THE PHYSICAL PROPERTIES OF LYSOLECITHIN AND ITS SOLS

PART IV. SOLUBILISATION

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The solubilisation of triolein, cholesterol and monostearin in water by lysolecithin has been investigated by the optical density method. These substances solubilised to a considerable extent, increasing in the order—triolein, cholesterol and monostearin. An increase in temperature of 15° had little effect on solubilisation of any of the three substances. The change in optical density with concentration of the solubilised component showed a similar pattern for the three substances, but the mixed sols formed had different stabilities. Lysolecithin-triolein sols contained only small proportions of triolein and were fluid and stable for no longer than 12 hours whilst lysolecithin-monostearin sols containing high proportions of monostearin formed gels and were stable for at least a month. Lysolecithin-cholesterol sols were unstable showing an initial rise and then a steady decrease in optical density over a period of 72 hours for given concentrations as slow separation of cholesterol was taking place. Factors operative in the solubilisation of each of these three substances by lysolecithin have been suggested.

The clearing action of lysolecithin on aqueous homogenates of whole rat brain and other biological substances has recently been reported[1]. The property of lysolecithin to solubilise in heterogeneous systems might be very different from its action on the pure components in these systems. We thought it desirable, therefore, to investigate more closely the solubilisation of certain pure biological substances by lysolecithin in an aqueous medium and some factors operative in bringing about this solubilising action. The following substances were examined—cholesterol, chemically reactive and insoluble in water; triolein, representative of a typical fat, chemically inactive (except at ethenoid linkages) and also insoluble in water; monostearin, chemically reactive and dispersible in water.

EXPERIMENTAL

Methods of preparation of the sols are described elsewhere[2]. All mixed sols contained a constant amount of 0·5 per cent w/v of lysolecithin. The sols were centrifuged before each optical density reading.

The optical densities of the sols were measured in a Spekker Absorptiometer (Hilger 506) incorporating H508 neutral density filters and a filament lamp. The sols and pure solvent were contained in matched cells of 5 cm. path length. The absorption spectrum of an aqueous 4 per cent w/v pure lysolecithin sol and the absorption spectra of 4 per cent w/v cholesterol, triolein and 0·4 per cent w/v monostearin solutions, using chloroform as solvent, were measured at room temperature with a Hilger Uvispek.

RESULTS

The variation of optical density of lysolecithin-triolein sols with concentration of triolein is shown in Figure 1. The variation of optical density of lysolecithin-cholesterol sols with concentration and time is shown in Figures 2 and 3, and for lysolecithin-monostearin sols in Figures 4 and 5.

Pure 0·5 per cent w/v lysolecithin sols (approximately 10^{-2} molar) themselves showed an optical density of less than 0·001 in the 5 cm. cell.

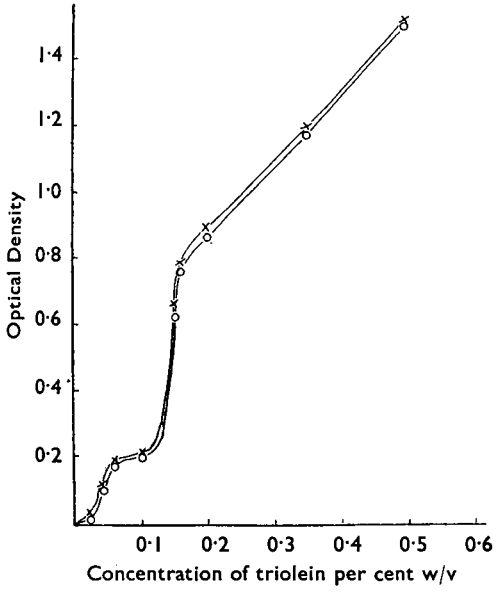

FIG. 1. Lysolecithin-triolein sols. ×, 25°: ○, 40°.

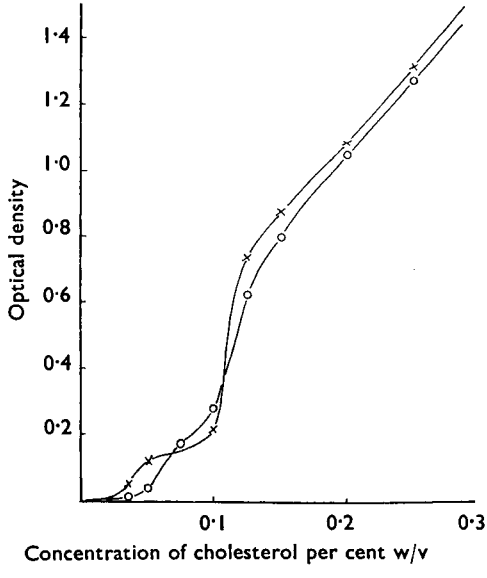

FIG. 2. Lysolecithin-cholesterol sols. ×, 25°: ○, 40°.

Spectrophometric readings for a 4 per cent w/v aqueous pure lysolecithin sol contained in a 4 cm. cell are shown in Table I. Readings for 4 per cent w/v cholesterol and triolein and 0·4 per cent w/v monostearin solutions using chloroform as a solvent are also given. The absorption spectrum within the visible region of the lysolecithin sol showed that light was not predominantly absorbed at any particular wavelength; the spectra of cholesterol, triolein and monostearin showed a similar behaviour.

