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Comparative study of 'in vitro' release of anti-inflammatory drugs from polylactide-co-glycolide microspheres

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Abstract

A differential scanning calorimetry study has been carried out on the effect exerted by three anti-inflammatory drugs, Biphenylacetic Acid, Naproxen, and Ketoprofen, released from polylactide-*co*-glycolide (50:50 w:w) microspheres (loaded with two different quantities of drug) on the thermotropic behaviour of dimyristoylphosphatidylcholine liposomes. The aim of this work was to study the release rate of a NSAID agent from polylactide-*co*-glycolide microspheres, by evaluation of the drug effect on the thermotropic behaviour of dimyristoilphosphatidylcholine unilamellar vesicles, as a model membrane representing the targeting surface where the drug should be delivered. Polylactide-*co*-glycolide microspheres loaded with NSAID drugs were prepared by the spray drying method. The lipid samples were unilamellar vesicles charged with increasing amounts of free drugs or added to weighed amounts of drug-loaded microspheres. Free drugs were found to interact with the phospholipidic bilayer modifying its thermotropic behaviour. In fact, increasing amounts of drugs in DMPC vesicles shift the peak temperature, assigned to the gel to liquid–crystal phase transition of pure phosphatidylcholine, toward lower values. The amount of drug released from the microparticulate drug delivery system versus time was quantified by comparing the T_m shift caused by the drug released from the polymeric system with that caused by known increasing amounts of the free drugs. The calorimetric technique detects changes occurring directly on the adsorption sites, constituted by DMPC vesicles. The release kinetics of these drugs have been reported and compared with the 'classical' in vitro release studies executed by a dissolution test. Good agreement was found between the two experimental methods. By calculating the drug partition between aqueous phase and lipidic phase, it should be possible to evaluate the amount of drug present at the surface of the lipidic membrane and the uptake kinetics. The data were explained in terms of physico-chemical characterisation by differential scanning calorimetry and scanning electron microscopy.

Keywords: Phosphatidylcholine; Differential scanning calorimetry; Membranes; NSAIDS; Poly-lactide-*co*-glycolide; Microspheres; Liposomes

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1. Introduction

The dispersion of non-steroidal anti-inflammatory drugs (NSAIDs) into biodegradable polymeric matrices (poly-lactide-*co*-glycolide microspheres, PLGA) is a good approach to obtain the therapeutic effect at a predetermined time (controlled release) and at the same time minimising the side effects of drugs. 'In vitro' studies on these matricial systems are extensively reported in the literature (Wise et al., 1979; Lewis, 1990; Whateley, 1993; McGee et al., 1994; O'Hagan et al., 1994; Okada and Toguchi, 1995; Kamijo et al., 1996), but these studies on drug release from PLGA microspheres do not take into account that 'in vivo' drug release is affected by the presence of biological membranes that absorb lipophilic molecules.

In our previous studies we investigated the drug release from micromatrices employing liposomes as model membranes able to accept drug molecules. Differential scanning calorimetry (DSC) was chosen to follow the variations of the thermotropic behaviour of a liposomal dispersion in the presence of drug delivery systems (Castelli et al., 1994, 1996, 1997).

Liposomes, made by single species of phospholipid, when heated, exhibit a gel-to-liquid ($L_\beta \rightarrow L_\alpha$) crystal phase transition that can be easily revealed by DSC by measuring the associated thermodynamic parameters (transitional temperature, T_m, and enthalpy changes, ΔH). Drug interaction with the lipid bilayer should cause a destabilisation of the lipid chain packing resulting in a change of the transitional thermodynamic parameters, like a depression of T_m (Lee, 1977; Sturtevant, 1982; Bach, 1984; Jain, 1988; Castelli et al., 1989, 1992, 1994, 1996, 1997). This behaviour can be analysed by the Van't Hoff model of the freezing point depression of solutions, verified for several classes of chemical compounds, such as anaesthetics (Lee, 1977; Suezaki et al., 1990), and applied on a theoretical basis by some researchers (Guggenheim, 1952; Lee, 1977; Jorgensen et al., 1991). This model was previously applied to compare the effects caused by free drugs to that of drugs released from different delivery systems (Castelli et al., 1994, 1996, 1997).

The present work is aimed to investigate the release of three NSAIDs, Biphenylacetic Acid (BPAA), Naproxen (NAP) and Ketoprofen (KPF), from PLGA microspheres loaded with two different quantities of drug. In this study, the acceptor site is represented by L-α-dimyristoylphosphatidylcholine (DMPC) large unilamellar vesicles (LUV). The capability of these drugs to interact with a model membrane was previously reported (Castelli et al., 1989, 1992).

To confirm the suitability of this model for the evaluation of the 'in vitro' release kinetics, a comparison between kinetic curves obtained by DSC study and by classical 'in vitro' release study was carried out. A satisfactory agreement was found between the two techniques employed.

A physico-chemical characterisation of the examined therapeutic delivery systems was also carried out, because, as reported in the literature (Bodmeier et al., 1989; Izumikawa et al., 1991; Dubernet, 1995), morphological properties and drug dispersion nature can affect drug release. As a consequence of the physico-chemical characterisation, a better understanding of the obtained release kinetics can be achieved, showing that significant differences between the examined drug-loaded microspheres can be due to different drug interaction with polymeric matrices and to the microparticle morphology.

2. Materials and methods

2.1. Materials

Synthetic 1,2-dimyristoyl-sn-glycero-3-phosphocholine was obtained from Fluka (Buchs, Switzerland). The lipid solution was chromatographically pure as assessed by two-dimensional thin-layer chromatography. Lipid concentrations were determined by phosphorous analysis using the method of Bartlett (1959).

Copolymer Poly-Lactide-co-Glycolide, 50:50 w/w, RG 502H, 3000 MW (MHE determination), was supplied by Boehringer Ingelheim (Ingelheim am Rhein, Germany). Biphenylacetic acid, Naproxen and Ketoprofen were supplied by Sigma-Aldrich (Milan, Italy).

