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THE INFLUENCE OF SILICA GEL G ON THE QUANTITATIVE ANALYSIS OF LIPID PHOSPHORUS

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SUMMARY

During the determination of phospholipids separated on thin layers of Silica Gel G (Merck) by means of phosphomolybdate reaction it was necessary to use a strongly acidic medium (pH from 0.5 to 0.7), in order to prevent autoreduction of molybdate which occurs at lower acidities and interferes with the true phosphomolybdate complex. Silica Gel G seems to act as a buffer; in its presence a substantially higher acidity is necessary for suppressing the autoreduction of molybdate solutions.

There are two methods for quantifying phospholipids separated on chromatoplates: firstly by means of quantitative densitometry, which is especially useful with very small samples, and secondly by means of the determination of phosphorus after preparative chromatography and scraping off the spots. The latter method has the advantage that the results are proportional to the amounts of phosphorus on a molar basis. In both procedures, numerous modifications have been developed. According to ROUSER *et al.*[1], the maximum reproducibility in the determination of lipid phosphorus is obtained when the amount of thin-layer chromatographic (TLC) adsorbent is kept constant, when variable loss of perchloric acid is prevented by the neutralization with hydrochloric acid of silicate in the adsorbent and the avoidance of loss of fumes during digestion, and when spurious colour production after the addition of a reductant (ascorbic acid) is prevented by immediate and thorough mixing.

In our laboratory[2], phospholipids and neutral lipids are separated by means of two-step one-dimensional chromatography on Silica Gel G. The first run is carried out up to 14 cm in chloroform–methanol–water–*n*-heptane (65:25:4:9), and the second run in *n*-heptane–diethyl ether–acetic acid (95:4:1). After detection with ammonium sulphate[3] and charring, semi-quantitative densitometry is carried out with the Vitatron UDF apparatus.

In phosphorus assays by means of the FISKE–SUBBAROW[4] method with BARTLETT's modification[5] after preparative TLC on Silica Gel G (Merck), we often observed autoreduction of samples, inclusive of blanks. After many experiments we found that a very acidic medium with pH from 0.5 to 0.7 is essential for the development of the blue phosphomolybdate complex. With lower acidities (pH above 1), autoreduction

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of molybdate occurs and the resulting dye can interfere with the determination of phosphomolybdate. Fig. 1 shows a typical absorption curve for phosphomolybdate (0.05 μmole of phosphorus) in comparison with the absorptions of blanks with and without Silica Gel G prepared under insufficiently acidic conditions, leading to autoreduction. The absorption spectra were measured with an Opton spectrophotometer from 325 to 1000 nm.

The autoreduction of molybdate is suppressed by increasing the acidity in the final volume of the sample. The experiment shown in Fig. 2 indicates the development of autoreduction in blanks and in samples containing 0.05 μmole of phosphorus with and without Silica Gel G and the suppression of colour formation as the acidity of the final solution is increased. Silica Gel G seems to function as a buffer: a substantially higher acidity is necessary for suppressing the autoreduction of the solutions (standards and especially blanks.) Colour development in samples with standards in the presence of Silica Gel G differs from that in samples without Silica Gel G. The optimal reaction medium is shifted to 6 % sulphuric acid concentration in the final volume. After charring with perchloric acid, the pH changes in a different

