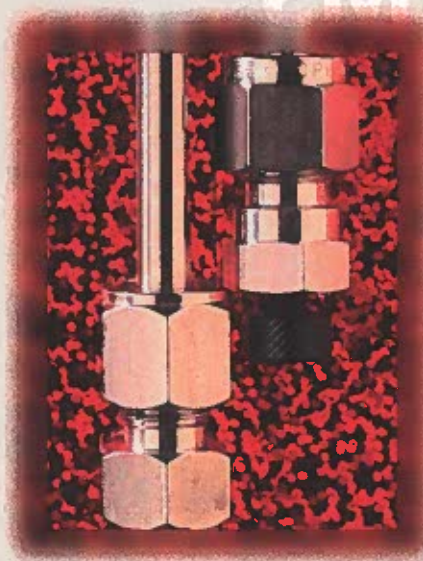


Ion Chromatography Using Anion Exchangers Modified with Anionic Polysaccharides



The authors investigated the retention behavior of anions and cations on anion exchangers modified with anionic polysaccharides such as chondroitin sulfate, heparin, and dextran sulfate. The modified stationary phases possess residual anion-exchange sites of the substrate as well as cation-exchange sites due to free anionic groups of the modifier. The authors observed unusual retention behavior of inorganic anions and simultaneous separation of both anions and cations. The pore size of anion exchangers and the molecular mass of modifiers strongly affected the retention behavior of analyte ions.

Many packing materials have been developed for ion chromatography (IC) since its introduction by Small and co-workers (1) in 1975. In terms of detection, packing materials with lower ion-exchange capacities are preferable in both suppressed and nonsuppressed IC because they enable the use of dilute eluents. However, ion-exchange capacity generally is difficult to control. Coating conditions can regulate the ion-exchange capacity in reversed-phase packing materials permanently coated with hydrophobic ions (2–4). Chromatographers have tried several approaches besides the ion-exchange capacity to manipulate the selectivity, analysis time, and sample capacity (5–7).

Dionex (Sunnyvale, California) developed latex-based anion exchangers (8). These materials comprised a surface-sulfonated poly(styrene-divinylbenzene) substrate with 5–25 μm particle diameters on which aminated latex particles with 0.1– μm diameters are adsorbed. The substrate and the latex particles are bound through 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 because of Donnan exclusion (6). Latex-based cation exchangers can be prepared if

the latex-based anion exchangers are covered with a second layer of porous sulfonated latex beads (9).

Other modification methods control the ion-exchange capacity, including electrostatic modification. It is expected that high-molecular-mass polyionic compounds can be used for the modification instead of latex beads. Various types of commercially available anionic polysaccharides can be candidates for this purpose. This article describes the retention properties of anion exchangers modified with anionic polysaccharides, which include chondroitin sulfate, heparin, and dextran sulfate.

Experimental

Apparatus: Our microcolumn liquid chromatograph comprised a model MF-2 microfeeder (Azumadenki Kogyo, Tokyo, Japan) equipped with a 0.5-mL model MS-GAN 050 gas-tight syringe (Ito, Fuji, Japan), a model ML-522 microvalve injector (Jasco, Tokyo, Japan) with an injection volume of 0.2 μL , a laboratory-prepared 100 mm \times 0.32 mm microcolumn, and a model UV-970 UV detector (Jasco). The data were handled by Computer Aided Chromatography (Nippon Filcon, Tokyo, Japan) or Chromatopac C-R4A (Shimadzu, Kyoto, Japan) data processors. A conventional IC system was assembled from a model 880-PU HPLC

**Toyohide Takeuchi, Safni,
and Tomoo Miwa**

Department of Chemistry, Faculty
of Engineering, Gifu University,
1-1 Yanagido, Gifu 501-1193,
Japan, e-mail takeuchi@apchem.
gifu-u.ac.jp

*Address correspondence to
T. Takeuchi.*

Table 1: Physical properties of the silica-based anion exchangers

Anion Exchanger	Specific Surface Area (m ² /g)	Pore Diameter (nm)	Pore Volume (mL/g)	Anion-Exchange Capacity (mequiv/mL)
1	181	14	0.79	0.16
2	238	16	1.06	0.11
3	197	23	1.27	0.15
4	197	36	1.10	0.066

pump (Jasco), a 50 mm × 4.6 mm column, a valve injector with an injection volume of 21 μL, and a model CM-8000 conductivity detector (Tosoh, Tokyo, Japan).

