CHROM. 5316

Preparative cylindrical thin-layer chromatography

Preparative thin-layer chromatography (TLC) carried out on glass plates requires special apparatus[1] to apply a uniform narrow band of sample along the line of origin. Although ingenious in their design, the generally high cost of these applicators limits their wide use. Sample application manually, the alternative, is tedious and does not produce a uniform band. Preparative TLC on 20 × 20 cm plates also requires a large developing chamber of moderate cost which is not readily saturated with solvent vapor and requires moderate amounts of solvent.

The advantages of TLC on cylindrical surfaces have been described[2,3]. Foremost of these are that application of a uniform layer by dipping is accomplished easily without the use of special spreaders and a cylinder of diameter d is equivalent in capacity to a flat plate of width $\pi \cdot d$, and thus much smaller inexpensive developing chambers such as beakers can be employed with the attendant marked reduction of solvent quantities used. Edge effects are eliminated by the use of cylindrical plates.

Preparative cylindrical TLC, especially when test tubes are employed, offers another advantage: the facile application of a quite uniform band of sample without the use of specially designed sample applicators. Herein a simple but novel technique for the application of a narrow sample band to an adsorbent-coated test tube is described. An alternate technique may be the subject of a future paper.

Experimental

The procedure described is for the separation of the components of the dye Sudan Black B (Fisher Certified) used because it produces bands, some of which are readily visible in photographs.

A washed and dried test tube (in this instance, 38 mm O.D. × 300 mm, Fig. 1) is dipped into a well-shaken slurry prepared to the desired thickness with Silica Gel 7GF (Baker Analyzed) and a 2:1 by volume mixture of chloroform and methanol. (PEIFER[4] reports that suspensions containing less than 25 g or more than 40 g of Silica Gel G per 100 ml liquid phase are not sufficiently uniform for TLC analysis.) The slurry is stored in a tall narrow jar having a mouth diameter about 25 mm greater than the test tube diameter. Ideal in this case for both slurry container and developing chamber is the 60 × 240 mm 200-S developing chamber marketed by Research Specialties Co.* The dipped test tube is smoothly withdrawn from the slurry and held in a vertical position so that the last drop of draining slurry remains centered on the tube end until most of the solvent has evaporated. The plate may then be activated if desired. Uniform thin layers as thick as 240 μ (measured by microscope) have been prepared by dipping.

The coated tube is clamped in a vertical position, closed end up. Sample is slowly applied to the center of the end by a syringe (Fig. 2). In this instance a 250 μl syringe is employed to deposit the sample solution (0.3 mg Sudan Black B in 50 μl methanol). (In the largest scale separation performed on a 38 mm test tube, a sample of 3.5 mg of Sudan Black B was separated satisfactorily.) The amount of solvent used to apply the

* Research Specialities Co., 200 South Garrard Blvd., Richmond, Calif., U.S.A.

sample is immaterial, as long as it is not applied so fast that the wetted area loses its circular shape.

When the solvent used to apply the sample spot has evaporated, sufficient amounts of a solvent with high elution ability (*e.g.* methanol) are applied to the center

