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Nanoencapsulation of a crystalline drug

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Abstract

The aim of this work was to assess the influence of various formulation parameters on the incorporation of a poorly water-soluble crystalline drug into nanoparticles. For this purpose, the influence of the polymer (polylactic acid, polysebacic acid terminated with lithocholic acid, and polysebacic acid-co-lithocholic acid) as well as the effect of the dispersion medium (aqueous phases at different temperatures, saline medium and ethanol) on the encapsulation was investigated. ^{3}H-labelled drug was used in order to determine the loading efficiency by liquid scintillation counting. The solubility of the drug in the various polymer materials was assessed by differential scanning calorimetry (DSC). The solubility of the drug in the different dispersion media was then determined by gas chromatographic–mass spectrometric measurements. The highest loading ratios were obtained using poly (lactic acid) (PLA). However, the drug solubility in the polymers, determined by DSC analysis, cannot be considered as predictive for encapsulation efficiency. The study of the influence of the liquid outer phase showed that the encapsulation efficiency increased when the drug solubility in the dispersion medium (before acetone evaporation) decreased. These experiments made it possible to propose a mechanism to account for the leakage of the crystalline drug during the nanoprecipitation process. So, when acetone is eliminated by evaporation, the drug solubility in the dispersion medium decreases, leading to the formation of crystals. During nanoparticles storage, the crystals continue to grow, the nanoparticles serving as drug reservoirs. These findings highlight the importance of using a polymer with a specific affinity for the drug, and a dispersion medium with the lowest drug solubility to achieve an efficient encapsulation of a crystalline drug.

Keywords: Nanoparticles; Poorly water-soluble crystalline drug; PLA; Polyanhydrides; DSC

A large number of pharmaceutical substances administered by the oral route are crystalline. In many cases, polymorphism might cause several problems related to drug bioavailability and stability (Clas,

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2003). Efforts have been made towards the formulation of crystalline drugs to achieve preselected and desired properties (Lin et al., 1999; Nikolakakis et al., 2000). Because of their poor bioavailability, several crystalline drugs have to be administered by the intravenous route. Nanoparticles have shown their ability to entrap and protect drug (Alonso, 1995). These vehicles are small enough not to embolize the smallest capillaries. Additionally, when their surface is conveniently modified to avoid capture by the mononuclear phagocytic system, controlled release into the vascular system or targeting of specific tissues or organs can be achieved (Gref, 2003). However, to our knowledge, only a few examples in the literature deal with the entrapment of crystalline drugs within nanoparticles and the entrapment efficiencies are in those few cases very poor (Brigger et al., 2001; Dong and Feng, 2004).

The aim of this work was to assess the influence of various parameters on the incorporation of a poorly water-soluble crystalline drug into nanoparticles. The parameters investigated were the nature of the polymeric phase and the nature of dispersion medium. We have tried here to establish a relationship between the efficiency of the incorporation of a crystalline drug into the nanoparticles and its solubility in the polymers as well as in the dispersion medium.

The polymers evaluated were poly(lactic acid) (PLA) with an average weight molecular weight (Mw) of 50,000 Da (Phusis, Saint-Ismier, France), poly(sebacic) acid terminated with lithocholic acid (p (SA)-term-LA)) with Mw = 6500 Da, and poly(sebacic acid-co-lithocholic acid) (p (SA-co-LA)) with Mw = 30,600 Da (Krasko et al., 2002). The dispersion media evaluated were ethanol, milliQ water at 20 and 2 °C, potassium chloride (0.1 M) and sodium sulphate (0.1 M).

The nanoparticles were produced by the nanoprecipitation process, as described elsewhere (Fessi and Devissaguet, 1992). The formulations were prepared as follows: 20 mg of polymer and 2 mg of drug were dissolved in acetone (1 ml), which was injected under magnetic stirring (1250 rpm) into an aqueous or ethanolic dispersion medium (2 ml) containing one of the following compounds: water at 20 or 2 °C, KCl, Na_2SO_4 and ethanol. Acetone was eliminated using a rotative evaporator (Rotavapor®) at room temperature. The suspensions were purified by centrifugation (5 min at 630 × *g*), prefiltration (Acrodisc, Gelman Laboratories, Glass fiber membrane, 1 μm), and finally filtration (Millex®-HV, Millipore, 0.45 μm) in order to eliminate drug crystals, which might form during acetone evaporation step. The nanoparticles were collected by centrifugation (at 30,000 × *g* for 30 min). The loading efficiency was determined by liquid scintillation using tritium-labelled drug (1.6 *Ci/mg drug) (RC TRITEC, Teufen, Switzerland) and expressed as the amount of the drug in nanoparticles divided by the weight of nanoparticles collected (Eq. (1)):

$$\text{Loading efficiency} = \frac{\text{amount of drug in nanoparticles}}{\text{weight of nanoparticles}} \times 100 \quad (1)$$

