Journal of Pharmaceutical & Biomedical Analysis
Vol. 5, No. 3, pp. 191–203, 1987
Printed in Great Britain

0731–7085/87 $3.00 + 0.00
Pergamon Journals Ltd.

Review

Overpressured layer chromatography and its applicability in pharmaceutical and biomedical analysis

E. TYIHÁK

Plant Protection Institute, Hungarian Academy of Sciences, Budapest P.O. Box 102, H-1525 Hungary

Abstract: The principles and potential of overpressured layer chromatography (OPLC) are presented and illustrated by some applications in the field of pharmaceutical and biomedical analysis. The efficiency of analytical and preparative OPLC systems depends on the optimum solvent front velocity, the quality of sorbent layer and sample application. OPLC as an instrumentalized version of planar liquid chromatography offers distinct advantages in the analysis of various chemical substance groups over TLC and HPTLC. The full exploitation of these advantages in future work is an expected development.

Keywords: *Thin-layer chromatography (TLC); high-performance thin-layer chromatography (HPTLC); overpressured layer chromatography (OPLC); high-performance liquid chromatography (HPLC).*

Introduction

The essential features of classical thin-layer chromatography (TLC) as a basic planar liquid chromatographic technique are that the solvent mixture migrates through the sorbent layer by capillary forces and there is a vapour phase of solvent above the sorbent layer [1–3]. These phenomena have an important influence on the resolving power and peak capacity which are significantly smaller in classical TLC than in modern column liquid chromatographic methods [4–6].

It is well-known that column and planar liquid chromatographic techniques — as supplementary techniques due to their arrangement — have characteristically developed in constant mutual interaction. Hence it is not surprising that the intensive development of high-performance liquid chromatography (HPLC) entailed the need for the fundamental renewal of thin-layer chromatography (TLC) as the most popular planar liquid chromatographic technique. It is known that planar liquid chromatography in general has real advantages (e.g. application of a relatively large number of samples during one separation; possibility of two-dimensional separation; use of multi-layer

systems; applicability of specific reagents for the detection of various substance groups) over column liquid chromatographic techniques. The exploitation of these advantages is a logical development.

Modern methods of column liquid chromatography employ constant flow rates [4–7] which have not hitherto been achievable in TLC [8–10]. However, the problems of greatly increased development time on a fine-particle size (5–6 μm) sorbent layer [11–13] (high-performance thin-layer chromatographic (HPTLC) chromatoplates) made it necessary to employ forced flow. The need for forced flow is also caused by the problems of optimization of the flow rate and reproducibility of development.

These aims were realised by development of overpressured layer chromatography (OPLC) [14–16] using in this case a pressurized ultramicro chamber and an additional overpressure for the admission of the eluent.

Apparatus

Characteristics of the pressurized ultramicro chamber

The essential feature of this chamber system is that the sorbent layer is completely covered with a flexible membrane under an external pressure so that the vapour phase above the layer is virtually eliminated [14]. In this chamber system it is possible to optimise the flow velocity of the eluent by means of a pump.

The principle of a pressurized ultramicro chamber made of polymethylmethacrylate, and used mainly for circular separation is illustrated in Fig. 1. The sorbent layer with the sample spots is placed on the polymethylmethacrylate support plate. The cover plate also made of polymethylmethacrylate is equipped with a pressure-gauge, water inlet and O-ring, to hold the plastic foil, plastic membrane and inlet for the developing solvent. The two plates are joined with clamps, and the cushion is filled with water.

Based on the experiences gained with the experimental pressurized chambers, the LABOR Instrument Works (Budapest–Esztergom, Hungary) developed CHROMPRES 10 and CHROMPRES 25, the first commercial pressurized ultramicro chambers.

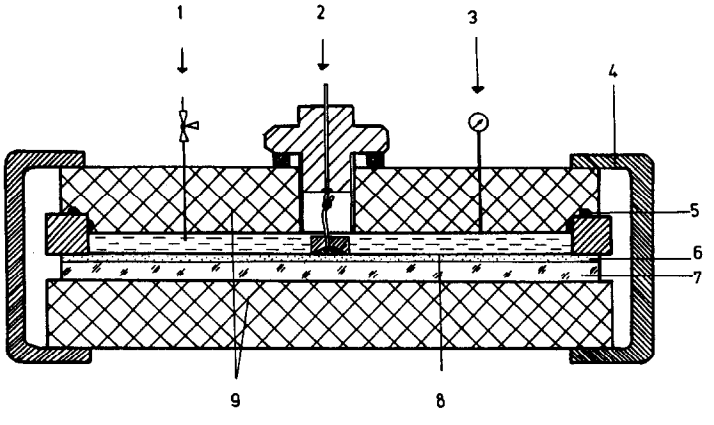

Figure 1
Schematic drawing of the circular type pressurized ultramicro chamber. (1) Water inlet; (2) inlet of developing solvent; (3) pressure gauge; (4) screw fastener; (5) rubber O-ring; (6) sorbent layer; (7) support plate; (8) cushion system of plastic foil; (9) polymethacrylate support blocks.

