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A NEW PREPARATIVE THIN-LAYER CHROMATOGRAPHIC TECHNIQUE FOR USING THICKER LAYERS

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SUMMARY

A method has been developed for the production and development of thick layers ranging from 1/8 in. to 1/2 in. thick. These layers do not have a supporting back plate, but are contained within a stainless-steel framework with thin stainless-steel wires stretched across the frame to help support the adsorbent layer. In this manner both surfaces of the plate are available for application of the sample and for the observation of separated zones. The layers were formed of silica gel with 20% gypsum binder based on the weight of the silica gel. A special chamber was constructed for development of the layers. The method was demonstrated by the separation of a number of dyes. In contrast to running numerous thin-layer plates to obtain preparative amounts of colorless compounds, the thick layers provide a means for spraying the surface with a visualizing reagent, and then removing the thin surface layer once the zones are located and still leave adequate amounts of the unsprayed compounds for recovery.

INTRODUCTION

Because of the speed and separations obtained with thin-layer chromatography (TLC), it has become increasingly popular for use in preparative work. In this case, thicker layers are used to increase the capacity. However, because of the adhesion of the layer to the supporting plate and the differences in coefficient of expansion of the layer and the supporting plate, thick layers have a tendency to crack. HONEGGER[1] has shown that it is very difficult to prepare layers thicker than 2 mm. To offset this disadvantage, plates as large as 20 cm × 1 m have been used[2] and multiple plates are usually run to increase the quantity of available separated material.

After introducing the present-day system of TLC in 1951 (ref. 3), the author realized the need for a TLC system which could separate larger quantities, and in 1951 (ref. 4) the details of the "chromatobar" were published. In essence, this was a column of silica gel bound with gypsum around a glass rod for support. No containing envelope was used so that the surface of the adsorbent was available (as with the thin-layer plate) for observation at all times. Although useful, the "chromatobar" has the inherent disadvantage of being difficult to load uniformly with sample.

Recently it was decided that the principle of the "chromatobar" could perhaps

be applied to a thick layer, thus eliminating the supporting plate and the consequent cracking tendency. There are also a number of designs for streaking equipment, so that samples can be applied uniformly to layers.

AFFONSO[5-7] has used thick plates (1–5 mm) of calcium sulfate, but this limits the separation possibilities because of adsorption capabilities of the calcium sulfate. KALIS *et al.*[8] used what was designated as "box chromatography", in which loose adsorbent was contained in a metal box. Development was carried out by inclining the box in the developing chamber. The maximum thickness reported was 0.7 cm. This method suffers from the usual disadvantage of loose layers[9], including the fact that the zones progress at an angle rather than perpendicular to the plate; this is particularly noticeable with thicker layers. CLARK[10] also used flat metal boxes to hold the adsorbent using layers up to 5 mm thick. Here again, the layer is accessible from only one side.

EXPERIMENTAL

Initial work in producing a self-supporting thick layer of silica gel bound with gypsum was indeed successful, and Fig. 1 shows the results of a separation of six dyes on a 1/4-in. thick layer. 100 mg of each of the dyes Yellow OB, Sudan I, II, and III, Crystal Violet, and Methyl Red* were applied to the layer with a modified Radin–Pelick streaker. One half of the material was applied to one side and the remainder to the other side of the plate. After allowing the sample solvent to evaporate, the plate was developed as follows:

(1) In an unsaturated chamber to the top with benzene–chloroform (10:1),
(2) In an unsaturated chamber to a height of 120 mm with the same solvent,
(3) In an unsaturated chamber with benzene for a distance of 120 mm,
(4) In a saturated chamber with benzene–chloroform (10:1) to the top,
(5) Two times in an unsaturated chamber with benzene to the top,
(6) Two times in an unsaturated chamber for a distance of 63 mm with the benzene–chloroform mixture to which ethanol had been added in the ratio 10:3.

Despite the excellent separation results, these layers demonstrated by Fig. 1 had one drawback: They were somewhat fragile and had to be handled carefully between glass plates at all times except when under actual development. It was relatively easy to lose a corner of the plate especially when wet with solvent.

The possibility of using a stainless-steel framework around the adsorbent layer was then investigated with excellent results. Because the mold for casting the unsupported 8 × 8 in. layers was already in existence, it was also used for the preparation of the stainless-bound layers. This mold consisted of a square aluminum plate 10 × 10 × 3/8 in. Around the outer edge of the base aluminum plate, a framework of stainless steel milled as in Fig. 3a was fastened by brass flathead machine screws. The accurate milling of the distance "e" is critical since this determines the uniformity of layer thickness and the flatness of the surface. These two factors are essential in streaking the sample and in uniform development of the chromatogram. The aluminum base plate was drilled and tapped to receive the machine screws. It is

* All dyes were previously purified by chromatography. The Sudan II was the larger middle band in the commercial dye when chromatographed on silica gel with benzene–chloroform (20:1) in ascending development (Fig. 2).

