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Preparation and evaluation of anion exchange open tubular column

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ABSTRACT

An anion open tubular column (OTC) by coating cationic latex particle onto the silica capillary with surface sulfonate functionalized has been prepared and evaluated. The performance of the OTC was observed to be superior to that of those prepared by the bare silica capillary without sulfonation in term of separation efficiency. To increase the surface area of the capillary, acid and base-based etching method for treating silica capillary wall was carried out and compared. An OTC with multiple layers was prepared by alternatively coating cationic and anionic latex particles aiming to further increase the column capacity. The anion OTC with size of 50 μm i.d. × 1 m long demonstrated good separation of common inorganic anions with high efficiency (e.g. for NO_2^-, its theory plate number is 11655 plate/m).

1. Introduction

Since introduced in 1975 by Small, ion chromatography (IC) has been a well-established technique for the analysis of ionic species[1]. As a branch of IC, open tubular IC (OTIC), in which very thin layer of a liquid or active solid held on the capillary inner wall acts as the stationary phase and the rest of capillary bore is open, dates back to the early 1970s and now is still confined to research laboratories due both to theoretical and practical limitations [2,3]. Since 1980s, much attention have been paid to OTIC due to their some desirable features such as high efficiency, fast analysis and extremely low operation pressure required.

Open tubular column (OTC) is one of the critical components for OTIC. Knox et al. pointed out that the optimal capillary inner diameter should be 0.26 μm to fully demonstrate the merits of OTC [4]. Regardless of the availability of such capillary at that time, other related components (e.g. micropump, injector and detector) are also the limiting factors even to the present. Thus the development of the OTCs with bigger bore would be more practical, which makes the preparation and running process much easier and also make the demanding requirements for other components less. Ishii and Takeuchi pioneered the preparation of cation OTC for IC by coating phenyltriethoxysilane into drawn soda-lime glass capillaries with 40 μm diameter [3]. The column demonstrated effective separation of nucleosides while no application for small inorganic cations was provided. By bonding 3-sulfopropylsilane and 3-(2-aminoethyl-amino)-propylsilane onto the fused silica capillary, Muller et al. have prepared cation and anion OTC with inner diameter of 4.6 μm, respectively, which demonstrated fast separations of inorganic ions [5,6]. It should be noted that the preparation process of such kinds of OTC was very tedious, e.g. 2–6 weeks was needed for the columns that Miller reported [5,6].

Relative to packed capillary column, a common drawback is inadequate column capacity. To address this, a typical way is to increase the surface area of the capillary by suitable treatment method. HF–HNO_3 and NaOH etching are widely used for treating the silica capillary [2,5–7] prior to column preparation. Although some reports claimed that either way could effectively increase the surface area, no report presented a clear comparison of these two methods, thus it needs to check which one is better? The other way to increase the column capacity is the use of multiple layers of stationary phase. Petr et al. presented anion OTC by the condensation polymerization reaction between diepoxide and primary amine, producing a branched, tree-like structure stationary phase in multiple layers [7]. The column capacity was observed to increase with the increase of coating layers and a OTC with size of 75 μm i.d. × 5 m long (25 layers) demonstrated effective separation of common inorganic anions with the theoretical plates ranging from 7000 to 14,000. In their subsequent work, a cation OTC with multiple layers coating was prepared by thermally copolymering poly(butadiene-maleic acid) [8]. The column capacity was found to successively increase with the number of coated layers. By alternatively coating anion and cation polyelectrolyte, Petr et al. also prepared another kind of anion OTCs [9]. Effective separation of inorganic anions by the OTC was achieved by sodium benzoate in less than 35 min with theoretical plates ranging from 2000 to 9000. More recently, Huang et al.

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presented a multilayered anion OTC fabricated by alternately coating chitosan and glutaraldehyde [10]. For a 4.5 m long × 75 μm OTC with 24 successive layers, effective separation of several inorganic anions with average theoretical plates of 12,000 was achieved. Fabrication of OTCs with multiple layers coating is a time-consuming procedure.

