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Primidone-loaded poly-ε-caprolactone nanocapsules: incorporation efficiency and in vitro release profiles

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Abstract

This paper describes the preparation of primidone-loaded poly-ε-caprolactone nanocapsules according to the interfacial deposition technique. The colloidal suspension obtained showed a monomodal size distribution with a mean diameter ranging from 308 to 352 nm. By adjusting the process parameters, the encapsulation efficiency was about 74% with good reproducibility. Primidone release from the nanocapsules was found to be slower as compared to the oily control solution despite an important burst-effect. The release profile was not influenced by the pH of the release medium. © 1999 Elsevier Science B.V. All rights reserved.

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1. Introduction

Primidone (5-ethyldihydro-5-phenyl-4,6(1H,5H) pyrimidinedione) is a deoxybarbiturate commonly used as anticonvulsant in the treatment of epileptic seizure in man and animals. Previous investigations have shown that, after orally administration at therapeutic levels to humans and animals, primidone is partly metabolized into two active metabolites: phenobarbitone (PB) and phenylethylmalondiamide (PEMA). The predictable known metabolites of phenobarbitone, para-hydroxy phenobarbitone and α-phenyl-γ-butyrolactone have been identified as minor metabolites of primidone.

In the case of intoxications (i.e. overdose), PEMA and α-phenyl-γ-butyrolactone are responsible of severe toxicities (Goodman and Bliss, 1953; Andresen et al., 1977). On the other hand, it has been reported that low doses of PEMA potentiated the anticonvulsant activity of PB in rats (Baumel and Mattson, 1972). Thus, it is difficult to affirm if primidone, PEMA and PB have additive or synergistic anticonvulsant action.

Therefore, it was of interest to develop a colloidal drug carrier in order to study the influence of the encapsulation of primidone on its metabolism in animals. In the last years, several researchers have shown the potential of the poly-

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ε-caprolactone nanocapsules as drug delivery systems (Marchal-Heussler et al., 1993; Losa et al., 1993; Calvo et al., 1996). Fessi et al. (1989) have developed an original method called interfacial deposition technique to obtain nanocapsules which are made of an oily core containing the drug surrounded by a thin polymeric wall. This technique was chosen because of the lipophilic nature of the primidone.

The aim of this study was to prepare nanocapsules of poly-ε-caprolactone containing primidone according to the procedure previously described by Fessi et al. (1989). The physicochemical characteristics of the nanocapsules (particle size and encapsulation efficiency) were evaluated and the in vitro release profiles were assessed.

2. Materials and methods

2.1. Materials

The poly-ε-caprolactone (PCL) was supplied by Aldrich Chimie (St Quentin-Fallavier, France). The molecular weight was 64 000 (Aldrich data). Primidone (PRM) was extracted from Mysoline® tablets (Zeneca, Cergy, France). Synperonic® PE/F68 (polyoxyethylene-polyoxypropylene copolymer) was purchased from ICI (Clamart, France). Benzyl benzoate and benzyl alcohol were obtained from Sigma (L'isle d'Abeau, France). Acetone (Rectapur® grade), acetonitrile and methanol (HPLC grade) were supplied by Prolabo (Paris, France). All other materials were analytical grade and used as received.

2.2. Primidone extraction

After grinding Mysoline® tablets with a mortar, the powder was dissolved in boiling water until saturation limit. The hot solution was rapidly filtered through fluted filter paper and primidone cristallization occurred as soon as the temperature of water decreased. The suspension was cooled overnight and the primidone was obtained by filtration. The purity of this compound was verified by microanalysis. The observed results were within $\pm 0.2\%$ of the theoretical values.

2.3. Preparation of PCL nanocapsules

Primidone-loaded PCL nanocapsules were prepared by the interfacial polymer deposition procedure. Typically, 1.5 g of PCL were dissolved in 250 ml of acetone at 50°C. Thereafter, 4 ml of a PRM solution in benzyl alcohol (60 g/l) were added to the acetonic solution. The resulting organic solution was poured under moderate magnetic stirring in 500 ml of distilled water containing 1 g of Synperonic® PE/F68, a nonionic surfactant used as stabilizing agent. The aqueous phase immediately turns milky with bluish opalescence as a result of the formation of nanocapsules. The acetone, which rapidly diffused towards the aqueous phase, was then removed under reduced pressure (Rotavapor® RE-140, Büchi, Switzerland). Finally, the colloidal suspension was concentrated to the desired volume (20 ml) by remove water under the same conditions.

2.4. Determination of the size of nanocapsules

The mean diameter and the nanoparticle size distribution were measured by photon correlation spectroscopy using a Coulter N4MD submicron particle analyzer (Coultronics, Margency, France).

