Molecular Crystals and Liquid Crystals
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gmcl16

Phase Transitions in the Lyotropic System Dipalmitoyl Phosphatidyl Choline (DPPC) Doped with the Antileprosy Drug, Dapsone

K. U. Deniz [a], P. S. Parvathanathan [a], E. B. Mirza [b], V. Amirthalingam [b], K. V. Muralidharan [b] & S. Gurnani [c]

[a] Nuclear Physics Division, Bhabha Atomic Research Centre, Trombay, Bombay, 400085, India

[b] Reactor Chemistry Section, Bhabha Atomic Research Centre Trombay, Bombay, 400085, India

[c] Biochemistry and Food Technology Division, Bhabha Atomic Research Centre, Trombay, Bombay, 400085, India

Version of record first published: 17 Oct 2011.

Mol. Cryst. Liq. Cryst., 1983, Vol. 98, pp. 163–173
0026-8941/83/9804–0163/$18.50/0

Printed in the United States of America

Phase Transitions in the Lyotropic System Dipalmitoyl Phosphatidyl Choline (DPPC) Doped with the Antileprosy Drug, Dapsone†

K. U. DENIZ,‡ P. S. PARVATHANATHAN,‡ E. B. MIRZA,§
V. AMIRTHALINGAM,§ K. V. MURALIDHARAN§ and S. GURNANI¶

Bhabha Atomic Research Centre, Trombay, Bombay 400085, India

(Received January 19, 1983)

We have carried out DSC investigations of the ice-water [*iw*, *wi* and (*wi*)′], chain-melting (CM) and chain ordering (CO) transitions in DPPC-water (model membrane) systems for a range of water concentrations for both pure and DDS-doped samples. The temperatures, T_{CM} and T_{CO}, are hardly affected by the inclusion of DDS, showing that DDS does not get embedded in the acyl chain layers. Increase in CM and CO transition widths indicates varied environments for the DPPC molecules in the presence of DDS. The enthalpies for the ice-water transitions point to the fact that DDS (a) reduces "free" water content of the membrane, (b) affects the population of the different bound water differently, perturbing the structure of the vicinal water. X-ray diffraction results show that the chain-melting transition which is strongly first order for the pure system is broadened by the inclusion of DDS, supporting the DSC data.

I. INTRODUCTION

Hardly any information is available on the interaction of DDS (Fig. 6b) either with proteins or with membranes. We have started a program to study the influence of DDS on model membranes. Our earlier results[1] on DPPC-H_2O systems with low water concentration show that the pretransition

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‡Nuclear Physics Division; §Reactor Chemistry Section; ¶Biochemistry and Food Technology Division

†Presented at the IX International Liquid Crystal Conference, Bangalore, December 6–10, 1982.

disappears with the inclusion of the drug in the membrane and for small drug concentration, the DDS molecules predominantly occupy sites in the interfacial region of the membrane. If this be so, DDS is likely to perturb the vicinal water in the membrane. The present work explores how DDS affects (1) vicinal water structure and (2) the chain melting transitions in (DPPC-H_2O) systems with a large range of water concentration.

It is known[2] that when heated, DPPC-water systems having the ratio of weight of water to that of DPPC, $x \leq 0.5$, show the following phase transitions:[3]

$$L_{\beta'} \underset{}{\overset{T_{PT}}{\rightleftarrows}} P_{\beta'} \underset{T_{CO}}{\overset{T_{CM}}{\rightleftarrows}} L_{\alpha}$$

CM = chain melting; CO = chain ordering; PT = pretransition. The chain melting and chain ordering transitions occur for all water concentrations used to date. However, the ordered phases preceding the L_α phase, when these systems are heated, are not known for $x \geq 1.0$.

II. EXPERIMENTAL DETAILS

DPPC and DDS were obtained from Sigma and Burrough's Welcome respectively. The sample preparation has been detailed earlier.[1] The molar ratios, R_m, of DDS to DPPC used in the present work were 0.05 and 0.30 for DSC experiments and 0.07 for the X-ray diffraction studies. The range of water concentration, x, varied from 0.5 to 2.5. Samples weighing from 8 to 12 mg were hermetically sealed in aluminium pans for use in the DSC experiments. The sealed pans were weighed before and after the scans to check for loss of water. Scans during which a weight loss (of water) of 0.2 mg or more occurred were not used in our analysis. X-ray diffraction experiments were done using Cu$K\alpha$ radiation. The sample cell was an aluminum sample holder, sealed by a very thin (<3 μm) mylar cover, mounted on a heater. The temperature stability of the sample was ± 0.5°C during the experiments. The high angle Bragg peak at 4.2 Å, corresponding to reflection due to 2-d order in the lamellae, was recorded as a function of temperature.

