Light scattering studies of the solubilization of some esters of *p*-aminobenzoic acid by lysophosphatidylcholine

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Light scattering equipment has been constructed using a vertically polarized helium-neon laser and a phase-sensitive detection system. The apparatus has been used to determine the micellar weight of lysophosphatidylcholine (LPC), giving values of $1·33 \times 10^5$ in water and $1·47 \times 10^5$ in 0·05 M sodium chloride; the sols showed some degree of polydispersity. The light scattering apparatus has also been used to study the solubilization by LPC of three n-alkyl esters of paraminobenzoic acid. These compounds caused a considerable increase in the scattered light, particularly at higher LPC concentrations.

It has been reported previously (Hunt & Saunders 1975) that LPC solubilized three esters of *p*-aminobenzoic acid, solubilization increasing in the order ethyl, n-propyl, n-butyl ester. N.m.r. studies have also indicated that hydrocarbon interiors of the lysophosphatidylcholine (LPC) micelles are primarily involved in the binding.

In this paper light scattering studies of LPC sols, and of LPC solubilization of ethyl, n-propyl and n-butyl paraminobenzoate are described. The light scattering apparatus had as a light source a low power vertically polarized helium-neon laser, to give a monochromatic beam of low divergence. The photomultiplier output was amplified and a phase-sensitive detector used to reduce noise.

Light scattering apparatus
The apparatus was based on the Brice-Phoenix model 2000 light scattering equipment, modified to use a 632·8 nm laser light source, and to improve the scattered light detection system. The validity of using a low power laser for this type of work has been established by Leite et al (1965) who showed that light scattered from organic liquids by a helium-neon laser was in agreement with the Rayleigh theory, and by Block (1968) who studied the light scattered by suspensions of polystyrene spheres in water. Conventional light sources have been compared with helium-neon lasers and shown to produce similar results for light scattering by colloidal solutions (Sherman et al 1968).

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The laser used in this work was a Scientifica and Cook B19 helium-neon laser, giving 3mW of vertically polarized light at 632·8 nm wavelength. A phase-sensitive detector comprising a switching circuit, switched by a reference signal was used to reduce noise in the photomultiplier output signal. The major parts of the apparatus were mounted on a channel section steel beam, which ensured freedom from vibration and prevented any movement once the apparatus was aligned. The laser was rotated on its mounts so that the beam was exactly vertically polarized, this adjustment being facilitated by the fact that the Brewster windows in the plasma tube of the laser reflect, in the plane of polarization, some of the light incident upon them.

Treatment of data
The light scattering instrument was calibrated by relating the scatter of solutions of colloidal silica to their turbidity. A secondary standard, made from a Perspex block was the same shape as the light scattering cell and measurements were made relative to the scatter of his block at 90°. The ratio of the scatter of a solution at angle θ to the direction of the laser beam to scatter of the block at 90° was called S_θ.

$$R_\theta = C.S \quad .. \quad .. \quad .. \quad (1)$$

where R_θ is the Rayleigh ratio and C is the instrument constant.

By measuring turbidity and S_{90} on a range of solutions of colloidal silica, and extrapolation to zero concentration, a value for C was obtained.

Normally S_θ is estimated by subtracting the scatter for pure solvent from the solution reading. This

procedure introduces inaccuracies, particularly when the scatter of solution is low. A better method in these cases is that described by Pugh (1970) and Pugh & Saunders (1971).

Solutions were clarified by filtration through Millipore PSWP 450 nm membranes, and measurements of Δn the refractive index difference between solution and solvent, and of the specific refractive index increment dn/dc were made using a Rayleigh interference refractometer, at the same wavelength as the laser.

As a check on technique, and on the alignment of the apparatus, a plot was made of $S_\theta \sin \theta$ against θ, for a colloidal solution of silica, which behaves as a Rayleigh scatterer. $S_\theta \sin \theta$ was found to be independent of θ, as expected. Measurements made of the molecular weight of silico-tungstic acid and bovine plasma albumin, gave results in agreement with values obtained by others (Hunt 1972).

