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Application of amino acid-bonded silicas as ion exchangers for the separation of anions by single-column ion chromatography

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ABSTRACT

Different silica-immobilized amino acids were examined for use as stationary phases in single-column ion chromatography. Cation-exchange properties were established for phases with secondary amino groups (L-Arg-SiO_2; L-Val-SiO_2; L-Tyr-SiO_2) and anion-exchange properties for phases with tertiary amino groups (L-Hypro-SiO_2; L-Pro-SiO_2). The correlations between retention times of inorganic anions, concentrations of carboxylic acid (oxalic, citric, acetic, succinic, tartaric) and pH of the eluents were determined.

INTRODUCTION

Although single-column anion-exchange chromatography has been widely used in practice, there are several demands connected with this technique. One constraint is the requirement for further improvements in the efficiency and selectivity of separations. The most progress in this direction has ensued from the application of agglomerated ion exchangers with an opposite charged surface layer [1]. Such an ion-exchange structure provides rapid mass transport and consequently high efficiency, in addition to good selectivity towards anions.

Another promising approach is in the utilization of stationary phases with immobilized zwitterionic molecules on the surface of a suitable matrix, *e.g.*, silica gel. α-Amino acids are the simplest available molecules of this type which are easily attached to a silica surface. Amino acid-bonded silicas are widely used in ligand-exchange [2], metal chelate [3] and affinity chromatography [4]. However, their ion-exchange properties have not been investigated in detail. A detailed study of the use of iminodiacetic acid-bonded silica for the ion-chromatographic separation of alkali, alkaline earth and transition metal ions has been described [5,6]. In a previous paper [7] the ion chromatographic separation of inorganic anions on an L-hydroxyproline-bonded silica gel column was reported. In this study, several silica-based materials were examined for use as stationary phases in anion-exchange chromatography and the eluent variables that affect the retention of anions were also studied.

EXPERIMENTAL

The equipment used included a Beckman Model 114M pump, a Rheodyne Model 7125 injection valve with a 20-μl loop and an LKB conductivity detector. The chromatographic columns (250 × 4 mm I.D.) were all slurry packed. The column packings were L-valine, L-tyrosine- and L-arginine-bonded silica (Silasorb Si 300, 7.5 μm; Lachema, Brno, Czechoslovakia) and L-hydroxyproline- and L-proline-bonded silica (KSK-1, 5 μm; Reakhim, Mos-

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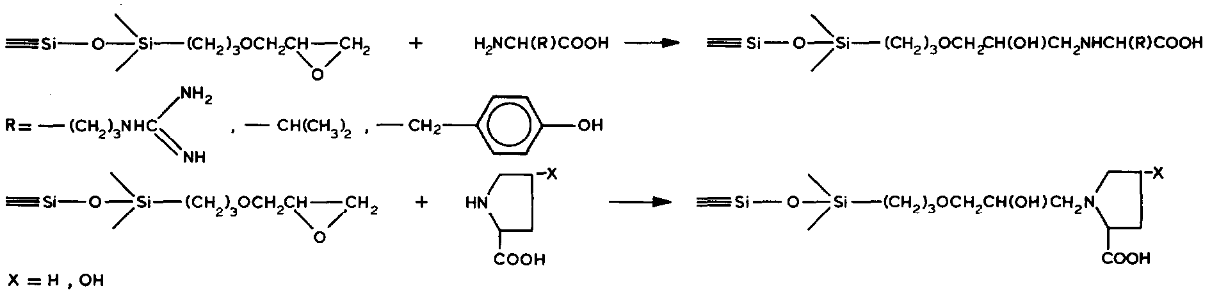


Scheme 1.

cow, Russia). They were prepared via an initial 3-glycidoxypropyltriethoxysilane surface modification followed by amino acid attachment as described [8]. The eluents were citric, oxalic, acetic, succinic and malonic acids; 1 *M* sodium hydroxide was added to adjust the pH.

RESULTS AND DISCUSSION

Ion-exchange properties of amino acid-bonded silicas

It is evident that the main point for consideration of amino acid-bonded silicas as stationary phase in ion chromatography will be the acid–base properties of the ion-exchange sites. Unfortunately, their evaluation is difficult for the following reasons:

(1) It has been established that the basicity of the silica-bound nitrogen-containing ligands decreased drastically in comparison with homogeneous analogues owing to interaction with silanol groups [9].

