MICELLAR SIZE OF MIXED LYSOLECITHIN-LECITHIN SOLS

BY N. ROBINSON

From the Department of Physical Chemistry, School of Pharmacy, University of London, Brunswick Square, London, W.C.1

Received September 7, 1960

Lysolecithin and lecithin can be expected to occur together in biological systems—aggregates formed by mixed lysolecithin-lecithin sols were found to have a molecular weight of nearly 1·5 million and to be unsymmetrical, probably closest to a rod shape of length 1,500 Å. The particles were thought to be mixed micelles and not solubilised lecithin within the micelles of the highly surface-active lysolecithin according to the classical Hartley concept.

LYSOLECITHIN is completely soluble in water and possesses considerable surface activity. Robinson and Saunders (1959) have shown it to form large symmetrical aggregates in water having a micellar weight of 100,000.

Lecithin possesses an additional unsaturated fatty acid chain occupying the α-position of the glycerol nucleus. This confers on lecithin a greater lipophilic character which causes the substance to form a dispersion in water. Lecithin forms large asymmetric particles (Robinson, 1960) in water having a micellar weight of $2{\cdot}7 \times 10^6$.

Since lysolecithin is an enzymatic breakdown product of lecithin these substances would be expected to occur together in biological systems. It is thought that the co-existence of these phosphatides will be in the formation of mixed micelles. The effect of the presence of the lecithin component on the micellar size and shape of lysolecithin aggregates, which will have some solubilising effect on the lecithin component, has been studied by the light-scattering method.

EXPERIMENTAL

Materials

The preparation of lecithin from the yolks of eggs has been reported (Robinson, 1960).

Lysolecithin was prepared by Saunder's (1957) modification of the method of Hanahan, Rodbell and Turner (1954). Analytical figures for the phosphatides are given in Table I below.

TABLE I

ANALYTICAL FIGURES FOR THE PHOSPHATIDES

	N (per cent)	P (per cent)	$[\alpha]_D^{20}$	Double bonds per molecule from iodine uptake
Lecithin	1·73	3·82	+7·6	2·3
Lysolecithin	2·83	5·98	+2·26	0·12

Preparation of Mixed Sols

Measured quantities of ethanolic solutions of known concentrations of lysolecithin and lecithin were placed in the same conical flask and gently

warmed to obtain a clear solution. This was then evaporated to dryness under vacuum leaving a film of intimately mixed phosphatides. A measured volume of ion-exchanged water was added to the residue and the flask attached to an automatic shaking machine and placed in a water bath at 40°. The flask was shaken in the water bath until the sol gave a minimum turbidity.

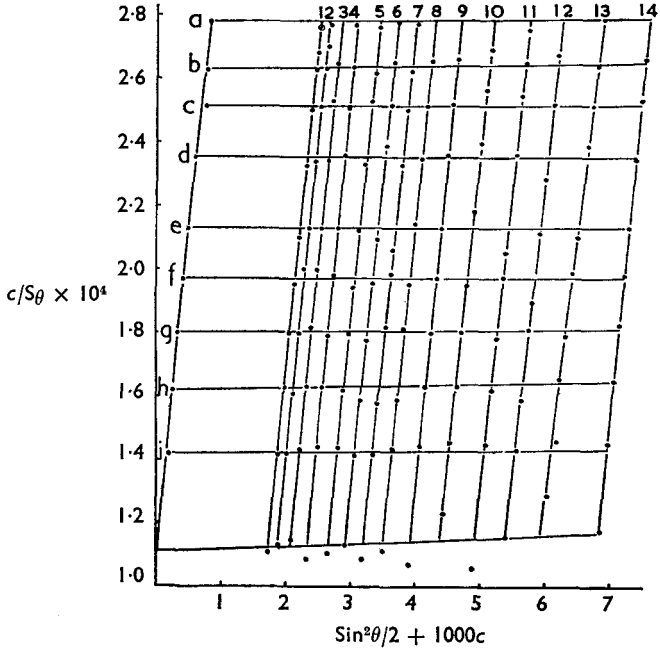

FIG. 1. Zimm plot for mixed lysolecithin-lecithin sols at 20°. Figures along the top of the plot denote total phosphatide concentrations (c) in g. ml.$^{-1}$ × 10^3 as follows.