The main structural elements of CHROMPRES 10 are shown in Fig. 2. This chamber can be connected to liquid micro pumps of varying performance. The cushion is pressurized up to a given external pressure with a liquid pump, while the solvent is fed by another pump, having a smaller performance. The maximum cushion pressure permitted in this chamber is 1.0 MPa.

CHROMPRES 10 can be used with plastic, aluminium or glass chromatoplates of max. 20 × 40 cm, coated with fine-particle (5–6 μm) sorbent, although good separations can also be obtained on smaller size chromatoplates (e.g. 10 × 10 cm and 10 × 20 cm). Special plastic inserts permit the use of glass chromatoplates of different sizes.

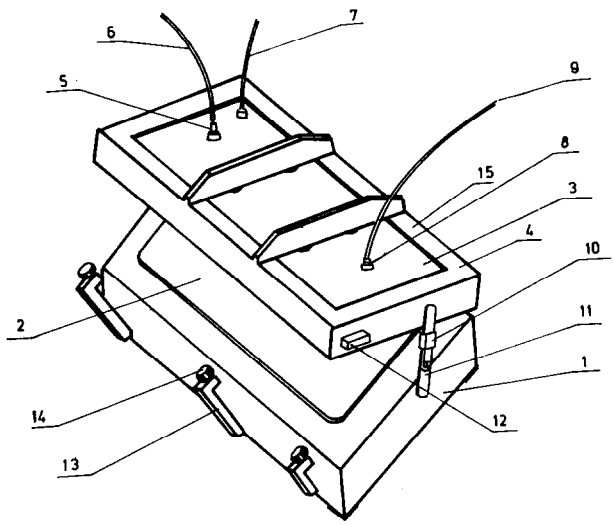

Figure 2
CHROMPRES 10 pressurized ultramicro chamber (LABOR MIM, Esztergom–Budapest, Hungary). (1) Bottom support block; (2) working place; (3) upper block with polymethacrylate support plate; (4) external frame; (5) solvent inlet; (6) solvent connection; (7) air-valve; (8) water inlet; (9) water inlet tube; (10) weight lock; (11) support; (12) handle; (13) clamping stirrup; (14) stirrup fixing; (15) countersunk screw.

The main structural elements of CHROMPRES 25 are shown in Fig. 3. The maximum cushion pressure permitted in this chamber is 2.5 MPa. This higher external pressure on the elastic membrane permits both the use of superfine-particle size (2–3 μm) sorbent layers and at the same time an increase of the solvent front velocity. There is need for these possibilities because the eluent inlet pressure increases in CHROMPRES chambers linearly with decreasing particle size and increasing development distance. In addition the optimum eluent front velocity is higher in a super-fine particle (2–3 μm) sorbent layer than in a fine-particle (5–6 μm) sorbent layer and the increase of eluent front velocity means a higher solvent inlet pressure. It is preferable to use a chamber operating under high external pressure than one using high eluent inlet pressure.

CHROMPRES 25 can be used with different chromatoplates of max. 20 × 20 cm, coated with fine-particle sorbent, but in this chamber system smaller size chromatoplates can also be used similar to CHROMPRES 10.

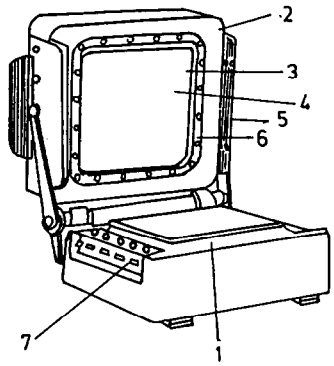

Figure 3
CHROMPRES 25 pressurized ultramicro chamber (Labor MIM, Esztergom–Budapest, Hungary). (1) Bottom support block; (2) cover plate; (3) closing ring; (4) cushion system; (5) cover plate handle; (6) screw; (7) locking device.

Preparation of chromatoplates for OPLC

The OPLC technique of linear development requires a special chromatoplate sealed at the edge. This prevents the eluent from flowing off the chromatoplate in an unwanted direction. The linear migration of solvent in an OPLC chamber using a linear developing mode can be achieved by placing a narrow plastic sheet on the layer or making a narrow channel in the layer ahead of the solvent inlet. The function of the solvent trough is to direct the solvent and to form a linear solvent front.

In practice a polythene or teflon insert with solvent trough is placed between the sorbent layer and the water cushion to protect the cushion and to direct the solvent in a linear front. When a polythene or a teflon insert is used without solvent trough, the trough should be scraped into the sorbent layer. It is obvious that for one-directional development a plate sealed on three sides should be used (Fig. 4).

