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COMMUNICATION

Entrapment Efficiency and Initial Release of Phenylbutazone from Nanocapsules Prepared from Different Polyesters

H. Marchais,* S. Benali, J. M. Irache,
C. Tharasse-Bloch, O. Lafont, and A. M. Orecchioni

Laboratoire de Pharmacochimie et Biopharmacie (EA DRED 1295), UFR de Médecine et de Pharmacie, Université de Rouen, B.P. 97, 76803 Saint Etienne du Rouvray Cedex, France

ABSTRACT

Several formulations of poly(ε-caprolactone) (PCL), poly(lactic acid) (PLA), and poly(lactic-co-glycolic acid) (PLGA) nanocapsules containing phenylbutazone were prepared according to the interfacial deposition technique. These formulations differed in the type of polymer used to form the shell of the nanocapsules. Analysis of particle size distribution and encapsulation efficiency of the nanocapsules revealed that the type and molecular weight of polyester used were the main factors influencing these properties. PLA had the highest encapsulation efficiency with the best reproducibility. From in vitro release studies, a small amount of drug release was observed at pH 7.4. However, in the gastric medium, an important burst effect occurred and was highest with the PLGAs and lowest with PCL, suggesting that drug release from these systems is affected by the type of polymer and the environmental conditions. The two formulations of phenylbutazone-loaded nanocapsules should be evaluated based on PCL and PLA in vivo in order to determine to what extent they are able to reduce the local side effects of this drug.

* To whom correspondence should be addressed. Fax: (33) 02.35.66.55.75. e-mail: Herve.Marchais@univ-rouen.fr

INTRODUCTION

Increasing the efficiency and efficacy of drugs by suitable delivery systems is a major objective of pharmaceutical technology. One possible strategy is the use of nanoparticulate delivery systems. Nanoparticles are solid polymeric colloidal particles ranging in size between 10 and 1000 nm. According to the process used in the preparation of nanoparticles, nanospheres or nanocapsules can be obtained. Nanospheres are matrix systems formed by a polymeric network, and nanocapsules are vesicular systems consisting of an internal, liquid core surrounded by a polymeric membrane (1,2). Drugs are usually entrapped, dissolved, or dispersed in the matrix or in the liquid core, but they can also be adsorbed at the surface of nanoparticles.

These systems can be manufactured from a large number of starting materials and by different preparation methods which generally employ liquid systems from which a solid phase containing the polymer and the drug is obtained by physical or chemical methods (3). Among the biodegradable polymeric materials, polyesters have been widely used for the preparation of nanoparticles. These polymers, including poly (ε-caprolactone) (PCL), poly(lactic acid), (PLA), poly (glycolic acid), and their copolymers, have received increasing attention because they are suitable for producing well-tolerated and bioresorbable microparticles (4,5).

In recent years, nanoparticles as drug colloidal carriers were found to increase the efficacy and/or to reduce the side effects of potent drugs (6). For example, nonsteroidal anti-inflammatory drugs (NSAIDs) are known to be responsible for gastrointestinal side effects such as inflammation and ulceration (7). Although drug formulation has little or no effect on inherent systemic adverse effects of these drugs, the effectiveness of colloidal drug delivery system such as nanocapsules in reducing gastric mucosa irritation following oral administration has been demonstrated for indomethacin (8,9). Several other NSAIDs such as ibuprofen (10) and diclofenac (11) have been encapsulated into nanoparticles, but phenylbutazone has not, to our knowledge.

The aim of this work was to prepare and characterize phenylbutazone nanocapsules made from four different polyesters according to the interfacial deposition technique. The influence of these polymers on the entrapment efficiency, and the capacity of a nanoparticulate system to retain the drug are discussed.

MATERIALS AND METHODS

Materials

Poly(DL-lactide-*co*-glycolide) (PLGA, 75:25, molecular weight 75–120,000), PLGA 65:35 (molecular weight 40–75,000), benzyl benzoate, and phenylbutazone were obtained from Sigma (St. Louis, MO). PLA (molecular weight 150,000) were purchased from PHUSIS (Le Versoud, France) and PCL (molecular weight 64,000) and from Aldrich-Chemie (Steinheim, Germany), respectively. Synperonic® PE/F68 was supplied from I.C.I. (Kortenberg, Belgium) and double-distilled water was obtained from Biosedra (Malakoff, France). Acetonitrile, perchloric acid, acetone, sodium chloride, and all other chemicals were of analytical grade and were purchased from Prolabo (Paris, France).

