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Calorimetric studies on tolmetin release from poly-DL-lactide microspheres to lipid model membrane

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Summary

The aim of this work was to study the rate of release of an NSAI agent from poly-DL-lactide (PDLLA) microspheres, by evaluating the effect of the drug on the thermotropic behaviour of dimyristoylphosphatidylcholine liposomes (DMPC), selected as a model membrane. Polylactide microspheres loaded with 1-methyl-5-*p*-toluoylpyrrole-2-acetic acid (tolmetin) were prepared by the spray-drying method. Samples made of liposomes charged with free drug and suspensions of blank liposomes added to weighed amounts of tolmetin-loaded microspheres were analyzed by DSC. Calorimetric analyses were performed on samples previously incubated at temperatures below and above the polymer glass transition temperature (T_g). Free drug was found to interact with the phospholipidic bilayer by modifying its thermotropic behavior. The amount of drug released from the microparticulate to void liposomes was quantified by comparing the T_m shift caused by drug release from the polymeric system with that due to free drug. The results demonstrate the extent to which the release process is affected by temperature throughout the polymeric structure. In conclusion, the calorimetric technique detects changes occurring directly on the adsorption sites and can thus be applied to study slow kinetics directly at the site of drug uptake.

Introduction

The preparation of polymeric microspheres has received much attention in recent years (Marcotte and Goosen, 1989 and references cited herein; Ammoury, 1990; Tomlinson, 1990; Izumikawa et al., 1991; Rosilio, 1991). Biodegradable and biocompatible polymers such as poly-DL-lactide (PDLLA) may be suitable as carriers for controlled release dosage forms.

Non-steroidal anti-inflammatory drugs (NSAIDs) are usually very slightly soluble in water and often gastrolesive after oral administration. For this reason dispersion into polymeric matrices is a good approach to obtain a therapeutic effect during a predetermined period of time (controlled release) while minimizing the side effects of NSAIDs. These systems allow the programmable release of drugs by diffusion and/or erosion mechanisms. The goal is to slow drug release as well as to prolong the therapeutic activity of drugs (Nixon, 1981; Leelarasamee et al., 1986; Uchida et al., 1989).

The preparation and in vitro dissolution be-

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haviour of drug-loaded polylactide microspheres have previously been described (Pavanetto et al., 1992; Conti et al., 1993).

In the present work, we studied the release of tolmetin from PDLLA microspheres. Tolmetin is a pyrrole-acetic acid derivative belonging to the class of non-steroidal anti-inflammatory agents, which has been shown to possess remarkable analgesic and anti-inflammatory efficacy in man (Berkowitz, 1974; Mainbach, 1976; Bachmann et al., 1977). As well as other NSAIDs, tolmetin displays a marked irritating effect on gastrointestinal mucosae. The interaction with lipidic membranes, of either free drug or of drug dispersed into PDLLA microspheres, has been never examined. Moreover, the characterization by DSC of such phenomena, with the aim of evaluating the influence of polymer structure variations on the release process, has scarcely been investigated. In fact, it has been reported that morphological properties such as particle size, surface area and porosity strongly affect drug release from microspheres (Rosilio, 1981; Benita et al., 1984; Benoit et al., 1986; Jalil and Nixon, 1990). Furthermore, drug release is also modulated by polymeric glass transition.

To study the release of drug from PDLLA matrices we employed L-α-dimyristoylphosphatidylcholine (DMPC) liposomes which exhibit a change in thermotropic behavior in the presence of molecules dissolved in their ordered structure.

The presence of drug in ordered bilayers lowers the melting temperature of lipid chains with respect to the lipid alone (Guggenheim, 1952; Sturtevant, 1982; Bach, 1984; Jain, 1988 and references cited herein).

This phenomenon is often analyzed according to the Van't Hoff model of the freezing point depression. The validity of this model has been verified for several classes of chemical compounds such as anaesthetics and insecticides (Lee, 1977; Buff and Berndt, 1981; Suezaki et al., 1990) and it has been applied on theoretical bases by some researchers (Sturtevant, 1982; Jorgensen et al., 1991).

