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Surface drug removal from ibuprofen-loaded PLA microspheres

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Abstract

The preparation, characterisation and drug release behaviour of ibuprofen loaded poly(D,L-lactic acid) (PLA) microspheres are described. Depending on the gelatin concentration in the aqueous external solution (1, 0.5, 0.1% w/v), microspheres with three different sizes (2.2, 4.1, 7.5 μm) were obtained. The properties of microspheres washed with water (Untreated microspheres) (Un-Ms) were compared to those of the microspheres washed with a sodium carbonate solution in order to remove the surface drug (treated microspheres) (T-Ms). The results indicate that the removal of the surface drug did not induce any change in the size of the microspheres whereas the morphology of the smallest T-Ms appeared to be modified. The release profiles of both Un-Ms and T-Ms resulted in biphasic patterns. The initial burst effect (first release phase) of the T-Ms was lower than that of the Un-Ms. The rate of the second release phase did not change for the microspheres with the biggest size but increased for the smallest microspheres probably owing to the modification of the matrix porosity.

Keywords: PLA microspheres; Ibuprofen; Microencapsulation; Controlled release; Solvent evaporation method

1. Introduction

Despite the considerable efforts invested in the development of new anti-inflammatory molecules, the anti-inflammatory treatment available currently may produce gastrointestinal adverse effects (Lanza et al., 1984; Goodman Gilman et al., 1990). In order to overcome such as problems, the controlled release dosage forms as microparticles remain the predominant way to achieve a reduction of the NSAIDs adverse effects (Bodmeier and Chen, 1989; Bakan et al., 1992).

Being the most part of NSAIDs lipophilic drugs, the solvent evaporation process is generally used for their microencapsulation (Watts et al., 1990). However, during the microencapsulation technique, a partial crystallisation of the drug in the dispersing phase and/or on the microspheres surface may occur (Dubernet et al., 1991; Guiziou et al., 1996). These free drug crystals are undesired since their release is not controlled by the polymer matrix. Therefore, various strategies have been studied in order to prevent the drug crystallisation during the solvent evaporation process

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(Benita et al., 1984) or to remove the free drug crystals from the microsphere surface after their preparation (Lalla and Snape, 1993).

On the other hands, the unencapsulated drug fraction can be considered as the priming dose necessary to start rapidly the therapeutic effect especially when the analgesic action is required.

In this work, poly(D,L-lactide) (PLA) microspheres with different sizes were prepared using the solvent evaporation technique in order to embed a model NSAID, ibuprofen. This drug is indicated for the relief of mild to moderate pain and inflammation in conditions such as dysmenorrea, migraine, postoperative pain, dental pain, in which disorders an immediate available dose is request. Ibuprofen is used also in chronic disorders as ankylosing spondylitis, osteoarthritis and rheumatoid arthritis for all of which a sustained release is desirable (Adams and Buckler, 1983).

Realising the importance for a diversification of the drug release in the anti-inflammatory therapy, the aim of this work was to investigate the ibuprofen-loaded PLA microspheres with and without surface drug fraction in order to obtain immediate and/or controlled dosage forms to satisfy different therapeutic requirements.

To remove the surface drug fraction from the microspheres a treatment with a sodium carbonate solution was used (Guiziou et al., 1996). Hence, ibuprofen-loaded PLA microspheres untreated or treated with the carbonate solution have been characterised with regard to their size and morphology. The physical state of the loaded drug and the in vitro drug release were also evaluated.

2. Materials and methods

2.1. Materials

Poly (D,L lactide) (PLA) (Resomer® 203; mean MW 16 000) was supplied from Boehringer Ingelheim (Ingelheim, Germany). (±) ibuprofen was kindly purchased from Laporte Organics Francis (Varese, Italy). Gelatin (from porcin skin, 250 Bloom) was obtained from Fluka (Milan, Italy). Methylene chloride (DMC) obtained from Carlo Erba (Milan, Italy) was used as the polymer solvent. All the solvent and chemicals (Carlo Erba, Milan, Italy) were of analytical grade and were used as obtained from the manufacturers.

