
5 Formulation Engineering of Food Emulsions

A.K. Pawlik¹, P.J. Fryer² and I.T. Norton¹

¹*School of Chemical Engineering, University of Birmingham, UK;* ²*Centre for Formulation Engineering, University of Birmingham, UK*

5.1 INTRODUCTION

Obesity is now a global problem. The World Health Organisation reports that currently the majority of the world's population lives in a country where being overweight and obesity kills more people than being underweight. Globally, there are 1 billion overweight adults and 300 million obese, with an estimated 2.6 million deaths annually as a result of obesity-related diseases (WHO, 2010). A fundamental cause of obesity and overweight is an energy imbalance between calories consumed and expended. Thus, the demand for a balanced diet, but with low energy density, and functional food products that address specific health benefits, is a pressing issue. With health and well-being as major driving forces of the modern food industry, it is now the consumer who tells food manufacturers what they prefer to eat: healthy, nutritionally balanced food with all of the taste and convenience of currently produced unhealthy food products. In this respect, it is a part of food engineering to provide structures that are both generally acceptable and stable enough to resist changes during the shelf-life of the product.

Food products are structurally complex (Dickinson, 2010) and may contain many different components, whose functions are still not fully understood. For instance, sodium chloride is present to deliver flavour, but also ensure microbial and structural stability of the product. Moreover, food products usually have complex rheological behaviour, which may change when the composition of the product is altered (Frasch-Melnik, 2011). For these reasons, reducing the amount of fat, sugar and salt in a food product is not as simple as taking out a portion of the respective ingredient. First, the effect that adding less of certain ingredients has on the microstructure of the original product must be fully

understood, whilst new strategies of including increased concentrations of low-calorie components, such as water or air, must be investigated. Therefore, the challenge for food engineers is to generate microstructures with desired properties, based on fundamental knowledge (e.g. colloid science), use materials whose individual properties are understood, apply processing variables (i.e. shear and temperature) in a controlled way, and to do this in a cost-effective manner (Aguilera and Stanley, 1999; Aguilera, 2005).

Many everyday foods are emulsions: dispersions of two immiscible liquid phases, one of which is the continuous phase, while the other is the dispersed phase. Simple emulsions are one of two different kinds: direct emulsions (e.g. milk, cream, mayonnaise, cake batter) are dispersions of oil into water (O/W), whereas inverse emulsions (e.g. margarine, butter) are dispersions of water into oil (W/O). More complex emulsions, termed *multiple* emulsions, have also been produced and investigated (Garti, 1997a). The simplest multiple emulsion, a *duplex emulsion*, is in fact a ternary system having either water-in-oil-in-water ($W_1/O/W_2$) or oil-in-water-in-oil ($O_1/W/O_2$) structures, where the dispersed droplets contain smaller droplets of a different phase.

There are some promising strategies in formulation engineering of healthy emulsion-based food products. One of them is substituting part of pure fat with water, which can effectively reduce the caloric value of food emulsions. Traditional mayonnaise, for instance, is an emulsion containing up to 80% vegetable fat, dispersed in the water continuous phase. Simply taking out a large portion of the fat would compromise rheological properties and sensory characteristics (such as flavour, mouthfeel and texture) of the product. Effective fat elimination can be achieved via $W_1/O/W_2$ duplex emulsion design, where the included fat is an emulsion itself (i.e. W_1/O emulsion). The internal water phase may be effectively shielded from consumer perception during oral processing, and then later trigger-released in, for example the stomach or small intestine. Additionally, the internal water phase may contain a functional additive (e.g. water-soluble vitamins, peptides), whose unacceptable taste prohibits its incorporation into the external water phase. Moreover, the complex structure of a duplex $W_1/O/W_2$ emulsion offers the possibility of salt reduction (e.g. NaCl, Malone *et al.*, 2003) and/or encapsulation (e.g. KCl, Frasc-Melnik *et al.*, 2010b). Despite a great potential for encapsulation and controlled release, there are no duplex emulsion-based foods available on the market to date. This is due to the many forms of instability occurring in such complex structures.

Another current promising approach, aiming at extending kinetic stabilities of simple and duplex emulsions, is through stabilising emulsion interfaces with particles (i.e. Pickering stabilisation). Interfaces covered with colloidal particles provide an excellent steric barrier to

droplet coalescence (Pichot *et al.*, 2009) and/or mass transfer across the interface (Frasch-Melnik *et al.*, 2010a), thus greater emulsion stability.

There is a strong drive within the food industry for utilisation of emulsion-based delivery systems for encapsulation of poorly bioavailable hydrophobic flavours (McClements *et al.*, 2007) and functional components, as consumers prefer to tackle certain health issues (e.g. obesity and cardiovascular disease) by using food products rather than drugs (Velikov and Pelan, 2008). Particularly suitable for this purpose are nano-emulsions, where the droplet size is below 100 nm (Li *et al.*, 2012), offering a great surface-area-to-volume ratio. The limitations of such systems, as well as the efforts to design them in order to control the extent, rate and specific site of delivery of the entrapped components within a human GI track (Troncoso *et al.*, 2012) will be discussed.

Another emerging fat-reduction strategy is the possibility of choosing particular fat substitutes in a specific concentration, whose rheological properties are comparable to the conventional full-fat products. An example of such a structure is a triphasic emulsion (Tchuenbou-Magaia *et al.*, 2009), where protein-stabilised air bubbles were used as a fat mimetic. Challenges in designing air-filled emulsions are discussed later.

Entrapping large quantities of water in matrices is one strategy in the development of low-calorie foods. For centuries, polysaccharides and proteins have been used as gels to immobilise large amounts of water (Aguilera, 2005). The uniqueness of such biopolymer structures comes from their inherent tendency towards phase separation, which offers the possibility of kinetically trapping structures of chosen rheological properties (Wolf *et al.*, 2000). Some examples of biopolymer mixtures, also termed water-in-water emulsions, will be further discussed.

The aim of this chapter is to report on current understanding of the ways that the state-of-the-art microstructural design methods can be employed in the formulation engineering of food emulsions. Novel formulation and processing approaches are discussed focusing on their advantages, limitations and potential applications in the modern food industry.

