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An automatic eight-channel spot applicator for thin-layer chromatography

Thin-layer chromatography has become a common procedure in almost all routine and research laboratories. In its practical application it is relatively simple, but some of the steps involved are time consuming if done manually. Particularly tedious is the spotting of samples on the thin-layer plate.

A survey of automatic TLC applicators developed in recent years was published by STAHL AND DUMONT[1]. These efforts primarily concentrated on improving the driving force used to transfer the sample onto the TLC plate. Most recent devices use gas pressure[1] or syringes[2-4]. We have developed an automated spot applicator in which the samples are transferred in parallel through thin elastic tubing directly from eight sample cups onto the TLC plate using a multi-channel peristaltic pump as the driving force.

Description

The samples are filled into eight disposable Oxford pipet tips*, each of which is connected with press-fit nipples to thin Autoanalyzer pump tubing**. The peristaltic pump we use is an Autoanalyzer pump I which can carry up to 15 tubes. Each of the output ends of the pump tubing is directly connected to a 70 mm long 22-gauge hypodermic needle (Fig. 1, A). The eight needles are supported by 18-gauge stainless steel tubing inside 15-gauge tubes 60 and 55 mm long, respectively (B). These are fixed in teflon rods (C), 20 mm long, 7 mm diameter, having threads for vertical adjustment in the teflon block 150 × 30 × 25 mm (D). This block is hinged with a support frame to a 23 × 23 cm stainless steel base plate (E) and is tilted up during insertion and removal of the TLC plate (G) to prevent damage of the applicator needles.

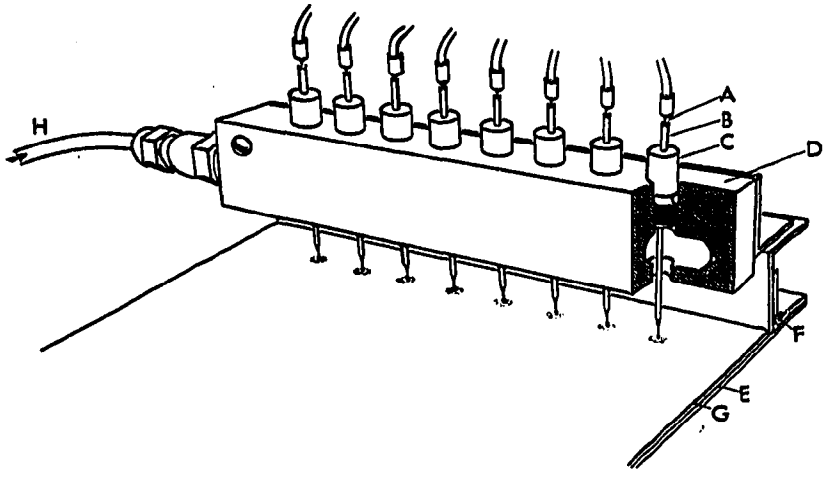

Fig. 1. Schematic drawing of applicator and spotted TLC plate. For further explanation see description in text.

* Oxford Sampler Tips 815 (1968), Oxford Sampler® Micro Pipetting System (brochure), Oxford Laboratories, 107 North Bayshore Blvd., Sam Mateo, Calif. 94401, U.S.A.

** Autoanalyzer Guide (1969), Technicon Corporation, Tarrytown, N.Y. 10591, U.S.A.

The entire applicator is placed on a temperature-controlled hot plate to keep the preheated (activated) TLC plate warmed to a constant temperature during sample application and to thus speed up evaporation of the solvent. The rate of evaporation is further increased by a stream of heated compressed air (or inert gas when it is necessary to prevent oxidation of the sample solute) directed toward the spot of application. The gas is heated by passing it through a coil made of copper tubing (2 m