Materials

LPC was prepared from egg yolk lecithin as described by Hunt & Saunders (1975). The ethyl, n-propyl and n-butyl esters of p-aminobenzoic acid were laboratory reagent grade, recrystallized from ether or ethanol before use.

RESULTS

The aggregate weight of LPC

Measurements were made of the scatter of an approximately 20 mM sol, and various dilutions, at seven angles from 45° to 135° to the incident beam. For each angle, straight line plots were obtained of n against S_θ. From these, values of $\Delta n/S_\theta \sin \theta$ were obtained, which were then plotted against $\sin^2\theta/2$. The intercept, calculated by regression analysis gave $(\Delta n/S_\theta \sin \theta) \Delta n \rightarrow 0, \theta \rightarrow 0$ which was then used to give an average micellar mass of $1\cdot33 \times 10^5$, with $P = 0\cdot95$ limits of error $5\cdot6\%$. The dissymmetry, S_{45}/S_{135} was $1\cdot08$. The micellar mass of LPC in $0\cdot05$ M sodium chloride solution was measured as $1\cdot47 \times 10^5$, with $P = 0\cdot95$ limits of error $3\cdot3\%$, the dissymmetry in this case being $1\cdot14$.

Measurements on LPC sols containing solubilized esters

LPC sols of up to 15 mM were made up in purified water, a known amount of ester added and the mixture allowed to equilibrate for 24 h with shaking. The mixed sol was then filtered into the light scattering cell and S_θ measured at angles θ from 45° to 135° to the incident beam. The refractive index increment

Δn was determined with respect to water. The mixture was diluted and left to reach a new equilibrium, values of S_θ and Δn were again measured after filtration to remove dust, and in this way a series of values over a range of concentrations were obtained. The specific refractive index increment dn/dc with respect to the concentration of LPC in the sol was determined for each ratio of LPC to ester investigated. n was found to be a linear function of LPC concentration for these sols, in the ranges measured.

Preliminary experiments were conducted to establish the best method of dilution of a mixed sol of ester and LPC to maintain a constant ratio of solubilized ester to LPC. Two methods were investigated, dilution with a saturated solution of the ester, and dilution with purified water.

It was found that when a mixed sol containing less than the maximum of solubilized ester was diluted with a saturated solution of that ester, the scattered light at first decreased, but as further dilution was carried out, it increased again to a maximum. Still further dilution produced a decrease in scattered light as would be expected. This phenomenon was interpreted as meaning that during dilution of a mixed sol with a solution of the ester, some of the ester in solution became bound to the LPC micelles, causing an increase in the amount bound per mole of LPC, and an increase in micellar size. As a result of these experiments it was decided to dilute ester-LPC mixtures with water to give a range of concentrations for the light scattering measurements.

A series of experiments was performed to measure the light scattered by LPC sols containing various ratios of solubilized ester to LPC. The mixed sols were diluted with water and values of S_θ and Δn measured for each dilution after allowing 2 h to come to equilibrium. The esters of p-aminobenzoic acid, when solubilized by LPC caused a very large increase in the light scattered, especially at higher concentrations of LPC. The dissymmetry was also increased, as shown by Table 1. Calculation of the micellar mass from light scattering measurements at seven angles relative to the incident beam gave results of around $1\cdot1 \times 10^5$ for the various molar ratios of the three compounds to LPC, when θ and Δn are extrapolated to zero.

For all three n-alkyl esters, above a certain concentration of LPC, the scattered light increased rapidly with increasing concentration. The curve of S_{90} against LPC concentration for LPC containing a maximum of n-propyl p-aminobenzoate, and with dilution by a saturated solution gave a straight line at low LPC concentrations. Analysis of the results at

Table 1. *Increase in dissymmetry on solubilization of n-propyl p-aminobenzoate by LPC sols of 5 mM concentration.*

Moles of compound per mole of LPC	Dissymmetry S_{45}/S_{135}
0	1·085
0·216	1·26
0·396	1·42
0·441	1·51
0·544	1·73

seven angles relative to the incident beam from which this curve was taken, by extrapolation to zero Δn and θ gave a micellar mass of $3\cdot33 \times 10^6$. The dissymmetry was 2·00.

