo formulation. This proved much more cost effective than Formulation A of Chapter 4.

CHAPTER 6: FORMULATION CAMPAIGN C

6.1 INTRODUCTION

The Department of Plant and Soil Sciences at the University of Pretoria hypothesised that one of the plant extracts, BS, may have SPF boosting capabilities. To test the extent of the SPF boosting property this plant extract needs to be incorporated into a sunscreen formulation. This chapter deals with the development of this sunscreen formulation which is referred to as Formulation C. The lotion formulations developed in Chapter 4 (Formulation A) and Chapter 5 (Formulation B) were used as the basis for Formulation C but were modified to incorporate physical UV filters.

Since a sensory evaluation had already been conducted on these lotion formulations, the main concern in developing the sunscreen was its stability. Long term stability was conducted on the three developed sunscreens in its placebo form and with the addition of the BS plant extract. This involved coalescence analysis, microscopy and cycle testing. A costing analysis was also done on all three placebo sunscreens.

6.2 FORMULATION DEVELOPMENT

The formulations of this chapter were based on Formulation A of Chapter 4 and Formulation B of Chapter 5. A broad-spectrum physical UV filter was selected in each formulation and a theoretical SPF of 15 was chosen. All the formulations in this chapter underwent high shear mixing to assist in the dispersion of the UV filter within the sample – as discussed in Chapter 3.

The first sunscreen formulation, Sunscreen A, was a modification of Formulation A of Chapter 4. In this formulation a Titanium Dioxide dispersion was used. This UV filter

was sourced through Croda (SA), contains 55 % solids content and has an approximate Titanium Dioxide content of 47 % by weight. To help with the incorporation of the UV filter a thickening agent was added to Formulation A. This was done to prevent solid particles from settling out of the sample. The complete formulation for Sunscreen A and its preparation method is shown in Table 6-1.

Table 6-1: The complete formulation of Sunscreen A

Ingredient	**%**	**Application**	**INCI/Chemical Name**
		Phase A	
Water	67.2	Diluent/solvent	Aqua
EDTA	0.10	Chelating agent	Tetrasodium EDTA
Glycerin	1.60	Humectant	Glycerin
Xanthan Gum	0.30	Thickener	Xanthan Gum
		Phase B	
Crodex M	5.60	Emulsifier	Cetostearyl Alcohol (and) Potassium Cetyl Phosphate
Crodamol STS	1.80	Emollient	PPG-3 Benzyl Ether Myristate
Crodamol SFX	1.80	Emollient	PPG-3 Benzyl Ether Ethylhexanoate
Solaveil™ XT-100	10.6	UV filter	Titanium Dioxide, C12-15 Alkyl Benzoate, Polyhydroxystearic Acid, Stearic Acid & Alumina
		Phase C	
Active ingredient	10.0	Active ingredient	Ethanolic Plant Extract
Germaben II	1.00	Preservative	Propylene Glycol (and) Diazolidinyl Urea (and) Methylparaben (and) Propylparaben

Method

1. Mix ingredients of Phase A together in a clean beaker and heat to 65 °C.
2. Mix ingredients of Phase B together in a clean beaker and heat to 65 °C.
3. Slowly add Phase B to Phase A while stirring using a Silverson® high shear mixer at 5000 rpm for 15 minutes.
4. Allow to cool to 40 °C with stirring using a magnetic stirrer.
5. Add the active ingredient and preservative, stirring after each addition.
6. Mix for 15 minutes until homogeneous.

Sunscreen B was based on Formulation B. When formulating Sunscreen B, Titanium Dioxide and Zinc Oxide powder were considered as possible UV filters. For both these UV filters, a loading of 1 % by weight corresponds to approximately 1 SPF unit (Making Cosmetics, 2017). Trial formulations were made using Titanium Dioxide alone, Zinc Oxide alone, and combinations of Zinc Oxide and Titanium Dioxide. The formulation containing Zinc Oxide alone showed the best feel properties amongst the trial formulations. Subsequently, the UV filter used in Sunscreen B was Zinc Oxide BP which has the INCI name Zinc Oxide. The complete formulation for Sunscreen B is shown in Table 6-2.

Sunscreen C was also based on Formulation B. It used the same ingredients and loadings as Sunscreen B with the exception of the UV filter. Sunscreen C incorporated a UV filter dispersion in natural esters and oils. The UV filter was called SO60MZJ and had the INCI name Zinc Oxide (and) Helianthus Annuus (Sunflower) Seed Oil (and) Jojoba Esters. It was sourced from Kobo Products through a local agent, Cosmetic Ingredients (Pty) Ltd. This UV filter has an active ingredient percentage of 57 % and an average particle size of 371 nm. Table 6-2 shows the complete formulation for Sunscreen C.

Table 6-2: The complete formulation of Sunscreen B and Sunscreen C

Ingredient	%	Function	INCI/Chemical Name
		Phase A	
Water	77.0	Diluent/solvent	Aqua
EDTA	0.10	Chelating agent	Tetrasodium EDTA
Propylene Glycol	1.80	Humectant	Propylene Glycol
Carbopol 940	0.10	Thickener	Carbomer
		Phase B	
Palmerol 6830	3.18	Emulsifier	Cetostearyl Alcohol
ERCAWAX CS 20 V/FD	3.18	Emulsifier	Ceteareth 20
ERCAREL AB V	3.64	Emollient	C12-15 Alkyl Benzoate
UV filter	13.5	UV filter	UV Filter
		Phase C	
Active ingredient	10.0	Active ingredient	Ethanolic Plant Extract
Germaben II	1.00	Preservative	Propylene Glycol (and) Diazolidinyl Urea (and) Methylparaben (and) Propylparaben
		Method	

1. Mix ingredients of Phase A together in a clean beaker and heat to 65 °C.
2. Mix ingredients of Phase B together in a clean beaker and heat to 65 °C.
3. Slowly add Phase B to Phase A while stirring using a Silverson® high shear mixer at 5000 rpm for 15 minutes.
4. Allow to cool to 40 °C with stirring using a magnetic stirrer.
5. Add the active ingredient and preservative, stirring after each addition.

6. Mix for 15 minutes until homogeneous.

6.3 LONG TERM STABILITY TESTING

In the development of the sunscreens, long term stability testing was conducted on Sunscreen A, Sunscreen B and Sunscreen C in their placebo forms and with the addition of 10 % (by mass) of the BS plant extract. Long term stability testing involved three testing procedure namely, coalescence analysis, microscopy and cycle testing.

6.3.1 Coalescence Analysis

Coalescence analysis was conducted on each of the sunscreens with and without the addition of the BS plant extract. Sunscreen A underwent a testing period of 12 months, Sunscreen B had a testing period of 7 months and Sunscreen C had a testing period of 6 months. All samples were stored under three storage conditions of 4 °C, 25 °C, and 40 °C.

The coalescence plots of Sunscreen A in its placebo form and with the BS plant extract are shown in Figure 6-1 and Figure 6-2 respectively. Similar trendlines under all three storage conditions were observed. This indicated that storage temperature had little effect on the stability of Sunscreen A.

Figure 6-3 and Figure 6-4 shows the coalescence plots for Sunscreen B in its placebo form and with the addition of the BS plant extract respectively. The coalescence plot for the samples stored at 40 °C consist of only three data points. This is due to the fact that these samples failed at oven conditions after 10 days when the UV filter settled out of the formulation. Later it separated into two liquid phases and a separate solids layer of the Zinc Oxide. It was therefore removed from long term stability testing.

Finally, the coalescence plots for Sunscreen C in its placebo form and with the addition of the BS plant extract is shown in Figure 6-5 and Figure 6-6 respectively. In both these plots, a clear direct proportionality between storage temperature and droplet size was noticed.

A summary of the trendlines, rates of coalescence, projected shelf life and minimum theoretical shelf life of each of the sunscreens in its placebo form and with the addition of the BS plant extract is shown in Table 6-3. Only Sunscreen A in both its forms showed a shelf life of more than 1 year at 25 °C and more than 6 months at 40 °C. Sunscreen B in both its forms stored at 40 °C showed similar, very short, projected shelf-lives and this was confirmed by its failure due to phase separation. Finally, Sunscreen C showed an inversely proportional relationship between storage temperature and shelf life. This sunscreen with the addition of the BS plant extract showed longer shelf-lives than in its placebo form. Considering the shelf-lives of the sunscreen samples stored at room temperature, Sunscreen C showed the shortest shelf-lives in both its forms.

Figure 6-7 shows the rate of coalescence as a function of the storage temperature. This is shown for each of the three placebo sunscreens and the three sunscreens with the addition of the BS plant extract. Here the slowest rates of coalescence were observed for Sunscreen A in its placebo form and with the addition of the BS plant extract. Sunscreens B and C showed similar rates of coalescence possibly due to the fact that both these formulations were based on Formulation B. As expected, the oven samples of Sunscreen B in its placebo form and with the BS plant extract had much faster rates of coalescence which resulted in the early failure of these formulations.

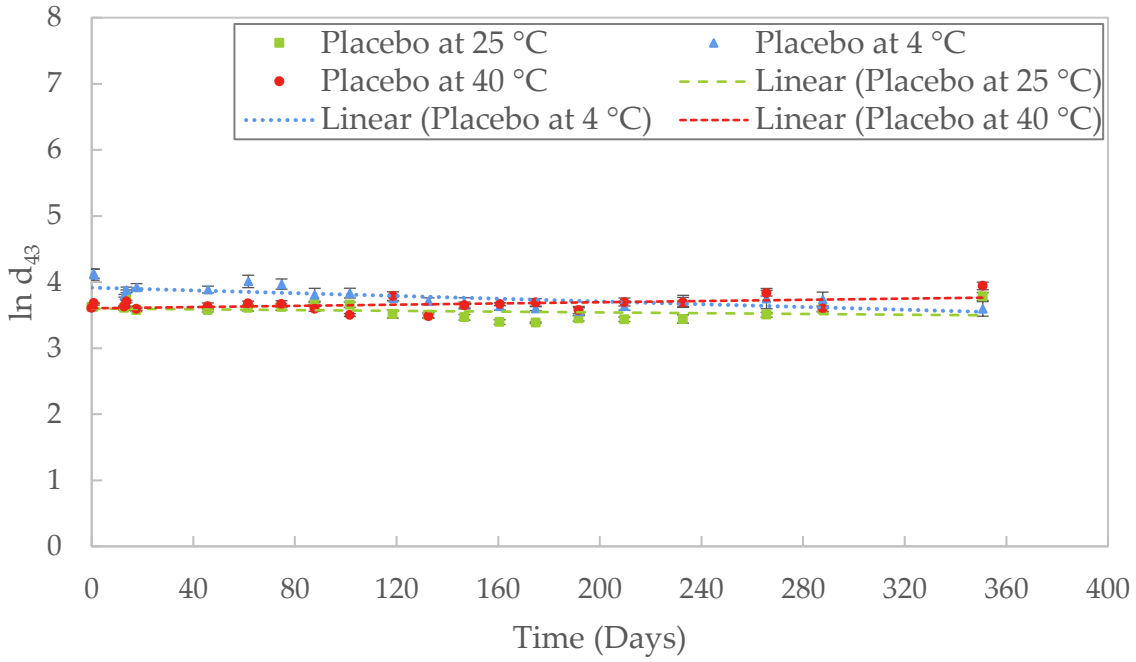


Figure 6-1: Coalescence plot of Sunscreen A in its placebo form

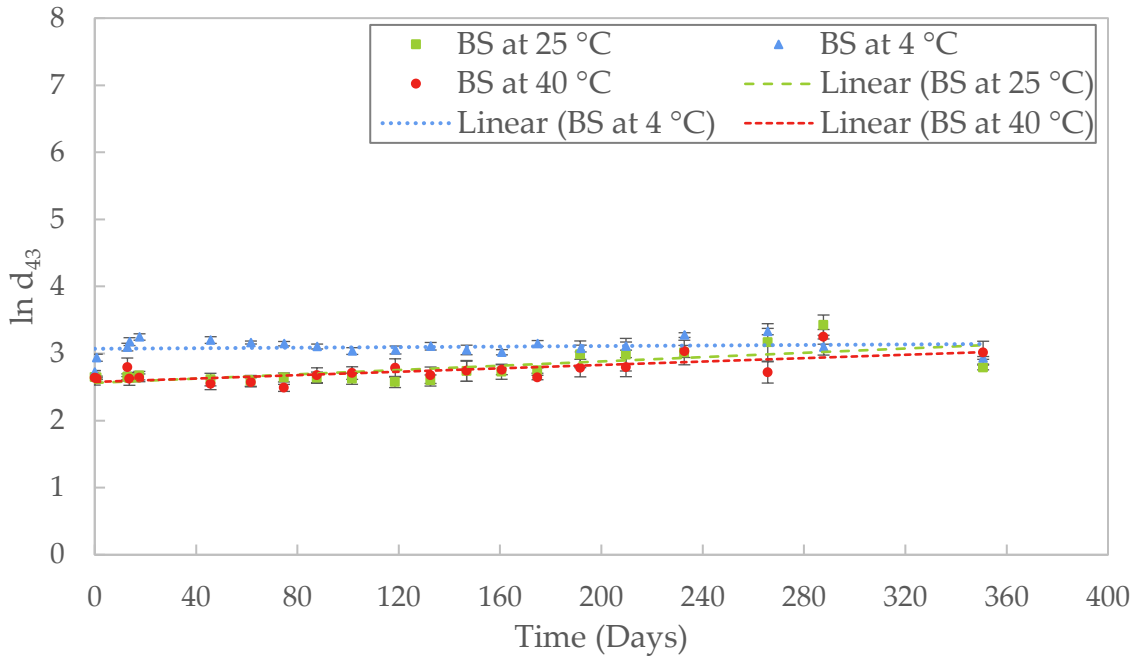


Figure 6-2: Coalescence plot of Sunscreen A with the addition of the BS plant extract