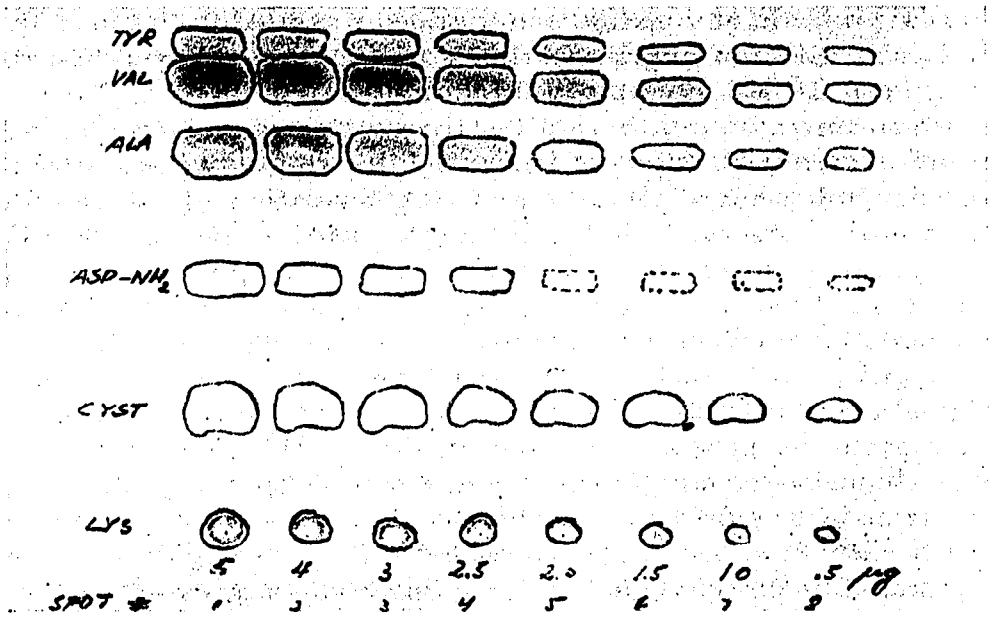

Fig. 2. Separation of a mixture of six amino acids in aqueous solution (0.1 µg/µl) on silica gel TLC plate. Staining with ninhydrin spray. Volumes spotted automatically range from 300 µl (spot 1, left) to 30 µl (spot 8, right). Application time: 11 min. Plate and gas temperature: 60°.

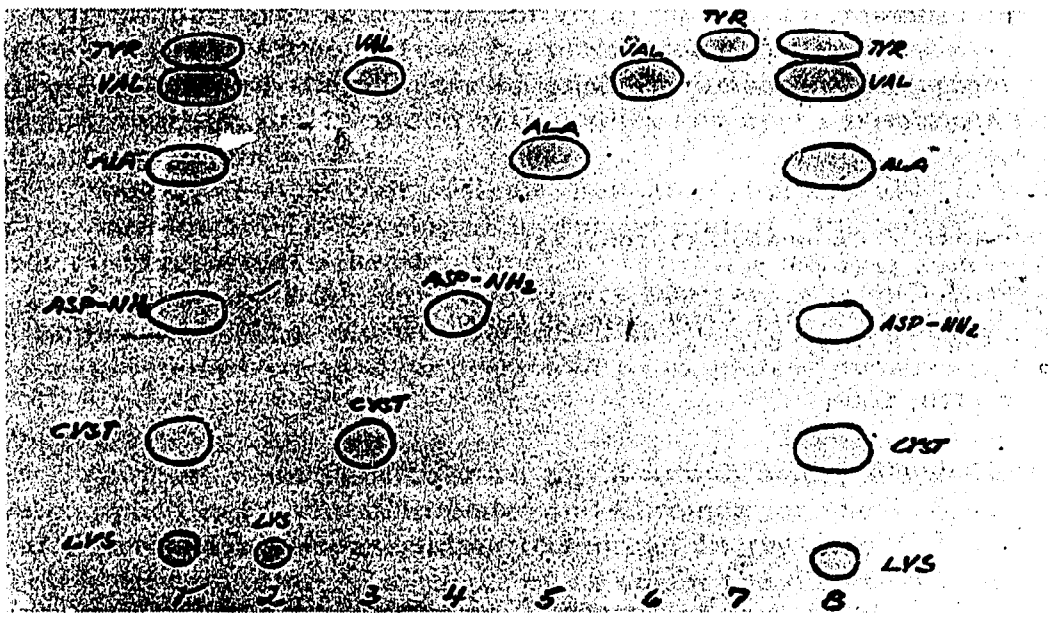

Fig. 3. Separation of amino acids in aqueous solution (0.1 µg/µl), 25 µl of each spotted separately (spots 2, 4–7) and in mixtures (spots 3 and 8). Stained with ninhydrin. Silica gel plate. Application time: 6 min. Temperature of plate and gas: 60°.

long, 8 mm diameter) immersed in a temperature-controlled water bath. The heated gas (H) is introduced through the longitudinal bore in block (D) where it is distributed through separate holes (8 mm diameter) as illustrated in the cross section (Fig. 1).

Discussion

Examples of chromatographic separation of aqueous solutions of amino acids after automatic spot application are shown in Figs. 2 and 3. Standard pump tubing (Technicon®) delivering 0.03 ml/min at constant pump speed was used (0.075 in. I.D., color code orange-red). Lysine does not migrate in the developing solvent system used (96% ethanol–water, 70:30). It was noted upon staining, as described by RADIN[5], that the sample solute is concentrated in a ring rather than a disc when large quantities of sample solvent are evaporated. In accordance with his results we found that chromatographic separation takes place regardless of ring formation of the sample spot (Fig. 2, left). But we also observed that substances with low R_F values may appear in ovals after separation when ring formation of the sample spot is very pronounced. In any case it is not usually necessary to spot such large solvent volumes. Both plates illustrated were spotted with standard hypodermic needles. Drop hold-up at the recessed needle openings has since been reduced by cutting a reverse bevel, symmetrical to the original bevel on each needle point as recommended by SAMUELS[2]. Somewhat smaller and more uniform spots were obtained after this modification.

The application time consists of sample transfer and sample spotting time. The first is the time used to transfer the first part of the sample from the sample cup to the tip of the applicator needle. It depends upon pump speed and tubing dead space. The spotting time is principally determined by the solvent vapor pressure (which limits the rate of solvent evaporation) and the desired spot size. Organic solvents have lower vapor pressure than water and can therefore be spotted faster. Care must be exercised so that any interfering substances from the pump tubing are not extracted by the organic solvent.

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