Separation of sugars on polyamide layers

Thin-layer chromatography (TLC) is becoming increasingly popular for the rapid separation and identification of sugars. Separation is usually achieved on layers of Kieselguhr G or Silica Gel G, but magnesium silicate[1], calcium silicate[2], Hyflo-Super-Cel and Filter-Cel[3] have also been used. However, low capacity of the plates ($\pm$ 5 μg of each component) and poor resolution are generally experienced with these adsorbents. Separation has been improved by preparing the adsorbent layers in buffer solutions[4–7].

Increased loading capacity has been obtained on cellulose layers[8–10] but, in order to obtain complete resolution of sucrose, glucose, and fructose from plant extracts on these layers, the plates have to be developed twice in the same direction[11].

Polyamide supports have recently been successfully used for the separation of many types of compounds, among them quinones, hydroxyquinones and phenols[12], sulphonamides[13], carotenoids[14], and DNP-amino acids[15,16]. Separation is based on the reversible formation of hydrogen bonds between the amide bonds of the polymer and the compound being separated[17]. The separation of sugars on polyamide has not, to the author's knowledge, been reported.

A procedure for the separation of various sugars on polyamide layers is described and it is shown that complete resolution of high loads of sucrose, glucose and fructose can be achieved with a single development.

Preparation of chromatoplates

Polyamide powder is usually applied to the plates as a slurry in methanol or chloroform[18]. In this laboratory thick layers ($\pm$ 0.6 mm) prepared with these solvents cracked and peeled off the plates. WANG, HUANG AND WANG[16] obtained durable thick layers by spreading the plates with polyamide dissolved in 75 % formic acid and keeping them in a horizontal position for two days at 26° or 29° in a chromatographic cabinet saturated with water vapour. The layers were subsequently dried in an oven at 100° for 15 min to eliminate the last traces of formic acid.

In the present study, thick layers which did not crack or peel and which could be used soon after spreading were obtained by making up the polyamide (Merck, for thin-layer chromatography) in benzene. The slurry (10 g of polyamide powder in 35 ml benzene) was spread on glass plates (20 × 20 cm) in a home-made applicator to give layers 0.6 mm thick, and the plates were air-dried in a fume cupboard. After excessive spraying with spray reagent fine cracks appeared in these layers during heating.

Standard solutions of all the sugars were made up in 80 % ethanol in order to wet the plates during the spotting procedure and to facilitate quick drying. After spotting the plates with standard sugar solutions, they were air-dried at room temperature (heat was avoided in order to prevent sugars from reacting with the polyamide).

Development of chromatoplates

Plates were developed at 23° without prior equilibration, in a glass tank (8 × 38 × 21 cm in height) using ethyl formate (B.D.H., purified and redistilled)–methanol (8:1) as solvent for 3.5 h. The temperature at which the plates were de-

veloped was carefully controlled since it had a marked influence on the R_F values obtained (Table I).

