Formation of Flavor Oil Microemulsions, Nanoemulsions and Emulsions: Influence of Composition and Preparation Method

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ABSTRACT: This study aimed to establish conditions where stable microemulsions, nanoemulsions or emulsions could be fabricated from a nonionic surfactant (Tween 80) and flavor oil (lemon oil). Different colloidal dispersions could be formed by simple heat treatment (90 °C, 30 min) depending on the surfactant-to-oil ratio (SOR): emulsions ($r > 100$ nm) at SOR < 1; nanoemulsions ($r < 100$ nm) at 1 < SOR < 2; microemulsions ($r < 10$ nm) at SOR > 2. Turbidity, electrical conductivity, shear rheology, and DSC measurements suggested there was a kinetic energy barrier in the oil—water—surfactant systems at ambient temperature that prevented them from forming metastable emulsion/nanoemulsion or thermodynamically stable microemulsion systems. High energy homogenization (high pressure or ultrasonic homogenizer) or low energy homogenization (heating) could be used to form emulsions or nanoemulsions at low or intermediate SOR values; whereas only heating was necessary to form stable microemulsions at high SOR values.

KEYWORDS: lemon oil, Tween 80, nanoemulsion, microemulsion, emulsion, SOR, low energy homogenization

■ INTRODUCTION

There is considerable interest within the food industry in the development of food-grade delivery systems to incorporate lipophilic functional components (such as flavors, colors, micronutrients, and antimicrobials) into foods and beverages.[1−4] A number of different colloid-based delivery systems have been shown to be particularly suitable for this purpose, including microemulsions, nanoemulsions, and emulsions.[5] These colloidal systems differ in their compositions, physicochemical properties, and thermodynamic stabilities, which lead to differences in their functional performances.[6] Colloidal delivery systems can be fabricated entirely from food-grade ingredients using simple processing operations.[7,8] Each type of colloidal delivery system has specific physicochemical properties that may give it advantages or disadvantages for particular applications.

Emulsions contain droplets with mean radii between about 100 nm and 100 μm. They are thermodynamically unstable systems because the contact between oil and water molecules is unfavorable, and so they will always break down over time.[9] Emulsions tend to be optically turbid or opaque because the droplets have a similar size to the wavelength of light and so strongly scatter light.

Nanoemulsions can be considered to be emulsions that have relatively small droplet sizes, i.e., mean radii <100 nm.[10] The relatively small size of the droplets compared with the wavelength of light means that nanoemulsions tend to be either transparent or only slightly turbid.[6] In the food industry, emulsions and nanoemulsions are usually produced using high-energy methods, such as high pressure valve homogenization, microfluidization, and sonication.[11−15] These methods generate intense disruptive forces that mechanically break up the oil phase into tiny droplets that are dispersed within the aqueous phase.[16] Nevertheless, a number of low-energy methods have also been utilized in other industries that may have application within the food industry, such as phase inversion and spontaneous emulsification methods.[17,18]

These low-energy methods are based on the spontaneous formation of tiny oil droplets under certain system conditions, which are then trapped in a metastable state.[19,20]

Unlike emulsions and nanoemulsions, microemulsions are thermodynamically stable systems that form spontaneously under a particular set of composition and environmental conditions.[21−24] Microemulsions tend to have very small droplets ($r < 25$ nm) compared to the wavelength of light, which means that they tend to appear either transparent or translucent.[25,26] It should be noted that the terminology historically developed to describe colloidal dispersions has become confusing, with the particles in microemulsions usually being smaller than those in nanoemulsions even though "micro-" means $10^{−6}$ and "nano-" means $10^{−9}$. The major distinction between these two systems should be their thermodynamic stability: with microemulsions being stable and nanoemulsions being unstable.

There are a number of food and beverage products that should be either transparent or only slightly cloudy in appearance (e.g., some fortified waters and beverages), and therefore it is important to utilize a delivery system that does not increase their opacity. For this reason, there has been growing interest in the development of stable food-grade nanoemulsions or microemulsions that can be used as delivery systems for lipophilic active components.[27,28] The purpose of the present study is to examine the conditions where stable microemulsions, nanoemulsions or emulsions can be formed using a food-grade nonionic surfactant (Tween 80) and flavor oil (lemon oil). Specifically, we focus on the influence of surfactant-to-oil ratio (SOR) and homogenization method (low- or high-energy) on the formation and stability of different colloidal dispersions. The information obtained from

this study would facilitate the rational design of food-grade delivery systems for applications in transparent or slightly turbid food and beverage products.

■ MATERIALS AND METHODS

Materials. Lemon oil ($1 \times$ fold) (SC036081) was kindly donated by International Flavors and Fragrances (Union Beach, NJ). Polyoxyethylene (20) sorbitan monooleate (Tween 80), propylene glycol and sodium chloride NaCl (purity >99.5%) were purchased from the Sigma-Aldrich Co. (St. Louis, MO). Double distilled and deionized water was filtered prior to use.

Preparation of Surfactant Solutions. A surfactant solution was prepared by mixing distilled water with propylene glycol (PG) in a 2:1 mass ratio, and then adding different amounts (0.5 to 23% w/w) of Tween 80, followed by stirring for 30 min to ensure dispersion and dissolution of surfactant.

Preparation of Colloidal Dispersions. A number of different approaches of forming colloidal dispersions using lemon oil and Tween 80 were examined: thermal treatment; high shear mixing; high pressure homogenization; and sonication. In all cases, the colloidal dispersions consisted of 10% w/w oil phase (lemon oil) and 90% w/w aqueous phase (0.5−23% Tween 80 in 2:1 water−PG solution).

Thermal Treatment. The oil and aqueous phases were placed in a glass beaker and mixed at ambient temperature ($\approx$ 23 °C) using a magnetic stirrer for 30 min. These systems were then transferred to test tubes, placed into a temperature controlled water-bath, heated from 23 to 90 °C at about 0.8 °C min^{-1}, and then kept at 90 °C for 30 min. The resulting systems were then cooled to ambient temperature.

High Shear Mixer. The oil and aqueous phases were weighed in a glass beaker, mixed at ambient temperature ($\approx$ 23 °C) using a magnetic stirrer for 30 min, and then homogenized for 2 min using a high-shear mixer (Tissu-Tearor, Biospec Products, Bartlesville, OK).

Microfludizer. The oil and aqueous phases were weighed in a glass beaker, mixed at ambient temperature ($\approx$23 °C) using a magnetic stirrer for 30 min, and then homogenized for 2 min using a high-shear mixer (Tissue-Tearor, Biospec Products, Bartlesville, OK). The resulting systems were then passed through a high pressure homogenizer for three passes at 9,000 psi (model M-110 L Microfluidizer Processor, Microfluidics, Newton, MA).