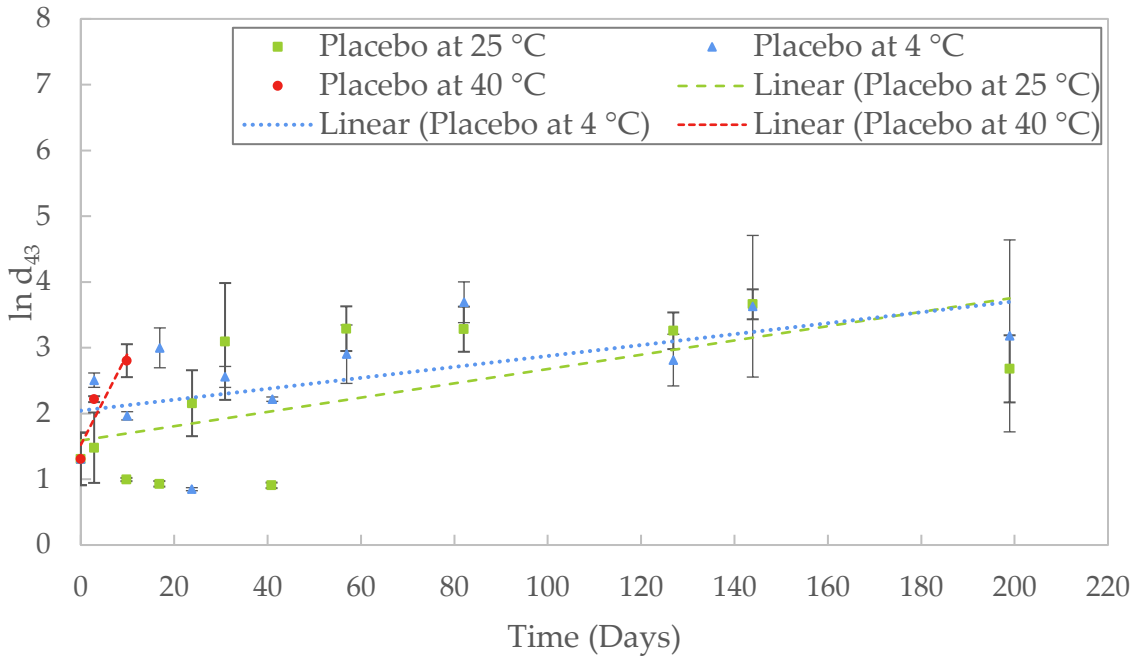


Figure 6-3: Coalescence plot of Sunscreen B in its placebo form

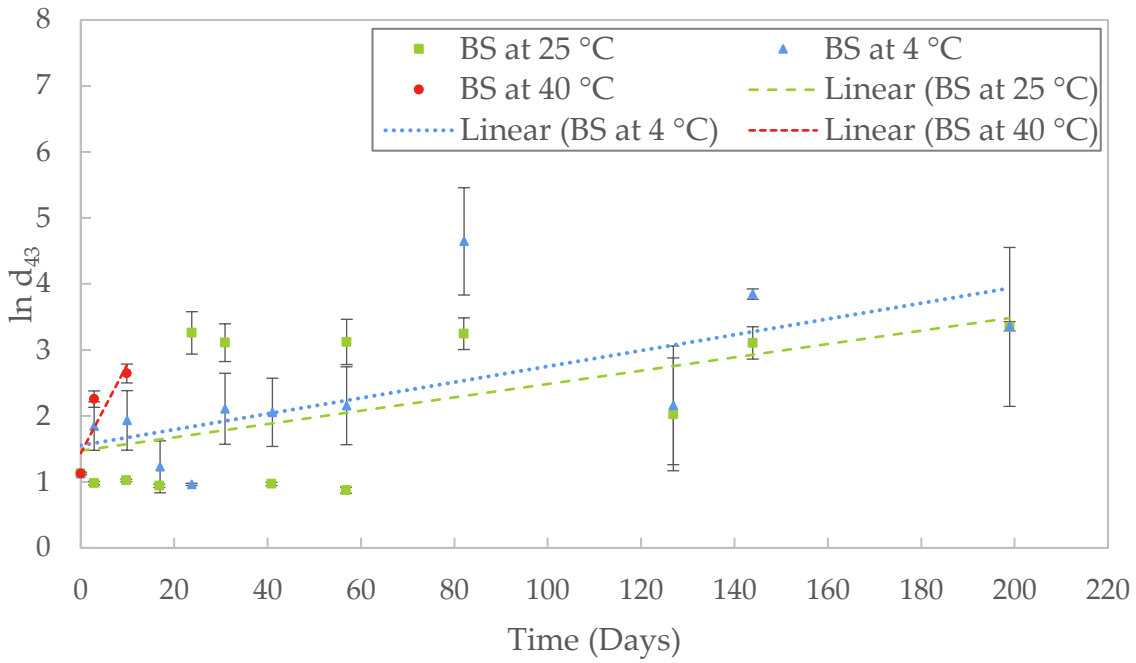


Figure 6-4: Coalescence plot of Sunscreen B with the addition of the BS plant extract

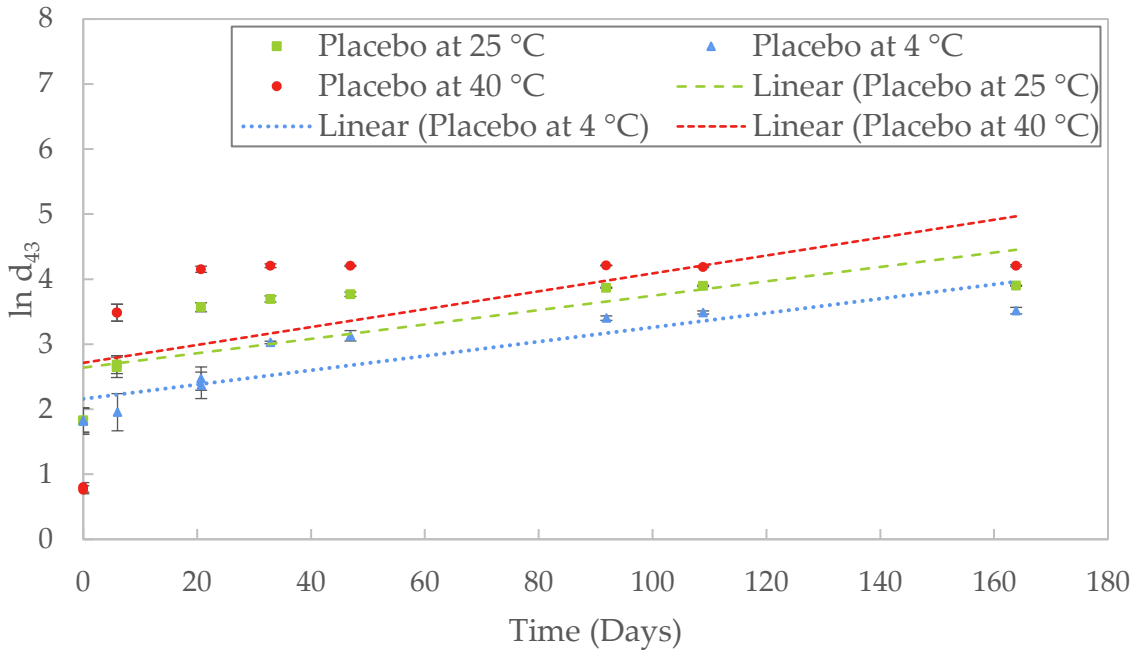


Figure 6-5: Coalescence plot of Sunscreen C in its placebo form

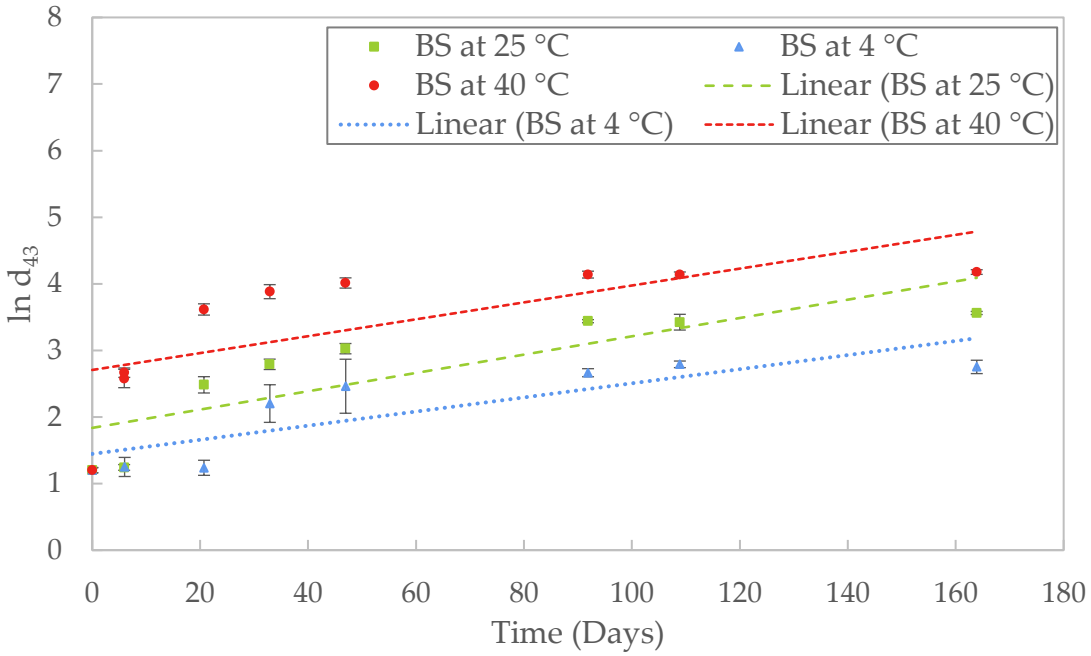


Figure 6-6: Coalescence plot of Sunscreen C with the addition of the BS plant extract

Table 6-3: Summary of the trendline equations, calculated K_C values, projected shelf life and minimum theoretical shelf life of the sunscreen formulations in its placebo form and with the incorporation of the BS plant extract

Temperature (°C)	Trendline Equation	K_C (Day^{-1})	Shelf Life (Years)	Minimum Shelf Life (Years)
Placebo Sunscreen A				
4	$y = 0.0001x + 3.7148$	0.3×10^{-3}	5.40 ± 1	4.55
25	$y = 0.0003x + 3.5987$	0.9×10^{-3}	2.86 ± 1	1.75
40	$y = 0.0005x + 3.6027$	1.5×10^{-3}	1.70 ± 1	0.769
BS in Sunscreen A				
4	$y = 0.0002x + 3.0712$	0.6×10^{-3}	11.5 ± 3	8.46
25	$y = 0.0016x + 2.5623$	4.8×10^{-3}	2.31 ± 1	1.09
40	$y = 0.0013x + 2.5766$	3.9×10^{-3}	2.81 ± 2	0.821
Placebo Sunscreen B				
4	$y = 0.0083x + 2.0429$	24.9×10^{-3}	0.617 ± 0.3	0.278
25	$y = 0.0109x + 1.5892$	32.7×10^{-3}	0.589 ± 0.3	0.257
40	$y = 0.1385x + 1.5231$	416×10^{-3}	0.0473 ± 0.02	0.0203
BS in Sunscreen B				
4	$y = 0.012x + 1.553$	36.0×10^{-3}	0.539 ± 0.3	0.229
25	$y = 0.0101x + 1.4728$	30.3×10^{-3}	0.662 ± 0.3	0.314
40	$y = 0.1352x + 1.4355$	406×10^{-3}	0.0502 ± 0.02	0.0263
Placebo Sunscreen C				
4	$y = 0.011x + 2.1586$	33.0×10^{-3}	0.437 ± 0.3	0.122
25	$y = 0.0111x + 2.6399$	33.3×10^{-3}	0.314 ± 0.2	0.123

Temperature (°C)	Trendline Equation	Kc (Day^{-1})	Shelf Life (Years)	Minimum Shelf Life (Years)
40	$y = 0.0138x + 2.7131$	41.4×10^{-3}	0.238 ± 0.1	0.122
BS in Sunscreen C				
4	$y = 0.0106x + 1.4453$	31.8×10^{-3}	0.638 ± 0.4	0.213
25	$y = 0.0138x + 1.8363$	41.4×10^{-3}	0.412 ± 0.2	0.143
40	$y = 0.0127x + 2.706$	38.1×10^{-3}	0.260 ± 0.1	0.112

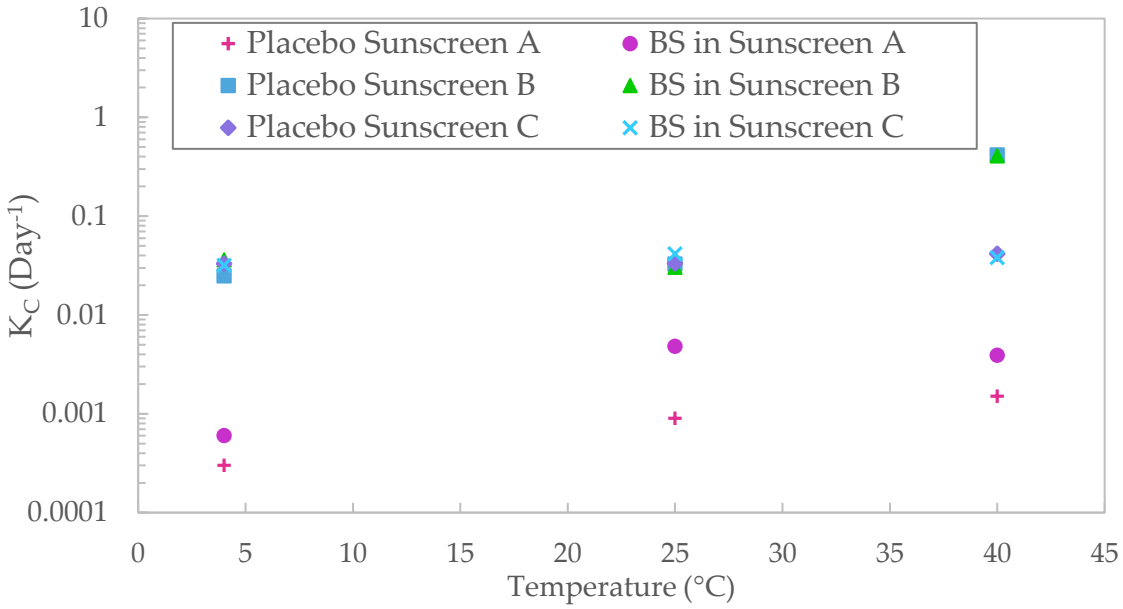


Figure 6-7: Rate of coalescence as a function of storage temperature for each of the sunscreen formulations

Overall Sunscreen A in both its forms showed slow rates of coalescence and acceptable shelf-lives. For this reason, Sunscreen A is recommended as the best performing sunscreen formulation for the addition of the BS plant extract.

