ON THE PHASE BEHAVIOR OF TRIGLYCERIDE/ETHANOL/WATER-SYSTEMS

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Master's Thesis in Pharmaceutical Technology

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Abstract

For personnel working in areas where hand disinfection is important, skin irritation on hands is a large and real problem. A solution may be a hand cream, containing ethanol, with moisturizing properties by using a Pickering emulsion. However, the effect of ethanol on a Pickering emulsion is unknown. The objective of this work was to gain understanding of this by mapping the phase diagram of triglyceride-ethanol-water, using tricaprin and triolein and 55-99.8% concentration of ethanol, mixed in ratios of 30:70, 50:50 and 70:30 (lipid:aqueous ethanol). The composition of the phases was then analyzed by high performance liquid chromatography (HPLC) and thermogravimetric analysis (TGA). The effect of ethanol on the melting point of the triglyceride was examined by differential scanning calorimetry (DSC). In most samples, only a few percent triglyceride was found in the ethanol phase, but at 40°C and 99.8% ethanol, tricaprin and ethanol formed one single phase. The melting point was lowered slightly with increasing ethanol concentration. The results settle concerns that large amounts of oil dissolves into the continuous phase and disrupts the emulsion, and increase awareness of handling temperatures. Next steps would be investigating the properties of the Pickering emulsion and the antimicrobial effect of the ethanol when contained in a cream.

Acknowledgements

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Abbreviations

CMC	critical micelle parameter
CN	carbon number
CPP	critical packing parameter
DSC	differential scanning calorimetry
ECN	equivalent carbon number
FA	fatty acid
GC	gas chromatography
HLB	hydrophilic-to-lipophilic balance
HPLC	high performance liquid chromatography
NMR	nuclear magnetic resonance
O/W	oil-in-water
RI	refractive index
RP-HPLC	reversed-phase high performance liquid chromatography
RT	room temperature
TG	triglyceride
TGA	thermogravimetric analysis
UV	ultra violet
W/O	water-in-oil

1. Introduction

1.1 Background

Anti-bacterial soaps and topical formulations are a common feature in the society of today. The importance of clean hands is substantial in certain occupations and the chance of preventing common diseases and colds is becoming increasingly popular. Formulations such as gels based on ethanol are the most common, occasionally combined with a hand-wash of soap and water. However, their effect on the skin is not always positive. People who use these products daily, such as surgeons, nurses and laboratory personnel, tend to have dry hands at the end of the day because of the drying effect ethanol has on skin, and these problems may turn into more severe consequences such as irritant contact dermatitis[1]. According to one study, 85% of the nurses reported a history of skin problems on their hands, and 25% suffered from contact dermatitis[2]. The reason for these problems is found to be an excess of washing and using of detergents in soaps, but there is a general belief that ethanol-based agents are the cause of concern. However, ethanol has been shown to be more gentle and less cause for contact dermatitis or other skin problems, compared to anti-bacterial soaps [3]. The burning sensation experienced may instead be due to pre-irritated skin [1, 4].

To assure patient safety in health care, the compliance of the employees regarding hand hygiene needs to be considered. Staff will be less compliant to use a product that induces an uncomfortable feeling. The product also needs to be easy accessible and work its function quickly, *e.g.* not leave residues for a long time, as time is usually short in these groups [5].

Ethanol has a considerable effect on both gram-positive and –negative bacteria, fungi and some viruses, probably by denaturation of proteins[6]. A concentration of 60-95% is recommended by the FDA for maximum efficacy. 70% is an accepted concentration that is commonly used, as it keeps cost down and reduces skin drying while still having the germicidal effect. Other antimicrobial agents except alcohol are available but less frequently used and might have other effects on the skin. Some of these are chlorhexidine, hexachlorophene, iodine or iodophores [6, 7].

A solution to the above mentioned problems of skin irritation could be an ethanol-containing hand cream, which offers the moisturizing and caring effects of a regular cream in combination with the anti-bacterial properties of *e.g.* an alcogel. Examples of this have been shown in some previous patents but are rare [8-12] and are not commonly found in stores.

As a hand cream is generally an oil-in-water emulsion, and ethanol dissolves both water and some common oils, the alcohol may interfere with the liquid-liquid interface. This gives rise to the question whether mixing ethanol into this composition will result in a functioning cream. In this thesis, a Pickering emulsion with starch particles is imagined as the final product. Is it possible for the emulsion to be stable while containing ethanol and will the separate components keep their inherent properties? How will the final product look and feel? Is it possible to add the high concentration of ethanol required for antibacterial effect?

To answer these questions, knowledge of the behavior of the oil-ethanol-water-system is needed. This can be obtained by mapping a ternary phase diagram of the components involved, enabling

prediction of the behavior in an emulsion. Investigating the melting point of the oil in the mixture can provide further information on any effect the ethanol might have, or about polymorphism that may introduce undesired effects.

1.2 Objective

The objective of this master's thesis was to answer two important questions;

- whether the oil dissolves from the dispersed phase into the continuous ethanol-water phase, which might affect the antimicrobial properties of the ethanol-phase

- whether any ethanol dissolves into the dispersed oil phase, which might affect the melting point of the oil and thus some properties of the cream

This was done by mapping the phase diagram of triglyceride/ethanol/water for two triglycerides, tricaprin and triolein. From these diagrams, some predictions about the suitability of these products in an emulsion were made. Furthermore, a possible effect of the ethanol on the melting point of the oil was investigated. The melting point is interesting from a formulation point of view, both for manufacturing steps and to predict the behavior on application on the skin. As the melting point also can be affected by polymorphism in the triglycerides, this phenomenon was also investigated.

The thesis does not comprise evaluating the microbiological effects of the mixture on skin or testing any final product of a hand cream containing further excipients.

1.3 Current research

As mentioned above, some hand creams have been made with ethanol as active ingredient, and gels with ethanol as active ingredient are common. Some creams are made with other antimicrobial agents. However, none of them are based on Pickering emulsions.

A patent by Lee [8] claims an antimicrobial gel containing skin moisturizers in the form of different emollients and humectants, using a high alcohol content (65-70%) as antimicrobial agent. Leece [9] has invented a topical formulation to be used with different antimicrobial agents, not limited to ethanol, with the intention of being skin-friendly. Sawan *et al.* claimed a more general topical formulation with antimicrobial properties, presented to be more gentle to the skin [10]. One of their examples states a hand cream that is designed to reduce skin irritation from latex gloves, and silver iodide and ethanol was added for antimicrobial function. Shick [11] has presented a formulation where the emollient is not miscible with the aqueous alcohol composing the antimicrobial base of the formulation, but instead contained in a delivery material, releasing the emollient upon skin application. Finally, a hand sanitizing lotion, based on an emulsion, is patented by Stack [12], where 2,4,4'-trichloro-2'-hydroxydiphenyl ether is chosen as the antimicrobial agent.

Pickering emulsions is an up-and-coming field and its suitability for drug delivery and topical formulations has been recognized by the scientific society[13]. The main advantage for topical formulations is the absence of surfactants, an ingredient that may cause skin irritation due to inherent toxicity.

2. Theory

2.1 Emulsions

Emulsions can be found in everyday life; milk, mayonnaise and ice cream are all examples. Emulsions arise when two immiscible liquids are mixed and the result is a dispersed phase (droplets) in a continuous phase, often with a cloudy appearance which is due to light scattering at the high number of interfaces [14]. If the dispersed phase is oil in a continuous phase of water, the emulsion is termed oil-in-water (O/W). The droplets usually range from about 100 nm to 100 μm in size. Emulsions with droplets smaller than ~100 nm exist, and are then called nanoemulsions [15].

2.1.1 Interfacial forces

The emulsion environment can be regarded as three regions; continuous phase, dispersed phase and the interface between these, as depicted in Figure 2.1. The interface is normally a few nanometers thick [16]. The polarity of a molecule decides which region it tends to stay in. However, it is a dynamic environment and small changes in the surroundings, such as temperature, can cause molecules to transfer between the regions. Surface-active molecules mainly adsorb to the interface, aligned to fit the polarity regions of the molecule to the region of the emulsion.

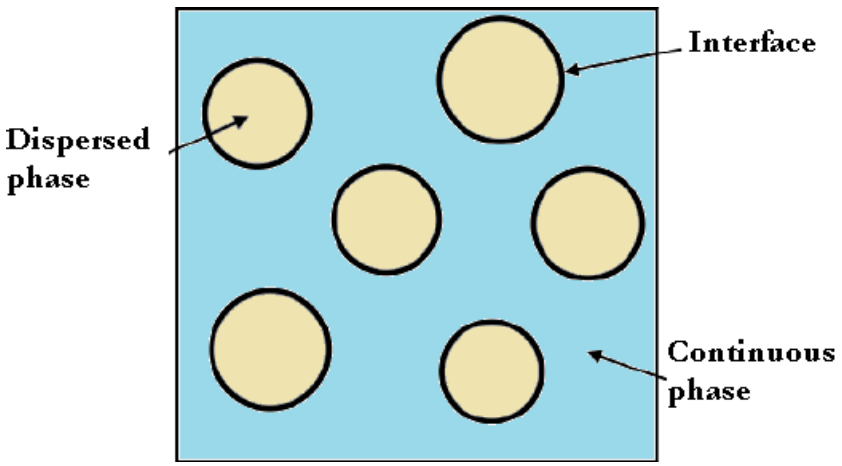

Figure 2.1. An emulsion can be simplified to contain three regions; the continuous phase, the dispersed phase, and the interface between these. Surfactants have a key role in the critical interface region.

Emulsions are thermodynamically unstable, a fact that is based on the thermodynamically unfavorable contact between water and oil molecules, which will eventually cause phase separation when the droplets merge with their neighbors [16]. This concept is shown in Figure 2.2.

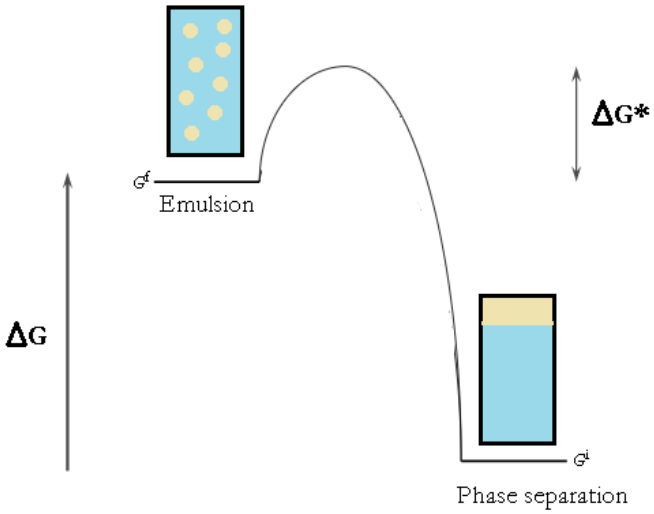

Figure 2.2. The emulsion exists in a thermodynamically unstable state, and if an activation energy ΔG^* is applied, it can transform into the more favorable phase-separated state.

Hence, both the formation and the stabilization of emulsions often require the addition of a surface-active component, called an emulsifier, which can be *e.g.* a surfactant, phospholipid, biopolymer or even a particle (see Section 2.1.3). The emulsifier has two important roles; it both acts by facilitating the formation of an emulsion and increasing stability as it prevents droplets from coalescence.

A surfactant works at the interface between the two phases by lowering the surface tension, enabling small droplets with a large surface area to form. Surfactants are amphiphilic, containing one end that is hydrophilic and one end that is hydrophobic. The hydrophilic part can position itself in the more polar region as it has higher affinity for water and the hydrophobic part in the non-polar region as it has higher affinity for lipids (or air). This is depicted in Figure 2.3.

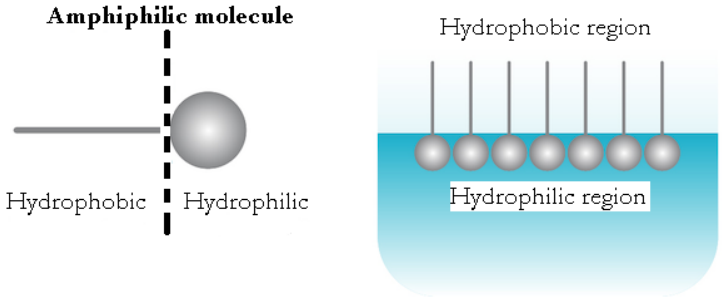

Figure 2.3. A surfactant is usually amphiphilic, meaning it has one hydrophobic and one hydrophilic part, and therefore aligns in the interface between two separated phases.

When a sufficient amount of surfactant is added to water, the surfactants will start to form micelles. This amount is called the critical micelle concentration (CMC), and below this point the surfactant molecules will only exist as monomers.

To predict which emulsion will be formed by a specific surfactant, the hydrophilic-to-lipophilic balance (HLB) number can be used [16]. A low HLB number (3-6) indicates that the molecule is slightly more hydrophobic, and thus forms water-in-oil emulsions. On the other hand, a surfactant with a HLB value of 10-18 will dissolve better in water and an addition of such a surfactant gives rise to oil-in-water emulsions. In the absence of a surfactant, the type of emulsions is usually determined by the amount of oil or water in the emulsion; a water-rich mixture will result in an oil-in-water emulsion.

The actual process of creating an emulsion is called homogenization, as the result will have the appearance of a homogenous solution. After a primary homogenization where the droplets are created, further homogenization can be carried out to decrease droplet size. An external mechanical force is needed during homogenization to increase the contact area between the two immiscible phases, as the forming of such a thermodynamically unfavorable state is non-spontaneous [16, 17]. The mechanical force which induces droplet disruption and creates more and smaller droplets will balance against the interfacial forces that keep the droplets together. The addition of an emulsifier during homogenization will further work against the interfacial forces and induce droplet formation.

As the system still wants to keep down the interfacial forces, it will create droplets in a spherical shape, which is the shape that minimizes surface tension[17]. The difference in pressure between the inside and outside of the droplet can be described by the Laplace pressure ΔP_L which is expressed as **Eq. 2.1**:

$$\Delta P_L = \frac{2\gamma}{r} \qquad \textbf{(2.1)}$$

Where r is the radius of the droplet and γ is the interfacial tension between the oil and water. This pressure is what keeps the droplet in its spherical form.

2.1.2 Stability – what does it mean to be stable?

The concept of stability generally connects to an ability to resist changes and keeping the initial properties over a longer time. Stability can be evaluated in both a physical and chemical sense; physical stability concerns the physical properties of the emulsion, such as appearance, texture, phase separation etc., whereas the chemical stability refers to a constant kind of molecules, without any chemical reactions occurring.

As mentioned before, an emulsion is always thermodynamically unstable because of the unfavorable large contact area between the two phases. Hence, the sense of stability actually depends on kinetics and the question is rather how long time will be required before the emulsion falls apart; not if, but when. Nevertheless, as can be seen in Figure 2.2, an activation energy ΔG^* is required to return the emulsion to the thermodynamically stable, phase separated state. If this activation energy is sufficiently high, the emulsion can be said to be kinetically stable as it takes too much energy to change state. In this case, sufficiently high means definitely higher than the thermal energy of the system, kT, preferably around $20kT$ [16]. However, it must be considered that there can be a number of metastable states in between, with lower activation energy, which can cause the emulsion to change and lose its stable appearance without entering the state of phase separation. Here another role of the emulsifier appears; increasing the

activation energy to prevent the emulsion from falling apart. A good example is the mixture of pure oil and water. An emulsion can be obtained without any addition of emulsifier, but it will phase separate in a short time if not immediately. This can be explained by the low activation energy of the step returning the emulsion to a phase separated state.

There is a number of ways in which an emulsion can lose its properties, with different causes behind. However, they are not necessarily separate processes; they can occur simultaneously and independent of each other.

