

CHROM. 18 267

DETERMINATION OF INORGANIC ANIONS BY ION-PAIR CHROMATOGRAPHY

HETAERON ADSORPTION CHARACTERISTICS ON SOME ALKYL-BONDED SILICA STATIONARY PHASES*

MICHEL DREUX*, MICHEL LAFOSSE, PETRONILLE AGBO-HAZOUME and BADIA CHAABANE-DOUMANDJI

Laboratoire de Chimie Organique Physique et Chromatographies, Université d'Orléans, 45046 Orléans Cédex (France)

and

MICHEL GIBERT and YVES LEVI

Centre de Recherches de la Compagnie Générale des Eaux, Chemin de la Digue, 78600 Maisons-Laffitte (France)

(First received July 17th, 1985; revised manuscript received October 14th, 1985)

SUMMARY

A non-buffered ion-pair chromatographic system with alkyl-bonded silica was used to determine inorganic anions. Some octyl-bonded stationary phases showed unexpected behaviour such as a dependence on pre-wetting and on the flow-rate during the dynamic equilibration. On the other hand, octadecyl-bonded stationary phases were not influenced by these effects. The influence of an organic co-solvent and the efficiency with 3- μm particles in ion-pair chromatography were also studied.

INTRODUCTION

In a previous paper¹ we described the separation of inorganic anions by ion-pair reversed-phase liquid chromatography monitored by indirect photometry. A lipophilic ionic hetaeron ($\text{C}_8\text{H}_{17}\text{NH}_3^+$) is adsorbed on octyl-bonded silica and the ionic solute competes with the UV-absorbing counter ion. A non-buffered aqueous mobile phase is used in order to decrease the conductivity of the eluent and permit double detection by conductimetry and indirect photometry². We have previously shown³ that pre-wetting of the octyl-bonded silica influences the amount of the surfactant ($\text{C}_8\text{H}_{17}\text{NH}_3^+ \text{CH}_3\text{C}_6\text{H}_4\text{SO}_3^-$) adsorbed, although the concentration of the surfactant in the aqueous mobile phase is kept constant, and that an increase in the amount of surfactant adsorbed causes an increase in the capacity factors of inorganic

* Presented as a poster at the 9th International Symposium on Column Liquid Chromatography, Edinburgh, 1–5 July, 1985. The majority of the papers presented at the symposium have been published in *J. Chromatogr.*, Vols. 352 and 353.

TABLE I
 AMOUNT (A) OF ADSORBED SURFACTANT AND CAPACITY FACTORS (k') WITH AQUEOUS MOBILE PHASE, OA OR TBA HETAERON AND (PTS) COUNTER ION ON OCTYL- AND OCTADECYL-BONDED SILICA
 Columns: Zorbax C₈, 240 × 4 mm I.D.; Zorbax ODS, 90 × 4 mm I.D. Mobile phase: 5 mM, surfactant, pH 4.5. Flow-rate: 2 ml min⁻¹.

		Zorbax ODS								
Pre-wetting: methanol (60 ml) then water (ml)	OA-PTS		TBA-PTS		Pre-wetting: methanol (60 ml) then water (ml)		OA-PTS			
	A (μ mole)	k' (I^-)	k' (SO_4^{2-})	A (μ mole)	k' (I^-)	k' (SO_4^{2-})	A (μ mole)	k' (I^-)	k' (SO_4^{2-})	
0	800	4.36	18.6	370	10.3	1.17	0	390	1.6	3
5	515	2.9	12.3	170	4.3	0.6	20	390	1.6	3
20	55	0.4	1.2	80	1.6	0.16	40	390	1.6	3
60	52	0.4	1.2	30	0.3	0.01	60	390	1.6	3

solutes. Other workers⁴⁻⁷ have found that the amount of surfactant adsorbed depends on the concentration of either the pairing ion or the ionic and/or organic modifier in the eluent.