5.2 EMULSION TYPES

5.2.1 Pickering emulsions

It is well known that solid particles of colloidal size can, like surfactant molecules, be used to kinetically stabilise emulsions (Binks, 2002;

Hunter *et al.*, 2008; Frascch-Melnik *et al.*, 2010a; Pichot *et al.*, 2010; Kargar *et al.*, 2011; Murray *et al.*, 2011). Many food products are, in fact, entirely or partially, stabilised by submicron particles; such as mayonnaise (O/W emulsion partially stabilised by mustard particles), homogenised and reconstituted milk (O/W emulsion stabilised by casein micelles), margarine and fatty spreads (W/O emulsions stabilised by fat crystals), whipped cream, batter, ice creams, etc.

5.2.1.1 Mechanism of Pickering stabilisation

Pickering stabilisation differs fundamentally from surfactant stabilisation as particles and surfactants, either in the bulk phase or at the interface, behave differently. For instance, particles do not aggregate to form micelles as surfactant molecules do (Binks, 2002). Moreover, unlike low-molecular-weight surfactants, solid particles in Pickering emulsions are thought to be irreversibly adsorbed at the oil–water interface, providing a mechanical barrier against coalescence (Arditty *et al.*, 2004) and, in some cases, Ostwald ripening (Timgren *et al.*, 2011). This is a consequence of a very high energy (E) required to remove a spherical particle from the interface ($\sim 2750 kT$), relative to thermal energy (Binks, 2002). Energy of desorption (E) is related to the size of the particle (r), interfacial tension between the two phases ($\gamma_{\alpha\beta}$) and the contact angle (θ), which the particle makes with the interface:

$$E = \pi r^2 \gamma_{\alpha\beta} (1 \pm \cos \theta) \quad (5.1)$$

There are several factors determining whether particles will stabilise an emulsion in a desired manner. These include particle wettability, size, shape and concentration, as well as particle–particle interactions (Frascch-Melnik, 2011). Amongst these, the most important aspect determining the behaviour of particles at the interface is probably wettability, which is measured by the contact angle that the particle assumes at the oil–water interface (Schulman and Leja, 1954; Binks, 2002). Similar to the hydrophilic–lipophilic balance (HLB) value for surfactants, the contact angle (θ) is a relevant parameter in defining whether the particle prefers to reside in the polar or the non-polar phase. For hydrophilic particles, the contact angle measured in the water phase is usually $<90^\circ$ (see Fig. 5.1) and the particle is largely immersed in the water phase. When $\theta = 90^\circ$, the particle is equally wetted by water and oil, and in a similar way to surfactant molecules, such particles will not enhance formation of either water- or oil-continuous emulsions. On the other hand, W/O emulsions may be stabilised by particles if their contact angle is $>90^\circ$ (see Fig. 5.1).

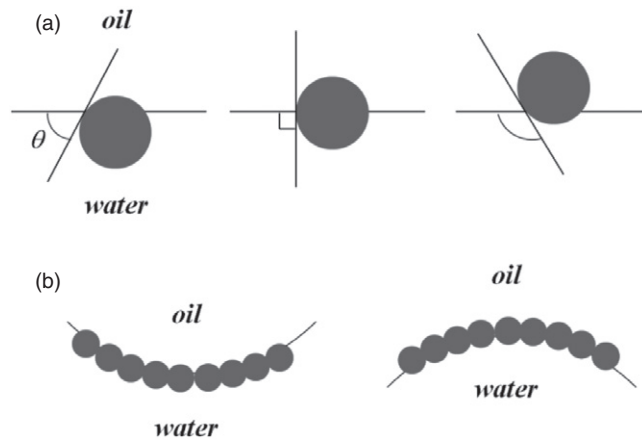


Fig. 5.1 (a) Contact angles of a small particle at a planar oil–water interface; (b) The position of solid particles at a curved interface: for $\theta < 90^\circ$ (left) an O/W emulsion can be formed, for $\theta > 90^\circ$ (right) a W/O emulsion can be formed. Courtesy of Dr R. Pichot. From Pichot, R. (2010) Stability and characterisation of emulsions in the presence of colloidal particles and surfactants. PhD thesis, University of Birmingham.

Apart from the wettability, an important factor in controlling the adsorption energy of solid particles is their size ($E \propto r^2$). In practice, in order to achieve effective Pickering stabilisation, the adsorbed particles need to be at least an order of magnitude smaller than the emulsion droplet they coat. This means that nano-sized particles would be suitable for stabilisation of a submicron-sized emulsion. However, even very small particles (~ 10 nm) can irreversibly adsorb at the interface, as long as their contact angle does not deviate too much from 90° (Dickinson, 2012).

5.2.1.2 Contact-angle measurements

Several methods of measuring particle wettability have been developed, depending upon the type of particle and/or the liquid(s). The contact angle of non-porous particles is commonly measured by *goniometry* or *tensiometry*. Goniometry involves the observation of a sessile drop of liquid on a solid substrate, while force tensiometry involves measuring the forces of interaction when a solid is in contact with a liquid. The contact angle of porous particles is most commonly measured using the *Washburn method*, where the time for a liquid to penetrate through a bed of compressed particles is measured. However, these techniques suffer from strong dependence on the degree of particle polydispersity and mechanical compression. On the other hand, a direct *microscopic observation* is limited to large particles (with $\sim 20 \mu\text{m}$ as the lower practical limit). A novel *gel-trapping technique* developed by Paunov (2003) allows visualisation of particles monolayer posi-

tioned on the air–water or oil–water interface. After particles adsorb at the interface, the water phase is gelled with non-adsorbing polysaccharide (e.g. gellan) and then the opposite phase is removed and replaced by a curing poly(dimethylsiloxane) (PDMS) elastomer. Next, a particle monolayer is “peeled off” with PDMS from the gelled aqueous phase and imaged with high-resolution scanning electron microscopy (SEM). The contact angle at the replicated interface is determined by measuring contact line radius and fitting the particle shape with a circle to determine the radius of curvature. Using this method, Paunov *et al.* (2007) measured the contact angle of irregularly shaped fat crystals, calcium carbonate particles coated with stearic acid and spray-dried soy protein/calcium phosphate, and found that the wettability data correlated well with the type of emulsion stabilised by these food-grade particles. Particles of intermediate wettability had the ability to invert emulsions from W/O to O/W, depending on the volume fraction of oil used.

