

PRECOATED SHEETS FOR THIN-LAYER CHROMATOGRAPHY*

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This paper describes a new material for thin-layer chromatography. The material is a precoated flexible sheet that can be used in place of the usual hand-coated glass plates. Some of its properties will be discussed, and its performance will be compared with that of thin layers on glass plates.

The technique of thin-layer chromatography has made a strong impact on separations chemistry. The popularity of this technique has grown rapidly in the past few years and at this point it has become perhaps the most widely used separations technique next to gas chromatography. Thin-layer chromatography is simple and rapid to use, and better suited to a greater variety of separations than paper chromatography. The large number of adsorbents available and the ease of changing conditions give the chemist a considerable number of parameters which can be varied to obtain a desired separation. The usefulness of the technique for rapid diagnostic separations perhaps has been best demonstrated by the wide acceptance the technique has had among the synthetic organic chemists who, for the first time, can follow the course of synthetic reactions, by using a simple chemical procedure not involving elaborate instrumentation.

The greatest deterrent to an even more rapid acceptance of the technique of thin-layer chromatography is the fact that the preparation of glass plates containing the thin-layer adsorbent is time-consuming. In the usual practice, the user must purchase adsorbents, glass plates, and a coating apparatus. The adsorbent must be mixed with water or other solvents in the right proportions, coated on a glass plate, and dried before it can be used. Glass plates also require careful handling, since the adsorbent tends to powder off easily. In addition to this, the size and weight of the plates make them inconvenient to handle, impractical to store, and unsuitable for record purposes.

Several supply houses have recently begun to sell precoated glass plates as an aid to those who do not want to prepare their own plates. These materials are conventional plates; hence they are fragile, and create the usual handling problems.

A chromatography medium combining the handling advantages of paper chromatography and the separation quality of thin-layer chromatography has been lacking up to this writing. The advantages of these two separation methods have now been combined by the development in the laboratories of Kodak-Pathé in France and the Eastman Kodak Company in the U.S.A. of a precoated flexible sheet, composed of an

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adsorbent material coated on a thin, inert base. This material was first described at the Thin-Layer Chromatography Conference in Brussels in September, 1964, by LESTIENNE¹. The adsorbent layer contains a small amount of a polyvinyl alcohol binder which results in a coating that is highly porous and allows the solvent to penetrate quickly. The support is a solvent-resistant polyester and is of the proper thickness to be self-supporting. At the same time the coated support is flexible and can be cut to any size for use.

A small amount of polyvinyl alcohol has been incorporated in the thin layer as a binder. A binder is necessary to make the adsorbent layer adhere to the support and to provide flexibility and abrasion resistance for normal handling. However, the binder chosen is unique, in that it does not alter the properties of the adsorbent. Furthermore, the binder is not affected by most organic solvents. This permits the use of this material in almost every solvent system employed in the development of conventional silica gel coatings on glass plates.

The advantages of this new material are obvious. Sheets of the precoated material can be activated if necessary, cut to the desired size with a cutting board or a pair of scissors, and used just as one would use a normal thin-layer plate. After the separation has been made, the chromatogram can be stored conveniently in a notebook, thereby retaining the actual record of what has been done. Of course, other commonly used methods of chromatogram documentation such as photography can also be used.

The performance of the thin-layer chromatographic sheets in analytical separations is a matter of real interest and is the subject of the remainder of this paper. Since the thin-layer adsorbent contains small quantities of a polyvinyl alcohol binder, it is not immediately apparent that behavior equivalent to that obtained on conventional thin-layer glass plates could be expected. From our experience, however, the nature of the binding of the silica particles is such that it does not significantly interfere with adsorption processes occurring on the surface of the silica gel.

A number of experiments were carried out to illustrate the performance of the new thin-layer chromatographic sheets.

Initial comparisons of the migration rate of various solvents through the thin-layer adsorbent were made. In these experiments conventional thin-layer plates of commercially available silica gel were coated at a thickness of 250 μ . These were air-dried, then activated prior to use. All chromatographic experiments, whether on plates or on sheets, were carried out in a "sandwich-type" chromatographic chamber. These chambers require very short solvent equilibration times and thus are preferred for use with either glass plates or chromatographic sheets. More details concerning the advantages of using these solvent chambers will be given later in this paper.