Ultrasonic. The oil phase and aqueous phases were placed in a glass beaker and mixed at ambient temperature ($\approx$23 °C) using a magnetic stirrer for 30 min. The resulting systems were then sonicated (Sonic Dismembrator 500, Fisher Scientific) for 1 min at an amplitude of 70% and a duty cycle of 0.5s using an ice−water bath to prevent excessive heating.

Electrical Conductivity Measurements. Electrical conductivity measurements were used to determine whether phase inversion (i.e., from oil-in-water to water-in-oil) occurred in the samples during heating. A small amount of salt (10 mM NaCl) was incorporated into the samples to be tested so as to increase their electrical conductivity. The samples were then continuously stirred using a mechanical stirrer (Field ESA, Partikel Analytik GmbH, Frechen, Germany) during temperature-scanning electrical conductivity measurements (Field ESA, Partikel Analytik GmbH, Frechen, Germany). The emulsions were heated gradually using a water bath, and the conductivity was measured as a function of temperature.

Turbidity Measurements. Temperature-scanning turbidity measurements were used to obtain information about potential changes in the microstructure of the samples during heating. Turbidity versus temperature scans were then performed on the samples 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.). The sample temperature, heating/cooling rate and data recording were handled through computer software (Swift 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 to blank the cells.

Differential Scanning Calorimetry. Different scanning calorimetry (DSC) was used to identify any thermal transitions in the samples during heating. An aliquot of the sample (8−10 mg) was placed in an aluminum pan and hermetically sealed. An empty pan was used as a reference. The sample and reference pans were placed into the measurement block of a differential scanning calorimeter (DSC; Q 1000, TA Instruments, New Castle, DE). Two heat−cool cycles were used to identify the critical temperatures and reversibility of any thermal transitions. Samples were placed in the DSC at room temperature, cooled to 10 °C at 5 °C/min, and then heated to 90 °C at 5 °C/min.

Rheological Behavior. The rheological behavior of selected colloidal dispersions was measured using a dynamic shear rheometer (Kinexus Rotational Rheometer, Malvern Instruments, Malvern, U.K). A concentric cylinder (code number: C25) measurement system was used: rotating inner cylinder, diameter 25 mm; static outer cylinder, diameter 27.5 mm. The samples were loaded into the rheometer measurement cell and allowed to equilibrate to 20 °C for 5 min. The samples were then heated from 20 to 90 at 5 °C/min at a fixed shear rate (i.e., 10 rpm). The apparent viscosity of the samples versus temperature was recorded using the instrument software.

Particle Size Determination. The mean particle radius ($r_{4,3}$ and $r_{3,2}$) and particle size distribution (PSD) of emulsions were measured using a static light scattering instrument (Mastersizer 2000 Malvern Instruments): $r_{4,3} = \Sigma n_i r_i^4 / \Sigma n_i r_i^3$ and $r_{3,2} = \Sigma n_i r_i^3 / \Sigma n_i r_i^2$, where n_i and r_i are the number and radius of the droplets in the ith size category. A few drops of sample were dispersed in approximately 125 mL of distilled water in the sample chamber with agitation until approximately 11−13% obscuration was obtained. The mean particle radius and particle size distribution of nanoemulsions or microemulsions were measured using dynamic light scattering (NanoZS, Malvern Instruments, Malvern, U.K.). The nanoemulsion samples were diluted 20× in appropriate phosphate buffer solution at ambient temperature prior to analysis to avoid multiple scattering effects. During these measurements we noticed that the turbidity of a number of the samples increased after they had been diluted and stored for 30 min, which suggested that there was an increase in mean particle radius induced by dilution. We found that this effect could be inhibited by heating the samples to 35 °C in a water bath for 5 min before measurement, and therefore we used this approach to prevent dilution-induced particle growth.

Statistical Analysis. All measurements were performed at least twice using freshly prepared samples, and are reported as means and standard deviations (calculated using Excel, Microsoft, Seattle, WA).

■ RESULTS AND DISCUSSION

Influence of Surfactant-to-Oil Ratio and Thermal Treatment. Initially, we prepared a series of 10% w/w lemon oil systems containing different amounts of Tween 80 so as to vary the surfactant-to-oil ratio (SOR). These samples were mixed, and then either maintained at ambient temperature or subjected to a thermal treatment (90 °C for 30 min) as described in Materials and Methods. The nature of the colloidal dispersions formed was initially determined by turbidity measurements and visual observations (Figure 1). Turbidity measurements were made immediately after samples had been vigorously mixed to make them homogeneous, whereas visual observations were made after the samples had been stored overnight.

Without thermal treatment, all samples were opaque and had relatively high turbidities after mixing (Figure 1a), and they
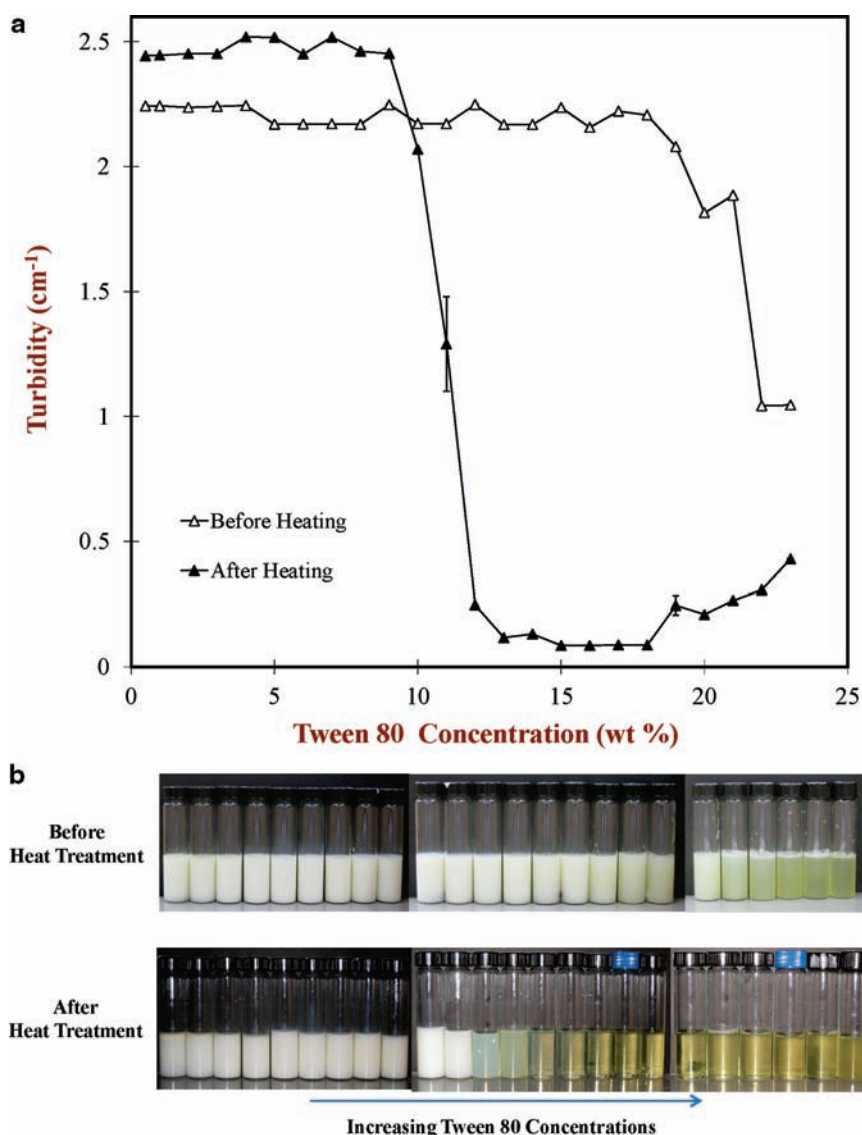
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Figure 1. (a) Dependence of turbidity on Tween 80 concentration in lemon oil (10 wt %)−Tween 80−buffer (mass ratio 1:2 PG:water) systems. "Before Heating": Samples were blended with a high shear mixed at ambient temperature. "After Heating": Samples were blended with a high shear mixed at ambient temperature, then heated to 90 °C for 30 min, and then rapidly cooled to ambient temperature. (b) Dependence of sample appearance on Tween 80 concentration in lemon oil (10 wt %)−Tween 80−buffer (mass ratio 1:2 PG:water) systems before and after thermal treatment. The Tween 80 concentration was increased from 0.5 wt % to 23 wt %; see panel a for actual concentration values.