We performed size-exclusion chromatography of anionic polysaccharides using two 300 mm × 7.8 mm TSKgel GMPW_{XL} columns (Tosoh) and 0.2 M sodium nitrate as the eluent. The microcolumns were prepared in the laboratory from fused-silica tubing, as reported previously (10). In our study we used four kinds of silica-based, strongly basic anion-exchange media with different pore distributions. We measured the pore size distribution, pore volume, and specific surface area of the anion exchangers using a model SA 3100 analyzer (Beckman Coulter, Inc., Fullerton, California), and the data are shown in Table 1. The anion-exchange capacities before modification were measured in situ by passing 10 mM sodium nitrate through the columns.

Figure 1 shows the pore diameter distributions of the anion exchangers. Anion exchanger 1 has a narrow distribution, whereas anion exchanger 4 has a very wide distribution with the largest mean pore diameter.

Reagents: Chondroitin sulfate A, chondroitin sulfate C, and heparin were obtained from Nacalai Tesque (Kyoto, Japan). We purchased sodium salts of dextran sulfate with different molecular mass distributions from Sigma Chemical Co. (St. Louis, Missouri) and Tokyo Chemical Industry (Tokyo, Japan). Figure 2 shows the molecular mass distributions of the polysaccharides used in our study. Other reagents were of reagent-grade and were obtained from Nacalai Tesque. We used these reagents without any further treatment. Purified water was prepared in our laboratory using a Milli-Q Plus system (Millipore Corp., Bedford, Massachusetts). The eluents were prepared from the purified water.

Modification with anionic polysaccharides: The 100 mm × 0.32 mm anion-exchange columns were first washed with 0.05 mL of water, 0.2 mL of 10 mM sodium sulfate, and 0.05 mL of water at a flow rate of 4.2 μL/min. Then, an aqueous solution

of 1.0% anionic polysaccharide, unless otherwise noted, was passed through the column at the same flow rate for 2 h, and the column was washed with water until the baseline stabilized. For conventional size columns, the flow rate was 1.0 mL/min.

Physical properties of anionic polysaccharides: Figure 3 illustrates the expected structures of the sodium salts of anionic polysaccharides used in this study. Table II compares the weight-average molecular mass and the polydispersity of the anionic polysaccharides. These parameters were determined by size-exclusion chromatography using poly(ethyleneoxide) as the standard.

Results and Discussion

Effect of modification: We found that modification effects became more pronounced with increasing concentration and volume when chondroitin sulfate C was used as the modifier. Therefore, when using the 100 mm × 0.32 mm columns, we kept the concentration and the volume of the modifier constant at 1% (w/v) and 0.5 mL, respectively.

Because the polysaccharides we used possess sulfate, aminosulfonate, or carboxyl groups, as illustrated in Figure 3, the polysaccharides could be introduced into anion-exchange media primarily by electrostatic interaction. When anion exchangers were modified with these anionic polysaccharides, the anion-exchange capacity was decreased (11–13). In other words, the retention of analyte anions decreased after the modification with anionic polysaccharides. Moreover, because it is reasonable to expect that all of the anionic groups do not interact with the anion-exchange sites of the substrate, free anionic groups of the polysaccharides adsorbed on the modified stationary phase can affect the retention of analyte ions. These free anionic groups can repel anions and attract cations.

Figure 4 shows the retention factor (*k*) of nitrate on anion exchanger 1 modified with various anionic polysaccharides versus the eluent concentration. The water dip was generally adopted as the nonretention position. When nitrate was excluded from mod-

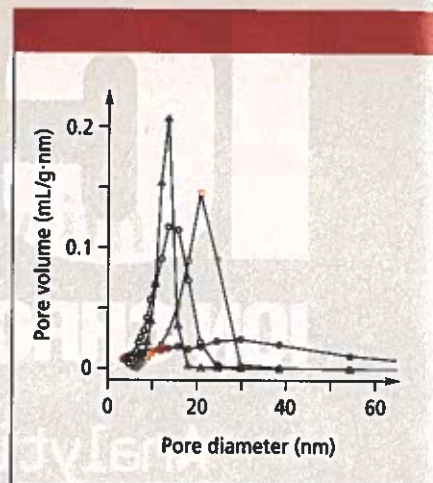


Figure 1: Pore distributions of the anion-exchange media. Plots: ▲ = 1, ○ = 2, ■ = 3, ● = 4. (Reprinted with permission from reference 15.)

