UNIVERSITAT DE BARCELONA
FACULTAT DE FARMÀCIA
DEPARTAMENT DE FARMÀCIA I TECNOLOGIA FARMACÈUTICA

ESTUDI DE LA FORMACIÓ DE NANO-EMULSIONS DE FASE EXTERNA AQUOSA I SOLUBILITZACIÓ DE FÀRMACS LIPÒFILS

Núria Sadurní Gràcia, 2006

Available online at www.sciencedirect.com
SCIENCE DIRECT
European Journal of Pharmaceutical Sciences 26 (2005) 438–445
EUROPEAN JOURNAL OF PHARMACEUTICAL SCIENCES
www.elsevier.com/locate/ejps

Studies on the formation of O/W nano-emulsions, by low-energy emulsification methods, suitable for pharmaceutical applications

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Received 22 February 2005; received in revised form 4 July 2005; accepted 3 August 2005
Available online 8 September 2005

Abstract

The formation of O/W nano-emulsions suitable for pharmaceutical application and the solubilisation of a practically non-water-soluble drug, lidocaine, have been studied in water/non-ionic surfactant/oil systems. Nano-emulsions were prepared by using low-energy emulsification methods, changing the composition at constant temperature. Kinetic stability was assessed by measuring droplet diameter as a function of time. Lidocaine solubilisation was studied in nano-emulsions with high water content. In the water/Cremophor EL/Miglyol 812 system the lowest droplet sizes, from 14 to 39 nm at 10/90 and 40/60 oil/surfactant ratios, respectively, and 90% of water content, were obtained with an emulsification method consisting of stepwise addition of water to oil/surfactant mixtures at 70 °C. Nano-emulsions of this system showed high kinetic stability. Droplet diameters did not exceed 67 nm after a period of at least 7 months. The maximum lidocaine concentration solubilised in nano-emulsions of the water/Cremophor EL/Miglyol 812 system with 90 and 95% of water content was 3.5 and 2.1%, respectively. These values are within the therapeutic range of lidocaine.

Keywords: Nano-emulsions; Surfactants; Low-energy emulsification methods; Drug solubilisation

1. Introduction

Nano-emulsions are a type of emulsions with uniform and extremely small droplet size, in the range 20–200 nm (Solans et al., 2003). Due to their characteristic size, some nano-emulsions are optically transparent. These properties together with low viscosity, high kinetic stability against creaming or sedimentation and a large interfacial area (Buszello and Müller, 2000; Solans et al., 2003) make nano-emulsions of increasing use in many different applications (Solans et al., 2003). In the literature, this type of liquid/liquid dispersions are also referred to as submicron emulsions (Benita and Levy, 1993; Lundberg, 1997; Sznitowska et al., 2001), miniemulsions (Ugelstad et al., 1973; El-Aasser et al., 1984; El-Aasser and Miller, 1997), ultrafine emulsions (Nakajima et al., 1993; Nakajima, 1997), unstable microemulsions (Rosano et al., 1981), etc. The term nano-emulsion is increasingly used because it gives a clear idea of the nanoscale size range of the droplets, avoiding misinterpretations with other kinds of dispersions such as microemulsions, which are thermodynamically stable systems (Dinielsson and Lindman, 1981; Solans et al., 1997).

Nano-emulsions are non-equilibrium systems. Therefore, energy input, generally from mechanical devices or from the chemical potential of the components, is required for their formation (Walstra, 1983). The emulsification methods using mechanical energy, so-called dispersion or high-energy emulsification methods (such as high-pressure homogenisation), are extensively used in industry to obtain emulsions with small and uniform droplet size. The emulsification methods making use of the chemical energy stored in the

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doi:10.1016/j.ejps.2005.08.001

components, also named condensation, low-energy or "spontaneous" emulsification methods (Gopal, 1968) are receiving increased attention. In these methods, nano-emulsions are obtained as a result of phase transitions produced during the emulsification process which is carried out, generally, at constant temperature changing composition (Forgiarini et al., 2001; Usón et al., 2004) or at constant composition changing temperature (Izquierdo et al., 2002; Morales et al., 2003), the well-known PIT method (Shinoda and Saito, 1968). In practice, a combination of high-energy and low-energy emulsification methods has proved to be an efficient way to obtain nano-emulsions with small and very uniform droplets (Nakajima, 1997).

