Indomethacin-Dipalmitoylphosphatidylcholine Interaction. A Calorimetric Study of Drug Release from Poly(Lactide-co-glycolide) Microspheres into Multilamellar Vesicles

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A comparative study of indomethacin controlled release from poly(lactide-co-glycolide) (50:50, molecular weight 3000) (PLGA) microspheres loaded with two different amounts of drug (10.9 ± 1%, and 34.1 ± 1% w/w) and pure free indomethacin, considering the effects exerted by the drug on the thermotropic behavior of dipalmitoylphosphatidylcholine multilamellar vesicles, was carried out by differential scanning calorimetry (DSC). The release was monitored by comparing the effect exerted by the free indomethacin on lipid thermotropic behavior with that of the drug released by the microspheres and relating these effects to a lipid aqueous dispersion containing the molar ratio of drug able to cause it. By DSC measurements, the pure free indomethacin was found to be able to have a fluidifying effect on the model membrane, causing a shift toward lower values of the transitional temperature (T_m), characteristic of phospholipid liposomes, without variations in the enthalpic changes (ΔH). This shift was found to be modulated by the drug molar fraction with respect to the lipid concentration in the aqueous dispersion. Successively, calorimetric measurements were performed on suspensions of blank liposomes added to weighed amounts of unloaded and indomethacin-loaded microspheres as well as free powdered indomethacin, and the T_m shifts of the lipid bilayer caused by the drug released from the polymeric system, as well as by the free drug, were compared with that caused by free drug increasing molar fractions dispersed directly on the membrane, employed as a calibration curve to obtain the fraction of drug released. This drug release model could be employed to determine the different kinetics involved in the drug transfer from the microspheres to a membrane. This in vitro study suggests that the kinetic process involved in drug release is influenced by the amount of drug loaded in the microspheres. This calorimetric study shows that the PLGA microspheres are a good delivery system able to sustain drug release. Moreover, the DSC technique applied to the drug interaction with biomembranes constitutes a good tool for determining the drug release representing an innovative alternative in vitro model.

Keywords Differential Scanning Calorimetry, Indomethacin, Membranes, Microspheres, Phosphatidylcholine, Poly-lactide-co-glycolide

Biodegradable polymeric matrices have been employed as drug control release systems to obtain sustained bioavailability of a drug and/or better solubility for poorly soluble drugs as well as an increased pharmacological effect due to more favorable contact with biological membranes (Kamijo et al. 1996; Lewis 1990; McGee et al. 1994; O'Hagan et al. 1994; Okada and Toguchi 1995; Whateley 1993; Wise et al. 1979); at the same time, the side effects of drugs can be minimized.

A class of drug widely studied as a model for in vitro release from polymeric microparticulate systems is represented by the nonsteroidal anti-inflammatory drugs (NSAIDs) dispersed in biodegradable polymeric micromatrices [poly(lactide-co-

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glycolide) microspheres]. In vitro studies of these systems are extensively reported in the literature (Kamijo et al. 1996; Lewis 1990; McGee et al. 1994; O'Hagan et al. 1994; Okada and Toguchi 1995; Whateley 1993; Wise et al. 1979), but such in vitro studies of drug release from drug-loaded PLGA microspheres do not take into account that in vivo drug release can be affected by the presence of biological membranes that adsorb lipophilic molecules, possibly increasing their release kinetic processes.

It is interesting to monitor the drug transfer to a biological model membrane as this gives more information on the drug release behavior. In our previous studies of the release of drugs from micromatrices (Castelli et al. 1994, 1996, 1997), we set up a system of L-α-dipalmitoylphosphatidylcholine (DPPC) multilamellar large vesicles (MLVs) whose thermotropic behavior is affected by the presence of molecules dissolved in their ordered structure.

