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Encapsulation of dexamethasone into biodegradable polymeric nanoparticles

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Abstract

The present paper concerns both the optimization of dexamethasone (DXM) entrapment and its release from biodegradable poly(D,L-lactide-*co*-glycolide) (PLGA) nanoparticles prepared by the solvent evaporation process. Since the addition of DXM induced the formation of drug crystals beside the nanoparticle suspension, the influence of several parameters on DXM encapsulation was investigated such as the type of organic solvent and polymer, the DXM initial mass, the evaporation rate of the solvent, the continuous phase saturation and the incorporation of a lipid in the polymer. Nanoparticle size and zeta potential were not modified in the presence of DXM and were respectively around 230 nm and −4 mV. The highest drug loading was obtained using 100 mg PLGA 75:25 in a mixture of acetone-dichloromethane 1:1 (v:v) and 10 mg of DXM. The drug was completely released from this optimized formulation after 4 h of incubation at 37 °C. Neither the evaporation rate of the organic solvent, nor the aqueous phase saturation with salt or the incorporation of 1 mg 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) within the nanoparticles modified the encapsulation efficiency. Differential scanning calorimetry (DSC) and X-ray diffraction (XRD) demonstrated that the drug was molecularly dispersed within the nanoparticles whereas the non-encapsulated DXM crystallized. These results demonstrate the feasibility of encapsulating dexamethasone and its subsequent delivery.

Keywords: Nanoparticles; Dexamethasone; PLGA; Crystalline drug

1. Introduction

Corticoids have demonstrated to be efficient anti-inflammatory drugs for the treatment of acute and chronic posterior segment eye diseases such as uveitis (Munoz-Fernandez and Martin-Mola, 2006). They may also have an important role in the management of ocular diseases that involve neovascularization, wound healing, and fibrosis, such as proliferative vitreoretinopathy and subretinal neovascularization (Machemer et al., 1979; Tshibashi et al., 1985; Kertes and Coupland, 2005). However, their use is often hampered by the induction of side effects mainly when administered by the systemic route. Topical application is preferred to avoid the deleterious effects of corticoids. Nevertheless, this mode of administration does not allow a sufficient drug passage to the posterior segment. Periocular injections can achieve therapeutic levels in the vitreous/retina, but may produce periocular fibrosis, globe perforation and ptosis (Ferrante et al., 2004).

In these conditions, a therapy allowing direct release of drugs into the vitreous is often required for the effective treatment of posterior segment diseases. Direct injections of corticoids into the vitreous have been shown to provide therapeutical concentrations and minimize the systemic side effects (Young et al., 2001; Tamura et al., 2005). However, depending on the drug rate of clearance from the vitreous, large boluses and repeated injections may be required to ensure therapeutic levels over an extended period of time (Velez and Whitcup, 1999). In addition, multiple intraocular injections can reduce patient compliance or lead to an increased likelihood of complication (vitreous hemorrhage, retinal detachment, etc.). Moreover, administration of drug solution often leads to a peak of concentration that can induce toxic effects (Kwak and D'Amico, 1992).

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Sustained drug delivery devices like implants offer an excellent alternative to multiple intravitreous injections (Hainsworth et al., 1996; Jaffe et al., 2006). Nevertheless, their disadvantages can include: large surgical incision to be implanted (Jaffe et al., 2000), difficulty if removal of the device is required and potential endothelium damage due to physical migration of the implant (Tan et al., 1999).

To overcome these problems, the incorporation of drugs into nano/microparticulate delivery systems represents a therapeutic opportunity. It provides a more efficient mean of improving the retention of the drug in the vitreal cavity (Martinez-Sancho et al., 2003), and may help to target the phagocytic cells (retinal pigment epithelium) (Kimura et al., 1994), along with improved patient compliance owing to reduce frequency of injection. Particles can be formulated from a wide variety of synthetic and natural polymers. However, biodegradable polymers such as poly (D,L-lactide-*co*-glycolide) (PLGA) have been the most frequently used for ocular drug delivery (Herrero-Vanrell and Refojo, 2001) due to their low ocular toxicity (Moritera et al., 1991). Although the use of microparticles may have some limitations such as visual impairment (Colthurst et al., 2000), these systems are the most commonly used. Although, nanoparticles have several advantages as compared to microparticles, only a few studies report the use of nanoparticles for intravitreal administration (Merodio et al., 2002; de Kozak et al., 2004; Irache et al., 2005; Gomes dos Santos et al., 2006). Nevertheless, they are very interesting carriers, since they may be internalized within retinal cells (Bourges et al., 2003) and do not sediment, leading to a more homogenoeous distribution in the vitreous.

