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Influence of sebacate plasticizers on the thermal behaviour of dipalmitoylphosphatidylcholine liposomes

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Abstract

Sebacic acid esters are a promising class of low toxicity plasticizers for PVC. They are lipophylic substances and thus, their interaction with biomembranes must be considered.

DPPC liposomes in the presence of increasing amounts of dimethylsebacate (DMS), diethylsebacate (DES), dibutylsebacate (DBS) and di-(2-ethylhexyl)sebacate (DEHS) have been studied by DSC, both on heating and cooling.

As a general feature, the melting point decreases and the enthalpy ΔH increases along with the half-width and asymmetry of the transition in most of the systems. The observed effects are strongly dependent on the length and the shape of the ester-chains suggesting that both polar interactions between carboxylic groups of sebacates and the polar heads of the lipids as well the hydrophobic interactions involving penetration into the bilayer are important. The effects are noticeable also in the presence of small amounts of plasticizers.

In the presence of large amounts of DES and DBS, a complex structure of the transition arising from the coexistence of aggregates of different types and also the appearance of new phases were observed.

The behaviour of sebacates to model biomembranes is quite different for other classes of lipophylic PVC plasticizers, such as phthalates and we explain the differences on the basis of the greater flexibility of the sebacic acid chain. © 2002 Elsevier Science B.V. All rights reserved.

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1. Introduction

In the manufacturing of plastics and rubber, plasticizers are commonly used to enhance the workability and flexibility of high molecular weight polymers. Plasticizers are generally stable organic high boiling liquid materials that penetrate the polymeric matrix and become an integral part of it. For this purpose, the esters of bicarboxylic organic (mainly phthalic, sebacic and adipic) acids with aliphatic alcohol of medium linear or branched chains lengths are widely used. They interact with the polymer molecules by means of van der Waals or also dipole–dipole forces and act as an internal lubricant allowing the polymer chains to move in relation to each other, i.e. providing flexibility [1].

Because plasticizers are generally not chemically bonded to the polymeric matrix, they can slowly migrate to the surface, thus, contaminating the environment. The loss rate of plasticizers from a plastic

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depends upon many factors, the most important being plasticizer type, temperature, environment characteristics and exposure time.

In the manufacturing of polyvinyl chloride (PVC), one of the most widely used plastics in the world, very large amounts of plasticizers are added to modify the hard bulk polymer to a soft, flexible material extensively used for industrial and household products as well as toys and biomedical devices [2]. To this end, phthalic acid esters and bis-(2-eyhylhexyl)-phthalate (DEHP) have been used as the primary plasticizers for more than 40 years.

Because of their widescale use as plasticizers, large quantities of phthalates have been released into the environment, resulting in a high risk of contact to humans and other living organisms. Their toxicology has been extensively studied. Recently, attention has focused on whether the wide use of plasticizers in food packaging, toys and biomedical devices pose a human health hazard [3,4]. Despite their low acute toxicity, noticeable reproductive effects have been observed in mammals and consequently they have been included in some lists of endocrine modulators. It has been suggested that they function as xenoestrogens [5,6].

In addition, studies on phthalic acid esters and mainly on DEHP have shown an increased incidence of liver tumors in rats and mice, consistent with their well documented action as peroxisome proliferators [7,8]. Whether peroxisome proliferator substances are also carcinogenic for humans remains not proved, because humans and primates, unlike rodents, seem to be particularly non-responsive to the peroxisome proliferators, and thus, a different mechanism of action has been postulated [9,10]. Nevertheless, many countries have adopted strict legislation and banned the use of phthalate plasticizers in children's toys and biomedical devices.

Therefore, other classes of plasticizers, without the negative characteristic of phthalate are currently being investigated in the manufacture of PVC, one of the most promising being the esters of sebacic acid. As with phthalates, sebacates are liquid, with low polarity, water insoluble lipophylic substances and thus, their interaction with the lipid fraction of biomembranes plays a key role in their biological and toxicological effects. As with phthalates, they show low acute toxicity and the study on long-term effects are still underway, but up to now the reported results seem to exclude tumor induction in rodents by peroxisome proliferation [11,12].

Differential scanning calorimetry (DSC) is a useful technique to study changes induced in model biomembranes by foreign substances. Indeed, multilamellar vesicles (liposomes) of pure DPPC show a sharp endothermic gel to liquid–crystal transition whose peak temperature and shape are strongly modified by interactions with other substances. The modifications also indicate if the interaction is mainly superficial or if penetration into the hydrophobic core of the bilayer takes place [13].