Preparation of Phenylbutazone-Loaded Nanocapsules

Nanocapsules were prepared by an interfacial deposition process following solvent displacement as previously described (12). Briefly, a variable amount of polymer (between 80 and 200 mg) was dissolved in 40 ml acetone containing 0.4 ml benzyl benzoate and the drug (40 mg). This organic phase was then poured in a constantly stirred aqueous phase (80 ml) containing 0.5% w/v Synperonic PE/F68 as stabilizer. Acetone was then eliminated and the final volume of the suspension was adjusted to 20 ml by evaporation under reduced pressure.

Nanocapsule Evaluation

Photon Correlation Spectroscopy

Particle size distribution, average size, and polydispersity index were measured by laser light scattering using a monochromatic laser ray diffusion counter (Coulter® N4MD submicron particle analyzer, Coultronics, Margency, France).

Phenylbutazone Entrapment Efficiency

One milliliter of each phenylbutazone-loaded nanocapsule batch was dissolved in 200 ml acetonitrile and total phenylbutazone content was then assayed by reversed-phase HPLC under the following conditions. The HPLC system consisted of an isocratic solvent delivery pump (110A pump, Beckman Instruments, Berkeley, CA), a 20-µl sample loop injector (model 7125 Rheodyne, Latek, Eppelheim, Germany), a reversed-phase col-

umn Nucleosil C18 (250 × 4 mm i.d., 5 μm particle size, Macherey-Nagel, Hoerdt, France), and a variable-wavelength UV detector (model 166, Beckman). The data recording system consisted of an IBM personal computer PS/2 model 8550.z with system Gold software (version 5.1, Beckman). The chromatography was performed at room temperature. The mobile phase was a mixture of acetonitrile and water (55:45 v/v) containing 70% perchloric acid (0.2% v/v) at a flow rate of 1.0 ml/min. The peaks were monitored at 240 nm.

The aqueous phase containing free phenylbutazone was separated after centrifugation of 5 ml nanocapsule suspension at 19,000 × *g* for 45 min. The supernatant was removed and the free drug content was measured by HPLC as described above.

The entrapment efficiency, expressed as a percentage, was then calculated as follows:

$$\text{Entrapment efficiency } (\%) = \frac{(C_1 - C_2)}{C_1} \times 100 \quad (1)$$

where C_1 is the total phenylbutazone content in the nanocapsule suspensions, and C_2 is the amount of free phenylbutazone (data from HPLC measurements).

To evaluate the influence of the type of polymer on the encapsulation efficiency, a one-way ANOVA was performed.

In Vitro Drug Release Studies

To investigate the initial release of phenylbutazone, samples of different batches of phenylbutazone-loaded nanocapsules were diluted with different buffers at 1/5, 1/10, 1/50 and 1/100 v/v. These studies were performed with gastric medium (pH 1.2) (USP 23), phosphate-buffered saline (PBS, pH 7.4) and pH 2 buffer (Pharmacopée Française X). Incubations were maintained at 37°C and stirred at 200 rpm for 10 min. Aliquots (1 ml) were collected and centrifuged for 45 min at 19,000 × *g*. The supernatants were immediately analyzed for phenylbutazone content by HPLC. The percentage release of phenylbutazone was calculated from the ratio of drug concentration in the supernatant versus the total concentration of phenylbutazone in the release solution.

RESULTS AND DISCUSSION

Nanocapsule Size Determination

The method used to prepare phenylbutazone-loaded polyester nanocapsules in this study is based on the interfacial deposition of a synthetic polymer in solution around oily droplets previously dispersed in the nonsolvent phase. It was previously demonstrated that both solvent (acetone in our case) and nonsolvent phases (water containing Synperonic as stabilizer) must have low viscosities and high mixing capacities in all proportions (13).

Table 1

Physicochemical Properties of Different Phenylbutazone-Loaded Polymer Nanocapsule Batches Obtained with 150 mg Polymer (n = 3); Data Expressed as Mean ± Standard Deviation

Polymer	Molecular Weight[a] (×10³)	Particle Size (nm)	Entrapment Efficiency (%)
PCL	64	293 ± 17	89.0 ± 1.8
PLA	150	236 ± 8	99.5 ± 0.1
PLGA 75:25	75–120	250 ± 13	99.0 ± 0.4
PLGA 65:35	40–75	271 ± 8	98.4 ± 1.2

[a]Data from manufacturers.

The influence of the type of polymer used during the preparation on the mean nanocapsule size is reported in Table 1. The different nanocapsule batches were prepared with 150 mg of each polymer. The colloidal suspensions showed a unimodal size distribution with a narrow size dispersion. It was especially interesting that the mean particle size of nanocapsules appeared to depend on the type and molecular weight of the polyester used. Thus, PLA and its copolymers enabled nanocapsules with smaller mean sizes to be obtained than did PCL. It also appeared that by increasing the polyester molecular weight (irrespective of the type of polymer) the mean nanocapsule size decreased. Finally, no evidence of an influence of polymer concentration on the mean nanocapsule size was found (data not shown). Under the experimental conditions used here, for all polyesters, increasing the polymer concentration used to prepare nanocapsules did not affect the mean particle size of the systems ($p < 0.05$).

