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## Novel stationary phase with regulated anion-exchange capacity

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### Abstract

A novel stationary phase — phenylaminopropyl (PLA) bonded silica — is proposed for anion-exchange chromatography. Low basicity ( $pK_a$  about 2.5) attached to silica phenylaminopropyl groups allows a variation of surface density of protonated sites in the pH range from 2 to 5. This enables us to use the same column for the separation of anions having different affinity to anion-exchangers. The effect of mobile phase pH on conditional capacity of PhA-silica was studied. The hypothesis on dependence of ion-exchange selectivity on the column capacity is discussed. Suitability of PhA-silica for ion-chromatographic separation of organic and inorganic anions at different pH values of eluent was demonstrated. © 2001 Elsevier Science B.V. All rights reserved.

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### 1. Introduction

As a rule, the retention of anions in ion chromatography (IC) is dependent on the nature/charge of the eluent and can be additionally regulated by change of concentration or pH, or can be varied with column temperature changes. Less attention has been paid to a possibility of variation of the retention by change of the ion-exchange capacity of ion exchangers. Usually the anion-exchange capacity is defined at the stage of stationary phase synthesis. As in the case of anion exchangers, the item functional groups are quaternary ammonium [1], the ion-exchange capacity of a particular phase is constant. To achieve the separation of different anions the analyst should have a set of anion exchangers with different

capacities and a few commercially available columns with specified properties.

A new approach to optimization of the column capacity — capacity gradient ion chromatography — was proposed recently by Yamamoto et al. [2]. This method is based on the ability of hydroxyl residues at the resin surface to form complexes with attached amino groups, thus reducing conditional anion-exchange capacity. At the beginning of the analysis, hydroxyl residuals are blocked in the complexes with dihydroxyphenylborane added to the eluent. Gradual increase of eluent pH causes their dissociation, increasing interaction between hydroxyl residues and bonded amino groups and thus forces decrease of resin's conditional anion-exchange capacity. Thus the capacity gradient IC allows the efficient separation of anions with very different affinity to the anion exchanger.

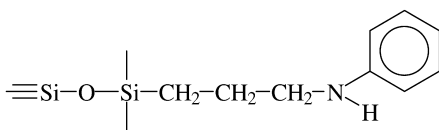
The anion-exchange capacity manipulation can be also accomplished by a simple change in protonation degree of primary, secondary or tertiary amino groups in case of a weak anion exchanger. However,

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there is a restricted possibility to do it for silica-based materials because of relatively high  $pK_a$  values of alkylamino groups, which are out of pH range 0–7.5 of hydrolytical stability of silica backbone. There are only a few papers on the application in IC of silica bound amines with  $pK_a < 7.5$ . Tetraethylenepentamine bonded silica with monotonically distributed  $pK_a$  values of primary and secondary amino groups in the range from 2.7 to 9.9 was used for the separation of transition metal ions by a chromatofocusing technique or isoconductive pH gradient IC system [3,4]. As a part of separation mechanism a linear pH gradient, formed on-column, produced the increase of anion-exchange capacity due to protonation of amino groups and consequent elution of metal cations due to electrostatic repulsion. Recently, a weak anion-exchange polystyrene–divinylbenzene (PS–DVB) resin, functionalized with diethylenetriamine having a similar structure of bonded layer and acid–base properties (set of  $pK_a = 4.3, 9.1$  and  $9.9$ ) was used for anion chromatography [5], while the changes of pH of the eluent were applied for the regulation of column capacity and retention times.

The covalent attachment of phenylaminopropyl functional groups to the silica surface results in a new weak anion exchanger [phenylaminopropyl-bonded silica (PhA-silica)]:



which may be a possible solution to the above problem. Due to the presence of a phenyl substitute, aromatic amino groups in PhA-silica are less basic than any other alkyl substituted amino functional groups of common anion-exchangers. It allows regulating the PhA-silica anion-exchange capacity by change of the eluent pH in weak acid pH range that is in accordance with the hydrolytical stability of the matrix. Thus, a single column packed with PhA-silica can be exploited for various separations of weakly and strongly retained anions.

