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Formation of Thermally Reversible Optically Transparent Emulsion-based Delivery Systems Using Spontaneous Emulsification

Amir Hossein Saberi [1], Yuan Fang [2] and David Julian McClements [1,3]*

[1] Biopolymers and Colloids Laboratory, Department of Food Science,
University of Massachusetts Amherst, Amherst, MA 01003, USA

[2] PepsiCo Global R&D, 100 Stevens Ave, Valhalla, NY 1059, USA

[3] Department of Biochemistry, Faculty of Science, King Abdulaziz University,
P. O. Box 12 80203 Jeddah 21589 Saudi Arabia

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

Abstract

Transparent emulsion-based delivery systems suitable for encapsulating lipophilic bioactive agents can be fabricated using low-energy spontaneous emulsification methods. These emulsions are typically fabricated from non-ionic surfactants whose hydrophilic head groups are susceptible to dehydration upon heating. This phenomenon may promote emulsion instability due to enhanced droplet coalescence at elevated temperatures. Conversely, the same phenomenon can be used to fabricate optically transparent emulsions through the phase inversion temperature (PIT) method. The purpose of the current study was to examine the influence of oil phase composition and surfactant-to-oil ratio on the thermal behavior of surfactant-oil-water systems containing limonene, medium chain triglycerides (MCT), and Tween 60. Various types of thermal behavior (turbidity *versus* temperature profiles) were exhibited by these systems depending on their initial composition. For certain compositions, thermoreversible emulsions could be formed that were opaque at high temperatures but transparent at ambient temperatures. These systems may be particularly suitable for the encapsulation of bioactive agents in applications where optical clarity is important.

Keywords: nanoemulsion; microemulsion; thermoreversible; limonene; emulsion; delivery systems

1. Introduction

Emulsion-based delivery systems are being developed to encapsulate, protect, and release numerous types of bioactive agents, such as lipophilic drugs, vitamins, nutraceuticals, flavors, colors, and preservatives [1-3]. The bioactive agent is usually dissolved within an oil phase, which is then homogenized with an aqueous phase containing an emulsifier to form either a conventional emulsion (d > 100 nm) or a nanoemulsion (d < 100 nm). The type of delivery system used depends on the requirements of the specific application, *e.g.,* opacity, stability, and release profile. Nanoemulsions are particularly suitable in low- to high-viscosity products where high optical clarity, high physical stability, and rapid release are required. Conversely, conventional emulsions are more suitable for application in viscous products that are cloudy or turbid, since the large droplets have a tendency to cream/sediment and scatter light strongly. In the remainder of this article, we use the term "emulsions" to refer to both nanoemulsions and conventional emulsions. Emulsions can be fabricated using high-energy approaches (such as high pressure valve homogenization, microfluidization, or sonication) or low-energy approaches (such as spontaneous emulsification, phase inversion temperature, or emulsion inversion point) [4-6]. High-energy methods require specialized mechanical devices ("homogenizers") to form emulsions, but are fairly versatile in the type of oils and emulsifiers that can be utilized [7-10]. On the other hand, low-energy methods are simple and inexpensive to implement and can produce emulsions without the need of any specialized equipment [5, 11, 12]. However, they are often only suitable for specific combinations of oil and surfactant, and typically require much higher surfactant-to-oil ratios (SOR) than high-energy methods [13]. Consequently, there is a need to optimize the composition and preparation methods required to produce stable emulsions using low-energy methods.

In this study, we focused on the production of emulsions from a commonly used lipophilic flavoring agent (limonene) and a food-grade non-ionic surfactant (Tween 60). In particular, we examined the influence of oil phase composition and SOR on emulsion formation and thermal stability. The information obtained in this study is useful for optimizing the formulation of emulsion-based delivery systems for lipophilic active ingredients.

2. Materials and methods

2.1. Materials

Medium chain triglyceride (MCT) oil (MIGLYOL® 812) was purchased from Warner Graham Company (Sasol Germany GmbH). Limonene, non-ionic surfactant (Tween 60), citric acid, and sodium benzoate were purchased from Sigma–Aldrich Co. (St. Louis, MO). Double distilled water was used in the preparation of all solutions and nanoemulsions. All concentrations Aare expressed as a mass percentage (wt%).

2.2. Emulsion preparation

Emulsion formation was carried out using a spontaneous emulsification method explained in our previous study [14]. In brief, spontaneous emulsification was performed by addition of an organic phase to an aqueous phase while continuously stirring the system using a magnetic stirrer. The composition of the buffer solution was designed to imitate the aqueous phase of many commercial beverage products. Unless otherwise stated, the experiments were carried out using standardized conditions: (i) *composition* - 10% total oil, 15% surfactant, and 75% aqueous phase; (ii) *stirring* - magnetic stirrer speed of 600 rpm; (iii) *temperature* - 45 ºC. In these samples, the oil (10 g) and surfactant (15 g) were first mixed together and then the mixture was slowly poured into 75 g of aqueous phase over a 15 min period with continuous stirring. In a number of experiments the ratio of limonene to MCT in the oil phase was varied and the total amount of surfactant (5, 10, 15, or 20%).

2.3. Particle size measurements

Particle size distributions were measured using a dynamic light scattering instrument (Zetasizer Nano ZS, Malvern Instruments, Malvern, UK). This instrument determines the particle size from intensity-time fluctuations of a laser beam (633 nm) scattered from a dilute emulsion. Each individual measurement was an average of 13 runs. To avoid multiple scattering effects, samples were diluted before the particle size measurements using acidic buffer solution (pH 3.0). The mean particle diameter (Z-average) was calculated from the particle size distribution. All measurements were conducted at ambient temperature.

2.4. Turbidity measurements

The influence of thermal treatment on the turbidity (absorbance at 600 nm) of the samples was determined using a UV–visible spectrophotometer with temperature scanning capabilities (Evolution Array, Thermo Scientific). Temperature scanning measurements were carried out by measuring the turbidity of the emulsions as the temperature increased from 25 to 95 °C at 1 °C per minute, and then decreased back to 20 and/or 25 °C at different cooling rates. Isothermal measurements were carried out by measuring the turbidity of the emulsions over time (1 min intervals) when they were held at the constant temperatures for 60 min.

2.5. Statistical analysis

All experiments were carried out at least twice using freshly prepared samples and the results are reported as the calculated mean and standard deviation of these measurements.

3. Results and discussions

3.1 Influence of organic phase composition

Initially, we examined the influence of organic phase composition on the formation and stability of the emulsions by varying the ratio of limonene-to-MCT, while keeping the total oil content fixed (10%). MCT was used as a carrier oil because it has previously been shown to be capable of forming stable nanoemulsions using the spontaneous emulsification method [14]. This stability has been attributed to the ability of MCT to act as a ripening inhibitor that slows down the rate of droplet growth due to Ostwald ripening [15, 16]. Ostwald ripening is the process whereby polar lipids (such as limonene) are transported from small to large droplets through the intervening aqueous phase [17]. The driving force for this process is the increase in the water-solubility of oils with increasing droplet curvature, *i.e.,* decreasing droplet size. Emulsions were formed using four different surfactant concentrations (5, 10, 15, and 20 %), which correspond to four different SOR values (0.5, 1, 1.5, and 2).

In general, there was initially a decrease in mean particle diameter with increasing limonene concentration until a minimum value was attained, after which the diameter increased upon further limonene addition (**Figure 1**). This kind of phenomenon has been observed previously for emulsions formed by spontaneous emulsification using different kinds of oils, such as vitamin E acetate and MCT [14, 18]. The relatively low molecular weight and polar limonene molecules may enhance the formation of small droplets at relatively low concentrations because they alter the optimum curvature, tension, and flexibility of the surfactant monolayer, thereby facilitating the spontaneous generation of oil droplets at the boundary between the organic and aqueous phases. However, when the limonene concentration is too high, the droplet size increases because of Ostwald ripening. Consequently, there is an optimum limonene level (6 to 7%) required to form stable nanoemulsions containing small droplets, which corresponds to 60 to 70% limonene within the organic phase. The smallest droplets that could be produced were for the systems containing 15 or 20% surfactant and 6% limonene, which had mean droplet diameters around 25 nm.

The size of the droplets produced by spontaneous emulsification also depended strongly on the surfactant-to-oil ratio (Figure 1). At low limonene levels, the droplet size decreased with increasing SOR, but at intermediate limonene levels droplet size was minimum at an intermediate SOR. A minimum in the droplet size at an intermediate surfactant concentration has also been reported in other studies using spontaneous emulsification [14, 19]. This effect can be attributed to the fact that a certain amount of surfactant is required to achieve the surfactant-oil-water compositions required at the boundary between the organic and aqueous phases to spontaneously form small droplets [5, 20]. The precise physicochemical mechanism responsible for this phenomenon is currently unknown, but it may be associated with the formation of a bi-continuous microemulsion phase that has nanostructured lipid domains that breakdown into small droplets in the presence of water [12, 20].

For certain practical applications, it is important that emulsion-based delivery systems have high optical clarity, *e.g.,* beverages fortified with lipophilic vitamins and nutraceuticals [21]. We therefore examined the influence of oil phase composition and surfactant-to-oil ratio on the turbidity of the emulsions produced by spontaneous emulsification (**Figure 2**). The turbidity was measured before and after a thermal heat treatment (25 to 90 to 25 ºC, at 1 ºC/min). Prior to heating, a number of the emulsions already had relatively low turbidities, which tended to be clustered at intermediate limonene levels and high surfactant levels (**Figure 2a**). After heating, the range of conditions where low turbidities could be achieved was reduced appreciably: 6-8%

limonene for 20% surfactant; 7-8% limonene for 15% surfactant; and 8-9% limonene for 10% surfactant (**Figure 2b**). These results suggest that emulsions that were optically clear and stable to heating could be produced under certain conditions.

When the turbidities were plotted against the droplet diameters of the same systems it was clear that emulsions with relatively low turbidities (<0.5 cm^{-1}) could only be formed for the systems containing very fine droplets, *i.e.,* $d < 50$ nm (**Figure 3**). This effect can be attributed to the fact that the scattering of light by colloidal particles decreases appreciably when the droplet dimensions are much smaller than the wavelength of light, *i.e.,* $d < \lambda/10$ [22].

3.2 Influence of composition on thermal behavior of nanoemulsions

In commercial applications, emulsion-based delivery systems are often exposed to variations in temperature, which may affect their particle size, turbidity, and physical stability. We therefore measured the influence of a well-controlled heating-cooling cycle on the turbidity of the emulsions: 25 to 95 to 25 ºC at 1 ºC per minute (**Figure 4**). In general, the behavior of the emulsions could be divided into a number of different categories depending on their turbidity *versus* temperature profiles (**Table 1**). Selected examples of SOW combinations that produced different kinds of thermal behavior are outline below.