Drug molecules change the ordered packing of lipids in model membranes. They can act as spacers or impurities, in the same way as a solute acts on the properties of the solvent in which it is dissolved, reducing the temperature of the transitional process (from a gel phase to a liquid crystalline phase) exhibited by hydrated phospholipids when heated. The drug interaction with lipid bilayers causes destabilization of the ordered structure of lipid chains, resulting in a shift of their transitional temperature toward lower values with respect to the lipid alone (Bach 1984; Castelli et al. 1989, 1992, 1994, 1996, 1997; Jain 1988; Lee 1977; Sturvetant 1982).

The thermodynamic parameters (transitional temperature and enthalpic changes) were determined by DSC analysis of the interaction of drug (free or released from microspheres) with lipidic membranes. This behavior can be analyzed by the van't Hoff model of the freezing point depression of a solution. This model has been verified for several classes of chemical compounds, such as anesthetics (Lee 1977; Suezaki et al. 1990), and it has been applied on a theoretical basis by some researchers (Guggenheim 1952; Jorgensen et al. 1991; Lee, 1977). The van't Hoff model was successfully applied in previous work (Castelli et al. 1994, 1996) to compare the effects exerted by free tolmetin and by tolmetin released from PDLLA microspheres on the thermotropic properties of L-α-dimyristoylphosphatidylcholine vesicles.

Evaluation of the melting temperature depression of pure DPPC liposomes caused by the transfer of indomethacin from matricial systems to void liposomes can be used to study release kinetics.

The interaction of indomethacin, which belongs to the class of nonsteroidal anti-inflammatory agents, with model membranes was previously described (Bonina et al. 1994), as well as indomethacin encapsulation and release from controlled drug delivery systems (Por Li et al. 1994; Rowe and Carless 1981; Suryakusuma and Jun 1984a,b). These previous studies (Bonina et al. 1994) are now extended by including the release of indomethacin from PLGA microspheres loaded with two different amounts of drug (10.9 ± 1 and 34.1 ± 1%) stored for 2 months at 20°C and 60% relative humidity and relating this release to that shown by free indomethacin. The acceptor site is represented by a lipid multilamellar vesicle, which, like a biological membrane, is able to accept drug molecules, showing saturation effects; the amount of drug released by the drug delivery system is thus determined directly at the acceptor site.

The aim of this work is to confirm the suitability of DSC for the evaluation of in vitro release kinetics from PLGA microparticulate systems and to evaluate the influence of different amounts of drug, loaded into the microspheres, on the polymer structure and on the drug release process. Both morphological properties and amount of drug loaded affect drug release from microspheres (Le Corre et al. 1994; Pradhan and Vasavada 1994; Rosilio et al. 1991).

MATERIALS AND METHODS

Chemicals

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

Indomethacin was supplied by Sigma Chemical Co. (St. Louis, MO, USA). Poly-lactide-co-glycolide (50:50, w/w), RG 502H, molecular weight (MW) 3000 (MHE determination), intravenous 0.2 g/dL was supplied by Boehringer Ingelheim (Ingelheim am Rhein, Germany).

Microsphere Preparation and Characterisation

Two batches (1 and 2) of microspheres of the same poly(lactide-co-glycolide) containing significantly different amounts of indomethacin were prepared. The preparation was performed by the spray drying method as reported previously (Pavanetto et al. 1993), and the microspheres obtained were morphologically characterized.

The PLGA microsphere drug content was determined by ultraviolet spectrophotometry. Weighed amounts (about 18 mg) of drug-loaded microspheres were dissolved in CH_2Cl_2 and indomethacin was analyzed with a Uvikon spectrophotometer (Kontron Instruments, Zurich, Switzerland) at 260 and 319 nm after extraction. Microsphere drug content values were 10.9 ± 1% (batch 1) and 34.1 ± 1% (batch 2) w/w.

Microspheres were morphologically characterized by scanning electron microscopy (SEM), using a microscope Leica-Cambridge S 360 at 20 kV. The samples, previously desiccated, were coated with a thin layer of Au and photographed under argon (Figure 1A and B).

Physicochemical characterization 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 (Figure 2).