One category of changes that can be introduced into an emulsion is gravitational changes [16]. These include creaming and sedimentation and arise due to density differences between the oil and water phase. Creaming occurs when the droplets have a lower density than the continuous phase, and so the droplets will pack together in a top layer, as can be seen in Figure 2.4. This is most common in food emulsions, as oils tend to have a lower density than water and most food emulsions are oil-in-water emulsions. Sedimentation, on the other hand, may happen when the droplets are heavier than the continuous phase and thus falls to the bottom. To avoid these changes, it is critical to ensure the densities of the separate phases are matched, as smaller differences in density may not introduce this problem. Another solution is to strive for smaller droplets in the emulsion, as smaller droplets are not as affected by gravity as larger droplets.

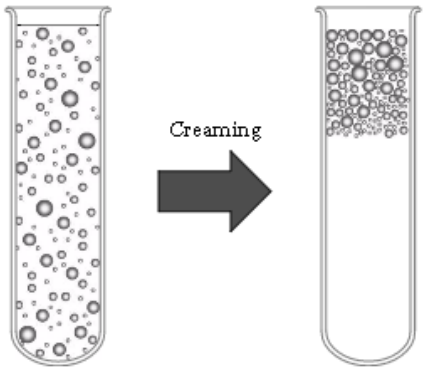

Figure 2.4. Creaming in an emulsion, where the droplets have gathered in a top layer. Figure adapted from [16].

There are more complex causes for changes in droplet size, number and distribution, such as flocculation, coalescence and Oswald ripening. These may induce unwanted properties of the emulsion if not controlled, but can sometimes be used to gain specific properties of *e.g.* a cream.

Flocculation is the accumulation of droplets without merging [16]. Two droplets come in contact with each other and stay attached, but keep their integrity as two separate droplets. As this can lead to a change in texture of the emulsion, it may be undesirable. The collisions of droplets leading to flocculation can be due to Brownian motion, the random movement of molecules in space, or gravitational separation, as discussed above, which enables the encounter of droplets. Flocculation can be prevented by ensuring steric hindrance of the collision of droplets. In this case, functional groups can be attached to the surface of the droplets, preventing droplets from coming in contact with each other. Moreover, mechanical agitation can assist flocculation, so measures can be taken to restrict this during *e.g.* transportation. Droplet size can also be increased to avert collision of droplets.

Similar, but not identical, to flocculation is coalescence of droplets. In this case, two droplets merge into a single droplet, a process that eventually will cause entire phase separation [16]. The physical basis for this phenomenon is the decrease in contact area between the two phases that comes with larger droplets, so as droplets coalesce, surface tension decreases. The processes leading to droplet coalescence begin with a droplet encounter, upon which film thinning may occur, the film being the infinitesimal interface of surfactant between the dispersed and continuous phase. Film thinning eventually causes film rupture, which results in coalescence of droplets as there is no longer an interface between them. The merging of droplets, causing increase in size, may also lead to sedimentation because of the formation of larger droplets. A typical reason for droplet coalescence is an insufficient amount of emulsifier in the emulsion, so the emulsifier does not cover the entire droplet. Impurities such as gas bubbles, solid particles or crystals are also common sources for coalescence as they promote film rupture. Uncontrolled freezing or drying of the emulsion may have the same effect.

A less intuitive alteration of emulsion properties occurs due to Ostwald ripening. This is a process during which larger droplets grow at the expense of smaller droplets. The key to this phenomenon lies in the solubility of the dispersed phase in the continuous phase, as molecules diffuse from the dispersed phase, through the continuous phase to another droplet of dispersed phase. Furthermore, the solubility of a substance increases with decreasing particle size in spherical droplets, so smaller droplets will have higher solubility and therefore their molecules will diffuse towards the larger ones. Ostwald ripening is usually not an issue in emulsions where the oil is nearly insoluble in water, such as long-chain triglycerides, which is the dispersed phase in this work. However, some additives may improve the solubility of oil in water, therefore increasing the rate of Ostwald ripening. Such additives can be alcohols or surfactant micelles. As ethanol is added in the solution in this work, this may be noteworthy.

As mentioned before, instability does not only refer to physical state but also chemical stability, as in, the molecular species remaining the same over time. Chemical reactions that should be taken into consideration especially when dealing with emulsions containing lipids are oxidation and hydrolysis of triglycerides [18].

2.1.3 Pickering Emulsions

Pickering emulsions are emulsions that use solid particles adsorbed to the liquid-liquid interface for stabilization instead of surfactants. They were first discovered by Pickering in 1907 [19]. Surprisingly, this discover lay dormant for decades, before proceedings in materials science allowed for a larger variation of small particles to be produced, and the field rose again.

There are many similarities between Pickering emulsions and surfactant-stabilized emulsions; however, they differ in important ways. The key factor for particles as stabilizers is their wettability, which can be compared to the HLB-value of surfactants, mentioned in Section 2.1.1. A particle that is more wetted by water than oil will reside to a larger extent in the water phase, thus favoring oil-in-water emulsions. This is better described by the contact angle θ at the oil-water-interface, as seen in Figure 2.5.

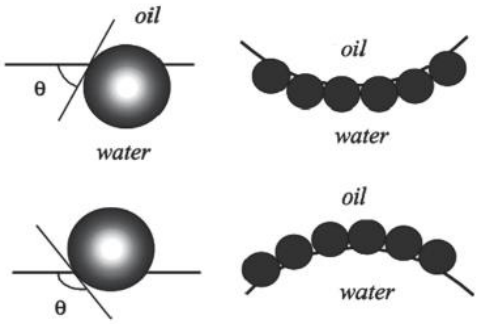

Figure 2.5. The influence of the contact angle on the wettability and emulsion created. Reprinted with permission from [20].

If the contact angle on the water side is smaller than 90°, the particle is poorly wetted by oil and the arrangement of particles will thus form oil droplets in a continuous phase of water, an O/W-emulsion. It follows that if the contact angle is larger than 90°, the particles promote a W/O-emulsion, because they are better wetted by oil [20, 21]. In this way, particles can be described as hydrophilic or hydrophobic depending on their wettability and contact angle. An interesting development is the application of so called Janus particles in Pickering emulsions[22]. Janus particles display opposing wettability on either side of their surface area, resulting in a stronger alignment of the particle in the interface between the oil and water phases, so the emulsion obtains even higher stability.

The high stability of Pickering emulsions compared to surfactant-stabilized emulsion can be understood by considering the energy required to remove the particle from its position in the interface. This energy E can be described by **Eq. 2.2** below [21].

$$E = \pi R^2 \gamma_{ow} (1 + \cos\theta)^{\,2} \qquad \textbf{(2.2)}$$

Where R is the particle radius, γ_{ow} is the interfacial tension and θ is the contact angle. From this equation it can be deducted that an angle of 90° will give the highest desorption energy, and that any deviations from 90° will make E decrease significantly, to the point where angles of 0-20° or 160-180° results in very low values of E. However, for reasonable contact angles E is high enough so that the particles can be said to be irreversibly adhered to the interface. Thus, a very high activation energy is needed for transferring the emulsion to its phase separated state, as discussed above. The consequence of this is an almost thermodynamically stable emulsion.

Pickering emulsions can be finely tuned to achieve desired properties by selecting specific stabilizing particles. The size, shape and concentration of particles all affect the final product, besides the important choice of particle material. Too small particles can display an insufficient desorption energy as it scales to the square of the particle radius. However, too large particles may lead to slow adsorption kinetics and inefficient packing at the droplet interface. The surface of the particles also need to be considered, as the large surface area that follows with small particles on this scale have a tendency to accumulate. This may require surface modifications to induce steric hindrance of aggregation.

The advantages for Pickering emulsions are several. They are normally surfactant-free which can be advantageous when working with pharmaceutical applications, as the toxicity from surfactants

can be avoided. The high desorption energy offers high long term stability of the emulsion, averting many of the above mentioned stability issues. The vast diversity of particles provides better variety; the emulsion can be designed and tailored to a specific function.

However, these emulsions face some problems as well. The toxicity is not as well investigated yet, as most research today on Pickering emulsions focus on tuning emulsions to a desired function. The safety and biocompatibility of nanoparticles, commonly employed in Pickering emulsions, is readily discussed and this might be an issue for some applications. Moreover, the particle wall on the interface may show high permeability and poor barrier properties as a gap emerges between the particles, which may be calamitous *e.g.* for applications where the droplets protect a precious drug. However, high permeability can also be beneficial in some applications. Recently, Sjöö *et al.* demonstrated an elegant solution with starch granules as stabilizing particles. Upon heating, the starch particles gelatinize, creating a more impermeable barrier [23].

In this work, the final target (outside the project outline) is a Pickering emulsion using starch particles as stabilizing agents. As starch is a natural material, this kind of emulsion could favorable to a classic surfactant-stabilized emulsion, from economic, environmental and safety aspects.

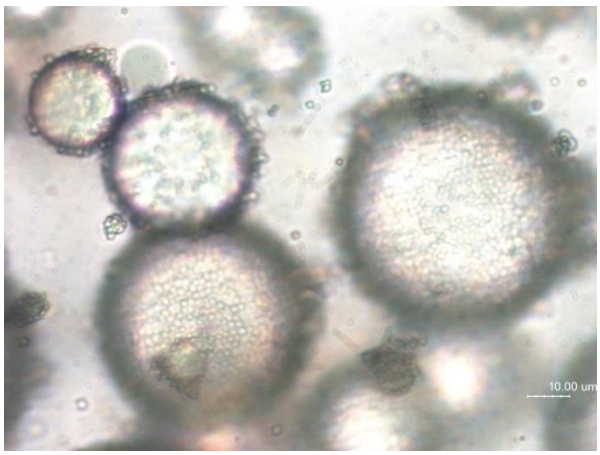

Figure 2.6. A Pickering emulsion with visible starch granules adhered to the surface of the oil droplets. The scale bar is 10 μm.

2.1.4 Emulsions in Topical Formulations

Emulsions are very well represented among skin care products and medical topical formulations, including *e.g.* sun screens, face creams, make-up and wound healing creams. In such products, the stability of the emulsion is of highest importance, both for shelf life but also for facilitating usage by the customer. To their advantage, emulsions allow mixing of desired ingredients that otherwise would not be compatible, and facilitates spreading and absorption[24]. An emulsion can be finely tuned to have the desired properties concerning viscosity, rheology, release of drugs and behavior on application. To achieve the desired properties, it is crucial to be aware of the behavior of the separate components but also of the combination of ingredients and how they affect each other. Solubility, melting points and chemical interactions are facts that help understanding these complex creations.

Other ingredients include humectants, co-solvents, surfactants, thickening agents for viscosity and texture, preservatives *etc.* Surfactants may be a source of skin irritation [1].

2.2 Triglycerides

Triglycerides (TGs) are some of the most common lipids. They are classified by Small [25] as "swelling amphiphiles", insoluble in water, that "spread to form a stable monolayer". Another name is triacylglycerol, relating to the structure which is three fatty acids esterified on a glycerol, as can be seen on the pictures in section 2.2.1. Unsaturated TGs have at least one double bond between two carbon atoms on one of the fatty acid chains, as opposed to saturated TGs which only have single bonds on the fatty acid chains. TGs are of great biological relevance as they are involved in many important processes in the body, but are also widely used in the food and pharma industry.

2.2.1 Structure

As mentioned above, TGs are esters composed of three fatty acids and a glycerol backbone. The fatty acids present in the TG are used for denomination. TGs that contain only one species of fatty acid are called monoacid TGs, and named after that fatty acid[26]. It then follows that a TG composed of *e.g.* three chains oleic acid can be called triolein, and the TG of capric acid termed tricaprin (or tridecanoin). However, most natural oils contain TGs that are made up of different fatty acids, so called mixed-acid TGs. The nature of these are complicated due to the differences between the fatty acids concerning chain length, saturation *etc.,* affecting physiochemical properties.

The triglycerides considered in this work were tricaprin, triolein and tristearin, all monoacid TGs. The structures of these TGs are shown below in figures 2.7, 2.8 and 2.9.

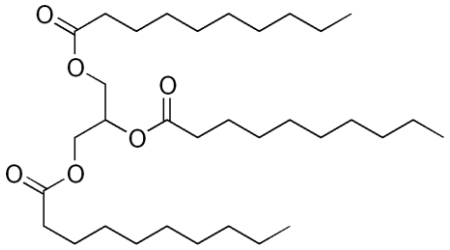

Figure 2.7. The structure of tricaprin, which is the triglyceride of capric acid (C10). Tricaprin is saturated as it has no double bonds between any carbon atoms.

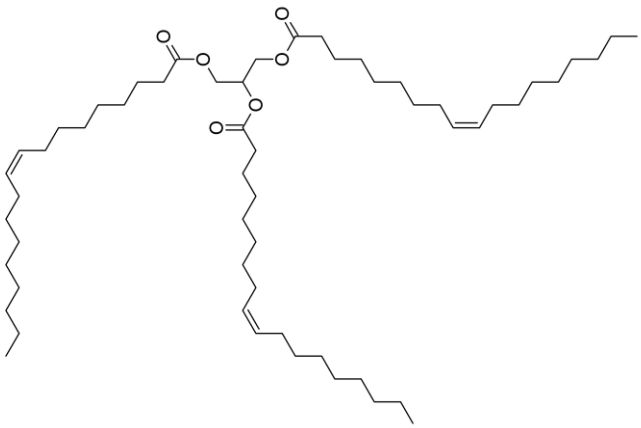

Figure 2.8. The structure of triolein, which is the triglyceride of oleic acid (C18:1). Triolein is unsaturated as it has a double bond between carbon atom 9 and 10 in the fatty acid chains.

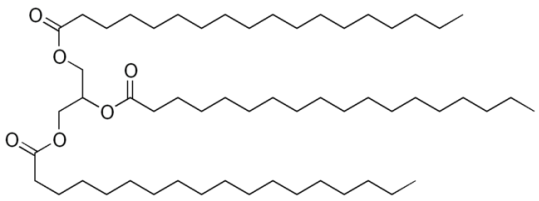

Figure 2.9. The structure of tristearin, which is the triglyceride of stearic acid (C18:0). Tristearin is saturated as it has no double bonds between any carbon atoms.

What can be noted about the TGs relevant for this work is that they differ in important ways. Triolein and tristearin differ from tricaprin in chain length of the fatty acids; 18 carbon atoms for triolein and tristearin as opposed to 10 carbon atoms for tricaprin. Furthermore, triolein differ from the other two in that its fatty acids contain a double bond between two carbon atoms, which makes it an unsaturated triglyceride. These differences will affect the melting point, density and other physiochemical properties of the TG, as will be discussed in the next section.

2.2.2 Properties

Several physical and chemical properties are relevant for working with these triglycerides. Density will determine the layering of mixtures with other liquids. The density of triglycerides tends to decrease with increasing temperature[27], and since this work studied TGs at two different temperatures, both values will be included. Solubility of TGs in various substances is of great interest, especially tricaprin which is soluble in warm ethanol, a component of the imagined emulsion this work strives towards. Finally, refractive index is useful to know for working with HPLC with a refractometer. These properties are presented in table 2.1.

Table 2.1. Physicochemical properties of the triglycerides used in this work.

Triglyceride	Melting point (°C)	Density (g/cm^3) at 20°C	Density (g/cm^3) at 40°C	Soluble in	Refractive index
Tricaprin	32 [28]	0.9304[29]	0.9203[30]	Warm ethanol	1.44466 [30]
Triolein	4 [28]	0.91[16]	0.8991[30]	Chloroform, ether, carbon tetrachloride, slightly soluble in alcohol [31]	1.46220[30]
Tristearin	73 [28]	0.87 at 70°C [32]	-	-	-

Generally, melting points of TGs are usually higher for those with longer chain length on the fatty acid (see C18:0 compared to C10 in Table 2.1), but also higher for saturated TGs (C10 compared to the unsaturated C18:1). A straight-chained TG will also have a higher melting point than a branched. Density is correlated to the packing efficiency of the molecule; an unsaturated TG will generally have a lower density than a saturated, as it is not as easy to pack these molecules. It is noteworthy that tricaprin has a melting point at 32°C, which is normally considered the temperature of the skin. This makes tricaprin interesting in topical formulations.

