# Nano-emulsions and nanocapsules by the PIT method: An investigation on the role of the temperature cycling on the emulsion phase inversion

Nicolas Anton [a], Pascal Gayet [a], Jean-Pierre Benoit [a,b], Patrick Saulnier [a,*]

[a] *University of Angers, Inserm U646, Ingénierie de la vectorisation particulaire, 10 rue A. Boquel, F-49100 Angers, France*
[b] *École pratique des hautes études (EPHE), 12 rue Cuvier, F-75005 Paris, France*

## Abstract

This paper focuses on the phenomenological understanding of temperature cycling process, applied to the phase inversion temperature (PIT) method. The role of this particular thermal treatment on emulsions phase inversion, as well as its ability to generate nano-emulsions have been investigated. In order to propose a general study, we have based our investigations on a given formulation of nano-emulsions classically proposed in the literature [Heurtault, B., Saulnier, P., Pech, B., Proust, J. E., Benoit, J.P., 2002. A novel phase inversion-based process for the preparation of lipid nanocarriers. Pharm. Res. 19, 875; Lamprecht, A., Bouligand, Y, Benoit, J.P., 2002. New lipid nanocapsules exhibit sustained release properties for amiodarone. J. Control. Release 84, 59–68], using a polyethoxylated model nonionic surfactant, a polyoxyehtylene-660-12-hydroxy stearate, stabilizing the emulsion composed of caprilic triglycerides (triglycerides medium chains), salt water (and also phospholipidic amphiphiles neutral for the formulation). Characterization of nano-emulsions was performed by dynamic light scattering (DLS) which provides the hydrodynamic diameter, but also the polydispersity index (PDI), as a fundamental criteria to judge the quality of the dispersion. Another aspect of the characterization was done following the emulsion inversion and structure by electrical conductivity through the temperature scan. Overall, the role such a temperature cycling process on the formulation of nano-emulsions appears to be relatively important, and globally enhanced as the surfactant concentration is lowered. Actually, both the hydrodynamic diameter and the PDI decrease as a function of the number and temperature cycles up to stabilize a steady state. Eventually, such a cycling process allows the generation of nano-emulsions in ranges of compositions largely expanded when compared with the classical PIT method. These general and interesting trends emerge from the results, are discussed and essentially explained by regarding the behavior of the nonionic surfactants towards the water/oil interface, linking partitioning coefficients, temperature variation, and surfactant water/oil interfacial concentration. In that way, this paper proposes new insights into the phenomena governing the PIT method, by originally investigating the temperature cycling process.
© 2007 Elsevier B.V. All rights reserved.

*Keywords:* Nano-emulsion; PIT method; Emulsion; Temperature cycling; Nonionic

## 1. Introduction

Nanometric-sized emulsions until 300 nm, so-called nano-emulsions or miniemulsions, were widely studied for their numerous potential applications. Nano-emulsions are transparent or translucent systems, kinetically stable (in contrast with microemulsions which show a thermodynamic equilibrium between components present in the different phases). These nano-emulsions are also characterized by a great stability in suspension due to their droplet sizes, *i.e.* the fact that the principal cause of instability is due to Ostwald ripening (Taylor, 1998). It is only in recent years that this subject has seen a real explosion in research activity partly due to cosmetic and pharmaceutical applications that have initiated the formulation of novel systems generating nanoparticles. Their property of immune privilege has generated considerable interest. Another important advantage of nano-emulsions comes from the fact that their high stability in suspension is fundamental for many industrial applications.

Processes used to generate nano-emulsions can be separated in two fundamentally different families: the first one gathers high-energy emulsification methods (utilizing either high pressure homogenizers or ultrasound generators) and can be in

---

* Corresponding author.
  *E-mail addresses:* nicolas.anton@univ-angers.fr (N. Anton),
p_gayet@hotmail.com (P. Gayet), jean-pierre.benoit@univ-angers.fr
(J.-P. Benoit), patrick.saulnier@univ-angers.fr (P. Saulnier).

certain cases out of our specifications (drug degradation during processing). The second group includes Low-energy emulsification methods, where intrinsic physico-chemical behavior is diverted to reach nanometric-sized emulsions, with a minimum of energy. Among low energy methods, we can find: (i) spontaneous emulsification (Rubin and Radke, 1980; El-Aasser et al., 1986; Miller, 1988), or the solvent-diffusion method (Quintanar-Guerrero et al., 1999; Trotta et al., 2001; Bouchemal et al., 2004), and (ii) the phase inversion temperature (PIT) method (Forster et al., 1990, 1992; Forgiarini et al., 2001; Sing et al., 1999; Izquierdo et al., 2002, 2004; Morales et al., 2003). We definitely focused here on this latter PIT approach for formulating oil-in-water nano-emulsions.

This low energy and solvent-free method was introduced in the last decades by Shinoda and Saito (1968, 1969), using the particular ability of emulsions stabilized by polyethoxylated (PEO) nonionic surfactants to undergo a phase inversion following a variation of temperature. The so-called *transitional* phase inversion occurs, when, at a fixed composition, the relative affinity of the surfactant for the different phases is changed and controlled by the temperature. As a result, oil-in-water (o/w) macro-emulsion undergoes a phase inversion to a water-in-oil (w/o) one during a temperature increasing, and *vice versa*. Within the transitional region between both macro-emulsions, for the temperatures at which the nonionic surfactants show very close affinities for the two unmiscible phases, the ternary system shows bicontinuous structure. The phase diagrams have commonly shown a typical Winsor IV configuration in the *caudal fin* of the Kahlweit-fish diagram (Salager et al., 2004; Solans et al., 2005; Kahlweit et al., 1985; Forster et al., 1995; Kunieda et al., 1996; Morales et al., 2003; Izquierdo et al., 2004, 2005), corresponding to the emulsion inversion zone. The emulsion inversion characterization, and as well the PIT, are determined by the follow-up of the emulsion electrical conductivity. However, it is noteworthy that, the emulsion phase inversion does not occurs for any nonionic polyethoxylated surfactant: in fact, even if the presence of OE groups in the surfactant molecules is a necessary condition for inducing a phase inversion, it true to underline that some POE nonionic surfactants, for which the POE headgroup is either too short or too long, or *vice versa* the oily chain is too long or too short, are not sensitive enough to the temperature, to induce a phase inversion. To summarize, only nonionic polyethoxylated surfactants will allow to perform the emulsion inversion, but the affinities of the surfactant for the aqueous and oily bulk phases have to be relatively balanced, right from the start.

