Key features of nano-emulsion formation by the phase inversion temperature (PIT) method

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Abstract

Nano-emulsion formation by the phase inversion temperature (PIT) method is reviewed. Characteristic features of low-energy emulsification are first summarized and then follows a description of the main characteristics of the PIT emulsification method. The main focus is on the relation between phase behaviour and nano-emulsion formation. Emphasis is put on the key role of phase transitions involving equilibrium phases such as lamellar liquid crystalline and/or bicontinuous microemulsion phases to obtain nano-emulsions with minimum and controlled droplet size.

Keywords: nano-emulsions, PIT, phase behavior, non-ionic surfactants

Introduction

Nano-emulsions are submicron-size emulsions with droplet diameters typically in the range 20-200 nm¹, although a size range cannot be exactly established as there is no sharp change in the physicochemical properties of emulsions when passing from micrometer to nanometer range. These colloidal systems have been designated in the literature with different terminology, such as miniemulsions²⁻⁴, ultrafine emulsions⁵, submicron emulsions⁶ or nanoemulsions^{7,8}. This last term has been increasingly adopted because it indicates unequivocally the size range and it is concise. Due to the misunderstandings that they are still prevailing in the literature, in spite of the efforts^{9,10} to clarify the difference between nanoemulsions and microemulsions (which are not emulsions as they are thermodynamically stable systems), the authors prefer the notation “nano-emulsion” to emphasize their emulsion nature.

The small droplet size confers nano-emulsions characteristic properties such as high surface area/volume ratio, stability towards sedimentation or creaming and transparent/translucent aspect. These properties are very relevant for a wide range of applications and consequently they are receiving increasing attention.

High surface area/volume ratio is important in applications where interfacial properties are decisive (e.g. their use as solubilization or reaction media)^{6,11,12}. Although nano-emulsions are stable against creaming or sedimentation (because the gravity forces are considerably reduced and the Brownian motion overcomes gravity) they may undergo other breakdown mechanisms (flocculation, coalescence and/or Ostwald ripening). It has been shown and it is widely accepted that nano-emulsion main destabilization process is Ostwald ripening^{13,14} which consists on the diffusion through the continuous phase of molecules of the disperse phase from small to big droplets, as a consequence of their different Laplace pressure. Nevertheless, nano-emulsions with high kinetic stability can be formulated by a proper choice of system components. In addition to the other characteristic properties, the visual transparent aspect of nano-emulsions confers them an esthetical character of interest in cosmetic, personal care, food (e. g. beverage) and pharmaceutical applications^{15–17}

As nano-emulsions are non-equilibrium systems, an energy input is required for their formation and properties such as droplet size and stability depend on the preparation method^{18,19}. Nano-emulsions, as all types of emulsions, can be prepared by dispersion and by condensation methods, generally known as high- and low-energy methods, respectively^{18–21}. The energy input of the former is external and nano-emulsion formation is generally achieved using mechanical devices (e.g. high-pressure homogenizers, ultrasound generators) able to produce intense disruptive forces. In these methods, generally the higher the energy input the smaller the droplet size; above certain energy input the

size tends to level off and does not decrease anymore. Only a small amount (as low as 0.1%) of the energy supplied is used for emulsification as the rest is dissipated as heat¹⁴. Therefore, high-energy methods are cost-inefficient for producing nano-emulsions. In contrast, low-energy methods take advantage of the internal energy of the system to achieve emulsification. The resulting droplet size is independent on the external energy applied as it depends on the physicochemical properties of the system. Moreover, simple equipment (i.e. of relatively low cost) is needed and emulsification can be carried out in mild conditions with high yield and reasonable process time. Therefore, low-energy emulsification can be considered more cost-efficient than high-energy methods and this is the main reason why they are focussing considerable attention from a practical viewpoint^{1,22,23}.

This review deals with nano-emulsion formation by the phase inversion temperature (PIT) method. Characteristic features of low-energy emulsification and the PIT method are first summarized. It follows a discussion on the relationship between phase behaviour and nano-emulsion formation, exemplified by a description of a water/mixed pure nonionic surfactants/oil system.

