Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1073 (2005) 147–153
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

New stationary phase for anion-exchange chromatography

Lúcia M.L.A. Auler[a], César R. Silva[b], Kenneth E. Collins[b], Carol H. Collins[b,*]

[a] *Centro de Desenvolvimento da Tecnologia Nuclear/Comissão Nacional de Energia Nuclear (CDTN/CNEN), Caixa Postal 941, 30123-970 Belo Horizonte, MG, Brazil*
[b] *LABCROM—Laboratório de Pesquisas em Cromatografia Líquida, Instituto de Química, Universidade Estadual de Campinas, Caixa Postal 6154, 13084-971 Campinas, SP, Brazil*

Abstract

This work describes the preparation of an anion-exchange phase based on silica, using a two-step modification process. First, 10 μm Davisil silica particles were silanized with chloropropyltrimethoxysilane to yield chloropropyl silica. The modified silica was then reacted with pyridine to produce positively charged propylpyridinium groups on the surface, the anion-exchange sites. The phase was characterized by thermogravimetric analysis and infrared and solid state ^{13}C and ^{29}Si NMR spectroscopies. HPLC separations of common inorganic anions, including chloride, nitrite, bromide and nitrate, were performed using 150 × 3.9 HPLC columns packed with the phase, using a phthalate buffer solution as mobile phase with non-suppressed conductivity detection. Efficiency and resolution were calculated and the results show that the new phase has significant promise for the analysis of these anions in environmental samples.

Keywords: Propylpyridinium; Silica; Anion chromatography; Stationary phase

1. Introduction

Ion chromatography, developed by Small et al. [1] allows the rapid separation and quantitative determination of inorganic and organic anions. Despite its many advantages ion chromatography still has some drawbacks. If large particles based on polymeric materials are employed, column efficiencies are low and, consequently, broad peaks result in decreased detectability. In addition, organic supports are generally not pressure resistant and swell in certain organic solvents. However, they still offer much better chemical stability than silica supports over a wide pH range, even when using sodium hydroxide as eluent [2].

Studies have been made to improve overall chromatographic performance of stationary phases, such as modifying the silica with organofunctional groups covalently bonded onto the surface [3,4]. However, the number of chemically modified silica-based phases for anion chromatography is very limited [5] and the development of new stationary phases for more difficult separations is of great importance [6]. Stationary phases for anion-chromatography based on aminopropyl [7] and phenyl-aminopropyl [8] silicas have been extensively used for the analysis of various organic and inorganic anions. More recently, successful approaches involving the use of reversed-phase particulate [9] and monolithic columns based on silica, permanently coated with quaternary ammonium salts, to perform ultrafast separations of some common inorganic anions have been reported [10–12].

The anion-exchange properties of a silica gel surface modified with covalently bonded pyridinium salts were first described by Tundo et al. [13]. Following this publication, several papers have described the use of these modified silica gels for the adsorption [14] and pre-concentration [15] of some inorganic anions and applications in electrochemistry. However, to the present date, the use of these pyridinium-modified silicas as stationary phases for anion chromatography has not been explored.

Based on these considerations, the primary goals of this research were to synthesize and characterize a novel sta-

* Corresponding author. Fax: +55 19 3788 3023.
E-mail address: chc@iqm.unicamp.br (C.H. Collins).

0021-9673/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2004.10.012

tionary phase with propylpyridinium groups attached on the silica surface for the separation of some inorganic anions.

2. Experimental

2.1. Materials

HPLC-grade methanol, hexane and dichloromethane (Merck) and Milli-Q water (Millipore, Bedford, USA) were filtered (0.22 μm membrane) before use. The reaction solvent, toluene, was also purchased from Merck (Rio de Janeiro, Brazil) and was dried over sodium and distilled before use. Pyridine and triethylamine were dried over KOH pellets and purified by distillation. The silylant agent, 3-chloropropyltrimethoxysilane (97%) was obtained from Aldrich (Milwaukee, WI, USA). The compounds used in normal phase chromatographic tests (benzene, benzyl alcohol, anthracene, benzonitrile, and nitronaphthalene) were analytical grade and were used without purification. The anions for the anion-exchange experiments were standard solutions from Merck. Davisil silica from Alltech Associates (Deerfield, IL, USA), having 10 μm irregular porous particles an average pore diameter of 15 nm and a specific surface area (S_{BET}) of 215 $m^2\ g^{-1}$, was used as support.

