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DSC and Raman study on the interaction between polychlorinated biphenyls (PCB) and phospholipid liposomes

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Abstract

Polychlorinated biphenyls (PCB) are very toxic lipophilic substances widely used in the past as non flammable dielectric fluids. PCB accumulation in the environment appears to be a great risk for human health. Dipalmitoylphosphatidylcholine (DPPC) and dimyristoylphosphatidylethanolamine (DMPE) liposomes with increasing amounts of Aroclor 1254 have been studied by DSC and Raman spectroscopy.

Noticeable changes take place in thermograms and Raman spectra even in the presence of small amounts of Aroclor, suggesting the existence of strong interactions due to the insertion of the PCB molecules into the hydrophobic core of liposomes.

In DPPC liposomes, a 'solution like' system is observed and the main effects are the decrease of both the melting temperature and ΔH of transition, with a simultaneous increase in the half width and asymmetry of the peak.

On the contrary, in DMPE liposomes, a complex structure of the thermograms, which comes from the coexistence of different phases, is observed in most of the analysed systems.

The behaviour is explained on the basis of a different penetration depth into the bilayer due to the polar interactions involving the polar head of the lipid.

The existence of an interdigitate phase in DMPE–PCB liposomes is also evident in the experimental results.

Keywords: Phospholipids; Polychlorinated biphenyls; Phase transition; DSC; Raman

1. Introduction

Polychlorinated biphenyls (PCBs) are synthetic organic compounds made by the chlorination of biphenyl. Because of their lack of flammability, the heat stability and the electrical insulating properties, PCBs were widely used as dielectric fluids in transformers and electrical capacitors as well as heat transmitting fluids.

As a result of their stability and low biodegradation, PCBs were accumulated in the environment, also entering in the food chain. Actually, their use is banned since they are dangerous to humans, but in the past a great amount of these compounds were released into the environment. Moreover, small amounts of PCBs arise also from the incineration of municipal and industrial wastes [1].

As a consequence of the non-selectivity of the aromatic chlorinating reaction, PCBs used for industrial purposes were complex mixtures of isomers, characterised by the average percent of chlorine or by the number of chlorine atoms present. For example, the

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Table 1
Some commercial, widely used, polychlorinated biphenyls

Commercial name	% Cl	Typical formula
Aroclor 1242	42	$C_{12}H_6Cl_4$
Aroclor 1254	54	$C_{12}H_4Cl_6$
Aroclor 1260	60	$C_{12}H_3Cl_7$

Aroclor 1254, used in this work as representative PCB, is a mixture of isomers with a percentage of chlorine of 54% and an average number of chlorine atoms per molecule equal to 6. Table 1 collects the most widely used Aroclor with their characteristics.

PCBs are lipophilic–hydrophobic substances and accumulate in both animal and human muscle fat, although they can also be found in the brain, liver and lung. Measurements of PCB concentration in animal and human samples have shown that, with the exception of fish, brain tissues are better protected against their accumulation than liver or muscle tissues, probably as a consequence of the blood–brain barrier, that is considerably less efficient in fish than in mammals [2–5].

All PCBs are toxic compounds and the more toxic appear to be the symmetric coplanar 'dioxin-like' isomers [2]. No peculiarities were found in regard to the tissue distribution of the coplanar PCBs over other congeners. There is clear evidence of carcinogenicity in several widely used products, including Aroclor 1254 and 1260. Lighter PCBs, like Aroclor 1242, seem to play a role on developing lung injuries [2].

Like other lipophilic substances, both PCBs and their metabolites could interact with biomembranes and the modifications induced in the structure of membranes could play a role in the transport of PCBs to the target tissues and in the development of their toxicity. Indeed, PCBs have been observed to bind the human erythrocyte membrane, causing haemolysis [6].

Preliminary Raman measurements suggested that Aroclor 1254 could interact with the apolar region of dipalmitoylphosphatidylcholine (DPPC) liposomes [7].

In this paper, we studied the interactions between Aroclor 1254 and multilamellar vesicles (liposomes) of DPPC and dimyristoylphosphatidylethanolamine (DMPE) using differential scanning calorimetry (DSC) and Raman spectroscopy.

The Aroclor 1254 was chosen as a representative PCB since it was probably the compound most widely used as dielectric fluid and many coplanar symmetric 'dioxin like' isomers are present in its composition, thus explaining the great toxicity.

DPPC liposomes are widely used as a model system of biomembranes because lecithins are the major component of most mammalian biomembranes, and their thermal transition occurs close to physiological temperature; DMPE liposomes are a useful model for nervous tissue cell membranes because cephalins are present in this type of tissue in a significant amount.

