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Ion chromatography on anion exchangers modified with mucopolysaccharides

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Abstract

Anion exchangers modified with mucopolysaccharides, such as chondroitin sulfates and heparin, were used for the stationary phase in ion chromatography. Unusual retention behavior of anions was observed for the modified stationary phases. A 50-μ*M* concentration of tartaric acid could separate inorganic anions in a reasonable time. The retention of analytes could be changed by changing the eluent composition. © 1998 Elsevier Science B.V.

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1. Introduction

Various types of ion exchangers have been developed for ion chromatography [1,2] since its introduction [3]. Surface-functionalized ion exchangers are superior to totally porous packing materials in terms of mass transfer resistance [4–6]. Pellicular-type or latex-agglomerated ion exchangers are designed so that analyte ions can interact with the functional groups on the surface of the substrate. An anionic, surface-sulfonated core particle can attract and bind cationic aminated latex particles with diameters of 0.1 μm, and the resultant material works as an anion exchanger. A latex-agglomerated cation-exchanger can be produced by using a sulfonated core particle, which is coated firstly with an aminated latex and secondly with a sulfonated latex.

Ionic polymers are also used for the modification of ion exchangers. Polymer-coated silica-based cation exchangers for ion chromatography can be synthesized by depositing pre-polymers of varying film thickness by in-situ crosslinking reactions using radical initiators or γ-radiation [7,8]. Poly(butadiene–maleic acid) is an anionic polymer and can be used to separate inorganic cations using weak acid eluents.

Nafion, a cation-exchange material, can be coated onto an octadecylsilyl reversed-phase column by hydrophobic interaction [9].

In preliminary work [10], we reported that a silica-based anion-exchange column modified with chondroitin sulfate C achieved unusual retention behavior; the retention of anions slightly decreased with decreasing eluent concentration. It is well-known that chondroitin sulfate is a mucopolysaccharide with various ionic groups and that its molecular mass is around $(4–10)\cdot10^4$.

Chondroitin sulfate contains sulfate and carboxyl groups, which are possibly strongly adsorbed to the charged surface by the strong ionic interaction with the cationic groups of the stationary phase. The

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hydroxyl and acetamide groups of the polysaccharides may also interact with the surface by hydrogen bridge bonding. The ion-exchange capacity of the stationary phase could, therefore, be changed, depending on the number of ion-exchange groups in the mucopolysaccharide skeleton and the thickness of the coating layer formed. The negative charges of the mucopolysaccharides can repel anionic analytes and, therefore, decrease the retention. We expected to obtain novel selectivities or retention behaviors by using stationary phases that possess both positive and negative charges.

This paper will describe in detail the retention behavior of anion exchangers modified with various mucopolysaccharides or other ionic compounds.

2. Experimental

2.1. Apparatus

The ion chromatograph was assembled from an 880-PU HPLC pump (Jasco, Tokyo, Japan), a 50× 4.6 mm I.D. column, a valve injector with an injection volume of 21-μl, an 870-UV detector (Jasco) and a CM-8000 conductivity detector (Tosoh, Tokyo, Japan), coupled in series, and a Chromatopac C-R4AX data processor (Shimadzu, Kyoto, Japan). Microcolumns (100×0.32 mm I.D.) were also operated by using a system comprising an MF-2 microfeeder (Azumadenki Kogyo, Tokyo, Japan) equipped with a 0.5-ml MS-GAN 050 gas-tight syringe (Ito, Fuji, Japan) as a pump, an ML-522 microvalve injector (Jasco) with an injection volume of 0.011 μl and a UVIDEC-100-V UV detector (Jasco). Size-exclusion chromatography was performed by using two TSKgel $GMPW_{XL}$ columns (Tosoh; each 300×7.8 mm I.D.) and 0.2 *M* sodium nitrate as the eluent.

The anion exchanger employed was TSKgel IC-Anion-SW, silica-based anion exchanger with an ion-exchange capacity of ca. 0.4 mequiv. g^{-1}. The molecular mass of completely excluded poly(ethyleneoxide) is $(5–6)\cdot10^4$ for the substrate of the packing.

