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Research paper

Composite microparticles with *in vivo* reduction of the burst release effect

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ABSTRACT

The aim of this study was to develop microparticles containing nanoparticles (composite microparticles) for prolonged drug delivery with reduced burst effect *in vitro* and *in vivo*. Such composite microparticles were prepared with hydrophobic and biodegradable polymers [poly(ε-caprolactone), poly(lactic-co-glycolic) acid]. Ibuprofen was chosen as the model drug, and microparticles were prepared by the extraction technique with ethyl acetate as the solvent. Nanoparticles and microparticles and an ibuprofen solution (Pedea®) were administered subcutaneously at the dose of 1 mg of ibuprofen per kg to overnight-fasted rats (male Wistar). Composite microparticles showed prolonged ibuprofen release and less burst effect when compared to simple microparticles (without nanoparticles inside) or nanoparticles both *in vitro* (PBS buffer) and *in vivo*. Moreover, ibuprofen was still detected in the plasma after 96 h with composite microparticles. Consequently, it has been demonstrated that composite microparticles were able to reduce burst release and prolong the release of ibuprofen for a long period of time.

1. Introduction

Various drug delivery systems, such as liposomes, micelles, emulsions and micro/nanoparticles have broad applications in controlled and/or targeted delivery. Several biodegradable polymers [such as poly(glycolic acid) (PLA), poly(lactide-co-glycolide) (PLGA), poly (ε-caprolactone) (PCL), poly(3-hydroxybutyrate), gelatin, alginate] are popularly used for this purpose. The main techniques for preparing polymer micro/nanoparticles include solvent evaporation/extraction, emulsion polymerization, interfacial polycondensation and spray drying.

Drug release from micro/nanoparticles depends on numerous factors including nature of polymer, physicochemical properties of drug and/or formulation factors. It has to be pointed out that the composition and manufacturing process of micro/nanoparticles, not mentioning the particle size, can strongly affect the underlying release mechanisms [1].

In many controlled release formulations, immediately upon placement in the release medium, an initial large bolus of drug is released before the release rate reaches a stable profile. This phenomenon, typically referred to as 'burst release', may have dramatic consequences in case of low therapeutic index drugs.

It is well known that the oil-in-water (O/W) or water-in-oil-in-water (W/O/W) emulsion–solvent evaporation technique is a complex process in which the organic solvent generates pores in the micro/nanoparticles structure during its evaporation/extraction [2]. Furthermore, the accumulation, through partitioning, of drug crystals on the surface of microspheres or the adsorption of drug crystals onto the surface during the encapsulation process generally produces burst release of the drug after administration [3–5].

The degree of burst release generally depends upon the nature of the polymer, drug nature (molecular weight), polymer/drug ratio [6–8] and/or the relative affinities of the drug for the polymer and the aqueous phase [4,9].

Multiple approaches have attempted to alleviate the burst by varying the polymer chemistry [10,11], adding excipients to the polymer phase [12–14], utilizing new polymers [4,15,16] or encapsulating particulate forms of the drugs into microparticles [17,18].

Attempts to reduce the burst effect have mainly involved macromolecules or hydrophilic drugs because burst control is one of the major challenges of parenteral administration. Although usually not so important as the burst release of hydrophilic drugs, attempts to control the burst with lipophilic drugs [14,19] may also be of interest in order to decrease the toxic side effects of high potent drugs or to prolong the drug release time *in vivo*.

Non-steroidal anti-inflammatory drugs (NSAIDs) are poorly water-soluble drugs, and solvent evaporation techniques are generally used for their microencapsulation [20]. However, during microencapsulation, a partial crystallization of the NSAIDs in the dispersing phase and/or on the microspheres surface may occur [4,20,21]. These free NSAIDs crystals are undesired because their release is not controlled by the polymer matrix. Therefore, various

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strategies have been tested in order to prevent NSAIDs crystallization during the solvent evaporation process [22] or to remove the free NSAIDs crystals from the microparticles surface after their preparation [19,20].

One promising method for reducing the release rate and suppressing the initial burst consists of encapsulating nanoparticles directly inside microparticles [21,23,24]. Encapsulation of nanoparticles inside microparticles has already been used with different goals such as (i) plasmid DNA intestinal mucosal delivery [25], (ii) targeting drugs to the inflammation site of inflammatory bowel disease and/or prevention of premature uptake or degradation of nanoparticles during their passage through the small intestine [26] and (iii) lung protein delivery [27]. Moreover, the idea of encapsulating nanoparticles into microparticles was also used to reduce the burst effect *in vitro* with different hydrophilic drug models such as dexamethasone sodium salt (low molecular weight drug) [18] and macromolecules i.e. bovine serum albumin (high molecular weight) [23,24].

Encapsulation of NSAIDs into nanoparticles followed by their encapsulation in microparticles could be of great help to reduce the undesired burst effect with NSAIDs (lipophilic drugs). Therefore, we have previously successfully encapsulated ibuprofen-loaded PCL nanoparticles inside ethylcellulose/Eudragit RS polymeric microparticles [21] and demonstrated a control of the burst effect.