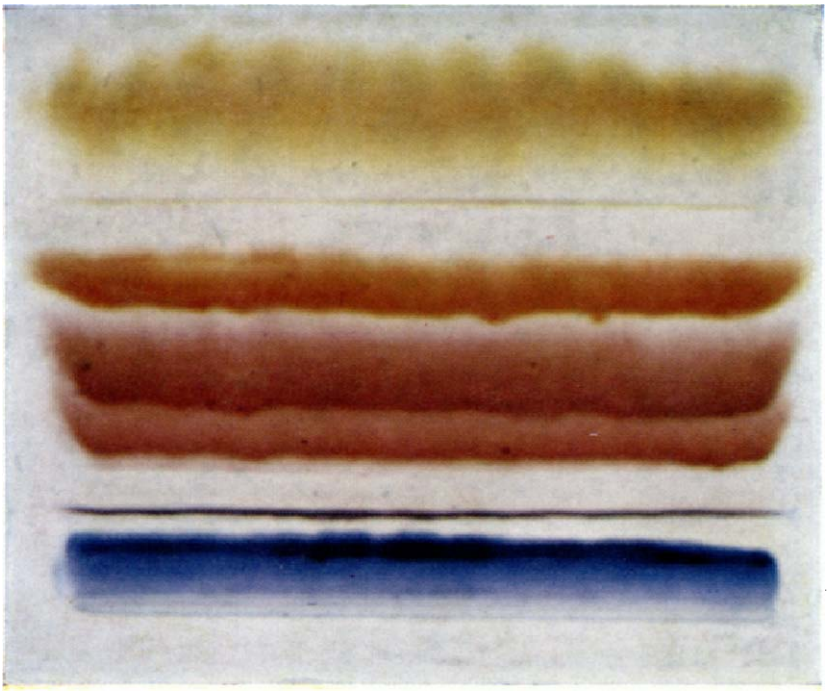

Fig. 1. Separation of 100 mg each of six dyes on a $\frac{1}{4} \times 8 \times 8$ in. silica gel self-supporting layer. Top to bottom: Yellow OB, Sudan I, Sudan III, Sudan II, Methyl Red and Crystal Violet. (See text for details).

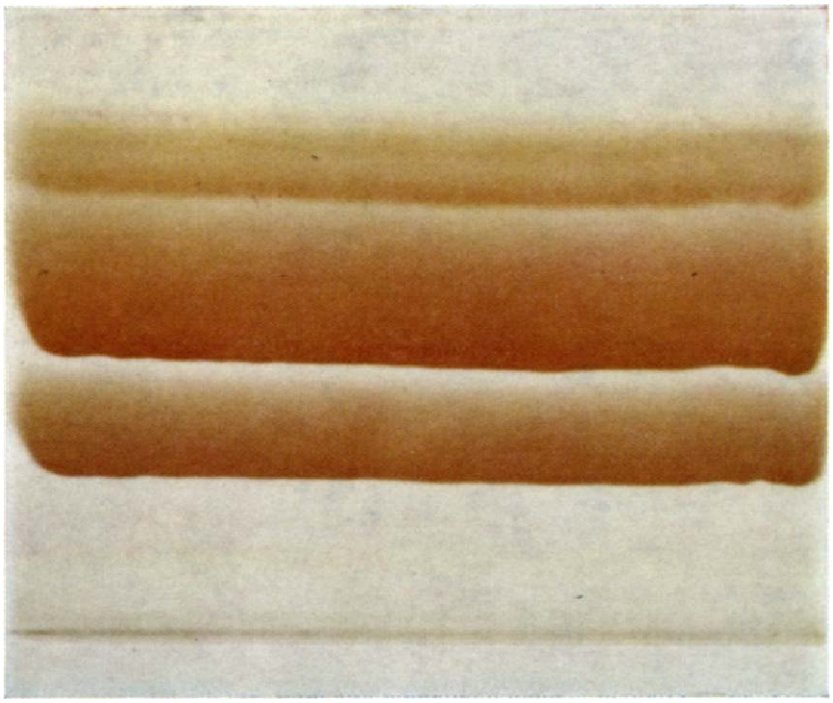

Fig. 2. Purification of 500 mg of Sudan II on $\frac{1}{4} \times 8 \times 8$ in. silica gel layer (SilicAR® + 20% gypsum binder) contained in a stainless-steel framework. The layer has no backing plate and is accessible from both sides. Developed in an unsaturated chamber twice with benzene–chloroform (20:1).

