

Stabilization of Phase Inversion Temperature Nanoemulsions by Surfactant Displacement

JIAJIA RAO AND DAVID JULIAN McCLEMENTS*

Biopolymers and Colloids Laboratory, Department of Food Science, University of Massachusetts, Amherst, Massachusetts 01003

Nanoemulsions are finding increasing utilization in the food and beverage industry to encapsulate and protect lipophilic functional components. Low-intensity methods, such as the phase inversion temperature (PIT) approach, are of particular interest for forming food-grade nanoemulsions because of their ease of formation and relatively low energy costs. Nevertheless, this type of emulsion tends to be highly unstable to droplet coalescence after preparation. In this study, we develop a potential solution to this problem using model water/surfactant (Brij 30, C₁₂E₄)/oil (tetradecane) systems. The PIT and system morphology were determined by monitoring the temperature dependence of the electrical conductivity, turbidity, and microstructure of the emulsions. Nanoemulsions were formed by holding water/surfactant/oil mixtures at their PIT and then rapidly cooling them. The influence of storage temperature on emulsion stability was investigated, which indicated that the optimum temperature (13 °C) was about 27 °C lower than the PIT (≈40 °C). Higher storage temperatures resulted in an increase in droplet growth rate due to coalescence, while lower temperatures led to gelation. Nanoemulsions that were relatively stable to coalescence could be formed at ambient temperatures by adding either Tween 80 (0.2 wt %) or SDS (0.1 wt %) to displace the Brij 30 from the droplet surfaces. We propose that these surfactants increase nanoemulsion stability by changing the optimum curvature of the interfacial layer, as well as by increasing the repulsive interactions (steric or electrostatic) between the droplets. This study may lead to a novel approach to create stable nanoemulsion-based delivery systems that are suitable for utilization within the food industry.

KEYWORDS: Nanoemulsion; phase inversion temperature; nonionic surfactant; surfactant displacement

INTRODUCTION

There is increasing interest in the utilization of nanoemulsions in the food, beverage, cosmetic, and pharmaceutical industries because they have a number of advantages over conventional emulsions (1–3). Nanoemulsions are thermodynamically unstable systems consisting of two immiscible liquids, with one of the liquids being dispersed as small spherical droplets (radius ≈10–100 nm) in the other liquid (4). The fact that the droplets are extremely small means that they exhibit different physicochemical and functional characteristics to conventional emulsions: optical transparency; high stability to droplet aggregation and gravitational separation; large interfacial area-to-volume ratio (5). The bioavailability of lipophilic components encapsulated within nanoemulsions may be appreciably higher than the same components encapsulated within conventional emulsions (6, 7).

Two main approaches are currently used for the preparation of nanoemulsions: high energy and low energy. *High-energy methods* used intense mechanical energy to break up macroscopic phases or droplets into smaller droplets and typically involve the

use of microfluidization, high-pressure homogenization, or sonication (8, 9). A drawback of this approach is the extremely high intensities required to generate nanoemulsions with very small droplets, making this preparation route unfavorable for many industrial applications (10). In contrast, many *low-energy methods* rely on the spontaneous formation of nanoemulsions under specific system compositions or environmental conditions as a result of changes in the optimum curvature of surfactants (11, 12). This change of curvature can be achieved through a number of different routes; e.g., the composition can be kept constant while the temperature is changed (phase inversion temperature or PIT method) or the temperature is maintained constant and the composition is changed (phase inversion composition or PIC method) (13, 14).

The PIT approach is usually based on changes in the molecular geometry of nonionic surfactants with temperature (15). Nanoemulsions are produced by bringing an oil, water, and surfactant mixture to the PIT where the affinity of the surfactant for the water and oil phases is balanced, and then the mixture is rapidly cooled (16). The temperature dependence of the behavior of a surfactant can be interpreted in terms of its packing parameter (p), which equals the effective surfactant tail group area divided

*To whom correspondence should be addressed. Tel: (413) 545-1019. Fax: (413) 545-1262. E-mail: mcclements@foodsci.umass.edu.

by the effective surfactant headgroup area: $p = A_T/A_H$ (17). At low temperatures, the cross-sectional area occupied by the hydrated surfactant headgroup is larger than that of the hydrophobic tail group ($p < 1$), thus favoring the formation of O/W emulsions. At high temperatures, the area of the dehydrated surfactant headgroup is less than the hydrophobic tail group ($p > 1$), thus favoring the formation of W/O emulsions. At an intermediate temperature (the PIT), the areas of the head and tail groups are nearly balanced ($p = 1$). At this temperature, an intermediate oil/water/surfactant phase is formed that typically consists of either a lamellar or a bicontinuous microemulsion structure (18). Around the PIT, the interfacial tension is ultralow, which promotes droplet disruption (thereby favoring the formation of small droplets) but also promotes droplet coalescence (thereby favoring droplet growth). To form stable nanoemulsions with small droplets, it is necessary to rapidly move through the PIT with continuous stirring, e.g., by quench cooling to a storage temperature of 25–30 °C below the PIT (19, 20). The formation of nanoemulsions using the PIT method can be monitored using a variety of analytical instruments, including electrical conductivity, interfacial tension, light scattering, X-ray scattering, and microscopy (21–23).

One of the major problems associated with the PIT approach of forming nanoemulsions is that they are not very stable to droplet coalescence after formation (24, 25). If the temperature approaches the PIT, then the droplets become highly unstable to coalescence because of the corresponding reduction in interfacial tension. This severely limits the application of this kind of nanoemulsion in many food and beverage products that are stored at elevated temperatures or that undergo some form of thermal processing during their manufacturing, e.g., cooking, pasteurization, or sterilization. Consequently, it is important to identify strategies to improve the kinetic stability of nanoemulsions formed by the PIT method after they have been prepared.