III. RESULTS AND DISCUSSION

A. DSC

Chain melting and Chain Ordering Transitions:

Figure 1 shows the variation with x, of the transition temperatures, $T_{CM,CO}$, and the full width at half maximum ($\Delta_{CM,CO}$) of the transition peaks.

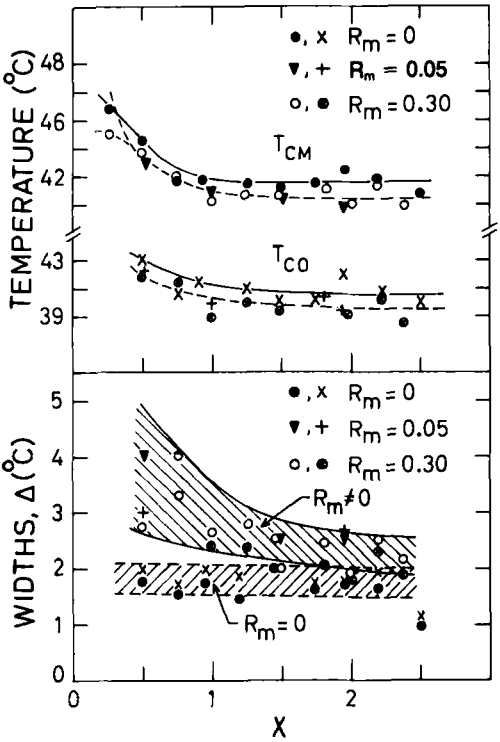


FIGURE 1 Variation of transition temperatures, T_{CM} and T_{CO} and corresponding widths, Δ_{CM} and Δ_{CO}, as a function of x. (●, ▼, ○) correspond to the chain melting transition and (x, +, ⊗) correspond to the chain ordering transition. The solid and dashed lines for $T_{CM,CO}$ are guides to the eye for the cases $R_m = 0$, and $R_m \neq 0$, respectively.

As expected, T_{CM} and T_{CO} decrease somewhat in the range $0.5 \leq x \leq 1.0$ and remain almost constant for $x > 1.0$. There is a sure but slight decrease in $T_{CM,CO}$ when the drug is added ($R_m \neq 0$) to the membrane. In the case of additives which are well embedded in the acyl chain layer, $T_{CM,CO}$ are known to decrease to a greater extent.[4] This suggests that DDS becomes only slightly embedded in the hydrocarbon (hc) chain region.

$\Delta_{CM,CO}$ are almost independent of x for $R_m = 0$. However, for $R_m \neq 0$, $\Delta_{CM,CO}$ increase with decreasing x and increasing R_m. In general, $\Delta_{CO} > \Delta_{CM}$ for $R_m = 0$ and $\Delta_{CM} > \Delta_{CO}$ for $R_m \neq 0$. The increase in width with non-zero R_m possibly points to some variations in the environment of the DPPC molecules.

The values of the transition enthalpies, $\Delta H_{CM,CO}$, obtained for different values of x and R_m, are given in Table I. These enthalpies are almost independent of both x and R_m. The values for $R_m = 0$ agree well with those quoted elsewhere.[5]

Pretransition: The scans carried out for drug-free samples show pretransition for all values of x that we have studied. However, we are not certain whether the ordered gel phases correspond to $L_{\beta'}$ and $P_{\beta'}$ for $x \geq 1.0$.

Values of T_{PT} and ΔH_{PT} are also given in Table 1. x-dependence of T_{PT} seems to follow that of T_{CM}. These values compare well with other values.[5] Pretransitions disappear even for small values of R_m. It therefore appears that pretransition is sensitively dependent on the physicochemical status of the interface.