2.2. Methods

2.2.1. Preparation of ibuprofen-loaded microspheres

The microspheres were prepared using the 'solvent-evaporation method' (Bodmeier and McGinity, 1986). Briefly, PLA (200 mg) and ibuprofen (100 mg) were dissolved in 2.5 ml of DCM and then emulsified by an Ultra Turrax® (Janke and Kunkel-Ika-Laboratortechnich, Staufen, Germany) with an aqueous gelatin solution (20 ml). Three lots of microspheres using three different gelatin concentrations in the aqueous phase (1, 0.5, 0.1% w/v) were prepared. After the complete evaporation of the solvent at room temperature (about 2 h), the microspheres were recovered by centrifugation (4200 rpm for 20 min) (model 4235, A.L.C., Milan, Italy).

2.2.2. Untreated microspheres (Un-Ms)

The microspheres recovered by centrifugation were washed three times, each time using 50 ml of deionised water. After the recovery, the washed microspheres were freeze-dried (Lyovac GT 2; Leybold-Heraeus, Hanau, Germany). According to the gelatin concentration (1, 0.5, 0.1% w/v) used in the preparation process, three different lots of microspheres (Un-Ms) were obtained (lots A, B, and C, respectively) (Table 1).

2.2.3. Treated microspheres (T-Ms)

The microspheres recovered by centrifugation were treated in a beaker with an aqueous solution of sodium carbonate (0.1% w/v; pH 11; 50 ml) under magnetic stirring (800 rpm) for 10 min. After this time period, the microspheres were recovered by centrifugation (4200 rpm for 20 min) and washed two times, each time using 50 ml of deionised water in order to remove the residual sodium carbonate. After the recovery, the treated microspheres were freeze-dried. According to the gelatin concentration (1, 0.5, 0.1% w/v) used in

Table 1
Dimensions, drug content, and drug encapsulation efficiency of untreated and treated ibuprofen loaded-PLA microspheres (Un-Ms and T-Ms) prepared using different gelatin concentrations in external aqueous phase

Lot	Gelatin concentration in the aqueous phase (%, w/w)	Mean diameter (μm)	Drug content (%, w/w)	Encapsulation efficiency (%)
Un-Ms				
A	1.0	2.2	26.6 ± 0.2	90.1 ± 0.7
B	0.5	4.1	25.9 ± 1.4	89.6 ± 4.9
C	0.1	7.5	25.8 ± 0.7	90.2 ± 2.3
T-Ms				
At	1.0	2.1	11.5 ± 0.5	39.6 ± 1.9
Bt	0.5	4.1	13.9 ± 0.3	48.7 ± 1.1
Ct	0.1	7.5	16.7 ± 0.4	58.5 ± 0.3

the preparation process, three different lots of microspheres (T-Ms) were obtained (lots At, Bt, and Ct, respectively) (Table 2).

2.2.4. Drug content

An exactly weighted amount of ibuprofen-loaded PLA microspheres (Un-Ms or T-Ms) (10 mg) were introduced in a capped glass tube and dissolved in DMC (0.5 ml). Then, 10 ml of phosphate buffer (20 mM, pH 7.4) were added and the system was magnetically stirred for 2 h. After this time period, the system was centrifuged (4200 rpm for 10 min) to separate the aqueous phase. The ibuprofen concentration in the buffer solution was determined spectrophotometrically at 264 nm (model Lambda 3B, Perkin–Elmer, Norwalk, USA) from a standard calibration curve.

2.2.5. Particle size and morphology evaluation

Particle size distribution of the freeze dried T-Ms and Un-Ms was determined using a Coulter Multisizer II (Cultronics, Margency, France) dispersing the microspheres in deionised water. A scanning electron microscope (SEM) (XL-40, Philips, Eindhoven, The Netherlands) was used to evaluate both the morphology and surface characteristics of the microspheres.

