Studies of seawater analysis by capillary isotachophoresis

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神戸大学博士論文

Studies of Seawater Analysis by Capillary Isotachophoresis

（細管式等速電気泳動法による海水分析に関する研究）

昭和64年1月

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Contents

Introduction

The sea is very important considering from the standpoints of the earth science, the environmental science and the resources science. At present, eutrophication is rapidly progressing in whole sea-area. Several problems (e.g. increase of deoxygenated sea-area and ecotypic change) arise along with the eutrophication.

On the other hand, development of ocean resources dissolved in seawater has attracted attention recently. For this purpose, simple analytical methods with high sensitivity and precision are required. Thus it is important to develop new analytical methods for determination of various components in seawater.

Further, chemical speciation of an element dissolved in seawater is very important as well as its determination of the total amount. Because the chemical behavior is dependent on its chemical form. Two kinds of method are carried out for the speciation of the element. One is the estimation method from information obtained by the following analytical methods: solvent extraction, dialysis, ultrafiltration, chromatography, electrochemical methods (e.g. polarography and voltammetry), etc. Another is the calculation method developed by L. G. Sillén; thermodynamic equilibrium in seawater is assumed, and known equilibrium constant, stability constant of complex, pH, redox potential, temperature, pressure and ionic strength are used for the calculation. The latter method is mostly used for estimation of inorganic species.

In recent years, capillary isotachophoresis (abbreviated as CITP) has been proposed by A. J. P. Martin and F. M. Everaerts; it is said to be the 3rd analytical method for rapid separation and determination of various ionic species following gas chromatography and high performance liquid chromatography. CITP has the following characteristics; i.e. various ionic species which are difficult to be determined by conventional analytical methods are determined with relative ease; pretreatment is not generally required; wide molecular weight range of samples can be separated. Thus CITP may be suitable for determination of various chemical species in seawater. CITP has so far been widely used for determination of various components ranging from inorganic ions with low molecular weight to protein with high molecular weight in all branches of natural science. However, it has not ever been applied to seawater analysis.

Therefore, in this study, applications of CITP to seawater analysis were investigated for characterization of chemical species dissolved in seawater. As generally well known, seawater is not a pure 3 % sodium chloride solution; it is a mixed solution containing chlorides and sulfates of alkali and alkaline-earth metals as major constituents, 10^{-6} M organic matters and $10^{-10} \sim 10^{-7}$M trace elements. Thus, in the applications of CITP to seawater analysis, a suitable pretreatment is necessary for the separation or the enrichment of a primary component from high concentrations of coexisting substances. In this study, coprecipitation enrichment, gas-liquid separation with a tubular microporous polytetrafluoroethylene (abbreviated as PTFE)membrane and ion-exchange separation were used as the pretreatment method. This thesis consists of 7 chapters.

In Chapter 1, preliminary studies of CITP were described. At first, the electrolyte system and the quantity of sample injected into the isotachophoretic analyser were examined for the simultaneous determination of some sulfur oxoacid ions. The established conditions were applied to the investigation of decomposition behavior of peroxodisulfate. Then the interaction between potassium ion and 18-crown-6 was applied to the simultaneous determination of low concentrations of ammonium and potassium ions. At last, the following conditions were examined for the determination of some metal-ethylenediaminetetraacetic acid (abbreviated as EDTA) complexes: effects of the length of the precolumn and the main column, kinds of viscous agents, counter-ions and terminating electrolytes, pH of leading electrolyte and the volume of sample.

In Chapter 2, studies on the determination of halide ions in seawater by CITP were described. At first, a CITP method for fluoride ion was developed; preliminary coprecipitation with magnesium hydroxide was used for the separation and enrichment of the fluoride ion from high concentrations of chloride, sulfate ions, etc. Then a CITP method for bromide ion was developed; a gas-liquid separator was used for the separation and enrichment of bromide ion.

In Chapter3, a CITP method for sulfide in seawater was developed; the gas-liquid separator was used for the separation and enrichment of sulfide.

In Chapter4, the coprecipitation with magnesium hydroxide was applied to the determination of various phosphate ions such as triphosphate, diphosphate and orthophosphate ions in seawater.

In Chapter 5, studies on the determination of alkali metal ions in seawater by CITP were described. At first, a CITP method for potassium ion was developed; ion-exchange separation using ammonium chloride solutions as the eluents was used. In this method, a new electrolyte system was used for the simultaneous determination of potassium and sodium ions in a solution containing a large amount of ammonium ion. Then a CITP method for ammonium ion was developed; an improved gas-liquid separator was used for the separation and enrichment of ammonium ion. The electrolyte system for the determination of ammonium and potassium ions proposed in Chapter 1 was adopted with a slight modification in this method.

In Chapter 6, studies on the determination of alkaline-earth metal ions in seawater by CITP were described. At first, a CITP method for magnesium and calcium ions was developed; ion-exchange separation using EDTA solution as the eluent was used. The analytical conditions for the determination of some metal-EDTA complexes established in Chapter 1 was adopted in this method. Then a CITP method for strontium ion was developed; ion-exchange separation using EDTA and glycoletherdiamine-N, N, N′ , N′ -tetraacetic acid (abbreviated as GEDTA)solutions as the eluents was used.

In Chapter 7, a CITP method for total carbon dioxide (abbreviated as TCO_2)in seawater was developed;the same gas-liquid separator used in Chapters 2 and 3 was used for the separation and enrichment of all carbonate substances. Further, concentrations of various forms of carbonate compounds were determined by calculations.

1 Preliminary studies of capillary isotachophoresis

1.1 Introduction

There are several reports[1,2] concerning the isotachophoretic determination of some sulfur oxoacid ions, but no investigation concerning the determination of peroxodisulfate ion which has relatively high mobility is reported;i.e. the equivalent ionic conductivity of the ion is 86 Scm^2mol^{-1} at 25℃ in aqueous solution[3].

Therefore, the electrolyte system and the quantity of sample injected into the isotachophoretic analyser were examined to establish the optimum conditions for the simultaneous determination of peroxodisulfate and sulfate ions by CITP. Further, the established conditions were applied to the determination of disulfate, tetrathionate ions, etc. which had not ever been determined by CITP.

A heating digestion method of various phosphorus compounds with peroxodisulfate[4] is commonly used for the determination of total phosphorus in aqueous samples. An autolysis rate of peroxodisulfate in this method was examined by Namiki et al.[5]

Therefore, the decomposition behavior of peroxodisulfate was studied in detail by using CITP; i.e. the effects of the kind and the concentration of peroxodisulfate, temperature, presence of sulfuric acid and kind of organic phosphorus compound on the decomposition rate of peroxodisulfate were investigated.

The separation of ammonium and potassium ions by CITP is possible with methanolic electrolytes, but not with aqueous electrolytes(6). However, it is difficult to use a longer migration tube and to apply a higher migration current with the methanolic electrolytes(7). For these reasons, when the methanolic electrolytes are used a large volume of sample solution cannot be injected into the isotachophoretic analyser for the purpose of determination of low concentration of ions. Tazaki et al.(8) studied the isotachophoretic behavior of mM concentrations of alkali and alkaline-earth metal ions in the presence of larger amounts of 18-crown-6 and evaluated its usefulness for their separation.

Therefore, the author has studied the simultaneous determination of low concentrations of ammonium and potassium ions by CITP with smaller amounts of 18-crown-6, for the purpose of determination of trace amounts of ammonium ion in seawater. By using the method proposed in this paper, the author has performed the separation of mixtures containing ammonium and potassium ions at concentrations less than 2.0mg/l, within 18 min, with injection of a large volume of sample solution.

There are several papers dealing with the use of complex-forming equilibria for metal analysis and the behavior of some complexes in CITP(9~17). Some alkaline-earth metals have been determined simultaneously using complex-forming equilibria between 1, 2-cyclohexanediamine-N, N, N′ , N′ -tetraacetic acid (abbreviated as CyDTA)and their ions by Nukatsuka et al.$^{15)}$ The isotachophoretic behavior of some bivalent and trivalent metal-EDTA complexes have been studied by Yoshida et al.$^{11)}$, and it was found that the mutual separation of the former was difficult owing to their similar mobility. There is no report concerning the isotachophoretic separation of Mg(II)-EDTA and Ca(II)-EDTA complexes and their simultaneous determination.

Therefore, the author has studied the effects of the length of the precolumn and the main column, viscous agents, counterions, terminating electrolytes, pH of leading electrolyte and the volume of sample injected into the isotachophoretic analyser on the separation of EDTA, Mg(II)-EDTA and Ca(II)-EDTA complexes to establish the optimum analytical conditions for their simultaneous determination. Under the established conditions, Cu(II)-EDTA and Mn(II)-EDTA were also determined successfully. The error in the determination of these EDTA complexes was less than ± 20.0 %.

1.2 Experimental

1.2.1 Apparatus

A Shimadzu IP-1B and IP-2A isotachophoretic analysers were used, with potential gradient detectors. The main column was a fluorinated ethylene-propylene (abbreviated as FEP) copolymer tube, 20~ 50 cm long, 0.5 mm inner diameter; the precolumn was a

PTFE tube, 0~ 20 cm long, 1.0 mm inner diameter. A Terumo MS-10 and a Hamilton 1725-N microsyringes were used for the injection of samples. A Tokyo-Rikakikai SB-35 water-bath was used for heating peroxodisulfate solutions. A Philips PW 9421 pH meter was also used for adjusting the pH of solutions.

1.2.2 Reagents

All solutions were prepared from analytical reagent grade chemicals. Standard solutions of some sulfur oxoacid ions were prepared by dissolving the following salts in water from a Yamato-Kagaku WAR-30 automatic still: potassium peroxodisulfate, ammonium peroxodisulfate, sodium peroxodisulfate, potassium sulfate, ammonium sulfate, sodium sulfate, potassium disulfate, sodium dithionate and potassium tetrathionate. The following organic phosphorus compounds were used: disodium p-nitrophenylphosphate, triphenyl phosphite, disodium phenylphosphate, adenosine-5′ -triphosphate, disodium D-glucose-6-phosphate. 18-crown-6 and hydroxypropyl methyl cellulose(abbreviated as HPMC) were obtained from the Aldrich Chemical Co. EDTA complexes obtained from Dojin Chemical Laboratory were used for the preparation of 0.1 M solutions. These solutions were used after proper dilution.

1.3 Results and Discussion

1.3.1 Determination of some sulfur oxoacid ions

1.3.1.1 Electrolyte system

The mobility of peroxodisulfate ion is relatively high. The leading ion with higher mobility than that of the ion should be used for the isotachophoretic determination of the ion. Ten mM sodium hydroxide was used as the leading electrolyte for the determination of dithionate ion with higher mobility3) than that of peroxodisulfate ion18). Therefore, the above leading electrolyte and 10mM hexanoic acid or 10 mM sodium acetate as the terminating electrolyte were adopted, but peroxodisulfate ion was not separated from sulfate ion, where the temperature of the thermostat was adjusted at 20 °C and the migration current was maintained at 50 μA. In general, separation of ions can be expected to succeed by using electrolytes containing organic solvents when they are not separated by using aqueous electrolytes19). Thus peroxodisulfate ion was separated from sulfate ion by using 10 mM sodium hydroxide containing 50 % acetone as the leading electrolyte. Further, the separation of these ions was greatly improved by adding 0.1 % Triton X-100 to this leading electrolyte20). Shiogai et al. 21) described that the sharpness of the zone boundary between glutamine and glycine was reduced when the leading ion concentration was lowered. Therefore, the effect of the leading ion concentration was examined for the separation of peroxodisulfate and sulfate ions by using 10 and 5 mM sodium hydroxide. The sharper separation was obtained by using the former concentration (10mM), while the analysis time was 2 times longer than that of the latter (5 mM).

On the other hand, the following terminating electrolytes were examined: 10 mM hexanoic acid and 10 mM sodium acetate. The carbonate ion in the leading electrolyte was separated from the hexanoate ion, but was not from the acetate ion. Therefore, 10 mM sodium hydroxide containing 50 % acetone and 0.1 % Triton X-100, and 10 mM hexanoic acid were used as the leading electrolyte and the terminating electrolyte, respectively in the following experiments.

1.3.1.2 Quantity of injected sample

Four-μl volumes of mixed solutions containing the same amounts of 100～500 mg/l peroxodisulfate and sulfate ions were injected into the isotachophoretic analyser. The total quantities of sulfur injected into the analyser were 0.8, 1.0, 1.6, 2.4, 3.2 and 4.0 μg. A mixed zone of peroxodisulfate and sulfate ions was observed when the total quantity was 2.4～4.0μg, while the mixed zone was not when the quantity was 0.8～1.6 μg, as shown in Fig. 1-1, and its zone length was increased with quantity of sulfur. Therefore, the total quantity should be less than 1.6 μg.

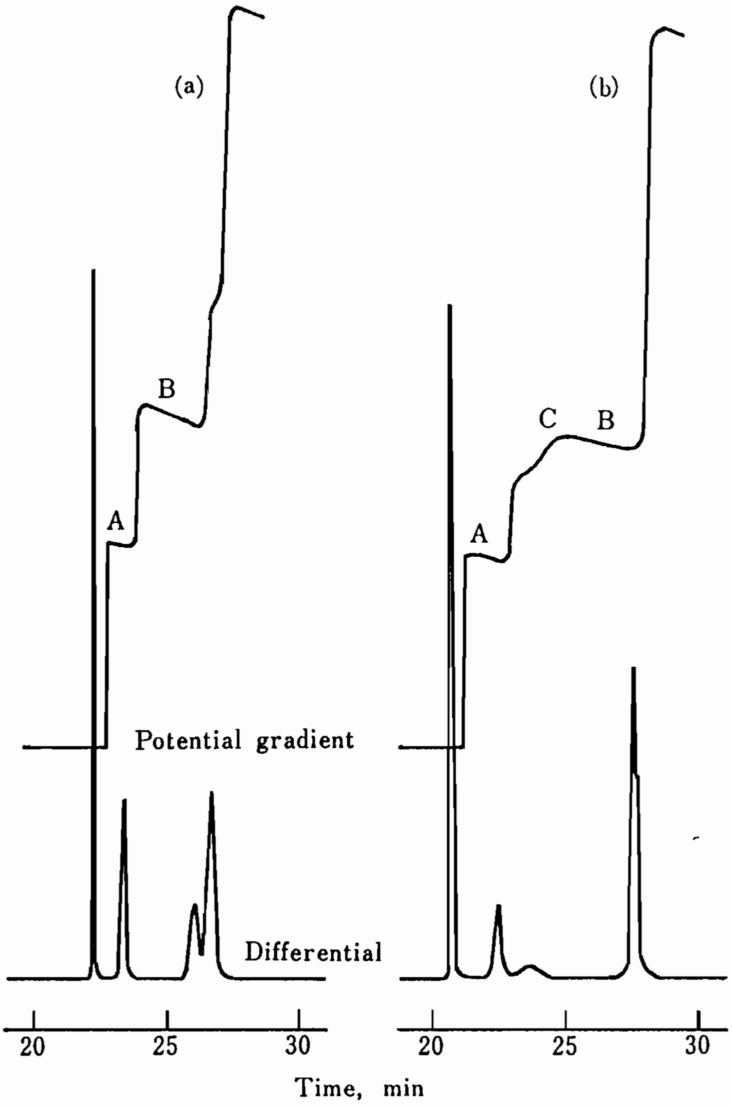

Fig. 1-1 Isotachopherograms for the separation of peroxodisulfate and sulfate ions. A, $S_2O_8{}^{2-}$; B, $SO_4{}^{2-}$; C, Mixed zone; (a), Total S=1.0μg; (b), Total S=2.4 μg.

1.3.1.3 Determination of peroxodisulfate and sulfate ions

Regardless of such kinds of cations as potassium, ammonium and sodium ions in peroxodisulfates and sulfates, linear working curves with similar slopes were obtained for peroxodisulfate and sulfate ions up to 250 mg/l. The regression equations for peroxodisulfate and sulfate ions were $y=10.1x+0.4$ and $y=21.6x+0.9$, respectively. The correlation coefficients were 1.000 and 0.998, respectively. In these equations, x is the quantity of sulfur in μg and y the zone length in mm when the recording speed is adjusted to 5 mm/min. The lower determination limits for peroxodisulfate and sulfate ions were 9.9×10^{-3} and $4.6\times 10^{-3}\,\mu$g, respectively, corresponding to a 0.1-mm zone length. Further, linear working curves were obtained for these ions up to 0.5 and 1.5 μg sulfur injected into the isotachophoretic analyser. When 3 or 4-μl volumes of mixed solutions containing peroxodisulfate and sulfate ions in various concentrations were injected and analysed by use of the working curves, the error in the determination of these ions was less than ± 20.0%, as shown in Table1-1. Similarly accurate results of these ions were obtained by using any salt such as potassium, ammonium or sodium.

1.3.1.4 Determination of other sulfur oxoacid ions

Similarly linear working curves were obtained for dithionate,

Table 1-1 Analytical results for peroxodisulfate and sulfate ions

Mixture	Added, μg		Found, μg		Error, %	
	A	B	A	B	A	B
1	0.10	0.40	0.12	0.40	+20.0	± 0.0
2	0.20	0.30	0.20	0.29	± 0.0	-3.3
3	0.25	0.25	0.24	0.24	-4.0	-4.0
4	0.30	0.20	0.29	0.17	-3.3	-15.0
5	0.40	0.10	0.36	0.08	-10.0	-20.0
6	0.20	0.80	0.16	0.82	-20.0	+2.5
7	0.40	0.60	0.38	0.59	-5.0	-1.7
8	0.50	0.50	0.48	0.51	-4.0	+2.0
9	0.60	0.40	0.54	0.39	-10.0	-2.5
10	0.80	0.20	0.75	0.16	-6.3	-20.0
11	0.30	1.20	0.29	1.20	-3.3	± 0.0
12	0.60	0.90	0.61	0.89	+1.7	-1.1
13	0.75	0.75	0.75	0.73	± 0.0	-2.7
14	0.90	0.60	0.90	0.57	± 0.0	-5.0
15	1.20	0.30	1.15	0.24	-4.2	-20.0

A, $S_2O_8^{2-}$; B, SO_4^{2-}.

tetrathionate and disulfate ions up to 250mg/l. The regression equations for dithionate, tetrathionate and disulfate ions were $y=8.9x+0.2$, $y=5.2x+0.3$ and $y=19.2x+0.6$, respectively. The correlation coefficients were 0.999, 0.994 and 0.999, respectively. In these equations, x and y are the same described above. The lower determination limits for dithionate, tetrathionate and disulfate ions were 1.1×10^{-2}, 1.9×10^{-2} and 5.2×10^{-3} μg, respectively, corresponding to a 0.1-mm zone length. The error in the determination of these ions was less than ± 20.0%, as shown in Table1-2.

1.3.2 Measurement of decomposition rate of peroxodisulfate

1.3.2.1 Effect of temperature

Two hundred and fifty ml of solutions containing 250 mg/l peroxodisulfate were heated at 75 °C in a water-bath. During the heating, 4μl of the solution was periodically taken and injected into the isotachophoretic analyser to determine peroxodisulfate and sulfate formed from peroxodisulfate by its decomposition. Decomposition ratios of potassium peroxodisulfate and ammonium peroxodisulfate after 5 h heating were 90 and 80 %, respectively, as shown in Fig.1-2. The ratio of sodium peroxodisulfate was approximately equal to that of potassium peroxodisulfate. When the peroxodisulfate solutions were similarly heated at 90 °C, potassium peroxodisulfate and sodium peroxodisulfate were almost completely

Table1-2 Analytical results for dithionate, tetrathionate and disulfate ions

Mixture	Added, μg			Found, μg			Error, %		
	A	B	C	A	B	C	A	B	C
1	0.20	—	0.80	0.20	—	0.78	± 0.0	—	-2.5
2	0.40	—	0.60	0.42	—	0.60	+5.0	—	± 0.0
3	0.50	—	0.50	0.50	—	0.49	± 0.0	—	-2.0
4	0.60	—	0.40	0.64	—	0.39	+6.7	—	-2.5
5	0.80	—	0.20	0.84	—	0.18	+5.0	—	-10.0
6	—	0.20	0.80	—	0.18	0.80	—	-10.0	± 0.0
7	—	0.40	0.60	—	0.44	0.59	—	+10.0	-1.7
8	—	0.50	0.50	—	0.51	0.49	—	+2.0	-2.0
9	—	0.60	0.40	—	0.57	0.38	—	-5.0	-5.0
10	—	0.80	0.20	—	0.79	0.17	—	-1.3	-15.0
11	0.20	0.80	0.50	0.22	0.71	0.48	+10.0	-11.3	-4.0
12	0.40	0.40	0.60	0.43	0.32	0.58	+7.5	-20.0	-3.3
13	0.50	0.60	0.40	0.51	0.53	0.39	+2.0	-11.7	-2.5
14	0.80	0.50	0.20	0.83	0.40	0.17	+3.8	-20.0	-15.0

A, $S_2O_6^{2-}$; B, $S_4O_6^{2-}$; C, $S_2O_7^{2-}$.

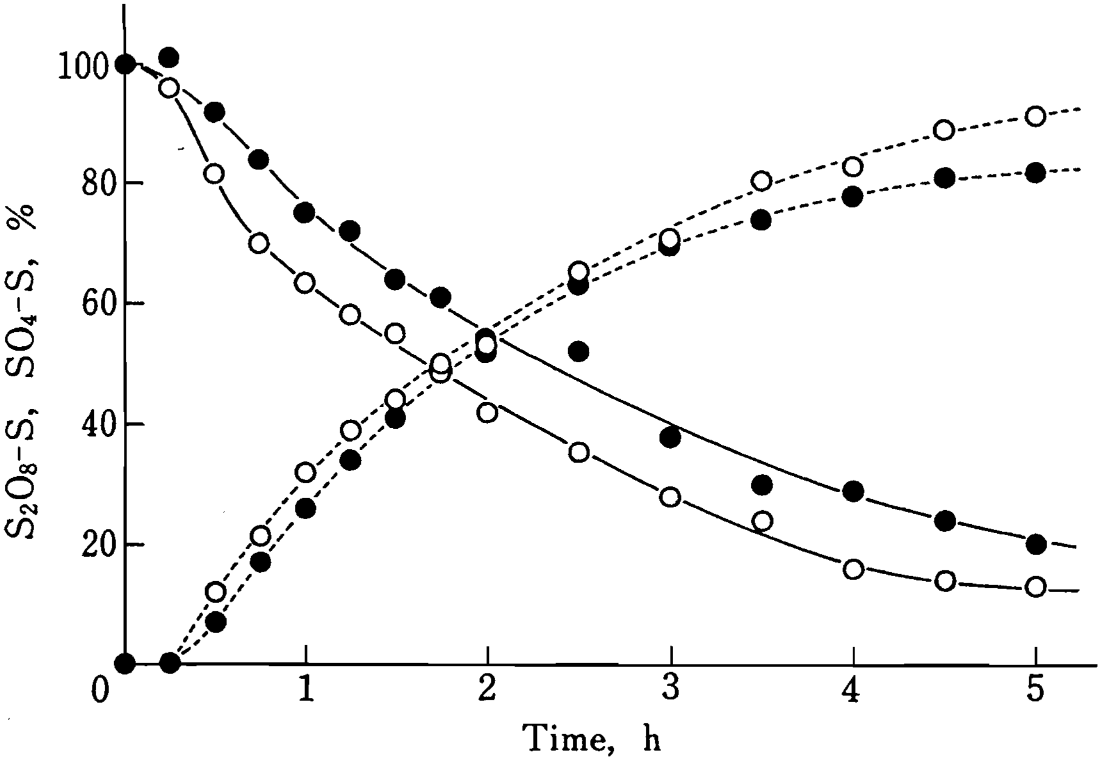

Fig. 1-2 Decomposition rates of potassium peroxodisulfate and ammonium peroxodisulfate. —○—, $K_2S_2O_8$; ···○···, K_2SO_4; —●—, $(NH_4)_2S_2O_8$; ···●···, $(NH_4)_2SO_4$.

decomposed within 1.25 and 1.5 h, respectively, and 90 % of ammonium peroxodisulfate were decomposed within 2.5 h. Recently a digestion method of aqueous samples by heating in a pressure bottle under pressure has been widely used for the decomposition of various phosphorus compounds to determine the total phosphorus[22]. Hiiro et al.[23] prepared an automated apparatus for the decomposition of various phosphorus compounds applying this method. By using this apparatus, 124 ml of the solution containing 250 mg/l peroxodisulfate were heated at 120 ℃ under the pressure of 2 kg/cm^2, and peroxodisulfate and sulfate in the solution were periodically determined. Both potassium peroxodisulfate and sodium peroxodisulfate were almost completely decomposed within 20 min, and 90% of ammonium peroxodisulfate were decomposed within 30min. Then, 50 ml of 250 mg/l potassium peroxodisulfate solutions were allowed to stand for 30 days at 17℃ in a laboratory, at 15 ℃ in a dark room and at 5 ℃ in a refrigerator. Potassium peroxodisulfate was little decomposed in these cases. The above results are summarized as follows;i.e. the decomposition rate of peroxodisulfate increased with temperature, and its order was approximately potassium peroxodisulfate $\geq$ sodium peroxodisulfate> ammonium peroxodisulfate.

1.3.2.2 Effect of concentration

When 250 ml of solutions containing 250, 1580 and 9490 mg/l

(4 %) potassium peroxodisulfates were heated at 90 ℃, potassium peroxodisulfates were almost completely decomposed within 1.25, 1.75 and 3 h, respectively, as shown in Fig. 1-3. That is to say, the decomposition rate of peroxodisufate decreased with increasing its concentration.

1.3.2.3 Effect of sulfuric acid

By using the automated apparatus, 120 ml of solutions containing 9490 mg/l peroxodisulfate added 4 ml of 6 M sulfuric acid or water were heated under pressure. Potassium peroxodisulfate was almost completely decomposed within 15min in the former case, and it was within 25 min in the latter, as shown in Fig.1-4. Both sodium peroxodisulfate and ammonium peroxodisulfate were almost completely decomposed within 15 min in the former. Sodium peroxodisulfate was almost completely decomposed, and 90 % of ammonium peroxodisulfate was decomposed within 30 min in the latter. That is to say, the decomposition of peroxodisulfate was accelerated by the addition of sulfuric acid. This agreed with the result obtained by Namiki et al.5)

1.3.2.4 Effects of various phosphorus compounds

Solutions containing 9490 mg/l potassium peroxodisulfate and 10 mg/l various phosphorus compounds were mixed and heated at 90

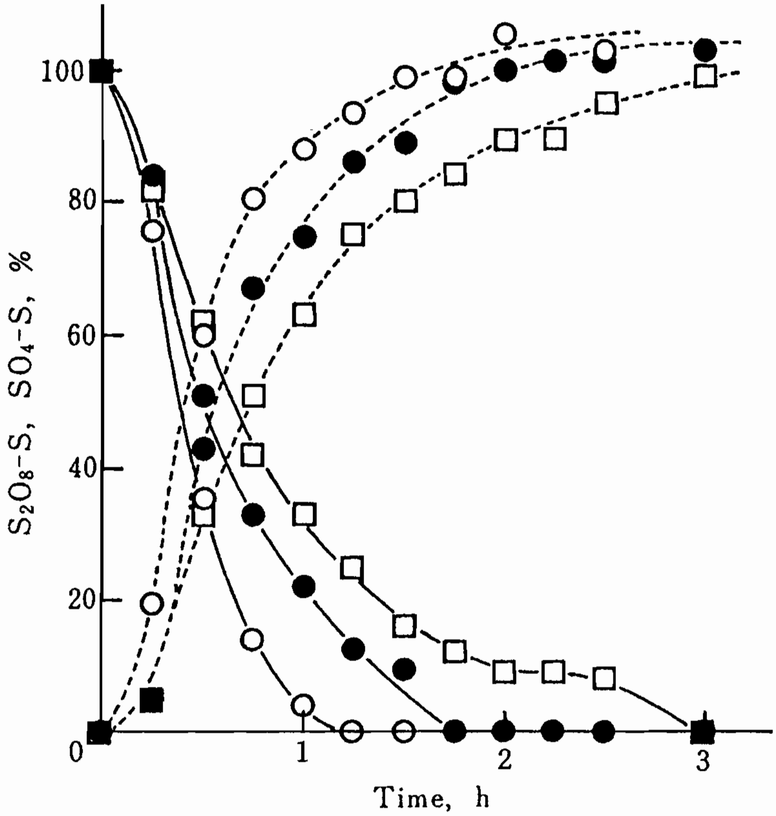

Fig. 1-3 Decomposition rates of potassium peroxodisulfate. —, $K_2S_2O_8$; ···, K_2SO_4; ○, 250 mg/l; ●, 1580 mg/l; □, 9490 mg/l.

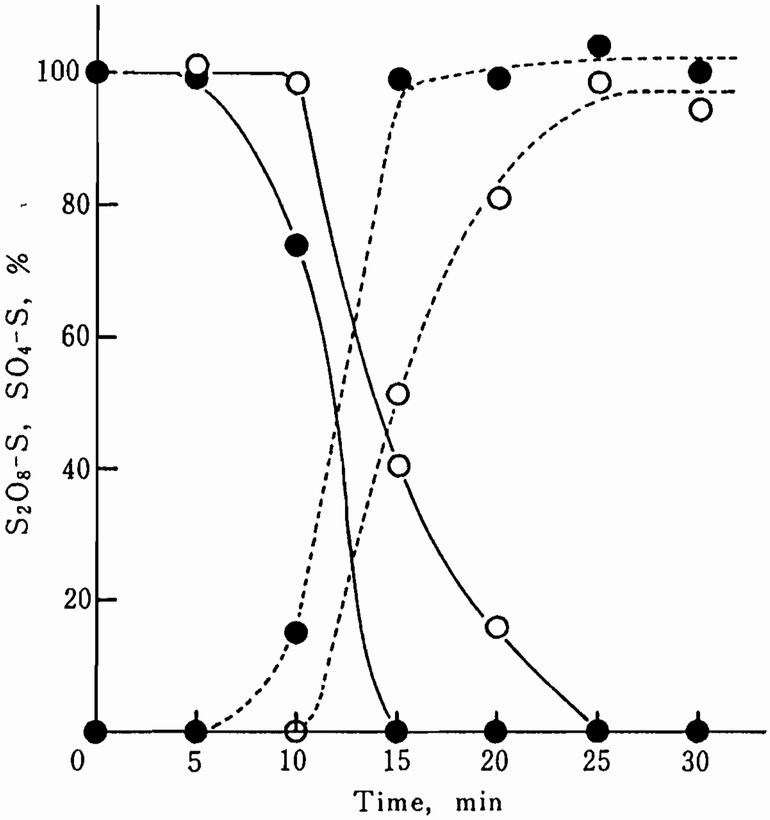

Fig. 1-4 Decomposition rates of potassium peroxodisulfate. — , $K_2S_2O_8$; ··· , K_2SO_4; ○ , Addition of 4 ml of water; ● , Addition of 4 ml of 6 M H_2SO_4.

°C . These results are shown in Fig.1-5. When disodium p-nitrophenylphosphate was added, potassium peroxodisulfate was almost completely decomposed within 3 h as well as in the case of no addition of disodium p-nitrophenylphosphate. On the other hand, when triphenyl phosphite was added, potassium peroxodisulfate was almost completely decomposed within 2.25 h, shorter time than that in the case of the addition of disodium p-nitrophenylphosphate. Then, when adenosine-5′ -triphosphate was added, a similar result was obtained as that of the addition of disodium p-nitrophenylphosphate. Further, when disodium phenylphosphate or disodium D-glucose-6-phosphate was added, a similar result was obtained as that of the addition of triphenyl phosphite. It was presumed that these differences were caused by the following reasons; i.e. the decomposition of peroxodisulfate was accelerated by the reaction of peroxodisulfate with triphenyl phosphite etc., while the autolysis of peroxodisulfate was a main reaction in the case of disodium p-nitrophenylphosphate etc. There is still great room for examination in this cause.

