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Ion-pair chromatography of polythionates and thiosulfate with detection based on their catalytic effects on the postcolumn azide–iodine reaction

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Abstract

The reduction of iodine with azide, catalyzed by polythionates (tri-, tetra-, penta- and hexathionate) and thiosulfate, has been utilized as a postcolumn reaction for chromatographic determination of these sulfur oxyanions. The method is based on the separation of polythionates and thiosulfate on an octadecylsilica column with an acetonitrile–water (20:80, v/v) mobile phase (pH 5.0) containing 3 m*M* tetrapropylammonium hydroxide and 6 m*M* acetic acid, followed by photometric measurement of the residual iodine (as triiodide) from the catalytic postcolumn azide–iodine reaction after mixing a reaction solution containing azide and iodine with the column effluent. Chromatograms obtained for the sulfur oxyanions showed negative peaks as a result of the decrease in absorbance of background. The conditions for the catalytic postcolumn reaction of the sulfur oxyanions in the column effluents were established by varying the concentrations of azide, iodine, iodide and acetic acid in the reaction solution, and varying the flow-rate, reaction temperature and length of the reaction tube. The detection limits (defined as $S/N = 3$) were 4.3 μ*M* for trithionate, 0.10 μ*M* for tetrathionate, 2.7 n*M* for pentathionate, 5.0 n*M* for hexathionate and 1.1 n*M* for thiosulfate. When compared with earlier methods, the proposed method gave a much higher sensitivity for the determination of two polythionates (penta- and hexathionate) and thiosulfate. This method was applied successfully to the analysis of polythionates and thiosulfate added to hot-spring water samples.

Keywords: Detection, LC; Azide–iodine reaction; Polythionates; Sulfur compounds; Thiosulfate

1. Introduction

When hydrogen sulfide is passed slowly and intermittently into an acidic solution of sulfite over a period of days, polythionates ($S_xO_6^{2-}$, $x = 3$, 4, 5 or 6) are known to appear as intermediate products together with thiosulfate [1,2]. However, detailed reaction processes of polythionate and thiosulfate formation are far from being understood, in part because analysis of individual polythionates in solution is often difficult. It is therefore desirable to develop a rapid and sensitive method for the determination of a polythionate and thiosulfate mixture. Ion chromatography is one of the most powerful techniques for the rapid determination of multiple sulfur species. Various ion chromatographic methods have been reported, describing the analysis of mixtures of sulfur anions containing two or three polythionates and thiosulfate, using UV detection [3–7], conductivity detection [8–13] and polarography [14]. However, most of these methods cannot be used for determining all four polythionates ($x = 3$–6) as well

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as thiosulfate in trace amounts ($<10^{-6}$ *M* levels) [3,5,6,8–13]. Wolkoff and Larose [15] reported the sensitive determination of polythionates ($x = 3$–6) and thiosulfate by cerium(III) fluorescence, the cerium(III) being produced in a postcolumn reaction of cerium(IV) with thiosulfate and sulfite formed from polythionates by their alkaline decomposition in the postcolumn effluent stream. Story [16] reported the determination of polythionates and thiosulfate in various samples such as mine waters and industrial wastewaters without any matrix interferences. In that study, the sulfur oxyanions in the effluent were allowed to react with bromine and the resulting sulfate was measured photometrically as $FeSO_4^+$. With these methods, use of the postcolumn reaction plays a critical role in obtaining high sensitivity and selectivity in polythionate and thiosulfate determinations. The present authors [17] have previously described an ion chromatography for the highly sensitive determination of sulfide, thiosulfate and thiocyanate in a mixture, based on the photometric measurement of the excess of iodine (as triiodide) for a postcolumn azide–iodine reaction catalyzed by each sulfur anion in the effluent stream. If polythionates are able to catalyze the reaction between azide and iodine, this reaction can be expected to be useful as a postcolumn reaction for the sensitive determination of polythionates by ion chromatography. No consideration has been given in the literature to the chromatographic determination of polythionates utilizing the catalytic postcolumn reaction between azide and iodine.

In this study, conditions have been established for the resolution of polythionates ($S_xO_6^{2-}$, $x = 3$–6) and thiosulfate in their mixtures on an octadecylsilica (ODS) separating column with an acetonitrile–water mobile phase containing an ion-paring reagent of tetrapropylammonium salt (TPAOH), and then for photometric measurement of the excess iodine (as triiodide) for the postcolumn azide–iodide reaction catalyzed by each sulfur oxyanion separated in the column effluent. The proposed method gave highly sensitive determination of higher polythionates (penta- and hexathionate) and thiosulfate without any preconcentration. This method was applied successfully to the determination of polythionates and thiosulfate added to hot-spring water samples. The recoveries ranged from 94.5 to 105.2%.

