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Ion chromatography of anions on stationary phases modified with chondroitin sulfate

Toyohide Takeuchi*, Safni[1], Tomoo Miwa

Department of Chemistry, Faculty of Engineering, Gifu University, 1-1 Yanagido, Gifu 501-11, Japan

Abstract

Inorganic anions were separated on anion exchangers modified with chondroitin sulfate-C. Retention of anions was not significantly affected by the eluent concentration for the TSKgel IC-Anion-SW anion exchanger modified with chondroitin sulfate-C. Lower concentrations of eluent could be used as the eluent. The system was applied to the determination of UV-absorbing anions in saliva. © 1997 Elsevier Science B.V.

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1. Introduction

Various types of stationary phases have been developed for ion chromatography since its first introduction by Small et al. [1]. Pellicular types of ion exchangers have also been introduced by the same authors [1] in ion chromatography because they are superior to totally porous packing materials in terms of mass transfer resistance, leading to achievement of higher column efficiency and faster separation.

Dionex (Sunnyvale, CA, USA) has developed latex-based anion exchangers. They are comprised of a surface sulfonated poly(styrene–divinylbenzene) substrate with particle diameters of 5–25 μm on which aminated latex particles with diameters of 0.1 μm are adsorbed. The substrate and the latex particles are bound by both electrostatic and Van der Waals interactions. Analyte anions interact with the functional groups on the porous latex beads and do not diffuse into the substrate owing to Donnan exclusion [2]. In case the latex-based anion exchangers are covered with a second layer of porous sulfonated latex beads, latex-based cation exchangers can be prepared [3].

The above Dionex approach suggests that high-molecular-mass polyionic compounds can be employed for the modification instead of latex beads. It is expected that the ion-exchange property of the modified stationary phases could give rise to novel selectivity in ion chromatography.

In this paper, chondroitin sulfate-C was used as the modifier and the retention behavior of the modified anion exchangers has been examined.

2. Experimental

2.1. Apparatus

Microcolumn and conventional LC systems were

*Corresponding author.

[1]On leave from the Department of Chemistry, Faculty of Mathematics and Natural Sciences, Andalas University, Padang, West Sumatra, Indonesia.

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used in this work. The former system was comprised from an MF-2 Microfeeder (Azumadenki Kogyo, Tokyo, Japan) equipped with an MS-GAN 050 gas-tight syringe (0.5 ml; Ito, Fuji, Japan), a 7520 microvalve injector (Rheodyne, Cotati, CA, USA), a 100×0.32 mm I.D. microcolumn, and a Uvidec-100 UV detector (Jasco, Tokyo, Japan). The latter system comprised an 880-PU HPLC pump (Jasco), a loop injector with an injection volume of 21 μl, a 50×4.6 mm anion-exchange column and an 870-UV detector (Jasco). The loop injector was prepared in the laboratory from a Model 7000 six-way valve (Rheodyne). A CM-8000 conductivity detector (Tosoh, Tokyo, Japan) was also used for the comparison.

The UV detectors were operated at 220 nm, and the data were handled by using a Chromatopac C-R4AX (Shimadzu, Kyoto, Japan) or a Computer Aided Chromatography data processor (Nippon Filcon, Tokyo, Japan).

The microcolumns were prepared in the laboratory from fused-silica tubing as reported previously [4]. The packing materials examined in this work were TSKgel IC-Anion-SW and IC-Anion-PW (Tosoh). The former packing is a silica-based anion exchanger with an ion-exchange capacity of ca. 0.40 mequiv. g^{-1}, whereas the latter is a polymer-based anion exchanger with an ion-exchange capacity of ca. 0.03 mequiv. ml^{-1}.

2.2. Reagents

Sodium salt of chondroitin sulfate-C and other reagents were obtained from Nacalai Tesque (Kyoto, Japan) and used without any further treatment, unless otherwise noted. Purified water was prepared in the laboratory by using a Milli-Q Plus system (Millipore, Molsheim, France). Eluents for conventional-size columns were degassed under vacuum before use. Saliva samples were diluted ten times with purified water before injection. The molecular mass of sodium chondroitin sulfate-C was measured by using TSKgel $GMPW_{XL}$ columns (300×7.8 mm I.D., ×2) and 0.2 *M* sodium nitrate as the eluent.

