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International Journal of Pharmaceutics 177 (1999) 247–257

international journal of pharmaceutics

The characterization and release kinetics evaluation of baclofen microspheres designed for intrathecal injection

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Received 31 July 1998; received in revised form 13 October 1998; accepted 23 October 1998

Abstract

Baclofen, a water soluble drug advocated for the treatment of spinal spasticity, was microencapsulated, using the oil/water emulsion extraction process in an attempt to identify the appropriate experimental conditions capable of producing microspheres releasing baclofen over 2–4 weeks. Individual microspheres ranging in size from 15 to 30 μm were formed exhibiting smooth surfaces at low drug payload (12.8% w/w), irregular and rough surface at high drug content (33.9% w/w). The microencapsulation yield remained practically unchanged (85–90%) up to theoretical payloads of 37.5% w/w, and decreased markedly to 70% when the initial theoretical payload was 50% w/w. The in vitro release profile of baclofen from the poly(D,L-lactide-co-glycolide) microspheres was biphasic only for the high drug payload microspheres with a rapid release of 70% within 48 h, followed by a slower release rate over at least 25 days. In contrast, the microspheres containing low baclofen contents (12.8% w/w) exhibited a gradual and progressive release rate over the course of the experiment. The baclofen release data did not fit either the general equation which describes the diffusional release of dispersed tiny drug particles from spherical micromatrices, or to the kinetic equations which describe the release of dissolved drug from monolithic microspherical devices. It appears that the release of baclofen from the present microspheres is not governed by a unique mechanism. This should be attributed either to the presence of some uncoated drug particles or to the large size of the embedded drug particles compared with the relatively small size of the spherical micromatices, or to some polymeric erosion occurring after several days incubation in the release medium.

Keywords: Baclofen; Microparticle; Oil/water emulsion extraction process; Drug payload; In vitro release; Kinetics

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0378-5173/99/$ - see front matter
PII: S0378-5173(98)00350-0

1. Introduction

Baclofen, a water soluble drug, has been advocated for the treatment of spinal spasticity. However, side-effects associated with its oral administration have limited the clinical efficacy (Hugenholtz et al., 1993). The direct application of baclofen into the spinal subarachnoid space avoids the dose-limiting adverse effects of its oral administration and can eliminate spasticity of spinal cord origin even at low efficient doses (Zierski et al., 1988; Loubser et al., 1991; Hugenholtz et al., 1992). The spasmolytic effect of intrathecal baclofen is dose related (Dralle et al., 1988). However, the experience of the different clinical studies shows that effective therapy can be maintained for almost all of the patients over the long term (chronic intrathecal infusion), but that technological problems, especially with the catheter system, may interrupt drug delivery and must be corrected (Ochs, 1989; Penn, 1992). Most of the patients are ambulatory and active, and therefore vulnerable to catheter dislodgement (Zierski et al., 1988, Ochs, 1989). Thus, to avoid the problems associated with the dislodgement of the catheter, it is intended to develop biodegradable (in vivo) microspheres of baclofen which can be injected directly into the subarachnoid space and release the drug over a period of time exceeding at least 2–4 weeks. Preliminary work has shown that blank poly(D,L-lactide-co-glycolide) (PLGA) microspheres, prepared according to the method presented in this study, have been stereotactically implanted in rat brains and were found to be biocompatible to the brain tissue (Menei et al., 1993). Furthermore, they are currently being investigated following injection in the subarachnoid space of rabbits (Menei et al., 1998). The direct administration of PLGA microspheres in the subarachnoid space appears, therefore, feasible and will probably allow the progressive sustained release of baclofen concentrations at the site of injection, where it can exert efficiently its action.

The aim of this work was to microencapsulate baclofen using the oil/water (o/w) emulsion extraction process in an attempt to identify the appropriate experimental conditions capable of producing microspheres meeting all the requirements for intrathecal administration and release of baclofen over 2–4 weeks.

2. Materials and methods

2.1. Materials

Baclofen (4-amino-3 (p-β chlorophenyl) butyric acid) was kindly provided by Novartis (Rueil-Malmaison, France). Poly(D,L-lactic acid-co-glycolic acid) copolymer 50/50 (PLGA) Resomer® 505 (MW 52 000) was purchased from Boehringer (Le Vésinet, France) and used as received. Polyvinyl alcohol (hydrolyzed to 88%, Rhodoviol® 4/125) and methylene chloride were purchased from Prolabo (Paris, France).

2.2. Microsphere preparation

The microencapsulation technique was based on the formation of an o/w emulsion under controlled stirring (RGH 500, Heidolph, Prolabo, Paris, France). A solution of 3 ml PLGA in methylene chloride (6.3% w/w), in which baclofen crystals were suspended, was poured in 170 ml polyvinyl alcohol aqueous solution (10% w/v).

A stable emulsion was obtained by mechanical stirring (700 rpm) at 10°C. After 2 min, the emulsion was added to 500 ml deionized water and stirred (500 rpm) over 2 min at 10°C. The resulting solvent extraction allowed the formation of microspheres which were collected by filtration (Millipore® filter, 8 μm pore size) after different washings with deionized water at room temperature (3×30 ml).