Hence, the PIT method consists in suddenly break-up such a microemulsion network by performing, at the PIT, a rapid cooling (Forgiarini et al., 2001; Izquierdo et al., 2002, 2004) and/or a sudden water dilution (Forster et al., 1995; Pons et al., 2003). This stage is considered as an irreversible process since it leads to the generation of the kinetically stable oil droplets, *i.e.* the o/w nano-emulsions. This great stability is due to the fact that the steric stabilization prevents the droplets flocculation and therefore coalescence. Thus, the de stabilization of such an emulsion only remains governed by the inter-droplets oil diffusion (so-called Ostwald ripening), widely discussed for instance by Taylor (1998).

The present paper focuses on the nano-emulsions formed by this PIT method, following a classical formulation based widely described on the literature (Heurtault et al., 2002; Lamprecht et al., 2002), exclusively using components acceptable by a parenteral administration route: the emulsion is composed of a polyethoxylated model nonionic surfactant, a macrogol 15 hydroxystearate (major component: polyoxyethylene-660-12-hydroxy stearate), stabilizing the emulsion caprilic triglycerides (triglycerides medium chains as oily phase), salt water, and additional phospholipidic amphiphiles neutral for the formulation. The originality of this formulation process, is that it includes an additional stage which consists in performing a cycling on the temperatures crossing the phase inversion zone (PIZ) (Heurtault et al., 2001, 2002; Lamprecht et al., 2002; Hoarau et al., 2004; Salager et al., 2004; Malzert et al., 2006; Béduneau et al., 2006). This temperature cycling process has been linked with improving quality of the nanometric dispersions, that is to say decreasing the size and polydispersity index (PDI). In certain cases, it even has been shown as a condition for generating such a nanometric suspension. This temperature cycling process is not well documented, thus we focused here on its phenomenological understanding. The purpose of this study is to grasp the place and the role of this process on the PIT method, and as well, to determine the conditions for which it appears beneficial for the formulation. Here we have chosen to follow, by dynamic light scattering (DLS), the quality of the dispersion, *i.e.* the droplets size and PDI, as a function of the temperature cycles number. An additional characterization and follow-up of the emulsions electrical conductivity during the cycling process was undertaken as well, disclosing significant trends coherently with the new ideas proposed, in this paper, from the DLS results.

## 2. Materials and methods

### 2.1. Materials

The nonionic highly polyethoxylated surfactant, Solutol® HS 15, is a macrogol 15 hydroxystearate with a number of ethylene oxide (EO) group given around 15 (major component: polyoxyethylene-660-12-hydroxy stearate). This surfactant is of technical grade and it should be (as it is in most cases Salager et al., 2004) a mixture of different oligomers of molecular weights centered on the one of announced by the manufacturer, say around $960 \, g \, mol^{-1}$. It was kindly provided by BASF (Ludwigshafen, Germany). Medium chains triglycerides, and more precisely caprilic triglycerides (Labrafac®) as the hydrophobe component was gift from Gattefossé (Saint-Priest, France). The choice of the Labrafac® as oily phase was initially done to be compatible with a parenteral administration route, and therefore it is the nano-emulsion formulation which has been adapted to the physicochemical properties of the oil. Ultrapure® water was obtained by MilliQ filtration system (Millipore, Saint-Quentin-en-Yvelines, France). Additional phospholipids, Lipoïd S75-3® (soybean lecithin at 69% of phosphatidylcholine) was obtained from Lipoïd Gmbh (Ludwigshafen, Germany) and

sodium chloride from Prolabo (Fontenay-sou-Bois, France). In all experiments, the fractions of NaCl and Lipoïd S75-3® are taken constant, so we have only studied the influence of the components directly involved in the emulsion phase inversion, thus the ternary system: aqueous phase, nonionic surfactant, oil.

### 2.2. Methods

#### 2.2.1. Nano-emulsion preparation and temperature cycling

We based our investigations on the formulation of Heurtault et al. (2002), the first work presenting such cycling technology, using systems acceptable for parenteral administration. It is to be noted that the sodium chloride concentration as to be considered as a formulation variable, and thus potentially influencing the emulsion phase inversion. Its effects on the PIT was reported through a comprehensive study elsewhere (Anton et al., 2007) along the temperature cycling process, and the NaCl concentration choice in the present study, was given by the earlier works presenting this formulation (Heurtault et al., 2002; Lamprecht et al., 2002), it corresponds to the suitable experimental conditions to perform the nano-emulsions generation. Furthermore, in addition to the common formulation with the ternary system {salt water; nonionic surfactant; oil}, it is also introduced phospholipidic surfactants neutral in both the emulsion inversion and the nano-emulsion formulations, but involved in the framework of the interfacial layer. The role such phopholipidic amphiphiles in the formulation has been investigated in detail by Minkov et al. (2005a,b,c), to disclose that they appreciably improve the nanoparticle stability, creating a framework within the shell. Similarly to the NaCl concentration, the choice of their concentration was optimized and fixed by the earlier works we take here as models.

Each sample was prepared by the sudden dilution of the ternary system taken at $T = $ PIT, with water at room temperature, and at least with two times the volume of the microemulsion. During the dilution step (as well as during all the experiment), a constant stirring was maintained, in fact only to homogenize the flack. The water addition rapidly cools and dilutes the system: the nano-emulsion is instantaneously created as a result. Besides, the systematic investigation of the influence of the temperature cycling involved to fix a given number of temperature cycles for each formulation. The PIT of this system arising around 85 °C, the temperature scanning windows was chosen to clearly wrap the emulsion inversion zone, thus 60 °C < $T$ < 90 °C. The system was constantly, magnetically stirred during the cycling process, without notable influence of the stirring rate on the emulsion phase inversion. The temperature variation rate ($\partial T/\partial t$) was fixed at $\pm 4$ °C min$^{-1}$, basing once again our process, on the one described on the literature by our team, for generating nano-emulsions. Likewise, the temperature variation rate could have an influence on the emulsion phase inversion morphology (as it is shortly discussed in Ref. Anton et al., 2007), and surely on the nano-emulsion generation, but our choice in this study was to keep it as a constant in order to evaluate the influence of the temperature cycling process.

