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Simultaneous Determination of Inorganic Anions by Sequential Injection Chromatography System Constructed from a Monolithic Column and a Microfluidic Polymer Chip with an Embedded Ion-Selective Electrode

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Abstract

A sequential injection chromatography (SIC) constructed from a short monolithic column and a microfluidic polymer chip with an embedded Cl^- ion-selective electrode (Cl^--ISE) as a detector was developed for simultaneous determination of common inorganic anions. Under the optimal flow conditions of the SIC system, the determination range for Cl^-, NO_2^-, Br^-, NO_3^- ions was as follows: For Cl^- ion in a concentration range from 1.0×10^{-3} to 5.0×10^{-2} mol dm^{-3}, for NO_2^- ion in the concentration range from 1.0×10^{-3} to 5.0×10^{-2} mol dm^{-3}, for Br^- ion in the concentration range from 1.0×10^{-3} to 5.0×10^{-2} mol dm^{-3}, and for NO_3^- ion in the concentration range from 3.0×10^{-4} to 1.0×10^{-2} mol dm^{-3}, respectively. Limit of detection, defined as S/N=3, was ca. 3.0×10^{-4} mol dm^{-3} for Cl^-, NO_2^-, Br^-, NO_3^- ions.

Keywords Sequential injection chromatography, monolithic column, inorganic anions, microfluidic polymer chip, ion-selective electrode detector.

1. Introduction

Much attention to the microfluidic chip technologies [1-9] have been attracted because of lower consumption of reagents and lower sample volumes. However, the detection systems for the microfluidic chips still remain large, compared with the chips themselves. Potentiometry using ISEs has been expected to be a good detection method in microfluidic chips because minimization of ISEs is possible with keeping the same selectivity and sensitivity, that is, the sensitivity of ISEs does not depend on their size. However, only a few studies regarding microfluidic chips with embedded miniaturized ISEs have been reported. [1,2,5-9] We have already developed a new microfluidic polymer chip with an embedded NO_3^--ISE as an indicator electrode and a Na^+-ISE as a reference electrode by using a small-diameter wire as a template for the channel. [9]

Recently, sequential injection analysis (SIA) has been proved to be an effective technique to satisfy the requirement of routine water analysis because the SIA methodologies have distinguished characteristics such as automatic operation, high sample throughput and low consumption of a reagent. [10-12] The SIA system has the advantages of high sample throughput and automatic operations of every procedure over the electrochemical detectors such as ISEs in SIA systems has been reported in recent years. [13-19] From the advantages of the SIA system and microfluidic chips with embedded miniaturized ISEs, the combination of the SIA system with the microfluidic chip with an embedded NO_3^- ISE, which we have developed as a detector, would be expected as an ideal automated analytical method for the determination of NO_3^- ion. Indeed, we have already reported the SIA system for the determination of NO_3^- ion using our microfluidic chip with an embedded NO_3^--ISE as a detector. [20]

A sequential injection chromatography (SIC) system constructed by incorporating a monolithic chromatography column into a SIA manifold provides a new possibility of implementation of a separation step in SIA. [21-23] In recent years, many papers [24-27] have been published on the use of ISEs for a detector in ion chromatography. Therefore, several inorganic anions would be determined simultaneously by using our SIA system with combination with a monolithic chromatography column for ion chromatography because an ISE in a plastic microfluidic polymer chip responds to several anions, depending on the selectivity of an ISE. [24-27]

In this paper, we wish to propose the SIC system for the

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*Corresponding author. E-mail address: masadome@sic.shibaura-it.ac.jp simultaneous determination of inorganic anions using our microfluidic chip with an embedded ISE as a detector.

2. Experimental

2.1 Chemicals

Tetradodecylammonium bromide (TDAB) from Sigma-Aldrich Japan Co. was used as an anion exchanger for Cl^- ISEs because the TDAB is cheaper than tetradodecylammonium chloride. Sodium tetrakis [3,5-bis(trifluoromethyl)phenyl] borate (TFPB) and bis (12-crown-4) (B12C4) were obtained from Dojindo Laboratories and used as a cation exchanger and a Na^+ ionophore for Na^+-ISEs, respectively. Poly (vinyl chloride) (PVC) (degree of polymerization: 1100) was obtained from Wako Pure Chemicals Co. 2-Nitrphenyl octyl ether (NPOE) from Dojindo Laboratories was used as a plasticizer for Cl^--ISEs and Na^+ -ISEs. All other reagents were of analytical grade.

