dungen in der Citraconsäure angesehen werden; durch sie wird eine stärkere Adsorption und damit ein kleinerer R_F-Wert bewirkt. Die geringere Konjugation der Itaconsäure wird im grösseren R_F-Wert deutlich. Die Aconitsäuren haben drei Carboxylgruppen in der Molekel; die R_F-Werte sind nur noch sehr klein.

Die stark adsorbierende Wirkung von Hydroxygruppen ist auch bei Carbonsäuren zu erkennen. So hat die Citronensäure in beiden Fliessmitteln die R_F-Werte 0.02 und die Dihydroxyfumarsäure bleibt völlig am Startpunkt zurück.

Auch zwei Säureanhydride konnten in die Versuche einbezogen werden, Maleinsäureanhydrid und Citraconsäureanhydrid.

Maleinsäureanhydrid zeigt im Chromatogramm neben Maleinsäure einen Fleck mit den R_F-Werten 0.67 (BME) und 0.56 (BDE). Ein käufliches Citraconsäureanhydrid liess vier Flecke erkennen. Neben Citraconsäure zeichnet sich vor allem ein deutlicher Fleck bei 0.77 (BME) und 0.86 (BDE) ab. Daneben konnten noch drei weitere Flecke erkannt werden, von denen einer eventuell der Mesaconsäure zugeordnet werden kann. Es ist unklar, ob die anderen Flecken durch Verunreinigungen bedingt wurden.

Erkennbar wird jedoch die geringere Adsorption der Säureanhydride am Kieselgel im Vergleich zu den Carbonsäuren.

Anmerkung

Während der Drucklegung veröffentlichten E. KNAPPE UND D. PETERI (*Z. Anal. Chem.*, 190 (1962) 380) eine Arbeit über die dünnschicht-chromatographsche Trennung ungesättigter Dicarbonsäuren.

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A simple saturation chamber for thin layer chromatography

It has often been reported that the R_F of a substance spotted near the edge of a thin layer chromatogram[1] is different from that when spotted at the centre. This *edge effect* was at first attributed to a difference in the layer thickness between the edges and the centre of the plate[1,2]. The effect, however, persisted even when adsorbent layers of constant thickness were prepared, so the phenomenon was reinvestigated. It has been found that the critical factor is the degree of saturation of the air in the

chromatography chamber with solvent vapour; the less saturated the atmosphere, the more pronounced is the effect. The effect can be reduced by running the ascending chromatogram in a chamber fitted with layers of thick filter paper which soak up the solvent which then quickly saturates the gaseous phase. A straight solvent front is obtained when the adsorbent is scraped from the edges of the plate prior to chromatography[3]. The *edge effect* can also be significantly reduced, if not eliminated, by reducing the size of the chamber[4].

