

Influence of Nanoemulsion Addition on the Stability of Conventional Emulsions

L. Salvia-Trujillo¹ · D. J. McClements^{1,2}

Received: 24 March 2015 / Accepted: 30 April 2015 / Published online: 10 May 2015
© Springer Science+Business Media New York 2015

Abstract Interest in using nanoemulsions as delivery systems for lipophilic food ingredients is growing due to their high optical clarity, good physical stability, and ability to increase bioavailability. Nanoemulsion-based delivery systems may need to be incorporated into food matrices that also contain conventional emulsions. The aim of this work was to evaluate the effect of adding nanoemulsions ($d < 200$ nm) to conventional emulsions ($d > 200$ nm) on the creaming stability and microstructure of the mixed systems. Droplet flocculation and rapid creaming was observed when the nanoemulsion concentration exceeded a particular level: the critical flocculation concentration (CFC) was 3.75 % and 0.25 % (v/v) for conventional emulsions with average droplet diameters of 350 and 250 nm, respectively. Confocal microscopy indicated that there was appreciable droplet flocculation, and the fraction of individual droplets with diameters < 100 nm decreased after 14 days storage, which was probably due to Ostwald ripening and/or coalescence. The results of the present study might have important implications for the incorporation of nanoemulsion-based delivery systems into food products containing larger fat droplets, such as dressings, sauces, or beverages.

Keywords Nanoemulsions · Emulsions · Depletion flocculation · Stability · Creaming · Confocal microscopy · Food matrix

✉ D. J. McClements
mcclements@foodsci.umass.edu

¹ Department of Food Science, University of Massachusetts, Amherst, MA 01003, USA

² Department of Biochemistry, Faculty of Science, King Abdulaziz University, P. O. Box 80203, Jeddah 21589, Saudi Arabia

Introduction

The development of nanoemulsion-based delivery systems for functional lipids (such as flavor oils, antimicrobials, vitamins, and nutraceuticals) is rapidly growing [1–3]. The main reason for the considerable interest in this type of delivery system is that optically transparent and physically stable systems can be formed, and that the bioavailability of encapsulated substances can be increased [4, 5]. Nevertheless, nanoemulsions must be carefully formulated to ensure that they will remain physically and chemically stable during storage. In particular, nanoemulsions containing fairly polar lipids (such as flavor or essential oils) are highly prone to droplet growth through Ostwald ripening, which is the process whereby oil molecules migrate from small to large droplets due to differences in droplet curvature. This process can be retarded by mixing the polar lipids prior to homogenization with highly non-polar lipids (such as long-chain triglycerides) that act as ripening inhibitors [6]. It is also important to select an appropriate emulsifier that will facilitate both the formation and stability of the nanoemulsion. Considerable progress has been made in designing and fabricating nanoemulsion-based delivery systems with physicochemical and functional properties suitable for utilization by the food industry.

However, there is currently an important knowledge gap in understanding the behavior of nanoemulsions once they are incorporated into complex food matrices. Food products vary appreciably in their compositions, structures, and processing requirements, which may alter the stability and performance of any nanoemulsion-based delivery systems introduced into them. An important category of food products where nanoemulsion-based delivery systems may be incorporated are conventional emulsions, such as dressings, sauces, dips, desserts, and beverages. Conventional emulsions contain lipid droplets with mean droplet diameters greater than about

200 nm, whereas nanoemulsions have mean droplet diameters less than this value. Various destabilization phenomena may occur when two colloidal dispersions containing different-sized particles are mixed together, including depletion flocculation [7] and accelerated Ostwald ripening [8].

Depletion flocculation arises in mixed colloidal dispersions when the concentration of smaller particles in the continuous phase exceeds a certain value [9]. The origin of this effect is an osmotic pressure that arises due to exclusion of smaller particles from a narrow region surrounding the larger particles [8]. The osmotic pressure increases as the concentration of smaller particles increases, which eventually causes the larger particles to flocculate to reduce the volume from which the smaller particles are excluded. There are several types of particles capable of inducing depletion flocculation in emulsions, including polysaccharides [10], surfactant micelles [11], and proteins [12]. Nevertheless, certain kinds of nanoparticles may also promote depletion flocculation in emulsions [13], with the depletion interaction depending of the nature of the particles [14].

The rate of Ostwald ripening may also be accelerated in mixed colloidal dispersions containing small and large droplets. The main driving force for Ostwald ripening is the difference in particle dimensions, and therefore this process may be accelerated in emulsions containing different-sized droplets [15]. In addition, the direct contact of flocculated oil droplets might promote Ostwald ripening in emulsions by reducing the diffusion path length [16]. In principle, destabilization mechanisms associated with mixed colloidal dispersions may have detrimental effects on both the stability of the conventional emulsion (food product) and the nanoemulsion (delivery system).

The objective of the present work was therefore to investigate the influence of addition of nanoemulsions to conventional emulsions on their stability to droplet growth and flocculation. In particular, we aimed to elucidate the origin of any destabilization mechanisms caused by mixing nanoemulsion droplets with emulsion droplets. This information is important for designing delivery systems that can be successfully incorporated into a wide range of commercial food products.

Material and Methods

Materials

Lemon oil (SC 020207, Lot: 2894308) was kindly donated by International Flavors and Fragrances (Union Beach, N.J.). Corn oil was purchased from a local supermarket. Tween 80, monobasic and dibasic phosphates and Nile Red dye were purchased from Sigma-Aldrich (St. Louis, MO). All aqueous solutions were prepared using purified water from a Milli-Q

filtration system (EMD Millipore, Merck KGaA, Darmstadt, Germany).

