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Fat crystal-stabilised double emulsions

by

Sarah Frasch-Melnik

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To my parents

To Hannah

and

To Sajid,

My favourites and

The most important people in my life

Abstract

Double emulsions in the application of low-fat foods have great potential in the battle against growing obesity levels throughout the world. A benefit of double emulsions is that they allow the encapsulation of solutes – e.g. salt or micronutrients. Despite more than two decades of intensive research, however, double emulsions with sufficient stability for commercial applications have yet to be formulated. One of the main issues is that two types of emulsifiers are required to stabilise the oppositely curved interfaces in double emulsions. Diffusion of emulsifiers between the two distinct interfaces destabilises the structure. This work focuses on $W_1/O/W_2$ emulsions to allow investigation of the effect of the two interfaces on the transport of solutes between the two aqueous phases.

In this work, fat crystals are used to stabilise the primary emulsion interface in order to control the diffusion of solutes from the W_1 primary to the W_2 continuous aqueous phase. These fat crystals are seeded at the interface during emulsion production using monoglycerides. Subsequently they sinter to form tight crystalline network “shells” around the water droplets. It is shown that these “shells” are capable of retaining salt encapsulated within the aqueous phase despite the application of osmotic pressure gradients. The effect of temperature on the emulsion structure is also investigated. Emulsions stability against coalescence, measured using NMR, is in line with the melting profile of the fat crystals. While stable against coalescence and salt release at temperatures below the melting range, the emulsions inverts when the crystals melt.

The W_1/O primary emulsions are incorporated into double structures using either membrane emulsification or a rotor/ stator device. When employing the technique of membrane emulsification it was found that the high viscosity and a pressurised feed system prevent good yield. Therefore the emulsions were made in the more conventional rotor/ stator mixer (Silverson). It was found that primary emulsion droplets retain their structure during the secondary emulsification step, although the shear may cause some damage to their protective “shells”.

The choice of secondary emulsifier is important to double emulsion stability. The double structure is not stable if monomeric emulsifiers or globular proteins are used to stabilise the secondary interface. Protruding fat crystals from primary emulsion droplets cause coalescence of double globules and lead to phase separation. Non-globular proteins such as Na-caseinate, on the other hand, can prevent this phase separation. Double emulsion stability is shown to depend on the direction of the osmotic pressure gradient. Tensile loading, i.e. higher osmotic pressure in the encapsulated aqueous phase, of the crystalline “shells” destabilises the shells. Compression, i.e. higher osmotic pressure in the continuous aqueous phase, helps maintain the structure.

Stability can be further increased by placing small particles at the secondary interface. In this case, the double emulsions release no salt for a period of at least 2 months, and are even stable against shear. The use of OSA-starch as secondary emulsifier also results in long-term storage and shear stability.

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Table of Contents

List of Figures

List of Tables

Nomenclature

Symbols

d	Droplet radius (m)
$d_{50,l}$	Geometric mean diameter (m)
$d_{3,2}$	Surface-weighted (Sauter) droplet diameter (m)
$d_{4,3}$	Volume-weighted droplet diameter (m)
D_s	Self-diffusion coefficient (m^2s^{-1})
g	Gradient strength (T)
σ	Standard Deviation from mean
Δ	Time between the starting points of two fgp (ms)
δ	Length of a fgp (ms)
M_g	Echo attenuation with application of fgp in NMR measurement
M_0	Full-size peak without application of fgp
R	M_g/M_0
$\Delta\pi$	Osmotic pressure gradient (atm)
θ	Contact angle
γ	Interfacial tension (N.m)

ΔE	**Interfacial Energy (J)**
A	**Surface area (m^2)**
P_L	**Laplace Pressure (N)**
P_c	**Trans-membrane pressure (membrane emulsification), bar**
d_p	**Pore diameter (m)**

Abbreviations

W	**Water (subset 1 referring to internal encapsulated, subset 2 to external continuous aqueous phase)**
O	**Oil**
fgp	**Field gradient pulse**
HLB	**Hydrophilic Liphophilic Balance number**
SPG	**Shirasu Porous Glass (material used for membranes)**
A-unit	**Scraped surface heat exchanger for production of fat crystal-stabilised W/O emulsions**
C-unit	**Pin stirrer for production of fat crystal-stabilised W/O emulsions**
DSC	**Differential scanning calorimetry**
PGPR	**Polyglycerol of polyricinooleates**

1 Introduction

1.1 Background

Obesity is a growing problem in the Western world and, increasingly, in many parts of Asia. Figure 1.1 illustrates how obesity has become more prevalent among U.S. adults between 1990 and 2009. Obesity is linked to many health problems such as cardiovascular disease, type II diabetes, and hypertension (Eastwood, 2010; Mokdad et al., 2003; Dietz, 1998). In England 6 1% of the population, and a third of all children, was overweight (i.e., body mass index (BMI) bigger than 25 kg m^{-2}) in 2009 (Eastwood, 2010). In New Zealand 63 % of the population is overweight. A large proportion of those who are overweight are obese with a BMI greater than 30 kg m^{-2}. In some states of the USA more than 30 % of people are obese according to a recent survey (Centers for Disease Control and Prevention, 2008). Being overweight reduces life expectancy, so that reducing the number of overweight people brings substantial benefits to individuals as well as Society as a whole.

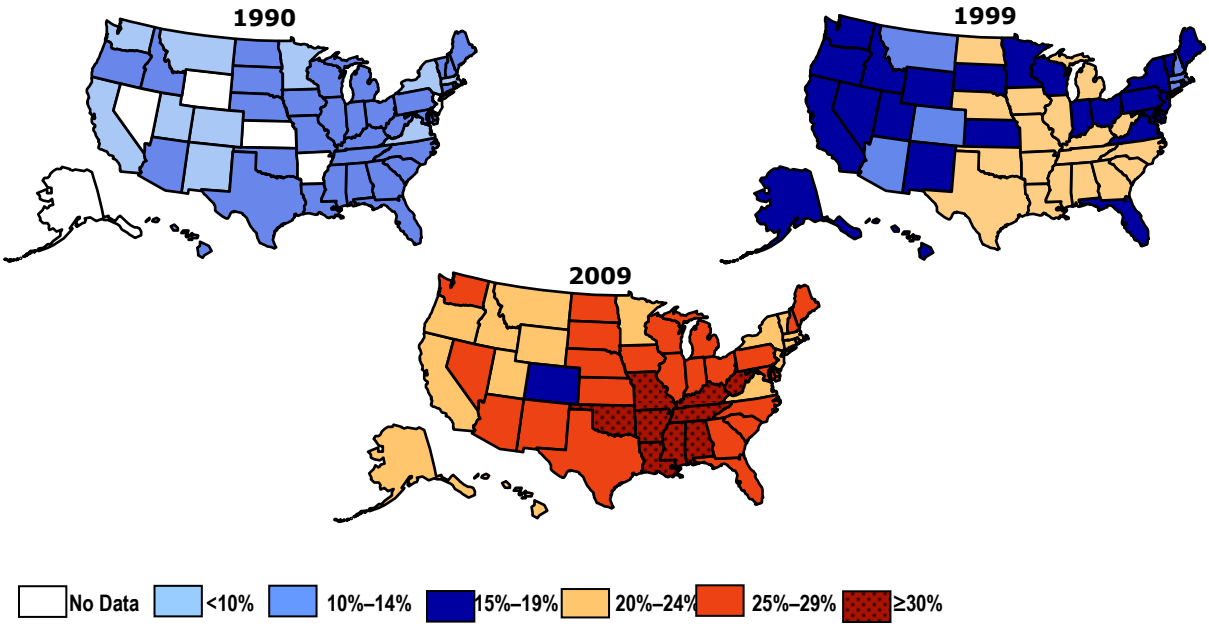

Figure 1.1: Obesity trends (BMI ≥ 30) among U.S. adults (Centers for Disease Control and Prevention, 2009)

Rising levels of obesity can be linked to an increasingly sedentary lifestyle and unhealthy eating (Eastwood, 2010). In the Western world, convenience foods and snacks are increasingly replacing home-cooked meals as lifestyles have become increasingly busy (Dixon et al., 2006). However, processed foods tend to contain high levels of salt, fat and sugar in order to meet consumer demand for flavour and texture (Norton et al., 2007).

Rising obesity rates have placed increasing pressure on producers of processed foods to lower the concentration of fat, especially the saturated kind, as well as salt and sugar. “Low fat” or “low sodium” alternatives to the original products, however, are usually of inferior taste and texture and hence less favoured by consumers (Norton et al., 2006).

A “processed food” does not, by any means, have a simple (micro-) structure. It may contain many different ingredients whose function within the food has yet to be fully

understood. Sodium, for instance, may be present not only to convey flavour, but also to ensure microbial as well as structural stability of the product. Furthermore, food products usually have a rheological behaviour that is complex and may change when the composition of the food product is altered. For these reasons, reducing the amount of fat, sugar and salt in a food product is not a matter of simply adding less of the respective ingredient. The effect of removing certain ingredients on the microstructure of the original food product must be understood, while new methods of incorporating increased concentrations of low calorie ingredients such as water or air must be investigated.

Oil-in-water emulsions are often used as models for the investigation of fat (and sugar and salt) reduction. Such emulsions are often consumed as part of a meal in the form of mayonnaise or salad dressings. Numerous ways of replacing fat from (O/W) emulsions have been investigated. Fat reduction has been achieved by incorporating air bubbles to replace up to 50 % of oil droplets (Tchuenbou-Magaia et al., 2009). A structured water phase has been used to replace some of the fat: The addition of hydrocolloids to the aqueous phase increases emulsion viscosity, thus masking the reduction in viscosity resulting from the reduction in oil volume. This is relatively simple, widely practiced and relatively inexpensive. However, the existence of a new “network” of starchy or gelling particles may negatively affect the sensory and organoleptic properties of such a product (Norton et al., 2007). Another way of partially replacing oil is by using water to create water-in-oil-in-water, or double, emulsions (Pawlik et al., 2010; Muschiolik, 2007; Garti, 1997a; Matsumoto et al., 1976). Water can also be used to replace the oil completely, creating so-called water-in-water emulsions (Spyropoulos et al., 2010; Norton & Frith, 2001). The applicability of the various approaches depends on the food product; Is it heated prior to

consumption? What is the expected shelf-life? Is it usually refrigerated during storage?

An important advantage of double emulsions is that they offer the possibility of achieving significant salt and sugar reductions in addition to fat reduction in the final food product. Food products with a “reduced” salt content are often perceived as bland by the consumer. It has been shown that the perception of a solute (e.g. salt) within the mouth strongly depends on its concentration in the (outer) water phase, but not on its overall content in an emulsion (Malone et al., 2003). One way of reducing the overall salt concentration without affecting salt perception would thus be to reduce the (outer) aqueous phase volume (effectively increasing the amount of fat in the emulsion) while keeping the salt concentration in the aqueous phase constant (and thus decreasing the overall salt content in the emulsion). However, this method would require an increase of the fat content of the emulsion, which is not in line with consumer demands for lower fat products. A double emulsion would enable a large amount of the fat to be replaced with water, thus achieving an overall reduction in continuous phase volume (and associated possibility of reduction of overall salt content) coupled with a decrease, or at least not increase, of the overall fat content.

As a further benefit potassium chloride, a salt which is often lacking in modern diets but necessary in small quantities, could be encapsulated within the primary emulsion droplets. This encapsulated salt could function to match the osmotic pressure between the two aqueous phases, enhancing emulsion stability (see section 2.2.3.1) while effectively “hiding” its bitter flavour upon consumption (Morris et al., 2010).

However, the major problem encountered in the use of double emulsions is their inherent thermodynamic instability (Muschiolik, 2007). This is the result of the

presence of two oppositely curved interfaces requiring two different species of surfactant. Additionally, the presence of two water phases may cause the diffusion of water and solved substances. Most research has focused on monomeric and polymeric emulsifiers. Although reasonably stable double emulsions have been reported, long-term encapsulation of solutes within the W_1 aqueous phase continues to pose a major challenge (Benichou et al., 2007; Fechner et al., 2007; Taki et al., 2007; Mezzenga et al., 2004; Pays et al., 2001; Garti & Aserin, 1996).

1.2 Objectives

A central aim of this thesis is to find alternative ways of stabilising $W_1/O/W_2$ double emulsions in order to solve the problem of long-term encapsulation of solutes. The problem with using monomeric emulsifiers is that these are not fixed at the interface and have a tendency to diffuse to the opposite interface. This destabilises the double emulsion, causing coalescence and the loss of internal phase.

The approach chosen for this work was to fix the emulsifiers at the respective interfaces to prevent emulsifier migration as well as the release of encapsulated solutes. For this purpose, polymeric emulsifiers in the form of proteins, but especially small particles have been used. Pickering emulsions, i.e. formulation stabilised by small particles at the interface, are characterised by their extremely good resistance to coalescence (Binks, 2002). The use of fat crystals at the primary emulsion interface constitutes a special form of Pickering stabilisation: Once seeded at the interface they subsequently sinter to form a solid “shell”, i.e. a very tight network of fat crystals, around the water droplets (Norton et al., 2009).

This stabilisation mechanism has been applied for hundreds of years, in butter and, more recently, margarine and low-fat spreads. The present work investigates the

effect of crystallisable fat concentration, temperature and osmotic pressure gradients on the ability of the fat crystal “shells” to retain water and solutes encapsulated within the structure.

Double emulsions containing fat crystal-stabilised primary emulsions were produced using conventional as well as novel emulsification techniques. Various materials were used for stabilising the secondary interface. The effect of using different proteins or small particles on the overall stability of the double structure was tested. The effect of temperature was also assessed by measuring double emulsion stability against coalescence, shear and salt release at various temperatures.

Specifically, the main objectives of this study are:

- To formulate and characterise a W/O emulsion that is capable of resisting the transport of water and solutes between the encapsulated and a continuous aqueous phase

- To incorporate this emulsion into a continuous aqueous phase using an emulsification technique gentle enough not to break the primary structure.

- To find an optimal food-grade emulsifier to stabilise the secondary interface.

- To investigate the effect of various emulsifiers on the stability of the double emulsion against coalescence, shear and salt release.

1.3 Thesis Layout

Chapter 2 gives a detailed review of published literature in the field of fat crystal stabilised emulsions as well as double emulsions. The first part gives an overview of factors affecting the stability of simple emulsions, with emphasis on emulsions

stabilised by fat crystals. The second part gives a detailed account of $W_1/O/W_2$ double emulsions, including stability issues and the role of osmotic pressure gradients.

Chapter 3 gives a detailed description of the process used for making the fat crystal stabilised primary emulsions as well as the techniques used subsequently to create double emulsions. Techniques used to characterise the stability of simple as well as double emulsions are also discussed.

Chapter 4 investigates the ability of fat crystal-stabilised W/O emulsions to retain solutes encapsulated within the water phase, as functions of applied osmotic pressure gradients, temperature, and fat crystal composition respectively.

Chapter 5 shows how a fat crystal-stabilised W/O emulsion can be incorporated into a double emulsion using rotor/ stator as well as membrane emulsification techniques. The potential of different emulsifiers to stabilise the secondary interface is investigated. The effect of osmotic pressure gradients on double emulsion stability is also assessed.

Chapter 6 demonstrates how placing particles on the external interface increases the stability of the double emulsions against shear, salt release and coalescence. The effect of temperature on double emulsion stability is highlighted, and the influence of protein impurities on the structure of the secondary interface is discussed.

The main findings of the thesis are summarised in **Chapter 7**. Recommendations for further investigation are also given here.

The results obtained during this study have been published as follows:

1. Frasch-Melnik, S., Spyropoulos, F., & Norton, I. T. (2010). $W_1/O/W_2$ double emulsions stabilised by fat crystals - Formulation, stability and salt release. *Journal of Colloid and Interface Science, 350,* 178-185

2. Frasch-Melnik, S., Norton, I. T., & Spyropoulos, F. (2010). Fat-crystal stabilised W/O emulsions for controlled salt release. *Journal of Food Engineering, 98,* 437-442.

3. Frasch-Melnik, S.*,* Spyropoulos, F.*,* Bakalis, S. & Norton, I.T. (2009). Fat crystal-stabilized W/O emulsions for controlled salt release*.* In*: Proceedings of the 5th International Symposium on Food Rheology and Structure,* pp. *530–531*, ETH Zurich, Zurich

The results obtained during this study have been presented as follows*:*

1. Frasch-Melnik, S.*,* Spyropoulos, F.*,* Bakalis, S. & Norton, I.T: *Fat-crystal stabilised W/O emulsions for controlled salt release*. **5th International Symposium on Food Rheology and Structure***.* Zurich, Switzerland, June 2009

2. Frasch-Melnik, S., Spyropoulos, F., & Norton, I. T.: *Double emulsions stabilised by fat crystals*. **Formula VI**, Stockholm, June 2010

3. Frasch-Melnik, S., Spyropoulos, F., & Norton, I. T: *W/O/W double emulsions stabilised by fat crystals and hydrophilic particles.* **10th International Hydrocolloids Conference,** Shanghai Jiao Tong University, Shanghai, China, June 2010.

A patent application (Application number GB0922626.7) has also been filed on 24.12.2009 protecting the fat-crystal stabilised double emulsions.

2 Literature Review

This chapter gives an overview of the current knowledge in the field of emulsions. Simple emulsions are introduced and discussed as an understanding of these is essential to formulating more complex emulsion structures. Double emulsions are then described and discussed in detail to show their potential as well as the problems that currently prevent double emulsions from being commercially adopted.

2.1 Emulsion-based foods

A lot of everyday foods are emulsions: either water dispersed in a continuous oil phase (W/O) or oil dispersed in a continuous aqueous phase (O/W). Examples of W/O emulsions include butter or margarine; milk, mayonnaise and salad dressings are examples of O/W emulsions of various concentrations (mayonnaise is a very concentrated system containing around 80% fat, while salad dressings are much more dilute and contain around 30% fat). Before a reduction of fat in these kinds of products can be attempted the original full-fat versions need to be fully understood.

The following section gives an overview of the vast field of emulsion science, including formulation, formation and analysing techniques.

2.1.1 Emulsion formation

Emulsion formation requires energy as well as the presence of a surfactant to lower the interfacial tension (McClements, 2005a). Usually, emulsions do not form

spontaneously because their interfacial energy ΔE (eqn 2.1) makes them inherently unstable in thermodynamic terms. A reduction in droplet size, which leads to an increase in surface area (A), leads to an increase in energy in the system (Walstra, 1993). Using a surfactant can lower the interfacial tension γ, and therefore the interfacial energy in the system, but ultimately the system tends towards minimisation of the interfacial area (i.e., a separation of the two phases).

$$\Delta E = \gamma \cdot \Delta A \qquad \textbf{Eqn 2.1}$$

Moreover, the energy required to deform and break a droplet, detailed in eqn 2.2), depends on the Laplace pressure of the droplet (P_L), or the pressure gradient between the concave and convex side of the interface (Walstra, 1993).

$$P_L = \gamma \cdot \left(\frac{1}{R_1} + \frac{1}{R_2} \right) \qquad \textbf{Eqn 2.2}$$

R_1 and R_2 are the principal radii of curvature, so that for a spherical drop of radius r the equation becomes:

$$P_L = \frac{2\gamma}{r} \qquad \textbf{Eqn 2.3}$$

As can be seen from equation 2.3, increasingly large pressures are required to counteract the Laplace pressure as the droplet size is reduced (Walstra, 1993).

As small droplets are more stable than larger ones with respect to coalescence, and creaming, a large amount of energy must be dissipated into the system in order to create emulsions droplets of a satisfactory size. This energy can be conveyed to the emulsion droplets by means of shear or pressure gradients.

The function of surfactant is not only to lower the interfacial tension in order for emulsion break-up to occur. More importantly, surfactants stabilise the interface and prevent newly formed droplets from coalescing (McClements, 2005a).

2.1.1.1 Conventional Emulsification Equipment

Conventional means of emulsion processing include high shear devices that incorporate rotor/stator systems and high pressure homogenisers, as shown in Figure 2.1. In rotor/ stator devices the close proximity of the driven mixing element (rotor) to the fixed mixing element (stator or screen) creates extremely high shear rates in the region of 20,000 to 100,000 s^{-1} (Atiemo-Obeng & Calabrese, 2004). These high shear rates are created as the mixture is forced through perforations in the screen (stator) by the centrifugal force applied to the liquid by the rotor.

High pressure homogenisers (shown schematically in Figure 2.1) are based on the principle of emulsifying a mixture by forcing it through narrow orifices at high pressure (5 - 35 MPa). This causes droplet break-up generated by highly turbulent streams of fluid and cavitation effects (van der Graaf et al., 2005).

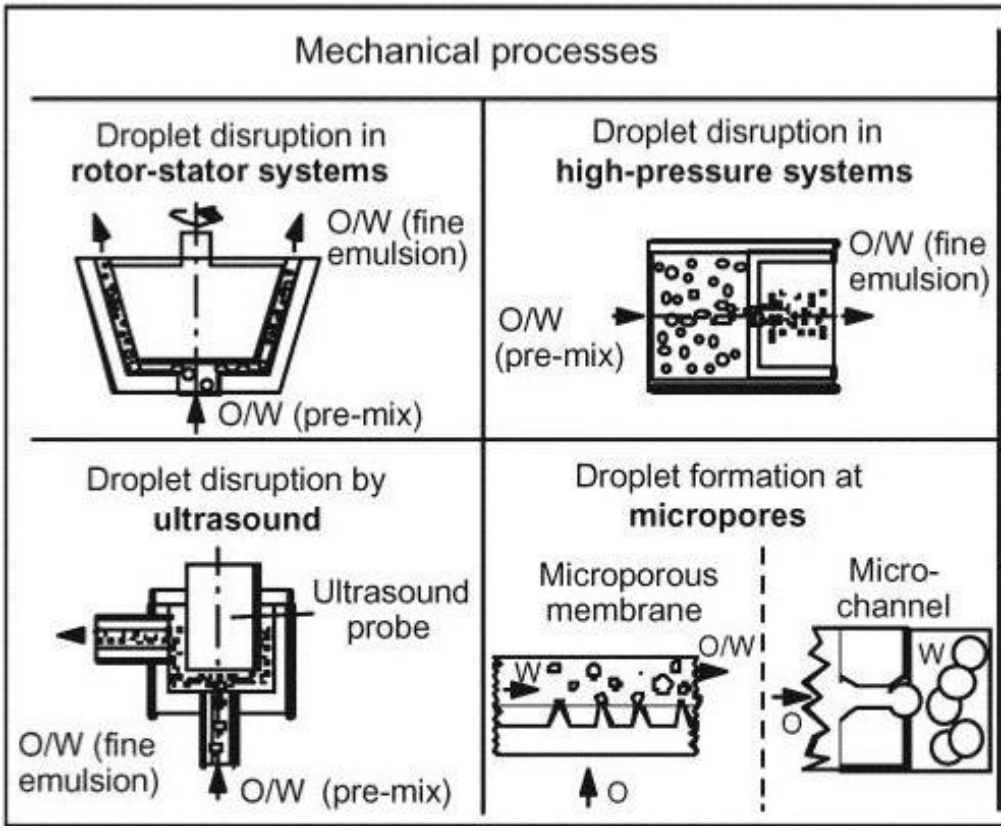

Figure 2.1: Different emulsification techniques (Schubert & Ax, 2003)

Although these devices are commonly used in the food industry to create emulsions, they are not very efficient in view of the fact that most energy supplied to the mixture is dissipated as heat (McClements, 2005a). Furthermore, it is important to control the back-reaction (i.e., coalescence), because the time it takes for surfactants to diffuse to the newly created interface may be too long to prevent re-coalescence of neighbouring droplets (Niknafs et al., 2011).

2.1.1.2 Novel emulsification methods

The lack of control of droplet formation and coalescence phenomena in conventional emulsification technology makes the control of droplet size difficult. In order to overcome this and the inefficiencies in terms of heat dissipation, novel methods for emulsion formation have been developed. These include membrane emulsification,

and multichannel apparatuses such as T-shaped channels (Okushima et al., 2004) and microcapillary devices (Utada et al., 2005). The fundamental difference between the latter and the conventional techniques is that with the new methods drops are not broken but formed from a liquid that is pressed through either a membrane (van der Graaf et al., 2005), through small orifices or passed through a number of T-junctions in small channels. Used on a laboratory scale, all of these techniques give good control over droplet size and narrow droplet size distribution.

In membrane emulsification several parameters influence droplet size. These include interfacial tension, applied trans-membrane pressure and wall shear stress, pore size as well as the diffusivity of the emulsifier (Vladisavljevic et al., 2006). Emulsion droplet size is dependent not only on membrane pore size d_p but also other operating parameters.

It was shown, for instance, that increasing the wall shear stress had a higher influence on final droplet size than the size of the membrane pores. A larger shear stress along the membrane increased the rate of detachment of droplets, preventing their coalescence at the surface (Joscelyne & Tragardh, 1999).

In order to make the disperse phase flow across a membrane, a minimum pressure needs to be applied to the dispersed phase-to-be (Equation 2.5). The magnitude of this trans-membrane pressure depends on the interfacial tension γ as well as the contact angle θ between the dispersed phase and the membrane surface and the pore diameter d_p (Joscelyne & Tragardh, 2000).

$$P_c = \frac{4\gamma \cos\theta}{d_p}$$ **Eqn 2.5**

As the trans-membrane pressure is increased beyond the minimum required for flow across the membrane, droplet size increases. This is because coalescence at the membrane surface increases when the rate of droplet formation is faster than the rate of emulsifier adsorption (Schroder et al., 1998).

The commercial use of these novel techniques has so far been limited due to the low flux associated with them. The trade-off for increasing the flux is the widening of the droplet size distribution. This decreases emulsion stability and hence the appeal of the technique for producing near-uniformly sized droplets.

An advantage of membrane emulsification over conventional emulsification techniques is that energy densities demanded by this application are 100 times lower than in high pressure homogenisation, and 10 times lower than in rotor/stator systems.

The major drawback of these novel emulsification techniques, and the reason why membrane emulsification has not yet been widely implemented on an industrial scale, is the difficult scale-up (Charcosset et al., 2004). In principle, scale-up should be relatively simple, requiring the use of many devices in parallel. In practice, however, the low fluxes associated with membrane emulsification do not justify the installation of expensive equipment except for use in creating high value products (some pharmaceuticals, for instance). Furthermore, the relatively rapid and frequent

fouling of membranes and difficult cleaning procedures also hinder industrial application of the technique (Joscelyne & Tragardh, 2000).

2.1.2 Emulsion (in) stability

Emulsions are inherently thermodynamically unstable (Dickinson, 1992) due to the large surface area between the two phases and, as such, will eventually show signs of physical or chemical instability. Furthermore, droplets constantly move within the continuous phase due to Brownian motion and density differences, thereby increasing the opportunities for droplet encounters.

If the physicochemical properties of emulsions remain largely unchanged for extended periods of time an emulsion is said to be kinetically stable (Friberg 1997). Kinetic stability is one of the most important attribute of emulsions. Processes leading to physical instability include creaming/ sedimentation (Walstra 1996; Dickinson 1992; Hunter 1989; Dickinson & Stainsby 1982), flocculation (Dukhin & Sjoblom, 1996; Lips et al. 1993), (partial) coalescence (Walstra 1996; Boode & Walstra, 1993; Mulder & Walstra 1974), Ostwald ripening (Taylor, 1995) and phase inversion (Campbell et al. 1996; Dickinson 1992; Shinoda & Friberg 1986), while chemical instabilities relate to oxidation (Nawar, 1996) or hydrolysis (Dalgleish 1996). Kinetic stability is influenced by emulsion properties such as droplet size, stabilisation mechanism and the physical properties of the immiscible phases.

2.1.2.1 Creaming/ sedimentation and flocculation

A density difference between the two phases causes the emulsion dispersed phase to sediment (W/O emulsions) or cream (O/W emulsions). Although this is a reversible process (simple stirring will disperse the cream or sediment), this phenomenon causes a concentration of dispersed phase droplets (Dickinson & Stainsby 1982). Unless the droplets are adequately stabilised, irreversible processes such as coalescence may subsequently occur.

The rate of creaming/ sedimentation may be influenced by droplet flocculation. In dilute systems the formation of flocs may enhance creaming rates as individual droplets essentially increase in size (Luyten et al., 2003). On the other hand, in more concentrated systems, flocculated droplets may form 3-dimensional networks, thus effectively preventing droplet movement caused by gravity (Bijsterbosch et al. 1995).

As already mentioned, the creaming/ sedimentation and/or flocculation of droplets increase the effective concentration of droplets, and therefore increase the likelihood of such processes as coalescence that are detrimental to emulsion stability. Different approaches to minimise the effect of gravity on emulsions have been suggested. These include matching the density of the two immiscible phases as closely as possible (Tan 1990), reducing the droplet size and therefore gravitational movement of these in accordance with Stokes' law (e.g. homogenisation of raw milk, Swaisgood 1996), increasing the viscosity of the continuous phase to slow or arrest droplet movement (van Vliet & Walstra 1989) and by altering the weighting of the adsorbed layer to influence droplet-droplet interactions (Dickinson, 1992).

2.1.2.2 Droplet aggregation and coalescence

When two droplets approach each other they may either repel each other or remain joined together as aggregates. This depends on the relative magnitude of attractive and repulsive forces – which is influenced by such things as interfacial coverage and emulsifier type.

The rate at which droplets collide is determined by the predominant process responsible for droplet movement: for instance, within a mixing vessel it may be shear (Walstra 1996; Dickinson 1992) or, in undisturbed samples, it may be gravity or Brownian motion (Zhang & Davis 1991; Melik & Fogler 1988), depending on the size of the dispersed droplets.

Droplets aggregate in two distinct ways: They may weakly flocculate, in which case droplets remain individual entities. Strong flocculation, or coagulation, on the other hand, usually leads to droplet coalescence, an irreversible process. Particle aggregation largely depends on the balance between attractive and repulsive forces. Attractive forces between droplets include, principally, van der Waals forces (Hunter, 1986) while repulsive forces may be electrostatic in the case of charged surfaces (Hunter 1986; Kitakara & Watanabe 1984), or steric if polymers are present at the interface (Dickinson 1992; Damodaran 1989).

2.1.2.3 Electrostatic stabilisation

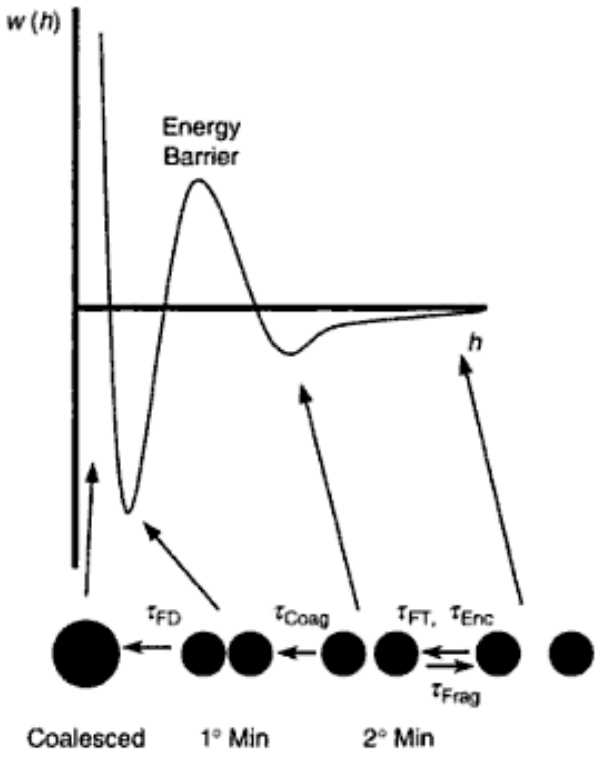

Figure 2.2: Theoretical interaction potential between two spherical emulsion droplets (taken from McClements. 2005)

Electrostatic repulsion occurs between two identically charged droplets and is a result of an overlap of the electric double layer surrounding the spheres. If the droplets are not charged, they immediately aggregate and may subsequently coalesce because there is no repulsive force opposing the van der Waals force. When the two droplets approach each other, ions of the opposite charge are increasingly attracted to the gap between them. This causes an osmotic pressure gradient in the gap which leads to a net repulsive force between the two droplets. This repulsive force opposes the van der Waals force that attracts the droplets to

each other. The balance of the two forces depends on droplet size and net charge of the droplets, as well as on the ionic strength of the aqueous medium surrounding the oil droplets. High ionic strength thins the electric double layer and thus weakens electrostatic repulsion between droplets (Hunter 1986; Kitakara & Watanabe 1984).

For fine droplets van der Waals forces are only important when the droplets are very close together (less than 1 nm), while for larger droplets these forces are important even at further separations (tens of nm).

The electrostatic repulsion and the attractive van der Waals forces are added together algebraically to produce the overall potential, which is termed DLVO (Derjaguin, Landau, Verwey, Overbeek) potential (Derjaguin 1989; Derjaguin et al. 1987; Hunter 1986). An example of this can be seen in Figure 2.2.

When the droplets are very small and highly charged in an aqueous medium of low ionic strength, repulsive forces dominate at all separation distances over van der Waals forces. The interaction potential remains positive and has the shape of an exponential decay. Such an emulsion is said to be electrostatically stabilised (Dickinson 2009).

When the droplets are in the μm size range or their surfaces are not highly surface charged, their resistance to flocculation and coalescence will be determined by the separation distance. As illustrated in Figure 2.2, van der Waals forces dominate at small and large separations, while repulsive forces are most important at intermediate distances. The relative size of the two forces determines the size of the

maxima and minima in the interaction potential, and thus the stability of the system. A large maximum on this plot (“energy barrier”) prevents droplets from approaching each other closely, so that droplets will repel each other. If the secondary minimum is also large, droplets will become loosely flocculated. If short-range repulsion is very high, but the energy barrier is comparatively low, droplets may become strongly flocculated (coagulation) with a very thin film separating individual droplets.

Strong flocculation may occur immediately upon droplet encounters. However, it is more common that strong flocculation evolves from a weakly flocculated network with time. Droplet coalescence can ensue when the short-range repulsion between droplets is low. Rapid coalescence ensues as soon as droplets fall into the primary minimum due to lack of a resisting force (Hunter 1989).

Coalescence can be prevented in several ways. Droplets may be physically stopped from close encounters with other droplets, e.g., by increasing the viscosity of or by gelling the continuous phase to prevent creaming/ sedimentation and flocculation. Alternatively, the interfacial tension and the viscosity of the interfacial film layer (i.e. by using polymeric emulsifiers or particles to stabilise the system) may be increased. Emulsion droplet size is also important: the big contact area between two large droplets increases the likelihood of film rupture and coalescence (Dickinson 1992).

2.1.2.4 Steric stabilisation

An emulsion may become sterically stabilised when polymers adsorb at the O/W interface even in the absence of charged emulsifiers. In order to adsorb at the

surface, polymers must be amphiphilic, i.e., contain hydrophobic as well as hydrophilic parts. The existence of hydrophilic and hydrophobic sections on the molecules makes their adsoption at the O/W interface thermodynamically favourable. Depending on their configuration, polymers may adsorb in two ways: loop-and-train or globular (Figure 2.3). This is to maximise contact of the hydrophobic and hydrophilic parts of the protein with the oil and water phase, respectively.

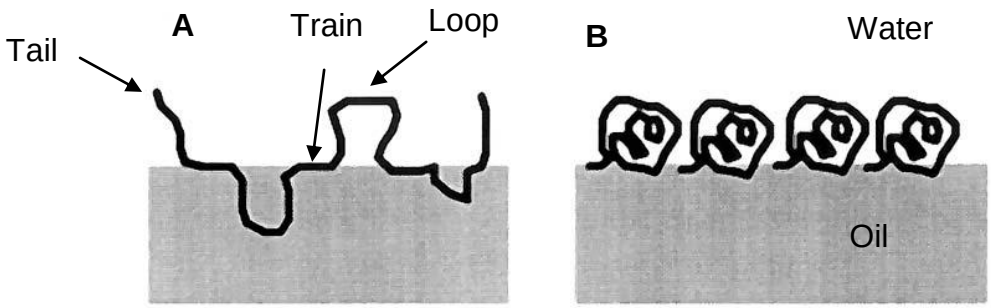

Figure 2.3: Adsorption configurations of proteins at the oil/water interface: Loop-and-train ("A") and globular ("B"). Adapted from McClements, 2005

The adsorption of polymers to oil/water interfaces causes stabilisation because the overlap and/ or compression of polymer layers on approaching droplets is entropically unfavourable. The layers overlap when the droplets have approached each other to a certain distance ("intermediate separation distance"), and protein interactions may result in either an attractive force (leading to flocculation of the droplets) or a repulsive force. The mixing of proteins determines their interaction at this distance and the solvent may influence the mixing behaviour. If the protein segments that protrude into the surrounding continuous phase are solubilised well, the overall mixing contribution to the interaction potential is positive, resulting in the droplets being repelled from each other.

When the droplets are even closer, the layers additionally become compressed. Compression usually results in a strong repulsive interaction (Hiemenz 1986; Hunter 1986). Compression is unfavourable because it restricts the volume available for each polymer chain and thus removes them from their equilibrium form (Dickinson 2009). Sterically stabilised emulsions are thus very stable against coalescence, but may flocculate if the intermediate interaction potential is positive. The strength of these steric interactions depends on droplet size and polymer concentration, as a thicker polymer layer increases the range of interaction. A sufficiently high polymer concentration is also important for preventing bridging flocculation. This is the mechanism by which polymers adsorb at several droplets simultaneously, causing these to flocculate (Everett, 1988).

2.1.2.5 Depletion interaction

The presence of colloidal particles, such as free emulsifier micelles, polymer aggregates or nanoparticles can cause emulsion destabilisation by a mechanism called depletion interaction (Jenkins & Snowden 1996; McClements 1994; Aronson 1992). These colloidal particles are excluded from the gap between two approaching droplets (Sperry 1982) because they are too large to fit inside. This causes an osmotic pressure gradient between the fluid in the gap and the surrounding fluid (Hiemenz 1986). In order to counteract the concentration difference of colloidal particles, the fluid drains from the gap, causing the emulsion droplets to aggregate. An increase in the concentration of colloidal particles causes an increase in the osmotic pressure gradient and therefore a stronger attractive interaction between emulsion droplets.

2.1.2.6 Particles at the interface

It was first discovered more than a century ago that particles can act as stabilisers of emulsions (Pickering 1907). So-called Pickering emulsions are characterised by their excellent stability against coalescence. This is a result of the strong adsorption of particles at the O/W interface.

Due to their great potential in various applications such as pharmaceuticals, food and agrochemicals these emulsions have been extensively investigated over the past 20 years (e.g. Binks, et al., 2007; Arditty et al., 2004; Aveyard et al. 2003; Midmore, 1999) and several comprehensive reviews have been published on this subject (e.g. Dickinson 2010b; Leal-Calderon & Schmitt 2008; Binks 2002;).

There are several factors determining whether particles will stabilise an interface in a satisfactory manner. These include particle wettability, size, shape, concentration as well as particle-particle interactions. Of these, the single most important aspect defining the behaviour of particles at an interface is probably wettability, measured by the contact angle of the particle with the oil and water phase (Binks 2002). If the particle is completely wetted by either oil or water phase (contact angle measured through the water phase = 0 or 180°) it is not likely to adsorb at the interface. However, a contact angle between around 90 and 140° means that, although preferentially wetted by the oil phase, the particle is also partially wetted by the water phase (similarly, if the contact angle is between 40 and 90°, the particle is preferentially wetted by the water phase). The partial wettability of the particles in both phases means that their position at the interface is thermodynamically

favourable. It is generally accepted that the liquid that wets the emulsion less well is usually the dispersed phase (Binks 2002). Particle wettability can be altered by chemically modifying the particle structure, e.g. by applying specific coatings, or by adding surfactants to the system (Pichot et al., 2009).

The energy E required to remove particles of radius r from the interface depends on interfacial tension γ between water and oil phase and contact angle θ of the particle (Binks 2002):

$$E = \pi \cdot r^2 \cdot \gamma \cdot (1 \pm \cos\theta) \qquad \textbf{Eqn 2.6}$$

As can be seen from equation 2.6, particles have the highest energy of attachment when the contact angle is close to 90°. Furthermore, larger particles attach more strongly, although there is a trade-off as very large particles will not be able to cover the surface evenly.

Particles, compared with monomeric surfactants, have the advantage of providing extreme stability to the droplets. Particle-stabilised droplets do not coalesce even after one year storage. This is due to the very rigid interfacial film (Tambe & Sharma, 1994) that is created by the adsorption of particles, and also by the high energy barrier obstructing the detachment of particles. This means that particles are almost irreversibly adsorbed to the interface (Leal-Calderon & Schmitt, 2008).

Surfactants cover an interface more rapidly than particles (Pichot et al, 2009). Particles are slow to diffuse to “naked” interfacial areas because of their large size

(which ranges from the 10s of nm to several μm). A lot of new surface area is created during emulsification as droplets are broken up. The fact that particles are slow to adsorb to these newly created interfaces can result in partially covered interfaces for lengths of time long enough to leave the droplets exposed to impacts from neighbouring droplets (Nienow et al., 2000). “Naked” interfaces cannot repel such approaching droplets so that coalescence is likely to occur. The formation of an emulsion with small particle size using only particles is therefore difficult because the rate of droplet coalescence may exceed the rate of droplet break-up (Niknafs et al., 2011).

It has been reported that surfactants can be used in conjunction with particles in order to compensate for this (Pichot et al., 2009). The primary role of the surfactant has been described as lowering the interfacial tension between oil and water, thus aiding droplet break-up, and stabilising the newly created interfaces immediately by means of fast adsorption. The presence of particles ensures long-term stability of the emulsions (Pichot et al., 2009). Once attached to the interface they remain there, due to their high energy of attachment. They also form a rigid layer as described above.

Monomeric surfactants may also be adsorbed to particle surfaces, thereby changing particle surface properties and hence their wettability. Depending on the conditions (pH, ionic charge, etc) and the type of particle, this may or may not be beneficial (Leal-Calderon & Schmitt 2008): Adsorption of surfactants on particle surfaces may affect the particle surface charge and change its hydrophobicity. Experiments have been performed which showed that silica particles, which are negatively charged at pH 9, can become positively charged by addition of cationic surfactant. The

surfactant electrostatically adsorbs in a monolayer at the particle interface and makes the particles increasingly hydrophobic. However, if chain-chain interactions cause adsorption of a second layer of surfactant, the charge is reversed and the particles become hydrophilic once again (Binks et al., 2007).

Such changes in the surface properties of the particles may lead to their aggregation. If the electrostatic attraction between particles is strong, droplets stabilised by particles may flocculate and form a 3-dimensional network within the sample. Such a network can prevent droplet movement and therefore inhibit coalescence by preventing droplets from approaching each other. Creaming is also prevented by the existence of a 3-dimensional network. This has been shown for mixtures of cationic surfactants with silica particles (Binks & Rodrigues 2007) as well as those of anionic surfactants and silica particles (Binks et al., 2007).

The adsorption of charged surfactants to particle surfaces can also lead to the destabilisation of emulsion droplets stabilised by particles. The surfactants can alter the wettability of particles as discussed above. Cationic surfactant adsorbing at the surface of hydrophilic particles stabilising to O/W emulsions can render the particles hydrophobic. This destabilises emulsions because hydrophobic particles have a contact angle which favours the stabilisation of W/O emulsions (Binks et al., 2007).

2.1.2.7 Fat crystals as emulsifiers

Fat crystals can be used as Pickering particles to stabilise emulsion droplets. Fat crystals have long been used to stabilise W/O food emulsions such as butter,

margarines and low fat spreads which are stable during storage at ambient temperatures but rapidly melt and then phase-invert at temperatures found within the mouth (Norton et al., 2006).

Fat crystals have significant, and different, impacts on the stability of oil-in-water (O/W) and water-in-oil (W/O) emulsions (Rousseau & Hodge 2005; van Boekel & Walstra 1981). Most fat crystals are hydrophobic in nature which means they preferentially reside in the oil phase. They may not be not present at the interface In the absence of a suitable emulsifier. Fat crystals in the dispersed phase have been reported to be detrimental to stability when the emulsion is sheared (van Boekel & Walstra 1981) because they may protrude from oil droplets, causing coalescence. Crystals in W/O emulsions, on the other hand, may impart stability on emulsions in two ways: individual crystals may, as particles, stabilise the oil/water interface (Pickering stabilisation) while the presence of a fat crystal network in the continuous oil phase serves to arrest water drop movement, thus preventing drop coalescence and providing long-term emulsion stability (Rousseau & Hodge 2005). The following section will only review the role of fat crystals in W/O emulsions where they have a stabilising effect.

a. Factors affecting the formation of fat crystals

Fat crystals are formed in solutions that have reached the saturation limit with respect to the crystallising ingredient (supersaturated solutions). This saturation limit is temperature dependent. Supersaturation is achieved when the activity of the crystallising component is higher than the activity of the saturated solution. This results in a chemical potential difference between the supersaturated and the

crystalline materials which is only minimised with crystallisation (Kloek et al., 2000a). Crystallisation is also achieved when the solution is supercooled, i.e. cooled below the melting point of the bulk fat.

The crystallisation process consists of two main processes – nucleation and growth. Nucleation is the process of crystal formation which can either be a spontaneous process (homogeneous or primary nucleation) or consist of crystal formation around an impurity (heterogeneous nucleation). Two competing factors determine the rate of nucleation: the energy required to create a new surface area, which hinders nucleation, and the energy gained from the transferal of a molecule from its metastable liquid state to its thermodynamically more favourable solid state, which drives nucleation. A nucleus is an arrangement of a number of molecules in a crystalline structure that is large enough to be thermodynamically favourable by leading to a decrease in Gibbs free energy (Kloek et al., 2000b). Impurities act as catalysts for the formation of crystals by lowering the activation energy required for nucleation and providing a “template” for crystals to form on (Campbell et al., 2002).

Impurities (e.g., in the form of dust particles) invariably exist in many types of solution and hence provide potential surfaces on which crystals can grow. The surface energy requirement for the creation of new surfaces is high, so that homogeneous crystallisation is unlikely to occur. Homogenous nucleation requires large degrees of undercooling and very pure solutions (Mullin 1993). It has been demonstrated in emulsion droplets where the triglyceride oil had been washed several times using methanol to remove impurities in the form of mono- and diglycerides (Skoda & Van den Tempel 1963). Although some impurities remained, these were shown to be

randomly distributed in the oil droplets. Some droplets were impurity-free. The triglycerides in such impurity-free droplets crystallised around 20 K below the crystallisation temperature of the bulk fat (Skoda & Van den Tempel 1963).

The cooling rate and concentration of crystallising fat, as well as concentration of impurities, all influence the nucleation rate of crystals. It has been shown that a rapid cooling rate (in excess of ~5 °C/min) results in a crystallisation rate 100 x higher than a slow cooling rate of 0.1 °C/min (Campos et al., 2002). This was attributed to the high thermodynamic driving force for crystallisation in rapidly cooled systems. More nucleation events occur in a shorter period of time because the instantaneous degree of undercooling is larger in rapidly cooled solutions. A short induction time for crystallisation was observed at rapid cooling rates, indicating that the free energy barrier to nucleation is lower (Campos et al., 2002) and thus nucleation events are more frequent than in slowly crystals. As a result, many crystal nuclei are formed that have a high surface free energy and are formed in a less stable polymorph (see below). On the other hand, a slow cooling rate reduces the frequency of nucleation events and only few nuclei are formed. A slow cooling rate results in the formation of the stable β-polymorph.

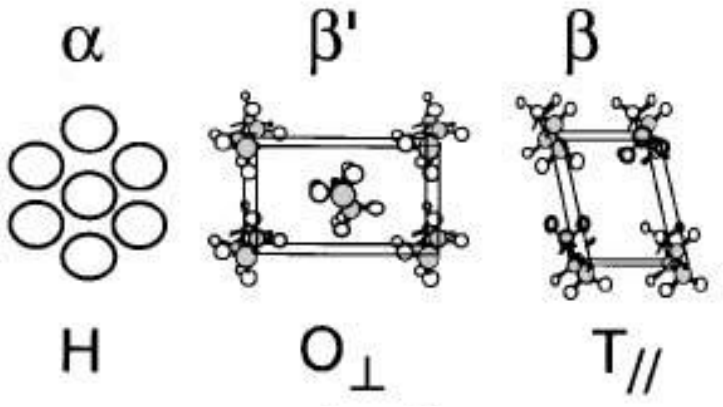

Figure 2.4: Examples of the structure of different polymorphs in triacylglycerols (from Sato 2001)

The cooling rate also influences the polymorphic forms of the crystals. Generally, 3 different crystal polymorphs in triglycerides are distinguished between (see Figure 2.4): α, which is unstable and has hexagonal subcells; β', which is more stable than the α-form and consists of orthorhombic subcells; and β, the most stable which consists of triclinic subcells (Akita et al., 2006; Lutton 1950). The different subcell structures define the arrangement of the crystals in crystal lattices (Sato 2001). For instance, in tripalmitoylglycerol the α-form is disordered, while it is densely packed in β-form. The degree of order within the crystal lattices is also reflected by the higher Gibbs Free Energy contained within crystals of the α—Polymorph (see Figure 2.5).

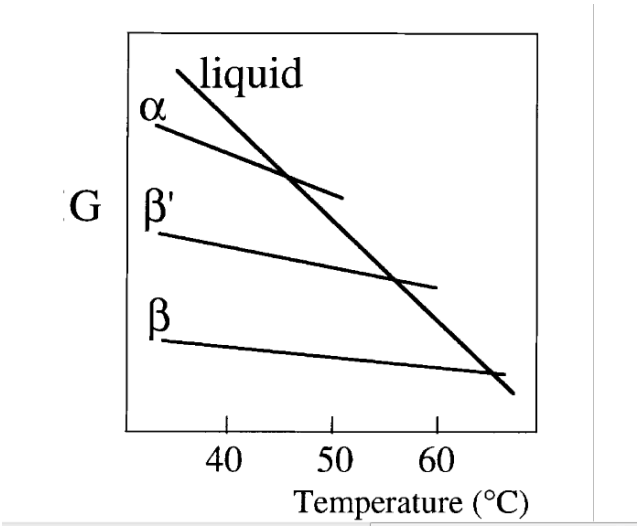

Figure 2.5: Gibbs free energy of different polymorphs of tripalmitoylglycerol (from Sato, 2001)

The crystal form is usually determined using DSC, and it has been shown that fast cooling rates favour the formation of unstable α crystals, while slow rates result in the crystallisation of the fat in its most stable form (Cebula & Smith 1991; Norton et al., 1985). Faster cooling increases the crystallisation temperature of the triglyceride fat (Norton et al., 1985). The least stable α-polymorph has the lowest free energy (ΔG_n) of nucleation as well as the lowest activation free energy ($\Delta G^{\#}_n$) of nucleation (Marangoni 2002, see

Figure 2.6), although it is finally the most energetic form. The low activation energy for nucleation means that the α-form crystallises as soon as the solution is cooled below the melting temperature of the crystals. That the solution does not require a great degree of supercooling for α-form crystals to form has been reported by Sato & Kuroda (1987). The low activation energy for nucleation encourages the formation of many α-form crystals in a short period of time. It has indeed been shown that when a

triglyceride fat is rapidly cooled a large number of small crystals, predominantly of the α-polymorph, are formed (Chapman 1962).

When a rapidly cooled sample is melted its heat capacity, measured by the DSC, is higher than if it was slowly cooled. α-form crystals exhibit a greater degree of freedom in the sample so that more energy is required to raise the temperature than in a slowly-cooled sample in which the crystals are arranged in a more orderly manner (Norton et al., 1985).

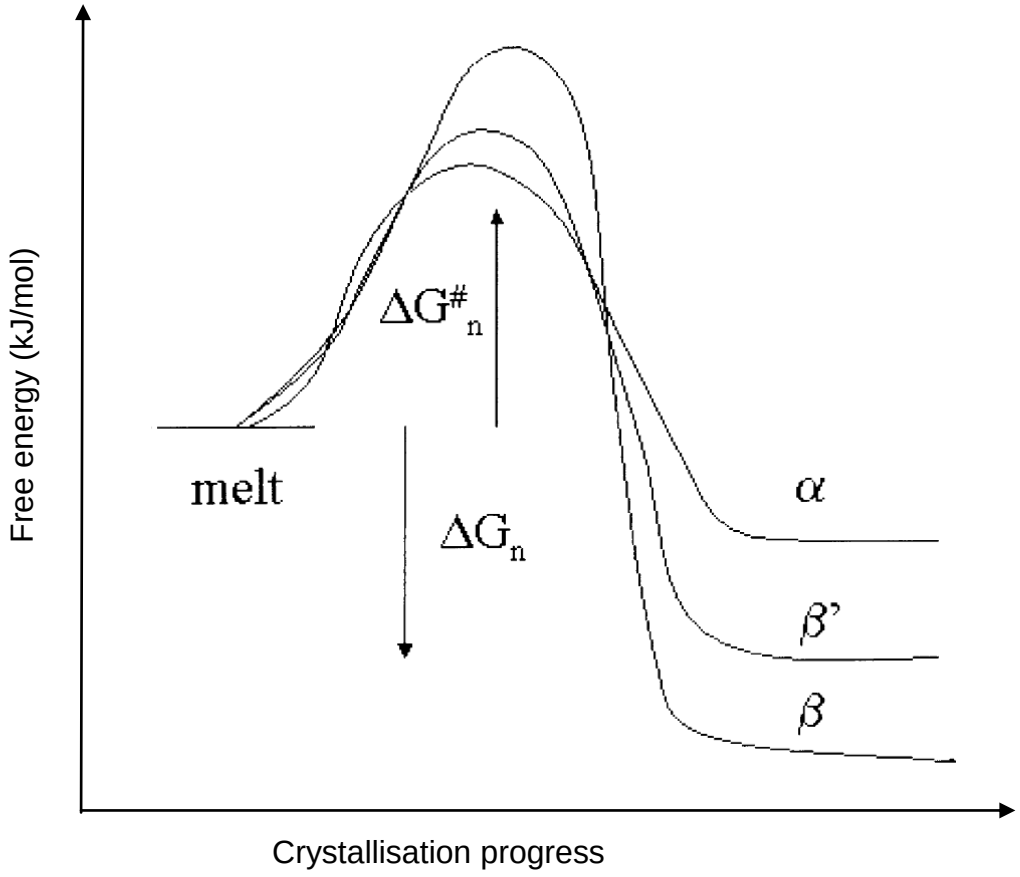

Figure 2.6: Overview of free energies for activation ($\Delta G^{\#}_n$) and free energies of formation (ΔG_n) of nuclei for the different polymorphs in triglyceride fats (taken from Marangoni 2002)

A thermodynamic driving force exists for the α-polymorphs to change to the more stable β-form because of the high free energy of the α-crystals (Campos et al., 2002; Norton et al., 1985). The molecules re-arrange over days of storage to minimise the free energy of the sample. α-crystals are no longer observed in a rapidly cooled fat that has been stored for several days (Campos et al., 2002).

The rate of nuclei formation influences crystals growth; the properties of crystallised fat can therefore be influenced by controlling the cooling rate. It was observed that slow cooling resulted in large crystal aggregates irregularly placed in a sample (Campos et al., 2002; Foley & Brady 1984; deMan 1964; deMan 1961). A slow cooling rate results in long induction time for nuclei formation. In slow cooling regimes nuclei form slowly. Those that have formed tend to grow so that a solution containing large crystals is formed. The time scale for nuclei formation is long compared to the time scale for diffusion and mass transfer onto a newly formed crystal. Crystallisation in slowly cooled samples therefore proceeds predominantly by the growth of existing crystals rather than the nucleation of new ones. It was shown that in rapidly cooled solutions the induction time for formation of crystals is short. A large number of crystals are formed in a short period of time (Campos et al., 2002). These crystals are evenly distributed throughout the solution and remain small because growth is restricted by the rapid increase of viscosity associated with the nucleation of crystals. The steep increase in viscosity slows mass transfer to the crystals (Campos et al., 2002).

A further factor influencing crystallisation rate is shear (Marangoni & Narine 2002, Mullin, 1993). Shear can enhance the crystallisation rate by distributing locally-

formed crystal nuclei within a sample. Nucleation occurs first at locations with the greatest degree of supercooling. In jacket-cooled vessels, this is often the inside wall. When a solution is sheared at the same time as it is cooled nucleation is enhanced by bringing nuclei that have formed at the wall of the vessel into contact with the bulk solution. When this occurs they may function as “seeding crystals” for the formation of more nuclei (Campbell et al., 1996).

b. Fat crystals at the interface of W/O emulsions

As discussed in section 2.1.2.6, particles provide a mechanical barrier to coalescence (Binks 2002). This can also apply to fat crystals, which are also particles. As discussed previously, a precondition for particles to be present at the interface is that they are wettable by both phases. A particle’s wettability is expressed by its contact angle (which should be greater than ~40 but less than ~130°, see section 2.1.2.6). Contact angles are measured using optical methods: the angle of contact between solid and at least one liquid phase is determined directly by measuring their physical angle of contact. Most triglyceride fat crystals are not naturally amphiphilic but it was shown that they have the tendency to adsorb at the oil/water interface. The contact angle of triglyceride crystals was shown to depend on the triglyceride as well as the polymorphic form of the crystals: Tristearin β-crystals, for instance, are not wetted by the water phase (contact angle ~0°), while the α-crystals have a contact angle between 20 and 30°. Palm stearin β’-crystals are slightly polar, with a contact angle of between 20 and 40° (Johansson et al., 1995). If the triglyceride fat crystals show a slight polarity they have a tendency to adsorb at the oil/water interface (Johansson & Bergenstahl 1995b).

In addition to their polarity, crystal size is an important parameter in determining whether crystals have a stabilising effect on emulsion droplets. The size of the crystals depends on the processing parameters, especially the cooling rate, as was discussed in the previous section (Campos et al., 2002). These processing parameters determine the crystal polymorphs: α-crystals, formed with rapid cooling, are small platelets, while β-crystals tend to be larger and needle shaped (van Boekel & Walstra 1981). A large number of small crystals will have the effect of forming a tight network of crystals around emulsion droplets, while good surface coverage is difficult to achieve with large crystals. The number of impurities in a crystallising solution influences the nucleation rate and thereby the crystal size: the more impurities are present, the more nuclei are formed (Kloek et al., 2000a). In W/O emulsions, the presence of emulsifier micelles in the continuous phase as well as the presence of oil-water interfaces may act as such catalysts for the formation of nuclei. If emulsifiers are present at concentrations above their critical micelle concentration (cmc), micelles may act as “seeds” for nucleation (Skoda & Van den Tempel 1963). If present at concentrations below the cmc, some emulsifiers may still have a catalysing effect on triglyceride nucleation by ordering the triglyceride molecule in a crystalline-like structure at the interface. Saturated monoglycerides have been shown to have such an effect (Skoda & Van den Tempel 1963).