If we seal two opposite sides of the chromatoplate and the solvent inlet is in the middle of the sorbent layer in a channel, this system is suitable for a two-directional separation with a large number of samples (Fig. 5).

Using a two-dimensional separation in an *off-line* system we have to seal the four sides of the chromatoplate beforehand and the seal opposite to the actual inlet should be covered with a strip of filter paper.

Analytical OPLC

The main part of the OPLC separation unit is the pressurized ultramicro chamber. One pump delivers water to the pressurized chamber. The solvent pump delivers solvent

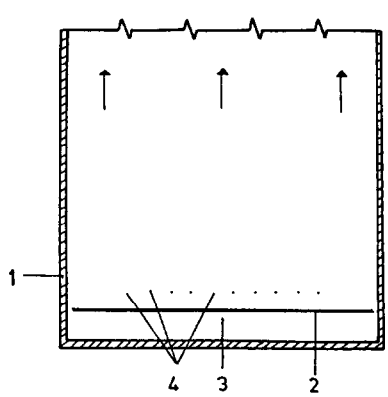

Figure 4
Special precoated plate for linear OPLC for one-directional development. Schematic drawing. (1) Impregnated edge; (2) channel in the layer; (3) solvent inlet; (4) place of the samples.

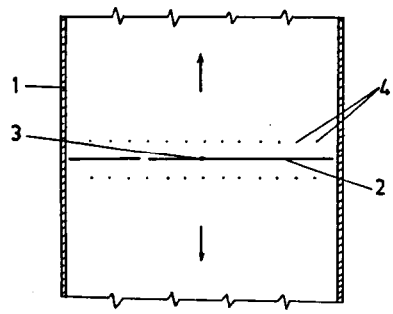

Figure 5
Special precoated chromatoplate for linear OPLC for two-directional development. Schematic drawing. Captions as in Fig. 4.

with overpressure into the pressurized ultramicro chamber. The sample applicator or micrometer syringe is used for sampling.

Figure 6 illustrates the analytical OPLC system which has three main parts: OPLC separation unit, detection unit and data processing.

The main procedures of analytical OPLC are sample application, chromatogram development in the pressurized ultramicro chamber, densitometric chromatogram scanning, result computation and result presentation. The auxiliary procedures are, e.g. sample preparation, selection of the chromatogram layer and UV-inspection, and, there are also optional steps as layer prewashing, in-situ derivatization, layer preconditioning and post chromatography derivatization.

Preparative OPLC

The OPLC system is applicable for analytical and preparative investigations, alike. According to our preliminary experiences it seems that the ratio between the two application modes will be about 1:1. Figure 7 demonstrates the preparative OPLC system.

In preparative OPLC one can isolate from the layer only the components of preparative interest, but in the case of preparative HPLC — similar to analytical HPLC — the instrumentation is occupied by the sample until all components have been eluted. Every component, preparatively relevant or not must run through the entire system.

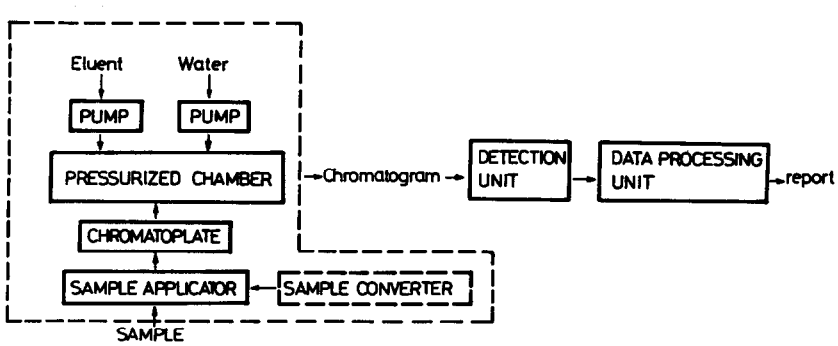

Figure 6
Schematic drawing of the analytical OPLC system.

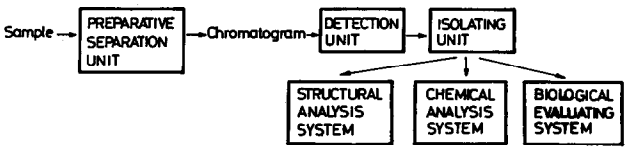

Figure 7
Schematic drawing of the preparative OPLC system.

Theory of OPLC

Characteristics of solvent migration in OPLC

It is known that in classical TLC and also in HPTLC a parabolic flow velocity law is valid as in these cases the area on the sorbent layer to be wetted increases quadratically with the development distance. The relationship between the development distance (z_f) and development time (t) can be described by parabolic equation:

$$z_f^2 = kt,$$

where k is a constant depending on the chromatographic system (mobile phase, sorbent) and the size of sorbent particles [8, 10]. Thus, the rate of migration of the solvent mixture in the TLC and HPTLC systems, caused by capillary forces under control of the vapour atmosphere above the sorbent layer, decreases with the advance of the solvent front.

