

## Chapter 2

# Nano and Micro Food Emulsions

### 2.1 Methods of Formation

Emulsions with microdroplets, sometimes called *conventional emulsions*, and nanodispersions, or thermodynamically stable emulsions (surprisingly called *micro-emulsions*), can be easily manufactured on an industrial scale up. Due to their satisfactory stability over a certain storage time and high bioavailability, they have attained particular interest as delivery systems for bioactive substances, such as carotenoids, phytostetol, polyunsaturated fatty acids, g-oryzanol, lipophilic vitamins, and numerous other compounds. Garti and co-workers (Amar et al. 2003; Spornath et al. 2002), for example, prepared food-grade conventional emulsions containing carotenoids with considerable success. Recently, studies have shown the successful approach of using nanoemulsions to improve stability in food applications. Tan (2005) and Yuan et al. (2008) prepared  $\beta$ -carotene nanodispersions using high-pressure homogenization and studied their physicochemical properties. Other applications include the encapsulation of limonene, lutein, omega-3 fatty acids, astaxanthin, and lycopene (Chen et al. 2006), the encapsulation of  $\alpha$ -tocopherol to reduce lipid oxidation in fish oil (Weiss et al. 2006), and the use of nanoemulsions to incorporate essential oils, oleoresins, and oil-based natural flavors into food products such as carbonated beverages and salad dressings (Ochomogo and Monsalve-Gonzalez 2009).

#### 2.1.1 Nanoemulsions

In the last two decades, nanotechnology has rapidly emerged as one of the most promising and attractive research fields. The technology offers the potential to significantly improve the solubility and bioavailability of many functional ingredients. The high hydrophobicity of some bioactive substances makes them insoluble in aqueous systems, and they therefore have a poor intake in the body. To improve

carotenoids' dispersibility in water, for example (Horn and Rieger 2001), and also to increase their bioavailability during gastrointestinal passage (Deming and Erdman 1999), carotenoid crystals must be formulated, that is, to incorporate them in the fine particles of oil-in-water (O/W) emulsions.

Nanoemulsions are nonequilibrium systems and cannot be formed spontaneously. They can be produced using two different approaches: high-energy and low-energy methods. High-energy methods use intense mechanical forces to break up macroscopic phases or droplets into smaller droplets and typically involve the use of mechanical devices known as homogenizers, which may use high-shear mixing, high-pressure homogenization, or ultrasonification. In contrast, low-energy methods rely on the spontaneous formation of emulsions under specific system compositions or environmental conditions as a result of changes in interfacial properties (McClements and Li 2010). Spontaneous emulsification is a less expensive and energy-efficient alternative that takes advantage of the chemical energy stored in the system (Bilbao Sáinz et al. 2010). High-energy methods are effective in reducing droplet sizes but may not be suitable for some unstable molecules, such as proteins or peptides.

One of the most used low-energy methods is the phase inversion temperature (PIT) method, which is based on the changes in solubility of polyoxyethylene-type nonionic surfactants with temperature. The surfactant is hydrophilic at low temperatures but becomes lipophilic with increasing temperature due to dehydration of the polyoxyethylene chains. At low temperatures, the surfactant monolayer has a large positive spontaneous curvature forming oil-swollen micellar solution phases (or O/W microemulsions), which may coexist with an excess oil phase. At high temperatures, the spontaneous curvature becomes negative and water-swollen reverse micelles (or W/O microemulsions) coexist with an excess water phase. At a critical temperature—the hydrophilic–lipophilic balance (HLB) temperature—the spontaneous curvature is zero and a bicontinuous microemulsion phase containing comparable amounts of water and oil phases coexists with both excess water and oil phases. The PIT emulsification method takes advantage of the extremely low interfacial tensions at the HLB temperature to promote emulsification. However, the coalescence rate is extremely fast and the emulsions are very unstable even though emulsification is spontaneous at the HLB temperature. By rapidly cooling or heating the emulsions prepared at the HLB temperature, kinetically stable emulsions (O/W or W/O, respectively) can be produced with a very small droplet size and narrow size distribution. If the cooling or heating process is not fast, coalescence predominates and polydispersed coarse emulsions are formed (Ee et al. 2008).

