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Eudragit as controlled release system for anti-inflammatory drugs A comparison between DSC and dialysis experiments

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Abstract

A comparative study between the release of Ibuprofen (IBU) from Eudragit RS100® (RS) and RL100® (RL) nanosuspensions as well as the free drug to a biological model membrane, consisting of dimyristoylphosphatidylcholine (DMPC) multilamellar vesicles (MLV), was carried out by DSC technique. The aim was to assess the suitability of such calorimetric technique to determine the kinetics of drug release from a polymer system, compared with a classical release test by dialysis method. Nanosuspensions were prepared by a modification of the quasi-emulsion solvent diffusion technique (QESD), a particular approach to the general solvent-change method. This kind of system was planned for the ophthalmic release of non-steroidal anti-inflammatory drugs in ocular diseases associated with inflammatory processes (i.e. post-cataract surgery or uveitis). The drug release was monitored by differential scanning calorimetry (DSC), following the effects exerted by IBU on the thermotropic behaviour of DMPC multilamellar vesicles. IBU affects the main transition temperature (T_m) of phospholipid vesicles, causing a shift towards lower values, driven by the drug fraction entering the lipid bilayer. The obtained values have been used as a calibration curve. DSC was performed on suspensions of blank liposomes added to fixed amounts of unloaded and IBU-loaded Eudragit RS100® and RL100® nanosuspensions as well as to powdered free drug. The T_m shifts caused by the drug released from the polymer system or by the free drug, during incubation cycles at 37 °C, were compared to the calibration curve in order to obtain the fraction of drug released. The results were also compared with in vitro dialysis release experiments. The suitability of the two different techniques to follow the drug release as well as the differences between the RL and RS polymer systems was compared, confirming the efficacy of DSC for studying the release from polymer nanoparticulate systems. Explanation of the different rate of kinetic release could be due to void liposomes, which represent a better up-taking system than the aqueous solution phase in the dialysis experiments.
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Keywords: Eudragit; Uptake kinetics; DSC; Nanoparticles; Model membranes; Ibuprofen

1. Introduction

Controlled drug release from ophthalmic systems attained large attention during the last years [1,2]. The aim is often to obtain polymer systems well accepted by the eye tissues regarding their biocompatibility and immunogenical properties. Nanoparticles and nanosuspensions charged with anti-inflammatory agents can be proposed as carriers for such kind of release [1–4]. The drug release can be monitored either “in vivo” by considering the plasmatic concentration,

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or by "in vitro" experiments, using dissolution devices or dialysis methods. The "in vitro" model does not take into account the membrane penetration and, mainly, the uptake process occurring on cell surface, which withdraws the drug molecules from the solution were the drug delivery device (nanosuspensions or nanoparticles), is dispersed. In this paper, the transfer of Ibuprofen (IBU) from Eudragit RS100® and RL100® nanosuspensions to a biological model membrane consisting of dimyristoylphosphatidylcholine (DMPC) multilamellar vesicles (MLV) was investigated by DSC and the results were compared with the "classical" dialysis membrane release, to show the differences in the drug release profile.

Nanosuspensions were prepared by a modification of the quasi-emulsion solvent diffusion technique (QESD), a particular approach to the general solvent-change method [5,6]. This kind of system was planned for the ophthalmic release of non-steroidal anti-inflammatory drugs in ocular diseases associated with inflammatory processes (i.e. post-cataract surgery or uveitis) [4,7].

IBU is a diffused non-steroidal anti-inflammatory agent used in the treatment of ocular inflammatory conditions, e.g. to prevent the myosis induced by surgical trauma, like during cataract extraction [8]. In vivo tests in rabbit showed that IBU-loaded nanosuspensions are avoid of ocular toxicity and able to ensure a drug concentration in the aqueous humour and an anti-myotic activity higher than a reference eye-drop formulation [7].

