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Short communication

Application of microcolumn ion chromatography using anion exchangers modified with dextran sulfate for the determination of alkali and alkaline-earth metal ions

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Abstract

Microcolumn ion chromatography using anion exchangers modified with dextran sulfate has been applied to the determination of alkali and alkaline-earth metal ions contained in guinea pig serum and bovine serum. These serums contained Na^+, NH_4^+, K^+, Mg^{2+} and Ca^{2+} and they were indirectly detected at 200 nm. The determination was done without any pretreatment procedure other than dilution.

Keywords: Dextran sulfate; Cations

1. Introduction

Ion chromatography has proved itself to be a powerful technique for the determination of trace organic and inorganic ions in environmental, industrial and clinical samples [1,2]. For the determination of cations, a number of cation-exchange columns have been developed and a variety of cation-exchange columns are now commercially available for applications [2].

Many papers have reported stationary phases and detection methods for alkali and alkaline-earth metal ions [3–14]. In our previous work, it has been shown that inorganic monovalent and divalent cations could be separated on anion exchangers modified with ionic polysaccharides such as heparin [13] and dextran sulfate [14]. This modification could control the ion-exchange capacity. Dextran sulfate possesses sulfate groups in each D-glucopyranosyl unit, which are strongly adsorbed to the charged surface by strong ionic interaction with the cationic groups of the stationary phase. Silica-based anion-exchange columns modified with dextran sulfate retained cations and achieved simultaneous separation of monovalent and divalent inorganic cations [14]. A portion of the anion-exchange sites remained unmodified and separation of anions on the modified anion exchangers was also demonstrated [14].

In this work, micro-scale anion-exchange columns modified with dextran sulfate were applied to the determination of alkali and alkaline-earth metal ions contained in serum samples, e.g., guinea pig serum

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and bovine serum. The present stationary phase has different selectivity from that observed in ion chromatography [14]. The determination of alkali and alkaline-earth metal ions in blood or serum is necessary because they reflect mental and physical conditions of mammalians.

2. Experimental

2.1. Apparatus

A microcolumn liquid chromatograph was assembled from an MF-2 Microfeeder (Azumadenki Kogyo, Tokyo, Japan) equipped with an MS-GAN 050 gas-tight syringe (0.5 ml; Ito, Fuji, Japan), an ML-522 microvalve injector (Jasco, Tokyo, Japan) with an injection volume of 0.2 μl and a UV-970 UV detector (Jasco). A Develosil LOP ODS (30 μm; Nomura, Seto, Japan) microcolumn (10×0.25 mm I.D.) was connected to the inlet of an IC-Anion-SW microcolumn (100×0.32 mm I.D.). These microcolumns were prepared in the laboratory as reported previously [15]. The data were handled by a C-R4A data processor (Shimadzu, Kyoto, Japan).

2.2. Reagents

Sodium salt of dextran sulfate (8000) was from Sigma (St. Louis, MO, USA). Other reagents were of reagent-grade and were from Nacalai Tesque (Kyoto, Japan). The reagents were used without any further treatment. Purified water was prepared in the laboratory using a Milli-Q Plus system (Millipore, Molsheim, France). The eluents were prepared from purified water.

2.3. Modification with dextran sulfate

The IC-Anion-SW column (100×0.32 mm I.D.) was washed with 0.05 ml purified water, 0.2 ml 10 m*M* sodium sulfate, and 0.05 ml purified water at a flow-rate of 4.2 μl min^{-1}. Aqueous solution of 1.0% sodium dextran sulfate was then passed through the column at the same flow-rate for 2 h, followed by washing with purified water until the baseline was stabilized.

3. Results and discussion

3.1. Simultaneous separation of monovalent and divalent cations

It was found that monovalent and divalent cations were retained on the anion exchanger modified with dextran sulfate. Not all of the sulfate groups of dextran sulfate interacted with the anion-exchange sites of the IC-Anion-SW silica-based anion exchanger, a portion of the sulfate groups on the modifier remained free, and acted as cation-exchange sites. The retention of cations decreased as the eluent concentration increased, which is usually observed in ion chromatography.

It was possible to demonstrate the separation of cations on the anion exchanger modified with dextran sulfate [14]. Fig. 1 demonstrates the separation of 1 m*M* each for monovalent and divalent cations on the IC-Anion-SW modified with dextran sulfate using 5 m*M* copper sulfate as eluent. The cations were indirectly detected at 200 nm, owing to the depression of background signal due to the analyte ions. In other words, the cations appeared as negative peaks.

