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Preparation of nanoemulsions by phase inversion temperature (PIT) method

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ABSTRACT

Nowadays, there is an increasing interest in the utilization of nanoemulsions in many industries, such as pharmaceuticals, food and beverage, cosmetics and agrochemicals because of a number of potential advantages over conventional emulsions (i.e., increased bioavailability, enhanced long-term stability and high optical clarity) and microemulsions (i.e., relatively low amount of surfactant used). The productions of nanoemulsions are typically divided into high-energy and low-energy emulsification methods. High-energy methods involve the use of mechanical devices to break up particles into smaller sizes. In contrast, low-energy methods divert the intrinsic physicochemical properties of surfactants, co-surfactants and excipients in the formulation, leading to the generation of emulsion droplets in the nanometric range. Owing to the advantages of low-energy methods (i.e., low cost and ease of implementation), there is considerable interest in the productions of nanoemulsions using low-energy methods, namely spontaneous emulsification, phase inversion composition, and phase inversion temperature (PIT). Among these, the PIT method is the most widely used in industry and has evidenced the progress in the knowledge of the factors leading to nanoemulsions with minimum size and low polydispersity index. In this review, the principle of the PIT method is presented. The major factors influencing nanoemulsion formation using the PIT method and its applications to other lipid-based nanocarriers are reviewed.

1. INTRODUCTION

Over the last decade, nanoemulsion-based delivery systems have been gained interest in the pharmaceuticals[1-4], food and beverage[5-8], cosmetics[9-11], agrochemicals[12-14] and other[15] industries. The main purposes are to encapsulate, protect, and deliver lipophilic components such as drugs, vitamins, food supplements, antioxidants, antimicrobials, pesticides. In general, it is desirable to utilize nanoemulsions with very small droplet sizes, typically < 100 nm, since they have several potential benefits, including increased bioavailability[1,2], enhanced long-term stability[16] and high optical clarity[17]. These can be explained by the particle properties regarding surface area, kinetic property and optical property which are related to their particle sizes.

The percentage of surface molecules in particles, particle settling velocities, and Brownian motion of the particles are summarized in Table 1. It is interesting to see that compared to a 10-µm particle, a large percentage of the molecules are present on the surface of nanoparticles. Hence, the dissolution rate of nanoparticles is much higher than microparticles. When the particles are of nanometer length scale, surface irregularities can play an important role in adhesion. Therefore, the smaller diameter of nanoemulsions leading to an increase in surface area enables nanoemulsions to exhibit greater biological activity per given mass

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compared with larger nanoemulsions. An example of this can be seen in the potential of nanoemulsions to increase the bioavailability of lipophilic substances encapsulated within them [1,2].

In general, nanoemulsions are metastable systems that they have a tendency to break down over time due to gravitational separation (creaming or sedimentation), coalescence, flocculation and Ostwald ripening[18,19]. As demonstrated in Table 1, the particles with particle size, typically ≤ 100 nm have a low setting velocity and high Brownian motion. Therefore, the smaller size of the nanoemulsions corresponds to greater stability against gravitational separation and droplet aggregation[16].

The size of the colloidal particles present in the solution determines the appearance of the colloidal dispersion. Figure 1 illustrates the optical appearances of a colloid dispersions having different particle size. The relatively small droplet sizes compared to the wavelength of light ($r << \lambda$) mean that they only scatter light waves weakly. Therefore, the systems tend to be transparent or translucent [17], which are advantageous for incorporation into optically transparent products[21].

Table 1. Percentage of surface molecules in particles, particle settling velocities and Brownian motion of the particles[20]

Particle size (nm)	Surface molecule[a] (%)	Settling velocity[b] (nm/sec)	Brownian displacement[c] (nm in 1 sec)
1	100.00	0.00043	54,250
10	27.10	0.043	17,155
100	2.97	4.30	5,425
1000 (1 μm)	0.30	430.0	1,716
10000 (10 μm)	0.03	43,005.0	543

[a]The ratio of molecule on the surface monolayer: % surface molecule = $\frac{\left(\frac{4}{3}\right)\pi[d^3-(d-\sigma)^3]}{\left(\frac{4}{3}\right)\pi d^3}100$

