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NEW PLANAR LIQUID CHROMATOGRAPHIC TECHNIQUE: OVERPRESSURED THIN-LAYER CHROMATOGRAPHY

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SUMMARY

A novel planar liquid chromatographic technique using a pressurized ultramicro chamber (PUM chamber) has been developed. The sorbent layer is completely covered by a membrane under external pressure, so that the vapour phase above the layer is virtually eliminated. Solvent is admitted under pressure by means of a pump system.

The main advantage of this technique, termed overpressured thin-layer chromatography (TLC), is the substantially shorter time required for separation than in classical column chromatography and TLC, with velocities of the mobile phase about as stable as in high-performance liquid chromatography, and aggressive reagents being applicable as in classical TLC. The method appears to be suitable for the accurate modelling of column chromatographic methods.

INTRODUCTION

The rapid progress of column chromatographic (CC) methods in recent years has yielded a potent means for the efficient and rapid separation of organic substances. The various gas chromatographic methods that are available cover are applicable to only about 20% of all organic substances, and therefore the necessity arose to develop further classical liquid column chromatography, which, owing to the large sorbent particles applied, is much less rapid and efficient than gas chromatography (GC). The most spectacular achievement in this field was the development of high-performance liquid chromatography (HPLC)[1,2].

In addition to CC methods, planar methods, first paper chromatography[3] and subsequently, in the last 20 years, various methods of thin-layer chromatography (TLC) proved very useful, especially for micro-chromatographic studies[4]. The well known advantages of TLC are firstly its simplicity and great economy in time and materials; secondly, the separated substances can be detected visually, often by means of selective and specific colour reactions; thirdly, the technique allows the simultaneous study of a large number of samples; and finally, aggressive reagents may be applied. However, the TLC also has drawbacks, especially the limitation of the number of separations by the length of the plate, and the long time required for development. More recent demands made of planar chromatographic techniques include higher resolution, stable velocity of the mobile phase and serviceability for accurate column modelling.

The greatest advance in this field was the development of high-performance thin-layer chromatography (HPTLC). This method, using a U-chamber with a fine-particle sorbent layer, affords better resolutions than classical TLC[5,6]. However, very efficient separations can be achieved with HPTLC only with short running distances, as the performance of the liquid feeding system is insufficient to force the solvent over longer distances.

The relationship between the mobile phase and the stationary phase is different in TLC and CC, as the column is "closed" whereas the layer is "open", and the forces driving the mobile phase are also different. In earlier work, we developed a modified closed TLC chamber to study the relationship between classical TLC and conventional CC[7]. In this chamber [termed an ultramicro (UM) chamber] the sorbent layer is covered by a glass plate.

To effect further improvements, it appeared logical to attempt to develop a planar chromatographic method that would combine the advantages of conventional TLC and modern HPLC. Our recent work demonstrated that the combination of TLC and HPLC in a specially designed, totally closed, pressurized ultramicro chamber (PUM chamber) indeed yields important advantages regarding separation efficiency, time requirement, resolution, potential for column modelling, etc., compared with classical TLC.

In this paper we describe this new planar chromatographic method, termed overpressured TLC (OPTLC) and its advantages and prospects in comparison with other planar chromatographic methods.

EXPERIMENTAL

Apparatus

Ultramicro chamber (UM chamber)[7]. The sorbent layer is covered by a thin glass plate that is slightly smaller than the supporting glass plate, so that, *e.g.*, a strip of 1.5 cm of sorbent remains free. The two plates are clamped together and placed into a large Desaga tank, and the run is carried out in the usual manner. Once the desired distance has been reached, the plates are dismantled and detection of spots is carried out as usual in TLC.

Pressurized ultramicro chamber (PUM chamber). A circular-type PUM chamber is shown schematically in Fig. 1. (A linear-type PUM chamber based on essentially similar principles to the circular type is under development). Separation takes place on the sorbent layer F supported by the glass or plastic plate G. The upper side of the sorbent layer is covered completely by a plastic membrane I, which fits tightly to its surface owing the gas pressure within the space bordered by the membrane and the upper support block H and sealed by the O-ring E, so that this space acts as a gas pressure pad. The gas inlet C and the pressure gauge A, together with the syringe J

