ELSEVIER

Journal of Chromatography A, 803 (1998) 235–239

JOURNAL OF CHROMATOGRAPHY A

Distribution of cationic ion-pairing reagents on thin-layers after continuous overpressured layer chromatography

János Török[a], Katalin Kovács-Hadady[a,*], Emil Mincsovics[b]

[a]*Lajos Kossuth University, Department of Inorganic and Analytical Chemistry, H-4010 Debrecen, P.O. Box 21, Hungary*
[b]*OPLC-NIT Engineering Co. Ltd., H-1119 Budapest, Andor u. 60, Hungary*

Received 11 June 1997; received in revised form 1 December 1997; accepted 23 December 1997

Abstract

Ion-pair HPLC separations on layers were modelled by using continuous overpressured layer chromatography (OPLC). As a first step, the distribution of several cationic ion-pairing reagents on the layer was studied. The mobile phase containing different quaternary ammonium salts, such as tetramethylammonium (TMA), tetrabutylammonium (TBA), cetyltrimethylammonium (CTMA) and trioctylmethylammonium (TOMA) halides was continuously pumped through the stationary phase at a pressure of 40 bar. The volume of the mobile phase varied between 4.5 to 67.5 cm^3 which is equivalent to a 1 to 15 bed volume of the given silica-gel layer. The concentration of the reagents was measured after chromatography in ten samples of the stationary phase taken along the direction of the development. Silica F-gel and physically and chemically bonded reversed-phase layers (C_{18}) were used. In some cases the effluents also were analyzed. With 0.05 *M* CTMA in 50% methanol–water mixture, the layers acted as 'spread out columns' and they could be conditioned after pumping through 22.5 cm^3 mobile phase. This means that there was no concentration gradient on the layer and the remaining reagent quantities on the layer did not change with increasing volumes of the mobile phase. In some cases, the behaviour of TMA was different from that of CTMA. © 1998 Elsevier Science B.V.

Keywords: Overpressured layer chromatography; Thin-layer conditioning; Ion-pairing reagents

1. Introduction

In ion-pair thin-layer chromatography (IPTLC), similarly to HPLC, one of the most important factors determining the retention is the concentration of the ion-pairing reagent in the stationary phase. In recent studies, the concentration of different ion-pairing reagents has been investigated on different layers, such as silica-gel, physically and chemically bonded reversed-phase layers by using different techniques for the treatment of the stationary phase [1,2]. In a set of the experiments, the reagent concentration of the stationary phase was determined before the chromatographic run. In these experiments, the ion-pairing reagent was applied in a methanolic solution and the layers were dipped into it. Horizontal or vertical dipping techniques provided a homogeneous distribution of the reagent on the layer [1]. In another set of the experiments, the adsorbed quantities of the reagents bonded to the different layers either treated or not treated with the reagent were determined after a chromatographic run. Methanol–water mixtures were used as mobile phases and in some cases the ion-pairing reagent was also added [3,4]. The ad-

*Corresponding author. Fax: +36 52 329667

0021-9673/98/$19.00
PII S0021-9673(97)01286-7

sorption of various quaternary ammonium compounds on silica-HPLC columns has been studied by Hansen et al. [5]. They found that only the long-chain compounds are adsorbed on the silica surface in appreciable amounts. The maximum amount that can be adsorbed per g of silica is of the same order of magnitude for each of the long-chain compounds studied. The stationary phase is called as 'dynamically modified silica' [5].

The overpressured layer chromatographic (OPLC) technique developed by Tyihák and Mincsovics [6–11], is suitable to study the retention transfer from the layer to the column [12]. In IPTLC systems, Szepesi et al. [13,14] obtained a good correlation between the results of off-line OPLC and HPLC for different carboxylic acids using cetyltrimethylammonium bromide (CTMA) as ion-pairing reagent.

Our present experiments were designed to study the possible retention transfer from layer to column in the ion-pairing chromatographic separations by means of continuous (on-line) OPLC. During on-line OPLC, the layer is under pressure, and the mobile phase is continuously pumped through it. In this situation, the layer acts as a 'spread out' column. In view of modelling of retention transfer, the 'conditioning of the column' is an essential question, therefore as a first step, the distribution and absolute concentrations of the reagents were determined in the stationary phase after continuous OPLC runs. The Personal OPLC (P-OPLC) instrument used for the development of the chromatograms works at up to 50 bar pressure.