In the case of OPLC there is a linear relationship between the distance (z_f) of the solvent front from the starting point and the developing time (t) [17]:

$$u = \frac{z_f}{t},$$

where u primarily depends on the rate of solvent flow, on the external cushion pressure and on the size of the particles constituting the layer. In principle, u is constant throughout the development, and is usually higher at a given sorbent and solvent mixture than in a TLC or HPTLC development. Figure 8 illustrates clearly the fundamental difference between the conditions of reversed-phase chromatoplates. This relationship shows that the mobile phase flows at a constant velocity only in linear OPLC.

Theoretical plate height in OPLC

Figure 9 illustrates the relationship between average theoretical plate height ($\bar{H}$) and the development distance (z_f). In the case of fine-particle sorbent layer the average theoretical plate height increases with the development distance using, e.g. HPTLC chamber. On the contrary, in linear OPLC only a slight increase can be observed even over longer distances and also in the case of fine-particle sorbent layers [15]. The major advantage of OPLC over other planar liquid chromatographic techniques lies in this fact.

Optimum eluent flow rate in OPLC

It is known that in HPLC there is a characteristic relationship between average theoretical plate height and flow velocity (u). The variation of efficiency of OPLC can

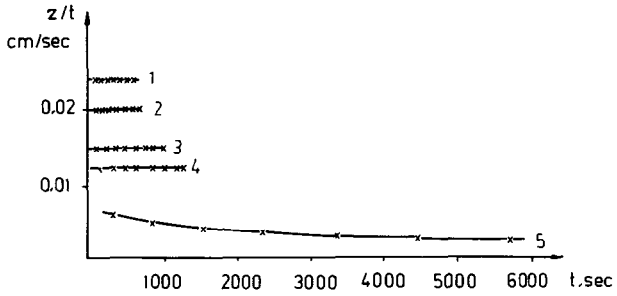

Figure 8
Variation of solvent front velocity ($z/t = u$) with time (t) in normal (N) and CHROMPRES 10 pressurized ultramicro chambers using Revsilpres N experimental reversed-phase chromatoplate. Eluent: acetonitrile–0.005 M KH_2PO_4 (1:9, v/v); external pressure on membrane at CHROMPRES 10 chamber; 0.25 MPa; migration distance: 15 cm; (1) CHROMPRES 10 chamber, 25 $cm^3\ h^{-1}$, 11.3 min; (2) same chamber, 20 $cm^3\ h^{-1}$, 12.2 min; (3) same chamber, 15 $cm^3\ h^{-1}$, 17 min; (4) 10 $cm^3\ h^{-1}$, 22 min; (5) normal chamber, 95.2 min.

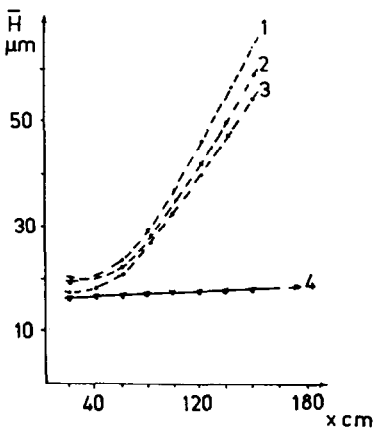

Figure 9
Correlation between average theoretical plate height ($\bar{H}$) and the distance (x) travelled by the eluent on silica gel layer and in various chamber systems. Solvent: methylene chloride; material: Butter Yellow. (1) N saturated chamber; (2) N unsaturated chamber; (3) UM chamber; (4) CHROMPRES 10 chamber; d_p = 5–5 µm.

also be determined by adjusting the solvent flow by means of a pump system, and the optimum solvent front velocity [18] is always higher than that of TLC or HPTLC. Figure 10 demonstrates such a relationship.

This is the basic analogy between OPLC and HPLC and at the same time this is the basic difference between OPLC and TLC (and HPTLC).

Some Applications of OPLC in Pharmaceutical and Biomedical Analysis

OPLC offers distinct advantages in the analysis of different substance groups over TLC or HPTLC and partly HPLC:

simultaneous efficient separation of a large number of samples;
accurate modelling of column chromatographic conditions;
minute consumption of developing solvent;
improved possibilities for quantitative evaluation;
possibility for the optimization of resolution as a function of solvent front velocity, development distance and temperature.

