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Rapid communication

The universality of low-energy nano-emulsification

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ABSTRACT

Extensive studies have been done on nano-emulsions and emulsification methods to provide nanometric-scaled templates for the formulation of nanoparticles. The so-called “low-energy” methods are of particular interest as they prevent the potential degradation of fragile encapsulated molecules. This work deals with new concepts in nano-emulsification using low-energy methods. Three-model ternary systems, water/nonionic surfactant/oil, were studied and compared. Nano-emulsions were generated using both spontaneous emulsification and the PIT method, so as to study the links between these two nano-emulsification methods. The influence of the composition and formulation variables on the nano-emulsion properties and emulsification procedures were thus investigated. This study pioneers the concept of the universality of low-energy nano-emulsification, proving that all these low-energy methods (*i.e.* spontaneous emulsification and the phase inversion temperature (PIT) method) are governed by a single unique mechanism. It thus provides a better understanding of low-energy nano-emulsification processes and notably the PIT method, useful in the fields of nanoparticle and nano-pharmaceutic formulations. These results are fundamental in establishing experimental procedures for the incorporation of drugs in nano-emulsions.

1. Introduction

Nanoparticles, defined as polymeric or lipid particles smaller than 300 nm, are of increasing interest in nano-medicine and nano-pharmaceutics today. They are considered as a tool to help open up a new dimension of treatments, penetrating cells and tissues, targeting diseased zones. However, despite their great potential, nanoparticles are as yet little known and controlled. One way of channelling this potential may be to generate nano-objects with very controlled surface and morphological properties in order to mimic “ideal” natural examples (*e.g.* viruses), along with a significant loading in active molecules. This would be intimately related to the control and understanding of their generating processes. Nanoparticles are mainly formulated through the generation of nano-emulsions (Anton et al., 2008) which serve as templates for polymerization, nano-precipitation and lipid nano-crystallization. It therefore follows that understanding the mechanisms of nano-emulsification is of prime interest in all of these processes. Hence the interest of the current study which presents a new point of view on nano-emulsification, disclosing a general and universal mechanism of nano-droplet generation.

Nano-emulsions consist of a mixture of immiscible liquids, where one of the liquids is dispersed in the form of nanometric-scaled droplets (20–300 nm) into the other one. The generating processes for nano-emulsions are divided into (i) high-energy and (ii) low-energy methods (Anton et al., 2008). High-energy methods involve the use of specific devices such as high-pressure homogenizers or sonifiers, and only a very low amount (~0.1%) of the mechanical energy produced is used for emulsification (Tadros et al., 2004). Low-energy methods divert the intrinsic physicochemical properties of the surfactants, co-surfactants and excipients in the formulation, leading to the generation of emulsion droplets in the nanometric range. Owing to the real advantages of low-energy methods in terms of formulation yields, potential industrial scale-up and non-aggressive features (*e.g.* against encapsulated fragile active molecules), there has been a real research interest in the development of such methods and techniques over the last twenty years. Numerous works lead to “classifying” the low-energy methods into *very distinct* emulsification procedures, *i.e.* distinct in the protocols (which depend on the nature of the excipients), but also distinct in the proposed mechanisms for the formation of nano-emulsion droplets. The two commonly reported low-energy nano-emulsification methods are (Anton et al., 2008) the spontaneous emulsification method and the phase inversion temperature method (PIT method). This study reveals the universality of these low-energy nano-emulsification processes, showing that in fact, all such methods are very close to each other and are governed by a single, common mechanism.

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The first method, spontaneous emulsification, is carried out very simply: The emulsion is created as a result of a mixture of two liquids at room temperature. One is a pure aqueous phase; the other is a mixture of oil, surfactant and a water-miscible solvent. A comprehensive review of Miller (1988) underlines the general principles and mechanisms governing this emulsification process, basically showing that it is the solvent displacement itself from the oily to the aqueous phase that induces great turbulence at the water/oil interface. The two liquids, thermodynamically stable alone, are brought to a non-equilibrium state when they are mixed. Thus, the rapid transfer of hydrophilic materials from the oil to the water phase results in a dramatic increase of the interfacial area, giving rise to the metastable emulsion state. In order to obtain nanometric-scaled droplets with this spontaneous emulsification method, the experimental conditions commonly reported in literature (El-Aasser et al., 1986; Trotta et al., 2001; López-Montilla et al., 2002; Bouchemal et al., 2004) are related to a very high solvent/oil ratio (*e.g.* a tiny percentage of oil in the organic phase before mixture). The solvent diffusion is hence even quicker and the turbulence thereby generated causes nano-scaled droplets to form. According to the type of nanoparticle required, the formulation can also include additional components such as surfactants, monomers, polymers, macromolecules and/or various other drugs. Although these additions may cause a slight interference with the process, the mechanisms will globally remain unchanged.

