# Selectivity of chemically bonded zwitterion-exchange stationary phases in ion chromatography

Pavel N. Nesterenko*, Alexandr I. Elefterov, Dmitry A. Tarasenko, Oleg A. Shpigun

*Department of Analytical Chemistry, Lomonosov State University, Leninskie Gory, GSP-3, 119899 Moscow, Russian Federation*

## Abstract

A number of zwitterion-exchange stationary phases were prepared by immobilization of amino acids of different structure (Asp, Glu, Val, Tyr, Pro, Hypro, Arg and Lys) on a silica surface. The occurrence of oppositely charged groups in a single ion-exchange site provided cation-, anion- and zwitterion-exchange properties. The chromatographic behaviour of these stationary phases was evaluated by the retention of a series of test organic compounds of different ionogenic nature. The role of the type and structure of the ion-exchange site was considered. The structure of the bonded molecules and the pH of the eluent were shown to be key parameters influencing the ion-exchange properties of amino acid-bonded silicas. The cation-exchange selectivity of phases containing primary or secondary amino groups was investigated for alkali and alkaline earth metal ions. The application of amino acid-bonded silicas to the ion chromatographic separation of anions and cations is described.

## 1. Introduction

There are two basic advantages of zwitterionic stationary phases (ZSP) which attract the attention of chromatographers. First, the existence of oppositely charged layers on the surface of these ion exchangers provides short diffusion paths and excellent mass transfer characteristics. As a result, more efficient separations were achieved for ZSP. Second, the use of these phases with fixed concentrations of ligands and varying pH and ionic strength of mobile phases provides a wide range of selectivity changes. Most recently the zwitterionic phases proved to be very useful for the efficient and selective separations of inorganic anions [1,2], cations [3] and ampholyte

molecules [4–6] and for the simultaneous separation of both anions and cations [7–9].

There are several types of zwitterionic stationary phases used in high-performance liquid chromatography (HPLC) and ion chromatography (IC). The zwitterionic properties of ion exchangers have been realized on different varieties of delocalization of positive and negative charges in stationary phases. First, there are agglomerated ion exchangers [10] and "centrally localized" ion exchangers [11] in which two oppositely charged layers occur at the surface. The distance between the oppositely charged layers on the surface of agglomerated ion exchangers depends on the diameter of the latex microbeads. The thickness of the negatively charged layer at the surface of the "centrally localized" anion exchangers is defined by the

---

* Corresponding author.

time of the treatment of the polymeric anion-exchange resin with sulphuric acid. Second, the oppositely charged sites can be distributed in a random order on the surface of inorganic oxides such as amphoteric alumina [12]. The distance between the positive and negative charges is determined by the structure of the oxide. Lastly, the permanently bonded matrix zwitterionic molecules [1–3] and corresponding dynamically modified ion exchangers [7,8] form a third group of ZSP in which the oppositely charged groups are very close together in one molecule. Amino acid-bonded silicas are widely used in ligand-exchange [13], metal chelate [13] and affinity chromatography [14]. However, their ion-exchange properties have not been systematically investigated.

The main purpose of this study was to characterize the ion-exchange properties of amino acid-bonded silicas and to study the selectivity of these types of ZSP for the separation of organic and inorganic anions and cations.

# 2. Experimental

## 2.1. Chromatographic system

The chromatographic system consisted of a Model 114M high-pressure pump (Beckman, Palo Alto, CA, USA), a Model 7125 injection valve (Rheodyne, Cotati, CA, USA) equipped with a 50-$\mu$l loop and Conductolyzer 5300 and Uvicord 2238 conductivity detectors (both from LKB, Bromma, Sweden). A Spectra-Physics (San Jose, CA, USA) DP-700 chromatographic data system was used for data collection and processing.

## 2.2. Eluents

Solutions of perchloric, nitric and citric acids (Reakhim, Moscow, Russian Federation) were used as eluents. Deionized, distilled water was used for the preparation of eluents. Solutions of 1 $M$ LiOH and 1 $M$ NaOH were used to adjust pH of the eluent. The pH values of eluents were

measured with a pH-340 pH meter (ZIP, Moscow, Russian Federation) with a glass electrode.