The rate-determining step in thin-layer chromatography, or for that matter in all types of chromatography, is the rate at which the eluant moves through the adsorbent. Certainly one of the attractive features of thin-layer chromatography has been the fact that most solvents migrate rapidly, so that normal development times are between 15 and 45 min, depending upon the solvents and adsorbents used and the degree of separation desired. Table I shows a tabulation of migration times for the new flexible chromatographic sheet in solvents of varying polarity. It can be seen that as the polarity of the solvent increases, the solvent travel rate decreases somewhat. In any case it is clear that the migration rates approximate those normally associated

with thin-layer coatings on glass plates. Generally the rates are slightly slower and this is believed to be caused by the presence of the binder.

Activation of the thin-layer sheets has an effect similar to that normally found in thin-layer chromatography on glass plates. To illustrate this, separations of a test dye mixture containing 4-dimethylaminoazobenzene, Sudan Red G, and indophenol, were carried out with chloroform on both activated and nonactivated supports.

TABLE I
SOLVENT TRAVEL RATE

Solvent	Sheet (cm*)	Glass TLC plate with silica adsorbent (cm*)
Chloroform	9.5	11.2
Ether	11.4	13.1
Benzene	10.2	12.8
Methanol	7.0	11.0

* Centimeters solvent traveled in 20 minutes.

This test dye mixture (Desaga, Heidelberg) is one that is sold by Brinkmann, Inc., Great Neck, New York, to test the activity of thin-layer chromatographic plates. Fig. 1 shows a separation of a test dye mixture on a thin-layer glass plate which was allowed to equilibrate under ambient conditions and thin-layer chromatographic sheets without activation. Separation of the dye mixture was obtained on both materials, the separation on the thin-layer plate being slightly superior. When the same separation was carried out on supports which were activated for one hour at 110°, the results shown in Fig. 2 were obtained. The R_F values obtained for the three dyes

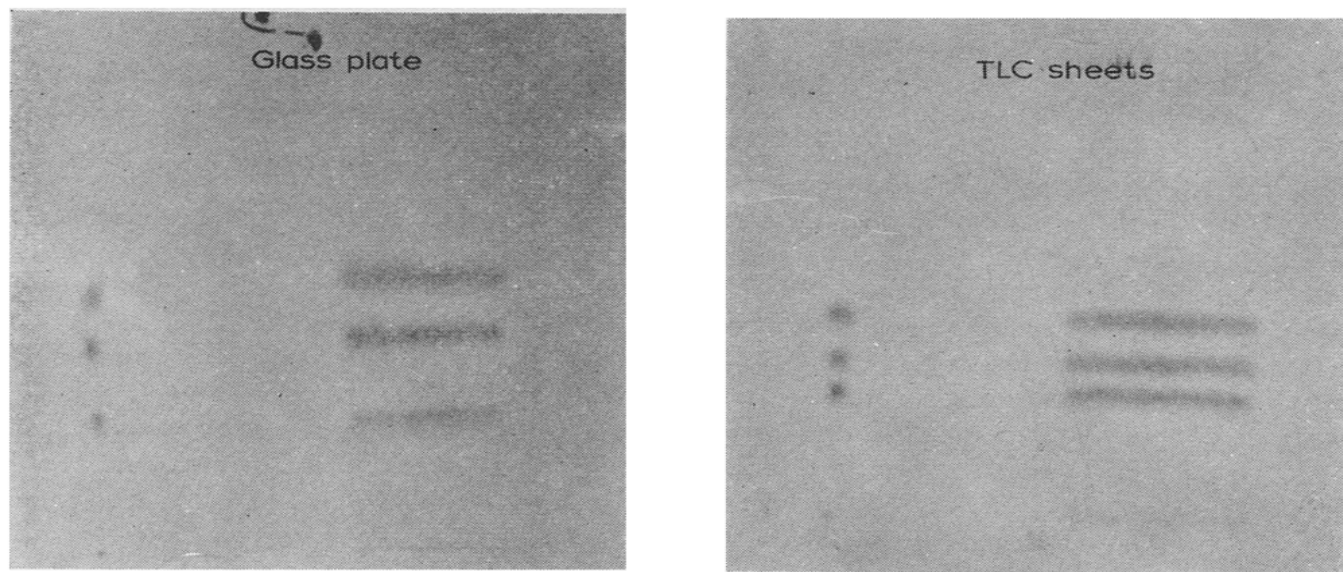


Fig. 1. Separation of test dye mixture by unactivated silica gel on glass base and polyester base. Solvent: chloroform. Dyes separated in order of decreasing R_F : 4-dimethyl-aminoazobenzene, Sudan Red G, indophenol.

