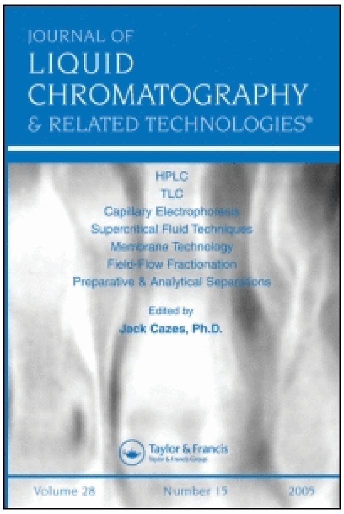

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OVERPRESSURED THIN-LAYER CHROMATOGRAPHY AND ITS APPLICATIONS

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In recent years, a rapid progress can be observed both in column and planar liquid chromatographic techniques. In the field of liquid column chromatography the most spectacular achievement was the development of high-performance liquid chromatographic /HPLC/ systems by means of several special instruments and sorbents /1, 2/. As regards planar techniques, the most significant break-through is the development of highperformance thin-layer chromatography /HPTLC/ /3/ based on the application of fine-particle sorbents. Both techniques proved to be very useful in many fields of chemical analysis, although the use of the latter is more restricted, mainly to micro chromatographic studies.

It appeared, thus, logical to develop such a planar liquid chromatographic technique that would approach HPLC as regards stability and standardization of chromato-

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graphic conditions but still would conserve the well known advantages of TLC and HPTLC such as visual evaluation, the use of selective and specific reagents, simultaneous study of a large number of samples and topographic identification of the separated substances.

The pressurized ultramicro chamber

Earlier, we developed a closed TLC chamber in order to study the relationship of conventional TLC and column chromatography /CC/ /4/. In this simple chamber /termed as ultramicro /UM/ chamber/, the sorbent layer is covered by a glass plate so that the end of the cover plate is not immersed into the solvent, in order to eliminate interfering capillary effects. The elimination of solvent vapour was first realized by this chamber. However, the advantages of a totally closed chamber were realized only later, when we developed the pressurized UM chamber /PUM chamber/ /5-8/.

The essential feature of PUM chamber of circular and linear types is that the sorbent layer is completely covered with a flexible membrane under an external pressure so that, in the closed chamber, a layer of water /a water-cushion/ forms between the Plexiglass cover-plate and the flexible and fixed membrane, and the vapour space above the layer is virtually eliminated. Solvent admission under overpressure has been solved by means of a pump system /Fig. 1/.

By adjusting the solvent, by means of a pump system in PUM chamber, it is possible to separate substances with

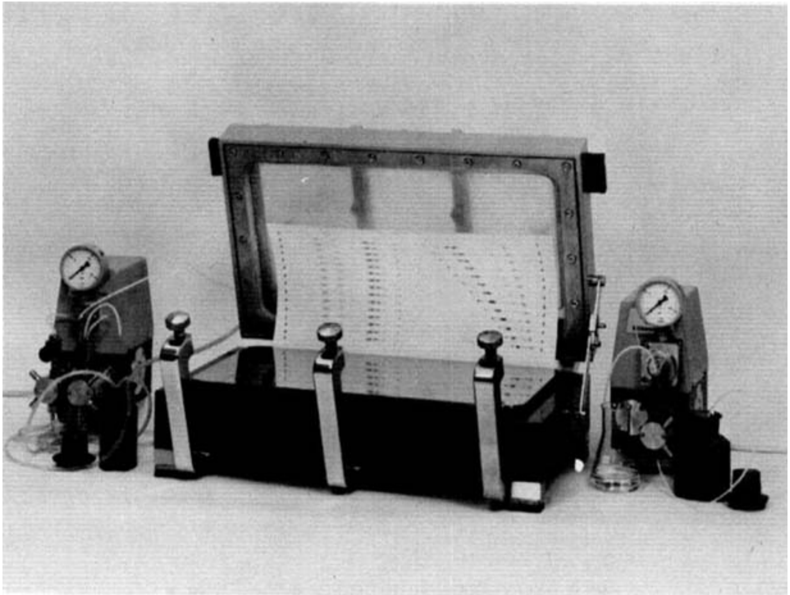

Fig.1

Top views of the CHROMPRES 10 chamber /LABOR Instrumental Works, Esztergom-Budapest, Hungary/ in open form after a two-directional separation of dyes.

optional development distances. This active form of the planar liquid chromatography is the so-called overpressured thin-layer chromatography /OPTLC/ which integrates the advantages of the classical TLC /9-10/, modern HPTLC /11-12/ and HPLC /1-2, 13/.