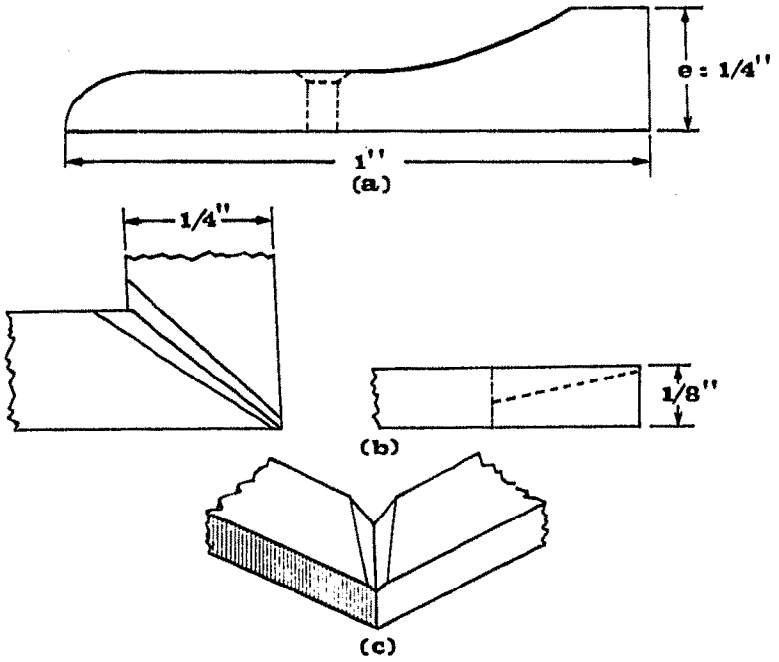

Fig. 3. (a) Cross-section details of side of mold for preparing ¼ in. layers. (b and c) Details of notches in corners of framework for ⅛ in. layers. Stainless-steel wire (0.025 in.) is soldered in the notches to support the layer.

convenient to have the holes drilled all the way through the plate to facilitate cleaning out any adsorbent which may fall in while removing the sides of the mold. The base plate was covered by a sheet of smooth plastic; a sheet of cellulose acetate 0.05 in. in thickness was satisfactory.

The individual square 1/4 in. thick frames were welded together from 1/4 × 1/8 in. stainless bar stock in a size such that they would just fit within the large 8 × 8 in. framework. Care was taken so that the edges of the 1/4 in. square frames did not project above the milled surface of the 8 × 8 in. mold, thus assuring that the completed adsorbent plate would be uniform and flat.

At first, to aid in supporting the adsorbent, four stainless-steel pins 1/16 in. in diameter and 3/8 in. long were inserted along each side of the frame. However, the adsorbent layer had a tendency to chip out at these points so the final solution to this problem was the use of 0.025 in. stainless-steel wires strung across the frame. These were soldered in holes drilled in the center of the 1/4 in. metal. Two patterns (Figs. 4, 5) of supporting wires were used: (a) four parallel wires with two wires 7/8 in. from the sides of the frame, and the remaining two equal distances apart (approximately 2 in.), (b) two diagonal wires running from corner to corner. The latter system was preferred, because it was easier to obtain the same degree of tension on these two wires than on the four parallel wires. However, either frame worked satisfactorily once the wire tension was uniformly adjusted. The wires and the edges and inside surface of the frames were sprayed with the bonding type Teflon spray obtainable from laboratory supply houses. This was done in the event there was shrinkage in the adsorbent layer it would not cling to the frame and cause cracking of the adsorbent layer. The stainless-steel wires did not cause any interference with the separation of compounds.

A 10 × 10 × 3/8 in. plate glass was used for the top side of the mold. This was

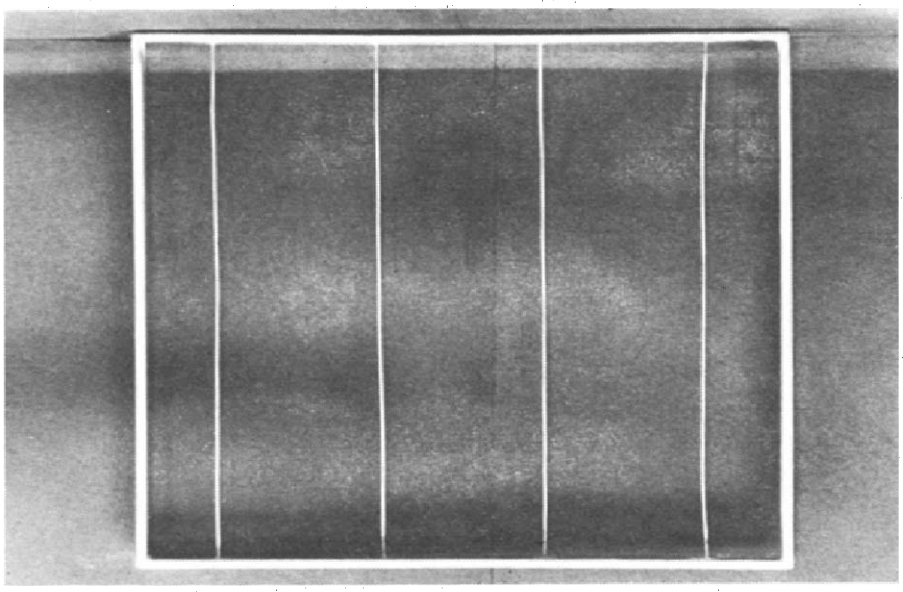

Fig. 4. Stainless-steel frame with parallel supporting wires.

covered with a sheet of smooth plastic thin enough so that it could be stretched and folded over on the edges and corners so as to provide one side with a smooth even surface. To hold it in place the edges of the plastic were fastened with Scotch tape to the back of the glass plate. The purpose of the two plastic sheets, one on the top and one on the bottom, was to permit the removal of the top and bottom plate, leaving the plastic sheet in place; the plastic could then be rolled back from one edge, leaving a smooth surface on the adsorbent layer.

For the 1/2-in. layers, a mold was formed by inserting 1/4 × 1 in. aluminum bars between the bottom plate and the sides of the square mold. The individual frames for the 1/2-in. thick layers were similar to the 1/4-in. frames except that they were made from 1/8 × 1/2 in. stainless bar stock.

