11th International Congress on Engineering and Food (ICEF11)

W/O/W emulsions stabilized by fat crystals - Their formulation, stability and ability to retain salt

Fotios Spyropoulos*, Sarah Frasch-Melnik, Ian T. Norton

School of Chemical Engineering, University of Birmingham, Birmingham, B15 2TT, UK

Abstract

The potential of fat-crystal stabilised W1/O emulsions containing salt to be incorporated into a W1/O/W2 double emulsion was studied. 30% W1/O emulsions stabilised by monoglyceride and triglyceride crystals, and containing 1.6% KCl in the W1 phase, have been mixed with equal amounts of oil and incorporated into double emulsions (W1/O/W2), where the W2 phase contained different levels of different types of emulsifiers (e.g. Na-caseinate). The resulting double structures were monitored over the course of 8 weeks. It was found that double emulsions remain relatively stable during this time depending on the species used to stabilise the external o/w2 interface.

Keywords: w/o emulsions; w/o/w double emulsions; salt release; fat crystals; Pickering emulsions.

1. Introduction

The great potential of double emulsions has been long recognised in many industrial sectors, including the pharmaceutical, agricultural, and especially the food industry. Although numerous applications can be envisaged, these systems are especially of interest in the creation of low-fat foods such as mayonnaise, soups and salad dressings. Current low fat food technologies rely on replacing some of the oil phase with water-soluble ingredients, such as biopolymers, in order to increase the viscosity of the emulsion’s aqueous phase in the “hope” that the mouthfeel that was lost upon fat expulsion can be regained. However reducing the fat content of foods via this approach can significantly affect their physical and organoleptic properties and can lead to an inferior product in terms of mouthfeel and flavour [1].

An alternative approach to reducing the effective oil content of an emulsion, while retaining its sensory properties, is to incorporate water droplets into the oil phase – i.e. replace the oil phase with a water-in-oil (W1/O) emulsion. This creates a W1/O/W2 double emulsion, and, depending on the volume fraction of the inner W1 aqueous phase, a potentially large amount of oil can be replaced this way. The benefit of this

* Corresponding author. Tel.: +44-0-12-414-5284
E-mail address: f.spyropoulos@bham.ac.uk.
approach is that the oil phase potentially appears unchanged to the consumer and thus sensory properties should remain close to those of the full fat product. In order to achieve this, care should be taken to ensure that the oil droplets (containing the primary emulsion) in the double structure and those in the simple emulsion are of very similar sizes.

However, the main drawback of double emulsions is their inherent thermodynamic instability. The existence of two oppositely-curved interfaces within the same structure requires the presence of two different emulsifiers; one lipophilic and one hydrophilic. The two different emulsifier species tend to diffuse from the one interface to the other, thus changing the curvatures of both and subsequently destabilising the structure which collapses to give a simple O/W emulsions. This is especially so when small-molecule emulsifiers are used, as these have higher mobility than large molecules such as proteins [2–4]. Another factor affecting the stability of double emulsions is the existence of a difference in the osmotic pressure between the two aqueous reservoirs. This difference either causes water from the external (W₂) aqueous phase to be transported to the internal W₁ phase, or vice versa, depending on the direction of the osmotic pressure gradient. In the first case, the internal W₁ droplets swell due to the increased phase volume of encapsulated water, while in the latter case, W₁ droplets gradually empty. In both cases, the double emulsion structure is gradually converted to a simple O/W emulsion [5, 6].

In the present study, W₁/O/W₂ double emulsions, where the primary W₁/O emulsion is stabilized by fat crystals, while the O/W₂ interface is stabilised by sodium (Na-) caseinate, were investigated. It is demonstrated that by controlling the fat crystal concentration in the W₁/O emulsions these can be successfully incorporated within a double structure. Furthermore, it is shown that these double emulsions are able to resist osmotic pressure gradients and can effectively retain an encapsulated solute within the primary emulsion droplets (W₁).

2. Experimental

2.1. Materials

Distilled saturated monoglycerides (MG), an equal mixture of Dimodan HP and Dimodan P Pel/B and spray-dried sodium-caseinate, were obtained from Danisco, UK. Glycerin Tripalmitate (tripalmitin), D+ -glucose, sodium azide, potassium chloride and sodium chloride were all obtained from Sigma, UK. Distilled water and commercially available sunflower oil were used as the water and oil phases, respectively, of all prepared emulsions. All materials were used as received, without further purification.