2.2 Fabrications of Cl^--ISEs, Na^+-ISEs and microfluidic polymer chip integrated with a Cl^--ISE detector

A Cl^--ISE as an indicator electrode and a Na^+-ISE as a reference electrode were fabricated and were integrated into a microfluidic polymer chip in the same manner as reported in the previous paper. [9] A sensing membrane solution for the Cl^--ISE was prepared by dissolving NPOE (2.0 mL), PVC (0.4 g), TDAB (0.1554 g) and TFPB (0.0886 g) in 10 mL of tetrahydrofuran (THF) in a beaker. A Cl^--ISE was fabricated by coating the bare metal part of the BEAMEX-S wire (diameter of bare metal part: 0.65 mm, o.d. 1.45 mm, standard type irradiated polyethylene wire from Furukawa Electric Co. Ltd.) with a plasticized PVC membrane containing the anion exchanger by dipping it into the above sensing membrane solution several times. The fabricated Cl^- ISE was conditioned in 0.1 mol dm^{-3} NaCl solution for one day. A sensing membrane solution for the Na^+-ISE was prepared by dissolving NPOE (0.20 mL), TFPB (4.6 mg), B12C4 (10 mg) and PVC (0.10 g) in 3.0 mL of THF. The Na^+-ISE was fabricated in the same manner as that of the NO_3^--ISE by using the BEAMEX-S wire.

The chip design of a polymer-based microfluidic chip detector with the embedded Cl^--ISE as an indicator electrode and the Na^+-ISE as a reference electrode was same as that described in our previous papers. [6-9] Diameter and effective length of a channel in the polymer-based microfluidic chip are 0.5 mm. and 4.0 cm. The distance between the Cl^--ISE and the Na^+-ISE is 1.5 cm. Channels of the polymer-based microfluidic chip were fabricated using polystyrene plates (ITEM 70128 500, Tamiya Co. Ltd., Japan) and stainless-steel wires (diameter ca. 0.5 mm, SUS304-W1, Waki Industrial Co. Ltd., Japan) according to our method. [4, 6-9]

2.3 Procedures for the determination of several inorganic anions using the SIC system

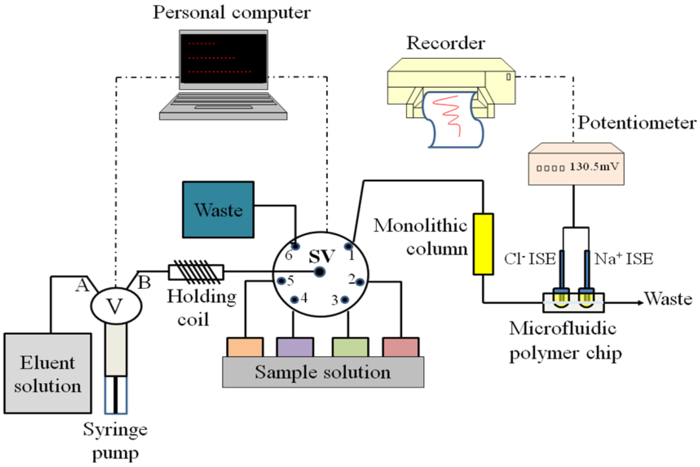

Figure 1 Schematic diagram of sequential injection chromatography system using a microfluidic polymer chip with an embedded chloride ion-selective electrode as a detector.

V: two-way port valve, SV: six-port selection valve, Holding coil: i.d. 0.5 mm x 480 cm, Monolithic column: Chromolith Flash RP-18e, 4.6 mm. i.d. x 25 mm, Merck.

Figure 1 shows a schematic diagram of an SIC system used in this work, which substitutes a popular LC system equipped with an autosampler. Table 1 shows the protocols of the experimental procedures. The SIC system is composed of a syringe pump (Carvo X Calibur Pump, Tecan Japan Co.), a two-way port valve (Carvo Smart Valve, Tecan Japan Co.), a holding coil (i.d. 0.5 mm x 480 cm), a six-port selection valve (Carvo Smart Valve, Tecan Japan Co.), a monolithic column (Chromolith Flash RP-18e, 4.6 mm i.d. x 25 mm, Merck), a polymer-based microfluidic polymer chip detector, a potentiometer (COM-20R, TOA-DKK), a recorder (LR 4120, Yokogawa Co.) and a personal computer. Acetonitrile solution was flowed into the monolithic column at the flow rate of 4.17 μL s^{-1} for 1.5 h and next, a 5 mmol dm^{-3} cetylpyridimium chloride solution was flowed into the monolithic column at flow rate of 4.17 μl s^{-1} for 2.5 h in order