2.2. Reagents

The reagents used were of guaranteed reagent grade and were obtained from Nacalai Tesque (Kyoto, Japan), unless otherwise noted. Purified water was prepared using a Milli-Q Plus system (Millipore, Molsheim, France). Sodium salts of chondroitin sulfates and heparin were from Nacalai Tesque. *N*-Acetylglucosamine 6-sulfate was from Sigma (St. Louis, MO, USA) and sodium tartrate was from Wako (Osaka, Japan). Sodium salts of poly(styrenesulfonate) with various weight-average molecular masses were from Tosoh. The eluents for the conventional liquid chromatography (LC) system were degassed in a vacuum under ultrasonic vibration before use.

2.3. Modification

An aqueous solution containing 0.1–1.0% (w/v) of each modifier was passed through the column for 2 h at a flow-rate of 1.0 ml min^{-1} for conventional-size columns and at 4.2 μl min^{-1} for microcolumns. The eluent was then passed through the modified column.

Fig. 1 illustrates the structures of the modifiers used in this work, whereas Table 1 compares the weight-average molecular masses (or molecular masses) of the modifiers.

3. Results and discussion

3.1. Effect of modifiers

As demonstrated in a previous study [10], the retention of anions was remarkably reduced when the IC-Anion-SW column was modified with chondroitin sulfate C. The negative charges of the modifier can repel anionic analytes and, therefore, decrease the retention time. The decrease in the retention time was also attributed to the decrease in the anion-exchange capacity, due to the modification. Fig. 2 shows the logarithm of the retention factor (k) versus the tartaric acid concentration, which was used as the eluent for the IC-Anion-SW column modified with 0.1% (w/v) chondroitin sulfate C. Unusual retention behavior can be seen in the figure; the retention factor decreased slightly with decreasing concentrations of tartaric acid. The unusual retention behavior is related to the composition and the concentration of the eluent. Repulsion is a function of