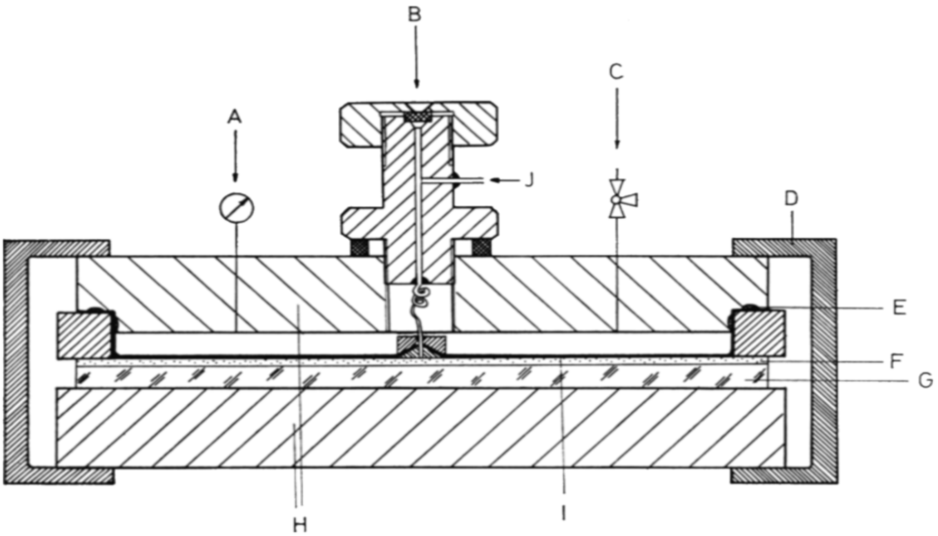

Fig. 1. Cross-section of circular-type PUM chamber.

for solvent admission and the inlet B for introducing the sample under pressure, are installed in the upper block H. The diameters of the upper and lower blocks H are 230 and 235 mm, respectively. The area of the sorbent layer may vary, *e.g.*, 100 × 100 mm or 200 × 200 mm standard sizes can be used. The upper and lower support blocks are locked together with several clamps D. Fig. 2 is a top view of a circular-type PUM chamber with a 100 × 100 mm HPTLC plate.

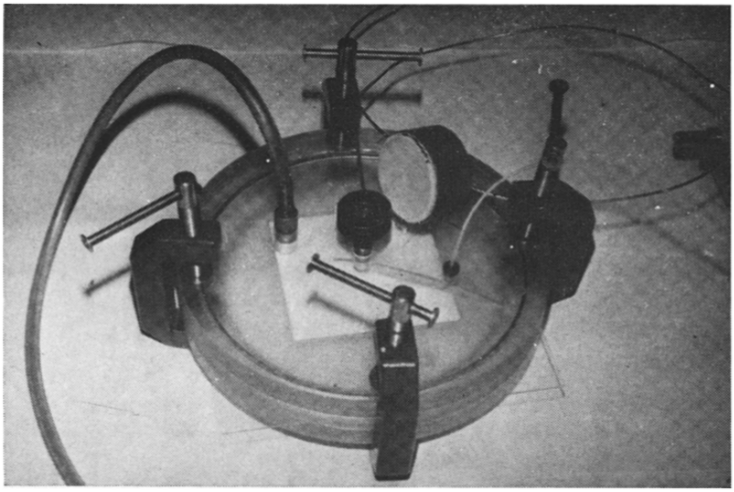

Fig. 2. Top view of the circular-type PUM chamber in operation.

In situ quantitative evaluation of the developed plates was accomplished with a Zeiss PMQ II chromatogram spectrophotometer. A Desaga chromatography tank was used. The solvent was pumped into the layer with an Infumator (Kutesz, Budapest, Hungary).

Chromatographic plates

Pre-coated silica gel glass plates ("conventional chromatographic plates") without an indicator, 200 × 200 mm, with a layer thickness of 0.25 mm, and silica gel 60 HPTLC plates with a fluorescent indicator, 100 × 100 mm (Merck, Darmstadt, G.F.R.) were used without prior washing.

Chemicals

All chemicals were of analytical-reagent grade and were used without further purification.

RESULTS AND DISCUSSION

In our earlier UM chamber, with the sorbent layer covered by a glass plate[7], the free volume of the chamber results from the interstices between the particles and the glass cover. A significant feature of this design is that the cover plate is not immersed in the solvent, and hence no capillary effects will interfere.