DISCUSSION

Solution of the three substances examined must be mainly attributed to the solubilising property of lysolecithin since they do not themselves dissolve appreciably in water. The systems showed an increasing tendency to solubilisation by lysolecithin in the order triolein, cholesterol and monostearin. The ageing of the sols varied and did not follow a pattern; these effects and possible mechanisms of solubilisation are discussed below.

Lysolecithin-Triolein. Triolein showed the least tendency to solubilisation by lysolecithin, requiring, for example, a seven-fold ratio of lysolecithin to obtain an arbitrary optical density value of 0·2. The optical

TABLE I

EXTINCTION COEFFICIENTS (E) FOR DIFFERENT WAVELENGTHS WITHIN THE VISIBLE REGIONS FOR EACH COMPONENT. TEMP. 20°

λ(Å)	Aqueous lysolecithin sol*	Cholesterol in chloroform*	Triolein in chloroform*	Monostearin in chloroform†
4200	0·242	0·097	0·382	0·024
4400	0·212	0·088	0·274	0·022
4600	0·192	0·079	0·206	0·020
4800	0·172	0·074	0·152	0·018
5000	0·155	0·069	0·117	0·017
5200	0·140	0·066	0·092	0·016
5400	0·128	0·059	0·074	0·015
5600	0·117	0·056	0·059	0·014
5800	0·108	0·053	0·049	0·013
6000	0·097	0·050	0·038	0·012

* Concentration 4 per cent † Concentration 0·4 per cent

density thereupon increased rapidly for increasing concentrations of triolein (Fig. 1). The solubilising power of lysolecithin in this system was influenced very little by an increase in temperature of 15°, and attempts to solubilise more triolein by shaking the sols for periods of up to 18 hours

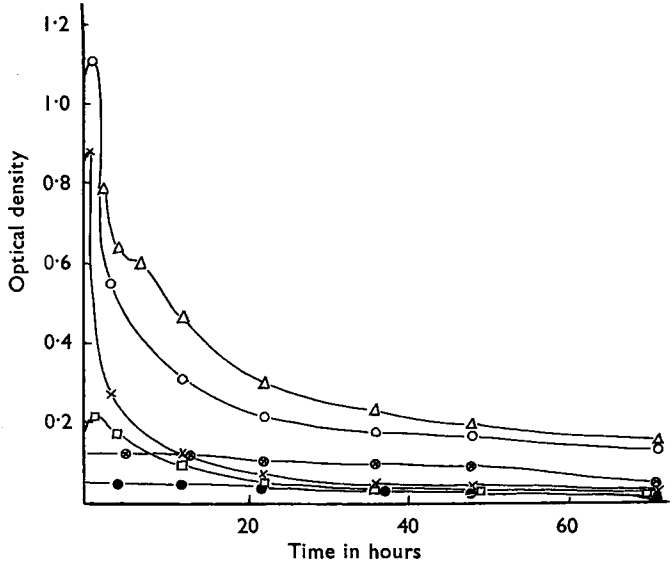

FIG. 3. Lysolecithin-cholesterol sols. Temp. 25°.
●—0·025 per cent w/v cholesterol
⊗—0·05 ,, ,, ,,
□—0·10 ,, ,, ,,
△—0·125 ,, ,, ,,
×—0·15 ,, ,, ,,
○—0·20 ,, ,, ,,

at 40° were unsuccessful. All sols were stable for 12 hours but a film of emulsified triolein formed on the surface of sols containing more than 0·1 per cent w/v triolein after 24 hours, although there was no marked change in optical density during this period and the following 48 hours.

It is probable that steric factors mainly prevent ready solubilisation of triolein in the micelles of lysolecithin. Double bonds in each fatty acid chain of the molecule will inhibit formation of a close-packed micelle and weaken hydrophobic association with the hydrocarbon chain of lysolecithin. The unsaturated linkages situated midway along the hydrocarbon chains of the triolein molecule are probably not suitably placed to undergo dipole-dipole or dipole-ion interaction with the head group of lysolecithin within the micelle.

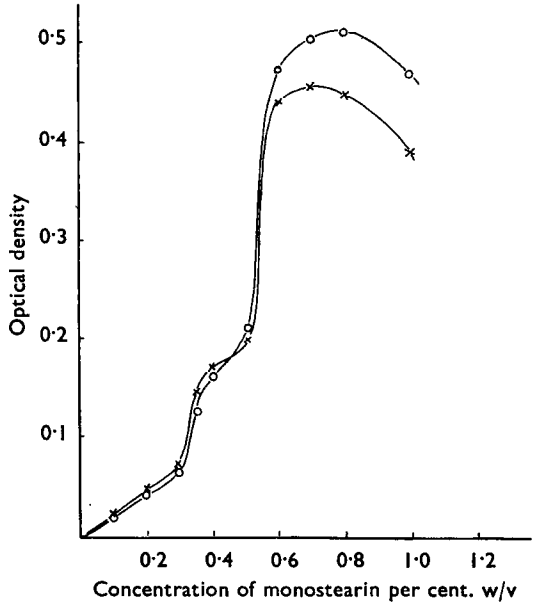

FIG. 4. Lysolecithin-monostearin sols. ×, 25°: ○, 40°

Although both substances were intimately mixed after evaporation of the mutual solvent it is probable that some particles of the triolein were many times bigger than a lysolecithin micelle and formed an emulsified phase which, on shaking and allowing to stand, produced the film observed after 24 hours.