As ethanol is an ingredient in the phase diagram that is the objective of this work, physiochemical properties of ethanol is of interest. Some of these are presented below in Table 2.2. It can *e.g.* be noted that the boiling point of ethanol is very close to the melting point of tristearin. Densities of four aqueous mixtures of ethanol are presented in Table 2.3, for comparison to the triglycerides. The density of 55% and 70% ethanol in water is rather close to the density of triolein.

Table 2.2. Physicochemical properties of ethanol, for comparison to the triglycerides.

Melting point °C	Boiling point °C	Density (g/cm^3) at 20°C	Density (g/cm^3) at 40°C	Refractive index
-114.5[33]	78.3[33]	0.78945 [34]	0.77244 [34]	1.3614[33]

Table 2.3. Density of ethanol in aqueous mixtures, in concentrations relevant for the project.

Concentration of ethanol in water	Density (g/cm^3) at 20/20 °C
55wt%	0.90418
70wt%	0.86920
85wt%	0.83242
99.5wt%	0.79383

The absorbance of UV-light is relevant for HPLC analysis with UV-detection. Triglycerides tend to absorb strongly at 200-230 nm because of the ester group chromophore [35]. Marini and Marini observes that low wavelengths, 190–237 nm, is favorable because of the ester carbonyl group and isolated double bonds that are present [36].

2.2.3 Polymorphism

An important phenomenon among triglycerides is polymorphism. The knowledge of the occurrence of polymorphs in a certain TG is crucial to predict its behavior in applications. If a triglyceride has polymorphs, it can crystallize into several different crystalline states, rendering disparate melting points, melting enthalpies and densities [37]. The polymorphs existing within a TG do not have the same stability, and the phase transition between these polymorphs is irreversible below the melting point; they are in other words monotropic. Polymorphs are divided into three groups based on their subcell structure: α, β' and β [26].

- The α-form is the least stable form, which almost never occurs in some triglycerides. Its subcell structure is hexagonal (see Figure 2.10). This polymorph provides the lowest melting point.
- The β'-form is the intermediate state, slightly more stable than the α-form, with a higher melting point. The subcell structure is orthorhombic perpendicular.
- The most stable form is the β-form, which has a triclinic subcell packing. It has the highest melting point, and once the TG has crystallized into this form, it cannot shift into one of the other forms unless melted again.

Most triglycerides display an unstable α-form, and all have a stable β-form. Not all, but some, also enter one or several intermediate β'-forms. Generally, saturated monoacid TGs actually have two or more β'-forms. Triolein has up to three β'-forms [26]. Very rapid cooling or supercooling can cause the TGs to crystallize in their most unstable polymorph, the α-form.

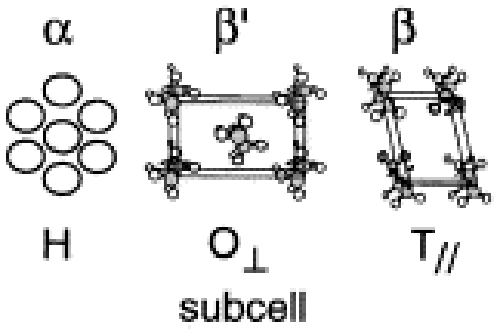

Figure 2.10. A schematic drawing of the three subcell structures: hexagonal (a), orthorhombic perpendicular (β') and triclinic parallel (β). Reprinted with permission from [38].

In Table 2.4, melting points are presented for the three different triglycerides used in this work, in their disparate polymorphs. As can be seen, the melting points differ quite a lot.

Table 2.4. Melting points in °C for the three triglycerides in their different polymorphs a, b' and b.[25, 28]

	α	β'	β
Tricaprin	-15	18	32
Triolein	-32	-13	4
Tristearin	54	64	73

The polymorphism of triglycerides can be studied by various techniques, such as X-ray diffraction and nuclear magnetic resonance (NMR). Furthermore, by DSC, it is possible to monitor the melting points, and from them assume which of the possible polymorphs that is present.

2.3 Phase Diagrams

To understand how the phase composition in a mixture changes with parameters such as temperature, pressure or components, phase diagrams can be employed. A phase is a part of a system (or the entire system) that is in a physically homogenous state, with a definite boundary towards other phases [39]. Phases can count *e.g.* as solid, liquid or gas phases, different crystal structures, or the dissolution of components in each other. Phase transitions are displayed in the diagrams as boundaries where a phase changes its physical state (*e.g.* from solid to liquid) or two phase separated components become one phase.

Phase diagrams are governed by Gibb's phase rule, based on thermodynamic equilibrium. It states the number of degrees of freedom, *i.e.* the number of parameters that affect the number of phases present. Gibb's phase rule gives the degrees of freedom, *f*, as **Eq. 2.3** [40]:

$$f = c - p + 2 \qquad \textbf{(2.3)}$$

where *c* is the number of components and *p* is the number of phases that can be present. The number 2 comes from the possibility to vary both pressure and temperature. Phase diagrams can be created as a function of *e.g.* composition-composition, composition-temperature or composition-pressure. Gibb's phase rule sets the criteria for the possibilities of drawing a specific phase diagram. For example, if three components are considered and a two-dimensional diagram

is to be employed, temperature and pressure needs to be fixed. Hence, for a ternary system, $c = 3$ and **Eq. 2** is written as $f = 3 - p + 2$. With temperature and pressure kept constant, two degrees of freedom are reduced and $f = 3 - p$. It follows that when two phases are present, f=3 -2 =1, monovariant, and when there is only one phase, f=3-1=2, bivariant.

The so called tie lines are lines drawn within a two-phase region towards the boundary of the region, with the ends representing the composition of the two phases [39]. The lever rule can be used in two-phase regions with tie lines to calculate the composition of each phase. The lever is imagined along the tie line:

$$\text{Phase percent} = \frac{opposite\ arm\ of\ lever}{total\ length\ of\ tie\ line} \cdot 100$$

A composition-composition phase diagram with three components and assuming constant temperature and pressure usually takes the form of an equilateral triangle, a ternary phase diagram [41]. In such a diagram, the corners of the triangle represent a pure component (100% of the component) and it follows that the amount of each component can be determined by measuring the height of a line perpendicular to the opposing base, from the base towards the point of interest. It is important to note that the percentages of the components must add to unity. This is demonstrated in Figure 2.11. The tie lines remain in the plane of the triangle as the temperature and pressure are defined.

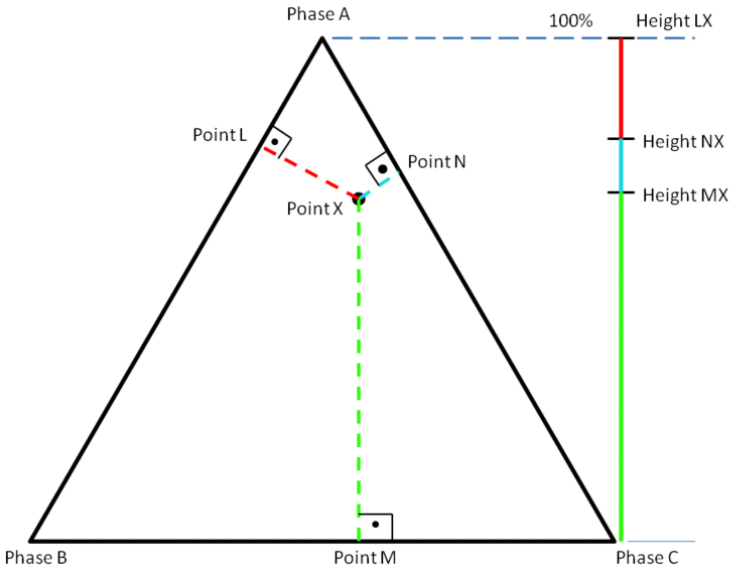

Figure 2.11 Calculations of the compositions of the components in a ternary phase diagram is made by measuring the height of a line perpendicular to the base opposing the point of the component of interest. The heights must add up to unity.

It is common to use ternary phase diagrams in systems where two of the components are miscible in the third, but not in each other. The result of such a diagram can be similar to the one in Figure 2.12. In Figure 2.12, there is a tie line in the two-phase region, and the composition of each phase in the green point can be calculated by using the lever rule towards the blue and red point, and reading the compositions at those points.

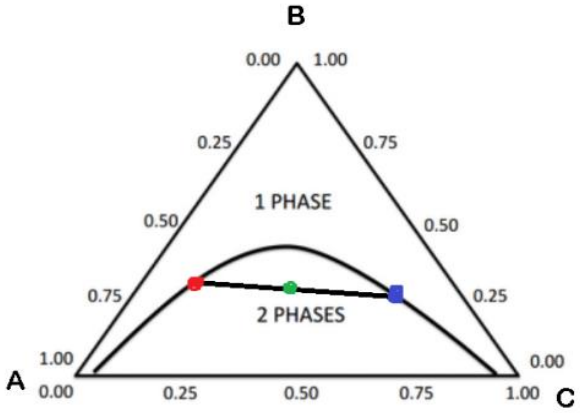

Figure 2.12. An example of a ternary phase diagram where both component A and component C are completely miscible in component B, but not in each other. The composition of the two phases in the green point can be read from the compositions in the blue and red points, using the lever rule.

2.4 Methods

Three main techniques were used in this work for analyzing the samples of oil-ethanol-water. Two of them fall within the group of thermal analysis, and the other was high performance liquid chromatography (HPLC) for content analysis.

2.4.1 Thermal Analysis

Thermal analysis is a general name for techniques used for studying how the properties of a material changes with temperature. One example, which was used in this work, is thermogravimetric analysis (TGA). The sample is placed on a pan, and a program is set to change the temperature. The instrument then measures the weight loss of the sample on the pan as temperature increases. This way, an indication of sample content can be obtained, if the boiling points of the constituents are known.

Differential scanning calorimetry (DSC) is a simple yet powerful instrument that can provide valuable information. It is based on measuring the heat flow of a sample as the temperature is altered in a controlled way. From these results, conclusions can be drawn about melting point, phase transitions and other thermal events. For triglycerides, it is further of interest for evaluating the crystalline state, as the melting point is changed when the triglyceride occurs as another polymorph.

Some definitions are useful when working with thermal analysis:

- The *onset* of a peak (*e.g.* an endo/exothermic melting peak) is defined as the point where the first tangent of the peak intersects with the extrapolated baseline (see point 1 in Figure 2.13). This point is generally used for pure samples as it shows the temperature where the start of *e.g.* a melting occurs.
- T_{max} is the temperature at the maximum of a peak. See point 2 in Figure 2.13.
- The enthalpy ΔH is found by integrating the peak and thereby retrieving the area. This provides information about the amount of energy required to melt or crystallize the sample.

- The *endset* of a peak can be used when dealing with a mixture, e.g. when monitoring a melting of a solid+liquid into a liquid, as it shows where the entire sample has become liquid. The endset is defined as the point where the second tangent of the peak intersects with the extrapolated baseline, see point 3 in Figure 2.13.

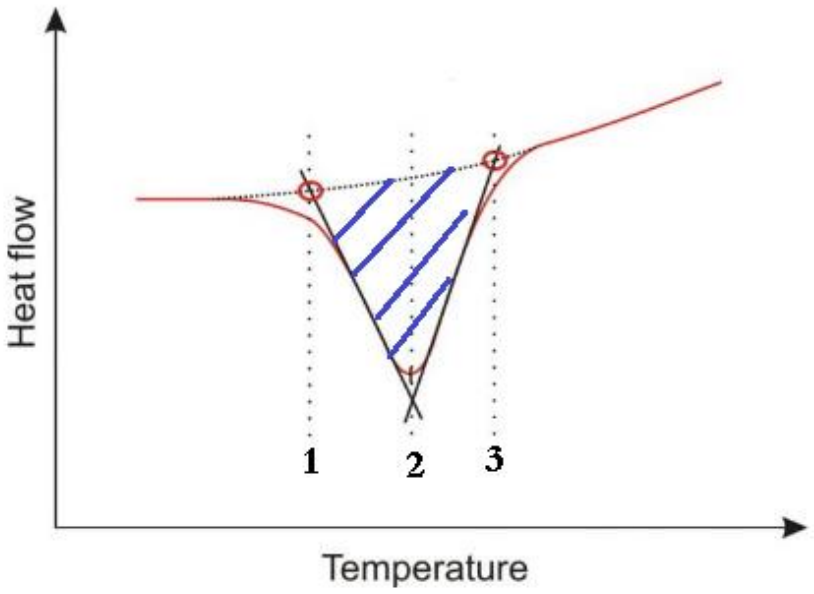

Figure 2.13. Point 1 represents the onset, i.e. *the intersection of the peak tangent and the extrapolated baseline. Point 2 is the peak temperature or* T_{max}. *Finally, point 3 corresponds to the endset, the intersection of the baseline and the second tangent of the peak. The enthalpy is defined as the area under the peak, marked with blue.*

2.4.2 High Performance Liquid Chromatography

High performance liquid chromatography (HPLC) uses the interactions between substances in a sample and the stationary phase (the inside material of a column) to separate the sample components from each other. [42] A pump is used to flush a mobile phase with high pressure through the column, carrying the sample. Substances that interact more with the stationary phase will stay longer in the column and therefore have a larger retention time, i.e. taking longer time to pass through the column. When the substance has passed through the column, it is detected by *e.g.* an ultra-violet (UV) photodetector, measuring the absorbance of the sample, or a refractive index (RI) detector, detecting the changes in refractive index in the passing stream. The detection is presented in a chromatogram, showing the signal from the detector over time, resulting in peaks when passage of a substance that is not the mobile phase.

HPLC was first developed with the stationary phase being more polar than the mobile phase, causing the more hydrophilic components of the sample to elute later than hydrophobic molecules [35]. When instead the mobile phase is polar (hydrophilic) and the stationary phase non-polar, the term is reverse-phase (RP) chromatography. In other words, hydrophobic compounds such as lipids will in RP-HPLC interact more with the stationary phase and have a longer retention time. For triglycerides, RP-HPLC is the most effective method[43].

Hence, the choice of column material and mobile phase is crucial for achieving adequate results. The mobile phase flow can be isocratic, i.e. having the same composition throughout the experiment, or a gradient can be chosen. However, a gradient flow is not appropriate for RI-detection. The UV-absorbance spectrum of the mobile phase also needs to be considered. Acetone, for example, is not suitable as mobile phase for UV-detection of triglycerides as it absorbs strongly in the same region as the TGs [35].

The usage of HPLC for triglycerides is widely documented. Gas chromatography (GC) is another popular method for triglyceride analysis, but suffers from the problem of decomposition of the TGs at the required high temperatures[36]. High temperatures are not required for HPLC which is an apparent advantage in this case.

Normally, the triglycerides will be separated based on their equivalent carbon number (ECN) which is calculated as[43]

$$ECN = CN - 2n$$

where *CN* (carbon number) is the total number of carbon atoms and *n* is the number of double bonds per triglyceride.

RI-detection appears frequently in the literature in analysis of triglycerides. However, this detector is generally more insensitive than a UV-detector, and needs to be operated at a specific temperature, but can be useful when experiencing that the ester chromophore in the TGs is too weak for UV-detection. Another advantage is that some solvents that cannot be used for UV-detection, such as acetone or chloroform, can be used in RI-detection, and these may be necessary when working with triglycerides of longer chain lengths that are difficult to dissolve. Common mobile phases for RI-detection of triglycerides are acetone/acetonitrile [44-46], propionitrile [36, 47] and acetonitrile/tetrahydrofuran/methanol[48, 49].

Triglycerides normally adsorb at 200-230 nm as mentioned in section 2.2.2, so this is an appropriate wavelength for UV-detection. The higher sensitivity of UV-detection is a strong advantage for using this detection method. Ethanol/acetonitrile, water/acetonitrile and tetrahydrofuran are common mobile phases for UV-detection[50]. The method used for this work was obtained by Phenomenex (CA, USA) as an application for identifying triglyceride in vegetable oil, and is further described in the Materials and Methods section.

