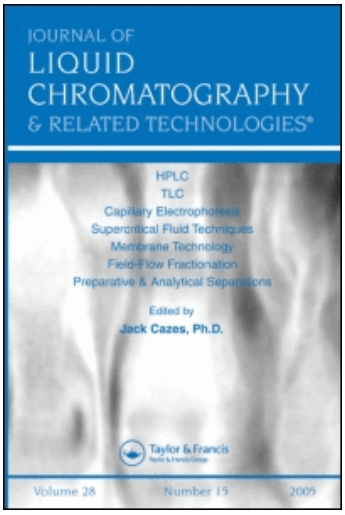

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CIRCULAR-DEVELOPMENT WITH OVERPRESSURED THIN-LAYER CHROMATOGRAPHY

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ABSTRACT

A novel type modification of circular thin-layer chromatography has been developed, in which the layer is tightly covered by a membrane, eliminating the vapour phase over the sorbent layer. The developing solvent is pumped through the apparatus.

INTRODUCTION

The two principal ways of performing chromatographic separation are the column method (closed system) and the planar technique (open system). When the same sorbent and solvent are used, the separation gives identical results in the two systems because the main separation process is the same. Otherwise, the results are different because in the planar technique the mobile phase is influenced by the vapour phase and different forces are operating

in the movement of the mobile phase. Both methods have their own advantages. The planar method first appeared as paper chromatography, but now is almost exclusively thin-layer chromatography (TLC), which has almost totally replaced paper chromatography. TLC has the same advantages as paper chromatography. Several sample spots can be investigated simultaneously by TLC and they can be compared quantitatively and qualitatively. The TLC technique does not require complicated and expensive instruments, and the separated spots can be detected with specific reagents. Ready-made TLC plates are manufactured and a wide choice of developing solvents and solvent-systems (individual strong acids and bases) can be used.

In TLC, as a consequence of the vapour-liquid equilibrium, the moving multi-component system of developing solvent is not the same as the developing solvent in column chromatography (CC). In CC the sample is usually applied after an exhaustive prewash (equilibration or conditioning) of the chromatographic column with a suitable solvent. The sorbent in TLC is not presaturated with the solvent used, but only with its vapours. As a consequence, in TLC multiple solvent front may occur. Tyihák and Held have discussed (2) these basic differences between CC and TLC, and developed the UM-chamber for TLC to cope with the problems arising when the results of TLC are transferred to CC and vice versa. In the UM-chamber (UM = ultramicro) the thin-layer of sorbent is covered by a glass plate, so that the vapour phase (over the sorbent layer saturated with the developing solvent) is greatly reduced in volume. When TLC chamber system was reduced to the UM-scale, there was a drastic change in behaviour even in the case of a single-component system. Our work on development of thin-layer chromatography under pressure in the pressurized ultramicro chamber (PUM-chamber) arose from the results of chro-

matography with the UM-chamber and from the relatively new technique of HPLC.

Some theoretical aspects of the effect of solvent velocity and of particle size on efficiency have been studied (3). For CC the efficiency can be given by the equation (3)

$$H = \lambda \, u^{\gamma} \, d_p^{\beta}$$

where H is the plate-height, d_p the particle diameter, u the carrier-liquid velocity, λ, β and γ are constants ($\beta \cong 1.8$; $\gamma \cong 0.4$). This rule shows that under the conditions of column liquid chromatography even a drastic increase in flow rate does not affect the resolution of the column if the particle size is reduced accordingly. Even a 100-fold increase in flow rate can be counterbalanced by a 3-fold decrease in the average particle size. The same rule holds for HPTLC; good separations can be achieved in a short time by simultaneously decreasing the particle size and the distance of development.

The practice of HPTLC realized two different kinds of solvent supply, namely the developing solvent system was transported by capillary action or a pump (4, 5) but in both cases the TLC or HPTLC plates that is the sorbent layers were not tightly covered, so the speed of solvent supply was restricted very closely to the value decided by the movement of solvent front. More exactly, any kind of solvent supply is only serving to compensate the amount of solvent, consumed by the capillary forces made progress. If the solvent supply were larger, the rest of solvent would move over the

