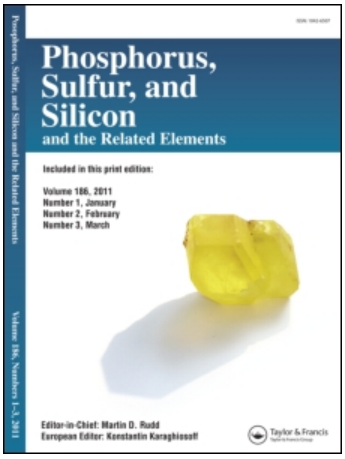

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RETENTION BEHAVIOR OF INORGANIC AND ORGANIC SELENIUM COMPOUNDS ON A POLYMER-BASED CATION EXCHANGE COLUMN

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The retention behavior of selenous acid, selenic acid, selenomethionine, selenoethionine, selenocystine, selenohomocystine, trimethylselenonium iodide, and (3-amino-3-carboxy-1-propyl)-dimethylselenonium iodide was studied on the polymer-based PRP-X200 cation exchange column with an aqueous solution of pyridine (20 to 100 mM) in the pH range 1.1-6.0 adjusted with formic acid as the mobile phase. An inductively coupled plasma mass spectrometer equipped with a hydraulic high pressure nebulizer served as selenium specific detector. The retention behavior was rationalized in terms of the pH-dependent deprotonation of the selenium compounds and of the pyridinium cation and of the formation of ion-pairs between hydrogen selenite, selenate, or the zwitterionic groups of the selenoamino acids with the pyridinium cation. A good separation of seven selenium compounds was achieved within 5.0 min at 40°C with 20 mM pyridine (pH 3.70). Selenoethionine eluted 15 min after injection. Peak areas and peak heights of the chromatographic signals provided linear calibration curves ($r^2 > 0.990$). Detection limits obtained were 1 μg/L with the exception of selenoethionine (5 μg/L).

Keywords Selenium; selenoamino acids; HPLC-ICP-MS

INTRODUCTION

The major selenium compounds in soils and in the aquatic environment are selenous acid and selenic acid[1]. Selenomethionine was identified in soils[2,3] and the selenoaminoacids selenomethionine, selenocystine, selenohomocystine, selenocystathionine, and the (3-amino-3-carboxy-1-propyl)dimethylselenonium cation in biological and environmental samples[1,4,5]. The trimethylselenonium ion was detected in human urine[6, 7, 8].

The identification and quantification of selenium compounds is best carried out with systems combining chromatographic separations with on-line, selenium-specific detection. Selenocystine and selenomethionine were separated on a reversed-phase column with sodium heptanesulfonate[9] or tetraethylammonium bromide[10] as ion pairing reagent and on an anion-exchange column with a solution of sodium salicylate/salicylic acid as the mobile phase[11]. Sodium pentanesulfonate served as ion pairing agent for the separation of the trimethylselenonium cation, selenocystine, and selenomethionine on a reversed-phase column[12]. On an anion-exchange column six selenium compounds[13] were separated with an aqueous solution of ammonium citrate, four selenium compounds[14] with an aqueous solution of sodium dihydrogen phosphate, and seven selenium compounds[4] with an aqueous solution of potassium hydrogen phthalate as mobile phase. The influence of the concentrations of carboxylic acids and of the hydrogen ion in the aqueous mobile phase on the retention behavior of six selenoamino acids, of trimethylselenonium iodide, of selenous acid,

and of selenic acid on a polystyrene-based anion-exchange column was investigated[15]. Selenous acid, selenic acid, (3-amino-3-carboxy-1-propyl)dimethylselenonium iodide, trimethylselenonium iodide, selenocystine, selenohomocystine, selenomethionine, and seleno-ethionine were chromatographed on a silica-based cation exchange column (Supelcosil LC-SCX)[16]. With aqueous 20 mM pyridine at pH 5.0 as the mobile phase seven selenium compounds were base-line separated; selenohomocystine and selenomethionine co-eluted. Recently, selenocystine, selenomethionine, selenoethionine, selenourea, selenous acid, and selenic acid were separated by vesicle-mediated high-performance liquid chromatography[17].

The potential of a polymer-based cation-exchange column (Hamilton PRP-X200) was now explored for the separation of inorganic and organic selenium compounds with an aqueous solution of pyridine as the mobile phase. The selenium compounds in the column effluents were detected selenium-specifically with an inductively coupled plasma mass spectrometer (ICP-MS).

EXPERIMENTAL

All commercial chemicals were of analytical grade and were not further purified. Sodium selenate was purchased from Fluka, sodium selenite pentahydrate from Merck, seleno-DL-ethionine (Seet), seleno-DL-methionine (Semet), and seleno-DL-cystine (Secys) from Sigma. Selenohomocystine (Sehcys)[18], trimethylselenonium iodide (TmSe)[8],

and (3-amino-3-carboxy-1-propyl)dimethylselenonium iodide (DmpSe)[19] were synthesized according to literature procedures.

Stock solutions of the selenium compounds (125 mg Se/L, 500 mg Se/L, or 1000 mg Se/L) were prepared with Milli-Q water (18.2 MΩ cm) and subsequently diluted to solutions with 10 mg Se/L. The 10 mg Se/L solutions were further diluted to solutions with concentrations in the range 1.0 μg Se/L to 50 μg Se/L.