The second low-energy method introduced by Shinoda and Saito (1968, 1969) is the so-called phase inversion temperature method (PIT method). Oil, water and nonionic surfactants are all mixed together at room temperature and slightly stirred. Next, the mixture is gradually heated up. As a result, the surfactant solubility progressively changes from the aqueous to the oily phase. Above the phase inversion temperature the surfactant is fully solubilized in oil and thus the mixture undergoes a phase inversion, from an oil-in-water (o/w) to a water-in-oil (w/o) emulsion. This phenomenon is well characterized and has been rationalized by relating the surfactant affinity difference, $\mathrm{SAD} = \mu^\circ{}_{\mathrm{water}} - \mu^\circ{}_{\mathrm{water}} = RT\ln\{C_{\mathrm{oil}}/C_{\mathrm{water}}\}$, with different formulation variables such as the electrolyte concentration, chemical structure of the surfactant, or temperature (Salager et al., 2000; Anton et al., 2008) ($\mu^\circ{}_i$ being the standard chemical potentials of surfactant in phase i, C the surfactant concentrations). At the PIT, the affinity of amphiphiles for each phase is similar, interfacial curvature is very low, and consequently nanometric-scaled microemulsions are formed. Unlike nano-emulsions, microemulsions are thermodynamically stable systems (Anton et al., 2008; Ott et al., 1991), exhibiting different structures at the nanometric scale, such as spherical, tubular or disklike micelles, lamella or sponge phases and presenting stability which only depends on thermodynamic variable change like temperature, composition and dilution. Nano-emulsions are instantly generated by performing an irreversible transformation—a rapid cooling or a sudden dilution with cold water, to this system maintained either at the PIT or higher than the PIT (Morales et al., 2003; Izquierdo et al., 2004; Solans et al., 2005). The nano-emulsions generated are kinetically stable for months.

As regards the mechanism of formation, it is widely believed that nanometric-scaled droplets originate from nanometric templates: the *PIT-microemulsions*. The latter are simply broken up by the rapid cooling and/or dilution, "freezing" the microemulsion network in the form of nano-emulsions. This would explain why our studies still correlate with the investigation at equilibrium of phase diagrams such as the Kahlweit-fish diagram (Salager et al., 2004; Izquierdo et al., 2004). Basically then, nano-emulsions are generated when the corresponding phase equilibrium is a Winsor III or IV system, related to the amount of surfactant.

The present study shows (supported by the experiment) that the generation of nano-emulsions using the "PIT method" can be explained much more simply, the above-mentioned spontaneous emulsification being triggered by a unique general mechanism. In the first part, the study deals with the characterization of three-model ternary systems water/nonionic surfactant/oil, leading to the generation of nano-emulsions by spontaneous emulsification methods. These three studied systems differ in their intrinsic surfactant/oil affinity at room temperature. This difference is shown to be the key point linking spontaneous emulsification and the PIT method. In the second part, nano-emulsions will be generated for the same systems, but this time using the PIT method. Here, we investigate the influence of the amount of water initially added to the ternary system on the generation and properties of the nano-emulsions. These experiments clearly show that a parallel can be drawn between both studies, and therefore between the two emulsification methods.

2. Experimental

2.1. Materials

Nonionic surfactants from BASF (Ludwigshafen, Germany), *i.e.* Cremophor ELP® (polyoxiethylated-35 castor oil, hydrophilic-lipophilic balance, HLB~ 12–14) and Solutol HS 15® (Macrogol 15 hydroxystearate, HLB~ 14–16) were kindly provided by Laserson (Etampes, France), and used as received. Hydrophobic phases, *i.e.* Labrafil M1944 CS® (Oleoyl Macrogolglycerides) and Labrafc CC® (medium chain triglycerides) were obtained by Gattefossé (Saint-Priest, France). HPLC grade ethanol was purchased from Sigma (Saint-Louis, USA). Ultrapure water was obtained using the MilliQ filtration system (Millipore, Saint-Quentin-en-Yvelines, France).