3. Materials and Methods

3.1 Reference Samples

The experimental procedure was divided into separate parts – preparation of reference samples, and later, analysis of these samples. The reference samples were prepared at an early stage of the work, to allow for equilibration, and in the meantime optimization of an analysis method was carried out. Ethanol (Ethanol absolute TechniSolv®, 99.8%, VWR Chemicals, Radnor, Pennsylvania, Lot 16H024012) was prepared in four different weight concentrations: 55%, 70%, 85% and 99.8% by mixing 99.8% ethanol and distilled water in appropriate amounts. The ethanol-water-mixture was then mixed, in room temperature, with triglyceride at a 50-50% weight ratio in NMR-tubes, as the visual inspection was believed to be facilitated in these long and thin tubes. Triglycerides of oleic acid (Captex® 1000, Abitec Corporation, Janesville, USA, Lot number 151207-6, 90.2% purity) and triglycerides of capric acid (Captex® GTO, Abitec Corporation, Janesville, USA, Lot number 140618-7, 98.5% purity) were used for this purpose. As the triglyceride of capric acid is solid at room temperature, it was heated in a water bath until melting occurred (at about 33°C), before it could be mixed with the ethanol.

After mixing, the samples were shaken by hand to facilitate contact between all phases of the sample. They were then placed in the dark in a specific temperature: room temperature (RT, 21°C), 32°C or 40°C. These temperatures were chosen for relevance; room temperature is the natural storage and handling temperature for a final product, 32°C resembles skin temperature where the product is to be applied, and 40°C is a typical stability study temperature.

The procedure was then repeated, using ampoules instead of NMR-tubes, because these had a larger volume (about 1.5 ml), facilitating proper mixing which was difficult in the narrow NMR-tubes. A set of samples were also created in 2 ml HPLC-vials with plastic screw lids, for quick use in the work of optimizing the analysis method. The composition and storage temperature of each tube is presented in the tables below. Note that the weight ratio and triglyceride used is specific and constant for each table.

Table 3.1. The composition and storage temperature of each tube, tube number in italics. For these samples, a 50:50% weight ratio of oil: ethanol/water was used, and the triglyceride was triolein (C18:1). This denomination was used both for reference samples and samples used for early analysis.

EtOH conc. / **Storage temp. °C**	**55wt%**	**70wt%**	**85wt%**	**100wt%**
RT	*1*	*2*	*3*	*4*
32	*5*	*6*	*7*	*8*
40	*9*	*10*	*11*	*12*

Table 3.2. The composition and storage temperature of each tube, tube number in italics. For these samples, a 50:50% weight ratio of oil:ethanol/water was used, and the triglyceride was tricaprin (C10). This denomination was used both for reference samples and samples used for early analysis.

EtOH conc. / **Storage temp. °C**	**55wt%**	**70wt%**	**85wt%**	**100wt%**
RT	*13*	*14*	*15*	*16*
32	*17*	*18*	*19*	*20*
40	*21*	*22*	*23*	*24*

A third oil, triglycerides of stearic acid (Dynasan® 118, IOI Oleochemicals, Hamburg, Germany), was initially a part of the project. However, due to its high melting point (73°C), which is very close to the boiling point of ethanol (78°C) the mixing of the two compounds was evaded to avoid boiling of ethanol. Furthermore, the oil in its solid form comes as a powder, which proved to be very difficult to transfer to small vessels such as NMR-tubes or small ampoules, so mixing at a lower temperature was close to impossible. For these reasons, the triglyceride of stearic acid was thereafter excluded from the project.

At a later stage, reference samples with a weight ratio of 30% oil and 70% ethanol/water, and furthermore, 70% oil and 30% ethanol/water and were created to obtain more points for the phase diagram. As it was later decided that the phase diagrams were only to be created for tricaprin at 40°C and 25°C, and for triolein at 25°C, the last samples were only mixed accordingly. The mixing procedure was identical to the above described, however only ampoules were used this time. The composition and storage temperature of each tube is presented in table 3.3-5. Note that the weight ratio and triglyceride used is specific and constant for each table.

Table 3.3. The composition and storage temperature of each tube, tube number in italics. For these samples, a 30:70% weight ratio of oil:ethanol /water was used, and the triglyceride was triolein (C18:1). This denomination was used both for reference samples and samples used for early analysis.

EtOH conc. / **Storage temp. °C**	**55wt%**	**70wt%**	**85wt%**	**100wt%**
RT	*25*	*26*	*27*	*28*
32	*29*	*30*	*31*	*32*
40	*33*	*34*	*35*	*36*

Table 3.4. The composition and storage temperature of each tube, tube number in italics. For these samples, a 30:70% weight ratio of oil:ethanol/water was used, and the triglyceride was tricaprin (C10). This denomination was used both for reference samples and samples used for early analysis.

EtOH conc. / **Storage temp. °C**	**55wt%**	**70wt%**	**85wt%**	**100wt%**
RT	*37*	*38*	*39*	*40*
32	*41*	*42*	*43*	*44*
40	*45*	*46*	*47*	*48*

Table 3.5. The composition and storage temperature of each tube, tube number in italics. For these samples, a 70:30 % weight ratio of oil:ethanol/water was used, and the triglyceride is noted in the left column. This denomination was used both for reference samples and samples used for early analysis.

EtOH conc. / Storage temp. °C & TG	55wt%	70wt%	85wt%	100wt%
RT, triolein	*49*	*50*	*51*	*52*
RT, tricaprin	*53*	*54*	*55*	*56*
40, tricaprin	*57*	*58*	*59*	*60*

3.2 Visual Inspection

Photos of the reference samples were taken with a mobile camera (Sony Xperia Z5 Compact) at several points;

- Directly after mixing
- After shaking of the samples
- 24h after preparation
- Weekly throughout the project

Changes in appearance were noted. When necessary, additional photos were taken of interesting events.

3.3 Content Analysis

The exact contents of the phases in the samples were analyzed by two methods; thermogravimetric analysis (TGA) and high performance liquid chromatography (HPLC). The reason for using two methods was to be able to compare the results and to some extent evaluate the use for TGA in content analysis. Before analysis, the ampoules were broken and the separate phases were transferred by pipette into 2 ml HPLC vials and kept at their specific temperature. The samples were expected to phase separate into one hydrophilic phase (water and ethanol) and one hydrophobic phase (triglyceride) or in some samples merge into one single phase.

3.3.1 Thermogravimetric Analysis (TGA)

As a first indicator of the amount of oil in the ethanol phase, thermogravimetric analysis of the ethanol phase was carried out. Pure ethanol and pure triglycerides were also analyzed for comparison. As the TGA provides a result that states the weight content remaining at a certain temperature, and the temperature at which ethanol, water and oil evaporates differs from each other, some information about the sample content could be obtained.

30 µl of sample was transferred by pipette from the ethanol phase in the vials to a platinum pan. In the case of the samples that were put in another temperature than room temperature, they were transferred in their specific temperature from the oven to the instrument and then the sample was taken and placed in the pan.

The following program was used on the instrument (TGA Q500, TA Instruments, New Castle, USA):

- Start at **25°C**
- Isothermal for 1 min
- Ramp 10°C/min to **600°C**
- Isothermal for 5 min

3.3.2 HPLC

To obtain more detailed information about the oil content in the ethanol phase, the ethanol phase was analyzed by HPLC with UV detection at 208 nm, the system consisting of a degasser, binary pump, column compartment and standard autosampler (1100 series, Agilent, Santa Clara, United States), photodiode array detector (G1315A – 1100, Agilent, Santa Clara, United States). A Kinetex® (Phenomenex, Torrance, California, United States) 2.6 μm C8 100 Å column (150 mm×4.6 mm) was used. The method was isocratic with a flow rate of 1.5 ml/min and the mobile phase comprised of acetonitrile/water (87:13). Sample injection volume was 10 μL. The procedure was carried out at room temperature for all samples.

A calibration curve for tricaprin was obtained by creating standard samples of oil in ethanol at 100, 50, 25, 12.5, 10.0, 5.0, 2.5, 1.25 mg/ml and measuring the peak area for each concentration. Dissolved tricaprin in ethanol was kept at 40 °C before put in sample holder at room temperature. A calibration curve was calculated by plotting the peak area against the concentration and running a linear fit on the curve, resulting in

$$a = r \cdot c$$

Where c is the concentration of oil, a is the response (peak area) and r, the slope, was calculated from the linear fit.

A calibration curve for triolein proved to be more difficult to create as triolein did not dissolve in ethanol, or in the mobile phase. It was found that it dissolved, in small amounts, in acetone/acetonitrile (60:40) and in isopropanol. However, continuous problems with the HPLC measurements such as peak tailing and fronting, combined with an inconsistency in peak appearance and retention time failed further measurements. A reference sample was bought and tested (Sigma-Aldrich) but the result from this sample differed from the earlier results of the used triolein to such an extent that the results were inconclusive.

Moreover, it should be mentioned that troubles with the column marked all measurements after the initial measurements on tricaprin. The same consistent and reliable results did not appear again, and suspicions arose that the column was somehow damaged or that the method was not adequate for this application. Unfortunately, there was not enough time to resolve these problems. This is further discussed in the Discussion section.

To determine the ethanol content in the hydrophilic phase, to be able to know whether any ethanol had dissolved into the lipophilic phase, RI-detection was used on the same system as before. The same mobile phase and column was employed, but the flow rate was decreased to 1.0 ml/min to avoid high pressure.

3.4 Construction of Phase Diagram

The data from HPLC and TGA measurements, combined with pictures of the ampoules, were used for plotting the phase diagrams, starting with separate points and using the lever rule (described in Theory section).

A phase diagram was created for tricaprin at 40°C and 25°C, and for triolein at 25°C.

3.6 Melting Point Analysis

To evaluate the effect of ethanol on the melting temperature of the oil, the oil phase of the samples was analyzed by differential scanning calorimetry (DSC). The pure triglycerides were also analyzed separately for comparison. Furthermore, information about polymorphism could be obtained from the DSC results.

The carrier gas was nitrogen at 80 ml/min. Calibration was done with indium. The samples were about 10-15 mg, placed in hermetically sealed 40 μl aluminum pans. An empty pan (air) was used as reference. The following program was used on the instrument (DSC 1, Mettler Toledo, Greifensee, Switzerland):

- Start at **25°C** for the RT samples and 40°C for the 40°C samples
- Cooling at 10°C/min to **-60°C**
- Isothermal for 2 min
- Heating at 10°C/min to **60°C**
- Isothermal for 2 min
- Cooling at 10°C/min to **-60°C**
- Isothermal for 2 min
- Heating at 10°C/min to **60°C**

The second round of heating was chosen to evaluate any changes of melting temperature when the sample was rapidly cooled.

4. Results

4.1 Visual Inspection

At the final point of visual inspection, after two months, all samples had separated into two phases except for samples 24, 40, 48 and 60. The samples that entered single phase all proved to have been mixed with 99.8% ethanol (no water) and tricaprin, in various ratios. It should be noted, however, that sample number 40 was in a single phase in the ampoule before entering a HPLC-vial for analysis, but the TG crystallized quickly after the transfer, as can be seen in Figure 4.6 and 4.7. This was also the only sample that was stored in room temperature that seemed to enter a single phase.

Furthermore, the samples that were of triolein mixed with lower concentrations (55% and 70%) of aqueous ethanol seemed to form some kind of emulsion after mixing and some shaking. In some samples, this disappeared after storage, but for other samples this behavior remained, which hindered separation of the phases by pipette for analysis. This feature is shown in Figure 4.1 below, in sample 1 (far left), sample 5 (ampoule number five from left) and sample 9 (ampoule number 9 from left).

What also can be noted is that all samples with tricaprin were mixed in melted state (above 35°C), but then samples 13-16, 37-40 and 53-56 were stored in room temperature, causing the triglyceride to recrystallize. This formed some interesting crystals, as seen in Figure 4.5-4.8, especially Figure 4.8 and sample 56 (far right).

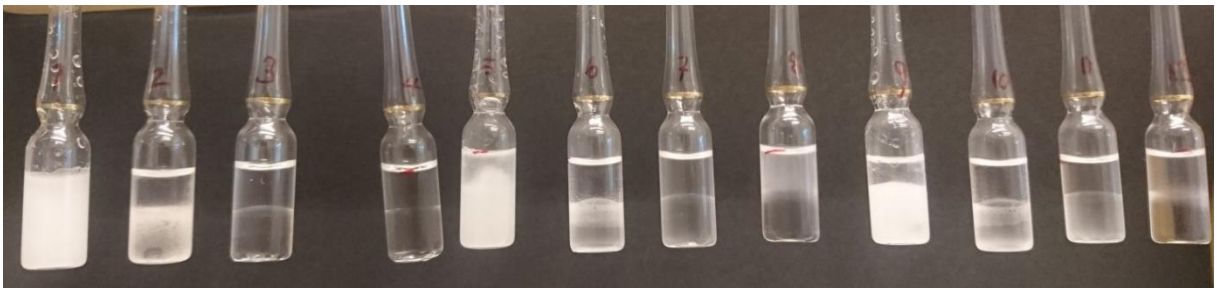

Figure 4.1. A picture of the emulsion-like mixtures that arose after mixing of triolein with lower concentrations of ethanol (55% and 70%). Note especially ampoule number 1, 5 and 9 from left, forming an emulsion-like state.

Below selected pictured of the samples in ampoules are presented. The single phase samples, 40, 24, 48 and 60, are noted in the text, in Figure 4.6 and 4.9-4.11.

Figure 4.2a. Samples 1-4(triolein, TG:aqueous ethanol ratio 50:50) just after mixing in ampoules. 4.2b. Samples 1-4 two months later, before analysis.

Figure 4.3a. Samples 25-28 (triolein, TG:aqueous ethanol ratio 30:70) just after mixing in ampoules. 4.3b. Samples 25-28 two months later, before analysis.

Figure 4.4. Samples 49-52 two months later, before analysis. Unfortunately, the picture just after mixing was lost.

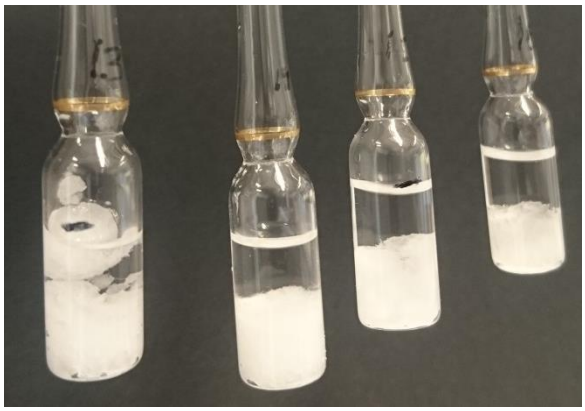

Figure 4.5 Samples 13-16 after two months, before analysis. Unfortunately, the picture just after mixing was lost, but the lipid was liquid during mixing.

Figure 4.6a. Samples 37-40 (tricaprin, TG:aqueous ethanol ratio 30:70) just after mixing in ampoules. 4.6b. Samples 38-40 two months later, before analysis. Sample 40 has one single phase.

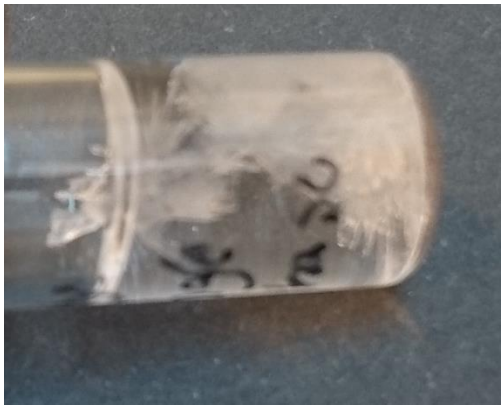

Figure 4.7. Sample 40 which crystallized after transfer from the ampoule to the HPLC-vial.

Figure 4.8. Samples 53-56 after two months, before analysis. Unfortunately, the picture just after mixing was lost. Note the crystal in sample 56.