In this work, we studied the effects of pre-wetting of two types of alkyl-bonded silica with two types of alkylammonium surfactants. The influence of the flow-rate of the mobile phase during the equilibration was investigated. With octyl-bonded silica, an irreversible modification was observed after several adsorption and desorption cycles and we propose a simple test to demonstrate this modification. Further studies indicated that the efficiency of a column in ion-pair chromatography is lower than that in reversed-phase chromatography, and that this efficiency is drastically reduced when 3- μm microparticles are used.

EXPERIMENTAL

The chromatographic equipment, eluent preparation, column loading and column desorption were as described elsewhere¹⁻³.

The columns were slurry packed in the laboratory with 7- μm Zorbax C₈ and ODS (DuPont, Wilmington, DE, U.S.A.), 5- μm LiChrosorb RP-8 and RP-18 (Merck, Darmstadt, F.R.G.) and 5- μm Spherisorb C₈ (Phase Separations, Queensferry, U.K.). For the study of the influence of flow-rate, an Ultrasphere C₈ column (Beckman, Fullerton, CA, U.S.A.) was used. Situated before the Rheodyne sample valve, a pre-column packed with octyl-bonded silica guarded the analytical column. In all experiments the following order of operations was adopted.

For the pre-wetting of the apolar phase, different finite volumes of liquid were pumped through the pre-column-analytical column system, first 60 ml of methanol and then from 0 to 60 ml of water. The analytical column was then disconnected and the pre-column was equilibrated separately with the eluent containing the surfactant. After equilibrium of the pre-column, the analytical column was switched into the same eluent flow line. The amount of surfactant adsorbed of the analytical column was calculated by the breakthrough method³ (Table I).

Aqueous eluents were prepared with octylammonium (OA) or tetrabutylammonium (TBA) hexaeron and *p*-toluenesulphonate (PTS), phthalate (P), chloride (Cl) or bromide (Br) counter ions. A comparative study of adsorption was carried out with hydro-organic eluents.

RESULTS AND DISCUSSION

Influence of pre-wetting of alkyl-bonded silica

Before pumping the surfactant (hexaeron-counter ion) dissolved in water through the column, the apolar stationary phase (Zorbax C₈ or ODS) was pre-wetted with 60 ml of methanol followed by water. The various volumes of water desorb a portion of the organic solvent adsorbed on the apolar moiety of the stationary phase and the lipophilic interactions between the surfactant and the stationary phase are decreased.

We have noted¹ that on Zorbax octyl-bonded silica the amount of OA-PTS (or TBA-PTS) surfactant adsorbed decreases with increase in the volume of water used in the pre-wetting stage. This decrease causes a decrease in the capacity factors of the solutes (Table I).

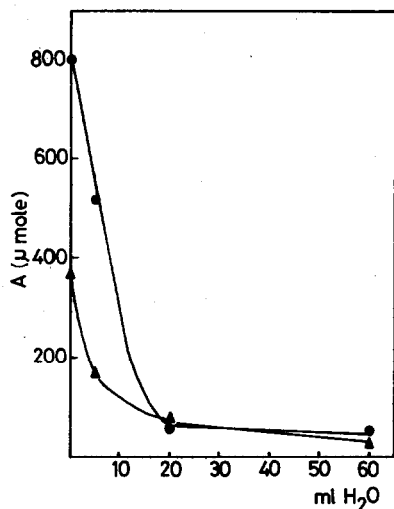


Fig. 1. Plots of amount of surfactant adsorbed as a function of the volume of water in the pre-wetting step following the methanol flushing. ●, 5 mM OA-PTS; ▲, 5 mM TBA-PTS.

Table I and Fig. 1 show a smaller amount of hetaeron adsorbed (A) for TBA than for OA, a greater decrease in A for OA than for TBA and a difference in the capacity factors of solutes for the two different adsorbed hetaerons (OA and TBA).

Two points can be made about the TBA-PTS system: first, the k' value of the sulphate dianion is lower than that of the iodide monoanion, and second, the k' value of the iodide monoanion is high.

