



A polar-copolymerized method to prepare silica-based anion exchanger for ion chromatography

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ABSTRACT

A novel silica-based strong anion exchanger was developed for ion chromatography by copolymerizing methyltrichlorosilane and 3-chloropropyltrichlorosilane. The method allows the column capacity to be easy control simply by adjusting the ratio of silanes. The unwanted residual silanol groups onto the surface of silica gel could also be greatly reduced by this strategy. The effective column capacity of the column used was measured to be 50.8 $\mu\text{equiv}/\text{column}$ (2.03 $\mu\text{equiv}/\text{cm}$). The exchanger was characterized by solid state CP/MAS ^{13}C NMR and elemental analysis and its separation performance was evaluated for the separation of common inorganic anions. The results showed that the column had good separation efficiency (e.g. the plate number of nitrite is 80,000/m) and the separation mechanism was observed to be dominantly governed by ion exchange mechanism. The utility of the column was demonstrated for the determination of nitrite and nitrate in saliva sample.

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1. Introduction

Ion chromatography (IC) is widely employed for the determination of ionic compounds. Since the introduction of IC, several types of ion exchangers for the separation of anions have been developed, including silica-based anion exchangers [1,2], poly(styrene-divinylbenzene) (PS-DVB)-based anion-exchangers [3–6], and macroporous hydroxyethyl methacrylate-based anion exchangers [7]. Among them, PS-DVB-based resins are widely used in present IC system due to their wide pH tolerance. Although the silica-based exchangers have the drawback of limited pH tolerance, they have some specific features, e.g., higher chromatographic efficiency, higher mechanical stability, no swelling or shrinking problems when contacting with high content of organic solvent in the eluent. In addition, the rich silanol groups onto the surface of silica gel are easily bonded chemically to introduce various kinds of functional groups. Since our aim is to develop anion exchangers, here only the work related with the anion exchanger was mentioned. Lin et al. developed a silica-based strong anion

exchanger (SAX) by immobilizing a quaternary ammonium salt of cyanuric chloride onto the silica surface [2]. Effective separation of anions using tartaric acid or o-phthalic acid as eluent was achieved while relative strong unwanted matrix effect was also observed. By immobilizing N-methylimidazolium ionic liquid onto silica gel, Liu et al. prepared an anion exchanger for the separation of anions [8]. They demonstrated good separation of organic anions and inorganic anions and multimode retention mechanism were observed. More recently, Takeuchi et al. demonstrated the effective separation of inorganic anions on a commercial available silica column designed for hydrophilic interaction liquid chromatography [9]. The pyridine functional group containing in the stationary phase served to be the effective ion exchange sites when operated in acid eluent. In addition, the use of commercial available silica monolithic column coated by cationic surfactant demonstrated great benefits for fast anion analysis [10–12]. Some silica-based anion columns with relative high efficiency have been commercial available and have found lots of specialized applications [13].

The problems associated with the common preparation method of silica-based ion exchanger are 1, difficult to control the content of the functional groups introduced onto the silica surface, always leading to extremely high column capacity. 2, unwanted effect from the residual silanol groups onto the silica surface, then leading to significant nonspecific interactions. In addition, the final prepared

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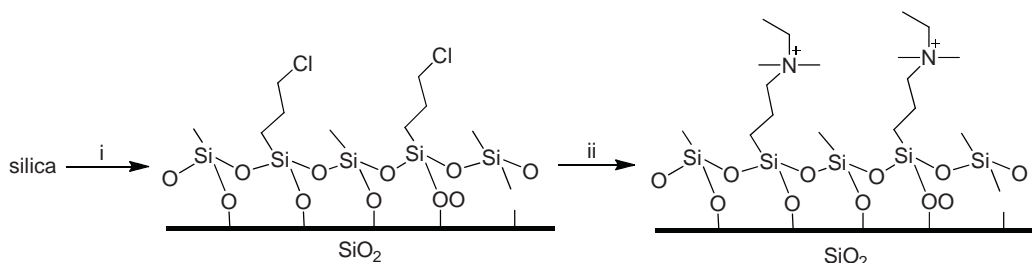


Fig. 1. Synthesis scheme for the preparation of PCP-SAX.

stationary phase with more hydrophilic characteristic would be favorable since it is beneficial to the effective elution in the ion exchange process.