6.3.2 Microscopy

Optical microscopy was conducted on each of the sunscreen formulations and the microscopy results are discussed in this section. The micrographs of Sunscreen A in its placebo form at the beginning and end of the 12 month testing period are shown in Figure 6-8. In these micrographs it can be seen that the UV filter tends to form agglomerates between the initial and final micrographs. Furthermore, the individual droplets become less visible between the initial and final micrographs.

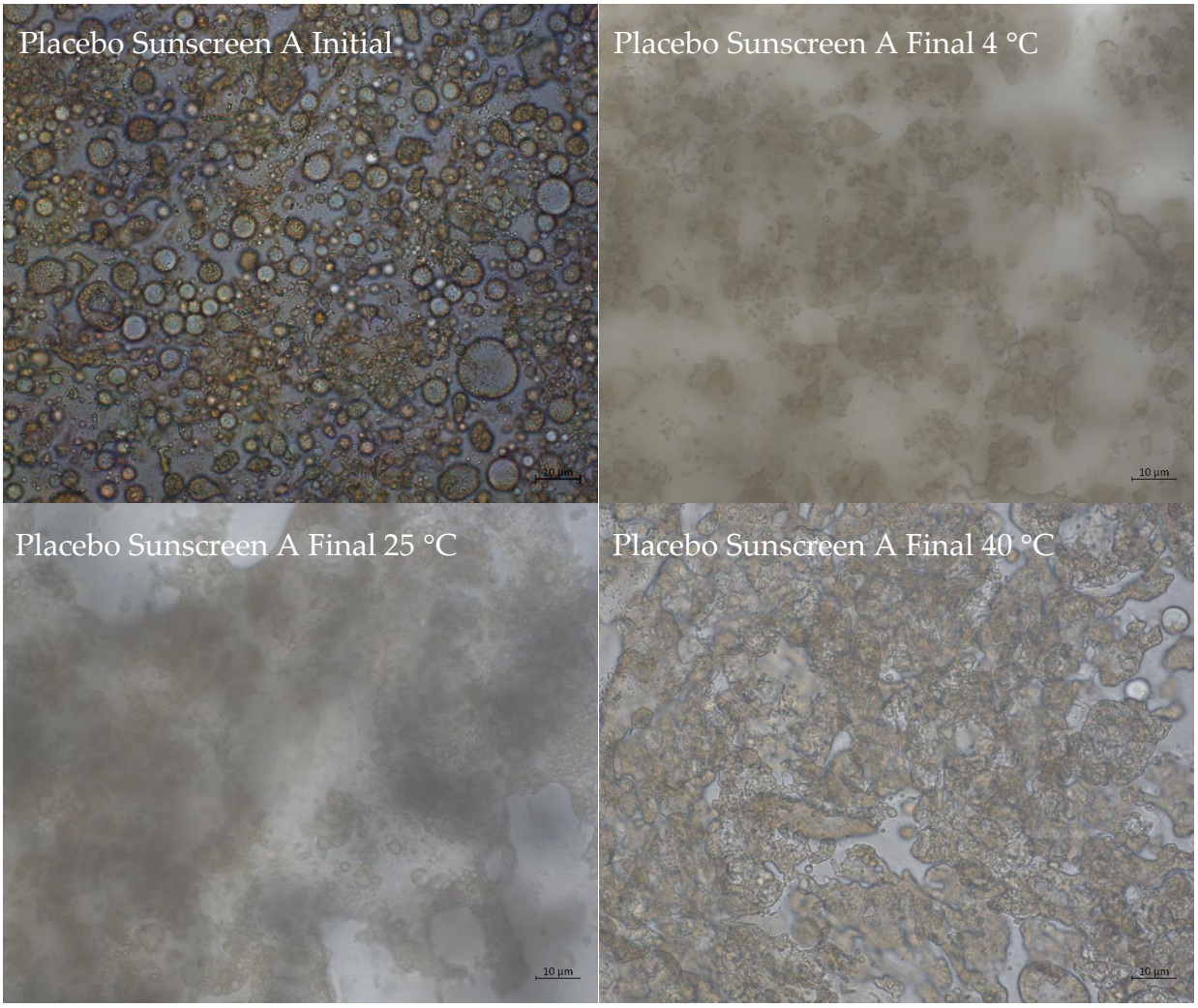


Figure 6-8: Micrographs of Sunscreen A in its placebo form

The micrographs of Sunscreen A with the addition of the BS plant extract is shown in Figure 6-9. Here similar droplet sizes are observed between the initial sample and the samples stored at room temperature and in the oven. This confirms the slow rates of coalescence observed in the previous section.

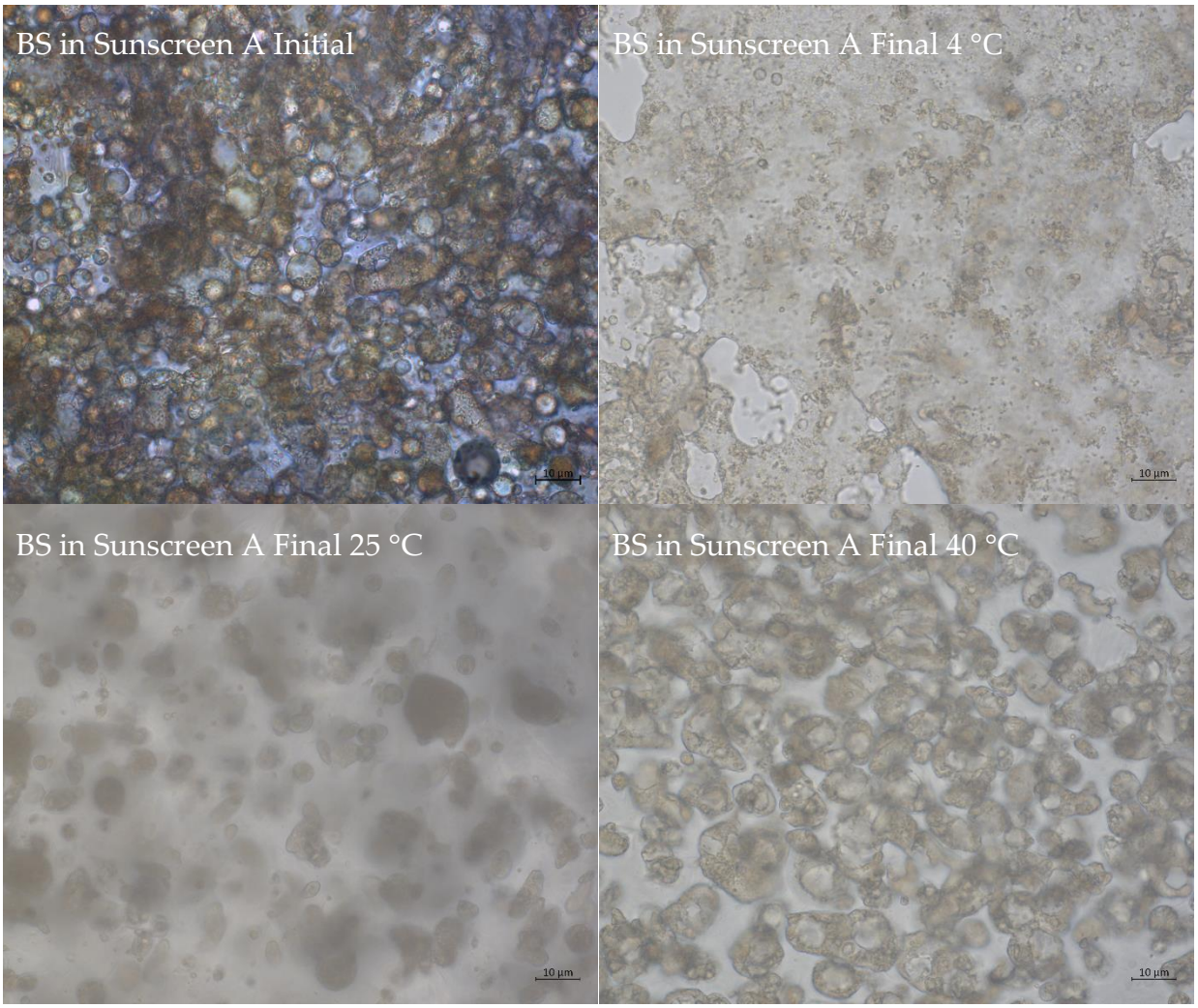


Figure 6-9: Micrographs of Sunscreen A with the addition of BS

The micrographs of Sunscreen B in its placebo form is shown in Figure 6-10 and Sunscreen B with the addition of the BS plant extract is shown in Figure 6-11. These micrographs were taken at the start and end of the 7 month testing period. Both these figures show the initial micrographs and the final micrographs of the samples stored at

4 °C and 25 °C. The sample stored at 40 °C separated before the end of the testing period and therefore microscopy was not done on the oven sample. In both versions of Sunscreen B, the UV filter formed agglomerates but very little variation was noticed between the final samples stored at room temperature and at 4 °C.

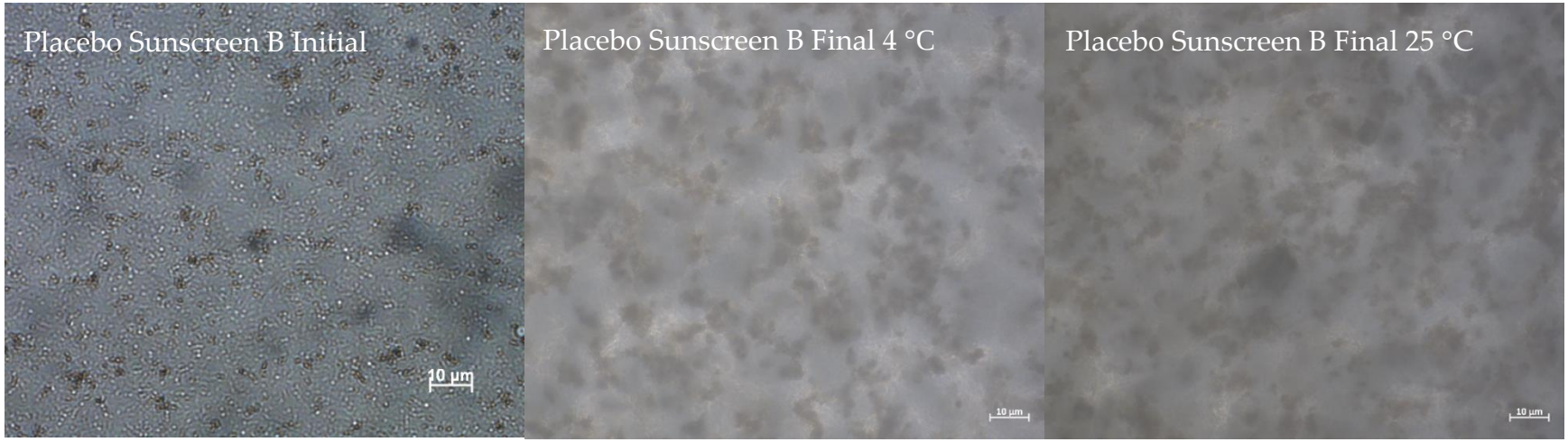


Figure 6-10: Micrographs of Sunscreen B in its placebo form

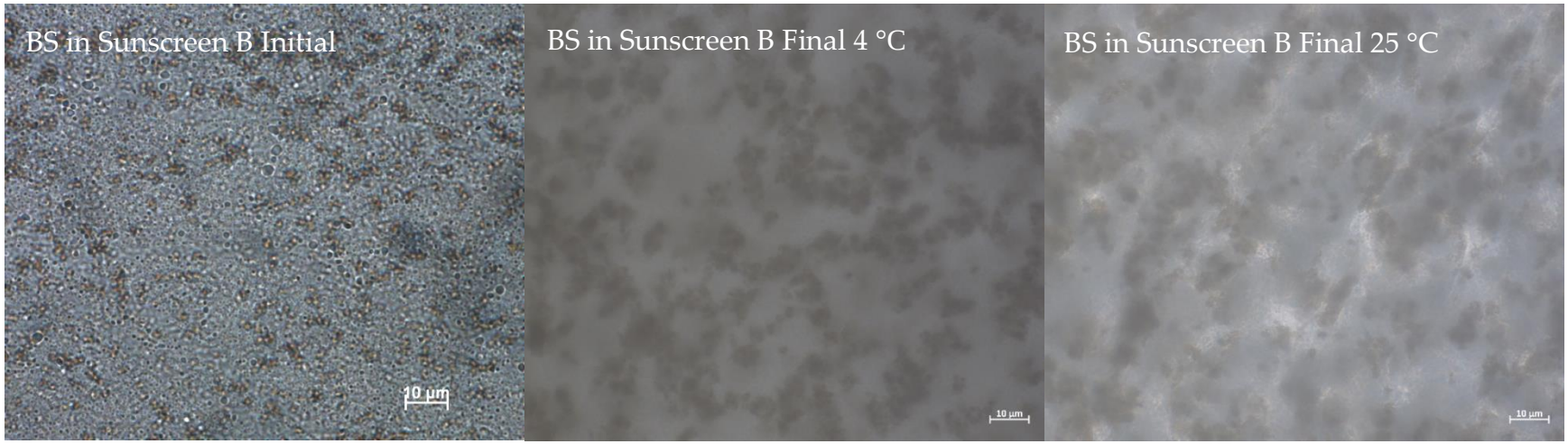


Figure 6-11: Micrograph of Sunscreen B with the addition of BS

The micrographs of the Sunscreen C in its placebo form is shown in Figure 6-12 and Sunscreen C with the addition of the BS plant extract is shown in Figure 6-13. The micrographs of the placebo formulation and the BS formulation were taken at the beginning and end of the 6 month testing period. The placebo sample showed the same droplet size between the initial and final samples. The samples with the addition of the BS plant extract showed a clear increase in droplet size between the initial and final micrographs.