## 2.3. Columns and sorbents

KSK-1 silica with a specific surface of 350 m$^2$/g and particle size 5 or 7.5 $\mu$m (Reakhim), Polyol-100 of 5 $\mu$m, Silasorb 600 of 7.5 $\mu$m and Silasorb 300 of 5, 7.5 or 10 $\mu$m particle size (Lachema, Brno, Czech Republic) were used as a matrix for synthesis. All amino acid-bonded phases were prepared by the initial reaction of amino acids with 3-glycidoxypropyltriethoxy-silane (Reakhim) followed by surface treatment of the silica with the amino acid (Scheme 1) according to a slightly modified method [15]. A weak acid cation exchanger containing propionic acid residues bonded to silica was purchased from JV BioChemMack. Stainless-steel or PEEK chromatographic columns were slurry packed. The values of the ion-exchange capacity were calculated from CHN elemental analysis data.
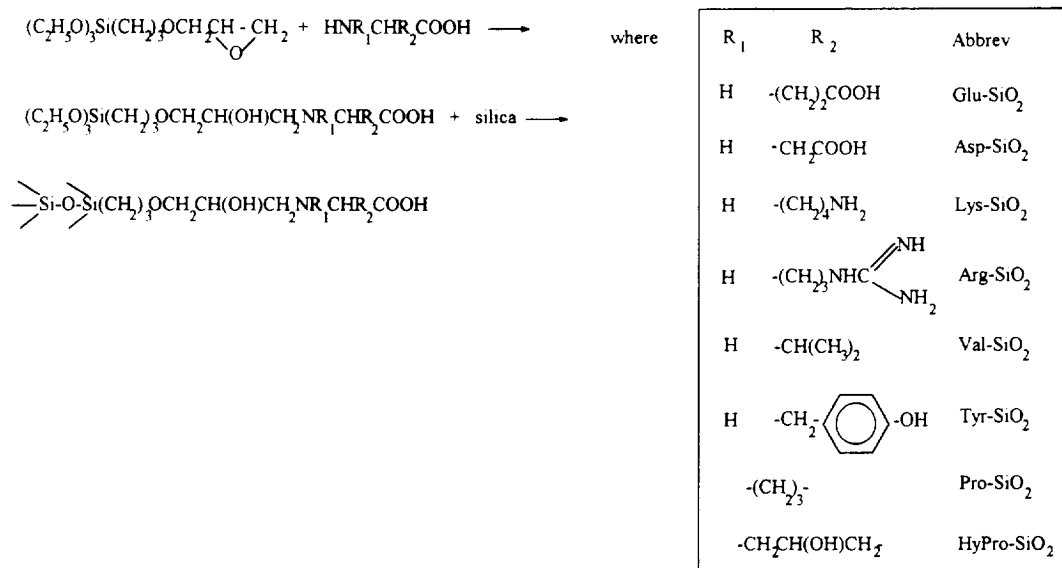
## 2.4. Chemicals

All chemicals were of analytical-reagent grade. Stock standard solutions of inorganic cations were prepared by dissolving appropriate amounts of alkali and alkaline earth nitrates in deionized, distilled water. Aqueous solutions of aniline sulphate, benzoic acid, benzenesulphonic acid, $p$-aminobenzoic acid, $p$-sulphanilic acid (all purchased from Reakhim) and N,N,N-trimethylphenylammonium bromide (Fluka, Buchs, Switzerland) were used for the characterization of the ion-exchange properties of bonded phases.

# 3. Results and discussion

## 3.1. Selection of amino acid-bonded silicas

$\alpha$-Amino acids are the simplest available zwitterionic molecules which can be easily attached to a silica surface. In accordance with their structure and presumably ion-exchange proper-

$(C_2H_5O)_3Si(CH_2)_3OCH_2CH-CH_2$ + $HNR_1CHR_2COOH$ $\longrightarrow$ where

$(C_2H_5O)_3Si(CH_2)_3OCH_2CH(OH)CH_2NR_1CHR_2COOH$ + silica $\longrightarrow$

$\equiv$Si-O-$Si(CH_2)_3OCH_2CH(OH)CH_2NR_1CHR_2COOH$

| $R_1$ | $R_2$ | Abbrev |
|-------|-------|--------|
| H | -$(CH_2)_2COOH$ | Glu-SiO$_2$ |
| H | -$CH_2COOH$ | Asp-SiO$_2$ |
| H | -$(CH_2)_4NH_2$ | Lys-SiO$_2$ |
| H | -$(CH_2)_3NHC\begin{smallmatrix}\nearrow NH \\ \searrow NH_2\end{smallmatrix}$ | Arg-SiO$_2$ |
| H | -$CH(CH_3)_2$ | Val-SiO$_2$ |
| H | -$CH_2$-C$_6$H$_4$-OH | Tyr-SiO$_2$ |
| | -$(CH_2)_3$- | Pro-SiO$_2$ |
| | -$CH_2CH(OH)CH_2$- | HyPro-SiO$_2$ |

Scheme 1

ties, all naturally occurring amino acids can be subdivided into four groups:

(1) monoaminocarboxylic acids containing one primary amino group and one carboxylic group;

(2) diaminocarboxylic acids such as lysine and arginine; it should be noted that the guanidino group containing several nitrogen atoms is considered as one basic group;

(3) monoaminodicarboxylic acids such as aspartic acid and glutamic acid;

(4) heterocyclic amino acids containing a secondary amino group and one carboxylic group, e.g., proline and hydroxyproline.

