

## **[<sup>31</sup>P] -Nuclear magnetic resonance spin lattice relaxation in lecithin reverse micelles**

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**Abstract.** [<sup>31</sup>P] -Nuclear magnetic resonance (NMR) spin lattice relaxation times ( $T_1$ ) have been measured for lecithin-nonpolar solvent-water as a function of added water for three solvents, namely, benzene, carbon tetrachloride and cyclohexane. In benzene and carbon tetrachloride systems, where spherical reverse micelles are formed, [<sup>31</sup>P]-NMR  $T_1$  values increase linearly with added water. However, in cyclohexane, the trends in the [<sup>31</sup>P]- $T_1$  values indicate very different micellisation processes. Even at the lowest concentration of added water, the [<sup>31</sup>P]- $T_1$  values in this solvent are substantially larger than the corresponding values in benzene and carbon tetrachloride, which is attributed to the intramolecular chlorine-phosphate interaction being the weakest in cyclohexane. At a higher water content of six mols of water per mol of lecithin in cyclohexane solvent, the [<sup>31</sup>P]- $T_1$  values show a sharp decrease indicating a sudden change in the dynamics of the phosphate group, and this confirms the on set of 'reverse micelle-to-liquid crystalline' phase transition observed in this system by other spectroscopic and physical techniques.

**Keywords.** [<sup>31</sup>P]-NMR; spin lattice relaxation; lecithin reverse micelles.

### **Introduction**

There has been a rapid growth of interest in recent years in the study of phospholipid aggregates in nonpolar media since these aggregated structures often 'model' faithfully the nature of cell membranes. Such aggregation leads to reverse micelles with a polar interior and a nonpolar outer surface. These aggregates can solubilize water in their interior as small water 'pools'. Several proton magnetic resonance studies on such reverse micellar systems are available, in which the phospholipid-water interactions have been studied (Davenport and Fisher, 1975; Henrikson, 1970; Shaw *et al.*, 1973; Walter and Hayes, 1971; Wells, 1974).

Two reports in the literature contains a specific reference to [<sup>31</sup>P]-spin lattice relaxation in lecithin reverse micelles. Klose and Stelzner (1974) have investigated the system lecithin-benzene-water, and the dependence of their [<sup>31</sup>P] - $T_1$  values on

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Abbreviations used: NMR, Nuclear magnetic resonance TLC, thin layer chromatography.

added water show a roughly sigmoidal (i.e., S-shaped) dependence, with unexplained plateau regions at either end of the water concentration scale which these authors refer to as 'breaks', and a sharp increase in between. On the other hand, by performing a similar [ $^{31}\text{P}$ ]-NMR relaxation experiment on lecithin-carbon tetrachloride-water system, Fung and McAdams (1976) have observed that the [ $^{31}\text{P}$ ]- $T_1$ 's increased linearly with added water, without any 'break'. Clearly, it would be desirable to have a further understanding of the lecithin [ $^{31}\text{P}$ ]-relaxation in these systems.

From our earlier physical (Kumar, *et al.*, unpublished observations) and spectroscopic (Kumar and Raghunathan, 1982) studies there is strong empirical evidence to show that in lecithin-cyclohexane-water system at a water concentration of six mols per mol of lecithin, the isotropic reverse micellar system changes to an anisotropic liquid crystalline state. Indeed, water diffusion in the above system, studied by the magnetic field gradient-spin echo method (Kumar and Raghunathan, unpublished observations), is reduced by as much as 30% compared with that of normal, bulk water. In this communication, we not only report a re-examination of the [ $^{31}\text{P}$ ]-spin lattice relaxation of lecithin in benzene and carbon tetrachloride as a function of the solubilised water, but also present the first observation of [ $^{31}\text{P}$ ]-NMR spin lattice relaxation times of the system lecithin-cyclohexane-water as a function of added water. As will become apparent, the cyclohexane system is a very interesting one in its own right.