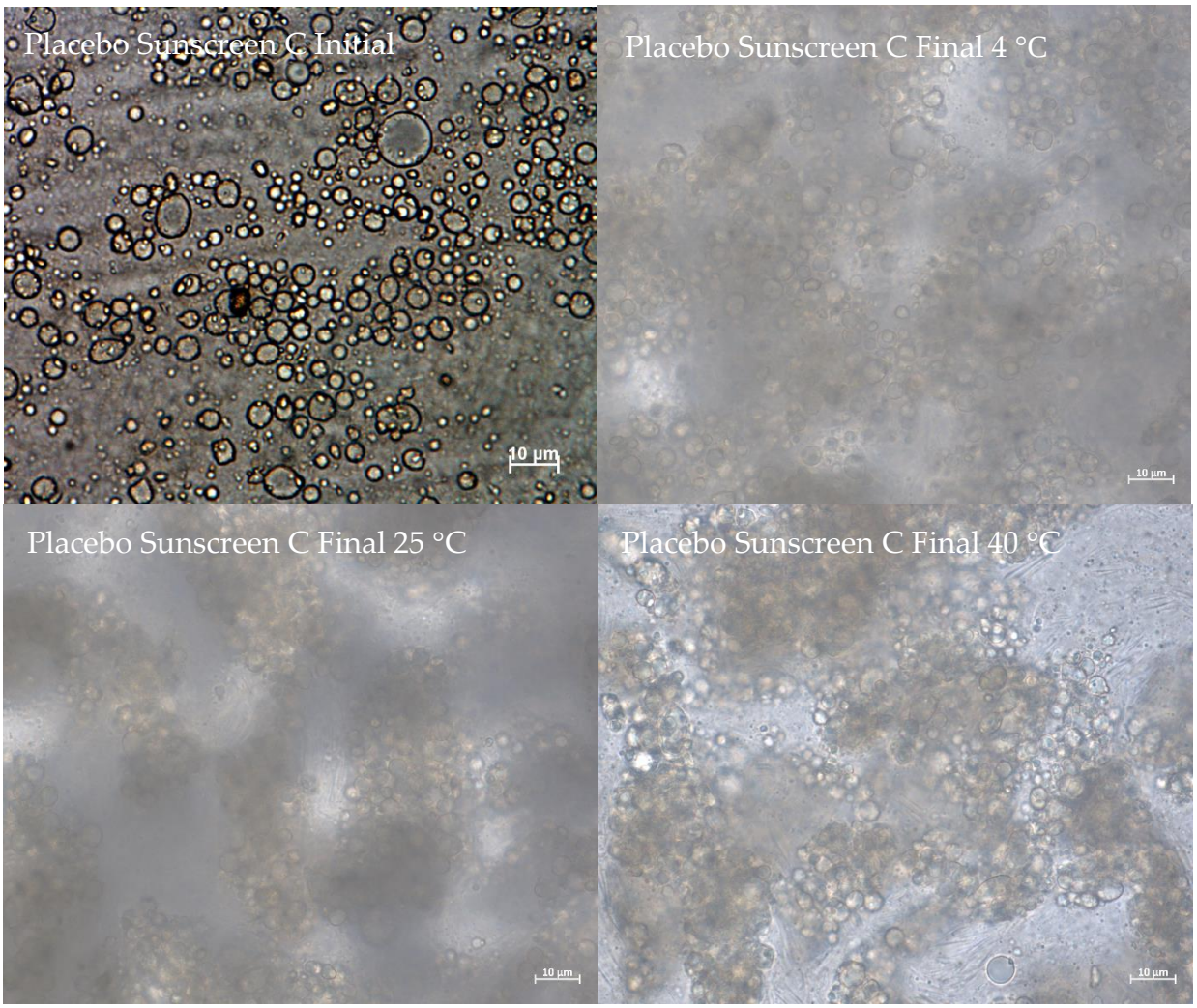


Figure 6-12: Micrographs of Sunscreen C in its placebo form

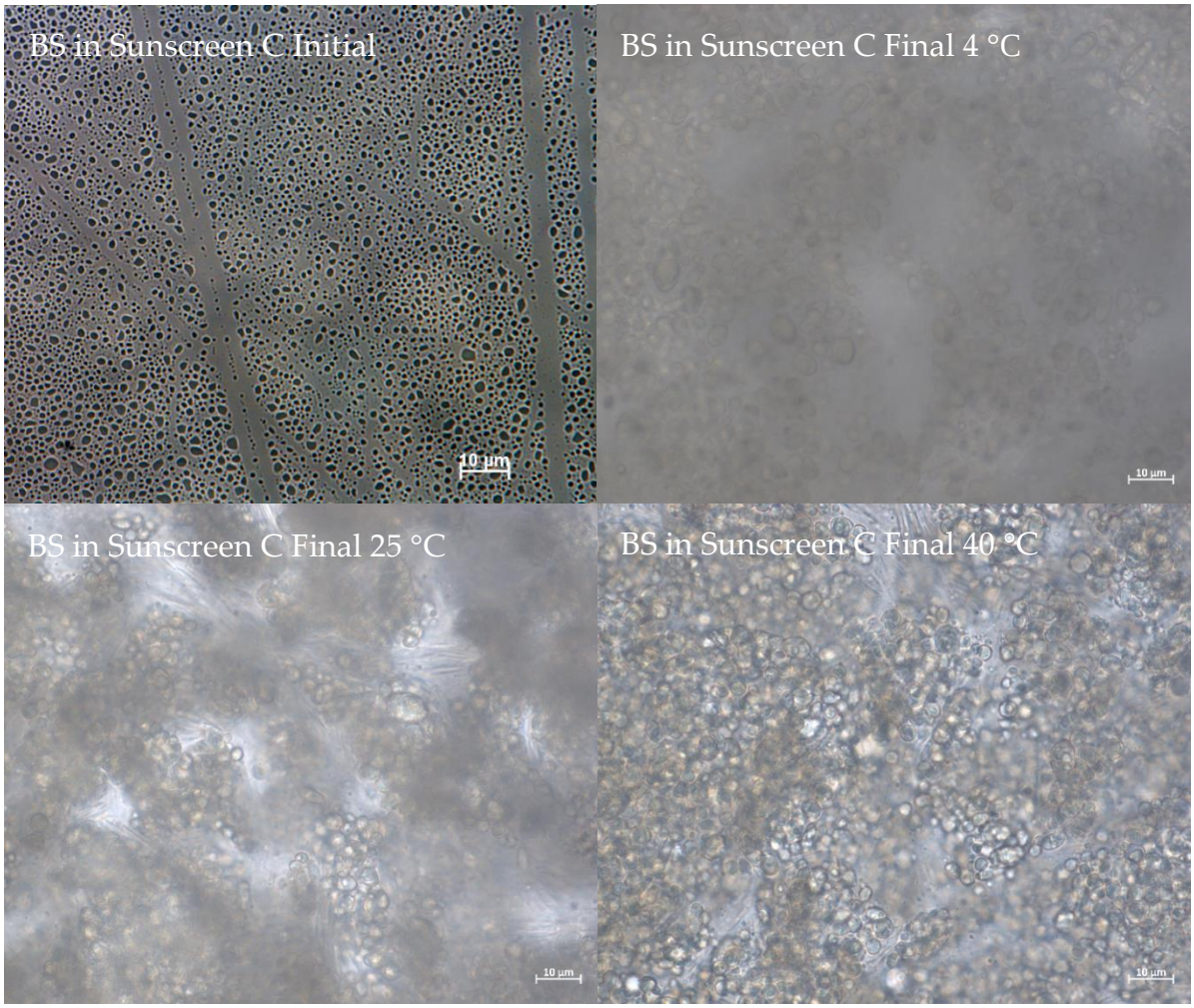


Figure 6-13: Micrographs of Sunscreen C with the addition of BS

6.3.3 Cycle Testing

Cycle testing was conducted on the sunscreen samples over six fridge-oven cycles for Sunscreen A and seven fridge-oven cycles for Sunscreen B and C, both followed by five freezer-oven cycles. The pH and droplet size were tested after each 48 hour cycle and the samples underwent centrifugation to observe any phase separation.

6.3.3.1 pH Analysis

The pH of the samples was measured at the end of each cycle. The change in pH over each cycle for the Sunscreen A in both its forms is shown in Figure 6-14. In both graphs of Sunscreen A, the pH increased between cycle 0 and cycle 1 and then remained constant across the remaining cycles. The pH of Sunscreen A in its placebo form and with the addition of the BS plant extract was 6.95 ± 0.09 and 6.87 ± 0.09 respectively.

Sunscreen B failed after the first cycle where the solids (the UV filter) settled to the bottom of the sample. Sunscreen B was excluded from testing after the first cycle therefore the pH over each cycle was not plotted. The pH of Sunscreen B in its placebo form decreased from 7.58 to 7.13 between cycle 0 and cycle 1. Similarly, the pH of Sunscreen B with the addition of the BS plant extract decreased from 7.62 at cycle 0 to 7.22 at cycle 1.

The cycle testing results for the pH of Sunscreen C in its placebo form and with the addition of the BS plant extract is shown in Figure 6-15. In the placebo sunscreen, the pH decreases over each cycle. A larger variation in the pH is noticed in the five freezer-oven cycles where larger error bars were obtained. The pH of Sunscreen C with the addition of the plant extracts decreases over the first few cycles and then stays fairly constant over the remaining cycles. The pH of Sunscreen C in its placebo form and with the addition of the BS plant extract was 7.35 ± 0.1 and 7.21 ± 0.08 respectively.

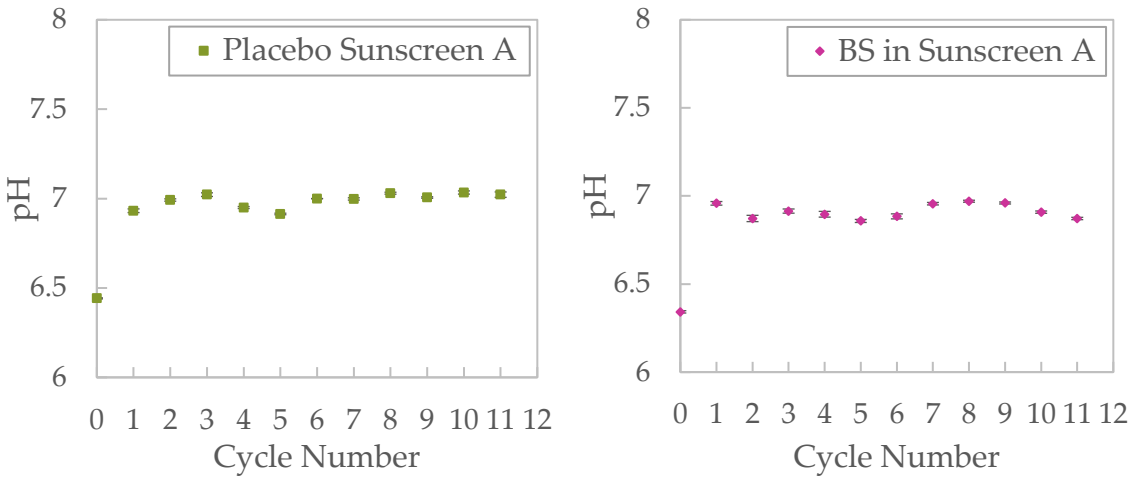


Figure 6-14: pH results for Sunscreen A over each cycle

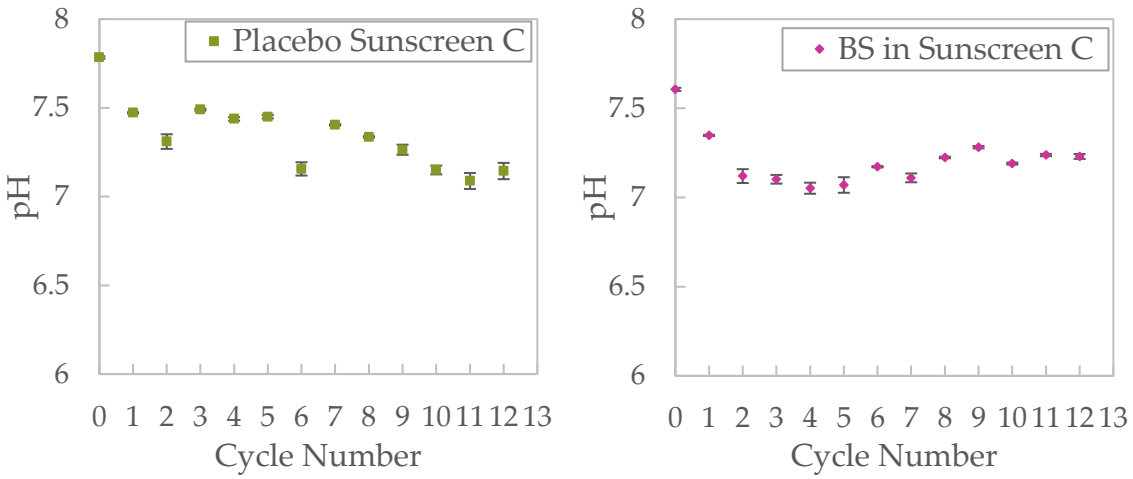


Figure 6-15: pH results for Sunscreen C over each cycle

6.3.3.2 Droplet Size Analysis

The results of the median droplet size at the end of each cycle for Sunscreen A in its placebo form and with the addition of the BS plant extracts is shown in Figure 6-16. From these graphs it was noticed that there was a decrease in the median droplet size over the six fridge-oven cycles. This was followed by an increase in droplet size over the first three freezer-oven cycles. The sample of Sunscreen A with the addition of the BS plant extract

showed a smaller variation in droplet size over the fridge-oven cycles than the placebo sample suggesting a possible stabilising effect.