Differential scanning calorimetry (DSC) appeared to be very useful as a technique to study the interactions between drugs and lipid-model membranes (Papahadjopoulos et al., 1975; Bach, 1984; O'Learly et al., 1986).

The depression of the melting temperature of pure DMPC liposomes caused by the transfer of tolmetin from a matricial system to void liposomes can be usefully employed to study the release kinetics. Such a process is found to be affected by polymer morphology, which, in turn, depends on the temperature below or above the polymer glass transition.

The reported method represents a new approach compared to the usual in vitro experiments (for example, dissolution tests or dialyzing membranes) to determine the amount of drug released by a drug delivery system. In fact, we have no sink model but a lipid multilayer, which, like a biological membrane, is able to accept drug molecules and shows saturation effects. By modifying the thermotropic properties of the bilayer, upon drug adsorption, we may also reveal the amount of substance penetrating within the lipid hydrocarbon core.

Materials and Methods

Chemicals

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

1-Methyl-5-*p*-toluoylpyrrole-2-acetic acid (tolmetin) was supplied by Aldrich Italia. Poly-DL-lactide (PDLLA), Res 206, Mol. Wt 109000, was supplied by Boehringer Ingelheim (Boehringer Ingelheim International GmbH, Ingelheim am Rhein, Germany).

Microsphere preparation

Microspheres were prepared by the spray-drying method and characterized by scanning electron microscopy (SEM), DSC and UV spectrophotometry as reported in a previous paper (Conti et al., 1993). Tolmetin content in PDLLA microspheres was 8.26% w/w.

Physicochemical characterization

Physicochemical characterization of microspheres and of the polymer was performed by DSC, with a Mettler TA 3000 system at a scanning rate of 10°C/min between 30 and 200°C.

Liposome preparation

Multilamellar liposomes were prepared in the presence and absence of free drug, at a temperature above the gel-liquid crystalline phase transition. Chloroform-methanol (1:1, v:v) stock solutions of lipid and drug were mixed in order to obtain the chosen mole fractions of drug. The solvents were removed under nitrogen in a rotoevaporator and the resulting film was kept overnight in a vacuum pump to remove the residual solvents. Liposomes were prepared by adding to the film 50 mM Tris buffer (pH 7.4), then heating at 55°C and vortexing three times for 1 min. The samples were shaken for 1 h in a water bath at 55°C to homogenize the liposomes. Thereafter, aliquots of 100 μl (4.5 mg of lipid) were transferred into 160 μl DSC aluminium pans and submitted to DSC analysis.

DSC

DSC was performed by using 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 2–55°C after an initial isothermal period of 15 min. The sensitivity was 1.72 mW, and the reference pan was filled with Tris buffer solution. Calibration in temperature and enthalpy was performed by using palmitic acid as 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 phospholipid by the phosphorus assay.

Release kinetic experiments

Tolmetin-loaded PDLLA microspheres or blank PDLLA microspheres were added to the DMPC liposomes in known amounts to obtain the same relative mole fraction of drug and polymer with respect to lipid.

After loading in DSC pans, samples were incubated in a Haake model F3-K ultrathermostat at a temperature (34°C) lower than the polymer glass transition temperature, found at 40°C. This temperature was lower than that detected for the dry polylactide ($\approx$ 54°C) (Conti et al., 1993), probably because the water absorbed has a plasticizing effect on the polymer (Siemann, 1985).

Only an initial sample was analyzed immediately after preparation, all other samples being analyzed, with increasing incubation times, at 2 day intervals. Each sample was submitted to the following procedures:

(1) An initial scan (from 2 to 55°C) to detect the drug release at 34°C; (2) a second scan (from 2 to 55°C) after a further incubation for 30 min at 55°C, to detect the drug released after a short incubation time at $T > T_g$; and (3) subsequent scans from 2 to 55°C after 2 hours incubation at 55°C, to detect drug released after a longer incubation time at $T > T_g$. This last procedure was repeated several times until no further drug release was observed (five times).