Ice-water transitions: DSC scans of ice-water transitions (identified by their dependence on x), obtained at 10°/min are shown in Figure 2. For $R_m = 0$ and $0.5 \leq x \leq 2.5$, a sharp peak was observed at $T_{iw} = 2.3$°C while heating. While cooling, however, two distinct transitions were seen: a sharp peak at T_{wi} in the temperature region, -15°C to -21°C and a relatively broad one at $T_{wi} = -43.5$°C. For $R_m \neq 0$, the same features were observed for $x \geq 1.0$. However, for $x < 1.0$, a hump, which becomes prominent with decreasing x, appeared during the heating cycle. More than one hump was seen between the (wi) and (wi)′ transitions. The appearance of humps (Figure 2) indicates alteration in the structure of vicinal water when DDS is present.

ΔH values for the three ice-water transitions are presented as a function of x in Figure 3, for all the R_m values. In general, all three transition enthalpies increase with increasing x. ΔH_{iw} (Cal/g H_2O) (Figure 3a) tends to saturate for $x > 2.0$. Inclusion of drug decreases ΔH_{iw}. In all cases ΔH_{iw} (membrane water) $< \Delta H_{iw}$ (free or bulk water), which is 79 Cal/g. ΔH_{wi}

TABLE I

ΔH_{CM}, ΔH_{CO}, ΔH_{PT} (kCal/Mole DPPC) and T_{PT} (°C)

	x	0.50	0.75	0.98	1.25	1.50	1.78	1.98	2.20	2.49
$R_m = 0$	ΔH_{CM}	8.88	7.74	7.86	8.72	9.5	9.02	8.03	8.67	7.50
	ΔH_{CO}	8.27	7.38	6.98	8.24	9.3	8.48	8.08	8.32	7.54
$R_m = 0.05$	ΔH_{CM}	8.31	—	8.54	—	8.45	—	8.35	—	—
	ΔH_{CO}	8.31	—	8.41	—	8.33	—	8.74	—	—
$R_m = 0.30$	ΔH_{CM}	8.53	7.71	7.64	8.97	9.04	8.62	7.53	10.14	10.23
	ΔH_{CO}	8.55	7.83	7.41	8.14	8.81	8.42	7.76	10.66	9.72
$R_m = 0$	T_{PT}	39.3	35.6	34.1	34.0	34.1	34.1	34.1	34.5	33.5
$R_m = 0$	ΔH_{PT}	1.65	—	1.55	—	1.11	1.72	1.60	1.63	1.12

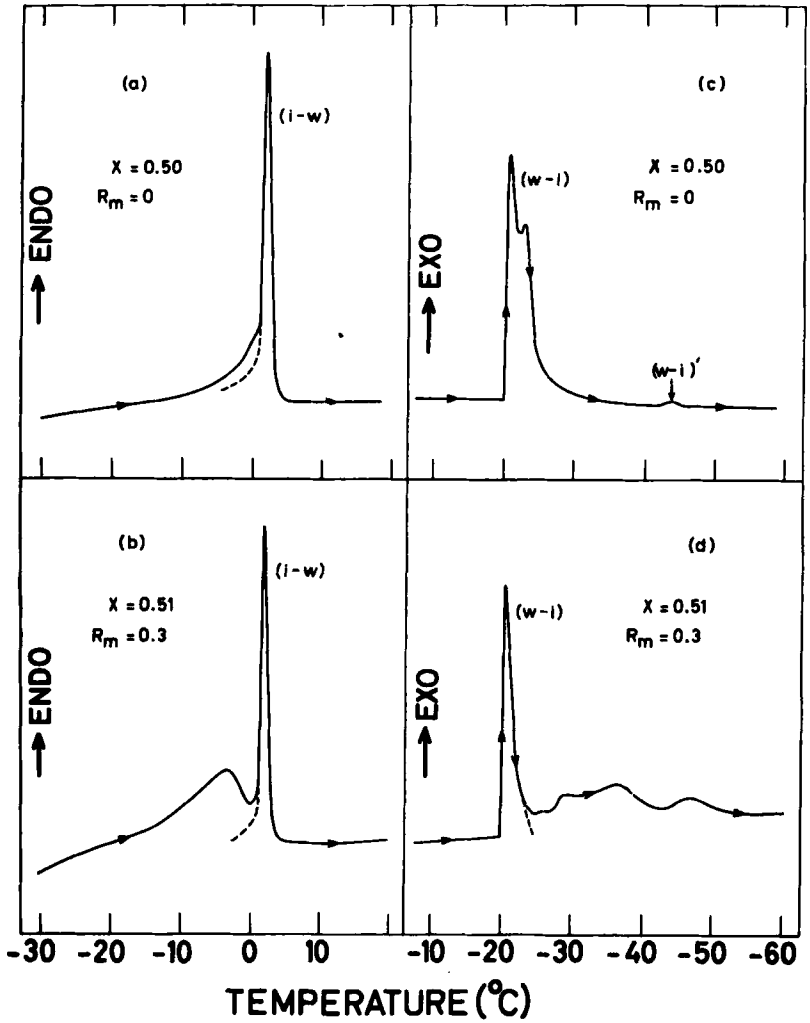


FIGURE 2 DSC scans at 10°/min for (ice-water) transitions. The dashed lines are extrapolations.