Surfactants have been used to increase the wettability of triglyceride crystals with water. These adsorb at the fat crystals surface and alter crystal surface properties and thus increase crystal polarity (Johansson et al., 1995). It was shown that a minimum concentration (greater than 0.2 %) of surfactants must be present to significantly change the crystal’s contact angle. The reason for this is the crystals

compete with the O/W interface for emulsifier adsorption and at low emulsifier concentrations there is insufficient emulsifier present to cover the crystal interfaces as well as the O/W one (Johansson et al., 1995).

Surfactants such as monoolein, Polyglycerol of Polyricinooleates (PGPR) and lecithin have been used for this purpose in the past (Hodge & Rousseau 2005; Garti et.al., 1999; Johansson & Bergenstahl 1995a). The HLB of the emulsifier determines the ultimate polarity of the fat crystals, and therefore whether they will preferentially stabilise W/O (contact angles smaller than 90°) or O/W (contact angle larger than 90°) emulsions. Low HLB emulsifiers such as PGPR have been used to make stable W/O emulsions containing fat crystals (Hodge & Rousseau 2005; Garti et al., 1999,). However, HLB of the emulsifiers do not determine emulsion stability: lecithin, another low HLB emulsifier had a destabilising effect on emulsions (Johansson et al., 1995). Very strong emulsifiers such as ethoxylated castor oil which reduces the interfacial tension between water and the oil phase to ~0 seem to displace the fat crystals from the interface (Johansson et al., 1995). Although the resulting emulsions are stable, the presence of fat crystals does not seem to contribute to this stability.

Saturated monoglycerides crystallise at temperatures above those of most triglycerides. Their surface activity means that they are present at the interface in their liquid state. As the sample is cooled the monoglycerides crystallise directly at the interface. This property gave rise to their description as surface active crystals (Krog & Larsson 1992). A high concentration of monoglycerides at the interface results in a high rate of nucleation there as the temperature is decreased below the monoglycerides' crystallisation point. The nucleation rate is high because the

monoglycerides are already aligned at the interface in a crystalline-like structure (Krog & Larsson 1992). The rapidly crystallising monoglycerides act as “seeds” (secondary nucleation), align the triglycerides at the interface and cause their crystallisation there (Mullin 1993).

The benefit of using monoglyceride crystals to seed triglyceride crystals at the interface is that sub-micron sized crystals are produced directly at the interface. As has been highlighted previously, crystal morphology and crystal size in particular are crucial for the effective stabilisation of the emulsion, with sub-micron sized crystals playing the essential role of ensuring dense surface coverage (Hodge & Rousseau 2005; Rousseau 2000). The small size of crystals seeded directly at the interface enables them to arrange themselves in such a way that curved structures are formed around water droplets. These structures are comprised of individual crystals, which are not curved, arranged in a tight network, or “shell”, around the oil/water interface

A further factor influencing the presence of crystals at an emulsion interface is the timing of their addition to the emulsion during the emulsification process. The presence of emulsion droplets prior to crystallisation may provide a further surface for rapid nucleation of crystals directly at the interface. Studies have been performed comparing the stability of W/O emulsions in which crystals were either present prior to the emulsification process (“pre-crystallised” systems) or formed once emulsification was complete (“post-crystallised”). It was shown for both paraffin-wax (Rousseau & Hodge 2005) as well as triglyceride fat crystals (Hodge & Rousseau 2005) that the presence of emulsion droplets encouraged the formation of crystals

directly at the interface, thus allowing tightly-fitting crystal “shells” to be formed around a water droplet (Hodge & Rousseau 2003).

It was observed that if the crystals are formed and added to the oil phase prior to emulsion formation (“pre-crystallised” systems) the resulting emulsions are not very stable with respect to coalescence and sedimentation, especially when larger concentrations of fat crystals (greater than 1 %) are present. In systems where the emulsion is formed first and crystals are subsequently formed by quench-cooling, increasing the concentration of crystallising fat had a positive effect on emulsion stability.

This observation was explained by the size and location of the crystals in the emulsion samples. In the case of pre-crystallised emulsions, large crystalline structures were observed in the continuous phase of the samples (Rousseau & Hodge 2005). The crystals had been formed by crystallising the oil solution for 24h at 5 °C and had already formed a weakly sintered network within the solution. The relatively slow rate of crystallisation meant that relatively few crystals were formed in the sample which subsequently grew to a large size (Hodge & Rousseau 2003). In post-crystallised emulsions crystals the cooling rate was faster so that more crystals formed. It is also possible that these were formed directly at the interface (Rousseau & Hodge 2005). The existence of many small crystals resulted in the formation of a strong crystal network because the strength of crystal-crystal interactions is dependent on the number of particles present in the sample (Campos et al., 2002). This strong crystal network in the continuous oil phase prevented droplet movement and therefore droplet coalescence. The formation of an aggregated crystal network at

the droplet interface provided a further barrier to coalescence by forming a very viscous, mechanically rigid surface layer or “shell” (Rousseau & Hodge 2005).

The reason for numerous fine crystals forming stronger networks than fewer and coarser ones is that the number of interactions between crystals is proportional to the number of crystals present (Campos et al., 2002). Forces stabilising the fat crystal network include van der Waals, Coulombic, steric as well as solid bridges (Johansson & Bergenstahl 1995a). Van der Waals, Coulombic and steric interactions are generally too weak to explain the formation of strong crystal networks in solutions containing fat crystals (Johansson & Bergenstahl 1995a). The formation of solid bridges, or sintering, is, in addition to nucleation and growth, a third process which may occur during crystallisation. Compared to nucleation, it is a slow process, so that it becomes especially important after crystallisation of the solution, e.g. by undercooling, has ceased (a so-called postcrystallisation process). Sintering causes the formation of tight networks of fat crystals. With time, fat crystals may have sintered to such an extent that individual crystals are no longer distinguishable. In this case, one may think of the network as a “shell”.

Studies have been performed on the extent of sintering in solutions containing fat crystals. Network formation can be quantified by measuring the extent of sedimentation of crystals in solution: the sediments of aggregated fat crystal networks tend to be large and bulky. Fat crystals that are not attracted to each other do not form aggregates. The absence of a 3-dimensional structure leads to the formation of more compact and dense sediments (Johansson & Bergenstahl 1995a). Sintering was also measured by rheology, for instance by measuring the stress

necessary to get the sample moving uniformly (Bingham yield stress). Solid bridges are discussed in more detail below. It was found that the process of sintering stands in competition with the nucleation of new crystals during crystallisation, so that it is favoured by slower cooling (Johansson & Bergenstahl 1995a). However, it can proceed once nucleation is no longer possible (i.e. when the thermodynamic driving force for new crystallisation has ceased) so that the strength of crystalline networks containing sintering fats increases with time (Johansson & Bergenstahl 1995a).

2.1.2.8 Partial coalescence

If fat crystals are present in the oil phase of an O/W emulsion at sufficient concentration, partial coalescence may occur causing destabilisation (van Boekel & Walstra 1981). This is the mechanism by which two droplets partially fuse together as a result of protruding fat crystals piercing through neighbouring droplets' interface. The following mechanism for partial coalescence has been proposed: Fat crystals within oil droplets may protrude from the surface. As a neighbouring droplet approaches, this protruding crystal may pierce the thin interfacial film separating the two droplets. Because the fat crystal is preferentially wetted by the oil rather than the water, an oil bridge between the two droplets will form, leading to partial coalescence. The droplets cannot fully coalesce because the fat crystals flocculate to form a rigid fat crystal network and fixing the structure of the oil droplets (van Boekel & Walstra 1981). The partial coalescence of emulsions is usually undesirable because it causes flocculation of oil droplets in emulsion samples (see Figure 2.7). However, in some cases, such as the production of butter or margarine, partial coalescence is an important processing step as it enables the formation of fine water

droplets by phase inversion (Rousseau et al., 2003). In this case, the partial coalescence of oil droplets gradually causes the formation of a continuous network of fat crystals. The oil phase therefore becomes the continuous phase in which water droplets have become dispersed.

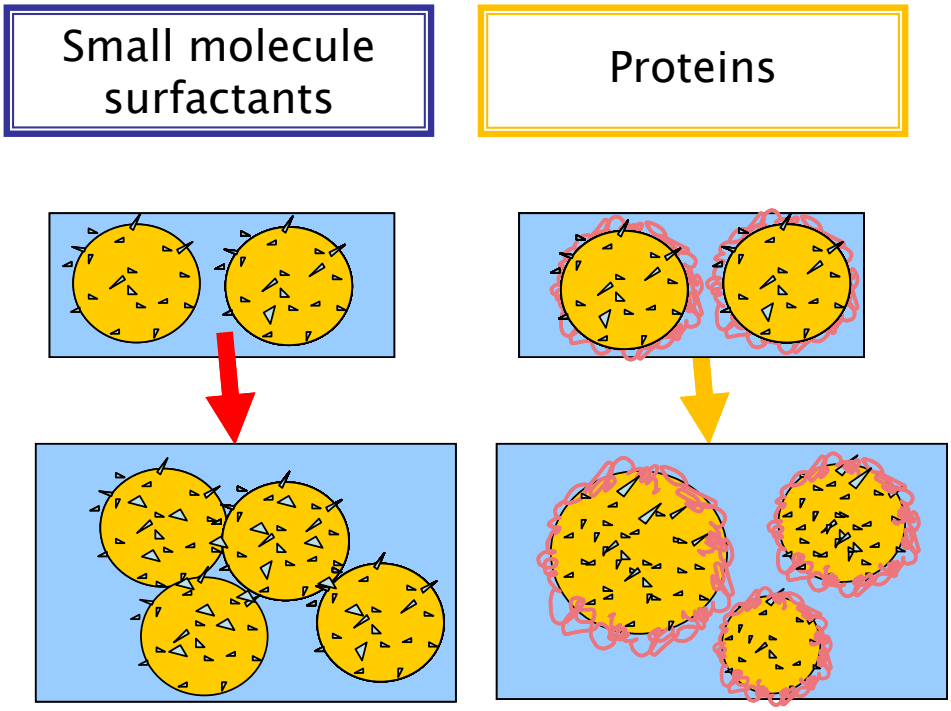

Figure 2.7: Influence of fat crystals in the dispersed phase on emulsions stability when stabilised with small molecule surfactants or proteins

Emulsion sensitivity to partial coalescence is increased as the concentration of solid fat is increased: more crystals increase the probability of protruding fat crystals piercing neighbouring interfaces (Boode et al., 1993). Partial coalescence can be controlled by the choice of emulsifier. This has been demonstrated by an experiment in which small molecular weight surfactants were added to protein-stabilised O/W emulsions containing fat crystals. It was shown that such addition resulted in the

destabilisation of previously stable emulsions. Low molecular weight surfactants have the ability to displace proteins from the O/W interface, reducing the film thickness and allowing protruding fat crystals to pierce the interfacial film between individual droplets (Davies et al., 2000).

Partial coalescence can be reduced if polymeric surfactants (proteins) are used instead of small molecule ones (see Figure 2.7). For instance, the distance of separation between two droplets stabilised by an ionic surfactant, SDS, is between 10 and 30nm (van Boekel & Walstra 1981). If Na-caseinate is used to stabilise the interface, this distance is increased to more than 100 nm (Dickinson 1998). The thicker interfacial film as well as steric stabilisation prevents the close approach of droplets stabilised with proteins, minimising contacts with protruding crystals.

Another factor that influences the stability of O/W emulsions containing fat crystals is the application of shear. Samples may be stable when they are stored in quiescent conditions, but destabilise once shear is applied. The reason for this is that shear decreases the minimum separation distance between droplets and increases collision events between droplets (Darling 1982; van Boekel & Walstra 1981; Mulder & Walstra 1974). It was shown, however, that the orientation of fat crystals is important in determining their shear stability: Samples containing crystals that are tangentially orientated at the interface are more stable than samples in which crystals protrude radially from the interface (Boode & Walstra 1993; Darling 1982). Radial orientation of crystals at the interface makes the emulsions sensitive to shear because they are more likely to pierce thin films between neighbouring droplets.

Partial coalescence can be reversed providing crystals are removed from the oil phase within a short time of time of the aggregation occurring. Fat crystals can be removed from the oil phase by changing the crystal wetting properties so that they are fully wetted by the water phase. This can be achieved by adding ionic surfactants, such as SDS, to the emulsion. Boode and Walstra (1993) have shown that if crystals are removed from the oil phase within 1 hour of the aggregation event partially coalesced droplets break apart. If the crystals are removed at later times partial coalescence becomes irreversible. This was ascribed to the fact that the fat crystals sintered over time, forming very strong networks that were hard to break (Boode & Walstra 1993).

2.1.2.9 Ostwald Ripening

Another mechanism for droplet size increase is Ostwald ripening. Ostwald ripening is the process of larger droplets growing at the expense of smaller ones due to a difference in chemical potential resulting from a difference in curvature between the two droplet sizes (Taylor 1995). The chemical potential increases with decreasing droplet size so that the solubility of the oil in the water also increases. Increased solubility causes small droplets to dissolve and the oil to diffuse and deposit on larger droplets, thus gradually increasing the average droplet size. Although droplet size changes due to Ostwald ripening are significant in most food emulsions, due to the solubility of most food-grade oils in water, coalescence is the biggest cause of instability for the emulsion systems investigated as part of this work. The influence of Ostwald ripening on changes in droplet size, and therefore on emulsion stability, is thus not investigated as part of this work.

2.2 $W_1/O/W_2$ Double Emulsions

Double emulsions are emulsions-of-emulsions, e.g. a water-in-oil emulsion is dispersed in an aqueous phase to give a water-in-oil-in-water emulsion. Double emulsions were first described at the beginning of the 20th century in a study investigating the influence of oil type on emulsion formation (Seifriz 1925). Their discovery was somewhat accidental in that the study was intended to investigate phase inversion.

Double emulsions have raised interest in many different industries (Muschiolik 2007) such as pharmaceutical, agrochemical as well as food, for their potential in particular to control the release of encapsulated ingredients (e.g., active ingredients in pharmaceuticals; nutritionally beneficial compounds or flavour in food). The potential for double emulsions to reduce fat in processed foods has already been discussed in Chapter 1. In pharmaceutical and agrochemical applications the main attraction of double emulsions is the potential for encapsulating oil and water active ingredients (in pharmaceutics, these could include vaccines, vitamins or hormones) in a structure which later permits controlled administration of this substance (Ficheux et al., 1998). Double emulsions are also attractive for cosmetic formulations as such structures can potentially combine the slow release of both water and oil soluble active ingredients, for example to the skin (Lin et al., 1999).

A desirable property of double emulsions is the controlled or triggered release of encapsulated substances such as flavour compounds in food products or active ingredients in pharmaceutical applications. One can imagine the potential particularly

for food applications of structures that deliver intense “bursts” of flavour in the mouth, or hide the unpleasant taste of some nutritional components during consumption but release these in the stomach. Triggered release of compounds has not yet been discussed in the literature on food-grade double emulsions. The challenge remains to formulate double emulsions that can retain compounds, e.g. salt, within the structure over extended periods of time, but release all encapsulated compounds near-instantaneously when the emulsion is consumed, delivering a “flavour burst”. Formulating such a structure is a major topic addressed by this thesis.

Effectively controlling the release of an encapsulated compound, however, poses challenges. For instance, if KCl or NaCl were to be encapsulated in a double emulsion structure it would need to remain encapsulated for a prolonged period of time (perhaps months) as the double emulsion is transported and stored. This has not yet been achieved because these salts are small and soluble species. Typically, between 30 and 80 % salt is released from double structures within 2 days (Pays et al., 2002; Garti, 1997b). Stability against salt release will be further discussed in section 2.2.3.

The practical use of double emulsions has so far been limited due to the poor thermodynamic stability of double structures. Instabilities can occur by many different mechanisms, such as coalescence between internal phase droplets or between double emulsion globules, or a loss of internal phase (see section 2.2.3). Formulating double emulsions that are kinetically stable over a period of several months is thus a challenge.

Numerous factors influence the stability of double emulsions, such as choice and ratio of the different emulsifiers, emulsification conditions and water: oil ratio (Ficheux et al., 1998). While early work (1970s - 1990s) has focused mostly on achieving double emulsion stability by finding an optimal balance between lipophilic and hydrophilic low molecular weight surfactants, more recent investigations have incorporated polymers such as proteins and colloidal particles into the double structure which has lead to the development of some double emulsions that are stable for up to 1 year (Barthel et al., 2003). The following section will give an overview of the current knowledge on double emulsions, especially formulation, production and stability issues.

2.2.1 Formation of double emulsions

Although originally formed in an uncontrolled one-step procedure close to the phase inversion point of oil and water, double emulsions are now commonly made using a two step procedure (Matsumoto et al., 1976): First, the primary emulsion is produced. This is subsequently emulsified to give the double structure. However, process parameters need to be controlled in order to make a stable and finely dispersed double emulsion. In food products, emulsion globules should be smaller than 15 - 20 µm to avoid negative influence on the product's sensory properties. Globules below this size range cannot be detected by the consumer, but larger globules tend to give the emulsion an "oily" mouthfeel (Norton et al., 2006). Primary emulsion droplets need to be small (less than 5 µm) so that they can be incorporated into double globules that do not exceed this size (Garti, 1997b). It is also important that the

primary emulsion droplets are capable of withstanding the shear required for the secondary emulsification step to create globules of the given size.

Droplet size is inherently difficult to control using traditional emulsification techniques, given that in any mixing vessel there are areas of higher and lower turbulence (and concomitant variations in energy density). This has led to investigations of double emulsions produced using membrane technology (Vladisavljevic et al. 2006, Nakashima et al., 2000; Mine et al., 1996). This technique has the advantage of good control of droplet size and relatively low shear rates compared to traditional emulsification techniques such as high pressure homogenisation or rotor/ stator mixing (see section 2.1.1) and also removes the risk of breaking the internal phase droplets upon encapsulation (Muschiolik 2007). Double emulsions can be produced by forcing a primary emulsion (which has either also been produced using membranes or by a more conventional emulsification process) through the membrane. The application of too much shear in conventional emulsification processes during the secondary emulsification step may damage or destroy the primary emulsion droplets (Muschiolik 2007). Using membrane emulsification for the secondary emulsification step is attractive because of the low shear applied to the dispersed phase (in this case the primary emulsion), as discussed in section 2.1.1. Double emulsions made using this technology tend to have uniformly-sized double globules which are evenly filled with primary emulsion droplets.

2.2.2 Formulation of double emulsions

2.2.2.1 Monomeric surfactants

The choice of emulsifiers on both interfaces is essential in order to achieve a stable double emulsion. Many early investigations (performed in the 1970s - 80s) into double emulsions used low molecular weight surfactants to stabilise both interfaces. Among the earliest investigators were Matsumoto et al. (1976); Matsumoto et al. (1980) and Magdassi & Garti (1984). Their reports showed the importance of obtaining a correct balance between the lipophilic and hydrophilic low molecular weight surfactants used for the stabilisation of the W_1/O and O/W_2 interfaces, respectively. A high concentration of hydrophilic emulsifier (stabilising the O/W_2 interface) was shown to be detrimental to the formation of double globules (Matsumoto et al., 1976). The authors suggested that 10x more lipophilic surfactant (in this case, Span 80) than hydrophilic surfactant (in this case, Tween 20) was necessary for the formation of double emulsions. The migration of hydrophilic emulsifier to the primary emulsion interface caused the dispersion of the primary emulsion water droplets to the continuous aqueous phase.

In apparent contradiction to these results, Garti et al (1985) reported that a concentration of lipophilic surfactant (stabilising the W_1/O interface) of up to 10 % w/w also resulted in rapid water transport between the two aqueous phases and therefore less stable double structures. Matsumoto et al (1976) had shown that the incorporation of up to 30 % of lipophilic emulsifier achieved a high yield of double emulsion droplets. An explanation for this apparent contradiction could be that a high

concentration of lipophilic surfactant such as Span 80 increases the viscosity of the oil phase and therefore reduces its permeability coefficient of water (Garti et al., 1985). At low concentrations of lipophilic surfactant, this effect is minimised so that the surfactant molecules readily move between the two interfaces.

In further investigations an optimisation of the lipophilic-to-hydrophilic emulsifier ratio was proposed in order to increase the stability in double emulsions when monomeric surfactants are used (Magdassi et al., 1984). The hydrophilic-lipophilic balance (HLB) number is a parameter that describes the affinity of emulsifiers for the oil or water phase. A high HLB number (larger than 7) describes an emulsifier that preferentially stabilises O/W emulsions, while a low (smaller than 6) number corresponds to an emulsifier preferentially stabilising W/O emulsions. In the food industry, monomeric emulsifiers with a high HLB include sorbitan monoesters (e.g. Tween 20, HLB = 16). The preferred emulsifier for W/O emulsions include monomeric surfactants such as polyglycerol of polyricinooleates (PGPR, HLB = 4) or Span 80, HLB = 4.3 (Muschiolik 2007).

Magdassi et.al. (1984) suggested that a modified HLB number can be calculated to optimise the ratio between lipophilic and hydrophilic emulsifiers in double emulsions. This is based on the optimum HLB number for the dispersed oil, so that an increasing fraction of lipophilic surfactant in the oil lowers the overall HLB of the formulation. The authors suggest that if the optimum HLB for a specific oil is known the relative concentrations of the two emulsifiers can be tailored to achieve the required HLB value (Magdassi et al., 1984).

Despite such attempts to stabilise double emulsions with an optimum ratio of hydrophilic and lipophilic emulsifiers long-term kinetic stability was not achieved through sole use of small molecule surfactants. The main reason for this is the ability of the small molecules to diffuse readily between the two interfaces. The different types of emulsifiers pack differently at the interfaces: lipophilic ones spontaneously curve around water droplets, while hydrophilic ones spontaneously curve around oil droplets (Pays et al., 2002; Pays et al., 2001). The reason why the concentration of hydrophilic surfactant affects emulsion stability negatively is that its inevitable presence at the primary emulsion interface encourages hole formation there, causing instability and phase inversion (Pays et al., 2001) and transforming the double emulsion into a simple O/W one (Pays et al., 2002).

2.2.2.2 Polymeric emulsifiers

The problem of double emulsion instability when using small molecule surfactants to stabilise the interfaces is widely recognised. It is now generally suggested that at least one of the two emulsifiers should be of polymeric nature (Pays et al., 2002), so that recent research has focused on using polymeric emulsifiers in double structures (e.g. Benichou et al., 2007; Su et al., 2006; Mezzenga et al., 2004; Garti & Aserin 1996). When polymeric emulsifiers are used on at least one interface, double emulsion stability against coalescence improves dramatically: no change in droplet size was observed after 1 week's storage when synthetic polymeric emulsifiers were used at both interfaces (Pays et al., 2002).

Natural proteins, such as Na-caseinate, BSA, whey protein isolate (WPI) and lecithin, have long been used to stabilise simple O/W food emulsions. It has been shown that when these are used in double emulsions, stability against coalescence is improved compared to when small molecule surfactants are used: Na-caseinate on the secondary interface stabilises double emulsion globules against coalescence for one month (Bonnet et al., 2009). Their large molecular size prevents proteins from migrating between interfaces. They will also not form reverse micelles transporting water and/or solutes through the oil phase. In addition, they provide steric stabilisation to the emulsion, which is very effective at preventing coalescence (see section 2.1.2.4).

Early experiments have shown that the addition of small quantities of BSA to double emulsions containing Tween 80 as hydrophilic emulsifier and Span 80 as lipophilic one is beneficial to the stability of the emulsion (Omotosho et al., 1986). BSA and Span 80 at the internal (W/O) interface has a synergistic effect on the stability and salt release profiles of the double emulsions, suggesting that the two species may have formed stabilising complexes. Moreover, addition of BSA to the external aqueous phase was also found to improve double emulsion stability, although it was less effective in preventing salt release from the encapsulated water phase than the previously mentioned combination. This difference in stabilisation mechanism is ascribed to the way that BSA at the external interface serves as a “protective colloid”, surrounding a layer of monomeric surfactant, while at the internal interface BSA was co-adsorbed with the monomeric emulsifier (Garti & Aserin 1996). Apparent synergy between a hydrophobic and hydrophilic emulsifier at the primary interface has also been observed when PGPR and Na-caseinate is used. The addition 0.5-1% Na-

caseinate to the primary emulsion increases the initial yield of double emulsions, although it had no effect on droplet size or emulsion stability (Su et al., 2006).

In an attempt to increase double emulsion stability even further, proteins have been used in combination with biopolymers such as dextran or xanthan gum. It has been shown that the combination of proteins, e.g. WPI, with biopolymers such as dextran or xanthan gum can lead to further improvements in double emulsion stability. This is explained by the formation of complexes at the interface between the two species (Benichou et al., 2007). These complexes form a rigid interfacial film and thus enhance double emulsion stability. Similarly, double emulsions stabilised by sodium caseinate-dextran conjugates result in increased stability with respect to coalescence (Fechner et al., 2007).

The benefits of using polymeric emulsifiers instead of monomeric ones are clearly seen in Table 2.1. In order to asses the stability of double emulsions, and their ability to retain water over an extended period of time, a solute (e.g., a salt, dye or water-soluble vitamin) is usually added to the primary emulsion aqueous phase (Bonnet et al., 2009; Fechner et al., 2007; Garti & Aserin 1996). The gradual release of the solute to the continuous phase is taken as a measure of its stability, and is assessed by various methods, e.g., by measuring conductivity (Sela et al., 1995) or by dialysis (Fechner et al., 2007).

The use of polymeric surfactants dramatically increases the ability of double emulsions to retain encapsulated solutes. This is due to the mechanisms described earlier in this chapter: the observation that polymeric surfactants sterically stabilise

the globules. Furthermore, water and/ or solute transport between the two phases is reduced. This is further discussed in section 2.2.3.

Table 2.1: Stability of double emulsions stabilised by monomeric or polymeric emulsifiers

Released substance	Lipophilic emulsifiers	Hydrophilic emulsifiers	% Release	Authors
NaCl	Monomeric (Span 80)	Monomeric (Span 80 and Tween 80)	After 5 hours: 35% After 25 hours: 53%	Garti 1997b
NaCl	Monomeric (Span 80) + protein (BSA)	Monomeric (Span 80 and Tween 80)	After 5 hours: 8% After 25 hours: 20%	Garti 1997b
$MgCl_2$	Monomeric (PGPR)	Protein (Na-caseinate)	After 30 days: 8%	Bonnet et al., 2009
NaCl	Synthetic polymer (Arlacel P135)	Synthetic polymer (Synperonic PE/F 68)	After 30 days: 5%, Carboxyl methyl cellulose is added to outer aqueous phase	Pays et al. 2002
NaCl	Monomeric (Span 80)	Monomeric (SDS) low concentration	After 17 hours: 20%	Pays et al. 2002
NaCl	Monomeric (Span 80)	Monomeric (SDS) high concentration	After 17 hours: 80%	Pays et al. 2002
Hydrated $MgSO_4$	Synthetic Polymer (Abil EM90) low concentration	Monomeric (PEG-25 soya sterol and Brij 58®)	After 25 hours: 30%	Geiger et al., 1998
Hydrated $MgSO_4$	Synthetic Polymer (Abil EM90) high concentration	Monomeric (PEG-25 soya sterol and Brij 58®)	After 25 hours: 8%	Geiger et al. 1998

2.2.2.3 Particles at the interface in double emulsions

In the last decade the use of particles in double emulsions has attracted increasing interest (Barthel et al., 2003). The mechanical barrier that particles provide against coalescence, along with their firm attachment at an interface (as discussed in section 2.1.2.6), makes them extremely attractive as candidates for stabilising double emulsions.

One of the earliest reports of double emulsions stabilised by particles was made by Oza & Frank (1989). Colloidal microcrystalline cellulose was added to the external aqueous phase of W/O/W emulsions and it was subsequently shown that these particles form networks at the interface. Small molecule surfactants were also present in the oil and external aqueous phase. The resulting double emulsions were stable for a period of around 1 month, and encapsulated salt was not released from the primary aqueous phase. Comparison with emulsions that did not contain the particles allowed the authors to conclude that the presence of particles at the interfaces was the reason for the good stability of the double emulsions.

Garti et al. (1999) showed that stable double emulsions containing α-tending triglyceride fat crystals could be formulated. These crystals were produced by flash-crystallisation of the triglyceride-emulsifier mixture and subsequently used to produce the W/O emulsion. It was found that emulsions containing such crystals along with a surfactant, PGPR, were stable against coalescence. Double emulsions containing this primary emulsion remained stable against coalescence for at least 2 weeks, although this depended on hydrophilic surfactant concentration (Garti et al., 1999).

The study did not clarify whether the presence of fat crystals at the primary emulsion interface improved emulsion stability compared with a primary emulsion that was solely stabilised by PGPR. Double emulsions containing only PGPR as primary emulsifier that were stable for at least a similar period of time have also been formulated by various researchers (Pawlik et al., 2010; Mezzenga et al., 2004).

Midmore & Herrington (1999) reported double emulsions containing a mixture of monomeric surfactants, polymer and colloidal silica particles that were stable against coalescence for up to 6 months. Double emulsions stabilised solely by silica particles, lipophilically and hydrophobically modified to stabilise the respective interfaces, have also been created and shown to be stable for up to 1 year (Aveyard et al., 2003; Barthel et al., 2003). This long-term stability against coalescence has not been achieved in double emulsions containing only small molecule surfactants or polymeric emulsifiers and shows the potential of particles in the creation of stable double emulsions.

2.2.3 Stability of double emulsions

Figure 2.8 illustrates the types of instabilities that exist in double emulsions (Ficheux et al., 1998):

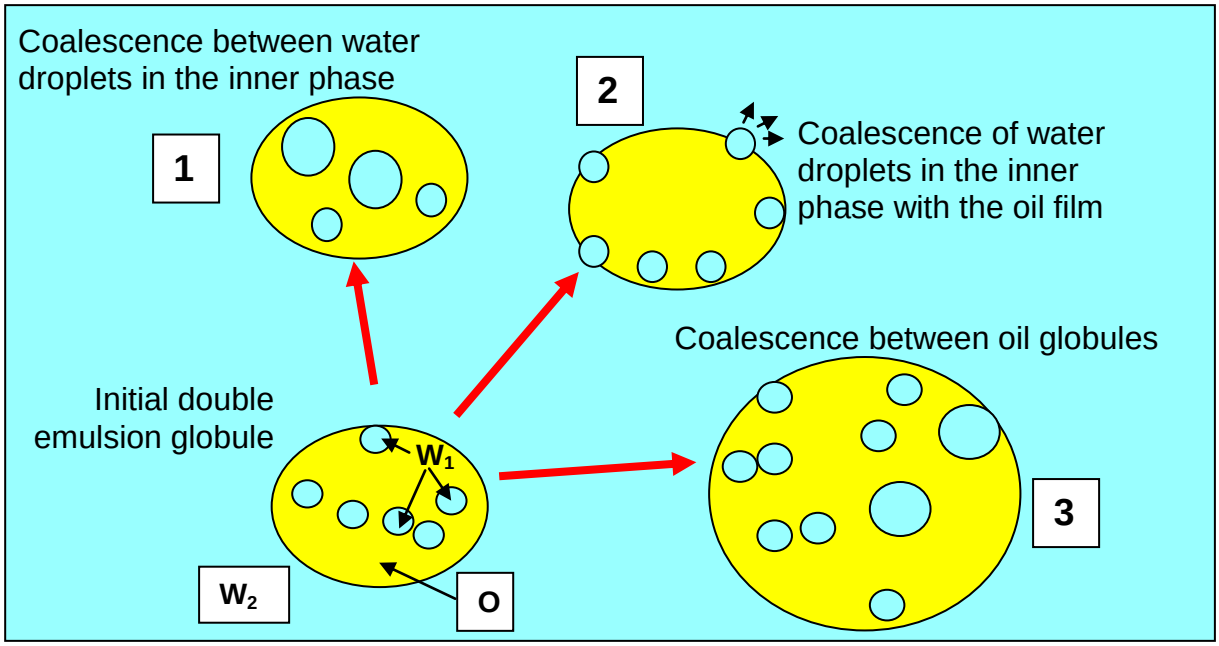

Figure 2.8: Types of physical instabilities in double emulsions

1. *Coalescence of primary emulsion aqueous droplets with each other*. This mechanism occurs if primary emulsion droplets are not sufficiently stabilised, and implies that the size of encapsulated water droplets increases. It does not directly lead to a loss of encapsulated water phase.

2. *Primary emulsion aqueous droplets attach themselves to and rupture the oil film separating the two aqueous phases*. If the primary emulsion interfacial structure is weak, it may merge with the secondary interfacial film. In this mechanism, the encapsulated water is emptied to the continuous aqueous phase, leading to a gradual loss of the double structure.

3. *Coalescence between double globules*. Coalescence between double globules may occur if the secondary interface is not sufficiently stabilised. This

process of destabilisation manifests itself in a gradual coarsening of the double emulsion. In the extreme, and if the primary emulsion is stable within the double structure, this process could lead to a separation of primary emulsion and continuous phase.

4. A further mechanism that affects double emulsion stability is the *diffusion of water across the oil boundary*. This is caused by the existence of an osmotic pressure gradient between the two aqueous phases and is discussed in detail in the next section.

2.2.3.1 Osmotic pressure gradients

The encapsulated water droplets have a Laplace pressure which creates an osmotic pressure gradient with the continuous phase (Mezzenga 2007). This osmotic pressure gradient may be enhanced by if the two distinct aqueous phases have different solute concentrations. Unless the osmotic pressure gradient = 0, water is transported from one phase to another in order to minimise the pressure difference. Depending on the direction of the osmotic pressure gradient, this may cause droplet swelling (and eventual rupture) or shrinkage (and eventual disappearance).

Several different mechanisms for water transport in double emulsions have been proposed (Benichou et al., 2004), as shown in Figure 2.9.

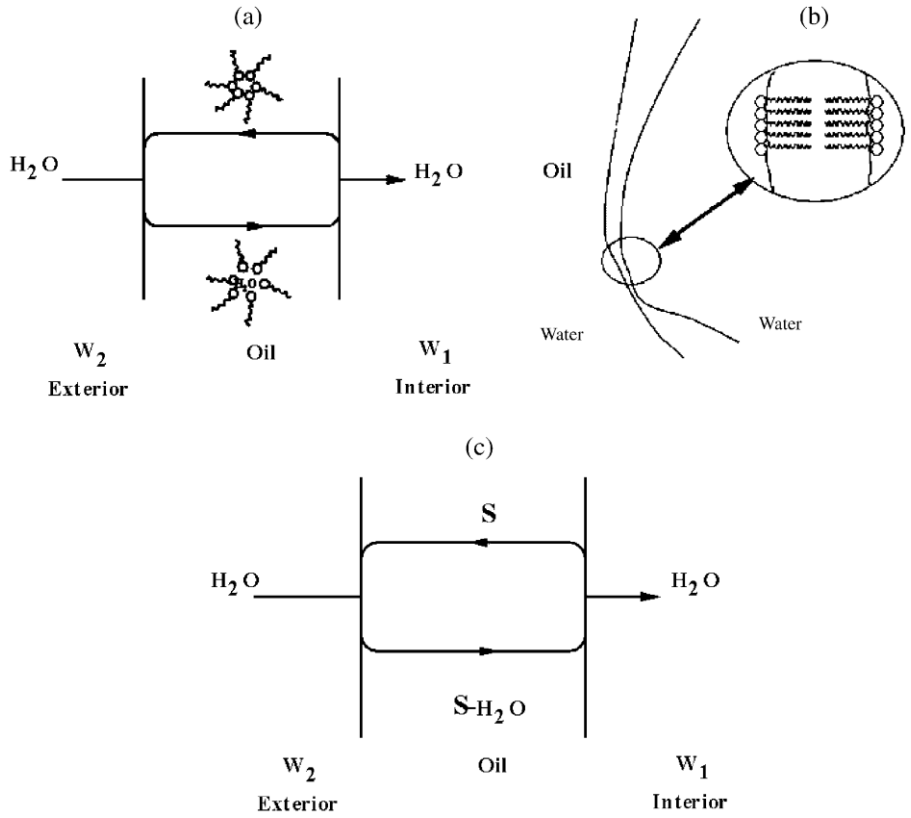

Figure 2.9: Forms of water transport in double emulsions, taken from Benichou et al., 2004. a) is indirect transport by reverse micelles, b) is direct transport through lamellae of surfactants and c) is direct transport of hydrated ions

1) Direct transport of ions or hydrated ions through lamellae of surfactants (Matsumoto et al., 1980), representing direct diffusion of these ions through the oil phase

2) Indirect transport of water by reverse micelles (Wen & Papadopoulos, 2001).

Single double emulsion globules have been observed using capillary video microscopy (Wen & Papadopoulos 2000). A double globule was created by injecting a water droplet directly into an oil globule present in a capillary filled with saline solution. This water droplet could be injected to be positioned directly at the interface

between the oil and the continuous water phase (“visually contacting”) or suspended in the oil globule away from the interface (“visually non-contacting”). The authors suggested that mechanism 1 occurs when oil films are sufficiently thin or “visually contacting”, as the rate of water transport in this case was several times higher than the rate of transport in “visually non-contacting” systems. For mechanism 2 to take place a minimum oil film thickness must be reached – “visually non-contacting” systems. Without this minimum film thickness reverse micelles cannot form and water transport does not take place (Cheng et al., 2007). In addition to transporting water, reverse micelles may also encapsulate water soluble substances.

When the osmotic pressure is matched and no gradient exists between the two aqueous phases, the driving force for water transport is low. This removes one factor of instability for double emulsions. The osmotic pressure gradient can be removed by adding solutes such as NaCl or glucose to the continuous aqueous phase to balance the osmotic pressure of the encapsulated droplets. It is even possible to tailor the final double emulsion morphology in terms of encapsulated droplet size by adjusting the osmotic pressure gradient of the external aqueous phase (Mezzenga et al., 2004). The internal droplets will swell or shrink until they their osmotic pressure is at equilibrium with that of the external aqueous phase.

However, it is likely that water transport does not cease entirely even as the osmotic pressure between the two aqueous phases is matched (Mezzenga 2007). Movement of water across the oil film boundary may continue in both directions, especially if small molecule surfactants are present to aid such transport. Such movement does not cause a change in the volume of the encapsulated droplets but can be observed

by the leakage of encapsulated solutes. Thus, such water/ solute movement can present a problem if the primary purpose of the double emulsion is to retain solutes for controlled release or delivery after a longer storage period. The only way of preventing such leakage between the two aqueous phases is to find a structure that does not enable the transport of water across the oil phase. Finding such a structure is the major aim of this thesis.

2.3 Summary of the literature review and outlook

Knowledge of the principles of emulsion science, such as emulsion formation and stabilisation, is important if stable double emulsions are to be created. The key stabilisation mechanisms are:

- electrostatic, usually provided by charged small molecule surfactants:
- steric, usually provided by polymeric surfactants or proteins
- Pickering, where small particles or fat crystals provide excellent stability against coalescence.

The formulation of stable double emulsions has attracted the attention of many researchers in recent years. The main problem associated with double emulsions is their poor stability: Coalescence between double globules as well as the gradual loss of the double structure hinders their industrial application. The replacement of small molecule surfactants with polymeric or particulate ones has improved the stability of double emulsions against coalescence from a few days (with small molecule

surfactants) to a few months (with particles at the interface). However, the encapsulation of solutes within the inner aqueous phase over extended periods of time remains difficult, especially when osmotic pressure gradients are applied.

The purpose of this work is to create double emulsions that remain stable and retain encapsulated solutes despite applied osmotic pressure gradients. As has been shown in the literature review, the most likely way of achieving this is by not including monomeric surfactants in the formulation. Instead, the primary W/O emulsion structures will be stabilised by tight networks of fat crystals, which have been shown to form “shells” around the water droplets. It will be investigated whether such “shells” can retain water and solutes despite applied osmotic pressure gradients.

3 Materials and Methods

3.1 Materials

The following materials were used in this work: Saturated monoglycerides (MG), an equal mixture of Dimodan HP and Dimodan P Pel/B, were obtained from Danisco, UK. Dimodan P Pel/B is derived from palm oil and its composition is therefore based on palm oil's natural fatty acid composition. Dimodan HP is also derived from palm oil, but has been distilled (V. Cole, personal communication, 2008). No attempt was made to characterise the individual components or to purify these commercially available products. Spray-dried sodium-caseinate (EM 7, milk protein type), was provided by DMV International. Glyceryl Tripalmitate (tripalmitin, purity of ≥85%, CAS number 555-44-2), D^+-glucose (purity ≥99.5%, CAS number 50-99-7), gelatine (from porcine skin, 80g bloom, CAS number 9000-70-8), colloidal microcrystalline cellulose (cmcc, Sodium Carboxymethyl Cellulose content 10.0 - 20.0%, CAS number 9004-34-6), guar (CAS number 9000-30-0), carboxymethylcellulose (cmc, medium viscosity grade (400-800 cP in 2% water), CAS number 9004-32-4), sodium azide (BioXtra, purity ≥99.5%, CAS number 26628-22-8), sodium chloride (BioXtra, purity ≥99.5%, CAS number 7647-14-5) and potassium chloride (BioXtra, purity ≥99%, CAS number: 7447-40-7) were all obtained from Sigma, UK. PGPR (Palsgaard 4150) was supplied by Palsgaard, Denmark. Hydrophilic silica particles (Aerosil 200), primary particle size 12 nm and surface area of 200 m^2/g, were kindly provided by Degussa, UK, in powder form. Mustard powder was purchased from the local supermarket. Sodium octenyl succinate (OSA) corn starch (N-creamer 46) was supplied by National Starch. Commercially available sunflower oil and double distilled water were used for the preparation of all emulsions. All materials were used without further

purification. All percentages of the water and oil phases listed below are weight/ total weight of the emulsion. The percentages of the components in the oil or water phase are given as percentage weight per total weight of the oil or water phase respectively.

3.2 Methods

The following section describes the methods used for simple and double emulsion preparation and subsequent characterisation. Most characterisation techniques were the same for simple and double emulsions, unless otherwise indicated.

3.2.1 Primary emulsion preparation

The oil phase components included varying concentrations of monoglycerides (usually 0.5 %, but in some formulations between 0.5 and 2.5 %) and triglycerides (between 0 and 2 %), and sunflower oil.

The aqueous phase components included salt (in the form of NaCl or KCl, 1.67%), and sometimes hydrocolloids, which were dissolved or dispersed in double distilled water as follows:

- gelatin: 1-5 % gelatin was dissolved in hot (70 °C) water
- cmc was dissolved in water at ambient temperature
- CMCC was dispersed in water using a high intensity ultrasonic vibracell (Jencons-PLC) operating at 20 kHz and 700 W for 6 minutes
- guar was dispersed at ambient temperature in water

The two phases were heated separately and combined at ~70 °C using a rotor stator mixer (Silverson). This pre-emulsion was then passed through a bench-scale scraped-surface heat exchanger (“A unit”), approximate volume = 25mL, and a pin stirrer (“C unit”), approximate volume = 210 mL, both were cooled with water at 5 °C. The emulsion was cooled at around 20 °C/min in the A-unit, which, along with the large surface area available for crystallisation and the good mixing of the newly formed crystals provided by the scraping blades, ensured a rapid crystallisation rate. The C-unit’s main purpose was to apply shear to cause phase inversion, and to cool the resulting emulsion to prevent the newly formed fat crystals from melting. The pump speed was set at 25 ml/min, and both stirrers were rotated at speed “8”, which corresponds to a shaft speed of 1230 rpm in the “A” unit and 1200 rpm in the ”C” unit (Pawlik, 2009, personal communication). Dimensions and set-up of these units are described in more detail in Figure 3.1, Figure 3.2 and Figure 3.3.

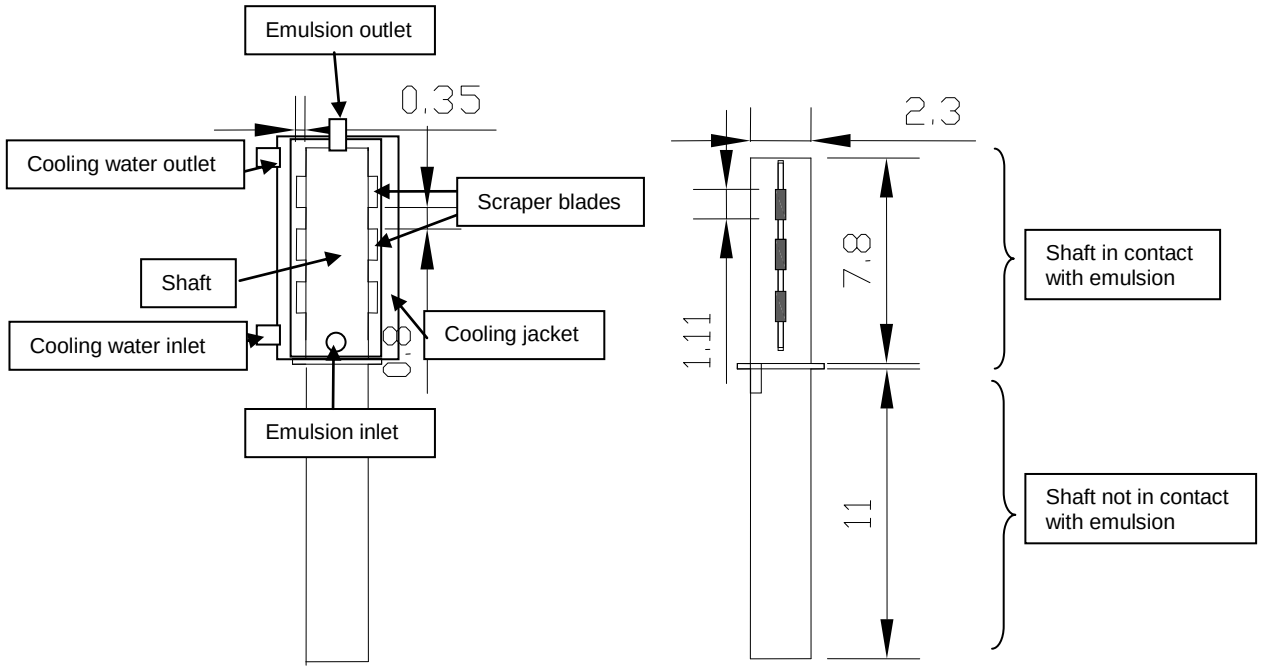

Figure 3.1: Scraped Surface Heat Exchanger, dimensions in cm

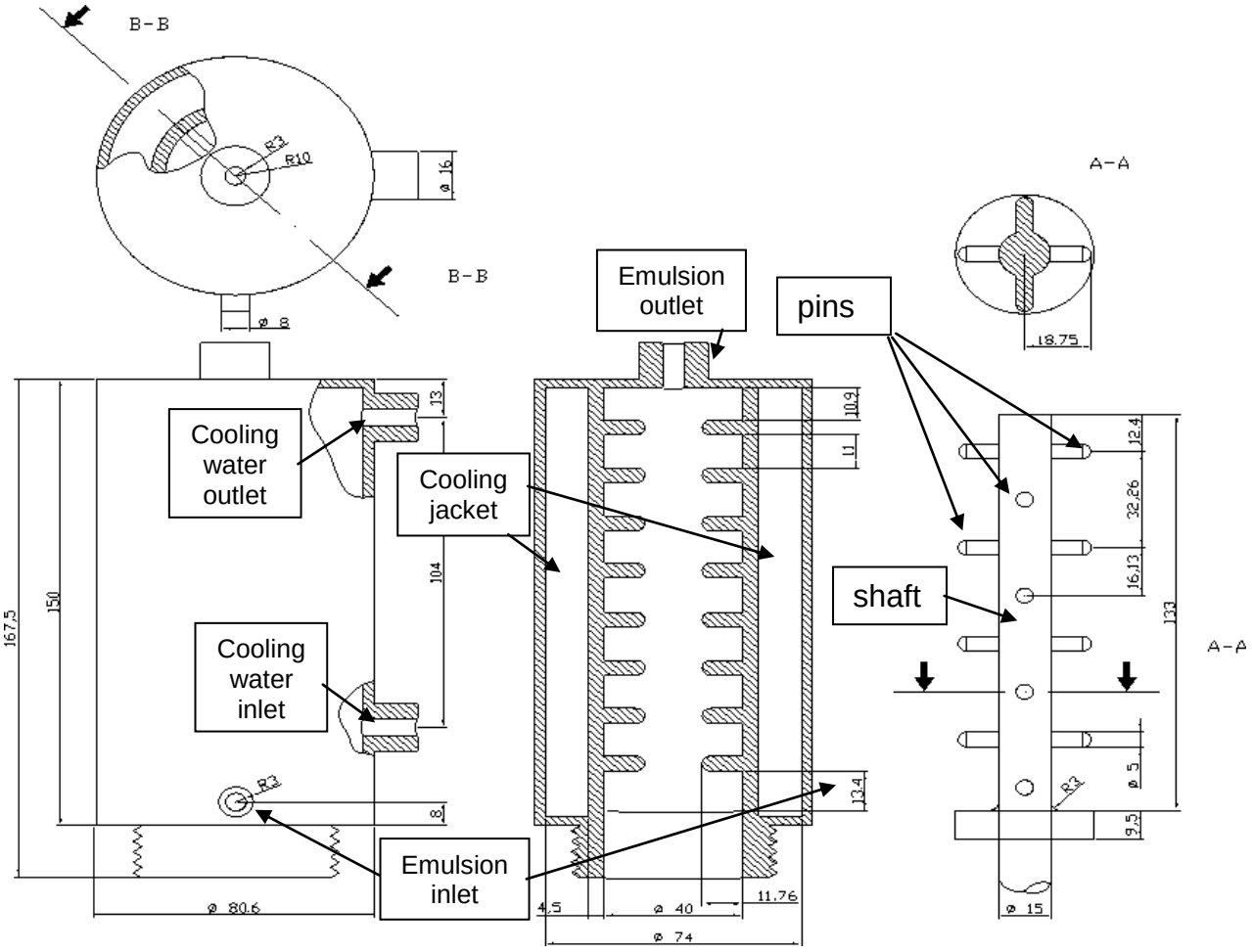

Figure 3.2: Pin stirrer, dimensions in cm (with permission from Gabriele, 2011)

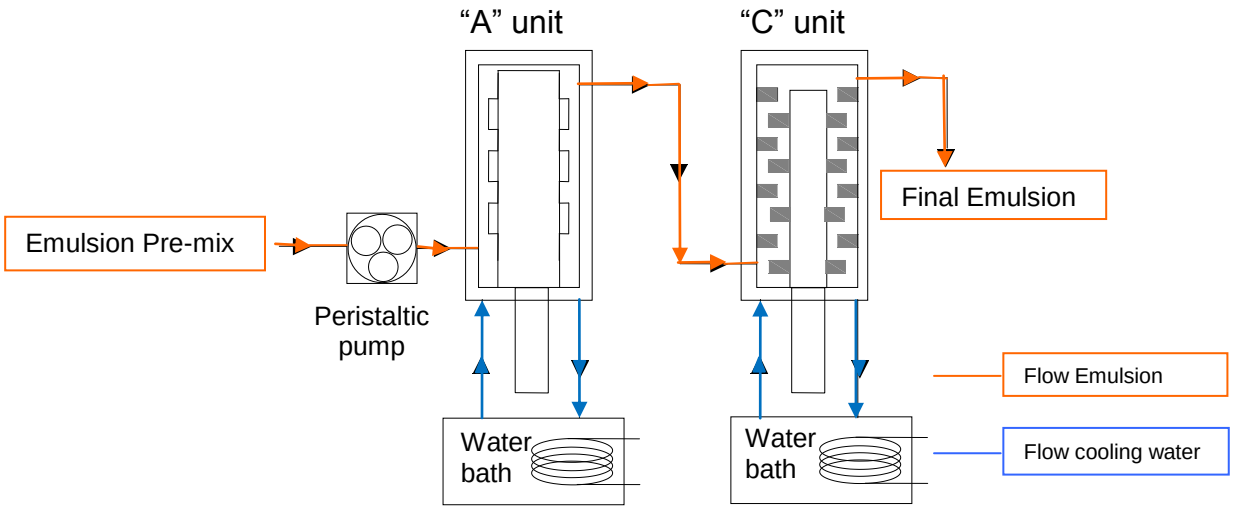

Figure 3.3: "A" and "C" unit set-up

The resulting emulsion was then passed a second time through the “A” and “C” units, in order to reduce droplet size and size distribution (Table 3.1). The final emulsion temperature at the outlet from the C-unit was measured at ~11 °C for all experiments.

Table 3.1: Overview of droplet sizes at various stages during production in a formulation containing 2 % tripalmitin and 0.5 % monoglycerides

1st pass A-unit	10.3 ± 0.9 µm
1st pass C-unit	5.1 ± 0.7 µm
2nd pass A-unit	3.0 ± 0.5 µm
2nd pass C-unit	4.1 ± 0.4 µm

3.2.2 Double emulsion Preparation ($W_1/O/W_2$)

In order to obtain double emulsions, sunflower oil was mixed with the W_1/O primary emulsion in a ratio of 1:1, thus reducing the W_1 water content to 15 %, before 20 g of this blend was slowly mixed with 80 g of the continuous W_2 aqueous phase. This W_2 aqueous phase also contained the secondary emulsifier:

- 1 % sodium caseinate which was dissolved in distilled water,
- 2 % or 5 % OSA starch dissolved in distilled water
- 0.2 % Bovine Serum Albumin (BSA) or 4 % WPI which were dissolved in distilled water
- 1 % or 2 % silica particles which were prepared as follows: A 2 % si-particle dispersion at pH 2, containing 0.1 % sodium azide as a preservative, was sonicated for 6 minutes to obtain finely dispersed silica particles (floc size ~100 nm), using a high intensity ultrasonic vibracell (Jencons-PLC) operating at 20 kHz and 700 W. When required this dispersion was subsequently diluted to obtain a 1% solution
- 1% mustard powder which was dispersed using the sonicator for 12 minutes. Acid (HCl) or base (NaOH) were added to obtain a pH of 2 or 7.2, respectively
- Various mixtures of Na-caseinate and silica particles (ratios 2:1, 1:1, 1:4, 1:10)

A small (0.01 %) amount of sodium azide was also added to the W_2 phase to prevent microbial contamination.

Varying concentrations of glucose (4-16 %), or NaCl (1-5 %) were added to the W_2 aqueous phase in order to create different osmotic pressure gradients (Δπ) between encapsulated (W_1) and continuous aqueous phase (W_2) (see Table 3.2). The osmotic pressure gradient between the aqueous phases was calculated based on the difference in the molar concentration of the solutes in the two phases.

Table 3.2: Directions of osmotic pressure gradients: A positive Δπ indicates the concentration of solute is greater in W_1 (inner) than in the continuous W_2 (outer) aqueous phase

Osmotic pressure gradient (Δπ)	Molar Concentration of solute	Concentration [NaCl] in W_2	Concentration [glucose] in W_2	Preferential transport of water
11 atm	$W_1 > W_2$	0 %	0 %	Into droplets
5.5 atm	$W_1 > W_2$	1.3 %	4 %	Into droplets
0 atm	$W_1 = W_2$	2.6 %	8 %	No preference
-11 atm	$W_2 > W_1$	5.2 %	16 %	Out of droplets

3.2.2.1 Double emulsion preparation using a Rotor/ Stator device

The formulation was subsequently sheared at 8000 rpm for 3 minutes using a Rotor/Stator apparatus (Silverson), while being placed in an ice bath to avoid an increase in temperature in the sample, which could melt the crystal network surrounding the W_1 primary emulsion droplets.

3.2.2.2 Double emulsion preparation using membranes

A cross-flow membrane system was set-up as detailed in Figure 3.4. A pressurised feed tank, containing primary emulsion, fed the membrane module. Continuous

aqueous phase was circulated around the membrane using a Cole-Parmer gear pump. 2 membranes were used: the 10 µm SPG membrane was purchased from SPG Techno (Japan). A 15 µm laser-drilled stainless steel membrane was custom made by Laser Micromachining Limited (Denbighshire, UK).

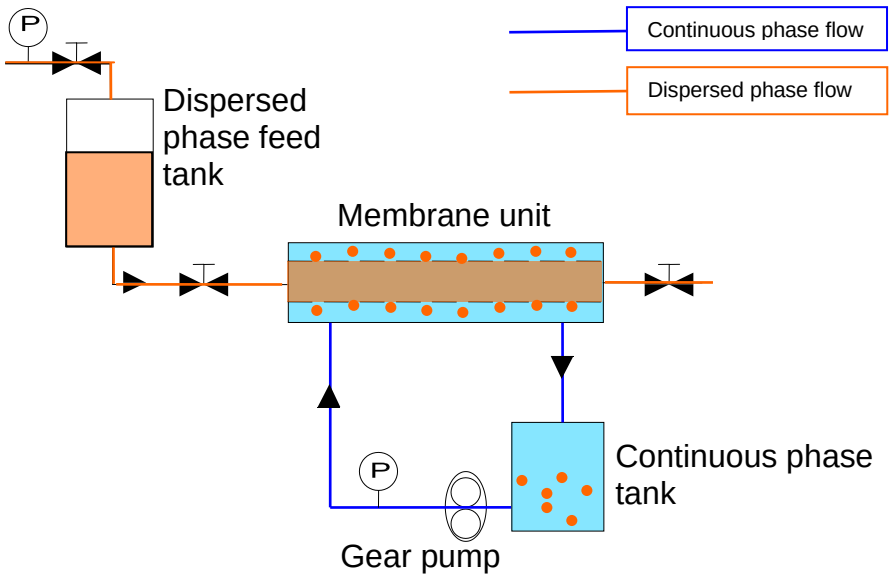

Figure 3.4: Membrane set-up

The trans-membrane pressure (i.e. the pressure which forces the emulsion through the pores) was set at 1 bar, which was the minimum pressure at which droplets formed. The cross-flow velocity was varied by varying the pump speed.

Further experiments were performed on a rotating laser-drilled stainless steel membrane (pore diameter: 80 µm) with a volume-controlled feed system. This system was set-up and used as described in Vladisavljevic & Williams (2006).

3.2.3 Emulsion droplet size and microstructure

The kinetic stability of emulsions was analysed using a variety of methods. Light microscopy was an invaluable tool used to visually determine emulsion microstructure. Further to this, cryo-SEM was employed in order to visualise the 3-dimensional structure. Droplet size distributions were obtained by measuring the size of individual droplets using image analysis software. This being a tedious and slow process, droplet size development was tracked using laser diffraction in emulsions deemed sufficiently stable to withstand shear. Laser diffraction was not a suitable technique for measuring the droplet size in fat crystal stabilised W/O emulsions, because it can not distinguish between individual droplets and droplet aggregates. For these emulsions pulsed-gradient NMR was used for measuring droplet size.