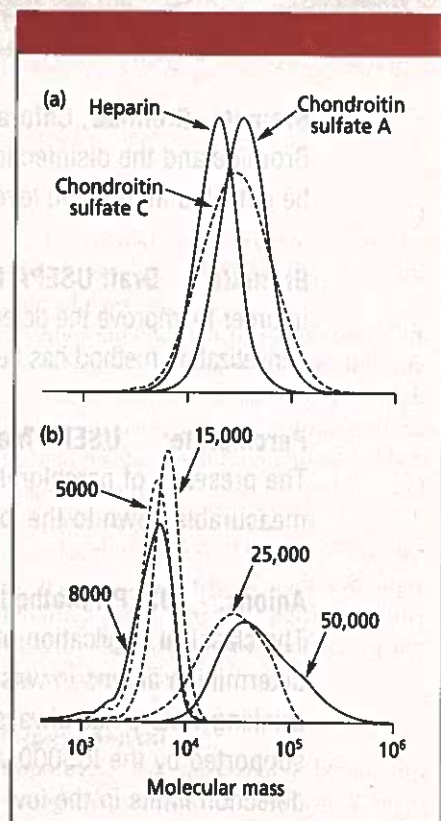


Figure 2: Molecular mass distributions of the anionic polysaccharides (a) heparin, chondroitin sulfate A, and chondroitin sulfate C and (b) dextran sulfate 5000, 8000, 15,000, 25,000, and 50,000.

ified stationary phases by using water as the eluent, its retention time was adopted as the nonretention time. In common IC, linear relationships between log *k* and the eluent concentration are observed, and log *k* decreases with increasing eluent concentra-

tion. In the case of the unmodified column, we actually observed this type of common relationship in Figure 4. However, in the case of anion exchangers modified with heparin and dextran sulfate 8000, $\log k$ increases with increasing eluent concentration (12,14–16).

The molecular mass of the modifier plays an important role in the retention behavior. The smaller the modifier, the more it is introduced into the anion exchanger and the more pronounced is the effect on the retention. As for the anion exchangers modified with heparin and dextran sulfate 8000,

k increased with increasing eluent concentration, and we achieved much smaller k values compared with those for the unmodified column. This unusual retention behavior is due to the fact that nitrate is repulsed by the free anionic groups of the modifier. The decrease in k also can be attributed to the decrease in the anion-exchange sites following modification. On the other hand, the column modified with dextran sulfate 50,000 showed usual retention behavior with slight decrease in k compared with that observed for the unmodified stationary phase. This outcome was obtained because only a portion of the pore of the substrate is modified with dextran sulfate 50,000.

The above results indicate that the pore size of anion exchangers also can influence the ion-exchange properties of modified stationary phases.

Effect of eluent cations: We examined the effects of the eluent composition on the retention of analyte anions for the anion exchanger (number 1 in Figure 1) modified with heparin (12). In common IC, k decreases with increasing eluent concentration, with a linear relationship between $\log k$ and eluent concentration. When we used sulfates with various counter cations as the eluent, we found that the retention behavior depended on the cation of the eluent (12). The retention factor of nitrate decreased with increasing eluent concentration when copper sulfate, aluminum sulfate, and sulfuric acid were used as the eluent, whereas it increased with increasing eluent concentra-

