

CHROM. 4611

New device for automatic streaking of samples for thin-layer chromatography

The following relates the development and improvements made to the applicator reported earlier¹. The semi-automatic spotter has been improved to adapt it for streaking as well as spotting. Microcapillary pipettes have been replaced by wicks which offer numerous advantages. The unit is now designed primarily for spotting and streaking of various sample solutions onto thin-layer plates. Interchangeable modular design of the sample reservoirs permits rapid conversion from various spotting to streaking modes. As currently designed, the unit will spot up to fifteen samples or streak up to four samples simultaneously on one 8 × 8 in. or four 2 × 8 in. plates respectively. Very little abrasion of the coating has been noted in the course of operation, a problem inherent in the earlier model as well as for many commercial models and in manual sample application by pipette.

Description

The basic unit is a rectangular frame, holding either one large 8 × 8 or up to four adjacently placed 2 × 8 plates, suspended by pivots at the rear. This frame is alternately raised and lowered by the action of a slotted cam drive wheel which is turned by a 2 r.p.m. electric motor (see Fig. 1). As a follower knob descends into a cut-out portion of the cam wheel, the frame descends lowering the TLC glass plate(s) into contact with the wicks which are upstanding from their respective reservoirs immediately below. When the follower encounters the raised portion of the cam, the frame is elevated and contact with the wicks is broken. The raised period constitutes the sample drying stage. The process is repeated continuously until the quantity of sample desired has been applied to the plate.

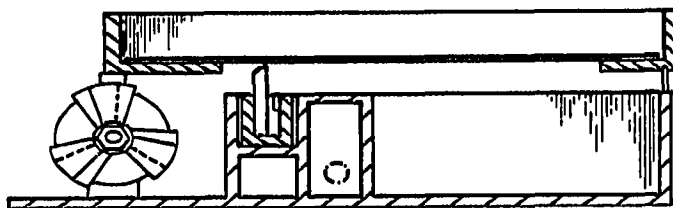


Fig. 1. Side view of applicator showing spotting wick juxtaposed immediately below TLC plate. As slotted cam wheel turns, the follower attached at the front of the plate carrier frame descends into the cut-out portion of the cam wheel lowering the TLC plate into contact with the wick.

The wick, which can be made of felt, is seen in outline in Fig. 1. The top of the wick is chisel shaped in the case where spotting is to be performed. It is flat, as can be seen in Fig. 3, in the case of routine streaking. The top edge of the felt wick comes into contact with the TLC coating, but, because it is so narrow and pliable, it bends slightly with the weight of the plate each time that the latter descends upon it. In doing so, it largely prevents abrasion of the coating on the plate. At the same time, it creates either a narrow spot or streak, depending on the width of the wick. In our model, the felt wicks used for spotting are 1/4 in. wide and are cut from material 1/8 in.

thick. The resulting spots produced are approximately $1/4$ to $3/8$ in. wide, side to side, and $1/8$ to $3/16$ in. thick, front to rear.

We allow $1/2$ in. width per spot and so are able to spot 15 samples simultaneously on an 8×8 in. glass plate, allowing indentation from the edges of the plate to reduce edge effects during development (see Fig. 2). The wicks must be of uniform height if all are to spot simultaneously. The wicks are placed in T-shaped reservoirs. Owing to their firm structure, they maintain their form for long periods despite the wetting action of the solvent and the pressure from the glass plate. Five separate T-shaped reservoirs are provided in each modular spotting unit which is readily removable from the track in which it rests.

Modular reservoirs for streaking are slightly shorter than those for spotting (Fig. 3) in order that four will fit end to end in the streaking position. Felt strips, $1 1/2$ in. wide and $3/32$ in. thick, are sandwiched between two glass plates of similar length. The felt extends about $1/8$ in. above the height of the supporting plates when the sandwich is positioned at the front of the reservoir. An L-shaped lid is then placed

