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THIN-LAYER CHROMATOGRAPHY AS A PILOT TECHNIQUE FOR THE OPTIMIZATION OF PREPARATIVE COLUMN CHROMATOGRAPHY

EDWARD SOCZEWIŃSKI* and TERESA WAWRZYNOWICZ
Department of Inorganic and Analytical Chemistry, Medical Academy, 20-081 Lublin (Poland)

SUMMARY

Thin-layer chromatography can be used as a convenient small-scale model of preparative liquid chromatography in the same adsorbent–eluent system. Large volumes of sample solution should be introduced from the edge of the thin-layer and then eluted continuously; these conditions, similar to column chromatography, can be secured using a horizontal sandwich tank with a glass distributor. The formation and separation of zones can be frequently observed under UV light. Specific chromogenic reagents can also be used to obtain preliminary information about the qualitative and quantitative composition of the partly separated mixture. A modified thin-layer chromatographic technique for two-dimensional preparative separations is described.

INTRODUCTION

Preparative liquid chromatography, owing to the large amounts of adsorbents and solvents used, requires preliminary optimization of the adsorbent–eluent system; analytical separations usually precede preparative runs, the sample size being gradually increased to estimate the effect of overloading on the separation[1–3]. Thin-layer chromatography (TLC) can also be used for the optimization of eluent composition[4]; the use of continuous elution and elimination of solvent demixing and preadsorption effects permits comparability of retention parameters in TLC and high-performance liquid chromatography (HPLC)[4–8].

It is also possible to produce in thin layers of adsorbent similar overloading to that occurring in preparative columns; in this way, the advantages of TLC (minimal loss of materials, rapidity, application of selective reagents) can be utilized for the choice of optimal systems for preparative separations.

The delivery of large volumes of sample solution to the thin layer cannot be carried out in the usual way, spotting (Fig. 1a) or streaking (Fig. 1b), owing to the formation of complex starting chromatograms. The sample should be introduced from the edge of the chromatographic bed as in column chromatography (Fig. 1c) so that the first stage of the process is equivalent to frontal chromatography, during which partial separation of the mixture is obtained[9]. This is followed by elution, which under favourable conditions results in full separation of the zones. The two

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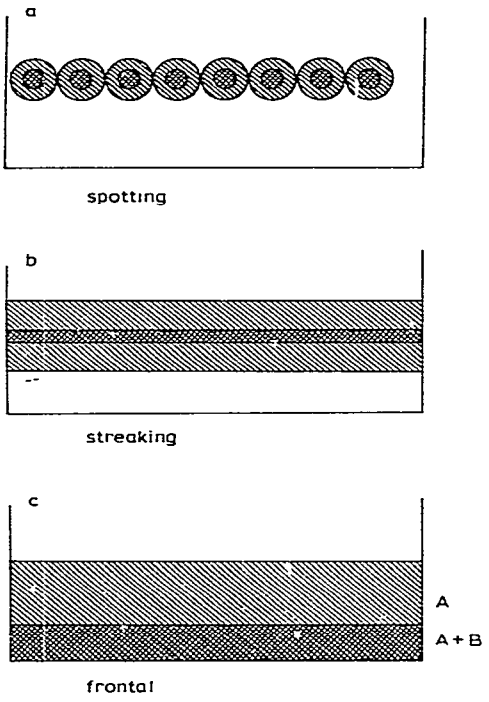

Fig. 1. Starting bands of large volumes of a binary sample (A + B) introduced by spotting, streaking and from the edge of the thin layer.

stages, frontal and elution chromatography, can also be performed using a modified sandwich tank of the Brenner–Niederwieser (BN) type with a glass distributer[9–11] which permits ready change of the solution delivered to the layer.

The separation of components in the overloaded zone can be observed by inspection under UV light or by use of several selective spraying reagents on narrow strips of the chromatogram. In this way the main components of the mixture can be established and information obtained on their molecular structures (*e.g.*, presence of functional groups). Irreversible adsorption of some sample components, which would lead to the destruction of the preparative column, can also be detected.

To investigate in more detail the formation and separation of zones during the frontal and elution steps[11], a modified sandwich tank has been constructed which permits elution of the zone at right angles to the first elution direction.

EXPERIMENTAL

A sandwich tank[10,11] with a modified glass distributer was used which permitted delivery of a 1 cm long zone of sample solution (dissolved in eluent), the remaining length of the plate being eluted with pure eluent. For 100 × 100 mm and 200 × 100 mm plates, the distributers were made from 1.2-mm glass (74 × 5 and 10 × 5 mm) attached with cyanoacrylic glue to the lower surface of the cover plate[10,11]. The sample solution and the eluent were delivered from separate 5-ml containers by si-

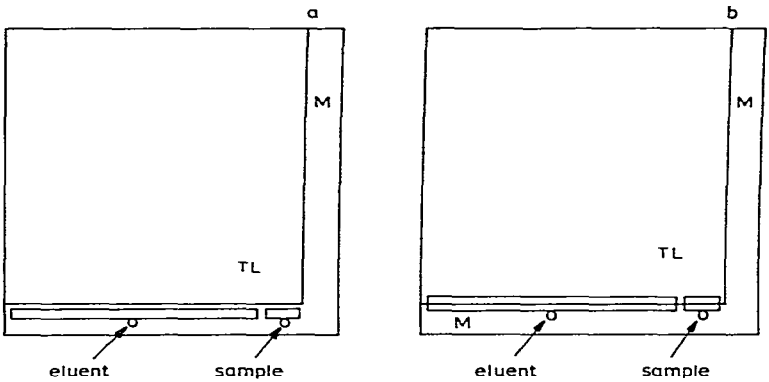

Fig. 2. Sandwich tank with two distributers for delivery of a 1 cm wide sample zone. M = Margin of the carrier plate cleaned of the adsorbent; TL = thin layer.

phons made of stainless steel capillaries (0.7 mm I.D.), the initial levels of the eluent being slightly lower than the thin layer[10]. The 2-mm gap between the two distributers prevents mixing of the sample and eluent. In model experiments the behaviour of coloured compounds was investigated. The flows of the two liquids were parallel and the boundary of the coloured zone was well defined and parallel to the edge of the plate. Only slight edge effects were observed.

