A microchromatograph for quantitative estimation of sugars using a paper strip as partition support

Some fifteen years ago FLOOD, HIRST AND JONES[1,2] developed a paper chromatographic method for the quantitative assay of single monosaccharides. In later methods columns of powdered cellulose[3], ion exchange resins[5], or starch[4] were used to obtain separation of the monosaccharides.

This paper describes a convenient paper chromatographic procedure for separation and quantitative colorimetric estimation of single sugars.

Experimental

Munktell filter paper OB was used with *n*-butanol–water–acetic acid (4:1:1) as the mobile phase for the separation of the individual sugars. The paper was cut into 2 to 3 cm wide strips, the length of the paper strip depending on the R_F of the sugars present in the test sample.

Fig. 1 shows the separation of five different sugars: fucose, mannose, glucose, galactose and glucosamine. The paper strip used was 3 cm wide, the temperature was 22° and a *n*-butanol–water–acetic acid mixture served as effluent yielding an average of 5 drops per hour. The values obtained from Fig. 1 serve as a standard which allows us to predict the time when a spot representing a certain sugar will leave the paper strip with the mobile phase. If the paper strip is 25 cm long it can be inferred from Fig. 1 that the individual sugars will leave the strip after the following number of hours: fucose 16, mannose 25, glucose 30, galactose 34 and glucosamine 45–50 h. The time interval between the centers of two subsequent sugar spots eluted from the strip will be at least 4 h. During this interval 20 drops of the effluent will have left the strip (64 drops = 1 ml). The procedure therefore even allows the separation of sugars with rather close R_F values as, for example, galactose and glucose.

The separation of single sugars from mixtures or biological hydrolysates was carried out by one-dimensional descending chromatography in a microchromatograph (see Fig. 2). The microchromatograph consists of a glass airtight chamber in which an atmosphere saturated with chromatographic solvent can be maintained. With a view to better saturation of the atmosphere, the chromatographic solvent was poured not only into the trough but also on the bottom of the chamber. However, the best results were attained when the inside of the chromatographic chamber was lined with filter paper.

Before use the strip of filter paper was washed carefully with chromatographic solvent and dried. A measured amount of the hydrolysate or the mixture of the sugars to be tested is applied at the central portion of the start line by means of micropipettes containing 10–25 μml. The spot of solution containing the sugars was dried and the chromatographic strip placed in the microchromatograph. When the effluent reached the end of the strip and began to form drops, the microchromatograph was connected to the fraction collector of the type described by CARLANDER AND GARDELL[6]. The required number of drops of effluent were collected in each test-tube. The effluent was then evaporated to dryness in a desiccator under reduced pressure. The residue was taken up in 0.5 ml destilled water and 0.5 ml 5 % phenol–water solution was added, followed by 3.0 ml concentrated sulfuric acid. The further steps in the assay procedure were the same as those described by DUBOIS *et al.*[7].

