A LIGHT-SCATTERING STUDY OF LYSOLECITHIN SOLS

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Sols of four samples of lysolecithin have been studied by means of a light-scattering apparatus. The results of a large number of measurements have been analysed statistically. They indicate that the mean molecular weight of the micelles in the sols is 92,400, the experimental error in this estimate being 7 per cent.

LIGHT-scattering measurements can be used to provide information about the size and shape of macromolecules, a method we have applied to the further studies of the physical chemistry of phosphatide sols.

The theoretical foundations of light-scattering phenomena were laid by Rayleigh in 1871[1]. Since Debye's[2,3] recent development of the theory the method has made a large contribution to the understanding of biological substances, high polymers and other macromolecules.

The apparatus constructed in our laboratory is based on that described by Hughes, Johnson and Ottewill[4]; it was calibrated with Ludox (a silica sol) and several organic solvents. The apparatus which is shown in plan, in Figure 1, was then tested by examining aqueous sols of some fractionated proteins and non-aqueous sols of high polymers; all gave molecular weights in agreement with values obtained by other workers using this technique. The instrument was found to be suitable for molecular weight determinations in the range 5,000 to 500,000.

Before commencing a series of light-scattering experiments on phosphatide sols we have examined the reproducibility of determinations of the molecular weight of lysolecithin by making a large number of light-scattering measurements with aqueous sols of four different preparations of this substance.

EXPERIMENTAL

Four different samples of lysolecithin (A, B, C and D) were prepared by the action of viper venom on hen egg lecithin. Aqueous sols of this substance were obtained as previously described[5] and centrifuged at 8,300 *g*. Before the light-scattering measurements were made each sol was filtered through a fine sintered glass frit (1 μ) under a pressure of 10 cm. of mercury, to remove traces of dust.

The sol was transferred to a rectangular glass cell ($5 \times 5 \times 1$ cm.^{3}) and placed in a thermostat jacket in the path of a beam of parallel light of wavelength 4,358 Å. The light was scattered in all directions by the micelles in the sol and the intensity of scattering at 90° to the incident beam was measured by means of an 11-stage photomultiplier tube connected directly to a mirror galvanometer. The symmetry of the light scattered about the 90° angle was also examined, all the scattered intensities being related to those obtained with a standard lead glass block.

The depolarisation factor for the scattered light at 90° was 0·0105. The dissymmetry I_{50}/I_{130} (where I is the intensity of scattered light and the subscript is the angle to the incident beam) for the samples A, B and D was 1·08 whilst for the sample C it was 1·17. All measurements were taken at a temperature of 20°.

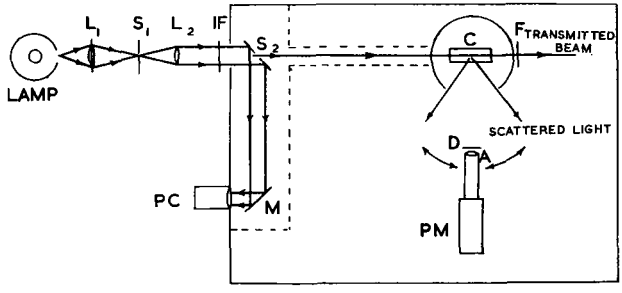

FIG. 1. Diagrammatic sketch of apparatus.

L_1 L_2 = Lenses. S_1 S_2 = Slits.
IF = Interference filter isolating $\lambda = 4358$Å.
F = Neutral density filter.
D = Polaroid disc for measuring depolarisation of scattered light.
A = Aperture. M = Mirror .
C = Cell immersed in thermostat-jacket.
PC = Photocell (connected via switch to galvanometer) for measuring intensity of incident beam.
PM = 11-stage photomultiplier (connected via switch to galvanometer) for measuring intensity of scattered light.

The specific refractive index increment of lysolecithin in water was determined by means of a Rayleigh interference refractometer modified for monochromatic light as described by Bauer[6]. This quantity, included in the constant K of the equation below, was found to be 0·1377.