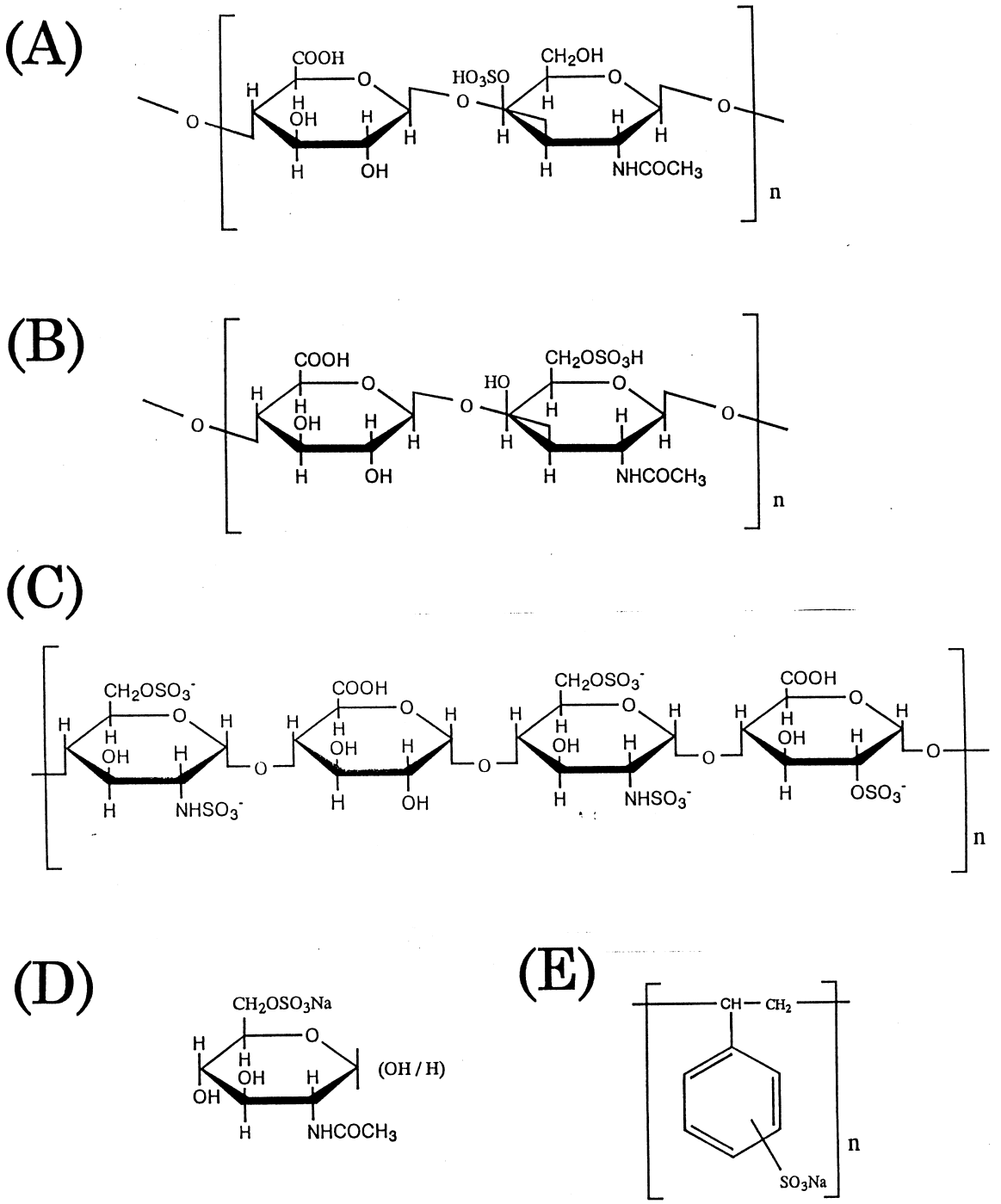

Fig. 1. Structures of the modifiers employed in this work. A=chondroitin sulfate A; B=chondroitin sulfate C; C=heparin; D=*N*-acetylglucosamine 6-sulfate; E=sodium poly(styrenesulfonate).

Table 1
Weight-average molecular mass (M_w) values of the modifiers

Modifier (sodium salt)	M_w (PEO)[a]	M_w (pullulan)[a]
Chondroitin sulfate A	$4.1 \cdot 10^4$	$6.4 \cdot 10^4$
Chondroitin sulfate C	$4.0 \cdot 10^4$	$6.5 \cdot 10^4$
Heparin	$2.2 \cdot 10^4$	$3.4 \cdot 10^4$
Poly(styrenesulfonate)	$2.6 \cdot 10^4$	–
Poly(styrenesulfonate)	$8.0 \cdot 10^4$	–
Poly(styrenesulfonate)	$1.5 \cdot 10^5$	–
N-Acetylglucosamine 6-sulfate	323	

[a]Calculated using poly(ethyleneoxide) (PEO) or pullulan as the standard, except for *N*-acetylglucosamine 6-sulfate.

the pH and ionic strength of the eluent. It is presumed that chondroitin sulfate C is primarily attached to the anion exchanger by electrostatic interaction via its sulfate groups and that a portion of the sulfate groups as well as the carboxyl groups remain free. The pH of the eluent used for the experiment shown in Fig. 2 was 3.2–4.3. Under the conditions in Fig. 2, the carboxyl groups were partially dissociated, whereas the free sulfate groups were completely dissociated. It is therefore expected that the higher the pH of the eluent, the larger the dissociation of the carboxyl groups and the larger the repulsion from the modified surface. This may be one of the reasons why retention of the analyte anions decreased with decreasing tartaric acid concentration.

Unfortunately, pK_a values for mucopolysaccharides were not available. The pK_a value of *N*-acetylglucosamine 6-sulfate was estimated to be around −1, by measuring the pH of the solution. It is expected that the pK_a values of the sulfate groups of mucopolysaccharides are approximately equal to that of *N*-acetylglucosamine 6-sulfate.

