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Flurbiprofen Release From Eudragit RS and RL Aqueous Nanosuspensions: a Kinetic Study by DSC and Dialysis Experiments

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ABSTRACT The present work investigated the release of Flurbiprofen (FLU) from Eudragit RS100® (RS) and Eudragit RL100® (RL) nanosuspensions to a biological model membrane consisting of Dimyristoylphosphatidylcholine (DMPC) multilamellar vesicles (MLV). This release was compared with those observed from solid drug particles as well as with dialysis experiments. Nanosuspensions were prepared by a modification of Quasi-Emulsion Solvent Diffusion technique. Drug release was monitored by the Differential Scanning Calorimetry (DSC). FLU dispersed in MLV affects the transition temperature (T_m) of DMPC liposomes, causing a shift towards lower values. The temperature shift is modulated by the drug fraction present in the aqueous lipid bilayer suspension. DSC was also performed, after increasing incubation periods at 37°C, on suspensions of blank liposomes added to fixed amounts of unloaded and FLU-loaded nanosuspensions, as well as to powdered free drug. T_m shifts, caused by the drug released from the polymeric system or by free-drug dissolution during incubation cycles, were compared with those caused by free drug increasing molar fractions dispersed directly in the membrane during their preparation. These results were compared with the drug release and were followed by a classical dialysis technique. Comparing the suitability of the 2 different techniques in order to follow the drug release as well as the differences between the 2 RL and RS polymer systems, it is possible to confirm the efficacy of DSC in studying the release from polymeric nanoparticulate systems compared with the "classical" release test by dialysis. The different rate of kinetic release could be due to void liposomes, which represent a better uptaking system than aqueous solution in dialysis experiments.

Key Words: Differential Scanning Calorimetry (DSC), Dimyristoylphosphatidylcholine (DMPC), Eudragit, Flurbiprofen (FLU), Membrane Model, Membrane Permeation, Nanosuspensions, Phosphatidylcholine

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INTRODUCTION

Polymer nanosuspensions of nonsteroidal anti-inflammatory agents (NSAIDs) have been often proposed as controlled drug delivery systems able to solve pharmacokinetic problems and/or the gastric damaging effects typical of most of these drugs [1, 2].

In previous yet unpublished studies we described Flurbiprofen (FLU) and Ibuprofen-loaded polymer nanoparticle suspensions, which were made of Eudragit RS100® (RS) or RL100® (RL) polymers and aimed at releasing drugs to eye tissues after ocular application. RS and RL polymers are copolymers of partial esters of acrylic and methacrylic

acids with alcohols containing a low amount of quaternary ammonium groups (approximately 5% and 10% for RS and RL, respectively). The resulting matrix shows water insolubility and a permeability profile independent of pH variations, thus representing a valid material for controlled drug delivery [3-9].

FLU [2-fluoro-α-methyl[1,1'-biphenyl]-4-acetic acid] is one of the few nonsteroidal anti-inflammatory agents used to treat ocular inflammatory conditions (eg, to prevent the myosis induced by surgical trauma, as during cataract extraction [10]). Rabbit in vivo tests showed that FLU-loaded nanosuspensions are void of ocular toxicity and are able to ensure drug concentration in the aqueous humor and an antimyotic activity higher than a reference commercial eye-drop formulation (unpublished results). Techniques employed to investigate drug-membrane interactions represent useful tools to obtain preliminary information about membrane permeation pathways or bioavailability of a drug [11].

The aim of the present study was to evaluate the efficacy of the Differential Scanning Calorimetry (DSC) technique in following the release of FLU from RS and RL nanoparticles and the forthcoming interaction of the released drug with a lipid membrane model. The results gain useful suggestions for the actual efficacy of the microparticulate in vivo formulation. The effects exerted by FLU on the thermotropic behaviour [12-15] of L-α-Dimyristoylphosphatidylcholine liposomes were examined by DSC technique [16-18]. The presence of foreign drug molecules dissolved in the ordered lipid bilayer, depending on their structural features, can induce significant variations in the thermodynamic parameters associated with the lipid phase transition such as the transition temperature (T_m) and enthalpy changes (ΔH) [19-22].

