

## Measurement of the Diffusion Coefficient in the Concentrated Phases of the Soap-Water System by Nuclear Magnetic Resonance

A MEASUREMENT of the lateral diffusion coefficient of spin labels in lecithin bilayers has been reported by McConnell *et al.*<sup>1</sup> This diffusion is found to be rapid, the consequences of which, in terms of the structure of biological membranes, cannot be overlooked. Here we report the result of nuclear magnetic resonance measurements on the diffusion coefficient in the concentrated phases of the potassium laurate-heavy water system. Our results strengthen the theory that diffusion in lipid water systems is rapid, magnetic resonance measurements have the advantage that it is the diffusion of the constituent molecule itself that is being observed and not the diffusion of an added "impurity" as is the case with spin label experiments.

The usual procedure for measuring diffusion by magnetic resonance is to observe the decay of the spin echo in the pulse sequence  $90^\circ, \tau, 180^\circ$  as a function of  $\tau$ , or as a function of the applied magnetic field gradient. This method is valid only for the inequality  $12/\gamma^2 G^2 D \leq T_2^2$  being satisfied where  $\gamma$  is the magneto-gyric ratio of the resonant nuclei,  $G$  is the applied magnetic field gradient,  $D$  the self diffusion coefficient and  $T_2$  the nuclear transverse relaxation time. This condition is normally satisfied for liquids of low viscosity where the diffusion is fairly rapid and the transverse relaxation time  $T_2$  is of the order of the longitudinal relaxation time  $T_1$ . In the concentrated phases of amphiphile-water systems the  $T_2$  is normally too short, owing to dipole-dipole interactions, for a liquid echo, and hence a diffusion coefficient to be obtained by this method. One method of overcoming this difficulty is measurement of the stimulated echo, first described by Hahn<sup>2</sup> and further developed for diffusion measurements by Tanner<sup>3</sup>.

To obtain a stimulated echo a sequence of three  $90^\circ$  pulses is required. The echo which occurs an interval after the third r.f. pulse equal to that between the first two pulses is unique in that its relaxation attenuation has a  $T_1$  dependence as a function of the interval between the second and third r.f. pulses. The attenuation of the stimulated echo in this case is given by

$$\ln(M/M_0) = -\frac{(\tau_2 - \tau_1)}{T_1} - 2\frac{\tau_1}{T_2} - \ln 2 - \gamma^2 D g \tau_1^2 (\tau_2 - \tau_1/3)$$

where  $M_0$  and  $M$  are the nuclear signal initially and at the time of the echo maximum respectively,  $\tau_1$  and  $\tau_2$  are the time intervals between the first and second, and first and third r.f. pulses. An additional condition discussed by Tanner<sup>3</sup> for optimizing the three  $90^\circ$  pulse sequence for minimum non-diffusional attenuation at fixed diffusional attenuation is that  $\tau_2/\tau_1 = T_1/T_2$ ; also, as the attenuation depends on  $\tau_1^2$ , this quantity cannot be made too small and in practice must remain a few milliseconds. Thus if the ratio of  $T_1/T_2$  is large, the interval  $\tau_2$  must be large and this can cause problems of signal to noise ratio and often, as in our case, demand that signal accumulation and averaging techniques be used.

The results on the potassium laurate-heavy water system prove interesting. In this system, X-ray work<sup>4</sup> has shown the existence of a cubic phase. This phase is unique in that the absorption lines are so narrow that the spectrum can be studied by high resolution n.m.r. The spectrum is in fact very similar to that obtained in the micellar phase. This liquid-like behaviour is due to the complete averaging of the static dipolar interactions. Thus in this phase a liquid echo may be obtained by the sequence  $90^\circ, 180^\circ$  and a diffusion constant measured. This has been