Methods

Conventional Emulsion Formation

Conventional emulsions were prepared using a relatively low surfactant-to-oil ratio (SOR) with pure corn oil as the dispersed phase. A mixture of 4 % (w/w) corn oil, 0.4 % (w/w) Tween 80 (SOR 0.1), and 95.6 % (w/w) aqueous buffer solution (5 mM phosphate buffer, pH 7) was blended with a high-speed blender at 10,000 rpm for 2 min to obtain a coarse emulsion. The final oil concentration in the conventional emulsions was selected to mimic a typical level found in some emulsion-based foods, such as beverages. This coarse emulsion was then passed three times through a two-stage homogenizer (LAB 1000, APV-Gaulin, Wilmington, MA) working at 5000 psi or 1000 psi to obtain conventional emulsions designated as “o/w₂₅₀” and “o/w₃₅₀”, respectively, according to their mean particle diameter.

Nanoemulsion Formation

Nanoemulsions were prepared using a relatively high SOR and a mixture of corn oil and flavor oil as the dispersed phase. Corn oil and lemon oil were mixed at a 1:1 ratio and stirred at room temperature for at least 1 h to obtain a homogenous dispersed phase. A blend containing a 20 % (w/w) oil phase, 15 % (w/w) Tween 80 (SOR 0.75), and 65 % (w/w) aqueous phase (5 mM phosphate buffer pH 7) was mixed by means of a high-speed blender at 10,000 rpm for 2 min to obtain a coarse emulsion. This coarse emulsion was then passed three times through a microfluidizer (model M110-P, Microfluidics, Newton, MA) working at 25,000 psi to form a nanoemulsion. The nanoemulsion was used as a stock solution in the mixing experiments to achieve different concentrations of lipid nanoparticles in the final mixtures.

Mixing Experiments

To study the effect of nanoemulsion addition on the properties of conventional emulsions we initially prepared a series of nanoemulsions with different droplet concentrations. The concentrated nanoemulsion containing 20 % (w/w) dispersed phase was diluted with an aqueous solution (5 mM phosphate buffer, pH 7) to obtain nanoemulsions containing 10, 7.5, 5, 2.5, 1, 0.5, 0.1 % of oil (w/w). Then, aliquots of 5-mL of conventional emulsions o/w₂₅₀ or o/w₃₅₀ were mixed with 5-mL aliquots of nanoemulsions with different oil concentrations with a magnetic stirrer for 5 min to produce mixed emulsions with different compositions (Table 1). We also carried out mixture experiments of conventional emulsions with

Table 1 Compositions of conventional emulsions and nanoemulsions before and after mixing. The homogenization conditions were three passes at 5 kpsi (o/w₂₅₀); 1 kpsi (o/w₃₅₀); or, 25 kpsi (Nanoemulsion)

Before mixing					After mixing (1:1)		
Conventional emulsions (o/w ₂₅₀ or o/w ₃₅₀)			Nanoemulsion		Final composition		
Mixed system	Oil concentration (% w/w)	Surfactant concentration (% w/w)	Oil concentration (% w/w)	Surfactant concentration (% w/w)	Oil concentration (% w/w)	Surfactant concentration (% w/w)	
1		4	0.4	20.0	15.0	12.0	7.60
2		4	0.4	10.0	7.50	7.00	3.85
3		4	0.4	7.5	5.63	5.75	2.91
4		4	0.4	5.0	3.75	4.50	1.98
5		4	0.4	2.5	1.88	3.25	1.04
6		4	0.4	1.0	0.750	2.50	0.475
7		4	0.4	0.5	0.375	2.25	0.288
8		4	0.4	0.1	0.075	2.05	0.138
9		4	0.4	0.0	0.000	2.00	0.100

surfactant solutions at equivalent surfactant concentration to the one used in the nanoemulsions. In this case, Tween 80 solutions were prepared and mixed with conventional emulsions after homogenization by magnetic stirring for 5 min. After mixing the conventional emulsions and nanoemulsions, samples were stored in the absence of light and at room temperature for 14 days.

Creaming Index

Creaming of mixed systems (10 mL) was monitored at room temperature by visual observation and measurement with a ruler of the height of the serum layer formed at the bottom of glass tubes (H_s), expressed as a percentage of the total height of the emulsions in the tubes (H_e):

$$CI = \frac{H_s}{H_e} \times 100$$

The thickness of the cream layer on top of the test tubes was also measured over 120 h to monitor the creaming over time.

Droplet Size Characterization

The particle size distribution of single and mixed emulsions was measured by dynamic light scattering (DLS) (Zetasizer NanoZS, Malvern Instruments Ltd, Worcestershire, UK) at a wavelength of 633 nm and temperature of 25 °C using a backscatter detector (173 °) and the particle was reported as the Z-average diameter (nm). Samples were diluted prior to analysis with buffer solution (1:10) to avoid multiple scattering effects and to achieve an attenuation of the laser beam between 5 and

10. Since the mixed systems had a bimodal size distribution due to the presence of small and large droplets, the data from the correlation function was analyzed using the method of multiple narrow modes to obtain the droplet size distribution of the two peaks with a higher resolution.