The isolated microspheres (250 mg batch size) were suspended in 1 ml water, frozen with liquid nitrogen and freeze-dried (RP2V Serail, SGD, Argenteuil, France).

The theoretical drug content in the microspheres was varied from 12 to 50%. Each experiment was performed in at least triplicate.

2.3. Drug content

Microspheres (10 mg) were dissolved in 1 ml of

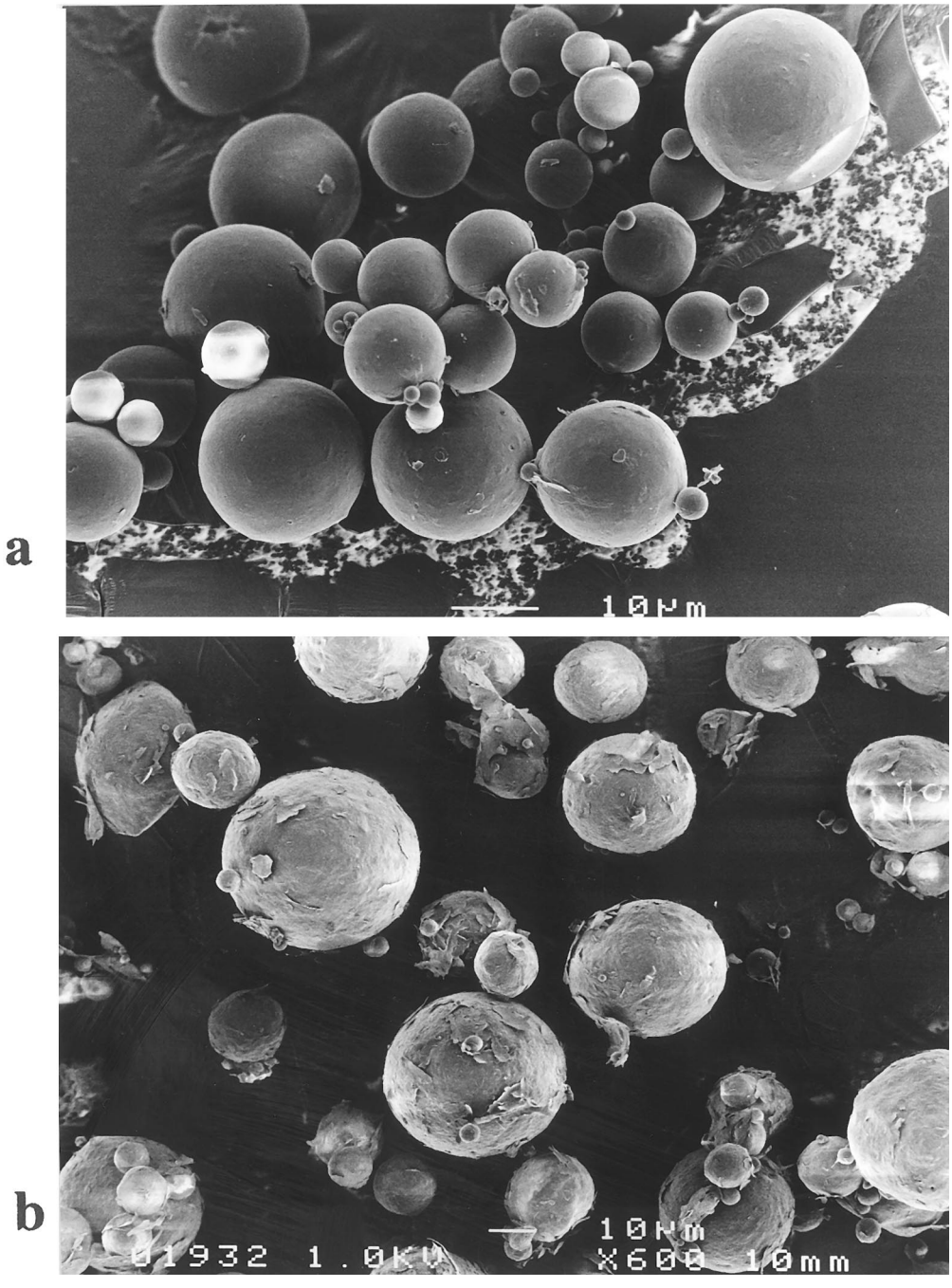

Fig. 1. Scanning electron micrograph of 12.8 % w/w (a) and 33.9 % w/w (b) baclofen-loaded PLGA microspheres.

Table 1
Baclofen-loaded PLGA microsphere characteristics

Theoretical payload (% w/w) (initial drug amount)	Mean particle size (μm ± S.D.)	Actual payload (%w/w ± S.D.)	Efficiency (% ± S.D.)
12.0	23 ± 3.7	10.3 ± 1.2	86.5 ± 14.1
16.7	20.7 ± 12.8	12.8 ± 2.4	85.0 ± 1.4
28.6	21.5 ± 3.1	26.1 ± 1.7	91.4 ± 5.9
37.5	24.2 ± 2.1	33.9 ± 2.8	90.4 ± 7.5
50.0	27.8 ± 0.3	35.2 ± 4.1	70.3 ± 8.3

methylene chloride, and baclofen was extracted with 20 g of water under constant stirring at 45°C to allow methylene chloride evaporation and precipitation of PLGA. The aqueous phase was allowed to cool, adjusted to initial weight (methylene chloride excluded) to compensate for water evaporation and then filtered. Baclofen concentration in the filtrate was determined by UV spectrophotometry (Kontron, Uvikon 922, France) at 266 nm against appropriate linear calibration curves.

