ANALYSIS OF SUGARS USING PAPER CHROMATOGRAPHY (HORIZONTAL MIGRATION)

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Paper chromatography has of late become a handy tool in the analysis of mixtures of organic as well as inorganic substances. After its evolution by Consden, *et al.* in 1944,[1] several modifications have been introduced in its technique. An important development in this direction is the Capillary Ascent (Upward Migration) Method of Williams and Kirby,[2] developed in connection with the analysis of amino acid mixtures. The scope of this method has very recently been extended by Rao and Beri[3] for the analysis of sugar mixtures. This method offers some definite advantages over the Downward Migration Method of Consden, *et al.* (*loc. cit.*) in the simplicity of the set-up of the apparatus and the ease of manipulation; yet it takes about 12 to 18 hours for the development of the chromatogram. Recently a modification has been introduced by Rutter[4] into the paper chromatography technique, whereby the substances under analysis are separated into circular zones instead of the usual spots or bands. Amongst the advantages claimed for this method may be mentioned the speed of separation of mixtures, the ease of manipulation and the simplicity and compactness of the apparatus. The suitability of this method for the identification of sugars has now been examined.

The procedure followed is essentially the same as that of Rutter with slight modifications. Whatman No. 1 circular filter-papers have been used for the development of the chromatogram. On irrigation with the solvent mixture the sugar, placed at the centre of the filter-paper, spreads itself into a circular zone of convenient size in about ½ to 2 hours, the size depending on the nature of the solvent, the duration of irrigation and the temperature. The zone is detected by spraying with a suitable reagent. In each case the R_F value (the ratio between the distance through which the solute moves and the distance through which the advancing front of the solvent moves) has been calculated. The values of the different common sugars with different solvents at different temperatures have been determined (Tables I and II). It is clear from the figures in Table I that the values as obtained by the present method are different from those reported by other methods.[3,5] It is, therefore, suggested that these values as determined by the present Horizontal Migration Method be referred to as "Circular R_F Values" to differentiate

them, thereby avoiding confusion, from the values recorded by the Downward or Upward Migration Method, which may be termed "Straight R_F Values".

An examination of the results (Tables I and II) shows that they vary with the solvent used and the temperature maintained, though the latter within close limits does not have a very pronounced effect. It is evident from the values recorded that each sugar has its own characteristic circular R_F value with a particular solvent and a particular temperature so that its identification becomes very easy. Since for Indian laboratories 35° is a convenient temperature which could be maintained conveniently by means of a oven without resort to the low-temperature incubators, the circular R_F values have been determined at 35° with different solvents (Table II). The results show that some of the sugars, though having the same or very close circular R_F values with one solvent, give very much differing values with a different solvent. Further, even if they are present in mixtures, they undergo separation into distinctly different zones, so that they can be identified by determining the circular R_F values in two or more different solvents.

Experimental

General Procedure.—Whatman No. 1 circular filter-paper was used for the development of the chromatogram. Perpendicular to the diameter and at equal distance from the centre two parallel cuts (about 2 mm. apart and 2 cm. in length) were made in the same direction on the filter-paper. The piece lying between the 2 parallel cuts was cut off at the ends away from the centre and folded along the diameter and perpendicular to the plane of the paper (Fig. 1). The " tail " so formed was cut down to about 1·5 cm. in length. For the formation of the "tail" Rutter (*loc. cit.*) used to make parallel cuts from the centre right to the edge. The present method has an advantage over that of Rutter in that the filter-paper retains its firmness, since the rectangular piece cut for the formation of the tail is very small. This facilitates the handling of the filter-paper in subsequent operations with ease and without any deformity. In the formation of the tail it is necessary to see that it is rectangularly perpendicular to the plane of paper, as otherwise the zones may not be truly circular. The sugar solution was placed with the help of a capillary tube as a microdrop (*c*, Fig. 1) on the joint of the tail and the paper, and air-dried. The solvent to be used for the irrigation was taken in a Petri-dish (15 cm. in diameter), and the filter-paper was placed over the dish in such a way that the tail hung down at the centre of the dish, to dip into the solvent below. A glass plate was placed over the filter-paper in order to retard evaporation of the solvent. The whole set-up (Fig. 2) was kept in a thermostat maintained at the required temperature.

