Methods for the quantitative application of extracts to thin-layer chromatograms

Thin-layer chromatography has hitherto been largely restricted to small amounts because of the absence of simple and reliable methods for the application of materials in broad bands[1,2]. Such methods are desirable for the chromatography of large amounts of material and improve the resolution obtained after chromatography of small amounts because the amount of material/unit area may be substantially reduced. The use of cotton and cotton wool wicks which do not damage the absorbent is described in this communication.

Known amounts of material in solution may be applied to a layer of absorbent from an "Agla" micrometer syringe which has a small cotton wick (3 mm) fixed in the needle by a stillette (Fig. 1). The Agla syringe containing the solution is placed over the chromatogram so that there is a gap of about 1 mm between the end of the wick and the surface of the absorbent. Spots are applied by lowering the barrel of the syringe until the wick touches the absorbent, and a straight band is obtained by depressing with the micrometer and moving the plate between guide rails after the application of each spot. The size of each spot is determined by the wetness of the wick and by the time during which contact between the wick and the absorbent is maintained. Solution may be applied to the absorbent at the rate of about 10 μl/min as a band about 1 mm wide.

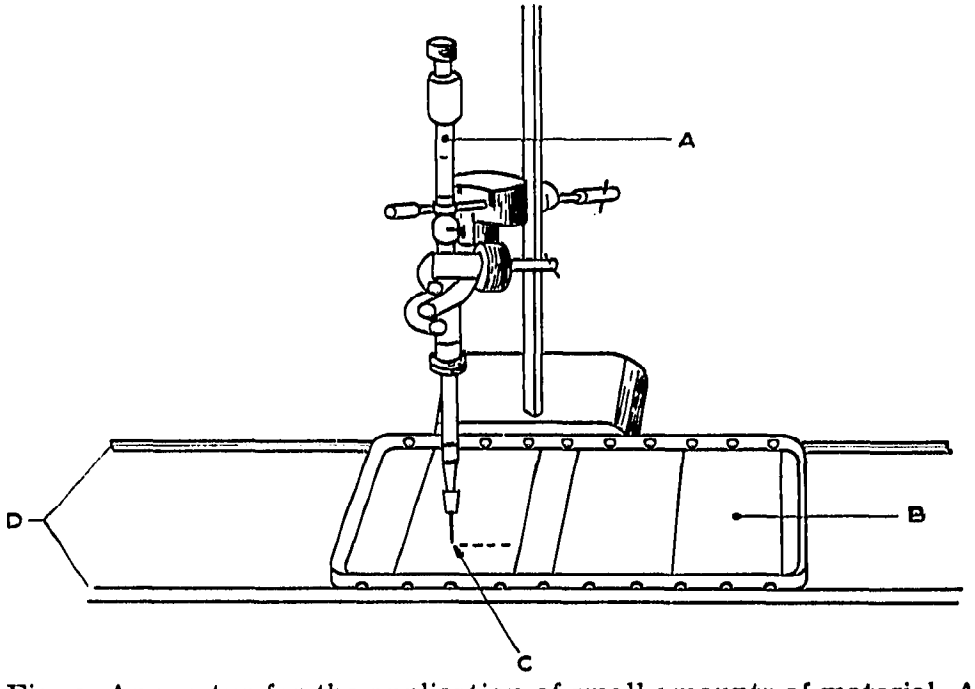

Fig. 1. Apparatus for the application of small amounts of material. A = "Agla" micrometer syringe; B = thin-layer plate on aluminium tray; C = cotton wick; D = glass rods.

A good example of the use of this method (Fig. 2) is the improved resolution obtained when separating γ-aminobutyric acid (GABA) from other amino acids in extracts of brain from albino rats[3]. The percentage recovery of GABA estimated by the method of VOIGT[3] after chromatography of ten portions of 0.5 μmoles GABA was 99 $\pm$ 2.5 (S.E.), although material to be eluted was removed from the plates by suction.

Although this method may be used also for the quantitative application, a more rapid method was developed for the application of large amounts of material. A 2 cm wick of non-absorbent cotton wool is inserted into a piece of thin-walled capillary tubing with a bore of about 1 mm. The wick should not be tightly packed as this prevents the flow of solution. The extract to be applied is dissolved in about 200 μl of

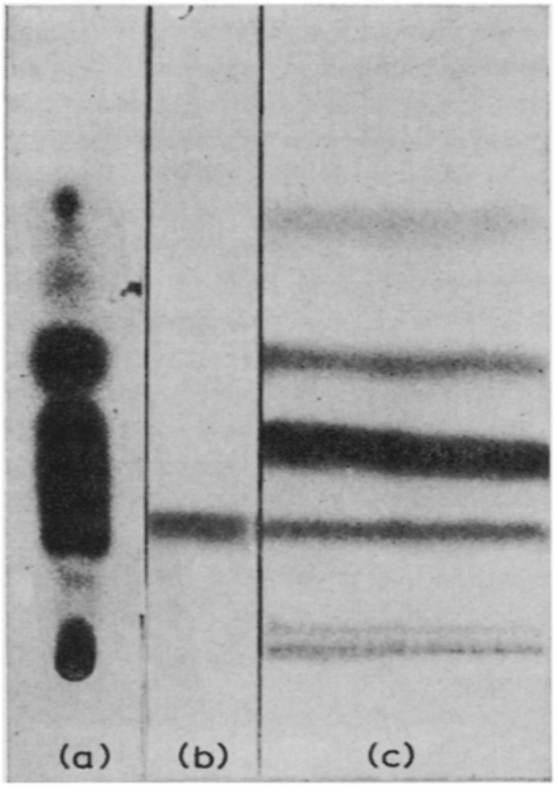

Fig. 2. Chromatography on silica gel of amino acids from the brain of an albino rat. (a) Application of 200 μl as a spot (corresponding to an extract of 20 mg brain). (b) GABA reference. (c) Application of 200 μl of extract as a band. The chromatogram was developed in isopropanol–water (70:30, v/v) and was sprayed with ninhydrin.

solvent and when the wick is placed in the solution a portion of the solution is drawn into the tubing. This is transferred to the absorbent by light brushing and the procedure is repeated until all the solution has been transferred. Washings are similarly applied. Although application results in some damage to the absorbent, a template to ensure the application of the extract as a straight band and to avoid damage to the absorbent is not necessary. Extracts are readily applied in even bands about 3 mm wide, and on subsequent chromatography good resolution of components is obtained.

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1 E. STAHL (Editor), *Thin-Layer Chromatography*, Academic Press, New York, London, 1965.
2 T. W. SCOTT AND J. W. U. BEESTON, *J. Lipid Res.*, 7 (1966) 456.
3 S. VOIGT, M. SOLLE AND K. KONITZER, *J. Chromatog.*, 17 (1965) 180.

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