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Research Papers

Effect of the complexation of some nonsteroidal anti-inflammatory drugs with β-cyclodextrin on the interaction with phosphatidylcholine liposomes

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Summary

The influence of β-cyclodextrin inclusion complexes with different anti-inflammatory agents on the main phase transition of dipalmitoylphosphatidylcholine vesicles was studied by differential scanning calorimetry. Drugs cause a lowering of the temperature of the gel to liquid crystal phase transition and a slight broadening of the calorimetric peak. Similar effects were found between the various free drugs, whereas noticeable differences were observed when drugs were employed as inclusion complexes. The ability of the inclusion complexes to act as drug carriers is discussed in terms of better release of drug with respect to the lipidic bilayer, chosen as model membrane. The interactions among inclusion complexes is explainable based on the values of their stability constants (K_c)

Introduction

Cyclodextrins (Cyds) are toroidal oligosaccharides which possess the remarkable property of forming inclusion complexes with a variety of small molecules of appropriate size. One of the most fascinating and far-reaching aspects of their chemistry is their model enzyme behaviour both in terms of substrate complexation and reactivity (Komiyama and Bender, 1978; Akkaya and Czarnick, 1988).

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In recent years, their application as drug delivery systems has been attempted in the pharmaceutical field (Bootsma et al., 1989; Hassan et al., 1990). A number of reports describe improvements in the pharmaceutically unfavorable properties of guest drugs, such as solubilization of a poorly water-soluble drug (Stadler-Szoke et al., 1985; Chow and Karara, 1986), stabilization of a labile molecule (Szejtli et al., 1983), reduction of irritancy (Irie and Uekama, 1985) and entrapment of a volatile drug (Gal-Fuzy et al., 1984), by means of the formation of inclusion complexes.

Non-steroidal anti-inflammatory drugs (NSAIDs) are usually very slightly soluble in water and often present a more or less gastrolesivity when

orally administered. For this reason a good approach to enhance their solubility and to minimize their ulcerogenic potency could be the inclusion into β-cyclodextrin (β-Cyd).

In a previous paper (Puglisi et al., 1990), the preparation, in vitro dissolution rate and solubility of an inclusion complex between β-Cyd and 4-biphenylacetic acid (BPAA) were described. Such a complexation improves biological activity, gastric tolerability and bioavailability of BPAA (Puglisi et al., 1991).

Moreover, we studied the interaction of the BPAA-β-Cyd complex, by means calorimetric techniques, with a lipid model membrane (Castelli et al., 1989).

In the present work we have extended the study to other anti-inflammatory agents, naproxen (NAP) and ketoprofen (KPF), with the aim to evaluate the ability of structure variations in modifying the drug release from inclusion complexes, in the presence of model membranes.

To study the release of drug from β-Cyd complexes we employed L-α-dipalmitoylphosphatidylcholine (DPPC) liposomes which show a change of their thermotropic behaviour in the presence of molecules dissolved in their ordered structure (see for references Jain, 1988). The technique employed was differential scanning calorimetry (DSC), which appears very useful in the study of interactions between drugs and lipid-model membranes (Cater et al., 1974; Papahadjopoulos et al., 1975; O'Learly et al., 1986).

Furthermore, attempts were made to correlate the thermotropic behaviour of DPPC liposomes in the presence of the complexed drugs to their stability constants.

Materials and Methods

Chemicals

Synthetic L-α-dipalmitoylphosphatidylcholine 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).

4-Biphenylacetic acid was obtained from Janssen (Belgium) (analytical grade) and was recrystallized from ethanol. Naproxen and ketoprofen were obtained from Sigma Chemical Co. (St. Louis, U.S.A.).

β-Cyclodextrin was purchased from Fluka Chemical Co. (Buchs, Switzerland) and was used after recrystallization from water and drying with P_2O_5 in vacuo.

Complexation with β-Cyd

Inclusion complexes of NSAIDs with β-cyclodextrin were prepared according to the coprecipitation solution method (Korozumi et al., 1975; Puglisi et al., 1990).