Other low-energy methods that can be used to prepare nanoemulsions are the PIC (phase inversion composition) method, in which the temperature is maintained constant and the composition is changed (the solvent quality is changed by mixing two partially miscible phases together). By using the PIC method, different nanomaterials such as colloidosomes (Dinsmore et al. 2002), cubosomes (Spicer 2004), and microfluidic channels (Xu et al. 2005) have been prepared. Liu et al. (2006) used polyoxyethylene (PEO) nonionic surfactants for the preparation of paraffin oil-in-water nanoemulsions also by using the PIC method. They reported the preparation of

stable nanoemulsions with diameters ranging from 100 to 200 nm. Surfactants used in this system were a combination of Span 80, a sorbitan monooleate with a low HLB (4.3), and Tween 80, an ethoxylated sorbitan monooleate ester with a high HLB. As these two surfactants possess the same backbone, they can mix easily, leading to a controlled change in the final HLB.

Low-energy emulsification also includes the catastrophic inversion method (CPI). In a typical phase inversion process, emulsification starts with a given emulsion morphology that inverts to an opposite emulsion by variations in emulsion properties. For example, an oil-in-water system (O/W) inverts into a water-in-oil system (W/O) and vice versa. Catastrophic inversion is induced by increasing the volume fraction of the dispersed phase. In CPI emulsification, the system usually begins with abnormal emulsions, that is, emulsions in which the surfactant has a high affinity to the dispersed phase. Abnormal emulsions are usually unstable and can only be maintained under vigorous mixing for a short period of time. The ultimate fate of an unstable emulsion is to invert to the opposite state. A CPI is triggered by increasing the rate of droplet coalescence so that the balance between the rate of coalescence and rupture cannot be maintained. This may be induced by changing the variables that increase the rate of droplet coalescence, such as the continuous addition of dispersed-phase volume, the most common variable used, or by adding a surfactant or a salt, or by altering the emulsification temperature or any parameter that can significantly enhance droplets' coalescence. Droplets formed via CPI are usually above micrometer. Submicrometer droplets may only form if CPI of abnormal to normal emulsion occurs in the vicinity of the locus of ultralow interfacial tension (Sajjadi et al. 2004; Jahanzad et al. 2010). Peng et al. (2010) adopted a low-energy method combining the PIT and CPI methods to prepare the water-in-oil nanoemulsion. The aim of their work was to gain a better understanding of the relationship among the ratio of surfactants, the water/oil ratio, and long-term stability. They found that the addition of a second surfactant to the formulation could provide more stable nanoemulsions with the minimum size than only one surfactant. This result was in agreement with other authors' findings, who reported that to form nanoemulsions, surfactant mixtures generally perform better than pure surfactants for various applications (Rees et al. 1999; Uskokovi and Drogenik 2005; Pey et al. 2006). Peng et al. (2010) also reported the optimum composition for the systems they studied.

