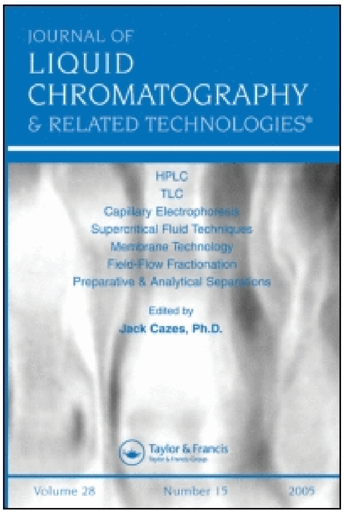

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Application of Alkylamide Phase to the Separation of Inorganic Anions by Reversed Phase HPLC

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ABSTRACT

Packing with an alkylamide chemically bonded stationary phase (AP) for high performance liquid chromatography (HPLC) was applied for the determination of inorganic anions in water samples. The conventional octadecyl (C_{18}) stationary phase was used as a reference column packing material for reversed-phase (RP) HPLC. The mobile phase composition under ion-pair RP HPLC conditions, was optimized by changing the concentration of the organic modifier (acetonitrile), the ionic strength of eluent (addition of TRIS), and the pH value. The data obtained were used for qualitative and quantitative determination of anions in samples of drinking water and water from the Vistula River.

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Key Words: Liquid chromatography; Chemically bonded phases; Alkylamide stationary phases; RP separation mechanism; Environmental analysis; Anions separation.

INTRODUCTION

Professional literature frequently provides information on new stationary phases for the separation of an increasingly larger number of complex analytes and mixtures. This concerns, especially, high-performance liquid chromatography (HPLC) whose exploitation materials, i.e., supports, packings, and columns, are also successfully applied in other related techniques, e.g., solid phase extraction (SPE), capillary zone electrophoresis (CZE), etc. It turns out that conventional packings or columns show low selectivity. This increases unit costs of determinations and extends the whole process. The optimum solution would be to prepare universal packings and columns, for a wide range of applications, enabling the determination of both organic and inorganic substances with high accuracy and reproducibility.

As regards organic solutes, the most popular phase (column), applied for various types of determinations, is octadecyl (C_{18}) phase (monomeric or polymeric, end-capped or not, with low- or high-coverage density).[1–5] This phase has been also successfully applied for the separation of inorganic anions.[6,7] However, different manufactures recommend the use of various silica-based ion exchanger packings.[8–11] There are also packings in which chemically bonded selective stationary phases are cyclic polyethers 18-crown-6 (1,4,7,10,13,16-hexaoxacyclooctadecane).[12,13] Igawa et al.[13] developed crown-based ether resins that could be used for the separation of numerous inorganic anions.

Organic polymers, such as totally porous latex particles and polystyrene–divinylbenzene copolymers prepared according to different methods, are commonly used for the separation of organic and inorganic anions.[14] Polymethacrylate and polyvinyl resins are employed for the separation of monovalent anions,[15] which permits reduction of peak broadening and tailing. For that reason, as well as to make the process shorter, these resins are used in ion chromatography and ion-pair or ion-suppression liquid chromatography under reversed-phase (RP) conditions, on chemically bonded phases with different terminal groups localized in hydrophobic ligands ($-$CN, $-NH_2$, $-$OH).[6,7,14]

Selective alkylamide phase (AP), already described in detail, is characterized by specific properties[3,16–18] (Fig. 1). Apart from hydrophobic C_{16} chains grafted to the amino groups, constituting terminations of short propyl chains, this packing contains also residual silanols and aminopropyl sites.[19] Our previous detailed study conducted using various organic solutes of a polar