exhibited phase separation after overnight storage, with an opaque cream layer forming on top of a turbid serum layer (Figure 1b). After thermal treatment, there was a distinct change in the appearance and stability of the systems depending on the Tween 80 concentration. From 0.5 to 10% w/w Tween 80, the heated systems remained optically opaque and had a high turbidity; from 10 to 13% w/w Tween 80, the turbidity decreased steeply; from 13 to 19% w/w Tween 80 the turbidity remained low and constant; and then the turbidity increased somewhat from 20 to 23% w/w Tween 80 (Figure 1a). However, the samples containing these high levels of surfactant did become transparent after being stored overnight (Figure 1b).

The appearance of the heated systems after being cooled to ambient temperature and stored overnight depended on surfactant concentration (Figure 1b). Systems containing 1 to 4% w/w Tween 80 separated into a cream layer on top of a milky white layer, suggesting that they contained relatively large droplets that scattered light strongly and creamed rapidly. Systems containing 6 to 10% w/w Tween 80 were homogeneous milky white liquids, suggesting that they contained intermediate-sized droplets that scattered light relatively strongly but were stable to creaming. Systems containing 10 to 14% w/w Tween 80 were homogeneous systems that were only slightly turbid or cloudy and stable to creaming. Systems containing >15% w/w Tween 80 were homogeneous transparent yellow liquids, which suggested that they contained very small particles that did not scatter light strongly or cream.[29] These experiments indicated that surfactant-to-oil ratio (SOR) and thermal treatment had a major impact on the type and stability of colloidal dispersions formed from lemon oil and Tween 80.

The mean particle radius (Figure 2) and particle size distribution (Figure 3) of selected 10 wt % lemon oil samples were also
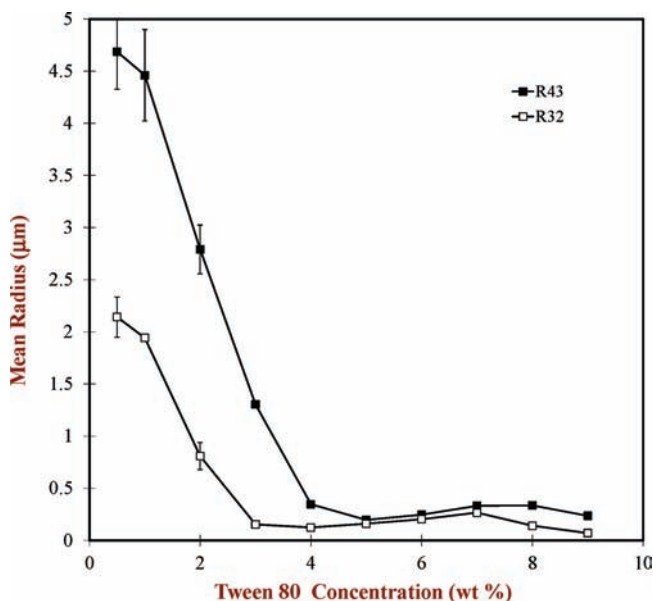
Figure 2. The dependence of the mean droplet radius (R_{32} or R_{43}) on Tween 80 concentration for lemon oil (10 wt %)−Tween 80−buffer (mass ratio 1:2 PG:water) systems after thermal treatment. Measurements at higher surfactant concentrations could not be made due to sample instability to particle growth upon dilution with water.
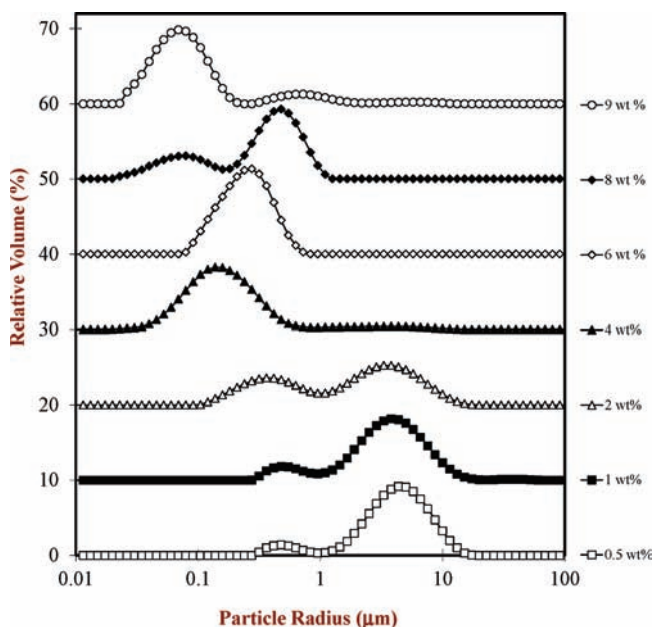

Figure 3. Particle size distributions of emulsions formed by thermal treatment of lemon oil (10 wt %)−Tween 80−buffer (mass ratio 1:2 PG:water) systems.