2.2. Methods

2.2.1. Primary Emulsion Preparation (W₁/O)

All primary water-in-oil (W₁/O) emulsions were formulated using a 30% aqueous phase (W₁), containing 1.6% KCl, and a 70% oil phase (O), which included 1.25% monoglyceride and 2.5% triglyceride. All primary emulsions were then prepared using the process described in [7].

2.2.2. Double emulsion preparation (W₁/O/W₂)

In order to obtain double emulsions, pure sunflower oil was mixed with the W₁/O primary emulsion at a ratio of 1:1, before 20 g of this blend was slowly mixed with 80 g of the continuous W₂ aqueous phase. The W₂ phase contained 1% sodium caseinate and also a small (0.01%) amount of sodium azide as an antimicrobial agent. Varying concentrations of glucose (4–16%), or NaCl (1–5%) were added to the W₂ aqueous phase in order to create various osmotic pressure gradients (Δπ) between encapsulated (W₁) and continuous aqueous phase (W₂). 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. The formulation was subsequently sheared at 8000 rpm for 3 min using a Rotor/Stator apparatus (Silverson), while being placed in an ice bath to avoid temperature increases, which could potentially melt the crystal network surrounding the W1 primary emulsion droplets.

2.2.3. Emulsion droplet size measurement and microstructure visualisation

The droplet sizes in the primary emulsions prior to incorporation within the double structure were measured using a nuclear magnetic resonance (NMR) device (Bruker Minispec NMR, Bruker Optics, UK), equipped with a gradient unit as previously detailed [7]. Measurements were performed on three different samples.

Light microscopy (Reichert Jung Polyvar) was used to visualize the double emulsion droplets and to calculate 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 obtained micrographs of the double systems. In order to obtain an accurate measure of the size distribution of the double emulsion globules and the W1 primary emulsion droplets, at least 2–3 different samples of each formulation were characterised by measuring between 500 to 1000 droplets. The standard deviation from the mean droplet size is also given as it provides an indication of the breadth of droplet size distribution (i.e. a small standard deviation value indicates a narrow size distribution).

Finally, cryo-SEM (Philips XL-30 FEG ESEM) was used to visualise the double emulsion microstructure. Samples were shock-frozen in liquid nitrogen, and dusted with gold particles prior to analysis in the SEM.

2.2.4. Conductivity measurements

In order to determine the effectiveness of the double emulsion structures in retaining a solute (KCl in this case) within the inner (W1) 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. Results & Discussion

3.1. Simple W1/O emulsions – Primary emulsions

Simple W1/O emulsions stabilised only by monoglycerides or triglycerides were found to be unstable against coalescence or sedimentation, eventually resulting in a fully phase separated system. Emulsions containing a mixture of mono- and triglycerides were subsequently produced. Monoglyceride concentration was kept constant at 0.5%, and triglyceride concentration was varied: 0.5%, 1% and 2%. Emulsions containing an equal mixture of mono- and triglycerides showed only a slight increase in the average droplet size after 25 days when stored at 5°C. Emulsions containing 1 and 2% triglyceride did not show any increase in droplet size within this time at 5°C. Emulsions containing 2% triglyceride did not show any increase in droplet size within this time at 5°C. Emulsions containing 2% triglyceride were also stable when stored at 22°C (Fig. 1), while emulsions containing 0.5 and 1% showed a significant increase in droplet size when stored under the same temperature conditions. When stored at 30°C, all emulsions destabilise within a day. It is suggested that the reason for the observed stability of the emulsions (at low temperatures) is the existence of a sintered fat crystal network, both at the droplets’ interface and also in the bulk continuous phase surrounding the droplets. The existence of these fat crystal “shells” was confirmed by SEM microscopy.
Fig. 1. Salt release from W1/O emulsions as a function of crystal composition at 22°C

Salt release from W1/O emulsions stabilised by a mixture of 0.5% monoglycerides and varying concentrations of triglycerides, was measured over time by submersing these in distilled water. Salt release profiles from these systems, as a function of fat crystal concentration (Fig. 1), temperature (Fig. 2) and applied osmotic pressure (Fig. 3), were obtained. Emulsion containing 0.5% monoglyceride only released around 50% of their total salt content after ~1 month. Adding 0.5% tripalmitin reduced release, over the same time, to less than 10%, while adding more than 1% tripalmitin almost completely arrested salt from releasing. Salt release from these structures seems to be related to the formation of crystalline sintered shells at the droplets’ interfaces, which would provide an effective Pickering stabilisation. A required condition for this to take place is the availability of sufficiently small crystals at the interface. The combination of high shear and fast cooling rate used for producing the emulsions seems to have the desired effect of producing very fine crystals at the interface. While the high cooling rate creates a high driving force for crystallisation, the simultaneous shear assists the distribution of the seeding crystals, with the result being a high crystallisation rate.