Charged nanometer latex particles have been widely used in the preparation of common ion exchangers [11,12]. There are plenty of ion exchange sites on the particle surface, which is helpful to the increase of the column capacity. In addition, since ion exchange process only occurs on the particle surface attached onto the capillary wall, it helps to get high separation efficiency. Such concept that agglomerated functionalized latex particle-based ion exchangers have been widely used in conventional IC system. Relative to above complicated chemically bonding method, it would be a simple way to prepare OTCs by dynamically coating the capillary with charged latex particle solution [13,14]. Pyo et al. firstly prepared an anion OTC by coating anion charged latex via the electrostatic binding with the oppositely charged surface of fused silica capillary ($-SiO^-$) [13]. The column showed much limited separation ability for four inorganic anions, though so much long column (6 m length × 50 μm i.d.) was used. Zhang et al. prepared a dual-layer latex-based OTC for capillary electrochromatography (CEC), in which cationic latex particles and anionic latex was used to alternatively coat the silica capillary [14]. The column was used for the separation of inorganic cations operated under CEC mode. It is known that the silanol groups existing on the fused silica capillary wall are an extremely weak acid, then leading to weak interaction with the latex particle. This is especially true in acidic eluent. Stripping of latex particle will of course lead to the column damage and poor retention reproducibility [7]. The use of silica capillary with strong acid functional groups (such as sulfonate or carboxylate) would potentially enhance the interaction with the charged latex particles.

Here we present an efficient anion OTC prepared by coating one or multiple layers of anion latex particles onto the silica capillary with surface sulfonate functionalized. Such kinds of OTCs were observed to be superior to that of without surface sulfonation in term of separation efficiency. The comparison of acid and base-based etching methods was investigated via the estimation of introduced sulfonate groups. The anion OTC with the size of 50 μm i.d. × 1 m long demonstrated good separation of common inorganic anions.

2. Experimental

2.1. Chemicals and materials

The chemicals were of analytical grade and used without purification. Sodium hydroxide, hydrochloric acid, toluene, hydrogen peroxide (30% aqueous solution), hydrofluoric acid, ethylenediamine tetraacetate acid disodium (Na_2EDTA), sulfuric acid, nitric acid, were all purchased from Lingfeng Chemical Corp. (Shanghai, China). (3-Mercaptopropyl)trimethoxysilane (3-MPTS) was obtained from silicone new material Corp. (Wuhan, China). Anion standards were courtesy provided by Tosh Corp. Both latex solution including AS18 latex (65 nm diameter, $-R_4N^+$ functionalized) and CS5A latex (140 nm diameter, $-SO_3^-$ functionalized) were obtained from Thermo Fisher Corp. Inc. Milli-Q ultrapure water (Milford, USA) was used throughout for solution preparation. Fused silica capillary was from Yongnian Corp. (Hebei, China).

2.2. Chromatographic system

The experiment was performed on a home-built setup which was described previously [15,16]. Briefly, a syringe pump (LSP01-1A, Baoding, China) was used to provide a constant flow rate required. A 100-nL internal injector (www.vici.com) was for injecting sample. In addition, a 4 nL internal injector from VICI Corp. was also used in some cases. Anion OTC was directly connected with the injector, aiming to reduce the extra-column dispersion as soon as possible. The conductivity of the column effluent was monitored by a capacitively coupled contactless conductivity detector (TraceDec, http://www.istech.at).

2.3. Preparation of anion OTC

Unless otherwise stated, the size of OTCs was 50 μm i.d. × 365 μm o.d. × 1 m long. The preparation procedures can be briefly summarized as three steps, capillary wall pretreatment, surface silanization and latex coating. Note: for the first two steps, much longer bare capillary could be treated for stockage.