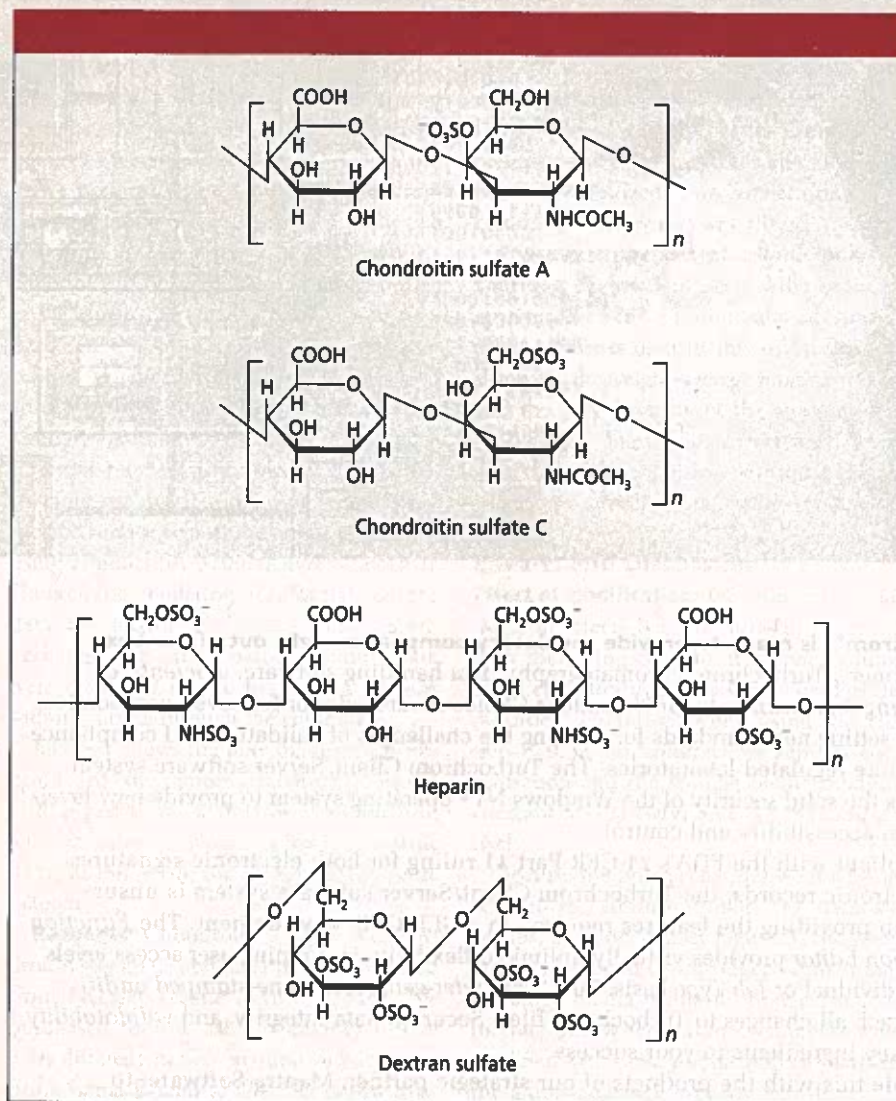


Figure 3: Structures of the anionic polysaccharides.

Table II: Weight-average molecular mass and polydispersity of the anionic polysaccharides

Polysaccharides	Weight-Average Molecular Mass*	Polydispersity
Chondroitin sulfate A	4.1×10^3	1.33
Chondroitin sulfate C	3.5×10^3	1.40
Heparin	2.2×10^3	1.24
Dextran sulfate 5000	4.9×10^3	1.23
Dextran sulfate 8000	5.1×10^3	1.35
Dextran sulfate 15,000	6.9×10^3	1.13
Dextran sulfate 25,000	3.5×10^4	1.67
Dextran sulfate 50,000	7.5×10^4	1.95

* Columns: two 300 mm \times 7.8 mm TSKgel GMPW_{XL}; eluent: 0.2 M sodium nitrate.

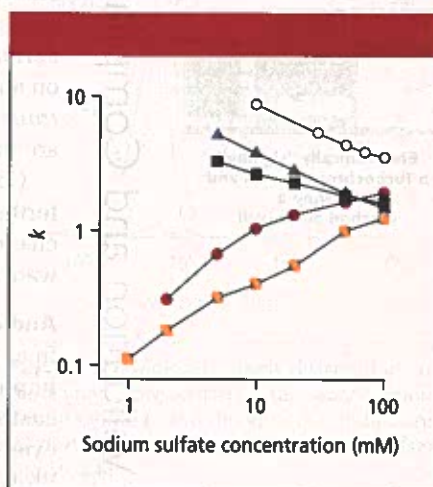


Figure 4: Comparison of retention behavior of nitrate on anion exchanger 1 modified with anionic polysaccharides. Columns: 100 mm \times 0.32 mm anion exchanger 1 modified with anionic polysaccharides; eluent: sodium sulfate. Modifiers: \circ = unmodified; \blacktriangle = dextran sulfate 50,000; \blacksquare = chondroitin sulfate C; \bullet = heparin; \blacksquare = dextran sulfate 8000. (Reprinted with permission from reference 15.)

tion for sodium sulfate and magnesium sulfate. The latter retention behavior is unusual in IC. The smallest k value was observed for sodium sulfate eluent. When we used water as the eluent, we found that nitrate was not retained on the modified stationary phase.