Regarding the first point, similar dianion elution has been observed previously in ion-exchange chromatography with a high concentration of salt in the eluent^{8,9}, with ion-pair systems¹⁰⁻¹² and with a particular surfactant^{2,13} (TBA-nitrobenzenesulphonate). In this instance sulphate is eluted as the chloride or bromide faster than as the nitrate or iodide.

The second point can be explained by the different mechanisms involved in TBA hetaeron ion pairing in the mobile phase and in the stationary phase and in OA hetaeron ion pairing in the stationary phase, and by the diffuse charge of the iodide anion, *i.e.*, strong hydrophobic adsorption of the solute on the alkyl-bonded surface is possible when a small amount of TBA surfactant is adsorbed.

Hence the pre-wetting of Zorbax C₈ has a marked effect on the retention of the solutes. The reproducibility and the prediction of retention are difficult without a detailed knowledge of the pre-wetting stage, which may be one explanation for the numerous problems encountered in many laboratories when using such techniques.

OA-PTS was adsorbed on octadecyl-bonded silica (Zorbax ODS) following the same pre-wetting procedure as for the octyl-bonded stationary phase. Table I shows that the amount of OA-PTS adsorbed and the capacity factors of mono- and dianions do not depend on the pre-wetting.

We can propose an explanation for the different behaviours of these apolar bonded silicas. Before flushing with water, the methanol molecules are intercalated between the apolar chains and can behave like a surfactant. When water (60 ml) is

TABLE II

CAPACITY FACTORS OF SUGARS USING AN OCTYL-BONDED SILICA COLUMN

Column: Zorbax C₈, 240 × 4 mm I.D. Mobile phase: water. Flow-rate: 0.8 ml min⁻¹.

Packing	<i>k'</i>		
	<i>Glucose</i>	<i>Sucrose</i>	<i>Raffinose</i>
Unmodified	0.14	0.14	0.17
Irreversibly modified	0.73	1.29	1.68

flushed through the column, methanol is easily removed from the short-chain octyl-bonded layer, which leaves a chaotic structure that collapses owing to increased hydrophobic association¹⁴. With water, which is a very bad wetting agent for these apolar chains, a disordered folded state is favoured like a dry packing¹⁵, and the surfactant is less adsorbed.

On the other hand, with ODS columns after the same flushing with water, some methanol molecules remain between the longer octadecyl chains, favouring an ordered "bristle" or chaotic state but non-collapsed. The residual methanol can be desorbed with a larger amount of water¹⁴ or by heating^{14,16}. With an octadecyl layer wetted by pure methanol or by methanol-water the same amount of surfactant can be adsorbed. Recently, spectral studies of ion-pair interactions have shown that a surfactant in water unfolds the octadecyl chain more than the octyl chain, as the shorter chains have less freedom to rearrange¹⁷.

To confirm this difference in structure of alkyl chains after flushing with water, we can compare the separation of hydrophilic compounds such as glucose, sucrose and raffinose on C₈ and C₁₈ unmodified columns with water as the eluent. On C₈ bonded silica (Table II) no selectivity is obtained and in this collapsed structure the carbohydrates have a retention volume close to the void volume. In contrast, on C₁₈ bonded silica a good separation of carbohydrates is obtained with a low-loaded octadecyl-bonded packing having residual silanol groups¹⁸ and also with a high-loaded Zorbax ODS packing¹⁹. For this last stationary phase residual methanol from the conditioning of the column may permit a chaotic structure to be preserved and the separation of carbohydrates by a sieve phenomenon.

Influence of organic co-solvent

With the same concentration of surfactant in the mobile phase, various amounts can be adsorbed and various capacity factors can be obtained by adding an organic solvent to the mobile phase.

