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Use of chromatographic system peaks for continuous quantitative analysis

Nira Mizrotsky, Luba Kristol, Eli Grushka*

Department of Inorganic and Analytical Chemistry, The Hebrew University, 91904 Jerusalem, Israel

Abstract

A study was made to establish whether the size of system peaks, which occur in chromatography with multi-component mobile phases, is a linear function of the concentration of the components. In the two chromatographic systems studied, it was found that the size of the system peaks is proportional to concentration. Using the fact that the injection of concentrations lower than that in the mobile phase results in negative system peaks whereas the injection of higher concentrations yields positive peaks, a simple method to determine the concentration of the components in the mobile phase is described. The approach seems to be robust although the retention times of some mobile phase components might be affected by the concentration of other components. The results presented indicate that system peaks can be used to monitor continuously various streams such as rivers, waste waters and physiological fluids.

1. Introduction

System peaks occur in chromatographic systems whenever the mobile phase contains more than one component. They are observed when at least one of the mobile phase components is detected by the detector. System peaks are not directly related to the injected solutes; they are a thermodynamic phenomenon, resulting from the perturbation in the chemical equilibrium at the column inlet which occurs when a sample of different composition to the bulk mobile phase enters the column. At the onset of this perturbation of equilibrium, the chromatographic system (i.e., column and stationary and mobile phases) begin to relax towards a new state of equilibrium by transferring mobile phase components, in addition to solutes, between the two phases. The net effect of the relaxation to a new state of equilibrium is the appearance of system peaks. System peaks move through the column as if they were injected solutes, i.e., they have definite capacity factors and peak shapes and they exhibit conventional adsorption isotherms that can be linear, Langmuir or of any other type normally encountered in chromatography.

System peaks have been studied extensively over the past decade or so. For example, Riedo and Kováts [1,2] developed the fundamental theory for the formation of system peaks. Melander et al. [3] also examined the process of system peak development. Knox and Kaliszan [4] used system peaks to obtain a better estimate of a column's void volume. Levin and Grushka [5–7] used experimentally determined system peaks to extract several chromatographic quantities such as void volumes and capacity factors. Arvidsson et al. [8] examined system peaks in ion-pair

* Corresponding author.

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chromatography. Golshan-Shirazi and Guiochon [9] solved numerically the mass balance equations relating to system peaks in linear chromatography. Levin and Abu-Lafi used system peaks to characterize the chromatographic behaviour of enantiomers in chiral liquid chromatography [10] and to study the distribution of several hydroxylated benzenes [11].

All of the above papers deal with fundamental aspects of system peaks. Very few publications can be found describing the use of system peaks. Phillips and McIlwrick [12] used system peaks in gas chromatography to characterize a catalytic bed. In another attempt, Westerlund and coworkers [14–17] demonstrated the use of system peaks to generate, in situ, mobile phase gradients which can produce sharper solute peaks.

In this work, we stress the practical utilization of system peaks. From a conceptual point of view it is of no, or little, consequence whether the sample is injected into the chromatograph in a small volume of the mobile phase, or whether it is passed through the column as part of the mobile phase and pure mobile phase (i.e., without the components to be separated) is injected. In both instances a chromatogram will be generated. However, from a practical point of view, the possibility of continuously passing the sample to be separated through the column opens the door to new modes of chromatographic operations. For example, a portion of a flowing stream, such as running waste effluents in factories, rivers and physiological fluids, can be diverted to pass continuously through the chromatographic column. At predetermined time intervals, a neat solvent is injected into the column, resulting in system peaks belonging to the components in the stream. In this manner, the stream can be monitored continuously, and automatically, for the level of one or more of the components in the stream.

To be a viable analysis method, the system peak approach must be analytically sound; it must be robust, precise and accurate. In this work, we investigated the dependence of system peak areas on the concentration of the solutes in the mobile phase and on the concentration of the same solutes which are injected into the column. This dependence establishes the quality of calibration lines that can be obtained for system peaks. The results also indicate the interrelationships between mobile phase components and thus the robustness of the analytical approach for the system concerned.