Determination of the complex stability constants

The stability constants (K_c) were determined using the phase solubility method of Higuchi and Connors (1965). Excess amounts of BPAA, NAP and KPF were added to Tris buffer (pH 7.4) solutions containing various concentrations of β-Cyd and shaken at 37 ± 0.5°C. After equilibrium was attained (approx. 2 days) an aliquot was filtered through a 0.45 μm Millipore filter. A portion of the sample was adequately diluted and analyzed by spectrophotometry at 272, 260 and 254 nm for NAP, KPF and BPAA, respectively. Apparent 1:1 stability constants were calculated from the slope and intercept of the linear portion of the phase solubility diagrams (reporting drug concentration vs β-Cyd concentration), using the equation:

$$K_c = \frac{\text{slope}}{S_0(1 - \text{slope})}$$

where S_0 is the solubility of the drug in pure buffer solution.

Preparation of liposomes

Multilamellar liposomes were prepared in the presence and absence of free drugs, drug-β-Cyd complexes and β-Cyd at a temperature above that of the gel-liquid crystalline phase transition.

Chloroform-methanol (1:1, v/v) stock solutions of lipid and drugs were mixed in order to obtain the chosen mole fraction of drugs. The solvents were removed under nitrogen and the resulting film was kept overnight on a vacuum pump to remove the residual solvents.

Drug-β-Cyd and β-Cyd were added to the lyophilized DPPC film in the calculated amount to obtain the same relative mole fraction of drugs with respect to lipid.

Liposomes were prepared by adding to the film 50 mM Tris buffer (pH 7.4), then heating at 60°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. Afterwards, aliquots of 120 μl (5 mg of lipid) were transferred to a 160 μl DSC aluminium pan 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 in the temperature range 10–70°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.

The samples were cooled and heated four times to achieve reproducibility of results. All samples, after calorimetric scans, were extracted from the pan and aliquots were used to determine the amount of phospholipids by the phosphorus assay.