Dexamethasone is a poorly soluble and crystalline corticoid that has been used for the treatment of diabetic macular edema administered as an implant (Posurdex, Oculex Pharmaceuticals, Phase III). It is active at the dose of 350–700 μg. The goal of this work was to optimize the encapsulation of dexamethasone into PLGA nanoparticles for intravitreal injection. Parameters such as the organic solvent, the polymer type (different lactide to glycolide ratio), the DXM initial mass, the evaporation rate of the organic solvent, the continuous phase saturation and the lipid incorporation, were investigated. Finally, an *in vitro* release of the optimized formulation was performed under sink conditions.

2. Materials and methods

2.1. Materials

Poly (D,L-lactide-*co*-glycolide) (PLGA) of different composition and molecular weight (PLGA 50-50, Resomer RG502 and PLGA 75-25 Resomer RG756) were purchased from Boehringer-Ingelheim (Germany). Poly (lactide) (PLA) was provided by Alkermes (USA). Poly (vinyl-alcohol) (PVA) (87–89% hydrolyzed, MW 30,000–70,000) and *N*-(2-hydroxyethyl) piperazine-*N*′-(2-ethanesulfonic acid) (HEPES) were obtained from Sigma–Aldrich (France). Dexamethasone (DXM) was provided by Chemos GmbH (Germany). 1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) was obtained from Genzyme (Switzerland). Acetone, ethyl acetate, chloroform and dichloromethane in analytical grade and acetonitrile in high-performance liquid chromatography (HPLC) grade were obtained from Carlo Erba Reagents (France). Water is purified using a Synergy system from Millipore (France).

2.2. Nanoparticle preparation

PLA, PLGA and PLGA-DPPC nanoparticles loaded with DXM were prepared by a solvent emulsion-evaporation technique. Briefly, 100 mg of PLGA, DXM and DPPC (when used) were dissolved into 5 mL of organic solvent. The organic solution was then pre-emulsified with 20 mL of a PVA aqueous solution (0.25%, w/v) by vortexing at 3200 rpm for 1 min (Mini Vortexer VWR, USA). The pre-emulsion was kept on ice and sonicated at 300 W for another minute using a Vibra cell sonicator (Bioblock Scientific, France). The organic phase was then evaporated at room temperature under gentle agitation (700 rpm). Nanoparticle suspension was then completed to 25 mL by weight. Amber vials were used throughout the process to provide protection against UV light, which degrades dexamethasone (The Pharmaceutical CODEX, 1994). Non-encapsulated DXM crystals were visualized by optical microscopy between cross polarizers. The crystals were eliminated by successive filtration of the nanoparticles suspension using PVDF filters (3.0, 1.5 and 0.45 μm consecutively).

2.3. Particle size and zeta potential

Particle size and polydispersity index were determined using a Malvern Zetasizer Nano ZS (Malvern Instrument, UK) based on quasi-elastic light scattering. Size measurements were performed in triplicate following a 1/100 (v/v) dilution of the nanoparticles suspension in water Milli-Q at 25 °C. Zeta potential was measured using the same instrument at 25 °C following a 1/50 (v/v) dilution in a 1 mM NaCl solution. The polydispersity index range was comprised between 0 and 1.

2.4. Optical microscopy

Suspensions of nanoparticles were placed between glass slides and observed with a Leitz Diaplan microscope equipped with a Coolsnap ES camera (Roper Scientific).