In this study, we examined the possibility of using a *surfactant displacement* approach to improve the stability of nanoemulsions to droplet coalescence during storage. In this approach nanoemulsions are formed using a nonionic surfactant with a relatively low PIT, and then another surfactant with a much higher PIT that is capable of generating strong repulsive forces between the droplets is incorporated into the system. The new surfactant displaces the original surfactant from the droplet surfaces, thereby increasing their stability to coalescence. A model oil/water/surfactant system was used in this study to simulate more complex food and beverage systems so as to obtain a better understanding of the underlying physicochemical mechanisms. Initially, we prepared nanoemulsions using a nonionic surfactant (Brij 30, C₁₂E₄) with a relatively low PIT. We then added either a nonionic surfactant (Tween 80) or an ionic surfactant (SDS) to displace the original nonionic surfactant (Brij 30) from the droplet surfaces after nanoemulsion formation. Tween 80 has a polyoxyethylene headgroup and a hydrocarbon (C18:1) tail group: it has a relatively high cloud point (65 °C) and would be expected to stabilize droplets through steric repulsion. Sodium dodecyl sulfate (SDS) has a sulfated anionic headgroup and a hydrocarbon (C12:0) tail group: it does not have a cloud point in the temperature range up to 100 °C and would be expected to stabilize droplets through electrostatic repulsion. Our overall objective was to determine the optimum protocol for producing nanoemulsions containing small stable droplets that might be suitable for application of the PIT technology in the food and beverage industries.

MATERIALS AND METHODS

Materials. *n*-Decane (99.5% purity), dodecane (99.5% purity), tetradecane (99.5% purity), hexadecane (99.5% purity), a nonionic surfactant, C₁₂E₄ (Brij 30, technical grade), sodium dodecyl sulfate (SDS, 99% purity), POE (20) sorbitan monooleate (Tween 80, Technical grade), and sodium chloride (NaCl) (purity >99.5%) were purchased from Sigma-Aldrich Co. (St. Louis, MO). Distilled water was deionized and milli-Q filtered prior to use.

Emulsion Preparation and Stability. Oil-in-water nanoemulsions were prepared using a one-step procedure. Preliminary experiments were carried out to determine the PIT of these emulsions using electrical conductivity measurements (see below). Weighed amounts of oil, surfactant, and water were mixed together in a glass beaker and then heated to a temperature of 15 °C above the PIT using a water bath. Once that temperature was reached, the mixture was held for 10 min and then rapidly cooled to the storage temperature by placing it in an ice bath. The emulsion was continuously stirred using a mechanical stirrer during the heating and cooling stages. The subsequent stability of the nanoemulsions was assessed by measuring the change in their particle size distribution when they were held at different storage temperatures using dynamic light scattering (NanoZS; Malvern Instruments, Malvern, U.K.). The samples were diluted 20-fold in water at room temperature. The data are reported as the mean droplet radius and particle size distribution.

Surfactant Displacement Method. Oil-in-water PIT nanoemulsions containing 20 wt % tetradecane, 6 wt % Brij 30, and 74 wt % aqueous phase were prepared using the PIT method described above and then diluted 10-fold using either SDS or Tween 80 solutions containing varying amounts of surfactant.

Determination of Phase Inversion Temperature. The phase inversion process was monitored using temperature-scanning conductivity, turbidity, and microscopy measurements as described below.

Conductivity Measurements. Coarse emulsions containing 20 wt % oil, 6 wt % Brij 30, and 74 wt % water phase (NaCl, 10⁻² mol/L) were prepared by simple mixing at ambient temperature. The emulsions were then continuously stirred using a mechanical stirrer (Fisher StedFast stirrer, Model SL 1200; Fisher Scientific), and their electrical conductivity was measured (Accumet AP85; Fisher Scientific, Pittsburgh, PA) as their temperature was increased at a controlled rate (1.5 °C min⁻¹). The PIT was obtained by measuring the change in conductivity of the emulsions upon heating (26).

Turbidity Measurements. Initially, a nanoemulsion was prepared using the PIT method described earlier. Turbidity versus temperature scans were then performed on the nanoemulsions using a UV/visible spectrophotometer at 600 nm (Ultraspec 3000 pro; Biochrom Ltd., Cambridge, U.K.) with a temperature control module (programmable heated cell holder, 80-2106-14; Biochrom Ltd., Cambridge, U.K.) (27). The sample temperature, heating/cooling rate, and data recording were handled through computer software (Swift TM Melting Temp v1.09, Data Capture Software v1.02; Biochrom Ltd., Cambridge, U.K.). The samples were contained within quartz cuvettes with a path length of 1.0 cm and heated from 20 °C at a rate of 1 °C min⁻¹. Distilled water was used as a reference.

Optical Microscopy. A Nikon optical microscope (C1 Digital Eclipse, Tokyo, Japan) with a 40× objective lens (NA 0.75) was used to capture the images of emulsions held at different temperatures during heating. An emulsion was placed between slide and coverslip and observed by optical microscopy equipped with a heating/cooling stage (Linkham PE 94; Linkam Scientific Instruments, Tadworth, Surrey, U.K.) attached to a filter pump (Eheim Professional III, Deizisau, Germany). The sample slide was loaded on the stage and heated from 20 °C to the desired temperature at a controlled heating/cooling rate of 5 °C/min. Images were acquired at specific temperatures using differential interference contrast (DIC) microscopy using a DIC polarizer and analyzer. The recorded images were analyzed with the image analysis software.

Statistical Analysis. All measurements were performed on at least two freshly prepared samples (i.e., new samples were prepared for each series of experiments) and were reported as means and standard deviations.