Low-energy emulsification

Depending on whether emulsification is triggered by a diffusion process^{24,25} or by inversion of the spontaneous curvature of the surfactant during emulsification^{26,27}, low-energy emulsification methods are classified as “self-emulsification” or “phase inversion”, respectively. For estimation of the spontaneous curvature, we refer to the seminal paper by Strey²⁸ and those that followed. In self-emulsification (also referred to as spontaneous-emulsification) energy is released by diluting with the continuous phase (typically

water) the rest of components (typically a mixture of oil and a solvent miscible with both oil and water). During the dilution process leading to oil-in-water (O/W) nano-emulsions, diffusion of water-miscible components (solvent, surfactant and/or cosurfactant) from the oil mixture into the aqueous phase is produced resulting in a metastable emulsion state^{24,25,29–32}. The so-called Ouzo effect is self-emulsification produced in systems with no surfactant molecules. A drawback is that nanosized droplets can only be obtained at very high solvent/oil ratios (i.e. low oil volume fractions). In phase inversion methods, energy is released when phase transitions occur due to changes in the surfactant spontaneous curvature during emulsification which inverts from negative (W/O emulsions) to positive (O/W emulsions) or viceversa (W/O emulsions)^{26,27}. Inversion processes can be triggered by changing temperature (keeping composition constant)²⁶ or composition (at constant temperature)²⁷, the so-called phase inversion temperature (PIT) and phase inversion composition (PIC) methods, respectively.

Low-energy methods were described long ago and have been used industrially. Self-emulsification was discovered by J. Gad in 1879³³ and the possible mechanisms were discussed more than eighty years later by J. T. Davies and E. K. Rideal³⁴. The Phase Inversion Temperature (PIT) emulsification method based on the changes of the lipophilic-hydrophilic properties of some type of surfactants with temperature was introduced by K. Shinoda and H. Saito in 1968³⁵. T. J. Lin et al. in 1975²⁷ described an emulsification method, designated years later as PIC method, consisting in the addition of water to a mixture of surfactant and oil. Interesting contributions to the different low-energy emulsification methods were produced in the 1980's and 1990's. C. A. Miller²⁴ introduced the “diffusion path theory” for self-emulsification, H. Sagitani⁵ described an emulsification method combining heating and dilution to obtain finely dispersed

droplets, J. L. Salager et al.³⁶ defined two kinds of emulsion inversion, transitional (induced by intermolecular interaction changes) and catastrophic (induced by changes in the water/oil ratio). T. Förster et al.³⁷ obtained fine emulsions of the order of 100 nm (i.e. nano-emulsions) by heating the sample above PIT and then cooling it down. They pointed out that transition through a bicontinuous microemulsion and/or lamellar liquid crystalline phase was required. Salager's group³⁸ described miniemulsion formation by transitional inversion and stated that a microemulsion phase was required to obtain nanosized droplets. The key role of bicontinuous and/or lamellar structures in emulsification were evidenced in earlier reports^{39–41}. In the last two decades, great research effort has been devoted to clarify the mechanisms of nano-emulsion formation^{42–51, 52–58} as well as on the study of their properties and applications^{59–65, 66–70} which has been reflected in diverse reviews^{1, 14–17, 71–79}.

Characteristics of the Phase Inversion Temperature (PIT) emulsification

PIT emulsification is based on the changes in the surfactant spontaneous curvature triggered by temperature. Therefore, it can be only applied to systems with surfactants sensitive to temperature changes, namely polyoxyethylene-type nonionic surfactants²⁶. The hydrophilic-lipophilic properties of these surfactants vary with temperature as it induces changes in the hydration of the poly(oxyethylene) chains^{35, 40}. At low temperatures, polyoxyethylene-type nonionic surfactants have a large positive spontaneous curvature and form direct type structures while at high temperatures, the spontaneous curvature becomes negative forming reverse type structures. At intermediate temperatures, the so-called hydrophilic-lipophilic balance (HLB) temperature^{26, 40}, the spontaneous curvature becomes zero or almost zero and structures such as bicontinuous microemulsions and lamellar liquid crystalline phases are formed (see Figure 1). At the HLB temperature, extremely low interfacial tensions (of the order 10^{-2} - 10^{-3} mNm⁻¹) are

achieved, which favour emulsification. However, coalescence is very fast. Therefore, emulsions are very unstable at the HLB temperature. Shinoda²⁶ showed that emulsions with small droplet size, narrow size distribution and high kinetic stability could be obtained by rapidly cooling or heating samples prepared at or close to the HLB temperature. If cooling is too slow, fast coalescence avoid the formation of nano-emulsions. During the cooling process, inversion from reverse type, W/O structures to O/W structures takes place, while for the heating process the reverse is true. Knowledge of the HLB temperature (i.e. phase inversion temperature or PIT) of the emulsion system is a requirement to apply this emulsification method. This parameter can be obtained by phase diagram determinations and also by electrical conductivity measurements. Although the PIT emulsification method is simple to perform it may not be suitable when using thermo-sensitive molecules.

