

VISCOSITY STUDIES WITH PHOSPHATIDE SOLS

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Received June 1, 1958

THE viscosities of mixed lecithin-lysolecithin sols pass through a high maximum as the relative proportions of the two phosphatides is varied, although sols of the separate components are found to have viscosities differing little from that of water¹. We have studied this effect further.

Preparations. Lecithin and lysolecithin were prepared from egg yolk¹. The mixed sols, containing a phosphatide weight fraction of lecithin less than 0.6, were prepared by weighing the dried solids obtained by evaporating to dryness under reduced pressure stock solutions in ethanol of the two materials. The solid mixture was dissolved in a minimum amount of warm ethanol which was then evaporated at 15 mm. pressure to leave a film of intimately mixed phosphatides. This was dispersed to give a clear sol by shaking with warm distilled water. Traces of electrolytes were removed by passing the sol through a column of mixed ion exchange resins.

Sols containing a weight fraction of lecithin greater than 0.6 were made by mixing lysolecithin sols obtained by dissolving the dried solid in warm water, with lecithin sols prepared as follows. A weighed quantity of dry lecithin was dissolved in a small volume of ether; to this, distilled water was added in successive small portions, with vigorous shaking. A smooth white emulsion of lecithin was at first formed which, with more water, became a thick gel and then a translucent sol. Nitrogen was then bubbled through to remove the ether and the sol was out-gassed on a filter pump. Finally, it was passed over mixed ion exchange resins and made up to volume with distilled water. This method could not be used in the presence of lysolecithin, because of the formation of stable foams during the nitrogen bubbling stage.

Viscosity studies with a capillary viscometer at 25°. *Effect of ageing.* Ageing of the sol had a considerable effect on the mixed sols of high viscosity. A sol of lecithin weight fraction 0.4 and total phosphatide content 0.4 per cent (w/v) had a relative viscosity of 10.0 one hour after preparation; this fell to 4.1 after 5 hours, 3.1 after 15 hours, and to a constant value of 3.0 after 24 hours. In view of these results, subsequent measurements were made with sols which had been aged for 24 hours.

Effect of lecithin weight fraction. A series of sols were prepared which contained 0.4 per cent (w/v) total phosphatides with varying weight fractions of lecithin. The results obtained were similar to those described previously¹, with a maximum relative viscosity of 3.22 at a lecithin weight fraction of 0.45. They are summarised in Table I.

Effect of total phosphatide concentration. The viscosities of the sols in which the total phosphatide concentration was varied between 0.1

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and 1 per cent at fixed lecithin weight fractions of 0.2, 0.4 and 0.6 are shown in Table II.

TABLE I
RELATIVE VISCOSITIES OF LECITHIN-LYSOLECITHIN SOLS AT 25°

γ	η	γ	η
0	1.02	0.580	1.92
0.20	1.11	0.600	1.37
0.325	1.62	0.650	1.31
0.375	2.87	0.800	1.22
0.449	3.22	0.900	1.17
0.500	2.43	1.000	1.11
0.550	2.20		

Total phosphatide concentration, 0.4 per cent w/v.
 γ = weight fraction of lecithin; η = relative viscosity.

TABLE II
VARIATION OF VISCOSITY WITH TOTAL PHOSPHATIDE CONCENTRATION AT 25°

C	$\gamma = 0.2$			$\gamma = 0.4$			$\gamma = 0.6$		
	$10^3\phi$	η_{sp}	η_{sp}/ϕ	$10^3\phi$	η_{sp}	η_{sp}/ϕ	$10^3\phi$	η_{sp}	η_{sp}/ϕ
0.10	0.92	0.05	54	0.93	0.28	303	0.93	0.11	118
0.21	1.93	0.05	26	1.94	0.66	340	1.95	0.28	144
0.31	2.85	0.08	28	2.87	1.06	370	2.89	0.50	173
0.44	4.04	0.14	35	4.07	1.78	437	4.10	0.83	203
0.58	5.24	0.16	31	5.27	3.11	590	5.31	1.78	335
0.69	6.34	0.19	30	6.38	4.06	636	6.42	3.44	535
0.83	7.63	0.22	29	7.68	5.67	738	7.73	5.50	712
1.00	9.19	0.28	30	9.25	55.2	5970	9.31	58.2	6250

γ = weight fraction of lecithin; c = total phosphatide concentration in g./100 ml. of sol.; η = relative viscosity; $\eta_{sp} = \eta - 1$; ϕ = volume fraction of total phosphatide.

These results are interesting because they give some idea of the shape of the macromolecules present. Values of specific viscosity $\eta_{sp} = \eta - 1$ are shown in Table II together with η_{sp}/ϕ where ϕ is the volume fraction occupied by the total phosphatide in the sol.

For the sols of lecithin weight fraction 0.2, the specific viscosity is low and apart from the first two results where the experimental error in η_{sp} is high, the value of η_{sp}/ϕ is independent of total concentration, having a mean value of 30. These results indicate that the macromolecules in these sols of low lecithin content are spherical or very nearly so, a conclusion which is supported by our diffusion measurements. The high value of η_{sp}/ϕ compared with Einstein's theoretical value of 2.5, suggests that the aggregates are hydrated.