The drug release was monitored by following the effects exerted by IBU on the thermotropic behaviour of DMPC multilamellar vesicles by DSC technique [9,10–14]. The thermodynamic parameters associated to the lipid phase transition, such as the main transition temperature (T_m) and enthalpy changes (ΔH) can be modified by the presence of foreign drug molecules dissolved in the ordered lipid bilayer [15–17]. IBU affects the transition temperature (T_m) of phospholipid vesicles, causing a shift towards lower values, which is related to the drug fraction entering the lipid bilayer. To study the transfer kinetics of the drug from the polymer delivery system to biological membranes, a suspension of blank liposomes was added to fixed amounts of unloaded and IBU-loaded Eudragit nanosuspensions, as well as to powdered free drug. Drug–liposomes interaction was allowed by keeping the vesicle suspension at 37 °C for variable incubation times and carrying out DSC analyses. In order to obtain the fraction of drug released, the T_m shifts caused by the drug delivered from the polymer system or by the free drug, during the incubation cycles, were compared to those caused by increasing free drug molar fractions dispersed directly in the membrane, employed as a calibration curve. The drug–membrane interaction (T_m shift) obtained when the drug is added to the lipids, in organic phase, during MLV preparation is considered the maximum drug–membrane interaction. Tending of the T_m shift of the system to the value observed for the preparation in organic phase, should indicate that the drug penetrated into the membrane, as previously reported in an experimental and theoretical study on diflunisal penetration through lipid membranes [18]. The results were also compared with dialysis release experiments. They represent a "classical" way to follow the release of a drug from a particulate drug delivery system but without an up-taking device able to capture the drug. The evaluation of all these results could explain the influence of different permeability of RL and RS polymers in the drug–membrane interaction, the suitability of DSC for studying the release from polymer nanoparticulate systems with respect to the classical release test by dialysis, and thus to speculate about the in vivo bioavailability of the investigated drug carrier systems [19–22].

2. Experimental

2.1. Chemicals

Synthetic L-α-dimyristoylphosphatidylcholine (DMPC) was obtained from Genzyme Pharmaceuticals (Liestal, Switzerland). Solutions of the lipid were chromatographically pure as assessed by two-dimensional thin-layer chromatography.

Eudragit RS100® and RL100® polymers were kindly gifted from Rofarma Italia S.r.l. (Gaggiano, Italy); Ibuprofen and Tween 80 were purchased from Sigma–Aldrich Chimica S.r.l. (Milan, Italy); both compounds were used as received. Absolute ethanol, chloroform and methanol were analytical or superior grade products. Phosphate buffer (pH = 7.4) was used to prepare the liposomes as well as for dialysis experiments.

2.2. IBU-loaded nanosuspensions

The QESD technique is a modification of the classical solvent-evaporation procedures [5,6]. A total amount of 200 mg of polymer and the drug (as the free acid; 33% in weight) were co-dissolved in ethanol (2 ml) and the solution was slowly added with a syringe connected to a thin Teflon tube, under high-speed agitation (23,500 rpm; Ultra-Turrax, IKA Labortechnik, Staufen, Germany) to 50 ml of a 0.02% (w/v) Tween 80 aqueous solution, kept in a water-ice bath. After 15 min, the counter-diffusion of water into the drug plus polymer solution and of ethanol into the aqueous dispersing medium was completed. As a consequence of the passage into the water phase, drug-loaded nanoparticles rapidly precipitated. A following slow stirring at room temperature for 4–8 h ensured the complete evaporation of the organic solvent. The system was analysed for mean particle size and ζ-potential (ZetaSizer, Malvern Instruments Ltd., Worcs, UK) and, after freeze-drying (Freeze-dryer Modulyo, Edward), morphologically characterised by X-ray diffraction, FT-IR and DSC [7]. IBU-RS nanosuspensions showed a mean size of 37.4 ± 4.3 nm (with a polydispersion index of 0.34) and a ζ-potential of $+41.6 \pm 0.2$ mV; RL systems gave a mean size of 40.0 ± 4.3 nm (0.22 P.I.) and a ζ-potential of $+40.3 \pm 0.2$ mV. Empty RS and RL nanosuspensions showed a similar positive ζ-potential ($+35 \pm 1$ mV), whereas the pure IBU powder suspension showed a negative value (-15.6 mV) [7]. It is noteworthy that all the spectroscopic analyses agreed in indicating that the drug maintains its crystallinity within the polymer network, without polymorph changes or amorphisation [7]. Therefore, comparison of dissolution behaviour from pure drug powder or nanosuspensions can be directly performed. In vitro IBU release was monitored by dialysing 5 ml of the nanosuspensions (containing 20 mg of drug) against 100 ml of pH = 7.4 phosphate buffer at 37 °C, using a Spectrapor membrane (cut-off: 3500 Da).