2.2. Synthesis of propylpyridinium silica—surface modification

First, Davisil silica was dried under vacuum at 100 °C for 6 h to remove adsorbed water. Then, the activated silica (20.0 g) was cooled to room temperature and placed in a reaction flask containing 250 ml of dry toluene. Under mechanical stirring, an amount of 680 μl of deionized water was added to this mixture. Then, 20 ml of 3-chloropropyltrimethoxysilane were added, followed by 2.0 ml of triethylamine (added as a catalyst) [16]. An excess of silylant agent was employed, assuming a maximum silanol concentration of 8 $\mu mol\ m^{-2}$ on the silica surface. The mixture was refluxed under nitrogen for 48 h. After refluxing, the reaction was stopped and the modified silica was cooled to room temperature, transferred to a vacuum glass filter, and washed with toluene (100 ml), an ethanol–water mixture (200 ml), deionized water (500 ml) and finally with methanol (100 ml). The chloropropyl silica, SilprCl, was dried under vacuum at 60 °C for 4 h, before reaction with pyridine.

In the second step, the chemically bonded chloropropyl groups on the silica surface were reacted with pyridine. Briefly, 20.0 g of dry chloropropyl silica were placed in a reaction flask containing 250 ml of anhydrous toluene and a large excess of pyridine was used (20 ml). The mixture was refluxed with stirring under a dry nitrogen atmosphere for 72 h. After refluxing, the reaction was stopped and the modified silica was cooled to room temperature, transferred to a vacuum glass filter, and washed with methanol (500 ml), water (300 ml), and again with methanol (200 ml). The propylpyridinium silica, denoted as SilprPy, was dried under vacuum at room temperature prior to packing and characterization procedures.

2.3. Thermogravimetric analysis

The thermogravimetric curves were obtained on a model 2050 Thermal Analyzer from TA Instruments (USA). The samples were heated at a rate of 5 °C s^{-1} in a nitrogen atmosphere.

2.4. Infrared spectroscopy

Diffuse reflectance infrared Fourier transformation (DRIFT) spectra were obtained on a Bomem spectrometer (Hartmann and Braun, Quebec, Canada) in the range of 4000–400 cm^{-1} with a resolution of 4 cm^{-1} and a scan rate of 20 scans min^{-1}, using a diffuse reflectance accessory.

2.5. Solid-state nuclear magnetic resonance spectroscopy

Solid-state ^{13}C and ^{29}Si NMR measurements for the SilprCl and SilprPy silicas were performed on a Brucker AC 300 spectrometer (Germany), using cross polarization and magic angle spinning (CP/MAS). For the ^{29}Si nucleus, a contact time of 5 ms and a pulse repetition time of 3 s were employed and for ^{13}C, a contact time of 3 ms and repetition time of 3 s were used. Frequencies of 75.5 and 59.6 MHz for carbon and silicon, respectively, were used.

2.6. Column packing

HPLC columns (150 mm × 3.9 mm) were made from type 316 stainless steel tubes. The internal surface was polished using a technique developed in our laboratory [17]. The columns were downward packed using 10% (w/v) stationary phase slurries in chloroform with methanol as propulsion solvent. A constant packing pressure of 34.5 MPa produced with a Haskel packing pump (Burbank, CA, USA) was used. After packing, the column was conditioned by passing 20 column volumes of isopropanol to remove the propulsion solvent and then a normal mobile phase, hexane–dichloromethane (95:5, v/v), at 0.2 ml min^{-1} prior to testing.

2.7. Chromatographic evaluation

The chromatographic evaluation of column performance were carried out using a mixture of benzene, benzyl alcohol, anthracene, benzonitrile and nitronaphthalene in the normal phase. For this test, separations were performed with modular HPLC equipment from Waters (Milford, MA, USA) composed of a Waters 510 pump, a Rheodyne 7125 injection valve (Cotati, CA, USA) with a sample loop of 5 μl and a Waters 486 UV–vis detector. The test mixture was separated

with a hexane–dichloromethane (95:5, v/v) mobile phase at different flow rates from 0.1 to 1.5 ml min^{-1} with detection at 254 nm. The optimal flow-rate was determined by a van Deemter plot. Data collection and treatment were carried out by using Chrom Perfect for Windows from Justice Innovations (Mountain View, CA, USA). Chromatographic performance was evaluated by means of efficiency (plates per meter, calculated at half peak height (*N*/*m*) and reduced plate height (*h*)), retention factor (*k*), resolution (Rs) and asymmetry (As) measured at 10% of peak height [18].