3.2.4 NMR measurements

The use of NMR for drop size determination in W/O emulsion is well documented (Balinov et al., 1994; van Duynhoven et al., 2002). It is non-invasive and does not require dilution for measurement and was pioneered by Packer & Rees (1972). The method has been compared to other techniques for droplet size measurement, such as laser diffraction (Fourel et al., 1994), and shown to give similar results. This is also the case with “solid” emulsions which are more difficult to measure using more conventional techniques (Fourel et al., 1995).

3.2.4.1 Theory of NMR measurement

A low field NMR (Nuclear Magnetic Resonance) equipped with a gradient unit is used for following the movement of protons in a sample. When a magnetic field is applied,

protons tend to align with the magnetic field, rotating around an axis parallel to the field lines, and showing a net magnetisation in the direction of the magnetic field (vector M).

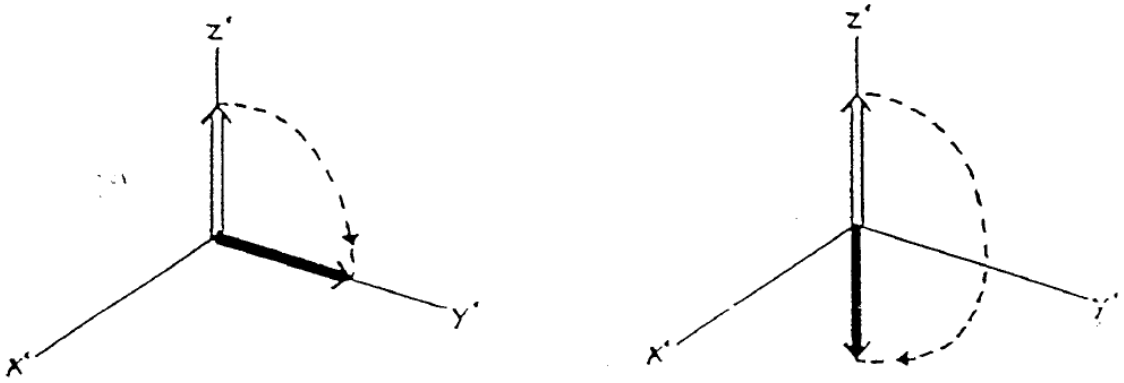

Figure 3.5: Examples of effect of 90° and 180° pulses on direction of vector M (all figures in this section taken from the Bruker Minispec mq Droplet size analysis manual, Version 1.0, Bruker Optics, Germany, 2001)

In the Hahn Spin-Echo sequence, developed to trace the movement of protons within a sample, radio waves of certain frequencies are applied as pulses to the magnetic field (either 90° or 180°). This sequence consists of a 90° pulse, followed, after a certain time τ with a 180° one. A 90° pulse rotates the vector M by 90°. For instance, if the vector was previously directed along the z axis, it would now be directed along the y axis (see Figure 3.5). However, because of inhomogeneity in the magnetic field, the magnetisation previously directed along a single axis will now start to spread out within the xy plane, as different protons rotate at different speeds (Figure 3.6, A and B). Once the 180° pulse is applied, this “fan” of magnetic moments is transposed by 180° (Figure 3.6, C). The movement of the protons, still moving at different speeds, is now reversed, so that the “fan” is closed (Figure 3.6, D). At time 2 τ, the signal re-

focusses as a single vector M, so that a sharp echo-signal is received on the oscilloscope. As the time increases beyond 2 τ, the signal strength decreases as the protons move past each other and begin to “fan out” once more (Dickinson & McClements, 1995).

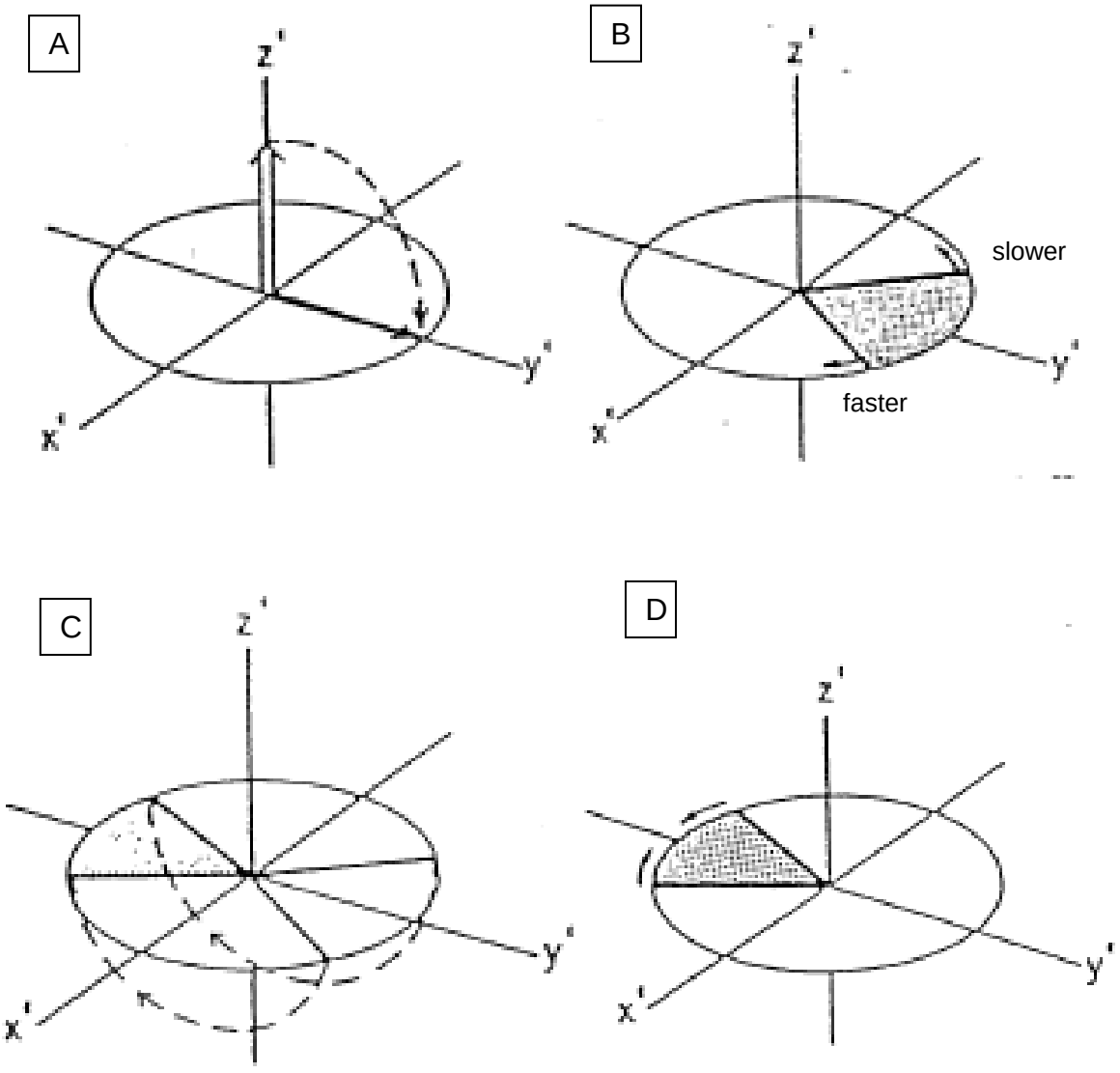

Figure 3.6: Effect of application of 90° and then 180° pulse on vector M

In order to measure the diffusion of protons within a sample, field gradient pulses (fgp) are applied for short times (Figure 3.7) within the Hahn Spin-Echo sequence described above. These fgps can move a vector within the xy plane over a specific angle to enable determination of diffusion coefficients. After the initial 90° pulse, the vector M starts to fan out. When the fgp is subsequently applied, any particular magnetic moment within the “fan” (say, all of those that are at that particular time rotating 30° faster than the average) will experience different field gradient strengths and will therefore be displaced at different angles. Once the 180° pulse and the second fgp is applied, the “fan” would only close again, and give the same signal as the sequence without fgp, if there was no diffusion. However because protons diffuse, they are displaced from different locations within the xy plane, so that at 2 τ, a loss of signal and a smaller peak, or attenuated echo, results (Callaghan 1984).

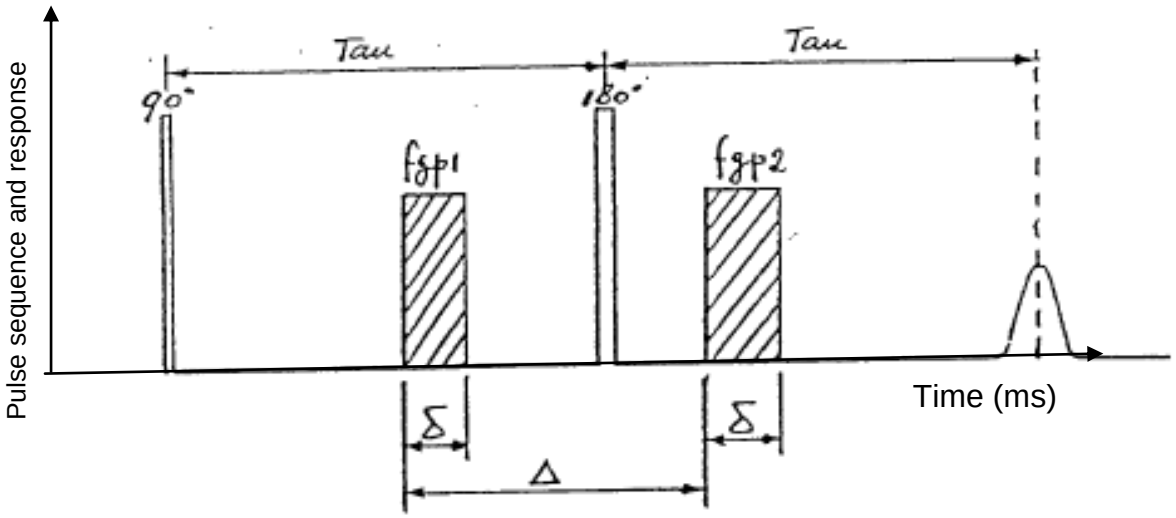

Figure 3.7: Pulse sequence for measurement with fgp

The relation of this echo attenuation (M_g) with the full-size peak obtained without the application of fgps (M_0), is known as the “R-value”, which is the ratio M_g / M_0. For

unrestricted diffusion, a plot of R vs Δ, which is the length of time between the starting points of the two fgps, gives a straight line, the slope of which gives information about the rate of diffusion of the protons. A steeper slope indicates increased diffusion rate (Dickinson & McClements 1995).

In W/O emulsion samples, the movement of water protons is restricted, so that a slope of R vs Δ will result in a plateau value at larger Δ (Figure 3.8). This can be related to droplet size. In order to find an expression for droplet size distribution, Δ is kept at a constant value within the plateau region, while δ (the length of time of each fgp) is varied. This set of experiments provides a "fingerprint" of an emulsion sample, from which the drop size distribution can be found (Packer & Rees 1972).

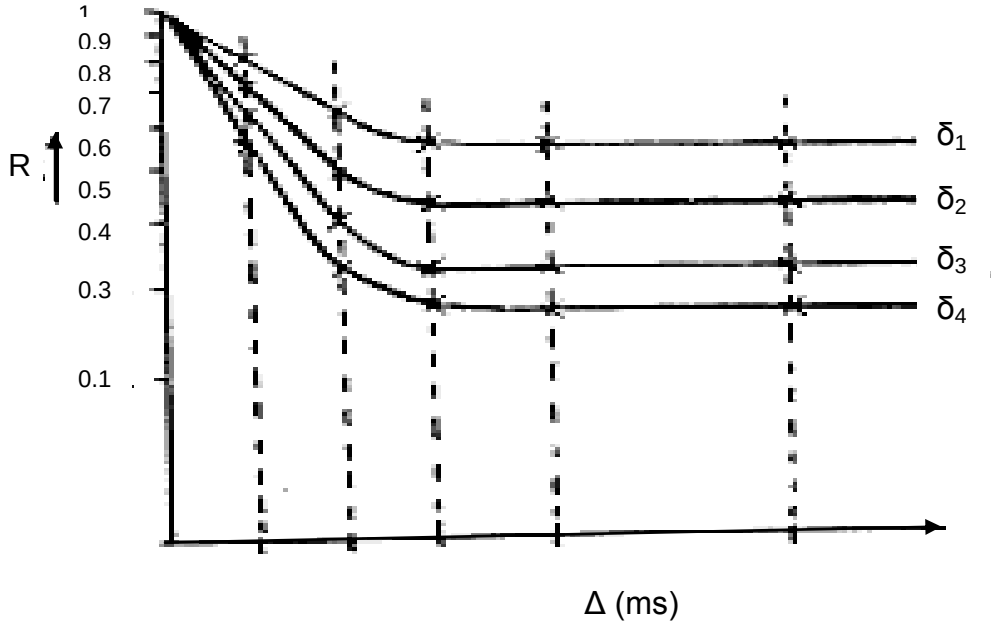

Figure 3.8: R vs Δ, at different δ, giving a "fingerprint" of the emulsions sample at any particular Δ within the plateau region

The time of measurement (Δ) required to gain the attenuated echo is quite long. This makes the signal too weak to detect. In order to “boost” the signal after fgp 2, the 180° pulse is split into 2 90° ones.

In W/O emulsions protons are present not only in the water phase, but also within the oil. It is therefore important that after the first 90° pulse, the protons comprised within the oil phase have no magnetisation, or the measurement of water diffusion could be compromised. The two different species have different relaxation times, so that oil protons relax more rapidly than water protons. If a 180° pulse precedes the initial 90° one at a certain time τ_o, when oil proton magnetisation is 0, only the magnetisation associated with the water protons is subsequently measured (Dickinson & McClements, 1995) .

3.2.4.2 Model for calculating drop size distributions

In order to calculate the droplet size distribution from the data acquired by the NMR two assumptions are made: a) the droplets are spherical and b) the distribution is log-normal. Experiments have shown that emulsions usually fulfil both conditions (Packer & Rees, 1972).

Equation 3.1 represents the model that is used to fit the diffusion data obtained in the NMR measurements to a droplet size distribution curve.

Eqn 3.1: Equation fitting diffusion data to a droplet size distribution curve (Packer & Rees 1972)

$$R(\Delta,\delta,D_s,g,d)=\exp\left[-2\gamma^2 g^2\sum_{m-1}^{\infty}\frac{\frac{2\delta}{\alpha_m^2 D_s}-\frac{2+e^{-\alpha_m^2 D_s(\Delta-\delta)}-2e^{-\alpha_m^2 D_s\Delta}-2e^{-\alpha_m^2 D_s\delta}+e^{-\alpha_m^2 D_s(\Delta+\delta)}}{\left(\alpha_m^2 D_s\right)^2}}{\alpha_m^2\left(\alpha_m^2 d^2-2\right)}\right]$$

2 parameters are important to fit a distribution curve to the diffusion data obtained during the NMR measurements: the geometric mean diameter, $d_{50,I}$, and the standard deviation as a measure of the width of the distribution. The ratio R has been shown to be a function of Δ, δ, the self-diffusion coefficient D_s, the gradient strength g and, because of the effect of restricted diffusion, the droplet radius d (Packer & Rees 1972). The model calculates average droplet size by varying the geometric mean as well as the standard deviation in order to fit to the mathematical description of a log normal curve. It was shown that this model accurately predicts real droplet size distribution in most W/O emulsions, making p-NMR a valuable tool for analysis of semi-solid W/O emulsions (van Duynhoven et al., 2002).

The drawback of NMR is the fact that any droplet size distribution that is not lognormal, is fit to a lognormal shape. Although it was shown that the droplet size distribution of most W/O emulsions is in fact lognormal, very coarse W/O emulsions may be bimodal. An indication that a given droplet size distribution is not lognormal is given in the form of the “free water” reading. “Free water” is the proportion of water that is not enclosed in droplets smaller than ca 15 µm. This “free water” is not included in the droplet size calculation but is listed separately as a percentage of the total water content. If emulsions had a large percentage of “free water”, further

checks (e.g. using light microscopy) were performed on the emulsion to verify the actual droplet size distribution.

When reporting droplet size in this work, it is always given as the average diameter $D_{3,2}$ along with the standard deviation from this value as an indication of the breadth of the droplet size distribution.

3.2.4.3 NMR measurements on W/O emulsion samples

Drop size measurements on the W/O emulsion samples were performed using self-diffusion measurements by nuclear magnetic resonance (NMR) (Bruker Minispec NMR, Bruker Optics, UK), equipped with a gradient unit. The parameters used for the measurements are listed in Table 3.3.

Table 3.3: Parameters used for drop size calculation in NMR measurements

Δ (ms)	δ (ms)	D_{water} ($m^2 s^{-1}$)
210	15	1.4×10^{-9}

δ was set at 15 ms. When lower values were used, results were found to be less accurate and reproducible. Higher settings, on the other hand, did not significantly increase the accuracy of results despite a significant increase in measurement time.

Δ was set at 210 ms as this is a value often used in the literature. In order to confirm that R-values had reached a plateau at this point, Δ was varied between 10 and 250 ms for six δ values. The resulting plot of R vs Δ showed that at 210 ms, all R-values

were in the plateau region, although at large δ (5 ms), a downward trend in the R-value continued (Figure 3.9). The likely reason for this is the relatively long time for the pulse, resulting in some signal deterioration especially at longer Δ times.

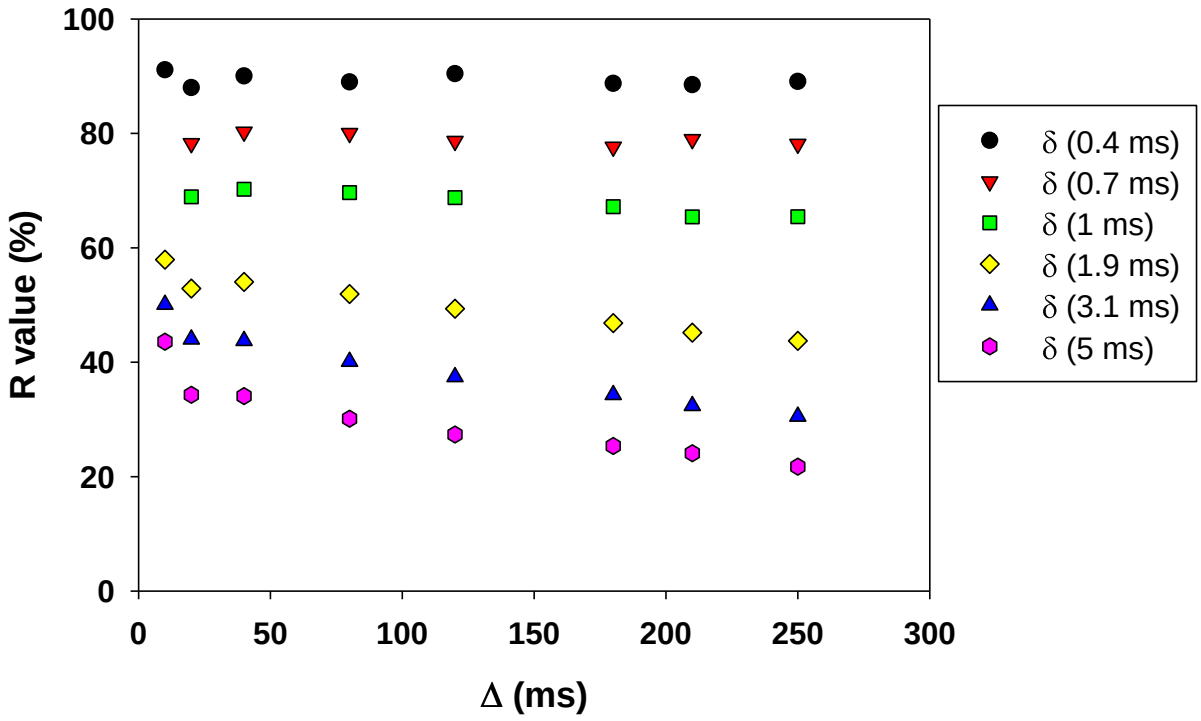

Figure 3.9: Plot of R value at various δ vs Δ

T_0 values varied depending on oil phase composition, as shown in Table 3.4. It was measured by analysing the respective oil phases using the application provided by Bruker Optics to measure the T_1 value of the oil phase. T_1 is related to T_0 as described in equation 3.2 (S. Ablett, personal communication, 2009).

$$T_0 = 0.69 \, . \, T_1 \qquad \textbf{Equation 3.2}$$

Table 3.4: τ_0 values for different tripalmitin concentrations

Oilmix containing 0.5 % monoglyceride +	τ_1 [ms]	τ_0 [ms]
2 % tripalmitin	79 ± 0.5	54.5 ± 0.5
1 % tripalmitin	73.3 ± 0.4	50.6 ± 0.4
0.5 % tripalmitin	76.6 ± 0.4	52.9 ± 0.4
0 % tripalmitin	74 ± 0.5	51.1 ± 0.5

Calculations for droplet size distributions were performed using the Minispec software, which fits the data to a log-normal curve (see section 3.2.4.2). In order to check the accuracy of the droplet size measurement in the W/O emulsion used in this work, average droplet sizes measured using light microscopy micrographs (see section 3.2.4.4) were compared to average droplet sizes obtained with NMR for selected emulsions.

Table 3.5: Comparison of droplet sizes measured using microscope pictures and NMR. All emulsions contained 0.5% monglyceride in the oil phase

	Microscope	NMR
After production	$D_{3,2}$ (µm)	$D_{3,2}$ (µm)
No Tripalmitin	37 ± 8	n/a
0.5 % Tripalmitin	7.4 ± 2	4.6 ± 0.6
1 % Tripalmitin	4.1 ± 1.9	4.3 ± 0.5
2 % Tripalmitin	3.5 ± 0.6	4.1 ± 0.5
After 1 week		
No Tripalmitin	18.7 ± 4.8	n/a
0.5 % Tripalmitin	17.3 ± 4.8	9.3 ± 0.8[1]
1 % Tripalmitin	5.6 ± 1.1	5.1 ± 0.7[2]
2 % Tripalmitin	4.8 ± 1	4.3 ± 0.5
After 2 weeks		
No Tripalmitin	22.1 ± 6.6	n/a
0.5 % Tripalmitin	21.6 ± 5.5	9.8 ± 0.9[1]
1 % Tripalmitin	4.5 ± 1.2	5.3 ± 0.8[2]
2 % Tripalmitin	3.2 ± 0.8	4.3 ± 0.5

[1]+~15 % free water; [2]+~20 % free water

Table 3.5 shows that values obtained with both techniques correspond well at lower droplet size. In less stable formulations with larger droplet sizes and broader size distributions, however, NMR measurements are consistently lower than values obtained by counting droplet sizes from microscopy micrographs. This is a consequence of the NMR method: the restricted diffusion measurement technique was designed for droplets smaller than 15 µm, preferably smaller than 10 µm (S.

Ablett, personal communication, 2009). In larger droplets, “restricted” diffusion path lengths become too long, so that droplet size can no longer be measured in the time interval available before the output signal becomes too weak.

The minispec program detects the presence of such larger droplets (larger than 15 µm), along with areas that contain “pools” of water, and excludes them from droplet size calculations. The fraction of water not included in the calculations is subsequently listed as “free water”. Consequently, the NMR-measured average droplet size decreased if the emulsion contained an increasing proportion of large droplets.

NMR measurements gave no reliable results in formulations where the size of a majority of droplets exceeded ~15 µm. In this case, only optical microscopy can give an idea of droplet size. However, such coarse W/O emulsions are usually unstable. For most W/O emulsions encountered in this work, droplet size ranged from 3-5µm, so NMR provided accurate, time-effective and reproducible size measurements.

3.2.4.4 Light microscopy

Light microscopy (Reichert Jung Polyvar) was used to visualise the primary emulsion as well as double emulsion droplets. Additionally, it was used to determine the size of the primary emulsion droplets post incorporation into the double structure and that of the double emulsion globules.

Image analysis software (ImageJ) was used to obtain size distribution data from the micrographs of the double systems. In order to obtain an accurate measure of the

size distribution of the double emulsion globules and the W_1 primary emulsion droplets, at least 2 - 3 different samples of each formulation were characterised by counting 500 - 1000 droplets in each case. The standard deviation (σ) from the mean droplet size is also given as it provides an indication of the breadth of droplet size distribution (i.e. a small σ value indicates a narrow size distribution).

3.2.4.5 Cryo-SEM

Cryo scanning electron microscopy (SEM; Philips XL-30 FEG ESEM) was used to visualise the (primary as well as double) emulsion microstructure. A small amount of sample was placed in perforated holes of a brass stage mounted on a steel rod. The sample was subsequently shock-frozen in nitrogen slush, and rapidly transferred to preparation chamber of the SEM instrument (-140 °C). Here the sample was fractured using a metal knife and then dusted with gold particles, to prevent damage by the electron beam. The sample prepared in this manner was subsequently transferred to the measurement chamber (-180 °C), and the sample analysed using a 2 keV beam to prevent excessive damage to the sample.

3.2.4.6 Laser Diffraction

A Malvern Mastersizer (Malvern, UK) was used for determining double emulsion globule sizes in shear-stable samples using laser diffraction. The principle behind this technique is that particles of different sizes scatter light in a distinct way. Scattering is induced using a laser beam directed through the dilute sample. The extent of light scattering is subsequently measured by an array of detectors. As can be seen in Figure 3.10, smaller particles scatter light at wider angle compared to larger ones.

Scattered light intensity, on the other hand, diminishes with decreased particle cross-sectional area.

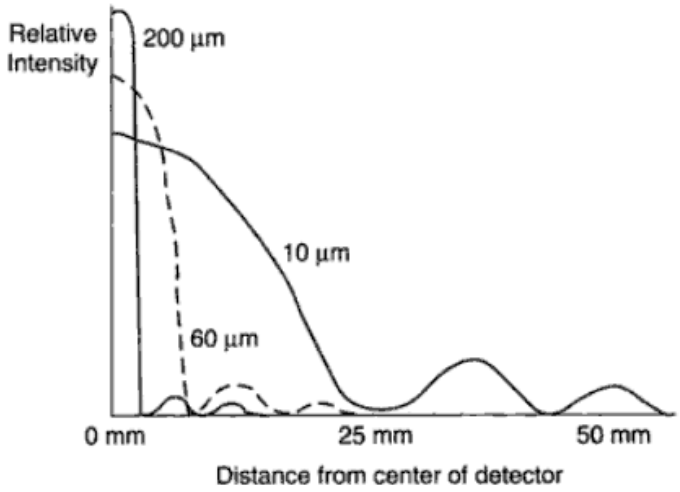

Figure 3.10: Principles of the laser diffraction technique for droplet size measurement (McClements 2005)

The extent of light scattering is related to emulsion droplet size using complex mathematical models and based on the theory of the propagation of an electromagnetic wave through an array of particles. The most commonly applied model (Mie theory) for measurement of food emulsion droplets is applicable only in very dilute regimes. For this reason, emulsions are diluted prior to measurement (McClements 2005).

Refractive index values of 1.5 (for samples containing OSA starch) or 1.46 (samples containing silica particles) were used. The stirrer speed in the sample cell was set to 2000 rpm, and emulsion was added by pipette until the laser obscuration reached ~15 % of its total value. Three measurements were performed per sample. The final

values ($D_{3,2}$) quoted were derived from the mean average values of these three measurements of three different samples.

This technique was only used to determine size distributions in double emulsions that were stable against shear (i.e. those stabilised by OSA starch and silica particles). When attempts were made to use this technique on samples that were not shear-stable, large aggregates of primary emulsions would form that were unevenly dispersed within the sample cell, leading to inaccurate measurements. For these samples light microscopy was used to measure globule sizes.

3.2.5 Thermal analysis

In order to determine melting points of the fat crystals, Differential Scanning Calorimetry (DSC) was performed on the emulsions, as well as on the oil pre-mix and individual oil-phase components, using a Perkin-Elmer Diamond apparatus (Perkin-Elmer, UK). An Intracooler III, capable of delivering cooling rates of 30 °C min^{-1} was used to cool the machine. 5-15 mg sample was filled into 50 µL aluminium pans and then sealed with aluminium covers. Empty pans were used as reference. Nitrogen was used as purge gas.

The following experiments were performed:

- W/O emulsions containing varying amounts of tripalmitin were heated from 0 to 70 °C at a rate of 10 °C min^{-1}

- Oil phase mixtures such as those used in W/O emulsions were heated to 80°C for 10 minutes, subsequently cooled to -10 °C, held there for 10 minutes and

subsequently re-heated to 80 °C at 10 °C min^{-1}. The sample was then cooled at 10 °C min^{-1} to -60 °C and finally, after remaining at this temperature for 10 minutes, re-heated to 80 °C at 10 °C min^{-1}.

- Pure tripalmitin was heated to 80 °C, held at this temperature for 10 minutes, and subsequently cooled to -10 °C at 10 °C min^{-1} or 1 °C min^{-1}, and held for a further 10 minutes. The cycle was repeated once, resulting in 2 crystallisation and 2 melting curves.

- The same procedure was repeated for the two monoglycerides used in this work, although the minimum temperature was lowered to -30 °C.

A comparison between the curves obtained for tripalmitin melting and crystallisation at the two different cooling rates revealed sharper and smaller peaks for the slower heating/cooling rates. It is well-known that a decrease in heating/cooling rate increases accuracy of the peaks, while faster rates increase sensitivity to small changes in heat capacity (Norton et al., 1985). As the latter was important in samples containing as little as 0.3 % crystallising material, the faster cooling rate was used in all experiments.

Analysis of the data could not be performed using available Perkin-Elmer Pyris software because peaks in most cases were too small for automatic peak detection. Instead, the baseline was adjusted by fitting a second order polynomial to the individual curves using MatLab's "cftool" programme and subtracting this from the original curve. Heat capacities were then estimated from the area under the crystallisation/ melting peaks. However, as these peaks were usually small and the

estimated area very dependent on the exact position of the baseline, data indicating melting/ crystallisation temperatures was almost exclusively used.

3.2.6 Conductivity

In order to determine the effectiveness of the primary as well as double emulsion structures in retaining a solute (NaCl or KCl) within the inner (W_1) aqueous phase, regular conductivity measurements were performed on samples where the osmotic pressure was regulated with glucose using a Mettler Toledo 7 Easy conductivity meter and probe (InLab 710).

3.2.6.1 Conductivity in W/O emulsions

For salt release measurements in primary emulsions, small samples of the prepared emulsions (~3 g) were placed in 150 g of distilled water in sealed containers. The emulsion sample would remain intact and float on top of the water. Conductivity measurements were taken at regular intervals in the water phase in order to determine salt release from the W/O emulsion sample. The water was gently stirred prior to every measurement in order to remove any concentration gradient that may have formed from previous measurements. All the experiments described above were carried out at controlled temperatures of 5, 22 and 30 °C.

The same measurements were then repeated by placing the emulsion samples into aqueous solutions (at 22 °C) of varied glucose concentrations, in order to monitor salt release under various degrees of osmotic pressure difference. The following concentrations were used: 0.56 M (matched osmotic pressure); 1.12 M (osmotic

between the glucose solution and the aqueous drops: 14.9 atm); 0.28 M (pressure difference: 7.5 atm). All measurements were performed at least in duplicate

The rapid release profile at 55 °C (within seconds) required a continuous measurement of conductivity. 200 g distilled water was placed in a jacketed and baffled, lightly stirred beaker. Conductivity was measured continuously by connecting the meter to a PC and recording the readings using an Eltima Freeware Data Logger.

3.2.6.2 Conductivity in W/O/W double emulsions

The concentration of an emulsion influences the conductivity reading (Pawlik et al, 2011). In order to minimise the impact of emulsion droplets on the conductivity measurements, samples were regularly taken from the serum phase of the creamed emulsion for conductivity measurement. The serum was largely devoid of emulsion droplets, as these were predominantly located in the cream. These experiments were not performed on samples containing NaCl in the W_2 aqueous phase due to sensitivity limitations of the instrument.

In the case of measurements at elevated temperatures (e.g. 30 - 45 °C), the double emulsion sample was placed in a water bath at 50 °C and constantly and gently stirred, in order to ensure homogenous composition of the sample. The conductivity was measured continuously for 400 seconds. The temperature in these samples was recorded at the same time using a Thermocouple (Fisher).

3.2.7 Rheology

The instrument used for rheology measurements was a Bohlin Gemini Nano Rheometer (Malvern, UK).

In primary W/O emulsions, the storage (G') and loss (G'') moduli were determined by oscillatory rheology in order to investigate the effect of the crystal concentration on the elastic properties of the emulsion. G' and G'' were measured using 40mm stainless steel parallel plate (1 mm gap) geometry. The parallel plate geometry was used because the sample was gradually expelled from the gap in a cone and plate geometry. The advantage of using a cone and plate geometry is that it allows tests to be performed on a well-defined and uniform shear field in the sample. With parallel plates, the shear rate varies across the sample so that an average value is given by the rheometer software.

The viscoelastic properties of a sample are determined by the magnitude of the viscous and elastic components. Their relative magnitude is indicated by the magnitude of the phase lag Φ. G' and G'' are related to Φ as follows:

$$G' = \frac{\tau \cdot \cos \Phi}{\alpha}$$

$$G'' = \frac{\tau \cdot \sin \Phi}{\alpha}$$

Where τ is the shear stress amplitude and α is the strain amplitude. The higher G', the more elastic is the substance.

The measurements must be performed in the linear elastic region in which the applied strain on the sample is small enough not to destroy the structure of the sample. In this region, the ratio between shear stress and shear strain is constant. To allow tests to be performed in the linear viscoelastic region, as previously determined in an amplitude sweep (see Appendix, Figure 8.12), the applied shear stress was varied with tripalmitin concentration; 50 Pa (2% Tripalmitin), 20 Pa (1% Tripalmitin) and 1 Pa (0% Tripalmitin). The shear stress was controlled, and the strain rate was measured.

In double emulsions, the viscosity of several formulations was measured at constant shear rate and increasing temperature in order to gain an understanding of the rheological behaviour of the double emulsions.

The shear rate was set at 5 - 10 s^{-1}, as higher shear rates had a detrimental effect on some formulations. The temperature was gradually increased by 1 °C/min from 15 to 55 °C, and viscosity measurements taken.

3.2.8 Interfacial Tension

A Wilhelmy plate method was used to determine the static interfacial (surface) tensions. Measurements were on a K 100 Tensiometer fromKrüss GmbH, Hamburg (Germany). Experiments were conducted at room temperature, except when the oil phase contained monoglycerides/ tripalmitin. In this case, the oil phase was heated to 90 °C to melt all solid components and achieve a homogenous mixture. The oil mixture was poured, while still hot, into vessel to calibrate the Wilhelmy plate. This oil was then discarded. To perform the measurement, hot oil (at around 70 °C) was

carefully pipetted onto the surface of the aqueous phase and measurement started. Measurements lasted between 30 and 60 minutes in order to ensure the system was at equilibrium.

All experiments were performed at least in triplicate on each different sample.

4 Fat crystal- stabilised W/O emulsions for controlled salt release

4.1 Introduction

One of the main problems affecting double emulsion stability, highlighted in the literature review, is the inability of the inner W/O emulsion to retain water and solutes against osmotic pressure gradients. The presence of two different types of emulsifiers (one lipophilic, one hydrophilic) causes water and/or solute transport between the two aqueous phases as the emulsifiers diffuse between the interfaces (Matsumoto et al., 1976). This results in gradual destruction of the double structure and, eventually, complete release of the encapsulated solutes. It is known that the use of polymeric emulsifiers greatly increases double emulsion stability compared to when monomeric ones are employed (Benichou et al., 2004; Pays et al., 2002). However, there are only a few polymeric food-grade emulsifiers that give good stability to W/O emulsions. Most recent investigations have included Polyglycerols of Polyricinooleates (PGPR) as emulsifier in the primary emulsion (Pawlik et al., 2010; Bonnet et al., 2009; Mezzenga et al., 2004). Although it is an excellent emulsifier and can give stable double emulsions, its use is tightly regulated and permitted only in chocolate and some spreads (Pawlik et al., 2010). Furthermore PGPR does not prevent water transport when osmotic pressure gradients are present between the two aqueous phases (Mezzenga et al., 2004). Due to the regulatory restrictions of PGPR, it is desirable to find a stabilising structure for W/O emulsions which does not contain the substance. The alternative stabiliser should also be food-grade and prevent coalescence between droplets. In addition, it should also be able to prevent

water transport between the aqueous phases. Preliminary experiments performed for this work have highlighted the fact that samples of commercially available low fat spreads in distilled water are able to retain solutes such as salt over extended periods of time. An emulsion stabilised by PGPR released encapsulated salt much more rapidly to the water phase into which the sample was immersed.

This chapter describes the production of stable fat crystal-stabilised W/O emulsions. It is shown that these emulsions are able to retain a solute, NaCl, encapsulated within the water phase when small emulsion samples are immersed in a water phase. NaCl was chosen as encapsulated solute due to its importance in food (see chapter 2). The influence of total fat crystal amount, storage temperature, hydrocolloid addition, as well as osmotic pressure gradients on the stability of the emulsions (during storage in sample pots as well as when immersed in water) was investigated. Furthermore, the fat phase components were studied in more detail using Differential Scanning Calorimetry (DSC) and contact angle measurements in order to determine the likely mechanism for the stabilisation of emulsions.

4.2 Emulsions containing monoglycerides and triglycerides

W/O emulsions containing only either monoglycerides or tripalmitin, as well as a mixture of both monoglycerides and tripalmitin were produced to find the optimum formulation for making stable emulsions. Monoglyceride-only emulsions were produced containing 0.5 %, 1.5 % and 2.5 % (wt/wt in the oil phase) monoglyceride. Tripalmitin-only emulsions contained 1 % tripalmitin (wt/wt in the oil phase). Mixed mono- and tripalmitin emulsions contained 0.5 % monoglyceride and between 0.5 and 2 % (wt/wt in the oil phase) tripalmitin.

As tripalmitin is crystalline at room temperature (see section 4.4) it can be placed at the interface and should provide Pickering stabilisation to the droplets. The benefits of Pickering stabilisation were discussed in chapter 2 and most importantly include the protection against coalescence that a particle-covered interface provides to individual droplets.

Emulsifiers are necessary in order to place tripalmitin crystals at the interface. Suitable food-grade W/O emulsifiers include lecithins and monoglycerides. These were shown to alter the polarity of the crystals sufficiently for the crystals to reside at the interface (Johansson et al., 1995). Monoglycerides were chosen as emulsifiers for the present work because they are themselves crystalline. As they have a higher crystallisation temperature than tripalmitin (see section 4.4) they can be used to "seed" tripalmitin crystals at the interface. As amphiphilic molecules, monoglycerides are present at the entire O/W interface where they initiate rapid crystallisation of many triglyceride crystals. As a result, these triglyceride crystals are tightly packed around the water droplets and form a "shell". The high degree of surface coverage ensures good emulsion stability because the "shell" forms a barrier that prevents two approaching droplets from coalescing.

4.2.1 Monoglyceride-only emulsions stability

Emulsions stabilised by monoglycerides were produced using the SSHE/ pin stirrer method described previously in chapter 3. These emulsions were formulated to contain 60 % (wt/wt) water (containing 1.6 % (wt/wt in the water phase) NaCl) and 0.5, 1.5 and 2.5 % (wt/wt in sunflower oil) monoglyceride in sunflower oil. The oil phase did not contain any tripalmitin. At least 2 different samples were produced of

each formulation and each measurement was repeated at least twice. As shown in Table 4.1, none of these emulsions showed good stability when stored for 4 weeks at 10 °C or 25 °C.

Table 4.1: W_1 droplet sizes of emulsions stabilised only by monoglyceride at 10 °C storage temperature, measured by NMR. The number after the ± sign gives an indication of the breadth of the drop size distribution ("span"). The average diameter $D_{3,2}$ varies by 0.1 - 0.3 µm in triplicate measurements.

	0.5 % monoglycerides		1.5 % monoglycerides		2.5 % monoglycerides	
	$D_{3,2}$ (µm)	free water	$D_{3,2}$ (µm)	free water	$D_{3,2}$ (µm)	free water
1 day	11 ± 1	0	5.1 ± 0.4	0	5.6 ± 0.3	0
1 week	22 ± 1	24 %	5.1 ± 0.4	8 %	19.6 ± 1	59 %
4 weeks	36 ± 1	30 %	5.2 ± 0.4	10 %	20 ± 1	60 %

Although the droplet size remained constant when the emulsions contained 1.5 % monoglyceride and were stored at 10 °C, sedimentation and separation of the oil and water phases occurred. This was observed by visual inspection of the samples (water sedimentation in bulk samples) and is also indicated by the increase of "free water" to 8 % after 4 weeks. At 25 °C (Table 4.2), on the other hand, droplet size nearly tripled within 1 month of storage. Emulsions containing 0.5 % or 2.5 % monoglyceride were unstable against coalescence, irrespective of whether the samples were stored at 25 °C or at 10 °C. The rapid increase in droplet size caused sedimentation and eventually resulted in full phase separation.

Table 4.2: W_1 droplet sizes of emulsions containing only monoglyceride at 25 °C storage temperature, measured by NMR. The number after the ± sign gives an indication of the breadth of the drop size distribution ("span"). The average diameter $D_{3,2}$ varies by 0.1-0.3 µm in triplicate measurements.

	0.5 % monoglycerides		1.5 % monoglycerides		2.5 % monoglycerides	
	$D_{3,2}$ (µm)	free water	$D_{3,2}$ (µm)	free water	$D_{3,2}$ (µm)	free water
1 day	12 ± 2	0	5.2 ± 0.4	0	5.1 ± 0.2	0
1 week	Unstable	-	7.3 ± 0.6	7 %	Unstable	-
4 weeks	Unstable	-	15 ± 0.6	7 %	Unstable	-

The presence of monoglycerides alone was apparently insufficient to create emulsions that were stable against coalescence and sedimentation. This was confirmed by obtaining SEM micrographs of these emulsions, an example of which is shown in Figure 4.1. The surface of emulsion droplets seems to be made up of crystalline fragments that do not form a continuous layer. It is possible that these crystalline fragments, indicated in Figure 4.1 by arrows, are artefacts that developed due to the method for preparation of the sample (which includes shock-freezing the sample in liquid nitrogen). However, the large size of some individual droplets ($D_{3,2}$ = 12 ± 2 µm) combined with the emulsion instability against coalescence during storage indicate that there is no tight fat crystal network surrounding the droplets to adequately stabilise the emulsion. Furthermore, a pronounced network of fat crystals that could arrest droplet movement and prevent coalescence seems absent in the continuous oil phase. A strong network of fat crystals in the continuous oil phase prevents the collision of droplets that is the pre-condition for coalescence. On the other hand, a strong interface hinders coalescence by preventing droplet deformation and film drainage. The combination of a weak interface and the relative freedom of

movement for the droplets explain the poor stability of the emulsions with low monoglyceride concentration.

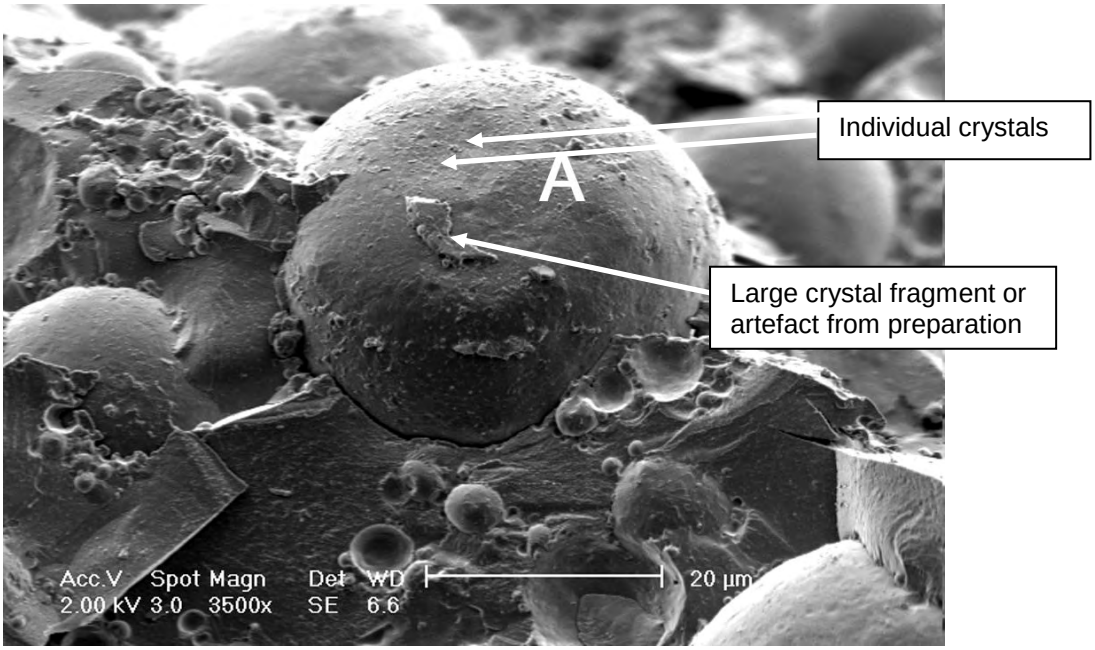

Figure 4.1: SEM micrograph of an emulsion stabilised by 0.5 % monoglyceride and containing no tripalmitin. The surface of a water droplet ("A") appears rough and uneven.

4.2.2 Tripalmitin-only emulsion stability

Emulsions containing 1.5 % (wt/wt in the oil phase) tripalmitin and without any additional emulsifier were produced using the A & C units (as described in Chapter 3) in order to test the ability of tripalmitin to stabilise emulsions.

The resulting emulsions were coarse (average droplet size was 13 ± 4 μm) and not stable against coalescence. Figure 4.2 shows a micrograph of such an emulsion immediately after production. This micrograph shows that the drop size distribution was broad with some droplets larger than 20 μm. The large globules are probably the result of the lack of emulsifier which prevents coalescence of droplets during processing (Niknafs et al., 2011).

After 1 month storage, the emulsion had partially phase separated due to large-scale coalescence. The poor stability of the emulsion is probably the result of the absence of a stabilising interface that prevents coalescence. The lack of monoglyceride crystals means that crystallisation is not induced at the oil/water interface. Tripalmitin crystals are therefore not likely to be tightly arranged around the droplet surface, although tripalmitin is present in crystalline form in the emulsions. The absence of emulsifier and a protective “shell” also means that two droplets can approach each other to a proximity that allows film drainage and subsequent coalescence to occur.

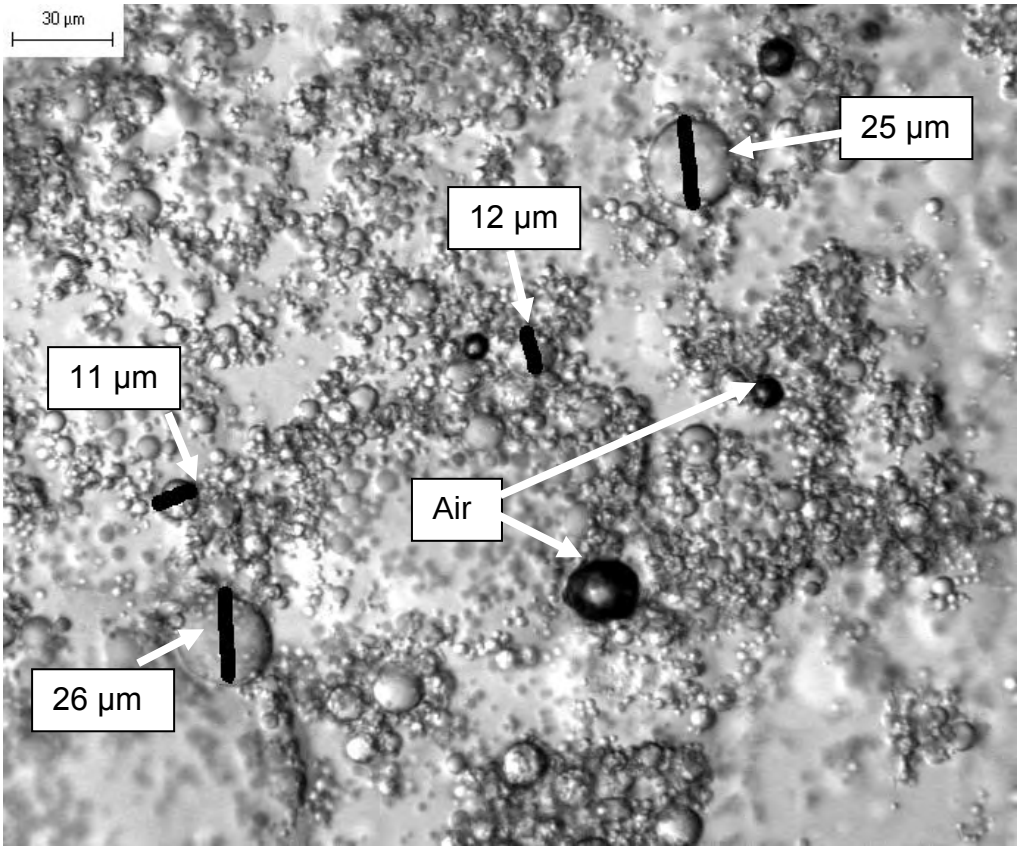

Figure 4.2: Emulsion containing only 1.5 % triglyceride (0 % monoglyceride)

Having determined that emulsions were not stable if they were stabilised by only either monoglyceride or tripalmitin, both were mixed and the effect of their joint presence on emulsion stability investigated. It was hypothesised that the presence of monoglyceride would allow the “seeding” of tripalmitin crystals at the interface, while the presence of tripalmitin would allow the formation of solid crystalline “shells” surrounding the droplets.

4.2.3 Emulsions containing mono- and triglycerides

The emulsions were produced using the method described in chapter 3. Monoglyceride concentration was kept constant at 0.5 %, and tripalmitin concentration was varied between 0.5 % and 2 % (wt/wt in the oil phase) in order to

investigate influence of tripalmitin on the structure and stability of the crystalline “shells”.

Tripalmitin, monoglyceride and sunflower oil were mixed and heated to ~80 °C and pre-emulsified with 40 % water (containing 1.6 % (wt/wt in the water phase) NaCl) at the same temperature. This pre-mix was then fed through the A & C unit, in which simultaneous cooling and shearing induced crystallisation of the monoglycerides and tripalmitin as well as droplet break-up. The product was recycled once again through the A & C unit in order to produce small droplets and a narrow drop size distribution (see chapter 3). Immediately after production the droplet sizes of the samples were measured (Table 4.3). Further samples were filled into suitable containers and stored at 5, 22 or 30 °C for later measurements designed to determine their stability.

Emulsions containing an equal mixture of mono- and triglycerides showed a slight increase (4.4 to 4.6 µm) in mean average droplet size after 25 days when stored at 5 °C, although this was within the experimental error. Emulsions containing 1 and 2 % triglyceride stored at 5 °C did not show any increase in droplet size (constant at around 4 µm) within this period (Table 4.3). Emulsions containing 2 % triglyceride were also stable when stored at 22 °C, while emulsions containing 0.5 and 1 % showed a significant increase in droplet size when stored under the same temperature conditions: The droplet size of the former doubled to 8 µm, while that of the latter rose to 7 µm within 25 days storage. When stored at 30 °C all emulsions destabilised within a day.

Table 4.3: Droplet sizes determined by NMR of emulsions stabilised by 0.5 % monoglyceride and increasing concentrations of tripalmitin at varying temperatures, in µm. Triplicate measurements give a variation of 0.1 - 0.3 µm from the $d_{3,2}$ presented. The emulsions containing less than 1 % Tripalmitin are unstable at 30 °C.

	Initial droplet size		5 °C (25 days)		22 °C (25 days)		30 °C (8 hours)	
% Tripalmitin	$d_{3,2}$ (µm)	σ	$d_{3,2}$ (µm)	σ	$d_{3,2}$ (µm)	σ	$d_{3,2}$ (µm)	σ
0 %	11.1	1.1	21.8	0.9	-	-	-	
0.5 %	4.4	0.4	4.6	0.5	7.9	0.7	-	
1 %	3.9	0.4	4	0.4	6.6	0.8	27	large
2 %	3.4	0.2	3.9	0.3	4.1	0.5	8.4	large

The monoglyceride and tripalmitin crystals jointly stabilise the water droplets by forming networks around the interface. With time, neighbouring individual crystals sinter, thus forming strong bonds between them (Johansson & Bergenstahl 1995a). Oscillatory rheometry showed that sintering and the strengthening of the fat crystal network occurred in the present emulsions (see section 4.5).

The existence of a tight fat crystal network, or “shells” was also indicated in SEM micrographs of an emulsion stabilised by 0.5 % monoglyceride and 1 % tripalmitin. An example of such a micrograph is given in Figure 4.3. In contrast to emulsion samples containing only 0.5% monoglyceride and no tripalmitin (Figure 4.1), where crystal fragments were visible on droplets, no individual crystals are visible at the droplet surface in emulsion samples containing 1% tripalmitin. This suggests that the crystals are sub-micron in size and have probably formed tight sintered crystal

networks around the water droplets, although it is also possible that the use of the laser for the SEM measurement alters the structure of the interface. However, crystalline “shells” which are visible in this image are present on all droplets and visible in several micrographs of different samples with the lowest strength laser (2 keV). The droplets are embedded in a continuous structure which may be a network of sintered tripalmitin crystals. Although crystallisation during the emulsification process was performed in a controlled way, it is unlikely that all tripalmitin crystallised directly at the interface. It is likely that tripalmitin crystals also formed in regions which large degrees of undercooling, such as at the walls of the vessels. The “free” crystals in the continuous phase also form a network, which contributes to emulsion stability by arresting droplet movement (Rousseau & Hodge 2005).

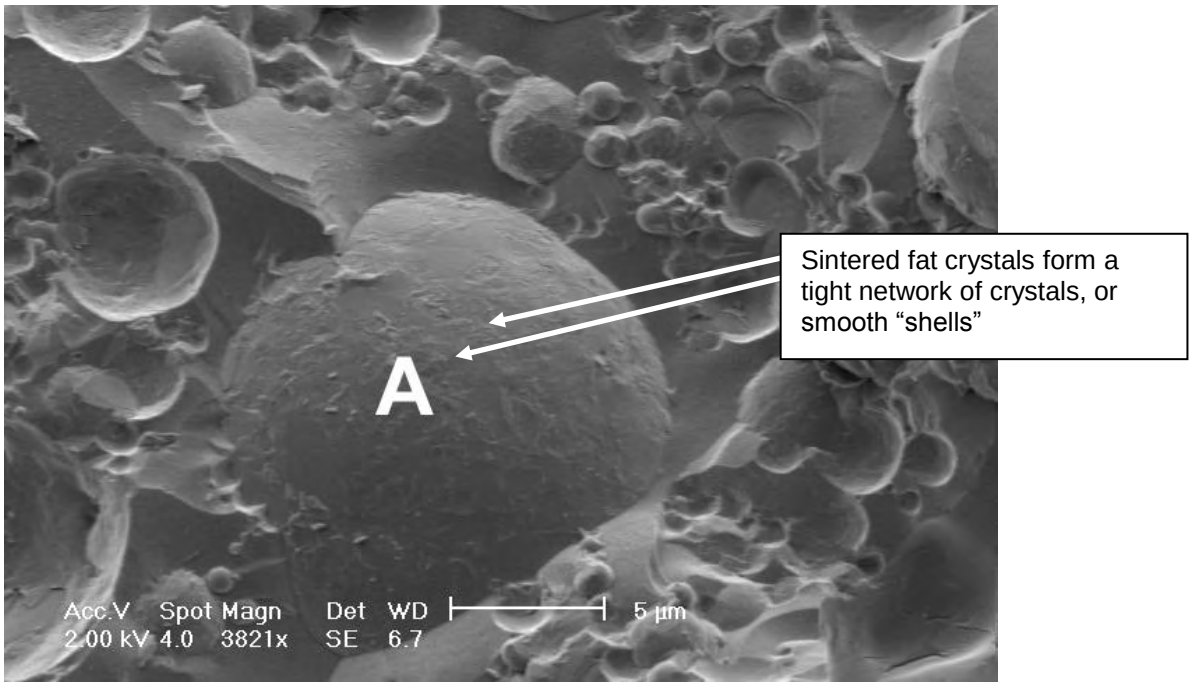

Figure 4.3: SEM micrograph of an emulsion stabilised by 0.5 % monoglyceride and 1 % Tripalmitin, after 3 weeks storage. A water droplet can be seen to be surrounded by a solid shell (“A”) of sintered fat crystals.

A required condition for the “shell” formation is the availability of small crystals at the interface. Small crystals are produced by a combination of high shear and a fast cooling rate (Mullin 1993) which are present within the A & C units. Crystal growth, on the other hand, is restricted due to the rapid increase of viscosity that accompanies the crystallisation in the continuous oil phase (Mullin 1993). The sub-micron sized crystals subsequently aggregate in flocs, and it is this close contact that encourages the formation of solid bridges (Johansson & Bergenstahl 1995a) and subsequent “shell” formation. The formation of solid bridges (sintering) is demonstrated in section 4.5 for emulsions containing 0, 1 and 2 % tripalmitin.

The results of the tests for stability also suggest that a minimum concentration of crystals is necessary for the formation of solid bridges within and between these flocs. At low tripalmitin concentration (0 or 0.5 %) emulsions have reduced stability when compared with formulations containing higher amounts of tripalmitin. It is proposed that this is because of a lack of sufficient numbers of crystals required for the formation of tight networks at the oil/water interface. There are also fewer crystals available in the continuous oil phase to form a stabilising crystal network for arresting droplet movement.

4.3 Melting behaviour of emulsions

In order to explain the dependence of emulsion stability on temperature, the melting behaviour of various emulsion formulations was investigated using DSC. Samples of the emulsions (5-10 mg) were loaded into aluminium pans, stabilised at 0 °C for 5 minutes and heated to 60 °C at a rate of 10 °C min^{-1}.