2.2.6. DSC analysis

The thermal analysis was performed using a DSC-4 differential scanning calorimeter equipped with a computerised data station (Perkin–Elmer). All the samples (ibuprofen, Un-Ms and T-Ms) (2.8–3.2 mg) were heated in crimped aluminium pans (Perkin-Elmer) using dry nitrogen as the effluent gas (30 ml min^{-1}). The analysis were performed with a heating rate of 10°C min^{-1} from 30 to 120 °C.

2.2.7. In vitro release study

Microsphere samples (25 mg) were incubated in 50 ml of phosphate buffer (20 mM, pH 7.4) at 37 ± 1°C under magnetic stirring. At fixed time intervals an aliquot of the suspension (3.5 ml) was withdrawn and centrifuged (4200 rpm for 10 min) to separate the microspheres from the aqueous

Table 2
Correlation coefficients and calculated slope of fitting ibuprofen release data to Eq. (2) for Un-Ms[a]

Lot	Correlation coefficient (r)	Slope (h^{-1})	Drug loading after correction (%, w/w)
A	0.9541	0.0321	3.1
B	0.9998	0.0303	3.9
C	0.9955	0.0270	8.8

[a] Drug loading after correction was calculated from intercept of the theoretical curves $Y = at$.

phase. The drug content in the solution was determined spectrophotometrically at 264 nm. After each spectrophotometric assay, supernatant and microspheres were mixed by a vortex (Zx3, Velp Scientifica, Milan, Italy) and putted in the release medium to maintain the volume and the amount of the microspheres constant.

2.2.8. Release kinetics

To evaluate the drug release kinetics, the microparticles were considered as heterogeneous spherical matrices and the release data were analysed according to the Higuchi model (Higuchi, 1963):

$$3 - 2M_t/M\infty - 3(1 - M_t/M\infty)^{2/3} = 6D(\varepsilon C_s/\tau A a_0^2)t \quad (1)$$

where M_t is the drug fraction released at time t; $M\infty$ is the drug fraction released at infinite time; C_s is the saturation concentration of ibuprofen in the release medium (27.3 mg ml^{-1}) determined experimentally in the pH 7.4 phosphate buffer at 37 ± 1°C; A is the initial concentration of the drug in the matrix in g cm^{-3}; a_0 is the initial radius of the matrix. Finally ε is the porosity of the matrix and τ is the tortuosity of the matrix; D is the ibuprofen diffusion coefficient in the release medium (cm^2 s^{-1}).

This model can be applied when the loading concentration, A, is much greater than εC_s in order to analyse the data until the first 60% of drug released.

According to Dubernet et al. (1990), Eq. (1) may be rewritten as below reported calling 'Y' the left side and 'a' the 't' coefficient:

$$Y = at \quad (2)$$

3. Results and discussions

3.1. Untreated microspheres (Un-Ms)

Three lots of Un-Ms were prepared using three different gelatin concentrations in the external aqueous phase. The mean diameter of the resulted ibuprofen-loaded microspheres are listed in Table 1 along with the drug loading and the encapsulation efficiency.

Among the several parameter influencing the particle size of microspheres, it is commonly known that surfactant concentration is the most important. Therefore, we studied the influence of gelatin concentration in order to obtain particles with different size. The highest was the gelatin concentration in the external aqueous phase, the smallest resulted the microsphere dimensions. As a high gelatin concentration corresponds to a high viscosity of the external solution, it is conceivable that the viscosity of the external aqueous phase may avoids the coalescence of the internal phase droplets. Thus, the final size of the microspheres is related to the gelatin concentration.

For all the microsphere preparation, the drug loading and the encapsulation efficiency were approximately the same (26 and 91% w/w, respectively), regardless of the particle size. According to the SEM analysis (Fig. 1a and b) both the lots A and B showed a spherical morphology and a smooth surface. In contrast, some irregular formations attributable to a fraction of unencapsulated drug were visible on the surface of microspheres of the lot C (Fig. 1c). The presence of a surface drug fraction on the microspheres could be due to the lowest surface area available for the drug deposition. However there is the possibility that gelatin concentration have some effect independent of particle size.