The Z-Average mean particle diameters were determined by laser light scattering using a nanosizer (Coulter® N4MD, Coulter Electronics, Margency, France). The drug solubility in the different polymers was determined using differential scanning calorimetry (DSC) (Theeuwes et al., 1974). For this, pure polymer films and polymer films containing the drug in variable amounts were cast from acetone. The polymer (20 mg) and the drug (in variable amounts) were dissolved in acetone (500 μl), and all acetone was then evaporated under atmospheric pressure at room temperature over 24 h. Accurately weighed samples of about 5 mg were analysed in aluminium pans (40 μl) on a differential scanning calorimeter (DSC7, Perkin Elmer, USA). The DSC runs were conducted over a temperature range of 0 at 130 °C at a rate of 10 °C/min. The so-called "acetone-dispersion medium" refers to the dispersion medium of the nanoparticles containing water or ethanol and acetone (1:2 v/v). This solution corresponds to the dispersion medium obtained immediately after pouring the medium containing the polymer and the drug into the aqueous or ethanolic medium, before acetone evaporation. In fact, the solubility of the drug was determined in this "acetone-dispersion medium" at room temperature. Equilibrium was reached within 4 h. The samples were purified by centrifugation (10 min at 16,100 × *g*), diluted by an appropriate factor, and assessed by GC–MS (GC: Hewlett Packard/HP5890 series II and detector: Hewlett Packard/MSD 5971A, Mass selective detector).

The values of the mean nanoparticle diameter and the loading efficiencies measured are summarized

Table 1
Z-Average mean diameters and loading efficiencies of nanoparticles (NP) dispersed in water at 20 °C

Polymers	Mean diameter ± S.D. (nm)			Loading efficiency ± S.D. (% mg drug/mg NP)
	Drug free-NP	Drug-loaded NP before purification	Drug-loaded NP after purification	
PLA[a]	186 ± 55	175 ± 47	162 ± 37	1.04 ± 0.07
p(SA-co-LA)[b]	187 ± 68	195 ± 36	184 ± 40	0.48 ± 0.22
p(SA) term LA[a]	170 ± 56	181 ± 36	174 ± 26	0.39 ± 0.14

[a] $n = 6$.
[b] $n = 5$.

in Table 1. All nanoparticles had a mean diameter lower than 200 nm, which is a relevant size for intravenous administration. The drug loading and the purification stage did not modify the nanoparticle size. The highest loading ratio was obtained using PLA (Table 1).

The drug solubility in the different polymers was determined using DSC. Fig. 1A shows the thermograms