Lysolecithin-Cholesterol. Cholesterol required only four times its molar quantity of lysolecithin to give the optical density value of 0·2 quoted for triolein. Again the optical density rose sharply for increasing quantities of cholesterol, an increase of 15° in temperature having small effect (Fig. 2). The ageing of lysolecithin-cholesterol sols was different, showing an initial rise in optical density within the first 5 hours followed by a gradual decline indicating slow separation which continued for another 67 hours (Fig. 3).

Strong dipole forces between the hydrophilic groups of each substance probably helped the lath-shaped cholesterol molecule to orient itself parallel to a lysolecithin molecule with its free hydroxyl group outwards, but its bulkiness ($7{\cdot}5 \times 4{\cdot}5 \times 20$ Å^3) would prevent close packing within a spherical micelle. In this mixed sol it is not possible to distinguish clearly between the lysolecithin as a solubilising substance and as a protective colloid.

The interfacial tensions between triolein and water and cholesterol and water are high and it is to be expected that a large excess of lysolecithin would be required to lower the interfacial tensions sufficiently to solubilise these substances completely. The latent heat of fusion involved in the transition of cholesterol from the solid into the liquid state when solubilised in the aqueous sol will also oppose its solubilisation.

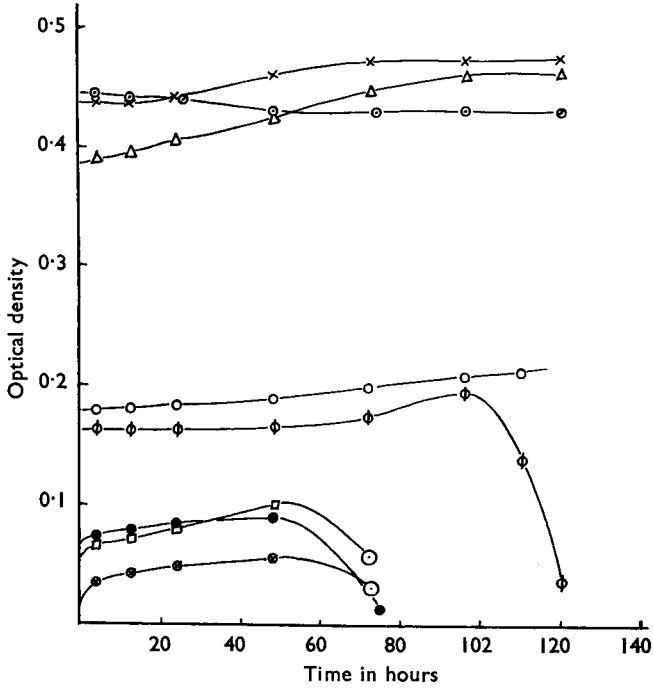

FIG. 5. Lysolecithin-monostearin sols. Temp. 25°.
⊗—0·1 per cent w/v monostearin
□—0·2 ,, ,, ,,
●—0·3 ,, ,, ,,
ϕ —0·4 ,, ,, ,,
○—0·5 ,, ,, ,,
×—0·6 ,, ,, ,,
⊙—0·8 ,, ,, ,,
△—1·0 ,, ,, ,,
⊙—denotes separation of monostearin.

Lysolecithin-Monostearin. Monostearin was solubilised in a mol ratio of 1·34 to 1 of lysolecithin at the optical density value of 0·2, the optical density subsequently increasing rapidly to a maximum for a mol ratio of 2:1. In this region the amount solubilised was slightly decreased by a 15° rise in temperature (Fig. 4). Sols containing small fractions (below 0·5 per cent) of monostearin were unstable after 48 hours when the monostearin separated quite rapidly (Fig. 5), but sols of higher proportions of monostearin formed gels which increased their stability and no separation was observed after a month.

Monostearin possesses a hydrophilic head group and a lipophilic region which gives the substance some degree of amphipathic character. In lysolecithin, which has a similar lipophilic region, this property is considerably increased by the presence of the phosphate-choline group giving the molecule complete water solubility and high surface activity due to the balance between the lipophilic and hydrophilic regions. The similarity in structure of these substances enables the monostearin molecules to orient themselves within the micelle, parallel and adjacent to the lysolecithin molecules, with maximum hydrocarbon chain adhesion by van der Waal's forces and dipole-dipole and dipole-ion interaction of the head groups exerting attractive Coulombic forces.

REFERENCES

1. Webster, *Nature, Lond.*, 1957, **180**, 660.
2. Robinson and Saunders, *J. Pharm. Pharmacol.*, 1959, **11**, 304.