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OVERPRESSURED THIN-LAYER CHROMATOGRAPHY

ZYGFRYD WITKIEWICZ* and JAN BŁĄDEK
Institute of Chemistry, Military Technical Academy, 01 489 Warsaw 49 (Poland)
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CONTENTS

1 INTRODUCTION

Thin-layer chromatography (TLC) has many advantages over other chromatographic methods, the most important including the simplicity of the equipment, low costs, small requirements as regards the training of personnel and, in many instances, a fairly short time needed for obtaining the results. TLC is also useful as a pilot technique for high-performance liquid chromatography (HPLC) as it facilitates the choice of a suitable chromatographic system and allows the prediction of separations that can be achieved.

Like other chromatographic methods, TLC is developing and improving, and continuously offers users new possibilities. The properties of the chromatographic plates are being improved and plates with new properties are being introduced including those for high-performance TLC (HPTLC)[1–4] and those with a concentrating zone[5,6]. There has also been progress in quantitative TLC analysis[7] and in the methods of revealing the chromatograms[8].

Particularly important is progress in the designs of chambers for developing chromatograms. Devices are known that allow very effective anticircular development[9,10] and continuous development, some permitting automatic recording of the chromatograms[11–13], and chambers for developing chromatograms on circular rotating plates[14,15].

In 1977–79 Tyihak and co-workers[16,17] published their first work on a new pressurized ultramicro chamber (PUM chamber) designed for TLC. This is an improved version of their ultramicro chamber (UM chamber)[18,19]. In the latter chamber an elastic foil adheres to the sorbent layer on a glass chromatographic plate. This

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foil is pressed on to the chromatographic plate by means of a sponge layer. The pressing system (foil–sponge–glass) is slightly shorter than the chromatographic plate so in effect a strip of sorbent about 1.5 cm wide remains uncovered. Both glass plates are pressed together and placed in a large chamber in which the developing procedure is conducted in the normal way.

Based on the work of Tyihak and co-workers[16,17] a new type of chromatography was founded, known as overpressured thin-layer chromatography (OPTLC), today often referred to as overpressured layer chromatography (OPLC). Kalasz[20] suggested calling it forced-flow TLC (FFTLC). This chromatographic procedure, which is being developed primarily in Hungary, is finding increasing applications in solving many problems encountered in chromatographic analysis.

In this review we outline the current state of the art in OPTLC. Other review on this subject have been published[19,21–24], but that was some years ago[21,22] or they were superficial[24] or in journals of very limited circulation[19,23]. We consider that OPTLC, in view of its properties and advantages, deserves wider dissemination and application in analytical practice.

2 GENERAL PROPERTIES OF OVERPRESSURED THIN-LAYER CHROMATOGRAPHY (OPTLC)

Although OPTLC has only recently been introduced into laboratory practice, it has already found wide use and has been applied to solve many analytical problems. Although the cost of the chamber for OPTLC is fairly high, the results obtained are very promising. Often it is possible to separate in the OPTLC chamber mixtures that cannot be separated in common TLC chambers. Also, the time of separation is usually shorter than in conventional chambers, so diffusion effects are reduced and the spots are small and compact, which contributes to an increased sensitivity of determination. The amounts of solvents required for determinations in OPTLC chambers is small, the volume of eluent necessary for developing a chromatogram on a 200 × 200 mm plate being less than 5 cm^3.

The flow-rate of the eluent fed to the plate can be controlled and optimized for a given chromatographic system and mixture to be separated. In OPTLC the height equivalent to a theoretical plate can be lower than in column chromatography and much lower than in classical TLC, in which the flow-rate of the eluent cannot be controlled.

The possibility of forcing the flow of the eluent through the adsorbent layer makes it possible to use solvents that wet the adsorbent poorly, so it is easier to select a suitable solvent or mixture of solvents.

The use of pressure for forcing the solvent on to the chromatographic plate extends the range of applicability of large chromatographic plates (*e.g.*, 200 × 400 mm) and of HPTLC plates (with fine-grained adsorbents of narrow mesh fractions) as it allows separations within reasonable times. The time required for developing chromatograms on HPTLC plates in conventional chambers is often very long, so in practice the development distance of the chromatograms is usually short. As the development distance of the chromatograms is increased, the resistance of flow of the eluent on the plate increases significantly, especially when viscous solvents are used. When the times of developing the chromatograms on HPTLC plates are long,

the spots are diffuse and the quality of the chromatograms is poor. Therefore, it is advantageous that chromatograms can be developed about five times quicker in OPTLC than in HPTLC. The time of developing chromatograms in OPTLC depends on the flow-rate of the eluent on the chromatographic plate. This rate must, however, be appropriately adjusted to the properties of the system. We can assume that for most systems good results are obtained when the eluent flow-rate is about 20 cm^3/h, which corresponds approximately to an eluent front migration velocity of about 1 cm/min[25].

The OPTLC chamber allows many samples to be separated simultaneously. As a result, good separations are obtained and the spots are well separated from the background (even at low concentrations) and the chromatograms can easily be registered by means of densitometers.

OPTLC, having many of the properties of HPLC, has several advantages over the latter: the possibility of continuous observation of the separation on the chromatographic plate, the use of various reagents for developing the chromatograms, which makes the identification of the particular components of the mixture possible, and the possibility of the simultaneous analysis of many identical or different samples. OPTLC often allows better separations to be obtained than with chromatographic columns, especially if two-dimensional development is applied.

In the chambers for OPTLC various single eluents can be used without difficulty However, the selection of a multi-component developing system must be made very carefully, as in some instances demixing of the solvents making up the system may occur. As a result, demixing regions may form on the chromatographic plate, which adversely affect the final separations. It is recommended, therefore, that the selected developing system is tested (preferably in an ultramicro chamber) prior to applying it in the pressure chamber, to ensure that its properties are suitable. Also, solvent mixtures of varying composition may successfully be used in the OPTLC chamber. In this way a gradient TLC system is obtained whose possibilities are similar to those of gradient HPLC[25]

3 METHODS OF DEVELOPING CHROMATOGRAMS

In pressure chambers, separations and chromatographic analyses can be carried out of mixtures of all substances that can be separated and analysed in other types of chambers and also of substances whose separation in conventional chambers is very difficult or even impossible. The general procedure and the methods of developing the chromatograms in pressure chambers are similar to those used in other types of chambers. However, pressure chambers allow for a greater variety of chromatographic separation methods, as follows[19]:

(i) One-directional development, where a square or rectangular chromatographic plate with three impregnated edges is used, and the samples are applied to the plate in the vicinity of its central impregnated edge. It is also from this edge that the solvent is fed (Fig. 1).

(ii) Two-directional development. This procedure is not used in non-pressure chambers for TLC Here a rectangular plate with the two longer edges impregnated is usually used. The solvent is fed to the centre of the plate between the impregnated edges so that two solvent fronts are formed perpendicular to the impregnated edges

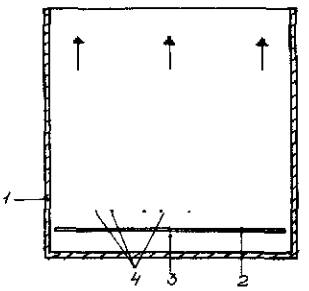


Fig. 1. Chromatographic plate for linear OPTLC for one-directional development. 1, Impregnated edges, 2, channel in the layer; 3, solvent inlet, 4, samples

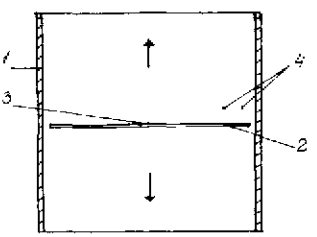
of the plate. The samples are placed on the start lines parallel to the solvent fronts. In this method of developing chromatograms one can chromatograph simultaneously twice as many samples as in one-directional development (Fig. 2).

(iii) Circular development. Here the edges of the plate (usually square, 200 × 200 mm) are not impregnated. The solvent is fed to the centre of the plate and the samples are placed around it. The solvent migrates from the centre of the plate to the circumference of the circle producing the development of the chromatogram in the same direction. In circular development it is also possible to feed the samples by means of a microsyringe to a feeder mounted on the conduit supplying the eluent. The separated components then appear on the chromatogram in the form of concentric circles[26] (Fig. 3).

(iv) Repeated development. In this instance two or more development operations are carried out, *e.g.*, according to methods (i)–(iii), with the use of the same or different eluents.

(v) Continuous development, a procedure which is easiest to apply in methods (i) and (ii) but also possible in (iii). It consists in supplying the solvent until the required separation effect is achieved. This method of developing the chromatogram can be used in preparative separations with on-line detection without the need to scrape off the adsorbent from the plate.

(vi) Two-dimensional development. Here square (200 × 200 mm) plates are used with all four edges impregnated. In the course of developing the chromatogram



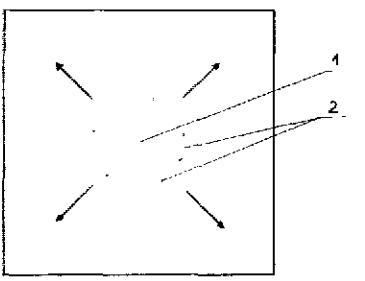


Fig. 2. Chromatographic plate for linear OPTLC for two-directional development. 1, Impregnated edges, 2, channel in the layer, 3, solvent inlet, 4, samples

Fig. 3. Chromatographic plate for circular OPTLC. 1, Solvent inlet, 2, samples

the edge of the plate opposite to the position where the eluent is supplied is covered with filter-paper. Usually one sample is applied to the plate and the eluent is supplied as in (i). After the first development the solvent is changed. The new solvent is supplied to the neighbouring edge of the plate (Fig 4).

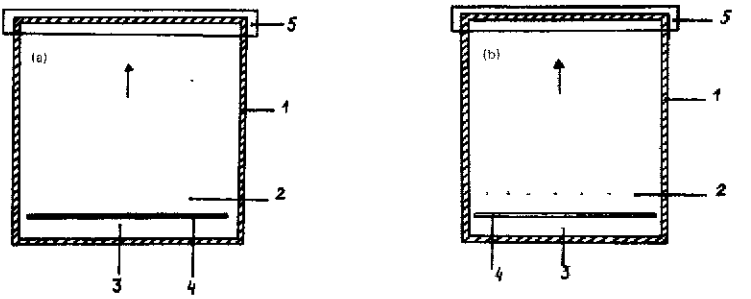


Fig 4. Schematic diagram of chromatoplate for two-dimensional separations 1, Impregnated edges; 3, solvent inlet, 4, channels, 5, sheet of filter-paper (a) First development; 2, sample (b) Second development; 2, spots after first development

(vii) Development in triangles (anticircular). Here plates in the form of isosceles triangles (as described by Issaq[27]) with impregnated edges are used. The solvent migrates from the base of the triangle to its vertex.