Table 1 Protocols of the SIC experimental procedures

Step	Position of two way port valve	Position of selection valve	Flow rate /μl s^{-1}	Volume /μl	Flow direction	Event
1	A	-	583.3	2420	R	Aspiration of eluent solution (1)
2	B	2-5	4.17	80	R	Aspiration of sample solution into the holding coil (2)
3	B	1	6.67	2500	F	Propelling a mixture of eluent and sample solution in the holding coil to the microfluidic polymer chip, and measurement (3)
4	A	-	583.3	2500	R	(1)
5	B	1	6.67	2500	F	(3)
6	A	-	583.3	2500	R	(1)
7	B	1	6.67	2500	F	(3)
8	A	-	583.3	2500	R	(1)
9	B	1	6.67	2500	F	(3)

to create anion exchanger on the column. [28] After washing the teflon tubing and the microfludic polymer chip with an eluent (a 1x 10^{-2} mol dm^{-3} Na_2SO_4 solution), 80 μL aliquot of a sample solution was aspirated into the holding coil. Only a solution is introduced into the flow line, and thus, the injection is not sequential. The syringe pump was pushed up and sample solution in the holding coil was flowed into the monolithic column and the eluent was introduced into the microfluidic polymer chip detector via a port 1 of the selection valve.

The protocols of the experimental procedures shown in Table 1 were performed by a personal computer using a homemade software program. During the sample solution was introduced into the Cl^--ISE detector in the microfluidic polymer chip, the potential difference between the Cl^--ISE and the Na^+-ISE was monitored with a potentiometer and recorded as a peak signal. Although flow of the eluent stops, flow rate of aspiration of eluent is a much higher than that of propelling a mixture of eluent and sample solution in the holding coil to the microfluidic polymer chip. Therefore, when the analyte is in the detection cell while in eluent aspiration, the signal response almost does not change and the flow in the column continues actually.

3. Results and discussion

3.1 Sensitivity of a polymer-based microfluidic polymer chip detector to several anions

In order to examine the sensitivity of a polymer-based microfluidic polymer chip detector to several anions, a sample solution containing a single ion in the concentration range from 1.0 x 10^{-5} to 1.0 x 10^{-2} mol dm^{-3} was introduced into the microfluidic polymer chip by the SIA technique. Table 2 shows the protocols of the experimental procedures. In this case, the sample solution was aspirated into the holding coil at the volume of 275 μL and was delivered to the polymer-based microfluidic polymer chip detector with the carrier solution (a 0.02 mol dm^{-3} KH_2PO_4 + 0.0133 mol dm^{-3} Na_2HPO_4 phosphate buffer solution, pH 6.7) at the volume of 975 μL. Figure 2 shows calibration curves for several anions by using the SIA system. The peak heights and Nernstian concentration range for several anions increase in the order, $F^- < HPO_4^{2-} < CH_3COO^- < SO_4^{2-} < Cl^- < NO_2^- < Br^- < NO_3^-$. The order is almost accord with Hofmeister series.

3.2 Selection of the eluent

An eluent should satisfy the following requirements. At first, the ISE detector should not respond to an anion in an eluent. Secondly, eluent for short separation time and good resolution is required. The sensitivity of Cl- ISE to SO_4^{2-} is the lowest among SO_4^{2-}, Cl^-, NO_2^-, Br^- and NO_3^- ions. F^-, HPO_4^{2-} and CH_3COO^- ions have lower elution power, compared with SO_4^{2-} ion. From these results, Na_2SO_4 solution is expected to be profitable eluent suitable for separation of Cl^-, NO_2^-, Br^- and NO_3^- ions. An effect of the concentration of the eluent (Na_2SO_4) on separation efficiency and sensitivity of the

Table 2 Protocols of the SIA experimental procedure

Step	Position of two way port valve	Position of selection valve	Flow rate /μl.s^{-1}	Volume /μl	Flow direction	Event
1	A	-	333.3	975	R	Aspiration of carrier solution
2	B	2-5	70.8	275	R	Aspiration of sample solution into the holding coil
3	B	1	4.2	1250	F	Propelling a mixture of carrier and sample solution in the holding coil to the microfluidic polymer chip, and measurement

polymer-based microfluidic polymer chip detector was examined. Figure 3 shows chromatograms of a mixed solution containing1.0 x10^{-2} mol dm^{-3} Cl$^-$,NO$_2^-$, Br$^-$ and NO$_3^-$ ions. In case that a 0.1 mol dm^{-3} Na_2SO_4 solution was used as the eluent, every anion was separated within 10 min.