Both DSC and Raman spectroscopy have been proved to be very useful techniques in studying changes induced in model biomembranes by foreign substances. Indeed, liposomes exhibit 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 reflecting changes induced in the bilayer structure [8–10].

Moreover, by plotting the intensities of some characteristic Raman bands as a temperature function, sharp changes are observed in correspondence to phase-change temperatures, related to the structural changes in the liposome structure and particularly in their inner hydrophobic core [11–13].

By combining DSC and Raman data, the type of interaction, the depth of the penetration in the bilayer, as well as the conformational changes in the hydrophobic chain structure can be investigated.

Liposomes have been extensively studied by these techniques, both in the presence of penetrating substances like cholesterol, anaesthetic and other lipophilic bioactive substances [8,14,15], and in the presence of non-penetrating molecules that mainly interact with the external hydrophilic surface of the bilayer, such as polyamines, ions and some classes of pesticides [16–18].

2. Materials and methods

Synthetic DL-DPPC and DL-DMPE were obtained from Sigma Chemical Co. (St. Louis, MO) with purity guaranteed greater than 99% (TLC) and thus used without further purification. Aroclor 1254 is a Supelco environmental standard neat product. Twice distilled

water, high purity 'pesticide analysis grade' chloroform and 'ACS reagent grade' Merck products were also used.

Samples were prepared according to [8,19], by mixing the appropriate amount of Aroclor and lipids in $CHCl_3$ solutions, and the subsequent removal of the solvent under nitrogen stream and then under vacuum.

We observed that the solubility of DMPC in $CHCl_3$ is greater than that of DMPE, confirming the higher polarity of ethanolamine residue compared to choline residue.

A NaCl 0.9% (w/w) solution buffered at pH = 7.0 with phosphate buffer (about 10^{-3} M) was added to the PCB–lipid mixture, to obtain a final lipid concentration of about 20% (w/w). Homogeneous gelatinous samples were obtained by gentle sonication (Vibra-cell from Sonics Materials; 3 min at 0.5 W of power) and then stored at −18 °C. Samples with Aroclor content ranging from 0.25% (w/w) to 30% (w/w) with respect to the DPPC and DMPE amount were examined.

DSC measurements were performed using a Mettler-Toledo DSC 821. A heating rate of 2.0 °C/min in the 25–50 °C range for DPPC and in the 30–60 °C range for DMPE liposomes was used. Temperature and enthalpy scales were calibrated with indium and capric acid samples. Thermal cycles were repeated at least four times to ensure constancy and good reproducibility of the data; the expected experimental errors in temperature and ΔH values were ±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 was weighed. After dissolving the dry material in $CHCl_3$, the amount of PCB was tested by GC–MS technique.

Raman spectra were recorded with a Jasco R-1100 Raman spectrometer equipped with an Ar^+ ion laser (typical laser power on the sample: 100 mW at λ = 448 nm). A variable temperature thermostatic Jasco cell holder with a ±1 °C accuracy was used to perform the spectra in the considered temperature ranges that were the same as in the DSC measurements. Raman intensities were measured as peak height [20].

3. Results and discussion

The thermal behaviour of pure DPPC and DMPE liposomes is well known. DPPC exhibit two endothermic transitions in the 25–50 °C temperature range: a broad pretransition at about 35 °C, with a low enthalpy change and a major, sharp transition at about 41.5 °C. On the contrary, DMPE exhibit only a main sharp transition near to 49.5 °C in the 30–60 °C thermal range. The shape of the DSC plot of the main transition peak in both lipids is only slightly asymmetrical, skewed slightly toward lower temperatures. In pure DPPC liposomes, the values we found for the main transition temperature (T_m), enthalpy of transition (ΔH) and half width of the peak ($\Delta T_{1/2}$) were 41.7 °C, 35.9 kJ m^{-1}, and 0.5 °C, respectively, whereas in DMPE liposomes the corresponding values were 49.9 °C, 27.8 kJ m^{-1} and 0.7 °C, in good agreement with [8,21,22]. In pure DPPC liposomes, we also observed a weak pretransition peak with maximum at 35.2 °C (T_{pr}).