5.2.1.3 Food-grade particles, and particle and emulsifier systems

A range of food-grade particles have been used for Pickering stabilisation. These include wax crystals (Binks and Rocher, 2009), CaCO_3 (Zhou *et al.*, 2009), ethyl cellulose (Campbell *et al.*, 2009), and protein–polysaccharide complexes (Schmitt *et al.*, 2009). For instance, naturally hydrophilic starch can be made partially hydrophobic (<3%) by chemical modification using octenyl succinic anhydride (OSA), and as such it has no limitations on its use as a food ingredient (i.e. E1450) (Timgren *et al.*, 2011). More interestingly, the ability of starches to gelatinise upon heating in the aqueous media offers the possibility of gelling its particles at the interface. By carefully selecting the temperature, an enhanced barrier to molecular transport across the interface can be formed. Timgren *et al.* (2011) used starch from quinoa, with a $D_{4,3} \sim 3.5 \mu\text{m}$, to stabilise 35–40 μm O/W emulsions (12.5–33.3 % oil). Droplet size decreased with particle concentration, and the emulsions showed increased resistance to lipolysis. When emulsions with adsorbed starch particles were heated to 70 °C, the lipolysis rate reduction (by up to 70%) could be observed, as compared to unheated emulsions. This was associated with partial gelatinisation of starch granules, which by swelling and leaching create a dense, cohesive layer at the interface.

Lipid oxidation in emulsions stabilised by solid particles was studied by Kargar *et al.* (2012). Microcrystalline cellulose (prepared from cellulose powder subjected to acid hydrolysis) and OSA-modified starch were used as sole stabilisers for O/W emulsions. Emulsion stability against coalescence was decreased at low pH due to loss of particle

surface charge, leading to droplet aggregation. pH-dependent lipid oxidation was hindered when a higher concentration of particles was used. This was associated with: (1) a thicker layer of adsorbed particles, but also (2) an increase in the viscosity of the system, resulting from the presence of non-adsorbed particles, and thus a decrease in mobility of pro-oxidants in the aqueous phase. When comparing the two particles, the potential of microcrystalline cellulose to reduce lipid oxidation to a greater extent than modified starch was linked with the particles ability to scavenge free radicals through their negative charge, and the ability to form a thicker layer around the oil droplets, due to their larger size (~ 415 nm with cellulose, ~120 nm with starch).

The contact angle of a particle at the oil–water interface is linked to surfactant adsorption onto the particle’s surface (Tambe and Sharma, 1994). According to Binks *et al.* (2007) and Binks and Rodrigues (2007) small-molecular-weight surfactants (both cationic and anionic) tend to adsorb at the particle surface (charged silica nanoparticles) and induce particle flocculation at the water–oil interface, which enhances emulsion stability towards creaming and coalescence. Surfactants can also displace particles from the interface (Pichot *et al.*, 2010; Vashisth *et al.*, 2010). Vashisth *et al.* (2010) mixed a silica-stabilised emulsion with an anionic surfactant (sodium dodecyl sulfate) solution, and observed that by applying shear the particles could be recovered from the interface without destabilising the emulsion. They concluded that desorption of particles from the interface was driven by energetically favourable adsorption of surfactant at the water–oil interface, rather than onto the particles’ surface. In other words, desorption of particles from the droplet interface is not determined by modification (if any) of their contact angle as a result of the surfactant adsorption onto the particles’ surface.

A study of a “*mixed emulsifier system*” containing both particles (hydrophilic silica) and oil-soluble surfactant (monoolein) was carried out by Pichot *et al.* (2009), who proposed a “two-part” mechanism resulting in long-term emulsion stability against coalescence. During emulsification, the role of low-molecular-weight monoolein is to initially reduce the interfacial tension, allowing further droplet break-up and delaying coalescence. This will ensure enough time for the less mobile colloidal particles to assemble at the oil–water interface, displace monoolein molecules and thus provide long-term O/W emulsion stability against coalescence. Moreover, both concentration of silica particles and surfactant affect the emulsion droplet size up to a set of respective “threshold” concentrations, above which the droplet size is limited by the emulsification process. When water-soluble emulsifiers (Tween 60, sodium caseinate and soybean lecithin) were used in combination with hydrophilic silica (Pichot *et al.*, 2010), emulsion micro-

structure was highly dependent on the emulsifier concentration. At low concentrations of Tween 60, for instance, silica particles adsorb at the oil–water interface (see Fig. 5.2a), resulting in stable emulsions with small droplet size. Upon increased surfactant concentration, particles were gradually displaced from the interface (see Fig. 5.2b), ultimately resulting in larger, surfactant-stabilised droplets (see Fig. 5.2c) at high silica concentrations. Such transformation from a Pickering stabilised emulsion to a surfactant-stabilised system was associated with particle–surfactant interactions and stronger competition for adsorption at the oil–water interface when surfactant concentration increased.

The study into the interactions between protein (sodium caseinate and whey protein isolate) and surface-active particles (cellulose and non-swelling starch) conducted by Murray *et al.* (2011) showed a synergistic effect of protein and particles at the interface. The relatively low visco-elasticity of the particle layer formed at the interface increased significantly when combined with proteins. Such a change in the interfacial properties of the adsorbed layer correlated with an increase in emulsion stability to coalescence and Ostwald ripening. With no evidence of specific particle–protein interactions, it was proposed that proteins enhance particle incorporation into the adsorbed layer and their *jamming* at the interface.

The idea of triggered release of encapsulated compound from Pickering-stabilised emulsions was explored by Frasc-Melnik *et al.* (2010a). W/O emulsions were constructed with a mixture of surface-active crystalline monoglyceride and network-forming triglyceride, with NaCl entrapped in the water phase. The purpose of such formulation design was to effectively stop salt migration during storage, but control its release under certain conditions (e.g. during consumption). By precisely selecting the ratio of monoglyceride to triglyceride and the processing conditions (combination of rapid cooling and high shearing rates applied in a scraped surface heat exchanger and a pin stirrer assembled in line), a sintered “shell” of fat crystals (see A in Fig. 5.3) at the interface, surrounded by network of fat crystals in the bulk continuous phase (see B in Fig. 5.3) could be obtained. Such emulsions effectively entrapped salt, independent of the magnitude of the applied osmotic pressure gradient. To trigger release of the salt, the temperature was modified and heat-induced melting or solvating of the fat crystals led to formation of cracks in the sintered fat crystal shells and leakage of water containing the encapsulated electrolyte. In their approach (Frasc-Melnik *et al.*, 2010a), monoglycerides were used to alter the polarity of the triglyceride crystals and also to seed formation of these crystals at the interface (Mullin, 1993).