(Figure 3b) saturates for $x > 1$. Inclusion of drug somewhat increases ΔH_{wi} for $x > 1$. The effect of drug is clearly seen in the case of $\Delta H_{wi)'}$ (Figure 3c) as a marked decrease when $R_m \neq 0$, for all x. All three transitions enthalpies for $R_m = 0$, extrapolate to zero for $x(\Delta H \rightarrow 0) \approx 0.25$. This accords well with the results of Chapman[6] and others, which indicate the presence of "free" water in membranes only for $x > 0.2$. When $R_m \neq 0$, $x(\Delta H \rightarrow 0)$ seems shifted to larger values, showing that DDS decreases the

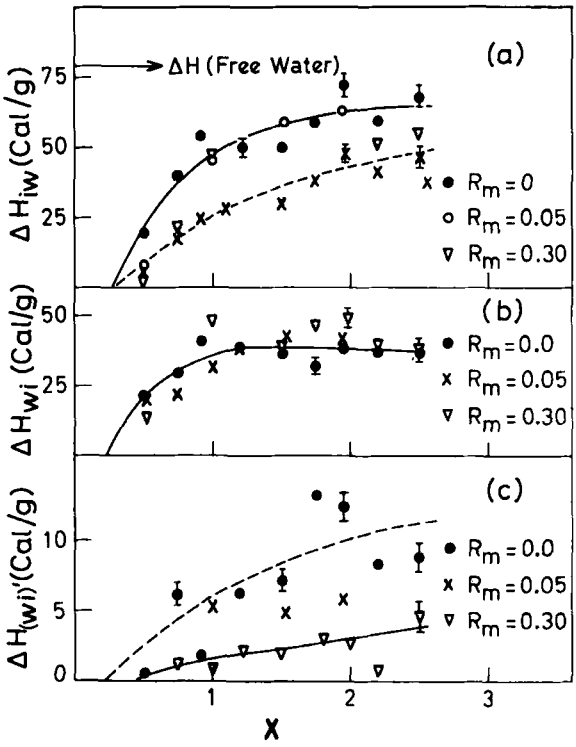


FIGURE 3 Enthalpies, ΔH, for the ice-water transitions, as a function of x. All the points give ΔH per gram of water, except the crosses in (a) which give ΔH_{iw} per gram of samples for $R_m = 0$. The lines are guides to the eye.

"free" water content in the model membrane. (wi) and $(wi)'$ correspond to transition in different types of structured water and DDS affects these types of water in different ways. These observations indicate that DDS probably competes with water for certain binding sites in the interface.

To take care of the effects of possible calibration changes, the ratio $R_1[\Delta H_{iw}(\text{Cal/g } H_2O)/\Delta H_{CM}$ (Cal/g DPPC)] was calculated and its x-dependence is shown in Figure 4. R_1 increases with increasing x, tending to saturate for large x, as expected. Doping the membrane with DDS clearly decreases R_1, supporting our preceding conclusion that "free" water does indeed decrease when DDS is added to the model membrane. R_2 $((\Delta H_{wi} + \Delta H_{(wi)'})/\Delta H_{iw})$ is also given as a function of x in Figure 4. All data points seem to fall on a single curve! R_2 decreases with increasing x, and $R_2 < 1$ for $x > 1$. $R_2 < 1$ indicates that not all the water taking part in the (iw) transition is involved in the (wi) and $(wi)'$ transitions.

