

Nuclear magnetic resonance and thermal studies of drug doped dipalmitoyl phosphatidyl choline-H₂O systems

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Abstract. The influence of the sulfone drugs, diamino diphenyl sulfone and diamino monophenyl sulfone on the phase transitions and dynamics of dipalmitoyl phosphatidyl choline-H₂O/D₂O vesicles have been investigated using differential scanning calorimetry and nuclear magnetic resonance. Our results show that diamino diphenyl sulfone interacts quite strongly with the headgroups of dipalmitoyl phosphatidyl choline whereas the diamino monophenyl sulfone-dipalmitoyl phosphatidyl choline interaction is quite weak. This is attributed to the difference in the structure and hydrophobic character of the two drugs.

Keywords. Dipalmitoyl phosphatidyl choline; drug-membrane interaction; differential scanning calorimetry; ¹H NMR; ³¹P NMR.

Introduction

Study of interactions of drugs with biomembrane is of great importance. Biomembranes being very complex, one studies interactions of drugs with model-membranes as a first step towards understanding drug-biomembrane interactions. In the present work, we have carried out differential scanning calorimetry (DSC) and nuclear magnetic resonance (NMR) (¹H and ³¹P) experiments at temperatures in the vicinity of the chain melting (CM) transition to observe the interaction of the sulfone drugs, diamino diphenyl sulfone (DDS or Dapsone) and diamino monophenyl sulfone (DMS or sulfanilamide) with the model membrane, dipalmitoyl phosphatidyl choline (DPPC)-H₂O/D₂O, in vesicle form. These drugs are expected to possess distorted tetrahedral structures (figure 1). Though DDS has been in use as an antileprosy drug for a few decades, hardly any information about its interactions with membranes was available until recently (Deniz *et al.*, 1983, 1989). While our main interest was in DDS-membrane interactions, DMS was used in order to understand how structural differences affect these interactions.

Materials and methods

DPPC and DMS were purchased from Sigma Chemical Company, St. Louis, Missouri, USA, while DDS was a gift from Burroughs Wellcome India. These were

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Abbreviations used: DSC, Differential scanning calorimetry; NMR, nuclear magnetic resonance; CM, chain melting; DDS, diamino diphenyl sulfone; DPPC, dipalmitoyl phosphatidyl choline; PT, pre-transition.

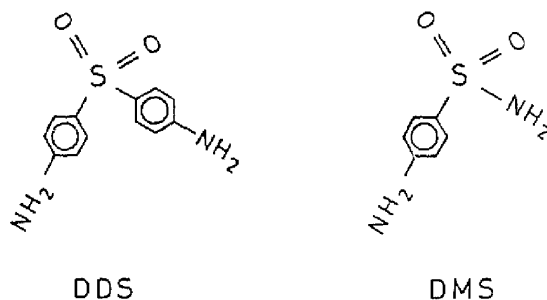


Figure 1. The structure of molecule, DDS and DMS.

used without further purification. The vesicles of DPPC and drug-DPPC were prepared by hydrating their films with H₂O/D₂O at 45°C, vorticing them and subsequently sonicating at 45°C for 45 min, using a microtip. DPPC concentration, [DPPC], of 50 mM and a molar ratio, R_m , of drug to DPPC of 0.1 were used. The DSC studies were carried out on a Perkin Elmer DSC-2C instrument. Sample weights ranged from 10–16 mg and the scans were carried out at rates of 2.5–10 K/min. ¹H NMR at 270 MHz was carried out on a Bruker WH 270 instrument with sodium-3-trimethylsilyl (2,2,3,3-²H) propionate (TSP) as reference and ³¹P NMR at 121.44 MHz was done on a Bruker MSL 300 instrument with phosphoric acid as reference.

Results and discussion

DSC

The model membrane, DPPC-H₂O/D₂O exhibits an ordered lamellar gel (L_β) phase at room temperature. On heating, a pre-transition (PT) occurs to a ripple phase (P_β) (Janiak *et al.*, 1976; Scott, 1981). This is followed by a CM transition into a disordered liquid crystalline (L_α) phase. This transition is of physiological importance, since the chain mobility (membrane function) is related to it.