2.2. Microsphere preparation and characterisation

PLGA microspheres loaded with two different amounts of NSAIDs (BPAA, NAP and KPF) were prepared by the spray drying method. The preparation was performed following the process conditions previously described (Pavanetto et al., 1992, 1993): briefly, the drug (10 and 30% w/w) was dissolved in a 2% w/w polymer solution in CH_2Cl_2. The polymer and drug solutions were sprayed through the standard nozzle of a Buchi Mini spray-dryer model 190 (Buchi, Flawil, Switzerland). Two batches of drug-loaded microspheres were obtained, for each drug used, with a theoretical drug content of 10 and 30%, respectively.

Drug content was determined by HPLC with the following procedure. The drug-loaded microspheres were dissolved in CH_2Cl_2 and filtered through a 0.22-μm Millipore membrane. Then, sample analysis was carried out by a Varian Model 5000 liquid chromatograph equipped with a 20-μl manual loop injector. A Hipersyl ODS, particle size 5 μm, 100 mm $\times$ 4.6 mm i.d. and a mobile phase of methanol/sodium acetate (pH = 5) (55:45) were used. Detection was made on a Vari-Chrom variable wavelength UV detector set at maximum absorption wavelength (λmax) for each of the three examined NSAID (respectively, BPAA: 254 nm; NAP: 270 nm; KPF: 260 nm). The microsphere drug content was $10 \pm 1\%$ (batch 1, 3, 5) and $30 \pm 1\%$ (batch 2, 4, 6) w/w.

Microspheres were morphologically characterised by scanning electron microscopy (SEM) using a Leica-Cambridge S 360 microscope at 20 kV. The samples, previously desiccated, were gold sputtered and photographed under argon.

Physico-chemical characterisation of microspheres and polymer was performed by DSC, with a Mettler TA 3000 system at a scanning rate of 5°C/min between 5 and 170°C.

2.3. Liposome preparation

Large multilamellar vesicles (MLV) were prepared, following the classical procedure, by vortexing dry lipid films (DMPC) in the presence of 50 mM Tris buffer (pH = 7.4). The resulting MLV dispersion went through an extruder (Sciema Technical Service) with a standard polycarbonate filter of 0.1-μm pore size (Nucleopore, Pleasanton, CA). MLV were injected into a central chamber above the polycarbonate filters, and nitrogen pressure applied via a standard gas cylinder fitted with a high pressure (0–4000 lb/in^2) regulator. The vesicles were extruded through the filter employing pressures of 100–500 lb/in^2 and were collected and re-injected. This procedure was repeated 10 times (Hope et al., 1985). Lipid concentration was determined by phosphorous analysis using the method of Bartlett (1959).

Afterwards, aliquots of 120 μl (4 mg of the lipid) were transferred to 150-μl DSC aluminium pans and submitted to DSC analysis.

2.4. Differential scanning calorimetry

DSC was performed by a Mettler TA 3000 system equipped with a DSC-30 cell and a TC-10 processor. The scan rate employed was 2°C/min, and the temperature range was 5–37°C after an initial isothermal period of 5 min. The sensitivity was 1.72 mW and the reference pan was filled with Tris buffer solution. After the factory-suggested calibration of the calorimetric system, a successive fine, in a narrow range, calibration temperature was carried out using indium, stearic acid and water. Indium was employed to calibrate the transitional enthalpies (ΔH). Temperature and enthalpies were also checked using palmitic acid. Enthalpies were evaluated from the peak areas using the integration program of the TA processor, permitting choice of different baselines and ranges of integration. The areas calculated with these different methods lie within experimental error ($\pm 5\%$). After calorimetric scans, all samples were extracted from the pans and aliquots were used to determine the phospholipid amount by the phosphorous assay previously mentioned.

2.5. Interaction between free-drugs and large unilamellar vesicles

Different aliquots of drug (BPAA, NAP, KPF) accurately ground, were weighed in DSC pans to ensure the presence of increasing drug molar frac-

tions, and added to the DMPC LUV prepared as described above. The calorimetric pans were hermetically sealed, then incubated for 1 h at 45°C. During this isothermal period, the samples were vortexed every 10 min, to permit the best interaction between drug and liposomes (to complete the drug dissolution and transfer through the aqueous lipidic dispersion to and inside the lipidic vesicles), which at temperatures above the glass transition temperature, are in a disordered state (L_α). Afterwards, the samples were submitted to DSC analysis that was carried out for several cycles until the DSC results showed that the equilibrium had been reached (observing no further drug–lipid interaction revealed as T_m shifts).

2.6. Drug partition between lipidic and aqueous phases

Samples (≈ 20 mg) of unilamellar vesicles loaded with different drug molar fractions ($X = 0.03$; $X = 0.06$; $X = 0.12$; $X = 0.18$) in Tris solution (pH = 7.4) were incubated at 37°C for at least 1 h with gentle shaking. Aliquots were submitted to DSC analysis to check the complete interaction (partition equilibrium reached) with the liposomes, by comparing the calorimetric results with those obtained following the experiments reported in the previous section. The samples were transferred to a centrifuge tube and centrifuged at $60 \times 10^3 \times g$ for 1 h using a Beckman L8-60M centrifuge. The supernatant was separated by the lipidic pellets, then both were dried and lyophilised. The amount of drug present in the two fractions (aqueous and lipidic) was detected by UV spectroscopy in $CHCl_3$ at λmax of the three NSAIDs, using a Perkin-Elmer 330 instrument equipped with a 3600 data station. The calculated partition coefficients were independent of the drug molar fractions. Each determination was made in triplicate and was corrected for the low fractions of water (carrying dissolved drug) remaining trapped within the lipid pellets. The amounts of drug found in the lipid phase were 55 ± 1, 40 ± 1 and $30 \pm 1\%$ respectively for BPAA, NAP and KPF.

2.7. Release kinetic experiments

Drug-loaded PLGA microspheres, as well as empty PLGA microspheres (to check eventual interaction between polymer and lipid membranes) have been added to the DMPC liposomes in known amounts to obtain the same relative molar fraction of drug and/or polymer with respect to the lipid.