In this study, we attempted to seek another route to the synthesis of a silica-based strong anion exchanger (SAX) based on polar-copolymerized approach [14]. This is an extension of our recent work in which a silica-based stationary phase for high performance liquid chromatography was prepared via polar-copolymerized approach [14]. Methyltrichlorosilane and 3-chloropropyltrichlorosilane has been copolymerized onto the surface of silica gel by using horizontal polymerization technique [15–17], followed by introducing quaternary ammonium functional groups. The effective ion exchange capacity could be easily controlled by adjusting the ratio of silanes, and the unwanted residual silanol groups could be effectively shielded by methyl group as well. The effective separation of common inorganic anions has been demonstrated and ion exchange mechanism was observed to dominate the separation process.

2. Experimental

2.1. Apparatus

A Waters HPLC system consisting of a 515 pump, a 20 μ L Rheodyne injector, and a 2487-UV absorbance detector was used to perform the chromatographic experiments. In some cases, an IC equipment (Techcomp Corp. Shanghai, China) was also used. Unless otherwise stated, the results were achieved at 30 °C with the flow rate of 1 mL/min. Solid state ¹³C CP/MAS NMR characterization was performed on a Bruker (USA) DSX 300 NMR Spectrometer (300 MHz, 7.0 T). Elemental analysis was measured on a Vario EL III elemental analysis system (Elementar, Germany). The void time was measured from the eluent signal in the UV detection. The plate numbers, N (N/m) of the separation to the tested anions were calculated from the equation $N = 5.54(t_r/W_{1/2})$ where t_r is the net retention time of the analyte and $W_{1/2}$ is the peak width at half peak height.

2.2. Synthesis of silica-based strong anion exchanger

A schematic diagram of the synthetic approach for the preparation of silica anion exchanger was outlined in Fig. 1. Firstly, silica gel (Fuji Silysia, 5 μ m particle size; 10 nm pore size; 315 m² g⁻¹ surface area) is silanized by the copolymerization between methyltrichlorosilane and 3-chloropropyltrichlorosilane to yield 3-chloropropyl silica. The pre-silanization procedures of the original silica particle were similar to our previous work [14]. After humidification, 10 g of silica was placed in a flask under a blanket of nitrogen, along with a stirring bar and 30 mL of anhydrous toluene. A solution of 1.2 mL of methyltrichlorosilane, 3 mL of 3-chloropropyl trichlorosilane and 20 mL of toluene was added to the pretreated silica gel. The reaction was allowed to continue for 24 h. The silanized silica was filtered and washed with dichloromethane,

methanol, water and methanol with 20 mL of each solvent successively. The silanized silica was dried at 80 °C overnight. Then 10 g of the above 3-chloropropyl silica was treated by 10 mL of N,N-dimethylethanamine in DMF at 80 °C for 24 h to produce positively charged groups on the surface of the silica particles. Stainless steel column (4.6 mm I.D. \times 250 mm) was packed in common slurry method, in which the column was upward packed using slurries of the stationary phase in methanol. 40 MPa packing pressure was used and methanol was also used as the propulsive solvent. In this study, the polar-copolymerized strong anion exchanger column was named as PCP-SAX.

2.3. Measurement of effective ion-exchange capacity of PCP-SAX column

The effective ion exchange capacity of silica-based PCP-SAX column was roughly estimated through the breakthrough method [10]. Briefly, the column was completely flushed with 50 mM NaCl eluent for 2 h at 1 mL/min and then flushed with water for 0.5 h at 1 mL/min, finally flushed with 5 mM NaNO₃ eluent at 0.4 mL/min and at the same time the effluent was monitored by UV absorbance detector operated at 210 nm until the breakthrough curve was achieved. The column capacity (Q) could then be calculated by the equation of $Q = CF(t_b - t_0)$. In the equation, C is the concentration of NaNO₃ eluent (mol/L), F is the flow rate of NaNO₃ eluent (mL/min), t_b is the time for complete breakthrough of NaNO₃ (min) and t_0 is the void time of the column (min).