2.5. Scanning electron microscopy

Scanning electron microscopy (SEM) was performed using a LEO 1530 (LEO Electron Microscopy Inc., Thornwood, NY) operating between 1 and 3 kV with a filament current of about 0.5 mA. After crystal filtration, liquid samples were deposited on carbon conductive double-sided tape (Euromedex, France) and dried at room temperature. They were coated with a palladium–platinum layer of about 4 nm using a Cressington sputter-coater 208HR with a rotary-planetary-tilt stage, equipped with a MTM-20 thickness controller. Particles were washed before imaging by centrifugation (around 7000 × *g* for 20 min) to remove the excess of surfactant that reduces the quality of images.

2.6. Differential scanning calorimetry

After crystal elimination the suspension was submitted to ultracentrifugation (138,800 × *g* for 30 min) in order to remove soluble DXM, the pellet containing nanoparticles was freeze-dried. Accurately weighed samples of about 15 mg were analyzed in aluminium pans (40 μL) on a differential scanning calorimeter (DSC7, Perkin-Elmer, USA). The DSC runs were conducted from 10 to 80 °C and back at a rate of 10 °C/min. The second order endothermic transition which appears as a step transition was analyzed as a glass temperature transition (T_g). Experiments were performed at least in duplicate.

2.7. X-ray diffraction

X-ray powder diffraction (XRD) patterns were measured on a Siemens D5000 diffractometer in Bragg–Brentano geometry using Ni-filtered Cu Kα radiation. Data were collected over an angular range comprised between 2 and 55° (2θ) with a step size of 0.1° and a counting time comprised between 150 and 500 s per step. Experiments were performed in duplicate.

2.8. Dexamethasone loading within nanoparticles

After crystal elimination by filtration as described previously, 2 mL of nanoparticle suspension was ultracentrifuged (138,800 × *g* for 30 min at 25 °C, ultracentrifuge Optima™ LE-80 K, rotor type 70.1, Beckman) to separate the soluble DXM in the supernatant from the nanoparticles. The temperature was kept at 25 °C to prevent recrystallization (Layre et al., 2005). Nanoparticles were then dissolved into 1.4 mL acetonitrile and filtered with a 0.45 μm PVDF filter prior to analysis to eliminate PVA that is not soluble in acetonitrile. PLGA-DPPC nanoparticles were dissolved first into 1 mL of dichloromethane and then 0.6 mL acetonitrile was added, finally the solution was filtered. The quantity of DXM in the particles was determined by injecting 20 μL of the filtered solution in a Waters™ liquid chromatograph (HPLC) equipped with a Waters™ 600 pump, a Waters™ 7956 interface, a Waters™ 2996 photodiode array detector, a Waters™ 717 autosampler and a Waters™ Empower Login software. The analysis was performed at 238 nm using an Interchrom™ Reverse Phase Nucleosil 5 C18 column (150× 4.0 mm) with a mobile phase composed of 40% acetonitrile and 60% water at 1 mL/min. All the analysis was performed at room temperature. The developed method shows satisfactory linearity between 0.3 and 26 μg/mL ($y = 43920x + 9731$; with $r^2 = 0.9995$). Experiments were performed at least in duplicate and some times more (up to $n = 5$).

2.9. In vitro release kinetics of dexamethasone

In vitro release of DXM from nanoparticles was carried out under sink conditions: drug concentration in the medium was kept 10 times lower than the saturation solubility of DXM in HEPES buffer (74 μg/mL). After crystal filtration, 3.5 g of nanoparticle suspension were ultracentrifuged at 6800 × *g* for 20 min at 25 °C using the same ultracentrifuge described above. The centrifugation was carried out at lower speed to allow resuspension of nanoparticles without any modification of their size as checked by light scattering. After removal of the supernatant, nanoparticles were resuspended into 2 mL of 10 mM HEPES buffer saline (150 mM NaCl, pH 7.4) by vortexing. A vial was prepared for each time point, protected from light and kept at 37 °C under tangential stirring (150 rpm) (Heidolph-Titramax 1000, Germany). At predetermined time intervals, the entire release medium was removed and ultracentrifuged at 138,800 × *g* for 30 min at 25 °C and the supernatant was collected. All centrifuged supernatants were stored at 4 °C until analysis by HPLC. DXM was checked to be stable in HEPES at 4 °C for 30 days. Experiments were performed in triplicate.