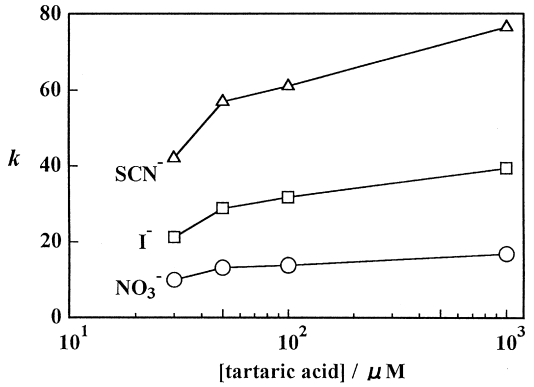

Fig. 2. *k* versus the concentration of eluent for the IC-Anion-SW column modified with chondroitin sulfate C. Column, IC-Anion-SW (50×4.6 mm I.D.) modified with 0.1% chondroitin sulfate C. Eluent, tartaric acid, 1 m*M* (pH 3.2); 0.1 m*M* (pH 3.9); 0.05 m*M* (pH 4.1) and 0.03 m*M* (pH 4.3). Flow-rate, 1.0 ml min^{-1}.

Tartaric acid, at concentrations down to 50 μ*M*, could be used without any problems. However, the peaks were skewed when the tartaric acid concentration was less than 30 μ*M*. It has been reported that, using stationary phases such as crown ether polymer [11], silica modified with polyamide crown ether [12] and octadecylsilica modified with micellar bile salts [13], very dilute solutions or water may be used as the eluent.

Nearly the same retention behavior was observed for the column modified with 0.1% (w/v) chondroitin sulfate A. Chondroitin sulfate A differs from chondroitin sulfate C in the position of the sulfate group, as depicted in Fig. 1.

In Fig. 3, a typical separation of eight components of inorganic anions using 50 μ*M* tartaric acid as the eluent and the IC-Anion-SW modified with 0.1% chondroitin sulfate C as the stationary phase is demonstrated. Conductimetric detection is displayed in Fig. 3A, whereas UV detection at 210 nm is shown in Fig. 3B. All of the analyte anions could be monitored by detection of the conductivity, whereas only UV-absorbing anions, such as iodate, nitrite, bromide, nitrate, iodide and thiocyanate, could be determined by measuring the absorbance at 210 nm. The system peak appeared close to that for iodide, which interfered with the determination of iodide under the conditions used in Fig. 3. The retention time of the system peak could be shifted by changing the eluent concentration. In addition, the background conductivity under the conditions shown in Fig. 3A was 0.03 mS cm^{-1}.

Retention of the analytes on the IC-Anion-SW column was much more remarkably reduced by modification with 1.0% (w/v) heparin. This may be because heparin has more anionic groups than chondroitin sulfate C, which, in turn, increases repulsion of anionic analytes. In Fig. 4, plots of the retention factor versus the concentration of sodium sulfate (as the eluent) for the IC-Anion-SW column modified with 1.0% (w/v) heparin are shown. The retention factor of the analyte anions decreased with decreas-