2. Experimental

2.1. Chemicals

The water used in this study was redistilled and then deionized with a Model Milli-QII instrument (Nippon Millipore, Yonezawa, Yamagata, Japan). All chemicals, except for potassium polythionates and sodium azide, were of analytical-reagent grade and were used without further purification.

Potassium trithionate ($K_2S_3O_6$) was prepared by the reaction of sulfurous acid with sulfur dichloride [18], and potassium tetrathionate ($K_2S_4O_6$) by the reaction of sulfurous acid with sulfur monochloride (disulfur dichloride) [18]. The tri- and tetrathionate so obtained were purified by recrystallizing in water at temperatures below 35 and 60°C, respectively. Potassium pentathionate ($K_2S_5O_6 \cdot 1.5H_2O$) was obtained by the reaction of thiosulfate with sulfur dichloride [19], and potassium hexathionate ($K_2S_6O_6$) by the reaction of thiosulfate with sulfur monochloride [19]. The pentathionate and hexathionate were recrystallized in 0.5 *M* hydrochloric acid at temperatures below 50°C and 2 *M* hydrochloric acid at temperatures below 60°C, respectively. The polythionate salts obtained were stored at -10 ± 2°C after drying at room temperature. A stock solution (1.0 m*M*) of each standard polythionate was prepared by dissolving 68.3 mg of the trithionate, 75.6 mg of the tetrathionate, 90.4 mg of the pentathionate or 92.0 mg of the hexathionate, respectively, in water and diluting to 250 ml. The concentration of each standard stock solution was determined by cyanolysis [20,21] and/or sulfitolysis [22] assays. These standard solutions proved to be stable for 6 weeks for trithionate, 6 months for tetrathionate, 4 months for pentathionate and 2 months for hexathionate, when stored at 5 ± 2°C after preparation. Working solutions of polythionates were obtained by appropriate dilution of the standard stock solutions with water.

A stock solution (about 0.1 *M*) of thiosulfate was prepared by dissolving sodium thiosulfate pentahydrate in water containing a small amount of sodium carbonate (0.01%) as a stabilizer, and was standardized by iodometry 1 week after preparation. Working solutions of thiosulfate were obtained by

suitable dilution of the standard thiosulfate with oxygen-free water.

The sodium azide used in the procedure was purified so as to obtain good reproducibility of the chromatographic peak height. Purification of the azide reagent was carried out as follows. Sodium azide (200 g) was added to 500 ml water, and the solution was heated to dissolve the salt. To the solution cooled to around 0°C, 500 ml methanol (0°C) was added. The sodium azide precipitate was filtered by suction with a sintered-glass filter (G3), washed with ethanol (0°C), and dried at room temperature. A 2 *M* azide solution was prepared by dissolving 26.0 g of sodium azide in water and diluting to 200 ml.

A 10 μ*M* iodine–0.1 *M* iodide–1.0 *M* acetic acid solution was obtained by adding 20 ml of 0.1 m*M* iodine in methanol to a solution containing 3.3 g potassium iodide and 20 ml of 10 *M* acetic acid, and diluting the mixture to 200 ml.

An acetonitrile–water (20:80, v/v) mobile phase (pH 5.0) containing 3 m*M* tetrapropylammonium hydroxide (TPAOH) was prepared by adding 6.1 ml of 0.49 *M* TPAOH to water containing 200 ml of acetonitrile and 30 ml of 0.2 *M* acetic acid, and then diluting the mixture to 1 l; acetic acid was used to adjust the pH of the mobile phase to 5.0. The mobile phase so obtained was used after filtering through a 0.2 μm membrane filter.