[b]Settling velocity: $\nu = \frac{d^2 g(\rho_s - \rho_l)}{18\mu_l}$

[c]Brownian displacement: $\chi = \sqrt{\frac{2k_B Tt}{\pi\mu d}}$

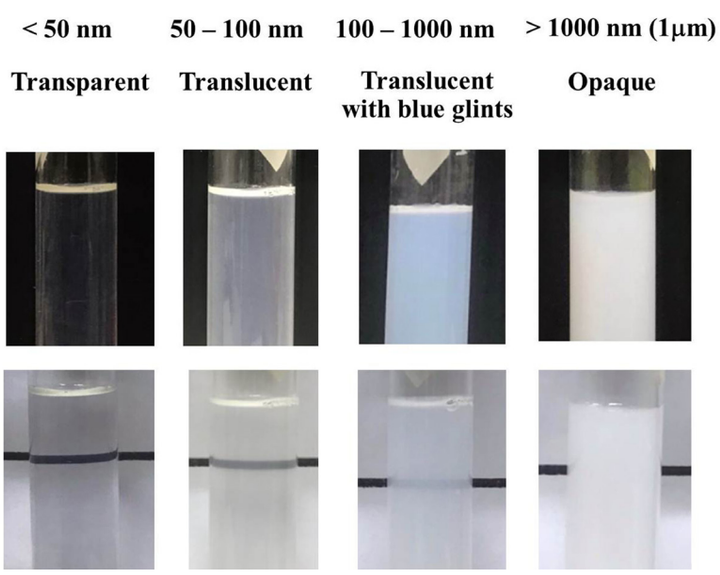

Figure 1. Optical appearances of colloid dispersions having different particle size.

Nanoemulsions can be prepared in a two-step process where macroemulsions is firstly formed and then converted to nanoemulsions in a second step which is typically categorized as high-energy and low-energy emulsification methods[16,22-24]. High-energy methods are based on the use of high energy for bringing down the large droplet size into small nano-size by using specific devices like ultrasound generators or high pressure homogenizers[25]. In contrast, low-energy methods allow the formations of nanoemulsions by utilizing the chemical potential of the components or environmental conditions, which makes changes in the optimum spotaneous curvature of surfactants by changing the temperature or the composition. These methods require significantly less input energy as only simple stirring is needed[26-28]. They can be classified as spontaneous emulsification (SE), phase inversion composition (PIC) and phase inversion temperature (PIT)[22,23].

Recently, there has been growing interest in producing nanoemulsions using low-energy methods because they need no expensive equipment and are more energy efficient. This review reports the main development in nanoemulsion formation by the PIT method. The attention is focused on the formation principle, preparation conditions and application of the PIT method to other lipid-based nanocarriers. Since nanoemulsion formation by PIT method involves the formation of microemulsions at its PIT before converting to nanoemulsions, differences and similarities between nanoemulsions and microemulsions are also reviewed.

2. NANOEMULSIONS AND MICROEMULSIONS

Nowadays, many researches prepare nanoemulsions by low-energy methods and self-emulsifying drug delivery systems generating nanodroplets. There is considerable confusion over the difference between nanoemulsions prepared by such methods and microemulsions. Differences and similarities between emulsions, nanoemulsions and microemulsions are summarized in Table 2

Table 2. Comparison of the properties of different emulsion types[16,22,24,29,30]

Properties	Emulsions	Nanoemulsions	Microemulsions
Diameter range	100 nm -100 μm	10 – 100 nm	2-50 nm
Shape	Spherical	Spherical	Spherical, lamellar
Polydispersity	Often high (> 40%)	Typically low (< 10 -20%), single or narrow peak that may be narrow or broad	Typically low (< 10%), single narrow peak
Stability	Thermodynamically unstable, weakly kinetically stable	Thermodynamically unstable, kinetically stable (metastable)	Thermodynamically stable
Surface-to mass ratio (m^2g^{-1})	0.07 – 70	70 – 330	130 – 1300
Appearance	Opaque / Turbid	Transparent / Translucent	Transparent
Method of preparation	High and low-energy methods	High and low-energy methods	Low-energy method
Stability analysis	Properties may change over time	Properties may change over time	Properties do not change over time

