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Edible Nanoemulsions: Fabrication, Properties, and Functional Performance

D. Julian McClements, *University of Massachusetts - Amherst*



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Edible nanoemulsions: fabrication, properties, and functional performance

David Julian McClements*

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There is increasing interest within the food, beverage and pharmaceutical industries in utilizing edible nanoemulsions to encapsulate, protect and deliver lipophilic functional components, such as oil-soluble flavors, vitamins, preservatives, nutraceuticals, and drugs. There are a number of potential advantages of using nanoemulsions rather than conventional emulsions for this purpose: they can greatly increase the bioavailability of lipophilic substances; they scatter light weakly and so can be incorporated into optically transparent products; they can be used to modulate the product texture; and they have a high stability to particle aggregation and gravitational separation. On the other hand, there may also be some risks associated with the oral ingestion of nanoemulsions, such as their ability to change the biological fate of bioactive components within the gastrointestinal tract and the potential toxicity of some of the components used in their fabrication. This tutorial review provides an overview of the current status of nanoemulsion fabrication, properties, and applications with special emphasis on systems suitable for utilization within the food industry.

Department of Food Science, University of Massachusetts, Amherst, MA, 01003, USA. E-mail: mclements@foodsci.umass.edu; Tel: +1 413 545 1019



David Julian McClements

David Julian McClements is a Professor at the Department of Food Science at the University of Massachusetts. He specializes in the areas of food biopolymers and colloids, and in particular on the development of food-based structured delivery systems for active components. Dr McClements received his PhD in Food Science (1989) at the University of Leeds (UK). He then did Post Doctoral Research at the University of Leeds, University of California (Davis) and University College

Cork (Ireland). Dr McClements is the sole author of the first and second editions of "Food Emulsions: Principles, Practice and Techniques", co-author of "Advances in Food Colloids" with Prof. Eric Dickinson, and co-editor of "Developments in Acoustics and Ultrasonics", "Understanding and Controlling the Microstructure of Complex Foods", "Designing Functional Foods" and "Oxidation in Foods and Beverages". In addition, he has published over 400 scientific articles as journal manuscripts, book chapters, encyclopedia entries and conference proceedings. Dr McClements has received awards from the American Chemical Society, American Oil Chemists Society, Institute of Food Technologists and University of Massachusetts in recognition of his scientific achievements.

1. Introduction

There has been growing interest in the utilization of *nano-emulsions* in the food, beverage, and pharmaceutical industries because they have a number of potential advantages over conventional emulsions for certain applications. Nanoemulsions have the ability to greatly increase the bioavailability of highly lipophilic substances encapsulated within them.¹ Nanoemulsions can be designed to have high stability to particle aggregation and gravitational separation, and therefore they may be used to extend the shelf life of commercial products.² The droplets in nanoemulsions are so small that they only scatter light waves weakly, and so they can be incorporated into optically transparent products without adversely affecting their clarity, e.g., fortified soft drinks and waters.^{3–5} Nanoemulsions can be made to be highly viscous or gel-like at much lower droplet concentrations than conventional emulsions,^{2,5,6} and could therefore be used to create products with novel textural attributes or reduced calories. This tutorial review provides an overview of the current status of nanoemulsion fabrication, properties and applications, with specific emphasis on edible systems that are applicable for application within the food industry. Only oil-in-water type nanoemulsions will be considered since these currently have the greatest potential for application within commercial products.

2. General characteristics of nanoemulsions

Generally, an emulsion consists of at least two immiscible liquids (usually oil and water, but not always), with one of the liquids being dispersed as small spherical droplets in the other.^{7–10} A number of different terms are commonly used to describe different kinds of emulsions, and it is important to clarify what these terms mean (Table 1).⁵ A conventional emulsion typically has droplets with mean radii between 100 nm and 100 μ m. This type of emulsion is thermodynamically unstable because of the relatively large positive free energy (interfacial tension) associated with the contact between the oil and water phases.

Table 1 Comparison of the properties of different emulsion types that can be prepared from oil, water and emulsifier

Emulsion type	Radius range	Stability	Surface-to-mass ratio/m ² g ⁻¹	Appearance
<i>Emulsion</i>	100 nm to 100 μm	Metastable	0.07–70	Turbid/opaque
<i>Nanoemulsion</i>	10–100 nm	Metastable	70–330	Clear/turbid
<i>Microemulsion</i>	2–50 nm	Stable	130–1300	Clear

Conventional emulsions tend to be optically opaque because the droplets have similar dimensions to the wavelength of light ($r \approx \lambda$) and so scatter light strongly—provided that the refractive index contrast between the droplets and surrounding liquid is not close to zero and the droplet concentration is not too low. A *nanoemulsion* can be considered to be a conventional emulsion that contains very small droplets, *i.e.*, mean radii between 10 to 100 nm.^{2,5} The relatively small droplet size compared to the wavelength of light ($r \ll \lambda$) means that they tend to be transparent or only slightly turbid (similar to microemulsions). In addition, the small droplet size means that they have much better stability to gravitational separation and aggregation than conventional emulsions.^{2,11} On the other hand, these systems are still thermodynamically unstable systems since the separated oil and water phases have a lower free energy than the emulsified oil and water phases (like conventional emulsions). A *microemulsion* is a thermodynamically *stable* system that typically contains particles with radii somewhere in the range of 2 to 50 nm. In this case, the free energy of the microemulsion is lower than those of the phase separated components from which it is comprised so it tends to form spontaneously. It should be stressed that a given system may only exist as a microemulsion under a particular set of environmental conditions (*e.g.*, composition and temperature), and if these conditions are altered then it may no longer be thermodynamically stable. A microemulsion may therefore change into a nanoemulsion, conventional emulsion, liquid crystalline system, or a phase separated system if the environmental conditions are changed. However, if the system is brought back to the original conditions, then it should turn back into a microemulsion, albeit at a rate that depends on any kinetic energy barriers. Microemulsions are usually optically transparent because the particle size is much smaller than the wavelength of light ($r \ll \lambda$) so that light scattering is weak. Microemulsions can also often be distinguished from nanoemulsions in terms of the particle shape. Nanoemulsions tend to have spherical droplets because the relatively high interfacial tension and small droplet size lead to a high Laplace pressure favoring the minimization of the oil–water interfacial area. On the other hand, microemulsions may contain spherical, ellipsoid, or worm-like shapes depending on the molecular geometry of the surfactants used because the interfacial tension is typically much lower. From both a scientific and a technical point of view, it is important to clearly distinguish between these different kinds of systems since this determines the best approach to optimize their formation, stability, physicochemical properties, and functional performance. In the remainder of this chapter, the focus will be primarily on nanoemulsions, but much of the material covered will also be relevant to conventional emulsions and microemulsions.

Nanoemulsions are thermodynamically unfavorable systems because of the positive free energy associated with creating the

oil–water interface, and so they have a tendency to break down over time, *e.g.*, due to gravitational separation, flocculation, coalescence, and/or Ostwald ripening.^{7–9} The rates at which these processes occur are often considerably different in nanoemulsions than in conventional emulsions because of particle size and curvature effects. For example, nanoemulsions are often more stable to gravitational separation, flocculation and coalescence, but less stable to Ostwald ripening (see later). A major focus of emulsion scientists is therefore to create nanoemulsions that have a sufficiently long *kinetic* stability for commercial applications. The kinetic stability of nanoemulsions can be improved by controlling their composition (*e.g.*, oil and water phase) and microstructure (*e.g.*, particle size distribution), or by incorporating substances known as *stabilizers*: such as emulsifiers, texture modifiers, weighting agents or ripening retarders.^{8,12,13} The type of stabilizers that can be used in food applications is restricted by government regulations, cost considerations, and practical factors (such as ease of utilization, reliability of source, and matrix compatibility). Common stabilizers that can be used by the food industry to formulate nanoemulsions are shown in Table 2.

Selection of the most appropriate stabilizer(s) to utilize within a nanoemulsion is one of the most important factors determining its fabrication and functional performance. In addition, the formation and stability of nanoemulsions are highly dependent on the nature of the oil phase used, *e.g.*, its polarity, water solubility, interfacial tension, and rheology. Finally, the ability to produce nanoemulsions containing small droplet sizes depends on the approach used to fabricate them, *e.g.*, high or low energy methods.^{2,3,8,14,15} In the following sections, methods used to prepare nanoemulsions, the physicochemical properties of nanoemulsions, and potential applications of nanoemulsions within the food industry are discussed.

3. Controlling nanoemulsion particle properties

The physicochemical properties of nanoemulsions, such as their optical properties, stability, rheology, and release characteristics, are mainly determined by the characteristics of the particles that they contain, *e.g.*, composition, concentration, size, physical state, and interfacial properties.⁸ The design of food-grade nanoemulsions with specific functional attributes therefore depends on an appreciation of how particle properties can be rationally controlled.

3.1. Particle composition

The composition of the particles in edible nanoemulsions can be controlled by careful selection of the food-grade ingredients used to fabricate them, *e.g.*, lipids, proteins, polysaccharides, surfactants, and minerals. The type of ingredients that can be used is

Table 2 Examples of some common stabilizers that can be used in the food industry to formulate nanoemulsions. Abbreviations: BVO = brominated vegetable oil; LCT = long chain triglycerides; WPI = whey protein isolate; soy = soy protein isolate

Stabilizer	Function	Examples
<i>Emulsifier</i>	Surface-active substances that adsorb to oil–water interfaces and form protective coatings around droplets that prevent droplet aggregation	Small molecule surfactants (<i>e.g.</i> , Tweens, Spans), phospholipids (<i>e.g.</i> , egg, soy or dairy lecithin), amphiphilic proteins (<i>e.g.</i> , WPI, caseinate), amphiphilic polysaccharides (<i>e.g.</i> , gum Arabic, modified starch)
<i>Texture modifier</i>	Substances that increase the viscosity or gel the aqueous phase. These may be used to provide desirable textural characteristics, or to stop gravitational separation.	Sugars (<i>e.g.</i> , sucrose, HFCS), polyols (<i>e.g.</i> , glycerol, sorbitol), polysaccharides (<i>e.g.</i> , xanthan, pectin, carrageenan, alginate) and proteins (<i>e.g.</i> , gelatin, WPI, SPI)
<i>Weighting agent</i>	Substances that are added to oil droplets to match their density to that of the surrounding continuous phase	Dense lipophilic materials (<i>e.g.</i> , BVO, SAIB, Ester Gums)
<i>Ripening retarder</i>	Highly hydrophobic substances that slow down or prevent Ostwald ripening when incorporated within oil droplets	Lipophilic materials with very low water-solubility (<i>e.g.</i> , LCT, Esters Gums)

often limited by the preparation method used (see later). For example, nanoemulsions can be prepared using the phase inversion temperature or spontaneous emulsification methods when surfactants are used as emulsifiers, but not when proteins or polysaccharides are used.

Conceptually, the droplets in nanoemulsions can be considered to consist of a *core* of lipophilic material surrounded by a *shell* of adsorbed material.⁸ The lipophilic core may be comprised of one or more non-polar components, including triacylglycerols, diacylglycerols, monoacylglycerols, flavor oils, essential oils, mineral oils, fat substitutes, waxes, weighting agents, oil-soluble vitamins, and nutraceuticals (such as carotenoids, phytosterols, curcumin, and co-enzyme Q). The shell surrounding the core may also be comprised of one or more materials, including surfactants, phospholipids, proteins, polysaccharides, and minerals. The physicochemical properties and biological fate of the droplets in nanoemulsions depend on the type, concentration, and location of these various components within the droplets.

In conventional emulsions, the thickness of the shell layer (δ_S) is much smaller than the radius (r) of the lipophilic core ($\delta_S \ll r$), and so the particles can be thought to consist predominantly of the oil phase. On the other hand, in nanoemulsions $\delta_S \approx r$ and hence the shell layer makes an appreciable contribution to the overall particle composition.^{2,5} To a first approximation this effect can be described by the following equation:

$$\Phi_S = \frac{(r + \delta_S)^3 - r^3}{(r + \delta_S)^3} \quad (1)$$

Here $\Phi_S (=V_S/V_{\text{effective}})$ is the volume of the shell divided by the effective volume of the overall particle (=core + shell). This equation can be used to estimate the importance of core radius and shell thickness on particle composition in nanoemulsions. For example, Φ_S is 25, 49, 70 and 88% for nanoemulsions containing oil droplets with core radius of 20 nm and shell

thicknesses of 2, 5, 10 and 20 nm, respectively. Whereas Φ_S is only 3, 7, 14 and 25%, for emulsions containing oil droplets with core radius of 200 nm and similar shell thicknesses. The thickness of the shell layer depends on the type of molecules adsorbed to the oil–water interface. In food systems, the shell thickness is typically 1–2 nm for small molecule surfactants, 2–10 nm for protein monolayers, and 10–50 nm for biopolymer multilayers. Thus the composition of the particles in nanoemulsions may be quite different from those in conventional emulsions made from the same ingredients.