2.2. Nano-emulsions produced by spontaneous emulsification

Spontaneous nano-emulsification was performed by slowly pouring the oil plus surfactant mixture (homogenized beforehand with a Vortex® mixer) into the slightly magnetically stirred (~300 rpm) ultrapure water phase. Nano-emulsions were rapidly formed. Each sample was further stirred for 15 min. The nano-emulsion properties, size, polydispersity index (PDI), and droplet concentration of the nano-emulsions were closely linked to the relative proportions between the different components. These formulation variables are defined as (i) solvent/oil weight ratio: $\mathrm{SOR} = 100 \times w_{\mathrm{surfactant}}/(w_{\mathrm{surfactant}} + w_{\mathrm{oil}})$, and (ii) surfactant-oil/water weight ratio: $\mathrm{SOWR} = 100 \times w_{\mathrm{surfactant+oil}}/(w_{\mathrm{surfactant+oil}} + w_{\mathrm{water}})$. The value of the SOWR was kept constant at 30% throughout this study, since its influence on nano-emulsion formation is negligible, its only influence being on droplet concentration.

2.3. Nano-emulsions produced by phase inversion temperature method

Instead of first mixing the oil and surfactant and then pouring the mixture into the water (as is the case for spontaneous emulsification) all the components were mixed together right from the start. To avoid spontaneous emulsification, water and surfactants were mixed beforehand and the oil was added subsequently. A milky macroscopic emulsion was formed. Temperature of this ternary system was rigorously monitored and controlled in a boiler and subjected to slight magnetic stirring. From room temperature, the system temperature was raised to 10 °C above the phase inversion temperature. Finally this hot system was suddenly diluted in water at room temperature (25 °C) using three times the volume of water to the hot w/o emulsion. Nano-emulsions were immediately generated as a result. The features, size, PDI and droplet concentration of nano-emulsions were also closely related to formulation variables: (i) SOR as defined above, and (ii) the water/oil weight ratio: $\text{WOR} = 100 \times w_{\text{water}}/(w_{\text{water}} + w_{\text{oil}})$. The PIT was determined beforehand using electrical conductivity measurements during the temperature increase (Konduktometer 703, Knick, Egelsbach, Germany). Finally, the different cloud points in water and oil were established by visual observation.

2.4. *Dynamic light scattering*

Hydrodynamic diameters were obtained by dynamic light scattering using a Malvern NanoZS instrument. The helium–neon laser, 4 mW, operates at 633 nm, with the scatter angle fixed at 173° and the temperature maintained at 25 °C. PDI is a mathematical definition that accounts for the relative error between curve fit and experimental values (Anton et al., 2007a). It shows the quality of the dispersion. Values ≤ 0.1 reflect a very good monodispersity and quality of the nanoparticulate suspensions. Measurements were performed three times for each point.

3. Results and discussions

3.1. *Spontaneous emulsification*

A mixture was prepared of hydrophobic (Labrafil M1944 CS®) and nonionic surfactants (Cremophor ELP®) freely soluble into one another at room temperature and homogenized, again at room temperature (25 °C). Nano-emulsions were generated immediately after pouring this organic phase into the aqueous one. The effects of SOR on hydrodynamic diameter and PDI were investigated (PDI is used here as a criterion for quality; Anton et al., 2007a). The results are reported in Fig. 1.

The first significant result here is the very easy method of nano-emulsion formulation, from SOR = 20% and thus without the use of solvents. Moreover, for this example (SOR = 20%), the surfactant amount in the final nano-emulsion suspension was of only 6 wt.%, the oil content being around 24 wt.%. It was relatively concentrated, thus requiring further dilution and consequently less surfactant in practical use. Bearing in mind the mechanism of spontaneous emulsification, it is clear that in the present case the hydrophilic material displacement, which plays the role of solvent, is ensured by the nonionic surfactant itself. Given that the major drawback of the solvent diffusion method is precisely the use of toxic solvents (acetone, ethanol) and that considerable effort has been put into dealing with this problem (Bouchemal et al., 2004), the present finding may open doors to new alternative formulations which get round this use of toxic solvent. Fig. 1 shows that the droplet diameters follow a net logarithmic decrease along with the SOR, and exhibit a very narrow size distribution (small PDI values). Hence the droplet sizes can be precisely controlled, indicating behaviour in keeping with the results obtained using the solvent diffusion method (López-Montilla et al., 2002).