Phospholipid liposomes have been extensively studied both in the presence of penetrating substances like cholesterol, anaesthetic and other lipophylic bioactive substances [14,15], and in the presence of non-penetrating molecules that interact mainly with the external hydrophilic surface of the bilayer, like polyamines or some pesticides [16–18]. DPPC liposomes are the most widely used model system of biomembranes because lecithins are the major component of most mammalian biomembranes and its thermal transition lies near physiological temperature.

This work studied the effects induced by the presence of different amounts of some sebacic acid esters on DPPC liposomes in order to characterize the type and the strength of the interaction.

2. Materials and methods

DL-DPPC was purchased from Sigma and used without further purification. Dimethylsebacate (DMS), diethylsebacate (DES), dibutylsebacate (DBS) and di-(2-ethylhexyl)sebacate (DEHS) were obtained from Fluka. They were chromatographically tested and used without further purification since their purity was greater than 99%. Twice distilled water, high purity 'pesticide analysis' grade chloroform and ACS reagent grade Merck products were also used.

Samples were prepared by mixing the appropriate amount of sebacates and DPPC in chloroform solutions. The solvent was removed under nitrogen stream and then by gentle heating (1 h at 60 °C) under vacuum.Multilamellar vesicles (liposomes) were prepared by adding an NaCl 0.9% w/w solution buffered at pH = 7.0 with phosphate buffer (about 10^{-3} M) to DPPC–sebacate anhydrous systems to obtain a final

lipid concentration of about 20% w/w. Homogeneous and gelatinous samples were obtained by sonication (3 min at 0.5 W power in an ice-cold bath) and stored at −18 °C. Samples with sebacate content ranging from 0.25 to 30% w/w with respect to the DPPC amount were examined.

DSC measurements were performed using a Mettler–Toledo DSC 821 using heating and cooling rates of 2.0 °C/min in the 25–50 °C range. Temperature and thermal response scales were calibrated with indium and caprilic acid samples. Both heating and cooling cycles were repeated at least four times to ensure constancy and good reproducibility of the thermal data; the experimental error in temperature and thermal response was ±0.1 °C and ±5%, respectively. After DSC measurements, all the samples were dried under vacuum at 80 °C for 12 h and the dry residue weighed. After dissolving the dry material in $CHCl_3$, the amount of sebacate was determined by gas-chromatography, using a Perkin-Elmer 8600 gas-chromatograph with a PTE5 (30 m length and 0.32 mm i.d.) capillary column, FID detector and He as carrier gas.

The amounts of sebacic acid esters as determined by GC measurements and those calculated theoretically by the weights were consistent within experimental error of a few percents, confirming that the losses of sebacates during the $CHCl_3$ evaporation, the preparation of the samples, the measurements and the final drying were negligible.

3. Results

The thermal behaviour of pure DPPC liposomes is well-known. On heating, they exhibit two endothermic transitions in the 25–50 °C temperature range: a pretransition at about 35 °C broad, with a low enthalpy change and a major, sharp transition at about 42 °C [14,19].

The pretransition arises from the conversion of a lamellar gel phase (L_β) to a rippled gel phase (P_β). This transition is only partially reversible and the sample needs to be held at a low temperature for at least 30 min. to obtain reproducible values if the sample is heated again. The pretransition is very sensitive to the presence of foreign substances, especially, if they penetrate into the hydrophobic core of the bilayer and frequently tends to disappear even in the presence of very small amounts (less as 1% w/w) of foreign substances.

The main transition arises from the conversion of the P_β gel phase to a lamellar liquid–crystal L_α phase. The transition is sharp, behaves with a very good reproducibility both in temperature of the maximum endotherm (T_m), in the half-width ($\Delta T_{1/2}$) and in enthalpy change, ΔH, giving similar values both on the heating and cooling. The shape of the DSC curve is slightly asymmetrical, skewed slightly toward lower temperatures, both on heating and cooling.

In pure DPPC liposomes T_m, ΔH and $\Delta T_{1/2}$ were 41.7 °C, 35.9 kJ m^{-1}, and 0.5 °C, respectively, in good agreement with the literature data [20]. In the presence of foreign substances interacting with the DPPC liposome changes T_m, $\Delta T_{1/2}$, ΔH as well as the symmetry of the peak, depending on the type and strength of the interaction. To study the asymmetry changes, an asymmetry index (A_s) is defined as:

$$A_s = \frac{[(T_{ons} - T_i)/(T_{end} - T_i)]\text{sample}}{[(T_{ons} - T_i)/(T_{end} - T_i)]\text{pure DPPC}}$$

T_{ons} (extrapolated onset temperature) is the temperature of the intercept between the base line and the inflection tangent on the peak in the increasing part, and T_{end} (extrapolated endset temperature) is the same referred to the decreasing part of the peak. The temperature T_i is of the interception point of the two previous tangents and its value is the same or very close to T_m.

