

## Frosted glass plates as an aid to thin-layer chromatography

Although STAHL *et al.*<sup>1</sup> were not the first to separate organic substances on thin-layer adsorbents<sup>2</sup>, they were responsible for standardizing and popularizing the application of the method. Currently, the potential of thin-layer chromatography is rapidly being explored and extended. However, the present form of the technique is not without its limitations.

The procedure of spreading and drying an adsorbent, such as silica gel, onto a clear glass plate, results in a thin, extremely fragile film (100–500  $\mu$ ). In an attempt to strengthen the applied film, various kinds of binders have been added to the silica gel. The use of binders, however, does not entirely solve the problem of fragility; solvents containing water tend to increase the delicacy of the silicic acid thin layer<sup>3</sup>, during and after chromatography. Also, the use of such binders presents other problems. For example, in the case of phospholipids<sup>4</sup> a load effect is evident.

In order to circumvent these difficulties, peeling and load effect, we sought to overcome the problem in another manner. The peeling and cracking of the silica gel layer is due partly to the fact that smooth glass plates do not offer an adhesive surface. During the course of our investigation, it was found that a more adhesive surface could be provided by etching the clear glass plates.

By sand-blasting clear glass with a variety of substances, it was possible to produce surfaces with varying degrees of roughness. The coarseness of the surface grain had an effect on the  $R_F$  values of compounds as well as providing a better adhesive surface. Very coarse surfaces resulted in  $R_F$  values lower than that of plain glass. A finer etched surface increased the  $R_F$  values. After experimenting and comparing a variety of different frosted plates, it was determined that the ideal surface was the one formed by blasting with a light medium grit of sand (U.S. Patent pending)\*.

When these finely etched plates were used for routine thin-layer chromatography, the cracking and peeling of the silica layer, normally exhibited on clear plates, especially in aqueous solvent systems, was not noticed. A comparison of clear and frosted plates may be seen in Fig. 1.

For the same solvent, it was demonstrated that  $R_F$  values were slightly higher when mixtures of compounds were chromatographed on etched plates in comparison to clear glass plates (Fig. 2). The increase mobility was most noticeable for compounds having a small  $R_F$  value. The effect was due to the capillary action of the frosted surface, itself.

Etched plates were particularly useful in the separation and quantitation of phospholipids. Previously, it has been shown that binders were undesirable, since load effects were evident<sup>4</sup>. Also, quantitating thin-layer chromatographic plates in the customary way presents some inherent problems. Recently, these were summarized by DOIZAKI AND ZIEVE<sup>5</sup>: loss of specimen in attempting to scrape sample area prior to quantitation, inhibiting effect of silica gel on the color development of phosphorus.

The problem of quantitating phospholipids on thin-layer plates was solved by scanning 1 × 8 in. plates directly with a double beam reflecting densitometer (Chromo-

\* Frosted glass plates are available from National Instrument Company, Rockville, Md., through Med-Chem. Laboratories, Detroit.