Nano-emulsions have experienced a growing interest as colloidal drug carriers for pharmaceutical applications. The use of nano-emulsions in topical administration of drugs is well documented (Friedman et al., 1995; Youenang Piemi et al., 1999; Fernandez et al., 2000). However, nano-emulsions reported in the literature are prepared by using high-energy emulsification methods as sonication, high-shear mixing or high-pressure homogenisation to form them (Walstra, 1996; Floury et al., 2003; Landfester et al., 2004). Drug penetration was reported to be strongly enhanced by solubilisation in small droplets (below 0.2 μm) (Schwarz et al., 1995). In this context, it has also been reported that when using oil-in-water emulsions as vehicles, the pharmacological activity is correlated to emulsion droplet size (Amselem and Friedman, 1998). Nano-emulsions are also used as ocular delivery systems to provide a reservoir for sustained release of drugs (Sznitowska et al., 1999). Oil-in-water nano-emulsions can be administered orally to increase the bioavailability of poorly water-soluble drugs (Wagner et al., 1966; Bates and Carrigan, 1975) due to an enhancement of the intestinal absorption of the drug (Constantinides, 1995). It has been also found that the absorption in the gastrointestinal tract is improved by a small droplet size (Toguchi et al., 1990). They are also used as intravenous delivery systems for the administration of lipids in parenteral nutrition and for their capacity to incorporate water non-soluble drugs directly to the veins (Lundberg, 1997; Mbela et al., 1998; Jumaa and Muller, 1998). Although, as it has been showed, there are several studies related to nano-emulsion applications in the pharmaceutical field, further effort is required in order to fully understand the mechanisms directly implied in nano-emulsion formation and stability and, therefore, optimise nano-emulsification processes.

The objectives of this work have been to study nano-emulsion formation with biocompatible components using low-energy emulsification methods and the solubilisation of a model non-water-soluble drug, lidocaine, a local anaesthetic of the amide type. For this purpose a water/polyoxyethyleneglycol castor oil derivative surfactant/medium-chain triglyceride system was chosen and its phase behaviour was determinated prior to study nano-emulsion formation and lidocaine solubilisation.

2. Materials and methods

2.1. Materials

Non-ionic surfactants, Cremophor EL and Solutol HS 15, supplied by BASF, were used. Cremophor EL is obtained by reacting castor oil with ethylene oxide in a molar ratio of 1:35. Its hydrophilic–lipophilic balance (HLB) lies between 12 and 14 (BASF Corporation, Technical literature). Solutol HS 15, whose HLB lies between 14 and 16, consists of polyglycol esters of the 12-hydroxystearic acid and free polyethylene glycols (BASF Corporation, Technical literature). The oils used, Miglyol 812, a medium-chain triglyceride was purchased from ROIG FARMA, and castor and soybean oils were purchased from SIGMA. Water was deionised by Milli-Q filtration. Lidocaine (2-diethylamino-*N*-[2,6-dimethylphenyl]-acetamide) was purchased from SIGMA.

ortho-Phosphoric acid 85%, acetonitrile and methanol, purchased from Merck, and triethanolamine, purchased from Fluka, were the analytical products used for HPLC analysis.

2.2. Methods

2.2.1. Phase diagrams

The phase diagram was determined at 25 °C by stepwise addition of one component to the mixture of the other components and homogenized with a vibromixer. After each addition the samples were stirred, homogenized and kept at 25 °C. The occurrence of phase separation was observed within a period of 48 h. To better define the phase boundaries some samples were prepared by weighting all components in ampoules, which were sealed, homogenized with a vibromixer and kept at 25 °C to equilibrate. Liquid crystalline phases were identified by using crossed polarisers and by optical microscopy (Reichter Polyvar 2, Leica) under polarized light.

2.2.2. Nano-emulsion formation

Nano-emulsions were prepared by addition of water to mixtures of the other two components (oil and surfactant), using a magnetic stirrer at approximately 2000 rpm. Independently of the temperature at which they were prepared, the samples were kept at 25 °C.

2.2.3. Small-angle X-ray scattering (SAXS)

Small-angle X-ray scattering equipment consisted of a Siemmens K-760 generator, Cu Kα anode (λ = 1.542 Å). The collimation was carried out with a Kratky camera and the scattering was detected with a linear position sensitive detector, OED 50 M, both from Heccus M. Braun. The temperature was regulated with an AP Paar K-PR temperature controller. Samples were placed in a sample holder for pastes between kallebrat films. Liquid crystalline phases were characterised by the SAXS peak ratios when plotting intensity as a function of the scattering vector ($q = 4\pi/\lambda \sin(\theta/2)$), which is the difference between the incident wave vector and the scattered one

to an angle θ. The interlayer spacing, d, of liquid crystalline phases was determined by the Bragg equation ($d = 2\pi/q$).