If A_s is greater than 1 (or less in cooling cycles), the peak is more asymmetric than for pure DPPC, with an increased skewness to lower temperatures; on the contrary A_s values lower than 1 (or greater in cooling) denote less asymmetry or, if $(T_{end} - T_i) > (T_{ons} - T_i)$, skewing to higher temperatures. Obviously, if A_s is equal to 1, no changes in skew or symmetry take place with respect to pure DPPC liposomes. It should be noted that the calculated A_s values are significant only in the presence of a single transition peak, although asymmetric. If the structure of the calorimetric peak becomes complex with the appearance of more than one component, a preliminary deconvolution process is performed. However, the choice of the deconvolution parameters is difficult and introduces uncertainty so that meaningless A_s values would be obtained.

In all DPPC liposomes, a low temperature shift of 0.8 °C between heating and cooling cycles was

Table 1

w/w (%)	DMS T_m (°C)	DES T_m (°C)	DBS T_m (°C)	DEHS T_m (°C)
0	41.7	41.7	41.7	41.7
0.25	41.7	41.5	41.4	41.5
0.5	41.6	41.4	41.2	41.3
1.0	41.5	41.2	40.8	41.2
2.0	41.3	41.0	40.4	41.1
3.0	41.2	40.5	39.9	41.0
5.0	41.1	40.0	39.3–37.7	40.9
7.5	41.1	39.7	38.6–37.7	40.7
10.0	41.2	38.8–37.5	37.7	40.3
15.0	41.4	38.4–37.5	37.5	40.1
30.0	41.5	37.5	37.4	39.6–38.0

observed. This T_m shift could be attributed both to the heating and cooling rate and to the finite equilibration and response time of the calorimeter. However, this fact does not modify the interpretation of the results since we are interested in the modifications rather than in the absolute values of the parameters.

Table 1 shows the value of T_m in all the DPPC–sebacate systems considered in heating (T_{mh}) cycles. The sebacate–DPPC molar ratio ranges approximately from 0.08 to 1.0 for DMS, from 0.07 to 0.9 for DES; from 0.06 to 0.7 for DBS and from 0.005 to 0.5 for DEHS-containing liposomes.

The A_s asymmetry index as well the half-width ($\Delta T_{1/2}$) of the transition and the ΔH in heating are summarized in Table 2.

Figs. 1–4 show the shape of the heating curves in multilamellar vesicles (liposomes) of pure DPPC and DPPC/DMS; DPPC/DES, DPPC/DBS and DPPC/DEHS mixed systems with a different sebacate content.

The pretransition appeared to be very sensitive to the presence of plasticizer but it was not detectable also in the presence of very small amounts of sebacates. The amount of sebacates able to make the transition disappear was related to the ester-chain length. In the DPPC/DMS samples with DMS equal to 0.5% w/w, the pretransition was still detectable, even broadened to some extent and with the maximum temperature lowered by about 0.5 °C. In the DPPC/DES and DPPC/DBS systems with 0.5% w/w and in the DPPC/DMS system with 1.0% w/w, the pretransition appeared very broadened and reduced in intensity. As a consequence, the true temperature of the maximum was very difficult to be measured, but it appeared to be lowered by about 0.8–1.0 °C compared to the pure DPPC liposomes. In the presence of DEHS,

Table 2

w/w (%)	DMS			DES			DBS			DEHS		
	$\Delta T_{1/2}$ (°C)	ΔH (kcal m^{-1})	A_s	$\Delta T_{1/2}$ (°C)	ΔH (kcal m^{-1})	A_s	$\Delta T_{1/2}$ (°C)	ΔH (kcal m^{-1})	A_s	$\Delta T_{1/2}$ (°C)	ΔH (kcal m^{-1})	A_s
0	0.5	8.3	1.0	0.5	8.3	1.0	0.5	8.3	1.0	0.5	8.3	1.0
0.25	0.5	8.3	1.0	0.6	8.3	1.1	0.6	8.1	1.3	0.7	8.3	1.9
0.5	0.5	8.2	0.9	0.6	8.4	1.1	0.7	8.4	1.8	0.9	8.4	2.2
1.0	0.5	8.4	0.9	0.7	8.2	1.2	0.8	8.3	2.2	0.9	8.2	2.6
2.0	0.6	8.5	1.0	0.8	8.4	1.6	1.1	8.2	3.0	1.0	8.3	2.8
3.0	0.6	8.6	1.0	0.9	8.5	2.0	1.4	8.2	3.5	1.0	8.4	2.8
5.0	0.6	8.8	1.0	1.0	8.6	2.7				1.0	8.2	3.0
7.5	0.6	9.2	1.3	1.4	9.0	3.2				1.1	8.3	3.0
10.0	0.8	9.4	1.8							1.3	8.2	3.1
15.0	1.2	9.6	2.9							1.3	8.5	3.5

