

Partridge S M. Aniline hydrogen phthalate as a spraying reagent for chromatography of sugars. *Nature* **164**:443, 1949.

A spraying reagent for filter paper partition chromatography of sugars which is soluble in moist butanol and can be used effectively with two-dimensional chromatograms for the separation of complex mixtures is described. [The SCI[®] indicates that this paper has been cited over 965 times since 1961.]

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"After returning in 1945 to the Low Temperature Research Station at Cambridge, I began a study of the proteins and polysaccharide components of animal connective tissue. Like others, I found the task rather daunting because of the paucity of means for isolating or identifying either sugars or amino acids from reasonably small quantities of hydrolysis products. I had known since 1940 that A. J. P. Martin and R. L. M. Synge had been doing some intriguing experiments. They used the relatively small differences in the partition coefficients of acetylated amino acids, between aqueous and solvent phases to effect separation by counter current distribution. The method used a large train of several hundred glass equilibration vessels.¹ This appealed to me as the first really systematic approach to separating the twenty or so amino acids obtained from the hydrolysis of proteins. Consequently, I was entirely entranced when

I attended a meeting in the north of England and heard R. Consden, A. H. Gordon, and A. J. P. Martin talk about the excellent qualitative separations of free amino acids they were able to obtain by allowing a water-saturated solvent to flow over moist filter paper in a closed container.²

"Unfortunately, sugars were not thought to be separable under these conditions; but some previous experience with phenol-water mixtures as solvents for polysaccharides convinced me that sugars would surely be soluble enough in wet phenol to allow their separation by the new paper partition chromatography. Of course, a spraying reagent for sugars was needed and I decided first to try ammoniacal silver nitrate. With the use of water-saturated phenol as the flowing solvent, almost at once, I thought I could detect the migration of a glucose spot: but the observation needed the eye of faith because spraying with an aqueous solution itself caused migration, and there was much blackening due to impurities in the phenol.

"In fact, quite a lot of work needed to be done to learn how to get clean results. The short 1949 paper, that came to be cited so often, described aniline hydrogen phthalate as a solvent-soluble spraying reagent, but was actually the third of a series of articles on paper chromatography of sugars beginning in 1946.^{3,4} The first two had already introduced butanol-acetic acid and s-collidine as second solvents for two-dimensional chromatograms, and the three papers together provided a convenient guide to identifying most of the common sugars."

1. **Martin A J P & Synge R L M.** The separation of the higher mono amino acids by counter current liquid extraction: the amino acid composition of wool. *Biochem. J.* **35**:91-121, 1941.
2. **Consden R, Gordon A H & Martin A J P.** Quantative analysis of protein partition chromatographic method using paper. *Biochem. J.* **38**:224-32, 1944.
3. **Partridge S M.** Application of the paper partition chromatogram to the qualitative analysis of reducing sugars. *Nature* **158**:270, 1946.
4. **Partridge S M.** Filter partition chromatography of sugars. General description of application to the qualitative analysis of sugars in apple juice, egg white and fetal blood of sheep. *Biochem. J.* **42**:238-50, 1948.