The median droplet size results of Sunscreen C at the end of each cycle is shown in Figure 6-17. Sunscreen C in its placebo form and with the addition of the BS plant extract increased in median droplet size over the seven fridge-oven cycles. The placebo sample of Sunscreen C showed very little variation in the median droplet size over the freezer-oven cycles whereas the sample containing the BS plant extract increased more drastically over these cycles.

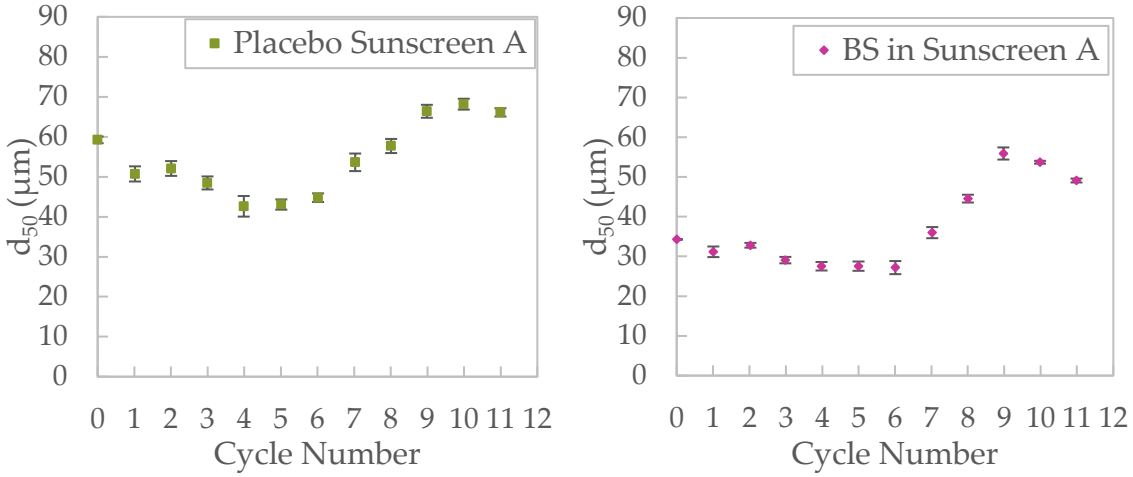


Figure 6-16: Median droplet size results for Sunscreen A over each cycle

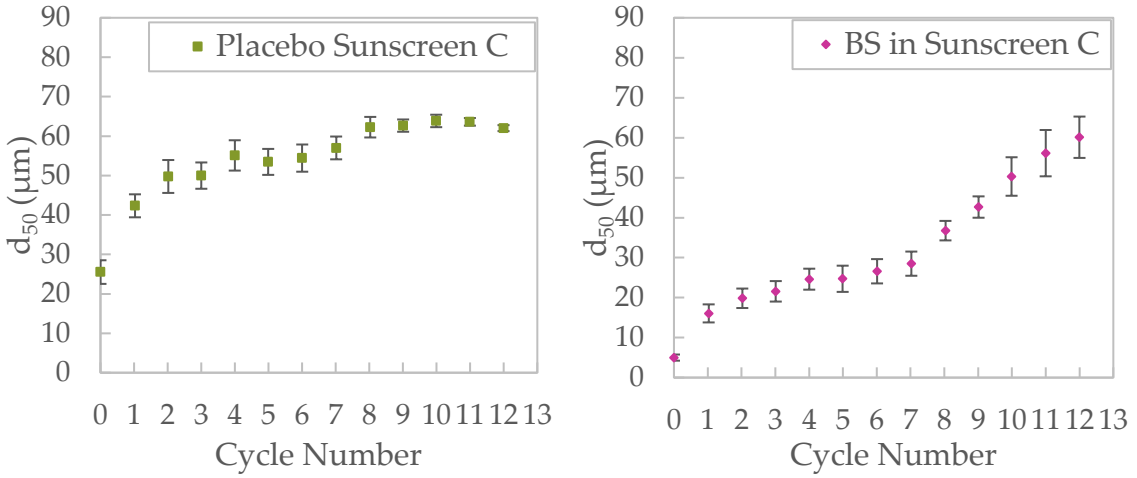


Figure 6-17: Median droplet size results for Sunscreen C over each cycle

6.3.3.3 Centrifugation

The samples underwent centrifugation at the end of each cycle. Table 6-4 shows the visual observation results. Here a range of abbreviations were used to describe the observations. NS indicates no separation occurred, LV indicates that the viscosity of the sample was lowered, LG indicates that the sample became less glossy, C indicates that creaming occurred where a dense layer formed at the top of the sample, and finally, PS indicates phase separation occurred where the solid phase separated from the liquid phase or the two liquid phases separated from each other. In this study, failure was determined by phase separation.

Sunscreen A showed no separation in both variations over all eleven cycles. In the second freezer-oven cycle, cycle 8, both variations of this formulation looked slightly less glossy but the samples showed no evidence of failure.

Sunscreen B showed phase separation at the end of cycle 1 where the solids separated out of the liquid phase. In cycle 2 the sample separated into two liquid phases and one solids phase which indicated complete failure and therefore was not tested any further.

Sunscreen C, in both variations, showed some phase separation at the end of the seventh fridge-oven cycle where some of the solids separated from the liquid phase. At the end of the first freezer-oven cycle, cycle 8, both samples showed signs of creaming where a foamy layer formed in top of the samples. At the end of the second freezer-oven cycle, Sunscreen C in both forms showed a decrease in viscosity. At the third, fourth and fifth freezer-oven cycles both Sunscreen C samples showed complete phase separation where the samples split into two liquid phases and one solids phase.

Table 6-4: Summary of the visual observations made after centrifugation

Cycle	**Sunscreen A**		**Sunscreen B**		**Sunscreen C**	
	Placebo	**BS**	**Placebo**	**BS**	**Placebo**	**BS**
1	NS	NS	PS	PS	NS	NS
2	NS	NS	PS	PS	NS	NS
3	NS	NS	-	-	NS	NS
4	NS	NS	-	-	NS	NS
5	NS	NS	-	-	NS	NS
6	NS	NS	-	-	NS	NS
7	NS	NS	-	-	PS	PS
8	LG	LG	-	-	C	C
9	NS	NS	-	-	LV	LV
10	NS	NS	-	-	PS	PS
11	NS	NS	-	-	PS	PS
12	-	-	-	-	PS	PS

6.4 COSTING

The costing of the placebo formulations of sunscreens A, B and C were conducted below. The cost breakdown of Sunscreen A is shown in Table 6-5. The cost of 100 g of Sunscreen A was calculated as R12.66. This is a fairly cost-effective sunscreen formulation. The UV filter used in this formulation was sourced from Croda (SA) and the minimum order quantity is 25 kg. This was the most expensive ingredient in this formulation and is used at a fairly a high loading. This is due to the fact that the loading of the UV filter is directly proportional to the SPF of the sunscreen product.

Table 6-5: Cost breakdown of placebo Sunscreen A

Ingredient	INCI/Chemical Name	%	Cost (R/kg)
Water	Aqua	74.8	0.00714
EDTA	Tetrasodium EDTA	0.10	34.41
Glycerin	Glycerin	1.80	14.45
Xanthan Gum	Xanthan Gum	0.30	30.28
Crodex M	Cetostearyl Alcohol (and) Potassium Cetyl Phosphate	6.20	67.00
Crodamol STS	PPG 3 Benzyl Ether Myristate	2.00	387.00
Crodamol SFX	PPG-3 Benzyl Ether Ethylhexanoate	2.00	368.00
Solaveil™ XT-100	Titanium Dioxide, C12-15 Alkyl Benzoate, Polyhydroxystearic Acid, Stearic Acid & Alumina	11.8	894.50
Germaben II	Propylene Glycol (and) Diazolidinyl Urea (and) Methylparaben (and) Propylparaben	1.00	143.00

Sunscreen B and Sunscreen C are based on the Formulation B and the loading of the UV filter in both formulations are the same. The costing analysis for both of these formulations are therefore shown in Table 6-6. In this cost breakdown the ingredient labelled UV Filter referrers to the two different UV filters used in each of these sunscreens.

Table 6-6: Cost breakdown of placebo Sunscreen B and Sunscreen C

Ingredient	**INCI/Chemical Name**	**%**	**Cost (R/kg)**
Water	Aqua	74.40	15.00
EDTA	Tetrasodium EDTA	0.10	1122.00
Propylene Glycol	Propylene Glycol	1.70	57.00
Carbopol 940	Carbomer	0.10	193.00
Palmerol 6830	Cetostearyl Alcohol	3.10	46.00
ERCAWAX CS 20 V/FD	Ceteareth 20	3.10	58.00
ERCAREL AB V	C12-15 Alkyl Benzoate	3.50	75.00
UV Filter	UV Filter	13.00	
Germaben II	Propylene Glycol (and) Diazolidinyl Urea (and) Methylparaben (and) Propylparaben	1.00	143.00

In Sunscreen B the UV filter used was Zinc Oxide BP which has an INCI name of Zinc Oxide. The cost of this UV filter was R 31.66 per kg. The cost of 100 g of placebo Sunscreen B was found to be R1.30. This is less expensive than Sunscreen A due to the fact that Sunscreen B was based on the more cost-efficient lotion formulation: Formulation B. Furthermore, the UV filter dispersion used in Sunscreen A was much more expensive than the Zinc Oxide powder used in this formulation.

The UV filter used in Sunscreen C was a Zinc Oxide dispersion called SO60MZJ. Its INCI names was Zinc Oxide (and) Helianthus Annuus (Sunflower) Seed Oil (and) Jojoba Esters and its cost was R 3229.00 per kg. The cost of 100g of Sunscreen C was found to be R42.86. This formulation is the most expensive formulation which is undesirable. In this formulation the UV filter was much more expensive than the UV filters used in Sunscreen A and B.

6.5 SPF TESTING

The SPF of Sunscreen A without any plant extract and with the addition of 10 % BS plant extract was tested through *in vitro* and *in vivo* SPF testing. These tests were conducted by the Photobiology Laboratory at Sefako Makgatho Health Sciences University.

6.5.1 *In vitro* SPF testing

The *in vitro* UVA SPF testing procedure was followed as described in ISO 24443. In this procedure the relevant UVA protection parameters were determined to provide a UV spectral absorbance curve. This curve was then used for the calculations of the Ultraviolet-A Protection Factor (UVAPF), critical wavelength and UVA absorbance proportionality (ISO 24443:2012(E), 2012).

The *in vitro* testing procedure is based on UV-transmittance. A thin film of sunscreen sample is spread on a roughened substrate. It is then exposed to a controlled dose of radiation from a defined UV exposure source. The UV-transmittance is assessed before and after exposure to the radiation. The Labsphere UV Transmittance Analyser 2000S was used to take these measurements.

6.5.2 *In vivo* testing

The *in vivo* SPF testing procedure was followed as described in South African Standard SANS 1557/ European Colipa Standard/ ISO 24444. The international standard ISO 24444 applies to products, intended to be placed in contact with human skin, that contains any component able to absorb, reflect or scatter UV rays.

The testing method determines the protection provided by sunscreen products on human skin against erythema induced by solar UV rays. It uses a xenon arc lamp solar simulator of a defined and known output to provide UV exposure. Three sections of each subject's skin are exposed to UV light. The first without any protection, the second after application of the sunscreen product being tested and the third after application of an SPF reference sunscreen formulation which is used to validate the procedure. An amount of 2.00 mg/cm^2 of sunscreen test product and reference formulation (before spreading) is applied to the skin (ISO 24444:2010(E), 2010).

An incremental series of delayed erythemal responses are induced on a number of small sub-sites on the skin. These responses are visually assessed for the presence of redness 16 and 24 hours after UV radiation exposure. The minimal erythemal dose (MED) referrers to the lowest dose of UV radiation that induces reddening of the skin. It is obtained for the unprotected skin (MED_U) and the product protected skin (MED_P). An individual SPF (SPF_I) for each test subject is then calculated using Equation (6-1). The arithmetic mean of all valid SPF_I results give the SPF of the product (ISO 24444:2010(E), 2010).

$$SPF_I = \frac{MED_P}{MED_U} \tag{6-1}$$

6.5.3 SPF results

A reference sunscreen of SPF 15 was used to validate the results. Regulations and guidelines complied with the World Medical Association Declaration of Helsinki. All volunteers signed informed consent before the study commenced. During the study 10 healthy human female volunteers were recruited. They were aged between 18 and 23 (mean: 19.5) and they all had skin of phototype II according to Fitzpatrick. The *in vitro* and *in vivo* SPF results obtained are shown in Table 6-7.