The second test mixture, containing some inorganic anions, was prepared by diluting commercially available 1000 μg ml^{-1} stock standards of chloride, nitrite, bromide and nitrate. Standard working dilutions of 10 μg ml^{-1} of each anion were prepared daily. These separations were performed with a Shimadzu system (Kyoto, Japan) equipped with a LC10A Shimadzu pump, a 7125 Rheodyne injection valve with a sample loop of 20 μl and a LC10A Shimadzu conductivity detector. The mobile phase was a 2.5 mmol l^{-1} phthalate buffer solution at 1.5 ml min^{-1}. The buffer was prepared by mixing equal volumes of a 0.25 mol l^{-1} phthalic acid solution with a 0.24 mol l^{-1} Tris solution. The resulting solution was diluted appropriately with deionized water and the pH was adjusted with acid or base, using a freshly calibrated pH meter.

To perform the separation of inorganic anions by ion-exchange chromatography, the mobile phase polarity from the organic solvent mixture was changed slowly. First, isopropanol was used, followed by methanol and a methanol–water (50:50, v/v) mixture. At least 20 column volumes of each solvent were passed through the column. The column was then used with the 100% aqueous phthalate buffer.

3. Results and discussion

3.1. Preparation of SilprPy silica

The procedure for surface modification of the silica is outlined in Fig. 1. In a first step, Davisil silica was silanized with 3-chloropropyltrimethoxysilane, in the presence of a trace of water, to yield chloropropyl silica (SilprCl). The addition of water was necessary to promote the polymerization of the trialkoxysilane, thus enhancing the surface coverage of the silica with the chloropropyl groups. The SilprCl silica was extensively washed with water to promote the total hydrolysis of the remaining methoxy groups. In the second step, a large excess of pyridine was used for reaction with chloropropyl groups to produce positively charged propylpyridinium chloride groups on the surface. These covalently bonded groups are responsible for the anion-exchange properties of the new phase.

3.2. Characterizations

Infrared spectroscopy is a useful tool to confirm the chemical modifications proposed in the reaction scheme of Fig. 1. The DRIFT spectra are shown in Fig. 2 for the bare silica before and after modifications with the chloropropyl silylant agent and with pyridine. In all spectra, a band around 1070 cm^{-1} is observed due to the stretching of the siloxane (≡Si–O) bonds of the silica backbone. The band at 1630 cm^{-1} in spectra A and B is associated to the bending of the water molecules, which are adsorbed on the surface of the bare silica by hydrogen bonding with the silanols. The broad band around 3300 cm^{-1}, due to the stretching of the O–H bonds of the geminal and vicinal silanols, as well as those for the

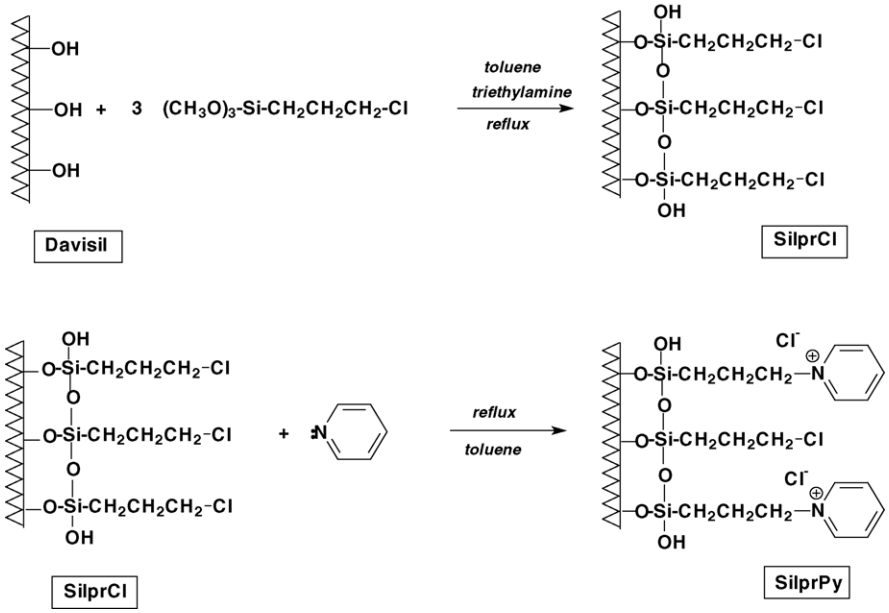

Fig. 1. Reaction scheme for surface modification to prepare the propylpyridinium modified silica.