METHOD

For approximately spherical molecules, the relation between light scattered at 90° to the incident beam, characterised by the term R_{90}, and concentration X per cent w/v is given by the equation[7]: $\frac{K.X}{R_{90}} = \frac{1}{M_w} + BX$, where K is a constant containing optical terms for a given system. R_{90} is calculated from the light scattered at 90° corrected for the scattering attributed to pure solvent and for depolarisation effects. Corrections for the dissymmetry and concentration of unassociated molecules[8] were small and are not included. M_w is the weight-average molecular weight of the solute and B is a correction term for non-ideality,—in ideal solutions it is zero. If $K.X/R_{90}$ is denoted by Y, then the plot of Y/X should give a straight line which, when extrapolated to X = O, gives an intercept Y_o equal to the reciprocal of the molecular weight of the solute.

RESULTS

When experimental values of Y were plotted against X they showed a random variation about the best straight line through the points (see Fig. 2). This is attributed to experimental error in the scattering measurements; relative to this, the error in the values X of concentration may

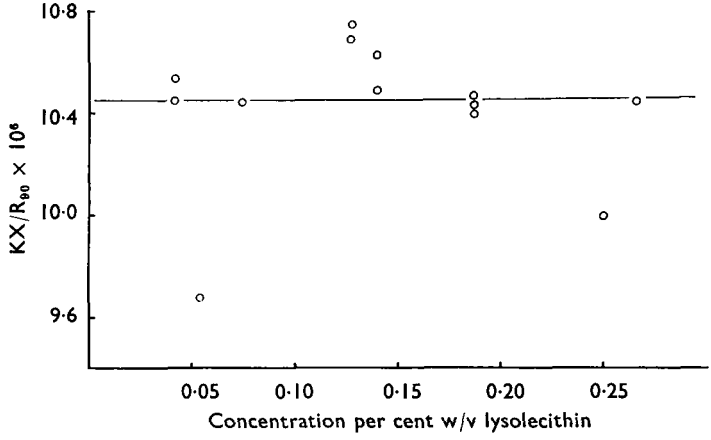

FIG. 2. Light-scattering of lysolecithin (B) in water at 20°.

be considered to be negligible. To obtain the best value of Y_o a regression analysis was made; this had the advantage of giving limits of error for the final estimate of molecular weight.

Y/X correlation coefficients were first computed for all the results obtained with each of the four samples of lysolecithin and these are given in Table I. For samples A, B and D the correlations were not significant,

TABLE I

Y/X CORRELATION AND REGRESSION COEFFICIENTS

Sample	N	Range of X	r	Theoretical r (P = 0·95)	Significance of r	b
A	53	0·06–1·00	−0·063	0·27	none	−0·306
B	13	0·04–0·25	−0·079	0·56	none	−0·403
C	13	0·02–0·12	0·793	0·56	significant	20·65
D	37	0·002–0·27	0·197	0·33	none	2·646

N is the number of scattering measurements made with each sample. X is the concentration of lysolecithin in g./100 ml. Y is the scattering quantity $K.X/R_{90}$ (see text). r is the correlation coefficient calculated from the results. The theoretical values of r for a probability of 0·95 were obtained from Fisher and Yates tables[9]. b is the regression coefficient[10] Y/X.

meaning that the slopes of the regression lines were not significantly different from zero. In these samples, therefore, the simple mean $\bar{Y}$ of the values of Y could be taken to be the reciprocal of the molecular weight. However, this treatment probably underestimates the experimental error in M_w and so in these samples the regression coefficient was also calculated and the extrapolated value $Y_o = \bar{Y} - b\bar{X}$ found. The values of Y_o were little different from $\bar{Y}$, as expected by the insignificant correlation of Y and X, but their limits of error which take into

account both the variance of Y and of the regression coefficient (see Appendix), are somewhat wider and probably more realistic.

The molecular weight estimates for samples A, B and D agree within their limits (see Table II) and so a grand mean total could be calculated. The limits for this were found to be 86,700 and 98,600 at P = 0·99 with a grand mean of 92,400. The experimental error in these molecular weight estimates from light-scattering appears to be about 7 per cent.