The size-dependence of particle composition has a number of important practical implications. The size of the droplets in nanoemulsions is often determined by instrumental techniques such as laser diffraction, spectro-turbidity, and dynamic light scattering.⁸ The mathematical models used by these instruments to calculate the particle size distribution of an emulsion from some measured physical property (such as a light scattering pattern) usually assume that the particles are homogeneous spheres with well-defined properties (such as refractive index and density). Consequently, there may be errors in the reported particle size distributions in nanoemulsions when the particles are core–shell structures rather than homogeneous spheres. In addition, the size-dependence of the particle composition is also important because it impacts the physicochemical properties of the particle (such as refractive index and density) that alter the bulk physicochemical properties of emulsions (such as optical properties and creaming rate).

3.2. Particle concentration

The concentration of the particles in edible nanoemulsions can be controlled by altering the initial amount of oil used to prepare the system, or by diluting or concentrating the system after production. The *effective concentration* of particles in a nanoemulsion may be considerably different from that in

a conventional emulsion with the same oil content because of the small particle dimensions.^{2,5} The *effective* volume fraction ($\phi_{\text{effective}}$) of the coated oil droplets in a nanoemulsion can be related to the volume fraction (ϕ) of the uncoated oil droplets:

$$\phi_{\text{effective}} = \phi \left(1 + \frac{\delta_s}{r} \right)^3 \quad (2)$$

In conventional emulsions ($\delta_s \ll r$) the effective particle concentration is similar to the oil concentration ($\phi_{\text{effective}} \approx \phi$), but in nanoemulsions ($\delta_s \approx r$) it may be appreciably larger than the oil concentration ($\phi_{\text{effective}} > \phi$) due to the influence of the shell layer. Calculations made using this equation indicate that the effective particle concentration may be much larger than the oil phase concentration when the droplets are small or the shell layer is thick (Fig. 1). These increases in effective concentration are important and may cause appreciable changes in the bulk physicochemical properties of nanoemulsions, such as rheology and stability (see below). Indeed, it may be possible to utilize this effect to create nanoemulsion-based food products with novel characteristics, such as highly viscous or gelled products (such as dressings or mayonnaise) with much lower oil contents than in conventional emulsions.

The effective thickness of the shell layer (δ_s) may be considerably larger than the physical dimensions of the molecules adsorbed to the droplet surfaces in electrostatically stabilized systems.^{2,5} In these cases, the thickness depends on the range of the electrostatic interactions (Debye screening length, κ^{-1}) of the system, which depends on the ionic strength of the surrounding aqueous phase.⁸ For aqueous solutions at room temperatures, $\kappa^{-1} \approx 0.304/\sqrt{I}$ nm, where I is the ionic strength expressed in moles per litre.¹⁶ For example, the ranges of the electrostatic interactions in salt solutions with different ionic strengths are: $\kappa^{-1} = 0.3, 0.96, 3, 9.6$ and 30 nm for $I = 1, 10^{-1}, 10^{-2}, 10^{-3}$ and 10^{-4} M, respectively. At low ionic strengths, the Debye screening

length may therefore be of the order of the radius of the droplets in nanoemulsions, which can have a major impact on the bulk physicochemical properties of the system, such as the optical or rheological properties.^{5,17–19}

3.3. Particle size

The size of the particles within a nanoemulsion is important because it influences its optical, rheological, stability, and release characteristics. The particle size distribution (PSD) of an emulsion can usually be controlled by varying emulsion preparation conditions and/or system composition. For example, in high-energy methods the droplet size depends on the intensity and duration of the energy input, the type and concentration of emulsifier used, the interfacial tension, and the relative viscosities of the disperse and continuous phases.^{8,20} Smaller droplets can usually be produced by increasing the intensity or duration of homogenization, by increasing the concentration of emulsifier used, or by controlling the viscosity ratio.^{11,21,22} In low-energy methods, the droplet size depends on factors such as system composition (such as surfactant–oil–water ratio, surfactant type, ionic strength) and environmental conditions (such as temperature–time history, stirring speeds).^{15,23} Different strategies are therefore needed to optimize the droplet size depending on the preparation method used.

As mentioned above, the radius of the particles in a nanoemulsion is the sum of the radius of the oil droplet core and the thickness of the shell layer: $r_{\text{effective}} = r + \delta_s$. One would therefore expect that smaller particles could be formed by using an emulsifier that forms a thinner layer around the oil droplets. There are appreciable variations in the thickness of the layers formed by food-grade emulsifiers: typically, small molecule surfactants (such as Tweens and Spans) < globular proteins (such as egg, whey or soy proteins) < flexible proteins (such as caseinate or gelatin) < polysaccharides (gum Arabic or modified starch). In addition, the thickness of the shell layer may be increased by adsorbing additional materials to the droplet surfaces after they have been formed, *e.g.*, by electrostatic deposition of one or more layers of charged biopolymers onto the surfaces of oppositely charged emulsifier-coated oil droplets.^{24,25} As discussed above, the fact that the shell layer makes a significant contribution to the overall particle dimensions in nanoemulsions has important consequences for the measurement and interpretation of particle size distributions.

3.4. Particle charge

The droplets in nanoemulsions often have an electrical charge because of adsorption of ionized emulsifiers, mineral ions, or biopolymers to their surfaces.⁸ The sign and magnitude of the electrical charge on the droplets play an important role in the functional performance and stability of nanoemulsions, *e.g.*, aggregation stability, interaction with other food components, and ability to adhere to biological surfaces such as the tongue, mucous layer, or other locations within the gastrointestinal tract. The electrical characteristics of nanoemulsion droplets can be controlled by careful selection of particular emulsifier types. In principle, droplets stabilized by non-ionic surfactants (*e.g.*, Tweens and Spans) should have no droplet charge, but in

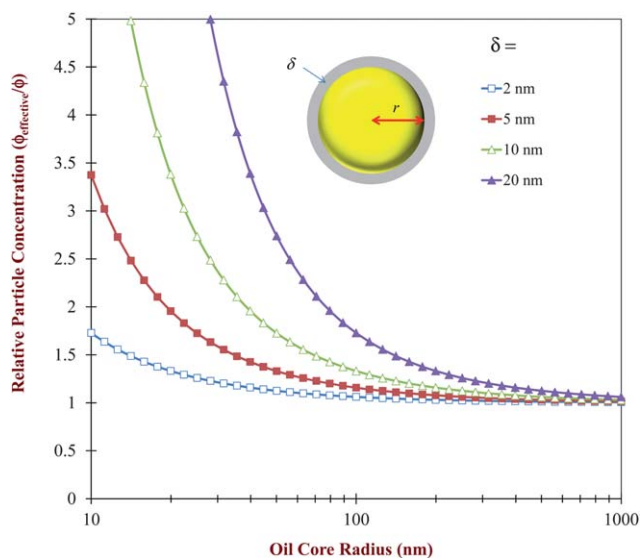


Fig. 1 Predicted influence of shell thickness on the effective concentration of core–shell particles in nanoemulsions. As the core radius decreases or the shell thickness (δ) increases, the effective particle concentration in a nanoemulsion increases.

practice they often have a significant negative charge which may be due to the presence of free fatty acids or other ionic impurities within them.²⁶ Droplets stabilized by anionic surfactants have a negative charge (*e.g.*, lecithin, DATEM, CITREM, fatty acids),²⁷ whereas those stabilized by cationic surfactants have a positive charge (*e.g.*, lauric arginate).²⁸ It is possible to vary the magnitude of the droplet charge in surfactant-stabilized systems by using different ratios of ionic and non-ionic surfactants to prepare them. Droplets stabilized by commercial polysaccharide emulsifiers tend to have a net negative charge (*e.g.*, gum Arabic and modified starch) due to the presence of anionic groups (*e.g.*, sulfate or carboxyl) on the polymer chains.²⁹ Oil droplets stabilized by proteins (*e.g.*, whey protein, casein, soy proteins, egg proteins) have a charge that depends on the solution pH relative to the isoelectric point (*pI*) of the protein.³⁰ Protein-coated droplets have a net positive charge for $\text{pH} < \text{pI}$, no net charge at $\text{pH} = \text{pI}$, and a negative charge at $\text{pH} > \text{pI}$. However, it should be noted that protein molecules have regions of both positive charge and negative charge on their surfaces, and this heterogeneous charge distribution can greatly impact their behavior.³¹ The charge on emulsifier-coated droplets in nanoemulsions can also be altered by adsorption of other charged substances onto their surfaces, such as proteins, polysaccharides, phospholipids, or multivalent ions.^{8,25,32–34}

3.5. Interfacial characteristics

Earlier it was assumed that the interfacial layer formed a homogeneous shell around the oil droplet core, but in reality this layer consists of various molecules with particular structures, organizations and interactions. The precise nature of the molecules at the interface will determine interfacial properties such as effective thickness, charge, permeability, rheology and environmental responsiveness. Controlling the interfacial characteristics is one of the most powerful methods of designing nanoemulsion systems with specific functional performances.^{5,8} The interfacial characteristics of nanoemulsion droplets can be controlled by selection of specific emulsifier types, *e.g.*, surfactants, phospholipids, proteins or polysaccharides. For example, caseinates are fairly flexible disordered proteins that tend to form relatively thick fluid-like interfacial layers, whereas whey proteins are fairly rigid globular proteins that tend to form thin elastic-like layers.^{9,35} Depositing successive layers of charged biopolymers onto oppositely charged lipid droplets can also be used to alter interfacial properties by forming nanolaminated coatings around them with different thicknesses, charges or permeabilities.^{25,32–34} The surfaces of nanoparticles can also be modified to alter their “stealth” character within the human body by attaching hydrophilic polymers to them, *e.g.*, polyethylene glycol (PEG).³⁶ Particles with highly hydrophilic surfaces tend to be less prone to removal by the bodies’ natural defenses, which increases the residence time of the particles in the systemic circulation. Alternatively, nanoparticles can be engineered so their surfaces have specific ligands attached that are capable of binding to specific biological entities or organs within the human body.^{37,38} These surface-modified nanoparticles can be used for targeted or sustained release applications.

3.6. Particle physical state

Nanoemulsions are normally prepared from oils that are liquid, but it is possible to form them from oil phases that are able to fully or partially crystallize at the application temperature.^{39,40} In these cases, the oil phase is usually maintained in the liquid state during nanoemulsion formation, which is achieved by keeping the temperature above the melting point of any crystalline material or by dissolving any crystalline material in a suitable organic solvent. The oil droplets can then be made to fully or partially crystallize at the application temperature by controlling their composition and/or the preparation conditions, such as temperature and triglyceride composition.^{8,41–43} For example, the oil droplets in an O/W nanoemulsion can be made to crystallize by reducing the temperature sufficiently below the melting point of the oil phase. This approach is often used to form *solid lipid nanoparticles* (SLNs) or nanostructured lipid carriers (NLCs), which are nanoemulsions where the oil phase has been fully or partly solidified.³⁹ An SLN contains lipid droplets that are fully crystallized and have a highly ordered crystalline structure. An NLC contains lipid droplets that have a less ordered crystalline structure or an amorphous solid structure, which can aid in the encapsulation and retention of highly lipophilic components. The crystallization temperature of an emulsified fat in a nanoemulsion may be appreciably below that of the same fat in a bulk phase because of supercooling effects.⁴⁴ In addition, the nature of the crystals formed in a nanoemulsion may be different from those formed by a bulk fat because of curvature affects, the limited volume present for crystal growth in droplets, and the lack of secondary nucleation sites.^{42,43} The concentration, nature and location of the fat crystals within the lipid droplets in an emulsion can be controlled by careful selection of oil type (*e.g.*, solid fat content *versus* temperature profile, polymorphic forms), thermal history (*e.g.*, temperature *versus* time), emulsifier type (*e.g.*, tail group characteristics) and droplet size.^{41,42,45} A major potential advantage of SLN and NLC systems is that the rate of molecular diffusion through the lipid phase can be reduced, which can slow down chemical degradation reactions and improve the stability of encapsulated lipophilic components.⁴⁶ In the remainder of this review article, we will only focus on nanoemulsions that have liquid–oil phases.