2.2.4. Dynamic light scattering (DLS)

The mean droplet sizes of nano-emulsions were determined using a Malvern 4700 photon correlation spectrometer (Malvern Instruments, Malvern, UK). An argon laser ($\lambda = 488$ nm) with variable intensity was used. Measurements were always carried out at a scattering angle of 90°. The DLS data was analysed by the cumulants method obtaining the z-average mean of nano-emulsion droplet diameter and the polydispersity index, which is a dimensionless measure of the width of size distribution, and the CONTIN method. Polydispersity indexes lower than 0.2 indicate that polydispersity maintains its significance as an accurate measure.

2.2.5. Drug solubilisation

Lidocaine was incorporated to oil/surfactant mixtures prior to the addition of water to form the nano-emulsions. The samples were first homogenized with a vibromixer, then, placed in an ultrasound bath for 15 min and they were finally kept in a water bath at 25 °C. They were examined by visual and optical observation after, at least, 12 h of preparation. The appearance of crystals observed by optical microscopy indicated that the maximum concentration of solubilised drug had been exceeded. The concentration of solubilised drug was determined by HPLC.

2.2.6. HPLC

The concentration of lidocaine solubilised was determined using a Waters LC-Module I instrument, operated at ambient temperature, consisting of an automatic autosampler system equipped with a 200 μl loop injection valve, a variable wavelength UV–vis detector and a Spherisorb ODS 2 (150 mm × 4.6 mm, 5 μm). The mobile phase consisted in a mixture of acetonitrile/methanol/triethanolamine/85% *ortho*-phosphoric acid 200/150/3/2 up to 1000 ml of water, adding 85% *ortho*-phosphoric acid until pH 2.5. The flow rate was 1 ml/min, UV detector 205 nm, injection volume was 10 μl, the retention time was 2.75 min, and calibration curves, 30–1025 μg/ml, were constructed from linear plots of peak area versus concentration. The calibration was carried out by diluting lidocaine in methanol. It was determined experimentally that lidocaine is very soluble in methanol (>50 wt.%).

3. Results and discussion

Nano-emulsion formation was achieved in different water/non-ionic surfactant/oil systems. Phase behaviour of the water/Cremophor EL/Miglyol 812 system at 25 °C, nano-emulsion formation and properties in different systems, and solubilisation of lidocaine in selected compositions are described.

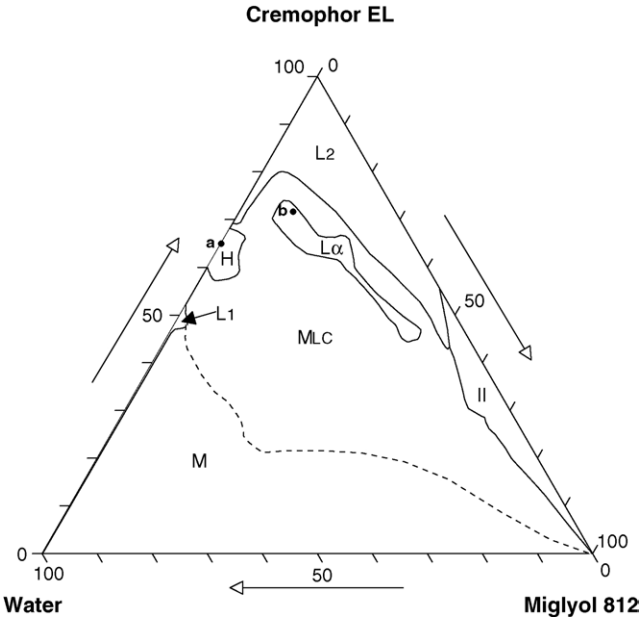

Fig. 1. Pseudoternary phase diagram of the water/Cremophor EL/Miglyol 812 system, at 25 °C. L_1: isotropic liquid phase (direct micellar solution or O/W microemulsion); L_2: isotropic liquid phase (reverse micellar solution or W/O microemulsion); II: two isotropic liquid phases; L_α: anisotropic phase (lamellar liquid crystalline phase); H: anisotropic phase (inverse hexagonal liquid crystalline phase); M_{LC}: anisotropic multiphase region (equilibrium not determined); M: isotropic multiphase region (equilibrium not determined). a and b: samples which SAXS spectra are shown in Fig. 2.