The mobile phases with 20 mmol/L of pyridine were prepared by dissolving 1.60 mL of pyridine (99.5%, Merck p.a.), 50 μL of a RbCl solution (1000 mg Rb/L), and appropriate volumes of formic acid (~98%, Fluka puriss. p.a.) to 1000 mL with Milli-Q water (mL formic acid, pH of mobile phase, molarity of formic acid in mobile phase given): 365, 1.10, 9.48; 200, 1.50, 5.19; 45, 2.00, 1.17; 8.0, 2.60, 0.21; 2.95, 3.00, 0.076; 1.63, 3.40, 0.042; 1.18, 3.65, 0.030; 1.11, 3.70, 0.029; 1.03, 3.75, 0.027; 0.83, 4.00, 0.022; 0.032, 5.00, 0.008; 0.030, 6.00, 0.0008.

The chromatographic system consisted of a Hewlett Packard-1050 series pump and an HPLC column heater, a PRP-X200 pre-column, and a PRP-X200 cation exchange column [250x4.1 mm, 10-μm particles of poly(styrene-divinylbenzene)sulfonic acid, stable between pH 1-13]. The column was kept in a thermostated chamber, the temperature of which can be controlled to 0.1°C in the range from 10 to 85°C. Most of the chromatographic experiments were carried out at 40°C. An inductively coupled plasma mass spectrometer (VG Plasma Quad II Turbo Plus, VG Elemental, Winsford, UK) equipped with a hydraulic

high pressure nebulizer (HHPN, Knauer, Berlin, Germany) served as selenium-specific detector[16].

RESULTS AND DISCUSSION

Anions derived from selenous acid and selenic acid are major selenium compounds in rocks, soils, freshwater, and seawater[1]. Organic selenium compounds occur frequently in biota (Figure 1).

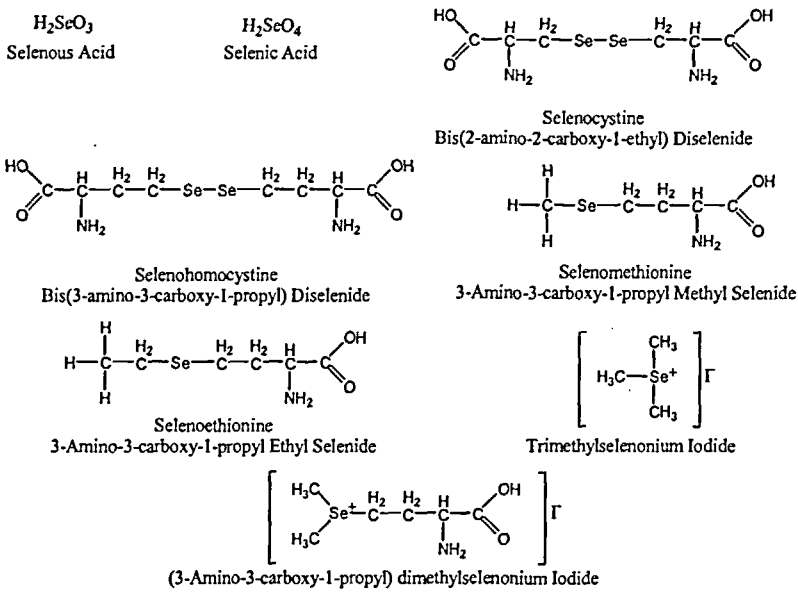


FIGURE 1 Formulae for inorganic and organic selenium compounds

Of special importance among these selenium compounds are the selenoamino acids that become incorporated into proteins. The reliable

identification and quantification of selenium compounds in inorganic and biological samples, for instance in food, selenium supplements, and urine, will become possible only, when selenium compounds can be determined simultaneously with methods characterized by short analysis times, robustness, and low detection limits. Although several methods for the chromatographic separation of selenium compounds on anion-exchange columns[11,13,14] or reversed-phase columns[9,10,12] were developed, only very few systematic investigations of the retention behavior of selenium compounds were carried out. Surprisingly, cation-exchange chromatography was almost completely neglected as a method for the separation of selenium compounds[8,16] in spite of the fact that several organic selenium compounds are permanent cations and the selenoamino acids are cationic under appropriately acidic conditions. The successful separation of seven selenium compounds on a silica-based cation-exchange column under isocratic conditions within seven minutes[16] prompted us to explore separations on a polymer-based cation-exchange column.

Each of the eight selenium compounds (Figure 1) was separately chromatographed on the Hamilton PRP-X200 polymer-based polystyrene-divinylbenzene cation exchange column with a 20 mM aqueous solution of pyridine, the pH of which had been adjusted in the range from 1.1 to 6.0 with formic acid. Each injected solution (100 μL) contained one of the selenium compounds at a concentration of 20 μg Se/L (0.25 μmol/L) (for Seet 50 μg Se/L). The retention times of most of the selenium compounds change appreciably with the pH of the

mobile phase (Figure 2) and decrease with an increase in the concentration of total pyridine in the mobile phase. The observed retention behavior should be explainable in terms of the deprotonation of selenium compounds, the protonation of pyridine, electrostatic interactions, hydrophobic interactions, ion-pair formation, and ion-exchange equilibria.

