Separation of simple saccharides and oligosaccharides by Circular Paper Chromatography.

Methods for the separation of sugars by paper chromatography have been described by several investigators. The circular paper chromatographic technique has been used in

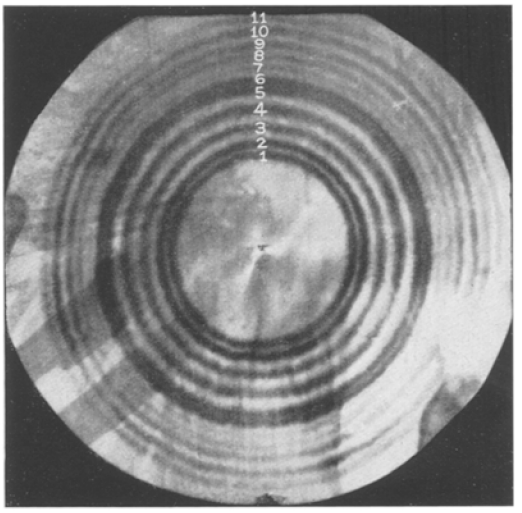

Fig. 1. Circular Paper Chromatogram showing the separation of sugars. (80 µl of the solution containing 1 gm. of each sugar in 100 c.c. of water were spotted at the centre.) *1* Raffinose; *2* Melibiose; *3* Maltose; *4* Sucrose; *5* Galactose; *6* Glucose; *7* Sorbose; *8* Arabinose; *9* Xylose; *10* Ribose; *11* Rhamnose.

this laboratory[1]) and by others[2]),[3]),[3a]), for the separation of sugars. We have, however, found that the solvent mixtures suggested by several investigators were not suited to the resolution of large number of sugars from a mixture and to the separation of oligosaccharides. After examining the various solvents, the solvent system finally chosen for development was n-butanol-acetone-water (20:70:10), which gave the best band separation of mono and disaccharides as well as oligosaccharides. While the work was in progress, BAYLY and BOURNE[4]) published a note in *Nature* describing a new method for the separation of oligosaccharides by paper chromatography involving the conversion of the saccharides on the chromatogram into the corresponding N-benzyl glycosylamines, thereby increasing the R_f values of the oligosaccharides. Our experience with the application of the circular paper chromatographic technique using the above solvent system to the separation of oligosaccharides formed during

the hydrolysis of starch by amylases show that these higher saccharides can be easily separated without any further treatment[5]).

The method briefly consisted in spotting 50—100 µl of the solution containing the mixture of saccharides at the centre of a circular filter paper (38·5 cm diam.) and developing the chromatogram with the solvent n-butanol-acetone-water (20:70:10) according to the procedure described by GIRI and RAO[6]). After drying the paper, the reagent aniline-diphenylamine phosphate[7]) was applied to the paper and heated at 90–100° C for about five minutes. The saccharides appear on the paper as blue, bluish-green or brown coloured circular bands, depending on the type of sugar, against the white background which turns slowly to blue after 24 hrs. One of the typical chromatograms showing the separation of eleven sugars from a mixture is shown in the accompanying fig. 1. The identification of the sugars can be made by running mixed chromatograms with the test sample and the known sugars spotted side by side on the circumference of a circle drawn at the centre of the paper according to the procedure described before[6]).

Full details of this method will be published elsewhere.

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