OPTLC also allows for temperature programming as in gas chromatography. In some instances better results can be obtained in this way than when the separation is conducted at ambient temperature. This improvement may be due to advantageous changes in the transport of the chromatographed substance in the mobile phase and in the viscosity of the eluent. Some influence may also be exerted by the vapour pressure of the developing solvent, which is meaningless at normal temperatures of OPTLC chambers but may have a significant effect on the separations obtained in other types of TLC chambers.

In OPTLC it is possible to predict the retentions of the substances obtained in the course of chromatography under conditions of a multi-step gradient, developing on the basis of the retention (R_F) under isocratic conditions.

4 APPARATUS FOR OPTLC

Apparatus (chromatographs) for OPTLC is now manufactured on a commercial scale and distributed by Labor Mim (Hungary). The principal element of these devices designed for developing chromatograms under increased pressure is the chromatographic PUM chamber, shown schematically in Fig. 5. The chamber consists of a metal base (8), an organic glass transparent cover plate (4), a flexible transparent foil membrane (9), a rubber washer (6) and holders (7). The free space (10) is filled during operation with water or gas under high pressure (of the order of 0.1–2.5 MPa), constituting a pressure cushion owing to the elasticity of the membrane. In this way the chromatographic plate (11) placed on the base is tightly covered with the membrane as in the classical UM chamber. The holders serve for connecting the base to the cover plate in order to prevent their mutual displacement when pressure is gen-

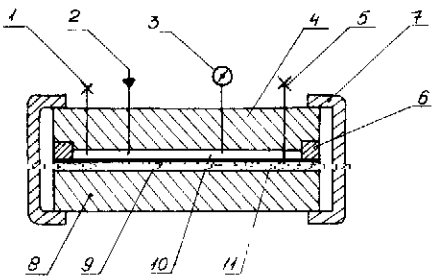


Fig 5 Schematic diagram of the pressurized ultramicro (PUM) chamber 1, Connector pipe feeding water or gas, 2, safety valves; 3, manometer; 4, plate of organic glass, 5, solvent dosing system, 6, rubber washer, 7, holders; 8, metal basis, 9, transparent foil membrane, 10, pressure cushion; 11, chromatographic plate

erated in the cushion. The complete isolation of the chromatographic plate from external space makes it necessary to provide the chamber with a solvent dosing system (5) There is also a connector pipe (1) feeding gas or water to the cushion, and manometers (3) and safety valves (2), indispensable when working under increased pressure

The essential feature of the PUM chamber 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 or gas is formed between the cover plate and the fixed flexible membrane, the vapour space above the layer being virtually eliminated. The admission of solvent under pressure has been solved by applying a pump system or gas pressure. The external pressure on the flexible membrane must always be higher than the input pressure of the solvent. By adjusting the solvent flow in the PUM chamber by means of a pump system, it is possible to separate substances at an optional development distance.

The today PUM chambers differ in design, the differences concerning primarily their shape, method of dosing the eluent and kind of connection between the cover plate and base. In ref. 28 a simple chamber was described in which air is used for generating pressure on the chromatographic plate and for forcing the eluent on to the sorbent layer.

In Fig. 6 a rectangular PUM chamber is shown which constitutes an element of the commercial Chrompres 10 device[19]. Here the cover plate is connected with the base by means of a clamping stirrup. The manometer and safety valve are located outside the chamber. The conduit with the air valve serves for removing air from the cushion and filling its whole volume with water The solvent is fed to the chromatographic plate at one point by the system shown in Fig 7. The chamber of the commercial Chrompres 25 device[29] and the circular-type PUM chamber[17] are built analogously, the system for solvent admission of the latter being located in the centre of a circular cover-plate made of organic glass.

A rectangular PUM chamber of the drawer type[30] is shown in Fig. 8. Here the cover-plate together with the flexible membrane and washer are fixed permanently to the base. The chromatographic plate is placed in a drawer which is pushed into the space between the membrane and base The cavity in the drawer, whose dimensions correspond to those of the chromatographic plate, keep that plate in place when displacing the drawer.

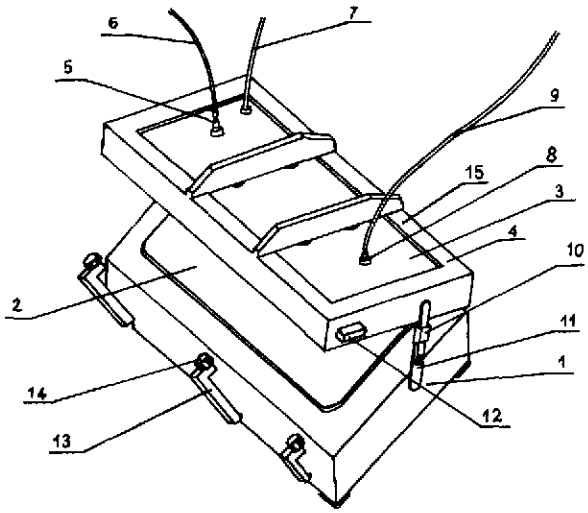


Fig 6 Chrompres 10 PUM chamber. 1, Bottom support block, 2, working area, 3, upper block with polymethacrylate support plate, 4, external frame, 5, solvent inlet, 6, solvent connection, 7, pipe with air valve; 8, water inlet, 9, water inlet tube, 10, weight lock, 11, support, 12, handle, 13, clamping stirrup; 14, stirrup fixing, 15, countersunk screw[19]

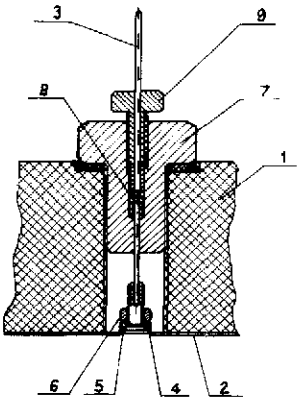


Fig. 7. System for solvent inlet 1, Supporting plate; 2, plastic foil, 3, solvent connection, 4, underplate to foil; 5, clamping ring, 6, stretching nut, 7, closing head-piece; 8, fitting, 9, stretching screw[19]

In addition to the method of leading a solvent on to the chromatographic plate from the upper side (as shown in Fig 8), it is also possible to carry it from the bottom through the hole in the drawer and chromatographic plate.

Another version of a sliding rectangular chamber is shown in Fig. 9 Here the cover-plate is connected to the membrane and washer It is provided with projections fitted to guide the slit in the base. As a result, the whole cover-plate can be shifted with respect to the base. The chromatographic plate is placed in a cavity in the base and the cover-plate is pushed over it. In this chamber the eluent is supplied in a different way than in the case described above The eluent is fed here from beneath

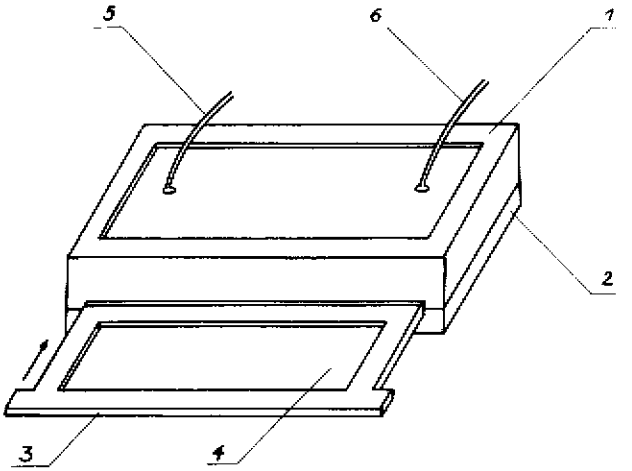


Fig. 8. PUM chamber of drawer type. 1, Plate of organic glass with metallic frame and foil membrane with washer, 2, metal support block, 3, drawer; 4, working area, 5, inlet solvent system, 6, connector pipe feeding gas to pressure cushion

through a stub pipe mounted in the base. The channels in the cavity of the base supply it to a groove which constitutes the front of the eluent. When the chromatographic plate is placed on the base it covers these channels, the edge of the plate lying along the groove. In this way the eluent fills the whole groove and next migrates on to the sorbent on the plate, generating a straight front whose width is equal to the

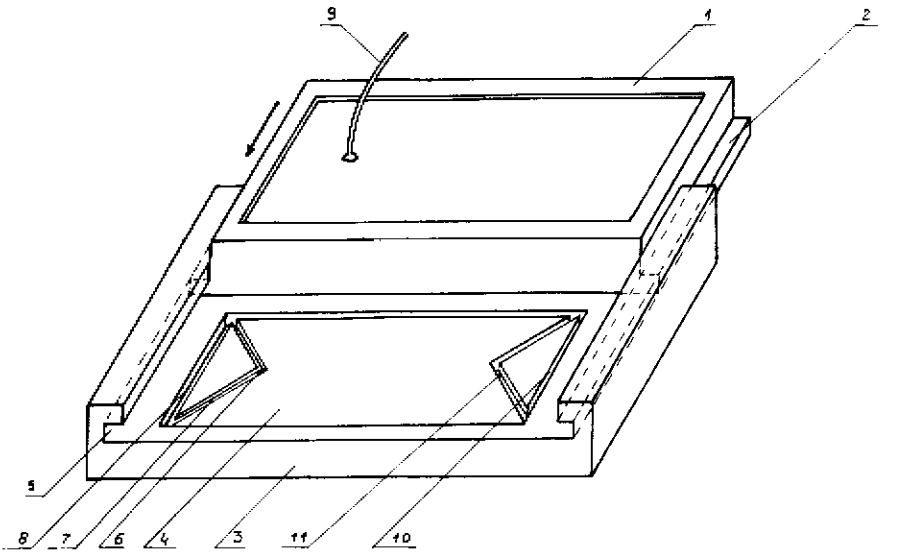


Fig. 9. PUM chamber with sliding upper block. 1, Cover of organic glass with metallic frame and foil membrane with washer; 2, cover projection; 3, support, 4, place for chromatographic plate; 5, guide; 6, solvent inlet; 7, channel feeding solvent to ditch, 8, ditch, 9, connector pipe feeding gas to pressure cushion, 10, channels for outlet of solvent, 11, solvent outlet

length of the groove. This method of feeding the eluent requires the space between the edges of the cavity in the base and the chromatographic plate to be sealed. Nevertheless, it is less complicated than the system shown in Fig. 7 and also it is not necessary to make a hole in the flexible membrane, which extends its life significantly. The channels and draining groove collect the eluent leaving the chromatographic plate and discharge it outside the chamber. This design allows the development of the chromatogram for any arbitrarily long time.

An important element of the devices for developing chromatograms under increased pressure are the pumps for supplying and compressing water in the space between the organic glass cover plate and the flexible membrane, and the pumps feeding the eluent. If compressed air is used for generating pressure in the cushion and forcing the eluent, pumps are not necessary and the device can operate without supply of electric power.