2.3. Modification with chondroitin sulfate-C

Aqueous solution of 0.1% (w/v) sodium chondroitin sulfate-C was passed through the column at a flow-rate of 4.2 $\mu l\ min^{-1}$ for microcolumns or 1.0 $ml\ min^{-1}$ for conventional-size columns. The modification was carried out for ca. 2 h. Sodium sulfate as the eluent was then passed through the column until the baseline was stabilized.

3. Results and discussion

3.1. Breakthrough of chondroitin sulfate-C

Chondroitin sulfate is a mucopolysaccharide, and its molecular mass is around $(5–10)\times10^4$. Chondroitin sulfate-C possesses an acetamide group, a sulfonic group and a carboxyl group as ionic groups in its disaccharide unit, as depicted in Fig. 1. It is expected that the sulfonic and carboxyl groups of chondroitin sulfate-C can interact with ion-exchange sites of an anion exchanger and affect the chromatographic property of the anion exchanger. Sodium chondroitin sulfate-C employed in this work was analyzed by size-exclusion chromatography. Its weight-average molecular mass was calculated to be 4.1×10^4 with poly(ethyleneoxide) used as calibration standards or 6.5×10^4 with pullulan used as calibration standards.

Two types of anion exchangers were used as the stationary phase in this work, involving silica-based (TSKgel IC-Anion-SW) and polymer-based (TSKgel IC-Anion-PW) anion exchangers. The pore size of the former packing is smaller than that of the latter packing. The molecular mass of poly(ethyleneoxide) completely excluded is $(5–6)\times10^4$ for the substrate of the former packing, whereas that for the latter substrate is estimated to be $(1–10)\times10^5$. This means that chondroitin sulfate-C molecules can permeate into the pore of the latter packing, whereas they are

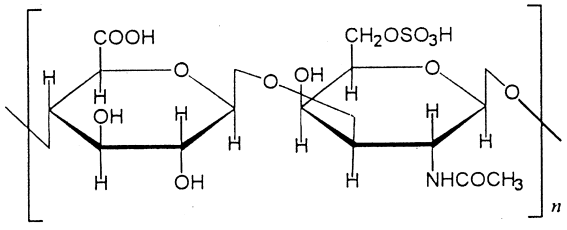

Fig. 1. Structure of chondroitin sulfate-C.

almost completely excluded from the pore of the former packing.

Breakthrough curves were monitored by passing 0.1% (w/v) sodium chondroitin sulfate-C aqueous solution into conventional-size columns at 1.0 ml min^{-1}. The breakthrough times observed were 40 and 10 min for the IC-Anion-SW and IC-Cation-PW columns, respectively. The shorter breakthrough time of the latter packing is due to its smaller ion-exchange capacity even if the chondroitin sulfate can permeate into the pore. It can be estimated from the above breakthrough time that around one-third of the ion-exchange sites of the IC-Anion-SW column are modified with chondroitin sulfate-C assuming that the sulfonic groups of the modifier exchange with the ion-exchange sites of the packing.

3.2. Effect of modification with chondroitin sulfate-C on retention of anions

Fig. 2 shows the logarithm of the retention factor (log *k*) versus the eluent concentration for the IC-Anion-SW column. Nitrate is selected as the analyte and sodium sulfate is employed as the eluent in the figure. Before the modification with chondroitin sulfate-C, log *k* decreased with increasing eluent concentration, whereas it is slightly increased with increasing eluent concentration after the modification with chondroitin sulfate-C. The latter retention behavior is unusual in ion chromatography. It is also found that the retention factors of nitrate decreased after the modification.

On the other hand, log *k* decreased with increasing sodium sulfate concentration for the IC-Anion-PW column in both cases before and after the modification with chondroitin sulfate-C, as illustrated in Fig. 3. It is also seen that the retention factor of nitrate slightly increased after the modification.

The big difference in the retention behavior observed between the results in Figs. 2 and 3 may be due to the differences in the physical property of the packing materials employed. TSKgel IC-Anion-SW and IC-Anion-PW packings employed in Figs. 2 and 3 differ in the pore size [exclusion limit, $(5–6)\times10^4$ vs. $(10–100)\times10^4$], ion-exchange capacity (0.40 mequiv. g^{-1} vs. 0.03 mequiv. ml^{-1}) and substrate (silica-based vs. polymer-based). Unfortunately, it is not clear which parameter contributed to the difference in the retention behavior observed in Figs. 2 and 3.