3.1. Phase behaviour

The phase diagram of the water/Cremophor EL/Miglyol 812, at 25 °C, is shown in Fig. 1. Four distinct one-phase regions are observed, L_1, L_2, H and L_α. L_1 and L_2 are isotropic, liquid colourless phases. L_1 region extending along the water/Cremophor EL axis, from a water/surfactant weight ratio of 48/52 to the water vertex consists of aqueous micellar solutions or O/W microemulsions. The maximum oil solubilised is 2 wt.% at a 51/49 water/surfactant weight ratio. The L_2 region, which extends along the Miglyol 812/Cremophor EL axis up to an oil/surfactant weight ratio of 45/55, corresponds to reverse micelles or W/O microemulsions. The maximum water solubilised in this region is 31 wt.%. The other monophasic regions, H and L_α, are anisotropic. H, a hexagonal liquid crystalline phase, extends along the water/Cremophor EL axis, from water/surfactant weight ratios of 32/68 to 40/60. The maximum oil content solubilised in this region is 8 wt.%. L_α region, which corresponds to lamellar liquid crystalline structures, extends from an oil/surfactant weight ratio of 9/91 to 50/50 in a limited range of water concentration between 12 and 23 wt.%. The rest of the diagram consists of: (1) a two-liquid phase region (II), which appears along the oil/surfactant axis, from an oil/surfactant weight ratio of 45/55 to the oil vertex; and (2) a multiphase region occupying most of the diagram, which has been divided in two subregions, M_{LC}, with liquid crystalline phases present, and M, which phase equilibria consist of liquid phases.

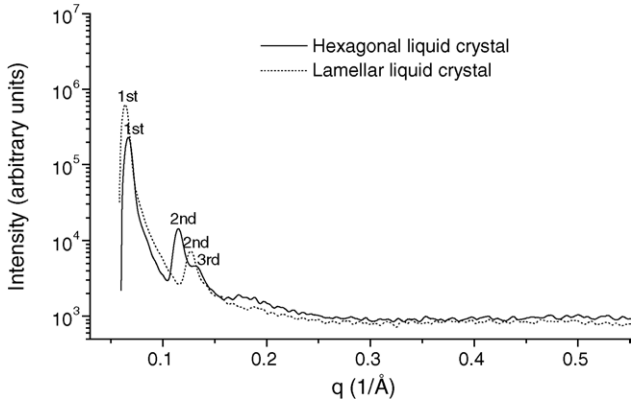

Fig. 2. Intensity vs. scattering vector, q, at 25 °C of samples belonging to region H (—) and L_α (. . .). The compositions of the samples are: (a) 35% water and 65% surfactant; (b) 18% water, 72% surfactant and 10% oil.

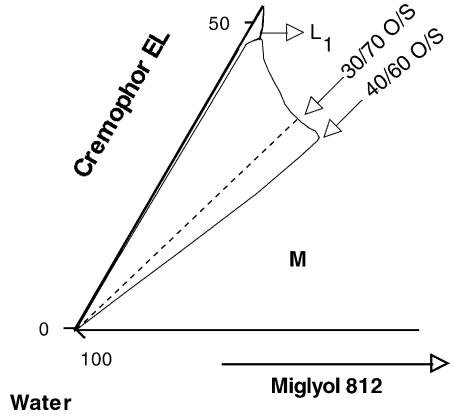

Fig. 3. Nano-emulsion region formed by using emulsification methods A (up to 30/70 O/S) and B (up to 40/60 O/S); O/S = oil/surfactant ratio.

The type of liquid crystal structures could not be identified by polarized optical microscopy because of their fragility. They were characterised by SAXS determinations, at 25 °C. As an example, Fig. 2 shows the spectra corresponding to samples (a) and (b) of region H and L_α, respectively (indicated in Fig. 1). For sample (a) the ratios of interlayer spacings from first, second and third peaks are $1:1/\sqrt{3}:1/2$ which correspond to a hexagonal structure (Larsson, 1976) and for sample (b) the ratios from first and second peaks are 1:1/2 which agree with a lamellar structure (1:1/2:1/3) (Larsson, 1976). The interlayer spacings, d, measured were 9.5 and 9.8 nm for samples (a) and (b), respectively.

3.2. Nano-emulsion formation and properties

Nano-emulsions were prepared in the water/Cremophor EL/Miglyol 812 system by addition of water to oil/surfactant mixtures, previously homogenised. First, they were prepared at 25 °C and water was added stepwise (method A) or at once (method B).

Transparent or transparent-bluish liquid dispersions appeared after addition of approximately 50 wt.% of water for oil/surfactant (O/S) ratios from 1/99 to 30/70 in method A and up to 40/60 in method B (as shown in Fig. 3). These transparent colloidal dispersions are not microemulsions but nano-emulsions because they are formed in a multiphase region, M (Fig. 1). Moreover, when samples were prepared by mixing all the components at the final composition or by adding oil to water/surfactant mixtures they showed a milky appearance.

