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ScienceDirect
International Journal of Pharmaceutics 344 (2007) 53–61
INTERNATIONAL JOURNAL OF PHARMACEUTICS
www.elsevier.com/locate/ijpharm

Effect of the microencapsulation of nanoparticles on the reduction of burst release

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Received 5 February 2007; received in revised form 27 May 2007; accepted 29 May 2007
Available online 13 June 2007

Abstract

The initial burst release is one of the major problems in the development of controlled release formulations including drug-loaded micro- and nanoparticles, especially with low molecular weight drugs. The objective of the present work was to encapsulate, by the W/O/W emulsion, polymeric nanoparticles into polymeric microparticles by using non-water soluble polymers and appropriate organic solvents for the preparation of these composite microparticles. They were characterized in vitro (encapsulation efficiency, mean diameter and release kinetics) and compared with nanoparticles and classical microparticles prepared by the same method. Poly-ε-caprolactone (PCL) dissolved in methylene chloride was used to make nanoparticles, whereas ethylcellulose and Eudragit RS dissolved in ethyl acetate, a non-solvent of poly-ε-caprolactone, were used for the preparation of microparticles. Ibuprofen and triptorelin acetate were chosen as lipophilic and hydrophilic model drugs, respectively. High entrapment efficiencies were obtained with ibuprofen whereas lower amounts of triptorelin acetate were encapsulated, mainly with formulations prepared with poly-ε-caprolactone and Eudragit RS used alone or blended with ethylcellulose. The burst was significantly lower with composite microparticles and may be explained by the slower diffusion of the drugs through the double polymeric wall formed by the nanoparticle matrix followed by another diffusion step through the microparticle polymeric wall.

Keywords: Burst release; Nanoparticles; Microparticles; Controlled release

1. Introduction

One major persistent problem in the development of injectable polymeric delivery systems is the drug initial burst release of drug which occurs during the first minutes of contact with the external medium (Yeo and Park, 2004). This burst release can be useful for the penetration of a drug, especially in the case of dermal applications, but sustained release is important for active drugs that are toxic at high concentrations or those that need to be present over a prolonged period. Burst release is often observed with microparticulate systems; it is unpredictable and generally difficult to control, but may be prevented by changing the drug distribution within the polymer matrix (Fu et al., 2003) or by developing more sophisticated drug delivery systems. Examples of the latter are liposomes encapsulated inside dextran (Stenekes et al., 2000) and alginate microcapsules allowing the release of the drug in a controlled way and eliminating the burst effect (Dhoot and Wheatley, 2003). A pentamidine-loaded hydrogel has also been microencapsulated within PLGA microparticles using the solvent evaporation technique, leading to a significant reduction of the burst effect (Mandal et al., 2002). Double-walled microspheres (Lee et al., 2002), double-layered minipellets (Maeda et al., 2003) and coated microspheres (Huang et al., 1999; Hurteaux et al., 2005) have all been developed to reduce the initial burst and provide sustained release profiles of the drug. A substantial reduction of the initial burst was also obtained by crosslinking the microparticle surface, thus creating an additional barrier to diffusion which prevented easy dissolution of the drug in the external medium (Thote et al., 2005). Microparticles prepared with blends of polymers characterized by different viscosity, molecular weight and swelling properties may also modify the

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doi:10.1016/j.ijpharm.2007.05.066

release of the drug compared with microparticles prepared from a single polymer. Indeed, the release of ibuprofen from microspheres prepared with a blend of ethylcellulose and polystyrene was prolonged over 24 h with a reduced burst compared with microspheres prepared with ethylcellulose alone (Saravanan et al., 2003). The use of surfactants (Bouissou et al., 2006) or hydrophilic additives such as glycerol (Yamaguchi et al., 2002) added to the organic dispersion of methylene chloride containing PLGA and crystalline insulin results in a drastically reduced initial burst of insulin, which is preferentially located inside microcapsules rather than at the surface. Moreover, the addition of glycerol decreased both the glass transition temperature of PLGA and the porosity of microspheres: thus the diffusion of insulin was effectively prevented. The microencapsulation of lipid (Lee et al., 2003) or polymeric nanoparticles prepared from hydrophilic polymers (Grenha et al., 2005; Bhavsar et al., 2006) can lead to the controlled release of the encapsulated drug. Nanostructured protein particles produced by spray-freezing and encapsulated within lactide/glycolide copolymer and poly(lactic acid) microspheres decreased the burst release and maintained high structural integrity (38%) of the encapsulated protein (Leach et al., 2005).

The goal of our study was to encapsulate, by the double emulsion method, polymeric nanoparticles within polymeric microparticles by using non-water soluble polymers and an appropriate organic solvent for the preparation of these composite microparticles, thus preventing the dissolution of the nanoparticulate suspension used as internal phase. Poly-ε-caprolactone (PCL) dissolved in methylene chloride was used for the manufacture of nanoparticles, whereas ethylcellulose and Eudragit® RS dissolved in ethyl acetate, a non-solvent of PCL, were used for the preparation of microparticles. Ibuprofen and triptorelin acetate were chosen as lipophilic and hydrophilic model drugs, respectively. Microparticles containing nanoparticles, called composite microparticles, were characterized in vitro (encapsulation efficiency, mean diameter, release kinetics) and compared with nanoparticles and simple microparticles prepared by the same double emulsion method.