In order to study the transfer kinetics of a drug from a polymer release system to biological membranes, the variations in the thermodynamic parameters of lipid vesicles, caused by the drug (free or loaded in a polymer carrier) left in contact with empty multilamellar vesicles (MLVs), were compared with the maximum interaction. The drug-membranes maximum interaction is obtained by adding the drug to the lipid, in organic phase, during MLV preparation. If the perturbative effect (T_m shift) of the system tends to the same value observed for the preparation in organic phase, it should represent a sign of the occurred membrane penetration, as was previously reported in an experimental and theoretical study on Diflunisal penetration through lipid membranes [23]. The results were also compared with dialysis release experiments, which represent a "classical" way of following the release of a drug from a drug delivery system but mimicking a "sink" method with the lack of a cell membrane able to capture the drug. Evaluation of all these results should give useful indications for understanding the influence of different permeability of RL and RS polymers in the drug-membrane interaction and, furthermore, the suitability of DSC for studying the release from polymeric nanoparticulate systems compared with the classical release test by dialysis, thus allowing speculations about the in vivo bioavailability of the investigated drug carrier systems [24-27].

MATERIALS AND METHODS

Liposomes Preparation

MLVs were prepared in both the absence and presence of increasing molar fractions of FLU by following the classical procedure: dissolution of the compounds in organic solvents, lipid film preparation, and rehydration with 50 mM Tris buffer (pH = 7.4) at a temperature above the gel-liquid crystalline phase transition (37°C) by vortex. The samples were left at this temperature for 1 hour to allow them to reach the partition equilibrium of the drug between the lipid membranes and the aqueous medium.

Aliquots (5 mg of lipid in 120 µL) of MLV, loaded with different molar fractions of FLU, were transferred in 160 µL DSC aluminium pans. Afterwards, the samples were submitted to DSC analysis.

Differential Scanning Calorimetry

A Mettler Toledo STARe system (Schwerzenbach, Switzerland) equipped with a DSC-822^e calorimetric cell and Mettler TA-STAR^e software was used. The scan rate employed was 2°C/min in the temperature range 5°C to 37°C. The resolution of the signal was smaller than 0.04 μW, and the reference pan was filled with Tris buffer solution. The calculations were performed using the Mettler STAR^e version 6.10 software. After the calorimetric scans, all samples were extracted from the pan and aliquots were used to determine the amount of phospholipids by the phosphorous assay [29].

Permeation Experiments

To study the capacity of FLU—released from a solid free drug or released from the polymeric matrices—to permeate the model membrane, kinetic experiments were carried out leaving in contact DMPC liposome suspensions in Tris buffer with (a) a known amount of pure, finely powdered drug; or (b) FLU-loaded or blank RS and RL nanosuspensions, placed in the bottom of the DSC crucible, in order to obtain the same relative molar fraction (X = 0.12) with respect to the lipids of free drug, dispersed in the polymer or pure polymer. Samples were submitted to the following step protocol: 1) a first scan (5°C-37°C) to detect drug uptake by the membrane; 2) an isothermal period of 1hour at 37°C to allow the drug to permeate the lipid layer(s); 3) a cooling scan from 37°C to 5°C to restart the heating program.

The whole procedure was performed at least nine times until a near constant drug-MLV interaction was reached (no further peak temperature variation observed), indicating a drug concentration equilibrium existing between the aqueous buffer and the lipid membrane.

Dialysis Experiments

FLU release from nanosuspensions was evaluated over 24 hours by a dialysis system, consisting of a Spectrapor membrane (cut-off: 3500 Da) loaded with 5 mL of nanosuspensions and soaked in a 0.15 M phosphate buffer solution (pH 7.4) at room temperature and under slow magnetic stirring. At determinate intervals, aliquots of 1 mL of the dissolving medium were withdrawn and immediately restored with the same volume of fresh buffer. The amount of drug released was determined spectrophotometrically at 247 nm vs a calibration curve realized in the same phosphate buffer.

RESULTS AND DISCUSSION

The calorimetric measurements evidenced as pure FLU. When incorporated in the MLV during the lipid film preparation in an organic phase, FLU is able to interact with model membranes, by decreasing the calorimetric peak and depressing the transitional temperature but leaving the enthalpy changes almost constant (Table 1). This effect has been expressed as $\Delta T_m/T°_m$ ($\Delta T_m = T°_m - T_m$, where $T°_m$ and T_m are the transition temperatures of pure DMPC and FLU-loaded DMPC liposomes, respectively) and plotted vs drug molar fractions present in the aqueous lipid dispersion (Figure 1). Such interaction is explained by the largely reported "fluidising" effect exerted when a foreign molecule is dispersed in the lipid "sea" causing destruction in the ordered lipid layer structure [30,31].

