CHROM. 6350

Thin-layer gel chromatography in organic liquids

Thin-layer gel chromatography is a technique which until now has found only limited application. It was first described in 1962[1,2]. The practical examples mentioned in the literature pertain mainly to the separation of proteins in aqueous media[3–5]. In a single instance, the separation of dyes in the strongly polar solvent dimethylformamide is described[6]. In the literature, some cases are also described in which the separation is not based on the molecular sieve principle but on adsorption[7,8]. The thin-layer technique can be used with advantage together with normal gel permeation chromatography on columns in order to test the possibility of separating a sample in advance. An important advantage is that different samples can be compared simultaneously.

The separations described previously pertain only to polar compounds. In order to adapt this method to less polar substances, it will be necessary to use gel materials that are accessible to apolar liquids. However, the number of gel materials that are suitable for these organic solvents is limited, especially for application in thin layers, in which the required particle size is an additional restrictive factor. Gel materials with the required particle sizes are commercially available, but some of them can be used only in polar systems such as water. These gels, polydextran gels of the Sephadex G Superfine (10–40 μm) type, however, can be modified by a simple alkylation, so that they become accessible to more apolar liquids[9–11]. In our experiments we used methylated Sephadex gels, and the methylation was carried out with dimethyl sulphate in aqueous sodium hydroxide solution. By using these modified polydextran gels, separations in organic solvents have been achieved. Moreover, some substances have been separated with gels of the Merckogel type (particle size <53 μm).

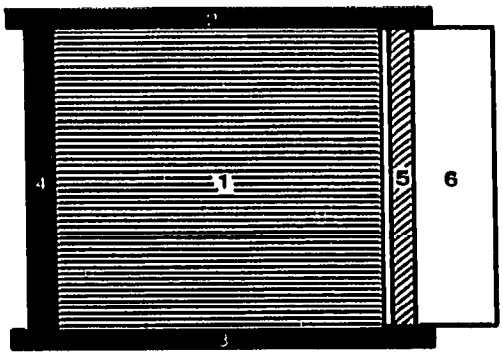
Experimental

For the application of this technique in practice, some problems have to be solved. The most important of these problems are as follows:

(1) Separations must be carried out in a closed vessel in order to prevent evaporation of the solvent, which may alter the properties of the gel. This holds especially for volatile organic solvents. It is preferable to use a sandwich chamber (see below).

(2) As the gel does not exert a capillary action on the eluent, as in normal thin-layer chromatography, a descending technique must be used. However, there is little attraction between the gel and the glass plate, so that the angle to the horizontal should not be too great, otherwise the gel will slip away. Also, wide angles cause the eluents to flow over the surface of the gel layer without sufficient interaction, which results in poor separations. It is preferable to use angles of less than 45°.

(3) Detection is more difficult than in normal thin-layer chromatography, and it is not possible to spray the wet plates directly. The plate must be dried under very mild conditions so as to prevent the gel from losing its structure. Spraying the plates that have been so dried can be carried out only with specific non-aggressive reagents, because the gel itself should not be degraded. The alternative of making a paper print by placing a piece of filter-paper on the wet plate[12,13] is in our experience not attractive owing to the rapid evaporation of the volatile organic solvents.



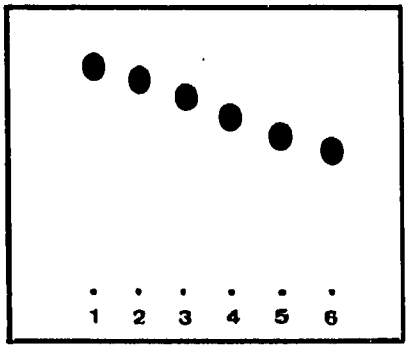
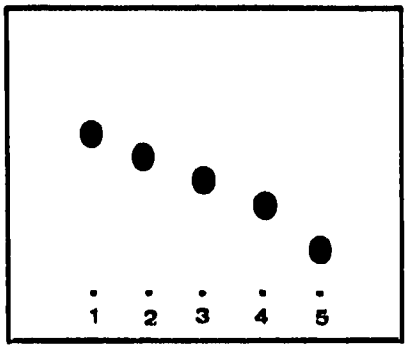


Fig. 1. Thin-layer gel chromatography apparatus. 1 = Bottom plate with grooves; 2, 3 and 4 = fixed glass bars; 5 = loose glass bar; 6 = filter-paper. The apparatus can be closed with a top plate.