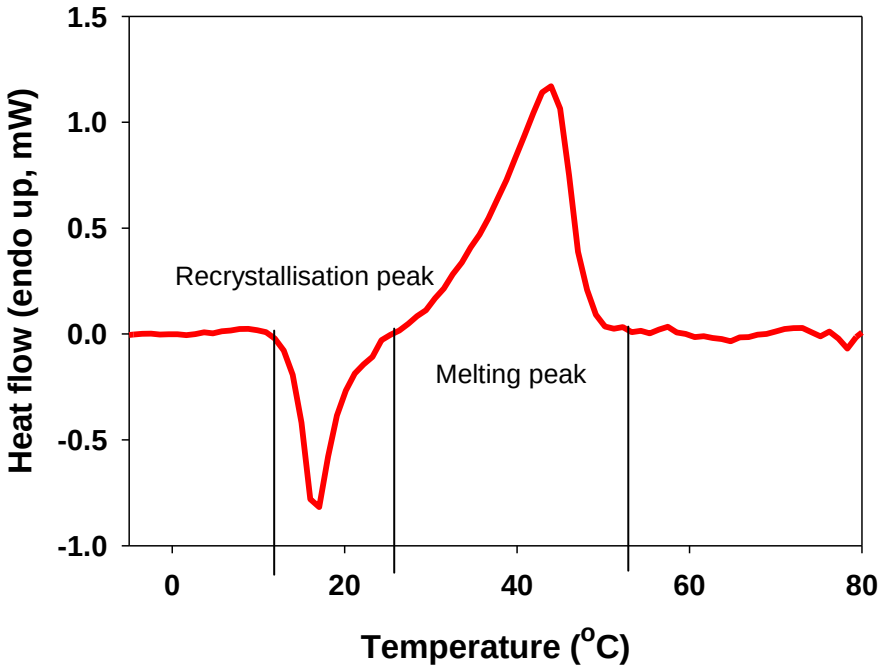

Figure 4.4: DSC curve of an emulsion stabilised by 0.5 % monoglyceride and 2 % tripalmitin, heating rate 10 °C min^{-1}

Figure 4.4 shows the melting profile of an emulsion containing 0.5 % monoglyceride and 2 % tripalmitin. The curve represents the average of three measurements with a heating rate of 10 °C/min (typical error: 0.01 mW). The shape was similar in emulsions with 0.5 and 1 % tripalmitin (shown in the appendix, section 8.3), although the melting temperatures varied according to the tripalmitin concentration. (see Table 4.4).

The melting peak of each emulsion formulation was preceded by a re-crystallisation event. Previous literature reports (Norton et al., 1985; Hale & Schroeder 1981) have shown that tripalmitin mixtures melt in a single peak in the presence of triolein (a major component of sunflower oil). The nature of this re-crystallisation peak will be further investigated in section 4.4.

The melting ranges given in Table 4.4 are defined as being between the first point (temperature) at which deviation from the baseline occurs and the point the curve returns to the baseline. The melting (crystallisation) range was deemed to be a better representation of the melting/ crystallisation properties than defining a “peak” temperature. Defining such a peak was not possible given the broadness of the peak and the small quantities of melting/ crystallising material. The values quoted in Table 4.4 are averages obtained from 3 different samples.

Table 4.4: Melting properties of emulsions stabilised by 0.5% monoglyceride and different tripalmitin concentrations

Emulsions	$Peak_{start}$	$Peak_{end}$
0.5 % tripalmitin	19.5 °C ± 0.7 °C	38.8 °C ± 0.4 °C
1 % tripalmitin	22.7 °C ± 0.8 °C	44.7 °C ± 1.2 °C
2 % tripalmitin	24 °C ± 1 °C	49.3 °C ± 1.2 °C

The main melting peak of the emulsions shifts to higher temperatures, from 19.5 to 24 °C, as the tripalmitin concentration increases. The melting point of a mixture of different triglycerides was previously shown to depend on the ratio of the different fats (Norton et al., 2009; Campbell et al., 1996; Norton et al., 1985). The increase in solid fat (tripalmitin)/ liquid (sunflower) oil ratio shifts the melting point of the mixture towards the melting point of the solid fat, which is at 42 °C (see Appendix 8.2 for the characterisation of the tripalmitin). Complete melting of the emulsions occurred when temperatures exceeded ~50 °C.

The melting profile of the W/O emulsions therefore corresponds with their increasing instability at higher temperatures. Emulsions showed high stability when stored at temperatures below the melting range of the fat crystals, but became increasingly unstable as the storage temperature was increased and entered the melting range (see Table 4.3). At a temperature close to the melting peak (~30°C), emulsions destabilised rapidly (within hours), while at a temperature beyond the melting range (~50°C), emulsions could not be stabilised (see section 4.6.2).

The temperature dependence shown above indicates that the crystals are responsible for stabilising the emulsions. At temperatures below the melting range (colder than 15°C) they provide Pickering stabilisation to the emulsion. When the temperature is increased to 25°C and beyond, the crystals become increasingly soluble (indicated by the broad melting range of the tripalmitin/ monoglyceride component within the emulsions). This coincides with the emulsions becoming less stable, as indicated by the increase of average droplet size in the emulsions. The decreased emulsion stability at elevated temperatures can therefore be explained by the fact that there are fewer crystals available at the interface, so that it is no longer fully covered with solid particles. .

These observations confirm that the emulsions owe their stability to Pickering stabilisation provided by a network of fat crystals forming solid “shells” around water droplets.

4.4 Melting/crystallisation behaviour of oil phase components

In order to investigate the exothermic peak that precedes the melting of the emulsion, the individual oil phase components, their melting behaviour in sunflower oil and the influence of the presence of water in the mixture were analysed using DSC.

4.4.1 The effect of water on the melting behaviour of monoglyceride and tripalmitin mixtures in sunflower oil

Monoglyceride (0.5%) and tripalmitin (0, 0.5, 1 or 2 % wt/wt) were mixed with sunflower oil and heated to ~80 °C to melt the solid fat components and ensure a homogenous mixture. This mixture was then weighed into aluminium pans. The heating/ cooling profiles were measured using the procedure described in chapter 3.

Figure 4.5 shows a broad single peak at temperatures larger than 0 °C when a sample stabilised by 0.5 % monoglyceride and 2 % tripalmitin was heated. This melting peak was preceded by a re-crystallisation event similar to that observed in emulsions containing both components in the oil phase. The figure is the average of 3 different measurements.

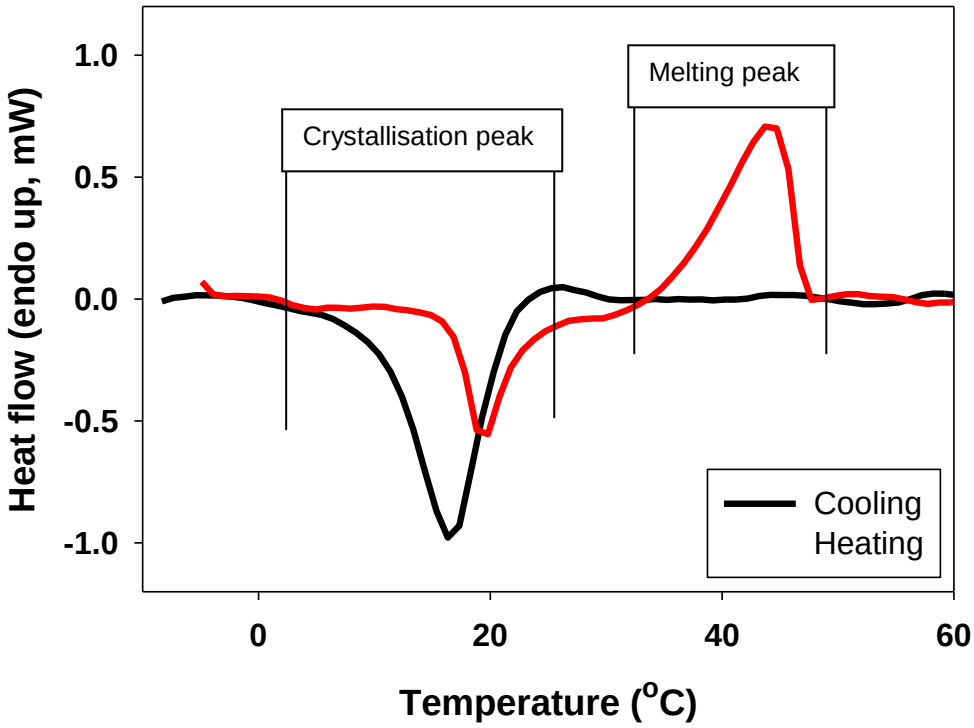

Figure 4.5: DSC curve (average of 3 measurements) of sunflower oil containing 0.5 % monoglyceride and 2 % tripalmitin, heating/ cooling rate: 10 °C min^{-1}

Table 4.5 shows the temperature ranges in which the melting or crystallisation occurs. The values quoted are averages obtained from 3 different samples. Melting ranges were defined in the same way as for the emulsion samples (see section 4.3). The melting/ crystallisation curves for samples with 0, 0.5 and 1% tripalmitin concentration are given in the Appendix (section 8.4)

Table 4.5: Temperatures at which oil mixtures containing 0.5% monoglyceride and various concentrations of tripalmitin melt and crystallise, heating/ cooling rate 10°C/min

	Heating		Cooling	
	$Peak_{start}$ (°C)	$Peak_{end}$ (°C)	$Peak_{start}$ (°C)	$Peak_{end}$ (°C)
0 % tripalmitin	19.5 ± 2.5	32.3 ± 2.3	21 ± 3	6.9 ± 0.3
0.5 % tripalmitin	20 ± 0.5	44 ± 1.4	22.8 ± 2.9	4.5 ± 2.5
1 % tripalmitin	24.5 ± 3	44.5 ± 0.5	20.7 ± 1.8	3.7 ± 0.4
2 % tripalmitin	31.7 ± 2	49 ± 1.1	23.8 ± 1.3	4.8 ± 1.2

A comparison of Table 4.5 with Table 4.4 shows that the presence of water from the emulsion does not influence the oil phase melting characteristics. The temperature at which the melting begins depends on the concentration of tripalmitin in both cases, while the location of the exothermic peak preceding the melting one does not. The exothermic peak is caused by the presence of polymorphic crystal forms of tripalmitin in the mixture. The data suggests that a limited fraction of crystal polymorphs are soluble within the sunflower oil. These crystallise, possibly as part of a polymorphic transition, at ~18 °C. The exothermic peak is further examined in the next section. Melting follows directly after the exothermic peak for formulations containing 1 % or less tripalmitin. The melting temperature of samples containing 2 % tripalmitin is 15°C higher than with lower concentrations. The explanation for the higher melting temperature at the higher tripalmitin concentration is the increased solids concentration in the sample. This was already discussed in section 4.3.

The data shows that tripalmitin concentration does not influence the initial crystallisation temperature (Table 4.5), indicating that the monoglyceride component

is the dominant factor in determining crystallisation. The crystallisation peak broadens with increasing tripalmitin concentration because of the larger amount of crystallising material present. It was shown in previous sections that the crystallisation temperature of monoglycerides is higher than that of tripalmitin (compare section 4.1. and 4.2). This result supports the theory that monoglycerides can be used to seed tripalmitin crystals. Due to their surface activity (Krog & Larsson 1992) monoglycerides reside preferentially at the interface. It is therefore likely that the monoglycerides crystallise directly at the interface, where they will seed tripalmitin crystals.

4.4.2 Characterisation of the exothermic peak preceding the melting peak

DSC analysis of a mixture of monoglycerides with sunflower oil in the absence of tripalmitin revealed that no exothermic peak preceded the melting peak in these samples (see Appendix 8.1.1 for detailed DSC analysis of monoglyceride/ sunflower oil mixture, Figure 8.2). The exothermic peak must therefore be the result of the presence of tripalmitin in the sunflower oil. It is likely that the less stable polymorphic forms crystallise rapidly at the interface when the oil mixture is cooled rapidly during emulsion production. Temperature cycling during production, as well as extended storage times will cause a shift towards the more stable polymorphic forms of tripalmitin crystals.

A DSC scan of pure tripalmitin revealed the presence of 3 different polymorphic forms (see Appendix 8.2, Figure 8.3). The melting behaviour of a mixture of a small quantity (0.5-2.5 %) of tripalmitin and sunflower oil was measured in order to

investigate whether the presence of different polymorphic forms of tripalmitin were the reason for the exothermic peak observed during the melting of emulsion samples (described in section 4.3). 1.5 % or 2.5 % tripalmitin was mixed with the sunflower oil and heated to 80 °C in order to melt the tripalmitin and obtain a homogenous mixture. The percentages were chosen to be equal to the total concentration of crystallising material used in the emulsion formulations. The mixture was then weighed into aluminium pans while hot and measured in the DSC (see chapter 3). At least 3 different samples of each formulation were characterised.

The melting/ crystallisation curves thus obtained are shown in Figure 4.6 (the full curve is shown in the Appendix 8.5, Figure 8.9). Crystallisation of the tripalmitin component in the mixture occurs at 18 °C. This crystallisation temperature is lower than when monoglycerides are present in the mixture, highlighting the role of monoglycerides to seed the tripalmitin crystallisation. Upon heating the mixture, an exothermic peak is observed at ~18 °C, while melting occurs at 35 °C. While the location of the exothermic peak remains constant, the melting temperature shifts to higher temperatures compared with the samples in which monoglycerides are present.

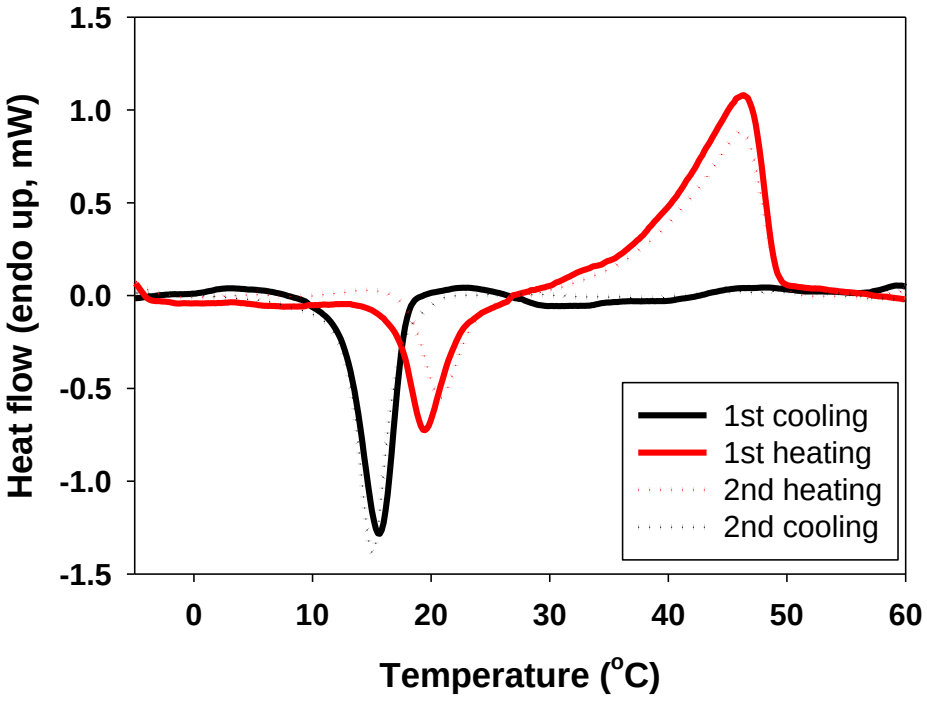

Figure 4.6: DSC curve of a mixture of 2.5 % tripalmitin and sunflower oil cooled and heated twice at 10 °C min^{-1}.

The melting/ crystallisation properties of this mixture are also summarised in Table 4.6 for tripalmitin concentrations of 1.5 and 2.5 %. When the sample was heated an exothermic (crystallisation) peak preceded the endothermic (melting) peak in each repeat measurement. This peak is similar to that observed in the DSC analysis of the emulsions containing tripalmitin (see 4.3). Similar curves were also obtained when the heating and cooling cycles were repeated once on the same sample (see Figure 4.6). The low concentration of tripalmitin investigated in this work requires a rapid heating/ cooling rate because of the enhanced sensitivity of the instrument towards small changes in differential heat capacity of the samples at rapid heating rates. At lower heating/ cooling rates the melting/ crystallisation peaks are too small to be distinguished from the baseline.

Table 4.6: Influence of different tripalmitin concentrations on melting and crystallisation properties of sunflower oil solutions

	1.5 % triglyceride		2.5 % triglyceride	
	Temperature (°C)	ΔH (mW.mg^{-1})	Temperature (°C)	ΔH (mW.mg^{-1})
	Heating		Heating	
Exothermic Peak (Re-crystallisation)	18±2	0.6±0.2	19±1.5	0.4±0.2
Endothermic Peak (Melting)	35±3	0.7±0.1	34±2	1.4±0.3
	Cooling		Cooling	
Exothermic Peak (Crystallisation)	14±0.5	0.6±0.1	18±0.5	1±0.3

It should be noted that even when heating rates of 10 °C min^{-1} are used, the areas are very small (ΔH less than 1 mW mg^{-1}) and can only be seen once a baseline has been subtracted. The small size of the peaks (due to the low concentration of tripalmitin in the sunflower oil) required the peak areas to be manually determined by fitting the DSC curve to a second-order differential (to remove the inclined slope of the baseline). The area underneath the curves was then calculated from this modified curve (see Appendix 8.6, Figure 8.11 for an example of the raw data). The value of ΔH thus depends on how the baseline is fitted to the curve (Cebula & Smith 1992). In

some cases this resulted in a 30-50 % error margin[1] between the values obtained in three repetitions of the experiment.

Compared to the melting/ crystallisation behaviour of pure tripalmitin, the addition of sunflower oil decreased the melting temperature of the tripalmitin component by around 20 degrees. The melting curve was also significantly broadened. This has been reported before and was ascribed to the solubility of tripalmitin in the sunflower oil (Norton et al., 1985; Hale & Schroeder 1981).

The melting peak represents tripalmitin melting in its most stable β form (Norton et al., 1985). It is preceded by an exothermic peak. The presence of the exothermic peak can be explained by the presence of various polymorphic forms of tripalmitin. The presence of polymorphic forms has been observed before in the first and second heating/ cooling cycles of tripalmitin/ triolein mixtures at low (0.1-1°C min^{-1}) rates (Norton et al., 1985; Hale & Schroeder 1981). If the sample is rapidly cooled it is likely that crystals re-form in less stable polymorphic forms for reasons explained below.

The formation of the less stable α and β' polymorphic forms are enhanced by rapid cooling/ heating rates. The reason for this is that a fast cooling rate increases the driving force for crystallisation, forcing it to occur in a short period of time. Nucleation occurs near instantaneously, and crystals are more likely to form in a less stable form because the free activation energy for nucleation is lower for the less stable

[1] Because the areas concerned are so small, and their exact values so dependent on several parameters, most evaluations of DSC curves take into account only the melting temperatures (start and end of melting/ crystallisation peaks). These were more reproducible than the ΔH.

polymorphic forms (Campos et al., 2002). Crystal forms shift to the most stable β form (Norton et al., 1985) as the solution is aged. This is because the surface energy of crystals of the less stable polymorphic forms is high (Marangoni 2002), and the system seeks to reduce its total free energy.

In the work presented here only 1st time crystallisation is of interest. This is because a temperature increase above the melting point of the solid fat components results in a destruction of the emulsion formulations (see section 4.2.3). For this reason, temperature cycling was not performed on the samples investigated and the exothermic peak that precedes the melting peak is due to the presence of the less stable polymorphic forms in the sample.

4.5 Sintering of crystal networks

Stability tests of the fat crystal stabilised W/O emulsions had revealed their stability against coalescence. It was argued that individual crystals had sintered to form tight networks around water droplets, which acted as effective barriers to prevent coalescence.

In order to quantify the degree to which the crystals sinter into networks, the elastic and viscous properties of the emulsions directly after preparation and after 2 weeks of storage were investigated. To enable such measurement the samples were loaded into a rheometer fitted with a 40 mm parallel plate geometry (gap size 1mm) kept at 15°C. First, the linear elastic region was determined by performing an amplitude sweep at constant frequency. The amplitude sweep was performed using the same geometry (40mm parallel plate, gap 1 mm) at constant frequency of 1 Hz (see

Appendix 8.7, Figure 8.12 for amplitude sweeps of the emulsion samples). Then, a frequency sweep was performed on different samples in order to find the elastic and viscous moduli of the emulsions (G' and G'', respectively). Depending on the concentration of tripalmitin, the applied stress was chosen to be 50 (2 % tripalmitin), 20 (1 % tripalmitin) or 1 Pa (0 % tripalmitin) so as to allow experiments to be performed within the linear elastic region (see Appendix 8.7). The frequency range was chosen because the sample containing no tripalmitin became unstable at lower and higher frequencies, while the samples containing more than 1% tripalmitin showed no change.

The data obtained from frequency sweeps for tripalmitin concentrations of 0, 1 and 2% tripalmitin in freshly prepared samples and those stored for 2 weeks are presented in Figure 4.7. Each curve represents the average of measurements on at least 3 different samples. It is shown that the G' of emulsions depends on tripalmitin concentration: the higher the number of crystals, the higher the elastic modulus. This is because the higher crystal concentration leads to more crystal interactions (Campos et al., 2002a), and thus more rigid networks (Rousseau & Hodge 2005). G' increases as the emulsions containing at least 1% tripalmitin are aged because of post-crystallisation processes such as crystal growth and sintering of the crystals (Johansson & Bergenstahl 1995a).

Furthermore, the G' of samples containing 1% tripalmitin increased by approximately 20%, and of emulsions containing 2% tripalmitin by 30%, over a two-week period. The samples are predominantly solid, as G'' remains well below G' at all frequencies in all 3 cases (values for G'' are not shown are not shown in the graph for clarity).

The value of G' is an indication of fat crystal network strength: a higher G' indicates higher elasticity of the samples, which is a sign of stronger interactions between crystals within the emulsions.

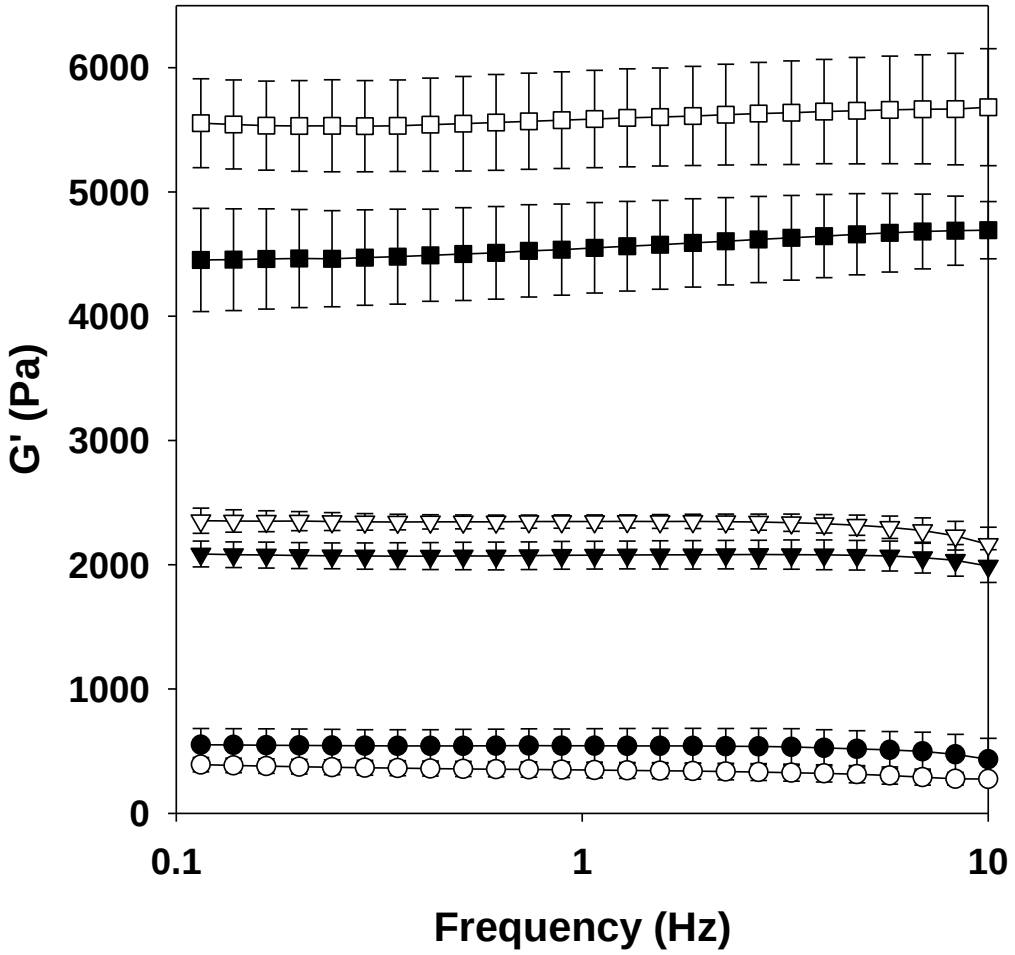

Figure 4.7: Storage moduli (G') of emulsions (stored at 5°C) containing various tripalmitin concentrations in a frequency sweep. All emulsions are stabilised by 0.5% monoglyceride and also: ○,● = No tripalmitin; ▽,▼ = 1% Tripalmitin; □,■ = 2% Tripalmitin. Closed symbols are data from 1 day old systems while open symbols are data from emulsions stored for 2 weeks.

For emulsions containing no tripalmitin, the value of G' did not change significantly over a two-week storage period. It has been discussed in previous sections that

monoglycerides, as surface-active crystals, are mainly situated at the interface. Monoglyerides form small crystals which surround the droplets in a more or less smooth layer, i.e. crystals do not protrude far from the interface (see section 4.2). It is likely that their concentration in the continuous oil phase is not high enough to form a pronounced crystal network there. Post-crystallisation interactions are therefore limited by the small number of crystals in the continuous oil phase, which explains why the value of G' does not change with storage.

These results show that post-crystallisation processes harden the crystal network in the continuous oil phase as well as at the interface, providing that a minimum crystal concentration is present. It is likely that one such post-crystallisation process is sintering. It is also likely that a further post-crystallisation exists: platelet-shaped α-form crystals that may have formed during production due to the fast cooling rates (see section 4.4) undergo transition to the stable β-form as the emulsion is aged. The transition to the β-form would lead to a more stable structure due to the lower overall free energy of the system. However, due to spatial restrictions because of the large number of nuclei, the crystal growth is limited, and post crystallisation processes occur in the form of sintering. The increasing rigidity of the crystal networks enhances emulsion stability as individual crystals merge to form "shells" around water droplets and a rigid crystal network in the continuous oil phase minimises droplet movement.

4.6 Salt release

The water phase of the fat crystal-stabilised emulsions was formulated to contain NaCl so as to allow investigation of the capability of these structures to encapsulate solutes. It is desirable that the fat crystal-stabilised structures can retain a solute

encapsulated within the aqueous phase because they will be incorporated into double emulsions in this study. If the structures can retain small salt molecules such as NaCl against an osmotic pressure gradient, it is likely that they can also do this when incorporated in a double emulsion, and with larger solutes than NaCl, as the actual structure of the primary emulsion is not changed. The release rate of a small solute such as NaCl is expected to be faster than of larger solutes. In double emulsions there are two additional factors: one is the presence of a secondary emulsifier, and one is the additional production step which potentially damages the primary emulsion “shells”. This section investigates the response of the primary emulsions exclusively to the application of osmotic pressure gradients, while the additional factors in double emulsions will be investigated in the next chapter. Controlling the release of small salt molecules has many potential applications in pharmaceuticals as well as in low fat and/or low salt food products (see chapter 1).

In order to investigate salt release from the W/O emulsion samples, small amounts of the various W/O emulsions were placed into water containing various concentrations of KCl to generate different osmotic pressure gradients (see chapter 3 for details). Salt release rates were monitored over time by measuring the conductivity of the water. The effect of fat phase composition, temperature and osmotic pressure were investigated in this way.

4.6.1 The effect of fat phase composition

Small samples (~3 g) from emulsions containing 0-2% tripalmitin and 0.5% monoglyceride were submersed in distilled water. No attempt was made to disperse the sample so that it remained as one piece in the water. Salt release from the

encapsulated water droplets to the continuous water phase was measured as a function of time and fat crystal concentration in order to characterise emulsion stability under a large applied osmotic pressure gradient, 14 atm (for the significance of the size of the osmotic pressure gradient refer to chapter 2).

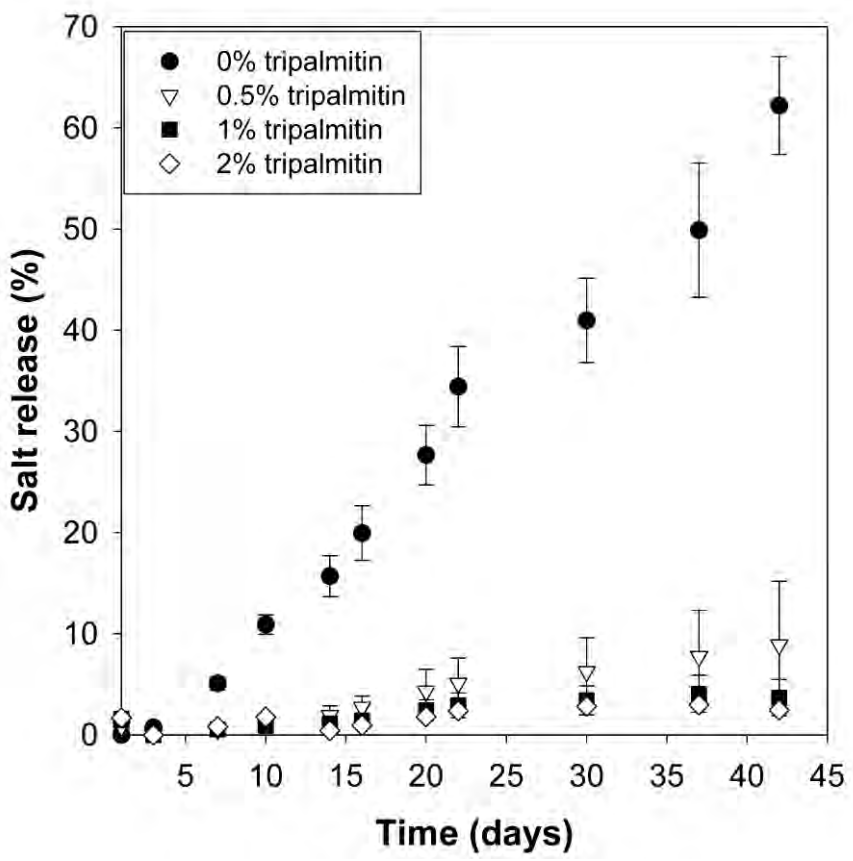

Figure 4.8: Salt release as monitored by measuring the conductivity of a series of emulsions within a bulk distilled water phase at 22 °C. All formulations contained 0.5% monoglyceride and 0, 0.5, 1 or 2 % tripalmitin.

Figure 4.8 shows how salt was released from 2 g W/O emulsion samples (containing 0.5% monoglycerides and varying concentrations (0-2 %) of tripalmitin) in 150ml distilled water, stored at 22 °C. The conductivity of the distilled water was measured

to trace the salt release from the emulsion samples. The graph shows that salt release from the W/O emulsions depended on the tripalmitin concentration of the emulsion. The emulsion containing 0.5 % monoglyceride and no tripalmitin released around 50 % of its total salt content after 1 month. Adding 0.5 % tripalmitin reduced this release to less than 10 %, while adding more than 1 % almost completely stopped any release within a one-month storage period.

These results show that crystalline “shells” are capable of retaining salt within the encapsulated water droplets despite an applied osmotic pressure gradient of 14 atm.

The mechanisms by which water transport occurs across an oil boundary in the presence of emulsifiers were discussed in Chapter 2. The most important ones include the formation of lamellae or hydrated surfactants in the oil boundary (Wen & Papadopoulos 2001) and the diffusion of reverse micelles of emulsifiers, which may contain water/solutes, between the two aqueous phases (Garti et al., 1985).

As monoglycerides are incorporated into a solid crystal network they do not aid water transport with such mechanisms. Their crystalline nature prevents them from forming reverse micelles and diffusing between the two aqueous phases while their rigid structure prevents them from forming lamellae in the oil phase. Although water may still diffuse directly through the oil, this is not possible for salt ions (Cheng et al., 2007). The broad melting range of monoglyceride in sunflower oil suggests that crystals start melting as the temperature is increased above 20 °C (see section 4.4). It is possible that liquid monoglycerides molecules aid water/ solute transport across the oil phase, e.g. by forming reverse micelles. The slow (almost negligible) release

rates of emulsions containing 1 or 2 % tripalmitin is probably the result of the fraction of liquid monoglycerides present in the sample.

The emulsion containing only 0.5 % monoglyceride and no tripalmitin released more than 50 % of the total encapsulated salt within the first month. This emulsion sample was less stable in terms of coalescence than emulsions containing more than 1% tripalmitin (see section 4.2), which may be one reason for the relatively rapid release. Several other factors could also affect the release rate in this emulsion sample: The fat crystal network is not very pronounced in this formulation containing only 0.5 % monoglyceride, so that water and solutes can leak through cracks and imperfections.

Finally, the crystalline “shell” structure is comparatively weaker than when triglycerides are present. Section 4.5 showed that emulsions containing only 0.5 % monoglycerides and no tripalmitin did not develop strong crystal interactions, as opposed to emulsions containing more than 1 % tripalmitin: while G’ increased with time in samples containing 1 % tripalmitin, no change was observed in samples that contained only monoglycerides. The post-crystallisation processes that increased G’ included the formation of a sintered network in samples containing 1 % tripalmitin. This sintered network is likely to have also incorporated monoglyceride molecules. The lack of such sintered crystal network formation in emulsions containing no tripalmitin enables monoglyceride molecules to move more freely in the oil phase between the two aqueous interfaces. This could contribute to the rapid release observed in such formulations.

4.6.2 The effect of temperature on salt release

It was previously shown that temperature influences emulsion stability by affecting the tight network structure encapsulating the water droplets. In order to highlight the effectiveness of these fat crystal networks in retaining water and encapsulated solutes the structure was systematically weakened by increasing the storage temperature of around 2 g emulsion samples in distilled water. Temperatures higher than 22 °C cause the crystals to gradually melt, as was shown in the DSC analysis of the emulsions (see section 4.4). Once the crystals melted the stabilisation mechanism changes from Pickering to electrostatic. It is well-known (Matsumoto et al., 1980 and Frenkel et al., 1983) that emulsions stabilised by monomeric surfactants cannot retain encapsulated solutes against an applied osmotic pressure gradient (see chapter 2), so that salt release is expected to be rapid in emulsions no longer stabilised by crystals.

Experiments were performed at 10 °C (below the onset of crystal melting for all emulsions), 22 °C (onset of crystal melting for emulsions, T_{onset}), 32 °C (close to the peak temperature for emulsions, T_{peak}) and 55 °C (end of crystal melting, T_{end}). Figure 4.9 shows the salt release, measured as described previously, at these storage temperatures in a sample containing 0.5% monoglyceride and 1% tripalmitin.

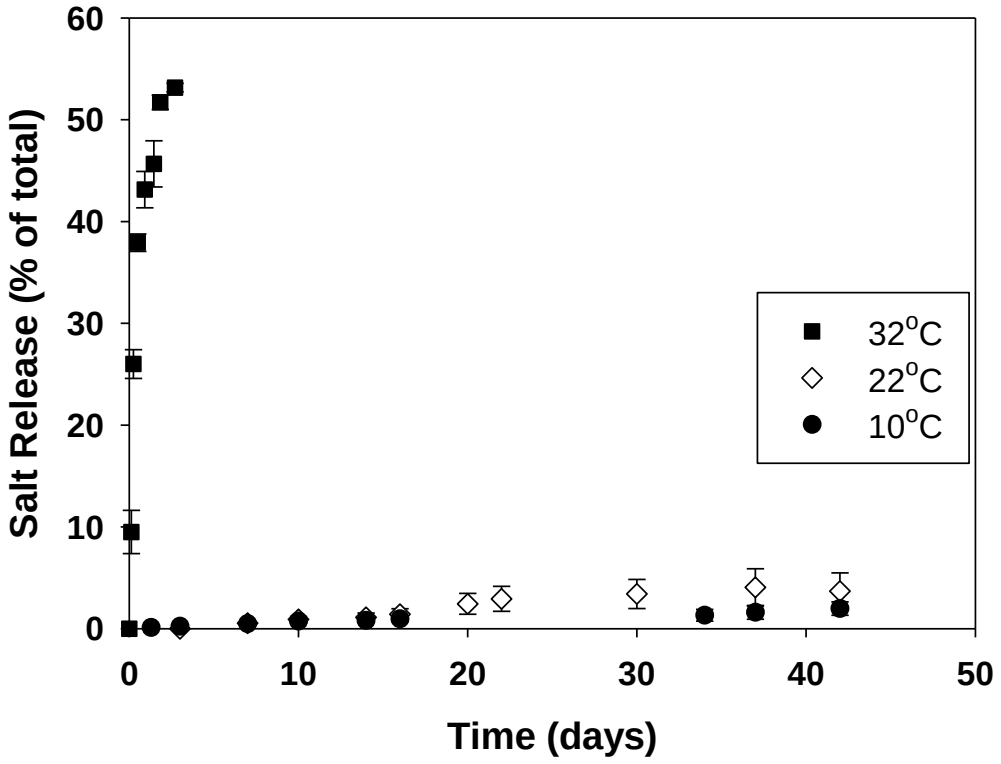

Figure 4.9: Salt release from samples of an emulsion stabilised by 0.5 % monoglyceride and 1% tripalmitin at different temperatures (10-32 °C)

As expected, salt release from the emulsions was in line with the crystal melting temperatures: After 1 month storage at 10 °C less than 2 % of salt has been released. Storage at 22 °C resulted in a similar release (5 % of the total). By contrast, all encapsulated salt was released within 5 days when the sample was stored at 32 °C. It takes more than 100 days in order for 5 % of salt to be released from an emulsion containing 1% tripalmitin and 0.5 % monoglyceride at 5 °C, but less than an hour at 32 °C (Table 4.7). After 1 month at 5 °C the emulsions containing 1 % tripalmitin released less than 2 % of their total salt content. Similar results were obtained with 2 % tripalmitin, although the emulsion was stable for a longer period at

30 °C, releasing 5% of total salt only after 4 hours. The higher tripalmitin in these samples increases the melting temperature of the mixture, as shown and discussed in section 4.4.

Table 4.7: Overview of the time it takes to release 5 % of total salt from an emulsion with 0.5 % monoglyceride and Tripalmitin in distilled water at different temperatures

Temperature	Time, 1 % tripalmitin	Time, 2 % tripalmitin
5 °C	> 100 days	> 100 days
22 °C	70 days	80 days
30 °C	< 1 hour	4 hours
50 °C	20 seconds	30 seconds

The increased salt release mirrors increased structural instability at higher temperatures. Enhanced solubility and subsequent melting of the crystals at the interface create imperfections in the tight crystal networks surrounding the water droplets. Increasingly soluble monoglycerides, as well as imperfections, allow for water to migrate from the encapsulated to the continuous aqueous phase and therefore for salt to be released.

The salt release at 55 °C was measured continuously by recording the conductivity measurements of the external water phase every 2 seconds, starting when the emulsion sample (~3 g) was placed in the water as described in chapter 3. Figure 4.10 shows that at 55 °C salt release was almost instantaneous in all emulsion samples (containing 0-2 % tripalmitin and 0.5 % monoglyceride), with complete release taking place within 3 - 4 minutes. At this temperature neither tripalmitin nor

monoglyceride are crystalline so all emulsions are no longer Pickering-stabilised. As a result, the interface is no longer able to withstand the large applied osmotic pressure difference. Droplets coalesce and phase inversion occurs. All salt is released to the continuous aqueous phase.

There is a small difference in the rate of release in the different formulations. It is fastest for the sample containing only 0.5 % monoglyceride. This formulation has released 90 % salt after 100 seconds. An emulsion containing 1 % tripalmitin has released 40 % salt after 100 seconds, while a sample containing 2 % tripalmitin and 0.5 % monoglyceride released 30 % salt within this time. The reason for this difference is that the tripalmitin concentration affects the melting point of the emulsion. The melting point is lower when the tripalmitin concentration is lower (see section 4.4). This means that an emulsion sample with 0.5 % tripalmitin that is placed in hot water reaches its melting point more quickly relative to a sample containing 2% tripalmitin so that coalescence commences faster in samples with no tripalmitin. An emulsion containing only monoglyceride melts fastest because the melting point of that emulsion is lowest: monoglyceride melts at a lower temperature than tripalmitin (see Appendix section 8.1).

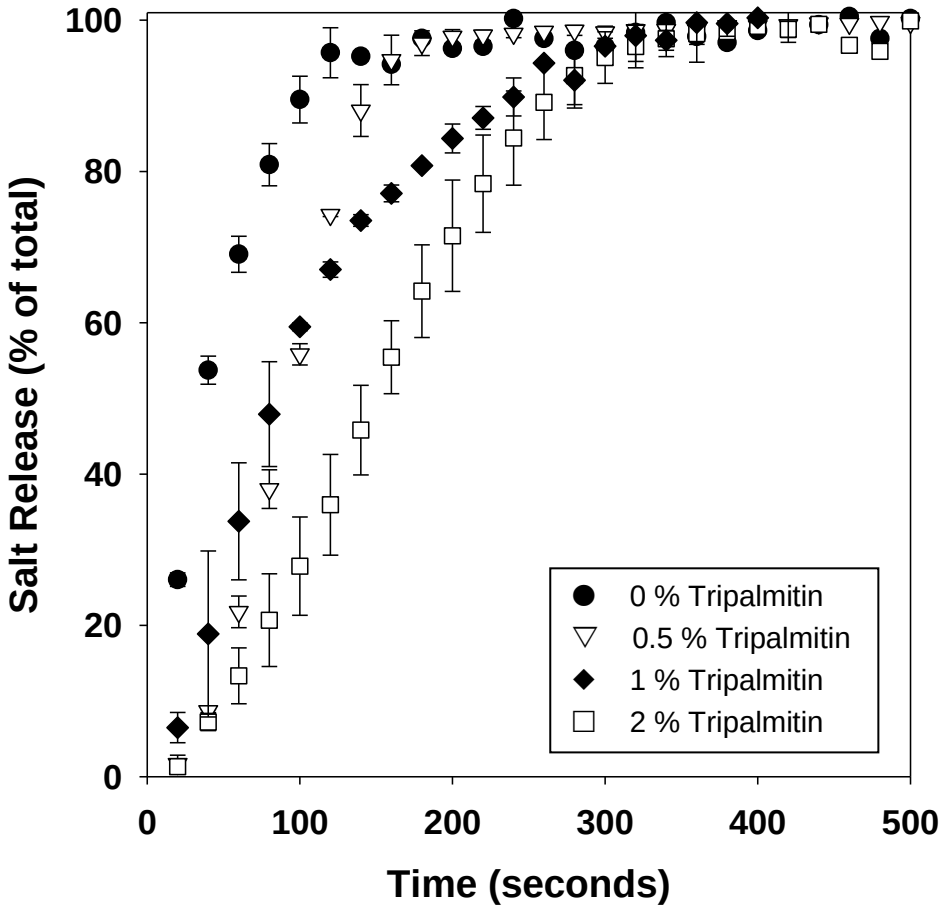

Figure 4.10: Salt release at 55 °C from emulsions stabilised by 0.5 % monoglyceride and 0, 0.5, 1 or 2 % tripalmitin.

4.6.3 The effect of osmotic pressure

Finally, the effect of different osmotic pressure gradients on salt release was investigated (Figure 4.11). Osmotic pressure gradients are a major cause of instability in double emulsions (see section 2.2.3.1). A study of the ability of fat crystal stabilised W/O emulsions to retain a solute in spite of an applied pressure gradient shows their potential to retain solutes even when incorporated into double emulsions.

The initial salt concentration within the emulsion water droplets of all samples was 1.6 %. Osmotic pressure is calculated using the equation:

$$\pi = M R T$$

Where M is the molar concentration of the solute, R is the universial gas constant (0.0821 L atm mol^{-1} K^{-1}) and T is the absolute temperature (298 K).

Osmotic pressure was varied to different gradient strengths by adding glucose to the external water phase (up until a gradient of 14 atm was achieved).

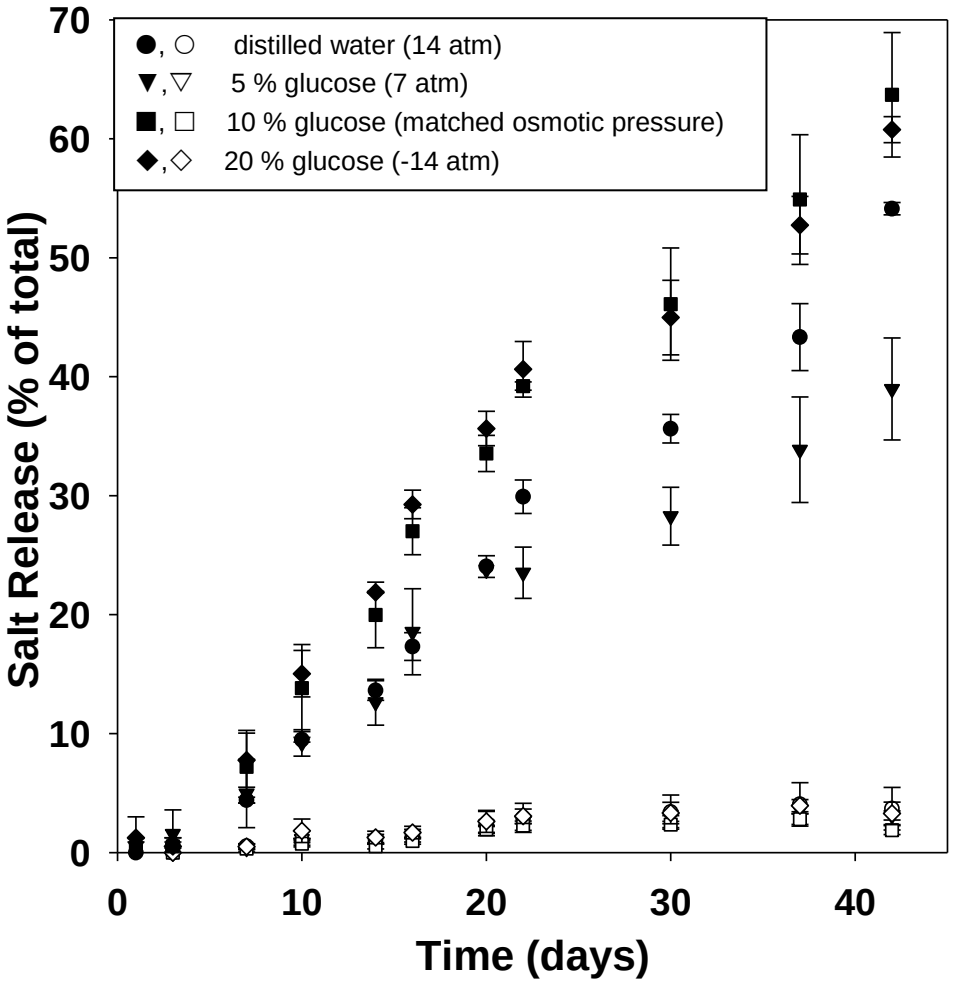

Figure 4.11: Influence of osmotic pressure gradients on the salt release from emulsions stabilised by 0.5% monoglycerides and 1 % Tripalmitin (open symbols) or no tripalmitin (filled symbols). The salt concentration in the droplet was always 1.6 % and the glucose concentration in the external water phase was varied. Storage temperature = 22 °C.

In formulations containing 1 % tripalmitin and 0.5 % monoglyceride, less than 5 % salt was released after 1 month (Figure 4.11), independent of the strength of the osmotic pressure gradient. In fat crystal-stabilised W/O emulsions osmotic pressure gradients therefore seem to have no significant effect on salt release. It was shown in double emulsions that the rate of salt release was faster the larger the osmotic pressure gradient between the two aqueous phases (Wen & Papadopoulos 2001).

The reason for this is that the osmotic pressure gradient drives water transport as the system seeks to remove the osmotic pressure gradient. If water transport occurs through reverse micelles, solutes within the water phase may also be “encapsulated” within the reverse micelle and therefore released to the other aqueous phase (Cheng et al., 2007).

Given the stability these samples have shown in distilled water, where the osmotic pressure gradient is large at 14 atm, it can be expected that lower osmotic pressure gradients have no influence on the salt release. This is because there are few amphiphilic molecules readily available to form reverse micelles to transport water and solutes between the two aqueous phases. Some salt is released, however, due to the solubility of water in sunflower oil which causes some diffusion through the tight networks made of sintered crystalline triglycerides (Guery et al., 2009).

The release data (Figure 4.11) for the emulsion containing no tripalmitin and 0.5 % monoglyceride, on the other hand, shows relatively fast release under all osmotic pressure gradients: after 10 days, between 10 and 20 % has been released, while between 20 and 40 % of the total salt content was released after 20 days. The reason for the fast release in all cases is the reduced stability of the emulsion droplets against coalescence, as well as the lower melting point of the emulsion sample (see previous section). Coalescence between water droplets may lead to salt release if the droplets are in contact with the external aqueous phase.

It has already been shown that crystalline monoglyceride molecules are part of a sintered network with crystalline tripalmitin that forms thight networks around water

droplets. Surfactant molecules bound in this way cannot freely move to transport water molecules through the oil phase. It was shown that the monoglyceride as well as tripalmitin molecules are crystalline at 20 °C (see Figure 4.4). Water molecules and salt ions must thus diffuse as insoluble species through a semi-solid oil phase, an invariably slow process, as this study clearly demonstrates. The crystal networks around emulsion droplets thus prevent the passage of water across the oil/ water interface even when osmotic pressure gradients are applied.

These results have shown that salt release in this system is controlled by temperature, not by osmotic pressure gradients: Emulsions are only stable when crystalline shells surround the water droplets. It occurs due to imperfections in the shells caused by melting or solvating of crystals as a function of temperature. These imperfections can create “cracks” through which water can leak from the inner to the outer aqueous phase.

4.7 Addition of hydrocolloids or lecithin

In many commercial low fat spreads, hydrocolloids are added in order to aid structure formation in such highly concentrated water/oil emulsions and to achieve desirable properties, such as good spreadability, stability at room as well as fridge temperature and a rapid but sustained flavour release upon ingestion (Cain et al., 1987). For instance, gelatin is added because it melts at temperatures similar to those in the mouth (Norton et al., 1996).

Guar, CMC, CMCC, lecithin and gelatin were added to the internal aqueous phase to investigate their effect on droplet size and stability in the model W/O emulsions

investigated as part of this work. The additives were dispersed in the aqueous phase as described in chapter 3 prior to emulsification. The aqueous phase also contained 1.6% NaCl. The oil phase contained 0.5% monoglycerides and 1% tripalmitin. Emulsification was performed in the same way as with emulsions that did not contain any such additives.

The emulsions, containing 1 or 5 % (wt/ wt in the aqueous phase) gelatin, 1 % CMC, 1 % CMCC or 0.5% lecithin, were produced in triplicate and samples stored at 5 °C for 4 weeks. The 5% gelatin solution gelled rapidly once cooled, but was liquid during the preparation of the emulsion pre-mix (when the temperature was around 80 °C). Droplet size was measured on fresh and stored samples using the NMR.

Table 4.8: Droplet sizes (μm) of W/O emulsions containing various hydrocolloids or lecithin

	1 % gelatin	5 % gelatin	1 % CMC	1 % CMCC	0.5 % lecithin
1 day	3.95 ± 0.29	7.48 ± 0.73	3.99 ± 0.35	5.1 ± 0.90	3.84 ± 0.24
1 week	3.95 ± 0.31	7.77 ± 0.73	4.02 ± 0.38	6.1 ± 1.02	5.38 ± 0.51
4 weeks	3.96 ± 0.33	unstable	4.04 ± 0.4	6.4 ± 1.0	unstable

Table 4.8 shows that the addition of 1 % CMC, 1 % CMCC or 1 % gelatin did not have a significant effect on initial droplet size or droplet stability against coalescence.

The addition of 5% gelatin, however, resulted in a poorly structured emulsion, in which the water droplets seemed to contain inclusions of oil, and some very large droplets were observed in the formulation (Figure 4.12). A 5 % gelatin solution gels rapidly once cooled. This means that a gel is formed inside the A – unit during

production of the primary emulsion, possibly before the formation of fat crystals causes the inversion of the O/W emulsion to produce fine, fat crystal stabilised water droplets. This would explain why some water droplets seem to include oil droplets – these have become trapped in the gel structure before fat crystals were formed.

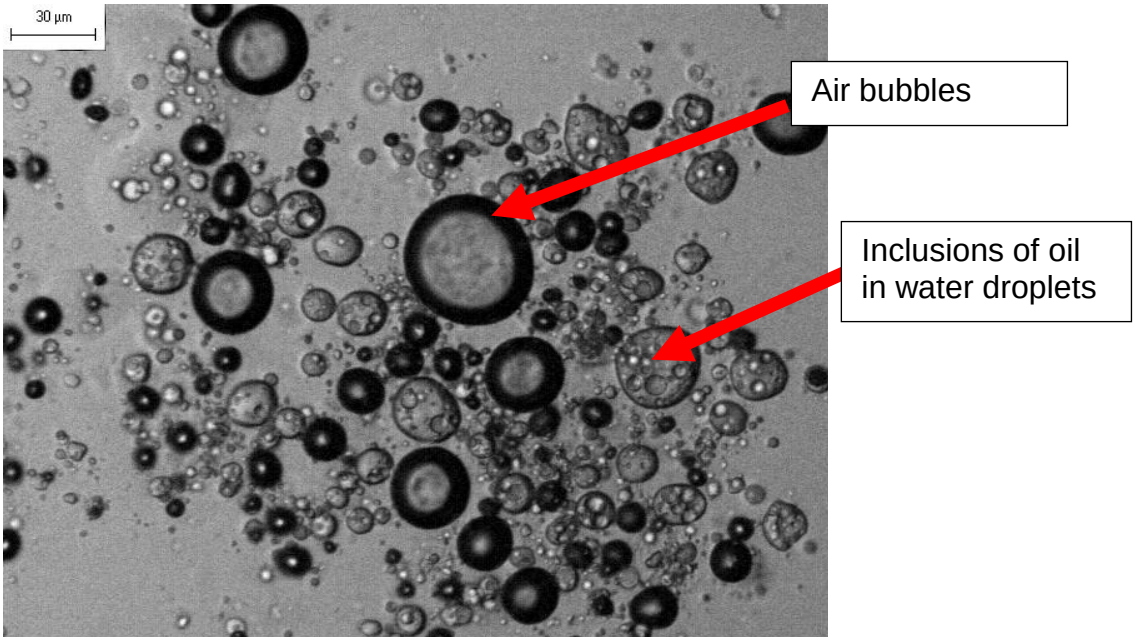

Figure 4.12: Emulsion containing 5% gelatin in the aqueous phase

The addition of lecithin resulted in a poorly stabilised emulsion which phase-separated within a few days. The addition of guar resulted in emulsions which were not stable against coalescence.

The poor storage stability against coalescence of emulsions containing guar or lecithin can be ascribed to the presence of surface active substances. Guar contains ~2.5% surface active protein (Anderson 1986). Lecithins are natural amphiphiles (Dickinson 2003) and may compete for interfacial space with the monoglycerides

during the emulsification process. This competition may prevent the formation of a tight fat crystal network so that coalescence is not as effectively prevented. Furthermore, it is possible that proteins adsorb to the fat crystals and change their surface properties. This might prevent them from adsorbing at the surface of water droplets because adsorption to proteins may have changed the crystal wetting behaviour; or caused large crystal aggregates to be formed that cannot effectively stabilise the droplet surface.

The addition of CMC or CMCC has little effect on the stability of the W/O emulsion. Although these hydrocolloids increase the viscosity of the aqueous phase, they do not gel or seem to interact with the interface. As a result, tight fat crystal networks can form. It is shown in Figure 4.13 that the addition of these hydrocolloids to the aqueous phase affects salt release at room temperature in distilled water slightly negatively. As the presence of CMC, CMCC or 1% gelatin has no effect on the stability against coalescence compared to an emulsion containing no hydrocolloids, it was expected that salt release would not be much different in the emulsion samples. The observed slight increase of salt release in samples containing hydrocolloids might be due to storage at a slightly higher temperature (the temperature of the room in which the samples were stored fluctuated; the samples containing hydrocolloids were prepared at a different time to those not containing hydrocolloids).

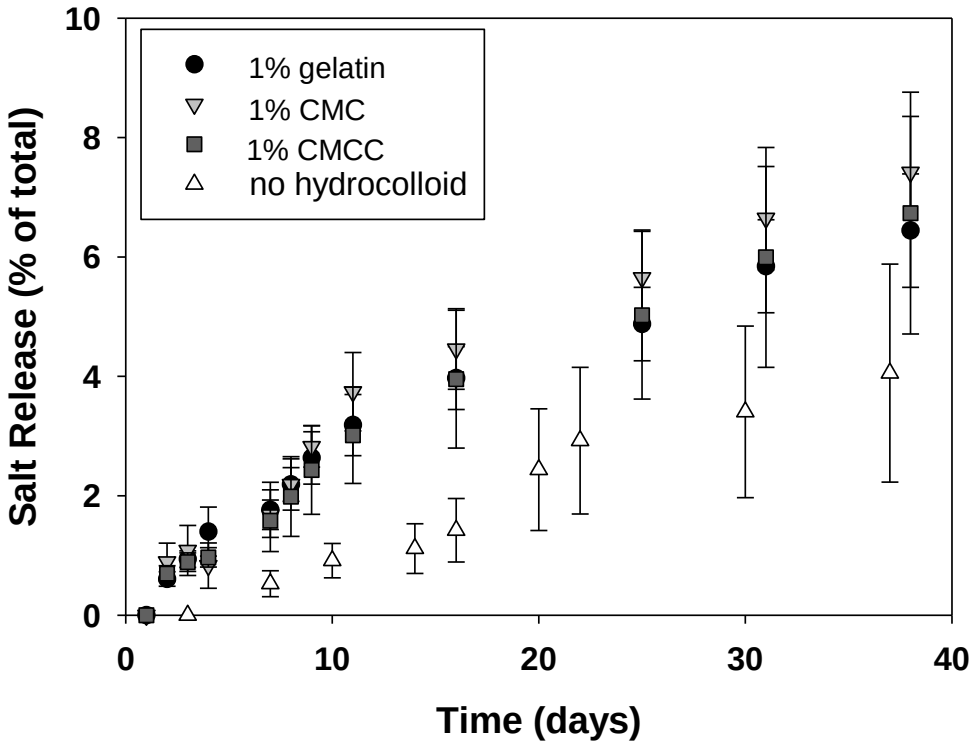

Figure 4.13: Salt release in formulations stabilised by 0.5 % monoglycerides and 1 % tripalmitin and containing hydrocolloids in the aqueous phase stored at 20 - 22 °C.

