Wax Hardening in Melt Emulsification

By

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Abstract

Wax-in-water emulsions can be defined as a mixture made up of one or more waxes in water. Although wax and water are immiscible materials, wax-in-water emulsions can be stabilised by using surface-active agents such as surfactants (these can be either ionic or non-ionic or a combination of both) to reduce the interfacial tension between droplets and to prevent instability scenarios such coalescence, creaming, flocculation, aggregation, etc occurring within the emulsion. Wax emulsions as they are commonly known are generally used in a wide range of applications, from personal care to coatings to surface care applications to cosmetics, etc. As versatile as they are, wax emulsions are most effective and desirable when they are stable and have a long shelf life.

This body of work employed the use of a non-ionic surfactant (Tween 20) and a Fatty Alcohol (Stearyl Alcohol or 1-Octadecanol as the wax); various compositions of these two in the presence of water were explored while evaluating how the different compositions influenced the Rheology and the particle size distribution. It was found that the compositions with lower surfactant amounts had a more polydisperse particle size distribution and consequently, reduced viscosity over time as a result of the effective packing of droplets that accompanies polydispersity. This research explores potential scenarios resulting from the use of high surfactant concentrations and the surpassing of Critical Micelle Concentration. These scenarios include micellar phase transition at ambient temperatures, the release of surfactant molecules from micelles during aging leading to the formation of new phases, and the development of lamellar phases under heat exposure.

This thesis is dedicated to my children, my husband, my immediate family and to the memory of my late father.

Continue to Rest in Perfect Peace

Major Bolarinwa Bonyameen Sadiq

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My EngD journey has been one filled with ups and downs, highs and lows, good times and not-so-great times but in all, I am glad to have taken on the challenge and proved to myself that ‘*Impossible is Nothing’*.

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Chapter 1

INTRODUCTION

1. Introduction

This project was sponsored by the BASF (*The Chemical Company*) & the ESPRC, the creation of the research topic came about as a result of the company setting out to build a decision tree of the steps and parameters required in the formulation of a stable wax-in-water emulsion with a long shelf life. Despite careful formulation by employing the use of multiple surfactants and adhering to processing parameters, emulsions can still phase separate quickly, rendering them unsuitable for their desired purpose.

1.1 Aims and Objectives of Research

- To investigate the mechanisms involved in wax hardening in melt emulsification in order to achieve a faster and more target-oriented process and product development.
- To develop a decision tree of process and product parameters for the formulation of stable, desired wax emulsions. Essentially, developing a methodical process in the formulation of wax emulsions by identifying crucial parameters that could influence the stability and properties of the emulsion.
- To cut costs involved in the Industrial formulation of these wax dispersions as a result of *trial-and-error approaches*.

1.2 Industrial Applications of Wax Emulsions

Wax emulsions are used extensively in several industrial applications (particularly surface-focused applications), this is due to the versatility and the low toxicity, of waxes making them desirable for use in a variety of industries. Waxes when used in formulations are known to improve certain properties in coatings, polishes, and paints. They are also commonly used to improve slip properties, water-repellency, matting & glossability, scratch resistance, and lubrication. The main different between waxes and oils is that they are typically harder in texture and less slippery.

According to industry standards (AZOM, 2019), the ideal wax to be used in formulations to optimise the properties listed above should have the following properties:

- Generally, has a low viscosity and is not fibrous above its melting temperature to allow for ease of application.
- Solid at approximately 20°C and have a melting temperature of at least 40°C – this is the main property that differentiates waxes from oils, this ensures ease of handling in comparison to liquids and means that end products containing wax formulations are stable at room temperature.

As previously mentioned, wax emulsions are used to improve surface properties and are typically used in water-based formulations. They are known to have relatively small particle size dimensions ($\approx \leq 1\mu m - 2\mu m$), which is favourable as it ensures a uniform and thorough incorporation within other ingredients present in the formulation by simple mixing, thereby maximising their desired functionality (Special Chem, 2023). The small droplet size and low inertia exhibited by wax droplets also means that wax emulsions generally have low Stokes numbers (Haj-Shafiei, 2011), which in turn influences the rate of movement of the droplets resulting in increased stability in the emulsion, making it desirable for several applications in industry.

A good example of waxes used in wax emulsions are fatty alcohols. Fatty alcohol emulsions are used in formulations as emulsifiers and thickeners in a plethora of industries, including Pharmaceuticals, concrete additives, cosmetics & personal care, and agrochemicals, to name a few. In the medical and pharmaceutical industry, wax emulsions are used in formulating lotions, creams, and ointments. The use of wax emulsions in pharmaceutical creams aid in optimising the stability of the dispersion of active pharmaceutical ingredients, thereby improving the emolliency and texture of the product (British Wax, 2024).

In construction, they are used to optimise the efficiency of concrete mixes by minimising water content and enhancing the ease of handling them when used as additives. They are also known to aid in decreasing compressive strength and absorption, thereby improving durability, and reducing early depreciation of the concrete mix (Al-Jabiri, 2006).

They are popularly used in the formulation of cosmetic and personal care products as they are known to improve the following: Emolliency, texturability, consistency/Spreadability, thickening, and stability/shelf life. Wax emulsions are used in formulations such as hair conditioners, shampoos, and moisturising lotions to improve the stability of the formulations and optimise certain desirable properties in these personal care products such as conditioning and detangling. Waxes are also used to improve thickness in oil-in-water emulsions when combined with surfactants with a high Hydrophilic-Lipophilic balance (HLB), resulting in the formation of liquid crystalline gel networks that can adhere to water molecules and increase viscosity (Deckner, 2018).

They are also used in Agrochemicals due to their ability to optimise the absorption of active ingredients present within a formulation onto surfaces and improve the uniform distribution of pesticides onto plants due to their emulsifying ability. They are also known to have the ability to form a protective barrier on crop/plant surfaces to prevent scenarios such as pest damage or moisture loss to ensure effective crop management and adequate hydration for the crops (Byrd, 2024).

1.3 Melt Emulsification of Wax-in-Water Emulsions

Emulsification involves the conversion of two immiscible liquids into a homogenous product. This process disperses droplets of one immiscible liquid into a continuous phase using mechanical agitation/energy to initiate droplet deformation of the mixture and its stability by impeding the chance of creaming or coalescence through the process of breaking up large droplets into smaller droplets and ensuring an even dispersion of said droplets in the continuous phase. Using mechanical agitation allows the distribution of the droplets to occur, while the presence of surfactants/emulsifiers at the interface prevents coalescence of the droplets, and therefore, enhances emulsion stability.

For this study, the use of melt emulsification was adopted, where the dispersed phase (wax) was melted before being added to the continuous phase (water and emulsifier mixture) (Köhler, 2011), and the wax-in-water emulsion was achieved using a rotor-stator device and the mixture cooled down, intending to obtain perfectly solid particles. The melt emulsification process has been suggested in literature to be the chosen method for this research as it is known to support the formation of droplets with uniform shapes and sizes, an important factor to consider when formulating emulsions, where reliable product quality is desirable. In addition, the melt emulsification process aids in the production of spherical droplets, which are generally more stable and less likely to coalesce or phase separate (Fanselow et al., 2016).

It has been observed in previous experiments that unwanted developments could arise during liquid-solid phase transition, i.e., whilst cooling the emulsion. Emulsion destabilising situations that could occur include full coalescence of large droplets due to unsuitable emulsifiers, partial coalescence leading to egg-shaped particles as well as flocculation and aggregation. This could result in changes in droplet number density, the viscosity of the colloidal system or the formation of a new interface due to insufficient emulsifier concentration.

Thus, this study aims to investigate the fundamental mechanisms of wax hardening during melt emulsification and, the material and process parameters required to overcome the challenges being faced when working with such emulsions in order to achieve spherical-shaped particles of the desired size.

That is, being able to adjust the size and distribution of spherical particles using the melt-emulsification method, requires understanding the interplay between numerous influencing factors and how the factors might affect the stability of the emulsion. Some of such factors include:

- Emulsifier Concentration
- Volume of the continuous phase
- Phase Volume Ratio (Wax: Water)
- Shear Rate during Cooling

- Cooling Rate
- Process Timescales
- Presence of liquid crystals in the system

To date, there has been little or no extensive work done in literature to investigate the factors affecting the rheology or particle size measurements of wax-in-water emulsions, a lot of literature materials focus on investigating such factors for oil-in-water emulsions, which of course behave similarly to a wax emulsion, however, the latter has to be formulated using the melt emulsification process, which is not always the case for an oil-in-water emulsion. According to Abramov S, et al., (2016), it was found that decreasing the processing temperature of emulsion resulted in several coalesced crystallised droplets. Generally, emulsions prepared at high temperatures have a better stability in comparison to emulsions prepared at low temperatures, this is because the viscosity and binding forces are reduced at elevated temperatures, making droplet breakup efficient and a stable wax emulsion is expected to be obtained following this.

The characterisation of the wax-in-water emulsions formulated in this research was done by taking particle size measurements, measuring rheology and observing the liquid crystalline states obtained at certain compositions, using microscopy. Based on rheology, the stearyl alcohol-in-water emulsion used in this research showed gelling for compositions with high surfactant amounts and very low viscosities in some cases for compositions containing low surfactant amounts, the peculiar viscosity differences observed have been put down as to whether this behaviour is occurring as a result of the emulsion composition or whether processing plays a part in the peculiar viscosities being seen. Also as mentioned above, factors such as the cooling rate, and storage time can influence rheology and in turn the particle size. Extensive evaluations and detailed side by side comparisons would have to be carried out over a period of time to build a process map that gives clear cut direction on the formulation of a stable wax-in-water emulsion.

1.4 Liquid Crystal Phases

Liquid crystals can be described as a mesophase state between the solid and the liquid phase, liquid crystals are known to be flexible like liquids but have the molecular orientation of a solid. The relationship between emulsions and the formation of liquid crystals was also explored. While surfactants are commonly used as stabilising agents in emulsions, using excess surfactant can lead to the formation of micellar structures and liquid crystals in an emulsion system. Certain ordered molecular arrangements indicate The liquid crystals formed, which can either be present in the emulsion bulk or surrounding the droplets. This behaviour has been seen in different emulsion systems and influences certain physicochemical factors such as rheology, particle size, and shelf life. Therefore, it is important to have a comprehensive understanding of micellar transitions, liquid crystal behaviour, and how they influence emulsion formulation & the desired product. Liquid crystal phases can be formed due to the self-assembly of the surfactant molecules, wax molecules or a combination of both molecules in an emulsion to form organised structures. The composition and arrangement of the liquid crystals formed is dependent on a few elements including molecular concentration, temperature, pressure and processing parameters (Zhang and Liu, 2013).

When the liquid crystals surround the wax or oil droplets in the system, they are characterised by an anisotropic structure known as the Maltese Cross when looking at them under a polarising microscope, these are known as multilamellar phases. However, the other type of liquid crystal exists in the continuous phase, this is simply known as the lamellar phase, however, this does not show up under the polarising microscope, it can be seen under an electron microscope, such as the Scanning Electron Microscope or the Transmission Electron Microscope (TEM) (Noweck et al., 2006). Both structures influence the rheology and general behaviour of the emulsion.

Owing to their structural networks, both the lamellar phase and the multilamellar layer are viscoelastic (Noweck et al., 2006) and can help reinforce the droplet network of emulsion, thereby providing stability and reducing the chance instability scenarios such as creaming/sedimentation developing within an emulsion. The effect of varying the dispersed phase composition and the surfactant composition has been briefly explored in this body of work, however the effect the presence of liquid crystals has on the rheology and particle size measurement of an emulsion is yet to be explored extensively.

Using a polarising microscope, different structures were observed (such as maltese crosses, needle-like crystals) with the different compositions that were investigated, some of which confirm the presence of the multilamellar phase, others showing biphasic structures where the system shows a pure surfactant phase. It is yet to be determined whether this phase comes under the liquid crystal phase category or can be described as a pseudophase that is formed when the surfactant concentration exceeds the CMC beyond micelle formation.

In addition, some of the structures observed in this research showed Lyotropic crystal phase characteristics, while others showed thermotropic qualities. A thermotropic liquid crystal is a mesophase where the orientation of its structure occurs at certain temperatures. A lyotropic liquid crystal is a mesophase where the orientation of its structures is determined by concentration and temperature.

In summary, the composition of the emulsion as well as other factors can influence the emulsion behaviour significantly. Thus, the selection process for optimal parameters to create a decision tree for formulating stable wax-in-water emulsions with a prolonged lifecycle includes a comprehensive understanding of the chemical structures of the materials being used and their interactions. In addition, other factors to consider include processing methods, environmental conditions, and additives that could influence emulsion behaviour. A good understanding of process timescales is also important as they can directly impact the rheology, particle size, and droplet morphology.

1.5 Thesis Outline

Chapter 1 (Introduction)

Sets the scene on areas that have been covered in this research, while briefly highlighting the industrial relevance of the research, the aims and objectives of the research, the definition of liquid crystals, and how they are presented in the emulsion system this research evaluates.

Chapter 2 (Literature Review)

Covers extensive literature investigations on past to present documentation of the micellisation of emulsion systems, lamellar gel systems – what they are, how they are formed, and micellar shape transition. It also reviews the literature on emulsions, surfactants, fatty alcohols, instability scenarios that can cause an emulsion to be unstable and how to stabilise such emulsions.

Chapter 3 (Materials & Methods)

Materials and Methods used in this research are covered. All the analytical methods are explained and documented as well as a detailed explanation of how each experiment was carried out. Information on the materials used and where they were obtained from as well as their purity percentages are also documented.

Chapter 4 (Investigating the Potential Presence of Lamellar Gel Networks and Liquid Crystals in a Wax-in-Water Emulsion)

The discovery of micelles and liquid crystals in this research is discussed, also how changes in the surfactant and wax concentrations influence the formation of liquid crystals. Maltese crosses are discussed; what they represent and how temperature changes might affect their formation.

Chapter 5 (Investigating Changes Occurring with Wax in Water Emulsions)

Here, six different compositions are explored, and the steady shear rheology, oscillatory rheology, and particle size measurement results are discussed as well as how the rheology and particle size change over time with ageing.

Chapter 6 (Conclusion and Future Work)

The summary and conclusion of the work is presented. Suggestions on future work is also highlighted.

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Chapter 2

LITERATURE REVIEW

2. Literature Review

Synopsis

This chapter summarises the understanding behind micellisation of surfactants, how micellar aggregates are formed and why they are formed. The competing forces that serve as the driving force behind amphiphilic aggregate formation are also briefly explored. Lyotropic liquid crystals are defined as well as some of the liquid crystals that could be obtained are also described. In addition to this, a brief insight into literature explanation for the formation of 'needle-like crystals' and 'Maltese crosses' is also documented.

In addition to the above, this section also describes what wax-in-water emulsion systems are - *focussing on Stearyl Alcohols as wax* (which was used as the dispersed phase throughout this study), scenarios that could occur during liquid-solid phase transitions (during formulation) resulting in unstable emulsions. A popular method used in the formulation of wax emulsions, Melt Emulsification is also described, the effect of this process on the stability of wax-in-water emulsions, and why it is one of the widely preferred methods used in these formulations. In addition to the above, terminologies and definitions which might be seen repeatedly throughout this thesis are also outlined and discussed in this chapter to guide the reader through this body of work.

2.1 Fatty Alcohols

Fatty Alcohols (FOHs) are a range of aliphatic hydrocarbons containing a hydroxyl (OH) group and with chain lengths ranging between $C_6 – C_{22}$. The functional OH group may be located at the terminal position or anywhere else within the aliphatic chain. Fatty alcohols are mostly linear and monohydric (i.e., containing one OH group), however, several branched-chain moieties can also be obtained in nature. Plants and animals contain natural raw materials that can be used in the production of fatty alcohol. Products of waxes, fats, and oils found in nature are generally used in the production of fatty alcohols and these tend to yield linear, primary, and even-numbered FOHs (Mudge , 2005).

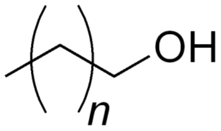


Figure 2.1: Generic structure of fatty alcohol, where n= 4-20 repeating number of carbon groups (Deckner, G, 2014)

As regards their degree of saturation, a number of fatty alcohols found in nature have no double bonds, which makes them saturated (Mudge , 2005). Nevertheless, there are fatty alcohols that have one double bond, making them mono-unsaturated or where there is more than one double bond, polyunsaturated. It is worth noting that the latter are rarely found in nature but can be easily created synthetically. The figure below shows two examples of branched and straight-chained monounsaturated fatty alcohols found in nature (Mudge, 2005).

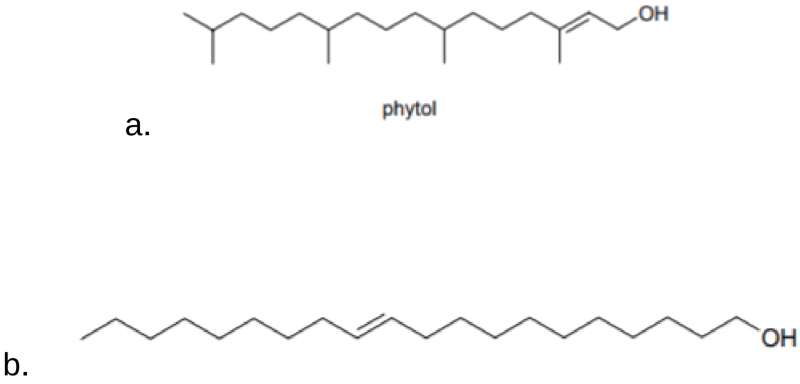


Figure 2.2: Examples of unsaturated fatty alcohols found in nature (a.) Phytol, a branched monounsaturated fatty alcohol obtained from a side chain (phytyl) of chlorophyll found in plants (Chikaraishi et al., 2005) and (b.) Eicos-11-en-1-ol, a C_{20} straight chain unsaturated fatty alcohol, found in a number of plant oils and nuts, these are used in the formulation of personal products (Kattner et al., 2003)

As seen above in figure 2.2, most linear fatty alcohols have their methyl (CH_3) groups on the opposite end to the terminal –OH group, however, in branched fatty alcohols, where the methyl group is one from the end of the molecule, this is known as an *iso* fatty alcohol and where the methyl group is two from the end molecule opposite to the terminal –OH group, this is known as a *anteiso* fatty alcohol (Mudge , 2005).

Examples are shown below:

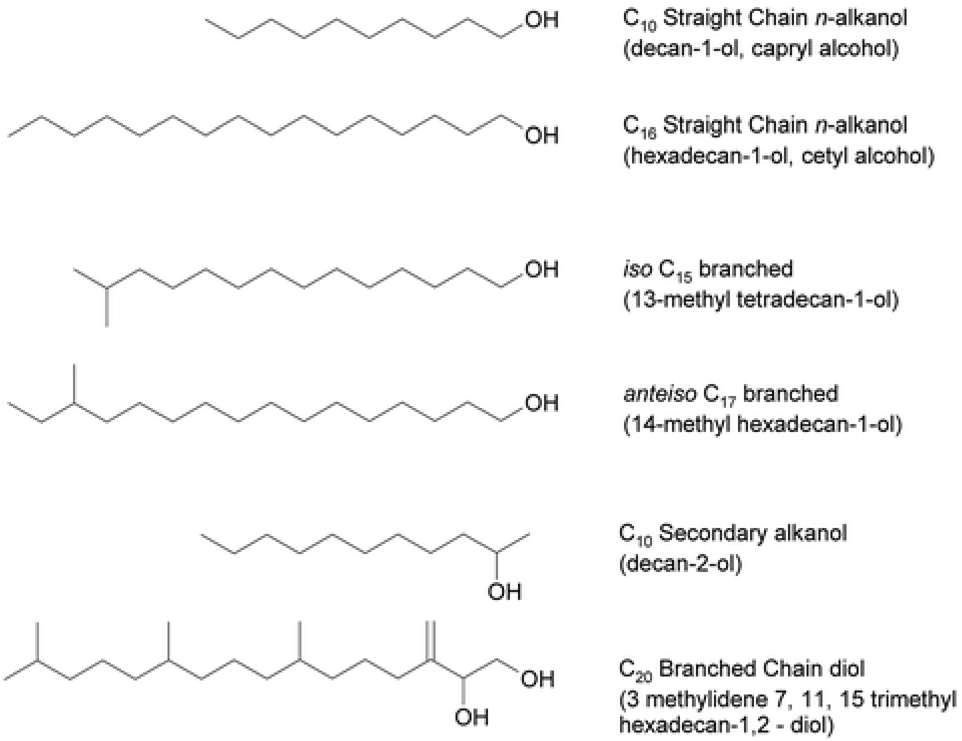


Figure 2.3: Examples of straight chained and branched chained fatty alcohols (Mudge et al, 2018)

The chain lengths for these fatty alcohols can range from 8 to over 38 carbons. Due to such varied chain lengths, degree of saturation, and different possible positions of the -OH group within the chain, whether straight or branched chain, fatty alcohols have a variety of chemical properties and environmental behaviour. Some properties of fatty alcohols include their amphiphilic nature – which means they have hydrophobic and hydrophilic parts. The structure of fatty alcohols can influence how they affect solubility, surface activity, foaming, and other properties. For example, longer-chain fatty alcohols demonstrate higher surface activity in comparison to their shorter-chain alternatives, this is due to increased hydrophobic interactions with nonpolar surfaces by the former which results in strong adsorption at the interface (Hill E.F. et al., 1954). The primary, straight-chain fatty alcohols can be used as surfactants due to their unusual surface-active properties, this is because branched fatty alcohols exhibit limited ability to reduce surface tension at the interface due to their branching, which alters the alignment of molecules at the interface. Some other properties found in other fatty alcohol derivatives such as lauryl sulphate and oleyl sulphate include good foaming action, low-temperature properties, and water solubility (Hill E.F. et al., 1954). Other factors that have an influence on the nature of fatty alcohols include the source they were obtained from, how they were manufactured as well as the raw materials used during the manufacturing process (Noweck et al., 2006).

2.1.1 Stearyl Alcohols (1-Octadecanol)

It is worth noting that although the title of this project is 'Wax Hardening in Melt Emulsification', making wax-in-water emulsions our choice of dispersion, stearyl alcohol was the fatty alcohol used as the dispersed phase to represent the 'wax'.

Stearyl Alcohol ($C_{18}OH$), the dispersed phase used throughout the course of this project is an example of an aliphatic, saturated fatty alcohol where the methyl group is on the opposite end to the –OH group (its chemical formula is seen below). It is typically in the form of water-insoluble waxy white flakes at room temperature and have a melting temperature of ≈59°C - 62°C. Due to its low toxicity and high latent heat properties it is used as one of the ingredients in a variety of industrial products such as cosmetics, personal care products, perfumes, lubricants, etc (Prime et al, 2012) and due to their ability to function as emollients, thickeners and in some cases, they also can be used as emulsifiers. Stearyl alcohols exhibit polymorphism – which means they can crystallise in multiple polymorphic forms under specific conditions, specifically, three forms α, sub-α and β, each having different molecular arrangements in the different forms, which in turn possess unique properties such as melting point and solubility. Stearyl Alcohols display different contact angle positions, depending on whether there are other additives present, surface texture and cleanliness. Due to its high hydrophobicity, Stearyl Alcohols generally show a high contact angle on hydrophobic surfaces (YanJu et al, 2016)

Figure 2.4: Chemical structure of Stearyl Alcohol (1-Octadecanol) (PubChem Compound Summary, Accessed 03/09/22)

2.2 Emulsifiers

Emulsifiers are characterised by their amphiphilic nature (i.e. having a hydrophilic head and a lipophilic tail), they serve as stabilising agents in thermodynamically unstable emulsion systems. They improve emulsion stability primarily by reducing interfacial tension between phases, thereby preventing physicochemical mechanisms such as flocculation, creaming, Ostwald ripening, and coalescence, which can lead to changes in both the continuous and dispersed phases of the emulsion (Marhamati et al., 2021).

Being a class of surfactants, emulsifiers are essentially surface-active agents. As previously mentioned, their main function is to lower interfacial tension between the continuous phase and the dispersed phase, they achieve this by coating the surface of the droplets to prevent aggregation, which in turn leads to the formation of steric or electrostatic barriers/repulsions that can prevent emulsion instability (CArancibia et al., 2017).

An emulsion is made up of two immiscible liquids mixed in the presence or absence of a surfactant to form a mixture made up of a dispersed and continuous/aqueous phase (this is discussed further later). For an emulsifier to effectively reduce surface tension between two phases, a suitable choice of emulsifier must be made for the emulsion system and there has to be a sufficient amount of emulsifier in the solution, such that the ratio of emulsifier present is adequate to cover the surface of droplets/particles in the disperse phase to slow down the process of possible particle aggregation. The ratio of the emulsifier in the emulsion system should be tailored to suit the specific needs of the desired application. Some factors to consider when deciding on the emulsion ratio include emulsion type, shelf-life requirements, the nature of the dispersed and continuous phase, and the desired particle size. In addition to this, rapid adsorption of the emulsifier at the interface of the phases present in the emulsion is essential for their optimal effectiveness to be demonstrated (Urrutia , 2006). Aside from emulsifiers lowering interfacial tension between phases, using the appropriate emulsifier also influences the ease of the emulsification process and the functional properties of the resulting formulation (McClements and Jafari, 2017).

Considering an oil-in-water emulsion, the interfacial tension at the dispersed and continuous phase interface is usually relatively high, this is because the contact between the water and oil molecules is thermodynamically unfavourable (McClements and Jafari, 2017). The role of the emulsifier is to then position its molecules at this interface to minimise these unfavourable interactions by lowering the interfacial tension between them. However, in order for an emulsifier to effectively lower the interfacial tension in the system, emulsifier properties such as molecular weight and the position of the hydrophilic & hydrophobic moieties, play a role in how effective the emulsifier would be. The driving force behind the emulsifier adsorption process is 'The Hydrophobic Effect', this is where water molecules interact through hydrogen bonding and exclude non-polar moieties, this leads to unfavourable hydrophobic interactions, increasing free energy in the bulk phase (Cosgrove, 2010). However, more energy is required to force a water molecule to the oil-water interface than it is to transport a surfactant molecule, this is because the interface is repellent to the water and therefore the adsorption of surfactant molecules to the interface happens simultaneously (Cosgrove, 2010). Surfactants reduce the interfacial tension between oil and water, aiding the transport of water molecules to the interface. However, it is important to note that surfactant molecules require less energy for transportation and adsorption at the interface compared to water molecules, which are repelled by the oil-water interface. Additionally, surfactant molecules are amphiphilic, with their hydrophilic regions orientated towards the water phase and their hydrophobic regions towards the oil phase. This amphiphilic nature contributes to stabilising the interface.

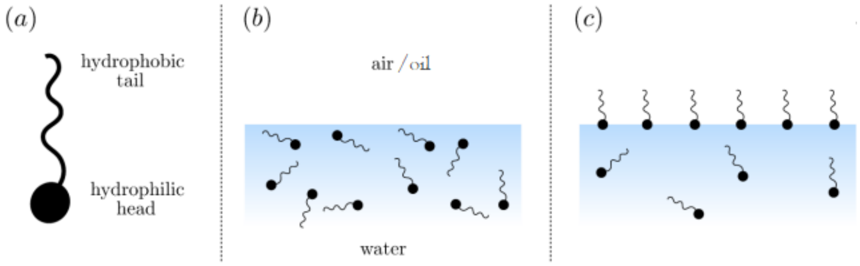


Figure 2.5: (a) Schematic representation of a surfactant, which is also the liking of a typical amphiphilic molecule, (b) Surfactant molecules in water, (c) Shows the

diffusion of the molecules to the oil-water interface and preferred orientation of the surfactant molecules at the interface (Manikantan and Squires, 2020)

When choosing an emulsifier for the formulation of specific emulsions, the HLB (Hydrophilic-Lipophilic Balance) number is usually considered a measure of the size and strength of the hydrophilic and lipophilic functional groups in the emulsifier molecule (Kralova and Sjöblom, 2009). As previously mentioned, emulsifiers are made up of two main parts, a hydrophilic head, and a hydrophobic tail, the percentage weight fraction of these two parts is a strong determinant of whether the surfactant molecule will be lipophilic or hydrophilic in behaviour. Using the HLB method allows for comparison between different surfactant/emulsifier types, it is a solid approach used in the selection of a suitable stabilising agent in the formulation of a stable emulsion. Hydrophilic (water-loving/ water soluble) emulsifiers have high HLB values, while lipophilic (oil-loving/ oil soluble) are assigned low HLB values (Gadhave, 2014). For example, emulsifiers with HLB values between 3-6 are used in water-in-oil emulsions, while emulsifiers with values between 8-18 are used for oil-in-water emulsions (Kinyanjui, 2003). Due to the crucial role emulsifiers play in improving the shelf life and stability of emulsions by influencing the viscosity, interfacial tension, repulsive/attractive forces between particles and particle size, it is therefore important to carefully select the right emulsifier for specific emulsions (Marhamati et al., 2021). Apart from consideration of the HLB value for the selection of an emulsifier, other factors to consider include chemical compatibility with the additives present in the emulsion mixture, the emulsifier's potential performance under the intended processing conditions, its ability to provide long-term stability to the emulsion, and availability.

Although HLB values can be determined by calculation or experimentally, the experimental method is thought to be laborious and its more common to calculate theoretical HLB values by using the formulas below:

For non-ionic surfactants such as polyoxyethylene (20) sorbitan monolaurate (Tween 20):

$$HLB = E/5$$ **Equation 2.1**

Where E = Weight percentage of ethyleneoxide

For surfactants that contain fatty acid esters of fatty alcohols

$$HLB = 20\left(1 - \frac{S}{A}\right)$$ **Equation 2.2**

S = Saponification number of fatty acid/esters – *this refers to the amount of the average chain length of all fatty acids in the sample presented as triglycerides*.

A = Acidity value of the fatty acid -

For example: Obtaining a HLB value for Tween 20

S= 45.5

A = 276

$$HLB = 20\left(1 - \frac{45.5}{276}\right) = 16.7$$

Tween 20 is regarded as a hydrophilic surfactant, therefore the value above agrees with the theory of high values for water-soluble surfactants.

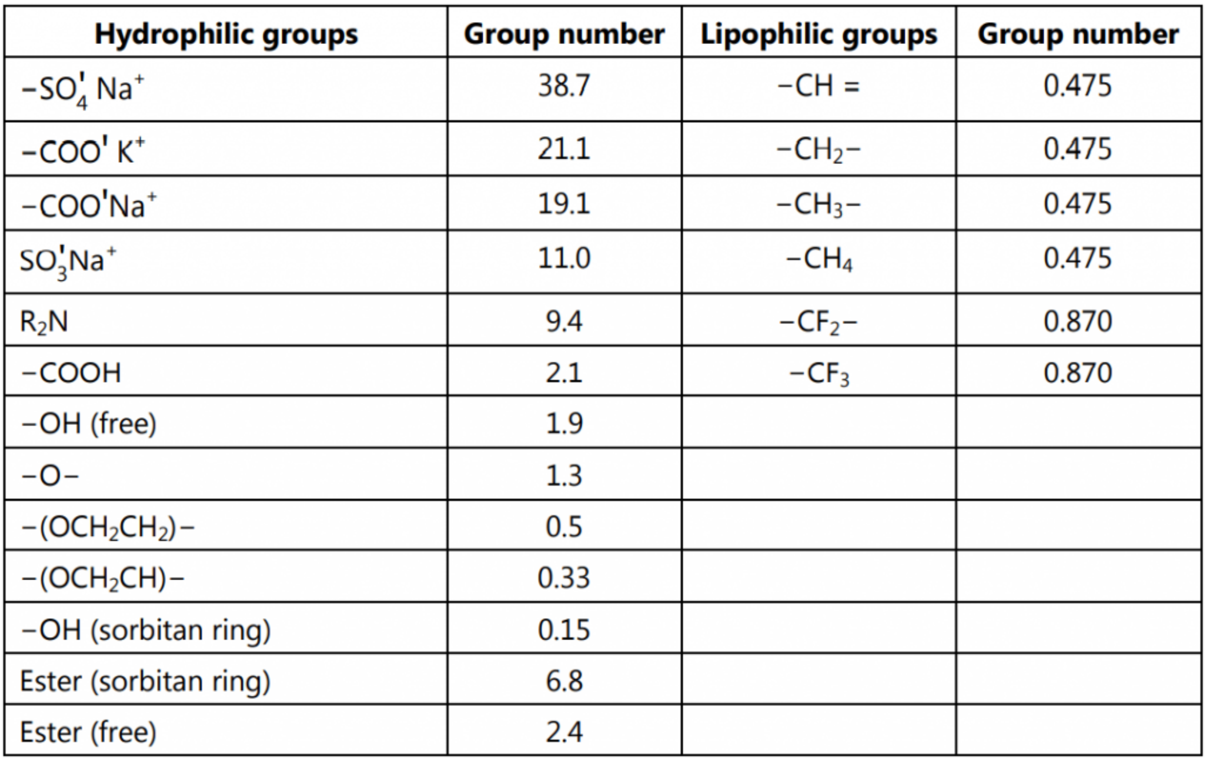
It is worth mentioning that there are limitations to this approach, particularly where polar surfactants or more complex surfactant systems are used and therefore a 3rd equation was proposed by Davies (1957).

$$HLB_{Davies} = 7 + \sum H_{h,i} - \sum H_{l,i}$$ **Equation 2.3**

Where $H_{h,i}$ = Hydrophilic group numbers

$H_{l,i}$ = Lipophilic group numbers

Theoretical $H_{h,i}$ and $H_{l,i}$ group value numbers can be seen in the table below:



Hydrophilic groups	Group number	Lipophilic groups	Group number
$-SO_4^{'}\ Na^+$	38.7	$-CH=$	0.475
$-COO^{'}\ K^+$	21.1	$-CH_2-$	0.475
$-COO^{'}Na^+$	19.1	$-CH_3-$	0.475
$SO_3^{'}Na^+$	11.0	$-CH_4$	0.475
R_2N	9.4	$-CF_2-$	0.870
$-COOH$	2.1	$-CF_3$	0.870
$-OH$ (free)	1.9		
$-O-$	1.3		
$-(OCH_2CH_2)-$	0.5		
$-(OCH_2CH)-$	0.33		
$-OH$ (sorbitan ring)	0.15		
Ester (sorbitan ring)	6.8		
Ester (free)	2.4		

Table 2.1: Table showing some hydrophilic and lipophilic groups and their numbers (Mistry, HLB Scale, Accessed 06/09/22)

Use	Example	HLB
Antifoaming agent	Oleic acid	1
	Sorbitan tristearate	2
	Glyceryl monostearate	3
Emulsifying agent (w/o)	Sorbitan mon-oleate (Span 80)	4
	Glyceryl monostearate	5
	Diethylene glycol monolaurate	6
Emulsifying (w/o), wetting and spreading agents	(None)	7
	Sorbitan monolaurate (span 20)	8
	Polyethylene lauryl ether (Brij 30)	9
Emulsifying agents (w/o)	Methyl cellulose (Methocel 15 cps)	10
	Polyxyethylene monostearate (Myrj 45)	11
	Triethanolamine oleate	12
Emulsifying agents (o/w) and detergents	Polyethylene glycol 400 monolaurate	13
	None	14
	Polyxyethylene sorbitan mon-oleate (Tween 80)	15
Emulsifying (o/w), solubilizing agent, detergents	Polyxyethylene sorbitan monolaurate (Tween 80)	16
Solubilizing agents	Polyxylene lauryl ether (Brij 35)	17
	Sodium oleate	18
	None	19
	Potassium oleate	20
Everything	Sodium lauryl sulfate	40

Table 2.2: Some common surfactants and their theoretical HLB values

(Mistry., HLB Scale, Accessed 06/09/22)

2.2.1 Tween 20 (Polysorbate 20)

Tween 20 is a non-ionic hydrophilic emulsifier with a short-length fatty acid chain with a high Hydrophilic-Lipophilic balance used mainly in food, cosmetics, cleaning products, and pharmaceuticals. They are generally derived from the reaction of sorbitol, ethylene oxide, and lauric acid. Tween 20 emulsifiers are soluble in water, ethanol, methanol, and ethyl acetate and are typically a preferred choice of emulsifiers for stabilising oil-in-water emulsions.

Although there are several other oil-in-water stabilising emulsifiers/surfactants out there, Tween 20 was the only surfactant used throughout this project. This is because a cloud point test was carried out where three non-ionic surfactants were compared to see which showed the most thermal stability and phase changes at high temperatures, of the three surfactants tested, Tween 20 in water remained clear at 69°C, which is the melting point of the wax of choice (Stearyl Alcohol).

2.3 Emulsions

Emulsions are classified as colloidal or dispersed systems used in the formulation of food, cosmetics, personal care, paint, oil, agrochemical, road construction, and pharmaceutical products (Silva et al, 2021). They are typically made up of two immiscible phases, where one phase (the disperse phase) is dispersed in the other (the continuous phase) to form spherical droplets. For example, in an emulsion where molten wax (disperse phase) is dispersed in water (continuous phase), in the presence of an emulsifier, this is referred to as a Wax-in-Water emulsion (W/W). However, there are other types of emulsions, but to mention a few notable ones, water-in-oil (W/O), oil-in-water (O/W), and oil-in-oil (O/O) (Tadros,2016).

The W/O formulation is an emulsion system where the oil is in the continuous phase and there are water droplets dispersed throughout the oil, in the presence of a stabilising agent. W/O emulsions are commonly used in the formulations of oil-based pharmaceuticals and oil-based personal care products, which are suitable for dry and mature skin. However, in oil-in-water emulsion systems, oil droplets are dispersed throughout the water (the continuous phase). O/W emulsions are typically used in the formulation of water-based products such as makeup, and sunscreen and in some food products such as vinegarette, milk, mayonnaise etc. On the other hand, O/O emulsions are non-aqueous colloidal systems made up of two immiscible oils, one being non-polar; for example – butane, propane, octane, and the other oil being polar aprotic/protic, for example – acetone or *N*,*N*-dimethylformamide. Where the previously mentioned colloidal systems can be stabilised using traditional emulsifiers such as Tween and Span, oil-in-oil emulsions can be stabilised using Pickering particles and blocked polymers (Aadarash et al., 2020). However, Pickering particles and blocked polymers are not limited to stabilising oil-in-oil emulsions alone, they can also be used in O/W, W/O, and oil-in-gas emulsions.

Although emulsions are used in a wide range of commercial applications, they are thermodynamically unstable systems due to the immiscibility of water and oil molecules. In order to formulate fairly stable emulsions, external mechanical force (e.g., homogenisation, mixing) is introduced to combine both phases, as well as a stabiliser (e.g. emulsifier, thickening agents) to elongate the shelf life of these emulsions and reduce instability (McClements, 2016). The two immiscible phases that were investigated in this study are wax and water.

2.3.1 Emulsion Formation

Emulsion formation or emulsification processes typically involve the deformation and breaking up of the droplets in the dispersed phase into smaller units using a shearing or simple mixing method. This process is nonspontaneous and requires energy for the breakup process. The break-up of these droplets occurs when the shear applied exceeds the cohesive forces holding the liquid droplets together. In addition to the droplet breakup process, the choice and concentration of emulsifier also influences the emulsion formation process where it plays a role in lowering interfacial tension between droplets and therefore reducing Laplace pressure (p) – (discussed later) and consequently, the energy required for the breakup is reduced (Tadros, 2013). Coalescence of droplets, which can occur simultaneously during emulsification can also be minimised when an emulsifier is introduced into an emulsion system, therefore the combination of these aforementioned factors can influence the resulting droplet size distribution in an emulsion (Fryer et al., 2009). In essence, droplet breakup, the fusion of droplets, and the adsorption of the emulsifier onto the droplet surface can influence the behaviour of the resulting emulsion.

To achieve the desired physical and chemical properties in an emulsion, - the choice of surfactant, the right amount of input energy, and the type of processing device must be carefully selected to produce a formulation with the ideal droplet size distribution (Santana et al., 2013). In choosing the right amount of input energy, the following should be considered, the desired droplet/particle size, the chosen emulsification method and the rheological behaviour of the formulation. The resulting droplet size from the formulation of an emulsion is believed to play an important role in emulsion properties such as rheology, long shelf-life stability, texture, appearance etc. (Jafari et al., 2008)

During emulsification, the energy (i.e., the stress/strain) required to produce an emulsion must be greater than the Laplace pressure gradient (dp/dr)

$$\frac{dp}{dr} = \frac{2\gamma}{r^2}$$ **Equation 2.4**

Where:

r = radius of the droplet

γ = interfacial tension between disperse and continuous phase

The Laplace pressure (Δp_L) which is inversely proportional to the radius of the droplet increases with smaller droplets, this simply means – that an emulsion formulation containing small droplets would require high energy input for the emulsification process due to the resisting interfacial forces between them (Santana et al., 2013).

$$\Delta p_L = \frac{2\gamma}{r}$$ **Equation 2.5**

However, the equation indicates direct proportionality between Laplace pressure and interfacial tension i.e., Δp_L decreases with decreasing interfacial tension, this suggests that an emulsion containing the right amount of surfactant/emulsifier to lower the interfacial tension between droplets would require low external energy for its emulsification (Santana et al., 2013). The concentration and choice of surfactant used in an emulsion influence the interfacial tension value. For example, Cambiella et al., (2006) confirmed in their experimental results using non-ionic surfactants in the preparation of oil-in-water emulsions, that high emulsifier concentrations reduced the rate of coalescence between droplets because of the presence of steric barriers between them.

