\bigcirc Review \bigcirc

Nano-Emulsions; Emulsification Using Low Energy Methods

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Nano-emulsions are emulsions with droplet sizes from 20 nm up to 200-500 nm, thermodynamically unstable, and appear transparent or translucent to the naked eye. Nano-emulsions are very useful in many industries; such as food, pharmaceutical, cosmetic, and chemical. Forming nano-emulsions using a method that requires energy input from a mechanical device is known as a 'high-energy method', while a method using chemical energy stored in the components is referred to as a 'lowenergy method'. The low-energy method can be classified into three categories: the Phase Inversion Temperature method, Phase Inversion Composition method or Emulsion Inversion Point method and the Spontaneous Emulsification method. To study nano-emulsification using the low-energy methods, phase diagrams are used to determine the phase behavior of the components and specify the temperature and concentrations at which various structures exist at equilibrium with desired properties. Most research focuses on droplet size and polydispersibility of nano-emulsions, because these properties influence physicochemical properties, appearance and stability of nano-emulsions. There have been many studies on the effect of composition and the major determining factors in the emulsification process on droplet size and polydispersibility of nano-emulsions such as surfactant type, concentration, location, oil type, surfactant-to-oil ratio, salt, stirring speed, and temperature. **Keywords:** preparation of nano-emulsion, phase inversion, spontaneous, phase diagram, droplet size

1. Introduction

Nano-emulsions are emulsions with droplet sizes from 20 nm up to 200 - 500 nm [1-6], thermodynamically unstable, and appear transparent or translucent to the naked eyes. Nano-emulsions have advantages over conventional emulsions due to their small droplet size which possess stability against sedimentation or creaming [1]. Nano-emulsions are usually very stable against gravitational separation due to the relatively small particle size resulting in Brownian motion effects dominating gravitational forces. Moreover, nano-emulsions can be diluted with water without changing the droplet size distribution [2]. The change in average number of published literature for nano-emulsions per month as obtained from Scopus is shown in Fig. 1. The number increased remarkably for the past decade. Nano-emulsions are very useful in industries such as pharmaceutical, cosmetic, food and chemical [3].

The formation of nano-emulsions, a non-equilibrium system, requires energy from either mechanical device or the chemical potential of the components. There are two main methods for preparing nano-emulsions: one is the so-called 'dispersion or high-energy method' which utilizes mechanical power and the other using the chemical energy stored in the components is referred to as the 'condensation or low-energy method'.



Fig. 1 Trends in the average number of published articles on nano-emulsions per month as retrieved from Scopus. The number of nano-emulsion articles includes those for both nano-emulsions and nano-emulsification methods.

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In high-energy methods, mechanical devices used include high-shear stirring, high-pressure homogenizers, or ultrasound generators capable of generating intense disruptive forces. It has been shown that the apparatus supplying the available energy in the shortest time and having a homogeneous flow produces the smallest sized droplets. The high energy is able to break the droplets into smaller ones, provided the Laplace pressure is overcome. An increase in surfactant content at the interface reduces the Laplace pressure [1,4]. However, the level of energy required for the production of nanometer-scaled droplets is very high and therefore costinefficient, especially considering that only a small amount (around 0.1%) of the energy produced is used for emulsification [3,5]. This review mainly focuses on lowenergy methods.

The low-energy method, or condensation method, can be classified into two categories. One is based on phase inversion taking place during the emulsification process where stored chemical energy is used to obtain a small droplet-sized distribution. The other low-energy emulsification method is the spontaneous emulsification method. Mechanisms involved in this method are diffusion, dilution and phase inversion, as described later.

These phase inversions result from the spontaneous change in the curvature of the surfactant from negative to positive (to obtain an O/W emulsion) or vice versa. As a result, phase inversion releases chemical energy that is used to form the nano-emulsion, and can be achieved (i) by changing temperature at constant composition, the so-called Phase Inversion Temperature (PIT) or (ii) by varying the composition of the system at constant temperature, the so-called Emulsion Inversion Point (EIP) method or Phase Inversion Composition (PIC) method [2–6].

2. Phase Inversion Temperature (PIT) emulsification

PIT emulsification method was introduced by Shinoda and Saito in 1968 [7]. PIT emulsification method is based on the change in surfactant curvature induced by temperature, or on the change in lipophilic-hydrophilic balance of some nonionic surfactants with temperature (HLB-temperature emulsification method). Therefore, this method can only be applied to surfactants sensitive to changes in temperature, *i.e.* polyoxyethylene-type nonionic surfactants [3].