A preliminary mixing of all components was done at room temperature before the first heating, under magnetic stirring. The first crossing of the PIZ was then considered as the origin of the cycling process, *i.e.* 'number of cycle = 0', therefore in the same conditions than the *classical* PIT method. Afterwards, we considered as a new period incrementing the number of temperature cycles, each time that $T = $ PIT for $\partial T/\partial t > 0$. When the chosen temperature cycle was attained, the nano-emulsion was generated from $T = $ PIT.

As a last remark, it is to be underlined that such a process, involving multiple thermal cycling across 80 °C, should be dedicated to the encapsulation of non-thermosensitive drug. At least, the integrity of the active molecule encapsulated has to be checked after the encapsulation. Actually, many works precisely utilizing this system and process (three temperature cycles across 80 °C...), have proved the drug degradation to be prevented; For instance the works of Lamprecht and Benoit (Lamprecht and Benoit, 2006) have even shown not only to preserve the integrity, but also the activity of a drug encapsulated.

#### 2.2.2. Dynamic light scattering

The nano-emulsions characterization, hydrodymanic diameter as much as poly-dispersity index measuring, was carried out by dynamic light scattering using a Nano ZS apparatus, Malvern Instrument (Orsay, France). The Helium-Neon laser, 4 mW, operates at 633 nm, with the scatter angle fixed at 173°, and the temperature at 25 °C. The polydispersity index (noted PDI) only appears as a mathematical definition, accounting for the relative error between curve fit and experimental values (Pusey and van Megen, 1989; Linder and Zemb, 2002; Sandkühler et al., 2004). The PDI discloses the quality of the dispersion, from values lower than 0.1 for suitable measurements and good-quality of the colloidal suspensions, to values close to 1 for poor-quality samples, which in concrete terms either do not present droplets sizes in the colloidal range, or exhibit a very high polydispersity.

Moreover, all measurements were performed in the standard suitable conditions to validate the results, in terms of sample dilution and at a the constant temperature of 25 °C. Furthermore, the apparatus we used (Malvern Nano ZS) systematically and automatically adapts to the sample, the intensity of the laser as well as the attenuator (diaphragm) of the photomultipher. Therefore, the reproducibility of the experimental measurement conditions is insured.

#### 2.2.3. Electrical conductivity measurements

A conductimeter (Cond 330i/SET, WTW, Germany) was used in nonlinear temperature compensation mode, according to EN 27888. It allowed to determine the location of the emulsion inversion zone following the conductivity variations according to the temperature. In fact, a conductivity value lower than 10 µS cm$^{-1}$ and essentially zero on the illustrated scales, means that the continuous phase is oil, whereas high steady state reached reflects that a water continuous phase is established. The experiments were performed three times.
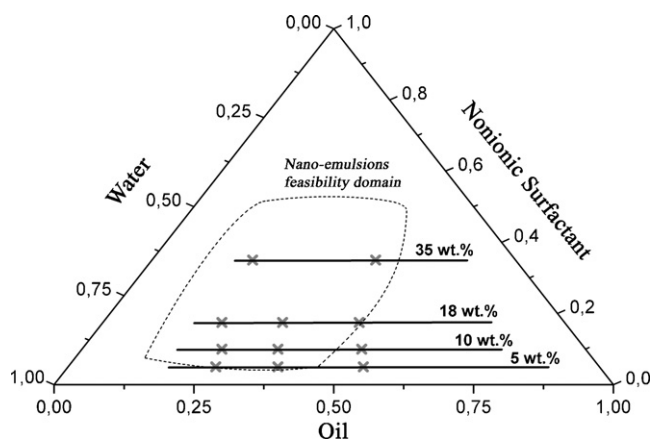
Fig. 1. Presentation of the selected representative compositions studied as a function of the number of temperature cycles. The dashed lines gather the compositions leading to the formation of nano-emulsions meeting both PDI ≤ 0.1 and a diameter < 300 nm.

## 3. Results and discussion

### 3.1. The influence of the temperature cycling on the nano-emulsions formulation

In order to compare the respective effects of each component composing the emulsion (but at a electrolyte and phospholipid wt.% maintained constant), the experiments were performed on systems, selected on the ternary diagrams. They are presented in Fig. 1, following the iso-concentration lines of nonionic surfactant (the representative cases of 5, 10, 18 and 35 wt.%) and varying the water-to-oil weight ratio (noted WOR, equal to $100 \times$ water/(water + oil) weight ratio). Moreover, the dashed lines bring out the nano-emulsions forming domain, after the cycling treatment, that gather together the formulations showing a PDI ≤ 0.1, and a diameter < 300 nm. Outside of this domain, the systems formed are essentially highly polydispersed and far from the nano-emulsion state.

The selected studied systems are marked with crosses in Fig. 1 and their characterizations as a function of the number of temperature cycles, are respectively, reported in Fig. 2. For the given surfactant amounts fixed in the formulations, different WOR are tested. It is to be noted that two points are chosen out of the feasibility domain, precisely {WOR = 44; 10 wt.% in surf.} and {WOR = 44; 5 wt.% in surf.}, in order to appreciate the system behaviors in the different cases.

In each graph in Fig. 2, the size and the polydispersity index of the droplets, created after water dilution, are simultaneously reported. The hatched parts (in certain cases, essentially for the lowest surfactant amounts), indicate that the criteria of PDI quality are not met (i.e. PDI ≥ 0.7), the suspension cannot be considered in any way as nano-emulsions, only PDIs are reported in the graphs. Since a similar study has never been undertaken, the first result confirms the important role of the temperature cycling, on both favoring the formation of nano-emulsions, and improving the quality of the dispersion. In concrete terms, the consequences

of such a cycling process are a simultaneous decrease of the droplet hydrodynamic diameters, and of the polydispersity index.