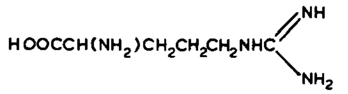
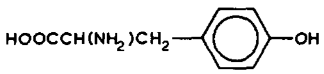
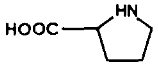
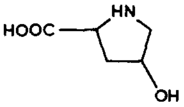
(2) There is not much difference in protolytic ability between silica-bound carboxylic acids and their homogeneous analogues [10].

(3) The basicity of the α-amino groups in amino acids after amino linkage to a 3-glycidoxypropyl spacer anchored to the silica surface as shown in Scheme 1 can also be altered. The primary amino groups (L-Val, L-Arg, L-Tyr) become secondary and the secondary amino groups (L-Pro, L-Hypro) become tertiary in attached form.

(4) The possible formation of an inner salt be-

TABLE I

p*K* VALUES FOR IONIZABLE GROUPS OF SOME α-AMINO ACIDS

Amino acid	Structure	pK_1	pK_2	pK_3
Arginine	 $HOOCCH(NH_2)CH_2CH_2CH_2NHC(=NH)NH_2$	2.18	9.04	12.48
Valine	$HOOCCH(NH_2)CH(CH_3)_2$	2.32	9.62	
Tyrosine		2.20	9.11	
Proline		1.93	10.60	
Hydroxyproline		1.92	9.73	

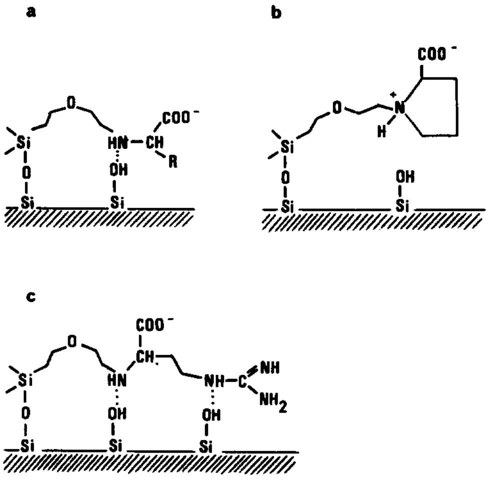


Fig. 1. Possible interaction of bonded amino acids with silanols. (a) L-Val-SiO$_2$; (b) L-Pro-SiO$_2$; (c) L-Arg-SiO$_2$.

tween amino and carboxyl groups can occur in neutral or weakly acidic media at low ionic strength.

L-Valine, L-arginine, L-tyrosine, L-proline and L-hydroxyproline were chosen as ion-exchange sites in accordance with the different acid–base properties of ionogenic groups and side-chain structure (Table I). L-Arginine, L-proline and L-hydroxyproline when bonded to silica can be expected to reveal strong anion-exchange properties owing to the high value of pK_2 for secondary amino groups and pK_3 for a side-chain guanidino functional group. L-Val-SiO$_2$ and L-Tyr-SiO$_2$ were used for comparison with the above.

As would be expected, L-Pro-SiO$_2$ and L-Hypro-SiO$_2$ confirmed our supposition. On the other hand, it was established that L-Arg-SiO$_2$ can be considered mainly as a cation exchanger and only a poor separation of chloride, perchlorate and sulphate was achieved with dilute malonic acid as eluent. It was possible to separate alkali metal ions on a column packed with this type of bonded phase. According to our results, L-Val-SiO$_2$ and L-Tyr-SiO$_2$ can be characterized as pure cation exchangers. This can be explained from the point of view of the effect of the α-amino group on ion-exchange properties. The main peculiarity of α-amino acids is in the presence of a definite internal acid–base relationship between carboxyl and amino groups: the greater is the protonation constant of the α-amino group in a free amino acid, the lower is the dissociation constant of the carboxyl group. There is one other compensation which may be characterized as "push–pull". The basicity of secondary amino groups after amino acid attachment to the silica surface (L-Arg, L-Val and L-Tyr) is decreased owing to interaction with silanol groups (Fig. 1a and c). Hence some of the carboxyl groups are replaced by intramolecular interaction with α-amino groups by silanols providing cation-exchange properties to L-Arg-SiO$_2$, L-Val-SiO$_2$ and L-Tyr-SiO$_2$. Owing to the same interaction with silanol groups, the effect of the side-chain amino groups on the anion-exchange properties of Arg-SiO$_2$ can be evaluated as weak but sufficient for the mentioned poor separation of chloride, perchlorate and sulphate.