In the commercial Chrompres 10 device a D 167 micropump (type LS-244/1) has been applied for supplying water and a micropump S 13 (type LS-232) for dosing the eluent. The pumps are supplied with electric current (220 V), are provided with manometers with a measuring range of 0–2.5 MPa and have an accuracy of 4%. The remaining parameters of the pumps are as follows. D 167: maximum operating pressure, 1.4 MPa; time necessary for filling the cushion, 2 min. S 13: maximum eluent inlet pressure at the end of development, 1 MPa, control range of eluent flow-rate, 0.1–13 cm^3/min.

The Chrompres 25 device (Fig. 10), built similarly to the Chrompres 10, is designed for developing chromatograms with the use of eluents of high viscosity. Here a much higher eluent pressure is required to ensure an adequate flow-rate. Hence the pressure in the cushion has to be increased accordingly (to 2.5 MPa). This requires the use of pumps with adequate parameters.

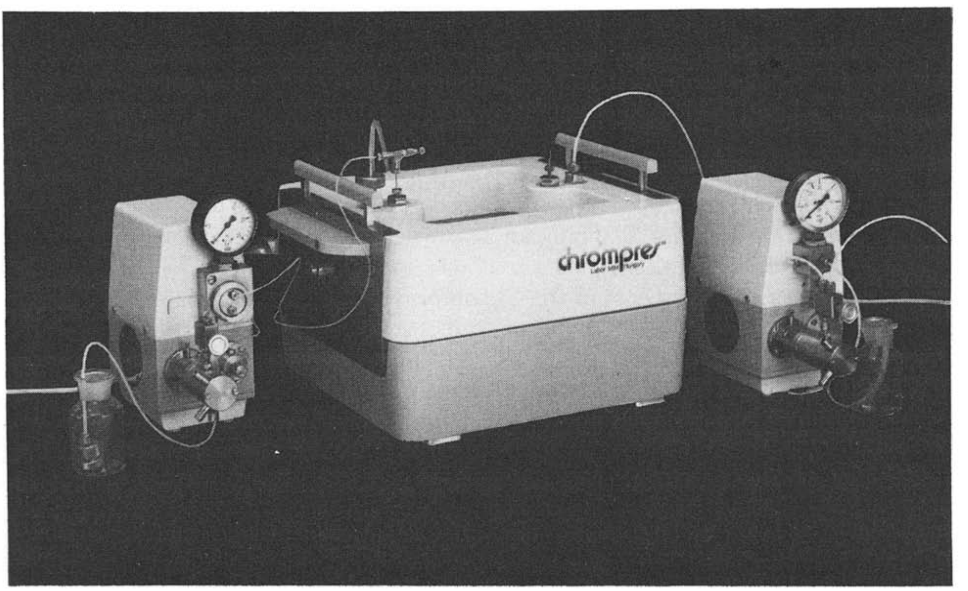


Fig. 10. Chrompres 25.

An LS-235/1 pump was applied to force water into the water cushion and an LS-232/2 pump to transport a solvent on to the chromatographic plate. The LS-232/2 pump makes possible an eluent flow-rate of 0.03–2.5 cm^3/min.

The devices provided with PUM chambers shown in Figs. 8 and 9 are supplied with compressed air. The gas fed to the device is separated into two independent streams (Fig. 11) The required pressure in the particular streams is adjusted by means of reducing valves One stream feeds the cushion and the other the eluent container. The eluent is forced out of this container according to the syphon principle into the capillary, channels and groove (Fig 9). The application of compressed gas makes it possible to use the device not only in remote laboratories but also under field conditions. As the source of compressed gas one can use a gas cylinder or any compressor

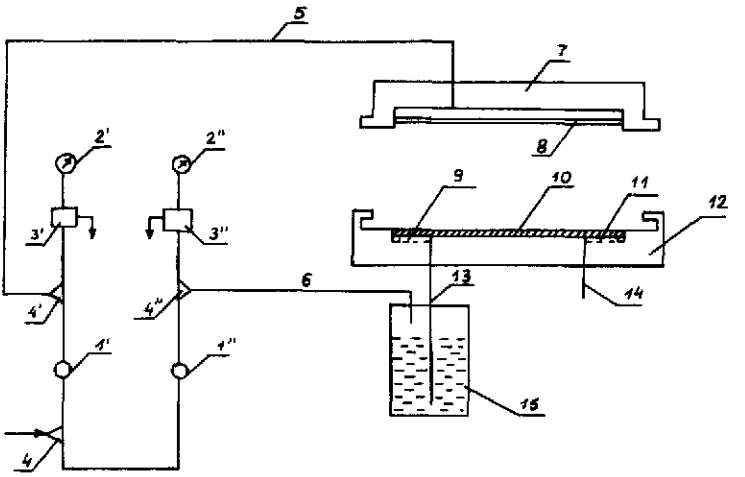


Fig 11 Schematic diagram of device working with compressed gas 1', 1", Reduction valves; 2', 2", manometers, 3', 3", degassing valves, 4', 4", T-pipes, 5, 6, capillaries feeding gas, 7, upper block, 8, membrane, 9, channels and ditch feeding solvent, 10, chromatographic plate; 11, channels and ditch for outlet of solvent, 12, support, 13, pipe feeding solvent, 14, pipe for outlet of solvent, 15, eluent tank.

It is possible to develop chromatograms with a continuous flow of eluent in all the chambers described. All of them can also work at temperatures varying from 5 to 40°C using an external thermostat.

The rectangular chambers of the Chrompres 10 and 25 devices are adapted for maximum plate sizes of 200 × 400 mm[19] and 200 × 200 mm[29], respectively. In the circular PUM chamber, 100 × 100 mm or 200 × 200 mm plates are used[17,26]. The chambers shown in Figs. 8 and 9 are adapted for 100 × 200 mm plates[30].

Glass, aluminium or plastic foil support chromatographic plates with an adsorbent layer thickness from 0.1 to 3 mm are applied in OPTLC chambers.

The feeding system shown in Fig. 7 introduces the eluent on to the chromatographic plate at a given point. Such a system of supplying the eluent is satisfactory with circular chambers In this instance the eluent migrates radially and its front has the form of a circle with a diameter that increases as the development proceeds. In rectangular chambers it is required that the front of the mobile phase be linear. In

this instance the chromatographic plates must be appropriately prepared. This consists in making a channel (Fig. 1) in the sorbent layer and in impregnating the edges of the plate which ensures a flow of the eluent in the required direction. Impregnation is carried out with paraffin or different suspensions of plastics, *e.g.*, Teflon[22]. Labor MIM company recommend the use of IMPRES 1 impregnating solution, supplied together with the devices, which is used for coating the chromatographic plate edges to a width of about 3 mm after removal of the sorbent. The plate prepared in this way is subjected to thermal treatment at 80°C for 5 min. Good results were obtained when an aqueous latex solution or a silicone lubricant were used as impregnants[30]; it should be noted that the latter can be used only with a limited number of developing solvents.

5 TWO-DIMENSIONAL COLUMNS

Two-dimensional chromatography is a variant of OPTLC[31]. In this technique the known possibilities of two-dimensional TLC have been developed, some of its disadvantages having been eliminated at the same time. This technique may be useful for separating multi-component mixtures which are difficult to separate by standard column chromatography in the isocratic mode and even with gradient systems.

Fig. 12 shows a view from above of a column filled with two different adsorbents, and Fig. 13 presents a scheme of the column with its equipment. The column, which may be planar, square or rectangular, is filled with one or two different stationary phases as used in HPLC or TLC. When two stationary phases are used (as in Fig. 12), one is applied in the form of a narrow strip.

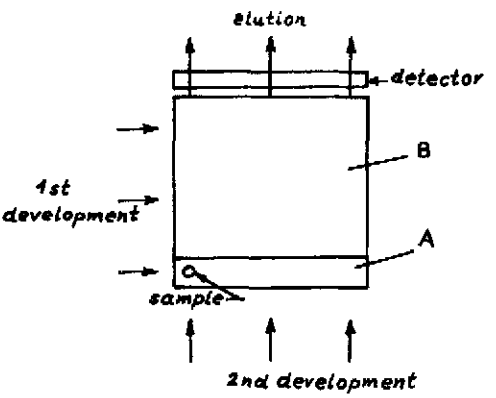


Fig 12 Principle of development in a two-dimensional column[31].

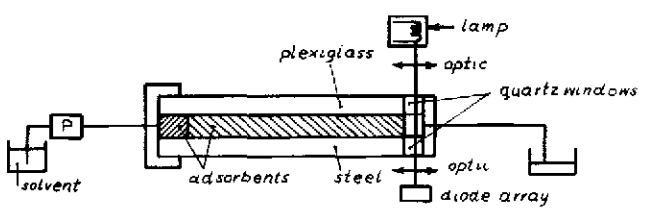


Fig 13 Two-dimensional column with its equipment[31]

The column was made by cutting a square (100 × 100 mm) cavity 1 mm deep in a steel block. The cavity was filled with a suspension of the stationary phase in one of the solvents used for applying the thin layers on the chromatographic plates. Silica gel, a reversed-phase material, a size-exclusion material or an ion-exchange resin may be used as stationary phases.

The steel block has a system of grooves by means of which the eluent is supplied to and removed from the column. The stationary phase is covered with a thin, chemically neutral foil (*e.g.*, Mylar foil) on which an organic glass plate is placed and accurately fixed to the steel block with bolts. The organic glass plate has a cavity 5 mm deep into which water is pumped, spreading over the foil. The water pressure is kept constant at the required level, up to 1 MPa. The organic glass plate is provided with a water supply system.

The samples are introduced into the column with a microsyringe through a conventional sampling port with a septum as in conventional liquid chromatographic columns.

The separation of the sample is carried out by pumping the first solvent parallel to the narrow adsorbent strip (A) on which the sample has been deposited (Fig. 12). The solvent is pumped until a satisfactory preliminary separation of the sample is obtained. After the pump has been switched off and the eluent has ceased to flow, the second pump starts, supplying a different solvent in a direction perpendicular to that of the first solvent. The second solvent passes through adsorbent B and then through the UV detector (254 nm) where the substances eluted from the column are detected in a 0.5 × 90 mm slit. The detector is linked with a computer, so the chromatogram may be recorded on magnetic tape and subsequently processed.

In two-dimensional column chromatography it is important that the chromatographic systems be selected so as to ensure that the following two conditions are fulfilled. Distinct differences occur in the column in the retention mechanism with respect to the components of the separated mixture (*e.g.*, in one mechanism the separation takes place according to the polarity or polarizability of the molecules and in the other mechanism according to the size of the molecules). Second, the solvents should be selected so that the first does not damage the adsorbent to be used in the second step and either mix with the second solvent or be easily removed, *e.g.*, with a neutral gas or a third solvent of low elution strength.

The peak capacity of the two-dimensional column is about ten times greater than that in other liquid chromatographic systems used hitherto. This is achieved because composite mixtures can be separated in the two-dimensional column according to different retention mechanisms. Theoretically there may be more than two such mechanisms, leading to multi-dimensional column chromatography[32].

