THE PHYSICAL PROPERTIES OF LYSOLECITHIN AND ITS SOLS

PART III. VISCOSITY

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The viscosity of pure lysolecithin sols increased approximately linearly with concentration and had a very small negative temperature coefficient. The viscosity of the sols increased in alkaline conditions when distinct ageing effects became apparent which were found to be irreversible on neutralisation of the sols. The viscosities of mixed sols of lysolecithin-cholesterol, lysolecithin-triolein and lysolecithin-monostearin have also been investigated; the two former systems did not show marked viscosity changes. In contrast, the lysolecithin-monostearin sols became very viscous with increasing monostearin concentration and with a rise in temperature gels were eventually formed which were stable for at least a month. Cholesterol introduced as a third component into the mixed lysolecithin-lecithin sol was found to lower the viscosity of the latter system.

AN examination has been made of the viscosity of lysolecithin sols and of sols of lysolecithin in combination with three other biologically important substances of different chemical structures, namely, cholesterol, triolein and monostearin. The effect of temperature on lysolecithin sols was studied, and also the increase in their viscosity in alkaline conditions, the ageing and the irreversibility of the viscous behaviour due to the alkaline conditions.

Investigations by Thomas and Saunders[1] on mixed lysolecithin-lecithin sols have shown that these phosphatides interact to form quite viscous systems depending on the ratio of the two components. It was thought that the viscosities of mixed sols of lysolecithin and other biological substances and their ageing effects would indicate the nature and strength of the different intermolecular forces between polar groups and hydrophobic regions which contribute to membrane structure in biological systems.

EXPERIMENTAL

Materials

Lysolecithin was prepared by treating lecithin with Russell viper venom by the method previously reported[2]. Analysis of the sample showed a nitrogen content of 2·72 per cent, a phosphorus content of 5·98 per cent and an iodine value of 4·5; the mean molecular weight calculated from the nitrogen and phosphorus contents was 516. Lecithin was prepared from egg yolk by the method outlined by Saunders[2]. Analysis of the sample gave a nitrogen content of 1·73 per cent, a phosphorus content of 3·82 per cent and an iodine value of 72; the mean molecular weight was calculated to be 809. Cholesterol (B.D.H. commercial) was recrystallised

twice from absolute ethanol. M. p. 147·6°. Triolein (B.D.H. commercial) was not redistilled on account of the likelihood of breakdown; after treatment with activated charcoal it retained a slight amber colour, its boiling point being 242°/18 mm. Monostearin (B.D.H. commercial) was recrystallised twice from ether. M. p. 81·5°.

Pure lysolecithin sols were prepared as previously described[3]. Mixed sols of lysolecithin and the second (lipid) component were prepared by adding the required amount of lysolecithin, dissolved in ethanol, to the

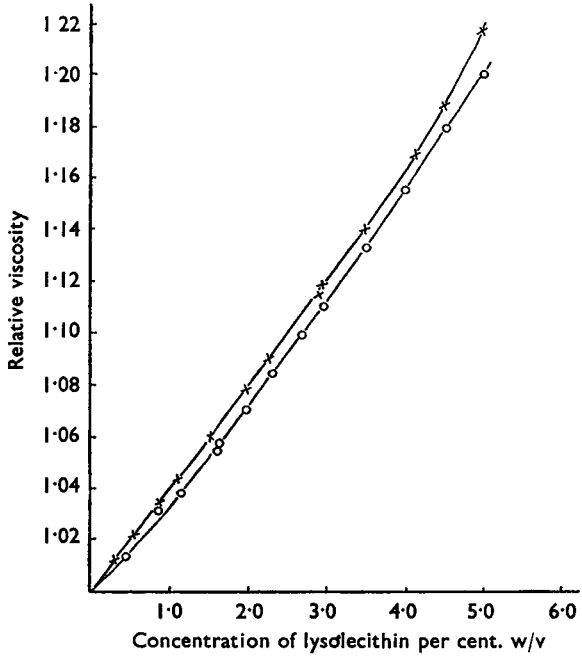

FIG. 1. Pure lysolecithin sols. ×, 25°: ○, 40°.

weighed quantity of second component. The solutes were mutually dissolved with the aid of a few drops of chloroform where necessary and the solution evaporated to dryness at 15 mm. pressure to leave an intimately mixed film of the two components. This was dispersed in distilled water, agitated on a Microid flask shaking machine at 40° for 1 hour and passed down an ion exchange column to remove traces of electrolytes. All the mixed sols contained a constant amount of 0·5 per cent w/v of lysolecithin.

