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Los Angeles

Controlling and Imaging
Multi-Component Dispersed-Phase Nanoemulsions

A dissertation submitted in partial satisfaction
of the requirements for the degree of
Doctor of Philosophy in Chemistry

by

Michael Matthew Fryd

2012

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ABSTRACT OF THE DISSERTATION

Controlling and Imaging Multi-Component Dispersed-Phase Nanoemulsions

by

Michael Matthew Fryd

Doctor of Philosophy in Chemistry

University of California, Los Angeles, 2012

Professor Thomas G. Mason, Chair

Oil-in-water nanoemulsions are aqueous dispersions of oil droplets having radii $a < 100$ nm and serve as interesting model systems for studying basic colloidal science. The nature of such small droplet sizes gives rise to a new range of physical properties that are potentially beneficial to a wide range of industries. In this dissertation, methods of controlling droplet sizes and morphologies through manipulation of dispersed-phase components are presented. An evaporative ripening technique is developed in which one of the components in a multi-component dispersed-phase nanoemulsion is evaporated out of the system to create nanoemulsion droplet sizes approaching the micellar scale. Foundational groundwork is then provided for cryogenic transmission electron microscopy (CTEM) of oil-in-water nanoemulsions; expansion of water upon freezing may cause nanoinclusions to appear in nanoemulsion droplets depending on the phase of ice and oil type. Finally, a method of controlling coalescence using oppositely charged surfactants is presented and extended to produce anisotropic emulsions and nanoemulsions using multiple immiscible liquids.

The dissertation of Michael Matthew Fryd is approved.

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To Mom, Dad, and Rachelle

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Wilking, J.N.; Chang, C.B.; Fryd, M.M.; Porcar, L.; Mason, T.G. Shear-Induced Disruption of
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Mayoral, K.; Kennair, T.P.; Zhu, X.; Milazzo, J.; Ngo, K.; Fryd, M.M., Mason, T.G. Rotational
Fourier Tracking of Diffusing Polygons. *Phys. Rev. E* **2011**, *84*, 051405.

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Phys. Chem. Chem. Phys. **2012**, *14*, 2455-2461.

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Chapter 1

Emulsions and Nanoemulsions

1.1 Emulsion Constituents, Classification, and Description

1.1.1 Basic Emulsion Components and Characteristics

From lotions to food to medicine, emulsions are ubiquitous in our everyday lives. Unlike other two-phase colloids, such as liquid-in-gas aerosols or gas-in-liquid foams, emulsions consist of one liquid, the dispersed phase, dispersed in a different immiscible liquid, the continuous phase.¹⁻³ Emulsions do not form spontaneously and are therefore not thermodynamically stable. They require a route of fabrication that involves energy input, usually provided by fluid flow. Examination of the Gibbs free energy of droplet formation $G_f = \sigma A - TS_f$, where σ is the interfacial tension between the two immiscible liquids, A is the interfacial area between the two immiscible liquids, T is the temperature, and S_f is the entropy associated with droplet formation, reveals the need for energy input. For emulsion formation, the large positive energy contribution σA from the total increase in interfacial area between the two immiscible liquids as droplets form typically dominates the entropic contribution leading to $\Delta G_f > 0$. Thus applied fluid stresses are needed to overcome the interfacial tension between the two immiscible liquids and rupture larger droplets down to a smaller size.⁴⁻⁷

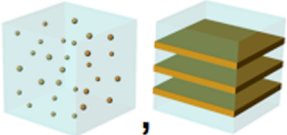
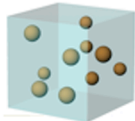
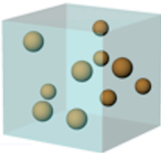
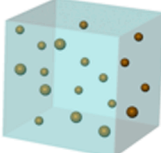
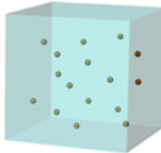
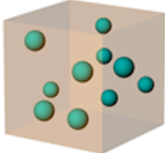
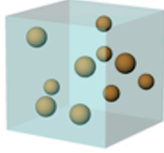
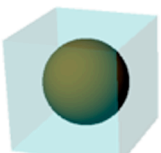
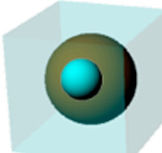
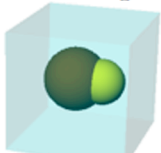
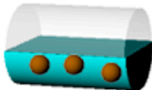
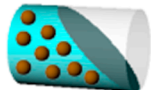
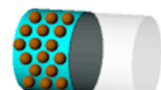
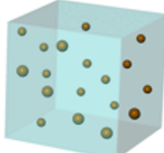
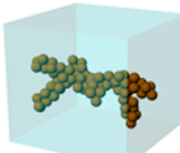
Because emulsion formation has $\Delta G_f > 0$ and therefore is “non-spontaneous,” emulsion destruction can have $\Delta G_f < 0$ and without any other additives, the two immiscible liquids macroscopically phase separate. To prevent this phenomenon from occurring, a surfactant is

added. Whether ionic (*e.g.* anionic sodium dodecyl sulfate (SDS) and cationic cetyltrimmonium bromide (CTAB)) or nonionic (*e.g.* poly-vinyl alcohol (PVA) and pluronics), surfactants are amphiphilic and preferentially reside at droplet interfaces.^{8,9} They are typically composed of a hydrophilic head group and a hydrophobic tail and lower interfacial tension. Surfactants provide either electrostatic or steric repulsion between droplets that prevents coalescence, a process by which two dispersed phase droplets penetrate a layer of the immiscible continuous phase liquid to create one larger dispersed phase droplet. If enough sequential coalescence events occur such that only one “droplet” remains, the emulsion will have both achieved complete macroscopic phase separation and reached a thermodynamically stable state. Good surfactants can provide emulsions that are very long-lived metastable systems as they are stable against coalescence for decades.

1.1.2 Emulsion Classification and Description

1.1.2.1 Emulsions vs. Microemulsions

Emulsions may be classified by droplet size, phase composition, morphology, rheology, and interaction potential (Figure 1.1), but energy input is needed to create the emulsion regardless of the classification. Systems in which $\Delta G_f < 0$ and droplets form spontaneously are lyotropic liquid crystalline phases called microemulsions.^{10,11} Although microemulsions may resemble emulsions, and therefore the two are commonly confused, microemulsions are a distinctly different system having vastly different stability criteria. They are self-assembled, thermodynamically stable systems subject to temperature and concentration dependent phase changes.¹² Phase morphologies can vary, for example, from swollen micellar phases, resembling

Classification	Schematic		
Stability	microemulsion (thermodynamic) 		emulsion (kinetic) 
Droplet Size	microscale emulsion 	miniemulsion 	nanoemulsion 
Phase Composition	W/O 		O/W 
Morphology	single 	double 	anisotropic 
Rheology	viscous 	viscoelastic 	elastic 
Interaction Potential	repulsive 		attractive 

 water droplet
  oil 1 droplet
  oil 2 droplet
 aqueous surfactant solution
  oil surfactant solution

Figure 1.1. Schematic of different emulsion classifications: droplet size, phase composition, morphology, rheology, and interaction potential. Microemulsions are thermodynamically stable systems that are not emulsions.

emulsion droplets, to planar lamellae.¹³⁻¹⁵ Typically microemulsions form when a significant amount of surfactant is used and a high level of relative solubility of molecular components exists such that the interfacial tension effectively vanishes and ΔG_f becomes negative. By contrast, emulsions are kinetically stable and can maintain the same size distributions and spherical droplet morphology under a wide range of temperatures, volume fractions, and dispersed phase, continuous phase, and surfactant compositions.⁴⁻⁷

1.1.2.2 Droplet Size

From smallest to largest droplet sizes, emulsions are classified as either nanoemulsions, miniemulsions, or microscale emulsions (*i.e.* emulsions). Although the exact size range limits corresponding to different terminology are debatable, common definitions state that nanoemulsions have approximate radii $1 \text{ nm} < a < 100 \text{ nm}$, miniemulsions have $a < 1 \mu\text{m}$, and microscale emulsions have $0.5 \mu\text{m} < a < 10 \mu\text{m}$.¹⁰ As nanoemulsions are predominantly the system of interest for this dissertation, the remainder of this chapter will place emphasis on nanoemulsions; much of the discussion also applies to miniemulsions and microscale emulsions as well.

1.1.2.3 Phase Composition

A nanoemulsion may be described by its dispersed and continuous phase materials. A nanoemulsion is called oil-in-water (O/W) if the dispersed phase material is oil and the continuous phase is water. Conversely, a nanoemulsion is called water-in-oil (W/O) if the dispersed phase is water and the continuous phase is oil. Simply reversing the dispersed and continuous phases may not appear to have a large effect, however, the shear and materials

required to obtain a particular phase composition and droplet size distribution can differ drastically between these two types.

1.1.2.4 Morphology

Single nanoemulsions contain spherical droplets composed of a single homogeneous phase, whether heterogeneous in composition or not. This is currently the only easily obtainable nanoemulsion morphology using simple surfactants. Microscale emulsions, however, have other morphologies such as double emulsions¹⁶⁻¹⁸ and anisotropic emulsions.¹⁹⁻²¹ A double emulsion consists of dispersed liquid phase droplets in a continuous phase, wherein each dispersed droplet contains one or more internal droplets of a different immiscible liquid phase. For example, a water-in-oil-in-water (W/O/W) emulsion consists of an aqueous dispersion of oil droplets, each of which contains at least one internal water droplet. Beyond double emulsions, multiple emulsions that have even greater hierarchies of internal droplets exist.¹⁷ Currently, the difficulty of simultaneously stabilizing both the inner water and outer oil droplets having such high curvature is a barrier to double nanoemulsion formation. Using carefully chosen synthetic surfactants may be a way to produce double nanoemulsions,²² however, more studies to establish the origins of formation and stability are needed.

The most common anisotropic emulsions contain a single “droplet” composed of two immiscible phases that are also mutually immiscible with the surrounding continuous phase. These droplets oftentimes resemble a snowman morphology and are commonly called Janus droplets. Most often these droplets are produced in low-throughput microfluidic devices under controlled flow or electric fields, making them difficult to produce on the nanoscale.

1.1.2.5 Rheology

Nanoemulsions are complex fluids that can exhibit liquid-like to solid-like behavior.²³ At dilute ϕ where a nanoemulsion exhibits Newtonian flow and does not have an elastic modulus (G'), it is a viscous liquid. At high ϕ beyond the volume fraction at which nanoemulsion droplets are randomly close packed, ϕ_{RCP} , the nanoemulsion becomes much more elastic as G' is on the order of many thousands of dyn/cm^2 , and it no longer exhibits perceptible flow on appreciable time scales (*i.e.* months to years). At this point, droplets are deformed from an applied osmotic pressure comparable to the Laplace pressure and this sets the scale for the elastic storage modulus of a nanoemulsion.

1.1.2.6 Interaction Potential

Many nanoemulsions are repulsive systems having droplets that diffuse through Brownian motion in the continuous phase. In this case, the surfactant provides a repulsive interaction potential between droplets, which precludes coalescence and keeps the droplets well dispersed. However, some nanoemulsion systems can be made attractive by fine-tuning the interaction potential between nanoemulsion droplets. For example, addition of an appropriate amount of salt to a negatively charged repulsive nanoemulsion system can create a secondary interaction potential well having a depth much greater than $k_B T$. Droplets are then attracted to each other and form large-scale droplet networks, yet do not coalesce.²⁴ Thus nanoemulsions may be referred to as either repulsive or attractive depending on the form of the droplet interaction potential.

1.1.2.7 Full Description

Although nanoemulsions may appear to be simple systems (after all, in their most basic form they only consist of three components (*i.e.* two immiscible liquids and surfactant)), they can be difficult to completely describe. Nanoemulsions are characterized by both macroscopic and microscopic properties and may have complex compositions that impact both these properties. One practical way to describe the relative concentrations of dispersed and continuous phases, which we will use frequently throughout the dissertation, is to use volume fraction, ϕ . It is defined as $\phi = V_{\text{dp}}/(V_{\text{dp}}+V_{\text{cp}}) = V_{\text{dp}}/V_{\text{tot}}$ where V_{dp} = dispersed phase volume, V_{cp} = continuous phase volume, and $V_{\text{tot}} = V_{\text{dp}}+V_{\text{cp}}$ = total emulsion volume.

1.2 Advantages of Nanoemulsions

Decreasing an emulsion's droplet size from the microscale to the nanoscale can drastically change the physical properties of an emulsion.¹⁰ For example, common microscale emulsions having $a > 0.2 \mu\text{m}$ typically appear milky-white, owing to multiple scattering of polychromatic light. Some nanoemulsions, however, have droplet radii that are much less than the wavelength of light, and they can be translucent even at high ϕ .²⁵ Because many of these physical properties are highly desirable in the pharmaceutical, food, and cosmetic industries,²⁶⁻²⁸ nanoemulsions are beginning to replace microscale emulsions in a growing number of applications. Recent improvements in formulation, emulsification, and characterization, as discussed in Chapter 2, showcase many of the physical property changes that are leading to advanced nanoemulsions having desirable functionality and well-controlled physical properties. Mechanical, optical, and transport properties of long-lived, metastable nanoemulsions can be tuned and controlled, and other desirable materials, such as biomolecules and chemical additives, can simultaneously be incorporated.

1.3 Stability of Nanoemulsions

For most nanoemulsion research and applications, long-lived nanoemulsion stability is desired. This means nanoemulsions should retain their original droplet size distributions and should remain homogeneous (*i.e.* not develop gradients in ϕ) for long periods of time (*i.e.* months to years). There are four main mechanisms by which emulsion instability can occur: creaming and sedimentation, depletion flocculation, coalescence, and Ostwald ripening (Figure 1.2). Creaming, sedimentation, and depletion flocculation only directly affect stability through gradients in ϕ , whereas coalescence and Ostwald ripening are irreversible processes that directly affect the droplet size distribution.

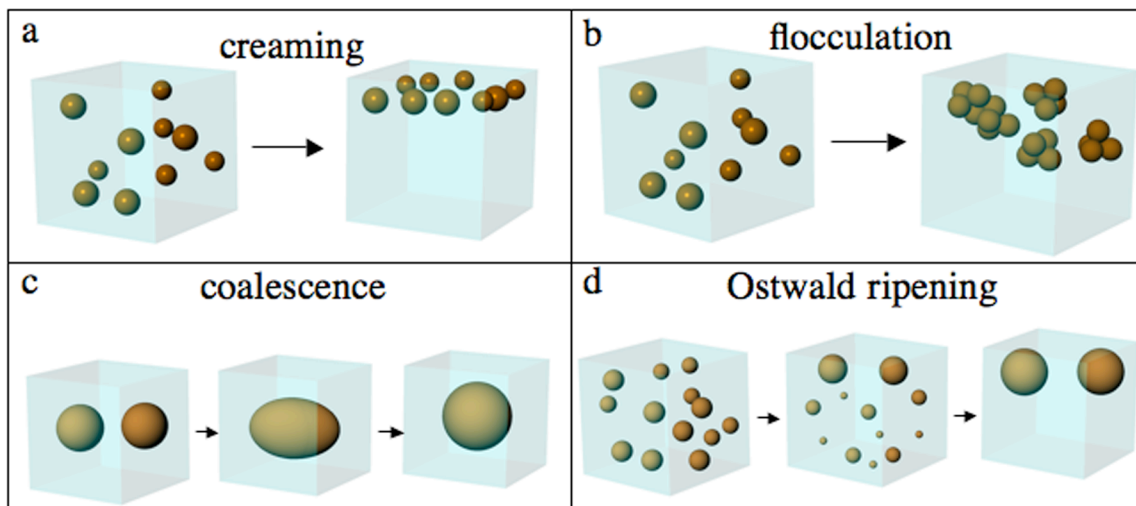


Figure 1.2. Schematic of emulsion instability mechanisms in O/W emulsions: creaming, flocculation, coalescence, and Ostwald ripening.

1.3.1 Creaming and Sedimentation

For most nanoemulsions, creaming and sedimentation do not play a major role. Creaming or sedimentation occurs when droplets rise or fall, respectively, in the emulsion container due to

the net gravitational forces. This net force may be written as $F = \frac{4}{3}\pi a^3(\rho_2 - \rho_1)g$ where a is the droplet radius, g the gravitational acceleration, and ρ_1 and ρ_2 are the continuous phase and dispersed phase mass densities, respectively. Droplets cream if $\rho_1 > \rho_2$ and droplets sediment if $\rho_1 < \rho_2$. Because $F \propto a^3$, the net force quickly diminishes for smaller droplets and forces due to thermal Brownian motion begin to dominate. Assuming droplets sediment, the probability of finding a droplet at height h above the bottom of the container is proportional to the Boltzmann factor. The relevant energy scale to compare with that of thermal energy, $k_B T$, for a dispersion of droplets is the potential energy mgh , where m is the buoyant mass of the droplet. The probability distribution is then given by the classic barometric result: $p(h) = \frac{mg}{k_B T} e^{-\frac{mgh}{k_B T}}$. By equating the potential energy with the thermal energy at room temperature for $a = 30$ nm and $\Delta\rho = 0.065$ g/cm³ and solving for $h_g = k_B T/(mg)$, the gravitational height h_g is found to be ≈ 6 cm. This means nanoemulsion droplets having $a < 30$ nm remain relatively evenly dispersed over long times when kept in typical laboratory glassware, unlike their creaming or sedimenting micron-sized counterparts. Nanoemulsions whose droplet sizes approach the upper limit of the conventional definition, however, can slowly begin to cream or sediment over time, especially for large $\Delta\rho$.

1.3.2 Depletion Flocculation

Depletion flocculation, also known as depletion attraction, is insignificant for nanoemulsions. In order for a depletion attraction to exist, an emulsion must contain a non-interacting depletion agent having radius a_m (*e.g.* surfactant micelles of only a few nanometers that form above the critical micelle concentration (CMC)) in addition to the emulsion droplets

having a much larger length scale of radius a . Due to the increased volume available to the depletion agent when emulsion droplets “touch”, the emulsion experiences an entropically-induced attraction.⁷ The attractive potential of this effect scales with the ratio of a to a_m , and the attraction becomes weaker as a_m approaches a .²⁹ Therefore, the lower emulsion droplet size range for which the attractive potential is large enough relative to $k_B T$ to provide significant creaming, where SDS is the depletion agent having $a_m = 2$ nm, for example, is approximately $150 \text{ nm} < a < 200 \text{ nm}$. As such, the classic depletion-attraction instability that induces flocculation of microscale and larger sub-micron emulsions using surfactant micelles is negligible for nanoemulsions because the droplet sizes are too close to the micellar dimensions.

1.3.3 Coalescence

The phenomenon in which two droplets having a higher total combined interfacial energy fuse to form one larger droplet having a lower total interfacial energy is called coalescence. When this happens, both the droplet number density and the droplet size distribution change. For coalescence to occur, a critical disjoining pressure must be overcome such that the thin continuous phase film separating two droplets that are in close proximity ruptures. Typically coalescence of a nanoemulsion only occurs if the nanoemulsion is depleted of surfactant, the osmotic pressure becomes large enough to overcome the critical disjoining pressure, or if the nanoemulsion is subjected to high-flow conditions.³⁰ Nanoemulsions can, however, become unstable to coalescence immediately upon formation if the surfactant is not dissolved in the continuous phase and available to newly formed interfaces in accordance with the Bancroft rule.³¹

Although nanoemulsion coalescence is not typically desired, it is commonly used to determine ϕ for O/W nanoemulsions. By massing an initial amount of nanoemulsion, m_i , at a known surfactant concentration, C , subsequently evaporating all the water to cause phase separation, and re-massing the final amount of oil and surfactant that remain, m_f , the volume fraction may be calculated by using³²: $\phi = \frac{V_d}{V_d + V_c} = \frac{(m_f - CV_c M)/\rho_{oil}}{(m_f - CV_c M)/\rho_{oil} + V_c}$ where M is the surfactant molecular weight, ρ_{oil} is the density of the oil, and $V_c = \frac{(m_i - m_f)}{(\rho_c - CM)}$ where ρ_c is the continuous phase density.

1.3.4 Ostwald Ripening

Ostwald ripening of a nanoemulsion occurs when dispersed phase molecules can diffuse from smaller, shrinking droplets to larger, growing droplets because of chemical potential differences induced by differences in their respective radii of curvature.³³ Thus, in order for Ostwald ripening to occur, the dispersed phase molecules must have finite solubility in the continuous phase. The solubility need not be high, however. Even dispersed phase materials with solubilities less than the ppm level can Ostwald ripen. Because solubility is a material property directly related to the energy cost per unit length of placing a dispersed phase molecule times its length in complete contact with the continuous phase in relation to $k_B T$, molecules having smaller hydrophobic regions of a given species are more susceptible to Ostwald ripening. For example, the Ostwald ripening rate of single-component alkane emulsions decrease exponentially with increasing carbon backbone length from nonane to hexadecane.³⁴

To better understand Ostwald ripening, consider the Helmholtz free energy of a single spherical droplet made of a dispersed phase species that Ostwald ripens³⁵ $F_d = F_b + 4\pi a^2 \sigma$ where

F_b is the free energy for the same volume of bulk-dispersed phase material (*i.e.* not as a droplet). The chemical potential is then given by differentiating with respect to the number of molecules in the droplet, $N = 4\pi a^3/3v$, where v is the volume of a dispersed phase molecule, and is $\mu_d = \mu_b + 2\sigma v/a$. The chemical potential difference $\Delta\mu = \mu_d - \mu_b$ may thus be directly associated with the spherical droplet's curvature $2/a$, where the chemical potential is larger for smaller droplets. Therefore, nanoemulsions are particularly prone to Ostwald ripening and care must be taken when choosing the dispersed phase material. Additionally, the pressure difference across the droplet interface, denoted the Laplace pressure, $\Pi_L = 2\sigma/a$, may be associated with $\Delta\mu$; differences in Laplace pressures effectively drive the migration of dispersed phase molecules from smaller droplets to larger ones through the continuous phase.

Although the origin of Ostwald ripening has been identified, the timescale of the phenomenon has yet to be mentioned. Assuming this is a diffusion-controlled process, the emulsion has a dilute volume fraction, and no barrier inhibits diffusion across the droplet interface, the long-time steady-state temporal dependence of the number-averaged droplet radius, $\langle a_N \rangle$, of a single molecular dispersed phase species that exhibits Ostwald ripening is predicted to be (*i.e.* according to LSW theory³⁶⁻³⁸): $\frac{da_N^3}{dt} = \frac{8D_{OR}\sigma V_m C_\infty}{9RT}$ where D_{OR} is the diffusion coefficient of the Ostwald ripening species in the continuous phase, V_m is the molar volume of the dispersed phase molecules, C_∞ is the bulk dimensionless solubility of the dispersed phase molecules in the continuous phase, and t is time. The experimental ripening rate obtained from dynamic light scattering (DLS) (after volume-weighted to number-weighted correction) for Ostwald ripening of decane droplets in a 3.5×10^{-5} M SDS solution at $\phi = 3.5 \times 10^{-5}$ is $da_N^3/dt = 350 \text{ nm}^3/\text{s}$.³⁹ Using the same assumptions above, the LSW theory also predicts the reduced

emulsion size distribution $p(a/a_c)$, where a_c is the critical droplet radius at which $da_c/dt = 0$, is time invariant and that $\langle a_N(t) \rangle = a_c(t)$. Thus, although a_N increases with time, the reduced form of the distribution remains the same.³⁶⁻³⁸

1.4 Nanoemulsion Formation

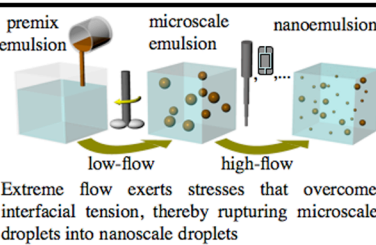
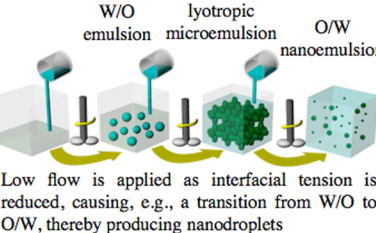
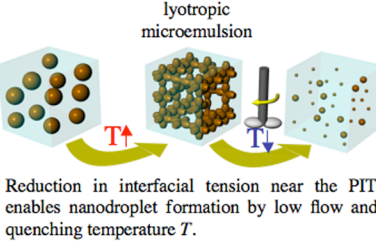
A wide range of emulsification methods can be used to produce nanoemulsions. High-flow emulsification, the most common method to date, provides a simple route to forming nanoscale droplets wherein externally applied shear, elongational flow, or a combination thereof overcome interfacial and internal viscous stresses to rupture bigger droplets into smaller droplets. This method is used to create all of the nanoemulsions described in Chapters 3-5 of this dissertation. Neglecting the dispersed phase viscosity, η_d , G.I. Taylor equated the viscous stress to the Laplace pressure in the case of dilute ϕ and captured the basic physics of droplet formation.⁴⁰ Although only fairly accurate under these conditions, the resulting equation $a \approx \sigma/(\eta_c \dot{\gamma})$, where η_c is the continuous phase viscosity and $\dot{\gamma}$ is the shear rate, provides a useful guide for nanoemulsion droplet formation. Thus, shear rates between 10^8 s^{-1} and 10^9 s^{-1} are needed to create nanoemulsions from 10-100 nm assuming $\sigma = 10 \text{ dyne/cm}$ and $\eta_c = 1 \text{ cP}$. Examples of emulsification devices with high enough strain rates to obtain nanodroplets include high-pressure microfluidic homogenizers^{41,42} and ultrasonicators.^{43,44} A combination of low ϕ , low η_d , high pressures, and high continuous phase surfactant concentrations give the smallest droplet sizes.