The species distribution diagrams for pyridine, selenous acid, selenic acid, selenocystine, and selenomethionine, compounds for which the pK values are available, are shown in Figure 3.

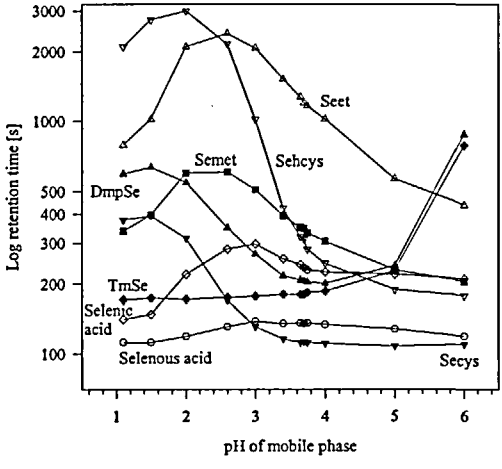


FIGURE 2 Dependence of the retention times of selenium compounds (Seet 50 μg Se/L; others 20 μg Se/L) on the pH of the mobile phase (20 mM pyridine, flow rate 1.5 mL/min; 22°C; PRP-X200 cation-exchange column; detection by HHPN-ICP-MS).

These diagrams were calculated with deprotonation constants valid for dilute aqueous solutions. However, the mobile phases are formic

acid/water mixtures with formic acid concentrations between 9.5 (pH 1.1) and 0.0008 mol/L (pH 6.0). Because formic acid (ε = 58.5, 16°C)/water (ε = 81.95, 15°C)[22] mixtures with high concentrations of formic acid have lower dielectric constants than pure water, and formic acid is a poorer hydrogen-ion acceptor than water, the deprotonation constants for the selenium compounds in formic acid/water mixtures could be smaller than in water.

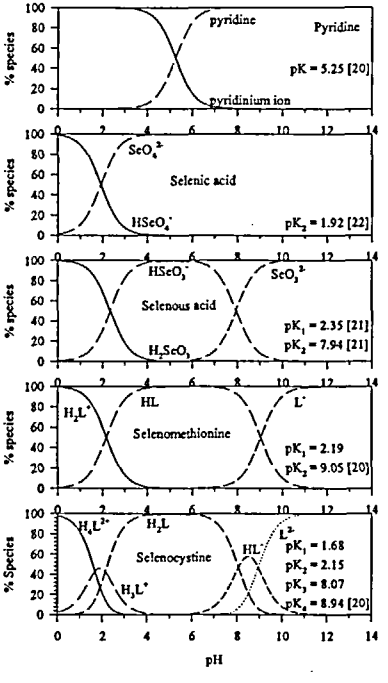


FIGURE 3 Species distribution diagrams for pyridine, selenic acid, selenous acid, selenomethionine, and selenocystine

Constants for the selenium compounds in formic acid/water mixtures

are not available in the literature. Therefore, the following qualitative discussions are based on the constants for dilute aqueous solutions with the awareness that at low pH (mobile phases with formic acid as a major component) the concentrations of the hydrogen ions are only operationally defined through the response of the glass electrode and that the constants used are very likely too large.

Retention behavior of selenous acid and selenic acid

Selenous acid is present in an aqueous solution of pH 1.1 predominantly (~90%) as neutral molecule. With increasing pH the proportion of the hydrogen selenite anion grows and above pH 4.0 this anion is the only selenium species in solution (Figure 3). Consequently, the largely anionic selenous acid incapable of interaction with the negatively charged sulfonate exchange sites has short retention times (112 to 138 seconds) with a weak maximum in the retention time/pH curve at pH 3.0 (138 seconds) (Figure 2).

Selenic acid, a much stronger acid than selenous acid, is present as an equimolar mixture of hydrogen selenate and selenate in aqueous solution with a pH of ~2 and exclusively as dinegative selenate above pH 4 (Figure 3). The retention time (148 seconds at pH 1.1) reaches a maximum at pH 3.0 (297 seconds) and decreases to 210 seconds at pH 6.0 (Figure 2). Selenic acid elutes later than selenous acid within the entire pH region (1.1 to 6.0) investigated.

The retention of selenous acid and of selenic acid is unlikely to be caused by interactions (for instance, formation of hydrogen bonds)

between the negatively charged sulfonate groups on the ion-exchanger and the anions of the selenium oxoacids. If hydrogen bonds were responsible for the retention of selenous acid and selenic acid, then the retention times of selenous acid and of selenic acid should decrease in the pH range 1.1 to 3.0, because the concentrations of H_2SeO_3 and $HSeO_4^-$, the compounds available and most likely to form hydrogen bonds, decrease in this pH range. However, the experimental retention times increase from pH 1.1 to pH 3.0 (Figure 2).