The samples were analysed immediately after preparation. Each has been submitted to the following procedure:

1. A first scan (from 10 to 37°C) to detect drug release bringing the sample to 37°C;
2. 60 min at 37°C, to detect drug release after a long incubation time at a temperature simulating 'in vivo' temperature;
3. A subsequent scan from 10 to 37°C, after incubation at 37°C followed by cooling of the sample to 10°C.

The whole procedure, performed on all batches of microspheres loaded with different amounts of drug, has been repeated several times until no further drug release was observed.

2.8. Dissolution tests

'In vitro' release studies were carried out by rotating paddle apparatus (Sotax AT 7) at 150 rpm and 37°C under sink conditions in 500 ml of 50 mM Tris buffer (pH = 7.4). Weighed amounts of drug-loaded microspheres were suspended in the dissolution medium and samples (1 ml) were withdrawn and filtered (Millex-HV 0.45 μM) at 1-h intervals.

The analyses of the amount of drug released at fixed time intervals were performed by HPLC, under the same conditions employed for drug content determination as previously reported. All samples were run at least in triplicate.

3. Results and discussion

3.1. Microsphere characterisation

This study is focused mainly on the evaluation of the mechanism of drug release from the micro-

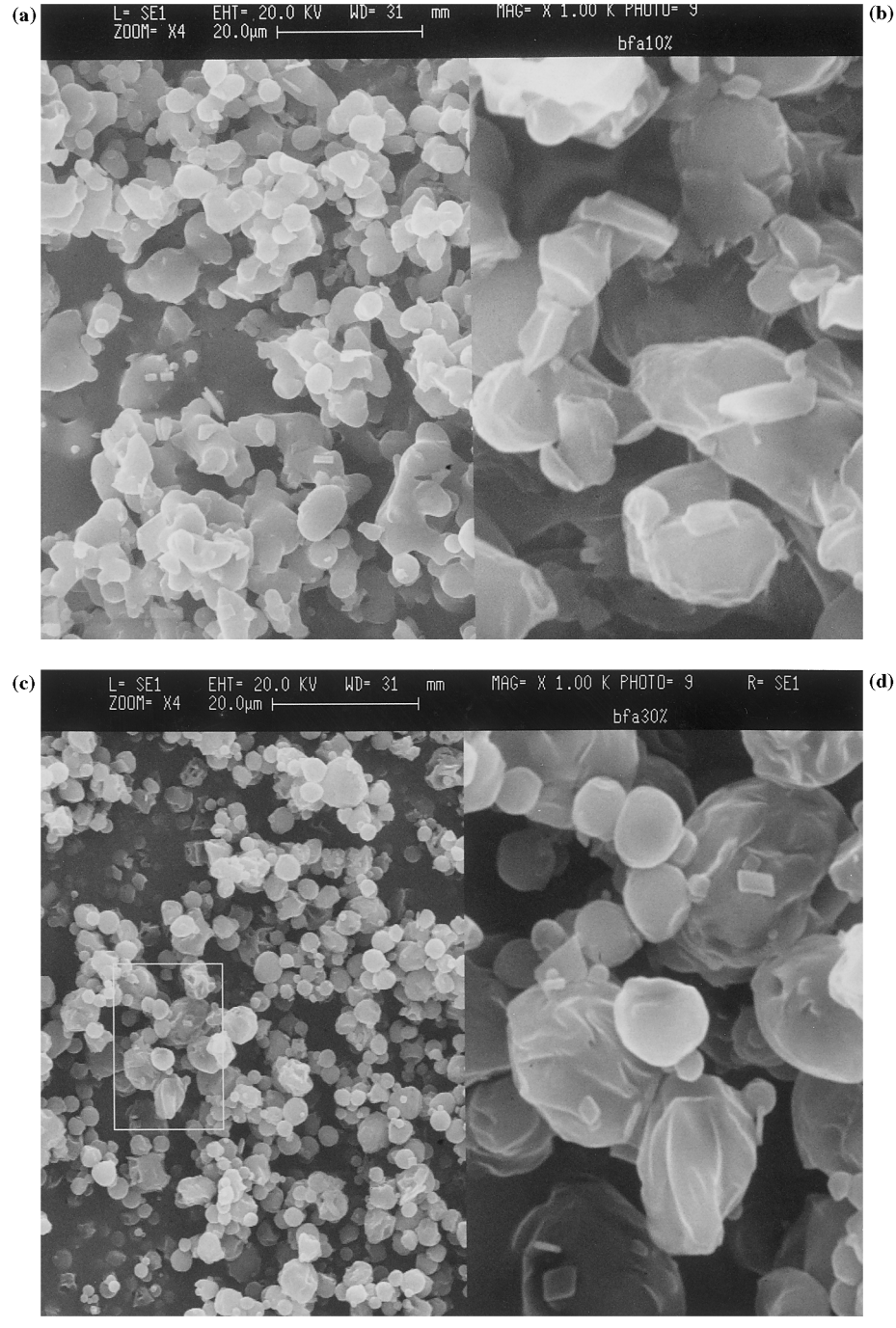

Fig. 1. Scanning electron micrographs of BPAA-loaded PLGA microspheres at two different magnifications: (a) $\times 1000$, (b) $\times 4000$, 10% drug-loaded microspheres; (c) $\times 1000$, (d) $\times 4000$, 30% drug-loaded microspheres.

spheres, and SEM and DSC characterization techniques were useful for this purpose. SEM analysis is an excellent tool for observation of the morphological features of the microspheres. Fig. 1 shows photomicrographs of microspheres, at two magnifications, loaded with 10% (Fig. 1a,b) and 30% BPAA (Fig. 1c,d). The microspheres appear to be not aggregated, well formed and separated. The size is about 5 μm for microspheres loaded with 10% BPAA, and about 2 μm for those loaded with 30% BPAA (Fig. 1a and c, respectively). The photomicrographs of the microspheres at higher magnifications (Fig. 1b,d) show a large number of drug crystals, either outside or adhering to the microsphere surface.

Fig. 2 shows photomicrographs of the microspheres loaded with 10% (Fig. 2a,b) and 30% (Fig. 2c,d) of NAP. The two differently loaded NAP microspheres appear very different; 10% loading gives single large microspheres of ≈ 200 μm size while 30% loading results in large aggregates of polymeric particulates of ≈ 50 μm. Small needle-shaped drug crystals are evident adhering to the microsphere surface or just below it (Fig. 2b,d).