1.3.3 Determination of low concentrations of ammonium and potassium ions

1.3.3.1 Concentration of 18-crown-6

The concentration of 18-crown-6 in the leading electrolyte (

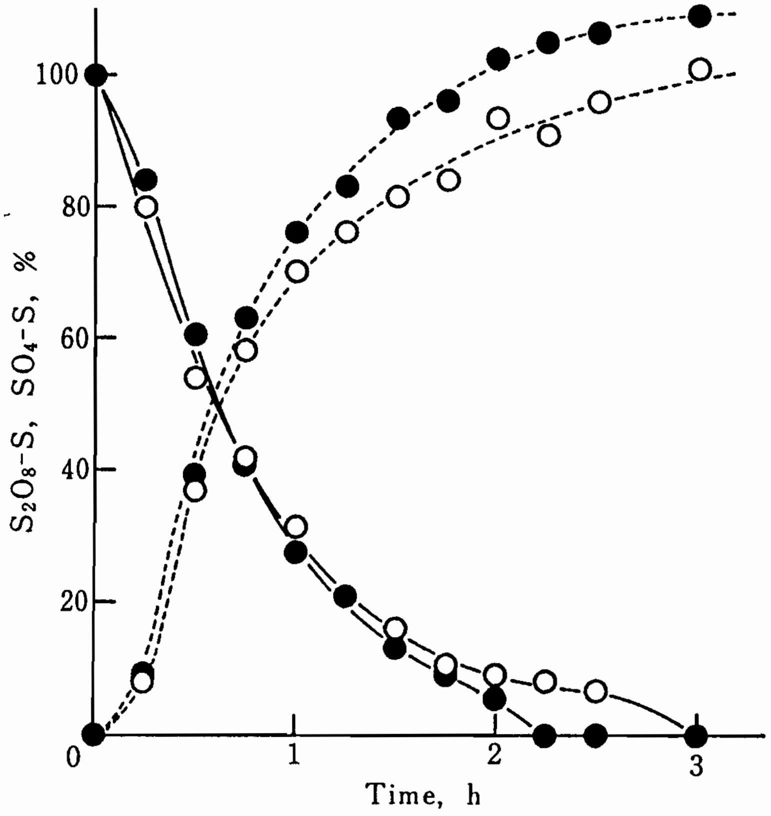

Fig. 1-5 Decomposition rates of potassium peroxodisulfate. —, $K_2S_2O_8$; ···, K_2SO_4; ○, Addition of disodium p-nitrophenylphosphate; ●, Addition of triphenyl phosphite.

5 mM hydrochloric acid containing 30 %v/v glycerol) was icreased up to 5mM. The terminating electrolyte was 10 mM lithium sulfate. A 250-μ l volume of a mixture containing 1.0mg/l ammonium and potassium ions was injected into the isotachophoretic analyser. The migration current was maintained at 200 μ A for the first 12 min and then reduced to 50 μ A. In general, the Potential Unit (PU) value is used for qualitative indication of the ions, and is defined by the following equation[24]:

$$PU=(PG_S-PG_L)/(PG_T-PG_L)$$

where PG_S, PG_L and PG_T are the potential gradients of sample ion, leading ion and terminating ion, respectively.

The PU value of the potassium ion increased with concentration of 18-crown-6 up to 3 mM, as shown in Fig. 1-6. On the other hand, the PU value of the ammonium ion remained almost constant, and complete separation of ammonium and potassium ions could be obtained with 1～3 mM 18-crown-6. The PU value of the potassium ion was the same as that of the sodium ion in the terminating electrolyte when its concentration was higher than 4 mM. In addition, it was found that the PU value of the sodium ion was smaller than that of the calcium ion when the glycerol concentration was 20～30 %, but greater than it when the glycerol concentration was less than 10%. Therefore, 3 mM 18-crown-6 was selected as the concentration in the leading electrolyte.

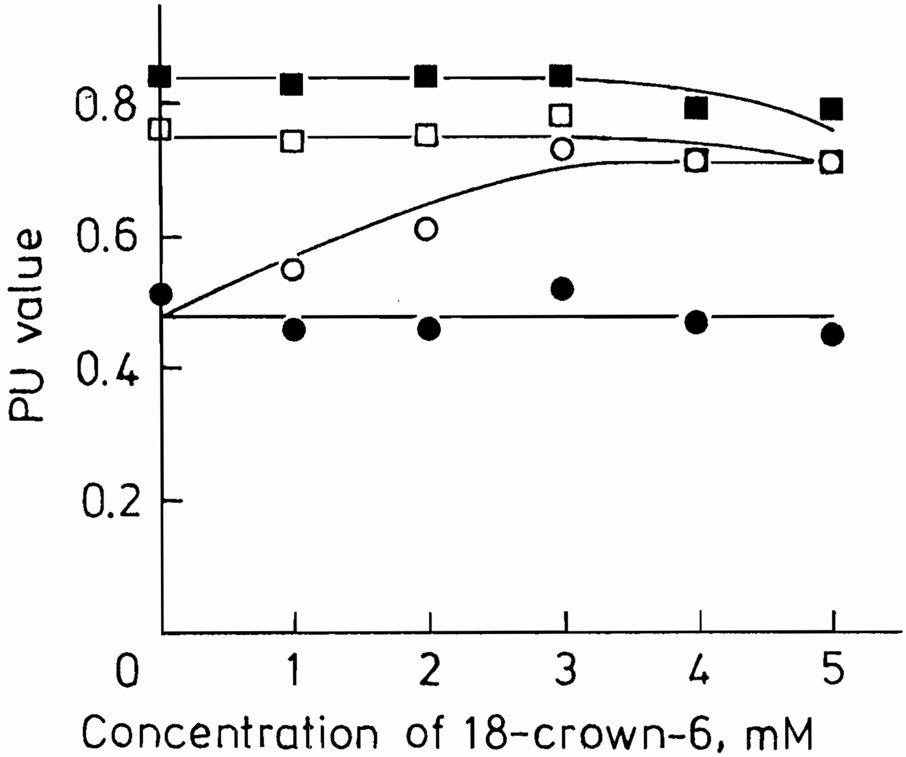

Fig. 1-6 Effect of the concentration of 18-crown-6 on the PU values. ●, NH_4^+; ○, K^+; □, Na^+; ■, Ca^{2+}.

1.3.3.2 Prediction of steady state concentrations

F.S. Stover$^{25)}$ presented a simplified theory for alkali metal-crown ether complexes in isotachophoresis as follows. The leading electrolyte is a non-complexed, 1:1 salt, L^+Y^-, at a concentration c_L with neutral ligand C added at a concentration $c_C{}^*$. The primary assumption of the model is that there is no mass transport of ligand; i.e. the total ligand concentration in zone of sample M^+ also is $c_C{}^*$. Formation of the complex in the metal zone, ($M^+ + C \longleftrightarrow MC^+$) is governed by

$$K = c_{MC}/c_M c_C \qquad (1\text{-}1)$$

where c_{MC}, c_M and c_C are the concentrations of complex, free metal and free ligand, respectively. This formation constant is equivalent to the thermodynamic constant since activity coefficients for metal and complex cancel. By substituting expressions for total metal concentration, $c_M{}^* = c_M + c_{MC}$, and total ligand concentration, $c_C{}^* = c_C + c_{MC}$, an equation for free metal concentration can be obtained.

$$Kc_M{}^2 + c_M(1 + Kc_C{}^* - Kc_M{}^*) - c_M{}^* = 0 \qquad (1\text{-}2)$$

The total metal concentration in the steady state is determined by the Kohlrausch regulating function and is dependent on leading ion mobility and leading zone specific conductance

$$c_M^*=\lambda_M^* c_L(\lambda_L+\lambda_y^L)/\lambda_L(\lambda_M^*+\lambda_y^M) \tag{1-3}$$

where c_L and λ_L are the concentration and single ion conductance of the leading ion, λ_y^L and λ_y^M are the single ion conductances of the counter-ion in the leading and metal zone, and λ_M^* is the effective single ion conductance of the metal

$$\lambda_M^*=(\lambda_M c_M+\lambda_{MC} c_{MC})/c_M^* \tag{1-4}$$

Substitution of eqn.(1-4) into eqn.(1-3) yields an expression for the total metal concentration in the steady state

$$a_1 c_M^{*2}+(a_2 c_M-a_3 c_L)c_M^*+a_4 c_M c_L=0 \tag{1-5}$$

where $a_1=\lambda_L(\lambda_y^M+\lambda_{MC})$, $a_2=\lambda_L(\lambda_M-\lambda_{MC})$, $a_3=\lambda_{MC}(\lambda_L+\lambda_y^L)$ and $a_4=(\lambda_{MC}-\lambda_M)(\lambda_L+\lambda_y^L)$. The single ion conductances at finite ionic strength can be obtained from the Onsager equation:

$$\lambda=\lambda^0-(A\lambda^0+B)c^{1/2} \tag{1-6}$$

where $c=c_M^*$ for λ_M, λ_{MC} and λ_y^M, $c=c_L$ for λ_L and λ_y^L, and A= 0.23 and B=60.65 for water at 25 °C .

Eqns. (1-2), (1-5) and (1-6) can be solved iteratively for given values of c_L, K, $c_C{}^*$, and λ^0. Initially $c_M{}^*$ and c_M are set equal to c_L. Single ion conductances then are corrected to finite ionic strength using eqn. (1-6). $c_M{}^*$ is found from eqn. (1-5) and c_M from eqn. (1-2). The procedure is repeated until successive values of $c_M{}^*$ agree within 0.1 %.

Therefore, calculated concentrations for potassium ion at various added 18-crown-6 concentrations were obtained by the above procedure and were shown in Table 1-3, where c_L=5 mM, K=107 l/mole, $\lambda_L{}^0$=349.8, $\lambda_y{}^0$=76.35, $\lambda_M{}^0$=73.5, $\lambda_{MC}{}^0$=25.3 Scm^2/equiv.

1.3.3.3 Determination of ammonium and potassium ions

Linear working curves were obtained for ammonium and potassium ions up to 1.0 and 2.0mg/l, respectively, by using the leading electrolyte containing 3 mM 18-crown-6. The regression equations for ammonium and potassium ions were $y=34.9x-0.1$ and $y=12.9x-0.1$, respectively. Both correlation coefficients were 1.000. In these equations, x is the concentration of the ion in mg/l and y the zone length in mm when the recording speed is adjusted to 40 mm/min. The lower determination limits for ammonium and potassium ions were 2.9 and 7.8μg/l, respectively, corresponding to a 0.1-mm zone length. When 250-μl volumes of mixed solutions containing ammonium and potassium ions in various concentrations were injected and analysed by use of the working curves, the error in the determination of these ions was less than ± 20.0 %, as shown

Table 1-3 Calculated concentrations for K^+/18-crown-6 complex

c_C^*, mM	c_M^*, mM	c_M, mM	c_{MC}, mM
0	2.96	2.96	0.00
1	2.88	2.66	0.22
2	2.81	2.40	0.41
3	2.73	2.17	0.56
4	2.67	1.97	0.70
5	2.61	1.80	0.81

in Table 1-4. An isotachopherogram of mixture 6 in Table 1-4 is shown in Fig.1-7. In view of these results, this electrolyte system (which uses a smaller amount of 18-crown-6) can be applied to the determination of trace amounts of ammonium ion in seawater after a suitable pretreatment.

1.3.4 Determination of some metal-EDTA complexes

1.3.4.1 Length of main column

The established analytical conditions are shown in Table 1-5. At first, the effect of the length of the main column on the separation of EDTA, Mg(Ⅱ)-EDTA and Ca(Ⅱ)-EDTA was examined. Their separation was much improved with increasing the length of the main column up to 40 cm; the separation was not improved further when even a 50-cm long main column was used. Therefore, a 40-cm long main column was adopted.

1.3.4.2 Length of precolumn

The effect of the length of the precolumn was examined. No appreciable improvement in the separation of Mg(Ⅱ)-EDTA and Ca(Ⅱ)-EDTA was observed when 0, 4, 8, 10 or 15-cm long precolumn was used. A 4-cm long precolumn was adopted, since larger separation capacity was obtained than without a precolumn.

Table 1-4 Analytical results for ammonium and potassium ions

Mixture	Added, mg/l		Found, mg/l		Error, %	
	A	B	A	B	A	B
1	0.10	2.00	0.12	2.00	+20.0	± 0.0
2	0.20	1.80	0.20	1.66	± 0.0	-7.8
3	0.40	1.40	0.39	1.36	-2.5	-2.9
4	0.60	1.40	0.58	1.34	-3.3	-4.3
5	0.60	0.60	0.56	0.59	-6.7	-1.7
6	0.80	0.40	0.75	0.39	-6.3	-2.5
7	1.00	2.00	1.01	0.18	+1.0	-10.0
8	1.00	1.00	1.02	1.03	+2.0	+3.0

A, NH_4^+; B, K^+.

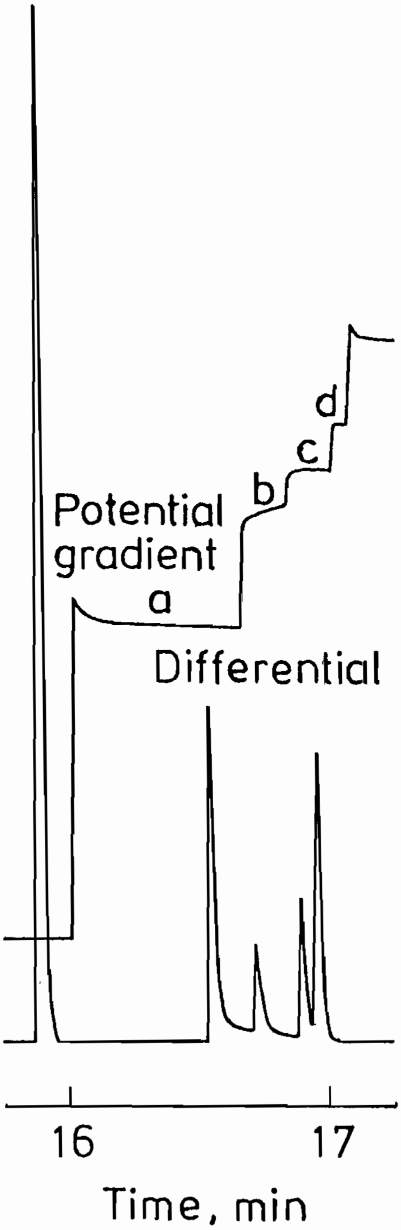

Fig. 1-7 Isotachopherogram of mixture 6. a, NH_4^+; b, K^+; c, Na^+; d, Ca^{2+}.

Table 1-5 Analytical conditions for EDTA, Mg(Ⅱ)-EDTA and Ca(Ⅱ)-EDTA

Precolumn	4cm long, 1.0mm inner diameter
Main column	40cm long, 0.5mm inner diameter
Migration current	initially 200μA for 13 min and then 100 μA
Leading ion	10 mM chloride
Counter-ion	tris(hydroxymethyl)aminomethane (abbreviated as tris)
pH	8.5
Viscous agent	0.5 % methyl cellulose
Terminating ion	10 mM hexanoic acid

1.3.4.3 Viscous agent

Hine[26) mentioned that the separation of anions was extremely sensitive to the kind of viscous agent added to the leading electrolyte. The separation of Mg(II)-EDTA and Ca(II)-EDTA was improved significantly by using 0.5 % methyl cellulose, as shown in Fig. 1-8. A similar result was obtained by using 0.1 % HPMC. The solutions of 0.1 % hydroxyethyl cellulose and 0.5 % polyvinyl alcohol were not suitable, because considerable amounts of impurities were included. Thus 0.5% methyl cellulose was adopted as the viscous agent.

1.3.4.4 pH of leading electrolyte

Mg(II)-EDTA seems to dissociate when the pH of the leading electrolyte is low; for example, it dissociated almost completely below pH 5, as Yoshida et al.11) reported. The degree of dissociation was determined by comparing the amounts of dissociated EDTA and of Mg(II)-EDTA. Mg(II)-EDTA dissociated when the pH of the leading electrolyte was< 8.5, as shown in Fig.1-9. Therefore, the leading electrolyte buffered at pH 8.5 was adopted.

1.3.4.5 Counter-ion

Yoshida et al.11) separated Cd(II)-EDTA and Pb(II)-EDTA based

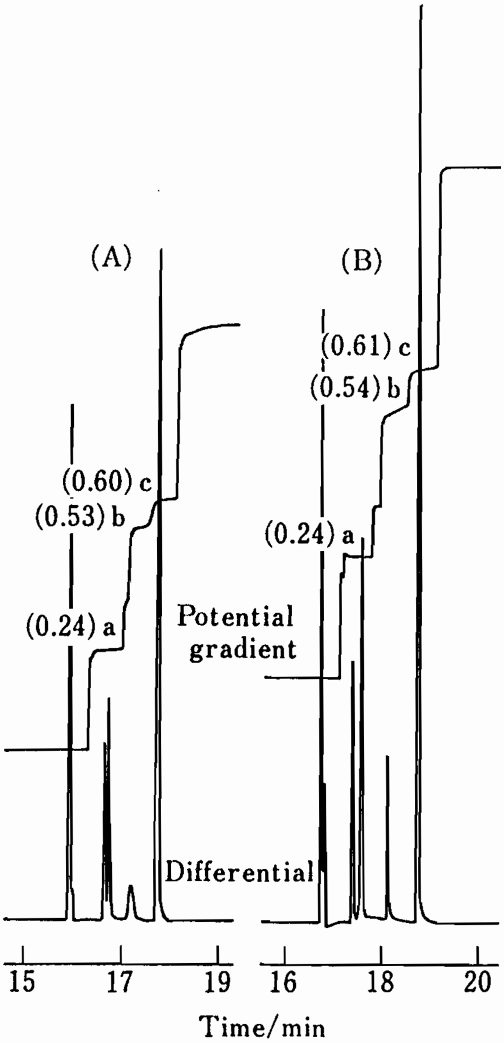

Fig. 1-8 Effect of kind of viscous agent on the mutual separation of EDTA, Mg(II)-EDTA and Ca(II)-EDTA. (A),0.1% Triton X-100; (B), 0.5 % methyl cellulose; a, EDTA; b, Mg(II)-EDTA; c, Ca(II)-EDTA; Values in the parentheses, PU value.

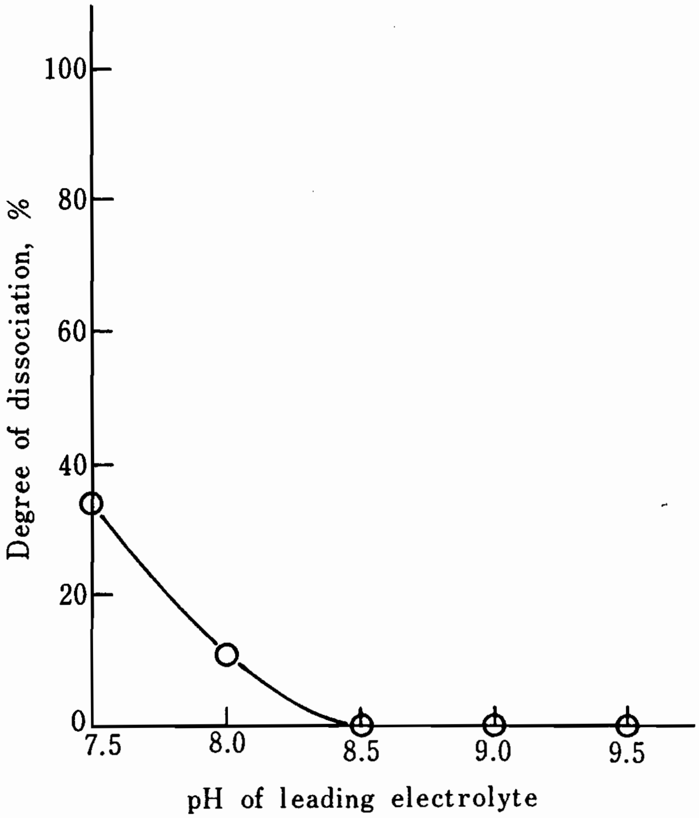

Fig.1-9 Effect of pH of leading electrolyte on the degree of dissociation of Mg(II)-EDTA.

on the difference of interaction characteristics between the central metal and the counter-ion in the leading electrolyte. In the author's experiments, separation of EDTA, Mg(II)-EDTA and Ca(II)-EDTA was almost the same when tris, triethanolamine, 2-amino-2-methyl-1,3-propandiol, 2,2′-iminodiethanol or 2-amino-2-methyl-1-propanol was used as a counter-ion; i.e. no appreciable differences in the interaction between metal and counter-ions were observed. Tris was adopted as the counter-ion.

1.3.4.6 Terminating electrolyte

Everaerts et al.[27] reported that a considerable amount of sample was lost if an inadequate terminating electrolyte was chosen. However, similar results were obtained when 10 mM hexanoic acid(pH 3.4), glutamic acid (pH 3.5) or sodium hexanoate (pH 7.1) was used as the terminating electrolyte for the separation of EDTA, Mg(II)-EDTA and Ca(II)-EDTA. When 10 mM β-alanine buffered at pH 10.9 or 10mM phenol buffered at pH 10.0 with barium hydroxide was used, the normal migration was not obtained because of the unusual increase of migration voltage, in spite of reducing the migration current to 100 μA. Ten mM hexanoic acid was adopted as the terminating electrolyte.

1.3.4.7 Determination of EDTA, Mg(II)-EDTA and Ca(II)-EDTA

Linear working curves were obtained for EDTA, Mg(Ⅱ)-EDTA and Ca(Ⅱ)-EDTA up to 6.0×10^{-3} M, by using the conditions shown in Table 1-5. The regression equations for EDTA, Mg(Ⅱ)-EDTA and Ca(Ⅱ)-EDTA were $y=2830x-0.5$, $y=2140x-0.1$ and $y=2440x+0.1$, respectively. The correlation coefficients were 0.999, 0.999 and 1.000, respectively. In these equations, x is the concentration of EDTA complexes in M and y the zone length in mm when the recording speed is adjusted to 10 mm/min. The lower determintion limits for EDTA, Mg(Ⅱ)-EDTA and Ca(Ⅱ)-EDTA were 3.5×10^{-5}, 4.7×10^{-5} and 4.1×10^{-5} M, respectively, corresponding to a 0.1-mm zone length. When the volume of sample was less than 4 μl, the mixed zone was not observed. When 4-μl volumes of mixed solutions containing EDTA, Mg(Ⅱ)-EDTA and Ca(Ⅱ)-EDTA in various concentrations were injected and analysed by use of the working curves, the error in the determination of these EDTA complexes was less than ± 20.0 %, as shown in Table 1-6.

1.3.4.8 Determination of Cu(Ⅱ)-EDTA and Mn(Ⅱ)-EDTA

Cu(Ⅱ)-EDTA and Mn(Ⅱ)-EDTA were also determined following the same procedure. Linear working curves were obtained for Cu(Ⅱ)-EDTA and Mn(Ⅱ)-EDTA up to 6.0×10^{-3} M. The regression equations for Cu(Ⅱ)-EDTA and Mn(Ⅱ)-EDTA were $y=2230x+0.1$ and $y=2150x$, respectively. The correlation coefficients were 0.999 and 1.000, respectively. In these equations, x and y are the same de-

Table1-6 Analytical results for EDTA, Mg(Ⅱ)-EDTA and Ca(Ⅱ)-EDTA

Mixture	Added, 10^{-3} M			Found, 10^{-3} M			Error, %		
	A	B	C	A	B	C	A	B	C
1	1.0	5.0	1.0	1.1	5.2	1.1	+10.0	+4.0	+10.0
2	1.5	3.5	2.0	1.6	3.6	2.1	+6.7	+2.9	+5.0
3	2.0	2.5	2.5	2.1	2.7	2.6	+5.0	+8.0	+4.0
4	2.5	1.5	3.0	2.5	1.6	3.1	± 0.0	+6.7	+3.3
5	3.0	0.5	3.5	3.0	0.6	3.6	± 0.0	+20.0	+2.9
6	3.5	2.0	1.5	3.6	2.2	1.5	+2.9	+10.0	± 0.0

A, EDTA; B, Mg(Ⅱ)-EDTA; C, Ca(Ⅱ)-EDTA.

Table 1-7 Analytical results for Cu(II)-EDTA and Mn(II)-EDTA

Mixture	Added, 10^{-3} M		Found, 10^{-3} M		Error, %	
	A	B	A	B	A	B
1	0.5	6.0	0.5	5.8	± 0.0	-3.3
2	1.0	5.0	0.9	5.0	-10.0	± 0.0
3	2.0	4.0	1.8	3.9	-10.0	-2.5
4	3.0	3.0	2.9	3.0	-3.3	± 0.0
5	4.0	2.0	4.0	2.1	± 0.0	+5.0
6	5.0	1.0	4.9	1.0	-2.0	± 0.0
7	6.0	0.5	6.1	0.5	+1.7	± 0.0

A, Cu(II)-EDTA; B, Mn(II)-EDTA.

scribed above. The lower determination limits for Cu(Ⅱ)-EDTA and Mn(Ⅱ)-EDTA were 4.5×10^{-5} and 4.7×10^{-5} M, respectively, corresponding to a 0.1-mm zone length. The error in the determination of mixtures of these EDTA complexes was less than ± 10.0 %, as shown in Table 1-7.

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2 Determination of halide ions in seawater

2.1 Introduction

Fluoride ion in seawater has been determined by means of spectrophotometry[1~3], ion-selective electrode method[2,4,5] and ion chromatography[6]. However, the interference by the diverse ions[7] is a problem in these methods.

On the other hand, CITP has not ever been applied to the determination of fluoride ion in seawater. When the method is applied to the determination of fluoride ion, a suitable pretreatment is necessary for the separation and enrichment of the ion from high concentrations of coexisting anions(e.g. chloride, sulfate and bromide ions).

Therefore, a preliminary coprecipitation enrichment method with magnesium hydroxide was examined for this purpose and the optimum analytical conditions were established; sodium hydroxide solution was added to seawater samples directly to obtain the magnesium hydroxide precipitate; a hydrogen cation-exchange resin was used for the dissolution of the precipitate[8], because acid could not be used due to the interference of high concentration of the anion of the acid for the isotachophoretic measurement. At first, an optimum electrolyte system was examined for the determination of fluoride ion and the working curve was prepared. Then the following analytical conditions were examined: the amount of

1M sodium hydroxide for coprecipitating fluoride ion and the concentration of sodium hydroxide solution for washing the precipitate. By the proposed method, a linear working curve was obtained for artificial seawater samples containing up to 2.5mg/l fluoride ion. The relative standard deviation for fluoride ion was 0.037. The lower determination limit for fluoride ion was 15 μ g/l. Finally, the method was applied to the determination of fluoride ion in surface seawater samples by use of the working curve method and the standard addition method.

It is important to determine the concentration of bromide ion in seawater for the following reasons; i.e. it is necessary to evaluate the environmental impact of the chlorination of a large amount of cooling seawater, carried out generally in steam power plants9), and the bromide ion affects on the corrosion of steel in seawater10).

Trace amounts of bromide ion in rain and river-waters have been determined by means of spectrophotometry using the catalytic effect11,12). Colorimetry13~ 15), iodometric titration16~ 18) and spectrophotometry19) have been used for the determination of bromide ion in samples in which a large amount of chloride ion coexists such as hot spring waters and oil well brines. Further, bromide ion in seawater has been determined by means of spectrophotometry20,21) and ion chromatography6,22). Most of these methods involve some problems such as the influence of interfering substances and the complexity in analytical processes.

On the other hand, there is no report concerning the determination of bromide ion in seawater by CITP. When the method is applied to the determination of bromide ion, a suitable pretreatment is necessary for the separation of the ion from high concentrations of coexisting anions. Gas-liquid separation with a tubular microporous PTFE membrane as a pretreatment has recently attracted the attention[23). For example, the gas-liquid separation has been used for the determination of nitrate ion[24) and ammonia [25~ 29) in environmental waters,blood and urine and of free chlorine[30) in tap-water. The gas-liquid separation appears to be useful for the separation and enrichment of volatile compounds in aqueous solutions containing large amounts of nonvolatile interfering substances.

Therefore, a new pretreatment procedure using a redox reaction in the gas-liquid separator was examined for the isotachophoretic determination of bromide ion in seawater and the optimum analytical conditions were established. Further, the behavior of chloride ion was examined simultaneously, because it was presumed that chloride ion in seawater was accompanied to a certain degree together with bromide ion from the gas-liquid separator. The method was also applied to the determination of bromide ion in surface and bottom seawater samples.

2.2 Experimental

2.2.1 Apparatus

The Shimadzu IP-2A isotachophoretic analyser was used, with a potential gradient detector. The main column was a FEP copolymer tube, 15 or 20 cm long, 0.5 mm inner diameter; the precolumn was a PTFE tube, 8~ 20 cm long, 1.0 mm inner diameter. A Hamilton 1710-N and the Terumo MS-10 microsyringes were used for the injection of samples. A Toyo MS-16B stirrer and a Kubota centrifuge were also used. The data on the determination of bromide ion was processed by a Shimadzu Chromatopac C-R2AX. A gas-liquid separator (Kimoto Electric Co.) was a double-tube structure; it consisted of an inner microporous PTFE tube (20cm long, 2.0 mm i.d., 2.8 mm o.d.) and an outer glass tube (3.5 mm i.d., 6.0 mm o.d.), as shown in Fig. 2-1. The following 2 kinds of PTFE tube (Japan Goretex Inc.) were used: 2 μm of maximum pore size, 50 % porosity, and 3 μm of maximum pore size, 70 % porosity. The flow system is shown in Fig.2-2. The gas-liquid separator was immersed in the same water-bath described in Section1.2.1. The inner tube was filled with hydrogen peroxide solution, and the sample solution to which potassium permanganate solution was added, was circulated through the outer tube by means of an Atto SJ-1220 peristaltic pump. A Denkikagaku-Keiki HPH-22 pH meter, a Yellow Springs Instrument 33 salinometer were used for the measurements of pH and salinity of seawater samples, respectively. A reagent bottle with a thin rope and a Rigosha Vandorn water sampler (2 l)

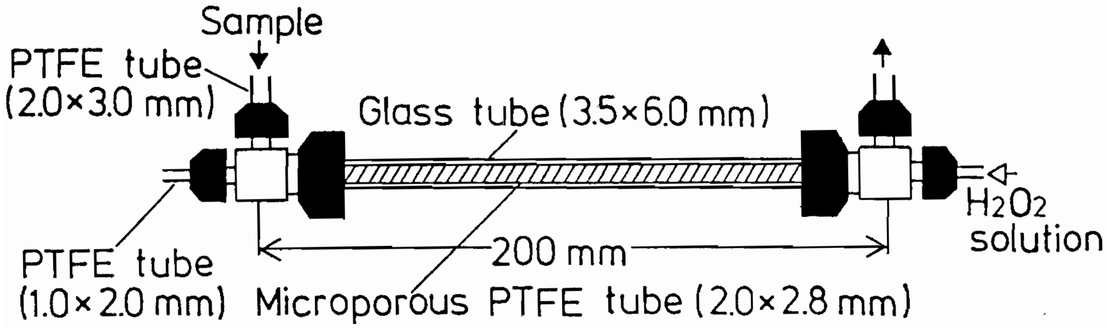

Fig. 2-1 Schematic diagram of gas-liquid separator.