The sulfonate exchange sites on the column conditioned with an aqueous solution of pyridinium formate must be paired with pyridinium cations that are continually exchanged for pyridinium cations from the mobile phase. The pyridinium-covered ion-exchanger presents a positively charged surface to anions in the mobile phase. Hydrogen selenite, hydrogen selenate, and selenate could interact electrostatically with the pyridinium cations bound to sulfonate groups and be retarded in their migration through the column. Because the apparent charge on the selenium oxoanions increases by approximately one unit in the pH range 1.0 to 3.0, the interactions between an anion and the pyridinium-covered stationary phase should become stronger and the retention times of the oxoanions should increase as observed experimentally (Figure 2). The decrease of the retention times between pH 3.0 and 6.0 cannot be attributed to any change in the nature of the selenium oxoanions. Selenate and hydrogen selenite are practically the only selenium species in this pH range. The sulfonate exchange sites should remain paired with pyridinium ions even at pH 6.0, because the mobile

phase at this pH still contains approximately 1.8 mmoles of pyridinium cations per liter, the pyridinium ion has a concentration 1000-times higher than the hydronium ions at pH 6.0, and no other cations are available to replace the pyridinium ions associated with sulfonate groups. When the surfaces of the exchange material remain loaded with pyridinium ions and the negative charge on the selenium oxoanions does not change, the retention times should not - but nevertheless do - decrease in the pH range 3.0 to 6.0 (Figure 2). Ion-pairing between the selenium oxoanions and the pyridinium cation in the mobile phase can - at least qualitatively - rationalize the observed trend in the retention times. The formation of such ion pairs is governed by equilibria (eqns. 1 and 2).

$$Py^+ + HSeO_3^- \rightleftharpoons Py^+HSeO_3^- \quad (1)$$

$$2\,Py^+ + SeO_4^{2-} \rightleftharpoons Py_2^+SeO_4^{2-} \quad (2)$$

Whereas the concentrations of the selenium oxoanions remain constant in the pH region 3.0 to 6.0, the concentration of the pyridinium cation decreases from ~19.80 mM at pH 3.0 to ~1.8 mM at pH 6.0. The lower the concentration of the pyridinium cation the more will the equilibria be shifted in disfavor of the ion pairs. Under the assumption that the uncharged ion pairs replace pyridinium ions associated with sulfonate exchange sites, the retention times of the selenium oxoanions must decrease in the pH range 3.0 to 6.0 with diminishing concentration of the ion pairs. In the pH range 1.1 to 3.0 the concentration of the

pyridinium cation is constant, but the concentrations of the anions $HSeO_3^-$ and SeO_4^{2-} and consequently the concentrations of the ion pairs increase leading to longer retention times with increasing pH. Thus, the ion-pair hypothesis explains the trend of the retention time with pH over the entire pH-range (1.1 to 6.0) investigated.

An explanation must now be sought for the observation, that selenic acid has longer retention times than selenous acid in the pH range 1.1 to 6.0 (Figure 2). The dinegative selenate must pair with two pyridinium cations to form an overall uncharged ion pair, whereas the mononegative hydrogen selenite requires only one pyridinium cation. Ion pairs with more than one pyridinium cation might be more efficient in replacing pyridinium ions associated with sulfonate exchange sites, might bind preferentially to the exchange sites because of the possibility of interacting with more than one sulfonate, and thus might "stick" to the stationary phase longer than an ion pair with only one pyridinium cation. Lipophilic interactions between the pyridinium cations of the ion-pairs and the organic backbone of the exchange material should also favor the retention of the ion-pairs with more than one pyridinium cation.

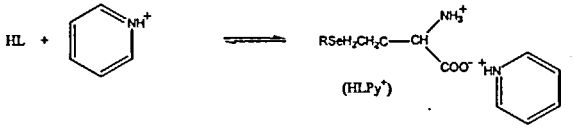
Selenomethionine and selenoethionine

The retention times for selenomethionine and selenoethionine reach flat maxima between pH 2.0 and 3.0 (Figure 2). The retention times for selenoethionine exceed those for selenomethionine at all pH values (1.1 to 6.0) investigated. At the maximum of the retention time/pH curve

selenoethionine has a retention time of 2400 seconds and selenomethionine of 607 seconds. These two selenoamino acids are 3-carboxy-3-amino-1-propyl alkyl selenides (alkyl in selenomethionine is methyl, in selenoethionine ethyl) that will exist in an aqueous solution of low pH as cations H_2L^+ (carboxyl group not deprotonated and uncharged, amino group protonated and positively charged), at intermediate pH as a zwitterion HL (carboxyl group deprotonated and negatively charged, amino group protonated and positively charged), and at high pH as anion L^- (carboxyl group deprotonated and negatively charged, amino group not protonated and uncharged). The deprotonation constants for selenoethionine are not known but are expected to be almost the same as the constants for selenomethionine, because methionine (pK_1 2.23, pK_2 9.08)[20] and ethionine (pK_1 unknown, pK_2 9.02),[22] the corresponding thioamino acids, have very similar constants.

The increase in retention times for selenomethionine and selenoethionine in the pH range from 1.1 to 2.0 cannot be rationalized by a cation-exchange mechanism. The concentrations of the cationic species of the two compounds decrease in this pH range. Consequently, the retention times - if caused by cation-exchange alone - should become shorter. The very slight change in the concentration of the pyridinium ion (19.998 mM at pH 1.0, 19.98 mM at pH 2.0) is unlikely to be responsible for the observed increase of the retention times (Figure 2). Only the concentration of the zwitterion changes in the same way as the retention time (Figures 2 and 3) in the pH range 1.1 to 2.6.