As previously mentioned, for droplet breakup to occur an external mechanical force larger than the interfacial forces at play between the droplets is introduced, this can be achieved using small-scale or industrial homegenisers. During the course of this project, a Silverson high shear mixer and an emulsifying stirrer were used. Although high-shear mixers are popularly used in melt emulsification processes due to their ability to apply high mechanical shear force to the emulsion and therefore form a dispersion with small droplets, the project wanted to give room for comparison between the two shear force intensities and to evaluate the effect they would have on rheology, particle size, and emulsion stability. Other mechanical devices used in emulsification processes include ultrasonic emulsifying machines, colloidal mills, combination mixers, high pressure homogenisers, microfluidic systems etc. High shear mixers are made up of two parts, a rotor/mixing part and a stator part, the former being rotational and the latter, the stationary part; simply put, its functionality involves the mixing of immiscible phases at high speeds (Vashisth et al., 2021), where material is pushed by the rotor head towards the stator where the material is then sheared, thereby causing a disruption, and mixing of interfaces and resulting in the formation of micron sized droplets. According to Vashisth et al. (2021), the stator geometry plays a huge role in achieving homogenous size distribution and dissipation of energy, this is because the stator geometry controls the flow pattern and strength of the mechanical shear force being applied to the mixture. Using the appropriate geometry will facilitate droplet breakup effectively, leading to a homogeneous size distribution of the dispersed phase. In addition to this, the dissipation of energy within the mixture is controlled by the stator geometry. Energy is dispersed in the mixture due to mechanical agitation and collision of droplets, however, the intensity and distribution of the dispersed energy and forces are determined by the stator geometry, which in turn affects the efficiency of the emulsification mixing process. However, the variability of rotor head speeds, allows for the ability to modify the shear force required in an emulsification process.

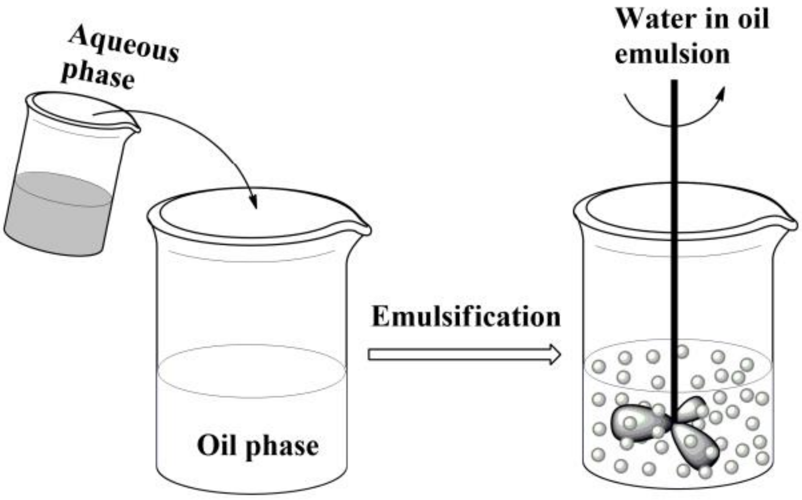


Figure 2.6: Schematic representation of a standard emulsification process showing the addition of the aqueous phase to the oil phase to form a water-in-oil emulsion while applying shear (Ganesan et al., 2018)

2.4 Instability Scenarios in Emulsion Systems

As discussed, the formation of an emulsion is a thermodynamically unstable process as a result of the increase in interfacial area post emulsification, therefore, naturally, the combined phases in the emulsion phase separate to avoid contact leading to emulsion instability. Emulsion instability scenarios can occur through five main mechanisms, coalescence, creaming/sedimentation, Ostwald ripening, flocculation, and phase inversion (Rousseau, 2000).

Because the droplets in the emulsion are in a constant collision and two competing interactions (attraction and repulsion) are at play within the emulsion system, there is bound to be emulsion instability. Attraction occurs due to the presence of London-van der Waals forces while repulsion arises with an increase in surfactant amount at the interface (Rousseau, 2000). In a case where the repulsion force is stronger, the dispersion remains stable, however, in a case where the attraction force supersedes the repulsion force, the droplets will coalesce, thereby resulting in a phase separated emulsion.

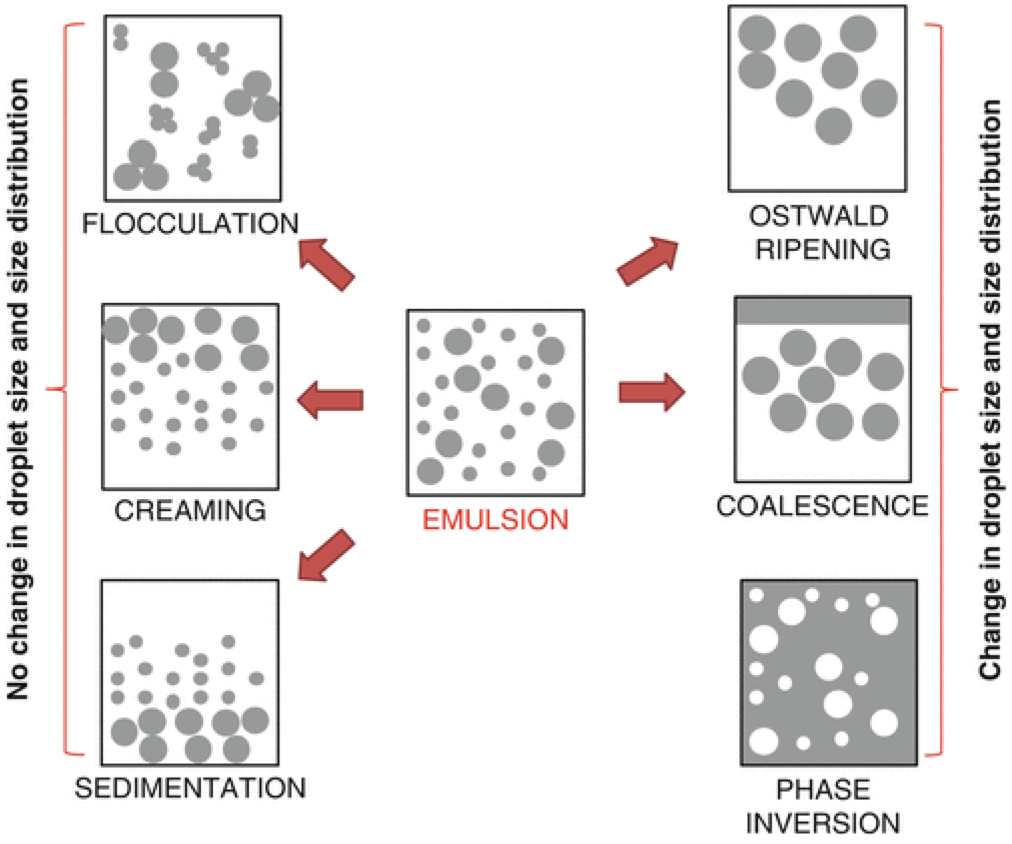


Figure 2.7: Image showing a polydisperse emulsion in the inset and the possible instability scenarios that could arise post emulsification and/or during storage (Piacentini, 2014).

2.4.1 Coalescence

Simply put, coalescence is when two droplets collide and form a single larger droplet (Rousseau D., 2000), this occurs when a thin film (from the continuous phase) between two droplets ruptures, causing them to merge and form one large droplet.

The rate of coalescence in an emulsion system is influenced by the choice of emulsifier/surfactant and the concentration of the surfactant. Coalescence arises as a result of a decrease in interfacial area, which in turn increases the likelihood of droplet collision. Another major driving force for coalescence is the thinning and rupturing of the thin film between droplets. The rupture occurs when the droplets approach each other very closely, perhaps due to Brownian motion, causing the thickness of the liquid film to reach a critical point due to the thermal instability it undergoes, leading to the breakdown of the thin film (Yamashita Y. et al., 2017). Holes can develop in the thin film during thermal instability, the rate at which these holes develop is dependent on the surface coverage of the droplets by the surfactant in the system, if the rate of surface coverage is slow, this could potentially reduce the growth of the holes in the thin film and essentially reduce the chance of film rupture (Pons., R, 2000). According to, emulsions stabilised by surfactants with high lipophilicity are thought to reduce the possibility of coalescence occurring in an emulsion. Other methods recommended in literature that could reduce the rate of coalescence include the use of polymeric emulsifiers to stabilise the emulsion as well as the use of liquid crystals, which could help strengthen the interfacial film between the droplets. (Yamashita et al., 2017).

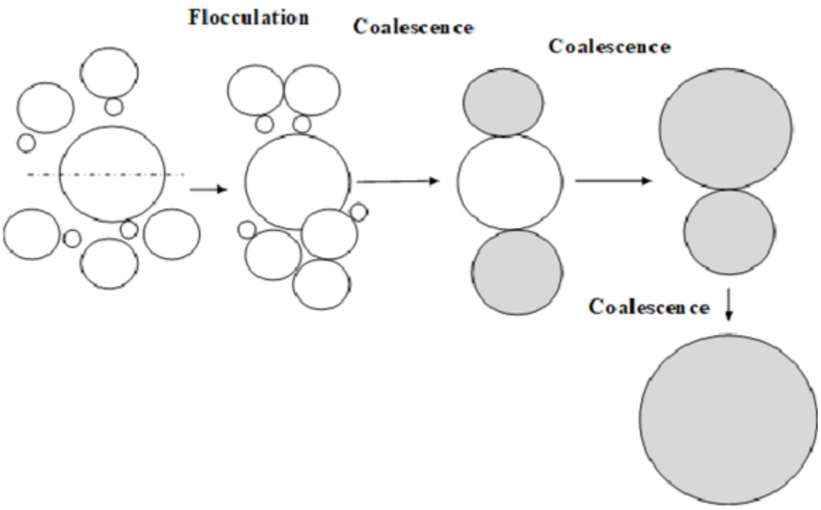


Figure 2.8: Schematic showing flocculation and coalescence of droplets (Parwignya, 2019)

2.4.2 Creaming/Sedimentation

Creaming occurs when the droplets in an emulsion or suspension move upwards due to their density being lower than that of the continuous phase, conversely, sedimentation refers to the downward movement of droplets when they are denser than the continuous phase. Both separation processes can be described are influenced by gravity. When this gravitational force surpasses the Brownian motion of the droplets, there is a build-up of a concentration gradient in the system which causes the transport of droplets to the top (in a case where the density of the droplets is lower than that of the medium) – *creaming* or to the bottom (in a case where the density of the droplets is higher than that of the medium) – *sedimentation* (Tadros T. F., 2013). Therefore, for an emulsion to be stable against creaming/sedimentation, the density differences between the dispersed and continuous phases must be paid attention to

(McClements, 2016). The creamed or sedimented droplets tend to form a tight network which can result in aggregated or coalesced droplets (McClements, 2002).

2.4.3 Ostwald Ripening

According to IUPAC, Ostwald ripening is defined as the dissolution of small particles or crystals and the redeposition of said dissolved particles onto larger particles, this process is called ripening (Hu X and Liu B, 2020). Ripening occurs because the larger particles/droplets are favoured energetically over the smaller droplets, which can cause the latter to have a higher solubility due to them having a higher Laplace pressure and a higher radius of curvature, giving room to the diffusion of loose molecules in the bulk emulsion or suspension to be added onto larger droplets. This phenomenon of the migration of molecules from smaller droplets to larger ones occurs through a solubility gradient between the droplets. For example, in the case of an oil-in-water emulsion, the smaller oil droplets increase the concentration in the emulsion system to above the equilibrium for larger oil droplets, so the smaller oil droplets precipitate onto the larger droplets. The consequence of this is a change in the particle size distribution of the emulsion system over time. Lord Kelvin gave an equation for the chemical potential difference between small and large droplets (Tadros, 2013):

$$S(r) = S(\infty) exp\left(\frac{\boldsymbol{2\gamma V_m}}{\boldsymbol{rRT}}\right)$$ **Equation 2.6**

Where,

$$\mathrm{S(r) = Solubility\ around\ droplet\ of\ radius(r)}$$

γ = Interfacial tension

V_m = Dispersed phase molar volume

R = Gas constant

T = Absolute Temperature

The opposite of this was observed during this work, where upon storage, larger droplets appeared to be smaller or disappear on the particle size distribution over time, this is to be discussed in the results section.

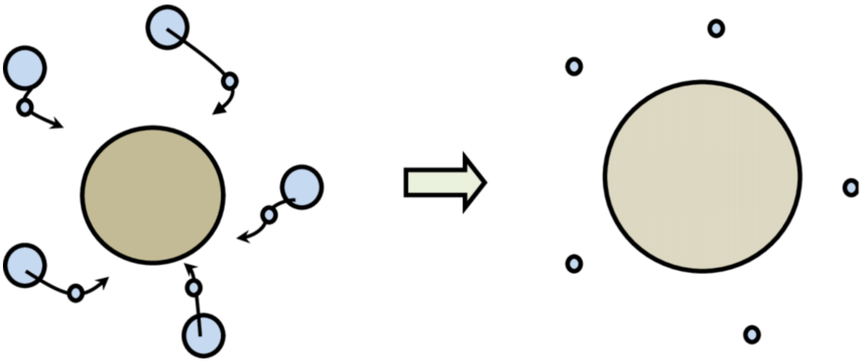


Figure 2.9: Basic schematic representation of the Ostwald ripening process (Visser et al., 2013)

2.4.4 Flocculation

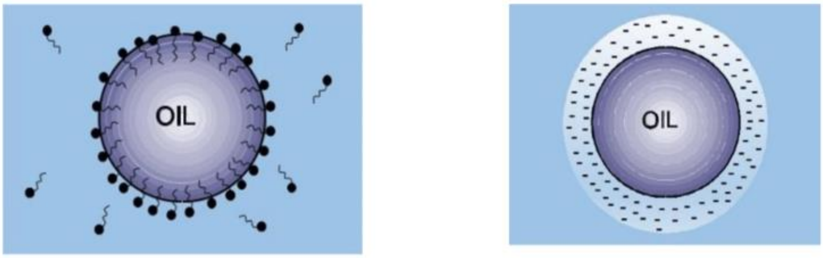
Brownian diffusion within an emulsion (particularly a polydisperse dispersion) brings about droplet collisions, the rate of these collisions can determine the rate of flocculation, (Gregory, 2013). Flocculation occurs when there is an aggregation of droplets driven by the domination of strong van der Waals attraction forces between them, where there has been a combination of both attraction and repulsion forces at play. As opposed to in the case of coalescence, there is no film rupture between the aggregated droplets. It is worth noting that during flocculation, the droplets keep their physical integrity and can form reversible or non-reversible flocs. This phenomenon can increase the potential of creaming within an emulsion system, this is because the flocs gravitate towards the top faster than single droplets (Tadros,2016).

2.4.5 Phase Inversion

Emulsions can also invert from o/w to w/o and *vice versa*, triggered by alterations in compositional or environmental variables, i.e. temperature, shear, emulsifier type and concentration, additives etc. It is a process desirable in the manufacturing of certain food products, such as butter and margarine to create a specific effect on appearance, stability and texture (McClements, 2016), it is also used in industrial applications as a low energy way of creating stable emulsions. Conversely, in colloidal science, phase inversion is deemed as an undesired, instability scenario. During emulsification, the dispersed phase is mixed into the continuous phase, typically in the presence of a surfactant. As the mixing progresses, the surfactant molecules in the mixture reduce interfacial tension between the two phases by adsorbing to the oil-water interface, thereby stabilising the emulsion. However, changes in either processing conditions or composition can disrupt the equilibrium between the dispersed and continuous phases, leading to a change in phase structure and resulting in phase inversion. During phase inversion, the surfactant molecules at the interface may reorient themselves to adapt to the new phase structure, which is more energetically favourable (Perazzo et al., 2015).

2.5 Stabilising Emulsions

Creaming, flocculation, coalescence and Ostwald ripening are constantly working to minimise the energy within the emulsion, causing the emulsion to ruin. As previously discussed, this is caused when droplets collide, therefore the basis of stabilisation is determined by these collisions. Since there are always strong, attractive van der Waals forces between similar droplets, it is necessary to provide repulsion between the droplets to impart stability. This repulsion should be at least as strong as the attractive force and comparable in range of the attractive interaction (McClements, 2005). Stability can be obtained by surrounding the droplets: with an electrical double layer (electrostatic stabilisation), with adsorbed or chemically attached polymeric molecules (steric stabilisation) or with free polymer in the dispersion medium (depletion stabilisation) (McClements, 2005). It can also be achieved by increasing the viscosity of the continuous phase which lowers the mobility of the dispersed phase resulting in fewer collisions of the droplets.



(a) (b)

Figure 2.10: Schematic representation of emulsion stabilisation mechanism showing (a) Steric stabilisation mechanism – here, a non-ionic surfactant is added to the system, and the hydrophobic part of the surfactant attaches to the oil phase and the hydrophilic part to the water phase, essentially to reduce droplet-droplet interactions, (b) Electrostatic Stabilisation – here, the emulsion droplets are covered

by an ionic surfactant, giving rise to droplets surrounded by the charged group of the surfactant (Aryanti et al., 2019)

2.6 Wax Emulsions

The formation of wax emulsions involves dispersing one or more waxes in water and typically in the presence of a suitable stabilising agent, e.g., an emulsifier/surfactant.

Waxes and water are typically immiscible materials, however, using a process called melt emulsification (discussed in the next section), where the mixture of molten wax, water, and a surfactant result in the formation of a wax emulsion – it is worth adding at this point that although melt emulsification is the most popular method of formulating wax emulsions, the choice of emulsification process used in the formulation of a wax emulsion, depends on the application and its intended use.

Some examples of wax emulsions include microcrystalline wax emulsions, paraffin wax emulsions, polyethylene wax emulsions etc.., this project focused on using Stearyl Alcohol (1-Octadecanol) as the wax component of the emulsion, although there was an intention to explore other wax options, however, due to extenuating circumstances and time constraints, this was the only wax that was focused on. Wax emulsions are so versatile that they are used in a variety of industries for different purposes, some of which include improving glossabilty in inks and paints, paper and wood coating, anti-abrasion in paints, anti-slip agents and water repellence in the food industry, to mention a few.

2.6.1 Melt Emulsification

The melt emulsification process is a well-known emulsion formation method used in the formulation of wax emulsions especially in applications requiring solid lipid nanoparticles (Fanselo et al, 2016) e.g. encapsulation of bacteria in lipid particles, and in other emulsions where the dispersed phase requires melting before undergoing droplet breakup. In this process, the dispersed phase is heated and melted at temperatures above its melting temperature, after which it is homogenised/ dispersed (subjecting the pre-emulsion to stress and shear) in the continuous phase. Consequently, the resulting emulsion is cooled after emulsification, the resulting droplets are expected to be solid and spherical (Fanselow et al, 2016). However, this is not always the case as emulsion instability scenarios (discussed above) could occur during the transition from liquid droplets to solid droplets. In a case where emulsifiers/surfactants are used to stabilise the emulsion, it is important to note that the melt-emulsification process is largely limited by the fact that said emulsifier must be particularly thermally stable at elevated temperatures i.e., not subject to denaturing or structural breakdown at such temperatures (Köhler et al., 2011). The emulsifier chosen must be one that is suitable throughout the emulsification process, in order to avoid colloidal instability during droplet breakup as well during and after cooling (Fanselow et al, 2016). On the other hand, the melt emulsification process is an easy, go-to method for wax emulsion formulation and is fairly cost effective in comparison to other methods such as ultrasonication, standard homogenisation and high-pressure homogenisation. At the start of this study and before deciding on a suitable emulsifier (Tween 20) for chosen waxy material (1-Octadecanol), a cloud point test was carried out, to determine which of the emulsifiers showed no cloudiness at 69°C and above, emulsifiers compared were Tween 20, Tween 80, and Span 80). The cloud point of a surfactant is the temperature above which a surfactant becomes insoluble in and separates from the aqueous solution as a result of high temperatures. With an increase in heat and insolubility, irreversible phase separation could occur. The physical evidence of a surfactant having gone past its cloud point is the appearance of a cloudy outlook of the solution,

2.6.1.1 Factors Affecting Formulation of Wax-in-Water Emulsions

In order to obtain a stable wax-in-water emulsion, it is important to have a full understanding of the processing parameters and processing design that influence the emulsion properties. Parameters such as processing temperature, choice of emulsifier, disperse phase concentration, continuous phase concentration, shear rate, cooling rate, etc. play an important role in the development of the physical and chemical properties of the emulsion (Koroleve et al., 2017).

As previously mentioned, the melt emulsification process, involves the preparation of the wax emulsion at a temperature above the melting temperature of the disperse phase. However, with increasing temperature, the viscosity of the disperse phase (wax) decreases, resulting in the formation of small particle droplets, after homogenisation. These droplets are prone to coalescence due to excessive collisions at high temperatures. According to Koroleva et al. (2017), the steady increase in homogenisation temperature of the wax emulsion was studied and it was found that, obtained particle size generally reduced with increase in processing temperature, where temperatures were increased from 60°C to 65°C and finally to 75°C and a shift to the left was observed on the particle size distribution, which suggests the disappearance of large droplets and formation of smaller droplets with increasing temperature, except for when temperature was increased to >20°C of the initial processing temperature (i.e. 80°C), here, a slight increase in particle size was observed, this was attributed to coalescence due to the very high temperature and therefore a higher chance of droplet collisions. Conversely, the preparation temperature increases between 60°C and 75°C revealed a reduction in particle size, and it was suggested this was as a result of decreased viscosity and lower molecular weights of the choice of wax used in the dispersed phase.

When considering how the dispersed phase affects resulting particle size in the emulsion, this relationship is very much dependent on the concentration or amount of material used in the dispersed phase. In theory, the lower the concentration of wax in the dispersed phase, the narrower the particle size distribution that will be obtained due to smaller, uniform wax droplets formed after cooling the emulsion (Koroleva et al., 2017). On the other hand, as mass fraction of the dispersed phased is increased to above 0.1, consequentially, the effectiveness of the emulsification process is reduced due to increase in viscosity when mass fraction of the wax is increased, resulting in larger droplets being obtained. Koroleva et al. (2017), tested the hypothesis by comparing low and high fractions of the dispersed phase and their influence on the resulting particle size, it was found that when the viscosity of the dispersed phase increased as a result of increased dispersed phase concentration, this led to a decrease in the efficacy of homogenisation and therefore, increase in the droplet size obtained.

Another important parameter to consider is the emulsifier concentration and the role in plays in the stability of a wax-in-water emulsion, having both hydrophilic and hydrophobic groups on the same molecule, examples of emulsifiers used in the preparation of emulsions include, proteins, phospholipids, polymers, surfactants etc (McClements and Jafari, 2017). A non-ionic surfactant was used as the emulsifier during this project, the processing of this involves the addition of the chosen surfactant to the aqueous phase, where it is expected to be mixed in ready for adsorption at the wax/water interface. However, there are a few factors that influence the adsorption of surfactants at the interface, which then influence the efficacy of homogenisation and in turn, the stability of the resulting emulsion. Factors such as pH, surfactant molecular weight, surfactant type, ionic strength, hydrophilic-lipophilic balance (HLB) value, adsorption kinetics and surfactant concentration can affect how effective the surfactant will be (McClements and Jafari, 2017). Typically, research has shown that with increasing surfactant concentrations, the interfacial tension in the emulsion system is decreased, allowing for easier droplet breakup during emulsification. According to Pichot et al (2010), it was found that when a non-ionic surfactant was used in the formulation of an oil-in-water emulsion at high concentrations, the emulsion appeared to be stable against coalescence, whereas at low concentrations of the non-ionic surfactant, some coalescence was observed after a period during storage. The effectiveness of a surfactant in reducing the interfacial tension in an emulsion system and therefore allowing efficient homogenisation that leads to small emulsion droplets is dependent on how rapidly the surfactant can adsorb onto the surface of the droplets before droplet breakup, this is because, after homogenisation, the surface area in the emulsion is increased, which means the surfactant may not be sufficient which would cause the interfacial tension in the system to increase making it difficult to generate small droplets during emulsification. It is therefore important to choose the right type of emulsifier and adequate emulsifier concentration that minimises interaction of the dispersed and aqueous phases. For example, small molecule surfactants adsorb onto droplet surfaces faster and are more effective at reducing the risk of coalescence than large biopolymers (McClements and Jafari., 2017). Further, the amount of emulsifier in the emulsion can also impact the rate of droplet coalescence, the mechanism of action follows the movement of free surfactant molecules in the system to areas where there is high interfacial tension to reduce potential coalescence between droplets. However, this is only possible if there is the right amount of surfactant in the system, sufficient surface coverage of the homogenised droplets, repulsive forces between droplets, and efficient & rapid adsorption of the chosen emulsifier onto the droplet surface. Even when the ideal surfactant is used alongside the right amount of surfactant, stable small wax/oil droplets are not always the result of an emulsification process, this is because very often, only a fraction of the emulsifier is adsorbed, leaving the droplets vulnerable to coalescence and/or other instability scenarios.

Although previous literature has quantified various factors that influence emulsion properties including phase concentrations and processing temperature, we theorise that these parameters may vary from one emulsion system to the next. This variability comes about because of additional factors that should be considered, such as specific materials being used and how their unique properties could affect their behaviour during and after emulsion preparation. For example, in an emulsion, the surfactant’s chemical composition, molecular weight, and surface activity may influence the rheology and stability of an emulsion. In addition to this, the physical properties of the dispersed phase such as its morphology and particle size distribution could affect the overall emulsion behaviour and its stability. As a result, it is crucial to have a comprehensive understanding of these material-specific factors to be able to enhance emulsion formulations and processing parameters to achieve desired product properties.

2.7 Micellisation of Surfactants

Micelles can be defined as the aggregation or self-assembly of amphiphilic molecules such as surfactants. Surfactant molecules, which can be defined as amphiphilic are typically made up of a hydrophilic head (e.g., phosphates and hydroxyl groups) and a hydrophobic tail (e.g., alkyl groups). When dissolved in water, surfactant molecules can spontaneously aggregate to form micelles, where the hydrophilic heads interact with the solvent and the hydrophobic tails orientate towards the interior. This thermodynamically unstable self-assembly process is stabilised by a few interactions forces such hydrophobic interactions, electrostatic forces, hydrogen bonding and weak van der Waals forces. It is worth mentioning that even after the micelles are formed, there is a continuous dynamic scenario where the individual surfactant molecules are exchanging with the surfactant molecules within the micelles, this constant exchange occurs as a result of the weak bonding between the surfactant monomers within the micelle.

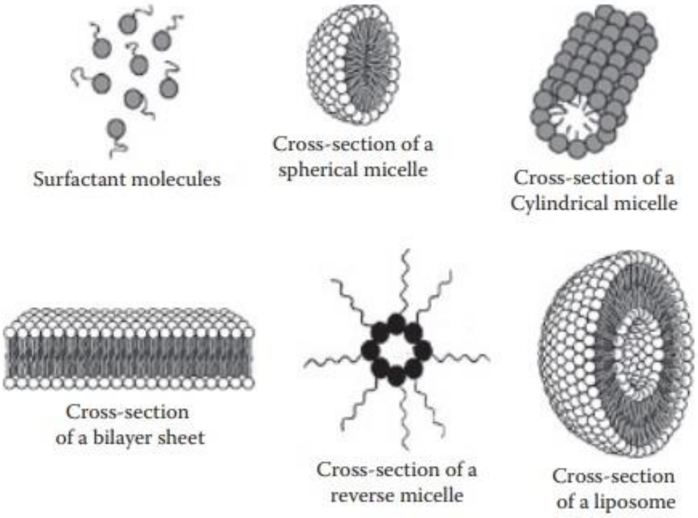


Figure 2.11: Schematic diagram showing some of the types of micelles that can be formed in an emulsion (Micelles, Pharmacy 180 -Accessed 20/09/22)

2.7.1 Critical Micelle Concentration

Micelle formation in solution is driven by the chemical potential of the surfactant molecules and is not just dependent on the surface concentration. When a surfactant is added to a solvent, the molecules are initially present as individual monomers, however, as the concentration of the surfactant increases, the chemical potential of the surfactant molecules increases until it gets to a point of saturation – this point is known as the critical micelle concentration (CMC). At the CMC, the chemical potential of the surfactant in the solution is at equilibrium with the surfactant in the micelles, this mechanism leads to the spontaneous formation of micelles (Krebs, Andersson and Morgner, 2006). At this point, surfactant self-assembly will occur, resulting in colloidal aggregates known as micelles. Naturally, CMC is defined as the concentration at, and above which surfactant molecules form micelles (Wang et al., 2022), it is the underlying property attributed to surfactants in an aqueous solution, it is important to define the CMC of a surfactant solution as it could potentially give an insight into the behaviour and performance of the system above and below the CMC, which in turn influences how specific surfactant in aqueous solution systems are used for industrial applications (Mousavi et. al, 2022)

In addition to the CMC, other key parameters that play a role in micelle formation include the Krafft Temperature and the aggregation number (N). Krafft temperature is defined as the minimum temperature at which micelles are formed, below this temperature, micellization cannot occur (Sheng, 2013), whereas the number of surfactant molecules present in a micelle when the CMC is reached or exceeded is known as the aggregation number.

According to Hassan, Verma & Ganguly (2012) some rapid and distinctive changes could present in the physicochemical properties of the surfactant solution system above the CMC. Some of these include viscosity changes, turbidity, solubility, changes to the osmotic pressure and diffusion coefficient, osmotic pressure, and interfacial tension. The occurrence of these changes is dependent on the change in concentration above the CMC, the changes do not present at specific concentration values, rather, each change in the figure below is seen over a short concentration period and seems to discontinue very swiftly.

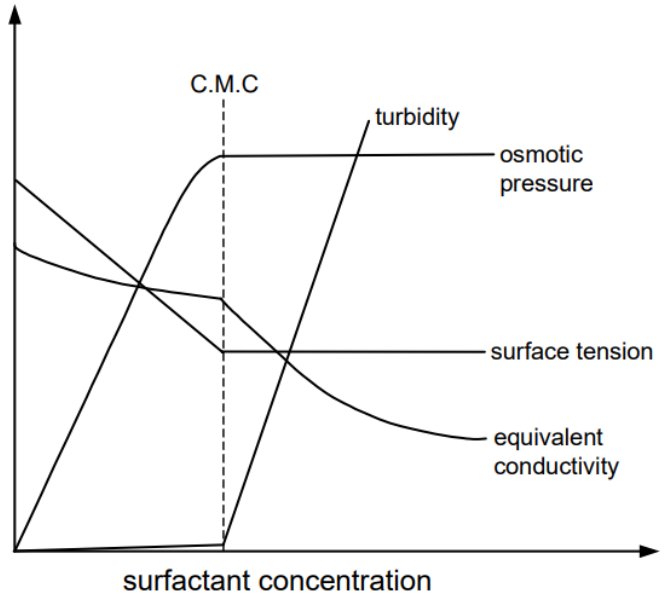


Figure 2.12: Schematic showing an increase in CMC with an increase in surfactant concentration and changes that can occur as surfactant concentration changes, (Eastoe, 2002a)

Generally, the measure of the changes to physical properties in the system can be used to determine the CMC, however, the widely used technique is based on the surface tension. Determining the CMC value is common practice in industry as it can help predict the effectiveness of a surfactant for its intended use (Rosen, 2004).

2.8 The Hydrophobic Effect

When a surfactant is added to a polar solvent such as water, the surfactant molecules reorder themselves to make room for the hydrophobic region, where the hydrophobic tails of the amphiphile orient themselves into a cavity and force water out, this leads to the formation of micelles and an increase in associated free energy in the system. Simply put, the hydrophobic effect refers to the likelihood of non-polar molecules (hydrophobic tails) in an aqueous medium to avoid water molecules (Kruse and Dinjus, 2006).

Hassan, Verma & Ganguly (2012) suggested that the driving force behind the amphiphile aggregation into structures such as vesicles, micelles, and bilayers, is dependent on the interplay between two main opposing forces, the hydrophobic effect and the solvation of the head groups, where the former brings the molecules closer together (aggregation), the latter keeps the hydrophilic parts apart from each other (e.g., surfactant monomers). Although unfavourable, the hydrophobic effect is the dominant force of the two opposing forces, leading to an increase in the entropy of the solvent molecules, which is the starting point of hydrophobic interaction. Further, it is noteworthy to mention that when a non-polar molecule is added to a polar solvent (such as water), the water molecules will have a strong affinity for each other and will therefore be held together by strong attractive forces, however when a polar molecule is added to an equally polar solvent, their molecules can form a bond with the water molecules. This strong interaction between the water molecules serves as the origin of the force that drives micellar formation as it increases the CMC, decreases the adsorption of the surfactant, and essentially, the association of hydrophobic tails to form a core that forces out water is activated (Kronberg et al., 1994).

It can be deduced from the mechanism above that the measure of the hydrophobic effect within the system is directly proportional to the surface area of the hydrophobic core formed by the tails of the non-ionic surfactant added to the solvent. In some industrial applications, this theory is commonly used in predicting the CMC value.

2.9 Micellar Structures

Although according to the subsection above, it has been established that micelle formation is driven by hydrophobic interactions that force the aggregation of surfactant molecules, however (Israelachvili et al., 1977) have postulated that in addition to this, amphiphilic aggregation is controlled by a balanced molecular geometry & the right thermodynamic treatment of the system.

Therefore, in order to fully understand the concept of micelle formation and the resulting shape/structures obtained, free energy interactions, geometry of the molecules (packing constraint) and degree of entropy must all be carefully considered.

During the micellisation process, the main structures that can be obtained include vesicles, bilayers and micelles. The resulting geometry is dependent on the surfactant chemical structure and the surfactant solution conditions such as pH, temperature, concentration etc (Lingafelter, 1948).

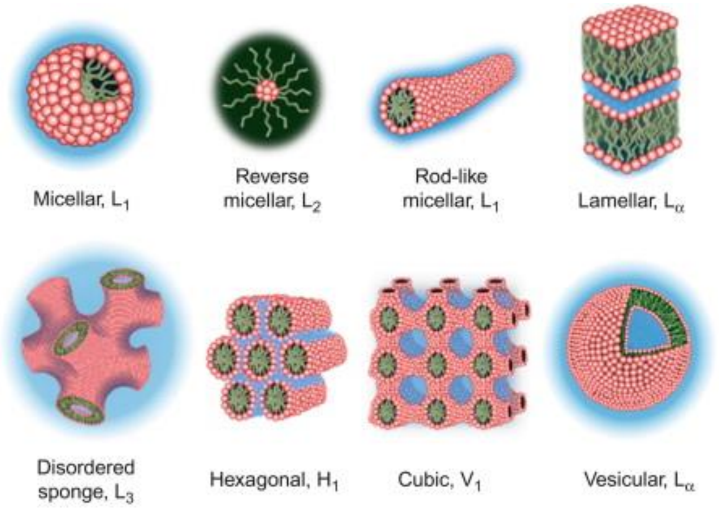


Figure 2.13: Schematic showing the different possible micellar structures that can be obtained (Eastoe J. & Tabor, 2014)

The concept of the packing constraint takes into consideration the packing of hydrophobic tails in the core of a micelle, it helps with understanding the relationship between the surfactant shape and the micellar shape by quantitatively measuring three main parameters (listed below)

The molecular geometry of a micelle is dependent on the minimum effective area used by the hydrophilic head group of the micelle (a_α), the entire length of the hydrophobic tail (l_t) and the volume of the hydrophobic tail (v)

The above parameters have been combined into a function known as the Critical Packing Parameter (P), this is defined as a hypothetical context used for determining the shape and size of amphiphilic aggregates formed by surfactants (i.e. whether they are micelles, bilayers or vesicles)

$$P = v/\alpha.l_t$$ **Equation 2.7**

Where

P= Critical Packing Parameter

v = Volume of Hydrophobic tail

α = Head group area

l_t = Hydrophobic Tail length

For instance, for a spherical micelle with surface area (A), hydrophobic volume (V), aggregation number (n), and radius (r),

$$A = n\alpha \qquad \textbf{Equation 2.8}$$

Therefore, $A = n\alpha = 4\pi r^2$

Hence, number of molecules = $4\pi r^2/\alpha$

$$V = nv = 4/3\pi r^3 \qquad \textbf{Equation 2.9}$$

Hence, number of molecules = $(4/3\pi r^3)/v$

Ratio of these two numbers = $3v/r\alpha$

Note r is l_t

This means the packing fraction is proportional to the ratio of the ‘number of molecules determined by head area’ to the ‘number of molecules determined by tail volume,

$$\text{Therefore, } \alpha = 3v/r \geq 3v/l_t \qquad \textbf{Equation 2.10}$$

Disregarding edge effects for rod and disc shapes, the resulting relationship for a **rod-shaped** micelle with radius r is:

$$\alpha = 2v/r \geq 2v/l_t$$ **Equation 2.11**

and the resulting relationship for a **disc shaped** micelle with thickness 2r is:

$$\alpha = v/r \geq v/l_t$$ **Equation 2.12**

In summary:

$\alpha \geq 3v/l_t$ (**sphere-shaped** micelle);

$\alpha \geq 2v/l_t$ (**rod-shaped** micelle);

$\alpha \geq v/l_t$ (**disc-shaped** micelle)

Micelles can take up three main shapes (spheres, rods, and bilayers/discs) of various sizes, the shape adopted is determined by the surfactant head group structure and alkyl chain length. Surfactants can adopt different micelle shape fluctuations listed above under certain conditions (Leermakers et al, 2005). Some of the conditions include temperature, concentration, chemical structure of the surfactant, type of solvent, and the presence of other additives in the mixture. For example, at certain concentrations, surfactants may form spherical micelles, but with an increase in concentration, the micelles get close in contact, and therefore, a change in shape may be adopted, the shape the micelles transition into is dependent on the chemical structure of the surfactant and other conditions within the emulsion/ dispersion. In addition, an increase in temperature in an emulsion will give rise to the formation of elongated micelle due to an increase in kinetic energy which in turn results in the change of the micelles from spherical to rod-shaped micelles. The change in micelle shape comes about as a result of two competing mechanisms - one mechanism involves a scenario where there are repulsive forces at play between the micelles, resulting in better micelle packing, and the other mechanism leads to the formation of larger-sized micelles with different geometries due to an internal driving force (Salarian et al, 2009).

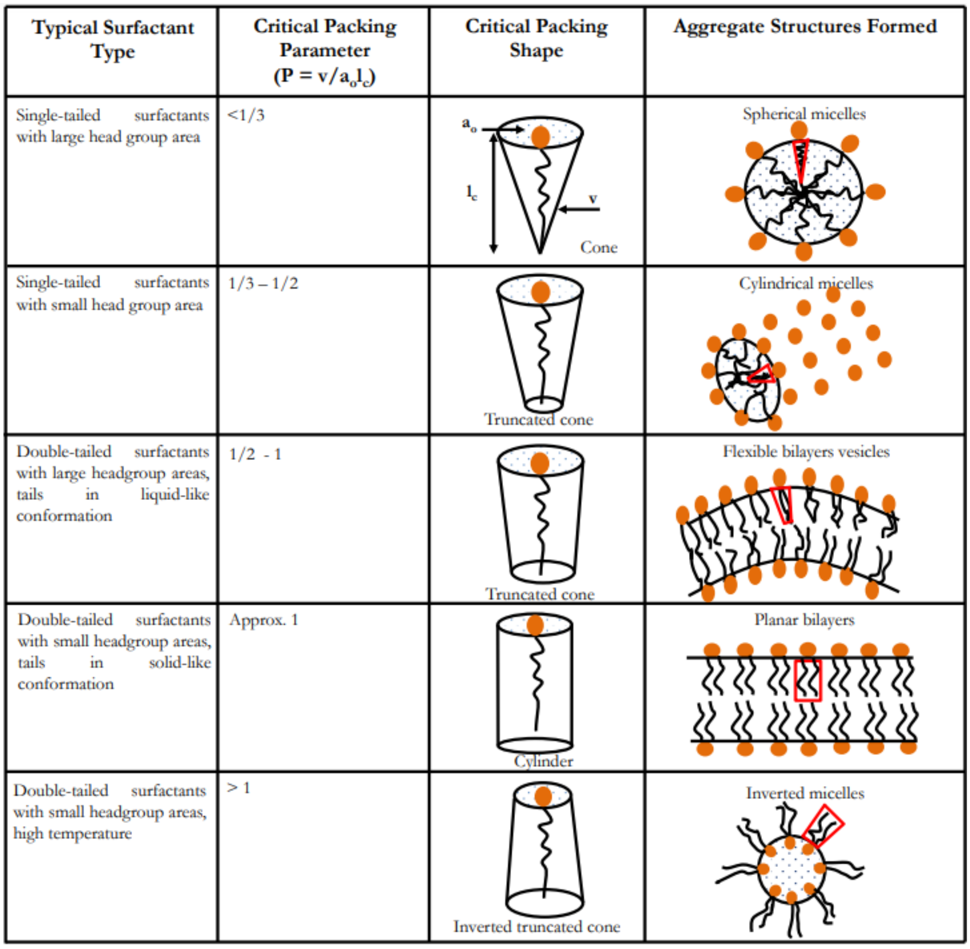
Table 2.3 below shows some of the characteristics of the different shapes and their packing constraints.