2.4. Size distribution analysis

Microsphere sizes were determined using a Coulter® Counter (Multisizer, Coultronics, Margency, France). Samples of microspheres were suspended by sonication (1 min) in an aqueous solution of polysorbate 80 (0.02% w/v) and size monitored after dilution in Isoton® II (Coultronics, Margency, France).

2.5. Microscopy studies

Optical microscopy was performed using an Olympus BH2 microscope (OSI, Paris, France). The surface and the internal morphology of the microspheres were investigated by scanning electron microscopy (SEM). The microspheres were mounted onto metal stubs using double-sided adhesive tape, vacuum-coated with a carbon film (MED 020, Bal-Tec, Balzers, Liechtenstein) and directly analyzed under SEM (JSM 6301F, JEOL, Paris, France). To characterize the internal morphology, the particles dried with absolute ethanol, were embedded in Epon 812 (SPI-CHEM, USA), which was polymerized at 37°C. After that, the inclusion was cut off using an Ultramicrotome (LEICA, Germany) and observed as previously described.

2.6. Differential scanning calorimetry studies

Differential scanning calorimetry (DSC) analysis of the different microspheres was carried out using a DSC TC II, Mettler apparatus (Toledo, Viroflay, France). Analysis was made by heating the samples from −50 to 250°C at a rate of 10°C/min in a sealed pan. The blank microsphere samples and the free drug and polymer (separately and in a mixture) were also examined.

2.7. In vitro release studies

Microsphere samples (10–50 mg) were placed into glass vials containing 400 ml of phosphate buffered saline (PBS) (pH 7.4) in a USP dissolution apparatus stirred at 100 rpm, 37°C (Sotax AT7, Switzerland). Supernatant was sampled at given time intervals over 2 to at least 25 days depending on the microsphere content. The baclofen concentration was determined by the high-performance liquid chromatography (HPLC) method.

The HPLC system consisted of a pump (Model 50l Waters, Paris, France) connected to a Lichrospher column (100 RP-18, 250 × 4.6 mm, Merck, Darmstadt, Germany) and an injector 4GK (Waters). The mobile phase consisted of a mixture of methanol and 50 mM ammonium phosphate (20:80) adjusted to pH 6.2 with phosphoric acid (Sallerin-Caute et al., 1988).