In the PUM, the external pressure on the flexible cover-membrane must always be higher than the input pressure of the solvent. The input pressure of the solvent increases linearly with increasing solvent migration distance. An increase in the solvent flow velocity always results in higher input pressures, which must be taken into account by choosing an appropriate external pressure on the membrane /14/.

The PUM chamber constitutes one system with the water-dosing pump of larger delivery and the eluent dosing pump of smaller delivery.

In the PUM chamber of linear type, linear migration of the solvent front is achieved by impregnating the edges of the layer and either placing a narrow plastic sheet on it or by making a narrow channel in the layer before attaching the solvent inlet. Paraffin or various plastic dispersions can be used for impregnation. In one-directional OPTLC, three edges of the plate are impregnated. Two-directional separation can, however, be carried out by impregnating only two edges of the chromatoplate and attaching the solvent inlet to the middle of the chromatoplate /Fig. 2/. This developing system is especially suitable for the efficient separation of a large number of samples.

Triangular /15/, anticircular /16/ and circular /3/ separations can be achieved, simply, by special impregnation of the respective shape.

By changing the inlet openings of the eluent, possibility is equally provided for one- or two-directional linear, circular, triangular /anticircular/ running.

It follows from this brief account that the succesful application of OPTLC requires a special instrument and precoated /impregnated/ chromatoplates from various inorganic and organic sorbents. Manufacturing of both products

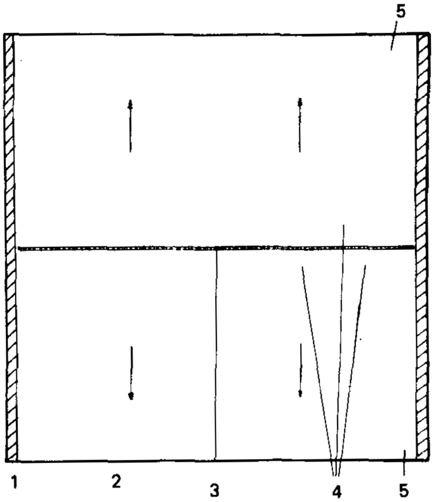

Fig. 2

Special pre-coated plate /e.g. SILPRES N/ to OPTLC for two-directional development. Schematic drawing 1 impregnated edges; 2 channel freed from sorbent; 3 solvent inlet; 4 place of samples; 5 front of solvent

is in progress, former at LABOR Instrumental Works /Esztergom-Budapest, Hungary/, latter at REANAL Fine Chemicals /Budapest, Hungary/ in cooperation with LABOR Instrumental Works.

Theoretical aspects

In classical TLC the distance between the solvent front and the solvent source /Z_f/ is related to separation time /t/ by a quadratic equation /$Z_f^2 = k.t$/ /17/ where the constant

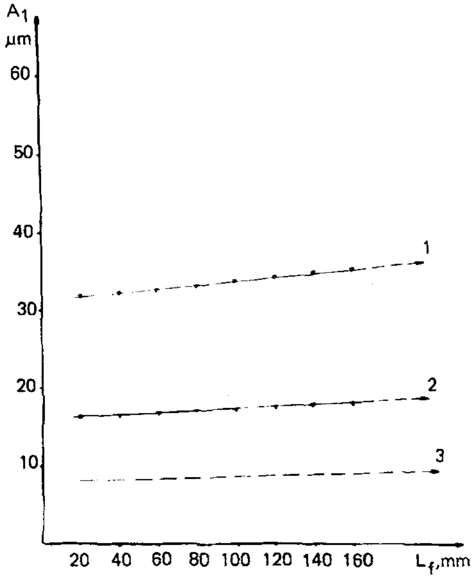

Fig. 3

Variation of the average plate height /H/ versus development length

Mobile phase, methylene chloride; investigated substance, Butter Yellow; external pressure on the membrane, 1.0 MPa; flow-rate of solvent, 20 cm^3/h; calculation of average plate height values according to Guiochon and Siouffi /18/

1 CHROMPRES 10 chamber, SILPRES N-1 experimental chromatoplate /d_p= 10-11 /um/; 2 CHROMPRES 10 chamber, SULPRES N-2 /d_p= 5-6 /um/; 3 CHROMPRES 10 chamber, SILPRES N-3 /d_p = 2-3 /um/

/k/ depends on the nature of stationary and mobile phases and also on sorbent particle size. The same quadratic is valid also in HPTLC /3/.