However, if the zwitterions were alone responsible for the retention (perhaps by lipophilic interactions with the polymer backbone), then the retention time should remain constant at pH values, at which the selenoamino acids are completely in zwitterionic form (for selenomethionine at pH ≥ ~4, Figure 3). The experimental results are not in agreement with this explanation. In the pH range from ~2.5 to 6.0, in which the retention times of the selenoamino acids decrease, only the concentration of the pyridinium cation follows the trend in the retention times. This trend can be reproduced by assuming that the cationic selenoamino acids H_2L^+ do not participate in the exchange process, that the negatively charged carboxyl groups in the zwitterions HL and the pyridinium cation form ion-pairs, and that the now positively charged ion pairs interact with the sulfonate exchange sites. In a solution containing zwitterions HL of the selenoamino acids and pyridinium cations the following equilibrium will be established (eqn. 3).



(3)

In the pH range 1.1 to 2.6, in which the retention times of the two selenoamino acids increase, the concentration of the pyridinium cation is constant (~20 mM), but the concentrations of the zwitterions HL increase. Consequently, the reaction between the zwitterions and the pyridinium ion is driven toward $HLPy^+$ with increasing pH. When the

:oncentration of $HLPy^+$ is high, the retention time will be long.

Beginning at approximately pH 2.6 the concentration of the)yridinium cation decreases, whereas the zwitterions are approaching heir maximal concentrations that are reached at pH 4 and remain :onstant to pH 6.0 (Figure 3). At constant HL concentration the lecreasing concentration of the pyridinium cation shifts the equilibrium n disfavor of $HLPy^+$. With decreasing concentration of $HLPy^+$ the 'etention times are expected to become shorter. This expectation was :onfirmed experimentally.

The interaction between the ammonium group of an $HLPy^+$ ion-pair ınd a sulfonate group on the stationary phase that appears to be 'esponsible for the retention of these selenoamino acids is made)ossible by the neutralization of the negative charge on the carboxylate ;roup by the pyridinium cation. Without such a charge neutralization)y ion-pair formation, the repulsion between carboxylate and sulfonate ;roups would have prevented or at least reduced the association)etween the ammonium group in the zwitterion and the sulfonate ;roup.

Lipophilic interactions between the organic backbone of the ;tationary phase and the carboxylate-pyridinium ion pair or the $RSeCH_2CH_2$ group could also be involved in the retention process. Only lipophilic interactions involving the $RSeCH_2CH_2$ group can :xplain, why selenoethionine ($R = CH_2CH_3$) has much longer retention imes than selenomethionine ($R = CH_3$) (Figure 2). The two ;elenoamino acids differ only by one CH_2 group.

Selenocystine and selenohomocystine

Selenocystine and Selenohomocystine are dialkyl diselenides, in which the Se-Se group connects two amino acid moieties (Figure 1). The species distribution diagram for selenocystine and pyridine, and the dependence of the retention times for selenocystine on the pH of the mobile phase are shown in Figure 4. The four deprotonation constants for selenocystine (Figure 3) indicate, that below pH 2.5 the dicationic (H_4L^{2+}) and cationic (H_3L^{+}) species account together for more than 50% of selenocystine. The di-zwitterion (H_2L) is practically the only form of selenocystine between pH 4.0 and 6.0 (Figure 4).

The retention time for selenocystine increases very moderately from 377 seconds at pH 1.1 to 393 seconds at pH 1.6 and then decreases rather precipitously to 116 seconds at pH 3.4. The retention time remains practically constant at ~108 seconds close to the dead time of the column (110 seconds) in the pH range 3.5 to 6.0 (Figure 4). Only cationic (H_4L^{2+}, H_3L^{+}) and the di-zwitterionic (H_2L) species are available to explain the trends in the retention times. The di-zwitterion increases in concentration in the pH range 1.6 to 4.0, in which the retention time of selenocystine decreases. Therefore, H_2L cannot be responsible for the retention. The di-cationic species H_4L^{2+} begins to decrease in concentration at pH 0 and has a concentration of almost zero at pH 3.0. If H_4L^{2+} were responsible for the retention, the retention time should have decreased in the pH interval 1.1 to 1.6 and should have reached the dead time (110 seconds) at pH 3.0. Experiment is not in agreement with this deduction (Figure 4).

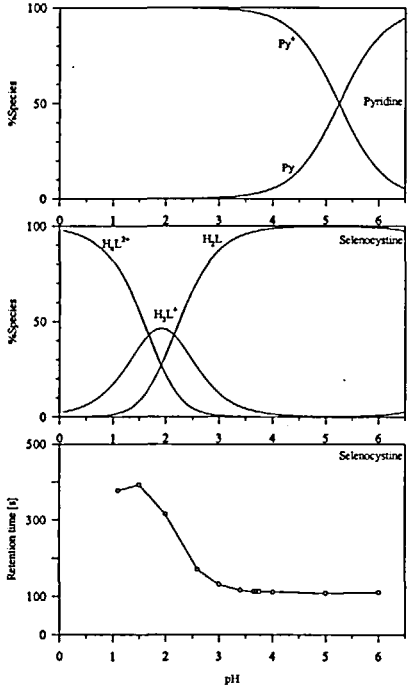


FIGURE 4 Species distribution diagrams for pyridine and selenocystine in the pH range 0 to 6.0 and the dependence of the retention times of selenocystine on the pH of the mobile phase (100 µL injected, 20 µg Se/L; mobile phase20 mM pyridine at pH 3.70; flow rate 1.5 mL/min; PRP-X200 cation-exchange column; detection by HHPN-ICP-MS).