Ibuprofen is indicated for the relief of mild to moderate pain and inflammation. To the best of our knowledge, there is no ibuprofen injectable sustained release dosage form on the pharmaceutical market. Based on the indications of ibuprofen, this would certainly be of interest. For instance, the intraarticular administration of ibuprofen in the management of chronic rheumatoid arthritis could be an alternative to corticosteroid administration, avoiding their devastating effects [14]. An ibuprofen intravenous solution has been marketed in Europe under the trade name of Pedea® for the treatment of ductus arteriosus in newborns [28]; this dosage form is a conventional aqueous solution allowing a fast therapeutic activity after intravenous administration. However, previous studies have indicated that ibuprofen shows both a high initial burst when formulated as nanoparticles [29] or microparticles [4] and a short half-life in biological medium (blood [30], synovial liquid [14], ocular [31]). Thus, controlling a potential ibuprofen burst effect could improve the therapeutic effect, prolong the biological activity, control the drug release rate and decrease the administration frequency.

However, previously used polymers [21] (ibuprofen-loaded PCL nanoparticles inside ethylcellulose/Eudragit RS microparticles) cannot be used for parenteral administration due to their non-biodegradable status. But, biodegradable polymers should be used instead of the two latter ones for subcutaneous administration. So in the following research work, we have prepared ibuprofen nanoparticles of a biodegradable polymer (PCL), which was then encapsulated in (PLGA) biodegradable microparticles with a view to reducing their burst effect and prolonging the ibuprofen–blood residence time after subcutaneous injection. Such nanoparticles in microparticles are called composite microparticles.

2. Materials

The acid form of ibuprofen [(R,S)-2(4-isobutylphenyl) propionic acid] (batch number 450025) generously supplied by Knoll Pharma Chemicals (Nottingham, UK), was used as the model drug. Poly(ε-caprolactone) (M_W 40,000 Da) and D–L poly(lactic-co-glycolic) acid 50:50 (m/m) Resomer® RG 504S end-capped (M_W 48,000; viscosity: 0.47 dL/g) were purchased from Sigma–Aldrich (Saint Quentin Fallavier, France) and Boehringer Ingelheim (Ingelheim, Germany) respectively.

Polyvinyl alcohol (PVA, M_W 30,000, 88% hydrolyzed) was supplied by Sigma–Aldrich and sorbitan monostearate, Span® 60, by Seppic (Paris, France). Ethyl acetate (water solubility = 8.3 g/100 mL at 20 °C) was purchased from Fluka Chemie GmbH (Buchs, 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, respectively. All other chemicals were of analytical grade and used without further purification.

3. Methods

3.1. Preparation of particles

3.1.1. Nanoparticles

Ibuprofen-loaded PCL nanoparticles were prepared by the W/O/W solvent evaporation method [32]. Briefly, 1 mL of aqueous internal phase was emulsified for 15 s in 5 mL of methylene chloride (containing 125 mg of PCL and 50 mg ibuprofen) with the help of an ultrasound probe (Vibra cell 72,434, BioBlock Scientific, Strasbourg, France) at 80 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 in the same conditions in order to create the W/O/W emulsion. Three milliliters (±1 mL) of nanoparticles 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 according to the methods described later, and the sedimented nanoparticles were then dispersed in 3 mL of purified water before freeze-drying. After lyophilization, a dry powder of nanoparticles was obtained. The nanoparticles preparation method was slightly modified for manufacturing the composite microparticles. Indeed, the only difference was that the solvent evaporation process was continued till 1.5 mL (±0.5 mL) of nanoparticles was obtained; this suspension was used directly (without freeze-drying) as the internal aqueous phase in the preparation of the composite microparticles. Due to the hydrophobic nature of ibuprofen, an O/W technique could have been used to prepare nanoparticles. However, with a view to comparing the dosage forms, a same preparation method (W/O/W emulsion) was used for all formulations i.e. PCL nanoparticles, PLGA simple microparticles (without nanoparticles) and composite microparticles (microparticles incorporating nanoparticles). This is the reason why 1 mL of purified water was used as the aqueous internal phase in the case of ibuprofen-loaded nanoparticles. Blank nanoparticles were prepared under the same conditions but without drug in the organic phase.

3.1.2. Microparticles

Microparticles containing ibuprofen–PCL nanoparticles (so-called composite microparticles) were prepared by the W/O/W solvent extraction method [33]. In the first step (W/O emulsion), the PCL nanoparticles suspension (1.5 mL) was used as the internal aqueous phase, which was emulsified (ultrasound probe at 80 W output for 15 s) in the organic solution of ethyl acetate (20 mL) containing PLGA (400 mg) and Span 60 (200 mg).

This primary emulsion was poured into 50 ml of 0.1% PVA aqueous solution in order to obtain a W/O/W pre-emulsion. After magnetically stirring for 30 s (1000 rpm) at room temperature, this pre-emulsion was added to 2 L of purified water and stirred mechanically (three-bladed propeller, 600 rpm) for 10 min to form the final W/O/W emulsion.

Upon solvent extraction, the polymers precipitated, and the microparticles cores solidified. Microparticles were collected by

filtration (Millipore® Type: 0.45 μm nitrate cellulose) and freeze-dried.

Blank PLGA composite microparticles (with blank PCL nanoparticles) and PLGA simple microparticles (with or without ibuprofen) were prepared according to the same conditions.