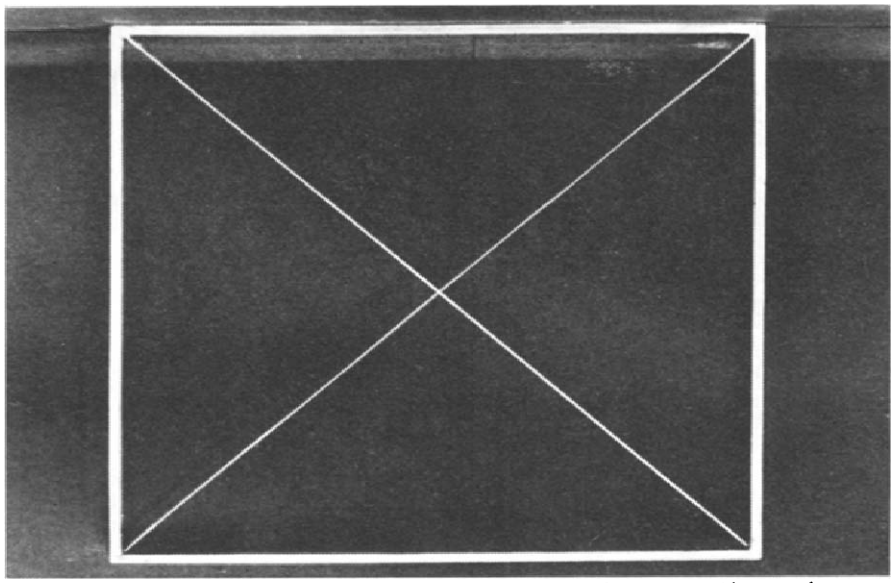

Fig. 5. Stainless-steel frame with diagonal supporting wires.

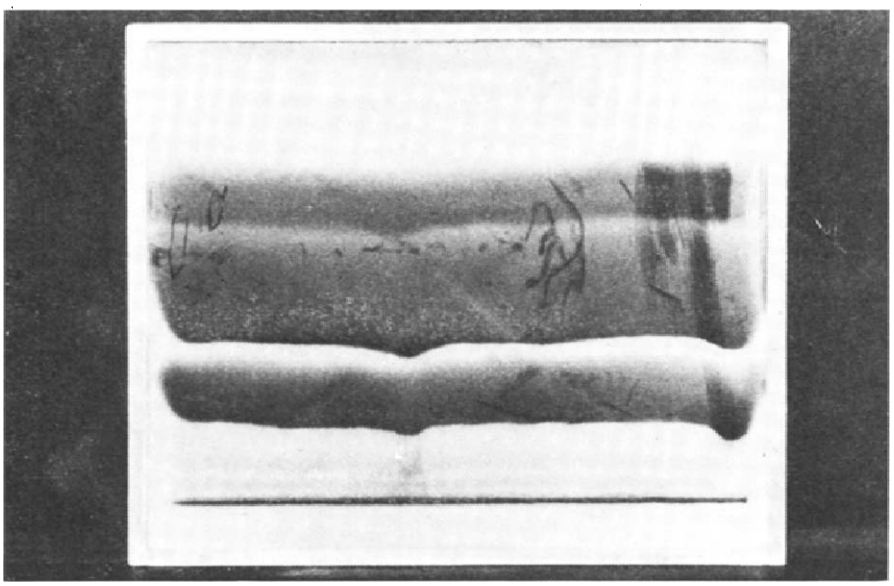

Fig. 6. Purification of 250 mg Sudan II on a ⅛-in. layer of silica gel (Mallinckrodt SilicAR®) bound with 20% gypsum in stainless-steel frame. Developed in an unsaturated chamber twice with benzene–chloroform (20:1).

Frames for a 1/8-in. layer were made from the 1/4 × 1/8 in. stainless metal laid flat to give the 1/8-in. thickness. Because the thinness of the metal made it difficult to drill for the stainless-steel wires, the following procedure was used: Notches were filed on a slant in the corners, in such a way that the wire on the inside of the frame was centered on the metal and on the exterior of the corner the wire was just beneath the surface of the metal (Figs. 3b and c). A special mold had to be constructed to hold the 1/8-in.-thick framework. This was identical with the mold for the 1/4-in. framework, except in the thickness of the sides of the mold. Fig. 6 shows the purification of 250 mg of Sudan II on the 1/8-in. layer. This was accomplished with benzene–chloroform (20:1) using two passes of the solvent.

For the preparation of the 1/4-in. adsorbent layers, 296 ml of water were placed in a 1-l beaker containing a variable speed stirrer. The beaker was placed on a support so that the support could be removed and the beaker taken quickly from the stirring apparatus; provision must be made to work rapidly because of the speed of setting of the gypsum binder. With the stirrer in fairly rapid motion, 226 g of a mixture of Mallinckrodt SilicAR 7 containing 20% gypsum binder based on the weight of the SilicAR 7 (this amounts to 16.67% of the total mixture) was added rapidly but at such a rate that there was no build-up of dry powdered material within the beaker. If this occurs, setting of the gypsum will be initiated rapidly and the mixture will become too stiff to work. As mixing progresses, the speed of the stirring should be increased; the important thing is to obtain thorough mixing as quickly as possible. The resulting mixture should be fluid enough to work with but should contain a minimum amount of water. Other brands of silica gel may require more or less water. For example, Camag silica gel, which is bulkier than SilicAR, required 434 ml of water for 226 g of mixture. On setting and drying, the plates made from Camag silica gel exhibited an air-gap of *ca.* 1/16 in. between the sides of the metal frame and the adsorbent layer. The supporting metal wires, however, held the adsorbent firmly and the layers could be used without difficulty. During development of this type of layer,

the solvent progressed uniformly and there was no evidence of any edge effect (Fig. 12).