2.1. Nanoemulsions

Nanoemulsions are emulsions that contain very small droplets, i.e., 10-100 nm[16], < 300 nm[29] or 20-500 nm[24]. Due to their small size, nanoemulsions often appear transparent or translucent similar to microemulsions. Since nanoemulsions can be formed at low surfactant-to-oil ratio, the particles in nanoemulsions tend to be spherical shape. These can be explained by high interfacial tension (γ) of the system and low particle radius (r), leading to a relatively large Laplace pressure ($\Delta P_L = 2\gamma/r$) favoring a reduction of the interfacial area. A sphere has the lowest interfacial area for a given volume of the material[30]. Nanoemulsions are a thermodynamically unstable system that will breakdown over time. However, because of the small size of nanoemulsions, they can be kinetically stable to gravitational separation and aggregation over long time scales[19]. Due to their thermodynamically unstable system, nanoemulsions cannot be formed spontaneously. They always require the input of external energy to convert the separate components into a colloidal dispersion.

2.2. Microemulsions

Microemulsions are a thermodynamically stable system (generally water, oils, and emulsifiers, with the optional addition of co-solvent) that typically contains high surfactant-to-oil ratio. In principle, microemulsions can be formed spontaneously by simply mixing all components together at a particular temperature without supplying any external energy. They may form one, two, three or more separate phases that are in equilibrium with each others. These phases may be water-continuous, oil-continuous or bicontinuous, depending on the concentrations, nature, and arrangements of the molecules present. Therefore, the structures of microemulsions may be spherical, lamellar structures or bicontinuous[29,30]. Although microemulsions are thermodynamically stable, they may become unstable if some of the components undergo chemical changes during storage or if the environmental conditions are altered into a range where the system is no longer thermodynamically stable[30].

3. LOW-ENERGY EMULSIFICATION METHODS

Low energy emulsification proceeds by the use of low energy derived from the constituents of the formulation, thus avoiding the use of external high energy equipment. This low energy from the formulation constituents transforms the emulsion droplets into nano-sizes. A number of different nanoemulsion preparation methods are based on this principle, including SE, PIC and PIT methods.

3.1. Spontaneous emulsification (SE)

This process is based on the diffusion upon the dilution of the system causing the movement of water-miscible components (solvent, surfactant and co-surfactant) from an organic phase into the aqueous phase. SE generally involves the addition of an organic phase (containing oil and hydrophilic surfactant) into an aqueous phase (containing water and co-surfactant)[26,27,31-34]. The rapid migration of water-miscible components into aqueous phase causes an immense turbulence in the interface of two phases and a large increase in the oil-water interfacial area. It leads to the spontaneous formation of oil droplets surrounded by aqueous phase through a budding process[16,22]. Nanoemulsions can also be prepared by dilution of microemulsions or cubic liquid crystals with water[14,35]. During dilution with water, the co-surfactants diffuse from the oil-water interface to an aqueous phase. These make the micelles no longer thermodynamically stable, obtaining nanoemulsions. Moreover, the SE method is used in the pharmaceutical industry to obtain nanoemulsions as carriers for lipophilic drugs in aqueous media. Systems prepared using this approach are usually referred to in the literature as self-nanoemulsifying drug delivery systems (SNEDDS)[36,37].

3.2. Phase inversion composition (PIC)

PIC method is based on a change in emulsion mixture phase (i.e., o/w to w/o or *vice versa*) due to a change in the composition of emulsion mixture at a constant temperature. It involves the addition of the components

(water or oil) over a mixture of the other two components (oil-surfactant or water-surfactant, respectively). For example, w/o microemulsions change into o/w nanoemulsions by slow addition of water. With the increase in water content in the system, the hydration of polyoxyethylene chain of surfactant also increases. This causes a change in the surfactant spontaneous curvature, turning from negative to zero. At this point, hydrophilic-lipophilic properties are balanced and further addition of water not only changes the surfactant curvature from zero to positive but also transforms w/o microemulsions into o/w nanoemulsions[22,23]. The preparation of nanoemulsions by PIC method can broadly be divided into 3 steps: mixing of the organic phase (oil + surfactant), titration of aqueous phase into an organic phase, and additional mixing[28,38,39].