2. Materials and methods

2.1. Materials

Ibuprofen [(R, S)-2(4-isobutylphenyl) propionic acid] (batch number 450025) generously supplied by Knoll Pharma Chemicals (Nottingham, UK) and triptorelin acetate, a gift of Debiopharm (Lausanne, Switzerland), were used as model drugs. Poly(ε-caprolactone) (MW 40,000 Da) was supplied by Aldrich, USA. An acrylic polycationic nonbiodegradable polymer (copolymers of acrylic and methacrylic acide esters with a low content of quaternary ammonium groups (0.5–0.8%) (4.48–6.77% ammonium methacrylate units by dry weight)) Eudragit® RS PO (MW 150,000 Da) (RS) and ethylcellulose powder (Ethocel viscosity 4) were donated by Röhm Pharma polymers, Degussa (Darmstadt, Germany) and The Dow Chemical Company (Michigan, USA), respectively.

Poly(vinyl alcohol) (PVA) (MW 30 kDa, 88% hydrolyzed) was supplied by Sigma–Aldrich (St. Louis, Missouri, USA). Ethyl acetate (water solubility = 8.3 g/100 ml at 20 °C)) was purchased from Fluka Chemie GmbH (Switzerland). Methylene chloride (water solubility = 1.3 g/100 ml at 20 °C) was supplied by Prolabo (Paris, France). Acetonitrile and orthophosphoric acid were obtained from Carlo-Erba (Val de Reuil, France) and Prolabo (Paris, France), respectively. All other chemicals were of analytical grade and used without further purification.

2.2. Methods

2.2.1. Preparation of particles

2.2.1.1. Nanoparticles (NP). Ibuprofen or triptorelin acetate-loaded PCL NP were prepared by the W/O/W solvent evaporation method (Hoffart et al., 2002). Briefly, 1 ml of aqueous internal phase was emulsified for 15 s in 5 ml of methylene chloride (containing 125 mg of PCL) using an ultrasound probe (Vibra cell 72,434, BioBlock Scientific, Strasbourg, France) at 50 W output. This primary emulsion was poured into 40 ml of a 0.1% PVA aqueous solution and sonicated again with the same ultrasound probe for 1 min under the same conditions in order to create the water in oil-in-water emulsion. Three to four milliliters of NP suspension were obtained after solvent evaporation under reduced pressure (Rotavapor, Heidolph, Germany). Nanoparticles were separated from the bulk suspension by centrifugation (Biofuge Stratos, Heraeus Instruments, Germany) at 42,000 × *g* for 20 min. The supernatant was kept for drug assay as described later and the sedimented nanoparticles were redispersed in 3 ml of purified water before freeze-drying. After lyophilization, the dried nanoparticles were resuspended in 2 ml of purified water shortly before preparing the composite microparticles.

Due to the lipophilic nature of ibuprofen, 50 mg of the drug were dissolved in the organic phase but, in order to keep the same preparation method for both drugs (W/O/W emulsion), 1 ml of purified water was used as the aqueous internal phase in the case of ibuprofen-loaded NP. On the other hand, since triptorelin acetate is water soluble, 1 ml of a triptorelin acetate aqueous solution (2.5 mg/ml) was used as the internal phase in the case of triptorelin acetate-loaded NP.

Blank nanoparticles were prepared under the same conditions without drug.

2.2.1.2. Microparticles (MP). Microparticles containing either ibuprofen or triptorelin acetate PCL NP (so-called composite microparticles) were prepared by the W/O/W solvent extraction method (Freytag et al., 2000). In the first step (W/O emulsion), the PCL NP suspension (2 ml) used as the internal aqueous phase was emulsified (ultrasound probe at 50 W output for 15 s) in an organic solution of polymer in ethyl acetate (5 ml). The polymers (250 mg) dissolved in ethyl acetate were (i) Eudragit® RS, (ii) ethylcellulose and (iii) a 1/1 blend of Eudragit® RS and ethylcellulose.

The primary emulsion was poured into 20 ml of 0.1% PVA aqueous solution in order to obtain a W/O/W pre-emulsion. After magnetically stirring for 1 min (1000 rpm) at room temperature, this pre-emulsion was added to 400 ml of a 0.1% PVA aque-

ous solution and stirred mechanically (three-bladed propeller, 1600 rpm) for 10 min to form the final W/O/W emulsion.

Upon solvent extraction, the polymers precipitated and the microparticle cores solidified.

Microparticles were collected by filtration (Millipore® Type: 0.45 μm nitrate cellulose for ibuprofen MP and cellulose acetate for triptorelin acetate MP) and dried at room temperature for 24 h.

Blank composite microparticles (with blank PCL NP) and simple microparticles (without PCL NP but containing one or the other drug) were prepared under the same conditions.

2.2.2. Mean diameter and zeta potential

2.2.2.1. Microparticles. Mean diameter and size distribution of MP were analyzed by laser diffraction in a particle size analyzer (Mastersizer S, Malvern Instruments, France). Each sample was measured in triplicate.

2.2.2.2. Nanoparticles. The mean diameter of NP and their surface potential were evaluated with a Malvern Zetasizer 3000 HSA (Malvern Instruments, France) using, respectively, photon correlation spectroscopy and electrophoretic mobility. Nanoparticles were diluted in 0.001 M NaCl prior to zeta potential measurements. The results were all normalized with respect to a polystyrene standard suspension (Malvern Instruments). Each sample was measured in triplicate.