In order to evaluate whether the drug loaded in microspheres was in amorphous or crystalline state, the differential scanning calorimetry (DSC) analysis was performed. The results are reported in Fig. 2. The thermogram of the plain drug crystals showed an endothermic peak at 74°C attributable to the drug melting (curve a). In the thermograms of the all microsphere samples the glass–rubber transition of the polymer at 42°C is evident. The same T_g value was observed for the unloaded microspheres (data not shown). The endothermic peak due to the drug melting resulted broad (curves b and c) or in the case of the biggest microspheres (curve d) practically superimposed with another peak. In addition for all the microspheres lots the drug melting point temperature (70–72°C) resulted smallest than that of the plain drug crystals. Obviously the thermogram of a drug in a amorphous state should be flat. There-

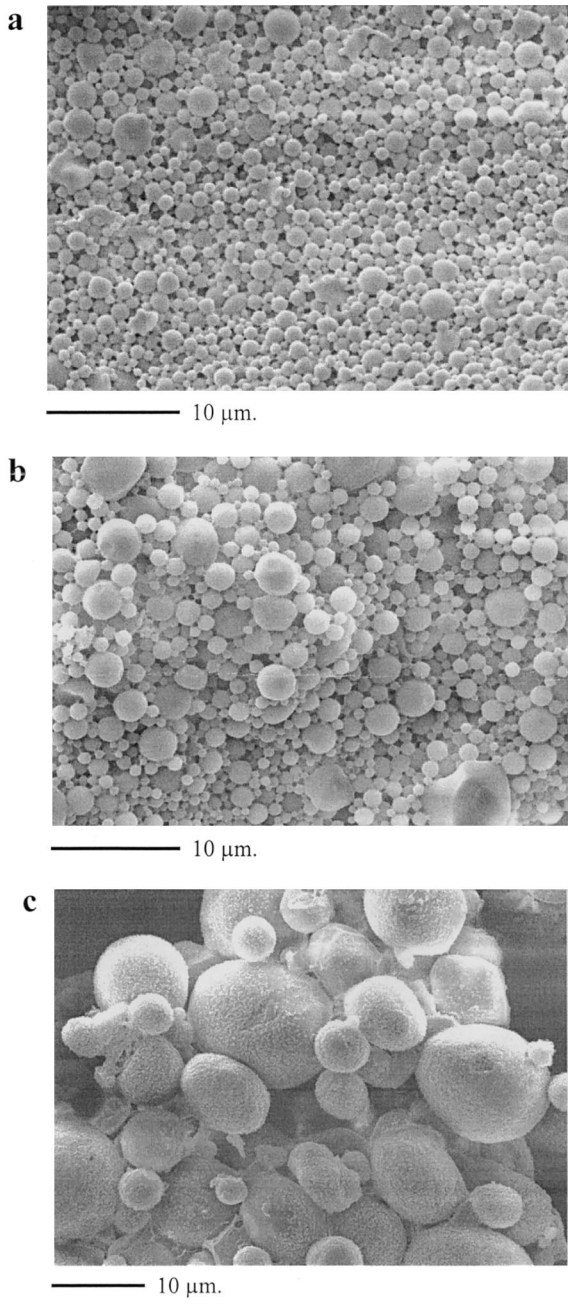

Fig. 1. Scanning electron photomicrographs of untreated ibuprofen loaded-PLA microspheres (Un-Ms). (a) lot A; (b) lot B; (c) lot C.

fore, as it was observed for microspheres a melting peak broader and a lower onset with respect of the pain drug, we hypothesised that a part of the drug associated to the microspheres becomes partially amorphous during the solvent evaporation process. This phenomenon could be produced by a hindering effect of the polymer chains on the drug crystallisation.