1.	1·72	6.	2·90	11.	4·89
2.	1·88	7.	3·17	12.	5·40
3.	2·08	8.	3·51	13.	6·04
4.	2·32	9.	3·92	14.	6·84
5.	2·63	10.	4·44		

Letters denote angles (θ) of scatter to incident beam.

a.	$\theta = 130°$	f.	$\theta = 80°$
b.	$\theta = 120°$	g.	$\theta = 70°$
c.	$\theta = 110°$	h.	$\theta = 60°$
d.	$\theta = 100°$	i.	$\theta = 50°$
e.	$\theta = 90°$		

Preliminary experiments on the turbidity of mixed lysolecithin-lecithin sols showed that sols having a mol ratio of lecithin to total phosphatide of less than 0·11 were optically clear, indicating that the lecithin component was completely solubilised. Sols having a mol ratio of lecithin to total phosphatide of 0·10 were prepared and used in the light-scattering studies.

Apparatus. The light-scattering apparatus has been previously described (Robinson and Saunders, 1959; Robinson, 1960).

Results and Discussion

Measurements were made in pure water which is close to the isoelectric point of lecithin (found experimentally by Chain and Kemp (1934) to be pH 6·7). The isolectric point of lysolecithin is probably very close to that of lecithin and the system in water was considered to possess minimum charge effects.

Diffusion studies of mixed phosphatide sols having a mol ratio of lecithin to total phosphatide of 0·5 or less followed the theory for a single solute (Thomas, 1958) indicating that, in selected ratios of mixed phosphatide sols, the micelles were fairly uniform in size and composition. These findings, probably due to the closely related structures of the lecithins, enabled the concentration term (c) to be taken as the sum of the two components.

The specific refractive index increment for the mixed lysolecithin-lecithin sols using the Raleigh interference refractometer method previously described (Robinson and Saunders, 1959) was 0·1361. The depolarisation of 90° scatter was 0·0331.

Light scattered by sols having total phosphatide concentrations between 6·84 and 1·72 $\times$ 10^{-3} g. ml.$^{-1}$ was examined between the angles 50° and 130° to the incident beam. Values for $c/S\theta$, where c is the concentration in g. ml.$^{-1}$ and $S\theta$ the scatter at angle θ to the incident beam, were plotted against $\sin^2\frac{\theta}{2} + 1{,}000\,c$ by the method of Zimm (Fig. 1). From the intercept $\left[\frac{c}{S\theta}\right]$ $c = 0$, $\theta = 0$ the molecular weight was calculated to be 1,484,000.

Since the shape of the particles was unknown the reciprocal of $P(\theta)$ (the particle scattering factor) was plotted against $\sin^2\frac{\theta}{2}$ (Stacey, 1956); by plotting theoretical curves with the same initial slope for a sphere, rod, random and polydisperse coil a comparison for the closest fit indicated that the particles were probably closest to a rod (Fig. 2), having a maximum dimension of 1,530 Å.

Results show that the molecular weight of the mixed micelles was nearly fifteen times greater than the micellar weight of the lysolecithin component although this substance constituted a mol fraction of nine-tenths of total phosphatide content. The dominating influence of lecithin to form asymmetric aggregates resulted in the mixed micelles showing a greater dissymmetry ($z = 2{\cdot}27$) than the dissymmetries shown by lysolecithin ($z = 1{\cdot}08$) and lecithin ($z = 1{\cdot}44$) independently; the shape was also closest to that of the lecithin micelles. The symmetrical lysolecithin micelle has been shown to possess a maximum dimension of less than 400 Å—this was extended in the presence of lecithin to the high value of 1,500 Å.