Confocal Fluorescence Microscopy

Confocal fluorescence microscopy images were taken to determine destabilization phenomena in the conventional emulsions after mixing with the nanoemulsions. Emulsions (single or mixed) were dyed with Nile red (a fat-soluble fluorescent dye) that was previously dissolved at 0.1 % (w/v) in ethanol. An air-cooled argon ion laser Model IMA1010 BOS (Melles Griot, Carlsbad, CA) was used to excite Nile red at 488 nm. A Nikon Confocal Microscope (Nikon D-Eclipse C1 80i, Nikon, Melville, NY) with a 60× oil immersion objective lens was used to capture the confocal images. The resulting fluorescent spectra of Nile red were detected in the 515 nm channel equipped with a narrow pass filter (HQ 515/30 m) with a pinhole size of 150 μm. The images generated had a size of 512×512 pixels, with a pixel size of 414 nm, and a pixel dwell time of 61.44 μs. All images were taken and processed using the instrument software program (EZ- CS1 version 3.8, Nikon, Melville, NY).

Statistical Analysis

All experiments were assayed in duplicate, and results were expressed as the mean and the standard deviation. A statistical analysis software program (JMP 8, SAS Institute Inc.) was used to perform the analysis of variance. The Student's *t* test

was run to determine significant differences at a 5 % significance level ($p < 0.05$).

Results and Discussion

Influence of Nanoemulsion Addition on Creaming Stability of Conventional Emulsions

Initially, we evaluated the effect of nanoemulsion addition on the stability of two conventional emulsions with different mean droplet diameters: *Emulsion o/w₂₅₀*, $d=250$ nm; *Emulsion o/w₃₅₀*, $d=350$ nm. There was some overlap in the size of the droplets in the two conventional emulsions, but *Emulsion o/w₃₅₀* had more large droplets than *Emulsion o/w₂₅₀* (Fig. 1). The nanoemulsion had a monomodal particle size distribution and a relatively small mean droplet diameter ($d=50$ nm), which can be attributed to the fact that it contained a high surfactant level (SOR=0.75) and was prepared using lemon oil (which has a low viscosity and interfacial tension). The particle size of the stock nanoemulsion (20 % w/w oil) remained constant throughout 30 days of storage (data not shown). The reason for the good stability of this system can be attributed to the ability of the corn oil to act as a ripening inhibitor, which retards Ostwald ripening through an entropy of mixing effect [15, 17].

After nanoemulsions and emulsions were mixed together and stored overnight, we noticed that phase separation occurred in some of the systems containing high levels of small droplets: a white cream layer was observed at the top, and a transparent serum layer at the bottom. The critical flocculation concentration (CFC) is defined as the minimum amount of added substance required to induce flocculation. For these samples, the CFC was determined by measuring the minimum amount of

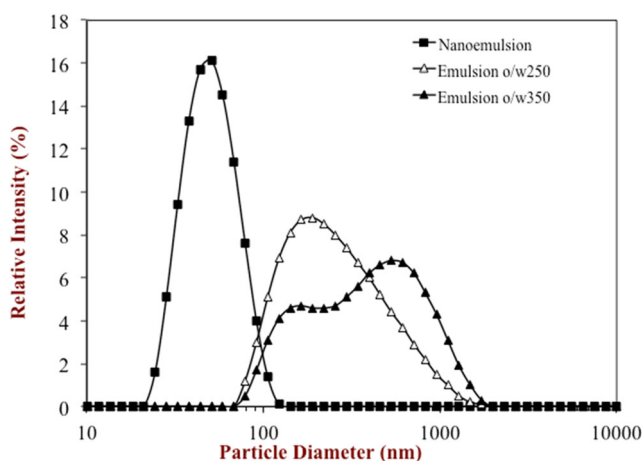


Fig. 1 Droplet size distribution of initial nanoemulsion (20 % oil, SOR 0.75 with Tween 80, microfluidization at 25 kpsi for 3 cycles), conventional emulsion o/w₂₅₀ (4 % oil, SOR 0.1 Tween 80, high-pressure homogenization at 5 kpsi, 3 cycles) and conventional emulsion o/w₃₅₀ (4 % oil, SOR 0.1 Tween 80, high-pressure homogenization at 1 kpsi, 3 cycles)

nanoemulsion required to promote rapid creaming, since flocculation is known to promote gravitational separation. The CFC depended on the initial mean droplet diameter of the conventional emulsions, being 3.75 % (v/v) for *Emulsion o/w₂₅₀* and 0.25 % (v/v) for *Emulsion o/w₃₅₀* (Fig. 2a). Therefore, the smaller the droplet size of the conventional emulsions, the higher the amount of nanoemulsion that had to be added to promote flocculation and creaming. Initially, we hypothesized that the origin of this effect was due to depletion flocculation of the large droplets in the conventional emulsions by the small droplets in the nanoemulsions. The small droplets act as non-adsorbed colloidal particles that can promote depletion flocculation due to the fact that they are excluded from a narrow region surrounding the larger droplets. Theoretical analysis and experimental measurements of mixed colloidal systems have shown that the amount of non-adsorbing small particles

