A device for simultaneous application of multiple spots on thin-layer chromatographic plates

The application of materials on thin-layer plates[1] with single capillary pipettes is tedious and frequently results in unsatisfactory separations due to inadvertent disturbance of the adsorbent layer at the point of application. These factors become particularly objectionable when mixtures are applied in a series of spots for preparatory separation of components. Preparation of such chromatograms is time consuming and the uneven distribution of sample across the plate and deviation of solvent flow around the attendant holes in the adsorbent layer often results in areas of overlap between adjacent bands on development of the chromatogram. A search for means of obviating these difficulties has resulted in the development of the sample applicator described herein.

Materials and construction

The apparatus as depicted in Fig. 1 consists of a multiple pipette holder made from an aluminum bar (1) with holes drilled near its end which permit it to slide freely

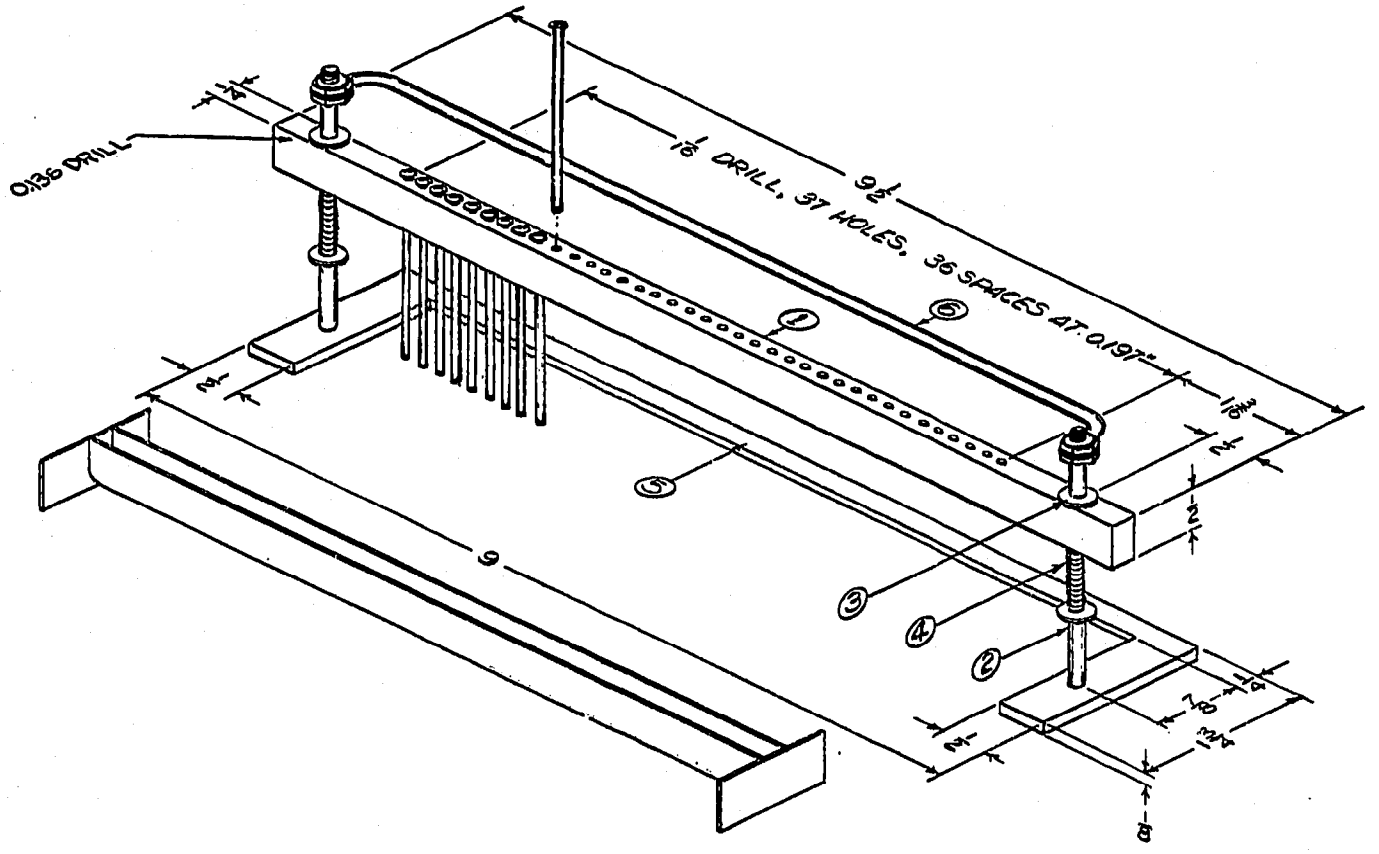

Fig. 1. Construction details for multiple spot applicator.

on two vertical 1/8 in. brass rods (2) and which is held up against adjustable stop washers (3) by partially compressed springs (4). The top and bottom stop washers are 1/4 in. O.D. and 3/32 in. I.D. and are enlarged slightly with a punch so that they may

be forced onto the vertical rods. The bottom washers which support the springs are soldered in place while the top washers remain in place by friction and may be adjusted by forcing them either up or down. The two vertical rods which are threaded, screwed into tapped holes, and soldered in place in the cutout base plate (5) and the upper horizontal tie rod (6) form the rigid support for the apparatus. Thirty-seven precisely drilled 1/16 in. holes spaced 0.197 in. (0.5 cm) on centers in the aluminum crossbar carry the spotting pipettes. These are made from pieces of 1.5 mm O.D. Pyrex melting point capillary tubing (Corning No. 9530) selected so that they will just pass through the holes in the crossbar with no friction. A funnel stop is blown at the upper end of each tube and the tubes cut at a length such that the tips will extend to within 1/16 in. of the surface of the chromatographic plate when placed in the crossbar. The delivery tips are fire polished and squared off by grinding on a flat piece of fine carborundum wetted with water. Progress in the latter operation should be observed under suitable magnification and the grinding continued only until the contact surface of the tip is smooth.