Results and Discussion

Interaction of free tolmetin-DMPC liposomes

DSC runs, in heating mode, of pure DMPC dispersions in Tris buffer (pH 7.4) containing different molar ratios of tolmetin are reported in Fig. 1. The main transition peak for pure DMPC was observed at 23.6°C. Tolmetin appears to interact with DMPC liposomes causing, by increasing its mole fraction in the aqueous dispersion, a considerable lowering of the transition temperature (T_m) of the calorimetric peak. This peak is associated with the well-known gel to liquid-crystal phase transition typical for PC multilayers (Fig. 1 and Table 1). The enthalpy changes (ΔH), related to the peak area, remained nearly constant (Table 1).

The interaction between drugs and PC liposomes was largely 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 destabilization of the lipid

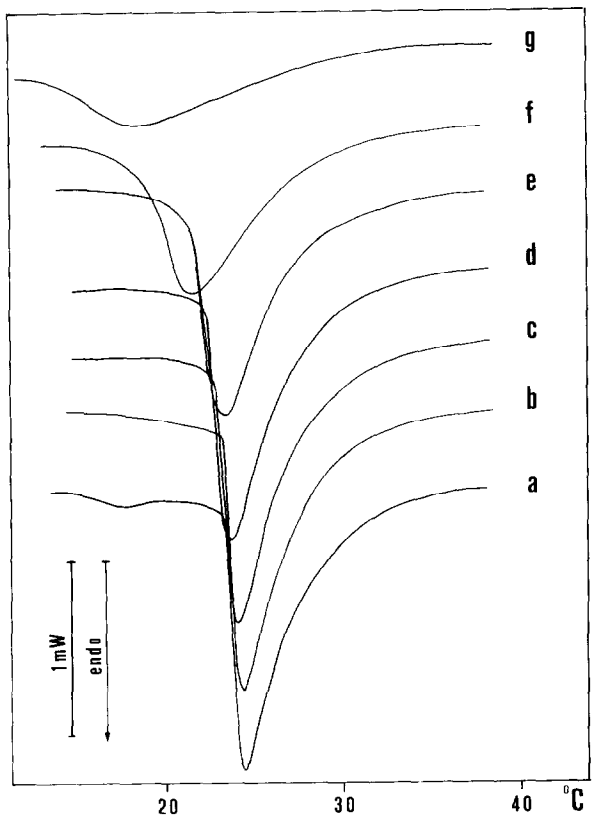

Fig. 1. Differential scanning calorimetry heating curves of hydrated DMPC containing tolmetin at drug mole fraction: (a) 0, (b) 0.03, (c) 0.06, (d) 0.09, (e) 0.12, (f) 0.18, (g) 0.24.

mosaic with decrease in the T_m of the gel to liquid-crystal phase transition. The negligible variation in ΔH is attributed to a surface interac-

TABLE 1

Main transition peak temperature (T_m, °C) and enthalpy changes (ΔH, expressed in kcal/mol) of DMPC aqueous dispersions at different mole fractions of tolmetin

Mole fraction	T_m (°C)	ΔH (kcal/mol)
0.00	23.6	6.6
0.03	23.4	6.4
0.06	23.3	6.0
0.09	22.8	6.0
0.12	22.6	6.0
0.18	20.5	5.8
0.24	19.7	5.9

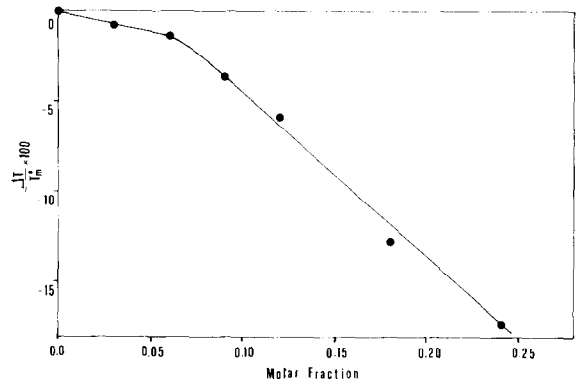

Fig. 2. Calibration curve relating the depression of DMPC lipid bilayer transition temperature T_m to the concentration of tolmetin dissolved in the lipid matrix.

tion between amphipathic molecules and DPPC polar heads, which occurs only at the surface of lipid layers without the acyl chains penetrating deeply (Estep et al., 1978; Bach, 1984; Castelli et al., 1992).