Due to the presence of a phenyl substituent in the silica bonded aminopropyl moiety, the combination of electrostatic, hydrophobic and  $\pi$ – $\pi$  interactions

should be taken into consideration of the retention mechanism of polar organic molecules. Selectivity of PhA-silica for the separation of phenol and its derivatives was studied in our previous work [6]. The similar stationary phase phenylpropanolamine (2-amino-1-phenyl-1-propanol or norephedrine) bonded silica was used in the reversed-phase HPLC separation of acidic, neutral and basic small molecules and in the ion-exchange separation of inorganic anions [7]. However, in the molecule of phenylpropanolamine the benzene ring is separated from the amino group by two carbon atoms which produces only a small change in the basicity of the amino group. For this reason the anion-exchange properties of the latter stationary phase correspond to those of aliphatic amine rather than those of aromatic amine, so protonation/deprotonation equilibrium takes place in alkaline media.

The aim of the present communication is to evaluate PhA-silica as a versatile stationary phase for the separation of anions with widely different affinities to anion exchangers. The chromatographic property of this novel anion-exchange functionality including the effect of pH on the retention of inorganic anions and on the separation selectivity is studied.

## 2. Experimental

The HPLC system consisted of an isocratic Altex 101A pump, an Altex 210 injection valve (Berkeley, CA, USA) equipped with a 100- $\mu$ l sample loop, an LKB 5300B conductolyzer, an LKB 2238 Uvicord spectrophotometric detector (Bromma, Sweden) set at 206 nm for the detection of UV absorbing anions ( $\text{Br}^-$ ,  $\text{NO}_3^-$ ,  $\text{I}^-$ ,  $\text{SCN}^-$ ,  $\text{BrO}_3^-$ ) with perchloric acid–sodium perchlorate eluent. The stainless steel guard column (40 $\times$ 3 mm I.D.) and the stainless steel column (150 $\times$ 4.6 mm I.D.) were slurry packed with PhA-silica from a 2-propanol–water mixture.

PhA-silica was prepared by treatment of silica particles (7  $\mu\text{m}$ , 300  $\text{m}^2/\text{g}$ ) KSK-G (Reakhim, Nizniy Novgorod, Russian Federation) with [3-(phenylamino)propyl]trimethoxysilane (Fluka, Deisenhofen, Germany) in dry toluene as described [6].

Solutions of perchloric acid and sodium perchlorate (HPLC grade, Fisher, USA), citric acid and

sulfosalicylic acid (Reakhim, Russia) in distilled water were used for preparation of the eluents. All tested compounds were of analytical grade.

### 3. Results and discussion

The nature, pH and ionic strength of the eluent must be responsible for the retention of inorganic anions on a column packed with PhA-silica.

#### 3.1. Effect of pH on retention of anions

The  $pK_a$  values of *N*-phenylaminopropyl group attached to the silica was expected to be close to 5.1 as compared to its homogeneous analog *N*-butylaniline [8]. However, it is well established that  $pK_a$  values of amino groups in grafted-to-silica state can be 2 units less than the  $pK_a$  values of corresponding homogeneous analogs because of the interactions with residual silanols at the silica surface [9]. In case of PhA-silica this change in basicity of amino groups may be less pronounced due to the steric hindrance from the bulk phenyl substitute.

The effect of pH on the retention of inorganic anions on PhA-silica was studied in the range from 2 to 6.5 under a constant concentration of eluting anion (Fig. 1). As expected, the increase of pH causes the deprotonation of the attached amino groups and the decrease of conditional anion-exchange capacity of PhA-silica. Accordingly, the retention of anions decreases. The obtained dependence looks like a part of an acid–base titration curve of protonated phenylaminopropyl groups with  $pK_a$  value about 2.5. It confirms the supposition about interaction of bonded amino groups with residual silanols at the PhA-silica surface unless the steric hindrance from the phenyl substituent.

