

Thin-layer chromatographic aids

The ever increasing use of thin-layer chromatography (TLC) has brought forth many ingenious and useful devices to aid in the application of this technique. Modifications such as sandwich chambers¹, round tanks and plate holders² for precoated plastic-backed TLC layers³, modified holders for conventional plates⁴, support spreaders⁵, spotting and streaking devices⁶⁻¹², apparatus for horizontal TLC^{13,14}, and microscope slide techniques^{15,16} have been reported. Recently a number of papers have described support spreader modifications to prepare gradient and mixed TLC layers¹⁷⁻¹⁹. Many of these and numerous other improvements are now described in standard TLC references (*e.g.* ref. 20). In addition many of the more practical accessories are now commercially available.

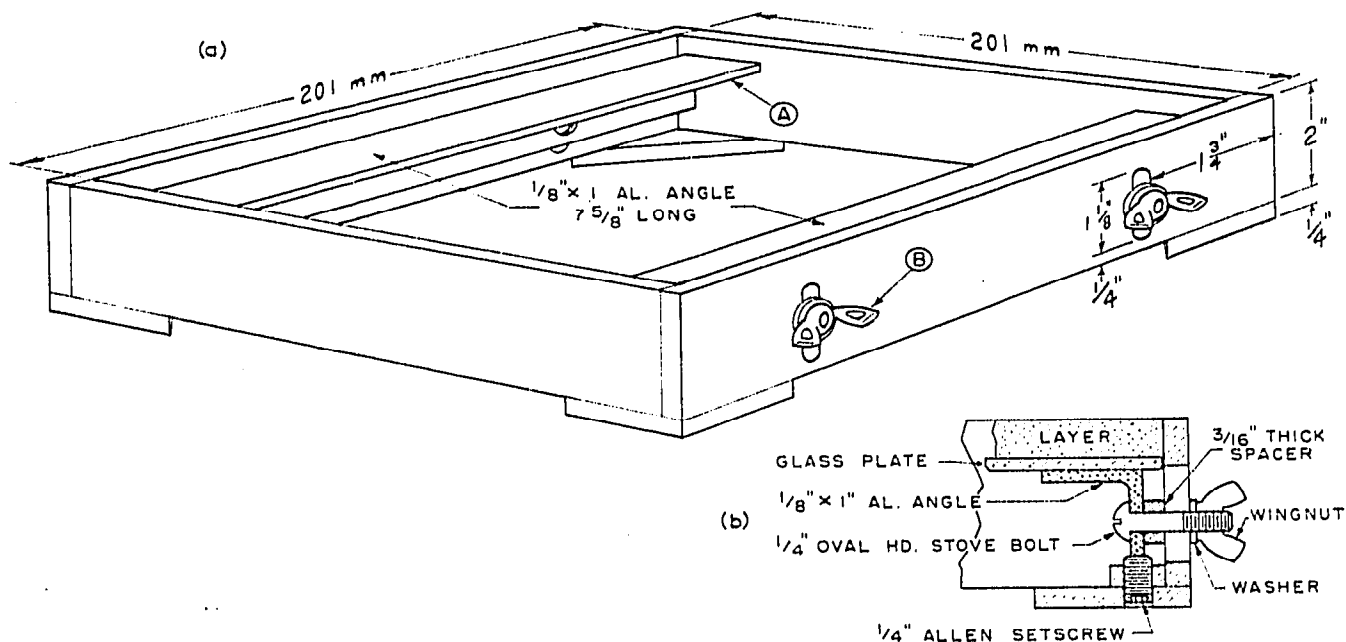


Fig. 1. Frame for preparing thick and wedge layers.

During the course of our work we found that the published devices and the commercially available equipment were not entirely adequate for our purpose. Therefore, we had occasion to devise and fabricate some apparatus which are described in this paper.

A. Preparation of thick-layer and wedge plates

The Desaga* and other commercially available support spreaders are widely used for preparing plates with sorption layers up to 2000 μ in thickness. Layers up to a thickness of 1000 μ can be prepared on precision channel ground glass plates**. BEKERSKY²¹ prepared layers up to 1100 μ thick with a variation of $\pm 40 \mu$, by spraying his support material onto plates. ABBOTT AND THOMSON^{22,23} elegantly prepared wedge layers ranging from 100 to 2000 μ by attaching a trapezoid gate to a Desaga

* C. Desaga GmbH, Heidelberg, Germany.