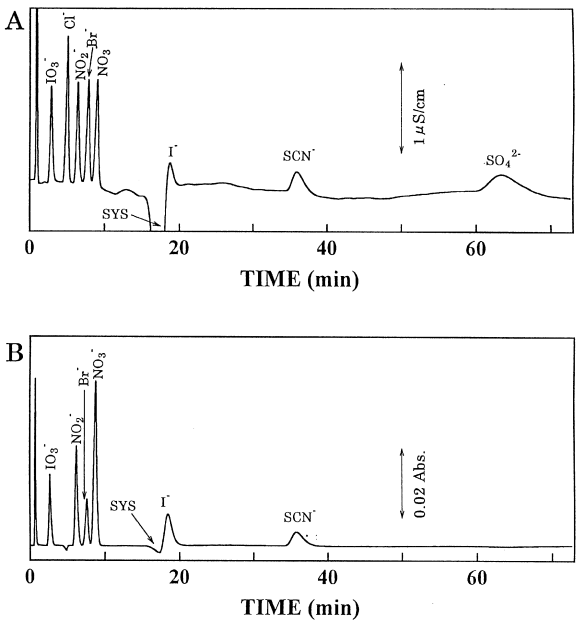

Fig. 3. Separation of inorganic anions on the IC-Anion-SW column modified with 0.1% chondroitin sulfate C. (A) conductimetric detection (background conductivity, 0.03 mS cm^{-1}); (B) UV detection at 210 nm. Column, IC-Anion-SW (50×4.6 mm I.D.) modified with 0.1% chondroitin sulfate C. Eluent, 0.05 m*M* tartaric acid. Flow-rate, 1.0 ml min^{-1}. Analyte, 0.1 m*M* each of iodate, chloride, nitrite, bromide, nitrate, iodide and thiocyanate. Injection volume, 21 μl.

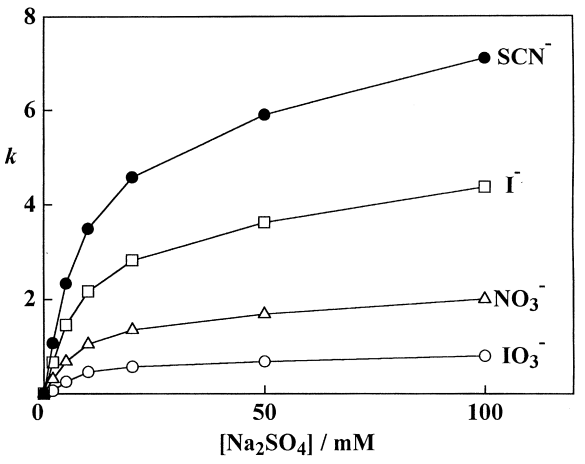

Fig. 4. Retention factor versus eluent concentration for the IC-Anion-SW column modified with heparin. Column, IC-Anion-SW (100×0.32 mm I.D.) modified with 1.0% heparin. Eluent, sodium sulfate. Flow-rate, 4.2 μl min^{-1}.

ing eluent concentration. It should be noted that when purified water was used as the eluent, the analyte anions examined were not retained on the heparin-modified stationary phase.

The chromatograms of iodate, nitrate, iodide and thiocyanate on an unmodified IC-Anion-SW column and on ones modified with chondroitin sulfate C or heparin are shown in Fig. 5. It can be seen that the retention times of the analytes are reduced after modification of the column with these mucopolysaccharides. Again, it should be noted that the retention of analytes was reduced remarkably by modification of the stationary phase with 1.0% heparin.

In Fig. 6, the molecular mass distributions of the mucopolysaccharides used as the eluent are shown. It can be seen that heparin has a smaller weight-average molecular mass than chondroitin sulfates A and C. It is reasonable to conclude that since the molecular mass of heparin is smaller than that of chondroitin sulfate C, more heparin could be introduced into the pores of the substrate than chondroitin sulfate C. The data shown in Fig. 6 indicate that a great part of chondroitin sulfate molecules are almost excluded from the pores of the IC-Anion-SW packing material. The observation that the retention of anions decreased after modification can be explained

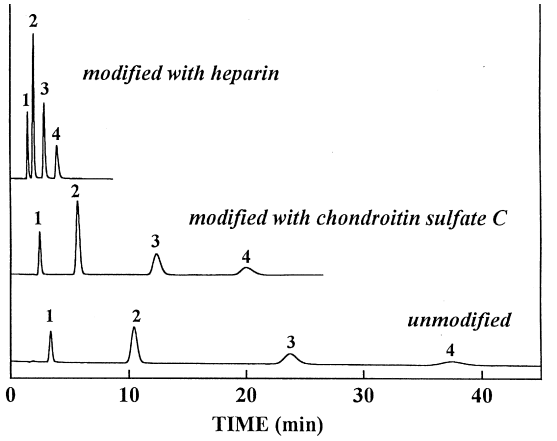

Fig. 5. Separation of anions on unmodified or modified anion-exchange columns. Columns, IC-Anion-SW (100×0.32 mm I.D.), unmodified or modified with 1.0% chondroitin sulfate C or 1.0% heparin. Eluent, 10 m*M* sodium sulfate. Flow-rate, 4.2 μl min^{-1}. Wavelength used for UV detection, 210 nm. Analytes, sodium salts of iodate nitrate, iodide and thiocyanate, 1.0 m*M* each. Injection volume, 0.011 μl.