Fig. 2. Chromatogram obtained with Merckogel OR-750 (<53 μm) in chloroform. Time, 3 h; angle, 15°. Detection: bromocresol purple. 1 = $C_{21}H_{43}COOH$; 2 = $C_{19}H_{39}COOH$; 3 = $C_{17}H_{35}COOH$; 4 = $C_{15}H_{31}COOH$; 5 = $C_{13}H_{27}COOH$; 6 = $C_{11}H_{23}COOH$.

Some special types of apparatus are available for thin-layer gel chromatography (Boehringer, G.F.R., and Pharmacia, Sweden) with which devices the angle of the plate can be adjusted. Unfortunately, they can be used only for aqueous systems because the plastic material from which they are constructed is soluble in organic solvents, so we therefore designed a sandwich chamber (Fig. 1).

The bottom plate (1) is a special glass plate with grooves*. On three sides of this plate, narrow rectangular glass bars are fixed (2, 3 and 4) by means of an aqueous suspension of sodium silicate and talcum powder. Because of the grooves in the bottom plate, some space remains between the last glass bar and the bottom plate for the discharge of eluent. The fourth side of the plate holds a loose glass bar (5). After the gel layer has been applied to the bottom plate (see below), a strip of filter-paper (6) is brought under this glass bar, and the other end of the filter-paper is connected with a



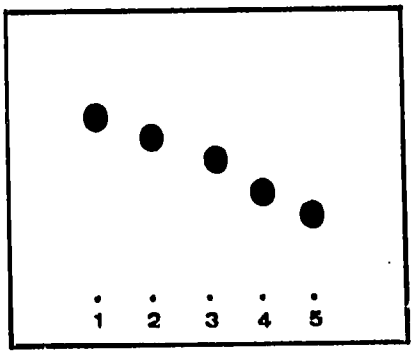


Fig. 3. Chromatogram obtained with Merckogel OR-1500 (<53 μm) in toluene. Time, 4 h; angle, 30°. Detection: fluorescence indicator. 1 = $C_9H_{19}\cdot C_6H_4$-p-$(OCH_2CH_2)_7OH$; 2 = $C_9H_{19}\cdot C_6H_4$-p-$(OCH_2CH_2)_5OH$; 3 = $C_9H_{19}\cdot C_6H_4$-p-$(OCH_2CH_2)_3OH$; 4 = $C_9H_{19}\cdot C_6H_4$-p-OCH_2CH_2OH; 5 = $C_9H_{19}\cdot C_6H_4$-p-OH.

Fig. 4. Chromatogram obtained with methylated Sephadex G-25 in methanol. Time, 1½ h; angle, 25°. Detection: Dragendorff reagent. 1 = Polyethyleneglycol (PEG) 2000; 2 = PEG 1500; 3 = PEG 1000; 4 = PEG 600; 5 = PEG 400.

* Desaga "Rillen-Platte" (G.F.R.).

solvent tank. The whole arrangement is closed with another glass plate. The bottom and top plates are held in position with two clamps. The samples can be spotted through holes drilled at regular distances in the top plate, and these holes can be closed with plastic caps.

As our laboratory is principally interested in the separation of substances with low molecular weights, we have to use gel materials with a low-molecular-weight range. To avoid the difficulties that occur on spreading thin layers of these gel types[14], a thin slurry of the swollen gel is poured on to the plate and spread evenly over the plate by tapping the sides. Apart from the interstitial liquid, the slurry contains about 25% of extra liquid. Evaporation causes the gel to thicken slowly. As soon as it has become sufficiently thick, but is not yet dry, the glass top is clamped in position so as to prevent further evaporation. The plate is then placed at the desired angle and the supply of eluent is started. Before the samples are spotted, the gel has to be equilibrated for some time. During the spotting, the plate must be horizontal.

Figs. 2–4 illustrate the applicability of the method.

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