Non-thermoreversible-Type 1 (NT-1): These emulsions were turbid prior to heating because spontaneous emulsification did not generate small droplets at these SOW compositions (**Figure 4a**). They were also turbid after heating suggesting that the heating-cooling cycle did not generate small droplets for these systems. The emulsions appeared physically stable after the thermal treatment, *i.e.,* they had a homogeneous appearance throughout. This kind of behavior can be attributed to the fact that the phase inversion temperature (PIT) was well above the measurement range for these surfactant-oil-water combinations.

Non-thermoreversible-Type 2 (NT-2): These emulsions had a low turbidity and homogenous appearance prior to heating because spontaneous emulsification successfully generated small oil droplets that did not scatter light strongly at these SOW combinations (**Figure 4b).** The turbidity of these emulsions remained relatively low as they were heated, until a critical temperature was reached after which it increased steeply. Upon cooling, the emulsions remained highly turbid (after they had been inverted a number of times to make them homogeneous), and they separated into a white cream layer on top of a watery serum layer when they were left to stand (**photo inset, Figure 4b**). This result suggests that extensive droplet coalescence occurred during the heating process, leading to the formation of large droplets that scattered light strongly and creamed rapidly. In this case, it is likely that these emulsions passed through a region just below the PIT (the "coalescence zone") where the droplets were highly susceptible to coalescence because of the change in their surfactant head group hydration. When non-ionic surfactants are heated their head groups become progressively dehydrated, which changes the monolayer packing parameter (p = tail group cross section / head group cross section) [23] and the relative surfactant solubility (oil-to-water partition coefficient) [20]. These changes alter the interfacial tension and flexibility, and may promote droplet coalescence over a certain temperature range [23].

Non-thermoreversible-Type 3 (NT-3): These emulsions initially had a low turbidity and homogenous appearance prior to heating because spontaneous emulsification generated small oil droplets that did not scatter light strongly (**Figure 4c).** When these emulsions were heated the turbidity remained relatively low, until a critical temperature was reached and the turbidity increased dramatically, which can be attributed to rapid droplet coalescence in the temperature range just below the PIT as discussed for the NT-2 systems. However, the turbidity of the emulsions decreased again upon further heating, which can be attributed to the formation of a bi-continuous microemulsion at the PIT that only contains small domains that do not scatter light strongly. Nevertheless, upon cooling below a critical temperature the turbidity increased rapidly, suggesting that extensive droplet coalescence occurred leading to stronger light scattering. Interestingly, after cooling these emulsions had a homogeneous appearance with no evidence of creaming, which suggests that the droplets formed were not too large (**photo inset, Figure 4c**).

Non-thermoreversible-Type 4 (NT-4): These emulsions initially had a high turbidity prior to heating because spontaneous emulsification could not produce small oil droplets, or because they were highly susceptible to Ostwald ripening (**Figure 4d).** Emulsions containing flavor oils (such as lemon or orange oils) have been reported to be highly unstable to droplet growth due to Ostwald ripening, which has been attributed to the relatively higher water-solubility of these oils in water [24, 25]. This process involves the diffusion of oil molecules from the small droplets to the large droplets through the intervening aqueous phase, and is driven by the increase in the water-solubility of the oil molecules with increasing curvature of the oil-water interface. When these emulsions were heated the turbidity remained relatively high, until a certain temperature was reached and then it fell to a relatively low level due to the formation of a bi-continuous microemulsion at the PIT. Upon further heating the turbidity increased due to phase inversion to a water-in-oil emulsion containing large water droplets that scatter light strongly. Upon cooling the turbidity remained relatively high until a certain temperature was reached, and then it fell steeply. This effect was probably an artefact associated with the creaming of the oil droplets to the top of the cuvettes, thereby decreasing the droplet concentration in the path of the light beam. Indeed, after cooling the emulsions had separated into a cream layer and a serum layer (**photo inset, Figure 4d**).

Thermoreversible (T): These emulsions initially had a relatively low turbidity and homogenous appearance because spontaneous emulsification generated small oil droplets that did not scatter light strongly (**Figure 4e**). Upon heating, the turbidity fell appreciably above a certain temperature, which suggested that a bi-continuous microemulsion phase was formed. Upon further heating, the turbidity increased again, which can be attributed to phase inversion and the formation of a water-in-oil emulsion containing relatively large water droplets that scattered light strongly. Upon cooling, the turbidity decreased steeply below a certain temperature, leading to the formation of a transparent emulsion with a homogeneous appearance (**photo inset, Figure 4d**). The formation of ultrafine emulsions using this heating-cooling process is that same as that by the PIT method that has been widely used to prepare nanoemulsions [12, 20].

It should be noted that the precise thermal behavior observed is specific for the limonene, Tween 60 and water system studied, and may be different when either the oil or surfactant type is changed. For example, we did not observe the same type of behavior when limonene was replaced with vitamin E acetate or when different surfactants were used (ongoing research). It seems that composition, structural organization, and physicochemical properties of the surfactant-oil–water mixtures are responsible for the complicated thermal behavior observed in this type of system. Further research is clearly needed to obtain more insight into the effect of system composition on the thermal behavior of emulsions produced using the spontaneous emulsification method, and to identify the molecular and physicochemical processes involved.

The influence of initial system composition (oil phase composition and surfactant level) on the mean particle diameter of the emulsions produced by this heating-cooling process were measured (**Figure 5**). These measurements indicated that nanoemulsions (*d* < 100 nm) could be formed over a range of intermediate limonene levels, particularly at high surfactant levels. As expected, the range of SOR compositions where small droplets are formed corresponds to the range where low turbidities are obtained (**Figure 2b**).

3.3. Influence of isothermal incubation on turbidity changes

These two SOW compositions were chosen because they had different optical properties after the heat-cool treatment: (i) *NT-3*: opaque (**Figure 4c**); (ii) *T*: transparent (**Figure 4e**).

For the SOW system containing 5% limonene, the turbidity remained relatively low and constant when it was held at 50 ºC for 1 hour, indicating that the droplets were stable to growth at this temperature (**Figure 6a**). The lack of droplet growth can be attributed to the fact that these emulsions were well below the phase inversion temperature (PIT ≈ 90 ºC, **Figure 4c**), and were therefore relatively stable to droplet coalescence. However, when this SOW system was held at 75 or 85 ºC there was a rapid increase in turbidity over time, which suggested that extensive droplet growth occurred, which can be attributed to coalescence just below the phase inversion temperature (**Figure 4c**). When this system was held at 90 ºC, there was an initial increase in turbidity during the first few minutes, but then the system became transparent. The initial turbidity increase can be attributed to some droplet growth occurring as the emulsion was heating up to the final temperature and passed through the coalescence zone. The high clarity at longer holding times suggests that a bi-continuous microemulsion was formed around the PIT that contained small domains that did not scatter light strongly (**Figure 4c**).

Quite different behavior was observed for the SOW system containing 7% limonene (**Figure 6b**). In this case, the turbidity remained relatively low and constant when the initial emulsions were held at 50 ºC for 1 hour due to the fact that they were well below the phase inversion temperature (PIT ≈ 80 ºC, **Figure 4e**). There was actually a decrease in turbidity when these SOW systems were held at 75 and 85 ºC for 1 hour (**Figure 6b**), which was around the temperature where they became clear during heating (**Figure 4e**). In this region we postulate that a bi-continuous microemulsion was formed around the PIT. There was a rapid increase in turbidity when these SOW systems were held at 90 ºC for 1 hour, which can be attributed to the formation of a water-in-oil emulsion above the PIT that contained water droplets that scattered light strongly (**Figure 4e**).

These isothermal incubation experiments therefore support the results of the temperature-scanning experiments discussed in the previous experiments. They highlight that the optical properties of a given SOW system depend strongly on its composition and holding temperature.

3.4. Influence of cooling rate on nanoemulsion formation

The purpose of these experiments was to examine the influence of cooling rate on the formation of transparent nanoemulsions, since previous studies have highlighted that this parameter can have a major impact on the size of the droplets formed by the PIT method [20, 26]. In this series of experiments, the thermoreversible (T) and non-thermoreversible (NT-3) SOW systems studied in the previous section were used again. The SOW systems were heated to 90 ºC and then cooled at different rates ranging from around 1 to 67 ºC/min (**Figure 7**).

For the SOW system containing 5% limonene, the final turbidity depended strongly on the cooling rate used to produce the final emulsions (**Figure 7a**). When the cooling rate was relatively slow (1 ºC/min), there was a large increase in turbidity when the temperature was reduced from 90 to 20 ºC, but when it was relatively rapid (67 ºC/min) the turbidity remained low indicating that the final systems contained relatively small droplets. Overall, the final turbidity of the emulsions after the heat-cool cycle increased with decreasing cooling rate. This phenomenon can be attributed to the fact that the SOW systems spent a longer time in the temperature range just below the PIT where rapid droplet growth occurred due to coalescence. However, emulsions containing very fine droplets could be produced by quench cooling the SOW systems from above the PIT to a temperature well below the PIT.

For the SOW system containing 7% limonene, the final turbidity was low for all cooling rate rates used to produce the emulsions (**Figure 7b**). Interestingly, the only emulsion that gave a slightly higher turbidity than the other samples was the one that was quench cooled (67 ºC/min). These results indicate that very different thermal behaviors were obtained depending on the initial SOW compositions of the emulsions used.

3.5. Storage stability

Finally, we measured the storage stability of selected emulsions by measuring the change in the mean particle diameter before and after holding them at ambient temperature for 30 days (**Figure 8**). There was little change in the particle size during the storage period, which suggested that the emulsions produced using this method had relatively good long-term stability. We also tested the stability of the emulsions to two sequential heat-cool cycles, and found that the particle size remained relatively constant after the repeated cycles (data not shown). In commercial applications, it would be important to test the stability of the emulsions under environmental conditions that the products would be expected to be utilized at, *e.g.,* pH, ionic strength and temperature fluctuations.

4. Conclusions

In this study, we examined the influence of initial system properties (oil phase composition and surfactant-to-oil ratio) on the thermal stability of emulsions produced by spontaneous emulsification. Emulsions containing very small droplets that do not scatter light strongly could only be produced over a limited range of system compositions using spontaneous emulsification at ambient temperatures: intermediate limonene-to-MCT ratios and high surfactant-to-oil ratios. After being subjected to a slow heating-cooling cycle (25 to 90 to 25 ºC at 1 ºC/min) some of the emulsions were optically transparent (small droplets), whereas others were opaque (large droplets) highlighting differences in their thermal stability. In addition, some of the emulsions were thermoreversible, whereas others were non-thermoreversible, depending on their initial composition. For non-thermoreversible systems, transparent emulsions could be formed by quench cooling the surfactant-oil-water mixtures to a temperature well below the phase inversion temperature. The main cause of high turbidity after heating-cooling was attributed to the length of time the emulsions remained in the temperature range just below the PIT where rapid droplet coalescence occurred: the longer the time, the higher the final droplet size and turbidity. Interestingly, for the thermoreversible systems there did not appear to be a pronounced droplet coalescence zone, as was observed in the non-thermoreversible systems. In this study, we used a relatively simple surfactant-oil-water system with a fixed total oil concentration. In future, studies it would be useful to examine the impact of oil concentration on the formation and properties of emulsions created by spontaneous emulsification since this parameter may also impact their fabrication and functional behavior. These results have important implications for the formation and stability of emulsion-based delivery systems for lipophilic active agents.