The hydrophilicity of nonionic surfactant depends on

hydration. Solubility in water of a nonionic surfactant decreases with increasing temperature. The molecular motion of water molecules hydrating to the polar heads of the nonionic surfactant increases with an increase in temperature. The number of water molecules hydrating to the surfactant decreases and the hydrophilicity also decreases. A solution of nonionic surfactant becomes turbid at elevated temperature, (referred to as the cloud point). To prepare a nano-emulsion using the PIT method, oil, water, and non-ionic surfactants are mixed at room temperature and slightly stirred. Next, the mixture is gradually heated. As a result, the HLB of surfactant progressively changes from more than 7 to less than 7. The phase of the emulsion may invert near PIT. Shinoda and Arai [8] reported that the cloud points in solutions of nonionic surfactants and the PIT in emulsions are parallel.

As shown in Fig 2, at temperatures below the PIT, the ternary system (water/nonionic surfactant/oil) shows a macro-emulsion and mostly hydrophilic heads of the nonionic surfactant are hydrated acquiring a relatively big volume, compared with the lipophilic part of the molecule. The surfactant monolayer has a large positive curvature to the water phase, forming an O/W emulsion. Increasing temperature causes dehydration of the hydrophilic heads and these surfactants gradually become lipophilic. When temperature increases further, the heads of the nonionic surfactant are dehydrated and the curvature becomes negative to the water phase, and a W/O emulsion appears. It means the changes in temperature induce a change in the hydration of the head group of the surfactant, and a consequent change in its curvature. At the PIT or intermediate temperature, the affinity of amphiphiles for each phase is similar, interfacial curvature is very low, (almost close to zero) and a thermodynamically stable planar structure with zero curvature appears, and lamellar liquid crystals, or bicontinuous microemulsions are formed. Saito and Shinoda [9] investigated the effect of temperature on the interfacial tension both between water and the surfactant phase, and between the surfactant phase and oil. They found that (1) the interfacial tension between the water and oil phase changed markedly with temperature and became nearly zero (below 0.01 mN m⁻¹) close to the PIT; (2) the mean droplet diameter was also small at the PIT but increased with temperature; and (3) the coalescence rate of the emulsion droplets was very fast close to the PIT [9]. Therefore, the temperature has to be quickly moved away from the PIT by rapid cooling or heating to obtain



Fig. 2 Schematic illustration for PIT and PIC/EIP emulsification.

kinetically stable nano-emulsions using this method.

Morales *et al.* [10] studied a water/hexaethylene glycerol monohexadecyl ether/mineral oil (d_{20} =0.85g/mL) system. Minimum droplet size was 40nm, when the phase type at PIT was single–D phase (flow birefrigent microemulsion) or two–phase (W phase+D phase). At 0.67 oil–to–surfactant weight ratio, droplet size (about 40nm) is independent of the water content. Izquierdo, *et al.* [11] investigated a water/mixture of polyoxyethylene 4–lauryl ether and polyoxyethylene 6–lauryl ether/isohexadecane system. Nano–emulsions with droplet radius of 60–70nm and 25–30nm were obtained at 20 wt% oil and surfactant concentrations of 4 and 8%, respectively. At 8 wt% surfactant concentration, D–phase microemulsions were observed.

Stability is an important property for nano-emulsions. Ostwald ripening would be the main mechanism for the instability of nano-emulsions. The mechanism is based on the different solubility of the dispersed phase to continuous phase between small and large droplets with different Laplace pressures. The Ostwald ripening rate is represented by the Lifshitz-Slezov-Wagner (LSW) theory. In this theory, the cube of the droplet radius and time show a linear relationship [1,12].

Izquierdo *et al.* [13] investigated the stability of nanoemulsions in a water/polyoxyethylene 4-lauryl ether(4.0-8.0 wt%)/oil (20 wt%) system. In this study, the stability of the emulsion decreased with an increase in surfactant concentration and also decreased with an increase in oil solubility to an aqueous phase. The stability of the emulsion in relation to the type of oil phase was hexadecane>tetradecane>dodecane>decane. Moreover, Izquierdo *et al.* [14] found that the decrease in nanoemulsion stability with an increase in surfactant concentration was attributed to the increase in the oil diffusion rate, as a consequence of higher surfactant excess. Ee *et al.* [15] also reported that the instability of nano-emulsions prepared by the PIT method depended on the surfactant concentration.