2. Experimental

2.1. Reagents and materials

Silica-gel layers (≠5554) were purchased from Merck (Darmstadt, Germany). Physically bonded reversed-phase layers were prepared by dipping silica-gel layers into a 7.5% solution of paraffin oil in dichloromethane for 3 min. C_{18} chemically bonded reversed-phase (Alugram RP-18 W/UV_{254}) layers were kindly provided by Macherey-Nagel (Düren, Germany). All chemicals were of analytical grade. Tetramethylammonium bromide (TMA) was the product of BDH Chemicals (Poole, UK) tetrabutylammonium bromide (TBA) was from Fluka (Buchs, Switzerland), cetyltrimethylammonium bromide (CTMA) was purchased from Reanal (Budapest, Hungary), and trioctylmethylammonium chloride (TOMA) was the product of Aldrich (Steinheim, Germany). The solvents were of HPLC grade (Carlo Erba Reagenti, Italy).

2.2. Apparatus

A P-OPLC instrument (OPLC-NIT, Budapest, Hungary) was used for the development of the chromatograms at 40 bar. The flow-rate of the mobile phase was 0.5 $cm^3\,min^{-1}$.

The apparatus used for potentiometric, spectrophotometric and capillary electrophoretic measurements were the same as described earlier [1–4].

2.3. Analytical methods

In the direction of the chromatographic run, ten samples were taken from a 20×20 cm layer at equidistance places. The preparation of the samples was the same as described earlier [1–4]. In some cases, the mobile phase leaving the layer was collected as 4-cm^3 fractions. For the determination of TBA and CTMA, a standard addition potentiometric method was applied by using laboratory-made ion-selective electrodes. For the determination of TMA, a capillary electrophoresis method, and for TOMA, a spectrophotometric method was used [1–4]. The effluent was analyzed after a hundred-fold dilution.

3. Results and discussion

In a recent work, in which the efficiency of different impregnation techniques was studied, a set of untreated layers were developed with methanolic solution of the ion-pairing regents by means of noncontinuous (off line) OPLC. After a run, the distribution of CTMA and TOMA was inhomogeneous, a decreasing concentration gradient formed on the layers in the direction of the development [1].

Similar experiments were performed by using the P-OPLC instrument, with mobile phases containing 0.05 *M* reagent (TMA, TBA, CTMA, TOMA) in methanol. The volume of the mobile phase was 67.5

cm^3 (approximately equivalent to fifteen bed volume of a 20×20 cm silica-gel layer, with a layer thickness of 0.2 mm). The bed volume of the layer was measured between the introduction of the mobile phase and the first drop of the eluent at the outlet. On all three kinds of layers studied, the same distribution was observed. After the treatment, the distribution of the different reagents was homogeneous in the stationary phase. The quantities of the reagents adsorbed on the layers, calculated in mg/g adsorbent and mmol/g adsorbent, are summarized in Table 1. The data show that the TMA concentrations calculated in mmol/g, on any layers were considerably larger than those of other ion-pairing reagents. This indicates that this molecule needs the smallest space when it bonds to the surface of the stationary phase. There are no significant differences between the quantities of TBA, CTMA and TOMA adsorbed on a given layer. The following sequence of the adsorbed concentrations was observed on different layers: silica-gel>physically bonded reversed-phase> chemically bonded reversed-phase (Table 1). In these experiments, the concentration of the reagent (TBA) was also measured from the effluent, however, the concentration of the effluent was less sensitive to the changes of the reagent concentration during the continuous flow. The mobile phase contained 0.05 *M* reagent, and $0.9–1.4\times10^{-4}$ *M*/g of TBA remained on the layers after the runs. For silica-gel layers, a considerable decrease could be measured in the reagent concentration in the mobile phase after pumping 4–8 cm^3 volumes, while only a slight concentration decrease was obtained in the first two fractions of the effluent for the other layers.

In HPLC separations based on ion-pairing, the usual way to perform the analysis is to incorporate the ion-pairing reagent into the mobile phase. The column is ready to be used when the surface of the packing material is saturated with the reagent. In ion-pair TLC, we have the following three possibilities in adding the reagent to the system: (i) the stationary phase is treated with the reagent before chromatography, (ii) the stationary phase remains untreated and the ion-pairing reagent is added to the mobile phase and (iii) the stationary phase is previously treated and the mobile phase also contains the reagent [15]. When in traditional and noncontinuous OPLC ion-pairing separation methods (ii), which is similar to HPLC, was used on silica-gel layers TBA and CTMA did not run with the solvent front, they were significantly retarded in the lower R_F region [3] after a chromatographic run. Using physically and chemically bonded reversed-phase layers, the same situation could be observed [4].