** Kontes Glass Company, Vineland, N. J., U.S.A.

spreader. Individual wedges of TLC supports ranging from 125 μ up to 1000 μ also can be prepared in wedge-type channel plates.*

The procedures described above are ideal for preparing thick and wedge layers to a thickness of up 2000 μ . However, no apparatus has been described for the preparation of even thicker layers and wedges which we often require in our work. For our requirements, we constructed a 201 mm square by 2 in. high (I.D.) frame from 1/4 in. perspex (Fig. 1a). If the 200 \times 200 mm plates employed by the analyst are made out of 24 oz. glass obtained locally, the inner dimensions of the frame should be increased by about 4 mm. This will accommodate the imprecise dimensions often encountered. Open spaces around the edges of the glass can be caulked easily and effectively with plasticene.

TABLE I

SILICA GEL G (MERCK) REQUIRED FOR THICK AND WEDGE LAYERS

Thickness (mm)	Silica Gel (g)	Water (ml)
2 to 2	40	80
4 to 4	80	160
6 to 6	120	240
8 to 8	160	320
2 to 9	120	240
2 to 10		
3 to 8		
4 to 7		

Formulas: Weight (g) Silica Gel G required = [bottom thickness of layer (mm) + top thickness of layer (mm)] \times 10.

Volume (ml) water required = weight of Silica Gel G (g) required \times 2.

The aluminum guides "A" permit the preparation of plates having a layer thickness of up to 12,000 μ and wedges tapering from 12,000 μ to 50 μ (allowing for a glass plate thickness of 4000 μ). The wedge angle in the latter case is about 3°. By employing a precision ruler and adjusting wing-nuts "B", intermediate thicknesses of sorption material can be prepared as desired.

Our use of the coating frame has shown that setting of layer thickness could be facilitated by modifying the frame to incorporate adjusting screws in the four corners, as shown in Fig. 1b. This would limit variation of layer thickness to a maximum of about $\pm 10 \mu$, somewhat better than the $\pm 35 \mu$ obtained with our present device.

To prepare thick-layer or wedge plates, the frame with a glass plate inserted is adjusted to the desired depth and angle. The required amount of slurry, as recorded in Table I or determined by the formulas therein, is added and excess material removed by drawing a glass rod across the top of the frame. If the layer is very thick, it is best to allow it to set for at least 4 hours before removing. Air-drying the thick layer or wedge overnight before activating has been found to minimize cracking of the layer surface. MA²⁴ has reported that a small amount of methanol added to the slurry is also effective in this. We observed, as did KABARA and co-workers²⁵, that the adhesive properties of the sorption layer to the glass are increased when the glass

* Kontes Glass Company, Vineland, N. J., U.S.A.

surface is frosted. However, instead of sand-blasting to prepare suitable surfaces we obtained the desired results by simply lapping glass plates together, with fine carborundum suspended in mineral oil.