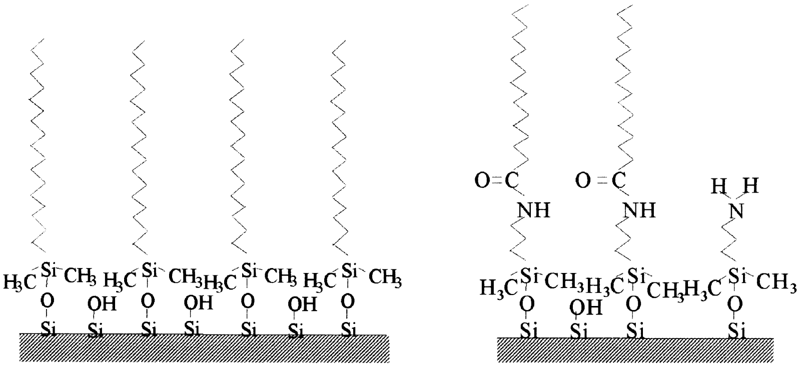

Figure 1. Structures of chemically bonded monomer phases, C18 and AP.

(basic or acidic) and non-polar character, shows that the retention of these compounds is governed by specific and non-specific interactions, which dominate in a mixed mechanism (adsorption, partition, and donor–acceptor interactions).[20,21]

Taking into account the specific properties of AP, we decided to employ it for the separation of anions present in water to widen its application range. We paid particular attention to the effects of pH, ionic strength of the mobile phase, and the concentration of organic modifier on the retention of anions and their quantitative and qualitative determination in samples of water from the Vistula River.

EXPERIMENTAL

Materials and Reagents

Bare spherical silica gel (SG) manufactured by J. T. Baker (Philipsburg, USA) was used as support of chemically bonded phases (CBPs). The physical parameters of SG were as follows: the particle diameter (d_p)—ca. 5 μm, the pore diameter (D)—ca. 16 nm, the pore volume (V_p)—ca. 1.86 cm^3/g, the specific surface area (S_{BET})—ca. 420 m^2/g.

The following reagents were applied for the preparation of CBPs: octadecyldimetyl chlorosilane (Petrarch System, Levitown, PA), γ-aminopropyldimethylmethoxysilane (Fluka, Buchs, Switzerland), stearoyl chloride (E. Merck, Darmstad, Germany) and specially prepared dry morpholine[2,22] (Riedel de Haën, Seelze, Germany). Methanol, toluene, and *n*-hexane (E. Merck) used in the experiment, and all the reagents were of analytical-reagent grade.

Retention measurements at different pH, ionic strength of hydro-organic mobile phase, and concentrations of TRIS [*tris*(hydroxymethyl)aminomethane] (Austranal Preparate, Wien, Austria), and boric acid (Chemapol, Prague, Czech Rep.) were carried out at 308K using a flow rate of 0.6 mL/min. Acetonitrile of gradient grade (J. T. Baker, Deventer, the Netherlands) and deionized water purified by the Milli-Q/RG water purification system (Millipore, Bedford, MA), were used for the preparation of hydro-organic mobile phases for chromatographic investigations. Deutered water (Nuclear Chemistry Institute, Swierk, Poland) was used as a marker for dead time determination.[21]

Apparatus and Physicochemical Investigations

The porosity parameters of bare silica (SG) (S_{BET}—specific surface area; V_p—pore volume; D—mean pore diameter) were determined by the low-temperature nitrogen adsorption–desorption method using a Sorptomatic instrument, Model 1800 (Carlo Erba, Milan, Italy). The details of this procedure have been described elsewhere.[17]

The degree of silica support surface coverage with bonded ligands was calculated from the carbon content,[18] determined with a CHN analyzer, Model 240 (Perkin Elmer, Norwolk, CT). Solid-state cross polarization magic-angle spinning (CP/MAS) NMR spectroscopy was performed on a Bruker MSL 300 spectrometer (Rheinstetten, Germany), on 200–300 mg samples of ^{29}Si and ^{13}C. CP/MAS NMR spectra were externally referenced to liquid tetramethylsilane; chemical shifts (δ) are given in parts per million (ppm).