$\Delta T_m/T_m^o$ ($\Delta T_m = T_m - T_m^o$, where T_m^o and T_m are the transition temperatures of pure DMPC and tolmetin-containing DMPC liposomes, respectively) representing the effect of increasing amounts of tolmetin was plotted vs the drug mole fraction (Fig. 2). This plot is used as a calibration curve to relate the T_m depression to the mole fraction of drug present on the membrane surface.

Interaction of tolmetin-loaded PDLLA microspheres with DMPC liposomes

DMPC liposomes were chosen, since their transition temperature is below the polylactide glass transition temperature (Fig. 3, curves a and c). This allows us to operate with the lipidic bilayer in a liquid-crystalline like phase, during the release of tolmetin. It is well known that hydrophobic molecules dissolve in the liquid-crystalline phase (where lipid chains are in a disordered conformation) more readily than in a gel phase (more ordered and rigid structure) (Lee, 1977). Consequently, the membrane model is in a favorable and constant condition to accept drug molecules released from the matricial system.

Moreover, the lipid phase transition temperature must be far from the polymer glass transition temperature, in order to avoid overlapping of the calorimetric peaks. These conditions also allow us to follow the endothermic process of the polymer glass transition, which disappears after heating over T_g (Fig. 3, curves a and b).

The kinetics of transfer of tolmetin from PDLLA microspheres to void lipid vesicles dispersed in Tris buffer was monitored by observing the shift of DMPC calorimetric curves and measuring the depression of the melting temperature of void DMPC liposomes after incorporation of drug molecules from the matricial system (Fig. 3, curves c–h).

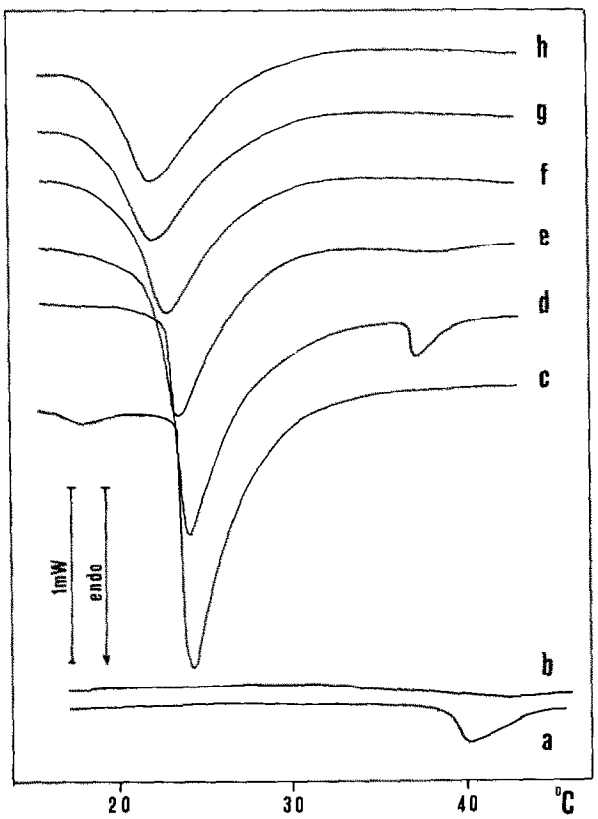

Fig. 3. Differential scanning calorimetry heating curves of: (a) PDLLA microspheres dispersed in Tris buffer after incubation at 34°C; (b) same sample as in curve A heated at $T > T_g$ (55°C) for 30 min; (c) DMPC liposomes; (d) DMPC liposomes in the presence of PDLLA microspheres containing 0.18 mole fraction of tolmetin after incubation at 34°C; (e) same samples as in curve E heated at $T > T_g$ (55°C) for 30 min; (f–h) samples of curve F after incubation for successive 2 h periods, at $T = 55°C$ ($T > T_g$).