On the contrary, the law of linear OPTLC development can be described by a simple equation:

$$Z_f = k^{OPTLC} \cdot t$$

The above relationship is valid not only in liquid-solid but also in liquid-liquid systems as well as in the "reversed phase" systems. In the circular OPTLC, however, the area to be wetted increases quadratically with the linear movement of the phase front. It is obvious from this fact that in circular OPTLC the classical law of TLC development is valid.

Optimum performance in linear OPTLC can be obtained with sorbent layers of very fine-particle and narrow size distribution as in HPLC. It is fact that the average plate height values in linear OPTLC diminish in direct proportion to decreasing of the particle size /Fig. 3/.

Due to the constant flow rate of the eluent in the linear OPTLC the theoretical plate number /N/ increases in a direct proportion to the distance /x/ while in the classical, normal saturated /N_s/ and unsaturated /N_{us}/, as well as in the ultramicro /UM/ chambers, an oppositional tendency dominates /Fig. 4/.

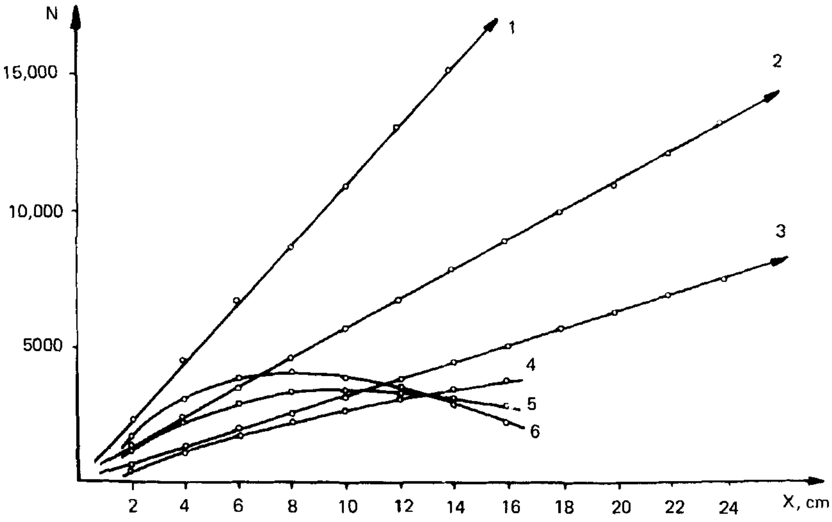

Fig. 4

Variation of the theoretical plate number /N/ values with the running distance /x/ in various chamber systems using various silica gel particle size Eluent, methylene chloride; materual, Butter Yellow 1 CHROMPRES 10, d_p = 2-3 µm; 2 CHROMPRES 10, d_p = 5 µm; 3 CHROMPRES 10, d_p = 11 µm; 4 N_s, d_p = 5 µm; 5 UM, d_p = 5 µm; 6 N_{us}, d_p = 5 µm

Application

A close fundamental relationship exists between the two alternatives of planar liquid chromatography: traditional TLC /and the modern HPTLC/ and OPTLC. There is, however, an essential difference between the two main techniques: in TLC /and in HPTLC/ a vapour phase is established above the sorbent layer in the normal /N/ chamber. In the sandwich /S/ chamber and also in so-called U-chamber /3/. The characteristics of the vapour phase essentially depend on the composition of the solvent mixture and on the chamber system.

Starting from the fact that there is no vapour phase above the sorbent layer in OPTLC, it follows that one-component solvents can be conveniently used for separation. In the case of solvent mixtures consisting of two or more components, there are two possibilities: one is a normal separation using one-component solvents. The other possibility consists of demixing of the solvent mixture on the sorbent layer; in this case fronts, zones will build up. In OPTLC, therefore, in some cases we have to choose a new eluent system from the solvent mixtures, which is suitable for the efficient separation of the given substance group without solvent demixing.

