Chromatography of sugars on plaster of Paris

The use of thick and thin strips of set plaster of Paris in the chromatographic separation of alkaloids[1] and amino acids[2] has already been reported. The present work deals with development of conditions suitable for chromatography of sugars on set plaster of Paris. To illustrate the efficiency of the method, it has been applied to the separation of sugars in urine and serum. The method has also been applied to the separation of pure known sugars. It has been found that the separation of sugars on set plaster of Paris strips is quite fast and efficient. Visualisation of the spots can easily and conveniently be done by silver nitrate reagent.

Experimental

Preparation of strips of set plaster of Paris. Strips, 1 mm thick and 15 cm × 3 cm, of set plaster of Paris were prepared as described previously[1]. It was found that if the set plaster of Paris, after removal from the glass plates, is allowed to dry overnight at room temperature (27°), the mechanical strength of the plaster is increased and better chromatographic separations are obtained. The strips were therefore cut off, as described previously, after the overnight drying time. They were then further dried before use, in an oven at 100° for 30 min.

Application of sample. Samples of material to be separated were applied to the strips, as spots, by means of a graduated capillary pipette. The strips were then dried for 15 min in a hot air oven at 100°. The following samples were chromatographed:

(1) A mixture of 0.4 g each of glucose, xylose, mannose and lactose in distilled water (100 c.c.).

(2) A mixture of 0.4 g each of glucose, arabinose, fructose in distilled water (100 c.c.).

(3) 24-h collections of urine from normal and diabetic individuals.

(4) Blood serum of a diabetic patient.

The size of sample applied to the strips was 3 μl in the case of (1) and 12 μl in the case of (2) and 3().

Solvent. While it is possible to devise solvent mixtures to suit experimental conditions other than those reported here, the solvent which gave good results consisted of chromatography grades of acetone (5 c.c.), butyl alcohol (4 c.c.) chloroform (1 c.c.), and acetate buffer pH 3.6[3] (1 c.c.). An alternative solvent is acetone (5 c.c.), isoamyl alcohol (4 c.c.), chloroform (1 c.c.), and acetate buffer pH 3.6[3] (1.2 c.c.).

Chromatography was carried out in a chamber for ascending chromatography. The minimum time required for complete separation in all cases was about 30 min. Shorter separation times could be achieved by reducing the size of the sample.

Visualisation of spots. After completion of the chromatographic separation, the strips were dried in a hot air oven at 100° till all the solvent had evaporated. The dried strips were then painted, on both sides, with a thick swab of cotton wool dipped in a mixture of acetone (20 c.c.) and an aqueous solution of N/10 $AgNO_3$ (2 c.c.). During this operation, the strips are held slantwise, like microscope slides, with the lower end resting against a glass plate. One or two strokes of the swab, in one direction only, suffice to cover strips with the solution. The strips were next dried in an oven at 100° till free from acetone. The strips were allowed to cool, and dipped vertically with a quick motion into a tall beaker filled to the brim with an aqueous solution of 5%

NaOH. The strips were held in this position for a minute. They were then removed and exposed to daylight till the spots acquired maximum intensity and then washed with distilled water. In order to fix the spots, the strips were bathed for 10 sec in an aqueous solution of sodium thiosulphate (2%) and then washed in distilled water. The spots appeared very clearly against the white plaster background.

Results

Fig. 1 shows the separation of a mixture of glucose, xylose, mannose and lactose.

Fig. 2 shows the separation of a mixture of glucose, arabinose and fructose.

Fig. 3 shows the separation of reducing substances present in normal urine (N) and urine of a diabetic patient (D). The position of the glucose spots in N and D was pinpointed by running a sample of glucose (G) simultaneously. The other spots observed in normal and diabetic urines have the R_F values of arabinose, fructose and xylose. These sugars have also been found present in normal urine by other workers[4–7]. Paper chromatography has been applied to the separation of urinary sugars[8] and seems to have a higher resolving power than paper electrophoresis[9]. Set plaster of Paris