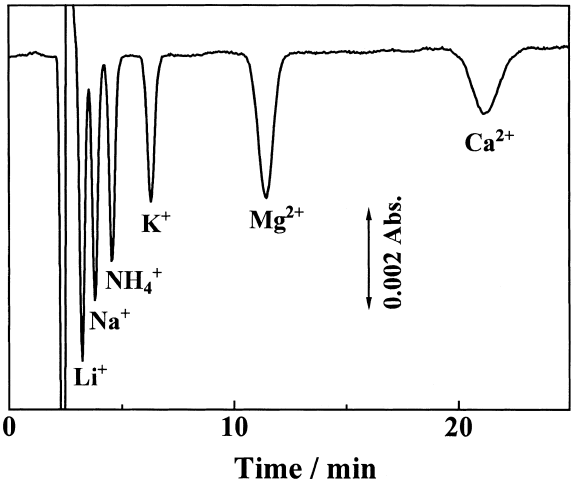

Fig. 1. Separation of monovalent and divalent cations. Column: TSKgel IC-Anion-SW (100×0.32 mm I.D.) modified with dextran sulfate 8000; pre-column: Develosil LOP ODS (10×0.25 mm I.D.); eluent: 5 m*M* $CuSO_4$; flow-rate: 4.2 μl min^{-1}; wavelength of UV detection: 200 nm; analytes: 1 m*M* each of 1=lithium, 2=sodium, 3=ammonium, 4=potassium, 5=magnesium, 6=calcium; injection volume: 0.2 μl.

3.2. Analytical figures of merit

When the column was modified with dextran sulfate 8000 and 10 m*M* aqueous copper sulfate solution was used as the eluent, the repeatability of the retention time, peak height and peak area were evaluated. The relative standard deviations of the retention time, peak height and peak area for six successive measurements were 1.9–2.7, 4.8–5.0 and 2.8–8.6%, respectively.

Good linear relationships between the concentration and the peak area were observed, as shown in Table 1. The linearity range was up to 5–10 m*M* depending on the analyte cation. Table 1 also shows the detection limits at $S/N=3$ under the conditions in Fig. 1, e.g., 26, 29, 39, 39 and 88 μ*M* for Na^+, NH_4^+, K^+, Mg^{2+} and Ca^{2+}, respectively, corresponding to 0.60, 0.52, 1.5, 0.94 and 3.5 μg ml^{-1}. On the other hand, the mass detection limits achieved by the present system were 0.12, 0.10, 0.30, 0.19 and 0.70 ng for Na^+, NH_4^+, K^+, Mg^{2+} and Ca^{2+}, respectively. The concentration sensitivity obtained by the present system was worse than that achieved by common ion chromatography with conductimetric detection, whereas the mass detection sensitivity of the present system is better or comparable to that of common ion chromatographic system.

3.3. Practical application

The present system was applied to the determination of monovalent and divalent cations present in guinea pig and bovine serums. It was possible to separate cations present in guinea pig and bovine serums on the anion exchanger modified with dextran sulfate. For this application, a Develosil LOP ODS pre-column was connected to remove hydrophobic species contained in serum samples that could be retained on the anion exchanger modified with dextran sulfate. Fig. 2A and B demonstrate the chromatograms. The serum samples were injected after 10-fold dilution with purified water. The cations detected in guinea pig and bovine serums were sodium, ammonium, potassium, magnesium and calcium ions. It took ca. 30 min for the determination of the cations contained in serum. The concentrations of sodium, ammonium, potassium, magnesium, and

Table 1
Detection limits and linearity range

Cation	Detection limits[a]		Linearity range (m*M*)
	m*M*	μg ml^{-1}	
Na^+	0.026	0.60	0.26–5
NH_4^+	0.029	0.52	0.029–10
K^+	0.039	1.5	0.039–8
Mg^{2+}	0.039	0.94	0.039–8
Ca^{2+}	0.088	3.5	0.088–10

[a] Detection limits at $S/N=3$.