3.7. Encapsulation of lipophilic components

One of the most important applications of edible nanoemulsions in the food and pharmaceutical industries is for the encapsulation of lipophilic components, such as vitamins, flavors, colors, preservatives, nutraceuticals and drugs.^{1,47} Encapsulation of a lipophilic component may be carried out for a number of reasons: to improve its ease of handling and utilization; to facilitate its incorporation within a product; to increase its bioavailability; to control the rate or location of its release; or to protect it from chemical degradation. Lipophilic components are usually blended with the oil phase prior to nanoemulsion formation, so that they are trapped within the droplets once the nanoemulsion has been produced. Nevertheless, it is possible to add them after formation of the nanoemulsion, *e.g.*, by incorporating them into a colloidal-delivery system that is mixed with the nanoemulsion. The physical location of lipophilic

components within nanoemulsions depends on their molecular and physicochemical properties, such as hydrophobicity, surface activity, oil–water partition coefficient, solubility, and melting point. Highly non-polar lipophilic components tend to be distributed within the hydrophobic core of the particles, whereas more polar lipophilic components may be incorporated within the amphiphilic shell. The physical location of the lipophilic components may have an important impact on the physical and chemical stability of the system. For example, some lipophilic components are susceptible to chemical degradation when they come into contact with particular water-soluble components in the aqueous phase. In this case, it may be important to ensure that the lipophilic components are trapped within the particle core, rather than within the shell. An example of this effect is the chemical degradation of citral molecules (a flavor oil component), which is promoted when these lipophilic components come into contact with protons in the aqueous phase.^{48–50}

In practice, it is often difficult to determine the precise location and orientation of lipophilic components within nanoemulsion droplets. The analytical methods that can be used are highly dependent on the nature of the lipophilic component used. Some of the methods that have proved useful for the *in situ* determination of the location of lipophilic components within emulsion droplets include nuclear magnetic resonance,^{51–54} fluorescence spectroscopy^{55,56} and Raman scattering microspectroscopy.⁵⁷

4. Nanoemulsion formation

Nanoemulsions can be fabricated using a variety of approaches, which are typically categorized as either *high-energy* or *low-energy* approaches.^{1,2,15,58,59} High-energy approaches utilize mechanical devices capable of generating intense disruptive forces that mix and disrupt oil and water phases leading to the formation of tiny oil droplets.^{3,4,58,60} Low energy approaches rely on the spontaneous formation of tiny oil droplets within mixed oil–water–emulsifier systems when the solution or environmental conditions are altered, *e.g.*, temperature or composition.^{2,23,61–64} The size of the droplets that can be produced depends on the approach used, the operating conditions, and the composition of the system. In this section, a brief overview of the most commonly utilized high-energy and low-energy approaches for fabricating nanoemulsions is given, and references to more detailed descriptions of the various approaches are highlighted where applicable.

4.1. High-energy approaches

Only mechanical devices that can generate extremely intense disruptive forces are capable of producing the tiny droplets present in nanoemulsions, *i.e.*, high-pressure homogenizers, microfluidizers, and ultrasonic devices.^{2,58} The reason such intense energy levels are needed is that the disruptive forces generated must exceed the restorative forces holding the droplets into spherical shapes.^{21,22,65} These restorative forces are determined by the Laplace pressure: $\Delta P = \gamma/2r$, which increases with decreasing droplet radius (r) and increasing interfacial tension (γ). The smallest size of the droplets that can be produced using a high-energy approach depends on the homogenizer type, homogenizer operating conditions (*e.g.*, energy intensity,

duration, and temperature), sample composition (*e.g.*, oil type, emulsifier type, relative concentrations), and the physicochemical properties of the component phases (*e.g.*, interfacial tension and viscosity).^{3,66} The three most commonly used high-energy approaches suitable for producing food-grade nanoemulsions are discussed below.

4.1.1. High pressure valve homogenizer. High pressure valve homogenizers (HPVHs) are currently the most popular method of creating fine emulsions in the food industry.^{21,65} These devices are more effective at reducing the size of the droplets in a pre-existing coarse emulsion, than at creating a nanoemulsion directly from the separate oil and water phases. A coarse emulsion is usually produced using a high shear mixer and then this is fed into the input of the HPVH (Fig. 2). The homogenizer has a pump that pulls the coarse emulsion into a chamber on its backstroke and then forces it through a narrow valve at the end of the chamber on its forward stroke. As the coarse emulsion passes through the valve it experiences a combination of intense disruptive forces (turbulence, shear and cavitation) that cause the larger droplets to be broken down into smaller ones. A variety of different nozzle designs is available to increase the effectiveness of droplet disruption within the homogenizer. Studies have shown that the droplet size tends to decrease when the homogenization pressure or number of passes increases, the interfacial tension decreases, the emulsifier adsorption rate increases, and the disperse-to-continuous phase viscosity ratio falls within a certain range ($0.05 < \eta_D/\eta_C < 5$).^{2,22,41} HPVH can be used to produce nanoemulsions provided the system composition is optimized and the device used is capable of generating intense disruptive forces.

4.1.2. Microfluidizer. In some respects microfluidizers are similar in design to high pressure valve homogenizers, since they use a pump to force a coarse emulsion pre-mix through a narrow orifice at high pressures to facilitate droplet disruption.^{20,66–68} Nevertheless, the design of the channels through which the coarse emulsion is made to flow within the device is different (Fig. 2). The coarse emulsion is forced into an inlet channel under

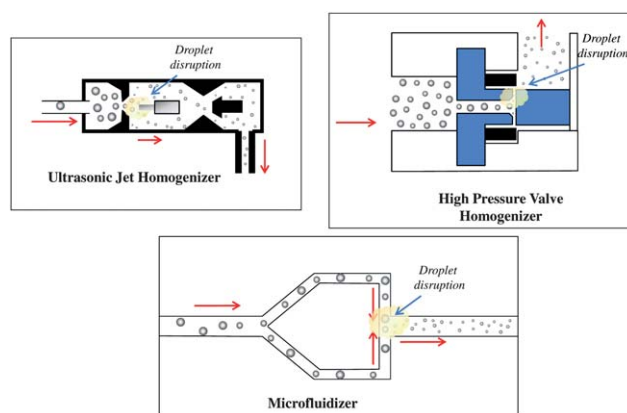


Fig. 2 Schematic representation of three mechanical devices that can be used to continuously produce food-grade nanoemulsions using the high-energy approach: high pressure valve homogenizer, microfluidizer, and ultrasonic jet homogenizer.

high pressure. The channel is designed so that it splits the coarse emulsion into two streams that are made to impinge on each other at high velocity in an interaction chamber. The intense disruptive forces generated in the interaction chamber are highly effective at breaking droplets up and forming fine emulsions. A number of researchers have examined the major factors that determine the size of the droplets produced by microfluidizers using ingredients suitable for preparing food-grade emulsions.^{20,58,66,67,69} These studies have shown that the droplet size tends to decrease with increasing homogenization pressure, increasing number of passes, increasing emulsifier concentration, and decreasing dispersed-to-continuous phase viscosity ratio. We examined some of these factors in a recent study aimed at elucidating the major factors influencing the formation of food-grade nanoemulsions using a microfluidizer.⁷⁰ The logarithm of the mean droplet radius decreased linearly as the logarithm of the homogenization pressure increased for both an ionic surfactant (SDS) and a globular protein (β -lactoglobulin) (Fig. 3). However, the slope of the $\log(d)$ versus $\log(P)$ relationship was appreciably steeper for the surfactant (-0.57) than for the protein (-0.29), which was attributed to the fact that the protein may adsorb more slowly to the droplet surfaces, and that it may form a viscoelastic coating that inhibits further droplet breakup. The dependence of the mean droplet radius on viscosity ratio (η_D/η_C) was also examined by preparing emulsions using different oil phase compositions (corn oil : octadecane) and aqueous phase compositions (water : glycerol) (Fig. 4). For the ionic surfactant there was a distinct decrease in mean droplet radius with decreasing viscosity ratio, which suggests that droplet disruption within the homogenizer became easier as the viscosity of the two phases became more similar. On the other

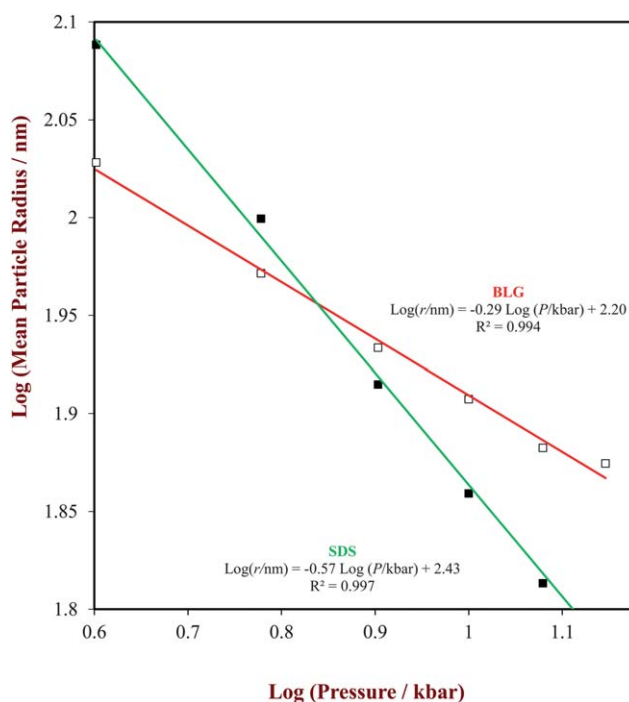


Fig. 3 Influence of homogenization pressure on the mean particle radius of droplets produced using a microfluidizer using either a protein (BLG) or a surfactant (SDS) as an emulsifier.⁷⁰

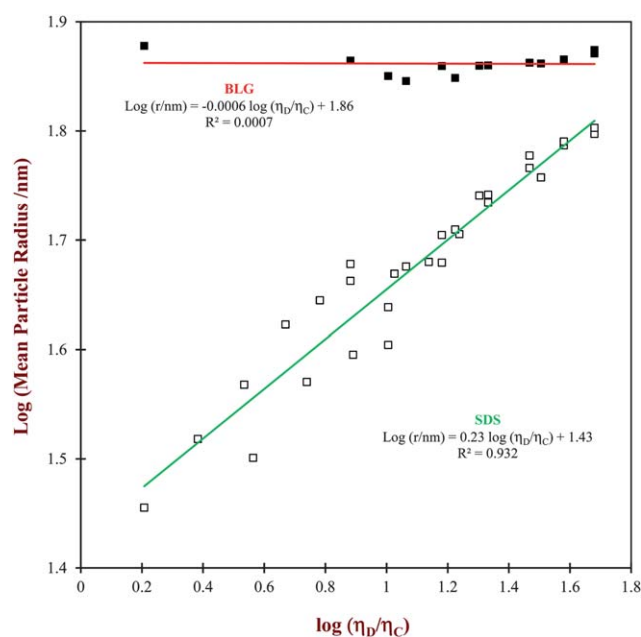


Fig. 4 Influence of the disperse-to-continuous phase viscosity ratio on the mean particle diameter of droplets produced using a microfluidizer using either a protein (BLG) or a surfactant (SDS) as an emulsifier.

hand, we found little change in mean droplet size with viscosity ratio when a globular protein was used as an emulsifier, which again may be due to the relatively slow adsorption of the protein and its ability to form a coating that inhibits further droplet disruption.

4.1.3. Sonication. Sonication methods utilize high-intensity ultrasonic waves (frequency > 20 kHz) to form emulsions containing very fine droplets.^{58,67,69,71,72} They can be used to homogenize separate oil and water phases, or to reduce the size of the droplets in a pre-existing coarse emulsion. Bench-top sonicators are widely used in research laboratories to produce small batches of nanoemulsions. These devices consist of an ultrasonic probe that contains a piezoelectric crystal that converts inputted electrical waves into intense pressure waves. The probe is dipped into the sample to be homogenized, and generates intense disruptive forces at its tip through a combination of cavitation, turbulence and interfacial waves.^{69,71} Large-scale continuous-flow homogenizers based on sonication are also available for commercial production of nanoemulsions (Fig. 2). In these devices, the sample to be homogenized is made to flow through a channel containing an element capable of generating intense ultrasonic waves. A number of studies have recently examined the major factors that impact the formation of nanoemulsions using sonication.^{20,58,71,105} Kentish and co-workers showed that it was possible to form nanoemulsions from food-grade ingredients using high-intensity ultrasound.⁷¹ They were able to prepare nanoemulsions with radii less than 70 nm using a food-grade oil (flax seed oil) and a food-grade non-ionic surfactant (Tween 40). The droplet size decreased with increasing sonication time, power level, and emulsifier concentration. In another recent study, it was shown that food-grade nanoemulsions (sunflower oil, Tween 80, Span 80) with droplet radii

as small as 20 nm could be produced using high intensity ultrasound.⁵⁸ To achieve these remarkably small sizes it was necessary to optimize the design of the ultrasonic reaction chamber, operating conditions, and product composition (*e.g.*, surfactant concentration and type, and oil content).