Alternatively, a different approach to nanoemulsion formation is through low-flow emulsification. One low-flow method that has been known for decades is the phase inversion temperature (PIT) method.⁴⁵ In this method, a composition is chosen for the interfacially active

component or components such that the interfacial properties of the emulsion change dramatically with only modest changes in temperature near the PIT. Moving through this temperature, a transition from an O/W to a W/O (or vice versa) may occur. Most often, nonionic surfactants are chosen to achieve phase inversion with changes in temperature. Within this surfactant class, ethoxylated surfactants have typically been chosen because of their inherent ability to become more hydrophobic with increasing temperature.⁴⁶ A microscale emulsion is then formed using the chosen surfactant and brought near the PIT to drastically lower the interfacial tension. At this temperature, the application of low flow, combined with a temperature quench, can be used to produce nanoemulsions.⁴⁷ A potential drawback of the low-flow PIT and microemulsion temperature quenching methods is that often the resulting nanoemulsion is not very stable over long periods of time because of the relatively high solubility of the components even at the lower final temperature. For this reason, surfactant displacement methods are being developed to stabilize nanoemulsions made using the PIT method.⁴⁸

Many other methods of nanoemulsion formation also exist. These include phase inversion composition (described in Figure 1.3a),⁴⁹ flow focusing,⁵⁰ satellite droplets,⁵¹ membrane emulsification,⁵² and liquid-liquid nucleation.⁵³ Figures 1.3a and 1.3b summarize and categorize methods of nanodroplet creation according to higher-throughput (bulk quantities (*e.g.* liters) at a given ϕ may be created within minutes) or lower-throughput (same bulk quantities at the same ϕ may be created within days or weeks), respectively. In flow focusing, the continuous phase is used to focus the dispersed phase into a jet that pinches off into droplets typically inside a microfluidic channel. Satellite droplets may be formed when droplets under shear stretch, undergo a capillary instability, and pinch-off to create multiple smaller droplets of a similar size along with nanodroplets. In membrane emulsification, low pressures are used to force the disper-

a

High-Throughput Nanoemulsion Formation Method	Process Schematic	Typical Advantages	Typical Disadvantages
Extreme Emulsification	 premix emulsion microscale emulsion nanoemulsion low-flow high-flow Extreme flow exerts stresses that overcome interfacial tension, thereby rupturing microscale droplets into nanoscale droplets	1. Appropriate for a wide range of compositions beyond the dilute ϕ limit 2. Droplet size distribution tailored through both flow rate and compositional control 3. Scalable to very large volumes	1. More energy input required compared to low flow methods 2. Apparatus can require significant up-front investment 3. Viscous heating can become appreciable at high flow rates
Phase Inversion Composition	 W/O emulsion lyotropic microemulsion O/W nanoemulsion Low flow is applied as interfacial tension is reduced, causing, e.g., a transition from W/O to O/W, thereby producing nanodroplets	1. Versatility of routes a. Vary liquid ratio b. Change [electrolytes] c. Mix surfactants 2. Low energy process 3. Not necessary to supply heat	1. More limited range of compositions, compared to high flow methods a. Limited ϕ range b. Limited [electrolyte] range c. Limited surfactant types 2. More prone to Ostwald ripening 3. Good mutual solubility of oil and water is needed
Phase Inversion Temperature	 lyotropic microemulsion Reduction in interfacial tension near the PIT enables nanodroplet formation by low flow and quenching temperature T.	1. Low flow process	1. Limited to mostly nonionic surfactants 2. Requires heat energy input 3. Limited stable T range 4. More prone to Ostwald ripening 5. Good mutual solubility of oil and water is needed

 = water droplet
 aqueous surfactant = solution

 = oil
 = oil droplet

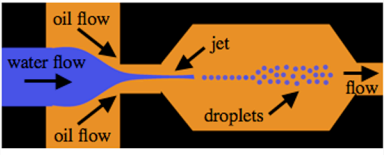
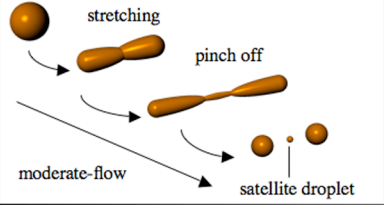
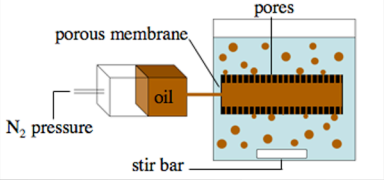
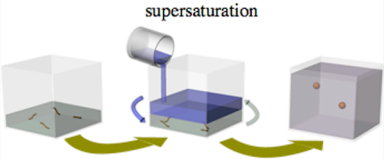
 = beaker
 = ultrasonic agitation

 = low-flow mixer
 = high-flow homogenizer

 = oil surfactant solution
 = oil droplet with surfactant

Figure 1.3a. Concept, schematic, typical advantages, and typical disadvantages of several important high-throughput nanoemulsification methods: extreme emulsification, phase inversion composition, and phase inversion temperature. Figure reprinted with permission by Michael M. Fryd and Thomas G. Mason, copyright 2011.

b

Low-Throughput Nanoemulsion Formation Method	Process Schematic	Typical Advantages	Typical Disadvantages
Micro/Nanofluidic Flow Focusing		1. Forms monodisperse droplets 2. Moderate flow	1. Requires microfluidic apparatus 2. Limited range of flow rate ratios that produce NEM-sized droplets 3. NEM-sized droplets demonstrated for W/O only
Satellite Droplets		1. Moderate flow	1. Satellite droplet size and production are limited by a. Viscosity ratio η_d/η_c b. Radius of drop before breakup c. Interfacial tension 2. Producing a NEM from satellite droplets requires separation from the larger droplets
Membrane Emulsification		1. Forms monodisperse droplets if a membrane having a narrow pore size distribution is used 2. Low pressure process	1. Requires Shirasu or other controlled porous glass membrane 2. Coalescence of droplets near pores can occur—wetting must be overcome and flow of outer liquid is needed to aid droplet detachment
Liquid-Liquid Nucleation (Ouzo Effect)		1. Low energy process	1. Limited compositions: continuous phase requires high mutual solubility. 2. Need small solubility of oil in one continuous phase component 3. Generates only very dilute ϕ

 = water
 = oil molecule
 = water-miscible solvent
 = oil surfactant = solution
 = water-miscible solvent + water

Figure 1.3b. Schematic, typical advantages, and typical disadvantages of low-throughput nanoemulsification methods: micro/nanofluidic flow focusing, satellite droplets, membrane emulsification, and liquid-liquid nucleation. Figure reprinted with permission by Michael M. Fryd and Thomas G. Mason, copyright 2011.

sed phase through membrane holes into the continuous phase while stirring to remove the newly created droplets from the proximity of the holes. Finally, liquid-liquid nucleation can occur, for example, when a water miscible solvent phase containing water-immiscible oil molecules is placed in contact with water. Diffusion of water into the water miscible solvent phase causes supersaturation of the oil, inducing droplet nucleation.

1.5 Energies, Forces, and Pressures

1.5.1 Individual Droplets

Each individual nanodroplet in a nanoemulsion has a relatively small interfacial area in contact with the continuous phase. However, the total collective interfacial area of all the nanodroplets can be quite large. Assuming monodisperse droplets, the total interfacial area A_{TI} is given by $A_{TI} = 3V_{dp}/a$. If $\phi = 0.2$, $V_{tot} = 100$ mL, and $a = 10$ nm, then the total number of droplets is $\approx 4.8 \times 10^{18}$ and the interfacial area becomes 6000 m^2 or 10% more than a football field! It costs energy to create this large amount of additional interface between two immiscible liquids during droplet formation; the energy cost per unit area to create additional interface between two immiscible liquids is known as interfacial tension, σ . Going back to the previous example, the energy cost to produce an interface of 6000 m^2 if $\sigma = 0.01 \text{ J/m}^2$ is $E = \sigma A_{TI} = 60 \text{ J}$, which is approximately equal to the kinetic energy of a 70 mi/hr baseball.

Because a spherical shape minimizes interfacial energy at a given volume, nanoemulsion droplets having ϕ below that which nanoemulsion droplet deformation occurs may be thought of as small, potentially deformable, spheres. Assuming an interfacial tension of 10 dyn/cm , the Laplace pressure for spherical nanoemulsions is between $\Pi_L \approx 2\text{-}20 \text{ atm}$. These enormous

pressure differentials, especially considering a typical car tire pressure of 30 psi is equivalent to ≈ 2 atm, explain why Ostwald ripening is such a common source of nanoemulsion instability; if the dispersed phase material has finite solubility, even beyond concentrations of ppm, smaller droplets will force molecules of the dispersed phase into the continuous phase.

Nanoemulsion droplets are not always composed of one dispersed phase liquid; two or more dispersed-phase-soluble liquids may be mixed together before emulsification. In this case, an osmotic pressure may develop. Consider the case where a droplet is composed of two soluble dispersed phase liquids in which one dispersed phase component Ostwald ripens (denoted L1) and the other dispersed phase component does not (denoted L2). The droplet interface may be thought of as a semi-permeable membrane and an osmotic pressure develops, due to the L2 trapped in the droplet. To first order, the osmotic pressure of L2 within a droplet is given by $\Pi_o = N_2 k_B T / V_d$ where N_2 is the number of molecules of L2 and V_d is the volume of the droplet. This phenomenon has important implications to Ostwald ripening behavior of nanoemulsions and is discussed further in Chapter 3.

1.5.2 Droplet-Droplet Interactions

Droplet-droplet interactions on the microscale dominate the macroscopic behavior of nanoemulsions. For example, nanodroplets having a repulsive interaction potential such as SDS-stabilized nanoemulsions, remain evenly dispersed by Brownian motion. An attractive droplet interaction potential, such as that caused by adding certain quantities of salt to a SDS-stabilized nanoemulsion, can cause aggregation, which can completely change the physical properties of a nanoemulsion. As the majority of nanoemulsions in subsequent chapters have a repulsive interaction potential due to the use of ionic surfactants, the major interaction contributions of

these repulsive nanoemulsions, namely van der Waals and the so-called electrostatic double-layer, are briefly discussed.

Within nanoemulsion droplets, fluctuations of electron density occur in the dispersed phase molecules relative to their nuclei. These fluctuations induce a fluctuating dipole moment of the molecule that influences neighboring molecules the net result of which causes attractive van der Waals (vdW) forces. If two droplets come in close enough contact such that fluctuations between molecules associated with two different droplets can interact, the droplets will experience an attractive force. The attractive vdW potential energy between two solid spheres having radii a_1 and a_2 separated by a surface-to-surface distance d is given by⁵⁴

$$U_{vdW}(d) = \frac{-A}{6} \left[\frac{2a_1a_2}{(2a_1 + 2a_2 + d)d} + \frac{2a_1a_2}{(2a_1 + d)(2a_2 + d)} + \ln \left(\frac{(2a_1 + 2a_2 + d)d}{(2a_1 + d)(2a_2 + d)} \right) \right] \quad (1)$$

where A is the dispersed phase material dependent Hamaker constant of order 10^{-19} J. Extending this to spherical liquid droplets, at a distance when this potential energy becomes greater than that of thermal energy, the two interacting droplets will cease to remain evenly dispersed as the vdW interaction dominates over Brownian motion (assuming no other interactions are present).

For a repulsive nanoemulsion due to ionic surfactants, however, the electrostatic repulsion exists in addition to the vdW attraction. Typical ionic surfactants are salts that dissolve into their positively and negatively charged ionic species in solution. The majority of a surfactant's active component will reside on droplet interfaces until the droplet interfaces are saturated and then the remaining surfactant will reside, along with all the counterions, in the continuous phase. The potential energy, for example, between two solid spheres due to this electrostatic repulsion where $a_1, a_2 \gg d$ and the spheres are coated with charged monovalent

ionic surfactant with counterion screening may be approximated as⁵⁴ $U_e(d) = \left(\frac{a_1 a_2}{a_1 + a_2} \right) Z e^{-\frac{d}{\lambda_D}}$

where Z is the so-called interaction constant and λ_D is the Debye screening length. Combining the vdW and electrostatic double layer interaction potentials (*i.e.* Derjaguin, Landau, Verwey, and Overbeek (DLVO) theory^{55,56}), the total droplet-droplet interaction potential $U_t(d) = U_{vdW}(d) + U_e(d)$ may be obtained. Typical nanoemulsion droplets stabilized with an ionic surfactant therefore experience a long-range vdW attraction that is less than $k_B T$ since aggregation does not usually occur, but also experience an electrostatic double layer short-range repulsion greater than $k_B T$ to preclude coalescence.

1.5.3 Droplet Kinetics

Nanoemulsion droplet kinetics in a repulsive nanoemulsion system without any applied flow (whether mechanical, thermal, or otherwise) is mainly driven by Brownian motion. Brownian motion is an entropically driven stochastic process that solute particles undergo due to thermally driven solvent molecule density fluctuations that provide random “kicks” to the solute. Because Brownian motion is a diffusive process, it may be described by Fick’s second law. If the probability density, $p(x,t)$, describes the probability of finding a nanoemulsion droplet at position

x at time t , then solving the 1-D diffusion equation $\frac{\partial p}{\partial t} = D \frac{\partial^2 p}{\partial x^2}$, where D is the diffusion

coefficient, under the boundary condition $p(x, t=0) = \delta(x)$ gives $p(x,t) = \frac{1}{\sqrt{4\pi Dt}} e^{\left(\frac{-x^2}{4Dt}\right)}$. The

average value for $\langle x \rangle = 0$, as Brownian motion is a stochastic process; it is equally likely for a particle to be pushed in one direction as the opposite direction. The mean square $\langle x^2 \rangle \neq 0$,

however. Instead, $\langle x^2 \rangle = 2Dt$ and reflects the average distance traveled by the nanodroplet. Extending to 3D, $\langle r^2 \rangle = 6Dt$ where $r^2 = x^2 + y^2 + z^2$. Thus a nanoemulsion droplet 50 nm in diameter, assuming a diffusion coefficient on the order of 10^{-12} m²/s, can travel an average distance equal to its diameter in ≈ 0.4 ms and can travel ≈ 2.5 μ m in one second. Given the high droplet number density for nanoemulsion droplets $\rho_d = 3\phi/(4\pi a^3)$, it is clear that “collisions” between nanoemulsion droplets occur much more frequently than between droplets in microscale emulsions.

1.6 Contact Angle, Spreading Coefficients, and Equilibrium Configurations

The contact angle describes the angle formed where three phases come into contact. For the traditionally defined case of solid, liquid, and vapor in contact at equilibrium, the contact angle is related to the surface tensions between each of the phases by Young’s equation $\cos \theta = (\sigma_{SV} - \sigma_{SL})/\sigma_{LV}$ where σ_{SV} , σ_{SL} , and σ_{LV} are the solid/vapor, solid/liquid, and liquid/vapor surface tensions, respectively (Figure 1.4a). If a drop of liquid placed on a solid is in equilibrium with the surrounding vapor and θ is small, then the liquid wets the surface of the solid and spreads over the surface. If θ is large, however, the liquid drop forms a raised bead.

Assuming only interfacial tension dictates the equilibrium configuration of three contacting liquids in which two immiscible liquid droplets come into contact with each other inside a mutually immiscible continuous third phase, three possibilities exist. Consider the change in the Gibbs free energy per unit interfacial area, G to separate two immiscible phases in contact and place each of them separately in contact with a third immiscible phase. This may be written generally as²¹ $G_i = (\sigma_{ij} + \sigma_{ik}) - \sigma_{jk}$ where i, j , and k are indices representing each immisc-

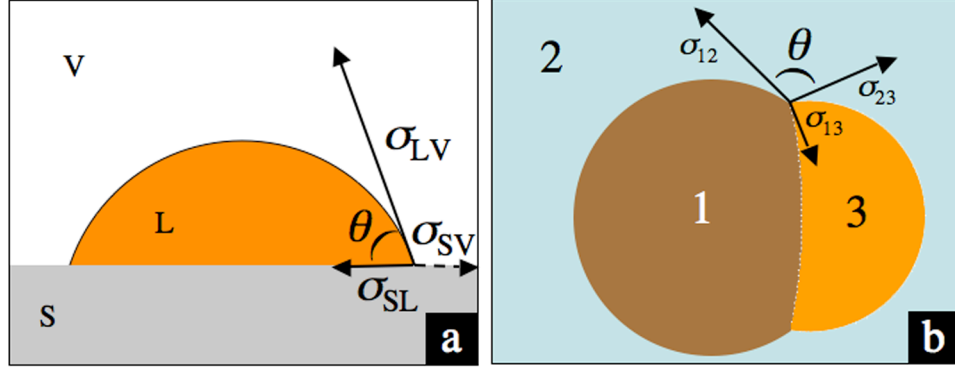


Figure 1.4. Schematic of contact angle between three different phases. (a) Traditional contact angle as defined between a solid (S), liquid (L), and vapor (V). Surface tension exists between the solid and liquid (σ_{SL}), solid and vapor (σ_{SV}), and liquid and vapor (σ_{LV}).⁵⁷ (b) Contact angle as defined for a three immiscible phase system of an asymmetric Janus O/W emulsion droplet. 1 represents oil type 1, 2 represents the aqueous continuous phase, and 3 represents oil type 2. Interfacial tension exists between liquids 1 and 2 (σ_{12}), liquids 1 and 3 (σ_{13}), and liquids 2 and 3 (σ_{23}).²¹

ible phase. For example, if we let $i = 2$ denote the continuous phase and $j = 1$ and $k = 3$ the other two immiscible phases, then $G_2 = (\sigma_{12} + \sigma_{23}) - \sigma_{13}$. If G_2 is negative, then the continuous phase will preferentially wet the interfaces of phases 1 and 3, and phases 1 and 3 will be separated by the continuous phase (*i.e.* phases 1 and 3 will not be in contact). It is perhaps more intuitive to think of this in terms of spreading coefficients $S_i = -G_i = \sigma_{jk} - (\sigma_{ij} + \sigma_{ik})$. In the case of our last example, if $S_2 > 0$, this means the continuous phase will preferentially wet phases 1 and 3 as this costs less energy per unit area than having phases 1 and 3 in contact. Letting $\sigma_{12} > \sigma_{23}$ for convention, S_1 must be less than 0 and only three possibilities exist for the final droplet configuration. If $S_2 < 0$ and $S_3 > 0$, then phase 3 will preferentially wet phase 1 until there is no contact of phase 1 with phase 2 (*i.e.* phase 3 will completely engulf phase 1). If $S_2 > 0$ and $S_3 < 0$, then phase 1 and phase 3 will remain as separate droplets, as in the earlier example. Finally, if $S_2 < 0$ and $S_3 < 0$, then there is no phase that will preferentially wet another. The energy balance then imposes an anisotropic Janus droplet (*i.e.* fusion of two immiscible droplets) configuration

(Figure 1.4b). In the Janus droplet case and neglecting any line tension, the contact angle is related to the interfacial tension as given by²¹ $\cos\theta = \frac{\sigma_{13}^2 - \sigma_{12}^2 - \sigma_{23}^2}{2\sigma_{12}\sigma_{23}}$ where σ_{12} , σ_{13} , and σ_{23} denote the interfacial tension between liquids 1-2, 1-3, and 2-3, respectively. This last possible configuration leads both to fascinating interfacial science and interesting geometrical possibilities for emulsions.

1.7 Characterization Techniques

A few of the ways to potentially characterize emulsions and nanoemulsions include dynamic light scattering (DLS), transmission electron microscopy (TEM), optical microscopy, rheology, microrheology, diffusing wave spectroscopy (DWS), optical tweezing, tensiometry, UV/vis spectroscopy, small angle x-ray scattering (SAXS), small angle neutron scattering (SANS), refractometry, atomic force microscopy (AFM), and scanning electron microscopy (SEM). Each technique, including variations on a given technique (*e.g.* cryogenic-TEM (CTEM)), provides different information about an emulsion system and offers its own advantages and disadvantages. DLS, CTEM, and optical microscopy are used extensively in Chapters 3, 4, and 5, respectively. Because optical microscopy is arguably the most familiar and widely used technique from those used in Chapters 3-5, we only include a brief synopsis of DLS and CTEM techniques and their sample preparation as applied to nanoemulsions.

1.7.1 Dynamic Light Scattering

Dynamic light scattering, also known as quasi-elastic light scattering (QELS) and photon correlation spectroscopy (PCS), is a powerful technique that can provide the size distribution of

nanoemulsions.⁵⁸ In a typical measurement, scattered light intensity fluctuations due to the Brownian motion-induced random shifts in relative particle position are measured as a function of time. How well these fluctuations are correlated in time provides information on a nanoemulsion's size. Because DLS depends on the random Brownian motion of droplets, correlated droplet movement due to creaming or sedimentation is typically the main factor that sets the upper size scale. The lower limit, on the other hand, typically depends on a variety of factors including the relative refractive index difference between dispersed and continuous phases, sample concentration, laser power, laser wavelength, and detector sensitivity. In the case of 10 cSt PDMS droplets in an aqueous continuous phase having $C_{\text{SDS}} = 5 \text{ mM}$ using a 633 nm HeNe laser and a Photocor Complex DLS system, the lower limit in radius is approximately 15 nm. Additionally, PDMS nanoemulsion droplets provide sufficient scattering contrast to obtain an accurate size in a matter of minutes. Therefore, as nanoemulsions fall directly in the useful size range for DLS, it is an indispensable technique for quickly measuring nanoemulsion sizes.

Static light scattering is performed at time scales many orders of magnitude longer than dynamic light scattering. The long-time averaged light-scattering intensity of nanoemulsions depends on the size of the droplets, the droplet number density, the angle of scattering, the incident intensity of light, the wavelength of incident light, the refractive index increment, and the distance from the point of scattering. In static light scattering experiments, these long-time averaged intensity measurements as a function of particle concentration and scattering angle are typically taken over several minutes at each scattering angle for a given particle concentration. On much shorter time scales (*i.e.* droplet diffusion time scales), the light scattering intensity fluctuates about the mean long-time averaged intensity. The diffusion coefficient of a nanodroplet D is related to these intensity fluctuations via the autocorrelation function, $G(\tau)$, for

monodisperse droplets by $G(\tau) = \langle I(t)I(t + \tau) \rangle = A + Be^{-2Dq^2\tau}$ where τ is a time step after time t , A and B are experimentally determined constants, and q is the scattering wavenumber $q = (4\pi/\lambda)\sin(\theta/2)$ where λ is the wavelength of light in the continuous phase and θ is the scattering angle. The diffusion coefficient is also related to the nanoemulsion droplet radius by the Stokes-Einstein relation $D = k_B T / (6\pi\eta a)$. Thus, by measuring $G(\tau)$ in a DLS experiment, the droplet size may be obtained.

In a DLS experiment, light scattered off of a dilute sample, typically $\phi \approx 10^{-5}$, is collected by a photon counter (*e.g.* photomultiplier tube) placed at 90° with respect to the incident laser light and is sent to a correlator having many channels (*e.g.* 64 channels). The correlator keeps track of how many photon counts occur during a minimum specified period of time called the sample time, α . It then uses each different channel that is present to compute the autocorrelation function for a specific integral number of α . In other words, for a 64 channel correlator with linear sample time spacing, channel 1 calculates $G(\alpha)$, channel 2 calculates $G(2\alpha)$, etc. Plotting each G for all the different multiples of α , $G(\tau)$ is obtained and takes the form of an exponential decay. By least squares fitting, A , B , and D may be obtained, thereby enabling the size of a nanoemulsion to be deduced from the Stokes-Einstein relation.

Nanoemulsions are rarely monodisperse unless they have been centrifuged and fractionated, so some way of interpreting the correlation function with a polydisperse sample is needed. Although a few ways of interpreting the autocorrelation function of a polydisperse nanoemulsion exist, the most common is the method of cumulants. For a polydisperse sample, the correlation function now becomes a sum containing contributions from droplets of all the different sizes⁵⁹ $G(\tau) = A + \left[\sum B(a)e^{-D(a)q^2\tau} \right]^2$ where A is a long-time correlation function decay

constant and $B(a)$ are constants depending on each individual droplet size. Koppel relates the natural logarithm of the moment generating function to the natural logarithm of the correlation function and expands with respect to τ .⁶⁰ Rewriting this result in terms of the correlation function, the correlation becomes $G(\tau) = A + Be^{-2K_1\tau + K_2\tau^2} \dots$ where K_1 is the first moment of the distribution of decay rate $\langle I \rangle = \langle Dq^2 \rangle$ and K_2 is the second moment $\langle (I - \langle I \rangle)^2 \rangle$. Thus, the average hydrodynamic radius (typically volume-weighted) and standard deviation of nanoemulsion droplet sizes may be determined through DLS by applying the method of cumulants to the autocorrelation function.