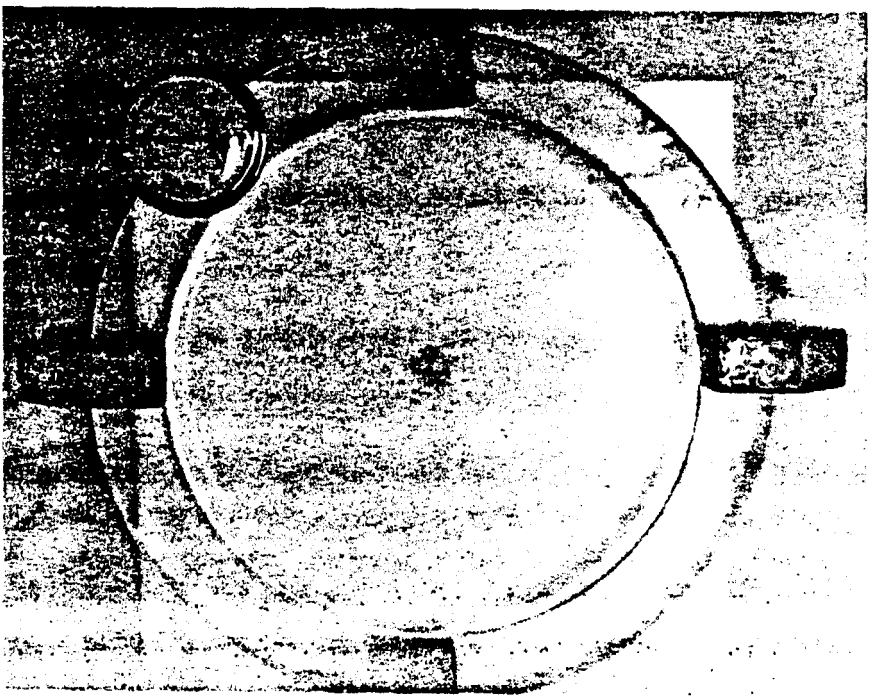

FIGURE 1. OPTLC of a dye-mixture in PUM-chamber by dichloromethane on Silicagel plate, 200 x 200 mm. a. The very beginning of development. The test-mixture to be separated had been spotted and the PUM-chamber had been set up. This is the 0 time of development.

sorbent layer; if the solvent supply were less, the shape of front would deform.

EXPERIMENTAL

Apparatus:

The equipment is a pressurized circular type of PUM-chamber (6). The thin-layer (traditional type or one suitable for HPTLC) is sandwiched between a glass

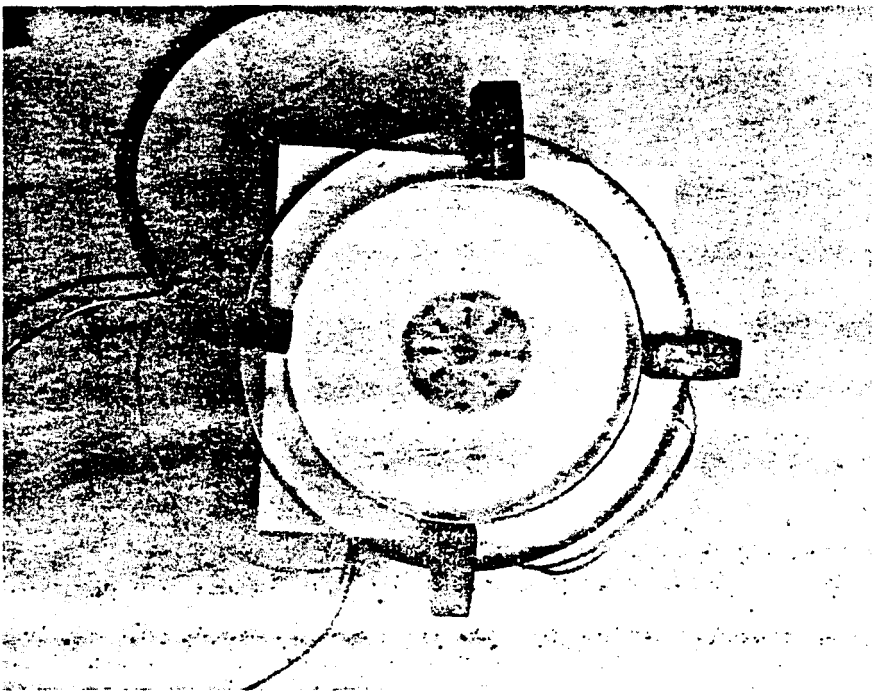

FIGURE 1b. After 90 seconds the radius of the circle is 33 mm, the substances have started to separate.