The increase in retention time between pH 1.1 and 1.6 could be caused by the increase in the concentration of H_3L^+ (selenocystine with one zwitterionic group and one cationic group) in this pH region. The decrease in retention time at pH > 1.6 could be connected with the decreasing concentration of H_3L^+ (beginning at ~pH 1.9). The retention

time reaches the dead time at pH ~4.0, the pH at which the concentration of H_3L^+ is practically zero. The concentration of the pyridinium cation is constant at 20 mM up to pH ~4.0. This rationalization of the trend of the retention time with pH in terms of the concentration change of H_3L^+ suffers from the not-coinciding maxima (difference of ~0.4 pH units) of the retention time/pH and H_3L^+ concentration/pH curves (Figure 4), but nevertheless reproduces the experimental facts best among all possibilities considered. Perhaps pK values for selenocystine in formic acid/water mixtures might remove the discrepancy. Because selenomethionine required the formation of an ion pair between the carboxylate group of the zwitterion and the pyridinium cation for the rationalization of the experimental retention times, a similar ion pair formation must be considered for H_3L^+ of selenocystine. The "ion-paired" end of the selenocystine molecule should then interact via its ammonium group with the sulfonate exchange sites with the other end [$-CH(NH_3)^+COOH$] exerting no detrimental but perhaps a beneficial influence on the retention.

The shape of the retention time/pH curve for selenohomocystine with its maximum at pH 2.0 (3000 seconds) can also be rationalized on the basis of the pH-dependent concentration of H_3L^+ of selenohomocystine and ion pair formation with pyridine. Unfortunately, the pK values for selenohomocystine have not yet been determined. However, they are not expected to differ appreciably from the pK values of selenocystine, because the analogous sulfur compounds cystine (pK_1 1.51, pK_2 2.79, pK_3 8.25, pK_4 8.97)[20] and homocystine (pK_1 unknown, pK_2 2.52, pK_3

8.68, pK_4 9.41)[23] have similar pK values.

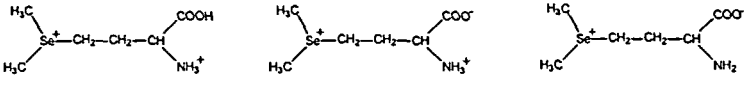
Selenohomocystine with two methylene groups more than selenocystine has retention times that are considerably longer than the retention times for selenocystine over the entire pH range investigated (Figure 2). These longer retention times can be attributed to the additional methylene groups that increase the lipophilic interactions between the organic backbone of the stationary phase and selenohomocystine.

Trimethylselenonium iodide and (3-amino-3-carboxy-1-propyl)-dimethylselenonium iodide

The retention time of the trimethylselenonium cation increases slightly from 171 seconds at pH 1.1 to 186 seconds at pH 4.0 and then sharply to 786 seconds at pH 6.0 (Figure 2). The trimethylselenonium ion is cationic irrespective of pH. The concentration of the pyridinium cation remains practically constant from pH 1.0 (19.998 mM) to pH 3.0 (19.80 mM), but changes drastically within two pH units from ~18.2 mM at pH 4.0 to ~1.8 mM at pH 6.0. The inverse relationship between retention time for the trimethylselenonium cation and the concentration of the pyridinium cation points toward the competition of two cationic species for the sulfonate exchange sites. The retention time under otherwise constant conditions increases with decreasing concentration of the pyridinium ion as expected for a straight-forward cation exchange process.

(3-Amino-3-carboxy-1-propyl)dimethylselenonium iodide (DmpSe,

Figure 1) is formed, when selenomethionine is methylated. DmpSe has a permanent cationic center on one end of the molecule and the α-aminocarboxylic acid functional group at the other end. Because the charge on this functional group (positive, zero on the zwitterion, negative) will change with pH, DmpSe may carry two positive charges (H_2L^{2+}), one positive charge (HL^+), or be uncharged (L^0).



The deprotonation constants for DmpSe are not known. The retention time/pH curve for DmpSe parallels the curve for selenocystine in the pH range 1.1 to 4.0 and for the trimethylselenonium cation in the range 4.0 to 6.0. Therefore, the interactions postulated for selenocystine (a molecule that also can have a cationic end and a zwitterionic end) should be applicable to the rationalization of the changes in the retention time for DmpSe in the pH range 1.1 to 4.0 and the cation-exchange mechanism postulated for the trimethylselenonium cation for the changes in retention times for DmpSe in the pH range 4.0 to 6.0. The species distribution diagram for DmpSe must then be similar to the diagram for selenocystine in the pH region 0 to 4.0 (Figure 3). Such a diagram for DmpSe could be obtained with pK values of approximately 1.4 and 2.0. Such low deprotonation constants for H_2L^{2+} and HL^+ might not be unreasonable in the presence of a permanent cationic center

close to the amino acid functional group.