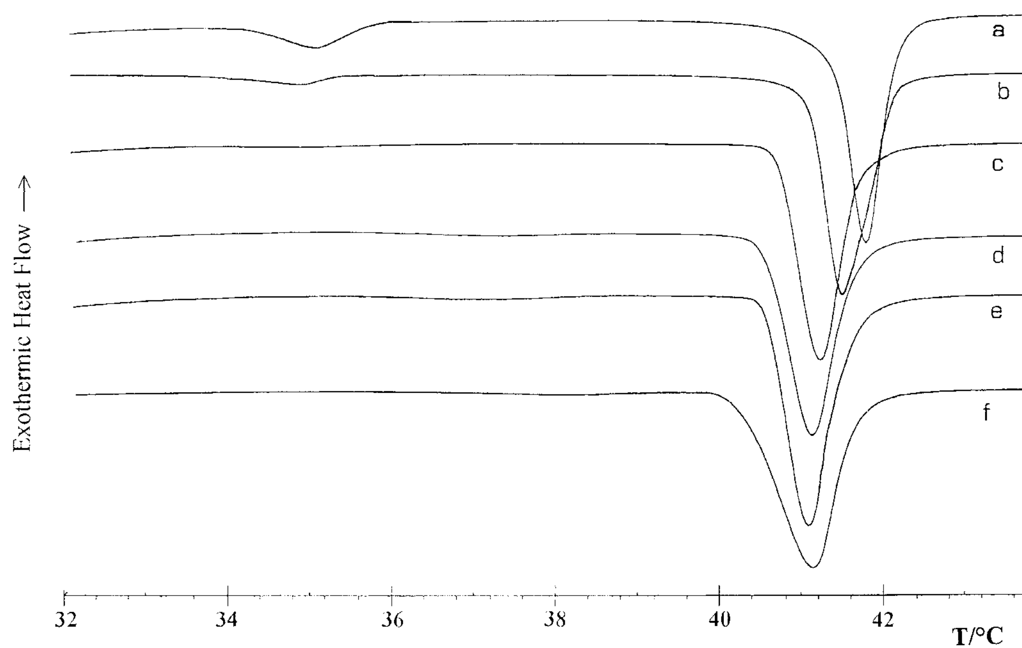

Fig. 1. DSC thermal response of hydrated multilamellar vescicles of DPPC/DMS mixtures with different DMS content: (a) 0%; (b) 1.0%; (c) 3.0%; (d) 5.0%; (e) 7.5%; and (f) 10.0%, on heating.

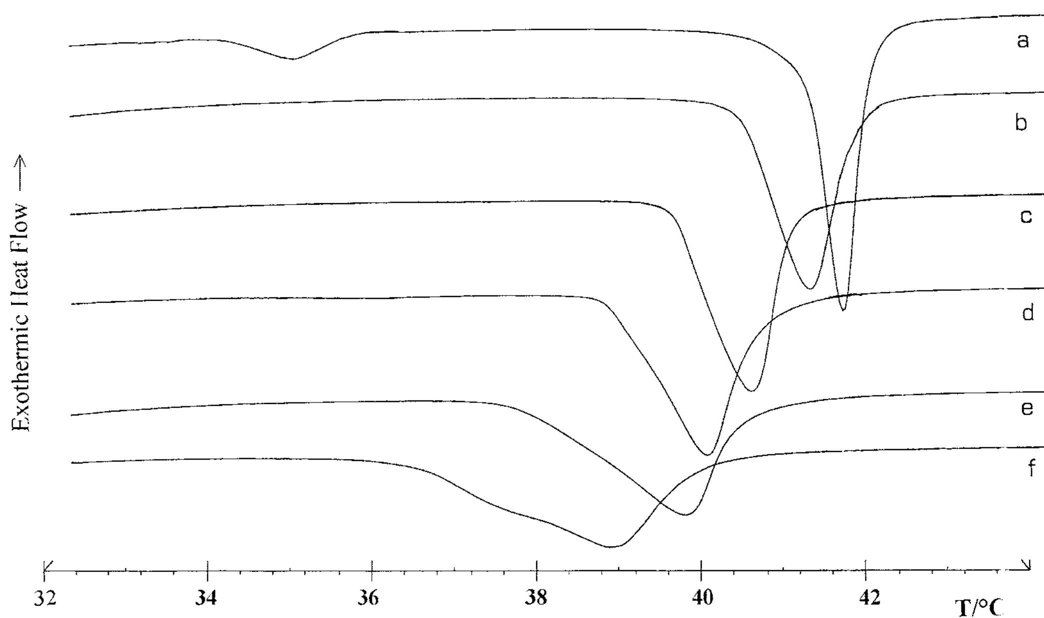

Fig. 2. DSC thermal response of hydrated multilamellar vescicles of DPPC/DES mixtures with different DES content: (a) 0%; (b) 1.0%; (c) 3.0%; (d) 5.0%; (e) 7.5%; and (f) 10.0%, on heating.