Chromatographic measurements were made using an isocratic LaChrom liquid chromatograph system (E. Merck-Hitachi, Darmstad, Germany), consisting of an isocratic pump L-7100, a column oven (Model L-7350), a variable UV–Vis detector (L-7420), and a Rheodyne 7125 injection valve (Barkaley, CA) with a 20 μL sample loop. The data were acquired and processed using a L-7500 integrator (E. Merck). Stainless-steel tubes (125 × 4.6 mm I.D.) and other column accessories were purchased from Bischoff AG. (Leonberg, Germany).

Preparation of Chemically Bonded Phases

Chemical modification of silica support was carried out in a specially constructed glass reactor, which prevents contact of reagents with the environment.[24] Prior to the modification, the silica sample was degassed under vacuum (10^{-3} Pa) at 459K. The procedure, mechanism, and conditions of C_{18}-phase synthesis have been described previously.[19,21] The phase, with a charge localized in hydrophobic ligands (SG-AP), was prepared by a two-stage reaction. The SG support was first heated at 458K under vacuum in 10^{-3} Pa. Next, portions of CBP support were treated with a mono-functional aminopropyl-silane modifier (SG-NH_2), followed by a reaction of the amino groups with the chloride derivative of stearyl acid, according to the procedure described in Ref.[22] The characteristics of the packing prepared in the experiment are listed in Table 1.

Column Packing Procedure

The columns (125 × 4.6 mm I.D.) were packed using an air-driven fluid pump, Model DST-122 (Haskel Inc., Burbank, CA) at 55 MPa, according to a modified upward slurry method described by Buszewski et al.[23] The slurry consisting of 35 mL of propanol-2 (8%, w/w) was shaken in an ultrasonic bath for 5 min. Methanol was used as a driving solvent.

RESULTS AND DISCUSSION

Characteristics of Stationary Phases

Figure 1 presents the structures of chemically bonded phases: conventional SG-C18 and SG-AP developed at our laboratory.[16] The structures of both phases were determined on the basis of data from an elementary analysis (Table 1) and spectroscopic measurements (^{29}Si and ^{13}C CP/MAS NMR). It was found that, in all cases, the percentages of carbon (P_C) and nitrogen (P_N) in bonded organic residues were high. This may suggest the formation of a surface with the so called high coverage density (which indicates that ca. 55% of residual silanols were converted).[1,2,6] This support surface was well

Table 1. Surface characterization of chemically modified silica gel support (SG).

Packing materials	P_C (%)	P_N (%)	α_{NH_2} (μmol/m^2)	α_{RP} (μmol/m^2)
SG-C18	20.24	—	—	2.77
SG-NH_2	5.80	1.43	2.64	—
SG-AP	12.72	1.43	0.77	1.87

shielded by stationary phases formed as a result of conversion of silanes with silanols.

The CHN data (Table 1) and the results of CP/MAS NMR show that the SG-C18 phase contains 53.5 ± 0.5% of bonded octadecyl (C18) ligands and 46.5 ± 0.5% of residual silanols. As concerns AP, its surface consists of residual amino groups (14.9 ± 0.5%), bonded acyloamido ligands (36.1 ± 0.5%) and residual silanols (49 ± 0.5%). This affects the mechanism of polar analyte retention, especially during separation under hydro-organic conditions in the RP system.[18] Due to the presence of free amino and acyloamino groups, ion and donor–acceptor interactions dominate in the mechanism of polar substance retention.[21,24] Therefore, we decided to apply these phases to the separation of anionic forms with UV detection. The optimization of the detection system for such compounds ($\lambda = 215\,\mathrm{nm}$), using the SG-C18 phase and a binary mobile phase typical of the RP system (methanol-water or acetonitrile-water) was discussed in our previous papers.[25,26] So, this time, we focused on the optimization of the mobile phase composition using stationary SG-AP.