(i) Capillary wall pretreatment. In order to increase the surface area and enhance the adhesion of the final stationary phase on the capillary surface, suitable pretreatment to the fused silica capillary is necessary. $HF–HNO_3$ and NaOH etching methods are widely used for the pretreatment of silica capillary. Here both manners were compared by measuring the introduced sulfonate numbers relative to bare capillary without special pretreatment. (1) $HF–HNO_3$ etching manner. The pretreatment procedure was same to that of previous report [8]. Briefly, the fused silica capillary was firstly rinsed with a mixture of HF and HNO_3 (2.5% v/v each) for 30 min, and then sequentially washed by 1% v/v HCl for 30 min, DI water to neutrality and then acetone for 30 min, finally dried by flowing nitrogen. (2) NaOH etching manner. The capillary was filled with 1 M NaOH for 2 h, followed by washing with DI water to neutrality, 0.1 M HCl for 1 h, then DI water again to neutrality and acetone for 30 min, respectively. At last the capillary was dried by flowing nitrogen. To facilitate the comparison, a bare fused silica capillary without any treatment was used for next step. Note: the fused silica capillaries in above three cases have the same size. Each case, three capillaries were tested in parallel.

(ii) Surface silanization. This step was basically similar to the previous report [10]. Briefly, the above pretreated capillary was filled with 10% 3-MPTS in toluene and then sealed at both ends with rubber septa, then heated to 110 °C for 24 h. In this process, thiol groups were introduced onto the capillary surface. After this, the capillary was sequentially rinsed with toluene, ethanol, DI water each for 30 min. At last, the thiol groups were oxidized to sulfonate groups by flushing 30% H_2O_2 aqueous solution for 20 h, followed by washing with DI water until neutrality. The synthesized route was shown in Fig. 1.

(iii) Latex coating. 10x dilute AS18 cationic latex particles solution was pneumatically driven to flush the treated capillary above at the flow rate of 10 μL/min for 1 h, followed by rinsing the capillary with pure water for at least 2 h. Then an anion OTC with one layer of AS18 latex was obtained. For coating two layers of AS18 latex, the prepared anion OTC was then flushed by 10x dilute CS5A anionic latex particles solution for 1 h. In this process, the anionic CS5A latex would be bounded onto the surface of cationic AS18 latex via electrostatic interaction. Then the capillary was rinsed by pure water for 1 h to remove the unbounded CS5A latex particles, followed by flushing 10x dilute AS18 latex particles solution for 1 h and then pure water for at least 2 h, and an anion OTC with two layers of AS18 latex could be achieved (SEM micrographs of the cross section of the column was provided in the support information). If more layers are needed, repeat the above coating procedure.

For comparison, anion OTC was prepared according to above procedure, except that no sulfonation treatment was performed.

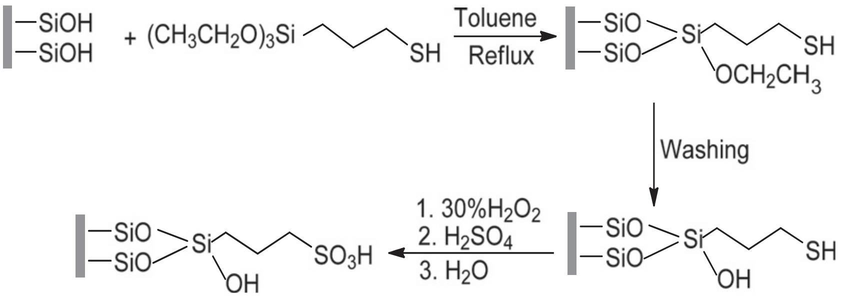

Fig. 1. Schematic diagram of sulfonation route of the capillary wall.

2.4. Estimation of –SO$_3^-$ capacity of the capillary surface

To evaluate the pretreatment effect of each manner, the $-$SO$_3^-$ numbers of the capillary surface were measured. Prior to surface silanization, a segment of the silica capillary (50 μm i.d. × 375 μm o.d. × 1 m long) was treated by acid or base. A segment of silica capillary was used to be reference, in which no acid or base pretreatment was employed and direct surface silanization was made. The capillary under different pretreatment manners was rinsed with 5 mM aqueous CuSO$_4$ solution for 2 h, followed by pure water washing for 30 h to remove any unbounded CuSO$_4$. Next, the capillary was flushed with 10 mM Na$_2$EDTA for 2 h to completely release the bounded Cu^{2+}, and at the same time the effluent was collected with a small vial. The collected solution was diluted to be 10 mL and then measured by inductively coupled plasma atomic emission spectroscopy (ICP) (Vanan710 Varian INC., USA). The Cu^{2+} amount released from the capillary wall was calculated according to the calibration curve made from CuSO$_4$ solutions with a given concentration.