With bonded L-Pro and L-Hypro we obtained another situation. The tertiary amino group is inaccessible for interaction with silanol groups owing to steric hindrance (Fig. 1b) and provides anion-exchange properties especially in acidic media when the carboxyl group is partially protonated. Hence further investigations were carried out with L-Pro-SiO$_2$ and L-Hypro-SiO$_2$.

The retention behaviour of anions in single-column ion chromatography depends on variables of the eluent such as the chemical nature of the carboxylic acid used, its concentration and pH. The chemical nature is determined by the value of the dissociation constant of the carboxyl group and defines the predominant ionic form in the eluent and the eluting power at equal concentration and pH of the eluent. A high percentage of ionization means that a higher concentration of the eluent anion is available to elute sample anions.

Effect of eluent concentration

Oxalic, citric, succinic and acetic acids at concentrations from 1 to 5 mM were used to elute anions without pH adjustment. According to our results (Table II and ref. 7, the elution power decreased in the order oxalic > citric > succinic > acetic acid. The elution order of equal-charged anions was similar for all eluents. As expected, a low concentration of the eluent caused a greater retention of anions than did higher concentrations, but only for L-Hypro-SiO$_2$ (Fig. 2b). Surprisingly, all the curves (Fig. 2a) for the relationship between log k' and log C_{oxal} contain a maximum in case of L-Pro-SiO$_2$. This means that the retention of anions was decreased by dilution of the eluent used. This could be caused by

TABLE II

LOG k' OF ANIONS FOR L-Pro-SiO_2 WITH 5 mM CARBOXYLIC ACID ELUENTS

Anion	Oxalic acid	Citric acid	Succinic acid	Acetic acid
Nitrite	0.197	0.436	0.545	0.520
Orthophosphate	0.151	0.386	0.428	0.367
Iodate	0.239	0.502	0.489	0.409
Bromate	0.369	0.663	0.612	0.547
Chloride	–	0.703	0.647	0.569
Bromide	0.601	0.932	0.835	0.713
Nitrate	0.651	0.993	0.874	0.745
Iodide	1.013	–	–	–
Perchlorate	1.270	–	–	–
Sulphate	0.772	>1	0.678	–

the possible formation of an inner salt at low eluent concentrations. It should be noted that as the eluent concentration decreases, the pH at which this interaction occurs increases and promotes inner salt formation. In the inner salt form some sites are not available for ion-exchange interaction with analytes and eluents.

Effect of eluent pH

The pH of the eluent acid should also be expected to affect the retention of various anions. It was demonstrated in an earlier study on L-Hypro-SiO_2 that a pH range of 2.5–4.0 in the eluent was optimum for the separation of singly charged inorganic anions [7]. In this range an increase in pH causes a decrease in retention for all inorganic anions. The effect of the eluent pH on the retention of anions on L-Pro-SiO_2 and L-Hypro-SiO_2 is shownin Fig. 3. The decrease in retention is attributed not only to the change in the degree of ionization of carboxyl groups in the amino acid moiety but also in the carboxylic acid eluent. On the one hand, an increase in the pH of the eluent led to decrease in the number of accessible ion-exchange sites owing to the enhancement of the degree of inner salt formation. The overall positive charge of ion-exchange sites is also reduced owing to dissociation of bonded amino acid carboxyl groups. On the other hand, the concentration and effective negative charge of eluent anions were increased to provide the growing competition with analyte anions for the ion-exchange sites. The elution order did not change for most of

