Automated quantitative spotting for analytical thin-layer chromatography*

Although a number of devices have been described for sample application for preparative thin-layer chromatography (TLC)[1-3], there has been little attention given to the problem of analytical TLC[4,5]. For two-dimensional separations, especially, the sample must be applied as a small circular spot of uniform size. Frequently heat cannot be used to accelerate the process and as aqueous solutions are often used, the operation can take a great deal of time. If performed manually, the procedure is not only laborious but it is likely to result in errors due to the tedium of the work. The apparatus described here is not designed to make the operation quicker, but to minimize human effort and error by largely automating the process.

The microsyringe

Of the various devices that can contain and deliver small volumes of liquid, the microsyringe** has been found most satisfactory since it is most easily automated and is available in a range of sizes. The most serious problem, however, is the shape

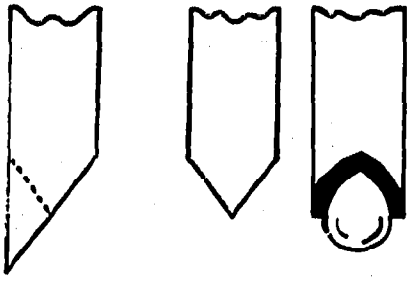

Fig. 1. Modification of needle point for TLC spotting.

of the needle point. The standard point is designed for piercing the septum of a gas–liquid chromatograph and is very sharp with a recessed opening. This is quite unsuitable for TLC spotting. The simplest modification of this would be to cut the needle tip square. In this case, however, there is a tendency for particles of the adsorbent to be picked up which often plug the orifice. Frequently, such blockage can only be corrected by cutting the needle shorter. An improved tip design has been found that largely eliminates this problem:

Using a small hand grinder with a fine abrasive wheel***, a reverse bevel, symmetrical to the original bevel, can be cut on the needle point (Fig. 1). In this way, the opening is centered at the tip of the needle, but recessed from the point. With this configuration there is no drop hold-up on the side of the needle and if the point should happen to touch the plate sufrace, adsorbent is not easily picked up or the opening plugged§. The same result can be approximated by cutting a piece of 0.38 mm I.D. polyethylene tubing§§ and slipping it over the needle. This is not wholly satisfactory, however, since there is a tendency for aspiration around the needle

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** Hamilton Company, Inc., Whittier, Calif., U.S.A.
*** Dremel Model No. 2, Dremel Manufacturing Co., Racine, Wis., U.S.A.
§ Syringe needles of this shape are available on special order from the Hamilton Company.
§§ Intramedic; PE-20, Clay-Adams, New York, N.Y., U.S.A.

unless the tubing is sealed in place. The plastic tip is less likely to mar the adsorbent surface, however, and permits the syringe to be used for other purposes.

The most satisfactory and versatile syringe pump available to us was the Sage* 237-1 variable speed pump with a range of 0.3 μl/min to 7.7 μl/min when fitted with a 10 μl syringe. The syringe must be precisely positioned in the holding clamp, however, since otherwise it will either not deliver completely or the plunger will be bent. Since the Sage pump is designed for horizontal operation, it was best

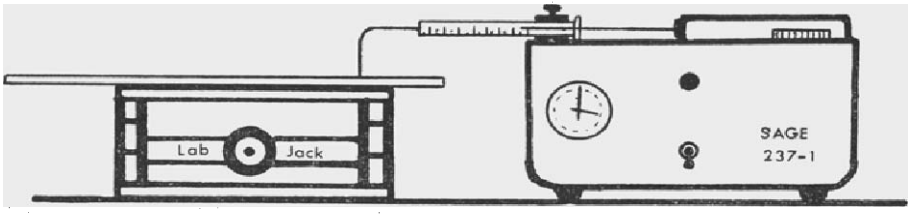

Fig. 2. Arrangement for TLC sample application.

adapted for our use by bending the syringe needles to a 90° angle taking care not to occlude the bore. The TLC plate could then be positioned under the needle tip by means of a laboratory jack so that the smallest hanging drop just touched the adsorbent surface. In practice, damage to the layer by the chisel-shaped point is minimal and a small scratch-mark aids in locating the origin point later (Fig. 2).

The syringes must be filled while they are clamped in the horizontal position. This is not difficult but it does place a restriction on the size and shape of the vessel holding the sample. Small pear-shaped flasks[6] have been found most suitable for this.

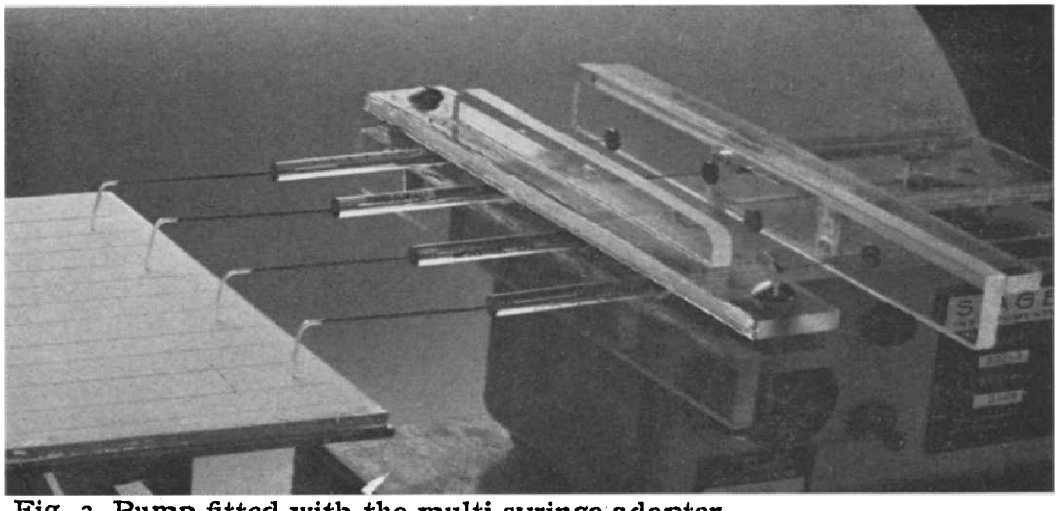

Fig. 3. Pump fitted with the multi-syringe adapter.