Table 1. Main Transition Peak Temperature (Tm K) and Enthalpy Changes (ΔH, °KJ/mol) of DMPC Aqueous Dispersions at Different Molar Fractions of Flurbiprofen

Molar Fractions	T_m	ΔH
0.00	298.0	29.8
0.030	297.8	29.8
0.045	297.5	29.8
0.060	297.2	29.7
0.090	296.6	28.1
0.120	295.5	27.9

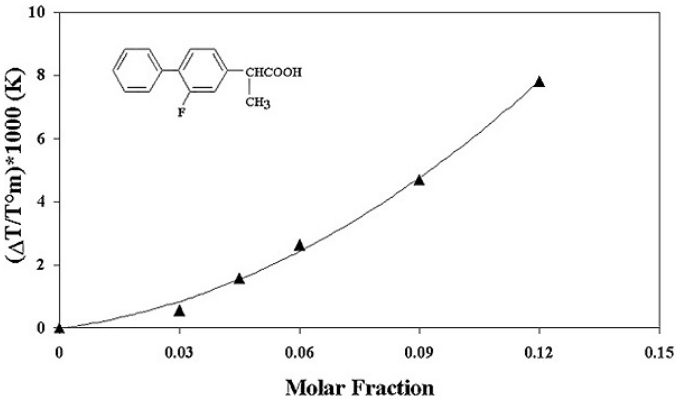

Figure 1. Effect of increasing molar fractions of FLU present in the aqueous dispersion of DMPC on the temperature shifts of the lipid phase transition.

To consider the ability of the pure drug to migrate through an aqueous medium and to demonstrate that RS and RL nanosuspensions are a system able to modulate the drug release, we compared the transfer kinetics of the drug, both free and after its release from nanosuspensions, with the maximum effect exerted on model membranes (preparation in organic phase).

No interaction was observed (data not reported) when an amount of pure RS or RL, equivalent to that employed for the release studies with drug-loaded nanosuspensions, was left in contact with a DMPC aqueous dispersion. Therefore, the effects observed on the lipid phase transition can be attributed only to FLU leaving the polymeric system and interacting with the model membrane.

Transfer phenomena of drug (0.12 molar fraction), free or released by the Eudragit matrices, through the aqueous medium were monitored at increasing incubation times, by observing the T_m shift of DMPC calorimetric curves (Figures 2, 3, 4). In these figures the calorimetric curves are compared with the curve (reference dotted curve) observed during "classical organic solvent preparation." Melting temperature values of void MLV, after drug molecules uptake from the accurately powdered solid drug or from nanosuspensions, were compared with the value observed by the interaction of a fixed molar fraction of FLU with the DMPC liposomes [24,27].

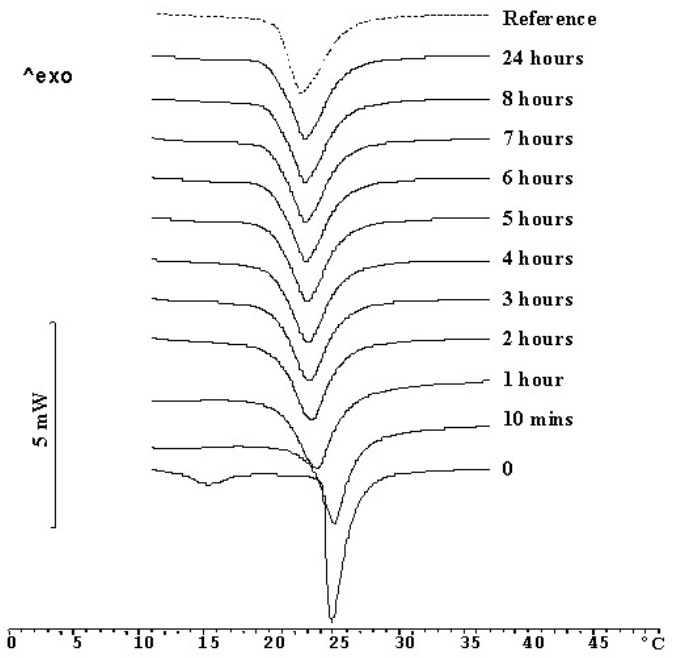

Figure 2. Calorimetric curves of FLU, as free drug, interacting with void MLVs, after migrating through the aqueous medium, for increasing periods of contact. The dotted reference curve represents the effect of a 0.12 molar fraction of FLU dispersed in the liposomes during the "classical" preparation.