We have shown^{2,3} that after methanol and water pre-wetting, an aqueous-organic eluent increases the amount adsorbed and the capacity factors, but the breakthrough curves show a diffuse shape. After pre-wetting with methanol without water, an increase in the percentage of organic solvent in the eluent (to more than 5–10%) decreases the amount adsorbed because the hydrophobic effect is decreased⁶. However, as can be seen in Fig. 2a, a small amount of organic co-solvent (0–2%) allows more surfactant to be adsorbed than the neat aqueous mobile phase. This small

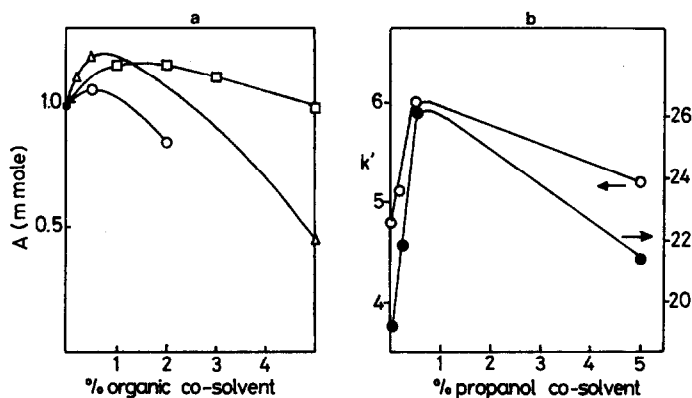


Fig. 2. (a) Amount of surfactant adsorbed as a function of the percentage of organic solvent in the mobile phase (5 mM OA-PTS): \circ , Tetrahydrofuran; Δ , propanol; \square , acetonitrile. (b) Capacity factors of solutes as a function of the percentage of propanol in the mobile phase (5 mM OA-PTS). \bullet , SO_4^{2-} ; \circ , I^- . Column: Zorbax C_8 , 250 \times 4.6 mm I.D. Mobile phase: 5 mM OA-PTS (pH 4.5) + organic co-solvent. Flow-rate: 2 ml min^{-1} . Pre-wetting: 60 ml of methanol.

modification to the mobile phase considerably increases the capacity factors (Fig. 2b).

In contrast a concentration of organic solvent higher than 2% decreases the amount adsorbed and also the capacity factors. In this instance the greater the affinity of the organic modifier for the apolar chain, the more it desorbs the lipophilic surfactant. This affinity can be characterized by the dispersion component, δ_d , of the Hildebrand parameter²⁰. Tetrahydrofuran ($\delta_d = 7.6$) is the most lipophilic (propanol, $\delta_d = 7.2$; acetonitrile, $\delta_d = 6.5$).

Fig. 2 shows that the same retention of a solute can be obtained with different amounts of surfactant using different percentages of propanol co-solvent (0.5 or 5%).

Finally, to increase the capacity factors it is possible to use a small amount of organic co-solvent, but the injection of aqueous solutions disturbs such systems².

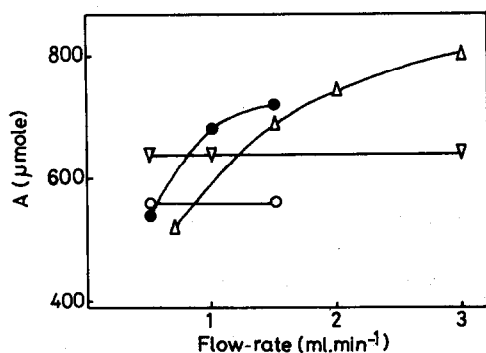


Fig. 3. Plots of amount of surfactant adsorbed [5 mM OA-P (pH 6.38)] as a function of the flow-rate of the equilibration. \bullet , Ultrasphere C_8 ; Δ , Zorbax C_8 ; \circ , Spherisorb C_8 ; ∇ , LiChrosorb RP-8.

Influence of flow-rate on equilibration

In their application notes, some manufacturers (e.g. Shodex) recommend changing the flow-rate of the mobile phase during the equilibration process, but the reason for this variation is not obvious. This fact, and the presence of some unexplained irregularities in the breakthrough curves³, prompted us to study the influence of flow-rate during the equilibration.