4.2. Low-energy approaches

Low-energy approaches rely on the spontaneous formation of tiny oil droplets within oil–water–emulsifier mixtures when either their composition or the environmental conditions are altered.^{2,15,23,61–64} A number of different nanoemulsion preparation methods are based on this principle, including spontaneous emulsification (SE), phase inversion temperature (PIT), phase inversion composition (PIC), and emulsion inversion point (EIP) methods.^{15,23,59,73,74}

4.2.1. Spontaneous emulsification. In this group of methods an emulsion or nanoemulsion is spontaneously formed when two liquids (usually an organic phase and an aqueous phase) are mixed together at a particular temperature.^{15,59,75} The practical implementation of this method can vary in a number of ways: the compositions of the organic and aqueous phases; the environmental conditions (*e.g.*, temperature, pH and ionic strength); and the mixing conditions (*e.g.*, stirring speed, rate of addition, and order of addition). For example, an organic phase consisting of non-polar oil and a hydrophilic surfactant and/or water-miscible organic solvent may be slowly added to water.¹⁵ Alternatively, water may be added to an organic phase containing non-polar oil, water-miscible organic solvent and surfactant.⁷⁶ The water-miscible organic solvent is typically ethanol or acetone, but it may be replaced with a water-miscible surfactant instead.^{15,23}

It has been proposed that the physicochemical mechanism underlying the formation of nanoemulsions using this method is the movement of a water-miscible component (either solvent or surfactant) from the organic phase into the aqueous phase.¹⁵ Initially, the organic and aqueous phases are mixed together. Upon mixing, the water-miscible solvent and/or surfactant move from the organic phase into the aqueous phase which creates a large turbulent force at the oil–water interface (Fig. 5). In

addition, the rapid movement of the water-miscible components into the aqueous phase causes a large increase in the oil–water interfacial area, which leads to the spontaneous formation of oil droplets surrounded by aqueous phase through a budding process. To prepare very small droplets, as is required in nano-emulsions, it is usually necessary to use a high ratio of water-miscible component-to-oil in the organic phase prior to mixing. The spontaneous emulsification method has found widespread utilization within the pharmaceutical industry, where it is used to form drug delivery systems to encapsulate and deliver lipophilic drugs.^{77–80} Systems prepared using this approach are usually referred to as either Self-Emulsifying Drug Delivery Systems (SEDDS) or Self-Nanoemulsifying Drug Delivery Systems (SNEDDS) depending on the droplet size produced. A great deal of research has gone into optimizing the formulation of SEDDS and SNEDDS in terms of identifying the most appropriate preparation method and compositions.^{77–80} The main drawback from using this approach in the food industry is that high concentrations of synthetic surfactants are normally needed, which may be undesirable due to regulatory, cost or sensory reasons. Nevertheless, there are certainly some applications where this approach may be useful, *e.g.*, incorporating small amounts of bioactive lipophilic components into beverages.

A practical example of the spontaneous emulsification method relevant to the food and beverage industry is the cloudiness that occurs when water is added to certain anise-flavored spirits, *e.g.*, Ouzo from Greece.^{81,82} When water is added to these alcoholic beverages the mixture turns into a cloudy white color due to the spontaneous formation of tiny oil droplets that scatter light. Ouzo consists of flavor oils (anise) dissolved in an aqueous ethanol solution (~40 to 45% ethanol). When water is added, some of the ethanol moves out of the organic phase into the aqueous phase, which causes the flavor oils to be no longer soluble and so small oil droplets spontaneously form.

4.2.2. Phase inversion methods. The phase inversion temperature (PIT) method relies on changes in the optimum curvature (molecular geometry) or solubility of non-ionic surfactants with changing temperature.^{14,15,60} For example, nanoemulsions can be spontaneously formed using the PIT method by varying the

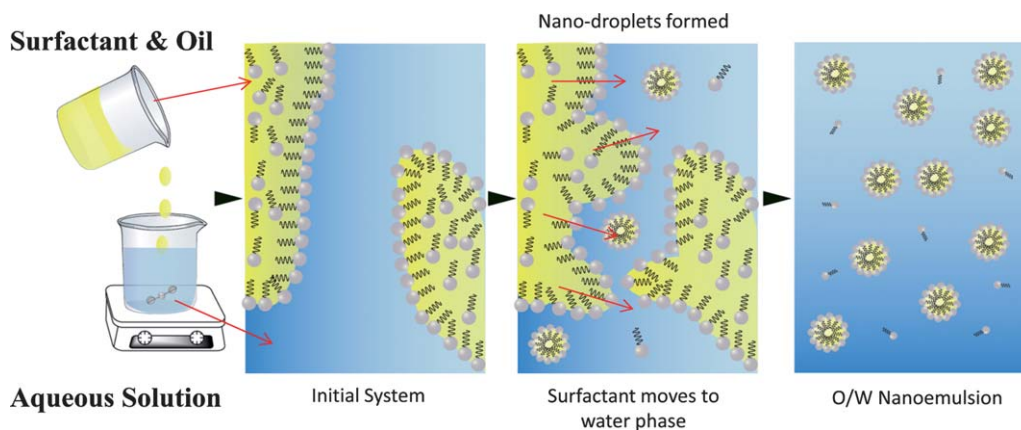


Fig. 5 Schematic representation of the proposed mechanism for spontaneous emulsification: oil droplets are spontaneously formed when an oil phase containing a water-dispersible substance is mixed with an aqueous phase. The underlying mechanism is the movement of the water-dispersible substance from the oil phase to the water phase (red arrows), leading to interfacial turbulence and oil droplet formation.

temperature–time profile of certain mixtures of oil, water and non-ionic surfactant. This type of phase inversion usually involves the controlled transformation of an emulsion from one type to another (*e.g.*, W/O to O/W or *vice versa*) through an intermediate bicontinuous phase. The driving force for this type of phase inversion is changes in the physicochemical properties of the surfactant with temperature (Fig. 6). The molecular geometry of a surfactant molecule can be described by a packing parameter, p :¹⁶

$$p = \frac{v}{la_0} \quad (3)$$

where, v and l are the volume and length of the hydrophobic tail, and a_0 is the cross-sectional area of the hydrophilic head-group. Due to the hydrophobic effect, surfactant molecules tend to spontaneously associate with each other in water and form monolayers that have a curvature that allows the most efficient packing of the molecules.¹⁶ At this *optimum curvature* the monolayer has its lowest free energy, and any deviation from this curvature requires the expenditure of free energy. The optimum curvature of a monolayer depends on the packing parameter of the surfactant: for $p < 1$, the optimum curvature is convex; for $p = 1$, monolayers with zero curvature are preferred; and for $p > 1$ the optimum curvature is concave (Fig. 6). In an emulsion or nanoemulsion, the surfactant monolayer is present in an oil–water interface. The type of emulsion that a particular surfactant tends to favor depends on its molecular geometry. Surfactants with $p < 1$ tend to favor the formation of O/W emulsions, those with $p > 1$ favor W/O emulsions, and those with $p = 1$ do not favor either O/W or W/O systems and instead lead to the formation of bicontinuous or liquid crystalline systems (Fig. 6). The physicochemical properties of non-ionic surfactants can also be described in terms of changes in their relative solubility in oil and water phases with changes in temperature.^{14,15} At low temperatures, the head group of a non-ionic surfactant is highly hydrated and so it tends to be more soluble in water. As the temperature is raised, the head group becomes progressively dehydrated and the solubility of the surfactant in water

decreases. At a particular temperature (PIT), the solubility of the surfactant in the oil and water phases is approximately equal. At higher temperatures, the surfactant becomes more soluble in the oil phase than in the water phase.

The temperature dependence of the packing parameter or solubility of non-ionic surfactants accounts for the ability to form nanoemulsions using the PIT method (Fig. 6). The temperature at which an oil–water–surfactant system changes from an O/W emulsion to a W/O emulsion is known as the *phase inversion temperature* or PIT. At temperatures well below the PIT ($\sim T < \text{PIT} - 30^\circ\text{C}$), the surfactant is soluble in water, its packing parameter is appreciably less than unity ($p < 1$), and it favors the formation of O/W emulsions. As the temperature is raised, the hydrophilic head-groups of the non-ionic surfactant molecules become progressively dehydrated, which causes its water solubility to decrease, and its packing parameter to tend towards unity. At the PIT the packing parameter equals unity ($p = 1$), and the emulsion breaks down because the droplets have an ultralow interfacial tension and therefore readily coalesce with each other. The resulting system may consist of excess oil, excess water, and a third phase containing surfactant molecules, oil and water organized into liquid crystalline structures. To form nanoemulsions it is important to control the overall system composition so that only this third phase is formed at the PIT. At temperatures sufficiently greater than the PIT ($\sim T > \text{PIT} + 20^\circ\text{C}$), the surfactant is more soluble in oil than water, its packing parameter is larger than unity, and the formation of a W/O emulsion is favored. A nanoemulsion can be formed spontaneously by rapidly cooling an emulsion from a temperature at or slightly above the PIT to a temperature well below the PIT. Recently, it has been proposed that the formation of nanoemulsions using the PIT method has a similar physicochemical basis as their formation using the spontaneous emulsification method.¹⁵ Above the PIT, a non-ionic surfactant/oil/water mixture consists of a W/O emulsion with the surfactant molecules being present predominantly within the oil droplets because they are more oil-soluble than water-soluble at this temperature. When this system is quenched below the PIT, the surfactant molecules rapidly move from the oil phase into the aqueous phase (just like the movement of water-miscible solvent or surfactant in the spontaneous emulsification method), which leads to the spontaneous formation of small oil droplets because of the increase in interfacial area and turbulent flow generated (Fig. 5).

An example of the phase behavior of a mixture consisting of oil (20 wt% tetradecane), water (74%), and non-ionic surfactant (6% Brij 30) upon heating is shown in Fig. 7.⁸³ Initially, a nanoemulsion ($d = 60\text{ nm}$) was formed by heating this mixture above the PIT ($\sim 36^\circ\text{C}$) and then rapidly cooling it to 13°C . The turbidity of this nanoemulsion was then measured as it was heated at a controlled rate. At $T \ll \text{PIT}$, the turbidity was low because the oil droplets in the nanoemulsion were so small that they did not scatter light strongly. As the temperature approached the PIT, there was an increase in the turbidity because the optimum curvature tended towards unity which increased the droplet coalescence rate and led to an increase in particle size. Above the PIT, the system was highly turbid since a water-in-oil emulsion was formed with relatively large droplets that scattered light strongly. If this emulsion was again cooled

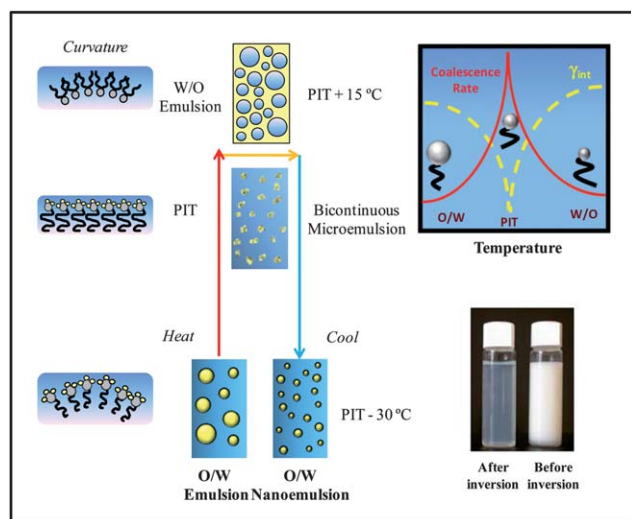


Fig. 6 Schematic diagram of the formation of nanoemulsions by the PIT method.

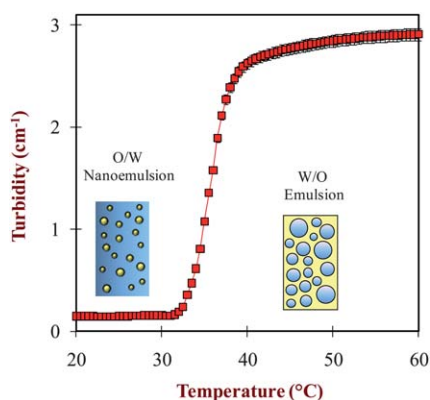


Fig. 7 Temperature dependence of the turbidity of tetradecane oil-in-water nanoemulsions. The turbidity increases above the PIT due to droplet coalescence and phase inversion.⁸³

rapidly to a temperature well below the PIT a nanoemulsion could be formed again. This figure highlights one of the major limitations of the PIT method of producing nanoemulsions—the droplets tend to be highly prone to droplet coalescence when the temperature of the system is raised. This could be a problem in many food and beverage applications that require some form of thermal treatment, such as pasteurization, sterilization or cooking. Recently, we developed an approach to overcome this problem by forming nanoemulsions using a non-ionic surfactant with a relatively low PIT (Brij 30), and then diluting the resulting nanoemulsions in a solution containing another surfactant (SDS or Tween 80) with no or a high PIT.⁸³

The *phase inversion composition* or PIC method is somewhat similar to the PIT method, but the optimum curvature of the surfactant is changed by altering the composition of the system, rather than the temperature.¹⁵ For example, an O/W emulsion stabilized by an ionic surfactant can be made to phase invert to a W/O emulsion by adding salt. In this case, the packing parameter is adjusted from $p < 1$ to $p > 1$ due to the ability of the salt ions to screen the electrical charge on the surfactant head groups.⁷⁴ Alternatively, a W/O emulsion containing a high salt concentration can be converted into an O/W emulsion by diluting it in water so as to reduce the ionic strength below some critical level.