The increase in the retention time in the pH range 1.1 to 1.6 (Figure 2) is then attributable to the increase in concentration of HL^+ and the decrease of the retention time in the pH range 1.6 to 4.0 to the decrease in the concentration of HL^+. The interaction with the sulfonate exchange sites should occur via the zwitterionic end of the molecule that had formed an ion-pair with the pyridinium cation, the concentration of which remains practically constant in the pH region 1.1 to 4.0. In a solution of pH ~4.0 the concentration of HL^+ should be practically zero and all of DmpSe should be present as L°. Because the amino group is not protonated any more and the carboxyl group is deprotonated and negative, the amino acid end of DmpSe cannot interact with the sulfonate exchange sites. However, the selenonium end of DmpSe can now compete with the pyridinium cation for the sulfonate exchange sites. At a constant concentration of L° and a pyridinium concentration that decreases in the pH range 4.0 to 6.0, the retention time of DmpSe should and does increase with increasing pH (Figure 2). That the interaction of the ammonium group in DmpSe with the sulfonate exchange sites is favored over the interaction with the selenonium center in the pH range, in which ammonium groups are present in DmpSe, can be rationalized on energetic grounds (ammonium group smaller than selenonium group).

Optimal Conditions for the Separation of Selenium Compounds on the PRP-X200 Column

Inspection of Figure 2 suggests, that an optimal separation of the eight selenium compounds is not possible with pyridine mobile phases at pH < 3.5, because the retention times for selenoethionine and selenohomocystine are too long. The signals for selenohomocystine, for selenic acid, for the trimethylselenonium cation, and for DmpSe overlap between pH 4.2 and 5.0 and the signals for selenomethionine and selenic acid between pH 5.0 and 6.0. The pH range 3.60 to 4.0 appears to be the best for the separation of the eight selenium compounds.

With a 20 mM pyridine solution at pH 3.70 seven selenium compounds can be separated in approximately 5.0 min (Figure 5). The signal for selenoethionine, the eighth compound, with a retention time of ~1100 seconds is very broad, but adequate for identification provided the concentration of this compound is higher than 10 µg Se/L. The exploration of the dependence of the retention times on the temperature, at which the cation exchange column was held during the separation, revealed, that the signal for selenoethionine moved from ~1200 seconds at 23°C to ~850 seconds at 60°C (Figure 5, Figure 6). Concomitantly with the shortening of the retention time, the width of the signal for selenoethionine decreased. However, at 60°C - the temperature desirable for the identification and quantification of selenoethionine - the signals for selenic acid and selenohomocystine that are almost baseline-separated at temperatures below 50°C overlap (Figure 5). Therefore, the separation should be repeated at 60°C for the quantification of selenoethionine. We observed that PRP-X200

columns, on which many separations had been carried out, could no longer separate selenic acid and DmpSe.

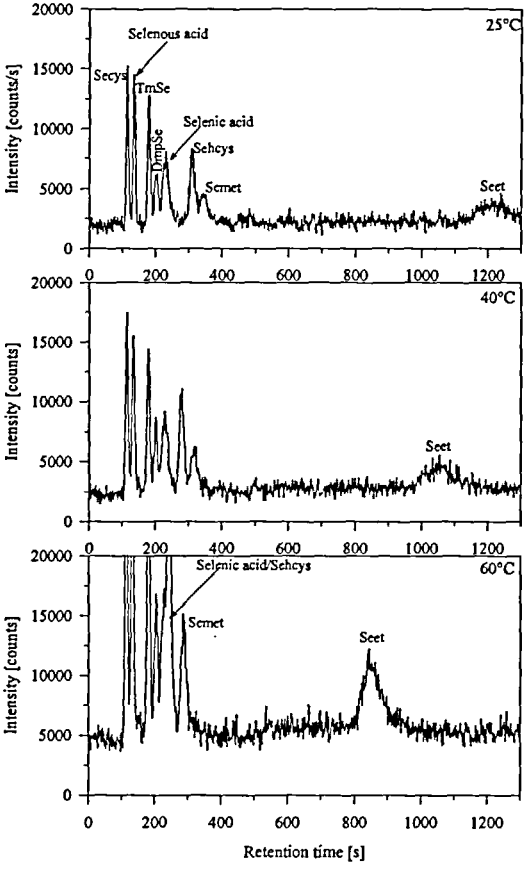


FIGURE 5 Chromatograms obtained with mixtures of the eight selenium compounds (20 μg Se/L each, Seet 50 μg Se/L) with a 20 mM aqueous pyridine at pH 3.70 as mobile phase at column temperatures of 25°C, 40°C, or 60°C (injection volume 100 μL, flow rate 1.5 mL/min; PRP-X200 cation-exchange column; detection by HHPN-ICP-MS).