3. Results and discussion

3.1. Effect of pretreatment manner

As mentioned above, an obvious drawback of OTC is inadequate capacity due to much limited surface area. Among many methods to address this, the pretreatment of the capillary wall is a simple and direct way. Actually, it is always a necessary step to greatly increase the surface area of the capillary, leading to more active sites to absorb or immobilize the stationary phase and thus higher capacity. Acid etching and base etching pretreatment manners are widely used in the majority of the published papers related with OTC [2,5–7,10,17]. Though some of authors claimed that the acid or base pretreatment could effectively increase the surface area of the capillary, there was no direct comparison of these two manners. Here we have made attempts to explore which one would be a better choice. The capillaries (50 μm i.d. × 1 m long) were treated according to the above procedures and the introduced $-$SO$_3^-$ numbers by silanization reaction on the capillary wall could be calculated by the measurement the amount of released Cu^{2+} mentioned above. The results were given in Table 1. For the bare fused silica capillary, the calculated $-$SO$_3^-$ numbers per nm^2 were ~5.6 × E14, corresponding to ~3.6 SO$_3^-$/nm^2. This value is in accordance with the known value of $-$SiOH numbers (4–8/nm^2) existing in the surface of silica or glass capillary [3]. It can be seen that both pretreatment manners could effectively enhance the surface area, indicated by much more $-$SO$_3^-$ numbers. Relative to the capillary without treatment, ~3.7-fold and ~40-fold enhancement for the introduced SO$_3^-$ numbers were obtained for acid and base treatment, respectively. Obviously, base etching manner is much more effective, almost 10-fold higher than that of acid treatment manner. This finding would provide a useful clue for pretreatment of silica capillary.

Table 1
Effect of treatment manner on the introduced SO$_3^-$ numbers in the capillary wall.

Type	Capillary treatment manner		
	HF/HNO$_3$	NaOH	No treatment
Calibrated Cu^{2+} concentration(μM)	0.17	1.88	0.047
SO$_3^-$ group numbers [a] (/nm^2)	13.2	143.8	3.6
Enhancement factor [b]	3.7	40	1.0

[a] SO$_3^-$ numbers per nm^2 on the surface of fused silica capillary was calculated from the data of the released Cu^{2+}—containing solution (10 mL).

[b] Enhancement factor was defined as the reference of the data calculated for the manner without treatment.

3.2. Effect of surface sulfonation of silica capillary

Fused silica capillary has been widely used for preparing OTCs. The flexible and inert characteristics of silica capillary are helpful to prepare column. Silanols in the surface of silica capillary renders easily the introduction of cationic nanometer latex particles through electrostatic attraction. While the silanol groups are not a good one to bind the cationic latex particles because silanol is an extremely weak acid, then easily leading to the stripping off the particles. From this point, the use of relative strong acid group such as carboxylate or sulfonate sites instead of silanol would be helpful to enhance the interaction with cationic latex particles. Based on these considerations, the silica capillary wall was firstly treated to introduce the sulfonate groups prior to coating the AS18 latex. The effect of sulfonation on the separation was shown in Fig. 2. The column prepared via the common way in which no sulfonation treatment was performed demonstrated much poor separation effect. By comparison, the sulfonation column demonstrated much better separation effect. The efficiency achieved by the column with size of 50 μm i.d. × 1 m long was even better than that of the column of 50 μm i.d. × 5 m long without sulfonation treatment [13]. Presently we do not know the clear reason of such significant effect of the introduced sulfonate groups on the separation. It probably results from much stronger interaction between latex particle with $-$R$_4$N$^+$ groups and $-$SO$_3$ groups existing in the capillary surface.