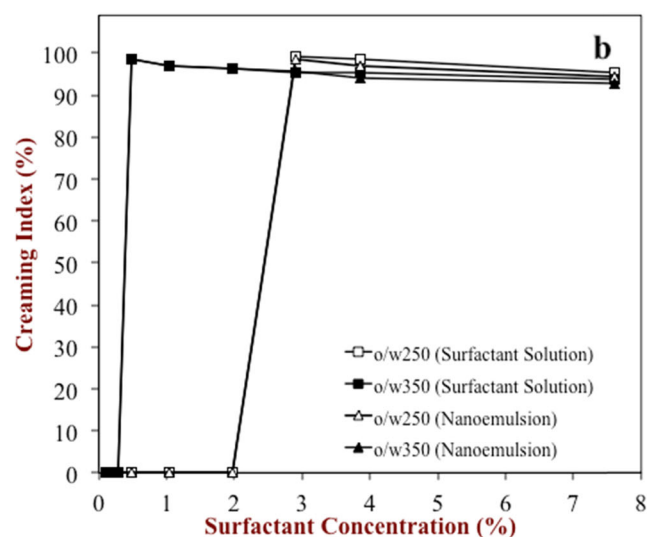
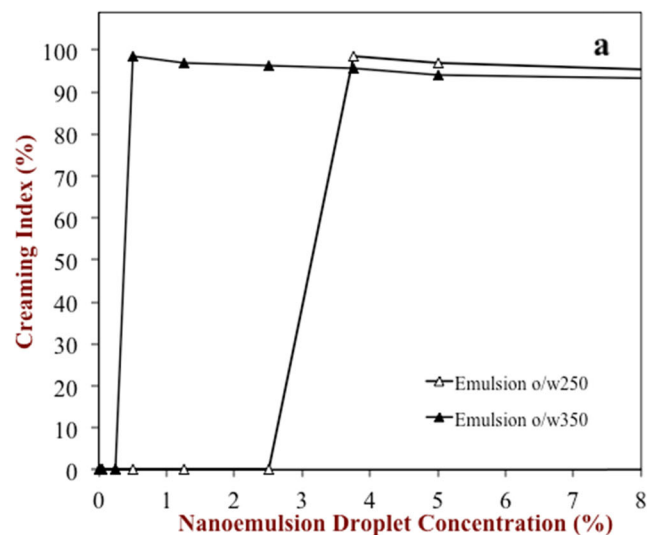


Fig. 2 Creaming index (%) of conventional emulsions o/w₂₅₀ or o/w₃₅₀ after addition of different concentrations of surfactant in nanoemulsions (a) or free surfactant without oil (b)

required to promote depletion flocculation increases with decreasing size of the larger particles [11].

Recently, Tabor and co-workers reported depletion flocculation in mixed colloidal suspensions containing large emulsion droplets and small silica nanoparticles [7]. Consequently, it is possible for small nanoemulsion droplets could promote depletion flocculation of larger emulsion droplets by a similar mechanism. However, it should be noted the nanoemulsions were prepared using a relatively high surfactant concentration (SOR=0.75) and so it is likely that there were appreciable amounts of surfactant micelles present, as well as nanoemulsion droplets. Surfactant micelles are small colloidal particles that have also been shown to promote depletion flocculation in emulsions when they exceed a particular concentration [18–20]. A number of authors have previously studied the flocculation of emulsions caused by non-adsorbed non-ionic surfactants, such as Tweens, which was attributed to depletion flocculation [18, 21]. It is therefore possible that it was the surfactant micelles (rather than the nanoemulsion droplets) that promoted depletion flocculation.

For this reason, we carried out an additional experiment where we added surfactant to the conventional emulsions, at levels equivalent to those found in the nanoemulsions. Interestingly, when we compared the flocculation results on the basis of the total amount of surfactant present in the surfactant solutions and the nanoemulsions, we observed very similar CFC values for both Emulsions o/w_{250} and o/w_{350} (Fig. 2b). These results strongly suggest that it is the surfactant micelles that promote depletion flocculation, rather than the nanoemulsion droplets. This result might have important

practical implications since nanoemulsions containing small droplets are often prepared using high surfactant concentrations, especially when they are formed using low-energy methods such as spontaneous emulsification. In the remainder of the experiments we only used mixtures containing nanoemulsions and Emulsion o/w_{250} because of the better uniformity of its particle size distribution (Fig. 1).

The thickness of the cream layer was measured over time in the mixed systems to provide information about the kinetics of the flocculation and creaming processes. In general, the thickness of the cream layer increased rapidly during the first few hours of storage, then reached a maximum value, and then decreased to a constant level (Fig. 3). The reduction in the thickness of the cream layer after prolonged storage can be attributed to rearrangement of the oil droplets to form a more compact structure [8]. The final thickness of the cream layer increased with increasing total surfactant concentration when either surfactant solution or nanoemulsion were mixed with the conventional emulsions. This effect can be attributed to the increase in the strength of the depletion attraction with increasing surfactant micelle concentration [8]. When the large droplets are held together by strong attractive interactions it is more difficult for them to rearrange themselves and so a more open particle network is formed. The creaming kinetics was fairly similar when either surfactant solution or nanoemulsion was added to the conventional emulsions at similar surfactant levels (Fig. 3), highlighting the fact that it is the surfactant micelles rather than the nanoemulsion droplets that promote depletion flocculation.