measured as a function of Tween 80 concentration after thermal treatment. Samples containing relatively low surfactant concentrations (SOR < 1) appeared to be stable to dilution with water (there was no change in the particle size distribution after dilution), but those containing relatively high surfactant concentrations (SOR ≥ 1) showed evidence of particle growth after dilution (as demonstrated by an increase in sample turbidity a few minutes after dilution). For this reason, we could not reliably

measure the samples with high surfactant-to-oil ratios (SOR ≥ 1). From 0.5 to 3 wt % surfactant (SOR ≤ 0.3), oil-in-water emulsions containing relatively large droplets were obtained (Figure 2): the particle size distributions were bimodal, with a small population of droplets with radii around 0.5 μm and a large population around 3 μm (Figure 3). As the emulsifier concentration was increased, the population of smaller droplets increased while the population of larger ones diminished until it eventually disappeared. This trend might be expected because there would be more surfactant present to cover the oil−water interface, thus enabling small droplets to be formed. From 4 to 10 wt % surfactant (0.4 ≤ SOR ≤ 1.0), the majority of droplets had radii <1 μm (Figure 3). The increase in sample turbidity that occurred after dilution with water at higher surfactant values (SOR ≥ 1) may be due to the fact that microemulsions moved to a composition where they were no longer thermodynamically stable. After dilution the overall concentration of propylene glycol in the aqueous phase would have decreased dramatically. In addition, there would have been a strong tendency for microemulsion particles to dissociate into monomers at high dilutions due to the increase in entropy of mixing (so the concentration may have fallen below the critical micelle concentration). This would pose a limitation on the commercial application of these high surfactant systems, since flavor oil emulsions are usually diluted extensively with an aqueous solution when they are incorporated into beverages. Possible physicochemical mechanisms responsible for the formation of the different types of colloidal dispersions are discussed in a later section.

Characterization of Thermal Behavior. As discussed above, thermal treatment was needed to facilitate the formation of stable colloidal dispersions from lemon oil and Tween 80. We therefore examined the influence of heating on the formation and properties of lemon oil colloidal dispersions in more detail by measuring the change in sample turbidity, electrical conductivity, enthalpy, and rheology when the temperature was changed.

Turbidity Measurements. Oil−surfactant−buffer mixtures were blended together, then heated from 20 to 90 °C, and then cooled from 90 to 20 °C at a heating/cooling rate of 1 °C/min, and the change in their turbidities (τ) and appearance was measured. The turbidity−temperature profiles of the mixtures depended strongly on surfactant concentration (Figure 4).

At a low surfactant concentration (7% Tween 80; SOR = 0.7), the samples were initially optically opaque ($\tau > 3$ cm^{-1}) after blending at ambient temperature. The turbidity remained high ($\tau > 3$ cm^{-1}) during heating and cooling over the whole temperature range studied (Figure 4a). Visual observation of the samples indicated that they were susceptible to phase separation (creaming) before heating, but were homogeneous and uniformly optically opaque after heating, i.e., no oiling off or phase separation occurred after heating.

At an intermediate surfactant concentration (13% Tween 80; SOR = 1.3), the samples were again initially optically opaque ($\tau > 3$ cm^{-1}) after blending at ambient temperature. Upon heating, the turbidity remained high from 20 to 54 °C, decreased appreciably from 54 to 83 °C, reached a value close to zero at 83 °C, and then increased appreciably at higher temperatures (Figure 4b). These measurements suggested that there was some change in the structure of the particles within these colloidal dispersions during heating, and that overheating promoted droplet aggregation (an increase in turbidity). The increase in turbidity observed at higher temperatures may have been due to
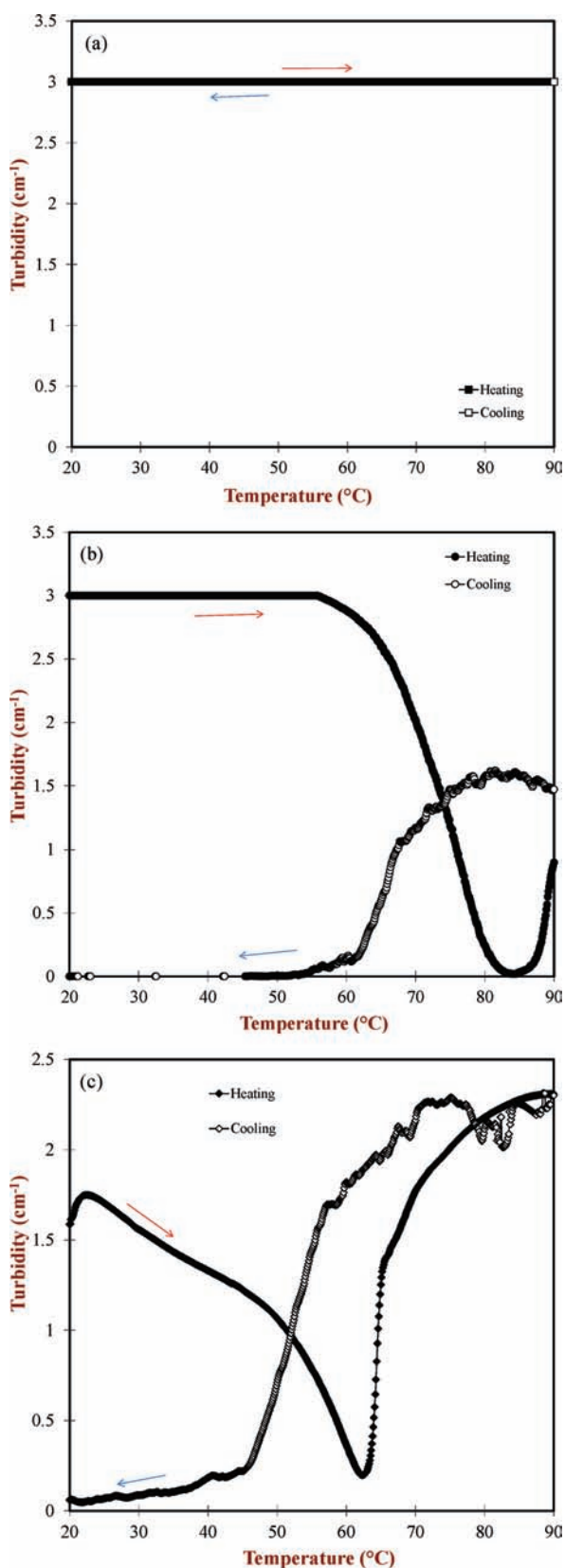
Figure 4. Temperature-dependence of turbidity of emulsions prepared by blending lemon oil (10 wt %)−Tween 80−buffer (mass ratio 1:2 PG: water) systems at ambient temperature and then heating and cooling. Samples contained different Tween 80 concentrations: (a) 7 wt %; (b) 13 wt %; (c) 20 wt %.

an increase in particle size caused by droplet coalescence. Upon cooling to ambient temperature, the turbidity of the sample gradually decreased from 90 to 54 °C, and maintained a value close to zero at lower temperatures (Figure 4b). We propose that the lemon oil−Tween 80−buffer solution spontaneously formed a nanoemulsion during the heat−cool cycle due to a physicochemical mechanism discussed later.

