Spot applicator for thin-layer plates

NORMAN S. RADIN

Mental Health Research Institute, University of Michigan, Ann Arbor, Michigan 48104

SUMMARY A device that lowers a micropipet into position just above a thin-layer plate is described. Use of a self-filling pipet and foot-operated bellows allows one to apply many portions of a sample to the same point at high speed without damaging the adsorbent coating.

KEY WORDS thin-layer chromatography · applicator

SEPARATIONS BY TLC are most effective and reproducible when the sample spots are applied without making a hole or indentation in the powder layer. While many people have a sufficiently steady hand to do this reasonably well, the operation becomes somewhat more difficult and undesirably slow when multiple portions of solution must be applied to the same point. When a relatively large micropipet is used for the multiple applications, the spots tend to be erratic in size and location. This factor, like holes in the powder, tends to give distorted, overlapping spots of erratic R_f.

This note describes a device that is easy to make and use, and that allows one to deposit many portions of sample in the form of a single small spot. The portions can be applied as fast as the solvent evaporates (about 10 2μl spots per min). Basically the device is a pipet holder that is readily lowered by a swinging motion so that the pipet tip is just above a specified point in the powder layer.

An important feature is the use of a platform (Fig. 1) which holds the TLC plate and air blower (not shown). The platform we use is part of a TLC sample streaker, designed in this laboratory and modified by Nicholas Pelick