3.3. Phase inversion temperature (PIT)

PIT is a low-energy emulsification method for preparing nanoemulsions which make changes in the optimum curvature of surfactants at constant composition by changing the temperature. It has the advantage over SE method in that its composition is without organic solvent which is an integral component of the SE method[16,23]. The PIT method also has superiority over the PIC method in that the nanoemulsion droplets have lower diameter and polydispersity index (PDI). The emulsification efficiency of PIT method has also been found higher (1) than that of PIC method (0.35)[40]. In the following sections, formation principle, preparation conditions and potential applications of the PIT method are discussed.

4. NANOEMULSION FORMATION BY THE PIT METHOD

4.1. Formation principle

The PIT method is firstly introduced by Shinoda and Saito[41,42], using the specific ability of non-ionic surfactants that their optimum curvature (molecule geometry) or solubility is temperature-dependent. Non-ionic surfactant behaves hydrophilic at low temperatures because of high hydration of the polar head group which tends to be more soluble in water. As the temperature is raised, it turns to lipophilic due to progressive dehydration of the polar head group. The amphiphilic character of surfactant is changed towards the lipophilic behavior and the solubility of the surfactant in water decreases. The surfactant becomes more soluble in the oil phase than in the aqueous phase. However, before changing from hydrophilic character to lipophilic character, it reaches a point called PIT or hydrophilic-lipophilic balance (HLB) temperature where it does not exhibit either lipophilicity or hydrophilicity. At this point, the solubility of the surfactant in the oil and aqueous phase is approximately equal (the mean spontaneous curvature of the surfactant molecules is zero) and extremely low interfacial tensions (10^{-2} - 10^{-5} mN·m^{-1})[16,23].

The temperature dependence of solubility or the packing parameter of non-ionic surfactants accounts for the ability to form nanoemulsions using the PIT method[16,22,23]. A schematic representation of the PIT method to form o/w nanoemulsions is illustrated in Figure 2. At relatively low temperature (T < ~ PIT – 30°C), the surfactant head groups are highly hydrated and have a large positive spontaneous curvature (molecule geometry $p < 1$) that favors the formation of o/w emulsions. At a critical temperature of HLB temperature (T ~ PIT), the surfactant head groups are partially dehydrated and the spontaneous curvature is zero (molecule geometry $p \approx 1$). It favors the formation of a planar monolayer or bicontinuous microemulsion phase containing comparable amounts of aqueous and oil phases coexists with both excess water and oil phases. In this region, the system appears translucent with blue glints[17,43]. At high temperature (T ~ PIT + 15 or 20°C), the polar head groups of surfactant are highly dehydrated and the spontaneous curvature becomes negative (molecule geometry $p > 1$) that favors the formation of w/o emulsions.

The PIT method takes advantage of the extremely low interfacial tensions at the PIT or HLB temperature to promote emulsification in which very small droplets sizes can be obtained.