Fig. 3a,b shows photomicrographs of 10% KPF-loaded microspheres that are not well formed, with irregular shape and without drug crystals on the surface. Increasing drug payload to 30% gives microspheres (Fig. 3c) that easily undergo surface modification during SEM analysis, thus making it difficult to obtain photomicrographs at higher magnifications. The appearance of KPF microspheres and their behaviour to SEM analysis suggest some interaction between the drug and the polymer. These data show that the three series of microspheres have very different morphologies depending on drug solubility in the casting solvent and in the polymer, and on drug–polymer interaction.

Calorimetric measurements on both drug–polymer physical mixtures and drug-loaded microspheres were useful to obtain more information on the nature of drug dispersion into the microspheres and to better explain the data shown by SEM analysis.

Fig. 4 reports the thermograms for the drug–polymer physical mixtures (curves A–C) for the three compounds examined and of the pure polymer (curves D). The (w/w) drug/polymer ratio was chosen equal to that found in the microspheres containing the highest amount of drug ($\approx 30\%$). This analysis was performed to examine if there was any interaction between the drugs and the polymer, by investigating the change in behaviour of their physical mixture when submitted to heating scans. Two heating scans were performed on the PLGA (curves D) because the first heating (curve D_1) is affected by the thermal history of the polymer, and therefore may not be reliable in indicating its original properties (Hausberger and DeLuca, 1995). During the second polymer scan, an abrupt change in slope was observed (curve D_2), meaning a change in sample heat capacity. This can be assigned to the glass transition, typical for amorphous copolymers like PLGA 50:50. The transition is characterised by a transitional temperature, T_g, defined as the temperature at which a rigid or 'glassy' polymer converts to a softer 'rubbery' polymer upon heating, with an increase in its free volume. In the first scan of polymer (curve D_1) the observed endothermic peak, which occurs in PLGA glass transition region is attributed to a kinetic overshoot due to the polymer structure. By heating the polymer at temperatures above T_g, then cooling below T_g at a faster rate than was used for heating, any polymer history was eliminated (Dubernet, 1995; Hausberger and DeLuca, 1995).

Calorimetric curves of heating scans of different drug–polymer physical mixtures show that KPF–PLGA mixture (Fig. 4: curves C) has a different thermotropic behaviour with respect to BPAA–PLGA (Fig. 4: curves A) and NAP–PLGA mixtures (Fig. 4: curves B). The first scans (curves A_1, B_1 and C_1) present similar thermotropic behaviour: all the drug–polymer mixtures show endothermic peaks. Comparison with the calorimetric profiles of polymer (curves D) suggests that the first endothermic process is due to kinetic overshoot that is superimposed on the polymer glass transition, while the second peak is attributable to drug melting (curves A_1, B_1, and C_1). The polymer present in the drug–polymer mixture lowers the melting temperature of drug by interactions between the two components. Subsequent heating (curves A_2, B_2, C_2) shows remarkable differences