RESULTS AND DISCUSSION

Dependence of PIT on Oil Type. The objective of the initial experiments was to identify the influence of hydrocarbon oil type

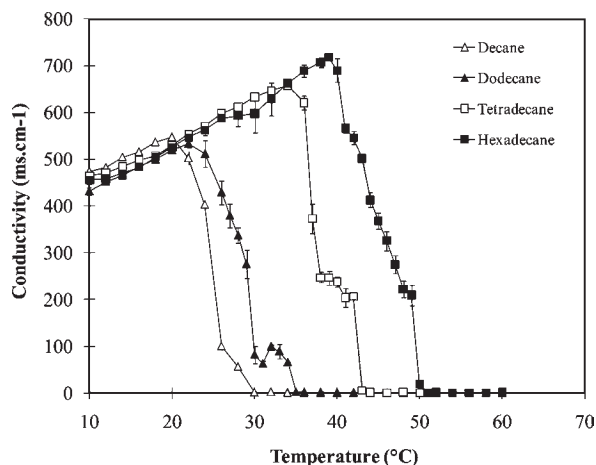


Figure 1. Temperature dependence of the electrical conductivity of hydrocarbon (20 wt % tetradecane), nonionic surfactant (6 wt % Brij 30), and water phase (10 mM NaCl) systems.

on the PIT for the nonionic surfactant (Brij 30) used in this study. Ideally, we wanted to identify a hydrocarbon oil that would give a PIT around 35–40 °C so as to facilitate the subsequent formation and characterization of nanoemulsions. A series of O/W emulsions were prepared using different kinds of hydrocarbons as the oil phase: 20 wt % oil (hydrocarbon); 6 wt % surfactant (Brij 30); 74 wt % aqueous phase (10 mM NaCl in water). These emulsions were created by blending the surfactant, oil, and aqueous phase together at ambient temperature using a high shear mixer. The electrical conductivity of these systems was then measured as their temperature was increased (**Figure 1**). Independent of hydrocarbon type, all emulsions exhibited similar general trends in the electrical conductivity–temperature profiles. With increasing temperature, there was initially a steady increase in conductivity and then a sharp decrease above a particular temperature, followed by a region where the conductivity remained close to zero (**Figure 1**). This behavior is indicative of phase inversion from an O/W emulsion with a watery continuous phase (high conductivity) to a W/O emulsion with an oily continuous phase (low conductivity) (11). In some emulsions, there was a range of temperatures where the electrical conductivity maintained an intermediate value before falling to zero. For example, in tetradecane emulsions the electrical conductivity had a value between the high (O/W) and low (W/O) levels in the temperature range from 38 to 42 °C. This intermediate zone is likely to correspond to the formation of structured oil/surfactant/water phases, such as bicontinuous microemulsions or lamellar phases (28).

The PIT was determined from the electrical conductivity versus temperature plots by defining it as the temperature where the measured conductivity was half the maximum value. The PIT of the mixed systems increased as the alkyl chain length of the hydrocarbon oils increased: 25.0, 29.5, 38.5, and 45.5 °C for decane, dodecane, tetradecane, and hexadecane, respectively. This result is in agreement with earlier studies of the effects of hydrocarbon type on the PIT of various nonionic surfactants (12). This effect can be attributed to the ability of oil molecules to penetrate between surfactant tails, thereby altering the optimum molecular geometry of the surfactant layer at the oil/water interface. Short chain hydrocarbon oils are able to penetrate more easily between the surfactant tails and thereby favor a curvature that is closer to planar than long-chain hydrocarbon oils. Emulsions containing short-chain hydrocarbons would therefore have to be heated to a lower temperature before the surfactant curvature tended toward unity and, hence, would have a lower

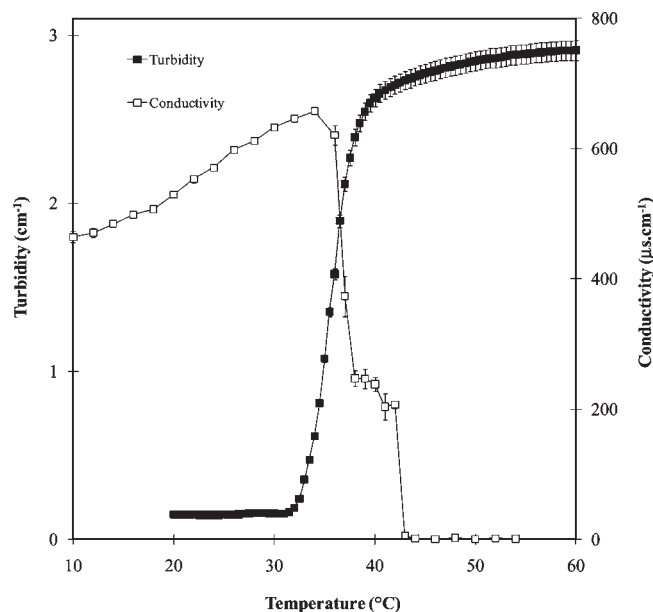


Figure 2. Temperature dependence of the electrical conductivity and turbidity of tetradecane (20 wt %), nonionic surfactant (6 wt % Brij 30), and water phase (10 mM NaCl) systems.

PIT (29). Following this initial study, tetradecane was selected as the oil source for the remainder of the experiments since it gave a PIT that was convenient for preparing and stabilizing nanoemulsions.

Changes in Optical Properties of Emulsions around the PIT.

