Aniline Hydrogen Phthalate as a Spraying Reagent for Chromatography of Sugars

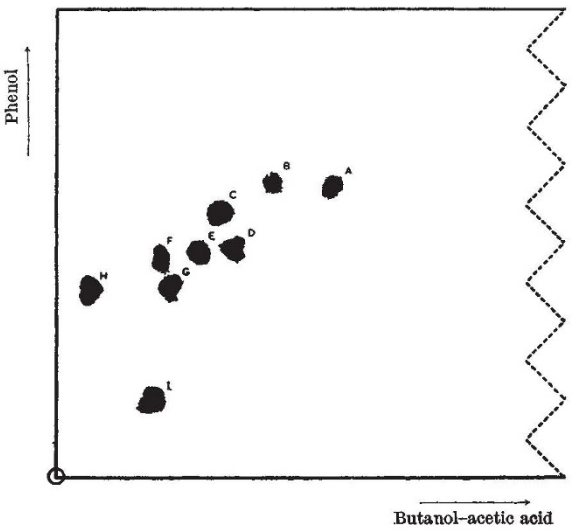
THE use of ammoniacal silver nitrate solution as a spraying reagent for revealing the presence of sugars on filter-paper chromatograms[1] has the advantage of general application; but it has a corresponding disadvantage in reacting with a very wide range of reducing substances other than the sugars, including various impurities commonly present in such solvents as phenol and collidine. Two-dimensional chromatograms are often rather unsatisfactory when ammoniacal silver nitrate is used, because (*a*) a rather large amount of the sugar mixture is needed and this increases the effect of interfering substances, (*b*) it is necessary to apply the spray as an aqueous solution, and unless the spraying is very rapid and uniform, the sugar spots migrate from wet to dry regions on the filter paper.

It has recently been found that two-dimensional chromatograms can be carried out very simply by use of aniline hydrogen phthalate as the spraying reagent. This reagent is much more selective for sugars than ammoniacal silver nitrate and is extremely sensitive for aldo-pentoses and aldo-hexoses. Furthermore, since it may be dissolved in moist butanol, migration of the sugar spots during the spraying process can be avoided. The reagent has also proved of value for use with one-dimensional chromatograms as a confirmatory test for pentoses and hexoses, and since interference from fortuitous reducing substances is largely eliminated, it often gives valuable results under otherwise unfavourable conditions.

The use of aniline hydrogen *oxalate* as a reagent for sugars was mentioned verbally during a contribution to a recent Biochemical Society discussion[2], but since the use of the acid phthalate rather than the oxalate has since proved advantageous (because of its ready solubility in organic solvents) the former is described here.

The reagent is prepared by adding aniline (0·93 gm.) and phthalic acid (1·66 gm.) to water-saturated butanol (100 ml.), and after spraying the chromatogram is heated for five minutes at 105° C. to develop the colour. The aldo-pentoses give a bright red colour, while the aldo-hexoses, desoxy-sugars and uronic acids give various shades of green and brown. With ketoses, colour development appears to depend upon the solvent used for developing the chromatograms, and on chromatograms irrigated with phenol or butanol–acetic acid mixture the reaction with fructose was either absent or very weak. Under these conditions, no colour was observed with raffinose, and reactions of intermediate strength were given by various disaccharides and amino-sugars. The reagent is very sensitive for both pentoses and aldo-hexoses, and will detect as little as 1 μgm. of either.

The accompanying photograph shows a two-dimensional chromatogram carried out with a mixture of nine common sugars using aniline hydrogen phthalate as the spraying reagent. It was prepared following generally the directions given by Consden, Gordon and Martin[3] for amino-acids. About 3 μl. of the mixed sugar solution (approximately 1 per cent with respect to each sugar) was applied to a standard sheet of Whatman No. 1 filter paper at the point marked by a circle. The chromatogram was irrigated first with phenol in the short direction of the paper (24 hr.), and was then turned through 90° and



Key: *A*, Rhamnose; *B*, ribose; *C*, arabinose; *D*, xylose; *E*, mannose; *F*, galactose; *G*, glucose; *H*, lactose; *I*, galacturonic acid

irrigated with butanol–acetic acid mixture[1] (24 hr.), the solvent being allowed to drip off the paper when the boundary reached the bottom edge. (In order to allow the solvent to drip uniformly the bottom edge of the paper was cut into a series of points at uniform intervals.) The reproduction was made by taking a reflex photograph of the chromatogram using Ilford Document Paper No. 50.

I wish to acknowledge the assistance of Mr. D. F. Elsden.

S. M. PARTRIDGE
Low Temperature Station for Research in Biochemistry and Biophysics,
Downing Street,
Cambridge.
March 7.

[1] Partridge, S. M., *Nature*, **158**, 270 (1946); *Biochem. J.*, **42**, 238 (1948).
[2] Partridge, S. M., *Biochem. J.*, **43** *Proc.*, xlviii (1948).
[3] Consden, R., Gordon, A. H., and Martin, A. J. P., *Biochem. J.*, **38**, 224 (1944).

Inorganic Paper Chromatography: the Qualitative Separation of Aluminium and Beryllium

R. P. LINSTEAD *et al.*[1] have described the separation by means of paper chromatography of aluminium, gallium, indium and zinc from solutions of their chlorides in *n*-butyl alcohol containing hydrochloric acid as a solvent. According to their work, aluminium scarcely moves at all from the original position, while the other elements move varying distances down the paper. We have recently followed their technique in an attempt to separate aluminium and beryllium, but instead of developing the aluminium with 'Aluminon', we have incorporated the technique of development by fluorescence first proposed by Pollard *et al.*[2]. After separation on the paper, the positions of the aluminium and beryllium spots are shown by spraying with an alcoholic solution of 8-hydroxyquinoline. Both the aluminium and the beryllium then fluoresce under ultra-violet light and can, indeed, be observed in strong sunlight. The fluorescence is intensified on drying the paper and exposing to ammoniacal vapours.