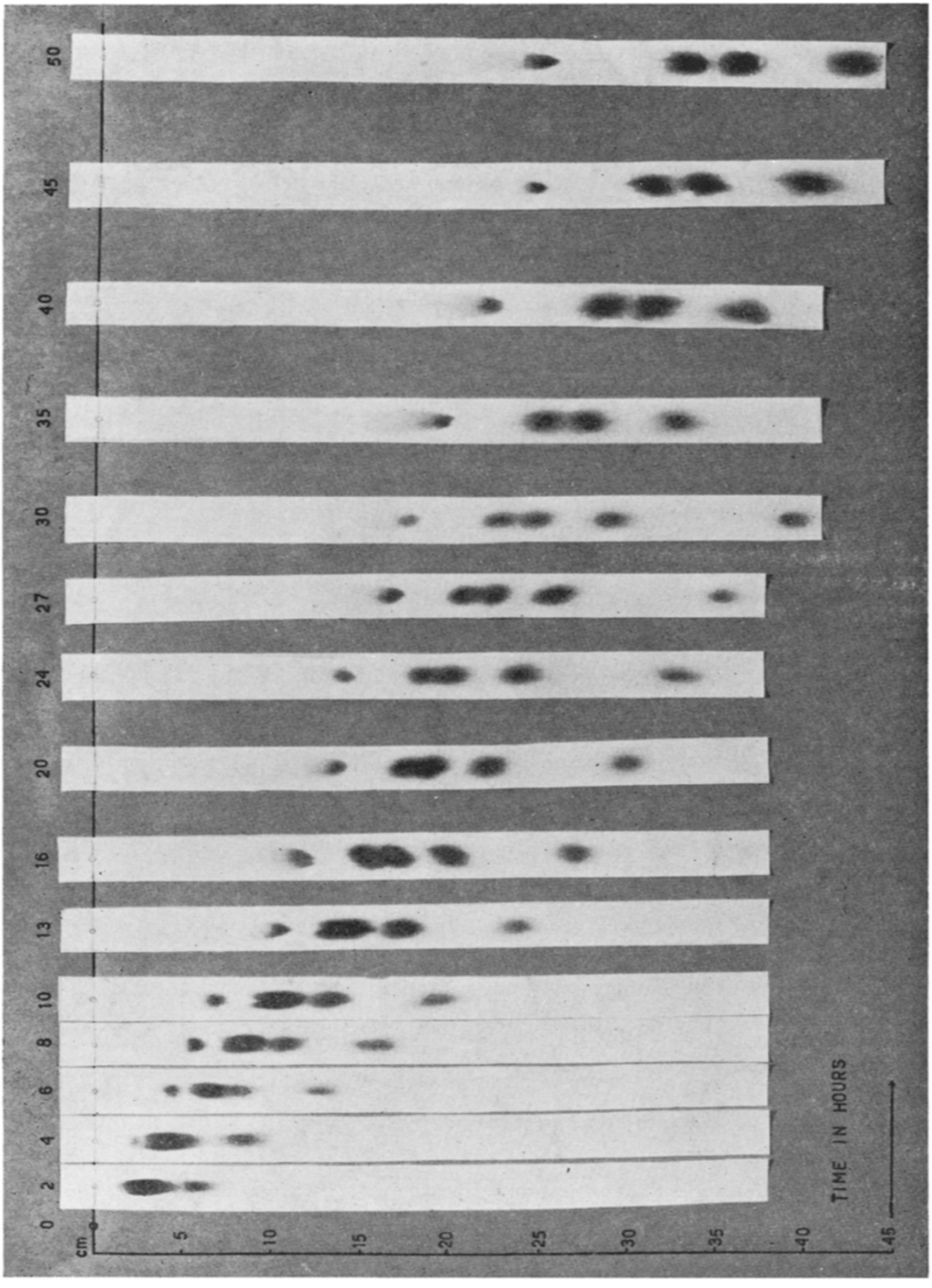

Fig. 1. The position of five sugars on the filter paper of 3 cm width and 45 cm length after different times of flow of the effluent. Further explanation in text.

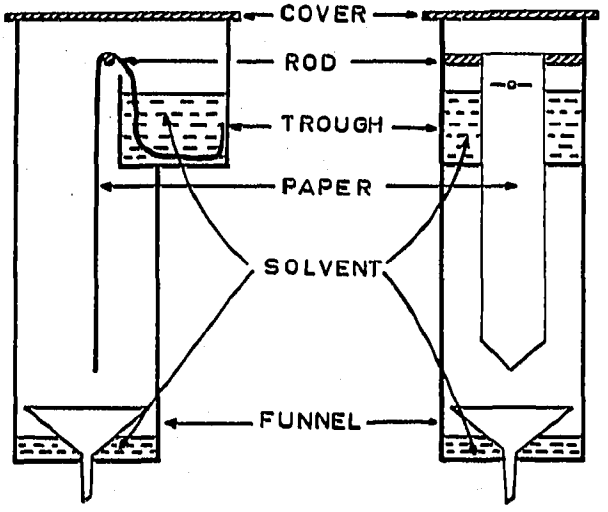

Fig. 2. Diagrammatic drawing representing the microchromatograph.