In aqueous solutions, amino acids behave as inner salts and are generally both weak acids and weak bases. At low pH the conjugated acid is the predominant form. At intermediate pH, amino acids exist in an equilibrium between the neutral molecules and the appropriate zwitterion form.

The attachment of amino acids to the surface of silica leads to a change in their ion-exchange properties owing to the interactions of the amino groups of the bonded molecules with residual silanols and an increase in the basicity of amino groups after linkage with a 3-glycidoxypropyl spacer [1]. Taking these and multiple acid–base equilibrium in bonded molecules into consideration, it is possible a priori to provide only an approximate evaluation of the relative affinities of amino acid-bonded phases for different ions. In previous work the anion-exchange properties of L-hydroxyproline-bonded silica and L-proline-bonded silica were carefully studied [1,2]. The cation-exchange properties were mentioned for silicas with attached arginine, valine and tyrosine.

For the investigation of the effect of the structure of the amino acid attached to silica on the ion-exchange properties, new sorbents with bonded lysine, aspartic acid and glutamic acid were synthesized. Hence each of the above-mentioned structural groups of amino acids were studied using two sorbents.

3.2. Selection of eluent

Evidently the main factor influencing the ionic state of bonded amino acids will be the pH and the concentration of eluent. Thus, citric acid ($pK_1 = 3.14$; $pK_2 = 4.66$; $pK_3 = 6.40$ [16]) providing a suitable buffer capacity over a wide range of pH was chosen for the investigation of

the chromatographic behaviour of these sorbents. For the study of the affinity of sorbents to cations of alkali and alkaline earth metals, aqueous solutions of perchloric and nitric acids were used as eluents to avoid possible complexation of alkaline earth metals with citric acid.

## 3.3. Ion-exchange properties of amino acid-bonded silicas

The retention of ionogenic solutes depends on both the ion-exchange capacity of the sorbents and the nature of bonded functional groups. The direct characterization of the ion-exchange properties of ZSP is difficult owing to the constant change in ionic state of bonded amino acids with changes in pH and ionic strength. The pH dependence of the retention of a set of test solutes of different acidity–basicity in an eluent of constant concentration would be more informative for the characterization of the different zwitterion exchangers. The difference in the surface concentrations of bonded molecules (Table 1) and the influence of residual silanol groups should be taken into consideration to explain the results obtained.

Six benzene derivatives were chosen for the characterization of the ion-exchange properties of sorbents. These were primary amine (aniline), quaternary amine (trimethylphenylammonium), two acids (benzoic and benzenesulphonic acid) and two ampholytes (sulfanilic acid and

p-aminobenzoic acid). Fig. 1 shows the dependence of the capacity factors for test solutes on the pH of the eluent for different columns, illustrating the ion-exchange properties of the studied ZSP supports.

The retention of the strongly acidic benzenesulphonic acid and weakly acidic benzoic acid decreased as the pH of eluent increased from 2.85 to 7.0 for all sorbents. This is in accordance with the increase in negative charge of bonded zwitterionic molecules owing to the dissociation of carboxylic groups and the increase in the eluting power of citric acid. Pro-SiO$_2$ and Hypro-SiO$_2$ exhibit a stronger anion-exchange ability at acidic pH. Evidently, this is connected with the presence of a more basic tertiary amino group in the ion-exchange sites of these sorbents. The application of these ion exchangers for the separation of inorganic anions was shown earlier [1,2].

The retention of zwitterionic solutes sulphanilic acid and p-aminobenzoic acid on Pro-SiO$_2$ and Hypro-SiO$_2$ showed a maximum corresponding to the isoelectric point for both bonded molecules (Fig. 1). At the respective isoelectric points of zwitterionic solutes, both the anionic and cationic groups are charged and these bipolar molecules are mainly retained via a quadruple interaction with doubly charged ion-exchange sites of sorbents [5,6]. Of course, the maximum of retention for sulfanilic acid, having a lower pK$_a$ value of 3.20 for the sulpho group, would

Table 1
Characterization of the stationary phases investigated

| Sorbent | Specific surface area (m$^2$/g) | Particle size ($\mu$m) | Capacity[a] ($\mu$mol/g) |
|---|---|---|---|
| Glu-SiO$_2$ | 300 | 5 | 140 |
| Asp-SiO$_2$ | 300 | 7.5 | 60 |
| Lys-SiO$_2$ | 600 | 7.5 | 130 |
| Arg-SiO$_2$ | 300 | 7.5 | 160 |
| Pro-SiO$_2$ | 100 | 5 | 150 |
| Hypro-SiO$_2$ | 350 | 5 | 120 |
| Tyr-SiO$_2$ | 300 | 7.5 | 750 |
| Val-SiO$_2$ | 100 | 5 | 180 |
| COOH-SiO$_2$ | 350 | 10 | 980 |