1.7.2 Transmission electron microscopy

Transmission electron microscopy is a powerful technique used most frequently to obtain real-space micrographs of a sample where length scales range from the nanoscale to the microscale.⁶¹ Because optical microscopes use visible wavelengths (*i.e.* $\approx 400 - 700$ nm), their minimum resolvable distance, $d_{\min} \propto \lambda / (n \sin \theta_a)$ where n is the refractive index of the medium between the lens and specimen and θ_a is the aperture angle, is not low enough to obtain direct images of a nanodroplet. However, the wavelength of an electron having an accelerating voltage of 200 kV (as is used for the CTEM micrographs in Chapters 3-5) is on the order of 0.003 nm. Despite the small wavelength of an electron, sub-angstrom resolution has not been realized because an electron microscope is typically limited by the ability to which spherical aberration may be corrected. Currently, resolution is limited to a few tenths of a nanometer. Thus, electron microscopes are an invaluable resource for viewing real-space images of nanoemulsion droplets.

A typical transmission electron microscope is composed of an electron source system, a condenser lens system, a specimen stage, an objective lens system, a projection lens system, an

imaging system, and a vacuum system. Each electron microscope is different, but the basic principles are as follows. The two main electron source systems are thermionic guns, made with, for example, a LaB₆ crystal, which eject electrons by applying heat through a current and field-emission guns made with, for example, a fine needle-point tungsten tip that ejects electrons by applying a large voltage. Once electrons are ejected by the gun, they travel to the condenser lens system which adjusts the beam to be parallel upon hitting the specimen. The degree of coherent illumination is controlled along with the total electron intensity. Unlike an optical microscope in which lenses are typically made of glass and focused by changing the relative lens positions and magnified by changing lenses, an electron microscope's lenses are electromagnetic and thus both focusing and magnification are controlled by changing the current through lenses.

The electrons now arrive at the specimen. The specimen is held by the specimen holder, which must be inserted into the microscope. The specimen must be held stable and must be able to be translated and potentially rotated. Here, electrons may exhibit a number of different scattering events including forward, back, elastic, and inelastic. The objective lens system then collects the electrons that pass through the specimen (whether scattered or not), and forms an intermediate image. Because this image is next magnified by the projection lens system, the quality of the objective lens typically dictates the resolving power of the microscope. Although some projection lens systems have the ability to magnify on the order of 1,000,000x, high magnifications quickly become detrimental to the sample because the illumination intensity, $I \propto M^2$ where M is the magnification.⁶² Hence, beam damage occurs more easily at high magnifications. The final image produced by the projection lens system is viewed either on a fluorescent screen, which fluoresces visible light when bombarded by electrons, or by some photographic method (*e.g.* film or digital camera). Because electrons must travel through the

length of the microscope column from the gun to the imaging system without collisions with air molecules, transmission electron microscopes are equipped with many vacuum pumps (*e.g.* roughing, diffusion, turbomolecular, etc.) that pump down the column to increase the mean free path of the electron. Typical electron microscopes operate at pressures p on the order of $p = 10^{-10}$ atm.⁶¹

Although real-space images of nanoemulsions may be obtained by traditional transmission electron microscopy, care must be taken in micrograph interpretation. The ability to view a sample depends directly on the amount of contrast present. Depending on the electron cross-sectional scattering area of the atoms present in the sample, more or less contrast may be observed; greater contrast is observed for samples containing elements of higher atomic number Z . For example, silicone oils, which contain silicon, scatter more strongly than hydrocarbon oils, such as squalene, thereby providing readily measurable differences in scattering contrast. Most nanoemulsions, like most biological samples, do not have enough inherent contrast. For this reason, a negative stain (*e.g.* uranyl acetate) is typically used to enhance the contrast of nanoemulsions.⁶³ Research must be done, however, to ensure the stain does not interact with the sample. Additionally, because of the low column pressures at which the electron microscope operates, the solvent must be evaporated prior to viewing thereby immobilizing the specimen on a grid. Therefore, the nanoemulsion morphology and structure as viewed from TEM micrographs may appear very different from the solvated sample.

CTEM mitigates these traditional TEM shortcomings. In CTEM, a small aliquot on the order of microliters is placed on a grid, blotted by filter paper to create a thin layer of sample (< 300 nm), and frozen in place by submerging the grid in a cryogen (*e.g.* liquid ethane). Stains are typically omitted in CTEM so contrast can be a problem depending on the composition of the

dispersed and continuous phases. In this case, phase-contrast, in which the objective lens is underfocused and Fresnel fringes are formed around the outside of the droplets, can be used to enhance contrast. Freezing the sample in its natural solvated state significantly increases the chances of obtaining a more accurate morphology and overall droplet structure of a given nanoemulsion. For this reason, CTEM is a valuable technique for directly viewing nanoemulsion morphology. Still, changes that can occur during the freezing process can greatly affect the appearance of nanoemulsion droplets, as we discuss in Chapter 4, along with their collective droplet structure.

1.8 Summary of Chapters

This dissertation focuses primarily on emulsions in which droplets are composed of more than one dispersed phase component. The basic principles of emulsions and nanoemulsions necessary for the discussion within subsequent chapters have been presented in this chapter. In Chapter 2, recent advances in the physical chemistry of advanced nanoemulsion systems are presented. The technique of evaporative ripening to reduce nanoemulsion sizes is presented in Chapter 3. The time-dependence, predictions, limitations, and physical principles thereof are presented therein. Although a means of easily creating double nanoemulsions and anisotropic nanoemulsions is not yet available, it would be highly desirable from an applications perspective (*e.g.* for pharmaceuticals and materials). To this effect, Chapter 4 provides foundational groundwork for understanding and interpreting CTEM micrographs of O/W nanoemulsions. Vapor nanoinclusions, caused by expansion of water upon freezing around the nanodroplets during the cryogenic temperature quench of the sample, can appear in nanoemulsions depending on the phase of ice and oil type. Finally, in Chapter 5, a method of controlling coalescence using

oppositely charged surfactants is presented and extended to produce anisotropic emulsions and nanoemulsions using multiple immiscible liquids. Janus droplets, composed of two immiscible oils in a third immiscible aqueous continuous phase, Cerberus droplets, composed of three immiscible oils in a fourth immiscible aqueous continuous phase, and potentially higher order droplets are able to be formed using this method.

Chapter 2

Recent Advances in Nanoemulsions

2.1 Abstract

Recent advances in the growing field of nanoemulsions are opening up new applications in many areas such as pharmaceuticals, foods, and cosmetics. Moreover, highly controlled nanoemulsions can also serve as excellent model systems for investigating basic scientific questions about soft matter. Here, we wish to highlight some of the most recent developments in nanoemulsions, focusing on surface modification and material properties. These developments, many of which preceded the work in this dissertation, provide insight into the substantial advantages that nanoemulsions can offer over their microscale emulsion counterparts.

2.2 Controlling Droplet Size and Polydispersity

When designing nanoemulsions, controlling the droplet size distribution, especially the average droplet radius $\langle a \rangle$ and the polydispersity $\delta a / \langle a \rangle$ (associated with the effective width δa of the distribution), is of vital importance. In particular, much research has been devoted to inhibiting Ostwald ripening, fabricating smaller nanoemulsion droplets because of their potentially beneficial physical property changes (*e.g.* increased translucence), and increasing monodispersity for basic scientific studies.

The main route to droplet size reduction in nanoemulsions is through direct manipulation of the relevant variables that affect droplet breakup: σ , $\dot{\gamma}$, η_c , η_d , and ϕ where η_d is the dispersed phase viscosity. For example, the viscosity ratio η_d / η_c (ratio of dispersed to continuous phase viscosities) can be altered to maximize droplet rupturing. Several studies have shown that

smaller nanoemulsions can be produced as η_c is increased while η_d is fixed.^{41,64} Maintaining high surfactant concentrations in the continuous phase reduces the interfacial tension, increases droplet stability, and increases η_c all of which lead to smaller droplets. Additionally, higher $\dot{\gamma}$ ⁶⁵ and lower ϕ lead to smaller droplets. Thus, emulsifying at lower σ and ϕ , higher $\dot{\gamma}$, and optimizing η_d / η_c produces the smallest droplets. Even after optimizing conditions for the smallest nanodroplet production, however, droplets may not be made arbitrarily small. A lower bound, typically flow-imposed, on droplet size still exists for a given system.

Droplet polydispersity of nanoemulsions can be reduced by two main methods. Ensuring that all droplets experience the peak shear rate generated by a flow-producing device during emulsification will reduce polydispersity. This can be achieved, for instance, by recirculating the nanoemulsion multiple times (*i.e.*, increasing the number of passes) in a homogenizer⁴¹ or by ultrasonication for longer times.⁶⁵ A second way of reducing a nanoemulsion's polydispersity is through post-emulsification fractionation using a physical process that separates larger from smaller nanodroplets. For instance, by applying a high effective acceleration through centrifugation, a solid plug having gradients in ϕ and a may be formed.⁶⁶ By repeating the process of cutting these plugs cross-sectionally, combining sections of plugs having similar ϕ and a , redispersing to lower the droplet volume fraction, and centrifuging multiple times, nanoemulsions with a polydispersity approaching $\delta a / \langle a \rangle \approx 0.1$ have been achieved. This polydispersity is low enough that the resulting nanoemulsions are suitable for scientific studies that require near-monodispersity.

2.3 Virus-Like Droplets: Curved Nanodroplet Templates for Molecular Self-Assembly

Because some types of proteins, notably amphiphilic proteins, contain both hydrophobic and hydrophilic segments, they adsorb on droplet interfaces and therefore have commonly been used as emulsifiers and stabilizers.⁶⁷ However, some proteins are not strongly amphiphilic; they can coexist in the continuous aqueous phase along with oil droplets yet do not adsorb significantly onto oil-water interfaces. Among these types of hydrophilic proteins are the coat proteins of certain plant viruses. For instance, a cowpea chlorotic mottle virus consists simply of a shell, or capsid, composed of coat proteins, which protects the internal genetic material (*e.g.*, RNA) from enzymatic degradation. This protein shell is typically composed of ordered multiprotein ring-like subunits, called capsomers, which self-assemble to form a water-permeable, often icosahedral, capsid cage.⁶⁸ By altering the pH and ionic strength, viruses can be disassembled, and RNA separated from coat proteins, yielding purified protein.⁶⁹

Purified capsid proteins have been self-assembled around anionically stabilized silicone oil nanodroplets to create virus-like droplets (VLDs), the dispersed liquid analogue of virus-like particles (VLPs) that have a solid nanoparticle rather than a liquid droplet as the core,⁷⁰ as shown in Figure 2.1a.⁷¹ The proteins assemble to encage (*i.e.*, encapsidate) the nanodroplets through droplet-templated self-assembly, not through standard amphiphilic adsorption. Negatively charged droplets effectively act as surrogates for RNA, and the capsid proteins assemble in the aqueous phase around the droplets' anionic surfaces when pH and ionic strength are set using dialysis. This process creates protein-caged VLDs that have liquid cores; these cores can be loaded with drug molecules, and even the carrier oil can be chosen for biocompatibility or bioavailability. This self-assembly of viral protein cages around nanodroplets is distinctly different from a simple polymer adsorption process of amphiphilic proteins at the oil/water inter-

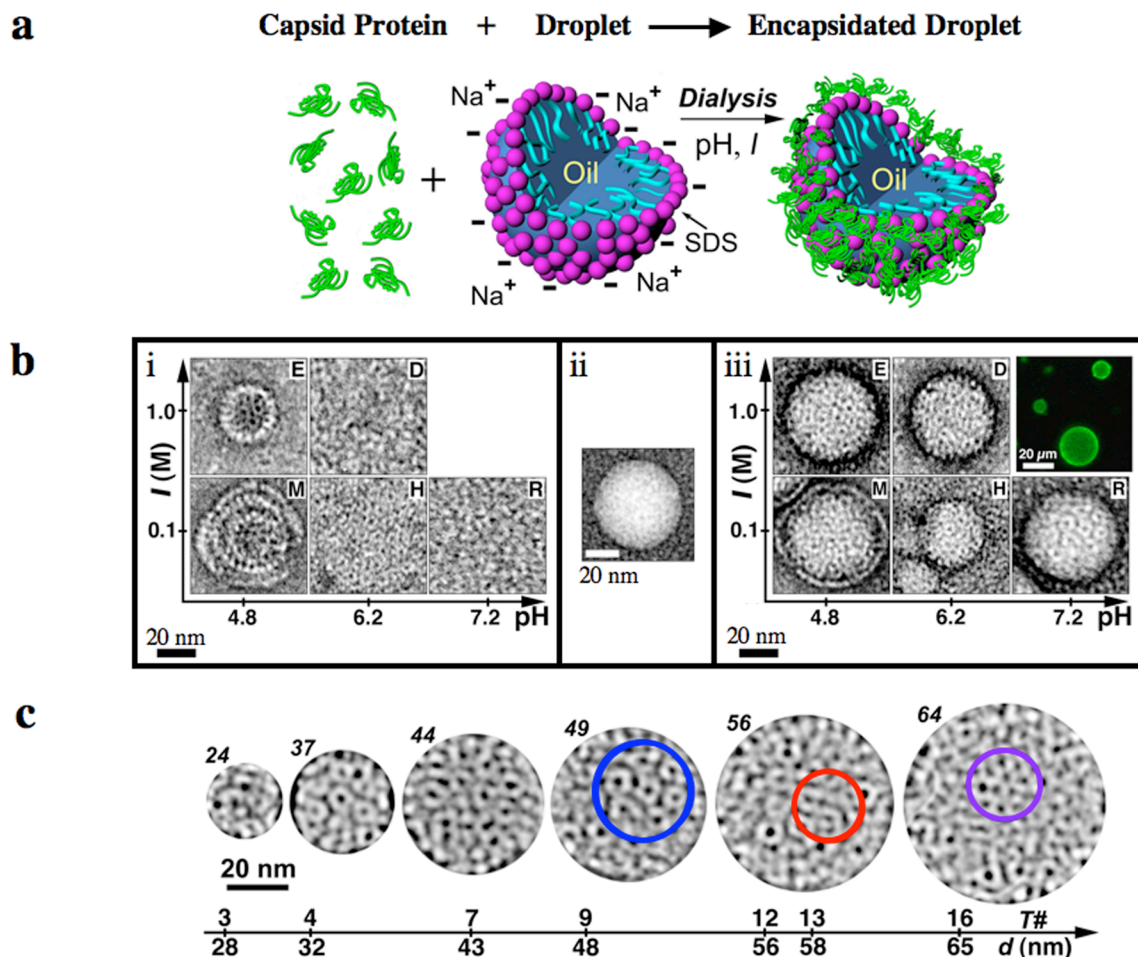


Figure 2.1. (a) An anionically stabilized nanoemulsion droplet can be encapsidated (*i.e.*, caged) by purified cowpea chlorotic mottle virus (CCMV) protein that self-assembles around the droplet interface as a result of changes in ionic strength, I , and pH. (b) Negatively stained transmission electron micrographs of isolated CCMV protein (*i*), a bare nanoemulsion droplet (*ii*), and encapsidated nanoemulsion droplets (*iii*). Subpanels *i* and *iii* show isolated protein self-assembly and self-assembly of the CCMV protein around a droplet, respectively, under different I and pH buffer conditions: E = empty shell, D = dimer, M = multishell, H = hexagonal sheet, R = reassembly. The subpanel *iii* inset shows green fluorescing fluorescein isothiocyanate-labeled CCMV protein coating microscale emulsion droplets. (c) Structural surface features of the CCMV protein self-assembled on nanoemulsion droplets as a function of droplet diameter. Allowed Caspar-Klug T -numbers for perfect icosahedral virus geometries and corresponding estimated diameters are shown in the bottom scale. As droplet sizes approach that of the native CCMV ($T\# = 3$, diameter $d = 28$ nm), more completely ordered protein capsomers (white rings) are found. These capsomers may order into sixfold arrangements, as shown within the blue circle. For larger droplet sizes, scars (*red circle*) and hexagonal web-like structures (*purple circle*) are also more frequently observed. Figure reprinted with permission from Reference 71, copyright 2008 by the American Chemical Society.

face, and it represents a new route to a decorated nanodroplet structure.

Figure 2.1b shows transmission electron microscopy (TEM) micrographs of purified cowpea chlorotic mottle virus (CCMV) protein that has been self-assembled around SDS-stabilized silicone oil nanodroplets for various ionic strength and pH levels.⁷¹ Native CCMV is an icosahedral-shaped virus composed of five-fold and six-fold protein rings called capsomers. Perfect icosahedral symmetry occurs when the number of proteins in the shell coincides with integer multiples of 60 (*i.e.*, allowed Caspar-Klug T-numbers).⁷² In the observed VLDs, varying degrees of order and disorder in the protein cages are observed for nanodroplets having different radii. Certain buffer conditions even lead to the self-assembly of multi-shell protein structures around nanodroplets. However, the dominant structural motifs (*i.e.*, ordered capsomers or disordered non-wild-type configurations) in the CCMV cages around droplets appear to be dependent on droplet size and therefore curvature, as well as buffer conditions. For larger nanodroplets, well beyond the native size of the virus, however, these ordered structures give way to other structures such as incomplete capsomers, scars, and hexagonal webs, as shown in Figure 2.1c. The degree of order and disorder in the presentation of multiple assembled proteins that encage the nanodroplets is likely to have an important impact on the effectiveness of VLDs in vaccine, targeting, and therapy applications.

2.4 Attractive Nanoemulsions

For dilute nanoemulsions composed of nanodroplets that interact only through short-range repulsions, the nanodroplets diffuse through Brownian motion in the continuous phase. However, not all nanoemulsion compositions yield repulsive nanodroplets. Deep secondary attractive wells in the interaction potential between droplets can be created by modifying the

composition of a repulsive nanoemulsion. This fundamental change in the droplet-droplet interaction potential can produce large macroscopic and microscopic changes in a number of important physical properties, as shown in Figure 2.2a. In the attractive regime, the secondary minimum is deep enough relative to thermal energy, $k_B T$, that the droplets do not unbind once they have encountered each other, but not so deep that the secondary minimum merges with the primary minimum, thereby leading to droplet coalescence. In some attractive systems, however, once a critical temperature, T_c , has been reached or exceeded, thermal energy can become comparable to or greater than the secondary potential well depth, and the droplets redisperse (*e.g.* this behavior is known for salt-induced aggregation of droplets).⁷³

Attractions between dispersed objects can lead to a variety of aggregation scenarios such as diffusion-limited aggregation (DLA) and diffusion-limited cluster aggregation (DLCA). In classic DLA, a cluster grows from spheres that diffuse one at a time and stick rigidly to the cluster.⁷⁴ In a scenario more closely related to experimental quenching of an attractive interaction in a uniform dispersion of spheres, aggregating clusters can themselves diffuse and join together to form a shear-rigid-bonded network through DLCA.⁷⁵

A related type of aggregation process, called slippery diffusion-limited cluster aggregation (S-DLCA), has been found while studying dynamics of gel formation in attractive nanoemulsions.²⁴ In S-DLCA, clusters of four droplets, each of which retains the ability to rearrange or slip around another, will form a tetramer before jamming together with other tetramer clusters to form a gel. Time-resolved small angle neutron scattering (SANS) studies of the structure factor, $S(q)$, on S-DLCA of nanoemulsions reveal the mechanism of formation of the salt-induced gel network. As time increases, the low- q value for $S(q)$ increases as larger-length-scale features form; meanwhile, in the mid- q range, the $S(q)$ minimum decreases as small-

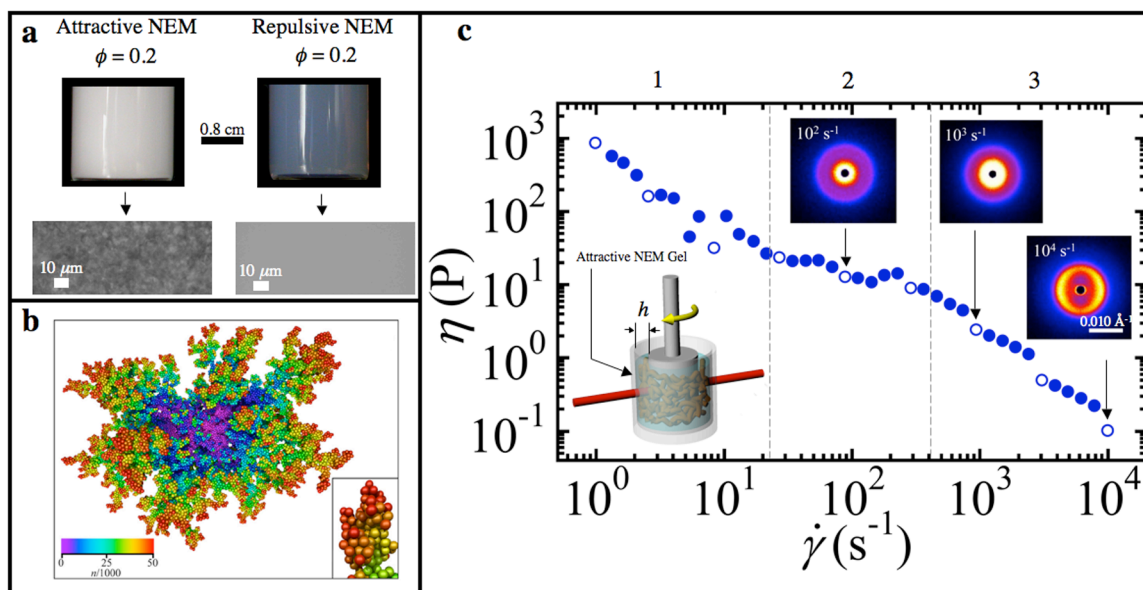


Figure 2.2. Attractive droplet interactions that create a secondary minimum in the pair potential can significantly alter the structure and properties of nanoemulsions (NEMs). (a) Macroscale (*upper*) and microscale (*lower*) images of an attractive ($C_{\text{salt}} = 400$ mM NaCl) and a repulsive nanoemulsion ($C_{\text{salt}} = 0$) having $\phi = 0.2$ and $C = 270$ mM sodium dodecyl sulfate. The attractive nanoemulsion appears white owing to multiple scattering of light from microscale inhomogeneities in the gel of aggregated nanodroplets, whereas the repulsive nanoemulsion appears bluish-white owing to scattering from droplets having length scales that are smaller than the wavelength of visible light. (b) Computer simulation results of slippery diffusion-limited aggregation (S-DLA). Shown is an example of an S-DLA aggregate that has been formed as a result of slippery aggregation of 50,000 spheres, each added one at a time through a random diffusion process to the growing cluster. The color scale indicates the order in which the n -th sphere was added. (c) Nanoemulsion gels can be disrupted by high shear flow. Shown are the structure and rheology of an attractive nanoemulsion gel made under steady shear: during (*open blue circles*) and independent of (*filled blue circles*) simultaneous SANS measurements. Gel is formed with $\langle a \rangle = 40$ nm, $\phi = 0.44$, $C_{\text{SDS}} = 10$ mM, and $C_{\text{salt}} = 700$ mM NaCl. The lower left inset shows a schematic of an attractive nanoemulsion gel network under shear in a Couette geometry with a gap, h . The shear direction of the gel is normal to the incident neutron beam (*red bar*). The upper right insets show two-dimensional scattering intensity profiles for $\dot{\gamma} = 10^2$, 10^3 , and 10^4 s^{-1} . Panel *a* reprinted with permission by Reference 112, copyright 2012 by Annual Reviews. Panel *b* reprinted with permission from Reference 76, copyright 2007 by the American Physical Society. Panel *c* adapted with permission from Reference 77, copyright 2011 by the American Chemical Society.

er individual droplets aggregate to form larger clusters. At high q , cluster peaks are seen in $S(q)$; these peaks have a higher intensity corresponding to more nearest neighbors than those of classic DLCA. This behavior of $S(q)$ has motivated the creation of the S-DLCA model for describing this system. Subsequent computer simulations of S-DLA in the limit of extremely dilute ϕ confirm that interlocking tetramers within a single isolated cluster confer rigidity, as shown in Figure 2.2b.⁷⁶

Rheological small angle neutron scattering (rheo-SANS) has also been used to probe a similar attractive nanoemulsion system.⁷⁷ Three different regimes are found according to shear rate at $\phi = 0.44$ (Figure 2.2c). At low shear rates, the originally jammed gel network is broken up into large clusters, leading to shear-thinning (regime 1). At intermediate shear rates, the clusters break and reform while tumbling under shear and increasingly become smaller as $\dot{\gamma}$ is increased (regime 2). Finally, at high shear rates, strong shear-thinning is again seen, corresponding to droplet clusters breaking into individual droplets (regime 3). An effective volume fraction argument related to a model of tumbling clusters is presented to explain the cluster breakup.