Figures 2–4 show changes in the retention (*k*) of selected anions: Cl^-, NO_2^-, NO_3^-, and I^- depending on the concentration of organic modifier (acetonitrile) in the hydro-organic mobile phase, changes in pH (boric acid), and the ionic strength of eluent (addition of TRIS). Good selectivity and resolution of particular solutes were obtained for the mobile phase consisting of 1.4 mM TRIS + boric acid (pH = 5.21) + 3% v/v ACN, and a flow rate of

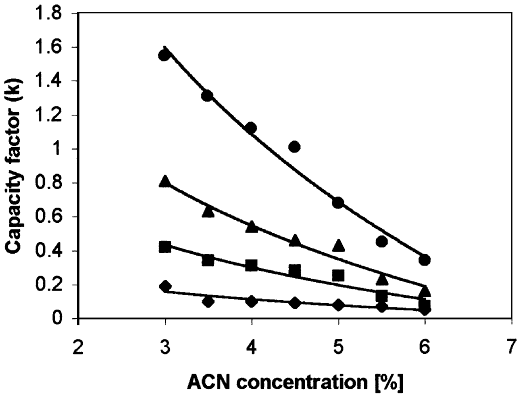

Figure 2. Dependence of the capacity factor (*k*) on the organic modifier content of the mobile phase.

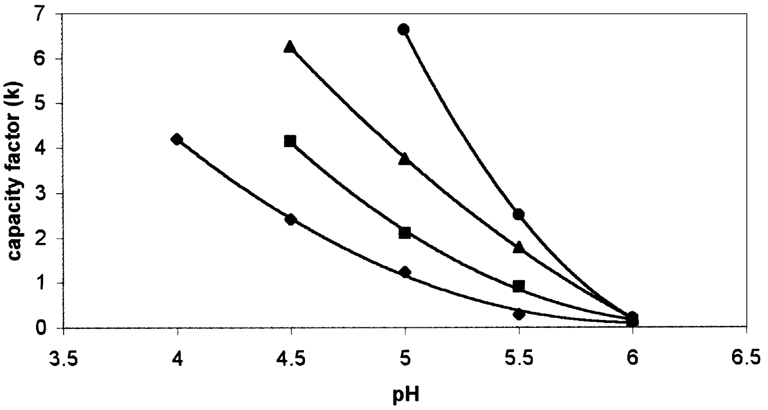

Figure 3. Dependence of the capacity factor (k) on pH values.

0.5 mL/min. The concentrations of standard mixture components varied from 10 to 20 ppb (Table 2).

Table 2 also shows a comparison between the data characterizing the retention and selectivity of separation obtained on columns with SG-C18 and SG-AP. The advantages of different parameters, k, α, and R_S, of SG-AP are plain to see (Table 2). This is also confirmed by resolution expressed by changes in the mobile phase pH (addition of boric acid). At pH = 5.63 (Fig. 5)

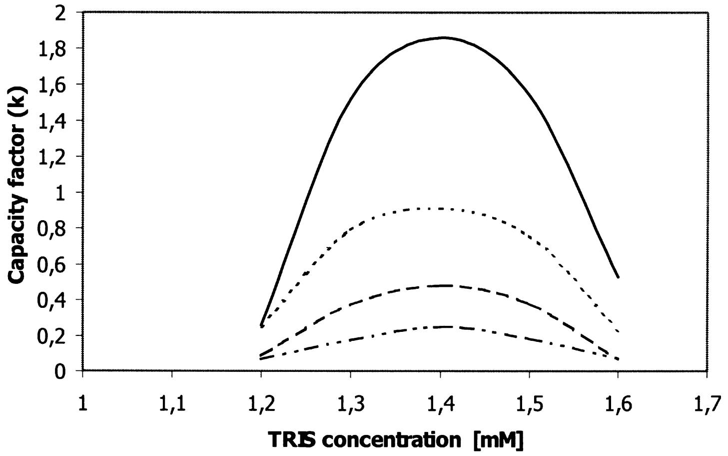

Figure 4. Dependence of the capacity factor (k) on the ionic strength of eluent (addition of TRIS) in the mobile phase.