In Fig. 3 are reported the release profiles of the three lots of microspheres obtained in phosphate buffer at 37 ± 1°C. The smallest microspheres (lots A and B) presented a very fast release rate. In fact, the entire loaded drug was released in 3 h from the two lots likely owing to the highest surface area. The biggest microspheres (lot C) showed the slowest release rate. In fact, the entire loaded drug was released in a time longer than 24 h. All the three microsphere lots presented a biphasic release profile. The first phase (burst effect) is more evident for the smaller microspheres (lots A and B) than for the bigger one (lot C). This immediate release period can be attributable to a fraction of unencapsulated drug or of drug encapsulated nearly the microsphere sur-

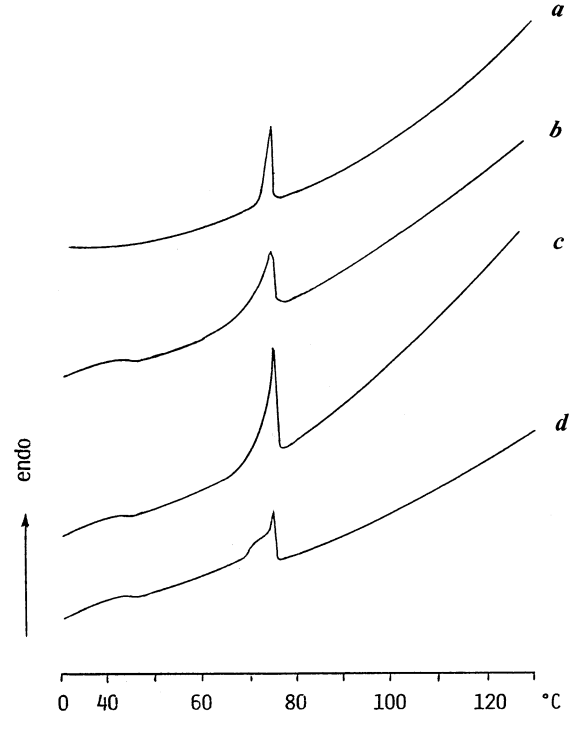

Fig. 2. DSC thermograms of: (a) plain drug crystals (ibuprofen) and of untreated ibuprofen loaded-PLA microspheres (Un-Ms) (b) lot A; (c) lot B; (d) lot C

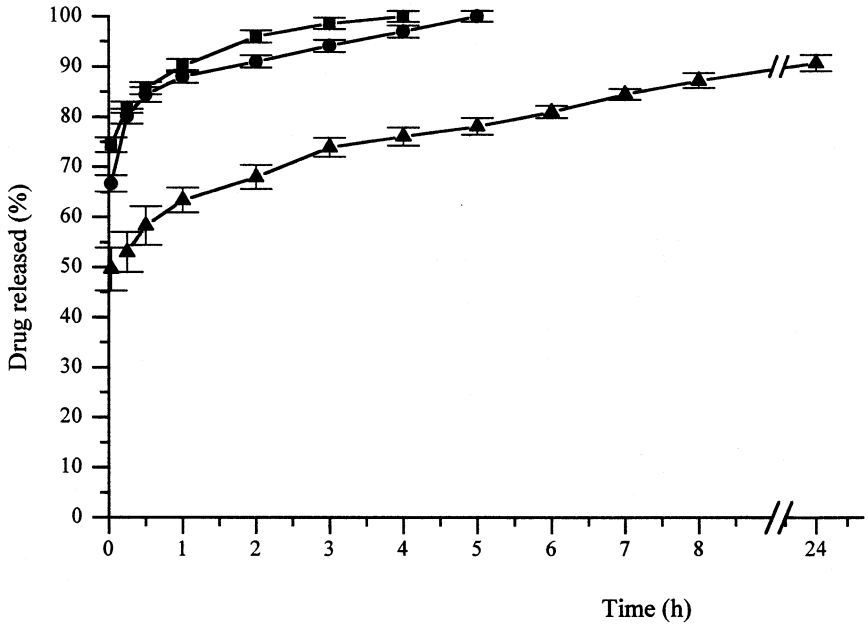

Fig. 3. Dissolution profile of ibuprofen from untreated ibuprofen loaded-PLA microspheres (Un-Ms) in 20 mM (pH 7.4) phosphate buffer at 37 ± 1°C. (■) lot A; (●) lot B; (▲) lot C.

face. As the surface area value of the smallest microspheres (lots A and B) is clearly the highest, these microspheres presented obviously the highest burst effect although the presence of surface drug is not evident using the SEM observation. The second phase of the release process is the slowest and it can be attributable to the diffusion of the encapsulated drug from microspheres.

