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Vibrational and calorimetric study on the effect of di-*n*-propylsulfoxide (DPSO) on DMPC, DPPC and DMPE liposomes

S. Bonora [a,*], M. Di Foggia [a], S.A. Markarian [b], V. Tugnoli [a]

[a] Department of Biochemistry 'G. Moruzzi', University of Bologna, Via Belmeloro 8-2, I-40126 Bologna, Italy
[b] Department of Chemistry, Yerevan State University, 375049 Yerevan, Armenia

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ABSTRACT

In this paper, the effect of increasing amounts of DPSO on the behaviour of lecithins (DMPC, DPPC) and cephalin (DMPE) liposomes was investigated by means of Raman, FT-IR and DSC techniques. The results show that DPSO strongly affect, but in a different way, both lecithins and cephalin liposomes. In presence of low DPSO amounts, the main effect was a small decrease followed by an increase in the transition temperatures of all lipids; on the contrary, marked differences between the two classes were observed at higher sulfoxide content and the thermograms are strongly affected by memory effects. The partial dehydration of the lipid surface and the modification induced on the water structure might explain the effects observed in presence of low DPSO content, whereas, at higher DPSO concentrations, the main role is played by the direct interactions between the sulfoxide and the liposome surface. Spectroscopic data exhibit that the DPSO interact with —CO, $-NH_3^+$ and —PO groups in cephalin and only with $-N(CH_3)_3^+$ groups in lecithins.

The extension of the study to the concentrated DPSO containing systems presents a relevant biological significance; as a consequence of the affinity to biomembranes, the sulfoxide concentration near the lipid surface increases noticeably.

1. Introduction

Di-*n*-propylsulfoxide (DPSO) has been recently characterized by vibrational spectroscopy, discussing a detailed analysis of its IR and Raman spectra [1], exhibiting, like DMSO and DESO, potential biomedical interest [2–4]. In addition, an intense protection activity against the thermal denaturation of lysozyme, in low concentration of DPSO, has been observed [5].

^{1}H NMR data showed that DPSO is able to form self-associative structures stronger than DESO and DMSO [6]. Evidence for highly self-associated structure in liquid DPSO arises from its very high Kirkwood correlation factor **g** ($\mathbf{g}_{DPSO} = 1.42$), that suggest the occurrence of strong parallel association between the dipoles [7]. Also recent volumetric and calorimetric measurements on DPSO in water suggest that strong interactions take place between DPSO and water, stronger than those of DMSO and only little weaker than those of DESO.

The molecular mechanism of DPSO biological action is not well clear; however, the interactions with biomembranes and the effect on their structure and permeability are considered to play a key role, like for smaller dialkylsulfoxides [8]. All dialkylsulfoxides show, to a varying degree, an amphipathic character, due to the presence of both a polar hydrophilic S=O group as well as of two hydrophobic (although able to take part in H-bonds formation, as pointed out in the literature [9,10]) alkyl groups. Consequently, both hydrophilic and hydrophobic interactions are responsible for the behaviour of sulfoxides; for example, the ability of DMSO to penetrate biological membranes was ascribed mainly to its amphipathic character [11].

The role of hydrophobic interactions in the biological properties of the sulfoxides is crucial. In fact, it has been demonstrated that hydrophobic interactions are involved both in the toxic effects exerted by DMSO at high temperatures on isolated proteins [12], as well as in the protein precipitation and denaturation [13]. Moreover, it has been postulated that the hydrophobic interactions of dialkylsulfoxides with biomembranes play a role in the cryoprotective effect [14].

The present paper is the continuation of a previous work on the effect of smaller dialkylsulfoxides on liposomes [15]; we now present a detailed study by means of spectroscopic (Raman and FT-IR) and thermal (differential scanning calorimetry – DSC) techniques on multilamellar DMPC (1,2-dimyristoyl-*sn*-glycero-3-phosphocholine), DPPC (1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine) and DMPE (1,2-dimyristoyl-*sn*-glycero-3-phosphoethanolamine) liposomes in presence of increasing amounts of DPSO.

DMPC and DPPC liposomes are widely used as a model system of biomembranes because lecithins are the major compo-

* Corresponding author. Tel.: +39 51 2094280; fax: +39 51 243119.
E-mail address: sergio.bonora@unibo.it (S. Bonora).

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nent of most mammalian membranes; DMPE liposomes are a useful model for nervous tissue cell membranes since large amounts of cephalins are present in this type of tissues. Both DSC and vibrational spectroscopy have been proved to be very useful techniques to study the changes induced in model membranes by foreign substances. Liposomes exhibit a characteristic thermal behaviour by heating, showing a sharp endothermic gel to liquid crystal transition whose peak temperature and shape are strongly modified by interactions with other substances, thus reflecting the changes induced in the bilayer structure [16,17]. Vibrational spectroscopy is a useful tool to examine the existence and the strength of localized interactions between molecules; moreover, the I_{2880}/I_{2850} and I_{1130}/I_{1090} Raman intensity ratios are probes of the molecular order in the lipid bilayer. Indeed, the I_{1130}/I_{1090} ratio is related to the average number of '*trans*' bond in the acylic chain, giving thus a measure of the order in the intrachain structure, whereas the I_{2880}/I_{2850} ratio is related to the vibrational coupling between the adjacent chains and gives a semi-quantitative measurement of the lateral interactions between the lipid chains [18].

2. Materials and methods

Synthetic DL-DMPC, DL-DPPC and DL-DMPE were Sigma (Sigma–Aldrich Europe group) products (purity guaranteed >99% by TLC), and thus used without further purification. Before the use, the compounds were held 24 h at room temperature in a drier with P_2O_5 to reduce the amount of the hydration water to the H_2O molecules heavily bonded only (about 2% w/w by thermogravimetric analysis). DPSO was prepared and purified according to the literature [1] and dried on molecular sieves. Its purity, tested by GC, was >99.5% and the residual water content was <0.01%. Twice distilled water and 'analytical grade' Merck chemical products were also used.