3. Results and discussion

The aim of this paper was the optimization of DXM encapsulation within nanoparticles of PLGA. A nanosphere system was developed using an emulsion evaporation technique based on a single oil-in-water emulsion.

3.1. Effect of the organic solvent on dexamethasone encapsulation

Several organic solvent or mixtures of organic solvent were considered for solubilization of both the DXM and the polymer. Dichloromethane was initially evaluated since it is widely used for drug encapsulation into polymeric nanoparticles. However, DXM has a poor solubility (125 μg/mL) in this organic solvent. Despite this low solubility, DXM encapsulation using dichloromethane as solvent was tested (100 mg PLGA 75:25 into 5 mL organic solvent). Drug loading was found to be rather low: 20 ± 1 μg/100 mg polymer, corresponding to an encapsulation efficacy of only 3% meaning that drug solubility in the solvent is a main parameters for DXM encapsulation. To further increase the encapsulation efficacy of a drug, the use of cosolvents is often reported in the literature (Cascone et al., 2002). Methanol and acetone are the best organic solvents for DXM, the later being the best one. To optimize DXM encapsulation within the nanoparticles, mixtures of organic solvents were tested (Table 1). A constant mass of polymer (100 mg) and dexamethasone (10 mg) were fixed. Although the DXM concentration was low, optical microscopy observations reveal the presence of DXM crystals which size varies between 1 and 10 μm (data not shown). It means that a fraction of the active principle is not encapsulated. As the organic solvents diffuse into the continuous phase due to their miscibility with water, the drug solubility in the continuous phase can temporarily increase. However, when the organic solvent is fully evaporated, the solubility decreases and some of the free drug can crystallize in the continuous phase (Layre et al., 2005).

The amount of crystals present was evaluated qualitatively by optical microscopy before the actual drug loading was quantified by HPLC. Independently of the mixture used, DXM crystals were observed in all suspensions (data not shown). The amount of crystals seemed qualitatively lower using acetone:dichloromethane (1:1) (Fig. 1). After elimination of the

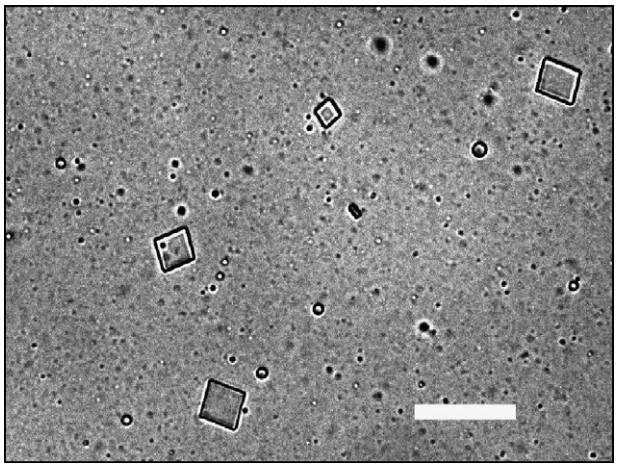

Fig. 1. Optical microscopy image of DXM crystals present in nanoparticle suspension (scale bar = 20 μm). Nanoparticles were prepared with 100 mg PLGA 75-25 and 10 mg DXM in 5 mL acetone:dichloromethane 1:1 (v:v). The aqueous solution was PVA 0.25%.