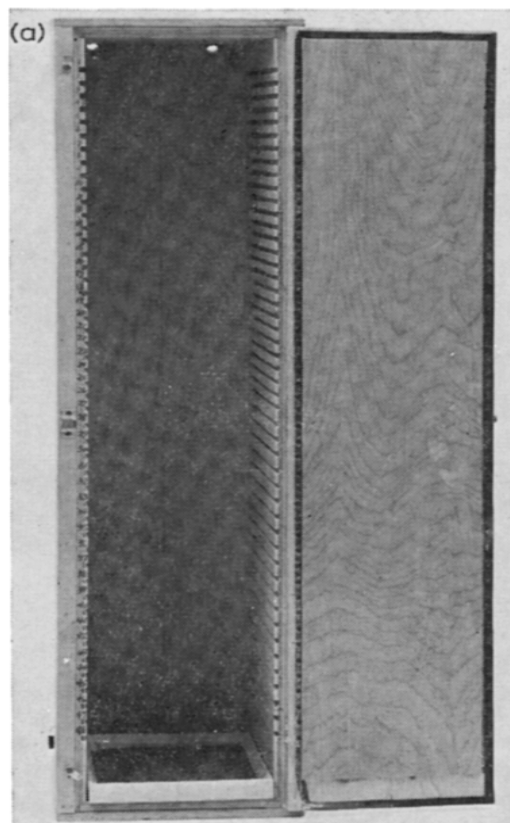
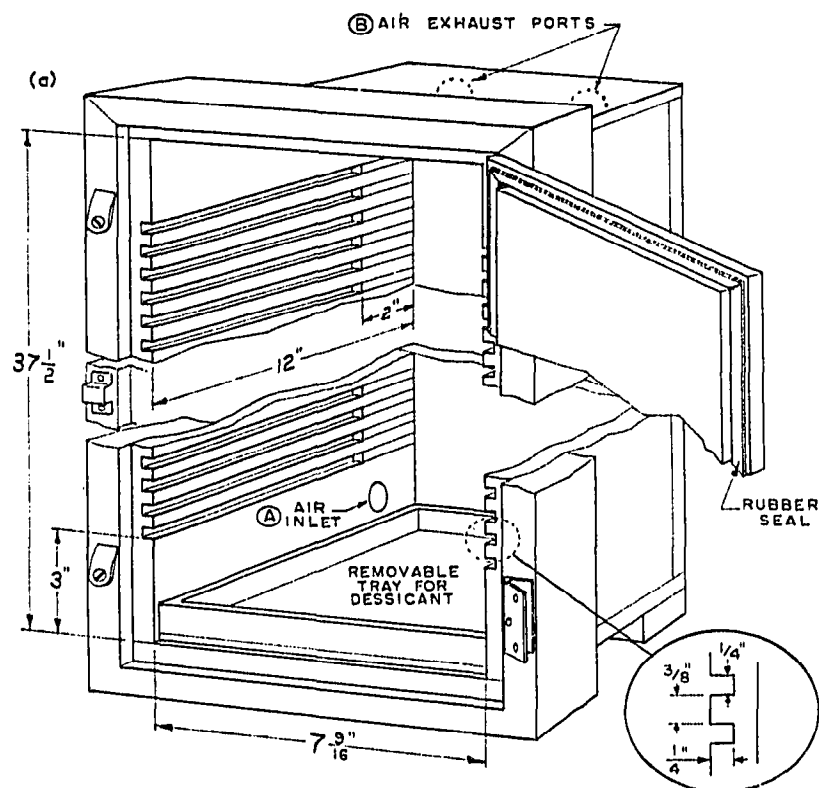


Fig. 2. TLC plate storage cabinet.

B. Storage cabinet

Researchers employing TLC are well aware of the effects of moisture levels on the activity of sorption media^{20, 26}. To maintain the desired level of activity, WOLLISH and co-workers⁵ prepared a stainless steel box for storing plates on their edges. SAHASRABUDHE²⁷ designed a portable stainless steel horizontal drying rack in which plates can be activated. After activation the entire unit is fitted into a wooden chest lined with stainless steel, containing desiccant and sealed for storage. These units represent a marked improvement with regard to both economy and convenience over the storage of racks of plates in oversized vacuum desiccators.

We have used the SAHASRABUDHE unit²⁷ and found it quite useful for storage of up to twelve 20 × 20 cm plates. However, many of our TLC studies required a large number and variety of plates prepared at the outset of a project. To accommodate them we were faced with fabricating many of these bulky 12-plate units, purchasing a smaller number of slightly larger commercially available cabinets, or designing more serviceable and economic units. Figs. 2a and b depict the storage cabinet, accommodating forty-eight 20 × 20 cm TLC plates, which we now have in use. The plates are kept in filtered dry air which enters the cabinet at port "A" above the desiccant tray, and escapes through port "B". The efficiency of the system is such that "self-