2.2.3. Determination of drug content

2.2.3.1. Ibuprofen. The amount of ibuprofen entrapped within polymeric particles was determined spectrophotometrically at 222 nm (UV-160 1PC, UV-visible spectrophotometer, Shimadzu, Kyoto, Japan) by measuring the amount of non-entrapped ibuprofen in the external aqueous solution (indirect method) which was recovered after filtration and washing of microparticles. In the case of nanoparticles, the external aqueous solution was obtained after centrifugation of the colloidal suspension for 20 min at 42,000 × g. A standard calibration curve was performed with the ibuprofen solution (aqueous solution of 0.1% PVA with 1% acetone). The established linearity range was 2–10 μg/ml ($r > 0.99$).

In order to validate the indirect assay for routine purposes, the results were compared with those obtained after measuring the amount of ibuprofen within Eudragit® RS microparticles (without PCL NP) directly by an established but slightly modified HPLC method (Fernandez-Carballido et al., 2004).

Briefly, 20 mg of particles were accurately weighed and dissolved in 20 ml of mobile phase (water/acetonitrile: 40/60 acidified with orthophosphoric acid pH 2.7). Fifty microliters of this solution were injected into the HPLC system (Model Shimadzu HPLC 10A vp, Shimadzu, Japan) with UV detection (SPD-10 A VP, Shimadzu, Japan). The separation was achieved by using a reversed phase column (Uptisphere ODB, 3 mm i.d., 150 mm long, 12 nm porosity, 5 μm particle size, Interchim, France). The detection wavelength was set at 264 nm. The flow rate of the mobile phase was 0.8 ml/min. Under these conditions, the polymer did not interfere with the drug at this specific wavelength. The ibuprofen calibration curve was linear from 1 to 100 μg/ml ($r = 0.999$).

2.2.3.2. Triptorelin acetate. Triptorelin acetate content was determined by an established reversed phase HPLC method previously described (Schuetz et al., 2005). The separation was achieved by using a C_{18} Whatman Partisphere WVS column (4.6 mm i.d., 125 mm long, 5 μm particles size, Interchim, France). The detection wavelength was set at 278 nm. The triptorelin acetate curve was linear from 1 to 500 μg/ml ($r = 0.999$) in an aqueous solution of PVA 0.1% and NaCl 0.1 M. The amount of triptorelin acetate entrapped within polymeric particles was also determined by two methods. In the indirect method, the amount of non-entrapped triptorelin acetate in the external aqueous solution was measured by injecting 20 μl directly into the HPLC system (Model Shimadzu HPLC 10A VP, Shimadzu, Japan) with UV detection (SD-10 A VP, Shimadzu, Japan). In the direct method, the triptorelin acetate amount was directly determined after extraction from the particles followed by injecting 20 μl of the extract directly into the HPLC system. To extract the drug, about 10 mg of particles were accurately weighed and dissolved in 0.5 ml of methylene chloride. Triptorelin acetate was extracted 6 times (vortex 10 min) from the organic phase with 2 ml of an aqueous solution of Tween 80 (0.1%), followed by a 20 min centrifugation at 800 × g.

2.2.4. In vitro drug release from both nanoparticles and microparticles

Fifty milligrams of freeze-dried or dried ibuprofen or triptorelin acetate loaded particles were suspended in 20 ml of saline phosphate buffer (KH_2PO_4 0.0044 M, $Na_2HPO_4 \cdot 0.0451$ M, NaCl 0.1 M, pH 7.4 adjusted by orthophosphoric acid) or NaCl 0.1 M, respectively. The particles suspension was gently stirred (200 rpm) at 37 °C into a water bath. One milliliter of suspension was withdrawn at appropriate intervals (5, 15, 30, 45 min, 1, 2, 3, 4, 5, 6, 8, 24 h) and filtered with a 0.22 μm nitrate cellulose filter (Millipore®) in the case of ibuprofen. For triptorelin acetate, due to established filter adsorption during the preliminary trials, each sample was centrifuged at 42,000 × g for 10 min. The filtrate (ibuprofen) or the supernatant (triptorelin acetate) was replaced by 1 ml of fresh buffer. The amount of ibuprofen in the release medium was determined by UV at 222 nm as previously described. For triptorelin acetate, the HPLC method described previously was used. Each particle batch was analyzed in triplicate.

3. Results and discussion

Burst release is a critical problem with currently marketed injectable microparticles, especially when slow release for a few weeks or months is required. The encapsulation of nanoparticles within microparticles as an alternative way to decrease the burst has been proposed by only a few groups (Lee et al., 2003; Sheikh Hasan et al., 2004; Grenha et al., 2005; Leach et al., 2005; Bhavsar et al., 2006). For example, Bhavsar et al. encapsulated gelatin nanoparticles in poly(ε-caprolactone) microparticles; whereas Grenha et al. encapsulated chitosan