[a] Calculated from elemental analysis data.

correspond to a lower value of the pH of the eluent than is the case for *p*-aminobenzoic acid, having a carboxylic group with $pK_a = 4.89$ [16]. The above property of silica-bonded proline and hydroxyproline allows their use for the simultaneous separation of anions and cations with a single eluent [9]. It is also interesting that this bell-shaped dependence was not observed for the other sorbents investigated in which bonded amino acids had a free proton in amino group and hence gave the possibility of interaction with residual silanol groups.
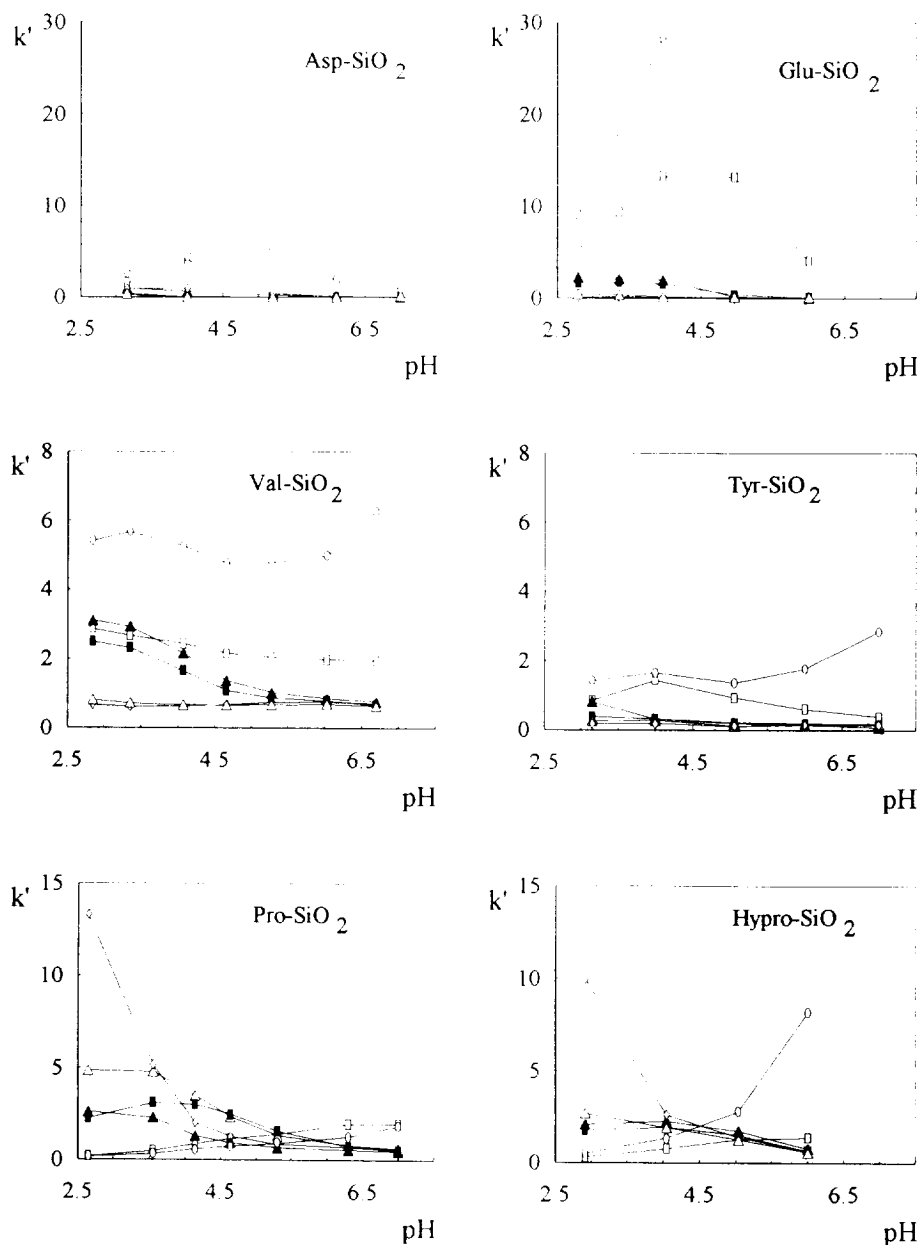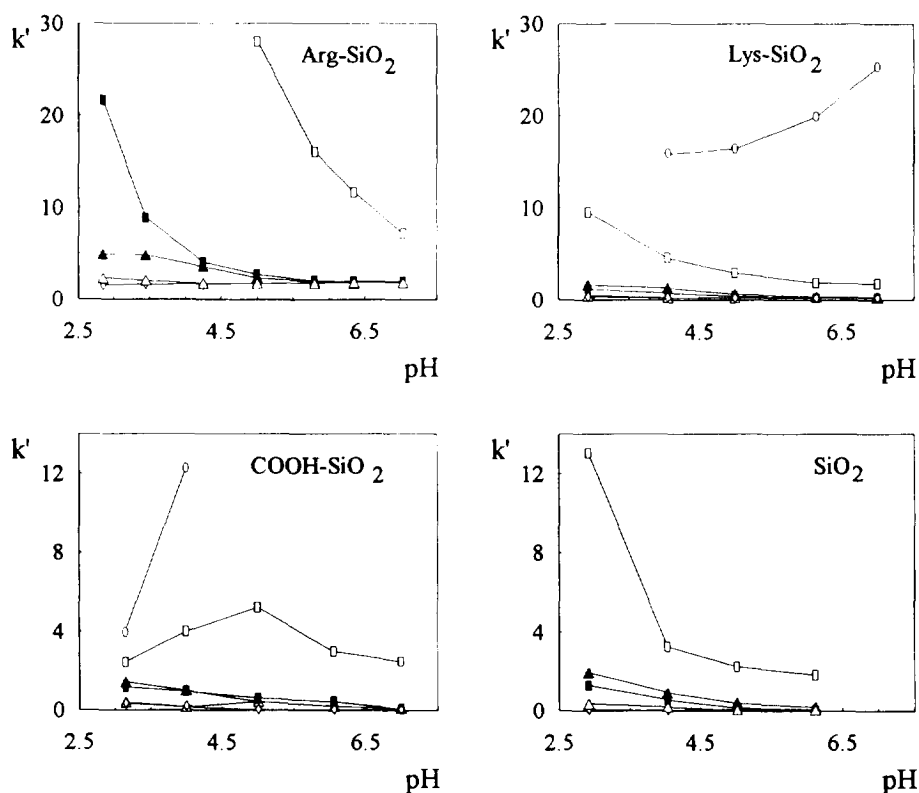