SilicAR produced an adsorbent layer which filled the frame completely. These also developed uniformly without any edge effect. Occasionally an adsorbent plate was prepared which exhibited a capillary crack between the metal frame and the adsorbent layer. In attempting to develop a plate of this type, the solvent would travel faster on the edges of the plate because of this capillary crack. This situation could be corrected prior to development by running a thin stainless-steel spatula blade between the metal frame and the adsorbent layer. This enlarged the crack sufficiently to destroy the capillary action.

After obtaining the slurry as described above, the beaker was covered with a sheet of plastic and rapped firmly on a pad of paper or rubber placed on the table top. This effectively removed most of the air bubbles that had been whipped into the mixture. The mixture was then poured into the mold and spread quickly with a spatula so that the entire mold was filled. The mold was rapped sharply on the table top in order to work to the surface any additional air bubbles that might be present. The bubbles could be broken by blowing on the surface. Once the air bubbles were removed, the plastic covered glass top was placed on the mold. This was done by placing the plate in contact with the mold on one edge and then lowering the plate so that no air bubbles were entrapped beneath the surface. Then by moving the glass cover plate around, and pressing firmly, the excess adsorbent mixture was squeezed out of the mold.

The plate was allowed to stand for ½ h for the gypsum to set, and then the Scotch tape was removed from the back of the glass plate. This now allowed the latter to be removed, still leaving the plastic covering the layer. The plastic was then peeled back from one edge to leave a smooth surface. If a thin film of adsorbent adhered to the plastic, this was an indication that the mixture had too much water in it.

The excess adsorbent was scraped away and the side plates of the mold were then removed by taking out the screws. The adsorbent layer was covered by a glass plate, so that it could be turned over with the base plate on top. The base plate was then lifted off leaving the plastic in place. The latter was similarly rolled back from one edge. The adsorbent layer could then be picked up and handled by means of the stainless-steel rim. Excess adsorbent was removed from the edges of the frame. (Excess or unused adsorbent–gypsum mixture may be recovered and re-used by first drying, powdering in a Waring type blendor, and then heating for 2 h 30 min at 160°.)

The 1/4-in. adsorbent layer was dried overnight at room temperature in a vertical position and then for 3 h at 110°. The plate may be dried from the wet state in a 110° oven for 5 h. All drying is done in a vertical or near vertical position, so that the plate dries uniformly from both sides. A 1/2-in. plate requires considerably longer drying, *e.g.* 9 h 45 min at 110° after drying overnight at room temperature. The dried plates may be kept in a desiccator over silica gel until used.

For application of the sample, a modified Radin–Pelick streaker was used. This modification consisted in raising the supporting posts 3/4 of an in. by replacing the 1/4 in. bushings at the bottom with two identical 1-in. aluminium discs. These must be carefully machined so that the syringe needle will travel in a plane exactly parallel to the base plate. Also the side-retaining bar against which the normal plate is placed

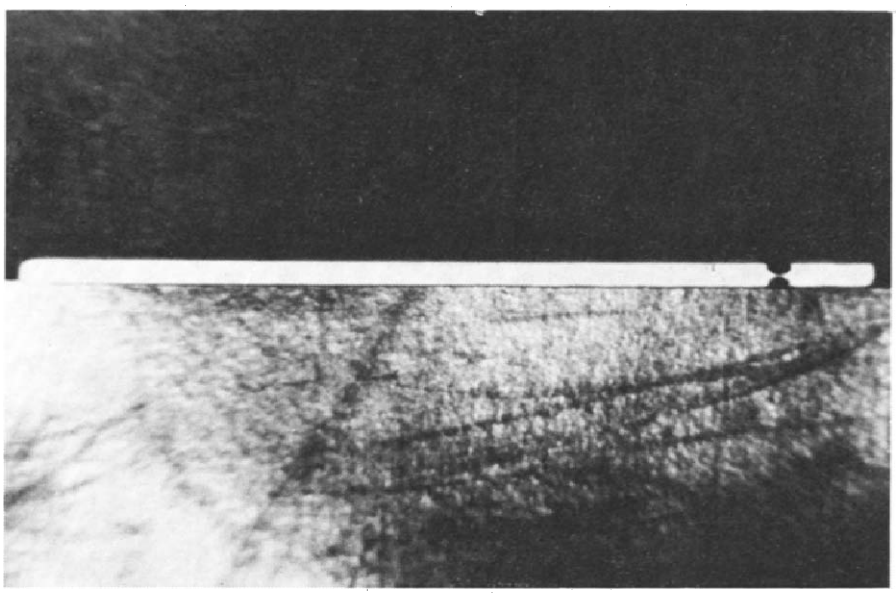

Fig. 7. Cross-section of a ¼-in. layer after sample application, one half to each side of the layer.