Additional information about the phase behavior of the system during heating was obtained using turbidity and optical microscopy measurements. Initially, a nanoemulsion was formed by heating a mixture of oil (20 wt % tetradecane), surfactant (6 wt % Brij 30), and aqueous phase (10 mM NaCl) above the PIT and then rapidly cooling to around 13 °C. The temperature dependence of the turbidity of the nanoemulsion formed was then monitored as it was heated at a controlled rate (**Figure 2**). The turbidity remained relatively low and constant from 13 to 31 °C, rose steeply from 31 to 39 °C, and then remained relatively high and constant upon further heating. The changes in turbidity corresponded closely to changes in electrical conductivities; i.e., when the conductivity fell steeply, the turbidity increased steeply (**Figure 2**). The PIT was determined from the turbidity measurements as the inflection point in the turbidity versus temperature curves. The transition temperature determined using the turbidity method (PIT = 36 °C) was fairly close to the value determined using the conductivity method (PIT = 38.5 °C). The differences between the two methods may have occurred for a number of reasons: (i) they are based on different physicochemical principles; (ii) different heating rates were used; (iii) different mathematical approaches were used to calculate the PIT from the curves. The observed change in sample turbidity during heating can be attributed to changes in the degree of light scattering as the system changes from an O/W nanoemulsion, to an intermediate phase, to a W/O emulsion. Information about changes in emulsion morphology was obtained at selected temperatures using optical microscopy (**Figure 3**). At temperatures well below the PIT (i.e., ≤31 °C), the samples appeared homogeneous, and no individual droplets could be observed because their dimensions were below the resolution of the microscope (**Figure 3**, top left). Around the PIT (i.e., 35 and 38 °C), some microscopic objects were present within the samples (**Figure 3**, top middle and right). These structures may be the result of the increased coalescence

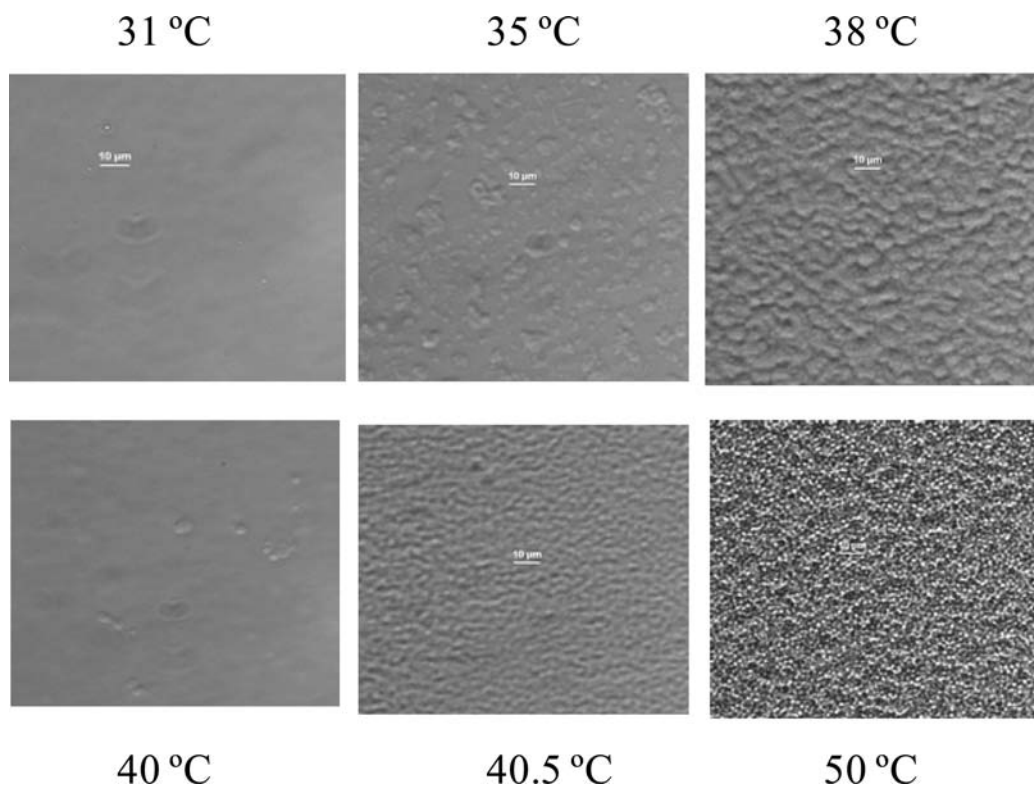


Figure 3. Optical microscopy images of emulsions (20 wt % tetradecane, 6 wt % Brij 30, 74 wt % water) held at different temperatures during heating. The scale bars indicate 10 μm .

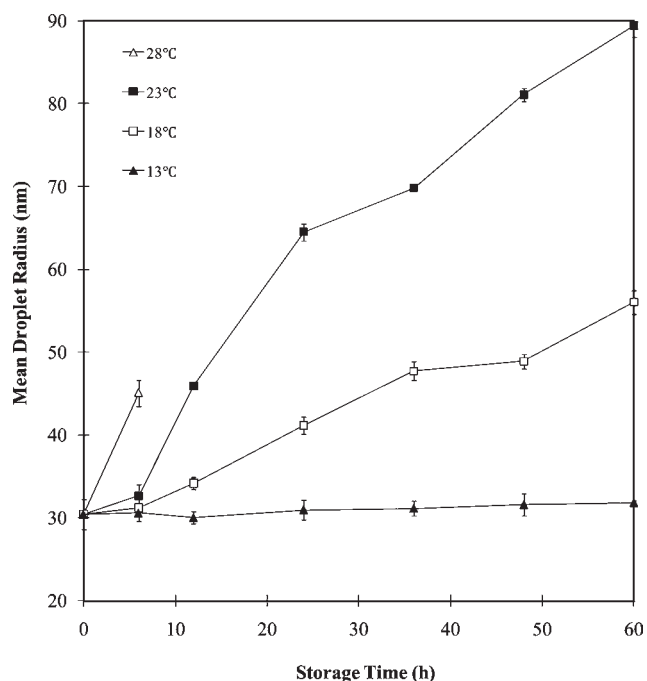


Figure 4. Growth of droplets during storage at different temperatures in nanoemulsions formed using the PIT method (20 wt % tetradecane, 6 wt % Brij 30, and 74 wt % water phase).

rate of the oil droplets just below the PIT and would account for the steep rise in emulsion turbidity around this temperature (Figure 2). At slightly higher temperatures (i.e., 40 °C), these structures disappeared, and the system again looked homogeneous (Figure 3, bottom left), which suggested that any microstructures formed were too small to be observed by optical microscopy. Interestingly, we did not see a corresponding decrease in

the turbidity of the samples at this temperature (Figure 2), which may have been because the heating rate was too fast in the turbidity scanning measurements to maintain the intermediate phases observed in the microscope under isothermal conditions. Nevertheless, this temperature did correspond to the intermediate zone observed in the electrical conductivity measurements, where structured surfactant/oil/water phases are believed to form (Figure 2). At a temperature well above the PIT (i.e., 50 °C), the system appeared to consist of large closely packed droplets dispersed within a continuous medium, presumably representing a W/O emulsion (Figure 3, bottom right).