2. Experimental

2.1. Instrumentation

Two chromatographic systems were used. (A) A Perkin-Elmer (Norwalk, CT, USA) Series 4 liquid chromatograph equipped with a 10-μl loop injection valve (Rheodyne, Cotati, CA, USA), a Perkin-Elmer Model 85B variable-wavelength spectrophotometric detector having a 1.4-μl flow cell and a D-2000 integrator (Merck–Hitachi, Darmstadt, Germany) was used. The detector was operated at 254 nm. (B) A Merck–Hitachi liquid chromatograph with a variable-wavelength detector and a 20-μl injection loop was used. The detector was operated at 235 nm. The detector was connected to a Merck–Hitachi D-2000 integrator. Two columns were used, 25-cm and 12.5-cm LiChrosorb RP-18 cartridges, held by a Hiber manual holder. The columns were thermostated with the aid of a laboratory-built water-bath.

2.2. Materials

Two mobile phases were prepared: (A) acetate buffer (0.02 *M*, pH 5.6) containing 0.001 *M* heptanesulphonate, $4 \cdot 10^{-4}$ *M* copper acetate and various concentrations of valine (Val), histidine (His), hydroxyproline (Hyp) and glycine (Gly); and (B) phosphate buffer (sodium dihydrogenphosphate and disodium hydrogenphosphate, 0.01 *M*, pH 6) containing various concentrations of tyrosine (Tyr) and phenylalanine (Phe). All amino acids were purchased from Sigma (Tel-Aviv, Israel).

2.3. Procedure

Four batches of mobile phase A were prepared containing the amino acids at concentra-

tions of 0.02, 0.04, 0.06 and 0.072 m*M*. Into each mobile phase was injected the same buffer containing various concentrations of the four amino acids.

With mobile phase B we prepared fifteen combinations of tyrosine and phenylalanine concentrations. Three phenylalanine concentrations were used, $5 \cdot 10^{-4}$, $1 \cdot 10^{-3}$ and $5 \cdot 10^{-3}$ *M*. At each phenylalanine concentration, five tyrosine concentrations were prepared: $1 \cdot 10^{-5}$, $5 \cdot 10^{-5}$, $1 \cdot 10^{-4}$, $2.5 \cdot 10^{-4}$ and $5 \cdot 10^{-4}$ *M*. The injected samples consisted either of pure buffer or buffers containing any of the above combinations of the two amino acids.

Owing to noisy detector signals, resulting from the use of strongly UV-absorbing mobile phases, it was decided to measure peak heights rather than peak areas.

3. Results and discussion

3.1. Behaviour of mobile phase A

The first system examined was with mobile phase A. Although a complicated system, we chose it for our initial studies owing to our familiarity with its behaviour [5–8]. The Cu–amino acid complexes absorb radiation at 235 nm, so we can monitor the behaviour of all amino acids.

Fig. 1a shows a chromatogram resulting from the injection of a pure buffer into the column equilibrated with mobile phase A containing 0.02 m*M* of Gly, Hyp, His and Val. The chromatogram has four negative peaks, one for each amino acid. The identification of these peaks was established by injecting the amino acids at higher concentrations than that in the mobile phase. Fig. 1b shows the chromatogram obtained from the injection of 0.05 m*M* of the four amino acids. The two positive peaks in Fig. 1a are system peaks belonging to other components of the mobile phase.

The injection of the amino acids gives either positive or negative peaks depending on whether the concentrations of the injected amino acids are higher or lower than those in the mobile phase. An example of the dependence of peak size and direction on the concentration of the injected amino acid is shown in Fig. 2 for valine. Similar behaviour was observed for all four amino acids.