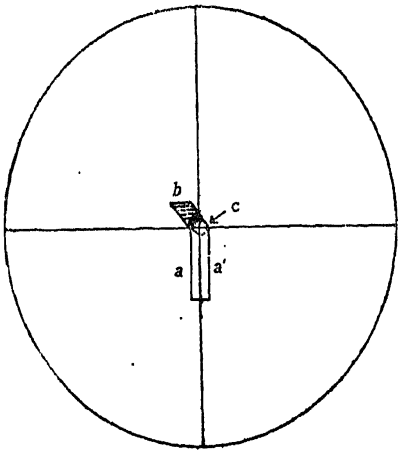


FIG. 1

a and *a'* = Parallel cuts
b = "Tail"
c = Microdrop of the sugar solution

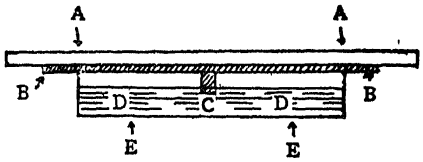


FIG. 2

A = Glass plate
B = Filter paper
C = "Tail"
D = Solvent
E = Petri dish

Through capillary effect the solvent gradually rose from the tail and began to irrigate the filter-paper, uniformly spreading itself in a halo. When the irrigation advanced sufficiently long (10 to 12 cm. in diameter) the paper was removed, placed on another Petri-dish serving as a support and dried in an oven at 105° for 5 minutes, the position of the solvent boundary having been marked with a pencil prior to transfer into the oven. It was then sprayed rapidly and evenly with a solution of aniline hydrogen phthalate in butyl alcohol[6] and dried again at 105° for 5 minutes as before, when a brownish purple zone corresponding to the position taken up by the sugar appeared on an almost white background. From the centre 3 radial lines in 3 different directions were drawn. Along each radial line the distances through which the sugar on the one hand and the solvent on the other moved were measured and the ratio between them was calculated in each case. The average value provided the circular R_F value.

Solvents.—Different solvents were tried for the irrigation:—phenol, *n*-butyl alcohol, *s*-collidine, methyl ethyl ketone, *p*-cresol, *n*-butyric acid and acetophenone. In each case the solvent was saturated with water before its use for the irrigation. Butyric acid and acetophenone were not found suitable, since with anhydrous butyric acid and even water-treated acetophenone, obviously on account of the latter's immiscibility with water, the solutes did not show any movement, while with moist butyric acid the zones were broad and diffused. The other solvents were found to be satisfactory.

Phenol was purified as recommended by Williams and Kirby (*loc. cit.*) by vacuum distillation over zinc dust, and collidine by treatment with bromine

and sodium thiosulphate and final distillation as was done by Partridge.[5] The other solvents were purified by simple distillation under vacuum. A stock solution of phenol was prepared as recommended by Partridge by mixing 100 g. of water with 900 g. of the purified phenol.

Sugar Solutions.—The solutions used were roughly 1 per cent. in concentration except in the case of lactose and maltose wherein a higher concentration was required (about 5 per cent.). In each case 3 to 4 μl. was taken.

Circular R_F Values of the Common Sugars.—Following the general procedure described above, the circular R_F values were determined at 20° with phenol and at 35° with all the solvents mentioned. For the sake of comparison the straight R_F values as determined by Partridge[5] at 20° are also included in the following table:—

(*a*) *At* 20° *with moist phenol as solvent* (time taken: 2 hours)

TABLE I

	Circular R_F values	Straight R_F values
d-Glucose ..	0·50	0·39
d-Galactose ..	0·54	0·44
d-Mannose ..	0·58	0·45
d-Fructose ..	0·63	0·51
d-Xylose ..	0·58	0·44
d-Arabinose ..	0·64	0·54
l-Rhamnose ..	0·72	0·59
Lactose ..	0·50	0·38
Maltose ..	0·50	0·36

(*b*) *At* 35° *with different solvents.*—With methyl ethyl ketone the development of the chromatogram took about ½ hour, while with the other solvents the time required was 1¼ to 1½ hours.