nanoparticles within mannitol or lactose microparticles: however, these hydrophilic polymers used for nanoparticles might favor water uptake by the microparticles and consequently induce a faster release and/or a faster hydrolysis of the non-soluble polyester polymer, as was shown in the case of the PCL microparticles prepared by Bhavsar et al. Furthermore, Grenha et al. observed a very high release of their model drug (insulin) after 20 min in a dissolution test, although their insulin nanoparticles were incorporated within mannitol microparticles. In order to prevent such rapid release, we have prepared composite microparticles based on non-water soluble polymers such as PCL, ethylcellulose and Eudragit® RS. Since the double emulsion technique was used for the preparation of composite microparticles, our original approach consisted in using a polymer for the nanoparticles (PCL) which is insoluble in the organic solvent used to manufacture the microparticles in the second step of the double emulsion method. Indeed, PCL was dissolved in methylene chloride because of the low boiling point and poor water solubility of this solvent. Microparticles were prepared by dissolving the two other polymers (ethylcellulose, Eudragit® RS and 1/1 blend of each polymer) in ethyl acetate which is a poor solvent for PCL: in our conditions of microparticle preparation the maximum solubility of PCL added directly to ethyl acetate is 5%. Therefore, it was possible to use the PCL nanoparticle suspension as the internal aqueous phase in the preparation of the composite microparticles.

In order to test the effect on burst release, two model drugs differing in their water solubility were selected. One was a peptide drug. Indeed, most of the currently marketed injectable microparticles are peptide dosage forms for which a reduction of the burst would be highly desirable. Triptorelin acetate is a decapeptide (MW = 1311.5) which is highly water soluble. Consequently, the only way to obtain sufficiently high incorporation in nanoparticles to use the W/O/W emulsion technique (Nicoli et al., 2001). Ibuprofen was the second model drug; with contrasting properties since it is very poorly water soluble (35.89 μg/ml) substance with a low molecular weight (MW = 206.3). Due to the very low aqueous solubility of ibuprofen, it would have been possible to prepare ibuprofen nanoparticles according to the simpler O/W emulsion technique. However, with a view to comparing the drug release with that of triptorelin acetate, ibuprofen nanoparticles and control simple microparticles were also prepared by the double emulsion technique. In this case, ibuprofen was dissolved in the organic phase and plain water (1 ml) was used as the internal aqueous phase.

Tables 1–3 summarize the main physicochemical parameters (mean diameter, zeta potential and encapsulation efficiency) of the three types of particles prepared with the various polymers.

Table 1
Mean diameter, drug encapsulation efficiency and zeta potential of blank or ibuprofen and triptorelin acetate loaded PCL nanoparticles ($n = 3 \pm$ S.D.)

	Blank NP	Ibuprofen NP	Triptorelin acetate NP
Mean diameter (nm)	355 ± 6.0	341 ± 9.0	380 ± 5.0
Zeta potential (mV)	−3.1 ± 2.9	+15.1 ± 5.5	+19.8 ± 12.4
Encapsulation efficiency (%)	–	95 ± 2.0	38 ± 7.0

Both drug-loaded NP preparations had diameters ranging from 340 to 380 nm (Table 1). Blank nanoparticles were similar. Unloaded NP showed a slightly negative zeta potential which became positive after the incorporation of either drug. Due to its low water solubility, ibuprofen was very efficiently incorporated into PCL nanoparticles (mean encapsulation efficiency 95%) (Table 1). On the other hand, the encapsulation efficiency was only 38% for triptorelin acetate nanoparticles. This is probably the result of its higher water solubility; it would diffuse more readily through the aqueous continuous phase of the second emulsion before polymer precipitation. For both triptorelin acetate and ibuprofen, the average diameter of PCL nanoparticles obtained by the double emulsion technique was in the usual range for this polymer and this method of preparation. Indeed, Hoffart et al. (2002) obtained nanoparticle diameter ranging from 380 to 500 nm with the same polymer and preparation method. With the same double emulsion technique and also using methylene chloride, Nicoli et al. (2001) obtained triptorelin acetate nanoparticles in a slightly larger range (574–743 nm) when the polymer was various types of PLGA.

Simple microparticles are very small whatever the drug or the polymers used, except ibuprofen-loaded MP prepared with ethylcellulose (Table 2). Indeed, the diameter of all MP was close to 15 μm whereas ibuprofen-loaded MP prepared with ethylcellulose were larger (136 μm). In addition, simple blank ethylcellulose MP are slightly larger (32 μm) than blank MP prepared with Eudragit® RS alone or blended with ethylcellulose. The difference in encapsulation efficiency of the two drugs in simple ethylcellulose MP is not significant. Indeed, similar encapsulation efficiency (Table 2) was observed for ibuprofen and triptorelin encapsulated inside ethylcellulose MP (89 and 87%, respectively). A more significant difference between the two drugs was obtained after encapsulation in MP prepared with Eudragit® RS and the blend of EC and Eudragit® RS (around 80% for ibuprofen and 60% for triptorelin). The encapsulation

Table 2
Mean diameter and drug encapsulation efficiency of blank, ibuprofen (IBU) and triptorelin acetate (TRP) simple microparticles (SMP) prepared with ethylcellulose (EC), Eudragit® RS (RS) and a blend 50/50 of ethylcellulose and Eudragit® RS (EC/RS) ($n = 3 \pm$ S.D.)