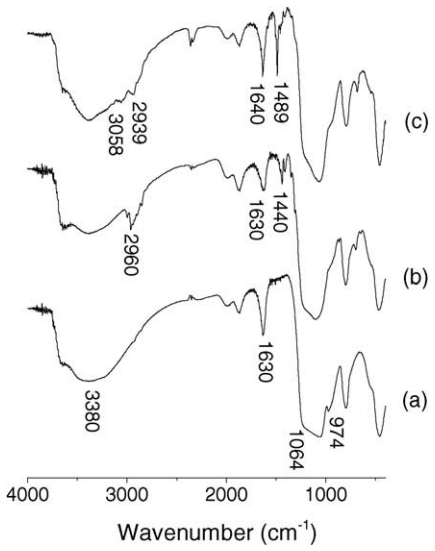

Fig. 2. IR spectra of (a) unmodified silica, (b) silanized silica and (c) pyridinium functionalized silica.

water molecules in the bare silica, decreases in the spectra of the modified silicas.

Another important feature is that, after silanization, the intensity of the shoulder at 974 cm^{-1}, attributed to free silanols on the silica surface, is greatly reduced and two new bands around 2960 cm^{-1} are observed in spectrum of Fig. 2B, due to the C–H stretching of the chloropropyl groups. C–H bending is also observed at 1440 cm^{-1}. The spectrum of Fig. 2C, for SilprPy silica, also shows bands at 2939 and 3058 cm^{-1}, associated with aliphatic and aromatic C–H stretching, respectively.

Two other new bands, at 1640 and 1489 cm^{-1}, are due to the presence of the pyridine ring. These bands are in close agreement with those reported in the literature for the propylpyridinium chloride group [14]. According to Silverstein et al. [19] and Pavia et al. [20], these bands are related to the aromatic C=C stretching and to the C=N vibration modes, respectively.

Fig. 3 shows the ^{29}Si CP/MAS NMR spectrum for the SilprCl, obtained after the modification with the trialkoxysilane. In addition to the peaks at −101 and −110 ppm, which are attributed to the silanol (Q^3) and siloxane (Q^4) groups; T^2 and T^3 species were also observed at −55 and −65 ppm [21]. The absence of T^1 species and higher peak intensities for T^2 and T^3 when compared to Q species suggest a high degree of crosslinking between the polymeric chloropropyl siloxane layer and the silica surface.

Fig. 4 shows the ^{13}C CP/MAS NMR spectrum for the SilprCl silica. The three signals observed at 10, 26 and 46 ppm are related to the chloropropyl group bonded onto silica. The absence of a signal at 50 ppm suggests that all methoxy groups (CH_3O–) were totally hydrolyzed during the washing procedure, subsequent to the derivation reaction [22].

After reaction with pyridine, ^{13}C NMR spectroscopy was again performed. Fig. 5 shows that three new signals, at 61,

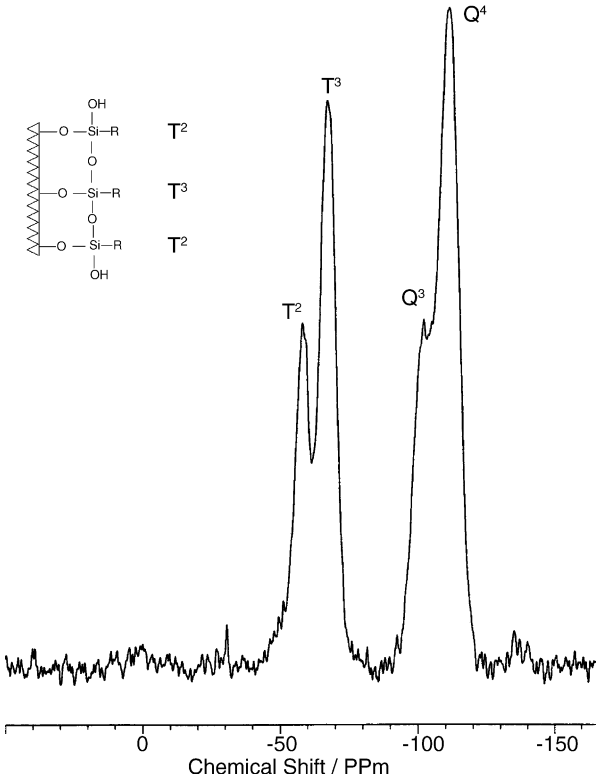

Fig. 3. Solid-state ^{29}Si CP/MAS NMR spectrum for the chloropropyl silica from the silanization reaction step.

