CCLXIX. THE ISOELECTRIC POINTS OF LECITHIN AND SPHINGOMYELIN.

By ERNST CHAIN and IAN KEMP.

From the Biochemical Laboratory and the Department of Colloids Science, Cambridge.

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The latest work on the isoelectric point of lecithin is that of Price and Lewis [1933] investigating the macrocataphoresis of lecithin dispersions. As in previous work it was noted that the electrical properties of lecithin show a gradual change with the time. Thus the (presumably negative) mobility of a freshly prepared dispersion "increased, at given p_H, with the age of the lecithin used, reaching a stable value after 3 weeks," when the isoelectric point was found to be at p_H 2·7.

Price and Lewis attempted to obtain the theoretical value of the isoelectric point from a consideration of the dissociation constants of glycerophosphoric acid and choline. The value of p_{Ka} was estimated as 1·4 by analogy with the dissociation constants for glycerophosphoric acid and for phosphoric acid. The value of k_b was determined from the degree of hydrolysis of choline chloride: it was obtained by measuring the p_H of solutions of the salt, and the resultant value was p_{Kb} 5·06, giving a theoretical isoelectric point of p_H 5·17.

Theoretical considerations and the direct measurements described by Fischgold and Chain [1934, 1] make it certain that the dissociation constant of choline is considerably greater than the value obtained by Price and Lewis; the latter is open to the serious objection that small amounts of free hydrochloric acid in the neutral salt may shift the p_H of the salt solution towards the acid side, thus giving a low value for the dissociation constant of the base. This point is further discussed by Fischgold and Chain [1934, 1].

Price and Lewis attribute the discrepancy between the calculated and observed isoelectric points to the adsorption of hydroxyl ions on the fatty acid portions of the lecithin. This hypothesis however is, in our opinion, unnecessary.

Thus it has been demonstrated by Fischgold and Chain [1934, 1] that freshly prepared lecithin rapidly becomes acid on standing, owing to the separation of free fatty acids, and it is therefore virtually impossible to work with a sample of lecithin which is entirely free from acid. The results of previous observers are likely to be in error on this account. Only by using freshly prepared lecithin may some idea be formed of its true isoelectric point.

Experimental.

The lecithin was prepared as previously described [Fischgold and Chain, 1934, 2] and had P 3·99 and N 1·8 %. Fresh solutions of lecithin in absolute alcohol were diluted with buffer solutions to form dispersions in media of known p_H. The measurements of cataphoretic mobility were made by means of a microcataphoresis apparatus of the type described by Mattson [1928; 1933].

The accuracy was of the order $0{\cdot}05 \times 10^{-4}$ cm.^{2}/volt. second. The p_H of the solutions was checked by indicators and if necessary by the hydrogen electrode and the quinhydrone electrode. The accuracy is about $\pm 0{\cdot}2$ p_H unit.

Results.

The relation between the mean cataphoretic mobility of aqueous lecithin dispersions and the p_H of the solution is shown in Fig. 1. In five different

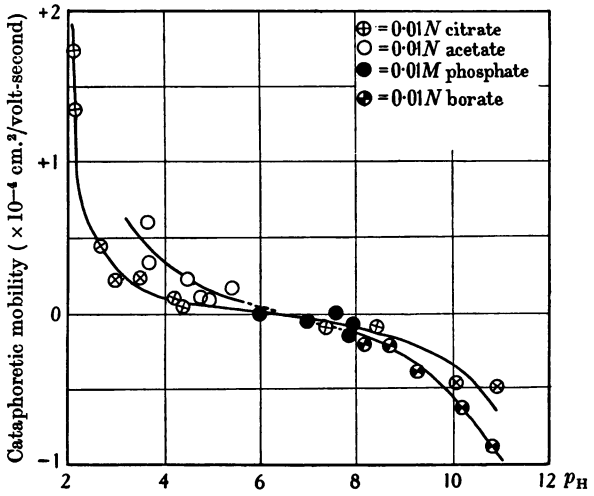

Fig. 1. Isoelectric point of lecithin.

buffers of varying ionic strength, the isoelectric point was found to be unchanged, within the limits of experimental error:

Buffer solution	Maximum ionic strength	Isoelectric point in p_H units
0·01 *N* acetate	0·01	6·5 ±0·3*
0·01 *N* citrate	0·06	6·5 ±0·2
0·01 *N* borate	0·01	7·0 ±0·3*
0·01 *M* phosphate	0·03	6·5 ±0·2
NaOH–HCl	0·001	7·1 ±0·3
	Mean isoelectric point	6·7 ±0·2

* Extrapolated.