Buffer solutions ranging from pH 9·2 to pH 12·66 were prepared by the method of Sörenson-Walbum.[4] Above pH 12·66 pure NaOH was used and neutralisation was by 10 N HCl. Inorganic and organic reagents used were Analar grade; the organic reagents for density determinations were redistilled and dried.

Reversibility of Sols in Alkaline Solution

Eight 10 ml. portions of 0·1 per cent w/v lysolecithin sols, made up in N NaOH, were examined for reversibility of the viscous state on neutralisation. 10 N HCl was added to each sol using an Agla syringe, the sols having stood for different lengths of time. Addition of the acid was sufficiently slow to allow stirring and observation of any separation which took place.

Density of Dry Lysolecithin

The density of dry lysolecithin was determined by the pyknometer method, using benzene and acetone as displacement liquids.

TABLE I

EFFECT OF TEMPERATURE AND TIME ON THE VISCOSITY (ηREL.) OF PURE LYSOLECITHIN SOLS

Sol of concn. 1 per cent w/v				Sol of concn. 2 per cent w/v			
Temp.	ηrel.	Time in hours	ηrel. at 25°	Temp.	ηrel.	Time in hours	ηrel. at 25°
17½	1·039	0·5	1·038	17½	1·080	0·5	1·077
25	1·038	1·5	1·038	25	1·078	1·5	1·078
32½	1·036	2·0	1·040	32½	1·074	2·0	1·078
40	1·033	6·0	1·041	40	1·070	6·0	1·077
		9·0	1·038			9·0	1·078
		24	1·039			24	1·077
		48	1·040			48	1·076

Apparatus

Ostwald capillary viscometers (Cannon-Fenske Nos. 50, 100 and 200) were used, supported in a water thermostat controlled to $\pm$ 0·05°; a constant volume (10 ml.) was delivered into the viscometers for each reading. The pyknometer made for density measurements had a capacity of approximately 5 ml. and was ground and stoppered at both ends; displacement liquids used were benzene and dry acetone. A Cambridge Bench pH meter was used to follow changes in the pH of lysolecithin sols.

TABLE II

VARIATION OF VISCOSITY OF PURE LYSOLECITHIN SOLS WITH pH. TEMP. 25°

C.	pH	ηrel.	C.	pH	ηrel.	C.	pH	ηrel.	C.	pH	ηrel.	C.	pH	ηrel.
	9·22	1·003		9·22	1·024		9·22	1·026		9·22	1·028		9·22	1·029
	10·32	1·004		10·32	1·025		10·32	1·028		10·32	1·031		10·32	1·033
0·01	11·14	1·007	0·04	11·14	1·025	0·07	11·14	1·030	0·1	11·14	1·033	0·25	11·14	1·033
	12·66	1·020		12·66	1·028		12·66	1·036		12·66	1·044		12·66	1·046
	13·10	1·025		13·10	1·056		13·10	1·075		13·10	1·080		13·10	1·086
	13·95	1·070		13·95	1·140		13·95	1·156		13·95	1·169		13·95	1·180

C. = Concentration per cent w/v lysolecithin
ηrel. = relative viscosity of sol

RESULTS

The density of dry lysolecithin by the pyknometric method using benzene as displacement liquid was 1·024, and with dry acetone 1·0184, giving a mean of 1·0212.