The I_{2880}/I_{2850}, I_{2930}/I_{2850} and I_{1130}/I_{1090} Raman intensity ratios and their plot as a function of the temperature in the considered thermal ranges, were used to study intrachain changes and lateral interactions of acylic chains. Sharp changes in the previously defined intensity ratios were observed in nearly all the considered samples in the correspondence of the main transition temperature. The order parameter S_T and S_L were calculated according to [11]. According to [11], S_T refers to the relative intensity of the 1130 cm^{-1} Raman band that is related to the average number of 'trans' bond in the acylic chain and gives a measure of the order due to intrachain structure. On the contrary, S_L refers to the relative intensity of the 2880 cm^{-1} Raman component that is related to the vibrational coupling between the adjacent chains, and gives a semi-quantitative measurement of the lateral interactions between the lipidic chains. The parameters are normalised so that $S = 1$ indicates the highest possible order and $S = 0$ no order (not necessarily the lowest possible) [11].

The main transition arises from the conversion of the P_β gel phase to a lamellar liquid-crystal L_α phase. It is sharp and strong, and it exhibits very good reproducibility.

The origin of the pretransition observed in pure DPPC liposomes is not very well established; it has been supposed to arise from the conversion of a lamellar gel phase (L_β) to a rippled gel phase (P_β).

It is well know that pretransition is very sensitive to the presence of foreign substances, especially if they penetrate into the bilayer hydrophobic core, and they

can disappear in the presence of very small amounts (≤1%, w/w) of foreign substances [8].

To study the asymmetry changes of the thermal peaks, an asymmetry index (A_s) was used according to [8,9]. As reported, A_s values are significant only in the presence of a single transition peak, even if asymmetric [8].

3.1. DPPC–PCB liposomes

In the presence of PCB, the thermograms of DPPC liposomes noticeably changed, showing disappearance of the pretransition, decrease in T_m, increase of $\Delta T_{1/2}$ and strong increase in the skewing of the peak with consequent A_s changes.

Table 2 summarises the values of T_m, ΔH, $\Delta T_{1/2}$ and A_s, measured in all the DPPC–Aroclor systems in the heating and cooling cycles. DPPC–PCB % (w/w) ranges from 0.25% to 30% and the corresponding molar ratios range approximately from 6×10^{-3} to 7×10^{-1} by considering an average molar weight of 326.4 for Aroclor 1254 [3].

Fig. 1 shows the heating curves of multilamellar vesicles (liposomes) in pure DPPC, and in some DPPC–PCB mixtures with a different Aroclor content.

Fig. 1 shows that the pretransition is very sensitive to the presence of Aroclor, disappearing as the PCB content is ≥1% (w/w). In the 0.25 and 0.5% (w/w) samples, the pretransition is still detectable, even if broadened and of a reduced intensity, making it difficult to determine the true peak value of the pretransition temperature, particularly when the 0.5% (w/w) sample is considered. However, T_{pr} appears to be lowered by ∼0.6 °C in the 0.25% (w/w) sample and of ∼1.0 °C in the 0.5% (w/w) DPPC–PCB liposomes.

The main transition temperature T_m noticeably decreases in the presence of PCB, and a rough linear correlation can be obtained by plotting T_m values as a function of the Aroclor content up to 15% (w/w) liposomes. Further PCB addition causes only small T_m decrease and a ‘saturation like’ situation is reached.

A contemporary increase in the half width $\Delta T_{1/2}$ of the main transition is observed from 0.5 °C in pure DPPC liposomes up to about 2.5 °C in the presence of 10% (w/w) of PCB. In addition, the peak becomes more asymmetric, with skewing toward lower temperatures. The A_s increase is strong in the presence of low Aroclor content, indeed it reaches a value of about 3.0 in the presence of only 2% (w/w) of added PCB. Further PCB addition induces only small A_s increases, that is ∼3.9 in the 10% (w/w) Aroclor containing liposomes.

In the more concentrated liposomes (30%, w/w, and to a lesser extent, also in 15%, w/w) the structure of the thermogram seems to become more complex, suggesting the presence of a weaker secondary peak, that, in any case, is poorly evidenced even in the 30% (w/w) liposomes. This fact suggests that in the presence of a high PCB content, another phase is present at lower level, and some phase segregation takes place.

In the PCB presence, a noticeable decrease in ΔH of the main transition is observed; in the presence of 10% (w/w) of Aroclor, ΔH is halved with respect to that measured in pure DPPC liposomes. A further

Table 2
Temperature of the maximum of the main calorimetric peak in heating and cooling process (T_m and T_{mc}), half width ($\Delta T_{1/2}$), enthalpy (ΔH) and asymmetry index (A_s) of the transition observed in pure DPPC and in the considered DPPC–PCB liposomes