Polymers	Mean diameter (μm)			Encapsulation efficiency (%)		
	Blank SMP	IBU SMP	TRP SMP	Blank SMP	IBU SMP	TRP SMP
EC	32 ± 5	136 ± 28	20 ± 5	–	89 ± 5	87 ± 8
RS/EC	13 ± 4	18 ± 5	16 ± 3	–	80 ± 8	61 ± 4
RS	15 ± 2	14 ± 7	10 ± 5	–	79 ± 5	65 ± 7

Table 3
Mean diameter and drug encapsulation efficiency of blank, ibuprofen (IBU) and triptorelin acetate (TRP) composite microparticles (CMP) prepared with ethylcellulose (EC), Eudragit® RS (RS) and a blend 50/50 of ethylcellulose and Eudragit® RS (EC/RS) ($n = 3 \pm$ S.D.)

Polymers	Mean diameter (μm)			Encapsulation efficiency (%)		
	Blank CMP	IBU CMP	TRP CMP	Blank CMP	IBU CMP	TRP CMP
EC	83 ± 10	161 ± 35	27 ± 6	–	87 ± 3	89 ± 8
RS/EC	23 ± 7	68 ± 18	24 ± 3	–	85 ± 4	66 ± 8
RS	16 ± 2	45 ± 9	21 ± 8	–	74 ± 5	59 ± 7

efficiency of triptorelin acetate was much higher in MP prepared with the two polymers used alone or blended than in NP prepared with PCL.

The PCL nanoparticle suspension of each drug was directly used as the internal aqueous phase in the preparation of the composite microparticles. Three types of composite microparticles were manufactured with either ethylcellulose, Eudragit® RS or the blend (50/50) of these 2 polymers. Composite microparticles had a larger diameter than simple microparticles (Table 3). This effect was more marked for ibuprofen composite microparticles. Encapsulation efficiencies remained fairly high and did not show major differences compared with simple microparticles. The high encapsulation efficiency of ibuprofen could again be explained by the lipophilic nature of the drug which has no affinity for the external aqueous phase, compared with the hydrophilic triptorelin acetate. The similar encapsulation efficiency observed with ethylcellulose simple and composite microparticles may be explained by the overall viscosity of the system. Indeed, it was observed (although not measured) that the solution of ethylcellulose in ethyl acetate was more viscous than with Eudragit® RS or with the blend ethylcellulose/Eudragit® RS. It would consequently be more difficult for the hydrophilic drug present in the internal dispersed phase to diffuse into the outer water phase (Bodmeier and McGinity, 1988). The triptorelin acetate encapsulation efficiency was higher in simple and composite microparticles than in nanoparticles: this is probably the effect of a faster precipitation of polymers after solvent (ethyl acetate) extraction during microparticle manufacturing compared with solvent evaporation (methylene chloride) during NP preparation.In order to demonstrate conclusively that nanoparticles were effectively entrapped in the microparticles, we performed the following experiment with unloaded micro- and nanoparticles. Blank composite EC/RS microparticles (containing PCL nanoparticles), blank simple EC microparticles, blank simple Eudragit® RS microparticles and PCL nanoparticles were dispersed in ethyl acetate. It should be noted that ethylcellulose and Eudragit® RS are soluble in ethyl acetate while the PCL polymer is not (or very slightly as stated above). Therefore, if PCL nanoparticles had really been encapsulated, the resulting suspension in ethyl acetate should have been very turbid, as should the blank PCL nanoparticle suspension dispersed in ethyl acetate. Fig. 1 clearly displays a similar turbidity in ethyl acetate of both PCL nanoparticles and composite microparticles showing the dissolution of ethylcellulose and Eudragit RS but the presence of PCL nanoparticles. In contrast, blank simple EC microparticles and blank simple RS microparticles are totally dissolved in ethyl acetate and produce a clear solution. Consequently, Fig. 1 shows that the manufacturing process allows the encapsulation of PCL nanoparticles within microparticles and demonstrates the composite character of these microparticles.

Most nano- and microparticulate systems are characterized by an initial burst which is generally difficult to control. Such a burst may be due to a number of phenomena, including (i) heterogenous drug distribution including surface-associated drug (Huang and Brazel, 2001); (ii) temperature, which affects drug distribution and morphology of particles (Guiziou et al., 1996); (iii) the physico-chemical nature of the polymeric matrix (Fernandez-Carballido et al., 2004; Thompson et al., 2007) and (iv) porosity of particles which is generally higher for the solvent evaporation method (methylene chloride) than for the extraction method (ethyl acetate) (Yeo and Park, 2004). There are two ways to determine whether the burst is controlled: by an in vitro dissolution test or by an in vivo approach after subcutaneous or intramuscular administration. As a first approach, we have used the in vitro dissolution test. These tests were carried out under sink conditions, in phosphate buffer (pH 7.4) and NaCl 0.1 M for ibuprofen and triptorelin acetate, respectively. The resulting release profiles are presented for each drug (ibuprofen or triptorelin acetate) and type of polymer (ethylcellulose, ethylcellulose/Eudragit RS and Eudragit RS). Fig. 2A–C displays the release profiles of ibuprofen from ethylcellulose, ethylcellu-