6 CHARACTERISTIC PROPERTIES OF OPTLC CHAMBERS

The specific properties of OPTLC chambers are the result of the elimination of solvent vapours from over the sorbent, allowing one to conduct chromatography in a closed system with controlled parameters and with the possibility of influencing the flow-rate of the solvent.

6.1. Flow of the solvent on the chromatographic plate

The idea of applying a forced flow of the eluent in OPTLC is not new. Pumps for forcing the eluent on to HPTLC plates have been used before[33,34]. However, the sorbent on the plates was not covered tightly, so the rate of supply of the solvent was limited to a value imposed by the migration of the front of this solvent. A danger then existed that the excess of the solvent would migrate over the surface of the sorbent layer and not in its volume. This relates largely also to a recently proposed procedure that does not require the use of pumps[35].

When the membrane adheres tightly to the sorbent layer on the chromatographic plate, the solvent forced on to the plate in OPTLC chambers can only move in the volume of the sorbent layer. Its migration velocity depends on the feeding pump used and on the shape of the plate. If the velocity of the eluent on a rectangular plate is to be constant, the output of the pump applied must also be constant. However, as the flow resistance of the eluent on the plate increases with distance, the working pressure of the pump must also increase gradually. For rectangular plates we then have a linear dependence of the distance of the eluent front from the start point (z_f) on time (t), *viz.*, $z_f = kt$, where k is a coefficient dependent on the solvent flow-rate, on the pressure pressing the membrane to the plate and on the particle size of the adsorbent layer. The value of k is constant during a chromatographic process conducted under the given conditions and higher than that in the same system under conventional TLC or HPTLC conditions. In Fig. 14 (curve 1) the dependence $z_f = kt$ for OPTLC is shown.

The procedure of developing chromatograms characterized by such a dependence and conducted in Chrompres 10 and 25 devices is referred to as linear OPTLC[23].

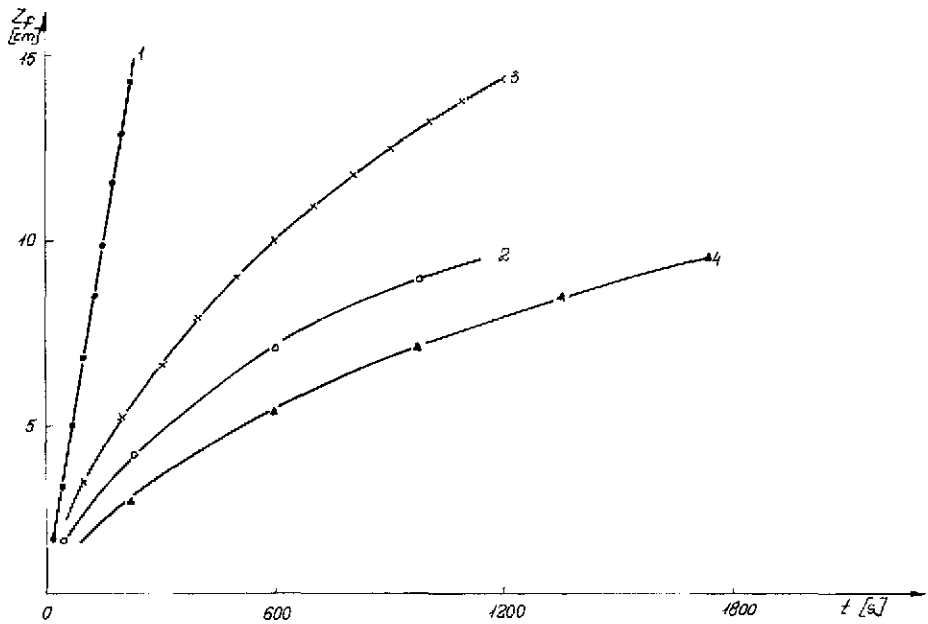


Fig. 14 Range of migration of the mobile phase front as a function of time 1, Linear OPTLC; 2, circular OPTLC, 3, linear OPTLC with decreasing solvent flow-rate; 4, conventional TLC

The ratios characterizing migration of the eluent on the plate for migration distances X_1 and X_2 and the corresponding times t_1 and t_2 are $k_1 = X_1/t_1$ and $k_2 = X_2/t_2$. In ideal linear OPTLC, $k_1 = k_2 = k_3 \ldots$ The value of k is independent of the solvent migration distance even if the chromatographic process is conducted on adsorbent layers with a bounded phase[23]. This corresponds to the conditions encountered in columns in HPLC.

If circular plates are used (circular OPTLC), the solvent expands over an increasing surface area in the direction of migration. Then the equation $z_f^2 = kt$ characterizing the flow of the solvent in conventional TLC and HPTLC remains in force. This is shown in Fig. 14 (curve 2).

When pumps of constant working pressure are used for forcing the eluent on to the chromatographic plate, the flow-rate of the eluent decreases with increasing resistance of flow even if rectangular plates are used[30] (see curve 3 in Fig. 14).

As follows from the relationships shown in Fig. 14, the properties of OPTLC with a decreasing solvent flow-rate are intermediate between those of linear OPTLC and conventional TLC or HPTLC.

6.2. Interaction of the eluent with the sorbent

In conventional TLC the composition of the vapour phase over the adsorbent may vary in an uncontrolled manner, especially if the chromatograms are developed with a solvent mixture, because full saturation of the chamber is impossible. The problem is of less importance in small-volume chambers. The components of the vapour phase are absorbed in the course of the chromatographic process by the sorbent, whose porosity decreases accordingly. This produces an increase in the velocity of migration of the solvent front and affects the migration of the front and complete wetting of the plate. These effects influence the R_F values of the chromatographed substances.

In view of the specific conditions of flow of the eluent, OPTLC reveals some characteristic and specific features that do not occur in conventional TLC[23]. The properties of the dry sorbent do not change in the course of the chromatographic process because of the absence of the vapour phase. It may happen, however, that the flexible membrane covering the sorbent layer will absorb in different ways the components of a multi-component eluent, which may affect the resulting separations. In some instances this effect could possibly be positive. Insufficient experimental material does not allow us, however, to derive theoretical relationships that would allow us to predict the separations, account being taken of the interactions of the solvent with the flexible membrane.

A distinct region may be distinguished on the chromatographic plate in the vicinity of the solvent front which differs from the remaining surface of the adsorbent even when single solvents are used for developing the chromatograms. This difference consists in incomplete wetting of the adsorbent layer near the solvent front. However, longer contact of the adsorbent with the solvent results in its complete wetting (filling of all its pores). In practice this does not hinder the obtaining of good separations.

6.3. Efficiency of OPTLC

The specific conditions of solvent migration in OPTLC have an influence on the efficiency of the system as measured by the number of theoretical plates. The

theoretical problems related to the effect of solvent velocity and of sorbent particle size in chromatographic efficiency have been studied by Martin *et al.*[36].

The height equivalent to a theoretical plate (H) in column liquid chromatography can be found from the relationship

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

where d_p is the particle diameter, u is the carrier liquid flow-rate and λ, γ and β are constants. It follows from the above relationship that the efficiency will not change when the particle size of the sorbent decreases with increasing flow-rate of the eluent. This relationship may also be applied to OPTLC.

Fig. 15 shows the dependence of the average height equivalent to a theoretical plate calculated according to Guiochon and Siouffi[37] on the migration of the solvent front, $H = f(z_f)$. In the linear OPTLC H is nearly constant over the whole chromatogram development range (curve 2 in Fig. 15). When in OPTLC the eluent migration velocity decreases, H also decreases (curve 1 in Fig 15).

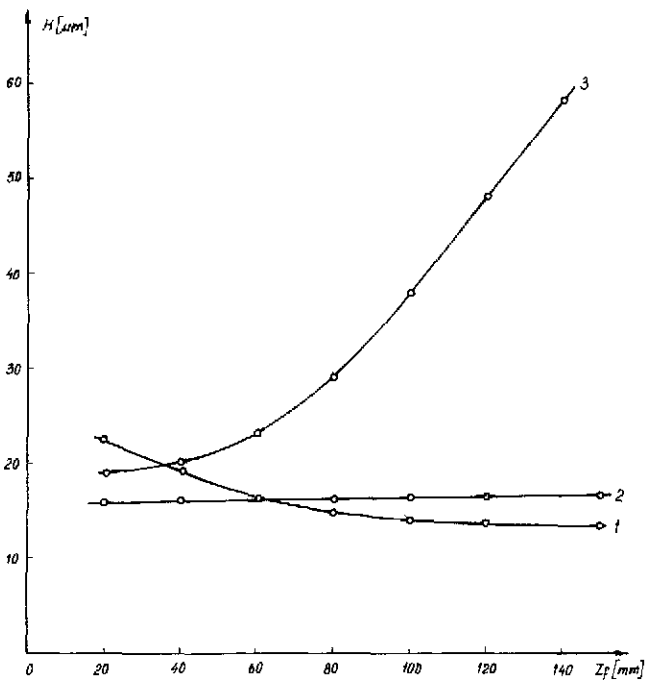


Fig. 15 Dependence of average height equivalent to a theoretical plate (H) on the range of migration of the solvent front (z_f) 1, OPTLC with decreasing solvent flow-rate; 2, linear OPTLC; 3, conventional TLC.

Curve 3 in Fig. 15 shows the relationship $H = f(z_f)$ for conventional TLC. Comparison of curve 3 with those for OPTLC shows that their shapes are completely different and that OPTLC is in reality a variant of column chromatography. This leads to the conclusion that only OPTLC may fully serve for modelling columns in HPLC. This is a great advantage of OPTLC over the TLC procedures known hith-

erto. In the latter it was only the application of a continuous flow of the eluent across the plate that made possible correct predictions of the separations in the column.

From the relationship between the height equivalent to a theoretical plate and the flow-rate of the solvent and the particle size of the sorbent it follows that both of the latter factors may be used for optimizing OPTLC separations. By taking account of the particle size of the sorbent one can determine the reduced plate height (h), which is given by the equation $h = H/d_p$, where d_p is the mean particle diameter. In OPTLC sorbents may be used with particle sizes varying over a wide range, including those with very fine particles.

Fig. 16 shows the dependence of H on the particle size of the sorbent. It can be seen that H decreases with decreasing particle size. Hence the efficiency of OPTLC is the greater the smaller is the particle size of the sorbent on the chromatographic plate. This emphasizes the particular usefulness of pressure chambers for chromatography on HPTLC plates.