Influence of Nanoemulsions on the Microstructure of Conventional Emulsions

We assessed the influence of adding nanoemulsions or surfactant solutions on the microstructure of conventional emulsions by confocal microscopy after 24 h storage (Fig. 4). The distribution of oil droplets in the conventional emulsions was fairly uniform and the oil droplets appeared to be in a size range consistent with that measured by DLS (Fig. 4a). The oil droplets were not visible in the nanoemulsions because they were below the detection limit of the confocal microscope (Fig. 4b). Nevertheless, the confocal images had a relatively high fluorescent intensity (red color) throughout the aqueous phase, which might be attributed to the high concentration of small oil droplets or surfactant micelles creating a red background in the microscope image. There were notable differences in the structure of the flocs formed in the emulsions at the same surfactant level (7.5 %) when they were mixed with nanoemulsion (Fig. 4c) or surfactant solution (Fig. 4d). The flocs formed in the nanoemulsion-emulsion system were larger than those formed in the micelle-emulsion system. This difference

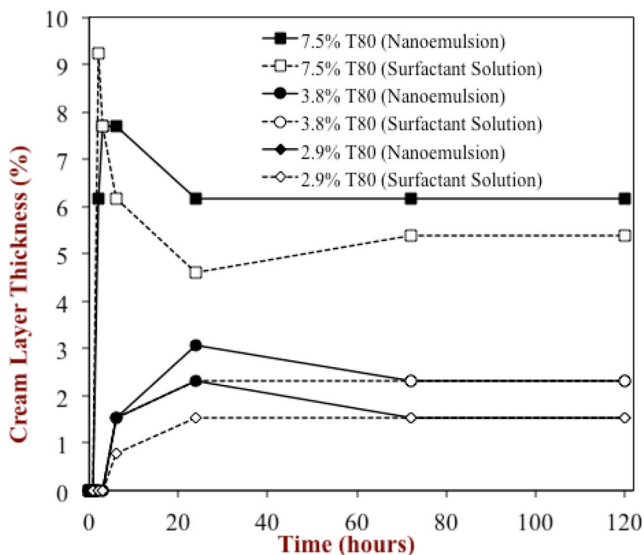


Fig. 3 Thickness of the cream layer of conventional emulsion o/w_{250} (4 % oil, SOR 0.1 Tween 80, high-pressure homogenization at 5 kpsi, 3 cycles) over time after addition of nanoemulsion (nE) (20 % oil, SOR 0.75 with Tween 80, microfluidization at 25 kpsi for 3 cycles) at different concentrations (%) or the equivalent free surfactant (Tween 80) concentration (%)

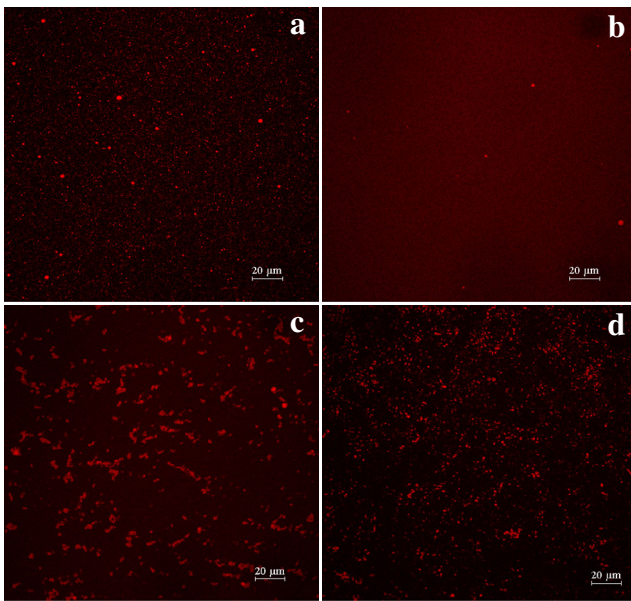


Fig. 4 Confocal microscopy images of conventional emulsion (**a**), nanoemulsion (**b**), mixed system of conventional emulsion containing a 10 % of nanoemulsion (**c**) and mixed system of conventional emulsion containing an equivalent concentration of surfactant present in the nanoemulsion, being 7.5 % (**d**)

might be related to the contribution of nanoemulsion droplets to the formation of the flocs. The nanoemulsion droplets had a larger diameter ($d \approx 50$ nm) than the surfactant micelles ($d \approx 10$ nm) as measured by dynamic light scattering. The nanoemulsion droplets may have altered the strength of the attractive forces between the conventional oil droplets, or they may themselves have been incorporated into the flocs. Previous studies have reported that a non-ionic surfactant concentration exceeding 7 % can induce depletion flocculation in oil-in-water emulsions containing small droplets [18]. Other authors have observed depletion flocculation in emulsions due to the presence of non-adsorbed polysaccharides [22] or proteins [23]. However, the current study appears to be the first to examine the influence of nanoemulsions on depletion flocculation in emulsions.

Influence of Nanoemulsions on the Droplet Size of Conventional Emulsions

We postulated that the addition of nanoemulsions to conventional emulsions may also change the size of the individual droplets in the mixed systems due to Ostwald ripening or coalescence processes. We therefore measured the influence of nanoemulsion concentration on the initial particle size distribution of the mixed systems to establish an appropriate particle ratio to use in subsequent storage studies (Fig. 5). The concentration of the conventional emulsion in these mixtures was kept constant at 2 % (v/v) whereas the concentration of the