When chlorides of alkali metal ions were used as the eluent, the retention of nitrate increased in the order of sodium, potassium, rubidium, and cesium (16). We found that the eluent cation also influences the retention of analyte anions, because the modified stationary phase possesses both anion- and cation-exchange sites.

Pore size effect: Table I and Figure 1 compare the physical properties of the anion exchangers. We evaluated four kinds of silica-based anion exchangers with mean pore diameters of 14, 16, 23, and 36 nm. After the modification of each anion exchanger with heparin, the anion-exchange properties were reduced, whereas cation-exchange properties were acquired. We found that nitrate was excluded from the columns after the modification of anion exchangers 3 and 4. In other words, the anion-exchange capacities of the modified anion exchangers 3 and 4 were very small. Modified anion exchangers 1 and 2, on the other hand, still exhibited anion-exchange properties after the modification when 1 mM sodium nitrate was used as the eluent. When appropriate eluents were selected, unmodified anion exchangers 1 and 2 exhibited both anion- and cation-exchange properties. On the other hand, nearly the same cation-exchange capacities were acquired for the anion exchangers after modification.

Table III compares the anion- and cation-exchange capacities. We found that the anion-exchange capacities were reduced after the modification, whereas cation-exchange capacities of 21–32 $\mu\text{equiv/mL}$

were observed for the modified stationary phases (15).

Retention of anions and cations: When the size of a pore is smaller than the size of a modifier, the anion-exchange sites existing on the pore remain unmodified. It also is expected that all of the anionic groups of the modifier do not interact with the anion-exchange sites of the anion exchanger, so a portion of the anionic groups on the modifier remain free and act as the cation-exchange sites (12,14). Therefore, it is expected that both anions and cations are retained simultaneously on the modified stationary phases.

We selected copper sulfate as the eluent to visualize cations by indirect photometric detection and inherently UV-absorbing anions as the test analyte anions. Figure 5 shows a plot of the logarithm of the retention factor versus the logarithm of the copper sulfate concentration (15). We found that both anions and cations are simultaneously retained on the anion exchanger modified with dextran sulfate 8000 (14,15). The retention of potassium and calcium ions decreased with increasing eluent concentration, whereas $\log k$ of anions slightly increased with increasing eluent concentration. In both cases, we observed linear relationships between the parameters.

The separation of cations on anion exchanger 1 modified with dextran sulfate 8000 is demonstrated in Figure 6, in which 10 mM copper sulfate was used as the eluent and the cations were detected indirectly at 210 nm because of the depression of copper ion (15). The elution order observed under the conditions in Figure 6 is the same as that observed in common IC. After modification with dextran sulfate 8000, the cation-exchange capacity was 64 $\mu\text{equiv/mL}$ (14).

Figure 7 demonstrates the simultaneous separation of anions and cations on anion

exchanger 1 modified with heparin (12). We used 1 mM copper sulfate as the eluent and a detection wavelength of 200 nm. Sodium, magnesium, and calcium ions appeared as negative peaks because of the depression of copper ion, whereas the anions appeared as positive peaks. The chromatogram also includes a system peak

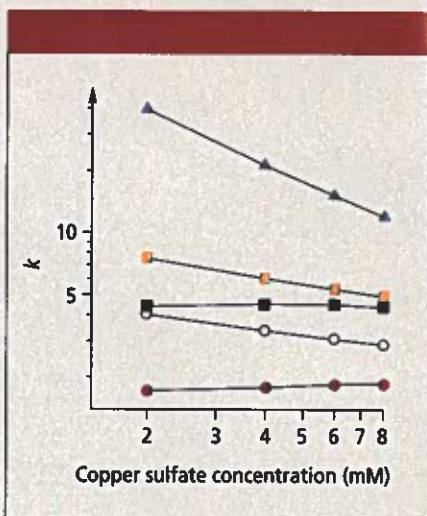


Figure 5: Plots of the logarithm of the retention factor versus the logarithm of the copper sulfate concentration for various anions and cations. Column: 100 mm \times 0.32 mm anion exchanger 1 modified with dextran sulfate 8000; eluent: copper sulfate. Analytes: \blacktriangle = calcium, \blacksquare = potassium, \blacksquare = nitrate, \circ = sodium, \bullet = iodate. (Reprinted with permission from reference 15.)