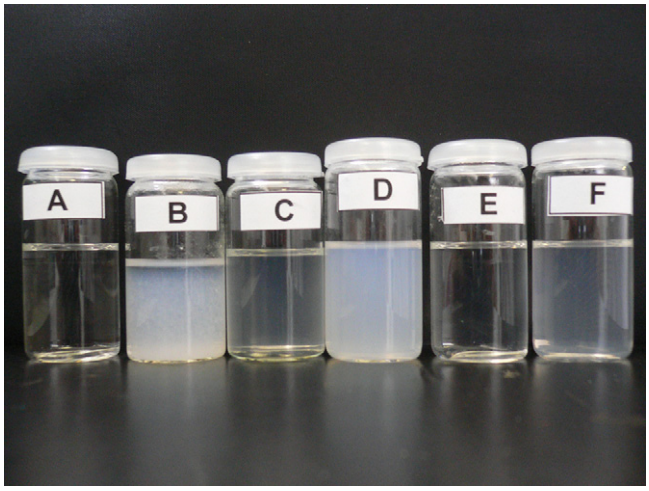

Fig. 1. Picture showing the macroscopic solubility in ethyl acetate of different nano- and microparticles prepared with different polymers: (A) ethyl acetate, (B) blank PCL nanoparticles, (C) EC/Eudragit® RS microparticles, (D) composite EC/Eudragit® RS microparticles, (E) simple Eudragit® RS microparticles, (F) simple EC microparticles.

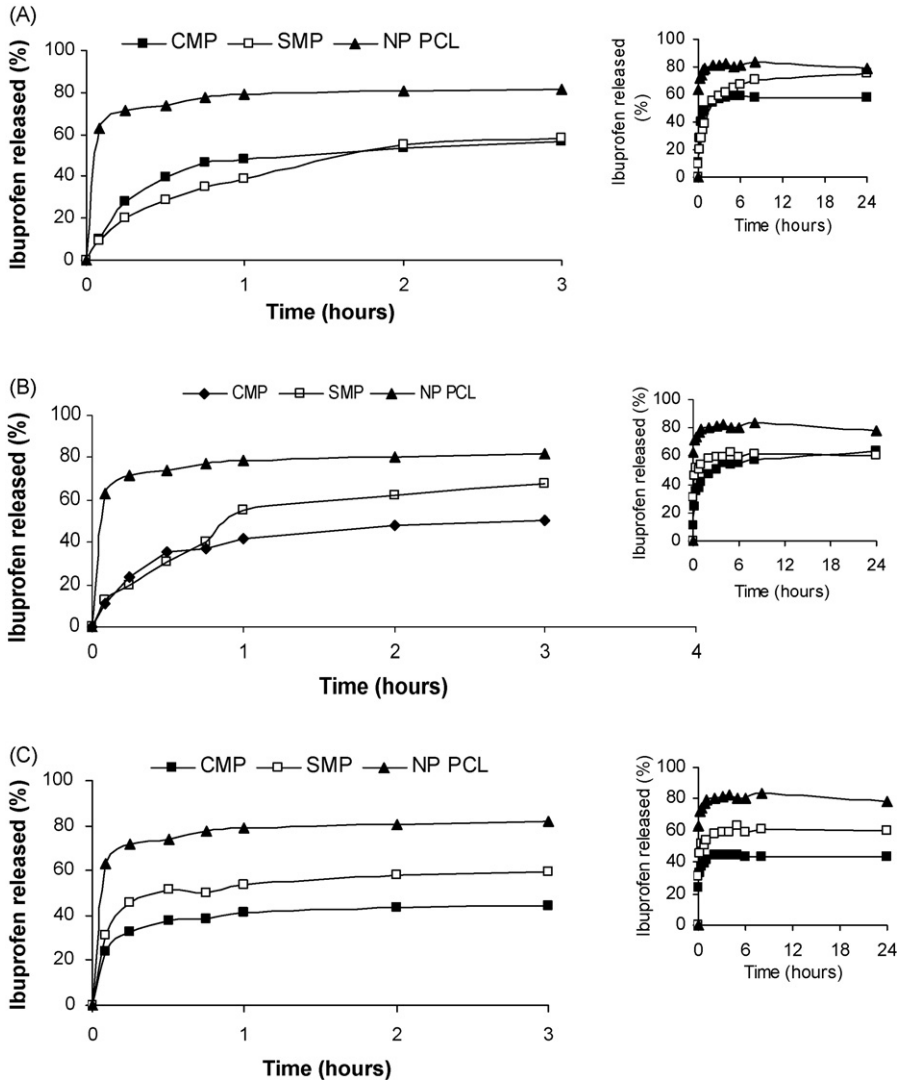

Fig. 2. Release kinetics of ibuprofen from PCL nanoparticles (solid triangles), simple (SMP, open squares) and composite microparticles (CMP, solid squares) prepared with (A) ethylcellulose, (B) Eudragit RS/ethylcellulose (50/50) and (C) Eudragit RS. Experiments were performed in phosphate buffer pH 7.4 at 37 °C. Data shown as mean ± S.D. ($n = 3$).

lose/Eudragit RS and Eudragit RS microparticles, respectively. Fig. 3A–C displays the release profiles of triptorelin acetate from ethylcellulose, ethylcellulose/Eudragit RS and Eudragit RS microparticles, respectively.