At a high surfactant concentration (20% Tween 80), the samples appeared homogeneous and highly turbid ($\tau > 1.5\,cm^{-1}$) at ambient temperatures, but their turbidity decreased steadily when the temperature was raised from 22 to 60 °C and they became transparent around 62 °C. However, the turbidity increased appreciably when the temperature was increased from 62 to 90 °C. Upon cooling back to ambient temperature, the turbidity of the samples decreased steeply from 90 to 44 °C and then gradually decreased and remained low at ambient temperature (Figure 4c). These measurements again indicated that heating promoted appreciable changes in the structure of the colloidal dispersions, presumably leading to the formation of smaller particles at ambient temperature that only scattered light weakly.

Electrical Conductivity. Electrical conductivity measurements are often used to follow phase inversions of emulsions and microemulsions, i.e., from oil-in-water (O/W) to water-in-oil (W/O) or vice versa. The electrical conductivity of water is much higher than that of oil so the conductivity of an O/W emulsion is much greater than that of a W/O emulsion. The temperature dependence of the electrical conductivity of a series of oil−surfactant−buffer systems with different Tween 80 concentrations was measured to determine if they underwent a phase transition during heating: 10 wt % lemon oil; 7, 13, or 20 wt % Tween 80; 83, 77, or 70 wt % buffer (containing 10 mM NaCl). Initially, the surfactant, oil and buffer were blended together at ambient temperature to form a coarse emulsion. The electrical conductivity of these coarse emulsions was then measured as their temperature was increased (Figure 5).

Independent of surfactant concentration, all of the emulsions exhibited a fairly similar general dependence of the electrical conductivity on temperature. Initially, the electrical conductivity of the samples was relatively high, which can be attributed to the fact that the continuous phase of the initial O/W emulsions was aqueous. There was a steady increase in electrical conductivity with increasing temperature, which is to be expected for aqueous solutions (Figure 5). Notably, we did not observe a temperature where there was an appreciable decrease in electrical conductivity, which suggested that oil continuous W/O emulsions or microemulsions were not formed. This may have been because the total oil concentration (10% lemon oil) was relatively low, and so it was not possible to form an oil continuous system. Nevertheless, we did observe an appreciable change in the electrical conductivity of the sample containing 13% Tween 80 around 80 °C and the sample containing 20% Tween 80 around 59 °C (Figure 5), which suggests that there may have been some change in their structures at these temperatures. Further studies using other analytical methods (such as temperature scanning light scattering or polarized microscopy) would be needed to identify the origin of these structural changes.

Rheology. The rheology of an oil−surfactant−buffer system would be expected to change if it underwent a major alteration in structure or if a phase inversion occurred (O/W to W/O). We therefore used temperature-scanning viscosity measurements to determine whether the samples underwent any significant
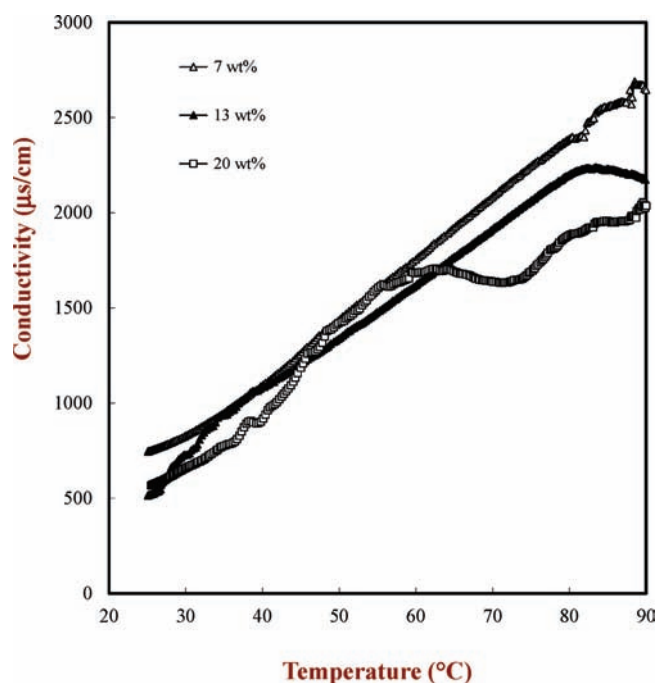
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Figure 5. Temperature-dependence of the electrical conductivity of emulsions prepared by blending lemon oil (10 wt %)−Tween 80−buffer (mass ratio 1:2 PG:water) systems at ambient temperature and then heating. Samples contained different Tween 80 concentrations (7, 13, 20 wt %).

Figure 6. Temperature-dependence of the relative viscosity of emulsions prepared by blending lemon oil (10 wt %)−Tween 80−buffer (mass ratio 1:2 PG:water) systems at ambient temperature and then heating. Samples contained different Tween 80 concentrations (7, 13, 20 wt %).

changes in rheological properties during heating. We measured the shear viscosity of oil−surfactant−buffer systems (η), as well as the shear viscosity of surfactant/buffer systems in the absence of oil (η_0), so as to identify any effects associated with the colloidal particles themselves.[30] The relative viscosity ($\eta_{relative} = \eta/\eta_0$) was then plotted as a function of temperature. The Einstein equation for dilute nonaggregated emulsions predicts that $\eta/\eta_0 = (1 + 2.5\phi)$, which is approximately equal to 1.25 for our system since the disperse phase volume fraction (ϕ) ≈ 0.1.[6] The shear viscosity of the samples was measured as they were heated from 20 to 90 at 5 °C min^{-1}, using a constant shear stress of 1 Pa.