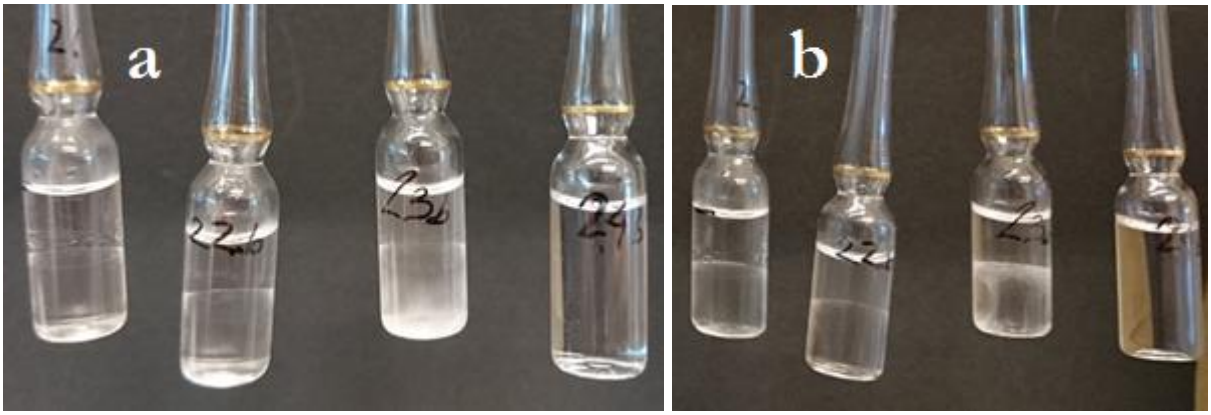

Figure 4.9a. Samples 21-24(tricaprin, TG:aqueous ethanol ratio 50:50) just after mixing in ampoules. 4.9b. Samples 21-24 two months later, before analysis. Sample 24 has one single phase.

Figure 4.10a. Samples 45-48(tricaprin, TG:aqueous ethanol ratio 30:70) just after mixing in ampoules. 4.10b. Samples 45-48 two months later, before analysis. Sample 48 has one single phase.

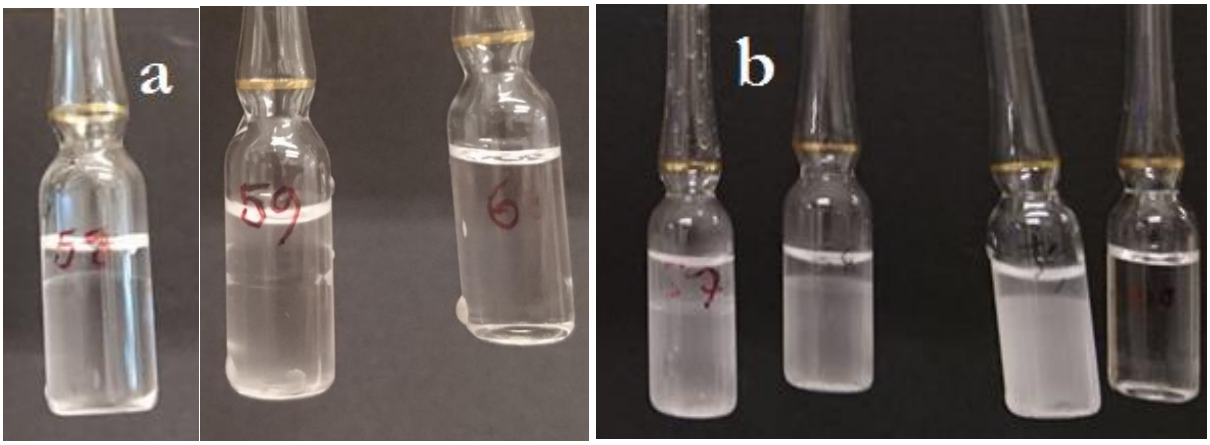

Figure 4.11a. Samples 58-60 (tricaprin, TG:aqueous ethanol ratio 70:30) just after mixing in ampoules. 4.11b. Samples 57-60 two months later, before analysis. Sample 60 has one single phase.

4.2 Content Analysis

After the visual inspection to determine the phase behavior, the separate phases were further examined by TGA and HPLC. The results from these measurements were used to create a phase diagram, showing the phase behavior of the triglyceride-ethanol-water system. Raw data from these measurements is available in Appendix.

From the TGA data, a typical result from one of the samples (ethanol phase) was a graph as in Figure 4.12, showing the remaining weight percent at a certain temperature. A rapid weight loss is evident below 100°C, and then there is another decrease in weight where the remains of the sample evaporates, at about 300-400°C. The weight percent remaining after the primary decrease was assumed to be the weight percent of oil in the sample.

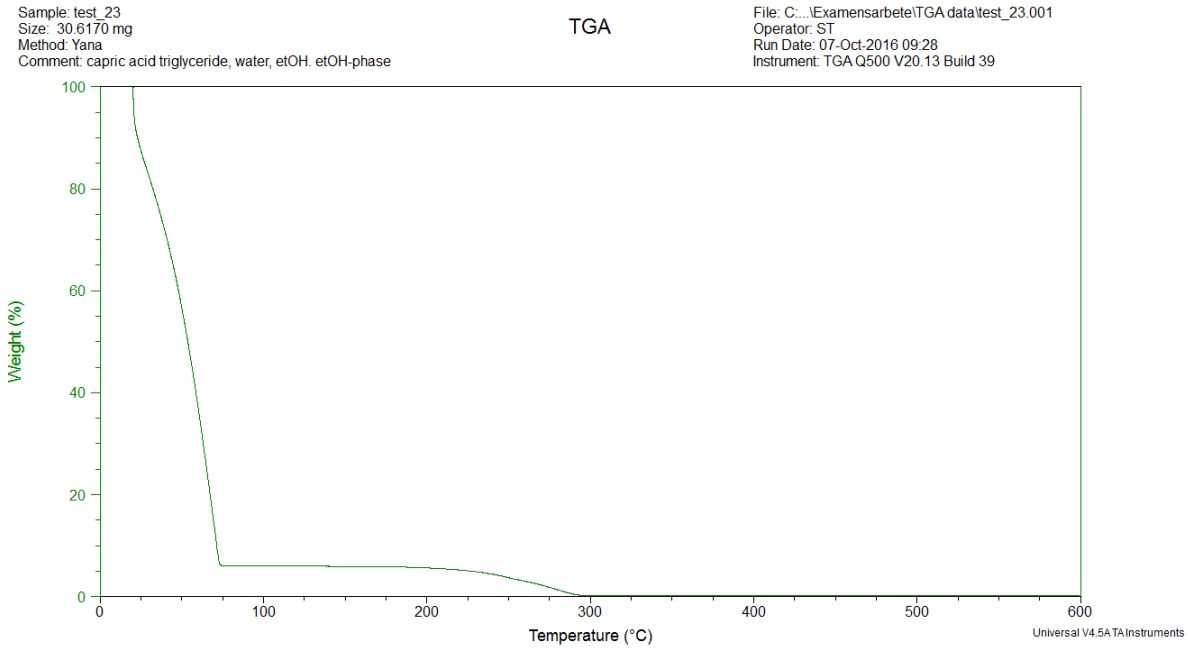

Figure 4.12. An example of the results obtained with TGA. A rapid decrease in sample weight is seen before 100°C, after which about 5% of the sample weight remains. At 300°C, the rest of the sample is evaporated.

This result can be compared with the graph of the weight loss of pure triglyceride, which is presented in Figure 4.13, in which all sample is evaporated at about 350°C.

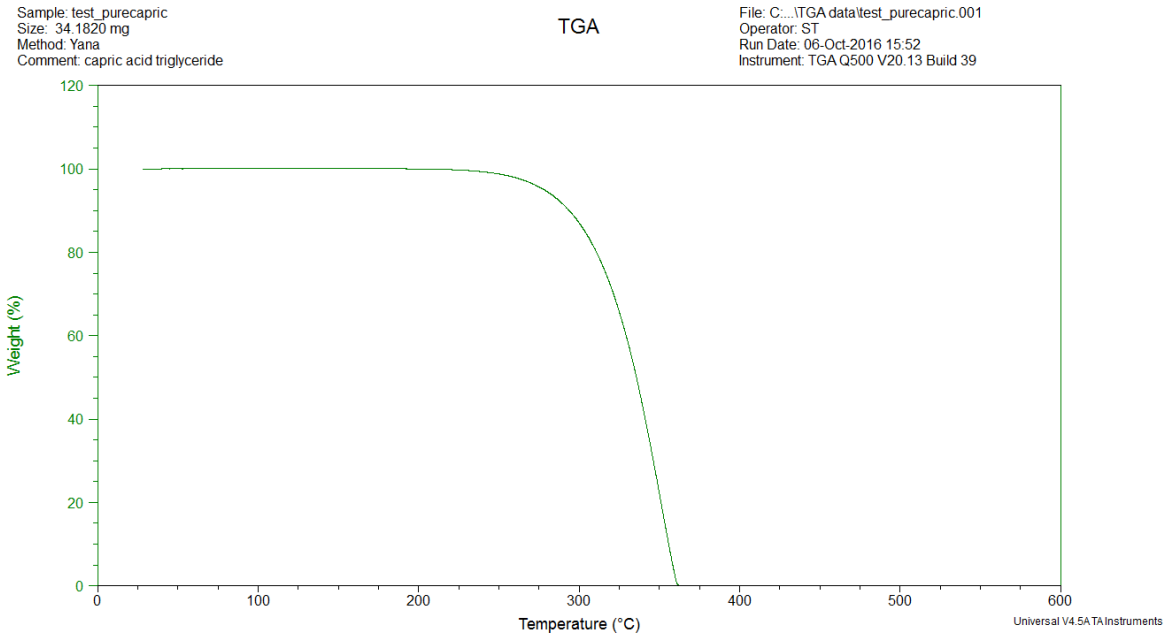

Figure 4.13. TGA analysis of a pure triglyceride, in this case tricaprin. Sample weight reaches zero at about 350°C.

As the HPLC analysis was not successful for the measurements of tricaprin at 40°C or for triolein, either due to column problems or inaptness of the method for this specific application, TGA data was used for creating the phase diagrams shown in Figure 4.14-16. The data from HPLC-UV and calibration curves are available in the appendix. Furthermore, the ethanol content determined by HPLC-RI was in many cases improbable, for example stating ~40% ethanol in a pure ethanol sample. The data from these measurements are included in the appendix. Hence instead of using the HPLC-RI values, the ethanol content in the hydrophobic phase is assumed to be the same as the lipid content in the hydrophilic phase, as the ratio between the phases in pictures 4.1-4.11 seem to be the same as in the initial mixtures. This is based on the lever rule and discussed further in the Discussion section.

In Figure 4.14, the phase diagram for triolein at 25°C is depicted. The majority of the phase diagram consists of a two-phase region, bur at high concentrations of ethanol, a single phase is formed. The points in light gray represent the composition of the reference samples, and the darker triangles represent the composition in the hydrophilic phase. All samples mixed in this project are in the two-phase region. The upper line is dashed as the exact curvature of the region boundary is not known.

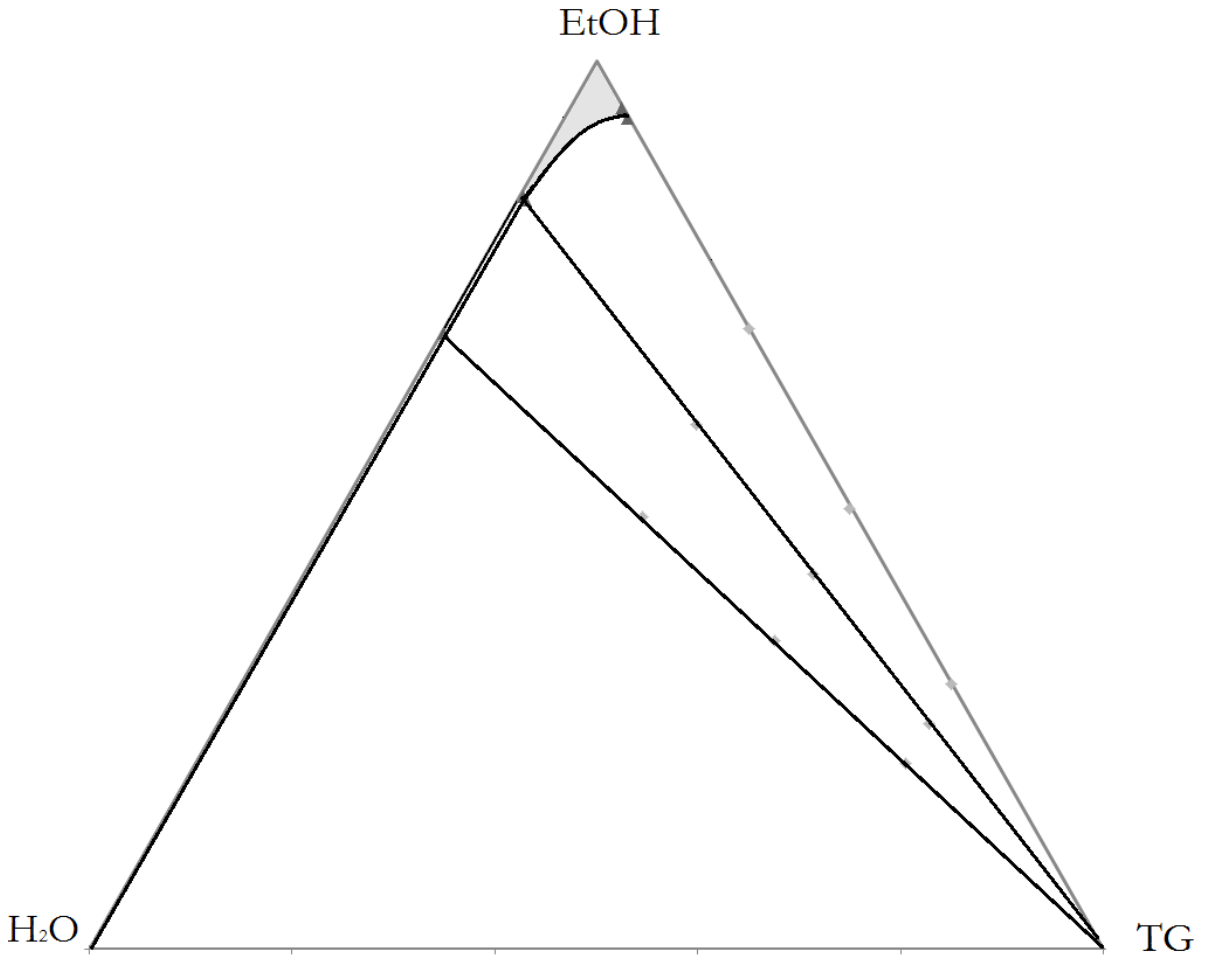

Figure 4.14. Phase diagram for triolein-ethanol-water at 25°C. All samples mixed in this project (points shown in light gray) showed phase separation. The single phase region is shown in light gray.

Tricaprin proved to be slightly more soluble. The phase diagram of tricaprin-ethanol-water at 25°C is shown in Figure 4.15. The points in blue represent the composition of the reference samples, and the green triangles represent the composition in the hydrophilic phase. The single-phase region (in light gray) is a little larger than for triolein, but still only exists for high concentrations of ethanol and small amounts of triglyceride. The upper line is dashed as the exact curvature of the region boundary is not known. Still, all mixed samples were in the two-phase region.

