Estimation of phosphorus on paper chromatograms

The sensitive method of phosphorus analysis described by BARTLETT[1] is particularly suitable for the estimation of organophosphorus compounds on paper chromatograms and electrophoretograms. Reasonable accuracy may be achieved without using special techniques or high individual spot concentrations. The method described here has been found to yield results accurate to better than $\pm$ 3 % with spots which contain from 0.5 to 15 μg of phosphorus.

The chromatogram, developed in purified solvents, is dried in the usual way and sprayed lightly with HANES–ISHERWOOD reagent[2]. After about 30 min the phosphorus-containing areas are detected by irradiating the paper with a high intensity mercury vapour lamp[3] (Hanovia 501/1, and the spots are then excised, allowing a small border round each to ensure full recovery of the phosphorus. A control "blank" of equal area, is cut from the same chromatogram, preferably from about the same R_F position. The pieces are placed in separate Pyrex tubes (120 × 15 mm) to each of which is added 0.6 ml 72 % perchloric acid. With this amount of acid it is advisable to limit the area of paper to 6 cm^2 per tube.

The tubes are first heated at 100° for 1 min (this moderates the vigour of the subsequent oxidation) and then transferred to a metal block[4] which is maintained at 190° $\pm$ 2°, and into which the tubes fit to a depth of 4 cm.

To prevent water, which is formed in the oxidation, from condensing inside the tubes, it has been found convenient to surround the tubes with a cylindrical shield of aluminium foil which rests on the block and reaches to within 1 cm of the top of the tubes. If the liquid in the tubes is not free of colour after heating for 2 h, the rate of clearing can be accelerated by the addition of a drop of 30 % hydrogen peroxide, followed by further heating.

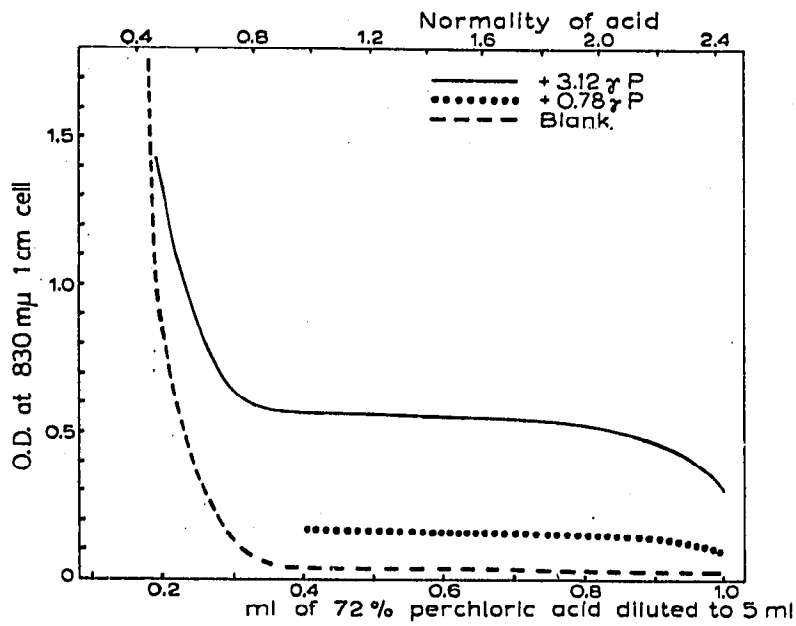

Fig. 1.

When the liquid is free of colour, the tubes are allowed to cool and the phosphate content of each is estimated as described by BARTLETT[1], a total volume of 5 ml being used during colour development.

Too high an acid concentration at this time will inhibit the formation of the blue colour, while insufficient acid gives rise to the production of considerable colour in tubes that contain no phosphorus. The useful concentration range for perchloric acid (determined in the absence of oxidised paper) was found to be from 0.9 *N* to 1.7 *N* (Fig. 1), and is similar to that found by BARTLETT for sulphuric acid[1]. BÖTTCHER *et al.*[4] recommend the use of 0.2 ml 70 % perchloric acid (equivalent to 0.48 *N* acid if diluted to 5 ml), but the total volume used during colour development is not stated.

The acid lost by the oxidation in the way described of 6 cm^2 Whatman No. 1 paper was found by titration to be equivalent to less than 0.05 ml 72 % perchloric acid. The final acid concentration is thus still within the recommended range.

Best results have been obtained using Whatman No. 1 paper which has been previously washed in 2 *N* acetic acid followed by distilled water and then dried[2]. Whatman No. 30 paper has also been found acceptable, but the phosphorus content of many untreated papers can be considerable.

The procedure described here has been applied largely to the determination of the rate of hydrolysis of phosphate diesters, and the analysis of the mixture of monoesters so obtained.

Note added in proof

Certain mixtures of concentrated perchloric acid with organic materials have been known to explode on heating or mechanical shock[5]. Whilst no explosion has resulted during more than five hundred phosphorus determinations using this method, it is emphasised that due precautions should be taken during the heating stage (see also ref. 6).

University Chemical Laboratory, Lensfield Road,
Cambridge (Great Britain) D. A. USHER

[1] G. R. BARTLETT, *J. Biol. Chem.*, 234 (1959) 466.
[2] C. S. HANES AND F. A. ISHERWOOD, *Nature*, 164 (1949) 1107.
[3] R. S. BANDURSKI AND B. AXELROD, *J. Biol. Chem.*, 193 (1951) 405.
[4] C. J. F. BÖTTCHER, C. M. VAN GENT AND C. PRIES, *Anal. Chim. Acta*, 24 (1961) 203.
[5] M. A. ELLIOT AND F. W. BROWN, *U.S. Bur. Mines, Rept. Invest.*, No. 4169 (1948) 17 pp.; C.A., 42 (1948) 2431d.
[6] R. M. C. DAWSON, *Biochem. J.*, 75 (1960) 47.

Received February 21th, 1963