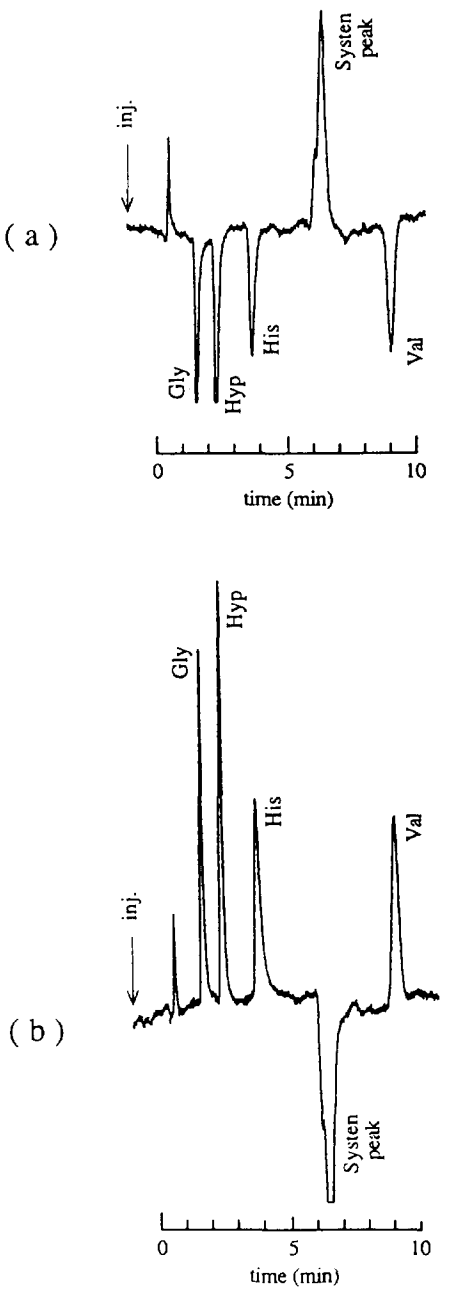

Fig. 1. System peaks resulting from the injection of (a) neat buffer and of (b) buffer and 0.05 m*M* Gly, His, Hyp and Val. Mobile phase, mobile phase A containing 0.02 m*M* Gly, His, Hyp and Val.

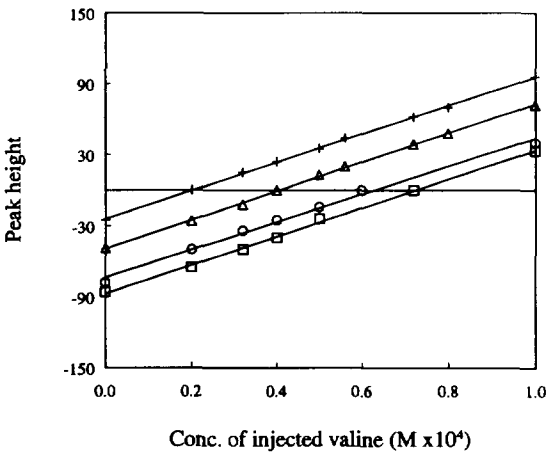

Fig. 2. Calibration lines obtained by injecting various concentrations of Val into mobile phase A. Each line corresponds to a different concentration of Val in the mobile phase: + = $2.0 \cdot 10^{-5}$; △ = $4.0 \cdot 10^{-5}$; ○ = $6.0 \cdot 10^{-5}$; □ = $7.2 \cdot 10^{-5}$ *M*. Peak heights are in arbitrary (integrator) units.

Each line in Fig. 2 represents a different concentration of the amino acid (valine in this instance) in the mobile phase. The linear dependence of the peak height on the concentration of the injected amino acid is not a function of the concentration of the same amino acid in the mobile phase. Each line is a calibration-type line that can be used to measure the concentration of the amino acid. The zero-height crossing point for each line occurs when the amino acid concentration in the injected sample equals the concentration in the mobile phase. Note also that as the concentration of the amino acid in the mobile phase is increased, the peak resulting from the injection of neat buffer is higher (more negative).

The linear dependence of the system peak height on the concentration coupled with the fact that each line crosses the zero-height line is of great practical importance. To characterize the amount of a solute in a stream all that we need to do is to divert a part of the stream through a chromatographic column and make two injections. One injection is of the stream effluent without the solute. This injection will result in a negative system peak associated with the solute. The second injection will include the solute at a relatively high concentration which will result in a positive peak. Interpolation between the two peak heights will give a line that will cross the zero-height line at the concentrations of the solute in the stream.

To check the linearity of the chromatographic system, some of the data in Fig. 2 were replotted so that the peak height was now a function of the concentration of Val in the mobile phase (Fig. 3). Each line in Fig. 3 belongs to a different injected concentration of the amino acids. Although each line has only four experimental points, the linearity is very good, with correlation coefficients of 0.999 and above. The bottom line is due to the injection of pure buffer without any Val. The extrapolation of that line to zero Val in the mobile phase shows, as expected, that the system peak vanishes. Extrapolation of all other lines to zero Val in the mobile phase gives positive peaks that would result from the injection of various concentrations of Val into a neat buffer. The extrapolated peaks heights should be the same as the absolute value of the system peak heights resulting from the injection of a neat buffer into mobile phases containing the same concentration of the amino acid, i.e., the absolute values of the points on the bottom line. Fig. 3 shows that such is the case. For