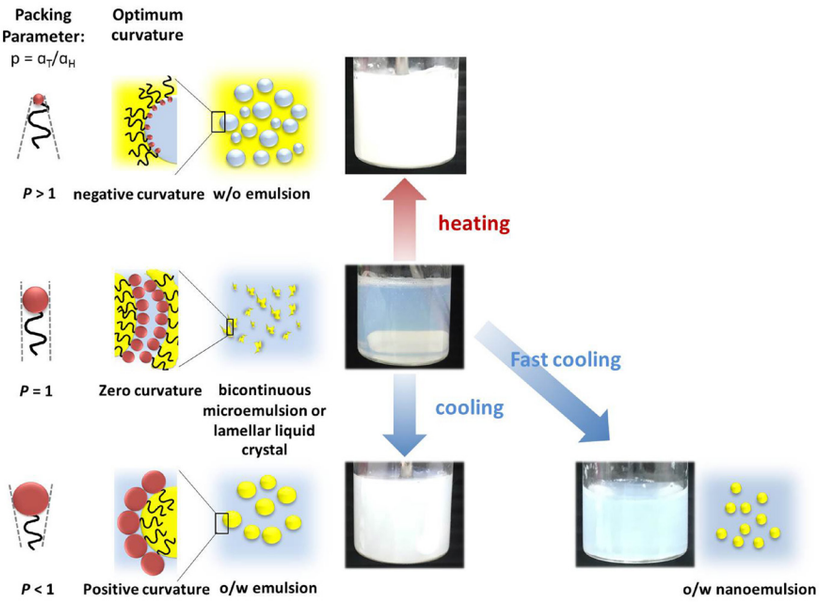

Figure 2. Schematic representation of the formation of nanoemulsions by the PIT method.

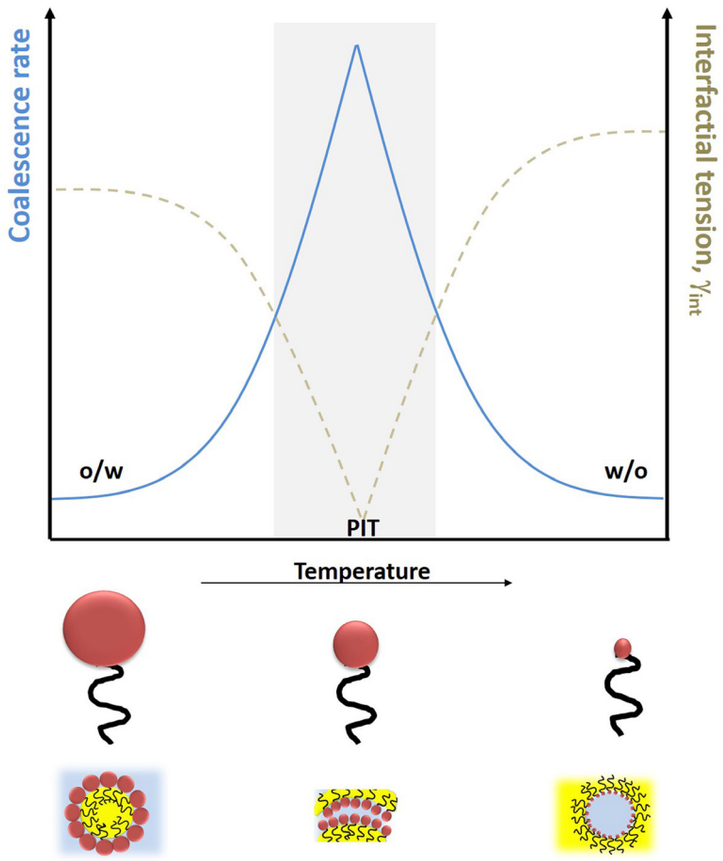

Figure 3. Schematic representation of temperature dependence of coalescent rate and interfacial tension of nanoemulsion formation by the PIT method.

However, it has been shown that the emulsions are very unstable and coalescence rate is extremely fast (Figure 3). If the temperature of the system is quickly moved away from the PIT by a rapid cooling or heating, kinetically stable o/w or w/o emulsions, respectively, can be produced [23,41,44,45]. During this fast cooling process, the surfactant molecules rapidly migrate from the oil phase into the aqueous phase, resulting in the spontaneous formation of small oil droplets due to the increase in interfacial area and turbulent flow generated [16].

4.2. Preparation conditions

In the PIT method, nanoemulsions are prepared by the formation of microemulsions at its PIT followed by immediate cooling to room temperature which can be divided into 3 main steps:

1. Non-ionic surfactant, oil and water are stirred at room temperature to form a coarse emulsion.

2. The mixture is then gradually heated up to around or above the PIT.

3. The solution is rapidly cooled to the room temperature with continuous stirring, resulting in the formation of o/w nanoemulsions.

In the fast cooling process, the mixture is rapid cooled either by immersing into an ice bath[44,46-49] or by diluting with cold water[17,27,34,43,50]. The influences of the formation parameters (i.e., preparation temperature, electrolytes, temperature cycling process and cooling rate) and composition parameters (i.e., oil type and surfactant concentration) have been investigated and reported.