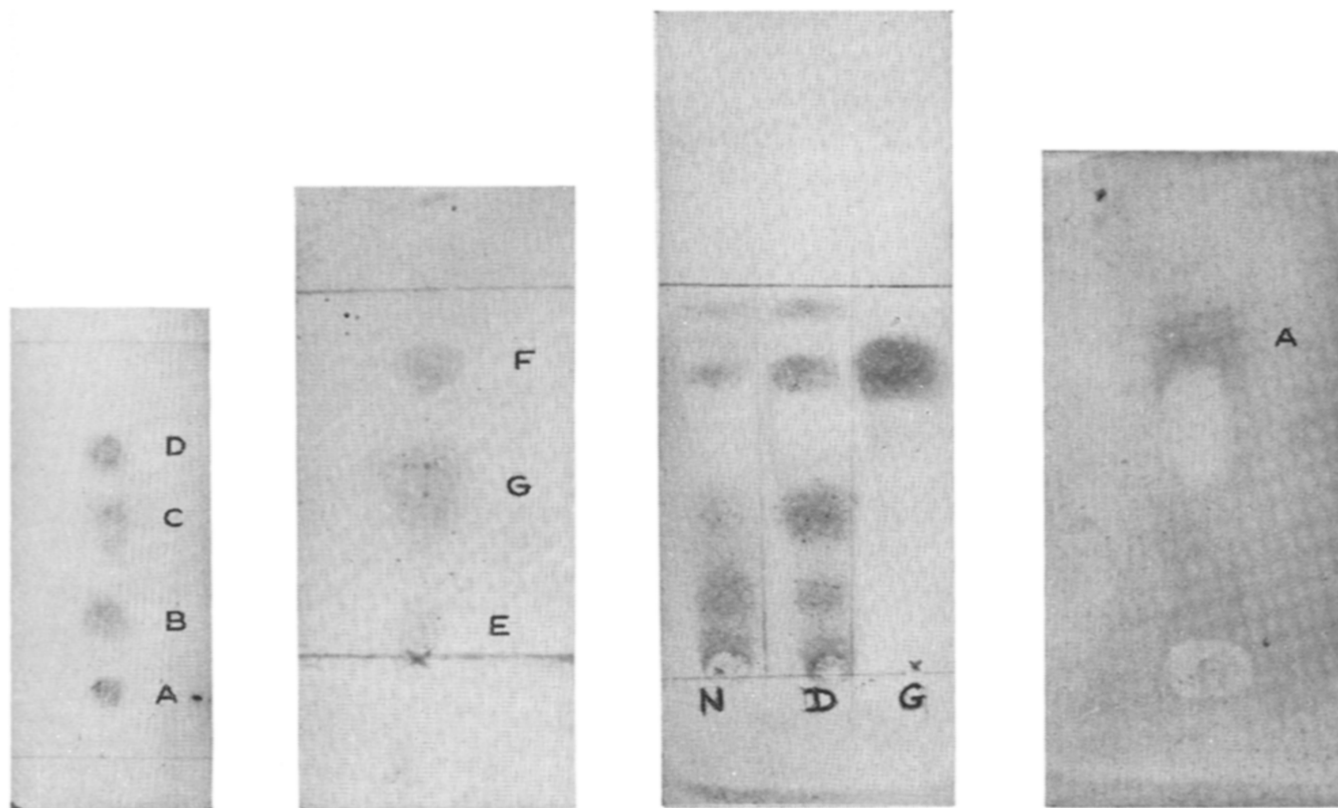

Fig. 1. Chromatogram of a mixture of four sugars on a plaster of Paris strip 15 cm × 3 cm × 1 mm thick. A = Lactose; B = mannose; C = glucose; D = xylose.

Fig. 2. Chromatogram of a mixture of three sugars on a plaster of Paris strip 15 cm × 3 cm × 1 mm thick. E = Fructose; F = arabinose; G = glucose.

Fig. 3. Chromatogram of samples of urine and glucose. N = Normal urine; D = urine of a diabetic patient; G = sample of glucose.

Fig. 4. Chromatogram of a sample of serum of a diabetic patient. A = Position of glucose.

strips have a clear advantage over paper because the size of sample which can be applied to them is far greater than that which is possible with paper.

Fig. 4 shows the separation of reducing substances present in the serum of a diabetic patient. The spot marked A has been identified as glucose by the glucose oxidase test.

Conclusion

Set plaster of Paris is a good medium for the quick separation of sugars. Using the solvent described, glucose, fructose, arabinose, lactose, xylose and mannose separate from each other in a matter of minutes. Glucose present in urine and blood serum separates from other known sugars and it is possible to break the plaster to isolate the glucose-containing zone for further quantitative estimation.

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Mehrfach-Kurzkeilstreifen- und 28-cm-Langkeilstreifentechnik in der Petrischale

Die Kurzkeilstreifentechnik unter Verwendung einer Petrischale als Chromatographiegefäss nach MATTHIAS[1] hat sich bei papierchromatographischen Einzeluntersuchungen von Stoffgemischen hervorragend bewährt, z.B. bei der Überprüfung des Reinheitsgrades von Ausgangsmaterialien, Zwischen- und Endprodukten, bei präparativen Arbeiten und zum Nachweis von Pflanzeninhaltsstoffen. Ein weiterer Vorteil dieser Methode ist, dass bei systematischen Untersuchungen zur Ermittlung der optimalen Laufmittelzusammensetzung für eine papierchromatographische Auftrennung von Stoffgemischen nur kleine Lösungsmittelmengen benötigt werden.

In Einzelfällen erwies es sich jedoch als Nachteil, dass kein Testgemisch unter gleichen Bedingungen mitchromatographiert werden konnte. Aus diesem Grunde modifizierten wir dieses Verfahren zu einer Mehrfachkeilstreifentechnik in der Petrischale gemäss Fig. 1a–d. Mit Hilfe dieser Methode ist es nun ebenfalls möglich,