must be heightened so as to take care of any size plate that may be used. The adsorbent layer is inserted in the streaker with a glass plate underneath it as a supporting medium. A sheet of glass protects the layer from moisture above the application line. After the addition of one half of the sample to the layer, the layer was turned over for the application of the remainder of the sample. For the purpose of locating the application line exactly on the opposite side of the plate, an adjustable stop was added to the Radin–Pelick streaker. In lieu of this stop, the position of the plate can be marked. It is advisable after adding the sample solution to apply a small quantity of pure solvent to the sample line in order to help carry the sample into the layer. Fig. 7 shows a cross-section of a 1/4-in. layer after application of the sample, and Fig. 8 shows a cross-section of a 1/2-in. thick layer after development. This latter demon-

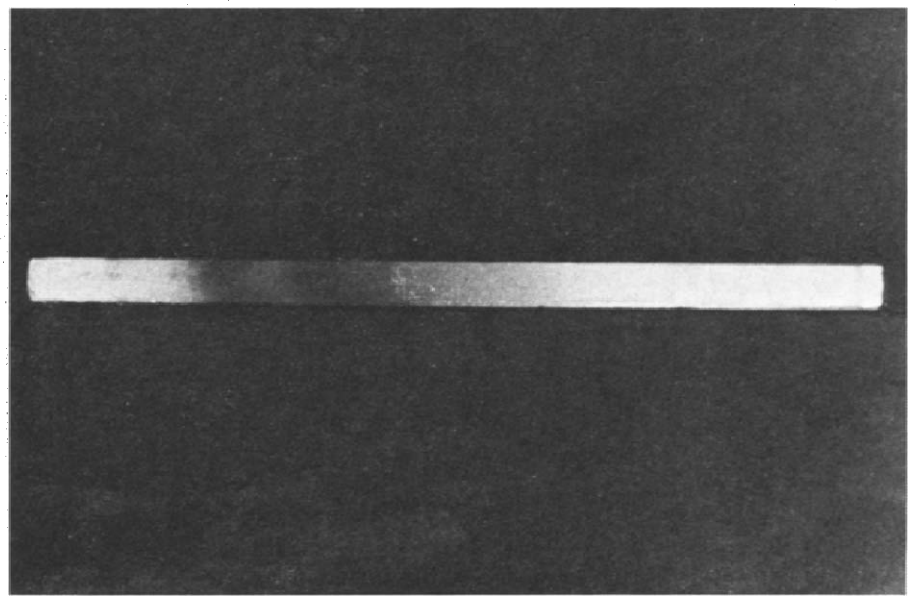

Fig. 8. Typical cross-section of a ½-in. layer after development showing distribution throughout the layer.

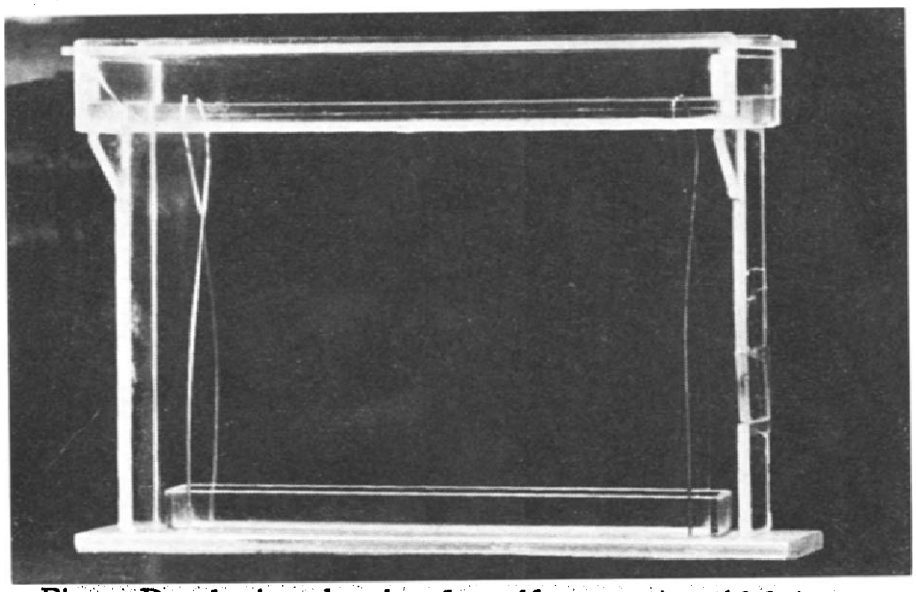

Fig. 9. Developing chamber for self-supporting thick layers.

strates how the "adsorbate" is distributed across the entire layer by diffusion even on a 1/2-in. thick layer.