In case that a 1.0 x 10^{-2} mol dm^{-3} Na_2SO_4 solution was used as an eluent, R_s, which is a measure of the ability of a column to separate two peaks, is higher than that obtained in the case that the 5.0 x 10^{-2} mol dm^{-3} Na_2SO_4 solution was used as the eluent. Retention times for Cl$^-$, NO$_2^-$, Br$^-$ and NO$_3^-$ ions obtained in the case that a 5.0 x 10^{-2} mol dm^{-3} Na_2SO_4 solution was used as the eluent, is shorter than those obtained in the case that the 1.0 x 10^{-2} mol dm^{-3} Na_2SO_4 was used as the eluent. By compromising retention time and Rs, we chose the 1.0 x 10^{-2} mol dm^{-3} Na_2SO_4 solution was found to be as the eluent for the most effective separation condition for present anions.

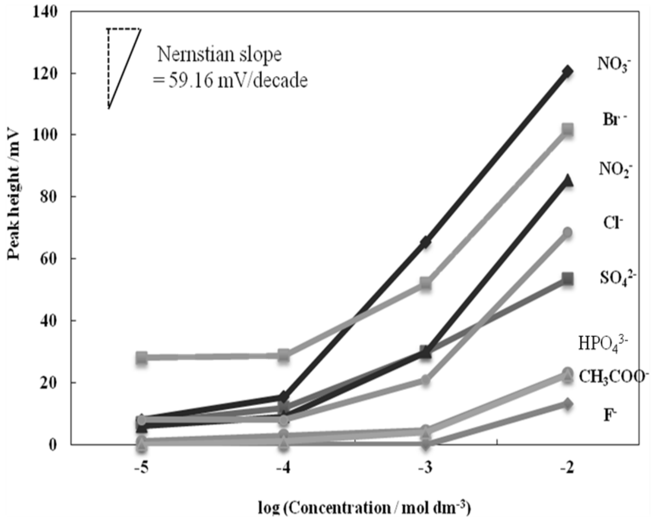

Figure 2 Calibration curves for several anions obtained by the SIA system. Experimental conditions are the same as that in Table 2.

3.3 Effect of flow rate of an eluent on separation efficiency

Effect of flow rate of an eluent on separation efficiency of several anions was examined. The flow rate was changed from 4.17 to 29.16 μl s^{-1}. The optimal flow rate of the eluent was found to be 6.7 μl s^{-1} from the $R_{s.}$

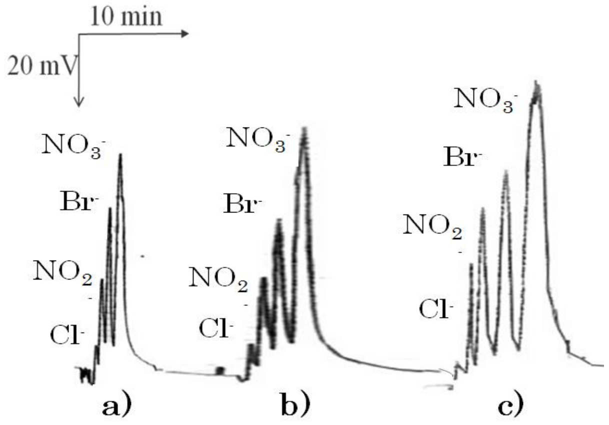

Figure 3 Chromatogram of a mixed solution containing 1.0 x 10^{-2} mol dm^{-3} Cl$^-$, NO$_2^-$, Br$^-$ and NO$_3^-$ ions obtained by using a microfluidic polymer chip with an embedded Cl$^-$ ISE as detector. Experimental conditions are the same as that in Table 1

Eluent : a) 1 x10^{-1} mol dm^{-3} Na_2SO_4 b) 5 x10^{-2} mol dm^{-3} Na_2SO_4 c) 1 x10^{-2} mol dm^{-3} Na_2SO_4