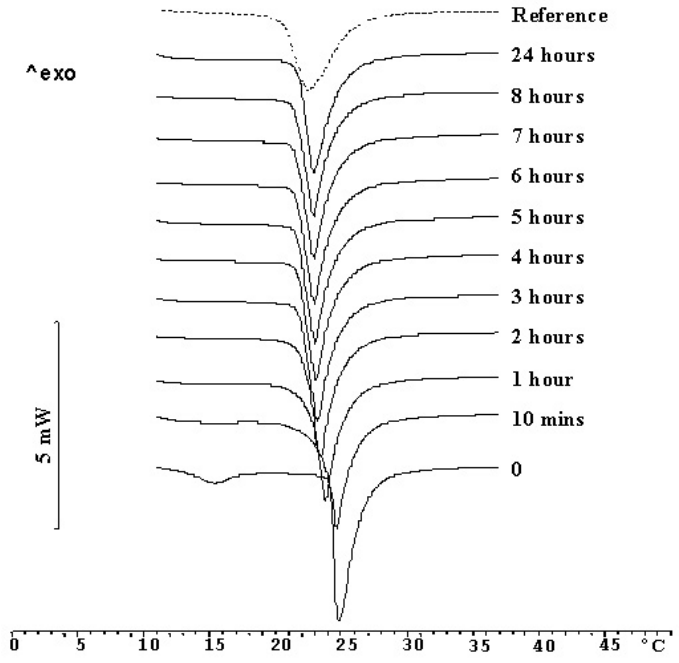

Figure 3. Calorimetric curves of FLU, released from Eudragit RL100® nanosuspension to void MLVs, for increasing periods of contact.

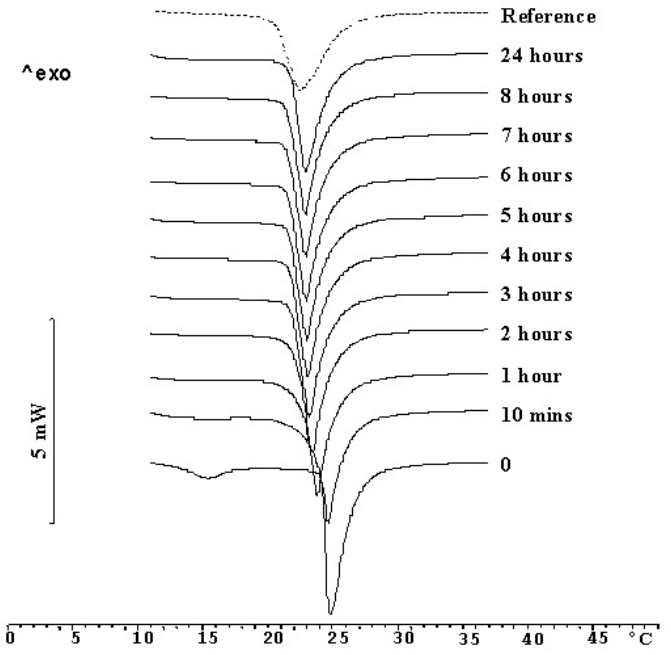

Figure 4. Calorimetric curves of FLU, released from Eudragit RS100® nanosuspension to void MLVs, for increasing periods of contact.

The maximum exerted effect, corresponding to the maximum amount of drug transferable from the polymer to liposomes, is visualized in Figure 5 as the point at t_{inf}. This value is obtained from FLU-MLV interaction after liposome preparation in the organic solvent and is considered as 100% of the released drug.

In Figure 5, the transfer kinetics of FLU as free drug or from RS and RL nanosuspensions to void MLVs are reported and compared with the kinetic transfer observed for dialysis experiments (at pH 7.4). Because of its acidic nature, FLU showed interaction with Eudragit matrixes, not only by a mechanical dispersion but also by virtue of electrostatic interactions with the ammonium groups present in the polymer backbone [32,33]. These interactions are stronger for drugs, like FLU, which bear a carboxylic moiety and show a lower pK_a value, and can significantly affect the drug release profile from the polymeric system.

For instance, in our previous research on solid dispersions of FLU and other NSAIDs with RS and RL matrixes [34], dissolution as well as absorption studies confirmed the complex relationship existing between drug molecule structure, mainly the presence of a dissociable acid group, and the RS or RL polymer amount in the systems. In particular the electrostatic nature of such interactions was evidenced.