Phenylbutazone Encapsulation Efficiency

Both the total phenylbutazone content in the nanoparticulate suspension and the amount of free drug were determined by the reversed-phase HPLC technique described. This technique requires the addition of an acid, such as perchloric acid, to the mobile phase to prevent the ionization of phenylbutazone; thereby producing better peak shapes and more reproducible retention times.

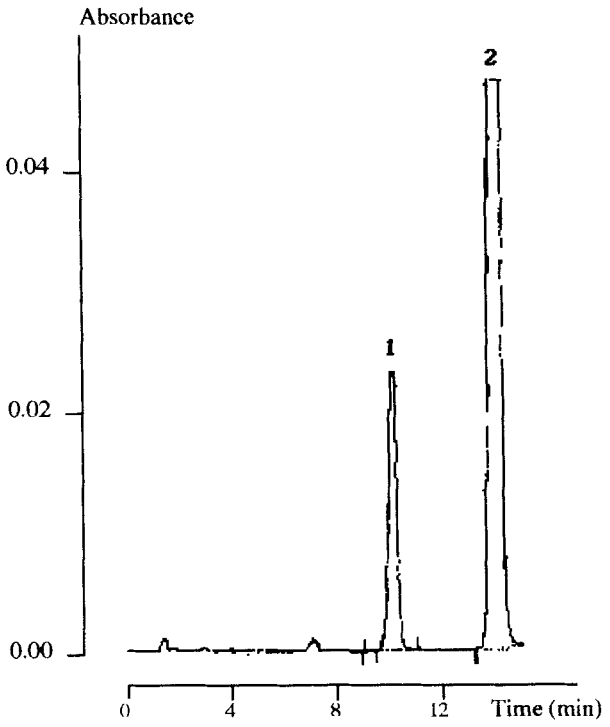

Figure 1. Chromatogram from the study of phenylbutazone content in a nanocapsule suspension. Retention times: (1) phenylbutazone: 10.4 min; (2) benzyl benzoate: 14.3 min.

The chromatograms obtained showed the peak corresponding to phenylbutazone at 10.4 min (Fig. 1). This technique also allowed us to detect benzyl benzoate as a peak appearing at 14.3 min.

From these results, the entrapment efficiency was calculated by means of Eq. (1) and this parameter was plotted against the initial amount of polymer used to prepare the phenylbutazone-loaded nanocapsules (Fig. 2). These results illustrate that the use of PLA and its copolymers enabled entrapment efficiencies close to 100% to be obtained. The efficiency of phenylbutazone loading was also high in nanocapsules prepared from PCL, but for the amount of polymer above 80 mg, the encapsulation efficiency was significantly lower in comparison with the three others polymers ($p < 0.05$). In all cases, PLA was the polymer that provided both higher drug entrapments and reproducible results.

In Vitro Release Examination

Nanocapsule batches, prepared at an initial polymer concentration of 150 mg, were tested for in vitro release

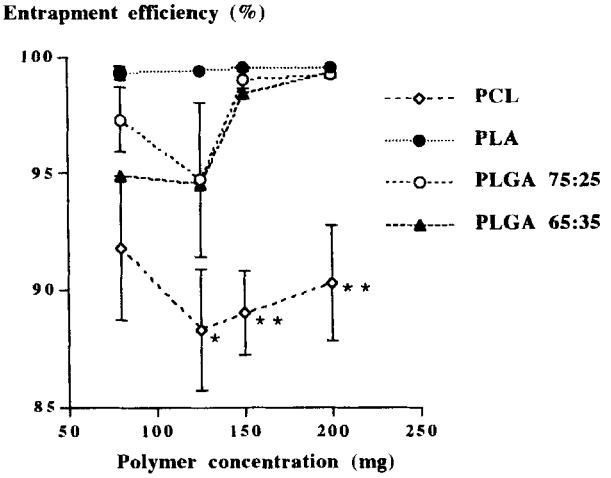

Figure 2. Influence of the initial amount of polymer on the entrapment efficiency of phenylbutazone. Data expressed as mean ± standard deviation ($n = 3$) (* $p < 0.05$, ** $p < 0.01$).

for 10 min at 37°C. As a general rule, drugs are released from nanoparticles in a biphasic way, characterized by an initial rapid and brief release period followed by a continuous and much slower release phase (14). Therefore, in order to predict the efficacy of a delivery system for the controlled release of drugs, it is interesting to investigate the in vitro release properties of these formulations during the initial period, usually called the burst effect. For nanocapsules, this burst effect can be attributed either to the desorption of the drug located on the nanocapsule surface (15) or to the degradation of the thin polymeric membrane surrounding the oily core containing the drug.