done by Charvolin (J. Charvolin and P. Rigny, to be published) who finds a diffusion coefficient at  $80^\circ \text{C}$  of  $2 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$ . Charvolin discusses the possibility of fast diffusion in the other concentrated phases. Here we have by means of the stimulated echo technique been able to measure directly the diffusion coefficient in both the hexagonal and the lamellar phases of the potassium laurate system. The results are a self diffusion coefficient of  $2.3 (\pm 0.3) \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$  in the hexagonal phase and  $2.4 (\pm 0.35) \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$  in the lamellar phase at  $80^\circ \text{C}$ . Concentrations were in the hexagonal phase 50%  $\text{D}_2\text{O}$ , and in the lamellar phase 28%  $\text{D}_2\text{O}$ . The temperature of  $80^\circ \text{C}$  was chosen because it is above the Krafft point for all phases. On decreasing the soap concentration and passing into the micellar solution, where a liquid echo is observed, the diffusion coefficient rises as a function of decreasing concentration. A factor 3 difference was observed in the diffusion coefficient at  $12 \text{ g l}^{-1}$  and at  $120 \text{ g l}^{-1}$ . This is to be expected, for what is now being observed is the diffusion of the micelles themselves becoming more rapid than that of their constituent molecules. A decrease is also observed in the activation energy from  $5.7 (\pm 0.2) \text{ kcal mol}^{-1}$  at  $120 \text{ g l}^{-1}$  to  $4.05 (\pm 0.1) \text{ kcal mol}^{-1}$  at  $12 \text{ g l}^{-1}$ . It is interesting to note that the activation energy in the cubic phase is  $5.5 (\pm 0.2) \text{ kcal mol}^{-1}$  (J. C. and P. R., to be published) and in the hexagonal phase is  $5.8 (\pm 0.2) \text{ kcal mol}^{-1}$ . This tends to show that whatever macroscopic structure (hexagonal, cubic and so on) the molecular aggregations may take, the motion of the individual molecules remains very similar. We have also examined the diffusion of sodium lauryl sulphate in the hexagonal phase (50%  $\text{D}_2\text{O}$ ) and found the extremely rapid diffusion coefficient of  $1.6 (\pm 0.3) \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$  at  $88^\circ \text{C}$ . An interesting point here which deserves further attention is that in the gel phase of the same concentration (that is at  $25^\circ \text{C}$ ) the measured diffusion coefficient was  $2.5 (\pm 0.4) \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$ . This shows a factor 10 difference, and if one assumes a similar activation energy for the process ( $5 \text{ kcal mol}^{-1}$ ) then there must be an increase in mobility on passing into the hexagonal phase.

A model which could explain n.m.r. data is that of a molecule whose polar head is constrained to move only in two dimensions, but the terminal methyl group has a completely liquid-like character. Spin-diffusion is not an effective process along the hydrocarbon chain. The  $T_1$  relaxation behaviour is governed primarily by rapid translational diffusion. A certain portion of the chain, however, near the polar head group remains relatively rigid; within this part of the chain, dipolar interactions exist. This gives rise to the  $T_2$  mechanism and also the  $T_{1\rho}$ .

These results are extremely interesting especially for biological membranes. An ordered system that looks viscous is on the molecular scale behaving almost as a liquid. Examination of diffusion in cell membranes as regards function may throw considerable light on the transport properties.

I thank the Science Research Council for a fellowship held during the course of this work.

R. T. ROBERTS

*Service de Chimie-Physique,  
Bat. 125,  
Centre d'Etudes Nucléaires de Saclay,  
BP No. 2, 91-Gif-sur-Yvette*

<sup>1</sup> McConnell, H. M., and Devaux, P. J., *Amer. Chem. Soc.*, **94**, 4475 (1972).

<sup>2</sup> Hahn, E. L., *Phys. Rev.*, **80**, 580 (1950).

<sup>3</sup> Tanner, J. E., *J. Chem. Phys.*, **52**, 2523 (1970).

<sup>4</sup> Luzzati, V., Jardey, A., Gulik-Krzywicki, T., Rivas, E., and Reiss-Husson, F., *Nature*, **220**, 485 (1968).