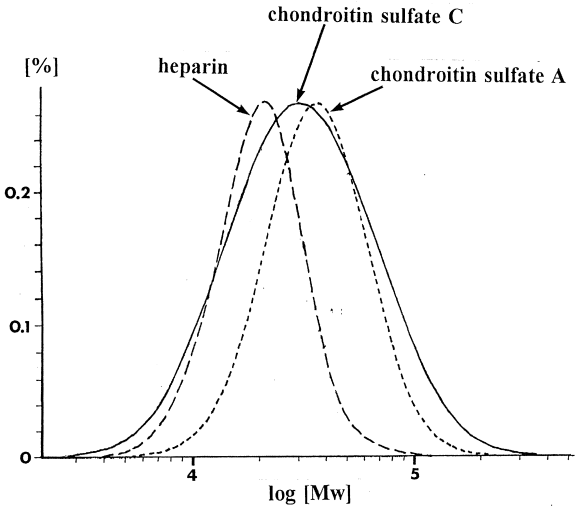

Fig. 6. Molecular mass distribution of mucopolysaccharides.

by a decrease in the number of anion-exchange sites as well as by the fact that free anionic groups, such as the sulfate or carboxyl groups of the modifier, can repel analyte anions. The more modifier that was introduced, the larger was the degree of repulsion caused by the free anionic groups.

The retention behavior varied when a different lot of chondroitin sulfate C was used. Chondroitin sulfate C with almost the same weight-average molecular mass but with slightly different dispersion characteristics gave rise to a different retention behavior. This implies that the retention behavior depends significantly on the size of the modifier. When the same modifier was used, variation in the retention of the analytes on the modified stationary phase was within 20%.

N-Acetylglucosamine 6-sulfate is a monosaccharide that is a constituent of chondroitin sulfates A and C, and heparin. When the IC-Anion-SW column was modified with *N*-acetylglucosamine 6-sulfate, the modifier that had been introduced into the column could be eluted out using an eluent such as 1 m*M* tartaric acid. Therefore, no modification effect was observed for the column modified with *N*-acetylglucosamine 6-sulfate.

Poly(styrenesulfonates) with different molecular mass distributions were also examined as modifiers in the same manner. IC-Anion-SW columns modified with three different poly(styrenesulfonates) displayed no distinct modification effect (Table 1). As in the previous case, it is presumed that the modifiers are likely to be eluted out of the column. On the other hand, when chondroitin sulfate C was introduced into the IC-Anion-SW column, it was very stable. Even after washing with 100 m*M* sodium sulfate for 12 h, the retention factor remained unchanged.

Considering the above results, it can be concluded that mucopolysaccharides strongly retained on the IC-Anion-SW packing produce modification effects due to their intrinsic structures.

3.2. Control of retention

In ion chromatography, the retention times of analytes are usually controlled by the concentration and/or the pH of the eluent. In contrast, it is difficult to control the retention time of analytes by changing the eluent concentration in the present system, as demonstrated in Figs. 2 and 4. The retention time could be changed by altering the composition of the eluent. Plots of retention time versus the composition of sodium tartrate and tartaric acid in the eluent are shown in Fig. 7. The eluents used were mixtures of 0.1 m*M* tartaric acid and 0.1 m*M* sodium tartrate. It can be seen that the retention time decreased when