Samples were prepared by adding to a amount of the lipid, previously weighted with a Mettler M-3 microbalance, the DPSO/H_2O mixtures of the appropriate molar fraction (χ). To more closely mimicking the physiological conditions, the DPSO/H_2O mixtures were prepared using a NaCl 0.9% w/w water solution buffered at pH 7.0 with phosphate buffer (about 10^{-3} M) and the final lipid concentration was 20% w/w. Homogeneous gelatinous samples were obtained by gentle sonication (Vibra-cell from Sonics Materials; 1 min at ~0.5 W of power in a ice cold bath). Under these conditions, the heating of the samples was negligible (<5 °C). We prepared liposomes in the presence of DPSO/H_2O mixtures, with a DPSO molar fraction χ ranging from 0.00 to 1.00 (DPSO/H_2O % w/w ranging from 0% to 100%).

DSC measurements were performed on a Mettler-Toledo DSC 821, using a heating and cooling rate of 2.0 °C/min in the 5–70 °C range for DMPC and in the 20–90 °C range for DMPE and DPPC liposomes. Temperature and enthalpy scales were calibrated with indium and tested in the considered thermal range by capric acid. Thermal cycles were repeated on three different samples to ensure constancy and reproducibility of the data; the experimental error in temperature and thermal (ΔH) response was ±0.1 °C and ±5%, respectively. Since we observed some differences, due to phase segregation or incomplete mixing, comparing the thermograms relative to the first and to the subsequent consecutive heating and cooling cycles, all reported calorimetric data refers to the second (or subsequent) run, as previously done for the smaller dialkylsulfoxides [15]. After DSC measurements, all samples were heated under vacuum to remove the liquid phase and the dry residue was weighted for ΔH evaluation. The absence of DPSO residues was confirmed by GC measurements, after dissolution of the dry residue in $CHCl_3$.

Raman spectra were obtained using a Jasco NRS-2000C instrument. All spectra were recorded in backscattering conditions with 4 cm^{-1} spectral resolution using the 488 nm [Ar^+] line. The laser power (Innova Coherent 70) on the sample was ~10 mW and the total number of scans averaged for each spectrum was 25. The detector was a 160 K frozen digital CCD (Spec-10:100B, Roper Scientific Inc.) and a variable temperature thermostatic sample holder (Linkam THM 600 with the Linkam CO 600 temperature controller) was used to collect the spectra in the gel phase (15 °C for DMPC; 25 °C for DPPC and DMPE).

IR spectra were recorded on a Nicolet 5700 FT-IR, equipped with a diamond attenuated total reflectance (ATR) accessory and a DTGS detector. The total number of scans averaged for each spectrum was 512 and measurements were made at the same temperatures of the corresponding Raman measures. Before vibrational spectra the samples were centrifuged to reduce the interferences.

3. Results

3.1. DMPC/DPSO and DPPC/DPSO systems

Literature data report that pure DMPC and DPPC liposomes exhibit, in the considered thermal ranges, a well-defined thermal behaviour characterized by a strong and sharp main transition near 24 °C, with $\Delta H \sim 26$ kJ m^{-1} for DMPC and near 41 °C, with $\Delta H \sim 37$ kJ m^{-1} for DPPC, arising from the conversion of the P_β gel phase to the lamellar liquid-crystal L_α phase [19]. A pretransition at about 13.5 °C in DMPC and 35 °C in DPPC liposomes, arising from the conversion of a lamellar gel phase (L_β) to a rippled gel phase (P_β) is also reported [19]. In pure DMPC and DPPC liposomes, the values of the main transition temperature (T_m) and enthalpy of transition (ΔH) we found were 23.8 °C, 25.9 kJ m^{-1} and 41.1 °C, 37.5 kJ m^{-1}, respectively, in good agreement with the literature data [19]. The transition is reversible and the small differences between the heating and cooling temperatures (~0.6 °C) can be attributed to the finite thermal response time of the calorimeter and to the different lateral mobility of acyl chains in the gel and in the liquid crystal phase. The shape of the peak is roughly symmetrical, with only a slight skewing toward lower temperatures. We also observed the weak pretransition with a maximum at 13.5 °C (T_{pr}), with a ΔH value of 4.1 kJ m^{-1} and 35.0 °C (T_{pr}), with a ΔH value of 5.6 kJ m^{-1}, in DMPC and DPPC liposomes, respectively; also in good agreement with the literature [19]. The pretransition peak is broad, nearly symmetrical and, because of the formation of an intermediate metastable phase that slowly interconvert into the L_β phase [20], the samples should be held at low temperature for at least 30′ to obtain reproducible pretransition thermograms.

Fig. 1 exhibits the trend of the main transition (T_m) temperatures and enthalpy (ΔH) values as a function of the χ_{DPSO} in the DPSO/H_2O systems where χ_{DPSO} ranges from 0.0 to 0.30, for both DMPC and DPPC liposomes. Tables 1 and 2 summarize the calorimetric data relative to heating and cooling cycles for all DMPC/DPSO and DPPC/DPSO considered systems. Fig. 2 shows the characteristic shapes of some significant thermograms of DPPC liposomes with different DPSO content.

For both lipids, the pretransition disappeared even with a very small amount of DPSO ($\chi_{DPSO} = 0.01$), as already observed in presence of substances able to insert within the lipid bilayer [16]. The main transition peak exhibits a complex T_m behaviour, resulting in a small decrease (up to $\chi_{DMPC} = \chi_{DPPC} = 0.05$), followed by a higher increase (Fig. 1). Contemporaneously (Tables 1 and 2), the heating–cooling hysteresis ($\Delta T_m = T_m - T'_m$) increases in presence of both lipids up to $\chi_{DPSO} = 0.30$ in the DMPC/DPSO and up to $\chi_{DPSO} = 0.20$ in the DPPC/DPSO systems, decreasing at higher DPSO content. On