Shape	**Micelle Size and Aggregation Number (n)**	**Limitations on size of head group (a)**
Sphere	Largest head group with the smallest alkyl chain length. Low aggregation number, n < 60	$P \leq 1/3$ $\alpha \geq 3v/l_t$
Rod	Smaller head group, large alkyl chain length. Large aggregation number, n > 60	$1/3 \leq P \leq 1/2$ $\alpha \geq 2v/l_t$
Bilayer/Disc	Smallest head group, largest alkyl chain length. low aggregation number, n < 300	$1/2 \leq P \leq 1$ $\alpha \geq v/l_t$

Table 2.3: Micelle shape classification and their packing constraints

At a given chain length and depending on head group size, surfactant molecules can pack into spheres, rods, or bilayers. Where the minimum area taken up by the head group at the micelle surface (α) is large, the three shapes are possible, however, where the values of ‘α’ is small, only disc-shaped micelles are possible (Tiddy, Hassan & Rowe, 2001). It is worth noting that different micellar structures can also be obtained where there are changes in the critical packing parameter of the molecules. Once ‘P’ is calculated using ‘P = $v/\alpha.l_t$, determining the shape and size of the liquid crystal structure into which a surfactant can pack would be possible.

Essentially, molecular packing and the structure of the micelle to be formed can be predicted if the molecular head area, volume of the molecules, and critical length is estimated/calculated. The table below shows the relationship between the volume of the hydrophobic tail (v), head group area (α), hydrophobic tail length (l_t), and the self-assembled structures, as derived by Israelachvili, (1991).



Typical Surfactant Type	Critical Packing Parameter (P = $v/a_o l_c$)	Critical Packing Shape	Aggregate Structures Formed
Single-tailed surfactants with large head group area	<1/3	a_o l_c v Cone	Spherical micelles
Single-tailed surfactants with small head group area	1/3 – 1/2	Truncated cone	Cylindrical micelles
Double-tailed surfactants with large headgroup areas, tails in liquid-like conformation	1/2 - 1	Truncated cone	Flexible bilayers vesicles
Double-tailed surfactants with small headgroup areas, tails in solid-like conformation	Approx. 1	Cylinder	Planar bilayers
Double-tailed surfactants with small headgroup areas, high temperature	> 1	Inverted truncated cone	Inverted micelles

Table 2.4: Table showing a schematic representation of the molecular shapes and critical packing parameters (P) of surfactants and the structure of self-assemblies formed (Salim, et al., 2014)

As described above, the value of the critical packing parameter determines the shape/structure of the resulting micelle in a solution. However, it is worth noting that factors such as chain packing, temperature, ionic concentrations etc can play a role in determining the structure of aggregate to be obtained, some of these are discussed below:

2.9.1 Chain Unsaturation:

An unsaturated surfactant is likely to have a curve or a bend in it, this is due to the presence of a double bond that makes the chain unsaturated. This means, that the more double bonds (unsaturation) present in a surfactant chain, the more pronounced the bend or curve, assuming its most available conformation. The conformation assumed by the surfactant chain will in turn affect the critical packing parameter. Generally, increasing chain unsaturation results in a decrease in the critical packing parameter value. For example, introducing unsaturated surfactants with branched chains will reduce the value of the critical packing parameter, leading to the formation of larger vesicles and eventually, inverted micelles (Wang et al, 2017).

2.9.2 Temperature:

Although not fully understood, Israelachvili, (1991) suggests that hydrophilic headgroups areas increase with increasing temperature due to steric repulsion between the headgroups. This leads to a decrease in critical packing parameters. While charged micelles shrink with increasing temperature, non-ionic-based aggregates do not shrink with increasing temperature, conversely, they increase in size and can adopt a cylindrical shape, due to a reduced steric repulsion between the head groups.

2.9.3 Headgroup area:

Surfactants with a small head group area (thus, high critical packing parameter can form inverted micellar phases and large vesicles Where there are anionic surfactant molecules present, the presence of salt can minimise the electrostatic interactions between the charged particles, resulting in the repulsive force between them being reduced, this, in turn, will cause the surfactant molecules to move closer in contact with each other and causing the head group area to reduce and have an effect on the packing behaviour of the surfactant molecules. Essentially, an increase in salt concentration is inversely proportional to the head group area (Israelachvili., 1991).

2.10 Lyotropic Liquid Crystalline Phase

Lyotropic liquid crystalline phases are mesophases formed by the interaction of amphiphiles with a polar solvent such as water at specific temperatures, pressure and concentrations. When the concentration of surfactant is increased (values much larger than the CMC, for example – 10^2 x CMC) in a solution that already contains micelles, a series of shapes/geometries may be obtained, these phases are known as mesophases or lyotropic liquid crystals. These come about as a result of repulsive interactive forces between micellar surfaces that grow close to each other due to the increasing number of aggregates in the solution, this interaction forces a change of shape and size in order to maximise the distance between the micellar surfaces (Eastoe, 2005).

It is worth noting that increasing the volume fraction of surfactant in a micellar solution typically results in the micellar structure transitioning into a shape with an increased critical packing limit and a lower aggregate curvature (Tiddy, Hassan & Rowe, 2001). These newly formed structures are known as lyotropic liquid crystals.

Owing to the liquid crystalline phase being a mesophase, liquid crystals are known to have properties such as their molecular arrangement and rheology to be intermediate between the solid (crystalline) phase and liquid (isotropic) phase. They are also known to display birefringence properties and have distinct textures that can be viewed under a polarising microscope as a result of the highly ordered direction within the mesophase (Eastoe, 2005).

Although there are a number of different liquid crystals that can be obtained, there are 6 main classes to that have been reviewed extensively in literature, these include, *Lamellar, hexagonal, cubic, gel, nematic,* and *intermediate phases* (Leigh et al., 1981). 4 out of these are discussed below.

2.10.1 Lamellar Gel Network

The lamellar gel network is a multi-phase colloidal assembly that is highly viscous and exhibits shear-thinning behaviour, this is due to 3D interlinked nature of the gel network and the rigidity of the bilayers present within the network. Due to the nature of its rheology, lamella colloidal systems are commonly used in the personal care industry in the manufacture of lotions, creams, conditioners etc., because they are deemed to be kinetically stable enough in their highly viscous state (Junginger, 1984).

Two constituents of the Lamellar gel network are highlighted and briefly discussed below; the L_α phase and the L_β gel phase; the former being a hexagonal packed crystal typically consisting of fatty alcohols and surfactants that make up a portion of the bilayers of the L_β gel phase. The latter is a complex network of evenly spaced bilayers of fatty alcohols, surfactants, an interlamellar water layer, hydrated crystals, and a bulk water phase (Iwata, 2017).

2.10.2 Lamellar (L_α) Phase

In this mesophase, the surfactant molecules are organised into stacked bilayers spread out over distances ranging from approximately a micron or more and separated by layers of water molecules (Tiddy, Hassan & Rowe, 2001 & Mitchell D.J et al, 1983). This phase has its uniquely distinct optical and rheological properties; the system tends to be of low viscosity, this is due to the parallel arrangement of the layers and the alkyl chains being in a semi-liquid state, which allows for ease of sliding movement of the flat layers whilst being sheared. It has an easily pourable consistency even at high concentrations.

In terms of its optical properties, the L_α phase when viewed under a polarising optical microscope (using cross polarisers), displays characteristic optical features (Tiddy, Hassan & Rowe, 2001) such as revealing “oily streaks” and “maltese crosses” (Iwata, 2017) when the bilayers are oriented and conversely, when non-oriented, a mosaic texture with birefringence of colours is seen.

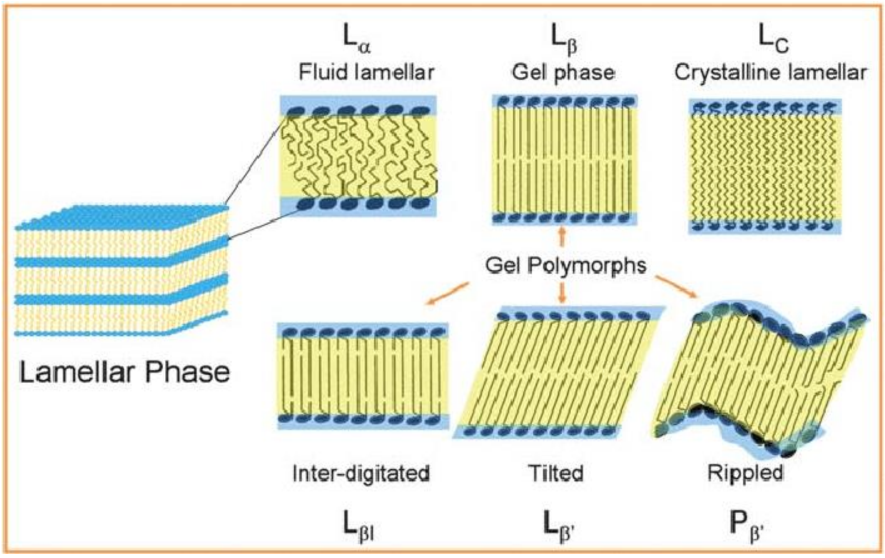


Figure 2.14: Schematic diagram showing common crystalline and Liquid crystalline lamellar phases (Chandrashekar, 2012)

The Lamellar phase can exist either as a planar lamellar phase layered as sheets (ULV) (Bleasdale & Tiddy 1991) or arranged as bilayers in closed concentric shells known as multi-lamellar vesicles (MLV) (Van de Pas,1991). Vesicles are very large bilayer aggregate structures that are made up of bilayers that self-close into spherical assemblies, giving room for an enclosure of water. The MLV is essentially one vesicle formed in another, adopting the structural arrangement of an ‘’onion’’.

Analysis using X-ray diffraction provides two main characteristic features distinct to the Lamellar phase; the first feature being the liquid-like behaviour of the hydrocarbon alkyl chains, seen using a diffuse wide-angle X-ray scattering diffraction (WAXS) peak equivalent to a Bragg reflection of 4.5Å (Luzzati, 1968a). The other feature is the repeat bilayer spacing - Molecules in the lamellar phase are ordered into well-defined layers and the x-ray diffraction analysis of this structural arrangement gives information regarding the spacing between the layers, characterised by ‘*d’* (where *d* = sum of the hydrophobic and hydrophilic layer dimensions) – Bragg’s Law, this would provide information important for gaining an insight into molecular packing within the layers and the distance between the hydrophobic and hydrophilic regions. Sharp reflections observed are displayed in the ratios *d*:*d* / 2:*d* / 3:d,....etc (Tiddy, Hassan & Rowe, 2001). The bilayer thickness can vary from approximately 1.0 to 2.0 times the surfactant chain length while the water layer thickness can vary over a bigger range, for example, 0 to 500 Å.

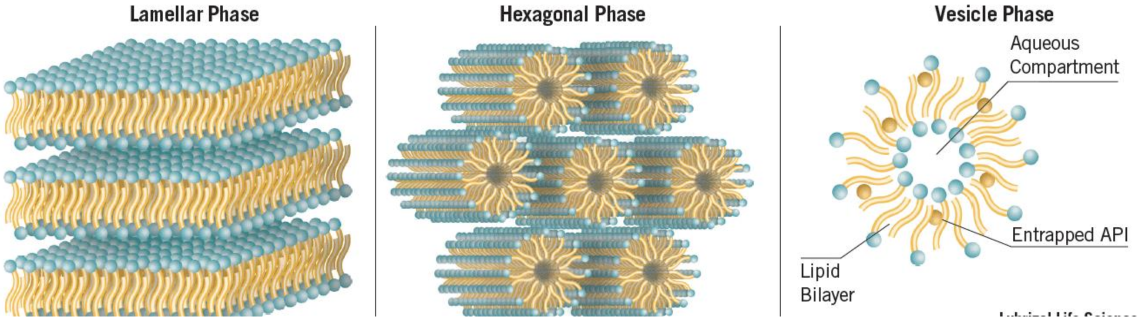


Figure 2.15: Schematic showing some surfactant liquid crystalline phases (Surfactants, *Lubrizol*, 2019 - Accessed 27/09/22)

2.10.3 Lamellar Gel Phase (L_β)

In this phase, the alkyl chains are hexagonally packed, in an all-trans configuration (Iwata T., 2017) with limited movement. This restricted movement accounts for the rheology of the system, making the L_β phase flowy at low concentrations. Although also made up of surfactant bilayers, however, this phase has a characteristic high viscosity as opposed to the L_α phase where there is more free movement of the layers and hence, low viscosity.

According to Sadtler et al, 2004, depending on the size of the hydrophilic head group, three probable states could be obtained in the L_β gel phase. The first state is a well-ordered gel phase where the bilayer is normal to the liquid crystal axis and can be found in lipid systems. Here, the thickness of the bilayer is found to be double that of the surfactant alkyl chain length (Chapman et al., 1967). The second state is the tilted gel phase, where the polar head group is significantly larger than the alkyl chains, an example of a system where this phase can be found is in monoglyceride systems. The third system is the interdigitated form, which is known for its long-chain monoalkyl systems, these can be found in potassium stearate systems (Vincent. et al, 1966). As previously mentioned, the formation of the above listed three phases is very dependent on the size of the headgroup and the stability of the L_β gel phase is very dependent on the packing of the alkyl chains.

2.10.4 Cubic Phase (I_1,I_2,V_1,V_2)

The cubic phase structure is made up of continuous curved bilayers and a pair of diffusing non-intersecting water channels separated by each other (Rajak et al., 2019). Two main aggregate structures are classed as cubic phases; one is made up of small micelles, and the other is based on 3D bicontinuous aggregates, these two main structures can present in a normal or reversed form, which means there are essentially four classes of aggregate structures in the cubic phase (Tiddy, Hassan & Rowe, 2001). With increasing micelle concentration in the cubic phase, micelles may be ordered into lattice patterns, and the set of structures obtained that are found between the micellar phase and hexagonal phase in the phase diagram are classed as I_1 or I_2, (1 or 2 denoting normal or reversed), while structures that appear between the hexagonal and lamellar phase are classed as V_1 or V_2 (Ekwall, 1975). It is worth mentioning that the cubic phase is characterised by spherical packing, where the polar part of the molecule interacts with the polar solvent and the non-polar (hydrophobic) part is oriented towards the core of the sphere, away from any interaction with the polar solvent (Rajak et al., 2019). Due to its highly viscous nature, cubic phases are also known as the viscous isotropic phase, the various structures that can be obtained in the phase could be based on any of the following, normal, face-centred or body-centred cubic lattice. The cubic phase has been reported to have a larger interfacial area than other phases, making it desirable in industry for use in controlled drug release because of its unique microstructure (Rajak et al., 2019).

2.10.5 Hexagonal Phase (H_1, H_2)

The hexagonal phase, also known as the middle phase, is presented as an arrangement of hexagonally close packed water layers sheltered by a surfactant monolayer (Rajak et al., 2019). It is referred to as the middle phase because it is positioned between the highly viscous cubic phase and the less viscous lamellar network.

There are two main classes of the hexagonal phase, the normal (H_1) and the inverse (H_2) hexagonal mesophase. H_1 is formed when surfactant is added to a polar solvent e.g., water, however in a system where the concentration of water is low, and surfactant concentration is high, an inverse hexagonal structure (H_2) may be obtained.

For the H_1 structure, the micellar diameter is in the region of 1.2-2.0 times larger than the trans-oriented alkyl chain length (l_t), with the inter-micellar separation estimated to be within 10-50Å. Conversely, H_2 structures have a micelle diameter 1.0-1.5 times alkyl chain length and inter-micellar separation similar to the above for normal hexagonal structures (Tiddy, Hassan & Rowe, 2001).

Optical textures of the hexagonal phase structures can be seen under a polarising microscope, with both normal and reverse phases exhibiting similar ‘fan like’ and ‘non-geometric’ textures (Tiddy, Hassan & Rowe, 2001).

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Chapter *3*

MATERIALS & METHODS

3. Materials & Methods

This chapter provides information on all the materials used throughout the course of this research and the methods used in the formulation of the emulsions as well as the data analysis techniques used and the theory behind them.

3.1 Materials

The materials used in the formation of the ternary system used for this research include:

Stearyl Alcohol (1-Octadecanol) was purchased from Sigma-Aldrich (Sigma-Aldrich, UK) – 95%, this was used as the dispersed phase.

Tween 20 (*HLB = 17*) was purchased from Pancreac-Applichem,UK

Distilled Water was from an Aquatron system

All materials listed above were used as received from the supplier and were used throughout the course of this study.

Stearyl Alcohol/ 1-Octadecanol

$CH_3(CH_2)_{16}CH_2OH$

(**Melting point:** 55 - 60°C)

(Sigma Aldrich UK, 95%)

Stearyl Alcohol is a long-chain primary fatty alcohol made up of 18 carbons, it has a hydroxy group at the C1 position of the unbranched saturated chain. It is usually in the form of white flakes that are not soluble in water. It can also be used as a surface-active agent (This is because stearyl alcohols are suitable for stabilising two immiscible phases at their interface due to their amphiphilic nature, which means they possess both hydrophilic and hydrophobic properties), as an emollient and ihe personal care industry and for many other applications in the industry including as an antifoam agent.

Tween 20

$C_{26}H_{50}O_{10}$

(Pancreac-Applichem UK, 99%)

Also known as Polysorbate 20, Tween 20 is a non-toxic, clear to greenish-yellow liquid. It is a neutral non-ionic surfactant soluble in water and in alcohols. It is typically used as an emulsifier in cosmetics, textiles and food.

3.2 Emulsion Preparation

The same materials were used for both results chapters, however with slightly different preparation methods. Each emulsion discussed and described was measured on a weight basis with the total emulsion weighing 700g.

The preparation method for the emulsions used in his research were carefully devised to ensure optimal conditions for emulsion formation and stability, the following factors were carefully considered:

- Pre-shearing Tween 20 in water ensured thorough mixing of the continuous phase.
- The use of molten wax in the formulation facilitated its seamless incorporation into the mixture, resulting in a smooth consistency in the final product.
- Employing the melt emulsification method ensured that all materials remained in a liquid state throughout the mixing process.
- Two different methods of agitation were selected, each aimed at achieving the same goal: the production of droplets.

3.2.1 Emulsion Preparation for Chapter 4

To prepare the emulsion, the continuous phase was first formed by slowly adding Tween 20 (Pancreac-Applichem,UK) (0.5 – 10 wt%) to the distilled water and then stirred for 1 minute using a magnetic stirrer on the hot plate at approximately 60°C (a digital thermometer monitored the temperature whilst stirring). The Tween 20 solution was then transferred to the main vessel of the Silverson, L4RT (Silverson Machines Ltd, UK) which was fitted with a heating jacket connected to a water bath (HAAKE Fisions- DC1) utilising a water circulation system and, the mixture was heated to 90°C, whilst stirring at 900rpm for 15 minutes. The Stearyl Alcohol was melted and heated to 90°C on a hot plate before being added to the main vessel using a 60ml syringe, the process of adding the stearyl alcohol to the continuous phase took 1min and whilst keeping the continuous phase stirring at 900rpm. Immediately after the addition of the

Stearyl Alcohol (5wt% and 10 wt%) to the main vessel, the vessel contents were emulsified at an increased speed of 5000rpm for 3 minutes. After emulsification, the hot water was quickly drained out of the water bath and replaced with iced water (~3°C). The emulsion was then cooled to 25°C at a cooling rate of ~3°C min^{-1} by circulating iced water around the jacketed vessel whilst stirring at 1400rpm, the translucent appearance of the mixture that was observed during preparation transitioned into a white, gel-like appearance upon cooling (this was especially the case where surfactant concentrations were above 5%), the cooling rate was monitored using a thermometer and a timer.

3.2.1.1 Emulsion Composition

The table below shows the composition of emulsions that were explored in this chapter at varying concentrations of stearyl alcohol & tween 20. BASF, the sponsoring company for this research, manufactures wax-in-water emulsions for various industries, that employ them in various applications. Many of BASF's wax-in-water emulsions contain wax concentrations ranging from 8% to 40% and utilise non-ionic surfactants (BASF, 2007). This composition range was chosen based on BASF's proprietary framework.

Stearyl Alcohol Content	**Tween 20 Content**
5%	0.5%
5%	1.5%
5%	5%

10%	1%
10%	3%
10%	10%

Table 3.1: Compositions used for emulsion preparation in Chapter 4

3.2.2 Emulsion Preparation for Chapter 5

A hot surfactant solution was first prepared by adding Tween 20 (0.5 – 10 wt%) (Pancreac-Applichem,UK) to hot water (≈60°C) in a large 1L beaker. The hot surfactant solution was stirred on a hot plate for 1 minute using a magnetic stirrer flea, Stearyl alcohol (wax) (5wt% and 10 wt%) was melted in a separate beaker, on a hot plate and added to the solution at 90°C, this melting process took ~2 minutes. The temperature of the surfactant solution upon addition of the Stearyl Alcohol was 90°C.

All three materials were then mixed using a VOS14 overhead mixer (VWR International, UK) for 5 mins at ~500rpm whilst still at 90°C on the hot plate. The resulting emulsion was subsequently cooled to room temperature (~25°C) quiescently by allowing it to stand without stirring**.**

3.3 Analysis

The main aim of the analytical methods selected was to look at the rheology and particle size of the emulsions to see how storage time and different compositions influenced emulsion behaviour. In order to do this, the use of Laser diffraction (Mastersizer 2000, Malvern Instruments, UK) and rheometry were employed.

The analytical techniques used are described in this section as well as how the samples are measured and analysed on each instrument.

The emulsions measured were stored at room temperature and analysed at the following storage times; 15 minutes, 1 hour, 2 hours, 4 hours, 24 hours, 48 hours, 1 week.

Please note: Not all compositions were measured at the storage time indicated above, this is because some samples had consistencies that were too thin to be measured, particularly on the rheometer. It was later deduced that using a different geometry to that which was used in this research would likely have been able to measure the samples with thin consistencies. In addition, the first measurement taken at 15 mins realistically allowed time to stop mixing and transfer product to the rheometer and mastersizer for testing.

3.3.1 Wet Laser Diffraction – Mastersizer 2000

The mastersizer uses a wet laser diffraction technique in generating particle size distributions (PSDs). In the Mastersizer, a laser beam shines through the dispersion and the angular variation in the intensity of the beam that has been scattered through the dispersion is measured. The angular variation intensity measurement is then processed to work out the particle size of the droplets in the dispersion as well as the volume. The Mastersizer uses the Mie theory in its analysis of the angular scattering data. The Mie theory assumes the shape of every droplet is spherical, which can sometimes be a disadvantage if there are crystalline structures or irregular spheres present in the dispersion (Laser Diffraction, 2024). The Mastersizer is often used for measuring particle sizes and analysing particle size distributions ranging from 0.1µm to 1000µm, this measurement technique has a direct correlation with the microstructural features of the system being investigated.

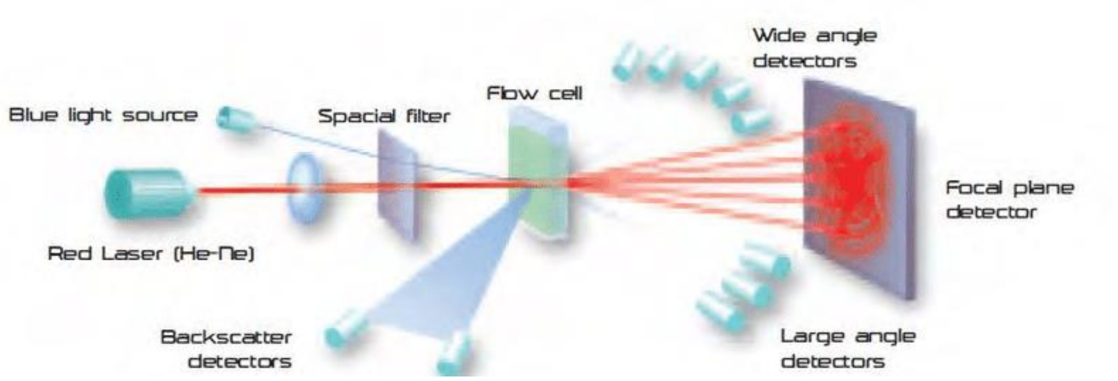

Figure 3.1: Standard operating principle of the Mastersizer 2000 according to Malvern Instruments, UK ('Mastersizer 2000', 2007)

Due to the dispersion that occurs during the measurement process in a mastersizer, there are often concerns as to whether drop breakup and hence, microstructural alteration could arise in the dispersion unit. Research suggests that the process follows a non-destructive and non-intrusive principle which ultimately allows the original properties of the sample being measured to be retained despite the gentle agitation of the particles whilst in the dispersion unit (Rawle, 2024). The process utilises the minimal agitation technique to break up aggregates and flocs to allow for accurate measurements whilst ensuring the sample's droplets remain intact – the agitations are needed to separate flocs but not destroy individual particles, there is likely to be a slight alteration to the microstructure due to the breaking of interparticle bonds that give the viscosity - but this must be done to obtain values for the underlying particle size distribution.

In addition, measuring emulsions, (such as the wax dispersion investigated in this body of work) in the mastersizer's recirculating cell has been known to give accurate and reproducible measurements, due to the presence of the surfactant in the emulsion which can aid in the even distribution of the droplets and therefore deciding of primary particle size measurement easier and likely to produce accurate results (Particle Size, 2019). Essentially, this non-destructive principle ultimately allows the original properties of the sample being measured to be retained despite the gentle agitation of the particles whilst in the dispersion unit. Dispersing the particles before measurement is essential to achieve an accurate particle size distribution. According to (Malvern, K. 2016), several studies have been carried out in different capacities and it has been confirmed that this process has a non-destructive effect on the chemical and physical properties of the particle being measured. The Mastersizer is typically set under precise conditions and stringent calibrations, which require standard operating procedures to ensure accurate and consistent measurements. It has been reported that the use of the laser diffraction method is known to produce reproducible and reliable results, which reduces its chance of producing errors or interfering with the sample's microstructure (Blott et al, 2004).

Measurement

The sample bottle containing the emulsion was swirled lightly, enough to allow droplets to be distributed evenly throughout the sample but not too much so as not to cause any damage or disruption to the particles, it also ensures that the measurement obtained represents the average particle size distribution within the sample, rather than representing localised variations in particle size. An aliquot of the sample is taken from the sample bottle and added to the dispersion unit attached to the mastersizer (Hydro SM, 2022), which contains some amount of distilled water, the sample was then dispersed in water at ~2000 rpm until it reached an obscuration of 10-30%, which is deemed sufficient for telling the particles apart from the background measurement. For the sake of consistency, every measurement taken was done 3 minutes after the addition of the sample to the dispersion unit. The refractive index for paraffin wax was input as the optical settings as it was very close in value to that of the stearyl alcohol. The surface average values and the width of the distribution were measured and automatically produced (D3,2 and Span values respectively).

3.3.2 Rheology- Bohlin CVO- 50 Rheometer

Simply put, rheology refers to the measurement of how materials flow and deform. The rheometer measures rheology and can help track and store information about the flow properties of a sample. The CVO-50 (Malvern Instruments, UK) allows the measurement of shear rate, strain, and stress under controlled conditions and has an inbuilt sensor that makes it suitable for a range of measurements including gap size measurements, steady shear measurements, and oscillatory measurements.

Measurement

The Bohlin CVO-RO rheometer was used in oscillatory mode as well as in steady shear mode, it was equipped with a double gap parallel plate configuration, the diameter of the plates was 40mm and the gap size between the plates was ≈1000μm, and all measurements carried out at 25°C. The gap size used was deemed to be the best fit as in order to prevent slip and particle jamming, the gap size between the plates must be at least ten times greater than the average particle size, of which the average particle size measured was approximately 10µm.

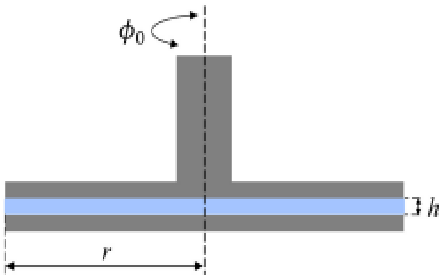

Figure 3.2: Schematic representation of the parallel plate geometry (Barnes et al, 1998)

Steady Shear Measurements

In order to test for hysteresis in the sample, steady shear tests were carried out by ramping up the shear rate between 0.5 to 200 s^{-1} to test up and down shear ramps. Approximately 50 measurements were taken in the space of 100 seconds.

Oscillatory Measurements

In order to take oscillatory measurements, the viscoelastic region (LVR) must be determined by running an amplitude sweep -the LVR is the point at which the elastic modulus begins to tail off. Determining the LVR allows the rheological properties of the sample to be measured without destroying the microstructure. An amplitude sweep was therefore completed with a stress range of 0.03 to 30Pa and at a fixed frequency of 1Hz. After the LVR value was obtained, the oscillatory frequency sweep was conducted by running a frequency range of 0.1-100Hz, a delay time of 2 seconds with a shear stress value selected from the amplitude sweep.

3.3.3 Leica DM-500 Optical Microscopy

Most optical microscopes are made up of at least 2 lenses, which is why they are also known as compound light microscopes, they gather visible light from where the sample is on the stage and send it up through the objective lens, which magnifies the sample for it to be viewed easily through the Ocular (eyepiece)

Measurement

Samples were viewed under the microscope 24 hours after preparation, where the samples had a very viscous consistency (for example where the concentration of wax is high), the drop of the sample placed on the microscope slide was diluted with a drop of water to ensure ease of viewing the particles. Samples were viewed at 40x magnification, a drop of the sample was placed onto the microscope slide, and a coverslip was placed on top to help spread the sample to view under the microscope**.**

3.3.4 OLYMPUS BX51 – Polarising Optical Microscope

Polarising optical microscopy is commonly used in the identification of anisotropic images and mesophase (Corradini and McClements, 2019) (Gary and Goodby,1984), it reveals detailed properties of mesophases by giving information about their composition, shape, size, and structure. It provides in-depth information by utilising the optical properties of anisotropy by revealing their characteristic optical textures.

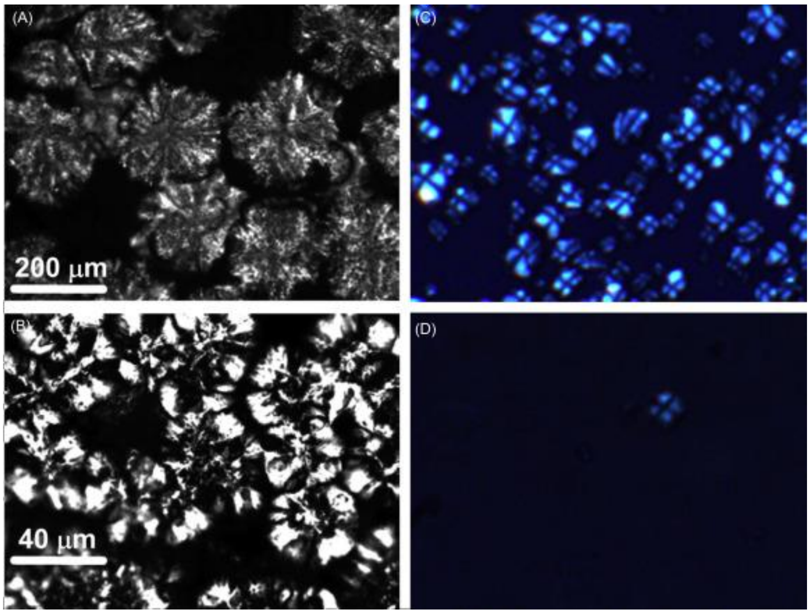

Figure 3.3: Sample polarised micrograph of solid fat contents, showing unique textures such as Maltese crosses and fan-like geometry suggesting the presence of lamellar layers and hexagonal micelles respectively (Corradini and McClements, 2019)

Liquid crystals are naturally anisotropic and therefore they exhibit birefringence, i.e. when light travels through a birefringent material, it does so with different velocities giving rise to different refractive indices.

The refractive index is given by

$$\boldsymbol{n = \frac{c}{v}}$$ **Equation 3.1**

Where;

n = refractive index

c = speed of light in a vacuum m/s

v = speed of light in the substance m/s

Birefringence (birefraction) arises as a result of polarised light passing through a mesophase and splitting into two parallel rays, known as ‘ordinary’ and ‘extra-ordinary’ rays.

Measurement

All POM images in this research were taken using the LMSCOPE lens attached to the Olympus BX51 polarising optical microscope. A Linkham hot stage was coupled to the microscope, making it easy to run temperature ramps as well as take microscopic images.

Figure 3.4: Image showing OLYMPUS BX51 microscope stage.

Figure 3.5: Image showing LiNKAM hot-stage.

A drop of the sample is placed on the microscope slide and a cover slip is placed on top of it to spread the sample evenly, images are then taken at room temperature before starting a temperature ramp that runs a heating cycle from 25°C to 90°C and a cooling cycle that runs from 90°C back down to 25°C at (10°C /min). Images taken during the cycle are captured at 25°C, 62°C (Melting temperature of the stearyl alcohol) at 90°C and 25°C again after the cooling cycle ends.

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Chapter 4

INVESTIGATING THE POTENTIAL PRESENCE OF LAMELLAR GEL NETWORKS AND LIQUID CRYSTALS IN A WAX-IN-WATER EMULSION

4. Investigating the Potential Presence of Lamellar Gel Networks and Liquid Crystals in a Wax-In-Water Emulsions

Synopsis & Introduction

The use of Polarising Microscopy, laser diffraction, and rheometry were employed in a bid to characterise and analyse the needle-like crystals and Maltese crosses observed in the emulsions prepared during this research. The effect of heat/temperature, storage, and material composition on the formation of these mesophases was explored. Lamellar networks are very often seen in emulsions where surfactants have been used to stabilise the droplets and therefore it was important to investigate the different structures that were observed during this study. It is worth mentioning that this chapter focused primarily on microscopy as they were a qualitative representation of changes occurring within the system.

As previously discussed in Chapter 1, Wax-in-water emulsions are used in several industrial applications such as food, pharmaceuticals, and cosmetic products due to some properties that make them desirable for use (Lansdaal, 2018). However, within the microstructure of these emulsions, some phases and networks are formed because of the emulsion system composition, emulsification parameters etc. A commonly found network in wax emulsions (particularly where fatty alcohols are used as the wax component in the emulsion) is the lamellar gel network. Increasingly, in recent years, industrial and academic interest has been piqued to explain the potential presence of lamellar gel networks in emulsions and the effect they have on their viscoelastic properties and stability due to their unique phase behaviour (Yang et al., 2023). Lamellar gel networks are complex systems that form the base of several personal care cream formulations. This is due to their exceptional ability to improve the stability of formulations in comparison to standard oil/water, and wax/water formulations. In addition to their shelf-life-enhancing ability, they are also known to possess desirable rheological characteristics due to their interconnected structure, making them especially coveted in product development industries (Cunningham et al., 2021).

Where emulsions are stabilised by surfactants, there is a chance that the dispersions could contain lamellar networks at a certain ratio to water (Hioko et al., 2023), particularly where the dispersed phase in the emulsion system is a fatty alcohol (e.g. stearyl alcohol, which was used extensively in this research) and where the surfactant is water-soluble (e.g. Tween 20) (Iwata, 2017). These lamellar networks are a product of a structural arrangement of wax (oil) and surfactant molecules created at the oil-water interface after the emulsification process and upon cooling (Iwata, 2017).

This research was carried out in a bid to gain a deeper understanding of the parameters and conditions that bring about the formation of lamellar crystals/ networks, thermotropic behaviours were observed through features such as Maltese crosses and needle-like crystal formation, which were seen during this research.

Exploring the complex behaviour of these microstructural features mentioned above seen in the wax-in-water emulsion system, provided the opportunity to delve into how they might influence emulsion stability, the physicochemical properties of the emulsion, and the overall behaviour of the emulsion. In addition to gaining knowledge for this research, it was also imperative to understand how these mesophases are formed in wax-in-water emulsions and how their presence in emulsions is relevant to different industrial applications.

This research aligns with literature research where the influence of mesophase microstructure on emulsion stability and rheological behaviour is of utmost importance (Engel, 1998). The combined use of analytical methods such as rheometry, laser diffraction and microscopy were employed, which is also seen in literature to gain insights into the presence of mesophases in wax emulsions and their effects (Friberg S.,1971), and in the process contributing to knowledge in this subject area through the collection of data, analysing the data and presenting the findings in this thesis. For example, Ahmadi D. et al (2020), explored the idea of working with a ternary system similar to the one used in this thesis (water, a water-soluble surfactant and octadecanol) to gain a molecular-level understanding of how the system behaves with a change in the choice of fatty alcohol and how the microstructure, rheology and stability of the system can be affected by a change in the overall system composition.

One of the long-term aims of the research carried out in this chapter was to explore different ternary systems (using different materials with similar properties), compare a bigger range of system compositions and utilise other analytical methods some of which include, Small & Wide-Angle X-ray Scattering (**WAXs**), Scanning Electron Microscopy (**SEM**), Differential Scanning Calorimetry (**DSC**) and Dynamic Light Scattering (**DLS**) to evaluate the lamellar and overall system behaviour, morphology and consistency.

Also, in Ahmadi D. et al (2020), viscosity and structural changes were observed with different system compositions and with thermal treatment of the emulsion. This is reminiscent of what is discussed later in this chapter where emulsions containing lower concentrations of surfactant showed a decrease in viscosity over time as well as an obvious phase separation (upon physical examination). It was also observed that running a heating and cooling ramp in combination with a polarised microscope showed an obvious structural transition upon heating well above the melting temperature of the dispersed phase and cooling down to room temperature, this could be because of the transporting of water between the lamellar crystal network and the bulk, causing structural changed with the heating and cooling process.

The investigation into the microstructural makeup and influence of lamellar crystal networks in our ternary system of interest (or similar) is of ongoing interest in literature and, new findings are being documented to unpack its relevance fundamentally and in industrial applications.

While the different mesophases observed in this chapter were discussed and they largely appear to support findings in literature, however, there is room for gaining a better understanding of the kinetics of lamellar gel network formation, which is also under-researched in the existing literature. A lot of research has focused on trying to underpin processing conditions and compositions that yield the formation of these networks as well as characterising them, however, much is left to do about having a structured framework that focuses on how various factors affect the rate of lamellar gel network formation.

4.1 Polarising Optical Microscopy

This technique was used as it is designed to capture material that can only be seen as a result of their optically anisotropic behaviour as well as provide information on the optical textures, mesophase orientation, and structural arrangement of the material being evaluated (Robinson et al, 2022). More importantly, it is typically used in the detection of non-cubic liquid crystal phases that may be present within a system, these crystalline phases can be detected when looked at between crossed polarisers (Raja, 2022). The operating function of the polarising optical microscope (POM) runs by polarising light that passes through the microscope using a polariser, this filter allows light to pass through in a specific direction. In a polarising optical microscope (POM), there are two OF such polarisers oriented at a right angle to each other, this positioning is known as crossed polars. One of the polarisers is angled horizontally towards the incident wave and the other is positioned vertically to the incident beam (Robinson, 2022). Examining a sample under the POM follows by placing an aliquot of the sample between a microscope slide and a cover slip to obtain a thin layer of the sample. The proximity between both surfaces allows for an orientation of the mesophase layers, which is required to obtain the expected optical information. Diat et al., (1993) suggested that the characteristic oily streaks associated with the presence of lamellar liquid crystalline phases can be seen under a POM due to the perpendicular arrangement of the liquid crystalline phase to the microscope slide and/or the coverslip.