2.5. Analytical determination of primidone

PRM was analyzed by using some modifications of high-performance liquid chromatography (HPLC) procedures previously reported (Liu et al., 1993; Moriyama et al., 1994). The HPLC system (Beckman Instruments, Berkeley, USA) consisted of an isocratic solvent delivery pump (Beckman 110 A) equipped with a 20 μl sample loop injector (Rheodyne 7125). Samples were chromatographed using a 5 μm (15 cm × 4.6 mm i.d.) ODS-Ultrasphere column (Beckman) thermostated at 40°C. The mobile phase, filtered through a 0.45 μm filter prior to use, consisted of a mixture of potassium phosphate buffer (0.01 M, pH 7), acetonitrile and methanol (110:50:30, v/v/v) at a flow-rate of 0.4 ml/min. The column effluent was monitored using a UV detector set at 227 nm (Beckman 166). The data recording sys-

tem consisted of an IBM personal computer PS/2 Model 55 SX with system Gold software (Beckman).

A stock solution of PRM (400 μg/ml) was prepared by dissolving the drug in methanol. Calibration curves were obtained from the methanolic PRM working solutions over the range 25–200 μg/ml. The curves were linear and passed through the origin ($r = 0.999$). The validation results were established for three injections per concentration and six concentrations. The repeatability and the reproducibility of the method were expressed by the relative standard deviation at a concentration of 100 μg/ml PRM ($n = 6$); the values were respectively 0.40% and 0.45%. The detection limit was calculated to be 2 μg/ml.

2.6. Evaluation of the encapsulation efficiency

Total PRM was determined following complete dissolution of a specific amount (1 ml) of primidone-loaded PCL nanocapsules suspension in 20 ml of acetonitrile. Twenty-five microlitres of methanol were then added in order to precipitate the polymer which was eliminated by filtration on ground-glass filter. After drying on sodium sulphate, solvents were removed by evaporation under reduced pressure to dryness and the white residue obtained was dissolved in 80 ml of methanol. Finally, an aliquot (20 μl) of this solution was injected into the HPLC system.

Free drug was measured in the clear supernatant following separation of nanocapsules from aqueous medium by centrifugation of 5 ml colloidal suspension at 19 000 rpm for 30 min (Beckman J2-21). Thereafter, 20 μl of this solution were analysed by the HPLC method described above. For the determination of the entrapped PRM, the separated nanoparticles were resuspended in 5 ml of distilled water and bath sonicated for 10 min. The aqueous medium of the resulting nanocapsules suspension was evaporated under reduced pressure to dryness and the particles were dissolved in 80 ml of acetonitrile. Then, 100 ml of methanol were added to precipitate the polymer and after filtration on ground-glass filter, the solvents were evaporated to dryness. The residue was dissolved in 80 ml of methanol and 20 μl of this solution was injected in the HPLC system.

The drug encapsulation efficiency was calculated according to the difference between the total amount of PRM in the nanocapsules suspension and the free amount in the supernatant.

2.7. In vitro drug release studies

Samples of 12 ml of nanocapsules suspension were placed in 1-l beakers of a dissolution apparatus (Sotax) containing 363 ml of simulated gastric fluid (pH 1.25) or simulated intestinal fluid (pH 7.4) (USP XXIII) without enzymes and incubated at 37°C under constant stirring (70 rpm). At appropriate time-intervals, 200 μl of the release medium were collected and a clear supernatant was obtained by a combined ultrafiltration centrifugation technique (11 000 × g for 10 min) using an Ultrafree® MC unit (30 000 MW, Millipore SA, St-Quentin-Yvelines, France). PRM concentration in the supernatant was determined using the HPLC method as described above. A primidone standard solution in benzyl alcohol was assessed in the same conditions as control. The in vitro experiments were performed under sink conditions.

3. Results and discussion

3.1. Characterization of primidone-loaded PCL nanocapsules

According to the manufacturing process previously described, the diffusion rate of the water miscible organic solvent (e.g. acetone) was very fast and the spontaneous nanodroplet formation resulted to the turbulence of the interface of the emulsion droplets as a result of the so-called Marangoni effect. Nanocapsules were formed immediately by precipitation of the coating polymer because of the reduced solubility of PCL at the interface of the oily resultant emulsion droplets containing PRM. In preliminary studies, PRM was dissolved in benzyl benzoate as lipophilic core and nanocapsules with an average diameter of 264 nm were obtained. However, only 63% of the initial amount of PRM used was recovered in the nanocapsules suspension. This could be explain