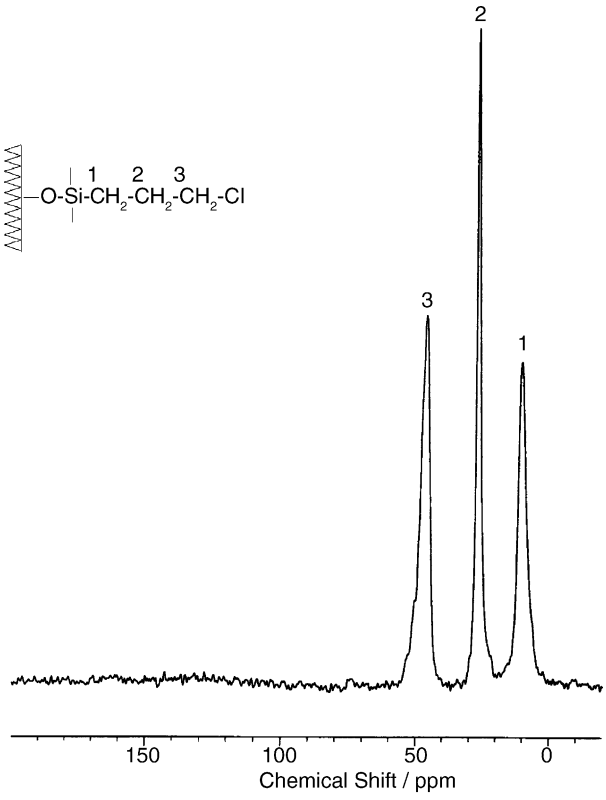

Fig. 4. Solid-state ^{13}C CP/MAS NMR spectrum for the chloropropyl silica, from the silanization reaction step.

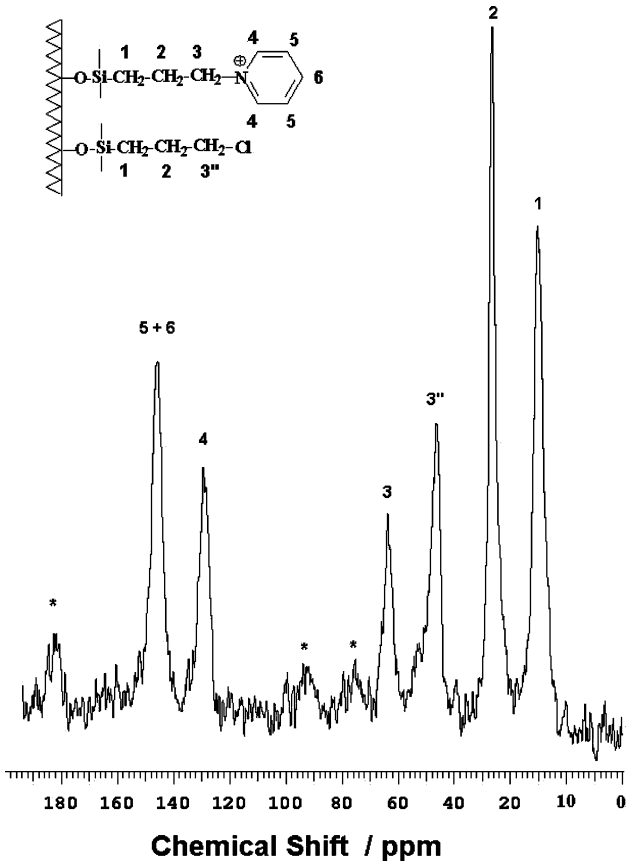

Fig. 5. ^{13}C NMR spectrum for the propylpyridinium silica after reaction with pyridine. The peaks marked with an asterisk are spinning side bands.

