Short Communications

A simple technique for the automatic application of solutions to chromatography paper

A technique has been developed for the automatic application of relatively large volumes of dilute aqueous solutions to absorbent paper in such a way as to produce "spots" of a suitable size for chromatography and paper electrophoresis.

The apparatus is shown in Fig. 1. The paper (F), held clear of the bench, is pierced by a needle bearing a length of thin cotton thread (No. 50) with a knot (E) at one end, at the point (D) where application of the solution is to be made. The fibres of the thread and of the paper are then matted together at their point of junction by scratching with the needle, and the thread is cut at a predetermined distance above the paper. The sample to be applied is contained in a pipette (A) supported above the paper and the upper end of the thread is attached by surface tension to the meniscus of the solution in the tip (B).

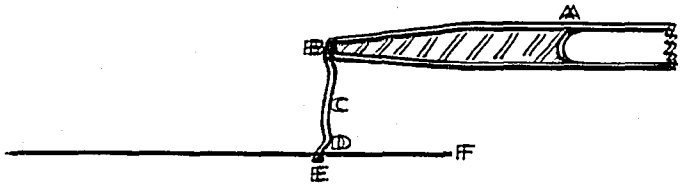

Fig. 1.

The solution soaks down the thread (C) and onto the paper, and when the rate of this process is balanced by evaporation, the area of the "spot" reaches a steady-state diameter and the solute is deposited in a fine ring at the circumference. The ring grows from its inner edge and all the sample is eventually drawn from the pipette. It should be noted that if the initial diameter of the ring on the paper is too small for the sample being used, it will eventually close in giving a uniform disc and subsequent application of solution is extremely slow. An estimate of the amount of solution contained in the thread can be made by observing the levels of the liquid in the pipette before and after the thread is saturated with the solution. This is retained at the end of the process, and if considered significant it can be washed onto the paper using pure solvent from a second pipette. The thread is then removed.

The size of the "spot" was observed to depend on the temperature, humidity and the length of the thread. This length must be determined by experiment under the prevailing conditions. In this laboratory lengths of 1.8 to 2 cm gave spots 0.8 to 1.0 cm in diameter with aqueous solutions, and 0.1 ml was applied in approximately 7 h. More volatile solvents require shorter threads. Mr. J. Ashworth has found here that

0.1 ml of a dioxane solution was applied through a 0.8 cm thread in 50 min and yielded a 1.5 cm diameter "spot".

Even though the "spots" produced by this technique were somewhat larger than those obtained on the most favourable occasions using the very much more time-consuming manual technique, equally good, if not better, resolution of urinary phenolic and indolic acids has been obtained[1] on chromatography papers 23 cm square.

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[1] R. M. ACHESON, J. COLE, D. P. DEARNALEY AND P. GLEES, *Neuro-Psychopharmacology*, Vol. 2, Symposium No. 5, Elsevier, Amsterdam, 1961, p. 455.

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Separation of monosaccharides, disaccharides and trisaccharides on carbon–aluminium oxide columns

A systematic study of the separation of carbohydrates on carbon columns was carried out by TISELIUS[1] in 1940. This method was not widely used until 1950, when WHISTLER AND DURSO[2] found that columns of carbon–Celite proved to be very effective. Thus, the separations of oligosaccharides[3–7] and methylated sugars[8–10] were successively achieved. In most cases carbon Darco G 60 and Celite 535 were used[1,5,7,10]. Celite alone was used for the separation of sugars[11] but it was usually added to carbon in order to increase the rate of flow of the eluate. The use of powdered cellulose instead of Celite was suggested by JERMYN[7]. The separations of sugars were performed on active carbon (40–60 mesh)[12]; columns of cocoanut charcoal (50–200 mesh) were used for separating methylated sugars and uronic acids[13] and the methylated sugars were separated on Al_2O_3 columns as well[14–16].

Since Celite has the tendency to contaminate the fractions[17], while carbon itself decreases the rate of flow of the eluate, we have investigated the separation of glucose, mannose and raffinose on a column containing carbon and aluminium oxide in ratio 3:4. It has been found that the three sugars could be recovered without any loss. The separations were controlled by paper chromatography[18], the results obtained showed that the separations were complete. The purity of the sugars recovered was checked by comparing their electric resistance with that of a mixture of pure sample and 0.5 % sodium chloride. The resistance of the sugar recovered was found to be greater than that of the mixture which meant that the impurities could not surpass 0.5 %. By using an aluminium oxide–carbon column we have succeeded in isolating the α,α-trehalose from baker's yeast[19] and β-gentiobiose from the reaction mixture obtained by the action of emulsin on glucose solution[19].