In all the lots, hence, there is a drug fraction (unencapsulated or distributed near the surface) that is responsible of the burst effect observed in the release process.

Therefore, in order to eliminate the surface drug, three lots of microspheres treated by a sodium carbonate solution were prepared.

3.2. Treated microspheres (T-Ms)

Size, drug content and drug encapsulation efficiency of the T-Ms are reported in Table 2. No effect of the carbonate solution treatment on microsphere size was observed, but a remarkable reduction in the drug content and in the encapsulation efficiency occurred (Table 2). Obviously, this reduction was more important for the lots with the smallest size (At and Bt) owing to the higher surface area available to the drug removal.

As concerns the microspheres morphology, the SEM analysis reveals that both lots At and Bt resulted modified with respect to the lots A and B (Fig. 4a and b). The spherical morphology of the smallest microspheres (lots At) was modified while the lot Bt presents a porous structure and a wrinkled surface. Finally, for the lot Ct, no effect of the treatment with the carbonate solution on microsphere morphology was observed (Fig. 4c).

As far as the thermal analysis (Fig. 5) of lots At and Bt is concerned (curves a and b), the peak of the drug melting appeared less broad compared to those of the lots A and B. For the lot Ct (curve c) two different peaks were visible probably owing to the contemporaneous presence of the amorphous and crystalline drug fraction. According to these experimental data, it is conceivable that the carbonate treatment removed the surface fraction of amorphous drug since it is obviously more soluble than the crystalline drug.

The release profiles of ibuprofen from all the T-Ms (Fig. 6) resulted in a biphasic pattern. As a consequence of the surface drug removal, for the three lots of microspheres a reduction of the burst effect was observed. In order to compare the steady state phase of the drug release from the T-Ms and Un-Ms, the Higuchi equation for spherical matrix was applied.