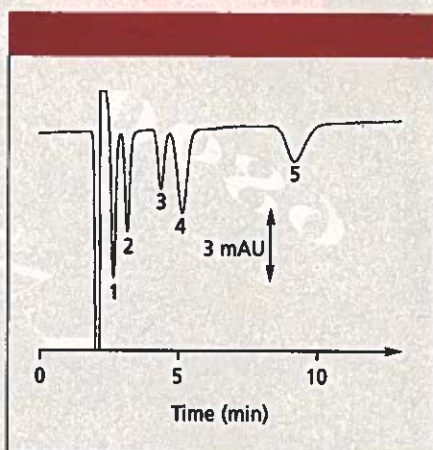


Figure 6: Separation of monovalent and divalent cations using copper sulfate as the eluent. Column: 100 mm \times 0.32 mm anion exchanger 1 modified with dextran sulfate 8000; eluent: 10 mM copper sulfate; flow rate: 4.2 $\mu\text{L/min}$; analyte concentration: 2 mM for each; injection volume: 0.2 μL ; detection wavelength: 210 nm. Analytes: 1 = sodium, 2 = ammonium, 3 = potassium, 4 = magnesium, 5 = calcium. (Reprinted with permission from reference 15.)

Table III: Comparison of ion-exchange capacities^a

Anion Exchanger	Before Modification		After Modification	
	Anion-Exchange Capacity ($\mu\text{equiv/mL}$)	Anion-Exchange Capacity ($\mu\text{equiv/mL}$)	Anion-Exchange Capacity ($\mu\text{equiv/mL}$)	Cation-Exchange Capacity ($\mu\text{equiv/mL}$)
1	160	2.1	32	
2	110	3.9	21	
3	150	Excluded	30	
4	66	Excluded	31	

^a Columns: 100 mm \times 0.32 mm anion exchangers modified with heparin; anion exchanger numbers refer to those in Figure 1 and Table I. Eluents for measurement of ion-exchange capacities after the modification: 1 mM sodium nitrate for anion-exchange capacity; 2 mM copper sulfate for cation-exchange capacity.

caused by copper sulfate. Under the conditions in Figure 7, the selectivity of cations is unsatisfactory; for example, other alkali metal and ammonium ions were eluted close to sodium ion. All of the anions except chloride moderately absorb at 200 nm, but the absorption of chloride at 200 nm is very weak. In addition, sulfate cannot be determined because the eluent contains sulfate. The copper sulfate peak is the system peak.

It is reasonable to conclude that chloride is indirectly detected by copper ion, although the detection mechanism is not yet elucidated (12). Both anions and cations are simultaneously visualized by using a single UV-absorbing eluent when both anions and cations are simultaneously retained on the stationary phase (17). When salicylate was used as the eluent for an amphoteric stationary phase such as alumina, cations appeared as positive peaks and anions appeared as negative peaks (17).

Retention mechanism: Figure 8 shows an imaginary illustration of a modified surface, where the counterions provided by the eluent are omitted. We have presumed that residual anion-exchange sites exist underneath the cation-exchange sites, and the analyte anions should pass through the zone near the cation-exchange sites, where the analyte anions undergo repulsion. It is believed that the repulsion from the cation-exchange sites increases with decreasing elu-

ent concentration, which leads to the decrease in the retention of analyte anions. On the contrary, the retention of analyte anions caused by ion exchange increases with decreasing eluent concentration. The net retention is determined by both contributions. We concluded that the contribution of the effect of repulsion on the retention is dominant for the columns modified with heparin and dextran sulfate 5000, 8000, 15,000, and 25,000 when sodium sulfate is used as the eluent. We can attribute the unusual retention behavior of anions on the modified stationary phase to the repulsion from the cation-exchange sites. On the other hand, the analyte cations interact with the cation-exchange sites without undergoing repulsion from the anion-exchange sites. Because of this situation, we observed usual retention behavior for analyte cations.