Table 2. Retention of selected anions on columns with SG-C18 and SG-AP.

Solutes	Concentration (ppb)	SG-C18			SG-AP		
		k	α	R_s	k	α	R_s
Cl^-	15	0.148	1.074	0.652	0.252	2.36	1.43
NO_2^-	10	0.159	0.987	0.599	0.595	1.57	0.954
NO_3^-	10	0.157	1.045	0.633	0.934	3.06	2.04
I^-	20	0.164			2.857		

the peaks are sharper and the retention is shorter (ca. 50%), but the resolution of both nitrate forms (NO_2^- and NO_3^-) decreases.

Due to its specific properties, we used SG-AP for the determination of anionic forms in samples of water from the Vistula River (Fig. 6). Apart from standard monitoring of the concentrations of particular anions (in the range

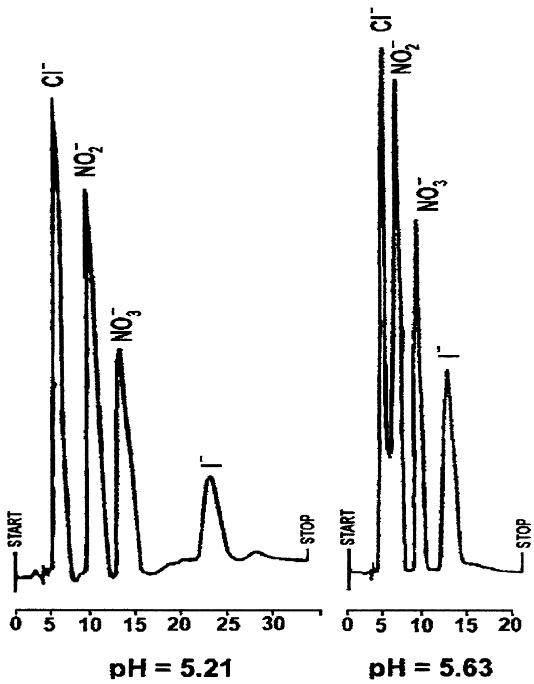

Figure 5. Chromatograms showing the separation of a mixture of chloride, iodide, nitrate and nitrite ions at different pH values.

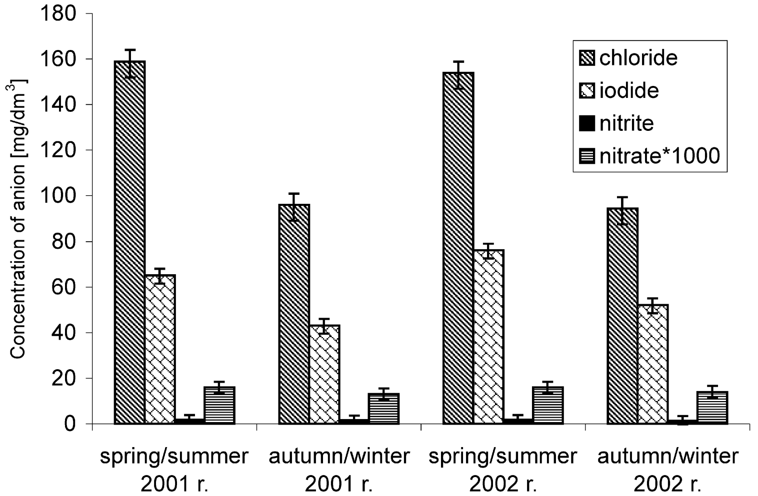

Figure 6. The content of the ions analyzed in samples of water from the Vistula River at various seasons of the year.

of 94–159 mg/dm^3 for chloride ions, 43–76 mg/dm^3 for iodide ions, 1.3–1.8 mg/dm^3 for nitrate ions and 0.013–0.016 mg/dm^3 for nitrite ions), our thorough investigations show high reproducibility of retention data, confirmed by standard deviations (SD) below 3%.

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