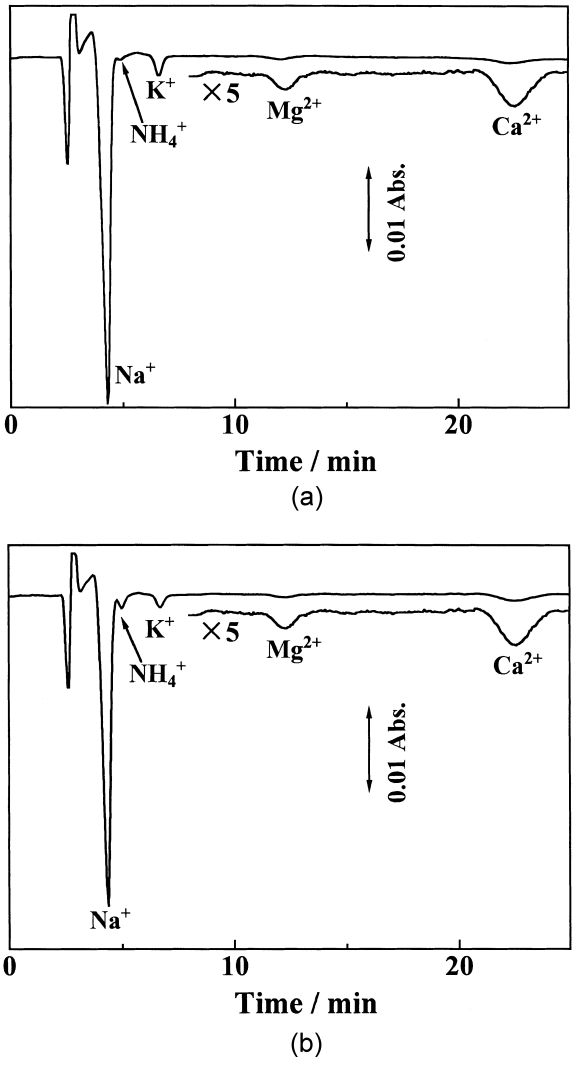

Fig. 2. Separation of monovalent and divalent cations in guinea pig serum (A) and bovine serum (B). Operating conditions as in Fig. 1 except for the sample.

calcium ions were 0.14 *M*, 0.55 m*M*, 4.9 m*M*, 0.97 m*M*, 2.6 m*M*, respectively, for guinea pig serum and 0.11 *M*, 2.5 m*M*, 3.6 m*M*, 0.87 m*M* and 4.4 m*M*, respectively for bovine serum, as shown in Table 2. For the determination of sodium ion contained in serum samples, the serums were 200-fold diluted with purified water considering the linearity range.

In addition, the lifetime of the pre-column was around 50 injections of serum samples.

Even though anions were also retained on the present column, there were no interferences from anions because the concentration of UV-absorbing anions contained in serums was negligibly small. The anions detected in guinea pig and bovine serums were nitrate and iodide. These anions were determined by anion-exchange chromatography with direct UV detection. The concentrations of nitrate and iodide were 0.19 and 0.61 μ*M*, respectively for guinea pig serum whereas they were 0.62 and 0.13 μ*M*, respectively for bovine serum. In addition, nitrate and iodide eluted in 2.45 and 3.08 min under the conditions in Fig. 2, respectively.

In conclusion, the anion exchanger modified with dextran sulfate was successfully applied to the separation of alkali and alkaline-earth metal ions contained in serum with the aid of indirect photometric detection. The determination was done without any pretreatment procedure other than dilution. The use of the Develosil LOP ODS pre-column extended the lifetime of the main column.

Table 2
Alkali and alkaline-earth metal cations contained in serum

Cation	Guinea pig serum (m*M*)	Bovine serum (m*M*)
Na^+	143	109
NH_4^+	0.55	2.5
K^+	4.9	3.6
Mg^{2+}	0.97	0.87
Ca^{2+}	2.6	4.4

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References

[1] P.K. Dasgupta, Anal. Chem. 64 (1992) 775A.
[2] P.R. Haddad, P.E. Jackson, in: Ion Chromatography – Principles and Applications, Journal of Chromatography Library, Vol. 46, Elsevier, Amsterdam, 1990, p. 485.
[3] U.S. Hong, H.K. Kwon, H. Nam, G.S. Cha, K.-H. Kwon, K.-J. Paeng, Anal. Chim. Acta 315 (1995) 303.
[4] K. Ito, T. Kumamaru, J. Chromatogr. A 850 (1999) 247.
[5] M. Trojanowicz, E. Pobozy, M.E. Meyerhoff, Anal. Chim. Acta 222 (1989) 109.
[6] K. Suzuki, H. Aruga, T. Shirai, Anal. Chem. 55 (1983) 2011.
[7] K. Watanabe, K. Tohda, H. Sugimoto, F. Eitoku, H. Inoue, K. Suzuki, S. Nakamura, J. Chromatogr. 566 (1991) 109.
[8] K. Ohta, M. Sando, K. Tanaka, P.R. Haddad, J. Chromatogr. A 752 (1996) 167.
[9] K. Ohta, H. Morikawa, K. Tanaka, Y. Uryu, B. Paull, P.R. Haddad, Anal. Chim. Acta 359 (1998) 255.
[10] K. Ohta, H. Morikawa, K. Tanaka, J. Chromatogr. A 850 (1999) 229.
[11] P. Kolla, J. Kohler, G. Schomburg, Chromatographia 23 (1987) 465.
[12] E. Munaf, R. Zein, T. Takeuchi, T. Miwa, Anal. Chim. Acta 341 (1997) 225.
[13] T. Takeuchi, Safni, T. Miwa, Y. Hashimoto, H. Moriyama, Analusis 26 (1998) 61.
[14] T. Takeuchi, Safni, T. Miwa, Y. Hashimoto, H. Moriyama, Chromatographia 50 (1999) 70.
[15] T. Takeuchi, D. Ishii, J. Chromatogr. 213 (1981) 25.