2.2. Apparatus

A schematic diagram of the ion chromatographic system used is shown in Fig. 1. A pump with dual pistons (Model LC-10A, Shimadzu, Kyoto, Japan) was used to run the mobile phase through the sample injection valve (Rheodyne, Berkeley, CA, USA) equipped with a 300-μl loop and an ODS separating column (TSK gel ODS-Ts, 150 mm×4.6 mm I.D., Toso, Tokyo, Japan). An additional pump (Model DMX-2000, SNK, Tokyo) was used to mix the azide solution and the iodine solution at a T-union, and successively to pump the resulting reaction solution through a mixing tee. The absorbance of iodine (as triiodide) was measured with a photometric detector (Model LIC-10-U1, Hitachi, Tokyo, Japan). A thermostat (Model BT-15, Yamato, Tokyo, Japan) was employed for adjustment of the desired temperature

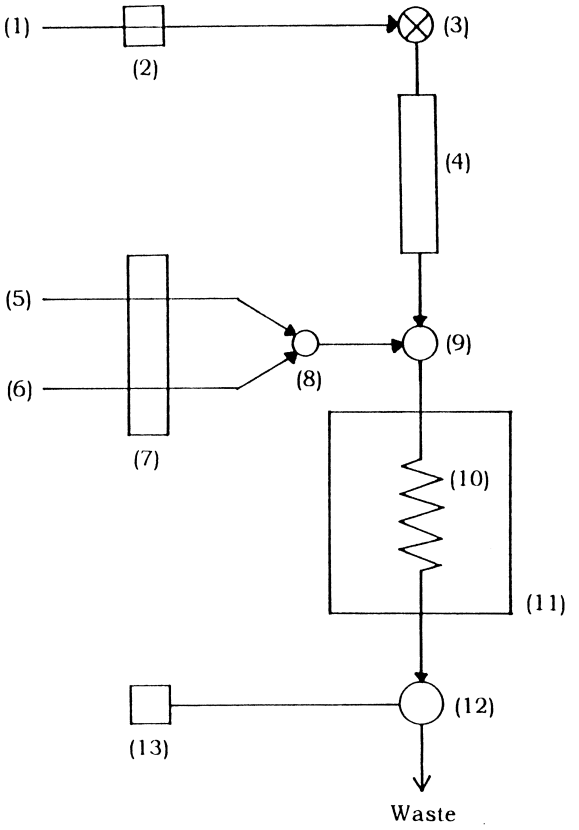

Fig. 1. Schematic diagram of an ion chromatography system. (1) Mobile phase, (2) pump, (3) injection valve, (4) separating column, (5) azide solution, (6) iodine solution, (7) pump, (8) T-union, (9) mixing tee, (10) reaction tube, (11) thermostat, (12) UV–visible photometer, (13) recorder.

of the catalytic postcolumn reaction between azide and iodine, and the chromatograms were recorded on a Shimadzu Model U-135 recorder.

2.3. Recommended procedure

The acetonitrile–water (20:80, v/v) mobile phase (pH 5.0) containing 3 m*M* TPAOH was allowed to flow at a rate of 0.6 ml min^{-1}, and then a 300-μl sample solution containing polythionates ($S_xO_6^{2-}$, $x = 3–6$) and thiosulfate was injected into the separation column, maintained at 23±2°C. The column effluent was pumped through a mixing tee, to which a reaction solution was added at 0.6 ml min^{-1} and mixed. The reaction solution was obtained by passing a 2 *M* azide solution and a 10 μ*M* iodine–0.1 *M*

iodide–1.0 *M* acetic acid solution at 0.3 ml min^{-1} through a T-union. The mixture obtained at the mixing tee subsequently flowed through a reaction tube (7 m×0.5 mm I.D.) thermostated at 30°C, in which the sulfur oxyanions catalyzed the reaction between azide and iodine. The absorbance of the residual iodine (as triiodide) for the catalytic reaction in the stream was monitored by photometry at 350 nm.

3. Results and discussion

3.1. Calibration graphs

A 300-μl aliquot of a sample solution containing polythionates ($x = 3–6$) and thiosulfate was treated exactly according to the procedure detailed in Section 2.3. Chromatograms obtained for the sulfur oxyanions are shown in Fig. 2. Thiosulfate and polythionates ($S_xO_6^{2-}$, $x = 3$, 4, 5 and 6) were detected at elution times of 9.1, 10.5, 11.9, 13.3 and 20.8 min, respectively. Using the height of the chromatographic peaks plotted versus concentration, each calibration graph gave a straight line in the range from the origin up to 0.10 μ*M* for thiosulfate, 0.20 m*M* for trithionate, 8.0 μ*M* for tetrathionate, 0.20 μ*M* for pentathionate and 0.40 μ*M* for hexathionate, respectively, with correlation coefficients greater than 0.999. From five replicates obtained for a mixture of 60.0 n*M* (6.72 ppb) thiosulfate, 0.100 m*M* (19.2 ppm) trithionate, 2.40 μ*M* (538 ppb) tetrathionate, 60.0 n*M* (15.4 ppb) pentathionate and 0.120 μ*M* (34.6 ppb) hexathionate, the proposed method afforded a mean value of 59.8 n*M* (6.70 ppb) for thiosulfate with a relative standard deviation (RSD) of 1.0%, 0.101 m*M* (19.4 ppm) for trithionate (RSD 2.3%), 2.42 μ*M* (542 ppb) for tetrathionate (RSD 1.6%), 59.8 n*M* (15.3 ppb) for pentathionate (RSD 1.4%) and 0.118 μ*M* (34.0 ppb) for hexathionate (RSD 1.8%), respectively. The detection limits (defined at $S/N = 3$) were 1.1 n*M* (0.12 ppb) for thiosulfate, 4.3 μ*M* (830 ppb) for trithionate, 0.10 μ*M* (22 ppb) for tetrathionate, 2.7 n*M* (0.69 ppb) for pentathionate and 5.0 n*M* (1.4 ppb) for hexathionate. This method gave much lower detection limits for higher polythionates (penta- and hexathionate) and thiosulfate as compared to earlier methods [3–5,7–16].