Other discussion: We measured the repeatability of the retention time and the peak heights of cations using anion exchanger 1 modified with dextran sulfate 8000 as the stationary phase and 10 mM copper sulfate as the eluent. The relative standard deviation of the retention time and peak height for six successive measurements were 1.9–2.7% and 4.8–5.0%, respectively. The modifier was partially washed out of the column when sodium perchlorate or chlorides of alkaline-earth metals were used

as the eluent. Chemically bonded stationary phases seem to be preferable.

We also examined poly(styrenesulfonate) with various molecular mass distributions as the modifier in the same manner. However, anion exchanger 1 modified with poly(styrenesulfonate) displayed no distinct modification (13). We also demonstrated that a poly(methacrylate)-based anion exchanger modified with chondroitin sulfate C did not show any distinct modification effect (11).

Conductivity detection without suppression will be the best way to achieve simultaneous detection of anions and cations using organic acids, such as tartaric acid, as the eluent. Simultaneous separation of both anions and cations will be useful for the analysis of acid rain.

Since Small and Miller's proposal (18), other researchers have developed various approaches for the simultaneous determination of anions and cations, including using mixed anion and cation exchangers, using anion- and cation-exchange columns in tandem, unifying the sign of ions by complexation, using amphoteric or zwitterionic stationary phases, and combining cation exchange and ion exclusion in a single column. Our present system will be an option for this purpose.

Because the ionic polysaccharides we used are chiral, we can perform enantiomeric separation of amine derivatives such as chloroquine. The result of this study will be reported elsewhere (19).

Application: We applied our present system to the determination of inorganic ions in various water samples. Figure 9 shows the conductimetric detection of components contained in saliva. We diluted the saliva sample 10-fold with purified water before injection and injected 21 μ L. The concentrations of chloride, nitrite, bromide, nitrate, and thiocyanate were 7.6, 0.02, 0.03, 0.43, and 0.31 mM, respectively. Simultaneous determinations of both anions and cations have not been successful because the selectivity of the present system still is unsatisfactory.

Conclusion

The retention behavior of inorganic anions on the anion exchanger modified with anionic polysaccharides was different from that of an analysis under conventional IC conditions. The retention of anions was remarkably reduced after modification. Very low eluent concentration, therefore, can be used for the separation of inorganic

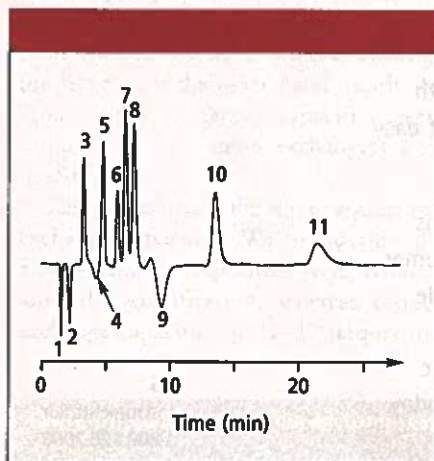


Figure 7: Simultaneous separation of anions and cations on anion exchanger 1 modified with heparin. Column: 100 mm \times 0.32 mm anion exchanger 1 modified with heparin; eluent: 1 mM copper sulfate; flow rate: 4.2 μ L/min; injection volume: 0.2 μ L; detection wavelength: 200 nm. Analytes: 1 = 2.4 mM sodium, 2 = 2 mM magnesium, 3 = 0.4 mM iodate, 4 = 2 mM calcium, 5 = 4 mM chloride, 6 = 0.4 mM nitrite, 7 = 0.4 mM bromide, 8 = 0.4 mM nitrate, 9 = 2 mM sulfate, 10 = 0.4 mM iodide, 11 = 0.4 mM thiocyanate. (Reprinted with permission from reference 12.)

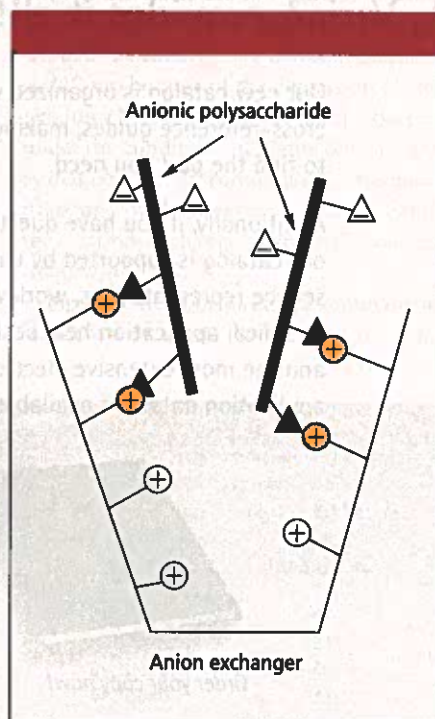


Figure 8: Diagram of the modified surface.

anions, and this procedure will lead to the achievement of high sensitivity. The system discussed in this article also allows the simultaneous separation of anions and

cations. The selectivity of the modified stationary phase will be improved by careful selection of the modifier and its size, as well as the pore size of anion exchangers. Future work will clarify the retention and detection mechanisms.