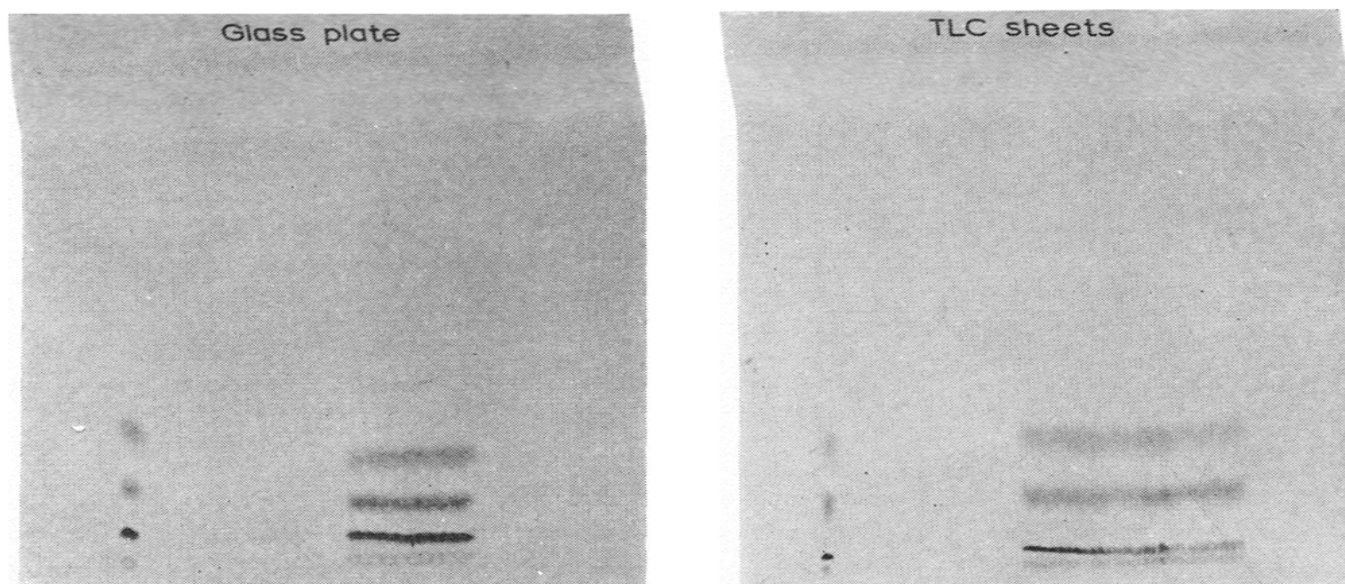


Fig. 2. Effect of activation with same dye mixture, solvent, and techniques as in Fig. 1.

on both supports are essentially identical. This comparison (Fig. 2) indicates that thin-layer chromatographic sheets are essentially equivalent to the conventional thin-layer glass plates when used either with or without activation.

Another example which shows that thin-layer chromatographic sheets are equally efficient as conventional glass plates involves the separation of a series of related dithiooxamide derivatives. The following compounds were studied in this series: didodecyl-, dibenzyl-, dimethyl-, and di-(2-hydroxyethyl)-dithiooxamides. This mixture was separated in the solvent system containing benzene-carbon tetrachloride (1:1). Visualization of the chromatogram was carried out by spraying the sheet with a 10% nickel chloride solution in one molar ammonia. Fig. 3 shows a