Rao and McClements [16] showed that the stability of a nano-emulsion in a water/polyoxyethylene 4-lauryl ether/tetradecane system increased by adding either Tween 80 (0.2 wt%) or SDS (0.1 wt%) to the prepared nano-emulsion. They proposed that these surfactants might change the optimum curvature of the interfacial layer. Mei et al. [17] studied the effects of inorganic salts on nano-emulsions prepared from a system of water/ mixture of Tween80 and Span 80 (0.56: 0.44)/paraffin oil using the PIT method. The inorganic salts reduced the PIT. The order of stability is AlCl₃≈NaCl>KCl>CaCl₂. Salts reduced the absolute value of the zeta potential. Some salts reduced the long-term stability of nanoemulsions. They described that adding salting-out salts in water could reduce the PIT of the system to an optimum temperature and thus allow nano-emulsions to be formed more easily.

For PIT emulsification methods, the temperature of the water/surfactants/oil emulsification mixture essentially crosses the PIT by heating or cooling the mixture. Roger et al. [18] reported that metastable nano-emulsions were produced through sub-PIT cycles with stirring in water/polyoxyethylene 8-cetyl ether/hexadecane system. The sub-PIT was a few degrees below PIT and above a threshold called the clear boundary determined by a minimum in the turbidity of the stirred mixture, which resulted from solubilization of all the oil into swollen micelles. The phenomenon of sub-PIT is similar to the results reported by Tomomasa et al. [19]. They prepared nano-emulsions by rapidly cooling the solubilization phase in a nonionic surfactant-oil-water system to room temperature. The droplet size of the nano-emulsion was about 50nm. The stability of the nano-emulsion depended on the nature of the oil and surfactant. The stability rose with an increase in the carbon number of the oil. The droplet size for liquid paraffin and squalane emulsion did not change for over a year at 25°C.

Usually, when using the PIT method, the temperature of the sample at PIT is lowered to optimum temperature by dipping the vessel containing the sample into cool water bath or ice bath. In some cases, the sample is cooled by dispersion in water [20,21]. In this method, it is considered the dilute effect also occurs when preparing the nano-emulsion. The dilute effect is the main mechanism of spontaneous emulsification as described later.

3. Phase Inversion Composition (PIC) method or Emulsion Inversion Point (EIP) method

For the PIC or EIP method, the basic concept is similar to the PIT method. When the O/W emulsion is prepared by the PIC or EIP methods, the surfactant is dissolved in oil and then water is added gradually as described later. In this system, a W/O type emulsion occurs in the oil phase. By adding water, the water phase volume increases gradually, and the W/O emulsion inverts to an O/W emulsion at a certain composition determined by the composition of the system used. When the composition of a system is appropriate, the O/ W nano-emulsion is easily prepared. This method is the same as the agent-in-oil emulsification method [22,23]. Sagitani and Takeuchi [22] reported that homogeneous O/W emulsions were obtained by conversion of the W/O emulsion to the O/W emulsion through the lamella phase and gel like O/D emulsion phase.

Fogiarini et al. [24] studied the formation of nanoemulsions using low-energy emulsification methods at constant temperature for a water/Brij 30 (polyoxyethylene(4) lauryl ether)/decane system. They compared three types of low-energy methods: (1) stepwise addition of oil to a water-surfactant mixture, (2) stepwise addition of water to a solution of the surfactant in oil, and (3) mixing all the components at once in the final composition. In this study, nano-emulsions with an average droplet size of 50 nm and high kinetic stability were obtained only at oil weight fractions lower than 0.3 using emulsification method (2). They described that phase behavior studies have revealed that compositions giving rise to nano-emulsions consist of Wm, (O/W microemulsion), La (lamellar liquid crystalline), and O (oil) phases, at equilibrium. It was shown that equilibrium properties could not fully explain nano-emulsion formation. Low values of equilibrium interfacial tensions and phase equilibrium involving a lamellar liquid crystalline phase were probably required but not sufficient to obtain nano-emulsions in this system. The key factor for nano-emulsion formation was attributed to the kinetics of the emulsification process.