2.5 Optical Properties: Tailoring Visual Appearance and Characterization

One of the appealing aspects of nanoemulsions is that their optical properties can be tuned to yield an appearance that ranges from nearly transparent to white primarily by controlling $\langle a \rangle$, ϕ , and the refractive index difference between the continuous and dispersed phases Δn (Figure 2.3a). This tunable appearance of nanoemulsions contrasts with common emulsions having $a > 0.2 \mu\text{m}$, which typically appear milky white, owing to multiple scattering of polychromatic light. The visual appearance of a repulsive nanoemulsion changes based on the viewing angle with respect to the light source (Figure 2.3b) in a manner reminiscent of Rayleigh

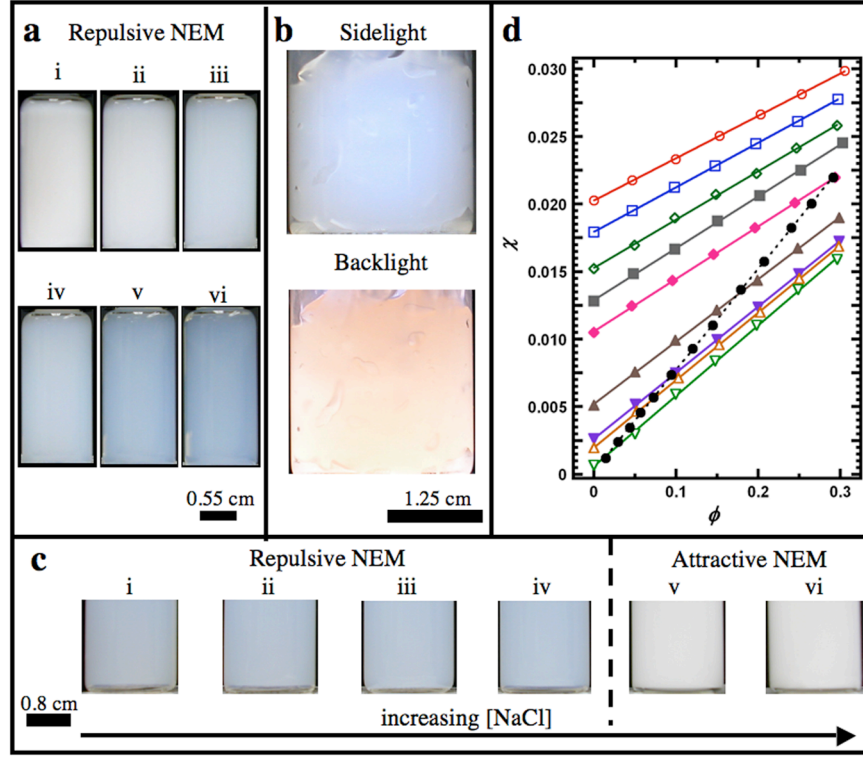


Figure 2.3. (a) A series of nanoemulsions (NEMs, *i*–*vi*) at $\phi = 0.3$ having decreasing droplet sizes $\langle a \rangle \approx 101, 75, 59, 51, 42$, and 35 nm and increasing $C_{\text{SDS}} = 10, 25, 50, 100, 200$, and 400 mM, respectively. Even at high ϕ , nanoemulsions become increasingly transparent and preferentially scatter blue light owing to scattering by droplets having very small $\langle a \rangle$ relative to the shortest visible wavelengths. (b) A nanoemulsion's visual appearance can change dramatically when viewed at different angles relative to the direction of incident light. A nanoemulsion when illuminated from the side appears bluish-white, whereas when illuminated in transmission, it appears reddish-white. (c) A series of nanoemulsions at $\phi = 0.2$ and $C_{\text{SDS}} = 270$ mM having increasing C_{salt} from left to right: $0, 50, 100, 200, 300$, and 400 mM NaCl, respectively. Between 200 and 300 mM NaCl, the nanoemulsion becomes dominantly attractive (*i.e.*, forms a gel network of aggregated nanodroplets), and the nanoemulsion's appearance turns from blue to white because multiple scattering from larger-microscale aggregate and gel structures occurs. (d) The normalized refractive index difference, $\chi = (n - n_o)/n_o$, where n is the measured refractive index of a polydimethylsiloxane (PDMS) oil-in-water nanoemulsion and n_o is the measured refractive index of pure water, plotted as a function of ϕ for a PDMS nanoemulsion having $C_{\text{SDS}} = 25$ (∇), 75 ($\triangle$), 100 ($\blacktriangledown$), 200 ($\blacktriangle$), 400 ($\blacklozenge$), 500 ($\blacksquare$), 600 ($\diamond$), 700 ($\square$), and 800 ($\circ$) mM. Linear fits are to the equation, $\chi(\phi, C) = \chi_{C^*}(C/C^*)(1 - \phi) + \chi_\phi\phi$, where C^* is the critical micelle concentration and χ_{C^*} and χ_ϕ are coefficients inherent to the type of surfactant and oil used, respectively. A serial dilution curve (*filled black circles and dashed line*) of $\chi(\phi, C)$ can be used to determine both ϕ and C of a previously uncharacterized nanoemulsion. Panels *a*–*c* reprinted with permission by Michael M. Fryd and Thomas G. Mason, copyright 2011. Panel *d* reproduced by permission of the PCCP Owner Societies from Reference 32.

scattering. Moreover, by changing a repulsive nanoemulsion into an attractive one (Figure 2.3c), its appearance can be varied from translucent to white, because light is multiply scattered from larger microscale aggregates and gel droplet network structures in attractive nanoemulsions.

Optical techniques have long been used to characterize colloidal dispersions, and nanoemulsions of undeformed droplets having optical properties that mimic dispersions of polymer latex spheres. For instance, dynamic light scattering from a highly dilute, non-attractive nanoemulsion is commonly used to characterize its effective average hydrodynamic radius and polydispersity. Moreover, the optical transmission as a function of wavelength of a nanoemulsion loaded in a thin optical cuvette has been used to measure the effective scattering mean free path, l , which depends primarily on $\langle a \rangle$, ϕ , and n .²⁵ These measurements compare reasonably well with predictions given by Mie theory. Either very dilute or very concentrated nanoemulsions having the smallest droplet sizes provide the greatest degree of transparency and largest l for visible light. Significant scattering can still be present in the UV, since UV wavelengths are closer to the droplet size.

An optical refractometer can be used to obtain the refractive index of a homogeneous nanoemulsion to a very high degree of accuracy and precision, provided the droplets are small enough that effective medium theory holds. The normalized refractive index difference, $\chi = (n - n_o)/n_o$, where n is the measured refractive index of a nanoemulsion and n_o is the measured refractive index of pure water, of an SDS-stabilized silicone O/W nanoemulsion is plotted as a function of ϕ for a series of different SDS concentrations in Figure 2.3d.³² Volume-weighted effective medium predictions of χ explain the measured trends, independent of $\langle a \rangle$, provided $\langle a \rangle < 100$ nm. Moreover, an unknown nanoemulsion's surfactant concentration C and

also its ϕ may be deduced simultaneously from a series of nondestructive measurements of χ after diluting repeatedly with the pure continuous phase.

The visual appearance of nanoemulsions can also be altered using additives. For instance, incorporating a molecular additive that aids in refractive index matching will reduce scattering from the droplet interfaces and make a nanoemulsion more transparent. Alternatively, dyes, whether absorbing or fluorescing, can be included in the dispersed or continuous phases to color the emulsion. Thus, a wide range of desirable and attractive appearances of nanoemulsions can be achieved by varying their composition and size distribution.

2.6 Mechanical Properties: Controlling Rheology

Nanoemulsions can exhibit stronger elasticity than microscale emulsions because the elastic storage modulus G' of a concentrated repulsive emulsion is proportional to the Laplace pressure of an undeformed droplet, $\Pi_L = 2\sigma/a$ and therefore is inversely proportional to droplet size. Moreover, a microscale emulsion at ϕ well below random close packing, which flows like a viscous liquid, can be transformed into a strongly elastic nanoemulsion without altering the composition by applying high flow rates that cause extreme droplet rupturing that leads to jamming.²³ This dramatic and irreversible flow-induced elastification (*i.e.*, termed vitrification because the resulting positional structure of nanodroplets is disordered) is an interesting development in the field of soft matter, both from scientific and applied viewpoints. Figure 2.4a illustrates this process, and macrophotographs, shown in Figure 2.4b,⁷⁸ demonstrate the increase in elasticity as $\langle a \rangle$ is reduced further into the nanoscale regime. Measurements of $G'(a, \phi)$ of direct silicone oil nanoemulsions stabilized by anionic SDS confirm that a Debye-screened repulsion between droplet interfaces plays an essential role in the nanoemulsion's elasticity and

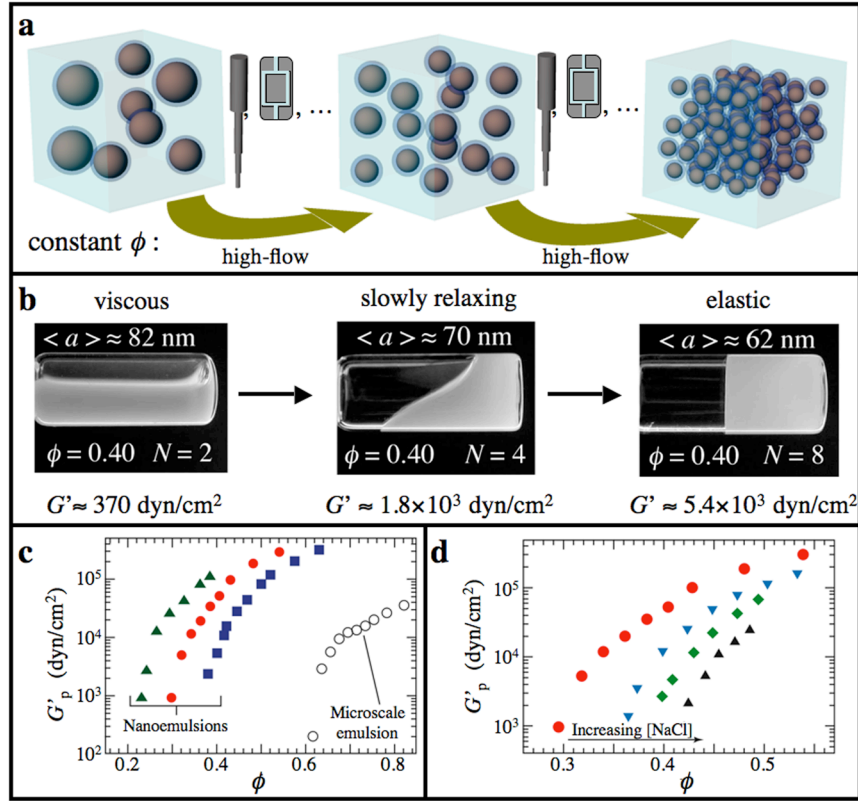


Figure 2.4. (a) Schematic of flow-induced vitrification (*i.e.*, solidification into a disordered elastic solid) of an ionically stabilized nanoemulsion. At constant ϕ , high flow and droplet rupturing cause the distances between droplet interfaces to approach the Debye screening length (represented by the *dark blue* spherical shell around the *orange* oil droplets), thereby causing droplets to repulsively jam in a disordered configuration. (b) Photographs of nanoemulsions made at constant $\phi = 0.40$ as a function of an increasing number of passes N through a microfluidic homogenizer. The images, which show the influence of gravity on a vial filled with a nanoemulsion that has been tipped sideways, illustrate the increasing elasticity as the droplet size decreases toward the Debye screening length. Measurements of the elastic shear modulus G' are reported at frequency $\omega = 10$ rad/s. (c) The plateau storage modulus G'_p as a function of ϕ for polydimethylsiloxane (PDMS) nanoemulsions having $\langle a \rangle = 28$ nm ($\blacktriangle$), 47 nm ($\bullet$), and 73 nm ($\blacksquare$) at $C_{\text{SDS}} = 10$ mM and, for comparison, a microscale emulsion having $\langle a \rangle = 740$ nm ($\circ$). A nanoemulsion's elastic onset occurs at much lower ϕ than that of a similar microscale emulsion and well below the maximal random jamming point (*i.e.*, random close packing) of monodisperse spheres at $\phi_{\text{MRJ}} \approx 0.64$. (d) G'_p as a function of ϕ for PDMS nanoemulsions at $C_{\text{SDS}} = 10$ mM and $\langle a \rangle = 47$ nm, with added sodium chloride salt concentrations $C_{\text{salt}} = 0$ mM ($\bullet$), 10 mM ($\blacktriangledown$), 40 mM ($\blacklozenge$), and 90 mM ($\blacktriangle$). As $[\text{Na}^+]$ and $[\text{Cl}^-]$ increase, the Debye screening length decreases, and the once-solid nanoemulsion is effectively melted into a liquid-like material. Panel *a* reprinted with permission by Reference 112, copyright 2012 by Annual Reviews. Panels *b* and *d* reprinted with permission from Reference 78. Panel *c* reprinted with permission from Reference 23, copyright 2007 by the American Physical Society.

the elastic vitrification effect. Later experiments using a PDMS, glycerol, ethanol, fatty acid, anionic surfactant system have also shown elastification.⁷⁹ Consequently, a stable, repulsive O/W nanoemulsion's composition can be mostly water and yet exhibit elasticity characteristic of microscale emulsions having much higher oil content, as shown by the plateau storage modulus $G'_p(\phi)$ in Figure 2.4c. Emulsions that are made elastic by attractive interactions often suffer from gravitational instabilities, so repulsive nanoemulsions offer a route to higher elasticity at lower ϕ while maintaining good stability against gravitational separation over long periods of time.

The elasticity of charge-stabilized repulsive nanoemulsions can be strongly reduced by adding modest concentrations of ionic species, thereby further screening the charge interactions between droplet interfaces. For instance, a solid direct nanoemulsion can be effectively melted by adding aqueous solutions of NaCl, as shown from the $G'_p(\phi, [\text{NaCl}])$ in Figure 2.4d.⁷⁸ Electrolyte control thus provides another mechanism for tailoring a nanoemulsion's mechanical properties when ionic concentrations are varied in the continuous aqueous phase. As $[\text{NaCl}]$ is increased beyond the range associated with melting, then a nanoemulsion can form a gel due to attractive droplet interactions. If the attractive nanoemulsion remains stable and is not destroyed by droplet coalescence, one observes re-entrant elasticity in $G'([\text{NaCl}])$.

2.7 Conclusions

We have exhibited some of the recent advances in the physical chemistry of nanoemulsions including control over their size and polydispersity, creation of virus-like nanodroplets, attractive nanoemulsions, optical properties and optical characterization of nanoemulsions, and mechanical properties of nanoemulsions. Although recent achievements in controlling a combination of composition, structure, and physical properties of nanoemulsions

via a number of synthetic routes are impressive, such advances provide access to yet other new, exciting frontiers in nanoemulsion research and applications. Ideally, it would be desirable to systematically design nanoemulsions that optimize certain desired end-uses, such as for fundamental scientific investigation, medical science, and advanced building materials based on rules that govern, for example, their transport, stability, rheological, and optical properties, while maintaining wanted chemical compositions and functionalities. To this end, much future nanoemulsion research is anticipated.

Chapter 3

Time-Dependent Nanoemulsion Droplet Size Reduction By Evaporative Ripening

3.1 Abstract

To overcome limitations of flow-induced rupturing and obtain nanodroplet sizes approaching the micellar scale, we make oil-in-water nanoemulsions having a dispersed phase that contains lower- and higher-molecular-weight oils. After nanoemulsion formation by extreme flow-induced droplet rupturing, the nanodroplets become even smaller as molecules of the lower-molecular-weight oil migrate out of the droplets into the aqueous continuous phase and are removed by evaporation while stirring. We control the degree of reduction primarily through the volume fraction of higher-molecular-weight oil originally present in the dispersed phase. We study the droplet size reduction rate using time-dependent dynamic light scattering. This evaporative ripening method is useful for making extraordinarily fine nanodroplets containing extremely viscous high-molecular-weight liquids that would be difficult to emulsify otherwise. Additionally, we recover the evaporated lower-molecular-weight oil by distillation and reuse it in subsequent emulsification, thus demonstrating an environmentally friendly method for mass-producing extremely small nanodroplets.

3.2 Introduction

When forming aqueous dispersions of extremely small oil nanodroplets that approach micellar dimensions through top-down rupturing processes, several different aspects of emulsion stability play a role. Among these, Ostwald ripening, driven by differences in the Laplace pressure, $2\sigma/a$, of undeformed spherical droplets having an interfacial tension, σ , and different

radii, a , can give rise to a coarsening instability.^{33,80,81} If oil molecules in the droplets have a small yet notable solubility in the aqueous continuous phase, then these molecules tend to diffuse from smaller droplets having higher Laplace pressures to larger ones having smaller Laplace pressures, causing the average droplet radius to increase. A theory of Ostwald ripening, developed by Lifshitz, Slezov, and Wagner (*i.e.*, LSW theory) for an oil that has a single molecular type and solubility, predicts that the number-average radius of the coarsening emulsion grows as the cube root of time.³⁶⁻³⁸ If droplet interactions are dominantly repulsive and not strongly attractive,²⁴ then other mechanisms of instability, such as creaming, coalescence, and flocculation, are usually not of concern because Brownian motion dominates buoyancy.

Ostwald ripening can be arrested by adding a component that is insoluble in the aqueous continuous phase to the dispersed phase, as proposed by Higuchi and Misra.⁸² They suggested that entropy of mixing could be used to arrest Ostwald ripening and thereby create an emulsion having a stable droplet size, even if soluble dispersed material continuously migrates between droplets in equilibrium. In this method, a dispersed-phase liquid prone to Ostwald ripening is mixed with a second dispersed-phase liquid that does not Ostwald ripen. This method of stabilizing an emulsion is sometimes called the two-component dispersed phase method or trapped species method (TSM).^{35,83,84} Indeed, it has been experimentally shown that Ostwald ripening may be slowed and even arrested with a second insoluble dispersed phase component.^{64,85} Solid nanoparticles can also be created through a similar process, called the solvent evaporation method.⁸⁶⁻⁸⁸ In this method, an O/W emulsion is created using a dispersed oil phase that typically consists of a polymer solution in which the solvent has a high vapor pressure. The emulsion is subsequently heated, and the solvent evaporates, thereby yielding a dispersion of solid nanoparticles that may or may not be spherical.

Although a variety of solvent evaporation methods exist for producing dispersions, the ultimate lower bound on the sizes of the smallest nanoemulsion droplets has not been fully explored in general or in specific applications.⁸⁹ Moreover, detailed time-dependent studies of the progression of the ripening of the droplet size distribution for different compositions and evaporation conditions are lacking. Past efforts in droplet size reduction have centered around optimizing emulsification parameters such as surfactant, flow, interfacial tension, and dispersed and continuous phase viscosities.^{43,64,65}

In the present study, we combine high-flow emulsification with solvent evaporation to produce extremely small nanodroplets that contain relatively few dispersed phase molecules and have sizes only slightly larger than surfactant micelles. We present detailed time-dependent dynamic light scattering (TD-DLS) measurements of the mean radii of droplets as a function of time after emulsification while imposing a flow-evaporation process. We examine how the rate of droplet size reduction is influenced by both the dispersed phase volume ratio of volatile lower-molecular-weight oil (*i.e.*, solvent) to higher-molecular-weight oil insoluble in the aqueous continuous phase (*i.e.*, nonvolatile oil) and the viscosity of the nonvolatile oil. We find a quadratic relation between the dispersed phase volume ratio and the rate of reduction, and we find that the rate of change in the average droplet size is largely independent of the viscosity of the nonvolatile oil species. Because large volumes of solvent must typically be evaporated to achieve significant reductions in droplet sizes, which could potentially limit the practical use of this approach, we further show that distillation can be used to recover the evaporated solvent for reuse in subsequent production of nanoemulsions, thereby achieving a “green” feedback process for creating the smallest possible droplets while minimizing environmental impact.

3.3 Experimental Procedure

We use the following procedure to make two-component dispersed phase O/W nanoemulsions and to study the evolution of their size distributions during subsequent evaporation. The solvent, denoted L1, is first mixed with a nonvolatile oil, denoted L2, at varying initial oil volume fractions of L2 in the dispersed phase, $\psi_{2,i}$. For our experiments, L1 is polydimethylsiloxane (PDMS) having molecular weight ≈ 160 g/mol and kinematic viscosity 0.65 cSt, and L2 is one of the following PDMS oils having a much higher viscosity and corresponding molecular weight: 100 cSt ($\approx 6,000$ g/mol), 1000 cP ($\approx 40,000$ g/mol), 60,000 cP ($\approx 80\,000$ g/mol), 300,000 cSt ($\approx 204,000$ g/mol), or 1,000,000 cSt ($\approx 308,000$ g/mol). This PDMS oil mixture is added to an aqueous surfactant solution of 100 mM sodium dodecyl sulfate (SDS) at an initial overall oil droplet volume fraction of $\phi_i = 0.2$ and then dispersed with a rotary mixer to form a microscale polydisperse O/W “premix” emulsion. Next, extreme-flow emulsification,⁴¹ using a high-pressure homogenizer (Figure 3.1) with an inlet air pressure $p_a = 75$ psi, corresponding to a liquid pressure inside the interaction chamber (Figure 3.1 inset) of $\approx 18,000$ psi, is applied to create a nanoemulsion. A cooling coil surrounded by an ice bath is used to minimize the loss of the volatile oil during emulsification. The nanoemulsion is cycled through the homogenizer $N = 6$ times to narrow the size distribution and decrease droplet size. The size distribution of nanoemulsions just after emulsification is monomodal with a polydispersity of approximately 0.3 to 0.35, as previously shown.⁴¹ Approximately 70 mL of nanoemulsion is then heated in an 80x40 mm crystallizing dish at temperature $T \approx 60$ °C while stirring to deplete L1 from the system. If left uncovered without stirring, a gel-like layer forms hindering the evaporation process. Water also evaporates but is replenished every hour during the evaporation to keep the total volume of the nanoemulsion constant. $T \approx 60$ °C was chosen so

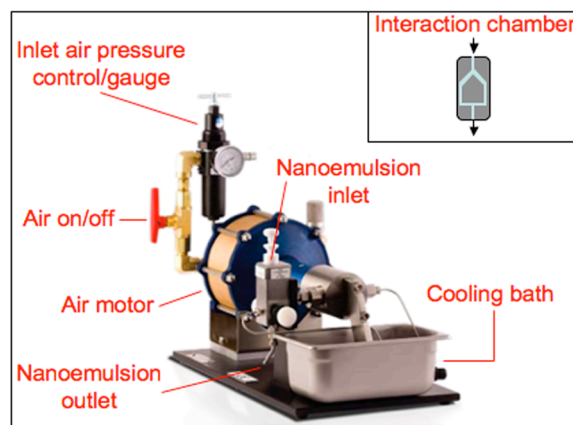


Figure 3.1. Photograph of a Microfluidics M-110S small volume pneumatic high-pressure homogenizer used to produce nanoemulsions. The M-110S is a gas-driven pulse-action device that forces liquids at amplified pressures $\leq 26,000$ psi through an interaction chamber (diagrammed in the inset), creating enough flow to rupture emulsion droplets down to the nanoscale. The cooling bath is used to counteract viscous heating due to the high flow. Photograph used with permission from the Microfluidics Corporation.

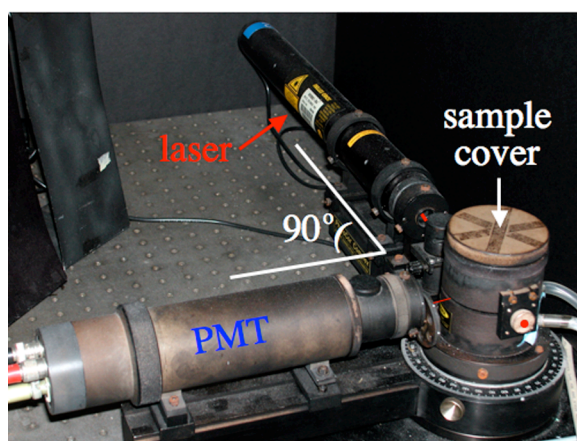


Figure 3.2. Photograph of a Photocor Complex dynamic light scattering system. A 5 mL vial of a dilute nanoemulsion (*e.g.* $\phi \approx 10^{-5}$) is placed directly beneath the center of the sample cover. Upon covering, a HeNe $\lambda = 633$ nm laser is turned on and focused to a small scattering volume in the center of the sample vial. A photomultiplier tube (PMT), placed at 90° with respect to the incident beam (*i.e.* $\theta = 90^\circ$), converts the scattered photons into a current and passes this information to a correlator. The correlator then computes the autocorrelation function enabling the volume-weighted average hydrodynamic radius to be determined from the Stokes-Einstein equation.

that the water need not be replenished multiple times an hour, but the evaporation rate is high enough to complete the evaporative ripening process in less than a day. The size of the nanoemulsion droplets is followed by TD-DLS (apparatus shown in Figure 3.2) each hour thereafter.