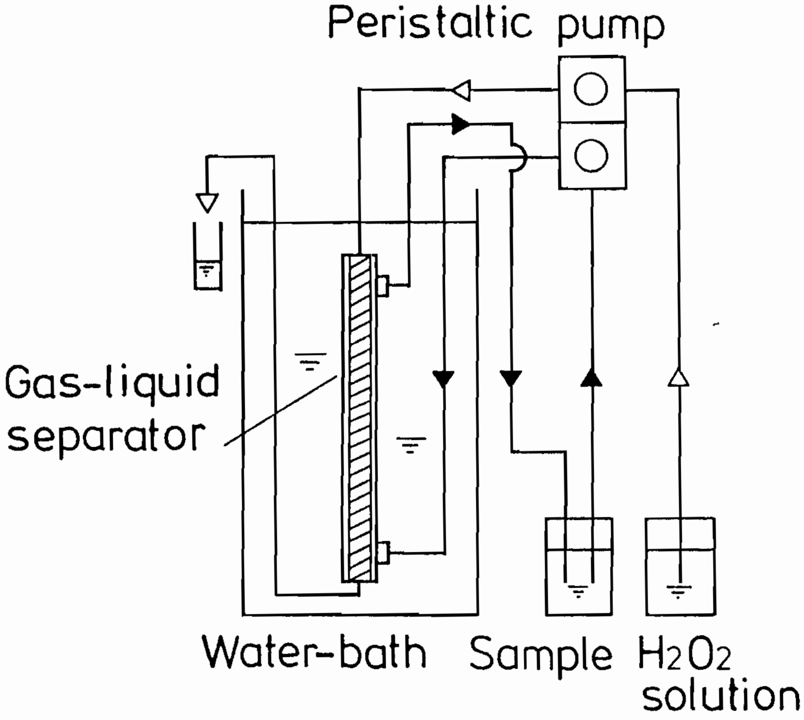

Fig. 2-2 Schematic diagram of flow system.

were used for sampling of surface and bottom seawaters, respectively. Seawater samples were filtered through a 0.45-μm membrane and stored in polyethylene bottles inside a refrigerator as soon as possible after collection.

2.2.2 Reagents

All solutions were prepared from analytical reagent grade chemicals. Standard solutions of fluoride or bromide ion were prepared by dissolving sodium fluoride or potassium bromide in water from a Yamato-Kagaku WA-22 automatic still and a Nihon Millipore-Kogyo Milli-QII system. Dowex 50W X8 (100~ 200 mesh) cation-exchange resin in the hydrogen form was used for dissolving the magnesium hydroxide precipitate. The resin was conditioned as follows: 200ml of water was added to 50 ml of the resin and stirred for 15 min; the supernatant solution was discarded; this treatment was repeated 3 times; a similar treatment was successively carried out with 150 ml of 1 M sodium hydroxide, 200 ml of water, 150ml of 0.5M sulfuric acid and 200 ml of water; finally, the resin was washed thoroughly with water on a filter paper and stored in water. A solution of 0.02 M potassium permanganate was prepared by the method which was used for titration31), and 35 % hydrogen peroxide was diluted with water to 3 %. The preparation of the artificial seawater was based on a Japanese Standard32), but no fluoride or bromide ion was included.

2.2.3 Procedure

The procedure for the determination of fluoride ion is as follows. Add 1.5 ml of 1 M sodium hydroxide to 50 ml of seawater sample to obtain the magnesium hydroxide precipitate. Centrifuge the suspension with 3000 rpm for 5 min. Discard the supernatant solution and wash the precipitate as follows: blow 50 ml of 10^{-3} M sodium hydroxide on the precipitate through a washing flask; centrifuge the suspension with the above conditions; discard the supernatant solution. After washing the precipitate 3 times, add 1.5ml of water and 1.3 g of the hydrogen cation-exchange resin on the precipitate and stir for 5 min to dissolve the precipitate. Adjust the total volume to 5.0 ml with water after filtration under reduced pressure. Inject 50 μl of the treated solution into the isotachophoretic analyser. Maintain the migration current at 300μA for the first 8 min and then reduce it to 75 μA. As leading electrolyte use 10 mM histidine hydrochloride/0.1 % Triton X-100, and as terminating electrolyte 10 mM hexanoic acid. Prepare a working curve by applying the method to synthetic standards.

The procedure for the determination of bromide ion is as follows. Add 0.3 ml of 9 M sulfuric acid and 0.7 ml of 0.02 M potassium permanganate to 10 ml of seawater sample, mixing after each addition, to oxidize bromide ion. With the water-bath temperature set at 40 ℃, circulate this solution through the outer tube at a flow-rate of 7.0 ml/min for 20 min. Bromine permeates through the wall of the inner tube and dissolves in 1.5 ml aliquots of a mixed solution of 10 ml of water, 0.5 ml of 0.1 M acetic acid and 2.0 ml of 3 % hydrogen peroxide previously placed in the inner tube. Pump out the solution containing bromide ion produced by the reduction of permeated bromine. Inject 10 μ l of the solution into the isotachophoretic analyser. Maintain the migration current at 200μ A for the first 18 min and then reduce it to 50μ A. As leading electrolyte use 5mM perchloric acid/10 mM tris//methanol, and as teminatig electrolyte 10 mM sodium fluoride/water. The length of the precolumn was 20 cm. Prepare a working curve by applying the method to synthetic standards. During the circulation, a small amount of manganese dioxide precipitate was generated and attached on the outside of the PTFE tube. The precipitate was easily removed by sequentially passing a small volume of 6 M hydrochloric acid and water through the outer tube.

2.3 Results and Discussion

2.3.1 Determination of fluoride ion in seawater

2.3.1.1 Electrolyte system

Fifty ml of artificial seawater sample containing 1.5 mg/l fluoride ion was treated by the proposed method. The treated solution was injected into the isotachophoretic analyser. The following 2 kinds of electrolyte system were examined: the leading electrolyte was 6mM cadmium nitrate, and the terminating electrolyte was 10 mM citric acid; the leading electrolyte was 10 mM histidine hydrochloride containing 0.1 % Triton X-100, and the terminating electrolyte was 10 mM hexanoic acid. The migration current was maintained at 250 or 300μA for the first 17 or 8 min and then reduced to 75 μA. These isotachopherograms are shown in Fig. 2-3. A mixed zone of sulfate and fluoride ions which interfered with the determination of fluoride ion was formed when the former electrolyte system was used, but was not when the latter was used. Therefore, the latter electrolyte system was selected for subsequent experiments.

2.3.1.2 Working curve of fluoride ion

A linear working curve was obtained for fluoride ion by injecting 50 μl of standard solutions containing 2.5~25 mg/l the ion into the isotachophoretic analyser. The regression equation was $y=0.95x$ (correlation coefficient 1.000) where x is the concentration of fluoride ion in mg/l and y the zone length in mm when the recording speed is adjusted to 10 mm/min. The lower determination limit for fluoride ion was 0.11mg/l, corresponding to a 0.1-mm zone length.

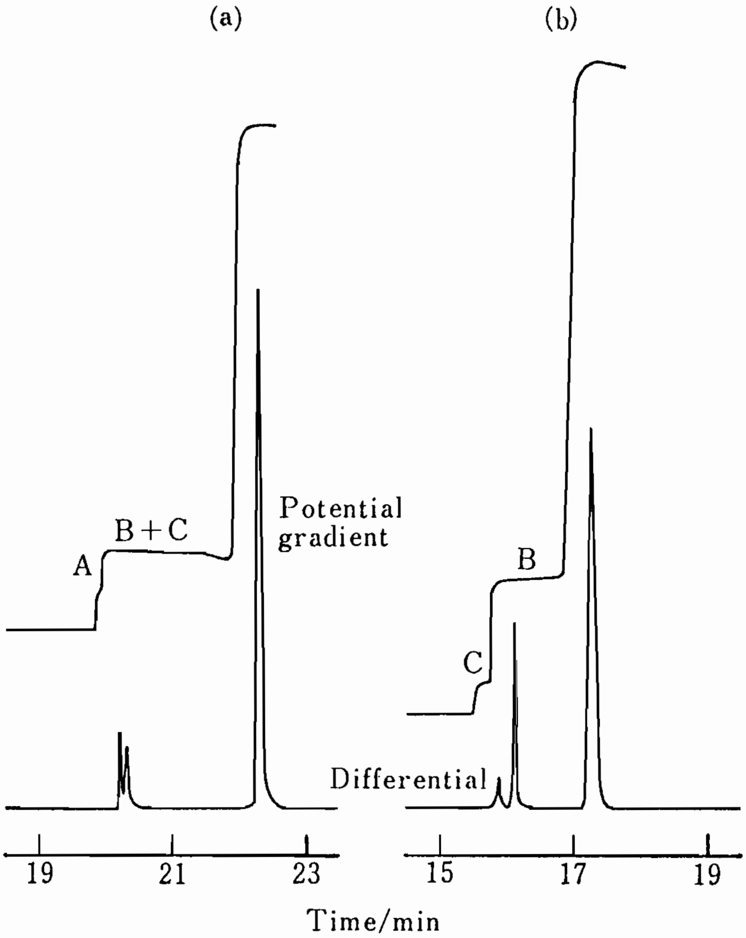

Fig. 2-3 Isotachopherograms of artificial seawater sample treated by the proposed method. (a) Leading electrolyte: 6 mM cadmium nitrate, Terminating electrolyte: 10 mM citric acid; (b) Leading electrolyte: 10 mM histidine hydrochloride/0.1% Triton X-100, Terminating electrolyte:10mM hexanoic acid; A, Cl^-; B, F^-; C, SO_4^{2-}.

2.3.1.3 Volume of 1 M sodium hydroxide for coprecipitating fluoride ion

The volume of 1 M sodium hydroxide was varied in the range 0.25~ 2.0ml for coprecipitating fluoride ion. As the sample, 50ml of artificial seawater containing 1.5 mg/l fluoride ion was used. The zone length for fluoride ion increased with volume up to 1.5 ml, but then almost levelled off, as shown in Fig.2-4. Therefore, 1.5ml was selected as the suitable volume of 1M sodium hydroxide.

2.3.1.4 Concentration of sodium hydroxide solution for washing precipitate

The concentration of sodium hydroxide solution was varied in the range 10^{-5}~ 10^{-1} M for washing the precipitate. The zone length for fluoride ion had the maximum value at 10^{-3} M, as shown in Fig.2-5. Therefore, 10^{-3} M was selected as the optimum concentration of sodium hydroxide solution.

2.3.1.5 Working curve of fluoride ion by coprecipitation enrichment

A linear working curve was obtained for 5 artificial seawater samples containing 0.5~ 2.5 mg/l fluoride ion. The regression equation was $y=6.57x+0.3$ (correlation coefficient 0.999) where x

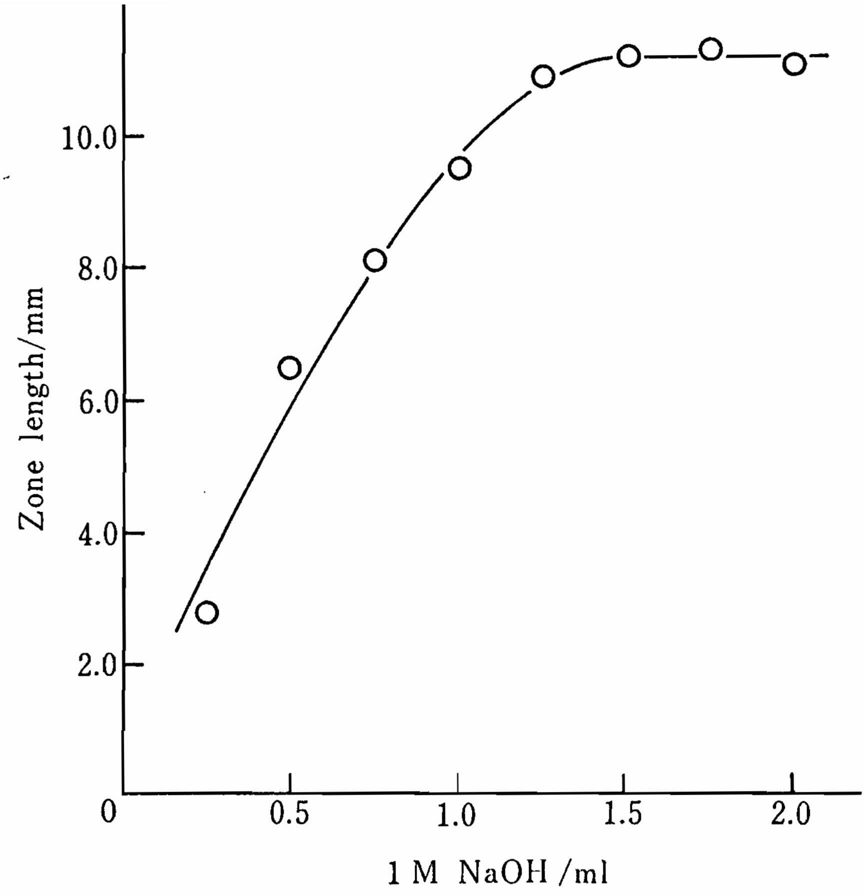

Fig. 2-4 Effect of 1 M sodium hydroxide volume.

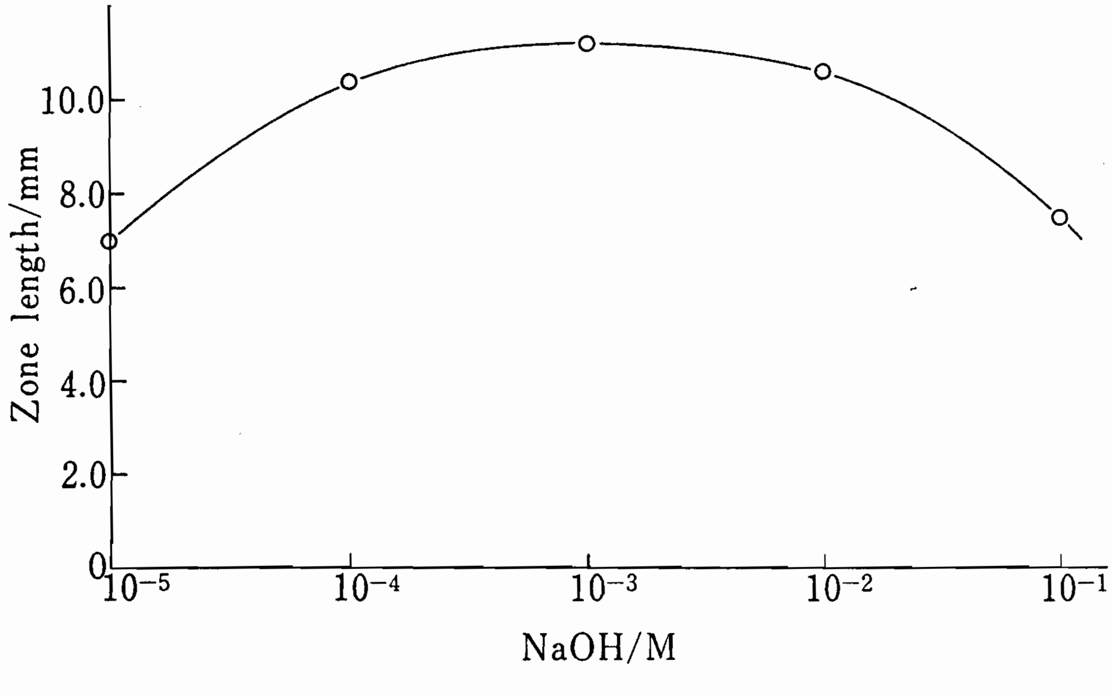

Fig. 2-5 Effect of sodium hydroxide concentration.

and y are the same described above. The relative standard deviations were obtained by calculating the zone length per 1.0mg/l at each point on the working curve or by similarly treating 4 samples containing 1.5 mg/l fluoride ion. Both relative standard deviations were found to be 0.037 (n=5 or 4). The lower determination limit for fluoride ion was 15 μ g/l, corresponding to a 0.1-mm zone length. The recovery of fluoride ion was 72± 3 %. This is mainly because the coprecipitation of fluoride ion is incomplete. It does not matter in practice, because the recovery is almost constant in the range 0.5～ 2.5 mg/l fluoride ion.

2.3.1.6 Analysis of seawater samples

The proposed method was applied to the determination of fluoride ion in surface seawater samples collected around the coastal area of Osaka Bay between Port of Amagasaki and Suma beach on 4 April 1984. An isotachopherogram of surface seawater from the outside of the pond at KUMM is shown in Fig. 2-6. The results obtained by the working curve method agreed closely with those obtained by the standard addition method, as shown in Table 2-1. Further, the concentrations of fluoride ion listed in Table2-1 agreed approximately with those obtained by Greenhalgh et al.$^{1)}$, Okutani et al.$^{3)}$ and Rix et al.$^{4)}$ (1.371 mg/l, 1.03 mg/l and 1.35 ± 0.05 mg/l, respectively). The recovery of fluoride ion in the standard addition experiment was approximately constant (71± 2 %)

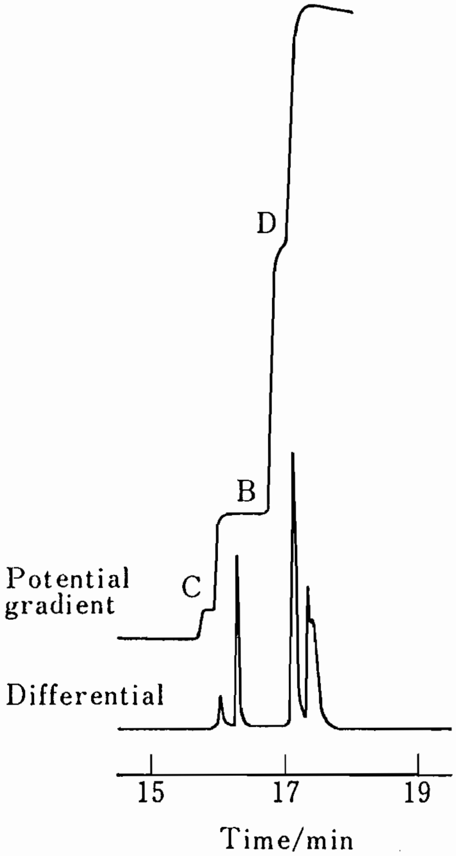

Fig. 2-6 Isotachopherogram of seawater sample treated by the proposed method. B, F^-; C, $SO_4{}^{2-}$; D, $PO_4{}^{3-}$.

Table 2-1 Results for fluoride ion in seawater

Sampling site	Temp. (°C)	pH	Salinity (‰)	F^- found (mg/l)	
				W.C.b)	S.A.c)
Port of Amagasaki	12.8	8.59	15.3	1.0	1.0
Koshien ferry terminal	9.4	8.49	27.1	1.2	1.1
Nishinomiya harbor	11.4	8.48	25.4	1.2	1.4
Fukaehama-machi	10.6	8.53	25.2	1.2	1.1
Pond at KUMMa)	9.5	8.24	26.8	0.9	1.0
Outside of the pond at KUMMa)	9.3	8.29	26.5	1.2	1.3
Uozakihama-machi	9.3	8.49	26.6	1.3	1.3
Sumiyoshihama-machi	9.7	8.52	25.9	1.2	1.3
Maya pier	9.8	8.58	26.0	1.2	1.1
Port of Kobe	9.4	8.58	27.0	1.3	1.3
Nagata harbor	9.7	8.44	27.3	1.2	1.2
Suma beach	8.8	8.54	28.0	1.2	1.2

Sampling date:4 April 1984; a), Kobe University of Mercantile Marine; b), Working curve method; c), Standard addition method.

as well as that described above.

2.3.2 Determination of bromide ion in seawater

2.3.2.1 Electrolyte system

In the proposed method, it is presumed that chloride ion in seawater is also oxidized to a certain degree and absorbed in the solution placed in the inner tube20). Therefore, the optimum leading electrolyte was examined for the isotachophoretic separation of bromide and chloride ions. It is known that these ions are separated with methanolic electrolytes in which their mobilities are different, but not with aqueous electrolytes in which they are almost equal33). Thus the concentration of tris in the leading electrolyte described above was varied in the range 5~ 20 mM to examine their separation. A 10-μ l volume of a mixture containing 10^{-3} M bromide and chloride ions was injected into the isotachophoretic analyser. The 10-cm long precolumn was used, and the migration current was maintained at 150 μ A for the first 13 ~ 16min and then reduced to 75 μ A. The difference between the PU values of bromide and chloride ions was less than 0.07 when the concentration of tris was lower than 7.5 mM, and it was almost constant, 0.14 when the concentration was higher than 10 mM, as shown in Fig. 2-7. Therefore, 10 mM tris was selected as the concentration in the leading electrolyte.

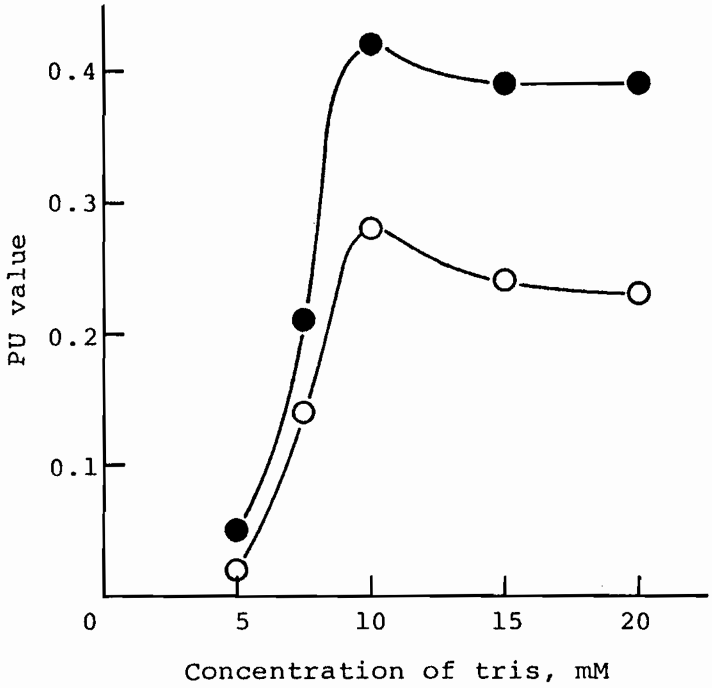

Fig. 2-7 Effect of tris concentration on the PU values of bromide and chloride ions. ○ , Br^-; ● , Cl^-.

2.3.2.2 Redox condition

A potassium permanganate solution containing sulfuric acid 20), a sodium hypochlorite solution containing nitric acid22), etc. have been used to oxidize bromide ion. The former solution was adopted in this study, because it was presumed that significant amounts of chloride ion derived from sodium hypochlorite were detected in the isotachophoretic analyser when the latter was used. That is to say, 0.3 ml of 9 M sulfuric acid and 0.7 ml of 0.02 M potassium permanganate were added to 10 ml of the seawater samples. On the other hand, the reducing condition of bromine to bromide ion was examined in detail by Katoh22) using hydrogen peroxide solution containing acetic acid. Therefore, the following solution was adopted as the reducing solution of bromine: a mixed solution of 10 ml of water, 0.5 ml of 0.1 M acetic acid and 2.0 ml of 3 % hydrogen peroxide. These reactions are as follows.

$$10Br^- + 2MnO_4^- + 16H^+ \longrightarrow 5Br_2 + 2Mn^{2+} + 8H_2O$$

$$Br_2 + H_2O_2 \longrightarrow 2Br^- + 2H^+ + O_2$$

2.3.2.3 Microporous PTFE tube

Artificial seawater samples containing 68 mg/l bromide ion were analysed by use of the gas-liquid separators with the 2 kinds of PTFE tube described above. The zone lengths for bromide or chloride ions in both isotachopherograms were almost equal, respectively; i.e. there was no difference in the permeabilities of bromine and chlorine by these PTFE tubes. The former PTFE tube was used in the following experiments.

2.3.2.4 Flow-rate of sample circulation

The flow-rate of sample solution was varied in the range 3.0~ 13.0 ml/min. Both zone lengths for bromide and chloride ions increased with flow-rate up to 7.0 ml/min, but then almost levelled off, as shown in Fig.2-8. Therefore, 7.0 ml/min was adopted as the optimum flow-rate for sample circulation.

2.3.2.5 Duration of sample circulation

The circulation time was varied in the range 5~ 40min. These results are shown in Fig.2-9. The zone length for bromide ion increased with circulation time up to 20 min, but then almost levelled off. On the other hand, the zone length for chloride ion increased with circulation time up to 40min. Therefore, 20min was adopted as the circulation time.

2.3.2.6 Effect of temperature

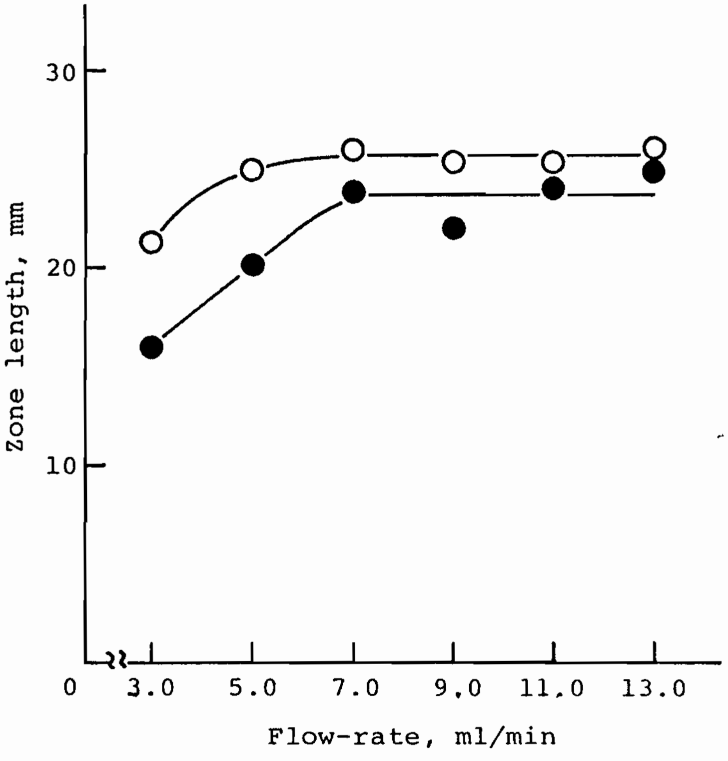

Fig. 2-8 Effect of the flow-rate of sample solution. ○ , Br^-; ● , Cl^-.

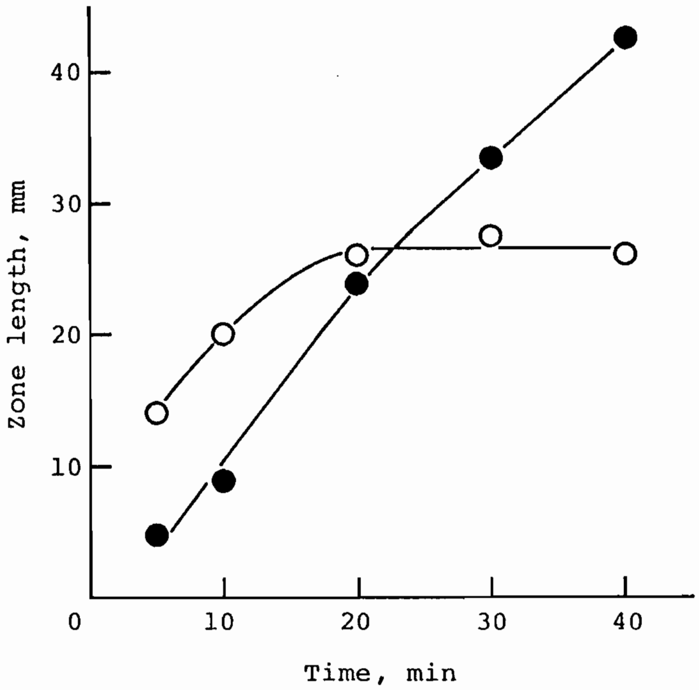

Fig. 2-9 Effect of circulation time. ○ , Br^-; ● , Cl^-.

The temperature of the water-bath was varied in the range 12.5~ 50 ℃. These results are shown in Fig.2-10. The zone length for bromide ion increased with temperature up to 40 ℃, but then almost levelled off. On the other hand, the zone length for chloride ion gradually increased with temperature up to 30 ℃, and then rapidly when this was> 30 ℃. Iwasaki et al.$^{20)}$ described that the degree of the oxidation for coexisting chloride ion slightly increased with temperature in the range 15~ 30 ℃ when bromide ion was oxidized. Therefore, 40℃ was adopted as the temperature of the water-bath.

2.3.2.7 Working curve

A linear working curve was obtained for 7 artificial seawater samples containing 10~ 100 mg/l bromide ion. The regression equation was $y=0.332x+0.6$ (correlation coefficient 0.998) where x is the concentration of bromide ion in mg/l and y the zone length in mm when the recording speed is adjusted to 20mm/min. The relative standard deviation was found to be 0.050 (n=7). The lower determination limit for bromide ion was 2.0×10^{-1} mg/l assuming 6.7×10^{-2}-mm zone length could be read by the Chromatopac C-R2AX. The recovery of bromide ion, at 42± 3 %, was less than complete. An artificial seawater sample containing 68 mg/l bromide ion was analysed according to the procedure, except the solution in the inner tube was increased to 3.0 ml in volume and circulated at a

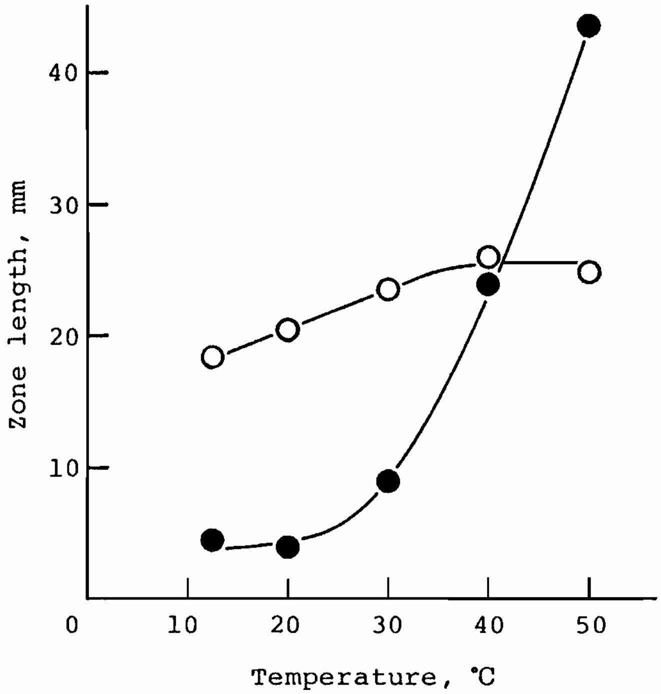

Fig. 2-10 Effect of temperature. ○ , Br^-; ● , Cl^-.

flow-rate of 1.0 ml/min. The recovery of bromide ion increased to 65 %, but did not to more than that when the solution was increased to 4.5 ml. In these cases, the zone lengths for bromide ion decreased, because the degrees of enrichment decreased. Since the recovery is almost constant in the range 10~ 100 mg/l bromide ion, the incomplete recovery does not matter in practice.