To find out if nano-emulsions could be formed in this system at higher oil/surfactant ratios, the samples were prepared at higher temperatures. Table 1 summarizes the methods studied and the maximum O/S ratios at which nano-emulsions could be formed.

Nano-emulsion region could not be extended at O/S ratios higher than 40/60 in any of the methods studied. At 50 °C, the extension of nano-emulsion formation was as that at 25 °C. At both temperatures the maximum O/S ratio at which they formed was 40/60 when water was added at once and 30/70 when it was added stepwise. However, at 70 °C, nano-emulsions with O/S ratio up to 40/60 could also be obtained by stepwise addition of water. The most striking result was that the maximum O/S ratio reached, 40/60, was obtained when water was added at once, independently of the temperature of preparation (methods B, D and F), which is of the outmost interest for practical applications. Provided that surfactant and oil are previously mixed nano-emulsions are formed "spontaneously" in this system.

In previous studies formation of nano-emulsions by low-energy methods has been related to phase transitions during the emulsification process involving lamellar liquid crystalline phases (Forgiarini et al., 2001; Izquierdo et al., 2002). The properties of the lamellar liquid crystalline phase were studied in the water/Cremophor EL/Miglyol 812 system by SAXS determinations, at 25, 50 and 70 °C. Although the phase behaviour of the system was only determined at 25 °C, some preliminary phase behaviour studies as a function of temperature confirmed the presence of lamellar liquid crystalline phases in the system at 50 and 70 °C. The interlayer spacings, measured at 25 °C, of samples with different O/S ratios and water concentrations between 26 and 32% were of the order of 17 nm. These values are higher than those reported for systems with polyoxyethylene non-ionic surfactant with similar degree of ethoxylation as that of Cremophor EL (Kunieda et al., 1999) which are of the order of 9 nm. Large interlayer spacings imply high flexibility of the lamellar structure, which could disorder easily on addition of water leading to the formation of nano-droplets. This would explain the "spontaneous" nano-emulsification that takes place in this system.

Droplet diameter was determinated in nano-emulsions with 90 wt.% of water after preparation, at 25 °C. Samples were prepared according to the methods described in Table 1. The results are plotted as a function of oil/surfactant ratio in Fig. 4.

For all methods studied, nano-emulsion droplet size increases with the increase of oil/surfactant ratio. This fact

Table 1
Maximum O/S ratios of nano-emulsion formation with different methods (O: oil; S: surfactant)

Method	Water addition	Temperature (°C)		Maximum O/S ratio of nano-emulsion formation
		Preparation	Storage	
A	Stepwise	25	25	30/70
B	At once			40/60
C	Stepwise	50	25	30/70
D	At once			40/60
E	Stepwise	70	25	40/60
F	At once			40/60

suggests that the oil constitutes the inner structure of the nano-droplets, which is consistent with a direct O/W-type structure (Attwood and Ktistis, 1989). Droplet diameters ranged from 14 nm (lowest O/S ratio) to 51 nm (highest O/S ratio). It was found that polydispersity indexes also increase with the increase in O/S ratio. It is noteworthy that for low O/S ratios, nano-emulsion droplet diameter is independent of the method of preparation. However, at high O/S ratios droplet size decreases with the increase in the temperature of preparation. Although the maximum O/S ratio, 40/60, was obtained by methods B, D, E and F, the lowest droplet diameter (38.9 nm) and polydispersity index were obtained with method E.

Nano-emulsion formation was also studied by emulsifying with method E in water/Cremophor EL/castor oil, water/Solutol HS 15/Miglyol 812 and water/Solutol HS15/soybean oil systems, which are suitable for pharmaceutical purposes. Transparent or transparent-bluish liquid dispersions corresponding to nano-emulsions appeared after addition of water to mixtures of oil and surfactant from the ratio O/S 1/99 to 40/60 in the water/Solutol HS 15/Miglyol 812 and from 1/99 to 20/80 in the water/Cremophor EL/castor oil and water/Solutol HS 15/soybean oil systems.

Nano-emulsion droplet diameter was determined in the different systems. The results obtained for nano-emulsions with 90 wt.% of water are indicated in Fig. 5. Droplet size increases with the increase of O/S ratio. These results are similar to those of the water/Cremophor EL/Miglyol 812 system, plotted in Fig. 4. Nano-emulsion droplet diameters are bigger than those measured in the water/Cremophor EL/Miglyol 812 system. However, they are lower than 45 nm, which is the droplet size corresponding to the nano-emulsion of the water/Solutol HS 15/Miglyol 812 system, with a 40/60 O/S ratio. It should be noticed that nano-emulsion droplet sizes of water/non-ionic surfactant/oil systems studied are exceptionally small compared to those reported in the literature (Bouchemal et al., 2004).