Although an ordinary chamber may be used for development, a special chamber was constructed that would have a small volume and in which the layer could be held in a vertical position. This vertical position is necessary so that the solvent will advance across the thickness of the layer at the same rate. The chamber is shown in Fig. 9. It was constructed with an aluminium base plate 3/8 × 2½ × 10¾ in. This had a shallow groove milled in the center of it of such size as to receive the glass solvent trough from a Camag sandwich-type apparatus. This was merely to keep the solvent reservoir from sliding around. Four 1/16-in. stainless-steel spring wire supports were inserted in the plate as close to the solvent reservoir as possible and extend 8¼ in. above the plate. These are bent so that they contact the adsorbent layer at the top only and hold it in a vertical position. The remainder of the chamber was constructed of 5/32-in. glass cemented together with epoxy cement. The main portion of the chamber measures 2 × 9⅜ × 7⅞ (height) in. outside dimensions. One end of the chamber has a small trap door hinged to it with Scotch tape; this permits the addition of solvent to the reservoir when necessary. The upper part of the chamber is a rectangular glass box 3¼ × 9⅞ × 1¾ (depth) in. such that there is additional space alongside the main chamber in order to take a second solvent reservoir. This reservoir is used whenever a saturated atmosphere is desired. Saturation is achieved by inserting filter paper sheets between the main chamber wall and the stainless-steel supporting springs. The upper ends of the filter paper dip into the upper solvent reservoir. The amount of solvent flow on these sheets can be controlled by the size of the sheet where it dips into the solvent. The top of this glass box is removable. The entire glass chamber can be conveniently lifted away from the chromatographic plate. Conversely, an ordinary thin-layer developing chamber could be used if a supporting framework is inserted so the adsorption plate is held in a strictly vertical position.

As an illustration of the method using the stainless-steel bound layers, a number of runs were made. Fig. 2 shows the purification of 500 mg of Sudan II on a 1/4-in.

thick plate where the dye was applied in 2 ml of alcohol-free chloroform. One half of the dye solution was applied to each side of the plate. After the dye solution had been applied, 0.5 ml of solvent was applied to each side of the plate along the sample streak to wash any dye that had deposited on the surface by evaporation of the solvent. During the application of the sample, the upper part of the layer was covered by a glass plate to prevent the adsorption of moisture. The solvent was allowed to evaporate and the plate was then placed in the developing apparatus. This was most easily accomplished by holding the adsorbent layer between two glass plates approximately 8½ in. long. After placing this "sandwich" between the supporting wires, the glass plates could be removed one at a time, being careful not to allow the springs to snap into place against the adsorbent layer, thus damaging the layer.

The developing chamber was then put in place and the developing solvent (benzene–chloroform, 20:1) was added to the lower solvent reservoir. The solvent was allowed to run to the top of the plate during a period of 2 h. The plate was removed from the chamber and placed under a hood to evaporate the solvent and was then returned to the chamber for a second development in the same solvent. In this particular example the sample was applied to within 1/4 in. of the edge of the adsorbent layer. This demonstrates the uniformity of development across the layer; in fact, if great care is taken to smooth the edge of the adsorbent where it meets the metal framework, the entire width of the plate could probably be used.

RIMMER[11] has investigated the sample distribution in the thin layer. He found that there was a concentration in the surface layer. In the present work with the thick-layer plates, it was noted that there was a thin coating of adsorbed material on the surface of the layer which gave the appearance that the separation was not as good as it really was. This is demonstrated in Figs. 10 and 11.

100 mg each of Yellow OB, Sudan I, II, and III were dissolved in 4 ml of alcohol-free chloroform and applied to a 1/4-in. thick layer prepared from SilicAR.

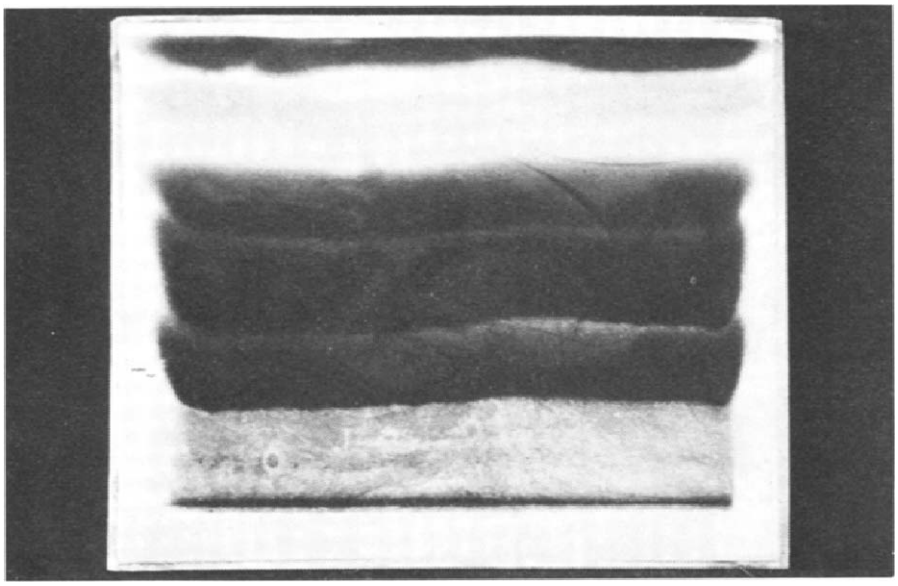

Fig. 10. Appearance of ¼-in. silica gel plate immediately after separation of 100 mg each of (from top to bottom) Yellow OB, Sudan I, Sudan III and Sudan II. Developed in an unsaturated chamber with benzene–chloroform (20:1).