Impact of Storage Temperature on Nanoemulsion Stability. After nanoemulsions have been formed using the PIT method they are highly prone to droplet coalescence at certain storage temperatures because of the relatively low interfacial tension (12). We therefore examined this effect by measuring the time dependence of the mean droplet radius (Figure 4) and particle size distribution (Figure 5) of emulsions stored at different temperatures. Initially, nanoemulsions were formed by heating surfactant/oil/water mixtures above their PIT and then quench cooling them to a particular storage temperature. The rate of droplet growth decreased appreciably with decreasing storage temperature from 28 to 13 °C (Figure 4). At 13 °C, the emulsions had a monomodal distribution that moved slightly to larger sizes during storage (Figure 5b), probably because of Ostwald ripening (see later). At 23 °C, the emulsions also remained monomodal during the first 60 h of storage, but there was evidence of rapid droplet growth (Figure 5a). The emulsions stored at the lower temperatures (13 °C) appeared stable to creaming whereas distinct creamed layers were observed at the top of the emulsions stored at higher temperatures (23 and 28 °C). These effects can be attributed to the fact that emulsions stored at higher temperatures were closer to their PIT and, therefore, had low interfacial tensions, which made them more prone to droplet coalescence (18). Interestingly, when the emulsions were stored at temperatures

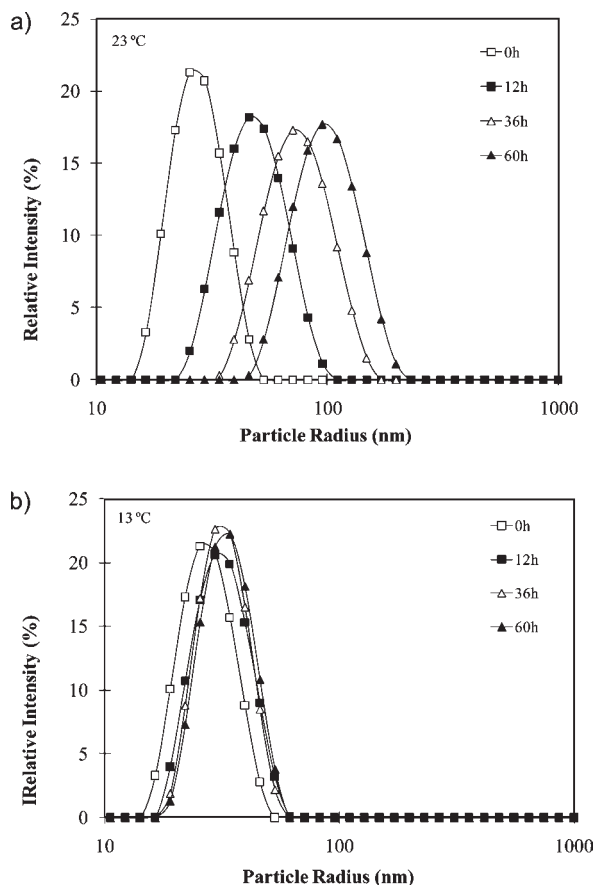


Figure 5. Evolution of particle size distributions of nanoemulsions formed using the PIT method (20 wt % tetradecane, 6 wt % Brij 30, 74 wt % water) during storage at the different temperatures indicated.

below 13 °C, they formed a gel, and so they could not be analyzed by dynamic light scattering. Early studies by Shinoda and Sato showed that stable O/W emulsions containing small droplets could be obtained using the PIT method when the storage temperature was 20–65 °C below the PIT (19). In our study, the optimum storage temperature of 13 °C was around 27 °C below the PIT for the surfactant/oil/water system used. However, further cooling promoted gelation, which may be either desirable or undesirable depending on the application.

Improvement of Nanoemulsion Stability by Surfactant Displacement. The tendency for the droplets in PIT nanoemulsions to grow during storage due to droplet coalescence is a major limitation to their practical application. Many industrial and commercial products need to remain stable during storage at ambient temperatures or after thermal processing, e.g., foods, cosmetics, personal care products, and pharmaceuticals. It is therefore important to identify effective strategies to prevent droplet coalescence occurring in PIT nanoemulsions. We examined the possibility of using a *surfactant displacement* approach. In this approach, the nanoemulsions are formed using a nonionic surfactant with a relatively low PIT, and then another surfactant with a much higher PIT is incorporated into the system. The new surfactant displaces the original surfactant from the droplet surfaces, thereby increasing their stability to coalescence. In this study, we used either a nonionic surfactant (Tween 80) or an ionic surfactant (SDS) to displace the original nonionic surfactant (Brij 30) from the droplet surfaces after nanoemulsion formation.