To achieve optimum emulsification knowledge on the phase behaviour of emulsion components is of utmost interest in low-energy emulsification^{1,5}

Relation between phase behaviour and nano-emulsion formation

Studies on the phase behaviour of water/poly(oxyethylene) nonionic surfactant/oil systems as a function of temperature have been crucial to elucidate the mechanisms of nano-emulsion formation by the PIT method^{43,44,46,47,48}. The so called fish diagram²⁸, i.e. the section of the phase diagram prism at constant water/oil ratio (Figure 1, left) is very useful to get insight on those mechanisms. An example of such a diagram for a ternary system water/non-ionic surfactant/oil is shown in Figure 2⁸⁰. Herewith, poly(oxyethylene) nonionic surfactants are abbreviated as CiEj, where i and j are the length of the alkyl and poly(oxyethylene) chains, respectively. For the case of a single surfactant, such as the one depicted in Figure 2, the fish diagram is approximately symmetric above and below the PIT, i.e. if an isotherm at PIT is taken as the axis of symmetry. In practice, however, many

products contain a mixture of surfactants, and the fish diagram get distorted or asymmetric as a result of changes in the composition of the interfacial layer with total surfactant concentration. The characteristic features of this type of systems are exemplified by the phase behaviour of the water/C₁₆E₄+C₁₂E₆/decane system, which is the result of adding a more lipophilic surfactant (C₁₆E₄) to the system corresponding to Figure 2. The phase diagram of the water/C₁₆E₄+C₁₂E₆/decane system is plotted in Figure 3 as a function of temperature and total surfactant concentration (S_T) at a constant water/decane (W/O) weight ratio of 80/20. This ratio is more relevant to nano-emulsion formation as compared to that of Figure 2, which is the typical used for fish diagrams. In Figure 3 only the three-liquid phase region (W+D+O), the microemulsion phase region (D) and the multiphase region with liquid crystalline phase present (LC) are shown. The structure of the liquid crystalline phase is of the lamellar type (L_α). The intersection of the microemulsion phase region, D, with the three-phase region, W+D+O, indicates the lowest concentration of total surfactant to form a microemulsion, which in this system is produced at a S_T value of 4 wt% and at 29°C. It should be noted that the three-phase region is shifted towards higher temperatures as S_T value is decreased. This is a characteristic behavior of mixed (technical) grade surfactant systems which is due to the different partition of the surfactant molecules between the bulk phases and the interface with the change in surfactant concentration. The PIT at each surfactant concentration falls in the middle of the (W+D+O) region for the lower surfactant concentration and in the middle of the D+LC region for higher surfactant. As shown in Figure 3, the system evolves from a W/O to an O/W structure by decreasing the temperature. The transition takes place through the three-phase region (W+D+O) at low surfactant concentrations (4.4wt% in this system), while at higher surfactant concentrations transition takes place through either D or D+ L_α phases.

An early report³⁹ on the influence of the phases present in the system on droplet size of emulsions obtained by the PIT method showed that starting emulsification in the three phase region (W+D+O) and rapidly cooling at a temperature far below the PIT resulted in emulsions with small droplet size. However, emulsions with smaller droplet size and narrower size distribution were obtained when emulsification was started from the single D-phase (bicontinuous microemulsion phase) and brought to the same low temperature. From these results, it was concluded that emulsification in the bicontinuous microemulsion D phase is the mechanism responsible for achieving small droplet size when emulsifying by the PIT method³⁹. It could be argued that the increase in surfactant concentration is mainly responsible for the decrease in droplet size. Figure 4 shows droplet size of emulsions of the water/C₁₆E₄+C₁₂E₆/decane system obtained by the PIT method as a function of the total surfactant concentration. Emulsification started at the three-phase region (W+D+O) and finalized in the two phase (W_m+O) region where the O/W structure is formed. A sharp decrease in droplet size from 180nm to 80nm, for 2 wt% and 4wt% surfactant, respectively, is observed. Similar results were obtained by Izquierdo et al.^{43,46,47} using systems with technical grade nonionic surfactants in which nano-emulsion droplet size and polydispersity decreased with the increase in surfactant concentration. The smallest sizes were obtained when emulsification started in a single D microemulsion phase or in a two-phase region (D+ L_α) consisting of D and lamellar liquid crystalline (L_α) phases.