Similar experiments were undertaken, changing first the nature of the surfactant and then the oil, *i.e.* changing the surfactant/oil affinities. Cremophor ELP® was substituted for Solutol HS 15®, and both Labrafil M1944 CS® and Labrafac CC® were used as hydrophobic phases. Unlike previous case results, the surfactant in these experiments was neither freely soluble nor miscible in oils at room temperature. Cloud points of Solutol HS 15®*in oil* were determined around $\text{CP}_{\text{oil}}^1 = 28\,°\text{C}$ and $\text{CP}_{\text{oil}}^2 = 87\,°\text{C}$, respectively for Labrafil M1944 CS® and Labrafac CC®. In pure water the cloud point was at $\text{CP}_{\text{water}} = 80\,°\text{C}$.

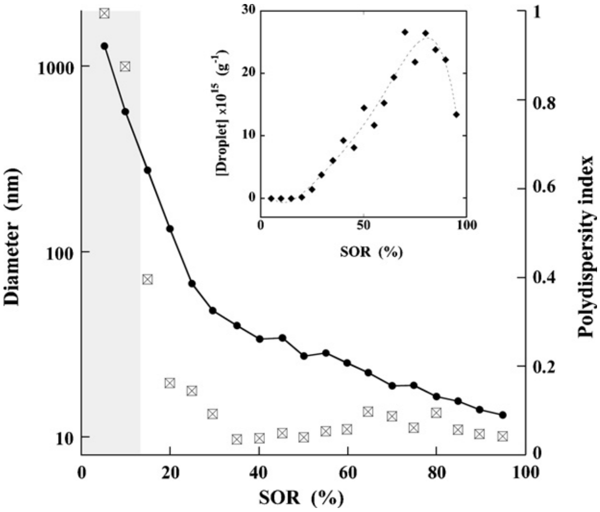

Fig. 1. Nano-emulsions formulated with oil and surfactant freely soluble at room temperature (25 °C). Surfactant = Cremophor ELP®, oil = Labrafil M1944 CS®. Hydrodynamic diameter (filled circles) and PDI (open squares) are plotted against the surfactant oil weight ratio. The hatched parts indicate that the criteria of PDI quality are not met, the suspension cannot be considered as a nano-emulsion. *Inset*: Evaluated droplet concentration *vs.* SOR.