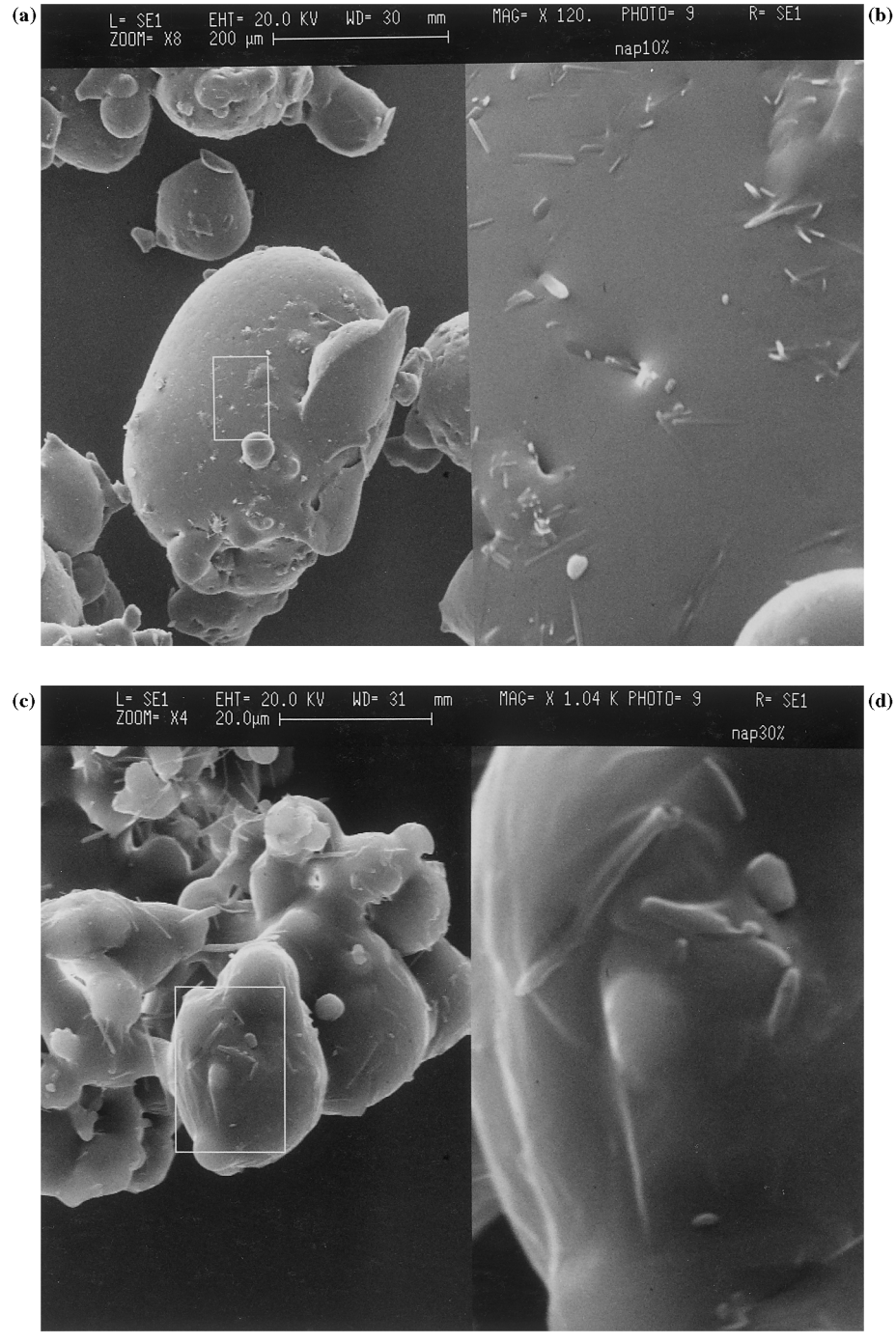

Fig. 2. Scanning electron micrographs of NAP-loaded PLGA microspheres at two different magnifications: (a) $\times 120$, (b) $\times 960$, 10% drug-loaded microspheres; (c) $\times 1000$, (d) $\times 4000$, 30% drug-loaded microspheres.

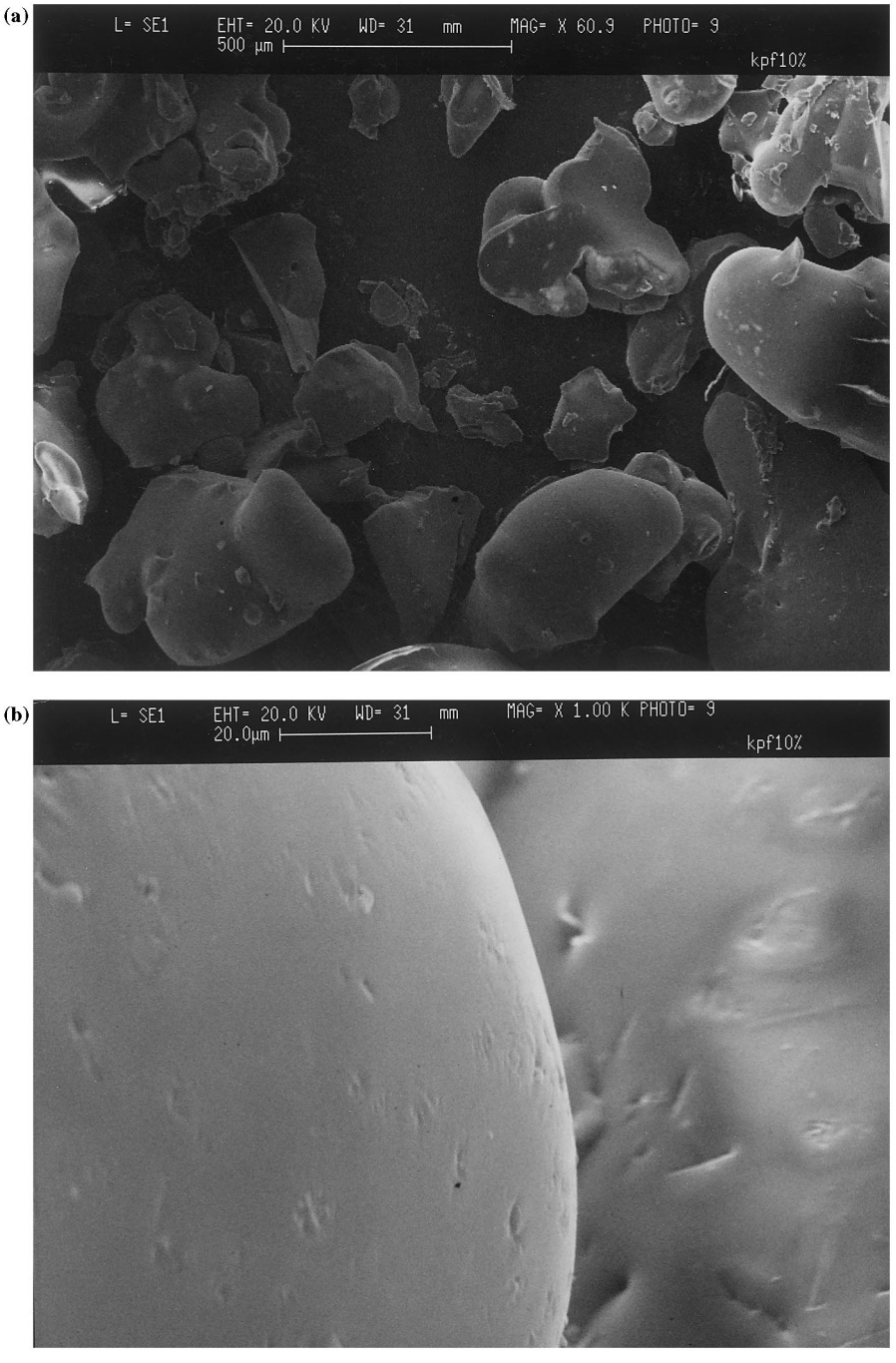

Fig. 3. Scanning electron micrographs of KPF-loaded PLGA microspheres at two different magnifications: (a) $\times 60$, (b) $\times 1000$, 10% drug-loaded microspheres; (c) $\times 60$, 30% drug-loaded microspheres.