The interplay of the two mechanisms of fat crystal stabilisation, surface-active (Pickering stabilisation), surface-inactive (network

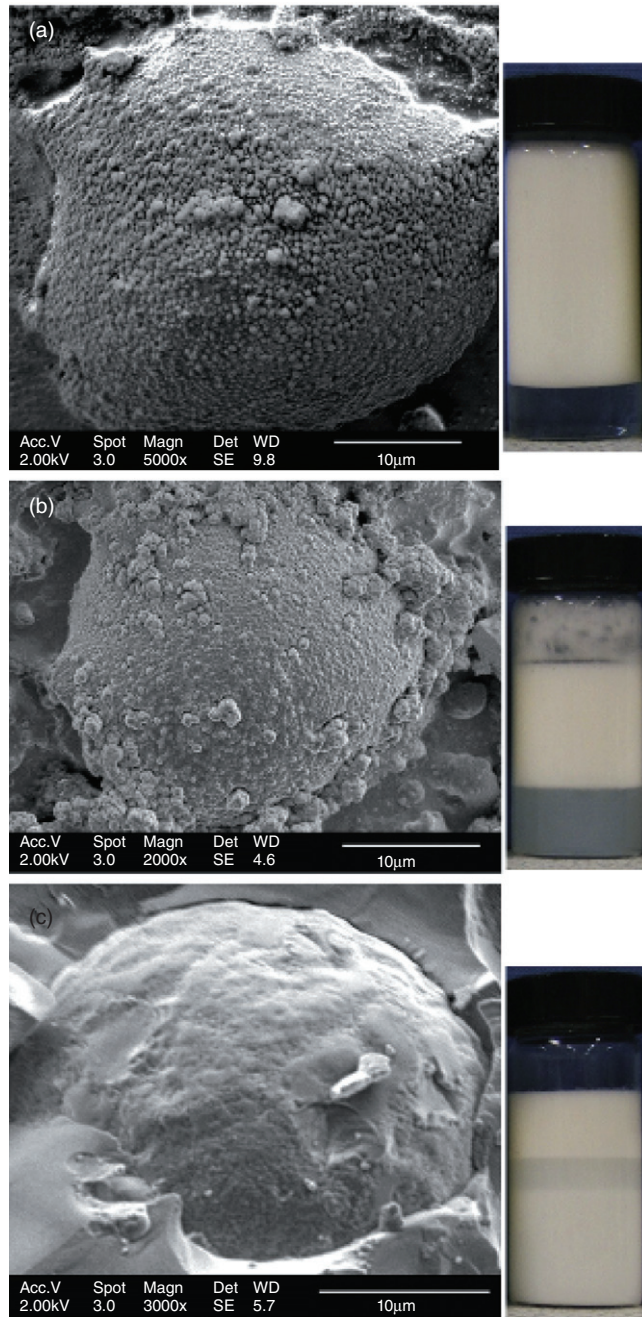


Fig. 5.2 SEM micrographs of O/W emulsion droplets stabilised by mixed emulsifier systems with 1% silica particles and: (a) 0.4%, (b) 1% and (c) 1.5% Tween 60. Reproduced from Pichot *et al.*, (2010) O/W emulsions stabilised by both low molecular weight surfactants and colloidal particles: The effect of surfactant type and concentration. *Journal of Colloid and Interface Science* 352, 128–135. With permission from Elsevier.

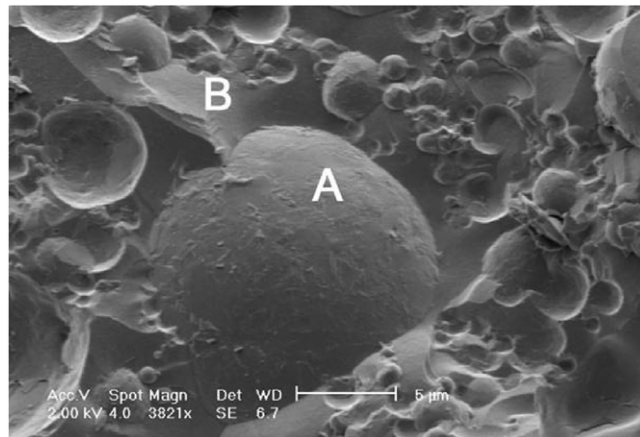


Fig. 5.3 SEM micrograph of an emulsion with tripalmitin. A solid shell (label A) of sintered fat crystals surrounds the water droplet. Surrounding these droplets is a continuous network of fat crystals (label B). Reproduced from Frasc-Melnik *et al.*, (2010a) Fat-crystal stabilised w/o emulsions for controlled salt release. *Journal of Food Engineering* 98, 437–442. With permission from Elsevier.

stabilisation), and a combination of both, on sedimentation and coalescence stability of emulsions, was investigated by Ghosh *et al.* (2011). Pickering stabilisation was achieved with glycerol monostearate and network stabilisation was due to simultaneous use of hydrogenated canola oil and glycerol monooleate. Authors concluded that Pickering crystals were more effective than network crystals for emulsion stabilisation. Upon thermal treatment, diffusion of molten emulsifier from the interface led to emulsion breakage. Frasc-Melnik *et al.* (2010a) showed that 40% more salt was released from W/O emulsions over a month when monoglyceride was used and less than 5% when the mixture of monoglyceride and triglyceride was used for emulsion stabilisation.

As most fat crystals are inherently lipophilic, their polarity can be altered by the addition of surfactants, which modify crystal surface properties (Johansson *et al.*, 1995). Surfactants such as monoolein, polyglycerol polyricinoleate (PGPR) and lecithin have been used to increase triglyceride wettability with water (Johansson and Bergensstahl, 1995; Garti *et al.*, 1999; Hodge and Rousseau, 2005). Whether fat crystals will stabilise an emulsion depends not only on their wettability but also their size, with submicron size crystals playing a crucial role (Hodge and Rousseau, 2005). The size of the crystals depends on processing parameters such as rate of cooling (Campos *et al.*, 2002) and shearing (Mullin, 1993). In the flash-cooling process Garti *et al.* (1998) produced the α polymorph of tristearin crystals, which were more hydrophilic than the β polymorph, thus more easily drawn to the interface (Johansson and Bergensstahl, 1995). Nevertheless, production

of non-flocculated, submicron α fat crystals required addition of a surfactant (PGPR). Also, the presence of small amount of PGPR during emulsification enhanced adsorption of α tristearin at the interface, resulting in increased emulsion stability to coalescence (Garti *et al.*, 1998).