Hydrocolloids are usually added in order to provide or increase structure to the aqueous phase of low fat spreads. The addition of hydrocolloids such as CMC, CMCC or 1% gelatin to the fat crystal-stabilised W/O emulsions investigated as part of this work did not increase emulsion stability. In the system investigated here, it is the crystallisation of monoglycerides and tripalmitin that drive emulsification. The presence of CMC, CMCC or 1% gelatin did not change the crystallisation properties so that tight crystal networks formed surrounding water droplets in all cases and emulsion properties such as droplet size and storage stability did not change. The presence of guar, lecithin and 5% gelatin was detrimental to the stability of the emulsions.

4.8 Conclusions

In this chapter it has been shown that a combination of mono- and triglyceride crystals can provide Pickering stabilisation to W/O emulsions without addition of another emulsifier. These emulsions are effective in encapsulating salt, irrespective of the magnitude of applied osmotic pressure. It is argued that this is a result of the crystals sintering to form solid shells around the water droplets.

As would be expected the stability against coalescence as well as salt release was found to depend on the temperature of the system. When the samples were stored in conditions below the melting temperature of the crystals (colder than 20 °C), almost no coalescence and release occurred as the shells around the droplets remain intact. At higher temperatures, crystals melted and as a result emulsion stability was compromised. This eventually led to complete salt release and phase separation.

The ability of the crystalline shells to retain salt encapsulated within water droplets suggests that it might be possible to incorporate them into a double structure if this shell structure can be retained in the secondary emulsification step. The resulting double emulsion would be capable of retaining encapsulated solutes during storage, while their rapid release could be triggered as the temperature is increased above the crystal melting temperature.

The addition of various hydrocolloids to the encapsulated aqueous phase did not enhance the stability of these fat crystal-stabilised emulsions.

5 $W_1/O/W_2$ double emulsions stabilised by fat crystals – formulation, stability and salt release

5.1 Introduction

Chapter 4 demonstrated that fat crystal-stabilised W/O emulsions have the ability of retaining solutes encapsulated within the aqueous phase due to the fat crystal "shells" around the water droplets.

One of the main problems in $W_1/O/W_2$ double emulsions is that of retaining solutes within the W_1 encapsulated phase. As has been discussed in chapter 2, the presence of two different types of emulsifiers in double emulsions facilitate the transport of water and/or solutes from one aqueous phase to the other (depending on the direction of the osmotic pressure gradient) by forming reverse micelles or "holes" in the oil layer (see chapter 2). In the case of fat crystal-stabilised emulsions, on the other hand, the emulsifiers are fixed at the interface so that they form tight crystalline "shells" around the water droplets. It is expected that when such emulsions are incorporated into a double structure water/ solute transport can be much reduced or even prevented despite the presence of a hydrophilic surfactant. This chapter treats the incorporation of fat crystal-stabilised primary emulsions into double structures in order to test this hypothesis.

The sintered, solid nature of the crystalline "shells" surrounding primary emulsion water droplets makes them sensitive to high shear rates and temperature changes. Both could damage the structure of the "shells" and compromise emulsion stability. This creates the need for a "gentle" secondary emulsification step, i.e., one that does

not require high shear rates and where temperature increases are kept to a minimum. Double emulsions were produced for this purpose using two different processing techniques:

1) A standard rotor/stator (Silverson), where processing is kept to a minimum by operating at a medium speed for a relatively short time.

2) Membranes, which have the advantage that droplets are formed at the membrane pore and hence not broken up by the application of shear, thus making this a very "gentle" processing technique well-suited for the production of double emulsions

Although emulsions can be formed without the application of high shear, membrane emulsification has many drawbacks, including low throughput and high fouling rates (see chapter 2), which makes the use of more conventional emulsification techniques, such as rotor/ stator systems, more practical.

In the first part of this chapter, the two processing techniques are compared for their respective potentials for producing the fat crystal-stabilised double emulsions. The influence of various parameters on double emulsion stability, such as emulsifier type and operating parameters are also investigated.

The second part of this chapter focuses on double emulsions stabilised by Na-caseinate in W_2 produced using the rotor/ stator for the secondary emulsification step. The effect of different osmotic pressure gradients on stability against coalescence and the ability of the double emulsion to retain water/ solutes encapsulated within the primary aqueous phase was investigated.

5.2 Double Emulsion Formation

5.2.1 Double emulsions made using a rotor/ stator device

Fat crystal-stabilised 30:70 W_1:O primary emulsions were prepared as previously described in chapter 3. These emulsions contained 0.5 % monoglyceride and 1 % triglyceride (wt/wt) in the oil phase, and 1.6 % (wt/wt) KCl in the aqueous phase. These emulsions were shown to be stable for at least 3 months at 10 °C: Average droplet size, as measured using NMR, was 3.7 ± 0.3 µm and remained constant during this time (see Table 5.1).

Table 5.1: Comparison of W_1/O emulsion stability of emulsions stabilised by 1% monoglyceride and 1% tripalmitin in the oil phase and either 30 or 60% water

	30:70 W:O emulsion	60:40 W:O emulsion
after production	3.7 ± 0.3 µm	4.3 ± 0.4 µm
1 week	3.5 ± 0.3 µm	4.3 ± 0.4 µm
2 weeks	3.7 ± 0.3 µm	4.3 ± 0.5 µm
4 weeks	3.8 ± 0.3 µm	4.3 ± 0.5 µm

Double emulsions were then prepared using this primary emulsion or a blend of 50% (wt/wt) primary emulsion and 50% sunflower oil, and containing 1% Na-caseinate as the emulsifier in the W_2 aqueous phase. The continuous aqueous phase also contained 10% (wt/wt in W_2) glucose to match the osmotic pressure gradient between W_1 and W_2. The primary emulsion (or primary emulsion/ sunflower oil mixture) was added slowly to the aqueous phase while the Silverson apparatus was set at 3000 rpm for slow mixing of the two phases during addition. Once all the

primary emulsion had been added, the rotor speed was increased to 8000 rpm for three minutes to allow reduction of double emulsion globule size while not applying shear for too long to the sample. The sample was homogenised at this speed for 3 minutes while being cooled in an ice bath. The resulting emulsions were analysed for droplet size and emulsion stability against coalescence.

Double emulsions containing undiluted primary emulsion made using this procedure were not stable: coalescence of double emulsion globules occurred within 1 day. This was also the case if a primary emulsion was prepared that contained 15% (wt/wt) water. There are a number of explanations for this. One is the high viscosity of the primary emulsion due to the existence of a fat crystal network within the continuous oil phase. The high viscosity of the primary emulsion prevented good mixing conditions within the vessel so that clusters of primary emulsion were not incorporated into a double structure. Another reason is that poor mixing resulted in extended periods of high shear for some primary emulsion clusters. An elevated heat level in localised high shear regions may have melted and damaged the fat crystal netword surrounding primary emulsion water droplets.

Mixing the primary emulsion with sunflower oil prior to the secondary emulsification step reduced the viscosity of the primary emulsion by breaking up the fat crystal network in the continuous oil phase (though not that directly surrounding the water droplets, this continued to stabilise the emulsion, see Table 5.2) and hence allowed effective dispersion of the primary emulsion required for the creation of double emulsions. The double emulsion that was formed retained its structure for at least 6 weeks (see section 5.3).

NMR was used to measure the average droplet size of W_1 droplets before these were incorporated into a double structure. W_1 droplet size was measured again once the primary emulsion had been incorporated into the double structure by using microscopy micrographs taken immediately after the double emulsion had been prepared. An average droplet size of 3.5 ± 0.3 µm was measured using NMR. This was, given the different measurement techniques, in good agreement with the droplet size after incorporation into the double structure of 2.5 ± 0.8 µm (see section 5.3.3). Table 5.2 shows that the dilution of the primary emulsion with sunflower oil has no influence on the droplet size even after 4 weeks of storage.

Table 5.2: Influence of dilution with sunflower oil on 30:70 W_1:O primary emulsion droplet size (measured by NMR prior to incorporation into double structure)

Dilution ratio emulsion: sunflower oil	1:0	3:1	1:1	1:5
Droplet size after production	3.5 ± 0.3 µm	4.4 ± 0.3 µm	4.0 ± 0.3 µm	3.8 ± 0.3 µm
Droplet size after 4 weeks storage	2.5 ± 0.8 µm	3.8 ± 0.3 µm	3.9 ± 0.3 µm	4.2 ± 0.2 µm

These results indicate that the W_1 droplets remained intact during processing in the rotor/ stator device. The tight fat crystal networks surrounding the water droplets prevented coalesce and break-up of primary emulsion droplets.

5.2.2 Membrane emulsification

Membrane technology was used to produce double structures from primary emulsions stabilised by fat crystals. The same fat crystal-stabilised 30:70 W_1:O primary emulsions as described in the previous section were used.

The size of the primary emulsion water droplets required a membrane with relatively large pore size (~ 10 µm), so that droplets could pass the pores unhindered (the assumption being that solid “shells” prevent deformation of the water droplets). This would reduce damage to the droplet “shells” as well as blockage of the membrane pores.

5.2.2.1 SPG membranes in a cross-flow system

A 10 µm Shirasu Porous Glas (SPG) membrane was prepared and mounted in a cross-flow system as described in chapter 3. A 1 % Na-caseinate (wt/wt) and 10 % (wt/wt) glucose solution was used as the continuous aqueous phase, unless otherwise indicated. The primary emulsion was filled into the feed tank and the tank was pressurised to 0.5, 1 or 2 bar.

The high viscosity of the semi-solid emulsion required a high feed tank pressure in order make the emulsion flow through the pores. No double emulsion globules could be produced when the trans-membrane pressure was less than 1 bar. No primary emulsion passed the pores even after circulating the continuous phase across the outside of the membrane for 2 hours. When the pressure was increased to 2 bar the final product contained a large number of air bubbles, relatively few oil droplets and

almost no double globules. The air used to pressurise the feed tank channelled through the primary emulsion and was released through the pores. The primary emulsion was not pushed through the pores because pressure was released following the path of least resistance.

Furthermore, formation of double globules was hindered by the build-up of a fat crystal network around individual pores, which gradually reduced effective pore size. This process seemed to be exacerbated at higher pressures, as it was not possible to produce any double globules with trans-membrane pressures greater than 1 bar.

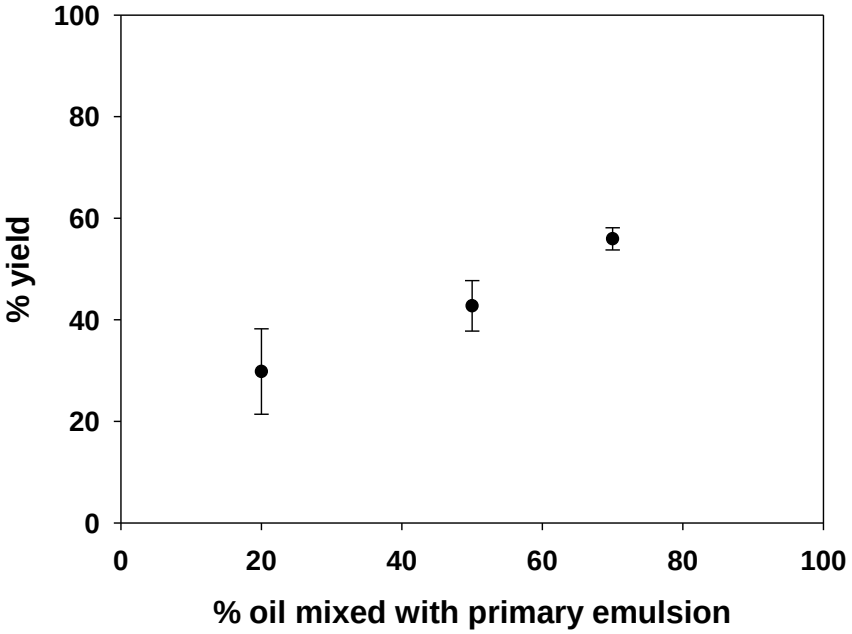

Figure 5.1: Influence of sunflower oil mixed with the primary emulsion prior to the passage through the membrane on the % yield (number of double globules containing primary emulsion droplets, measured by light microscopy)

In an attempt to prevent air channelling and pore fouling, the viscosity of the primary emulsion was reduced by adding sunflower oil (see Figure 5.1). For this purpose, the primary emulsion was mixed with the relevant amount of sunflower oil until the mixture was homogenous. A 20 % dilution (i.e., a mixture of 20 % sunflower oil and 80% primary emulsion) prevented air from channelling through the emulsion allowing double globules to be formed at the membrane surface. However, the membrane still fouled rapidly due to a build-up of fat crystals around the pores. As a result the number of globules containing water droplets ("yield") was below 40 % of the total number of globules (Figure 5.1). As the primary emulsion was mixed with increasing amounts of sunflower oil, the "yield" increased. When the primary emulsion mixture contained 30 % W_1/O emulsion and 70 % sunflower oil, the number of globules containing at least one water droplet was increased to 56 %. However, with this concentration of sunflower oil the total amount of encapsulated water is 1.8 %. Each double globule therefore contained only a few water droplets.

Despite the addition of large quantities of sunflower oil the membranes still fouled rapidly and a maximum double emulsion concentration of only 1:20 $(W_1/O):W_2$ was achieved when sunflower oil was added to the primary emulsion in a ratio 1:1. More concentrated double emulsions would have required a longer run time or a faster throughput. The throughput depends on the trans-membrane pressure, which was fixed at 1 bar for reasons explained above. The run time was also limited: After around 20 minutes of running the membrane emulsification double globules were no longer produced. This was because the membrane pore size was effectively reduced as fat crystals aggregated around the pore openings, gradually preventing the passage of primary emulsion droplets.

5.2.2.1.1 Membrane processing parameters

Various processing parameters were tested in an attempt to increase double globule “yield”. Feed tank pressure remained fixed at 1 bar, as this was the minimum pressure required for the primary emulsion to be pushed through the membrane. A 1% (wt/wt) gelatin (80 g bloom) and 10 (wt/wt) % glucose solution was used as the continuous aqueous phase. The glucose was added to match the osmotic pressure between internal and external aqueous phase. Gelatin was used as an emulsifier. At a concentration of 1 %, this type of gelatin did not gel, but increased the viscosity of the aqueous phase slightly, which might to aid the formation of smaller droplets at the membrane surface. The cross-flow pressure was varied by changing the speed of the pump used to circulate the continuous aqueous phase, as described in chapter 3. The droplet size and percentage of globules containing water droplets were evaluated using light microscopy.

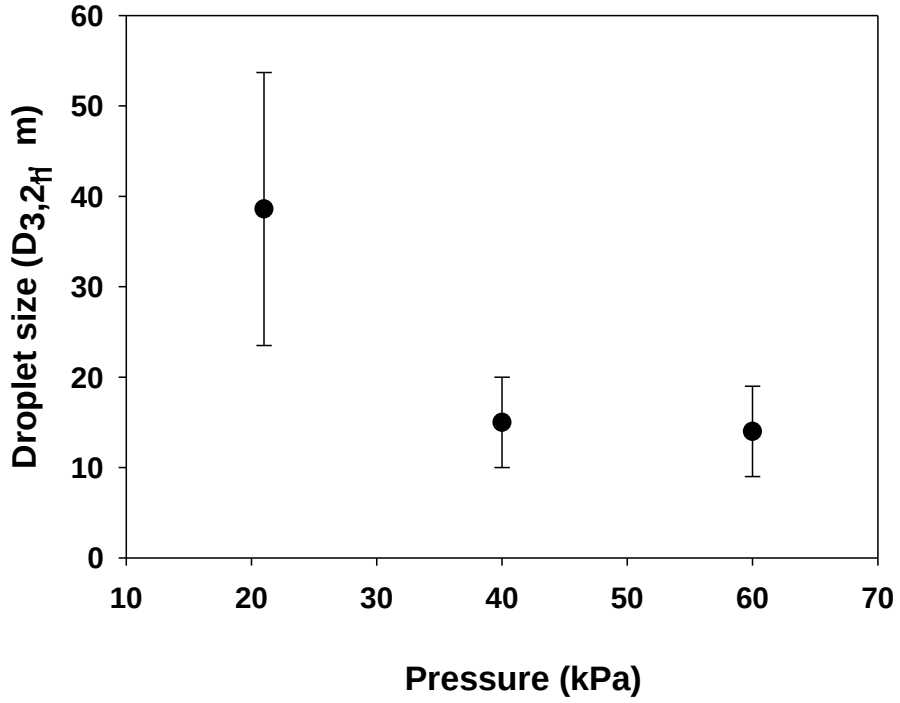

Figure 5.2: Influence of cross-flow pressure on droplet size and distribution. The breadth of the droplet size distribution is indicated by the bars. Droplet size measured by light microscopy.

Increasing the cross-flow pressure resulted in a decrease in double emulsion globule size, as expected and previously reported (Joscelyne & Tragardh 1999). A low cross-flow pressure (21 kPa) caused the formation of large double globules. The average globule size was 4 x the pore diameter (Figure 5.2). At this pressure individual double globules could grow at the membrane surface. Their large size led to coalescence with globules forming at neighbouring pores before detachment from the membrane surface. This was in line with results of previous research (Joscelyne & Tragardh 1999).

From this data it can be seen that as the cross flow pressure was increased to 40 kPa, globules were removed from the membrane surface before they could coalesce

with neighbouring droplets. This gave an average droplet size that was close to the pore size diameter and a narrower distribution. Even higher pressure (60 kPa) therefore did not result in a further decrease of globule size.

However, at 60 kPa, the double emulsion “yield” (as defined in section 5.2.2.1) was much reduced, resulting in many empty oil droplets in the samples studied (Figure 5.3). It seems as if the high pressure may have prevented the incorporation of aqueous phase droplets by not giving them much time to form at the membrane pore.

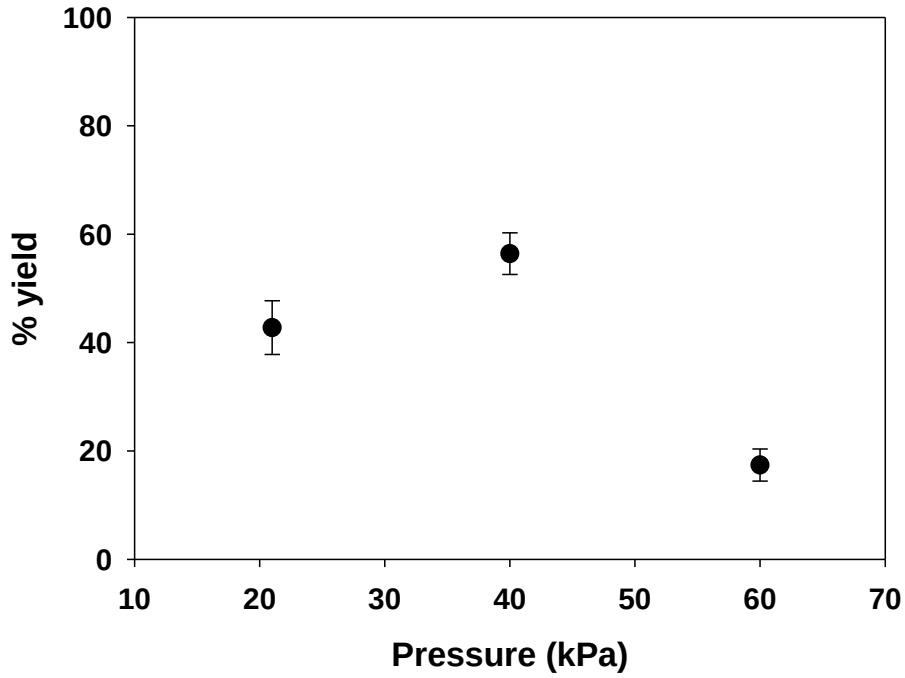

Figure 5.3: Comparison of yield of double emulsions containing 1% gelatin made at different cross flow pressures

In summary, the cross-flow pressure seems to influence the quality of double emulsions produced using membrane technology. However, in the case of fat crystal

stabilised primary emulsions the main problem remains achieving an acceptable throughput without the fouling of membrane pores. The throughput cannot be improved by any particular setting of cross-flow pressure.

5.2.2.2 Laser-drilled steel membranes

The previous sections highlighted that one of the major obstacles to the creation of fat crystal-stabilised double emulsions using membranes is the fouling rate. In an attempt to increase the number of globules containing water droplets as well as the throughput before fouling occurs membranes with larger pore size were sourced and tested. SPG membranes were not available in larger pore sizes. Custom-made laser drilled stainless steel tubes made by Laser Micromachining Limited (Denbighshire, UK) were used instead. 2 sizes were tested: 15-20 μm and 80 μm diameter.

5.2.2.2.1 15-20 μm pore size in a cross-flow system

The 15-20 μm drilled tube was tested using the cross-flow system. Only 1/3rd of the tube area contained drilled holes, so that this tube contained significantly fewer pores than the SPG membrane. The parameters used in this experiment (tank pressure, primary emulsion and continuous aqueous phase composition) were identical to those described in section 5.2.2.1. The cross-flow pressure was set at 40 kPa, determined previously to be the optimum setting for double emulsion production.

It was not possible to produce double emulsions using this set-up. The relatively small area available for double emulsion production fouled rapidly, so that, despite mixing the primary emulsion with equal parts sunflower oil, the product contained few oil droplets, no double globules and a significant amount of air bubbles. The increase

of pore size (diameter = 15 - 20 μm as opposed to 10 μm for the SPG membrane) therefore seemed to have no effect on the fouling rate.

The pressure-controlled feed system caused a large number of air bubbles in the product and prevented primary emulsion from being pressed through the pores. Replacing the pressurised feed tank with a volume-controlled feed system (such as a syringe pump) might improve results.

5.2.2.2.2 80 μm membranes in a volume-controlled rotating membrane system

Following these unsatisfactory results, a membrane with pore diameter of 80 μm was tested using a volume controlled membrane set-up. The membrane was rotating, which means that the cross-flow pressure is supplied by the rotation of the membrane. This is in contrast to the cross-flow set-up, where the external aqueous phase flows across a stationary membrane unit. The system used is described in Vladisavljevic & Williams (2006). The primary emulsion (containing 30 % water and 80 % oil phase) was the same as described in section 5.2.2.1 and was used without adding any sunflower oil. The continuous aqueous phase contained 4% Na-caseinate and 10 % glucose. Na-caseinate was used as an emulsifier because double emulsions were produced using this protein using rotor/stator technology (see section 5.2.1) could be produced. The membrane unit was rotated at 1500 rpm to produce double globules, and the primary emulsion was pushed through the membrane at a rate of 1 ml/min.

Double emulsions containing globules of uniform size distribution were produced (Figure 5.4). Although fouling remained a problem, each production run could last up

to 15 minutes before a significant reduction in droplet size and double emulsion yield was observed. However, the double emulsions were unstable due to the large globule size (~ 150 µm). Unfortunately, tests on the volume-controlled feed system using smaller pore diameters could not be performed, because no smaller pore size membrane was available in the volume-controlled set-up. The results nevertheless showed the potential of using a volume-controlled feed system for overcoming some of the problems described for a pressure-controlled one.

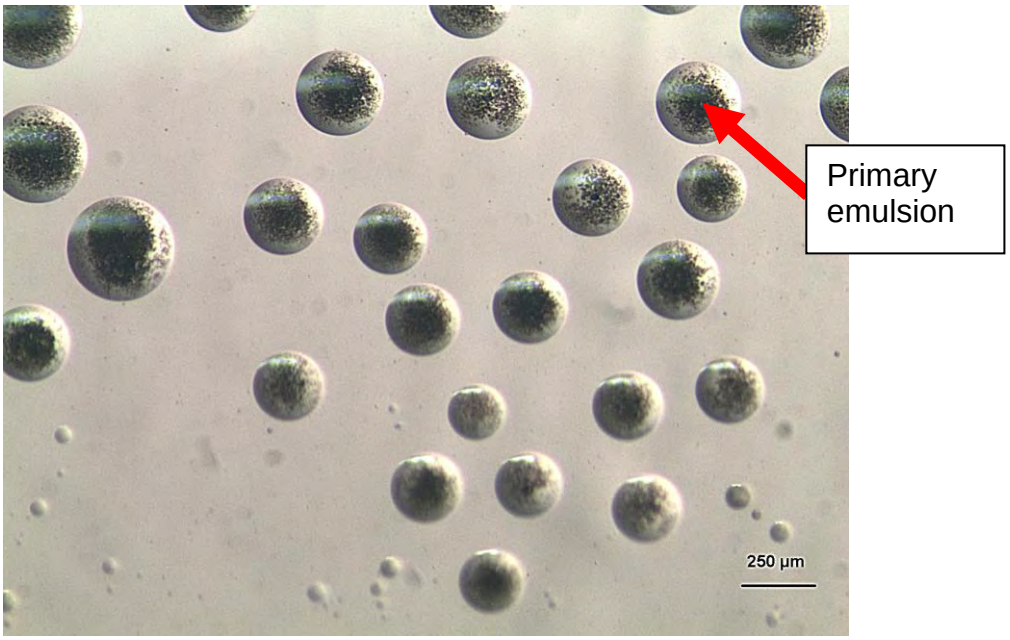

Figure 5.4: Double emulsion produced in a laser drilled stainless steel membrane (pore size = 80 µm); volume controlled feed system

Generally speaking, membrane emulsification cannot be ignored as a promising technique for the production of double emulsions mainly because it promises the creation of evenly-sized globules in a “gentle” manner. However, rapid membrane fouling and concomitant poor control over most processing parameters indicate that

membrane technology is unsuitable for the production of double emulsions containing fat crystals.

5.2.3 Comparison of rotor/ stator and membrane emulsification

The effectiveness of the rotor/ stator technique over membrane emulsification is demonstrated by directly comparing production of double emulsions with the same compositions using either of the two techniques. Double emulsions were produced using either the 10 µm SPG membranes in cross-flow or the rotor/ stator system in the same manner as was described in previous sections. The primary emulsion composition remained identical to the one described previously. 1% Na-caseinate was used as the external emulsifier, and the continuous aqueous phase also contained 10 % glucose to match the osmotic pressure between the two aqueous phases.

Table 5.3: Comparison of double emulsions produced using membranes or a rotor/ stator. Globule size determined using light microscopy.

	Average droplet size ($D_{3,2}$), µm		"yield" (%)	
	Membrane	Rotor/ Stator	Membrane	Rotor/ Stator
After production	22 ± 8	23 ± 8	74.1 ± 0.2	98
After 1 week	35 ± 11	31 ± 10	42.9 ± 3.4	98

When 1 % Na-caseinate was dispersed in the continuous aqueous phase (containing 10 % glucose to match the osmotic pressure) the samples retained their double structure for extended periods of time. This was regardless of the method used for

secondary emulsification (Table 5.3). Figure 5.5 shows such a formulation containing 1 % Na-caseinate in the continuous W_2 aqueous phase after production and after 10 days' storage, produced using membrane emulsification. The double globules were not tightly packed with aqueous droplets: Due to the limitations of membrane emulsification, only around 74 % of globules contained at least some water droplets. After 1 weeks' storage at 10 °C more than half of these globules had retained their water droplets.

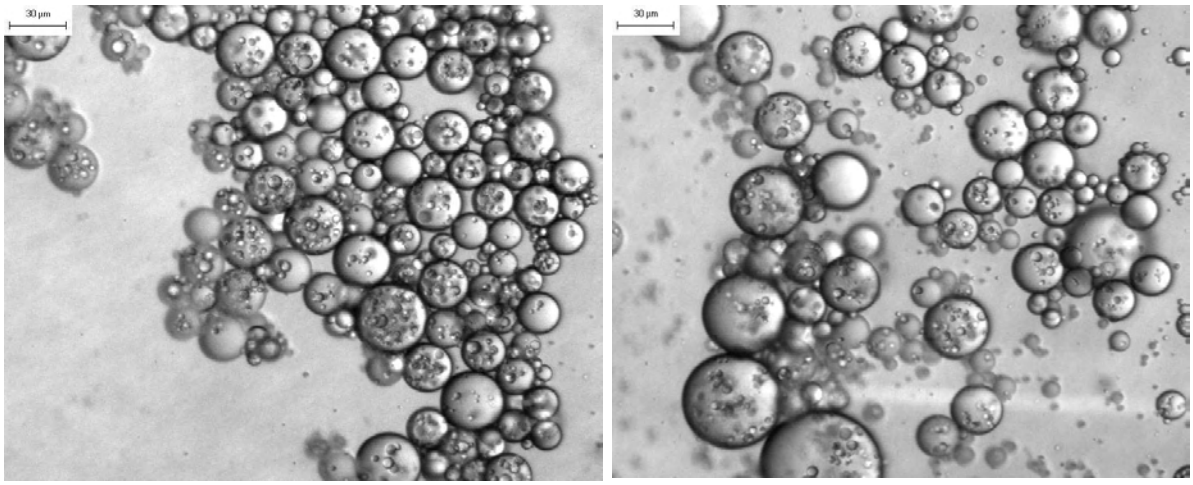

Figure 5.5: Double emulsion containing 1% Na-caseinate in the W_2 phase; the primary emulsion contains 30 % water (and is subsequently diluted by 50 % sunflower oil).

When the samples were produced using the rotor/ stator almost all globules contained water droplets, which were tightly packed, and retained these for at least one week (see Table 5.3). Despite the application of high shear, which could potentially damage the crystalline "shells" surrounding primary emulsion water droplets, these emulsions showed a slightly higher stability than those produced using the "gentle" membrane emulsification method. However, this difference might

be down to the light microscopy method used to determine the droplet size. Having fewer droplets in any given sample to analyse increases the potential for error in the measurement technique. Having only few droplets to analyse in each sample is a consequence of the problems associated with the processing of the fat crystal-stabilised primary emulsion in the membrane unit, which was discussed in section 5.2.2.1.

5.2.4 Effect of monomeric surfactants on double emulsions

The following section investigates the effect of using different secondary emulsifiers on double emulsion stability. The primary emulsion in all cases was a 30:70 W_1:O emulsion described in section 5.2.1. The primary emulsion was mixed with equal parts of sunflower oil prior to the secondary emulsification step. The secondary emulsifiers were small-molecule surfactants such as Tween 20 and SDS.

Small-molecule surfactants were added to the W_2 aqueous phase in order to investigate their effectiveness in stabilising double structures containing fat crystals. Emulsions were produced using 10 µm SPG membranes in a cross-flow set-up as described in section 5.2.2.1. The cross-flow pressure was set at 40 kPa, and the tank pressure at 1 bar. An example of a double emulsion containing 0.5 % Tween 20 in the W_2 phase is depicted in Figure 5.6. Although the sample contained a significant proportion of empty globules, a result of the membrane fouling as discussed in section 5.2.2, double globules can be clearly identified. These had an average size of 17±6µm and an average of 37 ± 3 % contained at least one water droplet (see Table 5.4).

Table 5.4: Droplet size (measured using light microscopy) and yield of membrane-produced double emulsions containing SDS or Tween 20

External Emulsifier	$D_{3,2}$ (μm)	% "yield"
0.5 % SDS	15 ± 5	32 ± 5
0.5 % Tween 20	17 ± 6	37 ± 3

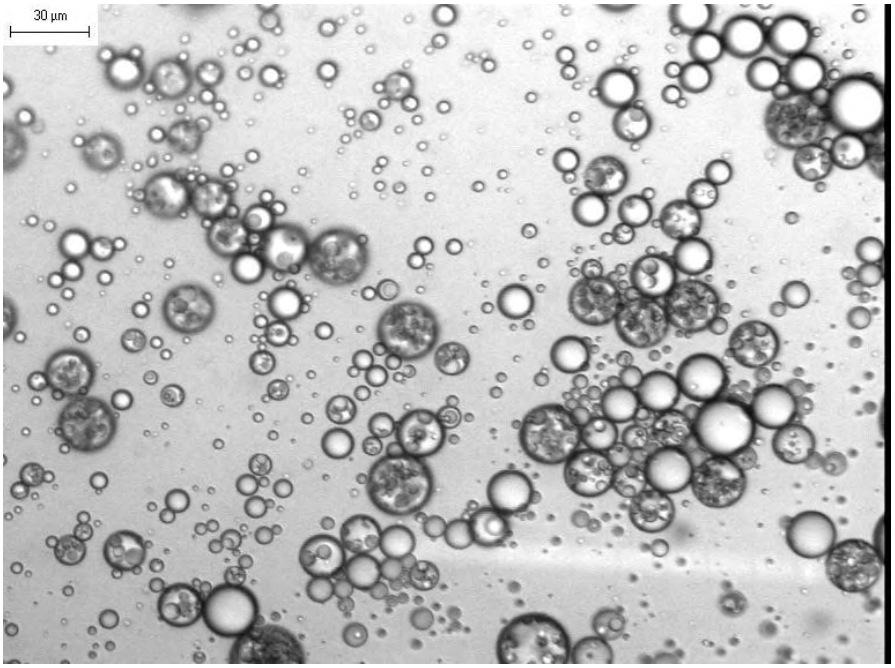

Figure 5.6: 0.5 % Tween 20 in W_2 phase; primary emulsion mixed with equal parts sunflower oil, after production

When the sample was stored for 1 day at 10 °C double globules were no longer detected. The sample had separated into two phases: the top ("cream") phase contained primary emulsion water droplets no longer incorporated into double globules, while the bottom phase contained mostly W_2 aqueous phase and some very small oil droplets (Figure 5.7). The concentration of Tween 20 in the secondary

aqueous phase did not seem to influence this phenomenon: it occurred at 0.1, 0.5, 1 and 2 % concentration. The formulation behaved similarly when a different small molecule emulsifier, SDS, was used.

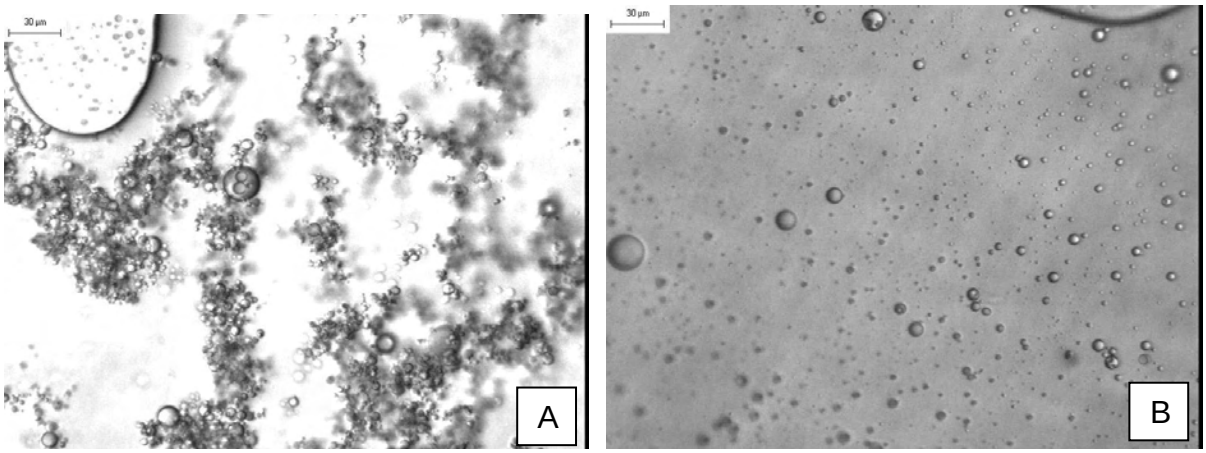

Figure 5.7: 0.5 % Tween 20 in W_2 aqueous phase, after 1 days storage at 10 °C. The emulsion has phase separated into a cream layer ("A"), containing primary emulsion water droplets, and a serum phase ("B"), containing small empty oil droplets.

The primary emulsion droplets remained intact even though they were no longer part of the double structure. As was discussed in chapter 2 double emulsions usually lose their double structure because of emulsifier migration between the interfaces. This destabilises the primary emulsion droplets stabilised by proteins or monomeric surfactants. In unstable double structures, primary emulsion droplets therefore usually disappear (see chapter 2).

Figure 5.7 shows that fat the primary emulsion droplets remain intact when they are incorporated into a double structure. The stability of the primary emulsion does not seem to be compromised by the existence of a hydrophilic emulsifier in W_2, so it is likely that fat crystals continue to stabilise the primary emulsion. The reason for the

instability of the double structure must lie with the small molecule surfactant used as hydrophilic emulsifier.

It is the presence of fat crystals that is the likely cause for dissolution of the double structure. While most fat crystals are likely to be located at the interface, where they have sintered to form smooth crystal “shells”, a number of crystals are inevitably present in the bulk oil phase where they may also form networks. Dilution with sunflower oil and the subsequent secondary emulsification step may break up this network. However, as a double emulsion sample is aged, “free” crystals tend to aggregate into clusters. Such crystal aggregates may protrude from the globule surface to the continuous W_2 phase. As two droplets approach each other, the protruding crystals can “pierce” the thin interfacial film created by the monolayer of small-molecule emulsifiers. As the crystals are wetted preferentially by oil the two droplets will subsequently remain aggregated. This mechanism has been termed “partial coalescence” (Boode & Walstra 1993) in simple O/W emulsions.

Small-molecule emulsifiers cannot provide an interfacial layer thick enough to prevent this process. This leads to extensive coalescence between oil globules and subsequent loss of the double structure. Primary emulsion water droplets, however, remain stabilised by the crystal shells leading to phase separation as schematically depicted in Figure 5.8.

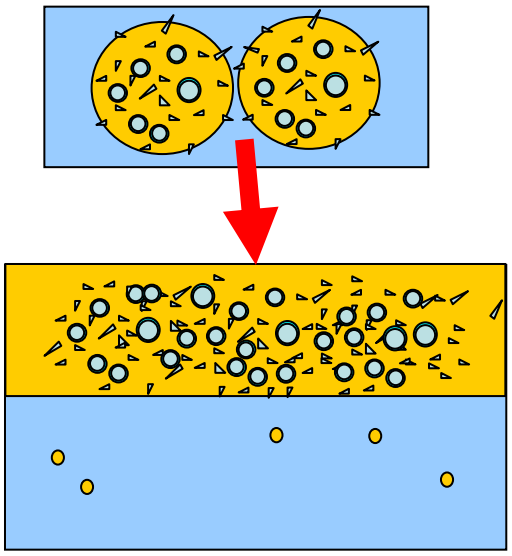

Figure 5.8: Schematic of how partial coalescence leads to phase separation of primary emulsion and secondary aqueous phase

These results demonstrate that small-molecule surfactants are not suitable for use as secondary emulsifiers in double emulsions containing fat crystals because they cannot keep globules separated over sufficiently long distances to prevent contact of the globules with protruding fat crystals.

5.2.5 Use of further proteins as the secondary emulsifier

Further experiments were performed on formulations containing different proteins as secondary emulsifiers, in the form of Bovine Serum Albumin (BSA) and Whey protein isolate (WPI), using the rotor/ stator for the secondary emulsification step. Typical concentrations of the proteins were chosen based on reports that these have previously yielded stable emulsions (Benichou, et al., 2007b; Rangsansarid & Fukada 2007; Waniska et al., 1981). Osmotic pressure was matched in all

formulations by adding 10 % glucose (wt/wt) to the W_2 aqueous phase. The double emulsions were prepared by mixing the primary emulsion with the continuous aqueous phase and shearing at 8000 rpm for 3 minutes while cooling the sample in an ice bath. Results were compared with a double emulsion containing 1 % Na-caseinate produced using the same method.

Initially, emulsions stabilised by either BSA or WPI showed a similar average droplet size to those stabilised by Na-caseinate (Table 5.5). Initial globule size seems to be largely dependent on hydrodynamic conditions in the rotor/ stator.

Differences in stability became apparent when the different formulations were stored for one week at 10 °C. Formulations containing BSA were unstable. The primary emulsion aggregated so that the double structure was no longer preserved in the same way as when monomeric surfactants were used as secondary emulsifiers. When WPI was used as secondary surfactant the double structure was largely maintained. However, secondary emulsion droplet size nearly doubled and the breadth of the droplet size distribution increased. The use of 1 % Na-caseinate as secondary emulsifier yielded the most stable formulations.

Table 5.5: Droplet size of emulsions stabilised by various proteins (concentrations are wt/wt in W_2), measured by light microscopy

$D_{3,2}$ (µm)	After production	After 1 week
0.2 % BSA	21 ± 7	Not stable
4 % WPI	27 ± 9	41 ± 12
1 % Na-caseinate	23 ± 8	31 ± 10

The reason for the difference in the stability of the double emulsion formulations depending on the choice of protein in the W_2 aqueous phase could be related to the structure of the protein molecules. Their structure influences the way that proteins adsorb at the interface. The major constituents of Na-caseinate are β-casein and α_{s1}-casein. They adsorb at the interface in a loop-and tail conformation, giving an extended interfacial layer that is up to 10 nm thick. WPI and BSA, on the other hand, are (or, in the case of WPI, are a mixture of) globular proteins that adsorb in a dense but, at 2 nm, comparatively thin monolayer (Dickinson, 2010a). In simple O/W emulsions, both are usually good emulsifiers. However, it has been shown that in a system containing fat crystals stabilising the primary emulsion, neither WPI nor BSA are able to stabilise the secondary interface.

This section has shown that rotor/stator technology is suitable for the production of double emulsions containing fat crystal stabilised primary emulsions. It was also shown that the choice of secondary emulsifier is an important parameter determining whether the double emulsions are stable structures. The investigation into the suitability of various commonly-used food-grade emulsifiers in double emulsions containing fat crystal-stabilised primary emulsions has shown that not all proteins are equally suitable for stabilisation of the secondary interface. One of the emulsifiers with good results was Na-caseinate. Further investigations were performed with this protein as secondary emulsifier as its use is common in the food industry and its effects on (simple) emulsion stability have been well documented.

5.3 Double emulsion stability and osmotic pressure gradients

The influence of the osmotic pressure gradient between the two aqueous phases on the stability of double emulsions has been discussed in chapter 2. The ability of double emulsions to retain their stability despite the existence of an osmotic pressure gradient is important because in many food products, sugar, salt and other solutes may be added to the continuous water phase. The effect of applied osmotic pressure gradients on double emulsion stability is examined in the next section.

Double emulsions containing 1 % Na-caseinate as secondary emulsifier and 20 % W_1/O emulsion were produced using the rotor/ stator system in order to investigate the influence of osmotic pressure gradients on emulsion stability. The W_1/O emulsion was the same as described in section 5.2.1. It was mixed with equal amounts of sunflower oil prior to the secondary emulsification step. The presence of 1.6 % KCl in the W_1 aqueous phase caused an osmotic pressure gradient that was controlled by adding different concentrations of salt (NaCl) or sugar (glucose) to the continuous W_2 phase.

Several formulations of double emulsions were prepared to give different osmotic pressure gradients ($\Delta\pi$) between W_1 and W_2 as listed in Table 5.6. These emulsions were stored at 10 °C for around 6 weeks in order to investigate their ability to retain double structure and resist coalescence. In order to assess the stability of these formulations, light microscopy and SEM were used to measure coalescence of oil globules as well as of internal W_1 droplets. The ability of W_1 droplets to retain a solute, KCl, was measured using conductivity experiments. The method for measuring the conductivity of double emulsion samples was described in chapter 3.

Table 5.6: Directions of osmotic pressure gradiets (Δπ): A positive Δπ indicates the concentration of solute is greater in W_1 than in the continuous W_2 aqueous phase

Osmotic pressure gradient (Δπ)	Molar Concentration of solute	Concentration [NaCl] in W_2	Concentration [glucose] in W_2	Preferential transport of water
11 atm	$W_1 > W_2$	0 %	0 %	Into droplets
5.5 atm	$W_1 > W_2$	1.3 %	4 %	Into droplets
0 atm	$W_1 = W_2$	2.6 %	8 %	None
-11 atm	$W_1 < W_2$	5.2 %	16 %	Out of droplets

All formulations had an initial double globule size of approximately 26 ± 8 μm. This size was independent of whether salt or sugar was present in the W_2 aqueous phase (Table 5.7), and not dependent on the osmotic pressure gradient. This demonstrates that the initial size distribution is determined by the processing conditions, which were the same for all formulations. The relatively large globule size and wide size distribution were the result of the short shearing time. Processing was kept to a minimum in order to preserve the integrity of the fat crystal network surrounding W_1 droplets.

Table 5.7: Globule size (μm) of various double emulsion formulations, measured by light microscopy. σ indicates the breadth of the droplet size distribution (1 standard deviation from the $D_{3,2}$).

Δπ (atm)	Δπ regulated using	after preparation		1 week		4 weeks		6 weeks	
		$D_{3,2\ (outer)}$	σ	$D_{3,2}$ (outer)	σ	$D_{3,2}$ (outer)	σ	$D_{3,2\ (outer)}$	σ
0	glucose	23	8	31	10	42	13	46	14
5.5	glucose	27	9	49	14	20	6	41	10
-11	glucose	25	8	26	9	32	10	25	7
0	NaCl	25	9	35	8	34	11	44	12
5.5	NaCl	31	10	34	10	53	15	45	13
-11	NaCl	31	10	37	12	39	12	37	12

5.3.1 Double emulsion stability – no osmotic pressure gradient

When NaCl or glucose was used to match the osmotic pressure in W_1 and W_2 the double structure was retained for a period of at least 6 weeks. However, creaming of the emulsions was not prevented in this formulation. The close proximity of double emulsion globules in the cream layer resulted in coalescence between double emulsion globules, with average globule size approximately doubling after 1 months' storage at 10 °C (Figure 5.9).

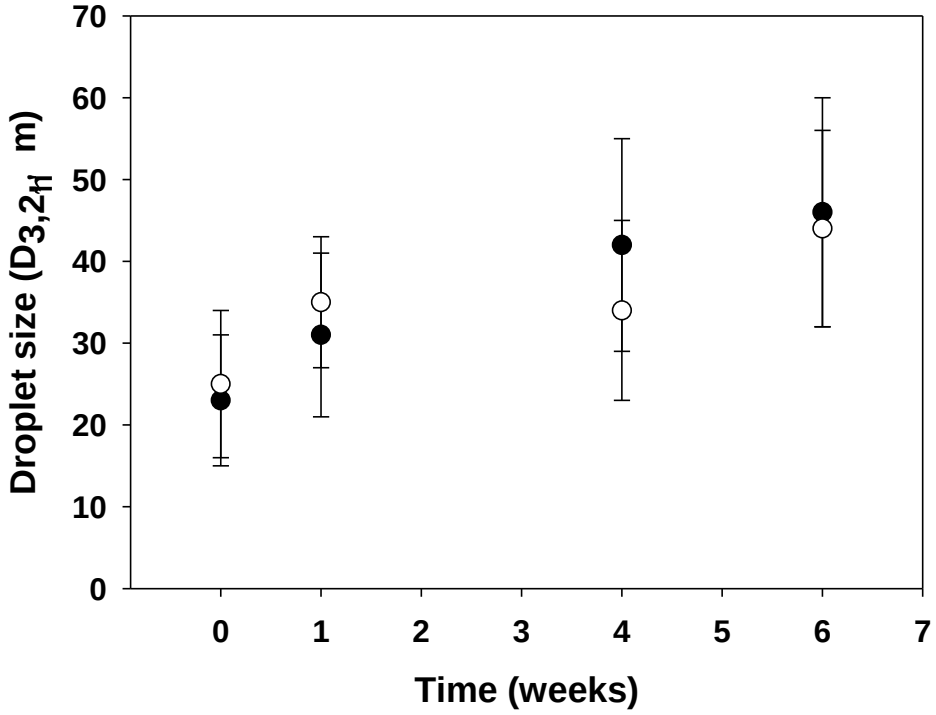

Figure 5.9: Double emulsion droplet sizes, Δπ = 0, measured using light microscopy. Osmotic pressure is matched with NaCl (open symbols) or glucose (filled symbols). The bars indicate the breadth of distribution.

The coalescence observed in these double emulsion formulations may have been exacerbated by the presence of fat crystals in the oil phase. Although most fat crystals are located at the W_1/O interface, where they form smooth “shells”, some are invariably present in the oil phase, as discussed in section 5.2.4. Several authors have reported on the destabilising effect of fat crystals in Na-caseinate stabilised O/W emulsions (Davies et al., 2000; van Boekel & Walstra, 1981). It is therefore possible that coalescence is enhanced by the presence of “free” fat crystals that can “pierce” interfaces of neighbouring droplets present in close proximity due to creaming.

The double emulsions were visualised using cryo-SEM in order to gain a better understanding of their microstructure. Figure 5.10 shows SEM micrographs of emulsions where the osmotic pressure has been matched using NaCl in the W_2 phase. Multiple W_1 primary emulsion droplets can be seen inside double emulsion globules (~15-20 μm in diameter). It seems as if the water droplets have largely remained intact during the secondary emulsification step demonstrates that the fat crystal network surrounding the water droplets can resist the high shear rates (~20,000 – 100,000 s^{-1}) experienced in the rotor/ stator mixer (Atiemo-Obeng & Calabrese, 2004).

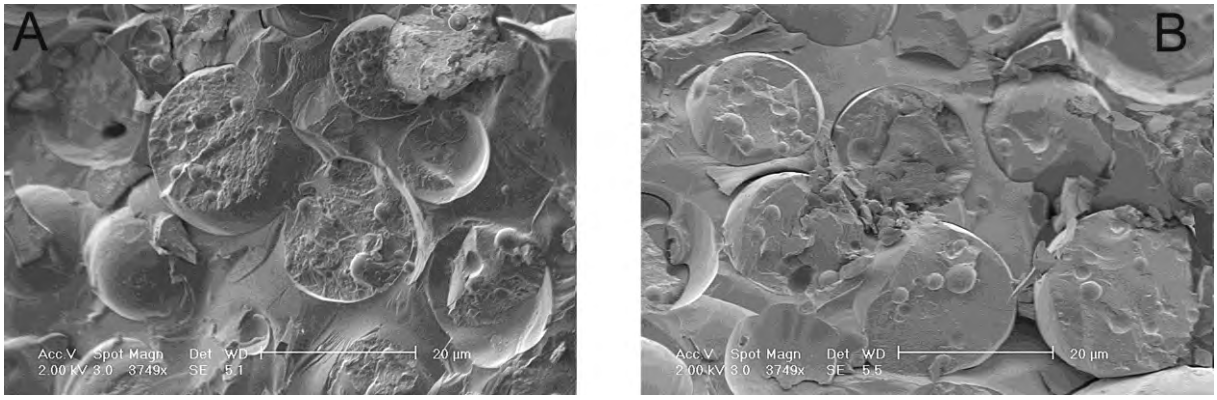

Figure 5.10: SEM micrographs of a double emulsion where Δπ = 0 (matched with NaCl in W_2), after production (A) and after 1 week (B). Little change in droplet size and globule size is observed. Scale bar = 20 μm

5.3.2 Influence of osmotic pressure gradient (Δπ) on double emulsion stability

Having established that double emulsions containing fat crystals and Na-caseinate as secondary emulsifier were stable for around 6 weeks with respect to the retention

of the double structure when the osmotic pressure was matched between the two aqueous phases, the role of the osmotic pressure gradient was analysed in more detail. Various concentrations of either salt (NaCl) or glucose were added to the W_2 aqueous phase in order to obtain selected positive or negative osmotic pressure gradients, as listed in Table 5.6. All other formulation parameters remained the same as described in the previous section.

In this work, a positive $\Delta\pi$ indicates the concentration of solute is greater in W_1 than in the continuous W_2 aqueous phase. In double emulsions this usually results in water being transported preferentially into the W_1 droplet, so that pressure on the interfacial "shell" is exerted from the *inside* of a W_1 droplet by an increasing volume of encapsulated water. A negative $\Delta\pi$, on the other hand, indicates that the concentration of solute is greater in the continuous W_2 phase. In such a system, preferential water transport occurs from W_1 to W_2 so that pressure on the "shell" acts on the *outside* of the W_1 droplets.

In double emulsions containing fat crystals, the stability of double globules against coalescence depended on the osmotic pressure gradient (Table 5.7). Double emulsion structure was not retained in formulations with large positive $\Delta\pi$ (e.g. 11 atm). Within one day the double emulsion had separated into two distinct phases (Figure 5.11): the "cream" layer consisting of primary emulsion droplets that were intact but no longer incorporated into a double structure, and the serum phase consisting mainly of W_2 aqueous phase. The same phenomenon was observed when monomeric emulsifiers were used as secondary stabilisers (see section 5.3).

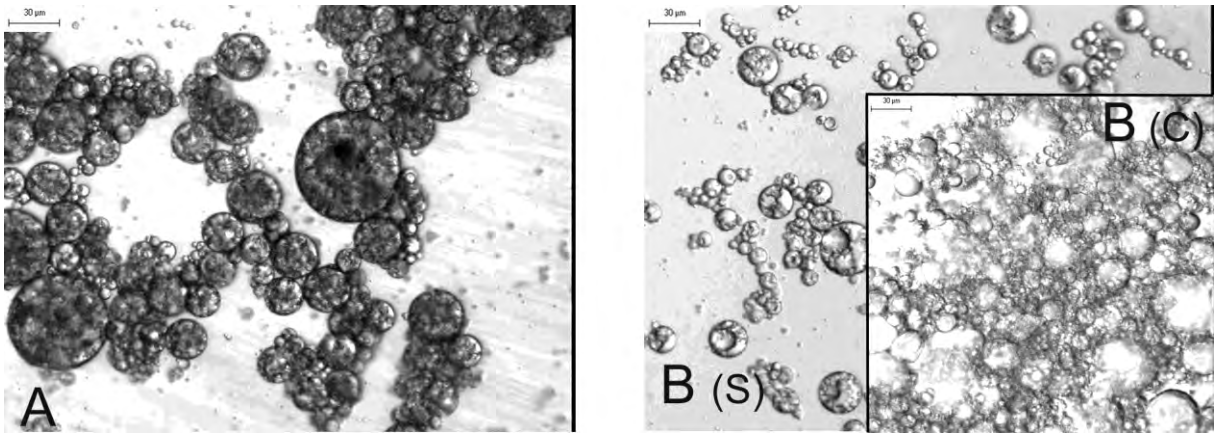

Figure 5.11: Double emulsion, Δπ=11atm, after production (A) and after 6 weeks (B). In (B), most primary emulsion has flocculated, leaving only a few filled double emulsion globules in the serum phase (S), while the cream phase (C) consists of partly swollen primary emulsion droplets. Scale bar = 30 µm

The large positive osmotic pressure gradient (+11 atm) caused crystal fragments to protrude from previously smooth crystal shells surrounding primary emulsion droplets, as discussed in detail in section 5.4.3. These protruding crystals increased the coalescence events between neighbouring double emulsion globules, as discussed in section 5.3.

When the osmotic pressure gradient was reduced to 5.5 atm, double emulsion globules became more stable to coalescence compared to formulations where Δπ= 11atm. However, they were also less stable than samples with no osmotic pressure gradient. Although double structure was largely retained for about 4 weeks, some large aggregates of primary emulsion droplets formed within this time-span. These aggregates were no longer incorporated into the double structure. Smaller double emulsion globules, on the other hand, were able to retain the double structure. This is reflected in the broad globule size distribution, as illustrated in Table 5.7. The rate of

coalescence in formulations containing glucose or NaCl seems to diverge after 1 week of storage: In the formulation containing glucose, globule size diminishes sharply after 4 weeks of storage, while in the one containing NaCl the globule size increases and globule size distribution exceeds 20 μm. A possible explanation for the apparent divergence in globule size when NaCl or glucose was used to regulate the osmotic pressure is the measurement method (manual counting and sizing of globules in light microscopy micrographs). Large aggregates formed in both formulations which were distinctly visible in some of the images studied. Although measurements were made using several micrographs of several different samples, accurately capturing the entire range of globule sizes would have required the evaluation of many more micrographs. In light of the results described in section 5.3.2 (stability induced by negative osmotic pressure gradients), further study of this issue was not deemed necessary for the purposes of this study. Lack of stability was encountered when osmotic pressure was positive irrespective of whether NaCl or glucose had been used. As in formulations where $\Delta\pi$ = 11atm, the destabilisation of the double structure by the formation of primary emulsion aggregates can be linked to crystal protrusions from the W_1 droplets. This will be discussed in detail in the next section (5.3).

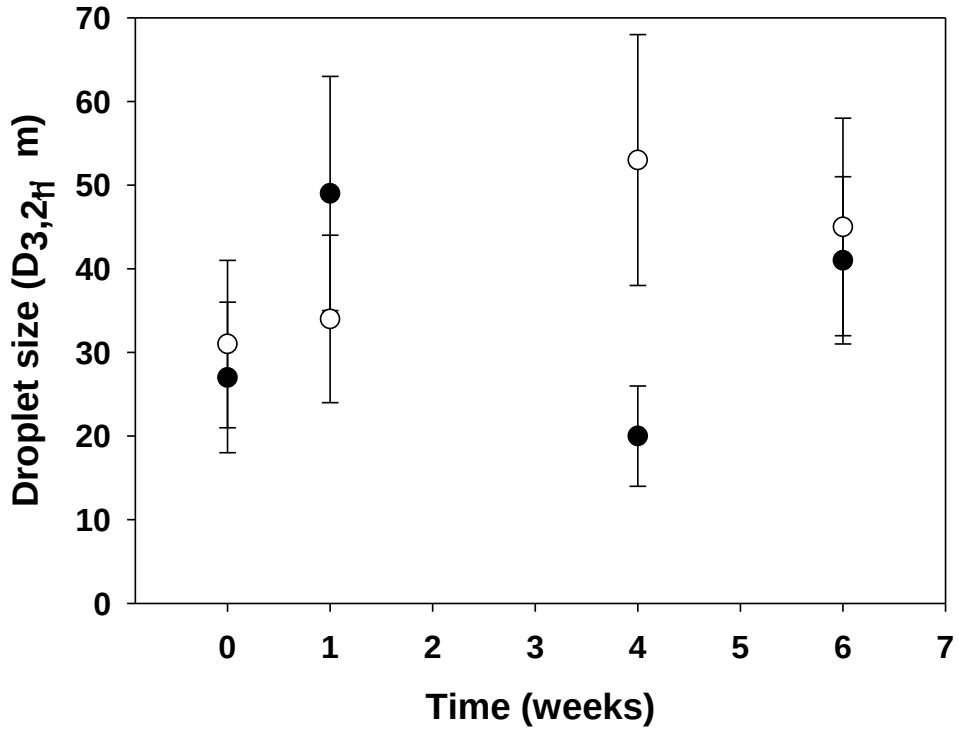

Figure 5.12: Globule size of double emulsions where Δπ = 5.5 atm, determined using light microscopy. Osmotic pressure is matched with NaCl (open symbols) or glucose (filled symbols)

The most stable double emulsions according to Table 5.7 had a negative osmotic pressure gradient (Δπ = – 11 atm). These formulations showed a reduced rate of coalescence of double globules compared to all other samples. A negative osmotic pressure gradient allows for the “shells” surrounding primary emulsion droplets to remain smooth. The number of protruding crystals is thus limited to the number of “free” crystals in the oil phase, so that coalescence between globules is reduced. This will be discussed in detail in section 5.4.3.