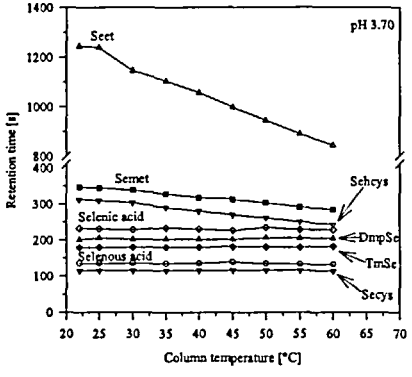


FIGURE 6 Influence of column temperature on the retention times of selenium compounds (100 µL injected, 20 µg Se/L, Seet 50 µg Se/L; mobile phase 20 mM pyridine at pH 3.70; flow rate 1.5 mL/min; PRP-X200 cation-exchange column; detection by HHPN-ICP-MS).

Calibration curves for the eight selenium compounds were constructed with the areas and the heights of the chromatographic signals for ^{77}Se, ^{78}Se and ^{82}Se. The best results were achieved with ^{78}Se, the second most abundant selenium isotope. Unfortunately, the signals from ^{80}Se, the most abundant selenium isotope, suffer from polyatomic interference by $^{40}Ar_2$. The regression coefficients (r^2) for the linear four-point calibration curves were never smaller than 0.99. The maximal relative standard deviations (n = 5) of the signal areas/heights at 1.0 µg Se/L were ~9%/17% (trimethylselenonium cation), at 5.0 µg Se/L ~7%/11% (selenous acid), and at 10 µg Se/L ~4%/5% (selenic acid).

REFERENCES

[1.] A. Läuchli, *Bot. Acta*, **106**, 455 (1993).
[2.] M. M. Abrams and R. G. Burau, *Commun. Soil Sci. Soc. Plant Anal.*, **20**, 221 (1989).
[3.] M. M. Abrams, R. J. Zasoski, C. Shennan, and R. G. Burau, *Agron. J.*, **82**, 1127 (1990).
[4.] G. Kölbl, *Mar. Chem.*, **48**, 185 (1995).
[5.] K. Pyrzynska, *Analyst*, **121**, 77R (1996).
[6.] R.J. Kraus, S. J. Foster, and H. E. Ganther, *Anal. Biochem.*, **147**, 432 (1985).
[7.] S. J. Hill, M. J. Bloxham, and P. J. Worsfold, *J. Anal. At. Spectrom.* **8**, 499 (1993).
[8.] G. Kölbl, *Determination of Selenium Compounds in Environmental and Biological Samples with High Performance Liquid Chromatography Using Selenium-Specific Detection, Ph.D Dissertation,* Institute for Analytical Chemistry, Karl-Franzens University, Graz, Austria, 1994.
[9.] N. Gilon, A. Astruc, M. Astruc, and M. P. Gautier, *Appl. Organomet. Chem.*,**9**, 623 (1995).
[10.] M. P. Gautier, C. Boucharat, A. Astruc, and M. Astruc, *Appl. Organomet. Chem.*, **7**, 593 (1993).
[11.] H. M. Crews, P. A. Clarke, D. J. Lewis, L. M. Owen, P. R. Strutt, and A. Izquierdo, *J. Anal. At. Spectrom.*, **11**, 1177 (1996).
[12.] R. M. Olivas, O. F. X. Donard, N. Gilon, and M. P. Gautier, *J. Anal. At. Spectrom.*, **11**, 1171 (1996).
[13.] H. Ge, X. Gai, J. F. Tyson, P. C. Uden, E. R. Denoyer, and E. Block, *Anal. Comm.*, **33**, 279 (1996).
[14] M. A. Quijano, A. M. Gutierrez, M. C. P. Conde, and C. Camara, *J. Anal. At. Spectrom.*, **11**, 407 (1996).
[15] M. Abegaz, W. Goessler, and K. J. Irgolic, *Appl. Organomet. Chem.*, in preparation.
[16.] W. Goessler, D. Kuehnelt, C. Schlagenhaufen, M. Abegaz, and K. J. Irgolic, *J. Chromatogr. A*, **789**, 233 (1997).
[17.] J. M González LaFuente, M. Dlaska, M.L. Fernández Sánchez, and A. Sanz-Medel, *J. Anal. At. Spectrom.*, **13**, 423 (1998).

[18.] K. Schachl, *Synthese und Physikochemische Charakterisierung von Selenohomocystin, Diplomarbeit*, Institute for Analytical Chemistry, Karl-Franzens-University, Graz, Austria, 1993.

[19.] M. Abegaz, *Investigation of the Chromatographic Retention Behavior of Organic and Inorganic Selenium Compounds and Determination of Total Selenium and of Selenium Compounds in Environmental and Biological Samples with Selenium Specific Detection, Ph.D Dissertation,* Institute for Analytical Chemistry, Karl-Franzens University, Graz, 1997.

[20.] M. R. Silva, R. M. Olivas, O. F. X. Donard, and M. Lamotte, *Appl. Organomet. Chem.*, **11**, 21 (1997).

[21.] A. E. Martell and R. M. Smith, *Critical Stability Constants, Volume 1, Amino Acids*, (Plenum Press, New York, 1974), p. 50.

[22.] Handbook of Chemistry and Physics, Vol. 60, CRC, Boca Raton, Florida, 1979.

[23.] A. Braibanti, E. Leporati, F. Dallavalle, and G. Mori, *Inorg. Chim. Acta*, **6**, 395 (1972).