After pre-wetting with methanol, the stationary phase was equilibrated using various flow-rates (3, 2, 1.5, 1 and 0.5 ml min⁻¹) compatible with the maximum pressure of the columns. For each value of these flow-rates the amount of surfactant adsorbed was measured.

This was tried with various packing materials (LiChrosorb RP-8 and RP-18, Ultrasphere C₈, Spherisorb C₈ and Zorbax C₈ and ODS) using OA as the hetaeron and P as the counter ion. The results presented in Fig. 3 for C₈ columns show a difference between two columns for which the amount of surfactant adsorbed is independent of the flow-rate (Spherisorb and LiChrosorb) and the other two columns (Zorbax C₈ and Ultrasphere C₈) for which there is a clear dependence on these parameters. An increase in the flow-rate induces an increase in the amount of surfactant adsorbed. The similarity between these two columns confirms the observations of Majors²¹ and Goldberg²², who found a similarity in the capacity factors of either apolar or acidic or basic polar compounds on these two stationary phases. Similar studies with the C₁₈ phases indicated complete independence of these parameters.

In order to verify the influence of the nature of the hetaeron and counter ion on this effect, the same test was carried out using TBA and OA as the hetaeron and PTS, P, Cl and Br as counter ions. Table III gives the results with the organic counter ions. A similar variation with flow-rate was obtained in all four instances. Replacing the organic with inorganic counter ions³ gave the same results.

It was found that this effect is not constant during the life of the column, being noticeable only when the column is either new or has been never used in ion-pair chromatography. After a few adsorption and desorption cycles it becomes saturated and eventually the amount of surfactant adsorbed becomes independent of the

TABLE III

INFLUENCE OF EQUILIBRIUM FLOW-RATE ON THE AMOUNT OF SURFACTANT ADSORBED AND ON THE CAPACITY FACTORS

Column: Zorbax C₈, 7 μm, 240 × 4 mm I.D. Mobile phase: 1 mM surfactant. Pre-wetting: methanol.

Surfactant	Flow-rate (ml min ⁻¹)	A (mmole)	k' (NO ₃ ⁻)	k' (I ⁻)	k' (SO ₄ ²⁻)
OA-PTS	3	0.57	5.1	6.2	28.5
OA-PTS	0.5	0.18	1.5	1.8	6.4
OA-P	3	0.45	3.6	5.3	10.8
OA-P	0.5	0.28	2.2	2.9	6.6
TBA-PTS	2.5	0.40	7.2	20.9	11.3
TBA-PTS	0.5	0.27	4.7	13	7.8
TBA-P	2.5	0.2	12.9	44	20.7
TBA-P	0.5	0.14	8.7	29	14.4

flow-rate used in equilibration (the same value as at a high flow-rate). Therefore, after utilization of these columns, the stationary phase becomes modified. We have devised a simple test to demonstrate the state of these columns. As can be seen from Table II, there is a great difference between the separation of a mixture of three specific sugars (glucose, sucrose and raffinose) on a new and on a modified column, the separation being impossible on a new column and very easy when it is modified. Hence the simple injection of a mixture of these sugars immediately indicates whether the modification to the column has occurred.

We think that a small amount of surfactant remains adsorbed after the desorption process with methanol-water (1:1). We have tried to desorb it by cleaning the column with solvents other than this methanol-water mixture, *viz.*, ethanol-propanol (1:1) at 50°C, methanol-chloroform (1:2) and 1 mM perchloric acid at 50°C, but the test with sugars showed that the column was still modified. A small amount of surfactant may be irreversibly adsorbed. In fact, Bij *et al.*²³ have shown that amines with a bulky alkyl chain such as octylamine are associated with residual silanol groups of bonded silica. These silanophilic interactions are stabilized by hydrophobic interactions between the alkyl chain of the amine and the alkyl moiety of the stationary phase.