These results have shown that double emulsion stability is dependent on the magnitude and direction of the osmotic pressure gradient between W_1 and W_2. Stability is maintained when a large negative $\Delta\pi$ is applied between the two phases. On the other hand, a large positive $\Delta\pi$ destabilises the double emulsion.

5.3.3 Influence of osmotic pressure gradient on primary emulsion stability

To answer the question why the direction of osmotic pressure gradient had an influence on double emulsion stability, the effect of osmotic pressure on the primary emulsion droplets once these were incorporated into a double structure was studied. In order to do this, the W_1 droplet size was measured within the double globules using light microscopy micrographs, and the structure observed using SEM. Figure 5.13 shows that the size of primary W_1 droplets was affected by the osmotic pressure gradient in formulations with a positive osmotic pressure gradient.

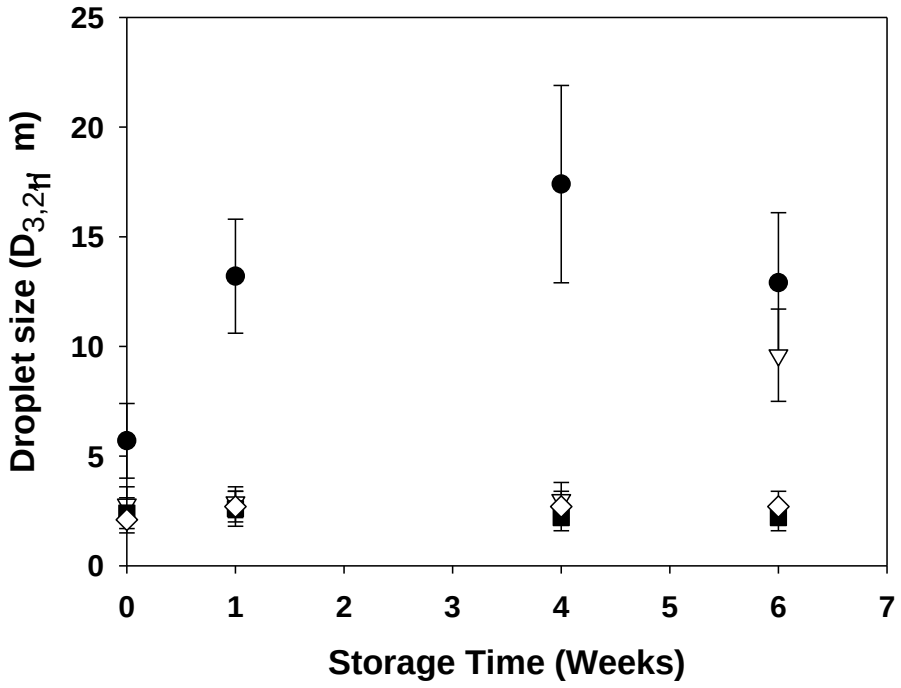

Figure 5.13: *Internal* W_1 droplet sizes in various double emulsion formulations stored at 10 °C. The W_1/O emulsion was stabilised by 0.5% monoglycerides and 1% tripalmitin, and contained 30% water (subsequently diluted with sunflower oil). The double emulsion was stabilised by 1% Na-caseinate and glucose was used to regulate the osmotic pressure gradient. ●=11atm; ▽= 5.5 atm; ■= 0 atm; ◊ = - 11atm. Droplet size was determined using light microscopy.

In a double emulsion with Δπ=5.5 atm, the average W_1 droplet size was constant during the first four weeks of storage, but tripled between week 4 and 6 of storage. When the osmotic pressure gradient was further increased (e.g. Δπ = 11 atm), the W_1 droplet size immediately after double emulsion production was, at 5.7 ± 1.7 μm, nearly twice as large as the size of the simple W/O emulsion (3.5 ± 0.3 μm). However, droplet size did not increase evenly in all droplets, as is exemplified in Figure 5.14. Only a small number of droplets, less than 10 % of the total, increased to a size greater than 10 μm within 4 weeks, while the majority remained a near-constant size. In double emulsions where the osmotic pressure was matched or the

gradient was negative (0 atm; -11 atm), on the other hand, droplet size and size distribution did not change during storage.

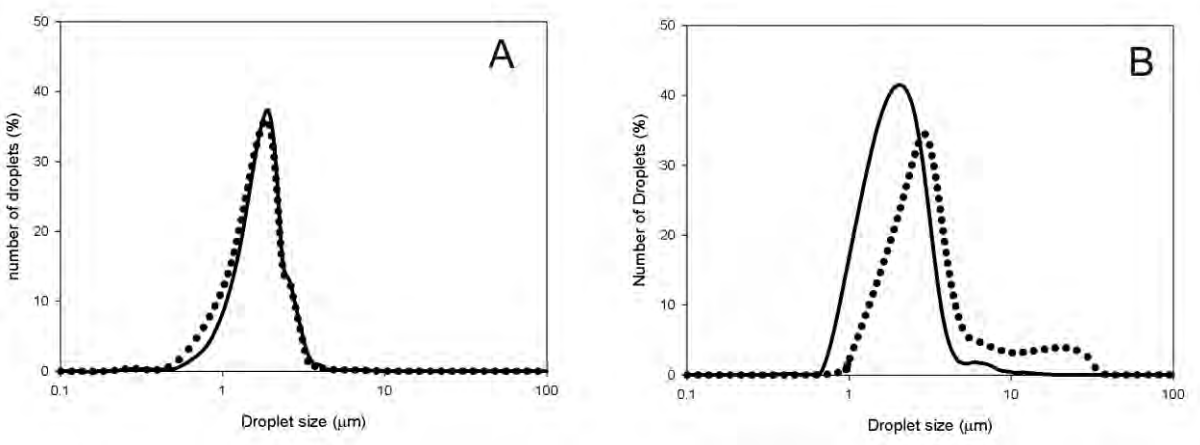

Figure 5.14: Number distribution of W_1 droplets in a double emulsion with (A) Δπ = 0atm and (B) Δπ = 11atm; after production (solid line) and after 4 weeks (dotted line). Droplet size determined using light microscopy.

A positive Δπ causes water to be preferentially transported into W_1 droplets at a rate dependent on the size of Δπ. Consequently, all encapsulated water droplets within a double emulsion structure are expected to swell at a similar rate. However, this was clearly not the case in double emulsions containing fat crystals. The fact that most droplets retained a constant size demonstrates the ability of the fat crystal "shells" surrounding W_1 droplets to resist water transport driven by the osmotic pressure gradient. The swelling of individual droplets could be the result of damage incurred during the secondary emulsification step. As a result of imperfect mixing regimes within the production vessel these droplets may have experienced higher shear and/ or temperatures than most. The sintered fat crystal "shells" may thus have been damaged. The resulting weakened interface would allow some water transport into the W_1 droplets during storage.

Damaged “shells” permit water transport driven by the osmotic pressure gradient. A positive gradient causes water to be transported into the W_1 droplets, which increases the pressure inside these droplets. The increased pressure weakens the sintered crystal structure by forcing the crystals to break apart. These crystal fragments then protrude from the water droplet interface, and the number of imperfections (“cracks”) increases. SEM micrographs (Figure 5.15) seem to visualise these protrusions from the surface of W_1 droplets in double emulsions where $\Delta\pi$ = 11 atm.

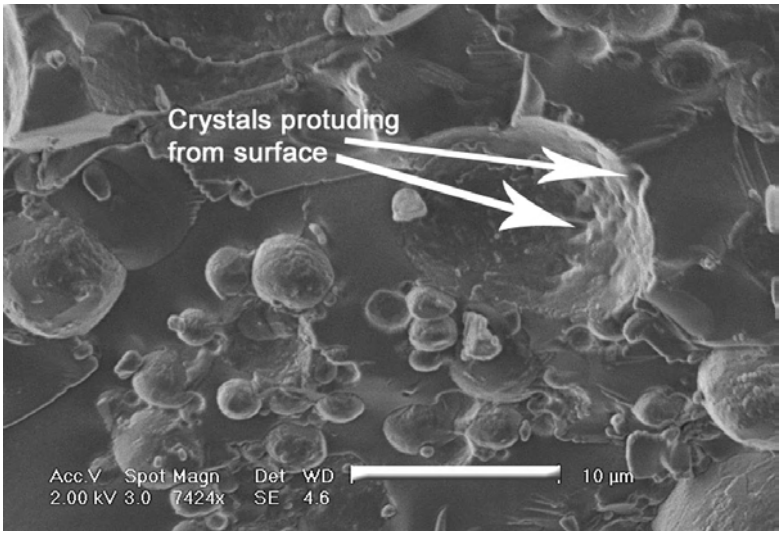

Figure 5.15: SEM micrograph of a W_1 water droplet in a sample where $\Delta\pi$=11atm. Scale bar = 10µm

In those formulations where the osmotic pressure is matched between the two aqueous phases or the gradient is negative, the primary emulsion water droplets retain a constant size in the double structure during 6 weeks of storage. In both cases there is no pressure increase inside the W_1 droplets. Although the fat crystal network surrounding the primary emulsion droplets may incur damage during the

secondary emulsification step similar to that described above, the lack of internal pressure means that this is not damaged further. Consequently, the number of crystals protruding through the secondary oil/water interface remains low, resulting in reduced coalescence compared to formulations with a positive osmotic pressure gradient (as discussed in section 5.4.2).

These results indicate that the direction of the applied osmotic pressure gradient is an important factor affecting the stability of the crystal “shells” surrounding the primary emulsion water droplets. These “shell” structures are compressed when the $\Delta\pi$ gradient is negative. On the other hand, when the osmotic pressure gradient is positive, the “shells” experience tensile loading. The effect of these two different loads is shown schematically in Figure 5.16. Taking certain rock structures described by Price and Cosgrove as an analogy (N.J. Price and J.W. Cosgrove, 1990), a weaker structure is attributable to tensile loading whereas compressive loading increases structural integrity. Furthermore, the existence of imperfections in the crystal “shells” may actually increase the strength of the structure in compressive loading, if crystals find room to re-arrange themselves in order to adjust to the pressure. The compression may even help the crystals to re-sinter, closing any imperfections in the fat crystal networks surrounding the primary emulsion droplets.

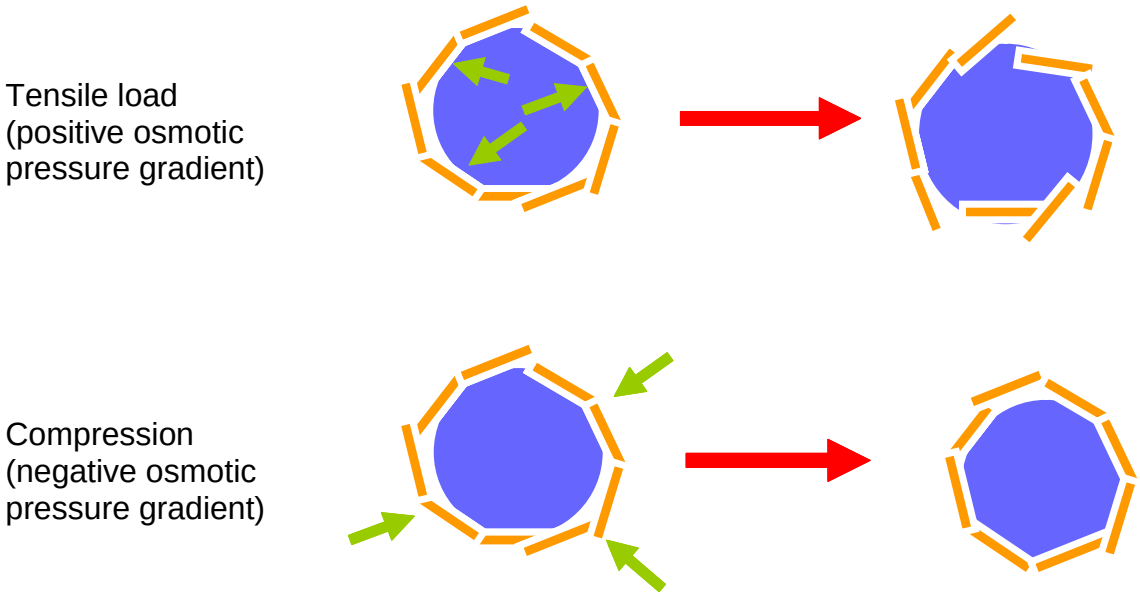

Figure 5.16: Schematic of influence of tensile load/ compression on primary emulsion droplet

These results have shown that it is not essential to balance osmotic pressure exactly in order to achieve good emulsion stability. On the contrary, the presence of a higher osmotic pressure gradient in the W_2 than in the W_1 phase was shown be beneficial to double emulsion stability, the explanation being that the crystal network structure surrounding the W_1 droplets was strengthened by compression induced by a negative pressure gradient.

5.3.4 Salt Release from W_1 to W_2 aqueous phase

As a further measure of double emulsion stability the ability of the formulations containing primary emulsions stabilised by fat crystals to retain a solute, KCl, was assessed by measuring the change in conductivity of the continuous aqueous phase. The primary emulsion contained 30 % water, including 1.6 % KCl, and 1.5 % tripalmitin and 0.5 % monoglyceride in the oil phase. The primary emulsion was mixed with equal parts sunflower oil and emulsified using the rotor/ stator at 8000

rpm for 3 minutes with the W_2 aqueous phase, containing 1 % Na-caseinate and various amounts of glucose to regulate the osmotic pressure gradient. Conductivity measurements were made of samples taken from the serum phase in creamed formulations (as described in chapter 3).

Salt was only slowly released from the encapsulated W_1 phase when the osmotic pressure was matched or the gradient was negative, as is shown in Figure 5.17. In both cases, only around 20 % of the KCl in the primary emulsion had been released after 6 weeks of storage. This salt release is probably the result of water transport through imperfections in the crystal “shell” structure as well as direct diffusion. In the absence of an osmotic pressure gradient between the two aqueous phases, there is no net water/ solute transport between these. However, some movement of water, including transport of solutes, inevitably occurs in both directions due to the chemical potential of the aqueous phases. The reason for the apparently linear nature of the transport is the comparatively low concentration of encapsulated salt and the very slow release rate over time. It is likely that at even longer storage times, the rate of release will slow down as the chemical potential between the two aqueous phases approaches equilibrium.

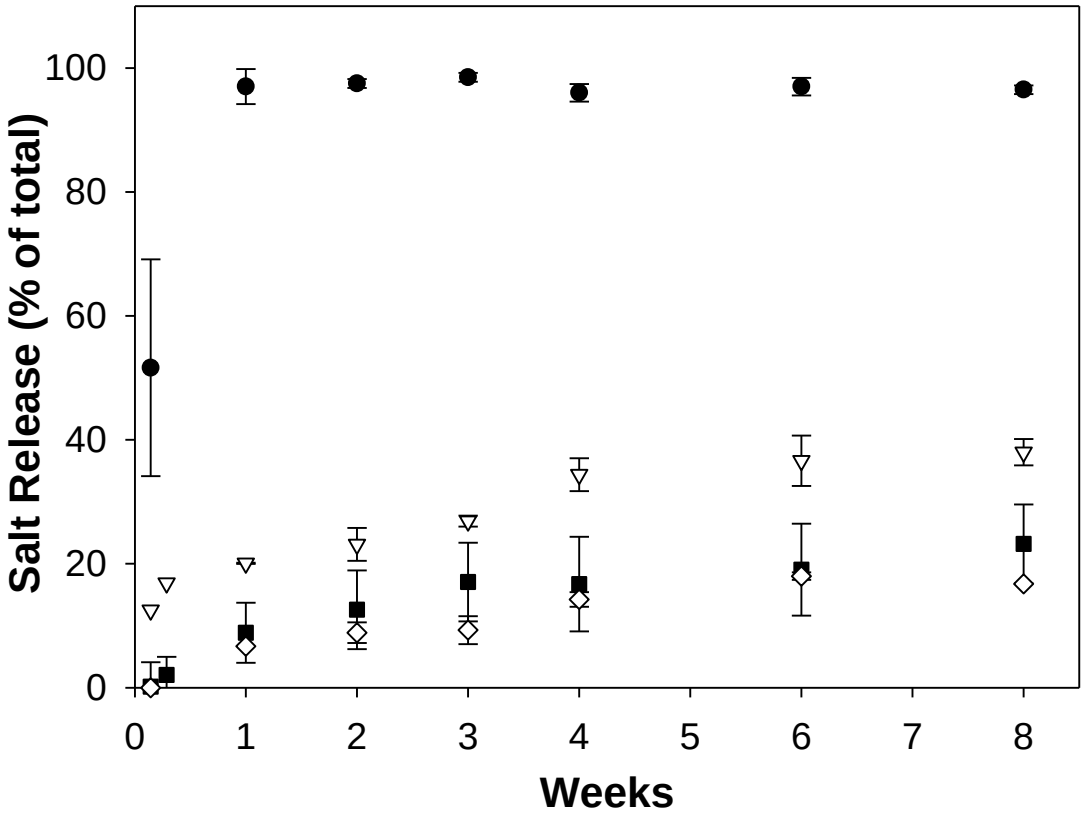

Figure 5.17: Influence of osmotic pressure gradients obtained by adding glucose to the W_2 aqueous phase on salt release. ●=11atm; ▽= 5.5 atm; ■= 0 atm; ◊ = - 11atm

When the osmotic pressure gradient was increased to Δπ = 5.5 atm, salt release also increased, so that almost 40 % of KCl was released within 6 weeks. This is in line with the observation that formulations at this Δπ were less stable in terms of coalescence and crystal network structure than formulations where Δπ = 0.

When Δπ was further increased to 11 atm, all salt was released within one week, as seen in Figure 5.17, and more than 50 % was released within the first day.

In chapter 4 the ability of W_1 emulsion droplets to retain salt encapsulated despite applied osmotic pressure gradients was demonstrated. However, when the primary

emulsions are incorporated into a double structure, the additional shear applied during the secondary emulsification step tends to rupture or damage the fat crystal "shells" of W_1 droplets, as previously described. This weakens the crystal structure so that a large osmotic pressure gradient can drive the rapid passage of encapsulated solutes to the W_2 aqueous phase.

5.3.5 Influence of the addition of PGPR on emulsion stability

In order to illustrate the effectiveness of the W_1 droplet "shells" in providing stability against osmotic pressure gradients, as well as to further elucidate the mechanism of water transport between the two aqueous phases, 1 % PGPR was added to some formulations.

Two formulations containing PGPR were directly compared with each other: The primary emulsion of one contained only 1 % (wt/wt) PGPR in the oil phase (no fat crystals). The ratio of water to oil was 30:70. The primary emulsion of the second formulation was a 30:70 fat crystal-stabilised one (containing 1 % tripalmitin and 0.5 % (wt/wt) monoglyceride in the oil phase). After the primary emulsion was produced, 1 % (wt/wt of the oil phase) PGPR was mixed with this emulsion using a low shear mixer. The double emulsion was then produced by dispersing the primary emulsion/ PGPR mixture in a continuous aqueous phase containing 1% Na-caseinate (wt/wt in the W_2), and emulsifying at 8000 rpm for three minutes (the ratio of primary emulsion: continuous aqueous phase was 20:80). Both formulations had an osmotic pressure gradient between W_1 and W_2 of 11 atm.

Table 5.8: Droplet size of W_1 primary emulsion droplets and double emulsion globules, Δπ = 11atm. Droplet sizes measured by light microscopy.

	after production		1 week		4 weeks	
	$D_{3,2}$	σ	$D_{3,2}$	σ	$D_{3,2}$	σ
	PGPR only					
internal droplet size	1.9	0.5	3.7	0.8	4.4	0.8
external droplet size	38.6	12.5	56.1	19.8	>>	>>
	PGPR + fat crystals					
internal droplet size	2.8	0.7	3.8	0.9	7.3	2.8
external droplet size	26.7	8.1	126.4	36.2	102.8	37.2

The data presented in Table 5.8 show that the W_1 droplets in formulations containing PGPR but no fat crystals in the primary emulsion swelled immediately and rapidly after production. Similar behaviour was observed when PGPR was present and the primary emulsion contained fat crystals. In both formulations the volume of primary emulsion droplets more than doubled within 1 month. Hence the oil globules expanded to fit the increased volume of the primary emulsion droplets, as exemplified in Figure 5.18. Although the double structure was largely retained, it became increasingly difficult to distinguish individual globules after around 4 weeks of storage. After 6 weeks, double globules seem to have merged to form a continuous oil phase.

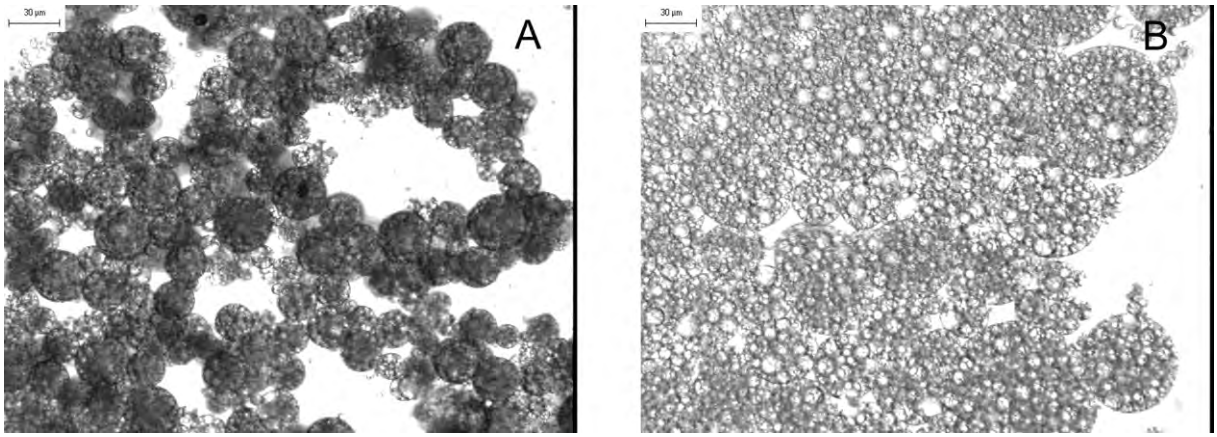

Figure 5.18: Double emulsion, containing W_1 droplets stabilised by fat crystals and 1% PGPR, $\Delta\pi$ = 11 atm, after production (A) and after 6 weeks (B): W_1 droplets have noticeably swollen, but the secondary interface has largely expanded to fit the higher phase volume of primary emulsion. Scale bar = 30 µm

The effect of the presence of PGPR on a fat crystal-stabilised primary emulsion is also evident in the droplet size distribution of the W_1 droplets within the double emulsion (Figure 5.19). The distribution shifts to a higher average size, but retains its shape, which shows that all droplets swell at a similar rate. This is in contrast to the observation made for double emulsions where fat crystals stabilised the W_1 droplets and PGPR was not present (see section 5.3.3).

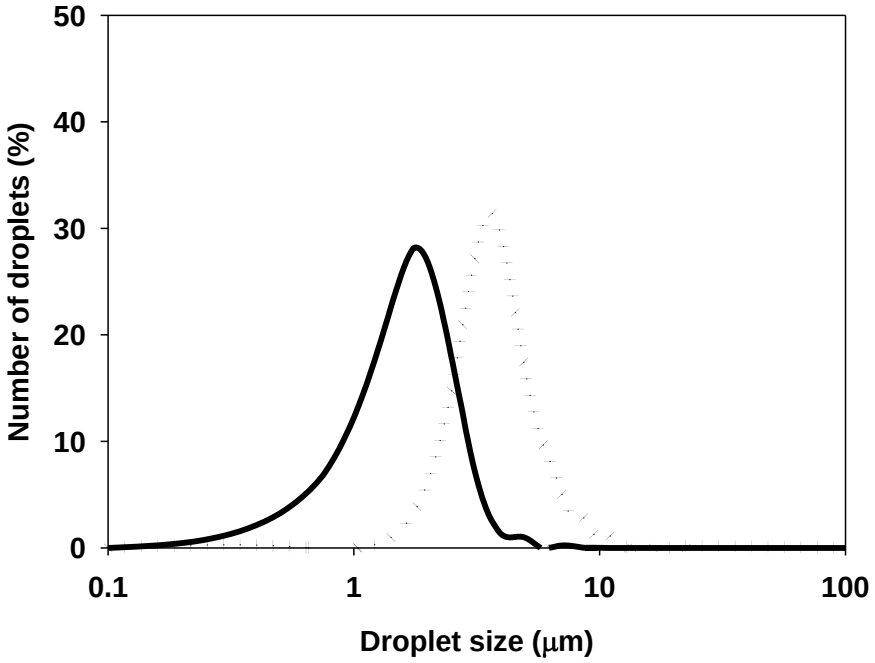

Figure 5.19: Number distribution of W_1 droplets in a double emulsion after production (solid line) and after 4 weeks (dotted line). The primary emulsion droplets are stabilised by fat crystals and contain 1% PGPR. Droplet size measured using light microscopy.

The effect of PGPR on crystal network structure was further investigated with SEM. Figure 5.20 shows SEM micrographs of a double emulsion containing PGPR as well as fat crystals. Water droplets in the double globules are seen to have a pronounced ripple-like structure (indicated on the figure). These ripples probably represent clusters of fat crystals that in formulations without PGPR formed smooth crystal "shells" around the water droplets. The addition of PGPR seems to have disrupted this crystalline structure and caused rearrangement of crystals into random aggregates.

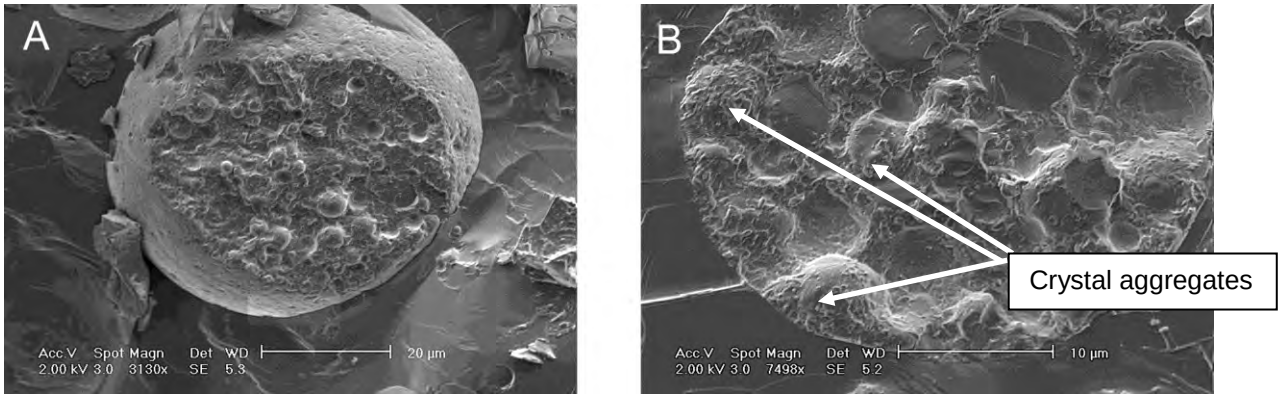

Figure 5.20: Double emulsion, containing W_1 droplets stabilised by fat crystals and 1 % PGPR, Δπ = 11 atm, after production (A) and after 1 week (B). PGPR seems to affect the interface of primary emulsion droplets

PGPR is well-known for its crystal-modifying properties. For instance, it is utilised in chocolate manufacture to reduce yield stress (Weyland & Hartel 2008) and has been shown to influence fat crystal form (Garti et al., 1999). It is also a widely used emulsifier in W/O emulsions.

The above results indicate that the addition of PGPR causes a modification of the crystal structure at the primary emulsion interface, thus counteracting the role of crystals as droplet stabilisers. Their displacement by PGPR results in a “patchy” interface only partially covered by crystals and, for the main part, by a PGPR monolayer. The permeability of this monolayer facilitates the rapid swelling rate induced by the osmotic pressure gradient. The elasticity of a PGPR-covered interface allows it to expand in order to accommodate the increased volume of water within the encapsulated phase. Rigid fat crystal-stabilised interfaces, on the other hand, are much less permeable, so that the increase in droplet size due to large osmotic pressure gradients is much reduced.

5.4 Conclusions

Double emulsions containing W_1 droplets stabilised by fat crystals were produced using two different processing methods. While membrane emulsification using a pressurised feed system was not suitable for making double structures, they could be produced using standard rotor/stator technology.

Double emulsion stability was found to depend on emulsifier type: Monomeric surfactants could not stabilise the emulsions. Fat crystals present within the oil globules “pierce” the thin interfacial layer between two neighbouring globules causing large-scale coalescence and destroying the double structure. Proteins were better suited for stabilising the double structures, although the type of protein used influenced stability: globular proteins seemed to be less suitable than those that adsorbed at the interface in a loop-and-tail conformation.

It was also confirmed that the crystalline “shells” surrounding the W_1 droplets reduced or stopped water transport between the W_1 and W_2 aqueous phases in a double structure. This was independent of the type of solute in W_2 used to regulate the osmotic pressure gradient between the two aqueous phases. Although it was not necessary to exactly balance the osmotic pressure gradient between the two phases, stability (against coalescence as well as release of encapsulated salt) was dependent on the direction of this gradient. If the osmotic pressure was higher in the external W_2 water phase, the stress exerted on the “shells” by an increased volume of water inside the W_1 droplets resulted in increased W_1 droplet size and salt release. However, if the osmotic pressure was higher in the internal W_1, the “shells” were able to withstand pressure exerted from without and the double emulsion remained stable.

A comparison was made of the formulations containing fat crystals in the primary emulsion with ones in which the primary emulsion was stabilised using PGPR, an emulsifier which surrounds water droplets in monolayers. This highlighted the ability of the fat crystal “shells” to withstand water transport caused by the osmotic pressure gradient. In formulations containing PGPR the primary emulsion droplets grew to accommodate increasing volumes of water within them. When fat crystals stabilised W_1 droplets, on the other hand, water droplet size remained constant, indicating that little water transport took place between the two aqueous phases.

6 Pickering in Pickering Duplex Emulsions

6.1 Introduction

Chapter 5 showed that proteins such as Na-caseinate could be used to stabilise double emulsions containing fat crystals. Although the double structure was retained the proteins did not prevent coalescence between double globules. Such coalescence makes the product unsuitable for industrial application, e.g. commercially available food products, as product sensory properties as well as microbial stability would be affected. It was proposed that one reason behind the increase in globule size was partial coalescence, caused by protruding fat crystals piercing the interfacial film of neighbouring globules (Boode & Walstra 1993).

Small particles adsorbed at droplet interfaces give a much thicker and more rigid surface film than proteins (Binks 2002). This thick and rigid film effectively protects oil globules from coalescence by physically preventing contact of the oil phase in each globule. If the film were thicker than the length of fat crystals likely to protrude from the globules, particles could prevent partial coalescence between double emulsion globules containing fat crystals.

The following chapter investigates whether the resistance of fat crystal-containing double emulsions to coalescence is improved when particles also stabilise the secondary interface. Furthermore, the effect of the presence of impurities on the stability of double emulsions was studied.

6.2 Influence of different particles at the secondary interface

Silica particles have often been used in emulsion science due to their advantage of being well-defined impurity-free chemically synthesised products. For this reason silica particles were also used in this study as stabilisers for the secondary interface in a formulation in which the primary emulsion was stabilised by fat crystals. However, regulators disfavour the use of silica particles in food products and thus it was also decided to investigate alternative food-grade particles as secondary emulsifiers. A major obstacle to this approach is finding naturally occurring edible submicron-sized particulate materials.

6.2.1 Silica-particles as secondary stabilisers

The ability of silica particles to stabilise the secondary interface in double emulsions containing fat crystals was assessed. 1 % or 2 % (wt/wt in W_2) silica particles were dispersed at pH 2 in a 10 % (wt/wt in W_2) glucose solution using the sonicator as described in chapter 3. At the pH level chosen – pH 2 – the particles carry no charge (Pichot et al., 2009). This eliminates electrostatic inter-particle repulsion thus ensuring that the interfacial layer is tightly packed with silica particles (Pichot et al., 2009). These silica particles are hydrophilised, with a contact angle of 14° when measured between water and toluene (Yan et al., 2000). The low contact angle means they are not preferentially located at the oil/ water interface. However, it was previously shown that vegetable oil in water emulsions can be stabilised against coalescence using 1% silica particles at pH 2 (Pichot et al., 2009). It is likely that impurities, e.g. mono- and diglycerides, in “natural” oils (e.g. rapeseed and

sunflower) modify the wetting properties of these particles sufficiently for them to be attracted to the oil/ water interface.

The primary emulsion (30 % (wt/wt) water in oil) was stabilised by 0.5 % monoglyceride and 1 % (wt/wt in the oil phase) tripalmitin, and was prepared in the A & C unit as previously described. The W_1 phase also contained 1.6 % (wt/wt in W_1) KCl. The primary emulsion was mixed with equal parts of sunflower oil and then slowly added to the silica particle dispersion in the rotor/ stator mixer, the stirring speed was set at 3000 rpm. The double emulsion was then prepared by shearing the sample at 8000 rpm for 3 minutes while cooling in an ice bath.

Both 1% and 2% silica particle concentration stabilised the double emulsion against coalescence, with little variation in drop size distribution over time when the samples were stored at 10°C (Figure 6.2). The average globule size was slightly smaller in samples containing 2% silica particles (17 μm) compared with those containing 1% particles (21 μm), but the difference is small and within the range of experimental error. The high level of stability of the globules against coalescence found to exist confirms the results of previous studies on the stability of silica particle-stabilised O/W emulsions containing at least 1 % silica particles (Pichot et al., 2009). All subsequent experiments were performed using the lower concentration of particles because this was demonstrably enough to prevent coalescence between globules.

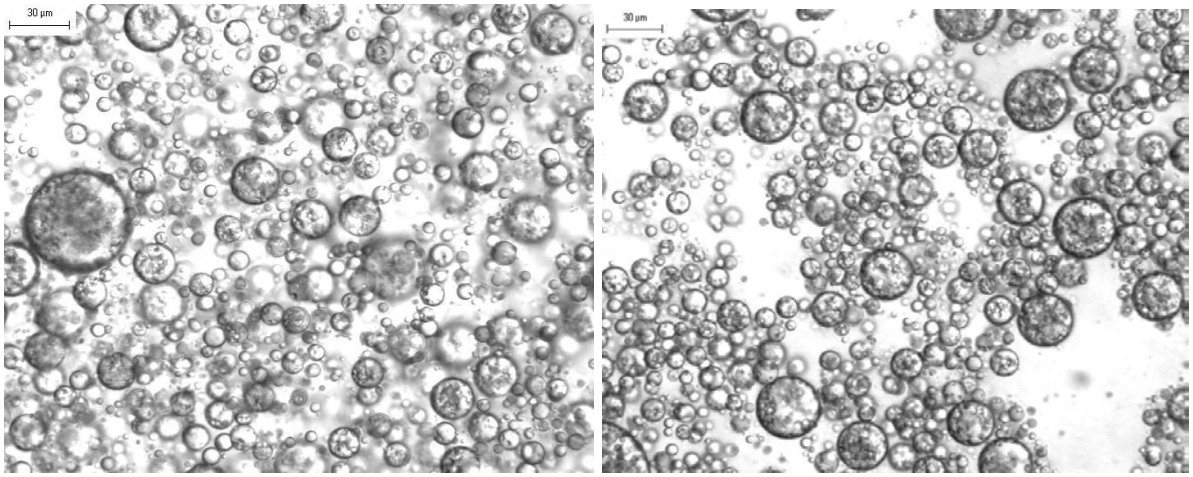

Figure 6.1: Light microscopy micrographs of double emulsions stabilised by 1 % silica particles stored at 10 °C, 2 days after production (left) and after 1 month's storage (right).

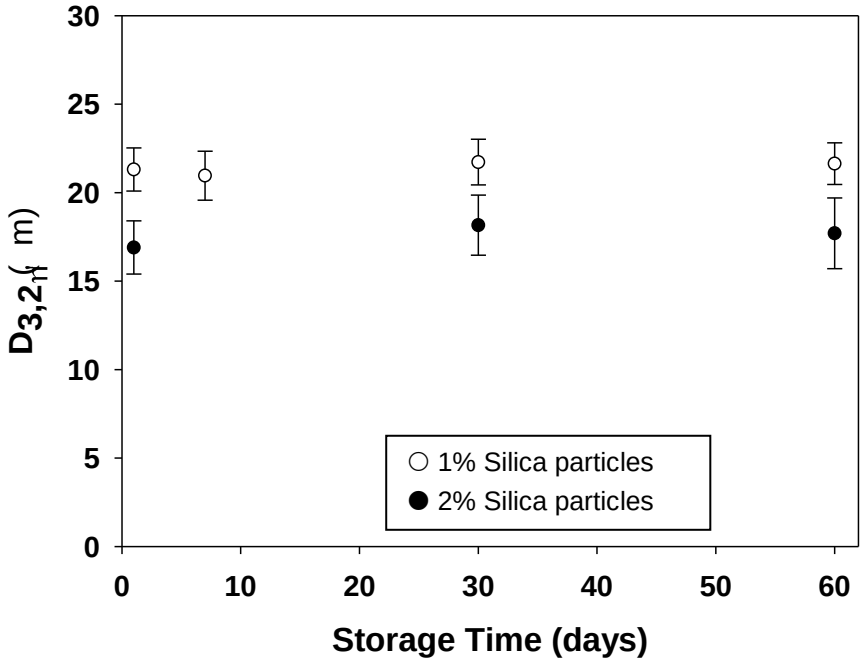

Figure 6.2: Comparison of droplet sizes in double emulsions stabilised by 1 % and 2 % silica particles in the W_2 aqueous phase, stored at 10 °C. Measured using laser diffraction.

The next step was to study stability over time when the double emulsion containing 1% silica particles was stored at 25 °C. At this temperature a small shift in average

globule size occurs during storage ($D_{3,2}$ after production ~ 20 µm ± 1 µm, after 8 weeks ~ 22 µm ± 1 µm) which is within the experimental error (see Figure 6.3). These results show that it is possible to prevent coalescence between double globules containing fat crystals when silica particles are used to stabilise the secondary interface.

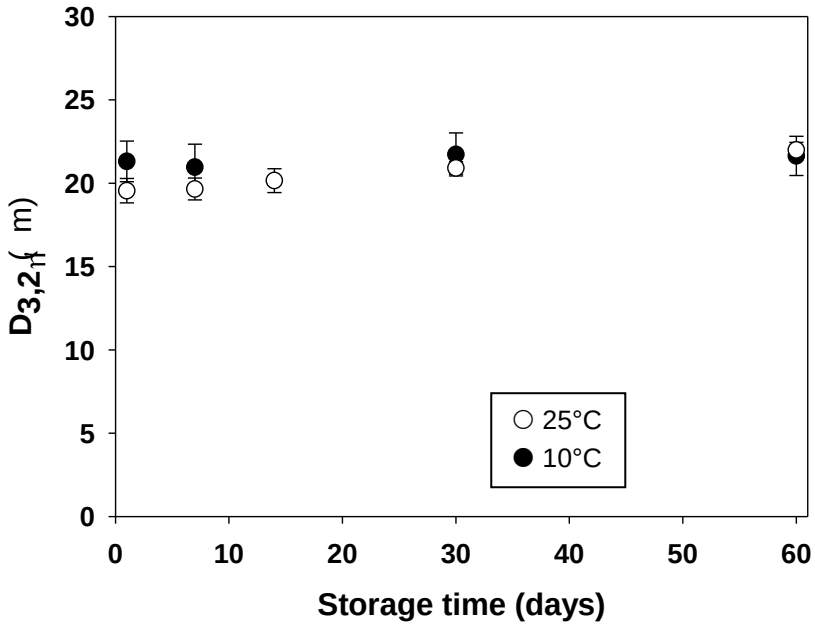

Figure 6.3: Size distribution after storage at 10 or 25 °C for double emulsions stabilised by fat crystals (primary W_1/O interface) and 1 % silica particles (secondary O/W_2 interface). Measured using laser diffraction

6.2.2 Colloidal Microcrystalline cellulose (CMCC) at the secondary interface

In the search for alternatives to silica particles CMCC was identified as a potential candidate, given reports that colloidal microcrystalline cellulose can be used to

stabilise W/O/W double emulsions (Oza & Frank, 1989). An attempt to replicate the results was undertaken as part of the present study. To test the ability of CMCC to stabilise simple O/W emulsions oil and water were homogenised at a ratio of 1:4 in a high shear rotor/ stator mixer for 5 minutes at 8000 rpm. The water contained 1 % (wt/wt) CMCC, dispersed by sonicating the aqueous mixture for 12 minutes. The formulation was adapted to use sunflower oil instead of paraffin oil, and no additional surfactants were used (Tween 20 and Span 80 were used in the study of Oza and Frank, 1989).

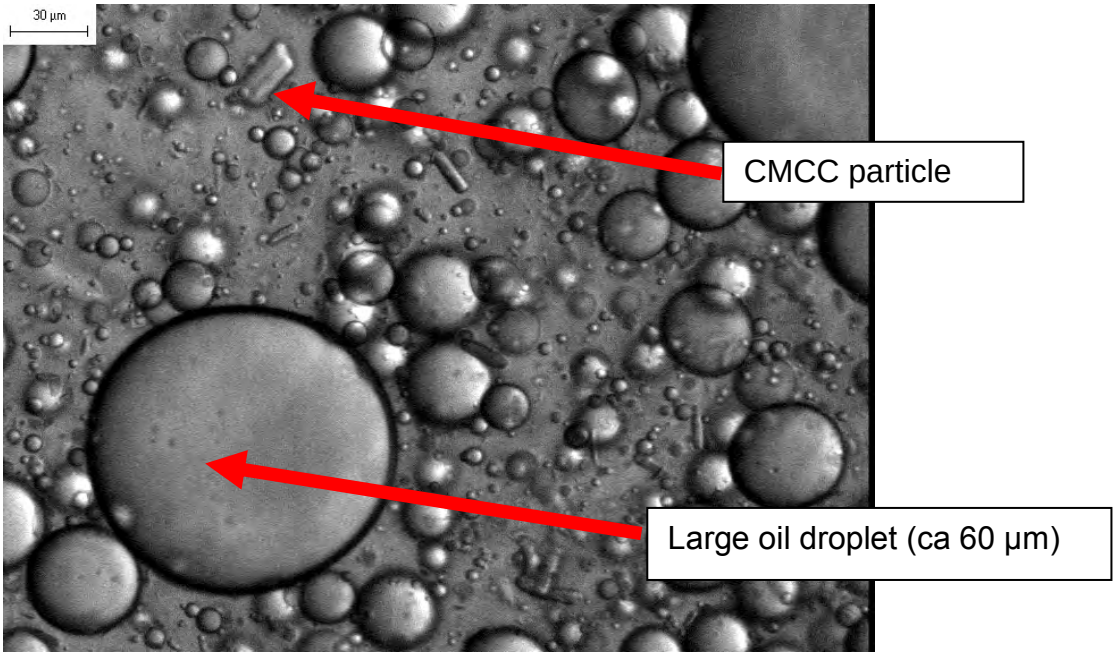

Figure 6.4: Light microscopy micrograph of a 20/80 O/W emulsion containing 1 % CMCC in the W phase

The resulting emulsion was unstable. Despite a long period of sonication, CMCC formed particle aggregates that could not be broken apart using this procedure. Visual inspection of the CMCC solution using microscopy revealed the existence of

rod-shaped particles of around 5 - 10 μm in length (Figure 6.4). After a day large (100 μm) oil droplets were observed. The formulation phase separated within a few days. As the CMCC particles are hydrophilic, it is likely that they require additional surfactants to modify their surface properties in order to place them at the O/W interface. It is also conceivable that the addition of monomeric surfactants would have aided the break-up of the CMCC aggregates. However, it has been demonstrated that the presence of small molecule emulsifiers tends to destabilise fat crystal-stabilised primary emulsions by displacing the crystals from the interface (see chapter 5). Given the poor stability of double emulsions containing CMCC as a stabiliser for the secondary O/W emulsions this line of inquiry was not pursued any further.

6.2.3 Use of OSA starch at the secondary interface

The potential of sodium octenyl succinate (OSA) starch as the secondary emulsifier was investigated as an alternative to colloidal starch particles. This hydrophobised starch is soluble in water and has been shown to be a good emulsifying agent (Tesch et al., 2002). 2 % (wt/wt in W_2) starch was dispersed in a 10 % (wt/wt in W_2) glucose solution until dissolved. This concentration was chosen to ensure a surface layer of starch particles at the secondary interface (see below). The viscosity of this solution was in the order of 600 mPa s (see Figure 6.5) – in line with findings from (Tesch et al., 2002).

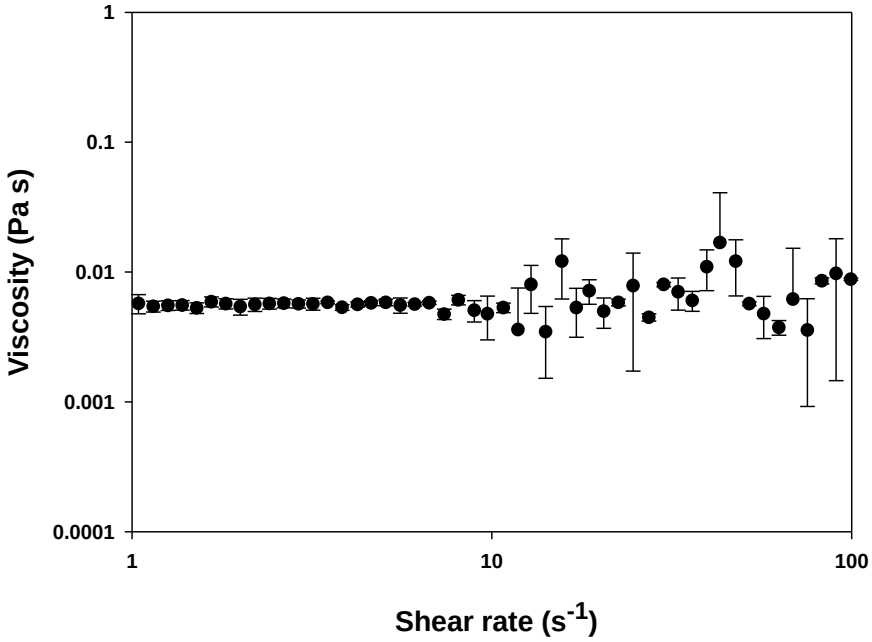

Figure 6.5: Viscosity of a solution containing 2 % OSA-starch at 15°

Double emulsions were prepared using the rotor/ stator high shear mixer as described in section 6.2.1. The primary emulsion composition was also the same: the 30:70 W/O emulsion contained 0.5 % monoglyceride and 1 % tripalmitin in the oil phase and 1.6 % KCl in the aqueous phase and was diluted with 50 % sunflower oil prior to the secondary emulsification step. The phase volume (primary emulsion): continuous W_2 aqueous phase was 20:80.

Formulations containing 2 % OSA starch were assessed for their stability against coalescence when stored at 10 and 25 °C. Double globules were small, in the order of 10 ± 0.5 µm (Figure 6.6). Globule size distribution remained constant at a storage temperature of 10°C, as shown in Figure 6.7. Light microscopy confirmed that the double emulsion structure was retained during this time.

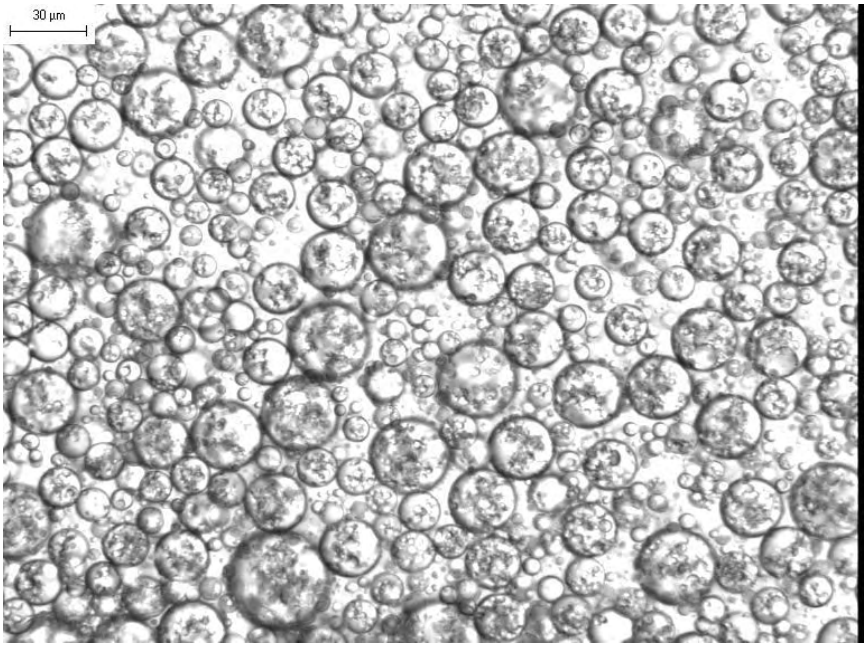

Figure 6.6: Double emulsion containing 2 % OSA-starch in the W_2 phase

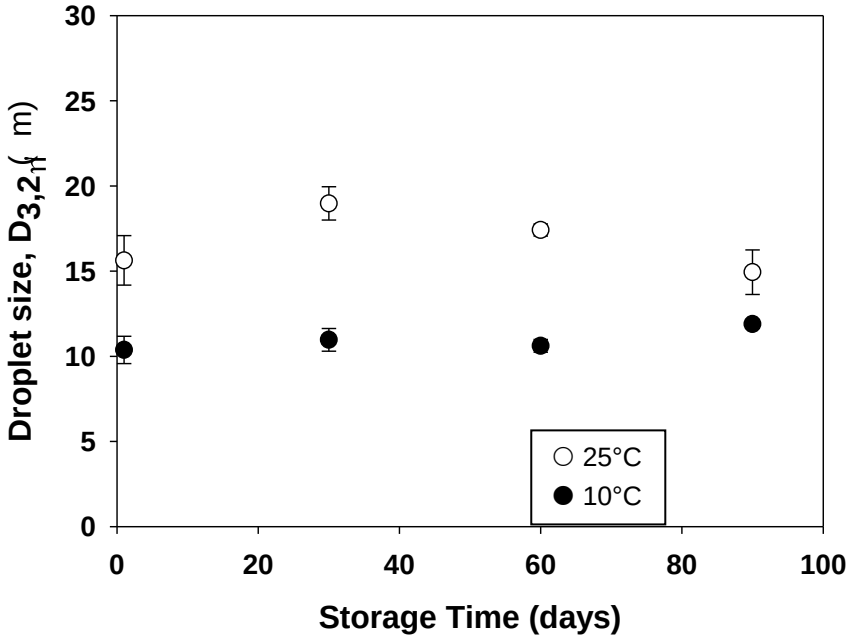

Figure 6.7: Droplet sizes of double emulsions stabilised by 2 % OSA starch in W_2, stored at 10 or 25 °C, measured using laser diffraction.

It is possible that the surface loading of the steric barrier is the defining parameter in determining stability against coalescence. This is because the surface loading determines whether crystals protrude from the oil globules and pierce neighbouring globules or not. According to Tesch et al. (2002) colloidal solubilised OSA starch molecules sterically stabilise oil globules. Steric stabilisation creates a stable barrier against coalescence as reported in chapter 2, although this mechanism does not involve a rigid shell as in the case of adsorbed fat crystals. Nilsson & Bergenstahl (2006) have shown that the surface coverage of OSA-starch varies between 3 $mg.m^{-2}$ to as high as 16 $mg.m^{-2}$.

The surface loading of the interface is governed by the amount of OSA starch available at the interface during emulsification and its adsorption rate (Nilsson & Bergenstahl 2006). When large surface areas are created (e.g. high energy input) the relative adsorption rate in comparison to the rate of surface area creation is low. The adsorbed starch has time to re-arrange at the interface and forms monolayers with loading of 1 - 3 $mg.m^{-2}$. When the energy input is low, the relative adsorption rate is high compared to the rate of surface area creation. The adsorbing starch does not have time to re-arrange itself into monolayers, resulting in high surface loading (Nilsson & Bergenstahl 2006).

In the case of double emulsions, energy input in the secondary emulsification step is low (to preserve the primary emulsion structure) and a large amount of starch is available at the interface, making it likely that the adsorption rate is rapid and heavily loaded interfacial layers are formed. The specific surface area of the double emulsion globules, as measured using the Mastersizer, was 0.6 $m^2 g^{-1}$. The potential maximum

surface coverage at 2 % OSA starch concentration is therefore 27 $mg.m^{-2}$ if all starch were adsorbed at the interface (1 g emulsion sample contains 80 % aqueous phase of which 2 % (16 mg) is starch. Assuming only starch is at the secondary interface, this covers the specific surface area with a coverage of 16/0.6 = 27 $mg.m^{-2}$). This data indicates that there is sufficient starch available to potentially create an interface of 16 $mg.m^{-2}$ starch coverage, even if not all available starch adsorbs at the interface.

The effect of storage temperature on the stability of the double emulsions containing OSA-starch was studied in an attempt to gain a better understanding of the roles of the various emulsifiers in the double emulsion. Samples of the same double emulsion described above were stored at 25°C and regular globule size measurements were performed using the Mastersizer. Droplet size appeared to increase from 15 to 21 μm within the first month of storage and then decrease (Figure 6.7). The reason for the decrease was the gradual formation of large aggregates of primary emulsion in the continuous aqueous phase. These were no longer part of the double structure and were not be accurately measured using the Mastersizer because they were not suspended within the water phase. The formation of such aggregates was, however, reflected in the changing shape of the droplet size distribution curves (Figure 6.8) and confirmed using light microscopy (Figure 6.9).

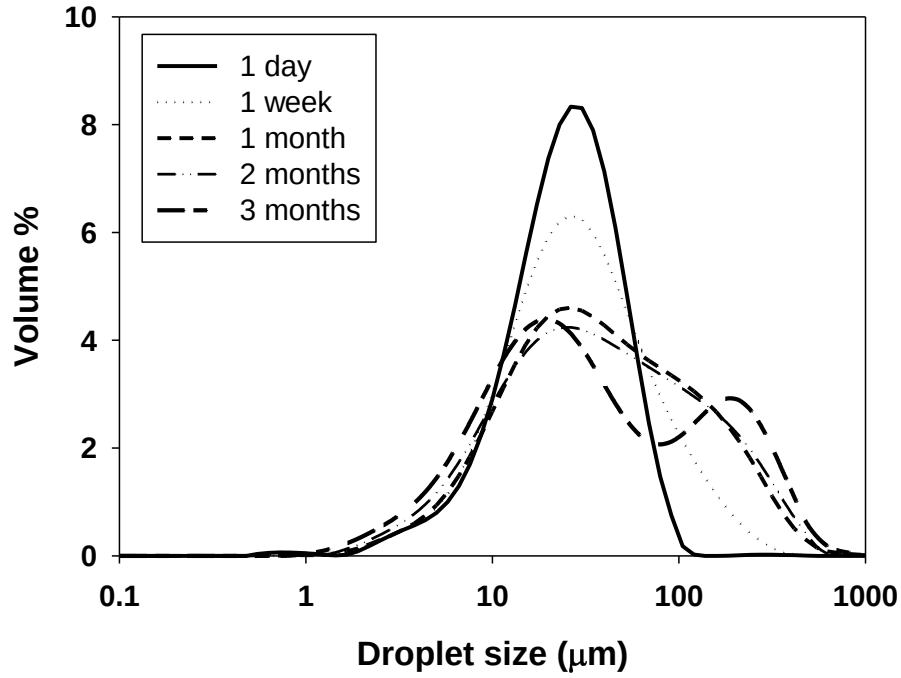

Figure 6.8: Size distributions of double emulsions containing 2 % OSA starch as secondary emulsifier at 25 °C. Measured using laser diffraction

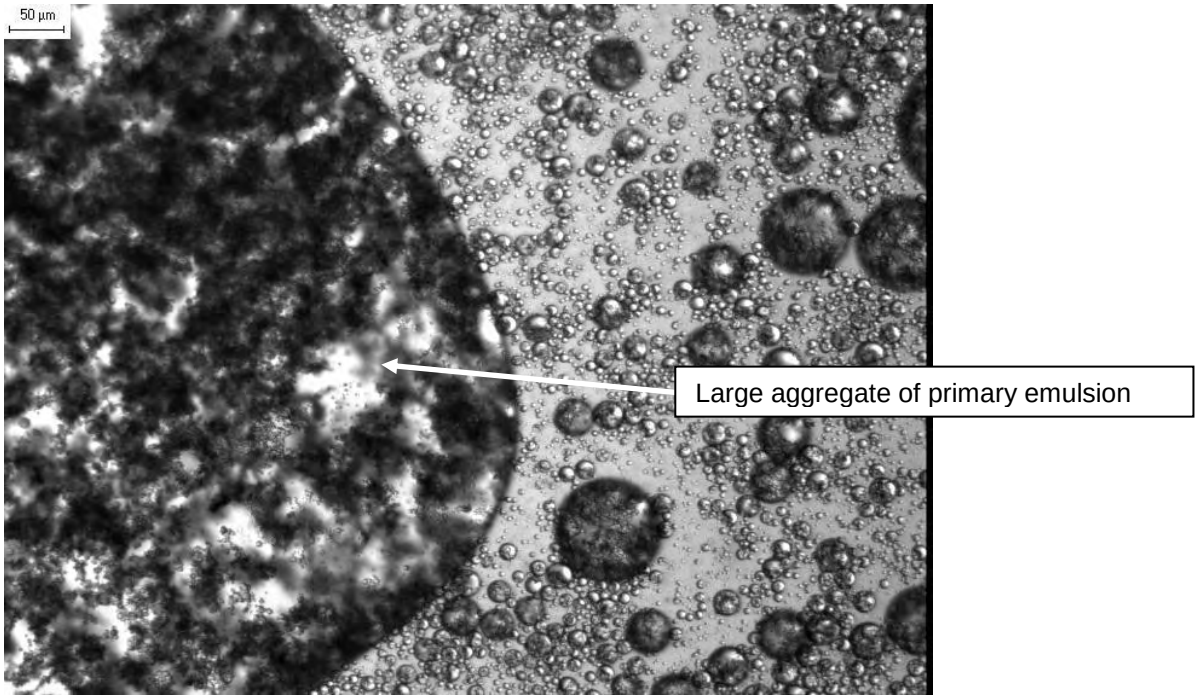

Figure 6.9: A double emulsions containing 2 % OSA in W_2 after 2 months' storage at 25 °C.

It is likely that the reason behind the observed instability for samples stored at room temperature is the presence of liquid, and therefore mobile, monoglyceride molecules within the oil phase. At 10 °C monoglycerides are in their crystalline state and therefore incorporated into the “shell” structure surrounding primary emulsion droplets. This prevents their migration between interfaces and subsequent destabilisation of the secondary interface as observed in chapter 5. However, monoglycerides become increasingly soluble in the oil phase at temperatures greater than 20 °C (see chapter 4) and this increases their mobility between the two interfaces.

