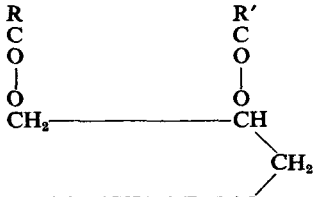
DETECTION OF LYSOLECITHIN IN A SAMPLE OF EGG LECITHIN

BY L. SAUNDERS AND J. PERRIN

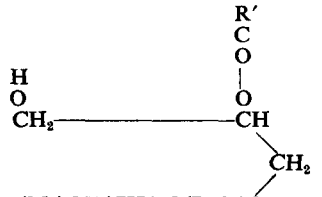
*From the Department of Chemistry, School of Pharmacy, University of London, Brunswick Square, W.C.*1

Received May 23, 1960

SMALL quantities of lysolecithin alter the physical properties of lecithin sols. This may have disadvantages if the latter is used as a suspending or emulsifying agent; or if it is injected, any lysolecithin impurities may cause unpleasant reactions. It was therefore decided to develop a method for detecting the lyso compound.



Lecithin



Lysolecithin

Methods involving molecular weight determinations are of little use for this purpose because a molecular weight variation of egg lecithin up to 10 per cent may be caused by altering the diet of the hens. Thus, methods such as osmotic pressure determination, and nitrogen and phosphorus percentage determinations are valueless. Dietary variation may also cause substantial variation in the iodine value of lecithin. The difficulty of reacting the primary alcoholic group of lysolecithin with any of the usual reagents is enhanced by possible hydrolysis of the phosphatides during reaction so liberating more primary alcoholic groups, and also because the phosphatides are thermolabile. Attempts to detect lysolecithin in lecithin samples by infra-red studies were also unsuccessful. A successful method has been developed from flocculation studies. These were based on observations made by Thomas[1] and Saunders[2]. Small quantities of lysolecithin protect a lecithin sol from flocculation by electrolytes to varying degrees. By measuring the optical density of a sol flocculated by the addition of electrolytes the presence of lysolecithin can be detected and the amount estimated.

Preparation of lecithin. The ninhydrin reacting impurities were removed by Dowex 1 × 2 50–100 mesh ion exchange resin in a bicarbonate form[3]. The lysolecithin impurities were removed by silicic acid chromatography as in reference 2. The product was recrystallised six times from methylethylketone-acetone (1–3) to give a white solid of 1·0 nitrogen: phosphorous ratio and iodine value 71.

Preparation of lysolecithin. This was prepared by the action of an aqueous solution of Russell viper venom on an ethereal solution of lecithin[2]. After four chloroform-ether precipitations the product was recrystallised twice from warm anhydrous ethanol. The crystallised product had a nitrogen : phosphorous ratio of 1·0 and an iodine value of 2 (approx.).

Infra-red studies. Potassium bromide discs containing 1 per cent lecithin or 1 per cent lysolecithin were prepared and the absorption curves determined by an Infra-cord machine. The curves were very similar and so no simple analytical procedure could be developed.

Coagulation studies. Concentrations of potassium and calcium chloride were chosen similar to those used by Thomas[1]. The optical density of the sols increases as the degree of flocculation increases if measurements are made before the precipitated phosphatides settle. All sols and solutions were prepared using ion exchanged *distilled* water.

TABLE I

TOTAL PHOSPHATIDE CONCENTRATION = 0·1 PER CENT FACTOR FOR KCl SOLUTIONS = 1·035. PERCENTAGE OF LYSOLECITHIN IN THE SOL

KCl conc. × 10^3 M	0	1	2	3	4	5	7·5	10
30·0	0·255	0·225	0·220	0·188	0·170	0·164	0·154	0·152
15·0	0·328	0·281	0·225	0·169	0·152	0·140	0·129	0·128
9·0	0·258	0·238	0·178	0·128	0·122	0·120	0·111	0·109
7·5	0·236	0·219	0·166	0·122	0·188	0·114	0·102	0·103
6·0	0·200	0·184	0·155	0·114	0·102	0·100	0·092	0·088
4·5	0·182	0·158	0·142	0·106	0·098	0·095	0·083	0·081
3·0	0·178	0·118	0·108	0·100	0·082	0·088	0·078	0·072
—	0·105	0·087	0·085	0·086	0·072	0·073	0·070	0·050

Preparation of lecithin sols. The material was dried overnight *in vacuo* and then dissolved in the minimum amount of ether. The requisite amount of water was added to give a sol of twice the strength required in the actual coagulation test. Nitrogen was then bubbled through the mixture (warmed to 30°) for 15 minutes to remove the ether and to give an even dispersion. The sol was then shaken for 30 minutes.

Preparation of lysolecithin sols. The material was dried overnight and then shaken for 30 minutes with the requisite volume of water to give a sol of twice the strength of that required in the test.

Preparation or mixed sols. In each group of tests the total phosphatide concentration was kept constant and sols of known lysolecithin content were prepared by mixing the correct ratio of lecithin and lysolecithin sols of this same concentration.

For example, if a sol containing 90 per cent lecithin and 10 per cent lysolecithin is required with a total phosphatide content of 0·4 per cent then 90 ml. of a 0·4 per cent lecithin sol is mixed with 10 ml. of a 0·4 per cent lysolecithin sol. In the tests the prepared mixed sols were diluted with an equal quantity of electrolyte solutions and thoroughly mixed. The optical densities developed after known time intervals were measured.

In the preliminary investigations $30 \rightarrow 3{\cdot}0 \times 10^{-5}$ M $CaCl_2$ and $30 \rightarrow 3{\cdot}0 \times 10^{-3}$ M KCl concentrations and the total phosphatide concentration was varied to give a suitable optical density range. KCl was found to be more suitable than $CaCl_2$ and Thomas[1] also found that lysolecithin was more effective in protecting lecithin sols against the flocculating action of potassium chloride than against the flocculating action of calcium chloride. 0·25 and 0·125 per cent phosphatide sols gave optical densities (up to 1·2) which tended to vary with time. 0·1 per cent sols were found to have suitable optical densities which reached a constant value in 2 hours (up to 0·3).

Measurements of optical density. Table I was compiled by measuring the optical densities of 0·1 per cent phosphatide sols 2 hours after preparation by means of a Spekker absorptiometer. The monochromatic source was a mercury vapour lamp together with a Hilger 605 filter (yellow-green). Five ml. samples were used in 0·5 cm. cells. All experiments were carried out at room temperature (20° $\pm$ 1·0°).

As can be seen from the Table up to 4·0 per cent lysolecithin appears to give incomplete protection to the lecithin against the electrolyte concentrations used and so the optical density tends to vary according to the protection afforded. No further protection appears to be afforded by further additions of lysolecithin and thus the optical density remains almost constant. $15 \rightarrow 7{\cdot}5 \times 10^{-3}$ M KCl concentrations give the largest changes of optical density, with small lysolecithin additions and so they are more suitable for the detection of small lysolecithin impurities in a lecithin sample.

References

1. Thomas, Ph.D. Thesis London University. 1958.
2. Saunders, *J. Pharm. Pharmacol.*, 1957, **9**, 834.
3. Saunders and Perrin, *ibid.*, 1960, **12**, 253.

After Mr. Perrin presented the paper there was a DISCUSSION.