2.3. Liposomes preparation

Multilamellar liposomes were prepared in the absence and presence of increasing molar fractions of IBU by the following procedure. Dissolution of drug and lipids in organic solvents, formation of a film and hydration with 0.15 M phosphate buffer (pH = 7.4) to originate multilamellar vesicles, by vortexing at a temperature above the gel–liquid crystalline phase transition (37 °C) by vortex. The samples were left at this temperature for 1 h to reach the partition equilibrium of the drug between the lipid membranes and the aqueous medium. Aliquots (5 mg of lipids in 120 μl) of blank MLV or loaded with different molar fractions of IBU, were transferred into 160 μl DSC aluminium pans. Afterwards, the samples were submitted to DSC analysis.

2.4. Differential scanning calorimetry

A Mettler Toledo STAR^e system equipped with a DSC-822^e calorimetric cell and Mettler TA-STAR^e

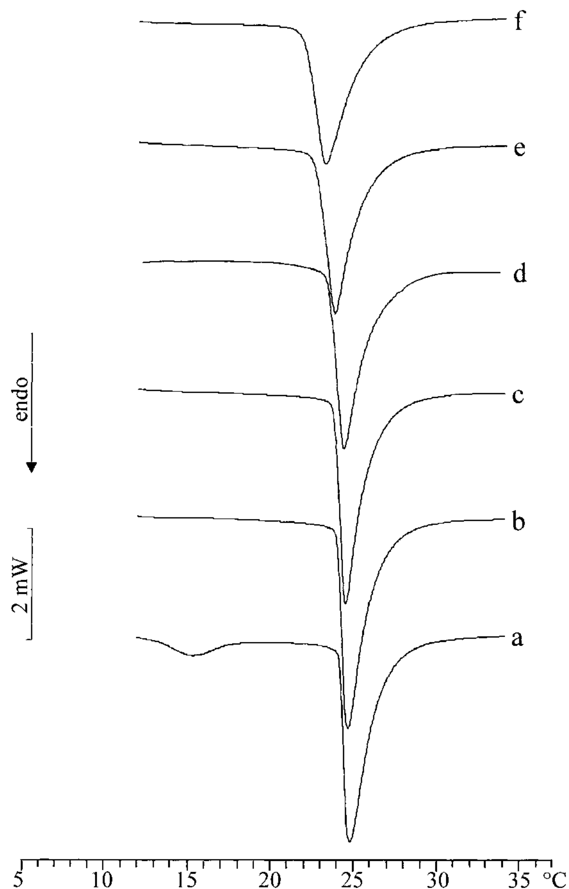

Fig. 1. Differential scanning calorimetry heating curves of hydrated DMPC containing IBU, obtained starting from organic solvent solutions, at different molar fractions: a = 0.0; b = 0.030; c = 0.045; d = 0.06; e = 0.09; f = 0.12.

software was used. The scan rate employed was 2 °C/min in the temperature range from 5 to 37 °C. The resolution of the signal was smaller than 0.04 μW, and the reference pan was filled with pH 7.4 phosphate buffer. The calculations were performed by the Mettler STAR[e] version 6.10 software. All samples, after the calorimetric scans, were extracted from the pan and aliquots were used to determine the amount of phospholipids by the phosphorous assay [23].