Nano-emulsion stability was determined by measuring droplet size as a function of time, at 25 °C. Nano-emulsions prepared by method E were selected for this study because this method produces the lowest droplet size and polydispersity index at all O/S ratios. The stability results obtained for nano-emulsions of the water/Cremophor EL/Miglyol 812 system with 90 and 95 wt.% of water are plotted in Fig. 6.

In nano-emulsions with an oil/surfactant ratio of 10/90, no significative changes were observed in droplet size within the period studied (230 days for those with 90 wt.% and up to 300 days for those with 95 wt.% of water). As mentioned above, if the samples were prepared by changing the order of addition of the components they showed a milky appearance, an indication that the colloidal dispersion was not thermodynamically stable. For higher O/S ratios than 10/90, nano-emulsion droplet diameters and polydispersity indexes increase with time. However, droplet size did not exceed 67 nm during at least 7 months. In addition, no phase separa-

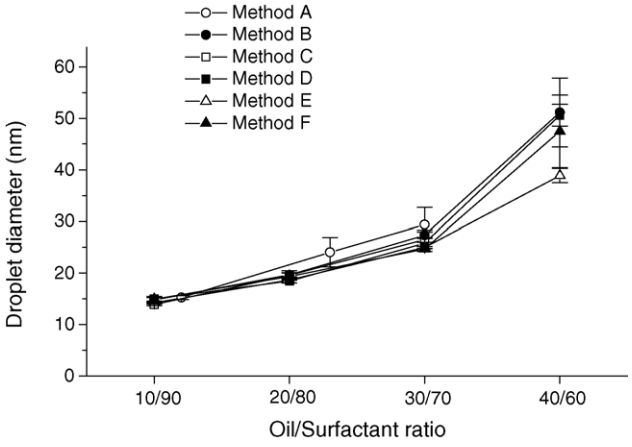

Fig. 4. Droplet diameter of nano-emulsions at 90 wt.% of water content as a function of O/S ratio, at 25 °C. The methods A–F are described in Table 1.

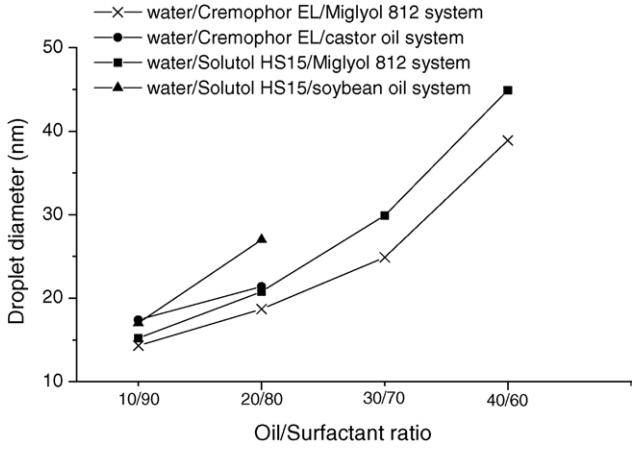

Fig. 5. Droplet diameter of nano-emulsions prepared by method E as a function of O/S ratio with 90 wt.% of water content, at 25 °C.

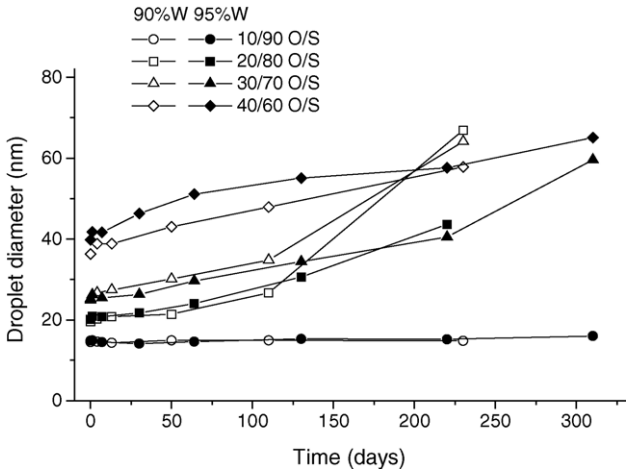

Fig. 6. Droplet diameter of nano-emulsions of the water/Cremophor EL/Miglyol 812 system prepared by method E as a function of time with 90 wt.% (empty symbols) and 95% (filled symbols) of water content, at 25 °C.

tion took place in this period of time. It should be pointed out that neither freeze–thaw cycles (from −18 to 25 °C) nor ultra-centrifugation at 25 °C (25 h, 22,912 × *g*) produced complete phase separation of these nano-emulsions.