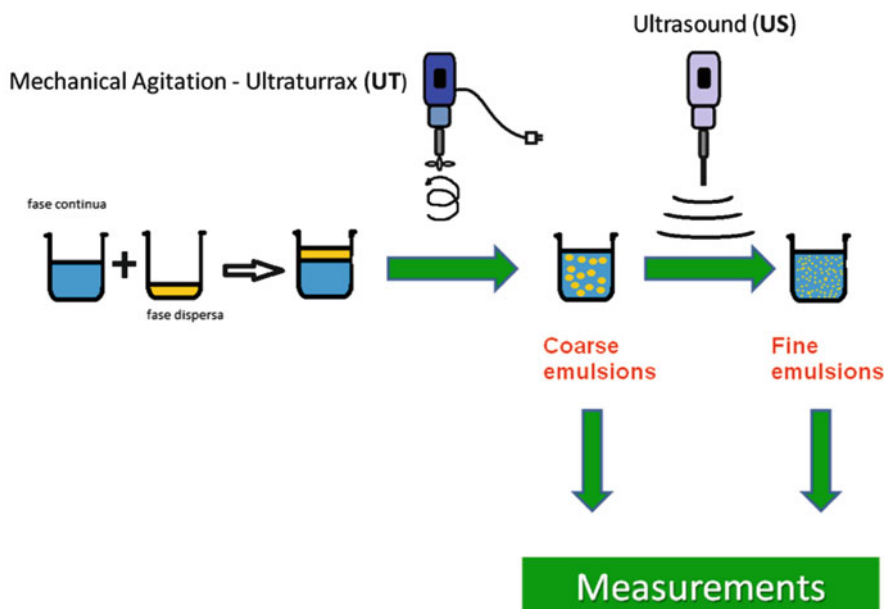
Another commonly used inversion method is transitional phase inversion (TPI) (Jahanzad et al. 2010). Before TPI can occur, the surfactants in both phases must diffuse toward the interface, adsorb at the interface, and conform into a mixed surfactant layer at the optimum conditions. The rate of diffusion of the surfactants depends on many parameters, including their size, the viscosity of the phase, and the intensity of mixing, etc. For oil-in-water emulsions containing a pair of water-soluble and oil-soluble surfactants, it was found that the addition of the water phase containing the water-soluble surfactant to the oil phase containing the oil-soluble surfactant may produce very fine emulsions if it is associated with interfacial tension lowering in the course of addition. The rate of addition of the second phase is of great importance in achieving an emulsion with the desired properties. This is

because the dynamic of phase inversion emulsification is very fast, and the emulsion properties change quickly with further addition, contrary to some conventional emulsification methods. Therefore, it is important to find and maintain a semi-equilibrated state in the course of emulsification during which sufficient surfactant diffusion/adsorption can occur, and thus droplet rupturing is enhanced. TPI occurs when the curvature of the oil–water interface gradually changes from positive to negative, passing through a zero curvature at the inversion point. This is associated with a shift in the surfactant nature from water-soluble to oil-soluble, or vice versa. At the inversion point, the surfactant has a similar affinity toward both phases. As a result, the interfacial tension passes through an ultralow value. This results in the formation of emulsions with a very small drop size, sometimes called *miniemulsions* and *nanoemulsions* (Jahanzad et al. 2010).

In a recent work, Ribeiro et al. (2008) produced  $\beta$ -carotene-loaded nanodispersions containing poly(D,L-lactic acid) (PLA) and poly(D,L-lactic-coglycolic acid) (PLGA) by the solvent displacement method. Nanoparticles containing  $\beta$ -carotene were produced by interfacial deposition of the biodegradable polymer, due to the displacement of acetone from the dispersed phase. Gelatin or Tween 20 was used as stabilizing hydrocolloids in the continuous phase.  $\beta$ -carotene was entrapped in the polymeric matrix in the absence of any oily core material. In this kind of formulation, polymers assumed the function of protective colloids and possibly also chemical stabilization of the nanodispersed phase.

### 2.1.2 Conventional Emulsions

Conventional emulsions, that is, emulsions with microdroplets, are thermodynamically unstable liquid–liquid dispersions. Emulsions with particle sizes higher than 100 nm are usually prepared by high-energy methods. As in the case of nanoemulsions, the O/W systems consist of lipid droplets dispersed in an aqueous medium, with each lipid droplet being surrounded by a thin emulsifier layer. The initial droplet concentration and size distribution of this type of delivery system can be controlled, as can the nature of the emulsifier used to stabilize the droplets. A careful selection of emulsifier type enables one to control interfacial properties such as charge, thickness (dimensions), rheology, and response to environmental stresses (such as pH, ionic strength, temperature, and enzyme activity) (McClements and Li 2010). To form conventional emulsions, usually a preemulsion with coarse particles, obtained by a mechanical device such as an Ultra-Turrax, is prepared. This system is further homogenized to obtain a microdroplet emulsion. In general, a variety of homogenizers are available to prepare emulsions, including high-shear mixers, high-pressure homogenizers, colloid mills, ultrasonic homogenizers, and membrane homogenizers. The choice of a particular kind of homogenizer and of the operating conditions used depends on the characteristics of the materials being homogenized (e.g., viscosity, interfacial tension, and shear sensitivity) and of the required final properties of the emulsion (e.g., droplet concentration, droplet size, and viscosity) (McClements