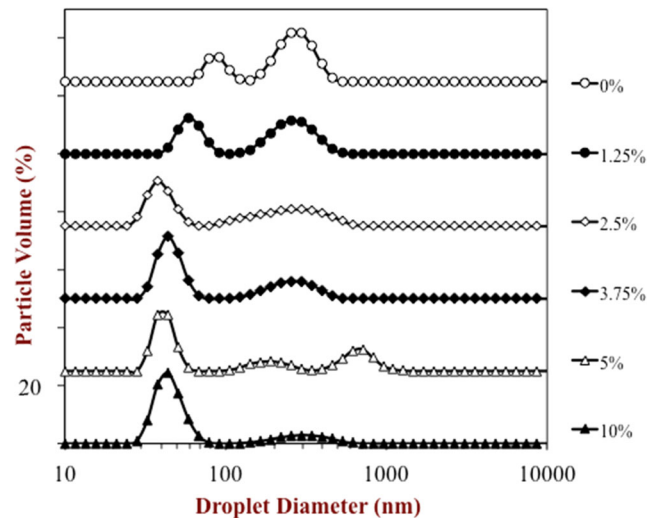


Fig. 5 Droplet size distribution of mixed systems containing conventional emulsion and different concentrations of nanoemulsion immediately after mixing

nanoemulsion ranged from 0 to 10 % (v/v). In this case, the conventional emulsion initially had a broad particle size distribution with the majority droplets being relatively large ($d > 100$ nm). As expected, the particle size distribution changed as increasing amounts of nanoemulsion were added to the conventional emulsions, which can be attributed to an increasing amount of light scattering by the small nanoemulsion droplets. At the highest nanoemulsion concentration (10 % (v/v)), there was a large population of small particles ($d \approx 44$ nm) corresponding to the nanoemulsions and a small population of large particles ($d \approx 300$ nm) corresponding to the emulsions. This mixed system was therefore used to determine if there were any changes in the particle size of the nanoemulsions during storage (Fig. 6).

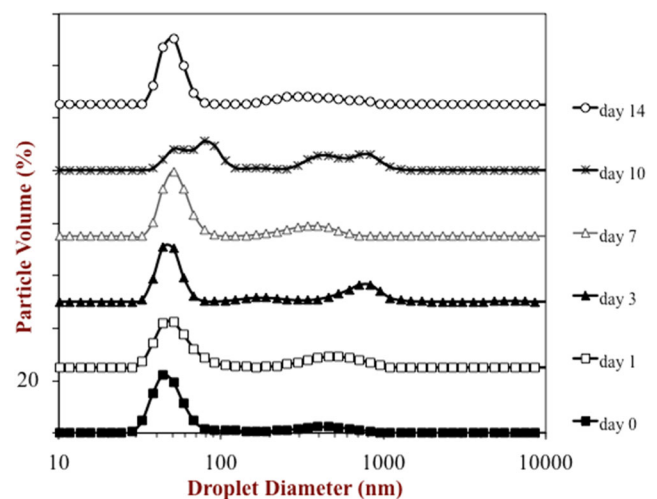


Fig. 6 Droplet size distribution of mixed systems containing conventional emulsion and nanoemulsion at 10 % (v/v) after up to 14 days of storage time at room temperature

The total volume of the peaks corresponding to the larger oil droplets increased over time, whereas the total volume of the peaks corresponding to the smaller nanoemulsion droplets decreased (Fig. 6). This effect depended on the initial concentration of nanoemulsion added to the conventional emulsion (Fig. 7). This figure shows that the volume percentage of droplets smaller than 100 nm significantly decreased after 14 days storage at room temperature in the mixed systems. This effect was more pronounced when the concentration of nanoemulsion in the mixture was greater or equal to 3.75 (v/v), which is similar to the value where depletion flocculation was observed (Fig. 2a). We therefore attribute the observed change in particle size distribution to accelerated droplet growth when oil droplets are brought into close contact through depletion flocculation. Droplets that are in close proximity to each other are more likely to undergo coalescence [8]. In addition, the Ostwald ripening rate may increase because the diffusion path between the droplets is reduced [16]. In this work, nanoemulsions were formulated with lemon oil, which has a relatively high solubility in the aqueous phase, and so the diffusion of lemon oil molecules from smaller to larger droplets might have occurred [24]. Other authors have also reported this fact, observing a higher stability for nanoemulsions containing more non-polar flavor oil components [25].

Calculation of Depletion Attraction Between oil Droplets in Emulsions

The aim of this section was to develop a more quantitative understanding of the potential impact of nanoemulsion

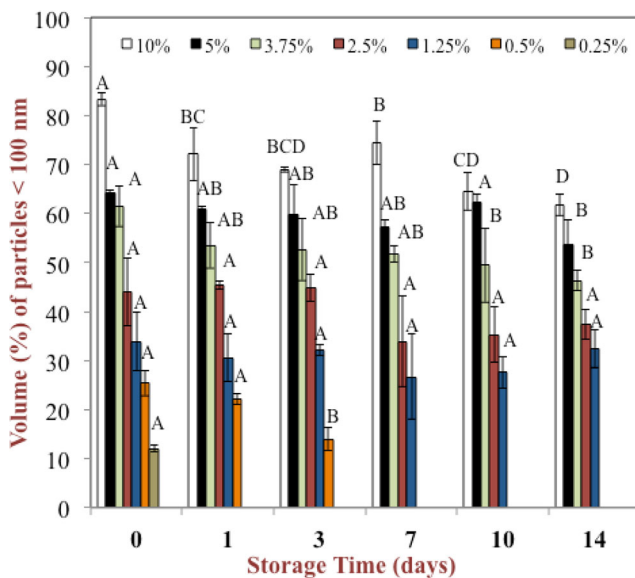


Fig. 7 Percentage of the volume area (%) corresponding to particles below 100 nm during storage time in mixed systems containing conventional emulsion and nanoemulsion at different concentrations. Different capital letters for the same nanoemulsion concentration mean significant differences in storage time

droplets and surfactant micelles on depletion interactions in conventional emulsions. The magnitude of the attraction between large particles (emulsion droplets) due to the presence of non-adsorbed small particles (nanoemulsion droplets or surfactant micelles) can be calculated with the following equation [8]:

$$w_{depletion}(h) = -\frac{2}{3}\pi r^3 P_{OSM} \left(2\left(1 + \frac{r_c}{r}\right)^3 + \left(1 + \frac{h}{2r}\right)^3 - 3\left(1 + \frac{r_c}{r}\right)^2 \left(1 + \frac{h}{2r}\right) \right) \tag{1}$$

$$P_{OSM} = \frac{ckTN_A}{M} \left(1 + \frac{2c}{\rho_m} \right) \tag{2}$$

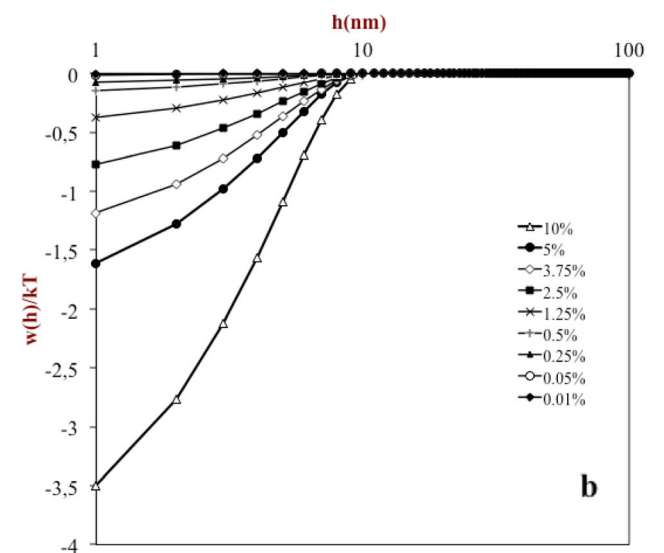
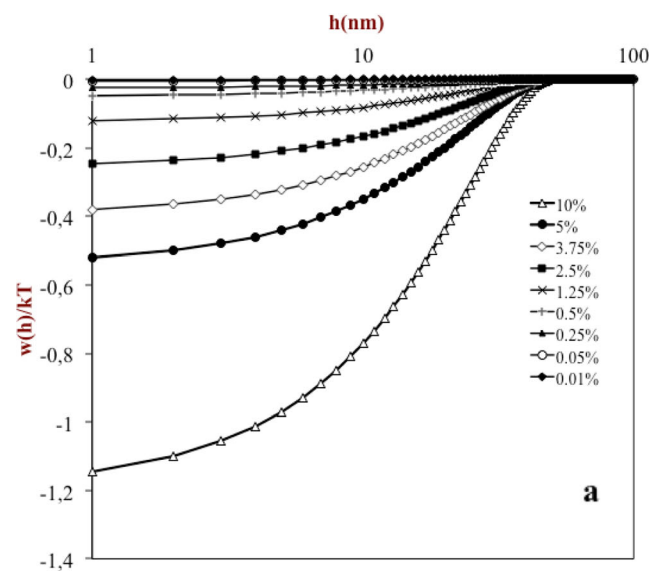


Fig. 8 Prediction of the depletion attraction of nanoemulsion (r_c 25 nm) at different concentrations of nanoemulsion (a) or surfactant micelles (b) between oil droplets of conventional emulsions ($r=125$ nm)

Here, $w_{\text{depletion}}(h)$ is the depletion attraction potential, h is the surface-to-surface separation of the large particles, P_{OSM} is the osmotic pressure arising from exclusion of the small particles from a narrow region surrounding the large particles, r is the radius of the large particles, and r_c is the effective radius of the small particles. This equation is applicable for $h < 2r_c$, for higher particle separations $w(h) = 0$. Moreover c , M and ρ_m are the mass concentration (in kg m^{-3}), weight of the particle (in kg mol^{-1}) and molecular density (in kg m^{-3}) of the small particles, N_A is Avogadro's number ($6.022 \times 10^{23} \text{ mol}^{-1}$), k is Boltzmann's constant ($1.3807 \times 10^{-23} \text{ J}$), and T is the absolute temperature. The concentration of particles (c) was calculated from the percentage of particles included in the mixture, being 100, 50, 37.5, 25, 12.5, 5, 2.5, 0.5, 0.1 (kg m^{-3}). In this section, we theoretically assessed the influence of the nanoemulsion droplets (r_c of 25 nm; ρ_m of 876.5 kg m^{-3}) or surfactant micelles (r_c of 5 nm; ρ_m of 1060 kg m^{-3}) on the depletion attraction of the larger oil droplets (r of 125 nm). The weight of nanoemulsion droplets or surfactant micelles was calculated using the following equation:

$$M = N_A \frac{4}{3} \pi r_c^3 \rho \quad (3)$$

The M values for lipid or surfactant particles were 34,520 or 334 kg/mol, respectively. The variation of the depletion attraction with droplet separation was calculated with Eq. 1 using Excel software for different nanoemulsion and surfactant micelle concentrations (Fig. 8). As expected, the depletion attraction between the larger oil droplets increased as the concentration of nanoemulsion droplets or surfactant micelles increased. However, the magnitude of the depletion attraction was relatively low ($\approx kT$) for the nanoemulsion droplets, even at the highest concentration used (Fig. 8a), which suggests that they are unlikely to promote depletion flocculation. On the other hand, the magnitude of the depletion attraction was appreciably larger than the thermal energy for the surfactant micelles at the highest concentrations used (Fig. 8b), which suggests that they could promote depletion flocculation. These calculations therefore support the results of the experimental observations, and suggest that it is the surfactant micelles rather than the nanoemulsion droplets that promote depletion flocculation in the mixed emulsion/nanoemulsion systems. These kinds of calculations are therefore useful for predicting the influence of particle size and concentration of a colloidal delivery system on the stability of a pre-existing emulsion. The results observed in the present work are in agreement with previous studies using theoretical calculations to predict the depletion induced in emulsions by biopolymers, observing that the magnitude and range of the attractive depletion interactions increased with increasing biopolymer molecular weight [26].

