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Thin-layer chromatography of simple sugars in the presence of large quantities of sucrose

In most of our investigations we find it necessary to analyze mixtures of monosaccharides in the presence of an overwhelming proportion of sucrose. In some cases the sucrose is present in concentrations of one hundred or more times that of the other sugars. Under such conditions, using either paper or thin-layer chromatography, most of the solvents and stationary phases we have tried failed to separate the sugars from each other[1]. The solvent system used by CHAN AND CAIN[2] to separate mono- and oligosaccharides from plants by paper chromatography has proved excellent for separating mixtures of galactose, glucose, fructose, raffinose and sucrose, even when the concentrations of the sugars vary by a factor of 100. The only disadvantage we have found is that the separation takes from two to four days to be completely effective.

Experimental

Layers of Cellulose MN 300 were spread on 8 in. × 8 in. glass plates using an adjustable applicator (Kensington Scientific Corp., Oakland, Calif. 94608). The layers were 0.275 mm thick for normal sample loading and 0.500 mm thick for sample applications above 20 mg. The layers were allowed to dry overnight at room temperature. No special storage procedure was required but the layers were kept from laboratory fumes by storage in a closed wooden box. Aqueous mixtures containing 5–15 mg of dissolved sugars were applied as a continuous streak 2 cm from one edge of the layer and 1 cm from each side. The water is evaporated from the layer after application using a forced air heater. The eluting solvent consists of a mixture of 1-butanol (H_2O saturated)–trichloroethylene–95 % ethanol (3:1:1, v/v). The B-N chamber (Fig. 1) is

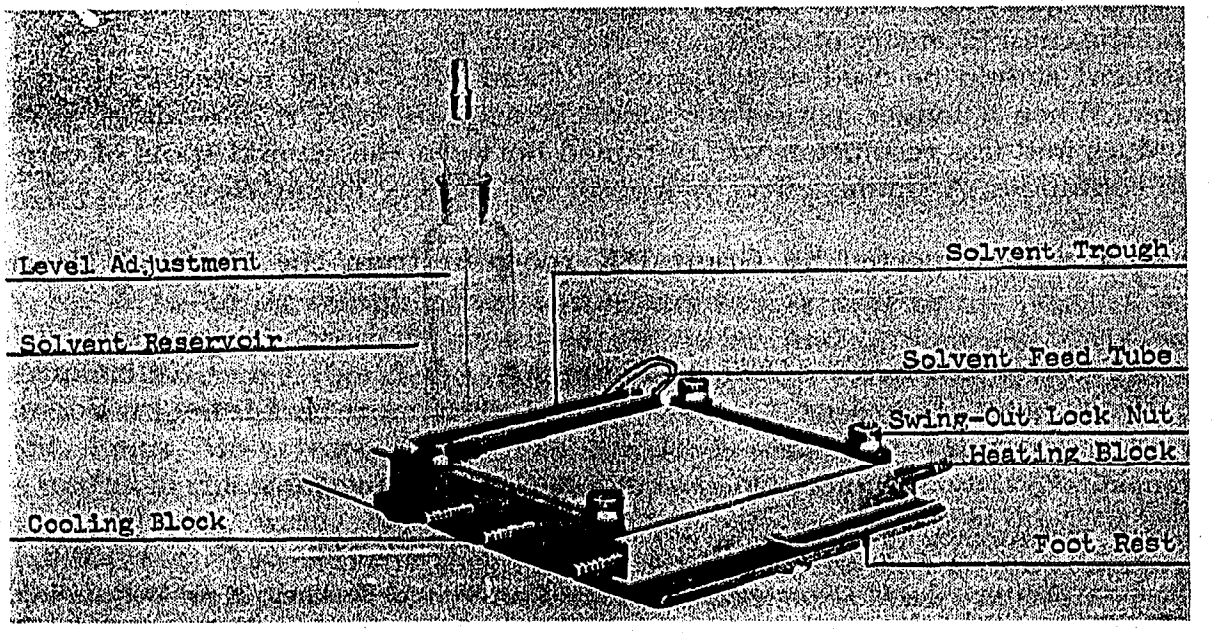

Fig. 1. Apparatus for continuous horizontal development of thin-layer plates. B-N chamber obtained from Brinkman Instruments, Westbury, N.Y., U.S.A.

used according to the manufacturer's instructions. The temperature of the cooling water was 18° and the heating block was 78°. After development for 19 h the layer is dried at 105° for 15 min. It is then sprayed with a mixture of 1 g aniline and 1 g diphenylamine in 100 ml acetone. Just before spraying 10 ml of 85 % H_3PO_4 was added to the detecting reagent. The layer is heated for 5–10 min at 105° to develop the colors for the various bands.