The temperature-dependence of the relative viscosity of a series of samples with different surfactant concentrations (7, 13, or 20 wt % Tween 80) was measured (Figure 6). At 7 wt % Tween 80 (SOR = 0.7), the relative viscosity remained low and fairly constant across the entire temperature range, as would be expected for a nonaggregated oil-in-water emulsion. If there had been an O/W to W/O phase inversion, then one would have expected an appreciable change in relative viscosity since oil has a different viscosity than buffer solution. At 13 wt % Tween 80 (SOR = 1.3), the relative viscosity decreased appreciably from 20 to 34 °C, remained relatively constant from 34 to 77 °C, and then increased somewhat from 77 to 90 °C. This increase in relative viscosity at high temperatures is consistent with the increase in turbidity observed over a similar temperature range (Figure 4b). At 20 wt % Tween 80, the relative viscosity was appreciably greater than 1.25 at ambient temperature, which suggested that the effective particle volume fraction was higher than the actual amount of lemon oil present. This effect may be due to the fact that the surfactant made up an appreciable fraction of the particles in a nanoemulsion, so that their effective volume
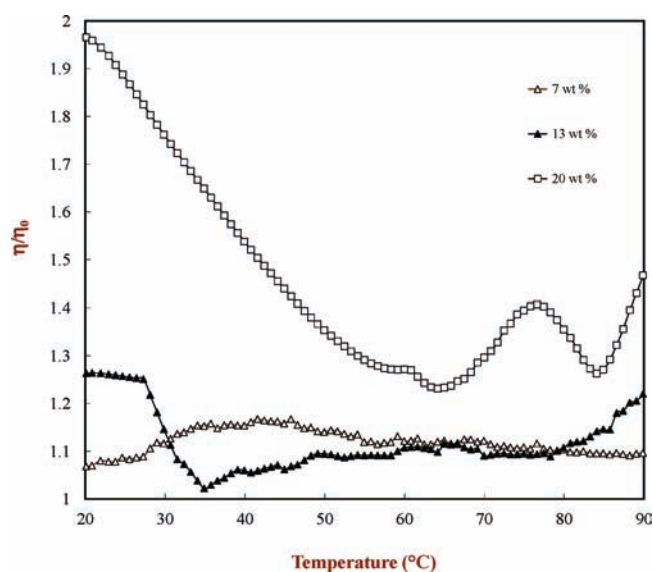
fraction was appreciably higher than the lemon oil volume fraction, or it may have been because of some particle−particle interactions at lower temperatures. When the samples were heated, the relative viscosity decreased from 20 to 62 °C, then increased from 62 to 75 °C, then decreased again from 75 to 83 °C, and then increased again from 83 to 90 °C (Figure 6). The complex nature of the relatively viscosity versus temperature profile of this sample suggests that there were a number of physicochemical changes in the system during heating. The first minimum in the η/η_0−T profile at around 64 °C corresponds to the minimum in the turbidity−T profile (Figure 4c) and decrease in the electrical conductivity−T profile (Figure 5) observed at a similar temperature.

Enthalpy Changes. Finally, we used differential scanning calorimeter (DSC) measurements to monitor changes in the enthalpy of the samples during heating. The absorption or release of heat by a sample during heating can provide valuable information about phase transitions. The temperature dependence of the enthalpy changes of a series of oil−surfactant−buffer systems with different Tween 80 concentrations was measured: 10 wt % lemon oil; 7, 13, or 20 wt % Tween 80; 83, 77, or 70 wt % buffer. DSC experiments were also carried out using surfactant solutions in the absence of lemon oil so as to identify the influence of the oil droplets on the overall enthalpy changes. Initially, the oil, surfactant, and buffer were blended together at ambient temperature to form a coarse emulsion. The enthalpy changes associated with these samples were then measured as their temperature was increased from 20 to 90 °C at a heating rate of 5 °C/min (Figure 7). The transition temperature (T_m) and enthalpy change (ΔH) of the different systems were calculated from the resulting thermograms (Table 1).

In the absence of lemon oil, none of the surfactant solutions showed any thermal transitions across the whole temperature range studied (Figure 7). On the other hand, in the presence of lemon oil, a broad single endothermic peak was observed from about 30 to 65 °C for each of the surfactants (Figure 7). The transition temperature and enthalpy change depended on the
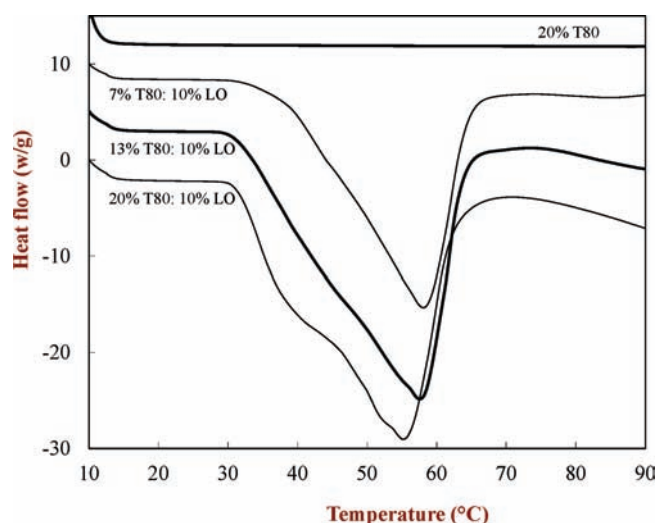
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Figure 7. Temperature-dependence of the enthalpy changes of aqueous surfactant solutions (20% Tween 20) or colloidal dispersions prepared by blending lemon oil (10 wt %)—Tween 80 (7, 13, or 20 wt %)—buffer (1:2 PG:water) systems at ambient temperature and then heating.

Table 1. Thermal Transition Temperature and Enthalpy Change for Oil—Surfactant—Buffer Systems Consisting of 10 wt % Lemon Oil and Different Tween 80 Concentrations

Tween 80 (wt %)	T_m (°C)	ΔH (J/g)
7	55.1	739
13	57.5	715
20	58.0	520

surfactant concentration in the lemon oil—surfactant—buffer systems (Table 1). As the Tween 80 concentration increased from 7 to 20 wt %, the transition temperature increased slightly from ≈55 to 58 °C; but the enthalpy change per unit mass of sample decreased appreciably from ≈740 to 520 J/g (Table 1). The observation of a fairly similar transition peak in all oil—surfactant—buffer samples suggests that a similar physico-chemical phenomenon was involved. The fact that no enthalpy peaks were observed in the samples containing only surfactant and buffer indicated that lemon oil played an important role in the thermal transitions. We postulate that the enthalpy changes observed in the emulsion systems were due to a change in the overall contact area between nonpolar and polar components during heating. This enthalpy change can be attributed to an alteration in the overall molecular interactions in the system (mainly hydrogen bond formation) due to the hydrophobic effect. Isothermal titration calorimetry (ITC) measurements on surfactant solutions have shown that the enthalpy change associated with an increase in oil—water contact area is endo-thermic at temperatures above ambient.[31] Consequently, the endothermic peak observed in the lemon oil—surfactant—buffer systems may have been due to an increase in the contact area between nonpolar and polar components with increasing tem-perature. A possible physicochemical mechanism for this in-crease in contact area is the greater intermingling of the oil and aqueous phases that occurs when the surfactant head groups become dehydrated and the surfactant molecules move from the aqueous phase into the oil phase. The enthalpy changes asso-ciated with the transition occurred over a temperature range where there was an appreciable decrease in turbidity (Figure 4), electrical conductivity (Figure 5) and viscosity (Figure 6) of the samples containing higher surfactant concentrations.