For the PIC or EIP method, ionic surfactants can be used for low-energy emulsification while only nonionic surfactants can be used for the PIT method. Solè et al. [25] compared a water/potassium oleate-oleic acid-polyoxyethylene (10) lauryl ether/hexadecane system for the EIP emulsification method with high-energy emulsification methods (ultra-sound emulsification and Ultra-Turrax emulsification). The droplet sizes obtained with the EIP method were as low as 17nm which is much lower than those obtained by high-energy methods. In this system, crossing a direct cubic liquid crystal phase along the emulsification pass was necessary to obtain a small droplet size. In addition, they described that the smaller droplet sizes were obtained when the liquid crystal zone was wide and extended to high water contents, because in this case, during the emulsification process, the system remains long enough in the liquid crystal phase to allow incorporation of all the oil. Around the optimal formulation variables, the liquid crystal zone crossed during emulsification was wide enough to incorporate all the oil whatever the mixing or stirring rate used, and then the resulting droplet size was independent of the preparation variables.

One application of nano-emulsions is preparation of nanoparticles. Sadtler *et al.* [26] investigated the preparation of PEO-coated polymer nanoparticles synthesized by miniemulsion polymerization of nano-emulsions prepared by the EIP method. They used Brij 98 (polyoxyethylene (20) stearyl ether) as the surfactant. Styrene and hexadecane were used for the oil phase. They made a partial phase diagram of the water/Brij 98/styrene system to determine the suitable region and start point (ratio of oil to surfactant) for the EIP method. They also studied the effect of the rate of adding water on the preparation of nano-emulsions. The particle sizes after polymerization were as low as 36 nm. They concluded that the EIP method could be very attractive in industrial applications for nanoparticle synthesis since the nanoemulsion formation did not require a high concentration of surfactant.

Ostertag et al. [27] studied the effects of SOR (surfactant-to-oil ratio), oil type (MCT, orange, limonene, olive, grape, sesame, peanut, canola and mineral), surfactant type (Tween 20, 80, 85 and the mixture) and the percentage of surfactant (Tween 80) added to the aqueous phase before emulsification for edible nano-emulsions system. They described that the droplet size depended on (i) oil type: medium chain triglycerides (MCT) < flavor oils (orange and limonene) < long chain triglycerides (olive, grape, sesame, peanut and canola oils); (ii) surfactant type: Tween 80<Tween 20<Tween 85; (iii) surfactant concentration: smaller droplets (d<160 nm) were produced at higher SOR (more than 0.75); and (iv) surfactant location: surfactant initially in oil<surfactant initially in water. They also compared the droplet size prepared by the EIP method with that prepared by a high-energy method (microfluidization). It was shown that small droplets (d<160 nm) could be produced by both methods, but much less surfactant was needed for the highenergy method (SOR \geq 0.1) than the low energy method (SOR≥0.7).

Sadtler *et al.* [26] also showed that nanoparticle sizes depended on SOR and increased with SOR. These results are consistent with the study by Fernandez *et al.* [4] who reported that droplet size distribution depends only on the weight ratio between the surfactant and oil rather than on the water concentration.

Nano-emulsions are usually prepared at room temperature (ambient temperature) with the PIC and EIP methods. However, Yu *et al.* [28] investigated the effect of elevated temperature on the oil-in-water nano-emulsions prepared by the PIC method in the water/mixture of Span 80 and Tween 80/paraffin oil system. They found that the emulsion droplet diameter decreased from 10.3 mm to 51 nm with an increase in preparation temperature from 20 to 70°C. The interfacial tension between the water and surfactant-in-oil solution decreased with an increase in temperature. The decrease in the curve of interfacial tension to an increase in temperature was similar to that of droplet size. At a droplet volume fraction \leq 0.5, the size distribution of this emulsion could be kept unchangeable for more than five months at room temperature. The HLB-temperature of this system was above 70°C. They concluded that the increase in preparation temperature enhanced the surfactant adsorption at the O/W interface and reduced the viscous resistance of the oil phase. The insolubility of the oil phase in the continuous phase prevented the Ostwald ripening process.