5.2.2 Nano-emulsions

Gutiérrez *et al.* (2008) defined nano-emulsions as emulsions with a very small droplet size (less than 200 nm) and relatively high kinetic stability, regardless of their preparation method (i.e. obtained by either the shear or the condensation methods). The advantages of nano-emulsions, relative to conventional emulsions (for certain applications), are: optical transparency (or only slight turbidity), high surface-to-volume ratio and extreme stability against aggregation and gravitational separation (Kentish *et al.*, 2008; Troncoso *et al.*, 2012). Even though there is evidence of enhanced kinetic stability of nano-emulsions, their small droplets have extreme Laplace pressures (10–100 atm) (Velikov and Pelan, 2008), which make them exceptionally susceptible to Ostwald ripening (Wooster *et al.*, 2008).

Because nano-emulsions are non-equilibrium systems, they do not form spontaneously and energy input (from mechanical devices or from the chemical potential of the components) is required for their formation (Solans *et al.*, 2005). High-energy emulsification is usually carried out with high-pressure homogenisers and ultrasound generators (Anton *et al.*, 2007). However, the difficulty in formation of food-grade nano-emulsions in shear is the high viscosity of the triglyceride oil as compared to *n*-alkane oils (Wooster *et al.*, 2008), for example. On the other hand, low-energy emulsification methods utilise the physico-chemical properties of the components, and involve phase inversion of the system (Velikov and Pelan, 2008).

One of the key applications of nano-emulsions is the incorporation of a lipophilic compound (e.g. lemon oil as a flavouring agent) into aqueous media, which need to remain transparent (e.g. beverages) (Rao and McClements, 2011). However, their utilisation as food products is hindered by the limited choice of suitable food-grade surfactants and low sensory/regulatory/economic acceptance of synthetic ones (Rao and McClements, 2011). Henry *et al.* (2009, 2010) conducted a study where a range of low-molecular-weight surfactants, phospholipids and proteins were used to investigate the Ostwald ripening rates in nano-emulsions produced by impinging jet technology (Microfluidizer). Ostwald ripening rates increased in the presence of surfactant micelles (Henry *et al.*, 2009) and with protein-stabilised nano-emulsions due to increased solubility of decane in water caused by its binding to the

protein molecules. Interestingly, no ripening was found in phospholipid-stabilised nano-emulsions, which was attributed to emulsifier aggregation at the interface, which increases its elasticity (Henry *et al.*, 2010). As such, phospholipid-stabilised nano-emulsions can be utilised in the food industry where long-term stability is essential, in addition to increased bioavailability of lipophilic components that are usually poorly adsorbed (Qian and McClements, 2010; Li *et al.*, 2012).

5.2.3 Duplex emulsions

Production of low-energy dense, nutritionally enriched food-grade delivery systems can be achieved through microstructuring of the product matrix (Palzer, 2009). One way to do this is with duplex emulsions. First reported in 1923 (Seifriz, 1923), duplex (double) emulsions are complex systems also termed “emulsions of emulsions” or “emulsified emulsions”, as droplets of a dispersed liquid are further dispersed in another liquid. There are two main types of duplex emulsions: water-in-oil-in-water ($W_1/O/W_2$) and oil-in-water-in-oil ($O_1/W/O_2$), as shown in Fig. 5.4. Other types of duplex emulsions such as oil-in-water-in-water ($O/W_1/W_2$) have also been prepared and investigated (Kim *et al.*, 2006). The main advantage of multiple emulsions over simple emulsions is a direct consequence of their complex microstructure. As discussed earlier, such constructions could potentially act as microcarriers of active ingredients (hydrophilic or hydrophobic) that could be encapsulated in the internal droplets. These active ingredients could be then delivered in a controlled way during a specific process (Lesmes and McClements, 2009). This property of multiple emulsions has been appreciated by the pharmaceutical, food, agrochemical and cosmetic industries (Muschiolik, 2007).

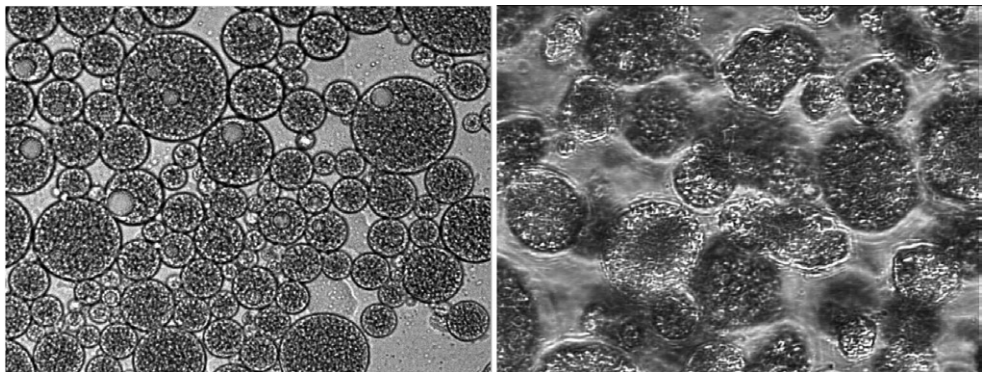


Fig. 5.4 Examples of duplex emulsions: $W_1/O/W_2$ emulsion (left), $O_1/W/O_2$ emulsion (right).

In food applications the possibility of salt reduction without compromising the taste of the product has been investigated (Malone *et al.*, 2003). Perception of saltiness of the food product increases with salt concentration in the water continuous phase. Reduction in the overall salt concentration can be realised by increasing the concentration of the dispersed phase while maintaining satisfactory, yet reduced salt levels in the external aqueous phase. With a $W_1/O/W_2$ duplex emulsion approach, the fat volume only appears to increase, as the fat phase is an emulsion itself (i.e. W_1/O emulsion). This means that the use of duplex $W_1/O/W_2$ emulsions allows for a substantial fat reduction, as the dispersed phase is made up by water contained in the internal aqueous phase (W_1). Therefore, a volumetric reduction in fat without a change in droplet size or phase content can be made. The rheological properties and microstructure of white cheese-like products based on duplex emulsion technology has been studied by Lobato-Calleros *et al.* (2006). Another potential benefit of duplex emulsions over conventional emulsions is taste masking of certain ingredients or additives, for example fish oils or bitter peptides (Leal-Calderon *et al.*, 2007).