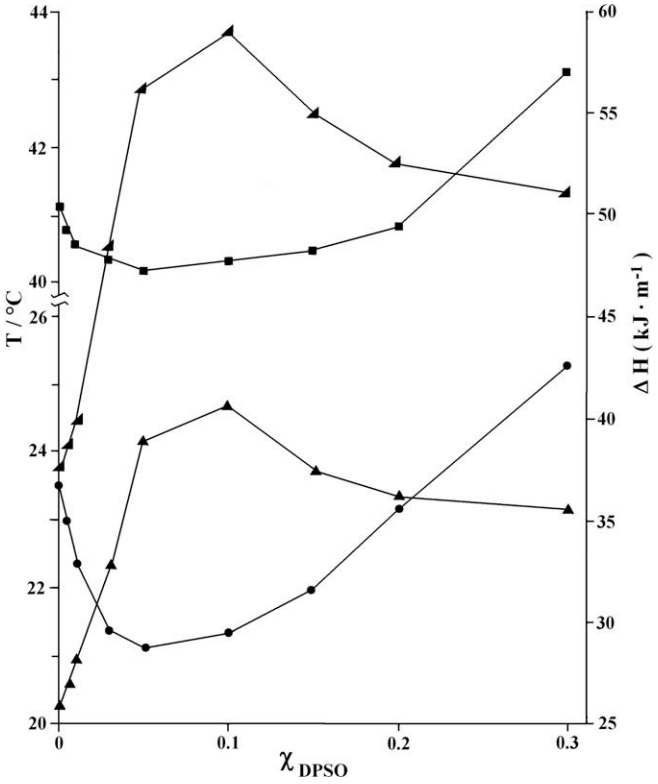

Fig. 1. ΔH value and main transition temperature (T_m) of DMPC and DPPC liposomes as a function of DPSO molar ratio (χ_{DPSO}) in the DPSO–H_2O mixtures (●, T_m DMPC; ▲, ΔH DMPC; ■, T_m DPPC; ◢, ΔH DPPC).

Table 1
Calorimetric data relative to the heating and cooling process of DMPC liposomes in H_2O/DPSO mixtures with increasing amounts of DPSO. 'χ' = molar fraction of DPSO; T_m and T'_m = main transition peak temperatures in the heating and cooling process; ΔH = enthalpy in the heating process; $\Delta T_{1/2}$ and $\Delta T'_{1/2}$ = half width of the main thermal peak in the heating and cooling process.

χ_{DPSO}	T_m (°C)	T'_m (°C)	ΔH (kJ m^{-1})	$\Delta T_{1/2}$ (°C)	$\Delta T'_{1/2}$ (°C)
0.00	23.5	22.9	25.9	0.5	0.5
0.005	23.0	22.4	26.9	0.5	0.5
0.01	22.4	21.7	28.0	0.5	0.6
0.03	21.4	20.4	32.8	0.6	0.6
0.05	21.2	19.8	38.9	0.5	0.6
0.10	21.4	20.0	40.7	0.7	0.6
0.15	22.0	20.5	37.2	0.6	0.6
0.20	23.2	21.3	36.4	1.4	1.2
0.30	25.8	23.7	35.6	2.3	2.0
0.50	34.9	33.3	34.4	2.6	2.5
0.70	45.6	44.3	32.5	3.0	2.9
1.00	54.2	52.9	31.4	2.9	2.6

the contrary, the $\Delta T_{1/2}$ of the transition increases monotonically with DPSO amount.

Fig. 1 and Tables 1, 2 show that even the ΔH of the main transition exhibits a complex behaviour; in fact, in presence of both lipids, an increase was observed up to $\chi_{DMPC} = \chi_{DPPC} = 0.10$, followed by a decrease at higher concentrations. In the samples with $\chi_{DPSO} \geqslant 0.5$, the heating thermograms show a broad endotherm, whose T_m lies at a noticeably higher temperature ($T_{mDMPC} > 30$ °C and $T_{mDPPC} > 50$ °C). The limited hysteresis between heating and cooling cycles ($\Delta T_m = T_m - T'_m < 1.5$ °C) suggests the lack of slow non-equilibrium processes.

As a consequence of the overlapping of strong DPSO Raman bands in both spectral regions that changes during the melting process (2800–2900 and 1050–1150 cm^{-1}), Raman spectroscopy

Table 2
Calorimetric data relative to the heating and cooling process of DPPC liposomes in H_2O/DPSO mixtures with increasing amounts of DPSO. 'χ' = molar fraction of DPSO; T_m and T'_m = main transition peak temperatures in the heating and cooling process; ΔH = enthalpy in the heating process; $\Delta T_{1/2}$ and $\Delta T'_{1/2}$ = half width of the main thermal peak in the heating and cooling process.

χ_{DPSO}	T_m (°C)	T'_m (°C)	ΔH (kJ m^{-1})	$\Delta T_{1/2}$ (°C)	$\Delta T'_{1/2}$ (°C)
0.00	41.1	40.4	37.5	0.5	0.5
0.005	40.8	40.0	38.5	0.5	0.5
0.01	40.6	39.7	39.9	0.5	0.5
0.03	40.4	39.4	48.7	0.5	0.5
0.05	40.2	39.2	56.4	0.6	0.5
0.10	40.3	39.2	58.9	0.6	0.5
0.15	40.5	39.2	54.8	0.7	0.6
0.20	40.8	39.1	52.5	0.9	0.8
0.30	43.1	41.6	51.3	1,4	1.4
0.50	50.8	49.3	49.8	2.2	2.0
0.70	55.8	54.4	47.2	2.4	2.2
1.00	63.9	62.7	45.4	2.3	1.7

cannot be used in the whole χ_{DPSO} range to evidence the average number of '*trans*' bond in the acylic chains and the lateral interactions between the lipid chains. Nevertheless, subtracting the DPSO spectra to the whole liposome Raman spectra, we obtained acceptable results up to $\chi_{DPSO} = 0.05$ (corresponding to a DPSO content equal to 28.1% w/w), showing that only a moderate diminution, not far from the limits of the experimental error, of both I_{2880}/I_{2850} and I_{1130}/I_{1090} Raman intensity ratios takes place. The I_{2880}/I_{2850} ratio ranges from 1.22 in pure DMPC liposomes to 1.17 in DMPC liposomes with $\chi_{DPSO} = 0.05$ and the I_{1130}/I_{1090} ratio ranges from 1.35 to 1.31 in the same samples, respectively, excluding thus the introduction of the DPSO molecules within the hydrophobic bilayer. Moreover, Raman spectroscopy provides useful information on the localized interactions of the S=O group, studying the DPSO $\nu_{S=O}$ bands in the 1000–1050 cm^{-1} range, free from noticeable lipid interferences. Fig. 3 shows the Raman spectrum of DPSO alone (Fig. 3a) and the difference spectra between DPSO in presence of the phospholipids and the corresponding phospholipid alone. In presence of both DPPC and DMPC, the 1024 cm^{-1} $\nu_{S=O}$ band splits in two components, at 1035 and 1017 cm^{-1} (Fig. 3b).