In spite of the constant concentration of chloride ion in the artificial seawater samples, the zone length for chloride ion increased linearly with the concentration of coexisting bromide ion. The regression equation of this curve was $y=0.257x+5.7$ (correlation coefficient 0.996). Shiota et al.[11] found out that when iodine was oxidized to iodate ion by using the potassium permanganate solution containing sulfuric acid, the oxidation was catalytically accelerated by a trace amount of coexisting bromide ion. Further, that was applied to the determination of a trace amount of bromide ion. The redox reactions and the standard redox potentials of bromine, iodate ion and chlorine are as follows[34]:

$$Br_2+2e \rightleftharpoons 2Br^- \qquad E^0=1.09\ V$$

$$IO_3^-+6H^++5e \rightleftharpoons 1/2I_2+3H_2O \qquad E^0=1.19\ V$$

$$Cl_2+2e \rightleftharpoons 2Cl^- \qquad E^0=1.39\ V$$

From these facts, the increase of the zone length of chloride ion probably is due to the catalytic acceleration of the oxidation of chloride ion by coexisting bromide ion.

2.3.2.8 Analysis of seawater samples

The proposed method was applied to the determination of bromide ion in surface and bottom seawater samples collected around the coastal area of Osaka Bay between Port of Amagasaki and Rokko Island on 6 April 1987. An isotachopherogram of surface seawater from the Nishinomiya harbor is shown in Fig. 2-11. Then 40～60 mg/l bromide ion was added to seawater from the Nishinomiya harbor diluted 2 times, and analysed. These results are shown in Table 2-2. There was a large difference owing to the influence of land-water between the concentrations of bromide ion in surface and bottom seawaters except those from the pond at KUMM and the Rokko Island. The recovery of bromide ion in the standard addition experiment was 103± 4 %.

Yamamoto et al.[9)] reported that an approximately linear relation existed between the chlorinity and the concentration of bromide ion in estuarine waters. Further, the relation between salinity S in ‰ and chlorinity Cl in ‰ is represented by the following equation[35)].

$$S=1.80655\times Cl$$

Therefore, the chlorinity was calculated from the salinity listed in Table2-2 and the above equation, and a linear relation was obtained between the chlorinity and the concentration of bromide

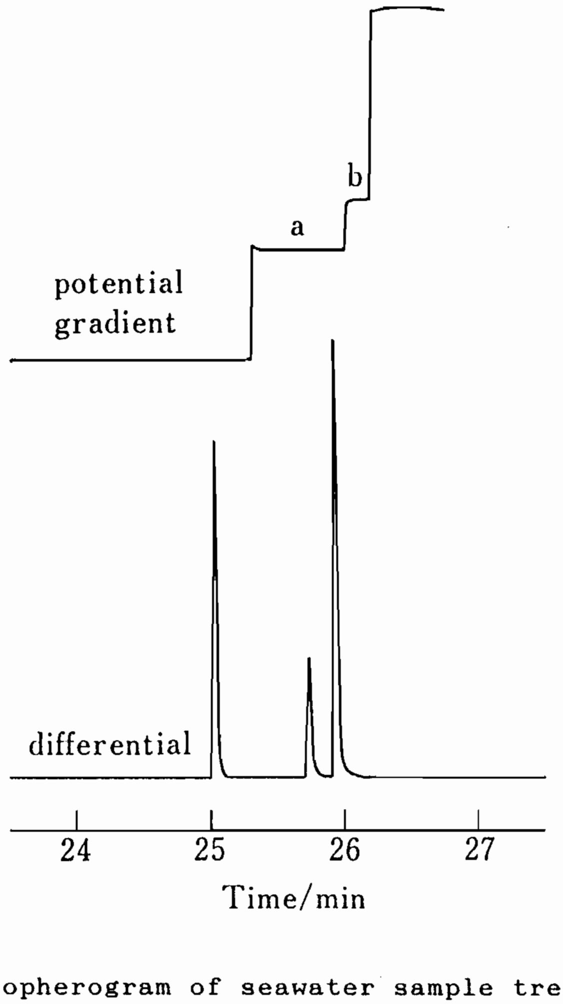

Fig.2-11 Isotachopherogram of seawater sample treated by the proposed method. a, Br^-; b, Cl^-.

Table 2-2 Results for bromide ion in seawater

Sampling site	Depth	Temp.	pH	Salinity	Br$^-$		
	(m)	(°C)		(‰)	Added (mg/l)	Found (mg/l)	Rec. (%)
Port of Amagasaki	0	16.5	8.52	9.0	—	18.4	—
Port of Amagasaki	4.8	11.2	7.96	28.0	—	61.8	—
Mouth of Muko river	0	14.6	7.91	21.1	—	49.4	—
Mouth of Muko river	3.7	10.5	7.90	24.5	—	66.0	—
Nishinomiya harbor	0	13.4	8.14	17.7	—	40.7	—
Nishinomiya harbor[a)]	0				—	19.3	—
Nishinomiya harbor[a)]	0				50.0	69.3	100
Nishinomiya harbor[a)]	0				60.0	83.4	107
Nishinomiya harbor	2.5	10.9	7.86	27.9	—	63.3	—
Nishinomiya harbor[a)]	2.5				—	30.7	—
Nishonomiya harbor[a)]	2.5				40.0	73.2	106
Nishinomiya harbor[a)]	2.5				60.0	93.4	105
Pond at KUMM	0	12.6	8.53	24.6	—	62.4	—
Pond at KUMM	5.5	11.0	8.15	28.1	—	65.1	—
Rokko Island	0	12.5	8.37	27.2	—	60.3	—
Rokko Island	5.5	10.5	8.23	29.6	—	59.9	—

Sampling date: 6 April 1987; a), 2 times diluted.

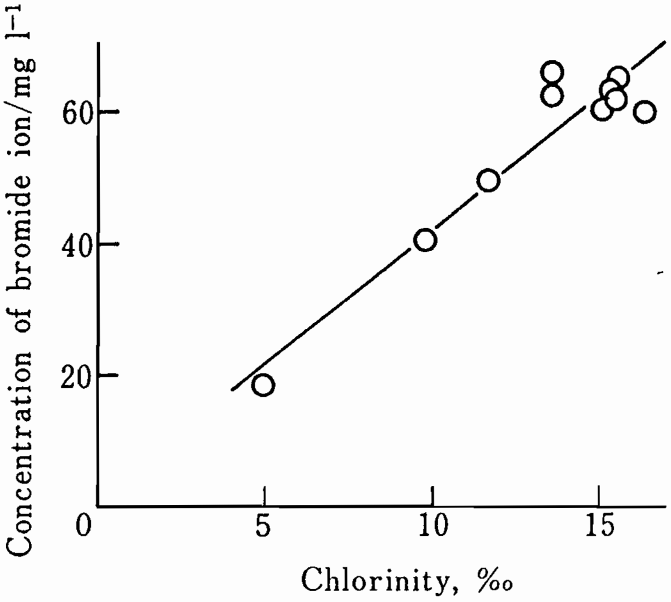

Fig.2-12 Relation between chlorinity and concentration of bromide ion.

ion listed in Table 2-2, as shown in Fig. 2-12. The regression equation of this curve was $y=4.04x+1.6$ (correlation coefficient 0.946) where x is the chlorinity in ‰ and y the concentration of bromide ion in mg/l. This also confirms the analytical results listed in Table 2-2.

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3 Determination of sulfide in seawater

3.1 Introduction

It is important to determine the concentration of sulfide in environmental waters in relation to water pollution. For example, it is said that hydrogen sulfide is generated upon eutrophication of seawater$^{1)}$. In addition,cloudiness in seawater or underground-water is considered to be caused by the presence of sulfide$^{2,3)}$.

Methylene blue spectrophotometry and iodometric titration are generally used for the determination of sulfide ion in aqueous solutions$^{4\sim 6)}$. Goda et al.$^{7,8)}$ determined sulfide ion in artificial aqueous samples and in deposits on the seabed by fluorescence analysis using the fluorescence quenching of mercuriofluoresceinates. However, these methods may lack sensitivity and be affected by interfering substances.

On the other hand, there is no report concerning the isotachophoretic determination of sulfide in seawater. For this purpose, it is necessary to separate sulfide from large amounts of coexisting anions, and to enrich prior to the isotachophoretic measurement.

Therefore, a new pretreatment procedure using an acid-base equilibrium in the gas-liquid separator described in Section2.2.1 was applied to the isotachophoretic determination of sulfide in seawater and the optimum analytical conditions were established.

The method was also applied to the determination of sulfide in surface and bottom seawater samples.

3.2 Experimental

3.2.1 Apparatus

The isotachophoretic analyser was the same as described in Section 2.2.1. The main column was a FEP copolymer tube, 15 cm long, 0.5 mm inner diameter; the precolumn was a PTFE tube, 10 ~ 30 cm long, 1.0 mm inner diameter. The Hamilton 1710-N and the 1725-N microsyringes were used for the injection of samples. The gas-liquid separator was the same as described in Section 2.2.1; the inner microporous PTFE tube, 2 μm of maximum pore size, 50 % porosity. The flow system was the same as described in Section 2.2.1, except the inner tube was filled with sodium hydroxide solution, and the sample solution to which sulfuric acid was added, was circulated through the outer tube. The dissolved oxygen (abbreviated as DO) content of seawater was measured by a Yellow Springs Instrument 58 DO meter. The pH meter, the salinometer and the water samplers were the same as described in Section 2.2.1. Seawater samples were taken in narrow-mouthed bottles (100 ml) fitted with ground stoppers cut at a 45-degree angle.

3.2.2 Reagents

All solutions were prepared from analytical reagent grade chemicals. Standard solutions of sulfide were prepared by dissolving sodium sulfide in oxygen-free water. Oxygen-free water was prepared by boiling water from the same apparatuses described in Section 2.2.2 for 30 min, and by quickly cooling to avoid absorption of air[5,9]. The standard solutions were prepared immediately before use.

The barium cation-exchange resin was prepared as follows:200 ml of 0.1 M barium chloride were added to 75 ml of Dowex 50W X8 (200~ 400mesh) cation-exchange resin in the sodium form and stirred for 15 min; the supernatant solution was discarded, and the resin was treated with 200 ml of water in a similar manner; these treatments were twice repeated; finally, the resin was washed thoroughly with water on a filter paper and stored in water. The preparation of the artificial seawater was based on a Japanese Standard[10].

3.2.3 Procedure

Seawater samples were analysed by the following procedure as soon as possible after collection. Add 0.5ml of 9 M sulfuric acid to 45 ml of seawater sample filtered through a 0.45-μ m membrane to generate hydrogen sulfide. Adjust the total volume to 50 ml with oxygen-free water. With the water-bath temperature set at 40 °C, circulate 10 ml of this solution through the outer tube at a flow-rate of 9.0 ml/min for 10 min. Hydrogen sulfide permeates through the wall of the inner tube and dissolves in 1.5 ml of 1.0×10^{-2} M sodium hydroxide previously placed in the inner tube. Pump out the sodium hydroxide solution. Large amounts of carbon dioxide in the seawater samples also permeates through the wall of the inner tube and dissolves in the sodium hydroxide solution. As carbon dioxide interferes with the isotachophoretic measurement of sulfide, remove it as barium carbonate; i.e. add 0.5 g of barium cation-exchange resin to the sodium hydroxide solution, shake the solution for 3 min, and then filter it under reduced pressure. Inject 150 μ l of the resulting solution into the isotachophoretic analyser. Maintain the migration current at 150 μ A for the first 15min and then reduce it to 50μ A. As leading electrolyte use 5 mM sodium hydroxide/0.1 % HPMC, and as terminating electrolyte 10 mM sodium acetate. Prepare a working curve by applying the method to synthetic standards.

3.3 Results and Discussion

3.3.1 Leading electrolyte

The following leading electrolytes were examined for the determination of sulfide using a 10-cm long precolumn: (I) 5mM hydrochloric acid containing 0.1% HPMC, adjusted to pH 10.5 with 2-amino-2-methyl-1-propanol; (II)5mM sodium hydroxide; (III) 5mM sodium hydroxide containing 0.1% HPMC. Both precolumn and main column were exchanged for new ones when the leading electrolyte (Ⅱ) was used, because isotachophoretic separation was generally affected by the HPMC that adhered to the column walls[11)]. A 100-μl volume of a standard solution containing 2.0 mg/l sulfide was injected into the isotachophoretic analyser. The leading electrolyte (Ⅲ) was selected for subsequent experiments, as sulfide could be detected most successfully.

3.3.2 Volume of sulfuric acid

The volume of 9 M sulfuric acid added to the artificial seawater sample containing 5.0 mg/l sulfide was varied in the range 0.1～1.5ml. A 100-μl portion of the solution treated by the proposed method was injected into the isotachophoretic analyser equipped with a 25-cm long precolumn. The zone length for sulfide in the isotachopherograms increased with the sulfuric acid volume up to 0.5 ml, but then almost levelled off, as shown in Fig. 3-1. Therefore, 0.5ml of 9 M sulfuric acid was selected and the subsequent mixture was diluted to 50 ml.

3.3.3 Concentration of sodium hydroxide solution

The concentration of the sodium hydroxide solution was varied in the range 0.5×10^{-2}～1.25×10^{-2} M. The zone length for sulfide

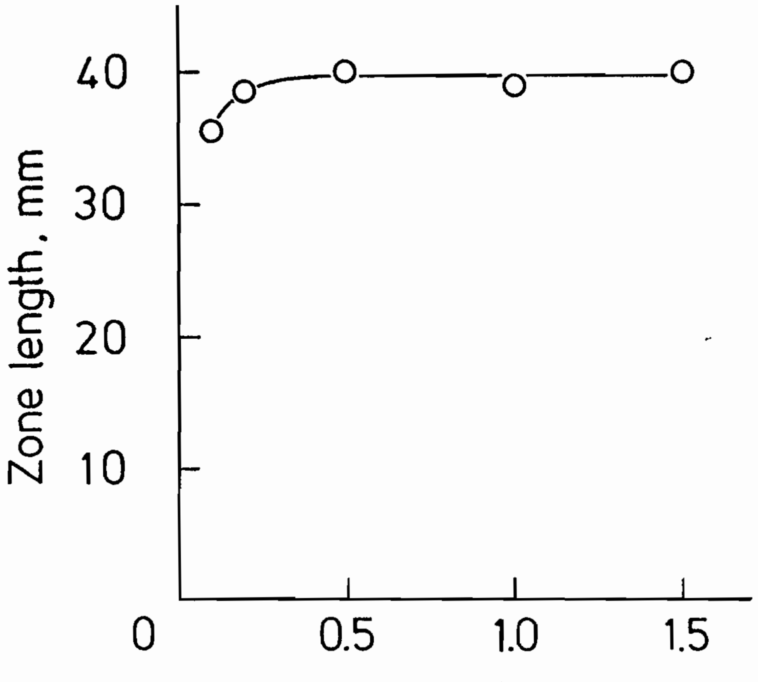

Fig. 3-1 Effect of 9 M sulfuric acid volume.

greatly increased with the sodium hydroxide concentration up to 1.0×10^{-2}M, and slightly up to 1.25×10^{-2}M, as shown in Fig. 3-2. On the other hand, the longer time for the isotachophoretic measurement with 1.25×10^{-2} M was due to the excess of hydroxide ion in the treated solution. Therefore, 1.0×10^{-2}M was adopted as the optimum sodium hydroxide concentration.

3.3.4 Flow-rate of sample circulation

The flow-rate of sample solution was varied in the range 1.0 ~ 10.0 ml/min. The zone length for sulfide increased with flow-rate up to 9.0 ml/min, but then almost levelled off, as shown in Fig. 3-3. Therefore, 9.0 ml/min was adopted as the optimum flow-rate for sample circulation.

3.3.5 Duration of sample circulation

The circulation time was varied in the range 5~ 30 min. The zone length for sulfide gradually decreased with increasing circulation time, as shown in Fig. 3-4. This decrease was considered to be due to the slight oxidation of sulfide over periods longer than 15min. Therefore, 10min was adopted as the circulation time.

3.3.6 Effect of temperature

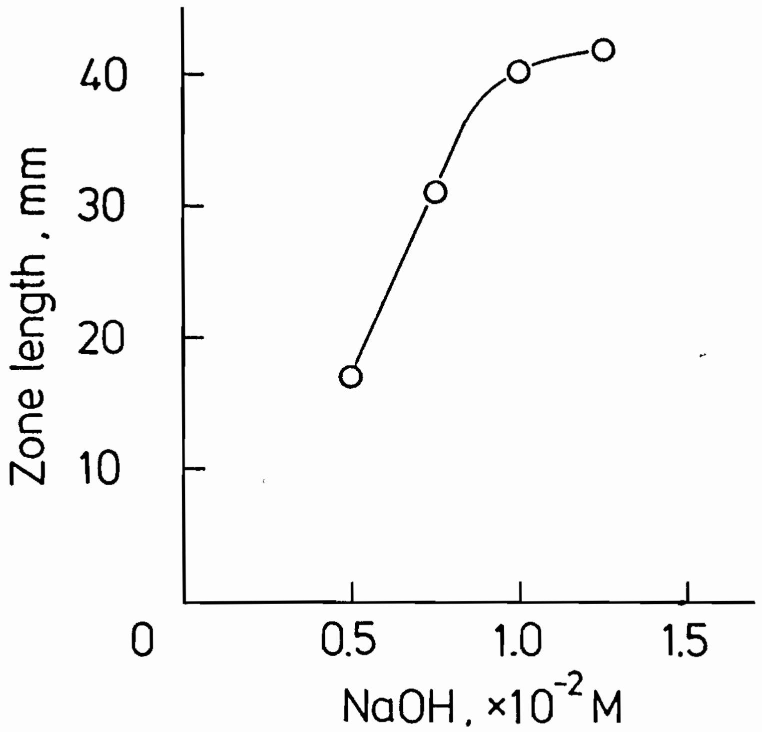

Fig. 3-2 Effect of sodium hydroxide concentration.

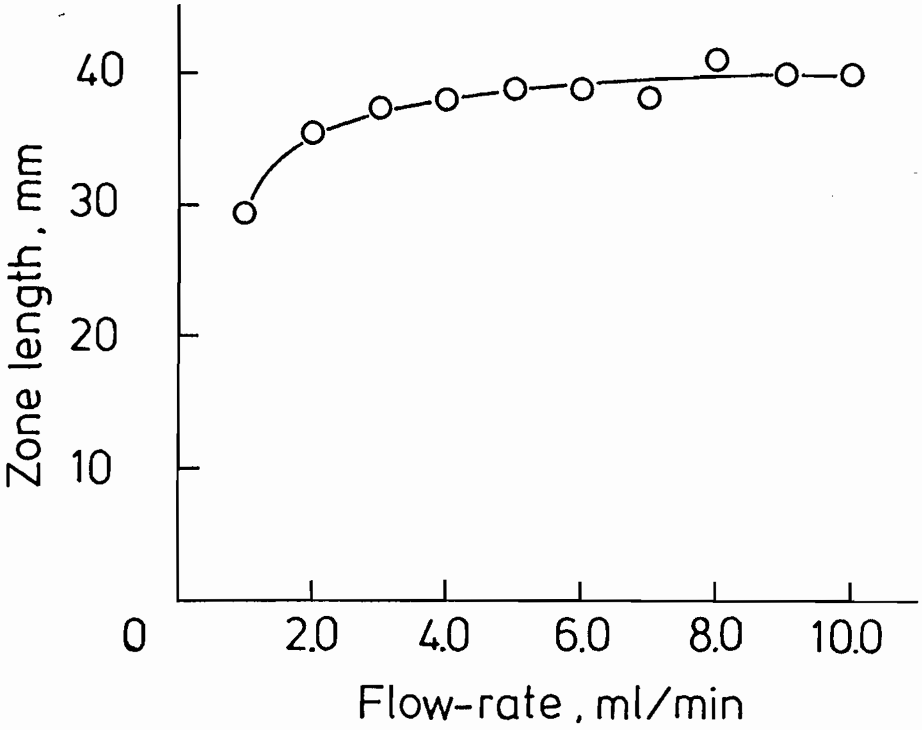

Fig. 3-3 Effect of the flow-rate of sample solution.

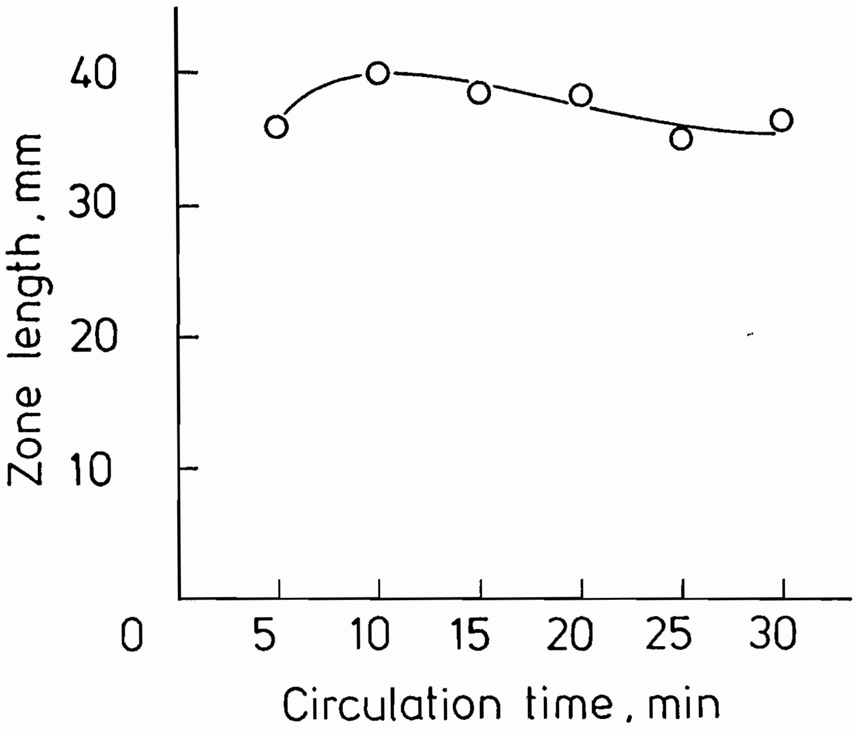

Fig. 3-4 Effect of circulation time.

The temperature of the water-bath was varied in the range 30 ∼ 50 ℃. The zone length for sulfide was almost constant over the whole range, as shown in Fig. 3-5. This constancy was considered to be due to the poor solubility of hydrogen sulfide, i.e.,2.014, 1.664 and 1.376 cm^3/cm^3 of water at 30, 40 and 50 ℃, respectively[12,13]. A temperature of 40 ℃ was adopted as temperature controle was easier.

3.3.7 Volume of injected sample

The volume of sample injected into the isotachophoretic analyser was varied in the range 50∼ 250 μl. The zone length for sulfide increased in proportion to the volume up to 150 μl, and then the inclination of the curve became gentle, as shown in Fig. 3-6. Therefore, 150 μl was adopted as the volume of sample injected.

3.3.8 Working curve

A linear working curve was obtained for 5 artificial seawater samples containing 0.25∼ 2.0 mg/l sulfide. The regression equation was $y=13.7x-0.1$ (correlation coefficient 1.000) where x is the concentration of sulfide in mg/l and y the zone length in mm when the recording speed is adjusted to 20 mm/min. The relative standard deviation was found to be 0.028 (n=5). The lower

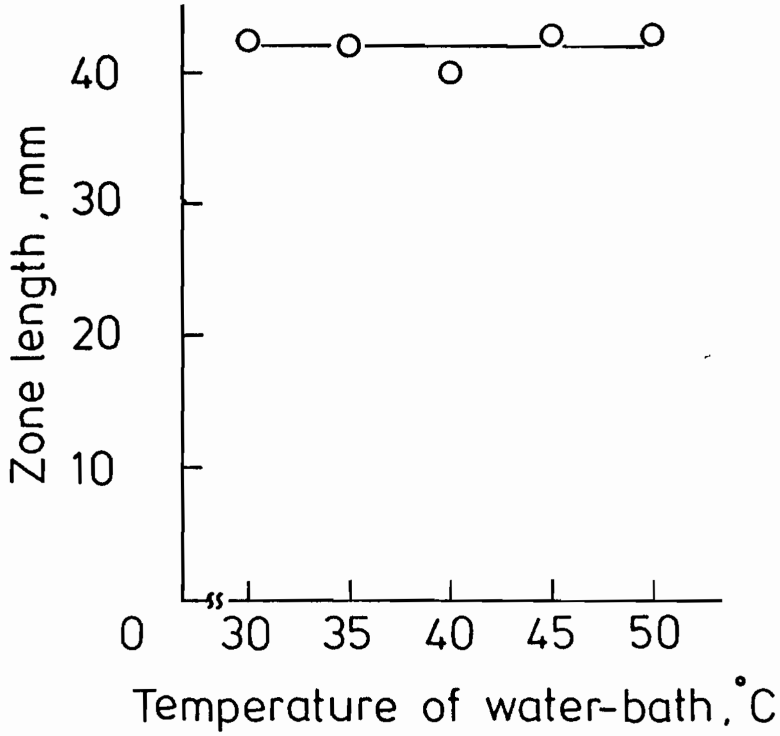

Fig. 3-5 Effect of temperature.

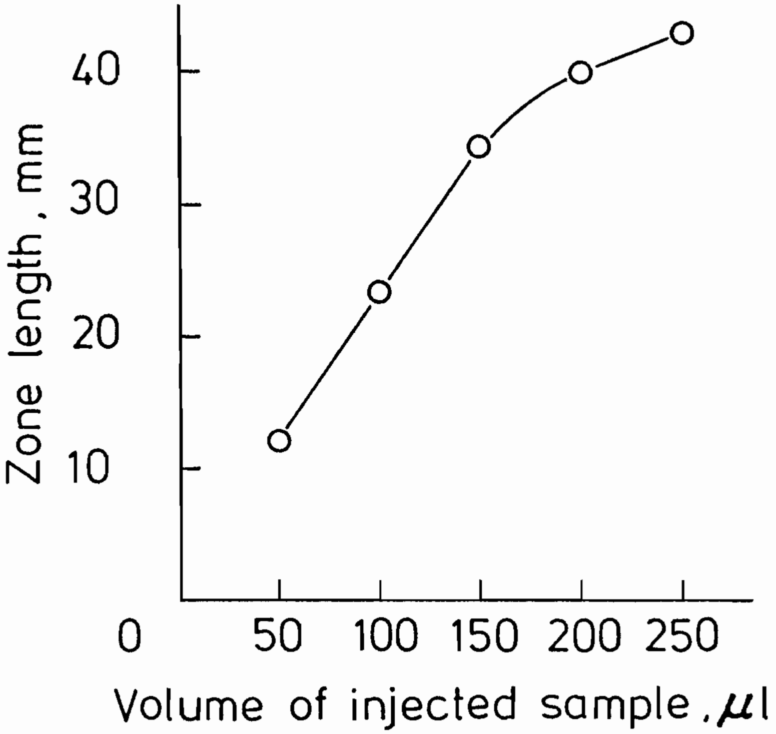

Fig.3-6 Effect of sample volume injected into the isotachophoretic analyser.

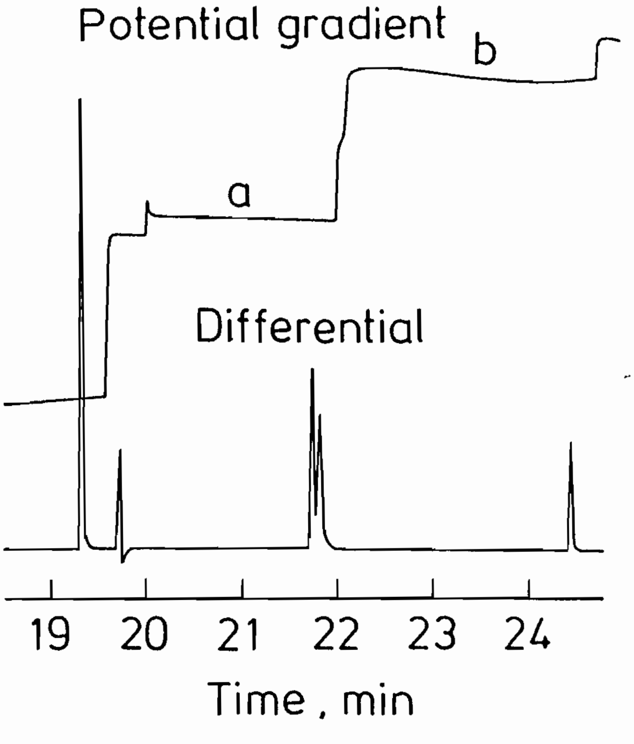

Fig. 3-7 Isotachopherogram of artificial seawater sample treated by the proposed method. a, HS^-; b, CO_3^{2-}.

Table 3-1 Results for sulfide in seawater

Sampling site	Depth	Temp.	pH	Salinity	DO	Sulfide		
	(m)	(°C)		(‰)	(mg/l)	Added (mg/l)	Found (mg/l)	Error (%)
Port of Amagasaki	4.5	24.5	7.53	23.8	1.72	—	N.D.a)	—
Koshien ferry terminal	4.0	24.4	7.63	28.6	3.38	—	N.D.a)	—
Nishinomiya harbor	0	24.9	8.76	24.3	10.51	—	N.D.a)	—
Nishinomiya harbor	3.0	25.4	7.82	28.9	3.11	—	1.6	—
Pond at KUMM	0	25.4	8.18	27.0	8.83	—	N.D.a)	—
Pond at KUMM	5.0	24.9	7.84	28.9	4.46	—	N.D.a)	—
Rokko Island	0	24.5	7.80	28.1	4.83	—	N.D.a)	—
Rokko Island	0					0.5	0.5	± 0.0
Rokko Island	0					1.0	1.1	+10.0
Rokko Island	0					1.5	1.6	+6.7
Rokko Island	5.5	24.2	7.80	29.1	3.32	—	0.1	—
Maya pier	0	24.4	7.95	23.4	6.57	—	N.D.a)	—
Maya pier	4.0	24.6	7.83	27.4	4.71	—	N.D.a)	—
Port of Kobe	0	24.1	7.95	29.0	5.39	—	N.D.a)	—
Port of Kobe	4.5	24.1	7.96	29.0	5.08	—	N.D.a)	—

Sampling date: 16～19 September 1986; a), Not detected.

determination limit for sulfide was 7.3×10^{-3} mg/l, corresponding to a 0.1-mm zone length. The recovery of sulfide, at 42± 2 %, was less than complete, but this does not matter in practice, because the recovery is almost constant in the range 0.25～ 2.0 mg/l sulfide. Fig.3-7 shows the isotachopherogram of 100μl of an artificial seawater sample containing 5.0 mg/l sulfide.

3.3.9 Analysis of seawater samples

The proposed method was applied to the determination of sulfide in surface and bottom seawater samples collected around the coastal area of Osaka Bay between Port of Amagasaki and Port of Kobe on 16～ 19 September 1986. Then 0.5～ 1.5 mg/l sulfide was added to surface seawater from the Rokko Island, and analysed. These results are shown in Table 3-1. Sulfide was not detected in these samples except bottom seawaters from the Nishinomiya harbor and the Rokko Island. The very high concentration of sulfide in bottom seawater from the Nishinomiya harbor was considered to be due to hydrogen sulfide generated in deposits in the enclosed area. The error in the standard addition experiment was less than ± 10.0 %.

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4 Determination of various phosphate ions in seawater

4.1 Introduction

It is important to determine the concentrations of various phosphate (e.g. triphosphate, diphosphate and orthophosphate) ions in environmental waters according to the prevention of eutrophication.