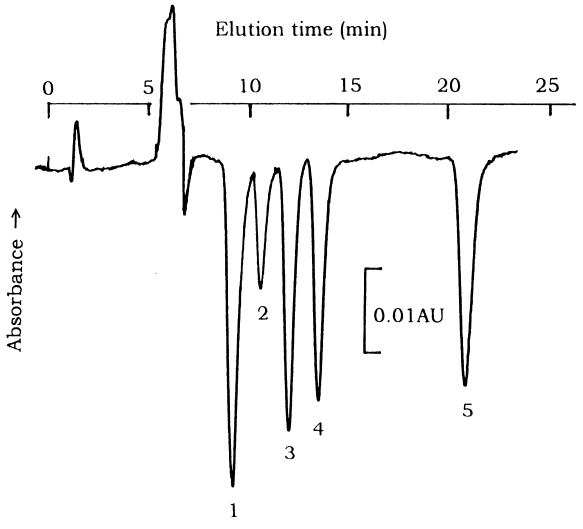

Fig. 2. Chromatograms of five sulfur oxyanions in a mixture. Peak identification: 1=$S_2O_3^{2-}$ (50 n*M*), 2=$S_3O_6^{2-}$ (70 μ*M*), 3=$S_4O_6^{2-}$ (3.5 μ*M*), 4=$S_5O_6^{2-}$ (0.10 μ*M*), 5=$S_6O_6^{2-}$ (0.15 μ*M*).

3.2. Resolution of sulfur oxyanions in a mixture

In order to separate polythionates and thiosulfate in their mixtures, two columns were investigated under the conditions described in Section 2.3; one was a TSK gel ODS-Ts column (Section 2.2) and the other was a Kaseisorb LC super column (150 mm× 4.6 mm I.D., Tokyo Kasei, Tokyo, Japan). The TSK column and the Kaseisorb column were packed with spherical ODS packings of 5 μm diameter and pore sizes of 80 and 120 Å, respectively. Separations of the sulfur oxyanions (except for trithionate) in a mixture are shown in Fig. 3. When compared with the Kaseisorb column, the TSK column gave a better resolution of sulfur oxyanions and complete separation of tetrathionate from pentathionate. The TSK column gave capacity factors of 0.52, 0.98, 1.23 and 2.47 for thiosulfate and polythionates ($S_xO_6^{2-}$, $x = 4$, 5 and 6), respectively. In addition, the effects of the concentration of each TPAOH ion-pairing reagent and acetonitrile in the mobile phase on the resolution of thiosulfate and polythionates were measured. First, acetonitrile–water (20:80, v/v) solutions (pH