2.4. Preparation of saliva sample

A 1.02-g amount of saliva drawn from a healthy male volunteer was diluted in 10 mL of ultrapure water by using a volumetric flask, and centrifuged at 3000 rpm for 5 min, followed by filtration with a 0.45- μ m membrane filter. The saliva sample was then stored in a refrigerator.

3. Results and discussion

3.1. Synthesis and characterization

The synthesis scheme of PCP-SAX was shown in Fig. 1. The copolymerization between 3-chloropropyl trichlorosilane and methyl trichlorosilane onto the surface of silica gel was designed to control the surface coverage of reactive chlorine groups and at the same time to increase the stability of the resulting stationary phase. Chloropropyl group was used to be changed into the quaternary ammonium group in the following reaction. Methyl groups introduced served as linkers of the horizontal chloropropyl groups and also covered the silanols of the silica gel surface to a great extent. The column capacity could be easily controlled easily by adjusting the coverage amount of chloropropyl group introduced, and then controlling the final amount of quaternary ammonium charged groups. In addition, the unwanted interaction from resid-

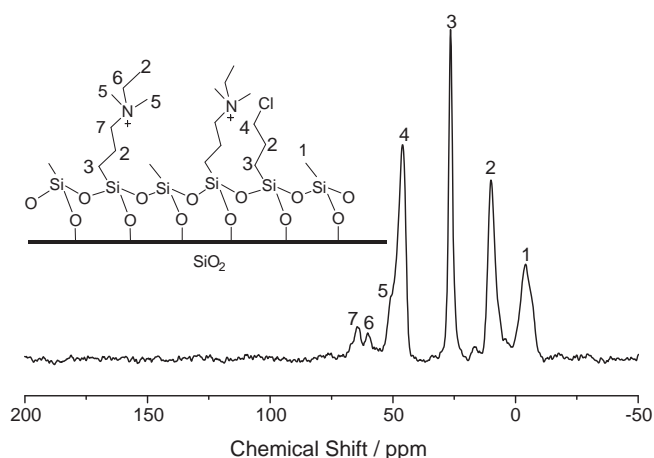


Fig. 2. ^{13}C -CP/MAS NMR spectra of PCP-SAX.

ual silanol groups could also be alleviated owing to the formation of thin layer of horizontal polymerization onto the silica surface.

Organic elemental analysis (EA) results indicated that the carbon and nitrogen content of the PCP-SAX was 5.85% and 0.2%, respectively. The apparent anion exchange capacity determined from the average nitrogen percentage obtained by elemental analysis was $\sim 0.14 \text{ mmol/g}$. While for the tested column (4.6 mm I.D. \times 250 mm long), its effective ion exchange capacity was measured to be $51.4 \text{ } \mu\text{eq/column}$ ($2.056 \text{ } \mu\text{eq/cm}$) referring to the above breakthrough method.

The ^{13}C CP/MAS NMR spectroscopy was used to investigate the bonded ligands onto the silica surface. As shown in Fig. 2, the resonance at 51.5, 60.2 and 64.5 ppm (peak 5, 6, and 7) relates to the carbon atoms attached to the ammonium group, which confirmed the presence of anion exchange groups bonded on the silica surface. The peaks at 4.1 ppm (peak 1) are assigned to the carbon atoms of copolymerized methylsilane. And the resonance at 10.0, 26.5 and 46.0 ppm relates to the other atoms on the propyl groups. The ^{13}C CP/MAS NMR spectrum proves the chemistry of the PCP-SAX.

The prepared column was checked to have good physical stability, as proved by the following experiment. Pure water flowed through the column driven by pump and the pressure drop across the column was measured as a function of the flow rate. In the tested range of 0.5–2 mL/min, the pressure drop of the column up to $\sim 150 \text{ bar}$ was linearly related with the flow rate with the correlation coefficient of 0.9998. This indicated that the exchanger was not damaged during the preparation process and the original mechanical stability of silica particles was well kept.