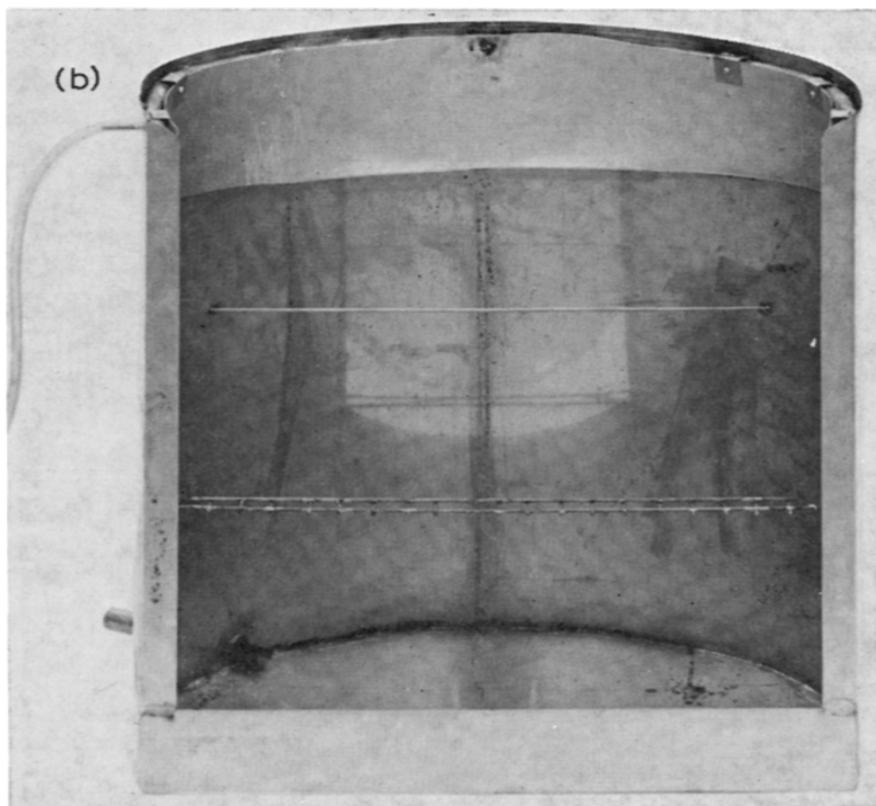
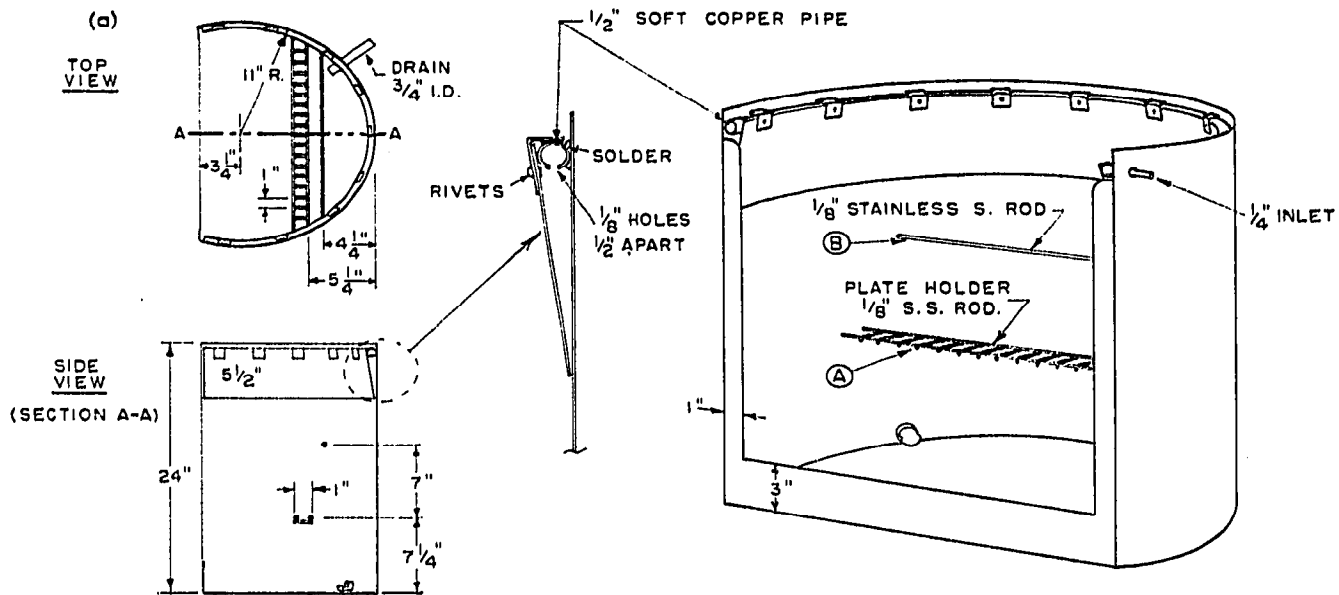


Fig. 3. TLC plate spraying cabinet.

indicating "silica gel in the desiccant tray in one of our units, shows no sign of moisture after 8 months of continuous service.