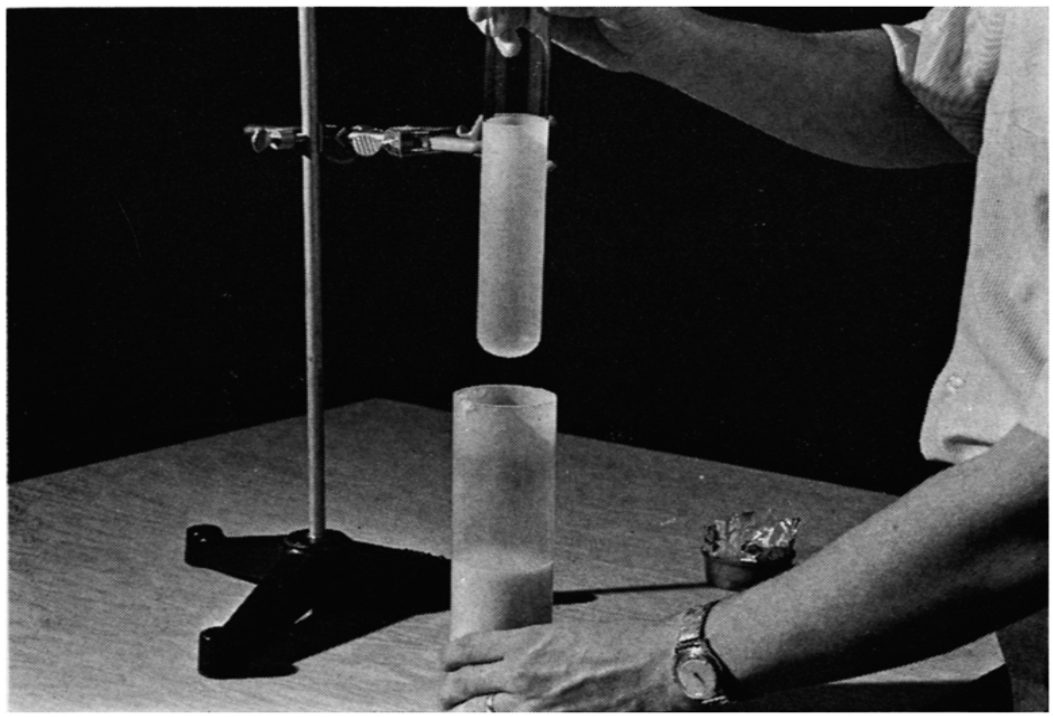

Fig. 1. Preparation of cylindrical plate by dipping.

Fig. 2. Spotting of sample by syringe.

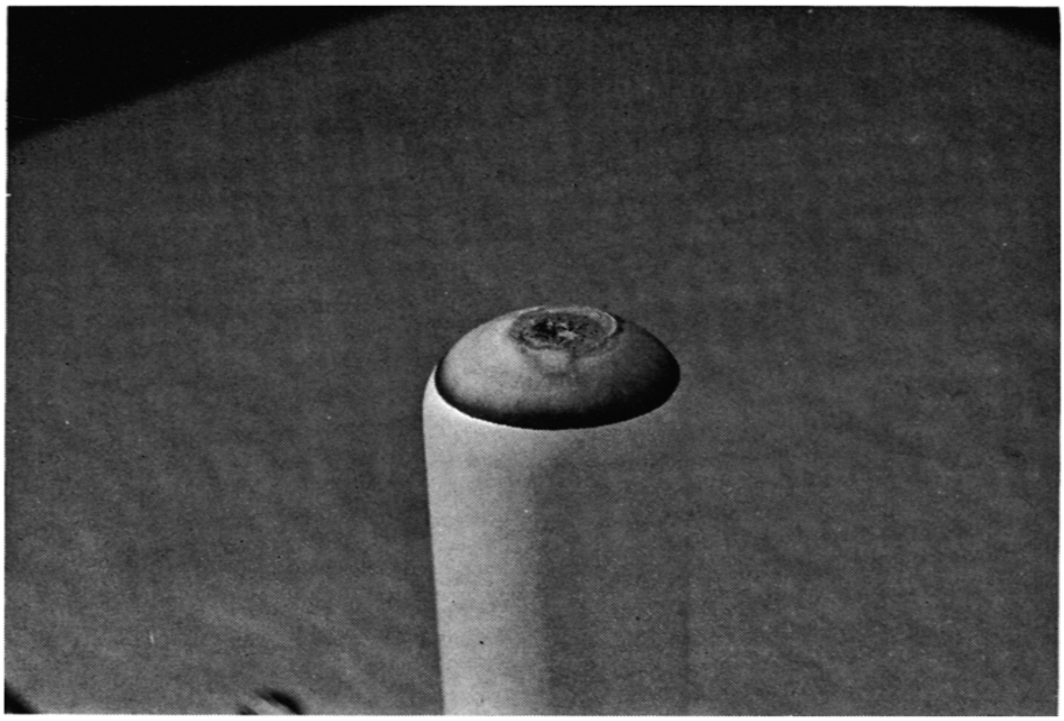

Fig. 3. Sample spot spread to a band by the use of solvent of high elution power.