Mobile monoglycerides may decrease the stability of an OSA-starch stabilised interface because monoglycerides have a higher surface activity than OSA starch. This is indicated by surface tension measurements. These measurements were performed at room temperature according to the protocol described in chapter 3. The respective samples were equilibrated for 17 minutes before the measurement was taken. When the water phase contained 2 % OSA starch and the oil phase was sunflower oil, the interfacial tension was measured at 20 ± 1 mN. On the other hand, when the oil phase contained 0.5 % monoglyceride and 1 % tripalmitin, and the aqueous phase was distilled water, the interfacial tension was 3.3 ± 0.1 mN. Thus, the presence of monoglyceride molecules at the secondary interface could displace the OSA starch from the secondary interface.

This competition for interfacial space could be the reason for the destabilisation of the double structure observed when the formulation was stored at 25 °C.

6.2.4 Mustard particles at the secondary interface

Mustard powder has long been used as an emulsifying aid in traditional food preparation (e.g. the stabilisation of mayonnaise). It is a naturally derived product that contains around 30 % protein, 20 % fat, some fiber and around 30 – 40 % starch (Bhattacharya et al., 1999). Interfacial tension measurements were performed using the protocol described in chapter 3 to measure whether commercially available mustard powder was surface active. The presence of 1% (wt/wt in W_2) mustard powder dispersed in the aqueous phase lowered the interfacial tension from 24.7 ± 0.2 to 15.0 ± 0.6 mN/m, confirming its surface activity (or that of one of its components). Figure 6.10 shows how the presence of 1 % mustard powder in water reduced the interfacial tension with sunflower oil by around 12 mN/m when compared with the interfacial tension between pure water and oil (25 mN/m).

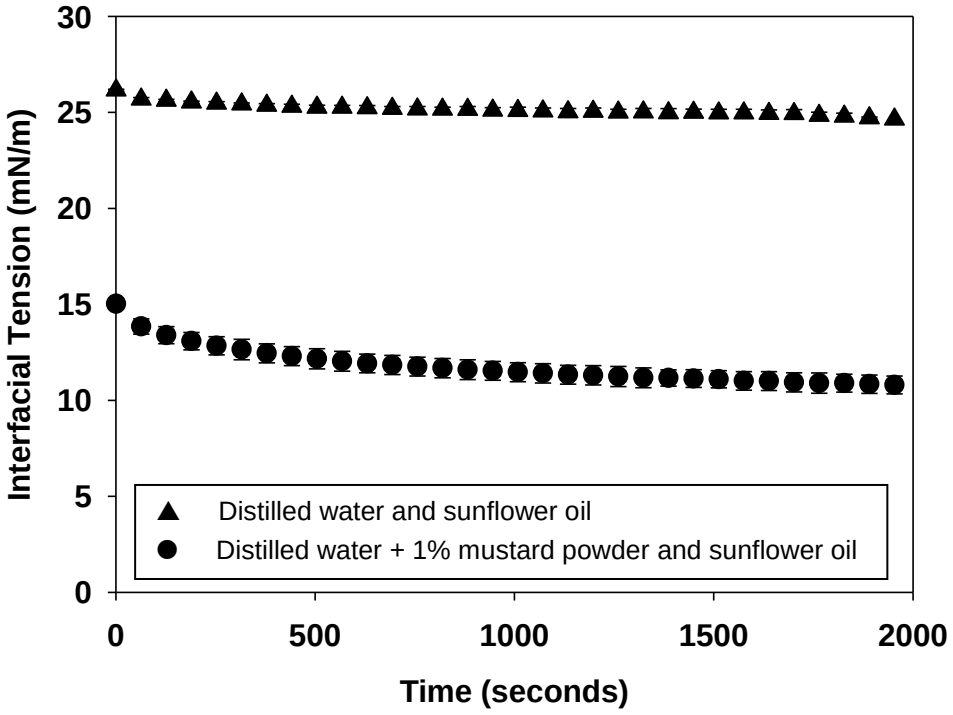

Figure 6.10: Interfacial tension of 1 % mustard powder (pH 5.4) in water with sunflower oil compared to interfacial tension between pure water and sunflower oil

Given this result, it was decided to further examine and test the use of mustard powder as a secondary emulsifier in double emulsions containing fat crystals.

1% mustard powder was dispersed in a 10 % glucose solution at pH 2, 5.4 and 7.2 using a sonicator. Aggregation between individual mustard particles occurred independent of pH despite long sonication (up to 12 minutes). This resulted in sedimentation of the mustard particles in the aqueous solution. The average aggregate size was measured using laser diffraction as being 17 ± 4 μm. Visual inspection of the mustard dispersion during gentle shear showed that aggregates

“disappeared” as long as the sample was stirred, suggesting that the aggregates could be broken during the application of shear.

Double emulsions were prepared using the same primary emulsion composition (0.5 % monoglyceride, 1 % (wt/wt in the oil phase) tripalmitin, 30 % (wt/wt) water containing 1.6 % (wt/wt) KCl) as was used in the section 6.2.3. The primary emulsion was mixed with sunflower oil in a ratio 1:1 and was slowly added to the aqueous solution containing 1 % (wt/wt in W_2) mustard powder and 10 % (wt/wt in W_2) glucose (to balance the osmotic pressure) at various pH. The double emulsion was then produced using the rotor/ stator mixer and the same procedure as described in section 6.2.3.

The resulting double emulsions rapidly creamed. The sedimentation of mustard aggregates was no longer observed once the particles were incorporated into a double structure. The lack of sediment formation in the double emulsion could indicate that the mustard particles were contained within the cream phase. The emulsions were gently stirred to disperse the cream before samples were taken for analysis in the microscope. Light microscopy micrographs taken within 1 day after production revealed the presence of large mustard particle aggregates as well as double emulsion globules (Figure 6.11).

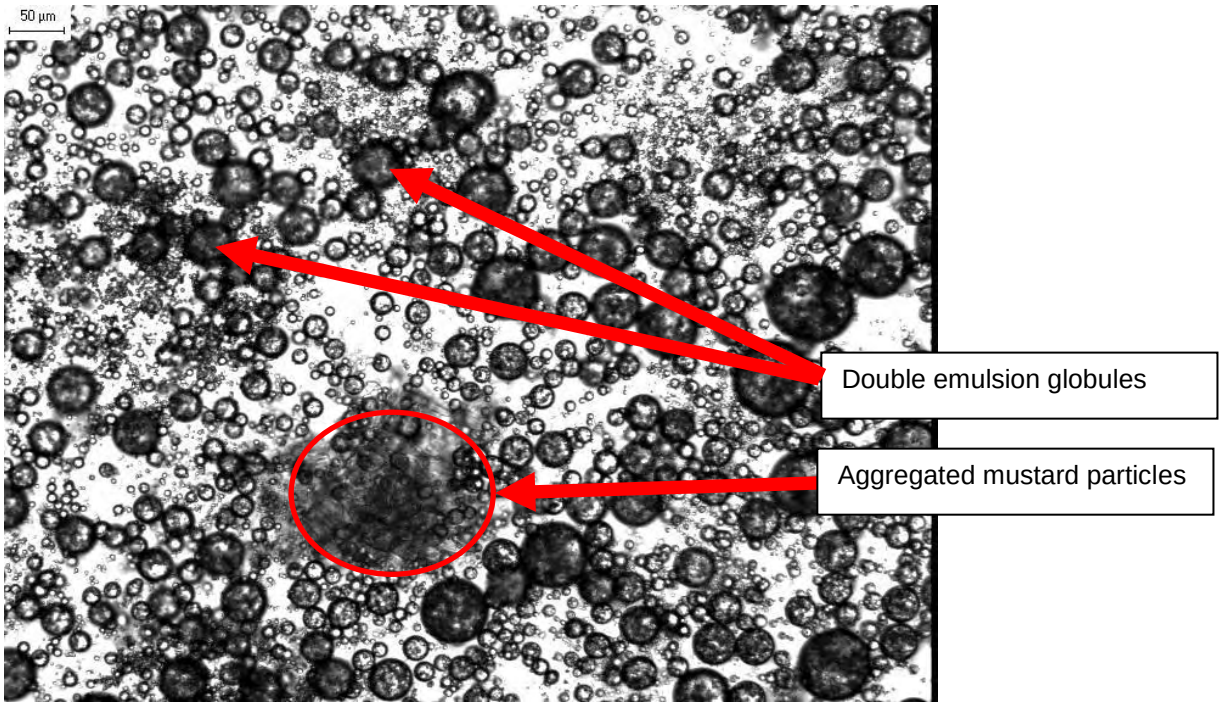

Figure 6.11: Light microscopy micrograph of a double emulsion containing 1 % mustard particles in the secondary aqueous phase (pH 7.2)

The stability of the formulations containing mustard powder at different pH was analysed using light microscopy and Laser Diffraction (Mastersizer) over a 1-month period. All 3 formulations (at pH 2, 4.5 and 7.2) were unstable against coalescence as large aggregates formed in all samples. Figure 6.12 shows the development of double globule sizes (measured in the Mastersizer) in formulations containing 1 % mustard powder (at different pH) as secondary emulsifier. At pH 2, globule size doubled from 60 to 120 µm within 1 month storage at 10 °C. Light microscopy confirmed the existence of large aggregates of primary emulsion in these samples. Coalescence rates were lower when the pH was raised to 5.4 (average globule size increased from 40 to 60 µm in 40 days). At pH 7, the average globule size almost doubled, from 43 to 75 µm, after 40 days storage.

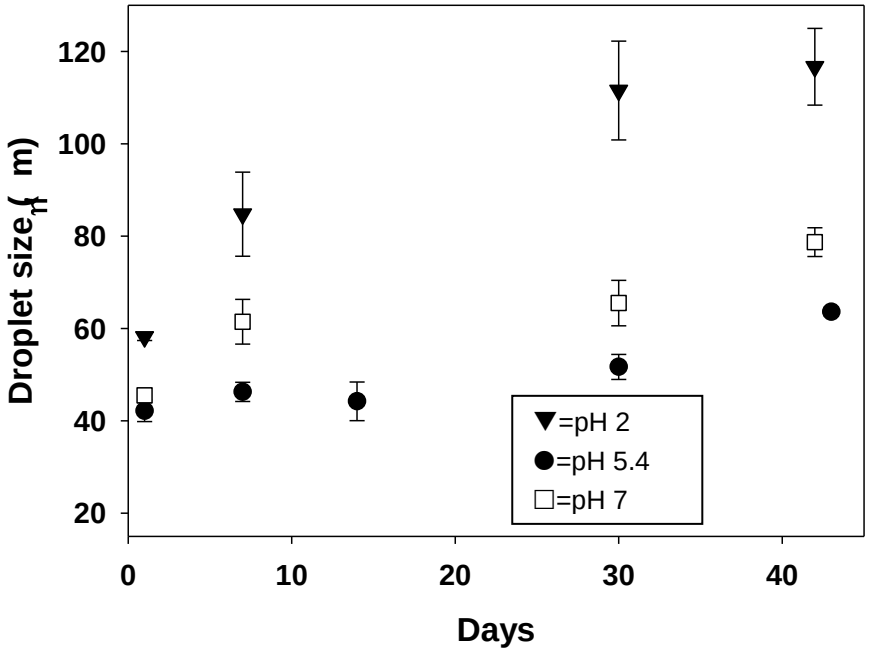

Figure 6.12: Double emulsion globule sizes of formulations containing 1 % mustard at various pH, storage temperature: 10 °C. Measurements performed using laser diffraction.

One likely reason for the observed instability of all formulations is the large size of mustard particle aggregates. Large aggregates cannot provide dense coverage at the interface so that parts of the droplet surfaces may be uncovered by particles (and therefore not protected against coalescence). Another reason is the presence of protein impurities in the mustard powder (up to 30% of the total). Proteins influence the adsorption of particles at the interface (see section 6.6) and may compete with the particles for the interface.

Although all formulations coalesce, the rate of coalescence depends on the pH of the double emulsion. The coalescence between double emulsion globules increases when the pH of the mustard particle solution is changed from initially 5.4 to either make it more alkaline (pH 7.2) or more acidic (pH 2). The formulation was least

stable at pH 2. One possible explanation for this phenomenon is the protein content of the mustard powder. As mentioned above, these proteins may be present at the interface and stabilise it. Reducing or increasing the pH alters the protein's surface charge. This causes a change in the protein structure, altering the adsorption properties of the protein. At pH values close to the pI, protein surface charge is at its minimum, which allows the proteins to pack together more tightly and associate more easily, giving a better coverage at the interface (Roth et al., 2000). The observations on emulsion stability indicate the pI to be around 5.

These results show that commercially available mustard powder in its present form is not suitable for the stabilisation of double emulsions. Purification of the mustard powder (e.g. removal of proteins) and/or milling of the powder to give smaller (submicron) particles might improve the potential of the use of such particles for the stabilisation of the secondary interface in double emulsions containing fat crystals.

6.3 Influence of the secondary emulsifier on salt release from emulsions

In order to assess the role of the secondary emulsifier on salt release, the formulations discussed in the previous sections stabilised at the secondary interface by silica (1 % at pH 2), OSA starch (2 %) or mustard powder (at pH 5.4) respectively were subjected to conductivity analysis (the method for this conductivity analysis was described in chapter 3). The primary emulsion water droplets contained 1.6 % KCl. In order to balance the osmotic pressure gradient between the two aqueous phases the continuous aqueous phase contained 10 % glucose in all formulations. Regular

conductivity measurements were performed on samples stored for several weeks at 10 or 25 °C.

Figure 6.13 gives an overview of the results. The formulations that were most stable against coalescence also showed the greatest resistance to releasing salt encapsulated within the primary emulsion water droplets: Formulations stabilised by 1 % silica stored at 10°C had released almost no salt to the continuous aqueous phase after 2 months of storage. When stored at 25 °C the same emulsion released around 25 % of the encapsulated salt. Formulations stabilised by 1% mustard powder and stored at 25 °C had released all salt within the first month. The double emulsion retained ~ 50 % of the total encapsulated salt when stored for 2 months at 10 °C. The formulation containing 2 % starch had released less than 20 % salt to the W_2 phase after two months storage at 10 °C. The same sample released 30 % salt when it was stored for the same time period at 25 °C.

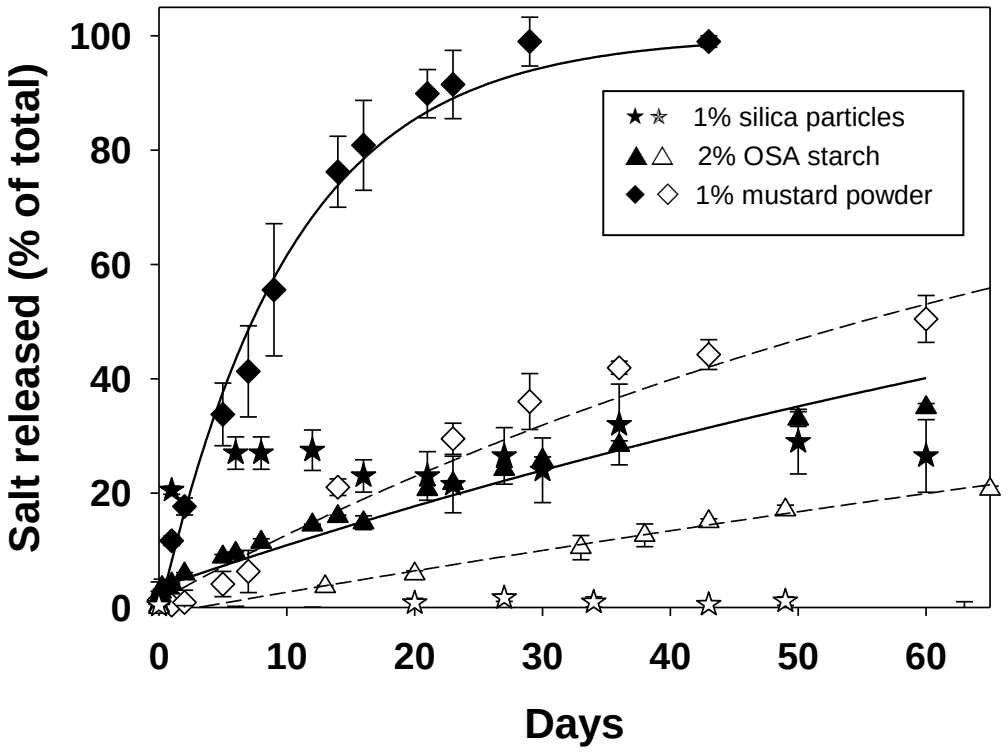

Figure 6.13: Release of KCl from the encapsulated W_1 phase to the continuous W_2 phase, when 1 % silica particles, 2 % OSA-starch or 1 % mustard particles were used as secondary emulsifiers. The open symbols denote release rates at 10 °C, and the closed symbols at 25 °C.

These results indicate that the secondary emulsifier has an influence on salt release. The primary emulsion composition was the same in all the formulations tested and there was no osmotic pressure gradient in any of the samples. The secondary emulsifiers that stabilised the double structure best also minimised salt release during storage.

The reason for salt release occurring despite the absence of an osmotic pressure gradient between the two aqueous phases is that chemical potential drives water/ solute transport in double emulsions. The existence of a crystalline “shell” surrounding primary emulsion water droplets minimises water transport in its own right because of the low mobility of the crystalline monoglycerides in the oil phase

(see chapter 5). However, some salt may nevertheless be released through imperfections in these “shells”. In order to be released to the continuous aqueous phase this “leaked” salt must pass the secondary interface

The important role of small-molecule surfactants aiding water/ solute transport across the oil boundary is demonstrated by the increased rate of release in all formulations at increased storage temperature (25 °C). This temperature lies above the melting temperature of monoglycerides (see chapter 4) so that monoglyceride molecules are mobile in the oil phase. These liquid small molecule emulsifiers can aid water/ solute transport either by the formation of lamellae (see chapter 2). The formation of reverse micelles in the oil phase is unlikely as the critical micelle concentration of monoglycerides, has been reported at around 2% wt/wt (Gaonkar & Borwankar, 1991).

When the monoglyceride is crystalline (storage temperature = 10 °C) the rate of release is slowed in all formulations. A secondary interface composed of a network of silica particles seems to prevent the migration of the salt released from W_1 to the continuous W_2 aqueous phase. A likely explanation for this is the lack of emulsifier which could facilitate the transport of water and solutes through the oil phase. This example demonstrates that salt release from W_1 to W_2 can be prevented when both interfaces are stabilised by particles (fat crystals at the primary interface, silica particles at the secondary one). Water has a limited solubility (less than 5 %) in sunflower oil (see sunflower oil MSDS). The movement of water/ solutes across the oil phase and the boundary of the secondary interface is limited due to an absence of

mobile amphiphilic molecules aiding such transport by, for example, the formation of reverse micelles.

Salt release is also slow (although not completely stopped) when the secondary interface is sterically stabilised (e.g. by OSA starch, but also Na-caseinate, see chapter 5). The transport of water/ solutes across the oil boundary is not facilitated by the formation of reverse micelles (described in chapter 2) because no small molecule emulsifiers that are able to move easily between the two interfaces exist. However, compared with formulations containing silica particles there is an increased rate of release (after 60 days of storage at 10 °C around 20 % of the total salt had been released to W_2 in formulations containing 2 % OSA starch). This indicates that the presence of a silica particle network at the secondary interface provides a better barrier to prevent the release of salt to the continuous aqueous phase.

A comparison with the release curves of formulations containing mustard particles further highlights the importance of the secondary interface to salt encapsulation. The rate of release at 10 °C is 3 - 4 times higher than that of formulations containing 2 % OSA starch. Poor particle coverage at the secondary interface permits water/ solute transport to take place at a faster rate. A possible mechanism for this is that the weakness of the secondary interface allows direct contact of the primary emulsion droplets with the continuous aqueous phase. Water/ solutes are therefore no longer limited by the rate of diffusion across the oil phase: Although the crystalline shells allow for the primary emulsion droplet structure to be maintained, salt leaks through the imperfections in the crystalline shells directly to the continuous aqueous phase (see chapter 5).

6.4 Salt release at elevated temperature

The ability of double emulsions stabilised by fat crystals to retain their structure and encapsulate salt at elevated temperatures (higher than 30 °C) was examined. In this way, the reaction of the double emulsions to conditions imitating those found in a human mouth upon consumption of the product could be tested.

In all formulations tested, the primary emulsion contained 30 % water (with 1.6 % KCl). The primary emulsion was stabilised by 0.5 % monoglyceride and 1% tripalmitin in the oil phase and was prepared according to the method described in chapter 3, and subsequently mixed with sunflower oil (ratio 1:1) to allow good dispersion in the secondary emulsification step. Double emulsions were prepared with 80 % continuous water phase, containing 2 % OSA starch, 1 % silica particles, 1 % Na-caseinate or 1 % mustard particles as secondary emulsifiers. In all samples, the osmotic pressure was balanced between the two aqueous phases by adding 10% glucose to the W_2 aqueous phase.

In order to measure salt release at elevated temperature in the various formulations the double emulsion samples were heated in a water bath set at 50 °C. These samples were stirred gently to ensure even temperature distribution. Continuous measurements of temperature and conductivity were taken in each sample until complete salt release was achieved.

Figure 6.14 shows that as the temperature rose beyond 32 °C, rapid and complete salt release occurred within 50 seconds in all formulations. When the double emulsion was stabilised by silica particles, approximately 25 % of encapsulated salt

was immediately released to the continuous aqueous phase as the temperature was increased beyond 20 °C. No more salt was released until the temperature reached 32 °C, at which point all remaining salt was released rapidly to the W_2. When the secondary interface was stabilised by other emulsifiers, the initial release was more gradual, but as the temperature rose beyond 32 °C, the release rate was just as rapid as in samples containing silica particles.

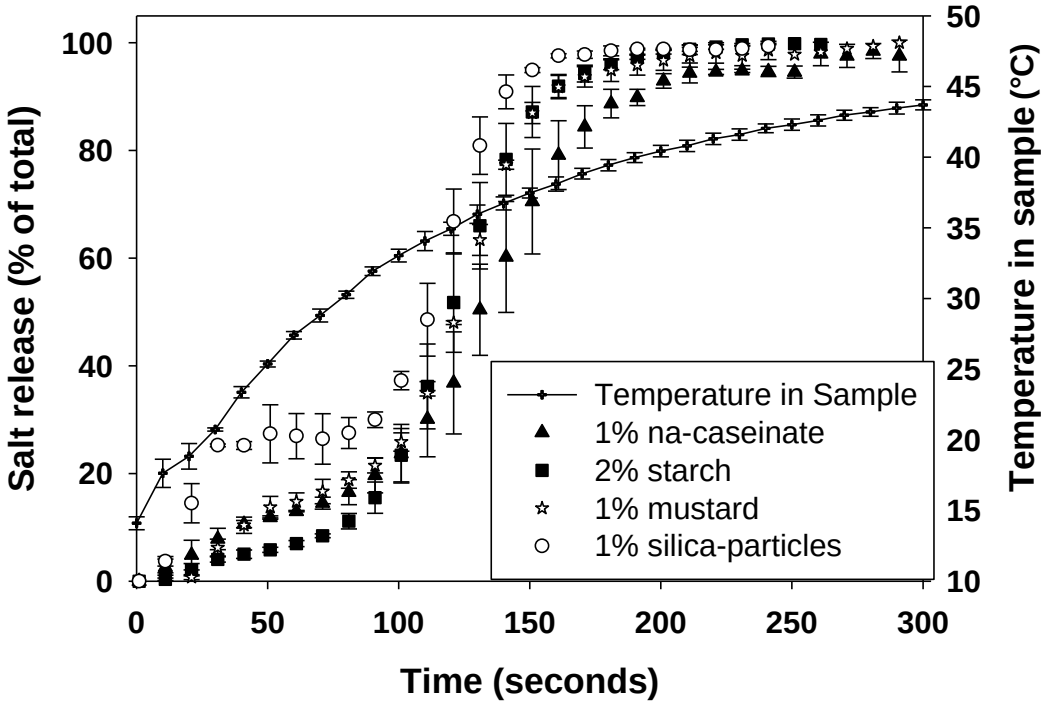

Figure 6.14: Salt release at elevated temperature: sudden and rapid release as the sample temperature rises above 30 °C

Figure 6.15 shows microscopy micrographs taken at 15 °C (before heating) and at 45°C (after heating), of an emulsion containing 1 % silica particles as secondary emulsifier. These micrographs confirm that the emulsion had lost its double character during the heating process. The emulsion was transformed into a simple O/W one. The secondary structure (i.e. the O/W_2 emulsion) is largely maintained when the

primary emulsion is destabilised in all formulations except those containing 1 % mustard particles, were all emulsion structure is lost entirely upon heating.

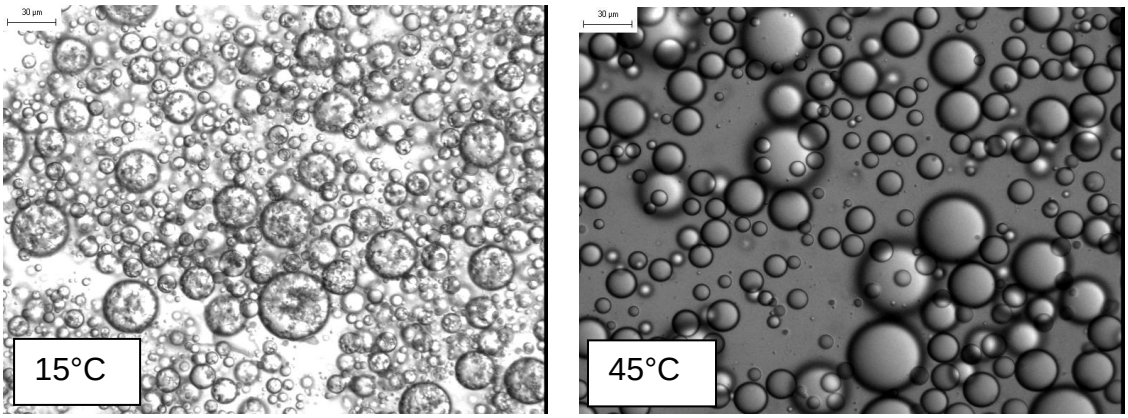

Figure 6.15: The emulsion microstructure changes from double to simple O/W character during heating

The reason for destabilisation of the primary emulsion at this temperature is that the stabilising fat crystal network surrounding the primary emulsion droplets melts (discussed in detail in chapter 4). The ensuing large-scale coalescence of the primary emulsion droplets leads to a rapid release of all water and encapsulated solutes to the continuous aqueous phase. The emulsion loses its double character.

These experiments show that the use of fat crystals to stabilise the primary emulsion enables direct control over the rate of release of an encapsulated substance to the continuous aqueous phase by varying the temperature. A storage temperature below the melting range of the crystals would result in long-term stable double emulsions, providing the secondary interface is adequately stabilised. On the other hand, an increase of temperature to the crystal melting point would cause complete destruction of the structure within seconds.

6.5 Shear stability of double emulsions

All stability measurements so far have been performed on double emulsions in quiescent conditions. However, knowledge of the behaviour of emulsions in a shear environment is also important in order to predict how stable a product will be in subsequent processing and transport or during use by the consumer. Moreover, double emulsions should have rheological properties similar to those of equivalent simple O/W emulsions, given that closely matched physical characteristics between full-fat and reduced-fat products are essential for consumer acceptance of reduced-fat products.

6.5.1 Shear stability of double emulsions containing silica particles

The flow properties of a simple and double emulsion stabilised by 1 % silica particles in the outer aqueous phase were compared with each other: Both emulsions contained 80 % aqueous phase (including 10 % glucose) and 20 % dispersed phase, but the double emulsion contained an additional 15 % water in form of primary emulsion droplets (stabilised by 0.5 % monoglyceride and 1 % triglyceride crystals). Both emulsions were produced by shearing for 3 minutes at 8000 rpm in the rotor/ stator mixer (for double emulsions, the primary emulsification step was described in chapter 3).

The behaviour of simple and double emulsions in sheared conditions was compared by shearing both samples at constant speed (10 s^{-1}) and gradually increasing the temperature from 15 to 55 °C. The geometry used was a 40 mm cone and plate. Increasing the temperature during the experiment allowed recording the influence of

temperature – and in double emulsions therefore the shift from double to single structure – on the viscosity of the sample. It is possible that the samples creamed slightly during measurement. However, the effect was deemed small because measurements were very reproducible and did not show obvious slip at any point.

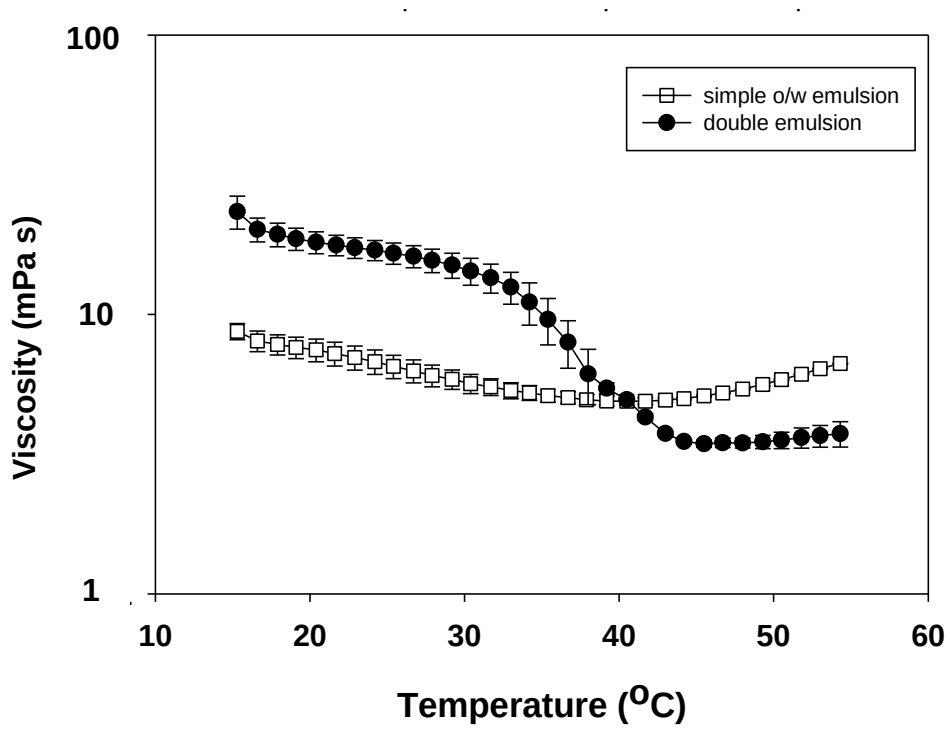

Figure 6.16: Comparison of viscosities at various temperatures in silica-stabilised simple (O/W) and double ($W_1/O/W_2$) emulsions. Shear rate = 10 s^{-1}

Figure 6.16 shows that in simple O/W emulsions stabilised by 1% silica particles, the viscosity was independent of temperature, with viscosity nearly constant at 9 mPas over the full range of temperatures investigated. In double emulsions, on the other hand, the viscosity changed with increasing temperature. Viscosity was constant at 20 mPas up to a temperature of 32 °C but dropped rapidly to a lower value of 7 mPas as the temperature was increased beyond 32 °C.

The viscosity of the double emulsion at temperatures below 32 °C was twice that that of the simple O/W one. There may be several reasons for this: The presence of the W/O emulsion droplets within the double globules could alter the emulsion's flow properties. Some of the stress experienced by the double globule may be transferred to the internal droplets. It was shown that in a double emulsion with a low internal emulsion viscosity, the internal droplets adsorb some of this stress applied to them in shear flow, resulting in a less distorted flow field of double emulsion globules. This means that less mechanical energy is dissipated into friction and that viscosity is reduced compared to simple O/W emulsions (Pal, 2008). As was shown in chapter 4, the primary emulsions have a very high viscosity compared to the continuous phase. The flow properties of double emulsions are influenced by the viscosity ratio of the W/O emulsion to the continuous phase (Pal, 2008). A high ratio of internal phase to continuous phase was shown to increase the viscosity of the double emulsion (Pal, 2008).

The presence of monoglycerides could also alter the network strength of silica particles in the water phase. It is possible that monoglycerides increase network strength of the silica particles by adsorbing at the particle surface and changing their surface properties, thus stabilising double emulsion globules by changing their packing properties at the interface (Pichot et al., 2009).

As the temperature was increased beyond 32 °C, the viscosity rapidly dropped to a new lower, but similarly constant value (7 mPas), slightly below the corresponding viscosity of the simple O/W emulsion. The temperature at which the viscosity drops coincides with the melting temperature of the fat crystals stabilising the primary

emulsion water droplets (see chapter 4). As was shown in section 6.4, the melting of the fat crystals invokes a destabilisation of the primary emulsion that results in loss of the double structure. The drop in viscosity can therefore be explained by an effective reduction of dispersed phase volume. This would also explain why the final viscosity of (previously) double emulsions is slightly lower than that of simple O/W emulsions.

6.5.2 Shear stability of emulsions stabilised by OSA starch

The experiment was repeated using simple O/W and double emulsions stabilised by 2 % OSA starch. The double formulations contained 80 % continuous aqueous phase (including 10 % glucose) and were prepared as described in section 6.2.3. The simple O/W emulsion (containing 20 % oil) was prepared by shearing in the rotor/ stator mixer for 3 minutes at 8000 rpm. The emulsion was then sheared at a constant shear rate 7 s^{-1} as a pure 2 % starch solution gave constant viscosity readings at this rate (see Figure 6.5). The temperature was gradually increased from 15 to 55 °C.

Figure 6.17 shows that the viscosity of simple O/W emulsions was independent of temperature up to around 45 °C. It is likely that the subsequent increase in viscosity is associated with the gelatinisation of the starch at the interface as well as in the bulk aqueous solution (Bao *et.al.*, 2003). The viscosity of the double emulsion, on the other hand, was constant up to 32 °C. As in silica-stabilised double emulsions, the viscosity fell as the temperature was increased above 32 °C. Again, as in formulations containing 1 % silica particles, this is associated with a reduction in the dispersed volume phase. Once all crystals had melted (~45 °C), the viscosity increased steadily from the minimum level registered at this point. Light microscopy

confirmed that all oil globules were devoid of primary emulsion droplets at 45 °C (similar to the double emulsion stabilised by 1% silica particles, shown in Figure 6.15).

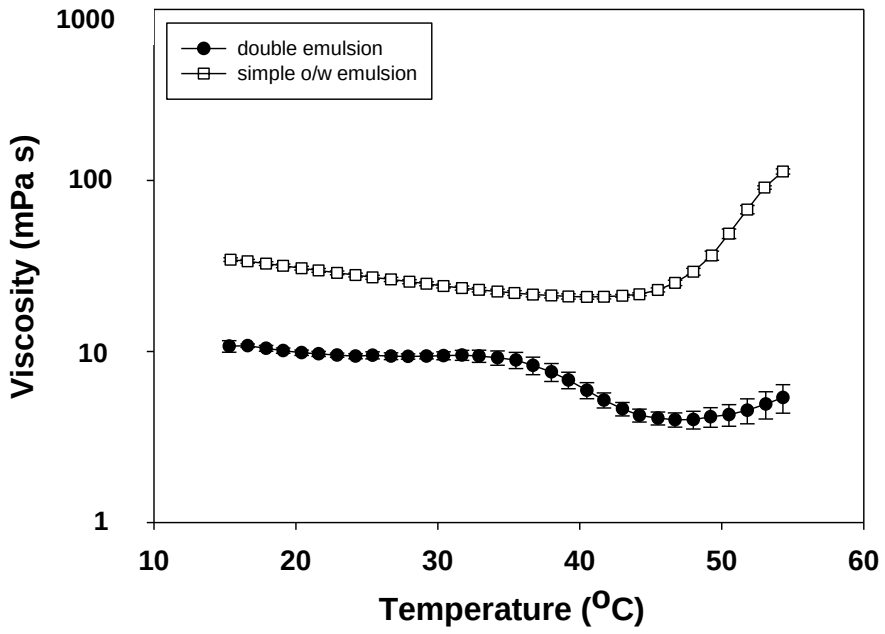

Figure 6.17: Viscosity at constant shear ($7s^{-1}$) and increasing temperature (15-55 °C) of simple and double emulsions containing 2 % OSA starch in the external water phase

These results indicate that OSA starch, like the silica particles, stabilised double emulsion globules in a sheared environment. However, as was shown in section 6.4, the stability of the primary emulsion is sensitive to temperatures exceeding 30 °C. This invokes a transformation of the double structure to a simple O/W one, and is visible in rheological measurements.

6.5.3 Shear stability of emulsions stabilised by Na-caseinate

Further experiments were performed with double emulsions stabilised by 1% Na-caseinate as the secondary emulsifier. The simple O/W emulsion also contained 1% Na-caseinate as emulsifier and was produced by shearing at 8000 rpm for 3 minutes in the rotor/ stator device. The emulsion was then sheared at a constant shear rate of 5 s^{-1}. This shear rate enabled reproducible measurements despite the shear sensitivity of the emulsion. The temperature was gradually increased from 15 to 55°C.

Figure 6.18 shows a comparison of the viscosity profiles of a simple O/W and a double emulsion stabilised by Na-caseinate. The viscosity of simple O/W emulsions decreases steadily as the temperature is increased. The reason for this is that the emulsion becomes destabilised under continuous shear and increasing temperature. Microscopy micrographs confirm that coalescence occurs in these samples.

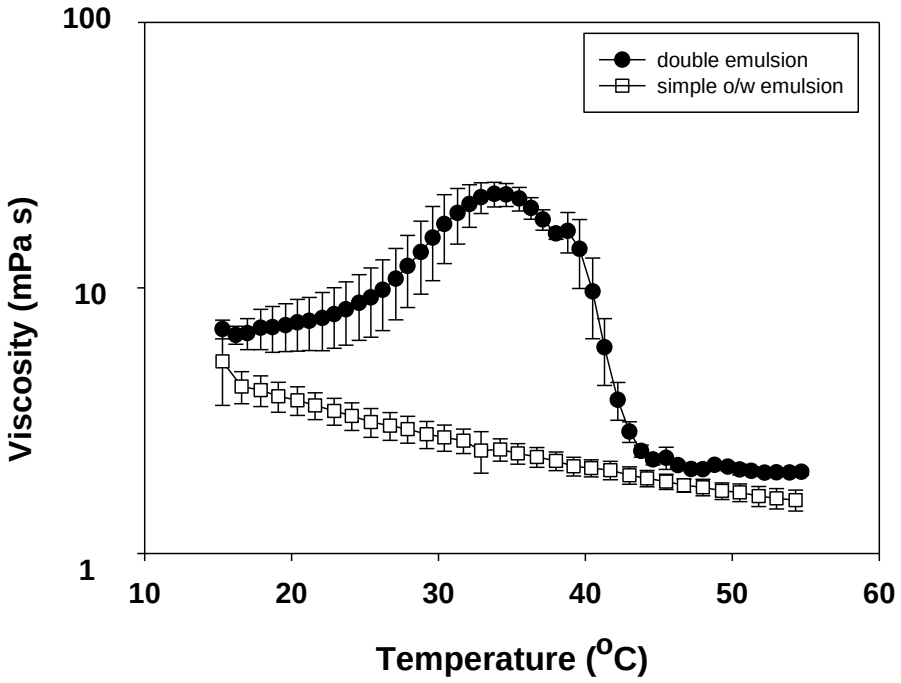

Figure 6.18: Influence of temperature on viscosity in simple and double emulsions containing Na-caseinate in the W_2 phase. Shear rate = 5 s^{-1}

The viscosity of double emulsions, on the other hand, increased steeply as the temperature was increased from 25 - 32 °C. Viscosity fell sharply as temperature was subsequently further increased to 45 °C. With further temperature increase, viscosity remained constant. The reason for the viscosity increase at temperatures less than 32 °C is partial coalescence of double emulsion globules – crystals protruded from globule interfaces and pierced the interfacial film of neighbouring globules, as discussed in chapter 5. The formation of aggregates limits the individual globule's ability to deform, which increases effective droplet size and thus emulsion viscosity.

As the temperature rose above the crystal melting point, partial coalescence was reversed. Partially coalesced drops, previously held together by a network of fat crystals, regained their deformability either by coalescing fully or breaking apart

under the applied shear. This loss of internal aqueous phase caused a reduction in dispersed phase volume which, along with the regaining of droplet deformability, caused a sharp decrease in viscosity.

The process of aggregate formation in line with increasing temperature, followed by de-aggregation and emptying of the internal W_1 phase, could be confirmed using optical microscopy. Samples were taken from the emulsion being sheared when the respective temperature was reached and analysed immediately in the microscope.

A series of micrographs demonstrating the effects of increasing temperature are shown in Figure 6.19. These micrographs show how aggregates are formed in double emulsions that were sheared at temperatures of 25 °C and above. Globules were loosely aggregated at 25 °C. At 30 °C these aggregates are very dense structures in which it is difficult to distinguish between individual globules. Once the temperature of the sheared sample has reached 40 °C the aggregates have disappeared and the emulsion no longer has a double structure.

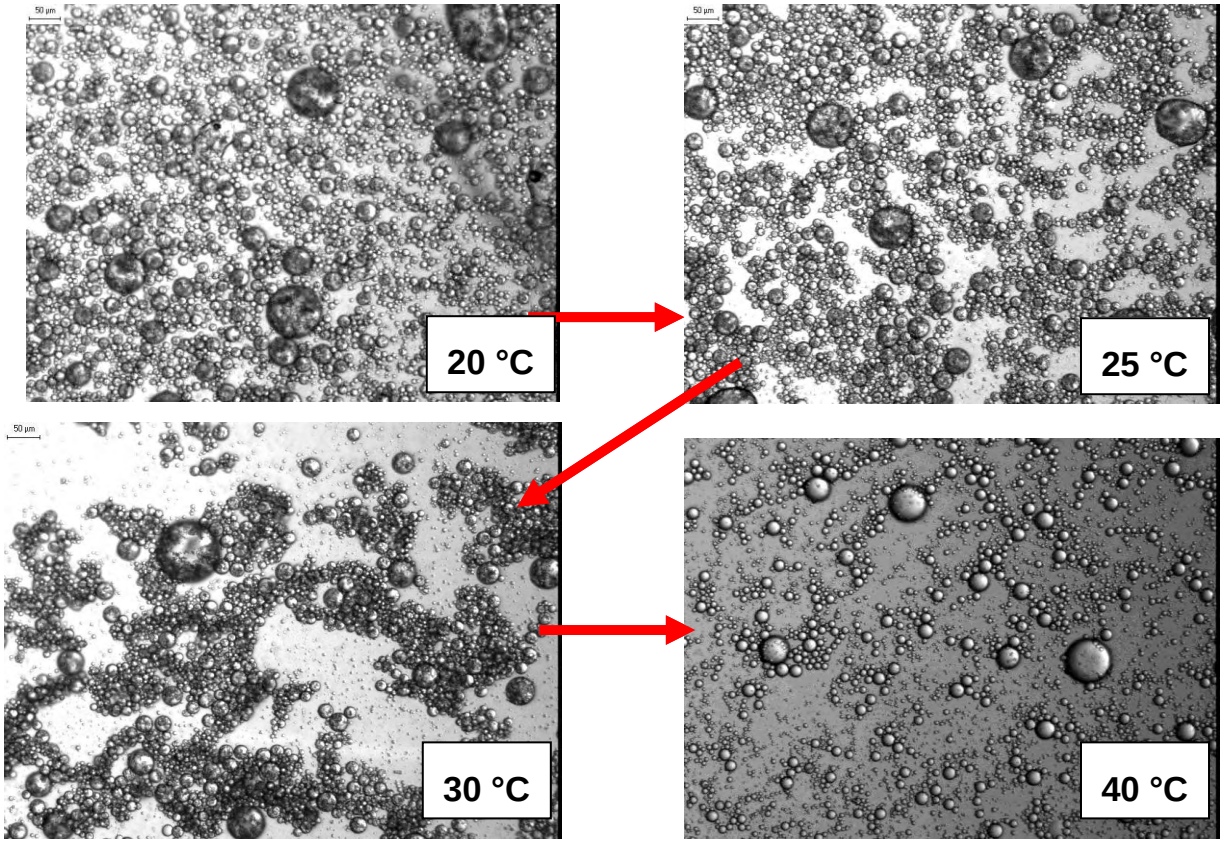

Figure 6.19: Different states of aggregation of double emulsions stabilised by 1% Na-caseinate in W_2 during constantly applied shear at increasing temperatures

When the behaviour of formulations containing Na-caseinate under shear is compared to those containing OSA starch or silica particles, it is clear that the choice of secondary emulsifier is crucial to the stability of the double emulsion. The presence of fat crystals can cause partial coalescence and thus destabilise the double structure. However, a sufficiently rigid or thick interface seems to be able to resist such destabilisation even when shear brings the double globules in close contact with each other. Such thick interfaces can be formed using small particles or modified starch, which has the ability of forming thick interfacial films of up to 16 $mg.m^{-2}$ (see section 6.2.3). Na-caseinate films, which have a maximum surface

coverage of 3 $mg.m^{-2}$ (Dickinson 1998) were apparently not loaded enough to prevent the partial coalescence of double globules during shear.

There is a clear relationship between the shear stability of double emulsions and their stability against coalescence in quiescent conditions. In quiescent conditions the presence of OSA starch or silica particles at the secondary interface prevents coalescence. Droplet sizes, however, increased when Na-caseinate was the secondary emulsifier (see chapter 5). When shear is applied to the formulations, OSA starch and silica particles stabilise the double emulsions up to a temperature of 32 °C, which is when the primary emulsion melts. Double globules in formulations containing Na-caseinate, on the other hand aggregate and partially coalesce when shear is applied at temperatures lower than 32 °C.

6.6 Particle/ protein interactions in double emulsions

The previous sections suggest that the presence of particles at the secondary interface can prevent coalescence in double emulsions containing fat crystals. However, “natural”, i.e. not chemically synthesised, particles often contain impurities in the form of proteins, and this may affect the stability of double emulsion. For instance, protein residues were thought to be one of the reasons for the poor stability of double emulsions stabilised by mustard powder. In order to assess the effect of the presence of protein impurities on double emulsion stability in a controlled way, Na-caseinate was added in various concentrations to the 1 % silica particle solution (ratios of 1:10, 1:4 and 1:1 Na-caseinate:silica particles).

The formulation and formation of the primary and double emulsions was the same as described in the previous sections. The continuous W_2 aqueous phase always contained 1 % silica particles and 10 % glucose. Various amounts of Na-caseinate (0.1 %, 0.25 or 1 %) were added after the particles had been dispersed in water and before the double emulsion was made by slowly adding the primary emulsion to the sheared continuous phase and then shearing at 8000 rpm for 3 minutes in the rotor/ stator mixer.

Double emulsions become increasingly unstable to coalescence as protein is added in increasing quantities to the formulation. This destabilisation was apparent at storage temperatures of 10 as well as 25 °C, but was more drastic at the higher temperature. Laser diffraction could not be used to assess the stability of double emulsions containing both particles and Na-caseinate as secondary emulsifier, the reason being that these formulations showed poor shear stability: the structures broke during measurement. Instead, the relative stability of the double structures was assessed using light microscopy micrographs taken of samples stored at 10 or 25 °C at regular intervals.

When 1% protein was added to the W_2 phase (ratio protein:particles 1:1), the double emulsion was almost immediately destabilised. Although the primary emulsion remained largely intact, it was no longer incorporated into a double structure thus resembling the results of formulations found to be unstable described in chapter 5.

Table 6.1: Globule sizes in double emulsions containing 0.25 % Na-caseinate and 1 % silica particles in W_2, measured using light microscopy

Storage temperature	$D_{3,2}$ (μm)			
	After 1 day	1 week	2 weeks	4 weeks
10°C	32 ± 5	40 ± 11	40 ± 5	54 ± 5
25°C	45 ± 2	81 ± 15	91 ± 13	n/a

When the protein: particle ratio was lowered to 1:4, the double structure was not retained for longer than 2 weeks when the sample was stored at 25 °C. Although the double structure was retained for a longer period than in a sample containing 1% protein, the average globule size increased rapidly as large aggregates of primary emulsion formed (see Table 6.1). At 10 °C, the double structure was barely retained after storage for 4 weeks and at which point primary emulsion aggregates became increasingly visible (Figure 6.20).

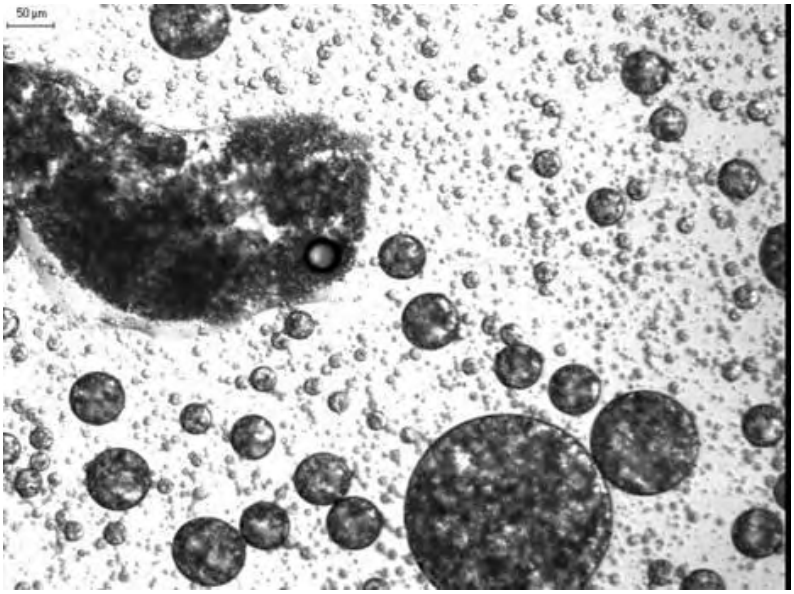

Figure 6.20: Double emulsion containing 0.25 % Na-caseinate and 1 % particles after storage at 10 °C for 4 weeks.

When the protein:particle ratio was further reduced to 1:10, the double structure was more stable than with the higher protein concentration, especially when stored at 10°C (see Table 6.2). However, stability against coalescence was lower than in a formulation containing only particles and no additional protein. The micrographs in Figure 6.21 clearly show that at 25 °C large primary emulsion aggregates had formed within a few weeks of storage. On the other hand, when the sample was stored at 10°C large aggregates were not observed and the double structure was retained – although some coalescence took place.

Table 6.2: Average globule size in double emulsions containing 0.1% Na-caseinate and 1% silica particles in W_2

Storage temperature	$D_{3,2}$ (μm)		
	After 1 day	1 week	4 weeks
10 °C	29 ± 1	35 ± 3	35 ± 5
25 °C	29 ± 1	32 ± 4	67 ± 4

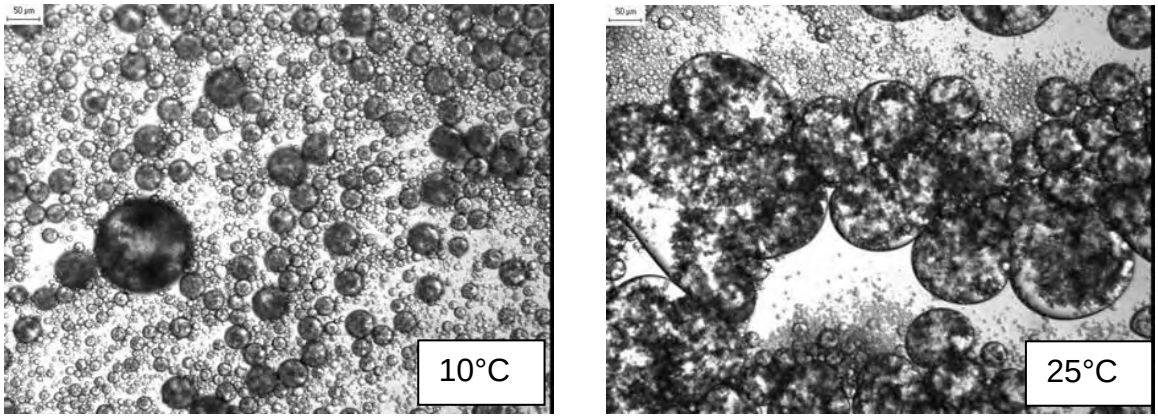

Figure 6.21: Micrographs of emulsions after 4 weeks' storage at 10 or 25°C

One of the reasons that protein addition causes instability in particle-stabilised double emulsions may be a weakening of the secondary interfacial structure due to competition of proteins with particles for the interface. Interfacial tension was measured between sunflower oil and an aqueous phase that contained silica particles, Na-caseinate or a combination of both according to the procedure described in chapter 3. Table 6.3 shows that sodium caseinate has a higher affinity for the interface than silica particles. While the addition of sodium caseinate to the oil

phase significantly lowers interfacial tension, the presence of silica particles has no effect on interfacial tension between the oil and water phase.

Table 6.3: Interfacial tension between W_2 aqueous phase and either pure sunflower oil or the mixture of mono-and triglycerides in sunflower oil used in the double emulsion oil phase

	Sunflower oil
Pure water	25.1 ± 0.2 mN m^{-1}
1% silica particles	24.9 ± 0.4 mN m^{-1}
1% Na-caseinate	13.2 ± 0.1 mN m^{-1}
0.1% Na-cas + 1% particles	17.4 ± 0.6 mN m^{-1}
0.25% Na-cas + 1% particles	15.5 ± 0.3 mN m^{-1}

This suggests that during emulsification Na-caseinate initially coats newly formed interfaces more rapidly than silica particles, although silica particles may subsequently become strongly attached to the interface (Pichot et al., 2009). The ultimate stability of the interface seems to depend on the concentration of Na-caseinate. The proteins could also attach to the silica particle surface and alter their surface properties.

At a protein:particle ratio of 1:10, particles are likely to cover most of the interfacial area because there is insufficient protein present to cover the interfaces fully. Fang & Dalgleish (1993) have shown that a minimum surface coverage of 1 $mg.m^{-2}$ is required for caseinate molecules to form monolayers and stabilise emulsions. A calculation including the specific surface area of the oil droplets and the oil fraction revealed that the minimum concentration of protein necessary to reach this surface

coverage is 0.12% in the aqueous phase, assuming all proteins adsorb at the interface. Therefore, a concentration of 0.1 % Na-caseinate does not give full surface coverage of proteins on the emulsion globule, allowing silica particles to become adsorbed at the surfaces. Network formation between silica particles could subsequently displace Na-caseinate molecules from the interface so that they cover a majority of the interfacial area.

On the other hand, protein concentrations above 0.1 % drastically reduce double emulsion stability. The presence of a higher concentration of unattached proteins and/ or silica particles in the W_2 aqueous phase may lead to depletion flocculation. Excess “free” particles and/ or micelles of proteins become excluded from the gap between two approaching droplets because of their size, causing an osmotic gradient between the pure fluid contained within the gap and the fluid containing particles and/ or protein micelles one outside. This will cause film drainage between the two droplets and cause flocculation, which may subsequently lead to coalescence. It is expected that a higher concentration of protein in the W_2 aqueous phase will lead to faster depletion flocculation due to the presence of more “free” protein micelles.

The competition between the two species results in “patchy” interfaces visualised using SEM. Figure 6.22 shows an example of a double globule containing 0.25 % Na-caseinate and 1 % silica particles. Although protusions that might constitute particles were seen at the interface, these did not form a continuous network. Furthermore, the double globules were not spherical, a probable result of fat crystals protruding from the weakened secondary interface. The combination of a weak

interface and protruding fat crystals would explain the observed instability of this formulation.

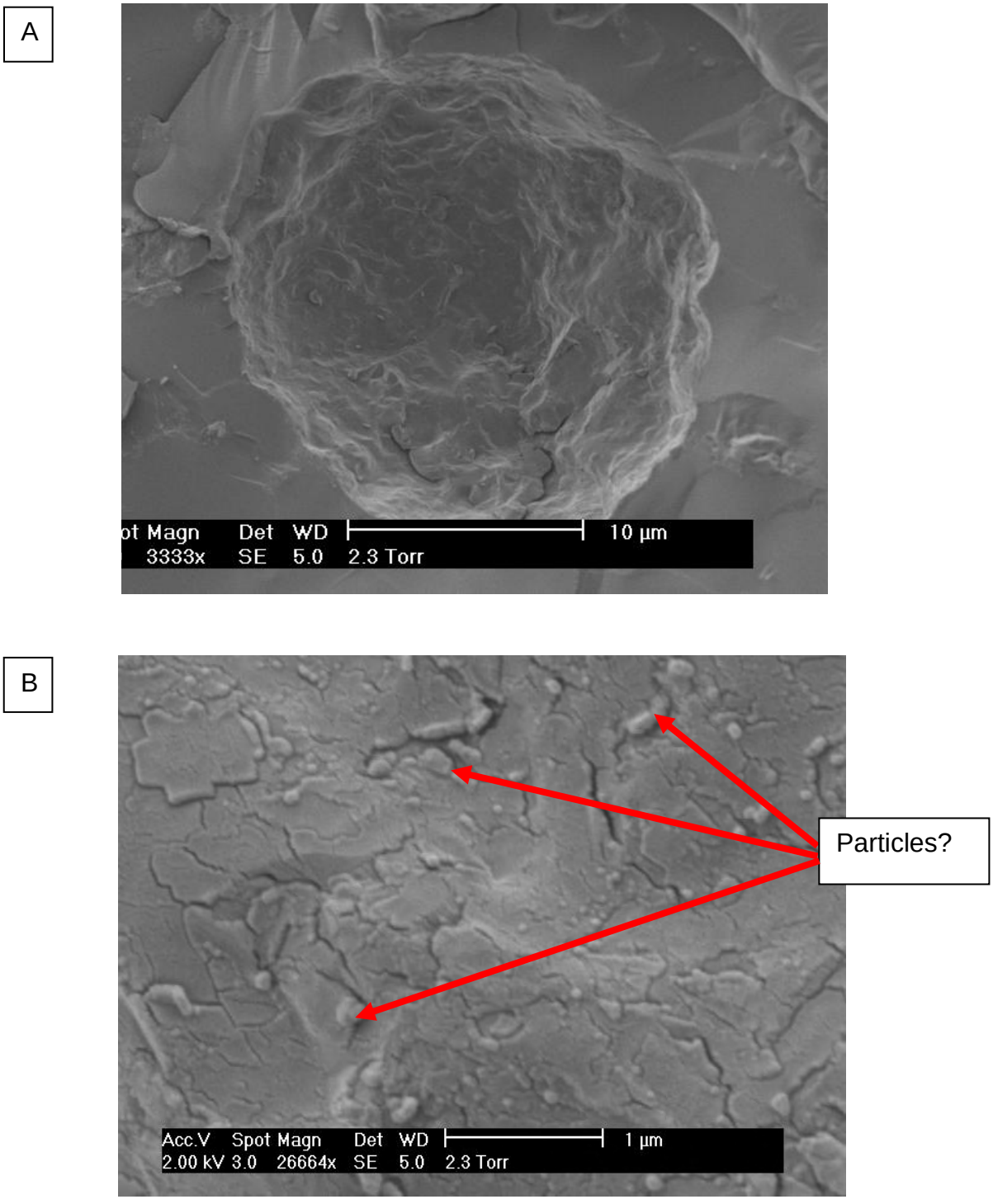

Figure 6.22: Secondary interface in 1-week old emulsion containing 0.25% Na-caseinate and 1% particles: the double emulsion oil globule structure (A) and a close-up of the interface (B)

6.7 Conclusions

This chapter has highlighted the importance of the secondary emulsifier in stabilising double emulsions containing fat crystals. Pickering stabilisation by particles can effectively protect double emulsions against coalescence at rest as well as during shear, and stop any salt from being transported across the secondary interface. However, it was shown that the size and purity of particles is important: large particles or particle aggregates prevent full surface coverage, while the presence of protein impurities can equally disrupt the interfacial structure by causing depletion flocculation.