Li et al. [29] prepared d-limonene nano-emulsions. In their articles, the PIC method was called a catastrophic phase inversion (CPI) method in the water/Tween 80/oil (d-limonene, olive oil etc) system. Even though the preparation name was different; the preparation method of the nano-emulsion was the same as the PIC method previously described. Nano-emulsions were prepared from a mixture of the oil phase and Tween 80 by slowly adding the water phase with gentle agitation, until a phase inversion occurred. They studied the effect of SOR and adding plant oil (olive oil, corn oil, sunflower oil and soybean oil) on turbidity, mean droplet diameter and stability of Ostwald ripening. Nano-emulsions were obtained at high concentration of surfactant (SOR=1.5). When the oil phase contained less than 15% (w/w) olive oil, the nanoemulsions could be prepared. D-limonene nano-emulsions stored at 28°C were more stable than those stored at 4°C. Adding olive oil to the oil phase increased the stability of the nano-emulsions against Ostwald ripening due to lower solubility of olive oil.

4. Phase diagram and nano-emulsification

In low-energy emulsification, the phase transition during the emulsification process in both PIT and PIC is governed by the same mechanism as shown in Fig. 2. Therefore, studying phase behavior is very important. The phase diagram, a graphical portrayal, of a system is used to study or determine the phase behavior of surfactants and to specify the temperature and concentrations at which various structures exist at equilibrium with desired properties.

Miyanoshita *et al.* [30] studied phase behavior when sucrose was applied in a ternary system: vegetable oil/ polyoxyethylen sorbitan monooleate (MOPS, Tween 80) or decaglycerol monolaurilester (DGML)/aqueous solution. Adding sugar altered the phase diagram of these systems. As shown in Fig. 3, the sponge phase (L₃) region in the MOPS with 40% sucrose system (Fig. 3 B) is obviously larger than the water system (Fig. 3 A) by changing H₁ (a hexagonal liquid crystalline structure) and I₁ (a cubic liquid crystalline structure) into L₃. Using small-angle X-ray scattering (SAXS) of the samples for (a), (b), (c) and (α), the microstructures were determined as H₁, I₁, L₃ and L₃, respectively. In the system in which DGML was used as a surfactant, adding sucrose enlarged the area of the La (lamella)+Wm+O phase (Fig. 4). Both (a) and (α) in Fig. 4 were determined as La by SAXS patterns. O/W emulsions were prepared as follows: 1) surfactant and water or 40 wt% aqueous sucrose solution were mixed at the specified weight ratios; 2) vegetable oil was then added dropwise while the mixture was stirred using a mechanical stirrer (300 rpm) at 25°C until the oil content reached 70 wt%; and 3) to prepare the O/W emulsion, 15 g of water was gradually added to 5 g of the mixture while being stirred using a magnetic stirrer. The arrows in Fig. 5 represent the course of compositional changes when oil is gradually added to mixtures of emulsifier and aqueous solutions.

In the case of the vegetable oil/MOPS/water system (Fig. 3A), the average diameters of the O/W emulsions



Fig. 3 Phase diagram of the vegetable oil/MOPS/water (A) and vegetable oil/MOPS/40 wt% sucrose solution system (B) at 25°C. Wm, L₃, I₁, H₁ and Om indicate micellar, sponge, cubic, hexagonal and reverse micellar phases, respectively. Wm+O, I₁+O, and L₃+I₁+O indicate two- or three-phase equilibriums consisting of Wm, I₁, and L₃ with an expelled oil phase. Om+Suc indicates two-phase equilibrium consisting of Om and recrystallized sucrose. (Reproduced by permission of the Japan Oil Chemists' Society, J. Ole Sci., 60, 355–362(2011).)





Fig. 4 Phase diagram of the vegetable oil/DGML/water (A) and vegetable oil/DGML/40 wt% sucrose solution system (B) at 25°C. Wm, La and Om indicate micellar, lamellar and reverse micellar phases, respectively. La+Wm indicates a two-phase equilibrium consisting of a lamellar phase and a micellar phase. Wm+O and La+Wm+O indicate two- and threephase equilibrium of Wm and La with an expelled oil phase, respectively. (Reproduced by permission of the Japan Oil Chemists' Society, J. Ole Sci., 60, 355-362(2011).)