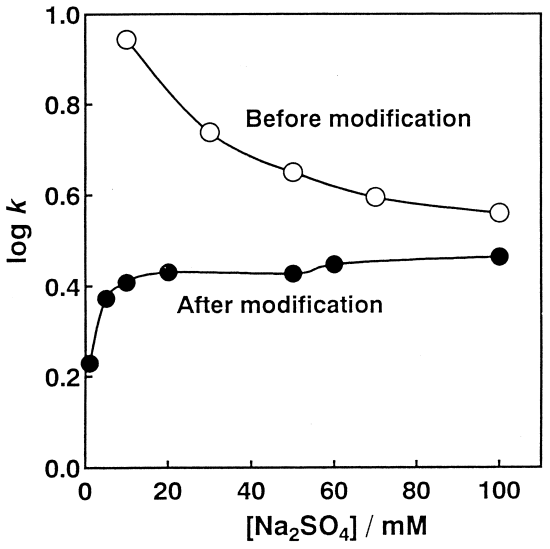

Fig. 2. log *k* versus the eluent concentration for the IC-Anion-SW column. Column, TSKgel IC-Anion-SW (100×0.32 mm I.D.); eluent, aqueous solution of sodium sulfate; flow-rate, 4.2 μl min^{-1}; injection volume, 0.2 μl; analytes, sodium nitrate.

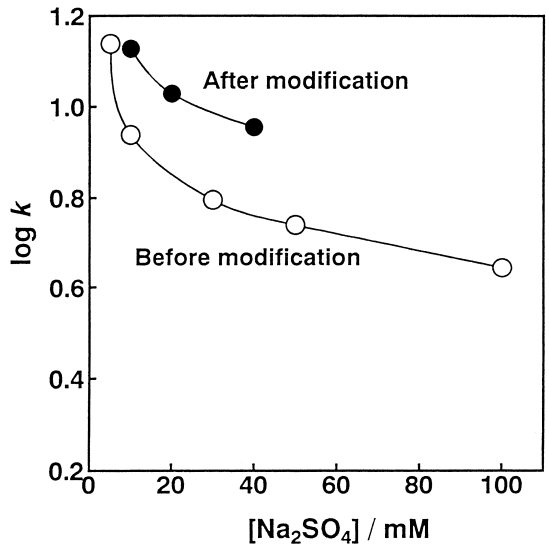

Fig. 3. log *k* versus the eluent concentration for the IC-Anion-PW column. Column, TSKgel IC-Anion-PW (100×0.32 mm I.D.); other operating conditions as in Fig. 2.

3.3. Analytical figures of merit

Fig. 4 demonstrates a typical separation of nitrate, iodide and thiocyanate on the IC-Anion-SW column modified with sodium chondroitin sulfate-C. The eluent is 10 m*M* sodium sulfate, and the analytes are detected at 220 nm. These three anions are separated in 5 min. Retention of other UV-absorbing anions was also examined. Iodate eluted before nitrate and completely separated from nitrate. Nitrite and bromide eluted close to nitrate, whereas thiosulfate was not eluted from the column under the conditions in Fig. 4. The calibration curves for nitrate, iodide and thiocyanate are shown in Fig. 5, where the eluent employed is 10 m*M* sodium sulfate. The peak heights were linear up to at least 0.1 m*M* for these anions, as demonstrated in the figure. The results indicate that the present system can be applied to the determination of UV-absorbing anions. The detection limits at $S/N=3$ under the conditions in Fig. 4 were 0.35, 0.23 and 1.4 μ*M* for nitrate, iodide and thiocyanate, respectively. The relative standard deviation of retention time for the successive five measurements under the conditions in Fig. 4 was 0.7–1.2%.

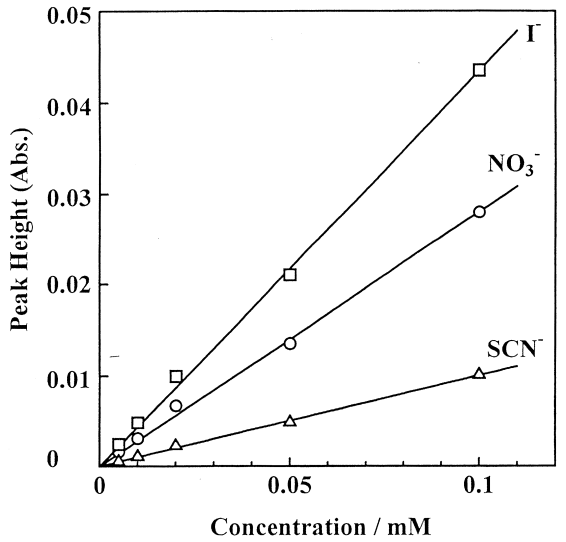

Fig. 5. Calibration curves for nitrate, iodide and thiocyanate. Operating conditions as in Fig. 4 except the analyte concentration.