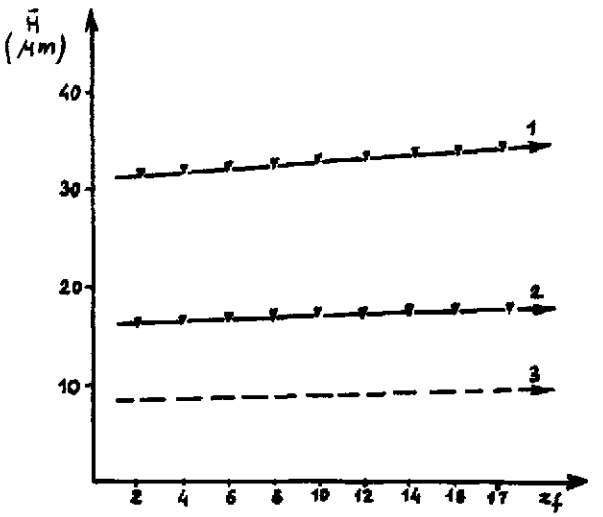


Fig 16 Dependence of H and migration distance of eluent front (z_f) on particle size of silica gel layers in the Chrompres 25 chamber Eluent, methylene chloride, substance chromatographed, butter yellow Particle size (d_p): (1) 11 μm, (2) 5 μm, (3) 3 μm[23]

In OPTLC the height equivalent to a theoretical plate depends on the flow-rate of the solvent at a given migration distance[38,39], which is shown in Fig. 17. H is at a minimum in a certain range of linear flow-rates. This optimal plate height varies with the migration distance according to the relationship shown in Fig. 18. Confirmation of this is provided by the results in Fig. 19, where the dependence of the number of theoretical plates on the development distance is compared, and it is shown that in OPTLC a particularly large number of theoretical plates is obtained. This number increases linearly with increasing development distance. Also, in an OPTLC chamber in which the migration velocity of the solvent decreases the variation of the number of theoretical plates with the distance of the mobile phase is close to linear.

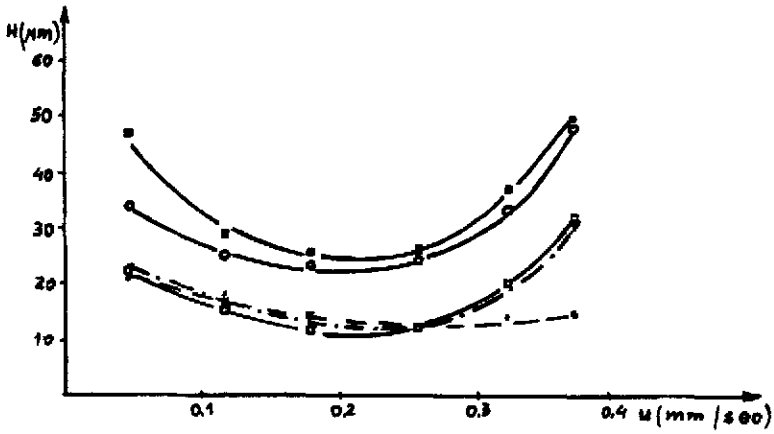


Fig 17 Plate heights (H) of a mixture of lipophilic dyes as a function of the linear flow-rate (u) of the eluent toluene Migration distance, 15 cm HPTLC plates, silica gel 60 F_{254}. (●) Ceres Violet BRN; (▼) Ceres Black G, (□) green component of Ceres Black G; (○) Blue VIF Organol, (■) Ceres Red G[39]

Hauck and Jost[39] studied the dependence of resolution (R_S) on the flow-rate of the solvent in a linear pressure chamber for several lipophilic dyes. They found that there exists a certain linear flow-rate at which there is maximum resolution (Fig. 20). The maximum resolution occurs at the flow-rate at which the number of theoretical plates is the greatest. Even in HPTLC an optimal flow-rate of the eluent can be achieved and hence the number of theoretical plates increased 2–5-fold, so more components of the mixture may be separated in one development. According to Hauck and Jost[39], the minimum plate height in OPTLC is obtained for HPTLC plates at a flow-rate of the solvent of 0.20–0.25 mm/s. Kalasz[20] found that for Fixion 50-X8 plates the optimum flow-rate of the eluent is 0.12 mm/s and for TLC plates with silica gel layers 0.16 mm/s.

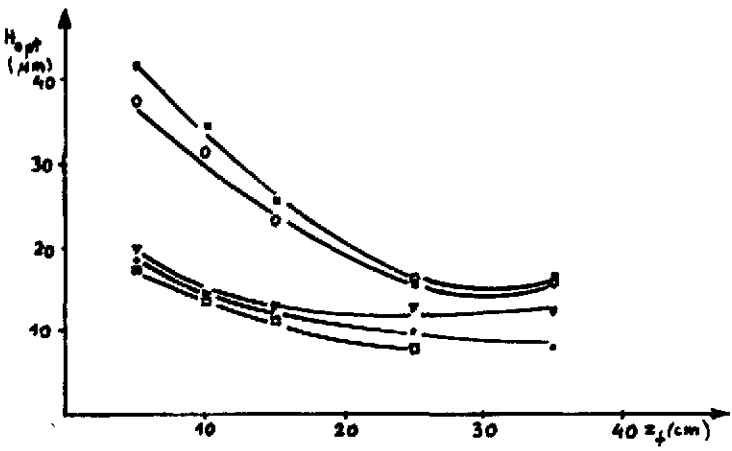


Fig 18 Optimal plate heights (H_{opt}) of a mixture of lipophilic dyes (linear flow-rate) *versus* migration distance (z_f) HPTLC plates silica gel, F_{254} (●) Ceres Violet BRN; (▼) Ceres Black G, (□) green component of Ceres Black G, (○) Blue VIF Organol, (■) Ceres Red G[39]

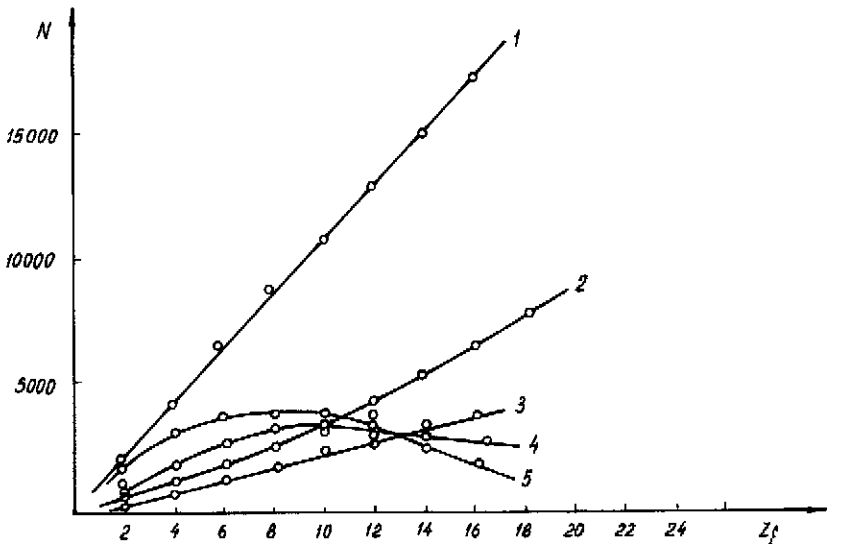


Fig 19 Dependence of theoretical plate number (N) on the development distance (z_f) 1, Linear OPTLC; 2, linear OPTLC with decreasing solvent flow-rate; 3, TLC in normal saturated chamber, 4, TLC in ultramicro chamber; 5, TLC in normal unsaturated chamber.

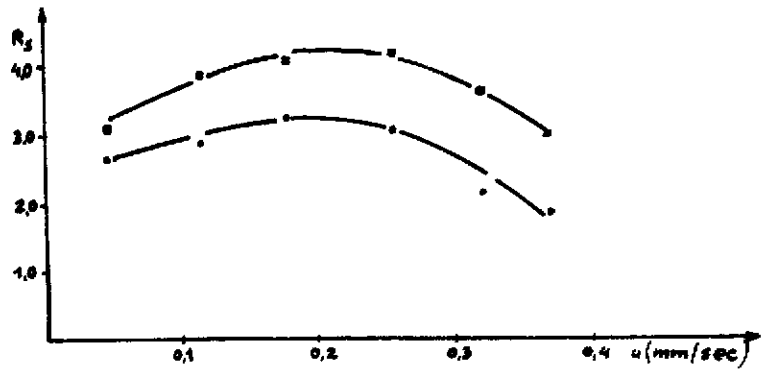


Fig. 20 Resolution (R_s) of a mixture of lipophilic dyes as a function of the linear flow-rate (u) of the eluent toluene. Migration distance, 15 cm HPTLC plates, silica gel 60 F_{254} (●) Ceres Black/Green; (■) Blue/Ceres Red[39].

7 PROPERTIES OF OPTLC AS AN ANALYTICAL METHOD AND EXAMPLES OF SEPARATIONS OF MIXTURES

The applications of pressure chambers in TLC are continuously expanding, especially with regard to the properties and applications of pressure chromatographic chambers for solving various analytical problems chiefly in biochemistry, agriculture,

foodstuffs and forensic medicine. OPTLC has been used for analysing, *e.g.**, dyes[17,40], amino acids and their derivatives[24,41–43], antibiotics, antioxidants, aromatic hydrocarbons, polyamines[44], quaternary ammonium compounds, purines, tobacco alkaloids[45], aliphatic aldehydes[22], organic amines and their derivatives[46], bile acids and their derivatives[47], doping agents[48], drugs and their metabolites, essential oils, glycolipids[25,49], glutathione, lipids, organic acids and their derivatives[50–52], peptides and their derivatives, phospholipids, steroids and their derivatives[53], volatile fatty acids, zearalenone, zearalenol, chemical warfare agents[54], digitalis glycosides, nucleosides[55,56], corticosteroids and poppy alkaloids[57] and aflatoxins[58].

Comparison of the results of the separation of the same mixtures (*e.g.*, of dyes)[40] in the pressure chamber and in different conventional (normal and ultramicro) chambers with the use of normal and high-performance chromatographic plates leads us to the conclusion that the separations are similar in all kinds of chambers but the diameters of the spots obtained in the pressure chamber are much smaller. This is due to smaller diffusion of the components of the mixtures separated in the same systems in pressure chambers compared with conventional chambers. This, together with the increased performance of the chromatographic system in the pressure chambers, yields significantly higher separability than that achieved so far in TLC.

Examples of the resolution of various mixtures by means of common and OPTLC chambers are shown in Figs. 21–23. In all instances the use of an OPTLC chamber gave better separations (in a shorter time) than those achieved with common chambers. It follows that considering the separations obtained the kind of chromatographic plate applied is of far less importance than the kind of chamber used. Examples given here, in addition to others (*e.g.*, in papers by Tyihak and co-workers[17,41], Szepesi *et al.*[47] and Horvath *et al.*[55]) showed that OPTLC gives better and often much better analytical results, including more efficient separations of mixtures, better detectability, and quantitative determinations and shorter analysis times than conventional TLC and HPTLC procedures.

OPTLC reveals its superiority over HPLC when a large number of samples have to be analysed, when some components of the samples are poorly detectable by the detectors used in HPLC so that it is necessary to use special reagents for their identification, when some components of the sample occurring in large amounts may cause overloading of the column and when some components of the sample remain at the start point.