4.2.3. Emulsion inversion point. In the emulsion inversion point (EIP) methods the change from one type of an emulsion to another (*e.g.*, W/O to O/W or *vice versa*) is through a *catastrophic* phase inversion (CPI), rather than a *transitional* phase inversion (TPI) as with the PIC or PIT methods.^{73,84} In this case a W/O emulsion with a high oil-to-water ratio is formed using a particular surfactant, and then increasing amounts of water are added to the system with continuous stirring (Fig. 8). Above a critical water content, the water droplet concentration is so high that they are packed very tightly together and the emulsion reaches a phase inversion point where it changes from a W/O to an O/W system. The size of the droplets formed depends on process variables, such as the stirring speed and the rate of water addition.⁸⁴ The emulsifiers used in catastrophic phase inversion are usually limited to small molecule surfactants that are able to stabilize both W/O emulsions (at least over the short term) and

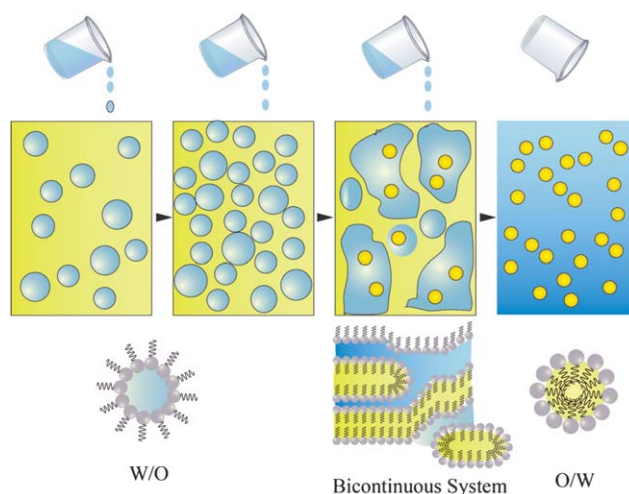


Fig. 8 Schematic representation of the proposed mechanism for low-intensity emulsification by the catastrophic phase inversion (CPI) method. The amount of water added to a W/O emulsion is progressively increased, until a phase inversion occurs and an O/W emulsion is formed.

O/W emulsions (long term). Recently, it has been shown that the emulsion inversion point (CPI) method can be used to produce nanoemulsions ($r < 100$ nm) from food-grade ingredients.⁸⁵

4.3. Finishing techniques

After a nanoemulsion has been formed using either a low-energy or high-energy approach it is possible to change the characteristics of the droplets formed using a variety of methods, which we refer to as *finishing techniques*. These techniques enable one to change a number of the nanoemulsion droplet properties discussed earlier.

4.3.1. Interfacial properties. In some situations it may be advantageous to alter the nature of the interfacial layer surrounding the oil droplets in a nanoemulsion after its formation. For example, one type of emulsifier may be particularly effective at facilitating the formation of small droplets during nanoemulsion preparation, but may not be particularly good at stabilizing the droplets once they are formed. This is true in nanoemulsions produced using the PIT method, where the non-ionic surfactants used to form the emulsions are not very effective at preventing droplet coalescence at elevated temperatures. Alternatively, a change in emulsifier type may be useful to change interfacial properties such as composition, electrical charge, rheology or thickness, which may enable one to tune the functional performance of a nanoemulsion to different applications.

The interfacial properties may be changed after nanoemulsion formation using a number of different approaches.

- *Emulsifier displacement:* a nanoemulsion may be mixed with a solution containing an emulsifier that competes at the oil–water interface thereby displacing some or all of the original emulsifier from the droplet surfaces.⁸³ The interfacial composition will depend on the relative concentrations and surface activities of the various emulsifiers present.⁸

- *Interfacial deposition:* the interfacial characteristics of the droplets in a nanoemulsion may also be changed using interfacial

deposition methods.^{25,86} In this case, the droplets in a nanoemulsion are mixed with a solution containing a substance that adsorbs to the surface of the existing emulsifier layer. For example, if the original droplets are coated by an emulsifier with an electrical charge, then oppositely charged substances (such as polyelectrolytes or mineral ion) can be deposited onto their surfaces using electrostatic deposition. This technique can be used to create multilayers around lipid droplets that have different functional properties than the original emulsifier layer.

- **Interfacial cross-linking:** for certain types of emulsifier it is possible to cross-link them after they have adsorbed to the oil–water interface, *e.g.*, physically, chemically or enzymatically. This approach has been used to covalently cross-link adsorbed protein layers^{87–89} and polysaccharide layers.⁹⁰

4.3.2. Droplet size. The size of the droplets in an emulsion can be reduced further using solvent displacement and/or solvent evaporation methods.^{81,91,92} In these methods, an oil-in-water emulsion is initially formed by homogenizing an organic phase (lipid and organic solvent) with an aqueous phase (water and hydrophilic emulsifier). The organic solvent is selected based on its water miscibility, boiling point, safety, and legal status. The lipid may be a liquid oil (such as a ω -3 or CLA rich oil), a solid fat (such as hydrogenated palm oil), a lipophilic nutraceutical (such as β -carotene), or a mixed system, but the lipid must be capable of being dissolved within the organic solvent prior to homogenization. The organic solvent may be hydrophilic, lipophilic or amphiphilic.⁸¹ After homogenization, the oil droplets within the emulsion contain a mixture of lipid and organic solvent. In the emulsification/solvent displacement method, an amphiphilic organic solvent that is partially miscible with water is used, and the aqueous phase of the emulsion is initially saturated with the solvent. When this emulsion is diluted with water then the organic solvent moves from the oil droplets into the aqueous phase, which causes the droplets to shrink in size. If necessary, the remaining organic solvent can then be removed by evaporation. In the emulsification/solvent evaporation method, a volatile organic solvent that is either immiscible (lipophilic solvent) or partially miscible (amphiphilic solvent) with water can be used.⁸¹ In this case, the organic solvent is removed from the oil droplets by evaporation, which causes the droplets to shrink in size. The final size of the droplets produced using these two methods depends on the particle size distribution of the initial emulsion, the amount of organic solvent present, and the water-solubility of the organic solvent.

We recently used this approach to form nanoemulsions containing protein-coated lipid droplets.⁹³ An oil-in-water emulsion was formed using a lipid phase that consisted of corn oil and ethyl acetate (an amphiphilic solvent), and an aqueous phase that contained water and a globular protein (whey protein isolate, WPI). The size of the droplets was then reduced by diluting these emulsions with pure water to promote movement of ethyl acetate into the aqueous phase (solvent displacement) and/or by heating them to remove the ethyl acetate (solvent evaporation). The droplet size produced using this combined homogenization/solvent removal approach could be controlled by varying the ratio of ethyl acetate to corn oil in the initial organic phase: the higher the fraction of ethyl acetate initially present in the organic phase, the smaller the final droplet size produced (Fig. 9).

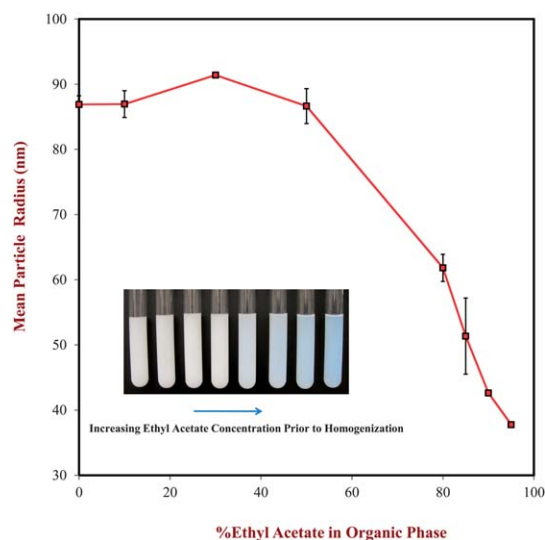


Fig. 9 Mean droplet radius and appearance of whey protein-stabilized oil-in-water emulsions produced using the homogenization/solvent evaporation method. As the % ethyl acetate in the organic phase prior to homogenization increases the size of the droplets produced after solvent evaporation decreases, and the system becomes less opaque.⁹³

One limitation of this approach is that the volume of a droplet is proportional to its radius cubed ($V \propto r^3$), so that to half the droplet radius one must reduce the droplet volume to 1/8th of its original value. This means that a large amount of organic solvent must be used compared to the amount of encapsulated component. The emulsification and solvent evaporation method have successfully been used to prepare dispersions of β -carotene nanoparticles from food-grade ingredients.^{64,91,92,94} Various food-grade emulsifiers can be used to prepare nanoemulsions based on this approach, including whey proteins, caseinate, and small molecule surfactants.^{91,92}

4.3.3. Droplet composition. The composition of the oil phase in a nanoemulsion may be changed after it has been prepared by mixing it with another emulsion or microemulsion system. If an oil molecule has a finite solubility in the aqueous phase, then it will be transported between lipid droplets through molecular diffusion.^{95,96} Alternatively, if the oil molecules can be solubilized within surfactant micelles they may also be transported between lipid droplets.⁹⁷ Consequently, it is possible to mix an emulsion containing droplets with one composition, with another emulsion or microemulsion containing droplets of a different composition. Compositional ripening effects will then lead to the transport of oil between droplets until a steady state condition is reached.⁹⁸ This method could be used to change the oil composition of the droplets in a nanoemulsion, *e.g.*, an oil-soluble antioxidant, antimicrobial or nutritional component could be introduced into a nanoemulsion after it has been formed.

4.3.4. Droplet physical state. The oil phase used in the preparation of nanoemulsions by either the high-energy or low-energy methods must usually be liquid. Nevertheless, it is possible to change the physical state of the oil phase after nanoemulsion formation.⁴⁶ As mentioned earlier, the oil phase

can be fully or partially crystallized after a nanoemulsion is formed by controlling its composition and the temperature–time history of the product. In this case, a lipid phase is used that is crystalline at the temperature at which the final product will be used. Before a nanoemulsion can be produced the lipid phase has to be heated above its melting point and maintained at this temperature throughout the manufacturing process to ensure that it is liquid. The nanoemulsion formed is then cooled at a controlled rate to crystallize the droplets, without promoting the product instability. By careful selection of oil type and thermal history, it is possible to control the type, concentration and location of the crystals within the droplets, which can lead to novel functional properties.^{46,99,100} It should be noted that the fat trapped within a nanoemulsion droplet may crystallize at a much lower temperature than the same bulk fat because of super-cooling and curvature effects.^{101,102}

5. Practical applications of nanoemulsions

In this section, a brief overview of the potential applications of nanoemulsions in the food, beverage and pharmaceutical industries is given. Where appropriate theoretical calculations or experimental data are used to demonstrate the structure–function relationships between particle characteristics and physico-chemical properties. For the sake of convenience, most of this information is taken from studies carried out in the author's laboratory.

5.1 Optically transparent systems

A major potential application of nanoemulsions in foods and beverages is the ability to incorporate lipophilic functional components (such as flavors, antioxidants, preservatives, and nutraceuticals) into transparent or slightly cloudy products.^{103,104} For this application, it is important that the droplet radius is small enough compared to the wavelength of light to prevent strong light scattering, and that it remains below this level throughout storage. The dependence of the specific turbidity on particle radius for emulsions containing monodisperse (spread = 1) and polydisperse (spread = 1.5) droplets was calculated using Mie theory (Fig. 10). The specific turbidity is defined as the calculated turbidity divided by the droplet concentration expressed as a volume percentage. Dilute emulsions ($\phi < 0.1\%$) were considered to be optically transparent when the specific turbidity was less than $0.5 \text{ cm}^{-1} \text{ \%vol}^{-1}$. For monodisperse emulsions, the predicted specific turbidity remained relatively low ($\ll 0.5 \text{ cm}^{-1} \text{ \%vol}^{-1}$) when the droplet radius was less than about 40 nm, but increased appreciably when the droplets were larger than this size. For polydisperse emulsions, the peak in the turbidity *versus* particle radius relationship was broadened somewhat compared to the monodisperse emulsions (Fig. 10). In this case, the mean particle radius had to be less than about 20 to 30 nm for the specific turbidity to fall below the critical level, which can be attributed to the presence of some larger particles in the distribution that scatter light more strongly. The precise particle-size dependence of the specific turbidity depends on the particle size distribution of the emulsion in question. These theoretical predictions are in agreement with the experimental measurements of Wooster and co-workers, who reported that the large majority of droplets in a nanoemulsion should have

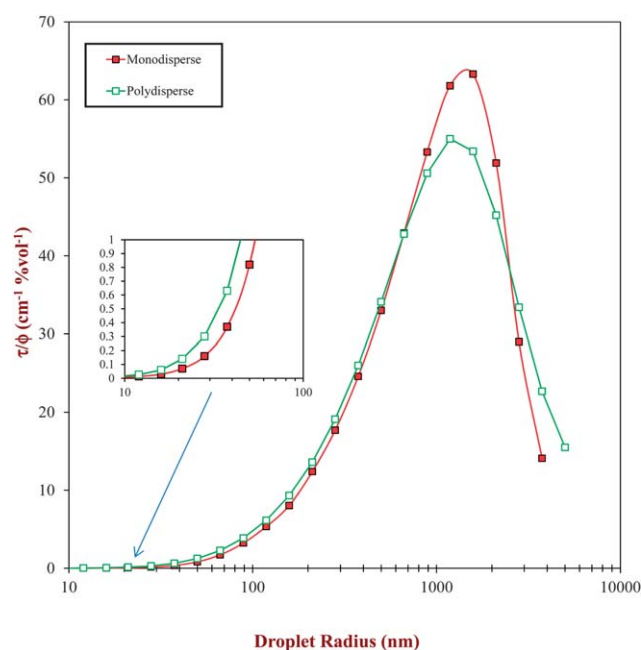


Fig. 10 Theoretical predictions (Mie theory) of the influence of mean particle radius on the specific turbidity of corn oil-in-water emulsions: $n_2 = 1.43$, $n_1 = 1.33$, $\lambda = 600 \text{ nm}$. The polydisperse emulsions were represented by their geometric mean. The region for small droplets is expanded in the inset.

a radius $< 40 \text{ nm}$ to obtain optically transparent systems.³ These predictions indicate that to prepare nanoemulsions that are suitable for application in transparent food products it is necessary to ensure that the majority of droplets fall below some critical value, which can be achieved by having a small mean radius and a narrow spread of the distribution. These calculations also demonstrate that it is important to prevent the droplets from growing during storage otherwise the emulsions will become cloudy. The influence of mean droplet size on the turbidity of nanoemulsions stabilized by whey protein is shown in Fig. 11—the turbidity increases as the droplet radius increases, especially above 75 nm.