Discussion

Both the paper chromatographic and the thin-layer procedures produce narrow discrete bands. The advantage of the thin-layer method is that it takes 19 h for com-

TABLE I

$R_{SUCROSE}$ OF VARIOUS SUGARS[a]

Sugar	*Thin-layer chromatography (19 h, 7 in. developing length)*	*Paper chromatography (4 days, 18 in. developing length)*
Raffinose	0.17	0.17
Sucrose	1.00	1.00
Galactose	1.63	1.85
Glucose	2.17	2.44
Fructose	2.94	3.46
Melibiose	0.37	0.39
Cellobiose	0.40	0.46
Arabinose	[b]	3.39
Xylose	[b]	4.46
Ribose	[b]	5.10

[a] Developing solvent: 1-butanol (H_2O saturated)–trichloroethylene–95 % ethanol (3:1:1, v/v).
[b] Migrates to solvent front.

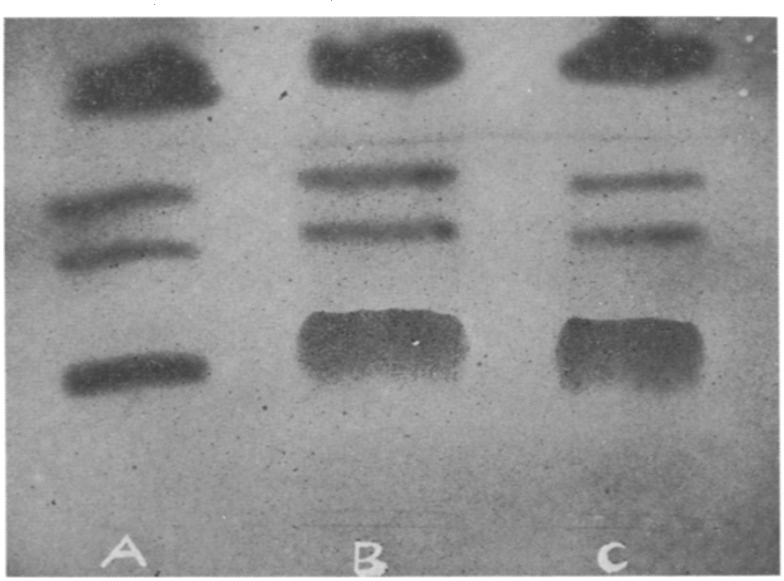

Fig. 2. Separation of sucrose, D-glucose, D-galactose and D-fructose on a 0.500 mm layer of Cellulose MN 300. Weight proportions of sugars are: (A) 1:1:1:1; (B) 10:1:1:1; (C) 100:1:1:1. Colors developed with aniline, diphenylamine, and H_3PO_4 are brown for sucrose, blue for D-glucose and D-galactose and yellow-brown for D-fructose.

plete separation while it takes four days for complete separation using Whatman No. 1 paper. The $R_{SUCROSE}$ values of various sugars tested are given in Table I. The separation of mixtures of galactose, glucose, fructose and sucrose is shown in Fig. 2. From left to right we have applied 0.1, 1.0 and 10 mg of sucrose. In all three samples, 0.1 mg of each monosaccharide was present. This gives corresponding ratios of 1:1, 10:1, 100:1 for the sucrose and each monosaccharide.

We have used the procedure for separating various monosaccharides from sugar syrups containing overwhelming concentrations of sucrose. We have also applied it successfully to the separation of disaccharide and trisaccharide hydrolysates. In one case we have hydrolyzed 15 mg of an unknown trisaccharide and have applied the resulting solution after neutralization to a 0.500 mm layer. Development for 19 h and subsequent visualization showed only galactose and fructose to be present. The identity of these two monosaccharides was confirmed by elution off the plate with water and subsequent derivatization with phenylhydrazine. Several procedures for non-destructive visualization prior to elution have been described by HORTON AND TSUCHIYA[3].

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