In the PUM chamber the free volume is even smaller than in the UM chamber, as the plastic membrane covering the sorbent layer is under external pressure, and hence fits closely to the unevennesses in the surface of the sorbent layer. The design of both circular- and linear-type PUM chambers is such that the pressure under which the solvent is admitted to the sorbent layer can be varied. The external pressure on the layer exerted by the gas pad must, however, always exceed the pressure of solvent admission.

Fig. 3 illustrates the quantitative evaluation of a separation of six lipophilic dyes in a circular PUM chamber at two different solvent flow-rates.

Comparisons of the overpressured and conventional TLC techniques were carried out with linear-type pressurized chambers, using both normal and HPTLC plates. Data obtained with three dyes in different chambers are shown in Fig. 4. It can

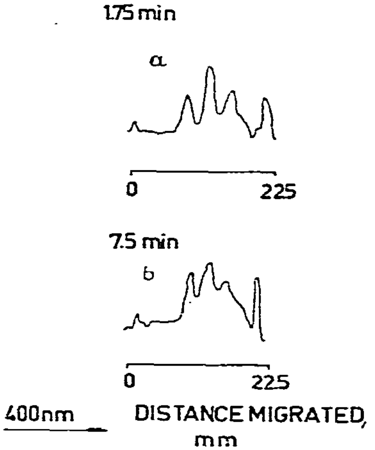

Fig. 3. Scans of the OPTLC separation of six lipophilic dyes on HPTLC silica gel 60, using a circular-type PUM chamber. $Z_f = 22.5$ mm. Mobile phase: methylene chloride. Flow-rate: (a) 60 cm^3/h; (b) 30 cm^3/h. Recorder chart speed: 150 mm/min. External pressure on membrane: 0.25 MPa.