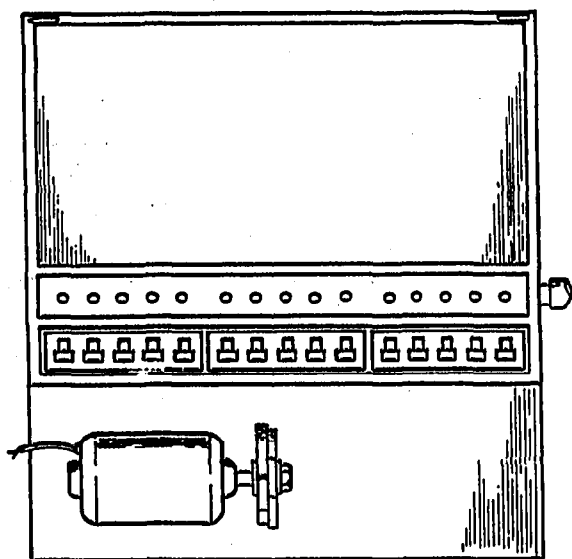


Fig. 2. Top view of applicator base assembly showing three spotting modular reservoirs, each containing five wicks. The wicks rest in the head of the inverted T-shaped reservoirs. To the rear of the reservoirs is a row of air holes, up through which air or inert gas is forced against the TLC plate. The base is approximately 8 in. wide.

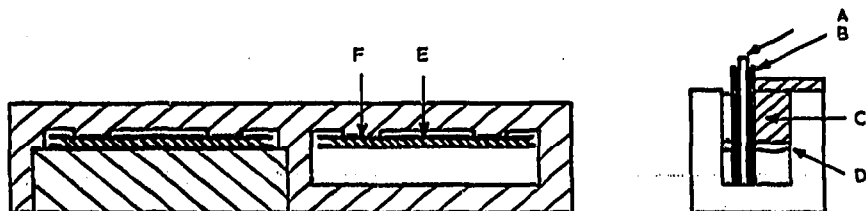


Fig. 3. To the left is seen a top view of a doublet reservoir used for streaking two 2-in. wide plates simultaneously. A wick-glass plate sandwich (E) is shown in place next to the anti-capillary struts (F) on the right half of the unit. In the left half, the reservoir cover has been positioned in place completing the assembly. The side-view drawing on the right shows a completely assembled streaking reservoir with the wick (A) projecting just above the supporting glass plates (B) and situated between the anti-capillary strut and the reservoir cover (C). The sample to be applied (D) rests in the bottom of the trough.

to the rear of the wick assembly for the purpose of pressing the assembly tightly against the two struts immediately to the front thereof, and also to retard evaporation of the sample extract from the reservoir.

An air or gas manifold is incorporated just to the rear of the line of sample application. By means of flexible tubing (Fig. 2) either air or an inert gas may be forced through the manifold and up against the TLC plate above it. This greatly accelerates the rate of solvent evaporation; thus, the spots are smaller with ultimately higher resolution, and time to re-application is shortened, thereby reducing the time required to spot the entire sample. An inert gas assists in reducing oxidative breakdown of the applied samples.

Inasmuch as the coated plate(s) is face down during the entire operation, the likelihood of accidental spillage of contaminating solutions onto the coating is substantially reduced. Additionally, little or no dust is able to settle onto the coating. Since the plate holder is easily removed by lifting directly upwards, and since its sides extend about 1/8 in. below the face of the average thickness coating, the plate holder may be placed down on any convenient flat surface without danger of scratching the coating. Operation during sample application may be interrupted at any time to remove and replace reservoirs as well as TLC plates. This may be desired in the case where individual reservoirs have been started on a sequential basis rather than at the same time, or in the event some reservoirs have less sample extract to spot or streak.

Commentary on wicks for streaking

Felt wicks containing varying amounts of wool have a tendency, owing to wool's amphoteric properties, to complex with certain compounds. Thus, other wick materials must be employed in certain instances, the selection of which is best determined by the investigator himself. We have found that Whatman papers Nos. 1 and 3 folded over to double thickness and placed in the glass sandwich, such that the folded edge is topmost, work quite satisfactorily in many applications. Glass fiber sheeting should similarly serve quite well when employed in the manner of the paper wicks, and would be non-reactive for the majority of purposes.

The fine fibrous nature of both the paper and felt wicks serves to filter out debris from even crude tissue extracts, and produces very clean spots on the TLC coating. This eliminates the problems often encountered due to debris interference during development of the chromatogram. Clogging is minimal and does away with the necessity of changing pipettes that have become plugged. It does, however, result on occasion in uneven streaking, which may be of only slight disadvantage since most streaking is for subsequent preparative elution of the large quantities of the separate components of the sample extract, rather than for analytical work.

Another advantage of wicks is that they may be pre-treated with a variety of known reference compounds and allowed to dry. Then as needed they may be inserted into the appropriate reservoir and their reference standard will co-chromatograph automatically with the unknown sample extract, thus providing accurate R_F value reference points.