3.2. Separation mechanism

The ion exchange mechanism could be confirmed by checking the change of the retention time of analytes with varying the eluent concentration. It is well known that plots of the logarithm of the retention factor ($\log k'$) of analyte ions versus the logarithm of the eluent concentration are linear if the separation mechanism is based on ion exchange. The separation of common several inorganic anions on PCP-SAX column was carried out using NaCl eluent. The effect of NaCl concentration on the retention time of analytes was demonstrated in Fig. 3. It can be seen that the retention times of several anions decreased with the increase of NaCl concentration. The plots of $\log k'$ versus $\log [\text{NaCl}]$ showed good linear relationship for all anions tested except IO_3^- . (It is probably due to the less retained property of IO_3^- on the column). The slopes were -1.340 , -1.115 , -1.103 , -1.081 , and -1.007 for IO_3^- , BrO_3^- , NO_2^- , NO_3^- , and I^- , respectively, which are well agreement with the theoretical

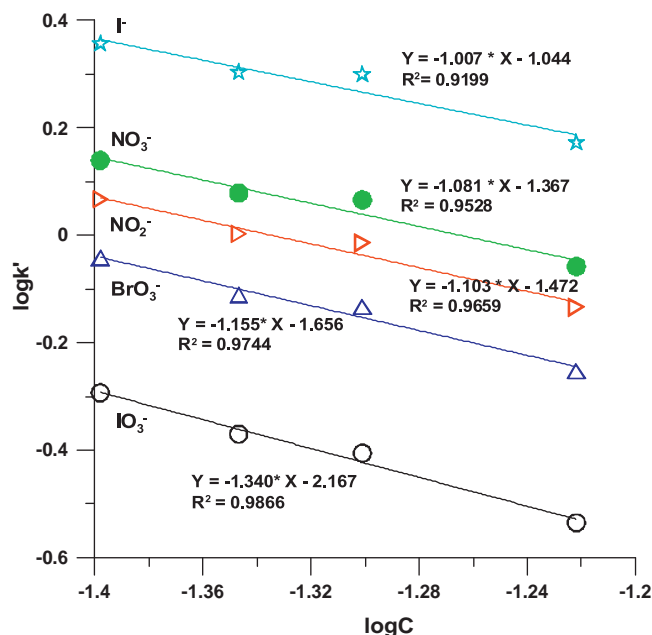


Fig. 3. Logarithm of the retention factor as a function of sodium chloride concentration. Conditions, PCP-SAX column, 4.6 mm I.D. \times 250 mm long (5 μm dia.); eluent, NaCl; analytes, 0.1 mM of IO_3^- , BrO_3^- , NO_2^- , NO_3^- , I^- ; column temperature, 30°C ; Injection volume, 20 μL ; flow rate: 1 mL/min; UV detection: 210 nm.

slope of -1.0 for anion-exchange elution of a singly charged anion (e.g. NO_3^-) with a singly charged eluent anion (Cl^-).

3.3. Temperature effect

The effect of temperature on the chromatographic separation has been paid much more attention in HPLC or in IC in recent years. The column operated in elevated temperature would enhance the kinetic and transport property of the analytes due to the decrease of the viscosity of eluent and the increase of the analyte diffusivity. Here, the effect of temperature on the performance of PCP-SAX column was also explored, as shown in Fig. 4. In the tested range of 30 – 80°C , good linear relationship between $\ln k'$ and reciprocal absolute temperature existed for all the tested analytes. For each plot, the standard enthalpy and entropy of the analyte transferring from the eluent to the exchanger could be calculated from the slope and the intercept, respectively.