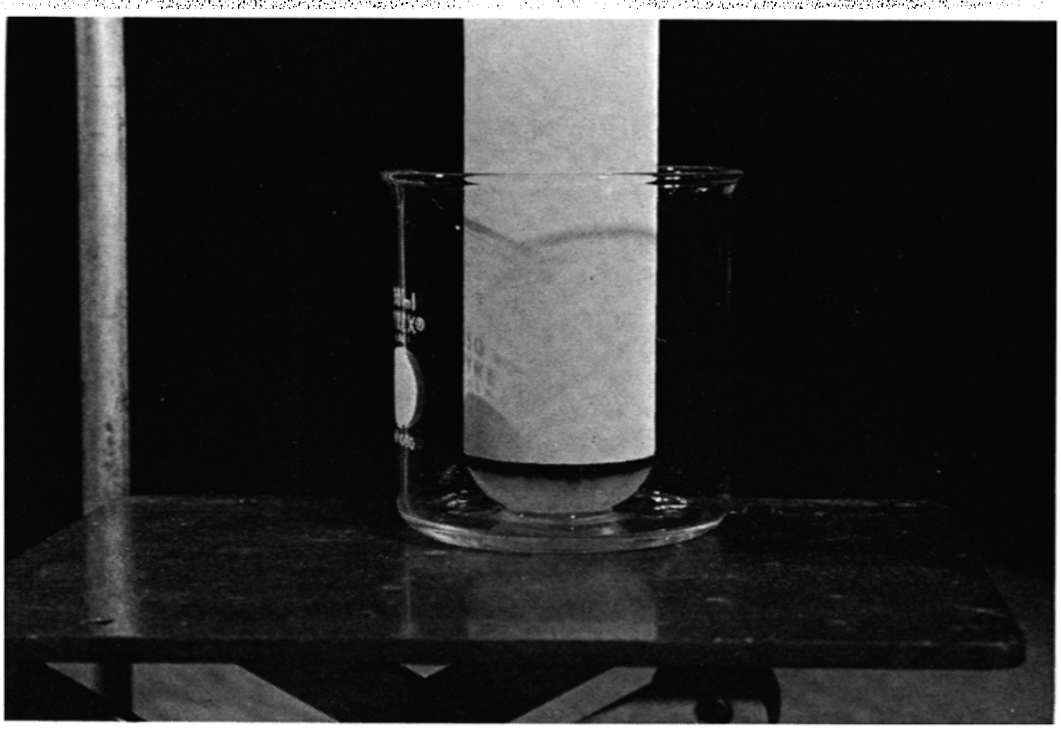

Fig. 4. Positioning and narrowing the sample band.

of the sample spot via syringe so as to cause the sample spot to spread radially. This should be continued until the band of sample has reached a distance of 5–10 mm measured vertically from the tip of the hemispherical test tube end (Fig. 3). It is again important (more so than previously) that the solvent be applied to the center and not

be applied so fast as to cause washing out of the thin layer or variance from a circular band shape. Positioning of the syringe is facilitated by use of a clamp above the test tube. In the example described, 150 μl of methanol were used to spread the spot to a ring.

The test tube is now clamped vertically (closed end down) above a beaker containing the solvent of high eluting power (solvent depth of 2–4 mm). The beaker is raised by a laboratory jack with a flat platform until the solvent touches the thin layer (Fig. 4). The solvent is allowed to rise until the sample band is approximately 5 cm above the hemispherical end of the tube. The sample band can, if necessary, be condensed to a narrower band by repeating this step one or two times allowing the solvent front to proceed no further than the top of the original band. A typical result is pictured in Fig. 5.

After thorough evaporation of the solvent used to position the sample band, the chromatogram may be developed. A suitable chamber would seem to be a cylindrical one as pictured in Fig. 6. Small chambers saturate more readily with vapor and require smaller amounts of solvent than do larger ones. Sudan Black B can be satisfactorily separated into seven components using benzene as developing solvent. Two of these are distinctly visible in the developing plate pictured in Fig. 4. To avoid damage to the thin layer (especially with narrow-checked chambers) and to assure a level solvent front, the developing chamber containing solvent should be raised to the plate by use of a laboratory jack. During development, the opening of the chamber may be closed with aluminum foil.

Removal of the silica gel from the plate for isolation of components seems best accomplished by scraping the powder off into a sintered glass funnel. The test tube is loosely clamped in a horizontal position, and the desired component is scraped off in