TABLE II

	Moist phenol	Moist butanol	Moist methyl ethyl ketone	Moist *p*-cresol	Moist *s*-collidine
d-Glucose ..	0·58	0·30	0·22	0·35	0·46
d-Galactose ..	0·60	0·26	0·19	0·37	0·42
d-Mannose ..	0·64	0·32	0·29	0·39	0·53
d-Fructose ..	0·68	0·30	0·30	0·45	0·52
d-Xylose ..	0·62	0·37	0·39	0·39	0·56
d-Arabinose ..	0·68	0·33	0·29	0·49	0·52
l-Rhamnose ..	0·76	0·48	0·47	0·53	0·67
Lactose ..	0·53	0·08	0·13	0·28	0·29
Maltose ..	0·54	0·10	0·15	0·25	0·36

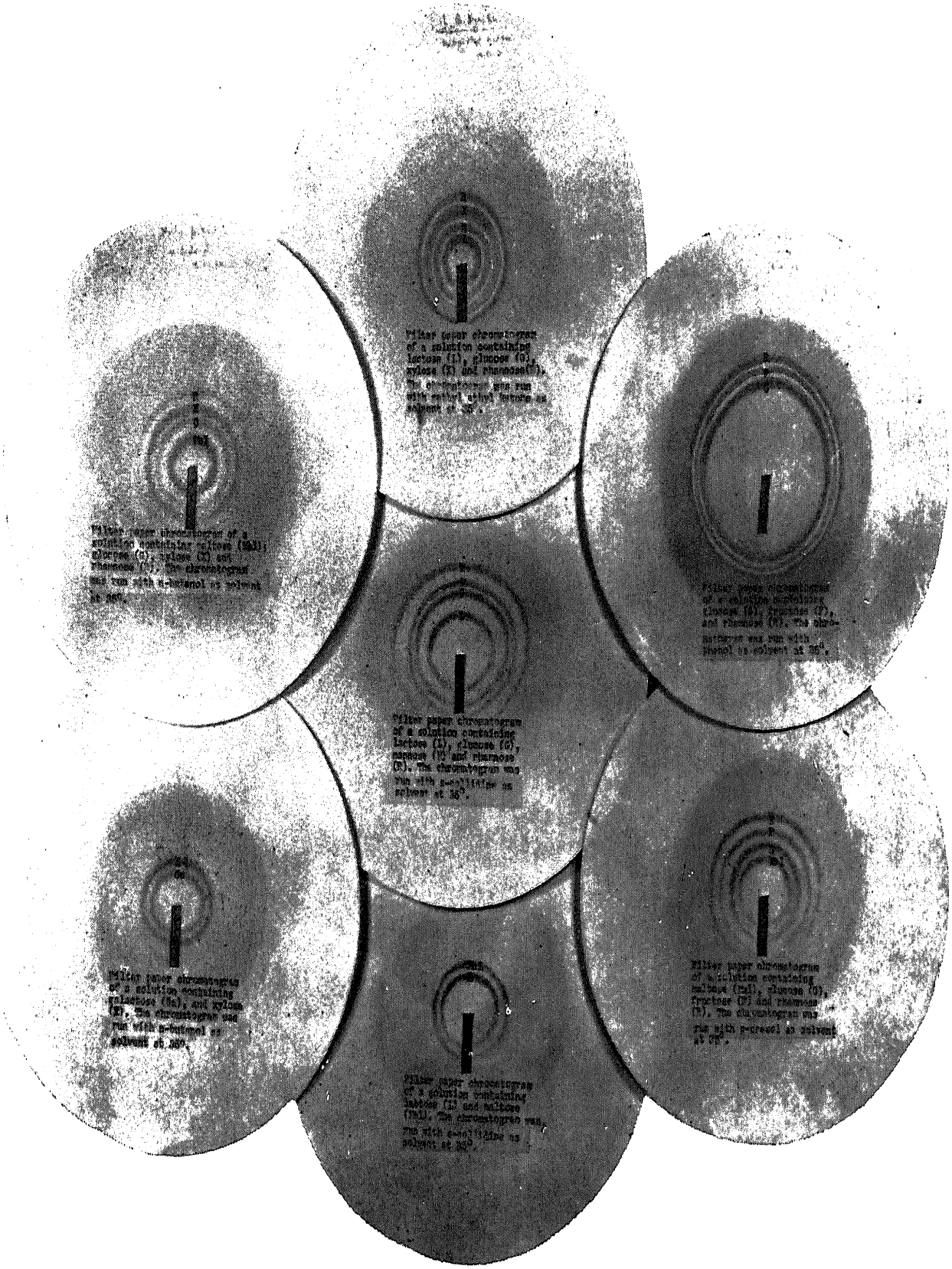
Identification of Sugars, when Present in Mixtures.—Mixed sugar solutions were prepared so that the concentration of the individual sugars was 1 per cent., except in the case of maltose and lactose, where the concentration was maintained at 5 per cent. Their separation into different zones on the chromatogram would naturally depend on the differences in the circular R_F values. From a large number of experiments it was found that a mixture could be separated provided the difference in the R_F values of any 2 components was not less than 0·06. The plate illustrates the separation of the several components of some sugar mixtures into distinct circular zones on running the chromatogram with the suitable solvent. It may be noted that it has not been found possible so far to separate *d*-glucose and *d*-galactose on the chromatogram, the difference in their circular R_F values being less than 0·06 with the different solvents tried.