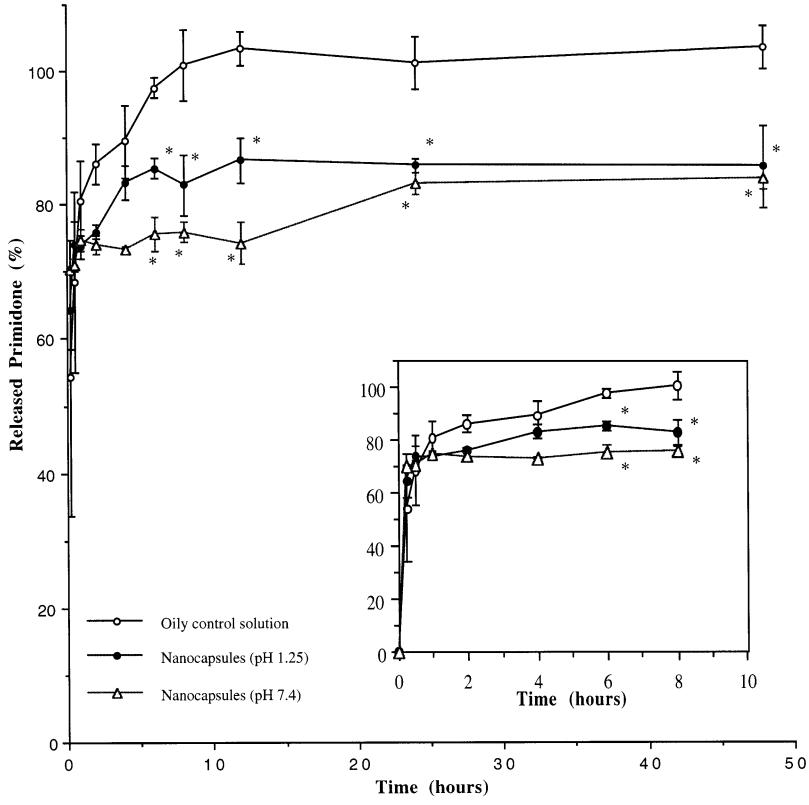

Fig. 1. In vitro release profiles of primidone from the nanocapsules (NC) in the two release media (simulated gastric fluid pH 1.25 and simulated intestinal fluid pH 7.4) and from the oily control solution (mean ± S.D., $n = 3$). The inset shows the release profiles over the first 8 h (* $P < 0.01$).

by the low solubility of the PRM in this oily compound (0.64 g/l). Several other lipophilic compound were tested and finally, the highest solubility of PRM was observed with benzyl alcohol (approximately, 15 g/l).

Several batches of nanocapsules were prepared with varying process conditions and the results are shown in Table 1.

The size of nanoparticles was higher (up to 352 nm) when the amount of polymer in the organic phase increased from 1.5 to 3.8 g. Calvo et al. (1996) have also found that the size of the PCL nanocapsules containing cyclosporin A prepared with the same method vary significantly with the amount of polymer. On the other hand, the mean particle diameter was not dramatically af-

Table 1
Physicochemical characteristics (mean particle size and encapsulation efficiency) of the formulations designed

Formulations	Polymer (g)	Oil (ml)	Acetone (ml)	PRM (mg)	Mean particle size (nm)	Encapsulation efficiency (%)
1	1.5	4	250	60	222	75
2	3.8	10	250	150	352	75
3	3.8	10	250	150	333	78
4	3.8	10	325	150	314	71
5	3.8	10	400	150	308	67

fected by the volume of acetone in the range 250–400 ml.

The encapsulation efficiency was also determined for the same batches. The results show an encapsulation efficiency varying from 67 to 78% but it appears not significantly influenced by the process conditions assessed.

3.2. In vitro drug release profiles

Drug release profiles from PCL nanocapsules in the two release media are shown in Fig. 1. The release profiles obtained from the nanocapsule suspensions were compared with that from the oily control solution.

At 8 h after the beginning, 100% of the primidone in the oily control solution was released. At the same time, the mean amount of primidone released from nanocapsules was $76.0 \pm 1.4\%$ at pH 7.4 and $83.0 \pm 4.5\%$ at pH 1.25. Despite the important initial drug release observed for the nanocapsules, a significant difference ($P < 0.01$) is noted with the oily control solution after 2 h and up to the end of the experiment. The amount of primidone released from the nanocapsules was significantly lower in comparison with control. A possible explanation could be an interaction between the drug and the polymer. In contrast, the rapid release of drug during the first hour suggests that an important part of primidone was adsorbed at the external surface of the nanocapsules. Similar results have been reported from the release of adsorbed ciproflexacin onto PIBCA nanoparticles (Fawaz et al., 1997).

4. Conclusion

Primidone-loaded poly-ε-caprolactone nanocapsules were prepared by an interfacial deposition technique. This process lead to obtain nanocapsules with a mean diameter ≈ 325 nm with an encapsulation efficiency $\approx 74\%$. The encapsulation of primidone into the nanocapsules permitted the drug release in a lesser extent in comparison with free drug. So, the primidone-loaded poly-ε-caprolactone nanocapsules would be useful for in vivo application in order to study the influence of primidone metabolism modification in animals.

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