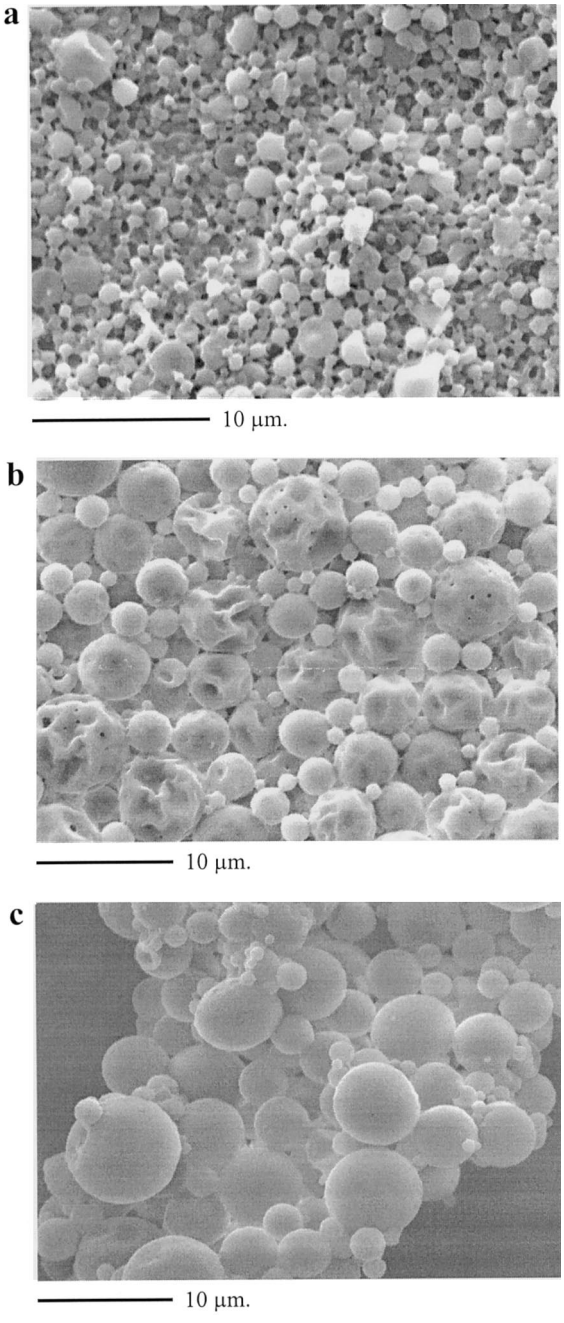

Fig. 4. Scanning electron photomicrographs of treated ibuprofen loaded-PLA microspheres (T-Ms). (a) lot At; (b) lot Bt; (c) lot Ct.

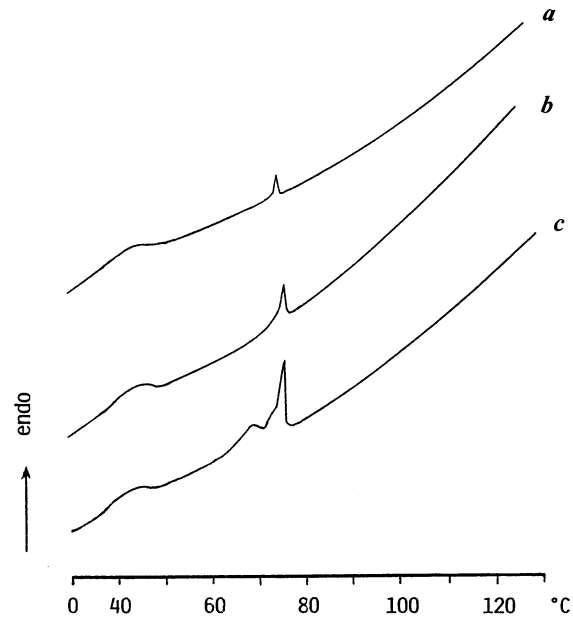

Fig. 5. DSC thermograms of treated ibuprofen loaded-PLA microspheres (T-Ms) (a) lot At; (b) lot Bt; (c) lot Ct.

3.3. Kinetics analysis

According to the literature (Dubernet et al., 1990) the mathematical model proposed by Higuchi (1963) was applied on the first 60% of the drug release after subtracting the drug fraction dissolved during the burst effect. After the correction of the experimental data, the release data of the Un-Ms fit well the Higuchi model as results from the coefficient correlation values (Table 2). On the contrary, the drug release from the T-Ms did not appear explained by the Higuchi model (Table 3) specially as far as the smallest microspheres (lots At and Bt) are concerned. In the attempt to explain these results, it is useful to observe the microspheres shape as showed by the SEM analysis. As previously reported the shape of the smallest T-Ms (lots At and Bt) did not

Table 3
Correlation coefficients and calculated slope of fitting ibuprofen release data to Eq. (2) for T-Ms[a]

Lot	Correlation coefficient	Slope (h^{-1})	Drug loading after correction (%, w/w)
At	0.9142	0.0614	1.4
Bt	0.9461	0.0441	2.4
Ct	0.9870	0.0266	8.5

[a] Drug loading after correction was calculated from intercept of the theoretical curves $Y = at$.

appear spherical. This observation can justify the correlation coefficient values of the release data to the Higuchi model (0.9142 and 0.9461 for the lot At and Bt, respectively).

Another important variable to observe is the slope 'a' of the theoretical curve $Y = at$. According to the Higuchi Eq. (1) the drug release rate is related to the matrix porosity. For the biggest microspheres (lots C and Ct) the slope value did not resulted modified by the carbonate solution treatment. On the contrary, for the smallest T-Ms the slope value increased significantly with respect to the Un-Ms (lots A and At, B and Bt) (Table 3). This behaviour can be a consequence of the treatment with the sodium carbonate solution. As above discussed, the SEM microphotographs showed the change of the microspheres structure produced by the treatment, justifying the changes in the drug release rate.