#### 3.2. Effect of conditional capacity on separation selectivity

The constancy of the ion-exchange selectivity under varied capacity of the ion exchanger is one of the fundamental statements in the theory of ion exchange and IC. In the ideal case the selectivity should not depend on the capacity. Several inves-

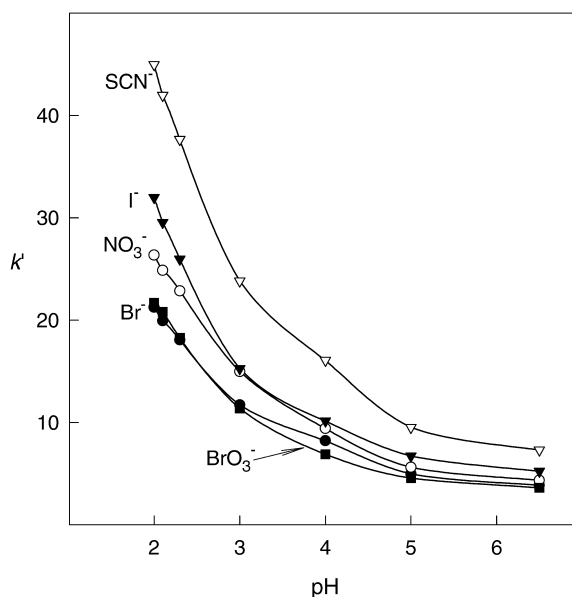


Fig. 1. The effect of pH on the retention ( $k'$ ) of inorganic anions on PhA-silica. Eluent: perchloric acid–sodium perchlorate,  $C(\text{ClO}_4^-) = 10 \text{ mM}$ .

tigations have been undertaken to study this carefully. Gjerde and Fritz synthesized a series of PS–DVB based strong anion exchangers with capacities ranging from 0.17 to 1.46 mequiv. of quaternary ammonium groups/g [10] and compared the retention of several inorganic anions. Their results provided evidence that the capacity of strong base anion exchanger did not affect the separation selectivity of  $\text{NO}_3^-$ ,  $\text{Cl}^-$ ,  $\text{F}^-$  and  $\text{OH}^-$  anions. The possible criticism of their approach is connected with the application of several separately packed columns and hence with the reproducibility of the packing procedure. Also the swelling of PS–DVB based ion-exchange materials in water based eluents depends on their ion-exchange capacity that should indirectly affect secondary interactions such as diffusion of ions inside of the resin corn and hence on the kinetics of mass-transfer.

From our point of view the use of a single column packed with non-swelling silica based weak base anion-exchange is looking more justified for checking the above-mentioned statement. In this case the regulation of the ion-exchange capacity can be achieved by simple adjustment of eluent pH. So, the

elution system water–HClO<sub>4</sub>–NaClO<sub>4</sub> with constant concentration of perchlorate was used for the investigation of the effect of eluent pH on the retention of anions. As the elution power of perchloric acid–sodium perchlorate solutions should be constant due to constant concentration of perchlorate anions in the eluent, the retention of analytes is defined by the conditional capacity of PhA-silica. The conditional capacity of PhA-silica at a given pH and pK<sub>a</sub> values of attached functional groups could be evaluated from the acid–base titration curve of the anion exchanger. As the acid–base potentiometric titration at low pH did not allow accurate quantitative determination of the deprotonation constant of phenylamino groups due to the absence of a noticeable titration jump at the corresponding curve, it was estimated indirectly from the pH dependence of retention times of anions of strong acids (Fig. 1). The pK<sub>a</sub> value of phenylaminopropyl groups estimated from the differential form of this curve was found close to 2.5.

The character of the dependence of the ion-exchange selectivity on the capacity of PhA-silica will not be changed under substitution of the conditional capacity of the anion exchanger by capacity factor or capacity coefficient  $k'$  of any particular anion. In our experiments the capacity factor of bromide  $k'_{\text{Br}}$  was used as reference. This substitution is correct, because these parameters are proportional [1]:

$$k' = \frac{CK_{\text{El}}^{\text{An}}}{RE} \quad (1)$$

where  $C$  is the ion-exchange capacity,  $K_{\text{El}}^{\text{An}}$  the selectivity coefficient between sample anion (An) and eluting anion (El),  $R$  the column phase ratio and

$E$  the eluent concentration. There is a single speculative supposition about the constancy of the selectivity coefficient  $K_{\text{El}}^{\text{An}}$  in experiments on checking the dependence of separation selectivity from the ion-exchange capacity.