The remaining question was if the decrease in droplet size with the increase in surfactant concentration was merely related to surfactant concentration or to the increase in volume fraction of the D microemulsion phase. The changes in droplet size keeping the surfactant concentration constant are very illustrative. Figure 5 shows the phase diagram of the water/C₁₆E₄+C₁₂E₆/decane system at constant water/decane (W/O) weight ratio of 80/20 and

total surfactant concentration of 5%, as a function of temperature and surfactant mixing ratio (W_1). A single-phase region (I) consisting of a shear-birefringent microemulsion exists at W_1 between 0.15 and 0.5 in the temperature range between 45°C and 25°C. A region containing liquid crystalline (LC) phases is observed at W_1 between 0.15 and 0.5 in the temperature range between 47°C and 28°C. It should be noted that the one phase (I) and LC-present phase regions are shifted towards higher temperatures as W_1 is decreased as a consequence of the presence of more hydrophilic surfactant ($C_{12}E_6$). Figure 6 shows droplet size of nano-emulsions obtained by cooling samples from the PIT to 25°C (indicated by arrows in Figure 5) as a function of W_1 . Emulsification started in the microemulsion phase region for samples with W_1 values between 0.2 and 0.4, and finalized, for all W_1 values, at the two-phase (W_m+O) region. Droplet size decreases from 100nm ($W_1=0.1$) to 70nm ($W_1= 0.2-0.4$). These results clearly show that emulsification from the bicontinuous microemulsion phase accounts for the effectiveness of the emulsification process.

The results of the research dealing with the relation between phase behavior and nano-emulsion formation^{39,42,44} evidenced the importance of phases with mean zero spontaneous curvature (e.g. bicontinuous microemulsions and lamellar liquid crystalline phases) to obtain very small droplet size⁸¹. It was also inferred that minimum droplet size was achieved when emulsification started from one single (bicontinuous microemulsion or lamellar liquid crystalline) phase. However, D. Morales et al.^{44, 48} showed that minimum droplet size can be achieved starting emulsification not only from a single-bicontinuous microemulsion phase but also from compositions displaying multi-phase equilibria, provided that all the surfactant and oil coexist in the same phase, (e.g. phase equilibria consisting of a bicontinuous microemulsion and excess water phase). This finding allowed

to conclude that droplet formation is controlled by the structure of the bicontinuous microemulsion and the excess water acts as mere dilution medium. The proposed mechanism of O/W nano-emulsion formation by the PIT method was thermal disruption of the bicontinuous microemulsion (or lamellar liquid crystalline) phase by the decrease in temperature which produces a change in curvature of the surfactant monolayer giving rise to oil droplet formation.

More recent contributions to the mechanisms of nano-emulsion formation are those of K. Roger et al.^{56,77}. They observed that a few degrees below the PIT, where a bicontinuous microemulsion coexists with excess oil, and under gentle shear, a homogeneous phase (“Clearing Boundary”), consisting of an O/W microemulsion, appeared as a metastable structure. By cooling from this “Clearing Boundary” (sub-PIT method), nano-emulsions with the same size as that obtained by cooling from or above the PIT were obtained (Figure 7). These results supported a mechanism of nano-emulsion formation through non-equilibrium self-assembly by disruption of the bicontinuous microemulsion coexisting with excess oil and water (Figure 8). It was concluded that this “Clearing Boundary” (or “Access State”) acts as template and determines nano-emulsion droplet size. Strictly, the Sub-PIT method should not be regarded as an inversion method since at the initial state (O/W microemulsion) the surfactant preferred curvature at the interface is of the same sign as that at the final state (O/W nano-emulsion).

It was reported^{56,77} that this sub-PIT emulsification can provide very similar results, in terms of particle size distribution, as “cross-PIT” emulsification in which PIT is crossed during the temperature change. These results confirmed that the nanostructure of the last phase crossed during emulsification greatly determines the final properties of the formed nano-emulsion. Moreover, it was also confirmed that the droplet size is directly controlled by the oil/surfactant ratio. The emulsification process is highly efficient in the sense that

most of the surfactant molecules are adsorbed on droplets, and consequently, the size of droplets is determined by the total interfacial area that can be covered by surfactant molecules. This allows a prediction of the final droplet size, as a function of composition and other known parameters such as the surfactant area per molecule⁷⁷.

In the recent years, there have been numerous publications on nano-emulsions, most of them dealing with formulation with different kinds of oils and on applications. However, contributions on the mechanisms of nano-emulsion formation by low-energy methods are missing. With the present knowledge it can be stated that transition through self-assembled structures with an average zero curvature (e.g. bicontinuous microemulsions or lamellar liquid crystalline phases) is essential to obtain nanosized droplets. It has also been demonstrated that nano-emulsion droplet size is controlled by the structure of the bicontinuous microemulsion or lamellar liquid crystalline phase present during the emulsification process.