3.2. Mean diameter and zeta potential

3.2.1. Nanoparticles

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

3.2.2. Microparticles

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

3.3. Determination of ibuprofen content

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) that 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 ($r > 0.998$).

In order to validate the indirect assay method for routine purposes, the results have been compared with those obtained after measuring the ibuprofen amount directly into PLGA simple microparticles and PLGA composite microparticles according to an established but slightly modified HPLC method [14].

Briefly, 10 mg of particles were accurately weighed and dissolved in 20 mL acetonitrile. Then, 50 μL of this latter solution was injected into the HPLC system (Shimadzu HPLC 10A VP, Shimadzu, Kyoto, Japan) with UV detection (SPD-10 A VP, Shimadzu, Kyoto, Japan) and a data-processing software (model Class VP). 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 222 nm. The flow rate of the mobile phase (water/acetonitrile: 40/60 acidified with orthophosphoric acid pH 2.7) was 0.8 mL/min. The ibuprofen curve was linear from 1 to 100 μg/mL ($r = 0.999$).

3.4. In vitro drug release from nanoparticles and microparticles

Freeze-dried loaded particles with a mass of 50 mg were suspended in 20 mL of saline phosphate buffer (KH_2PO_4 0.0044 M, Na_2HPO_4 0.0451 M, NaCl 0.1 M, pH 7.4 adjusted by H_3PO_4). Dissolution studies were carried out under sink conditions (ibuprofen solubility in saline phosphate buffer is 3.8 mg/mL). The particles suspension was stirred (200 rpm) at 37 °C in 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 through a 0.22 μm nitrate cellulose filter (Millipore®, Molsheim, France). The filtrate was replaced by 1 mL of fresh buffer. Released ibuprofen was determined by UV spectrophotometry at 222 nm as previously described. Each particle batch was analyzed in triplicate.

3.5. Differential scanning calorimetry

The glass transition temperature (T_g) of the polymers was analyzed by differential scanning calorimetry (DSC) using a calorimeter (DSC Q10®, TA Instruments, Guyancourt, France). Accurately weighed samples of approximately 3–5 mg were put in aluminium pans (Al-crucibes, 40 μL), first cooled down to −90 °C and then heated up to +250 °C at a constant rate of 10 °C/min under nitrogen atmosphere. The T_g was determined during the second-heating run.

3.6. In vivo studies of ibuprofen-loaded particles in rats

3.6.1. Treatment and plasma collection

Experiments were carried out in the compliance of the French legislation on animal experiments (authorization 54–68 from Direction Départementale des Services Veterinaires, Malzeville 54220, France). Male Wistar rats with a mean body weight of 300 ± 20 g were used. Ibuprofen–PCL nanoparticles, PLGA simple microparticles and PLGA composite microparticles aqueous suspension or an ibuprofen solution (Pedea®, Orphan Europe SARL, Paris, France, 5 mg/mL) were administered subcutaneously at the dose of 1 mg of ibuprofen per kg to overnight-fasted rats ($n \geqslant 3$). The administration volume was 300 ± 50 μL in a 2.25% (m/v) carboxymethylcellulose aqueous solution (for multiparticle systems). Rats were anesthetized by intraperitoneal injection of pentobarbital sodium (15 mg/kg). Blood samples (400 μL) were collected by cardiac puncture 15, 30, 45 min and 1, 2, 4, 6, 8, 10, 24, 48, 72 and 96 h after administration into 1.5 mL polypropylene vials containing 60 μL of 0.129 M sodium citrate solution. After centrifugation at 3000 *g* for 10 min at 18 °C, the plasma was immediately stored at −20 °C.

It has to be noticed that animals were divided in five groups of three rats each, so that only two blood samples per animal were collected for 24 h for each formulation. For the following times, blood was sampled from two groups leading to six samples at each time.

3.6.2. Ibuprofen–plasma assay

The same HPLC technique (cf. 3.3), but with little changes aimed to increase the sensitivity of the method, was used to measure ibuprofen in plasma [42]. Indeed, the detection was carried out with a spectrofluorimetric detector (model RF-10A XL, Shimadzu). The mobile phase consisted of a mixture of methanol (60%) and 0.05 M phosphate buffer pH = 6.5 (40%) filtered through a 0.45 μm filter, degassed before use and run at a flow rate of 0.6 mL/min. Spectrofluorimetric detection was operated at an excitation wavelength of 224 nm and emission wavelength of 290 nm. The five points calibration curve was built by spiking blank (drug-free) plasma samples (180 μL) with ibuprofen (20 μL of ibuprofen standard solutions prepared in mobile phase). Frozen plasma samples (200 μL) were thawed in a water bath at 37 °C, and proteins were precipitated by vortex mixing with 200 μL of a mixture of acetonitrile/1 M HCl (99:1 v/v) for 4 min. After centrifugation at 42,000 *g* for 10 min at 4 °C, the supernatant was transferred into HPLC sample vials and a 50 μL volume was injected into the HPLC system. The ibuprofen calibration curve was linear from 50 to 200 ng/mL ($r = 0.999$) in plasma.