Oil-in-water nanoemulsions containing 20 wt % tetradecane, 6 wt % Brij 30, and 74 wt % aqueous phase were prepared using the PIT method described above and then diluted 10-fold using

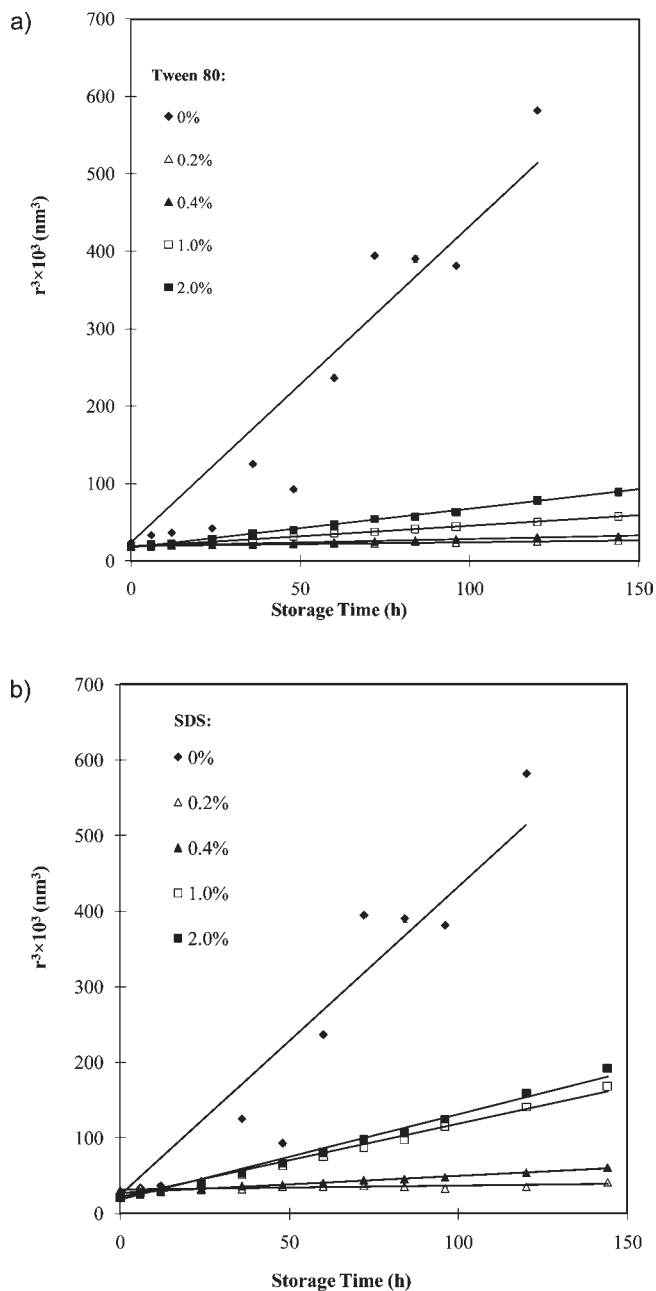


Figure 6. Dependence of droplet radius cubed on time for emulsions containing different amounts of added surfactant: (a) Tween 80 or (b) SDS system. The final composition of the emulsions was 2 wt % tetradecane, 0.6 wt % Brij 30, and 0–2 wt % additional surfactant.

either SDS or Tween 80 solutions containing varying amounts of surfactant. This led to emulsions with a final composition of 2 wt % tetradecane, 0.6 wt % Brij 30, and 0–2 wt % of either Tween 80 or SDS. The stability of these emulsions to droplet growth was then monitored by measuring the change in their mean particle radius cubed and particle size distribution during storage at 23 °C (Figures 6 and 7). The addition of either SDS or Tween 80 to the nanoemulsions clearly improved their resistance to droplet growth, by an amount that depended on surfactant concentration. An estimation of the droplet growth rate was obtained by fitting straight lines to droplet radius cubed versus time data during the first 100 h of emulsion storage (Figure 8). For the emulsions containing SDS, there was a distinct decrease in the droplet growth rate when 0.01 wt % additional surfactant was incorporated into the system. However, when higher amounts of SDS

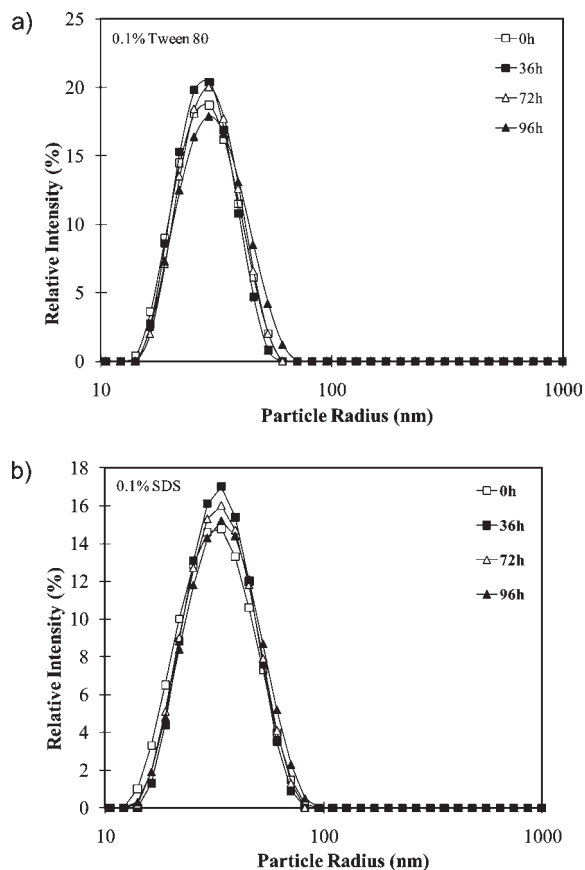


Figure 7. Dependence of droplet size distribution on time during storage at 23 °C for emulsions containing 0.1 wt % added surfactant: (a) Tween 80 or (b) SDS system. The final composition of the emulsions was 2 wt % tetradecane, 0.6 wt % Brij 30, and 0.1 wt % additional surfactant.