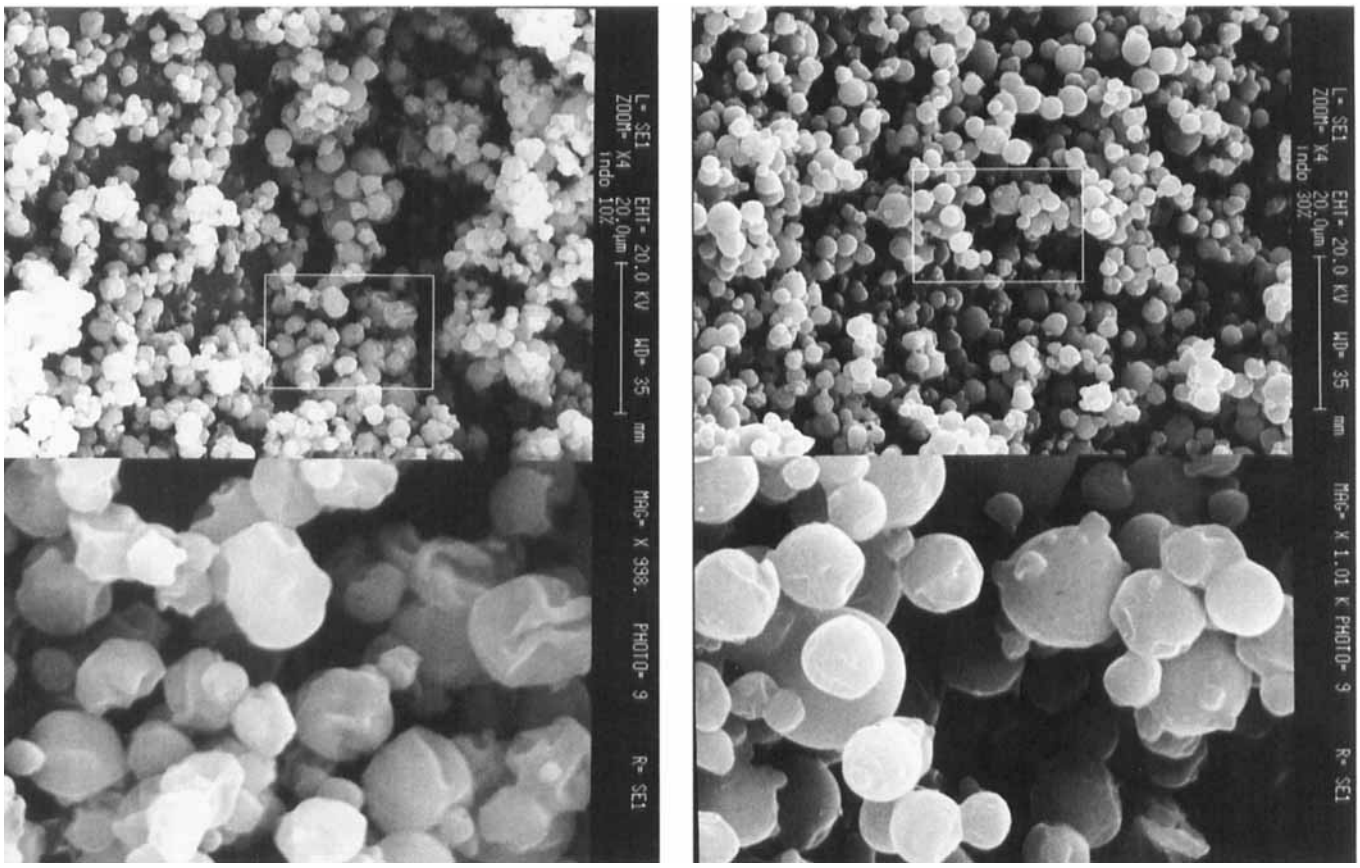

FIG. 1. Scanning electron micrograph of indomethacin-loaded microspheres. (A) 10.9%; (B) 34.1%

Liposome Preparation

Multilamellar liposomes (MLVs) were prepared in the absence of drug with a chloroform-methanol (1:1, v/v) stock solution of lipid. The solvents were removed under nitrogen in a rotoevaporator and the resulting film was kept overnight under vacuum to remove the residual solvents.