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Table 1. Effect of oil phase composition and surfactant concentration on thermal behavior (thermo-reversibility) of limonene loaded emulsions*.

	Limonene (%)										
	0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Tween 60 (5%)	NT-1 [a]	NT-1	NT-1	NT-1	NT-1	NT-1	NT-1	NT-1	NT-1	NT-4	NT-4
Tween 60 (10%)	NT-2	NT-2	NT-2	NT-2	NT-2	NT-3	NT-3	NT-3	T	T	NT-4
Tween 60 (15%)	NT-2	NT-2	NT-2	NT-2	NT-2	NT-3	NT-3	T	T	NT-4	NT-4
Tween 60 (20%)	NT-2	NT-2	NT-2	NT-3	NT-3	NT-3	T	T	T	NT-4	NT-4

[a] NT-1, -2, -3, -4: Not thermoreversible-type 1, 2, 3, and 4, respectively; T: Thermoreversible.

[*] Emulsions were prepared initially prepared by spontaneous emulsification using 10 wt% oil phase (limonene + MCT), different concentrations of surfactant phase (Tween-60; 5%, 10%, 15%, and 20%), and the remainder aqueous phase (pH 3 buffer solution) at a stirring speed of 600 rpm at 45°C. See Figure 4 for selected temperature-turbidity profiles representing different thermal behaviors.

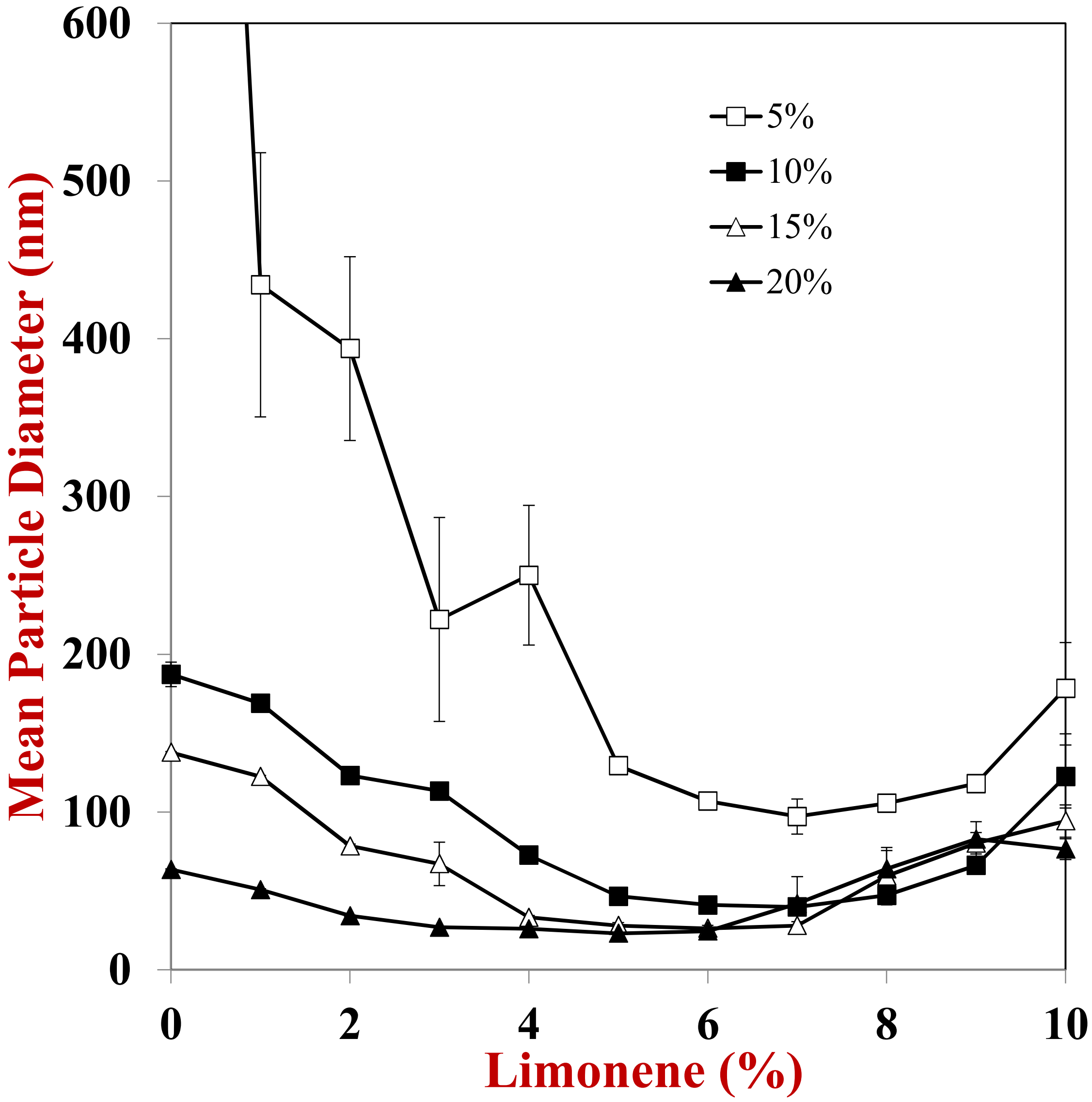


Figure 1. Effect of oil composition (wt.% of limonene in emulsion) on mean particle diameter of emulsion produced by spontaneous emulsification. Emulsions were prepared using 10 % oil phase (limonene + MCT), different concentrations of surfactant phase (Tween-60; 10%, 15%, and 20%), and aqueous phase (pH 3 buffer solution) at a stirring speed of 600 rpm at 45°C.

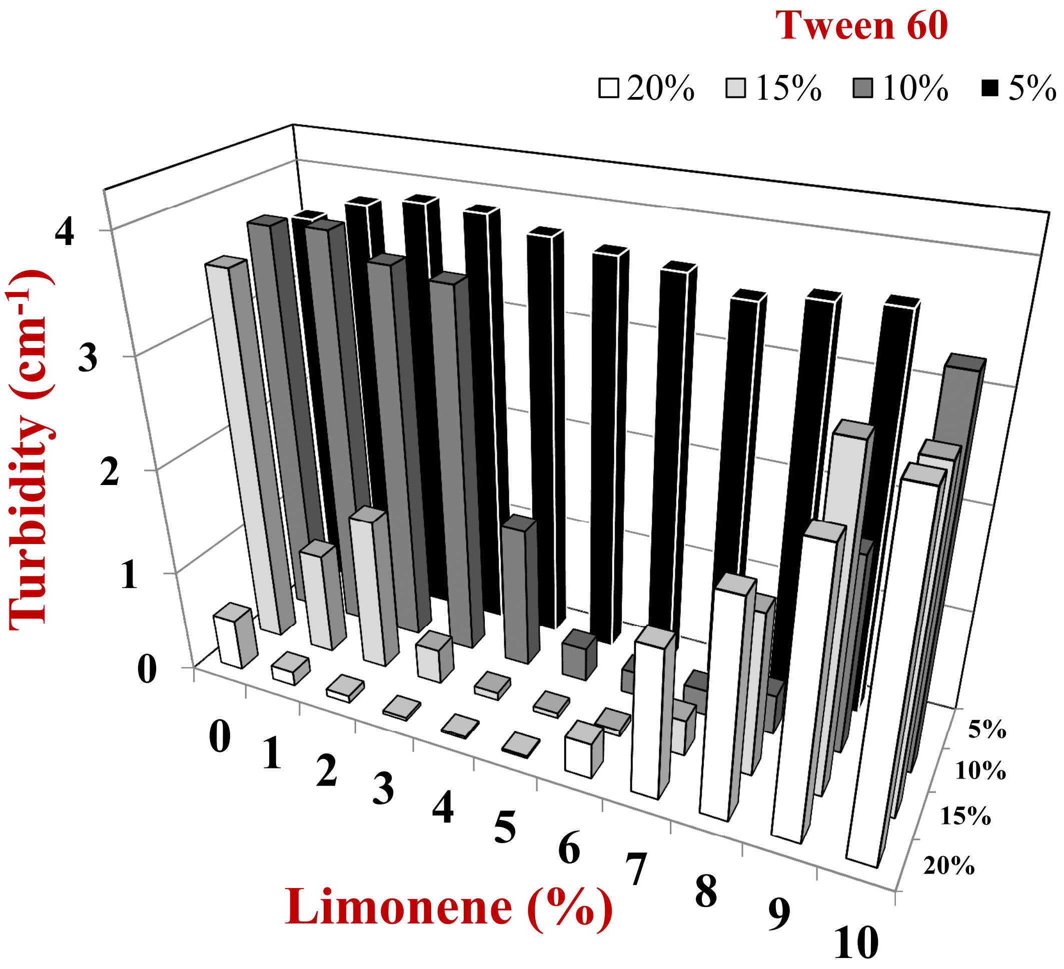


Figure 2a. Effect of oil phase and surfactant phase concentration on the turbidity of emulsion before thermal treatment. Emulsions were prepared by spontaneous emulsification approach using 10 % oil phase (limonene + MCT), different concentrations of surfactant phase (Tween-60; 5%, 10%, 15%, and 20%), and aqueous phase (pH 3 buffer solution) at a stirring speed of 600 rpm at 45°C.