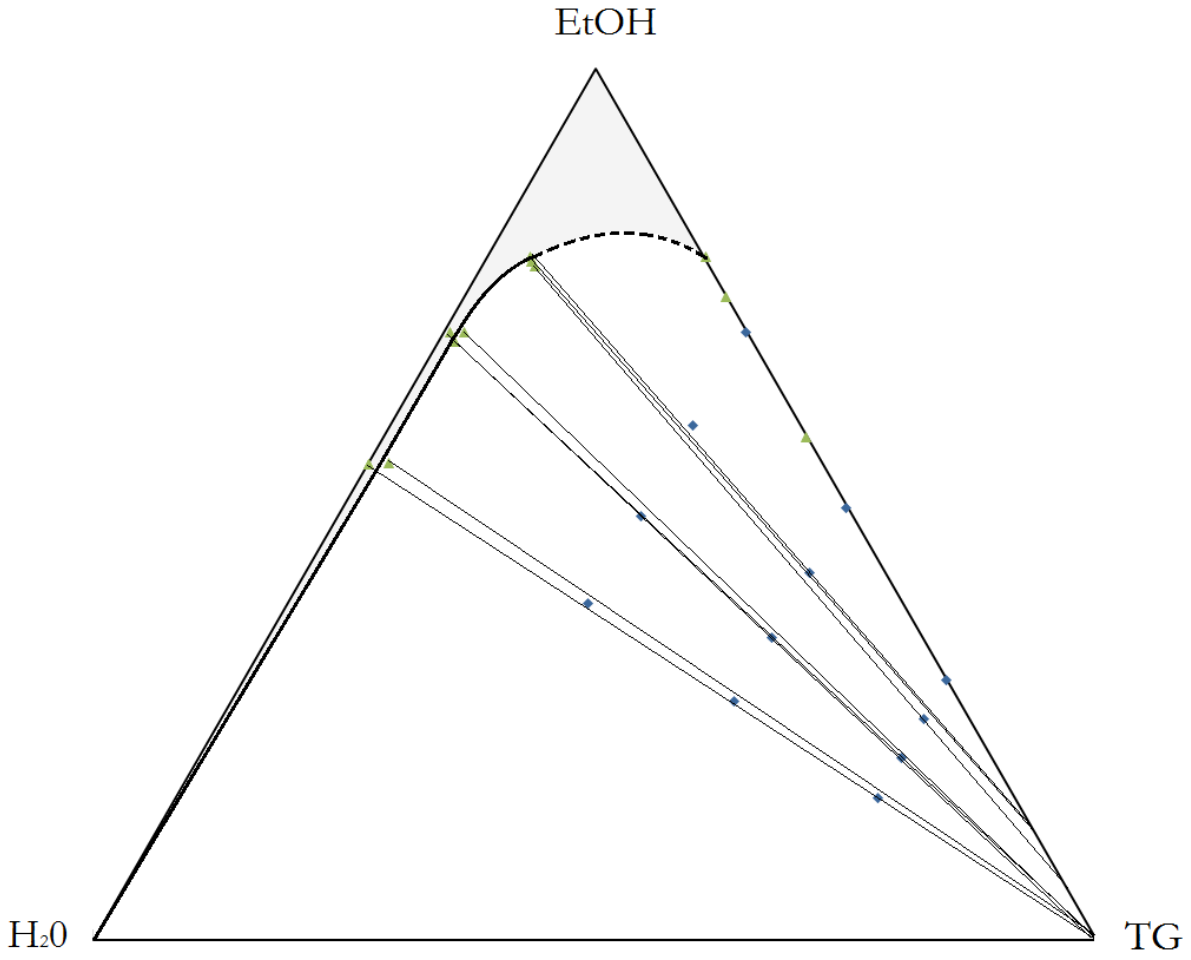

Figure 4.15. Phase diagram for tricaprin-ethanol-water at 25°C. All samples mixed in this project (points shown in blue) showed phase separation. The light gray region at the top of the triangle represents the single phase region.

When the temperature was elevated to 40°C, tricaprin was more soluble in ethanol. In Figure 4.16, the single-phase region (in light gray) has now extended along the right leg of the phase diagram. The line is dashed as the exact point where tricaprin is soluble in aqueous ethanol is not known. The three reference samples with 99.8% ethanol all formed a single phase, and all others phase separated.

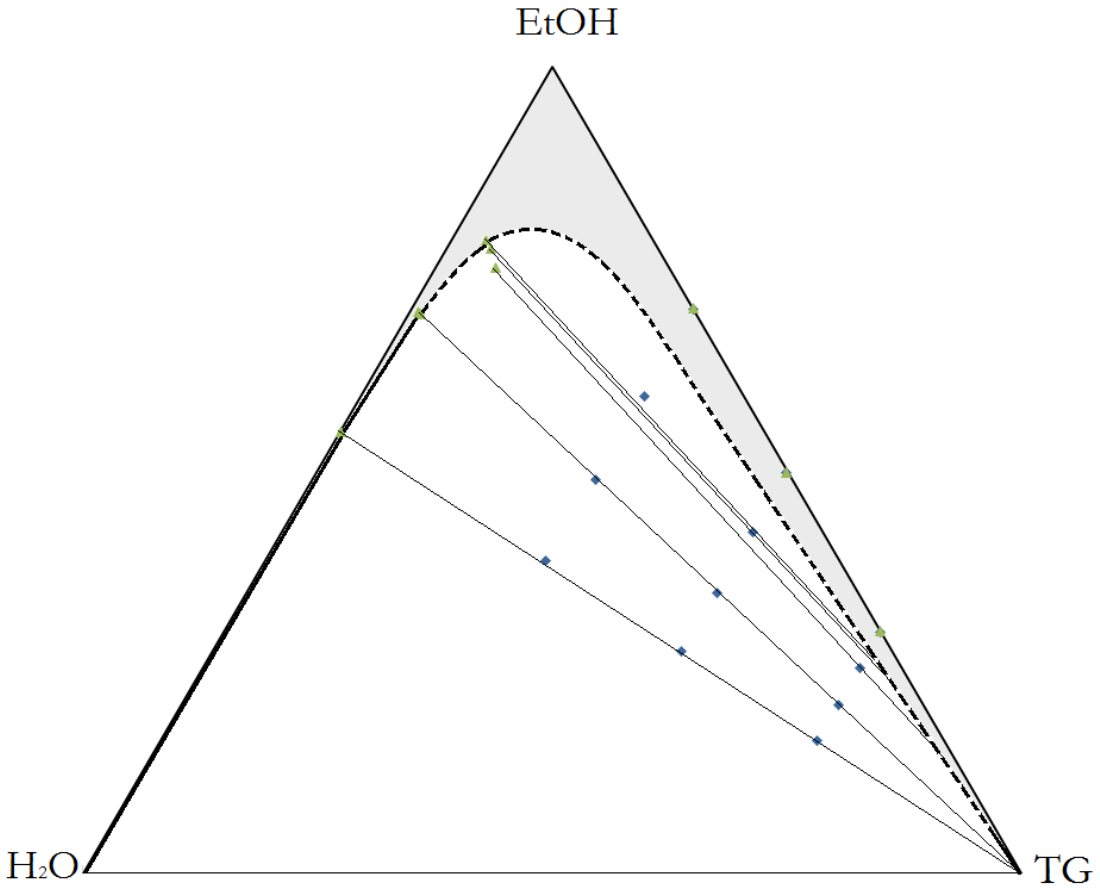

Figure 4.16. Phase diagram for tricaprin-ethanol-water at 40°C. Samples mixed in this work with 99.8% ethanol were in one single phase (light gray region), all others had phase separated into phases according to the diagram.

4.3 Melting Point Analysis by DSC

After DSC measurements, both onset and endset of the melting peaks were noted for comparison. The impact of the amount and concentration of aqueous ethanol on the melting point of the lipid phase was evaluated. In Figure 4.15-4.17 results are displayed as the melting point of the lipid phase as a function of the amount of triglyceride in the initial sample mixture. In each diagram, separate curves for different ethanol/water concentrations are presented for comparison. For some samples of 55% ethanol, no data is available as it was not possible to separate the phases from each other, so the lipid phase could not be analyzed.

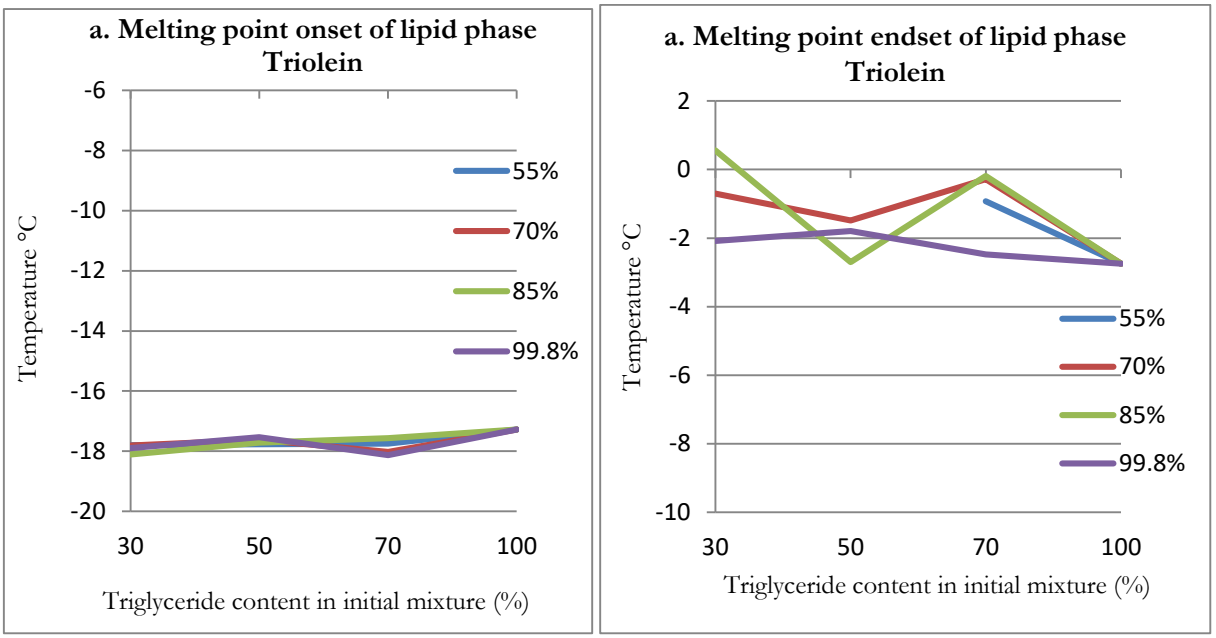

Figure 4.15a-b. Melting points of triolein in the lipid phase of the ethanol-water-TG-mixtures, at different ratios of TG:aqueous ethanol, Figure a showing onset and b endset. Separate curves are shown for different concentrations of ethanol in water (55%, 70%, 85% and 99.8%). The endset seems to vary to a greater extent than onset. In figure b, results are missing from the samples with 55% ethanol because the phases were impossible to separate.

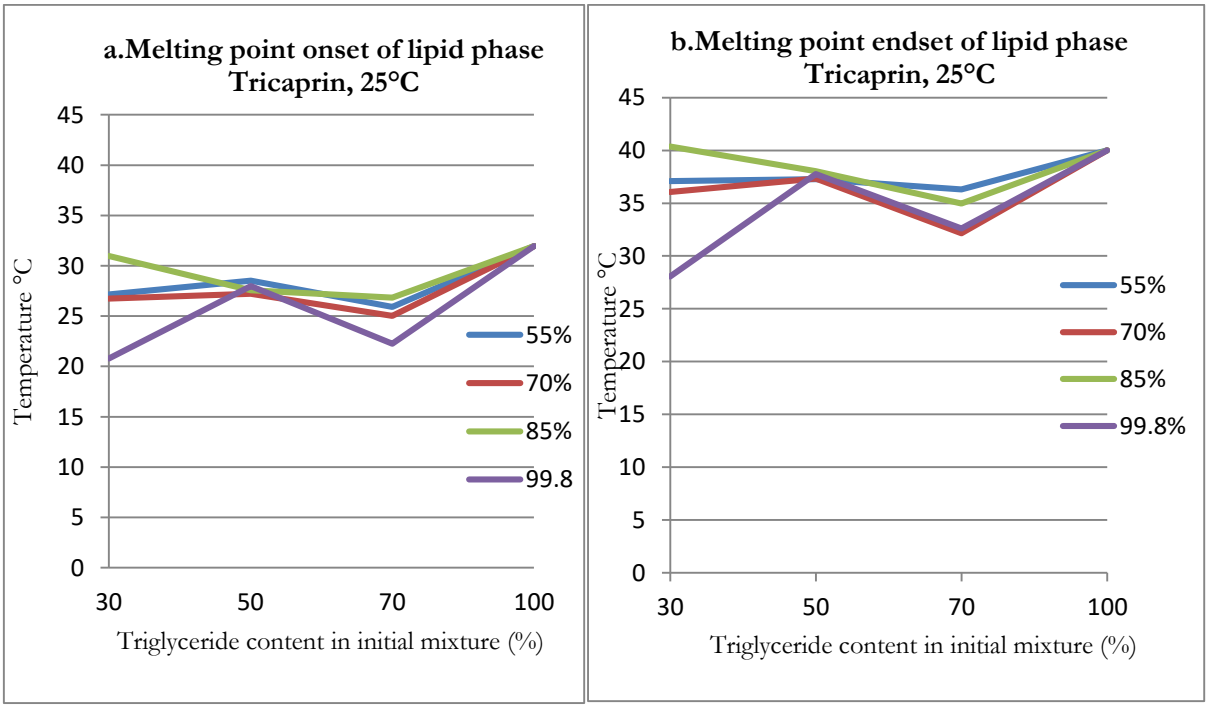

Figure 4.16a-b. Melting points of tricaprin (stored at room temperature) in the lipid phase of the ethanol-water-TG-mixtures, at different ratios of TG:aqueous ethanol, Figure a showing onset and b endset. Separate curves are shown for different concentrations of ethanol in water (55%, 70%, 85% and 99.8%). The endset seems to vary to a greater extent than onset, and a higher concentration of ethanol in the mixture seems to depress the melting point.

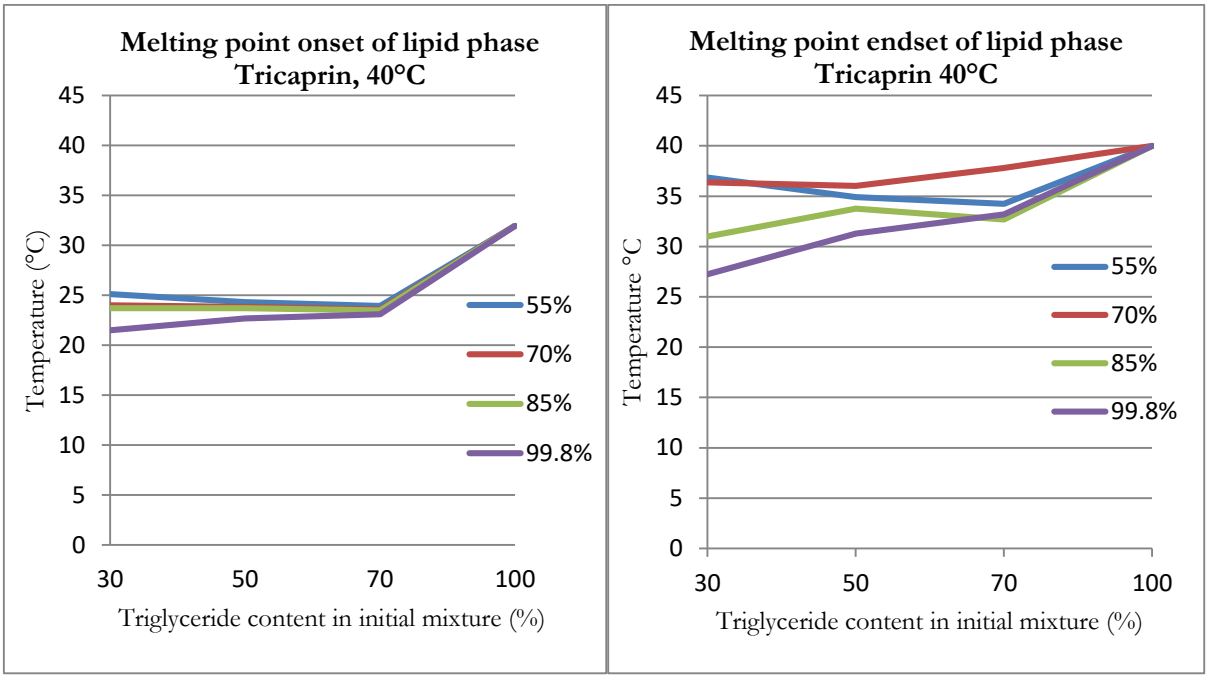

Figure 4.17a-b. Melting points of tricaprin (stored at 40°C) in the lipid phase of the ethanol-water-TG-mixtures, at different ratios of TG:aqueous ethanol, Figure a showing onset and b endset. Separate curves are shown for different concentrations of ethanol in water (55%, 70%, 85% and 99.8%). The endset seems to vary to a greater extent than onset, and a higher concentration of ethanol in the mixture seems to depress the melting point.