DPPC–PCB (%, w/w)	T_m (°C)	$\Delta T_{1/2}$ (°C)	ΔH (kJ m^{-1})	A_s	T_{mc} (cooling) (°C)	$\Delta T_{1/2}$ (cooling) (°C)
0	41.7	0.5	35.9	1.0	41.2	0.5
0.25	41.6	0.6	35.0	1.6	41.1	0.5
0.5	41.5	0.7	33.8	1.9	41.0	0.6
1.0	41.4	0.8	32.4	2.4	41.0	0.7
2.0	41.1	1.0	30.9	2.7	40.6	0.9
3.0	40.6	1.3	30.0	3.2	40.1	1.2
5.0	39.8	1.8	28.1	3.4	39.3	1.6
7.5	39.0	2.0	25.4	3.7	38.4	1.7
10.0	37.9	2.5	18.9	3.9	37.3	2.2
15.0	36.3	3.5	16.0	–	35.8	3.1
30.0	35.9	–	13.6	–	35.5	–

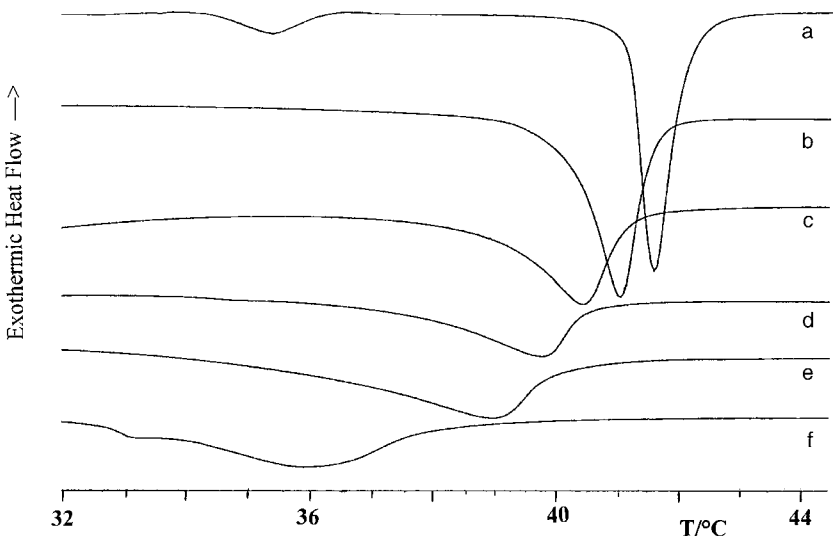

Fig. 1. DSC thermal response of hydrated multilamellar vesicles of DPPC–PCB mixtures with different PCB content (a: 0%; b: 1.0%; c: 3.0%; d: 5.0%; e: 7.5%; and f: 30%), on heating.

ΔH decrease is also observed at the highest Aroclor content, but the suggested presence of more than one calorimetric peak yields the measured ΔH values less significant. Indeed, the contemporary presence of an amount of the lipid in a different phase, that also gives rise to an endothermic transition, but with a different ΔH value, could explain the very low ΔH values measured in these two samples.

The calorimetric plots measured in the cooling process confirm the results obtained by heating, showing a similar trend. A slight and constant decrease in T_m of $\sim$0.5 °C in the cooling cycles was observed in all the samples, due to the finite response time of the calorimeter, whereas the $\Delta T_{1/2}$ and A_s values were generally 10–15% lower than the corresponding values in the heating process. On the contrary, similar ΔH values were measured both in the heating and cooling processes within the expected experimental errors.

Table 4 reports S_T and S_L order parameter deduced from Raman spectra in the gel phase (20 °C), and in the liquid crystal phase (50 °C) for some significant PCB contents.

Table 4 shows that the addition of Aroclor causes a noticeable decrease in both S_T and S_L in the gel phase that is roughly linearly related to the PCB content in the 0–15% (w/w) concentration range. Indeed, S_T and S_L decrease from 0.87 to 0.48 and from 0.42 to 0.29, respectively, as the PCB content increases from 0% (w/w) to 15% (w/w). On the contrary, the order parameters are poorly affected by the PCB content (both S_T and S_L do not change significantly) in the liquid crystal phase.

The disappearance of the pretransition even in the presence of small PCB content, confirms its marked sensitivity to the presence of foreign substances, as already observed in many systems characterised by penetration in some extent into the bilayer [8–10]. As a consequence of this insertion, the overall bilayer is forced in only one preferential conformation, probably the gel rippled phase [19].

Both DSC and Raman data suggest that the cooperativity of the main transition is strongly reduced by Aroclor addition, as deduced by the increase in $\Delta T_{1/2}$ value and by the behaviour of the plots of S_T and S_L as a function of the temperature.

In addition, Raman data show that the PCB presence is equally effective in reducing both S_T and S_L order parameter in the gel phase, suggesting a decrease in the lateral interaction between the acilyc chains which causes a more disordered structure [7].