The variation of viscosity of pure lysolecithin sols (relative to water) with concentration at 25° and 40° is shown in Figure 1. The effect of temperature and time on the relative viscosity of pure lysolecithin sols of concentrations 1 and 2 per cent w/v are shown in Table I. The tempera-

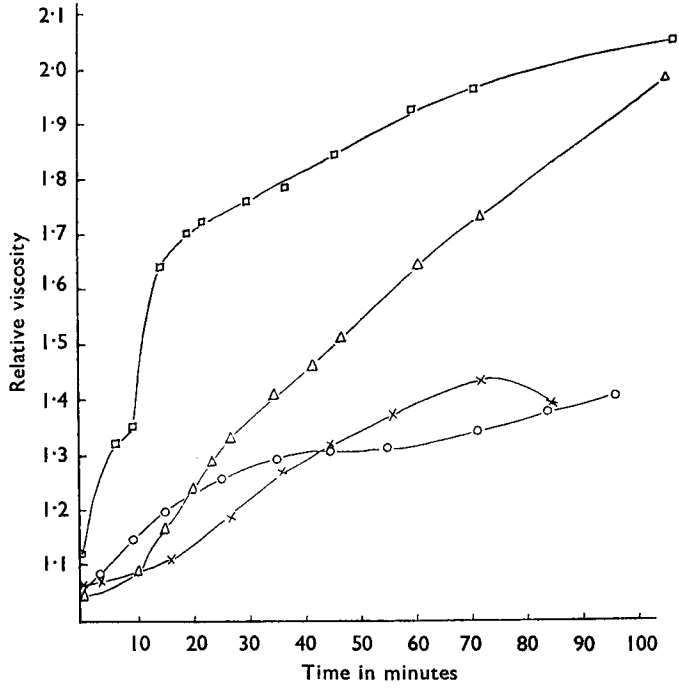

FIG. 2. Pure lysolecithin sols. pH = 13·1. ×, 20°: ○, 25°: △, 32·5°: □, 40·0°.

ture coefficient for the 1 per cent sol increased negatively from 8×10^{-5} to 5×10^{-4} and the 2 per cent sol from 2 to 6×10^{-4} between 17·5° and 40°. The activation energy for the viscous flow of these sols according to the equation[5] $\frac{\eta_0}{\eta} = \exp(E_A/RT)$ was 4 k.cal. mole^{-1}.

TABLE III

VARIATION OF VISCOSITY OF LYSOLECITHIN-MONOSTEARIN SOLS WITH CONCENTRATION OF MONOSTEARIN AT 25° AND 40°

	ηrel.			ηrel.			ηrel.	
C.	25°	40°	C.	25°	40°	C.	25°	40°
0·05	1·02	1·03	0·30	1·15	1·19	0·60	1·38	1·29
0·10	1·03	1·08	0·40	1·22	1·21	0·80	1·63	1·57
0·20	1·07	1·13	0·50	1·34	1·24	1·00	2·01	2·00

Concentration of lysolecithin: 0·5 per cent w/v
C. = Concentration per cent w/v monostearin
ηrel. = relative viscosity of sol

The increase in viscosity with an increase in pH from 7 to 14 for different concentrations of lysolecithin sols is shown in Table II. The ageing effect of a typical lysolecithin sol (0·1 per cent w/v) under strong alkaline conditions (pH 13·1) is shown in Figure 2.