Due to the small droplet size nano-emulsions are stable against creaming or sedimentation. However, irreversible destabilization can occur by Ostwald ripening and/or coalescence mechanisms. It has been reported that the main nano-emulsion destabilization mechanism is Ostwald ripening (Taylor and Ottewill, 1994): when nano-emulsions are polydisperse there are solubility differences between small and large droplets and diffusion of the dispersed phase through the continuous phase occurs. Then, large droplets grow at the expense of smaller ones. The LSW theory (Lifshitz and Slyozov, 1961; Wagner, 1961) gives an equation for the Ostwald ripening rate, W, which depends on r (the droplet radius at a given time, t), $C(\infty)$ (the solubility of a droplet with a infinite radius), V_m (the molar volume of the oil), D (the diffusion coefficient of the dispersed in the continuous phases), ρ (the density of the oil), and R (the gas constant) and T (the absolute temperature). The equation predicts a linear variation of r^3 with time. Coalescence (Walstra, 1996) is produced when two or more emulsion droplets fuse to form a unique larger one. Deminiere studies (Deminiere et al., 1998) predict a linear relationship of $1/r^2$ with time. To know if the mechanisms of destabilization were Ostwald ripening or coalescence, r^3 and $1/r^2$ were plotted versus time, respectively. Contrary to what the theories predict no linear variation was obtained, an indication that none of these mechanisms is predominant and maybe both breakdown processes occur simultaneously in this system.

Nano-emulsion stability was also studied for water/Cremophor EL/castor oil, water/Solutol HS 15/Miglyol 812 and water/Solutol HS 15/soybean oil systems up to 130 days. During this period of time nano-emulsion droplet diameter was lower than 53 nm.

3.3. Solubilisation

The solubilisation studies were carried out by incorporation of the drug to oil/surfactant mixtures prior to nano-emulsion formation in the water/Cremophor EL/Miglyol 812 system, as described in Section 2.2.5. Method E (stepwise addition of water at 70 °C) was not considered appropriate to prepare nano-emulsions for solubilisation studies because lidocaine suffers a slow degradation to 2,6-dimethylaniline and *N*,*N*-diethylaminoacetic acid already at 80 °C (Gröningsson et al., 1985; Powell, 1987). Instead, method D (addition of water at once at 50 °C) was selected for this purpose because those nano-emulsions showed lower polydispersity indexes than those prepared at 25 °C.

The results of maximum solubilisation of lidocaine at 90 and 95 wt.% of water as a function of O/S ratios are shown in Fig. 7. For comparative purposes, lidocaine solubilisation was also studied in samples without oil (O/S = 0/100), which correspond to micellar solutions.

The maximum concentration of lidocaine solubilised increases when oil/surfactant ratio increases at both water concentrations. Moreover, independently of oil/surfactant ratio, nano-emulsions with the higher water content showed lower maximum lidocaine solubilisation, as expected for a practically water-insoluble drug. The maximum lidocaine solubilisation values were 3.54 and 2.11 wt.%, for nano-emulsions with 90 and 95 wt.% of water content, respectively, and O/S ratios of 40/60. The theoretical values of lidocaine solubilisation were 2.71 and 1.35 wt.% for the corresponding samples. These values were calculated taking into account the solubility of lidocaine in nano-emulsion components, which were determined experimentally, at 25 °C. Lidocaine is practically insoluble in water (0.4 wt.%), and freely soluble in Cremophor EL (24.7 wt.%) and Miglyol 812 (30.5 wt.%). The higher experimental values of solubilisation as compared to the theoretical ones clearly show that the increase in interfacial area provided by the nanodroplets allows higher solubilisation (Florence, 1981). It is noteworthy that nano-emulsions with 90 wt.% and some with 95 wt.% of water

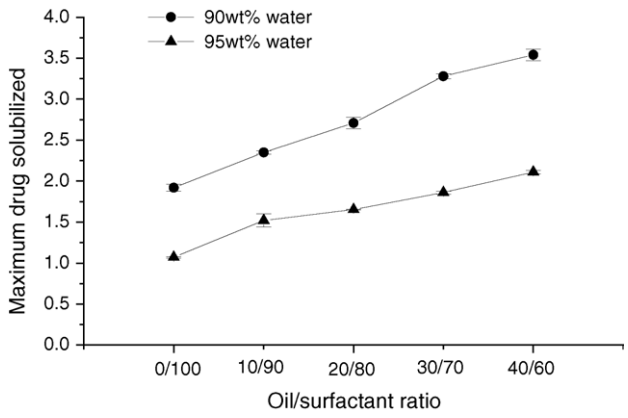

Fig. 7. Maximum concentration of lidocaine solubilised in nano-emulsions as a function of O/S ratio, at 25 °C.