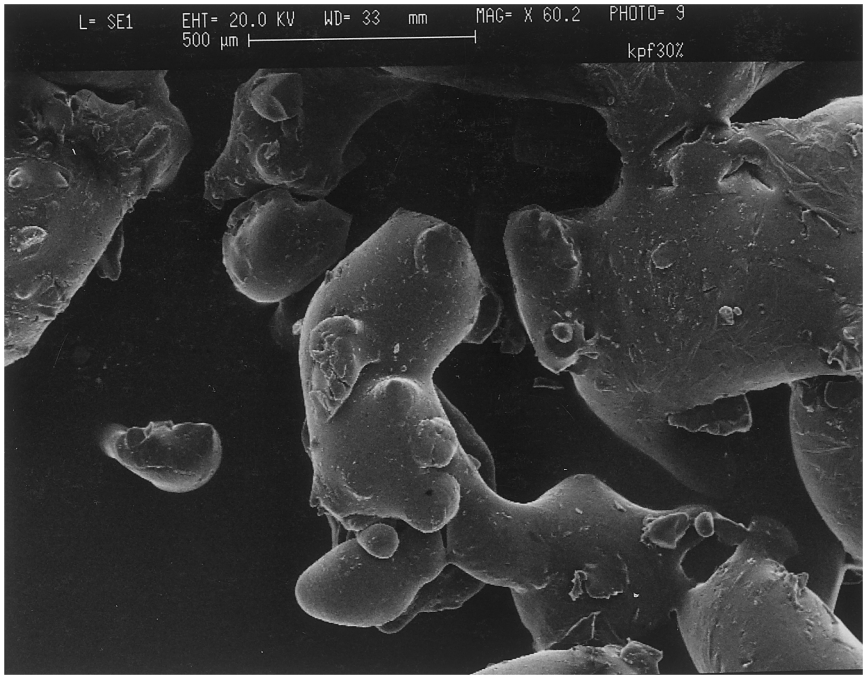

Fig. 3. (*Continued*)

among the drug–polymer mixtures. They can be summarised:

- BPAA–PLGA mixture thermotropic profile is reproducible to that of the first scan (curves A_1, A_2);
- NAP–PLGA mixture shows an exothermic peak before the endothermic one (curve B_2) that can be caused by a fraction of amorphousdrug that crystallises during heating before melting (endothermic peak) (Bodmeier et al., 1989; Izumikawa et al., 1991; Dubernet, 1995);
- KPF–PLGA mixture does not show any peak (curve C_2). The lowering of T_g value and the absence of a fusion event (compare curves C_2 and C_1) suggest the formation, after the first heating scan, of a solid solution of the drug in the polymer. This leads to plasticization of the mass due to strong interaction existing between the drug molecules and the polymer chains (Sanchez et al., 1993; Dubernet, 1995).

These considerations can be useful in explaining the thermal behaviour of PLGA microspheres loaded with two percentages of BPAA, NAP and KPF (Fig. 5: curves A, B, C and curves A′, B′, C′, respectively for 10 and 30% drug-loaded microspheres). By comparing the DSC profiles reported in Fig. 5 with those previously shown in Fig. 4 the following considerations can be drawn:

- There is a different interaction of the three drugs evaluated with respect to the polymeric matrix;
- The area corresponding to the drug melting process in the NAP- and BPAA-loaded microspheres increases proportionally to the percentage of drug loaded in the microspheres, as expected for crystalline dispersion in a polymeric matrix;
- No fusion event appears in KPF–PLGA thermograms, as expected for solid solution of the drug in the polymer.

These characterisations by SEM and by DSC lead to several assumptions:

- No homogeneity exists in shape and size of microspheres loaded with the three different NSAIDs;
- The nature of drug dispersion in the polymeric matrix is different for the three drugs: BPAA- and NAP-loaded microspheres are crystalline

dispersions, while KPF–PLGA is a solid solution;
- KPF exerts a plasticizing effect on the polymeric matrix.

3.2. Interaction between free-drug and DMPC large unilamellar vesicles

The heating scans of DMPC liposomes containing increasing amounts of free BPAA are reported in Fig. 6. Similar curves were observed studying the interaction with the other two drugs (NAP and KPF) (curves not reported). Free drugs were found able to interact with DMPC liposomes by depressing their transitional temperature but leaving their enthalpy almost constant. The interaction between drugs and DMPC liposomes is explained in terms of a 'fluidifying' effect due to the introduction of lipophilic drug molecules into the ordered structure of the lipidic bilayer. Drug molecules act as spacers in this structure causing a destabilisation of the lipid mosaic with a decrease of T_m of the gel to liquid crystal phase transition. The negligible ΔH variation is explained by surface interaction between amphipatic molecules and DMPC polar heads, which occurs only at the surface of lipid layers without deeply interacting with acyl chains (Estep et al., 1978; Sturtevant, 1982; Castelli et al., 1994, 1996).

In Fig. 7, the $\Delta T_m/T_m^o$ plots vs. drug mole fractions are reported ($\Delta T_m = T_m - T_m^o$, where T_m^o and T_m are respectively the transition temperatures of pure DMPC and drug-loaded DMPC

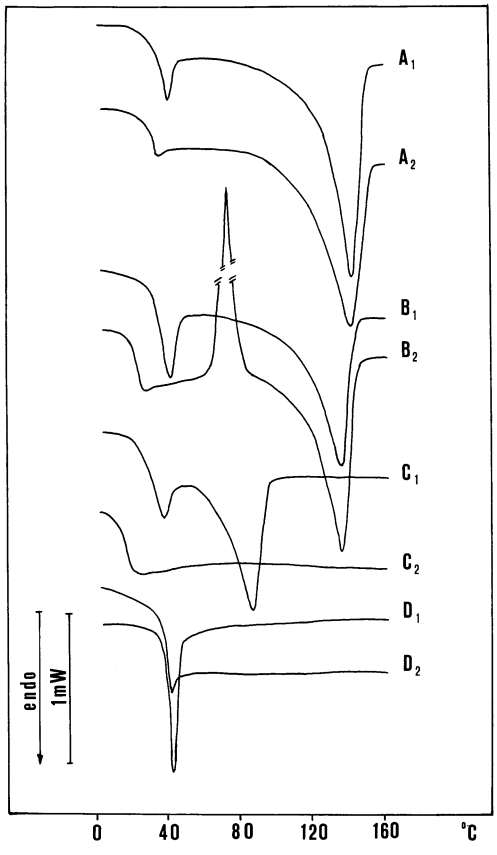

Fig. 4. Calorimetric scans of drug–polymer physical mixtures (A: BPAA–PLGA; B: NAP–PLGA; C: KPF–PLGA) and of the pure polymer (D: PLGA). Drug concentration was 30%. Subscript 1 refers to 1st scan, subscript 2 to 2nd scan.