Along two adjacent sides, 1-cm margins of the plate were cleaned of adsorbent. (It is essential that the edges of the layer are even and smooth.) The plate was activated, cooled in a desiccator and put in the horizontal tank with the distributers lying over the margin (Fig. 2a). By a slight temporary overpressure, the two siphons and the spaces under the short and long distributers were filled with sample solution and eluent, respectively. The cover plate was then moved along the carrier plate until the distributers lay partly over the adsorbent layer (Fig. 2b) and the two solutions were simultaneously delivered to the layer. After the formation of a sufficiently wide perpendicular starting zone, the container with the sample was replaced by another one containing pure eluent. Elution in the second dimension was carried out by placing another plate with a single distributer over the second, perpendicular, margin.

RESULTS AND DISCUSSION

A model mixture of dyes was investigated: azobenzene, dimethylaminoazobenzene, Sudan IV and Disperse Red 73. The solutes were dissolved in the eluent (1% each). The three stages of the run, formation of a wide starting zone (frontal chromatography), elution in the first direction and elution in the second direction, are illustrated in Fig. 3.

As illustrated previously[9], the movement of the zones in the sandwich tank with continuous elution is regular and permits estimation of the retention volumes of the wide zones leaving the chromatographic bed. Considering the analogy between equilibrium–sandwich TLC in BN chambers and in column HPLC, the behaviour of the zones in preparative column chromatography can be predicted taking into account the weight of adsorbent, column length and differences in the surface areas of the adsorbents, if any.

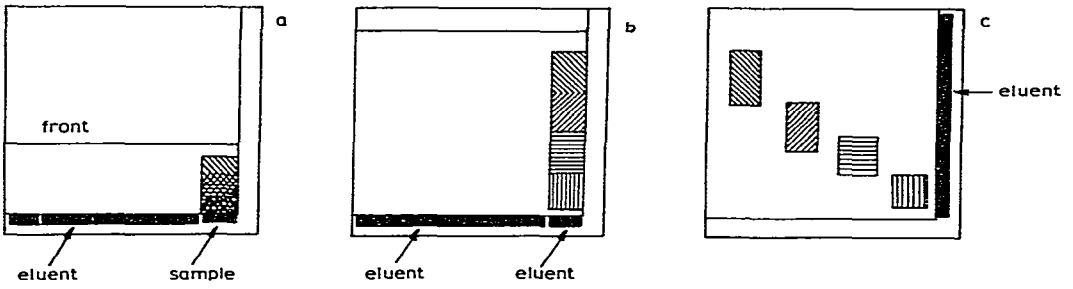

Fig. 3. Two-dimensional elution of a large sample volume: a, delivery of 25 μl of sample solution (3% solution of azobenzene, dimethylaminoazobenzene, Sudan IV and Disperse Red 73 in the eluent 50% benzene–heptane); b, elution in the first direction; c, elution in the perpendicular direction.

The pilot TLC experiments, which can also be carried out on narrow strips of TLC plates (*e.g.*, 10 × 1 cm), can provide information about essential parameters of the process, such as eluent strength and qualitative composition required to secure good selectivity, maximal concentration and sample loading (capacity of the adsorbent), irreversible adsorption and whether gradient elution is necessary. The loss of materials in these rapid preliminary experiments is minimal.

Sometimes the formation and separation of zones in the adsorbent layer can be observed directly (coloured solutes) or intermittently under UV light (which, however, may cause decomposition of certain solutes). This is difficult to achieve in column chromatography —even in glass columns the zones at the walls are distorted owing the the wall effect.

The pilot experiments may be especially useful in the separation of complex samples such as plant extracts. Preliminary equilibration of the layer with the eluent may be necessary for low-percentage solvent systems[12].

REFERENCES

1 J. J. DeStefano and J. J. Kirkland, *Anal. Chem.*, 47 (1975) 1193A.
2 J. H. Knox, *Course Manual of the Chemical Society Residential School*, London, 1980, p. 61.
3 L. R. Snyder and J. J. Kirkland, *Introduction to Modern Liquid Chromatography*, Wiley-Interscience, New York, 2nd ed., 1979, p. 615.
4 S. Hara, *J. Chromatogr.*, 137 (1977) 41.
5 S. Hara and K. Mibe, *J. Chromatogr.*, 66 (1972) 75.
6 D. Jaenchen, in A. Zlatkis and R. E. Kaiser (Editors), *HPTLC —High Performance Thin-Layer Chromatography*, Elsevier, Amsterdam, 1977, p. 141.
7 R. E. Kaiser and E. Oelrich, *Optimierung in der HPLC*, Hüthig, Heidelberg, 1979, p. 149.
8 E. Soczewiński and J. Kuczmierczyk, *J. Chromatogr.*, 150 (1978) 53.
9 E. Soczewiński, J. Kuczmierczyk and B. Psionka, *J. Liquid Chromatogr.*, 3 (1980) 1829.
10 E. Soczewiński and T. Wawrzynowicz, *Chromatographia*, 11 (1978) 466.
11 E. Soczewiński and W. Maciejewicz, *J. Chromatogr.*, 176 (1979) 247.
12 T. Wawrzynowicz and E. Soczewiński, *J. Chromatogr.*, 169 (1979) 191.