According to our preliminary results, linear and circular OPTLC methods are suitable for the separation of various groups for organic substances, similarly to TLC and HPTLC, but with reasonably shorter separation times. Owing to the short separation times and the stable flow velocity, resolution values, especially those obtained with fine-particle plates, are excellent even in the case of longer solvent migration distances /e.g. 200-350 mm/.

Fig. 5 illustrates the efficiency of the separation in PUM chamber of linear type. It can be seen that the diameter of the spots is small, owing to the low degree of diffusion. It is obvious that the decreased time of separation yields better resolution.

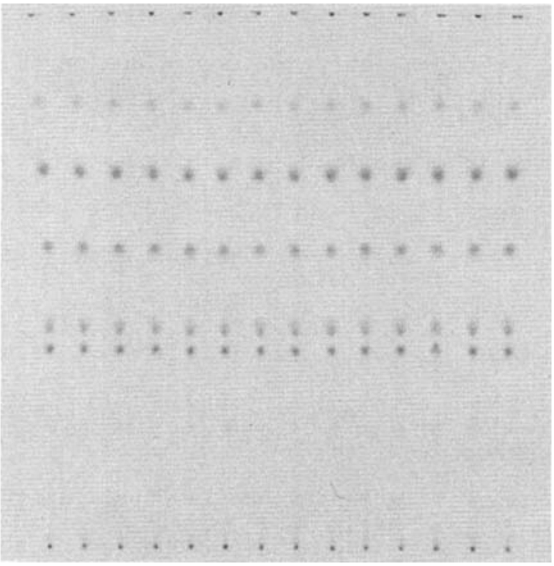

Fig. 5

One-directional separation of dyes onf fine-particle silica gel layer /d_p = 5 μm/
Eluent, methylene chloride; external pressure on the membrane, 1.2 MPa; flow-rate, 20 cm^3/h

It is known that reversed-phase chromatography, using chemically bonded phases, is one of the most frequently chosen separation modes in HPLC. The usefulness of the technique arises from the development of suitable packings and the wide variety of eluting solvents. Because of the complexity of the retention mechanism, it is possible to separate, simultaneously, compounds of a wide polarity range with excellent resolution and short retention times. The outstanding properties of chemically bonded phases on various TLC plates were used in OPTLC for the separation of dimedone adducts of aliphatic aldehydes. Owing to the substantially shorter separation time and stable flow rate in the RP-OPTLC, the resolution values obtained on reversed-phase plate were also good in the case of longer solvent migration distances

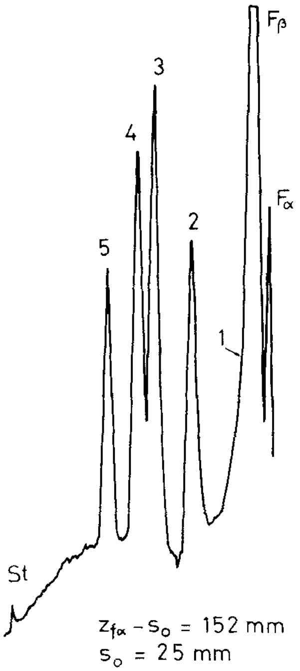

Fig. 6

Separation of dimedone adducts of formaldehyde and other aliphatic aldehydes on SILPEVPRES N experimental chromatoplate Eluent, acetonitrile - 0.005 M KH_2PO_4 /4:6,V/V/; s_o=start distance, 25 mm; $F_\alpha = \alpha$ -front; $F_\beta = \beta$ -front; Z_f^o= front distance
OPTON-ZEISS PM Q III chromatogram spectrophotometer; , 264 nm; slit width, 7.0x0.26 mm; chart speed, 10 cm/min; 500-500 ng/substance 1 dimedone; 2 acetoldomedone; 3 formaldomedone; 4 propionaldomedone; 5 butiraldomedone

so that the spots of the substances separated were suitable for quantitative evaluation /Fig. 6/. It follows from these results that RP-OPTLC together with quantitative evaluation may be a useful tool in routine analysis.

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