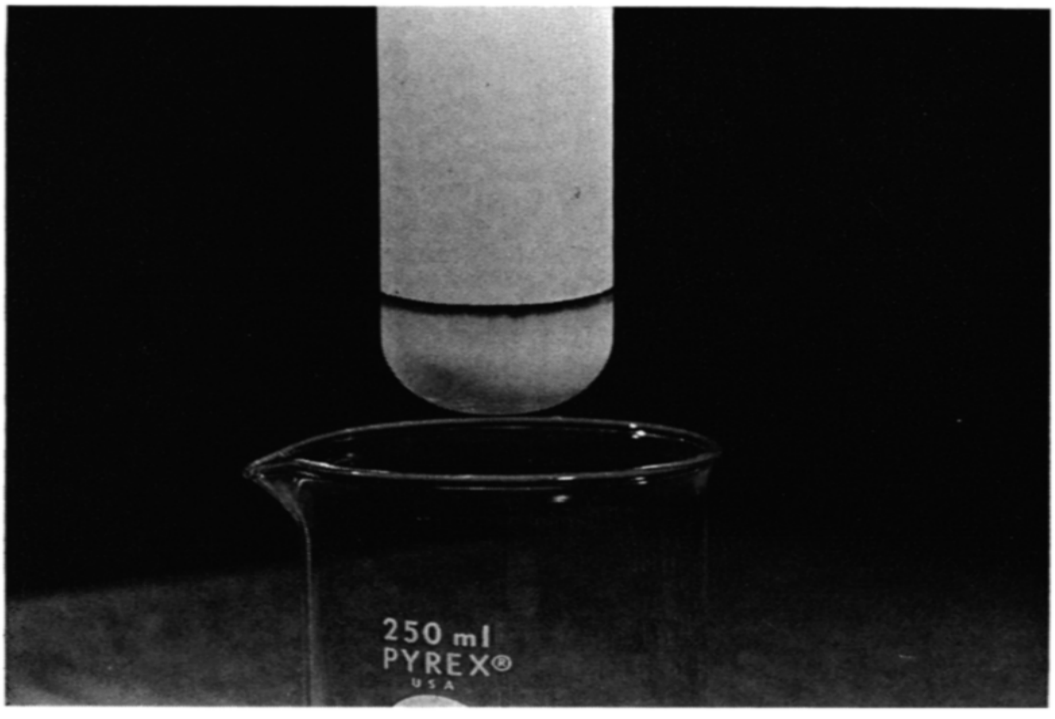

Fig. 5. A narrow sample band ready for development.

lathe fashion (Fig. 7). If scraping is restricted to the "underside" of the tube, all powder will fall immediately downward to the receiving funnel, and, therefore, not even minor contamination produced by powder falling over onto an adjacent band can occur. Hence neat removal of sample is easier with cylindrical plates than with

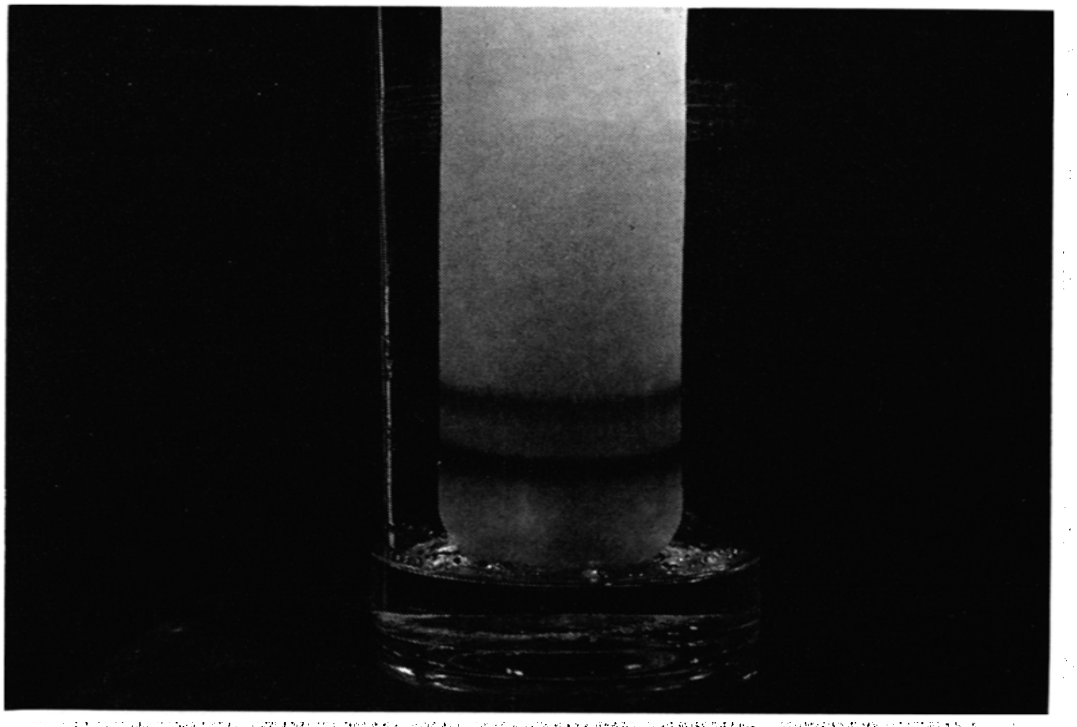

Fig. 6. Separation of Sudan Black B by benzene on Silica Gel 7GF layer.

