

RAPID SEPARATION OF MONOSACCHARIDES BY PARTITION CHROMATOGRAPHY
ON GLASS FIBER SHEETS.

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G. Jayme and H. Knolle (1956) reported that when glass fiber sheets are impregnated with Na_2HPO_4 various organic substances, among others, sugars can be separated with much greater speed than it is possible with conventional paper chromatography. The use of this procedure for chromatography of sugars, however, appeared very cumbersome as the glass fiber sheets these authors used had to be extracted with chromosulphuric acid before use which would make them brittle and difficult to handle. The organic solvents, furthermore, used by these authors were found in our laboratory not to be suitable for clean separation of various monosaccharides.

We describe here a modification of the procedure of Jayme and Knolle which eliminates its inconveniences.

Glass fiber sheets from Reeve and Angel, 834 A+ are immersed into a solution of 0.1 M Na_2HPO_4 which had been adjusted to pH 5 with 85% H_3PO_4 . The sheets are dried hanging at room temperature. After this treatment the sugars to be separated are applied the usual way and descending chromatography is carried out, without previous equilibration, for 4 hours.

The solvent is a pyridine, butanol water mixture (30 pyridine, 45 water,

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90 butanol). The chromatograms after being dried can be developed for sugar spots with the following reagents:

(1) Cysteine- H_2SO_4 spray: 0.75 g cysteine-HCl are dissolved in a mixture of 10 ml H_2O and 10 ml $\text{C}_2\text{H}_5\text{OH}$, then 5 ml H_2SO_4 are added with cooling in ice. The chromatogram is dried at 70°C after spraying.

With this reagent different sugars yield different colors (Table), which are fading within a few hours.

TABLE

Sugar	Rf	Color With The Cysteine Reagent	Color With The Thioglycolic Beta-Aminonaphthalide Reagent
galactose	0.31	violet	dark gray
glucose	0.42	blue	black
mannose	0.52	gray-brown	brown
fructose	0.76	light brick red	yellow
glucosamine	0.03	--	--
galactosamine	0.02	--	--
galacturonic acid	0.02	--	--
glucuronolactone	0.87	--	--
fructose	0.51	intense blue	
sorbitose	0.57	--	
ribose	0.70	violet reddish brown	
arabinose	0.54	violet red	
xylose	0.68	" "	
lyxose	0.74	" "	
thamnose	0.95	" "	

(2) Thioglycolic acid-beta-aminonaphthalide spray (Eastern Chemical Co., Newark, N.J.): 0.1 g of this reagent recommended for color reactions of sugars by Masamune and Sakamoto (1956) is dissolved in 5 ml H_2SO_4 conc. and diluted with a mixture of 10 ml H_2O and 10 ml $\text{C}_2\text{H}_5\text{OH}$ with cooling in ice. Development as described in (1). The colors are more stable and more differentiating than those obtained with cysteine. These two sprays do not react with hexosamines and hexuronic acids, which can be developed by aniline phosphate.

(3) Aniline phosphate spray: 50 cc of a stock solution of 3.6 g freshly distilled aniline, 3.6 g H_3PO_4 , 20 cc n-butanol, 80 cc H_2O and 100 cc acetone are diluted to 500 cc with acetone. The brown color of sugar spots is stable.

REFERENCES

1. G. Jayme and H. Knolle, *Angew. Chem.* 68, 243 (1956).
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