**Fig. 2.1** Scheme of conventional emulsions preparation procedure

and Li 2010). Figure 2.1 illustrates a preparation procedure. This is a common approach widely used in the literature. As an example, the typical conditions are as follows: The fat and aqueous phases may be mixed using an Ultra-Turrax high-speed blender operated at 20,000 rpm for 1 min. The resulting preemulsions may be further homogenized for 20 min using an ultrasonic liquid processing. It is advisable that the temperature of the sample cell is controlled by means of a water bath usually set at a temperature that prevents protein denaturalization and that does not increase the system viscosity very much, such as 15°C. By doing this, the sample temperature never rises higher than 40°C during ultrasound treatment. Then emulsions are typically cooled quiescently to ambient temperature (22.5°C) to perform physicochemical analysis. Particle sizes obtained with this protocol may vary from 0.2 to 1  $\mu\text{m}$  depending on the system under study (Álvarez Cerimedo et al. 2010).

## 2.2 Physical Chemical Properties

### 2.2.1 Nanoemulsions

The physical properties of nanoemulsions can be characterized by the combination of a wide variety of techniques. For example, the macroscopic properties, such as viscosity/viscoelasticity, conductivity, and interfacial tension, can be characterized

by rheometer, conductivity meter, and pendant drop tensiometer, respectively (Boonme et al. 2006). The size and shape of the emulsion droplets were routinely characterized by static and dynamic light scattering techniques (McClements 2005). The major drawback of light scattering techniques is that dilution of emulsion samples is usually necessary to reduce multiple scattering and interdroplet interactions. The dilution process may modify the structure and composition of the pseudoternary phases of the nanoemulsions; therefore, the obtained results do not accurately describe the actual system under study (Huang et al. 2010).

### 2.2.2 *Conventional Emulsions*

Conventional emulsions may also be characterized by the techniques mentioned above. However, there are other alternatives that allow emulsions to be described without the drawback of dilution. For example, particle size may be measured by low-resolution nuclear magnetic resonance (NMR). As will be explained in Chap. 3, the principle of this technique is completely different than that of light scattering techniques, and in some cases, a more accurate description of the system may therefore be made.

## 2.3 Structuring Food Emulsions

The structure of the different pseudoternary phases can be investigated by small-angle X-ray scattering (SAXS), small-angle neutron scattering (SANS), and microscopy-like cryotransmission electron microscopy (TEM) (Spicer et al. 2001; Borne et al. 2002; Boonme et al. 2006). These techniques may also be used to describe conventional emulsions' structure. By using SAXS measurements, Álvarez Cerimedo et al. (2010) proved that the role of trehalose in caseinate/fish oil emulsions went beyond the ability to form viscous solutions. For those systems, values of  $q$  (the reciprocal lattice spacing, with  $q=2\pi/d=4\pi \sin(\theta)/\lambda$ , where  $d$  is the interplanar spacing and  $2\theta$  is the Bragg angle) were significantly modified when the aqueous phase contained trehalose compared to emulsions without sugar in the aqueous phase. Values for emulsions with 10 wt.% fish oil and 5 wt.% sodium caseinate were 241, 0.248, and 0.252 nm<sup>-1</sup> for emulsions with 0, 20, and 30 wt.% trehalose, respectively. Some other aqueous phase components such as hydrocolloids proved to stabilize emulsions because they increase viscosity. The slightly increased  $q$  values with the trehalose addition suggested that trehalose had an effect beyond viscosity changes since modification of  $q$  values means that the aggregation state of the protein changed with the aqueous phase formulation. These results were in agreement with the small particle size as measured by dynamic light scattering found when trehalose was added to aqueous phase. These results were corroborated by confocal laser scanning microscopy (CLSM). CLSM is a more common technique to describe emulsion structure. It is widely used in food applications and provides a good description of the spatial distribution of different phases.

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