Fig. 2. Salt release from W1/O emulsions as a function of crystal composition at 55°C
In order to further establish whether the presence of crystals at the interface is responsible for the stability and salt release of the investigated emulsions, a series of salt release experiments were carried out at different temperatures; at 5°C (below the onset of crystal melting for all emulsions), 22°C (onset of crystal melting for all emulsions, \( T_{\text{onset}} \)), 30°C (close to the peak temperature for all emulsions, \( T_{\text{peak}} \)) and 55°C (end of crystal melting, \( T_{\text{end}} \)). The tested temperatures were selected after carrying out a series of differential scanning calorimetry (DSC) experiments to investigate the melting profiles of these emulsions. The reduced stability and also increased rate of salt release from the emulsions at increasing temperatures can be explained by the stabilising role of the crystals at the interface. The broad melting range of the emulsions shows that the crystals become more soluble in the oil phase with increasing temperature. Thus, both the shells and the surrounding crystal network are weakened at elevated temperatures, which results in an increase in the emulsions’ average droplet size. For instance, the average droplet size of an emulsion containing 2% Tripalmitin more than doubles after 4 hours of storage at 30°C. Melting of the crystals at the interface creates imperfections in the “shell” formed at low temperatures. It is these imperfections that now allow for water to migrate between different water droplets as well as from the encapsulated to the continuous aqueous phase and therefore for salt to be released. At 30°C, near the melting-peak temperature, there are insufficient solid crystals available to stabilise the interface. Without these crystals the emulsion no longer benefits from a rigid interface (provided by these particles due to Pickering stabilisation), which results in the rapid emulsion structure breakdown observed at 30°C. At 55°C, salt release was almost instantaneous, with complete release, depending on tripalmitin concentration, taking place within 3-4 minutes (Fig. 2). The DSC data revealed that the tripalmitin and monoglycerides are no longer solid at this temperature. Hence the emulsions become destabilised due to the lack of crystals.

![Fig. 3. Salt release from W_1/O emulsions as a function of an osmotic pressure difference induced between the W_1 water droplets of the emulsions and the bulk aqueous phases they were submerged into. Open symbols correspond to systems stabilised by 0.5% mono-G only while closed symbols to those stabilised by a mixture of 0.5% mono-G & 1% tri-G. [glucose]* denotes the glucose concentration required to balance the osmotic pressure created by the presence of salt in the W_1 water phase of the emulsion](image)

Finally, the effect of inducing different osmotic pressure gradients (between the W_1 water droplets of the emulsions and the bulk aqueous phases the emulsions were submerged into) on salt release was investigated (Fig. 3). Osmotic pressure was varied to different gradient strengths using glucose (up to a value of 14atm). The existence of an osmotic pressure gradient between two aqueous phases separated by
an oil phase is in general a source of instability, which leads to water migration from the one aqueous reservoir to the other. Water transport results in either the gradual shrinking or swelling of the internal water phase, depending on the direction of the osmotic pressure gradient (Matsumoto et al., 1980). Nonetheless in this study, no significant osmotic pressure effect can be detected in those samples containing Tripalmitin. On the contrary the release data for emulsions not containing tripalmitin shows relatively fast release under all osmotic pressure regimes. The large error bars are a result of differences in the initial sample shape and size (and hence surface area exposed to the aqueous solution), as well as local temperature fluctuations (which may be as high as ~3°C). No clear trend is seen in the results, indicating that the observed breakdown of the structure and associated salt release is independent of osmotic pressure.

3.2. Double emulsions W1/O/W2

The W1/O emulsions studied in the previous section were used as the primary emulsion for the formulation of W1/O/W2 double emulsions. The simple W1/O emulsions were diluted at a ratio of 1:1 using pure oil phase; double emulsions containing undiluted primary emulsion were not stable, with coalescence between the double emulsion globules occurring within 1 day after preparation, possibly due to the high viscosity of the W1/O emulsions. The outer aqueous phase (W2) contained 1% Na-caseinate (emulsifier) and in some cases either salt or sugar, to vary or match the osmotic pressures in the W1 and W2 aqueous phases.