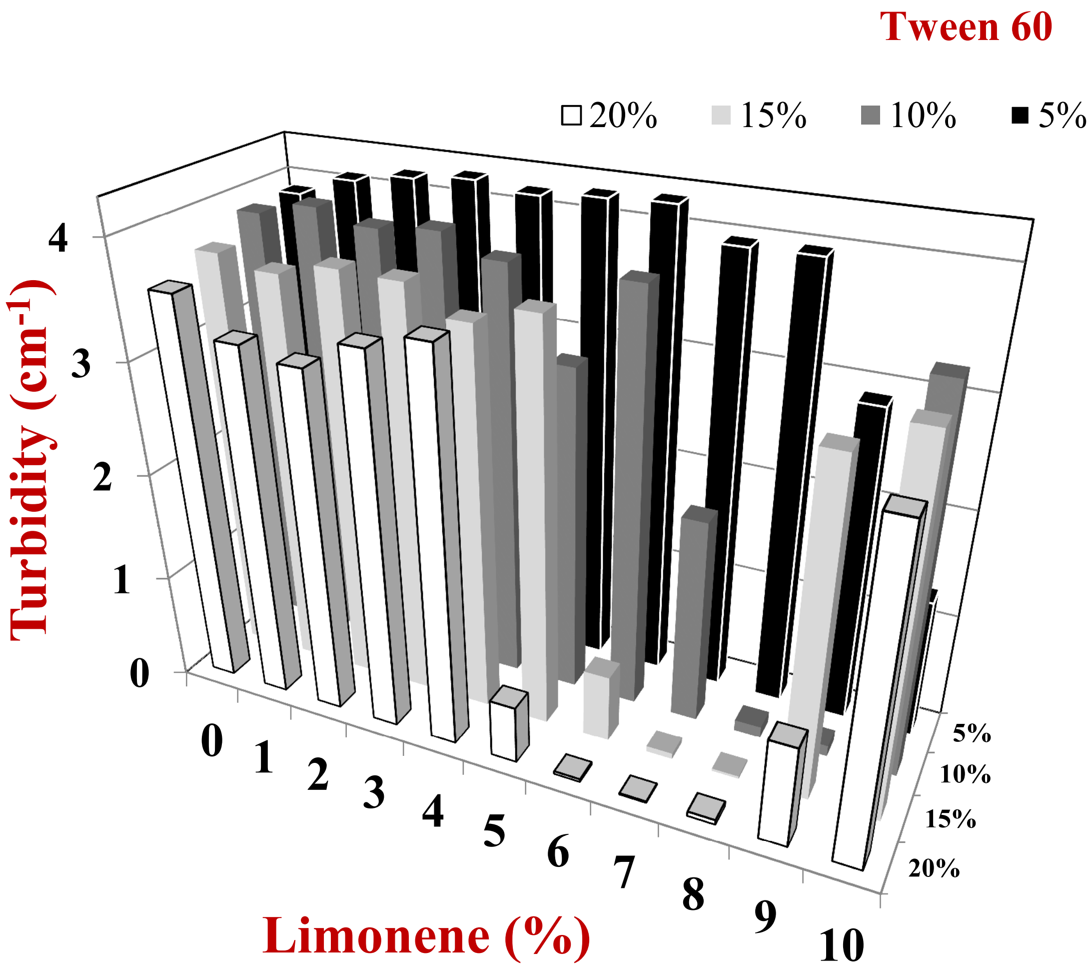


Figure 2b. Effect of temperature (heating from 25 to 95 °C followed by cooling from 95 °C to 25 °C using the thermal rate of 1 °C/min) on the turbidity of emulsion containing different oil phase compositions and surfactant concentrations. Emulsions were prepared by spontaneous emulsification approach using 10 % oil phase (limonene + MCT), different concentrations of surfactant phase (Tween-60; 5%, 10%, 15%, and 20%), and aqueous phase (pH 3 buffer solution) at a stirring speed of 600 rpm at 45°C.

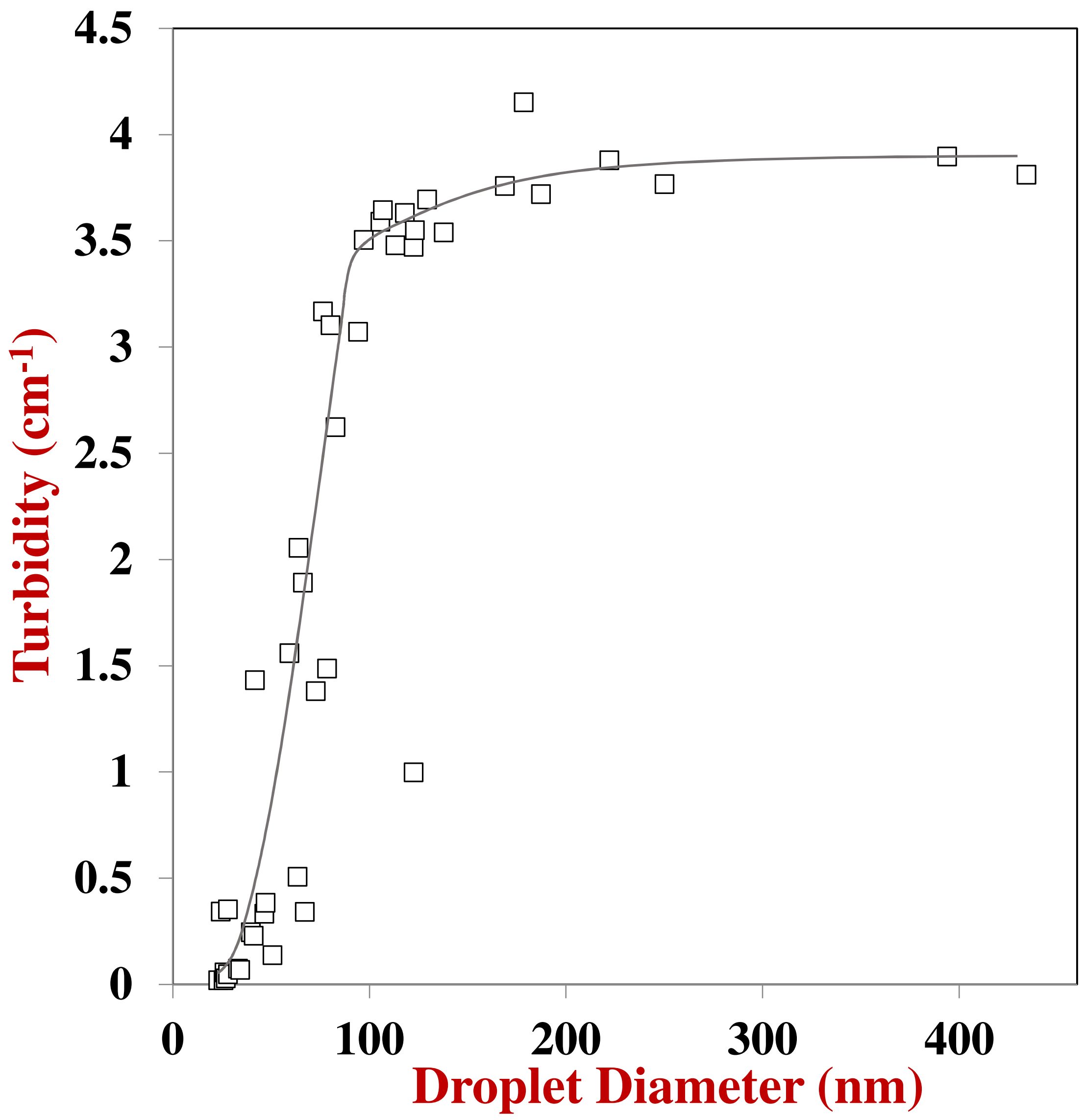


Figure 3. Plot of the turbidity of the nanoemulsions versus the droplet diameter, which shows that transparent systems can only be formed at very low particle sizes. The line is included to guide the eye.

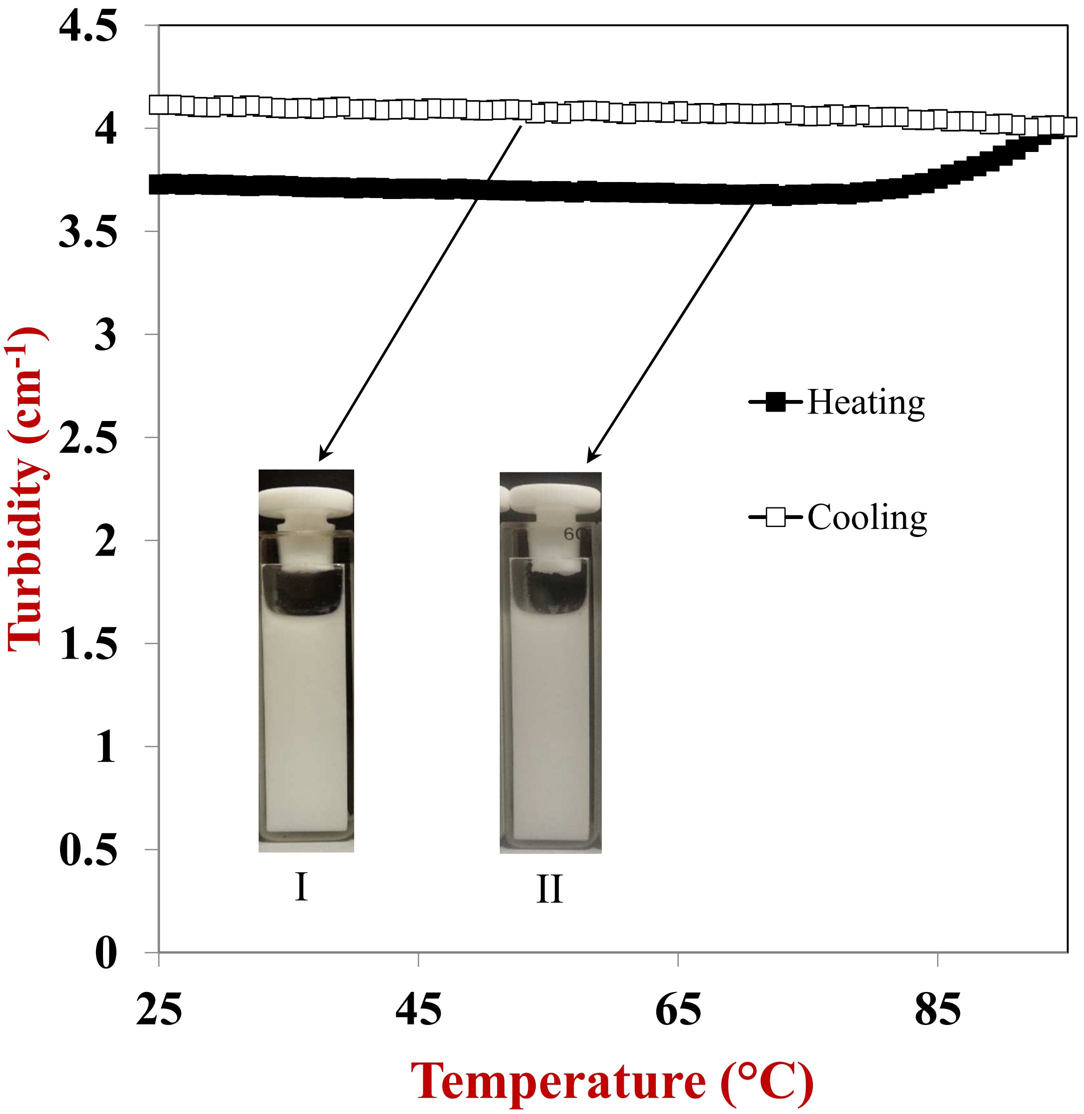


Figure 4a. Influence of temperature on turbidity of emulsions produced using 10% oil phase (4% limonene & 6% MCT), 5% surfactant phase (Tween 60), and 85% aqueous phase (pH 3 buffer solution) at a stirring speed of 600 rpm at 45°C. This figure is representative of *non-thermoreversible-type-1* (NT-1) emulsion. See Tables 1, 2, and 3. *Key*: I: Emulsion before thermal treatment. II: Emulsion after heating and cooling cycles.