4.2.1. Formulation parameters

4.2.1.1. Preparation temperature

It is well known that temperature plays an important role in nanoemulsion formation by the PIT method. Shinoda and Saito[41,45] reported that droplet size of a water-cyclohexane emulsion stabilized with polyoxyethylene nonylphenylether changes remarkably with temperature. The droplet size is very small but less stable towards coalescence close to the PIT or HLB temperature at which the interfacial tension between water and oil phases becomes nearly zero. Stable o/w emulsions are obtained when the PIT of the system is approximate 20-65°C higher than the storage temperature. On the contrary, stable w/o emulsions obtained when the PIT of the emulsions is about 10-40°C lower than the storage temperature[41,45]. Since the stability of nanoemulsions is sensitive to the temperature near the PIT, the stability of nanoemulsions could be improved by displacing surfactant with co-surfactants. It was found that adding either Tween 80 or sodium dodecyl sulfate in the poly(oxyethylene)-4-lauryl ether (Brij 30)/ tetradecane nanoemulsions improved nano-emulsion stability by increasing the PIT of the system and increasing the repulsive interactions between the droplets[47].

4.2.1.2. Electrolytes

Based on the fact that solubility of surfactant is related to alkalinity that affects the molecule geometry and spontaneous curvature of the surfactant as well as the PIT of the system. The principal effect of salts on the solution of non-ionic surfactant consists of salting in (i.e. all di- and tri-valent cations as well as H^+, Li^+ and anions such as I^-, SCN^-, ClO_4^-) or salting out (cations such as Na^+, K^+, NH_4^+, and anions such as F^-,Cl^-, SO_4^{2-}, PO_4^{3-}, OH^-) which raise or lower their cloud points. It has been reported that the salts which raise the cloud points of the aqueous solution of non-ionic surfactant also increase the PIT of the corresponding emulsion and *vice versa*[46,51]. Sharif et al.[52] investigated the effect of NaCl and Na_2SO_4 concentrations on the PIT of o/w nanoemulsions. The results showed that the PIT of nanoemulsions decreased with increasing concentration of NaCl and Na_2SO_4. At the same concentration, Na_2SO_4 could reduce the PIT of system stronger than NaCl[52]. Similar results were reported by Heurtault et al.[43].

The correlation between PIT in emulsions and cloud point in non-ionic surfactant solution has been investigated by Shinoda and Arai[53]. It was found that the cloud points in non-ionic surfactant solution saturated with hydrocarbons and the PIT in emulsions were parallel. This

phenomenon was confirmed by the studies of Sharif et al.[52]. From results obtained, the PIT of the system could be adjusted by adding electrolytes, especially NaCl. Zhang et al.[49] studied the preparation of β-carotene loaded nanostructured lipid carriers (NLC) by the PIT method. They found that the PIT of anhydrous milk fat/Tween 80 systems decreased from > 95 to 73°C when NaCl increased from 0 to 1.0 M in the aqueous phase[49]. Similarly, Hasan et al.[17] observed that the PIT of virgin coconut oil nanoemulsions using polyoxyl 40 hydrogenated castor oil as a surfactant was higher than 100°C and it can be adjusted to 75°C by adding NaCl at the concentration of 6%w/w[17].

4.2.1.3. Temperature cycling process

It has been reported that the particle size and PDI of nanoemulsions are influenced by a number of temperature cycling process (cycles of progressive heating and cooling through the PIT region) applied to the PIT method. The droplet size and the PDI of nanoemulsions decrease as a function of the number and temperature cycles up to stabilize a steady state[54]. Klaus et al.[55] reported that more than one cycle of heating and cooling in PIT method confers increased stability of Rapeseed oil nanoemulsions[55]. However, the effect of temperature cycling treatment is directly linked to the non-ionic surfactant amount in the system: the lower the surfactant amount, the higher the number of cycles required for stabilizing the nanoemulsions[54]. As described earlier, nanoemulsion formation takes place within the bicontinuous microemulsions at the PIT. To obtain stable nanoemulsions, all the oil needs to be stabilized within this phase [48]. Because of slow kinetics of the extended surfactants, more heating and cooling cycles are necessary to achieve long-term stability.