PCL-loaded nanoparticles of each drug displayed an immediate and important initial drug release in the first 15 min, followed by a plateau of around 80 and 70% at 24 h for ibuprofen and triptorelin acetate nanoparticles, respectively (Table 4). This immediate high release may be due to the small diameter of nanoparticles leading to a large exchange surface and probably to a more porous structure owing to the solvent evaporation method, favoring the release of the encapsulated drugs. Indeed, it has been already demonstrated that the slow precipitation of microparticles after solvent evaporation leads to more porous particles compared to the fast polymer precipitation obtained after solvent extraction (Jiang et al., 2002; Wang et al., 1991). This principle can be applied to the PCL nanoparticles which were prepared by solvent evaporation. It has also to be noted that, in the case of triptorelin acetate nanoparticles, the dissolution experiments were carried out in NaCl 0.1 M since the peptide was only slightly soluble in the PBS (solubility <0.25 mg/ml) used for ibuprofen. The higher ibuprofen release in PBS may be explained by its higher solubility in this buffer (3800 μg/ml) than in water (35.89 μg/ml). Indeed, ibuprofen is an acid (pK_a 4.5) and has better solubility in water with increasing pH (Levis et al., 2003). Not all the encapsulated ibuprofen and triptorelin acetate was released, since a plateau (around 80 and 70%, respectively) was obtained after 30 min and was stable up to 24 h (the end of the dissolution test). Similar behavior has already been observed with triptorelin acetate PLGA nanoparticles (Nicoli et al., 2001).

Although not all the encapsulated drug was released in 24 h, the dissolution test was limited to this time because the aim of this research was to demonstrate the influence of the encapsulation of nanoparticles within microparticles on the initial burst release. The initial burst and the dissolution profiles were

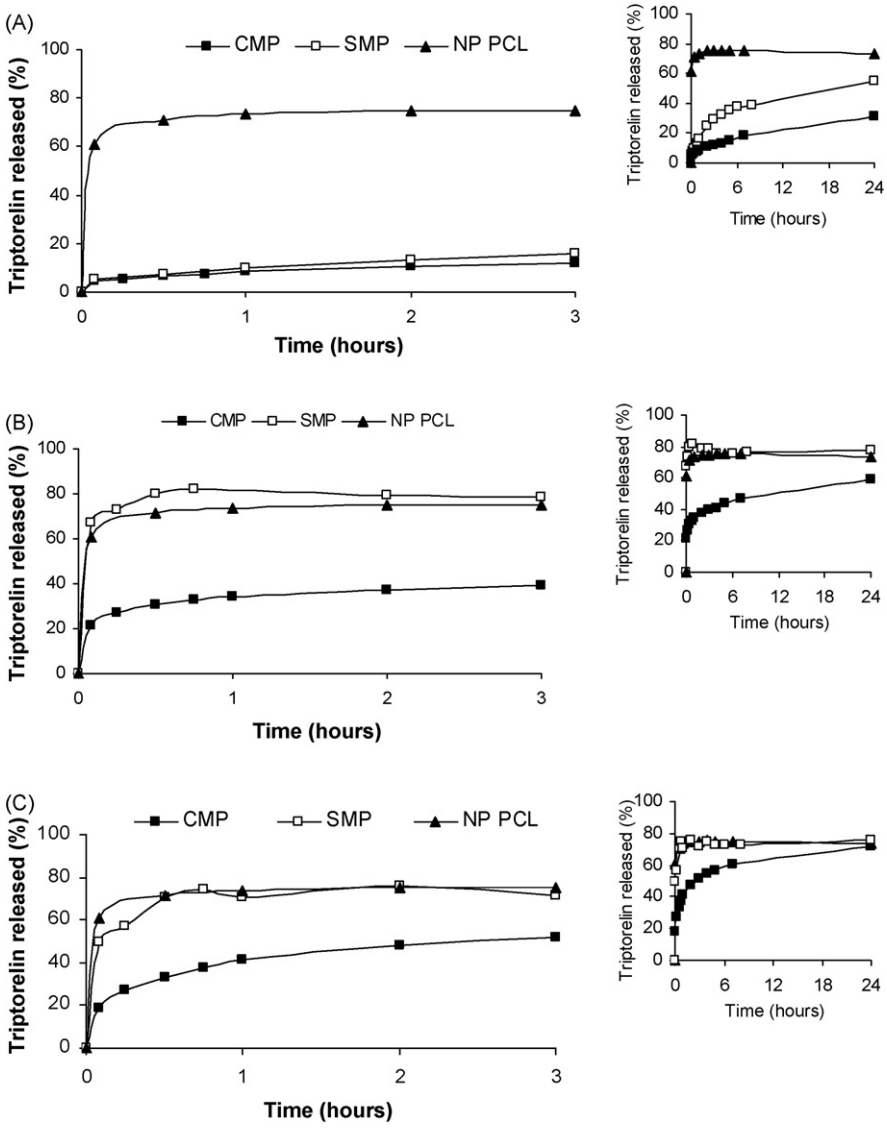

Fig. 3. Release kinetics of triptorelin acetate from PCL nanoparticles (solid triangles), simple (SMP, open squares) and composite microparticles (CMP, solid squares) prepared with (A) ethylcellulose, (B) Eudragit RS/ethylcellulose (50/50) and (C) Eudragit RS. Experiments were performed in NaCl 0.1 M at 37 °C. Data shown as mean ± S.D. ($n = 3$).

very different with all types of microparticles compared with nanoparticles, as shown in Figs. 2 and 3. The burst release was determined after 15 min, together with the percentage released after 24 h, as shown in Table 4.

For ibuprofen, the burst was higher for Eudragit® RS microparticles than for microparticles prepared with ethylcellulose or the blend of polymers (45.8 ± 1.2% when compared to 19.9 ± 5.3 and 19.8 ± 3.3%, respectively). This could be

Table 4
Mean percentage of ibuprofen (IBU) or triptorelin acetate (TRP) released after 15 min and 24 h from NP, simple and composite MP prepared with the different polymers used alone or blended ($n = 3 \pm$ S.D.)