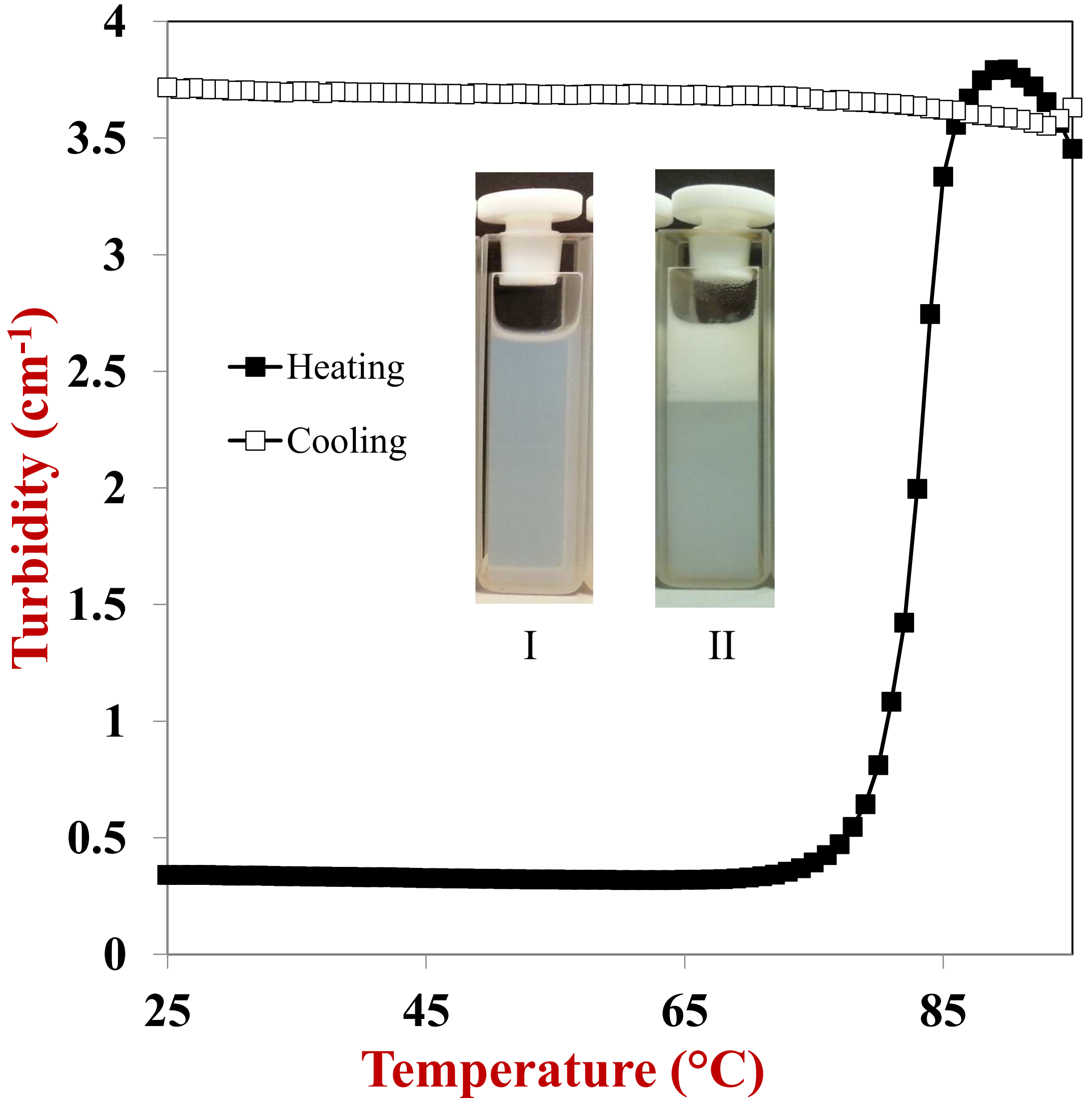


Figure 4b. Influence of temperature on turbidity of emulsions produced using 10% oil phase (3% limonene & 7% MCT), 15% surfactant phase (Tween 60), and 75% aqueous phase (pH 3 buffer solution) at a stirring speed of 600 rpm at 45°C. This figure is representative of Not thermoreversible-type-2 (NT-2) emulsion. See Tables 1, 2, and 3.

I: Fresh emulsion before thermal treatment.

II: Emulsion after heating and cooling cycles.

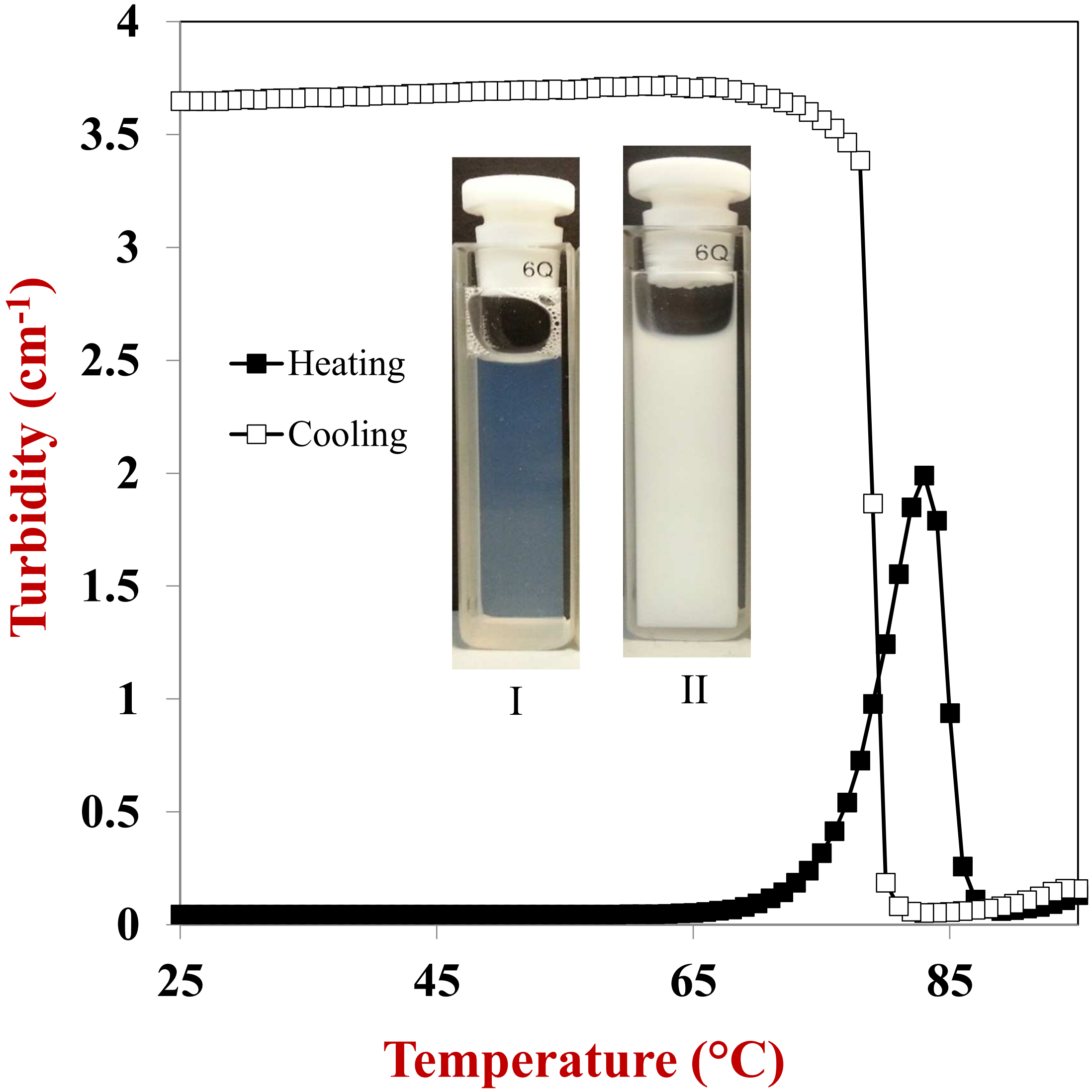


Figure 4c. Influence of temperature on turbidity of emulsions produced using 10% oil phase (5% limonene & 5% MCT), 15% surfactant phase (Tween 60), and 75% aqueous phase (pH 3 buffer solution) at a stirring speed of 600 rpm at 45°C. This figure is representative of non-thermoreversible-type-3 (NT-3) emulsion. See Tables 1, 2, and 3. *Key:* I: Fresh emulsion before thermal treatment. II: Emulsion after heating and cooling cycles.