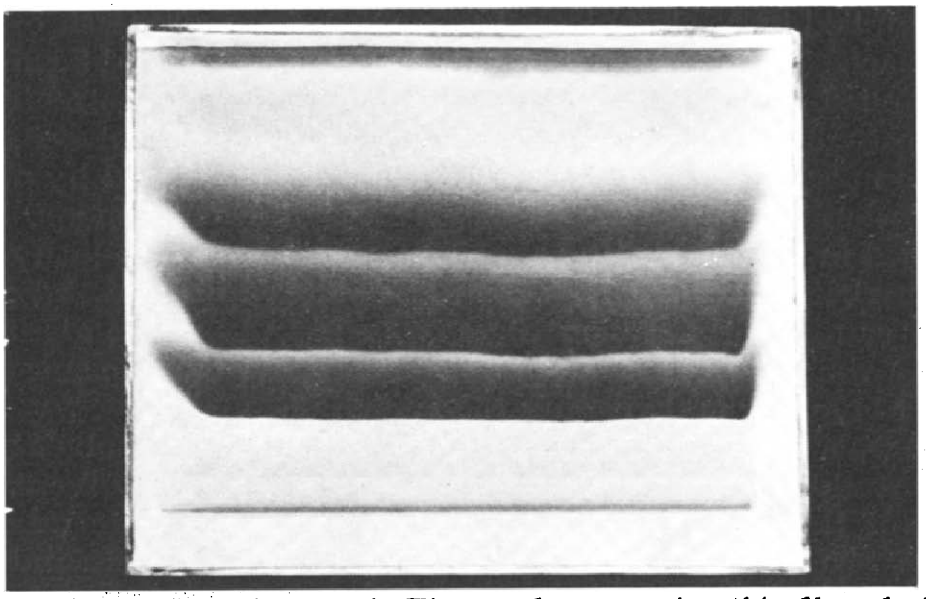

Fig. 11. Same layer as in Fig. 10 after removing thin film of adsorbent from the surface showing the actual separation obtained.

This was followed by 0.5 ml of solvent to each side of the layer to wash the dye into the layer. The chloroform was allowed to evaporate and then the plate was developed twice in benzene–chloroform (20:1). Fig. 10 shows the appearance of the plate immediately after separation, and Fig. 11 shows the same plate after scraping a thin film from the surface of the layer. In order to conserve as much material as possible, it is only necessary to remove the thin film at the junction of the bands to clearly delineate the bands. The surface material from two adjacent bands was checked and found to be chromatographically pure.

Fig. 12 shows how the plate is held in the chamber during development. This

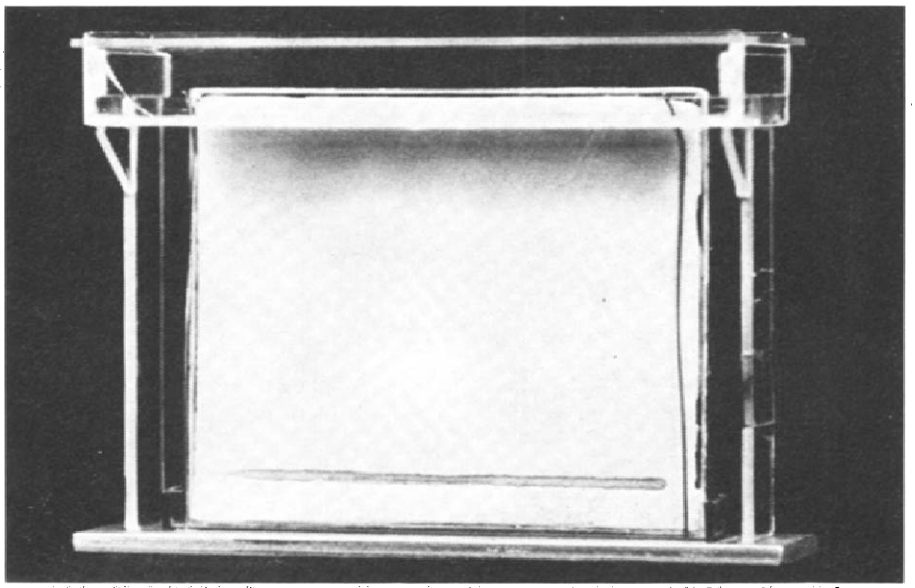

Fig. 12. ¼-in. layer in developing chamber. Purification of 500 mg of Yellow OB on Camag silica gel. Binder: 20% gypsum based on silica gel. Developed twice with benzene–chloroform (20:1).

was a purification of 500 ml of Yellow OB on a 1/4-in. layer prepared from Camag silica gel. Development was carried out twice in an unsaturated chamber with benzene–chloroform (20:1).

All the separations except that shown in Fig. 1 were carried out in an air-conditioned laboratory. This is important because a warm room increases the evaporation rate from the plate and thus extends the developing time. To offset this, a saturated chamber may be used. The air-conditioned laboratory also aids in holding down the evaporation of solvent during the application of the sample to the plate. In a warm laboratory there is a tendency for the sample to build up on the surface of the layer because of evaporation of the solvent.

A 1/4-in. plate contains 147 g of adsorbent–binder mixture(when prepared from Mallinckrodt SilicAR); in contrast an average 250-μ plate contains approximately 4.4 g of silica gel and binder. The 1/8- and 1/2-in. plates contain proportionately smaller and larger amounts of adsorbent. Of course, the actual amount of material that can be applied depends on the quantity of adsorbent and the degree of separation of the components.

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