A sample trough of suitable volume and design from which the pipettes may be filled for spotting preparatory plates may be constructed from metal, glass, or plastic depending upon the chemical nature of the samples and solvents employed. A trough of the design shown in Fig. 1 was constructed of No. 23 gauge Monel metal and has proven to be satisfactory for use with non-corrosive solvents.

By removing alternate pipettes from the applicator, the remaining pipettes then may be loaded with individual samples from micro-test tubes. For this purpose a support of suitable dimensions drilled with 19-1/4 in. (1 cm) on centers to accept small test tubes made from 6 mm glass tubing may be constructed of metal, wood, or plastic.

Operation

In replicate spotting of plates for preparatory separation of fractions in bands or spotting a series of individual chromatograms, the device is positioned over the trough or the test tubes in their support and the pipettes lowered into the test solutions by depressing the crossbar on its supporting springs by applying pressure with the index fingers placed at each end of the crossbar. The crossbar is then allowed to return to its normal position and the applicator with its pipettes charged with sample(s) is lifted and positioned over the chromatographic plate using the inside edge of the base plate to index the line of spot application at 7/8 in. from the bottom of the plate. The crossbar is then slowly depressed to slightly beyond the point at which the pipettes make contact with and begin to discharge sample on the adsorbent layer. When the spots have enlarged to the desired diameter, the crossbar is allowed to return to its normal position. The operation may be repeated when the solvent has evaporated from the spots should deposition of more material on the plate be desired.

Applicators of the design described above are in use in several laboratories at the University of Connecticut. These have been used successfully for the deposition of glycerides, phospholipids, hydrocarbons, steroids, substituted cyclohexanols, alkaloids, and 2,4-dinitrophenylhydrazones in bands on thin-layer plates with various appropriate solvents including ether, hexane, benzene, carbon tetrachloride, and chloroform. In addition to the great saving in the time of application, their use has resulted in little or no disturbance of the adsorbent layer and upon development of the

plates the separated bands have been remarkably straight with no areas of overlap between adjacent bands.

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Dünnschichtchromatographie von Lactonen, Lactamen und Thiol-lactonen

Für synthetische[1] und biochemische Arbeiten[2] auf dem Gebiet der Lactone, Lactame und Thiol-lactone war es notwendig, ein Nachweisverfahren auszuarbeiten, das es gestattete, Synthese- und Stoffwechselprodukte mit geringem Zeitaufwand zu untersuchen und zu identifizieren. Wegen ihrer einfachen Handhabung bedienten wir uns der Dünnschichtchromatographie nach STAHL[3]. Die Platten für die Chromatographie wurden nach STAHL[4] mit einem Streichgerät (Desaga, Heidelberg) mit Kieselgel G (Merck) beschichtet. Die Schichtdicke beträgt *ca.* 250 μ. Die Substanzen wurden in einem Abstand von $1^1/_2$–2 cm vom unteren Rand entfernt aufgetragen. Die Laufstrecke betrug durchschnittlich 10–13 cm.

Die Lactone lassen sich durch den Eisen–Chelat-Komplex ihrer Hydroxamsäuren nachweisen[5]. Sie werden zuerst durch ein Gemisch von gleichen Teilen 12.5 % NaOH in Methanol und 5 % Hydroxylamin-hydrochlorid-Lösung in Methanol in ihre Hydroxamsäuren überführt, die nach 10 Min. durch Besprühen mit Eisessig und einer 10 % Fe(III)-chlorid-Lösung in Wasser als braune Flecke sichtbar gemacht werden können.

Durch Auftragen verschieden grosser Mengen und anschliessender Chromatographie wird die Erfassungsgrenze bei Butyrolacton bestimmt. 0.07 μMol können noch nachgewiesen werden. Bei zu hoher Konzentration ($> 3\mu$Mol) tritt Schwanzbildung und eine Verschiebung der R_F-Werte zu höheren Werten ein.

An Papierchromatogrammen konnten Lactame mit Hilfe von Ninhydrin und anschliessender Jodbedampfung nachgewiesen werden[6]. Die besten Resultate an Dünnschichtchromatogrammen erzielten wir mit Hilfe des Dragendorff-Reagens. Dieses wird folgendermassen zubereitet[7]. Zu 850 mg Wismutnitrat in 50 ml 20 % Essigsäure werden 8 g KJ in 20 ml Wasser hinzugefügt. Man verdünnt 10 ml dieser Stammlösung mit 20 ml Essigsäure und 100 ml Wasser. Lactame reagieren rotbraun auf gelbem Hintergrund. Die untere Erfassungsgrenze lag für N-Methyl-pyrrolidon