3.6.3. Relative bioavailability after subcutaneous administration of the different dosage forms of ibuprofen

Ibuprofen-loaded particles were administered subcutaneously (1 mg/kg) to overnight-fasted rats. As a reference of immediate

dosage form, an ibuprofen solution (Pedea®) was administered subcutaneously at the same dose (1 mg/kg) in a second group of rats. The areas under the curves (AUCs) of the concentration–time profiles were calculated with the linear trapezoidal method. The relative bioavailability was calculated by the ratio of the respective AUC corrected by the administered doses. C_{max} and T_{max} were also observed as the kinetic parameters of absorption.

3.6.4. Statistical analysis

Results are presented as means ± standard deviation (SD). Multiple mean comparison was performed by Kruskal–Wallis Test, followed by the Student–Newman–Keuls test for group × group comparisons. A p value <0.05 was considered significant.

4. Results

The main physicochemical parameters (mean diameter, zeta potential and encapsulation efficiency) of the three types of ibuprofen dosage forms prepared with the various polymers were determined. Both unloaded PCL nanoparticles and ibuprofen-loaded PCL nanoparticles have a diameter around 350 nm (375 ± 6.0 and 341 ± 9.0 nm, respectively) with almost no charge (−1.3 ± 1.6 and + 2.9 ± 3.9 mV, respectively). The encapsulation efficiency is high (95 ± 2%).

Ibuprofen-loaded simple and composite microparticles are larger than unloaded ones. Indeed, the diameter of all ibuprofen-loaded microparticles is close to 35 μm, whereas unloaded microparticles exhibit a smaller size (25 μm). Composite microparticles have almost the same diameter as simple microparticles for both ibuprofen-loaded and ibuprofen-unloaded ones. The ibuprofen encapsulation efficiency in simple microparticles (84 ± 3%) and composite microparticles (89 ± 2%) is less than PCL nanoparticles (95 ± 2%) but still relatively high (Table 1).

Fig. 1 displays the release profiles of ibuprofen from ibuprofen bulk powder, PCL nanoparticles, PLGA simple microparticles and PLGA composite microparticles. Ibuprofen bulk powder was completely dissolved at the first sampling point (i.e. 100% at 5 min). Ibuprofen-loaded PCL nanoparticles display an important drug release (86 ± 7%) in the first 15 min corresponding to a significant ibuprofen initial burst effect followed by a plateau up to 24 h (Table 2). Different release profiles were obtained with PLGA simple and composite microparticles. The initial ibuprofen burst release was more important (21% after 15 min) with PLGA simple microparticles than PLGA composite microparticles (9% after 15 min) with a progressive and controlled release profile up to 24 h for both types of microparticles (62% and 39% after 24 h for simple and composite microparticles, respectively). Moreover, a common trend for all tested microparticles was the non-complete release of ibuprofen in 24 h as well as a controlled burst effect release with PLGA composite microparticles and, to a lower extent, with PLGA simple microparticles.

After subcutaneous administration of the ibuprofen solution (Pedea®) and the different multiparticular ibuprofen dosage forms, blood was collected till 96 h. Ibuprofen was only detected in the blood samples (limit of quantification: 50 ng/mL) during 24 h following the ibuprofen solution or ibuprofen–PCL nanoparticles

Table 1
Mean diameter and drug encapsulation efficiency of unloaded, ibuprofen simple microparticles (SMP) and composite microparticles (CMP) (n = 3 ± SD).

	Mean diameter (μm)		Encapsulation efficiency (%)	
	Unloaded	Ibuprofen	Unloaded	Ibuprofen
SMP	24.3 ± 4.7	37.9 ± 2	–	84 ± 3
CMP	27.9 ± 4	33.7 ± 7	–	89 ± 2

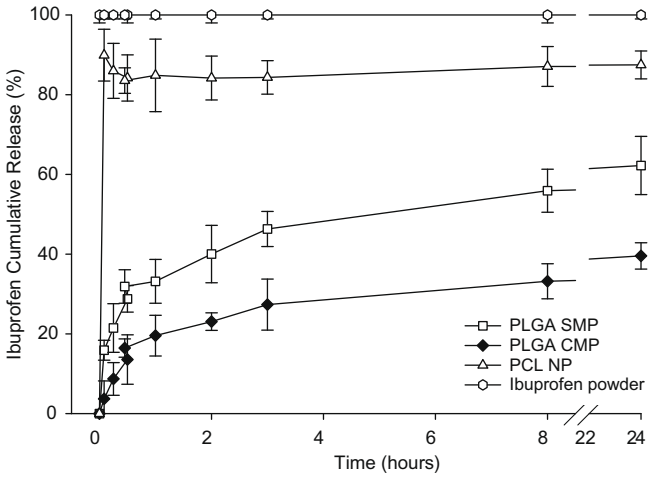

Fig. 1. Release kinetics of ibuprofen from ibuprofen bulk powder, ibuprofen PCL nanoparticles (PCL NP), ibuprofen PLGA simple microparticles (PLGA SMP) and ibuprofen PLGA composite microparticles (PLGA CMP) for 24 h. Experiments were performed in phosphate buffer pH 7.4 at 37 °C under sink conditions. Data shown as mean ± SD (n = 3).