plate or plastic sheet, and a membrane or foil, which is tightly pressed onto the thin-layer by gas pressure in the space between the membrane and the upper support block. The upper support block carries the gas inlet, the inlet for the developing system, a pressure gauge and an O-ring for securing the membrane. The upper and lower support blocks serve to adjust the distance between the thin-layer and the covering membrane which is under pressure. The dimensions are: upper and lower blocks 230 mm in diameter; membrane 200 mm diameter. The two

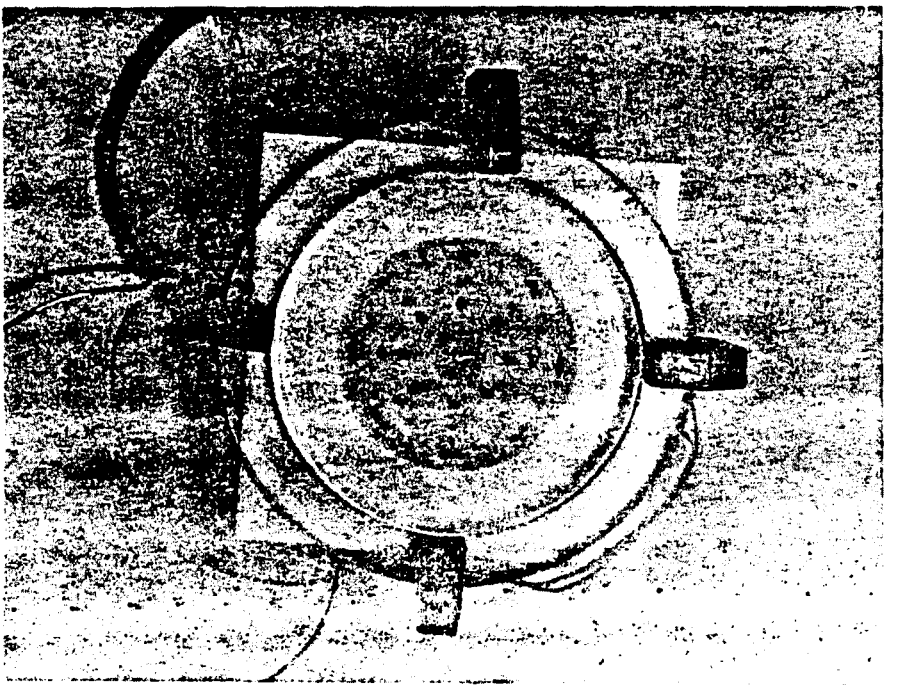

FIGURE 1c. After 180 seconds the radius of the circle (distance of solvent front) is 55 mm.

blocks also serve as an outer holder, and the whole is held together with four srew-clamps. The process of OPTLC (overpressured thin-layer chromatography) can be seen in Fig. 1. To give a better view, the PUM-chamber was turned over, so that the bottom was uppermost. Fig. 1a shows the sample after the spotting and before any development, and Figs. 1b, 1c and 1d show the different phases of the development.

The development is complete when the solvent front has travelled far enough to give adequate separation.