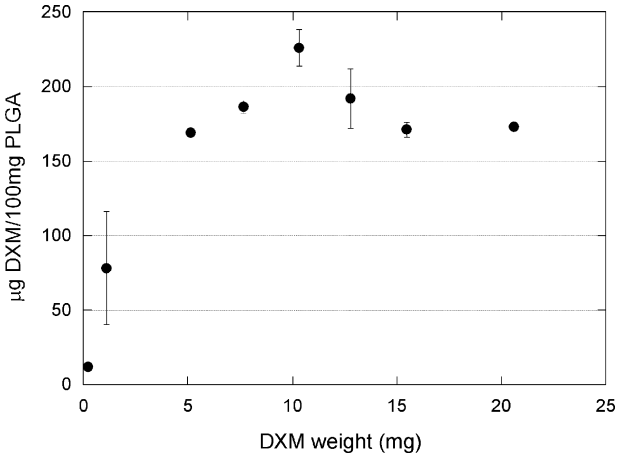

Fig. 2. Drug loading as a function of the initial mass of DXM ($n = 3$). Nanoparticles were prepared with 100 mg PLGA 75-25 and DXM in 5 mL acetone:dichloromethane 1:1 (v:v). The aqueous solution was PVA 0.25%.

crystals and the free DXM in the supernatant as described in Section 2, HPLC assay of the encapsulated DXM was performed. All solvent mixtures used allowed to increase drug loading by at least a factor 5 as compared to pure dichloromethane. HPLC also confirms that acetone:dichloromethane (1:1, v:v) leads to the highest drug loading with about 230 μg DXM for 100 mg polymer. Relatively monodisperse nanoparticles were obtained in all cases with a mean diameter comprised between 185 and 280 nm and polydispersity indices below 0.2.

The soluble amount of DXM in the continuous phase (PVA aqueous solution 0.25% (w/v)) was measured in all the previous formulations. No variation between the different formulations was found and the DXM solubility in PVA 0.25% (80 μg/mL, $n = 10$) does not differ significantly from the solubility in water (Amilne, 2002).

3.2. Effect of the lactide to glycolide ratio and polymer molecular weight on dexamethasone encapsulation

The influence of the polymer (composition and molecular weight) on the encapsulation of DXM into nanoparticles was investigated. A constant mass of polymer (100 mg) and dexamethasone (5 mg) were fixed. The values of the mean nanoparticle diameters and the loading efficacies are summarized in Table 2. All nanoparticles had a mean diameter around 220 nm. Drug loading in the nanoparticles changed slightly depending of polymer type. The lowest loading ratio (142 μg/100 mg of polymer) was obtained using PLA. The highest loading ratio (170 μg/100 mg of polymer) was obtained using PLGA 75:25, but the difference with PLGA 50:50 was not important. Although these polymers have a different molecular weight, this parameter should not influence the encapsulation rate as already observed by many authors (Görner et al., 1999; Fonseca et al., 2002).

These results suggest that encapsulation of DXM does not result from hydrophobic interactions since PLA is more hydrophobic than PLGA 50:50. Therefore, there is a molecular dispersion of DXM within the polymer and crystallization forces exclude the major part of the drug from the nanoparticles.

3.3. Effect of dexamethasone initial mass on encapsulation

Keeping constant the polymer type (PLGA 75:25), the effect of the initial mass of DXM on drug loading was investigated in the acetone–dichloromethane mixture. The DXM mass was varied between 220 μg and 21 mg just below the saturation limit in the organic solvent. Fig. 2 shows that the drug loading increases as the initial mass of DXM increases, until it reaches about 230 μg/100 mg PLGA for 10 mg DXM initial mass. The drug loading then decreases slightly and stabilizes around 170 μg/100 mg PLGA. As shown in Table 3, the nanoparticle size was not influenced by the initial DXM weight in the formulation.

These results suggest that there is a competition between molecular dispersion of the drug within the polymeric matrix and crystallization forces. Initially, DXM loading increases until

Table 1
Influence of the organic solvents on the amount of crystals, drug loading, nanoparticle size and polydispersity index (PDI) ($n = 3$)

Solvents (v:v)	Crystalline drug	Drug loading ± S.D. (μg/100 mg PLGA)	NP size mean ± width (nm)	PDI
Methanol:chloroform (1:4)	++	112 ± 2	276 ± 111	0.16
Acetone:ethyl acetate (2:3)	++	135 ± 6	185 ± 78	0.17
Acetone:chloroform (1:1)	++	170 ± 5	230 ± 72	0.11
Acetone:dichloromethane (1:1)	+	226 ± 12	218 ± 57	0.07

Nanoparticles were prepared with 100 mg PLGA 75-25 and 10 mg DXM in 5 mL organic solvent. The aqueous solution was PVA 0.25%.