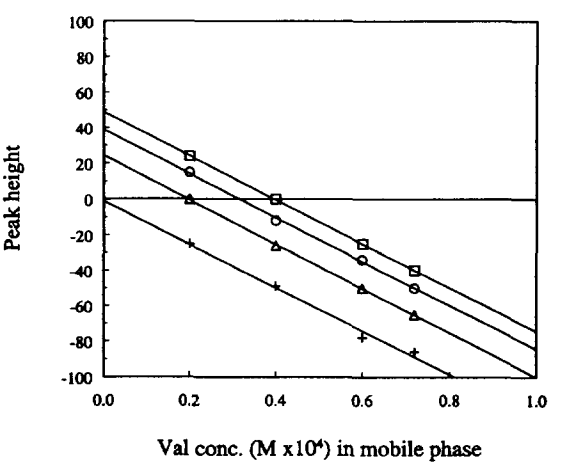

Fig. 3. Heights of Val system peak as a function of Val concentration in mobile phase A. Each line belongs to a different concentration of Val in the injected buffer: + = 0; △ = $2.0 \cdot 10^{-5}$; ○ = $3.2 \cdot 10^{-5}$; □ = $4.0 \cdot 10^{-5}$ *M*. Peak heights are in arbitrary (integrator) units.

example, the peak height resulting from the extrapolation of the line belonging to an injected concentration of 0.04 m*M* (top line) is 48.9 cm. The absolute value of the peak height resulting from the injection of a neat buffer into a mobile phase containing 0.04 m*M* Val is 49 cm. Hence system peaks can be calibrated and, therefore, can be used to determine species in the mobile phase.

3.2. Behaviour of mobile phase B

The mobile phase discussed above presents a very complex chromatographic system. To examine the feasibility of using system peaks with conventional mobile phases, we prepared a simple phosphate buffer and added to it Tyr and Phe. Injection of the neat phosphate buffer gave the system peaks associated with Tyr and Phe. Typical calibration lines resulting from the injection of various concentrations of Tyr in the phosphate buffer are shown in Fig. 4. Each line belongs to different concentrations of Tyr in the mobile phase. In all instances, the concentration of Phe was $5 \cdot 10^{-3}$ *M*. As in Section 3.1, the calibration lines cross the zero-height line when the concentrations of the injected Tyr were identical with those in the mobile phase. Extrapolation of each line to zero injected Tyr gives the system peak that would result from the injection of a neat buffer.

Fig. 5 shows the system peak heights as a function of Tyr concentration in the mobile phase. Each line corresponds to a different concentration of the injected Tyr. Again, in all instances the concentration of Phe in the mobile phase was $5 \cdot 10^{-3}$ *M*. The extrapolation of each line to zero Tyr concentration in the mobile phase gives the Tyr peak that would result from the injection of tyrosine into a mobile phase made up of the phosphate buffer and $5 \cdot 10^{-3}$ *M* Phe. The absolute values of the extrapolated peak heights discussed in connection with Fig. 4 should be identical with the extrapolated peak heights in Fig. 5. Table 1 gives the extrapolated peak heights. Also given in Table 1 are the absolute value of the actual Tyr system peak obtained from the injection of a neat buffer into each of the mobile phases containing various amounts of Tyr. All three values are very close, indicating the robustness of the approach; quantitative information about components of the

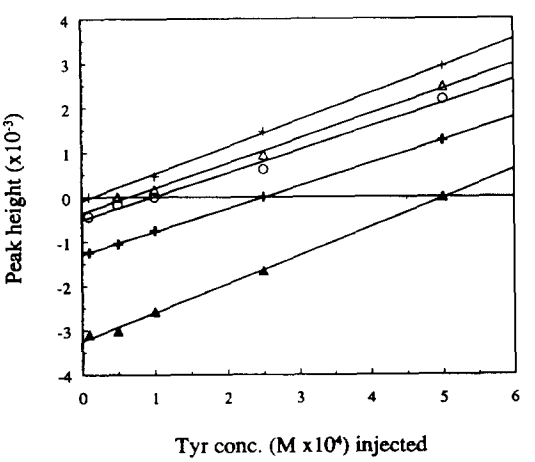

Fig. 4. Calibration lines obtained by injecting various concentrations of Tyr into mobile phase B containing Tyr and 0.005 *M* Phe. Each line corresponds to a different concentration of Tyr in mobile phase B: + = $1.0 \cdot 10^{-5}$; △ = $5.0 \cdot 10^{-5}$; ○ = $1.0 \cdot 10^{-4}$; + = $2.5 \cdot 10^{-4}$; ▲ = $5.0 \cdot 10^{-4}$ *M*. Peak heights are in arbitrary (integrator) units.