DISCUSSION

The micellar mass of LPC

Robinson & Saunders (1959) obtained a micellar mass for LPC in water of $0\cdot92 \times 10^5$ using light scattering, and a value of $1\cdot01 \times 10^5$ for LPC in 0·1 M sodium chloride solution. Perrin & Saunders (1964) found a value of $0\cdot95 \times 10^5$ using diffusion, viscosity and sedimentation measurements. Kellaway (1968) found $0\cdot98 \times 10^5$ using osmometry, whereas Pugh (1970) reported values of $1\cdot27 \times 10^5$ for LPC in water and $1\cdot38 \times 10^5$ for LPC in 0·1 M sodium chloride using osmometry, and similar values using light scattering. The results given here are larger than those quoted above, with the exception of those of Pugh with which there is reasonable agreement. Results using osmometry gave a number average micellar mass, which one would expect to be lower than the mass average given by light scattering, unless the LPC particles are monodisperse spheres. Robinson & Saunders (1959) used the scattered light at 90° only to calculate the micellar mass. Unless there is no angular variation this gives a low result. Results obtained using $(\Delta n/S_{90})_{\Delta n \to 0}$ only were $1\cdot25 \times 10^5$ in water and $1\cdot32 \times 10^5$ in 50 mM sodium chloride. Values given in this paper for the micellar mass of LPC thus appear to be consistent with the results of others, but indicate a degree of polydispersity.

In reports mentioned above for the micellar mass of LPC, the role of monomers is neglected. This is reasonable, as the critical micellar concentration (cmc) is very low. Robinson & Saunders (1959) have estimated from light scattering, surface tension and other methods that the cmc is in the range 20–200 μM.

Solubilization of the local anaesthetic esters

The three n-alkyl esters of p-aminobenzoic acid cause a large increase in the light scattered by LPC sols. For a given molar ratio of ester to LPC, the longer the n-alkyl chain on the ester the greater the increase in scattered light. It has been shown previously (Hunt & Saunders 1975) that the maximum amount of these compounds solubilized by LPC is also proportional to the chain length, and also that the longer
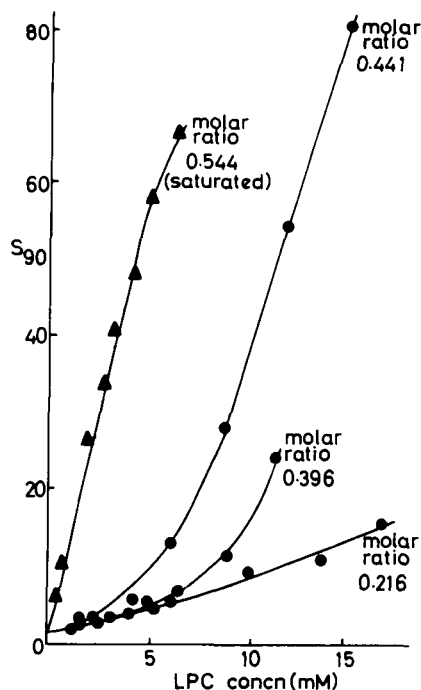

FIG. 1. Plot of S_{90} against LPC concentration for sols containing various ratios of solubilized n-propyl paraminobenzoate to LPC. ▲, Dilutions made with a saturated solution of the ester. ●, dilutions made with water.

the hydrocarbon chain the greater the n.m.r. line broadening effect on LPC. Thus it seems that the longer the n-alkyl chain on the ester molecule the stronger is its binding to LPC.

The plots of S_{90} against LPC concentration for the various mixtures show a sharp increase in slope at higher LPC concentrations. The concentration at which this effect occurred was found to be 7 mM for the ethyl compound, 5 mM for the n-propyl (Fig. 1) and 2 mM for the n-butyl ester. It was noticed that sols containing large amounts of LPC and ester were

quite viscous. These observations indicate a change in structure of the mixture at higher LPC concentration. From the slope of the plots of S_θ against LPC concentration at these higher concentrations the micellar masses are of the order of 10^6. The increase in dissymmetry on solubilization of n-propyl *p*-aminobenzoate, shown in Table 1, indicates the formation of large asymmetric aggregates at the higher ratios of ester to LPC.

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