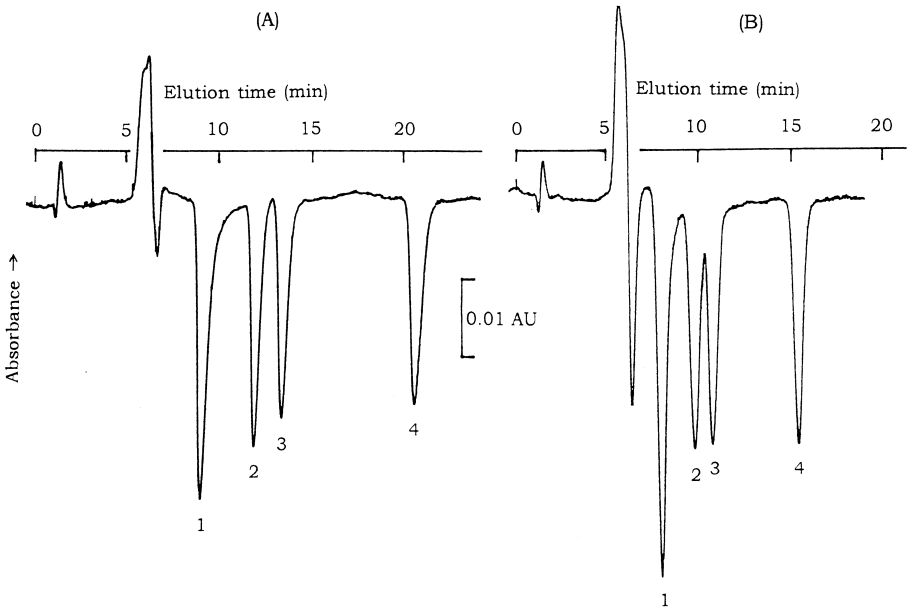

Fig. 3. Effect of separating column on the resolution of peaks for the sulfur oxyanions. (A) Obtained using a TSK gel ODS-Ts. (B) Obtained using a Kaseisorb LC super. Peak identification: 1=$S_2O_3^{2-}$ (50 n*M*), 2=$S_4O_6^{2-}$ (3.5 μ*M*), 3=$S_5O_6^{2-}$ (0.10 μ*M*), 4=$S_6O_6^{2-}$ (0.15 μ*M*).

5.0) containing various amounts of TPAOH were used as mobile phases. When TPAOH concentrations lower than 2 m*M* were employed, tri- and pentathionate could not be separated from thiosulfate and tetrathionate, respectively, because the elution time of trithionate was very close to that of thiosulfate and the elution time of pentathionate was very close to that of tetrathionate. TPAOH concentrations greater than 3 m*M* resulted in the complete separation of the five sulfur oxyanions, but the use of 5 m*M* TPAOH required about 27 min for the elution of hexathionate. Consequently, 3 m*M* TPAOH was used in the mobile phase. Next, various amounts (17 to 25%, v/v) of acetonitrile were added to the mobile phase (pH 5.0) of 3 m*M* TPAOH. A mobile phase (pH 5.0) containing 17% acetonitrile resulted in a long elution time for hexathionate (about 35 min). When the acetonitrile concentration was increased to 20%, all five sulfur oxyanions (polythionates and thiosulfate) could be separated completely within 21 min. However, a high concentration of acetonitrile (25%) failed to separate thiosulfate from trithionate and

Table 1
Effect of the concentration of azide solution on the height of chromatographic peaks for sulfur oxyanions

Concentration of azide (*M*)	Peak height[a] (mm) Mean±standard deviation			
	$S_2O_3^{2-}$, 60.0 n*M*	$S_4O_6^{2-}$, 4.00 μ*M*	$S_5O_6^{2-}$, 0.100 μ*M*	$S_6O_6^{2-}$, 0.200 μ*M*
1.0	66.1±1.05	17.1±1.10	15.3±0.83	16.0±1.00
1.5	89.6±1.00	42.9±0.98	37.2±0.89	41.0±0.97
2.0	100.7±1.06	72.9±0.96	66.3±1.10	72.8±0.96
2.5	111.5±1.00	110.5±0.80	103.1±0.90	110.5±0.95

[a] Peak height was obtained from four replicates at 0.1 AUFS and 350 nm.

tetrathionate from pentathionate. Subsequently, an acetonitrile–water (20:80, v/v) solution (pH 5.0) containing 3 m*M* TPAOH was used as the mobile phase throughout.

3.3. Effect of concentration of azide solution

To measure the effect of the concentration of azide solution on peak height for the sulfur oxyanions, various azide solutions (1.0 to 2.5 *M*) were employed (prepared according to Section 2.3). The results are shown in Table 1. The peak height for each sulfur oxyanion was increased greatly by increasing azide concentration. However, azide concentrations were limited to less than 2.5 m*M*, owing to the strongly toxic property of azide.

3.4. Effects of concentrations of iodine, iodide and acetic acid in iodine solution

When measuring the effect of iodine concentration, various amounts of iodine were added to a 0.1 *M* iodide–1.0 *M* acetic acid solution. Addition of iodine in the range 8–15 μ*M* gave the same maximal peak height for each polythionate ($S_xO_6^{2-}$, $x = 4$, 5 and 6) and thiosulfate. The concentration of iodine thereafter was 10 μ*M*.