An obvious advantage of elevating column temperature is the decrease of column backpressure. This was also confirmed in our experiment. When the column temperature increased from 30°C to 80°C , the pressure drop across the column decreased from 1041 bar to 660 bar, $\sim 36.6\%$ was reduced. Good linearity existed for the plot between the column backpressure and the column temperature ($R^2 = 0.9928$) in the tested temperature range. In addition, the effect of column temperature on the separation efficiency was also explored. The plate numbers of several tested analytes increased from 30 to 50°C while decreased when the temperature was over than 50°C , indicating that the optimal temperature was $\sim 50^\circ\text{C}$.

3.4. Performance evaluation of PCP-SAX column

The performance of PCP-SAX column was evaluated by the separation of the several common inorganic anions. To match the requirement of UV absorbance detector, the anions with strong UV adsorption were chosen. Fig. 5 showed a typical chromatogram. All the tested anions could be well separated using 30 mM NaCl eluent at the flow rate of 1 mL/min with high separation effi-

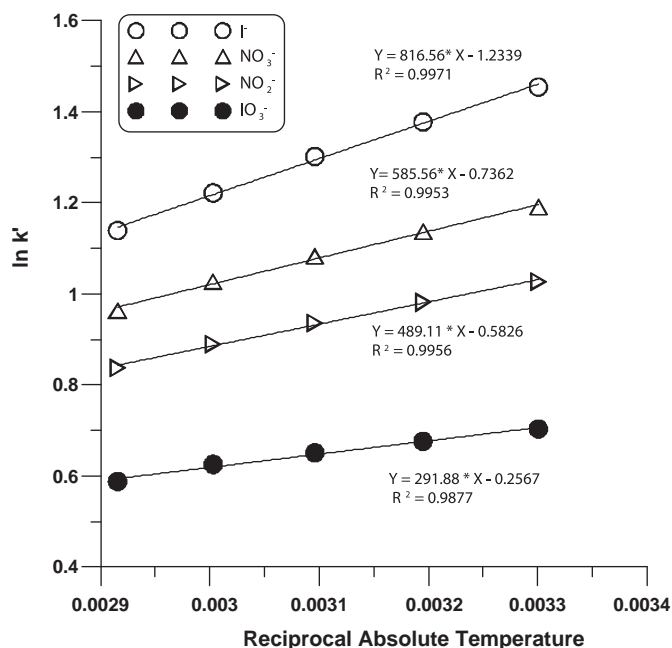


Fig. 4. Van't Hoff plots for inorganic anions separated on PCP-SAX column. Conditions, eluent, K_2SO_4 , 1 mM; analytes, 0.1 mM each of IO_3^- , NO_2^- , NO_3^- , I^- ; Other conditions same to Fig. 3.

ciency. Take NO_2^- as examples, the plate number was 80,000/m. The column showed similar selectivity to that of common PS-DVB polymer-based strong anion exchanger. It is known that t iodide and thiocyanate ions have strong retention on commercial PS-DVB anion exchanger and gradient mode is often required to make it be eluted in a suitable time. Obviously less retention of iodide facilitates its fast analysis on this PCP-SAX column. The tested anions mixture was well separated on the PCP-SAX column and the achieved peak heights of analytes were well in agreement with