DSC scans through the CM transitions for the 3 systems are shown in figure 2. We find that (i) T_{CM} is slightly increased in the presence of DDS but is slightly decreased by DMS and (ii) PT (not seen in the figure) is suppressed completely by DDS but only partially by DMS. This can be explained in the following way. Due to the tetrahedral structure of the DDS molecule and its amphiphilic nature, one would expect [in the light of De Verteuil *et al.* (1981) theory] a significant decrease in T_{CM} , if DDS were to be located in the acyl chain region. Since it is not observed, DDS must be located at the DPPC-H₂O interface, interacting with the polar head group of the lipid and enhancing the effective DPPC head group-head group interaction. This can account for the observed increase in T_{CM} (De Verteuil *et al.*, 1981). DMS being more polar than DDS, is less likely to enter the acyl chain region. However, it must be decreasing slightly, the effective interaction between DPPC head groups, leading to the observed decrease in T_{CM} . The values of T_{cm} and ΔH_{CM} measured with stacked bilayers (Deniz *et al.*, 1983) of these 3 systems, differ from those obtained in the present measurements with vesicles (table 1). It is observed that (a) ΔH_{CM} is drug-independent in stacked bilayers but is drug-dependent in vesicles and (b) in DPPC-H₂O, ΔH_{CM} (vesicle) $\ll \Delta H_{CM}$ (stacked

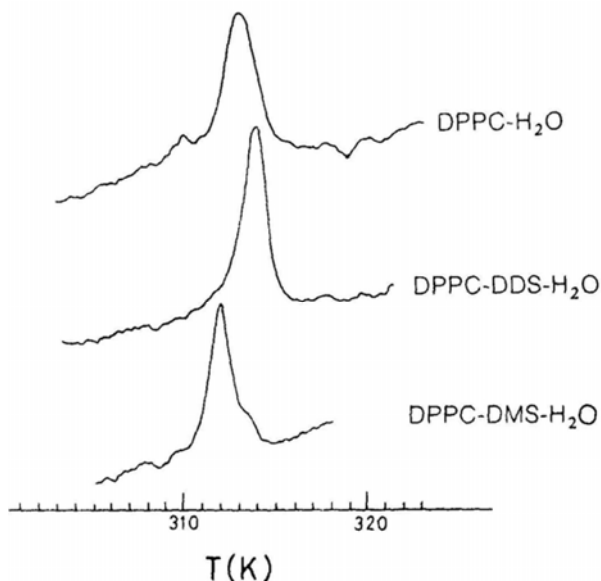


Figure 2. DSC scans of the 3 model membranes, DPPC-H₂O, DPPC-DDS-H₂O and DPPC-DMS-H₂O showing the CM transition (scan rate: 2.5 K/min).

Table 1. T_{CM} and ΔH_{CM} for various model membrane systems.

Systems	Stacked bilayers		Vesicles	
	T_{CM} (K)	ΔH_{CM} (KCal/mol)	T_{CM} (K)	ΔH_{CM} (KCal/mol)
DPPC-H ₂ O	313.7	8.44	312.8	4.42
DPPC-DMS-H ₂ O	312.4	9.56	314.8	5.39
DPPC-DDS-H ₂ O	312.9	8.41	314.0	7.01

bilayer). In vesicles which are unilamellar or made up of only a few lamellae (i) it is easier for the drug to have access to the DPPC-head group than in stacked bilayer and this could account for (a), (ii) increased fluctuations in the order parameter of the gel phase near T_{CM} reduces ΔH_{CM} resulting in (b) and (iii) these order parameter fluctuations might be reduced by the drug-DPPC interactions leading to ΔH_{CM} (DPPC-DDS-H₂O) > ΔH_{CM} (DPPC-H₂O) (observation (a)).