Potential Physicochemical Mechanism for Thermal Beha-vior. In this section, we propose a physicochemical mechanism to account for the observed thermal behavior of the various lemon oil—surfactant—buffer systems. Based on our experimen-tal observations we have drawn a schematic representation of the dependence of the colloidal dispersions formed on thermal

treatment and surfactant concentration (Figure 8).Our results suggest that there is a kinetic energy barrier at ambient tempera-ture that retards the transition of the mixtures from the initial state formed after blending to the more kinetically (emulsions or nanoemulsions) or thermodynamically (microemulsions) stable state formed after thermal treatment. This is most clearly seen at the highest surfactant concentrations where a metastable opaque nanoemulsion is formed after blending at ambient temperature, but a thermodynamically stable transparent microemulsion is formed after blending then heating (Figure 1). We propose that a kinetic energy barrier (ΔG^*) is related to the oil—water inter-facial tension (γ) of the system, e.g., $\Delta G^* \propto \gamma$. At high surfactant concentrations, the lemon oil molecules within the relatively large oil droplets formed by blending have to be transferred into the very small microemulsion droplets. This process may occur due to movement of lemon oil molecules through the continuous phase, spontaneous budding of microemulsion droplets from the oil—water interface, or collision of surfactant micelles with the oil—water interface and incorporation of lemon oil molecules. All of these processes would be expected to depend on the oil—water interfacial tension.

The nonionic surfactant used in this study (Tween 80) has a polar headgroup that consists of a number of polyoxyethylene chains. The physicochemical and functional properties of this type of surfactant are known to change appreciably with temperature.[20,32] These changes can be described in terms of the hydrophilic—lipophilic deviation (HLD) of the surfactant. The HLD number is a parameter that describes the relative thermodynamic affinity of a surfactant for a hydrophilic (aqueous) phase and a lipophilic (oil) phase.[33−35]

- HLD < 0: (i) The surfactant has a higher affinity for the water phase than the oil phase; (ii) the surfactant tends to form normal micelles or microemulsions in the aqueous phase; (iii) the interfacial tension is relatively high; (iv) the surfactant tends to stabilize O/W (rather than W/O) emulsions. The more negative the HLD number, the greater the affinity for the water phase.

- HLD = 0: (i) The surfactant has an equal affinity for the water and oil phases; (ii) the surfactant tends to form bicontinuous microemulsion or liquid crystalline phases; (iii) the interfacial tension tends to be very low; (iv) the surfactant tends to stabilize neither O/W nor W/O emulsions.

- HLD > 0: (i) The surfactant has a higher affinity for the oil phase than the water phase; (ii) the surfactant tends to form reverse micelles or microemulsions in the oil phase; (iii) the interfacial tension is relatively high; (iv) the surfactant tends to stabilize W/O (rather than O/W) emulsions. The more positive the HLD number, the greater the affinity for the oil phase.

At relatively low temperatures, the surfactant headgroup is highly hydrated, which means that the surfactant is more soluble in the aqueous phase than in the oil phase (HLD < 0). Under
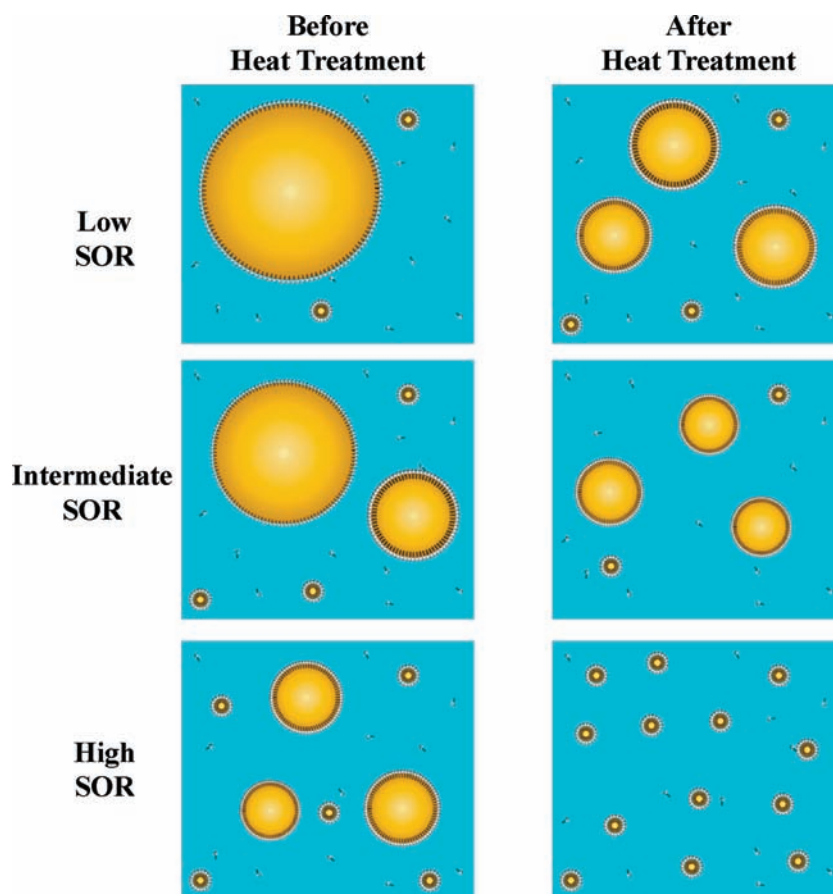
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Figure 8. Schematic representation of the various colloidal dispersions formed by lemon oil—Tween 80—buffer mixtures. The structures formed depend on thermal treatment and surfactant-to oil ratio (SOR).

these conditions the formation of an oil-in-water microemulsion, nanoemulsion or emulsion is favored. The type of colloidal dispersion formed will depend on the SOR: if there is sufficient surfactant present to solubilize all of the lemon oil, then a thermodynamically stable microemulsion is formed; but, if there is insufficient surfactant present, then an emulsion or nanoemulsion will be formed. As the temperature is increased the head-group becomes progressively dehydrated until HLD reaches 0, at which point the surfactant has an equal affinity for both phases. This temperature appears to be around 55 to 65 °C for the system used in this study (Figures 4, 5, 6, and 7). In this temperature range the oil—water interfacial tension will become extremely low, which will favor the intermingling of the polar and nonpolar components, thereby facilitating the spontaneous formation of microemulsion or emulsion droplets. At higher temperatures, the surfactant becomes more soluble in the oil phase than in the water phase (HLD > 0). At sufficiently high oil concentrations this would normally lead to the formation of a water-in-oil microemulsion, nanoemulsion or emulsion. However, at the low oil concentrations used here (10%), we would expect a highly unstable O/W emulsion or W/O/W emulsion to form containing relatively large oil droplets, which would account for the observed increase in turbidity that occurred at elevated temperatures in the samples containing high surfactant concentrations (Figures 4b and 4c).