2.5. Permeation experiments

To study the capacity of IBU to permeate the model membrane as a free drug or after its release from the polymer matrices, kinetic experiments were carried out leaving in contact blank DMPC liposomes suspensions in phosphate buffer with: (a) a known amount of pure, finely powdered drug; or (b) IBU-loaded; or (c) blank RS and RL nanosuspensions, placed in the bottom of the DSC crucible, so to obtain the same relative molar fraction ($X = 0.12$), with respect to the lipids, of the pure drug dispersed in the polymer or of pure polymer. Samples were submitted to the following step protocol:

(1) a first scan (from 5 to 37 °C) to detect drug uptake by the membrane;
(2) an isothermal period of 1 h at 37 °C to allow the drug to permeate the lipid layers;
(3) a cooling scan from 37 to 5 °C to restart the heating program.

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

2.6. Dialysis experiments

IBU release from nanosuspensions was evaluated over 24 h by a dialysis system consisting of a Spectrapor membrane (cut-off: 3500 Da), loaded with 5 ml of nanosuspension and soaked in a 0.15 M phosphate buffer solution (pH = 7.4), at room temperature and under slow magnetic stirring. At determinate time 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 265 nm (Shimadzu UV-1601) versus a calibration curve in the same buffer.

3. Results and discussion

The calorimetric measurements evidenced that IBU is able to interact with lipid model membranes by

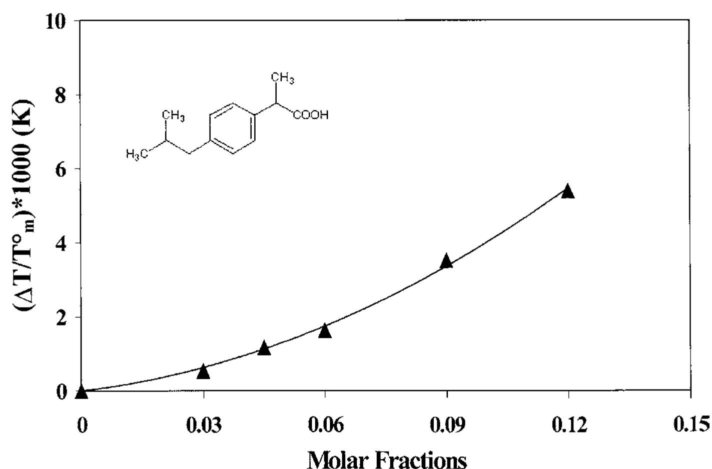

Fig. 2. Effect of increasing IBU molar fractions present in the aqueous dispersion of DMPC on the temperature shifts of the lipid phase transition.

shifting the peak of the calorimetric curves toward lower values (Fig. 1), depressing their transitional temperature but leaving the enthalpy changes almost constant. Drug effect on the thermotropic behaviour of DMPC liposomes has been expressed as $\Delta T_m/T_m^\circ$ ($\Delta T_m = T_m^\circ - T_m$, where T_m° and T_m are the transition temperatures of pure DMPC and IBU-loaded DMPC liposomes, respectively) and plotted versus drug molar fractions present in the aqueous lipid dispersion (Fig. 2). By increasing IBU molar fraction, a greater destabilising effect was exerted on the ordered lipid structure, giving indications of the drug ability to dissolve inside a model membrane and acting as a "fluidiser" molecule that causes destruction of the ordered lipid layer structure [24,25].

To follow the transfer of the drug from free finely powdered solid or from drug-loaded nanosuspensions, kinetic experiments were carried out. DMPC vesicles were put in contact with pure solid IBU or drug-loaded nanosuspensions, so to have the same amount of drug and the interaction between the drug and the vesicles was detected at different incubation times by DSC.

To demonstrate that the effects observed on the lipid phase transition can be attributed only to IBU leaving the polymer system and interacting with the model membrane, a control experiment was carried out by leaving in contact pure RS or RL nanosuspensions, whose amount was equivalent to that one employed for the release studies with drug-loaded particles, with a DMPC aqueous dispersion. In such experiments no interaction was observed (data not reported).