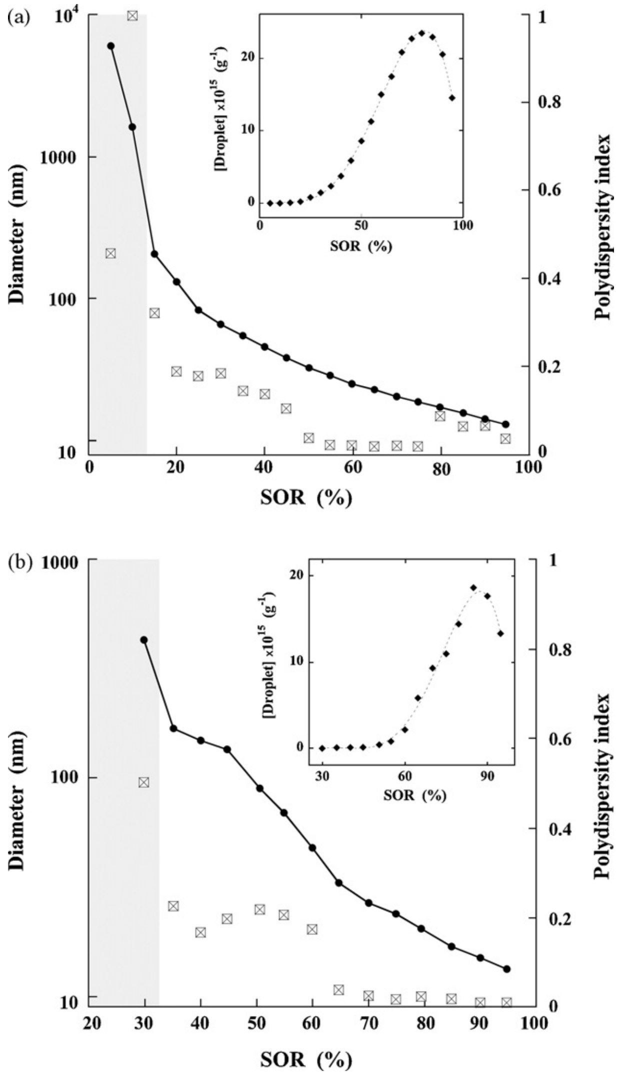

Fig. 2. Nano-emulsions formulated with oil and surfactant not freely soluble at room temperature, organic phases are maintained at 90 °C before pouring in water at 25 °C. (a) Surfactant = Solutol HS 15®, oil = Labrafil M1944 CS®. (b) Surfactant = Solutol HS 15®, oil = Labrafac CC®. Hydrodynamic diameter (filled circles) and PDI (open squares) are plotted against the surfactant oil weight ratio. The hatched parts indicate that the criteria of PDI quality are not met, the suspension cannot be considered as a nano-emulsion. *Inset*: Evaluated droplet concentration *vs.* SOR.

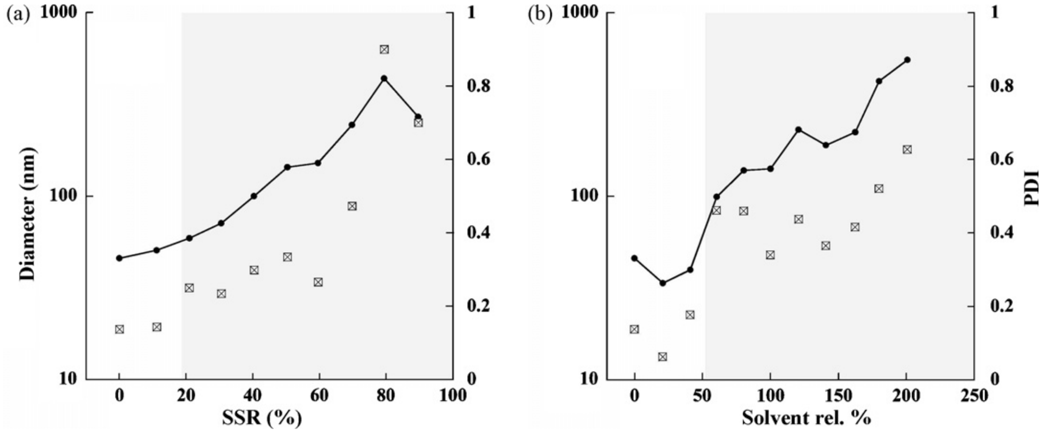

Fig. 3. Nano-emulsions formulated according to the spontaneous emulsification method, using oil, water and ethanol as a diffusing solvent. Surfactant = Solutol HS 15®, oil = Labrafil M1944 CS®. (a) Hydrophilic diffusing materials are kept constant ($m_{\text{surfactant}} + m_{\text{solvent}}$ = Constant, with $m_{\text{surfactant}} + m_{\text{solvent}}/(m_{\text{surfactant}} + m_{solvent} + m_{\text{solvent}}) =$ (S + S)OR = 40%), hydrodynamic diameter (filled circles) and PDI (open squares) are plotted against SSR. (b) Hydrodynamic diameter and PDI are plotted against the relative weight percentage of ethanol added to the formulation, with respect to the surfactant weight; the surfactant/oil weight ratio is kept constant at SOR = 40%. The hatched parts indicate that the criteria of PDI quality are not met, the suspension cannot be considered as a nano-emulsion.

In order to ensure the surfactant solubility in oil, the organic mixtures were heated in a boiler at a temperature T_b above CP_{oil}. Nano-emulsions were formed by pouring this hot mixture into water at room temperature. Above CP_{oil}, there was no real influence of T_b on the nano-emulsion properties (size, PDI); However, in order to compare this process with the second part of the study (the PIT method), T_b was fixed above the phase inversion temperature (PIT). In our case the aqueous phase used was pure water without electrolytes, and the PIT was found (by electrical conductivity monitoring *vs.* temperature (Anton et al., 2007b) to be close to CP_{water}, thus around 80 °C. Accordingly, T_b was fixed at 90 °C. The results are summarized in Fig. 2.