Overall, the process of temperature cycling appears beneficial for the nano-emulsion formulations. The general tendency shows that the evolution, and stabilizing behaviors, occurring with the number of cycles is nearly similar for a constant surfactant concentration (noted C). Indeed, when, for the highest surfactant amount presented, C = 35 wt.%, the nano-emulsions are easily formed without temperature cycles immediately exhibiting the final stabilized size, for C = 18 wt.% the stabilization of the sizes and of PDIs is acheived after N = 1 cycle. Likewise, for C = 10 wt.% whatever the WOR, the nano-emulsions only form after N = 1, and the stabilization is complete after N = 2 cycles. Finally, for the lowest represented surfactant amounts of C = 5 wt.%, this trend is further shifted, the droplet sizes and PDIs are stabilized from N around 3. Moreover, it is noteworthy that for the systems out (or at the frontier) of the feasibility domain, a very uncertain behavior is disclosed, showing a relatively high polydispersity index and fluctuations in the size after stabilization. On the other hand, in all the cases within the forming domain, their values remain very stable even when performing further temperature cycles (after stabilization). Furthermore, it clearly appears that the effects due to such a temperature cycling treatment are directly linked to the nonionic surfactant amount in the emulsion: the lower the surfactant amount, the higher the number of cycles required for stabilizing the nano-emulsion size and polydispersity. These results are of fundamental importance showing that very good quality nano-emulsions can be formed after a few temperature cycles, whereas according to the classical PIT method (i.e. without cycle for N = 0) no nano-emulsion is generated. It follows therefrom that such a cycling process gives another dimension to the PIT method, extending the feasibility domain of the nano-emulsions formation. The temperature cycling, crossing the PIZ, appears increasingly necessary for generating the nano-emulsions as the nonionic surfactant amount decreases, and overall whatever the WOR.

Besides, as regards the formulations including the temperature cycling technology (we have present in the introduction part), for which the whole process is essentially the same (similar experimental conditions, temperature variation rate, etc.), and the number of cycles fixed to N = 3, these new results disclose in which extent the parameter N = 3 is beneficial for the formulation. Thus in this context, three temperature cycles allows to expand in a non-negligible way the feasibility domain, and as well, for the higher surfactant amounts they do not appear really necessary.

### 3.2. Electrical conductivity measurements

Another aspect of this study is provided by the follow-up of the emulsion phase inversion by electrical conductivity measurements. On the one hand, Fig. 3(a) reports the conductivity as a function of the temperature for a representative case of WOR = 80, and varying the total surfactant amount in the formulation.
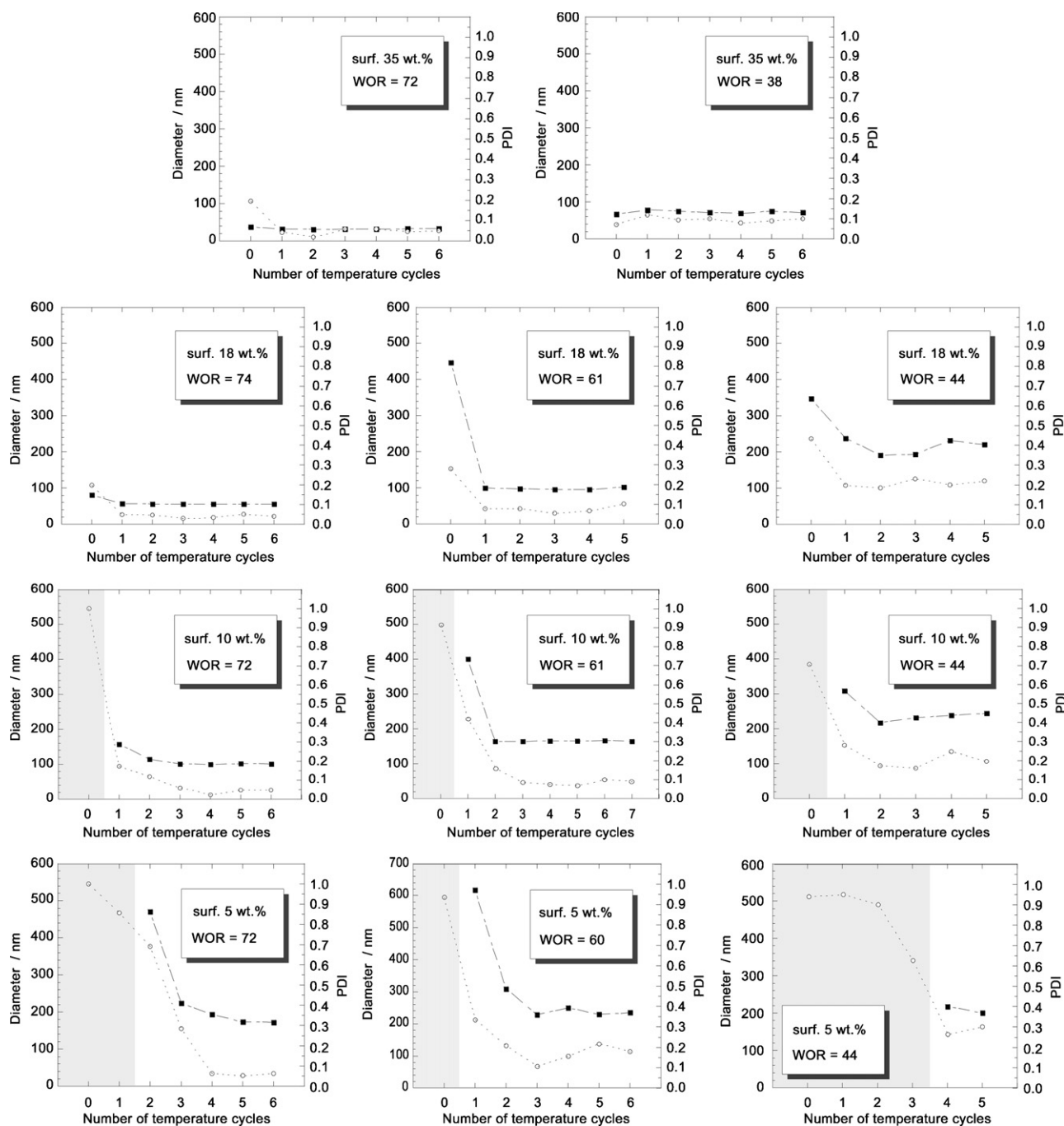
Fig. 2. Experimental values showing the effects of the temperature cycling on the nano-emulsion formed by the PIT method. The open circles (○) present the PDIs and the filled squares (■) report the hydrodynamic droplet diameters. Each representative composition is defined by both the surfactant amount and the WOR, and corresponds to those presented in Fig. 1.