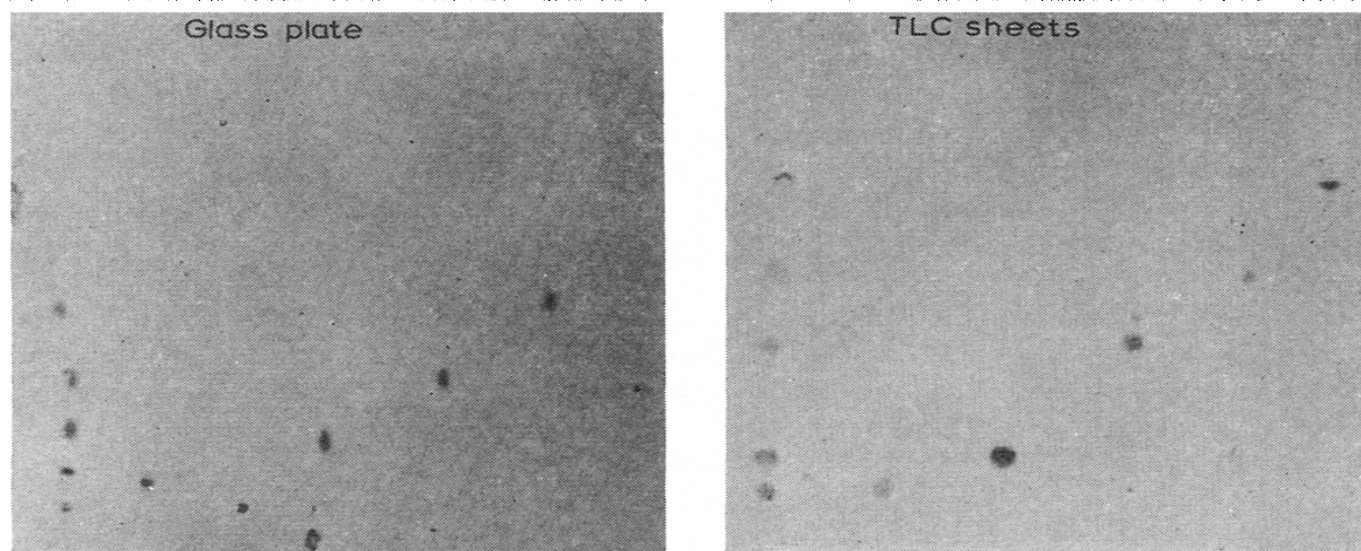


Fig. 3. Separation with 1:1 benzene-carbon tetrachloride solvent of dithiooxamides in order of decreasing R_F : didodecyl-dithiooxamide, dibenzyl-dithiooxamide, dimethyl-dithiooxamide, and di-(2-hydroxyethyl)-dithiooxamide. Column at far left of each chromatogram represents separation as components of mixture, followed by individual migrations.

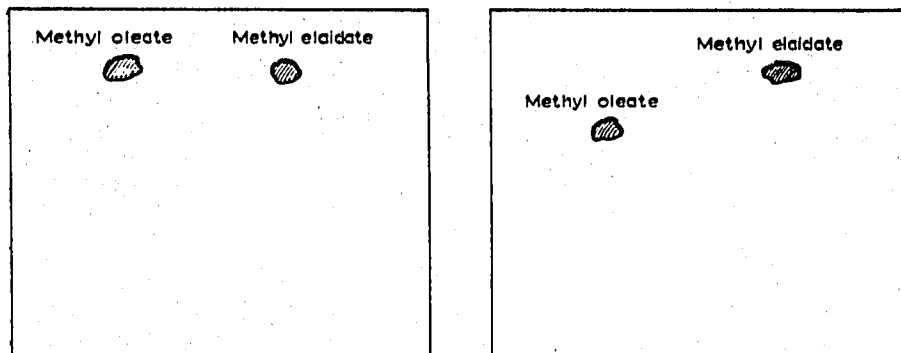


Fig. 4. Effect of pretreatment of TLC sheet with silver nitrate in differentiating R_F values of isomeric unsaturated esters. AgNO_3 -treated sheet is at right. Solvent: hexane-ether (9:1).

photograph of chromatograms developed on the two types of thin-layer supports. In this solvent system, the R_F values for the various dithiooxamide derivatives were not equivalent in the two methods. However, the separations obtained were equivalent. In any case, the example serves to illustrate the usefulness of this material for separation of multicomponent systems.

It is often desirable to modify the adsorbent layer chemically to achieve certain separations. Illustrative of this technique is the separation of the *cis-trans* isomers, methyl oleate and methyl elaidate, reported by MORRIS² in 1962. These two geometrical isomers are not separated on flexible chromatographic sheets (or on conventional silica-coated glass plates) by using a solvent of hexane-ether (9:1), for example (Fig. 4). Incorporating silver nitrate, which forms weak complexes with olefinic functions, into the adsorbent layer serves to differentiate these materials. In making thin-layer glass plates, it would be necessary to add the silver nitrate to the slurry of silica gel prior to coating. Usually, this results in metallic silver being deposited on the fingers of the operator and on the coating equipment. It has been our experience that coatings prepared in this way are unusually fragile. The flexible chromatographic sheets need only to be dipped into an alcoholic silver nitrate solution and then dried prior to use. Incorporation of the silver nitrate in this manner in no way affects the physical nature of the coating.