There are several reports concerning the separation and determination of various phosphate ions in river or lake-water$^{1\sim 3)}$, while there are not those in seawater.

On the other hand, when CITP is applied to the determination of various phosphate ions in seawater, a suitable pretreatment is necessary for the same reason described in Section 2.1. A coprecipitation has already been applied to the determination of phosphate ions in seawater as a pretreatment method$^{4\sim 8)}$.

Therefore, the CITP method for fluoride ion (see Chapter 2) was applied to the separation and determination of various phosphate ions in seawater; as the fluoride ion coprecipitated at the same time interfered with the isotachophoretic measurement, the ion was removed by passing the treated solution through the column packed with a lanthanum cation-exchange resin. At first, the pH of leading electrolyte was examined for the isotachophoretic separation of various phosphate ions. With the optimum analytical conditions, various phosphate ions in the mixed solutions were determined. In general, the volume of sample injected into the isotachophoretic analyser is less than several tens μ l. As the sensitivity is insufficient for the determination of low concentrations of ions with such volume of sample, a PTFE tube was connected to a usual capillary tube to increase the volume of sample up to 150 μ l. As a result, low concentrations of various phosphate ions could be determined. Then working curves of various phosphate ions were prepared. Finally, the method was also applied to the determination of various phosphate ions in surface seawater samples.

4.2 Experimental

4.2.1 Apparatus

The Shimadzu IP-1B isotachophoretic analyser was used, with a potential gradient detector. The capillary tube was a FEP copolymer tube, 20cm long, 0.5 mm inner diameter; a PTFE tube, 22.5 cm long, 1.0mm inner diameter was connected to the capillary tube when 150 μ l of sample was injected. The Terumo MS-10 and the Hamilton 1725-N microsyringes were used for the injection of samples. A Gilson Pipetman P-200 micropipet, a Furue peristaltic pump, an Omnifit column, 15cm long, 3mm inner diameter and a Toa-Denpa HM-18E pH meter were also used. The stirrer, the centrifuge, the pH meter and the water sampler were the same as described in Section 2.2.1. Seawater samples were stored according to the same procedure described in Section 2.2.1.

4.2.2 Reagents

All solutions were prepared from analytical reagent grade chemicals. Standard solutions of various phosphate ions were prepared by dissolving pentasodium triphosphate, tetrasodium diphosphate, and sodium dihydrogenphosphate in water from the same apparatus described in Section 1.2.2. The hydrogen cation-exchange resin was prepared by the same procedure described in Section2.2.2. The lanthanum cation-exchange resin was prepared similarly using 20 ml of Dowex 50W X8 (200∼ 400 mesh) cation-exchange resin in the hydrogen form, 60ml of 0.1 M lanthanum chloride and 100 ml of water. The preparation of the artificial seawater was based on a Japanese Standard9).

4.2.3 Procedure

Seawater samples were analysed by the following procedure as soon as possible after collection. Add 0.2 ml of 10 M sodium hydroxide to 200 ml of seawater sample to obtain the magnesium hydroxide precipitate. After standing for 40min, discard the supernatant solution. Centrifuge the residual solution containing the precipitate with 3000 rpm for 5 min. Discard the supernatant solution and wash the precipitate by the same procedure described in Section 2.2.3. Add 1.0 ml of water and 2.0 g of the hydrogen cation-exchange resin on the precipitate and stir for 5 min to dissolve the precpitate. Adjust the total volume by the same procedure described in Section 2.2.3. Pass 1.0 ml of the solution through the column packed with the lanthanum cation-exchange resin to remove the fluoride ion as lanthanum fluoride. Inject 150 μl of the treated solution into the isotachophoretic analyser. Maintain the migration current at 100μA for the first 18 min and then reduce it to 25μA with 25 ℃ of the thermostat temperature. As leading electrolyte use 10mM hydrochloric acid/0.1 % Triton X-100/histidine (pH 4.0), and as terminating electrolyte 10 mM hexanoic acid. Prepare working curves by applying the method to synthetic standards.

4.3 Results and Discussion

4.3.1 pH of leading electrolyte

Yagi et al.$^{10)}$ separated 7 kinds of phosphate ion using 10mM hydrochloric acid containing histidine and 0.1 % Triton X-100 as the leading electrolyte. Lucansky et al.$^{11)}$ separated 4 kinds of phosphate ion using 10mM hydrochloric acid containing β-alanine, creatinine or histidine as the leading electrolyte. Therefore, a 5-μl volume of a mixture containing 125 mg/l triphosphate, diphosphate and orthophosphate ions was injected into the isotachophoretic analyser equipped with only the capillary tube. The leading electrolyte proposed by Yagi et al. was used with varying its pH in the range 4.0～5.5. The terminating electrolyte was 10 mM hexanoic acid. The migratioin current was set at 50 μA. Triphosphate, diphosphate and orthophosphate ions were separated most successfully using the leading electrolyte with pH 4.0, as shown in Fig.4-1. Therefore, this leading electrolyte was selected for subsequent experiments.

4.3.2 Determination of various phosphate ions

Linear working curves were obtained for various phosphate ions by injecting 5 μl of standard solutions containing 50～250 mg/l these ions into the isotachophoretic analyser. The regression equations for triphosphate, diphosphate and orthophosphate ions were $y=7.04x-0.2$, $y=8.17x-0.1$ and $y=10.10x-0.1$, respectively. The correlation coefficients were 0.997, 0.999 and 1.000, respectively. In these equations, x is the quantity of phosphorus in μg and y the zone length in mm when the recording speed is adjusted to 5 mm/min. The lower determination limits for triphosphate, diphosphate and orthophosphate ions were 1.4×10^{-2}, 1.2×10^{-2} and 9.9×10^{-3} μg, respectively, corresponding to a 0.1-mm zone length. When 5-μl volumes of mixed solutions containing triphosphate, diphosphate and orthophosphate ions in various con-

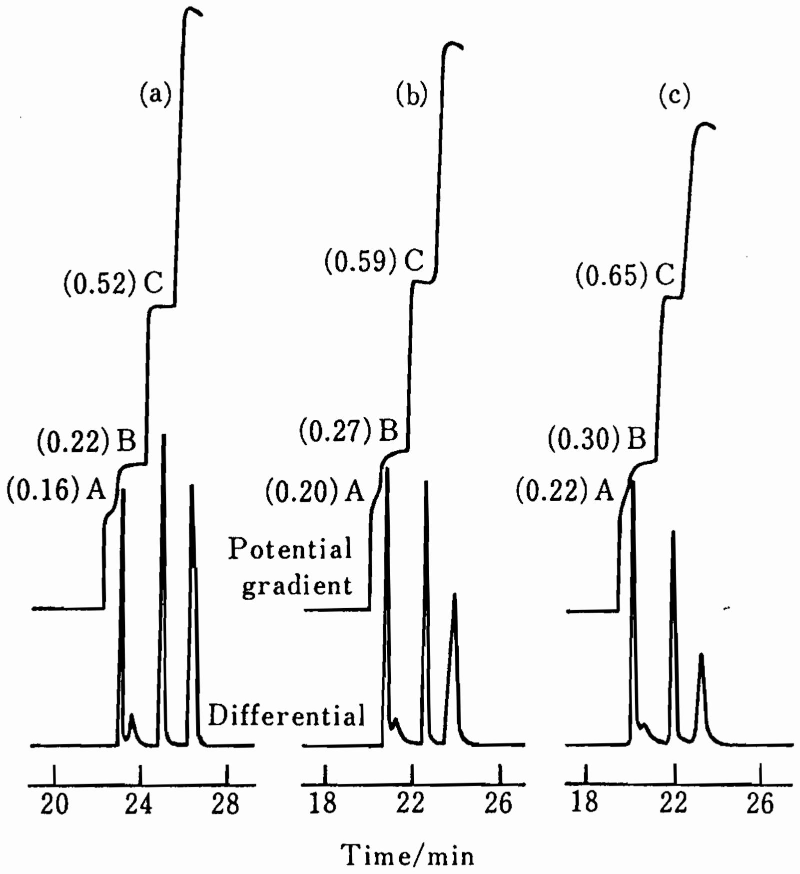

Fig. 4-1 Isotachopherograms for the separation of various phosphate ions. A, $P_3O_{10}{}^{5-}$; B, $P_2O_7{}^{4-}$; C, $PO_4{}^{3-}$; (a), pH 4.0; (b), pH 4.5; (c), pH 5.5; Values in the parentheses, PU value.

centrations were injected and analysed by use of the working curves, the error in the determination of these ions was less than ± 10.1 %, as shown in Table 4-1.

4.3.3 Injection method of large sample volume

As the concentrations of phosphate ions in seawater are relatively low, it is required to inject a large volume of sample into the isotachophoretic analyser for the determination of phosphate ions. An injection method of a 100-μ l volume of sample was proposed by Akiyama et al.$^{12)}$ as an injection method of large sample volume. An apparatus to which applied the method was marketed. The author tried to inject larger volume of sample than that of the method by Akiyama et al. That is to say, a PTFE tube, 22.5cm long, 1.0 mm inner diameter was connected to the capillary tube to increase the volume of injected sample up to 150 μ l. As the sample, a 150-μ l volume of a standard solution containing 1.0mg/l orthophosphate ion was injected into the isotachophoretic analyser. The migration current was varied in the range 10~ 100 μ A. When the migration current was below 75 μ A, it was maintained at 100μ A for the first 18 min and then reduced to respective values. Both the zone length for orthophosphate ion and the analysis time increased with decreasing migration current, as shown in Fig. 4-2 as the ratios of respective values to those of values with 100 μ A. Therefore, the procedure that the migration

Table 4-1 Analytical results for various phosphate ions

Mixture	Added, μg			Found, μg			Error, %		
	A	B	C	A	B	C	A	B	C
1	0.21	1.04	0.25	0.19	1.13	0.25	-9.5	+8.7	± 0.0
2	0.38	0.87	0.50	0.36	0.93	0.51	-5.3	+6.9	+2.0
3	0.47	0.79	0.63	0.43	0.87	0.63	-8.5	+10.1	± 0.0
4	0.54	0.71	0.75	0.52	0.77	0.75	-3.7	+8.5	± 0.0
5	0.69	0.56	1.00	0.72	0.59	1.04	+4.3	+5.4	+4.0

A, $P_3O_{10}^{5-}$; B, $P_2O_7^{4-}$; C, PO_4^{3-}.

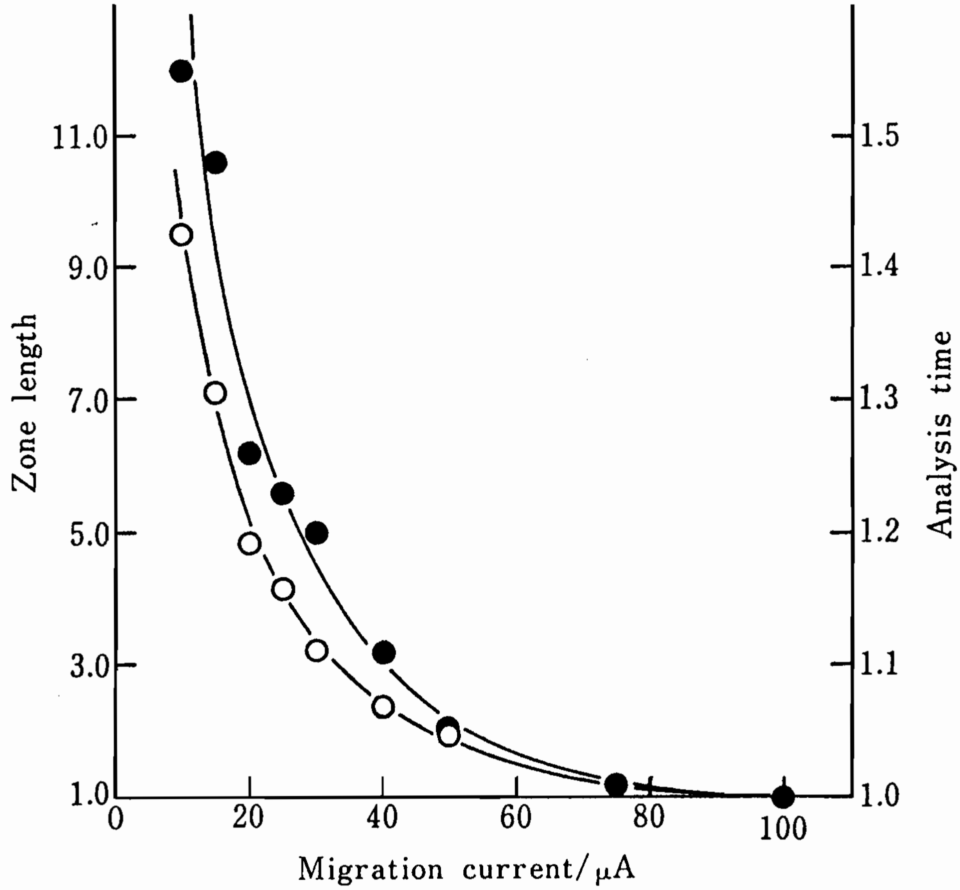

Fig. 4-2 Effect of migration current on zone length and analysis time. ○ , Zone length; ● , Analysis time.

current was maintained at 100μA for the first 18min and then reduced to 25 μA was adopted. This procedure made possible the injection of a 150-μl volume of sample.

4.3.4 Determination of various phosphate ions by injection method of large sample volume

Linear working curves were obtained for various phosphate ions by injecting 150 μl of standard solutions containing 0.2 ∼ 2.0 mg/l these ions into the isotachophoretic analyser. The regression equations for triphosphate, diphosphate and orthophosphate ions were $y=21.1x+0.1$, $y=29.8x+0.1$ and $y=39.7x+0.1$, respectively. The correlation coefficients were 0.995, 0.999 and 0.999, respectively. In these equations, x and y are the same described above except for 10 mm/min of the recording speed. The lower determination limits for triphosphate, diphosphate and orthophosphate ions were 4.7×10^{-3}, 3.4×10^{-3} and 2.5×10^{-3} μg, respectively, corresponding to a 0.1-mm zone length; it was found that 1/4∼ 1/3 of smaller amount of various phosphate ions could be determined compared with the injection of a 5-μl volume of sample. When 150-μl volumes of mixed solutions containing triphosphate, diphosphate and orthophosphate ions in various concentrations were injected and analysed by use of the working curves, the error in the determination of these ions was less than ± 20.0 % except for mixture 1, as shown in Table4-2. An isotachopherogram of mixture 8 in Table 4-2 is shown in Fig. 4-3.

4.3.5 Working curves

Linear working curves were obtained for 5～ 7 artificial seawater samples containing 5～ 213μ g/l various phosphate ions, 100, 200 and 500 ml in volume, as shown in Table 4-3. The relative standard deviations were obtained by calculating the zone length per 1.0μ g/l at each point on the working curves. When the volume of solutions were 100 and 500 ml, the volumes of 10 M sodium hydroxide to form the magnesium hydroxide precipitate were 0.1 and 0.5 ml, the times of standing to settle down the precipitate were 30 and 50 min and the amounts of hydrogen cation-exchange resin were 1.0 and 4.3 g, respectively. The precipitate was washed 4 times when the volume of solution was 500 ml.

4.3.6 Analysis of seawater samples

The proposed method was applied to the determination of phosphate ions in surface seawater samples collected around the coastal area of Osaka Bay between Port of Amagasaki and Suma beach on 4 April 1984. An isotachopherogram of surface seawater from the Nagata harbor is shown in Fig. 4-4. Triphosphate and diphosphate ions could not be detected from these samples by the proposed method, as shown in Table 4-4.

Table 4-2 Analytical results for various phosphate ions

Mixture	Added, μg			Found, μg			Error, %		
	A	B	C	A	B	C	A	B	C
1	0.07	0.26	0.09	0.06	0.23	0.12	-14.3	-11.5	+33.3
2	0.09	0.24	0.12	0.09	0.22	0.13	± 0.0	-8.3	+8.3
3	0.12	0.21	0.15	0.10	0.19	0.18	-16.7	-9.5	+20.0
4	0.13	0.20	0.18	0.13	0.19	0.20	± 0.0	-5.0	+11.1
5	0.15	0.18	0.21	0.14	0.16	0.24	-6.7	-11.1	+14.3
6	0.18	0.15	0.24	0.17	0.14	0.26	-5.6	-6.7	+8.3
7	0.19	0.14	0.27	0.18	0.13	0.29	-5.3	-7.1	+7.4
8	0.21	0.12	0.30	0.21	0.12	0.32	± 0.0	± 0.0	+6.7

A, $P_3O_{10}{}^{5-}$; B, $P_2O_7{}^{4-}$; C, $PO_4{}^{3-}$.

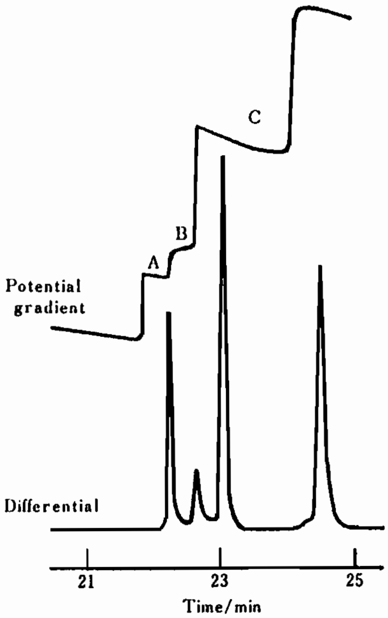

Fig. 4-3 Isotachopherogram of mixture 8. A, $P_3O_{10}{}^{5-}$; B, $P_2O_7{}^{4-}$; C, $PO_4{}^{3-}$.

Table 4-3 Working curves of various phosphate ions

Ion	Vol. (ml)	Concn. (μg/l)	Regression equation	Corrn. coet.	R.S.D.	L.D.L. (μg/l)	Rec. (%)
A	100	36~213	$y=2.9\times 10^{-2}x+1.0$	0.999	0.232(n=6)	3.4	58±14
A	200	16~112	$y=7.0\times 10^{-2}x$	0.998	0.087(n=6)	1.4	44±5
A	500	28~98	$y=8.1\times 10^{-2}x-0.1$	0.998	0.068(n=5)	1.2	23±5
B	100	15~150	$y=4.8\times 10^{-2}x-0.2$	0.988	0.323(n=6)	2.1	34±16
B	200	10~120	$y=9.8\times 10^{-2}x+0.1$	0.997	0.128(n=7)	1.0	53±11
B	500	20~70	$y=2.32\times 10^{-1}x$	0.997	0.070(n=6)	0.4	49±5
C	100	10~125	$y=7.7\times 10^{-2}x+0.4$	0.993	0.196(n=6)	1.3	69±8
C	200	10~60	$y=1.62\times 10^{-1}x$	0.997	0.050(n=6)	0.6	62±4
C	500	5~35	$y=4.05\times 10^{-1}x+0.2$	0.998	0.069(n=6)	0.2	64±6

A, $P_3O_{10}^{5-}$; B, $P_2O_7^{4-}$; C, PO_4^{3-}; x, Concentration of phosphate ion in μg/l; y, Zone length in mm; R.S.D., Relative standard deviation; L.D.L., Lower determination limit.

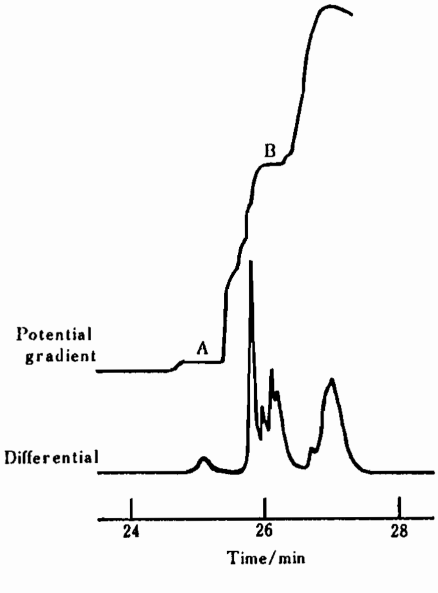

Fig. 4-4 Isotachopherogram of seawater sample treated by the proposed method. A, SO_4^{2-}; B, PO_4^{3-}.

Table 4-4 Results for phosphate ions in seawater

Sampling site	Temp. (℃)	pH	Salinity (‰)	PO_4^{3-} found (μg/l)
Port of Amagasaki	12.8	8.59	15.3	142
Koshien ferry terminal	9.4	8.49	27.1	46
Nishinomiya harbor	11.4	8.48	25.4	61
Fukaehama-machi	10.6	8.53	25.2	106
Pond at KUMM	9.5	8.24	26.8	18
Outside of the pond at KUMM	9.3	8.29	26.5	45
Uozakihama-machi	9.3	8.49	26.6	6
Sumiyoshihama-machi	9.7	8.52	25.9	13
Maya pier	9.8	8.58	26.0	14
Port of Kobe	9.4	8.58	27.0	21
Nagata harbor	9.7	8.44	27.3	32
Suma beach	8.8	8.54	28.0	13

$P_3O_{10}^{5-}$, $P_2O_7^{4-}$ were not detected. Sampling date: 4 April 1984.

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5 Determination of alkali metal ions in seawater

5.1 Introduction

Potassium ion in seawater has been determined by means of flame photometry$^{1)}$, gravimetric analysis$^{1)}$, potentiometric titration$^{2)}$, potentiometry$^{3)}$, γ-ray spectrometry$^{3)}$, spectrophotometry$^{4)}$, auto-analyser method$^{5,6)}$ and isotope dilution surface ionization mass spectrometry$^{7)}$.

On the other hand, there is no report concerning the isotachophoretic determination of potassium ion in seawater. Generally, the concentration of sodium ion in seawater is about 30 times high as that of potassium ion$^{8)}$. For the determination of potassium ion in seawater, it is necessary to separate potassium ion from a large amount of sodium ion to a certain degree prior to the isotachophoretic measurement.

Therefore, ion-exchange separation was applied to the isotachophoretic determination of potassium ion in seawater. While hydrochloric acid has been generally used as the eluent of alkali metal ions$^{9,10)}$, it cannot be used because a large amount of hydrogen ion interferes with the isotachophoretic measurement. Greenhalgh et al.$^{11)}$ reported that sharper elution curves for cations were obtained with ammonium chloride solution than with the same molarity of hydrochloric acid. On the other hand, an alkaline leading electrolyte has not been used for the isotachophoretic determination of potassium and sodium ions, while an acidic one has been generally used[12,13]. Therefore, the author expected that ammonium chloride solution could be used as eluent without interference if a strong alkaline leading electrolyte could be used for the isotachophoretic determination of potassium and sodium ions. Since only liberated molecular ammonia exists in the solution with pH> 11[14], high concentrations of ammonium ion in the eluate will probably not interfere with the isotachophoretic measurement. At first, a new electrolyte system satisfying the above conditions was examined. Then the concentration of ammonium chloride solution and flow-rate were examined for the elution of potassium and sodium ions adsorbed on the cation-exchange column. Thus the behavior of potassium and of sodium ion was observed simultaneously. Further, the effect of concentrations of other salts on the recovery of potassium ion and of the volume of sample injected into the isotachophoretic analyser was also examined. By the proposed method, a linear working curve was obtained for artificial seawater samples containing up to 700mg/l potassium ion. Finally, the method was also applied to the determination of potassium ion in surface and bottom seawater samples. The method has the advantage that a small sample of 0.5ml is sufficient for the simple operation.

It is important to determine the concentration of ammonium ion in seawater, because the ion is closely related to marine-life production[15].

The indophenol blue method[16~21] is widely applied for the purpose, but is complex and time-consuming and subject to several interferences.

On the other hand, there is no report concerning the isotachophoretic determination of ammonium ion in seawater. For this purpose, it is necessary to separate ammonium ion from large amounts of coexisting cations (e.g. sodium, magnesium, calcium and potassium ions), and to enrich prior to the isotachophoretic measurement. Recently, separation with a gas-permeable membrane has been applied to the determination of ammonium ion in river and lake-waters[22~24], blood and urine[25,26], and condensed steam and boiler feed-water[27], but has been little applied to the determination of ammonium ion in seawater[28,29].

Therefore, a similar procedure described in Section 3.2.3 was applied to the determination of ammonium ion in seawater and the optimum analytical conditions were established; a modified gas-liquid separator was used. The method was also applied to the determination of ammonium ion in surface and bottom seawater samples.

5.2 Experimental

5.2.1 Apparatus

The isotachophoretic analyser was the same as described in

Section 2.2.1. The main column was a FEP copolymer tube, 15 cm long, 0.5 mm inner diameter; the precolumn was a PTFE tube, 10 or 30cm long, 1.0 mm inner diameter. The Terumo MS-10 and the Hamilton 1725-N microsyringes were used for the injection of samples. The ion-exchange system was composed of the Atto SJ-1220 peristaltic pump, an Omnifit column, 5 cm long, 3 mm inner diameter packed with a cation-exchange resin in the ammonium form and an Isco Cygnet fraction collector. Two kinds of gas-liquid separator were used. One was the same as described in Section2.2.1. The second was similar, but had an inner microporous PTFE tube (100cm long, 1.0mm i.d., 1.8mm o.d.) and an outer FEP tube (2.4 mm i.d., 3.2mm o.d.), as shown in Fig. 5-1. Both PTFE tubes (Japan Goretex Inc.) had maximum pore size of 2 μ m, and 50 % porosity. The flow system is shown in Fig. 5-2. Both gas-liquid separators were immersed in the same water-bath described in Section1.2.1; the latter gas-liquid separator was wound round a cylindrical PVC former. The sample solution (the pH of which was raised by addition of sodium hydroxide solution) was circulated through the outer tube; sulfuric acid was circulated through the inner tube by means of the Atto SJ-1220 peristaltic pump. The pH meter, the salinometer and the water samplers were the same as described in Section 2.2.1; the DO meter was the same as described in Section 3.2.1. Seawater samples were stored according to the same procedure described in Section 2.2.1.

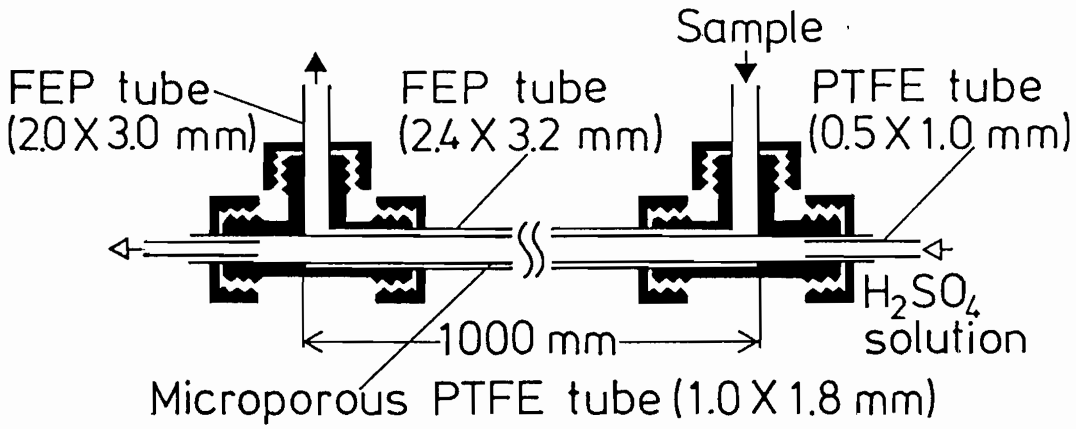

Fig. 5-1 Schematic diagram of gas-liquid separator.

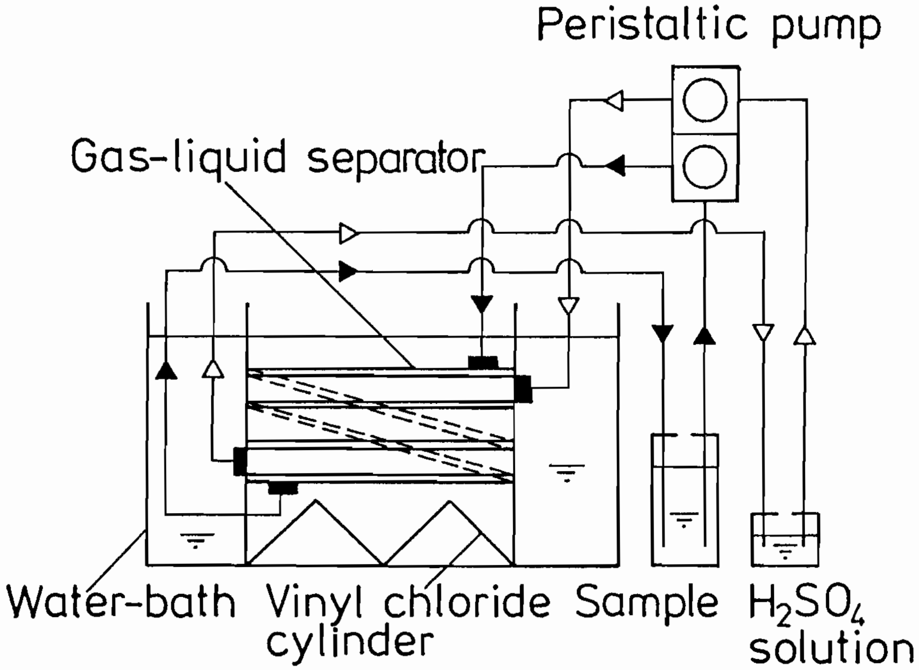

Fig. 5-2 Schematic diagram of flow system.

5.2.2 Reagents

All solutions were prepared from analytical reagent grade chemicals. Cesium hydroxide was obtained from Nakarai Chemicals. Standard solutions of potassium or ammonium ion were prepared by dissolving potassium chloride or ammonium chloride in water from the same apparatuses described in Section2.2.2. The ammonium form cation-exchange column was prepared as follows: Dowex 50W X8 (100 ~ 200mesh) hydrogen form cation-exchange resin was conditioned by the ordinary method; 10 ml of 10 % ammonium chloride was passed through the column packed with the conditioned resin at a flow-rate of 1.0ml/min; then 10ml of water was passed through the column at the same flow-rate. The preparation of the artificial seawater was based on a Japanese Standard30); no potassium ion or potassium ion was included.

5.2.3 Procedure

The procedure for the determination of potassium ion is as follows. Pass 0.5 ml of seawater sample through the column at a flow-rate of 1.0ml/min to adsorb potassium ion. Sodium, magnesium and calcium ions were also adsorbed at the same time. Wash the column with 5 ml of water. Pass 40ml of 2.0×10^{-2}M ammonium chloride through the column at a flow-rate of 2.0 ml/min to elute sodium ion. A part of the potassium ion was also eluted little by little. Pass 3.0×10^{-1}M ammonium chloride through the column at a flow-rate of 1.0 ml/min for the elution of the potassium ion remaining in the column. Reject the first 0.5 ml of the eluate. Inject 3μ l of the following 1.5ml of the eluate into the isotachophoretic analyser. Maintain the migration current at 150 μ A for the first 15min and then reduce it to 50 μ A. As leading electrolyte use 5mM cesium hydroxide/2mM 18-crown-6/0.01% HPMC/70% methanol, and as terminating electrolyte 5 mM tetrabutylammonium bromide/0.01% HPMC/70 % methanol. Similarly, make a blank test using water. Then subtract the zone length for potassium ion in the blank test from that of the seawater sample. Prepare a working curve by applying the method to synthetic standards.