Fig. 4 exhibits the IR spectra of DPPC liposomes in H_2O and in DPSO in the 1850–1600 cm^{-1} spectral region, in which the asymmetric $\nu_{C=O}$ stretching mode lies, and 1100–750 cm^{-1} region, in which some characteristic frequencies of the phospholipids polar groups appear [21]. Fig. 5 shows the IR spectra in the 3800–3000 cm^{-1} spectral region, in which lies the water ν_{OH} before and after the addition of anhydrous DPSO to DMPC. This band exhibits a narrowing and a shift towards the same frequency of the mixture water–DPSO (Fig. 3b).

3.2. DMPE/DPSO samples

In pure DMPE liposomes, the values of the main transition temperature (T_m) arising from the conversion of the L_β in the L_α phase and enthalpy of transition (ΔH) we found were 49.8 °C, and 27.8 kJ m^{-1}, in good agreement with the literature data (49.9 °C and 27.5 kJ m^{-1}) [19]. The peak is highly reproducible, sharp, with a nearly symmetrical profile and the calorimetric measurements of all considered systems are collected in Table 3. In pure DMPE liposomes, T_m showed a hysteresis ($\Delta T_m = T_m - T'_m$) of about 0.6 °C, arising from the causes previously pointed out; a small increase in the hysteresis was observed by increasing χ_{DPSO} ($\Delta T_m \sim 1.3$ °C if $\chi = 0.05$).

Fig. 6 shows the shape of some significant thermograms relative to DMPE liposomes with different amounts of DPSO. Up to a $\chi_{DPSO} = 0.05$, only a T_m decrease is observed, similarly in presence

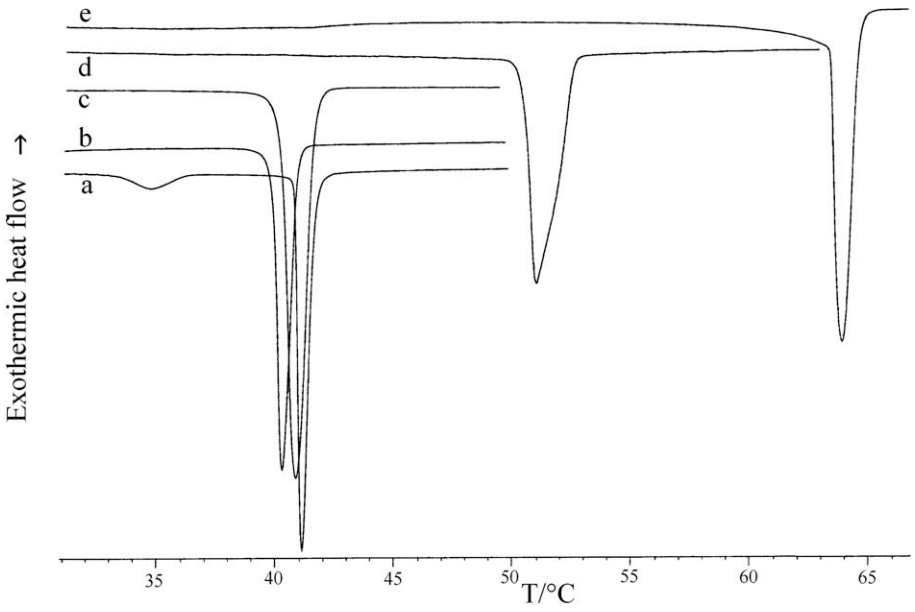

Fig. 2. DSC thermal response of DPPC liposomes in the presence of DPSO/H_2O mixtures. 'χ' = molar ratio of the sulphoxide; (a) χ_{DPSO} = 0.00; (b) χ_{DPSO} = 0.05; (c) χ_{DPSO} = 0.15; (d) χ_{DOSO} = 0.50; (e) χ_{DPSO} = 1.00.

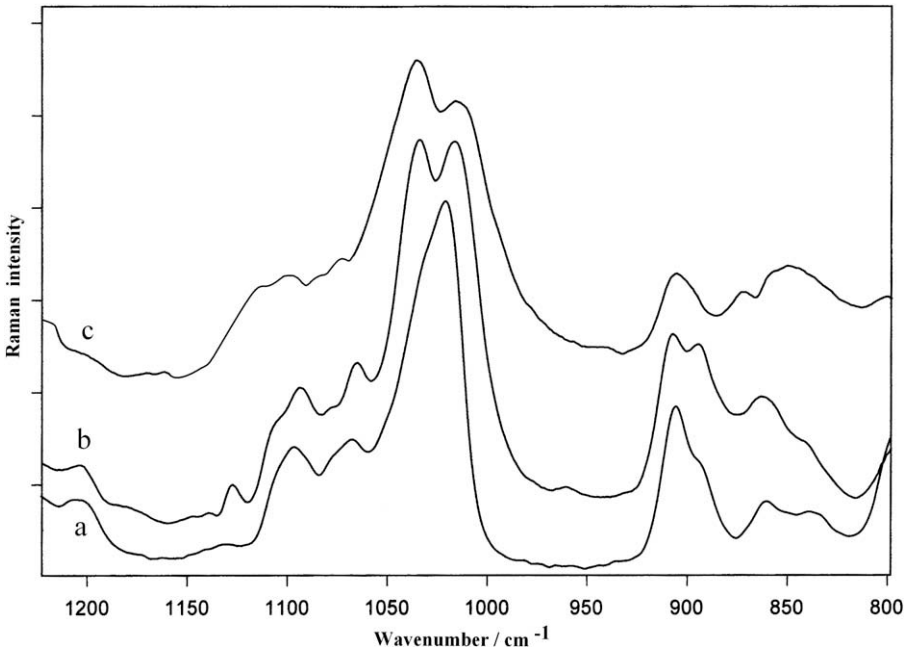

Fig. 3. Raman spectra in the 1200–800 cm^{-1} spectral region of DPSO alone (a) and in presence of DPPC (b) or DMPE (c). Spectra (b) and (c) are the difference spectra between those of the liposomes in DPSO and those of the same liposomes in H_2O.