The curve profiles appear very close to those disclosed in Fig. 1, indicating close comparability of the two methods of nano-emulsion formulation. It may be assumed that for all the experiments summarized by Figs. 1 and 2, close experimental conditions were recreated by experimenting with temperature variation. Figs. 1 and 2(a) are very similar, almost superimposable, whereas Fig. 2(a) and (b) are quite different. This emphasizes that, apart from the nature of the surfactants, the determining factor governing nano-emulsion formation may be the affinity of the surfactants for the hydrophobic phase. This would explain the huge difference in the surfactant/oil affinity for the two systems of Fig. 2, and the significant difference between CP^1_{oil} and CP^2_{oil}. The higher the amphiphile solubility in oil, the higher their dispersion and their "anchorage" in oil at the nanometric scale. Moreover, since the surfactants used are extremely hydrophilic, this affinity for the oily phase appears fully necessary to ensure a quick and complete diffusion from the organic to the aqueous phase, which is the very basis of the process. Besides, the graph inset report shows the droplet concentration in function of the SOR. Interestingly, this profile is continuous and shows a maximum SOR of 75–80%. It indicates that when the surfactant content reaches this limit, the decrease in the droplet diameter is stabilized. In the case of biological evaluation of these systems and in order to compare different formulations, the droplet concentration is also an important parameter.

A last series of experiments on spontaneous emulsification, reported in Fig. 3, show the influence of an additional solvent in the formulations. Traditionally, this spontaneous emulsification method, the so-called solvent diffusion method, involves the use of a hydrophilic solvent to act as hydrophilic diffusing material (whereas to date in our study nonionic surfactants were used in this capacity). Solvents remain useful in the formulation of polymeric nanoparticles, solubilizing preformed polymers and inducing their nano-precipitation in the form of nanocapsules after diffusion (Anton et al., 2008). The impact of the presence of a solvent in the formulation of nano-emulsions was evaluated (a) by a gradual substitution of the surfactant for ethanol at constant weight of hydrophilic materials in oil (Fig. 3(a)); and (b) by a progressive addition of solvent (Fig. 3(b)). The initial formulation was based on the one used in Fig. 2(a), with SOR = 40% (using Solutol HS 15®/Labrafil M1944 CS®).

In these solvent displacement methods, ethanol is recognized as a solvent-diffusing model for generating nano-emulsions (*e.g.* (Bouchemal et al., 2004; Garrigue et al., 2005)). These results show that, at a *constant* amount of material diffusion (Fig. 3(a)), increasing the proportions of solvent leads to a dramatic increase in nano-emulsion size and in a near loss of monodispersity and quality. This remains true for low amounts of solvent, such as SSR = 10–20%, but compared to the results where ethanol is not used, using it fully inhibits the nano-emulsion emulsification capabilities. Furthermore, the results of Fig. 3(b), wherein the surfactant amount is kept constant, highlight similar behaviour. However, nano-emulsification is achieved to a relative solvent proportion of ~50% (*i.e.* for the same weight of surfactant and ethanol in the formulation). These results emphasize that (i) the nano-emulsion size and PDI increase when surfactants are replaced by water-miscible solvents (ii) this behaviour is not due to the decrease in the amount of surfactant but rather to the presence of a solvent. It follows that self-emulsification is mainly induced by surfactant diffusion towards the aqueous phase.

3.2. Phase inversion temperature method

The selected system was the one used in Fig. 2(a), that is to say, Solutol HS 15®, Labrafil M1944 CS and ultrapure MilliQ Water. The PIT was determined at around 80 °C, and the boiler temperature T_b was thus fixed at 90 °C. First, the influence of both SOR and WOR on the size and polydispersity of the nano-emulsions generated by the PIT method was investigated. Secondly, these results were compared to the ones obtained by spontaneous emulsification in Fig. 2(a). This comparison is actually of fundamental importance in the present study, not only to reinforce the bridge between these two methods, but also to understand that the mechanism of nano-emulsion generation using the PIT method is very close to the one described for spontaneous emulsification (Fig. 4).

These results emphasize the bridge between the two methods, which, at a constant SOR, result in very similar nano-emulsions.