Fig. 1.

Fig. 1. Effect of eluent pH on capacity factor (log $k'$) of some ionogenic organic compounds for different amino acid-bonded silicas. Eluent: 5 m$M$ citric acid. $\triangle$ = Sulfanilic acid; $\diamond$ = benzenesulphonic acid; $\blacksquare$ = $p$-aminobenzoic acid; $\blacktriangle$ = benzoic acid; $\square$ = aniline; $\bigcirc$ = trimethylphenylammonium.

The retention of positively charged tri-methylphenylammonium increased dramatically with increase in the pH of citric acid eluent for both ZSP having bonded monoaminodicarbox-ylic acids and for a cation exchanger having only carboxylic functional groups. This corresponds to the growing dissociation of carboxylic groups at the surface of the ion exchanger. The retention of aniline shows an inversion point at pH 4.8–5.0 for the above sorbents. Such behaviour is a sum of two processes taking place in the column: the dissociation of carboxylic functional groups of the ion exchanger, which increases the retention, and the deprotonation of anilinium ion, which reduces its retention.

Unexpectedly, the most notable cation-ex-change properties were observed for the silica-bonded arginine and lysine. The isoelectric points of these amino acids are higher than those for other amino acids and are 10.76 and 9.74,

respectively (Table 2), so one can consider the related sorbents as stronger anion exchangers in acidic media. In contrast, the observed ion-ex-change affinity to the organic cations was found to be higher than that for silica-bound aspartic and glutamic acid. This may be connected with the self-organization of the three oppositely charged layers at the silica surface. The inner layer is formed by negatively charged residual silanol groups, the middle positively charged layer is formed by protonated amino groups of bonded amino acids and the last, external layer consists of the negatively charged carboxylic groups. The localization of the positively charged layer between two negatively charged layers causes repulsion of protons from carboxylic functional groups of bonded amino acids and leads to their acidification. It should be noted that the number of carboxylic groups is double that of the amino groups in the case of aspartic

Table 2
Acid–base and complexing properties of amino acids [16]

| Amino acid | $pK_1$ | $pK_2$ | $pK_3$ | $pI$ | Log $K_1$ | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | $Mg^{2+}$ | $Ca^{2+}$ | $Sr^{2+}$ | $Ba^{2+}$ |
| Asp | 1.88 | 3.65 | 9.66 | 2.77 | 2.43 | 1.60 | 1.48 | 1.14 |
| Glu | 2.16 | 4.32 | 9.96 | 3.24 | 3.44 | 1.43 | 1.37 | 1.28 |
| Lys | 2.18 | 9.12 | 10.53 | 9.82 | | | | |
| Arg | 2.17 | 9.04 | 12.84 | 10.76 | 1.30 | | | |
| Val | 2.32 | 9.62 | – | 5.96 | | | | |
| Tyr | 2.20 | 9.11 | 10.07 | 5.66 | 2.0 | 1.48 | | |
| Pro | 1.99 | 10.6 | – | 6.30 | | | | |
| Hypro | 1.92 | 9.73 | – | 5.83 | | 0.48 | 0.04 | |

and glutamic acid, so there is no similar phenomenon for simple amino acids such as valine or tyrosine. As a result, strong cation-exchange properties were observed for the ion exchanger with carboxylic functionality.