In order to recover L1 during the evaporative ripening process, we use a simple distillation apparatus consisting of a round bottom flask, well-insulated Y-adapter, condenser, and vacuum distillation adapter. The round bottom flask containing the sample to be recovered is placed in a sand bath on a hot plate, connected to the rest of the distillation apparatus, and heated while stirring to $T \approx 75$ °C. The distilled L1 is then collected until shortly after the foam disappears. Any water also collected is removed before reusing L1.

3.4 Results and Analysis

Photographic images of the appearance of the nanoemulsion during the evaporation process, beginning immediately after high-flow emulsification, are shown in Figure 3.3a. Initially, the nanoemulsion strongly scatters light and has a white appearance. Additionally, a cream quickly appears before any stirring due to the aggregation and creaming of larger droplets that have Ostwald ripened enough for depletion attraction to occur. The appearance becomes more transparent during the evaporation. The foam shown partway through the process results from the evaporation of L1 in the presence of excess surfactant in the aqueous continuous phase; the foam dissipates as L1 is removed from the emulsion. Figures 3.3b and 3.3c show microscopic and nanoscopic schematics of the emulsion size reduction evolution and the size reduction of a representative droplet as L1 is evaporated, respectively.

We use DLS to measure the mean radius for a series of different $\psi_{2,i}$ as a function of time

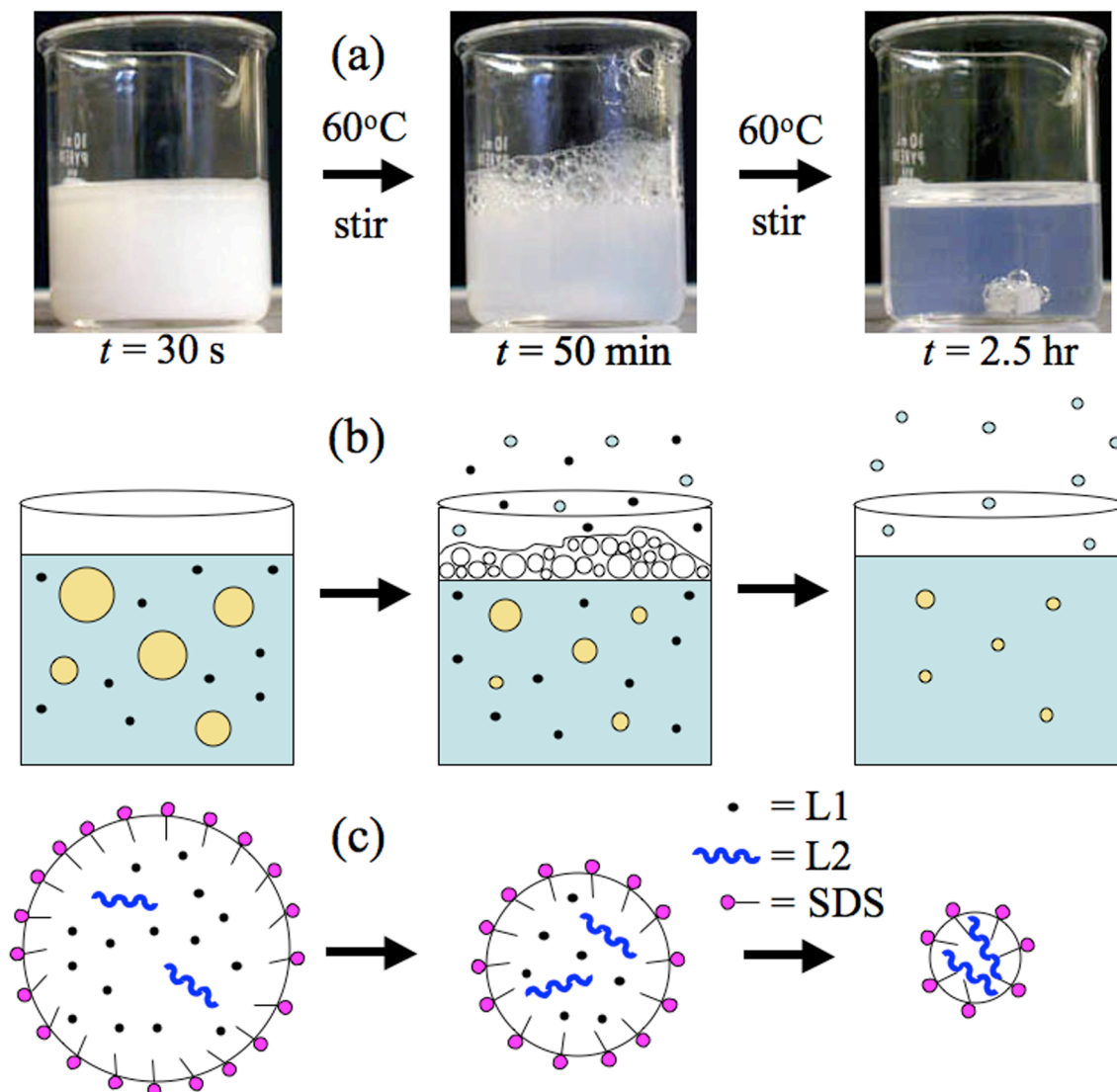


Figure 3.3. Time-dependent evolution of a nanoemulsion subjected to evaporation-driven ripening. The nanoemulsion is heated to $\approx 60^\circ\text{C}$ while stirring in a hood with 15 cm^2 of surface area for evaporation. The initial total volume fraction is $\phi_i = 0.2$, the initial volume fraction of nonvolatile oil in the dispersed phase is $\psi_{2,i} = 0.1$, the homogenizer's air pressure is $p_a = 75\text{ psi}$, the SDS concentration is $C = 100\text{ mM}$, and the number of passes is $N = 6$. (a) Photographs of the evolution of the appearance of a nanoemulsion as evaporation occurs. (b) Microscale schematic of nanoemulsion evolution (light blue = aqueous continuous phase, light blue circles = water vapor, yellow circles = surfactant coated oil droplets, black dots = solvent molecules (L1)). (c) Nanoscale schematic of nanodroplet evolution as evaporation proceeds. Blue wavy lines represent nonvolatile oil molecules (L2) and magenta circles with tails represent SDS molecules. Figure reprinted with permission by Reference 42, copyright 2010 by the American Chemical Society.

that exhibit negligible Ostwald ripening immediately after emulsification, as shown in Figure 3.4a. Many factors, such as the volume of emulsion, surface area available for evaporation, stirring rate, C , and T , can contribute to the evolution of the droplet size distribution. Because a full theory is lacking, in Figure 3.4a, we empirically fit the average measured radius to an exponential equation that captures the decrease in the time-dependent average droplet radius $\langle a(t) \rangle$ toward a final steady-state radius $\langle a_f \rangle$:

$$\langle a(t) \rangle = \langle a_f \rangle (1 + b \exp(-t/t_o)) \quad (1)$$

Here t_o is a time scale characterizing the decrease in droplet size and b is a dimensionless factor related to the composition and emulsification conditions. This empirical form reasonably describes the measured evolution. As $\psi_{2,i}$ approaches unity, the initial mean radius $\langle a_i \rangle = \langle a_f \rangle (1 + b)$ approaches $\langle a_f \rangle$; the lack of evolution in this limit of composition is expected because no solvent is used in making the emulsion. In Figure 3.4b, the plot of $\langle a_i \rangle$ and $\langle a_f \rangle$ against $\psi_{2,i}$ clearly shows this trend. We also use volume conservation to calculate the predicted final mean radius, $\langle a_{f,p} \rangle$, based on the initial mean radius determined from the fit using $\langle a_{f,p} \rangle = \langle a_i \rangle \psi_{2,i}^{1/3}$. All measured $\langle a_f \rangle$ are within 4 nm of the predicted $\langle a_{f,p} \rangle$. Additionally, we fit the measured $\langle a_f \rangle$ to $\langle a_f \rangle = d + m\psi_{2,i}$ over the range $0.3 < \psi_{2,i} < 1$, where m and d are fitting parameters that depend on experimental conditions. The value of d is related to a lower bound on the droplet size; in the limit when $\psi_{2,i} \rightarrow 0$, d approaches the radius of an SDS micelle. The observed linearity of $\langle a_f \rangle$ with $\psi_{2,i}$ is intriguing but may be fortuitous; this linear dependence could arise from a combination of factors that influence droplet breakup, such as oil viscosity within the droplet, interfacial tension, flow, and surfactant type and concentration. To show further the advantage of this method, we compare the nanoemulsion created under the con-

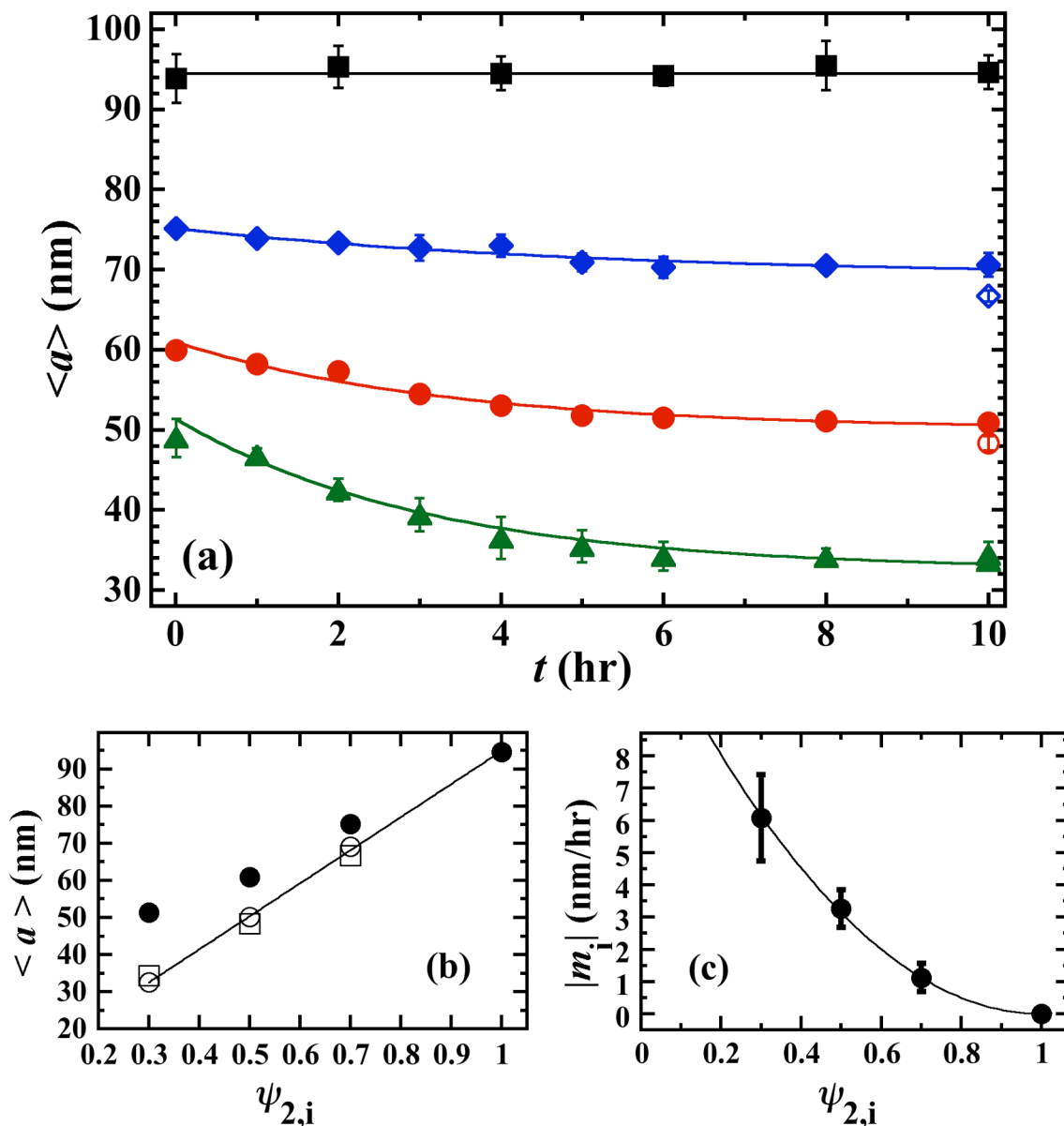


Figure 3.4. (a) Average droplet radius, $\langle a \rangle$, as a function of time, t , for initial dispersed phase L2 volume fractions of $\psi_{2,i} = 0.3$ ($\blacktriangle$), 0.5 ($\bullet$), 0.7 ($\blacklozenge$), and 1 ($\blacksquare$). Open symbols at $t = 10$ h indicate a predicted final radius $\langle a_{f,p} \rangle$ based on the initial radius, $\langle a_i \rangle$, using volume conservation. Bars denote standard deviation of the average radii, measured in three separate trials of the same evaporation experiment. Lines: fits using equation 1. Emulsification conditions: $p_a = 75$ psi, $C = 100$ mM, $\phi_i = 0.2$, $N = 6$. (b) Initial and final average droplet radii of data in part (a) as a function of $\psi_{2,i}$. Solid circles are $\langle a_i \rangle$, open circles are $\langle a_f \rangle$, and open squares are the predicted $\langle a_{f,p} \rangle$. Line: fit to the measured $\langle a_f \rangle$. (c) Absolute value for initial slopes, $|m_i|$, of data in part (a) as a function of $\psi_{2,i}$. Line: quadratic fit to $|m_i|$ (see text). Figure reprinted with permission by Reference 42, copyright 2010 by the American Chemical Society.

ditions $\phi_i = 0.2$, $\psi_{2,i} = 0.3$, $p_a = 75$ psi, $C = 100$ mM, and $N = 6$ to a nanoemulsion created with $\phi_i = 0.06$, $\psi_{2,i} = 1$, $p_a = 75$ psi, $C = 100$ mM, and $N = 6$. Although each nanoemulsion has the same total volume fraction after the evaporation process, the evaporative ripening method produces droplets having a mean radius of ≈ 33 nm, whereas the conventionally prepared nanoemulsion produces droplets with a mean radius of ≈ 88 nm.

As the mean radius decreases over time, L1 is removed from the system, so ϕ decreases with time while the volume fraction of L2, ψ_2 , increases with time. The final droplet volume fraction ϕ_f is given by $\phi_f = \psi_{2,i}\phi_i$; during the evolution, $\phi(t) = \phi_i(\langle a(t) \rangle / \langle a_i \rangle)^3$ and $\psi_2(t) = \psi_{2,i}(\langle a_i \rangle / \langle a(t) \rangle)^3$. As evaporation proceeds in a given nanoemulsion, $\psi_2(t)$ increases. We find that the instantaneous rate of decrease in the droplet size at a particular value of ψ_2 is equal to the corresponding initial rate of decrease we have measured in separate samples having different $\psi_{2,i}$, within the experimental uncertainty. As such, we plot the initial rate of droplet size reduction, $|d\langle a \rangle / dt|_i = |m_i|$, as a function of $\psi_{2,i}$ in Figure 3.4c. The reduction in the initial rate can be fit to a quadratic form: $|m_i| = c(1 - \psi_{2,i})^2$, where c describes the average shrinking rate of a droplet's radius and is $c = 12.5$ nm/h.

When we emulsify at low $\psi_{2,i}$, the nanoemulsions begin to Ostwald ripen rapidly immediately after droplet rupturing occurs. As $\psi_{2,i}$ approaches 0.1, droplet sizes comparable to the lower limit of our DLS instrument are reached after the evaporative ripening process. At $\psi_{2,i} \approx 0.1$, the time required to observe an autocorrelation function as well-defined as the one observed at $\psi_{2,i} > 0.3$ greatly increases, and the resulting mean radius observed corresponds to a size larger than that expected. This suggests that we may be measuring the tail of a distribution of sizes where the mean droplet radius is below the detection limit of the DLS apparatus. A cryo-

TEM image of such a nanoemulsion, as shown in Figure 3.5, verifies this. Similar results are seen for $\psi_{2,i} < 0.1$. Increasing the pressure as well as increasing the amount of surfactant, thereby optimizing conditions for even smaller droplets, also yields similar results.

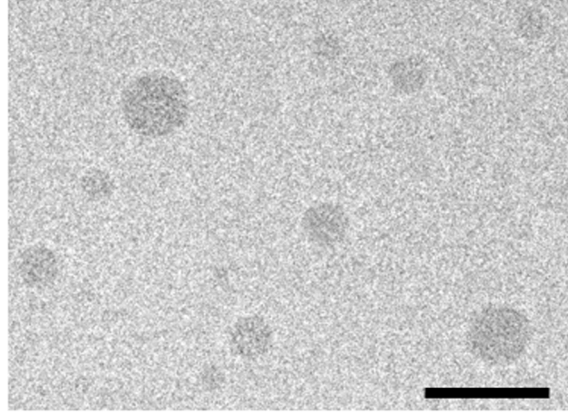


Figure 3.5. Cryogenic transmission electron microscopy micrograph of a PDMS nanoemulsion ($\Delta n \approx 0.06$) having droplet sizes smaller than standard DLS can resolve using a HeNe $\lambda = 633$ nm laser at $\theta = 90^\circ$. Emulsification conditions: $p_a = 75$ psi, $C = 100$ mM, $\phi_i = 0.2$, $N = 6$, $\psi_{2,i} = 0.1$. Scale bar is 50 nm.

Next, we investigate how the mean droplet evolution depends on the viscosity of L2. Figure 3.6 shows how $\langle a \rangle$ changes with time at $\psi_{2,i} = 0.3$ for several L2 having viscosities 100 cSt, 1000 cP, and 60 000 cP with $C = 100$ mM, $\phi = 0.2$, $p_a = 75$ psi, and $N = 6$ when heated and stirred at a temperature $T \approx 60$ °C. The exponential form in equation 1 provides reasonable fits to the data. The characteristic time scale $t_0 = 3.0$ h and the initial rate $m_i = -7.7$ nm/h, averaged over three experiments, are independent of the viscosity of L2 within experimental error. In addition, the final mean radius corresponds exactly to what would be expected by volume conservation within experimental error. Therefore, whereas L2 viscosity affects the emulsification process and therefore the initial droplet size, it does not appear to have much influence on the rate of droplet size reduction during the evaporation process. One would anticipate that the initial droplet radius

becomes larger as the L2 viscosity is increased because a higher dispersed phase viscosity provides more resistance against droplet rupturing.⁴¹ Furthermore, we have made size-reduced nanoemulsions having an even larger range of L2 PDMS viscosities to show that it is possible to obtain very small nanodroplets using much higher-molecular-weight oils. After emulsifying with $C = 100$ mM SDS, $\phi_1 = 0.2$, $\psi_{2,i} = 0.1$, $p_a = 75$ psi, and $N = 6$ over a very broad range of L2 viscosities up to 10^6 cSt, we still achieve very fine nanoemulsions, all of which have $\langle a_f \rangle < 26$ nm.

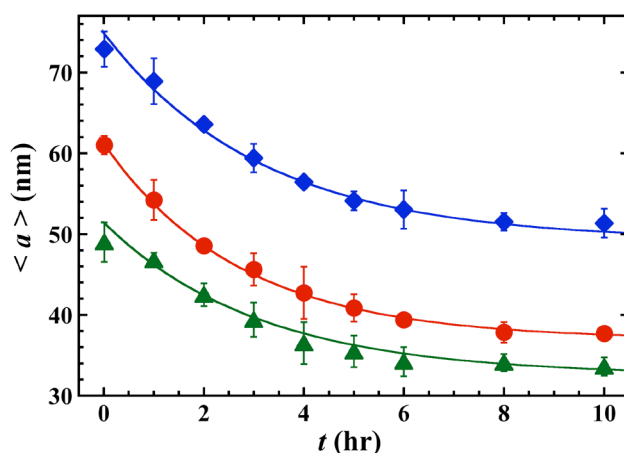


Figure 3.6. Average droplet radius, $\langle a \rangle$, as a function of time, t , for different L2 = 100 cSt (▲), 1,000 cP (●), and 60,000 cP (◆) emulsified at $\psi_{2,i} = 0.3$. Bars denote standard deviations based on three separate trials. Lines: fits to equation 1. Emulsification conditions: $p_a = 75$ psi, $C = 100$ mM, $\phi = 0.2$, $N = 6$. Figure reprinted with permission by Reference 42, copyright 2010 by the American Chemical Society.

3.5 Discussion

We have shown that combining a two-component dispersed phase with evaporation and aqueous continuous phase replenishment removes essentially all L1 from an emulsion. This process has several advantages: using a nonvolatile oil species mixed with solvent in the dispersed phase reduces its viscosity, yielding smaller droplets, stirring inhibits creaming due to Ostwald ripening, and evaporating removes any residual L1 left in the droplets that would tend to

remain because of entropy of mixing. When the osmotic pressure, due to L2 inside the droplets (which inhibits L1 from leaving), is at least as large as the driving interfacial Laplace pressure forcing L1 molecules into the aqueous continuous phase, $2\sigma/a \leq N_2 k_B T / V_d$, where N_2 is the number of molecules of L2 in a droplet, k_B is Boltzmann's constant, and V_d is the volume of a droplet, then the droplets will no longer Ostwald ripen. Indeed, this has been shown to be true in the general sense for a polydisperse emulsion with varying N_2 in each droplet.³⁵ This stability due to entropy of mixing may be overcome by evaporation because evaporating L1 that is in the aqueous continuous phase acts as a sink of L1 from the droplets, thereby effectively raising the osmotic pressure of L2 inside the droplets without bound, and drawing out the remaining L1.

By varying a combination of $\psi_{2,i}$, C , p_a , and N , we can create nanoemulsions having very small $\langle a_f \rangle$ over a desired range. The final mean radius may be predicted using volume conservation based solely on the initial radius and $\psi_{2,i}$. Volume conservation would require $\psi_{2,i} = (1/n)^3$ for a reduction in radius by a factor of n . In cases when Ostwald ripening occurs so rapidly after emulsification that accurate initial sizes are difficult to obtain (*i.e.*, $\psi_{2,i} < 0.2$), it is still possible to estimate $\langle a_{i,p} \rangle$ using $\langle a_f \rangle$ and assuming volume conservation.

Because large volumes of L1 compared with L2 are typically needed to obtain significant droplet size reduction, it would be desirable in terms of reducing cost and environmental impact to recover L1 and reuse it. Therefore, we use a distillation apparatus to condense the evaporated L1, thereby creating a green process that recovers and recycles L1 so that it can be reused in subsequent emulsifications by the same evaporative process. We have demonstrated this for relatively small 20 mL volumes with a recovery of L1 after separation from any immiscible water condensate above 80%, and higher recovery efficiencies above 90% could be achieved by simple improvements to the distillation apparatus. Therefore, substantial quantities of solvent

need not be wasted and discharged to achieve noticeable droplet size reduction by an evaporative process. This green process is scalable and could be used in a large-scale industrial emulsion manufacturing process.

Although the empirical equation we have used to describe the droplet size evolution provides a reasonable fit to our measurements over a fairly wide range of compositions and conditions, we know of no microscopic theory that predicts this form. A predictive theory, or at least complex modeling, is still needed to determine the origin of $\langle a(t) \rangle$ for this multi-phase convection process. Such a theory or complex model could provide a basic understanding of the observed quadratic dependence of $|d\langle a \rangle/dt|_i$ on $\psi_{2,i}$.

Smaller sizes beyond the limit for a given nanoemulsion system using a HeNe $\lambda = 633$ nm laser at $\theta = 90^\circ$ could be measured by changing the laser wavelength and θ . Additionally, alternative experimental techniques, such as X-ray or neutron scattering, could be used.

3.6 Conclusion

We have used evaporative ripening and high-flow rate emulsification to make extremely fine O/W nanoemulsions having sizes that approach micellar dimensions with a wide range of dispersed phase viscosities. Since a significant volume of solvent could be lost through evaporative droplet size reduction, we show recovery and reuse of the solvent by distillation, making this an environmentally friendly and cost-effective process.

Chapter 4

Nanoinclusions in Cryogenically Quenched Nanoemulsions

4.1 Abstract

Nanodroplets containing mixtures of silicone oil and squalene are dispersed in a simple aqueous surfactant solution, quenched in liquid ethane, and examined using cryogenic transmission electron microscopy (CTEM). Depending on the phase of ice that forms around the nanodroplets and on the composition of the oil mixture, nanoinclusions can be observed inside oil nanodroplets, independent of surfactant type. Our observations suggest that these nanoinclusions arise from nucleation of vapor cavities as the water freezes and expands while the oil remains liquid during the quench.