It is sometimes necessary to remove materials separated on a thin-layer chromatogram from the support material for characterization by other physical means. To test the inertness of the flexible chromatographic sheets for such applications, a 0.5 mg sample of 2,2'-dihydroxy-4,4'-dimethoxybenzophenone was chromatographed in the following solvents: methanol, acetone, ether, chloroform, benzene, and hexane. Removal of the benzophenone from the adsorbent was accomplished by leaching a cut-out of the film for five minutes with the same solvents. No filtration was necessary since the coating remained intact. Characterization was completed by comparing ultraviolet and infrared spectra. There were no significant differences in the spectra obtained under these conditions when compared with a spectrum of the pure compound. It was found that a four-hour leaching with the same solvents dissolved only trace amounts of the support material, with the exception of chloroform, which removes a small amount of low molecular weight polyester from the support. The flexible chromatographic sheet is therefore sufficiently inert to permit the characterization of separated materials after removal from the support.

In our experiments it is clear that this new flexible sheet material exhibits a greater sensitivity to chamber saturation effects than do conventional thin-layer glass plates. In practice, this is manifested by variable R_F values if the developing chamber is not adequately pre-equilibrated with the solvent. Fig. 5 illustrates this effect through the use of various types of chambers for a given separation. On the left is a regular tank-type chamber in which vapor equilibrium is usually difficult to maintain. In the center is an identical chamber which has been lined with a solvent-saturated paper to aid in equilibrating the chamber. As the chromatogram shows, this provides a marked improvement. On the right is a "sandwich-type" chamber which gives an even better separation.