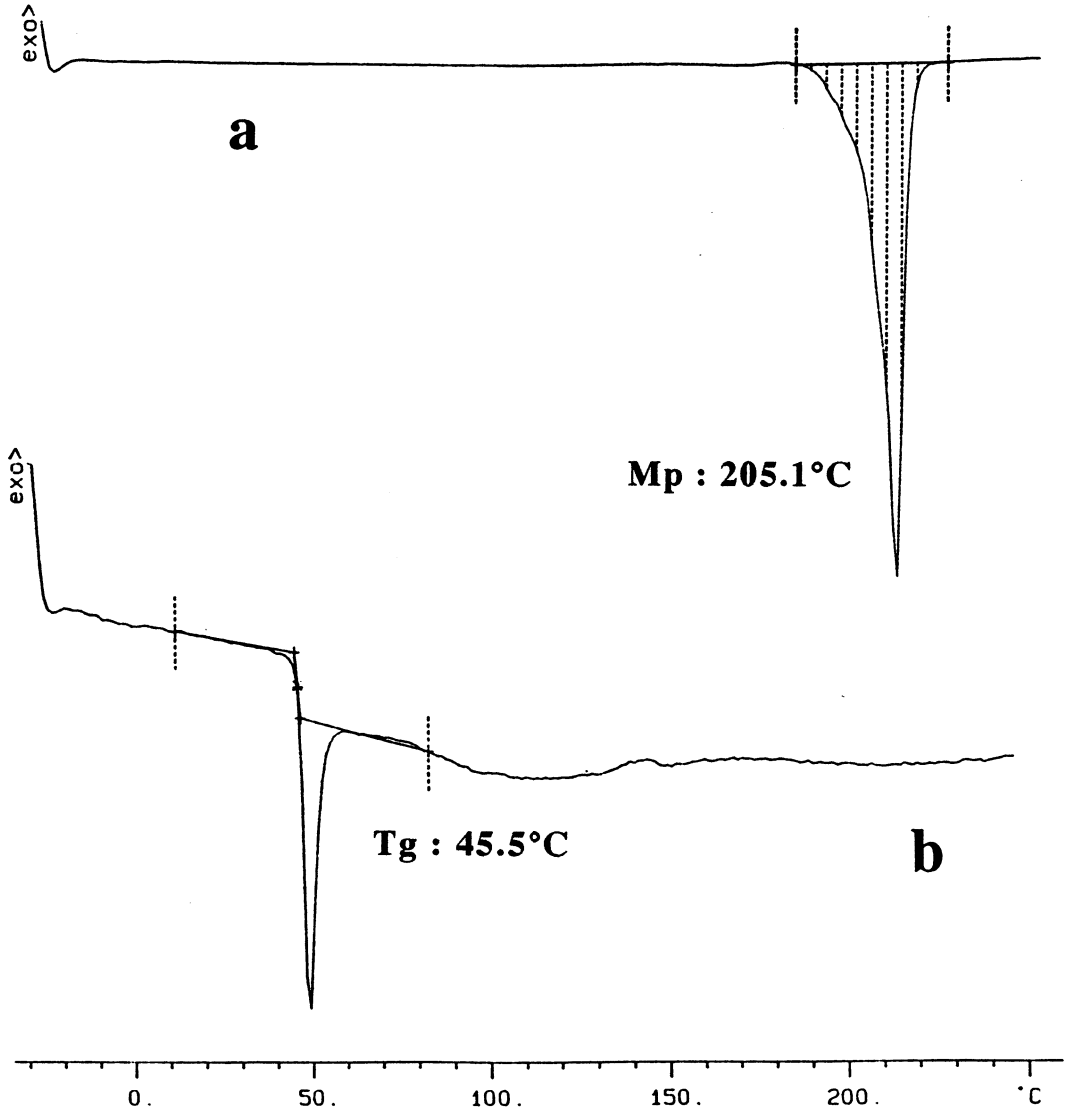

Fig. 2. DSC analysis of the pure baclofen (a) and the empty microspheres of PLGA (b).

3. Results and discussion

In preliminary studies already reported (Boisdron-Celle et al., 1995), optimal manufacturing conditions were identified for the preparation of empty PLGA microspheres. The incorporation of baclofen was carried out by suspending the drug particles in the organic phase. Individual microspheres ranging in size from 15 to 30 μm were formed, exhibiting smooth surfaces at low drug payload (12.8% w/w), and irregular and rough surface at high drug content (33.9% w/w) (Fig. 1). The results showed that baclofen particles did not dissolve markedly in external aqueous phase of the emulsion or in the aqueous extraction phase under the experimental conditions used. It was noted that the microencapsulation yield remained practically unchanged (85–90%) up to theoretical

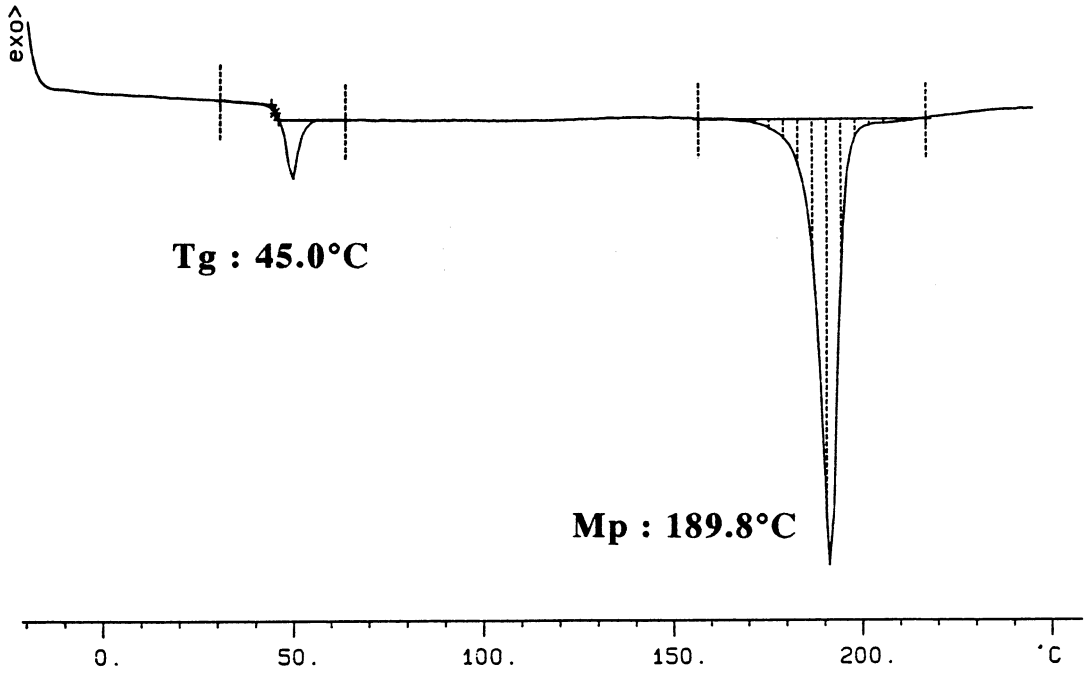

Fig. 3. DSC analysis of a simple mixture of the PLGA and baclofen.