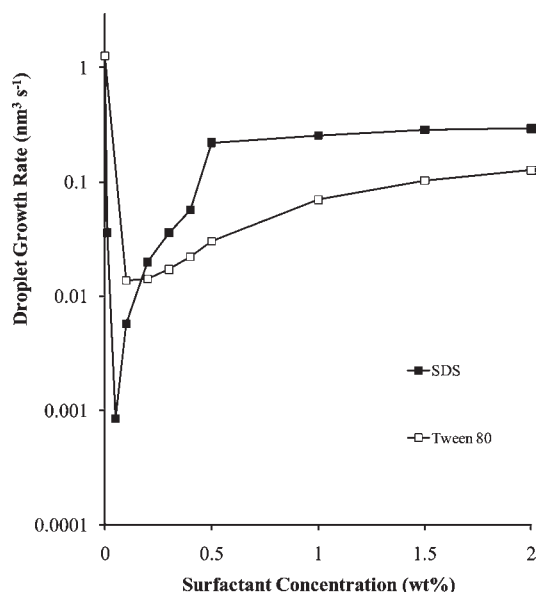


Figure 8. Dependence of droplet growth rate (ω) on the concentration of additional surfactant (either Tween 80 or SDS) added after emulsion formation. The final composition of the emulsions during storage at 23 °C was 2 wt % tetradecane, 0.6 wt % Brij 30, and 0.1 wt % additional surfactant.

were added, the droplet growth rate increased appreciably, particularly when the SDS concentration exceeded about 0.5 wt %. For the emulsions containing Tween 80, there was also an

appreciable decrease in the droplet growth rate when 0.1 wt % additional surfactant was added and a noticeable increase when more surfactant was added. The particle size distribution measurements indicated that the emulsions containing either 0.1 wt % SDS or 0.1 wt % Tween 80 were monomodal, with the peak radius slowly increasing with time (Figure 7). These results show that the addition of either nonionic or ionic surfactants to PIT nanoemulsions can improve their stability to droplet growth, particularly when used in an optimum concentration range. We postulate that the addition of either SDS or Tween 80 is able to decrease the droplet coalescence rate by altering the optimum curvature of the surfactant layer surrounding the droplets (thereby altering the interfacial tension), as well as increasing the repulsive interactions between the droplets (see later). Nevertheless, the addition of SDS or Tween 80 was unable to prevent droplet growth entirely, which may be attributed to Ostwald ripening (OR).

Ostwald ripening arises from the fact that oil solubility increases with decreasing droplet size, which leads to the growth of large droplets at the expense of smaller ones in polydisperse systems due to molecular diffusion of oil through the continuous phase (30). The droplet growth rate, ω , due to Ostwald ripening is described by the Lifshitz-Slezov and Wagner (LSW) theory:

$$\omega = \frac{dr^3}{dt} = \frac{8}{9} \left(\frac{C_\infty \gamma V_m D}{RT} \right) \quad (1)$$

where r is the number average droplet radius, t is the storage time, C_∞ is the bulk phase solubility, γ is the interfacial tension, V_m is the molar volume of the oil, D is the diffusion coefficient in the continuous phase, R is the gas constant, and T is the absolute temperature. Equation 1 indicates that the cube of the mean droplet radius should increase linearly with time for emulsions undergoing OR, with a slope given by $\omega = r^3/t$. The droplet growth rate due to OR has been predicted for tetradecane oil-in-water emulsions using this equation and the physicochemical parameters of the system to be $\omega = 0.14 \text{ nm}^3 \text{ s}^{-1}$ and measured to be around $1\text{--}1.5 \text{ nm}^3 \text{ s}^{-1}$ for SDS-stabilized systems (31). The time dependence of the particle size of the emulsions containing added surfactant used in our studies was characteristic of Ostwald ripening, i.e., a linear dependence of the number mean particle radius cubed on time (Figure 6) and a monomodal size distribution that moved up the size axis with time (Figure 7). The calculated ripening rates (ω) from our experimental data ranged from about 0.01 to $1.5 \text{ nm}^3 \text{ s}^{-1}$ (Figure 8), which largely agree with those reported previously. Nevertheless, at certain intermediate surfactant concentrations our measured ripening rates were about an order of magnitude lower than the predicted rates. We attribute this effect to the fact that we used a mixed surfactant system in our study, which may have led to a considerably lower interfacial tension than that of a single surfactant (SDS) used in the calculations of Wooster and co-workers (31). The ripening rate is proportional to the interfacial tension (eq 1), so a reduction in the interfacial tension should cause a reduction in ω .

As mentioned earlier, the initial decrease in droplet growth rates that were observed when small amounts of either SDS or Tween 80 were added to the system can be attributed to a reduction in droplet coalescence. On the other hand, the appreciable increase in droplet growth rates observed at higher surfactant concentrations can be attributed to the impact of surfactant on OR rates. SDS and Tween 80 can both form surfactant micelles that can solubilize hydrocarbon molecules in their hydrophobic interiors. These surfactant micelles may facilitate the transport of hydrocarbon oil between the droplets, thereby accelerating the Ostwald ripening process (30). In addition, surfactant micelles