Ammonium ion in seawater sample was determined by the following procedure as soon as possible after collection. Add 0.5 ml of 5M sodium hydroxide to 45 ml of seawater sample to convert ammonium ion into ammonia. Centrifuge the suspension at 3000rpm for 5 min to remove the magnesium hydroxide precipitate. With the water-bath temperature set at 60°C , circulate 40 ml of the supernatant solution through the outer tube at a flow-rate of 6.0 ml/min and 2.0 ml of 2.0×10^{-4} M sulfuric acid through the inner tube at a flow-rate of 1.0 ml/min, both for 20 min. Ammonia permeates through the wall of the inner tube and dissolves in the sulfuric acid. Pump out the sulfuric acid and inject 150μ l of it into the isotachophoretic analyser. Maintain the migration current at 200 μ A for the first 16 min and then reduce it to 50 μ A. As leading electrolyte use 5mM hydrochloric acid/2mM 18-crown-6/0.01% Triton X-100, and as terminating electrolyte 10mM lithium chloride/0.01% Triton X-100. Prepare a working curve by applying the method to synthetic standards.

5.3 Results and Discussion

5.3.1 Determination of potassium ion in seawater

5.3.1.1 Electrolyte system

An uncharged neutral ligand, 18-crown-6, was used in the isotachophoretic analysis of alkali and alkaline-earth metal ions [31,32]. The author has also used 18-crown-6 for the determination of ammonium and potassium ions (see Section 1.3.3). Tazaki et al. [31] suggested that the inclination of relation between PU value and concentration of 18-crown-6 was higher when the stability of the complexes was higher for alkali metal ions, and that cesium, potassium and sodium ions were isotachophoretically separated by using the aqueous leading electrolyte containing 20~ 40 mM 18-crown-6. Further, the stability constants of these complexes in methanol were larger than those in water[33]. Therefore, the author expected that the simultaneous determination of potassium and sodium ions was possible by using the strong alkaline leading electrolyte containing cesium hydroxide, methanol and lower concentration of 18-crown-6.

At first, the concentration of 18-crown-6 in the leading electrolyte described above was varied in the range 1～3mM. As the sample, a 5-μl volume of a standard solution containing 10^{-3} M potassium and sodium ions was injected into the isotachophoretic analyser. Complete isotachopherograms were obtained with the concentrations of 2 and 3mM, but migration voltage of the latter was higher than that of the former; the PU values of potassium and sodium ions were 0.69 and 0.27, respectively, at the concentration of 2 mM, and they were 0.75 and 0.28, respectively, at the concentration of 3 mM. An incomplete isotachopherogram was obtained with the concentration of 1mM, as shown in Fig.5-3. Therefore, 2 mM 18-crown-6 was selected to be added to the leading electrolyte.

Then the concentration of methanol in the leading electrolyte was varied in the range 60～80%. The concentration of methanol in the terminating electrolyte was varied corresponding to that of the leading electrolyte. Complete isotachopherograms were obtained with the concentrations of 70 and 80 %; the PU values of potassium and sodium ions were 0.81 and 0.34, respectively, at the concentration of 80 %. Normal migration was not obtained at the concentration of 60 % because of the unusual increase of migration voltage. Therefore, 70 % was selected as the optimum concentration of methanol in the leading electrolyte. Sharp separation of potassium and sodium ions was not obtained without the

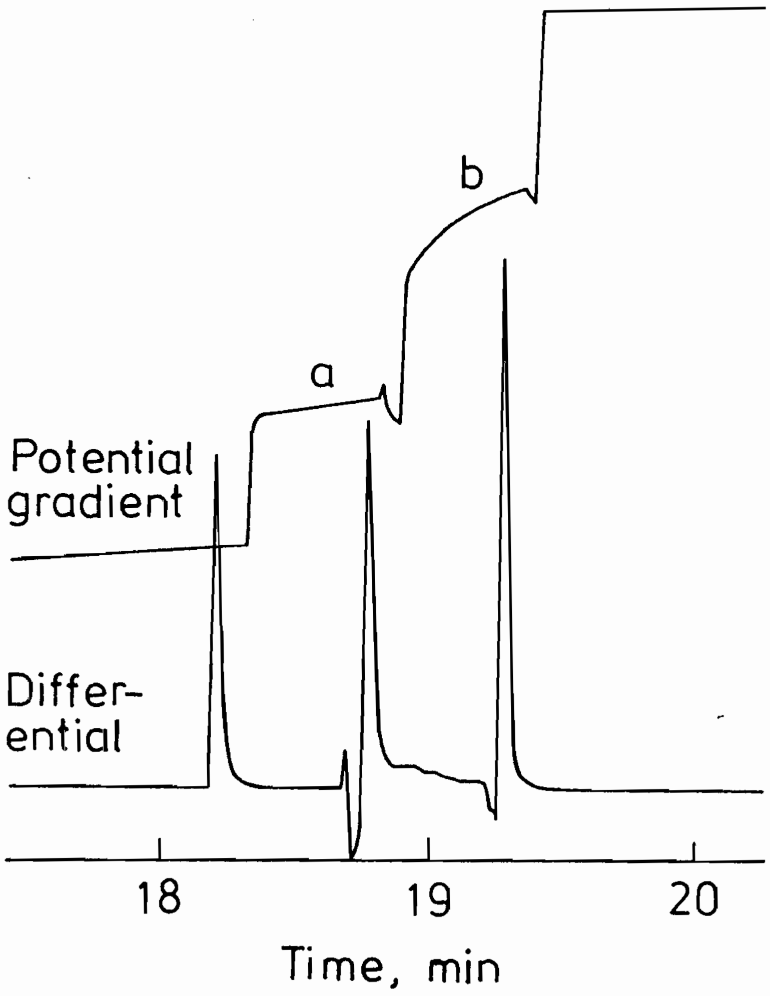

Fig.5-3 Isotachopherogram for the separation of potassium and sodium ions using the leading electrolyte containing 1 mM 18-crown-6. a, Na^+; b, K^+.

addition of HPMC.

5.3.1.2 Determination of potassium and sodium ions

Linear working curves were obtained for potassium and sodium ions up to 60mg/l, by using the above electrolyte system. The regression equations for potassium and sodium ions were $y=0.526x-0.2$ and $y=0.747x$, respectively. The correlation coefficients were 0.999 and 1.000, respectively. In these equations, x is the concentration of the ion in mg/l and y the zone length in mm when the recording speed is adjusted to 40mm/min. The lower determination limits for potassium and sodium ions were 0.19 and 0.13mg/l, respectively, corresponding to a 0.1-mm zone length. When 5-μl volumes of mixed solutions containing potassium and sodium ions in various concentrations were injected and analysed by use of the working curves, the error in the determination of these ions was less than ± 3.3 %, as shown in Table 5-1.

Various concentrations of potassium and sodium ions in 3.0×10^{-1} M ammonium chloride were similarly determined by injecting 2μl of samples. The error was less than ± 12.0%, as shown in Table 5-2. In spite of the presence of a large amount of ammonium ion, potassium and sodium ions were determined successfully using the new electrolyte system.

Table 5-1 Analytical results for potassium and sodium ions

Mixture	Added, mg/l		Found, mg/l		Error, %	
	A	B	A	B	A	B
1	10	60	10	61	± 0.0	+1.7
2	20	50	20	51	± 0.0	+2.0
3	30	40	30	40	± 0.0	± 0.0
4	40	30	39	30	-2.5	± 0.0
5	50	20	50	20	± 0.0	± 0.0
6	60	10	58	10	-3.3	± 0.0

A, K^+; B, Na^+.

Table 5-2 Analytical results for potassium and sodium ions in 3.0 × 10^{-1} M ammonium chloride

Mixture	Added, mg/l		Found, mg/l		Error, %	
	A	B	A	B	A	B
1	25	150	28	150	+12.0	± 0.0
2	50	125	55	124	+10.0	-0.8
3	75	100	80	100	+6.7	± 0.0
4	100	75	106	74	+6.0	-1.3
5	125	50	133	48	+6.4	-4.0
6	150	25	164	24	+9.3	-4.0

A, K^+; B, Na^+.

5.3.1.3 Concentration of ammonium chloride solution for eluting sodium ion

The concentration of ammonium chloride solution was varied in the range $1.0\times 10^{-2}\sim 3.0\times 10^{-2}$M for the elution of sodium ion. As the sample, 0.5 ml of artificial seawater containing 400 mg/l potassium ion was used. Two μ l of sample was injected. It took longer to elute sodium ion using 1.0×10^{-2} M solution. On the other hand, the amount of potassium ion eluted at the same time increased with the ammonium chloride concentration up to 3.0×10^{-2} M. Therefore, 2.0×10^{-2} M was adopted as the optimum ammonium chloride concentration for the elution of sodium ion.

5.3.1.4 Flow-rate of ammonium chloride solution for eluting sodium ion

The flow-rate of 2.0×10^{-2}M ammonium chloride was varied in the range 1.0～3.0 ml/min for the elution of sodium ion. More eluent was naturally required at a flow-rate of 3.0 ml/min than at 2.0 ml/min, and larger amount of potassium ion was eluted in the former than in the latter. Therefore, 2.0 ml/min was adopted as the optimum flow-rate for the elution of sodium ion.

5.3.1.5 Concentration of ammonium chloride solution for eluting potassium ion

After sodium ion was almost eluted using 40ml of 2.0×10^{-2} M ammonium chloride, the concentration of ammonium chloride solution was elevated in the range $2.0\times 10^{-1}\sim 4.0\times 10^{-1}$M for the elution of potassium ion remaining in the column. More eluent was required at the concentration of 2.0×10^{-1} M than at 3.0×10^{-1} M. Normal migration was not obtained at the concentration of 4.0×10^{-1} M because of the unusual increase of migration voltage. Therefore, 3.0×10^{-1} M was adopted as the optimum ammonium chloride cocentration for the elution of potassium ion.

5.3.1.6 Effect of other salts

The volume of twofold concentration of artificial seawater was varied in the range 5~ 30 ml in 50 ml of artificial seawater sample containing 400 mg/l potassium ion. Despite the difference in the concentrations of other salts, the recovery of potassium ion was almost constant.

5.3.1.7 Volume of injected sample

The volume of sample injected into the isotachophoretic analyser was varied in the range 1.5~ 4 μ l. The zone length for potassium ion increased proportionally up to 3.5 μ l. Slightly curved relation was observed at the larger injection volume. Therefore, 3 μ l was adopted as the optimum sample volume.

5.3.1.8 Working curve

A linear working curve was obtained for 7 artificial seawater samples containing 100～ 700 mg/l potassium ion. The regression equation was $y=4.12\times 10^{-2}x+0.2$ (correlation coefficient 1.000) where x and y are the same described above. The relative standard deviation was found to be 0.021 (n=7). The lower determination limit for potassium ion was 2.0 mg/l, corresponding to a 0.1-mm zone length. The recovery of potassium ion, at 39± 3 %, was less than complete, and this was attributed to the following reasons: a part of potassium ion was eluted with 2.0×10^{-2}M ammonium chloride together with sodium ion; when potassium ion was eluted with 3.0×10^{-1}M ammonium chloride, only the following 3 fractions were taken after rejecting the first fraction. It does not matter in practice, because the recovery is almost constant in the range 100～ 700 mg/l potassium ion.

5.3.1.9 Analysis of seawater samples

The proposed method was applied to the determination of potassium ion in surface and bottom seawater samples collected around the coastal area of Osaka Bay between Port of Amagasaki and Rokko Island on 6 April 1987. An isotachopherogram of surface seawater from the Rokko Island is shown in Fig.5-4. Then 200 mg/l potassium ion was added to seawater from the Rokko Island diluted

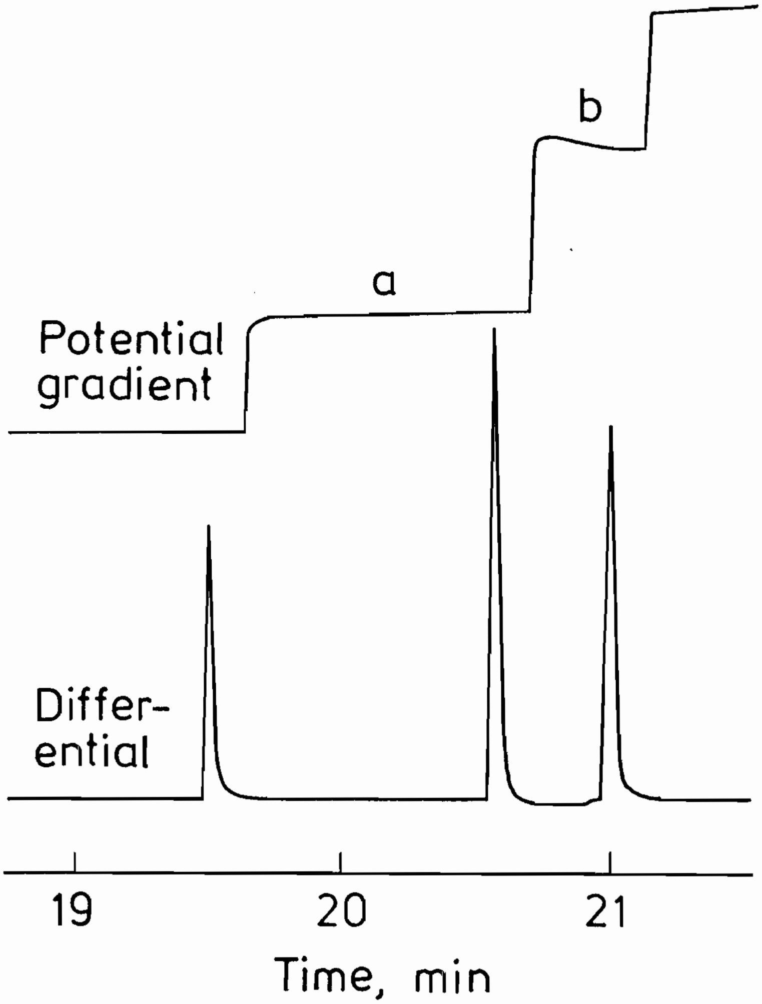

Fig. 5-4 Isotachopherogram of seawater sample treated by the proposed method. a, Na^+; b, K^+.

2 times, and analysed. These results are shown in Table5-3. There was a large difference owing to the influence of land-water between the concentrations of potassium ion in surface and bottom seawaters except those from the pond at KUMM and the Rokko Island. The error in the standard addition experiment was less than ± 22.1 %.

Soifer et al.$^{3)}$ reported that the correlation coefficients between the salinity and the concentration of potassium ion were 0.810~ 0.997 for the individual stations. Therefore, the data listed in Table 5-3 were plotted, and a linear relation was obtained between the salinity and the concentration of potassium ion, as shown in Fig. 5-5. The regression equation of this curve was $y=12.4x+28.4$ (correlation coefficient 0.947) where x is the salinity in ‰ and y the concentration of potassium ion in mg/l. This also confirms the analytical results listed in Table 5-3.

5.3.2 Determination of ammonium ion in seawater

5.3.2.1 Electrolyte system

It was necessary to select an electrolyte system by which the ammonium ion could be isotachophoretically separated from the potassium ion present as an impurity in the reagents. In Section 1.3.3, the author has already described how the isotachophoretic separation of low concentrations of these ions can be achieved

Table 5-3 Results for potassium ion in seawater

Sampling site	Depth (m)	Temp. (°C)	pH	Salinity (‰)	K^+ Added (mg/l)	K^+ Found (mg/l)	K^+ Error (%)
Port of Amagasaki	0	16.5	8.52	9.0	—	119	—
Port of Amagasaki	4.8	11.2	7.96	28.0	—	345	—
Mouth of Muko river	0	14.6	7.91	21.1	—	267	—
Mouth of Muko river	3.7	10.5	7.90	24.5	—	364	—
Nishinomiya harbor	0	13.4	8.14	17.7	—	272	—
Nishinomiya harbor	2.5	10.9	7.86	27.9	—	362	—
Pond at KUMM	0	12.6	8.53	24.6	—	372	—
Pond at KUMM	5.5	11.0	8.15	28.1	—	391	—
Rokko Island	0	12.5	8.37	27.2	—	369	—
Rokko Island^a)	0				—	195	—
Rokko Island^a)	0				200	432	+19.0
Rokko Island	5.5	10.5	8.23	29.6	—	364	—
Rokko Island^a)	5.5				—	204	—
Rokko Island^a)	5.5				200	449	+22.1

Sampling date: 6 April 1987; a), 2 times diluted.

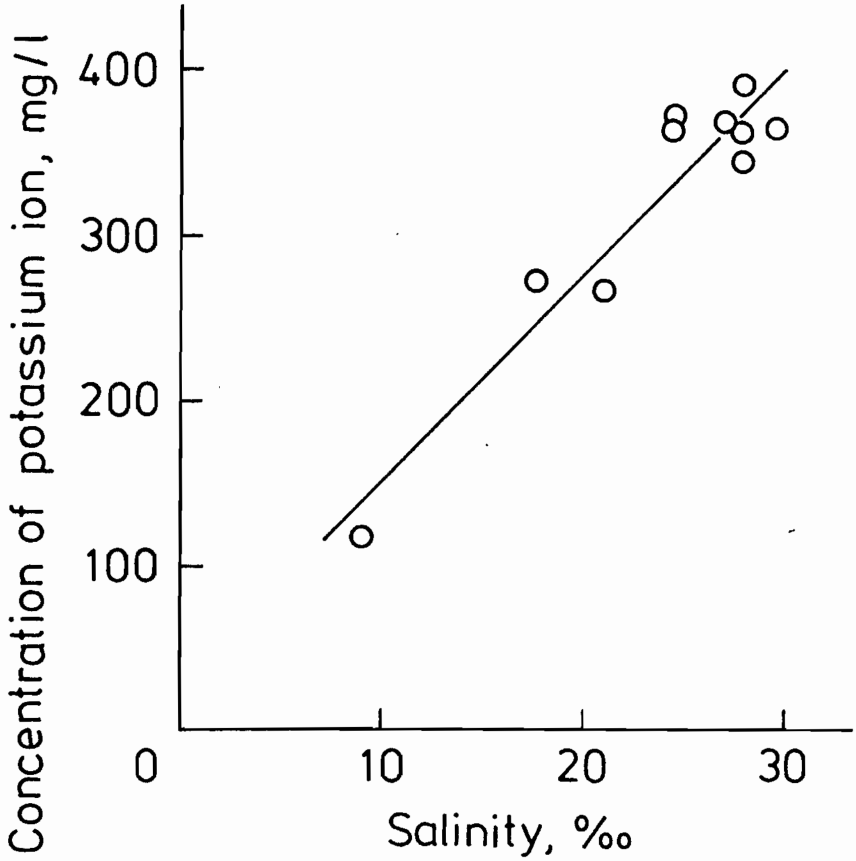

Fig. 5-5 Relation between salinity and concentration of potassium ion.

with a leading electrolyte containing 1～3 mM 18-crown-6, with linear response for ammonium ion up to 1.0 mg/l.

5.3.2.2 Gas-liquid separator

Artificial seawater samples containing 100μg/l ammonium ion were analysed by use of both the gas-liquid separators described above. The recovery of ammonium ion was 20% when the older(glass) type was used and 69% with the modified (FEP) type, i.e., the increase in the surface area of the inner tube resulted in a corresponding increase in the recovery factor. The modified gas-liquid separator was, therefore, used in all subsequent experiments.

5.3.2.3 Volume of sodium hydroxide solution

The zone length for ammonium ion in the isotachopherograms was almost constant irrespective of the volume of 5 M sodium hydroxide added (over the range 0.3～0.7 ml), as shown in Fig. 5-6. During the circulation, magnesium hydroxide precipitated when the volume of alkali added was below the range above, because significant amounts of the magnesium ion remained in solution, and calcium carbonate precipitated when volumes> 0.7ml were used. Therefore, 0.5ml of 5 M sodium hydroxide was selected as the volume to be added to the 45 ml of sample solution.

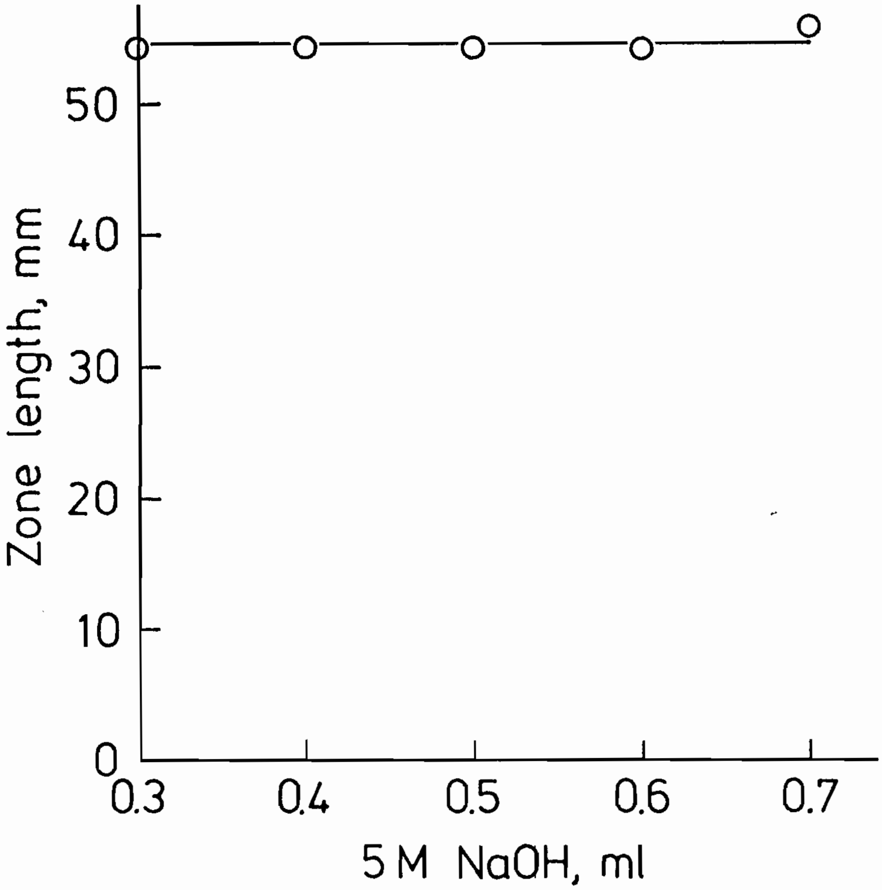

Fig. 5-6 Effect of 5 M sodium hydroxide volume.

5.3.2.4 Concentration of sulfuric acid

The concentration of the sulfuric acid was varied in the range $0.5\times 10^{-4}\sim 3.0\times 10^{-4}$M. The zone length for ammonium ion increased with the sulfuric acid concentration, but only slightly when this was$> 1.0\times 10^{-4}$ M, as shown in Fig. 5-7. The isotachophoretic measurement took longer when the sulfuric acid concentration was higher than 2.5×10^{-4} M, because of the excess of hydrogen ion in the treated solution. Therefore, 2.0×10^{-4} M was adopted as the optimum sulfuric acid concentration.

5.3.2.5 Flow-rate of sample circulation

The flow-rate of sample solution was varied in the range 2.0 ~ 10.0 ml/min. The zone length for ammonium ion increased with flow-rate, but only slightly when this was> 4.0 ml/min, as shown in Fig. 5-8. At higher flow-rate, sample solution as well as ammonia is liable to permeate through the wall of the inner tube, so 6.0ml/min was adopted as the optimum flow-rate for sample circulation.

5.3.2.6 Duration of sample circulation

The circulation time was varied in the range 10~ 30 min. The zone length for ammonium ion increased with circulation time, but

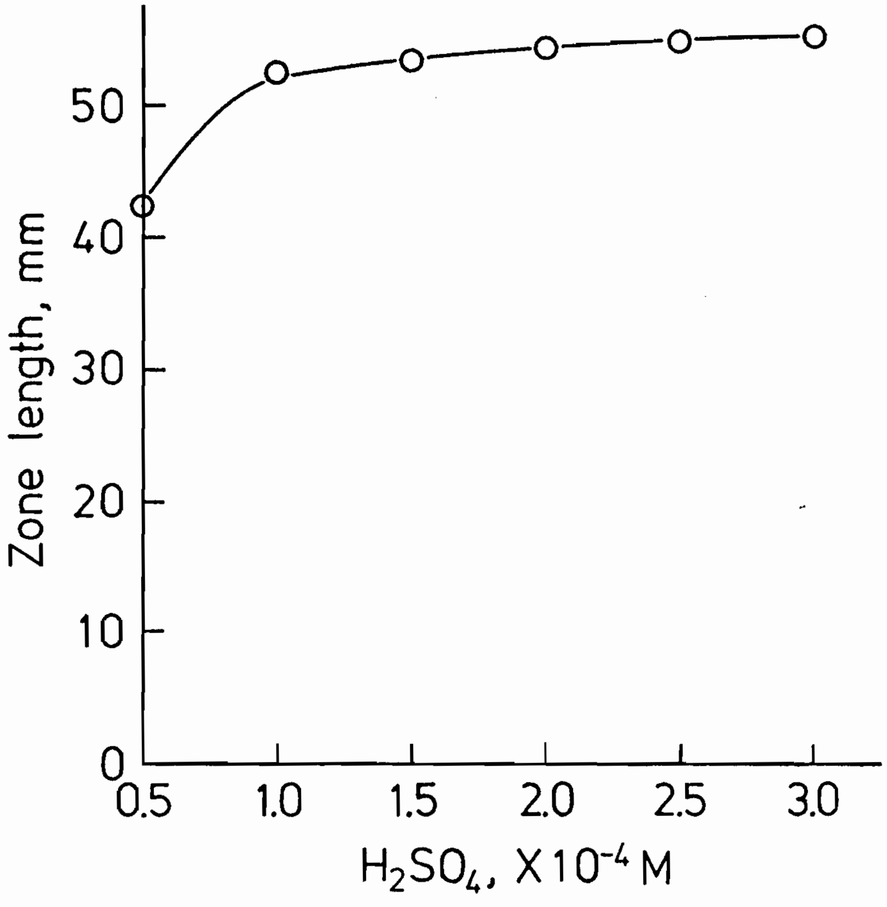

Fig. 5-7 Effect of sulfuric acid concentration.

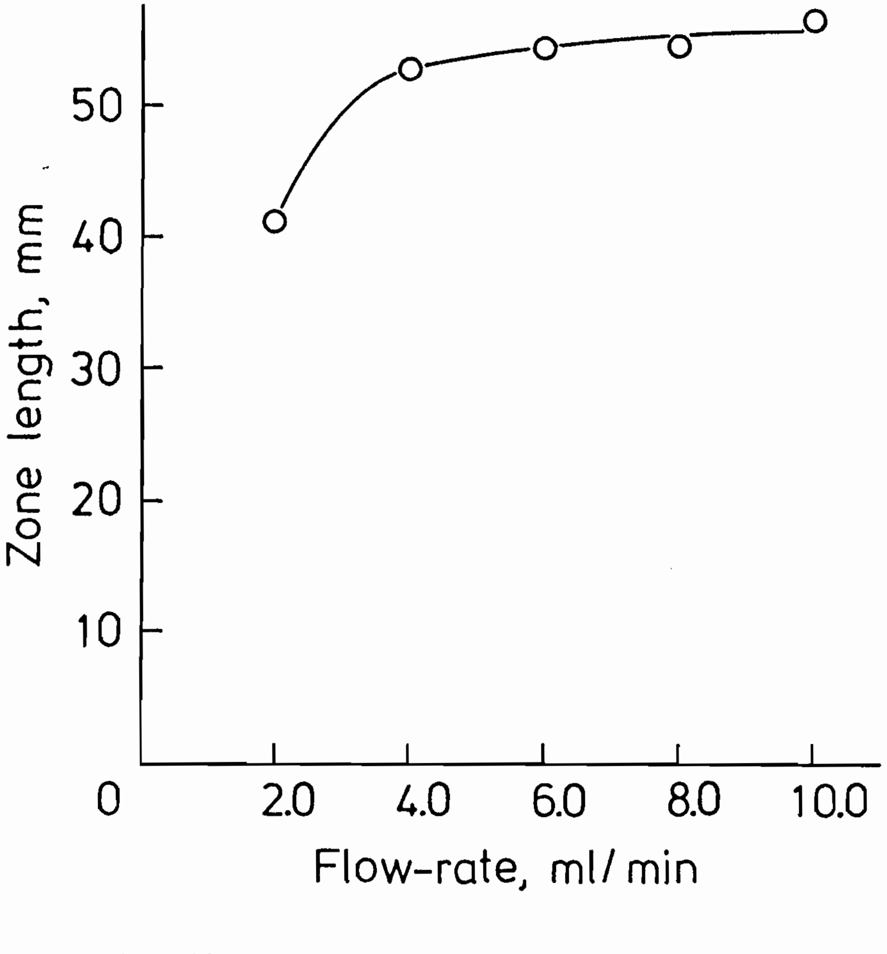

Fig. 5-8 Effect of the flow-rate of sample solution.

not linearly, as shown in Fig. 5-9. To shorten the analysis time, 20 min was adopted as the circulation time.

5.3.2.7 Effect of temperature

The temperature of the water-bath was varied in the range 30 ~ 70 ℃ . The zone length for ammonium ion increased linearly with temperature up to 60 ℃ , because of decreased solubility of ammonia in the alkaline solution, as shown in Fig. 5-10[34,35]. Although the recovery of ammonia was greater when a temperature of 70 ℃ was used, 60 ℃ was preferred because temperature controle was easier.

5.3.2.8 Working curve

A linear working curve was obtained for 12 artificial seawater samples containing 25~ 300 μ g/l ammonium ion. The regression equation was $y=0.395x-0.8$ (correlation coefficient 1.000) where x is the concentration of ammonium ion in μ g/l and y the zone length in mm when the recording speed is adjusted to 40 mm/min. The relative standard deviation was found to be 0.033 (n= 12). The lower determination limit for ammonium ion was 0.25 μ g/l, corresponding to a 0.1-mm zone length. The recovery of ammonium ion, at 67± 3 %, was less than complete, but this does not matter in practice, because the recovery is almost constant in

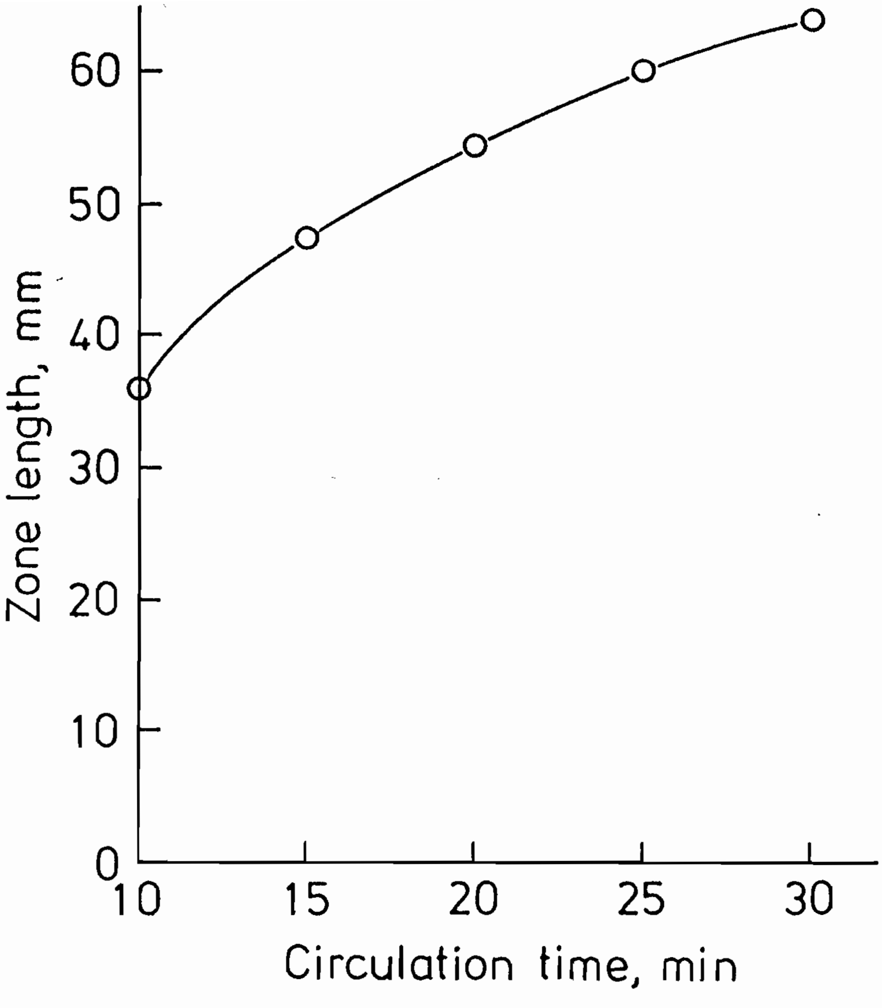

Fig. 5-9 Effect of circulation time.