Liposomes were prepared by adding 50 mM Tris buffer (pH 7.4) to the film, then heating at 60°C and vortexing three times for 1 min. The samples were shaken for 1 h in a water bath at 60°C to homogenize the liposomes. Afterward, aliquots of 120 µl (4 mg of the lipid) were transferred to 150-µl DSC aluminum pans and submitted to DSC analysis.

Differential Scanning Calorimetry

DSC was performed with 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 5–55°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. Calibration in temperature and enthalpy was done with palmitic acid as the reference. Enthalpy changes were calculated from peak areas by using the integration program of the Mettler processor. All samples, after calorimetric scans, were extracted from the pans and aliquots were used to determine the amount of the phospholipid by the phosphorus assay previously mentioned.

Indomethacin-DPPC Liposome Interaction

Different aliquots of indomethacin were weighed into DSC pans to give increasing molar fractions of drug, and, after addition of DPPC liposomes prepared as described, were incubated for 2 h at 60°C and vortexed every 15 min to permit interaction between drug and liposomes. The samples were then submitted to DSC analysis. The constancy in the DSC results showed that equilibrium was reached.

Release Kinetic Experiments

Free powdered indomethacin, indomethacin-loaded PLGA microspheres, and blank PLGA microspheres were added to the DPPC liposomes in known amounts to obtain the same relative molar fraction of drug and/or polymer with respect to the lipid.

The samples were analyzed immediately after preparation. Each sample was submitted to the following procedure:

1. A first scan (from 10 to 55°C) to detect drug release, bringing the sample to 55°C.
2. 60 min at 37°C to detect the drug release after a long incubation time at a temperature near the human temperature.

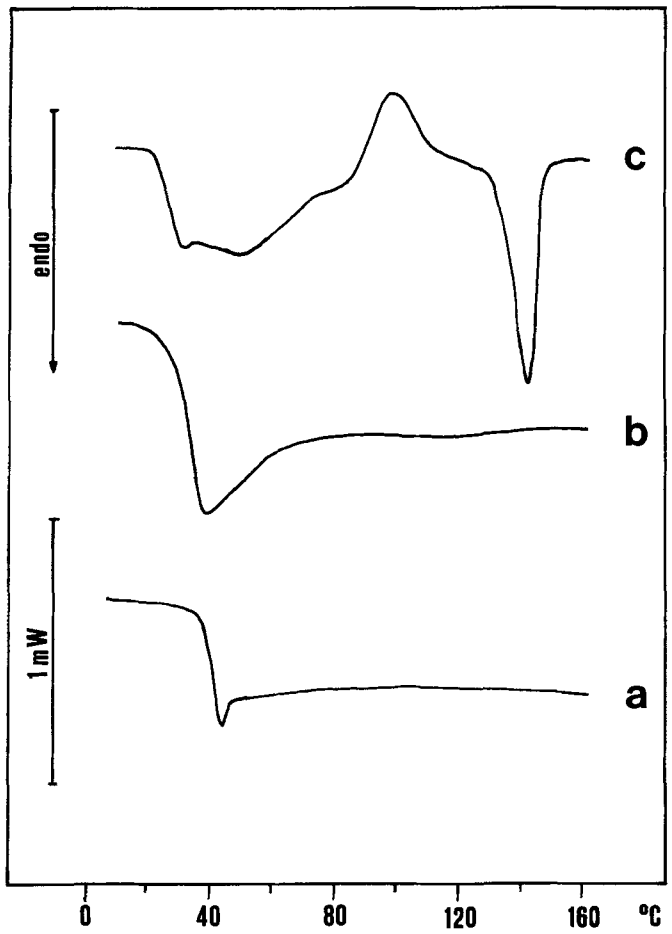

FIG. 2. Calorimetric scans of polymer and drug-loaded microspheres (a, polymer; b, 10.9% indomethacin-loaded microspheres; c, 34.1% indomethacin-loaded microspheres).