The analysis of all samples discussed in this chapter was carried out as described in Chapter 4 and all instruments used for the analyses are also listed and discussed in the same chapter.

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Stearyl Alcohol – Used interchangeably as ‘*Wax’/ ‘SA’*

Tween 20 – Used interchangeably as ‘*Surfactant’*

4.2 Results & Discussion

Various emulsion compositions with varying concentrations of wax and surfactant were investigated. This range allowed for a comprehensive comparison of dispersions to examine the presence of mesophases within the emulsions. The range of concentrations was chosen based on both trial & error (to explore new concentration regimes that have not been evaluated) and a review of existing literature on lamellar gels. Studies in literature have suggested morphological and structural changes were observed in formulations containing fatty alcohol and surfactant concentrations ranging from 5% - 15% (Iwata, 2017). Our selection builds upon these findings and also intends to explore new concentrations that could potentially yield fascinating novel results suitable for future work.

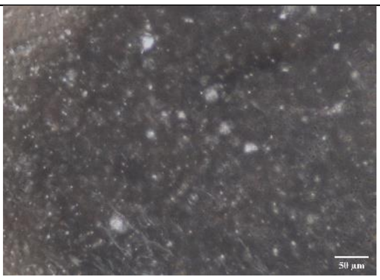
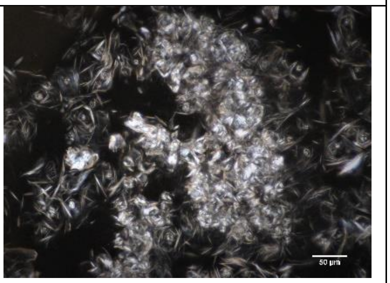
The physical appearance of the emulsions was also evaluated to assess the stability of the dispersions. Where phase separation or creaming was observed, the emulsion was deemed unstable, however upon physical examination, where no phase separation was observed, the emulsion was deemed stable. The phase separation seen in the emulsions formulated in this research had a characteristic of a white/milky upper layer and a clear bottom layer, which suggests creaming.

Emulsion Composition	**Physical Appearance**
2.5% Wax, 5% Surfactant	Milky, pourable
5% Wax, 5% Surfactant	Translucent, Not Resistant to flow
5% Wax, 7.5% Surfactant	Translucent, Not Resistant to flow
5% Wax, 15% Surfactant	Opaque, very resistant to flow
7.5% Wax, 5% Surfactant	Opaque, slightly resistant to flow
7.5% Wax, 15% Surfactant	Translucent, resistant to flow
10% Wax, 5% Surfactant	Opaque, slightly resistant to flow
10% Wax, 10% Surfactant	Translucent, Resistant to flow
15% Wax, 15% Surfactant	Translucent, Resistant to flow

Table 4.1: List of dispersions tested and their physical appearance after 1 Week of Room Temperature Storage

The emulsion with the lowest concentration of wax had a milky appearance and showed no resistance to flow when poured, this was also the only emulsion that showed signs of creaming after 1 week of storage of emulsions at room temperature. In addition, when observed under the POM, the sample containing 2.5% Wax, 5% Surfactant showed no irregular-shaped crystals, characteristic needle-like crystals nor Maltese crosses. It looked amorphous (had a runny consistency and it was difficult to obtain reliable results on the rheometer), it was decided that this composition would not be explored further as this study was focused on investigating the potential presence of lamellar crystal networks within the emulsion system, However, looking at this composition under the microscope did not suggest the presence of mesophases in the system. Micrographs were also taken for other emulsions listed above and it was found that optically anisotropic behaviour and textures that may suggest the presence of the liquid crystalline phases were mainly seen in compositions containing >10% Tween 20. This would suggest that the Critical Micelle Concentration (CMC) of Tween 20 in water with the wax in this emulsion system is somewhere between 10 and 15%w/v in comparison to CMC for pure Tween 20 in water which is 0.06 – 0.07% at room temperature, indicating a much higher concentration of Tween 20 in the mixture. At phase boundaries, surfactant molecules gather due to their amphiphilic nature, and they act as stabilising agents by lowering interfacial tension. However, the gathering of surfactant molecules near the phase boundary can result in the formation of micelles and have an effect on emulsion stability and overall behaviour. Going by the observation of micellar behaviour at surfactant concentrations above 10%, we, therefore, infer that the phase boundary of the emulsion system might have been reached or exceeded, and concurrently, CMC exceeded. This indicates that at surfactant concentrations >10%, the spontaneous formation of micelles will be observed.

Although fatty alcohols are also known to behave like non-ionic surfactants as a result of their amphipathic nature, the role the presence of stearyl alcohol might have played in the emulsion system of interest was never explored in detail for this research, it was mainly focused on as the wax/ dispersed phase. However, this body of work would need to be explored further and in detail to fully understand the role of stearyl alcohol in the mixture (i.e. could potentially not just be playing the role of the dispersed phase but also acting as a co-surfactant with Tween 20). While this is speculation in the case of this project, it has been shown in the literature that fatty alcohols including stearyl alcohols can act as co-emulsifiers (Maher et al., 2023) to improve the emulsifying properties of the emulsion system and strengthen the reduction of interfacial tension between the dispersed and continuous phases. This investigation aims to explain whether the surface-active materials in the mixture are exhibiting competing interactions or working together effectively – in doing this, the aim is to determine how the presence of stearyl alcohol and Tween 20 affect the overall performance and behaviour of the emulsion.

Emulsion Composition	**POM Image**	**Emulsion Composition**	**POM Image**
2.5% Wax, **5%** Surfactant		**7.5%** Wax, **15%** Surfactant	

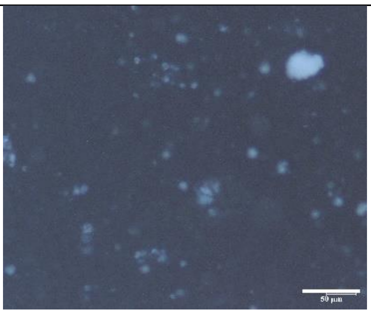
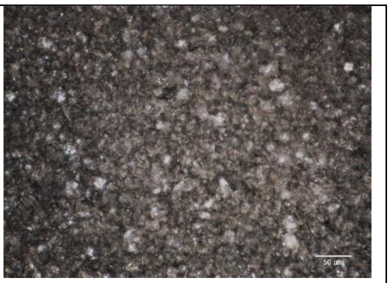
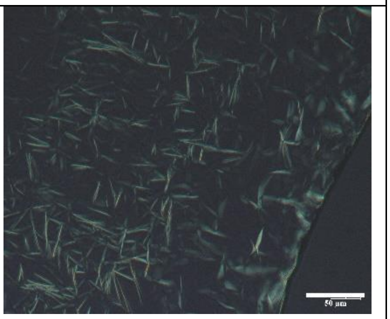
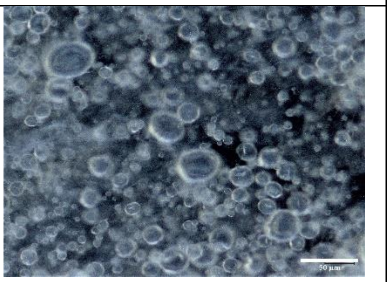
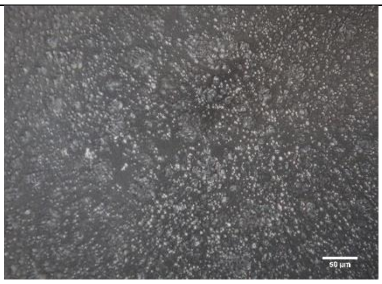
5% Wax, **5%** Surfactant		**10%** Wax, **5%** Surfactant	
5% Wax, **7.5%** Surfactant		**10%** Wax, **10%** Surfactant	
5% Wax, **15%** Surfactant		**15%** Wax, **15%** Surfactant	
7.5% Wax, **5%** Surfactant			

Table 4.2: POM images showing all dispersions at room temperature, 24 hours after preparation before heating and cooling the samples on the POM's heated stage.

In all the images, the scale bars represent a length of 50µm. However, variations in photo dimensions may result in the scale bars appearing larger or smaller in some images due to manual changes while acquiring the micrographs.

4.3 Effect of Surfactant Concentration

In a bid to gain a better understanding of how the concentration of Tween 20 affects the formation of liquid crystalline structures in the system, the experiment was repeated, this time focusing on compositions containing 7.5% of surfactant and above, this is because this chapter was focused on exploring the potential presence of lamellar phases present in the emulsion and because compositions containing surfactant concentrations below 7.5% did not indicate the presence of mesophases therefore it was decided that this study would focus on higher surfactant concentrations.

The following compositions were investigated:

Sample ID	**Water Content**	**Wax**	**Surfactant**
A	87.5%	5%	7.5%
B	85%	5%	10%
C	80%	5%	15%

Table 4.3: Concentration of Stearyl Alcohol, Tween 20 and the amount of water used in the testing of how the **amount of surfactant** influences the formation of liquid crystal phases in wax-in-water emulsions.

Figure 4.1: (L – R): Sample A – White, soft-gel like material and not resistant to flow, Sample B – Off-white, lumpy gel and slightly pourable, Sample C – Off-wite, Lumpy and Resistant to flow.

The emulsions were observed for 1 week, taking micrographs on Day 1, Day 5 and at 1 week. Bulk samples were stored at room temperature (~24°C) and on a countertop, a fraction of the bulk sample was then taken each time for the different observations recorded. When the sample was placed under the microscope, a heating ramp treatment was applied, this was done in a bid to see if the formation of the possible lamellar phase structures would be affected by temperature changes; this is because liquid crystals can either be thermotropic (i.e., phase transitions occur with an increase in temperature) or lyotropic (i.e., phase transitions occur with changes in temperature and concentration) (Garidel et al., 2015). Initial micrographs were then taken at room temperature, followed by the temperature ramp which heats the sample to 90°C (this was chosen to mimic the melt emulsification process temperature that was adopted at the time of preparation), whilst this was heating up, melting of the sample was observed under the polarising microscope at ~62°C, which agrees with the melting point of Stearyl Alcohol in literature. After the heating cycle, the sample is then cooled to room temperature at 10°C/min. Recrystallisation then began at ~54°C and continued until the cooling cycle ended at 24°C, also observed using polarised microscopy. Micrographs taken at the different temperatures during the heating and cooling cycle are shown below:

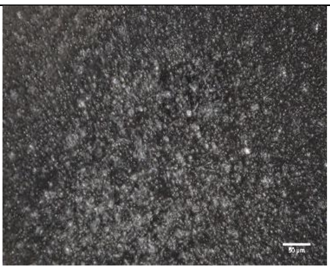
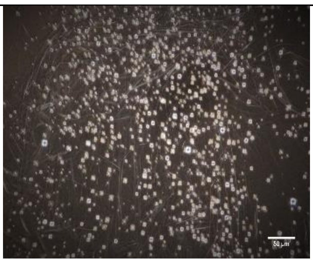
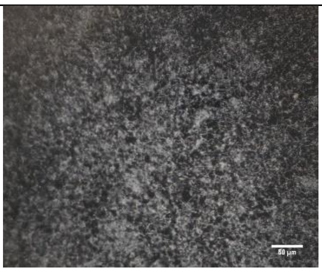
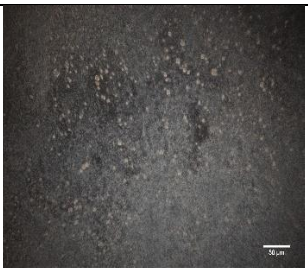
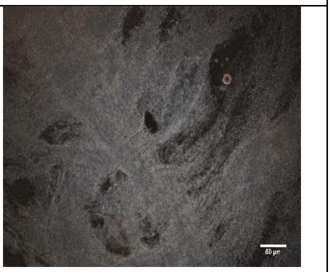
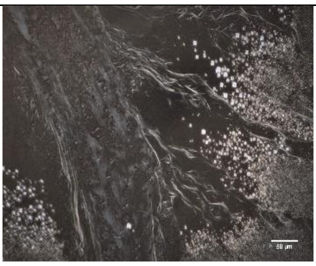
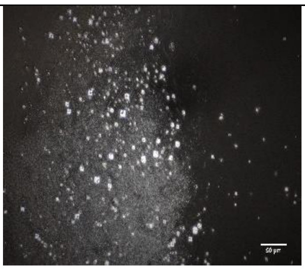
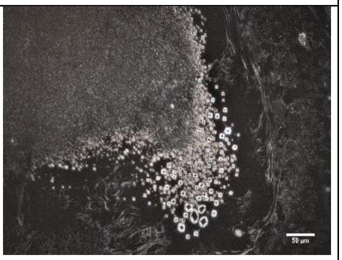
Emulsion / Ramp Temp.	*5% Stearyl Alcohol,* *7.5% Tween 20*	*5% Stearyl Alcohol,* *10% Tween 20*	*5% Stearyl Alcohol,* *15% Tween 20*
(Before Heating) *24°C*			
90°C (Melted)			
(After Cooling) *24°C*			

Table 4.4: Images showing the following compositions (i) 5% Stearyl Alcohol, 5% Tween 20 – A (ii) 5% Stearyl Alcohol, 10% Tween 20 – B (iii) 5% Stearyl Alcohol, 15% Tween 20 – C under the POM at specific temperatures during the heating ramp cycle. These micrographs were taken **1 day** after preparation. The scale bar seen in these micrographs represents 50µm.

The three samples revealed distinct structures at the temperatures they were observed at. At room temperature, before the heating ramp cycle started and as expected, with increasing surfactant concentration, the samples displayed different optical textures and characteristics. Sample A showed what appeared to be waxy crystalline droplets, many of them appearing to be spherical with some gel-like materials were also seen trapped between the droplets. This composition had the least amount of surfactant of the three and at this temperature, gave no indication of the presence of a lamellar phase, only showed the expected polydisperse solid wax droplets, where some appeared to be aggregated. This is expected of a wax-in-water emulsion and was stored at room temperature for a further few days to observe possible structural changes that could occur with ageing. On the other hand, and at room temperature, sample B displayed an extensive presence of Maltese crosses, surrounded by flexible needle-like material. A completely different behaviour to what was seen in the previous sample above, this strongly suggested that structural changes were occurring with the change in surfactant concentration. The characteristic Maltese crosses and needle-like crystals seen in sample B suggest a transition from one phase to the next in the liquid crystalline phase progression (see below) – although the exact phases being transitioned into are not fully known at this time, however theorise a structural buildup occurring due to increased surfactant concentration, further work and analysis would have to be carried out to gain a comprehensive understanding of what phase can be attributed to what morphological changes.

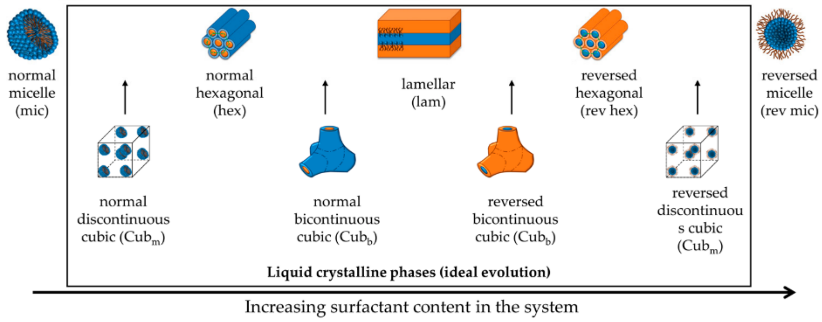
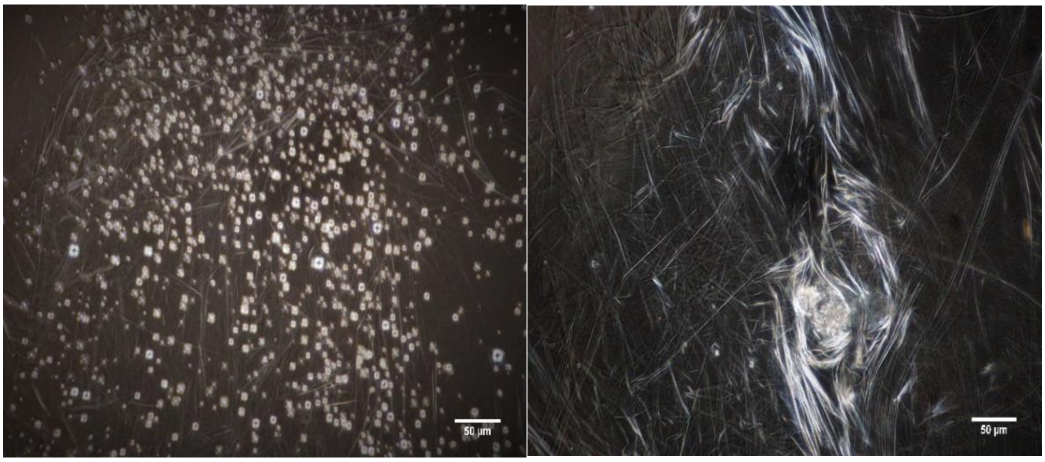


Figure 4.2.: A transitional representation of the different liquid crystalline phases with increasing surfactant concentration (Gonçalves et al., 2021)

It also confirms the suggestion in literature (Gonçalves et al., 2021) that with a change in the Critical Micelle Concentration (CMC), there is micellar growth, and the critical packing parameter (CPP) also changes.

The presence of Maltese crosses infers the formation of ordered liquid crystals in the lamellar phase – it is an indication of the oil/wax droplet in the system surrounded by multilamellar layers, this theory has been confirmed in literature by multiple research papers, notable ones being Iwata, (2017) and Eccleston et al. (2000). On the other hand, it has been theorised in literature (Kudla et al., 2010) that the presence of needle-like crystals, suggests a pure surfactant phase. In their experiments, they found that with increasing surfactant concentration, they had two scenarios, one where they observed Maltese crosses and needle-like crystals, this was described as a system containing Lamellar and pure surfactant crystal phase, and where they had the highest concentration of surfactant; just needle-like crystals were observed – this system was described as ‘*biphasic’,* where water bound to the surfactant phase is expunged, making the system one containing pure water and a pure surfactant phase, hence the term, biphasic. In agreement with what was observed in literature as stated above, we observed the presence of maltese crosses and needle-like crystals in the samples containing 5% wax, 10% surfactant (Sample B) & 5% wax, 15% surfactant (Sample C), respectively. Images below show a bolder image of these observations.

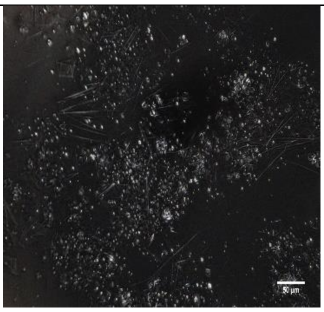
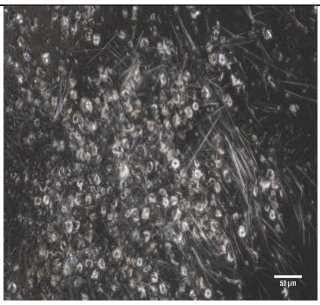

Sample B

Sample C

Figure 4.3: (i) Sample B (5% Wax, 10% Surfactant) showing a mix of maltese crosses and needle-like crystals (ii) Sample C (5% Wax, 15% Surfactant) showing predominately needle-like crystals before the heating and cooling cycle.

The latter scenario described is in partial agreement with what was observed in sample C in this study, in this sample, 15% surfactant was used, making it the composition with the highest surfactant amount in this series. Here, some flocculated wax crystals were surrounded by a strong presence of needle-like crystals. Although the needle-like crystals were not quantified in this study, upon visual inspection, they appeared to make up a larger portion of the optical structures seen in this emulsion, it is worth noting that the micrographs are representative of the general behaviour seen under the microscope as opposed to an individualistic portion of the unique behaviour seen being captured.

The micrographs taken at 90°C showed melting of the various structures seen i.e., solid spherical wax droplets, Maltese crosses, and needle-like crystals, however, it was expected that if there was complete melting of the wax phase in the system, there would be a dark appearance seen at 90°C, however some material was seen at 90°C, which is well above the melting temperature of the stearyl alcohol. The presence of these materials seen at this temperature, further confirms the theory above that suggests that needle-like crystals and MC can be attributed to the presence of a dominant surfactant phase and a multilamellar phase (Kudla et al., 2010). Note: We did not allow time for structural rearrangement after cooling, samples were just observed as they arrived at temperature. Any hysteresis has not been accounted for. After the heating cycle, the samples were cooled to room temperature on the heated stage attached to the microscope. In sample A, the initial solid wax droplets that were previously seen had converted into finer what appeared to be wax particles, this was as expected, suggesting a breakdown in structure upon heating and a build-up of structure upon cooling. In sample B, it was observed after recrystallisation that all the needle-like crystals present had melted during the heating cycle, this is because the resulting image post-recrystallisation shows Maltese crosses with wax particles, however, there was no sign of the crystals, which suggests that the pure surfactant crystal phase is a temporary and reversible phase, owing to its disappearance during the heating cycle. Sample C on the other hand showed peculiar behaviour in that the pure surfactant phase seen before going through the heating cycle had fully converted into an aggregated arrangement of Maltese crosses. Going by this observation, it was inferred that the Maltese crosses seen after heating and cooling are a result of an increased amount of surfactant in the system during heating, the excess surfactant in the system then rearranges and interacts with the existing wax droplets in the system, thereby forming multilamellar structures around the wax droplets, resulting in the formation of Maltese crosses. Again, here, the needle-like crystals are temporary and show that they can be subject to change when heated and cooled. The industrial relevance of the rearrangement occurring within these systems at high temperatures is not fully understood now, however, personal care companies & pharmaceutical companies formulate systems made up of lamellar gel networks. The keen interest they have in these multilamellar structures is due to the strength of bilayers in the multilamellar arrangements, which means that in practice, the dispersions are likely to be more stable and have a long shelf life.

Emulsion/ Ramp Temp.	*5% Stearyl Alcohol,* *7.5% Tween 20*	*5% Stearyl Alcohol,* *10% Tween 20*	*5% Stearyl Alcohol,* *15% Tween 20*
(Before Heating) *24°C*			

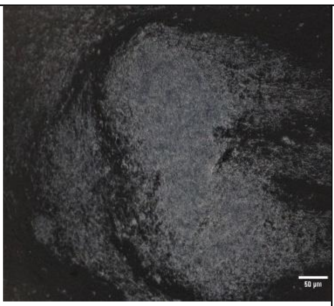
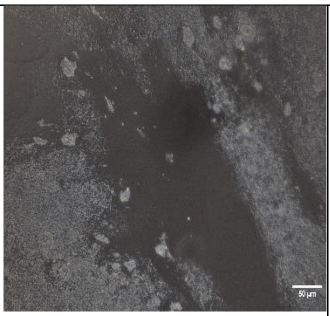
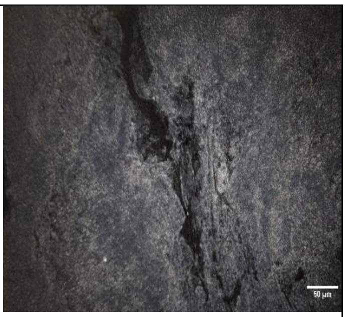
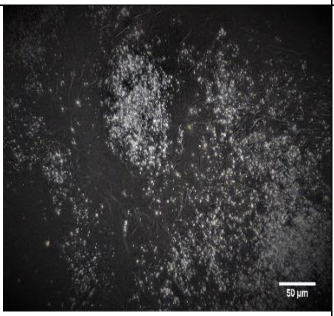
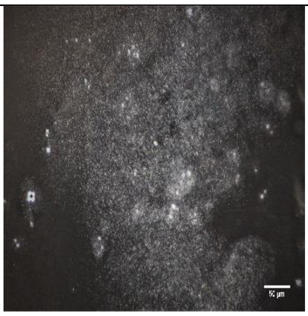
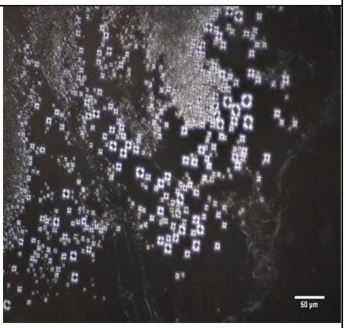
(Melted) *90°C*			
(After Cooling) *24°C*			

Table 4.5: Images showing the following compositions (i) 5% Stearyl Alcohol, 5% Tween 20 – Sample A (ii) 5% Stearyl Alcohol, 10% Tween 20 – Sample B (iii) 5% Stearyl Alcohol, 15% Tween 20 – Sample C under the POM at specific temperatures during the heating ramp cycle. These micrographs were taken **5 days** after preparation. The scale bar seen in these micrographs represents 50µm.

The samples were observed after 5 days to see how ageing might have influenced the liquid crystalline structures observed 24 hours after emulsion preparation. It was observed that sample A appeared to have transitioned from being made up of purely spherical solid wax droplets to a mix of spherical droplets and large needle-like crystals and with less flocculation, the formation of the large needle-like crystals is assumed to have come about as a result of the expulsion of surfactant molecules from the already existing micelles in the system through internal relaxation processes, this process is assumed to occur over time and during storage, according to (Kudla et al., 20105. This theory was supported by what was found in literature, where it was first postulated by Aniansson and Wall (1974) that over time, micelles are expected to go through an insertion and expulsion of surfactant molecules process in order to create a metastable equilibrium state with the other surfactant molecules (Jensen et al., 2013) in the emulsion (as it is known that emulsions are generally thermodynamically unstable systems) (Mysona et al., 2019). Therefore, the constant exchange of surfactant molecules within the micellar structure influences the stability and equilibrium of the emulsion during ageing. The complex behaviour of the emulsion system is indicated through the interplay between the reordering of micelles and surfactant concentration exchange. This kinetic mechanism of nucleation and growth supports the theory above where it was assumed that the transition to spherical droplets as well as a pure surfactant phase (represented by the needle-like crystals) came about as a result of surfactant molecules being expelled from already existing micelles in the system and therefore increasing surfactant concentration in the system, enough to create a surfactant phase which was observed after 5 days, however, it is possible that this phase is temporary as the emulsion system is always seeking to find equilibrium.

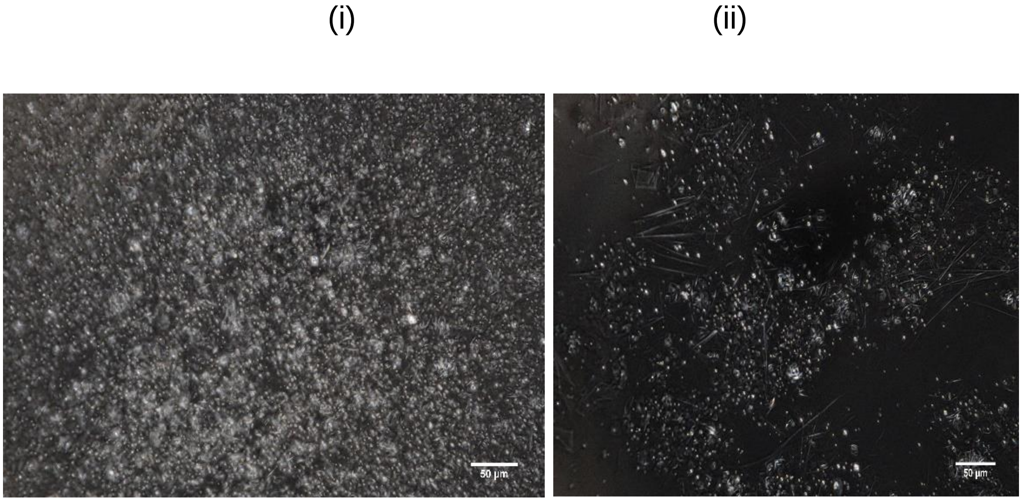


Figure 4.4: (i) Sample A showing solid wax droplets at 24°C after 24 hours (ii) Sample A showing a mix of solid wax droplets and needle-like crystals at 24°C after 5 days, both images were taken before the heating and cooling cycle.

After the heating and cooling cycle on the heated stage of the POM, sample A retained some needle-like crystals, which strongly indicates that some structural re-arrangement occurred during storage and may not be just a transient phase rearrangement if it was resilient enough to go through heating hysteresis without losing all its structure.

Samples B and C showed very similar behaviour to what was observed after 24 hours of storage.

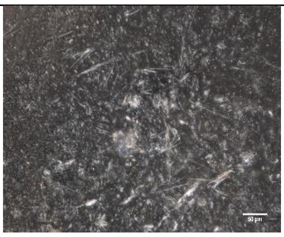
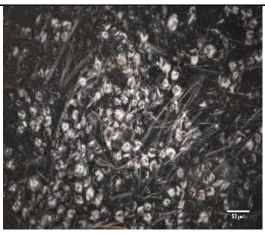
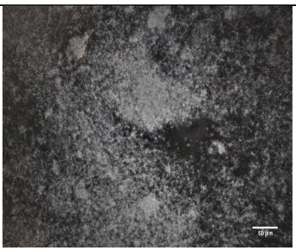
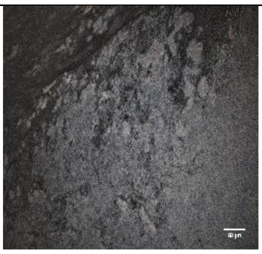
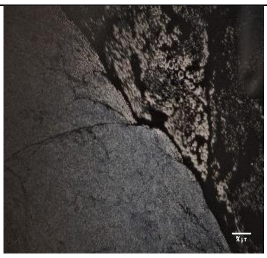
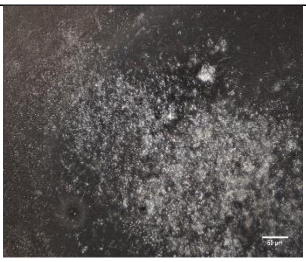
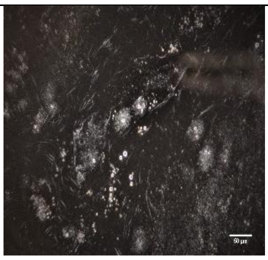
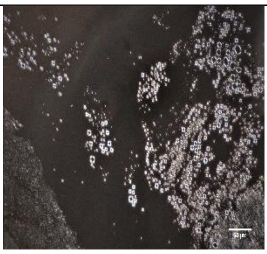
Emulsion/ Ramp Temp.	*5% Stearyl Alcohol,* *7.5% Tween 20*	*5% Stearyl Alcohol,* *10% Tween 20*	*5% Stearyl Alcohol,* *15% Tween 20*
24°C (Before Heating)			
90°C (Melted)			
(After Cooling) *24°C*			

Table 4.6: Images showing the following compositions (i) 5% Stearyl Alcohol, 5% Tween 20 – A (ii) 5% Stearyl Alcohol, 10% Tween 20 – B (iii) 5% Stearyl Alcohol, 15% Tween 20 – C under the POM at specific temperatures during the heating ramp cycle. These micrographs were taken **1 Week** after preparation. The scale bar seen in these micrographs represents 50µm

After 1 week of storage, samples all had the characteristic needle-like structures at room temperature, which suggests that the ageing of these samples favours the growth of the pure surfactant phase as well as the presence of lamellar structures. The formation of multilamellar units may have been as a result of the redistribution of surfactant molecules within the emulsion system. The process of redistribution can impact the availability of surfactant molecules at the interface and consequently, the composition, shape and size of the micelles would be altered. While the concentration of the surfactant remains unchanged, the distribution of surfactant molecules in the system would affect the emulsion behaviour and properties.

The samples have shown behaviours of being either thermotropic or lyotropic, this would explain the dependence on high temperatures for the formation of the maltese crosses and on high concentrations of surfactant to have emulsions that are multiphasic, this was seen in sample B, which contained 10% surfactant.

4.4 Effect of Wax Concentration

Keeping the concentration of the surfactant constant this time (at 5%) – to mirror a similar pattern of the compositions that were investigated in subsection ‘4.3 - ‘Effect of Surfactant Concentration’, the concentration of wax was varied to see how changes in the composition of the emulsion system might influence the structural rearrangements and potential micellar presence seen in section 4.1. The following compositions were tested and observed.

Sample ID	Water Content	Wax	Surfactant
D	87.5%	7.5%	5%
E	85%	10%	5%
F	80%	15%	5%

Table 4.7: Concentration of Stearyl Alcohol, Tween 20 and the amount of water used in the testing of how the amount of **wax content** influences the formation of liquid crystal phases in wax-in-water emulsions

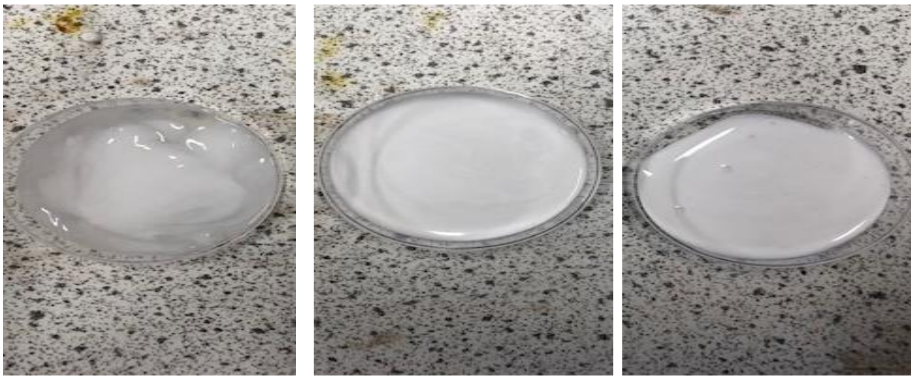


Figure 4.5: (L – R): Sample D (7.5% Wax, 5% Surfactant)– White, very viscous and translucent, Sample E (10% Wax, 5% Surfactant) – Milky Consistency, runny and very pourable, Sample F (15% Wax, 5% Surfactant)– White, Milky and Very easy to pour.

All samples were first examined under the microscope after 24 hours. Sample containing 7.5% Wax, and 5% Stearyl Alcohol appeared to contain large amounts of spherical wax droplets and some gel-like material when observed at room temperature. However, after going through the heating and cooling cycle, the particles appeared to have reduced in size and particles looked finer in appearance, not much phase change seemed to have occurred here.

On the other hand, the sample containing 10% Wax and 5% Tween 20 at room temperature showed large coalesced droplets as well as some aggregated wax droplets, which is as expected, this is because the ratio of surfactant concentration to wax concentration would not be sufficient to fully cover the surface of the wax droplets, and where there is Brownian motion in the emulsion system, there is likely to be droplet collisions which would eventually lead to the formation of coalesced, flocculated droplets within the Emulsion system, Interestingly, post the heating and cooling cycle, there was a sudden appearance of Maltese crosses and an apparent loss of the aggregated spherical droplets that had been previously seen at room temperature. It can be theorised that the formation of the Maltese crosses after the heating and cooling cycle could be a result of an increased amount of surfactant in the emulsion from heating and melting the already formed emulsified droplets leading to the formation of multilamellar structures (hence the Maltese crosses), the other working theory could be the fact that the fatty alcohol being used – stearyl alcohol Is an emulsifying wax in itself and is capable of forming gel networks in the presence of a surfactant and water[7], this would go to suggest that the although the interplay between the wax and tween can form complex networks, the mixture required thermal treatment to form a multilamellar phase.

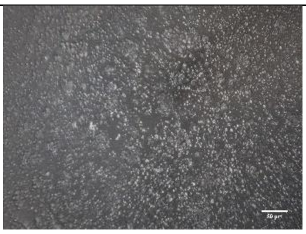
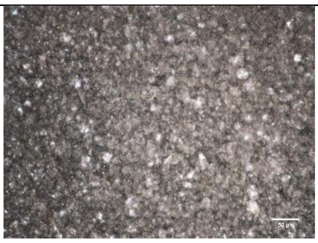
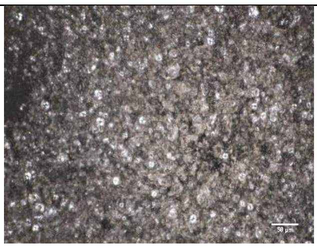
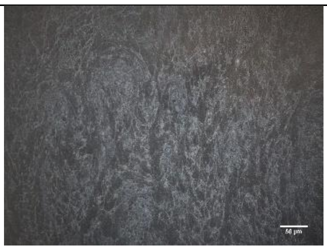
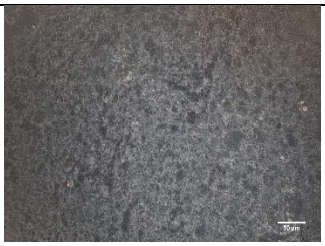
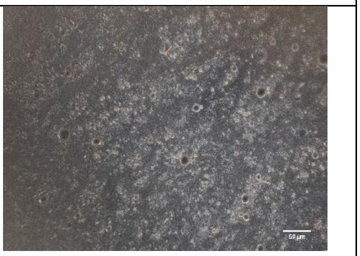
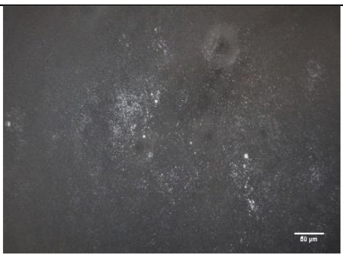
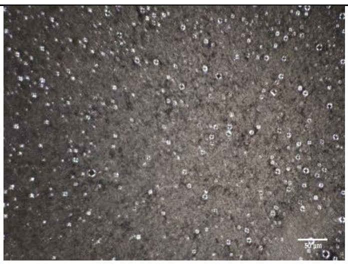
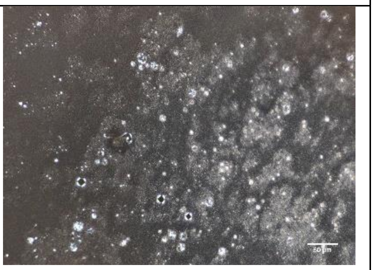
Emulsion/ Ramp Temp.	*7.5% Stearyl Alcohol,* *5% Tween 20*	*10% Stearyl Alcohol,* *5% Tween 20*	*15% Stearyl Alcohol,* *5% Tween 20*
(Before Heating) *24°C*			
90°C (Melted)			
(After Cooling) *24°C*			

Table 4.8: Images showing the following compositions (i) 7.5% Stearyl Alcohol, 5% Tween 20 – A (ii) 10% Stearyl Alcohol, 5% Tween 20 – B (iii) 15% Stearyl Alcohol, 5% Tween 20 – C under the POM at specific temperatures during the heating ramp cycle. These micrographs were taken **1 day** after preparation. The scale bar seen in these micrographs represents 50µm

The composition with the highest concentration of wax (15%) and 5% Tween 20, revealed many Maltese crosses at room temperature before the heating and cooling ramp. The appearance of these multilamellar structures and the pourability of the sample (Hyde, 2001) as indicated in Table 4.8 above would suggest the formation of multilamellar structures in this composition is influenced by the high concentration of wax in the system (Hyde, 2001). The reason why this would be seen at room temperature is not fully understood at this time, however, considering the trend of increasing wax concentration, it appears to play a significant role in the formation of multilamellar structures.