A steady state at a high conductivity value indicates that the water is the continuous phase, whereas a conductivity close to a zero value means that the continuous phase is oil. Through the phase inversion region, the conductivity gradually changes, continuously, linking the two macro-emulsions states. Such a continuous variation of the electrical conductivity suggests the existence of different microemulsion structures all along the emulsion phase inversion. We have discussed this aspect elsewhere (Anton et al., submitted for publication), the peak of conductivity reflecting some particular structures of the bicontinuous systems typically associated with the formation of nano-emulsions. The gradual shift of the curves towards the lower temperatures is also a behavior sometimes reported, which allows to draw a fine parallel between the conductivity curves and the equilibrium phase diagram (Izquierdo et al., 2004, 2005).

On the other hand, Fig. 3(b) reports the evolution of the conductivity curves during the temperature cycling process, for a representative system (WOR = 80 and $C = 15$ wt.%). A global shift towards the lower temperatures surprisingly arises with the cycling treatment, which appears similar to the behavior induced
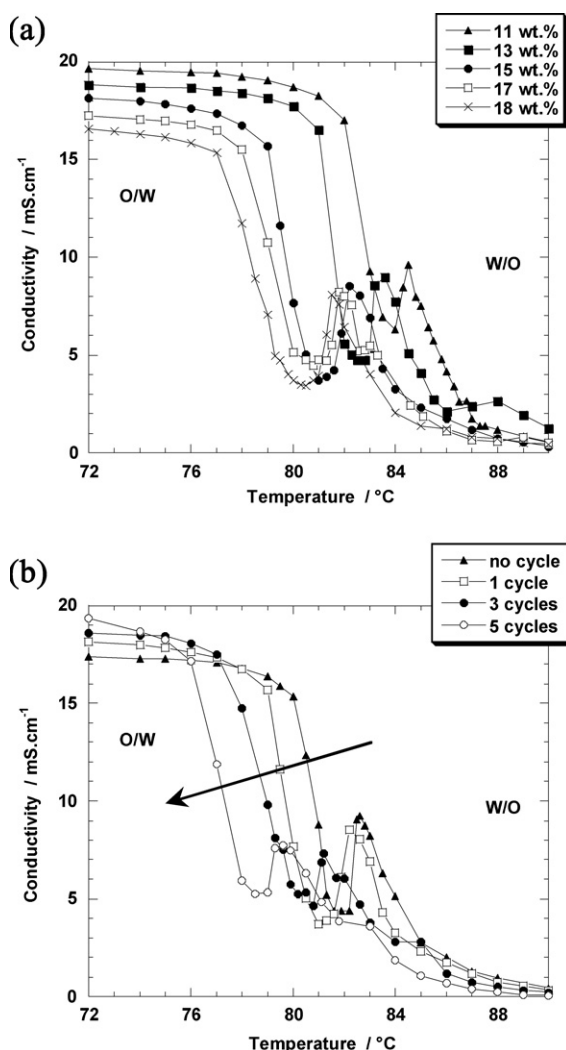
Fig. 3. Conductivity of the emulsion as a function of the temperature: (a) at different concentrations of surfactant and for a constant WOR = 80, and (b) following the number of temperature cycles at constant WOR = 80 and surfactant concentration C = 15 wt.%.

by the physical increase of the surfactant presented in Fig. 3(a). Hence, since the emulsion inversion is totaly governed by interfacial phenomena, intimately linked to the surfactant interfacial concentration, these observations suggest that the system mimicks a physical increase of concentration, along the cycling process.

Finally, Fig. 3 reports an experimental case illustrating these phenomena of curve shift with the cycling process. It is to be noted in fact, that the curve shit is more marked as the surfactant amount is increased, and the choice to present a concentration of 15 wt.% has been done in that way. Likewise it strongly depends on the expreimental conditions, electrolyte concentration, temperature variation rate... as it is discussed elsewhere (Anton et al., 2007). Actually, Fig. 2 clearly provides that size and PDI stabilization is achieved fastest as the surfactant amount is high. It means that the conditions to generate the nano-emulsions exhibiting their final characteristics (in size and PDI) are reach more quickly for high surfactant amounts than for low ones. From that angle, we can say that the effects of the cycling are not

greater at low surfactant amount (*i.e.* 5 wt.%), but are slower. The explanations could be provided by the fact that, low surfactant amounts involve higher efforts (through temperature cycling) to concentrate the surfactant at interface compared to high concentrations, to reach the stabilization of the nano-emulsions droplets size.