The syringe pump

The design of the Sage pump readily allows adaptation for holding four or more syringes (Fig. 3). The adapter consists essentially of three strips of acrylic plastic 6 × 25 × 230 mm long reinforced with narrower pieces of the same length to provide rigidity. One strip is welded (with chloroform) onto the face of the gear driven drive-block so as to extend its effective width to 230 mm. The second strip is drilled so that

* Sage Instruments Co., White Plains, N.Y., U.S.A.

it can be attached to the pump chassis in place of the regular syringe mounting clamp. It is then drilled and tapped 15 mm in from each end, and matching (slightly larger) holes are drilled through the third strip so that these two pieces of plastic can be bolted together to hold the syringes. One layer of 1/4 in. thick foam rubber (or a similar material) is used as padding between the clamping surfaces.

The syringes are positioned with respect to the drive block and each other, between the top plastic strip and the rubber pad underneath. The two bolts are tightened together to provide adequate uniform pressure on the battery of syringes. The clear plastic permits accurate reading of the syringe volumes. The modified pump is as easy to use as the basic unit and is particularly suitable for mono-dimensional TLC. Different volumes can be dispensed from the individual syringes since the plungers will be engaged by the driveblock at the appropriate distance of travel, and will disengage when all the syringes have been emptied.

In Fig. 3, standard Hamilton (No. 701) syringes were modified for spotting by means of 75 mm lengths of 0.38 mm I.D. polyethylene tubing bent to a right angle and cut as previously described. Due to potential leakage around the tube actual modification of the needle itself is to be preferred.

The apparatus can be used for streak application by using a slow speed motor to move the plate past the syringe needle. A second Sage motor unit has been used for this purpose, with a large rubber stopper replacing the drive gear. Heavy string was taped onto the underside of the plate and fastened to the rubber stopper which functioned as a windlass.

Application

The effectiveness of the technique can best be demonstrated by its use for quantitative thin-layer analysis by direct scanning since uniformity of spot size is critical for this procedure.

A 0.1 m*M* mixture of the amino acids histidine (His), taurine (Tau), and alanine (Ala) was spotted at 0.6 μl/min at 4, 6, 8 and 10 μl on a 0.25 × 200 × 200 mm-MN-300 cellulose plate for mono-dimensional high-voltage thin-layer electrophoresis (TLC); layers were prepared with the Desaga apparatus*. The TLC procedure of SAMUELS AND WARD[8] was followed using a Savant FP-18** plate and HV-5000 power supply with direct cooling by means of a Blue M*** PCC-24A refrigeration unit. TLC was for 15 min at 2000 V (25 mA), pH 1.9, 4°.

After drying at room temperature the plate was sprayed with 0.2 % ninhydrin, 0.5 % acetic acid, 0.5 % S-collidine in acetone and allowed to develop overnight in the dark. The circular spots were quantified by reflectance densitometry using a Joyce, Loebl§ "Chromoscan" with Thin-Layer Accessory.

The results are illustrated in Fig. 4. As expected slope values differed slightly for the different amino acids[8] and integrator readings increased with spot mobility.[9] The linearity of the values demonstrated the effectiveness of the spotting technique.

Advantages

The apparatus utilizes commercially available equipment with only slight

* Brinkman, Instruments, Westbury, N.Y., U.S.A.
** Savant Instruments Inc., Hicksville, N.Y., U.S.A.
*** Blue M Electric Co., Blue Island, Ill., U.S.A.
§ Joyce, Loebl and Co., Ltd., Gateshead, England.

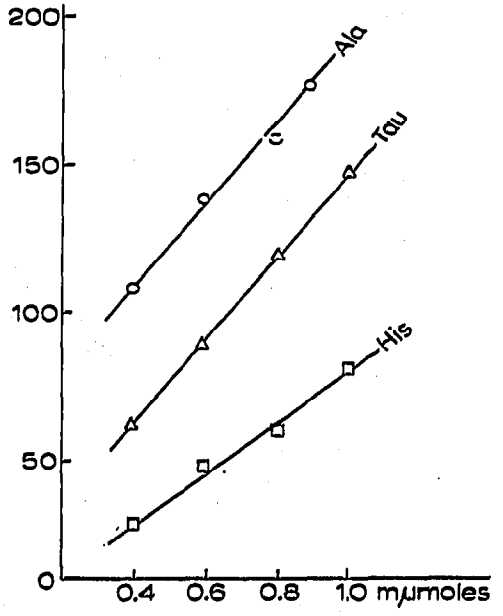

Fig. 4. Standard curves of three amino acids. Integrator readings *vs.* spot concentration. See text for details.

modification. It is essentially automatic and does not require frequent attention. Because micro-syringes are used, a considerable range of sample viscosity or application rate is permissable. The syringe drive pump is not basically altered, and it can still be used for such other purposes as may be required. The multi-syringe adapter is designed to be easily fabricated and none of the dimensions are critical. It can hold one or more syringes, spaced as may be convenient for multiple sample application. The device is useful for spotting two two-dimensional plates simultaneously as well as for mono-dimensional chromatograms. Syringe arrangements can be easily and quickly changed.

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