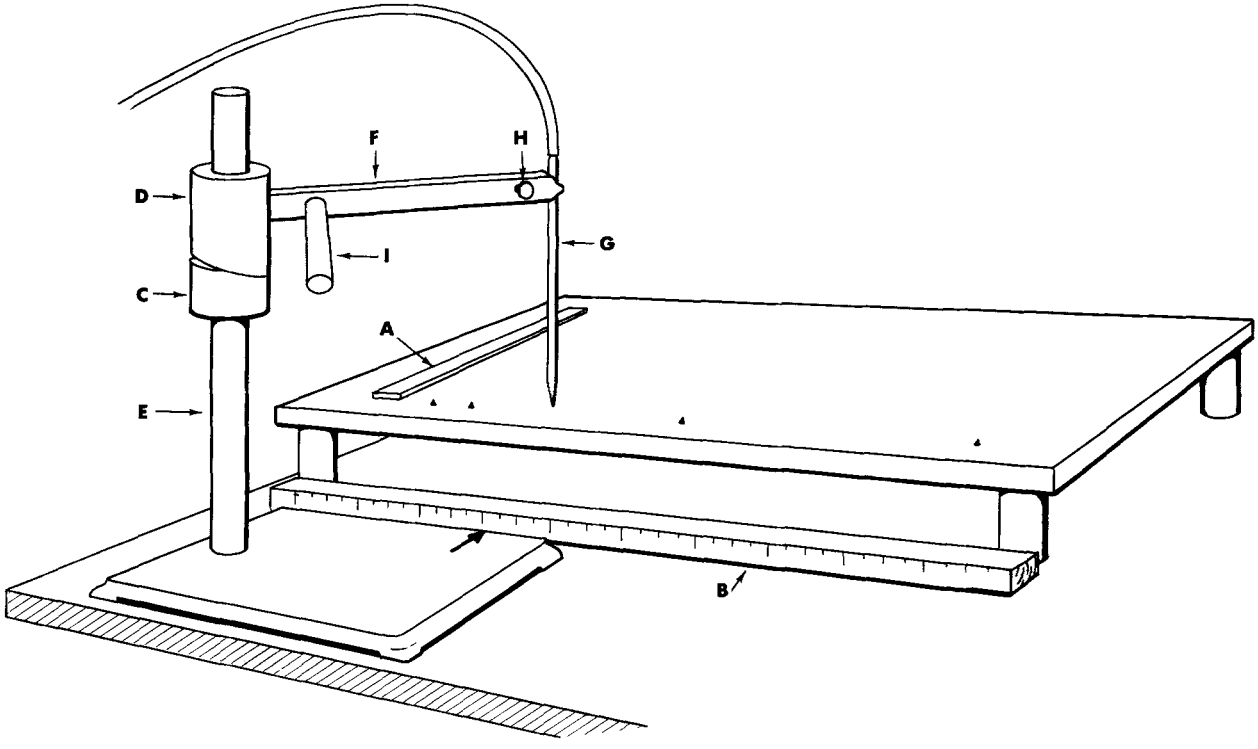

FIG. 1. TLC spot applicator, over-all view. *A* is a metal alignment bar, which keeps the TLC plate perpendicular to the front of the platform and standardizes the starting position of the spots. *B* is a ruled alignment bar, which keeps the pipetting stand parallel to the front of the TLC plate. The height-adjusting screws, with wide knurled heads below the platform, are shown near the front. They are spaced so as to support plates 5–40 cm wide; their positions are (measured from the right edge of *A*) 6, 44, 94, 194, and 394 mm. *C* and *D* are two pieces of nylon rod, 37 mm in diameter, with a hole drilled through the center to allow a close fit to *E*, an aluminum rod about 13/16 inch (20 mm) O.D. *E* is screwed at the bottom into an ordinary iron ring stand base. The two nylon rods are sawn from a single piece at an angle of about 25° and two portions are sawn off at right angles (see Fig. 2). Part *C* is fixed permanently in place by two set screws (not shown). Part *D* rotates freely on *E*, rubbing against *C*, which forces it up or down as it rotates. *F* is a metal bar, 230 × 12 × 12 mm, with a groove at the right end to hold the micropipet, *G*. *G* is a 2 μl pipet, the design of Paul Mills and Felix Sabatino (K-42252, Kontes Glass Co., Vineland, N. J.). *H* is a pair of screws, one on each side, which hold taut the two rubber bands that hold the pipet firmly against *F*. *I* is a metal rod, 125 × 12 mm, which is used as the handle for rotating arm *F*.

Abbreviation: TLC, thin-layer chromatography.

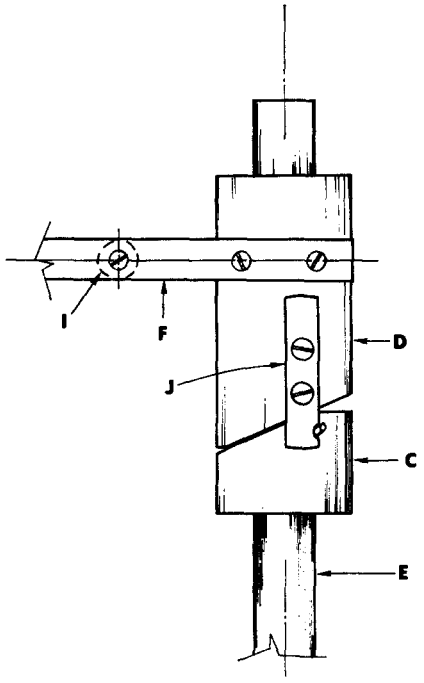

FIG. 2. Back view of rotating parts. The pipettor is in the lowered position, with bar *J* up against the limiting stop. If the set screws are set in *C* correctly, the pipet tip will always be at the correct position above the TLC plate (about 20 mm from the front).

for manufacture (Applied Science Laboratories Inc., State College, Pa.). The TLC plate rests at the front (bottom) end on two screws and the third point of suspension is a small "chromatography clamp" which has a piece of rubber $^1/_8$ inch thick glued to the lower jaw. The two screws raise the plate a trifle to accommodate plates and coatings of varying thickness; the plate as a whole should slope slightly down toward the front.

Fig. 1 shows the pipet in the lowered position. The pipet must be filled while in the raised position, swung forward beyond the edge of the table. It is filled by capillary action simply by raising the solution to the tip of the pipet. The pipet is next swung back to its lower position, which is fixed by a metal stop in the back of part *C* (Fig. 2). The liquid is expelled by a gentle squeeze of the narrow-bore rubber tube attached to the top end of the pipet. The tube runs down to the floor and the floor end is closed with a dowel and taped to a heavy board. Thus the foot can be used to expel the liquid, while the left hand serves to move the pipet and the right hand holds the remainder of the sample. The entire process of filling and expelling takes about 6 sec.