3. Subsequent scan from 10 to 55°C after the incubation at 37°C, followed by cooling of the sample to 10°C.

The whole procedure was performed on the two batches of microspheres loaded with different amounts of drug. This procedure was repeated several times until no further drug release was observed.

RESULTS AND DISCUSSION

Microsphere Characterization

Microsphere characterization was performed by the SEM and DSC techniques. Surface morphological analysis by SEM shows no substantial differences between the two differently loaded batches (Figure 1A and B). They appear to have similar sizes and shapes, without observable crystals at their surfaces. More information was obtained by the calorimetric measurements showing differences in the nature of the drug dispersion throughout the polymeric matrices. This knowledge is important for better understanding how the nature of the drug dispersion in the polymeric matrix can influence the release rate.

Thermograms of polymer and drug-loaded microspheres are shown in Figure 2. The abrupt change in the slope of the polymer curve (curve a), meaning a change in heat capacity, is usually assigned to the glass transition, typical for amorphous copolymers such as PLGA 50:50. This transition is characterized by a transitional temperature T_g defined as the temperature at which a rigid or "glassy" polymer is converted to a softer, "rubbery" polymer upon heating, with an increase in the free volume of the polymer (Hausberger and DeLuca 1995). In the presence of drug (10.9% indomethacin-loaded microspheres) this transition is slightly shifted toward a lower temperature (curve b). The lower T_g and the absence of a fusion event (due to the crystalline drug dispersed in the microspheres) suggest that indomethacin is dissolved in the polymer as a solid solution, where drug and polymer interaction with each other, leading to polymer plasticization (Dubernet 1995). When a higher amount of drug was loaded into the microspheres (34.1% indomethacin), three different process were observed (curve c). They appear as a large endothermic peak followed by two peaks, an exothermic followed by an endothermic process. The first large endothermic process should still be caused by a structural change (glass transition) upon heating of the polymeric matrix. The exothermic process could be caused by the fraction of drug dispersed in microspheres as "amorphous," which can crystallize during the heating (exothermic peak) before melting (endothermic peak). The melting process is observed to be at a lower temperature with respect to the pure drug, probably because of the interaction between the drug and polymer (Bodmeier et al., 1989; Izumikawa et al. 1991; Rosilio et al. 1991). These results show that the amount of the drug loaded in the matrix system influences the nature of the dispersion: a lower indomethacin content (10.9%) leads to formation of a drug solid solution in the polymeric matrix, whereas a higher drug content (34.1%) leads to amorphous drug dispersion in the polymer.

Effect of Free Indomethacin on the Thermotropic Behavior of DPPC Liposomes

The calorimetric curves for free DPPC compared with those obtained in the presence of increasing amounts of indomethacin on DPPC liposomes (Figure 3) highlight that the indomethacin is able to interact with the lipidic model membranes by shifting the calorimetric peak toward lower values, depressing their transitional temperature (Table 1) but leaving the enthalpy change almost constant. This shift appears to be related to the molar fraction of drug present in the liposomial dispersion (Figure 4).

The interaction between drugs and DPPC 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 destabilization 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