### 3.3. Surfactant behavior at the water/oil interface

The idea we propose in this paper concerns the concentration of surfactant at the water/oil interface, and precisely the following concept, that the quantity of non-ionic surfactant trapped at the interface increases with the temperature cycles. Firstly, it is to consider the behavior of the surfactant during the phenomenon of emulsion phase inversion, from the respective affinities of the surfactant for the different phases. Thus, the surfactant affinity difference (SAD) can be determined, according to the physicochemical definition given by De Donder of the two chemical potentials, of the surfactants, in the aqueous and oily phases:

$$\mu_{wat} = \mu_{wat}^{\circ} + RT \ln(a_{wat}C_{wat}) \tag{1}$$

$$\mu_{oil} = \mu_{oil}^{\circ} + RT \ln(a_{oil}C_{oil}) \tag{2}$$

where $\mu_{wat}$ and $\mu_{oil}$ are the chemical potentials (of the surfactants) in water and oil respectivelly, $\mu^{\circ}$ the standard ones, $a$ the activity coefficients, and $C$ is the surfactant concentrations. Accordingly, the SAD is given by the following relation (3), at the physicochemical equilibrium, and considering the activity coefficients close to the unity.

$$SAD = \mu_{wat}^{\circ} - \mu_{oil}^{\circ} = RT \ln\left(\frac{C_{oil}}{C_{wat}}\right) \tag{3}$$

The optimum formulation for ionic surfactants corresponds to SAD = 0, *i.e.* for a partitioning coefficient equal to the unity, but it is not true for nonionic surfactants. For this reason, it has been defined the so-called adimensional variable, hydrophilic–lipophilic deviation (HLD) (Salager et al., 2001), that use, as a reference SAD^ref, the value of the SAD taken for the optimum formulation (corresponding to the phase inversion). As a result, the definition of the HLD, thus the correlations for determining the emulsion phase inversion is given for nonionic surfactants and without the use of additional co-surfactant (such as alcohol), by

$$HLD = \frac{SAD - SAD^{ref}}{RT} = \alpha - EON + bS - kACN + C_T \Delta T \tag{4}$$

where $\alpha$ is a parameter, function of the nature of the surfactant lipophilic group, EON the number of ethylene oxide group per surfactant molecules, $S$ the salinity of the aqueous phase (NaCl wt.%), ACN the alkane carbon number, $\Delta T$ the difference between the temperature of the system $T$ and 25 °C, and finally $b$, $k$ and $C_T$ are other constant formulation parameters. Therefore, the HLD appears as a linear combination of the formulation variables (Salager, 2000; Rondon, 2006), allowing easily to follow their respective effects, notably in the present study (and in

all paper relating the PIT method), the HLD is controlled by the temperature.

It follows therefrom, considering Eqs. (3) and (4), that a modification of the temperature of the system directly induces a modification of the partitioning coefficient of the surfactants. A raise of *T* increases the surfactants solubility in the oily phase, whereas a fall of *T* induce an increase of their solubility in the aqueous one. Therefore, at each temperature cycle, the surfactant is forced to migrate towards the interface. Furthermore, in the case of HLD < 0 the affinities for the nonionic surfactants with water predominate to lead to the o/w emulsion, as opposed as HLD > 0 for which the interactions surfactants/oil outweigh the latter ones involving the formation of a w/o emulsion. For the temperatures at which HLD = 0, at the transitional region between both macro-emulsions, nonionic surfactants show very close affinities for the two unmiscible phases. These systems exhibiting ultralow interfacial tensions as well as a very low droplet curvatures, have been shown closely related to the destabilization of the droplets of macro-emulsion (Kabalnov and Wennerström, 1996). The conditions are gathered together to create microemulsion systems, bicontinuous, microemulsion-like, and nano-structured. This is precisely the link with the concept presented here, concerning the quantity of nonionic surfactant trapped at the oil/water interface and the effects of the cycling of temperature. Indeed, the notion of microemulsion is inevitably related to a huge interfacial concentration of surfactant, and therefore to an increased area of the oil/water interface. The chemical potential of the nonionic surfactants trapped at the microemulsion interface is lower than the ones at both, the macro-emulsions interface and in the bulk phases (Anton and Salager, 1986), the microemulsion clearly exhibits privileged interfaces for the surfactant adsorption.

Furthermore, changing the partitioning coefficient with the temperature and crossing the PIT, involves a forced migration of the nonionic amphiphiles towards the oil/water interface. In that way, the surfactants pass by a stabler state each time the microemulsions form. Accordingly, they are progressively trapped at the interface. The whole oil/water interfacial area is gradually increased with the amount of trapped molecules at interface and therefore with the number of temperature cycles, increasing the fineness of the bicontinuous network as a result.

As regards Fig. 2, such a conceptual approach appears fully coherent with the results: firstly, the general trend, a decrease and stabilization of the droplet sizes and PDI, is a consequence of the gradual structuring of the bicontinuous microemulsion with the temperature cycling, suddenly broken-up with the water dilution. The finer the bicontinuous system is, the smaller and monodisperse results the nano-emulsions. The droplet diameters reached with the stabilization are governed by the water/oil ratio, which also govern the microemulsion structure (say more or less swollen by oil). Secondly, the necessity to perform the cycles of temperature, for generating the nano-emulsions and attaining low PDIs, is gradually more pronounced as the total surfactant amount in the formulation is diminished. This is in agreement with the behavior suggested above: the higher the global quantity of amphiphile (*C*), the higher their ability to be trapped at the interface and to form fine bicontinuous microemulsion

structures. Thus, a cycling temperature treatment is required for lower values of the concentration, to finally generate a similar nano-emulsion system. Accordingly, this temperature cycling process appears as a solution to reduce the quantity of nonionic surfactant useful for the nano-emulsions formulation.

Nevertheless, the diameter of droplets after the cycling treatment appears only influenced by the WOR, without any effects on the stabilizing behavior of the cycles. Overall, increasing the initial oil amount in the formulation gives rise to an increase of the droplets diameter (still illustrated in Fig. 2). It is to be noted that investigations on the specific influence of the composition on the suspension size distribution have been undertaken elsewhere (Heurtault et al., 2003), and of course fully consistent with these results.