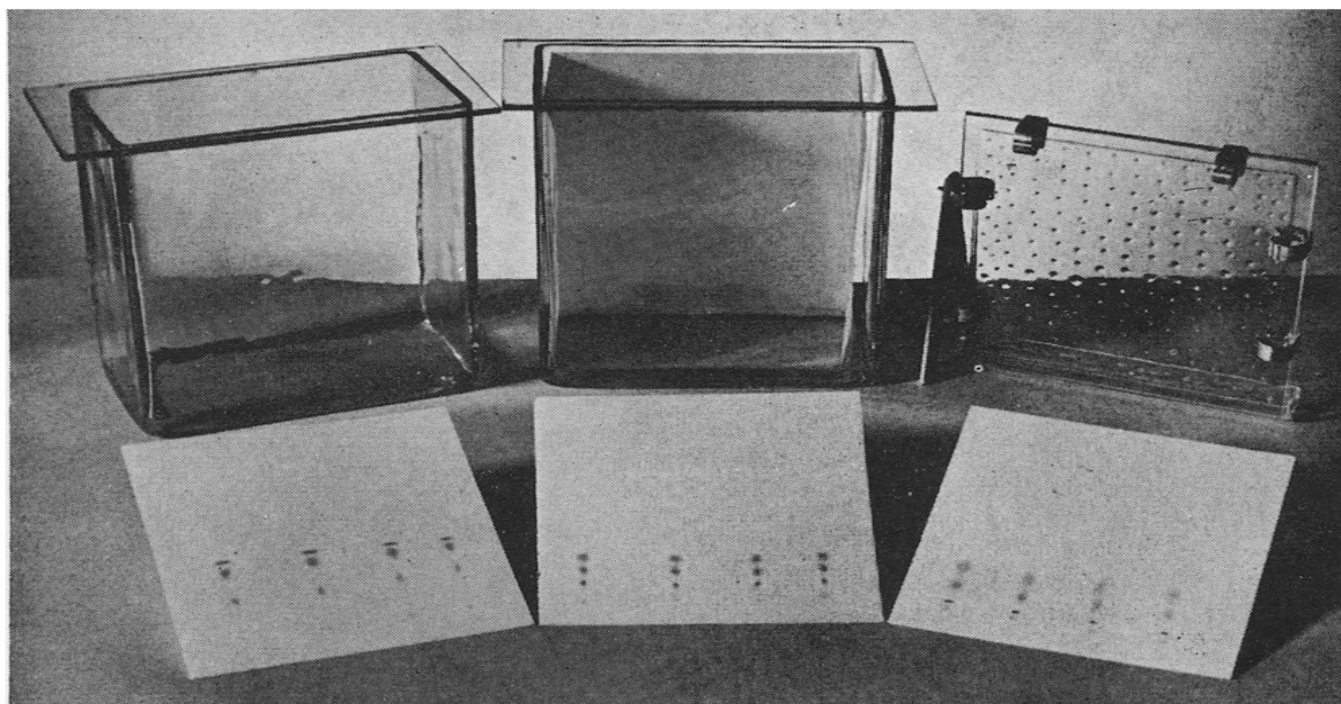


Fig. 5. Effect of vapor saturation in chamber on separation of test dye with chloroform as developing solvent. Left: Tank chamber with no prior equilibration. Fastest migrating dye has followed solvent front with little adsorption. Center: Tank chamber lined with blotter and equilibrated for several days with solvent. All three dyes display R_F less than one. Right: "Sandwich" chamber. No prior equilibration.

This last chamber drastically reduces the air volume that must be saturated with solvent and thus ensures rapid equilibration of the air space with the adsorbent layer. The sensitivity of thin-layer chromatographic sheets to these solvent saturation effects is not clearly understood. The effect has been reported on conventional glass plates but is of a smaller magnitude. It might be reasoned that since the adsorbent layer of the flexible sheet is about one-half as thick as a thin layer on a glass plate, it contains 50 % less solvent. Thus, the loss of a given amount of solvent through evaporation will be larger, percentage-wise on the flexible sheet, than on the thin-layer glass plate. In any event the system in Fig. 5 using a "sandwich-type" chromatographic chamber eliminates this problem.

For use with the flexible chromatographic film, a modified "sandwich-type"

chamber has been devised which will give optimum separations with this new flexible material and will readily accommodate all-sized sheets from 8 × 8 in. to smaller dimensions (Fig. 6). This chamber features pimples on the glass surface which keep the flexible sheets from lying against the glass surface, thus confining the solvent migration within the adsorbent layer. The pimples, even where they contact the adsorbent layer, do not interfere with the movement of the solvent nor do they affect the separation in any way. In our experience this type of chamber has given optimum results with this new flexible thin-film support. This does not preclude the use of other chambers commonly used for thin-layer chromatography.