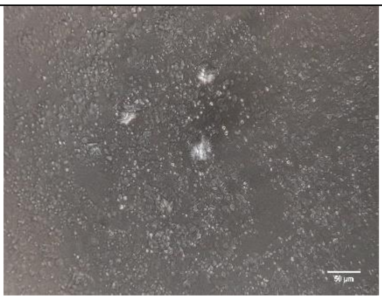
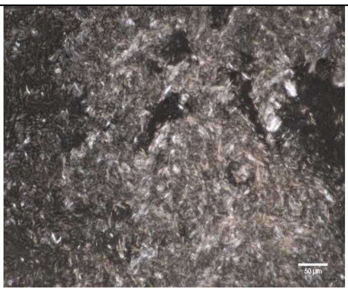
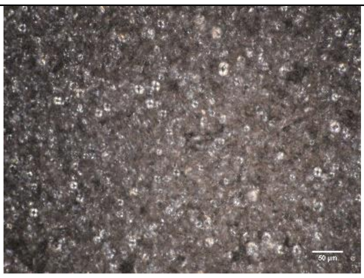
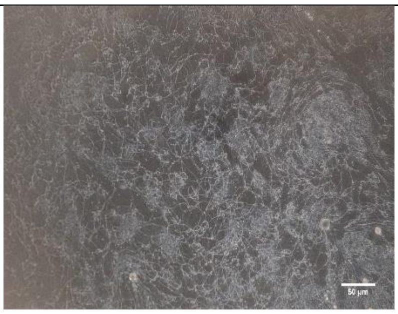
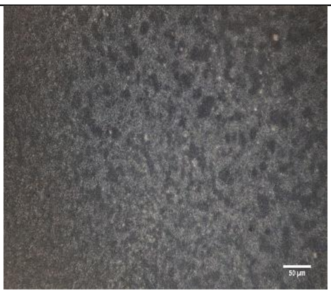
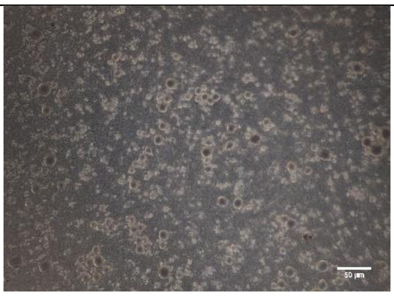
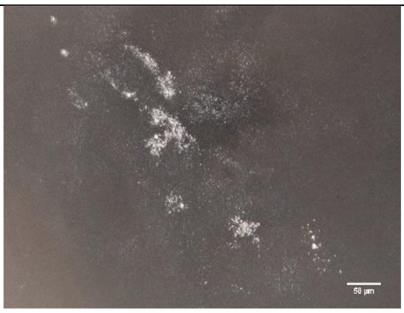
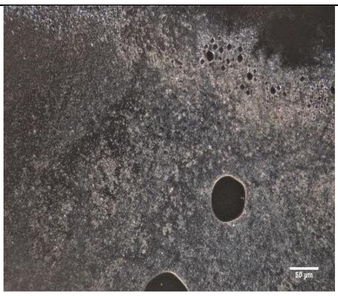
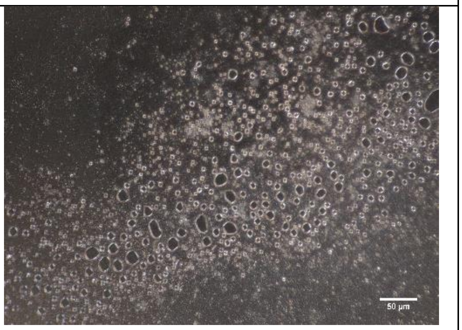
Emulsion/ Ramp Temp.	*7.5% Stearyl Alcohol,* *5% Tween 20*	*10% Stearyl Alcohol,* *5% Tween 20*	*15% Stearyl Alcohol,* *5% Tween 20*
(Before Heating) *24°C*			
90°C (Melted)			
(After Cooling) *24°C*			

Table 4.9: Images showing the following compositions (i) 7.5% Stearyl Alcohol, 5% Tween 20 – A (ii) 10% Stearyl Alcohol, 5% Tween 20 – B (iii) 15% Stearyl Alcohol, 5% Tween 20 – C under the POM at specific temperatures during the heating ramp cycle. These micrographs were taken **1 week** after preparation. The scale bar seen in these micrographs represents 50µm

Very similar behaviour is seen after 1 week of storage as was seen for 24 hours after sample preparation, this would suggest that a very well-structured system was formed because they all retained their morphology and change in structure after the heating & cooling cycle.

Due to time constraints, it was impossible to explore the other emulsion compositions in detail to gain a better understanding and meticulously investigate what factors are the key players in forming these multilamellar layers.

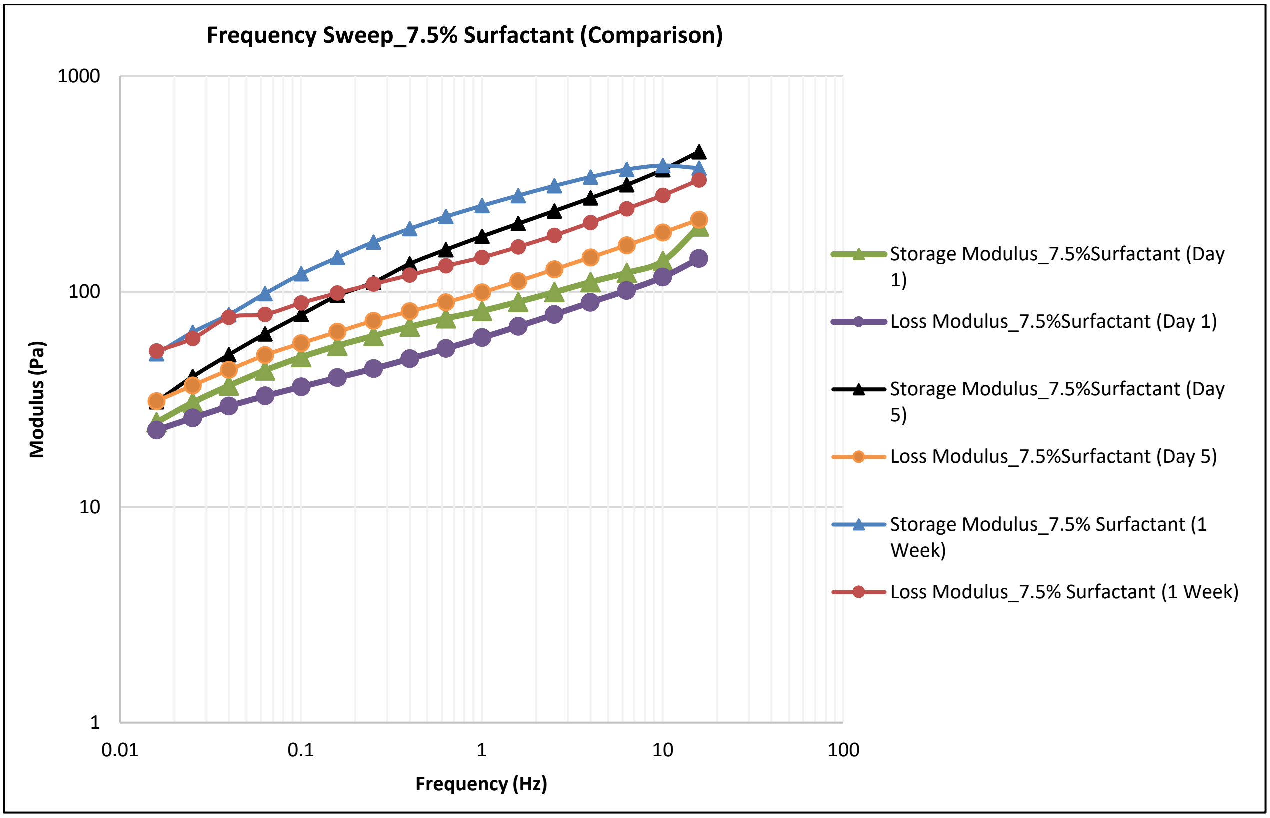
For completeness, images were taken at various temperatures, including room temperature (24°C), melting temperature (65°C), and freezing temperature (55°C). The full range of images taken during the heating and cooling ramps on the polarising microscope can be found in the Appendix.

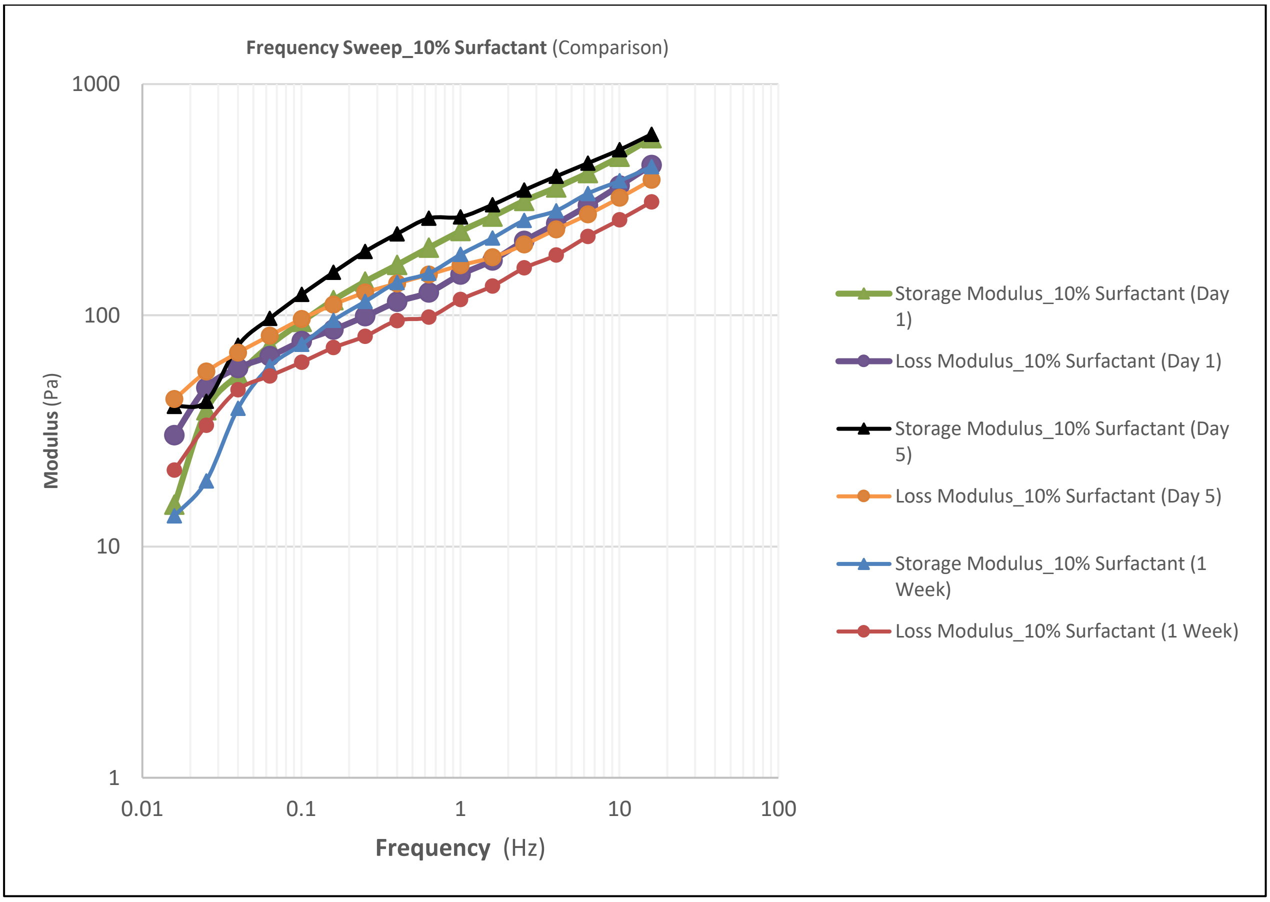
4.5 Rheology

In a short experiment, the rheology of the samples listed above was investigated, in order to see how the change in structural arrangement being seen under the microscope would reflect or agree with the rheology results.

Oscillatory rheology data was acquired on this occasion, the emulsion system of interest, has proven to be shear thinning each time a steady-shear measurement is carried out. The rheology of these compositions is worth exploring further in the near future as some of the results shown below suggest that there might be two kinds of systems at play, a well-structured one or weakly structured one.

Keeping the concentration of wax constant at 5%, three different concentrations of Tween 20 were explored; 7.5%, 10% and 15%.


Frequency Sweep_7.5% Surfactant (Comparison)
1000
100
10
1
Modulus (Pa)
0.01
0.1
1
10
100
Frequency (Hz)
Storage Modulus_7.5%Surfactant (Day 1)
Loss Modulus_7.5%Surfactant (Day 1)
Storage Modulus_7.5%Surfactant (Day 5)
Loss Modulus_7.5%Surfactant (Day 5)
Storage Modulus_7.5% Surfactant (1 Week)
Loss Modulus_7.5% Surfactant (1 Week)


Frequency Sweep_10% Surfactant (Comparison)
1000
100
10
1
Modulus (Pa)
0.01
0.1
1
10
100
Frequency (Hz)
Storage Modulus_10% Surfactant (Day 1)
Loss Modulus_10% Surfactant (Day 1)
Storage Modulus_10% Surfactant (Day 5)
Loss Modulus_10% Surfactant (Day 5)
Storage Modulus_10% Surfactant (1 Week)
Loss Modulus_10% Surfactant (1 Week)

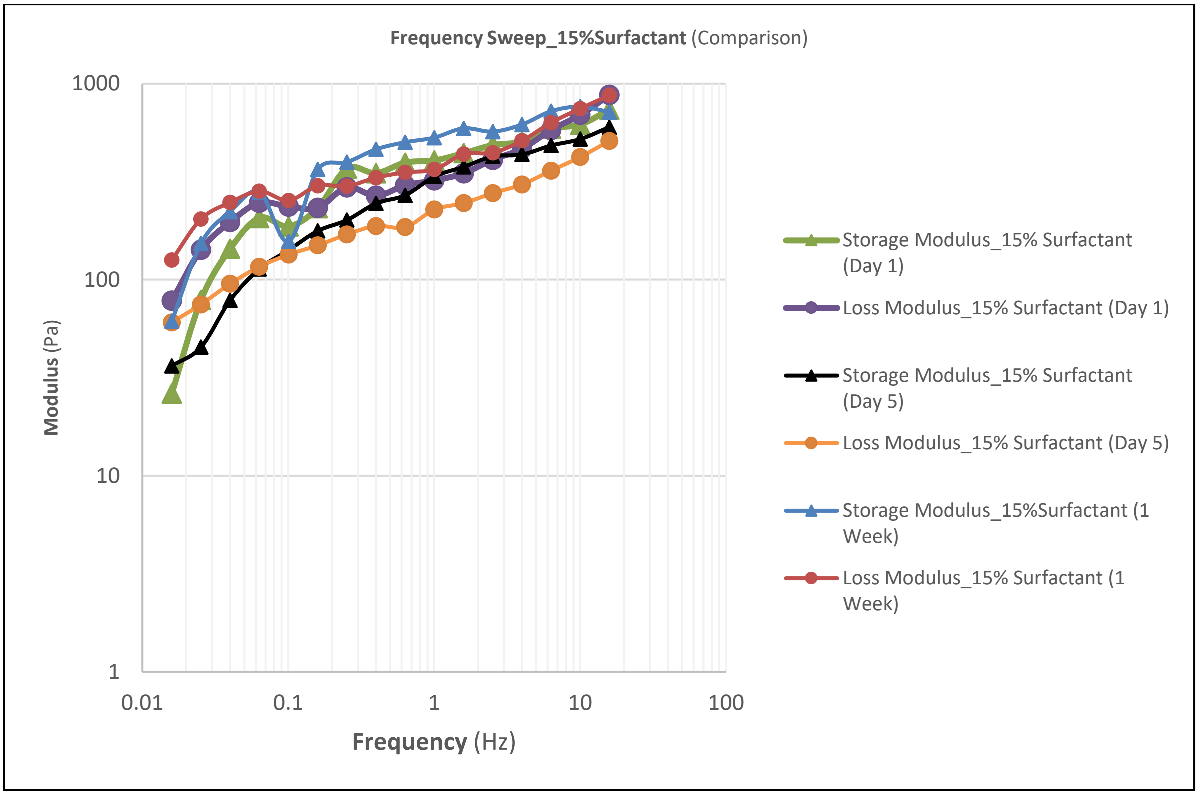
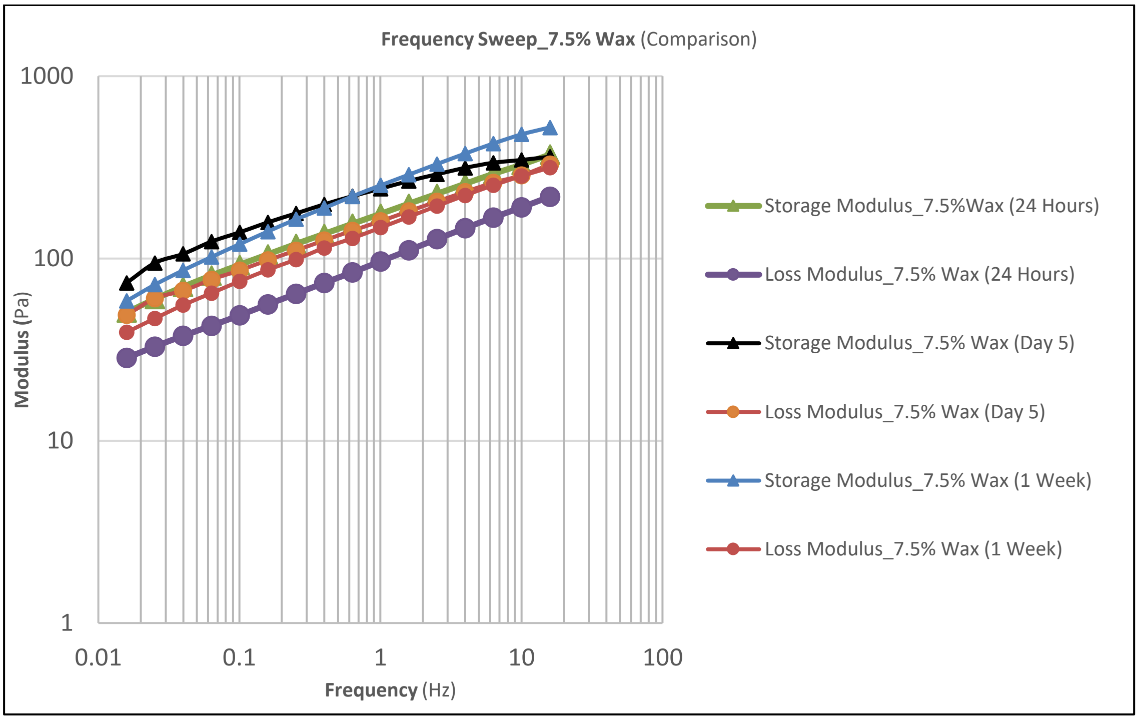
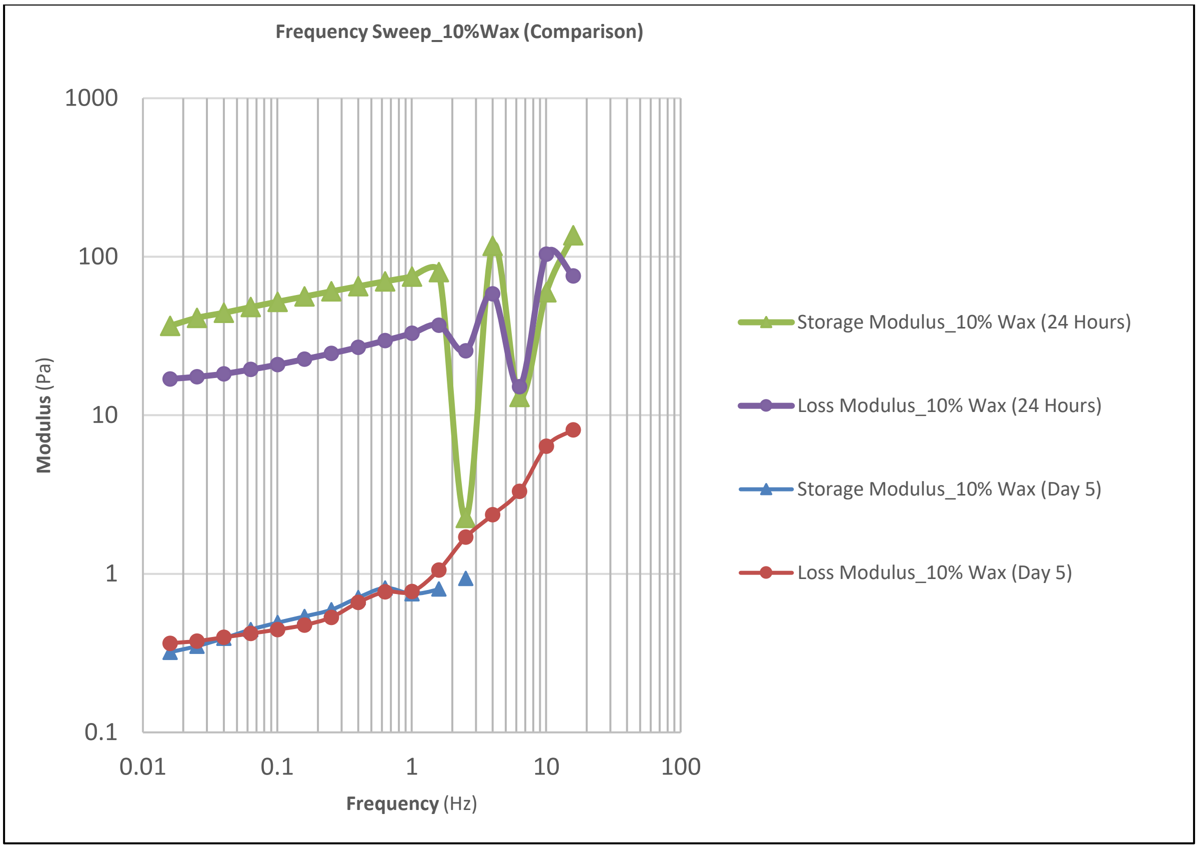


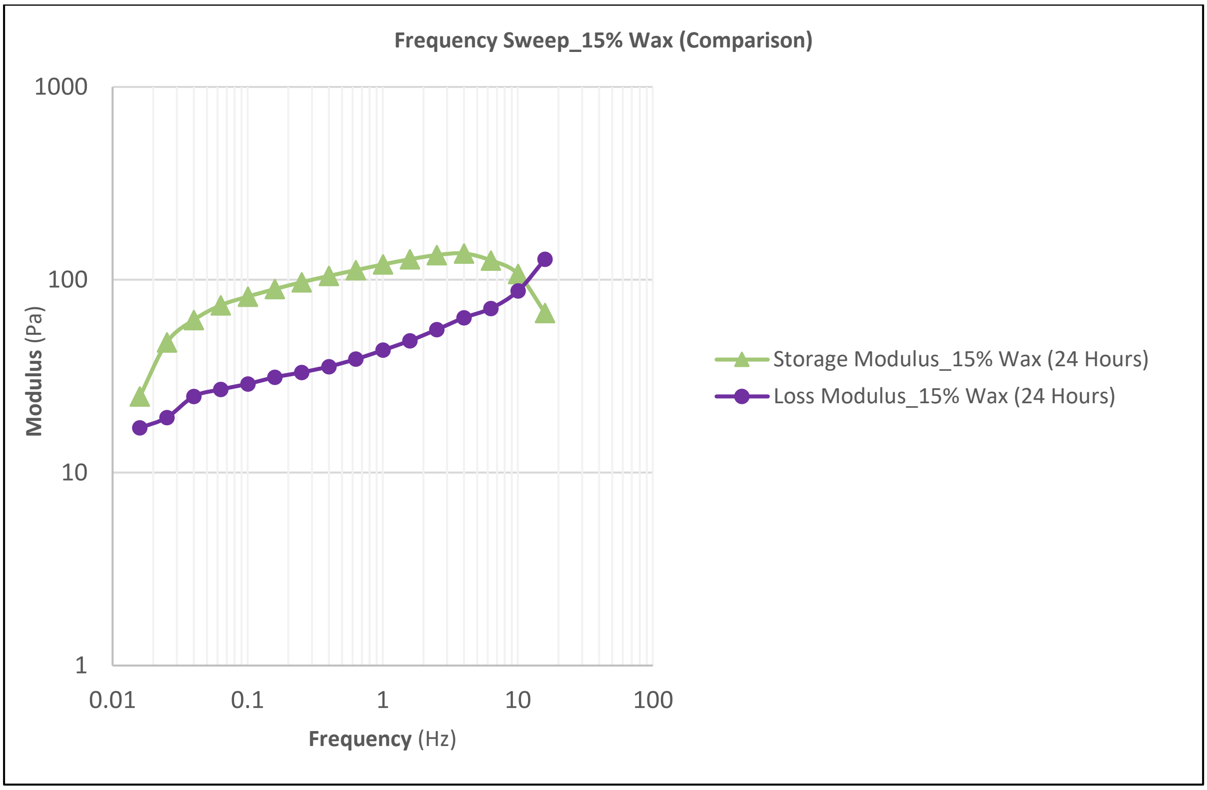
Figure 4.6: From top to bottom: Oscillatory frequency graphs showing the following compositins (i) 7.5% Tween 20, 5% Stearyl Alcohol (ii) 10% Tween 20, 5% Stearyl Alcohol (iii) 15% Tween 20, 5% Stearyl Alcohol

Sample containing 7.5% Tween, and 5% Wax showed a stable elastic behaviour over time, with the storage modulus greater than the loss modulus across all measurements. This is indicative of a well-structured gel system, this agrees with the microscopic images seen for this composition, which showed more spherical droplets than liquid crystals, although some needle-like crystals were seen after 1 week mixed with the spherical droplets, they showed a higher number of wax droplets and had a thick consistency, upon physical assessment.

The other two samples containing 10% Tween 20 and 15% Tween 20 also show that they are partially gelled systems. They appear to be more elastic at lower frequencies, which suggests it is partly liquid-like, this could be a result of the effective packing of the Maltese crosses and needle-like crystals in the systems, which would allow for free movement of fluid within the emulsion, in addition to this, emulsions containing mesophases have been said to have lowered viscosities (Hyde, 2001). Keeping the concentration of Tween 20 constant at 5%, three different concentrations of Stearyl Alcohol were explored: 7.5%, 10% and 15%.




Frequency Sweep_10%Wax (Comparison)
1000
100
10
1
0.1
Modulus (Pa)
0.01
0.1
1
10
100
Frequency (Hz)
Storage Modulus_10% Wax (24 Hours)
Loss Modulus_10% Wax (24 Hours)
Storage Modulus_10% Wax (Day 5)
Loss Modulus_10% Wax (Day 5)


Frequency Sweep_15% Wax (Comparison)
1000
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Modulus (Pa)
0.01
0.1
1
10
100
Frequency (Hz)
Storage Modulus_15% Wax (24 Hours)
Loss Modulus_15% Wax (24 Hours)

Figure 4.11: From top to bottom: Oscillatory frequency graphs showing the following compositions (i) 7.5% Wax, 5% Tween 20 (ii) 10% Wax, 5% Tween 20 (iii) 15% Wax, 5% *Tween 20*

Note: As seen in the plot showing 10% Wax , 5% Surfactant, the irregular line seen in the plot is indicative of the sample not being stable at high frequencies, therefore data 1Hz is unreliable

Here, the sample containing the least amount of wax at 7.5% showed a stable elastic behaviour over time, with the storage modulus greater than the loss modulus across all measurements. It is interesting to observe that the sample containing the least amount of wax shows stable elastic behaviour, this observation suggests that the network decomposes in the presence of higher wax concentrations, we infer that this could be as a result of insufficient surfactant in the system holding the network together. This is indicative of a well-structured gel system. However, for the other two compositions, comprehensive results could not be obtained since the samples had become so runny after just 48 hours and could not be measured on the rheometer.

In all, it is evident that there is a lot of structural rearrangement and phase formation occurring in these compositions, however, the interplay between the two phases is yet to be fully understood and would need to be explored further. Multilamellar layers and biphasic structures are being formed which agrees with what would be expected at certain concentrations of the dispersed and continuous phase, however, the main driving force behind these structural arrangements is yet to be confirmed.

4.6 Conclusion

The investigation carried out in this chapter was aimed at studying different emulsion compositions with varying concentrations of Stearyl Alcohol and Tween 20, to enable a broad comparison of the compositions to identify the presence of mesophases and complex structures in the emulsion mixtures. The concentration range chosen was based on 1) conducting experiments to evaluate new concentration regimes and 2) assessment of existing literature on lamellar gel networks, which indicated structural changes in formulations containing fatty alcohol and non-ionic surfactant in concentrations ranging from 5-15% (Iwata, 2017).

Assessing emulsion stability was dependent on visual observation and rheological measurements. Where the emulsions were examined physically, phase separation/ creaming suggested emulsion instability. Emulsions with lower wax concentrations showed signs of creaming over time, while samples containing higher surfactant concentrations showed micellar behaviour, which can be attributed to the potential presence of a phase boundary past the Critical Micelle Concentration (CMC). The observation where micellar growth was seen fits in with literature research (Gonçalves et al., 2021) where it was suggested that micellar growth occurs with a change in the CMC.

Though Stearyl Alcohol was used as the dispersed phase in this study, it has also been suggested in literature as a potential co-surfactant, however, its role as a surface-active agent was not explored. Further work should be carried out to investigate the role of fatty alcohols as co-surfactants, the mechanism of interplay between fatty alcohols and surfactants in a system, and how their presence in a mixture as co-surfactant can enhance emulsion behaviour and influence interfacial tension.

This chapter also investigated the effect of surfactant concentration on the formation of liquid crystals. Here, the focus was on compositions containing >7.5% surfactant concentration where mesophases were expected. Polarised microscopy revealed fascinating morphologies, where needle-like crystals indicated a pure surfactant phase, and Maltese crosses suggested a lamellar phase. These observations align with literature (Kudla et al., 2010) concerning CMC and lamellar phase formation. The effect of wax concentration was also explored, it was observed that the emulsions containing higher concentrations of wax showed the presence of multilamellar structures, highlighted by the presence of Maltese crosses. Rheology experiments were in agreement with these findings as they indicated the elastic behaviour of a well-structured gel. However, the role of the interaction between Stearyl Alcohol and Tween 20 in the formation of these structural arrangements is yet to be fully understood and should be explored further.

To summarise, this chapter highlights how complex the ternary system investigated is and the peculiar interplay between the different phases in impacting the formation of mesophases and the stability of the emulsion. Although, interesting findings have been documented, however, further work is required to gain a comprehensive understanding of the complex relationship between the different components to enhance stability in emulsion formulations.

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Chapter 5

INVESTIGATING CHANGES OCCURRING WITH WAX IN WATER EMULSIONS

5. Investigating changes occurring with Wax-in-Water Emulsions

Synopsis & Introduction

To formulate a wax-in-water emulsion, the use of a saturated fatty alcohol (Stearyl Alcohol (SA)/1-Octadecanol – $CH_3(CH_2)_{16}CH_2OH$) was employed throughout the course of this research to serve as the 'wax' - *the dispersed phase* and a widely used non-ionic surfactant, Tween 20 (also known as Polysorbate20) served as the surfactant – *the continuous phase*. Tween 20 was chosen during a cloud point test which was conducted at the start of the project during a surfactant selection process, during the cloud point test, three different non-ionic surfactants (Tween 80 and Span 80) , including Tween 20 were added to water and heated to 90°C to see which of the surfactants would remain clear in water at this temperature. This was an important test to conduct because a non-ionic surfactant's cloud point is the temperature at which said surfactant is in water and displays a turbid appearance due to liquid-liquid phase separation. It was important to select a surfactant that would be stable even at such high temperatures, this is because the melt emulsification process requires working at temperatures much higher than the melting temperature of the dispersed phase. 1-Octadecanol has a melting temperature of 65°C (Sigma, 2003).

Emulsions are typically made up of a dispersed phase and a continuous phase, this could either be as a single emulsion structure (e.g. Oil-in-Water emulsion, O/W) or as a multiple emulsion structure (e.g. Water-in-Oil–in–Water emulsion W/O/W). The choice of emulsion structure chosen is dependent on required properties and applications (Tadros, 2013). Examples of industries where selecting the appropriate emulsion has a direct influence on the stability, appearance, texture, functionality, and storage stability of the final product include – Cosmetics, Paint, Pharmaceuticals, Food, etc. Factors such as the volumetric ratio between the continuous and dispersed phase, the processing methods, processing time, and environmental factors – such as temperature, storage conditions, and choice of emulsifier have a significant influence on the type of emulsion formed. It is also important to note that, where a phase transition occurs during the preparation of an emulsion or colloidal dispersion, this can affect the performance and structure of the final product formed. For example in this research, the preparation of the emulsion begins with mixing molten wax with the continuous phase, however upon cooling, a phase transition occurs, where the molten wax is assumed to have become solid droplets with a different structure due to their post formulation interaction with the continuous phase.

As a result of their versatility and sustainability, wax emulsions are used in numerous applications in industry and on several surfaces, ranging from optimising textural properties in cosmetics by enhancing by enhancing spreadability and consistency of creams/lotions – through the use of different types of waxes that can offer a plethora of textural properties, thereby making it possible to create cosmetic formulations with preferred sensory properties and enhancing surface qualities in coatings, by impacting properties such as anti-blocking, slip control, water repellency, scratch resistance etc. For example. Some natural waxes (such as paraffin waxes) perform well on porous surfaces by releasing oils that absorb into the material’s pores, introducing hydrophobicity and thereby making the surface water-repellent (AZOM, 2019). Wax emulsions are also used for insulating fiberglass and ceramics to enhance the overall feature and robustness of the products, by providing properties such as moisture resistance, optimised binding, forming barriers, collectively, these can minimise heat transfer and enhance effective insulation, therefore improving overall thermal behaviour of fibreglass and ceramics (PMC Crystal, 2017). However, to achieve these desired effects, the structural properties of the emulsions must be adapted to induce them. Thus, it is important to have (a) a comprehensive understanding of the interplay between the continuous and dispersed phases when formulating emulsions to ensure a stable emulsion with the desired emulsion structure is formed and (b) gain insight into the intricate relationship between the industrial applications of wax emulsions and the structural properties that would have to be induced to enhance product quality and longevity. The main aim of the research carried out in this chapter was to gain a better understanding of the structural behaviour of wax-in-water emulsions (where fatty alcohol – **Stearyl Alcohol** served as the wax in the emulsion system) and the interplay between the dispersed phase (Stearyl Alcohol) and the continuous phase (**Tween 20** and water), particularly how the volumetric ratio of the surfactant would influence the short-term stability of the emulsion. Other parameters considered during the research include investigating the influence of surfactant concentration on the long-term stability of the emulsion as well as the influence of the dispersed phase concentration on the emulsion and how the particle size and rheology were affected by these varying factors.

Research into literature shows a limited number of findings on the viscoelastic behaviour of wax-in-water emulsions, while, conversely, there is a wealth of research on the complex rheology of oil-in-water emulsions. Due to the limited information available in this research area, it was deemed important to carry out some experiments, document the findings, and present the results as a way of contributing to the subject topic. The primary difference between oil-in-water and wax-in-water emulsions is post-preparation (after cooling), during storage and in their applications. Wax serves as the dispersed phase in wax-in-water emulsions, while oil droplets are dispersed in the aqueous medium in oil-in-water emulsions. The preparation of wax-in-water emulsions is typically carried out using a melt emulsification process where the formulation is prepared at a temperature above the melting point of the dispersed phase resulting in temporary oily droplets that transition into hardened wax upon cooling. While oil-in-water are prepared by slowly combining the different phases that have been mixed and (sometimes) heated separately, after combining, the different phases are then mixed continuously dispersing fine droplets of oil in water to achieve desired droplet size and enhance emulsion stability. The preparation method and temperature chosen for the emulsion is dependent on the desired application for the emulsion being formulated (O'Sullivan et al, 2015).

Giermanska et al, (2007), investigated the gelling process that can occur in wax-in-water emulsions when sheared & cooled, and how the resulting emulsion can be influenced in terms of droplet size, morphology, and rheology. This is especially probable where the dispersed phase is crystallisable oil/wax with its melting temperature above room temperature. The research also found that upon cooling, the initially spherical molten droplets formed during formulation undergo crystallisation which leads to irregular surfaces being formed and therefore, partial coalescence due to a jamming that occurs during the cooling transition. It is postulated that coalescence and partial coalescence can occur due to a process called bridging, where nearby droplets interact with each other and eventually form a rigid network, and the latter occurs when the film separating droplets potentially breaks due to jamming (Giermanska et al, 2007).

During the research reported in this thesis, peculiar rheological changes were observed upon storage, where formulations with higher surfactant concentrations showed an increase in viscosity over time, whereas those with low surfactant concentrations showed a loss in viscosity. This suggests a structural breakdown vs buildup could be happening in the emulsion system, which is influenced by the surfactant concentration used in the formulation. However, there is very little research on the rheological behaviour of wax-in-water emulsions, particularly where Stearyl Alcohol and Tween 20 are used as a case study. One of the aims of this study was to gain a better understanding of the rheological behaviour of wax-in-water emulsions and how it changes with storage to allow for better prediction of rheological changes that could affect shelf-life and stability.

The core aims of this project include understanding the underlying mechanisms behind:

- Full coalescence of large droplets due to not selecting the appropriate surfactant or wax.
- Partial coalescence resulting in the formation of irregular shaped droplets
- The formation of flocculates and aggregates in the emulsion

Essentially, the aim is to gain insight into the mechanisms that govern wax-in-water emulsion formulations and their behaviour. According to Giermanska et al. (2007), factors such as dispersed phase concentration, continuous phase concentration, cooling rate and emulsion preparation affect the resulting emulsion. In addition to this, colloidal properties such as particle size, rheology and morphology need to be fully understood to avoid trial-and-error methods but rather develop a systematic process that is cost-effective and designed to adapt products to meet exact application requirements.

5.1 Composition Case study

Before delving in, to look in-depth at the results and analyses of the different compositions and concentrations that were explored when studying how the viscosity of the wax-in-water emulsions changed with time. For the sake of simplicity and setting a tone for the trends observed, it is important to give a background example of the structural changes assumed to be going on in the emulsion system by looking at the composition containing 5% Stearyl Alcohol and 5% Tween 20. This sample composition was chosen as it appeared to have some changes in trend during storage that differed completely from the other compositions that were looked at. When this composition was doubled (whilst keeping it a 1:1 ratio), the same behaviour was seen.

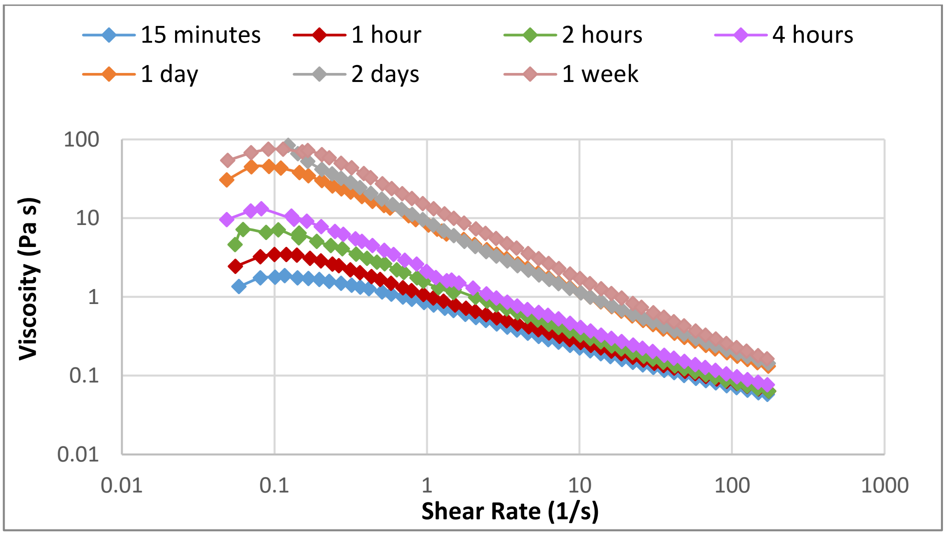


Figure 5.1: Change in Viscosity with Storage Time (*Steady Shear Time Comparison*) for 5% Stearyl Alcohol, 5% Tween 20

The emulsion containing 5% Stearyl alcohol and 5% Tween 20, generally showed a steady increase in viscosity over time. In the first 4 hours post-emulsion preparation, the increase in viscosity can be seen but appears to be a steady increase in viscosity, however, between 4 and 24 hours, a more obvious jump in viscosity is seen, which could be due to difference in the rate of flocculation/ coalescence of the droplets obtained, following this, the increase in viscosity appears to be a steady one as was observed in the first 4 hours. The particle size distribution also showed a shift to the right over the course of a week, suggesting an increase in particle size. Further along in this chapter, the increase in viscosity seen over time in some of the compositions has been attributed to a structural build-up of the droplet viscoelastic network or as a result of droplet aggregation occurring from the Brownian motion at play at low shear rates in the emulsion. It Is theorised that the structural build-up of the droplet network observed could be due to a change in micellar shape/structure from one form of micelle to tangled worm-like micelles. Research conducted by Yang, (2002), suggests that the transition from spherical micelles to worm-like micelles results in an increase in viscoelasticity and therefore an increase in emulsion viscosity. Micelles are typically formed when the surfactant in the system is sufficient and has covered the surface area of the dispersed phase droplets, the unused surfactant molecules between the droplets can form spherical micelles (when the CMC has been exceeded) due to the spontaneous curvature of surfactant molecules. Although the research mentioned above only discusses micellar shape transition for ionic surfactants, processing effects such as shearing and change in temperature can reduce the curvature of the spherical micelles and therefore result in the transition of micelles from spherical to other micellar shapes such as hexagonal micelles, lamellar structures, and worm-like micelles.