All DSC measurements were done in two following heating rounds. This was motivated by the interest in any existing polymorphs created by rapid cooling. However, the melting point did not differ significantly between the two rounds. Below are two examples of the result achieved from the DSC measurements, for sample 58-60. The curves in similar colors but different shades represent the same sample at the first and second round of heating, the darker shade being the first round and the lighter shade being the second. It is apparent that the melting event occurs at lower temperature for higher concentration of ethanol in the sample, but the melting event is very similar in the first and second round.

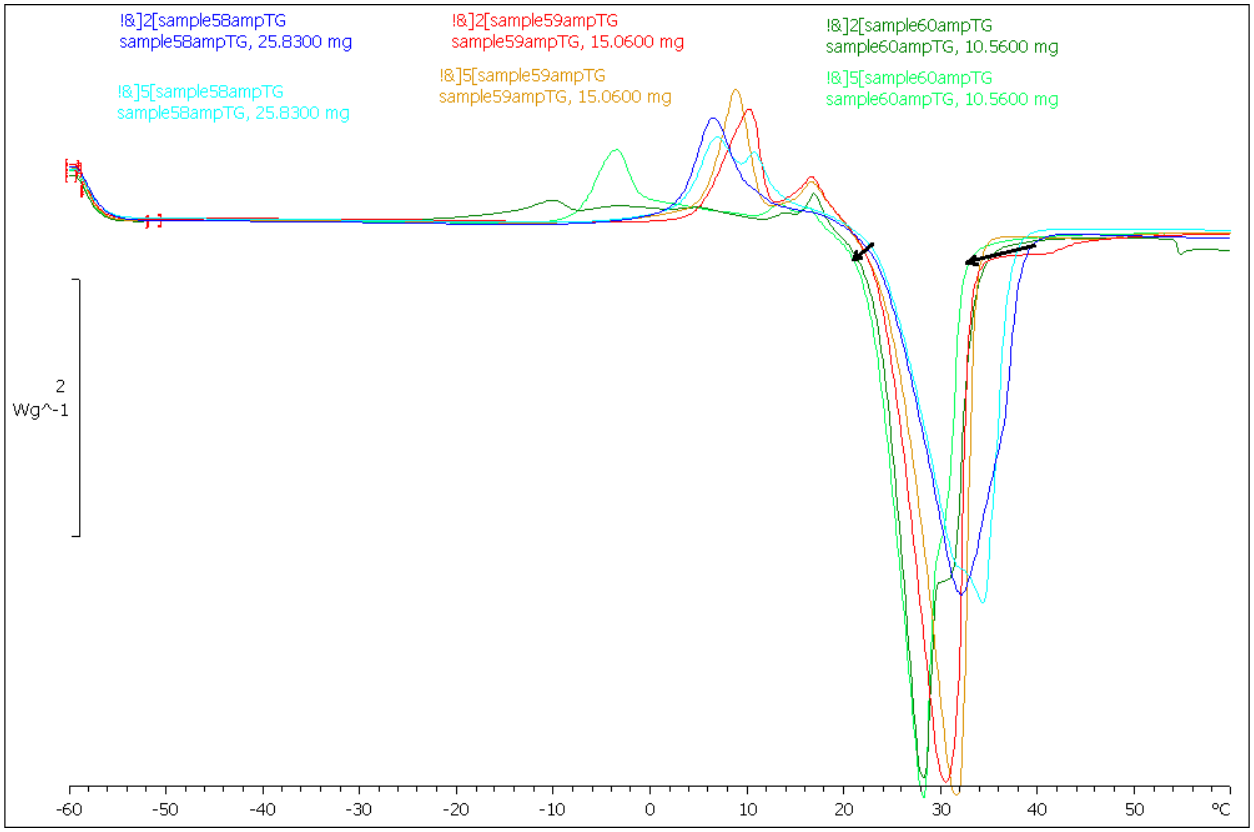

Figure 4.18. The endothermic event of melting of tricaprin. The samples represented are 58 (shades of blue, first round in dark blue and second round in turqouise), 59 (shades of red-orange, first round in red and second round in orange) and 60 (shades of green, first round in dark green and second round in light green), with 70, 85 and 99.8% ethanol concentration, respectively, and the initial mixture was 70:30 TG:ethanol. It is clear that the melting event is of similar shape during both rounds of heating and that the melting event occurs at a lower temperature with higher ethanol concentration, as indicated by the arrows.

In Figure 4.19, this effect on melting point becomes even clearer when only comparing sample 58 and 60.

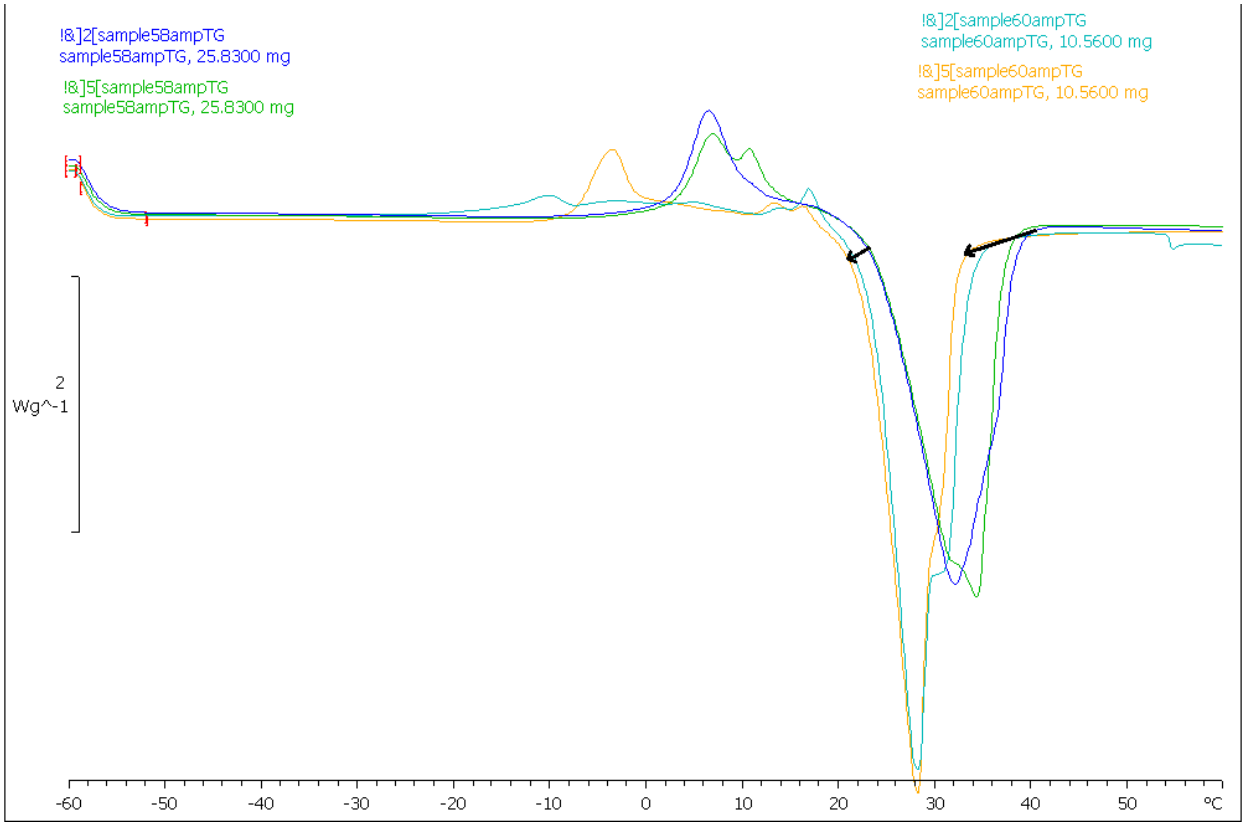

Figure 4.19. Same picture as above but with only sample 58 (70% ethanol) and sample 60 (99.8% ethanol) for even more clarification that the melting event is of similar shape both rounds of heating and that the melting event occurs at a lower temperature with higher ethanol concentration.

5. Discussion

5.1 Behavior of System

The overall results correspond well to literature; tricaprin dissolves in warm ethanol (99.8% is assumed to be comparable to pure ethanol), as mentioned in the Theory section. The solubility is higher for higher temperatures, as the single phase region is larger in the phase diagram at 40°C than at 25°C. Concerning triolein, only very small amounts are found in the hydrophilic phase. This can be related to the structure of the molecules. The longer chains in triolein are bulkier and contribute to the lower solubility.

Contemplating these results with a hand sanitizing product in mind, some observations are in order. The interesting part of the diagram is in the region with concentrations of ethanol at 60-80% and smaller amounts of triglyceride, about 10-30%. As the triolein dissolved in smaller amounts in the aqueous ethanol, at first sight this might be the more interesting alternative of the two. Moreover, the melting temperature of triolein is in a more suitable handling range, as it can be expected to be liquid at most handling temperatures, avoiding the introduction of polymorphs that can arise during supercooling. The melting range of tricaprin can on the other hand be problematic as it is around skin temperature, and also the solubility of tricaprin in ethanol seems to increase at these temperatures. If other triglycerides than triolein and tricaprin should be considered, two structural properties would be desirable; long chain length on the fatty acid to decrease solubility, and at least one double bond to keep down the melting point.

The boundaries between the single and two-phase regions are drawn with dashed lines in all phase diagrams. This is because assumptions were made about curvature and, in the case of tricaprin at 40°C, ethanol concentration at the phase boundary. However, it is known that it is somewhere between 85% and 99.8% ethanol, as these two samples were included in the work. Furthermore, triglycerides are insoluble in water according to Small [25], so it can be assumed that the tie lines do not intersect lower axis, as the lipid phase probably does not contain any water. It should be noted, however, that this merely an assumption.

With respect to equilibration in the system, reference samples were created at an early stage so that as much time as possible would pass before breaking them. Clearly there is still a question whether the system had enough time to equilibrate. For more accurate results, longer time would probably be proper. Samples 1, 25 and 49 (Figure 4.1-4.4) can be mentioned as examples. They were all stored in room temperature, containing triolein and 55% aqueous ethanol. It was impossible to separate the phases in these samples, as they never left the emulsion-like state that arose after mixing the components. It should be noted that all samples were emulsion-like directly after mixing but entered two phases after certain time had passed. It is therefore possible that this would have occurred in these samples as well, had longer time been given. The reason that just these samples require longer time might be that the density of triolein at room temperature and 55% aqueous ethanol are very similar (about 0.9g/cm^3, described in Table 2.1 and 2.3), so they do not separate easily with only gravitational force. A solution might have been to centrifuge them, however, this is difficult without breaking the glass ampoules.

Another strange observation occurred in sample 40 where at first a single phase was observed and then, upon transfer, the lipid crystallized, without other external change. An explanation may

be that the single phase solution was oversaturated and therefore the act of transfer induced enough change for the lipid to precipitate.

5.2 Melting Point Effects

The first question that arises in this contest is whether to use onset or endset of the endothermic melting peak to compare to the pure triglyceride. A common view, as revealed in the Theory section, is that onset is generally used for pure substances, whereas for mixtures, it is the endset that is monitored, as it gives the point where the entire sample has melted to liquid, even if not the whole sample melts at once. With this logic, it should in this case be the endset that is relevant. For triglycerides, however, it makes sense to discuss a melting *range* rather than a melting *point*, and the reason for this became apparent in this work; there is rather big difference between onset and endset. Nevertheless, the important point here is that the melting range, both endset and onset, seem to be lowered after the triglyceride has encountered ethanol. Since DSC measurements show a change in melting range, it can be assumed that some ethanol is present in in the oil phase, and more so in the samples with high concentration of ethanol, as they seemed to have the lowest melting range.

However, a comparison with literature values may be problematic as the triglycerides used were not entirely pure. Deviating melting points arises the question of polymorphism, which was the reason for running two identical rounds of DSC at each sample. For tricaprin the curves look similar for both rounds and the temperature corresponds well to the literature value of the β-form at 33°C [28], so there is no reason to suspect any presence of polymorphs from these measurements. Triolein, on the other hand, displays a rather deviating melting temperature, lower than the literature value of the most stable β-form at 4°C [28], but higher than the other polymorphs. Here as well, the curves have similar shape and events for both rounds. The same went for the triolein obtained from the manufacturer, without being mixed with aqueous ethanol. The explanation might be found in the specifications of the purchased triglyceride. Actually it only contained 90.2% oleic acid; 2.8% were stearic acid and 4.3% linoleic acid, and this might form an eutectic that lowers the melting point, as different triglycerides are present. Unfortunately, the melting point for triolein was not determined by the manufacturer so there is no reference value for this exact product.

Despite the shorter chain length of tricaprin, the fact that it is saturated gives it a significantly higher melting range than triolein. It is interesting to observe the melting point of tricaprin, as it is very close to skin temperature, around 30°C. For topical formulation purposes, this could result in a formulation that involves solid matter which melts upon skin application. However, if the ethanol decreases the melting point, this needs to be considered, as the product then may face a melting range around room temperature, especially in tropical areas.

Finally, some strange events were observed in some samples; an exothermic peak just before onset, and in a few samples there were "double" peaks in the melting event. The exothermic peak is probably a part of the sample that crystallized later for some reason. The double peaks could be due to a large sample size, thus the melting occurs far away and it takes time before the event is registered in the sensor.

5.3 Lab Procedure

To obtain a more detailed phase diagram, more mixtures of different ratios could have been created, and also a wider range of concentrations of ethanol could have been tested. However, these points were chosen with respect to the time frame. With the future application in mind, mixtures with a lower percentage of triglyceride content could be chosen.

The challenge in this project turned out to be the content determination of the separate phases. It was agreed upon to analyze the oil content in the ethanol phase as this seemed most feasible with the available equipment. The HPLC-UV method that was used was obtained from the company also providing the column, and had been used for analyzing triglyceride content in vegetable oils. However, it did not seem to be entirely appropriate for this application. After several successful measurements with tricaprin (successful referring to achieving expected retention times and concentrations, and clearly defined peaks with no tailing or fronting), triolein measurements were initiated. This was when the problems started; we experienced peak tailing and fronting, loss of peaks, unexpected peaks, low reproducibility and a significant increase in column pressure which sometimes lead to leaks. All following results were implausible and did not correlate to the initial successful results with tricaprin. This gives rise to suspicions that the triglyceride somehow interfered with the column material, maybe due to too high concentrations of triglyceride (a few percent) in the sample. Another explanation could be some kind of degradation of the analyte (triglycerides), as there in some measurements appeared a large number of peaks when the sample should only contain two or three components. One example may be hydrolysis of triglycerides into mono- and diglycerides. However, degradation should have appeared at the first measurements already, which makes this explanation seem less likely. Thirdly, the method may just need further development for this application concerning flow rate, mobile phase or calibration.

Further problems were then encountered with the HPLC-RI method for ethanol determination. In the end it was decided not to use the results from HPLC-RI for ethanol content, and there were several reasons for this decision. First of all, as mentioned in the Results, it can be compared with the height ratio between the phases in the pictures. If tie lines are drawn with the ethanol concentration values from HPLC-RI it would mean that a large amount of ethanol dissolves into the oil phase, and then the ratio between the phases would change dramatically from the ratio they were actually mixed in from the beginning. Looking at pictures 4.1-4.11 and measuring the height of the phases, it can be concluded that this has not happened. Thus it is reasonable to assume that the same amount of ethanol has dissolved into the oil phase as the amount of oil that has dissolved into the ethanol phase. However, it is difficult to evaluate the exact ratio of the phases in the samples that contain solid triglycerides (Figure 4.5-4.8), so this may be a source of error. Secondly, the method seemed especially inappropriate for higher concentrations of ethanol; the calibration curve (available in the appendix) showed a linear behavior up to about 70% ethanol, but thereafter the signal seemed to be saturated. Since many samples contained 70% ethanol or higher concentrations, the results for these samples were unreliable. Finally, it also comes down to evaluating the HPLC-RI data against both TGA and HPLC-UV. For example, in one sample, which contained only 99.8% ethanol and tricaprin, the triglyceride content in the hydrophilic phase determined by HPLC-UV was 11% (25% by TGA), and the ethanol content was 41%. This only adds up to 50-60%, and as there were no other

components, one of the results must be faulty. Taking the reasons listed above into consideration, it was decided against using the HPLC-RI data.