4.2 Introduction

Transmission electron microscopy (TEM) is a powerful technique that enables direct real-space imaging of small molecules,⁹⁰ particles,⁹¹ and assemblies.⁹² In classic TEM, the solvent, usually water, must first be evaporated before imaging, and stains containing heavy elements, such as uranyl acetate, are typically used to enhance contrast. By contrast, in cryogenic TEM (CTEM), the solvent is not evaporated; instead, a thin (typically < 300 nm) layer of water containing the objects to be imaged is quickly frozen by rapidly quenching the temperature, usually without any stain.⁹³ A desired outcome of this fast quench is vitrification of the water into amorphous ice, so nanoscale objects embedded in the ice can be imaged in an environment in which the water molecules remain disordered, reminiscent of water's structure at room temperature.⁹⁴ Because CTEM provides images of objects in an environment that can closely mimic how they appear at room temperature, it is a popular technique across a broad range of

disciplines, especially for biomolecules⁹⁵ and colloidal drug delivery systems.⁹⁶ CTEM has been used to study nanoparticles,⁹⁷ quantum dots,⁹⁸ virus morphologies,⁹⁹⁻¹⁰¹ anisotropy of self-associating RNA,¹⁰² vesicle morphologies,¹⁰³ vesicle size,¹⁰⁴ peptide hydrogels,¹⁰⁵ block copolymers,¹⁰⁶ phase transition dynamics,¹⁰⁷ microemulsion phases,¹⁰⁸ and many other self-assembled systems.¹⁰⁹⁻¹¹¹

Although CTEM is routinely used to study biological soft matter, the application of CTEM to nanostructured multi-fluid phase systems remains an emerging frontier. Nanoemulsions, sub-100nm dispersions of liquid droplets in another immiscible liquid, are one of the most important multi-phase systems.¹⁰ Whether oil nanodroplets are dispersed in water to form an oil-in-water (O/W) nanoemulsion or water nanodroplets are dispersed in oil to form a water-in-oil (W/O) nanoemulsion, nanoemulsions have industrial potential because they can exhibit desirable material properties (*e.g.* optical and mechanical) compared to their microscale counterparts, and monodisperse nanoemulsions provide a good scientific platform for studying soft matter composed of deformable objects.¹¹² Droplet size, structure, and morphology of nanoemulsions have been studied using scattering techniques, including dynamic light scattering⁴¹ and neutron scattering.^{113,114} Beyond scattering, O/W nanoemulsions have been probed by real-space imaging techniques, such as cryogenic scanning electron microscopy (CSEM), freeze-fracture scanning electron microscopy (FF-SEM), and freeze-fracture transmission electron microscopy (FF-TEM), in addition to CTEM.^{63,112} Although CTEM has been used to a limited degree to study nanoemulsions, such as complex formulations for personal care applications¹¹⁵ and formulations stabilized by molecularly-engineered diblock copolypeptides,²² a thorough CTEM study of a simple model O/W nanoemulsion system stabilized by a well-known surfactant is still lacking.

Water-in-oil-in-water (W/O/W) double nanoemulsions have been reported as an interpretation of CTEM observations, following temperature quenching, of emulsified silicone oils in aqueous solutions of certain single-component hydrophilic-hydrophobic diblock copolypeptides (DBC).²² This result, especially the aspect of a single-component stabilizer, is surprising, because creating long-lived double emulsions, regardless of the droplet size scale, usually requires stabilizing the outer water-oil interface using one type of surfactant while also stabilizing the inner oil-water interface using a different type of surfactant.¹¹⁶ Among possible mechanisms for stabilizing both interfaces using single-component DBCs, it has been conjectured that oil-phase intra- and inter-molecular hydrogen bonding of DBCs, enabled by the secondary structure of a racemic hydrophobic block, is the mechanism that leads to the formation of stable double W/O/W nanoemulsions.²² This conjecture, while possible, is not the only potential explanation for the internal structures within oil nanodroplets observed in CTEM images of DBC-stabilized nanoemulsions. In addition, DBC-stabilized W/O/W nanoemulsions made using polydimethylsiloxane (PDMS) oil are reported to exhibit a surprisingly narrow distribution of the ratio of the inner water nanodroplet radius to the outer oil nanodroplet radius, regardless of the method of emulsification. Moreover, only a single internal water nanodroplet inside each oil nanodroplet is typically observed for PDMS oil and most types of DBC stabilizers. From the perspective of emulsion science, these are quite unusual features, since one would expect different emulsification methods to yield a wide range of different nanodroplet sizes and a greater variation in the numbers of internal water nanodroplets. Thus, the nature of the internal nanoscale structures within oil nanodroplets in CTEM images remains mysterious, and further experiments on nanoemulsions using CTEM are necessary.

Here, we show that cavity-like nanoinclusions form as internal structures inside oil nanodroplets in common O/W nanoemulsions stabilized by simple anionic surfactants that have been cryogenically quenched for CTEM observation. We find that the appearance of the nanoinclusions depends on the physical properties of the oil in the nanodroplets and ice that forms during the temperature quench and is independent of the type of surfactant. Nanodroplets surrounded by vitreous ice do not contain nanoinclusions, whereas nanodroplets embedded in other phases of ice that are less dense, such as cubic or hexagonal ice, may or may not contain nanoinclusions, in a systematic manner that depends on the type of oil. These nanoinclusions are most likely internal bubbles of low-density vapor that have been nucleated in the oil nanodroplets during the cryogenic temperature quench as the water expands into frozen ice, yet the dispersed oil phase does not freeze as rapidly and can remain liquid for a longer period of time before ultimately solidifying. A simple relative thermal expansion model is presented, and this model provides estimates of the sizes of the nanoinclusions within oil nanodroplets that are in reasonable agreement with our observations.

4.3 Experimental Procedure

We fabricate simple O/W nanoemulsions in which the oil phase is a mixture of two miscible oils at room temperature (23 °C) as follows. The first oil is 10 cSt (*i.e.* average molecular weight $M_w \approx 1,250$ g/mol) polydimethylsiloxane (PDMS), having a glass transition temperature of $T_g \approx -130$ °C,¹¹⁷ and the second oil is squalene, having a freezing point of $T_f \approx -75$ °C. Prior to emulsification, we mix these two oils at varying oil mixing volume fractions $\psi = V_{\text{PDMS}}/(V_{\text{PDMS}} + V_{\text{sq}})$ where V_{PDMS} and V_{sq} are the volumes of PDMS and squalene, respectively. Thus, at $\psi = 0$, the oil mixture is pure squalene, and at $\psi = 1$, it is pure PDMS. The oil mixture is

then added to an aqueous sodium dodecyl sulfate (SDS) solution having $C_{\text{SDS}} = 10 \text{ mM}$ while stirring with a rotary mixer to form a microscale O/W premix emulsion having an oil volume fraction of $\phi = 0.05$. Nanoemulsions are then formed by subjecting the premix emulsion to extreme flows using a high-pressure microfluidic homogenizer⁴¹ at a liquid pressure $p_1 \approx 18,000 \text{ psi}$ (Y channel; $75 \text{ }\mu\text{m}$ width), causing the microscale droplets to be ruptured into nanoscale droplets. To reduce the droplet size distribution and make it more uniform, we pass the emulsion a total of $N = 6$ times through the homogenizer. An ice bath and cooling coil are used to dissipate viscous heating, so the nanoemulsion's ϕ does not change significantly as a result of water evaporation which can otherwise occur. After emulsification, the nanoemulsions are diluted by a factor of two to eliminate SDS micelles, since the critical micelle concentration of SDS is $C_{\text{SDS}}^* \approx 8 \text{ mM}$.

To prepare grids of frozen nanoemulsions for CTEM, we follow a standard procedure.⁶³ Equipment used for sample preparation and imaging is shown in Figures 4.1a-d. First, a grid holder receptacle and a cryogen cup are placed in a Styrofoam box. The box is then filled to the top of the cryogen cup with liquid nitrogen and allowed to equilibrate. Next, ethane gas is bubbled into the cup where it condenses into a liquid. After the liquid ethane reaches $T_f \approx -180 \text{ }^\circ\text{C}$, a tweezer holding a grid is then clamped vertically into a magnetically controlled plunger arm directly over the liquid ethane. A nanoemulsion volume of $5 \text{ }\mu\text{L}$ is placed on a Quantifoil R 2/1 grid and manually blotted using Whatman 1 qualitative filter paper for three seconds before the magnetic contact is released, and the loaded grid is gravity-plunged into the liquid ethane. The prepared grid is transferred to a grid storage device and stored in liquid nitrogen. When ready to image, a grid is transferred to an FEI Tecnai G² TF 20 transmission electron microscope

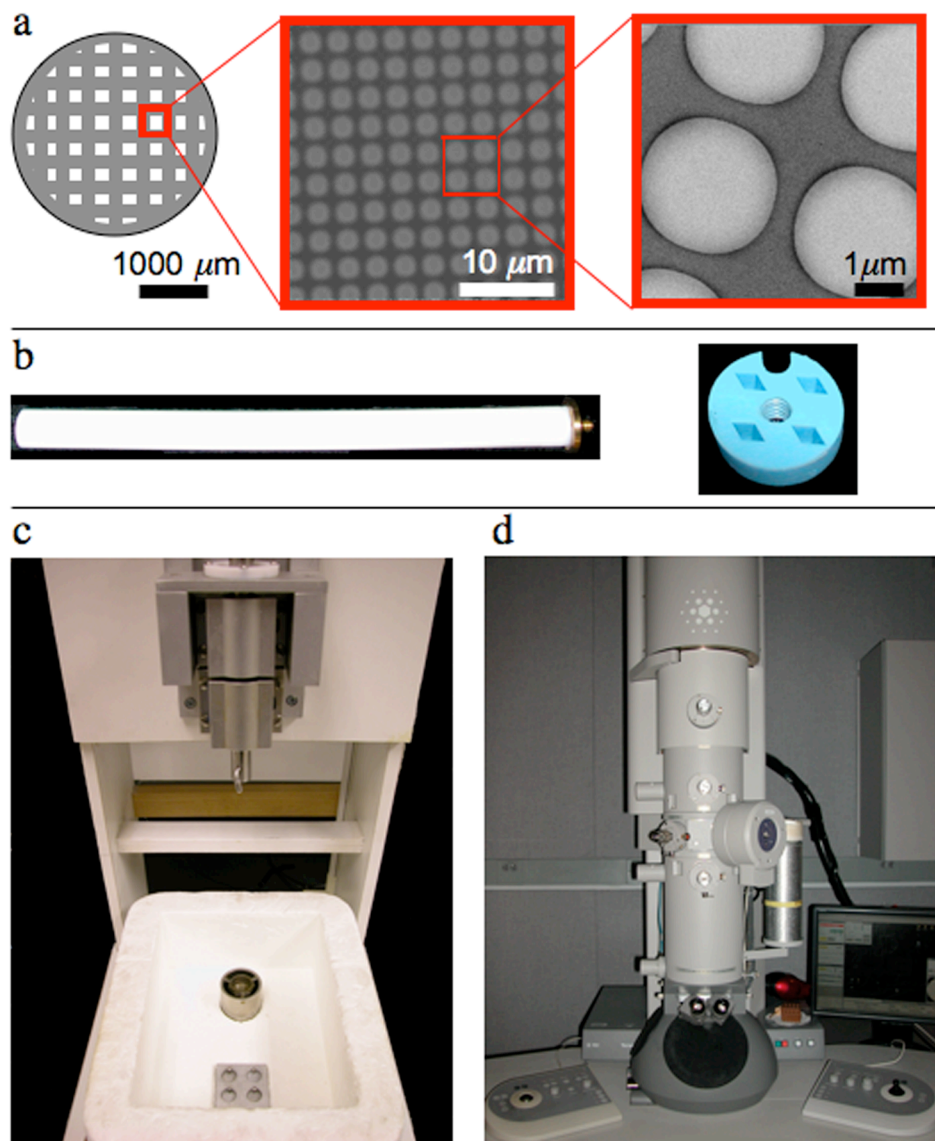


Figure 4.1. Equipment used for cryo-transmission electron microscopy (CTEM). (a) Magnified from left to right: schematic, optical microscopy micrograph, and TEM micrograph of a circular-hole R 2/1 Quantifoil transmission electron microscopy grid. (b) A cryogenic grid holder. The stem shown on the left screws into the top of the grid holder on the right, where each diamond-shaped slot holds one grid. This device is stored in liquid nitrogen until ready for imaging. (c) Gravity-plunging device used to freeze grids. (d) Photograph of a FEI Tecnai G² TF 20 transmission electron microscope.

using a Gatan 626 cryo specimen holder. Images are taken in low-dose mode (typically at < 300 e^-/nm^2 to avoid beam damage) at 200 kV with typical magnifications between 13,000x-15,000x.

4.4 Phases of Ice in CTEM

When water-based materials are quenched and viewed using CTEM, it is possible to observe different phases of ice. Common phases of ice include vitreous (also known as low-density amorphous), cubic, and hexagonal. These ice phases have reported densities between $\rho_v = 0.94\text{-}0.944$ g/cm^3 ($T = 77$ K),^{118,119} $\rho_c = 0.931\text{-}0.943$ ($T = 78$ K),^{119,120} and $\rho_h = 0.92\text{-}0.934$ g/cm^3 ($T = 73\text{-}250$ K),^{118,119,121} respectively, depending on the measurement method and means of ice-phase formation, where average density values imply $\rho_v > \rho_c > \rho_h$. Although the creation and measurement conditions under which these densities are reported do not exactly match the CTEM preparation quench conditions, only small changes in densities between the reported values and the true CTEM quench values are likely to occur. Because these ice phase densities are less than the density of water ($\rho_w \approx 1.0$ g/cm^3 at room temperature), expansion of the sample must occur as the aqueous solvent freezes, where the expansion becomes greater for decreasing ρ . In CTEM, these different ice phases can typically be distinguished in CTEM images by textures or patterns in the ice: amorphous ice is featureless, cubic ice has a speckled pattern, and hexagonal ice has black-streak (*i.e.* ribbon) pattern.^{92,94} Although amorphous ice is typically desired, sometimes the structures of interest in CTEM images are found in other forms of ice.

Standard CTEM sample preparation can yield many different phases of aqueous ice, even on the same grid, depending on the sample thickness, cooling rate, and any additives present. The use of robotics for performing the rapid quench, while helpful, does not remove all variations in the ice structures that can form; such variations can also be a result of the blotting

procedure and quench conditions. Examples of amorphous, cubic, and hexagonal ice phases, in the absence of any nanodroplets (*i.e.* at $\phi = 0$), using our preparation and quenching protocol are shown in Figures 4.2 a-c, respectively, and resemble what have been observed in prior CTEM studies of ice structures.^{92,94}

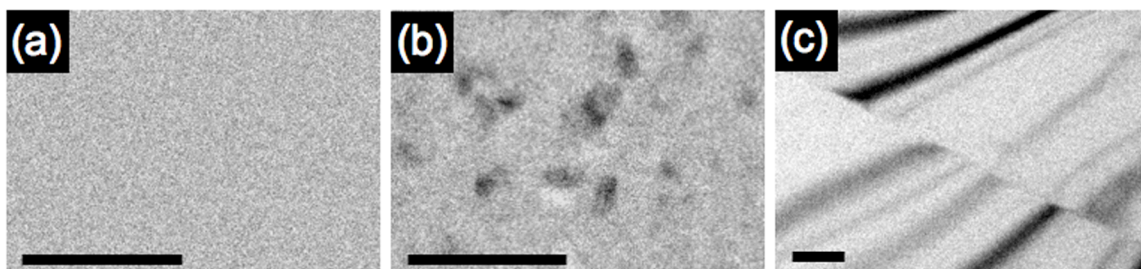


Figure 4.2. Cryo-transmission electron microscopy (CTEM) micrographs of (a) uniform grey appearance of vitreous ice, (b) mottled, speckled appearance of cubic ice, and (c) black-streak (*i.e.* ribbon) appearance of hexagonal ice. Scale bars are 200 nm.

4.5 Results and Analysis

Oil nanodroplets in CTEM images of simple O/W nanoemulsions can exhibit internal structures, depending on the phase of ice that has formed around the droplets. A CTEM micrograph of an SDS-stabilized PDMS O/W nanoemulsion at $\psi = 1$ is shown in Figure 4.3a. The ice in the upper half of the image is vitreous and contains oil nanodroplets that do not exhibit any internal structures; by contrast, the ice in the bottom half of the image is cubic and contains oil nanodroplets, each having a single, near-circular inner structure that is lighter in intensity than both the frozen oil phase and the frozen continuous phase surrounding the nanodroplet under the exact same beam conditions. Because these two different morphologies have been observed under the same beam conditions and the same exposure history, it is unlikely that beam damage (*e.g.* bubbling¹²²) is the source of the near-circular internal structures within PDMS nanodroplets in the cubic ice. Additionally, the imaging is done well below the irradiation at which biological

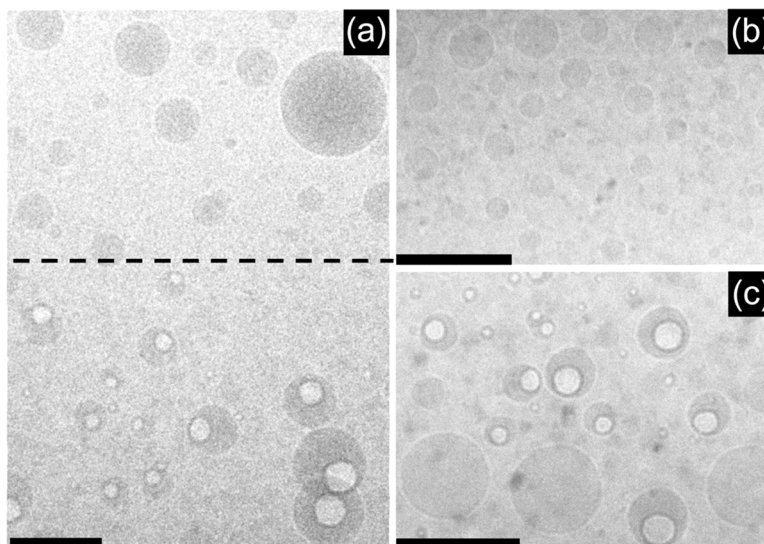


Figure 4.3. (a) CTEM micrograph of a polydimethylsiloxane (PDMS) oil-in-water (O/W) nanoemulsion stabilized by sodium dodecyl sulfate (SDS). Internal structures are not found within dispersed oil nanodroplets in vitreous ice (top region), whereas near-circular internal structures are seen within all oil nanodroplets in cubic ice (bottom region) (dashed line: separates vitreous from cubic ice regions). (b) CTEM micrograph of an SDS-stabilized squalene oil-in-water nanoemulsion in cubic ice. No internal structures are observed within the squalene nanodroplets. (c) CTEM micrograph of 1:1 volume mixture of a PDMS nanoemulsion with a squalene nanoemulsion in cubic ice. PDMS nanodroplets all have bright, near-circular internal structures, whereas squalene nanodroplets do not. All nanoemulsions (a-c) have SDS concentration $C_{\text{SDS}} = 5$ mM. Scale bars are 200 nm.

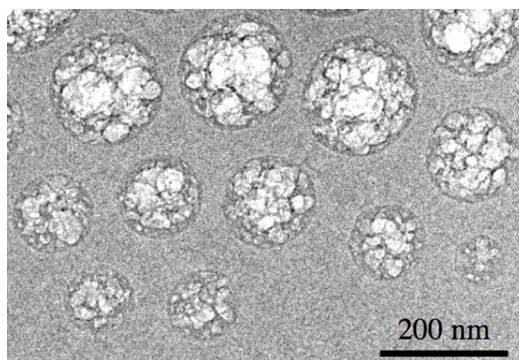


Figure 4.4. CTEM micrograph of bubbling in a SDS-stabilized ($C_{\text{SDS}} = 5$ mM) dodecane O/W nanoemulsion at ≈ 1000 e^-/nm^2 .

specimens begin to exhibit bubbling, namely, at total exposures of $\approx 2\text{-}10\text{ ke}^-/\text{nm}^2$.⁹⁴ An example of bubbling in dodecane nanoemulsions having $C_{\text{SDS}} = 5\text{ mM}$ is shown in Figure 4.4.

To investigate if droplet internal structures may be seen with other dispersed phase liquids, we have examined O/W nanoemulsions made using other oil types, maintaining the same surfactant concentration, emulsification conditions, and CTEM sample preparation protocol. In cubic ice, we routinely observe single internal structures within nanodroplets of simple viscous PDMS oils having $1,250\text{ g/mol} \leq M_w \leq 28,000\text{ g/mol}$. However, we do not observe internal structures in nanodroplets containing pure squalene, polyoctylmethyilsiloxane (POMS, supplied by Gelest), or soybean oil. As an example, an SDS-stabilized O/W nanoemulsion made using pure squalene, using the same conditions as for PDMS, is shown in Figure 4.3b. Although the squalene nanodroplets are in cubic ice, they do not exhibit any lighter, near-circular internal structures.

To show that the sample preparation, vitrification process, grid transfer, and beam conditions do not influence these results, equal volumes of an SDS-stabilized PDMS O/W nanoemulsion at $\psi = 1$ and an SDS-stabilized squalene O/W nanoemulsion at $\psi = 0$, each formed separately using the same emulsification protocol, have been mixed, loaded onto the CTEM grid, and quenched. A micrograph of the mixed PDMS nanoemulsion and squalene nanoemulsion in cubic ice is shown in Figure 4.3c. Since PDMS (which contains silicon) has a higher density of atomic number than squalene, PDMS nanodroplets appear darker than squalene nanodroplets of the same size in CTEM images taken using the same exposure conditions, provided the ice thickness is also about the same. Therefore, we can quantitatively differentiate PDMS nanodroplets from nearby squalene nanodroplets in a single CTEM image using intensity and size information. Based on this, each darker PDMS nanodroplet in Figure 4.3c contains a bright

near-circular internal structure, whereas each lighter squalene nanodroplet does not contain any internal structures.

Additionally, for PDMS O/W nanoemulsions formed by emulsification using other types of surfactants, such as polystyrene-*b*-poly(acrylic acid) and polyethylene oxide-*b*-polypropylene oxide copolymers, we find that the CTEM images of nanodroplets in amorphous and cubic ice appear to be the same as for SDS; no internal structures are found in amorphous ice and predominantly single, near-circular, internal structures are found in cubic ice. Thus, the type of surfactant appears to have little influence on the appearance of PDMS oil nanodroplets.

In cubic ice, PDMS nanodroplets and squalene nanodroplets show either the presence or the absence, respectively, of internal structures within oil nanodroplets for SDS-stabilized nanoemulsions. Because these two oils are miscible at room temperature, we focus on SDS-stabilized O/W nanoemulsions containing oil droplets that have mixtures of PDMS and squalene. We systematically examine the structure inside nanodroplets over a wide range of ψ in both vitreous and cubic ice. Representative micrographs showing typical number and size of internal structures as a function of ψ are shown in Figure 4.5. The overall background intensities in these images have been adjusted to be the same while maintaining the relative contrast between nanodroplets and the frozen continuous phase. In vitreous ice, no internal structures are observed within nanodroplets, independent of ψ . However, in cubic ice, both the number and size of internal structures can vary dramatically as ψ ranges from 0 to 1.