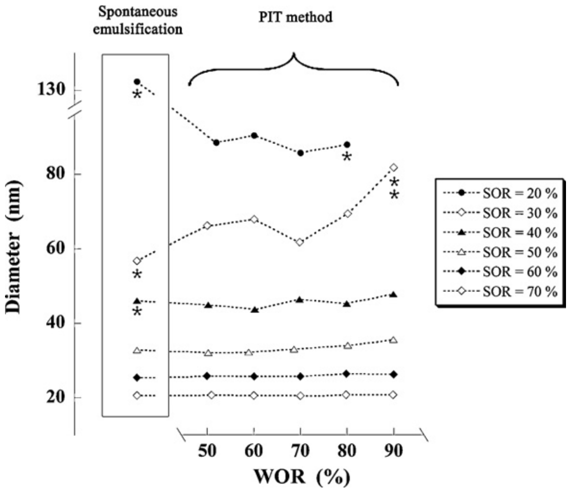

Fig. 4. A comparison between nano-emulsions generated by the PIT method and spontaneous emulsification. Surfactant = Solutol HS 15®, oil = Labrafil M1944 CS®, aqueous phase = ultrapure MilliQ water. Hydrodynamic diameter plotted against the WOR and the SOR. Two stars = PDI > 0.2, one star = $0.1 <$ PDI < 0.2, no star = PDI < 0.1.

The surfactant oil ratio alone has an influence on the nano-emulsion size, regardless of the WOR. It may mean that the same mechanism, closely related to the surfactant oil ratio, governs both processes. It follows that the concepts involved in the PIT procedure may in fact be the same as those involved in spontaneous emulsification, based on the rapid diffusion of one species from the oily to the aqueous phase. This mechanism, illustrated in Fig. 5, can be described as follows: below the PIT, Fig. 5(a), a milky macro-emulsion forms and most nonionic surfactants are solubilized in the aqueous phase, according to a surfactant partitioning coefficient shifted towards water solubility (Salager et al., 2000; Anton et al., 2008) (see below). As the temperature of the mixture is increased, Fig. 5(b), these nonionic amphiphiles gradually become hydrophobic, causing them to migrate within the oily phase. Then, given that the amounts of surfactant are of the same order of magnitude as those of oil, the volume fraction of the dispersed phase increases. In addition, interfacial curvature and interfacial tension are lowered, giving rise to propitious conditions for the establishment of Winsor III or Winsor IV microemulsions at equilibrium (Forster et al., 1995; Salager et al., 2004; Izquierdo et al., 2004; Solans et al., 2005; Anton et al., 2008), as shown in Fig. 5(c). Finally, when the temperature is further increased above the PIT, the system undergoes phase inversion and water is dispersed in the oil plus surfactant mixture (surfactants are lipophilic at this temperature). At this point, after rapid cooling, nano-emulsions are created. This last step simply recreates the emulsification conditions of the spontaneous emulsification procedure. The system is very rapidly set up at a temperature that dramatically changes the surfactant partitioning coefficients, making them fully hydrophilic. As a result, amphiphiles spontaneously move from the oil to the aqueous phase, just as occurs in spontaneous emulsification. Finally, as shown in Fig. 5(c), nano-emulsions are generated, due solely to this displacement of hydrophilic materials.

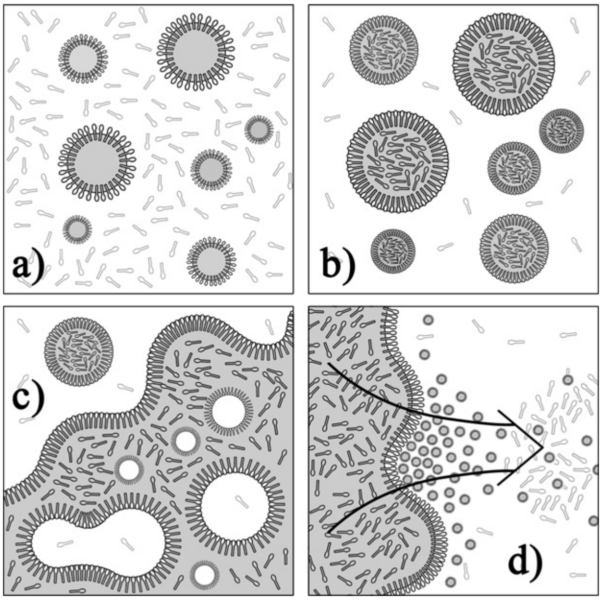

Fig. 5. Diagram of the mechanism of the generation of nano-emulsions using the PIT method. A water/nonionic surfactant/oil system undergoes a phase inversion. (a) *T* is below the PIT, the ternary system presents a macro-emulsion and nonionic surfactants are mostly hydrophilic. (b) *T* is increased; the surfactants gradually become lipophilic and are solubilized by the oily phase. (c) *T* is at the PIT, bicontinuous microemulsions form. (d) Temperature is brought above the PIT, the emulsion is inverted and then water is dispersed into the mixture of oil and lipophilic surfactant. The system is then rapidly cooled using water dilution, making the surfactant suddenly hydrophilic and inducing spontaneous and rapid migration to the aqueous phase. As in spontaneous emulsification, this turbulent displacement induces the generation of nano-emulsions.