A 0.18 mole fraction of drug dispersed in PDLLA microspheres was chosen to follow the release. The maximum amount of drug to be transferred from the matrix to liposomes should cause an effect similar to that observed for the same X_{Drug} of free tolmetin dispersed in DMPC liposomes and visualized in Fig. 4 (curve D).

The release process at 34°C ($< T_g$) appears to be quite slow (Fig. 4, curve A). After 8 days only the 0.07 mole fraction was transferred from the polymer to liposomes, indicating that the drug diffuses poorly through the polymeric structure in a rigid glassy phase (Omelczuck and McGinity, 1992). The calorimetric results are in keeping with the in vitro release studies, where in order to attain complete drug release, more than 15 days is needed (Conti et al., 1993).

It was sufficient to maintain the sample at 55°C ($> T_g$) for 30 min to increase the release rate (Fig. 4, curve B). The results in Fig. 4 show that a rubber-like structure of the polymer enables more rapid and greater release of drug (final amount of drug equal to 0.11 mole fraction). Successive experiments at 55°C for 2 h (curves C_1–C_5) demonstrated greater release of drug, however, in every case the quantity was lower than the maximum we could achieve for complete release (curve D).

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

Conclusions

The results obtained suggest that diffusion is probably not the only kinetic process involved in the release of tolmetin, the complete erosion of the polymer matrix and a longer time being needed to attain total release.

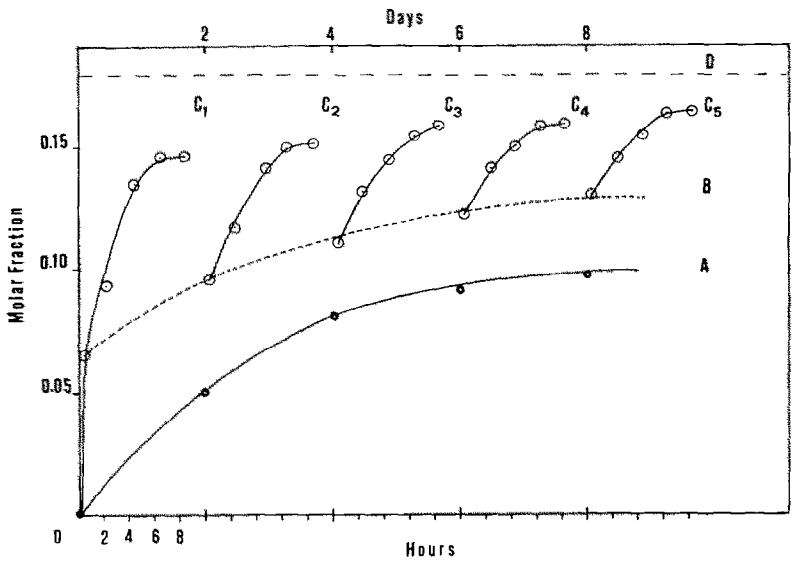

Fig. 4. Kinetics of uptake from drug-polylactide matrix to void liposomes ($X_{Drug} = 0.18$). Concentrations of tolmetin inside DMPC vesicles vs time. (A) Mole fraction of released tolmetin after incubation at 34°C ($T < T_g$) (days). (B) Same samples as in curve A heated at $T > T_g$ (55°C) for 30 min (days). (C_1–C_5) Samples of curve B after incubation for successive 2 h periods, at $T = 55$°C ($T > T_g$) (h). (D) Hypothetical amount of tolmetin to be released to DMPC liposomes.

The different kinetics, as determined from our calorimetric experiments (slower below T_g and faster above T_g), are explained by the change in the physicochemical properties of the polymer matrices. They are correlated with the methods of preparation (Izumikawa et al., 1991) and with the temperature at which the release process takes place. It is worth emphasizing that our calorimetric technique can be applied to follow such slow kinetics directly at the site of drug uptake instead of the usual in vitro determination.

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