Conclusions

The Phase Inversion Temperature (PIT) emulsification method is based on the changes in lipophilic-hydrophilic properties of polyoxyethylene-type nonionic surfactants with temperature. Oil-in water (O/W) nano-emulsions are obtained by rapidly cooling samples prepared at or close to the PIT. During the cooling process inversion from reverse type water-in-oil (W/O), structures and/or transitions through self-assembled structures with an average zero curvature (e.g. bicontinuous microemulsions or lamellar liquid crystalline phases) to oil-in water (O/W) structures takes place. These self-assembled structures with average zero curvature play a key role not only for the formation of nanosized droplets but also controlling the size of the resulting nano-emulsions. It has been shown that minimum droplet size is achieved when all the surfactant and oil are in the same phase when starting emulsification. Although the PIT emulsification method is simple to

perform it can be only applied to systems with surfactants sensitive to temperature changes, namely polyoxyethylene-type nonionic surfactants and it may not be suitable when using thermo-sensitive molecules.

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Figure 1: Scheme of the changes in the surfactant layer curvature with temperature in non-ionic surfactant/oil/water systems. The phase diagram prism is shown on the left with the section at constant water/oil ratio (the so called fish diagram)

Figure 2: Phase diagram of the water/ $C_{12}E_6$ /decane system at constant water/decane (W/O) ratio of 50/50 as a function of temperature and total surfactant concentration (S_T). W: Aqueous phase; W_m : direct O/W microemulsion phase; O: oil phase; O_m : Reverse W/O microemulsion phase; D: Bicontinuous microemulsion phase (D). I is an isotropic, single-phase region. Adapted with permission from Ref.80

Figure 3. Phase diagram of the water/ $C_{16}E_4+C_{12}E_6$ /decane system at constant water/decane (W/O) weight ratio of 80/20 as a function of temperature and total surfactant concentration (S_T). W: Aqueous phase; W_m: direct O/W microemulsion phase; O: oil phase; O_m: Reverse W/O microemulsion phase; ME: microemulsion phase, Lα: Lamellar Liquid Crystalline phase. The red arrows indicate the emulsification path. Feng and Solans, unpublished results.

Figure 4. Droplet size of nano-emulsions (at 25 °C) of the water/ $C_{16}E_4+C_{12}E_6$ /decane system at constant water/decane (W/O) weight ratio of 80/20, obtained by the PIT method as a function of the total surfactant concentration (S_T). Feng and Solans, unpublished results. The polydispersity index is below 0.2.

Figure 5. Phase diagram of the water/ $C_{16}E_4+C_{12}E_6$ /decane system at constant water/decane (W/O) weight ratio of 80/20 and total surfactant concentration of 5%, as a function of temperature and surfactant mixing ratio (W_1). LC: liquid crystal phase. I: isotropic, single-phase region. Feng and Solans, unpublished results.

Figure 6. Droplet size of nano-emulsions (at 25 °C) obtained by cooling samples from the corresponding PIT to 25°C (indicated by arrows in Figure 3) as a function of W_1 . Water/decane (W/O) weight ratio = 80/20. Total surfactant concentration = 5%. Feng and Solans, unpublished results. The polydispersity index is below 0.2.

Figure 7 Mean size and polydispersity (top) and appearance (bottom) of emulsions in the $C_{16}E_8$ /hexadecane/water system. The emulsions are stir-quenched from different initial temperatures to 25°C. The PIT of the system is 76.2 °C. Water/oil (W/O) ratio= 0.2, oil/surfactant mass ratio=3.5. For initial temperatures below the clearing boundary, a bimodal distribution is obtained after the quench, whereas for initial temperatures above the clearing boundary a unimodal distribution is obtained after quenching. Winsor I, Winsor III and Winsor II are equivalent to W_m+O , $W+D+O$, and O_m+W , respectively, according to the nomenclature of Figure 1. Reproduced with permission from Ref.52

Figure 8. Proposed scheme of the nano-emulsification through a “superswelled access state” by the sub-PIT method. O/W nano-emulsions are formed by a fast decrease of temperature while simultaneously applying agitation (red upper curve). Initial temperature is selected to achieve a disruption of the bicontinuous nanostructure, forming a transient swelled microemulsion (access state) that forms the final trapped state nano-emulsion. In contrast, nano-emulsions are not formed when temperature is slowly reduced in absence of shear (blue lower curve), leading to separated phases at the equilibrium state. Reproduced from Ref. 77, with permission