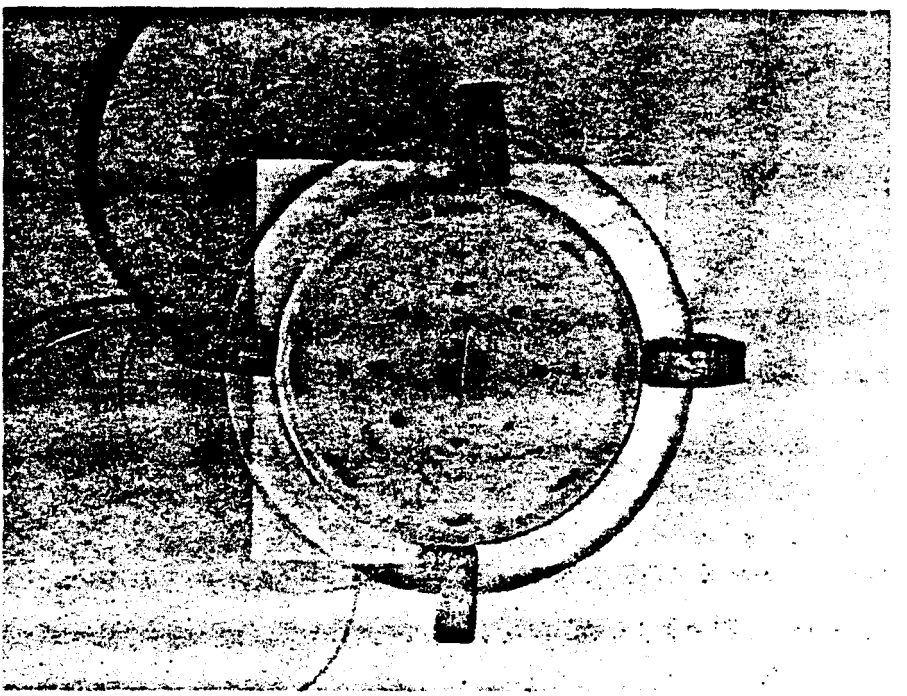

FIGURE 1d. After 290 seconds the distance of solvent front is 85 mm, the development has been finished.

The chamber can the be opened and the separated substances detected in the usual way.

We chose the circular system for the following reasons:

1/ The instrument is simpler. The solvent front of the developing solvent - released at a single point - is naturally circular. A linear front needs a special feed arrangement.

2/ The circular technique makes it possible to apply the sample during the development process, by means of

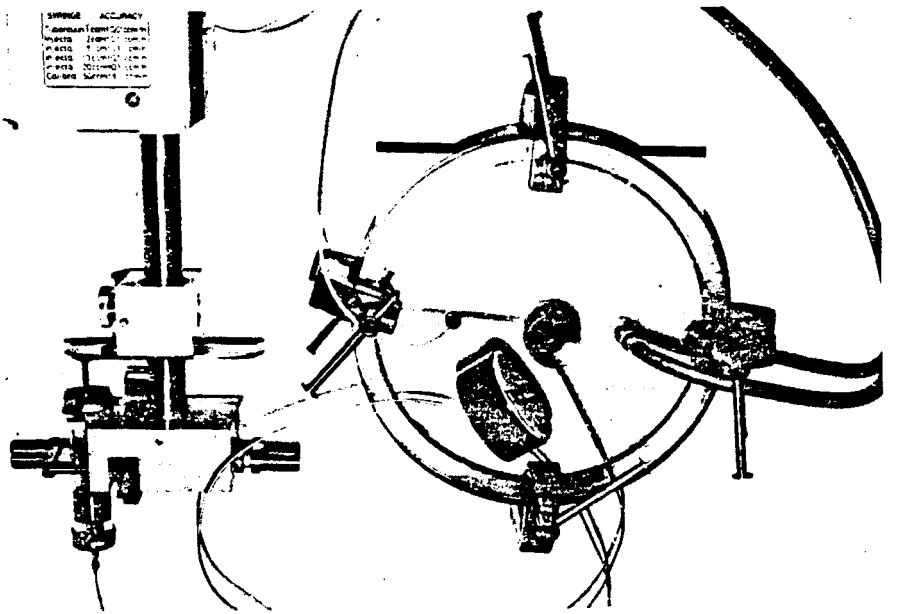

FIGURE 2. Applying the sample during the development process, the separated components appear in concentric rings.

an HPLC injection port, such an experiment can be seen in Figs. 2 and 3.

Naturally, the linear technique of OPTLC can be preferred when the results of column technique are to be studied in the case of TLC or OPTLC. Only the linear technique will be able to model the theoretical and practical rules - concerning:

1/ resolution vs. flow rate,

2/ resolution vs. particle size,

3/ resolution vs. plate dimension.

Although our circular set up (Fig. 4) has solved some of these problems, complex interactions like resolution, the effect of covering plate, temperature, length