The generally accepted hypothesis is that nano-emulsions are created from the PIT-microemulsions, but the results of this study show that the mechanism of nano-droplet formation could be explained as simply being based on that of spontaneous emulsification. Some studies actually link the structures and morphology of the equilibrium microemulsions directly to the formation and properties of nano-emulsions (Morales et al., 2003; Izquierdo et al., 2004). In these works, fluctuations of nano-emulsion droplet sizes are correlated with the PIT-microemulsion morphologies at equilibrium. But from the point of view adopted in the present study, the only key influencing parameter also appears to be the surfactant/oil ratio. Furthermore, Fig. 4 emphasizes that the WOR has no influence on the formulations, thus indicating not only that the initial water does not play a significant role in the process, but also that the mechanisms are governed by the behaviour of the organic components. This corresponds well to our mechanism. Finally, Fig. 6 reports complementary results of a blank experiment, showing the influence of the temperature of the emulsion before dilution on

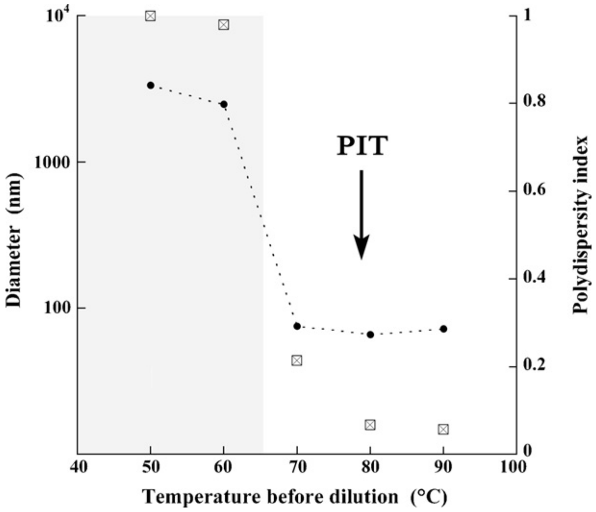

Fig. 6. The PIT method: Influence of the emulsion temperature before dilution (with water at room temperature), on the generation of nano-emulsions. Surfactant = Solutol HS 15®, oil = Labrafil M1944 CS®, aqueous phase = ultrapure MilliQ water. Hydrodynamic diameter (filled circles) and PDI (open squares) are plotted against the emulsion temperature. The hatched part indicates that the criteria of PDI quality are not met, the suspension cannot be considered as a nano-emulsion.

the generation of nano-emulsions. The selected system is identical to the one studied above, *i.e.* Solutol HS 15®/Labrafil M1944 CS®/ultrapure MilliQ water. The SOR is fixed at 30% and the WOR at 70%, as representative values.

These results show that below the PIT (around 80 °C) the nano-emulsification process does not occur and macroscopic emulsions form. When approaching the PIT, the emulsion droplet sizes and PDI are dramatically reduced, respectively below 100 nm and 0.1. This phenomenon also occurs just below the PIT at 70 °C, the droplet size is 75 nm but with a relative polydispersity, PDI = 0.21. The samples become extremely monodisperse at $T \geq$ PIT. Above the PIT, the mean size of the nano-emulsion droplets remains constant, still with a very low PDI of 0.06. These details are actually of fundamental importance and serve to corroborate the mechanism described in this paper, essentially based on the particular temperature controlled displacement of nonionic surfactants.

4. Conclusion

Understanding nano-emulsification processes is of prime importance for the generation of nanoparticulate systems. In the formulation of nano-pharmaceutics or cosmetics, low-energy methods appear to be favoured as a means to encapsulate fragile or thermosensitive drugs. To date, these methods were categorised into different methods themselves governed by different mechanisms. This study actually indicates that finally, all these low-energy methods including the PIT method follow a single, universal mechanism controlled by a rapid displacement of the surfactants from the oily to the aqueous phase, this phenomenon giving rise to spontaneous nano-emulsification.

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