OPTLC may be used in all analyses carried out by conventional TLC, including HPTLC, and gives better qualitative results in a shorter time. Separations of organic and inorganic compounds can be performed by OPTLC 5–20 times quicker than by ordinary TLC in the same chromatographic systems and with the same development distance[17]. Also, good separations of compounds with a wide polarity range are possible.

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* The analysis of compounds for which literature references are not given has been described either in advertisements from Labor Mim (*e.g.*, in application notes) or presented in the form of communications during chromatographic conferences. Data on the analytical conditions for some of these compounds are listed in Table 1 near the end of this review.

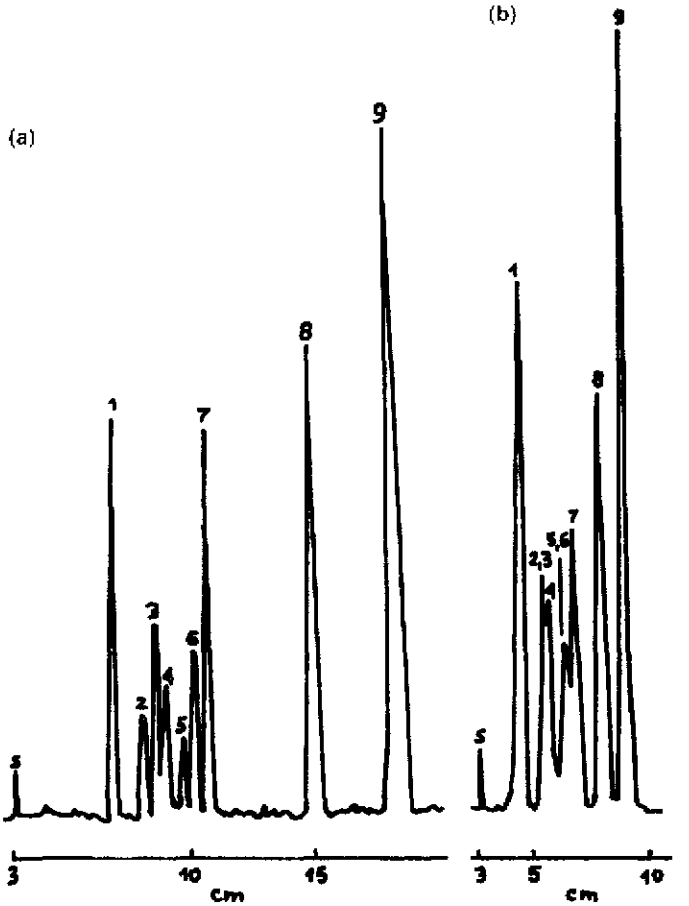

Fig 21. Densitogram of a mixture of doping agents in (a) a Chrompres 10 and (b) a conventional chamber 1 = Strychnine; 2 = ephedrine, 3 = methamphetamine; 4 = phenmetrazine, 5 = methylphenidate; 6 = amphetamine, 7 = desopimon; 8 = coramin, 9 = caffeine HPTLC silica gel 60 F_{254} (Merck). Eluent, *n*-butanol–chloroform–methyl ethyl ketone–water–acetic acid (25:17 8:4 6). OPTLC flow-rate, 0 85 cm/min[48]

The sensitivity of OPTLC determinations may be better by an order of magnitude or even more compared with conventional chambers The time of analysis of doping agents in urine as calculated per sample and per analyst is 8–10 min[48].

Some compounds can be chromatographed on plates with silica gel layers by using one-component eluents, *e.g.*, essential oils by using benzene or methylene chloride, corticosteroids by using nitromethane, ergot alkaloids by using acetonitrile, lipophilic dyes by using methylene chloride, bile acids and their derivatives by using ethyl acetate and dinitrophenyl hydrazones of aliphatic aldehydes by using diisoamyl or diisopropyl ether.

Many compounds are chromatographed on silica gel by using mixed eluents, *e.g.*, benzene–ethyl acetate (98:2) for essential oils and chloroform–ethyl acetate (95:5) for dimedone adducts of aliphatic aldehydes.

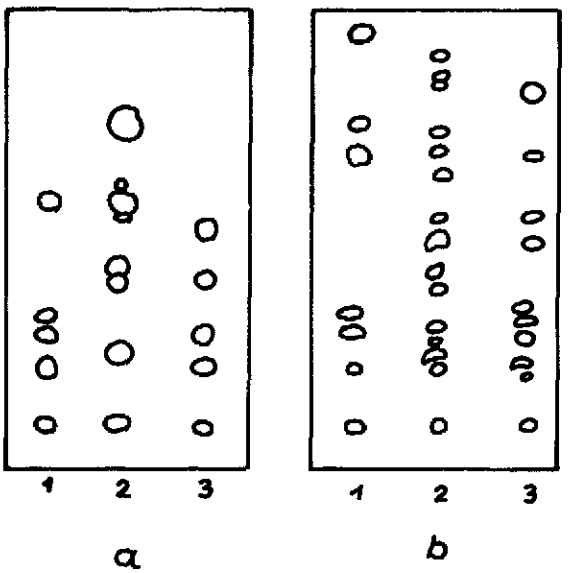

Fig 22 Separation of perfume components on HPTLC plates Eluent, chlorobenzene (a) In a common chamber; time of development 145 min (b) In a Chrompres 25 chamber, time of development 23 min[29]

When chromatographic plates with an adsorbent layer with a bonded phase are applied, use is made of commonly used aqueous eluents. For instance, acetonitrile–water and methanol–water mixtures are used for chromatographing poppy alkaloids[57], digitalis glycosides and nucleosides[55,56], and dioxane–water mixtures

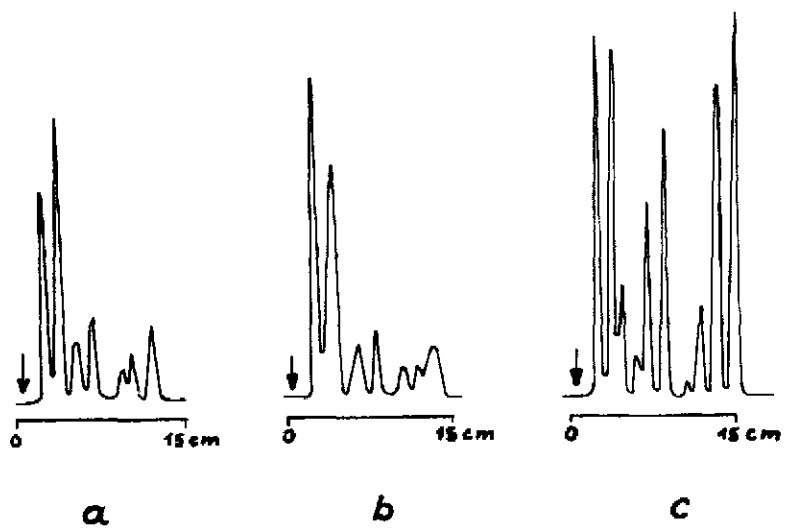

Fig. 23 Densitogram of dye mixture after separation on chromatoplates with silica gel layers Eluent, toluene (a) TLC plates in a common chamber, time of development 39 min (b) HPTLC plates in a common chamber, time of development 50 min (c) HPTLC plates in a Chrompres 25 chamber, time of development 12 min[29]

for amino acids*. Thin-layer cation-exchange chromatographic plates may also be used in OPTLC, for example for the separation of basic amino acids and neutral polyamines[44].

OPTLC may be used for carrying out separations by the ion-pair reversed-phase (IP-RP) technique on chromatographic plates with a silica gel layer or a bonded phase, *e.g.*, RP-18[46,50,51]. The times of these analyses are comparable to those achieved in HPLC. The plates must be treated prior to use with a solution containing an ion-pair reagent; it should be noted that the presence of this reagent in the solution has a strong effect on the relative retention of the chromatographed compounds. Ion pairs may be formed on plates with silica gel or a bonded phase treated with an ion-pair reagent even if the eluent does not contain this reagent. However, its presence in the eluent improves the shape of the spots and the separation of the mixtures.

A good separation of organic amines was obtained by reversed-phase ion-pair chromatography by using 10-camphorsulphonic acid as the ion-pair reagent[46]. The chromatographic plate with a silica gel layer was immersed prior to use first in cetrimide and then in 10-camphorsulphonic acid ethanolic solutions. The efficiency of the separation is increased in the presence of cetrimide (0.05 mole/l) and 10-camphorsulphonic acid (0.05 mole/l) in the eluent. The best eluent was methanol–aqueous phosphate buffer (pH 5.5).

An important feature of OPTLC is the possibility of predicting separations in a given chromatographic system and in seeking systems that will give the best HPLC results. The common TLC method is widely used for this purpose[59–62], but the properties of OPTLC make it particularly suitable[17].

The usefulness of OPTLC for predicting the effects of separations by HPLC has been demonstrated for organic acids on silica gel by the IP-RP technique[51]. The reverse procedure is also possible. This consists in predicting separations by OPTLC on the basis of tests carried out by HPLC. It has been found that a relationship exists between the relative retentions in OPTLC, $(R_{F0} - R_{F1})/R_{F0}$, where R_{F1} and R_{F0} are the values of R_F for the same compound in the presence and absence of the ion-pair reagent, respectively, and the capacity ratios in HPLC.

Reversed-phase ion-pair chromatography of ionizable compounds on silica using aqueous eluents may allow the separation of many mixtures that are very difficult to separate on bonded reversed phases.

With lipids the use of OPTLC gave better results than the use of common HPTLC[25], in which not all lipids are separated and development is extremely time consuming. In a single isocratic OPTLC development a neutral lipid extract obtained from human lymphocytes was separated into neutral glycolipids, cholesterol, glycerides and cholesterol esters. Initial development with chloroform (13 cm) followed by development with chloroform–methanol–water (65:25:4) (4 cm) made it possible to separate additionally the group of neutral glycolipids into tetrahexosyl, trihexosyl, dihexosyl and monohexosyl ceramide.

Two-dimensional separations of neutral lipid classes and simple glycolipids by developing in the first dimension with chloroform and in the second dimension with chloroform–methanol–water (65:25:4) have also been used. Good results have been obtained by separating neutral lipids first with chloroform (7 cm) and then with

* See footnote on p. 129.

chloroform in which the content of methanol was gradually increased to 50% (7 cm).

OPTLC has also been used for separating mixtures of acidic glycosphingolipids obtained from human blood[49]. Their separation into sulphatides and gangliosides can be obtained by isocratic development with chloroform–methanol (70:30). The separation of gangliosides in the presence of sulphatides was carried out by using chloroform–methanol–0.25% aqueous potassium chloride (55:36:9). Conditions of analysis were established such that the components of blood from patients with different leukaemias can be studied.

Fig. 24 shows a densitogram of phenylthiohydantoin amino acids after their separation by OPTLC, and some examples of analyses carried out with the use of OPTLC chambers are presented in Table 1.