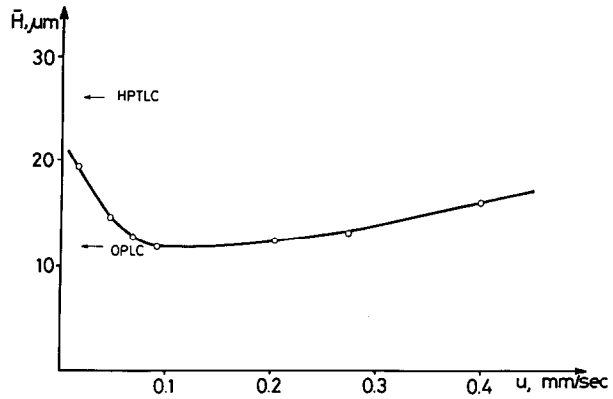

Figure 10
Relationship between average theoretical plate height ($\bar{H}$) and eluent front velocity (u) in OPLC. Sorbent: HPTLC silica gel 60 (Merck) with sealed edges; eluent: 0.9% NaCl solution; reagent: ninhydrin; substance: N^{ε}-formyl-L-lysine; CHROMPRES 10 chamber; external pressure on membrane: 1.4 MPa. For HPTLC separation on an unmodified fine-particle chromatoplate higher $\bar{H}$ and smaller average u values are characteristic than in OPLC.

Determination of formaldehyde and its reaction products

According to recent investigations formaldehyde can be produced in animals and man from various methylated drug compounds [19] and, e.g. from nitroseamines [20] by the effect of demethylase enzymes, or in the presence of aromatic hydrocarbons [21] or as a result of different stress effects (e.g. virus infections [22]). Thus, much needs to be done to improve analytical methods for the analysis of formaldehyde in various biological samples.

There are already GC, GC–MS and other methods [23–25] for the efficient determination of formaldehyde in biological samples but the exploitation of the advantages of planar liquid chromatography for the measurement of formaldehyde (e.g. short separation time, application of a large number of samples during one separation, possibility of perpendicular evaluation) is an important development.

Figure 11 demonstrates the separation of formaldemethone (a dimedone adduct of formaldehyde) in a fish blood sample by means of OPLC. It can be seen that the method is suitable for the measurement of formaldehyde level by means of a calibration curve. Other chloroform-soluble materials such as lipids, chlorophylls, etc. do not disturb the detection of formaldemethone.

This simple aliphatic aldehyde can take part in spontaneous reactions (without biological or chemical catalysts). Figure 12 illustrates the separation of the reaction mixture of formaldehyde and L-lysine using a special fine-particle ion-exchange chromatoplate and buffer system in an OPLC chamber. In this system the N^{ε}-formyl-L-lysine migrates with the highest R_f value and other reaction products can be found in the lower part of the chromatogram [26].

Separation of N^{ε}*-methylated lysines on silica gel layer*

N^{ε}-methylated derivatives of L-lysine but mainly N^{ε}-trimethyl-L-lysine have an important cell proliferation promoting effect on several normal and neoplastic cell

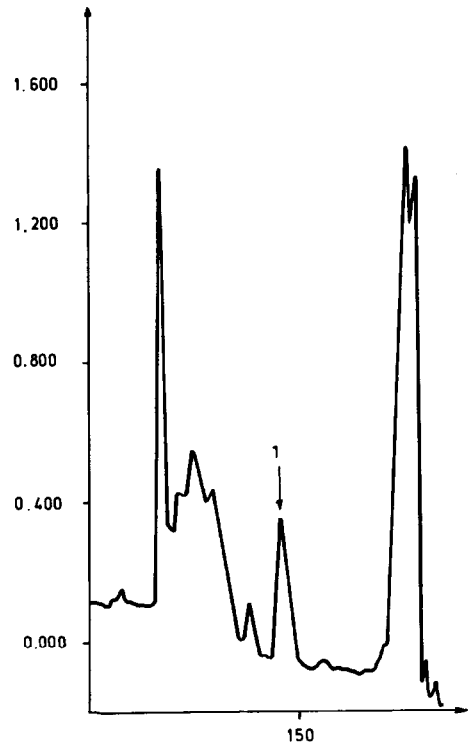

Figure 11
Measurement of formaldehyde as dimedone adduct in fish blood by OPLC. Sorbent: HPTLC silica gel 60 (Merck) with sealed edges; eluent: chloroform–methylene chloride (65:25, v/v); Shimadzu CS-930 (Japan), 270 nm; CHROMPRES 10 chamber; sample: 5 μl (1 ml fish blood + 2 ml 0.1% dimedone in 5% acetic acid solution; extraction with chloroform and taken up in 0.5 ml methanol); **1** formaldemethone.