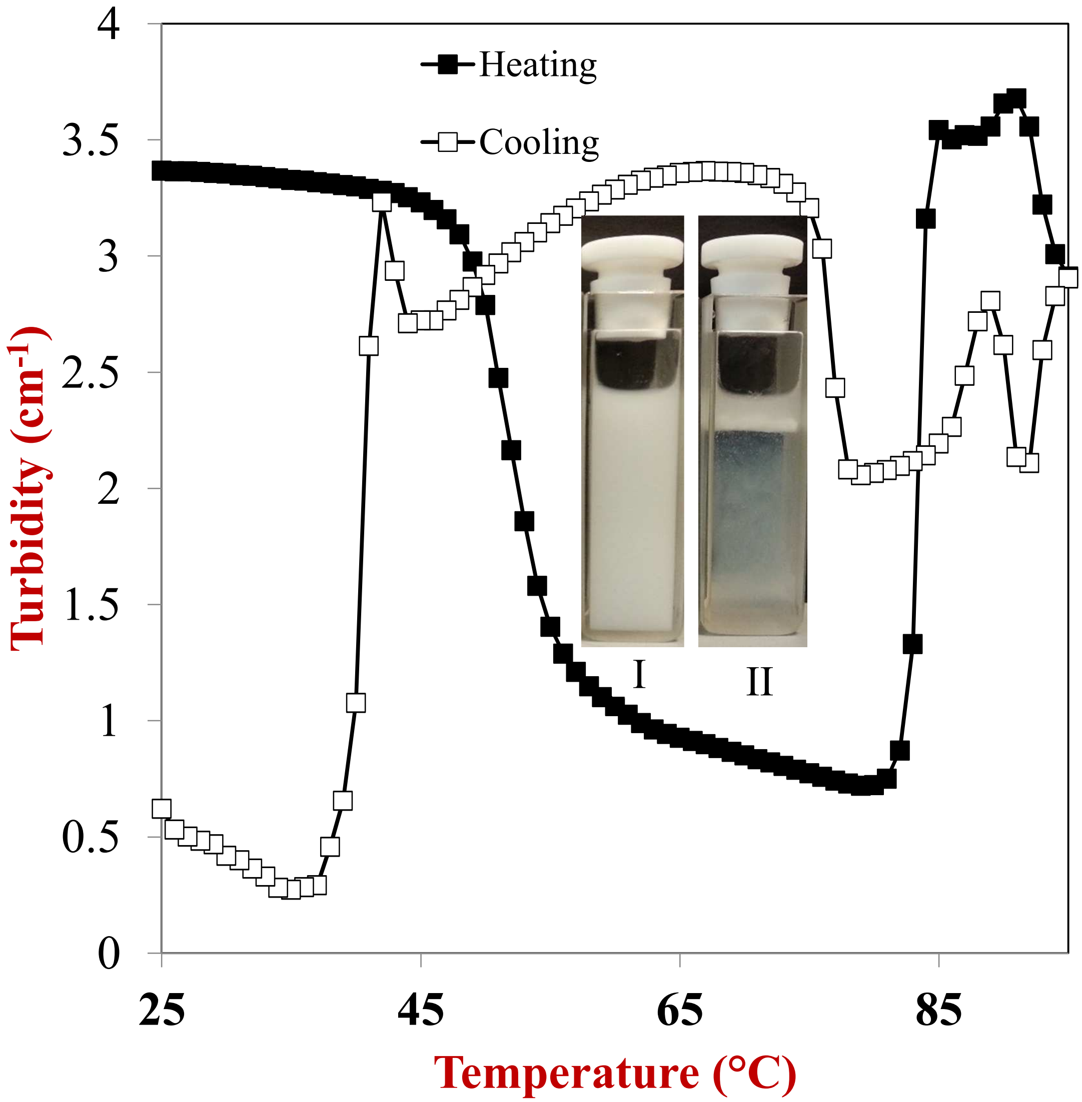


Figure 4d. Influence of temperature on turbidity of emulsions produced using 10% oil phase (limonene), 15% surfactant phase (Tween 60), and 75% aqueous phase (pH 3 buffer solution) at a stirring speed of 600 rpm at 45°C. This figure is representative of non-thermoreversible-type-4 (NT-4) emulsion. See Tables 1, 2, and 3. NT-4 underwent phase separation. I: Fresh emulsion before thermal treatment. II: Emulsion after heating and cooling cycles.

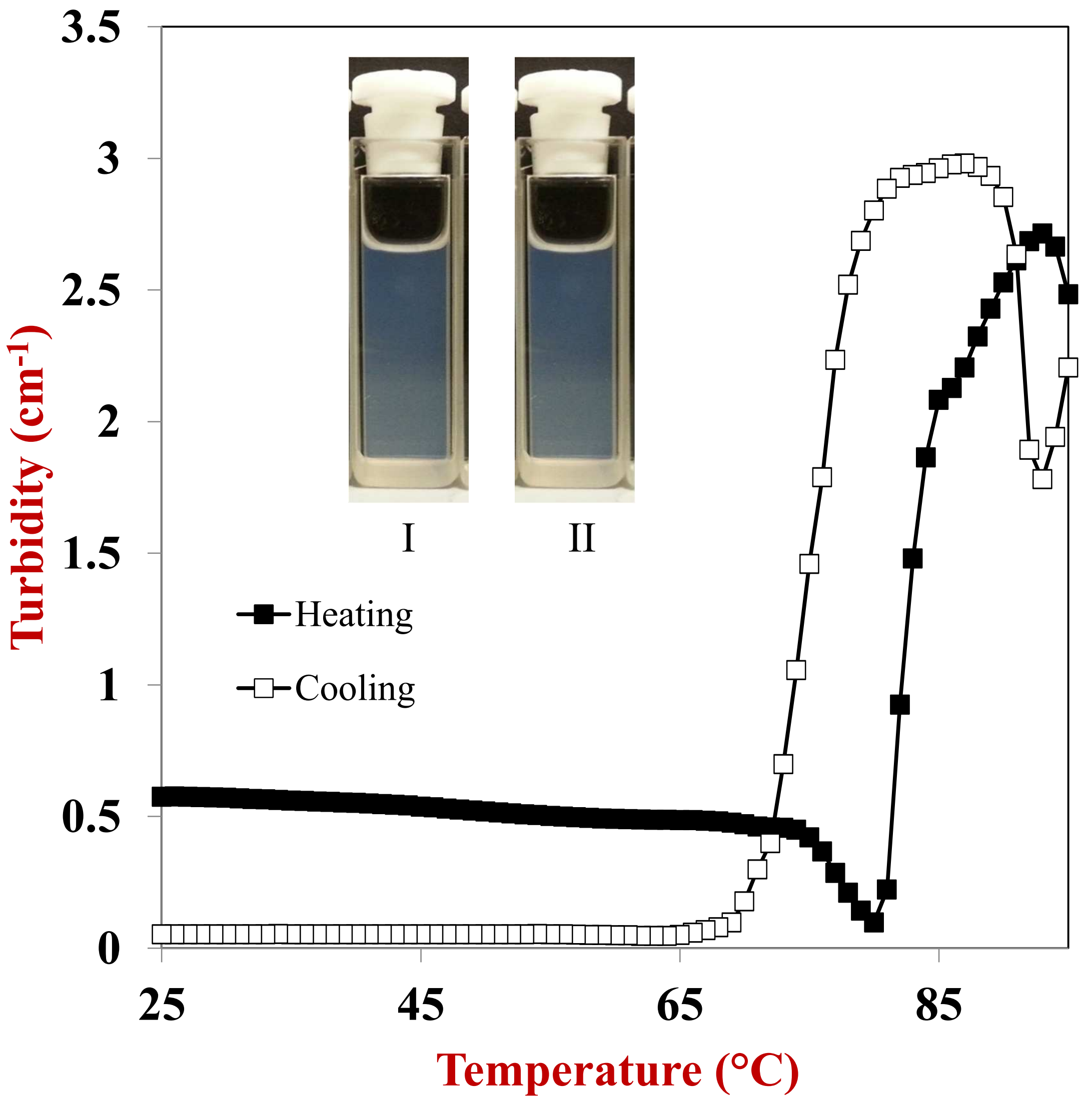


Figure 4e. Influence of temperature on turbidity of emulsions produced using 10% oil phase (7% limonene & 3% MCT), 15% surfactant phase (Tween 60), and 75% aqueous phase (pH 3 buffer solution) at a stirring speed of 600 rpm at 45°C. This figure is representative of thermoreversible-type-1 (T-1) emulsion. See Tables 1, 2, and 3. Key: I: Fresh emulsion before thermal treatment. II: Emulsion after heating and cooling cycles.

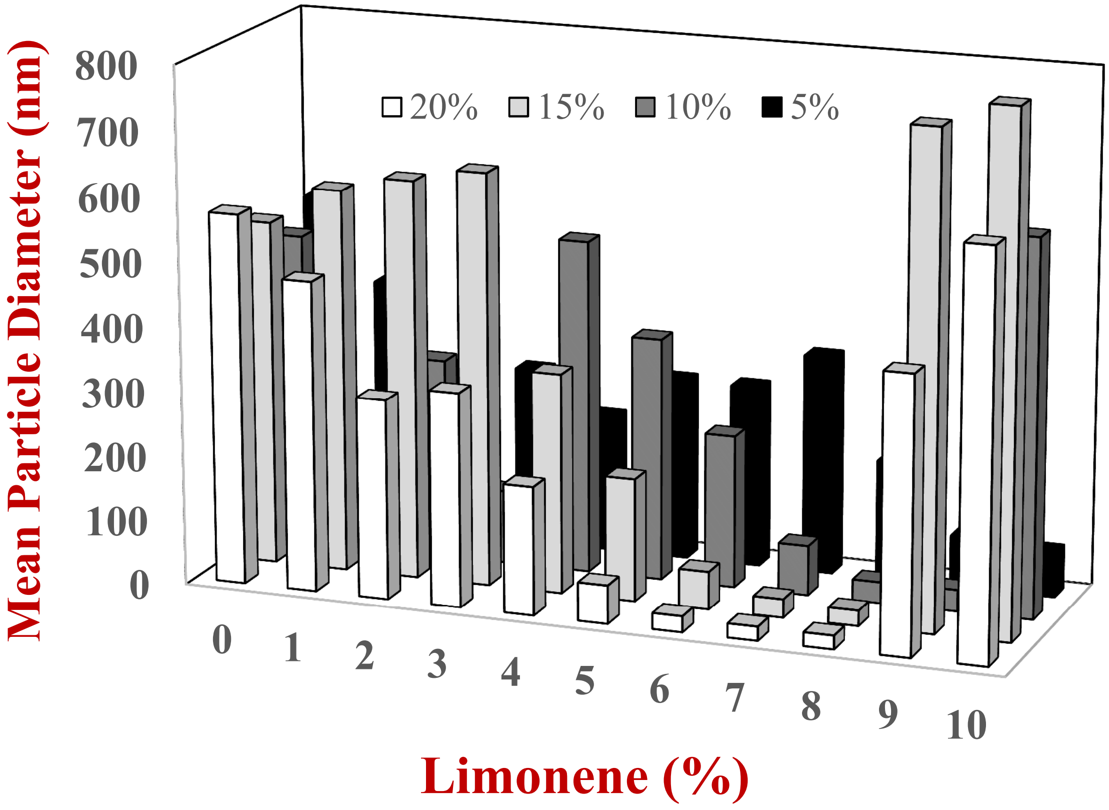


Figure 5. Effect of oil phase composition and surfactant concentration on the mean particle diameter produced after slow heating followed by cooling. Emulsions were initially prepared by spontaneous emulsification approach using 10 % oil phase (limonene + MCT), different concentrations of surfactant phase (Tween-60; 5%, 10%, 15%, and 20%), and aqueous phase (pH 3 buffer solution) at a stirring speed of 600 rpm at 45 °C.