Double emulsions also showed good stability (at 10 °C) when they were stabilised by OSA starch. Although salt release could not be stopped entirely, double emulsions were stable against coalescence at rest as well as during shear. Compared with Na-caseinate stabilised emulsions, which were unstable during shear and showed coalescence when stored in quiescent conditions, OSA starch provided much better stability. Both compounds sterically stabilise the double emulsions. It is therefore likely that the thickness of the interfacial layer, which in the case of OSA starch can be a multiple of that formed by Na-caseinate molecules, is important to the stability of double emulsions. Thicker interfacial layers can prevent any fat crystals present in the oil phase from protruding from the double globules.

Finally, the stability of double emulsions was compromised in all cases when the storage temperature was raised to 25 °C. At this temperature an increased amount of monoglycerides cease to be crystalline and become mobile within the oil phase. The

ensuing competition between different emulsifier species at the secondary interface contributes to more rapid destabilisation of the double structure.

7 Conclusions

This thesis has examined double emulsions containing fat crystal-stabilised primary emulsions. Specifically, this work consisted of

- Formulating and characterising W/O emulsions stabilised by fat crystals and investigating their ability to retain encapsulated salt at various temperatures and osmotic pressure gradients

- Formulating and characterising double emulsions containing fat crystal-stabilised primary emulsions using either membrane emulsification or rotor/stator technology and containing a range of monomeric and polymeric emulsifiers on the secondary interface

- Optimising the formulation to create double emulsions that are resistant to coalescence and stable in a sheared environment.

7.1 Fat crystal-stabilised W/O emulsions

W/O emulsions were prepared and stabilised by 0.5% monoglyceride and between 0 and 2% tripalmitin and with a water content of 40%. The emulsions showed excellent stability when they were stabilised by a combination of 0.5% monoglycerides and at least 1% tripalmitin for at least 3 months. The joint presence of tripalmitin and monoglyceride crystals at the interface caused the formation of a crystal network. In post-crystallisation processes the mono and triglycerides sintered to form very tight crystal networks, or “shells”, around individual water droplets. The role of surface-

active monoglyceride crystals was to aid droplet break-up during emulsification as well as to seed triglyceride crystals at the interface.

The tight crystalline networks surrounding the water droplets were able to retain salt encapsulated in the water phase even when relatively large osmotic pressure gradients (14 atm) were applied. Less than 5 % of total encapsulated NaCl was released to an external water phase after 45 days storage from emulsions stable at room temperature.

The stability of the emulsion structure with respect to coalescence as well as its ability to encapsulate salt was temperature dependent and decreased when the sample was stored at temperatures greater than 25 °C. The emulsion was completely unstable when it was heated to more than 30 °C. A link was established between the stability of the emulsions and the melting characteristics of the fat crystals, which had a melting range between 20 and 50 °C.

These results show that control of stability against coalescence and release of encapsulated salt is possible in fat crystal-stabilised emulsions by varying the temperature of the product. At temperatures below the melting point of the fat crystals it is possible to keep salt encapsulated within the water droplets, and thus to keep it separate from an external aqueous phase.

7.2 Formulation of double emulsions containing fat crystals

Double emulsions were produced using a rotor/ stator for the secondary emulsification step. These were able to retain salt encapsulated within W_1 and did not

release salt to W_2 despite the existence of an osmotic pressure gradient, although this was dependent on the type of secondary emulsifier used. Monomeric emulsifiers and globular proteins could not stabilise the secondary interface. This was attributed to their inability to provide interfacial layers sufficiently loaded to prevent the protrusion of fat crystals from the double globules and their subsequent coalescence.

When Na-caseinate was used as secondary emulsifier, the double structure was retained for at least 6 weeks when the osmotic pressure gradient was matched between W_1 and W_2, although coalescence occurred and globule size doubled from ~23 to ~46 µm. 20 % of the total KCl encapsulated within the internal aqueous phase was released during this time period.

The stability of the double emulsions depended on the direction of the osmotic pressure gradient between the two aqueous phases. Double structure was retained over the period of 8 weeks when the osmotic pressure gradient between internal and external aqueous phase was 0 or negative (i.e., the solute concentration being higher in the external than internal aqueous phase). This was attributed to a compression of the crystalline structure which strengthened the “shells” and prevented water/ solute transport across the boundary.

When the osmotic pressure gradient was positive, the double emulsions were unstable. This was due to tensile loading of the crystalline structure, which pushed the sintered crystal segments apart at imperfections and cracks sustained during the secondary emulsification step.

Membranes were assessed for their suitability for application in the secondary emulsification step but were found to be unsuitable for the secondary emulsification step.

7.3 Pickering-in-Pickering double emulsions

The effect of adding particles to the secondary interface was investigated. The use of 1 % silica particles effectively prevented the coalescence of double globules and ensured that average droplet size remained stable at 22 µm over the course of three months. When 2 % OSA starch was used as stabiliser for the secondary interface the globule size remained stable at 12 µm after three months storage at 10 °C. Both substances form rigid interfacial structures (Binks, 2002, Tesch et al., 2002) that are apparently resistant to fat crystals “piercing” the interface.

Both formulations were also stable when shear (between 7 and 10 s^{-1}) was applied, although a sharp fall in viscosity was observed at 30 °C, which coincided with the melting temperature of the fat crystals stabilising the primary emulsion. Temperature was thereby shown to be a crucial factor in determining the stability of the double emulsions investigated. It was also shown that 1 % Na-caseinate could not stabilise the secondary interface against shear as it induced partial coalescence.

Salt release can be controlled using temperature and complete salt release within seconds can be triggered at temperatures that are higher than the melting point of the fat crystals. For the large part, salt remained encapsulated within primary emulsion water droplets when the samples were stored at low temperatures (below 15 °C). The use of 1 % silica particles for stabilisation of the secondary interface was

sufficient to stop salt release at this storage temperature almost completely. When the storage temperature was increased to 25 °C salt release rates increased in all formulations. When the samples were exposed to temperatures greater than 30 °C, the melting point of the fat crystals, complete salt release occurred in all formulations within seconds.

The presence of protein impurities in the continuous aqueous phase of particle-stabilised double emulsions had a negative effect on their stability. This was ascribed to competition between the two species for interfacial space as well as the adsorption of proteins to the silica particles causing a change in their surface properties.

7.4 Future Work

Based on and proceeding from the findings of the work conducted for this thesis the following areas warrant further investigation:

- **Optimisation of the primary emulsion formulation**

The presence of fat crystals in the oil phase, and protruding from the double globule surface, contributed to instability in a number of the double emulsion formulations investigated in this work. Although the influence of fat crystal concentration on emulsion stability was investigated to some extent, the influence of other factors such as temperature oscillation, cooling rate or emulsifier concentration on the location of the crystals within the emulsion was not explored.

Changing the cooling rate and oscillating the temperature during processing might well affect crystal size and location. A faster cooling rate could well lead to the formation of smaller crystals while temperature oscillation might enhance post-crystallisation processes such as sintering. A more detailed characterisation of the crystallisation process within the “A” and “C” units might therefore lead to process improvements allowing control of the precise placement of crystals on the droplet interface. The final outcome would be a process in which the majority of crystals would be utilised for the formation of smooth crystal “shells” while minimising the number of crystals not bound to or protruding from the interfacial crystal network. A reduction of potentially protruding crystals from double globules would result in double emulsion stability that is less dependent on the thickness of the secondary interface.

Changing the monoglyceride-tripalmitin ratio, or using different solid fat components, might also affect the thickness of the crystalline shells and the location of fat crystals in the formulation. The monoglyceride content in this work was kept low at 0.5 % and the influence of increasing monoglyceride concentration in the presence of tripalmitin was not investigated. It is conceivable that the addition of more monoglyceride could induce faster crystallisation, given that crystal formation depends on concentration as well as temperature (Mullin, 1993). This would induce a more rapid seeding of tripalmitin crystals at the interface. Increased tripalmitin and monoglyceride concentration there would result in an increase in “shell” thickness and thus increase the resistance of the primary emulsion to the high-shear conditions of the secondary emulsification step. Here the assumption is that increased “shell” thickness would increase the rigidity and structural integrity of the crystalline interface. Further experimental work is required in order to determine whether this approach is feasible.

- **Reduction of primary emulsion droplet size**

Throughout the work performed for this thesis, the droplet size of primary emulsions was kept at a constant 3 - 4 μm, the major reason being the limitations imposed by the equipment used for processing the emulsions. The shear achievable within the A and C units, given that they were already run at maximum shaft rotation (maximum shear), could not be increased further. The emulsions were passed twice through the units and droplet size could not be further reduced in a third run. Primary emulsion droplet size was thus relatively large and implies a low packing efficiency of primary emulsion droplets within double globules. Furthermore, primary emulsion droplets in this size range require double globules that are at least greater than 5 μm to allow encapsulation of at least one water droplet within each double globule. However,

emulsions are more stable when their droplets are small because they are less likely to cream (McClements, 2005a). Creaming makes coalescence more likely as droplets are in close contact with each other. It would therefore be desirable to reduce primary emulsion droplet size.

For sensory reasons, droplet size in commercially available O/W emulsions is usually smaller than 10 µm. Furthermore, in order to achieve fat reductions of 40 % or more by adding water droplets into double globules, these must be as tightly packed as possible to accommodate the volume of water within. Reducing the size of primary emulsion droplets, preferably to less than 1 µm, is thus an important objective. An improvement to the design of the "A" and "C" units to remove dead zones (areas in the mixers that experience low or no shear[2]) and increase shear rates are an important preconditions for reducing droplet size to ~1 µm, thereby allowing for tighter packing and the inclusion of a larger aqueous phase volume to the oil phase.

- **Water loading of the double emulsions**

Throughout this study, the $(W_1:O):W_2$ ratio was kept constant at 20:80, in order to allow comparison of different formulations. As mentioned above the inclusion of more water in a double emulsion is a decisive part of the quest for fat reduction and potential commercialisation of the product.

Experiments have shown that it is possible to reduce the ratio of sunflower oil: primary emulsion from 1:1 to 1:4, and still achieve mixing in the rotor/ stator. Furthermore, such a 1:4 mixture was incorporated into a double structure containing

[2] Gabriele (2011) presents data that shows the existence of such areas in the C-unit

2% OSA starch at a ratio (primary emulsion) : (W_2 aqueous phase) of 1:1. This formulation was stable for at least 1 week despite the high concentration of double globules. This is an emulsion containing 50 % fat phase. However, the actual fat content is only 38 % because of the inclusion of water droplets within the oily globules. More concentrated double emulsions containing a higher proportion of encapsulated water require further and more detailed investigation. Further optimisation of the primary emulsion structure might further improve these results.

With smaller W_1 emulsion droplets and fewer “free” crystals in the primary emulsion, it might be possible to reduce the viscosity to an extent sufficient to remove the need for dilution of the primary emulsion with sunflower oil prior to the secondary emulsification step. This would enable investigation of the stability of primary emulsions containing up to 80 % water and of double structures containing equally large amounts of dispersed primary emulsion.

- **Crystallisation of the secondary interface**

In light of the stability of fat crystal-stabilised W/O emulsions against coalescence, it would be interesting to investigate crystal-stabilised O/W emulsions. Highly polar crystals such as monoglycerides could adsorb at the secondary interface and form tight networks similar to those found on the primary emulsion droplets. The main challenge for this approach will be to find a suitable crystalline material that is preferably wetted by the aqueous phase.

- **Membrane emulsification with volume-controlled feed mechanism**

This study exposed the difficulties of membrane emulsification using a pressure-controlled feed system. Most problems experienced in this work were related to the high viscosity of the primary emulsion causing air to channel through the emulsion. Further investigation into membrane emulsification using volume-controlled feed systems is desirable because this technique avoids application of high shear rates for the production of double globules.

Experiments utilising a volume-controlled feed system successfully produced double emulsion globules. However, the membrane associated with this system contained very large (80 μm) pores so that the samples were unstable. The double globules produced creamed rapidly and were unable to retain their spherical shape, causing phase separation.

Trials should be performed using membranes containing smaller pores (~10 – 15 μm) and a volume-controlled feed system. Such a set up would solve the problem of air channelling through the primary emulsion. At a conceptual level there is little reason to doubt the potential of this technique for the production of small double globules without significant damage to the primary emulsion crystalline “shells”.

- **Scale-up of the process**

The fact that the fat crystal “shells” surrounding primary emulsion droplets can apparently retain their structure in a high shear processing environment is promising for a future application of double emulsions stabilised by fat crystals in fat-reduced

food products. Investigation is required to ascertain whether primary emulsions of the kind investigated in this study can be made successfully employing larger scale A&C units (throughput in the order of tens of kg/hr).

Additional trials using a pin stirrer/ homogeniser combination for the secondary emulsification step are also needed. These are commonly used in pilot scale trials in the food industry with a minimum throughput of 4 kg/hr.

The capacity of such a device is large compared to the production volume of the “A” and “C” units that were used in this study to make the primary emulsion (~25 ml/min). An up-scaling of the primary emulsion production units is also essential. This can be done either by building bigger units or by finding alternative, more efficient ways of seeding fat crystals directly at the interface (e.g. by using membrane emulsification, a very cold water phase and hot oil phase) and would allow an examination of the issues involving alternative processing techniques and up-scaling of double emulsion production.

8 Appendix

8.1 Melting and crystallisation profiles of individual monoglyceride components Dimodan P Pel/B and Dimodan HP

The individual melting and crystallisation profiles of the two monoglycerides Dimodan P Pel/B and Dimodan HP were measured in the DSC. The measuring protocol was described in chapter 3.

Representative curves of the individual heating and cooling profiles are given in Figure 8.1. These show that the two monoglycerides used show different melting (red line) and crystallisation (black line) profiles, indicating that have a different composition. Both monoglycerides show two melting peaks (M1, at 10 - 20 °C) and M2, at 50 – 70 °C) upon heating and two crystallisation peaks (C1 and C2, onset at similar temperature to the respective melting peaks). Dimodan P Pel/B also has a component that, when it is heated, re-crystallises (Peak RC) before the first melting peak (M1). It also shows an additional crystallisation endotherm (C3).

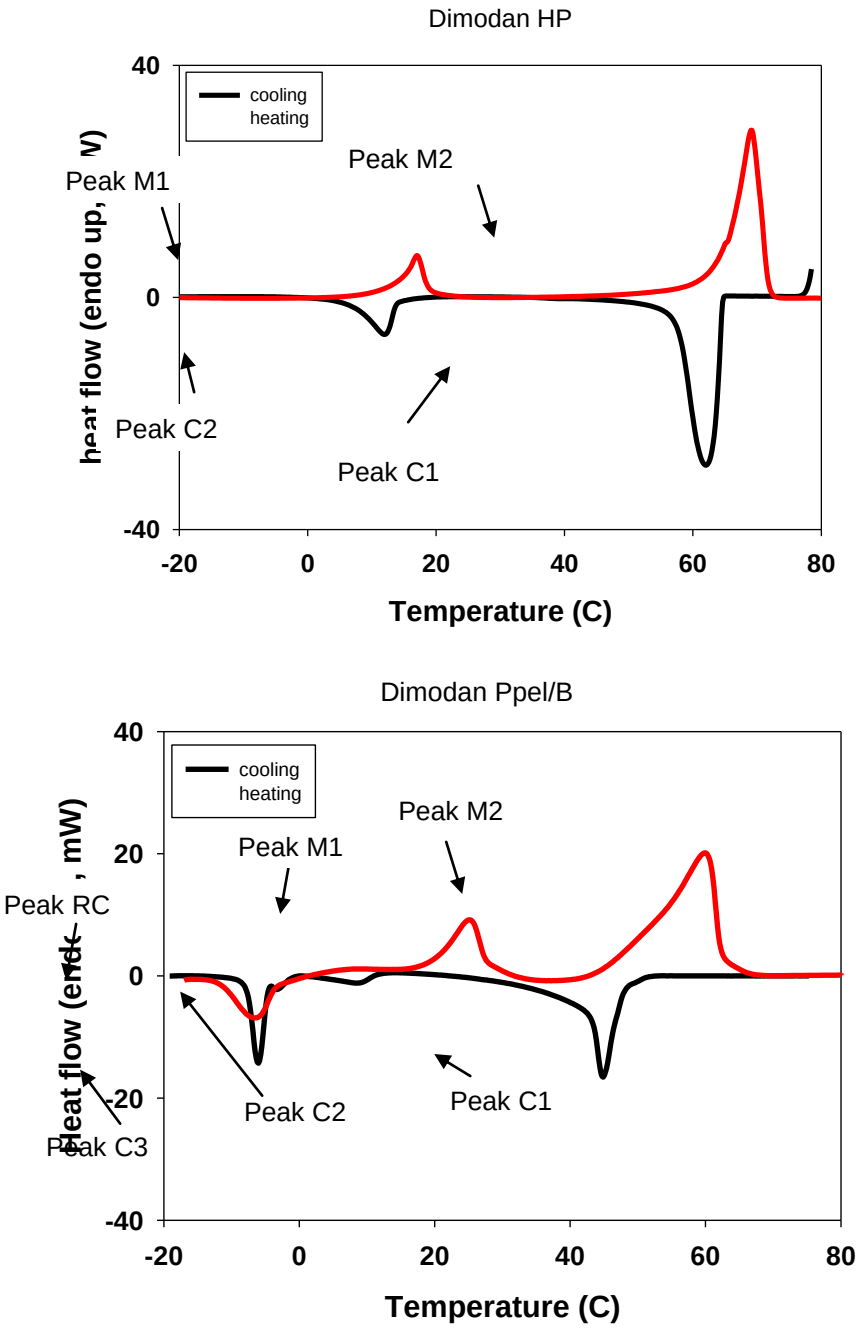

Figure 8.1: Example of melting and cooling curves of individual monoglycerides

Table 8.1 shows the melting/ crystallisation temperatures, averages of at least two measurements. The two monoglycerides have different melting/ crystallisation temperatures: The majority of Dimodan HP crystallises (melts) at just over 64 °C (62 °C, respectively). Melting (Peak 2) of Dimodan P/Pel B occurs at 44 °C, while it first crystallises at 49 °C. Furthermore, a majority of Dimodan HP crystallises at the

higher temperature (Peak C1), while in the case of Dimodan P/Pel B approximately equal amounts of components crystallise at the two different temperatures (peaks C1 and C3), and a small amount crystallises at ~15 °C (peak C2).

Table 8.1: Overview of melting (Peaks RC, M1 and M2) and crystallisation peak (Peaks C1 to C3) temperatures in two different monoglycerides

	Dimodan P/Pel B		Dimodan HP	
Peak	**Temperature (°C)**	**ΔH (mJ/mg)**	**Temperature (°C)**	**ΔH (mJ/mg)**
	Heating		**Heating**	
RC (exotherm)	**-9±1.1**	**1.1±0.3**		
M1 (endotherm)	**16.5±2**	**1.4±0.3**	**11.4±0.5**	**3.7±0.3**
M2 (endotherm)	**43.7±0.4**	**3.7±0.4**	**62.0±1**	**17±2**
	Cooling		**Cooling**	
C1 (exotherm)	**49±1.4**	**1.7±0.2**	**64.1±0.1**	**17±2**
C2 (exotherm)	**12.5±0.7**	**0.15±0.03**	**15.8±0.4**	**3.8±0.4**
C3 (exotherm)	**-1.25±0.4**	**1.6±0.2**		

DSC data does not allow for accurate analysis of the composition of the commercial monoglycerides because the melting and crystallisation properties of the individual constituents in a blend influence each others melting/ crystallisation behaviour (Norton et al., 1985). Dimodan P Pel/B is derived from palm oil and its composition is therefore based on palm oil's natural fatty acid composition (V. Cole, personal

communication, 2008). It is therefore proposed that oleic acid constitutes the small melting peak, while Peak 2 is mostly a result of the melting of saturated monopalmitin, which in its pure form has a melting point of 66 – 70 °C (Brokaw & Lyman, 1958). Dimadan HP is a distilled saturated monoglyceride derived from hydrogenated palm oil (V. Cole, personal communication, 2008). It is likely to have fewer constituents due to the distilliation, with a lower concentration of monoolein than Dimodan P Pel/B and more monopalmitin, so that the melting peaks are sharper compared to Dimodan P Pel/B.

8.1.1 Melting and crystallisation of a monoglyceride/sunflower oil mixture

The objective of this section is to gain an understanding of the melting behaviour of the monoglyceride in sunflower oil.

A mixture of two types of saturated monoglycerides was used as an emulsifier for the W/O emulsions. Early trials had shown that emulsions with good stability could be obtained when using this mixture, while use of the individual components did not result in stable emulsions. A possible explanation for this is that a solution requires certain crystallisation characteristics in order to crystallise in the A-unit (i.e. crystallisation should occur at temperatures 40 – 60 °C). These crystallisation characteristics could be achieved by mixing the two monoglycerides (see previous section for their individual melting and crystallisation profiles)

8.1.1.1 Monoglyceride crystallisation in sunflower oil

The monoglyceride (50 % Dimodan HP and 50 % Dimodan P Pel/B) was added to sunflower oil, heated to ~80 °C to melt the monoglycerides and obtain a homogenous mixture, and weighed into aluminium pans for measurement in the DSC. The percentage of monoglyceride in the sunflower oil was varied between 0.5 and 2.5 %. A large melting and crystallisation peak below 0 °C could be attributed to the sunflower oil by comparing the readings obtained with the DSC curve of pure sunflower oil (see section 8.3). The smaller peaks above 0 °C, shown in Figure 8.2, are not observed in pure sunflower oil samples and may therefore be attributed to the melting/ crystallisation of the monoglyceride mixture.

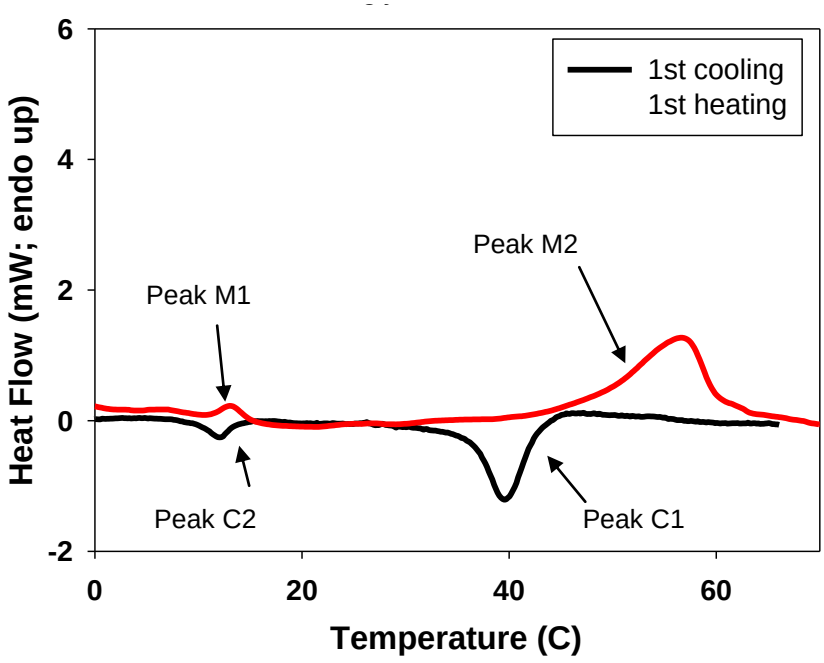

Figure 8.2: DSC curves for melting (red line) and cooling (black line) of a 2.5 % mixture of monoglcyerides in sunflower oil

The crystallisation temperature of the monoglyceride component was observed at around 40 °C (peak C1) and melting occurred at 34 °C (peak M2) for monoglyceride concentrations above 1.5 % (see Table 8.2). A smaller crystallisation peak (C2) was observed at around 13 °C. A smaller melting peak (M1) occurred at 9 °C. The melting as well as crystallisation peaks were broad, which is a result of impurities contained within the monoglycerides. The shift in crystallisation temperature is due to the increased concentration of crystalline material within the oil. The effect of increasing the concentration of crystals on melting temperature has already been discussed (see section 4.3).

At 0.5 % monoglycerides do not begin to crystallise or melt until the solution has cooled to around 20 °C. Moreover, only a single peak appears at this concentration. The component triggering this smaller peak is therefore probably soluble in sunflower oil at this concentration. A higher concentration of crystallising monoglyceride increases crystallisation temperature because the scope for nucleation and subsequent seeding of new crystals is increased (Mullin, 2003).

Table 8.2: Influence of monoglyceride concentration on melting/ crystallisation temperatures

	0.5% monoglyceride	1.5% monoglyceride	2.5% monoglyceride
	Peak Temperatures – Heating (°C)		
Peak M1		9 ± 1	9 ± 1
Peak M2	20 ± 3	34 ± 4	32 ± 2
	Peak Temperatures – Cooling (°C)		
Peak C1	21 ± 3	40 ± 1	42 ± 2
Peak C2		13±1	13±1

8.2 Characterisation of pure tripalmitin

The melting and crystallisation characteristics of the tripalmitin were investigated. A sample (between 7 and 10 mg) at room temperature was loaded into a 50µL aluminium pan and heated to 80 °C. After being held at this temperature for 10 minutes, the sample was cooled at 1 or 10 °C/min to -10 °C. Here it was held for another 10 minutes, and reheated at the same rate to 80 °C (see chapter 3). At least 2 measurements were performed in this way on different samples. Figure 8.3 shows the melting and crystallisation behaviour of the tripalmitin for a heating/ cooling rate of 10 °C/min. Differences between different samples were small and the curves obtained almost exactly superimposed on one another. Furthermore, the melting and crystallisation profile did not change when the heating/ cooling rates were reduced to 1 °C/min (Table 8.3).

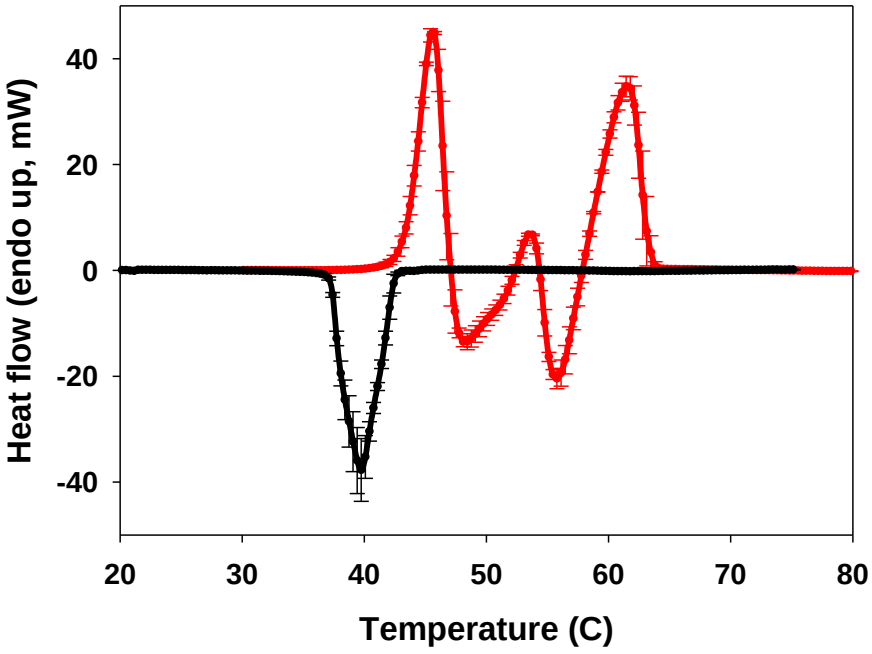

Figure 8.3: DSC heating (red) and cooling (black) curves of pure tripalmitin

Table 8.3: Overview of melting and crystallisation peak temperatures and areas of pure tripalmitin

		Peak Temperature (°C)	ΔH (mW/mg)
Melting	**1st peak**	**42.5 ± 0.2**	**11.6 ± 0.1**
	Re-crystallisation		**-4.7 ± 0.1**
	2nd peak	**52.8 ± 0.2**	**0.9 ± 0.1**
	Re-crystallisation		**-4.7 ± 0.2**
	3rd peak	**58.0 ± 0.1**	**12.8 ± 0.3**
Crystallisation		**42.8 ± 0.4**	**13.6 ± 0.2**

The melting curve shows three peaks; the first and the second are each followed by a crystallisation event. This behaviour compares to that reported in the literature (Siekmann & Westesen, 1994; Hale & Schroeder, 1981). Tripalmitin usually exists in 3 polymorphic forms: α (unstable), β' (metastable) and β (most stable). Upon heating, the tripalmitin in α (first peak) and β' forms (2. peak) convert to the β form (3rd peak) before finally melting, a process in which acyl chains are re-ordered (Siekmann & Westesen, 1994). This results in the triple peak observed in Figure 8.3 (Hale & Schroeder, 1981). Unless crystallisation occurs in well-controlled conditions, all 3 forms are usually present in the solid.

8.3 Melting profiles of emulsions stabilised by 0.5 or 1% tripalmitin and 0.5% monoglyceride

For completeness, the melting curves of emulsions stabilised by 0.5 or 1 % tripalmitin and 0.5% monoglyceride are shown in Figure 8.4 and Figure 8.5.

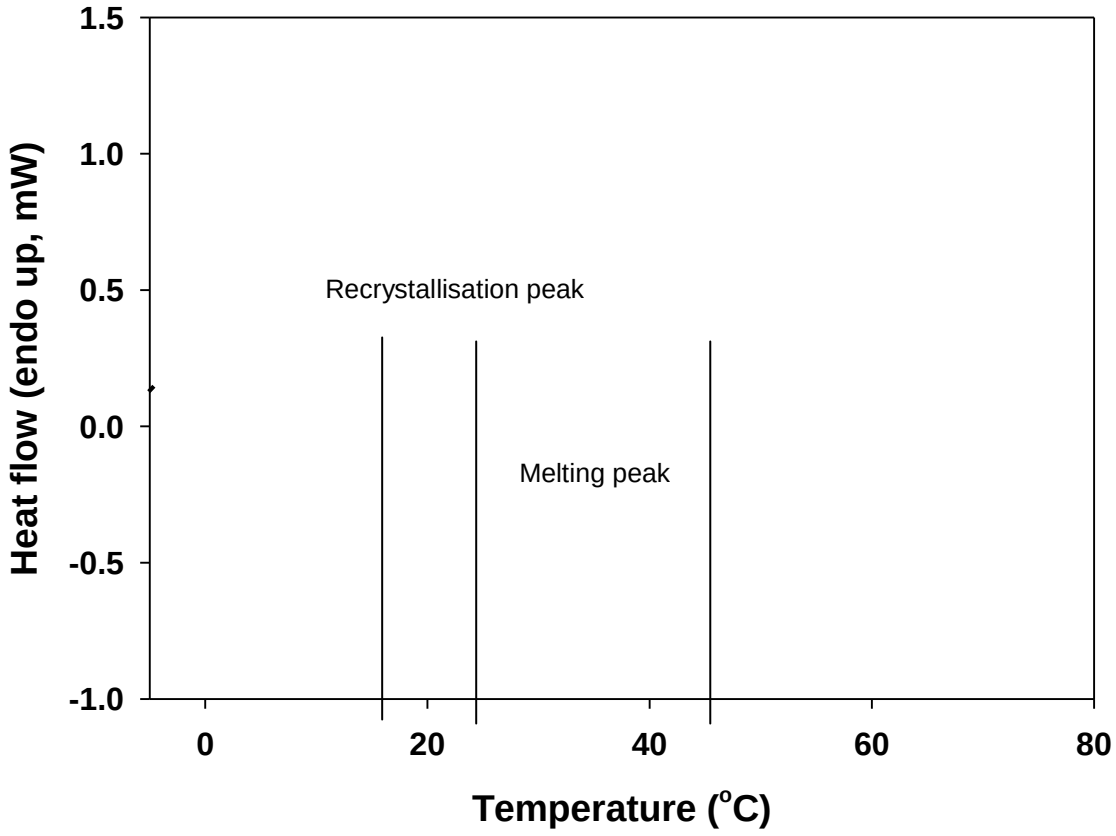

Figure 8.4: DSC curve of an emulsion stabilised by 0.5 % monoglyceride and 1 % tripalmitin, heating rate 10 °C/min

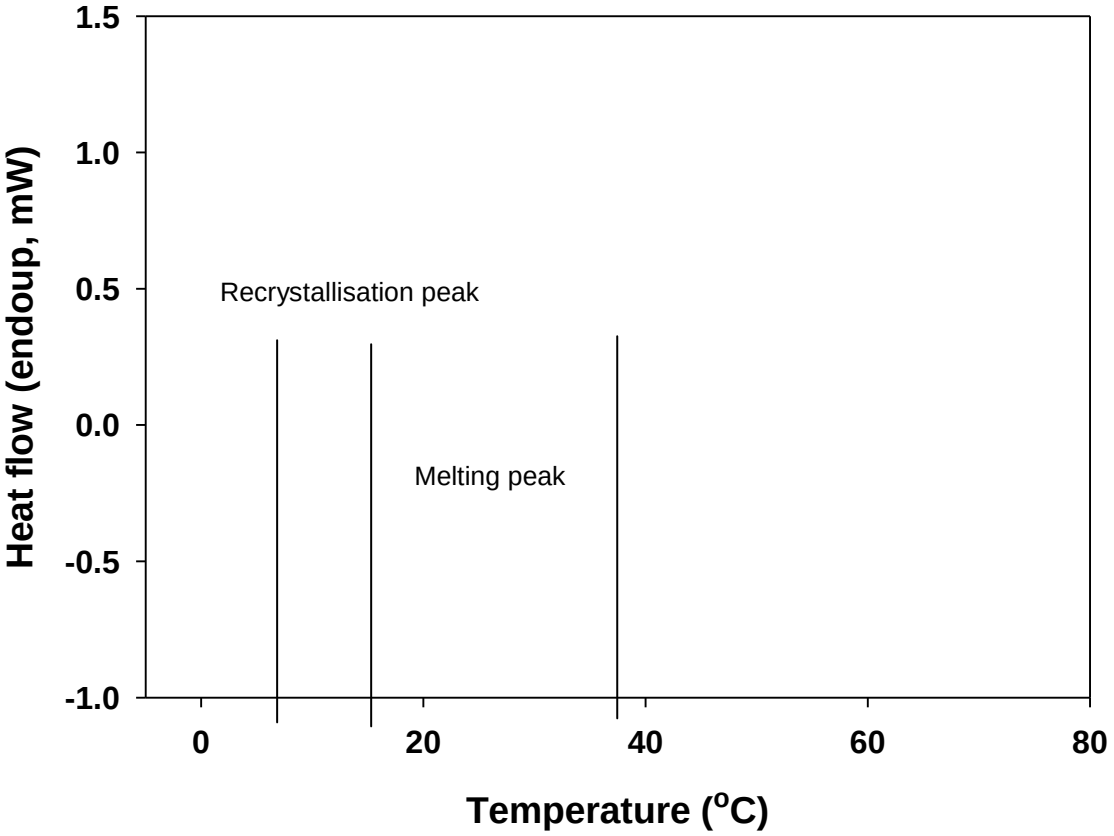

Figure 8.5: DSC curve of an emulsion stabilised by 0.5 % monoglyceride and 0.5 % tripalmitin, heating rate 10 °C/min

The recrystallisation peak can clearly be seen in both samples. The location of the peaks shifts with solid fat concentration.

8.4 Melting profiles of oil mixtures containing 0, 0.5 or 1 % tripalmitin and 0.5 % monoglyceride

The melting and crystallisation profiles of the oil mixtures of oil mixtures containing 0 (Figure 8.6), 0.5 (Figure 8.7) or 1 % tripalmitin (Figure 8.8) and 0.5 %

monoglycerides are given below. The data, in form of crystalisation and melting temperatures, is discussed in section 4.4.1.

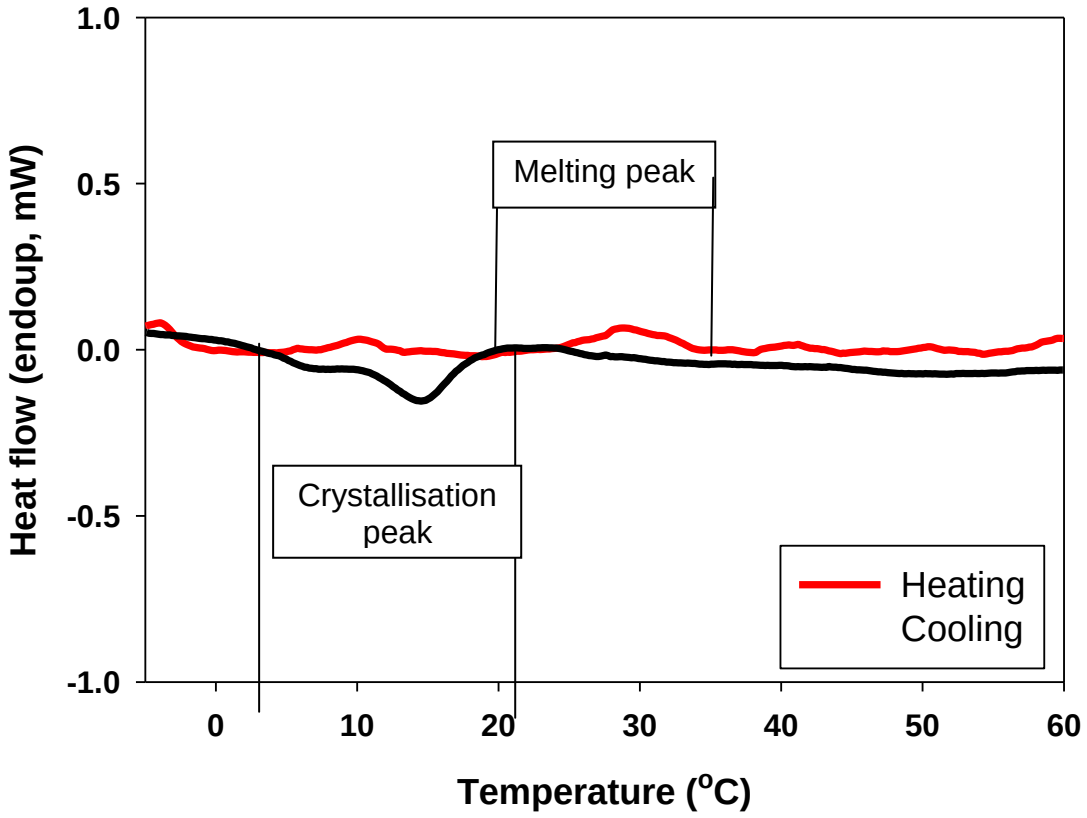

Figure 8.6: DSC curve (average of 3 measurements) of sunflower oil containing 0.5 % monoglyceride and 0 % tripalmitin, heating/ cooling rate: 10 °C/min

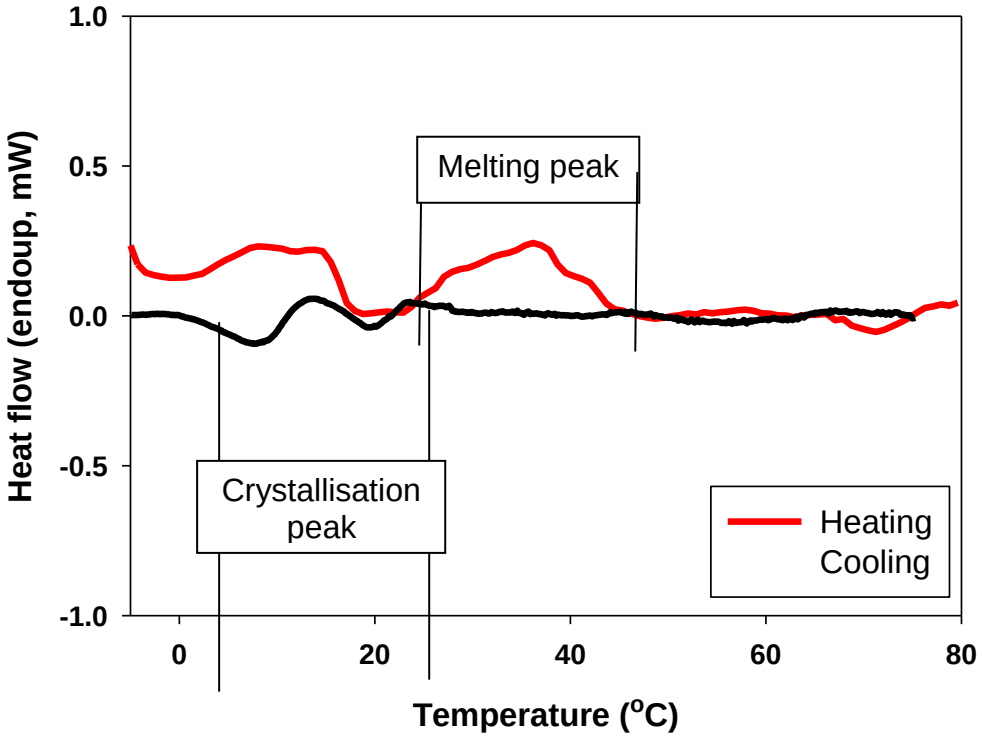

Figure 8.7: DSC curve (average of 3 measurements) of sunflower oil containing 0.5 % monoglyceride and 0.5 % tripalmitin, heating/ cooling rate: 10 °C/min

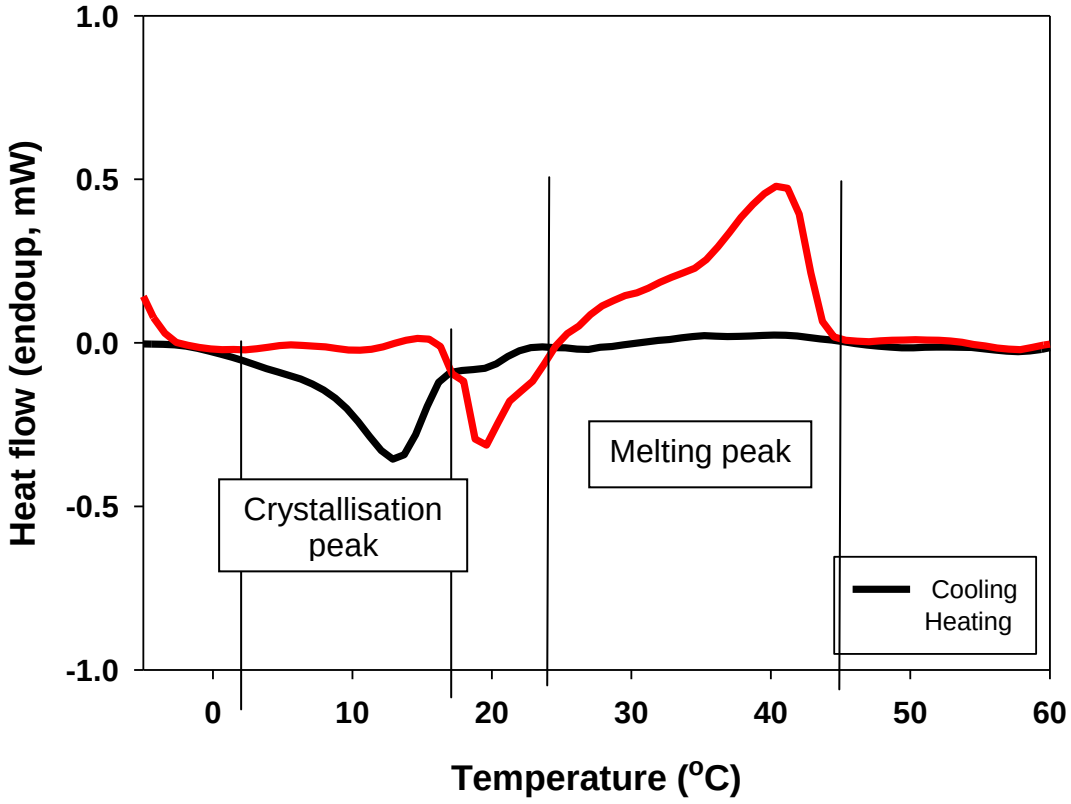

Figure 8.8: DSC curve (average of 3 measurements) of sunflower oil containing 0.5 % monoglyceride and 1 % tripalmitin, heating/ cooling rate: 10 °C/min

8.5 Full melting and crystallisation profiles of mixtures of monoglyceride, tripalmitin and sunflower oil

In order to confirm that the peaks above 0 °C were attributable to the monoglyceride and tripalmitin components in the oil mixture, a comparison was made between the DSC heating curves of the mixture and pure sunflower oil. The oil mixture contained 0.5 % monoglyceride, 2 % tripalmitin and sunflower oil. The samples were cooled at 10 °C/min to - 60 °C, equilibrated there for 10 minutes and heated at 10 °C/min to 30 °C (sunflower oil) or 60 °C (oil mixture).

It is seen in Figure 8.9 that the components in sunflower oil melt between - 40 and 0 °C. A similar melting peak is observed in the oil mixture, which suggests that this peak (peak 1) is attributable to the sunflower oil component of the mixture, given that the endothermic peak occurs at a similar temperature range as that of sunflower oil. A second endothermic peak (Peak 2) occurs between 20 and 50 °C in the oil mixture but is not present in the melting profile of pure sunflower oil. This peak is therefore attributed to the monoglyceride and tripalmitin components in the oil mixture.

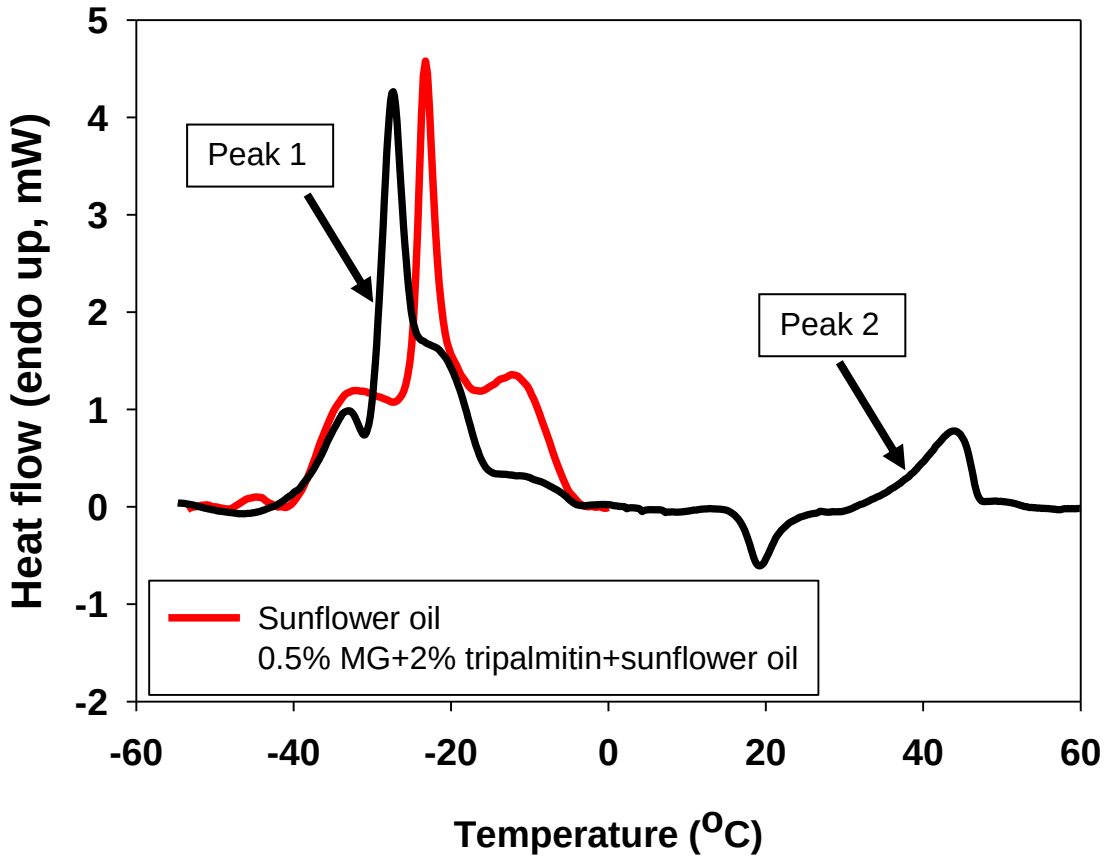

Figure 8.9: DSC of sunflower oil (red line) and a mixture of 0.5% monoglyceride and 2% tripalmitin in sunflower oil (black line) heated at 10°C/min.

8.6 Example of raw data obtained from the DSC for an oilmix sample.

Due to the small amounts of tripalmitin/ monoglyceride in the samples, automatic peak detection (using the Perkin-Elmer software supplied with the DSC) could not be used. Therefore, a baseline was fitted manually to each set of data. It was shown that

a 2nd order polynomial exactly fit the original baseline (see Figure 8.10). This baseline was obtained by heating 2 empty DSC pans from -30 to 70°C at 10°C/min.

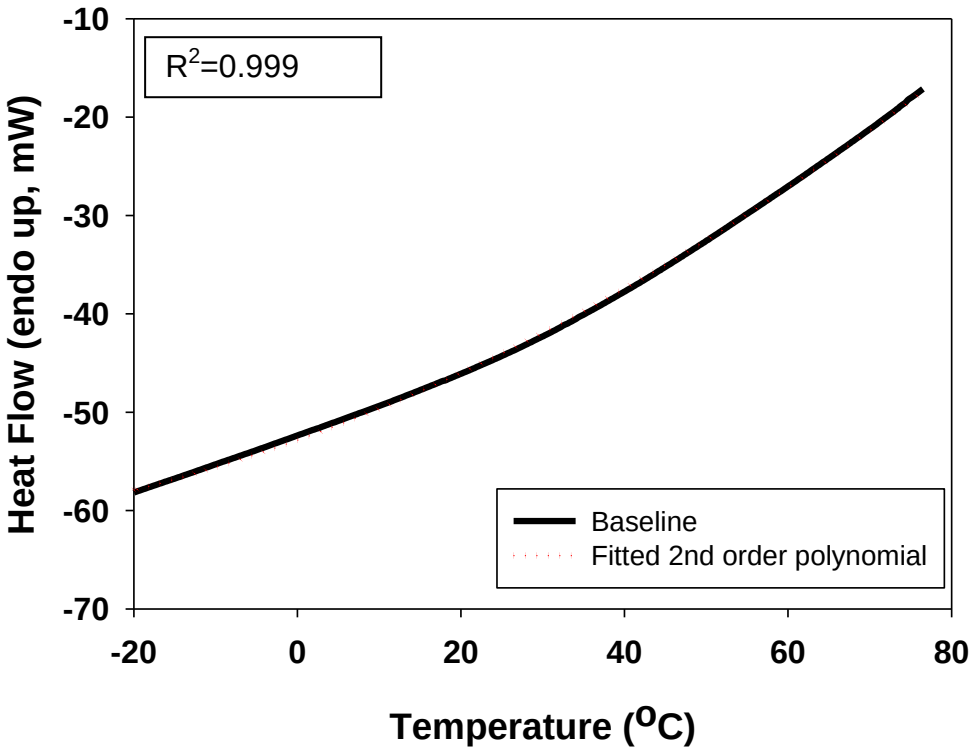

Figure 8.10: 2nd order polynomial fitted to the Baseline of the DSC

The absolute position of the curve shifts depending on the quantity of sample in the pans. It is hence more accurate to fit the second order polynomial to each sample plot. Figure 8.11 shows and example of the raw data obtained for a mixture of 0.5 % monoglyceride and 2 % tripalmitin in sunflower oil. The baseline obtained by fitting a 2nd order polynomial is also indicated. When the fitted curve is subtracted from the original data curve, the incline is removed. This allows for better illustration of the relative size and shape of the peaks. For clarity reasons, all DSC plots in this work show data in which the baseline was subtracted from the raw data.

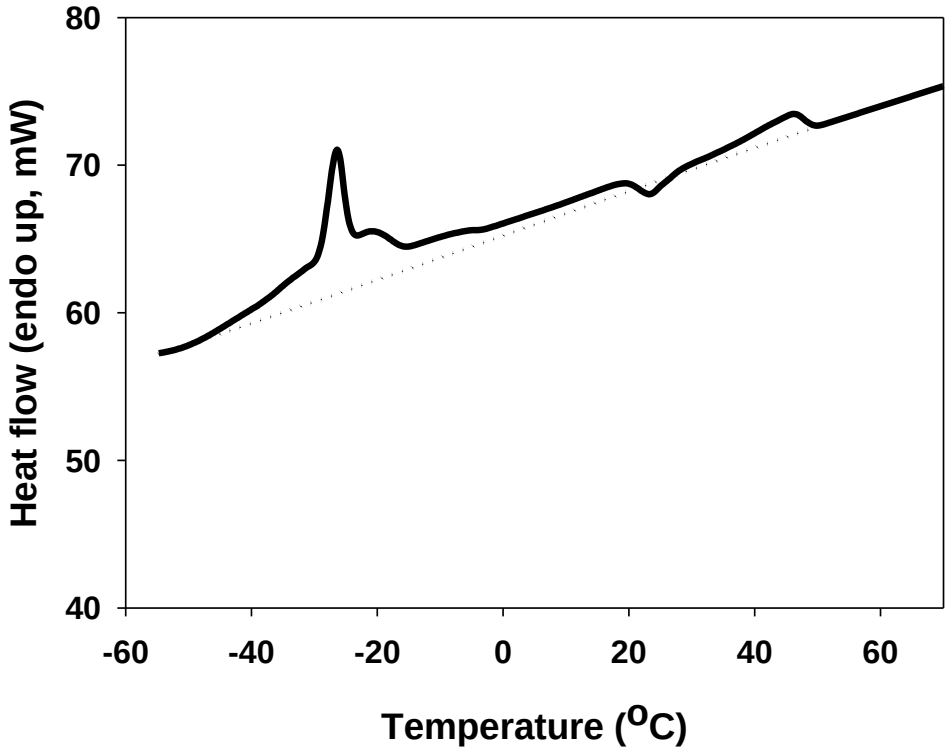

Figure 8.11: Raw data (solid line) of a DSC heating curve at 10 °C/min of a mixture of 0.5 % monoglyceride, 1 % tripalmitin and sunflower oil. The dotted line indicates the baseline obtained by fitting the raw date to a 2nd order polynomial.

8.7 Amplitude sweeps of emulsion samples

In order to perform frequency sweeps accurately, the experiments must be performed at a shear stress that is within the linear elastic region of the sample. Amplitude tests were performed to determine this region in the various emulsion samples.

Samples of fresh emulsions containing 60 % water and 0.5 % monoglycerides and between 0 and 2 % tripalmitin in the oil phase were analysed using a 40 mm parallel plate geometry with gap size 1 mm. The frequency was constant at 1 Hz.

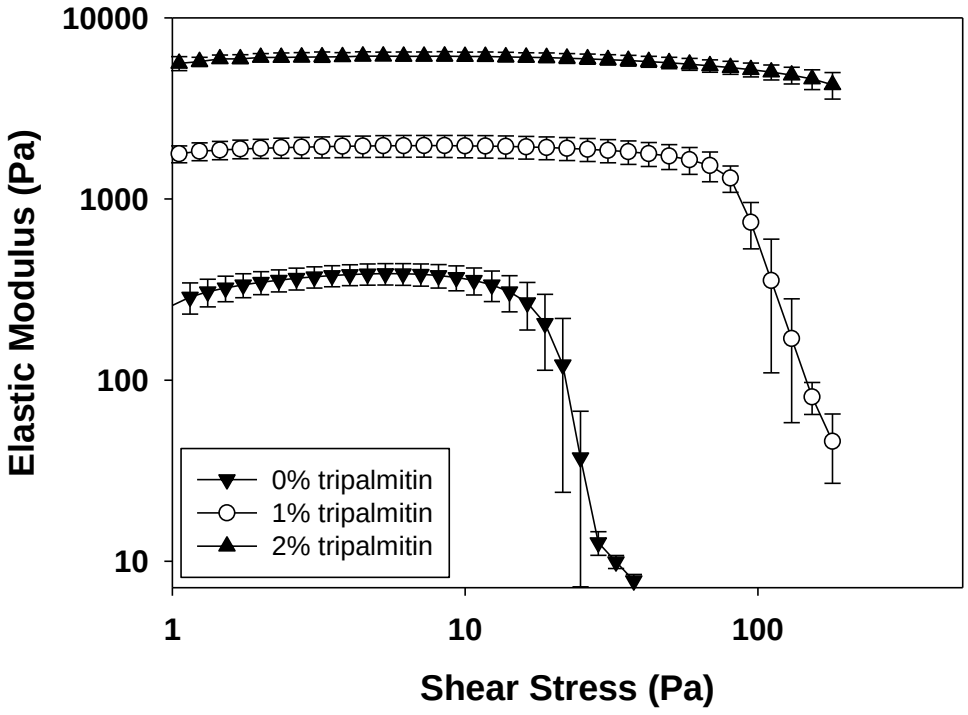

Figure 8.12: Amplitude Sweeps of various emulsion samples containing 0.5% monoglyceride and ▼= 0% tripalmitin; ○ = 1% tripalmitin; ▲ = 2% tripalmitin

Figure 8.12 shows how the length of the linear elastic region increases with increasing tripalmitin concentration. In a sample containing no tripalmitin, the linear elastic region ends at a shear stress of around 10 Pa, while for a sample containing 2 % tripalmitin it continues until above 100 Pa. For this reason, the shear stress at which frequency tests were performed was adjusted for each sample. The shear stress was set at 1 Pa for emulsions containing no tripalmitin; at 20 Pa for emulsions containing 1% tripalmitin; and at 50 Pa for emulsions containing 2 % tripalmitin.

9 References

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