The slope of the dependence of capacity coefficient  $k'_{\text{An}}$  of a selected anion from  $k'_{\text{Br}}$  at two different conditional capacities of PhA-silica ( $C_1$  and  $C_2$ ) with ClO<sub>4</sub><sup>−</sup> eluting ion must be equal to selectivity coefficient  $K_{\text{Br}}^{\text{An}}$ :

$$tg\alpha = \frac{(k'_{C_2} - k'_{C_1})_{\text{An}}}{(k'_{C_2} - k'_{C_1})_{\text{Br}}} = \frac{K_{\text{ClO}_4}^{\text{An}}}{K_{\text{ClO}_4}^{\text{Br}}} = K_{\text{Br}}^{\text{An}} \quad (2)$$

The linearity of dependence between  $k'_{\text{An}}$  and  $k'_{\text{Br}}$  in the whole range of  $k'_{\text{Br}}$  should mean the proof of constancy of selectivity coefficient  $K_{\text{Br}}^{\text{An}}$  for various capacities of PhA-silica.

The obtained results show that the separation selectivity of anions in case of PhA-silica is slightly decreased with eluent pH (Table 1). At a first look  $k'_{\text{An}} - k'_{\text{Br}}$  dependencies seem to be linear (Fig. 2) as can be concluded from calculated correlation coefficients. However, strong correlation does not always provide real evidence of linearity. It is more important how experimental points fitted the statistically “ideal” line. Only for the large anions such as thiocyanate and iodide the distribution of data points around the correlation line gave a small suspicion that the real type of dependence is different from linear. Unfortunately, the volume of obtained experimental data was not sufficient to prove this statement statistically and it cannot be stated with enough confidence whether this arrangement is casual or regular.

Table 1

The values of selectivity coefficients  $\alpha = \kappa'_2/\kappa'_1$  obtained for inorganic anions retained on PhA-silica at different pH values in eluents of the equal ionic strength created by variation of HClO<sub>4</sub> and NaClO<sub>4</sub> content

pH	$\alpha(\text{NO}_3^-, \text{Br}^-)$	$\alpha(\text{I}^-, \text{Br}^-)$	$\alpha(\text{SCN}^-, \text{Br}^-)$	$\alpha(\text{BrO}_3^-, \text{Br}^-)$
2.0	1.24	1.50	2.12	1.02
2.1	1.25	1.48	2.11	1.05
2.3	1.27	1.44	2.08	1.01
3.0	1.28	1.30	2.03	0.97
4.0	1.15	1.24	1.96	0.84
5.0	1.13	1.35	1.91	0.92
6.5	1.12	1.35	1.88	0.93

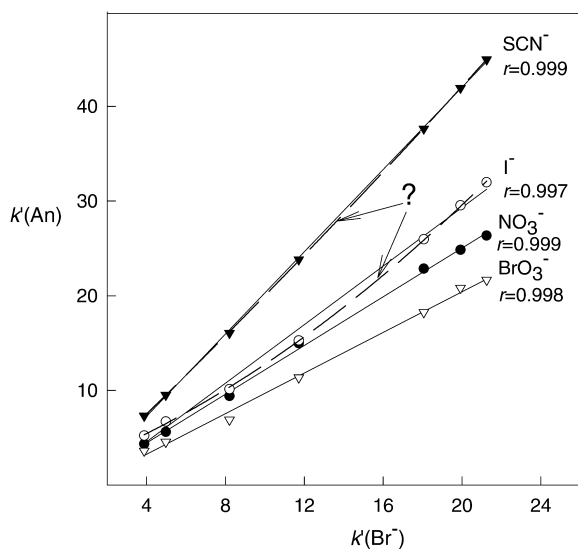


Fig. 2. The dependence of separation selectivity from the column capacity expressed as a function of capacity factor of one anion ( $k'_{An}$ ) from the capacity factor of bromide ( $k'_{Br}$ ).

### 3.3. Effect of eluent pH on separation efficiency

The column efficiency was found to be pH-dependent (Fig. 3). All experimental points of this depen-