¹H NMR

¹H NMR spectra for DPPC in the 3 model membranes are shown in figure 3. In DPPC-D₂O (figured 3A), the chain resonances, (1)-(4) and the choline group resonances, (5)-(7) are clearly visible at 315 K. The chain resonances are seen to be very broad and unresolved at 310 and 312 K. They begin to get resolved at 313 K, showing that 312 K < T_{CM} < 313 K. The N⁺(CH₃)₃ resonance, (5) is sharp even at 310 K, indicating that these methyl groups are quite mobile not only for $T > T_{CM}$ but also for $T < T_{CM}$. Resonance (5) consists of two peaks, (5)' and (5)'' corresponding to the choline groups of the outer and inner leaflets of the bilayer.

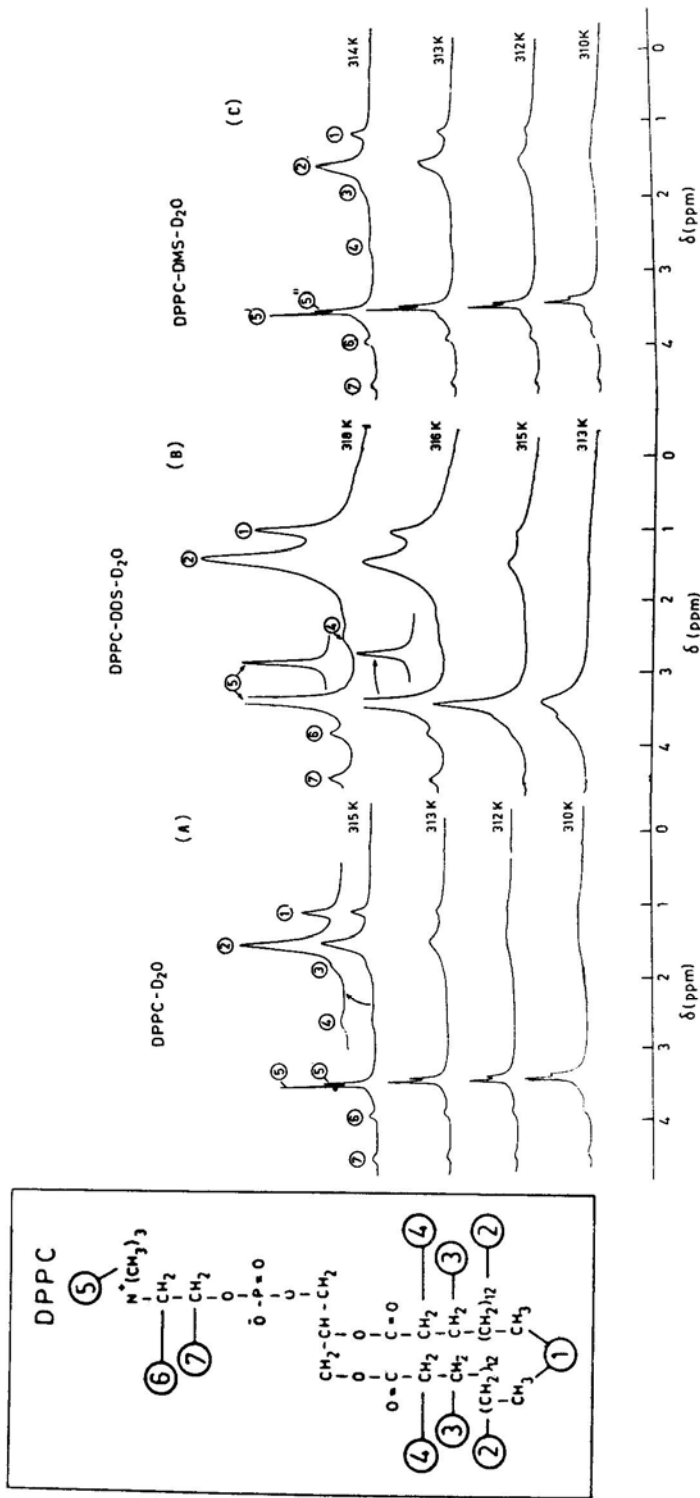


Figure 3. ¹H NMR spectra of (A) DPPC-D₂O, (B) DPPC-DDS-D₂O and (C) DPPC-DMS-D₂O taken at different temperatures in the neighbourhood of *T_{CM}*. The assignments to the various groups in DPPC are shown in the insert.