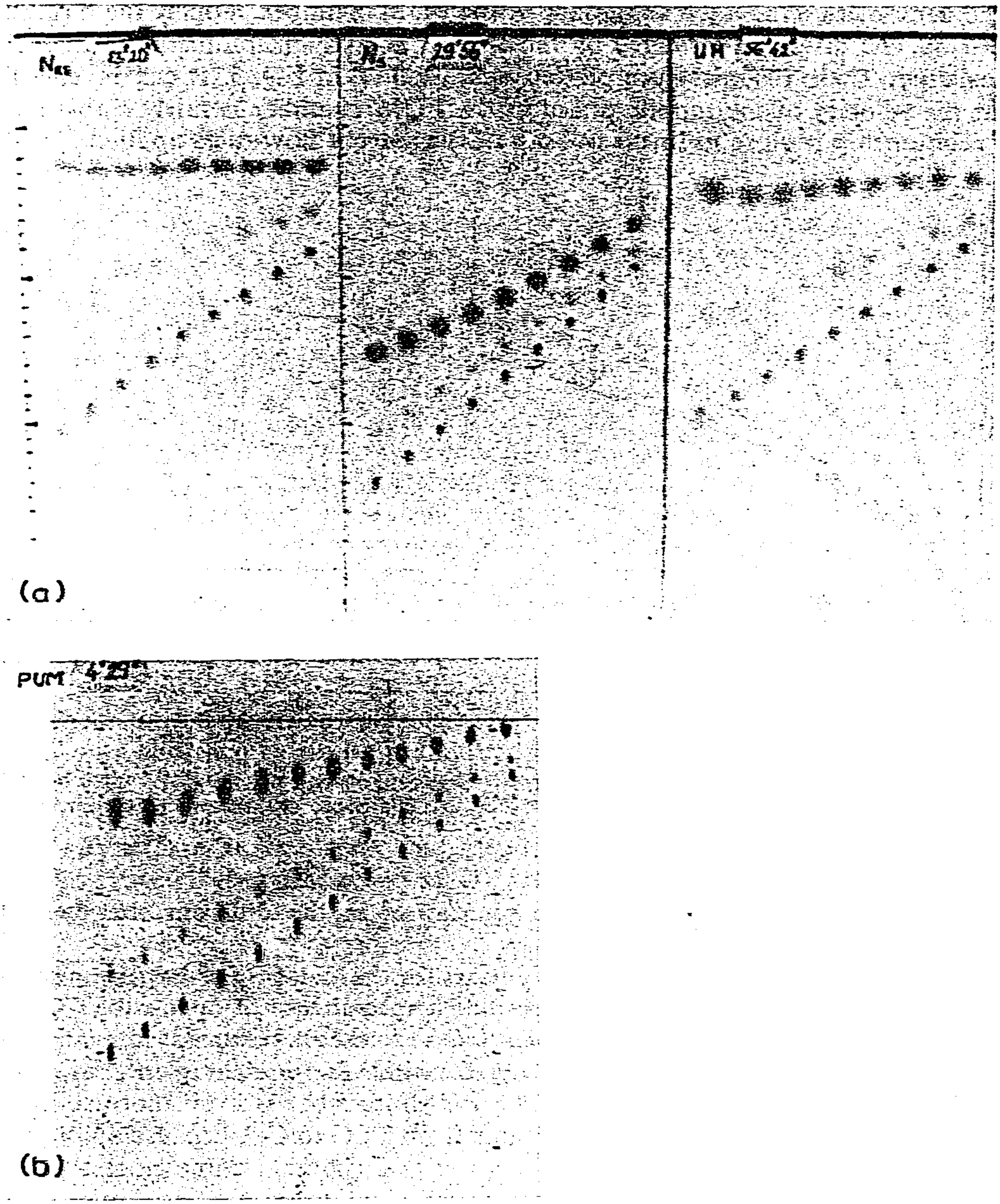

Fig. 4. Separation of three dyes in different chambers using silica gel 60 at different starting points (s_0). Mobile phase: methylene chloride. Z_f = 150 mm. (a) N_{us} = normal unsaturated chamber, 83.3 min; UM = ultramicro chamber, 56.7 min; N_s = normal saturated chamber, 29.9 min. (b) PUM = pressurized ultramicro chamber, 4.5 min; flow-rate of solvent, 80 cm^3/h; external pressure on membrane, 0.25 MPa.

be seen that the separation in the PUM chamber is similar to that in normal and UM chambers, but the diameter of the spots is much smaller, owing to the lower degree of diffusion. The results shown were obtained with conventional silica gel 60 (Merck) plates. Resolution on fine-particle HPTLC plates was obviously even better.

To demonstrate the main advantage of the PUM chamber, namely the rapidity of separation, the distances travelled by the solvent at different moments of the run were measured. These distances are plotted against time in Fig. 5. It can be clearly seen that the velocity of the mobile phase in the PUM chamber is fairly stable and and the relationship is close to linear at both of the solvent flow-rates applied.

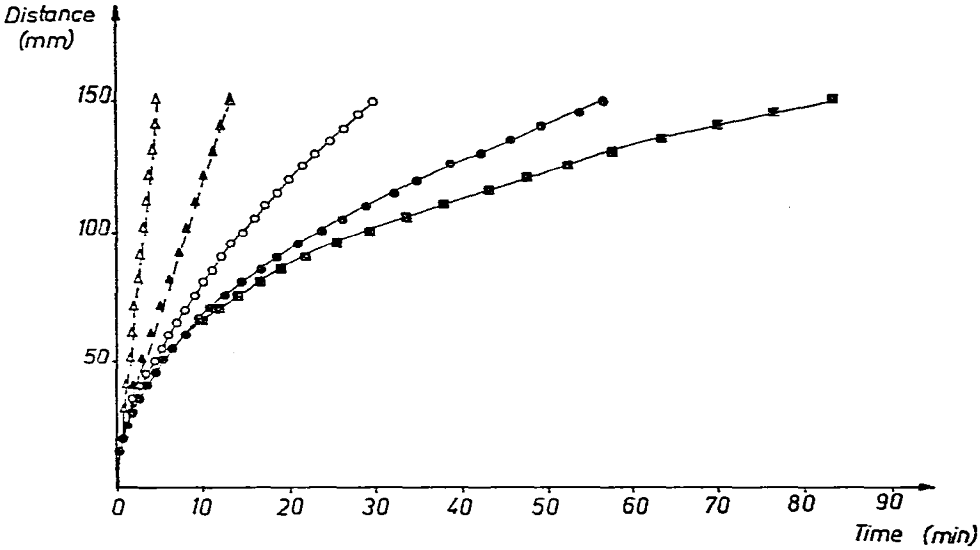

Fig. 5. Distance travelled by the solvent on silica gel 60 plate *versus* time. △, PUM chamber, 90 cm^3/h; ▲, PUM chamber, 60 cm^3/h; ○, normal saturated chamber; ●, ultramicro chamber; ■, normal unsaturated chamber.

The quantitative evaluation of the separation in the different chambers is shown in Fig. 6.