Figure 3 shows the experimental data plotted as the amount of phenylbutazone released from the polyester nanocapsules as a function of pH and the dilution ratio (v/v). At neutral pH, for all polyester nanocapsules, the dilution ratio had little effect on the initial phenylbutazone release. Nevertheless, under acidic conditions and with high dilution ratios (1/50 and 1/100) the initial release of phenylbutazone from nanocapsules increased considerably. This was particularly marked when experiments were carried out in synthetic gastric medium. It was also clear that in order to minimize this initial drug release, the optimal formulation was that obtained from PCL. In this case, at pH above 2 and for all dilution ratios, the amount of phenylbutazone released in the first 10 min was always less than 14% of the total loaded drug. PCL is a semicrystalline polymer, and crystallinity is known to play an important role in the polymer hydrolysis rate

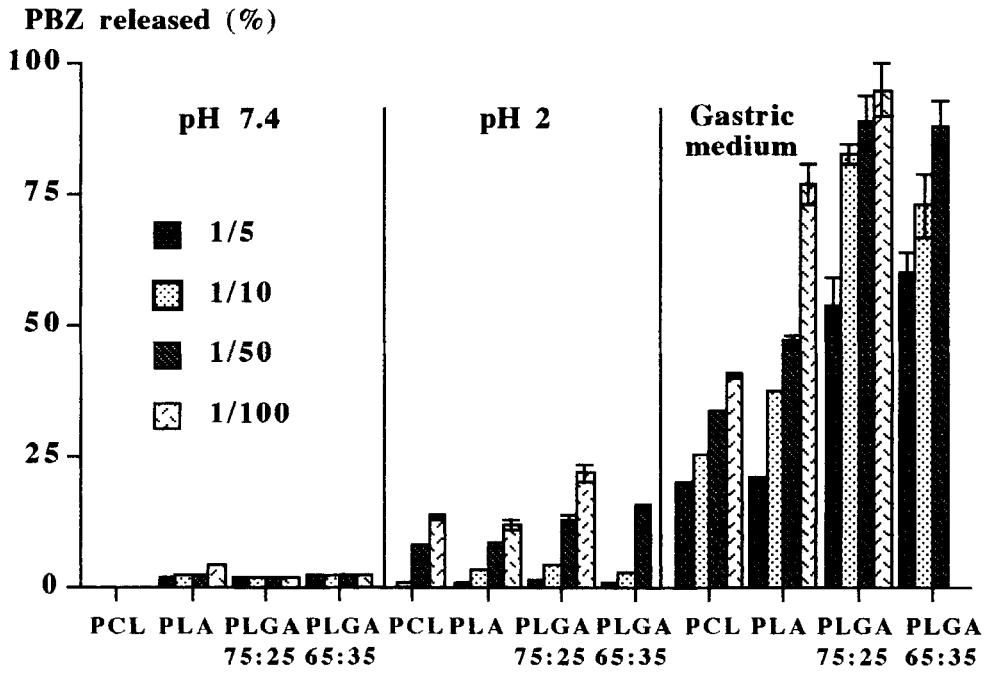

Figure 3. In vitro release of phenylbutazone from nanocapsules as a function of pH and dilution ratio. Experimental conditions: incubation time: 10 min at 37°C, $n = 3$.

because it is considered that the bulk crystalline phase is inaccessible to water. It has been reported that PLA and its copolymers show pH-dependent degradation which is accelerated under strongly acidic conditions (16). Another hypothesis could explain the important burst effect observed with the simulated gastric medium. The presence of pepsin may cause rapid uptake of phenylbutazone and enhance the solubilization of the drug. It is known that phenylbutazone binds to a high extent (>90%) to proteins in the blood and is poorly soluble in water. Additional experiments conducted in the same medium without pepsin have shown a twofold reduction of the burst effect for nanocapsules made from PCL.

In view of these results, phenylbutazone-loaded nanocapsules made from PCL exhibit the most reduced burst effect.

CONCLUSIONS

Colloidal nanoparticulate suspensions could be a suitable formulation for drugs with severe adverse effects such as NSAIDs. In order to assess the potential of phenylbutazone-loaded nanocapsules in vivo, it was necessary to prepare formulations with different types of polymers. In the present study, the highest encapsulation efficiency was obtained with PLA, but the lowest burst effect was observed under simulated gastric conditions with PCL. These two nanocapsule formulations based on PCL and PLA should be orally administered in vivo to determine the protective effect of these colloidal carriers against the local irritative effects of NSAIDs on the gastrointestinal tract.

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