Table 2
Mean percentages of ibuprofen released *in vitro* after 15 min and 24 h from bulk powder, PCL nanoparticles (NP), PLGA simple microparticles (SMP) and PLGA composite microparticles (CMP) (n = 3 ± SD).

Formulations	Polymers	Ibuprofen released (%)	
		15 min	24 h
Powder	–	100	100
PCL NP	PCL	86 ± 6.9	87.5 ± 3.5
PLGA SMP	PLGA	21.5 ± 6.1	62.2 ± 7.3
PLGA CMP	PCL/PLGA	8.7 ± 4.1	39.5 ± 3.3

subcutaneous administration, whereas it was detected for a much longer time (up to 48 h) for PLGA simple microparticles and PLGA composite microparticles. Fig. 2 shows the mean plasma concentrations of ibuprofen after subcutaneous administration (1 mg/kg) of the solution (Pedea®), the ibuprofen-loaded PCL nanoparticles, the PLGA simple and composite microparticles. During the first 8 h, the ibuprofen–plasma profiles appeared different with all dosage forms. The subcutaneous administration of ibuprofen solution (Pedea®) gave rise to an immediate peak. The highest mean plasma concentration (C_{max} 8691 ng/mL) for the Pedea® ibuprofen solution was obtained rapidly (15 min) and then decreased to 961 ng/mL after 8 h (Table 3). Administration of PCL nanoparticles suspension led to a high initial ibuprofen–plasma level of about 5070 ng/mL after 45 min. This initial peak was followed by a gradual decrease in plasma ibuprofen, which reached 1630 ng/mL after 8 h. C_{max} was much lower and much longer to achieve with PLGA simple microparticles (about 2 h) and PLGA composite microparticles (4 h) dosage forms (C_{max} 2033 and 1249 ng/mL, respectively). Then, a prolonged plateau, up to 6–8 h, was observed for composite and simple microparticles indicating a constant release rate for ibuprofen (Fig. 2B). Plasma concentrations decreased slowly till 48 h where the quantification limit was reached.

The pharmacokinetic parameters obtained from the plasma ibuprofen concentrations are summarized in Table 4. Based on the concentration–time profiles, areas under the ibuprofen concentration curves (AUCs) were calculated. The AUCs ranked according to the following order: Pedea® > PCL nanoparticles > simple microparticles > composite microparticles. For the first 24 h, the highest relative bioavailability with regard to the Pedea® solution is obtained with the nanoparticles suspension, whereas PLGA composite microparticles exhibit the lowest one. Moreover PLGA simple

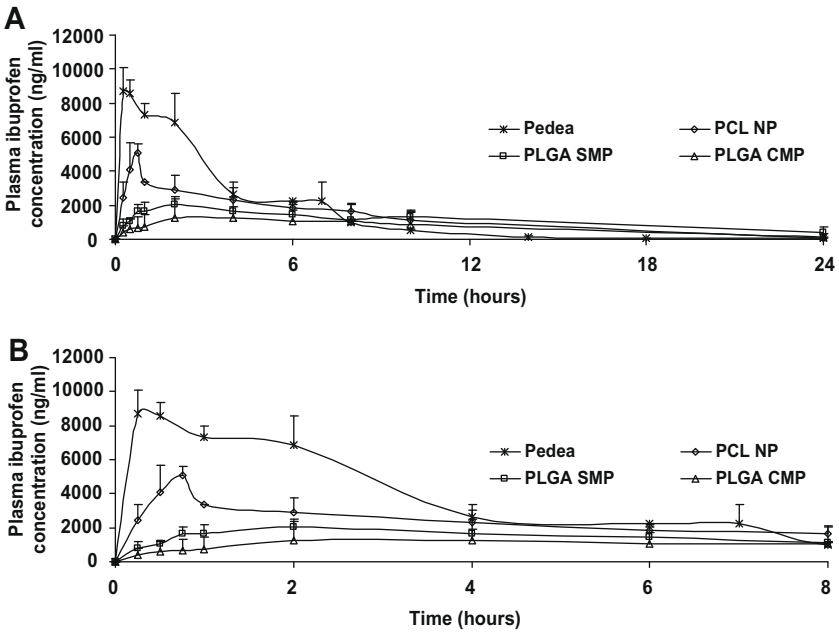

Fig. 2. Plasma ibuprofen concentrations as a function of time after subcutaneous administration in rats (1 mg/kg) of various formulations: ibuprofen solution (Pedea®), ibuprofen nanoparticles (PCL NP), ibuprofen PLGA simple microparticles (PLGA SMP) and ibuprofen PLGA composite microparticles (PLGA CMP). Data shown as mean ± SD ($n \geqslant 3$) either for 24 h (A) or 8 h (B).

Table 3
Mean ibuprofen–plasma concentrations (ng/mL) 15 min and 8 h after subcutaneous injection of Pedea® solution, PCL nanoparticles (NP), PLGA simple microparticles (SMP) and PLGA composite microparticles (CMP) in rats at the dose of 1 mg/kg ($n \geqslant 3 \pm$ SD).