Table 6-7: *In vitro* and *in vivo* SPF results for Sunscreen A in both its forms

Property	Placebo	10% BS plant extract
UVAPF mean	6.47 (standard deviation: 0.06; coefficient: 0.92 %)	6.45 (standard deviation: 0.06; coefficient: 0.99 %)
SPF *in vivo* / UVAPF ratio	2.32	2.33
UVA balance	39 %	39 %
Critical wavelength	379.19 nm	379.50 nm
SPF	15 ± 0.8 (mean: 15.8; standard deviation: 0.4)	16 ± 0.5 (mean: 16.1; standard deviation: 0.7)

From this it is clear that the BS plant extract provides no additional protection against UVA radiation. Sunscreen A containing 10 % BS plant extract was able to increase the SPF of placebo Sunscreen A by 1 SPF unit showing slight SPF boosting capability. Although the increase was small it can be owed to the high antioxidant activity of the plant extract. This activity allowed the ability to scavenge free radicals associated with erythema caused by exposure to UV radiation.

Under the current SANS 1557 standard, products may make the claim of "Broad Spectrum" should the product have a mean critical wavelength of 370 nm or above.

Products may make the claim of "UVA Protection" only if the product has a UVA balance of 33% and a mean critical wavelength of 370 nm or above. This implies that Sunscreen A in its placebo form and with 10 % of the BS plant extract, can make both these claims.

6.6 CONCLUSION

Although three sunscreen formulations were developed, Sunscreen A outperformed both Sunscreen B and C. Sunscreen B showed signs of separation at accelerated conditions and under extreme stress conditions during cycle testing. Sunscreen C on the other hand, did not fail under accelerated conditions but did show signs of separation under cycle testing conditions. Both these formulations showed minimum theoretical shelf-lives of less than 6 months which is undesirable.

Sunscreen A in its placebo form and with the addition of the BS plant extract showed good stability. This was proven by its slow rates of coalescence, long shelf-lives and good resistance to high stress conditions. Even though Sunscreen B was the most cost-effective formulation, it was not a stable formulation. Sunscreen A proved to be more cost-effective than Sunscreen C with a raw material cost of R 12.66 per 100 g. Sunscreen A was therefore selected as the best performing sunscreen for incorporation of the BS plant extract.

SPF testing was conducted on Sunscreen A in its placebo form and with the addition of 10 % BS plant extract. The SPF of placebo Sunscreen A was found to be 15 whereas Sunscreen A containing the BS plant extract was found to be 16. The BS plant extract therefore only showed slight SPF boosting capabilities which may be owed to its antioxidant activity.

CHAPTER 7: CONCLUSIONS AND RECOMMENDATIONS

7.1 INVESTIGATION CONCLUSIONS

Different formulation types, including gels, body milks, creams and lotions, were considered as candidate formulation types to incorporate the three ethanolic plant extracts for topical skin applications. These plant extracts were ethanol/water extracts. Since the plant extract is water soluble it will present itself in the water phase of emulsion formulations. Initial screening was done using formulations loosely based on guideline formulations of each type received from the suppliers of raw materials in the cosmetic industry. The most promising formulation type was identified by considering both sensory characteristics and preliminary stability testing of formulations without the addition of the plant extracts. The HO, PM and LSSJ plant extracts were then added to the selected formulation -Formulation A- upon which long term stability testing was conducted. This testing procedure involved coalescence analysis, microscopy and cycle testing.

In Chapter 4, the three most sensory appealing carrier formulations were a light lotion, a body milk and a light cream. The light lotion performed the best during preliminary stability testing and was therefore chosen as Formulation A. Coalescence analysis revealed that Formulation A with the plant extracts had an increase in coalescence rate suggesting a possible destabilising effect by the ethanolic plant extract. The storage temperature showed little effect on the rate of coalescence of the placebo formulation suggesting temperature resistance. The formulations with the addition of the plant extracts showed a direct proportionality between coalescence rate and storage temperature. The minimum theoretical shelf life of the placebo formulation and the formulations containing HO and LSSJ exceeded 1 year making these formulations viable

for long term application. The microscopy results verified the droplet size analysis and showed the change in the droplet size due to time and various storage conditions. The samples performed well during cycle testing where the stability of the Formulation A in its placebo form and with the addition of the plant extracts was substantiated by the pH and centrifugation results which remained constant and did not show signs of instability. The pH of all four samples was viable for skin care applications. Finally, the cost of 100 g of the placebo Formulation A was found to be R 2.16.

A second formulation, Formulation B, was developed in an attempt to reduce the raw material cost of Formulation A. The raw materials used in this formulation were commonly used cosmetic ingredients that are locally produced. Five formulations were included in an in-depth sensory evaluation where they were compared to a commercially available lotion which served as a benchmark. Preliminary stability testing was done on the five formulations and the sensitivity of the formulations to the dosage of the plant extract was tested using a mock extract. This mock extract was an ethanol/water mixture of the ratio 40:60. A dosage of 10 % and 20 % was tested and compared to the stability of the placebo formulations. Formulation B was selected based on sensory appeal and preliminary stability. This formulation was made including the HO, PM and LSSJ plant extracts which then underwent long term stability testing.

The sensory evaluation results showed that the two formulations that performed closest to the benchmark formulation were modifications of each other with the only difference being the presence of a thickening agent. The lotion formulation inclusive of the thickening agent showed the longest shelf life with the addition of the mock extract at 10 %. Since 10 % is the required plant extract dosage, the formulation inclusive of the thickening agent was selected for the addition of the three plant extracts. Long term stability testing showed acceptable shelf-lives for the placebo formulation and the

formulation with the LSSJ plant extract. Through cycle testing this formulation with and without the plant extracts showed pH readings close to 5.5 making it viable for skin care applications. All four samples showed no separation after undergoing centrifugation. Due to the short shelf-lives and variation in droplet size throughout the testing period, Formulation B is not recommended for the addition of the HO and PM plant extracts. This formulation with the addition of the LSSJ plant extract showed good long-term stability and performed well throughout cycle testing. The cost analysis of this formulation showed that the cost of 100 g of placebo formulation is R 0.99 which proved much cheaper than Formulation A of Chapter 4.

Three sunscreen formulations were developed based on Formulation A and Formulation B. These sunscreens, in its placebo form and including the BS plant extract, underwent long term stability testing. The best performing sunscreen was selected as Formulation C. This formulation with and without the BS plant extract was sent for *in vitro* and *in vivo* SPF testing to determine the extent of the SPF boosting capability of the BS extract.

In Chapter 6 the sunscreen based on Formulation A outperformed the other two. This formulation used a Titanium Dioxide dispersion as the UV filter. It showed good stability in its placebo form and with the addition of the BS plant extract. This was proven by its slow rates of coalescence and good resistance to high stress conditions. The projected shelf-lives of the placebo formulation and with the addition of the BS plant extract exceeded 1 year at storage temperatures of 4 °C and 25 °C, and exceeded 6 months at 40 °C. The cost of 100 g of Sunscreen A was calculated as R12.66. The SPF of placebo Sunscreen A was found to be 15 whereas Sunscreen A containing the BS plant extract was found to be 16. The BS plant extract therefore exhibited slight SPF boosting capabilities which may be owed to its antioxidant activity.

In conclusion a single formulation was recommended for each of the plant extracts. Formulation A, developed in Chapter 4, is recommended for use with the HO plant extract. Formulation B, developed in Chapter 5, is recommended for use with the LSSJ plant extract. In Chapter 6, Sunscreen A performed the best and is therefore recommended for use with the addition of the BS plant extracts. The PM plant extract did not perform well in any of the lotion formulations. For this reason, it is recommended that a gel formulation should be developed for the incorporation of this plant extract.

7.2 RECOMMENDATIONS FOR FUTURE WORK

The formulations suggested in this investigation will have to undergo further testing. Firstly, all suggested formulations should be sent for skin irritancy patch testing to confirm long term skin care applications. The formulations with the addition of the LSSJ and HO plant extracts should be sent for *in vivo* acne reduction efficacy testing. A gel formulation with the addition of the PM plant extract should be sent for efficacy testing to test its activity against acne. With these results the efficacy of the formulations containing the plant extracts can be quantified and the suggested formulations can be introduced into the commercial market.

REFERENCES

Aliano, J. (2008). *Four out of five sunscreens may be hazardous to your health*.

Almeida, I. F., Gaio, A. R., & Bahia, M. F. (2008). Hedonic and descriptive skinfeel analysis of two oleogels: Comparison with other topical formulations. *Journal of Sensory Studies*, *23*, 92–113.

Alvarado, V., Wang, X., & Moradi, M. (2011). Stability proxies for water-in-oil emulsions and implications in aqueous-based enhanced oil recovery. *Energies*, *4*, 1058–1086.

Alvarez, N. J. (2011). *An Experimental and Theoretical Study of Surfactant Dynamics at Microscale Interfaces*. Carnegie Mellon University.

André, V., Willenbacher, N., Debus, H., Börger, L., Fernandez, P., Frechen, T., & Rieger, J. (2003). Prediction of emulsion stability: facts and myth. *Cosmetics and Toiletries Manufacture Worldwide*, 1–7.

ASTM E1490-03. (2003). Standard Practice for Descriptive Skinfeel Analysis of Creams and Lotions. *Annual Book of ASTM Standards*.

Aust, L. B., Oddo, L. P., Wild, J. E., Mills, O. H., & Deupree, J. S. (1987). The descriptive analysis of skin care products by a trained panel of judges. *Journal of the Society of Cosmetic Chemists*, *38*, 443–449.

Baki, G., & Alexander, K. S. (2015). *Introduction to Cosmetic Formulation and Technology*. John Wiley & Sons, Inc.

Bendjaballah, M., Canselier, J. P., & Oumeddour, R. (2010). Optimization of Oil-in-

Water Emulsion Stability: Experimental Design, Multiple Light Scattering, and Acoustic Attenuation Spectroscopy. *Journal of Dispersion Science and Technology*, *31*(9), 1260–1272.

Binks, B. P. (1998). *Modern Aspects of Emulsion Science*. *Modern Aspects of Emulsions*.

Bjerregaard, S., Vermehren, C., Söderberg, I., & Frokjaer, S. (2001). Accelerated stability testing of a water-in-oil emulsion. *Journal of Dispersion Science and Technology*, *22*(1), 23–31.

Brugè, F., Tiano, L., Astolfi, P., Emanuelli, M., & Damiani, E. (2014). Prevention of UVA-induced oxidative damage in human dermal fibroblasts by new UV filters, assessed using a novel In Vitro experimental system. *PLoS ONE*, *9*(1), 1–11.

Castel, V., Rubiolo, A. C., & Carrara, C. R. (2017). Droplet size distribution, rheological behavior and stability of corn oil emulsions stabilized by a novel hydrocolloid (Brea gum) compared with gum arabic. *Food Hydrocolloids*, *63*, 170–177.

Chanchal, D., & Swarnlata, S. (2008). Novel approaches in herbal cosmetics. *Journal of Cosmetic Dermatology*, *7*, 89–95.

Coelho, S. G., & Hearing, V. J. (2009). UVA tanning is involved in the increased incidence of skin cancers in fair-skinned young women. *Pigment Cell and Melanoma Research*, *23*, 57–63.

Das, K. P., & Chattoraj, D. K. (1982). Kinetics of coalescence of polar oil/water emulsion stabilised by ionic detergents and proteins. *Colloids and Surfaces*, *5*, 75–78.

Davies, J. T. (1957). A Quantitative Kinetic Theory of Emulsion Type. I. Physical Chemistry of the Emulsifying Agent. In *2nd International Congress Surface Activity*

(pp. 426–438). Butterworths, London.

de Gruijl, F. R. (1999). Skin cancer and solar UV radiation. *European Journal of Cancer*, *35*(14), 2003–2009.

de Gruijl, F. R. (2000). Photocarcinogenesis: UVA vs UVB. *Methods Enzymol.*, *319*, 359–366.

Derler, S., & Gerhardt, L. C. (2012). Tribology of skin: Review and analysis of experimental results for the friction coefficient of human skin. *Tribology Letters*, *45*, 1–27.

Dickinson, E., Miller, R., & Akhtar, M. (2001). *Food Colloids Fundamentals of Formulation*. Cambridge: The Royal Society of Chemistry.

Filipe, P., Silva, J. N., Silva, R., Cirne De Castro, J. L., Marques Gomes, M., Alves, L. C., … Pinheiro, T. (2009). Stratum corneum is an effective barrier to TiO2 and ZnO nanoparticle percutaneous absorption. *Skin Pharmacology and Physiology*, *22*, 266–275.

Fitzpatrick, T. B. (1988). The Validity and Practicality of Sun-Reactive Skin Types I Through VI. *Archives of Dermatology*, *124*, 869–871.

Friberg, S., Jansson, P. O., & Cederberg, E. (1976). Surfactant association structure and emulsion stability. *Journal of Colloid And Interface Science*, *55*(3), 614–623.

Gambichler, T., Künzlberger, B., Paech, V., Kreuter, A., Boms, S., Bader, A., … Hoffmann, K. (2005). UVA1 and UVB irradiated skin investigated by optical coherence tomography in vivo: A preliminary study. *Clinical and Experimental Dermatology*, *30*, 79–82.

Garland, C. F., Garland, F. C., & Gorham, E. C. (2003). Epidemiologic evidence for different roles of ultraviolet A and B radiation in melanoma mortality rates. *Annals of Epidemiology*, *13*(6), 395–404.