Fig. 5 Compositional changes upon the addition of vegetable oil. The initial compositions of process (A), (B), (C), (D), (E) and (F) are 40, 50, 60, 70, 80 and 90 wt% of aqueous solutions of surfactant, respectively. (Reproduced by permission of the Japan Oil Chemists' Society, J. Ole Sci., 60, 355–362(2011).)

prepared from mixtures represented by courses (D) and (F) in Fig. 5 were 200 and 18,100 nm, respectively. In the case of the vegetable oil/polysorbate 80/sucrose aqueous solution system (Fig. 3B), the average diameters of the O/W emulsions prepared from mixtures represented by courses (C) and (E) in Fig. 5 were 220 and 23,500 nm, respectively. Similarly with the result of Ikeda *et al.* [31] who found that the phase diagram of the ternary system composed of MOPS, 40%w/w sugar solution (D-fructose, D-glucose or D-maltose) and vegetable oil showed the sponge phase region expanded toward lower MOPS contents and eliminate the highly viscose micelle cubic phase region.

In the case of the vegetable oil/DGML/water system (Fig. 4A), the average diameters of the O/W emulsions prepared from mixtures represented by courses (E) and (A) in Fig. 5 were 190 and 1,170 nm, respectively. In the case of the vegetable oil/DGML/sucrose aqueous solution system (Fig. 4B), the average diameters of the O/W emulsions prepared through courses (D) and (F) were 170 and 56,800 nm, respectively.

The results showed that nano-emulsions were obtained when the composition was altered to either cross the sponge phase (L_3) or lamella phase (L_a) in these system. Furthermore, emulsification was unsuccessful if the system corresponded to H_1 (a hexagonal liquid crystalline structure) phase. These results correspond to those obtained by Ikeda *et al.*, (2013) in which nano-emulsions were formed if the transitions from the L_3 region to the L_3+L_1+O regions are involved during

emulsification.

Wakisaka *et al.* [32] studied the relationship between phase behavior and formation of O/W nano-emulsions in a vegetable oil/mixture of polyglycerol polyricinoleate (PGPR) and polyglycerin fatty acid ester/water system. As shown in Fig. 6, the region of Lc (liquid crystal phase)+L₃ was very wide for vegetable oil/surfactant mixture of PGPR and hexaglycerol monolaurilester (1:1)/water system. Using both small-angle X-ray scattering (SAXS) and polarized microscopic images of the samples for (a), (b) and (c) in Fig. 6, the microstructures were determined as La+L₃, Lc+L₃ and La+L₃, respectively (Fig. 7).

O/W emulsions were prepared as follows: 1) surfactant mixture, water and vegetable oil were mixed before emulsification and 2) Water (7g) was added dropwise to 3g of the mixture with stirring using mechanical stirrer at 300 rpm and 25°C. The result indicated that L_3 or Lc (including La) or both were necessary to form O/W nano-emulsions whose average droplet diameter was about 30 nm with bluish and translucent appearance.

Most of the systems studied for emulsification used a nonionic surfactant and the results confirmed that it is



Fig. 6 Phase diagram of vegetable oil/surfactant HLP/water system at 25°C. L₃, Lc, La, Wm and O indicate a sponge phase, a liquid crystal phase, a lamellar phase, a micellar phase and an oil phase, respectively. Lc+L3 and L3+La indicate a two-phase equilibrium consisting of a liquid crystal phase and a sponge phase, a sponge phase and a lamellar phase, respectively. Wm+O and L3+Wm+O indicate twoand three-phase equilibrium of Wm and L3+Wm with an expelled oil phase, respectively. (Reproduced by permission of the Japan Oil Chemists' Society, J. Ole Sci., 63, 229– 237(2014).)



Fig. 7 Polarized microscopic images and SAXS patterns of samples consisting of 10.0 wt%, 75.0 wt%, and 15.0 wt% (a); 10.0 wt%, 65.0 wt%, and 25.0 wt% (b); 10.0 wt%, 50.0 wt%, and 40.0 wt% (c) of vegetable oil, surfactant HLP, and water, respectively. Compositions (a), (b), and (c) are indicated in Fig. 6 using the corresponding letters in parentheses. (Reproduced by permission of the Japan Oil Chemists' Society, J. Ole Sci., 63, 229–237(2014).)

necessary to select an appropriate starting composition leading to the formation of a lamellar phase (La) or sponge phase (L₃) prior to emulsification to form a nanoemulsion. However, preparation with an ionic surfactant showed different results. Solè *et al.* [25] found that a cubic liquid crystal phase that occurs during the emulsification process is main requirement to obtain a nanoemulsion when an ionic surfactant was used. It is considered that an appropriate liquid crystal for nano-emulsification might be different for the system.

