Application of Large Volumes in Small Spots on Paper Chromatogram Strips

SEVERAL devices have been designed for continuous application of large volumes up to 5 ml. on chromatogram strips[1,2]. The diameter of the spots thus obtained is usually 1 cm. or more, and considerable time is needed.

In order to apply relatively large volumes in very small spots on narrow paper strips in a reasonably short time, the following apparatus was constructed, in which a strong centripetal stream of warm air is concentrated around the place of application on both sides of the paper.

The apparatus is made of glass and consists of two parts (Fig. 1).

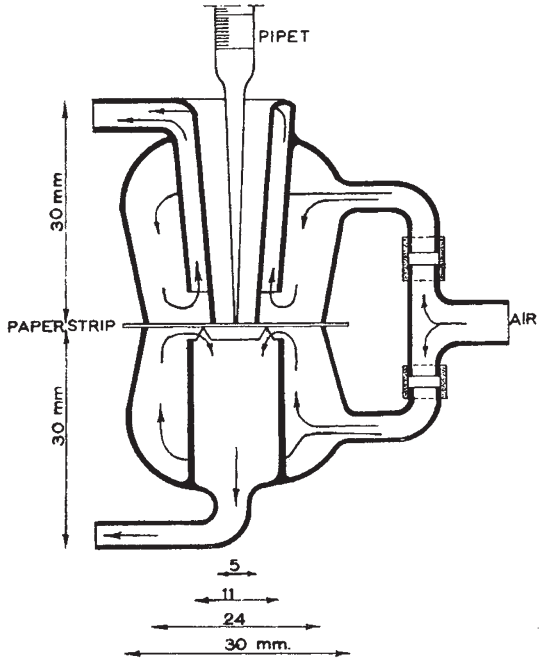

Fig. 1

The upper part is made of three concentric tubes, of diameters as indicated, fused together at the top. The middle tube is 2 mm. shorter than the other two, which have been ground down afterwards so as to end at exactly the same level. An air inlet is fused to the outer tube, the outlet is between the middle and the innermost tube.

The lower part is made symmetrical with the upper part, but without the innermost tube. The inner tube is again shorter than the outer one by 2 mm. and provided with four small points, the ends of which have been made level with the rim of the outer tube by grinding.

The paper is firmly held in its place by the rims of the two outer tubes of the innermost tube of the upper part, and the points of the inner tube of the lower part. The two parts are held together by slightly flexible rubber joints on the air inlet.

In this way even very narrow chromatogram strips 6 mm. wide can be used, when a paper mask is inserted. The tip of the applying burette or pipette is put on to the paper by entering it into the innermost tube of the upper part, and is protected by it from the air stream.

Air is blown through the apparatus from the compressed-air main after passing a filter and a heated copper spiral. A thermometer permits control of temperature.

The solution can be applied by means of a microburette of the Scholander or Rehberg–Kirk[3] type or by means of an Agla microsyringe. Thus outflow of solution, air stream and air temperature can all be controlled.

The time needed for application depends on the paper, the temperature used, and the volatility of the solvent. With various sorts of paper and solvents very small spots can be obtained in a reasonably short time: dilute solutions can be applied in a spot of 4–5 mm. diameter at a rate of 0·1 ml. in less than 10 min. for water, and less than 5 min. for butanol at 50° C. It is more convenient to condense volumes larger than 1 ml. by evaporation before application.

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[2] Urbach, K. F., *Science*, **109**, 259 (1949).
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Chromatography of Insulin on Calcium Phosphate Columns

Swingle and Tiselius[1] investigated the behaviour of the protein phycoerythrin when subjected to chromatography on columns packed with hydrated tricalcium phosphate supported on kieselguhr. It was found that the protein could be displaced by low concentrations of phosphate buffer. In a further study by Tiselius[2] it was found that other proteins could be displaced to give sharp elution zones when using aqueous solutions containing sodium phosphate.

The purpose of this communication is to record some results obtained when solutions of insulin stored under various conditions were subjected to chromatography on calcium phosphate–kieselguhr filled columns, eluting with sodium phosphate buffer of linearly increasing concentration.

The method used for the preparation of the calcium phosphate gel was essentially that of Keilin and Hartree[3], and each column contained 40 ml. gel supported on 26 gm. kieselguhr. The insulin was adsorbed at low ionic strength and eluted with the phosphate gradient increasing from zero to 0·05 *M* (*p*H 7·3) over a volume of 150 ml.; the concentration was then maintained at 0·05 *M*. The eluting solvent contained 0·5 per cent sodium chloride. Fractions of 3·5 ml. volume were collected and the optical density of each fraction at 276 mμ determined on a Unicam *SP* 500 instrument.

When the insulin solution was freshly prepared, the protein was eluted as a reasonably sharp peak (insulin 1) showing evidence of tailing. Any preservative such as phenol was only slightly, if at all, adsorbed and was eluted as a band ahead of the insulin. When the insulin solution was stored for a period of time in acid solution before subjecting to chromatography, it was found that a fraction of the insulin was transformed to a modification (insulin 2) which was eluted behind the band originally observed. On increasing the period of storage, the fraction of insulin changed increased still further. This effect is illustrated by