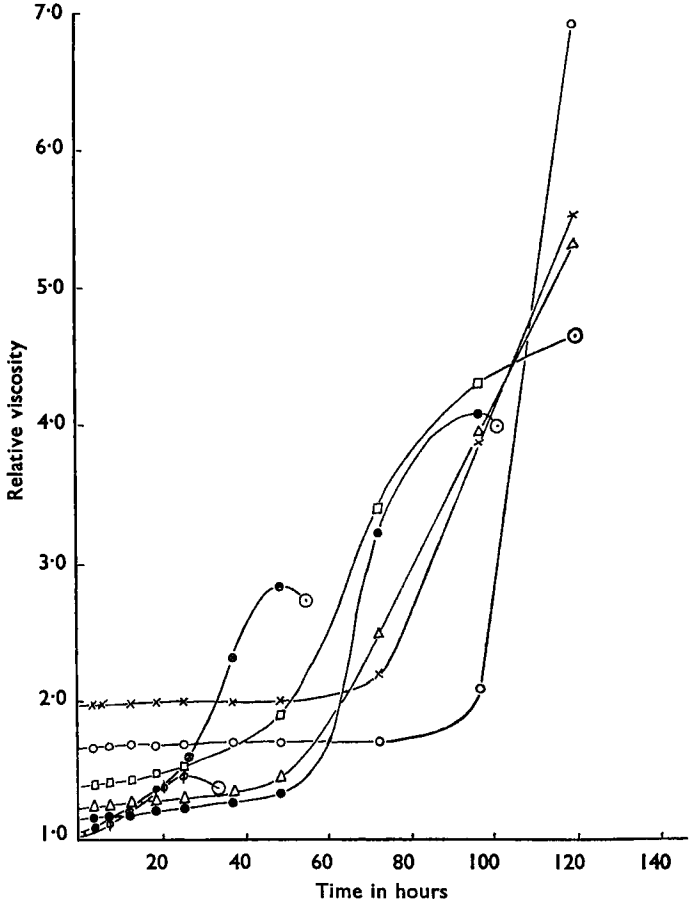

FIG. 3. Lysolecithin monostearin sols. Temp. 25°.

ϕ —0·1 per cent w/v monostearin
⊗—0·2 " " "
●—0·3 " " "
△—0·4 " " "
□—0·6 " " "
○—0·8 " " "
×—1·0 " " "
⊙ denotes separation of the sol.

The relationship between the viscosity of lysolecithin-monostearin sols and concentration of monostearin is shown in Table III. The effect of time on these sols is shown in Figure 3; when the sols begin to show separation the curves are terminated thus ⊙.

The variation of viscosity of lysolecithin-cholesterol sols with concentration of cholesterol and the variation of viscosity of lysolecithin-triolein sols with concentration of triolein is shown in Table IV.

TABLE IV

VARIATION OF VISCOSITY OF LYSOLECITHIN-CHOLESTEROL AND LYSOLECITHIN-TRIOLEIN SOLS WITH CONCENTRATIONS OF THE CHOLESTEROL AND TRIOLEIN COMPONENTS. TEMP. 25°

Lysolecithin-cholesterol sols			Lysolecithin-triolein sols					
	ηrel.			ηrel.			ηrel.	
C_{ch}	25°	40°	C_{tr}	25°	40°	C_{tr}	25°	40°
0·05	1·041	1·032	0·02	1·018	1·019	0·15	1·023	1·020
0·10	1·042	1·036	0·04	1·019	1·019	0·20	1·024	1·021
0·30	1·043	1·036	0·06	1·021	1·020	0·35	1·028	1·023
0·50	1·047	1·039	0·10	1·022	1·018	0·50	1·033	1·030
1·00	unstable							

Concentration of lysolecithin: 0·5 per cent w/v
C_{ch} = Concentration per cent w/v cholesterol
C_{tr} = Concentration per cent w/v triolein

TABLE V

EFFECT OF CHOLESTEROL AS A THIRD COMPONENT ON THE VISCOSITY OF LYSOLECITHIN-LECITHIN SOLS. TEMP. 25°

Lysolecithin-lecithin sols		Lysolecithin-lecithin-cholesterol sols	
Conc. of lecithin per cent w/v	ηrel.	Conc. of each of lecithin and cholesterol per cent w/v	ηrel.
0·25	7·50	0·25	1·20
0·45	7·75	0·45	1·21

Concentration of lysolecithin 0·5 per cent w/v

TABLE VI

EFFECT OF NEUTRALISING 0·1 PER CENT W/V SOLS OF LYSOLECITHIN MADE VISCOUS BY ALKALINE CONDITIONS. TEMP. 25°

Sample	Viscosity of alkaline sol	Remarks
1	1·27	*After* 3 *min.* on neutralising to pH 7 slight opaqueness appeared; after 10 min. at pH 6 separation took place.
2 & 3	1·25 & 1·27	*After* 3 *min.* on neutralising to pH 7 slight opaqueness appeared; immediate separation on acidifying to pH 4.
4	1·26	*After* 5 *min.* on adding HCl to pH 4 separation took place.
5	1·22	*After* 10 *min.* and acidifying to pH 6 viscosity dropped to 1·073.
6	1·27	*After* 10 *min.* alkalinity reduced to pH 8; 2 hours after, viscosity found to be unchanged but on neutralising it dropped to 1·06 and separation took place.
7	1·28	*After* 30 *min.* excess HCl added very slowly, drops passed through sol and formed acid layer at bottom. Lysolecithin separated on shaking.
8	1·24	*After* 90 *min.* on neutralising complete separation took place.