5.2.3.1 Duplex formation

Duplex emulsions are conventionally prepared in a two-step emulsification process. The primary emulsion is typically formed under intense homogenisation conditions in order to transform two immiscible fluids into an emulsion, or to reduce the size of a pre-existing emulsion. In the food industry this process is usually carried out using mechanical devices (e.g. high-speed blenders, high-pressure homogenisers, colloid mills), where the dispersed phase is broken up by turbulent shear stresses. Such processes should preferably generate the smallest possible primary emulsion droplets, as such emulsions are: (1) more stable during the subsequent secondary emulsification step, and (2) incorporated into duplex emulsions, which ideally would be smaller than $20\ \mu\text{m}$ due to unacceptable sensory properties (Norton *et al.*, 2006).

Contrary to the primary emulsification, the secondary emulsification step has to be carried out under mild shear conditions in order to avoid the rupture of the internal droplets (Dickinson and Akhtar, 2001; McClements, 2005). In the conventional turbulence-based emulsification techniques (such as those used for the primary emulsification step) the recurrent rupture of the emulsion droplets may lead to a substantial loss of the internal phase. Novel methods of duplex emulsion manufacture, based on individual formation of droplets (drop-by-drop), have been proposed. These include microchannel emulsification (Sugiura *et al.*, 2004), microcapillary devices (Utada *et al.*, 2005), microfluidic devices (Okushima *et al.*, 2004) and membrane emulsification (Mine

et al., 1996; Vladislavljević *et al.*, 2006). It was suggested that using membrane technology for the secondary emulsification step gives the advantage of: (1) a good control over droplet size and droplet size distribution (Vladislavljević *et al.*, 2004), (2) low energy consumption (essential for temperature-sensitive components and from an economical point of view; Schadler and Windhab, 2006) and most importantly (3) low shear in the process (van der Graaf *et al.*, 2005). It has also been proposed (Aserin, 2008) that membrane emulsification enables high encapsulation yields of the internal droplets in the final product. Even though much work has been done (Mine *et al.*, 1996; Vladislavljević and Schubert, 2003; Vladislavljević *et al.*, 2004, 2006; Scherze *et al.*, 2005; van der Graaf *et al.*, 2005), on the investigation of the influence of membrane emulsification parameters on the droplet size and droplet size distribution in $W_1/O/W_2$ duplex emulsions, the encapsulation and marker release from these, remains scarcely explored (Okochi and Nakano, 1997; Scherze *et al.*, 2005; Vladislavljević *et al.*, 2006). By using a rotating membrane technique (Aryanti *et al.*, 2006; Schadler and Windhab, 2006; Yuan *et al.*, 2009), where the centrifugal force pushes the droplets off the membrane surface, the coalescence process can be limited. This results in a droplet size smaller than obtained for similar cross-flow process conditions (trans-membrane pressure, membrane pore size and surfactant concentration; see Fig. 5.5; Le Révérend *et al.*, 2010).

5.2.3.2 Duplex (in)stability

Despite the potential usefulness, the application of multiple emulsion technology has thus far been hindered by the many forms of instability (Jiao and Burgess, 2008). Duplex emulsions are thermodynamically unstable, and frequently a large part of the encapsulated inner phase is lost during their formation, handling and storage. It has also been reported that the overall stability of these structures effectively depends on the stability of the primary emulsion (Su *et al.*, 2006), which in turn is determined by the type and concentration of the emulsifiers used, and the nature of the fat phase and any entrapped materials (encapsulant). This is particularly true if possible encapsulants are, or include, small electrolyte molecules that can rapidly diffuse across the oil layer (Dickinson *et al.*, 1994). Also, the presence of two different surfactants (for stabilisation of W/O and O/W interfaces) is a major source of instability, as they tend to migrate from one interface to another, thus considerably shortening the emulsion lifetime (Garti, 1997b; Michaut *et al.*, 2004).

Another source of instability comes from the large Laplace pressure of small water droplets (W_1) encapsulated in duplex $W_1/O/W_2$

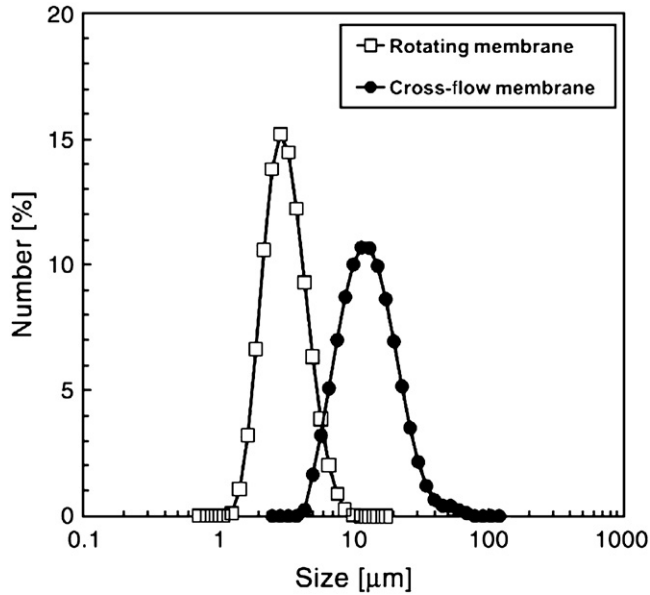


Fig. 5.5 Droplet size distributions for O/W emulsions manufactured using the rotating membrane (\square) and cross-flow membrane (\bullet) emulsification techniques. Both SPG membranes have $0.8 \mu\text{m}$ pores and both operate at a 60kPa trans-membrane pressure. Reproduced from Le Révérend *et al.*, (2010) Colloidal aspects of eating. *Current Opinion in Colloid and Interface Science* 15, 84–89. With permission from Elsevier.

emulsions, leading to water diffusion from the internal to the external water phase. Mass transfer rates in $W_1/O/W_2$ emulsions for the migration of water and/or water-soluble materials through the oil film are dominated by two major release mechanisms: (1) the “swelling-breakdown” mechanism and (2) diffusion/permeation through the oil membrane. In the second mechanism, the molecules either pass through thin lamellae of surfactant, which partially form in the oil layer due to fluctuations in its thickness, or diffuse across the oil layer via incorporation into “reverse micelles” (Cheng *et al.*, 2007) or via hydrated surfactants (Wen and Papadopoulos, 2001; Cheng *et al.*, 2007). Additionally, there is evidence suggesting that any osmotic pressure imbalance provides a driving force for the flux of water and water-soluble material between the two aqueous compartments (Mezzenga *et al.*, 2004). Such mass transfer could lead to emulsion destabilisation. However, for certain food applications, controlled release of an encapsulated substance could be considered beneficial.