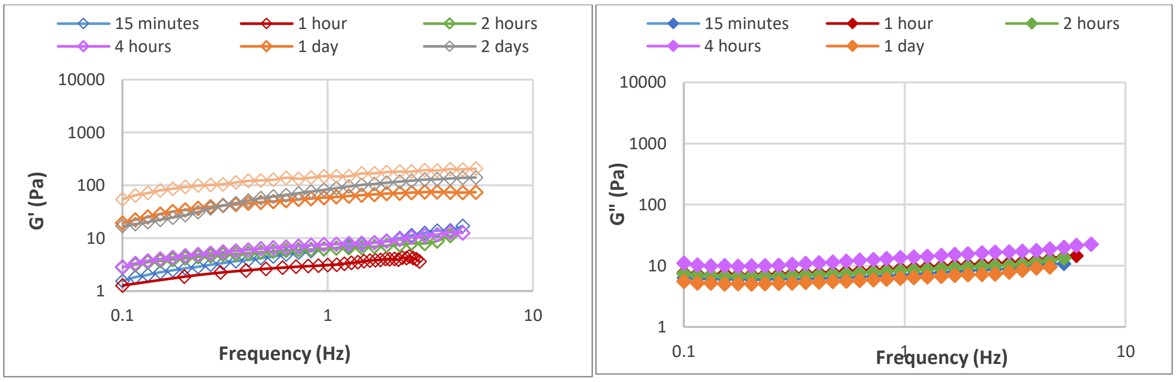


Figure 5.2: Change in Modulus (G' – Storage Modulus (Left) & G" – Loss Modulus (Right)) with Storage Time (*Oscillatory Time Comparison*) for 5% Stearyl Alcohol, 5% Tween 20

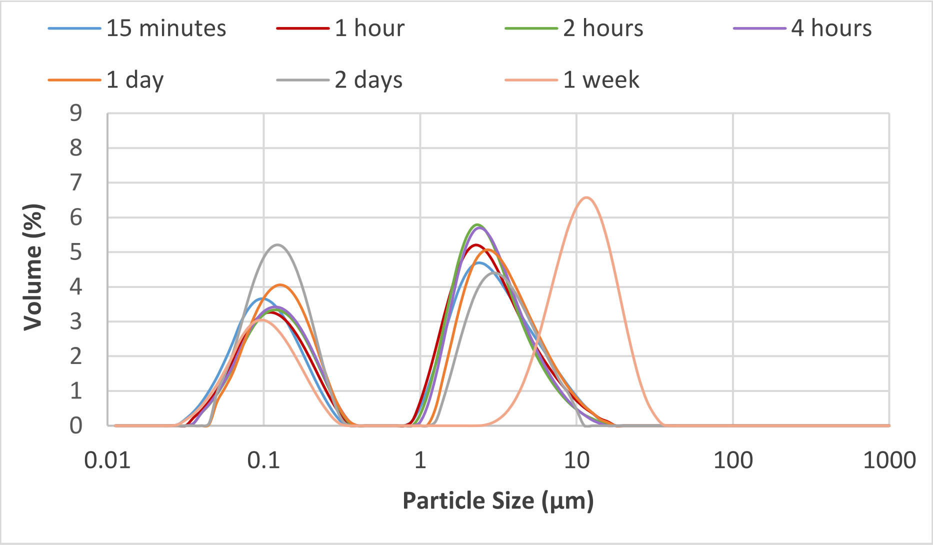


Figure 5.3: Particle Size Distribution for 5% Stearyl Alcohol, 5% Tween 20

Much like the behaviour seen in rheology above, the composition containing 5% Stearyl Alcohol, 5% Tween 20 showed an increase in particle size during storage. An initial bimodal peak was observed on the PSD, this could be a result of droplet aggregation or the presence of different particle types within the emulsion as this experiment was repeated and the same peaks were observed. This is indicated by the slow shift of the bimodal peaks from left to right after 4 hours and a strong appearance of a strong single peak at 1 week, suggesting particle size growth. This could potentially agree with the hypothesis suggesting the formation of entangled worm-like micelles above, (*Note: A worm-like micelle can go up to 10µm in length* (Dreis, 2017)). However in order to confirm the presence of worm-like micelles or spherical micelles, the use of Small Angle X-ray Scattering (SAXS)/ Wide Angle X-ray Scattering (WAXS) or Dynamic Light Scattering (DLS) would have to be employed.

It is also worth considering that the increase in particle size could be simply due to instability scenarios that can happen to emulsion systems upon storage and/or during ageing (Discussed below).

5.2 Steady Shear Rheology

The initial series of experiments carried out were aimed at understanding how the different wax: surfactant ratios vary with increasing shear and over time. Emulsions were prepared well above the melting temperature of the stearyl alcohol at approximately 80°C, to ensure the wax remained molten during the experiment.

As seen in the figures below, the dispersions are largely shear thinning within the first few days and become Newtonian after ~2 days, this is especially the case in compositions where there are lower surfactant concentrations. The length of time for which the shear thinning behaviour is seen appears to be dependent on the amount of surfactant in the system, emulsions with higher amounts of Tween 20 exhibited shear thinning for longer while on the other hand, emulsions with lower amounts of surfactants showed shear thinning behaviour for the first 48hours, followed by a sharp drop in viscosity and exhibiting Newtonian behaviour. Figure 5.4 below makes for an ideal example where a strong shear thinning pattern is seen initially within the first 4 hours, after which the viscosity appears to be independent of shear for the next 5 days, with no obvious change in viscosity.

Emulsions containing 5% of Stearyl alcohol and the lowest concentrations of Tween 20 (0.5% and 1.5%) show an initial increase in viscosity, however, after 4 hours, this increase was followed by a sharp drop in viscosity. Further, it was observed that with an increase in surfactant concentration, the preliminary increase in viscosity lasted longer and overall viscosity over the first 24 hours was higher.

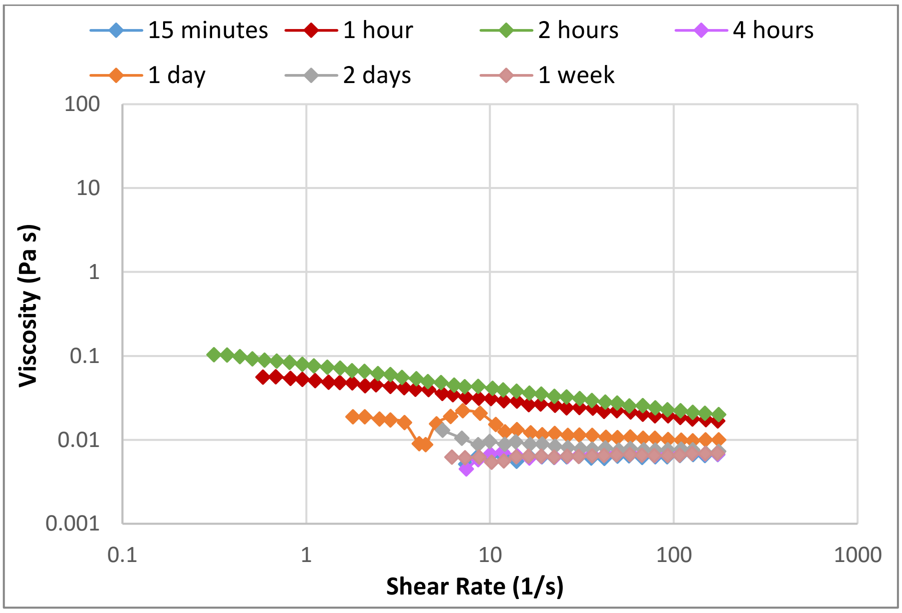


Figure 5.4: Change in Viscosity with Storage Time for 5% SA,0.5% Tween 20 Emulsion

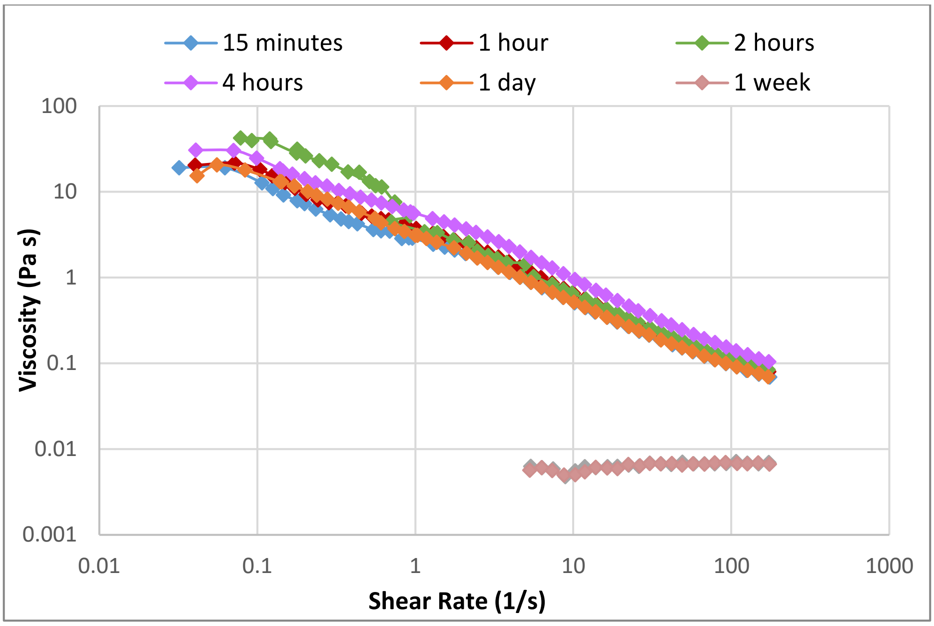


Figure 5.5: Change in Viscosity with Storage Time for 5% SA,1.5% Tween 20 Emulsion

Looking at figures 5.6 and 5.7 below, similar viscosity loss behaviour (as in emulsions containing 5% SA, 0.5% Tween 20 and emulsion containing 5% SA,1.5 Tween 20) is seen, however, it is worth noting that in the first 4 hours post emulsion preparation, an obvious fluctuation in viscosity is seen, which differs slightly from the aforementioned.

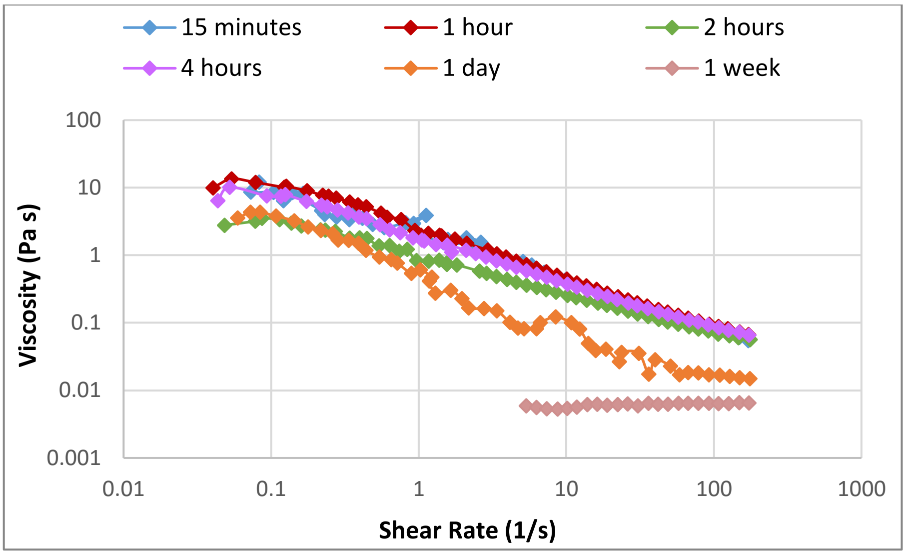


Figure 5.6: Change in Viscosity with Storage Time for 10% SA,1% Tween 20 Emulsion

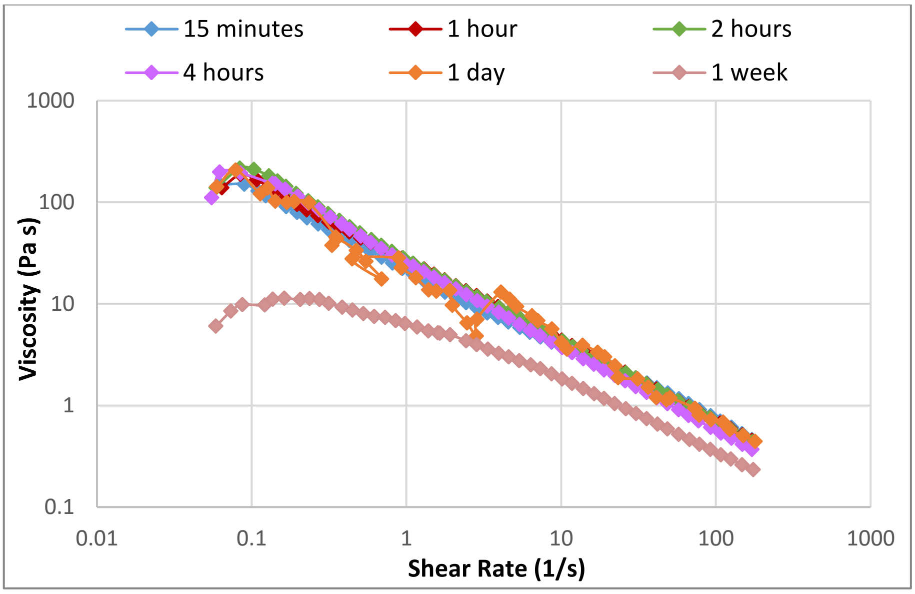


Figure 5.7: Change in Viscosity with Storage Time for 10% SA,3% Tween 20 Emulsion

On the other hand, emulsions with the 1:1 ratio (5% SA: 5% Tween 20 & 10% SA: 10% Tween 20 – In appendix) did not lose viscosity over the course of 1 week, instead, viscosity appeared to increase over time and upon storage.

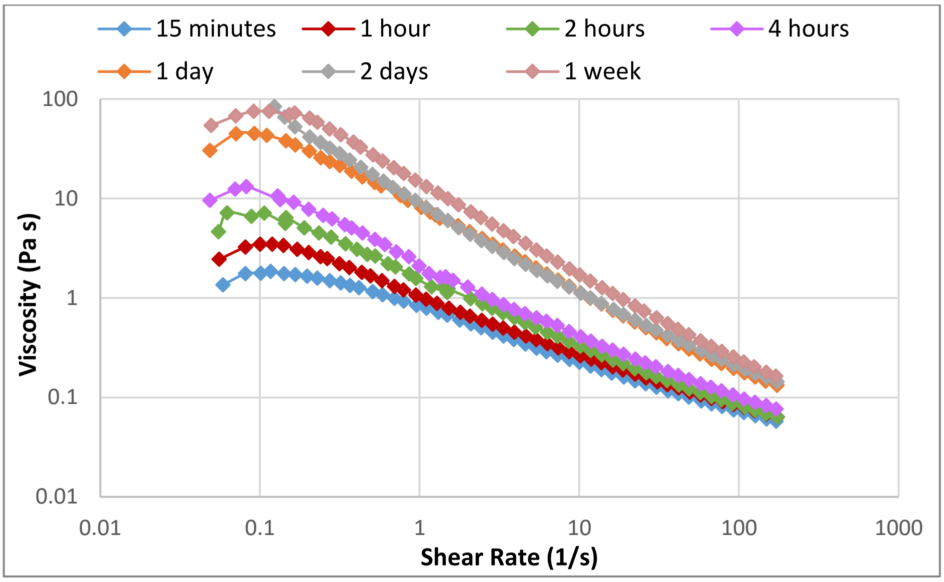


Figure 5.8: Change in Viscosity with Storage Time for 5% SA,5% Tween 20 Emulsion

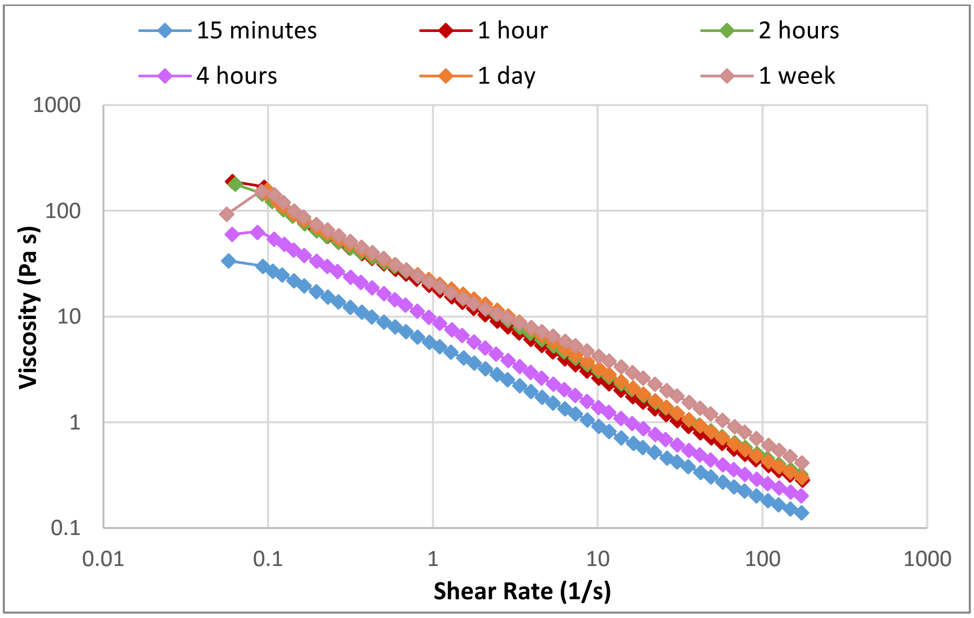


Figure 5.9: Change in Viscosity with Storage Time for 10% SA,10% Tween 20 Emulsion

The simultaneous reduced viscosity and increased viscosity behaviour observed overall suggests that there could potentially be two competing mechanisms at play in these systems; one mechanism involves the build-up of the droplet network, while the other involves the breakdown of the droplet network. Where there is a higher concentration of surfactant, the former is seen and where there is a lower concentration of surfactant, a breakdown of the droplet network is seen.

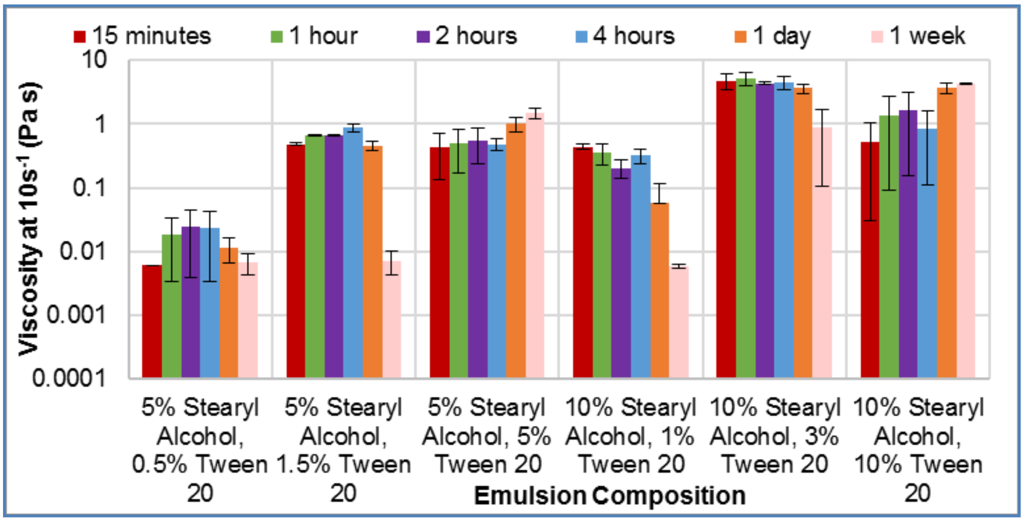
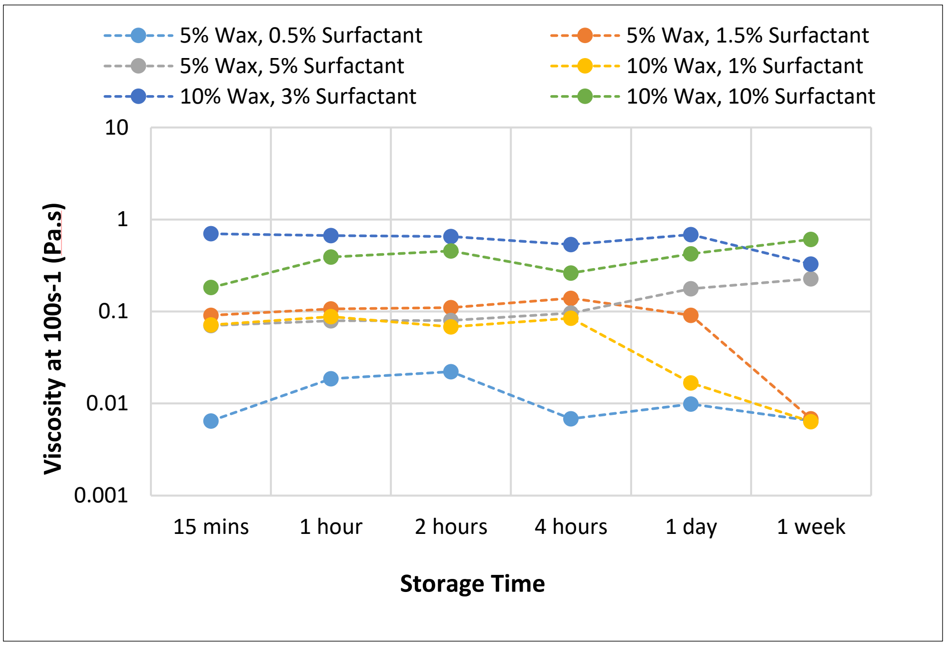


Figure 5.10: Graph showing change in instantaneous viscosity over time (Error Bars Show Standard Deviation from Mean of 2 Emulsions)

Figure 5.10 above highlights the instantaneous viscosity and key trends at a shear rate of 10 s^{-1} for each system at each measurement time. This shear rate was chosen because by analysing viscosity at shear rate 10 s^{-1} across different emulsion compositions, it is easier to home in on how variations in composition affect the flow behavior and rheological behaviour of the emulsions.

According to Pal, (1996), it is suggested that the more polydisperse an emulsion system is, the lower the viscosity would be for said emulsion. In general, previous studies hypothesise that droplet size, surfactant content and dispersed phase content have a strong influence on the viscosity scaling of an emulsion. In addition to these factors, some other previous work in literature also takes into account micellar depletion and creaming as factors that influence the rheological behaviour of certain emulsions (Jansen et al., 2001).

Across board, rheology results obtained for the compositions above are not in complete agreement with what is suggested in literature. Pal, (1996) found that in oil-in-water emulsions, there was a rapid decrease in viscosity over the course of 10 days, accompanied by a reduction in the shear-thinning effect observed. Although in some of the compositions above, a drop in viscosity is seen upon storage of the emulsion after a few days, and the strong shear thinning effect seen initially drops off to become almost Newtonian behaviour, this was not seen for every single composition. Emulsion systems with higher Tween 20 content showed an increase in viscosity over time and exhibited strong shear thinning properties over the course of a week.



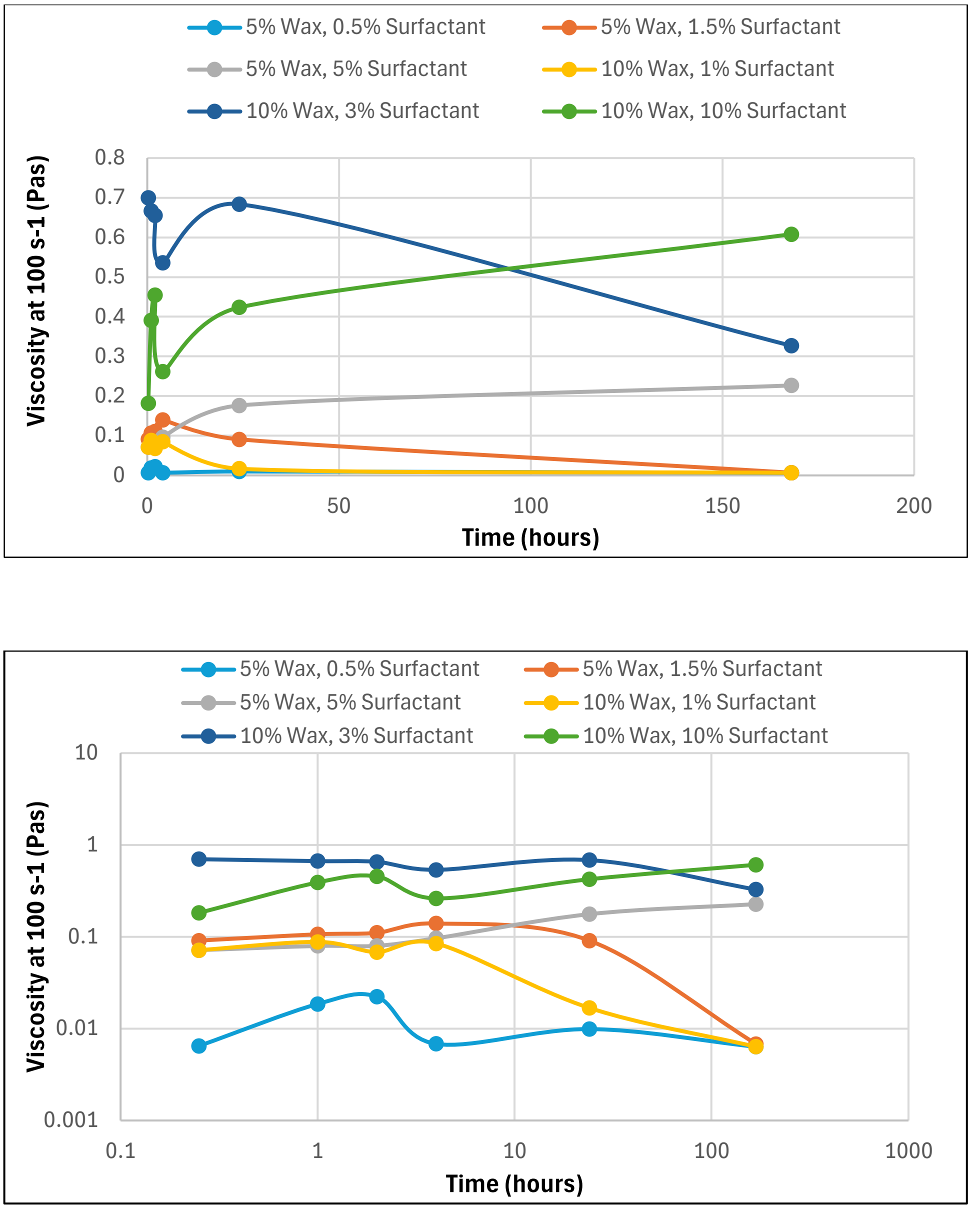


Figure 5.11: Combined graph showing viscosity at 100s^{-1} for the different compositions over time. *Different axes scales have been used in each plot to give better clarity.*

A note-worthy phenomenon that plays a role in the viscosity change seen in emulsions is Brownian force, (Shikata and Pearson, 1994). The shear-thinning effect seen in formulations above is as a result of an interplay between Brownian and hydrodynamic forces. Where Brownian force is in place, it causes random movements of the droplets within an emulsion system thereby making them susceptible to collision, these randomised collisions then lead to the formation of aggregates, flocs, coalesced particles etc. Conversely, hydrodynamic forces enable the arrangement of droplets/particles into layers, promoting droplet breakup and disorder of the flocs, and aggregates that might have been formed as a result of Brownian motion. At low shear rates, the Brownian force takes over and causes rotation of the flocs and consequently, the formation of aggregates, which results in increased viscosity and a higher dissipation of energy. However, as the shear rate increases, droplet breakup would occur and therefore there would be a reduced number of aggregates/flocs in the emulsion, thereby reducing the viscosity of the emulsion system (Pal, 1996).

On the contrary, Jansen et al., 2001 proposed a new scaling parameter for the viscosity of surfactant-stabilised emulsions, they suggested that the attraction between emulsion droplets comes about as a result of the small spherical surfactant micelles present in the continuous phase of an emulsion system. Micelles have been discussed extensively in the previous chapter; they are formed when the concentration of the surfactant in dispersion surpasses the critical micelle concentration (CMC). Aronson, (1989) postulated that unstable scenarios that are experienced in emulsion formulation such as flocculation, creaming, aggregation etc. arise due to the depletion of micelles in the gap between droplets. However, based on results obtained from experiments carried out, it is theorised that the spherical micelle between droplet dynamics is not the structure at play in this study.

This is because, spherical micelles give rise to Newtonian behaviour and a viscosity similar to that of water, which does not correlate with what has been observed so far. However, other micellar structures such as cylindrical and lamellar micelles have a greater viscosity that is shear-dependent. Therefore, a fitting hypothesis would be that a micellar shape change is occurring within the emulsion system due to some processing factors such as temperature, surfactant concentration etc.

Although past its CMC, Tween 20 tends to support the formation of spherical micelles, the critical packing parameter of Tween 20 could be altered during preparation giving rise to alternate micellar structures (Cardiel J et al, 2015). However, as its preferred structural orientation is the spherical micelle, upon storage, there could potentially be structural breakdown, detangling and relaxation, which would account for the transition from shear thinning to Newtonian behaviour.

Plotting viscosity at 10s^{-1} (Pa.s) vs storage time, compositions containing 5% Stearyl Alcohol and 10% Stearyl Alcohol are shown separately in the plots below.

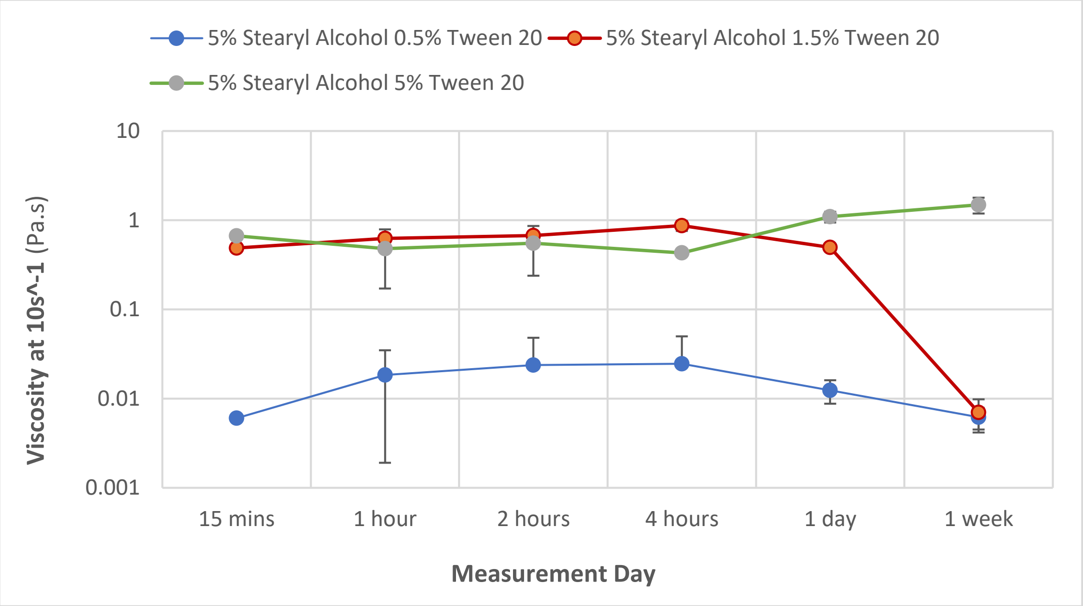


Figure 5.12: Plot showing viscosity at 10s^{-1} for emulsions containing 5% Stearyl Alcohol – with error bars

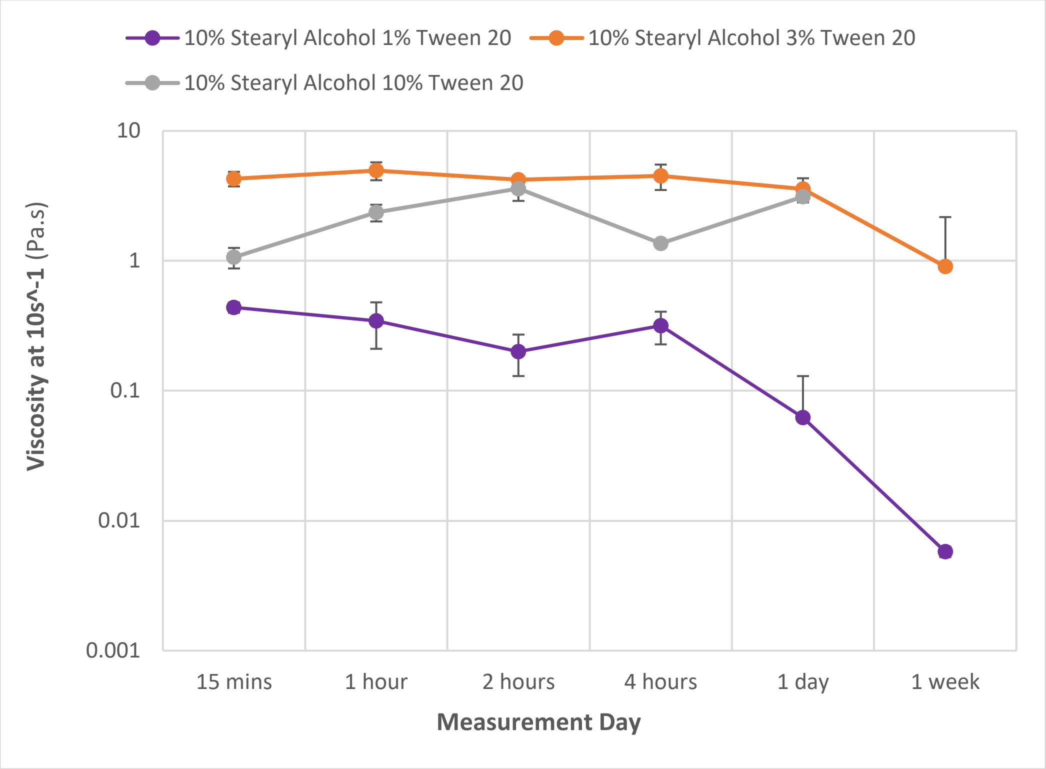


Figure 5.13: Plot showing viscosity at $10s^{-1}$ for emulsions containing 10% Stearyl Alcohol - with error bars.

A possible structural breakdown is seen after 4 hours across all compositions except where there is a 1:1 ratio of Stearyl Alcohol: Tween 20, which suggests a possible structural build-up. Judging by the physical appearance of 5%wax, 5% surfactant & 10% wax, 10% surfactant, these emulsions appeared to thicken over time, which would agree with the suggested structural build-up seen in the plots below. The emulsion preparation and measurements were repeated to ensure repeatability and reproducibility. The error bars in Figures 5.12 and 5.13 represent the standard deviation of three measurements. Generally, errors observed in these plots are minimal, with slightly larger errors observed in both plots. These larger errors may be attributed to calibration errors from the rheometer or the presence of different particle sizes and structural networks within the emulsion, rather than a lack of reliability and validity in the obtained results. Further repeats of all the experiments would need to be carried out to test the reliability of the results. In addition, efforts should be made to achieve homogeneity of the particle size in the emulsion.

5.3 Oscillatory Rheology

Even though the emulsion system being studied appears to be viscous, it is generally known that viscous materials can also show elastic behaviour. Therefore, as well as the steady shear tests conducted, oscillatory rheology tests were also carried out to examine changes occurring within the elastic modulus(G’) and the viscous modulus (G”), albeit being mindful not to damage the emulsion structure. These tests were conducted within the linear viscoelastic region (LVR) to investigate the stored energy in the elastic structure of the emulsion that can help estimate how much-said material can deform elastically when a certain amount of stress is applied as well as the *degree of viscous response of the material.*

Please Note: Results taken at high frequencies are not included as they were inaccurate due to inertia effects.

In agreement with the previously postulated theory of structural build-up and breakdown, the G’ and G” generally showed an increase and decrease with storage time, which could suggest that the initial increase in viscosity and decrease in viscosity over time is a viscoelastic process. Although not consistent, there is a trend of an initial increase in viscoelasticity with storage, which cannot be corroborated with what is suggested in the literature. However micellar structures such as wormlike micelles and other complex micelles can give rise to increased viscoelasticity in emulsion systems (Yang, 2002).

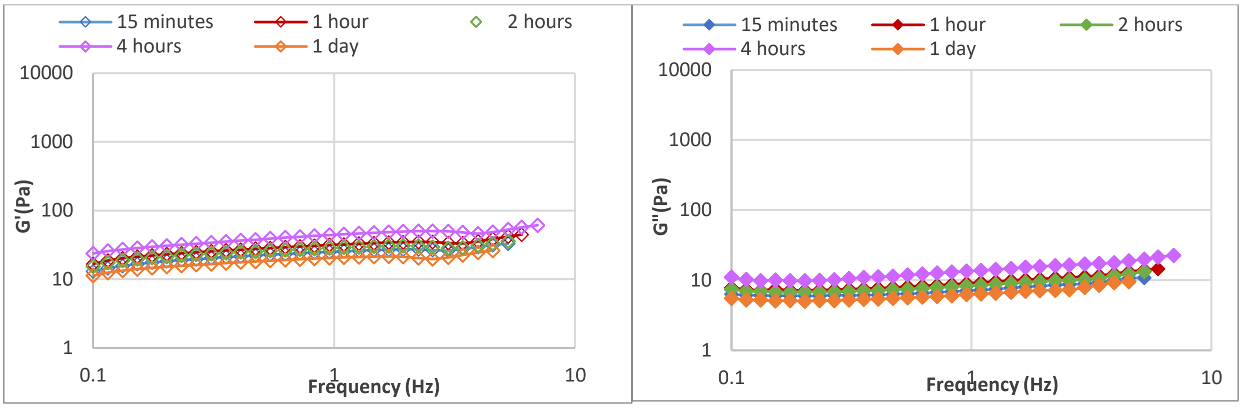


Figure 5.14: Change in Elastic & Viscous Modulus (G' & G") with storage time – 5%SA,1.5%Tween 20

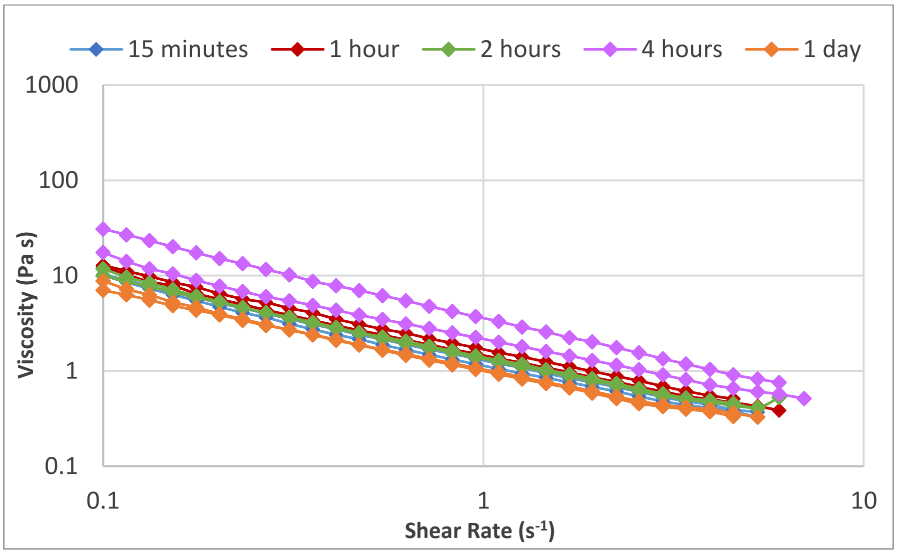


Figure 5.15: Change in viscosity with storage time – 5%SA,1.5%Tween 20

The phase angle (θ) helps predict where a material would fall within 0° and 90°, essentially whether a material behaves elastically like a solid (low phase angle) or flows like a liquid (high phase angle). It is defined by:

$$\text{Tan } \theta = \frac{G''}{G'}$$

Equation 5.1

When calculated, θ across board was always less than 45°, which suggests that the emulsions are more elastic than they are viscous at the frequencies indicated, which could be attributed to a structural build-up or a strong network of particles at the time of measurement, whose force is greater than that applied by the rheometer. In addition, for all emulsions, G' and G" appear to have a low dependency on the frequency which suggests gel-like properties in the emulsions.

In the emulsions containing low surfactant concentrations (5% Wax 1.5% Surfactant, 10% Wax 1% Surfactant and 10% Wax 3% Surfactant), viscosity appears to steadily increase within the first 4 hours after preparation, followed by a sharp decrease in viscosity after 24 hours. However, in the emulsion containing 10% Wax 3% Surfactant, a more continuous decrease in viscosity is seen during storage, this is consistent with what is generally documented in literature (Hemar et al., 2005), where a decrease in viscosity is recorded over time due to structural relaxation or structural breakdown. The compositions generally showed a higher storage modulus (G') in comparison to the loss modulus (G"), which is an indication of these emulsions being elastic and perhaps would be regarded as gel-like.