Recently, it has been shown that optically transparent nanoemulsions can be prepared using high-energy approaches, such as high-pressure homogenization³ or high-energy ultrasound.^{58,105} To produce the small droplet sizes required to prepare transparent nanoemulsions, it was important to optimize the homogenization conditions (*e.g.*, energy intensity and duration), system composition (*e.g.*, type and concentration of oil, surfactant and polymers), and the physicochemical properties of the component phases (*e.g.*, viscosities and interfacial tensions of continuous and disperse phases). Transparent nanoemulsions have also been formed using low-energy approaches, such as phase inversion methods.^{106–108} The system composition and preparation conditions must again be carefully controlled to produce small droplets in the first place, and to prevent them from growing over time due to coalescence or Ostwald ripening.

5.2. Increased bioavailability of lipophilic components

A number of studies have shown that the bioavailability of highly lipophilic functional components encapsulated within lipid

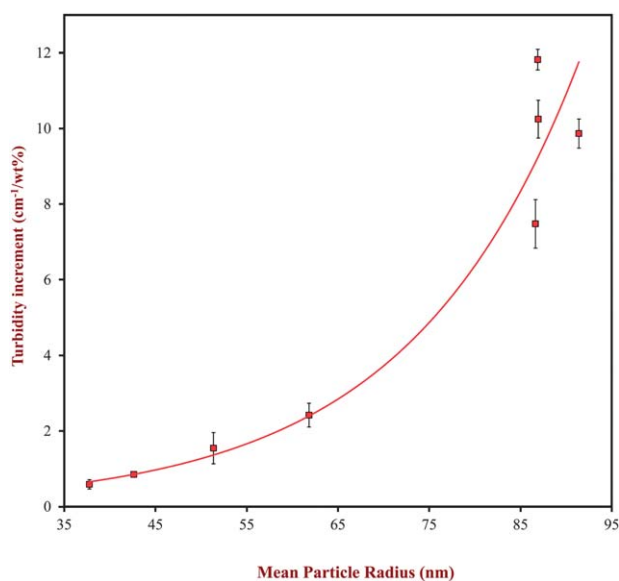


Fig. 11 Turbidity increment *versus* mean droplet radius for corn oil-in-water nanoemulsions stabilized by whey protein isolate produced using the homogenization/solvent evaporation method.⁹³

droplets is increased when the droplet size is decreased.^{1,99} There are a number of possible reasons for this increase in bioavailability. First, small droplets have a large surface area and may therefore be digested more quickly by digestive enzymes so that their contents are released and absorbed more easily. Second, the small droplets may penetrate into the mucous layer that coats the epithelium cells within the small intestine, thereby increasing their residence time and bringing them closer to the site of absorption. Third, very small particles may be directly transported across the epithelium cell layer by either paracellular or transcellular mechanisms. Fourth, the water-solubility of highly lipophilic components increases as the droplet size decreases, which may enhance absorption. At present there is not a good understanding of the relative importance of these different mechanisms for nanoemulsions with differing droplet sizes, compositions, and surface characteristics. Recently, it has been shown that the oral availability of curcumin can be increased by incorporating it within nanoemulsions.^{109,110} Nanoemulsions have also been shown to improve the bioavailability of various lipophilic nutraceuticals and pharmaceuticals^{111–114}

5.3. Improved emulsion stability

Emulsions are thermodynamically unfavorable systems that tend to break down over time due to a variety of physicochemical mechanisms (Fig. 12), including gravitational separation, flocculation, coalescence and Ostwald ripening.^{7–9} The physicochemical stability of nanoemulsions is different from that of conventional emulsions prepared from similar components. Compared to conventional emulsions, nanoemulsions tend to have better stability to gravitational separation and droplet aggregation, but worse stability to Ostwald ripening, due to their relatively small droplet size. In addition, nanoemulsions may also be more susceptible to chemical degradation than conventional emulsions due to their small particle size. In this section,

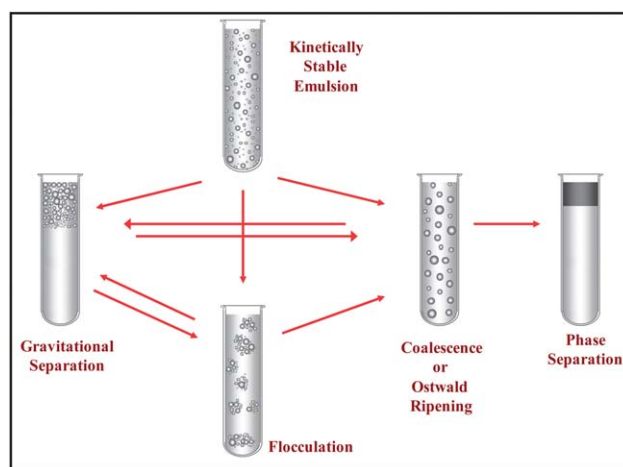


Fig. 12 Schematic diagram of most common instability mechanisms that occur in food emulsions: creaming, sedimentation, flocculation, coalescence, Ostwald ripening and phase inversion.

the influence of particle size on the stability of nanoemulsions to physical and chemical degradation is discussed.

5.3.1. Gravitational separation. Gravitational separation is one of the most common forms of instability in conventional emulsions, and may take the form of either *creaming* or *sedimentation* depending on the relative densities of the dispersed and continuous phases (Fig. 12). Creaming is the upward movement of droplets due to the fact that they have a lower density than the surrounding liquid, whereas sedimentation is the downward movement of droplets due to the fact that they have a higher density than the surrounding liquid. Liquid edible oils normally have lower densities than liquid water and so creaming is more prevalent in conventional oil-in-water emulsions, whereas sedimentation is more prevalent in water-in-oil emulsions. Nevertheless, this may not be the case in emulsions that contain fully or partially crystalline lipids because the density of oils usually increases when crystallization occurs.⁸ In addition, the lipid droplets in nanoemulsions may be surrounded by relatively thick and dense biopolymer coatings, which may impact their tendency to either cream or sediment.

The velocity that a droplet moves upwards in an emulsion due to gravitational separation is given by Stokes' law:

$$v_{\text{Stokes}} = -\frac{2gr^2(\rho_2 - \rho_1)}{9\eta_1} \quad (4)$$

where, v_{Stokes} is the creaming velocity, r is the radius of the particle, g is the acceleration due to gravity, ρ is the density, η is the shear viscosity, and the subscripts 1 and 2 refer to the continuous and dispersed phases, respectively. Gravitational forces cause a droplet to move either upwards or downwards depending on its density relative to the surrounding liquid. Hence, if only gravitational forces operated, then the droplets would accumulate at either the top or bottom of the sample. In practice, droplets may also move due to Brownian motion forces associated with the thermal energy of the system. The root mean square distance moved by a particle in a fluid due to Brownian motion is given by: $\Delta = \sqrt{(2Dt)}$, where D is the translational

diffusion coefficient of the particle and t is the time.⁴¹ The dependence of the creaming velocity on the droplet size for oil droplets ($\rho_2 = 920 \text{ kg m}^{-3}$) suspended in water ($\eta_1 = 1 \text{ mPa s}$, $\rho_1 = 1000 \text{ kg m}^{-3}$) is shown in Fig. 13a. The distance the droplets are predicted to move in one day due to gravitational forces and Brownian motion is plotted. The upward movement of droplets due to gravity increases with increasing droplet size for gravitational forces, but decreases with increasing droplet size for Brownian motion. Consequently, droplet movement is dominated by gravity for relatively large emulsion droplets, but

dominated by Brownian motion for smaller particles. A prediction of the equilibrium vertical distribution of droplets in oil-in-water emulsions with different mean droplet sizes is shown in Fig. 13b. These calculations indicate that we would not expect to see any creaming in nanoemulsions where the droplet radius fell below about 10 nm due to the domination of Brownian motion effects. In practice, gravitational separation may not be observed at even higher droplet sizes because the rate is so slow.

As mentioned earlier, the shell layer may form an appreciable fraction of the total volume of the droplets in nanoemulsions (Fig. 1). The overall density of a particle consisting of a core (oil) surrounded by a shell layer (emulsifier) is given by:

$$\rho_{\text{particle}} = \Phi_S \rho_S + (1 - \Phi_S) \rho_C \quad (5)$$

The shell layer usually has a higher density than the oil or aqueous phases, so that an increase in the volume fraction of the shell layer will tend to increase the overall particle density (Fig. 14). This may have important implications in preventing gravitational separation in emulsions since it will reduce the density contrast between the particles and surrounding fluid. In addition, very small droplets may actually sediment rather than cream if they contain sufficiently thick and dense emulsifier layers. A practical example of this effect is shown in Fig. 15, which compares the pH-stability of protein-stabilized conventional emulsions ($r_{43} = 163 \text{ nm}$) and nanoemulsions ($r_{43} = 33 \text{ nm}$) to gravitational separation.⁹³ The small droplets in the nanoemulsions tend to sediment because the overall particle density is greater than water (δ/r is relatively large, so the dense protein shell makes a large contribution to overall particle density), whereas the large droplets in conventional emulsions tend to cream because their overall density is less than water (δ/r relatively small, so the shell makes little contribution to particle density). This indicates that it should be possible to produce density matched particles by controlling the oil droplet size and the thickness of the adsorbed emulsifier layer.

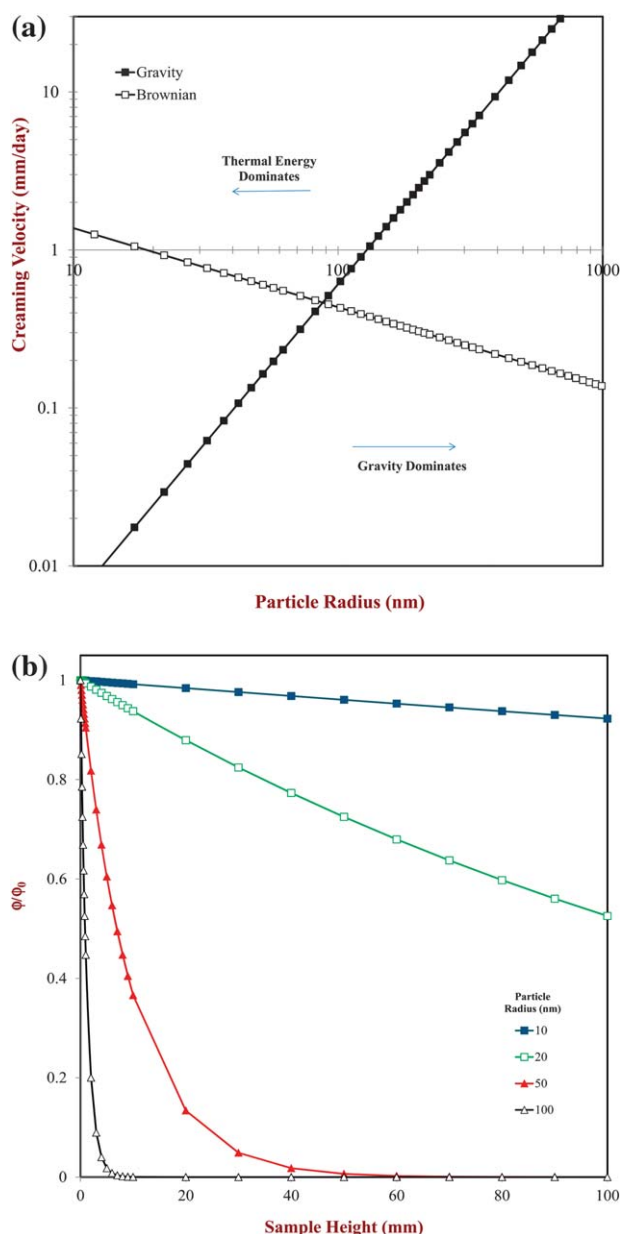


Fig. 13 (a) Predicted velocity of upward movement for oil-in-water emulsions with different sized oil droplets due to gravitational forces and Brownian motion. (b) Equilibrium vertical distribution of different sized droplets in oil-in-water emulsions calculated by balancing the gravitational and Brownian forces acting upon the droplets. The droplet concentration at a particular height is normalized to that at the bottom of the sample.