A physicochemical mechanism has recently been proposed to account for the spontaneous formation of emulsion or nanoemulsion droplets when a mixture of oil, surfactant and water are cooled from above to below a phase inversion temperature.[36,37] When the system is cooled below the phase inversion temperature, the surfactant molecules rapidly move from the oil phase to the water phase, which leads to a budding process at the oil—water interface and the spontaneous formation of small droplets. These small droplets only scatter light weakly accounting for the low turbidity of the samples after cooling back to ambient temperature.

In summary, our measurements suggest there is a kinetic energy barrier in the oil—surfactant—water system when they were first mixed together at ambient temperature, which prevented the system from moving into a more kinetically stable state (emulsions and nanoemulsions) or thermodynamically stable state (microemulsions). When the temperature was raised, this kinetic energy barrier was reduced, which facilitated this transition. This kinetic energy barrier is probably associated with the positive oil—water interfacial tension, which is known to decrease appreciable near the phase inversion temperature. Thus, a simple thermal treatment seems to be sufficient to form different kinds of lemon oil dispersions (microemulsions, nanoemulsions or emulsions) depending on the surfactant-to-oil ratio.

Influence of High Energy Homogenization. Currently, most emulsions and nanoemulsions used in the food industry are prepared using high energy methods, such as high pressure valve homogenization, microfluidization, or sonication.[38,39] It is
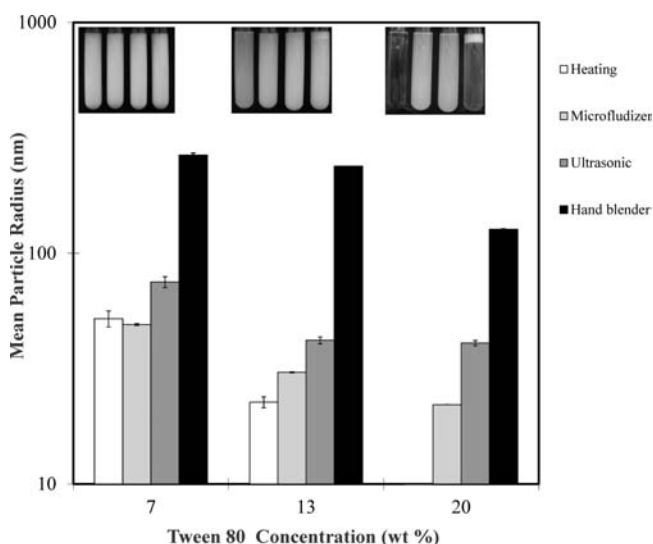
Figure 9. Influence of different homogenization methods and Tween 80 concentrations on the formation of colloidal dispersions consisting of lemon oil (10 wt %)−Tween 80−buffer (mass ratio 1:2 PG:water). Photographs of the samples were made after storage overnight.

therefore useful to contrast the low energy method of preparing lemon oil colloidal dispersions described in the previous section with some high energy methods, since each approach has its own advantages and disadvantages.

Three samples with different SOR ratios were homogenized using thermal treatment, a high shear mixer, a microfluidizer, or a sonicator, and then their mean particle radii and appearances were measured (Figure 9).

Thermal Treatment. After heat treatment, relatively stable nanoemulsions ($r < 100$ nm) were formed at 7 and 13 wt % Tween 80, whereas microemulsions ($r < 10$ nm) were formed at 20 wt % Tween 80 as discussed in detail in the previous sections.

High Shear Mixer. After homogenization using a high shear mixer, the samples were optically opaque, exhibited phase separation after overnight storage (with a cream layer forming on top of a turbid serum layer), and contained relatively large droplets ($r > 130$ nm) (Figure 9). Even the samples with the highest surfactant concentration (20 wt % Tween 20) contained relatively large droplets that were unstable to creaming. These results indicate that the energy input associated with high shear blending was insufficient to overcome the kinetic energy barrier required to form stable emulsions, nanoemulsions or microemulsions.

Microfluidizer. After microfluidization, all of the samples contained relatively small droplets ($r < 100$ nm) and could therefore be considered to be nanoemulsions, with the mean droplet radius decreasing with increasing Tween 80 concentration, i.e., from around 50 to 22 nm as the Tween 80 concentration was increased from 7 to 20 wt % (Figure 9). Microfluidization therefore appeared to be more effective than high shear mixing at forming nanoemulsions at ambient temperature. Nevertheless, the droplet size was still relatively large in the microfluidized samples containing the highest surfactant concentration (20 wt % Tween 80) when compared to the equivalent heat treated samples. This suggests that the energy input associated with microfluidization was also insufficient to overcome the kinetic energy barrier required to form microemulsions at ambient temperatures.

Sonication. Samples prepared by sonication had fairly similar properties to the samples prepared by microfluidization: nanoemulsions could be formed at all surfactant concentrations, but microemulsions could not be formed at the highest surfactant concentration where they could by thermal treatment (Figure 9).

In summary, these studies show that microemulsions can only be formed by heating at high SOR, but that nanoemulsions can be formed by heating, ultrasonication and microfluidization at medium SOR. Conventional emulsions could be formed by high shear mixing or thermal treatment at low SOR.

Conclusions. This study focused on understanding the nature of the colloidal dispersions that could be formed using Tween 80 as a nonionic surfactant and lemon oil as an edible lipid phase. Conventional emulsions ($r > 100$ nm, metastable), nanoemulsions ($r < 100$ nm, metastable) or microemulsions ($r < 10$ nm, thermodynamically stable) could be formed depending on the surfactant-to-oil ratio (SOR) and the homogenization method used. We have shown that a simple thermal treatment (90 °C, 30 min) can be used to form nanoemulsions (medium SOR) or microemulsions (high SOR) from lemon oil and Tween 80. Our results suggest that there is a kinetic energy barrier at ambient temperature that prevents the oil−surfactant−buffer system from reaching its most kinetically or thermodynamically stable state. The application of heating appears to be much more effective than the application of mechanical energy (blending, microfluidization or sonication) at overcoming this energy barrier. Overall, this study provides some useful insights into the formation and stability of different types of food grade colloid dispersions containing flavor oils. This information may be useful for designing colloidal dispersions for incorporating flavor oils or other lipophilic components into specific products, e.g., clear versus cloudy beverages.

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