When there was no osmotic pressure difference, primary emulsions remained within the double structure for at least 8 weeks. As the emulsions were not stabilised against creaming, globule size approximately doubled within this time, as a result of the almost infinite contact time between droplets in the tightly packed cream layer (Table 1). Despite the occurrence of coalescence of some of the globules, no significant increase in primary emulsion droplet size was detected (Table 2). However, an osmotic pressure gradient of 11atm, caused globules to coalesce rapidly, resulting in bridging between the primary emulsion droplets which formed a cream layer within a day. Although the primary emulsion droplets do not entirely coalesce, individual double globules were no longer visible.

<table>
<thead>
<tr>
<th>Δπ (atm) induced by</th>
<th>Δπ induced by</th>
<th>D1,2 (μm) after preparation</th>
<th>D1,2 (μm) after 1 week</th>
<th>D1,2 (μm) after 4 weeks</th>
<th>D1,2 (μm) after 6 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.5 Glucose</td>
<td></td>
<td>27.1 ± 9.1</td>
<td>48.8 ± 14.1</td>
<td>19.7 ± 6.0</td>
<td>40.7 ± 10.3</td>
</tr>
<tr>
<td>0 Glucose</td>
<td></td>
<td>23.0 ± 7.9</td>
<td>30.9 ± 10.2</td>
<td>41.6 ± 12.8</td>
<td>46.4 ± 14.3</td>
</tr>
<tr>
<td>-11 Glucose</td>
<td></td>
<td>25.0 ± 8.3</td>
<td>25.7 ± 8.5</td>
<td>32.4 ± 9.6</td>
<td>24.6 ± 7.2</td>
</tr>
<tr>
<td>5.5 NaCl</td>
<td></td>
<td>31.5 ± 10.3</td>
<td>33.6 ± 9.8</td>
<td>53.1 ± 14.9</td>
<td>44.5 ± 13.5</td>
</tr>
<tr>
<td>0 NaCl</td>
<td></td>
<td>25.1 ± 8.5</td>
<td>35.3 ± 8.4</td>
<td>33.8 ± 11.1</td>
<td>43.8 ± 12.4</td>
</tr>
<tr>
<td>-11 NaCl</td>
<td></td>
<td>31.3 ± 9.8</td>
<td>37.0 ± 11.8</td>
<td>38.6 ± 12.3</td>
<td>37.3 ± 11.5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Δπ (atm) induced by</th>
<th>Δπ induced by</th>
<th>D1,2 (μm) after preparation</th>
<th>D1,2 (μm) after 1 week</th>
<th>D1,2 (μm) after 4 weeks</th>
<th>D1,2 (μm) after 6 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>11 Glucose</td>
<td></td>
<td>5.7 ± 1.7</td>
<td>13.2 ± 2.6</td>
<td>17.4 ± 4.5</td>
<td>12.9 ± 3.2</td>
</tr>
<tr>
<td>5.5 Glucose</td>
<td></td>
<td>2.8 ± 0.8</td>
<td>2.9 ± 0.7</td>
<td>3 ± 0.8</td>
<td>9.6 ± 2.1</td>
</tr>
<tr>
<td>0 Glucose</td>
<td></td>
<td>2.4 ± 0.7</td>
<td>2.6 ± 0.8</td>
<td>2.2 ± 0.6</td>
<td>2.2 ± 0.6</td>
</tr>
<tr>
<td>-11 Glucose</td>
<td></td>
<td>2.1 ± 0.6</td>
<td>2.7 ± 0.7</td>
<td>2.7 ± 0.7</td>
<td>2.7 ± 0.7</td>
</tr>
</tbody>
</table>
In order to study the role of osmotic pressures on emulsion stability, various concentrations of salt or glucose, were used to obtain selected osmotic pressure gradients. A positive osmotic pressure gradient, i.e. the concentration of solute is greater in the encapsulated (W₁) rather than in the continuous (W₂) aqueous phase, usually results in swelling of double emulsion droplets. A negative osmotic pressure gradient, on the other hand, results in water being preferentially transported out of the encapsulated to the continuous aqueous phase, resulting in their gradual shrinking and eventual “disappearance”. The influence of both positive osmotic pressure gradients, where pressure on the crystal “shells” covering the W₁ water droplets is expected to be exerted from the “inside-out”, as well as negative osmotic pressure gradients, where pressure on the “shells” is expected to come from the “outside-in”, were investigated. As can be seen in Table 2, formulations that have an osmotic pressure gradient of 5.5atm show reduced coalescence behaviour of the primary droplets compared to those where the gradient is 11atm, but increased coalescence compared to samples where the osmotic pressure is matched. Primary emulsion droplet size remains constant for the first 4 weeks, and subsequently increases markedly, as some droplets visibly swell.