The shape and dimension of the particles suggest that the lecithin was not actually solubilised within the lipophilic region of the lysolecithin micelle in the conventional manner—this is restricted somewhat by the large hydrophilic phosphorylcholine head group of the lecithin molecule. Furthermore, the symmetry of the lysolecithin micelle would probably

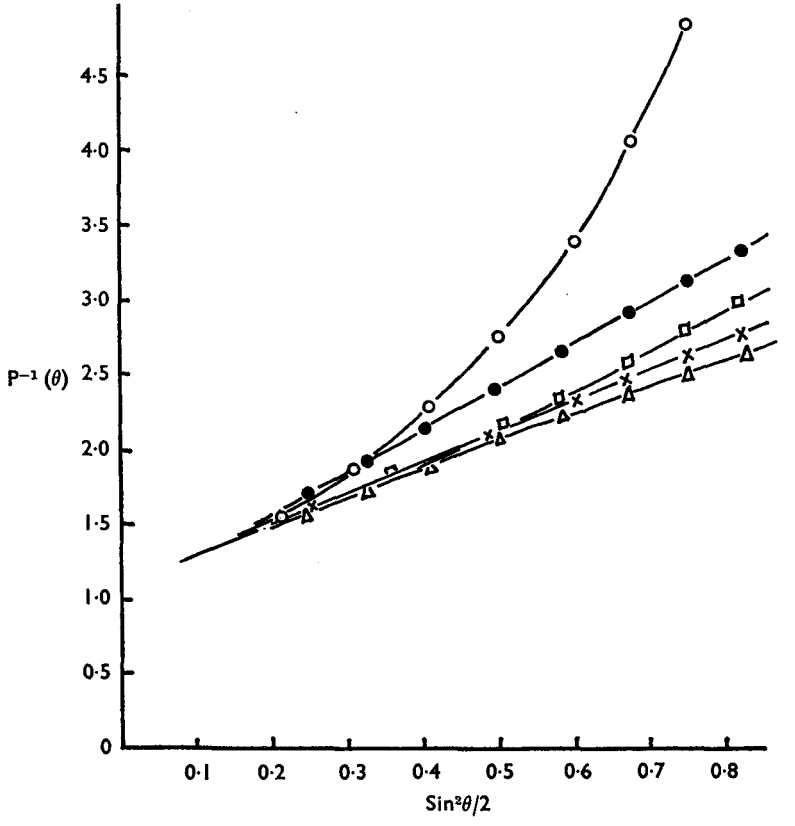

FIG. 2. $P^{-1}(\theta)$ functions for the sols and for models.
× Mixed lysolecithin-lecithin sols.
○ Sphere.
△ Rod.
□ Monodisperse coil.
● Polydisperse coil.

not have been disturbed significantly on solubilisation of a mol fraction of lecithin amounting to one-tenth of total phosphatide. It is more probable that the surface of a lysolecithin micelle was impregnated with single lecithin molecules orienting themselves in a manner adjacent to the lysolecithin molecules, though not without causing considerable distortion of the spherical shape. The particles of the sol were thus regarded as evenly distributed mixed micelles rather than macromolecules composed of a lecithin nucleus within a lysolecithin sheath surrounded by an aqueous medium.

If the assumption is made that the particles were composed of the mole fractions of phosphatide components used in the preparations, then the numbers of lysolecithin and lecithin monomers present in the mixed micelles were 2,630 and 280 respectively. This represents a 14-fold increase in the number of lysolecithin monomers in the mixed micelle over those present in a micelle of lysolecithin alone.

The increase in depolarisation of this system compared with pure lysolecithin sols resulted mainly from an increase in anisotropy, since the turbidity of the mixed sols was low and hence the secondary scatter minimised.

Acknowledgement. I thank Dr. L. Saunders for his continued interest throughout this work.

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