128 and 142 ppm, appear in the ^{13}C CP/MAS spectrum for the SilprPy silica. The signal at 61 ppm is related to the carbon bonded to the nitrogen atom from pyridine [14]. The other two bands are attributed to the carbons of the pyridine ring. The presence of the signal at 46 ppm, due to the C3 of chloropropyl moiety, is an indication that unreacted chloropropyl groups are still present on the silica surface after the chemical modification with pyridine. These unreacted groups do not affect the overall performance of the new phase for ion chromatography. However, a high yield for this second reaction is desirable to improve ion exchange capacity.

Thermogravimetry can be employed to determine the surface coverage of the chemically modified silicas since the weight loss observed between 200 and 600 °C was found to be associated to the loss of the organic groups attached on the surface [23]. Fig. 6 shows the thermogravimetric curves for pure silica, chloropropyl and propylpyridinium silicas. For the Davisil silica, mass loss is attributed exclusively to the loss of adsorbed water molecules as well as the condensation of the silanol groups at temperatures above 250 °C. The SilprCl silica obtained after the chemical modification with the trialkoxysilane shows a higher mass loss due to the loss of the chloropropyl moieties. After reaction with pyridine, the organic content has again increased and SilprPy exhibits an even higher mass loss. From the mass losses, the

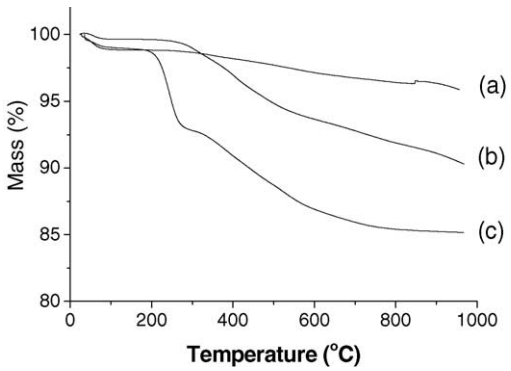

Fig. 6. Thermogravimetric curves obtained for (a) unmodified silica, (b) chloropropyl silanized silica and (c) propylpyridinium functionalized silica.

concentration of organic groups attached on the silica surface is calculated as 1.2 mmol g^{-1} (5.6 μmol m^{-2}) of chloropropyl groups for SilprCl and 0.56 mmol g^{-1} (2.6 μmol m^{-2}) of propylpyridinium groups for SilprPy, confirming that not all the chloropropyl groups reacted with pyridine, as previously indicated by ^{13}C NMR spectroscopy.

3.3. Chromatographic separations

Initially, 150 mm × 3.9 mm HPLC columns packed with the SilprPy phase were used to separate a test mixture composed of non-polar and polar compounds in the normal phase, to evaluate if the packing was adequate. Fig. 7 shows a chromatogram of this test mixture using hexane–dichloromethane (95:5, v/v) as mobile phase at the optimal flow rate of 0.4 ml min^{-1}. The chromatographic parameters were calculated and are presented in Table 1. The retention time of an unretained compound was taken from benzene, although, benzene might interact somewhat with the propylpyridinium stationary phase. A reduced plate height of 2.99 for the most retained compound, nitronaphthalene, and the low asymme-

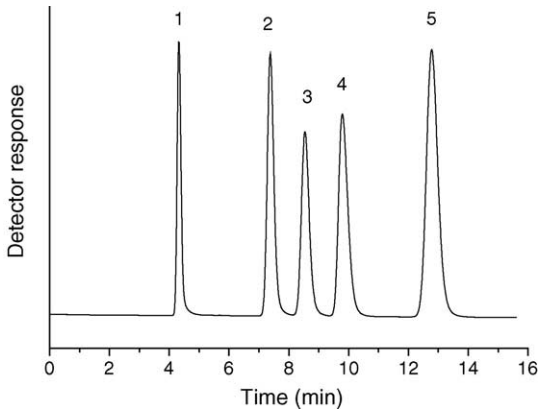

Fig. 7. Chromatogram obtained with stationary phases prepared with the SilprPy silica under normal phase conditions. Test mixture: (1) benzene, (2) anthracene, (3) benzyl alcohol, (4) benzonitrile and (5) nitronaphthalene. Chromatographic conditions: mobile phase: hexane–dichloromethane (95:5, v/v), flow-rate: 0.4 ml min^{-1}, injection volume: 5 μl and detection: UV at 254 nm.