5.2.3.3 Choice of emulsifiers

In the food industry, sorbitan monoesters are frequently used as high HLB surfactants (e.g. Tween(s) for O/W emulsion), and amongst low

HLB surfactants (for W/O emulsions) Span(s) and PGPR are the most common (Mezzenga, 2007). PGPR is a non-ionic, oil-soluble surfactant that is permitted for use in a number of food products in many countries, and has been frequently used in studies of duplex $W_1/O/W_2$ emulsions. Su *et al.* (2006) reported that the entrapment yield of polymeric dye was mostly dependent on the coalescence stability of the inner water droplets, which is controlled by PGPR concentration. Pawlik *et al.* (2010) showed that the visco-elastic properties of PGPR-adsorbed films at oil–water interfaces ensure long-term stability of the primary W_1/O emulsion, which in turn imparts overall duplex $W_1/O/W_2$ stability during preparation and storage.

Due to limitations of small-molecular-weight surfactants, polymeric emulsifiers have been used to improve stability, and to control sustained and prolonged release of active materials (Mezzenga *et al.*, 2004; Su *et al.*, 2006; Benichou *et al.*, 2007). By employing PGPR and sodium caseinate for the stabilisation of primary and secondary interfaces, respectively, Bonnet *et al.* (2009) showed that both primary and secondary emulsions were stable against coalescence for a month. Also bovine serum albumin was used in mixtures with monomeric surfactants (Tween 20 and Span 80) resulting in the improved stability of $W_1/O/W_2$ emulsions (Garti, 1997a).

Both $W_1/O/W_2$ and $O_1/W/O_2$ emulsions stabilised solely with solid particles have been prepared and claimed to be stable against coalescence for over a year (Barthel *et al.*, 2003). Duplex emulsions where the primary W_1/O emulsion was stabilised solely by fat crystals (of both monoglyceride and triglyceride, Frasc-Melnik *et al.*, 2010b) or mixture of fat crystals and a hydrophobic emulsifier (Garti *et al.*, 1999) showed good encapsulation properties associated with the formation of fat crystal “shells” around the internal water phase, which seal the water from releasing its addenda.

5.2.3.4 Control of the osmotic pressure

The effect of the osmotic pressure imbalance on the water transport rates in $W_1/O/W_2$ duplex emulsions is rather complex and to some extent still not completely explained. Wen and Papadopoulos (2001) showed, by observing single $W_1/O/W_2$ globules through capillary video microscopy, that only water may permeate through the oil phase while salt remains trapped in water compartments. In their later study, Cheng *et al.* (2007), through the same visual study method, found for the first time that ions of NaCl and AgNO₃ can migrate through thick oil films. The transport considerably slows when oil film thickness is below 1 μm and the transport of ions mainly takes place via “reverse micelles” instead of direct diffusion, and depends on the ion’s Pauling’s radius.

Jager-Lezer *et al.* (1997) found that there was no net transport of either water or electrolyte (MgSO_4) under iso-osmotic conditions in $W_1/O/W_2$ emulsions. Conversely, according to Garti (1997b) release of electrolytes takes place even if droplets are stable to coalescence and the osmotic pressures of the two phases have been equilibrated. It is therefore evident that knowledge of the interplay between water transport rates and the important system parameters is still insufficient for controlling water and encapsulant transport in multiple emulsions (Wen and Papadopoulos, 2001; Aserin, 2008).

5.2.4 Tri-phasic emulsions

One way of producing sensorially acceptable, low energy-dense emulsions is by employing small stable air cells designed to resemble oil droplets in terms of their size and physical properties. *Triphasic* emulsions contain two distinctive dispersed phases: oil and air mixed in the water continuous phase. Such aerated systems are mainly destabilised by gravitational drainage, coalescence and disproportionation, due to their high Laplace pressure and high air solubility in water. Tchienbou-Magaia *et al.* (2009) constructed a fat globule mimetic by stabilising air cells with a unique hydrophobin layer. Hydrophobins are fungi-derived small proteins (7–15 kDa) that self-assemble into an amphipathic, visco-elastic membrane at fluid interfaces (de Vocht, 2001). Bubble dispersions, termed “air-filled emulsions”, produced with hydrophobin HFBII from *Trichoderma reesei* in an ultra-sonication process, had sizes between 1 and 100 μm and showed little change in cell size up to 4 months. Mixing the “air-filled emulsions” with an O/W emulsion produced triphasic emulsions (see Fig. 5.6) consisting

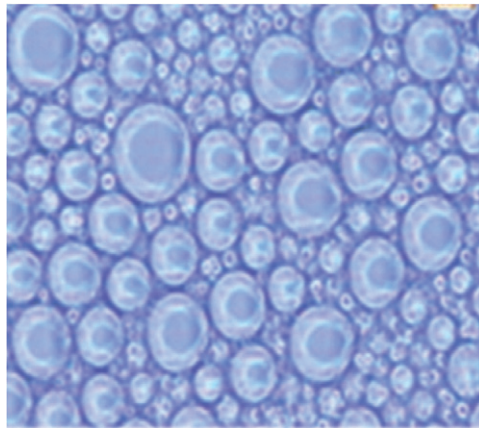


Fig. 5.6 Micrograph of an air- and oil-filled emulsion after four days storage (picture width 100 μm). Oil droplets are $\sim 8 \mu\text{m}$ in diameter and the air-filled droplets are $\sim 2 \mu\text{m}$ in diameter. Reproduced from Le Révérend *et al.*, (2010) Colloidal aspects of eating. *Current Opinion in Colloid and Interface Science* 15, 84–89. With permission from Elsevier.

of 20% air, 16% oil and 64% water. Their high capacity to survive high-shear mixing and their long-term stability (up to 45 days) were associated with the robust structure of the hydrophobin coating, which hinders coalescence and disproportionation. The authors demonstrated a close rheological match between emulsions with the same dispersed phase volume: a simple O/W (28% oil) and the tri-phasic emulsion (10% air and 18% oil). Nevertheless, specific properties of hydrophobins come at a high commercial cost, which may hinder their use in today's food industry. A much cheaper alternative, food-grade proteins have been used for the production of triphasic systems, making them financially promising for food and non-food products (Le Révérend *et al.*, 2010). This formulation engineering approach shows that the size, stability and elasticity of the air bubbles allow a substantial fat-replacement strategy, possibly without drastically compromising the oral behaviour of food emulsions, although this is yet to be investigated.