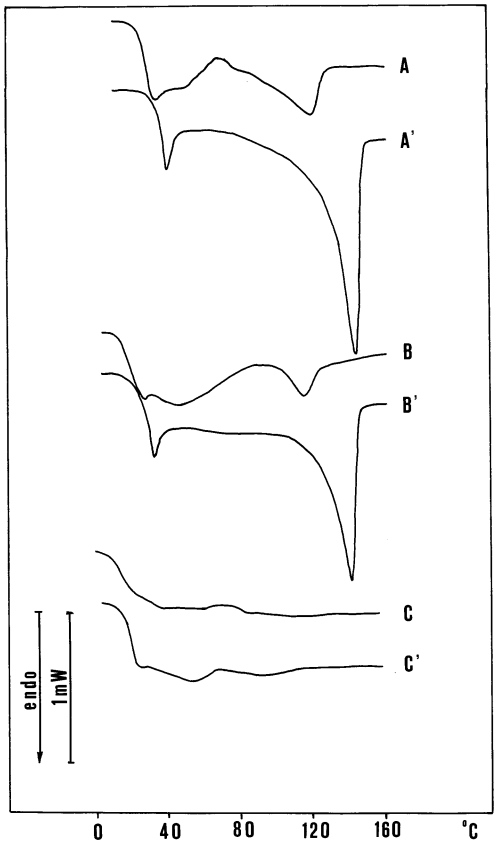

Fig. 5. Calorimetric scans of drug-loaded microspheres (BPAA–PLGA, NAP–PLGA and KPF–PLGA), respectively A, B and C for 10% drug-loaded microspheres and A′, B′ and C′ for 30% drug-loaded microspheres.

vesicles). This T_m depression represents the effect of increasing amounts of drug, present in the lipidic aqueous dispersion, on the transitional temperature. By relating this T_m depression to the molar fraction of drug present in the liposomal dispersion, the following equations were obtained: $y = -61.37x + 0.11$ ($R^2 = 0.991$); $y = -170.12x^2 - 12.86x - 0.117$ ($R^2 = 0.997$); $y = -120.11x^2 - 11.95x - 0.036$ ($R^2 = 0.996$), respectively for BPAA, NAP and KPF. These correlation equations are useful to quantify and follow the transfer of drug released from the microparticulate systems to empty membranes.

The correlation curves can be modified multiplying for the partition coefficient (drug distribution between lipidic and aqueous phases) to determine the real amount of drug (present in lipidic bilayer) exerting the 'fluidifying' effect on the thermotropic behaviour of liposomes.

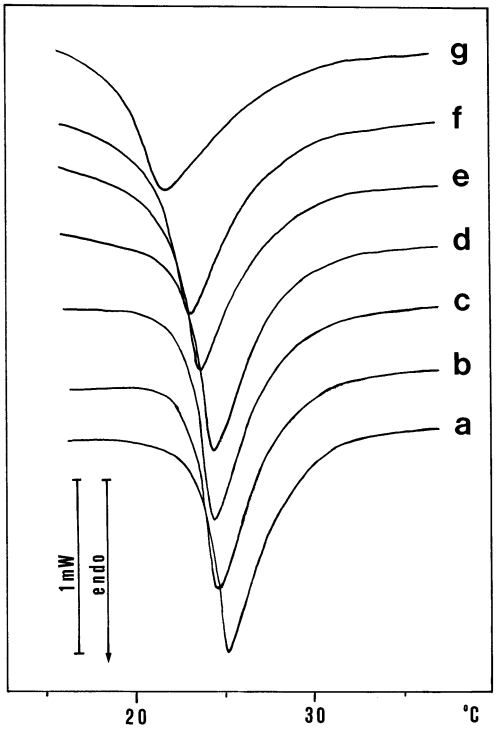

Fig. 6. Calorimetric curves of DMPC unilamellar vesicles, prepared in Tris (pH = 7.4), in the presence of increasing molar fractions of BPAA: a = 0; b = 0.03; c = 0.06; d = 0.09; e = 0.12; f = 0.18; g = 0.24.

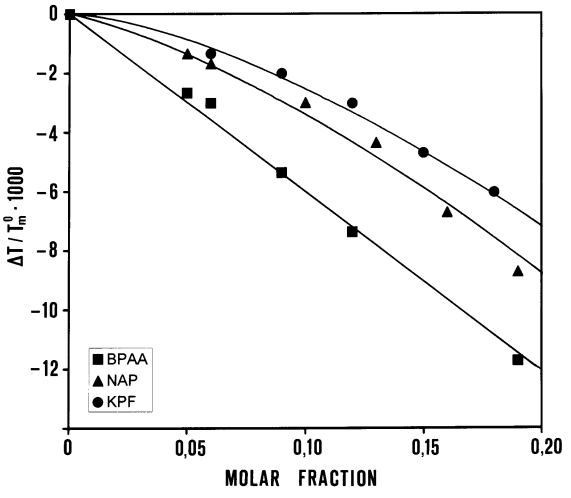

Fig. 7. Correlation curves relating the drug effect on the thermotropic behaviour of DMPC unilamellar vesicles (see Section 3) to drug concentration in the liposomal dispersion (■ = BPAA; ▲ = NAP; ● = KPF).

3.3. Release kinetics experiments

The release kinetics at 37°C from microspheres containing two different amounts of the three examined drugs to void lipid unilamellar vesicles

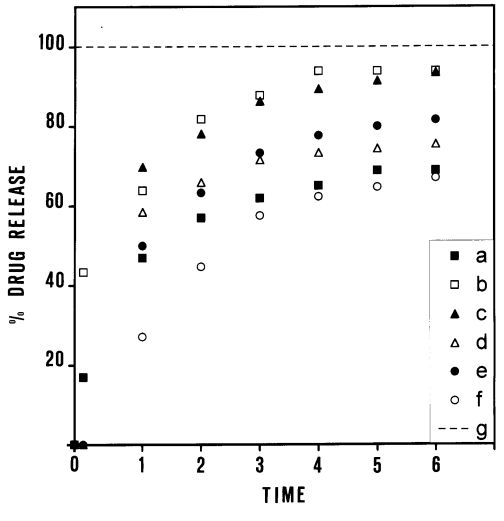

Fig. 8. Release kinetics at 37°C from different drug-loaded microspheres to DMPC unilamellar vesicles. The first values represent the released drug without incubating at 37°C; the successive values represent the same samples at 1-h incubation periods at 37°C.

are reported in Fig. 8. The release was monitored by calorimetric measurements, observing the depression of melting temperature, as a T_m shift, of DMPC unilamellar vesicles in the presence of drug-loaded microspheres, caused by drug molecules transferred from the matricial system to the liposomes. The comparison of this shift with that caused by the interaction of well known increasing molar fractions of drug with the DMPC liposomes, permits estimation of the amount of drug released. To calculate the exact amounts of drugs transferred to liposomes, the $\Delta T_m/T_m^o$ obtained from the kinetic experiments were substituted in the correlation equations (previously reported in Section 3.2) between thermotropic effect and drug molar fractions present.