In addition, various amounts of iodide were used in a 10 μ*M* iodine–1.0 *M* acetic acid solution in order to measure the effect of iodide concentration. The results are shown in Table 2. By increasing the iodide concentration from 0.01 to 0.15 *M*, the sulfur-oxyanion peak heights were enhanced. Iodide concentrations of more than 1.5 *M*, however, led to a decrease in stability of the iodine solution. Iodide in solution thereafter was adjusted to 1.0 *M*.

In preliminary experiments, polythionates were found to catalyze the reaction between azide and iodine in an acidic medium. To acidify the catalytic postcolumn-reaction mixture, various amounts of acetic acid were added to a 10 μ*M* iodine–0.1 *M* iodide solution. When acetic acid was used in the range 0.5–1.5 *M*, each sulfur oxyanion caused maximal peak height; 1.0 *M* acetic acid was used in the iodine solution.

Consequently, 10 μ*M* iodine–0.1 *M* iodide–1.0 *M* acetic acid was employed as the iodine solution in all subsequent work. This iodine solution was stable for at least 10 h at room temperature.

3.5. Effect of the flow-rate of the reaction solution

In another experiment, the effect of the flow-rate of the reaction solution on peak height for the sulfur oxyanions was assessed. When the reaction solution was pumped at a flow-rate in the range 0.6 to 1.0 ml min^{-1}, the highest peak height was obtained. Therefore, the reaction solution was used at 0.6 ml min^{-1}, that is the 2 *M* azide solution and the 10 μ*M* iodine–0.1 *M* iodide–1.0 *M* acetic acid solution were each pumped at 0.3 ml min^{-1}.

3.6. Effect of the length of the postcolumn reaction tube

An increase in length of the reaction tube might be expected to enhance peak height, because it increases postcolumn reaction time. Various tube lengths (0.5 mm I.D.) were employed in order to measure this

Table 2
Effect of iodide concentration in iodine solution on peak heights for sulfur oxyanions

Concentration[a] of iodide (*M*)	Peak height[b] (mm) Mean±standard deviation			
	$S_2O_3^{2-}$, 60.0 n*M*	$S_4O_6^{2-}$, 4.00 μ*M*	$S_5O_6^{2-}$, 0.100 μ*M*	$S_6O_6^{2-}$, 0.200 μ*M*
0.01	38.1±0.89	31.8±1.02	27.7±1.10	29.2±1.12
0.05	82.2±1.15	59.6±1.00	52.0±1.01	57.4±1.14
0.10	100.7±1.06	72.9±0.96	66.3±1.10	72.8±0.96
0.15	105.1±1.00	82.2±1.00	73.4±1.17	83.0±1.13

[a] Various concentrations of iodide were used in a 10 μ*M* iodine–1.0 *M* acetic acid solution.
[b] Peak height was obtained from four replicates at 0.1 AUFS and 350 nm.

Table 3
Effect of temperature of the postcolumn reaction on the height of chromatographic peaks for sulfur oxyanions

Temp. (°C)	Peak height[a] (mm) Mean±standard deviation			
	$S_2O_3^{2-}$, 60.0 n*M*	$S_4O_6^{2-}$, 4.00 μ*M*	$S_5O_6^{2-}$, 0.100 μ*M*	$S_6O_6^{2-}$, 0.200 μ*M*
15	93.3±1.01	23.6±0.98	20.4±1.03	22.6±1.02
25	100.2±1.22	49.3±1.09	42.8±1.01	45.1±1.06
30	100.7±1.06	72.9±0.96	66.3±1.10	72.8±0.96
35	101.1±1.08	100.1±1.28	100.0±1.21	94.6±1.14

[a] Peak height was obtained from four replicates at 0.1 AUFS and 350 nm.

effect. By increasing the reaction tube length from 2 to 9 m, the peak height for each polythionate increased, but that for thiosulfate decreased. This decrease was attributed to peak broadening after postcolumn reaction. Subsequently, a 7-m reaction tube was employed in further experiments.

3.7. Effect of temperature of the catalytic postcolumn reaction

To measure the effect of postcolumn reaction temperature on peak height, the thermostat temperature was increased from 15 to 35°C. Each polythionate showed a great increase in peak height (Table 3), but thiosulfate reached a maximum at 25°C, and remained constant in the range 25–35°C. The temperature was adjusted to 30°C in all subsequent experiments.