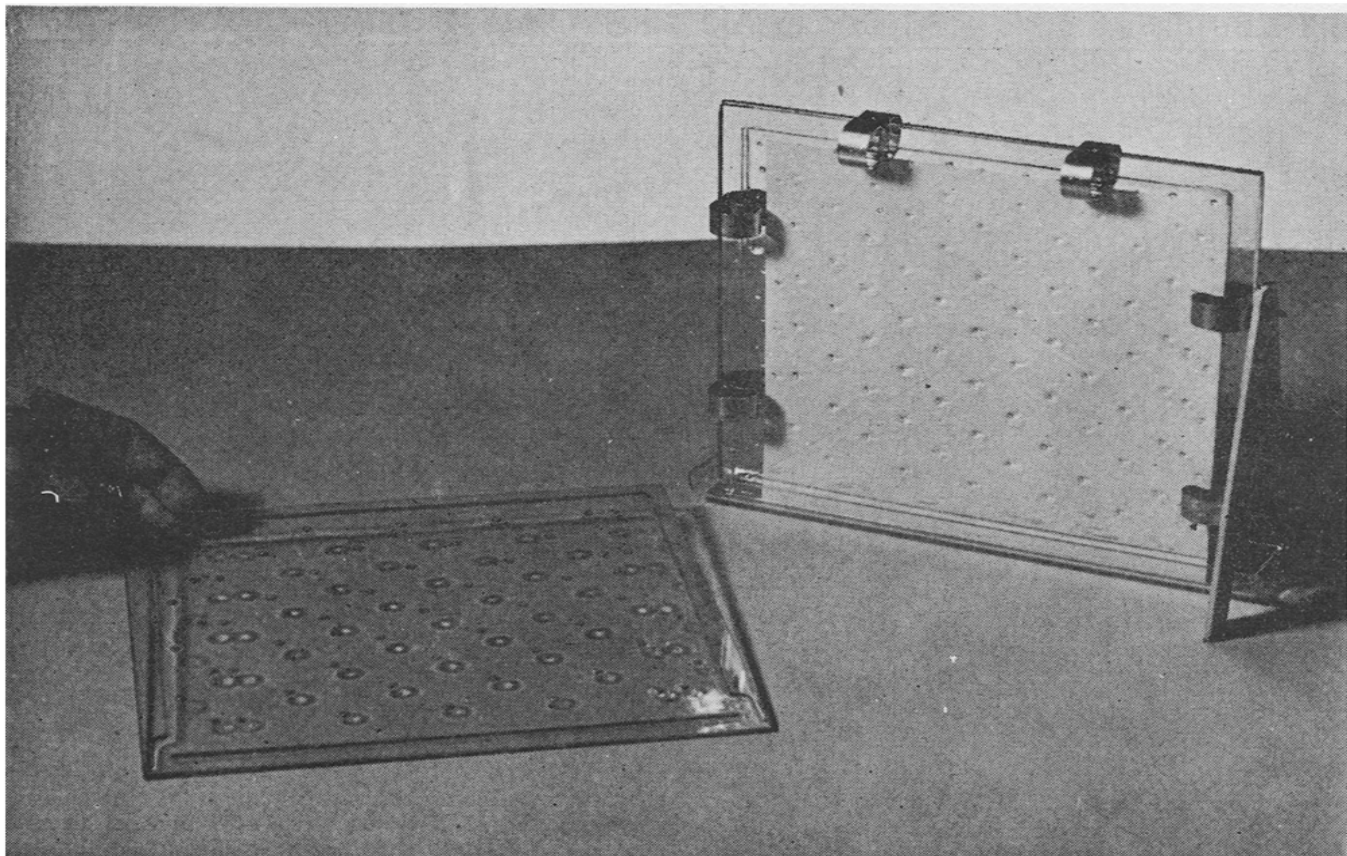


Fig. 6. "Sandwich" chamber with pimples on glass surface for use with flexible thin-layer sheets.

The developed chromatograms on thin-layer sheets can be visualized by most of the techniques used in conventional thin-layer chromatography. The notable exceptions are those visualization techniques based on charring with sulfuric or chromic acids. Iodine-vapor visualization can be used successfully, despite the presence of the organic binder. The flexible chromatographic sheet is supplied with an incorporated inorganic phosphor for visualization under ultraviolet light.

The combination of support and polyvinyl alcohol binder used in the thin-layer chromatographic sheet material is resistant to a variety of organic solvents. Table II gives a partial listing of solvents in which these materials have been tested for resistance and found to be essentially inert. Among these solvents are many commonly used in chromatographic separations.

It is our belief that thin-layer chromatographic sheets represent a significant contribution to the field of thin-layer chromatography. The sheets and the previously discussed developing apparatus are commercially available through Distillation Products Industries (Division of Eastman Kodak Company) as Eastman Chromagram Sheet and Eastman Chromagram Developing Apparatus. The fact that these materials are available in a form ready for use and that they have physical stability which will permit easy handling in laboratories, as well as in subsequent record-keeping, provide a type of support not yet known in the field of thin-layer chromatography. In tests of

TABLE II
USEFUL SOLVENTS TESTED

Methanol
Ethanol
Isopropanol
n-Butanol
Benzene
Hexane
Methylene chloride
Chloroform
Carbon tetrachloride
Diethyl ether
Dioxane
Acetic acid
Ethyl acetate
Acetone
Pyridine

these materials for the separation of a variety of chemical systems, they have given separations equivalent or superior to those of conventional thin-layer glass plates, with silica gel as the adsorbent material. Further experiments are contemplated to extend the use of these materials to a variety of other chemical systems and to check their usefulness for other types of chromatography.

SUMMARY

The characteristics of precoated sheets for thin-layer chromatography are described. Separation performance is compared with that of conventional thin-layer glass plates. The precoated sheets are shown to be equivalent or superior in performance to glass plates utilizing silica gel adsorbent.

REFERENCES

- 1 A. LESTIENNE, E. P. PRZYBYLOWICZ, W. J. STAUDENMAYER, E. S. PERRY, A. D. BAITSHOLTS AND T. N. TISCHER, Precoated sheets for thin-layer chromatography, *Thin-Layer Chromatography Conference, Brussels, September, 1964*.
- 2 L. J. MORRIS, *Chem. Ind. (London)*, (1962) 1238.