Adjusting the Plate Height. Before filling the pipet, one must raise the TLC plate so that it is just under the lowered pipet. This adjustment is greatly aided by use of a 12 volt, high intensity lamp set at the left edge of the platform with the reflector horizontal. This gives a very sharp shadow from the pipet. If room illumination is too intense, the shadow will be indistinct and it may be necessary to build a shade near the plate.

Several precautions are necessary for this step. (*a*) The plate should be pushed slightly to the left while adjusting the screws to maintain contact with the aligning bar, *A*. Some screws tend to make the plate slide to the side. (*b*) The operator must not lean on the pipettor handle (*I*) while adjusting or rotating the apparatus. The materials are not rigid and thoughtless pressure can produce a scratch in the TLC powder. A light push with one finger is all that is needed for the rotation, but the stop should be approached firmly to make sure the pipet is at its normal, "down" position.

The platform, if not already available as part of the streak applicator, can probably be substituted by a laboratory jack, but in our experience such jacks give rather erratic movements.

Notes on Fabrication. In making the apparatus, it is important to make a snug fit between the aluminum support rod *E* and nylon part *D*. A little heavy oil between the two helps.

The stop pin in the back of *C* (Fig. 2) should be placed so that the upper nylon bearing does not quite descend to its lowest point, otherwise there will be two stop positions—one gravitational, the other mechanical.

A weight on the support base is probably helpful in stabilizing the apparatus. We added nylon gliders to the base to reduce damage to the table, as the apparatus must be slid alongside the ruled alignment bar (*B*).

The pipet recommended (Fig. 1) is particularly suitable because of its length. Most micropipets of the self-filling type are too short to reach into the bottom of common test tubes or flasks.

While a hair dryer "gun" can be used to dry spots, there is the danger that this might differentially influence the adsorptive power of the coating from spot to spot. We use a long "squirrel cage" blower (STD-200-21881, The Torrington Manufacturing Co., Torrington, Conn.), which sends a much more uniform air flow over the plate; this is especially useful when the platform is used as a streak applicator. The blower is mounted on a sheet metal box which fits closely over the plate. Fastened on the top of the box, near the front, is a strip heater, which gives uniform heat over the front of the plate. A little patience may be substituted for the heater. The above-named blower is unusually economical and compact.

Note on Use. The pipet is readily washed between samples by immersion of its tip well below the surface of a solvent, which rises into the wide part of the pipet. The pipet drains rapidly except for the last 2 μl, which is expelled by foot pressure on the bellows.

When the sample drop is expelled from the pipet, the operator should hold his foot down for a few moments to give the solution time to soak into the powder. Otherwise

some liquid will return to the pipet and rise above the capillary segment when the foot pressure is released.

It is helpful to draw an aiming arrow on the base, pointing toward the ruled bar, *B*. The arrow shows the position of the pipet at its lowest point and helps one estimate where the spot will be deposited and the spacings between spots.

The 2-μl pipet makes spots about 4.5 mm wide. Smaller spots can be made with a 1 microliter pipet, also available from Kontes. It is easy to deposit adjacent multiple spots by sliding the pipet support stepwise along the alignment bar.

It becomes evident when colored materials are deposited by multiple spot application that the sample is actually concentrated in the form of a ring, rather than a disc, but it is not immediately obvious whether the effect is harmful. We compared a single spot containing 50 μg each of methyl stearate and cholesterol in chloroform with eight superimposed spots containing the same weights; after development there was no discernible difference. Using hexane as solvent and doubling the applied weights also showed no effects.

The possibility exists that oxygen-sensitive materials suffer more when applied in dilute rather than concentrated solution, because of the longer exposure to the air blower. Addition of an antioxidant to the sample solution seems advisable in such a case.

Curtis (1) has recently described a TLC spot applicator for simultaneous application of many samples. Our device has the advantages of allowing flexible spacing of the individual samples and of yielding more uniform spots. It probably requires less skill in use, especially in filling the pipets.

I am indebted to our Shop Division (Conrad H. Juchartz, William O. Strauch, and Paul L. Yoder) for valuable suggestions on design.

This work was supported in part by PHS Research Grant NB 03192 from the National Institute of Neurological Diseases and Blindness, U. S. Public Health Service.

Manuscript received 12 May 1967; accepted 5 July 1967.

Reference

1. Curtis, P. J. 1966. *Chem. Ind.* (*London*). 247.