Table 1
Chromatographic parameters for the column based on pyridinium functionalized silica stationary phase, using normal phase conditions

Compound	Chromatographic parameters[a]				
	N/*m*	*h*	As	*k*[b]	Rs
Benzene	33500	2.99	1.5	–	–
Anthracene	33800	2.96	1.3	0.7	9.3
Benzyl alcohol	32300	3.10	1.4	1.0	2.6
Benzonitrile	29800	3.36	1.7	1.3	2.3
Nitronaphthalene	33500	2.99	1.3	2.0	4.6

[a] Chromatographic conditions: mobile phase: hexane–dichloromethane (95:5, v/v), flow-rate: 0.4 ml min^{-1}, injection volume: 5 μl, detection: UV at 254 nm.
[b] Column dead time was measured with benzene.

try values for all compounds are good evidence for a well-packed column.

To achieve the highest ion exchange capacity by displacing the chloride counter ion from the propylpyridinium phase, the column was conditioned with a 0.25 mol l^{-1} aqueous phthalate buffered mobile phase at pH 4 at 0.5 ml min^{-1} for 2 h. After this procedure, the column was conditioned with more dilute buffer solutions in which the total buffer concentration was never higher than 10 mmol l^{-1}. The test mixture chosen for this study was composed of chloride, nitrite, bromide and nitrate at 10 μg ml^{-1}.

An organic buffer was chosen to delay column degradation since some inorganic buffers, such as carbonate and phosphate, cause enhanced silica dissolution [24]. Phthalic acid solutions offer good buffer capacity over the pH range recommended for silica based phases due to its two dissociation constants (pK_{a1} = 3.1 and pK_{a2} = 5.4).

A large number of separations were performed, varying the total buffer concentration from 2.5 to 10 mmol l^{-1}. At concentrations higher than 5 mmol l^{-1}, it was difficult to obtain a good baseline. The pH values of the mobile phase were also varied from 3.8 to 7.0. Separations at pH of 3.8 showed very long retention times even when using higher buffer concentrations. In addition to this, pH values lower than 3 should

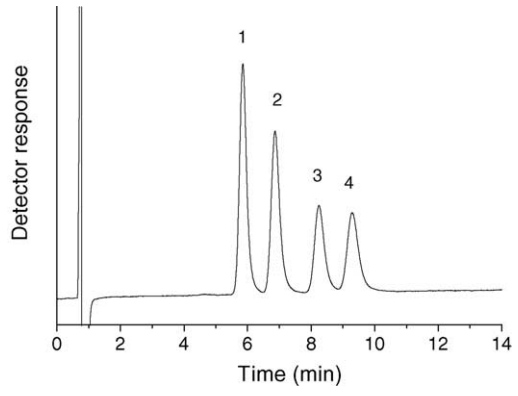

Fig. 8. Chromatogram obtained with the stationary phase prepared with the SilprPy silica using anion-exchange conditions. Test mixture: (1) chloride, (2) nitrite, (3) bromide and (4) nitrate. Chromatographic conditions: mobile phase: 2.5 mmol l^{-1} phthalate buffer solution at pH 4.2, flow-rate: 1.5 ml min^{-1}, injection volume: 20 μl and detection: non-suppressed conductivity.

Table 2
Chromatographic parameters calculated for the separation of some anions on the propylpyridinium stationary phase, using ion exchange conditions

Anions	Chromatographic parameters[a]				
	N/*m*	*h*	As	*k*[b]	Rs
Chloride	20500	4.88	1.29	7.7	–
Nitrite	20500	4.88	1.32	9.2	1.20
Bromide	21600	4.63	1.38	11	1.23
Nitrate	22200	4.50	1.31	13	1.24

[a] Chromatographic conditions: 150 mm × 3.9 mm column, mobile phase: 2.5 mmol l^{-1} phthalate buffer solution at pH 4.2; flow-rate: 1.5 ml min^{-1}; injection volume:20 μl, detection: non-suppressed conductivity.
[b] Column dead time was measured from the mobile phase signal in the conductivity detection.

be avoided to prevent the hydrolysis of siloxane bonds and consequently loss of the bonded phase. Separations at pH values of 5 were faster and the retention factors were lower, however, a system peak started to be observed. At pH 6 and 7, resolution was totally lost. The best separation was achieved when using a 2.5 mmol l^{-1} phthalate buffer solution at pH 4.2. Fig. 8 shows a chromatogram, where the four anions are separated with good resolution, low asymmetries and good reduced plate height values, as can be seen in Table 2, in which the chromatographic parameters for this separation are summarized.

