

pad cracks, the crack is tamped with a spatula in order to maintain an even flow of solvent through the pad). This wash was followed in order by 200 ml 25 % methanol; 300 ml isopropanol-acetic acid-water (60:20:20); 200 ml water; and 200 ml methanol. The full washing procedure took 25 min. The cellulose pad was filtered dry under full vacuum, and dried further, overnight at 40°, in an oven. The dried pad could readily be broken into a fine powder by shaking it in a bottle. The resulting powder was used in the usual way for spreading, though 5-10 % less water was needed if colloidal material had been removed by a washing treatment.

Bulk solvents were used—any impurities that they contributed were completely overshadowed by those they removed. Other effective solvent mixtures included: ethyl acetate-acetic acid-water (1:1:1) (too expensive for general use); 0.002M EDTA made to pH 11 with LiOH; and 0.1M LiOH (layers tended to blister when sprayed with aqueous solutions). Other solvents when required could be used equally well. However, the following details should be noted. Making the original slurry in 80 % methanol or a similar mixture yields a pad that filters much more freely than that obtained from an aqueous slurry. Aqueous solvents tend to remove particles of colloidal fineness from the cellulose powder: presumably this is one cause of the faster solvent flow in washed-cellulose layers. It is necessary to dry the cellulose after washing, as attempts to use the washed cellulose while it was wet (by adding further water or ethanol and homogenizing then spreading) yielded plates with a rough or broken surface. It was better to use a final methanol wash, instead of drying the cellulose in the oven directly after a water wash. The latter procedure yielded a cellulose block that was very difficult to powder, though the dried lumps could be broken up into a smooth suspension if they were homogenized with water in an efficient homogenizer (Virtis 45 at 20,000 r.p.m.). In spreading suspensions of washed cellulose, scrupulously clean plates were required, since surface active impurities present in the normal cellulose had been removed. An unexplained observation concerns the stability of cellulose layers when plates were dipped in water or sprayed with buffer solutions, as required in thin layer electrophoresis techniques. Freshly made layers of acid-washed cellulose blistered or pocked rather readily. However, when these same layers were kept for a week or two before they were dipped or sprayed with buffer, normal wet stability was obtained.

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Conical preparative thin-layer chromatography

Circular thin-layer chromatography has been used without the need for a tank¹⁻³, and has proven a very useful method for rapid determination of suitable eluants⁴ for ascending linear TLC. Various closed-chamber circular TLC systems have been developed⁵ for analytical purposes, but this method has not yet been widely used

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for preparative purposes. In this note we wish to report on a new circular device which is well suited for preparative adsorption chromatography.

Experiments with circular systems similar to those described by STAHL⁶ revealed advantages over linear systems, mainly because of better separation of compounds having close R_F values, or having a tendency to trail. However, application of the layer and even contact to the wicks used in these systems caused complications. The system to be described eliminates these difficulties.