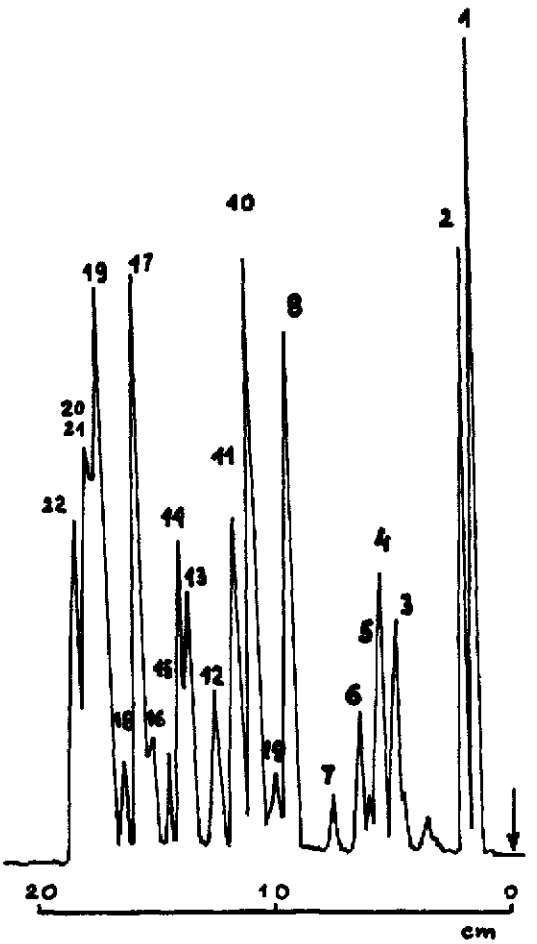

Fig. 24. Densitogram of phenylthiohydantoin amino acid mixture after separation of HPTLC silica gel F_{254}. Elution: First 16 cm with chloroform–ethanol (95%)–acetic acid (90:10:2), and then continuous development with dichloromethane–ethyl acetate (90:10). The total migration time was 60 min. 1 = Cysteic acid (K^+ salt); 2 = His; 3 = Asn; 4 = Gln, 5 = S-methylcysteine, 6 = Asp; 7 = Ser, 8 = Glu; 9 = Thr, 10 = Lys; 11 = Tyr, 12 = Gly; 13 = Trp; 14 = Ala; 15 = S-carboxymethylcysteine, 16 = Met; 17 = Phe, 18 = Val; 19 = Nle; 20 = Ile; 21 = Leu; 22 = Pro[45]

TABLE 1

EXAMPLES OF APPLICATIONS OF OPTLC

Compounds separated	*Eluent system*	*Chromatographic plates*	*Time of development*	*Ref.*
Dyes indophenol, sudan, butter yellow	Methylene chloride (80 cm^3/h)	TLC silica gel 60	4 5 min	17
Dimedone, acetaldomedone, formaldomedone, propionaldomedone, butyraldomedone	Acetonitrile–0.005 *M* KH_2PO_4 (4 6)	Silrevpress (bonded phase)		22
Neutral glycolipids· tetrahexosyl ceramide, trihexosyl ceramide, dihexosyl ceramide, monohexosyl ceramide, cholesterol, glycerides, cholesterol esters	Chloroform Chloroform methanol–water (65 25 4) (0 15–0 20 cm^3/min)	HPTLC silica gel 60, 100 × 200 mm (Merck)		25
Camag test dye mixture I· butter yellow, sudan G, indophenol	Methylene chloride (20 cm^3/h)	TLC and HPTLC aluminium sheets, silica gel 60 F_{254}	12.9 min (TLC), 13 3 min (HPTLC)	40
21 amino acids	*n*-Butanol–acetic acid–water (4 1 1) (10 cm^3/h)	HPTLC silica gel 60 F_{254}	47 min or continuous development, 70 min	41
2,4-Dinitrophenylamino acids, ether soluble	Chloroform–carbon tetrachloride–methyl ethyl ketone–propanol–methanol–acetic acid (30.30.20·30·15·2) (10 cm^3/h)	HPTLC silica gel 60 F_{254}	35 min	42
2,4-Dinitrophenylamino acids, water soluble	*n*-Propanol–conc. NH_3 solution (7 3)	HPTLC silica gel 60 F_{254}		42
Phenylthiohydantoinamino acids	Chloroform–ethanol (95%)–acetic acid (90 10 2) Dichloromethane–ethyl acetate (90·10) (0 30 cm^3/min)	HPTLC silica gel F_{254}	60 min, continuous development	43
Polyamines ornithine, arginine, putrescine. spermidine, spermine and tissue extract of mouse	300 mmol/l potassium phosphate buffer (pH = 7 5) containing 2 mmol/l sodium chloride	Fixion 50-X8, TL cation-exchange chromatosheet, 200 × 200 mm	30 min, continuous development	44

Tobacco alkaloids nicotine, nor-nicotine, anabazine, nicotyrine, 2,2'-dipyridyl	Ethyl acetate–methanol–water (12.3.5 3)	HPTLC silica gel 60 F_{254}		45
o-Phenylenediamine, *p*-phenylenediamine, aniline, *o*-nitroaniline, *p*-nitroaniline, 3,4-diaminobenzophenone, ethionamide	Methanol–aqueous phosphate buffer (35·65) containing 0 05 mole/l of cetrimide and 0 05 mole/l of camphorsulphonic acid (pH 5 5)	RP TLC plate KC-18 (Whatman)		46
Chenodeoxycholic acid and related compounds	Chloroform–ethyl acetate–glacial acetic acid–2-methoxyethanol (9:9.2.1)	HPTLC silica gel 60 F_{254}		47
Bile acids and their taurine and glycine conjugates	Isooctane–ethyl acetate–glacial acetic acid (5.4.1) Chloroform–*n*-butanol–glacial acetic acid–water (2.6 1.1)	HPTLC silica gel 60 F_{254}		47
Doping agents. strychnine, ephedrine, methamphetamine, phenmetrazine, methylphenidate, amphetamine, desopimon, coramin, caffeine	*n*-Butanol–chloroform–methyl ethyl ketone–water–acetic acid (25 17 8 4 6) (0 85 cm/min)	HPTLC silica gel 60 F_{254}, 200 × 200 mm	25 min, continuous development	48
Sulphatides and gangliosides class separation	Chloroform–methanol (70·30) (0.1 cm^3/min)	HPTLC silica gel 60	7 min	49
Gangliosides in the presence of interfering sulphatides	Chloroform–methanol–0 25% aqueous KCl (55 36·9) (0 1 cm^3/min)	HPTLC silica gel 60		49
Organic acids, phenol, cresol, resorcinol, pentachlorophenol	Different mixtures containing methanol, tetrahydrofuran, acetonitrile and cetrimide	Polygram Sil G/UV_{254} Silica gel 60 F_{254} RP TLC plate KC-18 (Whatman)		50
Quaternary ammonio steroids Morphine derivatives	Mixtures of water with acetone or methanol or dioxane in various concentrations	Silica gel impregnated with 5% paraffin oil in *n*-hexane Silica gel 60, silanized (Merck)		53

(Continued on p 136)

TABLE 1 *(continued)*

Compounds separated	*Eluent system*	*Chromatographic plates*	*Time of development*	*Ref*
Chemical warfare agents: chloroacetophenone, *o*-chlorobenzalmalononitrile (CS), adamsite, lewisite, soman, sarin, VX, mustard gas, BZ	Acetone–carbon tetrachloride (1:4)	TLC silica gel 60, 80 × 100 mm	75 s	54
Aflatoxins B_1, B_2, G_1, G_2 and M_1, quantitative at the nanogram level	Chloroform–ethyl acetate–tetrahydrofuran (8:12:0.6) (0 58 cm^3/min)	HPTLC silica gel 60, 200 × 200 mm	8 min	56
Human plasma lipids. gangliosides, simple neutral glycolipids, sulphatides, cholesterol, glycerides, cholesterol esters	Two-step gradient (1) chloroform–methanol (95:5), (2) chloroform–methanol–water (60 35:3) Three-step gradient: (1) chloroform–methanol (95:5), (2) chloroform–methanol–water (60:35:3), (3) chloroform–methanol–0 25% aqueous KCl (55.36:9) (0 1 cm^3/min)	HPTLC silicagel, 100 × 200 mm (Merck)		63
Chamomile oil components farnesene, chamazulene, *cis*-en-in-dicycloether, (−)-α-bisabolol, (−)-α-bisabolol-oxide A, (−)-α-bisabolol-oxide B	*n*-Heptane methylene chloride nitrobenzene (36.50:14) Methylene chloride–cyclohexane (70:30) Methylene chloride–carbon tetrachloride (50:50) Methylene chloride–nitrobenzene (70:30) Methylene chloride–nitroethane (80:20) Methylene chloride–nitromethane (80:20) (1 1 cm/min)	TLC aluminium sheets silica gel 60 F_{254} (Merck)		66

Digitalis glycosides. lanatoside A, lanatoside B, lanatoside C, desacetyl C, gitoxin, digoxin, digitoxin, acetyldigitoxin, digitoxigenin, digoxigenin, gitoxigenin, gitaloxigenin, gitaloxin	Ethyl acetate Methyl ethyl ketone Ethyl acetate–acetone (8·2) Methyl ethyl ketone–dichloromethane (8·2) (20 cm^3/h)	TLC and HPTLC aluminium sheets silica gel 60 F_{254} (Merck) TLC silica gel G (Merck)		67
Tobacco alkaloids	Ethyl acetate–chloroform–methanol–water (14 14 30·10)	HPTLC silica gel 60 F_{254} Kieselgel 60 silanisiert		64
Cannabinoids	Dichloromethane Dichloromethane–ethyl acetate (9 1) Cyclohexane (with layers impregnated with dimethylformamide)	HPTLC silica gel 60 F_{254}		65
Volatile fatty acids in fermented feed lactic acid, acetic acid, propionic acid, butyric acid, valeric acid	Acetone–methyl ethyl ketone–pyridine–cyclohexane–carbon tetrachloride (8:3·1:22·6) (0.25 cm^3/min)	HPTLC silica gel 60	15 min	Application Notes No. 1, Labor MIM (Hungary)
Stimulants· strychnine, gracidine, desopimone, ephedrine, amphetamine, caffeine, metamphetamine, coramine, centedrine	*n*-Butanol–chloroform–methyl ethyl ketone–water–acetic acid (12.5·8.5·5:4·2·3)	HPTLC silica gel 60 F_{254}	23 min (with overrunning)	Application Notes No. 2, Labor MIM
2,4-Dinitrophenylamino acids (ether soluble)	Chloroform–carbon tetrachloride–methyl ethyl ketone–propanol–methanol–acetic acid (30:30.20:30.15:2) (0 17 cm^3/min)	HPTLC silica gel 60	35 min	Application Notes No 3, Labor MIM
Amino acids in feed	*n*-Butanol–water–acetic acid (4 1:1) (0.07 cm^3/min)	HPTLC silica gel 60	70 min (with overrunning)	Application Notes No 4, Labor MIM

Apart from its analytical applications, OPTLC may also be used for preparative separations. The bands of the particular substances are well separated and reveal distinct edges.