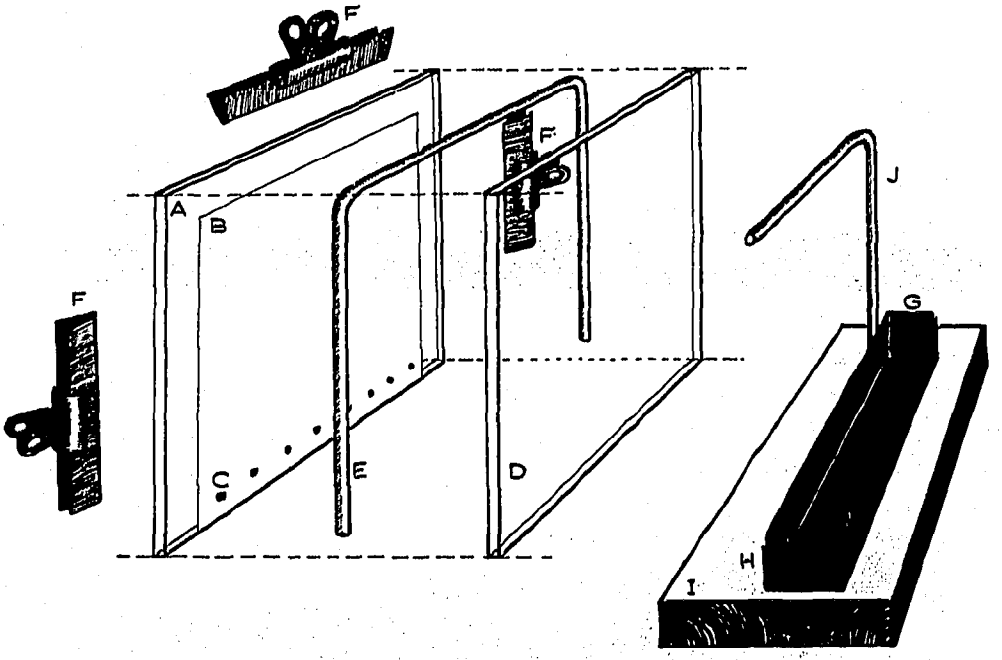

Fig. 1. Components of the chamber.

This communication describes the construction of a simple and inexpensive, yet highly efficient chamber for developing thin layer chromatograms under conditions of high chamber saturation with the solvent, brought about by a reduction of the size of the chamber. It is based on the principle of STAHL's *S-Chamber*[5].

Description of apparatus

The chamber is formed from two plates of 32 oz. glass (20 × 20 cm), one of which (A) is the plate supporting the solid phase, the thin layer of adsorbent (B) (see Fig. 1). The adsorbent is removed from three edges of the adsorbent layer to leave a clear margin 1.5 cm wide at the top and two sides. The samples (C) are now applied to the adsorbent layer along a line 1.5 cm from the bottom edge. The cover plate (D) is placed over the adsorbent layer, but is held just under 3 mm away from it by a glass spacer (E). This is made from a 55 cm length of 3 mm diameter glass rod which is bent at right angles 18 cm from each end so that it forms three sides of a rectangle. It is held between the two sheets of glass with its 19 cm middle section at the top. The two glass plates are held firmly by three large (5.5 in.) "Bulldog" clips (F). It is important that the bends in the glass rod should be flush with the glass plates; any projection of the glass can be removed with a file. The chamber is now open at only the lower end, and this end is placed in the solvent trough (see Fig. 2).

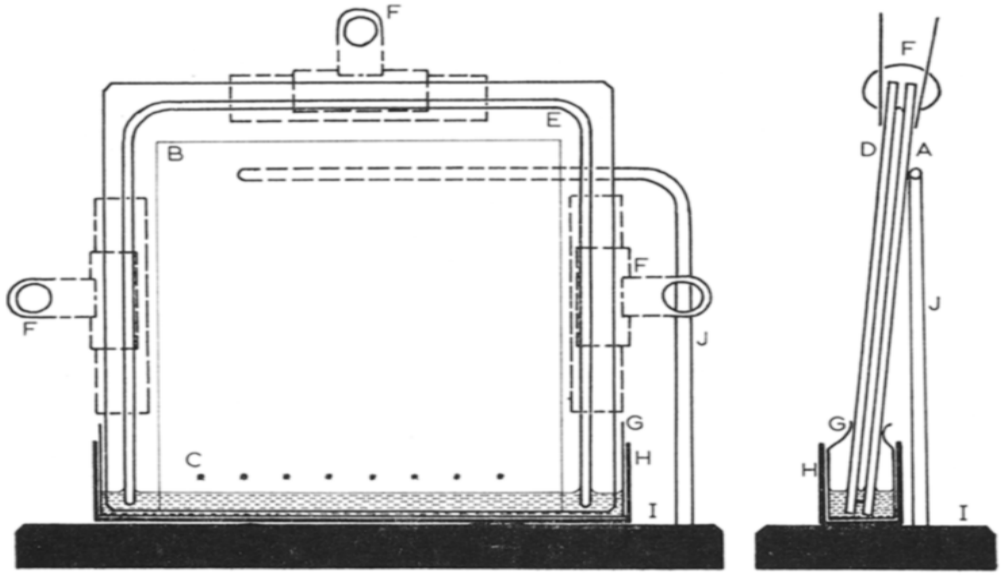

Fig. 2. Construction of the apparatus.