### Materials and methods

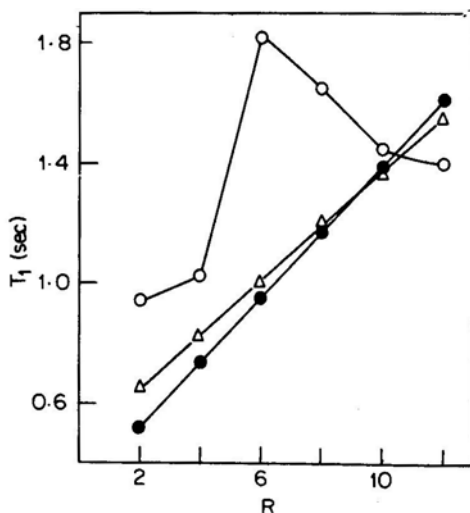
All the solvents used were of Analar grade, which were further dried and distilled. Chromatographically homogeneous egg lecithin was extracted by the method of Singleton *et al.* (1965). Thin layer chromatography (TLC) on silica gel G (Merck) was used for checking the purity of the extracted lipid. The phosphatide extractions from egg yolk in a 9: 1 chloroform-methanol mixture were first applied to the TLC plates and then developed by solvent system chloroform-methanol-water (65 :25 :4,v/v) in a solvent-vapour saturated chamber. Iodine vapour was used for identifying the chromatographed material. A single spot on the TLC plate confirmed the purity of the lipid. After evaporating away the solvent mixture, the residual solid lipid was dried under vacuum (~50 microns) for about 6 h and then dissolved in the required dry solvent. These solutions, as well as the triple-distilled water used in this work, were deoxygenated by purging with a current of dry nitrogen gas before the NMR measurements.

[ $^{31}\text{P}$ ] -NMR results were recorded on a Varian XL-100 FT NMR spectrometer at a [ $^{31}\text{P}$ ]-Larmor frequency of 40.5 MHz. phosphoric acid 85% in a capillary tube was used as a reference, with a  $\text{C}_6\text{D}_6$  external 'lock' for field-frequency control. The relaxation times ( $T_1$ ), determined by the standard  $180^\circ$ - $T$ - $90^\circ$  pulse sequence, were accurate to within  $\pm 5\%$ .

All measurements were carried out at  $25 \pm 1^\circ\text{C}$  over a range of R values, where R is defined as the molar concentration ratio ( $=[\text{H}_2\text{O}]/[\text{lecithin}]$ ).

## Results and discussion

Figure 1 shows the variation of  $[^{31}\text{P}]$ -spin lattice relaxation time of the choline headgroup with increasing water concentration for the three systems, namely, lecithin-benzene-water, lecithin-carbon tetrachloride-water and lecithin-cyclohexane-water. Even at first glance, the point which is striking here is that



**Figure 1.** Variation in  $[^{31}\text{P}]$ -NMR  $T_1$  values of lecithin head group in lecithin-benzene, lecithin-carbon tetrachloride and lecithin-cyclohexane systems as functions of added water. Benzene system, ( $\Delta$ ); carbon-tetrachloride system, ( $\bullet$ ); and cyclohexane system, ( $\circ$ ).

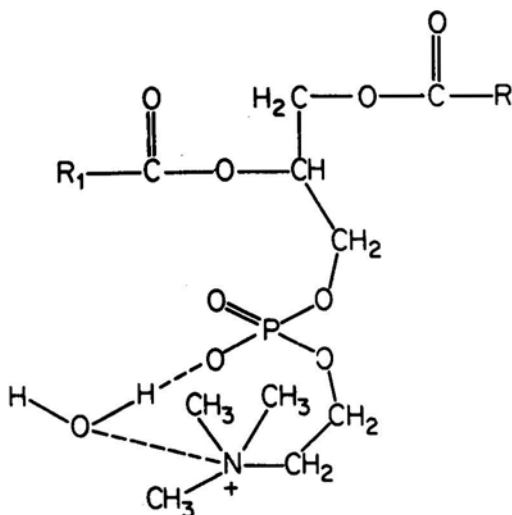
though all the three solvents are highly nonpolar (having almost the same dielectric constant of around 3), the cyclohexane system behaves entirely differently from the other two. This leads to the inference that micelles formation takes place in different ways in different solvents, and it is perhaps useful to make the cautionary remark here that one ought not to extrapolate the apparent physical state of the micelle from one solvent to another.

From figure 1 it is clear that  $[^{31}\text{P}]$ - $T_1$  in benzene and carbon tetrachloride systems increase linearly with added water. In the water concentration range studied, the slopes of our  $T_1$  curves for these solvents are in quantitative agreement with each other and with that found by Fung and McAdams (1976) in carbon tetrachloride (the only solvent these authors used). Like Fung and McAdams (1976), we do not also find the distinct 'breaks' reported by Klose and Stelzner (1974). It should also be noted here that in a subsequent study, Klose *et al.* (1978) have reported that the lecithin-benzene system shows considerable temperature-hysteresis and 'ageing' effects and that small amounts of water change the properties of their system drastically; in view of their findings, these authors have stated that the earlier conclusions of Klose and Stelzner (1974) are uncertain.