The viscosities of two lysolecithin-lecithin sols at 25° are shown in column (*a*) of Table V, and sols prepared in the same manner, but including cholesterol equal in concentration to the lecithin component, are shown in column (*b*).

Effects of neutralisation of strong alkaline (pH 13·9) 0·1 per cent w/v lysolecithin sols are shown in Table VI.

Discussion

Lysolecithin Sols

The relative viscosity of lysolecithin sols at 25° and 40° increases approximately linearly with increasing concentrations up to 5 per cent. The ratio $\frac{\eta_{sp}}{\phi}$ is greater than the value of 2·5 calculated by Einstein for spherical particles in dilute solutions, which is attributed to hydration and solvation of the particles. However, application of Guth and Simha's equation[6] ($\eta_{rel} = 1 + 2{\cdot}5\phi + 14{\cdot}1\phi^2$) which introduces a second virial coefficient to account for the mutual interactions of the disturbed flow region around each suspended sphere, gave a better relation; the second coefficient was calculated to be 14·5 and 12·1 at 25° and 40° respectively.

Using viscometers with different capillary bores gave inconsistent values of relative viscosity for a typical sol, indicating that the sols showed non-Newtonian behaviour. This property can be attributed to orientation effects of the particles because the particles, though spherical, are probably not rigid and distortion takes place to a different extent with different velocity gradients of flow. All subsequent experimental work on lysolecithin sols was done using the same viscometer.

The viscosity of the lysolecithin sols decreased slightly with temperature indicating that the thermal motion at higher temperatures caused a breaking down of some of the aggregates. It is suggested that little or no increase in hydration took place with an increase in temperature, the smaller particles formed after breakdown must have been hydrated to the same extent indicating uniform hydration of single molecules and aggregates. The temperature coefficient of a 1 per cent w/v lysolecithin sol was small, increasing negatively from 8×10^{-5} at 17·5° to 5×10^{-4} at 40°; the intrinsic viscosities of the sols at these temperatures were 3·38 and 3·16 respectively.

Application of the Arrhenius type of equation for viscosity of liquids suggested by Barrer[5] ($\frac{\eta_0}{\eta} = \exp(E_A/RT)$ where E_A is the activation energy of viscous flow) gives a value of 4 k.cal./mole which is slightly higher than that for pure water (3,940 cals./mole) within a similar temperature range. This activation energy, a prerequisite for viscous flow, agrees with the assumption that the particles are spherical and have a negligible degree of co-ordinated structure.

Effect of Alkaline Conditions. Increasing the pH of the lysolecithin sols from neutral to strongly alkaline, showed an initial slight increase in viscosity up to pH 12·6 followed by a rapid, steady rise in viscosity to pH 14 (Table II)—a parallel behaviour was shown by all concentrations of the sols. An examination of the ageing effect of a typical (0·1 per cent w/v) sol in alkaline solution (pH 13·1) at four different temperatures (Fig. 2) showed that a large rise in viscosity took place at each temperature the process being more rapid at higher temperatures.

Reversibility. The increase in viscosity of lysolecithin sols with increasing alkalinity was found to be irreversible when the sols were slowly

neutralised. On neutralisation, or lowering the pH still further, separation of a substance took place which would not re-dissolve on shaking. Alkaline hydrolysis was therefore slowly taking place and on reducing the pH below 7 free fatty acid separated out.

Lysolecithin-Cholesterol Sols

The viscosity of these sols was similar to that of pure lysolecithin sols. The results indicated that a higher rate of increase in viscosity took place in sols containing less than 0·05 per cent w/v cholesterol; between 0·05 and 0·5 per cent w/v cholesterol the viscosity appears to increase proportionately to the concentration of cholesterol; above 0·5 per cent w/v the cholesterol started to separate out before measurements could be completed (Table IV). The viscosity was slightly less throughout the concentration range of cholesterol at 40° than at 25°. The viscosity measurements indicate that there was no asymmetry; although the lath-shaped cholesterol molecules are bulky ($7{\cdot}5 \times 4{\cdot}5 \times 20$ Å^3) and are not solvated in the aqueous medium, they appear to have been solubilised within the lysolecithin aggregates which retained the spherical shape, with little interaction between individual spheres.