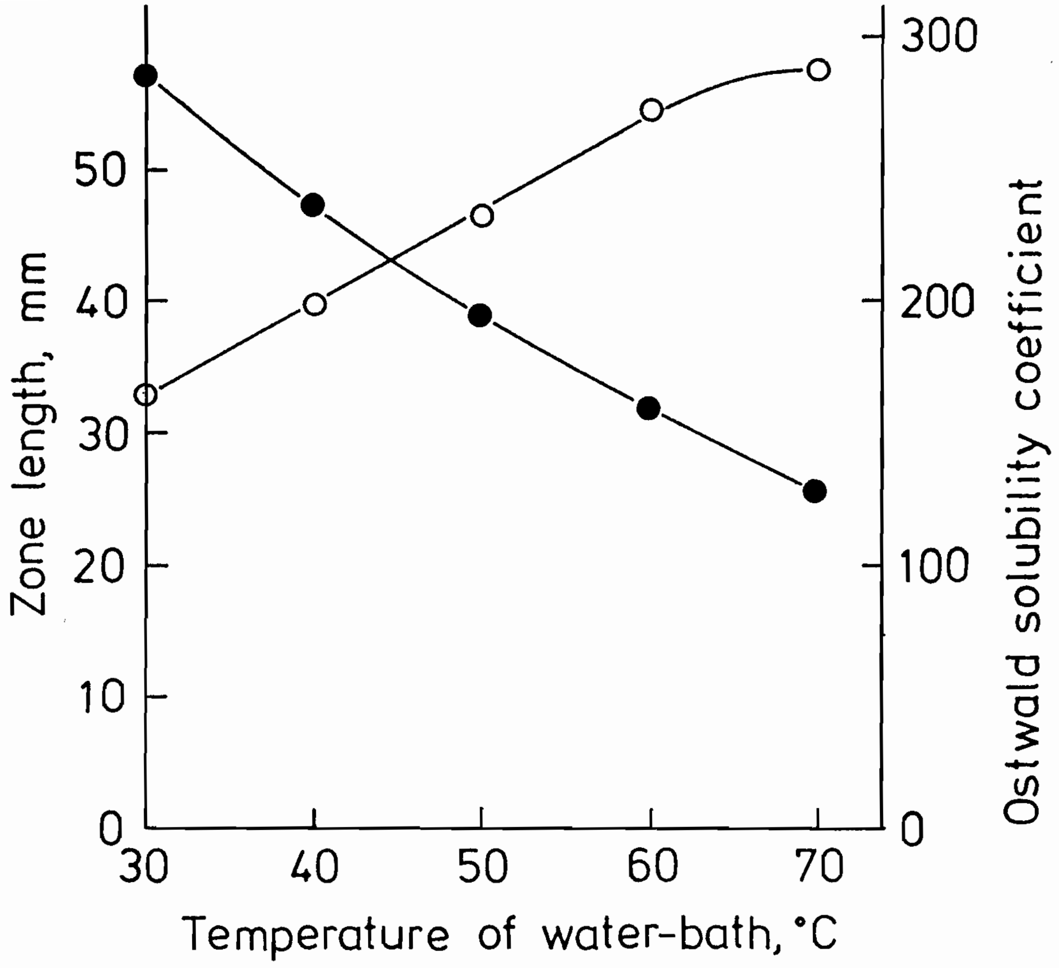

Fig. 5-10 Effect of temperature. ○ , Zone length for NH_4^+; ● , Ostwald solubility coefficient of NH_3.

the range 25~ 300 μg/l ammonium ion. It may, however, be increased by use of a longer microporous PTFE tube.

In addition, a similar working curve was obtained for seawater samples appropriately diluted with artificial seawater. The regression equation of this curve was $y=0.379x+0.6$ (correlation coefficient 0.999).

5.3.2.9 Analysis of seawater samples

The proposed method was applied to the determination of ammonium ion in surface and bottom seawater samples collected around the coastal area of Osaka Bay between Port of Amagasaki and Rokko Island on 6 February 1988. The concentrations of ammonium ion in these samples were so high that they were diluted with artificial seawater before analysis. An isotachopherogram of surface seawater from the mouth of the Muko river is shown in Fig.5-11. The concentrations of ammonium ion listed in Table 5-4 were calculated by use of the working curve prepared with artificial seawater samples. From these results, it was found that eutrophication was progressing rapidly in these sea-areas. Further, a linear relation was obtained between the salinity and the concentration of ammonium ion, except for those in surface seawater from the pond at KUMM, listed in Table 5-4, as shown in Fig.5-12. The regression equation of this curve was $y=-230x+7170$ (correlation coefficient -0.990) where x is the salinity in ‰ and y the

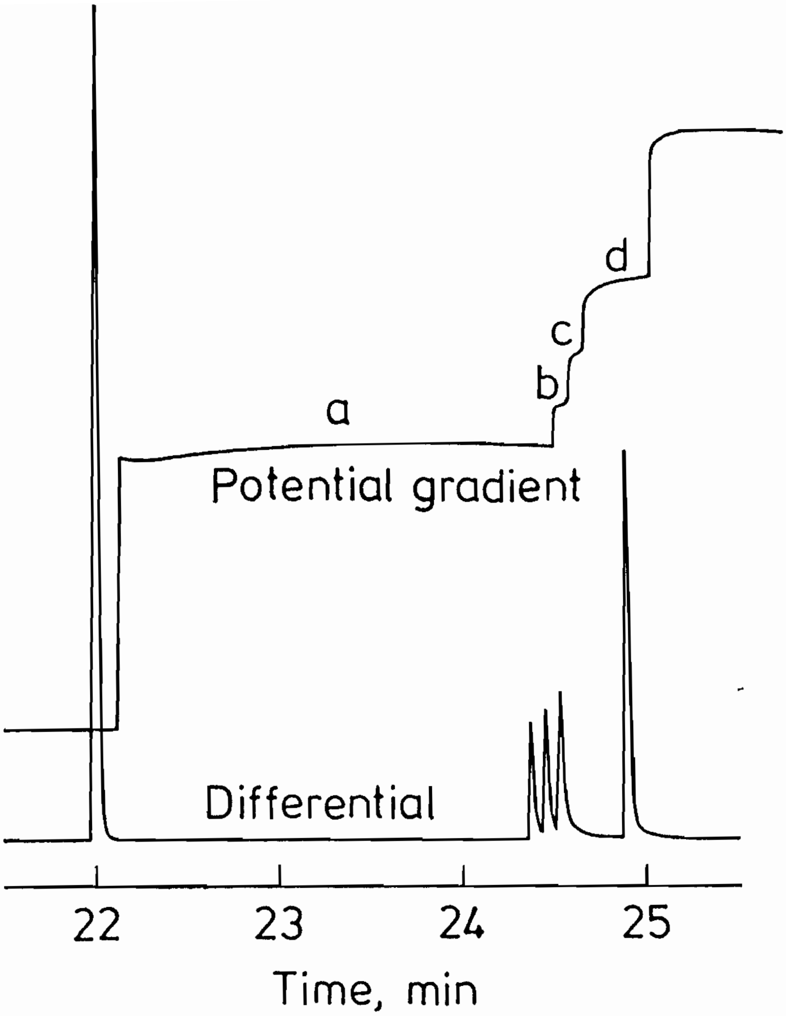

Fig.5-11 Isotachopherogram of seawater sample treated by the proposed method. a, NH_4^+; b, K^+; c, Ca^{2+}; d, Na^+.

Table 5-4 Results for ammonium ion in seawater

Sampling site	Depth (m)	Temp. (℃)	pH	Salinity (‰)	DO (mg/l)	NH_4^+ found (μg/l)
Port of Amagasaki	0	10.5	7.50	15.1	7.11	3750
Port of Amagasaki	5.0	10.7	7.64	24.0	7.00	1740
Mouth of Muko river	0	9.5	7.79	26.0	10.05	1060
Mouth of Muko river	3.0	9.2	7.85	26.5	9.09	1060
Nishinomiya harbor	0	10.1	7.78	25.6	8.85	1200
Nishinomiya harbor	1.5	9.6	7.83	26.6	8.25	990
Pond at KUMM	0	9.3	7.82	22.6	9.89	4580
Pond at KUMM	5.0	9.2	7.80	26.1	7.89	1040
Rokko Island	0	9.2	8.01	27.6	10.77	1010
Rokko Island	5.5	9.1	8.08	27.9	9.80	970

Sampling date: 6 February 1988.

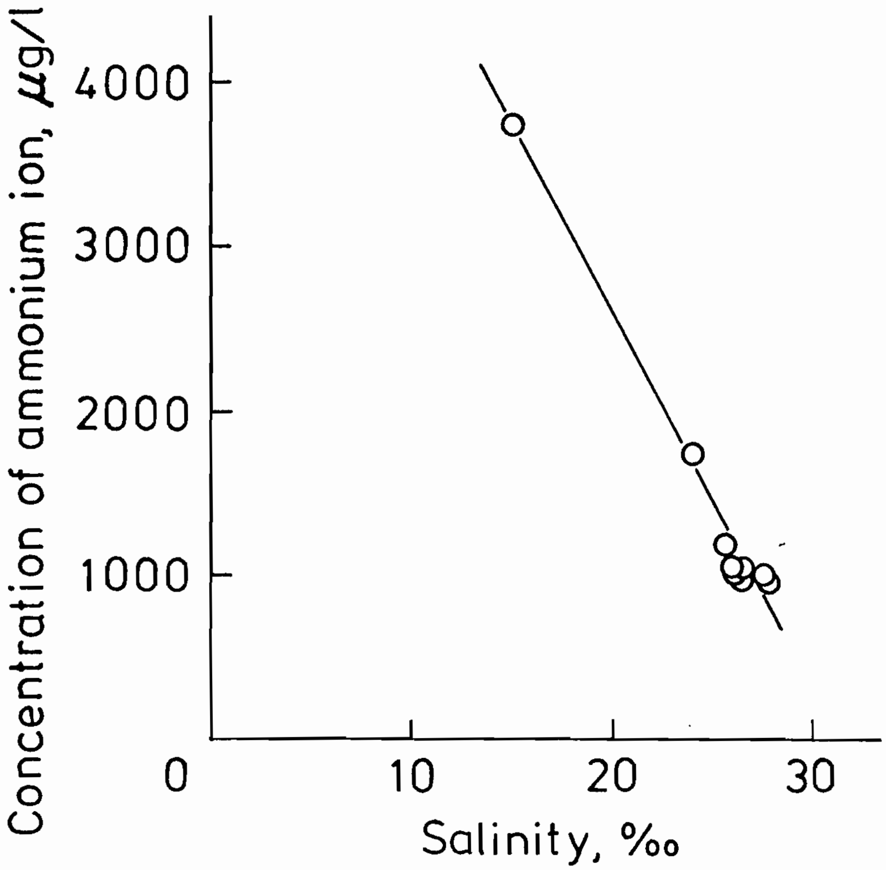

Fig. 5-12 Relation between salinity and concentration of ammonium ion.

concentration of ammonium ion in μg/l. It can be presumed that the negative correlation is due to the influence of rain-water.

Tanaka et al.$^{23)}$ found that methylamine interfered positively with the determination of ammonium ion by the automated measuring apparatus with a gas-permeable membrane. In the proposed method, this interference can be eliminated because methylammonium, dimethylammonium and trimethylammonium ions are separated from the ammonium ion by isotachophoresis with the electrolyte system described above$^{36)}$.

For the determination of ammonium ion in seawater, the proposed method is simple, has a high sensitivity and precision and no interferences.

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6 Determination of alkaline-earth metal ions in seawater

6.1 Introduction

Magnesium ion in seawater has been determined by means of colorimetry with thymol phthalein complexone and iodometric titration1). On the other hand, calcium ion in seawater has been determined by means of titration with GEDTA, EDTA or potassium permanganate solution and flame spectrochemical analysis1). However, magnesium and calcium ions can not be determined simultaneously by these methods. Further, these methods are affected by interfering substances.

On the other hand, there is no report concerning the isotachophoretic determination of magnesium and calcium ions in seawater. For this purpose, it is necessary to separate magnesium and calcium ions from a large amount of sodium ion prior to the isotachophoretic measurement. For their determination by the ion chromatography, EDTA solution was applied as the eluent by Yamamoto et al.2)

Therefore, the EDTA elution method was applied to the simultaneous determination of magnesium and calcium ions in seawater by CITP and the optimum analytical conditions were established. At first, the pH, concentration and flow-rate of EDTA solution were examined for the elution of magnesium and calcium ions adsorbed on the cation-exchange column. Then the volume of sample injected into the isotachophoretic analyser was examined. By the proposed method, linear working curves were obtained for 3% sodium chloride samples containing up to 2000 mg/l magnesium or 1250 mg/l calcium ion adjusted to pH 8.0 with 5.0×10^{-2} M sodium carbonate. The relative standard deviations for magnesium and calcium ions were 0.035 and 0.076, respectively. The lower determination limits for magnesium and calcium ions were 7.9 and 14.3mg/l, respectively. Finally, the method was also applied to the determination of magnesium and calcium ions in artificial samples and surface seawater samples. Reproducible values were obtained in the standard addition experiment.

The development of resources dissolved in seawater has been studied recently. Strontium in seawater seems to be one of the useful resources, since it has been used for the protection of radiant emissions from Braun tubes of color television and also used for the production of ferrite magnets3). In the development of techniques for collecting strontium in seawater, it is necessary to develop a simple analytical method with high sensitivity and precision for the determination of strontium ion.

Strontium ion in seawater has been determined by means of atomic absorption spectrometry4~6), flame photometry7~9), spectrophotometry10), radioactivation analysis11,12), isotope dilution analysis11) and a combination of flame photometry and radioactivation analysis13). In these methods, the presence of coexisting components or acids interferes with the measurement of strontium ion or some special equipment is required.

On the other hand, there is no report concerning the isotachophoretic determination of strontium ion in seawater. For the determination of strontium ion in seawater, it is necessary to separate strontium ion from large amounts of coexisting cations prior to the isotachophoretic measurement.

Therefore, ion-exchange separation using a chelating reagent as eluent was applied to the isotachophoretic determination of strontium ion in seawater and the optimum analytical conditions were established. That is to say, after the large amount of calcium ion adsorbed on the cation-exchange column was removed with EDTA solution, only strontium ion was eluted with GEDTA solution. At first, some chelating reagents were examined for the isotachophoretic separation of alkaline-earth metal ions. Then the conditions such as pH, concentration, flow-rate and volume of eluent were examined for the elution of strontium or calcium ion adsorbed on the cation-exchange column. Thus the behavior of strontium ion and of calcium ion was observed simultaneously. By the proposed method, a linear working curve was obtained for artificial seawater samples containing up to 16.0 mg/l strontium ion. The relative standard deviation for strontium ion was 0.046. The lower determination limit for strontium ion was 0.21 mg/l. It turned out that the proposed method had high sensitivity and precision. Finally, the method was also applied to the determination of strontium ion in surface seawater samples. Reproducible values were obtained in the standard addition experiment.

6.2 Experimental

6.2.1 Apparatus

The isotachophoretic analyser was the same as described in Section 2.2.1. The main column was a FEP copolymer tube, 40 cm long, 0.5 mm inner diameter; the precolumn was a PTFE tube, 8 or 10 cm long, 1.0 mm inner diameter. The Terumo MS-10 microsyringe was used for the injection of samples. The ion-exchange system was composed of the Atto SJ-1220 peristaltic pump, an Omnifit column, 10 or 15 cm long, 3 mm inner diameter packed with a cation-exchange resin in the sodium form and a Toyo SF-160K fraction collector. The same pH meter described in Section 1.2.1 was used for adjusting the pH of solutions. A Yamato-Kagaku M-66 stirrer was also used. The pH meter, the salinometer and the water sampler were the same as described in Section2.2.1. Seawater samples were stored according to the same procedure described in Section 2.2.1.

6.2.2 Reagents

All solutions were prepared from analytical reagent grade chemicals. Chelating reagents were obtained from Dojin Chemical

Laboratory. Standard alkaline-earth metal solutions were prepared by dissolving their chloride salts in water from the same apparatus described in Section1.2.2. EDTA solution was prepared by dissolving disodium salt of EDTA in water. Dowex 50W X8 (100~ 200 mesh) sodium form cation-exchange resin was prepared by the ordinary method. The preparation of the artificial seawater was based on a Japanese Standard[14)], but no magnesium and calcium ions or no strontium ion were included.

6.2.3 Procedure

The procedure for the simultaneous determination of magnesium and calcium ions is as follows. Pass 10 ml of 3 % sodium chloride adjusted to pH 8.0 with 5.0×10^{-2} M sodium carbonate through the column at a flow-rate of 1.0ml/min. Then pass a 1.0ml of seawater sample through the column at the same flow-rate to adsorb magnesium and calcium ions. Wash the column with 5 ml of water. Pass 5.0ml of 6.0×10^{-2}M EDTA adjusted to pH 10.5 with 2-amino-2-methyl-1-propanol through the column at a flow-rate of 1.0 ml/min to elute magnesium and calcium ions. Inject 1.4 μl of the eluate into the isotachophoretic analyser. Maintain the migration current at 200μA for the first 17 min and then reduce it to 100μA. Use the same electrolyte system established in Section1.3.4. Prepare working curves by applying the method to synthetic standards.

The procedure for the determination of strontium ion is as follows. Pass 10 ml of 3% sodium chloride adjusted to pH 8.0 with 5.0×10^{-2} M sodium carbonate through the column at a flow-rate of 1.0ml/min. Then pass 10.0ml of seawater sample through the column at the same flow-rate to adsorb strontium ion. Both calcium and magnesium ions were also adsorbed at the same time. Wash the column with 10 ml of water. Pass 100 ml of 5.0×10^{-3} M EDTA adjusted to pH 6.6 with 1 M sodium hydroxide through the column at a flow-rate of 2.0 ml/min to elute calcium ion. Magnesium ion was also eluted little by little. Wash the column with 10ml of water. Pass 5.0×10^{-3}M GEDTA adjusted to pH 10.0 through the column at a flow -rate of 1.0 ml/min to elute strontium ion. Magnesium ion was not eluted. Reject the first 1.0 ml of the eluate. Inject 10 μ l of the following 2.0 ml of the eluate into the isotachophoretic analyser. Maintain the migration current at 200μ A for the first 18 min and then reduce it to 100 μ A. As leading electrolyte use 10 mM hydrochloric acid/0.1 % HPMC/tris (pH 9.0), and as terminating electrolyte 10mM sodium hexanoate. Prepare a working curve by applying the method to synthetic standards.

6.3 Results and Discussion

6.3.1 Determination of magnesium and calcium ions in seawater

6.3.1.1 pH of EDTA solution

One ml of the solution containing 1300mg/l magnesium and 400 mg/l calcium ions was passed through the column to adsorb these ions on the column. This solution was a 3 % sodium chloride adjusted to pH 8.0 with 5.0×10^{-2}M sodium carbonate. Then magnesium and calcium ions were eluted with 1.0×10^{-2} M EDTA adjusted to pH 9.5, 10.5 or 11.5, respectively. The fractions were taken in the fraction collector (1.0 ml), and a 3 μ l portion of each fraction was injected into the isotachophoretic analyser. Because more eluent was required in pH 9.5 than in pH 10.5 or 11.5, 10.5 or 11.5 was found to be the suitable pH range of the eluent of magnesium and calcium ions.

6.3.1.2 Concentration of EDTA solution

Various concentrations of EDTA solutions: 1.0×10^{-2}, 2.0×10^{-2}, 4.0×10^{-2}, 6.0×10^{-2} or 8.0×10^{-2} M adjusted to pH 10.5 were used as the eluent of magnesium and calcium ions. A concentration of 6.0×10^{-2} or 8.0×10^{-2} M EDTA was found to be suitable for the elution of magnesium and calcium ions.

6.3.1.3 Flow-rate of EDTA solution

The flow-rate of 6.0×10^{-2}M EDTA adjusted to pH 10.5 was varied in the range 0.5∼ 1.5 ml/min for the elution of magnesium and calcium ions. A flow-rate of 1.0 or 1.5 ml/min was found to be suitable for the elution of magnesium and calcium ions. When CyDTA or diethylenetriamine-N, N, N′ , N″ , N″ -pentaacetic acid (abbreviated as DTPA) was used as the eluent, a satisfactory result was not obtained.

6.3.1.4 Volume of injected sample

The volume of sample injected into the isotachophoretic analyser was varied in the range 1.2～ 2.2μ l. As the sample, 3 % sodium chloride containing 900 mg/l magnesium and calcium ions was used. When it was 2.2μ l, sharp separation of Mg(II)-EDTA and Ca(II)-EDTA was not obtained. Since 1.4μ l was enough for the determination of magnesium and calcium ions, 1.4μ l was adopted as the sample volume.

6.3.1.5 Working curves

Linear working curves were obtained for 3 % sodium chloride samples(n=8 or 5) containing 250～ 2000mg/l magnesium or 250～ 1250 mg/l calcium ion adjusted to pH 8.0. The regression equations for magnesium and calcium ions were $y=1.26\times 10^{-2}x+0.2$ and $y=7.0\times 10^{-3}x$, respectively. Both correlation coefficients were 0.999. In these equations, x is the concentration of the ion and y the zone length when the recording speed is adjusted to 20mm/min. The relative standard deviations for magnesium and calcium ions were found to be 0.035 (n=8) and 0.076 (n=5), respectively. The lower determination limits for magnesium and calcium ions were 7.9 and 14.3 mg/l, respectively, corresponding to a 0.1-mm zone length. Both recoveries of magnesium and calcium ions, at 95± 6 %, were almost constant in the range 250～ 2000mg/l magnesium or 250～ 1250 mg/l calcium ion.

6.3.1.6 Analysis of seawater samples

At first, various concentrations of magnesium and calcium ions in 3 % sodium chloride were determined by the proposed method. The error was less than ± 12.9 %, as shown in Table 6-1. Then artificial seawater samples containing 300～ 1500 mg/l magnesium and calcium ions were analysed. The error was less than ± 6.7 %, as shown in Table 6-2. Further, the method was applied to the determination of magnesium and calcium ions in surface seawater samples collected around the coastal area of Osaka Bay between Port of Amagasaki and Suma beach on 4 April 1984. An isotachopherogram of surface seawater from the Suma beach is shown in Fig. 6-1. Most of the values listed in Table 6-2, except in the Port of Amagasaki, agreed approximately with the reported concentrations of magnesium and calcium ions, 1.326×10^6 and 4.22×10^5 μ g/l$^{15)}$, respectively. The lower values in the Port of Amagasaki would be due to the influence of land-water. Finally, 200～ 700 mg/l magnesium and calcium ions were added to seawater from the

Table 6-1 Analytical results for magnesium and calcium ions in 3% sodium chloride

Mixture	Added, mg/l		Found, mg/l		Error, %	
	A	B	A	B	A	B
1	700	1100	700	1190	± 0.0	+8.2
2	900	900	920	1000	+2.2	+11.1
3	1100	700	1100	790	± 0.0	+12.9
4	1300	500	1320	520	+1.5	+4.0
5	1300	400	1320	390	+1.5	-2.5
6	1500	300	1500	330	± 0.0	+10.0

A, Mg^{2+}; B, Ca^{2+}.

Table 6-2 Results for magnesium and calcium ions in seawater

Sampling site	Temp. (°C)	pH	Sal. (‰)	Added (mg/l)		Found (mg/l)		Error (%)	
				Mg^{2+}	Ca^{2+}	Mg^{2+}	Ca^{2+}	Mg^{2+}	Ca^{2+}
Artificial seawater				1100	700	1090	720	-0.9	+2.9
Artificial seawater				1300	500	1330	530	+2.3	+6.0
Artificial seawater				1500	300	1560	320	+4.0	+6.7
Port of Amagasaki	12.8	8.59	15.3	—	—	650	260	—	—
Nishinomiya harbor	11.4	8.48	25.4	—	—	1140	330	—	—
Fukaehama-machi	10.6	8.53	25.2	—	—	1100	350	—	—
Pond at KUMM	9.5	8.24	26.8	—	—	1180	380	—	—
Pond at KUMM[a)]				—	—	570	250	—	—
Pond at KUMM[a)]				200	700	780	920	+1.8	-12.0
Pond at KUMM[a)]				400	500	960	760	-1.8	+4.0
Pond at KUMM[a)]				600	300	1190	490	+3.5	-24.0
Port of Kobe	9.4	8.58	27.0	—	—	1190	450	—	—
Suma beach	8.8	8.54	28.0	—	—	1240	450	—	—

Sampling date: 4 April 1984; a), 2 times diluted.

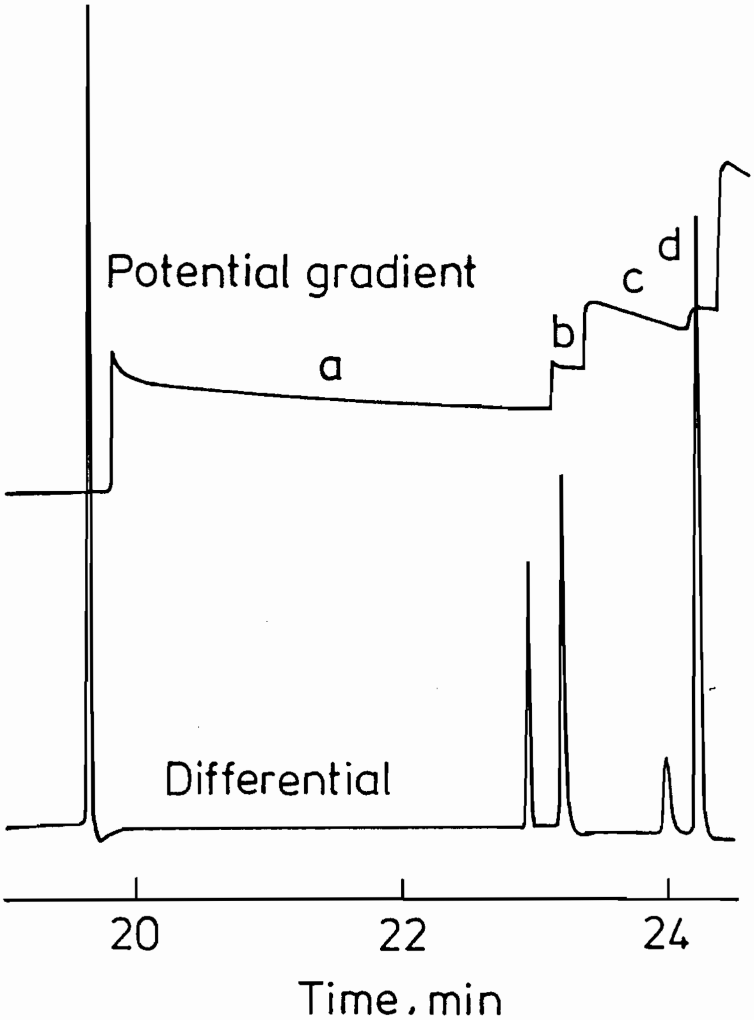

Fig. 6-1 Isotachopherogram of seawater sample treated by the proposed method. a, EDTA; b, Impurity; c, Mg(II)-EDTA; d, Ca(II)-EDTA.

pond at KUMM diluted 2 times, and analysed. The error was less than ± 24.0 %, as shown in Table 6-2.

On the other hand, linear relation was obtained between the salinity and the concentration of magnesium or calcium ion listed in Table 6-2, as shown in Fig. 6-2. The regression equations of these curves for magnesium and calcium ions were $y=46.4x-58.7$ and $y=13.4x+41.1$, respectively. The correlation coefficients were 0.999 and 0.851, respectively. In these equations, x is the salinity in ‰ and y the concentration of the ion in mg/l.

6.3.2 Determination of strontium ion in seawater

6.3.2.1 Kind of eluent

Five ml of solutions containing 4.0×10^{-3}M EDTA, CyDTA, DTPA, GEDTA, triethylenetetramine-N, N, N′ , N″ , N‴ , N‴ -hexaacetic acid (abbreviated as TTHA) or 1, 2-diaminopropane-N, N, N′ , N′ -tetraacetic acid (abbreviated as methyl-EDTA) was added to each 5.0ml of solution containing 2.0×10^{-3}M magnesium,calcium, strontium or barium ion. These solutions were adjusted to pH 10.0 with 1 M sodium hydroxide and then stirred for 15 min to form chelate compounds. Then 3 μl of these solutions containing the chelate compound was injected into the isotachophoretic analyser and the PU values were determined. It was found that GEDTA was the most effective chelating reagent for the separation of the alkaline-

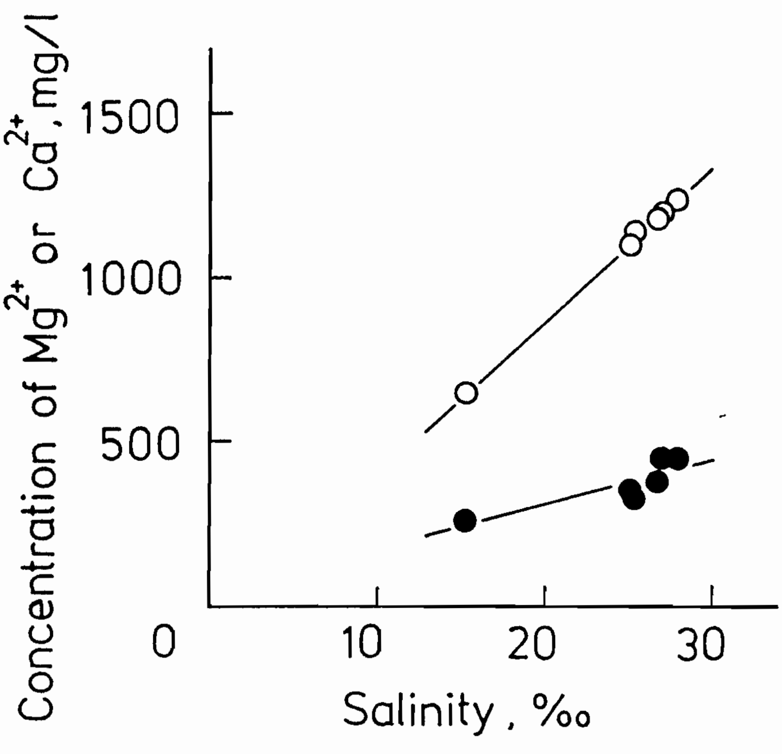

Fig. 6-2 Relation between salinity and concentration of magnesium or calcium ion. ○, Mg^{2+}; ●, Ca^{2+}.

earth metal ions except magnesium ion as their GEDTA chelate compounds, as shown in Table 6-3. Magnesium ion could not form the chelate compound with GEDTA[16~ 18]. Then the effect of some organic solvents on PU values of metal-GEDTA complexes was examined by adding 20 % solvents to the leading electrolyte. As a result, it was impossible to enlarge the differences in these PU values using these organic solvents. Further, it was difficult to elute strontium and calcium ions separately using GEDTA solution as the eluent. On the other hand, EDTA solution was used for the ion-exchange separation of calcium and strontium ions as the eluent, as reported by Honda[19], and as mentioned above, Ca(II)-GEDTA and Sr(II)-GEDTA could be separated but Ca(II)-EDTA and Sr(II)-EDTA could not by CITP. Therefore, the combination of EDTA and GEDTA solutions was adopted as the eluent for the elutions of calcium and strontium ions.