Figs. 3–5 compare the calorimetric curves of pure DMPC MLV respectively with the MLV left in contact with the drug (0.12 molar fraction), free or released by RL or RS Eudragit matrices. All the curves were also compared with that one (curve r) relative to the IBU-loaded MLV sample prepared in organic solvent and taken as reference curve, representing the maximum exerted effect, and thus corresponding to the 100% of amount of drug transferable to liposomes.

In all cases, IBU showed to be transferred to liposomes: in fact, shifts of the calorimetric curves toward lower temperatures were observed but at different extent. The data are better represented in Fig. 6, where the temperature shifts of the calorimetric curves were converted into the amount of drug released to liposomes. The conversion was obtained comparing the ΔT_m values to that showed by the preparation in

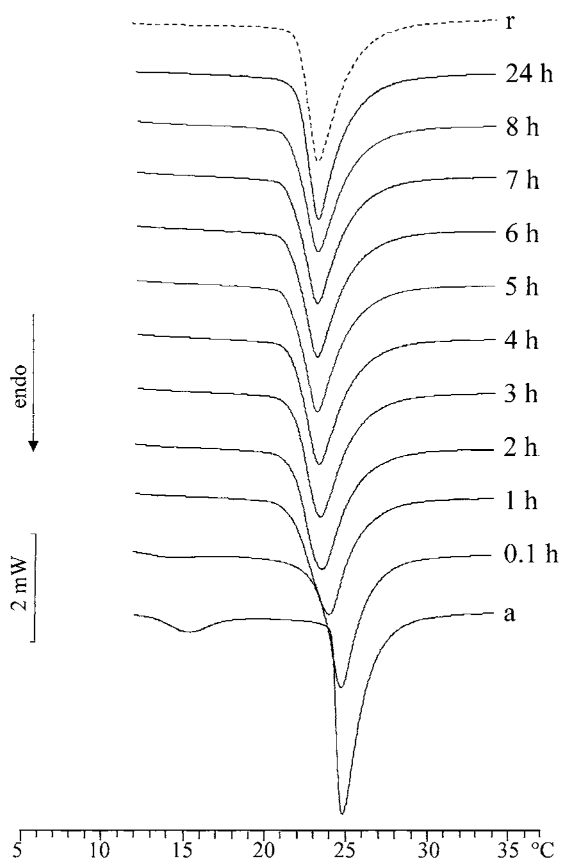

Fig. 3. Calorimetric curves of IBU, as a free drug, interacting with void MLVs, after migrating through the aqueous medium, for increasing periods of contact. The curve (a) represents the pure DMPC, instead the dotted reference curve (r) represents the effect of a 0.12 molar fraction of IBU dispersed in the liposomes during the "classical" preparation.

organic phase which, as stated before, can be considered as the 100% of releasable drug. It is evident as the uptake process of the released IBU by RL or RS polymers is enough fast reaching a plateau in 2 h. The different plateau position in the graph revealed that for the RL polymer about the 80% of the drug has been caught by the membrane, against the only 50% observed for the RS. These kinetics are faster also than the pure drug, being the drug molecularly dispersed in the Eudragit matrices and thus more ready to be dissolved in the aqueous medium.

In Fig. 6, the results of the transfer kinetics of IBU as free drug or from RS and RL nanosuspensions to void MLVs, are compared also with those of kinetic transfer