Formulations	Polymers	Ibuprofen–plasma concentration (ng/mL)	
		15 min	8 h
Ibuprofen solution (Pedea®)	–	8691 ± 1386	961 ± 183
PCL NP	PCL	2449 ± 932	1630 ± 386
PLGA SMP	PLGA	795 ± 395	1119 ± 100
PLGA CMP	PLGA (PCL)	398 ± 51	1043 ± 165

Table 4
Pharmacokinetic parameters of ibuprofen after subcutaneous administration in rats ($n \geqslant 3$): PCL nanoparticles (PCL NP), PLGA simple (SMP) and PLGA composite (CMP) microparticles and ibuprofen marketed solution (Pedea®).

	Ibuprofen solution	PCL NP	PLGA SMP	PLGA CMP
T_{max} (h)	0.25	0.75	2	4[a,b]
C_{max} (ng/mL)	8691	5071[a]	2033[a]	1249[a,b]
AUC 0 → 24 h				
AUC 0 → 48 h	35950	30491	26506	17955
AUC 0 → 96 h	–	–	35814	20151
(ng/mL h)	–	–	–	21450
Rel. F (%)				
(0–24 h)	100	84	73	49
(0–48 h)	100	84	99	56

[a] Significantly different from ibuprofen solution ($p < 0.05$).
[b] Significantly different from PCL nanoparticles ($p < 0.05$).

and composite microparticles show continuous ibuprofen release and stable plasma concentration for longer times leading to either 99% and 56% relative bioavailability after 48 h for simple and composite microparticles respectively.

Results in Table 4 also indicate that the ibuprofen burst release is significantly more reduced considering T_{max} ($p = 0.004$) and C_{max} ($p = 0.016$) values with PLGA composite microparticles than with both the ibuprofen solution, the PCL nanoparticles suspension and with PLGA simple microparticle *in vivo*. The same result was also observed *in vitro*.

5. Discussion

Burst release is a critical problem with currently marketed injectable microparticles, especially when slow release for a few weeks or months is expected. The encapsulation of nanoparticles into microparticles, as an alternative way to decrease the burst, has been proposed by only a few groups for hydrophilic drug [18,21–24,34].

As previously reported [21] composite microparticles made with hydrophobic polymers for both microparticles and nanoparticles (such as PLGA, PCL, PLA, Eudragit® RS, ethylcellulose) would certainly have the potential to eliminate or at least decrease the burst effect for hydrophilic and hydrophobic drugs when compared to hydrophilic polymers (such as gelatin, alginate, chitosan). But the encapsulation of a hydrophobic polymer (as nanoparticles) in another hydrophobic polymer (as microparticles) is really challenging with classical microparticles techniques.

In a previous work, we have already prepared ibuprofen–PCL nanoparticles that were incorporated into microparticles made of ethylcellulose, Eudragit® RS or their 1:1 blend. Such composite microparticles were able to control the ibuprofen burst *in vitro* [21]. However, with a view to injecting the composite microparticles subcutaneously, it was mandatory to use two biodegradable polymers. This is why PLGA was selected as the polymer-constituting microparticles. In our already-mentioned previous work, the original idea was to use a solvent (ethyl acetate) in which one of the polymers (PCL) was not soluble during the double emulsion process. Replacing the previous non-biodegradable polymers (ethylcellulose, Eudragit® RS) by PLGA did not change anything since the latter is also soluble in ethyl acetate. Indeed, microparticles were prepared by dissolving PLGA and span 60 in ethyl acetate, which is a poor solvent for PCL. In our conditions of microparticles preparation, the maximum solubility of PCL, when added directly in ethyl acetate, is 5%. Therefore, it was possible to use the PCL nanoparticles suspension as the internal aqueous phase in the

preparation of the composite microparticles since this polymer is mostly not dissolved in ethyl acetate.

Ibuprofen was selected as the model drug for the *in vivo* study. Indeed, (i) it corresponds to a small molecule (MW = 206.3), and it is poorly water soluble (35.89 μg/mL [29]), (ii) almost all ibuprofen microparticles and nanoparticles show initial burst release [4,20,29,35,36] and (iii) it has a short elimination half-life (about 2 h after oral administration in man [37]). Furthermore, it could be interesting to develop a new injectable and possibly once a day dosage form of ibuprofen for intraarticular administration [14].

Ibuprofen was encapsulated both in nanoparticles and microparticles with a high efficiency (simple or composite). This high encapsulation efficiency of ibuprofen could be explained by the lipophilic nature of the drug, which has no or very low affinity for the external aqueous phase. However, the encapsulation efficiency of ibuprofen in PCL nanoparticles (95%) is higher than in both types of microparticles (84% and 89% for simple and composite microparticles, respectively). This might be due to a solvent effect; indeed, methylene chloride was used during PCL nanoparticles preparation instead of ethyl acetate for the microparticles manufacturing. Ibuprofen is more soluble in methylene chloride (around 800 mg/ml at 4 °C) than in ethyl acetate (around 300 mg/ml at 4 °C), so diffusion of ibuprofen from ethyl acetate to water is probably easier than from methylene chloride. Indeed, this was already reported by Mainardes and Evangelista [38] who noticed that the encapsulation efficiency of praziquantel in PLGA nanoparticles increased when the solvent was methylene chloride compared to ethyl acetate. They attributed this phenomenon to the higher water solubility of ethyl acetate. Thus, there is a faster partitioning of ethyl acetate in the external phase accompanied by the polymer precipitation with decreasing drug incorporation into nanoparticles. In addition, it has also to be kept in mind that the volume of the external phase is much smaller during the nanoparticles preparation (40 mL) than during the microparticles preparation (2 L). The similar encapsulation efficiency observed with PLGA simple and composite microparticles may be explained by the overall fast PLGA precipitation when ethyl acetate is extracted in water [39]. Thus, it would consequently be more difficult for ibuprofen to diffuse towards the outer water phase very differently for the two types of microparticles.