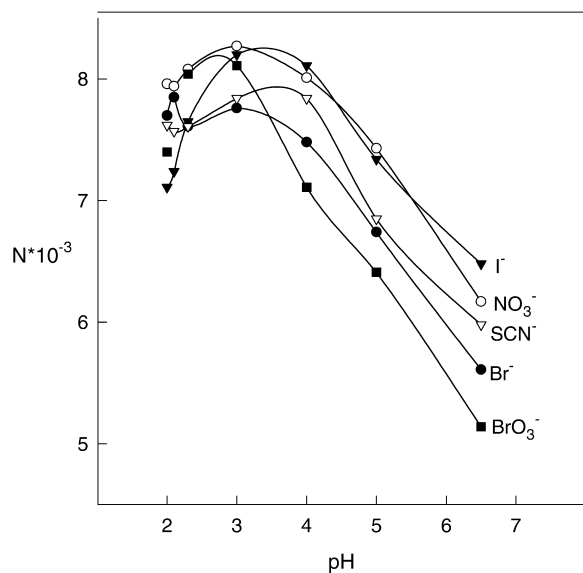


Fig. 3. The effect of pH eluent on column efficiency. Eluent: perchloric acid–sodium perchlorate,  $C(\text{ClO}_4^-) = 10 \text{ mM}$ .

dence were obtained in perchloric acid–sodium perchlorate based eluent at a constant concentration of perchlorate. There is a maximum efficiency for all of the studied anions at pH 3 which drops with an increase in pH. The calculated number of theoretical plates  $N$  per column at the maximum was equal to 8270 for nitrate. This corresponds to a reduced plate height  $h = 2.42$ , which is close to the theoretically possible maximum value. Obviously, the existence of the maximum of dependence  $N = f(\text{pH})$  is related to the ionic state of the functional groups on PhA-silica (see Section 3.1). At eluent pH below 3, phenylamino groups are protonated as well as the residual silanol groups. It means the absence of coordination of the amino groups on silanols and relatively free access of separated anions for electrostatic interactions. Further increase of pH leads to the dissociation of silanols, the part of which can strongly coordinate positively charged phenylamino groups. This type of coordination is responsible for the kinetically slow mass-transfer, probably due to a slow dissociation of this associate, as was established by Fornstedt et al. [11]. It is rejected in a drop of separation efficiency with increase of pH of the eluent (Fig. 3) as the separated anions have to compete with silanols for the coordinated phenylamino groups.

The nature of the eluent was found to affect the column efficiency too. The use of sulfosalicylic and citric acid eluents shows a worsening of column efficiency in 4 and 2 times correspondingly. The more pronounced effect in case of sulfosalicylic acid is connected with its possible  $\pi$ – $\pi$  interactions with phenyl groups in PhA-silica. However, water solutions of these acids were used as eluents in further experiments because of their buffering properties in the desired pH range and a more stable baseline of the conductivity detector.

### 3.4. Separation of inorganic anions

Variable capacity of PhA-silica allows the separation of both weakly and strongly retained anions at different eluent pH. At a low pH value, when the conditional anion-exchange capacity of PhA-silica is high, several phosphates and chloroacetic acid anions were separated with 1.5 mM sulfosalicylic acid as an

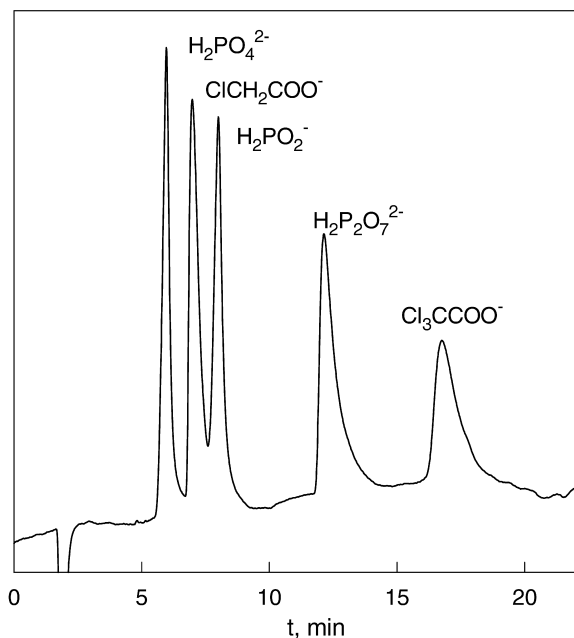


Fig. 4. Chromatogram of a standard mixture of inorganic anions. Column: PhA-silica, 150×4.6 mm with a 40×3 mm guard column. Eluent: 1.5 mM sulfosalicylic acid, pH 2.6. Flow-rate 1 ml/min. Detection: conductivity.