3.3. Characterization of anion OTC

A typical chromatogram for separating several common inorganic anions including F$^-$, Cl$^-$, NO$_2^-$, Br$^-$, NO$_3^-$, was provided in Fig. 3. The tested anions were well separated except F$^-$. And the theory plate number (N) for Cl$^-$, NO$_2^-$, Br$^-$, NO$_3^-$ was 11629, 11655, 10004, and 9516, respectively. F$^-$, a much less retained analyte on common anion exchanger, could not effectively be separated from water dip on

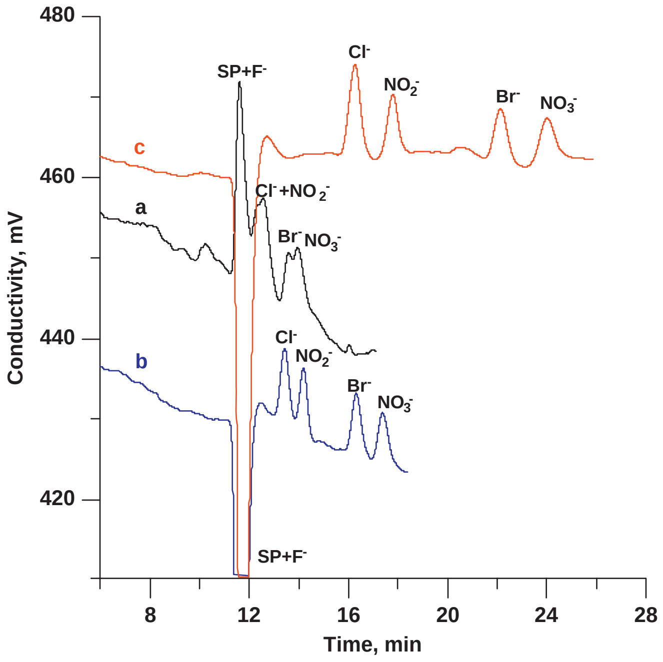

Fig. 2. Effect of the sulfonation treatment of silica capillary on separation. Conditions: column: 50 μm i.d. × 1 m long; eluent: 1 mM sodium benzoate; flow rate: 0.16 μL/min; injection volume: 100 nL injector with ∼10:1 split ratio; sample concentration: 0.1 mM. (a) Without sulfonation. (b) Sulfonation treatment with one layer AS18 latex. (c) Sulfonation treatment with two layers AS18 latex.

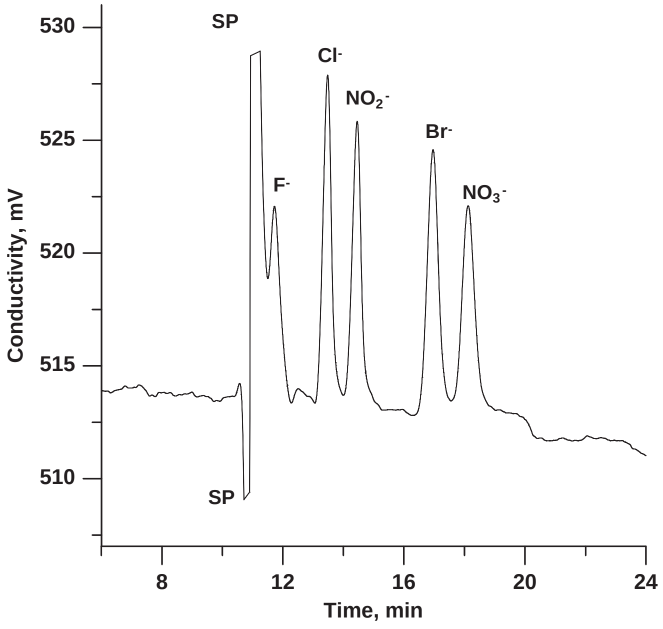

Fig. 3. Separation of five common anions on OTC. Conditions: column (one layer of latex): 1 m long × 50 μm i.d.; eluent: 1 mM sodium benzoate; flow rate: 0.18 μL/min; injection volume: 4 nL; sample concentration: 0.2 mM.