The T_m decrease and the contemporary increase in the gauche to trans conformer ratio, as well as the decrease of the lateral interaction between acilyc chains in the gel phase indicate that Aroclor molecules deeply

penetrate into the bilayer hydrophobic core. Moreover, the experimental data on T_m and ΔH suggest that Van der Waals interactions between PCB and DPPC molecules are weaker than those between the acylic chains in pure DPPC liposomes.

The structure of phospholipids bilayer has been the subject of many theoretical and experimental works and some models have been proposed to explain experimental data in pure (mainly DPPC) liposomes and in the presence of foreign substances [23–25]. It has been shown that in the presence of small, lipophilic substances, like short chain alcohol's, a simple solution model, assuming that during the melting or freezing processes the foreign substances distributes itself between the gel and liquid crystal phase, agrees well with the experimental data [23]. According to this model, both a decrease in the melting temperature and a contemporary increase in the half width of the transition peak are to be expected in a linear correlation with the concentration, as a consequence of the introduction of 'free volumes' into the structure of the bilayer. In addition, it has been observed, that the 'solution model' fails in the presence of medium or high molecular weight molecules, particularly if they are linear long chain molecules, without branching. The failure may arise from the rising of relatively strong Van der Waals interaction between the lipid acyl chains and the long, linear chains of the foreign substances [26].

Our experimental data agree well with the solution model, at least up to PCB concentration of about 10% (w/w) or little more, as confirmed from the linear trend in the T_m decrease and $\Delta T_{1/2}$ increase.

Despite their relatively high molecular weight, the hydrophobic PCB molecules deeply penetrate into the hydrophobic core and, as a consequence of its relative small size, they are able to upset only partially the overall thickness of the bilayer, thus explaining the observed behaviour.

Also ΔH decrease is consistent with the previously described model and confirms a deep penetration of PCB molecules in the bilayer, without any interaction with the polar groups of the lipid. Indeed, it has been observed that if such an interaction takes place, as in the presence of hydrophobic molecules with polar centres, ΔH does not decrease and also some increases were observed [9].

As stated before, the effect on A_s is also noticeable and a remarkable increase is observed even after addition of small amounts of Aroclor (Table 2). The increase in the A_s value in the main transition peak, indicates lack of symmetry with contemporary skewing toward lower temperatures, adding further evidence to the hypothesis of a deep penetration in the bilayer. Similar behaviour was previously observed in DPPC liposomes in the presence of penetrating lipophilic substances, like some plasticisers and explained on the basis of the cluster model of the lipidic bilayer [15,24,27].

According to this model, the melting process arises from the cooperative and contemporary change of phase of all the molecules within each domain, or cluster, in which the liposome can be subdivided. More homogenous and of comparable size is the cluster, greater is the cooperativity of the melting process and consequently sharp and symmetric the thermogram. In the presence of foreign substances able to penetrate into the bilayer giving a 'solution like' system, a modification in the shape and the size distribution of the clusters takes place and their number increases noticeably. At the same time, the clusters become smaller and their surface more ramified, as deduced from theoretical studies [27]. Consequently, the cooperativity of the melting process decreases and the thermograms broaden noticeably.

Moreover, the foreign substance distribution into the cluster is not homogeneous, tending to be localised preferentially near to the boundary surface thus producing concentration gradients. The observed skewing arises from these concentration gradients as well as from the spreading in the cluster sizes.

In the cooling process, the peak appears to be more symmetric and less skewed, as denoted by the A_s and $\Delta T_{1/2}$ values slightly smaller than those in the heating cycles. This behaviour is probably due to the higher lateral mobility of the chains in the liquid crystal rather than in the gel phase, giving thus smaller concentration gradients and, consequently, shorter equilibration times.

Only in the more concentrated liposomes (particularly in the presence of 30%, w/w, of PCB) the shape of the peak becomes more complex and the presence of more than one calorimetric peak is observed suggesting the coexistence of the aggregates with different structures. Nevertheless, the new peak has a weak intensity.

However, the ability of PCB to promote phase transition in DPPC liposomes seems to be very small; indeed the presence of a second peak in the thermogram is clearly suggested only in the 30% (w/w) PCB containing liposomes, corresponding to a molar ratio of about 1:1 (Fig. 1).

3.2. DMPE–PCB liposomes

Table 3 collects the values of T_m, ΔH, $\Delta T_{1/2}$ and A_s, measured in heating cycles, in all the considered DMPE–Aroclor systems. T_{mc} and $\Delta T_{1/2}$ measured in cooling cycles are also reported. DMPE–PCB % (w/w) ranges from 0.25% to 30% and the corresponding molar ratios range approximately from 5×10^{-3} to 6×10^{-1}.