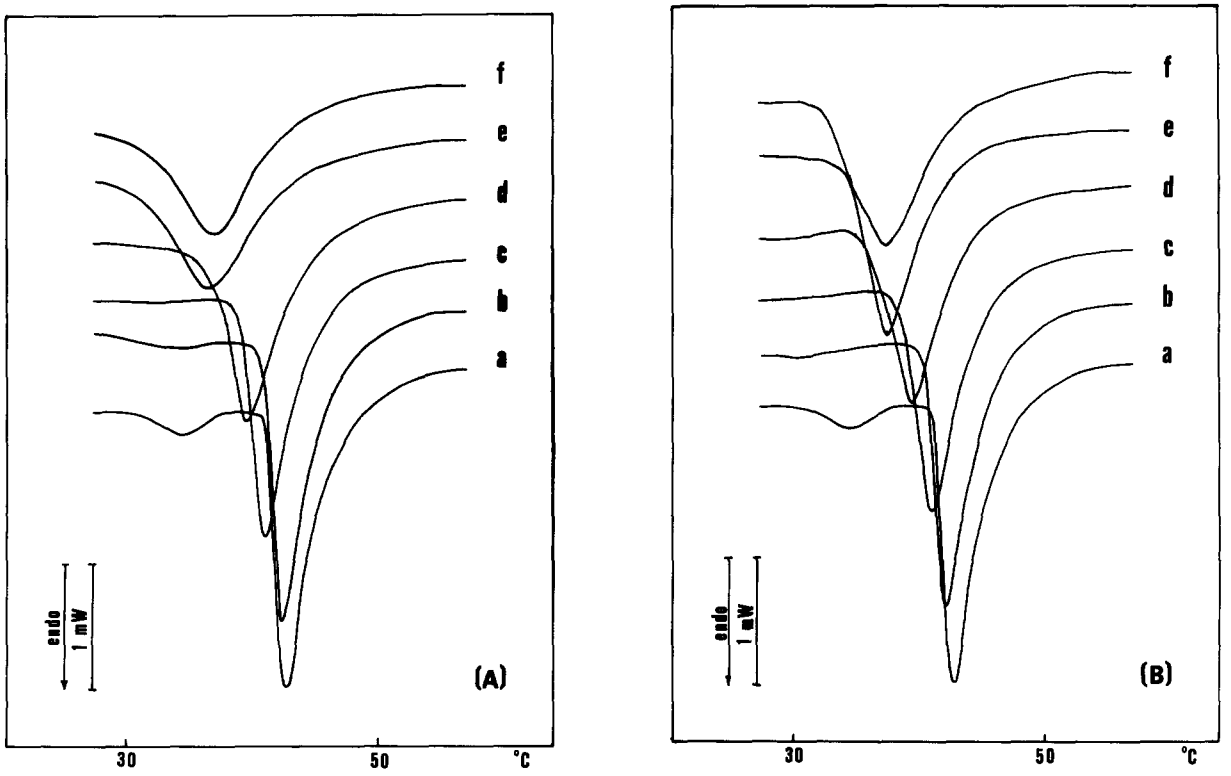

Fig. 1. Differential scanning calorimetry heating curves of hydrated DPPC containing (A) BPPA, (B) NAP and (C) KPF at drug mole fractions of: a = 0, b = 0.06, c = 0.12, d = 0.18, e = 0.24, f = 0.36.

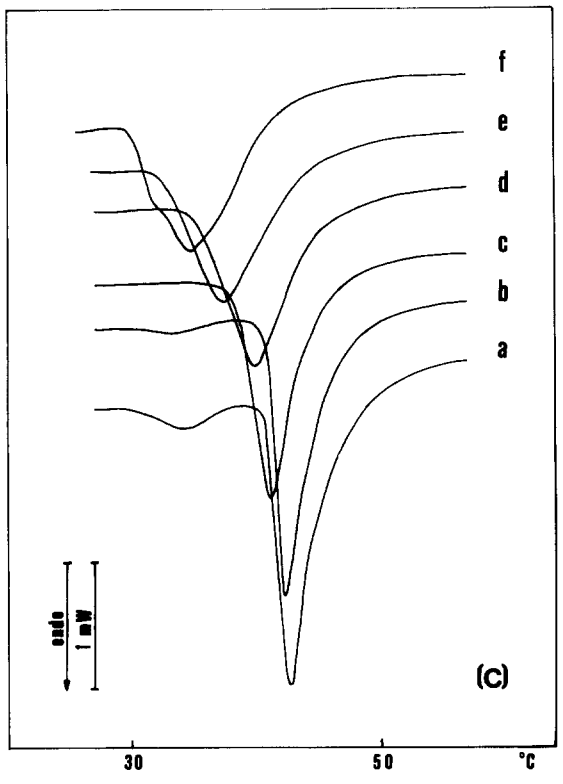

Fig 1 continued.

Results and Discussion

Fig. 1 shows the calorimetric heating curves of DPPC liposomes in the presence of different mole fractions of the following drugs: BPAA, NAP and KPF.

All drugs are able to interact with DPPC liposomes causing a considerable shift of the transition temperature (T_m) of the calorimetric peak associated to the well known gel to liquid-crystal phase transition typical for DPPC multilayers (Figs 3–5 and Table 1).

The enthalpy changes (ΔH), related to the peak area, remained nearly constant (Table 2).

As judged from the curves of Figs 3c–5c, the effects caused by the different drugs are of the same order of magnitude (effects are considered on the basis of T_m depression and ΔH values). This behaviour is justified by considering the formulae of the compounds. In fact, they show a short apolar group and a polar head allowing them to interact with the choline groups of DPPC liposomes.