eluent (Fig. 4). Increase of the eluent pH up to 4.2 allowed separation of seven inorganic anions including strongly retained  $I^-$ ,  $ClO_4^-$ ,  $SCN^-$  and  $SO_4^{2-}$  in 25 min. However, the buffering ability of sulfosalicylic acid ( $pK_a$  are 2.46 and 11.74) at pH about 4 was weak and injection of real samples could cause a strong distortion of baseline. So, citric acid ( $pK_a$  are 3.13, 4.76 and 6.40) water solution was found more suitable for such separation. In 4 mM citric acid (pH < 4) seven inorganic anions were separated in 28 min. Unlike traditional anion exchangers, retention of easily polarized anions, such as  $ClO_4^-$ ,  $SCN^-$  on PhA-silica is not too strong and they are eluted shortly after  $NO_3^-$  and  $I^-$  anions (Fig. 5).

#### 4. Conclusions

A novel stationary phase — 3-phenylaminopropylsilica — was studied for the separation of

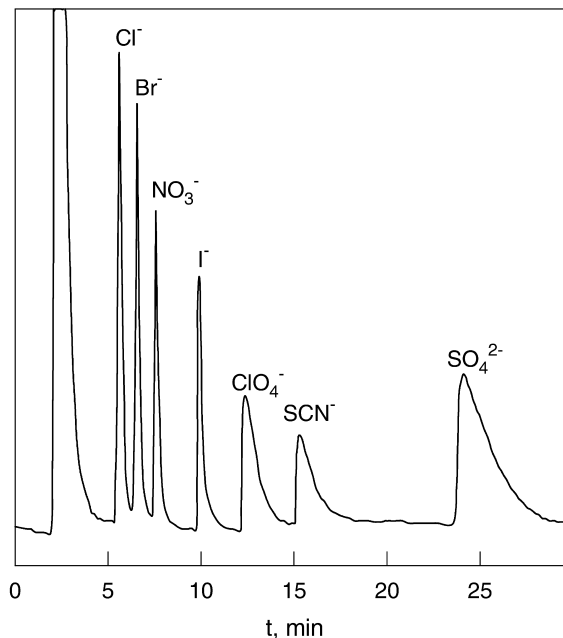


Fig. 5. Chromatogram of a standard mixture of inorganic anions. Column: PhA-silica, 150×4.6 mm with a 40×3 mm guard column. Eluent: 4 mM citric acid, pH 4. Flow-rate 1 ml/min. Detection: conductivity.

inorganic anions. The main feature of this anion exchanger is regulated conditional anion-exchange capacity. It enabled us to study the effect of stationary phase capacity on separation selectivity of anions with the help of the same column. Separation selectivity for large anions  $I^-$  and  $SCN^-$  appeared to be ion-exchange capacity-dependent. Variable capacity of PhA-silica affords us to use the same column for separation of anions with different affinities towards the anion exchanger.

#### References

- [1] D.T. Gjerde, J.S. Fritz, *Ion Chromatography*, Hüthig, Heilderberg, 1987, Chapter 4, p. 70.
- [2] A. Yamamoto, Y. Inoue, S. Kodama, A. Matsunaga, *J. Chromatogr. A* 850 (1999) 73.
- [3] P.N. Nesterenko, A.V. Ivanov, *Mendeleev Commun.* (1994) 174.
- [4] P.N. Nesterenko, A.V. Ivanov, *J. Chromatogr. A* 671 (1994) 95.

- [5] J. Li, J.S. Fritz, *J. Chromatogr. A* 793 (1998) 231.
- [6] M.G. Kiseleva, P.N. Nesterenko, *J. Chromatogr. A* 898 (2000) 23.
- [7] S. Wongyai, *Chromatographia* 38 (1994) 485.
- [8] P.H. Howard, W.M. Meylan, *Handbook of Physical Properties of Organic Chemicals*, CRC Press, 1997.
- [9] G.V. Kudryavtsev, G.V. Lisichkin, *Zh. Fiz. Khim.* 55 (1981) 1352.
- [10] D.T. Gjerde, J.S. Fritz, *J. Chromatogr.* 176 (1979) 199.
- [11] T. Fornstedt, G.M. Zhong, G. Guiochon, *J. Chromatogr. A* 742 (1996) 55.