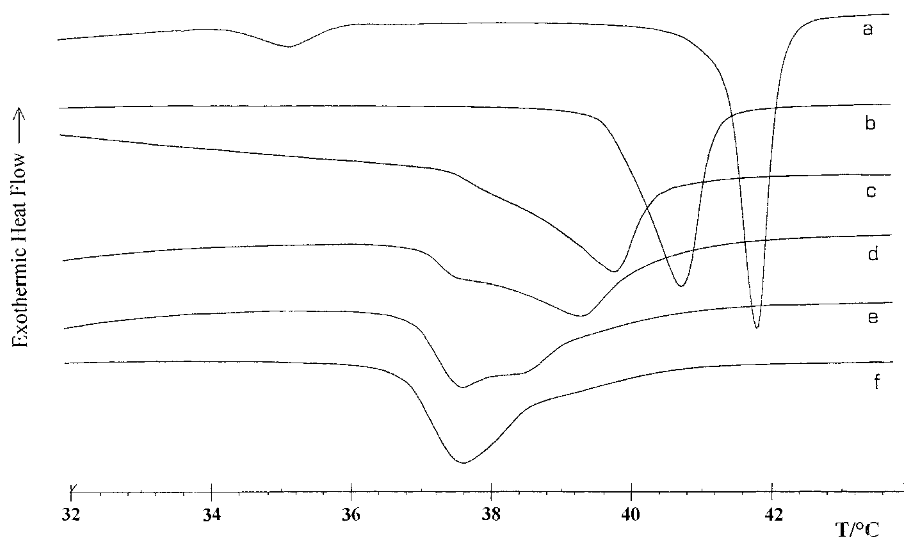

Fig. 3. DSC thermal response of hydrated multilamellar vescicles of DPPC/DBS mixtures with different DBS content: (a) 0%; (b) 1.0%; (c) 3.0%; (d) 5.0%; (e) 7.5%; and (f) 10.0%, on heating.

the pretransition was not detectable even in the presence of only 0.5% w/w DEHS.

The main transition temperature T_m appears noticeably affected in the presence of sebacic acid esters and, as a general feature, a lowering of its value was observed. By plotting the decrease of T_m as a function of the sebacate content a straight line was obtained, at least for low ester content, and the slope was related to the properties (i.e. length and branching) of the ester-chains. Long unbranched chains appear to be the most effective in decreasing T_m, indeed a decrease in T_m of 0.6, 1.7, 2.4 and 0.8 °C in the DPPC/DMS, DPPC/

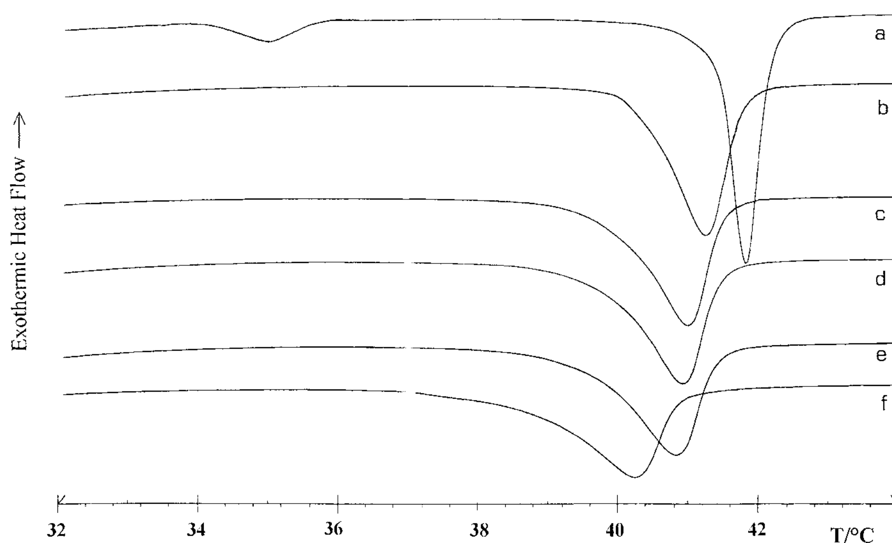

Fig. 4. DSC thermal response of hydrated multilamellar vescicles of DPPC/DEHS mixtures with different DEHS content: (a) 0%; (b) 1.0%; (c) 3.0%; (d) 5.0%; (e) 7.5%; and (f) 10.0%, on heating.