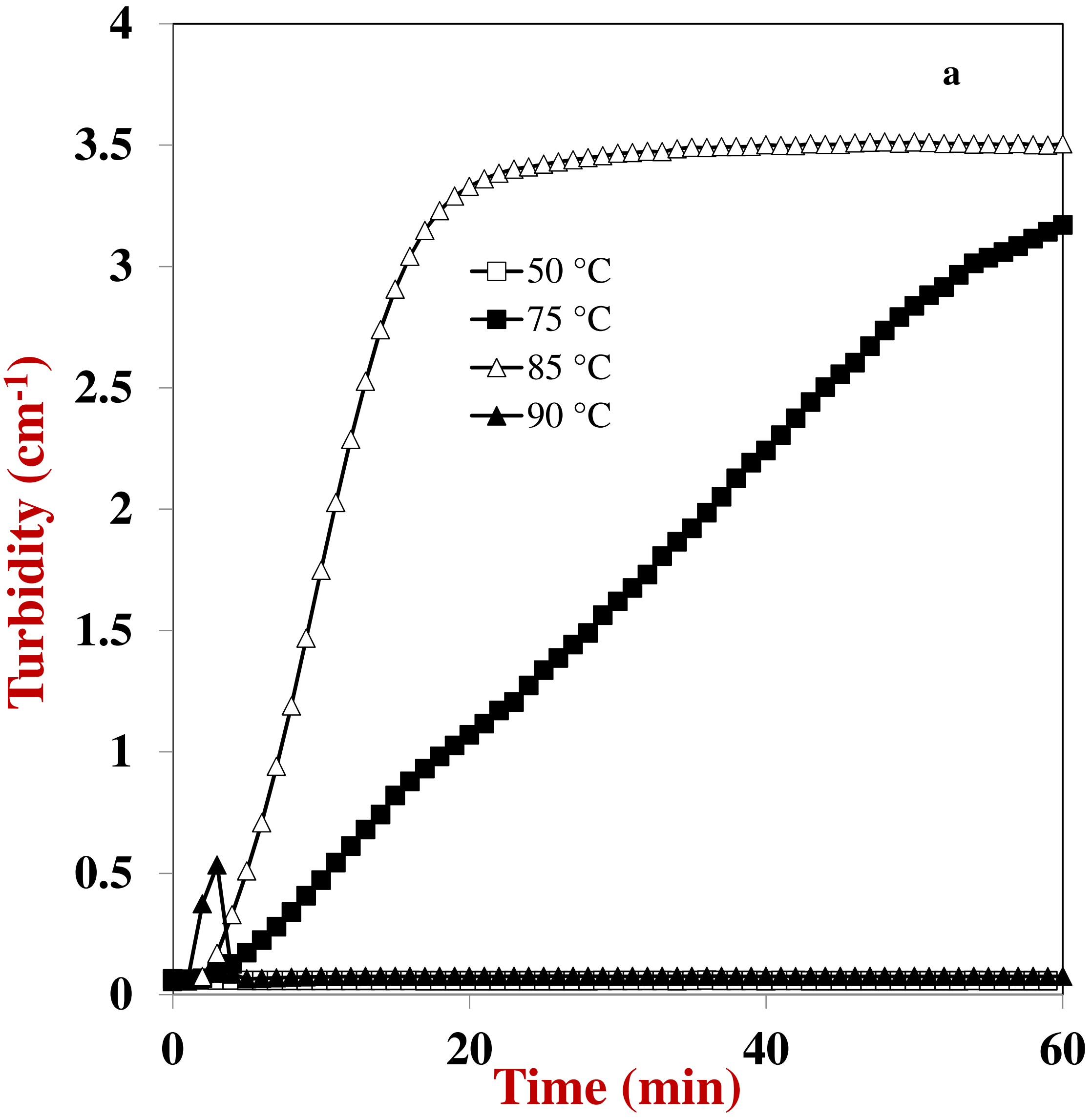


Figure 6a. Influence of holding time at different temperatures (different isothermal conditions) on the turbidity of emulsion produced using 10% oil phase (5% limonene & 5% MCT), 15% surfactant phase (Tween 60), and 75% aqueous phase (pH 3 buffer solution) at a stirring speed of 600 rpm at 45°C.

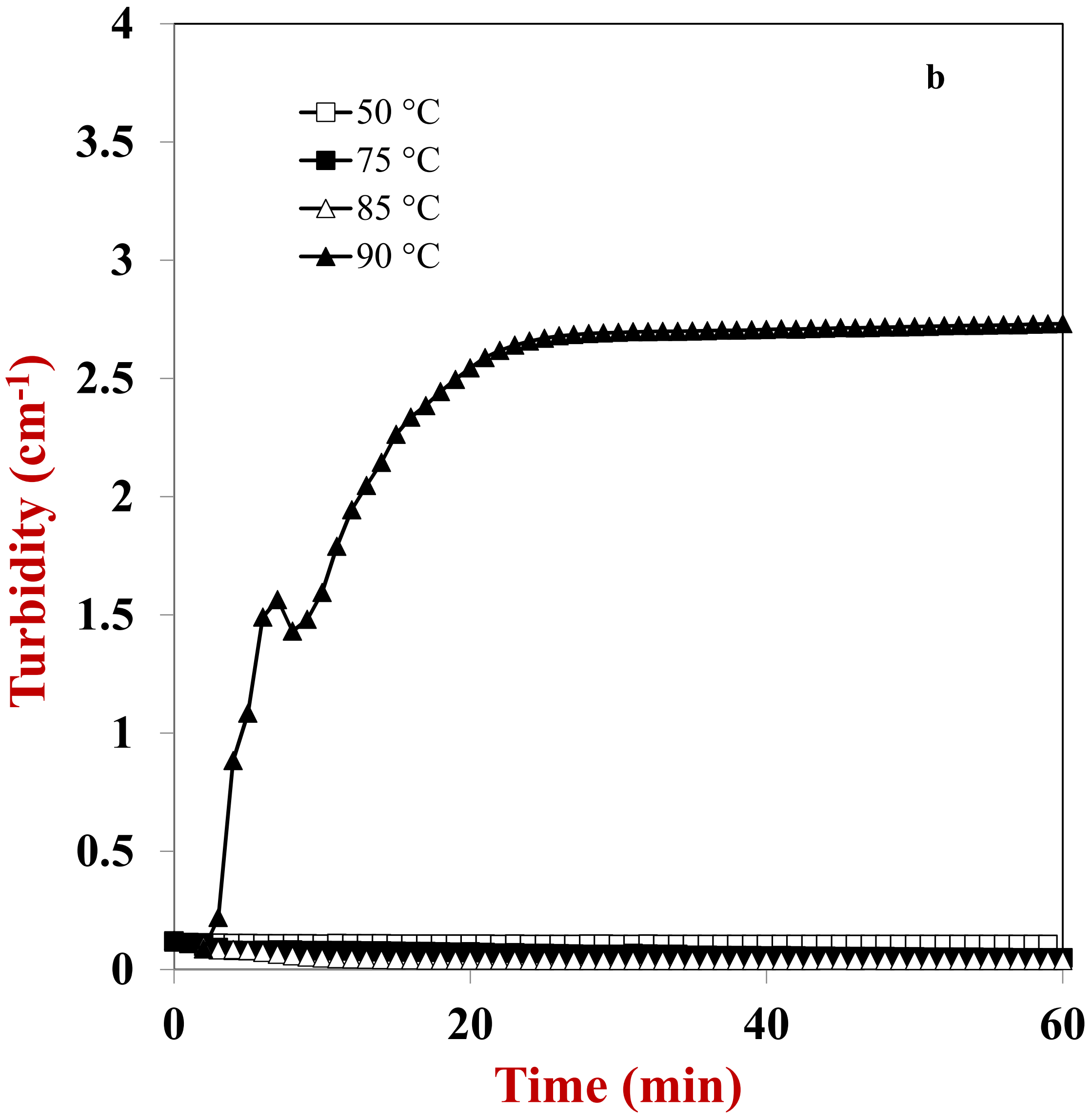


Figure 6b. Influence of holding time at different temperatures (different isothermal conditions) on the turbidity of emulsion produced using 10% oil phase (7% limonene & 3% MCT), 15% surfactant phase (Tween 60), and 75% aqueous phase (pH 3 buffer solution) at a stirring speed of 600 rpm at 45°C.

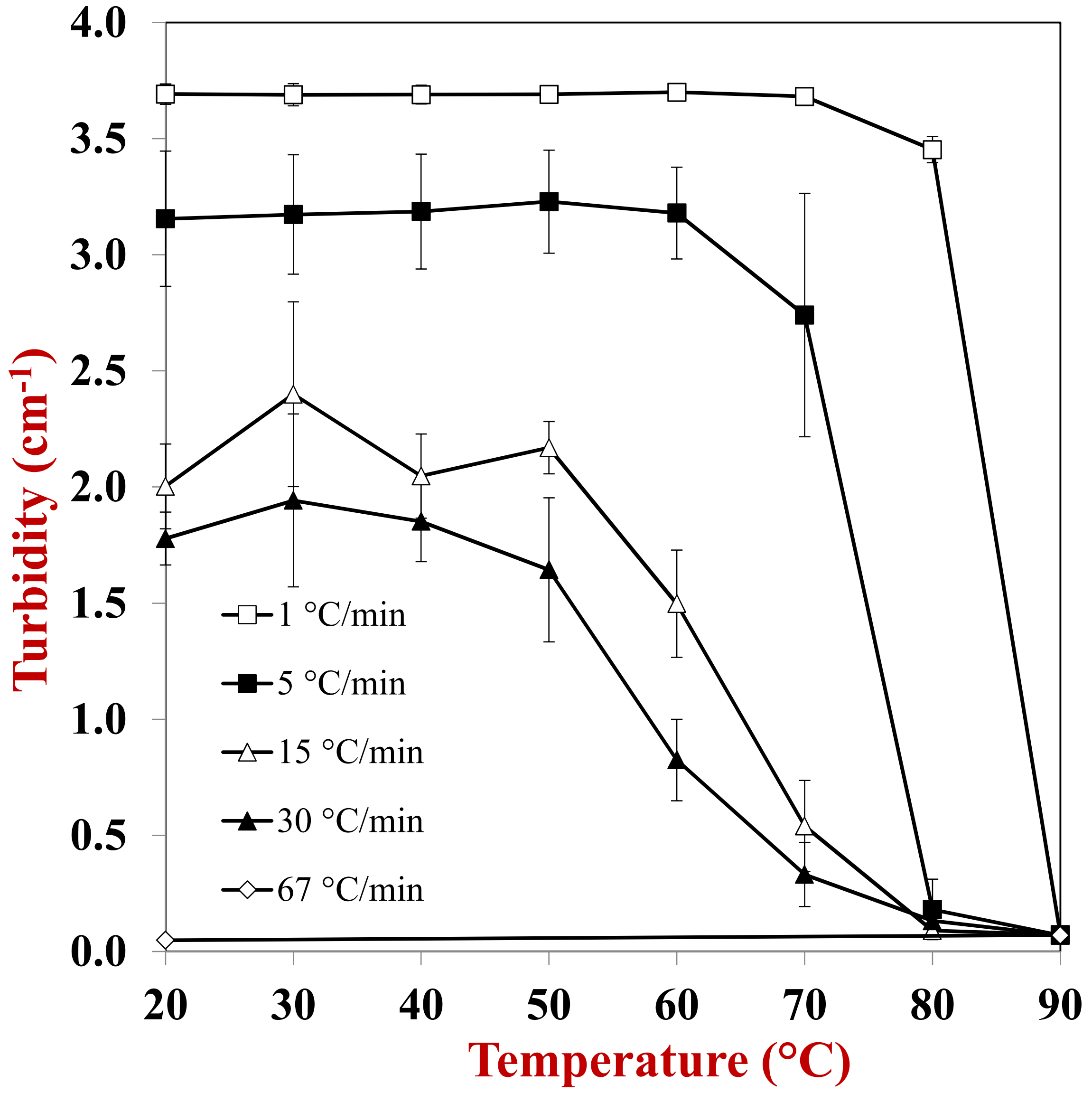


Figure 7a. Influence of cooling rate on the turbidity of emulsion produced using 10% oil phase (5% limonene & 5% MCT), 15% surfactant phase (Tween 60), and 75% aqueous phase (pH 3 buffer solution) at a stirring speed of 600 rpm at 45°C.