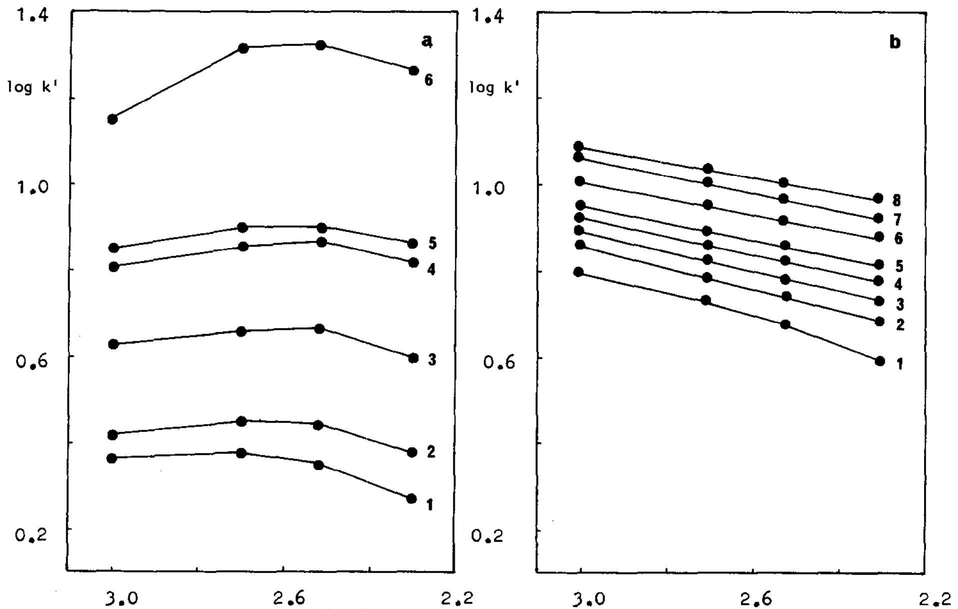


Fig. 2. Influence of oxalic acid concentration on the retention of anions. (a) L-Pro-SiO_2; (b) L-Hypro-SiO_2. Anions: 1 = orthophosphate; 2 = iodate; 3 = chloride; 4 = bromide; 5 = nitrate; 6 = iodide; 7 = perchlorate; 8 = thiocyanate.

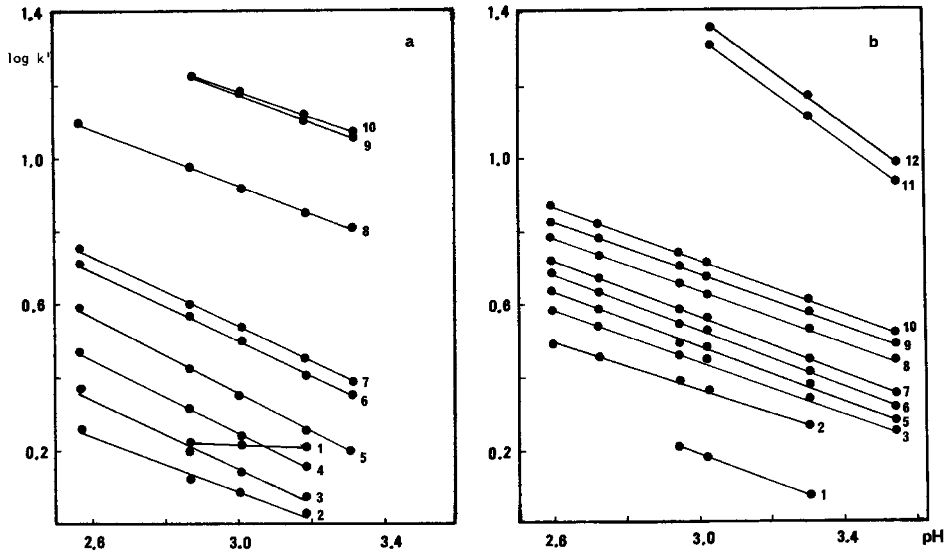


Fig. 3. Influence of pH on the retention of anions. Eluent: 5 m*M* oxalic acid. Columns: (a) L-Pro-SiO_2; (b) L-Hypro-SiO_2. Anions: 1 = nitrite; 2 = orthophosphate; 3 = iodate; 4 = bromate; 5 = chloride; 6 = bromide; 7 = nitrate; 8 = iodide; 9 = perchlorate; 10 = thiocyanate; 11 = sulphate; 12 = thiosulphate.

the anions in the pH range studied. The retention times of divalent anions decreased more rapidly than those of monovalent anions as the eluent pH increased. This may be used for the simultaneous separation of monovalent and divalent anions at high eluent pH. It should be noted, however, that a lower pH would provide a better separation from the point of view interference from the system peak.