of DMPC or DPPC; on the contrary in presence of a greater DPSO amount ($0.1 \leqslant \chi_{DPSO} \leqslant 0.30$), the behaviour of the thermograms become complex: the heating curve exhibits at first an endothermic transition followed by an exotherm, and finally by another endotherm, whereas the cooling cycle exhibits only one exotherm. Such behaviour is well reproducible, and is very clear in the sample with χ_{DPSO} = 0.15 (Fig. 6d). Finally, as $\chi_{DPSO} \geqslant 0.5$, a single and reproducible transition endowed with a great hysteresis is observed. In DPSO-containing DMPE liposomes, we observe a monotonic ΔH increase, at least until only one transition is present (Table 3). Nevertheless, the same trend is retained even in presence of $\chi_{DPSO} \geqslant 0.1$, by considering the algebraic sum of the ΔH of all transitions observed in the heating cycle. With great amounts of DPSO ($\chi_{DPSO} \geqslant 0.5$) a further increase of ΔH is observed and, if χ_{DPSO} = 1.0, its value is about three times the value measured at χ_{DPSO} = 0.0.

Fig. 3c exhibits that, in presence of DMPE, the Raman $\nu_{S=O}$ band splits in two components, at 1036 and 1015 cm^{-1}, whereas Fig. 7 shows the IR spectra of DMPE liposomes in H_2O and in DPSO in the 1850–1600 and 1150–750 cm^{-1} spectral regions. The ν_{OH} IR spectral region exhibit a behaviour similar to that previously reported in Fig. 5, relative to the DMPC/DPSO systems. Also the

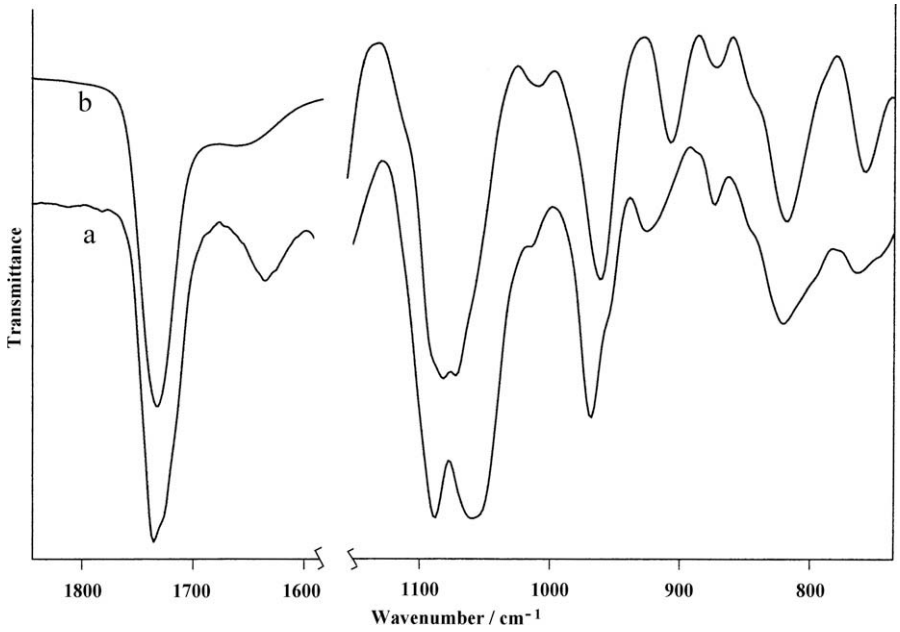

Fig. 4. FT-IR spectra in the 1850–1600 and 1150–750 cm^{-1} spectral regions of DPPC liposomes in H_2O (a) and in DPSO (b).

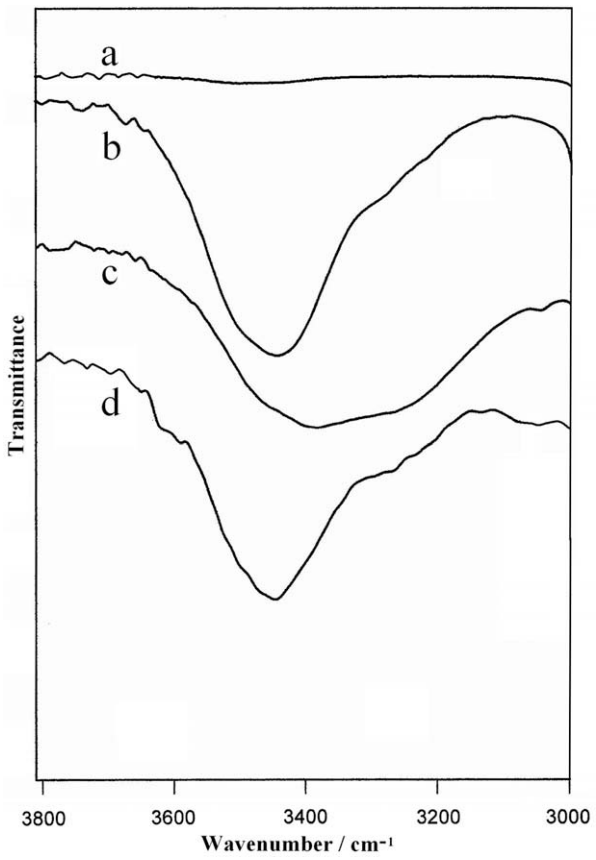

Fig. 5. FT-IR spectra in the 3800–3000 cm^{-1} spectral region of: pure DPSO (a); DPSO with 2.0% w/w added H_2O (b); DMPC (c); DMPC after addition of pure DPSO (d).

behaviour of I_{2880}/I_{2850} and I_{1130}/I_{1090} Raman intensity ratio supports the exclusion of the DPSO insertion into the hydrophobic bilayer, since both values does not change, within the limits of the experimental error, up to a $\chi_{DPSO} = 0.05$.