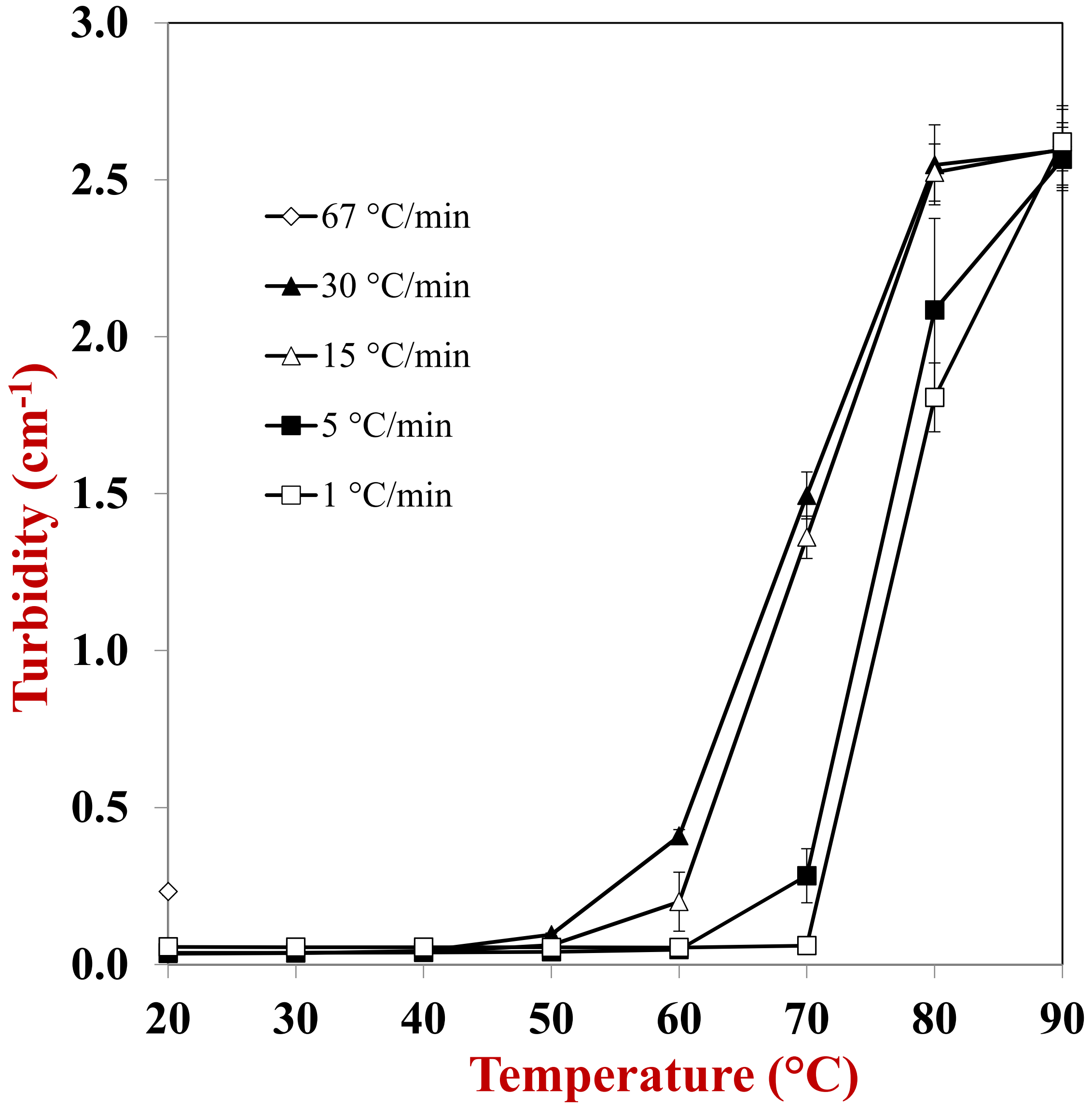


Figure 7b. Influence of cooling rate on the turbidity of emulsion produced using 10% oil phase (7% limonene & 3% MCT), 15% surfactant phase (Tween 60), and 75% aqueous phase (pH 3 buffer solution) at a stirring speed of 600 rpm at 45°C.

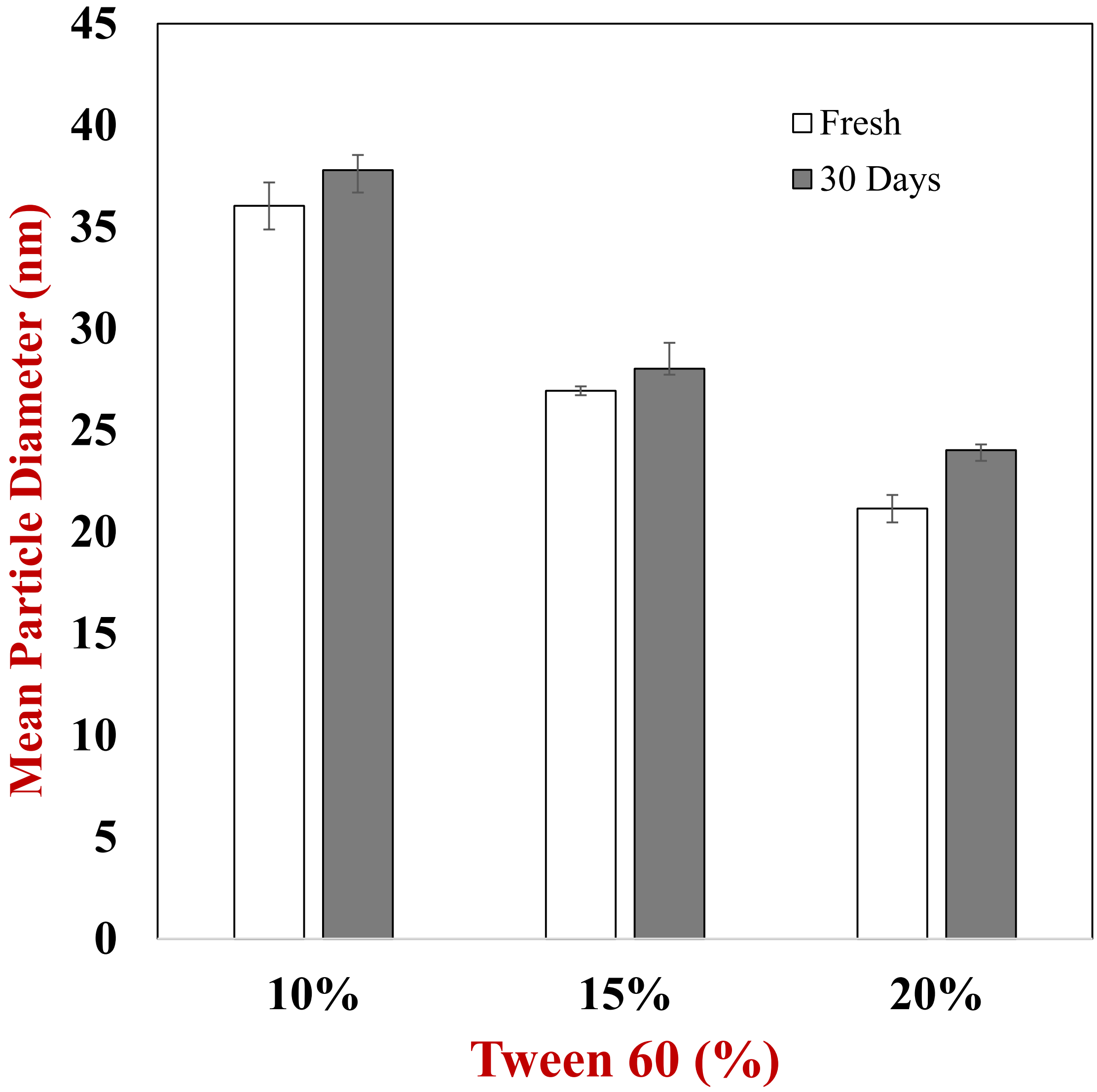
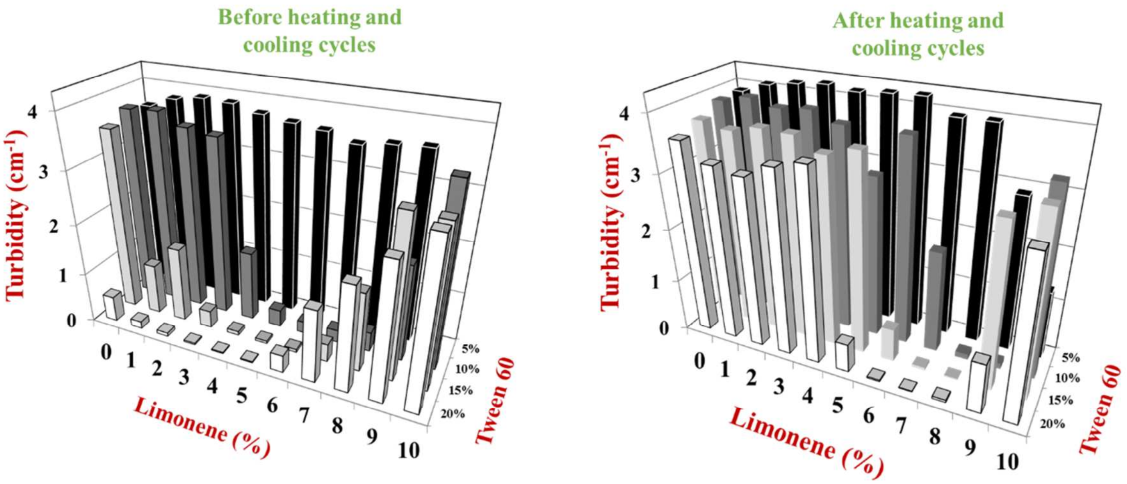


Figure 8. Effect of storage time on mean particle diameter of emulsion produced using spontaneous emulsification. Emulsions were produced using 10% oil phase (8% limonene & 2% MCT), 10, 15, and 20% surfactant phase (Tween 60), and aqueous phase (pH 3 buffer solution) at a stirring speed of 600 rpm at 45°C. Emulsions were subjected to thermal treatment (heating from 25 to 95 °C followed by cooling from 95 °C to 25 °C using the thermal rate of 1 °C/min) before storage study.

Factors Affecting Thermal Reversibility of Ultrafine Limonene-Enriched Emulsions Produced Using Spontaneous Emulsification, by Amir Hossein Saberi, Yuan Fang, and David Julian McClements *



Oil phase composition and surfactant-to-oil ratio have appreciable impacts on the transparency and thermal behavior of surfactant-oil-water systems containing limonene, medium chain triglycerides (MCT), and Tween 60.