Formulations	Polymers	IBU released (%)		TRP released (%)	
		15 min	24 h	15 min	24 h
NP	PCL	71.7 ± 12.1	78.3 ± 4.5	71.0 ± 11.2	73.5 ± 10.0
Simple MP	RS	45.8 ± 1.2	59.9 ± 1.4	56.9 ± 3.2	76.0 ± 2.4
	EC	19.9 ± 5.3	75.3 ± 4.8	7.1 ± 4.3	54.9 ± 3.6
	EC/RS	19.8 ± 3.3	58.2 ± 0.5	73.0 ± 6.0	77.3 ± 5.2
Composite MP	RS	32.9 ± 6.5	43.4 ± 4.2	26.7 ± 2.2	72.0 ± 2.3
	EC	27.7 ± 9.7	57.3 ± 8.0	5.4 ± 7.8	31.5 ± 5.8
	EC/RS	23.8 ± 3.7	63.8 ± 4.4	27.0 ± 6.1	59.5 ± 3.2

due to the physicochemical properties of Eudragit® which is a more hydrophilic polymer owing to its quaternary ammonium groups that favor water uptake and drug diffusion towards the dissolution medium (Huang et al., 2006). However, composite microparticles tend to reduce the initial burst effect especially for microparticles prepared with ethylcellulose used alone or blended with Eudragit® RS (27.7 and 23.8%, respectively, compared with 32.9% for Eudragit® RS alone composite microparticles). This can be explained by the rather lipophilic properties of ibuprofen does not favor its dissolution in aqueous media, but also to the high encapsulation ratio of PCL ibuprofen nanoparticles. Encapsulation of nanoparticles into microparticles also had a strong effect on the dissolution profile, especially for the ethylcellulose and Eudragit® RS/ethylcellulose microparticles. Indeed for the latter two types of microparticle, the total ibuprofen released was much lower after 24 h and a plateau of around 50% was obtained as early as 2 h. The presence of EC in the matrix of microparticles conferred a slower and more progressive release of ibuprofen during the time of the experiment. This could be explained by different interactions between the drug and either Eudragit® RS or ethylcellulose polymers.

For triptorelin acetate, the burst was the lowest for both the composite and the simple ethylcellulose microparticles compared with Eudragit® RS or blended microparticles (Fig. 3A) but the drug was more slowly released from the composite than from the simple microparticles in the 24 h period of the dissolution test ($54.9 \pm 3.6\%$ versus $31.5 \pm 5.8\%$). As for ibuprofen, except for composite Eudragit® RS microparticles which displayed a reduced burst, there was no major difference in terms of burst reduction between simple and composite microparticles for ethylcellulose and the blend of ethylcellulose and Eudragit® RS (from 54.9 to 31.5% and from 77.3 to 59.5%, respectively). Again, the burst was higher for Eudragit® RS microparticles compared with ethylcellulose or polymers blended microparticles. On the other hand, the effect of reducing the burst is much more marked with triptorelin acetate. Indeed, due its high hydrophilicity, this compound has a natural tendency to diffuse very rapidly towards the surrounding aqueous phase. Therefore, any mechanism which is able to restrict this diffusion of triptorelin acetate towards water would be easily observed. The influence of nanoparticle encapsulation in microparticles is obvious for each of the three types of microspheres. As a first observation, it can be noticed that simple microparticles prepared with Eudragit® RS (alone or blended with ethylcellulose) allowed a very fast release of triptorelin acetate. Basically the release profile was the same as for the triptorelin acetate PCL nanoparticles. It is only with simple ethylcellulose microparticles that the triptorelin acetate profile was intermediate between nanoparticles and composite microparticles when taking into account the whole 24 h of the experiment. This is probably due to the slow diffusion of water into the lipophilic EC matrix. However, in terms of burst reduction there is no difference between simple and composite ethylcellulose microparticles since the burst was under 10% in both cases (Table 4). When PCL triptorelin acetate nanoparticles were encapsulated in microparticles, there was a large decrease in the burst. Again, this decrease is much more marked than that for ibuprofen due to the hydrophilic nature of the drug. Therefore, the advantage of encapsulating nanoparticles in microparticles (composite microparticles) has been definitely demonstrated for hydrophilic drugs. The decrease in the burst is greater for ethylcellulose than for Eudragit® RS due to the lipophilic nature of ethylcellulose which slows down the entrance of water in the polymeric matrix. On the other hand, Eudragit® RS is more permeable to water which facilitates drug release from the encapsulated PCL nanoparticles.

4. Conclusion

More generally, the differences observed with the composite microparticles may be explained by the heterogeneous composition of the polymeric matrix. Indeed, in order to be released into the external dissolution medium, both drugs have to diffuse first through the PCL nanoparticles followed by another diffusion step through the ethylcellulose or Eudragit® RS matrix. The diffusion pathway takes longer for ethylcellulose due to the hydrophobicity of this polymer. The overall dissolution profiles show the potential of composite microparticles to dramatically change the burst effect and the release profile of drugs in vitro. This concept remains to be verified after subcutaneous or intramuscular administration, in this case using biodegradable polymers.

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