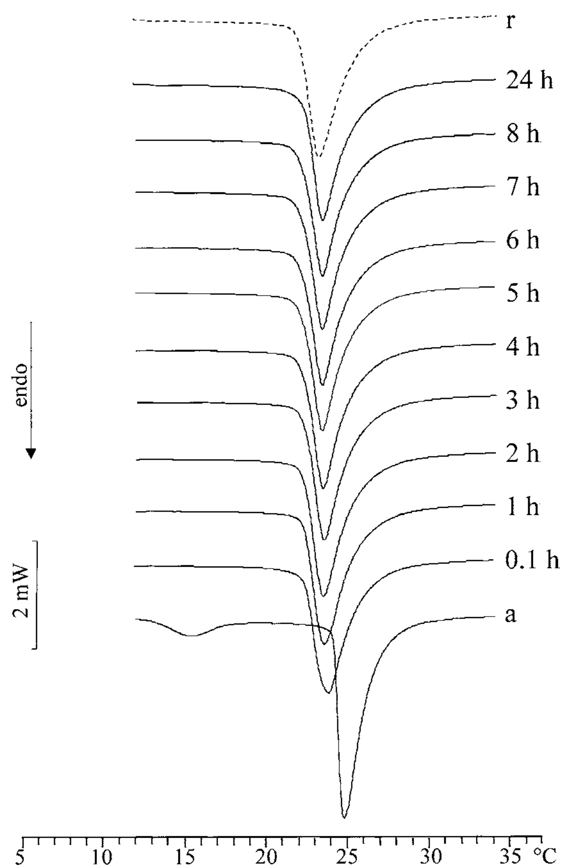

Fig. 4. Calorimetric curves of IBU, released from Eudragit RL100® nanosuspension to void MLVs, for increasing periods of contact.

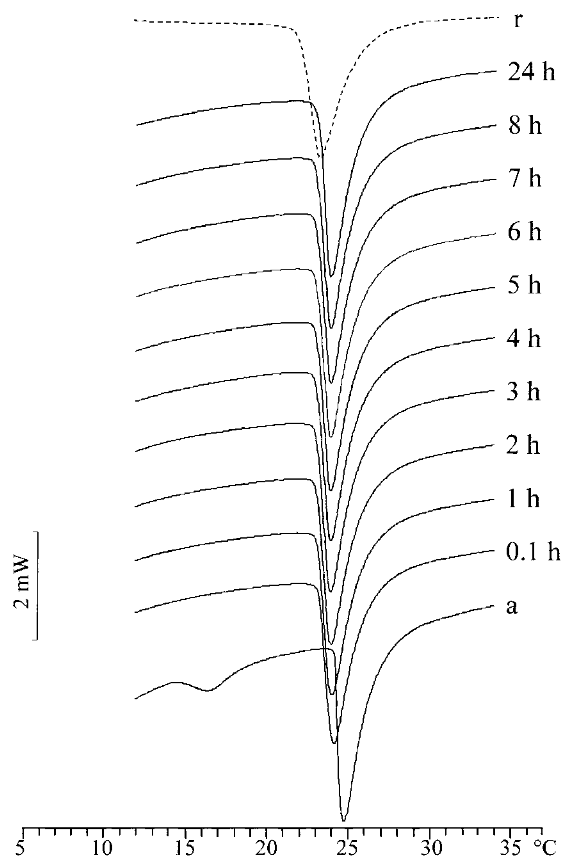

Fig. 5. Calorimetric curves of IBU, released from Eudragit RS100® nanosuspension to void MLVs, for increasing periods of contact.

followed by dialysis experiments (at pH 7.4). Due to its acidic nature, IBU is able to interact with Eudragit matrices, beside a mechanical dispersion, by virtue of electrostatic interactions with the ammonium groups present in the polymer backbone [26–28]. These interactions are stronger for drugs bearing a carboxylic moiety, thus having lower pK_a values, and significantly affected drug release profile from the polymer system.

For instance, in a previous research of some of us on solid dispersions of FLU and other NSAIDs with RL and RS matrices [28,29], dissolution as well as absorption studies confirmed the complex relationship existing between drug molecule structure, mainly for 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 uptake profiles of IBU from nanoparticles is related to equilibrium among drug release, its ionisation in the dissolution medium and the saturation of the binding sites on the surface of polymer particles. Such a behaviour can in fact be ascribed to the fact that the dissolved drug, becoming ionised in the neutral dissolution medium, is re-adsorbed back onto the polymer particles, because of the presence of opposite electrical charge [30].