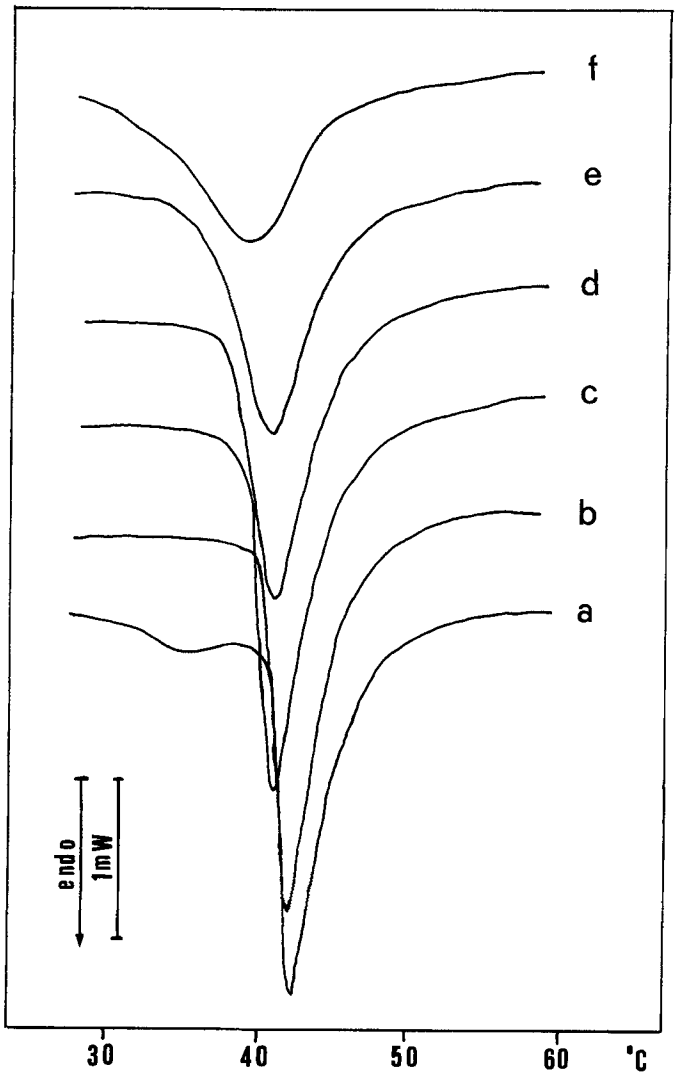

FIG. 3. Calorimetric curves of DPPC multilamellar vesicles in the presence of increasing molar fractions of indomethacin: a, 0.0; b, 0.03; c, 0.06; d, 0.09; e, 0.12; f, 0.18.

TABLE 1
Transitional temperatures and related temperature shifts obtained by interaction of increasing amounts of indomethacin with DPPC vesicles

$X_{indomethacin}$	T_m(K)	$\frac{\Delta T}{T_m^\circ} \cdot 1000$
0.00	314.86	0.0
0.030	314.26	−1.90
0.060	313.16	−3.49
0.090	313.06	−5.71
0.120	312.56	−7.29
0.180	311.66	−10.15

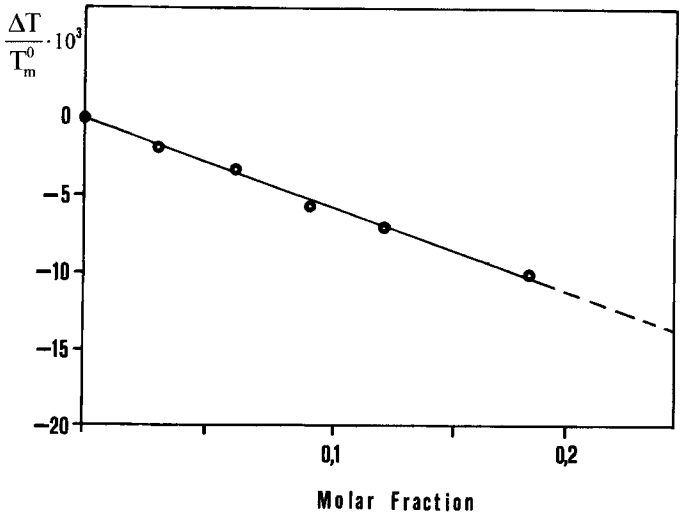

FIG. 4. Calibration curve relating the depression of the DPPC multilamellar vesicle transition temperature (T_m) to the concentration of indomethacin.

between amphipatic molecules and DPPC polar heads, which occurs only at the surface of lipid layers without the interaction of acyl chains (Castelli et al. 1994, 1996; Estep et al. 1978; Sturtevant 1982).