DES, DPPC/DBS and DPPC/DEHS systems with sebacic acid esters content equal to 5.0% w/w were observed respectively (Table 1). An increase in T_m was observed in the DPPC/DMS liposomes when the DMS amount was $\geq$7.5% w/w; in the presence of 30% w/w, the T_m value was close to that observed in pure DPPC liposomes, although, the peak had broadened and was more asymmetric as evidenced from the $\Delta T_{1/2}$ and A_s values.

With high sebacate contents in some of the systems considered, the shape of the peak was strongly modified, and more than one peak appeared (see Figs. 2 and 3), suggesting the concomitant presence of more than one phase. The sebacic ester content, which induces a complex peak structure, appeared to be strictly related to the ester-chain properties. Indeed, all the systems considered showed a single broad peak in the presence of DMS. On the contrary, the peak was complex in the systems with more than 10.0% w/w DES, more than 5.0% w/w DBS and 30.0% w/w DEHS. Similar results emerged considering the shape of the peaks in the cooling cycles.

In the DPPC/DES systems (DES $\geq$ 10.0% w/w), a new endothermic transition was present at about 37.5 °C (36.7 °C on cooling), whose intensity increased with increasing DES content. The maximum temperature appeared to be poorly or not altered by the DES amount.

In the DPPC/DBS system, the structure of the endotherm became complex when the DBS content was $\geq$5.0% w/w. A new well-defined endotherm peak was observed at about 37.7 °C (36.8 °C on cooling) and in the presence of DBS = 30.0% w/w, it was the only peak present. When the DBS content ranged from 5.0 to 10.0% w/w, it appeared together with the original main transition, although the latter decreased its intensity and T_m. Also, the temperature of the maximum of this new transition was poorly affected by the increasing amount of DBS (Table 1 and Fig. 3).

In the DEHS-containing liposomes, the appearance of a complex peak structure was evidenced only at the most concentrated sample (30.0% w/w) where a new poorly resolved transition in addition to the main endotherm appeared at about 38.0 °C by heating.

As a general feature, the half-width of the main transition appeared to increase in all the systems considered as the main peak did not show a complex structure. The observed increase was very close both on the heating and cooling cycles and the $\Delta T_{1/2}$ enlargement was roughly linearly related to the sebacate content. The $\Delta T_{1/2}$ increasing power was in the order: DBS > DES $\cong$ DEHS > DMS. On the contrary, the $\Delta T_{1/2}$ of the new transition at about 37.7 °C was detectable in the DBS/DPPC systems with DBS amounts greater than 10.0% w/w but appeared not to be greatly affected by the ester content.

The A_s asymmetry index of the heating peaks increased with increasing sebacate content and the peak was skewed to lower temperatures. There was a roughly linear correlation in liposomes containing DMS, DES and DBS (Table 2). The A_s was affected by the length of the ester-chain and increased in the order of DBS > DES > DMS. The effect on A_s was slightly different with branched chains present (i.e. DEHS). Indeed, a very small amount of DEHS causes a strong A_s increase, whereas, only small changes were observed on further addition of DEHS.

The skewness of the peak toward lower temperatures was also observed on cooling and A_s values lower than 1 were noted. The A_s behaviour was similar to that observed on heating although increases in A_s were less noticeable.

The ΔH values exhibited only small changes or were unchanged in any of the samples when only one peak was present. In the DMS/DPPC and DES/DPPC systems an increase in ΔH of about 15.0 and 10.0% was observed in the sebacic acid esters range 0.0–15.0 and 0.0–10.0% w/w, respectively (Table 2). On the contrary, in the DEHS/DPPC system (0.0–15.0% w/w) and in DBS/DPPC systems (0.0–3.0% w/w) no significant changes in ΔH were observed. Similar ΔH values were measured both in heating and in the cooling cycles within the expected experimental errors.

4. Discussion

The study of the effects of foreign substances in phospholipid bilayers have been addressed in many theoretical and experimental studies and some models have been proposed to explain the experimental data [21–23].

The solution model assumes that during the melting process the foreign substance, like a solute, distributes itself between the gel and liquid–crystal regions. This

model leads to the conclusion that a decrease in the melting temperature with a broadening of the peak are to be expected as a consequence of the induction of 'free volumes' into the bilayer structure, with only a minor dependence on the kind of the solute. The model agrees well with experimental results only if small, lipophilic substances, like anaesthetic or short chain alcohols are present in DPPC liposomes [21].

If large molecules, like long-chain alcohols, are present, this simple model fails and other effects, such as the lateral mobility into the bilayer and the increase of strong lateral van der Vaals interactions between the acyl chains of lipids and the foreign substances play an important role and increases in T_m have also been observed [24].