Gilbert, L., Picard, C., Savary, G., & Grisel, M. (2013). Rheological and textural characterization of cosmetic emulsions containing natural and synthetic polymers: Relationships between both data. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *421*, 150–163.

Gilbert, L., Savary, G., Grisel, M., & Picard, C. (2013). Predicting sensory texture properties of cosmetic emulsions by physical measurements. *Chemometrics and Intelligent Laboratory Systems*, *124*, 21–31.

Gioia, F., & Celleno, L. (2002). The dynamics of transepidermal water loss (TEWL) from hydrated skin. *Skin Research and Technology*, *8*, 178–186.

Griffin, W. C. (1954). Calculation of HLB Values of Non-Ionic Surfactants. *Journal of the Society of Cosmetic Chemists*, *5*(4), 249–256.

Harding, C. R., Long, S., Richardson, J., Rogers, J., Zhang, Z., Bush, A., & Rawlings, A. V. (2003). The cornified cell envelope: An important marker of stratum corneum maturation in healthy and dry skin. *International Journal of Cosmetic Science*, *25*(4), 157–167.

Henríquez, C. (2009). *W/O Emulsions: Formulation, Characterization and Destabilization*.

Husson, F., Lê, S., & Pagès, J. (2007). Variability of the representation of the variables resulting from PCA in the case of a conventional sensory profile. *Food Quality and Preference*, *18*, 933–937.

Imhof, R. E., De Jesus, M. E. P., Xiao, P., Ciortea, L. I., & Berg, E. P. (2009). Closed-chamber transepidermal water loss measurement: Microclimate, calibration and performance. *International Journal of Cosmetic Science*, *31*, 97–118.

ISO 24443:2012(E). (2012). Determination of sunscreen UVA photoprotection in vitro.

ISO 24444:2010(E). (2010). Cosmetics - Sun protection test methods - In vivo determination of the sun protetction factor (SPF).

Jacobi, U., Gautier, J., Sterry, W., & Lademann, J. (2005). Gender-related differences in the physiology of the stratum corneum. *Dermatology*, *211*, 312–317.

Jean-Louis Refrégier, M. (2004). Relationship between UVA protection and skin response to UV light: Proposal for labelling UVA protection. *International Journal of Cosmetic Science*, *26*, 197–206.

Kabalnov, A. S. (1994). Can micelles mediate a mass transfer between oil droplets? *Langmuir*, *10*(7), 680–684.

Kabalnov, A. S., & Shchukin, E. D. (1992). Ostwald ripening theory: applications to fluorocarbon emulsion stability. *Advances in Colloid and Interface Science*, *38*(C), 69–97.

Katepalli, H. (2014). *Formation and Stability of Emulsions : Effect of Surfactant- Particle Interactions and Particle Shape*. *Open Access Dissertations*.

Khan, B. A., Akhtar, N., Khan, H., & Braga, V. D. A. (2013). Development , characterization and antioxidant activity of polysorbate based O / W emulsion containing polyphenols derived from Hippophae rhamnoides and Cassia fistula. *Brazilian Journal of Pharmaceutical Sciences*, *49*(4), 763–773.

Khan, B. A., Akhtar, N., Khan, H. M. S., Waseem, K., Mahmood, T., Rasul, A., … Khan, H. (2011). Basics of pharmaceutical emulsions : A review. *African Journal of Pharmacy and Pharmacology*, *5*(25), 2715–2725.

Krause, W. J. (2005). *Krause's Essential Human Histology For Medical Students*. *Universal Publishers* (Third Edit).

Kucera, M., Barna, M., Kálal, J., Kucera, A., & Hladíkova, M. (2005). Topical Symphytum Herb Concentrate Cream Against Myalgia : A Randomized Controlled Double-Blind. *Advances In Therapy*, *22*(6), 681–692.

Kučera1, M., Barna, M., Horáček, O., Kováriková, J., & Kučera, A. (2004). Efficacy and safety of topically applied Symphytum herb extract cream in the treatment of ankle distortion: Results of a randomized controlled clinical double blind study. *Wien Med Wochenschr*, *154*(21–22), 498–507.

Lall, N., & Kishore, N. (2014). Are plants used for skin care in South Africa fully explored? *Journal of Ethnopharmacology*, *153*, 61–84.

Leal-Calderon, F. (2012). Emulsified lipids: Formulation and control of end-use properties. *OCL*, *19*(2), 111–119.

Luebberding, S., Krueger, N., & Kerscher, M. (2012). Age-related changes in skin barrier function - Quantitative evaluation of 150 female subjects. *International Journal of Cosmetic Science*, *35*, 183–190.

Luebberding, S., Krueger, N., & Kerscher, M. (2013). Skin physiology in men and women: In vivo evaluation of 300 people including TEWL, SC hydration, sebum content and skin surface pH. *International Journal of Cosmetic Science*, *35*, 477–483.

Luger, T. A. (2002). Neuromediators - A crucial component of the skin immune system. *Journal of Dermatological Science*, *30*, 87–93.

Madaan, V., chanana, A., Kataria, M. K., & Bilandi, A. (2014). Emulsion Technology and Recent Trends in Emulsion Applications. *International Research Journal of Pharmacy*, *5*(7), 533–542.

Mahmood, T., & Akhtar, N. (2013). Stability of a Cosmetic Multiple Emulsion Loaded with Green Tea Extract. *The Scientific World Journal*, *2013*, 1–7.

Making Cosmetics. (2017). How a SPF is Determined.

Malvern. (2017). *Mastersizer 3000 Smarter Particle Sizing*.

Moehrle, M. (2008). Outdoor sports and skin cancer. *Clinics in Dermatology*, *26*, 12–15.

Montenegro, L., Rapisarda, L., Ministeri, C., & Puglisi, G. (2015). Effects of Lipids and Emulsifiers on the Physicochemical and Sensory Properties of Cosmetic Emulsions Containing Vitamin E. *Cosmetics*, *2*, 35–47.

Morávková, T., & Stern, P. (2011). Rheological and textural properties of cosmetic emulsions. *Applied Rheology*, *21*(3), 1–6.

Moschakis, T., Murray, B. S., & Biliaderis, C. G. (2010). Modifications in stability and structure of whey protein-coated o / w emulsions by interacting chitosan and gum arabic mixed dispersions. *Food Hydrocolloids*, *24*, 8–17.

Moyal, D. D., & Fourtanier, A. M. (2008). Broad-spectrum sunscreens provide better protection from solar ultraviolet-simulated radiation and natural sunlight-induced immunosuppression in human beings. *Journal of the American Academy of*

Dermatology, *58*(5), S149-54.

Naranjo, J. D. (2011). *A 95 % Confidence Interval for Slope*.

Nemes, Z., & Steinert, P. M. (1999). Bricks and mortar of the epidermal barrier. *Experimental & Molecular Medicine*, *31*(1), 5–19.

Oblong, J. E. (2012). Comparison of the impact of environmental stress on male and female skin. *The British Journal of Dermatology*, *166*(2), 41–44.

Opedal, N. V. D. T., Sørland, G., & Sjöblom, J. (2009). Methods for Droplet Size Distribution Determination of Water-in-oil Emulsions using Low-Field NMR. *Diffusion-Fundamentals.Org*, *9*(7), 1–29.

Parente, M. E., Ares, G., & Manzoni, A. V. (2010). Application of two consumer profiling techniques to cosmetic emulsions. *Journal of Sensory Studies*, *25*, 685–705.

Parente, M. E., Gámbaro, A., & Solana, G. (2005). Study of sensory properties of emollients used in physicochemical properties. *Journal Of Cosmetic Science*, *56*, 175–182.

Particle Sciences, I. (2011). Emulsion Stability and Testing. *Technical Brief*, *2*.

Phillips, T., Demircay, Z., & Sahu, M. (2001). Hormonal effects on skin aging. *Clinics in Geriatric Medicine*, *17*(4), 209–216.

Pichot, R. (2010). *Stability and Characterisation of Emulsions in the presence of Colloidal Particles and Surfactants*.

Predel, H. G., Giannetti, B., Koll, R., Bulitta, M., & Staiger, C. (2005). Efficacy of a

Comfrey root extract ointment in comparison to a Diclo- fenac gel in the treatment of ankle distortions : Results of an observer-blind , randomized , multicenter study. *Phytomedicine*, *12*, 707–714.

Primavera, G., & Berardesca, E. (2005). Sensitive skin: Mechanisms and diagnosis. *International Journal of Cosmetic Science*, *27*, 1–10.

Ragnarsson, J. O., & Labuza, T. P. (1977). Accelerated shelf-life testing for oxidative rancidity in foods-A review. *Food Chemistry*, *2*, 291–308.

Ribeiro, A. ., Estanqueiro, M., Oliveira, M. B., & Lobo, J. M. S. (2015). Main Benefits and Applicability of Plant Extracts in Skin Care Products. *Cosmetics*, *2*, 48–65.

Rigel, D. S. (2008). Cutaneous ultraviolet exposure and its relationship to the development of skin cancer. *Journal of the American Academy of Dermatology*, *58*(5), S129-32.

Rodrigues, N. D. N., Staniforth, M., & Stavros, V. G. (2016). Photophysics of sunscreen molecules in the gas phase : a stepwise approach towards understanding and developing next-generation sunscreens. *Proc. R. Soc. A*, *472*(20160677), 1–29.

Rogiers, V. (2001). EEMCO guidance for the assessment of transepidermal water loss in cosmetic sciences. *Skin Pharmacology and Applied Skin Physiology*, *14*, 117–128.

Sadaf, F., Saleem, R., Ahmed, M., Iqbal, S., & Ul-Zafar, N. (2006). Healing potential of cream containing extract of Sphaeranthus indicus on dermal wounds in Guinea pigs. *Journal of Ethnopharmacology*, *107*, 161–163.

Saint-Martory, C., Roguedas-Contios, A. M., Sibaud, V., Degouy, A., Schmitt, A. M., & Misery, L. (2008). Sensitive skin is not limited to the face. *British Journal of*

Dermatology, *158*, 130–133. x

Sayre, R., Kollias, N., Roberts, R., & Baqer, A. (1990). Physical sunscreens. *Journal of the Society of Cosmetic Chemists*, *41*, 103–109.

Sayre, R. M., Agin, P. P., LeVee, G. J., & Marlowe, E. (1979). A comparison of in vivo and in vitro testing of sunscreening formulas. *Photochemistry and Photobiology*, *29*, 559–566.

Schulman, J. M., & Fisher, D. E. (2009). Indoor ultraviolet tanning and skin cancer: health risks and opportunities. *Current Opinion in Oncology*, *21*, 144–149.

Silverson. (2017). Mixing with a High Shear Laboratory Mixer.

Smijs, T. G., & Pavel, S. (2011). Titanium dioxide and zinc oxide nanoparticles in sunscreens: Focus on their safety and effectiveness. *Nanotechnology, Science and Applications*, *4*, 95–112.

Sobisch, T., & Urbansky, N. (2014). *Short communication : Mass balance and emulsion stability*.

Stechschulte, S. A., & Kirsner, R. S. . (2011). Sunscreens for non-dermatologists: What you should know when counseling patients. *Postgraduate Medicine*, *123*(4), 160–167.

Street, R. A., & Prinsloo, G. (2013). Commercially Important Medicinal Plants of South Africa : A Review. *Journal of Chemistry*, *2013*, 1–16.

Tadros, T. F. (2005). *Applied Surfactants Principles and Applications*. Wiley-VCH Verlag GmbH & Co. KGaA.

Tadros, T. F. (2013). *Emulsion Formation and Stability*. Wiley-VCH Verlag GmbH & Co. KGaA.

Trakranrungsie, N., Chatchawanchonteera, A., & Khunkitti, W. (2008). Ethnoveterinary study for antidermatophytic activity of Piper betle, Alpinia galanga and Allium ascalonicum extracts in vitro. *Research in Veterinary Science*, *84*, 80–84.

Twilley, D., Kishore, N., Meyer, D., Moodley, I., Kumar, V., & Lall, N. (2017). The Effect of Helichrysum odoratissimum (L .) Sweet on Cancer Cell Proliferation and Cytokine Production. *International Journal of Pharmacognosy and Phytochemical Research*, *9*(5), 621–631.

Twilley, D., & Lall, N. (2015). Extracts and Compositions of Helichrysum Odoratissimum for Preventing and Treating Skin Cancers.

Vardhan, A., Khan, S., & Pandey, B. (2014). Screening of Plant Parts for Anti-Tyrosinase Activity By Tyrosinase Assay Using Mushroom Tyrosinase. *IndianJ.Sci.Res*, *4*(1), 134–139.

Waqas, M. K., Akhtar, N., Shah, P. A., Danish, M. ., Shah, A. ., De Andrade Braga, V., & Khan, B. . (2016). Development of cetyl dimethicone based water-in-oil emulsion containing botanicals: Physical characteristics and stability. *Pakistan Journal of Pharmaceutical Sciences*, *29*(1), 173–178.

Watkinson, A. C., Brain, K. R., Walters, K. A., & Hadgraft, J. (1992). Prediction of the percutaneous penetration of ultra-violet filters used in sunscreen formulations. *International Journal of Cosmetic Science*, *14*, 265–275.

Watson, A., Fray, T., Clarke, S., Yates, D., & Markwell, P. (2002). Reliable Use of the

ServoMed Evaporimeter EP-2™ to Assess Transepidermal Water Loss in the Canine. *The Journal of Nutrition*, *132*(6), 1661S–1664S.

Wilson, B. D., Moon, S., & Armstrong, F. (2012). Comprehensive Review of Utraviolet Radiation and the current Status on Sunscreens. *The Journal of Clinical and Aesthetic Dermatology*, *5*(9), 18–23.