Reproducibility of results

Table I shows the results obtained for 2-in.-long streaks applied to silica gel

TABLE I

¹⁴C-LABELED SUCROSE IN 50% METHANOL
c.p.m. × 100 for number of applications.

Plate	1	2	3	4	5
1	31.84	58.36	69.25	76.64	89.25
2	34.62	57.58	70.16	71.61	90.08
3	34.27	60.67	62.79	81.88	96.00
4	33.98	60.33	68.17	72.45	94.74
5	30.67	60.81	70.23	81.89	99.21
6	31.45	55.85	69.80	73.49	90.63
7	30.70	53.26	64.58	79.45	96.58
8	34.21	54.52	61.47	78.72	90.49
9	28.68	55.25	—	—	89.80
10	—	—	—	—	88.52
11	—	—	—	—	100.02
12	—	—	—	—	96.98
13	—	—	—	—	89.08
14	—	—	—	—	99.34
\bar{X}	32.27	57.40	67.06	77.01	93.62
S.D.	0.697	0.945	1.259	1.457	1.156
S.D./ \bar{X}	0.021	0.016	0.018	0.018	0.012

TLC plates, using ¹⁴C-labeled sucrose in a 50 % methanol solution. Individual TLC plates were streaked from 1 to 5 times and at least eight plates were streaked in each case, *i.e.*, there were at least eight repetitions. After streaking, the silica gel was uniformly removed from each plate and analyzed for activity in a Beckman liquid scintillation counter. Activity levels were such that error in counts/min does not exceed 0.5 %. Three millilitres of extract were placed in the reservoir and produced about the first 50 streaks. A second sample of 3.0 ml was then added to the reservoir to wet the succeeding plates streaked.

The means and their respective standard deviations are shown at the bottom on the columns (Table I). An estimate of precision in reproducibility, for any given number of streaks, was then computed by dividing the standard deviation by its mean. It can be seen that this percentage was at its greatest, 2.1 %, for plates receiving only one streak. Part of this variation in uniformity of streaking is probably due to irregularities in coating thickness between plates. It can also be seen that there is a progressive reduction in the amount applied between applications. This may be due to a type of equilibrium between the material on the plate and that on the wick with some reduction in forward flow at each succeeding application, or saturation of the silica gel due to the extremely thin coating and narrow streak applied in each case.

It can be seen in Table I that while there is a progressive reduction in the values between means per number of applications, there is a partial reversal of this decreasing trend on those plates that received five applications. This may be due to delayed uptake of the second aliquot of sample added to the reservoir. It is, however, more likely due to an increase in the down-time (*i.e.*, length of time in which the wick is in contact with the plate) that may have resulted from an adjustment made to the drive wheel cam ratio to correct for slippage that developed during this lengthy set of applications. Streaks were scanned prior to elution under a Nuclear Chicago Acti-

graph III gas flow detector as well as visually, and it was determined that none of the streaks exceeded $3/16$ in. width, front to back.

Materials

The model and the reservoirs depicted were fabricated largely of $1/4$ and $1/8$ in. plexiglas. Because the reservoirs are of this material, only lower alcohols, methanol and ethanol and aqueous solutions have been successfully tested in them. Investigators desiring to use solvents reactive with plexiglas would have to fabricate the reservoirs from higher polymer plastics such as polypropylene, or from glass or stainless steel. In order to insure proper contact between the wicks and the TLC coating, the reservoirs and their track area must be perfectly parallel to that of the plate carrier frame. The wicks, also, must be of uniform height, shape and composition if the relative rates of application are to be similar between wicks.

It is most advantageous to use either a variable speed motor or a rheostat-controlled 2 r.p.m. motor, so as to permit fine adjustment of the period required for drying of the spots between applications. A more detailed discussion of these considerations is contained in ref. 1. A U.S. patent is pending on this device.

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Thin-layer chromatographic separation of steroids and their localization by diazo dyes

Steroid hormones generally occur in low concentrations in biological fluids and tissues as a result of which they present a difficult analytical problem. Several chromatographic purification techniques in combination with gas-liquid chromatography (GLC) have been described in the literature for the determination of these substances¹⁻⁷.

Recently we have developed a highly reproducible and sensitive GLC column, for the detection of steroids in the nanogram range⁸, for which it was desirable to have a simple prepurification procedure. The present paper describes a suitable method using marker azo dyes for the specific localization of each class of steroids on thin-layer chromatograms. A similar technique has been described earlier⁹.

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