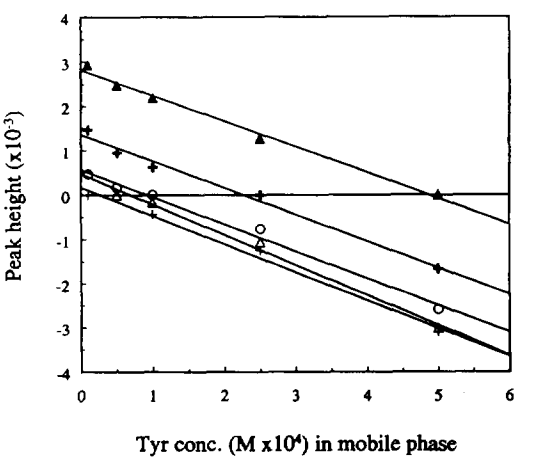

Fig. 5. Heights of Tyr system peak as a function of Tyr concentration in mobile phase B containing 0.005 *M* Phe. Each line belongs to a different concentration of Tyr in the injected buffer: + = $1.0 \cdot 10^{-5}$; △ = $5.0 \cdot 10^{-5}$; ○ = $1.0 \cdot 10^{-4}$; + = $2.5 \cdot 10^{-4}$; ▲ = $5.0 \cdot 10^{-4}$ *M*. Peak heights are in arbitrary (integrator) units.

Table 1
Heights of extrapolated system peaks from Figs. 4 and 5 and from injection of neat buffer

Tyr concentration either in mobile phase or injected (10^{-4} M)	Extrapolated peak height to zero Tyr in injected sample (Fig. 4)	Extrapolated peak height to zero Tyr in mobile phase (Fig. 5)	Peak height resulting from neat buffer injection
0.1	85	175	0
0.5	359	475	236
1.0	512	571	468
2.5	1288	1366	1417
5.0	3246	2828	3127

mobile phase can indeed be obtained via their system peaks.

We examined two other sets of mobile phase, each with a different concentration of Phe, to see if different concentrations of mobile phase components affect the peak height of Tyr. Table 2 shows that a ten-ten fold increase in Phe changes the Tyr peak height by at worst 30%. Hence the determination of Tyr by system peaks can be carried out without the need to control closely the concentration of Phe in the mobile phase.

The discussion up to this point was centred on the size of the peaks which were generated from various combinations of Tyr in the mobile phase and in the injected sample. In addition to peak size, the question of retention time stability is also of great importance. If the retention times are a strong function of concentration, then we might have difficulty with the actual identification of a peak. Fig. 6 shows plots of the capacity factor of Tyr as a function of injected Tyr concentration when the mobile phase contains $2.5 \cdot 10^{-4}$ M of the same amino acid. Each line corresponds to a different Phe concentration in the mobile phase. Similar results were obtained for other Tyr concentrations in the mobile phase. To a good approximation, the capacity factor is independent of the amount of Tyr injected. However, the concentration of Phe does have an effect of the retention of Tyr. As the concentration of Phe in the mobile phase is increased, the retention of Tyr becomes smaller, possibly because of the more successful competition of the former compound for the interaction sites in the stationary phase. At the higher concentrations Phe acts like a weak mobile phase modifier. Thus, although Phe does not have a large effect

Table 2
Effect of Phe concentration in mobile phase B on the height of the Tyr system peak

Injected Tyr concentration (M)	Phe concentration in mobile phase (M)		
	0.0005	0.001	0.005
$1.0 \cdot 10^{-5}$	−1692	−1385	−1240
$5.0 \cdot 10^{-5}$	−1324	−1151	−1053
$1.0 \cdot 10^{-4}$	−818	−866	−754
$2.5 \cdot 10^{-4}$	0	0	0
$5.0 \cdot 10^{-4}$	1726	1439	1269

The mobile phase contained $2.5 \cdot 10^{-4}$ M Tyr.