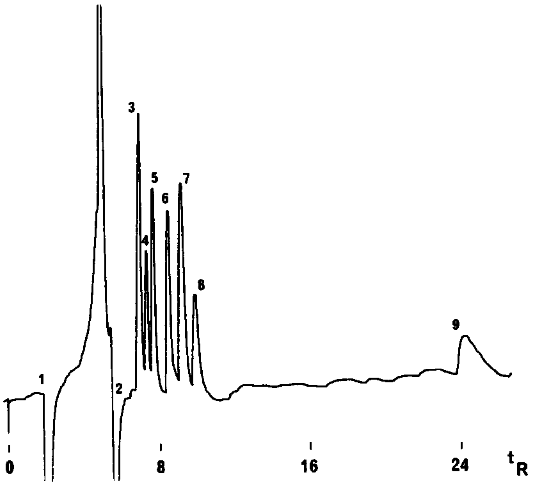


Fig. 4. Chromatogram of mixture of anions. Column: L-Pro-SiO_2, 5 μm (250 × 4 mm I.D.). Eluent: 5 m*M* succinic acid, pH 3.18. Flow-rate: 1.5 ml/min. Detection: conductimetric. Peaks: 1 = alkali metal cations; 2 = system; 3 = chloride; 4 = bromide; 5 = nitrate; 6 = iodide; 7 = perchlorate; 8 = thiocyanate ; 9 = sulphate. Concentrations of test anions are: 3 = 5 ppm; 4, 5, 7 = 10 ppm and 6, 8, 9 = 20 ppm. Retention time (t_R) in min.

The pH stability of silica-based ion exchangers is of great importance. Although the optimum pH range for the separation of anions is between 2.5 and 4.5, the columns studied were used from pH 2 to 7.5 without a noticeable change in performance during at least 1 year. A similar stability has been reported for analogous ligand-exchange chromatographic packings [2].

The concentration and pH of the eluents were optimized to give good separations of different anions tested. Figs. 4 and 5 show examples of the separation of standard anion mixtures on L-Pro-SiO_2 and L-Hypro-SiO_2. It was found that the anion-exchange properties of these bonded amino acids allow a wide variety of separations, including those of common anions such as orthophosphate, chloride, nitrate and sulphate.

The zwitterionic character of L-Pro-SiO_2 and L-Hypro-SiO_2 offers several advantages over traditional anion exchangers. The presence of carboxyl and tertiary amino groups in adjacent ion-exchange sites allows very small values of the ion-exchange capacity to be achieved for these phases by direct

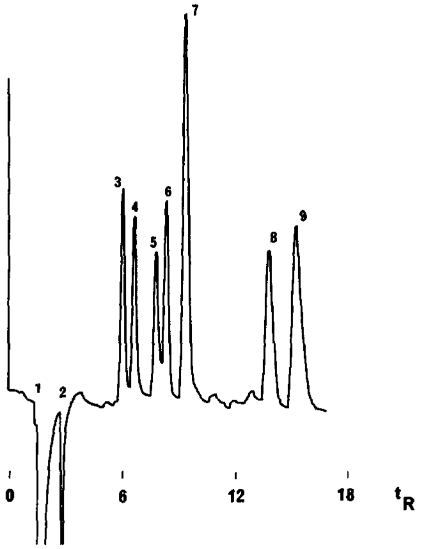


Fig. 5. Chromatogram of mixture of anions. Column: L-Hypro-SiO_2, 5 μm (250 × 4 mm I.D.). Eluent: 5 m*M* oxalic acid. Flow-rate: 1.0 ml/min. Detection: conductimetric. Peaks: 1 = alkali metal cations; 2 = system; 3 = orthophosphate; 4 = iodate; 5 = nitrite; 6 = bromate; 7 = chloride; 8 = bromide; 9 = nitrate. The concentrations of test anions are: 3–6, 8, 9 = 10 ppm and 7 = 5 ppm. Retention time (t_R) in min.

regulation of the eluent pH and concentration. For this reason it is possible to apply dilute eluents with low equivalent conductivity; in practice, this means sensitive detection and the possibility of working in the single-column ion chromatographic mode. Another advantage of the utilization of L-Pro-SiO_2 and L-Hypro-SiO_2 columns is the elution of system peak components before common anions. Finally, the structure of the ion-exchange sites in amino acid-bonded silicas provides good ion-exchange kinetics. The efficiency of the L-Pro-SiO_2 and L-Hypro-SiO_2 columns calculated for nitrate (eluent 5 m*M* oxalic acid) is equal to 7100 and 12 200 theoretical plates, respectively.

CONCLUSIONS

These studies demonstrate that an efficient separation of inorganic anions is possible by using amino acid-bonded silicas. The results confirm the alteration of acid–base properties in amino acids after attachment to the silica surface and demonstrate the dual nature of the bonded phases obtained. Other applications of amino acid-bonded silicas that could be used for cations are being studied.

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