The plateau observed in the dissolution profiles of FLU from nanoparticles is related to an equilibrium among drug release, its ionization in the dissolution medium, and the saturation of the binding sites on the surface of polymer particles. Such behavior can be ascribed to the fact that the dissolved drug, becoming ionized in the neutral dissolution medium, is readsorbed onto the polymer particles because of the presence of opposite electrical charges [35]. RL is known to be more permeable than RS in aqueous media, owing to the higher ammonium group content. In the case of dialysis tests, the driving force leading to FLU release from the nanoparticles is the volume and light-alkaline pH of the dissolution buffer; the absence of an up-taking system in the external medium thus made the observed release profile more strictly dependent upon the nature of the polymers. In fact, the 2 systems showed similar time-release curves, however, with a higher amount of drug released from the more permeable RL matrix (Figure 5). Conversely, when the biological model was assayed, the overall drug-release profile was conditioned by the ability of liposome bilayers to capture and retain drug molecules after their leakage from the polymeric network.

In these experiments the volume of dissolution medium is much smaller, and the equilibrium between the amount of drug bound to nanoparticle surface, the fraction dissolved in the buffer, and the amount captured by the MLVs is greatly conditioned by the affinity of RS or RL polymers for the drug. The behavior observed for FLU-loaded RL and RS nanosuspensions is then quite different (Figure 5). Although the maximum released amount was higher for the former system due to higher RL water permeability, the liposomes incubated with RS nanoparticles showed a quicker capture of the dissolved drug. Since the DMPC liposomes were able to retain all the amount of drug dispersed in the nanoparticles, ie, a quantity corresponding to a 0.12 molar fraction of FLU, the above DSC data can be

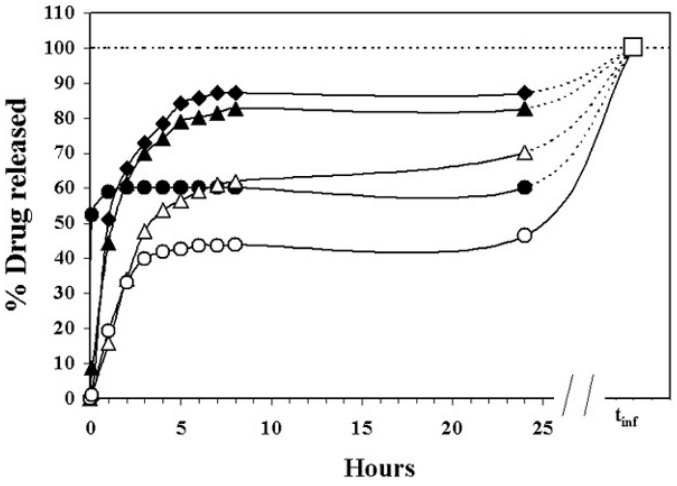

Figure 5. Drug release from free FLU (◆) as well as from Eudragit RL (▲) and RS (●) nanosuspensions to void MLVs, compared with the data observed in the dialysis experiments (Δ and ○). The (□) represents the maximum amount of drug releasable (value obtained from the interaction of MLV preparation in organic solvent in presence of 0.12 molar fraction of Flurbiprofen).

explained by considering a prompt release of the dispersed drug from RS polymeric matrix; conversely, RL particles would be able to retain the drug molecules longer on their ammonium "binding sites." In other words, whereas in the classical dialysis tests the permeability of RS and RL polymers represents the limiting step to the release of an acidic drug such as FLU, in the smaller space of the DSC pan the affinity equilibrium for particle surface or liposome bilayers plays a major role in determining the overall drug-release profile.

This in vitro study suggests that the kinetic process involved in drug release is influenced by the different kinds of polymers forming the nanosuspensions acting on drug dissolution rate and membrane disorder [24-27,36]. The different permeability between the 2 polymers justifies the entity of drug release and interaction with DMPC bilayers, whereas the affinity of the drug to the polymeric matrix leads the rate at which these phenomena occur.

The results confirm the suitability of DSC for studying the release from polymeric nanoparticulate systems compared with the "classical" release test by dialysis. The different rates of kinetic release could be due to void liposomes, which represent a better uptaking system than aqueous solution in dialysis experiments.

This drug release-uptake model evidences that a) Eudragit RL100 nanosuspensions is a better delivery system than RS100 to sustain the drug release; b) DSC technique applied to the drug interaction with biomembrane represents a good tool to follow the drug release; and c) this model, representing an alternative in vitro tool, could be employed to determine the different kinetics involved in the drug transfer from a drug delivery system to a membrane selected as an uptake site.

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