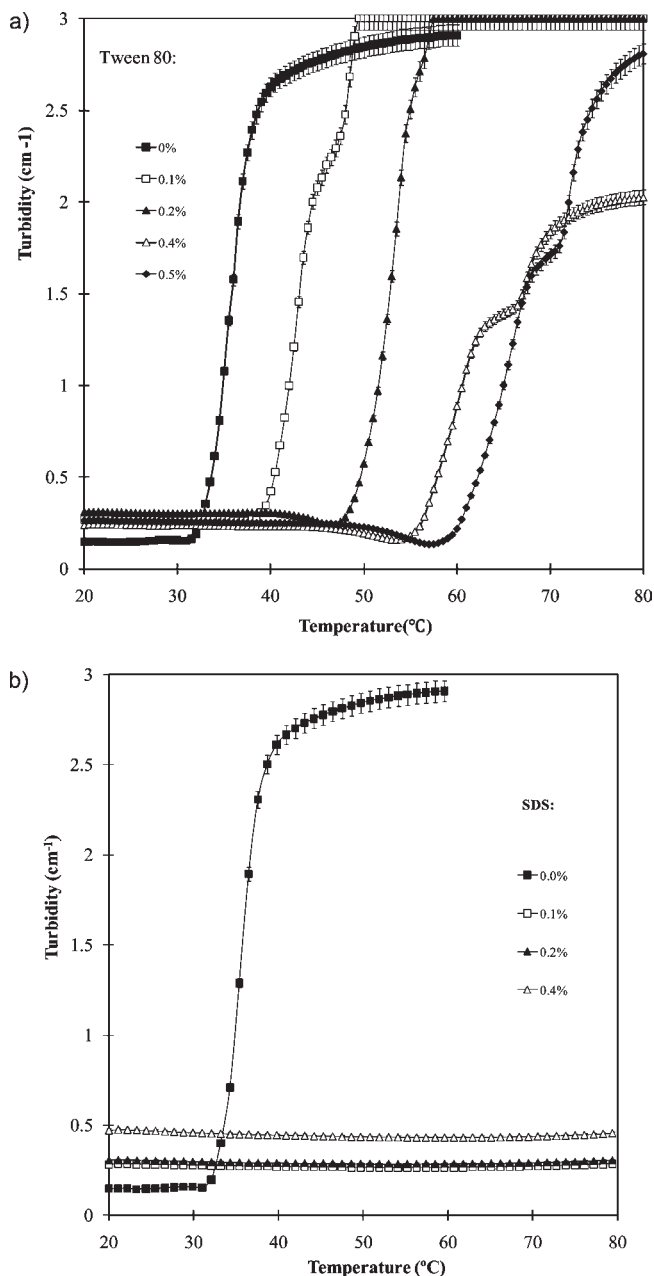


Figure 9. Temperature dependence of emulsion turbidity for emulsions containing different concentrations of additional surfactant: (a) Tween 80 or (b) SDS.

can promote droplet flocculation by generating an osmotic stress in the system through a depletion mechanism, thereby bringing the droplets closer together and facilitating the exchange of oil molecules between them (32).

Temperature Stability of Mixed Surfactant Nanoemulsions. Finally, we examined the impact of the presence of additional surfactants on the thermal stability of the nanoemulsions. For these experiments we only used surfactant concentrations (<0.5 wt %) where the nanoemulsions were relatively stable to droplet growth at ambient temperatures. The addition of increasing amounts of Tween 80 to the nanoemulsions had a distinct effect on their thermal stability. The turbidities of the nanoemulsions remained relatively low below their PIT but increased dramatically once this temperature was approached and exceeded (Figure 9), which can be attributed to extensive droplet coalescence as discussed earlier. At the higher Tween 80 concentrations

there appeared to be a slight dip in the turbidity just below the PIT, which suggested some structural change occurred in the emulsions around these temperatures. The estimated PIT increased appreciably as the Tween 80 concentration added to the nanoemulsions increased, being $\approx 38, 43, 54, 55, 60,$ and $66\text{ }^{\circ}\text{C}$ for 0, 0.1, 0.2, 0.3, 0.4, and 0.5 wt % Tween 80, respectively (Figure 9). The origin of this improved stability to heating can be attributed to the influence of Tween 80 on the molecular geometry of the surfactant layer surrounding the lipid droplets (33). Tween 80 (20 oxyethylene groups) has a larger headgroup than Brij 30 (4 oxyethylene groups), and therefore the packing parameter of the surfactant layer ($p = A_T/A_H$) is lowered when more Tween 80 is incorporated into the interface. The PIT corresponds to the temperature where the curvature of the surfactant layer becomes unity ($p = 1$). Thus an emulsion containing Tween 80 will have to be heated to a higher temperature than an emulsion containing Brij 30 before the packing parameter reaches unity and the PIT is attained (34). In addition, there will be a greater steric repulsion between the droplets in the presence of Tween 80 because of its bigger hydrophilic headgroup, which may also help to prevent coalescence.

The turbidity of the nanoemulsions containing added SDS (0.01–0.4 wt %) remained constant across the entire temperature range studied, which suggested that there was no change in their structure during heating (Figure 9). These experiments indicated that the addition of small amounts of SDS to the nanoemulsions was very effective at preventing their breakdown due to droplet coalescence, especially during heating. The adsorption of SDS molecules to the surfaces of the droplets in the nanoemulsions will lead to a strong electrostatic repulsion that prevents them from coming into close contact and therefore inhibits coalescence. Unlike nonionic surfactants, the SDS headgroups are not subject to extensive changes in their molecular geometry during heating, and therefore their presence at the interface does not lead to phase inversion.

Conclusions. Nanoemulsions have been prepared using the PIT emulsification method for an oil (tetradecane), nonionic surfactant (Brij 30), and water system. Electrical conductivity, turbidity and morphology measurements were used to characterize the phase inversion from O/W to W/O emulsions that occurred when their temperature was raised. The stability of the emulsions to droplet coalescence during storage increased as the storage temperature was decreased, but they tended to form gels when cooled below $13\text{ }^{\circ}\text{C}$. The stability of the emulsions to droplet coalescence at ambient temperatures or during heating could be improved by adding either nonionic surfactant (Tween 80) or anionic surfactant (SDS) to displace $C_{12}E_4$ (Brij 30) from the droplet surfaces. This effect was attributed to the ability of the added surfactants to change the optimum curvature of the oil/water interface and to increase the repulsive colloidal interactions between the droplets. Nevertheless, some droplet growth still occurred in the emulsions containing added surfactant due to Ostwald ripening. This form of emulsion instability would have to be arrested using alternative strategies, e.g., by incorporating highly water insoluble oils into the disperse phase. This study provides some useful insights into the formation and stabilization of nanoemulsions that can be used as delivery systems for lipophilic components for various industrial applications. For example, in the food and beverage industry one may be able to add food grade surfactants to improve the formation and stability of nanoemulsions utilizing the PIT method.

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