Fig. 2 shows the heating curves of liposomes of pure DMPE and DMPE–PCB mixed systems with a different Aroclor content.

The thermal behaviour of DMPE liposomes in Aroclor presence is quite different compared to that observed in DPPC liposomes. In the liposomes with a low Aroclor content (<1.0%, w/w) only a weak, roughly linear T_m decrease is observed, whereas, at the same time a small increase of the half width of the transition takes place.

When Aroclor content is ≥1.0% (w/w) the presence of a new transition peak exhibiting its maximum at 47.0 °C is well evident and traces of them can also be detected in the 0.5% liposomes.

When the PCB content ranges from 1% (w/w) to 7.5% (w/w) both the new, lower temperature transition, and the higher temperature transition are observed. By increasing PCB content, the higher temperature transition decreases in intensity and its maximum T_m shifts toward lower temperatures (i.e. it is at about 48.1 °C in the 7.5%, w/w, samples), even if to a lesser extent, compared to the corresponding DPPC–PCB systems. At the same time, the lower temperature transition increases its intensity, but its T'_m temperature is constant up to a PCB content of 10% (w/w), when the peak of the lower temperature transition is the only one detectable. The two transitions exhibit about the same intensity when the PCB content is 3% (w/w).

As PCB content is greater than 10% (w/w) only the low temperature transition is observed, whose T'_m shifts to a slightly lower temperature when more Aroclor is added; on the contrary, $\Delta T_{1/2}$ does not increase or increases slightly.

This behaviour suggests that a phase segregation to a new well defined and structured 'phase II', takes place even in the presence on only a very little amount of PCB. Indeed, only when the PCB content is <0.5% (w/w), the behaviour is similar to that observed in the corresponding DPPC–Aroclor system (although the T_m decrease and the peak shape modifications are less evident) and Aroclor seems to 'dissolve' effectively in the liposomes. In the presence of a high Aroclor content, the 'solution like' model fails and the liposomes could be described as a mixture of 'phase II' domains inserted within a structure of smaller and further ramified 'phase I' domains.

The changes in size and shape of the residual 'phase I' domains could explain the small decrease in T_m,

Table 3
Temperature of the maximum of the main calorimetric peaks in heating and cooling process (T_m, T'_m and T_{mc}), half width ($\Delta T_{1/2}$), enthalpy (ΔH) and asymmetry index (A_s) of the transition observed in pure DMPE and in the considered DMPE–PCB liposomes

DMPE–PCB (%, w/w)	$T_m - T'_m$ (°C)	$\Delta T_{1/2}$ (°C)	ΔH (kJ m^{-1})	A_s	T_{mc} (cooling) (°C)	$\Delta T_{1/2}$ (cooling) (°C)
0	49.9	0.7	27.8	1.0	49.4	0.8
0.25	49.8	0.8	27.6	1.2	49.3	0.9
0.5	49.6	0.8	27.7	1.3	49.2	1.1
1.0	49.7–46.9	0.9	27.9	–	49.1–45.7	1.2
2.0	49.1–46.8	–	28.5	–	48.5–45.5	–
3.0	48.9–46.7	–	28.7	–	48.0–45.3	–
5.0	48.4–46.8	–	29.5	–	47.8–45.5	–
7.5	48.1–46.9	–	30.2	–	47.5–45.4	–
10.0	46.7	0.9	31.6	1.7	45.4	1.4
15.0	46.6	0.9	30.5	1.8	45.4–44.8	–
30.0	46.5	1.0	27.8	1.7	45.7–44.7	–

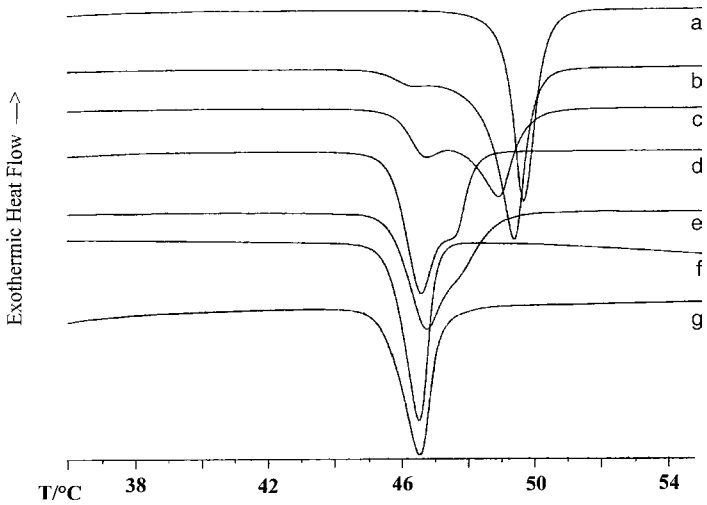

Fig. 2. DSC thermal response of hydrated multilamellar vesicles of DMPE–PCB mixtures with different PCB content (a: 0%; b: 1.0%; c: 3.0%; d: 5.0%; e: 7.5%; f: 10.0%; and g: 30%), on heating.