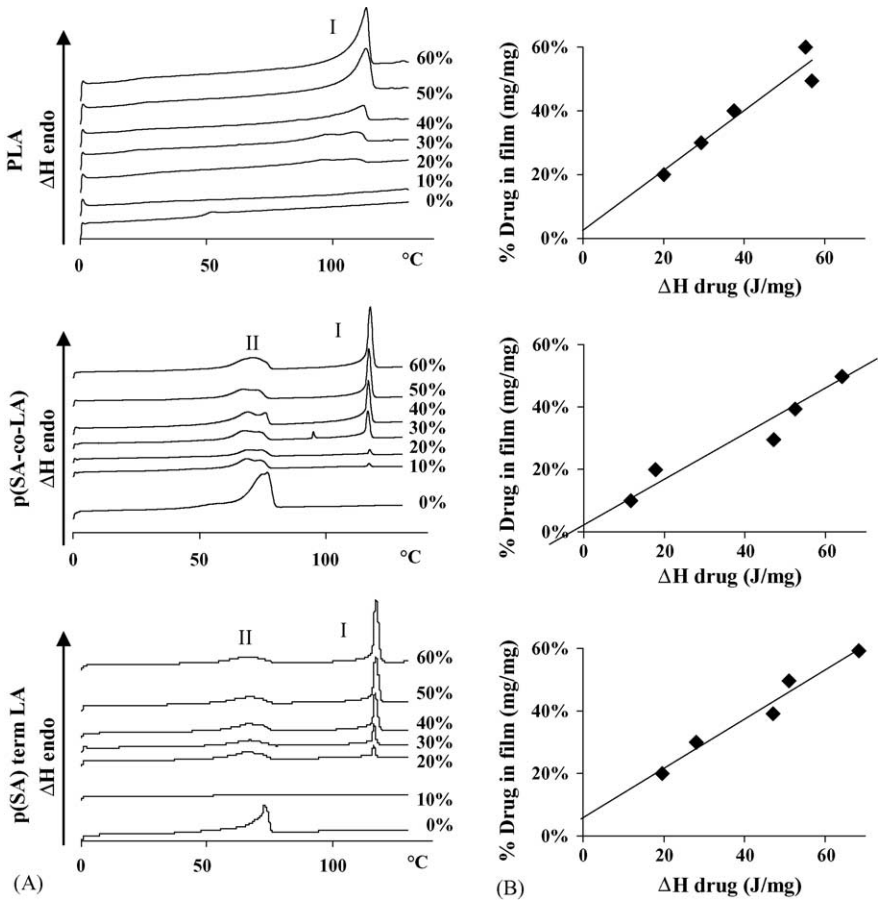

Fig. 1. (A) Differential scanning calorimetry (DSC) thermograms of polymer films loaded with the drug in variable amounts. (I) Fusion peak of the crystalline drug; (II) fusion peak of the polymers. (B) Relationship between drug concentration in the film samples and the heat of melting of the incorporated drug as determined by DSC.

Table 2
PLA nanoparticles Z-Average mean diameters and loading efficiencies

Dispersion medium	Mean diameter ± S.D. (nm)			Loading efficiency ± S.D. (% mg drug/mg NP)
	Drug free-NP	Drug-loaded NP before purification	Drug-loaded NP after purification	
Ethanol	132 ± 21	140 ± 49	142 ± 45	0.49 ± 0.13
KCl (0.1M)[b]	239 ± 49	257 ± 55	257 ± 55	0.88 ± 0.03
H_2O at 20 °C[a]	186 ± 55	175 ± 47	162 ± 37	1.04 ± 0.07
H_2O at 2 °C[a]	154 ± 33	172 ± 34	162 ± 35	1.10 ± 0.11
Na_2SO_4 (0.1M)[b]	166 ± 46	195 ± 38	214 ± 39	1.39 ± 0.24

[a] $n = 6$.
[b] $n = 4$.

of the polymer films loaded with variable amounts of the crystalline drug up to 60% (w/w). In these thermograms, polymer fusion peaks (II) appeared around 60 °C for the two polyanhydrides; therefore, these polymers were semi-crystalline. In all cases, the drug fusion peak (I) appeared around 115 °C. The diagrams in Fig. 1B were obtained by plotting the heats of melting of the drug against the concentration of drug into the films. The intercept of the lines correspond to the drug solubility in the polymer according to (Theeuwes et al., 1974). Therefore, the drug solubility was 2.5% (w/w) in PLA, 2.2% (w/w) in p(SA-co-LA), and 7%(w/w) in p(SA)-term-LA. Drug solubility in the different polymers was compared with the loading efficiency in nanoparticles made using the same materials. For all polymers evaluated, the drug solubility was higher than the drug loading. Moreover, whereas the p(SA)-term-LA had the highest drug solubility, it had also the lowest drug loading efficiency. Thus, the solubility values could not be considered as a predictive parameter for encapsulation. These unexpected findings could be related to the different drug incorporation conditions. Indeed, the polymer films were cast in anhydrous media, whereas the nanoparticles were prepared in a liquid medium. Thus, in this latter case, drug diffusion in the nanoparticle dispersion medium must also be taken into account.