Table 2
Effect of polymer characteristics on physical characteristics of dexamethasone-loaded nanoparticles

Lactide (%)	Molecular weight (Da)	NP size[a] mean ± width (nm)	PDI	Presence of crystals	Drug loading in NP ± S.D. (μg/100 mg PLGA)[b]
100	17,000	213 ± 60	0.08	+	142 ± 5
75	98,000	224 ± 65	0.08	+	170 ± 7
50	12,000	220 ± 50	0.09	+	162 ± 2

Nanoparticles were prepared with 100 mg polymer and 5 mg DXM in 5 mL acetone:dichloromethane 1:1 (v:v). The aqueous solution was PVA 0.25%; PDI: polydispersity index.
[a] $n = 3$.
[b] $2 \leq n \leq 5$.

the matrix is saturated with the drug (initial DXM weight <10 mg). Then, as the number of crystals increases, crystallisation becomes the driving force and contributes to reduce the amount of drug encapsulated. Finally, equilibrium is reached between the crystallized and the encapsulated DXM.

Altogether, these results suggest that the highest drug loading within the nanoparticles can be obtained using PLGA 75:25 in a 1:1 (v:v) mixture of acetone–dichloromethane and an initial mass of dexamethasone of 10 mg: the drug loading is then 230 μg/100 mg PLGA. To further increase the drug loading, several strategies were considered.

3.4. Optimization of DXM entrapment

Instead of slowly evaporating the organic solvent by gently mixing the emulsion with a stir bar, a drastic evaporation was performed. Just after the sonication, the emulsion was transferred into a round flask and evaporation was performed under vacuum using a rotating evaporator. After the evaporation, crystals were observed but their size was significantly smaller than with the gentle evaporation. However, these crystals underwent growth overnight and the drug loading was exactly the same as with the gentle evaporation. Another strategy was considered to increase DXM encapsulation: the aqueous phase was saturated with NaCl (0.9%, w/v). Indeed, saturation of the aqueous phase has been shown to reduce the solubility of some drug therefore increasing their encapsulation. The solubility of DXM in the continuous phase (after organic solvent evaporation) and the drug loading in the nanoparticle were not modified. Finally, to improve dexamethasone encapsulation, DPPC, a phospholipid was incorporated in the nanoparticles to favor hydrophobic interactions between DXM, phospholipids and the polymer matrix. After evaluation of DPPC solubility in the organic mixtures, 1 mg was added to the organic phase and emulsified in PVA aqueous solution as described. After evaporation, DXM crystals have been observed by optical microscopy (not shown). The size of nanoparticles did not vary with DPPC incorporation. The loading in the nanoparticle were not modified (234.2 ± 4.2 μg/100 mg polymer, $n = 2$). None of these strategies allowed to significantly improve the drug loading.

Table 3
Influence of the initial drug loading on nanoparticle size and distribution ($n = 3$)

Initial mass of DXM (mg)	NP size mean ± width (nm)	PDI
Unloaded	229 ± 86	0.14
5	222 ± 61	0.07
10	223 ± 63	0.08
20	224 ± 58	0.07

Nanoparticles were prepared with 100 mg PLGA 75-25 (±DXM) in 5 mL organic solvent. The aqueous solution was PVA 0.25%; PDI: polydispersity index.

3.5. Characterization of the optimal nanoparticle formulation

The optimized DXM loaded nanoparticle formulation therefore corresponds to an initial DXM weight of 10 mg, PLGA 75:25 and the acetone:dichloromethane mixture (1:1, v:v) as organic solvent. Electron microscopy (after filtration) showed nanoparticles with a mean diameter of 150–200 nm, a spherical shape and a smooth surface (Fig. 3). The DXM-loaded nanoparticles do not differ from the unloaded ones.