Bare silica shows a marked affinity only to aniline. This is in accordance with known specific interactions of aniline with residual silanol groups. Obviously, the degree of functionalization of the surface of silica should not be of great value for the resulting ion-exchange selectivity of the prepared stationary phases.

Therefore, the above results show that only cyclic amino acids bonded to silica can be considered as zwitterion exchangers. The sorbents containing bonded amino acids of other types behave as weak or strong cation exchangers.

### 3.4. Ion-exchange selectivity for cations

The other interesting property of amino acid-bonded silicas to be used in ion chromatography is the ion-exchange selectivity to alkali and alkaline earth metal cations. It is known that silica-based carboxylic cation exchangers having a functional groups with $pK_a < 3$ have the ability to separate effectively monovalent and divalent classes in an isocratic elution mode with a dilute strong acid or mildly acidic complexing eluents [17,18]. According to a previous report [19], the acidity of the carboxylic group in the molecules is not changed after their attachment to the silica

surface. Hence one could hope that attachment to silica of an amino acid having a carboxylic group with relatively low $pK_a$ value (Table 1) would produce such a kind of ion exchanger. However, this is not completely correct for the molecules having an $\alpha$-amino group. Owing to specific interactions with residual silanol groups, the basicity of amino groups is changed, which induces uncertain alterations in the acidity of the carboxylic group and in the selectivity of ion exchange. The sorbents with bonded arginine, lysine, aspartic acid and glutamic acid show relatively strong retention of organic cations such as aniline and trimethylphenylammonium and were chosen for further study of cation-exchange selectivity. Fig. 2 shows the retention of alkali and alkaline earth cations by the above sorbents as a function of concentration of the perchloric acid used as the eluent. The log $k'$–log $C$ dependences are nearly linear for all the cations studied. The slopes for monovalent and divalent ions are 0.8–0.85 and 1.7–1.75, respectively.

The data on the selectivity of chromatographic separation ($\alpha = k_2'/k_1'$) of pairs of cations are presented in Table 3. The ion exchanger with a functionality incorporating two carboxylic groups shows a group selectivity to monovalent and divalent cations (Fig. 2). It is interesting that the potential complexing ability of aspartic and glutamic residues (Table 2) for alkaline earth metals has a poor effect on the selectivity of separation of the above cations and is similar to the selectivity observed for ion exchangers with
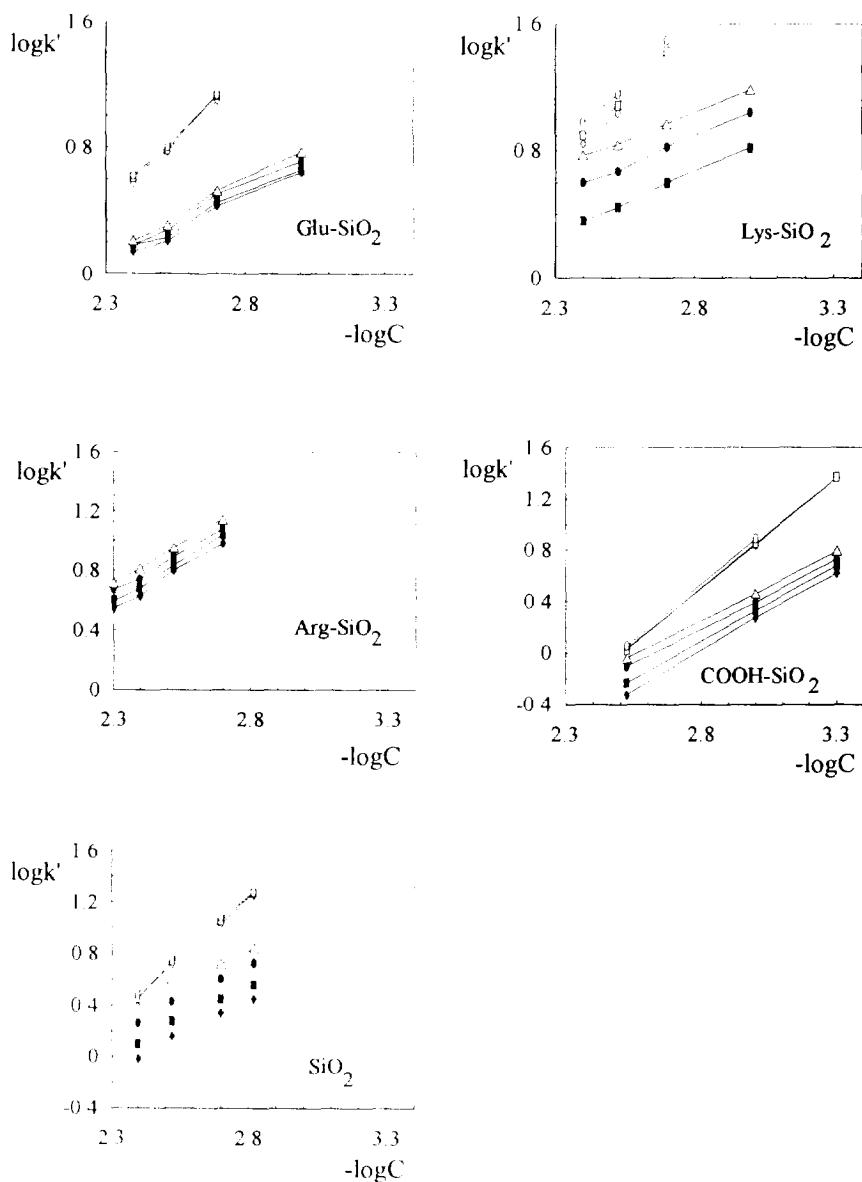
Fig. 2. Relationship between logarithm of capacity factor (log $k'$) of alkali and alkaline earth metal cations and logarithm of eluent concentration (log $C$). Eluent: perchloric acid. ◆ = Li⁺; ■ = Na⁺; ● = NH₄⁺; △ = K⁺; ◇ = Mg²⁺; □ = Ca²⁺; ○ = Ba²⁺.