On the other hand, formulations where the osmotic pressure gradient is -11atm, show a much reduced rate of coalescence for the double globules (Table 1), and a largely constant primary emulsion droplet size (Table 2). Whether the osmotic pressure gradient is obtained using salt or sugar does not seem to make a significant difference, as the same pattern is followed in both cases: a negative osmotic pressure gradient is beneficial to stability whereas the higher the positive osmotic pressure gradient, the less stable the double emulsions become. An osmotic pressure gradient that induces a driving force for water transport into the W₁ droplets, causes these to swell, thus increasing the stress experienced by their protective interfacial crystal “shells”, as previously discussed. However, the primary emulsion droplets show a certain degree of resistance to any swelling, which is most likely attributable to the strength of these crystal “shells” and is exemplified by the fact that primary emulsion droplet size remains near constant for the first 4 weeks in samples where the osmotic pressure gradient is 5.5atm. In the opposite case, when water is preferably transported out of the W₁ droplets, the forces are acting on the outside of the crystal “shells”, so that any defects in the interfacial crystalline structure may in fact be consolidated, which could slow water transport. The differences between systems without an osmotic pressure gradient and those with an osmotic pressure gradient of -11atm, however, are small and within experimental error, which indicates that the “shells” show a higher degree of resistance to pressure exerted from the outside rather than the inside of a droplet.

![Salt Release Graph](image_url)

**Fig. 4.** Salt (KCl) release from W₁/O/W₂ emulsions as a function of an osmotic pressure difference induced between the W₁ and W₂ aqueous phases. ■ = -11 atm; ◇ = 0 atm; ▲ = 5.5 atm; ○ = 11 atm
The ability of the double emulsion structure to retain KCl entrapped within their primary emulsion was studied by means of conductivity measurements. As shown in Fig. 4, when the osmotic pressure is matched with glucose, most salt is retained in the emulsions for a period of at least 6 weeks, when only around 20% of the KCl from the primary emulsion has been released to the continuous aqueous phase. The salt release that occurs is likely to be the result of water transport through imperfections in the crystal “shells”, although there is no net transport between the dispersed and continuous aqueous phases, some movement of water, including transport of solutes, inevitably occurs in both directions. The linear release curve indicates such a slow, diffusion-controlled water transport. When the osmotic pressure is 11atm, on the other hand, all salt is released within one week, and more than 50% is released within the first day. This is not surprising, as this formulation is not stable with respect to retaining a double emulsion structure.

It appears that the direction of the osmotic pressure gradient is also important for salt release; note that a sample with a -11atm gradient exhibits a much slower salt release than a sample with an osmotic pressure gradient of +11atm. As argued earlier, this is due to the fact that an osmotic pressure gradient favouring water transport out of the droplet, may contribute to a more stable shell, while an osmotic pressure gradient favouring water transport into the droplet may “amplify” the defects on the surface of the “shells” due to swelling of these droplets. However, when osmotic pressures of an opposite nature are applied, salt release is not significantly increased compared to a sample where the osmotic pressure is matched (Figure 4). These results indicate that it is not essential to exactly balance osmotic pressure in order to achieve good emulsion stability. However, it may be preferable to add rather more solute to the continuous aqueous phase, i.e. to “overbalance” the osmotic pressure in order to avoid possible aggregation of the primary emulsion in the sample.

4. Conclusions

It has been demonstrated that W1/O emulsions can be stabilised by a combination of mono- and triglyceride crystals (Pickering stabilisation) without the addition of another emulsifier. Salt can be successfully encapsulated within these emulsions, regardless of application of an osmotic pressure gradient. W1/O/W2 double emulsions containing these W1/O emulsions were then produced. The “shells” around the W1 droplets have been shown to greatly reduce water transport between the W1 and W2 aqueous phases, but in this case the existence of an osmotic pressure gradient does affect stability. The produced double emulsions could withstand osmotic pressures applied to induce a drive for water from W2 to migrate to the W1 phase, but this was not possible when osmotic pressure conditions applied drove the reverse migration.

References


Presented at ICEF11 (May 22-26, 2011 – Athens, Greece) as paper FMS1054.