With sols of lecithin fraction 0.4, the viscosity-concentration relationship is completely different. The specific viscosities are high and rise sharply with increasing concentration. The values of η_{sp}/ϕ vary with concentration, the plot of η_{sp}/ϕ against ϕ is linear for the more dilute solutions and can be extrapolated to give an intrinsic viscosity $[\eta]$, where $[\eta] = (\eta_{sp}/\phi)_{\phi=0}$ of 274.

The results for the sols of lecithin weight fraction of 0.6 are similar to those of fraction 0.4, the extrapolated intrinsic viscosity being 93 in this case.

The high viscosities of the sols with lecithin fractions of 0.4 and 0.6 and the variation of their values of η_{sp}/ϕ with ϕ , indicate that highly

asymmetric macromolecules are present in these sols. Application of the Simha equation², for the intrinsic viscosity of sols containing ellipsoidal particles indicates that if the macromolecules are rod-like entities, their axial ratios (length/diameter) would be 65 and 34 for the sols having lecithin fractions of 0.4 and 0.6 respectively.

If, as is more likely with these phosphatides which readily form film-like structures, the macromolecules are present as plates, then application of another form of the Simha equation² gives values of the ratios, plate diameter/plate thickness, of 404 and 137 for these two sols.

The above results are deduced without taking into account the undoubted hydration of the macromolecules, for example 30 per cent hydration would reduce the ratios given above by about 15 per cent for rod-like macromolecules and 25 per cent for plates. It will be interesting to see how these conclusions about particle asymmetry agree with studies of the sols by other physical methods. The decision whether the particles are in fact rod-shaped or disc-shaped cannot be made on the basis of the information available.

Measurements with a cone and plate viscometer at 25°. The apparent relative viscosity of the thick sols, measured by means of an Ostwald viscometer, was found to vary somewhat with the diameter of the capillary. In order to investigate the effect of rate of shear on the apparent viscosity, some measurements have been made with a Ferranti-Shirley cone and plate viscometer with which the shear rate could be varied between 20 and 20,000 sec.⁻¹.

A highly viscous sol was used for this study, having a lecithin fraction of 0.4 and a total phosphatide concentration of 2 per cent. Shear stresses at varying shear rates were measured. The plot of stress against rate showed that the viscosity was non-Newtonian at low rates but became Newtonian, with a linear relationship between stress and rate, at rates above 500 sec.⁻¹. The apparent relative viscosity was 35 at the lowest shear rate measured, 84.5 sec.⁻¹, and fell to a constant value of 4.2 at shear rates above 500 sec.⁻¹.

TABLE III
EFFECT OF TIME INTERVAL ON IMMEDIATE SHEAR STRESS OBTAINED IN A SECOND SHEARING OF A VISCOUS SOL, AT 25°

<i>t</i>	<i>S</i>	<i>t</i>	<i>S</i>
0	154	5	129
1	80	6	135
2	86	7	142
3	117	8	154
4	123		

Total phosphatide content of sol, 2 per cent w/v; weight fraction of lecithin, 0.4. Each shearing made at a rate of shear of 84.5 sec.⁻¹, for 2 seconds.

t = time interval in minutes between two shearings.

S = immediate shear stress in dynes cm⁻².

The change in the apparent relative viscosity on increasing the shear rate may be due to the large asymmetric particles present in the sol becoming orientated in the direction of flow, or it may be due to the

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fact that the association of micelles is quite loose and can be broken down to a large extent mechanically. The thixotropic nature of the sol was established by applying a low shear rate for 2 seconds to the sol at varying time intervals and observing the immediate stress produced. The results are shown in Table III. It is seen that at short time intervals, low stresses are obtained at the second shearing, some of the structure in the sol having been broken down by the first shearing. With longer time intervals, however, this structure is able to re-form and with an 8 minute interval, a stress equal to the immediate stress obtained in the first shearing, was found.

We thank Professor E. Shotton for the use of the cone and plate viscometer.

REFERENCES

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2. Simha, *J. Phys. Chem.*, 1940, **44**, 25.

DISCUSSION

The short communication was presented by DR. L. SAUNDERS.

THE CHAIRMAN. It might be helpful if the solutions were examined in polarised light between polarised screens. Had any progress been made with the purification of the phosphatides? Were they satisfied that the lysolecithins had the α and β structures quoted?

DR. F. HARTLEY (London). It was intriguing that after great precautions to remove electrolytes the proportion of lecithin and lysolecithin giving the highest relative viscosity was nearly equimolecular. Was this profoundly affected by electrolytes?

DR. SAUNDERS replied. He had emphasised throughout that these preparations must be regarded as fractions. The samples were not the same as those used last year. There were variations in the physical properties of some of the batches but the viscosities did not vary greatly. It was not possible to obtain the products as single pure substances. The effect of electrolytes was the next step, but preliminary results suggested that the effect would not be great. He was not so sure that the equimolecular ratio of lecithin and lysolecithin was very significant.