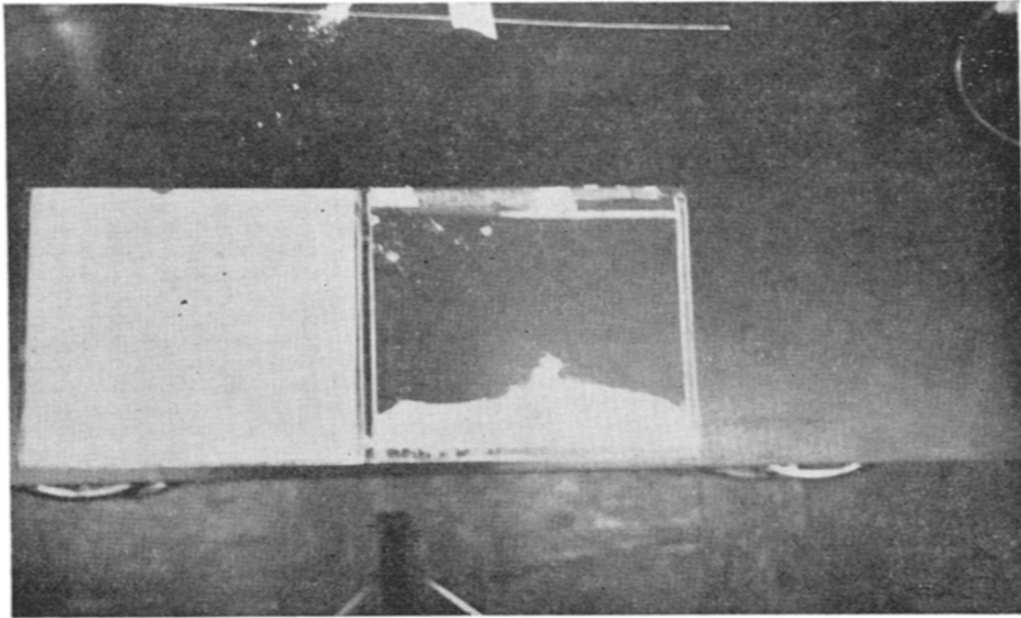


Fig. 1. On the right is an example of peeling and cracking of silica gel thin layer, without binder, covering a smooth glass surface. On the left, is silica gel thin layer, without binder, coated on an etched glass plate. The etched glass plate shows no peeling or cracking.

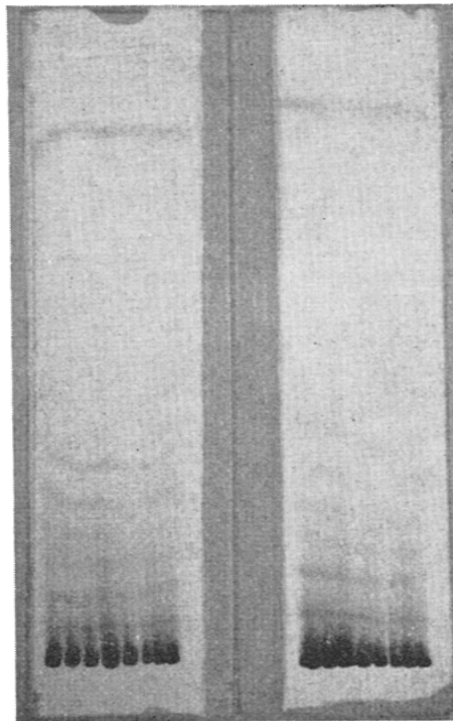


Fig. 2. Comparison of synthetic porphine samples (kindly supplied by A. Szutka, University of Detroit), separated on clear and etched glass. The silica gel on an etched glass surface (right) showed a greater number of component substances, especially those with small  $R_F$  values.

scan, National Instrument Company, Rockville, Md.). In a previous report, we demonstrated the usefulness of this instrument for agar-gel pherograms, as well as paper chromatograms<sup>6</sup>. Scanning of the thin-layer silica gels, in a similar manner, was now possible because of the greater adhesion between the gel and the etched glass plate. The per cent of each phospholipid fraction could be determined through scanning, and total phosphorus content could be measured on an aliquot of the same sample<sup>7</sup>. By this method, the amount of each phospholipid in a mixture could be calculated. Complete details of this method will be the subject of another report.

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### **Beitrag zum Problem der Sichtbarmachung von chlorierten Insektiziden am Dünnschichtchromatogramm**

Die Papier- und Dünnschichtchromatographie (DC) wurde in den letzten Jahren immer mehr zur Abtrennung geringer Mengen von Pestiziden herangezogen. In Arbeiten auf dem Gebiete der Papierchromatographie (ausführliche Literaturübersichten<sup>1-6</sup>) wurden die Bedingungen der Abtrennung festgelegt und Vorschriften für die quantitative und semiquantitative Bestimmung gegeben.

Die Sichtbarmachung der Phosphor- und Thiophosphorsäureester am Papierchromatogramm ist verhältnismässig einfach da die besondere Beschaffenheit dieser Verbindungen verschiedene Reaktionen ermöglicht. Schwieriger gestaltet sich die Anfärbung und Unterscheidung der chlorierten Kohlenwasserstoffe.

MITCHELL UND PATTERSON<sup>7</sup> und MITCHELL<sup>8</sup> behandeln zu diesem Zweck das Chromatogramm, nach Auftrennung, mit Monoäthanolamin um dann mit Silbernitrat anzufärben. WINTERINGHAM und Mitarb.<sup>9</sup> verwenden Monoäthanolamin, Silbernitrat und ultraviolettes Licht. In späteren Arbeiten verwenden MITCHELL<sup>10,11</sup> wie auch MILLS<sup>12,13</sup> Silbernitrat (in einer 2-Phenoxyäthanollösung). Ein neuer Vorschlag<sup>14</sup>