These hypotheses do not provide any explanation for the separation of carbohydrates on the modified columns. One can only postulate that the phenomenon is similar to that observed with C₁₈ bonded silica for the separation of the same compounds.

Efficiency of the ion-pair chromatographic system

The efficiency of apolar columns (C₈ and C₁₈) is usually assessed by chromatographing aromatic solutes with methanol-water as the mobile phase²⁴. However, we have found that this evaluation of the performance of an apolar packing material has no meaning when it is used in an ion-pair chromatographic rather than in a reverse-phase system.

Table IV illustrates the results obtained with 7- and 3- μ m Zorbax octadecyl-

TABLE IV

COMPARISON OF EFFICIENCY OF ZORBAX ODS PACKINGS IN REVERSED-PHASE AND ION-PAIR CHROMATOGRAPHIC SYSTEMS

System	Mobile phase	Solute ion	Column			
			Zorbax ODS 7 μ m (150 \times 4.6 mm I.D.)		Zorbax ODS, Golden 3 μ m (80 \times 6.2 mm I.D.)	
			Plates/m (N)	k'	Plates/m (N)	k'
Reversed-phase	Methanol-water (70:30)	Nitronaphthalene	40 400	6.20	65 950	4.75
		Naphthalene	55 440	9.74	95 920	8.11
		NO ₃ ⁻	17 430	1.73	14 420	2.49
Ion-pair	1 mM OA-PTS	ClO ₃ ⁻	18 680	2.54	16 310	3.39
		ClO ₄ ⁻	22 320	3.78	17 330	4.58
		SO ₄ ²⁻	27 150	9.51	22 100	10.58

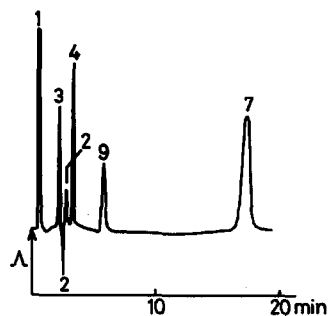
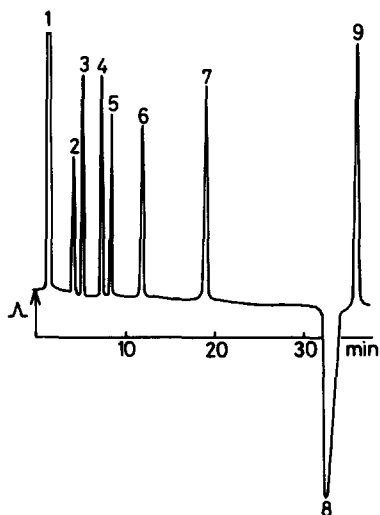


Fig. 4. Chromatogram of a test mixture of inorganic anions. Sample loop: 20 μ l. 1 = Cations; 2 = carbonate, 0.6 μ g; 3 = chloride, 0.36 μ g; 4 = nitrite, 0.92 μ g; 5 = bromide, 1.58 μ g; 6 = nitrate, 1.24 μ g; 7 = sulphate, 0.96 μ g; 8 = induced peak; 9 = iodide, 2.52 μ g. Column: Zorbax C₈, 240 \times 4 mm I.D. Eluent: 1 mM tetrabutylammonium phthalate, pH = 6.2, flow-rate = 1.5 ml min⁻¹. Conductivity detection.

Fig. 5. Chromatogram of a test mixture of inorganic anions. Solutes as in Fig. 4. Multiple peaks for carbonate. Column: Zorbax ODS, 90 \times 4 mm I.D. Eluent: 0.2 mM Tetrabutylammonium phthalate, pH = 6, flow-rate = 1.5 ml min⁻¹. Conductivity detection.

bonded stationary phases. Two observations can be made: first, the opposite variation of efficiency with granulometry in the reversed-phase (RP) and the ion-pair (IP) systems ($N = 55\,440$ at a 7 μ m particle size and 95 920 at 3 μ m in the RP system; $N = 27\,150$ at 7 μ m and 22 100 at 3 μ m in the IP system); and second, the decrease in efficiency in the ion-pair system (for the same k' value, $N = 55\,440$ in the RP system and 27 150 in the IP system at 7 μ m; $N = 95\,920$ in the RP system and 22 100 in the IP system at 3 μ m). This decrease in efficiency can be explained by the numerous equilibria involved in the retention process and by the small velocity of mass transfer in ion-pair chromatography.