All the results obtained agree with our previous works. The KPF/DPPC interaction which is

TABLE 1

Main transition peak temperature (T_m, °C) of DPPC dispersions at different molar fractions of BPAA, NAP, and KPF and their β-Cyd inclusion complexes

Molar fraction	BPAA	NAP	KPF	BPAA -β-Cyd	NAP -β-Cyd	KPF -β-Cyd	NAP -β-Cyd NH_3
0 0	42.2	42.2	42 2	42.2	42 2	42.2	42 2
0 06	41 6	41.6	41 6	42 1	41.6	41.6	42 2
0 12	40 3	40 6	40.6	41.0	41 0	40 6	41 9
0 18	39 0	39 1	39.1	40 8	40 0	39 5	41.7
0.24	36 2	37 1	36 6	40.0	38.3	37.5	41 3
0 36	36.1	36 6	34.0	39 0	37 7	34 8	40.6

TABLE 2

Main transition enthalpy changes (ΔH, kcal / mol) of DPPC dispersions at different molar fractions of BPAA, NAP and KPF and their β-Cyd inclusion complexes

Molar fraction	BPAA	NAP	KPF	BPAA -β-Cyd	NAP -β-Cyd	KPF -β-Cyd	NAP -β-Cyd NH_3
0 00	8.2	8.2	8 2	8 2	8 2	8 2	8 2
0 06	8.1	7.9	7.3	8 5	7.6	8.1	6.4
0.12	7.7	7 6	7 4	7 6	7.7	7.9	6.6
0 18	7 5	7 2	7 7	7.5	8 2	8 8	6.7
0.24	6 7	6 7	7.1	7.1	7 8	8 5	7 0
0 36	6.6	7 1	7 0	7 6	7.9	8.7	6 6

the only one among the three free drugs studied that had not been described before, appears coherent with the results already obtained for NAP and BPAA (Castelli et al., 1989, 1991).

The interaction between drugs and DPPC liposomes was largely explained by us as well as in the literature (Cater et al., 1974; Estep et al., 1978; Castelli et al., 1984) 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 a spacer in such a structure causing a destabilization of the lipid mosaic with a decrease in the T_m of the gel to liquid crystal phase transition. The negligible variation in the ΔH is explained in the literature as a surface interaction between amphipatic molecules and DPPC polar heads, which occurs only at the surface of lipid layers without strongly involving the acyl chains. The differences in the structure are thus able to influence the incorporation of drugs in the model membrane.

We wished to extend the investigation on the interaction between DPPC liposomes and BPAA-, KPF- and NAP-β-Cyd complexes. By considering our purpose, it was necessary to evaluate the interaction between pure β-Cyd and DPPC liposomes. No interaction was detected, as shown in Figs 3a–5a. In Fig. 2 we report typical heating curves of increasing amounts of drugs present as inclusion complexes in β-Cyd. Similarly to that observed for the free drugs, an interaction between drugs and the model membrane occurs, albeit less evidently, causing a shift of the calorimetric peak towards lower T_m values (Figs 3–5 and Table 1). The enthalpy changes remain unaffected by the presence of β-Cyd complexes (Table 2).

In Fig. 2D, the curves of the NAP-β-Cyd complex as the ammonium salt are reported. This