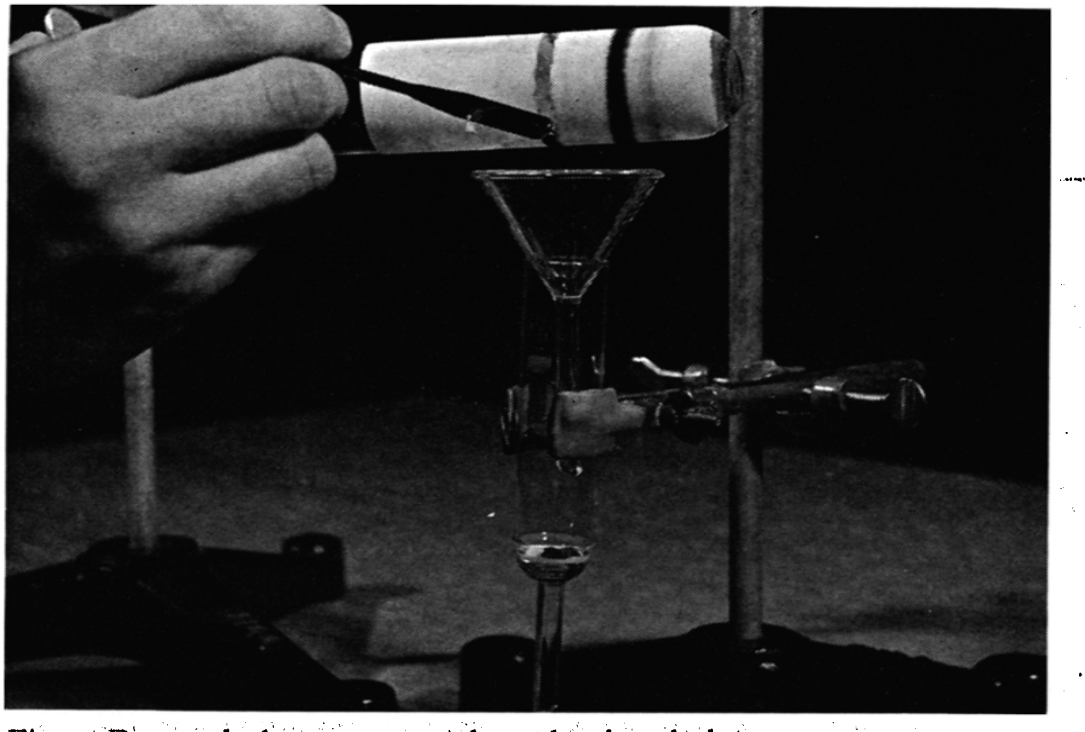

Fig. 7. Removal of a component from developed plate.

large flat plates which are difficult to scrape upside-down. Extraction of components from silica gel is performed by previously described techniques[5,6].

Discussion

The 38 × 300 mm test tube used in this illustration is equivalent in capacity to a flat thin-layer plate of dimensions 119 × 300 mm. The use of a 65 × 500 mm test tube (commercially available at nominal cost) would provide a thin-layer plate of greater capacity than the 20 × 20 cm flat plate frequently used for preparative TLC.

This technique would not be satisfactory for TLC work where layers much thicker than *ca.* 250 μ are necessary, where a fixed calibrated layer thickness is required or where two-dimensional development is desired. No technique has yet been devised for application of gradient TLC methods to cylindrical plates.

The chief advantages of this technique lie in its simplicity and low cost. All steps are easily performed. On the scale described here it permits the use of preparative TLC for separation of quantities up to approximately 10 mg in practically any laboratory.

Department of Chemistry, DAVID M. JORDAN
State University of New York, College at Potsdam,
Potsdam, N.Y. 13676 (U.S.A.)

1 E. STAHL, *Thin-Layer Chromatography*, 2nd ed., Springer Verlag, New York, 1969, pp. 98, 99.
2 R. IKAN AND E. RAPAPORT, *J. Chem. Educ.*, 44 (1967) 44.
3 D. JORDAN, *J. Chem. Educ.*, 45 (1968) 510.
4 J. J. PEIFER, *Mikrochim. Acta*, (1962) 537.
5 E. STAHL, *Thin-Layer Chromatography*, 2nd ed., Springer Verlag, New York, 1969, p. 101.
6 R. AMOS, *J. Chromatogr.*, 48 (1970) 343.

Received January 25th, 1971