4. Discussion

To explain the melting process in liposomes many models have been proposed [22,23]. According to the cluster model, the main transition arises from the cooperative and contemporary change of phase $P_\beta \rightarrow L_\alpha$ (for cephalines) or $L_\beta \rightarrow L_\alpha$ (for ethanolamines) of all the molecules within each domain in which the liposome is divided. In the presence of substances that insert, or even only penetrate to some extent into the hydrophobic core of the bilayer, concentration gradients are formed near the surface of the domains, that become smaller and more ramified, as deduced by theoretical studies [24]. As a consequence, the transition cooperativity decreases and calorimetric peaks broaden (ΔH decreases and $\Delta T_{1/2}$ increases) becoming more asymmetrical, with skewing towards lower temperatures [16].

Moreover, it was already observed that not only the substances penetrating into the lipidic bilayer, but even those interacting only with the external surface, can affect the liposomes thermal behaviour [25,26]. For example, in presence of some alkaline-hearth cations, like Ca^{2+} or Mg^{2+} and poly-cations, like poly-amines, an increase of both T_m and ΔH, besides the persistence of the pretransition, was observed. In that case, only moderate decreases or even increases on both Raman order parameter were observed [27]. Such behaviour was explained assuming that the changes at the liposome–water interface are able to modify the topology of the polar lipid surface, thus influencing the thermal parameters of the melting process but excluding the direct interaction with the inner bilayer [27,28].

The disappearance of the pretransition in all DMPC/DPSO and DPPC/DPSO systems as $\chi_{DPSO} \geqslant 0.01$ suggests that the interaction involves the hydrophobic moieties of the bilayer; on the contrary, the ΔH increases, the negligible or small changes observed in $\Delta T_{1/2}$ at the same DPSO concentration (Tables 1 and 2) and the small modification on the Raman order parameter up to $\chi_{DPSO} = 0.05$, suggest an opposite behaviour.

In the more diluted solutions ($\chi_{DPSO} \leqslant 0.10$), the relative number of DPSO molecules is small; consequently, the modifications of the inner lipidic structure are mainly mediated by changes in the water structure. In such conditions, the effect of DPSO occurs through the modification of the water–liposome interaction, play-

Table 3
Calorimetric data relative to the heating and cooling process of DMPE liposomes in H_2O/DPSO mixtures with increasing amounts of DPSO. 'χ' = molar fraction of DPSO; T_m and T'_m = main transition peak temperatures in the heating and cooling process; ΔH = enthalpy in the heating process; $\Delta T_{1/2}$ and $\Delta T'_{1/2}$ = half width of the main thermal peak in the heating and cooling process.

χ_{DPSO}		T_m (°C)		T'_m (°C)		ΔH (kJ m^{-1})		$\Delta T_{1/2}$ (°C)	$\Delta T'_{1/2}$ (°C)
0.00		49.8		49.2		27.8		0.5	0.7
0.005		48.8		48.2		29.9		0.6	0.8
0.01		47.9		47.0		32.7		0.6	0.9
0.03		46.7		45.7		36.9		0.7	1.0
0.05		46.5		45.2		38.1		0.9	1.1
0.10	47.1	55.1		45.3	42.3	12.3		–	1.2
0.15	47.6	49.2	57.4	45.8	15.8	−31.7	67.0	–	1.4
0.20	48.7	50.3	59.5	46.9	12.8	−30.7	69.8	–	2.1
0.30	52.6	55.0	65.6	51.5	9.0	−31.2	73.7	–	2.4
0.50		75.9		57.9		87.8		1.5	2.0
0.70		79.5		60.8		88.2		2.1	1.9
1.00		80.7		66.6		90.1		3.1	2.5

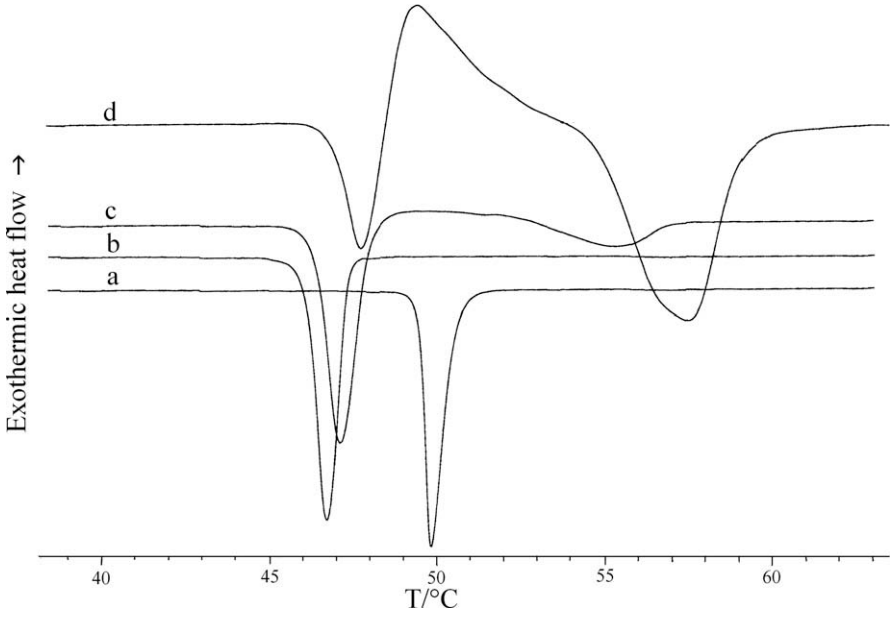

Fig. 6. DSC thermal response of DMPE liposomes in the presence of DPSO/H_2O mixtures. 'χ' = molar ratio of the sulphoxide; (a) χ_{DPSO} = 0.00; (b) χ_{DPSO} = 0.03; (c) χ_{DPSO} = 0.10; (d) χ_{DOSO} = 0.15.

ing in the direct interaction between the S═O group and the polar centres of the phospholipid only a minor role. On the contrary, by a further χ_{DPSO} increase, the direct DPSO–liposome interaction earns importance, becoming the most relevant in the more DPSO-concentrated systems. The direct DPSO–liposome interaction consists in polar interactions involving the S═O groups, whose importance in the structures of dialkysulfoxides-containing systems has been highlighted [9,10].