In order to definitely demonstrate that nanoparticles were effectively entrapped in microparticles, we have carried out the following experiment with ibuprofen-loaded micro and nanoparticles. Ibuprofen–PLGA composite microparticles, ibuprofen–PLGA simple microparticles, ibuprofen–PCL nanoparticles and also 50 mg of ibuprofen powder (same quantity used for manufacturing microparticles) were dispersed in ethyl acetate (Fig. 3). It has to be remembered that PLGA and span 60 are soluble in ethyl acetate but not the PCL polymer (or very slightly as stated before). Therefore, if PCL nanoparticles have effectively been encapsulated, the resulting suspension in ethyl acetate should be very turbid as should be the ibuprofen–PCL nanoparticles suspension also dispersed in ethyl acetate. Fig. 3 clearly displays a similar turbidity in ethyl acetate of both PCL nanoparticles and PLGA composite microparticles showing the dissolution of PLGA and Span 60 but the presence of PCL nanoparticles. At the opposite, ibuprofen–PLGA simple microparticles are totally dissolved in ethyl acetate and lead to the same clear solution as the ibuprofen powder. Consequently, Fig. 3 shows that the manufacturing process allows the encapsulation of PCL nanoparticles in PLGA composite microparticles and demonstrates the composite character of these microparticles.

There are two ways to verify that the burst is controlled i.e. either by an *in vitro* dissolution test or by an *in vivo* approach for instance after subcutaneous or intramuscular administration. We have used both approaches to verify the potential burst reduction with composite microparticles.

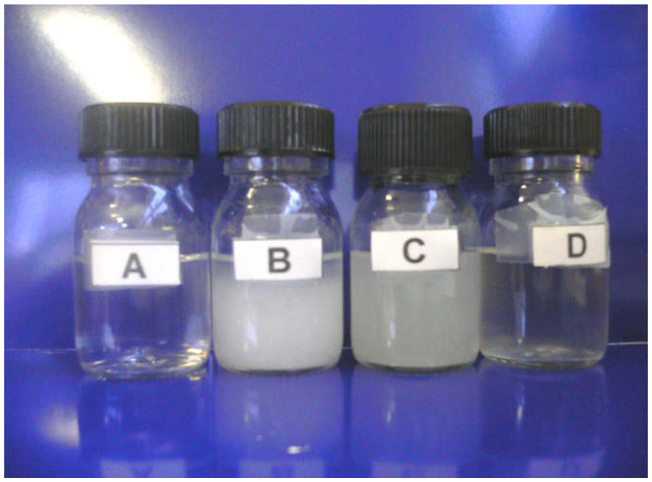

Fig. 3. Picture showing the macroscopic solubility in ethyl acetate of different nano and microparticles prepared with different polymers: (A) ibuprofen powder, (B) ibuprofen-loaded PCL nanoparticles, (C) ibuprofen-loaded PLGA composite microparticles, (D) ibuprofen-loaded PLGA simple microparticles.

In vitro, it was possible to rank the four dosages forms according to the burst: bulk powder > PCL nanoparticles > simple microparticles > composite microparticles (Fig. 1 and Table 2). It is obvious that the lowest burst was achieved with the composite microparticles. Indeed, the burst at 15 min was almost three times more with the simple microparticles and about 10 times more with the PCL nanoparticles and the bulk powder than the composite microparticles. The incomplete ibuprofen release (87.5 ± 3.5% at 24 h) may be explained by loss of nanoparticles during sampling due to retention on the 0.22 μm filter.

Differential scanning calorimetry was carried out with the three multiparticular dosage forms and demonstrated that ibuprofen was dispersed under amorphous form in the polymer matrix (data not shown). This is in favor of fast release dissolution in the dissolution medium, but there is no difference between the three dosage forms, and this factor is not able to explain the observed burst differences. In the case of PCL nanoparticles, the main factor driving ibuprofen dissolution is the large exchange surface area developed with the outer medium due to the very small diameter of nanoparticles. Since the diameter of both simple microparticles and composite microparticles is relatively close (around 35 μm), this parameter cannot explain the differences observed between the two types of microparticles.