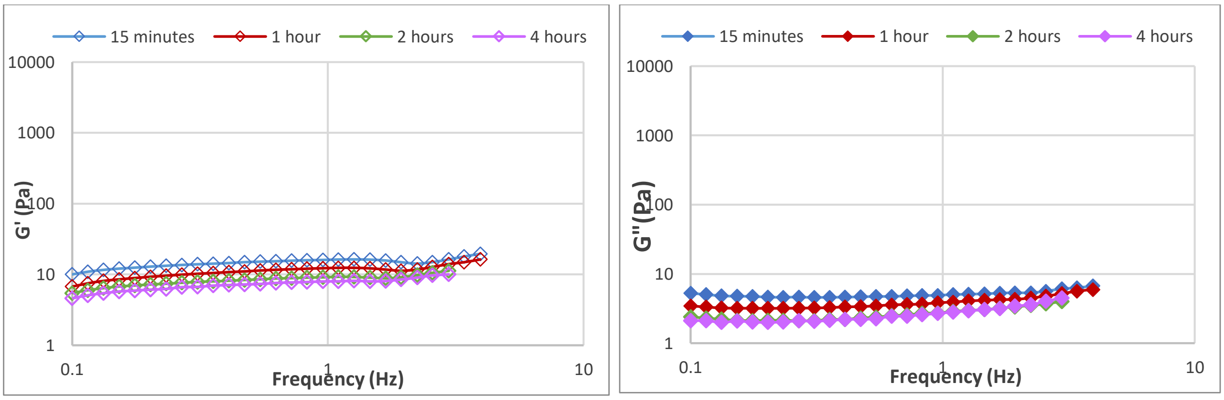


Figure 5.16: Change in Elastic & Viscous Modulus (G' & G'') with storage time: 10%SA,1%Tween 20

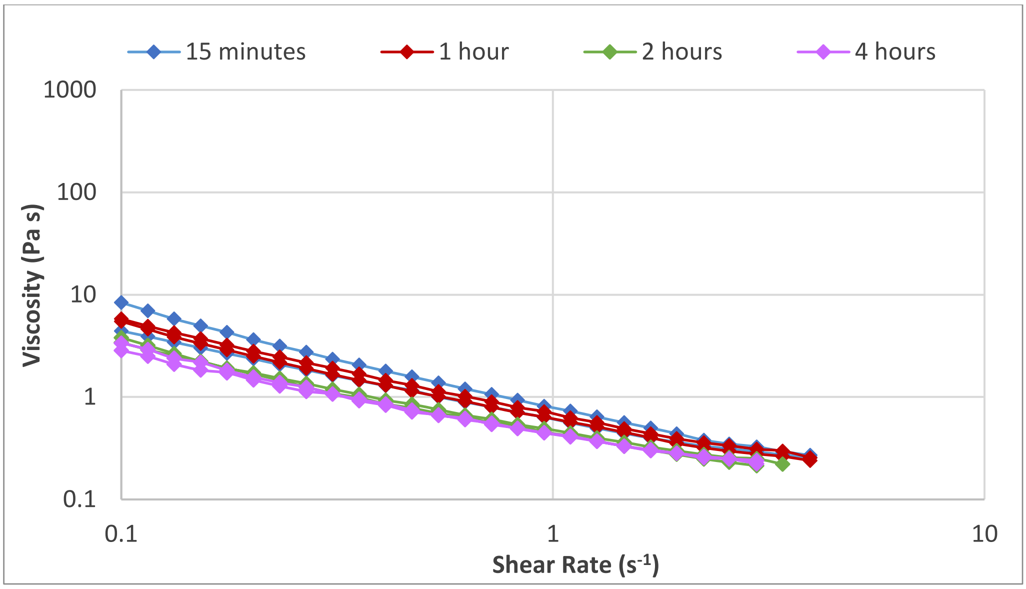


Figure 5.17: Change in viscosity with storage time – 10%SA,1%Tween 20

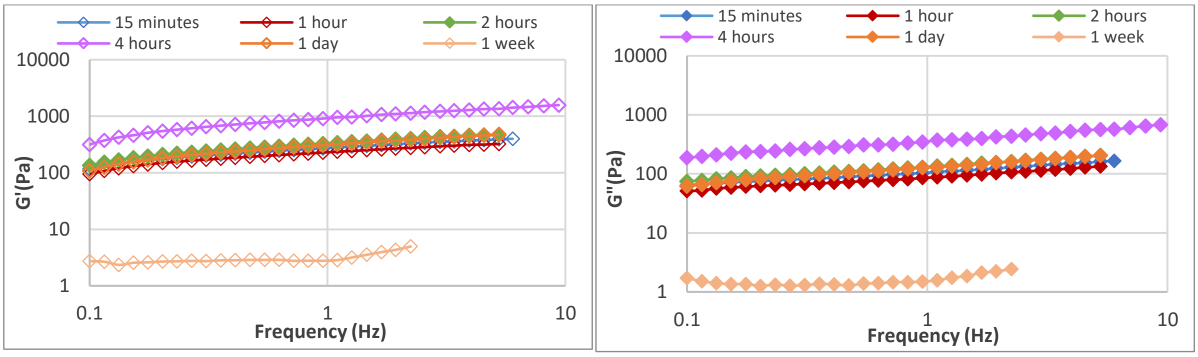


Figure 5.18: Change in Elastic & Viscous Modulus (G' & G'') with storage time: 10%SA,3%Tween 20

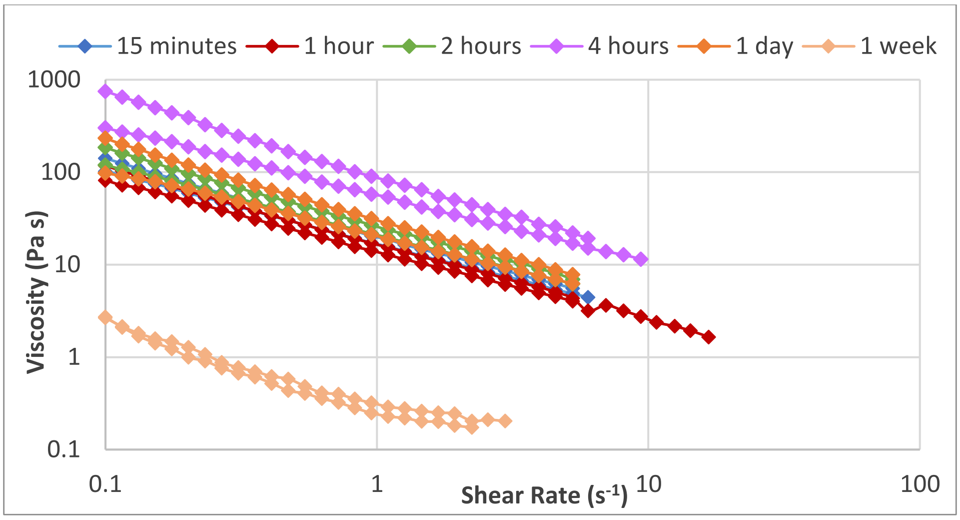


Figure 5.19: Change in viscosity with storage time – 10%SA,3%Tween 20

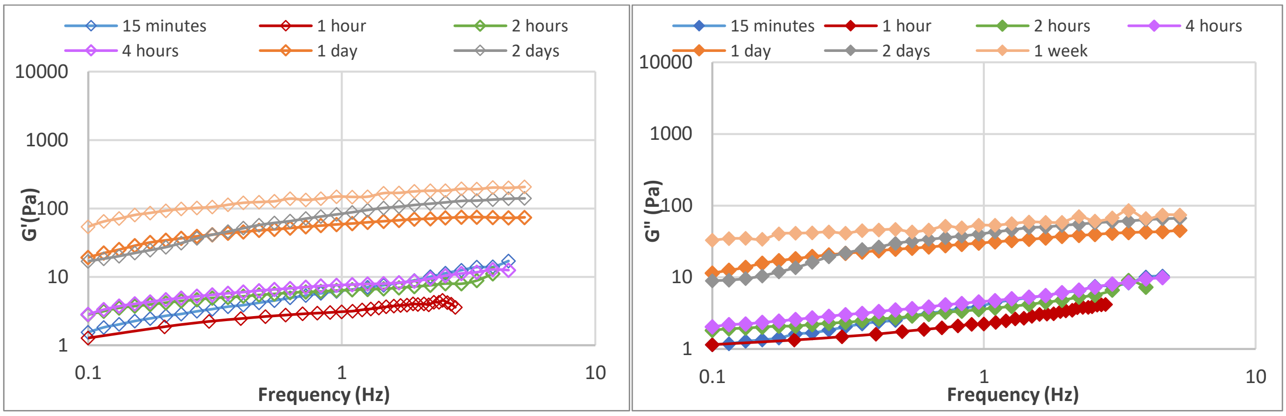


Figure 5.20: Change in Elastic & Viscous Modulus (G' & G'') with storage time – 5%SA,5%Tween 20

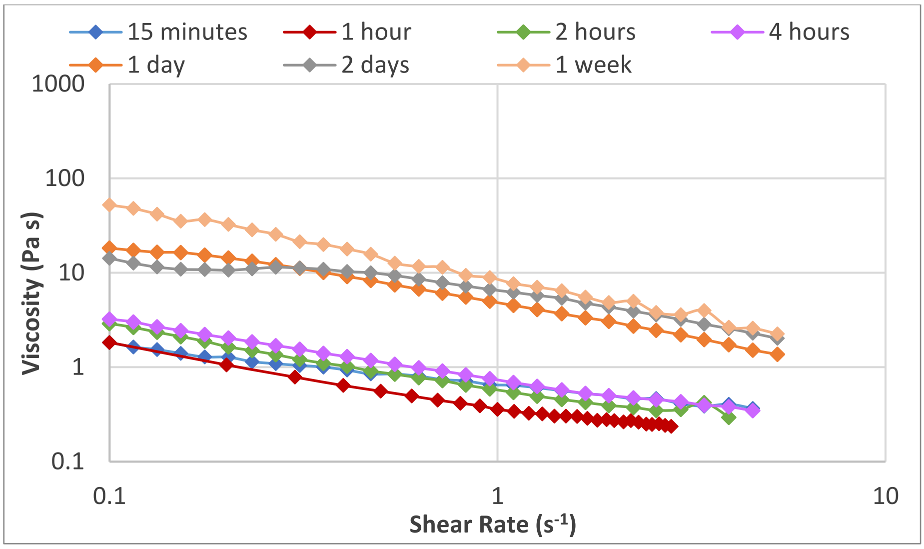


Figure 5.21: Change in viscosity with storage time – 5%SA,5%Tween 20

In emulsions containing high concentrations of surfactant, 5% Wax 5% Surfactant & 10% Wax 10% Surfactant, a steady increase in viscosity and moduli was observed. This could be due to droplet aggregation – where wax droplets aggregate to form large droplets resulting in a structural buildup within the emulsion and therefore changes to the rheological properties. Other possible scenarios that could have given rise to increased G’ and G” over time include phase separation or increased network build-up within the emulsion. The particle size distributions obtained for these compositions (discussed later) show an increase in particle size during storage, which is indicative of a structural buildup occurring during emulsion ageing.

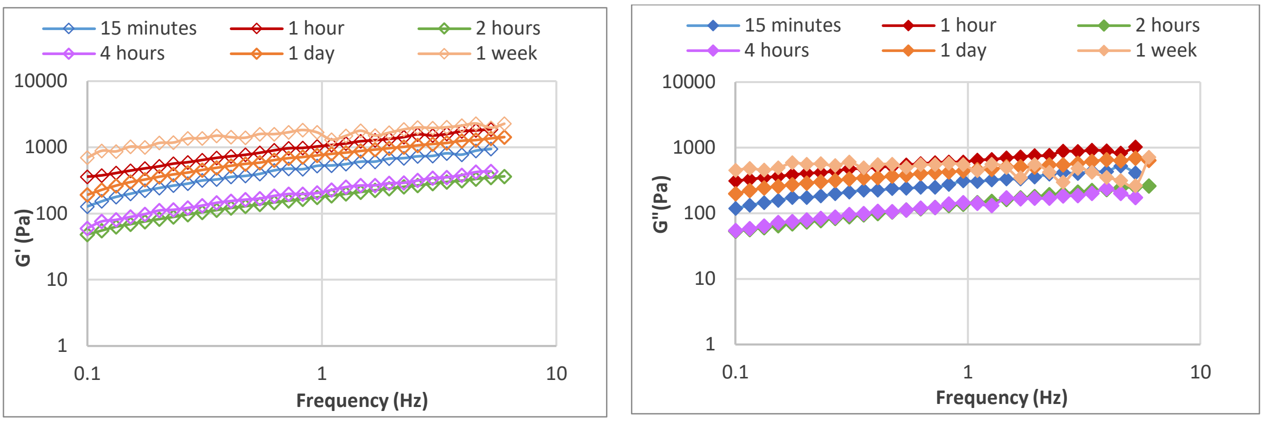


Figure 5.22: Change in Elastic & Viscous Modulus (G' & G'') with storage time – 10%SA,10%Tween 20

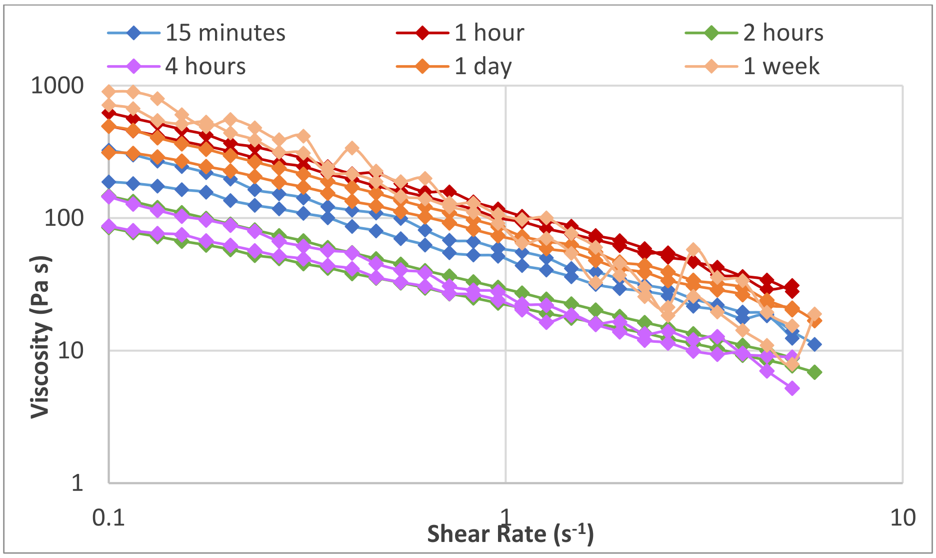


Figure 5.23: Change in viscosity with storage time – 10%SA,10%Tween 20

Although G' and G" do not appear to cross in any of the graphs, it is expected that once there is a change of structural arrangement due to ageing in the emulsions, the phase angle would be higher causing G' and G" to cross, however, real values could not be obtained using parallel plates on the rheometer as it flagged 'raw phase angle value' being too high. In the future, an alternative to parallel plates would be to use the

Couette geometry, this would allow obtaining a robust and bigger range of results at lower elasticities and lower shear rates.

A summary of trends seen in all emulsions is collated in the illustration below (figure 5.17), some measurements are not included as the phase angle was too large to be measured by rheometer, which therefore meant the data being obtained was unreliable and could not be used. With increasing elastic modulus, more stress would be required for the emulsion to flow, as seen in the graphs above, elastic modulus (G') is highest for emulsions that appeared to be most viscous, physically.

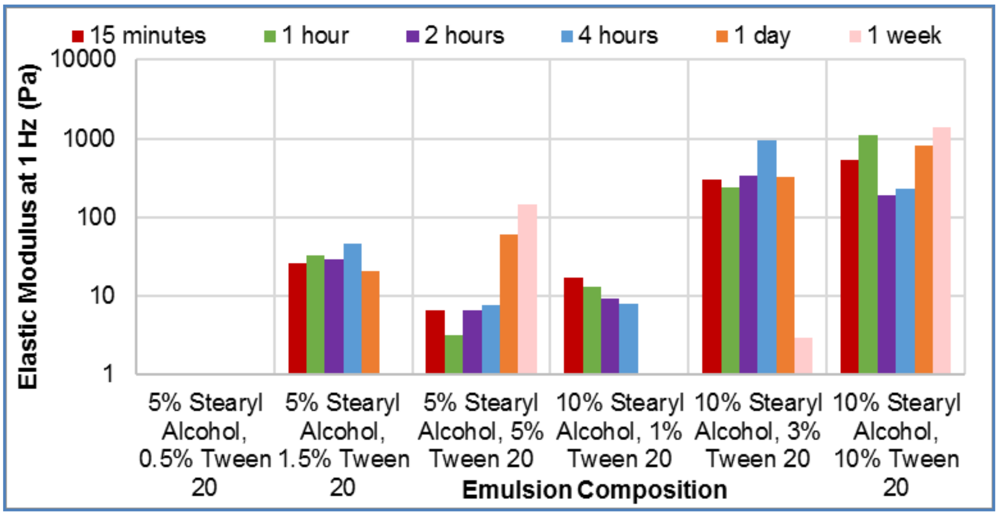


Figure 5.24: The graph above shows the change in elastic modulus with storage time (No error bars)

5.4 Particle Size Measurements

The particle size distributions (PSD) obtained across all the compositions were bimodal (i.e., showing two peaks), one peak was seen at around 0.3 µm and the second peak at approximately 5µm (See collated PSD below – Figure 5.25). Although the PSDs were generally bimodal, the compositions with 1:1 ratio (for example 5% Wax:5% Surfactant, as discussed above), revealed bimodal peaks in the first few days of the emulsion ageing and showed a distinct singular peak after of week of emulsion storage, suggesting a network build-up had occurred in that period.

In order to confirm the theory of the presence of micelles, particularly, the spherical micelles, the use of DLS would have to be employed, this is because micelles are usually seen in the submicron range, which cannot be scaled on a mastersizer's particle size measurement. Therefore, the peak seen at approximately 0.3µm is unlikely to be a micelle, as in theory, it would be deemed too large to be a micelle (10).

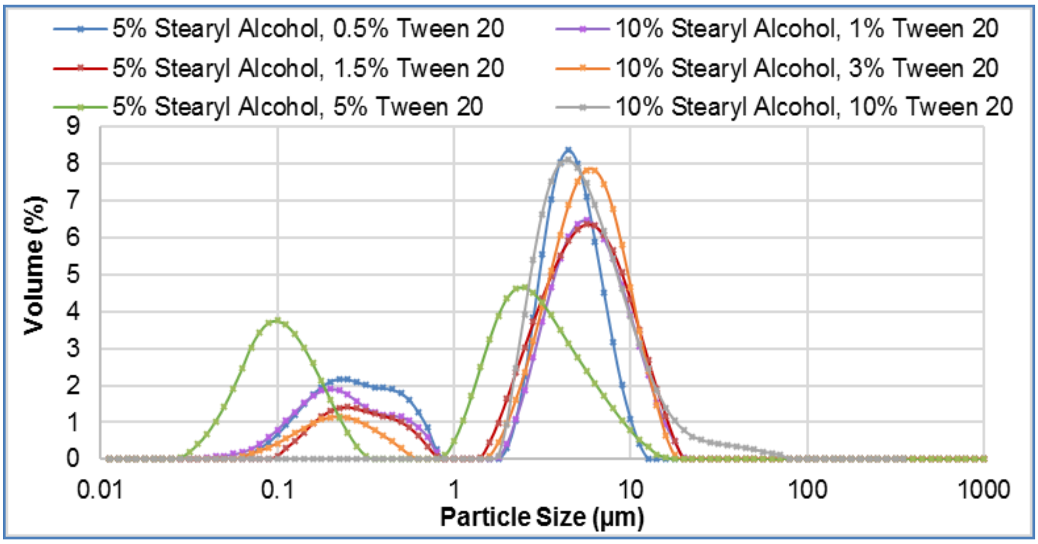


Figure 5.25: Particle Size Distribution for measurements taken 15 minutes after the emulsion had been cooled.

After one week of storage, the particle size of the emulsion is measured again and emulsions with less surfactant show distributions that go from bimodal to multimodal, a third peak is seen to have developed at 0.6 µm, between 0.3µm and 5µm, this could be owing to the fact that droplet networks that were formed post emulsion formulation and during storage (particularly within the first 4 hours) have started to break down, perhaps due to weak network bonding that can be easily broken with shearing during mastersizer measurement or flocs of droplets that can be broken by slight agitation of the sample bottles. This third peak appearance was only observed for emulsions with low amounts, meanwhile, emulsions with the 1:1 composition ratio showed a larger proportion of larger particles and no third peak after 1 week of storage.

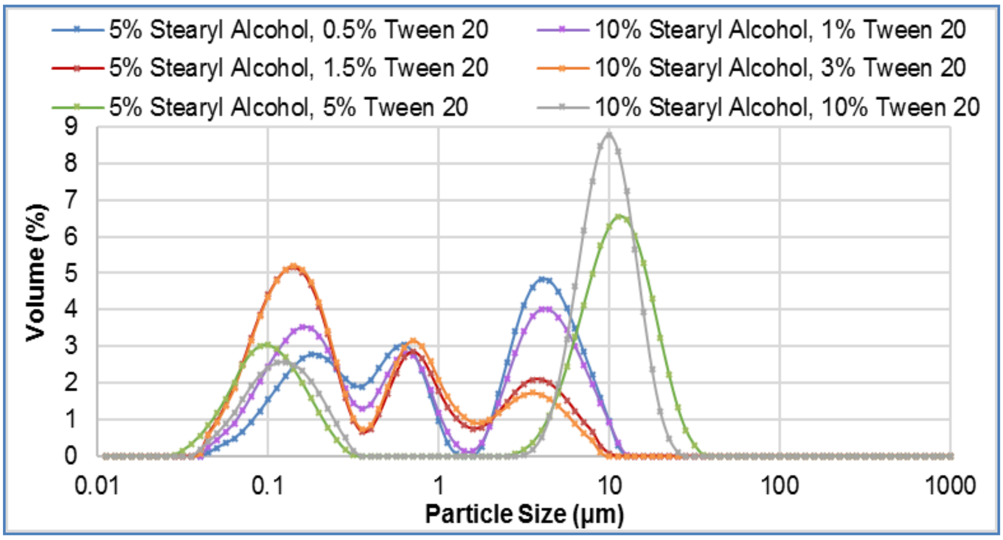


Figure 5.26: Particle Size Distribution for measurements taken after 1 week of storage.

Micrographs taken after 1 day of sample storage (Figures 5.27 - 5.32) revealed the presence of at least two distinct particle sizes, consistent with the bimodal peaks observed in the particle size distribution. These images not only provided a visual representation of the emulsion droplets but also served to validate the assumption that the smaller peak observed on the left side of the particle size distribution was not merely an artifact (e.g., bubbles in the Mastersizer measuring vessel).

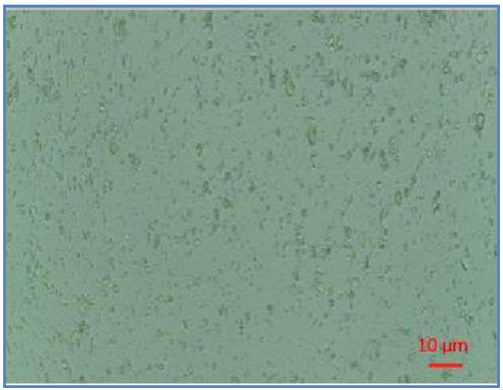


Figure 5.27: 5% Wax, 0.5% Tween 20

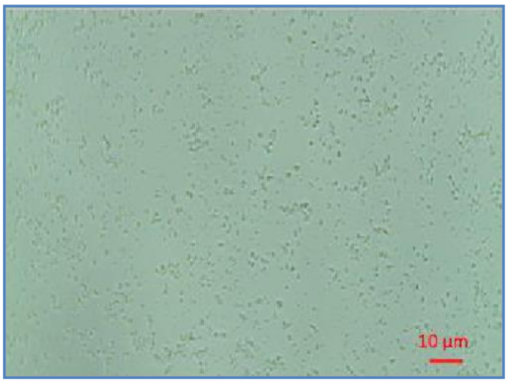


Figure 5.28: 10% Wax, 1% Tween 20

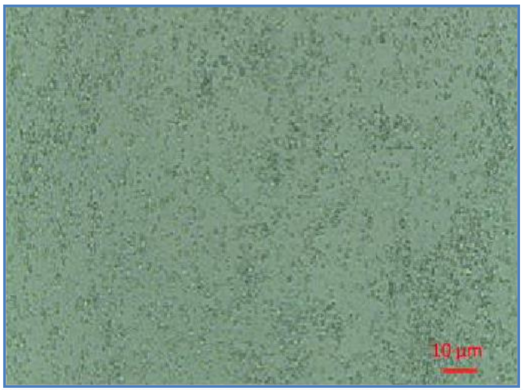


Figure 5.29: 5% Wax, 1.5% Tween 20

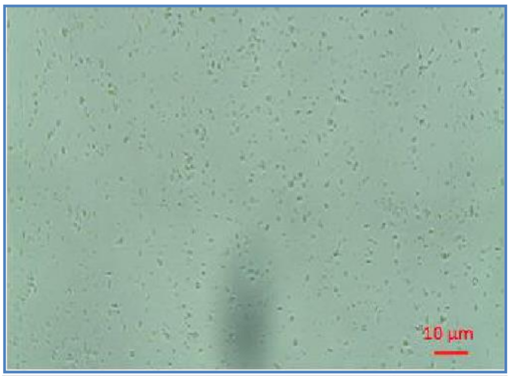


Figure 5.30: 10% Wax, 3% Tween20

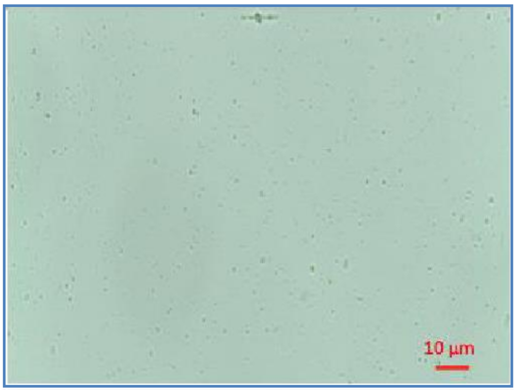


Figure 5.31: 5% Wax, 5% Tween 20

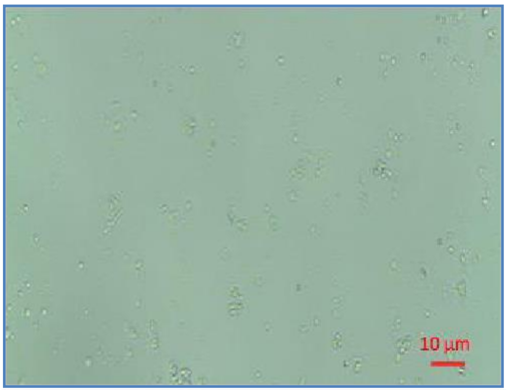


Figure 5.32: 10% Wax, 10%Tween 20

Droplets seen in the emulsions with lower surfactant concentrations appeared to be more elongated as opposed to those in the emulsions with higher surfactant concentrations. Further, there is a minimal number of flocs/ aggregates that can be seen across all compositions, which points in the direction of the theory suggesting a micellar network build-up and breakdown in the emulsion system being the driving force behind the increase and decrease in particle size and/or rheology that has been observed as opposed to it being due to the formation of flocculated and/or aggregated droplets.

In addition to the microscopic images, aliquots of the bulk emulsion sample were taken from different sections of the emulsion formulation vessel to establish whether the reason behind the bimodal/multimodal peaks that were observed are as a result of different islands of mixing being present in the bulk. A sample was taken from the top of the impeller and the other sample from the bulk and measured in the mastersizer, both showed multiple peaks in the PSD. This was therefore deemed not to be the reason behind the multimodality seen in the particle size measurements.

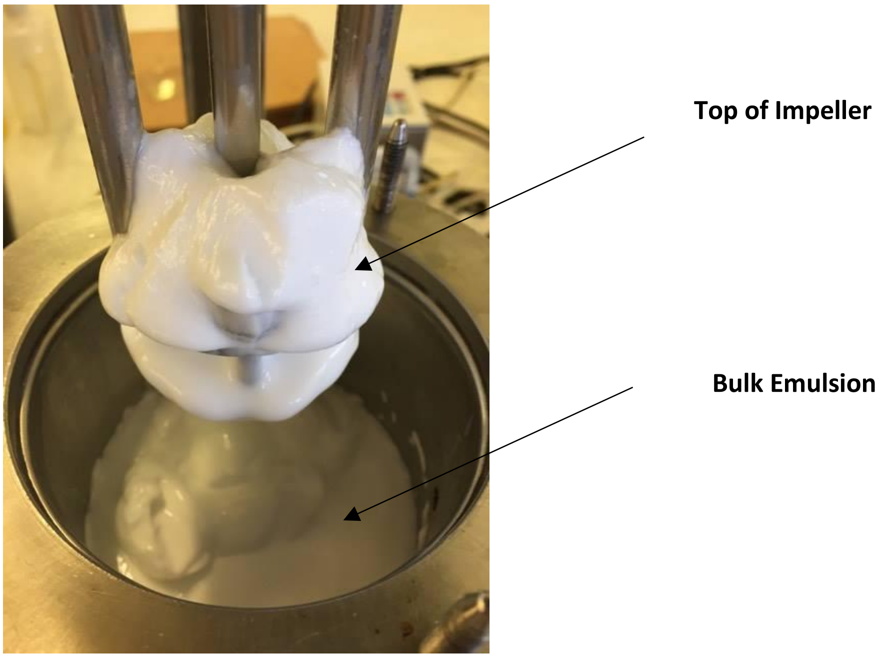


Figure 5.33: Image showing sections where aliquots were taken from to determine whether islands of mixing were the reason for polydisperity seen in the PSDs.

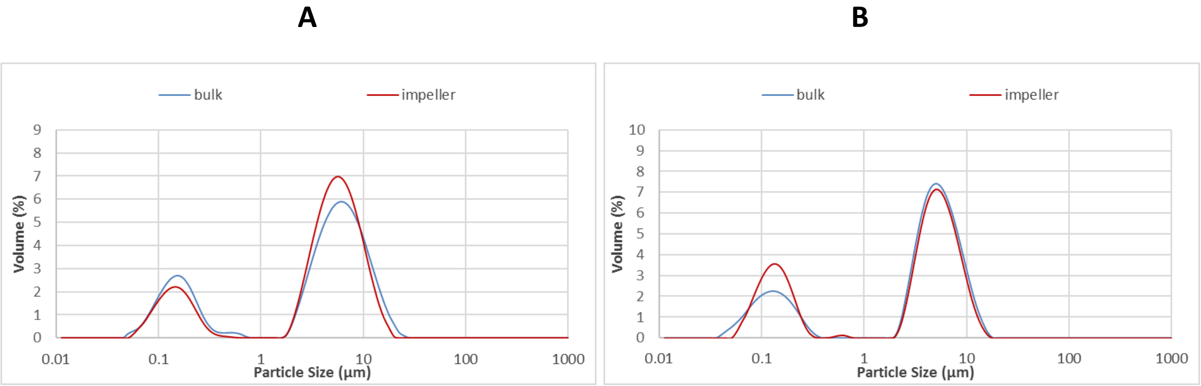


Figure 5.34: Bulk and Impeller Sampling particle size measurements after 24-hour storage

A: 10% Stearyl Alcohol, 3%Tween 20

B: 10% Stearyl Alcohol, 10% Tween 20

The rate of change seen in the Particle Size Distribution is very dependent on the quantity of the wax and surfactant used in the emulsion system, below is a histogram (fig 5.35) showing the changes in the Sauter Mean Diameters D[3,2] relative to the particle size after 15 minutes post preparation , to highlight the extent of changes occurring within each emulsion. As anticipated, the emulsions showed a greater D [3,2] value after 1 week of storage than after just 15 minutes of storage, this is expected as emulsions are prone to instability scenarios that can cause the emulsions to behave differently from the initial structure observed immediately after preparation. The emulsion containing 5% Stearyl Alcohol and 5% Tween 20 showed a peculiar increase in D [3,2] within the first 24 hours of storage and a drop in the Sauter mean diameter after 1 week, which could be a result of structural network building-up and breaking down respectively. This unusual change seen is unlikely to be an error in the measurement as it was repeated and going by the error bars obtained, the values obtained are reproducible. Conversely, emulsion containing 10% Stearyl Alcohol and 10% Tween 20 show significant error bars, this would have to be repeated multiple times in order to accurately theorise on what might be the reason behind the D [3,2] during storage.

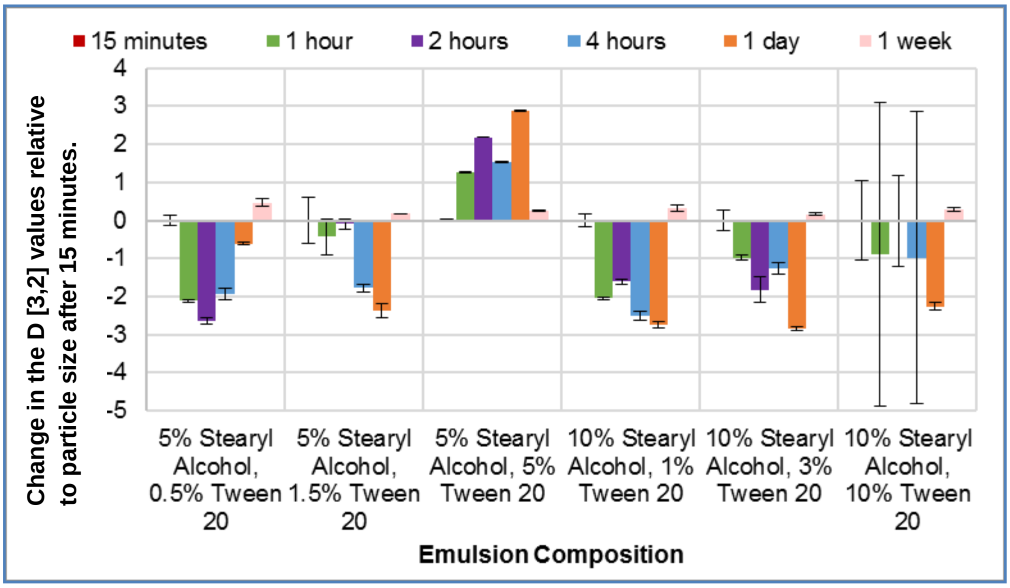


Figure 5.35: Change in the D [3,2] values relative to particle size after 15 minutes (*Error bars are based on the standard deviation mean of 2 formulations*)

It is not unusual to find that wax-in-water emulsions result in non-spherical (crystalline) droplets due to the crystallisation of wax during cooling. However, the mastersizer uses the laser diffraction technique to measure the particle size distribution of droplets, this means it uses the 'Mie theory', which assumes the volume of every material is equivalent to a sphere model. It is worth noting that the micrographs show droplets that are not perfect spheres; some looked elongated, and others appear to be irregular spheres. In order to obtain a more concise and accurate analysis of the particle size and shape, it would be beneficial to employ the use of an image analysis sensor such as 'QicPic' – this analyser considers every single particle/droplet and analyses based on its actual shape/size.

Ngee et al. (2009) found that samples with a low dispersed phase concentration and a low surfactant concentration showed a broader particle size distribution, which was true in this study for samples containing less than 3% Tween 20, they showed more polydisperity, with their particle size ranging from 0.1µm to approximately 20 µm. Conversely, samples containing a higher amount of Wax and Tween 20 showed a narrower PSD range and less polydisperity (e,g, sample containing 5% Stearyl Alcohol: 5% Tween 20). Rheology on the other hand is also influenced greatly by droplet size and polydisperity in the system. Ngee et al. (2009) also suggested that compositions with lower particle sizes and narrower PSDs resulted in a sample with increased viscosity, this again was true for emulsion containing 5% Stearyl Alcohol, 5% Tween 20. However, for emulsion containing 10% Stearyl Alcohol and 10% Tween 20, monodisperity was observed and in turn, increased viscosity but its particle sizes in the range of 50-80 µm, could not be described as 'low'. This composition could either be an anomaly that needs to be investigated extensively or perhaps low particle size and monodispersity are mutually exclusive and only either one is required in order to see viscosity changes. In this study, compositions that showed a drop in viscosity over time also showed an increase in polydisperity and according to Ngee et al. (2009), increased polydisperity allows for efficient packing, which consequently allows the particles/droplets to be more mobile and less resistant to flow (decreased viscosity), this could account for the viscosity changes seen during storage in the compositions containing a lower amount of surfactant.

5.5 Long Term Stability

The emulsions were stored at room temperature and tested after 12 weeks to test for phase separation and creaming. Samples were taken from the top, the middle and phase-separated layer of the emulsions and the particle size distribution was measured.

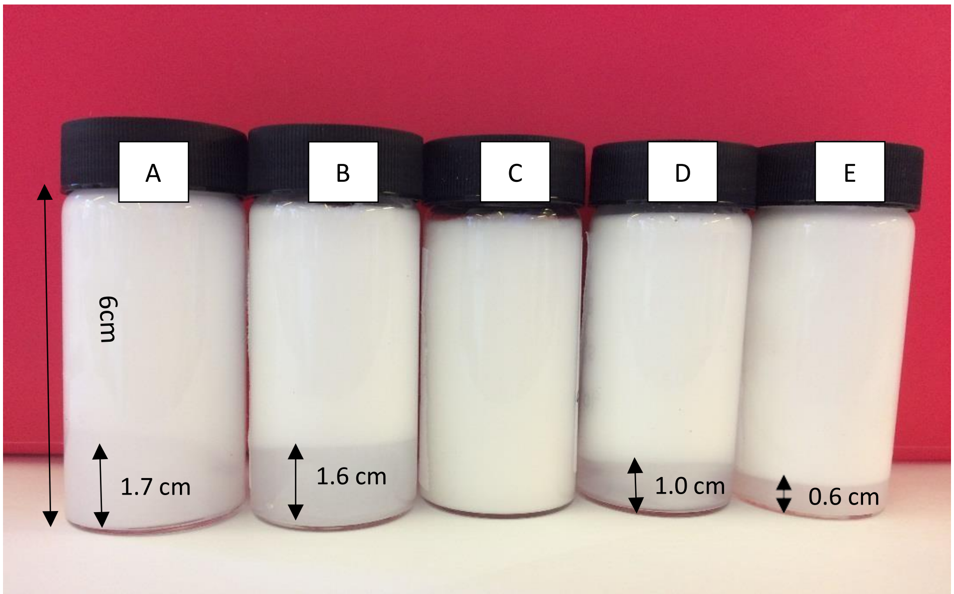


Figure 5.36: The image above shows 5 different emulsions and their degree of creaming; (A) 5% wax 0.5% surfactant, (B) 5% wax 1.5% surfactant, (C) 5% wax 5% surfactant, (D) 10% wax 1% surfactant, (E) 10% wax 3% surfactant

The figure above shows five different compositions and their level of creaming after 12 weeks of storage. The composition with the highest amount of surfactant (5% Stearyl Alcohol, 5% Tween 20) showed no creaming at all, while the composition with the lowest amount of surfactant (5% Stearyl Alcohol, 0.5% Tween 20) showed the most phase separation.

In general, the PSDs obtained showed no major differences for each emulsion layer, this suggests that the particle size measurement differences seen in the section above is unrelated to creaming/phase separation. However, in the emulsion containing 5% Stearyl Alcohol, 1.5% Tween 20, the top part of the emulsion showed more particles, which means it would be easy for flocculation/aggregation/coalescence to occur in this emulsion, which would result in large particles, and consequently, faster creaming.

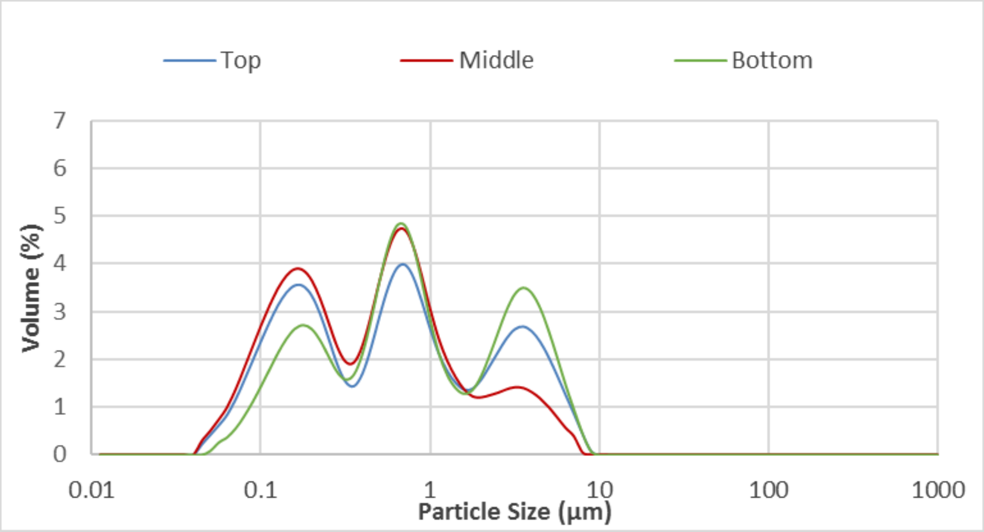


Figure 5.37: Top, Middle and Bottom PSDs for 5%SA, 1.5% Tween 20

The above Long-term stability experiment was a short test carried out and would need to be explored further in order to comment definitively on the accuracy of the results obtained from the different layers of the emulsion.