Glucosamine did not give a positive phenol–sulfuric acid color reaction, therefore the ELSON AND MORGAN[8] procedure as modified by IMMERS AND VASSEUR[9,10] and by IMMERS[11] was applied. The absorbancy was measured at 490 mμ for hexoses and at 480 mμ for pentoses or methyl pentoses and at 530 mμ for glucosamine. A Beckman spectrophotometer model DU with a 10 mm Cortex cell was used for these measurements.

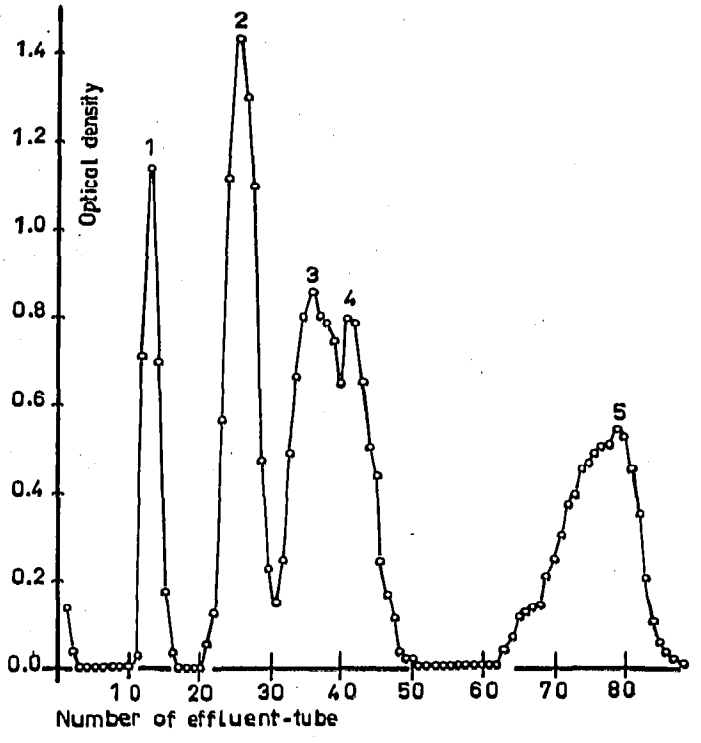

Fig. 3. The concentration (optical densities) of 445 μg fucose (1), 400 μg mannose (2), 290 μg glucose (3), 410 μg galactose (4) and 480 μg glucosamine (5) mixed in 25 μml water as function of effluent tube numbers. The width of filter paper is 2 cm, the length 25 cm. In each test-tube 4 drops of effluent. Absorbancy for fucose at 480 mμ, for mannose, glucose, galactose at 490 mμ and for glucosamine at 530 mμ.

TABLE I

RECOVERY OF ADDED AMOUNTS OF DIFFERENT SUGARS AFTER CHROMATOGRAPHIC PROCESSING

	Fucose			*Mannose*			*Glucose*			*Galactose*			*Glucosamine*		
Run. No.	*Added µg*	*Found µg*	*Recovery %*	*Added µg*	*Found µg*	*Recovery %*	*Added µg*	*Found µg*	*Recovery %*	*Added µg*	*Found µg*	*Recovery %*	*Added µg*	*Found µg*	*Recovery %*
1	445	451	101.3	400	450	112.5	290	253	87.2	410	423	103.1	480	443	92.2
2	190	187	98.3	204	199	97.5	—	—	—	210	202	96.1	156	148	94.2
3	54	53	98.0	55	46	83.6	60	62	103.3	—	—	—	—	—	—
4	116	120	103.4	202	—	—	154	139	90.2	136	130	95.5	166	157	94.6
5	380	361	95.0	408	462	113.2	360	328	91.1	420	397	94.5	259	232	89.7
6*	150	151	100.6	—	—	—	200	198	99.0	—	—	—	—	—	—

* The strip of filter paper was 2.5 cm wide and 5.0 cm long.