5. The spontaneous emulsification (SE) method

The SE method is one method of low-energy emulsification. SE was first reported in 1878 in a aqueous sodium hydroxide and fatty acid in oil system [33-36]. Davies and Haydon [33, 34] reported that SE was observed in the aqueous phase when toluene containing a sufficient amount of ethanol came into contact with water. Ruschak and Miller [37] proposed a theoretical mechanism of this type of SE using a ternary phase diagram. This theory is called the diffusion path theory [38]. Surfactant-free emulsions are prepared using this emulsification method. Greek alcoholic beverage ouzo appears the milky white when mixed with water. The anise oil in ouzo spontaneously nucleates by diffusion of alcohol to water. As a result, the mechanism of SE in a ternary liquid system (water, alcohol and oil) is called the ouzo effect [39, 40]. Nazarzadeh et al. [41] prepared nano-emulsions using ultrasound, PIC and the ouzo effect. For the ouzo effect, the nano-emulsions were obtained by adding 5.0 mL of 0.1 vol% oil in ethanol solution to 10.0 mL of water with stirring. The stability of the nano-emulsions obtained with the ouzo effect was very low.

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SE caused by diffusion of a co-surfactant was reported by Rang and Miller [42] and by Nishimi and Miller [43]. In the former case, 250 mL of an n-hexadecane/oleyl alcohol/liner alcohol ethoxylate ($C_{12}E_6$) mixture was injected into 75 mL of water at 30°C with gentle mixing by a magnetic stirrer. Oleyl alcohol gradually diffused out and water entered the mixture and the water content in the mixture increased. The state of the mixture is converted to lamella liquid crystal and also in some case to a microemulsion, followed by nucleation of oil within the mixture resulting in the formation of small droplets in the continuous water phase. In the latter case, SE was observed for n-octane/AOT (sodium bis(2-ethylhexyl) sulfosuccinate)/water mixtures injected into water when the initial AOT concentration exceeded approximately 25 wt% and initial water content was less than approximately 10 wt%. An intermediate lamellar liquid crystalline phase formed on contact of the mixture with water. Local super saturation occurred in the aqueous phase, leading to nucleation of oil droplets. As AOT continued to diffuse into the aqueous phase the lamellar phase was converted to a microemulsion, causing emulsification of much of its solubilized oil. It is considered that phase inversion, from a W/O microemulsion to an O/W microemulsion through the bicontinuous microemulsion or lamellar liquid crystal, may occur in these systems.

Bilbao-Sáinz et al. [44] prepared nano-emulsions by CPI method to apply to edible films. They described CPI method was one type of SE. W/O emulsions inverted to O/W emulsions by slowly increasing the water volume fraction in a lipid (Acetem)/water/nonionic surfactant (Tween 60) system and it was found that the stirring speed and surfactant concentration influenced droplet size distribution and emulsification time. The processing time was reduced 12-fold to reach droplet sizes of around 100 nm when compared with the direct method. They also found that at the beginning of the emulsification process, the emulsion droplet size decreased with increasing surfactant concentration, which was attributed to an increase in interfacial area and a decrease in interfacial tension. However, over time, the finest and quite stable emulsions were formed under the lowest surfactant concentrations. A slight increase in droplet size at higher surfactant concentration with mixing rate 1,300 rpm may be caused by Ostwald ripening.

Yang *et al.* [45] produced nano-emulsions using microfluidization and the SE method. For SE, the mixture of medium chain triglyceride (MCT) and surfactant (Tween 80, Tween 85 and a mixture of Tween 80 and Tween 85) was poured into a water phase at a rate of about 10 g per minute with continuous stirring (500 rpm) using a magnetic stirrer for 15min in this study. The 20 wt% MCT oilin-water emulsions were prepared by the SE method. If the oil-surfactant mixture was poured into the water phase too quickly (>20g per min), then large clots were formed that were difficult to disperse. For the Tween 80 and Tween 80/Tween 85 system, the particle size progressively decreased with increasing surfactant concentration. The smallest droplet was obtained at 20% surfactant: 77 nm for Tween 80 and 55 nm for the Tween 80/ Tween 85 mixture. For the Tween 85 system, a minimum droplet radius of around 87 nm was observed at 8% surfactant content. Chang and McClements [46] produced an orange oil nano-emulsion by SE method. The mixture of the oil phase (MCT+orange oil) and surfactant (Tween 20, 40, 60, 80, and 85 and Span 20) was titrated into an aqueous solution with continuous stirring (500 rpm). In this study, the ratio of orange oil to MCT was constant (10 wt%). The ratio of surfactant to emulsion was varied (2.5-20 wt%). Transparent nano-emulsions (droplet diameter was less than 30 nm) were obtained at 20% surfactant (Tween 40, 60, or 80) and 10% oil phase (4-6% orange oil+6-4% MCT). They also studied the thermal stability of nano-emulsions. Most of the nanoemulsions broke down after thermal cycling (from 20 to 90°C and back to 20°C). One system (20% Tween 80, 5% orange oil, and 5% MCT) remained transparent after thermal treatment. Although the droplet sizes of this particular nano-emulsion increased over time (40 days), the droplet growth rate was reduced appreciably by dilution (x 10).