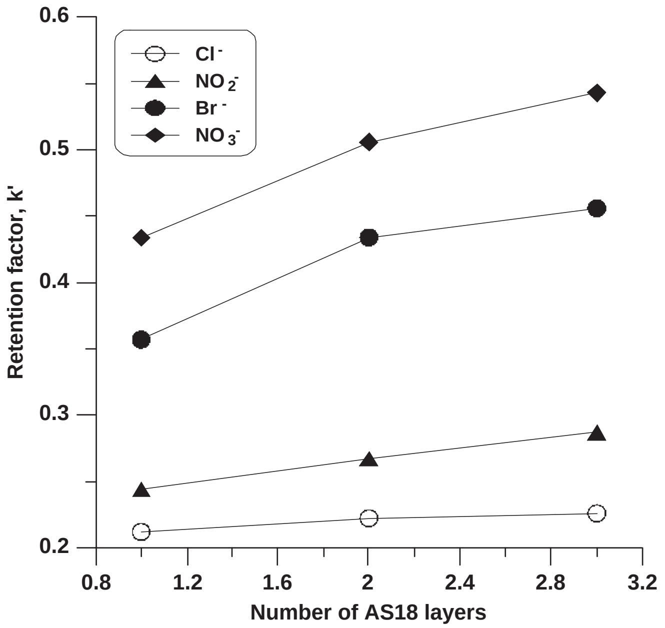

Fig. 4. Effect of the numbers of AS layer on the retention factor (conditions same as Fig. 2).

the OTC, which mainly resulted from the still-limited column capacity. As mentioned above, a common way to increase the column capacity is the use of two layer coating or more [14]. Here such a way was also used to improve the column capacity by the use of two layers of OTC. The results were demonstrated in Fig. 2b and c. The retention times of four anions on the OTC with two layers latex were obviously enhanced compared to those on the OTC with one layer latex, from 1.21 to 1.38-fold enhancement of retention times for Cl^-, NO_2^-, Br^-, and NO_3^- was achieved. To further increase the column capacity, the preparation of OTCs with multiple layers of AS18 latex particles was tried. The effect of the numbers of AS18 layers on the retention of the analytes was shown in Fig. 4. Obviously, the retention factors of all the tested analytes were increased with the increase of the number of AS18 layers. Such trend is more obvious for the strong retained analytes. Although the use of multiple layers of AS18 latex coating could significantly increase the column capacity, their long-term running stability was observed to be poor, indicated by the decrease of the retention times of the tested analytes during continuous running process. This probably resulted from the stripping of the coated particles. Generally the column with two layer AS18 latex particles demonstrated acceptable stability, proven by the fact that there was no appreciable change on the column efficiencies after intermittent use for 1 week. In addition, the running reproducibility of retention time was determined by separating a mixture of four inorganic anions. The relative standard deviation (RSD) of retention time for seven consecutive injections was less than 1.0%, indicating between run reproducibility well. In addition, column-to-column reproducibility for three batches was also evaluated and RSD of the retention times of above analytes were in the range of 3.4–5.7%.

For the anion OTCs with multiple AS18 latex layers (e.g. ≥ 2), a potential application is for simultaneous separation of anions and cations since cation exchange groups from CS5A anionic latex exist, as observed by the anion OTCs prepared by alternatively coating anion and cation polyelectrolyte [9]. While the data indicated that such anion OTCs had no separation ability for the cations (data not shown). Perhaps the cations could not penetrate into the below CS5A latex layer through the AS18 layer (with $-R_4N^+$ functional groups) due to Donnan exclusion.

In summary, we have described a simple way to prepare fused silica-based anion exchange OTC. The sulfonation of silica capillary was found to be helpful to the increase of the separation efficiency. Such strategy will be extended the development of other polymer-based capillary with sulfonate or carboxylate groups, which will greatly simplify the preparation procedure. In addition, NaOH etching manner to the silica capillary was found to be more effective for increasing the surface area of the capillary relative to acid etching manner, which will provide a clue for improvement of surface area of silica capillary.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2012.08.036.

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