To quantify the frequency at which internal structures appear within oil nanodroplets surrounded by cubic ice in the CTEM images, we count the number of internal structures N_i within a given nanodroplet, where the subscript *i* means internal, for all nanodroplets observed at a given ψ . We plot the normalized probability density (*i.e.* distribution) $p(N_i, \psi)$ in Figure 4.6. At

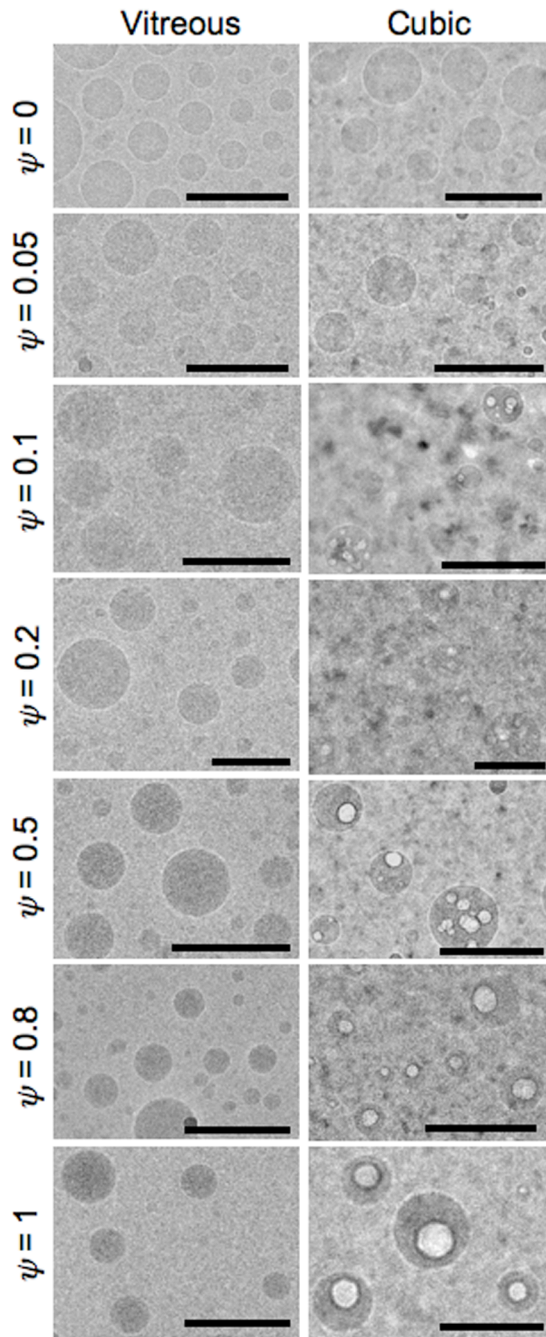


Figure 4.5. CT-TEM micrographs of SDS-stabilized O/W nanoemulsion droplets found in vitreous (left column) and cubic ice (right column). The oil inside nanodroplets is a mixture of PDMS and squalene. PDMS oil mixture volume fractions, $\psi = V_{\text{PDMS}}/(V_{\text{PDMS}} + V_{\text{sq}})$, in the dispersed oil nanodroplets are: 0, 0.05, 0.10, 0.20, 0.50, 0.80, and 1. The SDS concentration is fixed: $C_{\text{SDS}} = 5$ mM. Scale bars are 200 nm.

least 100 droplets have been analyzed for the distributions at each ψ . As ψ increases, the probability of finding a nanodroplet absent of any internal structures decreases from nearly 100% at $\psi = 0$ to nearly 0% at $\psi = 1$, whereas the probability of finding a droplet having one internal structure increases from nearly 0% at $\psi = 0$ to nearly 100% at $\psi = 1$. The probability crossover, where the probability of finding one internal structure is equal to the probability of finding no internal structures, occurs near $\psi_c \approx 0.1$. Although the most probable N_i for all ψ is either zero or one, two or more internal structures can also be commonly observed, especially for $0.1 < \psi < 0.5$.

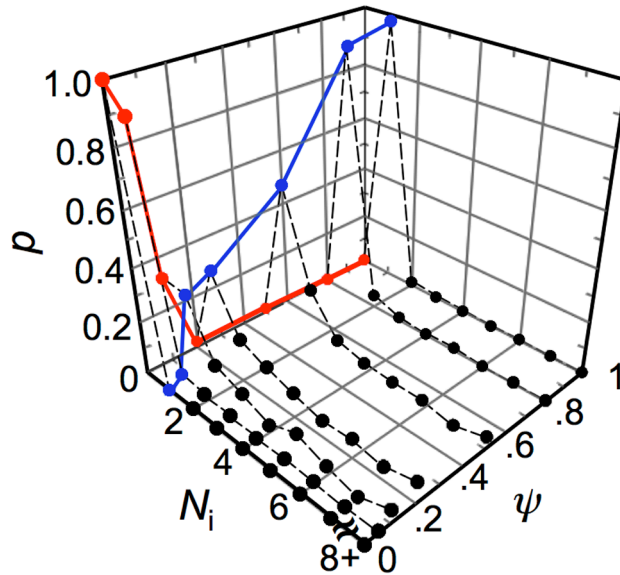


Figure 4.6. Normalized probability distributions p of observing N_i internal structures within a nanodroplet containing a PDMS-squalene oil mixture at a given ψ found in cubic ice, as observed using CTEM. Solid lines show probability trends for zero internal structures $N_i = 0$ (red) and for one internal structure $N_i = 1$ (blue). Dashed lines guide the eye at different fixed ψ . At least 100 droplets have been analyzed to provide distributions at each ψ . For all observations, the SDS concentration in the aqueous continuous phase is fixed at $C_{\text{SDS}} = 5$ mM.

Next, we quantify the size of the nanoinclusions as a function of ψ . Individual micrographs provide 2D images of nanodroplets in projection. Since nanodroplets are 3D objects, we choose to use volume as an appropriate measure of size. Although nanodroplets in

2D appear nearly circular and their volumes are easy to obtain, not all of their internal structures are nearly circular. So, we measure a major axis a and minor axis b of each internal structure, and we assume a prolate spheroid model to compute the volume $4\pi ab^2/3$ of each nanoinclusion. A prolate model was chosen over an oblate model because a prolate model leads to a smaller, and therefore more energetically favorable, internal droplet surface area. The median minor to major axis quotient b/a ranges from about 0.80 at lower $\psi \approx 0.1$ to 0.96 at higher $\psi \approx 1$. The dimensionless median quotient of the total internal structure volume divided by the total droplet volume V_i/V_d is plotted as a function of ψ in Figure 4.7. The median ratio is chosen over the average ratio, since distributions are not always peaked for all ψ . Our V_i/V_d results at $\psi = 1$ correspond well to the peak distribution value shown for PDMS nanoemulsions stabilized by diblock copolypeptides.²²

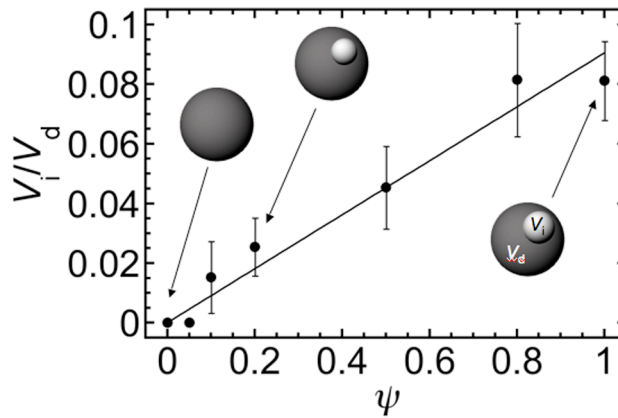


Figure 4.7. Median volume ratio, defined as the total internal structure volume divided by the total droplet volume, V_i/V_d , as a function of ψ in cubic ice for SDS-stabilized oil-in-water nanoemulsions as observed using CTEM. V_i is computed using a prolate spheroid model (see text). Error bars represent the median absolute deviation. Solid line: linear fit to $V_i/V_d = b\psi$, yielding slope $b = 0.090 \pm 0.005$.

The internal structures observed within predominantly PDMS nanodroplets in cubic ice are unlikely to be a result of emulsification using a special stabilizer. PDMS nanoemulsions

formed using a high hydrophilic-lipophilic balance (HLB) surfactant, such as SDS, are routinely known to be single O/W nanoemulsions, not double W/O/W nanoemulsions. At the concentrations we use, SDS by itself does not facilitate the formation of double W/O/W nanoemulsions because, as a high HLB surfactant, SDS cannot stabilize the inner droplet W/O interface, according to the Bancroft rule.³¹ Previous small angle neutron scattering (SANS) results for the form factor of PDMS O/W nanoemulsions stabilized by SDS support this view that the nanoscale oil droplets have no internal structures at room temperature.¹¹³ Thus, it is extremely unlikely that the bright near-circular internal structures, which we routinely observe by performing CTEM on quenched SDS-stabilized PDMS O/W nanoemulsions in non-amorphous ice, are smaller internal water nanodroplets that have been created within oil nanodroplets during the emulsification process.

The existence of internal structures within oil nanodroplets in cubic ice depends strongly on the oil type, so such internal structures most likely result from the temperature quench during CTEM sample preparation. We hypothesize that the internal structures within oil nanodroplets are nanoinclusions (*i.e.* nanocavities or nanobubbles containing a very low density oil vapor phase that is nearly a vacuum). As the liquid aqueous continuous phase freezes into cubic ice, it expands as the density changes in accordance with the phase change. For oils having low freezing points T_f or glass transition temperatures T_g , such as PDMS, it is possible for the oil to remain liquid and not undergo a significant density change as the liquid water becomes ice. In this case, as liquid water, which surrounds an oil nanodroplet having volume V_d , solidifies into cubic ice, the expansion creates a spherical ice chamber having a larger volume $(\rho_w/\rho_c)V_d$, inside which the liquid oil is still enclosed. If the volume increase $(\rho_w/\rho_c - 1)V_d$ is large enough, then stresses created by the expansion during ice solidification can nucleate a vapor cavity. Surface

tension then quickly causes the vapor cavity to become nearly spherical before the oil solidifies. A schematic depicting the hypothesized nucleation of a nanoinclusion during the temperature quench is shown in Figure 4.8. For oils that become solid at higher temperatures that are closer to the freezing point of water (or for oils that would also expand significantly as the temperature is quenched), then no vapor cavity would be seen because the relative volume expansion cannot apply a sufficient stress to the oil rapidly enough to nucleate a vapor cavity. Instead, it is likely that local stresses from the expansion are trapped both in the solid internal droplet network and the nearby ice that are not seen in CTEM. This hypothesis provides a physical mechanism that can explain why nanoinclusions are not observed inside squalene nanodroplets in cubic ice in CTEM images, whereas nanoinclusions are routinely observed inside PDMS nanodroplets in cubic ice.

As we have shown in Figure 4.5, no internal structures are observed in nanodroplets embedded in vitreous ice, independent of the oil type. Low density vitreous ice also has a density less than that of water and a similar mechanism of nanobubble nucleation might be expected. However, it is likely that the relative expansion stresses generated in the formation of cubic ice are sufficient to nucleate bubble-like nanoinclusions within the liquid oil nanodroplets, yet those stresses are not large enough to cause nanobubble nucleation within the oil nanodroplets when more dense amorphous ice is formed. This mechanism further assumes that there exists a critical relative volume expansion between the water and oil that is necessary to nucleate a bubble after the water has frozen but the oil has not. This hypothesis is also consistent with our additional observations that nanoinclusions can be found inside squalene droplets that are found in lower density hexagonal ice but not in higher density cubic ice.

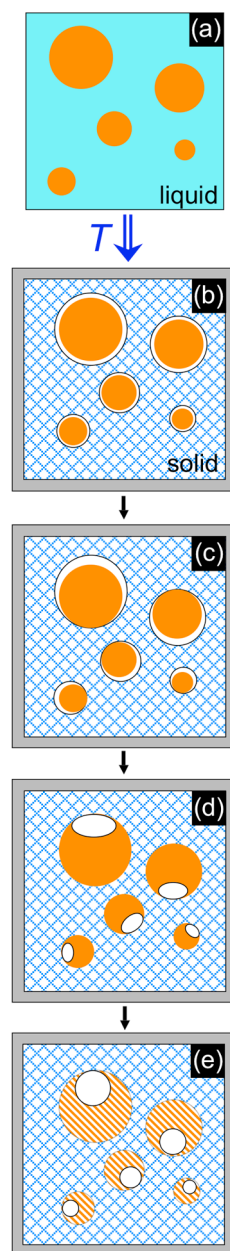


Figure 4.8. Schematic showing the hypothesized evolution of O/W nanoemulsion droplets subjected to a cryogenic temperature quench. As the liquid nanoemulsion (a) is quenched into liquid ethane and the continuous phase becomes solid, the aqueous continuous phase freezes first and expands, and the difference in thermal expansion coefficients between the dispersed phase and the continuous phase can be large enough to lead to the nucleation of a thin vapor cavity around the droplets (b). To minimize surface energy, the vapor shell quickly becomes a vapor bubble (c and d), after which the dispersed phase solidifies (e). light blue = liquid continuous phase, orange = liquid nanodroplets, blue cross-hatching = solid continuous phase, gray = liquid ethane, solid white = nucleated vapor cavity, orange stripes = solid nanodroplets.

Using the proposed ice expansion mechanism, the simplest expectation would be for V_i/V_d to depend linearly on the volume of PDMS in a nanodroplet and therefore ψ . Accordingly, the results in Figure 4.7 are fit to $V_i/V_d = m\psi$ where $m = 0.090 \pm 0.005$ is a dimensionless slope related to the volume expansion of cubic ice relative to pure PDMS at $\psi = 1$. This value for m is in reasonable comparison with the simple ice expansion model that predicts $m_p = \rho_w/\rho_c - 1 \approx 0.08$ using the crystallographically determined density of $\rho_c \approx 0.93$.¹²⁰ Because the simple model does not take into account any density changes of the oil (since this is unknown over the relevant temperature range), the excess relative volume expansion $m - m_p \approx 0.01$, while close to the uncertainty in m , could possibly indicate that there is also a small volume contraction of the PDMS as the temperature is quenched.

When nanodroplets contain a mixture of squalene and PDMS at lower but nonzero ψ , such as $\psi \approx 0.1$, several smaller nanoinclusions are commonly observed (see Fig. 4.5). We speculate that, inside the nanodroplets, squalene may freeze into a squalene-rich solid network first (*e.g.* perhaps by a process resembling spinodal decomposition), so the expansion of the ice can nucleate several cavities in different locations while the PDMS is still liquid. These multiple smaller nanoinclusions, which have a higher average anisotropy and are less circular than a single nanoinclusion at $\psi = 1$, may be hindered by the network from consolidating into a single nanoinclusion at low but nonzero ψ .

4.6 Discussion

The formation of nanoinclusions within certain types of pure oil nanodroplets that have been cryogenically quenched is a complex process. The occurrence of nanoinclusions depends on many factors: the difference in temperature-dependent coefficients of thermal expansion

between the water and the oil, temperature-dependent density changes, freezing points (or glass transition temperatures), ice structure, temperature-dependent heat capacities, and relative rates of solidification of the continuous and dispersed phases. As these factors are not yet understood quantitatively, more studies are needed to deduce the mechanism of nanoinclusion formation. Currently, a theory that would enable an *a priori* prediction of nanoinclusions within nanodroplets is lacking. Developing such a theory, as well as measuring thermodynamic properties of the continuous and dispersed phases down to cryogenic temperatures, could provide criteria that predict if expansion stresses in the water as it solidifies are sufficient to nucleate a vapor nanoinclusion in the oil nanodroplets. For a mixture of two oils, a theory would also have to incorporate the complexity of the time- and temperature-dependent solid network formation of the oil that has a higher solidification temperature. Thus, further theoretical and experimental work would provide additional insight into the formation of the nanoinclusions for a variety of oils and oil mixtures.

The simple expansion model presented here that predicts nanoinclusions inside nanodroplets could have many other implications for samples across a wide range of disciplines examined using CTEM. Extensible samples in water that are not extremely rigid could expand upon freezing, leading to sizes that may appear somewhat larger (*e.g.* 5-10%) than they actually are in water near room temperature. Potential examples include viruses, polymers, and porous particles. Rigid objects that are inextensible (*e.g.* polystyrene spheres), however, are likely to remain similar in size after freezing because of their strong cohesion and relatively small thermal expansion coefficient. Boundary conditions at the interface between the nanoscale object of interest and the solvent can also be important. Additionally, although some O/W nanoemulsions exhibit nanoinclusions, W/O nanoemulsions are unlikely to exhibit nanoinclusions, since most

oils will not solidify and expand considerably and, for most oils used as a continuous phase, the water inside the droplets will typically freeze and expand first during the quench.

Although a nucleated vapor nanoinclusion and a true double nanodroplet may appear similar in micrographs, potential ways of distinguishing between the two exist. For structure interpretation, as in this case, as well as image clarity, only micrographs of samples imbedded in amorphous ice should be used. First, the intensity of the internal structure must be analyzed with respect to the continuous phase nearby. A double nanodroplet's inner droplet intensity would be partway between that of the droplet and the nearby continuous phase because the electrons must pass through the dispersed phase in addition to the inner droplet. A vapor nanoinclusion, however, could have a higher intensity than that of the nearby continuous phase depending on the location of the nanoinclusion relative to the droplet, the phase contrast, and the electron scattering length density of all phases. Digital image analysis of a nanoemulsion in cubic ice showing evidence for a nanoinclusion in a nanodroplet is shown in Figure 4.9. Second, the shape of the inner structure should be considered. The inner droplet of a double nanodroplet should appear spherical unless stresses in the ice warp the entire droplet, but a nanoinclusion may or may not be spherical depending on the dynamic vitrification and droplet solidification process. Finally, cryo-electron tomography (CET), a technique in which micrographs of a single droplet with a nanoinclusion would be taken at a series of tilt angles α and then reconstructed to form a 3D image,¹²³ could potentially be used. The 3D reconstruction would identify the location of the inner structure and the overall morphology of the droplet. The inner droplet of a double nanoemulsion must be completely surrounded by the dispersed phase; however, a vapor nanoinclusion could be in direct contact with the continuous phase. If possible, all three distinguishing methods presented should be used in tandem.

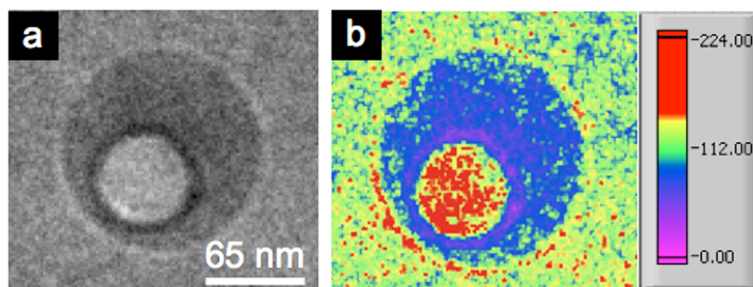


Figure 4.9. (a) Original CTEM micrograph of a PDMS nanodroplet stabilized by $C_{\text{SDS}} = 5 \text{ mM}$ in cubic ice. (b) Same droplet as in (a) with intensity information rescaled by color. The average inner structure intensity is higher than the average intensity of the surrounding ice.

4.7 Conclusion

Our CTEM experiments on simple SDS-stabilized O/W nanoemulsions lead us to conclude that internal structures within PDMS oil nanodroplets surrounded by lower-density forms of ice are nanoinclusions rather than inner water droplets. Thus it could be that PDMS nanodroplets emulsified using certain DBCs really contain nanoinclusions rather than inner water droplets stabilized by hydrogen bonding of racemic hydrophobic segments; however, further experiments on PDMS oils emulsified with both DBCs and simple surfactants such as SDS are needed.

Chapter 5

Controlled Coalescence of Emulsions Using Oppositely Charged Surfactants

5.1 Abstract

Anionically stabilized monodisperse O/W microscale emulsions are mixed in bulk with a cationic surfactant solution having the same surfactant concentration. Depending on the droplet volume fraction ϕ and relative mixing ratios of the two solutions, χ , in which the anionic surfactant remains in excess, varying amounts of droplet coalescence occur before the emulsions are re-stabilized by the unreacted anionic surfactant. By mixing multiple anionically stabilized emulsions, each of which is composed of a mutually immiscible oil, before adding the cationic surfactant solution, this coalescence technique may be extended to form Janus, Cerberus, and higher order anisotropic droplets both at the micro- and nano-scale.

5.2 Introduction

Emulsions are metastable systems of one liquid dispersed in another immiscible liquid. If no repulsive interaction potential exists between two miscible dispersed phase droplets, upon collision they combine to create one larger droplet having a reduced total interfacial area, thereby lowering the total interfacial energy, in a process called coalescence.⁸¹ To prevent this phenomenon from occurring, a surfactant is added to provide either steric or electrostatic repulsion between droplets. However, even emulsions containing a surfactant may exhibit coalescence if too little surfactant or a poor surfactant is used (*e.g.* solid stabilized pickering emulsions^{124,125}). Additionally, coalescence can occur in emulsions by having a high enough osmotic pressure to overcome the disjoining pressure,¹²⁶ flow-inducing coalescence,^{30,127,128}

applying electric fields,^{129,130} changing surfactant HLB,¹³¹ changing pH,¹³² or adding simple salts.¹³² For most applications, such as those in the food industry,¹³³ emulsion stability is desired, so the phenomenon of coalescence and the prevention thereof has been studied experimentally and theoretically for many years.¹³⁴⁻¹³⁹

Coalescence between two droplets each composed of a different immiscible dispersed phase species has been studied as well. When these two droplets come into contact, three possibilities exist depending on the lowest total energy configuration between the three phases (*i.e.* dispersed phase 1, dispersed phase 2, and the continuous phase):¹⁴⁰ 1. No fusion occurs and the droplets remain separate. 2. One droplet engulfs another and a double emulsion is created. 3. Partial engulfing occurs, producing an anisotropic snowman-like droplet. The latter case has been achieved under controlled shear or electrical field conditions,²¹ and more recently in microfluidic devices¹⁹ and through inversion processes.²⁰ Similar to their all-solid anisotropic colloidal particle¹⁴¹ or part solid, part liquid anisotropic counterparts,¹⁴² these liquid anisotropic droplets are intriguing as potential building blocks for new types of materials and for studying interfacial science.

Separate from emulsions, mixed surfactant systems have been studied both experimentally and theoretically as they are often used in applications typically due to their synergistic effects.¹⁴³ In particular, anionic and cationic mixtures have been shown to be useful due to the increase in surface activity beyond that of their individual components.¹⁴⁴ These mixed systems often exhibit rich phase behavior.^{145,146} For example, depending on the relative concentrations of anionic sodium dodecyl sulfate (SDS), cationic dodecyltrimethylammonium bromide, and water, either molecular dispersions, micelles, vesicles, crystal precipitate, or a combination thereof may be observed.¹⁴⁷

Here, we present a self-limiting reaction method in which a certain desired amount of coalescence can be induced in an emulsion before it restabilizes. Anionically stabilized monodisperse emulsions are induced to fuse by adding certain molar amounts of a cationic surfactant solution, however, the fusion reaction does not continue to occur indefinitely. We study the extent of coalescence as a function of volume fraction and relative molar amounts of surfactant. This method is then extended to produce anisotropic droplets upon fusion of two or more immiscible dispersed phase droplets both on the micro- and nano-scale.

5.3 Experimental Procedure

Two monodisperse 84-85 μm average diameter $\langle d \rangle$ soybean oil-in-water (O/W) master emulsions having a SDS concentration of $C_{\text{SDS}} = 50 \text{ mM}$ are first created using flow-focusing in a droplet junction chip by Dolomite Microfluidics as shown in Figure 5.1. Some satellite droplets much smaller than $\langle d \rangle$ are created; those having $d < 40 \mu\text{m}$ are not used for any of the studies herein. Next, the SDS concentration C_{SDS} of both master emulsions is reduced by waiting for the emulsion to cream, removing the continuous phase below the cream, replenishing with a lower C_{SDS} , and repeating until $C_{\text{SDS}} = 5 \text{ mM}$, below the SDS critical micelle concentration (CMC) of $\approx 8 \text{ mM}$ at room temperature. Optical micrographs are then taken of the emulsion and the size distribution of both master emulsions is then characterized using digital image analysis of at least 200 droplets.

The coalescence dependence on both volume fraction ϕ and SDS surfactant mole fraction after mixing $\chi_{\text{SDS}} = n_{\text{SDS}}/(n_{\text{SDS}} + n_{\text{CTAB}})$ where CTAB is cetyltrimmonium bromide is then systematically studied. To study the volume fraction effect at a fixed $\chi_{\text{SDS}} = 0.7$, one of the master emulsions is concentrated down to $\phi = 0.43$. 30 μL of a 5 mM CTAB solution (the CMC

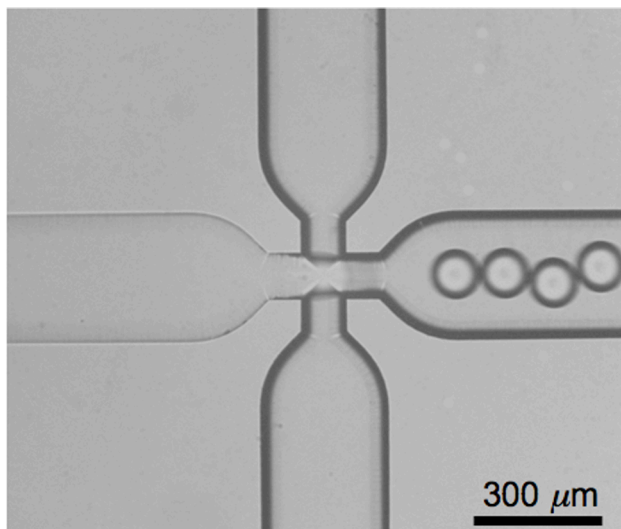


Figure 5.1. Micrograph of a Dolomite Microfluidics droplet junction chip. An aqueous surfactant solution in the two vertical channels flows towards the center, where it flow-focuses the oil coming from the left horizontal channel into a thread that pinches off to produce monodisperse oil-in-water emulsion droplets that are collected downstream.

for CTAB at room temperature is ≈ 0.9 mM) is taken and rapidly mixed with $70\ \mu\text{L}$ of the emulsion while shaking by hand to suppress any gradients in ϕ due to creaming. After addition, we continue to shake by hand for 3-4 seconds as coalescence occurs on the order of seconds. For subsequent experiments at lower ϕ , this master solution is diluted using $C_{\text{SDS}} = 5$ mM until the desired ϕ is reached. After five minutes, $3\text{-}5\ \mu\text{L}$ of the resulting coalesced emulsion is then placed in a microscope slide, 5 mM SDS is added to fill the remaining volume, the slide is sealed, and the size distribution is characterized as in the monodisperse case. Any small aggregates that may form due to a mixed surfactant system¹⁴⁸ are ignored. The other master emulsion is used to study the effect of χ_{SDS} on coalescence at a fixed $\phi = 0.078$.