SE is also prepared by diluting O/W microemulsions. Solè et al. [47] produced nano-emulsions by diluting the water-in-oil or oil-in-water microemulsions in the system of water/SDS/hexanol or pentanol/dodecane. In this study, four different emulsification procedures were used: 1) addition of microemulsion into water in one step; 2) stepwise addition of microemulsion into water; 3) addition of water into microemulsion in one step; and 4) stepwise addition of water into microemulsion. The water composition at the end of the emulsification procedure was always 98%. Starting emulsification from O/W microemulsions resulted in, nano-emulsions with droplet diameters of 20 nm, independent of microemulsion composition and the dilution procedure. On the other hand, when emulsification was started from W/O microemulsions, nano-emulsions were only obtained if the dilution procedure used allowed reaching equilibrium in an O/W

microemulsion domain during emulsification. These conditions were achieved by stepwise addition of water to the W/O microemulsion. The chain length of the alcohol for co-surfactant had an important effect on the size, polydispersity and stability of nano-emulsions. Nanoemulsions made with hexanol as a co-surfactant, which has a lower solubility in water and lower diffusivity than pentanol, were smaller in size, lower in polydispersity, and had lower Ostwald ripening rates than those with pentanol. They recommend that starting emulsification from a direct O/W microemulsion phase is preferred, since nano-emulsions are obtained by simply diluting this phase, no matter the dilution procedure used. Moreover, this process is easy to scale-up and has lowenergy consumption, as neither temperature changes nor high stirring rates (as no highly viscous phases are present during emulsification) are needed. However, if a concentrate with low water content is required, starting emulsification from a carefully selected W/O microemulsion would be the preferred choice.

As described above, there are several mechanisms for spontaneous emulsification. Consequently, Solans and Solè [3] claim that phase inversion does not occur in the self-emulsification (spontaneous emulsification) method, and Anton *et al.* [38] include the EIP method into the spontaneous emulsification method.

6. Microemulsion and nano-emulsion

As described previously, nano-emulsions are thermodynamically unstable while microemulsions are thermodynamically stable. The term microemulsion does not describe micrometer-sized emulsions. The structure of a microemulsion is described in various ways: micelles, reverse micelles, rod-like micelle, lamella structure, bicontinuous structure, and swollen micelle. McClements [48] proposed a new approach for distinguishing between nano-emulsions and microemusions based on free energy. According to him, the free energy of nanoemulsions, nanometer-sized droplets dispersed in a continuous phase (the disperse state), is higher than that of the separate state of oil and water. A nano-emulsion can be kinetically stable due to the energy barrier between the two states. On the other hand, the free energy of the dispersed state for microemulsions is lower than that of the separate state.

7. Conclusion

Nano-emulsions are very useful in many industries such as food, pharmaceutical, cosmetic, and chemical. Therefore, the number of articles for nano-emulsions increased remarkably in the past decade. The preparations of nano-emulsions are divided into two methods. the high-energy method and the low-energy method. This article summarized the low-energy method for nano-emulsion preparation. For the phase inversion temperature (PIT) method, phase inversion composition (PIC) method and emulsion inversion point (EIP) method, the phase structure of the water-surfactant-oil mixture is very important for the subsequent preparation of nano-emulsions. The phase structure is also important for the spontaneous emulsification (SE) method, even though phase inversion does not occur in the emulsification process. Consequently, identification and clarification of the phase structures existing in the pre-emulsification and determination of the relevant phase structure for producing the desired emulsion size during the emulsification process is very important when employing low-energy emulsification methods.

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