In the extreme-narrowing limit an increase in  $[^{31}\text{P}]$ -relaxation time ( $T_1$ ) means a decrease in the rotational correlation time of the phosphate group, i.e., an increase

in its mobility. At very low concentrations of water, the positively charged trimethylammonium group and the negatively charged phosphate group would interact with each other strongly, with consequent severe hindrance in the internal rotation of the phosphate group. Here we stress that, as distinct from the intermolecular interaction between choline and phosphate groups from adjacent lipid molecules in the case of bilayer vesicle structures in aqueous medium (Buldt and Wohlegemuth, 1981), the choline-phosphate interaction usually envisaged in reverse micellar systems is intramolecular, being brought about by a water molecule becoming hydrogen-bonded to the phospholipid head group (see below).

Extensive proton NMR chemical shift and  $T_1$  results for water added to lecithin in the three nonpolar solvents perdeuterobenzene, carbon tetrachloride and perdeuterocyclo-hexane (Kumar, 1982; Kumar and Raghunathan, unpublished observations) have established that, in the former two solvent media, one  $H_2O$  is tightly bound per polar headgroup at all water concentrations whereas it is much more loosely bound in the cyclohexane medium. The conclusion that one molecule of water becomes bound to the headgroup is in excellent agreement with the deuterium NMR  $T_1$  results of Fung and McAdams (1976) who have, however, worked only with the lecithin- $D_2O$ -carbon tetrachloride system. The proposed binding of water to the zwitterionic headgroup is represented in figure 2. This 'folded' arrangement derives support from the quantum mechanical studies of Pullman and Berthod (1974) on the energetically preferred conformations of the polar heads of phospholipids.



**Figure 2.** Model of interaction of water with the polar headgroup of lecithin reverse micelles.

With increasing  $R$ , this choline-phosphate interaction weakens progressively due to the exchange of the bound water with water in the central 'pool', thereby leading to greater relaxation of the headgroup. The water-binding has been

observed to be the weakest in cyclohexane in the proton NMR studies of Kumar and Raghunathan (unpublished observations), and this explains the observed difference in the  $[^{31}\text{P}]$ - $T_1$  values in figure 1 at initial R values (e.g.,  $R=2$ ) between the cyclohexane system on the one hand ( $T_1 = 0.90 \pm 0.03$  s) and the benzene and carbon tetrachloride systems on the other ( $T_1$  about 0.60 s). Further spectroscopic studies on the same systems in our laboratory using 'polarity' probes (Kumar and Raghunathan, 1982) have not only confirmed the exchanged between the bound and pool water at high R values in the above-mentioned solvents, but also the weak binding of the  $\text{H}_2\text{O}$  to the headgroup in cyclohexane solvent.

As already noted, the other remarkable feature of figure 1 is that phosphorus  $T_1$  values in cyclohexane increase upto the  $T_1$ 's upto  $R=6$  shows that the mobility of the phosphate group increases upto this point. At further concentrations of added water, a sharp decrease in the mobility of the phosphate group is heralded, suggesting that some molecular rearrangements involving the headgroup are taking place in cyclohexane medium at an R value of 6.

A possible mechanism that could be invoked to explain the above  $[^{31}\text{P}]$ -NMR  $T_1$  data is as follows: the change in  $T_1$  values at  $R=6$  can be rationalised as being due to the onset of an ordered liquid-crystalline phase, complemented by the intercalation of cyclohexane molecules between successive hydrocarbon chains of the lecithin —  $(\text{CH}_2)_n$ —'tail' in the hydrophobic region.

The results of our other spectroscopic investigations pertinent to this point are the following: our studies using 'polarity' probes such as the  $\text{NO}_3^-$  ion and anilinonaphthalene sulphonic acid (Kumar, 1982; Kumar and Raghunathan, 1982) show that (a) the amount of water present in the organic solvent phase is negligible and (b) in the cyclohexane system in particular, intercalation of the organic solvent could be occurring gradually even at lower R values, eventually 'triggering' the phase transition around  $R=6$ . If our proposed cyclohexane 'intercalation' mechanism is correct, then the protons of the lipid tail should be bathed in this organic solvent and therefore, not be in contact with the added water. Indeed, proton  $T_1$  values of the  $(\text{CH}_2)_n$ -tail in (perdeuterated) cyclohexane remain constant at 0.45 seconds for R values between 2 and 10 (Kumar and Raghunathan, unpublished observations), lending support to our proposal.

Our present study independently confirms the occurrence of the reverse micelle-to-liquid crystalline phase transition indicated by electron microscopy and a variety of other physical and spectroscopic measurements made in our laboratory (Kumar and Raghunathan, 1982).

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