Winter, R. M. S. (2009). *A Consumer's Dictionary of Cosmetic Ingredients* (Seventh Ed). New York: Three Rivers Press.

Ye, A., Hemar, Y., & Singh, H. (2004). Influence of polysaccharides on the rate of coalescence in oil-in-water emulsions formed with highly hydrolyzed whey proteins. *Journal of Agricultural and Food Chemistry*, *52*(17), 5491–5498.

Zhai, H., & Maibach, H. I. (2002). Occlusion vs. skin barrier function. *Skin Research and Technology*, *8*, 1–6.

Zografi, G. (1982). Physical stability assessment of emulsions and related disperse systems : a critical review. *Journal of the Society of Cosmetic Chemists*, *33*, 345–358.

APPENDIX A

FORMULATION SHEETS OF CARRIER FORMULATIONS

CF 1

A skin gel based on Carbomer as the gelling agent.

Ingredient	%	Application	INCI/Chemical Name
Water	95.2	Diluent/solvent	Aqua
Carbopol 940	1.50	Thickening agent	Carbomer
EDTA	0.10	Chelating agent	Tetrasodium EDTA
Glycerin	2.20	Humectant	Glycerin
Germaben II	1.00	Preservative	Propylene Glycol (and) Diazolidinyl Urea (and) Methylparaben (and) Propylparaben

Method

1. Add water into clean glass beaker and commence stirring with a magnetic stirrer until a vortex is formed.
2. Sprinkle the carbomer on the surface of the water and mix at a low speed until uniform.
3. Add the EDTA and glycerin, stirring after each addition.
4. Add the preservative, stirring after addition.
5. Mix for 15 minutes until homogenous.

CF 2

A thicker skin gel based on Carbomer as gelling agent.

Ingredient	%	Application	INCI/Chemical Name
Water	94.7	Diluent/solvent	Aqua
Carbopol 940	2.00	Thickening agent	Carbomer
EDTA	0.10	Chelating agent	Tetrasodium EDTA
Glycerin	2.20	Humectant	Glycerin
Germaben II	1.00	Preservative	Propylene Glycol (and) Diazolidinyl Urea (and) Methylparaben (and) Propylparaben

Method

1. Add water into clean glass beaker and commence stirring with a magnetic stirrer until a vortex is formed.
2. Sprinkle the carbomer on the surface of the water and mix at a low speed until uniform.
3. Add the EDTA and glycerin, stirring after each addition.
4. Add the preservative, stirring after addition.
5. Mix for 15 minutes until homogenous.

CF 3

A skin gel based on Xanthan Gum as gelling agent.

Ingredient	%	Application	INCI/Chemical Name
Water	95.9	Diluent/solvent	Aqua
Xanthan Gum	0.60	Thickening agent	Xanthan Gum
EDTA	0.10	Chelating agent	Tetrasodium EDTA
Glycerin	2.40	Humectant	Glycerin
Germaben II	1.00	Preservative	Propylene Glycol (and) Diazolidinyl Urea (and) Methylparaben (and) Propylparaben

Method

1. Add water into clean glass beaker and commence stirring with a magnetic stirrer until a vortex is formed.
2. Sprinkle the xanthan gum on the surface of the water and mix at a low speed until uniform.
3. Add the EDTA and glycerin, stirring after each addition.
4. Add the preservative, stirring after addition.
5. Mix for 15 minutes until homogenous.

CF 4

A white creamy skin gel based on Sodium Polyacrylate as gelling agent and a combination of Cyclopentasiloxane and Dimethicone, for ease of application and silky feel.

Ingredient	%	Application	INCI/Chemical Name
		Phase A	
Water	76.0	Diluent/solvent	Aqua
Sodium Polyacrylate	0.30	Thickening agent	Sodium Polyacrylate
EDTA	0.10	Chelating agent	Tetrasodium EDTA
Glycerin	2.20	Humectant	Glycerin
		Phase B	
Lipowax P	5.50	Emulsifier	Cetostearyl Alcohol (and) Polysorbate 60
Mineral Oil	4.40	Emollient	Mineral Oil
Afrisil 350 cSt Fluid	2.20	Emollient	Dimethicone
IPM	2.80	Emollient	Isopropyl Myristrate
		Phase C	
Afrisil D5	5.50	Emollient	Cyclopentasiloxane
Germaben	1.00	Preservative	Propylene Glycol (and) Diazolidinyl Urea (and) Methylparaben (and) Propylparaben
Sodium Hydroxide	QS	Neutralising Agent	Sodium Hydroxide
		Method	
1. Mix ingredients of Phase A together in a clean beaker and heat to 80 °C.			

2. Mix ingredients of Phase B together in a clean beaker and heat to 80 °C.
3. Slowly add Phase B to Phase A while stirring using a Silverson® high shear mixer at 5000 rpm for 15 minutes.
4. Allow to cool to 40 °C with stirring using a magnetic stirrer.
5. Add the Afrisil D5 and the preservative, stirring after each addition.
6. Add the sodium hydroxide while stirring and measuring the pH. At a pH of 5.8 to 6.4 the gel will thicken.
7. Mix for 15 minutes until homogeneous.

CF 5

A liquid body milk based on Xanthan Gum and as the gelling agent.

Ingredient	%	Application	INCI/Chemical Name
		Phase A	
Water	73.4	Diluent/solvent	Aqua
Xanthan Gum	0.20	Thickening agent	Xanthan Gum
EDTA	0.10	Chelating agent	Tetrasodium EDTA
Glycerin	5.50	Humectant	Glycerin
		Phase B	
Mineral Oil	15.8	Emollient	Mineral Oil
Dermofeel GSC	4.00	Emulsifier	Glyceryl Steareate Citrate
		Phase C	
Germaben II	1.00	Preservative	Propylene Glycol (and) Diazolidinyl Urea (and) Methylparaben (and) Propylparaben

Method

1. Mix ingredients of Phase A together in a clean beaker and heat to 70 °C.
2. Mix ingredients of Phase B together in a clean beaker and heat to 70 °C.
3. Slowly add Phase B to Phase A while stirring using a Silverson® high shear mixer at 5000 rpm for 15 minutes.
4. Allow to cool to 40 °C with stirring using a magnetic stirrer.
5. Add the preservative, stirring after addition.
6. Mix for 15 minutes until homogeneous.

CF 6

A light skin cream based Cyclopentasiloxane and Dimethicone for fast absorption and good spreading.

Ingredient	%	Application	INCI/Chemical Name
			Phase A
Water	75.2	Diluent/solvent	Aqua
EDTA	0.10	Chelating agent	Tetrasodium EDTA
Glycerin	2.00	Humectant	Glycerin
			Phase B
Lipowax P	9.70	Emulsifier	Cetostearyl Alcohol (and) Polysorbate 60
Mineral Oil	4.00	Emollient	Mineral Oil
Afrisil 350 cSt Fluid	3.00	Emollient	Dimethicone
Imex IPM 98	2.00	Emollient	Isopropyl Myristrate
			Phase C
Afrisil D5	3.00	Emollient	Cyclopentasiloxane
Germaben II	1.00	Preservative	Propylene Glycol (and) Diazolidinyl Urea (and) Methylparaben (and) Propylparaben
			Method

1. Mix ingredients of Phase A together in a clean beaker and heat to 80 °C.
2. Mix ingredients of Phase B together in a clean beaker and heat to 80 °C.
3. Slowly add Phase B to Phase A while stirring using a Silverson® high shear mixer at 5000 rpm for 15 minutes.
4. Allow to cool to 40 °C with stirring using a magnetic stirrer.
5. Add the Afrisil D5 and the preservative, stirring after each addition.

6.	Mix for 15 minutes until homogeneous.

CF 7

A light skin cream based on Mineral Oil.

Ingredient	%	Application	INCI/Chemical Name
			Phase A
Water	72.0	Diluent/solvent	Aqua
Mineral Oil	20.0	Emollient	Mineral Oil
Crodex M	7.00	Emulsifier	Cetostearyl Alcohol (and) Potassium Cetyl Phosphate
			Phase B
Germaben II	1.00	Preservative	Propylene Glycol (and) Diazolidinyl Urea (and) Methylparaben (and) Propylparaben

	Method
1.	Mix ingredients of Phase A together in a clean beaker and heat to 75 °C.
2.	Stirring using a Silverson® high shear mixer at 6000 rpm for 2 minutes.
3.	Allow to cool to 40 °C with stirring using a magnetic stirrer.
4.	Add the preservative, stirring after addition.
5.	Mix for 15 minutes until homogeneous.

CF 8

A light skin cream that has a thick luxurious feel

Ingredient	%	Application	INCI/Chemical Name
		Phase A	
Water	85.5	Diluent/solvent	Aqua
EDTA	0.10	Chelating agent	Tetrasodium EDTA
Xanthan Gum	0.40	Thickening agent	Xanthan Gum
Glycerin	2.00	Humectant	Glycerin
		Phase B	
Crodex M	7.00	Emulsifier	Cetostearyl Alcohol (and) Potassium Cetyl Phosphate
Crodamol STS	2.00	Emollient	PPG 3 Benzyl Ether Myristate
Crodamol SFX	2.00	Emollient	PPG-3 Benzyl Ether Ethylhexanoate
		Phase C	
Germaben II	1.00	Preservative	Propylene Glycol (and) Diazolidinyl Urea (and) Methylparaben (and) Propylparaben

Method

1. Mix ingredients of Phase A together in a clean beaker and heat to 75 °C.
2. Mix ingredients of Phase B together in a clean beaker and heat to 75 °C.
3. Slowly add Phase B to Phase A while stirring using a Silverson® high shear mixer at 5000 rpm for 15 minutes.
4. Allow to cool to 40 °C with stirring using a magnetic stirrer.
5. Add the preservative, stirring after addition.
6. Mix for 15 minutes until homogeneous.

CF 9

A light skin lotion based on Xanthan Gum as gelling agent and a combination of Cyclopentasiloxane and Dimethicone for ease of application, spreading and silky feel.

Ingredient	%	Application	INCI/Chemical Name
		Phase A	
Water	79.9	Diluent/solvent	Aqua
Xanthan Gum	0.50	Thickening agent	Xanthan Gum
EDTA	0.10	Chelating agent	Tetrasodium EDTA
Glycerin	2.00	Humectant	Glycerin
		Phase B	
Lipowax P	5.00	Emulsfier	Cetostearyl Alcohol (and) Polysorbate 60
Mineral Oil	2.00	Emollient	Mineral Oil
Afrisil 350 cSt Fluid	2.00	Emollient	Dimethicone
Imex IPM 98	2.50	Emollient	Isopropyl Myristrate
		Phase C	
Afrisil D5	5.00	Emollient	Cyclopentasiloxane
Germaben II	1.00	Preservative	Propylene Glycol (and) Diazolidinyl Urea (and) Methylparaben (and) Propylparaben

Method

1. Add water into clean glass beaker and form a vortex using a magnetic stirrer.
2. Sprinkle the xanthan gum on the water surface and mix until uniform.
3. Add the remaining ingredients of Phase A to the water and heat to 80 °C.
4. Mix ingredients of Phase B together in a clean beaker and heat to 80 °C.

5. Slowly add Phase B to Phase A while stirring using a Silverson® high shear mixer at 5000 rpm for 15 minutes.
6. Allow to cool to 40 °C with stirring using a magnetic stirrer.
7. Add the Afrisil D5 and preservative, stirring after each addition.
8. Mix for 15 minutes until homogeneous.

CF 10

A light skin lotion based on Caprylic/Capric Triglyceride.

Ingredient	%	Application	INCI/Chemical Name
		Phase A	
Water	72.0	Diluent/solvent	Aqua
Crodamol STS	5.00	Emollient	PPG-3 Benzyl Ether Myristate
Crodamol SFX	5.00	Emollient	PPG-3 Benzyl Ether Ethylhexanoate
Crodamol GTCC	10.0	Emollient	Caprylic/Capric Triglyceride
Crodex M	7.00	Emulsifier	Cetostearyl Alcohol (and) Potassium Cetyl Phosphate
		Phase B	
Germaben II	1.00	Preservative	Propylene Glycol (and) Diazolidinyl Urea (and) Methylparaben (and) Propylparaben

Method

1. Mix ingredients of Phase A together in a clean beaker and heat to 75 °C.
2. Stir using a Silverson® high shear mixer at 6000 rpm for 2 minutes.
3. Allow to cool to 40 °C with stirring using a magnetic stirrer.
4. Add the preservative, stirring after addition.
5. Mix for 15 minutes until homogeneous.

CF 11

A light skin lotion that absorbs easily and has a luxurious feel

Ingredient	%	Application	INCI/Chemical Name
		Phase A	
Water	85.9	Diluent/solvent	Aqua
EDTA	0.10	Chelating agent	Tetrasodium EDTA
Glycerin	2.00	Humectant	Glycerin
		Phase B	
Crodex M	7.00	Emulsifier	Cetostearyl Alcohol (and) Potassium Cetyl Phosphate
Crodamol STS	2.00	Emollient	PPG 3 Benzyl Ether Myristate
Crodamol SFX	2.00	Emollient	PPG-3 Benzyl Ether Ethylhexanoate
		Phase C	
Germaben II	1.00	Preservative	Propylene Glycol (and) Diazolidinyl Urea (and) Methylparaben (and) Propylparaben

Method

1. Mix ingredients of Phase A together in a clean beaker and heat to 75 °C.
2. Mix ingredients of Phase B together in a clean beaker and heat to 75 °C.
3. Slowly add Phase B to Phase A while stirring using a Silverson® high shear mixer at 5000 rpm for 15 minutes.
4. Allow to cool to 40 °C with stirring using a magnetic stirrer.
5. Add the preservative, stirring after addition.
6. Mix for 15 minutes until homogeneous.