For modelling the real situation in the column, 0.05 *M* reagent was dissolved in a methanol–water (1:1, by volume) mixture and this solution was used as a mobile phase for 'conditioning of the column'. The results obtained for silica-gel with TMA and CTMA are shown in Fig. 1. The concentration of the reagents was measured after pumping through mobile phase volumes 4.5, 22.5, 45.0 and 62.5 cm^3. In contrast to the results obtained for other ion-pairing reagents [3,4], in the case of TMA an almost homogeneous distribution of the reagent developed even after one run on silica-gel layers. When the volume of the mobile phase was equivalent to 5 to 15 bed volumes (22.5–67.5 cm^3), the distribution of TMA remained unchanged, but the absolute concentration of the reagent increased with increasing

Table 1
Quantities of TMA, TBA, CTMA and TOMA [calculated in mg/g sorbent and mmol/g sorbent (in parenthesis)] on different stationary phases obtained from a 0.05 *M* methanolic solution after continuous OPLC development (the volume of the mobile phase is 67.5 cm^3, number of samples=10)

Stationary phase	The concentration of the ion-pairing reagent and the R.S.D. values (%)							
	TMA		TBA		CTMA		TOMA	
Silica-gel	29.5 (0.19)	4%	44.1 (0.14)	3%	41.2 (0.11)	4%	54.7 (0.14)	4%
Physically bonded silica-gel	26.1 (0.17)	4%	31.1 (0.10)	2%	36.1 (0.10)	4%	43.6 (0.11)	4%
Chemically bonded silica-gel	22.6 (0.15)	4%	29.1 (0.09)	4%	29.9 (0.08)	6%	41.0 (0.10)	6%

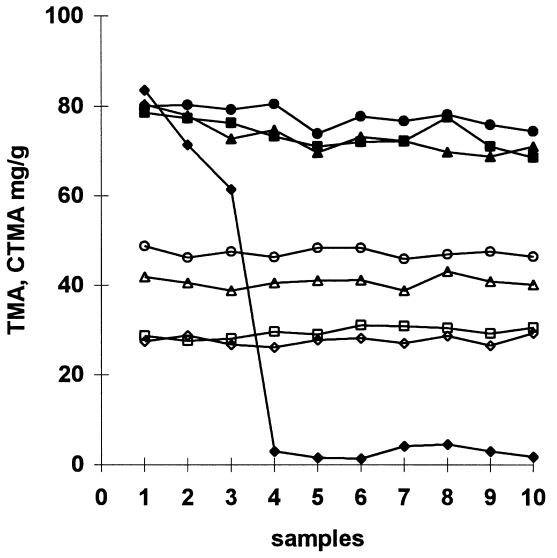

Fig. 1. Amount of TMA (empty) and CTMA (filled) adsorbed on silica-gel layers after continuous OPLC development. The mobile phase contained 0.05 *M* reagent in 50% methanol and water; the volume of the mobile phase was 4.5 (◇), 22.5 (□), 45.0 (△) and 67.5 (○) cm^3.

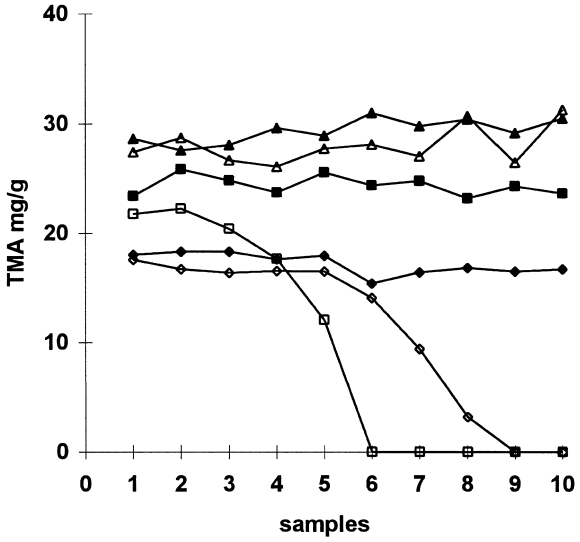

Fig. 2. Amount of TMA adsorbed on reversed-phase and silica-gel layers after continuous OPLC development. The mobile phase contained 0.05 *M* TMA in 50% methanol and water; the volume of the mobile phase was 4.5 (empty) and 22.5 (filled) cm^3. (◇) Chemically bonded reversed-phase layer, (□) physically bonded reversed-phase layer, (△) silica-gel layer.

volume of the mobile phase (Fig. 1). After pumping 4.5 cm^3 of mobile phase (Fig. 2) on reversed-phase layers, TMA was retarded on the lower half (physically bonded reversed-phase layers) or on the lower two-third part (chemically bonded reversed-phase layers) of the layer. After pumping 22.5 cm^3 of 0.05 *M* TMA in 50% methanol–water mixture, no concentration gradient of the reagent was observed (Fig. 2). The quantities of the reagent adsorbed on the layer were different depending on the polarity of the layer.

When CTMA was used under the same conditions, after one run CTMA was retarded in the region of lower R_F values, similarly to earlier observations [3]. After pumping 5 to 15 bed volumes from the mobile phase, the distribution of CTMA was homogeneous, and the adsorbed quantities were the same and independent from the volume of the mobile phase (Fig. 1). For reversed-phase layers a similar picture was obtained as for silica-gel (Fig. 3).