The spectra for DPPC-DMS-D₂O (figure 3B) closely resemble those of DPPC-D₂O except that T_{CM} is slightly decreased ($T_{CM} \approx 312$ K). The spectra for DPPC-DDS-D₂O (figure 3C) shows the following: (i) The chain resonance (1), (2) and (4) can be seen at 318 K, but resonance (3) is not observed, possibly due to the broadening of the (CH₂)_n resonance, (ii) The CM transition in this case, occurs between 315 and 316 K, *i.e.*, T_{CM} (DPPC-DDS-D₂O) > T_{CM} (DPPC-D₂O). (iii) Although all the 3 choline resonances, (5)–(7) can be seen, (5) is a single peak even at $T > T_{CM}$, unlike in the other two cases. This would mean that the chains and the N⁺(CH₃)₃ groups become more rigid in the presence of DDS. These results suggest that DDS interacts with the choline groups and perhaps also the carbonyl group of DPPC, the latter leading to decreased chain mobility.

The ¹H NMR spectra of the aromatic protons of (i) DDS in D₂O, (ii) DDS in DPPC-D₂O and (iii) DMS in DPPC-D₂O are shown in figure 4, the latter two for temperatures in the vicinity of T_{CM} . The aromatic proton spectra for DDS in D₂O and DMS in D₂O are similar, consisting of two doublets. This doublet structure (the spin coupling constant) is temperature independent for the temperature range used in these experiments. In DPPC-DMS-D₂O, the doublet structure is hardly changed. However, in DPPC-DDS-D₂O, these resonances are broadened and the doublets cannot be resolved, showing that the aromatic rings of DDS are also interacting with DPPC.

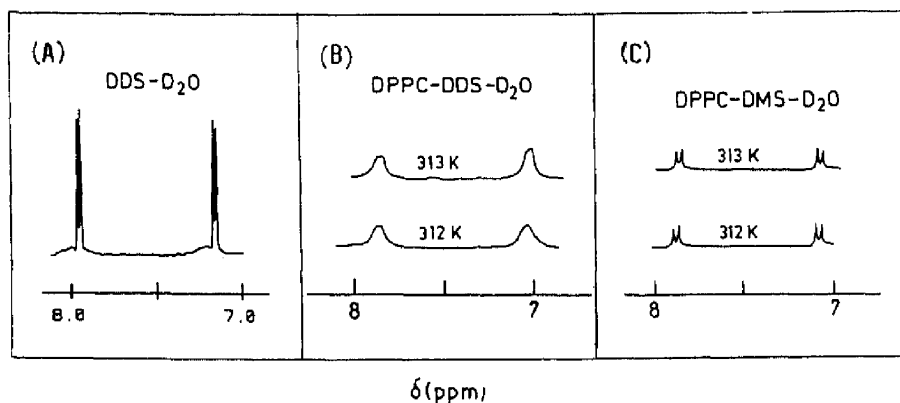


Figure 4. ¹H NMR spectra of the aromatic protons in (A) DDS-D₂O, (B) DPPC-DDS-D₂O and (C) DPPC-DMS-D₂O. (B) and (C) are given for temperatures $\approx T_{CM}$.

³¹P NMR

A ³¹P NMR study of DPPC-D₂O and DPPC-DDS-D₂O was carried out as a function of temperature. Typical spectra for $T < T_{CM}$ and $T > T_{CM}$ are shown in figure 5. For the drug-free system, the resonance is very sharp for both the temperatures, whereas in the presence of DDS, these resonances are broadened considerably even for $T > T_{CM}$. This indicates that DDS (NH₂ group) interacts with the phosphate group of DPPC.