payloads of 37.5% w/w, and decreased markedly to 70% when the initial theoretical payload was 50% w/w (Table 1). Indeed, the solubility of baclofen in water at room temperature was measured and found to be 6 mg/ml, close to the value reported in the literature (Ahuja, 1984), whereas at 10°C, it decreased to 4.6 mg/ml. Under the experimental conditions used, it could be expected that all the baclofen poured into the microsphere aqueous medium (i.e. 50 mg in 170 ml PVA 10% aqueous solution) should dissolve. This is not the case, since most of the drug particles are microencapsulated. It appears that the methylene chloride diffused immediately within the aqueous phase resulting in the precipitation of the polymer around the particles and marked solubility reduction of the active ingredient in the aqueous phase. However, a loss of 10% in the yield was observed due to some aqueous solubility extent of the active ingredient in the aqueous phase under the experimental conditions used. This is confirmed by the results of the high drug-loaded microsphere batches, which exhibited a clear decrease in the microencapsulation efficiency, mainly due to unencapsulated particles rather than to an increase in aqueous solubility of the compound. It should be emphasized that the presence of PVA in water does not markedly alter the aqueous solubility profile of the drug. This method of microencapsulation is, therefore, unable to entrap more than approximately 35% by weight of the drug particles calculated on the total weight basis of the drug and coating polymer. In the high payload microspheres, most of the unencapsulated particles are removed during the microsphere isolation process combining filtration and various consecutive washings at room temperature (3×30ml). This is confirmed by SEM observations of the various batches, which did not reveal the presence of uncoated baclofen particles in the low drug-loaded microsphere batches and provided some evidence of few uncoated particles in the high drug-loaded microsphere batches (Fig. 1).

The DSC analysis of the pure baclofen elicited a thermal event at 205°C, very close to the reported value of the melting point, which is 208°C,

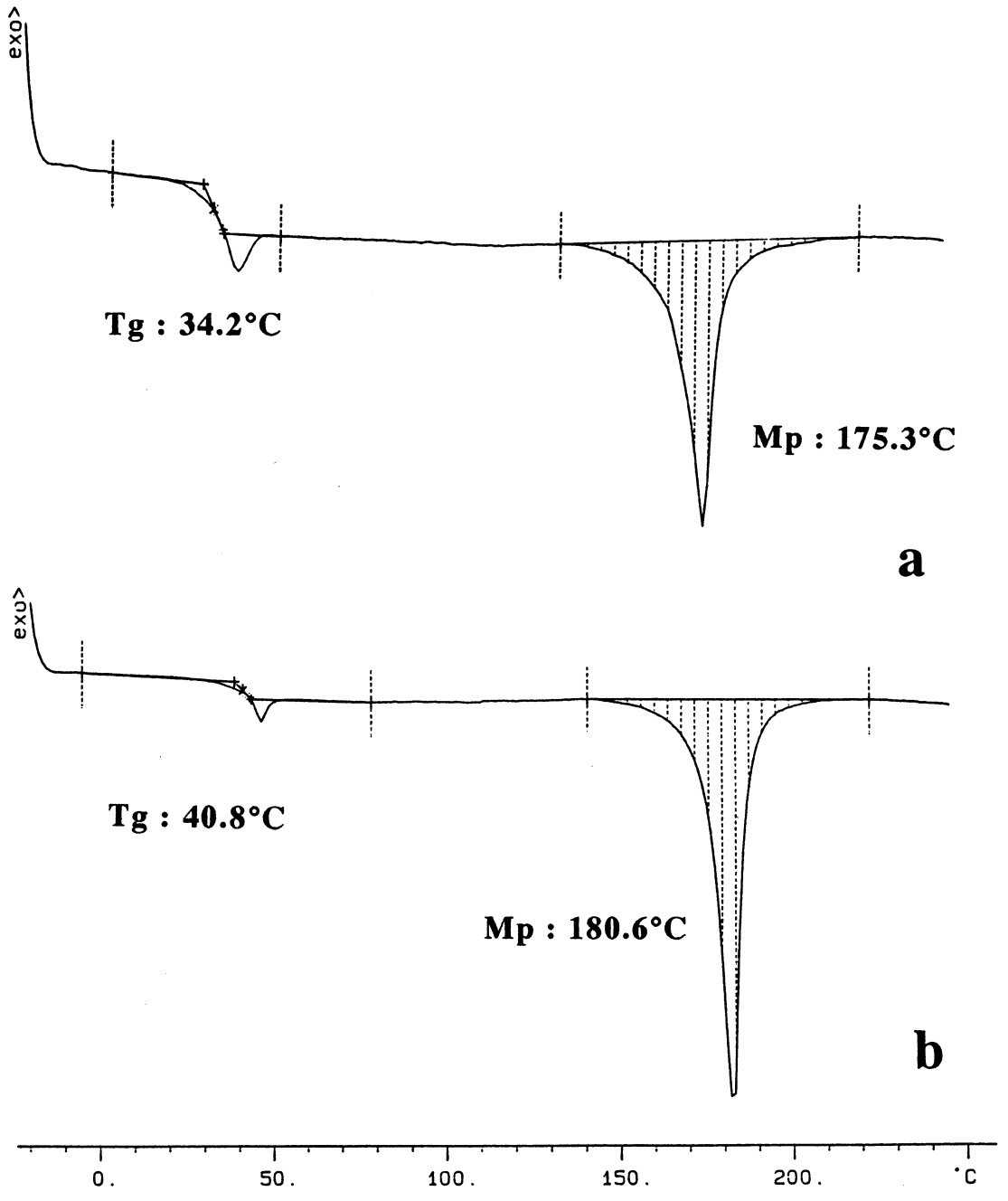

Fig. 4. DSC analysis of 12.8% w/w (a) and 33.9% w/w (b) baclofen-loaded PLGA microspheres.