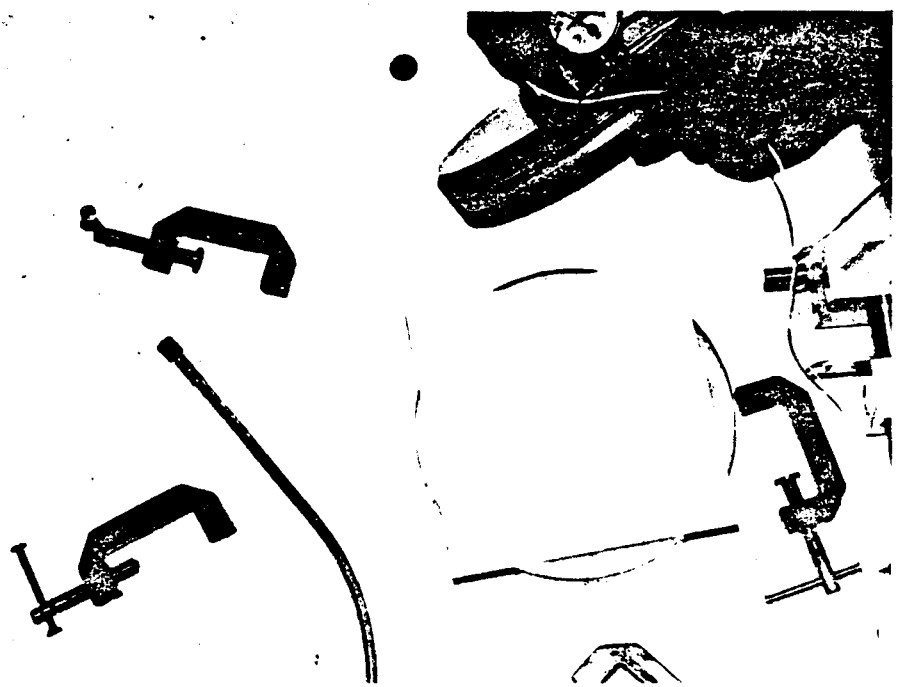

FIGURE 3. After setting out the PUM-chamber, the separated substances can be observed or visualized by colour reagents.

and width of chromatogram, flow rate, etc. can be investigated only in a system with the arrangement for linear front.

DISCUSSIONS

In this work our aim was to define the basic concept of overpressured thin-layer chromatography with circular technique. The thin-layer of sorbent is tightly covered by a membrane which is kept under pressure. By using the membrane, the vapour phase is eliminated

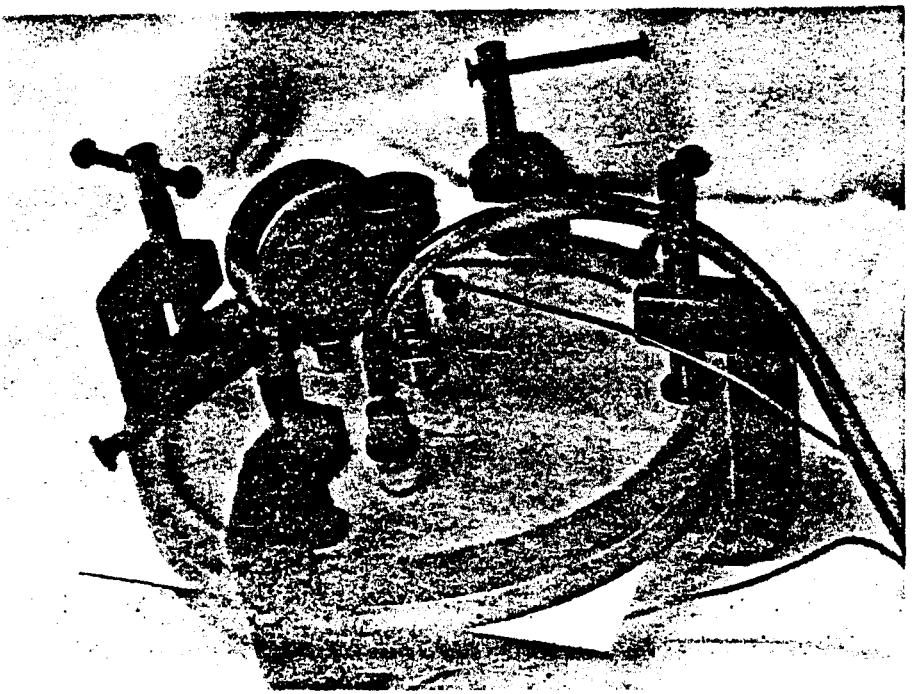

FIGURE 4. The picture of PUM-chamber (pressurized-ultra-micro-chamber).

above the thin-layer. At the same time the whole set up of the sorbent layer can exist as an openable planar column. The OPTLC has combined several advantages of both CC and TLC, namely:short time of chromatography, reproducibility, possibility of applying very fine particles, sampling during development, separation of several samples simultaneously and detection of spots by colour reagents.

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