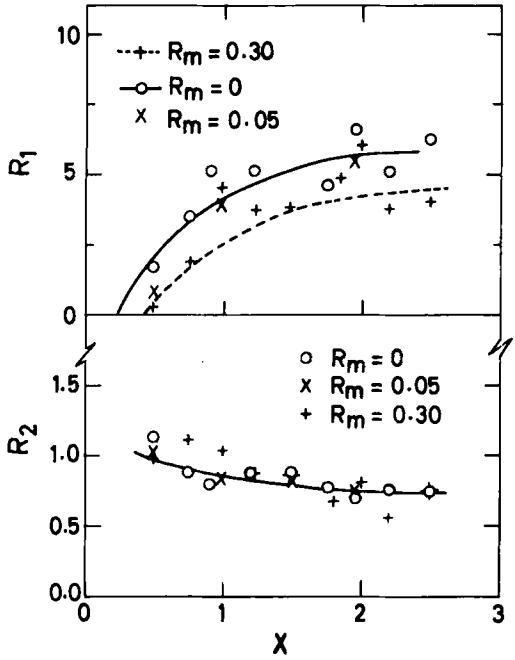


FIGURE 4 The ratios R_1 and R_2 as a function of x. Lines are guides to the eye.

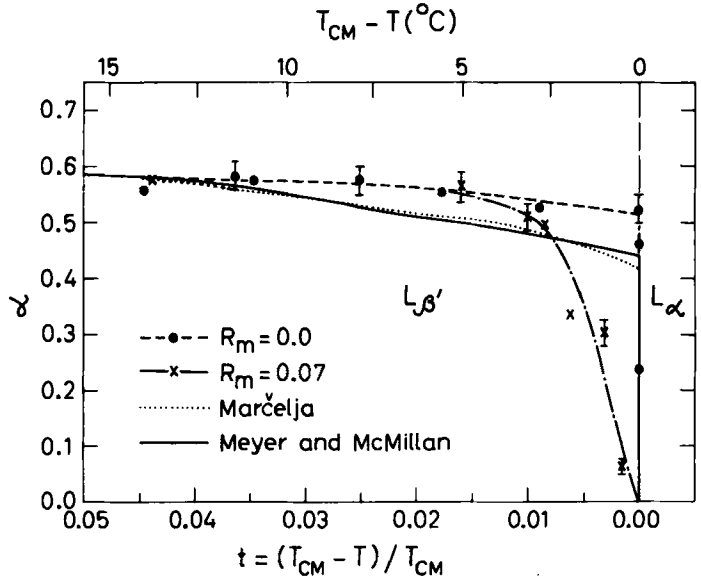


FIGURE 5 Order parameter α, as a function of the reduced temperature t. The dashed and dot-dashed lines are guides to the eye for α_{exp}.

B. X-ray diffraction

The integrated intensity of the high angle reflection at 4.2 $Å^{-1}$, $I \propto \alpha^2$, where α is the translational order parameter in the 2-d membrane lamella. The values of α (α_{exp}) obtained from I and normalized as explained below are shown in Figure 5 as a function of the reduced temperature, $t = (T_{Cm} - T)/T_{CM}$. α_{exp} shows (a) a sharp drop to zero at $t = 0$ for $R_m = 0$, while (b) for $R_m = 0.07$, α_{exp} gradually decreases to zero for $t \rightarrow 0$. This confirms our DSC result that the inclusion of drug broadens the CM transition, probably due to fluctuations in the order parameter. α_{exp} is compared with α calculated from (1) Marcelja's theory[7] for chain melting transitions, assuming that the chain order parameter $\propto \alpha$ (Marcelja) and (2) Meyer and McMillan (MM)[8] theory for the S_H (2-d crystal) $\rightarrow S_A$ (2-d liquid) transition. Normalization of α_{exp} and $\alpha_{(Marcelja)}$ have been done to MM's value of α, at $t = 0.045$. $\alpha_{(Marcelja)}$ and α_{MM} are very similar, but do not explain the t-dependence of α_{exp}.

C. DDS-DPPC-water interactions

Our results on the chain melting transition are well explained by the theory of De Verteuil *et al.*[9] which assumes that the drug molecules occupy interstitial sites in the triangular lattice formed by the lipid chains. Their theory predicts that (1) ΔH_{CM} varies little for small values of R_m and (2) T_{CM} is hardly changed by the inclusion of drug, if the lipid-lipid interactions are not much affected by the presence of the drug molecules. The above theory, however, does not explain pretransition which is predicted by Scott's model.[10] This model predicts a pretransition which is related to the onset of rotational disorder in the acyl chains. As we have seen above, the DDS molecules seem to be predominantly in the interfacial region. If this be true, it would not be possible to explain the disappearance of the pretransition in the doped membranes on the basis of Scott's model.