C. Spraying cabinet

Although some elaborate TLC spraying cabinets are described in reference

texts²⁰, and at least one spraying chamber is available commercially*, the presence of toxic and noxious chromatographic spray fumes and condensed spray solutions is still a problem in most laboratories. To obviate these difficulties while spraying paper chromatograms, FIEBERG AND SIEGEL²⁸ designed a spray cabinet** which sits in a normal fume hood. In their design, the back of the 30 in. high cabinet is covered with fiber glass cloth which is continuously washed with flowing water. Unfortunately, their apparatus is not suitable for glass plates (although satisfactory for plastic-backed ones). Since nothing similar appears to be available for TLC, we constructed an inexpensive semicircular spray cabinet out of 26 gauge stainless steel, with the inner surface washed continuously with a film of water (Fig. 3a).

The spray cabinet efficiently removes condensed corrosive chromatographic sprays. In addition, its use minimizes the amount of vapours which the fume hood must handle. The plate holder "A" (Fig. 3a) is long enough to accommodate two 20 × 20 cm plates, and broad enough to allow masking of thick-layer and wedge plates. The retaining rod "B" (Fig. 3a) holds plates at a convenient angle for spraying (Fig. 3b).

The apparatus described above have proven to be most useful accessories, and should be of value to other workers.

Acknowledgements

The authors gratefully acknowledge the assistance of L. O. DAVIES and his staff for fabricating the units described and Messrs. G. MORRIS and B. V. KORDA for the illustrations.

Research Laboratories, Food and Drug Directorate,
Ottawa, Ontario (Canada)

H. D. BECKSTEAD
W. N. FRENCH
S. J. SMITH

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Received June 5th, 1967

J. Chromatog., 31 (1967) 226-231

Fluorocarbons as solvents for thin-layer chromatographic analysis*

In a previous publication¹ we reported that fluorocarbons exhibit unusual properties when used as chromatographic solvents. For example, both a perfluoroalkane mixture and a mixture described as perfluorokerosene separated the sesquiterpene hydrocarbons (C₁₅) from the monoterpene hydrocarbons (C₁₀) on thin layers of Aluminum Oxide G. Under the conditions of the analysis the monoterpenes did not leave the origin. On the other hand, hydrocarbon solvents moved both the C₁₀ and C₁₅ terpenes but did not separate them. Consequently, the fluorocarbons appear more non-polar in their chromatographic behavior than the hydrocarbons.

To determine if other fluorocarbons exhibit this same behavior, samples of four additional aliphatic fluorocarbons and chlorofluorocarbons, and three aromatic

TABLE I

R_F VALUES OF REPRESENTATIVE TERPENES ON SILICA GEL G LAYERS USING AROMATIC SOLVENTS

Compound	Class of compound	<i>R_F</i>			
		Benzene	Fluoro-benzene	Penta-fluoro-benzene	Hexa-fluoro-benzene
<i>p</i> -Isopropenyltoluene	Aromatic hydrocarbon	0.98	0.98	0.92	0.94
Valencene	Sesquiterpene hydrocarbon	0.98	0.95	0.93	0.92
Thymyl methyl ether	Aromatic ether	0.92	0.93	0.86	0.83
Citronellal	Aldehyde	0.65	0.64	0.43	0.33
Linalyl acetate	Ester	0.56	0.53	0.37	0.30
Citral	Aldehyde	0.34	0.35	0.28	0.22
Linalool	Alcohol	0.26	0.23	0.14	0.12
α-Terpineol	Alcohol	0.15	0.13	0.10	0.07

* Cooperative research by the Florida Citrus Commission and the Florida Citrus Experiment Station.