From the data presented above, it can be seen that OPTLC may be used for various purposes both in research and in routine analyses.

The apparatus for OPTLC is expensive and the cost of one analysis is higher than with conventional TLC chambers. However, it is less expensive than HPLC. Nevertheless, the results of analyses obtained by OPTLC are so good that we should expect an extension of the range of applications of this method, which may take place at the cost of HPLC.

8 SUMMARY

The properties, apparatus and applications of overpressured thin-layer chromatography (OPTLC) have been reviewed. In the Introduction planar chromatography has been briefly characterized, with particular attention to TLC The general properties of OPTLC and methods of development of chromatograms in this technique have been then given. The construction of chambers and equipment for OPTLC has been described, paying attention to two-dimensional columns. The properties of chambers for OPTLC have been characterized considering the flow of eluent, sorbent–eluent interactions and the efficiency of various systems. OPTLC, TLC and HPTLC have been compared and also a comparison between OPTLC with a constant (linear) eluent flow-rate and with a decreasing eluent flow-rate has been made Analytical applications of OPTLC have been described and examples of separations of mixtures have been given.

REFERENCES

1 R E Kaiser (Editor), *Einfuhrung in die Hochleistungs-Dünnschicht-Chromatographie*, Institute of Chromatography, Bad Durkheim, 1976
2 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.
3 D. C. Fenimore and Ch M Davis, *Anal. Chem.*, 53 (1981) 252A.
4 J Sherma and B Fried, *Anal Chem* , 56 (1984) 48R
5 H Halpaap and K.-F Krebs, *J Chromatogr* , 142 (1977) 823
6 H. Halpaap, *Kontakte (Merck)*, No. 1 (1978) 32
7 V. G. Berezkin and A S Bochkov, *Kolichestviennaja Tonkoslomaja Khromatografia*, Nauka, Moscow, 1980
8 J Błądek and Z. Witkiewicz, *J. Chromatogr.*, in preparation
9 R E Kaiser, *J High Resolut Chromatogr Chromatogr Commun* , 1 (1978) 164
10 K. Kariko and J Tomasz, *J High Resolut. Chromatogr. Chromatogr Commun* , 2 (1979) 247
11 E Soczewiński, *J Chromatogr* , 138 (1977) 443
12 E Soczewiński and T Wawrzynowicz, *Chromatographia*, 11 (1978) 466
13 E Soczewiński, in R E Kaiser (Editor), *Planar Chromatography*, Vol 1, Hüthig, Heidelberg, 1986
14 A S Kamev, O. A Rysiev and V N Chechevichkin, *Zavod Lab* , 47 No 4 (1981) 13
15 Sz. Nyiredy, C A J Erdelmeier and O Sticher, *J High Resolut Chromatogr Chromatogr Commun.*, 8 (1985) 73
16 E Tyihak, H Kalasz, E. Mincsovics and J Nagy, *Proc. Annu. Hung Biochem Meet* , *Kecskemet*, (1977) 49, *C A* , 88 (1978) 15386u
17 E Tyihák, E Mincsovics and H. Kalász, *J. Chromatogr* , 174 (1979) 75.
18 E Tyihak and G. Held, in A Niederwieser and G Pataki (Editors), *Progress in TLC and Related Methods*, Vol II, Ann Arbor Sci Publ , Ann Arbor, MI, 1971, p. 183

19 E Tyihak, E. Mincsovics and F. Körmendi, *Hung Sci. Instrum*, 55 (1983) 33.
20 H. Kalasz, *Chromatographia*, 18 (1984) 628.
21 H. Kalasz, E Tyihak and E. Mincsovics, in A Frigerio and M McCamish (Editors), *Recent Developments in Chromatography and Electrophoresis*, Vol. 10, Elsevier, Amsterdam, 1980, p 289
22 Z Lengyel, E. Tyihak and E Mincsovics, *J Liq Chromatogr*, 5 (1982) 1541
23 E Tyihak and E. Mincsovics, *Hung Sci Instrum.*, 57 (1984) 1.
24 J M Newman, *Lab. Pract*, 34 (1985) 65
25 J Pick, J. Vajda and L. Leisztner, *J. Liq. Chromatogr*, 7 (1984) 2759.
26 H. Kalasz, J Nagy, E Mincsovics and E Tyihak, *J Liq Chromatogr.*, 3 (1980) 845
27 H. J. Issaq, *J Liq. Chromatogr*, 3 (1980) 789
28 Z Witkiewicz and S Rattay, *Farm Pol*, 39 (1983) 407
29 *Labor MIM Prospektus Chrompres 25*, Labor MIM, Budapest, 1984
30 J Błądek and Z. Witkiewicz, *Biul. Wojsk. Akad Tech*, 34, No 5 (1985) 117; *Chem. Anal*, in press.
31 G Guiochon, M F Gonnord, M Zakaria, L. A. Beaver and A. M. Siouffi, *Chromatographia*, 17 (1983) 121
32 G Guiochon, L A Beaver, M. F Gonnord, A M. Siouffi and M Zakaria, *J Chromatogr*, 225 (1983) 415
33 J Blome, in A Zlatkis and R. E Kaiser (Editors), *HPTLC —High Performance Thin-Layer Chromatography*, Elsevier, Amsterdam, 1977, p. 53.
34 R. E Kaiser, in A Zlatkis and R E. Kaiser (Editors), *HPTLC —High Performance Thin-Layer Chromatography*, Elsevier, Amsterdam, 1977, p 76
35 S J MacDonald and E F Barry, *J. High Resolut Chromatogr. Chromatogr Commun*, 8 (1985) 265.
36 M Martin, G Blue, C Eon and G. Guiochon, *J Chromatogr Sci*, 12 (1974) 438
37 G Guiochon and A Siouffi, *J Chromatogr Sci.*, 16 (1978) 470
38 H Kalasz and J Nagy, *J. Liq. Chromatogr*, 4 (1981) 985.
39 H. F. Hauck and W Jost, *J Chromatogr.*, 262 (1983) 113
40 E. Mincsovics, E. Tyihák and H Kalász, *J Chromatogr.*, 191 (1980) 293.
41 E. Tyihák, E. Mincsovics, H. Kalász and J Nagy, *J Chromatogr*, 211 (1981) 45
42 Ngo Tuan Cong, E Tyihák, M Vajda and E Mincsovics, *J. High Resolut. Chromatogr Chromatogr. Commun.*, 5 (1982) 511.
43 S. Fatér and E Mincsovics, *J Chromatogr.*, 298 (1984) 534
44 S. Bardocz, T Karsai and P Elodi, *Chromatographia*, 20 (1985) 23.
45 Sz. Nyiredy, I Tompa, S Y. Meszaros, E Tyihák and G Verzar-Petri, *4th Danube Symposium on Chromatography and 7th International Symposium on Advances and Application of Chromatography in Industry, Bratislava, Chechoslovakia, 29th August–2nd September, 1983, Abstracts*, Vol II, C 18
46 G Szepesi, M Gazdag, Zs. Pap-Sziklay and Z Vegh, *Chromatographia*, 19 (1984) 422.
47 G Szepesi, K Dudás, A Pap, Z Végh, E. Mincsovics and T Tyihák, *J Chromatogr.*, 237 (1982) 137
48 H Gulyás, G. Kemény, I. Hollósi and J Pucsok, *J. Chromatogr.*, 291 (1984) 471
49 J Pick, J Vajda, N Anh-Tuan, L. Leisztner and S R Hollan, *J. Liq. Chromatogr*, 7 (1984) 2777
50 G. Szepesi, Z. Végh, Z Gyulay and M Gazdag, *J Chromatogr.*, 290 (1984) 127.
51 M Gazdag, G. Szepesi, M Hernyes and Z Végh, *J Chromatogr*, 290 (1984) 135
52 Z. Végh, Z Gyulai, C. Szepesi and E Tyihák, *4th Danube Symposium on Chromatography and 7th International Symposium on Advances and Application of Chromatography in Industry, Bratislava, Chechoslovakia, 29th August- 2nd September, 1983, Abstracts*, Vol II, E 50
53 E. Janos, T. Cserhati and E Tyihák, *J High Resolut Chromatogr. Chromatogr Commun*, 5 (1982) 634
54 Z. Witkiewicz and S Rattay, *Biul. Wojsk Akad Tech*, 32, No. 2 (1983) 113.
55 P Horvath, G. Szepesi, M Hoznek, Z. Vegh and E Mincsovics, in R E. Kaiser (Editor), *Proceedings of the 2nd International Symposium on Instrumental High Performance TLC, Interlaken*, Institute for Chromatography, Bad Durkheim, 1982, p 295.
56 E Mincsovics, T Szekely, M Hoznek, Z Végh, I Zambo, G. Szepesi and E Tyihák, in S. Górog (Editor), *Advances in Steroid Analysis*, Elsevier, Amsterdam, 1982, p. 427.
57 E Tyihák, T J Szekely and E Mincsovics, in R E Kaiser (Editor), *Proceedings of the 2nd International Symposium on Instrumental High Performance TLC, Interlaken*, Institute for Chromatography, Bad Durkheim, 1982, p 159
58 H Gulyas, *J Chromatogr*, 319 (1985) 105
59 W Jost, H E Hauck and F Eisenbeiss, *Kontakte (Merck)*, No 3 (1984) 45

60 E Soczewiński and W. Gołkiewicz, *J Chromatogr*, 118 (1976) 91.
61 E Soczewiński, T. Dzido, W Gołkiewicz and K Gazda, *J. Chromatogr.*, 131 (1977) 408.
62 T Dzido and E Soczewiński, *J High Resolut Chromatogr Chromatogr Commun.*, 2 (1979) 88
63 J Vajda, L Leisztner, J Pick and N Anh-Tuan, *Chromatographia*, 21 (1986) 152
64 Sz. Nyiredy, I Tompa, K Robicsek, M. Barsony, S Y. Meszaros and G Verzar-Petri, *4th Danube Symposium on Chromatography and 7th International Symposium on Advances and Application of Chromatography in Industry, Bratislava, Czechoslovakia, 29th August–2nd September, 1983, Abstracts*, Vol II, C 17
65 P. Oroszlan, G Verzar-Petri, M Elek, E. Mincsovics and A Vastag, *4th Danube Symposium on Chromatography and 7th International Symposium on Advances and Application of Chromatography in Industry, Bratislava, Czechoslovakia, 29th August–2nd September, 1983, Abstract*, Vol II, C 19
66 E. Mincsovics, E Tyihak, J Nagy and H Kalasz, *Acta Horticulturae*, 96 (1980) 181
67 E Tyihak, E Mincsovics, P Tetenyi, I. Zambo and H Kalasz, *Acta Horticulturae*, 96 (1980) 113