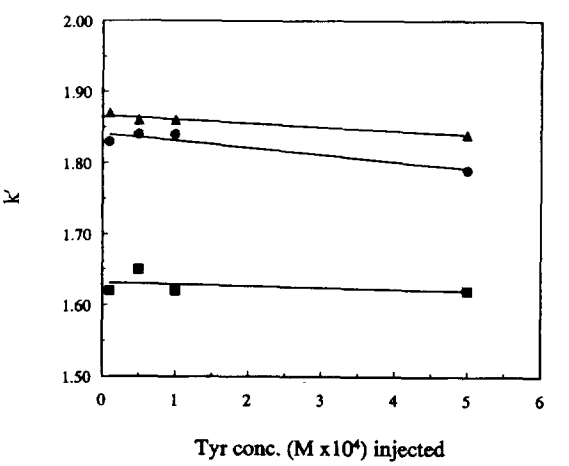

Fig. 6. Capacity factors of Tyr as a function of injected Tyr concentration. Mobile phase B contained $2.5 \cdot 10^{-4}$ M Tyr and Phe. Each line corresponds to a different Phe concentration: ▲ = $5.0 \cdot 10^{-4}$; ● = $1.0 \cdot 10^{-3}$; ■ = $5.0 \cdot 10^{-3}$ M.

on the peak size, it does have an appreciable influence on the retention of Tyr. If the amount of Phe in the mobile phase is not maintained over a narrow concentration range, the Tyr peak might be outside the time window of the integrator, and a misidentification might occur.

4. Conclusions

The size of system peaks is related to the amounts of the components responsible for these peaks. Hence system peaks can be used to monitor the concentration of the components in the mobile phase. This fact opens up the possibility of using the solutions to be analysed as the mobile phase. Injection of neat solutions, or of controlled concentrations of the components of interest, will give quantitative information about the concentrations of these components in the mobile phase. The attraction of this approach lies in the fact that the sample to be analysed can be passed continuously through the column and the analysis is carried out periodically by the injection of neat solvents. There is no need to remove samples for off-line analysis. The approach can be automated very easily. It can be utilized for continuous stream monitoring and also for continuous, on-line process control. We are applying the system peak method to monitor the progress of chemical reactions. Subsequent publications will describe the monitoring of an on-line titration and of a hydrolysis reaction.

References

[1] F. Riedo and E. Kováts, *J. Chromatogr.*, 239 (1982) 1–28.
[2] F. Riedo and E. Kováts, in F. Dondi and G. Guiochon (Editors), *Theoretical Advancement in Chromatography and Related Techniques (NATO ASI Series)*, Kluwer, Dordrecht, 1992, pp. 211–226.
[3] W.R. Melander, J.F. Erad and Cs. Horváth, *J. Chromatogr.*, 282 (1983) 229–248.
[4] J.H. Knox and R. Kaliszan, *J. Chromatogr.*, 349 (1985) 211–234.
[5] S. Levin and E. Grushka, *Anal. Chem.*, 58 (1986) 1602–1607.
[6] S. Levin and E. Grushka, *Anal. Chem.*, 59 (1987) 1157–1164.
[7] S. Levin and E. Grushka, *Anal. Chem.*, 61 (1989) 2428–2433.
[8] E. Arvidsson, J. Crommen, G. Schill and D. Westerlund, *J. Chromatogr.*, 461 (1989) 429–441.
[9] S. Golshan-Shirazi and G. Guiochon, *Anal. Chem.*, 62 (1990) 923–932.
[10] S. Levin and S. Abu-Lafi, *Chirality*, 6 (1994) 148–156.
[11] S. Levin and S. Abu-Lafi, *J. Chromatogr. A*, in press.
[12] C.S.G. Phillips and C.R. McIlwrick, *Anal. Chem.*, 45 (1973) 782–786.
[13] A. Sokolowski, T. Fornstedt and D. Westerlund, *J. Liq. Chromatogr.*, 10 (1987) 1629–1662.
[14] T. Fornstedt, D. Westerlund and A. Sokolowski, *J. Liq. Chromatogr.*, 11 (1988) 2645–2684.
[15] T. Fornstedt, D. Westerlund and A. Sokolowski, *J. Chromatogr.*, 506 (1990) 61–74.
[16] T. Fornstedt, D. Westerlund and A. Sokolowski, *J. Chromatogr.*, 535 (1990) 93–110.
[17] T. Fornstedt and D. Westerlund, *J. Chromatogr.*, 648 (1993) 315–324.