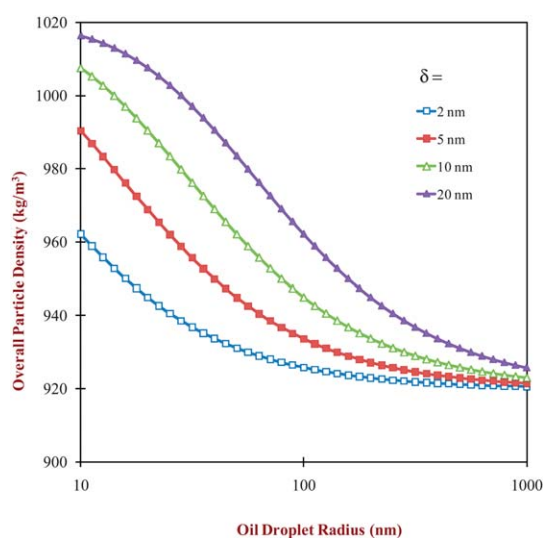


Fig. 14 Predicted influence of droplet size and shell layer thickness on the effective density of the core-shell particles in nanoemulsions. It was assumed that the density of the shell was 1200 kg m^{-3} and that of the oil phase was 920 kg m^{-3} .

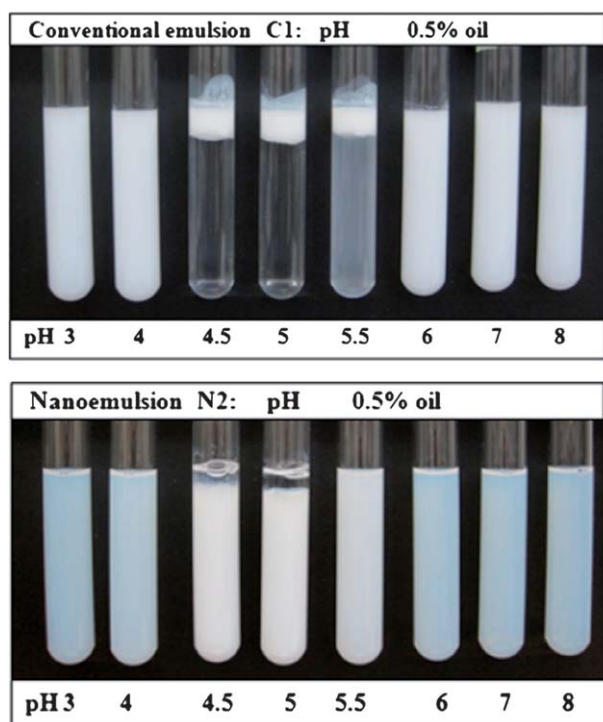


Fig. 15 Influence of pH on the stability of protein-stabilized 0.5 wt% corn oil-in-water conventional emulsions ($r_{43} = 163$ nm) and nanoemulsions ($d_{43} = 33$ nm) to gravitational separation. The particles in the nanoemulsions moved downwards because their overall density was greater than water (due to the thick adsorbed protein layer), whereas the particles in the conventional emulsions moved upwards because their density was less than water.⁹³

5.3.2. Droplet aggregation. Nanoemulsions typically have much better stability to droplet aggregation (flocculation and coalescence) than conventional emulsions because of the influence of their small particle size on the colloidal interactions.² To a first approximation the overall colloidal interactions between a pair of droplets can be described by the sum of the van der Waals (w_{VDV}), electrostatic (w_E), steric (w_S) and hydrophobic (w_H) interactions:⁸

$$w(h) = w_{VDV}(h) + w_E(h) + w_S(h) + w_H(h) \quad (6)$$

The van der Waals and hydrophobic interactions are attractive, whereas the steric and electrostatic interactions are usually repulsive. The steric interaction is a strong short range repulsive interaction, whereas the magnitude and range of the electrostatic repulsion depend on the electrical charge on the droplets (ζ -potential) and the ionic composition of the aqueous phase (I). In general, the magnitude of both the attractive and repulsive colloidal interactions tends to increase with increasing droplet size.⁸ Theoretical predictions of the influence of ionic strength on the overall interaction potential between protein-coated oil droplets are shown in Fig. 16 for nanoemulsions ($r = 20$ nm) and conventional emulsions ($r = 200$ nm). It was assumed that the droplets had a relatively high ζ -potential (25 mV) so that there was a strong electrostatic repulsion between them in the absence of added salt. In the conventional emulsions at low ionic

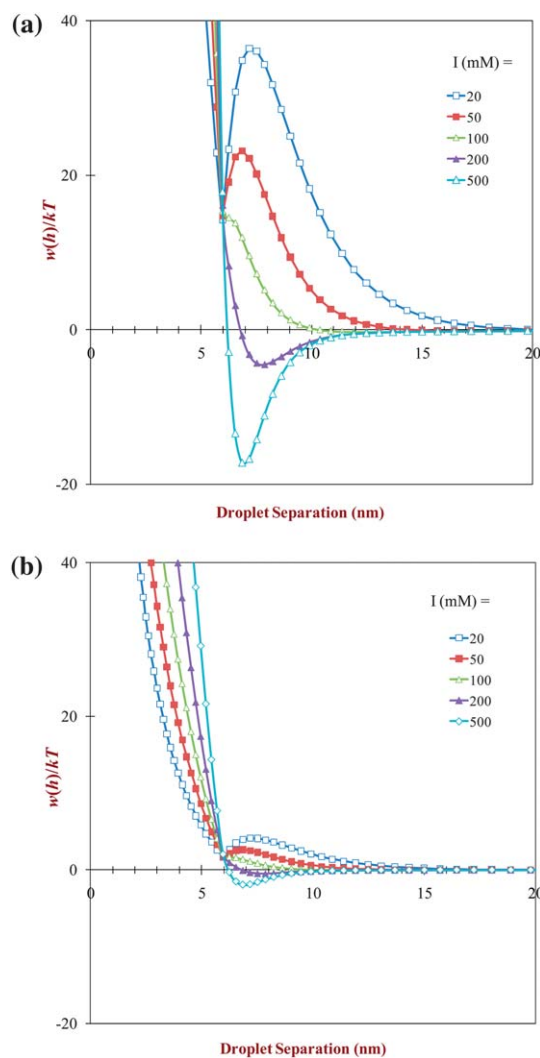


Fig. 16 (a) Predicted influence of changes in the ionic strength on the total interaction potential between oil droplets coated by a globular protein: conventional emulsion ($r = 200$ nm, $\delta = 3$ nm, $\zeta = 25$ mV). (b) Predicted influence of changes in the ionic strength on the total interaction potential between oil droplets coated by a globular protein: nanoemulsion ($r = 20$ nm, $\delta = 3$ nm, $\zeta = 25$ mV).

strengths ($I = 20$ and 50 mM), there is a high potential energy barrier (>20 kT) due to electrostatic repulsion that should prevent droplets from coming close enough to aggregate (Fig. 16a). On the other hand, at high ionic strengths (200 and 500 mM), this energy barrier is greatly diminished due to electrostatic screening effects and there is a deep secondary minimum. The droplets would therefore be expected to flocculate at high ionic strengths in conventional emulsions, which has been observed experimentally.^{115,116} A similar kind of behavior was observed in the nanoemulsions, but the attractive and repulsive interactions were much weaker, and hence the height of the energy barrier and the depth of the secondary minimum were much smaller (Fig. 16b). In this case, one would expect that there would be either little or no droplet flocculation. This effect has recently been observed in protein-stabilized corn oil-in-water nanoemulsions ($r_{43} = 30$ nm) in our laboratory, where they were found to be stable to droplet aggregation and creaming from 0 to

1000 mM NaCl at pH 7 (*data not shown*). Thus both theoretical predictions and experimental measurements suggest that nanoemulsion delivery systems can be designed that have much better stability to droplet aggregation and gravitational separation than conventional emulsions.

5.3.3. Ostwald ripening. Ostwald ripening is the process whereby the mean size of the droplets in an emulsion increases over time due to diffusion of oil molecules from small to large droplets.^{12,117} The driving force for this effect is the fact that the water-solubility of an oil contained within a spherical droplet increases as the size of the oil droplet decreases, *i.e.*, its curvature increases.^{8,12}

$$S(r) = S_{\infty} \exp\left(\frac{2\gamma V_m}{RT r}\right) \quad (7)$$

Here $S(r)$ is the water-solubility of the oil when it is present within a spherical droplet of radius r , S_{∞} is the water-solubility of the oil when it is present within a droplet of infinite curvature (*i.e.*, a planar interface), V_m is the molar volume of the oil, γ is the oil–water interfacial tension, R is the gas constant, and T is the absolute temperature. The increase in water-solubility of an oil with decreasing droplet size means that there is a higher concentration of solubilized oil molecules in the aqueous phase surrounding a small droplet than surrounding a larger one. Solubilized oil molecules therefore tend to move from around the smaller droplets to around the larger droplets because of this concentration gradient. Once steady state has been achieved, the rate of Ostwald ripening is given by:¹² $r^3 - r_0^3 = \omega t = \frac{4}{9} \alpha S_{\infty} D t$ Here, r is the number mean droplet radius at time t , r_0 is the initial number mean droplet radius, D is the translational diffusion coefficient of the oil through the aqueous phase, $\alpha = 2\gamma V_m / RT$, and ω is the Ostwald ripening rate. In practice, the main factor determining the stability of a nanoemulsion to OR is the water-solubility of the oil phase (S_{∞}). For this reason OR is not usually a problem for nanoemulsions prepared using oils with a very low water-solubility, such as long chain triglycerides (*e.g.*, corn, soy, sunflower, or fish oils). On the other hand, OR may occur rapidly for nanoemulsions prepared using oils with an appreciable water-solubility (*e.g.*, short chain triglycerides, flavor oils, and essential oils).^{3,118}

This effect can clearly be seen when comparing the droplet growth rate of a short chain triglyceride (tributyrin) and a long chain triglyceride (corn oil) (Fig. 17). Nanoemulsions prepared using corn oil were very stable to droplet growth, but those prepared using tributyrin exhibited rapid droplet growth and phase separation within the first few minutes after preparation (Fig. 17). Tributyrin is a small non-polar molecule that has a relatively high water-solubility, and is therefore highly prone to Ostwald ripening.^{3,118} On the other hand, corn oil contains large non-polar triacylglycerol molecules with a very low water-solubility, and therefore emulsions prepared from it are highly stable to OR.^{118,119}

The stability of nanoemulsions containing a high water-solubility oil prone to OR can be greatly improved by incorporating a low water-solubility oil into the oil phase prior to homogenization.¹² This approach has previously been used to improve the

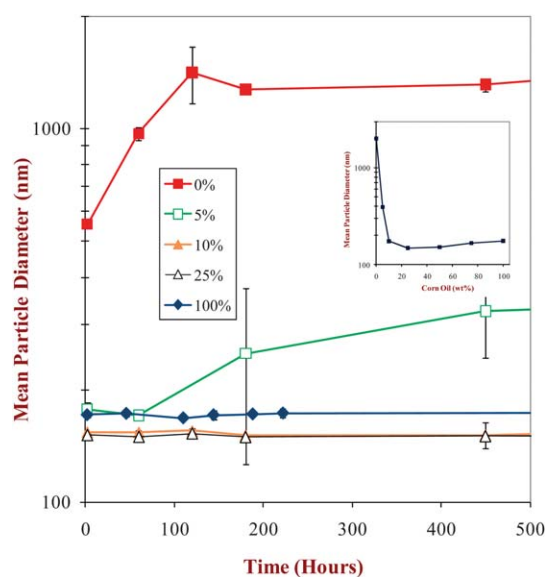


Fig. 17 Droplet growth in oil-in-water nanoemulsions containing different amounts of corn oil and tributyrin in the oil phase.¹¹⁸ The inset shows the mean particle size after 24 hours for different initial corn oil concentrations in the oil phase.

stability of food-grade nanoemulsions, such as those containing short chain triglycerides or essential oils.^{3,118} An example of this approach is demonstrated in Fig. 17, which shows that droplet growth can be retarded when 25% or more corn oil is included in the oil phase of a tributyrin/corn oil-in-water nanoemulsion. The ability of low water-solubility oils to retard OR in emulsions containing high water-solubility oils can be attributed to an entropy of mixing effect that opposes droplet growth due to differences in curvature.¹²⁰ Consider an oil-in-water emulsion that contains droplets comprised of two different lipid components: a water-insoluble component (like corn oil) and a water-soluble component (like tributyrin). The water-soluble molecules will diffuse from the small to the large droplets due to OR. Consequently, there will be a greater percentage of water-soluble molecules in the larger droplets than in the smaller droplets after OR occurs. Differences in the composition of emulsion droplets are thermodynamically unfavorable because of entropy of mixing: it is more favorable to have the two lipids distributed evenly throughout all of the droplets, rather than be concentrated in particular droplets. Consequently, there is a thermodynamic driving force that operates in opposition to the OR effect. The change in droplet size distribution with time then depends on the concentration and solubility of the two components within the oil droplets.