Conclusions

In the present study, we determined the influence of adding nanoemulsions to conventional emulsions on the overall stability of the mixed system. The addition of nanoemulsions to the emulsions promoted droplet flocculation and rapid creaming above a critical level. Moreover, there appeared to be some growth in the size of the individual oil droplets during storage particularly in those systems that contained flocculated droplets. We attributed this droplet growth to an accelerated Ostwald ripening and coalescence when the droplets were brought into close proximity. Experimental measurements and theoretical calculations suggested that it was the presence of non-adsorbed surfactant micelles in the nanoemulsions, rather than the nanoemulsion droplets themselves, that were responsible for promoting droplet flocculation in conventional emulsions. High surfactant levels are often used when forming nanoemulsions (particularly by low energy methods), and so this may be a limitation of this kind of delivery system. In summary, the current work highlights the importance of selecting an optimal nanoemulsion and surfactant concentration to maintain overall food matrix stability. In general, this study has important implications for application of colloidal delivery systems in foods. Depending on their particle size and concentration, colloidal delivery systems may promote instability in food products that already contain larger oil droplets, such as dressings, sauces, soups, or beverages.

Acknowledgments This material is based upon work supported by the Cooperative State Research, Extension, Education Service, United State Department of Agriculture, Massachusetts Agricultural Experiment Station (Project No. 831) and by the United States Department of Agriculture, NRI Grants (2011-03539, 2013-03795, and 2014-67021).

References

1. L. Salvia-Trujillo, M.A. Rojas-Graü, R. Soliva-Fortuny, O. Martín-Belloso, *Food Hydrocoll.* **30**(1), 401–407 (2013)
2. F. Donsì, M. Annunziata, M. Sessa, G. Ferrari, *LWT Food Sci. Technol.* **44**(9), 1908–1914 (2011)
3. J. Rao, D.J. McClements, *J. Agric. Food Chem.* **59**(9), 5026–5035 (2011)
4. L. Salvia-Trujillo, C. Qian, O. Martín-Belloso, D.J. McClements, *Food Chem.* **141**(2), 1472–1480 (2013)
5. L. Salvia-Trujillo, M.A. Rojas-Graü, R. Soliva-Fortuny, O. Martín-Belloso, *Food Control* **37**, 292–297 (2014)
6. K. Ziani, Y. Fang, D.J. McClements, *Food Res. Int.* **46**(1), 209–216 (2012)
7. R.F. Tabor, H. Lockie, D.Y.C. Chan et al., *Soft Matter* **7**(24), 11334–11344 (2011)
8. D.J. McClements, *Food emulsions principles, practices, and techniques* (CRC Press, Boca Raton, 2005)
9. P. Jenkins, M. Snowden, *Adv. Colloid Interf. Sci.* **68**, 57–96 (1996)
10. A. Meller, J. Stavans, *Langmuir* **12**(2), 301–304 (1996)
11. M.P. Aronson, *Langmuir* **5**(2), 494–501 (1989)

12. E. Dickinson, M. Golding, M.J.W. Povey, J. Colloid Interface Sci. **185**(2), 515–529 (1997)
13. S. Ji, J.Y. Walz, Langmuir **29**(49), 15159–15167 (2013)
14. S. Ji, J.Y. Walz, J. Phys. Chem. B **117**(51), 16602–16609 (2013)
15. T.J. Wooster, M. Golding, P. Sanguansri, Langmuir **24**(22), 12758–12765 (2008)
16. A.M. Djerdjev, J.K. Beattie, Langmuir **24**(15), 7711–7717 (2008)
17. D.J. McClements, L. Henson, L.M. Popplewell, E.A. Decker, S.J. Choi, J. Food Sci. **77**(1), C33–C38 (2012)
18. D.J. McClements, Colloids Surf. A-Physicochem. Eng. Asp. **90**(1), 25–35 (1994)
19. J. Bibette, D. Roux, B. Pouligny, J. Phys. Ii **2**(3), 401–424 (1992)
20. M. Wulff-Perez, A. Torcello-Gomez, M.J. Galvez-Ruiz, A. Martin-Rodriguez, Food Hydrocoll. **23**(4), 1096–1102 (2009)
21. E. Dickinson, C. Ritzoulis, M.J.W. Povey, J. Colloid Interface Sci. **212**(2), 466–473 (1999)
22. T. Moschakis, B.S. Murray, E. Dickinson, Langmuir **22**(10), 4710–4719 (2006)
23. E. Dickinson, S.J. Radford, M. Golding, Food Hydrocoll. **17**(2), 211–220 (2003)
24. J. Rao, D.J. McClements, Food Chem. **134**(2), 749–757 (2012)
25. J. Rao, D.J. McClements, Food Hydrocoll. **29**(2), 326–334 (2012)
26. U. Klinkesorn, P. Sophanodora, P. Chinachoti, D.J. McClements, Food Res. Int. **37**(9), 851–859 (2004)