and justify also that they are smaller than those observed in the corresponding DPPC–PCB liposomes, for which the 'solution like' model well agrees with the experimental results. The observed constancy of T'_m temperature up to a 10% (w/w) PCB content could be explained by supposing that at intermediate concentrations the 'phase II' domains increases only in number, but not in size or shape. When the Aroclor content is >10% (w/w) both T'_m and $\Delta T_{1/2}$ are affected only to a small extent, therefore we deduced that the 'solution like' model is not applicable for Aroclor dispersion in the 'phase II' liposomes, whose structure could be depicted as a dispersion of 'drops' or 'clots' of PCB within (or between) phase II domains. The presence of these PCB 'clots' could induce some distortion in size and shape in the existing 'phase II' domains thus explaining the very small changes observed in both T'_m and $\Delta T_{1/2}$.

Also the A_s and ΔH data are well consistent with the previously depicted model. Indeed, small A_s increases were measured in the presence of small amounts of Aroclor, although smaller than those measured in the corresponding DPPC liposomes, confirming that PCBs are able to dissolve themselves only slightly in DMPE liposomes. It should be noted that detectable A_s increases were already observed in systems in which $\Delta T_{1/2}$ seems not to increase within the experimental error, confirming that the asymmetry of the calorimetric peak is a more sensitive parameter than the half width in the presence of very small amounts of foreign substances.

The asymmetry index of the 'phase II' liposomes does not increase, supporting the previously described model in the presence of high PCB content.

Although the effects on ΔH are light, their trend is significant and further supports our model. Indeed, in the presence of small PCB amounts ΔH is constant or decreases slightly suggesting thus a very low solubility of the Aroclor in the 'phase I' liposomes. A further PCB addition up to 10% (w/w) induces a ΔH increase, that reaches its maximum value when only the 'phase II' liposomes are present. In the presence of a very high PCB content (30%, w/w) a small decrease of ∼10% is observed in ΔH. This fact could be due to a weakening of the lateral interchains interactions as a consequence of the insertion of the PCB 'drops' between the domains as well as to a little modification in the shape or size of the domains itself.

In Table 4, S_T and S_L order parameter in the gel phase (30 °C) and in the liquid crystal phase (55 °C) for some significant PCB contents are reported.

Raman data confirm the DSC suggestions. The observed decrease in the S_T and S_L values are smaller than those observed in the corresponding DPPC liposomes (Table 4). Indeed, S_T decreases of ∼17% and S_L of ∼10% in the 15% DMPC liposomes, whereas

Table 4
Order parameters as deduced from Raman spectra for some different DPPC–PCB and DMPE–PCB systems

DPPC–PCB (%, w/w)	S_T (20 °C)	S_L (20 °C)	S_T (50 °C)	S_L (50 °C)
0	0.87	0.42	0.31	0.14
1	0.81	0.40	0.29	0.14
5	0.60	0.34	0.29	0.12
10	0.54	0.31	0.30	0.13
15	0.48	0.29	0.28	0.13
DMPE–PCB (%, w/w)	S_T (20 °C)	S_L (30 °C)	S_T (55 °C)	S_L (55 °C)
0	0.75	0.40	0.28	0.12
1	0.73	0.40	0.27	0.12
5	0.70	0.38	0.29	0.13
10	0.64	0.38	0.30	0.12
15	0.62	0.36	0.29	0.13

the corresponding decreases in the DPPC liposomes are ∼45% and ∼31%, respectively. Also, the order parameter plots as a function of the temperature exhibit changes in the correspondence of the transition temperature that agrees with calorimetric data. Indeed, the transition is sharp in the presence of small amounts of PCB (1%) suggesting the existence of only one transition; broad at intermediates PCB content, in agreement with the coexistence of two phase transition; again sharp and a little shifted to lower temperature at high PCB content (10 and 15%), as only 'phase II' liposomes are present. The main transition temperature measured from Raman plots are consistent with those arising from DSC measurements within the limits of the experimental error.

4. Conclusions

The noticeable changes observed in both DPPC and DMPE liposomes when PCB is present, denote that Aroclor can strongly interact with the model membrane phospholipids and the interaction is dependent on the nature of the lipid.