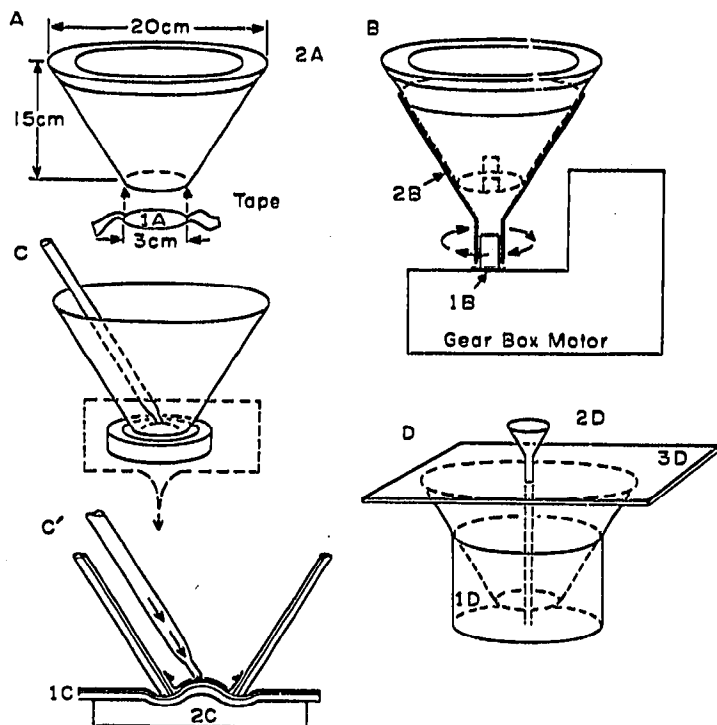


Fig. 1. (A) Cone frustum with polyethylene disc (1A) and rubber collar (2A); (B) mechanical thin-layer applicator (gear box motor) with vertical drive (1B) and polyethylene support funnel (2B); (C, C') sample application procedure and sample applicator with bilayered seal (1C) and base plate (2C); (D) development procedure in cylindrical vessel (1D) with funnel for solvent addition (2D) and cover plate (3D).

As a support for the layer we used a glass "frustum" of 15 cm height and 20 and 3 cm I.D., respectively, obtained from a funnel ("A" in Fig. 1). Such cone frustums can be prepared without difficulty by cutting off the necks of commercially available glass funnels with a glass saw. For the application of the layer, the smaller end of the frustum is closed with a polyethylene disc (1A) taped to the outside glass walls; the other end is guarded by means of a wide rubber collar (2A) (cut from a household rubber glove) slipped over one end. A slurry of 25 g Silica Gel GF₂₅₄ (according to STAHL) in 45 ml of water is poured into the frustum. With a little experience, one can produce an even layer over the inner surface by rotating the cone and tapping the sides to induce proper flow.

A mechanical device ("B" in Fig. 1) to achieve this purpose consists of a variable-speed gear-boxed motor (Eberbach Corp. con-torque), with its vertical drive (1B) fixed to a polyethylene funnel (2B) which is used as support for the glass frustum.

The speed was varied between 100–200 r.p.m. in order to evenly distribute the slurry on the inner surface. After the CaSO_4 has bound, the motor is turned off, and after drying at room temperature, the layer is cut to 12 cm actual length with a spatula. The mechanical device offers, however, little actual advantage over the manual procedure and the layers prepared in either way are comparable in uniformity.

After application of the layer the small opening of the glass frustum is placed onto the sample applicator ("C" in Fig. 1) which consists of a bilayer (1C) of 0.01 mm DuPont "Teflon" film and beneath that a 0.5 mm Silicon rubber sheet put on top of the base plate (2C) which was machined out of metal. The mixture to be separated is dissolved in about 2 ml of a solvent of low to medium eluting power, and is then applied in a rapid flow out of a pipet onto the little hill which has formed in the middle of the lower end of the "frustum". The "cone" is taken out after the solution has been absorbed by the layer and is air dried. It is again placed on the applicator and this time 2.5 ml of a solvent with a relatively high eluting power is pipetted onto the hill. This second solvent collects the sample into a narrow band. The cone is then dried again and the last procedure carried out for a total of three times, each time using exactly 2.5 ml solvent.

The dried cone bears the substance mixture in a narrow circular band 2 cm above the lower end. For development ("D" in Fig. 1) the cone is then placed into a cylindrical vessel (1D). The eluent is added to the vessel through a long necked funnel (2D) which runs through the cover plate (3D), until its level is about 5 mm below the substance band and well inside the cone. The eluent rises in the layer and when it reaches the upper edge of the silica gel, the cone is taken out, dried, and usually run a second time.

Detection and isolation of the separated materials is done with a U.V. light source (mineral light) and a long-tipped vacuum slurp gun. The wide opening of the cone makes detection and isolation no more difficult than that with glass plates.

We have used this method successfully in separating anomers of glycosides and other sugar derivatives and found that it can be of particular value whenever compounds are involved which tend to trail. The method frequently gave superior separation compared to ascending preparative TLC on 20 × 20 cm plates, and is of relatively low cost. Until now, we have not been able to prepare conical layers without binder. We suggest the fabrication of constant-thickness, precoated inert material cones as an improvement of preparative TLC on prefabricated layers.

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