whereas empty microspheres of PLGA exhibited a thermal event at 45.5°C, reflecting the phase transition temperature of the PLGA polymer after having been subjected to the microsphere formation process (Fig. 2). It should be pointed out that a simple mixture of the polymer with the drug elicited two thermal events at 45 and 189.8°C, indicating that the melting point of the

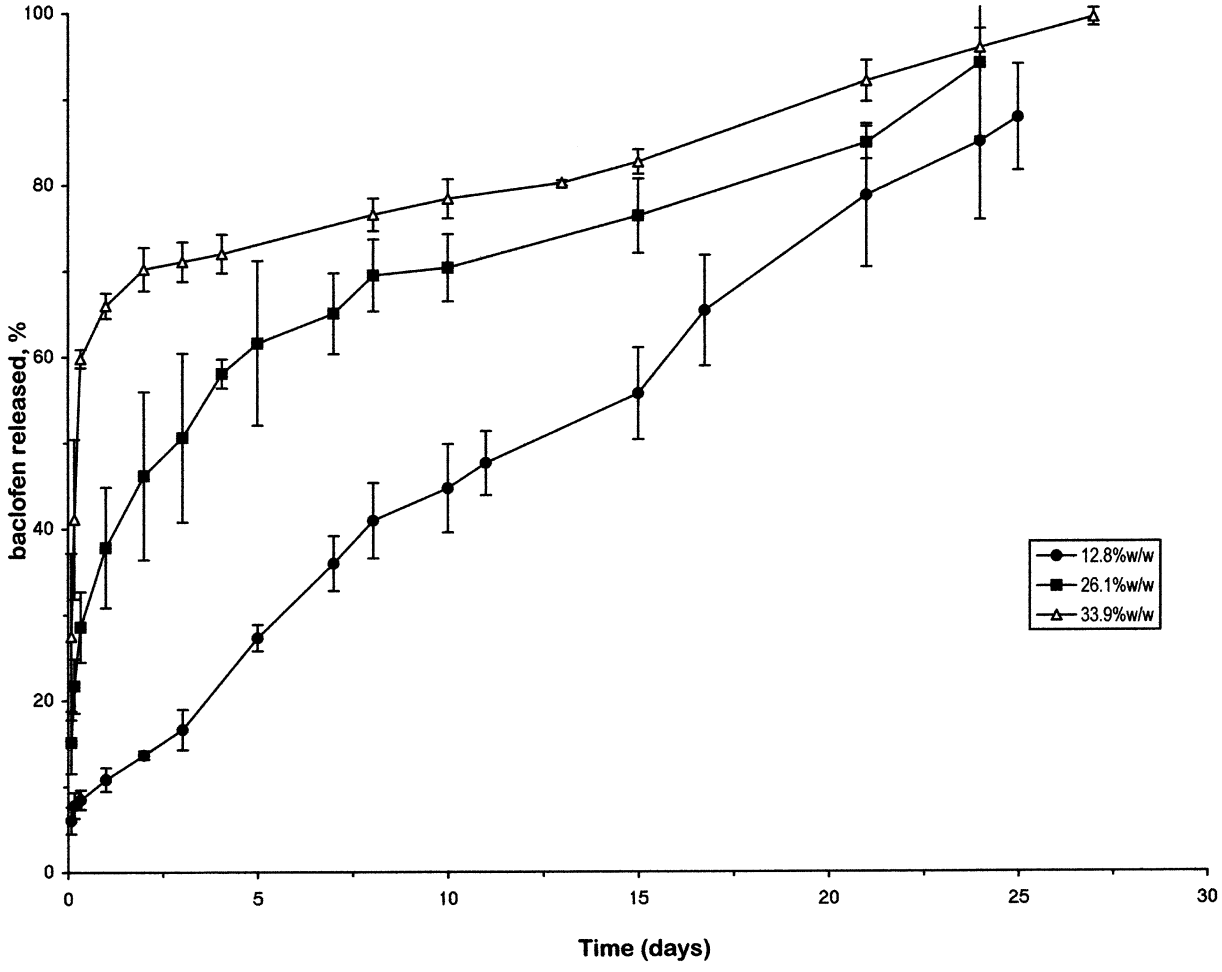

Fig. 5. Release profiles of baclofen from the microspheres with three drug payloads.

drug is affected by the presence of the polymer (Fig. 3).