5.3.4. Chemical stability. Nanoemulsions have very large specific surface areas so that any chemical degradation reaction that occurs at the oil–water interface may be promoted, such as lipid oxidation.¹²¹ In addition, the fact that nanoemulsions are transparent means that UV and visible light can penetrate into them easily, which may promote any light-sensitive chemical degradation reactions.¹²² Consequently, it may be necessary to take additional steps to improve the chemical stability of labile components encapsulated within nanoemulsions, *e.g.*, by adding antioxidants or chelating agents.^{121,123}

5.4. Texture modification

Nanoemulsions may exhibit very different rheological properties than conventional emulsions with the same oil content due to the very small size of the droplets. Consequently, there may be some novel applications of nanoemulsions for modifying the texture of food and other products. In general, emulsions can exhibit a wide variety of different rheological characteristics depending on their composition, structure and droplet interactions, *e.g.*, viscous liquids, viscoelastic liquids, viscoelastic solids, plastics, or elastic solids.^{8,41,124–126} Reviews of the rheology of nanoemulsions have previously appeared in the articles of Mason and co-workers,⁵ Tadros and co-workers² and Sonneville-Auburn and co-workers.⁶

To a first approximation the viscosity (η) of concentrated emulsions can be described by the following semi-empirical equation:

$$\frac{\eta}{\eta_0} = \left(1 - \frac{\phi}{\phi_c}\right)^{-2} \quad (9)$$

Here, η_0 is the continuous phase viscosity, ϕ is the disperse phase volume fraction, and ϕ_c is a critical disperse phase volume fraction above which the droplets are so closely packed together that they cannot easily flow past each other. Typically, ϕ_c has a value of around 0.4 to 0.6 depending on the nature of the system.⁸ For conventional O/W emulsions ϕ is usually taken to be the volume fraction of oil phase present, but for nanoemulsions ϕ should be replaced by the effective disperse phase volume fraction ($\phi_{\text{effective}}$) given in eqn (2). As discussed earlier, the effective disperse phase volume fraction may be much greater than the oil volume fraction because of the presence of the adsorbed layer (Fig. 1).

Generally, the viscosity of an emulsion increases with increasing droplet concentration, gradually initially and then steeply as the droplets become more closely packed (Fig. 18). Around and above the droplet concentration where close packing occurs (ϕ_c typically around 40–60% for a non-flocculated conventional emulsion), the emulsion exhibits solid-like characteristics, such as visco-elasticity and plasticity.^{8,125,126} For nanoemulsions, the oil concentration where the steep increase in viscosity occurs can be considerably less than that for conventional emulsions because the presence of the interfacial layer contributes appreciably to their effective volume fraction (Fig. 18). Experimental evidence of this effect is given in Fig. 19,¹⁸ which shows that hydrocarbon oil-in-water emulsions transition from a liquid-like form when the droplets are relatively large ($r > 100$ nm) to a gel-like form when the droplets are relatively small ($r < 80$ nm). Similar results have also been reported for silicone oil-in-water emulsions stabilized by SDS.¹²⁷ This effect can be attributed to the “shell layer” around each of the oil droplets resulting from the relatively strong electrostatic repulsion associated with the anionic head groups of the SDS molecules.

Consequently, it may be possible to promote the gelation of a nanoemulsion at a much lower oil concentration than for a conventional emulsion (Fig. 19). This may be desirable for producing reduced fat products that need to be highly viscous or gel like. At present, there is little work on utilizing nanoemulsions to create novel textural effects in edible products. Nevertheless, recent studies with non-edible systems have shown

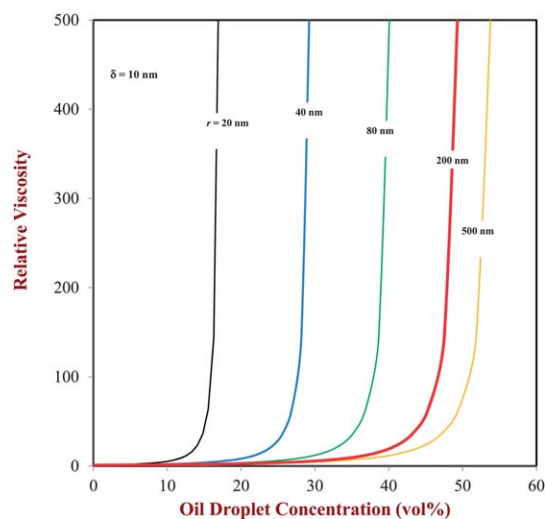


Fig. 18 Predicted relative viscosity (η/η_0) of oil-in-water emulsions with different mean droplet radii (r), and a shell thickness (δ) of 10 nm.

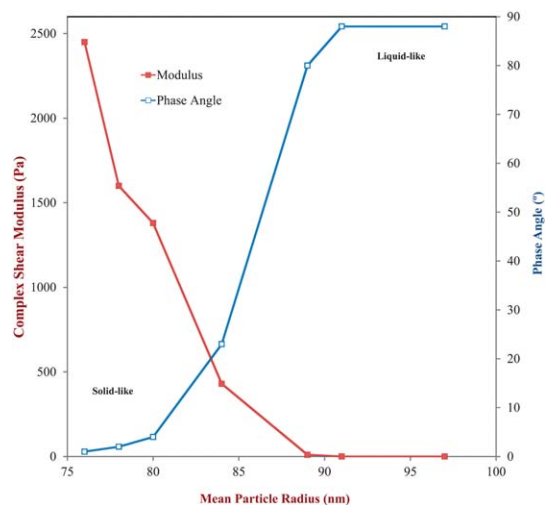


Fig. 19 Influence of particle radius on the rheology of SDS-stabilized oil-in-water nanoemulsions. The nanoemulsions gain solid like characteristics when the droplet size falls below a critical level.¹⁸

that transparent nanoemulsions with gel-like characteristics can be prepared based on electrostatic repulsion effects, *i.e.*, when the thickness of the electrical double layer is on the order of the radius of the emulsion droplets.^{19,127} Potentially, this approach could be used to produce highly viscous or visco-elastic nanoemulsions using much lower oil contents than is required to produce similar systems in conventional emulsions, *e.g.*, sauces, dips, dressings, or mayonnaise.¹²⁷

5.5. Encapsulation and controlled release characteristics

The ability of nanoemulsions to incorporate and release functional components, such as flavors, antimicrobials, vitamins, nutraceuticals and drugs, depends on the molecular characteristics of the functional components as well as the composition, properties, and microstructure of the nanoemulsions themselves.⁸ In this section, we consider the encapsulation and release

of lipophilic components that are (i) volatile; (ii) non-volatile with some hydrophilic character; and (iii) non-volatile and highly lipophilic.

For volatile components (such as aromas), their concentration in the headspace above a product is important since this determines the overall flavor perception. The initial concentration of a volatile component in the headspace above a nanoemulsion is governed by its air–water (K_{AW}) and oil–water (K_{OW}) equilibrium partition coefficients. The head-space concentration of a non-polar component decreases as the lipid concentration increases, which has important consequences for flavor perception.⁸ The time-release profile of non-polar flavor molecules from nanoemulsions is also strongly dependent on lipid concentration: a high fat product tends to give a more sustained release, whereas a low fat product tends to give an intense initial burst release profile.¹²⁸ The release profiles of flavor components from nanoemulsions can therefore be controlled by varying the total fat content of the system. One would expect the size of the droplets in nanoemulsions to have little effect on the release profile, since the rate of diffusion of flavor molecules out of them is so rapid that it is not the rate limiting step (see below).

For non-volatile components, one is interested in their release into the surrounding aqueous phase in the human body, *e.g.*, the mouth, stomach, or small intestine. In this case, it is usually important to establish any potential trigger mechanisms for release (*e.g.*, dilution, pH, ionic strength, temperature, enzyme activity, *etc.*), as well as the rate and extent of release. In an emulsion-based delivery system, the release is usually characterized in terms of the increase in concentration of the encapsulated compound in the surrounding aqueous phase or in some target organ (such as the mouth, nose, stomach or gastrointestinal tract) as a function of time.¹²⁹ The ability of nanoemulsions to encapsulate and release non-volatile components may be quite different from conventional emulsions made from similar components. The solubility of a component within an emulsion droplet usually increases as its droplet size decreases (eqn (7)). For a typical food oil contained within a droplet surrounded by water ($v = 10^{-3} \text{ m}^3 \text{ mol}^{-1}$, $\gamma = 10 \text{ mJ m}^{-2}$), the value of solubility increases by a factor of 2.24, 1.08, 1.01 and 1.00 for oil droplets with radii of 10, 100, 1000 and 10 000 nm, respectively. This effect may alter the oil–water partition coefficient (K_{OW}) of any substances encapsulated within a nanoemulsion, and therefore their bioavailability.

The fact that the droplets are very small in nanoemulsions also has important implications for the release rate of any encapsulated substances through mass transport processes. A convenient measure of the rate of release is the time required for half of the compound to diffuse out of the droplets, $t_{1/2}$:

$$t_{1/2} = \frac{0.0585r^2K_{OW}}{D} \quad (10)$$

Here D is the translational diffusion coefficient of the encapsulated substance through the oil phase. For nanoemulsions ($r < 100 \text{ nm}$), these times are typically less than 1 ms and therefore the release of the encapsulated components due to diffusion can be considered to be very rapid.⁸ Nevertheless, it may be possible to slowdown the release somewhat if there is a highly impermeable and thick coating surrounding the lipid droplets. Alternatively, it

may be possible to encapsulate nanoemulsion droplets within hydrogel particles that increase the path length that compounds must diffuse, and therefore slow down their release rate.¹²⁸

Non-volatile components with some hydrophilic character may be released from nanoemulsion droplets by simple dilution. When a sample is diluted, some of the component will move from the oil droplets to the surrounding aqueous phase in order to reestablish equilibrium according to the components K_{OW} value.⁸ On the other hand, highly lipophilic non-volatile components (*i.e.*, those having a high K_{OW}), may only be released from the droplets in nanoemulsions once the lipid phase has been digested, and they are incorporated into mixed micelles and liposomes. In this case, the release rate of the encapsulated components will depend on the rate of enzyme digestibility of the nanoemulsion droplets. Nanoemulsion droplets have a much higher oil–water interfacial area than conventional emulsions, and therefore they should be digested more rapidly. Nevertheless, there are currently few studies on the behavior of nanoemulsions within the human GI tract.

6. Conclusions

Conventional oil-in-water emulsions are currently the most widely used emulsion-based delivery systems in many industrial applications. These emulsions contain droplets that are relatively large ($r > 100 \text{ nm}$), and so they are optically opaque and susceptible to breakdown through gravitational separation and droplet aggregation mechanisms. In contrast, nanoemulsions contain relatively small droplets ($r < 100 \text{ nm}$) that do not scatter light strongly and that are highly stable to gravitational separation and droplet aggregation. In addition, the small size of the droplets in nanoemulsions means that they are able to greatly increase the bioavailability of encapsulated lipophilic substances. Nanoemulsions may therefore have applications in the food, beverage and pharmaceutical industries as delivery systems, particularly in products that need to be optically transparent, or when increased bioavailability of an active component is important.

7. Challenges and future trends

Nanoemulsions are likely to become increasingly the focus of research and development efforts because of their potential advantages over conventional emulsions for certain applications, *e.g.*, transparent foods and beverages, increased bioavailability, and improved physical stability. Nevertheless, there are a number of challenges that need to be overcome before nanoemulsions are more widely used. First, suitable food-grade ingredients must be identified for formulating food nanoemulsions. Currently, many of the components used to fabricate nanoemulsions using both low-energy and high-energy approaches are unsuitable for widespread utilization within the food industry, *e.g.*, synthetic surfactants, synthetic polymers, synthetic oils, or organic solvents. Ideally, the food industry would like to prepare nanoemulsions from legally acceptable, label-friendly and economically viable food grade ingredients (such as flavor oils, triglyceride oils, proteins, and polysaccharides). Second, suitable processing operations must be identified to economically and robustly fabricate food-grade

nanoemulsions on an industrial scale. Many of the approaches that have been developed within research laboratories may not be suitable for scale-up to industrial production facilities. In addition, it would be advantageous to utilize existing equipment and manufacturing lines, so as to reduce the costs of developing new manufacturing processes. High intensity approaches are widely used in the food industry at present to produce emulsions, but low intensity approaches have not been thoroughly investigated. Third, there may be some safety concerns associated with the utilization of very small lipid droplets in foods, e.g., they may change the extent or route of absorption of lipophilic components. Thus, the bioavailability or potential toxicity of a lipophilic component encapsulated within nanometre-sized lipid droplets may be considerably different from when it is dispersed within a bulk lipid phase. Further studies are clearly needed in this area.

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