The zeta potential and size of unloaded nanoparticles and DXM-loaded nanoparticles did not differ significantly. Both formulations demonstrated similar sizes (229 ± 86 nm for unloaded nanoparticles and 224 ± 58 nm for DXM loaded nanoparticles, $n = 3$) and negative zeta potential (−4.04 ± 0.40 mV for unloaded nanoparticles and −3.54 ± 0.25 mV for DXM loaded nanoparticles, $n = 3$).

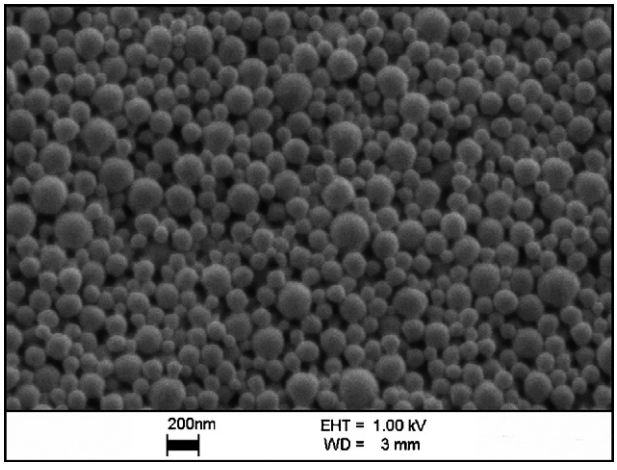

Fig. 3. Typical SEM image of DXM loaded nanoparticles. Unloaded nanoparticles look exactly the same (scale bar = 200 nm). Nanoparticles were prepared with 100 mg PLGA 75-25 and 10 mg DXM in 5 mL acetone:dichloromethane 1:1 (v:v). The aqueous solution was PVA 0.25%. The sample was filtered on 0.45 μm before observation.

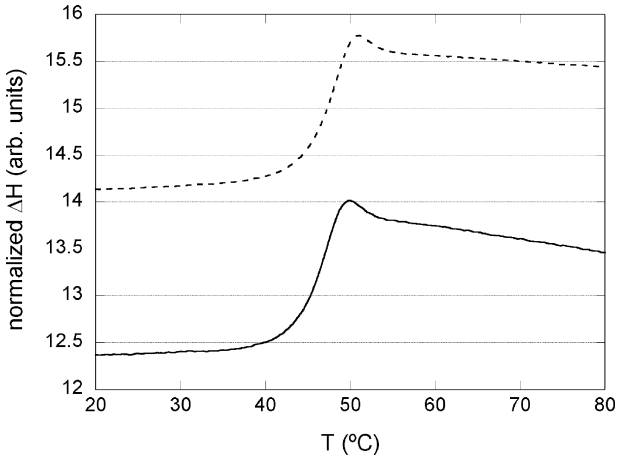

Fig. 4. Differential scanning calorimetry (DSC) thermograms of DXM-loaded nanoparticles (dotted line) and unloaded nanoparticles (solid line). Nanoparticles were prepared with 100 mg PLGA 75-25 and ±10 mg DXM in 5 mL acetone:dichloromethane 1:1 (v:v). The aqueous solution was PVA 0.25%.

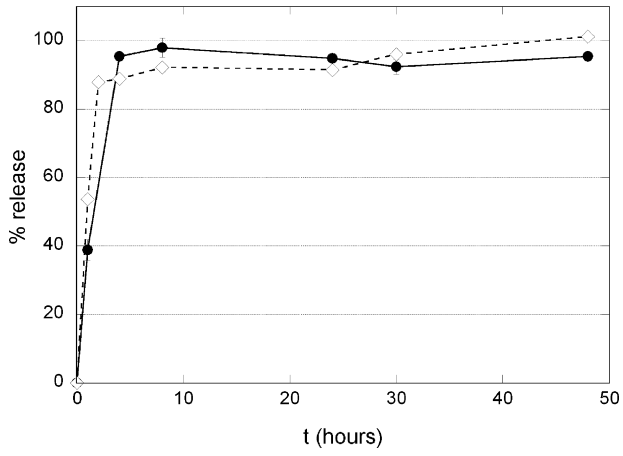

Fig. 5. *In vitro* release profile of dexamethasone in HEPES buffer (mean ± S.D., $n = 3$) from DXM nanoparticles (●) and DXM-DPPC nanoparticles (◊). Nanoparticles were prepared with 100 mg PLGA 75-25, 10 mg DXM and (±1 mg DPPC) in 5 mL acetone:dichloromethane 1:1 (v:v). The aqueous solution was PVA 0.25%.