Our results show that DDS-DPPC interaction is much stronger than that of

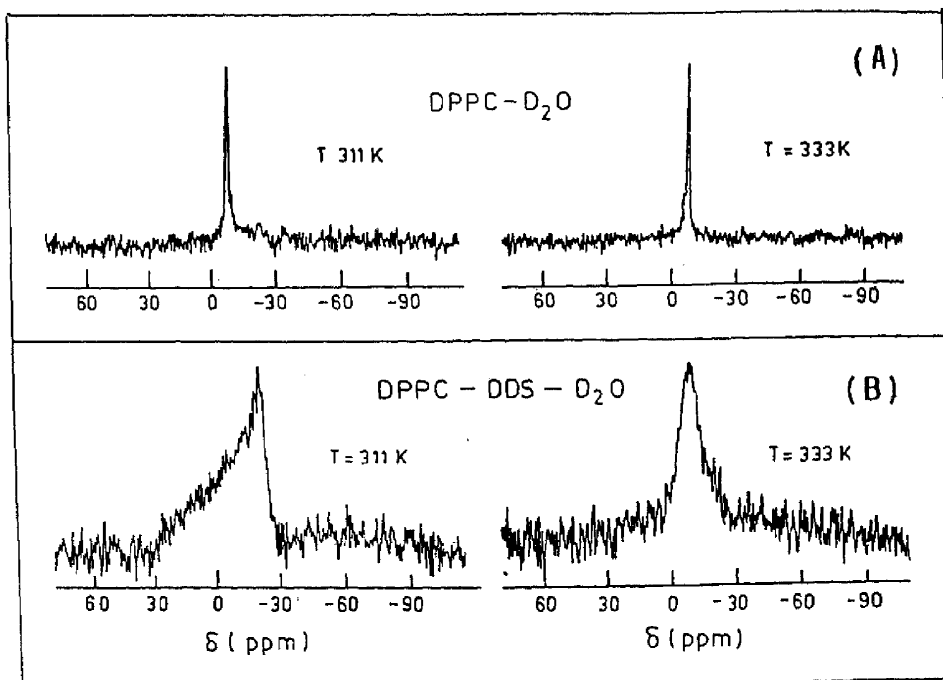
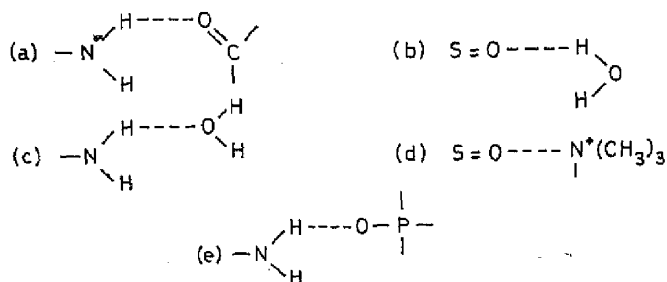


Figure 5. ^{31}P NMR spectra of (A) DPPC- D_2O and (B) DPPC-DDS- D_2O , for $T < T_{\text{CM}}$ and $T > T_{\text{CM}}$.

DMS with DPPC. The aromatic as well as polar groups of DDS interact with DPPC head group. The most likely interactions are shown below:



Probable combinations of these are (a) + (b), (c) + (d) and (a) + (d).

The difference between the DDS-DPPC and DMS-DPPC interactions could be due to (i) more polar character of DMS leading to its greater solubility in water and weaker interactions with DPPC and (ii) molecular structure of DDS which would allow its interactions with the head groups of neighbouring DPPC molecules whereas the same would not be possible with DMS.

Acknowledgement

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References

- Deniz, K. U., Parvathanathan, P. S., Mirza, E. B., Amirthalingam, V., Muralidharan, K. V. and Gurnani, S. (1983) *Mol. Cryst. Liq. Cryst.*, **98**, 163.
- Deniz, K. U., Parvathanathan, P. S., Datta, G. and Mirza, E. B. (1989) in *Surfactants in solution* (ed. K. L. Mittal) (New York: Plenum) vol. 8, p. 203.
- De Verteuil, F., Pink, D. A., Vadas, E. B. and Zuckermann, M. J. (1981) *Biochim. Biophys. Acta*, **640**, 207.
- Janiak, M. J., Small, D. M. and Shipley, G. G. (1976) *Biochemistry*, **15**, 4575.
- Scott, Jr., H. D. (1981) *Biochim. Biophys. Acta*, **643**, 161.