The changes in the lateral interaction between acyl chains of lipids as a consequence of the insertion of foreign substances into the bilayer are able to modify T_m. Indeed, the surface effects arising from the interaction of polar groups or ions with the polar heads of phospholipids can also induce in some systems a stiffening of the structure with increase in the T_m value [25].

In the sebacate–DPPC systems, we found that changes in T_m depend strongly on the length and shape of the ester-chain. Thus, the simple solution model does not explain all the observed results and we suppose that the hydrophobic interaction between lipidic chains as well the polar interaction involving carboxylic groups also play an important role. Indeed, C=O groups of sebacates could interact by means of dipole–dipole interactions with the polar groups of the phospholipid head. The flexibility of the acyl chain binding the two carboxylic groups in the sebacic acid esters favours conformational rearrangements thus, allowing the C=O groups to orient themselves to the best dipole–dipole interactions. As a consequence, the surface structure stiffens as the lateral mobility decreases. A similar behaviour was observed in DPPC bilayers in the presence of polyamine and divalent cations, with a T_m increase. [25].

In DMS/DPPC liposomes, the effects due to the penetration into the bilayer are small and surface interactions are the most important to explain the experimental behaviour. By increasing the length of the ester-chains up to butyl, penetration effects predominate and T_m decreases are observed, as in the presence of short chain alcohols [21].

In DEHS/DPPC liposomes, long and branched ester-chains are present. The depth of penetration into the hydrophobic core is great, but the T_m decrease is not so great as expected probably because of the presence of strong lateral Van der Waals interactions as in DPPC liposomes containing long chain alcohols [24].

The observed behaviour of other parameters considered (pretransition, ΔH, $\Delta T_{1/2}$ and A_s) can be explained on the basis of both dipole–dipole surface interaction, 'free volume' induction and hydrophobic interaction due to penetration into the bilayer. We observed a small increase in ΔH values in the presence of DMS and, to a lesser extent, DES whereas surface effects are supposed to play a key role showing thus that surface electrostatic interactions are able to increase ΔH value. On the contrary, in the presence of DBS and DEHS, whereas, the hydrophobic interaction effects were prominent, no changes in ΔH were observed as in the presence of phthalates [26].

The pretransition disappeared in most of the systems considered and was no longer present in any of the samples in which the sebacate content was greater than 1.0% w/w, confirming the marked sensitivity of the pretransition to the presence of foreign substances, as already observed in many different systems [13,24,26]. The disappearance of the pretransition led us to assume that the structural differences between rippled and lamellar gel phase were no longer present. The insertion of foreign molecules into the bilayer near to the surface forces the bilayer into one conformational phase only (probably the gel-rippled), thus, prevent the change in orientation of the acyl chains with respect to the normal orientation of the bilayer surface [19].

The experimental evidence that the pretransition is still detectable in DMS/DPPC systems at concentrations at which it disappears in all other systems supports the hypothesis of reduced penetration of DMS and adds further weight to the proposed interaction model.

The $\Delta T_{1/2}$ value increases roughly linearly with the sebacate content in all systems, both in heating and cooling, and its trend was strongly dependent on length and shape of the ester-chains in the sebacate molecule. In DMS/DPPC liposomes, $\Delta T_{1/2}$ changes very slightly up to a content of 7.5% w/w. On the contrary, significant and comparable $\Delta T_{1/2}$ increases,

also in the presence of small amounts of sebacate, were observed in the presence of both DES and DEHS. Even a greater $\Delta T_{1/2}$ increase was observed in DBS/DPPC liposomes.

It has been observed that, when the penetration into the hydrophobic core of the bilayer takes places, a lack of co-operativity in the fusion process was generally observed with increasing $\Delta T_{1/2}$ [15]. We explained the observed broadening efficiency (DBS $>$ DES $\cong$ DEHS $>$ DMS) as a consequence of the different degree of penetration. The lesser broadening capability of DEHS compared to DBS could reflect its capacity to induce a strong lateral van der Waals interaction. Another explanation is that the sterical hindrance of the branched chains reduces the penetration into the bilayer, but this seems to us to be scarcely probable because corresponding A_s values are high.

An A_s increasing value was generally observed in most of the system considered (Table 2).

The A_s changes were not observed in the DMS/DPPC systems up to a 7.5% w/w content. On the contrary, asymmetry increases were well-evident in liposomes containing more than 1% w/w of DES and A_s reached high values in DEHS/DPPC and DBS/DPPC systems even in the presence of very small amounts of sebacates.