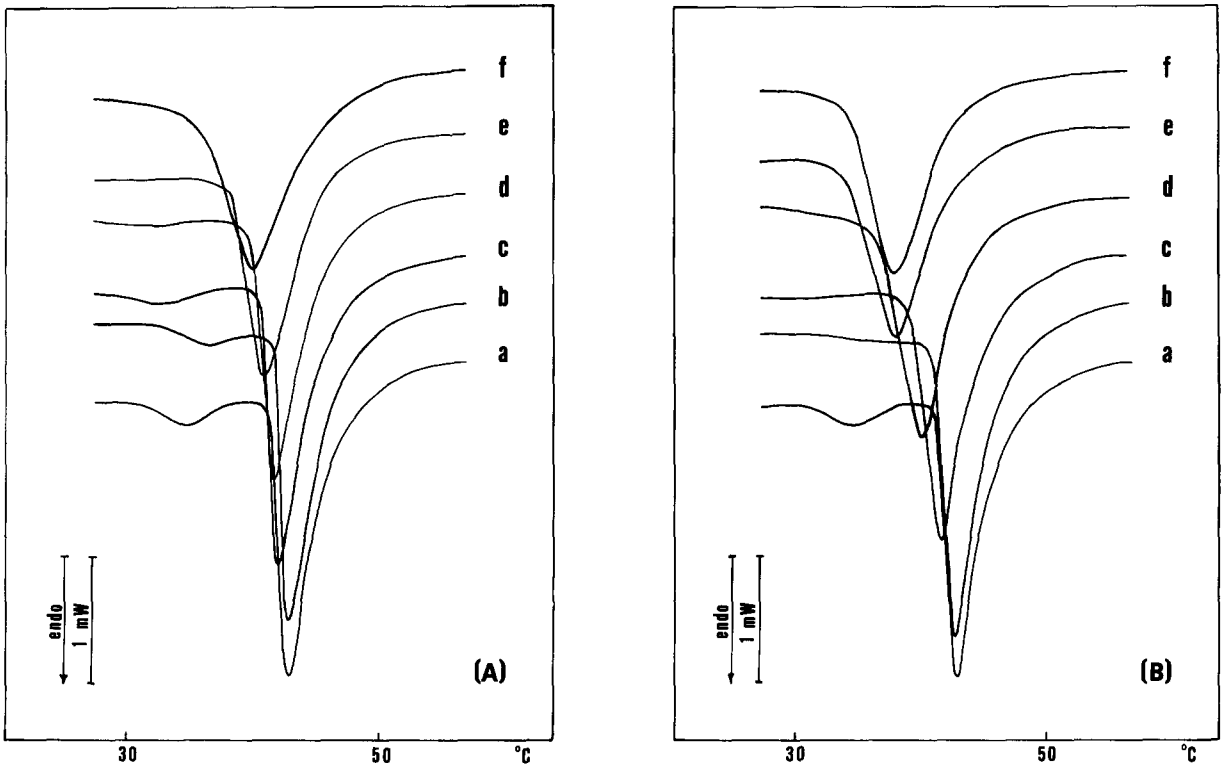

Fig 2. Differential scanning calorimetry heating curves of hydrated DPPC containing (A) BPAA-β-Cyd, (B) NAP-β-Cyd, (C) KPF-β-Cyd and (D) NAP-β-Cyd · NH_3 at drug mole fractions: a = 0, b = 0.06, c = 0.12, d = 0 18, e = 0.24, f = 0 36.