No effect of unloaded PLGA microspheres on DMPC liposomes was observed in our experimental conditions (data not reported). This led us to exclude free lactic or glycolic acid formation by polymer degradation, because preliminary experiments showed a shift to higher values of the DMPC transition temperature (until 40°C) and a broadening of the calorimetric peak, upon lactic acid addition to liposome suspensions.

A 0.18 molar fraction of drug (dispersed in PLGA microspheres) was chosen to follow the release; this molar fraction will represent the maximum amount of drug (100%) that can be transferred from the matrix to liposomes, causing an effect similar to that observed for the same X_{Drug} of free drug dispersed in DMPC liposomes (curve g in Fig. 8).

The drug release of samples loaded with the highest amount of BPAA (batch 2: curve b), appears to be quite fast with respect to the lowest loaded sample (batch 1: curve a). After 6 h, both samples reach a maximum in the drug released, but only batch 2 releases almost 95% of X_{Drug} and the curves show a significant burst effect. NAP (batch 1: curve c; batch 2: curve d) and KPF (batch 1: curve e; batch 2: curve f) release kinetics show a different behaviour, with respect to BPAA microspheres, because both a faster and a higher amount released from the lower loaded sample are observed with respect to high loaded microspheres.

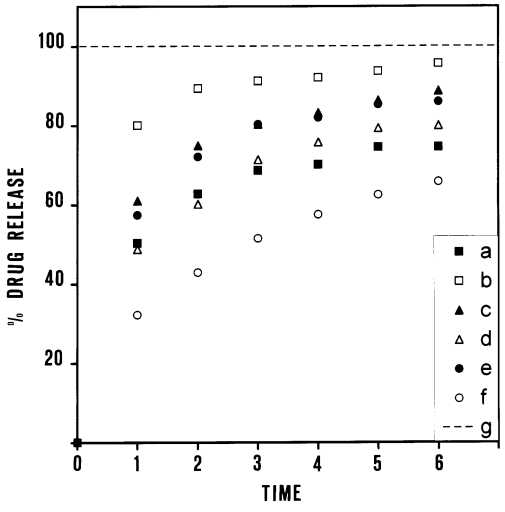

Fig. 9. Release kinetics at 37°C from different drug-loaded microspheres in Tris (pH = 7.4) determined by dissolution tests.

Fig. 9 reports the release kinetics at 37°C in Tris phosphate buffer (pH = 7.4) obtained by the USP 23 rotating paddle dissolution apparatus. The results obtained by the official dissolution method can be compared to those obtained by the calorimetric evaluation of drug release, showing good agreement between the data, for all the samples tested. A further advantage of the calorimetric method is represented by the possibility to determine the amount of drug released from the microspheres and actually transferred to the model membrane. These data can be obtained by the correlation curves referred to the drug in liposomal dispersion versus the effect on T_m corrected by the real distribution of the drug between the lipidic and aqueous phases.

The results obtained show that only BPAA-loaded microspheres (Fig. 8: curves a and b) have a release behaviour in keeping with the data in the literature (Spenlehauer et al., 1986; Whateley, 1993; Le Corre et al., 1994; Pradhan and Vasavada, 1994). Usually a faster release rate proportional to increasing amounts of drug in the microspheres is typical of crystalline dispersions of drug in the polymer matrix, and it can be explained by the assisted diffusion model (Whateley, 1993). This model, based mainly on drug

solubility properties, does not fit the release behaviour of NAP- and KPF-loaded microspheres because in these cases the drug release curves (Fig. 8: curves c–f) show faster release from those microspheres with lower drug payload. A satisfying comprehension of drug release kinetics can be achieved by DSC and SEM results. These studies revealed differences in the microsphere size, in the nature of drug dispersion inside the polymer matrix, and in the type of interaction between the drug and the polymer. On the basis of these data, and considering the release profiles shown in Figs. 8 and 9, it can be hypothesized that:

- BPAA release depends mainly on crystals dissolution, hence it is promoted by the small microsphere size and the large number of drug crystals.
- NAP release rate results from a dissolution process that is hindered by microsphere morphology; in fact NAP-loaded microspheres are large and have irregular shape, and a small number of drug crystals is observed, only on the microsphere outer surface.
- KPF release rate from the particulate system depends mainly on a diffusion, rather than a dissolution mechanism. The plasticizing effect exerted by the drug on the polymeric matrix produces irregular large masses with no crystal, slowing release kinetics.

The nature of drug dispersion through the polymeric matrix and morphology differences between the examined drug-loaded microspheres influence drug release rates more than the differences in water solubility between the three NSAIDs (Ventura et al., 1994; Fini et al., 1995).

4. Conclusions

We can conclude that:

1. Drug release rate from the microspheres is highly affected by the morphologic characteristics of microspheres and by the nature of drug dispersion in the microspheres;
2. Only BPAA release behaviour can be ascribed to the assisted diffusion model;
3. Drug release evaluation by a calorimetric technique establishing the interaction with a phospholipid bilayer is comparable to the traditional dissolution test by USP rotating paddle apparatus;
4. The application of calorimetric analysis to drug release study is an effective alternative to dissolution tests, and it represents a good model considering the important role of drug lipophilicity in drug absorption processes.

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