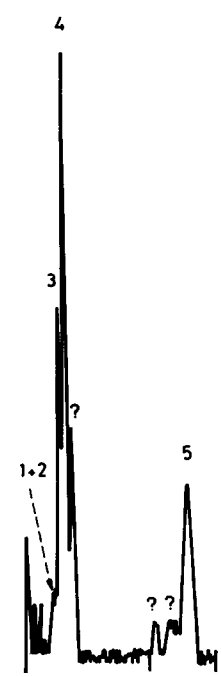

Figure 12
Quantitative evaluation of the OPLC for the reaction mixture (pH = 9) between formaldehyde and L-lysine after 12 h by the Shimadzu CS-920 zig-zag high-speed scanner at 525 nm. Sorbent: Ionpres™ 6; eluent: 0.1 M NaCl; external pressure on membrane: 1.2 MPa; development distance: 160 nm; *x*: 12 mm; rate: 20 mm min^{-1}.

systems [27, 28] and they occur in different proteins (e.g. myosin, cytochrome C [29]), so their efficient separation and quantitative measurement are important achievements.

Figure 13 shows the separation of these compounds using ninhydrin reagent for detection.

OPLC in peptide sequence analysis

It is generally considered that in the linear TLC and HPTLC development mode the maximum number of components that can be resolved in one development (15 and 4–5 cm, respectively) is *ca* 10, and this is valid for amino acids also. It is also true that an automatic amino acid analyser is suitable for the quantitative evaluation of the composition of *ca* 5–10 protein hydrolysates per day. It is a fact that in the different proteins about 20–30 amino acids are now known [30]. Therefore, in current practice it is necessary to separate these amino acids. In conventional TLC and HPTLC such separation is not possible in the linear developing mode. For a two-dimensional separation long analysis time and poor sensitivity are characteristic and in this case quantitative evaluation is difficult.

We have found that OPLC is suitable for the efficient separation of amino acids using fine-particle cellulose or silica gel chromatoplates. Figure 14 demonstrates the separation of 13 S-containing amino acids using a fine-particle cellulose layer.

It is known that the determination of the primary structure of peptide and proteins has been dependent to a considerable extent on techniques for identifying their NH_2 terminal residues. The first successful reagent used in studies of this type was Sanger's 1-fluoro-2,4-dinitrobenzene while a reagent which is greatly favoured at present is dansyl-

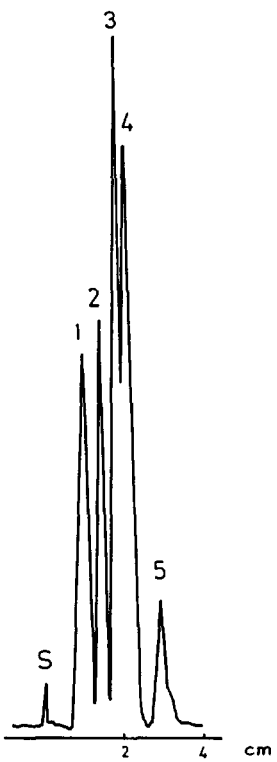

Figure 13
Separation of N^{ε}-methylated lysines. Sorbent: silica gel 60 (Merck) with sealed edges; eluent: *n*-propanol–methanol–0.1 M Na acetate in water (20:3:30, v/v); reagent: ninhydrin in acetone (0.1%) (100°C); Shimadzu CS-920, 530 nm. (S) start; (1) TML; (2) DML; (3) MML; (4) Lys; (5) TL.

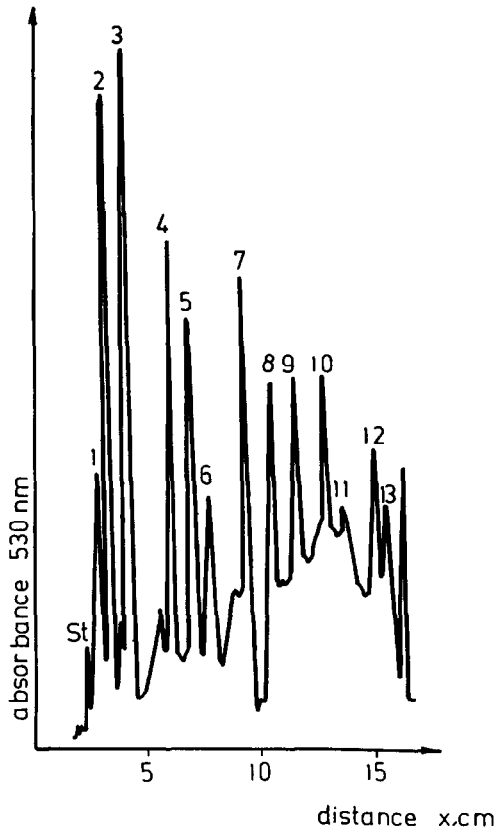

Figure 14
Separation of S-containing amino acids on HPTLC cellulose chromatoplate with sealed edges. Eluent: methyl ethyl ketone–acetonitrile–acetic acid–water (3:4:1:2, v/v); separation distance: 16 cm; absorbance: 530 nm (Shimadzu CS-920, Japan). (1) L-$(Cys)_2$; (2) L-Cys; (3) L-Homo-$(Cys)_2$; (4) DL-Homo-Cys; (5) DL-Met-S-methyl sulphonium bromide; (6) L-Met-sulfoxide; (7) S-methyl-Met; (8) S-methyl-Cys; (9) Met; (10) Ethionine; (11) β-front; (12) L-Homo-Cys-thiolactone HCl; (13) S-Benzyl-Homo-Cys.

chloride. The OPLC separation of ether-soluble DNP–amino acids was solved by multicomponent eluent and fine-particle silica gel [31].