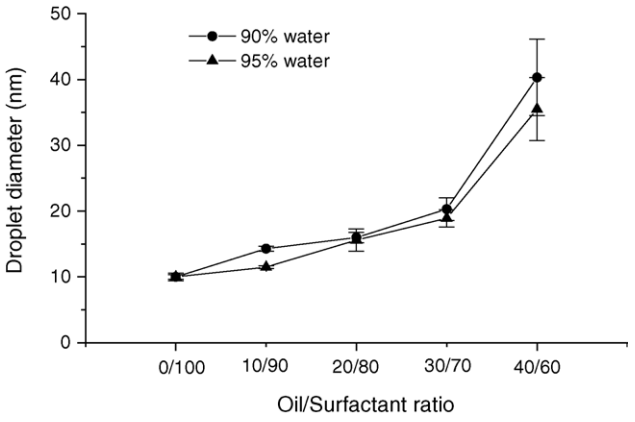

Fig. 8. Droplet diameter of nano-emulsion with 90 and 95 wt.% of water content as a function of O/S ratio, with 1 wt.% of lidocaine solubilised, at 25 °C.

content solubilised the therapeutic concentration of lidocaine to be applied in surface anaesthesia, which is in the range 2–10 wt.%.

It was considered of interest to determine the possible effect of lidocaine on nano-emulsion droplet size and stability. For this study, the concentration of lidocaine solubilised was kept constant (1 wt.%) at all O/S ratios.

The variation of nano-emulsion droplet diameter as a function of time was the same as without drug solubilised, during the experimental time of the study (1 month). Droplet size variation with the increase of O/S ratio (Fig. 8) also follows the same trend as without drug incorporated (Fig. 4). The values are slightly lower than those without lidocaine solubilised (Fig. 4). It should be noted that at high O/S ratios the difference in size is enhanced. It has been reported that solubilisation of 0.5% of lidocaine in O/W emulsions produce a slight decrease in droplet size (from 400 to 390 nm) (Gopal and Bhogi, 1999). In contrast, some authors have described an increase in droplet size when lidocaine was solubilised in propofol emulsions (Park et al., 2003). However, it is not clear if the increase in size reported was due to emulsion destabilization. The cause of the decrease in size with lidocaine incorporation could be attributed to the amphiphilic behaviour of the drug which chemical structure is composed of a hydrocarbon chain, an aromatic ring and an amide group.

In conclusion, O/W nano-emulsions with extremely small droplet sizes have been formed in water/non-ionic surfactant/oil systems suitable for pharmaceutical applications by using low-energy emulsification methods. In the water/Cremophor EL/Miglyol 812 system, the lowest droplet sizes and polydispersity indexes were obtained by the emulsification method consisting of stepwise addition of water at 70 °C. At 90 wt.% of water content, nano-emulsion droplet diameter ranged from 14 up to 39 nm for O/S ratios between 10/90 and 40/60, respectively. Surfactant and oil homogenisation prior addition of water to oil/surfactant mixtures and phase transitions during emulsification involving lamellar liquid crystal structures seem to be key factors for nano-emulsion formation in this system. By adding water stepwise at 70 °C, nano-emulsion formation was also achieved in different systems suitable for pharmaceutical applications, proving to be a good method of nano-emulsification in water/non-ionic surfactant/oil systems.

Nano-emulsions of the water/Cremophor EL/Miglyol 812 system show high kinetic stability: no phase separation occurred during the experimental time of at least 7 months. Moreover, droplet size of nano-emulsions with an oil/surfactant ratio of 10/90 was not changed within this period of time. At higher oil/surfactant ratios, although nano-emulsion droplet size increases with time, droplet diameter did not exceed 67 nm in the same period of time.

Solubilisation of therapeutic concentrations of a practically non-water-soluble drug, lidocaine, was achieved in O/W nano-emulsions with 90 wt.% of water content at O/S ratios from 10/90 to 40/60 and with 95 wt.% of water at 10/90 O/S ratio. The variation of droplet size with time of nano-emulsions with 1% of lidocaine was of the same order as without lidocaine. Considering the capacity of solubilisation of the system and the stability results, they can be considered as promising drug delivery systems, for topical and oral administration.

Acknowledgments

The authors acknowledge financial support by the Spanish Ministry of Science and Technology, DGI (Grant PPQ2002-04514-C03-03) and Generalitat de catalunya, DURSI (Grant 2001SGR-00357). N.S. also acknowledges the financial support by the “Fondo Social Europeo”, under a CSIC grant (Program I3P).

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