The baclofen-loaded microspheres at low (Fig. 4a) and high (Fig. 4b) drug payload exhibited two thermal events in each thermogram, corresponding to the phase transition temperature of the polymer (34.2 and 40.8°C, respectively) and to the baclofen melting phase transition (175.3 and 180.6°C, respectively) (Fig. 4).

The DSC analysis revealed, as expected, that drug particles were dispersed in the polymer matrix network since the loaded microspheres elicited a thermal event at 175°C (close to the baclofen melting phase transition) indicative of the crystalline domains in the microspheres.

The in vitro release profile of baclofen from the PLGA microspheres under perfect sink conditions was biphasic only for the high drug payload microspheres (Fig. 5), with a rapid release of 70% within 48 h (Fig. 6) followed by a slower release rate over at least 25 days. In contrast, the microspheres containing low baclofen contents (12.8% w/w) exhibited a gradual and progressive release rate over the course of the experiments (Fig. 5). The drug-loading extent did alter the release profile of baclofen from the microspheres. The

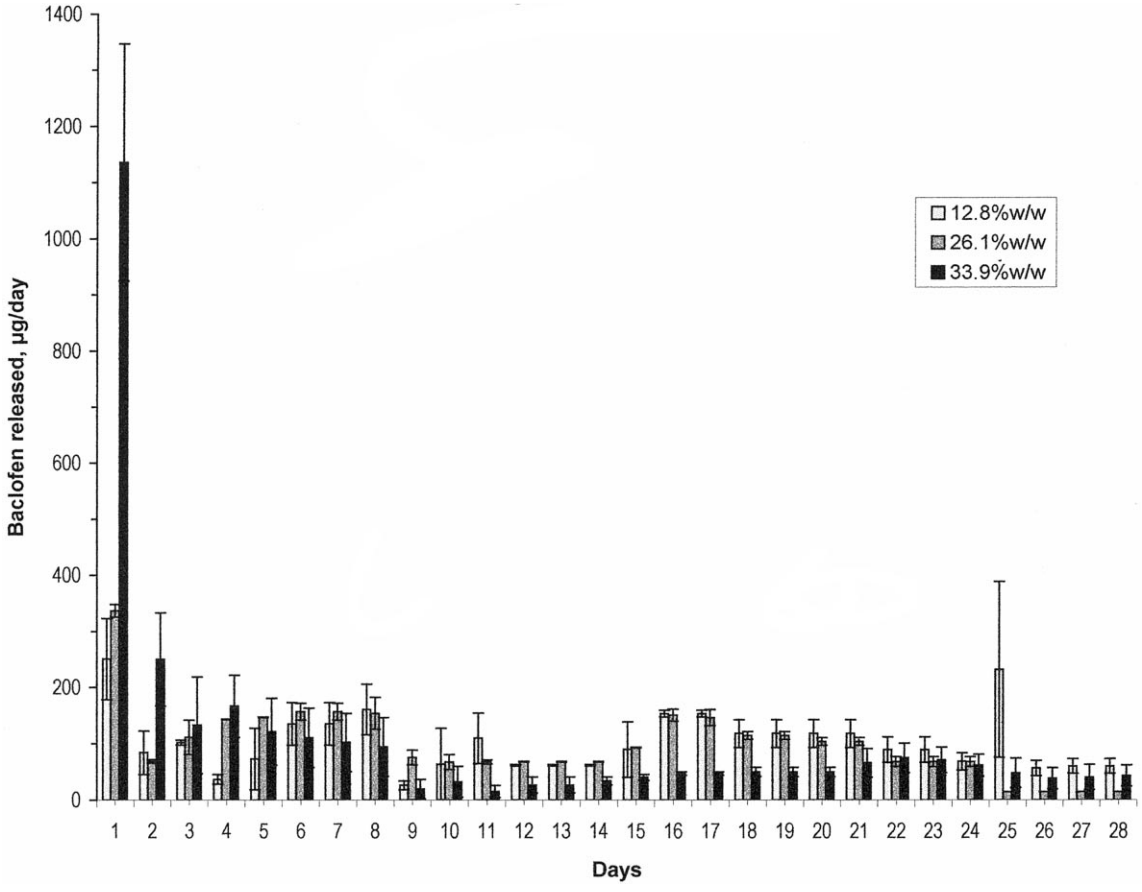

Fig. 6. Baclofen amounts released per day from PLGA microspheres at different drug contents using PBS (pH 7.4) as the release medium.

rapid initial release phase observed in the highly loaded microspheres (33.9% w/w) should be attributed either to the marked location of coated particles close to the surface of the microspheres or to the adsorption of small coated or uncoated particles onto the microsphere surfaces, as evidenced by SEM examinations. However, polymer–drug molecular interaction cannot be excluded.

The in vitro release kinetics pattern of a drug from microspheres should depend on the physical state of the drug molecules entrapped in the polymer network. Different kinetic behaviours are expected from a drug which is dissolved or dispersed in the polymeric matrix of the microspheres. In some cases, both states may coexist, thus complicating the identification of the release kinetic model.