4. Conclusions

A new stationary phase for anion-exchange chromatography, having chemically bonded propylpyridinium groups, was successfully prepared through silanization of silica with 3-chloropropyltrimethoxysilane, followed by reaction with pyridine. The addition of traces of water in the silanization step allows the formation of a polymeric layer of chloropropyl groups, enhancing the surface coverage and, consequently, improving ion exchange capacity and preventing silica support dissolution, especially in ion chromatography that requires the use of 100% buffered aqueous mobile phases.

The results obtained by infrared and ^{13}C NMR spectroscopies confirm the presence of the propylpyridinium groups covalently attached on the silica surface. According to the results obtained in normal phase chromatography, it can be concluded that the procedure chosen was adequate to achieve a good, high performance chromatographic phase.

The new stationary phase showed promising results for the separation and quantification of some inorganic anions often found in real world samples, while the good results obtained for the separations in normal phase do not exclude the possibility of using this new stationary phase in normal phase chromatography as well.

Acknowledgements

The authors wish to acknowledge the financial support of CNEN/CDTN, CNPq and of FAPESP. L.M.L. Auler also

acknowledges a travel grant from CNEN/CDTN to present part of this work at the HPLC 2004 conference.

References

[1] H. Small, T.S. Stevens, W.C. Bauman, Anal. Chem. 47 (1975) 1801.
[2] A. Woodruff, C.A. Pohl, A. Bordunov, N. Avdalovic, J. Chromatogr. A 997 (2003) 33.
[3] P.K. Jal, S. Patel, B.K. Mishra, Talanta 62 (2004) 1005.
[4] M.M. Collinson, Trends Anal. Chem. 21 (2002) 35.
[5] E. Sugrue, P. Nesterenko, B. Paull, J. Sep. Sci. 27 (2004) 921.
[6] C. Sarzanini, J. Chromatogr. A 956 (2002) 3.
[7] H.J. Cortes, J. Chromatogr. 234 (1982) 517.
[8] M.G. Kiseleva, P.N. Nestenko, J. Chromatogr. A 920 (2001) 87.
[9] D. Connolly, B. Paull, J. Chromatogr. A 917 (2001) 353.
[10] P. Hatsis, C.A. Lucy, Analyst 127 (2002) 451.
[11] P. Hatsis, C.A. Lucy, Anal. Chem. 75 (2003) 995.
[12] D. Connolly, D. Victory, B. Paull, J. Sep. Sci. 27 (2004) 912.
[13] P. Tundo, P. Venturello, E. Angeletti, J. Am. Chem. Soc. 104 (1982) 6547.
[14] S.T. Fujiwara, R.V.S. Alfaya, Y. Gushikem, Colloid Surf. A: Physicochem. Eng. Aspects 178 (2001) 135.
[15] M.S. Iamamoto, Y. Gushikem, Analyst 114 (1989) 983.
[16] H. Engelhardt, P. Orth, J. Liq. Chromatogr. Relat. Technol. 10 (1987) 1999.
[17] K.E. Collins, A.C. Franchon, I.C.S.F. Jardim, E. Radovanovic, M.C. Gonçalves, LC–GC 18 (2000) 106.
[18] U.D. Neue, HPLC Columns, Theory, Technology and Practice, Wiley-VCH, New York, 1997.
[19] R.M. Silverstein, G.C. Basser, T.C. Morrill, Spectrometric Identification of Organic Compounds, second ed., John Wiley & Sons Inc., New York, 1991.
[20] D.L. Pavia, G.M. Lampman, G.S. Kriz, Introduction to Spectroscopy, second ed., Saunders College Publishing, New York, 1996.
[21] S. Bachmann, L.F.C. Melo, R.B. Silva, T.A. Anazawa, I.C.S.F. Jardim, K.E. Collins, C.H. Collins, K. Albert, Chem. Mater. 13 (2001) 1874.
[22] K. Albert, E. Bayer, J. Chromatogr. 544 (1991) 345.
[23] B. Lumley, T.M. Khong, D. Perrett, Chromatographia 60 (2004) 59.
[24] H.A. Claessens, M.A. van Straten, J.J. Kirkland, J. Chromatogr. A 728 (1996) 259.