In DPPC liposomes, a deep penetration of the Aroclor molecules into the hydrophobic core of the bilayer takes place. The interactions are mainly hydrophobic involving only the lipidic chains in the bilayer. On the contrary, the interactions between the polar C–Cl bonds of PCB and the polar head of the lipid are not present or play a secondary role.

The 'solution like' model agrees very well with the experimental results confirming that the hydrophobic interactions and the 'free volume' induction in the bilayer play the main role to explain the behaviour of DPPC–PCB liposomes.

On the contrary, the DMPE–PCB systems show a totally different behaviour that can not be explained on the basis of the small decrease in the acylic chain length (from C_{16} to C_{14} in DPPC and DMPE liposomes, respectively) with the consequent small thinning of the hydrophobic region, indeed PCB molecules, despite a relatively high molar weight, are relatively small in size and compact molecules. We assume that the key role is played by the differences in the polar head structure (charge distribution and size) of the two lipids, that in DMPE lead to a more localised charge distribution in the ethanolamine residue, as it is confirmed from the low solubility in solvents like chloroform. Consequently, in DMPE–Aroclor liposomes, we suggest that dipole–dipole electrostatic interactions involving the polar C–Cl bonds in PCB and the polar head of the lipid play a significant role. As a consequence, Aroclor molecules are forced not to penetrate deeply into the bilayer and are confined in a region close to the polar surface, thus perturbing the inner core of the lipid to a lesser extent and explaining the smaller changes observed in the thermal parameters.

From the experimental data, some suggestions on the possible structure of 'phase II' liposomes can also be deduced.

The existence of other stable and metastable phases in addition to the gel and liquid crystal phases in some cephalins is well known [28]. In a recent work on DMPE liposomes, a complex structure of the thermograms in the cooling cycles has been observed and attributed to a liquid crystal to subgel phase transition [28]. However, the new transition was detected only at very low cooling rates (0.1–0.2 °C min^{-1}), and disappeared completely when the cooling rate was greater than 1.0 °C min^{-1}. Consequently, it seems reasonable to exclude any role of a subgel metastable phase to explain the complex peaks structures by us observed in most DMPE–PCB liposomes. Also, the existence of a 'mixed type bilayer' phase, as observed in some DPPC–anionic detergent mixed liposomes [29], seems to be excluded. Indeed, the shape of the PCB molecule is very different with respect to the linear structure

and the amphipatic character of the considered detergents.

On the contrary, the existence of a stable interdigitated gel phase $L_{\beta I}$, as reported in tetracaine containing DPPC liposomes [30], could be in accordance with our experimental results. Indeed, by comparing the heating and cooling DSC peaks, a hysteresis of about 1.4 °C is observed, 0.9 °C greater than that expected as a consequence of the finite time-response of the calorimeter at the used heating/cooling rate and hysteresis has been commonly observed when interdigitation takes place [30,31]. It could be due to a different receptivity to PCB partitioning between L_α and $L_{\beta I}$ phases as well to a slow kinetic of the interdigitated $L_{\beta I}$ phase formation by cooling.

Interdigitation also agrees with the previously suggested localisation of the Aroclor molecules, into the hydrophobic core but near to the polar surface. Indeed, as a consequence of interdigitation between acylic chains that bend also, the potential free volume induced in the hydrophobic core by the presence of PCB near to the polar surface strongly reduces or vanishes. Consequently, the 'solution like' model fails, according to experimental results.

Raman data support also this hypothesis. Indeed, the lower relative decrease observed for S_T and S_L in the gel phase, compared to corresponding DPPC liposomes, agrees well with the existence of a more compact hole less structure, as is to be expected in an interdigitated phase, where strong lateral interaction between acilyc chains are expected. The bending of the chains to interdigitate and the consequent decrease of 'free volumes' into the bilayer oppose to the gauche to trans ratio increase thus explaining the relatively high S_T values observed in DMPE–PCB liposomes.

The experimental data suggest that the role of biomembranes in the developing of biological PCBs effects could arise not only by a 'transport' effect to the target organs and sites but also, at least partially, to their capability to modify to some extent the bilayer structure. Consequently also the function of transmembrane proteins, which are very sensitive to the microenvironment for a correct biological role, could be affected [32].

Moreover, it has been observed that the PCBs effects are closely related to the class of the interacting phospholipids. Indeed, the interaction involves mainly the hydrophobic core of the bilayer, however both size and polarity of the polar head of the phospholipids seems to play a significant role.

The observed differences could be important to explain the biological and toxicological effects on organs and tissues.

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