Based on the data generated in the present study and previous knowledge in vitro kinetics of baclofen, release from the microspheres should be analyzed using the general equation of Higuchi (1963). This equation describes the diffusional release of dispersed drug particles from a spherical micromatrix according to:

$$1-\alpha+2(1-\alpha)(a'/a_0)^3-(3-4\alpha)\,(a'/a_0)^2 - \alpha(a'/a_0)+\alpha\,\ln(a_0/a')=6(DC_st/Aa_0^2) \quad (1)$$

where $(a'/a_0)^3$ is the residual drug fraction, $\alpha = C_s/A$, C_s is the solubility of the drug in the matrix, A the total amount of drug in the matrix, D the

diffusion coefficient of the drug in the matrix, and a_0 the radius of the microsphere.

In this equation, it is assumed that no interaction exists between the drug and the polymer; otherwise, major deviations would arise. Furthermore, the microspheres should not degrade, at least not during the kinetic experiment period of time. Nevertheless, as it cannot be excluded that part of baclofen could dissolve in the microsphere polymeric network, it was decided to also verify the validity of the kinetic equation, which describes the desorption kinetics of a dissolved drug from a monolithic spherical device. The full kinetic equation is too complex, but it can be reduced to two approximations which are valid for different portions of the desorption curve, and are reliable to more than 99% (Baker and Lonsdale, 1974). The early-time approximation, which holds for the initial portion of the curve, is expressed by Eq. (2), and the late-time approximation by Eq. (3).

$$(Q'/W_0) = (Dt/r^2\pi)^{1/2} - 3(Dt/r^2) \quad (2)$$

$$(Q'/W_0) = 1 - 6/\pi^2 \exp - (\pi^2 Dt/r^2) \quad (3)$$

where r is the radius of microsphere, Q' the amount of drug released, W_0 the initial quantity of drug and D is as previously explained.

Eq. (2) is valid for $Q'/W_0 = 0.4$ and Eq. (3) is a first-order equation valid for $Q'/W_0 > 0.6$.

The non-linear least-square regression search method was used to match the degree of fit of baclofen release data to the various kinetic equations described (Benita, 1984). The high χ^2 (ranging from 4.7 to 6.8) values obtained during the identification procedure clearly indicated that the release data of the three different microsphere batches did not conform to the general equation which describes, after dissolution of the tiny dispersed drug particles, the diffusional release from spherical micromatrices or to the kinetic equations, which describe the release of dissolved drug from monolithic microspherical devices. It is probable that the mechanism of drug release from microspheres is complex. Probably, some partial erosion of the PLGA microspheres occurred over time in the release medium. This hypothesis appears plausible and is supported by the kinetic results reported in Fig. 6. It can be seen from the results of Fig. 6 that the amount of baclofen released per day is neither constant nor decreasing with time, as it could be expected. It can be further noted that after 14 days, the rate of release increased again for all the batches tested, showing that a marked erosion of the matrices occurred under the kinetic experimental conditions used. These deductions can be supported by the SEM results depicted in Fig. 7, where cross-sections of PLGA microspheres are shown after 8-day immersion in the release medium PBS (pH 7.4). It can easily be noted that large holes are formed in the microspheres.

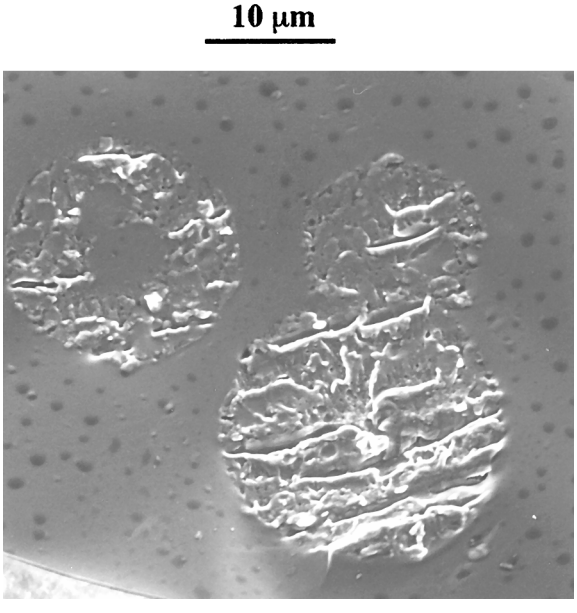

Fig. 7. SEM micrograph of a cross-section of empty PLGA microspheres after 8-day incubation in PBS (pH 7.4).

4. Conclusion

It appears, therefore, that the release of baclofen from the present microspheres is not governed by a unique mechanism but rather by a complex kinetic process involving a mixed release mechanism. This should be attributed either to the presence of some uncoated drug particles, the large size of the embedded drug particles compared with the relatively small size of the spherical

micromatices or some polymeric erosion occurring after several days incubation in the release medium. The in vitro release kinetic deductions are further confirmed by the observations yielded by DSC and SEM techniques. However, the kinetics exhibited by the baclofen microspheres at a loading of 12.8% w/w was interesting from a clinical standpoint since they were not far from linearity. In this particular case, the microspheres could be viewed as intrathecal minipumps.

Acknowledgements

The authors thank the Laboratory of Histology of the School of Medicine of the University of Angers for their electronic microscopy facilities.

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