The stability studies of such nano-emulsions formed with performing temperature cycling (number fixed at *N* = 3), has disclosed a very high stability, at least for a year with nearly any droplets growth (Heurtault et al., 2002). In comparison, the typically formed nano-emulsions with the classical PIT method (Izquierdo et al., 2004, 2005), are stable only for a few months. Such a difference of stability is attributed to the difference in the droplet structures, and more precisely to the thick interfacial layer potentially created with the temperature cycling process. In fact, since the surfactant is forced to over-concentrate at the interface during the cycling, it is legitimate to conceive that the resulting droplets are created with an over-concentrated interfacial zone. This thick surfactant layer is also reinforced (in our case) with the neutral phospholipidic framework. As a result it is created around the nano-emulsion droplets a real barrier against the passage of the oil across the interface, which significantly reduces the inter-droplet oil diffusion, *i.e.* the Ostwald ripening, as it is also found in the literature when the droplets are stabilized with macromolecular assemblies or thick polymeric species (Higuchi and Goldberg, 1969; Yotsuyanagi et al., 1973). It is actually for this reason, that these particular nano-emulsions formulated by temperature cycling, are often denominated as *nanocapsules* or *lipid nano-capsules* (LNC). Moreover, it is even legitimate to imagine that the microemulsions formed (being bicontinuous systems) before dilution, will exhibit a different water/oil interfacial area than the final nano-emulsion system (and obviously smaller for nano-emulsions). Therefore, interfacial surfactant quantity will not be comparable for both systems, and even, this contraction of interfacial area can explain the amorphous surfactant over-concentration shell surrounding the droplets, and forming the *nanocapsules*. Finally, several reported works concerning electronic microscopy of these objects, brought out the core–shell structure of such colloidal objects, see References (Saulnier and Benoit, 2006), a priori relatively rigid at room temperature, below the surfactant melting point which is given by the manufacturer (BASF) around 30 °C.

## 4. Conclusion

To a certain extent, the first aim of this study was to rationalized the impact of the temperature cycling process on the PIT method. Nevertheless, the systematic characterization of the

dispersions along the temperature cycling, not only emphasized the fact, that quality of the nano-emulsions and this process are closely related, but gives another dimension to the PIT method. Likewise, the interfacial behavior of nonionic surfactants along the temperature cycling is originally approached, considering that the amphiphiles are gradually trapped and concentrated in the interfacial zone. The thick interfacial layer acts as a barrier to the oil diffusion, reducing the Ostwald ripening and conferring to the suspension a great stability (even higher than usual nano-emulsions), that therefore allows the denomination of 'nanocapsules'. Such a cycling process gives another dimension to the PIT method, extending the feasibility domain of the nano-emulsions formation. Finally, this process is also relatively interesting for adapting the PIT method to components suitable for pharmaceutical applications, for reducing the surfactant amount needed to generate nano-dispersions.

## References

Anton, N., Saulnier, P., Benoit, J.-P. Origin of the electrical conductivity peak within the emulsion inversion, and links with the formation of nano-emulsions. Colloids Surf. A, submitted for publication.

Anton, N., Saulnier, P., Beduneau, A., Bénoit, J.P., 2007. Salting-out effect induced by temperature cycling on a water/nonionic surfactant/oil system. J. Phys. Chem. B 111, 3651–3657.

Anton, R.E., Salager, J.L., 1986. Emulsion instability in the three-phase behavior region of surfactant-alcohol-oil-brine systems. J. Colloid Interface Sci. 111, 54–59.

Béduneau, A., Saulnier, P., Anton, N., Hindré, F., Passirani, C., Rajerison, H., Noiret, N., Benoit, J.P., 2006. Pegylated nanocapsules produced by an organic solvent-free method: evaluation of their stealth properties. Pharm. Res. 23, 2190–2199.

Bouchemal, K., Briancon, S., Perrier, E., Fessi, H., 2004. Nano-emulsion formulation using spontaneous emulsification: solvent, oil and surfactant optimisation. Int. J. Pharm. 280, 241–251.

El-Aasser, M.S., Lack, C.D., Vanderhoff, J.W., Fowkes, F.M., 1986. Miniemulsification process—different form of spontaneous emulsification. Colloids Surf. 29, 103–118.

Forgiarini, A., Esquena, J., Gonzalez, C., Solans, C., 2001. Formation of nano-emulsions by low-energy emulsification methods at constant temperature. Langmuir 17, 2076–2083.

Forster, T., Schambil, F., Tesmann, H., 1990. Emulsification by the phase inversion temperature method: the role of the self-bodying agents and the influence of oil polarity. Int. J. Cosmet. Sci. 12, 217–227.

Forster, T., Schambil, F., von Rybinski, W., 1992. Production of fine disperse and long-term stable oil-in-water emulsions by the phase inversion temperature method. Disp. Sci. Techn. 13, 183–193.

Forster, T., von Rybinski, W., Wadle, A., 1995. Influence of microemulsion phases on the preparation of fine-disperse emulsions. Adv. Colloid Interface Sci. 58, 119–149.

Heurtault, B., Saulnier, P., Pech, B., Proust, J.E., Richard, J., Benoit, J.P., 2001. Lipidic nanocapsules, formulation process and use as a drug delivery system. *Patent*, No. W001/64328.

Heurtault, B., Saulnier, P., Pech, B., Proust, J.E., Benoit, J.P., 2002. A novel phase inversion-based process for the preparation of lipid nanocarriers. Pharm. Res. 19, 875.

Heurtault, B., Saulnier, P., Pech, B., Venier-Julienne, M.C., Proust, J.E., Phan-Tan-Luu, R., Benoit, J.P., 2003. The influence of lipid nanocapsule composition on their size distribution. Eur. J. Pharm. Sci. 18, 55–61.

Higuchi, W.I., Goldberg, A.H., 1969. Mechanisms of interphase transport. II. Theoretical considerations and experimental evaluation of interfacially controlled transport in solubilized systems. J. Pharm. Sci. 58, 1342–1352.

Hoarau, D., Delmas, P., David, S., Roux, E., Leroux, J.C., 2004. Novel long-circulating lipid nanocapsules. Pharm. Res. 21, 1783.

Izquierdo, P., Esquena, J., Tadros, T.F., Dederen, C., Garcia, M.J., Azemar, N., Solans, C., 2002. Formation and stability of nano-emulsions prepared using the phase inversion temperature method. Langmuir 18, 26–30.

Izquierdo, P., Esquena, J., Tadros, T.F., Dederen, J.C., Feng, J., Garcia-Celma, M.J., Azemar, N., Solans, C., 2004. Phase behavior and nano-emulsion formation by the phase inversion temperature method. Langmuir 20, 6594–6598.

Izquierdo, P., Feng, J., Esquena, J., Tadros, T.F., Dederen, J.C., Garcia-Celma, M.J., Azemar, N., Solans, C., 2005. The influence of surfactant mixing ratio on nano-emulsion formation by the PIT method. J. Colloid Interface Sci. 285, 388–394.