Lysolecithin-Triolein Sols

The viscosities of the lysolecithin-triolein sols increased approximately linearly with increasing fractions of triolein within the concentration range measured (Table IV). The maximum increase in viscosity was not very large and the uniform increase in viscosity indicated that spherical particles could be assumed. In this two-component system some expansion of the spheres was thought to take place but they were likely to retain their elasticity and distance apart, and thus not restrict the rate of shear.

Lysolecithin-Monostearin Sols

Aqueous lysolecithin-monostearin sols showed a distinctly different viscous behaviour from the two-component systems previously discussed, in some aspects resembling the viscosity of mixed lysolecithin-lecithin sols reported by Thomas and Saunders.[1]

Instantaneous values of viscosity increased with increasing concentration of the monostearin fraction, the viscosity being slightly greater at 40° than at 25° (Table III). This increase in viscosity with temperature may be due to distortion of the particles and since the particles have increased energy of Brownian rotation at higher temperatures more particles will consequently lie across the line of flow.

The effect of time showed a distinct ageing of the sols which increased the viscosities considerably at higher concentrations—clearly orientation was taking place. For small concentrations of monostearin the sols were unstable but the stability increased with the concentration of monostearin fraction to such an extent that the sol containing 0·8 per cent w/v monostearin (approaching equi-molecular fractions of each component) remained a gel for a week. This sol appeared homogeneous, was reversible and showed some thixotropic behaviour although reversion to a gel took

place within one or two days. The 1 per cent w/v monostearin sol showed greater elasticity but otherwise behaved in the same way. Both sols were stable for the time of keeping which was a month.

Monostearin possesses hydrophilic character due to the dipolar free hydroxyl groups and is dispersible in water whilst lysolecithin itself can be regarded as a derivative of a monoglyceride (probably closest to β-monopalmitin), the only essential difference between the two molecules being a phosphate-choline group conferring complete water solubility on lysolecithin. The viscous behaviour of the lysolecithin-monostearin sol therefore seems to be connected with the ionic head group of lysolecithin. Ion-dipole interaction between the lysolecithin head group and the monostearin free hydroxyl groups will probably be the greatest contributing factor in the viscous effect. Adhesion of the saturated hydrocarbon chains by van der Waal's forces will help to associate and orient the molecular species to form intermolecular links in a wide network in accordance with their film-forming properties. The intermolecular links may have been sufficiently large and rigid to force the complex out of solution, resulting in the gelated form.

Application of Simha's equation[7] for the intrinsic viscosity of sols containing elipsoidal particles indicates that, if the aggregates are plate-like as suggested, their axial ratios (plate diameter/plate thickness) would be, for sols containing 0·8 and 1 per cent w/v monostearin, 72 and 102 respectively at 25°, decreasing to 63 and 95 at 40°. Considering the aggregates to be rod-like in structure, another equation due to Simha gives axial ratios (length/diameter) for the same sols at 25° of 27 and 22 respectively decreasing to 26 and 20 at 40°.

Lysolecithin-Lecithin-Cholesterol Sols

Mixed sols of lysolecithin and lecithin have been shown to be quite viscous. A preliminary study of the introduction of cholesterol into this system showed that the viscosity of the previous two-component system could not be attained, and considerable deposition of the cholesterol took place immediately after the shaking action to solubilise the substances was stopped. It is quite possible that cholesterol can be introduced into the lysolecithin-lecithin system and the viscosity of the three component system retained using another technique. These results indicate that cholesterol initially inhibited the formation of lysolecithin and lecithin into a network of macromolecules which may be attributed to the dipole interaction of the free hydroxyl group or double bond of cholesterol, or to the bulky asymmetric condensed rings weakening van der Waal's forces binding the hydrocarbon chains of the phosphatide molecules.

Saunders[2] has shown that lysolecithin loses its lytic action when present in sols with certain weight fractions of lecithin; whether the penetration of cholesterol dipole linkages into the lysolecithin-lecithin macromolecules will restore the lytic properties of lysolecithin is to be investigated.

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