propionic residues. At the same time, the ion exchangers bearing two or more amino groups at the ion-exchange site show good selectivity both for alkali and alkaline earth metal cations.

Bare silica shows a higher affinity to the divalent cations than was observed for propionic acid-bonded silica. At the same time, the selec-

tivity of separation of alkaline earth metals is very poor.

There are two possible explanations for the results obtained. The first is an increase in acidity of the carboxylic group in the presence of two or more protonated amino groups. This improves the selectivity of carboxylic ion ex-

Table 3
Selectivity of separation for alkali and alkaline earth metal ions: $\alpha = k_2'/k_1'$ (3 m$M$ perchloric acid)

| Sorbent | NH$_4$–Na | K–NH$_4$ | Mg–K | Ca–Mg | Ba–Ca |
|---|---|---|---|---|---|
| Asp-SiO$_2$ | 1.91 | 1.03 | 0.80 | 1.02 | 1.04 |
| Glu-SiO$_2$ | 1.38 | 1.57 | 1.34 | 1.02 | 1.07 |
| Lys-SiO$_2$ | 1.67 | 1.36 | 1.72 | 1.12 | 1.17 |
| Arg-SiO$_2$[a] | 1.15 | 1.18 | – | – | – |
| COOH-SiO$_2$ | 1.34 | 1.17 | 1.17 | 1.01 | 1.05 |
| Silasorb 600 | 1.42 | 1.56 | 1.27 | 1.04 | 1.03 |

[a] Alkaline earth metals are not eluted.

changers, as shown by Jensen et al. [17] and Kolla et al. [18]. Evidently, the presence of a single protonated amino group in the attached molecule of amino acid cannot provide an effective repulsion of protons in a carboxylic functionality and hence does not produce a marked change in acidity and selectivity. Second, as the radius of the separated cations increases, their repulsion from protonated amino groups also increases. In this case the repulsive interactions between amino groups and cations are responsible for the changes in ion-exchange selectivity.

The simultaneous separation of a mixture of alkali and alkaline earth metal ions on column packed with Lys-SiO$_2$ is shown in Fig. 3. It should be noted the peak corresponding to potassium is broad, which restricted the application of this column. A column packed with silica-bonded arginine having a similar concentration of bonded molecules as Lys-SiO$_2$ (Table 1) and hence having an identical concentration of carboxylic functional groups shows strong cation-exchange properties. The alkaline earth metals were not eluted from this column under the optimum conditions for the separation of monovalent cations (Fig. 4).