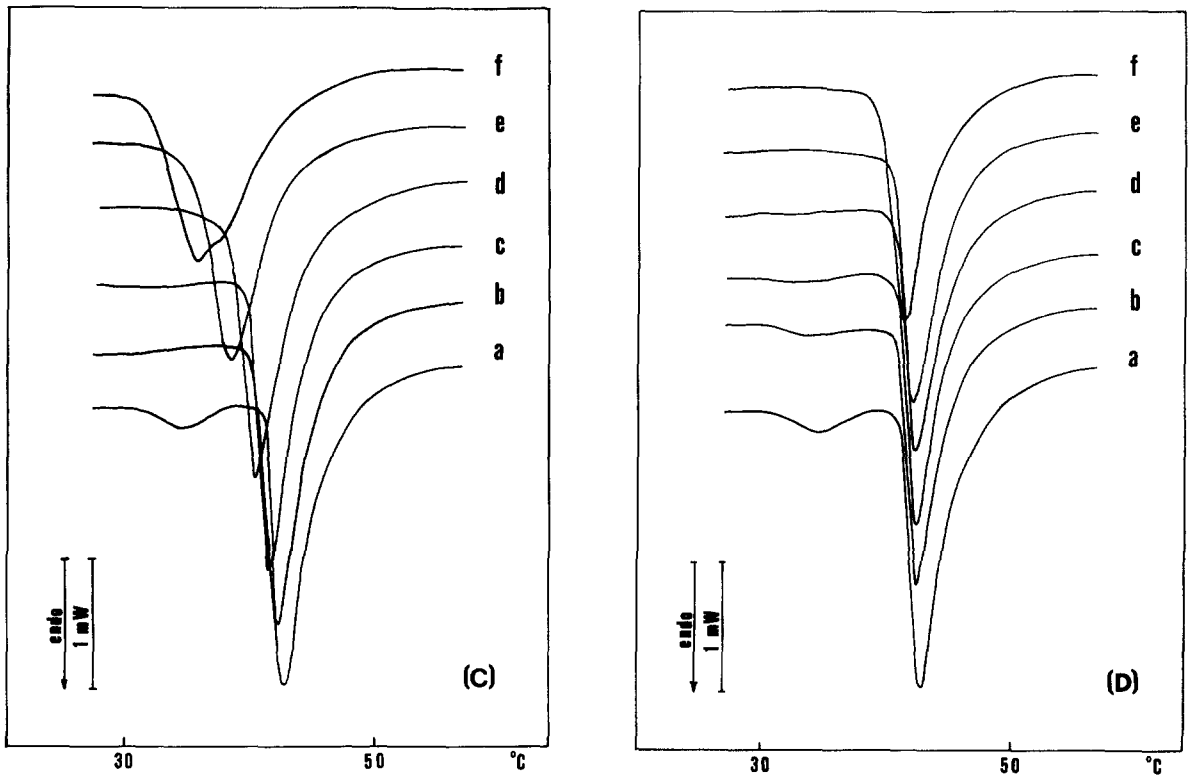

Fig. 2 continued

product was obtained during the preparation of NAP-β-Cyd and was considered to be worth studying as well (even though unimportant from the viewpoint of pharmaceutical utility), for the different lipophilicity that it could show with respect to the drug acid form. It should permit

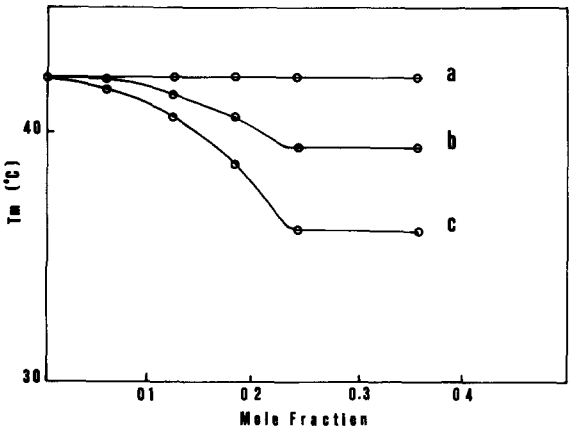

Fig. 3. Transition temperature (T_m, °C) values (average of at least four runs), in heating mode, as a function of mole fraction of a (β-Cyd), b (BPAA-β-Cyd), c (BPAA)

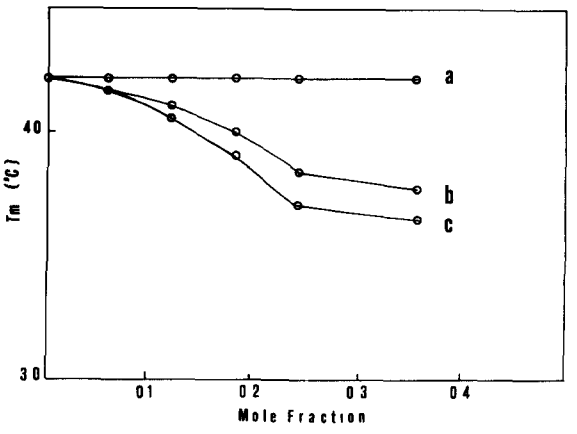

Fig. 4. Transition temperature (T_m, °C) values (average of at least four runs), in heating mode, as a function of mole fraction of a (β-Cyd), b (NAP-β-Cyd), c (NAP).