Summary

Employing filter-paper chromatography a convenient method (Horizontal Migration Method) has been developed for the identification of reducing sugars, when present either alone or in mixtures. Since only small amounts are required for the identification, the method may be useful both to the regular student and the research worker. Under the conditions of the experiment the components of mixtures separate in ½ to 1½ hours into circular zones, which can be easily detected by spraying with a suitable reagent like aniline hydrogen phthalate. Each sugar has its own characteristic circular R_F value (the ratio between the distances through which the sugar moves on the one hand and the solvent front on the other) with a particular solvent, and its determination enables the identification of the sugar. Since some of the sugars have the same or very close R_F values with a particular solvent, it is desirable to run the chromatogram with at least 2 different solvents to confirm the identification. In the case of mixtures it may be necessary to determine the R_F values with 3 or more different solvents in order to definitely identify the individual components. Some of the advantages of the method are the speed and ease of manipulation, simplicity and compactness of the apparatus, and accuracy and reproducibility of the results.

References

1. Consden, R., Gordon, A. H. and Martin, A. J. P. — *Biochem. J.*, 1944, **38**, 224.
2. Williams, R. J. and Kirby, H. .. *Science*, 1948, **107**, 481.
3. Rao, P. S. and Beri, R. M. .. *Curr. Sci.*, 1951, **20**, 99.
4. Rutter, L. .. *Analyst*, 1950, **75**, 37.
5. Partridge, S. M. .. *Biochem. J.*, 1948, **42**, 238.
6. ———— .. *Nature*, 1949, **164**, 443.



Filter paper chromatogram of a solution containing lactose (1), glucose (2), xylose (3) and rhamnose (4). The chromatogram was run with n-butanol as solvent at 30°.

Filter paper chromatogram of a solution containing glucose (2), fructose (3), and rhamnose (4). The chromatogram was run with phenol as solvent at 30°.

Filter paper chromatogram of a solution containing lactose (1), glucose (2), maltose (3) and rhamnose (4). The chromatogram was run with n-butanol as solvent at 30°.

Filter paper chromatogram of a solution containing galactose (1a), and xylose (3). The chromatogram was run with n-butanol as solvent at 30°.

Filter paper chromatogram of a solution containing maltose (3a), glucose (2), fructose (3) and rhamnose (4). The chromatogram was run with pyridine as solvent at 30°.

Filter paper chromatogram of a solution containing lactose (1) and maltose (3a). The chromatogram was run with n-butanol as solvent at 30°.