5.6 Conclusion

In this chapter, the rheological behaviour, structural changes, and stability of wax-in-water emulsions through different compositions and conditions were explored. Some of the key areas investigated include the Influence of the composition, presence of micellar structures, particle size distribution, and long-term stability.

A detailed case study was conducted on a composition that was found to have interesting changes in trend during storage and showed a difference in emulsion behaviour to the other compositions that were investigated. The emulsion containing 5% Stearyl Alcohol and 5% Tween 20 showed a steady increase in viscosity during storage and a distinct increase in viscosity between 4 hours and 24hours, which is suggestive of potential aggregation occurring within the system. Analysis of this behaviour pointed to the likelihood of a structural rearrangement occurring, theorising the possibility of a transition from spherical micelles to worm-like micelles as seen in literature (Yang, 2002).

Steady shear rheology was conducted on all the compositions, and what was discovered was an intricate interaction between the concentrations of the two phases and the shear applied. Evolution of shear thinning and Newtonian behaviour relative to ageing. It was found that surfactant concentration had a significant influence on rheological behaviour, where an initial viscosity increase was observed followed by viscosity loss or increase (depending on the composition). Although deemed unusual, it was evident that there was a possible presence of two competing mechanisms operating a structural buildup and structural breakdown within the emulsions. The storage and loss moduli were measured to obtain information on the viscoelastic behaviour of the emulsions through oscillatory rheology. The general take home from these measurements showed the presence of gel-like properties and low phase angles, which is in agreement with the theory that there is a continuous structural rearrangement process at play.

Particle size measurements were also carried out, where we inferred that surfactant concentration and emulsion composition have a complex interplay that influences particle size during storage, owing to the changes observed through bimodal peaks and peak shifts in the particle size distribution.

Finally, a long-term stability test was carried out to determine the relationship between surfactant concentration, creaming behaviour and emulsion homogeneity. As expected, the emulsions with higher surfactant concentrations showed better stability and those with lower surfactant concentrations showed a tendency to phase separate quicker. This chapter certainly sets the tone for interesting findings surrounding rheological behaviour and structural rearrangements; however, more work is required to delve in deeper for a better understanding of how the presence of higher wax concentrations results in loss of viscosity over time.

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Chapter 6

CONCLUSION & FUTURE WORK

6. Conclusion and Future Work

The investigation carried out in this work studied emulsions of Stearyl Alcohol and Tween 20 across a broad range of compositions. The aim was to characterise and understand the effect of composition on emulsion behaviour through observation of stability, characterisation of microstructure and measurement of rheology.

Different mechanisms and approaches to understanding the science behind stabilising wax-in-water emulsions were investigated and most of it explored, however, there is no definitive conclusion as to what the ideal, optimal conditions are for the formulation of wax emulsions using the melt emulsification method. However, some of the findings from the discussions in the results chapters are summarised below as well as crucial future work that ought to be carried out in order to gain further understanding of the research and to have answers to questions that have been left unanswered in this work.

6.1 Structure and Stability

In Chapter 4 it was revealed that composition has a significant effect on structure and stability. Emulsions with lower wax concentrations showed signs of creaming over time, while samples containing higher surfactant concentrations showed micellar behaviour, which can be attributed to the potential presence of a phase boundary past the Critical Micelle Concentration (CMC). Furthermore, polarised microscopy revealed the presence of mesophases and complex structures in the emulsion mixtures.

There was a focus on the effect of surfactant concentrations >7.5% on the formation of liquid crystals. Polarised microscopy revealed needle-like crystals indicating a pure surfactant phase, and Maltese crosses suggested a lamellar phase. These observations are similar to those of Kudla et al. (2010) concerning CMC and lamellar phase formation. The effect of wax concentration was also investigated, it was observed that the emulsions containing higher concentrations of wax showed the presence of multilamellar structures, highlighted by the presence of Maltese crosses. Rheology measurements agreed with these findings, showing the elastic behaviour of a well-structured gel. However, the interaction between Water, Stearyl Alcohol and Tween 20 in the formation of these structural arrangements is not fully understood and should be explored further.

6.2 Rheology and Stability

The emulsion system proved to be a transient one with the constant change in structure occurring particularly during storage. Compositions containing low surfactant amounts, showed an initial increase in viscosity, followed by a drop in viscosity over time, while compositions with higher surfactant amounts or 1:1 composition ratio showed an increase in viscosity over time. The constant change in viscosity is one that is curious and with investigating further. The constant change in rheology and particle size as discussed in Chapter 5 could be a result of structural relaxation (where there is loss of viscosity) and a structural build-up (potential formation of worm-like micelles), where there is an increase in viscosity over time. As well as the rheology measurements, the particle size distributions also show a change in particle size over the same period as the rheology.

Steady shear rheology was conducted on all the compositions, and what was discovered was an intriguing interaction between the concentrations of the two phases and the shear applied. It was found that surfactant concentration had a significant influence on rheological behaviour, where an initial viscosity increase was observed followed by viscosity loss or increase (depending on the composition).

The emulsion containing 5% Stearyl Alcohol and 5% Tween 20 for example showed continuous increase in viscosity during storage, suggestive of potential aggregation occurring within the system. Analysis of this behaviour pointed to the likelihood of a structural rearrangement occurring, perhaps suggesting a transition from spherical micelles to worm-like micelles as seen in literature (Yang, 2002).

It appears that there are possibly two competing mechanisms of structural buildup and structural breakdown of a complex viscoelastic network within the emulsions and the relative importance of each is somehow affected by composition as revealed by, respectively, increase or decrease in viscosity with time. The storage and loss moduli were measured to obtain information on the viscoelastic behaviour of the emulsions through oscillatory rheology. The general take home from these measurements showed the presence of gel-like properties and low phase angles, which is in agreement with the theory that there is a continuous structural rearrangement process at play.

Particle size measurements were also carried out, where we inferred that surfactant concentration and emulsion composition have a complex interplay that influences particle size during storage, owing to the changes observed through bimodal peaks and peak shifts in the particle size distribution.

Finally, a long-term stability test was carried out to determine the relationship between surfactant concentration, creaming behaviour and emulsion homogeneity. As expected, the emulsions with higher surfactant concentrations showed better stability and those with lower surfactant concentrations showed a tendency to phase separate quicker.

6.3 Future Work

There are certainly, without a doubt, liquid crystalline structures in some of the compositions explored, however the exact type of liquid crystalline structures need to be fully understood as well as the driving force behind the formation of these phases based on the emulsion system of interest.

Conductivity tests should be carried out to determine the CMC of tween 20 in the emulsion system, so it can be determined whether the phase changes are a result of the concentration being used or if there is excess surfactant molecules in the system

Other wax and surfactant types should be explored to give room for comparison.

The use of a Scanning Electron Microscope (SEM) should be considered to gain further insight into the structure being looked at.

A wider range of compositions should be looked at.

Although Stearyl Alcohol was used as the dispersed phase in this study, it has also been suggested in literature that it can act as a potential co-surfactant. Further work should be carried out to investigate the role of fatty alcohols as co-surfactants, the mechanism of interplay between fatty alcohols and surfactants in a system, and how their presence in a mixture as co-surfactant can enhance emulsion behaviour and influence interfacial tension.

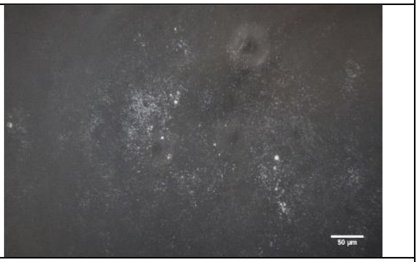
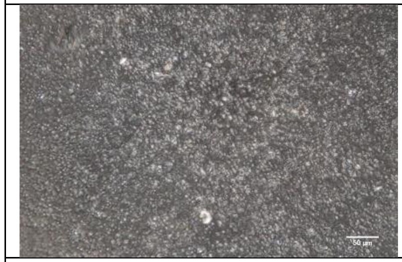
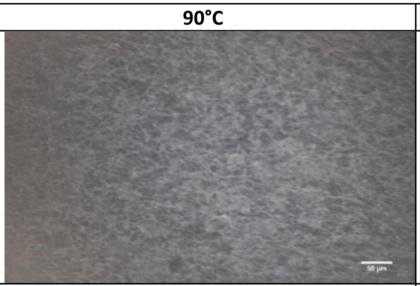
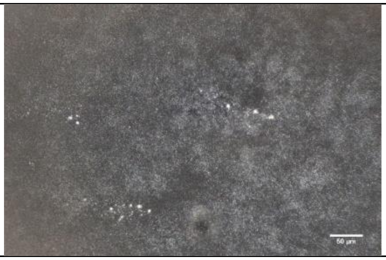
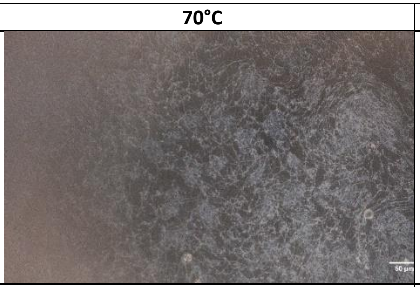
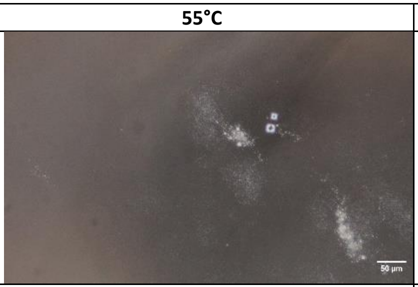
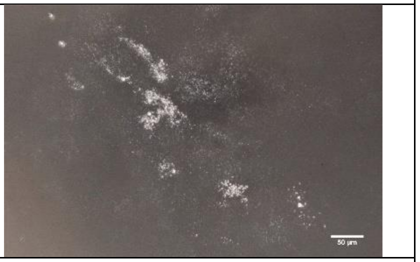
Small angle X-ray Scattering (SAXs) and/or Wide-angle X-ray scattering (WAXs) should be used to determine whether there are micelle structures in the system and what they are.

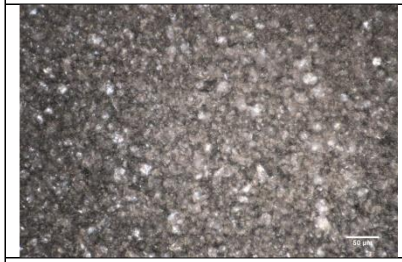
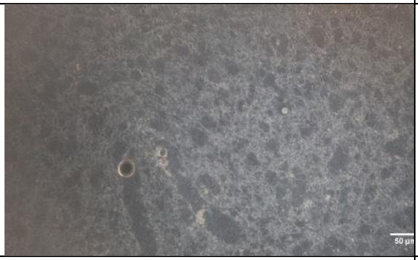
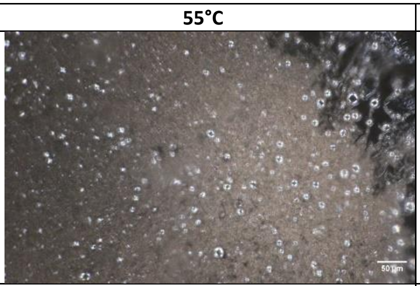
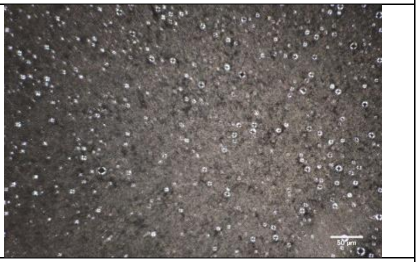
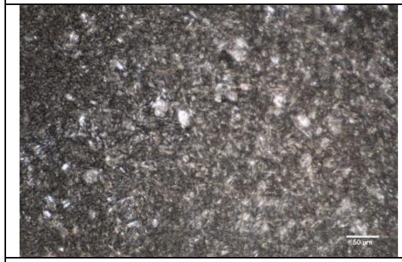
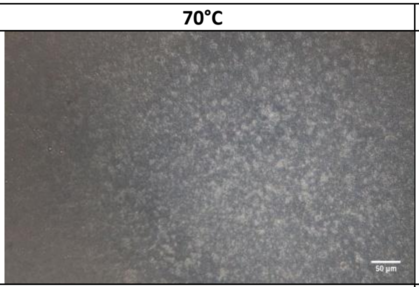
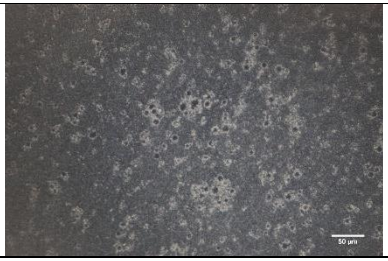
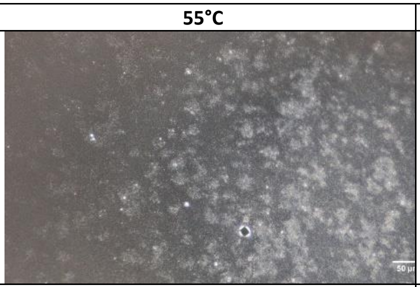
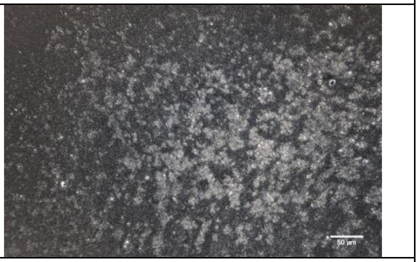
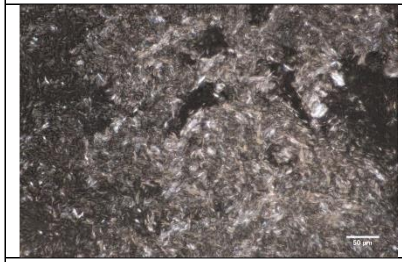
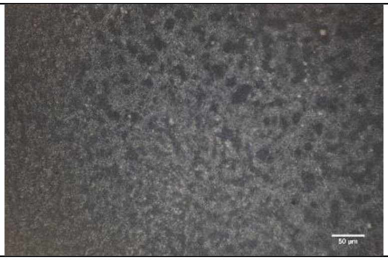
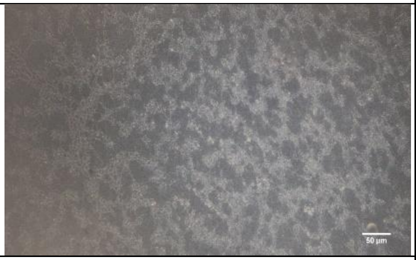
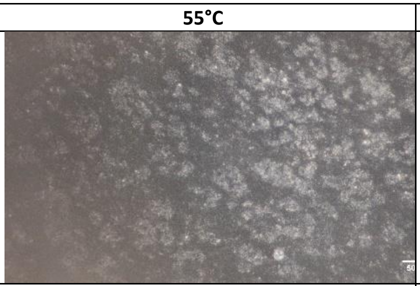
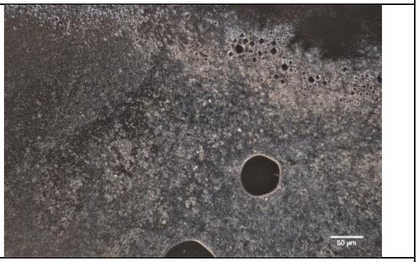
Dynamic light scattering (DLS) should be used to see if there are particles in the sub-micron region as they could potentially be micelles.

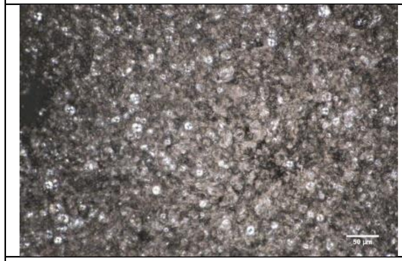
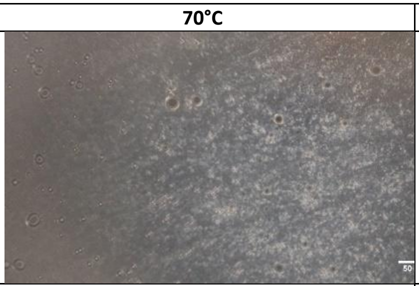
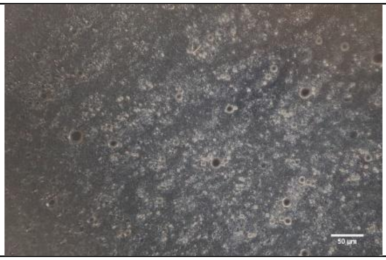
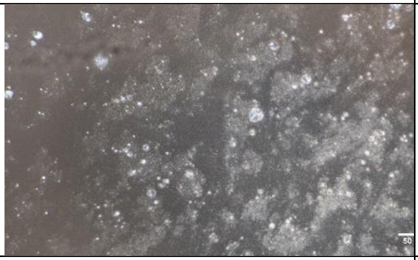
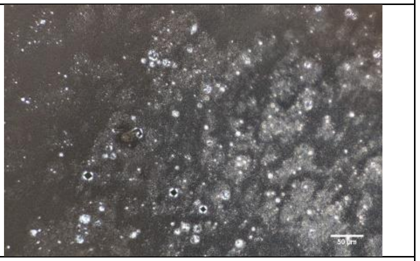
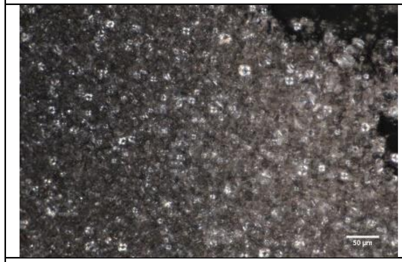
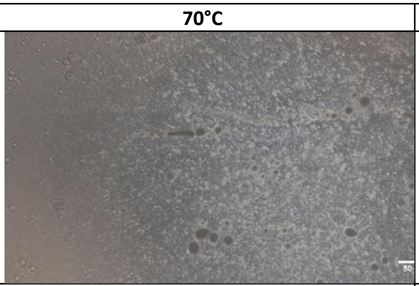
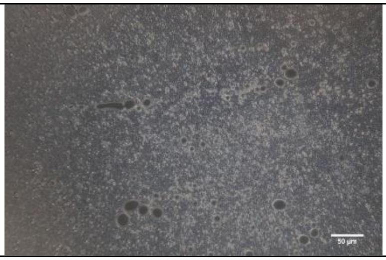
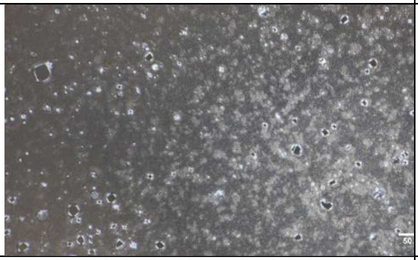
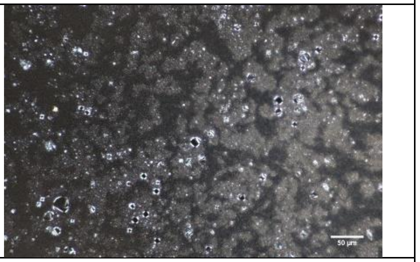
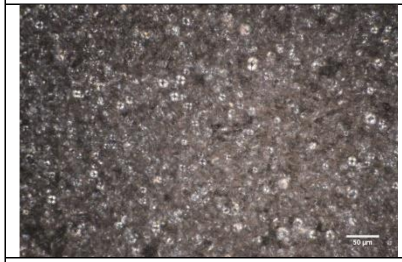
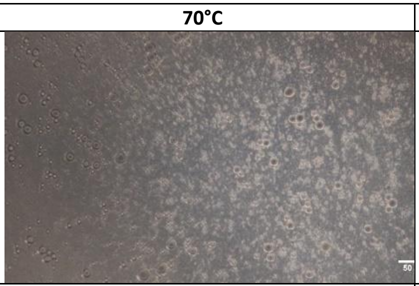
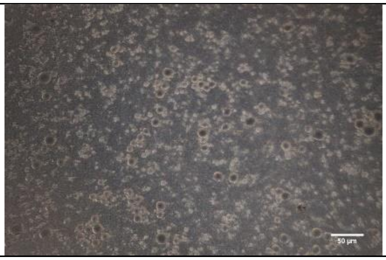
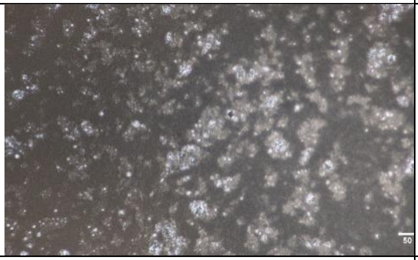
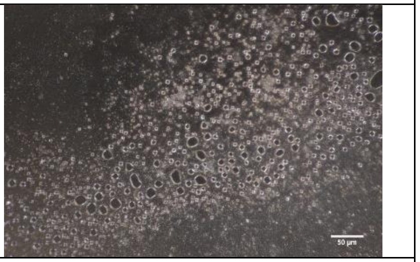
APPENDIX

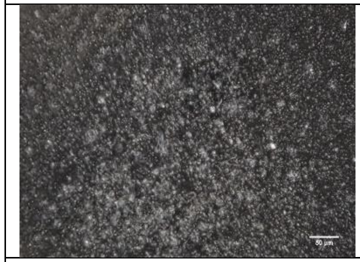
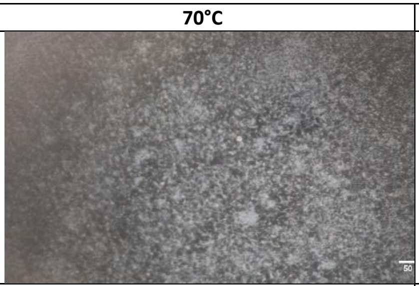
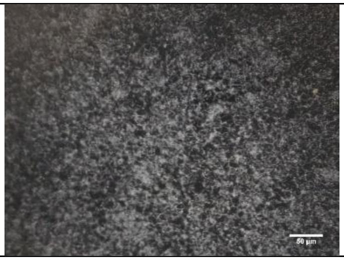
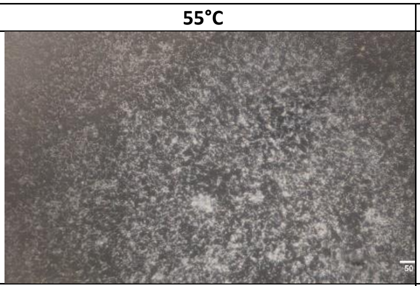
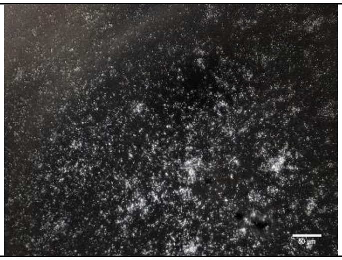
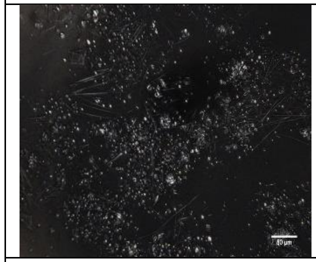
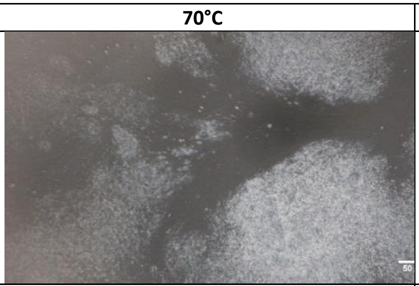
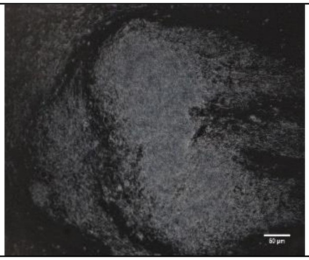
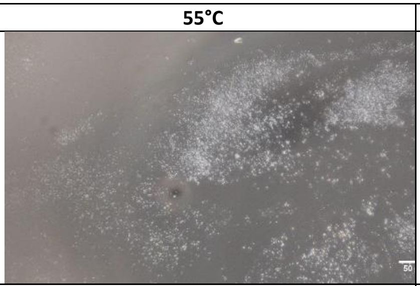
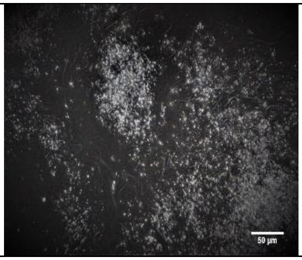
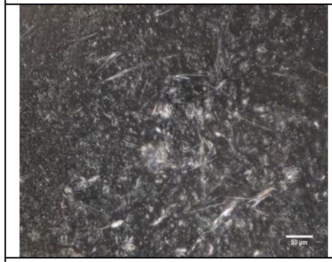
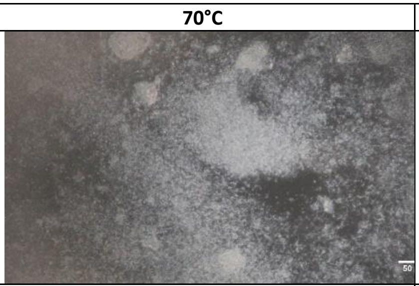
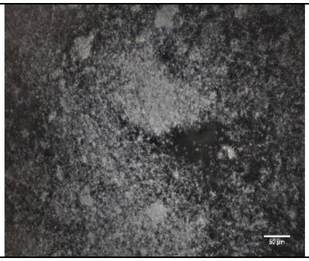
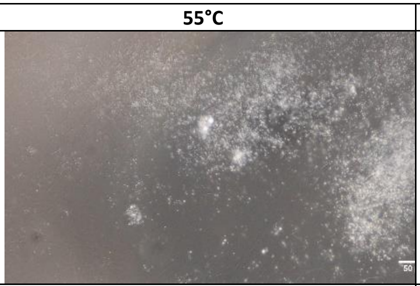
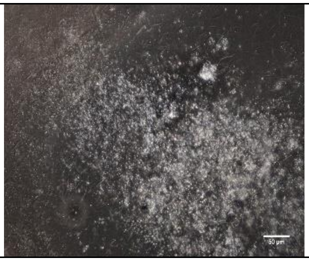
Compositions: 7.5% Wax, 5%, Tween 20| 10% Wax, 5% Tween 20 | 15% Wax, 5% Tween 20

Compositions: 5% Wax, 7.5%Tween 20 | 5% Wax, 10% Tween 20 | 5% Wax, 15% Tween 20

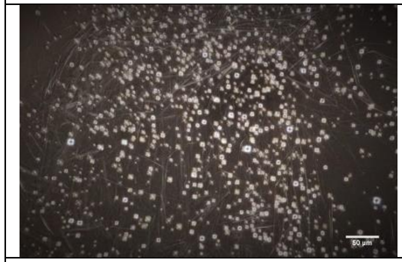
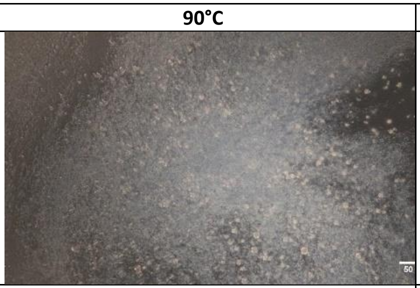
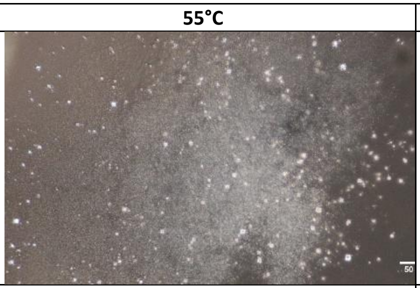
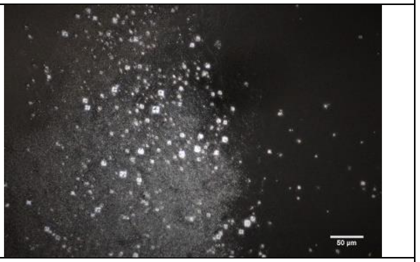
7.5% Wax, 5%, Tween 20 - **Day 1**				
25°C	**70°C**	**90°C**	**55°C**	**25°C**
				
Globby waxy droplets, lots of spherical droplets present.	Melted droplets with a string-like appearance.	Stringy melted emulsion	Hardened particle-like droplets.	Hardened particle-like droplets.
7.5% Wax, 5%, Tween 20 - **Day 5**				
25°C	**70°C**	**90°C**	**55°C**	**25°C**
				
Hardened droplets with irregular shapes and different sizes	Melted emulsion.	Melted emulsion.	Hardened particle-like droplets.	Hardened particle-like droplets with minimal Maltese crosses.
7.5% Wax, 5%, Tween 20 - **1 Week**				
25°C	**70°C**	**90°C**	**55°C**	**25°C**
				
Some hardened droplets and some globby droplets of irregular shapes and different sizes. Very small-sized Maltese crosses.	Melted droplets with a string-like appearance	Melted droplets with a string-like appearance	Hardened, aggregated, and non—aggregated particle-like droplets. Some crystalised Maltese crosses.	Hardened, aggregated, and non—aggregated particle-like droplets.

10% Wax, 5% Tween 20 - **Day 1**				
25°C	**70°C**	**90°C**	**55°C**	**25°C**
				
It appears to be a large cluster of coalesced wax droplets of different shapes and sizes.	Melted droplets with a string-like appearance	Melted droplets with a string-like appearance	A sudden appearance of multiple Maltese crosses was observed	A sudden appearance of multiple Maltese crosses.
10% Wax, 5% Tween 20 - **Day 5**				
25°C	**70°C**	**90°C**	**55°C**	**25°C**
				
Some spherical droplets as well as some short, sharp crystals.	Melted particles.	Melted droplets and particles were observed	Aggregated, hardened wax particles as well as some maltese crosses	Aggregated, hardened wax particles as well as some maltese crosses
10% Wax, 5% Tween 20 - **1 Week**				
25°C	**70°C**	**90°C**	**55°C**	**25°C**
				
The presence of short, sharp crystals appeared to have increased by 1 week and there are barely any spherical wax.	Melted particles.	Melted droplets and particles. Some string-like appearance	Aggregated, hardened wax particles, no maltese crosses.	Aggregated, hardened wax particles as well as some maltese crosses (substantially more than on Day 5)

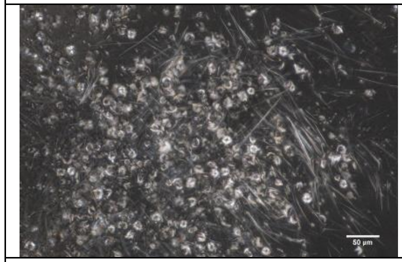
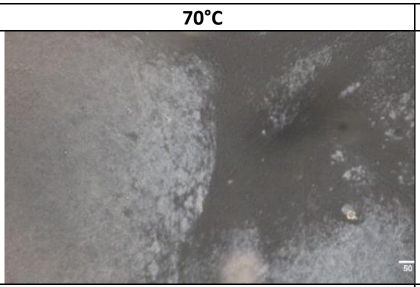
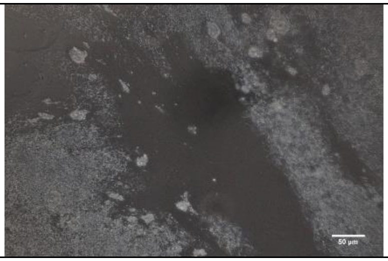
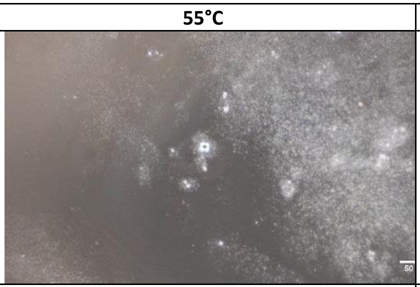
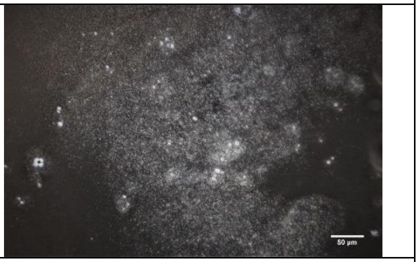
15% Wax, 5% Tween 20 - **Day 1**				
25°C	**70°C**	**90°C**	**55°C**	**25°C**
				
A mix of maltese crosses, aggregated wax and spherical droplets.	Melted wax droplets	Melted droplets and particles observed	Coalesced wax particles with some large wax droplets.	Aggregated wax particles observed with some Maltese crosses
15% Wax, 5% Tween 20 - **Day 5**				
25°C	**70°C**	**90°C**	**55°C**	**25°C**
				
A mix of maltese crosses, aggregated wax and spherical droplets.	Melted wax droplets	Melted droplets and particles observed	Coalesced wax particles with some large wax droplets. An increased amount of Maltese crosses.	Aggregated wax particles observed with some Maltese crosses.
15% Wax, 5% Tween 20 - **1 Week**				
25°C	**70°C**	**90°C**	**55°C**	**25°C**
				
A mix of maltese crosses, aggregated wax and spherical droplets. Maltese crosses here appear to be more and slightly larger than at Day 5	Melted wax droplets	Melted droplets and particles observed	Coalesced wax particles with some large wax droplets.	An increased amount of maltese crosses in comparison to day 5

5% Wax, 7.5% Tween 20 - **Day 1**				
25°C	**70°C**	**90°C**	**55°C**	**25°C**
				
Wax crystalline droplets, many of them appear to be spherical. Some gel-like material trapped between the droplets	Melted wax droplets	Entangled, string-like materials were observed upon melting	Upon recrystallization, fine, wax particles are observed with some string-like material present.	Upon recrystallization, fine, wax particles are observed
5% Wax, 7.5% Tween 20 - **Day 5**				
25°C	**70°C**	**90°C**	**55°C**	**25°C**
				
Large needle-like material surrounded by wax droplets which now appear to be less aggregated.	Melted wax droplets	Fine, wax particles	Fine, wax particles	Fine, wax particles with shorter needles seen embedded
5% Wax, 7.5% Tween 20 - **1 Week**				
25°C	**70°C**	**90°C**	**55°C**	**25°C**
				
Reminiscent of the Day 5 sample, however, more evident coalescence and much bigger needles observed here.	Melted wax droplets	Melted droplets	Fine wax particles	Fine, wax particles with shorter needles seen embedded between them

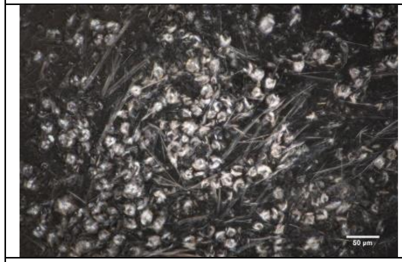
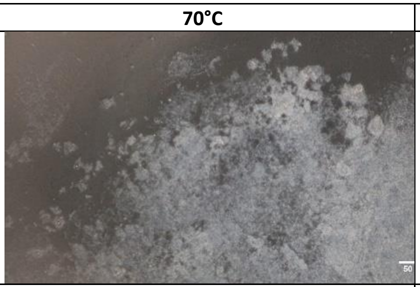
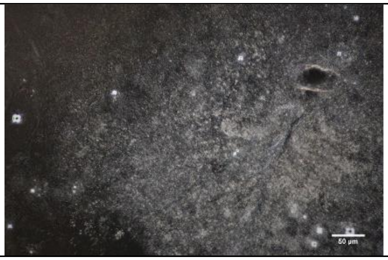
5% Wax, 10% Tween 20 - **Day 1**

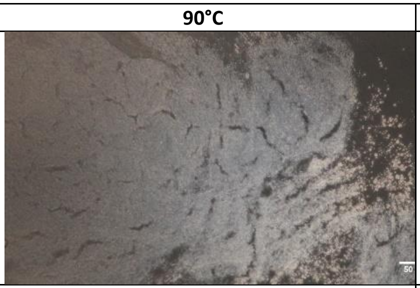
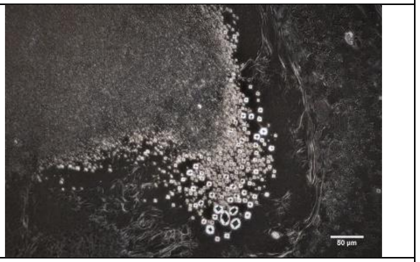
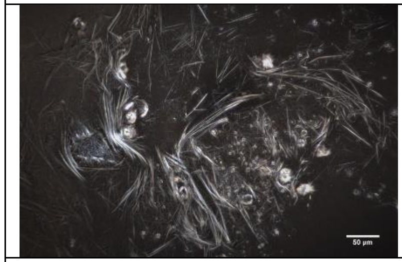
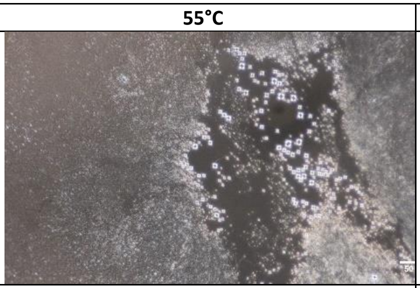
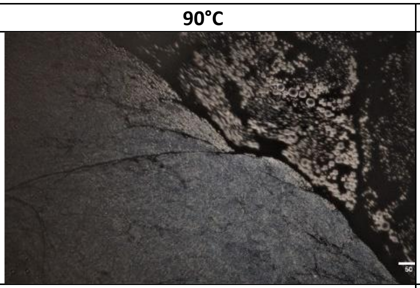
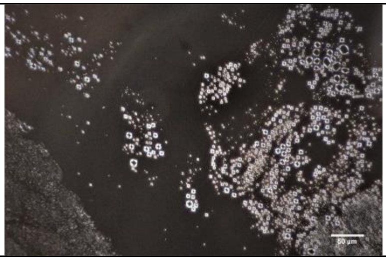
25°C	**70°C**	**90°C**	**55°C**	**25°C**
				
Extensive maltese crosses surrounded by flexible needle-like material	Melted doplets and needle-like material	Melted droplets and particles	A reappearance of maltese crosses surrounded by fine wax particles	Maltese crosses surrounded by fine wax particles

5% Wax, 10% Tween 20 - **Day 5**

25°C	**70°C**	**90°C**	**55°C**	**25°C**
				
Deformed maltese crosses surrounded by flexible needle material and agglomeration of wax crystals	Melted droplets and crystals	Melted droplets and crystals	A reappearance of maltese crosses surrounded by fine wax particles.	Maltese crosses surrounded by fine wax particles. Although a reduced number of maltese crosses

5% Wax, 10% Tween 20 - **1 Week**

25°C	**70°C**	**90°C**	**55°C**	**25°C**
				
The deformed maltese crosses surrounded by flexible needle material appear to be larger	Melted droplets and crystals	Melted droplets and crystals	A reappearance of maltese crosses surrounded by fine wax particles.	Maltese crosses, short needles and wax particles

5% Wax, 15% Tween 20 - **Day 1**				
25°C	**70°C**	**90°C**	**55°C**	**25°C**
				
Formulation containing highest amount of surfactant showed a strong presence of translucent gel material, with some wrapped around wax crystals.	Melted droplets and crystals	Melted droplets and crystals	A reappearance of maltese crosses surrounded by fine wax particles and some needle-like material	Upon cooling, a high number of maltese crosses next to a self-assembled arrangement of the translucent gel-like material seen at room temperature.
5% Wax, 15% Tween 20 - **Day 5**				
25°C	**70°C**	**90°C**	**55°C**	**25°C**
				
Similar in appearance to what was observed after 24 hours, however, some larger wax crystal droplets here.	Melted droplets and crystals	Melted droplets and crystals	A reappearance of maltese crosses surrounded by fine wax particles and some needle-like material	Upon cooling, a high number of maltese crosses
5% Wax, 15% Tween 20 - **1 Week**				
25°C	**70°C**	**90°C**	**55°C**	**25°C**
				
A staggering increase in the number of needle-like crystals.	Melted droplets and crystals	Melted droplets and crystals	Upon cooling, a high number of maltese crosses	Upon cooling, a high number of maltese crosses