As far as the intercept value is concerned, it is useful to calculate the drug loading after correction, i.e. after the dissolution of the drug fraction responsible of the observed burst effect (Tables 2 and 3). For the biggest Un-Ms (lot C) the drug loading after correction resulted very similar to that of the correspondent lot treated with the carbonate solution (lot Ct). This provides the evidence that in this case the carbonate solution did not remove the encapsulated drug. On the basis of this observation the modest burst effect

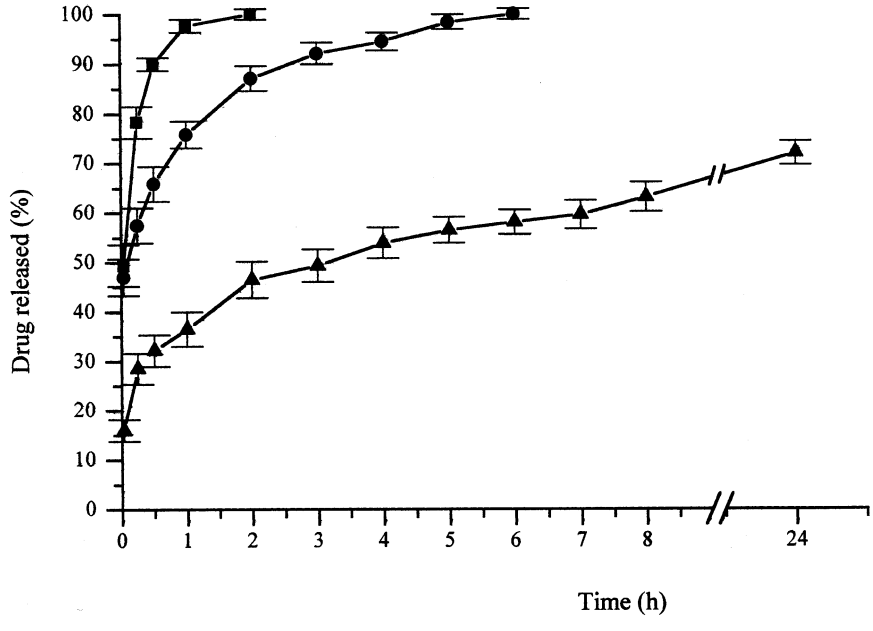

Fig. 6. Dissolution profile of ibuprofen from treated ibuprofen loaded-PLA microspheres (T-Ms) in 20 mM (pH 7.4) phosphate buffer at 37 ± 1°C. (■) lot At; (●) lot Bt; (▲) lot Ct.

observed for the lot Ct could be probably attribute to the drug encapsulated nearly the microsphere surface. On the contrary for the smallest T-Ms (lots At and Bt) the drug loading after correction is lower than that of the Un-Ms (lot A and B). Thus, following the treatment by the carbonate solution, the removal of a fraction of the encapsulated drug could be occurred simultaneously with the modification of the matrix porosity.

4. Conclusions

In conclusion, according to the microsphere size, different results were obtained from ibuprofen-loaded PLA microspheres. For the smallest microspheres (size lower than 4 μm) the treatment with the carbonate solution removed the unencapsulated drug and the drug encapsulated nearly the microsphere surface degrading partially the polymeric matrix. On the contrary, for the biggest microspheres (size higher than 4 μm) only the unencapsulated drug can be removed without modifying the microsphere morphology. Therefore, the different microsphere treatment (water or sodium carbonate solution) allows two different dosage forms to be prepared according to the therapy exigency. In fact, ibuprofen-loaded microspheres loading a surface drug fraction are useful in order to assure a priming dose of the drug. The microsphere treatment with an appropriate solution removing the surface drug allows a controlled therapeutic system to be obtained.

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