3.8. Determination of sulfur oxyanions added to hot-spring water samples

The effects of various ions on the determination of polythionates ($S_xO_6^{2-}$, $x = 3$–6) and thiosulfate were investigated. Ions such as F^-, Cl^-, Br^-, BrO_3^-, IO_3^-, NO_2^-, NO_3^-, CO_3^{2-}, S^{2-}, SO_3^{2-}, SO_4^{2-}, HPO_4^{2-}, $HCOO^-$, CH_3COO^-, NH_4^+, Na^+, K^+, Mg^{2+}, Ca^{2+}, Zn^{2+}, Co^{2+}, Cu^{2+}, Fe^{3+} and Al^{3+} all eluted at shorter retention times than thiosulfate. Except for BrO_3^-, IO_3^-, NO_2^-, S^{2-} and SO_3^{2-}, these ions, at concentrations as high as 1 m*M*, did not interfere with thiosulfate determination. BrO_3^-, IO_3^- and NO_2^- gave positive peaks as a result of the formation of iodine in their reactions from iodide in the reaction solution, while S^{2-} and SO_3^{2-} gave negative peaks because they reduced iodine. In large amounts (>0.5 m*M*) these five anions interfered with thiosulfate determination, because their large peaks partially overlapped with the thiosulfate peak. Concentrations below 0.1 m*M* did not give any interference. When nitrogen gas was bubbled through a sample solution containing S^{2-} and SO_3^{2-} after acidification according to previous methods [23,24], interferences of S^{2-} and SO_3^{2-} in amounts of up to 0.2 m*M* could be eliminated, because they were evolved as H_2S and SO_2, respectively. The proposed method was applied to the determination of polythionates ($S_xO_6^{2-}$, $x = 3$–6) and thiosulfate mixed in various ratios. Each

Table 4
Determination of sulfur oxyanions mixed in various ratios

Mixture					Found				
$S_2O_3^{2-}$ (n*M*)	$S_3O_6^{2-}$ (m*M*)	$S_4O_6^{2-}$ (μ*M*)	$S_5O_6^{2-}$ (μ*M*)	$S_6O_6^{2-}$ (μ*M*)	$S_2O_3^{2-}$ (n*M*)	$S_3O_6^{2-}$ (m*M*)	$S_4O_6^{2-}$ (μ*M*)	$S_5O_6^{2-}$ (μ*M*)	$S_6O_6^{2-}$ (μ*M*)
20.0	–	6.00	0.100	0.800	19.3	–	5.86	0.097	0.760
36.6	0.100	–	–	–	36.6	0.104	–	–	–
40.0	–	4.00	0.800	0.200	38.0	–	4.00	0.807	0.207
58.6	0.100	–	–	–	60.6	0.105	–	–	–
60.0	–	1.00	0.600	0.300	60.3	–	1.00	0.593	0.303
80.0	–	2.00	0.400	0.100	79.7	–	2.00	0.393	0.095

Table 5
Recoveries of sulfur oxyanions added to hot-spring water samples

Sample	Dilution (fold)	Added					Found					Recoveries				
		$S_2O_3^{2-}$ (n*M*)	$S_3O_6^{2-}$ (μ*M*)	$S_4O_6^{2-}$ (μ*M*)	$S_5O_6^{2-}$ (μ*M*)	$S_6O_6^{2-}$ (μ*M*)	$S_2O_3^{2-}$ (n*M*)	$S_3O_6^{2-}$ (μ*M*)	$S_4O_6^{2-}$ (μ*M*)	$S_5O_6^{2-}$ (μ*M*)	$S_6O_6^{2-}$ (μ*M*)	$S_2O_3^{2-}$ (%)	$S_3O_6^{2-}$ (%)	$S_4O_6^{2-}$ (%)	$S_5O_6^{2-}$ (%)	$S_6O_6^{2-}$ (%)
I	0	0	0	0	0	0	0	0	0	0	0	–	–	–	–	–
	1.5	38.6	40.0	4.70	0.65	0.200	40.6	40.0	4.68	0.630	0.200	105.2	100.0	99.6	96.9	100.0
II	25	0	0	0	0	0	[a]	[b]	0	0	0	–	–	–	–	–
		0	30.0	3.50	1.10	0.200	[a]	[b]	3.50	1.04	0.196	–	–	100.0	94.5	98.0
	500	0	0	0	0	0	12.8	0	0	0	0	–	–	–	–	–
		20.0	43.5	4.70	1.10	0.080	33.9	44.1	4.88	1.08	0.080	104.5	101.4	103.8	98.2	100.0
III	100	0	0	0	0	0	[a]	[b]	0	0	0	–	–	–	–	–
		0	0	5.00	1.50	0.200	[a]	[b]	4.85	1.50	0.202	–	–	97.0	100.0	101.0
	500	0	0	0	0	0	19.0	0	0	0	0	–	–	–	–	–
		19.5	27.5	0.53	1.05	0.100	39.5	27.3	0.525	1.01	0.093	105.1	99.3	99.1	96.2	101.1