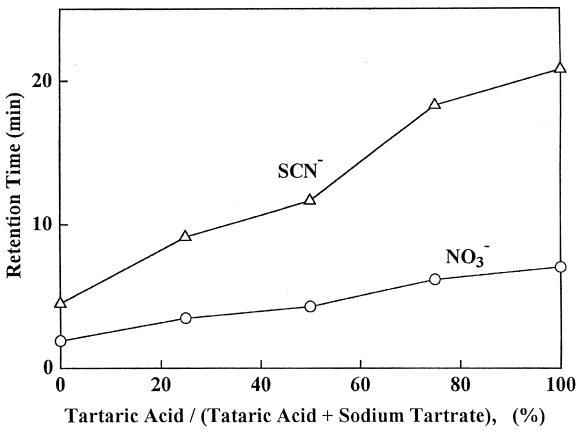

Fig. 7. Retention time versus eluent composition. Column, IC-Anion-SW (50×4.6 mm I.D.) modified with 0.1% chondroitin sulfate A. Eluent, mixture of 0.1 m*M* tartaric acid and 0.1 m*M* sodium tartrate. Flow-rate, 1.0 ml min^{-1}.

there was more sodium tartrate in the eluent. The pH varied slightly depending on the composition of the eluent, e.g. it was 4.1 for 0.1 m*M* tartaric acid and 5.7 for 0.1 m*M* sodium tartrate. The decrease in the retention of analyte anions may be attributed to the increase in repulsion caused by the carboxyl groups of the modifier with increasing pH.

3.3. Analytical figures of merit

The signal-to-noise (S/N) ratios obtained by both conductimetric detection and UV detection of iodate, chloride, nitrite, bromide, nitrate, iodide and thiocyanate were nearly the same for UV-absorbing anions. The limits of detection at $S/N=3$ under the conditions given in Fig. 3 were sub μ*M* to 2 μ*M* for both detection methods. Baseline drift, under the conditions given in Fig. 3, was slightly more severe with the conductimetric method than the UV detection method.

The relative standard deviations of the retention time for five successive measurements under the conditions given in Fig. 3 were 0.2–0.5%, whereas the R.S.D. values for peak height were 0.2–1%. The calibration curves were linear up to around 0.1 m*M*. The dynamic range was narrowed due to a decrease in the ion-exchange capacity compared with that of the unmodified column.

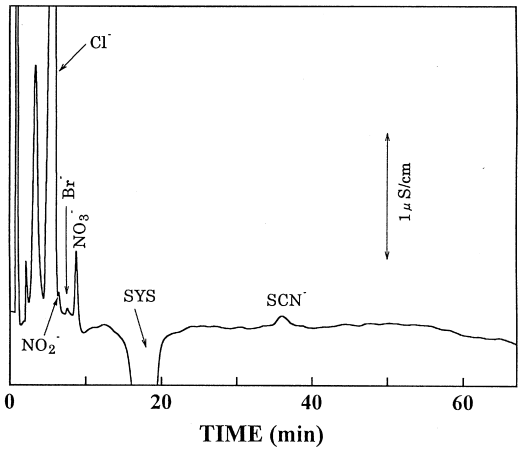

Fig. 8. Conductimetric detection of components contained in saliva. Operating conditions as in Fig. 3A except for the sample. Sample, saliva that was diluted tenfold with purified water. Injection volume, 21 μl.

3.4. Application

This system was used to determine the inorganic anions present in saliva. The conductimetric detection of components contained in saliva is demonstrated in Fig. 8. The saliva sample was diluted tenfold with purified water before injection, and 21 μl were injected. The concentrations of chloride, nitrite, bromide, nitrate and thiocyanate were 7.6, 0.02, 0.03, 0.43 and 0.31 m*M*, respectively.

4. Conclusion

The IC-Anion-SW anion exchanger modified with mucopolysaccharides could be used to separate anions when low concentrations of eluents were used. Unusual retention behavior was observed, due to the presence of both anionic and cationic sites. Sensitivity may be improved using this system because of the lower background noise. In recent work, we also found that cations were retained on the modified stationary phase and the simultaneous separation of anions and cations will be demonstrated elsewhere. The present method is superior to capillary electrophoresis, which allows the simultaneous separation of anions and cations, in terms of concentration sensitivity, sample capacity and peak shape.

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