In conclusion, a realistic test for an ion-pair chromatographic system needs to be devised. Moreover, one must remember that the arguments applied by chromatographers to reversed-phased systems are not always transposable to ion-pair chromatography.

CONCLUSION

Non-buffered ion-pair reverse-phase liquid chromatography was found to be a successful system for the determination of inorganic anions. The equilibration process is longer with octadecyl- than with octyl-bonded stationary phases, and requires particular attention with octyl-bonded stationary phases. The selectivity depends on the alkyl-bonded stationary phase. For example, Figs. 4 and 5 show that for the determination of carbonate, an octyl-bonded stationary phase must be selected.

Finally, we cannot choose between the C₈ and C₁₈ systems, and we consider that the efficiency of an ion-pair system must be measured by tests different from those used with classical reversed-phase systems.

REFERENCES

- 1 M. Dreux, M. Lafosse and M. Péquignot, *Chromatographia*, 15 (1982) 653.
- 2 P. Agbo-Hazoumé, *Thèse*, Université d'Orléans, Orléans, 1985.
- 3 M. Dreux, M. Lafosse and P. Agbo-Hazoumé, *Chromatographia*, 18 (1984) 15.
- 4 I. Molnár, H. Knauer and D. Wilk, *J. Chromatogr.*, 201 (1980) 225.
- 5 R. S. Deelder and J. H. M. van den Berg, *J. Chromatogr.*, 218 (1981) 327.
- 6 L. E. Vera Avila, M. Caude and R. Rosset, *Analisis*, 10 (1982) 36.
- 7 M. Denkert, L. Hackzell, G. Schill and E. Sjögren, *J. Chromatogr.*, 218 (1981) 31.
- 8 H. L. Rothbart, in B. Karger, L. R. Snyder and C. Horvath (Editors), *An Introduction to Separation Science*, Wiley, New York, 1973.
- 9 P. R. Haddad and C. E. Cowie, *J. Chromatogr.*, 303 (1984) 321.
- 10 G. Smuckler, B. Rössner and G. Schwedt, *J. Chromatogr.*, 302 (1984) 15.
- 11 R. E. Barron and J. S. Fritz, *J. Chromatogr.*, 316 (1984) 201.
- 12 F. J. Gustafson, C. G. Markell and S. M. Simpson, *Anal. Chem.*, 57 (1985) 621.
- 13 M. Dreux and M. Lafosse, in preparation.
- 14 W. E. Hammers and P. B. A. Verschoor, *J. Chromatogr.*, 282 (1983) 41.
- 15 L. C. Sander, J. B. Callis and L. R. Field, *Anal. Chem.*, 55 (1983) 1068.
- 16 D. E. Morel and J. Serpinet, *Chromatographia*, 18 (1984) 611.
- 17 S. D. Dowling and W. R. Seitz, *Anal. Chem.*, 57 (1985) 602.
- 18 G. Palla, *Anal. Chem.*, 53 (1981) 1966.
- 19 M. Lafosse and M. Dreux, unpublished results.
- 20 B. L. Karger, L. R. Snyder and C. Eon, *Anal. Chem.*, 50 (1978) 2126.
- 21 R. E. Majors, *J. Chromatogr. Sci.*, 18 (1980) 488.
- 22 A. P. Goldberg, *Anal. Chem.*, 54 (1982) 342.
- 23 K. E. Bij, Cs. Horváth, W. R. Melander and A. Nahum, *J. Chromatogr.*, 203 (1981) 65.
- 24 M. Verzele and C. Dewaele, *Chromatographia*, 2 (1984) 84.