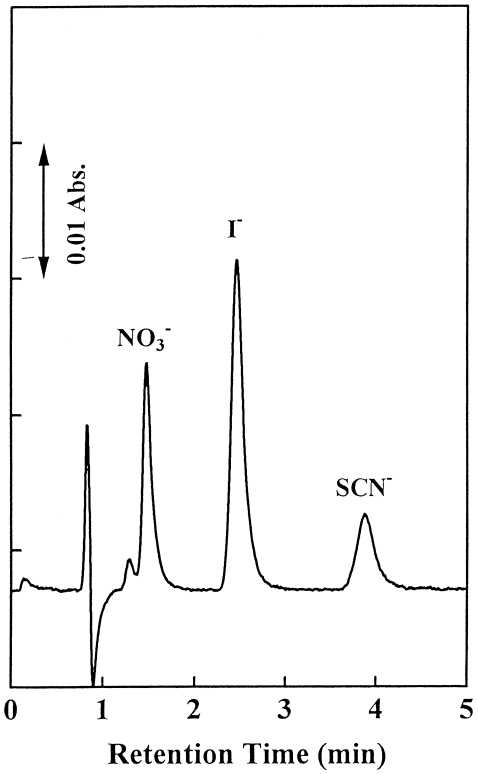

Fig. 4. Separation of nitrate, iodide and thiocyanate. Column, TSKgel IC-Anion-SW (50×4.6 mm I.D.); eluent, 10 m*M* sodium sulfate; flow-rate, 1.0 ml min^{-1}; injection volume, 21 μl; analytes, 0.01 m*M* each of sodium nitrate, sodium iodide and sodium thiocyanate, wavelength of UV detection, 220 nm.

3.4. Application to saliva samples

The present system was applied to the determination of anions contained in saliva. Fig. 6 demonstrates the separation of UV-absorbing components contained in saliva. The saliva sample was ten times diluted with purified water, and 21 μl of the diluted sample was injected. Nitrate, iodide and thiocyanate were determined to be 0.24, 0.002 and 0.37 m*M*, respectively.

3.5. Lower-concentration eluent

Fig. 2 suggests that nitrate can be eluted from the TSKgel IC-anion-SW column modified with chondroitin sulfate-C even if lower-concentration eluents

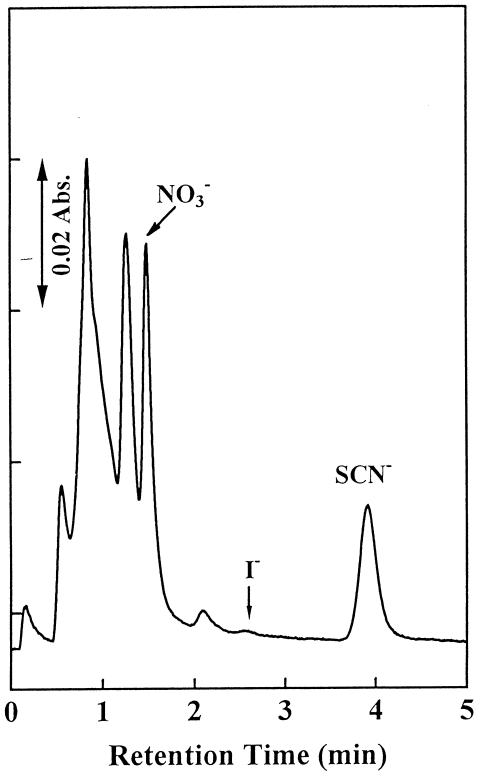

Fig. 6. Separation of UV-absorbing components in saliva. Operating conditions as in Fig. 4 except the sample.