5.2.5 Water-in-water emulsions

Proteins and polysaccharides are widely found in food products. When mixed together in solution, they are often thermodynamically incompatible and under certain conditions tend to phase separate into two liquid phases. Such a phenomenon gives a rise to an emulsion-like morphology, termed a water-in-water (W/W) emulsion (Spyropoulos *et al.*, 2010). The tendency towards the phase separation of the two solutes in water is determined by the Gibbs free energy of mixing, and thus its constituent components: the entropy and enthalpy of mixing. In general, the enthalpy term is positive and favors de-mixing (phase separation) of the two solutes in a common solvent, due to the molecules' preference for neighbours of the same kind. Therefore, the changes in the entropy become important in determining the overall energy of the system. A reduction in temperature will reduce the entropy of the biopolymer system and may also alter the interactions between their components, leading to their increased incompatibility. Frequently, both intermolecular ordering and molecular weight increase, resulting in a reduced entropy penalty for the formation of two separate phases, is the main driver for phase separation (Norton and Frith, 2001).

The phase behaviour of mixed biopolymer systems is complex and can be controlled by various internal and external factors, such as biopolymer composition, gelation of one or two phases, macromolecular characteristics, and chemical interactions between the macromolecules and solvent, temperature, pressure and shear (Frith, 2010).

When the structure of a mixed biopolymer system is formed without any applied flow, the included phase usually forms spherical domains

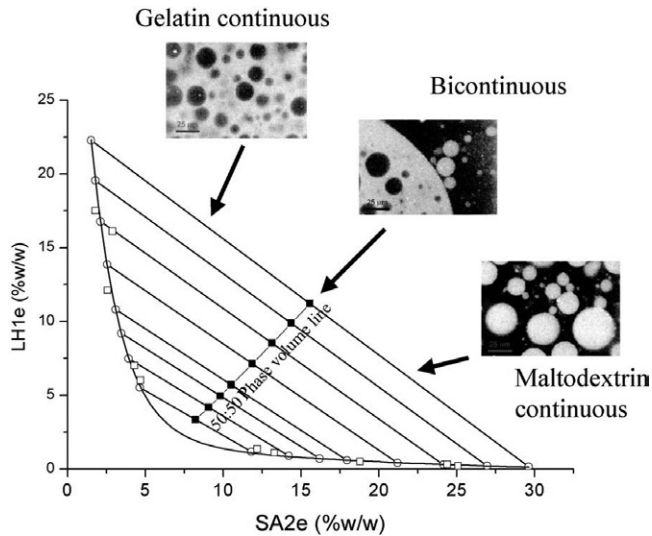


Fig. 5.7 Phase diagram of a mixture of gelatin (LH1e) and maltodextrin (SA2e). Insets show examples of the phase structures observed (in insets, light phase is protein rich). Reproduced from Norton and Frith (2001) *Microstructure design in mixed biopolymer composites*. *Food Hydrocolloids* 15, 543–553. With permission from Elsevier.

with sizes in the range of 2–20 μm (Norton and Frith, 2001). Fig. 5.7 shows examples of phase structures observed when the relative volume fraction of both phases change. In general, the equilibrium phases of a mixed biopolymer system show no inclination as to whether they want to be the included or the continuous phase (Frith, 2010). If the volume of one of the components increases, this biopolymer progressively stops being the dispersed phase, as the system goes through a bi-continuity stage (at 50:50 ratio of both components) and phase inverts (i.e. the dispersed phase now becomes continuous).

W/W emulsions are in certain aspects different to conventional O/W emulsions. For instance, due to the fact that both phases comprise 80 to 90% water, the interfacial tension between both phases is very small (Ding *et al.*, 2002), in the range of a few $\mu\text{N/m}$. In the absence of a non-polar phase, the addition of surfactant does not modify the interfacial properties of the system, as surfactant molecules tend to reside in the bulk water phases (Cox *et al.*, 2009). Also, unlike the conventional emulsions, both phases of W/W systems have similar densities, which lead to better stability against gravitational separation and make the phases very difficult to entirely separate without ultracentrifugation (Frith, 2010). Recently, advances in rheo-optical methodology allowed observation of the microstructural changes under flow (Pacek *et al.*, 1994; Wolf and Frith, 2003) and measurement of droplet deformation in a controlled flow (Wolf *et al.*, 2000; Ding *et al.*, 2002; Antonov

et al., 2004), which helps to better understand the properties of a number of systems.

Similarly to conventional emulsions, the structure of W/W emulsions is formed in shear by repeated processes of droplet break-up and coalescence (Foster *et al.*, 1997). The flow behaviour of a mixed biopolymer system can be influenced by factors such as the viscosity of the continuous phase, the viscosity ratio, the phase volume of both phases, the phase sense of both phases and their chemical composition (Portsch, 2011). The best way of stabilising the biopolymer structure is by halting the phase separation through gelation of either one or both components (Lorén *et al.*, 2001). This was done by Wolf *et al.* (2000), who by application of simple shear manipulated the phase morphology of W/W emulsions. By simultaneous cooling and shearing of biopolymer systems with at least one gelling component, microstructures with a wide spectrum of shapes were created. Moreover, by changing the flow stresses, control over the extent of anisotropy in biopolymer mixtures during gelation could be gained. Spherical particles were formed at lower shear stresses and elongated to irregular shapes when the shear stress was high. It has been shown that when the continuous phase contains the gelling agent or gels first, the phase sense of the system changes and it phase inverts. As with classical emulsions the application of shear to a biopolymer mixture during gelation leads to a situation where the more viscous phase is the included phase (Han *et al.*, 1998). Interest in kinetically trapped biopolymer structures derives from their potential as rheology modifiers (Wolf *et al.*, 2000), making them an effective tool for producing low-fat products with similar properties to high-fat products (Norton and Frith, 2001).

5.3 CONCLUSIONS

The large diversity of physiochemical and sensory properties of emulsion-based foods is the result of different kinds of ingredients and processing conditions applied during their production (McClements, 2005). Current lifestyles rapidly change the needs of the consumer, who demand cheaper, healthier, more palatable and more convenient food products. To satisfy this ever-increasing demand, the modern food industry requires new know-how on ways of designing colloidal food structures with the desired properties.

Current approaches to the formulation of microstructurally complex food emulsions have been considered, and various examples of differently stabilised emulsions have been discussed, with the emphasis on their potential applications.

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