Therefore, other hypotheses have to be taken into account to explain such differences in the burst. For instance, it has been shown for lipophilic drugs (NSAID, nifidepine, dexamethasone, lidocaine) that the burst is mainly dependent on the internal morphology of particles and drug distribution state including surface association [4,36,40], which is affected by process and formulation parameters (especially temperature) and drug/matrix interactions. Temperature was the same for preparing the simple microparticles and the composite microparticles, so this parameter can also be ruled out. Both types of microparticles being prepared according to the same double emulsion method, this should also not affect the overall porosity. It seems more likely that heterogeneous distribution and physicochemical nature of the polymer matrix are the two main different parameters. Indeed, for composite microparticles, it is not only the drug but the PCL nanosuspension, which is distributed in the PLGA polymer matrix. Thus, drug distribution could be different between simple and composite microparticles. In addition, PCL is a more hydrophobic polymer than PLGA, which may also slow down water diffusion in the incorporated nanoparticles. As for the polymer matrix, it is

also obvious that, in composite microparticles, there are two barriers for the drug to diffuse through. The first barrier is due to the PCL polymer and the second barrier is the outer PLGA matrix. It is reasonable to make the assumption that the double layer of polymers is the main reason to explain the dramatic *in vitro* burst reduction.

Similar ibuprofen burst reduction has been described by different authors but using different approaches. For instance, Wang et al . [5] deposited polysaccharides on ibuprofen-loaded poly(hydroxybutyrate-co-hydroxyvalerate) microspheres using layer-by-layer self-assembly to produce core–shell microparticles. Saravanan et al. [41] prepared ibuprofen microspheres with very lipophilic and non-biodegradable polymers such as ethylcellulose/polystyrene and Fernandez-Carballido et al. [14] added Labrafil® oil (non-ionic amphiphilic excipient) in PLGA microspheres loaded with ibuprofen.

As observed in Fig. 1, both simple and composite microparticles release ibuprofen very slowly since only around 60% and 40% of drug are found in the outer phase after 24 h, respectively. This is another feature of the composite microparticles whose very slow release pattern could also be of interest in the development of a long-lasting injectable dosage form.

Although the entire encapsulated drug was not released within 24 h, the dissolution test was limited to this time since the goal of this research work was to demonstrate the influence of the encapsulation of nanoparticles into microparticles on the initial burst release. These results from *in vitro* release study were confirmed with the *in vivo* study.

Due to their small average diameter, the suspension of nanoparticles was easily injected subcutaneously. As for the simple and composite microparticles, it was first necessary to disperse them in a relatively viscous aqueous solution prior to injection. The carboxymethylcellulose aqueous suspension of simple and composite microparticles also allowed an easy subcutaneous injection and was characterized by (i) a good stability [no sedimentation observed during preparation and injection (2–5 min)], (ii) good syringability (23 G) and (iii) a physiological pH of 7.4. As already observed, the ibuprofen solution and nanoparticles display a fast absorption, whereas absorption is much slower for simple and composite microparticles.

C_{max} and T_{max} reflect the absorption rate of a drug. Therefore, the initial burst is correlated to the values of the two latter parameters. In terms of burst effect, the four dosage forms can be ranked as: Pedea® solution > PCL nanoparticles > simple microparticles > composite microparticles. There is a 7-fold times difference between the solution and the composite microparticles without mentioning that the T_{max} values are also very different between the two dosage forms (0.25 and 4 h, respectively). The difference in C_{max} between the composite microparticles and simple microparticles is less spectacular but is still statistically significant (1.6 times more). Thus, it is confirmed that the microparticle systems, and more particularly the composite microparticles, have a dramatic influence on the initial burst release *in vivo*. Furthermore, the rank order in decreasing C_{max} is exactly the same as it is for the ibuprofen-dissolved percentage after 15 min in the *in vitro* release test. The same hypotheses, as already discussed for the *in vitro* dissolution results, may explain the observed *in vivo* results. Thus, it has been definitely confirmed that the composite microparticles present a strong potential in reducing the burst effect not only *in vitro* but also *in vivo*.

The *in vivo* study was performed for 96 h in order to evaluate the potential of the three multiparticular dosage forms as prolonged release compositions. Unfortunately, and despite the set-up of a new HPLC method with lower detection limits for ibuprofen [42], the ibuprofen–plasma concentrations were below the limits of quantification after 18 h and 24 h for the ibuprofen solution and the PCL nanoparticles, respectively. For the simple and composite microparticles, the limit of quantification was reached after 48 h. However, for composite microparticles, ibuprofen was still detected in the plasma till 96 h, which demonstrates that ibuprofen is released for longer periods of time with composite microparticles. However, taking into account the relatively low number of animals and the normal *in vivo* variability, the figures of relative bioavailability for nanoparticles and both types of microparticles are relatively close, and it can be considered that the bioavailabilities are the same and around 100% (there is no statistical significant differences between AUC values of the three multiparticular dosage forms). In the case of composite microparticles, about half of the incorporated ibuprofen has been released in 48 h. This shows the great potential of such dosage forms to act also as long release microparticles. One way to increase or decrease the release rate of drugs from such composite microparticles would be to play on the type of biodegradable polymers used in their manufacturing. Indeed, higher or smaller molecular weights of the two types of polymers could lead to tailor made release. Such an approach will be used in the continuation of this work.

6. Conclusion

When ibuprofen–PCL nanoparticles are encapsulated in microparticles, a strong decrease in the drug burst release is observed. Therefore, the advantage of encapsulating nanoparticles in microparticles (composite microparticles) is definitely demonstrated *in vivo* with hydrophobic matrix such as PCL and PLGA. The use of slower degrading polymers such as PLA or even PLGA or PCL with larger molecular weights could still increase the slow release potential of these formulations.

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