On the basis of the results, it can be concluded that for modelling IP–HPLC separations, the continuous OPLC technique seems to be a useful tool. The stationary phase can be ‘conditioned’ after

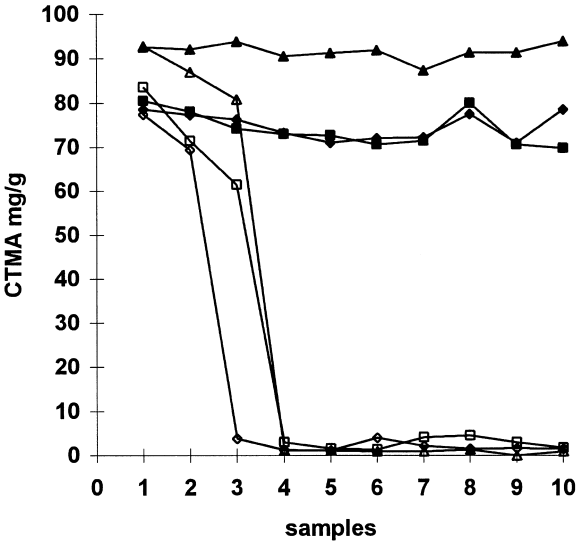

Fig. 3. Amount of CTMA adsorbed on reversed-phase and silica-gel layers after continuous OPLC development. The mobile phase contained 0.05 *M* CTMA in 50% methanol and water; the volume of the mobile phase was 4.5 (empty) and 22.5 (filled) cm^3. For abbreviations of the layers see Fig. 2.

pumping the mobile phase containing the ion-pairing reagent in a volume equivalent to several bed volumes of the sorbent. For this purpose, mainly CTMA or structurally related ion-pairing reagents are suitable, because the formation of the steady-state is faster than for TMA. In controlling the retention, the concentration of the reagent, the concentration and quality of the organic modifier may have an important role. However, the transfer of retention from layer to column is not free from problems, because of the contradictions observed in the effect of pH on thin-layers treated with ion-pairing reagents [16–20]. Experiments are in progress to explore further details.

Acknowledgements

This work was financially supported by a grant from the Hungarian Academy of Sciences, OTKA No. T 015486. The authors express their special thanks to Macherey Nagel for the Alugram RP-18 W/UV_{254} layers.

References

[1] K. Kovács-Hadady, T. Varga, J. Planar Chromatogr. 8 (1995) 292.
[2] K. Kovács-Hadady, J. Planar Chromatogr. 9 (1996) 174.
[3] K. Kovács-Hadady, J. Planar Chromatogr. 10 (1997) 18.
[4] K. Kovács-Hadady, Chromatographia 45 (1997) 81.
[5] S.H. Hansen, P. Helboe, U. Lund, J. Chromatogr. 240 (1982) 319.
[6] E. Tyihák, E. Mincsovics, H. Kalász, J. Chromatogr. 174 (1979) 75.
[7] E. Mincsovics, E. Tyihák, H. Kalász, J. Chromatogr. 191 (1980) 293.
[8] H. Kalász, J. Nagy, E. Tyihák, E. Mincsovics, J. Liq. Chromatogr. 3 (1980) 845.
[9] E. Mincsovics, E. Tyihák, J. Planar Chromatogr. 1 (1988) 309.
[10] E. Tyihák, E. Mincsovics, T.J. Székely, J. Chromatogr. 471 (1989) 375.
[11] L. Botz, Sz. Nyiredy, O. Sticher, J. Planar Chromatogr. 5 (1990) 352.
[12] E. Mincsovics, E. Tyihák, in: F.A.A. Dallas, H. Read, R.J. Ruane, I.D. Wilson (Eds.), Recent Advances in Thin-Layer Chromatography, Plenum Press, New York and London, 1988, p. 57.
[13] G. Szepesi, Z. Végh, Zs. Gyulay, M. Gazdag, J. Chromatogr. 290 (1984) 127.
[14] M. Gazdag, G. Szepesi, M. Hernyes, Z. Végh, J. Chromatogr. 290 (1984) 135.
[15] D. Volkmann, Top. Curr. Chem. 126 (1984) 51.
[16] G.P. Tomkinson, I.D. Wilson, J. Planar Chromatogr. 6 (1993) 224.
[17] K. Kovács-Hadady, K. Barna-Katona, J. Planar Chromatogr. 2 (1989) 133.
[18] K. Kovács-Hadady, J. Szilágyi, J. Planar Chromatogr. 4 (1991) 194.
[19] K. Kovács-Hadady, J. Pharm. Biomed. Anal. 10 (1992) 1025.
[20] K. Kovács-Hadady, J. Chromatogr. 589 (1992) 301.