It is well-known that the Edman procedure for determining amino acid sequences of proteins and peptides has assumed a central role in protein research. Therefore, the efficient and quick separation and identification of PTH(phenyl-2-thiohydantoin) derivatives of amino acids is a very important demand in the sequence analysis of proteins. The preliminary results in this field are promising [32].

Separation of some other substance groups

OPLC technique was used successfully for the fractionation of neutral lipid class [33], isolation of glycolipids from blood elements [34] and class fractionation of acidic glycolipids and further separation of gangliosides [35].

Ion-pair systems can be used in the field of OPLC as well [36], the application of this system is especially attractive for modelling column liquid separations [37].

OPLC gave a satisfactory separation of bile acids and related compounds by development of a HPTLC silica gel 60 chromatoplate with a four-component solvent mixture [38].

OPLC is suitable for routine analysis as well, e.g. in the case of doping agents [39], amino acids in cereals [40], essential oils [41] and glutathiones [42].

This technique has important applications in phytochemistry on both analytical and preparative scales [43–45].

The Prisma model which recently was developed for mobile phase optimization in normal and reversed-phase HPLC [46], in centrifugal layer chromatography (CLC) [47], can be used for OPLC as well [48]. The basic principle of this model involves the selection of suitable solvents with the help of the solvent strength classification of Snyder [49]. The model consists of an unlimited number of triangular solvent diagrams stacked to form a prism. Each plane has a different solvent strength. The diagrams thus form the horizontal functions of the Prisma model, and solvent strength gives the vertical one [50].

References

[1] E. Stahl (Ed.), *Dünnschicht–Chromatographie*. Springer, Heidelberg (1967).
[2] J. G. Kirchner, *Thin-Layer Chromatography*. Wiley, New York (1978).
[3] A. Niederwieser and G. Pataki (Eds), *Progress in TLC and Related Methods*, Vol. I–III. Ann Arbor Sci. Publ., Ann Arbor (1970–1972).
[4] P. R. Brown, *High Pressure Liquid Chromatography*. Academic Press, New York (1973).
[5] H. Engelhardt, *Hochdruck–Flüssigkeits–Chromatographie*, 2. Aufl. Springer, Berlin (1977).
[6] Cs. Horváth (Ed.), *High-Performance Liquid Chromatography — Advances and Perspectives*, Vol. 1. Academic Press, New York (1980).
[7] J. J. Kirkland (Ed.), *Modern Practice of Liquid Chromatography*. Wiley, New York (1971).
[8] F. Geiss, *Die Parameter der Dünnschicht–Chromatographie*. Vieweg, Braunschweig (1972).
[9] A. Siouffi, A. Guillemonat and G. Guiochon, *Chromatographia* **10**, 651–661 (1977).
[10] G. Guiochon and A. Siouffi, *J. Chromatogr. Sci.* **16**, 598–609 (1978).
[11] R. E. Kaiser (Ed.), *Einführung in die Hochleistungs–Dünnschicht–Chromatographie*, Institut für Chromatographie, Bad Dürkheim (1976).
[12] A. Zlatkis and R. E. Kaiser (Eds), *HPTLC High-Performance Thin-Layer Chromatography*. Elsevier, Amsterdam (1977).
[13] W. Bertsch, S. Hara, R. E. Kaiser and A. Zlatkis (Eds), *Instrumental HPTLC*, Hüthig, Heidelberg (1980).
[14] E. Tyihák, E. Mincsovics and H. Kalász, *J. Chromatogr.* **174**, 75–81 (1979).
[15] E. Mincsovics, E. Tyihák and H. Kalász, *J. Chromatogr.* **191**, 293–300 (1980).
[16] H. Kalász, J. Nagy, E. Mincsovics and E. Tyihák, *J. Liquid Chromatogr.* **3**, 845–855 (1980).
[17] E. Tyihák, E. Mincsovics, H. Kalász and J. Nagy, *J. Chromatogr.* **211**, 45–51 (1981).
[18] H. Kalász and J. Nagy, *J. Liquid Chromatogr.* **4**, 985–1005 (1981).
[19] A. Gescher and C. Raymont, *Biochem. Pharm.* **30**, 1245–1253 (1981).
[20] C. Turberville and V. M. Craddock, *Biochem. J.* **124**, 725–739 (1971).
[21] J. C. Arcos, A. H. Conney and Ng. Ph. Buu-Hoi, *J. Biol. Chem.* **236**, 1291–1296 (1961).
[22] E. Tyihák, J. Balla, R. Gáborjányi and E. Balázs, *Acta Phytopathol. Acad. Sci. Hung.* **13**, 29–31 (1978).
[23] É. János, J. Balla, E. Tyihák and R. Gáborjányi, *J. Chromatogr.* **191**, 239–244 (1980).
[24] H. d'A. Heck, E. L. White and M. Casanova-Schmitz, *Biomed. Mass Spectrom.* **9**, 347–353 (1982).
[25] F. F. Andrawes, *J. Chromatogr. Sci.* **22**, 506–508 (1984).
[26] E. Tyihák, L. Trézl and P. Kolonits, *J. Pharm. Biomed. Anal.* **3**, 343–349 (1985).
[27] B. Szende, E. Tyihák, L. Kopper and K. Lapis, *Neoplasma* (Bratisl.) **17**, 433–434 (1970).
[28] E. Tyihák, B. Szende and K. Lapis, *Life Sci.* **20**, 385–392 (1977).
[29] R. J. DeLange, A. N. Glazer and E. L. Smith, *J. Biol. Chem.* **244**, 1385 (1969).
[30] W. K. Paik and S. Kim, *Protein Methylation*. Wiley, New York (1980).
[31] N. T. Cong, E. Tyihák, M. Vajda and E. Mincsovics, *J. HRC and CC* **5**, 511–512 (1982).
[32] S. Fatér and E. Mincsovics, *J. Chromatogr.* **298**, 534–538 (1984).
[33] J. Pick, J. Vajda, L. Leisztner, *J. Liquid Chromatogr.* **7**, 2759–2776 (1984).
[34] J. Pick, J. Vajda, A. Anh-Tuan, L. Leisztner and S. P. Hollán, *J. Liquid Chromatogr.* **7**, 2777–2791 (1984).
[35] J. Pick, J. Vajda and L. Leisztner, *J. Liquid Chromatogr.* **6**, 2647–2660 (1983).
[36] G. Szepesi, Z. Végh, Zs. Gyulay and M. Gazdag, *J. Chromatogr.* **290**, 127–134 (1984).
[37] G. Szepesi, M. Gazdag, Zs. Pap-Sziklay and Z. Végh, *Chromatographia* **19**, 422–430 (1984).
[38] G. Szepesi, K. Dudás, A. Pap, Z. Végh, E. Mincsovics and E. Tyihák, *J. Chromatogr.* **291**, 471–475 (1984).
[39] H. Gulyás, G. Kemény, I. Hollósi and J. Pucsok, *J. Chromatogr.* **291**, 471–475 (1984).
[40] Zs. Fatér, G. Kemény, E. Mincsovics and E. Tyihák, *Dev. Food Sci.* **5A**, 483–488 (1983).