4.2.1.4. Cooling rate

Except for temperature cycling process, cooling rate at the PIT is another factor that can influence the stability of nanoemulsions produced by PIT method. At the slow cooling rate, there was an increase in droplet size, attributed to the particle growth as the emulsions passed through the droplet coalescence zone. On the other hand, at faster cooling rates, the small droplet size was observed which can be attributed to the fact that the emulsions spent less time in the droplet coalescence zone[8,56,57].

4.2.2. Composition parameters

4.2.2.1. Oil type

Rao and McClements[47] studied the effect of hydrocarbon oil type on the PIT of the Brij 30. It was reported that the PIT of the systems, containing 20 % w/w hydrocarbon oil, 6%w/w Brij 30 and 74 %w/w aqueous phase (10 nM NaCl solution), increased as the alkyl chain length of hydrocarbon oil increased. This effect can be attributed to the ability of oil molecule to penetrate between surfactant tails. Short chain hydrocarbon oils can penetrate more easily between the surfactant tails and thus favor a curvature that is closer to planer than long-chain hydrocarbon oil [47].

4.2.2.2. Surfactant concentration

The influence of surfactant concentration on the formation of nanoemulsions by the PIT method has been investigated. At 20 %w/w *n*-decane, an increasing in Brij 30 concentration decreased the PIT of the system and decreased the droplet size[58]. Similarly, Mei et al. reported that the PIT of paraffin oil/water nanoemulsions stabilized by Tween 80/Span 80 decreased with an increase in surfactant-to-oil ratio[46]. As the surfactant concentration increased, ethylene oxide chains tend to accumulate at the oil-water interface. Therefore, the positive spontaneous curvatures of the surfactant layer become negative at a lower temperature more easily, resulting in a reduction of the HLB temperature. However, the surfactant concentration optimization is necessary because at higher surfactant concentration gels may be formed while at lower surfactant concentration phase separation occurs.

4.3 Applications of the PIT method to another lipid- based nanocarriers

Since PIT method is a thermal approach

requiring a change in temperature to produce fine droplets, it also appears easily adaptable to the process of generating other lipid-based nanocarriers. The PIT method has been successfully implemented to form lipidic nanocapsules (LNC), an oil-in-water emulsion of a lipidic core surrounded by a shell of polyethylene glycol (PEG)-like surfactants and phospholipids, firstly reported by Heurtault et al.[43]. The size of these LNC was 51 ± 12 nm with PDI < 0.3 and stable for 18 months on storage at 4°C and 1.5 months at 37°C. The PIT method was used as a method for preparing LNC in many studies[59-62]. Zhang et al.[49] used PIT process to form milk-fat-based NLC for β-carotene delivery. Stable NLC with the size of ~ 25 nm would be prepared and showed to protect degradation of β-carotene compared to the soybean-oil based nanoemulsions[49]. Montenegro et al.[63] developed sunscreen loaded cetyl palmitate solid lipid nanoparticles (SLN) using the PIT method. All the SLN showed a mean size ranging from 30 – 95 nm with a single peak distribution[63]. Recently, other SLN prepared by the PIT method have been developed by Gao and McClements[64], indicating that SLN of Brij30/octadecane/water with a particle size < 200 nm can be formed and they were stable at a temperature below their melting point.

5. CONCLUSIONS

Nanoemulsions have been of continuous and growing interest due to their characteristic properties. They are advantageous over conventional emulsions and microemulsions for a wide range of applications. Low-energy emulsification methods, comprising SE, PIC and PIT methods have been gained interest over the past decade. Stable nanoemulsions with small droplet size and narrow size distribution can be obtained using the internal chemical energy of the system to produce fine small droplets. Among these, the PIT method is the most widely used in industry and has evidenced the progress in the knowledge of the factors leading to nanoemulsions with minimum size and low PDI. The PIT method is based on the changes in solubility of a non-ionic surfactant with temperature. It involves the formation of intermediate bicontinuous microemulsions at the PIT or HLB temperature that breaks down into fine droplets by the rapid cooling process. Presently, the PIT method has been successfully implemented to prepare other lipid-based nanocarriers including LNC, NLC and SLN.

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