3.4 Effect of sample volume on sensitivity to several anions

In order to examine the effect of sample volume on sensitivity (peak height) of the Cl$^-$-ISE detector to several anions, a mixed sample solution (Cl$^-$, Br$^-$, NO$_2^-$, NO$_3^-$) at 1.0 x 10^{-2} mol dm^{-3} was introduced into the monolithic column by the SIC technique. In this case, the sample solution was aspirated into the holding coil at the volume from 5 μL to 100 μL and was delivered to the monolithic column. Then, the eluent of the 1.0 x 10^{-2} mol dm^{-3} Na_2SO_4 solution was flowed at the flow rate of 6.7 μl s^{-1} to the microfluidic polymer chip detector. The peak height for four anions gradually increased as the sample volume increases up to 100 μL. However, in case that the sample volume was 80 μL, Rs is the maximum value. Since sample throughput decreased as the sample volume increased, an optimal sample volume was found to be 80 μL by a compromise among sensitivity, sample throughput and R_s.

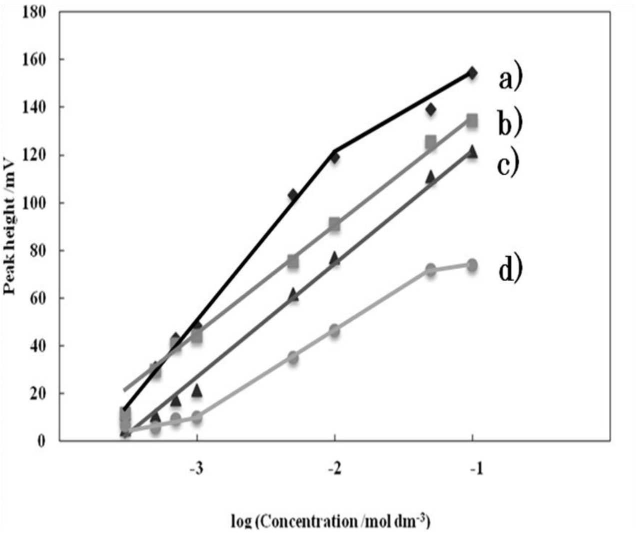

Figure 4 Calibration curves for several anions obtained under the optimal experimental conditions as shown in Table 1. a) NO_3^- ion, b) Br^- ion, c) NO_2^- ion, d) Cl^- ion.

3.5 Calibration curves for several anions obtained by using a microfluidic polymer chip with an embedded Cl- ISE in the SIC system

Figure 4 shows calibration curves for several anions obtained under the optimal conditions described above. A linear relationship was found to exist between peak heights and logarithmic concentration of the several anions under the optimal conditions described above. The graph equations are as follows: Y = 31.1 X + 104.9 for Cl^- ion in the concentration range from 1.0 x 10^{-3} to 5.0 x 10^{-2} mol dm^{-3} with a correlation coefficient of 0.995, Y= 45.0 X + 159.7 for NO_2^- ion in the concentration range from 1.0 x 10^{-3} to 5.0 x 10^{-2} mol dm^{-3} with a correlation coefficient of 0.991, Y= 40.7 X + 168.4 for Br^- ion in the concentration range from 1.0 x 10^{-3} to 5.0 x 10^{-2} mol dm^{-3} with a correlation coefficient of 0.996, Y= 55.1 X + 169.0 for NO_3^- ion in the concentration range from 3.0 x 10^{-4} to 1.0 x 10^{-2} mol dm^{-3} with a correlation coefficient of 0.986. Here, Y is peak height (mV) and X is logarithmic molar concentration of anions. Limit of detection, defined as S/N=3, was ca. 3.0 x 10^{-4} mol dm^{-3} for Cl^-, NO_2^-, Br^-, NO_3^- ions. Real water samples such as sea water and a ion supply drink often contain anions more than 10^{-3} M level. Therefore, anions in these real water samples can be determined without dilution by the proposed SIC system. The relative standard deviations of the peak heights examined by three injections of the 1.0 x 10^{-2} mol dm^{-3} anions were found to be 3.9 % for Cl^- ion, 2.3 % for NO_2^- ion, 0.87 % for Br^- ion, 1.1 % for NO_3^- ion, respectively.

4. Conclusions

A plastic microfluidic polymer chip with embedded Cl^--ISE was used as a detector for several anions in the SIC system. The present SIC system has the advantages of automatic operations over other analytical methods. Four inorganic anions were determined simultaneously by using the SIA system with combination with a monolithic column for ion chromatography because the Cl^--ISE in a plastic microfluidic polymer chip responds to several inorganic anions, depending on the selectivity of the Cl^--ISE.[24-27] The present SIC system may be useful for the determination of several anions in environmental water samples.

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