[41] E. Mincsovics, E. Tyihák, J. Nagy and H. Kalász, *Herba Hung.* **19**, 181 (1980).
[42] M. Ábrahám, B. Polyák, B. Szajáni and L. Boross, *J. Liquid Chromatogr.* **6**, 2635–2645 (1983).
[43] E. Tyihák, E. Mincsovics, P. Tétényi, I. Zámbó and H. Kalász, *Acta Horti.* **96**, 113–124 (1980).
[44] P. Horváth, G. Szepesi, H. Hoznek, Z. Végh and E. Mincsovics, in *Proc. 2nd International Symposium on Instrumental HPTLC* (R. E. Kaiser, Ed.), Interlaken, pp. 295–304. Institute for Chromatography, Bad Dürkheim (1982).
[45] E. Mincsovics, T. J. Székely, M. Hoznek, Z. Végh, I. Zámbó, G. Szepesi and E. Tyihák, in *Advances in Steroid Analysis* (S. Görög, Ed.), pp. 427–433. Akadémiai Kiadó, Budapest (1982).
[46] Sz. Nyiredy, B. Meier, C. A. J. Erdelmeier and O. Sticher, *J. HRC* and *CC* **8**, 186–188 (1985).
[47] Sz. Nyiredy, C. A. J. Erdelmeier, B. Meier and O. Sticher, *Chromatographie Suppl.* **4**, 24–30 (1985).
[48] Sz. Nyiredy, C. A. J. Erdelmeier and O. Sticher, in *Proc. on International Symposium on TLC with Special Emphasis on OPLC* (E. Tyihák, Ed.), Szeged, 1984, pp. 222–231. Labor MIM, Budapest (1986).
[49] L. R. Snyder, *J. Chromatogr. Sci.* **16**, 223 (1978).
[50] Sz. Nyiredy, C. A. J. Erdelmeier, B. Meier and O. Sticher, *Farm. Tijdschr. Belg.* **61**, 261 (1984).

[First received for review 10 February 1986; revised manuscript received 14 August 1986]