The $\nu_{S=O}$ Raman peak at 1024 cm^{-1} in pure DPSO splits in two components, at 1035 and 1017 cm^{-1}, in presence of both DMPC and DPPC (Fig. 3b), suggesting that DPSO replaces the H_2O molecules interacting with the lipid molecule in the phospholipid, as confirmed in a recent study [1] that assigned the second Raman band appearing at a frequency lower than 1024 cm^{-1} to the H-bonded S═O groups in the DPSO–H_2O systems. This hypothesis is confirmed by the ν_{OH} IR behaviour; indeed Fig. 5 exhibits that both wavenumber and shape of the band in DPPC in presence of anhydrous DPSO is quite similar to the band in DPSO in presence of small amounts of water ($H_2O \approx 2\%$). The impossibility of DPPC to form H-bonds arises from the lack of H-donor groups at neutral pH.

IR spectra offer explanations on the site of the DPSO–DPPC (or DPSO–DMPC) interaction. Fig. 4 shows that $\nu_{C=O}$ remains at about the same frequency both in H_2O and in DPSO (1734 cm^{-1} in H_2O; 1733 cm^{-1} in DPSO), indicating that C═O group interactions of the same type and strength occur both in H_2O and in DPSO. On the contrary, the IR spectra of DPPC (Fig. 4) exhibit noticeable changes in the 1100–900 cm^{-1} spectral regions, in which lies the polar head vibrations. The peak at 1058 cm^{-1}, attributed, according to the literature [21] to the P–O–C vibrations, shifts in DPSO to higher frequency at 1072 cm^{-1}, suggesting that the phosphate group is not accessible to the direct interaction with DPSO. On the contrary, the frequency decreases (from 968 to 960 cm^{-1} and from 926 to 907 cm^{-1}), that we attribute to the C—C—$N^+(CH_3)_3$ antisymmetric stretching modes, suggest the setting up of polar interactions between the $(CH_3)_3N^+$ group of the choline and the S═O group of DPSO.

The behaviour of DMPE liposomes is quite different; after a close resembling general trend up to χ_{DPSO} = 0.05, the calorimetric pattern changes noticeably with the contemporary appearing of both endothermic and exothermic peaks when $0.1 \leqslant \chi_{DPSO} \leqslant 0.3$. Finally, as $\chi_{DPSO} > 0.3$, a single transition at a noticeably greater T_m appears.

Like previously in lecithins, both the splitting of the ν_{SO} peak at 1024 cm^{-1} of the pure DPSO Raman spectrum in two components at 1036 and at 1015 cm^{-1} as well as the similar behaviour we observed in the ν_{OH} IR region, suggest that DPSO acts as a dehydrating agent even towards DMPE, replacing water molecules in direct

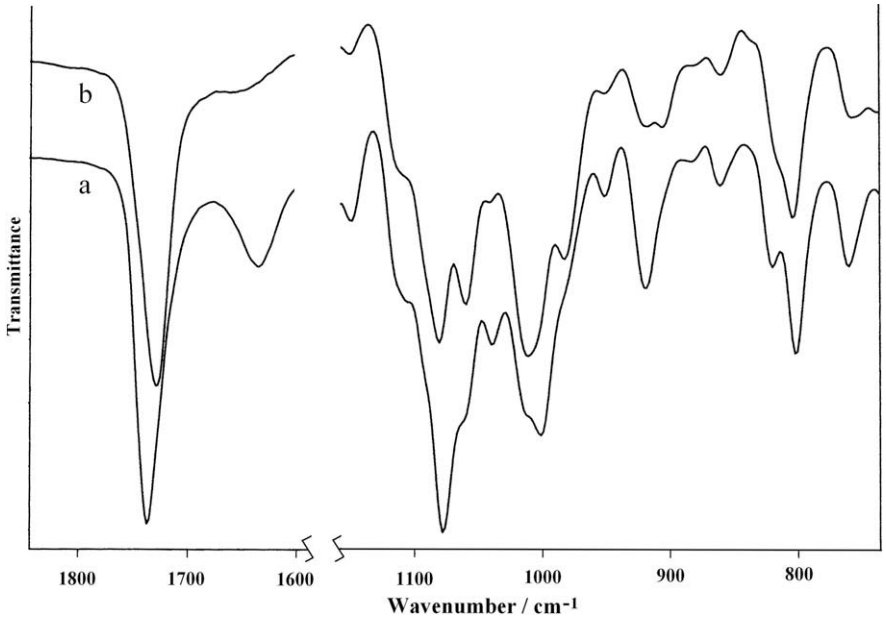

Fig. 7. FT-IR spectra in the 1850–1600 and 1150–750 cm^{-1} spectral regions of DMPE liposomes in H_2O (a) and in DPSO (b).

interaction with the lipid. However, owning the DMPE molecule a H-donor group, we hypothesize that even the setting up of hydrogen bonds between NH_3^+ and S═O groups plays a role.

The IR spectra of the DMPE/DPSO systems point out the existence of several interaction sites on the lipid. The ν_{CO} stretching frequency decrease from 1740 cm^{-1} in presence of water to 1732 cm^{-1} in presence of DPSO (Fig. 6), suggests the setting up of strong interactions between S═O and C═O groups. Also the 1050–850 cm^{-1} spectral region exhibits changes; the lower frequency shift from 1010 cm^{-1} (in H_2O) to 1001 cm^{-1} (in DPSO) of the peak attributed, according to the literature [21], to the C—C—N^+ antisymmetric stretching mode, confirms that the interaction of DMPE with DPSO involves even the amino group of the phospholipid, probably by means of the formation of hydrogen bond between the S═O and NH_3^+ groups. Moreover, the shift of the 820 and 802 cm^{-1} to 813 and 804 cm^{-1}, respectively, attributed to the O—P—O antisymmetric stretching mode [21], suggest that also phosphate groups on the lipidic molecule are involved, although to a lower extent, in the interaction with DPSO.

A detailed explanation of the complex behaviour observed in DMPE liposomes with intermediate DPSO content is not easy; in any case, we suppose that the hydration–dehydration process of the outer lipidic surface together with a relatively slow interchange rate between sulfoxides and water, help to explain the observed behaviour. We suppose that the lower temperature endothermic peak arises from the gel to the liquid crystal transition in liposomes with a higher degree of hydration, whereas the higher temperature endothermic peak is due to the same transition in dehydrated ones, but we cannot exclude that the interconversion between metastable phases plays a role [19,29].

If $\chi_{DPSO} \geqslant 0.5$ a single transition appears again, suggesting the existence of only one bilayer structure in the higher DPSO presence.