Figure 4 shows the $\Delta T/T_m^\circ$ plot versus drug mole fraction ($\Delta T_m = T_m^\circ - T_m^\circ$, where T_m° and T_m are the transition temperatures of pure DPPC and indomethacin containing DPPC liposomes, respectively), representing the effect of increasing amounts of indomethacin. By relating T_m depression to the molar fraction of drug present on the membrane surface, a calibration curve ($Y = -0.191 - 57.13X$, $r = 0.997$) is obtained to quantify and follow the transfer of indomethacin, released from the microparticulate system, to empty membranes.

Comparative Release from Free Solid Indomethacin and Indomethacin-Loaded PLGA Microspheres

In Figure 5 (curves a and b) the transfer kinetics of indomethacin from PLGA microspheres (batches 1, 2) to void lipid multilamellar vesicles is shown and compared with the that observed for the free drug (curve c). These transfer phenomena were monitored by observing the shift of the DPPC calorimetric curves, as the T_m shift, and by measuring the depression of the melting temperature of void DPPC multilamellar vesicles after drug molecules were taken up from the matrix system or from the powdered solid drug and comparing this shift with that observed for the interaction of increasing molar fractions of indomethacin with the DPPC liposomes, reported as a calibration curve in Figure 4.

A 0.18 molar fraction of drug (free or dispersed in PLGA microspheres) was chosen to follow the release. The maximum amount of drug transferred from the matrix to liposomes should cause an effect similar to that observed for the same X_{drug} of free indomethacin dispersed in DPPC liposomes and shown in Figure 5 (curve d).

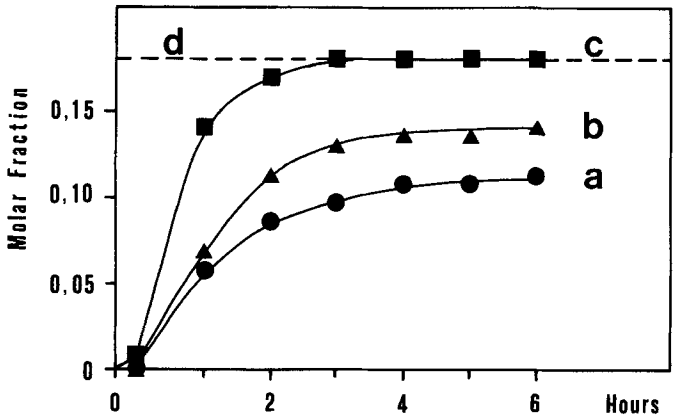

FIG. 5. Release kinetics for free solid indomethacin and indomethacin-PLGA matrices to void MLV (X_{drug} = 0.18): (a) batch A (10.9% drug loaded sample); (b) batch B (34.1% drug loaded sample); (c) free solid indomethacin. The curves represent the concentrations of indomethacin in the aqueous DPPC vesicle dispersions versus time. The first value represents the amount of indomethacin without incubation at 37°C; the successive values represent the same samples heated at 37°C at 1-h incubation periods. (d) Theoretical amount of indomethacin to be released to DPPC multilamellar vesicles.

The release process at 37°C for the sample loaded with the highest amount of drug (batch 2) is faster (Figure 5, curve b) than for the lowest loaded sample (batch 1, curve a). After 6 h both samples reach a maximum in drug released, and batch 2 releases almost 80% of the drug. The results obtained suggest that the process of drug diffusion through the polymeric matrix, before polymer erosion takes place, is affected by the amount of drug loaded.

Experiments carried out on unloaded PLGA microspheres with DPPC liposomes in our experimental conditions (37°C) gave no evidence for the presence of free lactic or glycolic acid, at least during the first 7 h of the kinetic experiments (data not reported).

Comparing these release profiles with those for the pure free drug, it appears evident that the release process is delayed, confirming that the polymeric system can constitute a good controlled-delivery system.

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