As HPLC measurements did not turn out as well as expected, more focus was turned towards TGA, which initially was thought of merely as an indication of interesting samples. There is an obvious error source with the method for content analysis in this context; the high volatility of ethanol. Before measurement starts, the sample is placed by pipette on the pan. It then takes some time before the sample is actually weighed, time during which a substantial amount of ethanol has evaporated. This leads to a seemingly higher percentage of oil in the sample, as even a small loss of ethanol will represent a significant percentage in the small sample sizes employed. This was clearly demonstrated in the samples which were only in one phase. For example, the sample where 50% was triglyceride and 50% was ethanol of 99.8% concentration, the result from TGA claimed an oil percentage of 61%, and where 30% was triglyceride and 70% was ethanol of 99.8% concentration the TGA showed an oil percentage of 55%. The exact error in the TGA measurements may be further investigated by weighing the samples before placing them on the pan, and then comparing this number to the starting weight achieved by the TGA scales. It would also be appropriate to use pans with closed lids, to avoid the evaporation of liquids.

5.4 Future studies

A continuation of this work would primarily include development of the HPLC-method for content determination. This might involve testing other variants of HPLC, such as refractive index detection for triglyceride analysis (not for the ethanol content determination), which has been frequently employed in the literature (see Theory section). To obtain more correct tie lines, either the method of determining ethanol amount in the ethanol phase must be developed, or a method for finding the ethanol content in the lipophilic phase can be created. The latter faces problems with very small amounts of ethanol in a high amount of triglyceride, which at least for HPLC poses problems with balancing detection limit against column interference by the lipid.

A more detailed mapping of the phase boundaries, in this work only assumed with dashed lines, would be appropriate. Creating more mixtures of different ratios in the interval of interest and analyzing the content of these would be a way towards this. This could also be of help in the work of closer determination of the tie lines.

Another interesting study would be to use X-ray diffraction to look at the triglycerides at different points in the phase diagram, to further evaluate the possibility of polymorphism and any phase transitions occurring concerning polymorphs.

Concerning the final cream, the first step would be creating a recipe for the Pickering emulsion containing ethanol and evaluate its stability and properties, such as rheology, viscosity and temperature dependence. The antimicrobial effect of this cream, and the ethanol concentration required to acquire this effect, needs to be examined.

6. Conclusions

The background of this work is the objective of studying the compatibility of triglycerides, ethanol and water in emulsions for hand sanitizing purposes. To obtain understanding of the interaction of the separate components, the goal was to create a phase diagram for two different triglycerides, tricaprin and triolein. As tricaprin had a melting range around 30°C, its phase behavior when liquid, at 40°C, was also studied.

All samples showed phase separation except for those containing tricaprin and ethanol of 99.8% concentration, stored at 40°C, which entered a single phase. It was found that only small amounts (a few percent) of triglyceride dissolved into the hydrophilic phase. Triolein had lower solubility in ethanol than tricaprin, thus being a more interesting candidate for the emulsion. Its melting range around 0°C is also more appealing and easy to handle than the, however interesting, possible problematic melting range of tricaprin at about 30°C.

The melting range of the triglycerides, or more correctly, the lipophilic phase, proved to lower slightly after being mixed with ethanol and then phase separated. This implicates that the lipid phase also contained some ethanol. No polymorphism was observed.

The work was marked by difficulties with the analytical techniques used. However, this provided the opportunity to evaluate the results of different techniques employed for the same purpose. The use of thermogravimetric analysis, where weight loss of the sample at increasing temperature, is promising for this use but faced problems with evaporation, providing a significant error source. The alternative, HPLC, is a more sensitive method which, tuned more correctly towards the specific application, could deliver accurate results. Unfortunately, the time to optimize the method was not enough during this work, hence the unreliable results from this technique. The results obtained from HPLC and TGA combined, however, provided enough indications to create a fair picture of the behavior of the triglyceride/ethanol/water-system.

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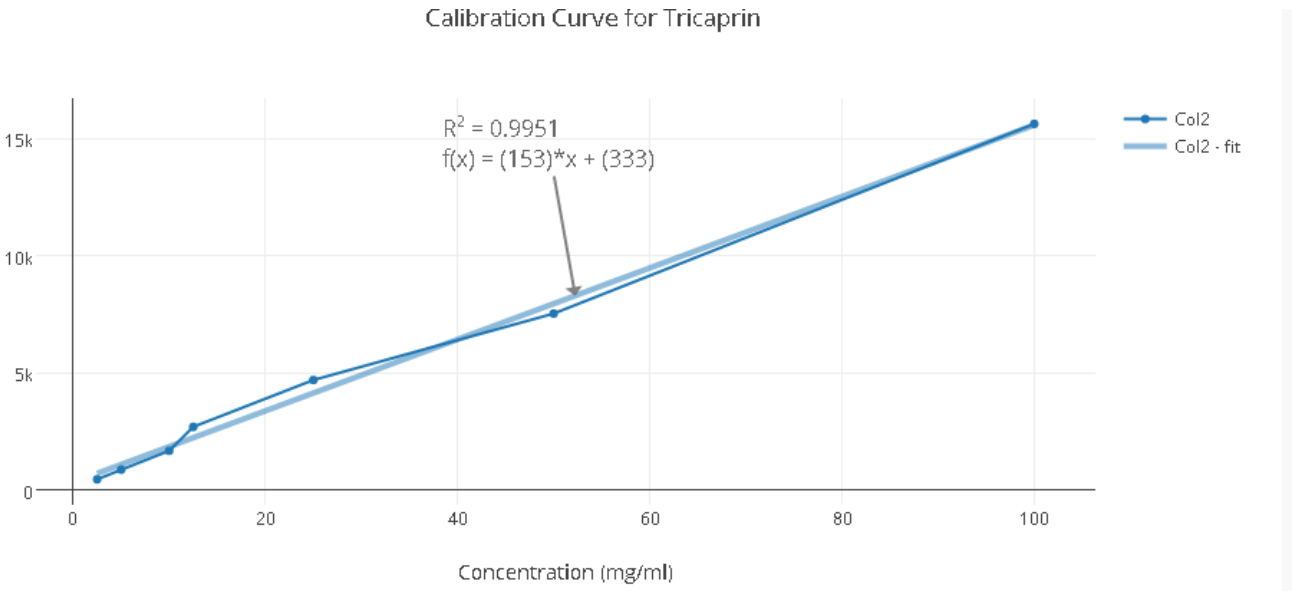
Appendix

TGA for triglyceride content

Sample	**% remaining at 100°C**
Pure tricaprin	100
13	0.1
14	0.5
15	4.2
16	25.0
21	0.1
22	1.0
23	4.9
24	61.0
37	1.5
38	1.5
39	3.2
40	19.0
45	0.1
46	0.9
47	5.7
48	55.0
53	0.3
54	2.2
55	4.7
56	45.0
57	0.1
58	0.7
59	3.7
60	**80**
Sample	**% remaining at 100°C**
Pure triolein	100
1	-
2	0.10
3	0.50
4	5.70
25	-
26	0.10
27	0.55
28	4.80
49	-
50	0.20
51	0.30
52	5.90

HPLC-UV for triglyceride detection

Calibration sample (mg/ml) tricaprin in ethanol	Peak Area
100	15640.2
50	7540.7
25	4703.1
12.5	2703.1
10	1683.36
5	876.8
2.5	462.6
1	-
0.5	-
Calibration Curve:	**153x+333**

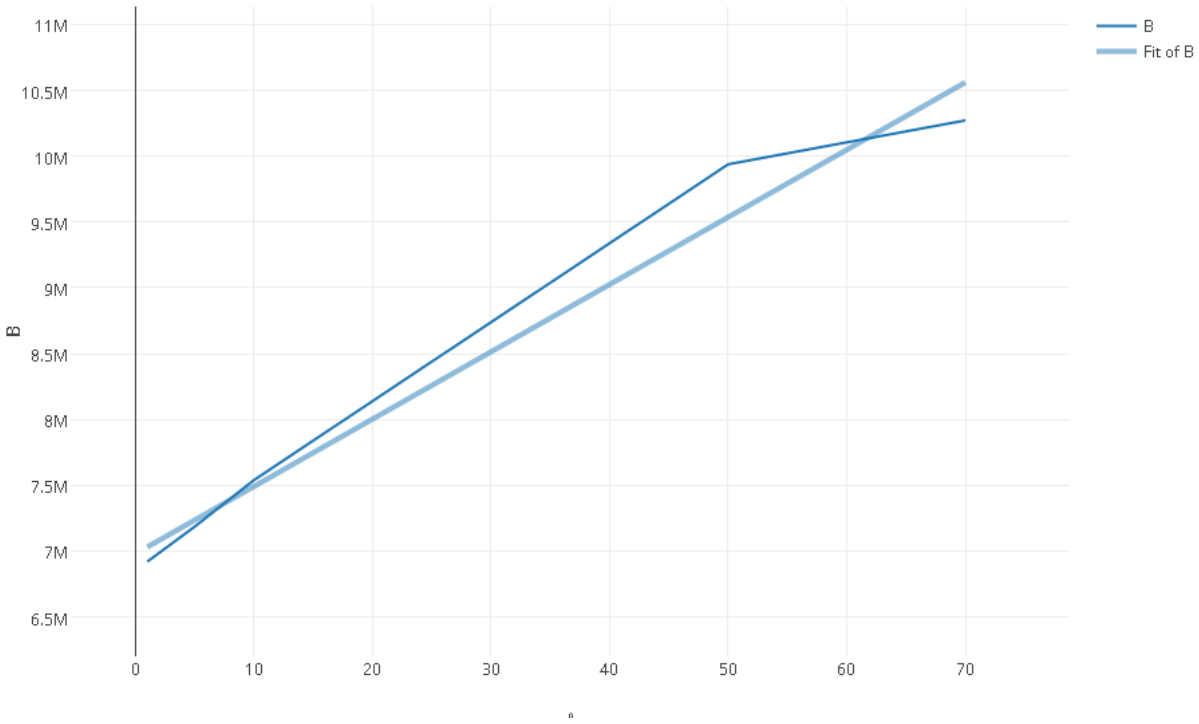


Sample	Peak Area	Conc. (mg/ml)	Conc. (%)
13	7.3	<0.05	<0.005%
14	198.9	1	0.1%
15	1035.2	4.5	0.45%
16	17396.8	111.5	11.5%
21	104.2	0.5	0.05%
22	470.8	1.5	0.15%
23	4749.4	28.9	2.89%
24 (single phase)	5834.2	350	35%
37	(178.8)	-	-
38	203.2	1	0.1%
39	1251.6	6	0.6%
40 (single phase)	-	-	-
45	163.1	0.5	0.05%

46	607.2	1.79	0.179%
47	5603	34.4	3.44%
48 (single phase)	3563.5	211	21.2%
53	No peak	-	-
54	No peak	-	-
55	1215.2	8.97	0.897%
56	25311.8	188.80	18.88%
57	3706	27.56	2.756%
58	12964.2	96.65	9.665%
59	3091.9	23.00	2.3%
60 (1:10)	7195.9(*10)	536.05	53.605%

HPLC-RI for ethanol detection

Calibration sample (mg/ml) ethanol in water	**Peak Area**
700	10271507
500	9938334
100	7540021
50	7186230
10	6920338
Calibration curve:	**y = 51133.8457x + 6980445.2978**



Sample	Peak Area	Conc. EtOH in hydrophilic phase (%)
1	9549212	50.24
2	9832356	55.77
3	9089099	41.24
4	9176936	42.96
25	-	-
26	9717044	53.52
27	10154900	62.08
28	9650400	52.22
49	-	-
50	9687825	52.95
51	9146193	42.36
52	9437509	48.05
13	10234022	63.63
14	9826912	55.67
15	10081960	60.66
16	9087595	41.21
21	9338069	46.11
22	8466374	29.06
23	8266266	25.15
24	9280105	44.97
37	9829911	55.73
38	9512000	49.51
39	10057278	60.17
40	-	-
45	9140336	42.24
46	8375615	27.30
47	8352567	26.83
48	8494652	29.61
53	9647430	52.16
54	9992954	58.92
55	9944958	57.98
56	8122332	22.33
57	9235616	44.10
58	9014385	39.78
59	8552514	30.74
60	-	-

Populärvetenskaplig sammanfattning

Varför behöva välja mellan rena och välmående händer?

Många personers yrke kräver noggrann rengöring av händer flera gånger dagligen, och det är tydligt att huden tar stryk av detta. En mjukgörande kräm som samtidigt innehåller alkohol för att döda bakterierna hade varit en välkommen lösning för dessa personer. Men för att veta om alkoholen kan påverka krämens stabilitet behöver man veta hur de separata komponenterna samspelar.

För bland annat läkare och sjuksköterskor tillhör det vardagen att desinficera sina händer flera gånger om dagen. Självklart är ju detta viktigt, inte minst för personalen själva, men även för att försäkra patientsäkerheten så att inte farliga sjukdomar och bakterier överförs mellan patienter. Denna ständiga tvättning har dock en baksida – torra och irriterade händer. Kanske har du själv märkt på vintern att upprepad tvättning efter toalettbesök och användning av handsprit för att undvika vinterkräksjuka får dina händer att kännas nariga och röda.

Handsprit eller alkogel är vanliga produkter som syns mycket i butikerna, och alkoholen – etanol – som ingår i dem är det som dödar bakterierna, men den har också oönskade effekter på huden på dina händer. En bättre idé hade kunnat vara en handkräm som dessutom innehåller etanol, det vill säga desinfektion i kombination med den vårdande funktion som en handkräm har

Utmaningen med att göra en sådan kräm ligger i själva uppbyggnaden av krämen. Krämer är baserade på så kallade emulsioner, blandningar av två ämnen som egentligen inte löser sig, till exempel olja och vatten. Mjölk är ett vanligt exempel på en emulsion, där fettet bildar pyttesmå droppar i vattnet och ger intrycket av en enhetlig lösning.

Om man tillsätter alkohol till en sådan blandning, kan oväntade saker uppstå om man inte vet exakt hur ämnena beter sig tillsammans. Till exempel finns det risk att etanolen löser upp oljan, vilket kanske förstör hela den komplexa uppbyggnaden av krämen då de små dropparna faller sönder. Konsekvensen kan då bli att du upplever krämen som rinnande vatten, medan allt fett som skulle smörja dina händer sitter kvar i förpackningen. Att blanda i etanol skulle även kunna leda till att smältpunkten på oljan ändras, och plötsligt kanske din kräm inte är en kräm längre utan snarare en peeling, med fasta små korn av olja som inte har smält vid den temperatur det var tänkt. En alltför stor andel olja löst i etanolen kan kanske också ha negativ effekt på den bakteriedödande förmågan hos etanolen.

För att undvika dessa scenarion har min uppgift varit att undersöka hur olja, vatten och etanol uppför sig i olika blandningar. Med hjälp av känsliga instrument har jag bestämt hur mycket olja och etanol som faktiskt löst sig i varandra, och det visade sig inte vara särskilt mycket, speciellt i rumstemperatur och den temperatur som huden har, det vill säga cirka 30-32 grader. Min slutsats var därför att etanol troligen bara kommer blanda sig med vattnet och inte påverka de små dropparna av olja särskilt mycket. Den enda mätningen där olja och etanol faktiskt löste sig i varandra var när jag blandade ren alkohol med olja, vilket aldrig kommer vara fallet i en kräm utan mer av vetenskapligt intresse. Mätningar visade också att etanolen gjorde att oljan smälter vid en lägre temperatur, vilket kan vara viktigt att veta vid tillverkning och inte minst för krämens egenskaper.