3.6. Differential scanning calorimetry (DSC) and X-ray diffraction (XRD)

In order to assess the possible interactions between DXM and the polymer, DSC analysis was performed on both unloaded and DXM-loaded nanoparticles. Thermograms are typical of PLGA, exhibiting a glass temperature transition around 50 °C (Fig. 4). No significant difference could be observed proving that DXM does not interact with the polymeric matrix or that the amount of DXM encapsulated is too low to observe a difference. The absence of interactions explains the tendency of DXM to crystallize: DXM molecules prefer to assemble with other DXM molecules instead of being dispersed within the PLGA nanoparticles. XRD analysis was used to determine the crystalline content of DXM in the nanoparticles. No crystalline DXM was detected, which suggests the presence of DXM dispersed at molecular state within the nanoparticles. These results were further confirmed by the absence of DXM crystalline peak at 255 °C on the DSC thermogram of DXM-loaded nanoparticles (not shown).

3.7. In vitro drug release from nanoparticles

Two *in vitro* release studies were performed under the same conditions for DXM nanoparticles and DXM-DPPC nanoparticles. These profiles show a fast release of the active drug during the first 4 h of incubation (Fig. 5). This type of profile is characteristic of a burst-release, which suggests that DXM may be in the vicinity of the nanoparticle surface. Incorporation of DPPC does not modify significantly the release profile of the drug. Nevertheless, this release profile could probably be modified when nanoparticles would be injected intravitreally since injected DXM concentration would be above the sink conditions. Therefore, DXM would probably reach quickly its saturation that would most probably limit its release from the nanoparticles.

Although several studies were conducted to encapsulate DXM within PLGA nanoparticles, none of them took into account the presence of crystals (Panyam et al., 2004; Kim and Martin, 2006). Whether these were not observed or not present remains to be elucidated. Very recently, other biodegradable polymers were used showing that the amount of DXM encapsulated can be increased probably due to specific interactions with the polymer (Zhang et al., 2006). Synthesis of modified polymers specifically for DXM encapsulation should therefore be considered (Layre et al., 2006). Other forms of DXM should also be considered for encapsulation such as DXM sodium phosphate, using a double emulsion method. Whether encapsulation of this form of DXM would allow one to increase the drug load and/or slow the drug release remains to be studied experimentally. In addition, encapsulation of DXM-loaded nanoparticles within another delivery vehicle that would quickly fall apart when administered could also be of interest for ocular delivery (Tsapis et al., 2002).

4. Conclusion

DXM encapsulation within PLGA nanoparticles was optimized for ocular delivery. The presence of crystalline drug along with the nanoparticles considerably reduces the ability to load the polymeric matrix. However, 230 μg DXM could be encapsulated in 100 mg PLGA 75:25 using a mixture of dichloromethane–acetone as the organic solvent and 10 mg DXM. Nanoparticle size was around 230 nm and the zeta potential was negative around −4 mV. All the attempts to improve the encapsulation such as modifying the rate of evaporation or incorporating a phospholipid within the particles failed DXM molecules prefer to assemble with themselves and crystallize. Although the DXM release under sink conditions is very fast *in vitro* (100% in 4 h), it would probably be slower *in vivo* since injected DXM concentration would be higher than the one used in sink conditions. In addition, the amount of DXM encapsulated might be sufficient for therapeutic purposes since nanoparticles internalization within retinal pigment epithelial cells should increase the drug efficacy. These nanoparticles could also be of

interest for other inflammatory diseases and could be used for example after intra-articular injection to treat arthitic disorders (Makrygiannakis et al., 2006).

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