The influence of the dispersion medium on the incorporation of the crystalline drug into PLA nanoparticles was investigated. The values of the mean nanoparticle diameters and the loading efficiencies measured are summarized in Table 2. The drug loading and the purification stage did not modify the nanoparticle size. All nanoparticles had a mean diameter ranging ∼200 nm, except those prepared in a potassium chloride medium. The drug loading values clearly depended on the nature of the dispersion media, and the highest loading value was obtained using the medium containing sodium sulphate. These data show the importance of the choice of the dispersion medium in the encapsulation process. Fig. 2 summarizes the drug loading, the drug solubility in the dispersion medium after acetone evaporation and the drug solubility in the acetone-dispersion medium. The drug solubility was low and practically the same in the different acetone-free dispersion media, while it varied in the different acetone-dispersion media. In all cases, the drug solubility in the acetone-dispersion medium was higher than that in the final dispersion medium (after acetone evaporation). In conclusion, it was clearly observed that the encapsulation efficiency increased when the drug solubility in the acetone-dispersion medium decreased, except for the sodium sulphate medium (Fig. 2).

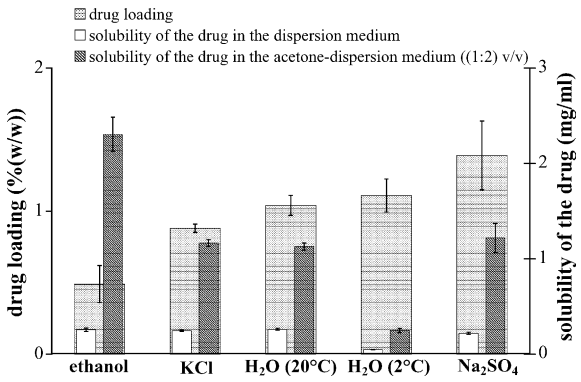

Fig. 2. Drug loading and drug solubility in the dispersion medium and in the acetone-dispersion medium.

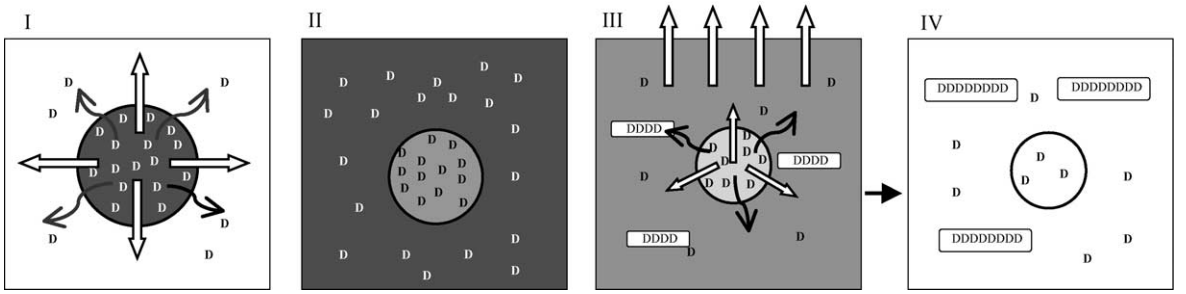

Fig. 3. Hypothetical mechanism of drug leakage in the nanoprecipitation process. (I) Injection of the acetone polymer phase. Acetone (grey) diffusion (empty arrows) and drug (D) leakage (full arrows). (II) End of acetone diffusion. (III) Acetone evaporation. Drug crystal (DDDD) formation. (IV) Drug crystal growth.

These data made it possible to propose a potential mechanism of drug leakage during the nanoprecipitation process (Fig. 3). To prepare nanoparticles, the polymer containing acetone medium was poured into the dispersion medium. Acetone, which is miscible with the aqueous or ethanolic dispersion medium, started to diffuse in this dispersing phase (empty arrows) and the drug started to leak out (full arrows) (I). When acetone diffusion was complete, nanoparticles were formed, suspended in an acetone-dispersion medium (II). The higher the drug solubility in this outer phase, the higher the amount of the drug in the dispersion medium (II). When acetone is eliminated by evaporation, the drug solubility in the dispersion medium decreases, leading to the formation of crystals (III). During nanoparticles storage, the crystals continue to grow, the nanoparticles serving as drug reservoirs (IV). These findings highlight the importance of using a polymer with a specific affinity for the drug, and a dispersion medium with the lowest drug solubility to achieve an efficient encapsulation of a crystalline drug.

Further investigations are in progress to determine the loading efficiency of the model drug with other biodegradable polymers, and other dispersing phases, especially acetone/water mixtures in various proportions. Indeed, previous studies in the literature (Yalkowsky and Roseman, 1980) reported that poorly water-soluble drug solubility decreased, when proportion of acetone in the acetone/water cosolvent decreased.

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