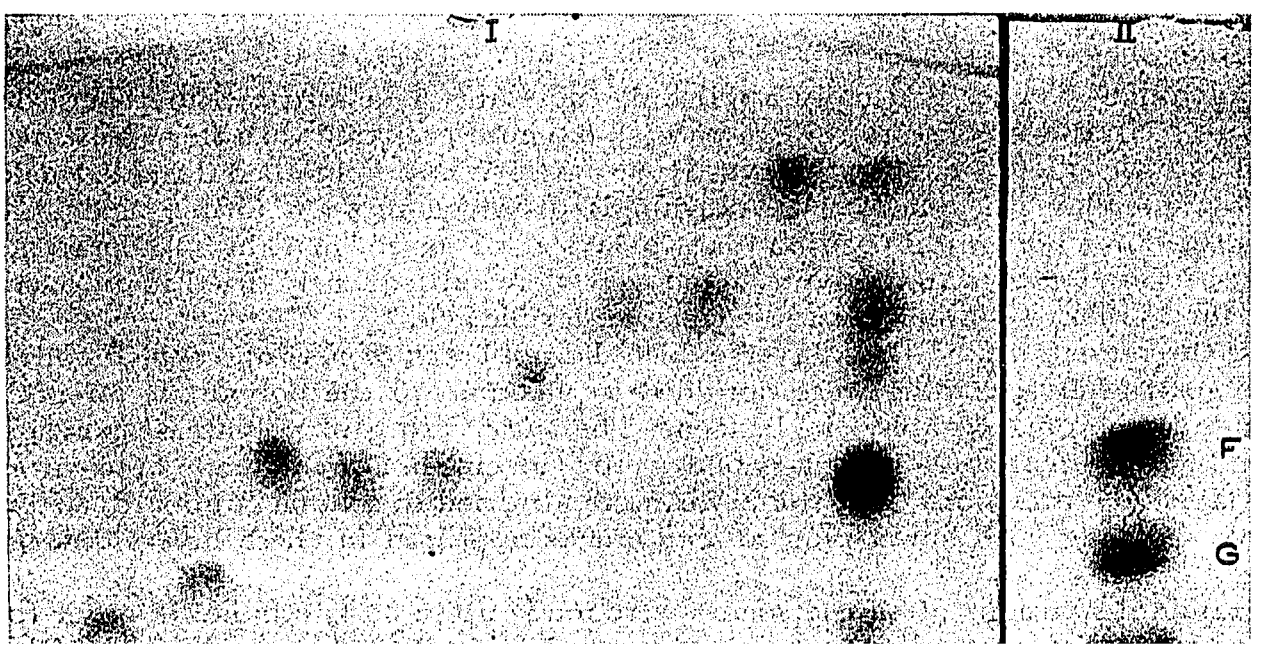
Spraying of chromatoplates

After development the plates were lightly sprayed with a modification of the

TABLE I

APPROXIMATE R_F VALUES OF SUGARS SEPARATED ON POLYAMIDE LAYERS AT 18° AND 23° USING ETHYL FORMATE–METHANOL (8:1) AS SOLVENT

Sugar	*R_F values*	
	18°	*23°*
Maltose	0.12	0.15
Sucrose	0.15	0.23
Glucose	0.28	0.39
Galactose	0.26	0.37
Mannose	0.27	0.38
Fructose	0.38	0.52
Arabinose	0.47	0.59
Xylose	0.48	0.61
Ribose	0.64	0.79



BRYSON AND MITCHELL[19] aniline–phosphate reagent, and heated at 110° until the spots appeared. In this reagent the butanol, which caused the polyamide layer to crack badly during heating, was replaced by benzene and ethanol. The reagent consisted of aniline–ethanol–phosphoric acid–benzene (0.1:20:1:75) made up in that order.

Fig. 1, Plate I illustrates the resolution obtained at 23° using 25 μg each of maltose, sucrose, glucose, galactose, mannose, fructose, arabinose, xylose, and ribose while approximate R_F values obtained at 18° and 23° are given in Table I.

Fig. 1, Plate II shows the resolution obtained at 18° for high loads (120 μg each) of sucrose, glucose and fructose.

The data indicate that thick polyamide layers present a means for the rapid resolution of various sugars at loads which are considerably higher than those generally encountered in TLC of sugars.

Department of Biochemistry, Natal Agricultural Research Institute, Pietermaritzburg (South Africa) J. P. MARAIS

1 H. GRASSHOF, *J. Chromatog.*, 14 (1964) 513.
2 P. T. JÖSSANG, *J. Chromatog.*, 12 (1963) 413.
3 J. L. GARBUTT, *J. Chromatog.*, 15 (1964) 90.
4 P. G. PIFFERI, *Anal. Chem.*, 37 (1965) 925.
5 S. ADACHI, *J. Chromatog.*, 17 (1965) 295.
6 H. JACIN AND A. R. MISHKIN, *J. Chromatog.*, 18 (1965) 170.
7 D. WALDI, *J. Chromatog.*, 18 (1965) 417.
8 D. W. VOMHOF AND T. C. TUCKER, *J. Chromatog.*, 17 (1965) 300.
9 M. L. WOLFROM, D. L. PATIN AND R. M. DE LEDERKREMER, *J. Chromatog.*, 17 (1965) 488.
10 M. L. WOLFROM, R. M. DE LEDERKREMER AND G. SCHWAB, *J. Chromatog.*, 22 (1966) 474.
11 D. W. VOMHOF, J. TRUITT AND T. C. TUCKER, *J. Chromatog.*, 21 (1966) 335.
12 W. GRAU AND H. ENDRES, *J. Chromatog.*, 17 (1965) 585.
13 Y.-T. LIN, K.-T. WANG AND T.-I. YANG, *J. Chromatog.*, 20 (1965) 610.
14 K. EGGER AND H. VOIGT, *Z. Pflanzenphysiol.*, 53 (1965) 64.
15 K.-T. WANG AND J. M.-K. HUANG, *Nature*, 208 (1965) 281.
16 K.-T. WANG, J. M.-K. HUANG AND I. S. Y. WANG, *J. Chromatog.*, 22 (1966) 362.
17 E. STAHL AND P. J. SCHORN, in E. STAHL (Editor), *Dünnschicht-Chromatographie*, Springer, Berlin, 1962, p. 383.
18 H. K. MANGOLD, H. H. O. SCHMID AND E. STAHL, in D. GLICK (Editor), *Methods of Biochemical Analysis*, Vol. XII, Interscience, New York, 1964, p. 393.
19 J. L. BRYSON AND T. J. MITCHELL, *Nature*, 167 (1951) 864.

Received August 22nd, 1966