The lower frequency shift of the IR components at ~1740, ~1010 and ~820 cm^{-1} suggest that DPSO exhibits a greater affinity toward ethanolamine than toward choline liposomes. This behaviour is attributed to the increased polarity of DMPE liposome surface, consequent to the unshielded positive charge on the nitrogen atom. Therefore, strong dipole–dipole, ion–dipole as well as H-bonding interaction involving the S═O group can be formed, involving both NH_3^+, O—P—O and C═O groups.

5. Conclusions

Our results show that DPSO strongly affects, although in a different way, lecithins and cephalins liposomes, involving different sites: the C═O, NH_3^+ and, to a lower extent, P═O groups in cephalins; mainly the —$N(CH_3)_3^+$ group in lecithins.

In the literature, it was suggested that the modifications induced in biomembranes by the presence of sulfoxides (DMSO) arise mainly from their ability to affect the water structure, excluding thus any role of the hydrophobic interactions [30]. In this frame, the behaviour of the sub gel transition in DPPC/DMSO-containing liposomes was explained by DMSO's ability to enhance the water structure [31]. On the contrary, our data clearly show that, in the presence of DPSO, the hydrophobic interactions play a role, as suggested by the initial T_m decrease.

Moreover, the importance of the polarity of the outer lipidic surface has been highlighted. Indeed, we observed, in presence of the more polar DMPE liposome surface, the setting up of a equilibrium able to produce a slow interconversion between metastable phases. It should be noted that the existence of metastable phases with consequent phase segregation of regions with different properties has been thought to play a role in membrane damage caused by severe external conditions [32].

As a consequence of the DPSO affinity to biomembranes, its concentration near the lipid surface increases noticeably, justifying thus the extension of our study to the systems with high DPSO content.

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References

[1] S.A. Markarian, L.S. Gabrielian, S. Bonora, Spectrochim. Acta A 68 (2007) 1296.
[2] S.A. Markarian, A.A. Poladyan, G.R. Kirakosyan, A.A. Trchounian, K.A. Bagramyan, Lett. Appl. Microbiol. 34 (2002) 417.
[3] S.A. Markarian, S. Bonora, K.A. Bagramyan, V.B. Arakelyan, Cryobiology 49 (2004) 1.

[4] S.A. Markarian, A.M. Asatryan, K.R. Grigoryan, H.R. Sargsyan, Biopolymers 81 (2006) 1.
[5] A. Torreggiani, M. Di Foggia, I. Manco, A. De Maio, S.A. Markarian, S. Bonora, J. Mol. Struct. 891 (2008) 115.
[6] S.A. Markarian, K.R. Grigoryan, Arm. Khim. Zhur. 41 (1988) 245 (Armen. J. Chem., in Russian).
[7] J.F. Casteel, P.G. Sears, J. Chem. Eng. Data 19 (1974) 196.
[8] A.M. Smondyrev, M.L. Berkowitz, Biophys. J. 76 (1999) 2472.
[9] W.N. Martens, R.L. Frost, J. Kristol, J.T. Kloprogge, J. Raman Spectrosc. 33 (2) (2002) 84.
[10] S.A. Markarian, L.A. Gabrielian, S. Bonora, C. Fagnano, Spectrochim. Acta A 59 (2003) 575.
[11] T.J. Anchordoguy, J.F. Carpenter, J.H. Crowe, L.M. Crowe, Biochim. Biophys. Acta 1104 (1992) 117.
[12] T. Arakawa, J.F. Carpenter, Y.A. Kita, J.H. Crowe, Cryobiology 27 (1990) 401.
[13] T. Arakawa, Y.A. Kita, S.N. Timasheff, Biophys. Chem. 131 (2007) 62.
[14] T.J. Anchordoguy, C.A. Cecchini, J.H. Crowe, L.M. Crowe, Cryobiology 28 (1991) 467.
[15] S. Bonora, S.A. Markarian, A. Trinchero, K.R. Grigorian, Termochim. Acta 433 (2005) 19.
[16] S. Bonora, A. Torreggiani, G. Fini, Thermochim. Acta 408 (2003) 55.
[17] R.L. Biltonen, D. Lichtemberg, Chem. Phys. Lipids 64 (1993) 129.
[18] B.P. Gaber, W.L. Peticolas, Biochim. Biophys. Acta 465 (1977) 260.
[19] B. Tenchov, Chem. Phys. Lipids 57 (1991) 165.
[20] D. Marsch, Handbook of Lipids Bilayers, CRC Press, Boca Raton, FL, 1990. p. 135.
[21] F. Parker, Applications of Infrared, Raman and Resonance Raman Spectroscopy in Biochemistry, Plenum Press, New York, 1983. p. 436.
[22] W.W. Van Osdol, Q. Ye, M.L. Johnson, R.L. Biltonen, Biophys. J. 63 (1992) 1011.
[23] K. Jorgensen, J.H. Ipsen, O.G. Mouritsen, D. Bennett, M.J. Zuckermann, Biochim. Biophys. Acta 1062 (1991) 227.
[24] O.G. Mouritsen, M.J. Zuckermann, Eur. Biophys. J. 12 (1985) 75.
[25] Y.A. Ermakov, A.Z. Averbakh, A.B. Arbuzova, S.I. Sukharev, Membr. Cell. Biol. 12 (1998) 411.
[26] G.L. Jendrasiak, R. Smith, A.A. Ribeiro, Biochim. Biophys. Acta 1145 (1993) 25.
[27] A. Bertoluzza, S. Bonora, G. Fini, M.A. Morelli, Life Chem. Rep. 9 (1991) 269.
[28] A. Bertoluzza, S. Bonora, G. Fini, M.A. Morelli, J. Raman Spectrosc. 19 (1988) 369.
[29] K. Kinoshita, M. Yamazaki, S.J. Li, Eur. Biophys. J. 30 (2001) 207.
[30] S. Tristram-Nagle, T. Moore, H.I. Petrache, J.F. Nagle, Biochim. Biophys. Acta 1369 (1998) 19.
[31] I.I. Vaisman, M.L. Berkowitz, J. Am. Chem. Soc. 114 (1992) 7889.
[32] P.J. Quinn, Cryobiology 22 (1985) 128.