The possible binding sites of DDS in the model membrane, are depicted in Figure 6. The probable interactions leading to this binding are visualised as shown in Table II. Binding sites a, b, f, g are the most likely ones. When DDS occupies these sites, the amino hydrogens and sulfonyl oxygens not only hydrogen-bond with water, but also compete with water for the binding sites in the polar heads. Other techniques such as NMR, infra-red and Raman spectroscopy would have to be used to determine with certainty the binding sites of DDS.

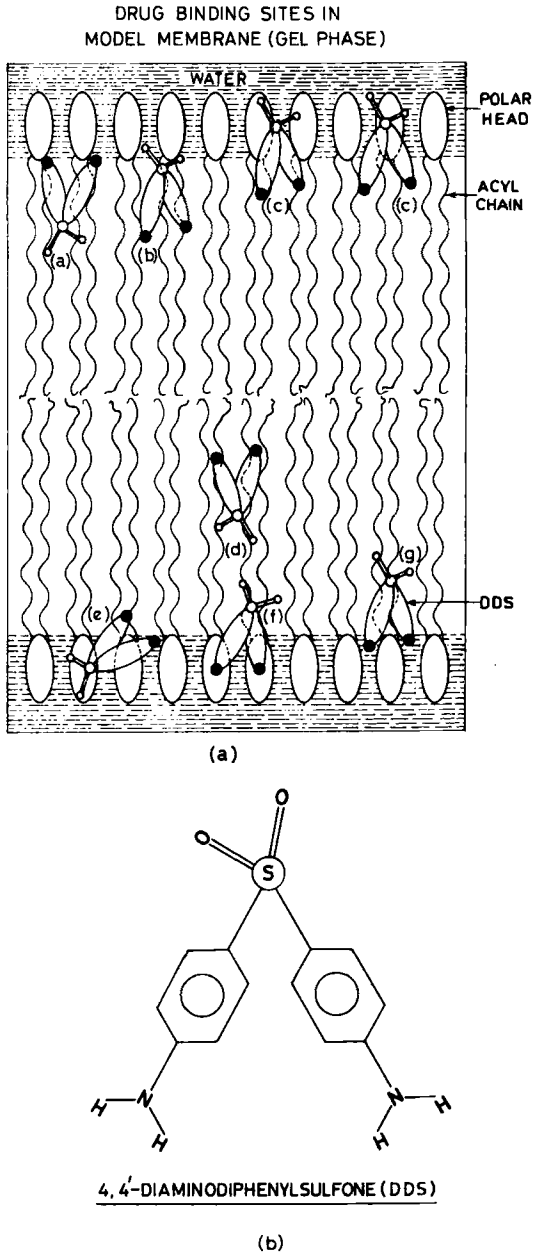
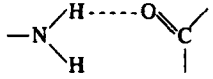
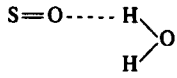
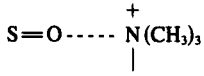
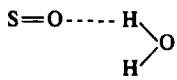
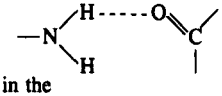
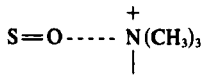
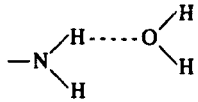
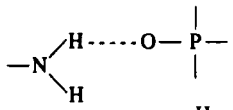
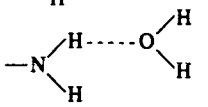


FIGURE 6 (a) Possible binding sites of DDS in the gel phase of the membrane, and (b) a schematic drawing of the tetrahedral DDS molecule.

TABLE II

Possible binding sites of DDS in the model membranes, showing the interactions possible at each binding site.

		Drug	Membrane	Remarks
a)				
b)				
c)	(i)	 or 		Unlikely because hydrophobicity of the benzene ring is not satisfied.
	(ii)			
d)		Embedded in the acyl chain layer		least probable (see discussion on $T_{CM,CO}$).
e)	(i)			Unlikely for reasons given in 'c(i).
	(ii)			
f)				
g)				

IV. CONCLUSIONS

From our results, we find that DDS (1) does not become embedded in the acyl chain layer, but stays near the interface, (2) creates varied environ-

ments for the DPPC molecules, and (3) perturbs the structure of vicinal water. This alteration in the water structure implies impairment of the biological functions of the membrane.

Acknowledgment

We are grateful to Burrough's Welcome India Ltd. for the gift of DDS.

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