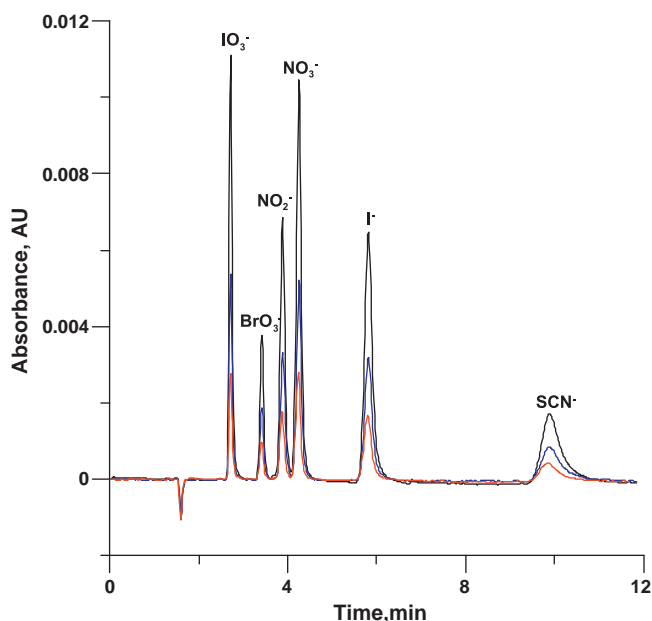


Fig. 5. Typical chromatogram of the analysis of inorganic anions. Conditions: NaCl eluent, 30 mM; analyte concentrations vary from upper to lower level: top, 0.05 mM each of IO_3^- , BrO_3^- , I^- , SCN^- and 0.025 mM each of NO_2^- , NO_3^- ; middle, 0.025 mM each of IO_3^- , BrO_3^- , I^- , SCN^- and 0.0125 mM each of NO_2^- , NO_3^- ; low, 0.0125 mM each of IO_3^- , BrO_3^- , I^- , SCN^- and 0.00625 mM each of NO_2^- , NO_3^- ; other conditions same to Fig. 3.

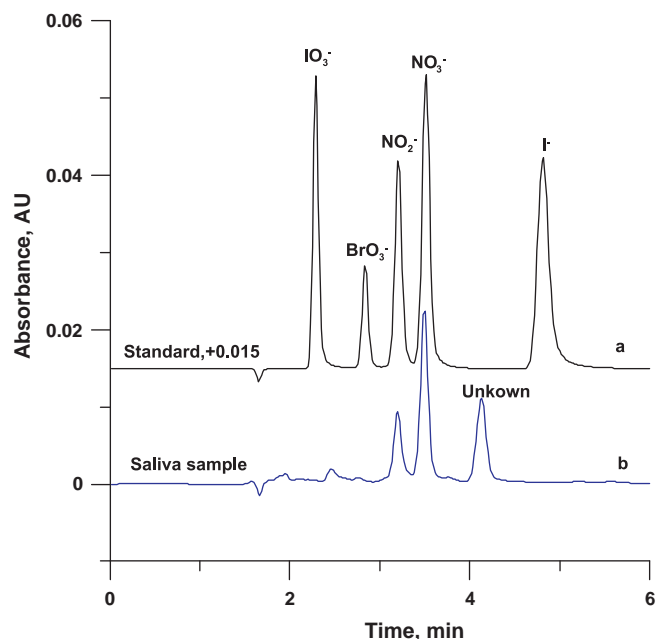


Fig. 6. Separation of inorganic anions contained in saliva sample. Conditions: analytes, 0.15 mM each of IO_3^- , BrO_3^- , I^- and 0.075 mM each of NO_2^- , NO_3^- ; NaCl eluent, 50 mM; other conditions same to Fig. 3.

their concentration. We did not check the separation of divalent anions such as phosphate and sulfate because they do not have UV absorption. Run-to-run reproducibility of the column was measured by the analysis of anions under isocratic mode. The relative standard deviation (RSD) of retention time and peak height over 10 consecutive analyses was 0.38 and 1.7%, respectively. Day-to-day reproducibility was also checked and RSD of retention times in three days was found to be less than 4%. In addition, the suitable pH working range of 2–8 is recommended for the column, similar to that of silica gel itself.

To demonstrate the utility of the silica-based anion column, the determination of inorganic UV-absorbing anions contained in a saliva sample was performed on the PCP-SAX column, as shown in Fig. 6. The saliva sample was taken from a healthy male volunteer. The concentration of nitrite and nitrate in the saliva was determined to be 1.45 and 2.93 mg/L, respectively.

4. Conclusions

A novel silica-based anion exchanger for IC has been prepared and evaluated. The exchanger demonstrated good hydrophilic property due to its very simple function groups onto the silica gel. By this polar-copolymerized method, the capacity could be easily controlled and at the same time the unwanted residual silanol effect could be reduced to a great extent. Effective separation of common anions can be achieved and the ion exchange mechanism dominates the separation process. It is believed that the column would find some applications for the separation of common inorganic anions.

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