## 4. Conclusions

The ion-exchange properties of eight sorbents prepared by attachment of different amino acids to a silica surface were studied and compared with those for bare silica and propionic acid bound to silica. A strong cation-exchange ability was demonstrated for bonded monoaminodicar-



Fig. 3. Separation of a mixture of alkali and alkaline earth metal ions. Column, Lys-SiO$_2$ (150 mm × 4 mm I.D.); injection volume, 20 $\mu$l; eluent, 5 m$M$ HNO$_3$; flow-rate, 1.5 ml/min. 1 = Li$^+$; 2 = Na$^+$; 3 = K$^+$; 4 = Ca$^{2+}$; 5 = Sr$^{2+}$; 6 = Ba$^{2+}$.
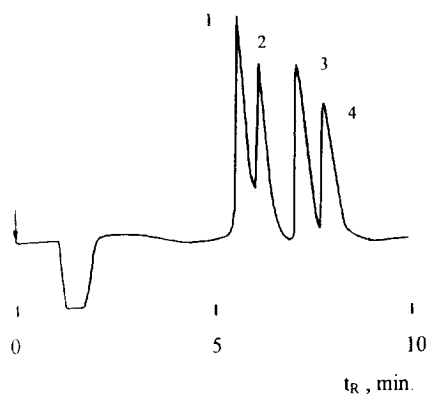


Fig. 4. Separation of a mixture of alkali metal ions and ammonium. Column, Arg-SiO$_2$ (250 × 4 mm I.D.); eluent, 5 m$M$ HClO$_4$; flow-rate, 1 ml/min; injection volume, 20 $\mu$l. 1 = Li$^+$; 2 = Na$^+$; 3 = NH$_4^+$; 4 = K$^+$.

boxylic acids (Asp, Glu) and, curiously, for bonded amino acids containing at least two basic groups (Lys, Arg). Evidently, the latter is connected with the formation of a multi-layer structure at the surface of silica, which causes a localization of charges in ion-exchange sites in a particular arrangement. Only bonded lysine and arginine show a reasonable selectivity for the separation of monovalent and divalent cations. The ability of bonded glutamic and aspartic acids for the group separation of alkali and alkaline earth metal cations was demonstrated. The data obtained from this study could be of special interest in those modes of HPLC using amino acid-bonded silica as a stationary phase.

## Acknowledgement

## References

[1] P.N. Nesterenko, *J. Chromatogr.*, 605 (1992) 199.
[2] P.N. Nesterenko, *J. High Resolut. Chromatogr.*, 14 (1991) 767.
[3] P.N. Nesterenko, O.A. Shpigun and Yu.A. Zolotov, *Dokl. Akad. Nauk*, 324 (1992) 107.
[4] W. Hu and H. Haraguchi, *Anal. Chim. Acta*, 285 (1994) 335.
[5] L.W. Yu and R.A. Hartwick, *J. Chromatogr. Sci.*, 27 (1989) 176.
[6] L.W. Yu, T.R. Floyd and R.A. Hartwick, *J. Chromatogr. Sci.*, 24 (1986) 177.
[7] W. Hu, T. Takeuchi and H. Haraguchi, *Anal. Chem.*, 65 (1993) 2204.
[8] W. Hu, H. Tao and H. Haraguchi, *Anal. Chem.*, 66 (1994) 2514.
[9] P.N. Nesterenko, R.V. Kopylov, D.A. Tarasenko, O.A. Shpigun and Yu.A. Zolotov, *Dokl. Akad. Nauk*, 326 (1992) 838.
[10] P.R. Haddad and P.E. Jackson, *Ion Chromatography—Principles and Application (Journal of Chromatography Library*, Vol. 46), Elsevier, Amsterdam, 1990, Ch. 3.
[11] A.M. Dolgonosov, *J. Chromatogr.*, 671 (1994) 33.
[12] T. Takeuchi, E. Suzuki and D. Ishii, *Chromatographia*, 25 (1988) 480.
[13] V.A. Davankov, J.D. Navratil and H.F. Walton, *Ligand Exchange Chromatography*, CRC Press, Boca Raton, FL, 1988, Ch. 5.
[14] A.V. Gaida, V.A. Monastyrskii, Yu.V. Magerovskii, S.M. Staroverov and G.V. Lisichkin, *J. Chromatogr.*, 424 (1988) 385.
[15] M. Gimpel and K. Unger, *Chromatographia*, 17 (1983) 200.
[16] L.G. Sillen and A.E. Martell, *Stability Constants of Metal-Ion Complexes (Special Publications* 17 and 25), Chemical Society, London, 1964 and 1971.
[17] D. Jensen, J. Weiss, M. Rey and C.A. Pohl, *J. Chromatogr.*, 640 (1993) 65.
[18] P. Kolla, J. Kohler and G. Schomburg, *Chromatographia*, 23 (1987) 465.
[19] D.V. Miltchenko, G.V. Kudryavtsev and G.V. Lisichkin, *Teor. Eksp. Khim.*, 27 (1986) 243.