Kabalnov, A., Wennerström, H., 1996. Macroemulsions stability: the oriented Wedge theory revisited. Langmuir 12, 276–292.

Kahlweit, M., Strey, R., Firman, P., Haase, D., 1985. Phase behavior of ternary systems: water-oil-nonionic surfactant as a near-tricritical phenomenon. Langmuir 1, 281–288.

Kunieda, H., Fukui, H., Uchiyama, H., Solans, C., 1996. Spontaneous formation of highly concentrated water-in-oil emulsions (gel-emulsions). Langmuir 12, 2136–2140.

Lamprecht, A., Benoit, J.-P., 2006. Etoposide nanocarriers suppress glioma cell growth by intracellular drug delivery and simultaneous P-glycoprotein inhibition. J. Control. Release 112, 108–113.

Lamprecht, A., Bouligand, Y., Benoit, J.P., 2002. New lipid nanocapsules exhibit sustained release properties for amiodarone. J. Control. Release 84, 59–68.

Linder, P., Zemb, T., 2002. Neutrons, X-rays and light: scattering methods applied to soft condensed matter. Elsevier, Amterdam.

Malzert, A., Vrignaud, S., Saulnier, P., Lisowski, V., Benoit, J.P., Rault, S., 2006. Formulation of sustained release nanoparticles loaded with a tripentone, a new anticancer agent. Int. J. Pharm. 320, 157–164.

Miller, C.A., 1988. Spontaneous emulsification produced by diffusion Ů a review. Colloids Surf. 29, 89–102.

Minkov, I., Ivanova, Tz., Panaiotov, I., Proust, J., Verger, R., 2005a. Reorganization of lipid nanocapsules at air-water interface: 3. Action of hydrolytic enzymes HLL and pancreatic PLA2. Colloids Surf. B 45, 24–34.

Minkov, I., Ivanova, Tz., Panaiotov, I., Proust, J., Saulnier, P., 2005b. Reorganization of lipid nanocapsules at air-water interface. Part 2. Properties of the formed surface film. Colloids Surf. B 44, 197–203.

Minkov, I., Ivanova, T., Panaiotov, I., Proust, J.E., Saulnier, P., 2005c. Reorganization of lipid nanocapsules at airŰwater interface: I. Kinetics of surface film formation. Colloids Surf. B 45, 14–23.

Morales, D., Guiterrez, J.M., Garcia-Celma, M.J., Solans, C., 2003. A study of the relation between bicontinuous microemulsions and oil/water nanoemulsion formation. Langmuir 19, 7196–7200.

Pons, R., Carrera, I., Caelles, J., Rouch, J., Panizza, P., 2003. Formation and properties of mini-emulsions formed by microemulsions dilution. Adv. Colloid Interface Sci. 106, 129–146.

Pusey, P., van Megen, W., 1989. Dynamic light scattering by non-ergodic media. Physica A 157, 705.

Quintanar-Guerrero, D., Allemann, E., Fessi, H., Doelker, E., 1999. Pseudolatex preparation using a novel emulsion-diffusion process involving direct displacement of partially water-miscible solvents by distillation. Int. J. Pharm. 188, 155–164.

Rondon, M., 2006. Influence de la formulation pgysico-chimique et des propriétés interfaciales sur la stabilité des émulsions asphaltènes-eau-huile. Application à la déshydratation du pétrole. Ph.D. Thesis. University of Pau.

Rubin, E., Radke, C.J., 1980. Dynamic interfacial tension minima in finite systems. Chem. Eng. Sci. 35, 1129–1138.

Salager, J.L., 2000. Formulation Concepts for the Emulsion Maker. Marcel Dekker, New York, Chapter 2, pp. 19–72.

Salager, J.L., Anton, R., Anderez, J.M., Aubry, J.M., 2001. Formulation des microémulsions par la méthode du HLD. Techniques de l'ingénieur, Génie des Precédés J2, 1–20.

Salager, J.L., Forgiarini, A., Marquez, L., Pena, A., Pizzino, A., Rodriguez, M., Rondon-Gonzalez, M., 2004. Using emulsion inversion in industrial processes. Adv. Colloid Interface Sci. 108–109, 259–272.

Sandkühler, P., Sefcik, J., Morbidelli, M., 2004. Kinetics of aggregation and gel formation in concentrated polystyrene colloids. J. Phys. Chem. B 108, 20105–20121.

Saulnier, P., Benoit, J.P., 2006. Lipidic core nanocapsules as new drug delivery systems. In: Nanoparticulates as Drug Carriers. Imperial College Press, London, pp. 213–224.

Shinoda, K., Saito, H., 1968. The effect of temperature on the phase equilibria and the types of dispersions of the ternary system composed of water, cyclohexane, and nonionic surfactant. J. Colloid Interface Sci. 26, 70–74.

Shinoda, K., Saito, H., 1969. The stability of O/W type emulsions as a function of temperature and the HLB of emulsifiers: the emulsification by PIT-method. J. Colloid Interface Sci. 30, 258–263.

Sing, A., Graciaa, A., Lachaise, J., Brochette, P., Salager, J.L., 1999. Interactions and coalescence of nanodroplets in translucent O/W emulsions. Colloids Surf. A 152, 31–39.

Solans, C., Izquierdo, P., Nolla, J., Azemar, N., Garcia-Celma, M.J., 2005. Nano-emulsions. Curr. Opin. Colloid Interface Sci. 10, 102–110.

Taylor, P., 1998. Ostwald ripening in emulsions. Adv. Colloid Interface Sci. 75, 107–163.

Trotta, M., Gallarate, M., Pattarino, F., Morel, S., 2001. Emulsions containing partially water-miscible solvents for the preparation of drug nanosuspensions. J. Controlled Release 76, 119–128.

Yotsuyanagi, T., Higuchi, W.I., Ghanem, A.H., 1973. Theoretical treatment of diffusional transport into and through an oil-water emulsion with an interfacial barrier at the oil-water interface. J. Pharm. Sci. 62, 40–43.