The quantity of an individual sugar in a sample can be estimated from a standard curve previously constructed for the particular sugars of the test samples (see GARDELL[4]).

The possibility of separating five single sugars may be illustrated by plotting the concentrations (optical densities) as a function of tube number, see Fig. 3. The total amount of the individual sugars could be calculated by summing the values found in the tubes corresponding to a complete fraction (1, 2, 3, 4 or 5).

The recovery from six series of chromatograms in which varying amounts of the sugars were tested is summarized in Table I.

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[1] A. E. FLOOD, E. L. HIRST AND J. K. N. JONES, *Nature*, 160 (1947) 86.
[2] A. E. FLOOD, E. L. HIRST AND J. K. N. JONES, *J. Chem. Soc.*, (1948) 1679.
[3] L. HOUGH, J. K. N. JONES AND W. H. WADMAN, *Nature*, 162 (1948) 448.
[4] S. GARDELL, *Acta Chem. Scand.*, 7 (1953) 201.
[5] J. X. KHYM AND L. P. ZILL, *J. Am. Chem. Soc.*, 74 (1952) 2090.
[6] A. TH. CARLANDER AND S. GARDELL, *Arkiv Kemi*, 4 (1952) 46.
[7] M. DUBOIS, K. A. GILLES, J. K. HAMILTON, P. A. REBERS AND F. SMITH, *Anal. Chem.*, 28 (1956) 350.
[8] L. A. ELSON AND W. T. J. MORGAN, *Biochem. J.*, 27 (1933) 1824.
[9] J. IMMERS AND E. VASSEUR, *Nature*, 165 (1950) 898.
[10] J. IMMERS AND E. VASSEUR, *Acta Chem. Scand.*, 6 (1952) 363.
[11] J. IMMERS, *Exptl. Cell Research*, 15 (1958) 595.

Received January 15th, 1964

J. Chromatog., 15 (1964) 252–256

Entfernung der Untergrund-Färbung nach Disc-Elektrophorese

Bei der Disc-Elektrophorese wird das Protein-Gemisch nach der Trennung im Polyacrylamid-Gel mit Amido-Schwarz gefärbt. Gemäss der Originalvorschrift von ORNSTEIN UND DAVIS erfolgt die Entfernung des überschüssigen, nicht gebundenen Farbstoffes mit der Trenn-Apparatur in vertikaler Stellung der Gel-Stäbchen durch Anlegen eines Stromes von 10–20 mA je Röhrchen. Dabei kommt es nach unseren Erfahrungen, je nach Dauer der elektrophoretischen Entfärbung des Untergrundes, zu einer Veränderung des Fraktionsmusters: durch Diffusion werden die Bänder nachträglich unscharf und/oder entfärbt; infolge des Auftretens von Gasblasen und der dadurch hervorgerufenen Widerstandserhöhung tritt Erwärmung auf. Diese Störungen können vermieden werden, wenn die kataphoretische Entfärbung *senkrecht* zur Trennrichtung erfolgt. Die zu diesem Zweck konstruierte Apparatur ist in Fig. 1 dargestellt.

In einer runden Plastik-Schale (B) werden in den Boden 12 Plastik-Stücke eingeschweisst, die längliche Schlitze (F) enthalten. Der Boden der Schlitze, die genau der Standard-Grösse der Polyacryl-Säulchen entsprechen, besteht aus einer Glasfritte P4. Nach dem Einlegen der zu entfärbenden Gel-Stäbchen in die Schlitze, werden diese mit Entfärbungsflüssigkeit aufgefüllt. Die Schalen A und B werden mit 7%iger Essigsäure beschickt und zusammengesetzt. Die Schale A enthält in der Mitte den