In the case of dialysis tests, the driving forces leading to IBU release from the nanoparticles are the volume and the light alkaline pH of the dissolution buffer. The absence of an up-taking system in the external medium thus made the observed release profile less strictly dependent upon the nature of the polymer and drugs. In fact, the two systems showed similar time–release curves. In the classical dialysis tests, the

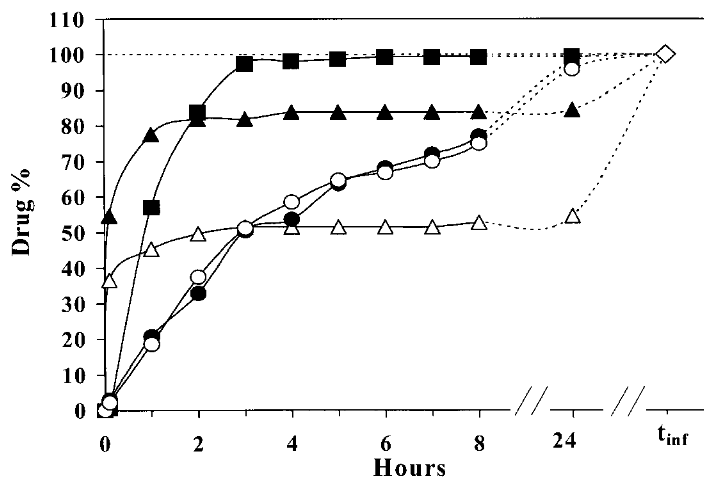

Fig. 6. Drug release from free IBU (■) as well as from Eudragit RL (▲) and RS (△) nanosuspensions to void MLVs, compared with the data observed in the dialysis experiments (● and ○). The (◇) represents the maximum amount of drug releasable (value obtained from the interaction of MLV preparation in organic solvent in the presence of a 0.12 molar fraction of IBU).

dissolution rate of IBU represents the limiting step, stating that RL and RS posses a remarkable different permeability to hydrophilic compounds. Conversely, when the biological model was assayed, the overall drug release profile was conditioned by the ability of liposomes bilayers of capturing and retaining drug molecules after their leakage from the polymer network. In the smaller space of the DSC pan, the affinity equilibrium between particle surface or liposomes bilayers plays a major role in determining the actual drug release profile.

In these experiments the volume of dissolution medium is much smaller, and the equilibrium between the amount of drug bound to nanoparticles surface, the fraction dissolved in the buffer and the amount captured by MLVs is greatly affected by the affinity of RS or RL polymers for the drug. The behaviour observed for IBU-loaded RL and RS nanosuspensions is then quite different (Fig. 6). The maximum released amount was higher for the former system, because of the higher water permeability of RL, whereas the liposomes incubated with RS nanoparticles showed a similar initial rate of capture of the dissolved drug. Since DMPC liposomes showed to be able to retain all the amount of drug dispersed in the nanoparticles, i.e. an amount corresponding to a 0.12 molar fraction of IBU, the above DSC data can be explained by considering a nearly complete release of the dispersed drug from RL particles, in respect to that observed for the RS polymer matrix. In other words, whereas in the classical dialysis tests the permeability of RS and RL polymers will represent the limiting step to the release of an acidic drug, like IBU, this “in vitro” study suggests as the kinetic process involved in drug release is influenced by the different kind of polymer forming the nanosuspensions, acting on drug dissolution rate and membrane disorder [19–22,31]. The different permeability between the two polymers justifies the entity of drug release and interaction with DMPC bilayers, whereas the affinity of the drug to the polymer matrix gives the rate at which these phenomena occur.

4. Conclusions

As already shown in previous similar studies [29,31], DSC technique appeared as a valid approach to follow the release profile and kinetics of a drug from polymer particulate systems, with respect to classical dissolution tests. The presence of three-dimensional uptake devices, represented by the MLV, better resemble the biological environment and reduces the influence of artful experimental parameters (like high volume of external medium) of dissolution tests.

In the particular contest used in the present study, the DSC technique allowed a better view of the complex equilibrium phenomena occurring between the drug-loaded nanoparticles and the MLV suspension, showing the influence of polymer permeability on the overall drug release process.

Acknowledgements

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