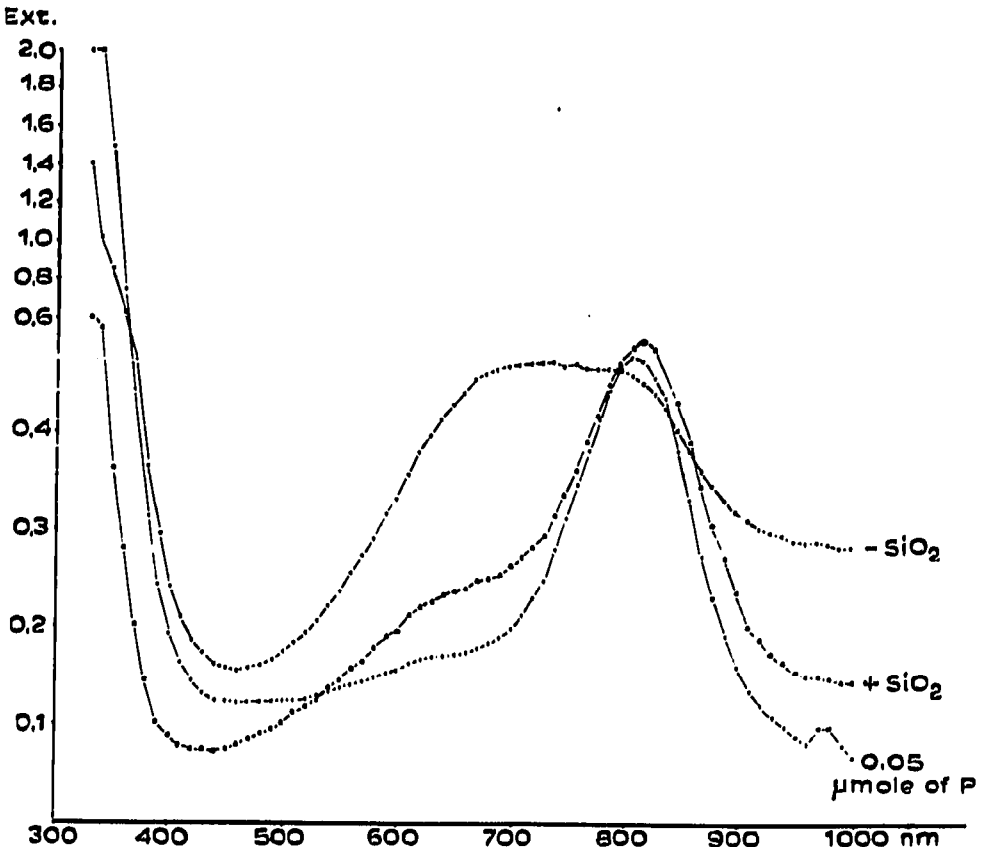

Fig. 1. Absorption spectrum of phosphomolybdate (0.05 μmole of phosphorus) (■—■); molybdate (autoreduction at lower acidity) blank without Silica Gel G (●—●); silicomolybdate blank (autoreduction at lower acidity) (▲—▲).

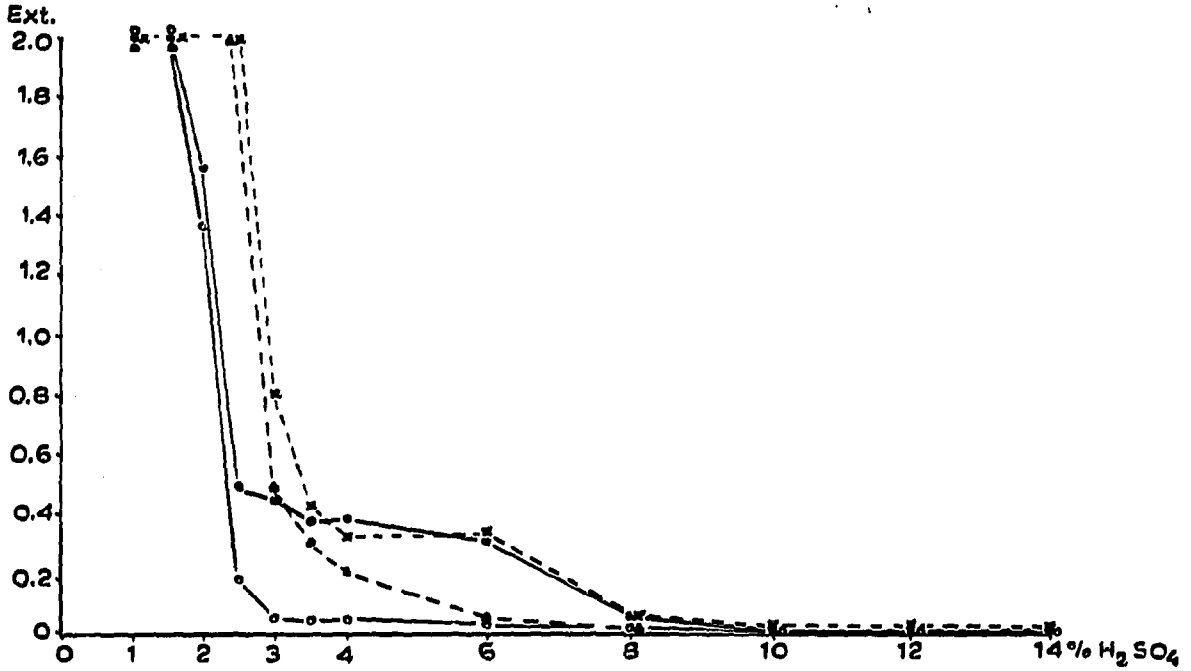

Fig. 2. Comparison of absorption spectra of phosphomolybdate with and without Silica Gel G at various acidities. ●—●, 0.025 μmole of phosphorus without Silica Gel G; ○—○, blank without Silica Gel G; ×---×, 0.025 μmole of phosphorus with Silica Gel G; ▲---▲, blank with Silica Gel G.

manner in samples with and without Silica Gel G. Silica Gel G acts as a catalyst in supporting the mineralization, probably because of its large surface area.

During the sedimentation of the Silica Gel G suspension, the pH value changes. Therefore, for reproducible colour development of phosphomolybdate in the presence of Silica Gel G, it is necessary to shake the samples continuously. Details will be presented in another paper.

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