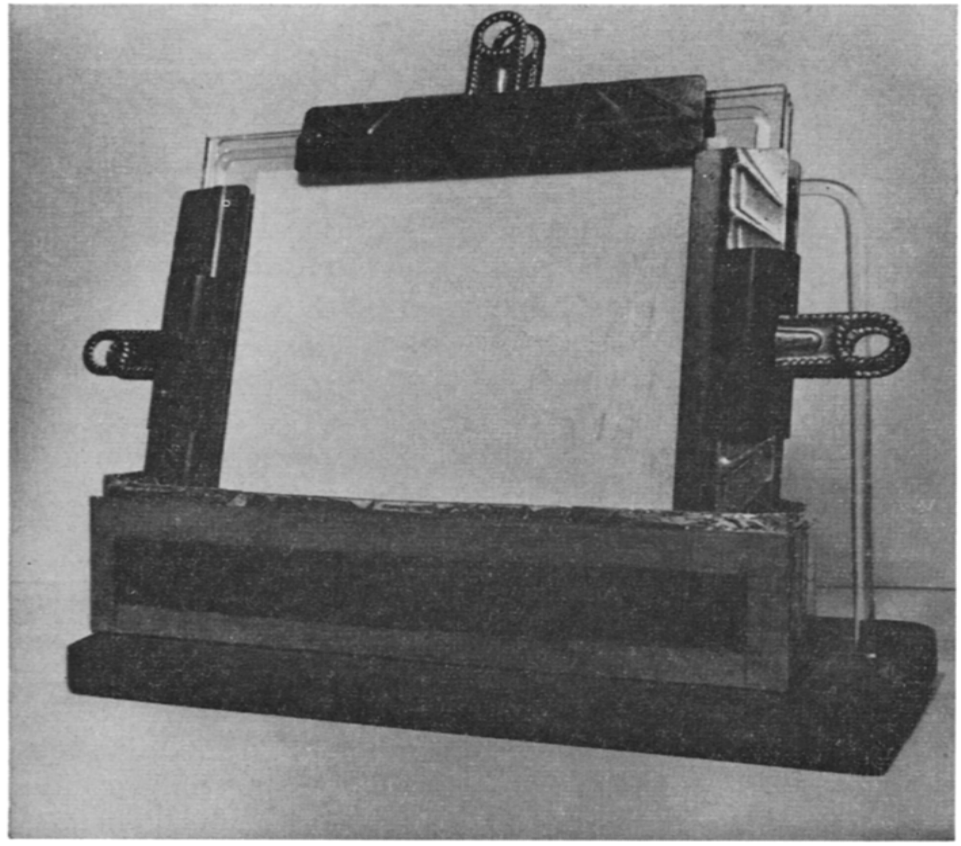

Fig. 3. Front view of the assembled apparatus.

The trough which has been used for organic solvents is made from several layers of aluminium foil (G). This is placed inside a strong cardboard trough (H) which is held to a wooden baseboard (I) with drawing pins. A 5 mm diameter glass rod with a single right angled bend (J), which is fitted into a hole drilled in the baseboard, is sufficient to hold the chamber vertically. The solvent is placed in the trough to a depth of 0.5–1.0 cm. The advantage of using an aluminium foil trough for organic solvents is that its edges can be bent over on to the chamber to reduce evaporation of the solvent from the trough (see Fig. 2). The foil trough can be replaced by a glass one for use with aqueous solvents.

The size of the chamber is, of course, not limited to 20 × 20 cm. Glass plates as long as 50 cm have been used to carry out the simultaneous chromatographic separation of up to 50 samples. In this case, a 60 cm glass trough was used to hold the solvent.

Discussion

The results obtained with this technique have been compared with those from experiments using a standard thin layer chromatography chamber (22 × 22 × 7 cm) fitted with filter paper. In the case of lipid samples chromatographed with non-aqueous solvents, the 3 mm chamber described above gave a much better separation. The spots are much smaller, and the complete separation of mixtures of carotenes[6] or of other isoprenoid hydrocarbons[7,8] can be accomplished in 15 min. The high chamber saturation with solvent vapour results in the absence of the *edge effect*, so that R_F values are constant over the whole length of the chromatograms.

This technique has also been used to separate substances requiring the use of aqueous solvents. In this case, there is little difference between these results and those obtained using the larger standard chamber, since the running time of chromatograms with aqueous solvents is necessarily longer than with organic ones.

The author is grateful to his colleagues who have thoroughly tested this technique for their various requirements, to Professor T. W. GOODWIN for his interest and encouragement, and to Professor E. STAHL for demonstrating the principle of his "S-chamber" on a recent visit to this laboratory.

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