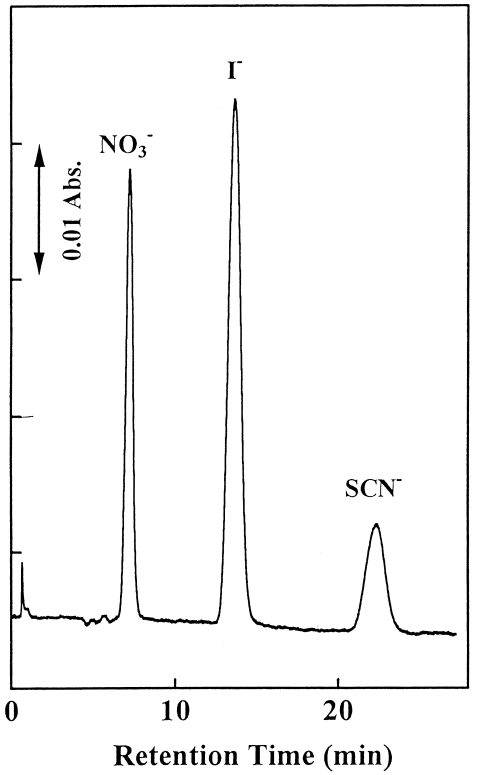

Fig. 7. Separation of sodium salts of nitrate, iodide and thiocyanate using water as the eluent. Eluent, water; other operating conditions as in Fig. 4.

are used. It was also found that after the experiments using sodium sulfate as the eluent water could elute the analyte ions from the column.

Fig. 7 demonstrates the separation of nitrate, iodide and thiocyanate using water as the eluent. When water was used as the eluent, the retention time of the analyte ions gradually increased during repeated chromatographic runs. However, conductimetric detection could not visualize the analytes under the conditions in Fig. 7.

On the other hand, when 50 μM tartaric acid was used as the eluent, the analyte ions could be detected by the conductivity detector, as demonstrated in Fig. 8. Although the system peak eluted close to iodide, the four peaks were well separated and detected. In addition, when 50 μM tartaric acid was used as the eluent, the repeatability of the retention time was comparable to that achieved under the conditions in Fig. 4. It should be noted that the retention time of the analytes were not significantly affected by the concentration of tartaric acid.

Considering these results, it could be concluded that water as the eluent in Fig. 7 was contaminated with a very low concentration of sodium sulfate when passing through the separation column, which allowed the elution of the analytes. Unfortunately, the preliminary results in the present work could not clarify the retention mechanism nor find the optimum conditioning procedure. Further investigation will be required to elucidate the modification and retention mechanism involved in the present separation system.

The use of water or lower-concentration eluent will have great potential to improve the detectability when a conductivity detector is used because the background of the eluent can be minimized. So far, crown ether polymer [5], silica modified with poly-

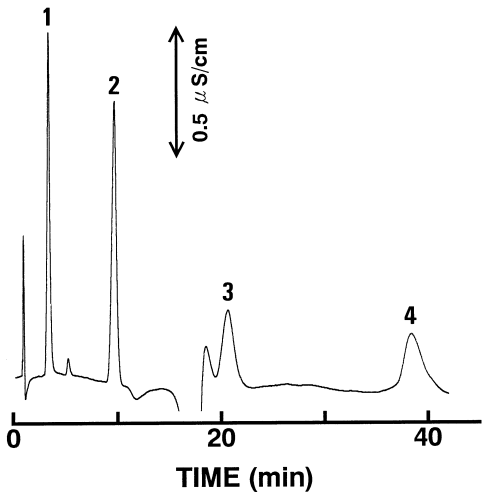

Fig. 8. Conductimetric detection of iodate, nitrate, iodide and thiocyanate using tartaric acid as the eluent. Eluent, 50 μ*M* tartaric acid; detector, conductivity; analytes, 0.1 m*M* each of iodate (1), nitrate (2), iodide (3) and thiocyanate (4); other operating conditions as in Fig. 4.

amide crown ether [6] and octadecylsilica modified with micellar bile salts [7] have been reported as the stationary phases which allow the use of water as the eluent.

4. Conclusion

Retention behavior of inorganic anions observed for the TSKgel IC-Anion-SW column modified with chondroitin sulfate-C was different from that observed under conventional ion-exchange chromatographic conditions. The retention of anions was not significantly varied by the eluent concentration after the modification, and very low concentrations of eluents could also be used as the eluent for the separation of anions. Elucidation of the modification and retention mechanism, retention of cations and the use of other types of polyanions are being investigated.

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