[a] The content was too much to be determined.
[b] The trithionate peak could not be separated completely from a large peak of thiosulfate of high concentration.

sulfur oxyanion could be determined with an error of less than 5%, as can be seen in Table 4. Moreover, this method was used for the determination of polythionates ($S_xO_6^{2-}$, $x = 3–6$) and thiosulfate in hot-spring water samples in order to confirm its utility. The results are shown in Table 5. Although thiosulfate in the two hot-spring waters, II and III, could be determined, polythionates were absent. Therefore, we attempted to recover the sulfur oxyanions added to the hot-spring waters. Recoveries ranged from 94.5 to 105.2%. Chromatograms for the sulfur oxyanions added to the hot-spring water II in Table 5 are shown in Fig. 4.

The peak for the added trithionate could not be

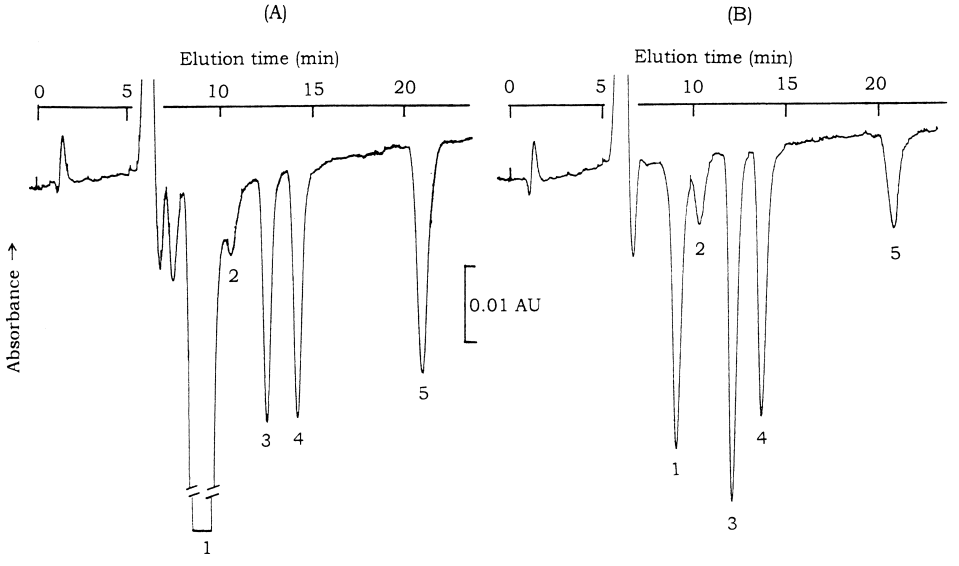

Fig. 4. Chromatograms of polythionates and thiosulfate added to hot-spring water samples. (A) Obtained for the hot-spring water (b) diluted 25-fold, to which $S_3O_6^{2-}$ (30.0 μ*M*), $S_4O_6^{2-}$ (3.50 μ*M*), $S_5O_6^{2-}$ (1.10 μ*M*) and $S_6O_6^{2-}$ (2.00 μ*M*) had been added. (B) Obtained for the hot-spring water (b) diluted 500-fold, to which $S_2O_3^{2-}$ (20.0 n*M*), $S_3O_6^{2-}$ (43.5 μ*M*), $S_4O_6^{2-}$ (4.70 μ*M*), $S_5O_6^{2-}$ (1.10 μ*M*) and $S_6O_6^{2-}$ (0.80 μ*M*) had been added. Peak identification: $1=S_2O_3^{2-}$, $2=S_3O_6^{2-}$, $3=S_4O_6^{2-}$, $4=S_5O_6^{2-}$, $5=S_6O_6^{2-}$.

separated from a large peak for thiosulfate because thiosulfate was present in large amounts, while added tetra-, penta- and hexathionate were completely separated and their peaks had a good shape (Fig. 4A). The separation of trithionate and thiosulfate was achieved by diluting 500 times (Fig. 4B). This demonstrates that unknown species in the hot-spring waters did not interfere with the determination of the sulfur oxyanions. The present method can be applied successfully to the determination of thiosulfate and polythionates in hot-spring waters.

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