The agreement between the isoelectric points in different buffers indicates that ionic adsorption is insignificant at these concentrations. This was confirmed by investigating the effect of the strongly adsorbed Ba^{++} ions. Thus in 0·09 *N* citrate buffer solution which contained Ba^{++} ions of concentration 0·01 *N*, the isoelectric point was found to be at p_H 6·5 ± 0·2. For higher concentrations of Ba^{++} ions strong adsorption was found to occur, no reversal of charge being obtained over a large p_H range when the concentration was 0·05 *N* (Fig. 2). Price and Lewis record a similar effect for high concentrations of Ba^{++} ions.

The values obtained for the isoelectric point of freshly prepared lecithin are sufficiently near to the theoretical value which would be expected to be 7·5 according to its dissociation constants. The fact that the observed isoelectric point

is still slightly acid may be attributed to the presence of a very small amount of free acid resulting from the rapid disintegration of the freshly prepared substance.

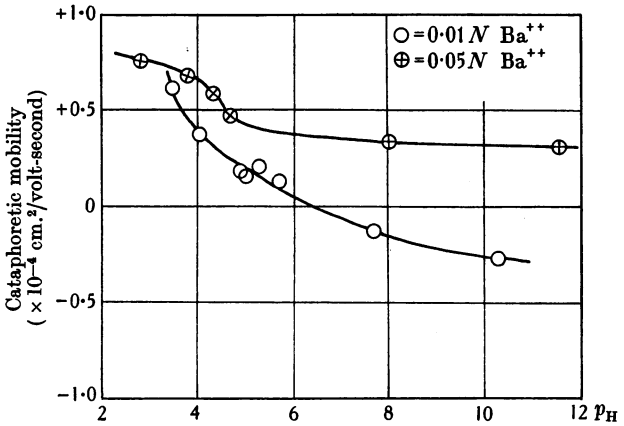

Fig. 2. Isoelectric point of lecithin.

The isoelectric point of sphingomyelin.

The structure of sphingomyelin is so similar to that of lecithin that one may reasonably expect the electrical properties of the two compounds to show close resemblances. In actual fact the acid and basic groups are identical in these two compounds, which should therefore have very similar dissociation constants and isoelectric points.

The preparation [*v.* Fischgold and Chain, 1934, 2] of sufficiently pure sphingomyelin was found to present certain difficulties; thus it contains small amounts of free acid which can be estimated by titration with sodium ethoxide in benzene solution.

The amount of impurity in different samples of sphingomyelin could be estimated by alkali titration, and it was found that on decreasing the amount of impurity (by successive purifications *via* the cadmium salt) the isoelectric point was shifted towards the alkaline side:

Numbers of purifications *via* the Cd salt	Amount of impurity ml. N/50 Na ethoxide per 50 mg. sample	Approximate isoelectric point in acetate buffer p_H
0	2·7	2
1	0·7	4
2	0·4	5
3	0·4	5

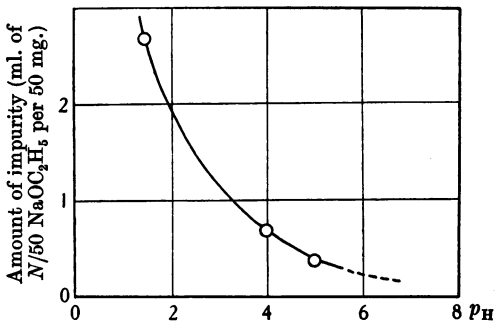

Fig. 3. Isoelectric point of sphingomyelin.

It appears that the cadmium purification is not sufficient to remove the last traces of impurity and hence, as in the case of lecithin, accurate determinations of the isoelectric point have little value, since the pure compound cannot be obtained. For this reason the determinations of the isoelectric point of sphingomyelin described above are only approximate ($\pm 0{\cdot}5\ p_H$). It is clear however that sphingomyelin with no acid impurity will have an isoelectric point above p_H 6 (see Fig. 3).

SUMMARY.

1. The observed isoelectric point of lecithin, from measurements of the cataphoretic mobility, is shown to be at p_H $6{\cdot}7 \pm 0{\cdot}2$. From the values of the acid and basic dissociation constants, the theoretical value should be p_H 7·5. The slightly acid value of the observed isoelectric point is attributed to the rapid spontaneous decomposition of lecithin, by which fatty acids are liberated.

2. The possible sources of error in the determination of previous observers are discussed.

3. Sphingomyelin contains small traces of an alkali-titratable acid impurity. By diminishing the amount of this impurity through successive purifications, the isoelectric point is shown to be shifted towards the alkaline side, and it is inferred that pure sphingomyelin will have an isoelectric point above p_H 6. This is in fair agreement with the theoretical value of the isoelectric point which for structural reasons should be the same as that of lecithin, namely at p_H 7·5.

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