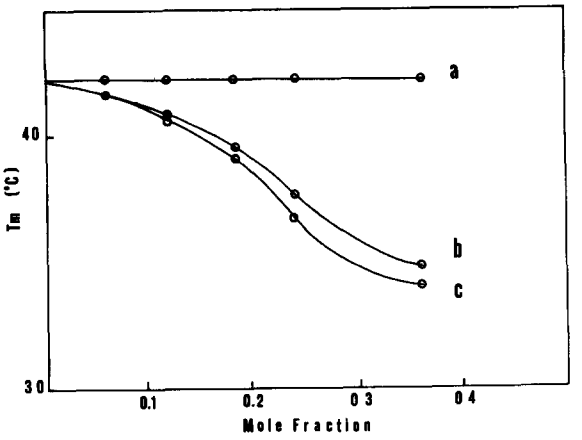

Fig. 5. Transition temperature (T_m, °C) values (average of at least four runs), in heating mode, as a function of mole fraction of a (β-Cyd), b (KPF-β-Cyd), c (KPF).

better clarification of the importance of drug structure in liposome interactions.

The effect of NAP-β-Cyd as the ammonium salt on the T_m was negligible (Table 1); in fact, only a slight shift of T_m occurs without variation in the enthalpic changes.

The effects exerted by the three drugs, both free or included into β-Cyd, are summarized in Figs 3–5. We focus our comparative discussion mainly on $X_{drug} \leqslant 0.24$, since it is evident from the calorimetric curves that phase separations for KPF (included as well as free) began at concentrations higher than this value. Consequently, these systems should be inhomogeneous, probably due to the formation of micro-domains richer in drugs, and hence the data are unreliable.

From the results obtained, it is possible to evaluate the differences, as regards the DPPC gel to liquid-crystal phase transition, among the three drug-β-Cyd complexes as well as with the respective free drugs.

In fact, by comparing Figs 3b–5b and 3c–5c it is evident as the KPF-β-Cyd profile is very close to that of free-KPF in contrast to what was shown by NAP and even more by BPAA. By considering that the effect on the T_m is due to the molecules of drug able to reach inside DPPC liposomes, leaving the host β-Cyd molecule when the complexes are dissolved in water, we can suppose the existence of a dynamic equilibrium between molecules entrapped in β-Cyd and those free to pass into bilayers.

The shift in the T_m observed for KPF-β-Cyd suggests that almost all KPF present in the complex leaves the cavity of Cyd to reach the DPPC liposomes. In contrast, for BPAA only a part of the complexed drug is able to move from the complex to the lipid bilayer.

An intermediate behaviour is observed for NAP. These results agree with those of the complex stability constants (K_c). The K_c values, determined as described in Materials and Methods, are 0.272, 0.039 and 0.019×10^3 mol^{-1} for BPAA, NAP and KPF, respectively.

The species interacting with the lipidic bilayer should be the protonated form of drugs, since comparing the T_m values of NAP free, complexed with β-Cyd and as ammonium salt, it appears evident as in the first two cases that the drug produces a greater effect than the last one where the drug is certainly present mostly in a deprotonated form. It should then be the protonated form that is responsible for the interaction with lipidic bilayers, confirming our previous results on the importance of pH in the BPAA-DPPC liposome interaction (Castelli et al., 1990).

Conclusions

Previously reported calorimetric data show a different interaction among NSAIDs-β-Cyd complexes and the DPPC liposomes, chosen as a simple model of a biological membrane. The complexed drugs show the following interaction order: KPF > NAP > BPAA.

These results could be correlated with the K_c values of the complex which determine the free drug amount in solution. In fact, the BPAA-β-Cyd complex shows weaker interaction with DPPC liposomes, owing to the higher K_c values, whereas for KPF-β-Cyd and NAP-β-Cyd complexes the absence of significant differences in the interaction with DPPC liposomes is due to their similar K_c values.

This approach represents a further means to judge the ability of β-Cyd inclusion complexes to

release the carried drugs near to biological membranes.

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