Classical CC has been used for many years for the isolation of larger amounts of various substances. TLC can be considered as a special case of open-tube CC, so that the close relationship between "open" and "closed" columns immediately becomes obvious. Our UM chamber has proved suitable for modelling column chromatography to find optimal conditions[7]. The PUM chamber is even more suitable for studies on the transfer of optimal conditions from TLC to CC, because among all available TLC chamber systems, the conditions in this chamber come closest to the conditions in column chromatography. Thus, OPTLC might be used as a pilot-scale technique for HPLC. We consider this to be an important advantage.

In conclusion, the results indicate that in planar liquid chromatography, with its advantages such as visual evaluation and the applicability of aggressive reagents, OPTLC appears to yield results comparable to those achieved in GC and HPLC with respect to reproducibility. In comparison with conventional TLC and partly even HPLTC, OPTLC has the advantage, which is of particular interest for routine tests, that rapid and effective separations of mixtures of various organic and inorganic substances are effected 5–20 times faster. The decreased time of separation yields better resolutions, but must be compensated or overcompensated by the use of smaller

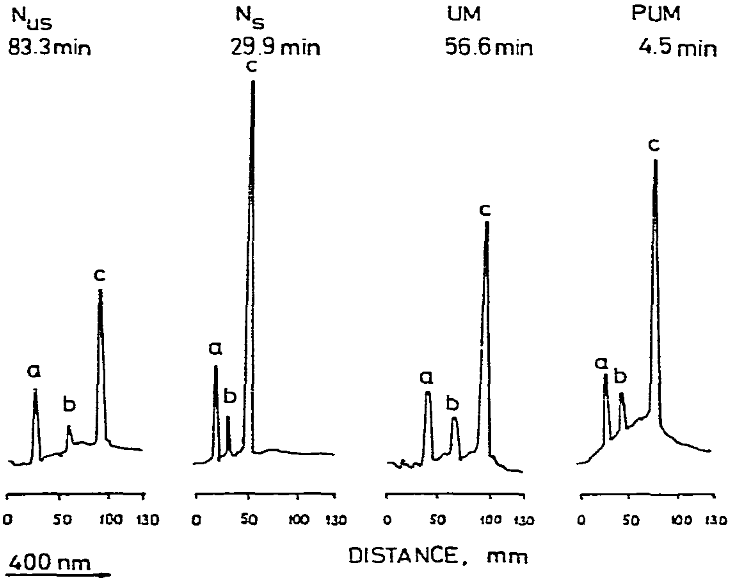

Fig. 6. Scans of the separation of three dyes in different chambers using silica gel 60 plate. Mobile phase, methylene chloride; recorder chart speed, 25 mm/min. a = Indophenol; b = Sudan G; c = Butter Yellow.

and more uniform sorbent particles. Hence the sorbent layer and its particle size, uniformity and quality are basic factors in OPTLC.

OPTLC allows one to use one plate for testing a large number of samples. It appears to be applicable to modelling CC and HPLC conditions.

Eventually it should be possible to increase solvent flow-rates further. It may also be expected that resolution in OPTLC might be improved by applying higher temperatures.

ACKNOWLEDGEMENT

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REFERENCES

1 L. R. Snyder and J. J. Kirkland, *Introduction to Modern Liquid Chromatography*, Wiley-Interscience, New York, 1974.
2 H. Engelhardt, *Hochdruck-Flüssigkeits-Chromatographie*, Springer, Berlin, Heidelberg, New York, 1975.
3 M. Hais and K. Macek, *Paper Chromatography*, Academic Press, New York, 1963.
4 E. Stahl (Editor), *Thin-Layer Chromatography, A Laboratory Handbook*, Springer, Berlin, Heidelberg, New York, 1st ed., 1962; 2nd ed., 1967.
5 R. E. Kaiser (Editor), *Einführung in die Hochleistungs-Dünnschicht-Chromatographie*, Institute of Chromatography, Bad Dürkheim, 1976.
6 A. Zlatkis and R. E. Kaiser (Editors), *HPTLC—High Performance Thin-Layer Chromatography*, Elsevier, Amsterdam, Oxford, New York, and Institute of Chromatography, Bad Dürkheim, 1977.
7 E. Tyihák and G. Held, in A. Niederwieser and G. Pataki (Editors), *Progress in TLC and Related Methods*, Vol. II, Ann Arbor Sci. Publ., Ann Arbor, Mich., 1971.