The lack of symmetry and the skewing of the peak to lower temperatures has previously been observed in many systems [15,17,22,27]. It appears that factors influencing the asymmetry are strictly related to those affecting the broadening of the peak and the $\Delta T_{1/2}$ increase.

In the presence of lipophilic substances able to penetrate into the bilayer, a modification in the size distribution of the molecular clusters, where all molecules melt co-operatively is observed. The number of the clusters increases and its average size decreases noticeably. The lipophylic foreign molecules are supposed to stabilize the boundary of the cluster domains, whose number increase and size reduces. Even its shape changes and becomes more ramified.

Literature studies using statistical methods on DPPC liposomes containing anesthetics concluded that foreign molecules tend to localize preferentially near to the boundary surface of the domains; these latter become smaller and more numerous and non-homogeneity in the composition takes place causing a broadening of the transition peak, explaining the observed increase in $\Delta T_{1/2}$ values [27]. The skewing to lower temperatures may arise from the gradient of concentration from the surface to the inner part of the domains or also from differences in the partition coefficient between fused and still in the gel phase domains.

The behaviour of A_s supports the hypothesis that dipole–dipole surface interaction plays an important role in some sebacate–DPPC systems. Indeed, the observed effects on A_s were low in the liposomes in which penetration into the bilayer occurs only slightly and greater if the penetration is noticeable.

Also in the cooling cycles asymmetric enhancement of the peak was observed; the peak started sharply and, after the maximum temperature, it decreased more slowly than for pure DPPC liposomes. The degree of asymmetry observed during cooling cycles appeared less than that during corresponding heating measurements, probably because the higher lateral mobility in the fused phase that results in shorter equilibration time in solidification than in fusion.

The presence of more than one calorimetric peak observed in DBS/DPPC systems as DBS content was greater than 5.0% w/w and in the DES/DPPC and DEHS/DPPC system with higher sebacate content suggests the coexistence of aggregates with different structures. These could be cylindrical or globular structures, as observed, for example, in laudan-containing DPPC liposomes [28] or mixed type bilayers as observed in some phosphatidylcholine–anionic detergent mixed liposomes [29]. The appearance of new calorimetric peaks has been explained also by supposing a transition from multilamellar vesicles to aggregate containing mixed micelles. The transition involves conformational changes trans-gauche of lipid chains in the mixed micelles [30].

The constancy of the hysteresis in the main transition peak between heating and cooling cycles seems to exclude the presence of interdigitated $L_{\beta I}$ phases observed, for example, in the presence of high concentrations of alcohols and local anesthetics [30,31].

5. Conclusions

The experimental data show that noticeable changes take place in DPPC liposomes when sebacic acid esters are present. They suggest that both electrostatic

and hydrophobic interactions are present in the surface region dipole–dipole interactions between the C=O groups of sebacates and C=O and P=O groups of DPPC occur, whereas, hydrophobic interactions due to the penetration of the ester-chains into the core of the bilayer take place.

As with sebacates, phthalates, another class of plasticizers, also induce marked changes on the thermotropic behaviour of DPPC liposomes [26]. In the presence of phthalates, changes are strongly dependent on the length and shape of the ester-chains and are also evident in the presence of very small amounts of plasticizers. Short chain phthalates, like dimethyl and diethylphthalate, appeared to be the most effective in perturbing the inner core of the bilayer; whereas, those with long chains, such as bis-(2-ethylexyl) and di-*n*-ottylphthalate, were less effective.

This behaviour indicates that the phthalates interact with phospholipids mainly by means of hydrophobic interactions with lipidic chains. The polar interactions seem to play a secondary role probably because of the aromatic ring rigidity that does not allow the C=O groups of phthalates for the best orientation to interact with the polar moieties of DPPC.

On the contrary, sebacates, whose two ester groups are bound to a flexible chain, can adhere better to the surface, so favoring dipole–dipole electrostatic interactions. As a consequence, the shorter chain sebacates, for which surface interactions are dominant, only slightly perturb the inner core, whereas, the longer chain sebacates where the hydrophobic interactions are stronger than the electrostatic ones appear to have a strong effect on the hydrophobic moieties of DPPC. Sebacates, like phthalates, are able to affect ΔH values only slightly, but they can enhance some systems. As a general feature, sebacates appear to be less effective in modifying the inner bilayer structure, supporting the hypothesis that DPPC–sebacates interactions are localized mainly in a more external region of the bilayer with a shallower penetration into the hydrophobic core. Their biological effect could be, at least partially due to such a capability: indeed, the structural changes in the bilayer are a possible tool to modify the structure and thus, the function of the transmembrane proteins, whose good working has proved very sensitive to its microenvironment.

The differences in the modalities of the interaction toward model biomembranes between the two classes of compounds could play a significant role in explaining a different behaviour from the biological and toxicological standpoint.

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