In order to create two-component anisotropic Janus microscale emulsion droplets composed of two immiscible oils, the following procedure is used. A Fisher Scientific Power Gen 125 mixer is used to create a polydisperse $C_{\text{SDS}} = 5$ mM soybean O/W microscale emulsion

at $\phi = 0.3$ and a separate $C_{\text{SDS}} = 5$ mM polydimethylsiloxane (PDMS) O/W microscale emulsion at $\phi = 0.3$. These two emulsions are mixed using equal volumes of $35 \mu\text{L}$ such that the total oil volume fraction is $\phi = 0.3$, but the individual oil volume fractions are reduced to $\phi_{\text{soybean}} = \phi_{\text{PDMS}} = 0.15$. Next, $30 \mu\text{L}$ of $C_{\text{CTAB}} = 5$ mM solution is added to the mixed emulsion to induce fusion.

In order to create a Janus nanodroplet, a microfluidic homogenizer is used to create a $\phi = 0.05$, $C_{\text{SDS}} = 5$ mM PDMS O/W nanoemulsion and a separate $\phi = 0.05$, $C_{\text{SDS}} = 5$ mM soybean O/W nanoemulsion using the procedures discussed in Chapter 3. These two nanoemulsions are then mixed at equal volumes and fused at $\chi_{\text{SDS}} = 0.7$ before freezing and viewing using the procedures for CTEM discussed in Chapter 4.

To create anisotropic Cerberus droplets composed of three mutually immiscible oils, an Ika mixer is used to create three individual O/W microscale emulsions using poly(2-phenylpropyl)methylsiloxane (PPPMS), polytetradecylmethylsiloxane (PTDMS), and PDMS (one for each individual emulsion) at $C_{\text{SDS}} = 5$ mM and $\phi = 0.4$. All three are mixed using equal volumes of $23.3 \mu\text{L}$ such that the total oil volume fraction is $\phi = 0.4$, but the individual oil volume fractions are reduced to $\phi_{\text{PPPMS}} = \phi_{\text{PTDMS}} = \phi_{\text{PDMS}} = 0.13$. $30 \mu\text{L}$ of a CTAB solution at $C_{\text{CTAB}} = 5$ mM is then added to fuse them.

5.4 Results and Analysis

5.4.1 Coalescence of Miscible Oils

We first study the dependence of coalescence on ϕ for a fixed $\chi_{\text{SDS}} = 0.7$. At this χ_{SDS} an appreciable amount of coalescence can occur without any observable macroscopic phase separation. The normalized probability distribution of finding a given dimensionless diameter ratio d/d_0 , where d_0 is the initial monodisperse average diameter, at a specific ϕ is plotted in

Figure 5.2. The volume fraction at $\phi = 0$ represents the initial monodisperse emulsion; it represents the case where the emulsion is so dilute no droplets coalesce upon addition of the CTAB solution. Distinct peaks are able to be resolved in each distribution corresponding to the number of monodisperse droplets that have fused; if N droplets have fused, then a peak at $d/d_0 = N^{1/3}$ will appear. Thus the peaks occur at $d/d_0 \approx 1, 1.26, 1.44, 1.59$, and 1.71 for 1, 2, 3, 4, and 5 droplets fusing, respectively. At a fixed ϕ , the area under each successive peak $d/d_0 \geq 1$ decreases, which shows the probability of N droplets fusing decreases with increasing N . As ϕ increases, the area under the peak at $d/d_0 = 1$ decreases, while the area under the peaks at $d/d_0 > 1$ increase indicating that more coalescence occurs at higher ϕ . At $\phi = 0.4$, macroscopic phase separation of the vegetable oil is seen; however, a lower ϕ emulsion still remains underneath, the analysis of which is shown in Figure 5.2.

Next, the effect of χ_{SDS} on coalescence at a fixed $\phi = 0.078$ is examined. At this ϕ an appreciable amount of coalescence can occur without any perceptible oil breakout. The normalized probability distribution of finding a given d/d_0 at a specific χ_{SDS} is plotted in Figure 5.3. The $\chi_{\text{SDS}} = 1$ represents the initial monodisperse emulsion as no CTAB solution was added to cause coalescence. Trends similar to those in Figure 5.2 are again seen, namely, peaks at distinct values of d/d_0 exist because of the discrete nature of the number of coalesced monodisperse droplets and the probability of N droplets coalescing decreases with increasing N at a fixed χ_{SDS} . As χ_{SDS} decreases, the peaks at $d/d_0 = 1$ show a decreasing trend, while peaks at $d/d_0 > 1$ exhibit an increasing trend; more coalescence occurs with additional CTAB solution added. Even though trends appear, the effect of any surfactant phase changes (*e.g.* crystal or vesicle formation), which may occur upon addition of CTAB, on coalescence remains unknown.

At $\chi_{\text{SDS}} = 0.6$, a thin film of oil breakout appears at the top of the mixture; however, a lower ϕ emulsion still remains underneath, the analysis of which is shown in Figure 5.3.

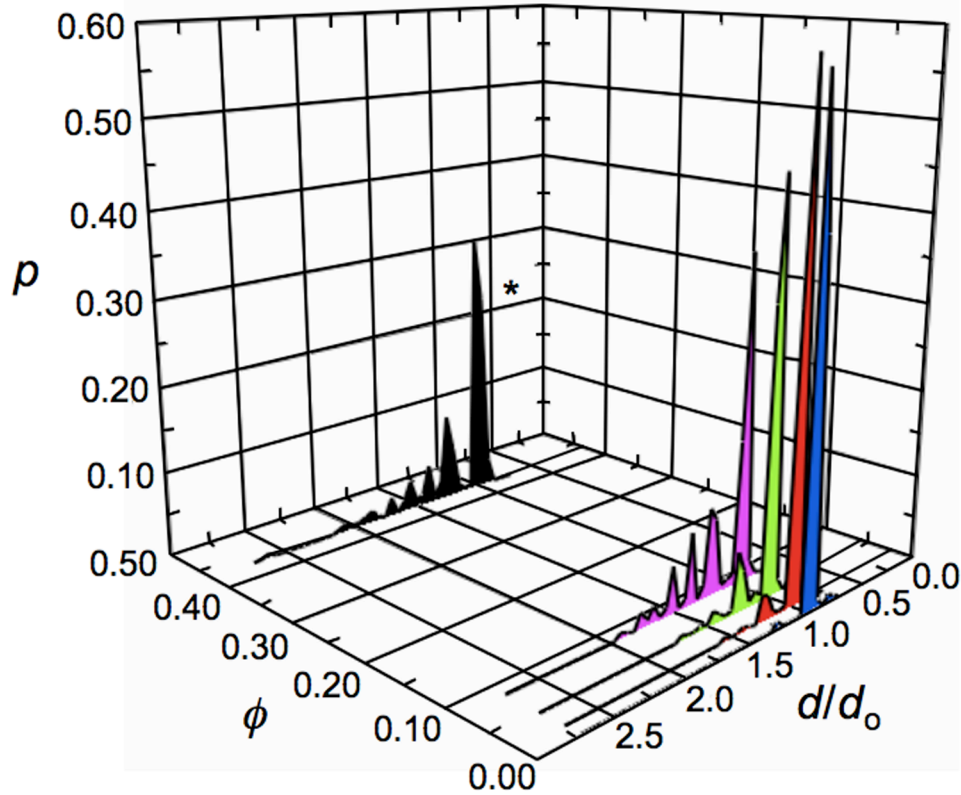


Figure 5.2. Number-weighted normalized probability distribution p as a function of the dimensionless diameter ratio d/d_0 at volume fractions $\phi = 0, 0.017, 0.043, 0.082$, and 0.43 after coalescence of a $C_{\text{SDS}} = 5$ mM soybean oil emulsion induced by mixing with $C_{\text{CTAB}} = 5$ mM surfactant solution at constant SDS-to-CTAB mole mixing fraction $\chi_{\text{SDS}} = n_{\text{SDS}}/(n_{\text{SDS}} + n_{\text{CTAB}}) = 0.7$. The data at $\phi = 0$ represents the initial monodisperse size distribution. * at $\phi = 0.43$ indicates macroscopic phase separation of oil, where the probability is normalized to the lower ϕ emulsion droplets underneath. If the rest of the oil volume associated with the breakout was accounted for, all peaks at $\phi = 0.43$ would be reduced.

5.4.2 Coalescence of Immiscible Oils

We now extend this coalescence technique to fuse multiple droplets composed of different mutually immiscible oils and explore the morphological possibilities thereof. Figures 5a-e show the majority of the morphology types when a soybean oil emulsion has been mixed

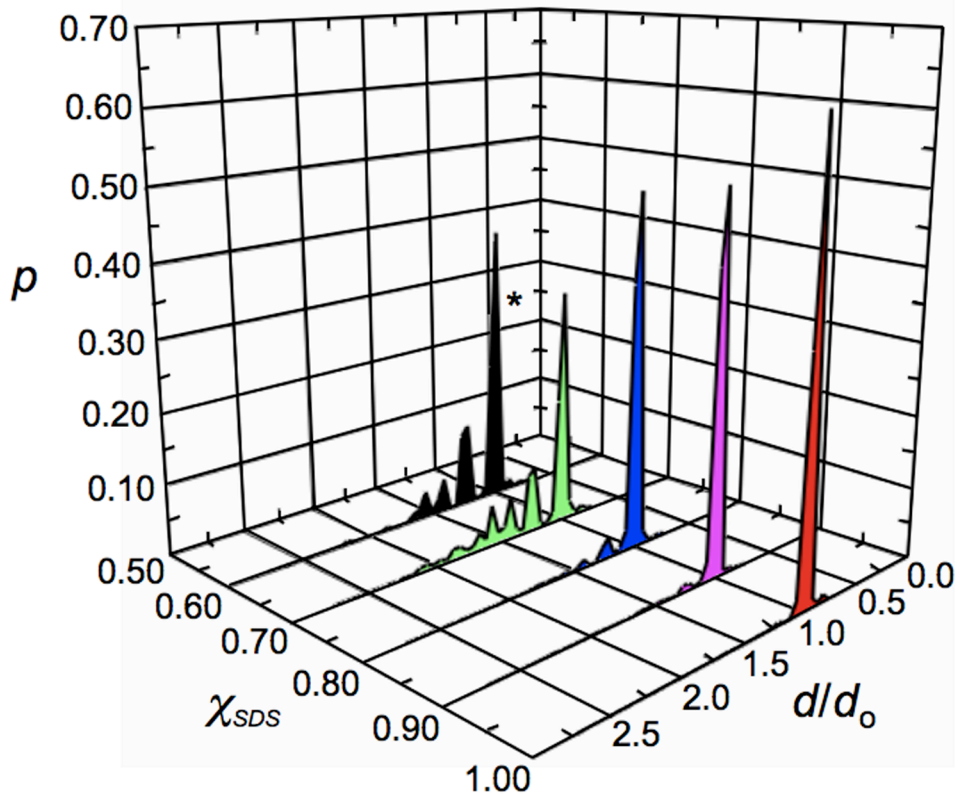


Figure 5.3. Number-weighted normalized probability distribution p as a function of the dimensionless diameter ratio d/d_o at $\chi_{\text{SDS}} = 0.60, 0.70, 0.80, 0.90$, and 1 after coalescence of a $C_{\text{SDS}} = 5$ mM soybean oil emulsion induced by mixing with $C_{\text{CTAB}} = 5$ mM at constant $\phi = 0.078$. The data at $\chi_{\text{SDS}} = 1$ represents the initial monodisperse size distribution. * at $\chi_{\text{SDS}} = 0.60$ indicates a thin layer of oil breakout, where the probability shown is normalized to the lower ϕ emulsion droplets underneath. If the rest of the oil volume associated with the breakout would be accounted for, all peaks at $\chi_{\text{SDS}} = 0.60$ would be reduced.

with a PDMS emulsion. Figure 5.4a shows the most common anisotropic droplet of two immiscible oils, also known as a Janus droplet. Although the interface between the two immiscible oils appears curved, this could be caused by droplet orientation induced by the different buoyant forces (*i.e.* densities and sizes) associated with the two oil droplets. Droplets having the opposite curvature are also seen, which supports this view. If desired, the two immiscible oils may be differentiated by adding a fluorescent dye that is soluble in only one of the oils. An example of this is shown in Figure 5.4b; the soybean oil part of the droplet in Figure

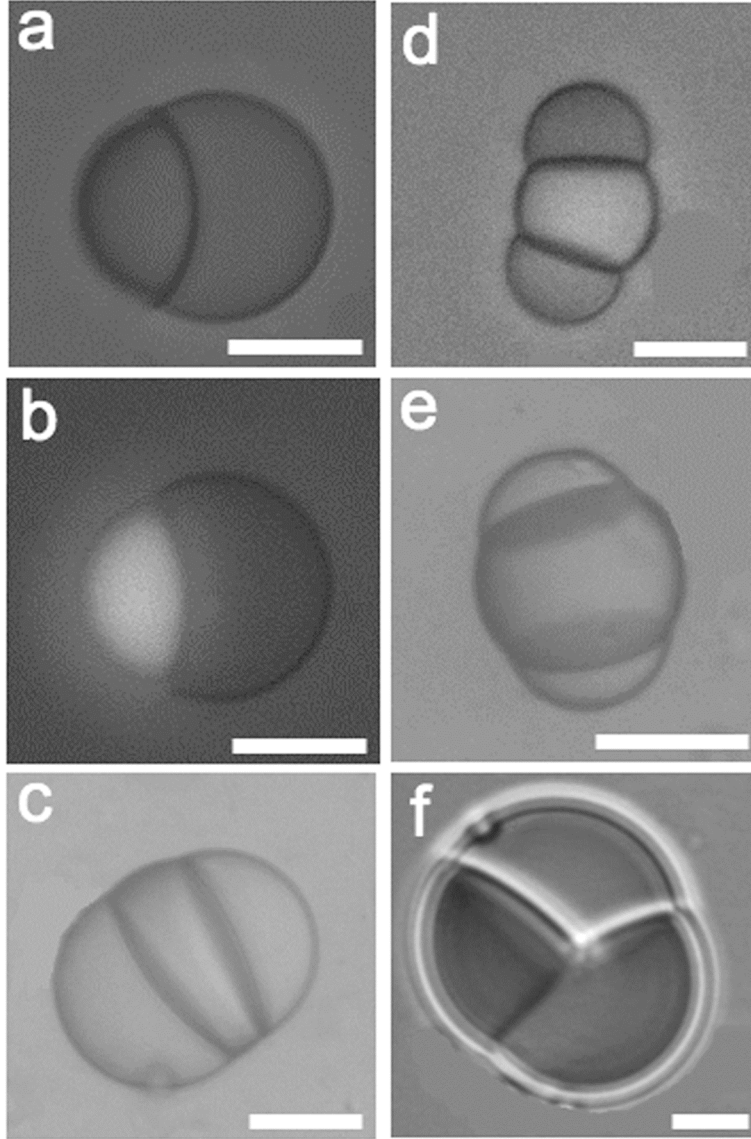


Figure 5.4. Micrographs of droplet morphologies upon coalescence of multiple droplets made of different immiscible oils. (a-e) Different morphologies observed after mixing a $\phi = 0.3$, $C_{\text{SDS}} = 5$ mM soybean oil emulsion with an equal volume of a $\phi = 0.3$, $C_{\text{SDS}} = 5$ mM PDMS emulsion and fusing at $\chi_{\text{SDS}} = 0.7$. (a) The most common morphology, the Janus droplet. (b) The same droplet as in (a) in which fluorescence of a fluorophore (100 mM pyrene) dissolved in soybean oil enables the two oils to be differentiated. (c-e) The second most common morphology in which two droplets having the same oil are separated by a droplet of a different immiscible oil. In (d), the outer two droplets are determined to be PDMS as the middle soybean droplet fluoresces with added pyrene. (f) A Cerberus droplet composed of three mutually immiscible oils (PTDMS, PPPMS, and PDMS). This is produced by making three separate emulsions each of which is made with a different mutually immiscible oil at $\phi = 0.4$ and $C_{\text{SDS}} = 5$ mM, mixing them at equal volumes, and fusing at $\chi_{\text{SDS}} = 0.7$. All scale bars are 10 μm .

5.4a fluoresces with 100 mM pyrene. Figures 5.4c-e show the second most common anisotropic droplet upon fusion of two separate immiscible oil emulsions. Each shows two droplets of one oil, whether PDMS or soybean, separated by the other immiscible oil with increasing relative volume of the droplet in the middle from (c) to (e). This technique is extended even further to produce droplets comprising three different mutually immiscible oils. Figure 5.4f shows such a droplet made of PTDMS, PPPMS, and PDMS. To the best of our knowledge, a droplet of this morphology has not been previously created. Continuing in the tradition of basing the names of anisotropic droplets after Greek and Roman mythology (*e.g.* Janus droplets because they have two different faces, like the Roman god of doorways), we call this a Cerberus droplet after the traditionally three-headed guard dog of the underworld.

We also verify that this technique works on the nanoscale. A cryogenic transmission electron microscopy micrograph of a fused droplet composed of PDMS and soybean oil is shown in Figure 5.5. The lighter part of the droplet is soybean oil as the silicon in the PDMS provides extra inherent contrast and thus appears darker in the image.

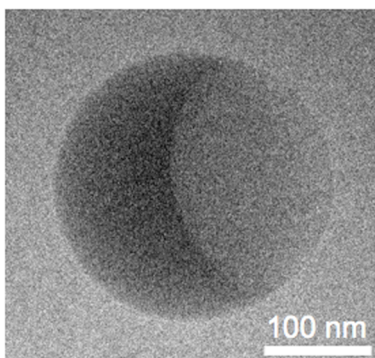


Figure 5.5. Cryogenic transmission electron microscopy micrograph of a Janus nanodroplet made by mixing equal volumes of a PDMS nanoemulsion with a soybean nanoemulsion and fusing at $\chi_{\text{SDS}} = 0.7$. Extreme emulsification conditions for both nanoemulsions: $p_a = 75$ psi, $C_{\text{SDS}} = 5$ mM, $\phi = 0.05$, and $N = 6$. PDMS comprises the darker part of the droplet as the silicon provides extra inherent contrast.

5.5 Discussion

Coalescence induced by mixing an emulsion stabilized by an ionic surfactant with an oppositely charged surfactant solution is a complex process. The extent to which coalescence occurs depends on factors such as ϕ and χ_{SDS} , but also likely depends on the rate of mixing, distance between the droplets (*i.e.* ϕ and d), and any surfactant phase changes that occur upon mixing. Further experiments are still needed to deduce a mechanism. It is possible that the CTAB complexes with enough SDS on the droplet interface such that momentarily there is not enough electrostatic repulsion to preclude coalescence. In this case, the amount of coalescence would likely depend on the probability of droplets undergoing one or more collisions during the transient destabilization. Stabilization would likely rapidly occur due to a combination of effects: 1. The excess SDS in the continuous phase diffuses to re-coat the droplet interfaces and 2. The interfacial charge density increases enough upon coalescence, due to the overall decrease in interfacial area as two droplets become one, to preclude further coalescence. It is also possible that the mixed surfactants cause crystals to form at the interface, thereby precluding any further coalescence, as crystals are seen forming in bulk depending on χ_{SDS} . Further time-lapse studies of this system after fusion are still needed to quantify stability. Also, although a particular coalescence procedure is given for the studies herein, variants on this procedure may be used to cause coalescence. For example, coalescence is produced by adding a CTAB-stabilized emulsion to an SDS-stabilized emulsion.

Although this method of coalescence may be extended to fuse multiple droplets composed of immiscible oils, the probabilities associated with creating an anisotropic droplet differ. The probability of fabricating a fused anisotropic droplet comprising N immiscible oils is less than finding coalescence of N droplets. In the case of creating an anisotropic droplet, a

fusion event must occur between two immiscible oil droplets and not between droplets comprising the same oil. For example, consider the case of two droplets fusing after mixing an SDS-stabilized PDMS emulsion with an SDS-stabilized soybean oil emulsion and adding CTAB. A PDMS oil droplet could fuse with another PDMS oil droplet to produce a larger PDMS oil droplet, or it could fuse with a soybean oil droplet to produce an anisotropic Janus droplet. This could be advantageous, however, as it may allow fusion at much higher ϕ without inducing macroscopic phase separation. Furthermore, this possibility may allow for droplets composed of four or more immiscible oils to be created.

This technique for creating anisotropic droplets works well for many mutually immiscible oils, but not all mutually immiscible oils will produce anisotropic droplets upon coalescence. Even if the electrostatic repulsion is reduced enough to cause droplets to “touch,” there still needs to be a driving force to cause the anisotropic droplet to form. Thus the three possible cases discussed above, namely, engulfing, no fusion, and anisotropic droplets still hold.

With this in mind, this mixed-surfactant self-limiting reaction technique could be used to combine droplets containing different solubilized reaction species for micro- or nano- reactor applications. Additionally, it may be possible to use this technique not only for coalescence between two liquids, but also between other phases such as a liquid and a solid or a liquid and a gas.

5.6 Conclusion

Self-limiting droplet coalescence reactions can be induced by mixing an ionically stabilized emulsion with an oppositely charged ionic surfactant solution. The quantitative discrete peaks observed in the droplet size distributions after fusion of monodisperse droplets

could be used to test theories of self-limiting reaction kinetics as a function of a variety of parameters (*e.g.* ϕ and χ_{SDS}). We have also used this technique to produce anisotropic droplets composed of two or more immiscible oils both on the micro- and nano- scale.

Chapter 6

Conclusions and Future Directions

Nanoemulsions have great potential for industrial applications and as model systems for studying the basic physics of soft-condensed matter. Herein, only a small subset of the latter has been explored, namely nanoemulsion droplet size control, real-space cryogenic transmission electron microscopy (CTEM) imaging of nanoemulsions, and coalescence induced by oppositely charged surfactants. These nanoemulsion experiments have led to advances by overcoming prior limitations of extreme flow-rupturing, interpreting CTEM images, and controlling coalescence.

In Chapter 3, we presented an environmentally friendly evaporative ripening method for mass-producing small nanoemulsion droplets approaching the micellar scale. Although rates as a function of $\psi_{2,i}$ and L2 viscosity were studied, it would be interesting to study rate as a function of both surfactant type and length. Larger surfactant molecules (*e.g.* polymers and proteins) that are intra- and inter- molecularly densely packed may inhibit or possibly arrest evaporative ripening. Longer-tailed surfactant molecules may interact more with L1 also inhibiting evaporative ripening and vice-versa for shorter surfactant molecules. Additionally, a study of the surfactant density on the surface of a droplet as a function of size at different stages during the evaporative ripening process would be revealing. When a nanoemulsion droplet is created, a certain surfactant density exists on the interface between the two immiscible phases. As a droplet decreases in a , the interfacial area available to the surfactant decreases as a^2 . Either the surfactant density could continue to increase with decreasing a or perhaps at some critical surfactant density, the surfactant could begin to be ejected from the surface, thereby increasing the bulk continuous phase surfactant concentration.

The real-space imaging of O/W nanoemulsions using CTEM was discussed in Chapter 4. Depending on the oil type and phase of ice, vapor nanoinclusions may appear in nanoemulsions due to the cryogenic sample preparation temperature quench. The mechanism of nanoinclusion formation may depend on a number of material and physical parameters that still remain unknown. Systematically studies examining and identifying which physical properties or combined properties of the oil, the ice, and their interface cause nanoinclusion formation would be revealing. It would also be interesting to explore the effect of different additives both to the dispersed and continuous phases; additives may enhance or suppress nanoinclusions by fine-tuning the physical properties of one or both phases.

A means of producing self-limiting coalescence in an emulsion has been introduced and explored in Chapter 5. This method has been extended to produce anisotropic Janus and Cerberus droplets using multiple mutually immiscible oils. Perhaps even more mutually immiscible oils could be used to create higher order anisotropic droplets. Quantitatively, it would be interesting to study the probability of forming Janus, Cerberus, and higher order droplets upon fusing an emulsion mixture containing a certain number of mutually immiscible oils. Although coalescence was studied as a function of ϕ and χ_{SDS} , the effect of the initial droplet diameter on coalescence at a fixed ϕ has yet to be determined. As droplets become smaller, the average distance between droplets decreases and, given more rapid rates of diffusion, more coalescence events may occur during the transient destabilization.

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