For sample C, different results were obtained. The Y/X correlation was clearly significant (Table I) and so $\bar{Y}$ could not be used in place of Y_o for estimating the molecular weight. The distribution of the X, Y points about the regression line was wider than in the previous samples

TABLE II

ESTIMATES OF MOLECULAR WEIGHT OF LYSOLECITHIN MICELLES

Sample	Section (i)			Section (ii)		
	$\bar{Y} \pm$ L.E. $\times 10^6$	M_w from $\bar{Y}$	Limits	$Y_o \pm$ L.E. $\times 10^6$	M_w from Y_o	Limits
A	11·37 ± 0·49	88,000	84,300–91,900	11·48 ± 0·80	87,100	81,400–93,600
B	10·41 ± 0·29	96,000	93,500–98,800	10·46 ± 0·67	95,600	89,900–102,100
C	r is significant			6·34 ± 1·19	157,700	132,800–194,200
D	10·28 ± 0·39	97,300	93,700–101,000	10·06 ± 0·63	99,400	93,500–106,000

In Section (i):

$\bar{Y}$ is the arithmetic mean of the values of Y for a sample. L.E. given by $\pm t\sqrt{(V/N)}$, are the P = 0·99 limits of error for $\bar{Y}$, where V is the variance of the values of Y about the mean and t is a theoretical quantity derived from tables. M_w is the reciprocal of $\bar{Y}$ and the limits are the reciprocals of ($\bar{Y}$ − L.E.) and ($\bar{Y}$ + L.E.) respectively.

In Section (ii):

Y_o is the extrapolated value of Y at X = O (see Appendix). L.E. are the limits of error for Y_o for P = 0·99, calculated as $\pm t\sqrt{(V_{Y_o})}$, the variance of Y_o is calculated as shown in the Appendix. M_w and the limits are the reciprocals of Y_o and its limiting values respectively.

and the limits for the molecular weight estimate were more than twice as wide as those for samples A, B and D. The mean molecular weight was very much higher and differed significantly from the other three, there being no overlapping of the limits. Sample C was recrystallised from a batch prepared some months earlier and it may have contained traces of fatty acids. This conclusion is supported by the observation that the dissymmetry factor for C was higher than for A, B and D. In view of the wide limits of error for the estimate of molecular weight obtained from sample C (−16 and +23 per cent) we have felt justified in omitting it from the grand mean molecular weight of 92,400.

The results for sample C illustrate the uncertainty of physical measurements on materials derived from biological sources; in such work it is desirable that many results should be obtained with different samples of the substance. The results can then be summarised by making a statistical analysis.

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Appendix

Variance of Extrapolated Value Y_o

From Figure 3 it is seen that if b is the slope of the regression line and $\bar{X}$, $\bar{Y}$ are the means of all the values of X and Y respectively, then since the regression line always passes through the point $\bar{X}$, $\bar{Y}$

$$Y_o = \bar{Y} - b\bar{X}$$

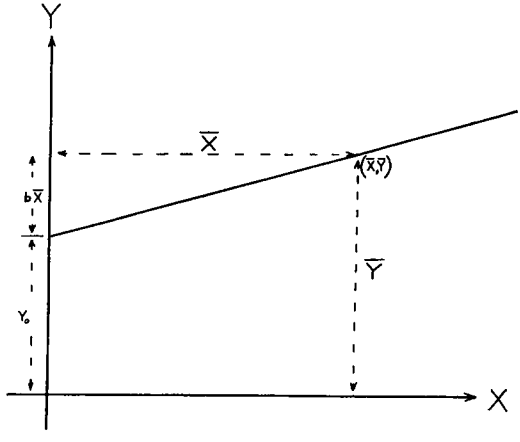

FIG. 3. Regression line Y/X.

$\bar{Y}$ is subject to random error and so is b, but the variance in X (the concentration of a solution) is small compared with the variance of values of Y; $\bar{X}$ can therefore be regarded as having no variance. The variance of Y_o is then given by $V_{Y_o} = V_{\bar{Y}} + \bar{X}^2 V_b$, both $V_{\bar{Y}}$ and V_b can be estimated from the set of values of Y and X.

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After Dr. Robinson presented the paper there was a DISCUSSION.