CCXLI. THE PHYSICO-CHEMICAL BEHAVIOUR OF LECITHIN.

III. THE ELECTROPHORETIC BEHAVIOUR OF LECITHIN-CHOLESTEROL DISPERSIONS.

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THE work described in this paper is a continuation of that commenced by H. I. Price and Lewis [1929] and extended by Jones and Lewis [1932].

The former measured, by means of a dynamic method, the surface tension of aqueous dispersions of pure lecithin as a function of p_H . It was found that a maximum value of the surface tension occurred at p_H 2.6 (approx.) in close agreement with the value of the isoelectric point (p_H 2.7) found by Fugii [1923-24] from distribution measurements¹. The surface tension falls to a very marked minimum on either side of the maximum referred to.

Jones and Lewis investigated the effect of the presence of pure cholesterol in the lecithin dispersion. It was found, using the same surface tension method as H. I. Price and Lewis [1929], that the addition of cholesterol, whilst leaving the general capillary behaviour of lecithin as a function of p_H unchanged, nevertheless introduces modifications of the surface tension values. The most important modification is a shift in the position of the maximum in the surface tension- p_H curve from p_H 2.6 to p_H 4.

This appears to indicate a corresponding shift in the position of the isoelectric point. But, as pointed out by H. I. Price and Lewis the evidence is not sufficient to make this certain. It is with this point, in the main, that the present work on the electrophoresis of lecithin-cholesterol mixtures deals.

EXPERIMENTAL.

Pure lecithin, prepared by the method of Levene and Rolf [1927], was used for these measurements.

Cholesterol itself does not disperse in water. It is, however, peptised by lecithin and is easily dispersed in the presence of this substance.

The method of preparation of dispersions of the mixed material was similar to that used by Jones and Lewis [1932]. Equimolecular weights of lecithin and cholesterol (approximately 2 g. of lecithin : 1 g. of cholesterol) were dissolved together in ether. The ether was then evaporated at room temperature. The whole of the mixture was dispersed by shaking with the correct quantity of water and the dispersion kept in an atmosphere of nitrogen. The dispersion was found to be quite stable and no separation of either of the constituents took place.

¹ The value of the effective isoelectric point of lecithin has since been determined directly by electrophoresis measurements and found to agree with the value p_H 2.7 [cf. C. W. Price and Lewis, 1933].

For the electrophoretic mobility measurements, aliquot parts of the above dispersion were diluted with citrate buffer to give a dispersion containing 0.05 % lecithin and the requisite proportion of cholesterol.

The apparatus and method of measuring the mobility and also the method of preparing and buffering the dispersions follow that recently described by C. W. Price and Lewis [1933]. The addition of 2 % of sucrose to the dispersions was found again to facilitate the adjustment and maintenance of a sharp boundary without affecting the mobility in any way.

The values of the velocities obtained in cm./sec. were corrected to 20° by multiplying by the factor η_x/η_{20} , where η_x and η_{20} are respectively the viscosities of water¹ at the temperature of measurement and at 20°.

As before, the p_H values below 7 were measured by the gold quinhydrone electrode [Corran and Lewis, 1924]; for higher values of p_H the glass electrode [Harrison, 1930] was used.

A representative record of an electrophoretic mobility determination is given:

Composition of the dispersion, 0.05 % lecithin; 0.025 % cholesterol (approx.); 2 % sucrose; $N/100$ citrate buffer.

Composition of the supernatant liquid: citrate buffer of the same ionic concentration and p_H (to within 0.01 p_H unit) as the dispersion.

Temp., 20.5°; $p_H = 5.87$; current, 3.0 milliamps; potential gradient, 2.80 volt/cm.

Movement of boundary:

Anode limb (upwards)	Cathode limb (downwards)	Time (mins.)
0.0	0.0	0
5.5	5.5	10
10.5	10.5	20
15.5	16.0	30

Mean movement, 15.75 scale divisions in 30 mins. (1 scale division = 0.043 cm.)

Electrophoretic velocity under a potential gradient of 1 volt/cm. at 20° = 13.0×10^{-5} cm./sec.

Table I shows the variation of the electrophoretic mobility with p_H of dispersions of mixtures of lecithin and cholesterol in equimolecular proportions.

Table I. *Showing the variation of electrophoretic mobility with p_H values of dispersions of mixed lecithin and cholesterol.*

(The sign denotes the sign of the charge.)

p_H	Electrophoretic velocity under a potential gradient of 1 volt/cm. at 20° cm./sec.
8.15	-28.5×10^{-5}
6.95	-23.6 "
6.48	-19.4 "
5.87	-13.0 "
5.22	-10.65 "
4.89	-9.9 "
4.05	-7.65 "
3.30	-4.6 "
2.51	+4.9 "
2.30	+10.4 "

¹ The ratio η_x/η_{20} for the solutions used is not sensibly different from that of water at the same temperature.

In order to compare the behaviour of the above mixture of lecithin and cholesterol with that of lecithin alone, the mobility- p_H curve for lecithin, reproduced from the above-mentioned publication of C. W. Price and Lewis, is plotted as a dotted line and the data of Table I as open circles in Fig. 1.

Fig. 1.

— — — Lecithin. ○ Lecithin and cholesterol.

From Fig. 1 it will be seen that the addition of an equimolecular proportion of cholesterol to lecithin has no effect on the electrophoretic behaviour of the latter substance.

DISCUSSION.

Combination of lecithin with glucosides, enzymes, cholesterol, proteins *etc.* has been described in the literature. It is not possible, however, in the majority of these cases, to differentiate between true molecular compounds, adsorption complexes (due to local interaction at certain positions on the mutual surfaces) or merely mechanical mixtures. Loewe [1912] considers that the majority of these so-called combinations are adsorption complexes.

Moravek [1927] claims to have shown that actual compounds are formed between lecithin and cholesterol and gelatin. No evidence, however, is available to show whether, in the absence of the third substance, combination between lecithin and cholesterol takes place, although the surface tension results of Jones and Lewis [1932] point to a union of some sort.

As previously pointed out, cholesterol will not form a dispersion in water. It is, however, peptised by lecithin and is easily dispersed in the presence of this material. It is quite possible, therefore, that cholesterol behaves merely as an "inert" body, its particles in a lecithin dispersion taking on the mobility of that dispersion in much the same way that an "inert" quartz particle in a protein

dispersion takes on the mobility associated with the protein itself. In other words, the two substances most probably exist together, in a dispersion, as an adsorption complex.

Further, it will be observed that no change in the position of the isoelectric point results from the addition of cholesterol to lecithin. It follows therefore that the points of union in the adsorption complex do not involve the amphoteric groupings of the lecithin or the groups which carry the charge due to adsorbed ions.

Clearly a shift in the position of the maximum in the surface tension- p_H curve does not necessarily imply a corresponding shift in the isoelectric point. This is borne out further by the effect of calcium chloride upon the surface tension maximum and electrophoretic speed respectively; the maximum being shifted towards the more acid region, the effective isoelectric point to the alkaline side.

SUMMARY.

The mobility- p_H relationship of dispersions of lecithin + cholesterol in equimolecular proportions has been determined. It is shown that no change in the mobility at various p_H values or in the position of the isoelectric point is produced by the addition of cholesterol to lecithin. It follows that the bonds or groups responsible for the formation of the lecithin-cholesterol complex cannot be the amphoteric groups of the lecithin or those on which ion adsorption takes place.

In conclusion the writer would like to express his thanks to Prof. W. C. M. Lewis, under whose direction this work was carried out.

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