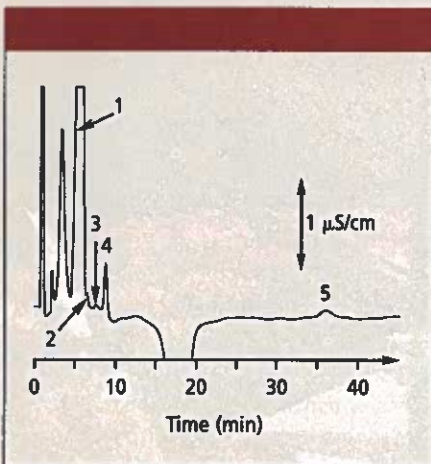


Figure 9: Separation of saliva components with conductimetric detection. Column: 50 mm \times 4.6 mm anion exchanger 2 modified with 0.1% chondroitin sulfate C; eluent: 0.05 mM tartaric acid; flow rate: 1.0 mL/min; sample: saliva diluted 10-fold with purified water; injection volume: 21 μ L. Analytes: 1 = chloride, 2 = nitrite, 3 = bromide, 4 = nitrate, 5 = thiocyanate. (Reprinted with permission from reference 13.)

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References

- (1) H. Small, T.S. Stevens, and W.C. Bauman, *Anal. Chem.* **47**, 1801 (1975).
- (2) R.N. Reeve, *J. Chromatogr.* **177**, 393 (1979).
- (3) R.M. Cassidy and S. Elchuk, *Anal. Chem.* **54**, 1558 (1982).
- (4) R.M. Cassidy and S. Elchuk, *J. Chromatogr.* **262**, 311 (1983).

- (5) J.S. Fritz, D.T. Gjerde, and C. Pohlandt, *Ion Chromatography* (Hüthig Verlag, Heidelberg, Germany, 1982).
- (6) P.R. Haddad and P.E. Jackson, *Ion Chromatography: Principles and Applications*, *J. Chromatogr. Library vol. 46* (Elsevier, Amsterdam, 1990).
- (7) J. Weiss, *Ion Chromatography* (VCH, Weinheim, Germany, 1995).
- (8) J. Weiss, *Ion Chromatography* (VCH, Weinheim, Germany, 1995), p. 43.
- (9) J. Weiss, *Ion Chromatography* (VCH, Weinheim, Germany, 1995), p. 173.
- (10) T. Takeuchi and D. Ishii, *J. Chromatogr.* **213**, 25 (1981).
- (11) T. Takeuchi, Safni, and T. Miwa, *J. Chromatogr. A* **789**, 201 (1997).
- (12) T. Takeuchi, Safni, T. Miwa, Y. Hashimoto, and H. Moriyama, *Analisis* **26**, 61 (1998).
- (13) T. Takeuchi, Safni, T. Miwa, Y. Hashimoto, and H. Moriyama, *J. Chromatogr. A* **804**, 79 (1998).
- (14) T. Takeuchi, Safni, T. Miwa, Y. Hashimoto, and H. Moriyama, *Chromatographia* **50**, 70 (1999).
- (15) T. Takeuchi, *Bunseki Kagaku* **48**, 69 (1999).
- (16) Safni, T. Takeuchi, T. Miwa, Y. Hashimoto, and H. Moriyama, *J. Chromatogr. A* **850**, 65 (1999).
- (17) T. Takeuchi, E. Suzuki, and D. Ishii, *Chromatographia* **25**, 480 (1988).
- (18) H. Small and T.E. Miller Jr., *Anal. Chem.* **54**, 462 (1982).
- (19) Safni, T. Takeuchi, N. Ito, and T. Miwa, *J. Chromatogr. A* **864**, 25 (1999). ■



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