6.3.2.2 pH of GEDTA solution

Ten ml of 8.5 mg/l strontium solution was passed through the column to adsorb strontium ion on the column. The strontium solution was a 3% sodium chloride adjusted to pH 8.0 with 5.0×10^{-2} M sodium carbonate. Then strontium ion was eluted with 5.0×10^{-3} M GEDTA adjusted to pH 9.0, 10.0 or 11.0, respectively. The fractions were taken in the fraction collector (1.0 ml), and a 10 μl portion of each fraction was injected into the isotachophoretic

Table 6-3 PU values of alkaline-earth metal chelate compounds

	Mg^{2+}	Ca^{2+}	Sr^{2+}	Ba^{2+}
EDTA	0.56	0.62	0.63	0.61
CyDTA	0.66	0.71	0.72	—
DTPA	0.37	0.38	0.39	0.38
GEDTA	—	0.70	0.75	0.79
TTHA	—	—	—	—
Methyl-EDTA	0.59	0.65	0.65	0.66

analyser. As shown in Fig. 6-3, 10.0 or 11.0 was found to be the suitable pH range of the eluent of strontium ion, because more eluent was required in pH 9.0 than in pH 10.0 or 11.0.

6.3.2.3 Concentration of GEDTA solution

Various concentrations of GEDTA solutions: 3.0×10^{-3}, 5.0×10^{-3} or 7.0×10^{-3} M adjusted to pH 10.0 were used as the eluent of strontium ion. As shown in Fig.6-4, 5.0×10^{-3} or 7.0×10^{-3}M GEDTA was found to be suitable for the elution of strontium ion.

6.3.2.4 pH of EDTA solution

As mentioned above, EDTA solutions with pH 6.6 and 10.0 were used for the ion-exchange separation of calcium and strontium ions as the eluents, as reported by Honda[19)]. For applying this method, 10.0ml of 3% sodium chloride containing 10.0 mg/l calcium and 8.5 mg/l strontium ions adjusted to pH 8.0 was passed through the column and calcium ion was eluted with 5.0×10^{-3} M EDTA adjusted to pH 6.0, 6.3 or 6.6, respectively. Then strontium ion was eluted with 5.0×10^{-3}M GEDTA adjusted to pH 10.0. More eluent was required in pH 6.0 or 6.3 than in pH 6.6, as shown in Fig. 6-5. The recovery of strontium ion was not affected by the pH of EDTA solution in the range pH 6.0～6.6. Therefore, 6.6 was adopted as the pH of the eluent of calcium ion.

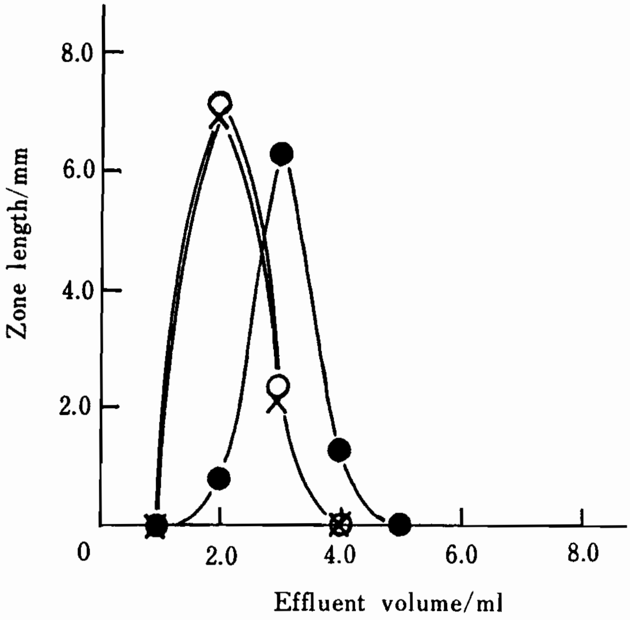

Fig. 6-3 Effect of pH of GEDTA solution on the elution of strontium ion. ●, pH 9.0; ×, pH 10.0; ○, pH 11.0.

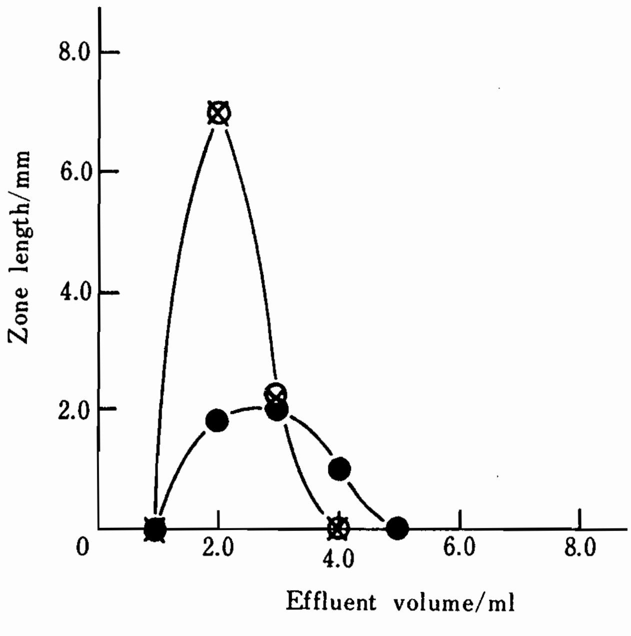

Fig.6-4 Effect of GEDTA concentration on the elution of strontium ion. ● , 3.0×10^{-3}M GEDTA; × , 5.0×10^{-3} M GEDTA; ○ , 7.0×10^{-3} M GEDTA.

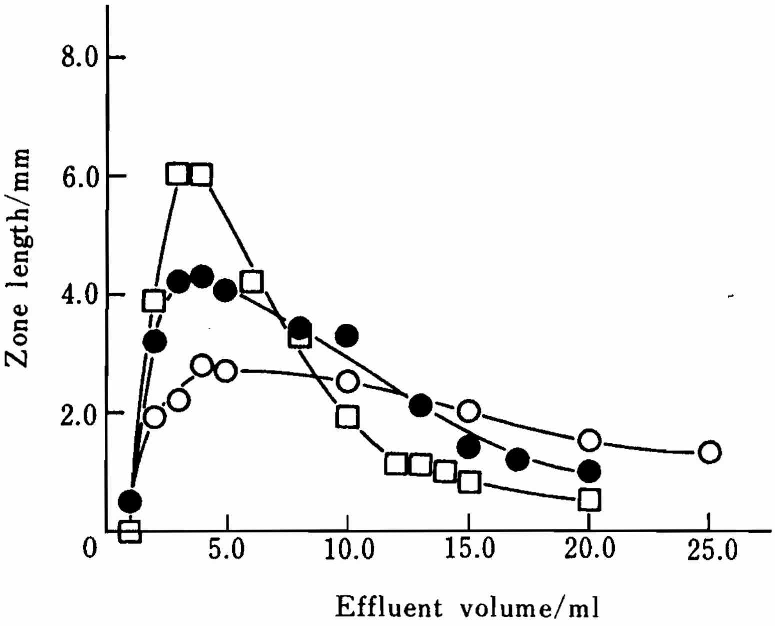

Fig. 6-5 Effect of pH of EDTA solution on the elution of calcium ion. ○, pH 6.0; ●, pH 6.3; □, pH 6.6.

6.3.2.5 Concentration of EDTA solution

Various concentrations of EDTA solutions:2.5×10^{-3}, 5.0×10^{-3} or 1.0×10^{-2} M adjusted to pH 6.6 were used as the eluent of calcium ion. In the concentration of 1.0×10^{-2} M, the fractions were twice diluted with water prior to the injection into the isotachophoretic analyser, because a high concentration of EDTA in the fractions interfered with the isotachophoretic measurement of calcium ion. When 1.0×10^{-2} M EDTA was used, strontium ion was eluted simultaneously with calcium ion. It took longer to elute calcium ion using 2.5×10^{-3} M EDTA, as shown in Fig. 6-6. Therefore, 5.0×10^{-3} M was adopted as the concentration of the eluent of calcium ion.

6.3.2.6 Flow-rate of EDTA solution

The flow-rate of 5.0×10^{-3} M EDTA adjusted to pH 6.6 was varied in the range 1.0~ 3.0 ml/min for the elution of calcium ion. More eluent was naturally required at a flow-rate of 3.0 ml/min than at 2.0ml/min, as shown in Fig.6-7. The recovery of strontium ion was not affected by the flow-rate of EDTA solution in the range 1.0~ 3.0 ml/min. Therefore, 2.0 ml/min was adopted as the flow-rate for the elution of calcium ion.

6.3.2.7 Elution of high concentration of calcium ion

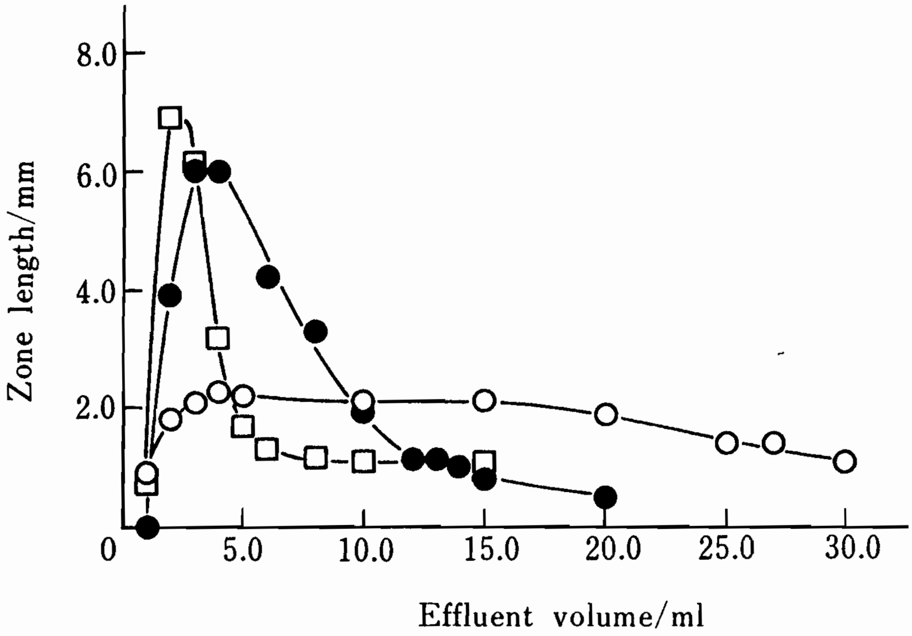

Fig. 6-6 Effect of EDTA concentration on the elution of calcium ion. ○, 2.5×10^{-3} M EDTA; ●, 5.0×10^{-3} M EDTA; □, 1.0×10^{-2} M EDTA.

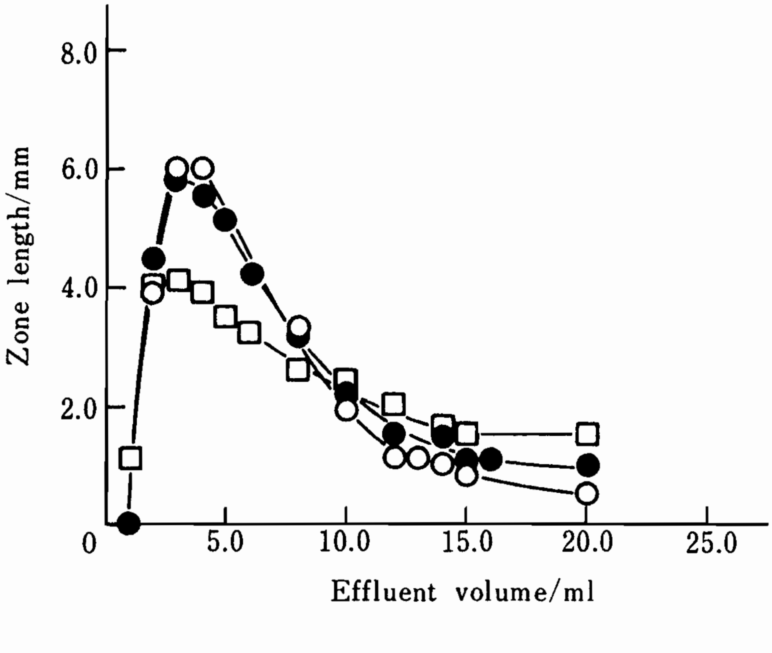

Fig. 6-7 Effect of the flow-rate of EDTA solution on the elution of calcium ion. ○ , 1.0 ml/min; ● , 2.0 ml/min; □ , 3.0 ml/min.

Ten ml of 3 % sodium chloride containing high concentrations of calcium ion:200, 300, 400 or 500mg/l and 8.5mg/l strontium ion was passed through the column and calcium ion was eluted with 5.0×10^{-3}M EDTA adjusted to pH 6.6 at a flow-rate of 2.0ml/min. Then strontium ion was eluted in the manner described above. When the solution contained 500 mg/l calcium ion, 100 ml of the eluent was required, as shown in Fig. 6-8. Regardless of the concentration of calcium ion, the recovery of strontium ion was approximately constant. A similar elution curve was obtained when calcium ion adsorbed from artificial seawater was eluted in the manner described above. Therefore, 100ml was found to be a sufficient volume for the elution of calcium ion in seawater.

6.3.2.8 Working curve

A linear working curve was obtained for 8 artificial seawater samples containing 2.0~ 16.0mg/l strontium ion. The regression equation was $y=0.483x$ (correlation coefficient 0.999) where x is the concentration of strontium ion in mg/l and y the zone length in mm when the recording speed is adjusted to 20mm/min. The relative standard deviation was found to be 0.046 (n=8). The lower determination limit for strontium ion was 0.21 mg/l, corresponding to a 0.1-mm zone length. The recovery of strontium ion, at 102± 7%, was almost constant in the range 2.0~ 16.0mg/l strontium ion.

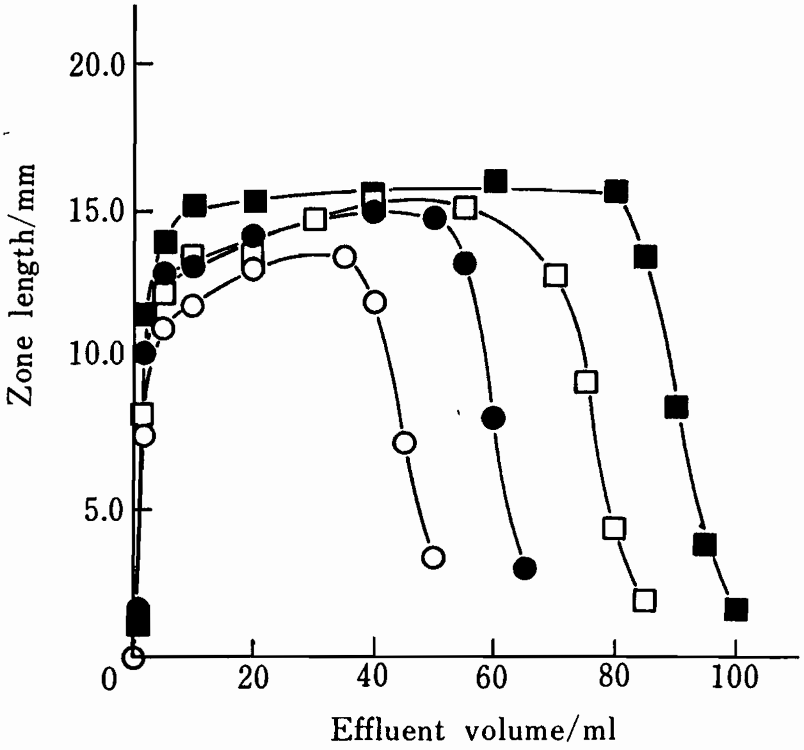

Fig. 6-8 Elution curves of calcium ion in 3 % sodium chloride solution. ○ , 200 mg/l Ca^{2+}; ● , 300 mg/l Ca^{2+}; □ , 400 mg/l Ca^{2+}; ■ , 500 mg/l Ca^{2+}.

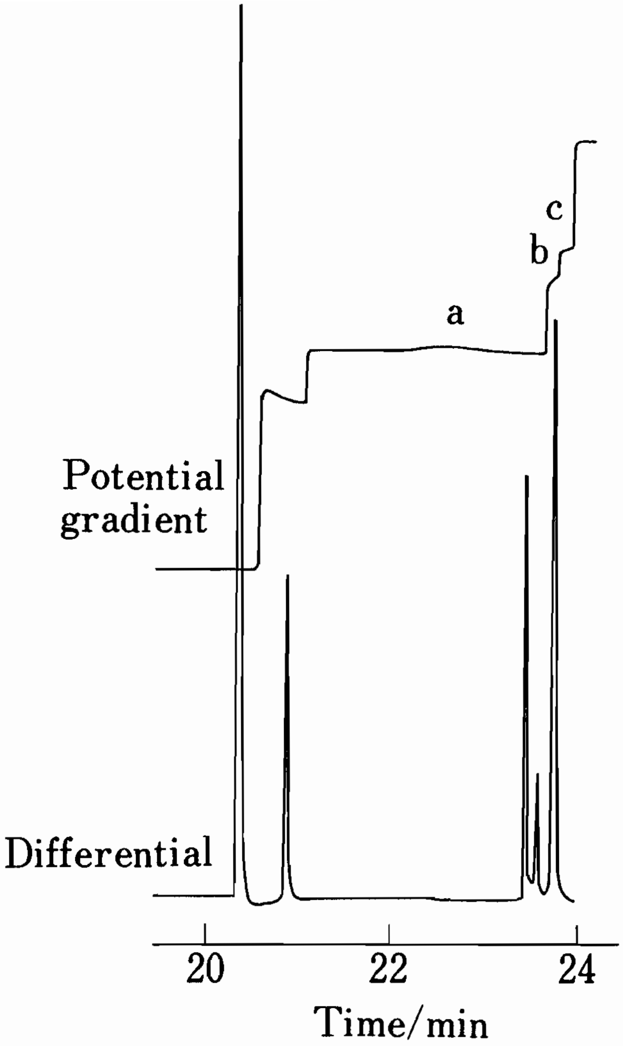

Fig. 6-9 Isotachopherogram of seawater sample treated by the proposed method. a, GEDTA; b, Ca(Ⅱ)-GEDTA; c, Sr(Ⅱ)-GEDTA.

Table 6-4 Results for strontium ion in seawater

Sampling site	Temp. (℃)	pH	Salinity (‰)	Sr^{2+} Added (mg/l)	Found (mg/l)	Error (%)
Port of Amagasaki	29.2	8.51	14.9	—	2.0	—
Nishinomiya harbor	29.7	8.82	18.0	—	4.1	—
Pond at KUMM	29.0	8.25	21.5	—	6.4	—
Rokko Island	28.9	8.03	24.4	—	6.4	—
Maya pier	29.5	8.05	24.5	—	6.4	—
Port of Kobe	28.6	8.15	25.7	—	6.4	—
Suma beach	28.9	8.46	27.0	—	6.4	—
Suma beach[a)]				—	3.2	—
Suma beach[a)]				4.0	6.4	-25.0
Suma beach[a)]				8.0	11.2	± 0.0
Suma beach[a)]				12.0	15.5	+9.4

Sampling date: 10 September 1985; a), 2 times diluted.

6.3.2.9 Analysis of seawater samples

The proposed method was applied to the determination of strontium ion in surface seawater samples collected around the coastal area of Osaka Bay between Port of Amagasaki and Suma beach on 10 September 1985. An isotachopherogram of surface seawater from the Suma beach is shown in Fig.6-9. Then 4.0~ 12.0 mg/l strontium ion was added to seawater from the Suma beach diluted 2 times, and analysed. These results are shown in Table 6-4. Most of the values listed in Table 6-4, except in the Nishinomiya harbor and the Port of Amagasaki, agreed approximately with the reported concentration of strontium ion inside of a bay, 6.7 mg/l5). The lower values in the Nishinomiya harbor and the Port of Amagasaki would be due to the influence of land-water. The error in the standard addition experiment was less than ± 25.0 %.

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7 Determination of total carbon dioxide in seawater

7.1 Introduction

Carbonate compounds in seawater which exist in 60 times higher quantity than carbon dioxide in the atmosphere, are most important fundamental components for breeding marine lives1). In addition, it will become a big problem that carbon dioxide is released to the atmosphere from the ocean owing to the acidification of seawater2). Considering such viewpoints, it is important to determine the total concentration of free carbon dioxide, carbonic acid, hydrogencarbonate and carbonate ions, i.e. TCO_2, in seawater.

TCO_2 in seawater has been determined in detail by means of titration3~6), potentiometric titration7,8), infrared absorption 8,9~13), gas chromatography8,14~18), coulometry19) and calculation8,15,20~22).

On the other hand, there is no report concerning the isotachophoretic determination of TCO_2 in seawater. For this purpose, it is necessary to separate TCO_2 from large amounts of coexisting anions prior to the isotachophoretic measurement. Recently, separation with a gas-permeable membrane has been applied to the determination of TCO_2. For example,Baadenhuijsen et al.23) determined TCO_2 in plasma by flow-injection analysis in which a silicone-rubber membrane was applied. Nagashima et al.24) determined TCO_2 in artificial seawater by a gas-sensitive electrode equipped with a microporous membrane. Separation with a gas-permeable membrane has not ever been applied to the determination of TCO_2 in seawater as pretreatment. Further, it was found that the established method in Chapter 3 could be applied to this purpose.

Therefore, a similar procedure described in Section3.2.3 was applied to the determination of TCO_2 in seawater and the optimum analytical conditions were established. That is to say, carbon dioxide was generated by adding sulfuric acid to seawater samples, permeated through the PTFE membrane and dissolved in sodium hydroxide solution. The following analytical conditions were examined: pH of the leading electrolyte, volume of sulfuric acid, concentration of sodium hydroxide solution, flow-rate, time and temperature of the circulating samples. By the proposed method, a linear working curve was obtained for artificial seawater samples containing up to 40 mg/l TCO_2. The relative standard deviation for TCO_2 was 0.035. The lower determination limit for TCO_2 was 7.8×10^{-2} mg/l. The method was also applied to the determination of TCO_2 in surface and bottom seawater samples. Satisfactory results were obtained in the standard addition experiment. Finally, concentrations of total of free carbon dioxide and carbonic acid, hydrogencarbonate and carbonate ions in the samples were calculated from the concentration of TCO_2, temperature, pH and salinity of samples measured in situ.

7.2 Experimental

7.2.1 Apparatus

The isotachophoretic analyser was the same as described in Section 2.2.1. The main column was a FEP copolymer tube, 15 cm long, 0.5 mm inner diameter; the precolumn was a PTFE tube, 10 cm long, 1.0 mm inner diameter. The Terumo MS-10 microsyringe was used for the injection of samples. The gas-liquid separator, the flow-system and the DO meter were the same as described in Section 3.2.1. The same pH meter described in Section 1.2.1 was used for adjusting the pH of solutions. The pH meter, the salinometer and the water samplers were the same as described in Section 2.2.1. Seawater samples were taken in the same bottles described in Section 3.2.1.

7.2.2 Reagents

All solutions were prepared from analytical reagent grade chemicals. Standard solutions of TCO_2 were prepared by dissolving sodium hydrogencarbonate in TCO_2-free water. TCO_2-free water was prepared on the basis of a Japanese Standard[25)] by using water from the same apparatuses described in Section 2.2.2. The preparation of the artificial seawater was based on a Japanese Standard[26)], but no hydrogencarbonate ion was included.

7.2.3 Procedure

Seawater samples were analysed by the following procedure as soon as possible after collection. Dilute seawater samples filtered through a 0.45-μm membrane 2 times with TCO_2-free water. Add 1.0 ml of 5.0×10^{-2} M sulfuric acid to 45 ml of the seawater sample to generate carbon dioxide. Adjust the total volume to 50 ml with TCO_2-free water. With the water-bath temperature set at 40 °C, circulate 10 ml of this solution through the outer tube at a flow-rate of 9.0 ml/min for 15 min. Carbon dioxide permeates through the wall of the inner tube and dissolves in 1.5 ml of 2.0×10^{-2} M sodium hydroxide previously placed in the inner tube. Pump out the sodium hydroxide solution. Inject 5 μl of the solution into the isotachophoretic analyser. Maintain the migration current at 150μA for the first 8min and then reduce it to 50μA. As leading electrolyte use 5 mM hydrochloric acid/tris (pH 8.5), and as terminating electrolyte 10 mM sodium acetate. Similarly, make a blank test using TCO_2-free water. Then subtract the zone length for hydrogencarbonate ion in the blank test from that of the seawater sample. Prepare a working curve by applying the method to synthetic standards.

7.3 Results and Discussion

7.3.1 pH of leading electrolyte

The pH of the leading electrolyte containing 5 mM hydrochloric acid was adjusted to 7.5, 8.0, 8.5 or 9.0 with tris. As the sample, 10 μl of standard solution containing 1 mM sodium hydrogencarbonate was injected into the isotachophoretic analyser. The zone length for hydrogencarbonate ion in the isotachopherograms increased with pH of the leading electrolyte up to 8.5, but then almost levelled off, as shown in Fig. 7-1. This result was explained by first and second apparent dissociation constants of carbonic acid, $10^{-6.35}$ and $10^{-10.33}$, respectively[27]. That is to say, carbonic acid completely dissociated into hydrogencarbonate ion in the leading electrolyte of which the pH was higher than 8.5, but incompletely in those of which the pH was 7.5 and 8.0. Therefore, 5 mM hydrochloric acid of which the pH was adjusted to 8.5 with tris was selected for subsequent experiments.

7.3.2 Volume of sulfuric acid

The volume of 5.0×10^{-2} M sulfuric acid added to the artificial seawater sample containing 28 mg/l TCO_2 was varied in the range 0.1~2.0 ml. A 5 μl portion of the solution treated by the proposed method was injected into the isotachophoretic analyser. The zone length for hydrogencarbonate ion increased with the sulfuric acid volume up to 1.0 ml, but then almost levelled off, as shown in Fig. 7-2. Therefore, 1.0 ml of 5.0×10^{-2} M sulfuric acid was selected and the subsequent mixture was diluted to 50 ml.

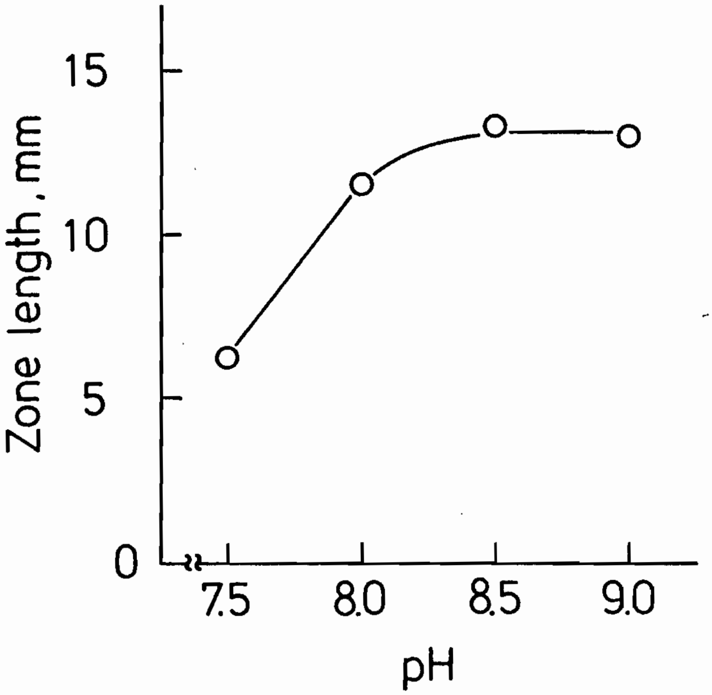

Fig. 7-1 Effect of pH of leading electrolyte.

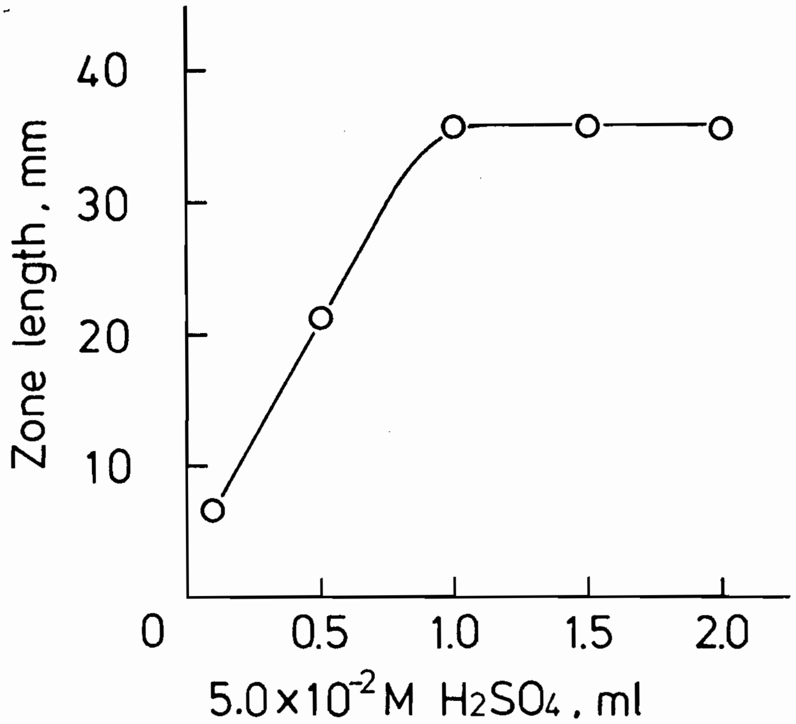

Fig. 7-2 Effect of 5.0× 10^{-2} M sulfuric acid volume.

7.3.3 Concentration of sodium hydroxide solution

The concentration of sodium hydroxide solution was varied in the range $1.0\times 10^{-2}\sim 4.0\times 10^{-2}$M. The zone length for hydrogencarbonate ion increased with the sodium hydroxide concentration up to 2.0×10^{-2}M, but then almost levelled off, as shown in Fig.7-3. Therefore, 2.0×10^{-2} M was adopted as the optimum sodium hydroxide concentration.

7.3.4 Flow-rate of sample circulation

The flow-rate of sample solution was varied in the range 1.0 ~ 9.0 ml/min. The zone length for hydrogencarbonate ion increased with flow-rate, and was almost constant at 9.0ml/min, as shown in Fig. 7-4. Therefore, 9.0 ml/min was adopted as the flow-rate for sample circulation.

7.3.5 Duration of sample circulation

The circulation time was varied in the range 5~ 20 min. The zone length for hydrogencarbonate ion increased with circulation time up to 15 min, but then almost levelled off, as shown in Fig. 7-5. Therefore, 15 min was adopted as the circulation time.

7.3.6 Effect of temperature

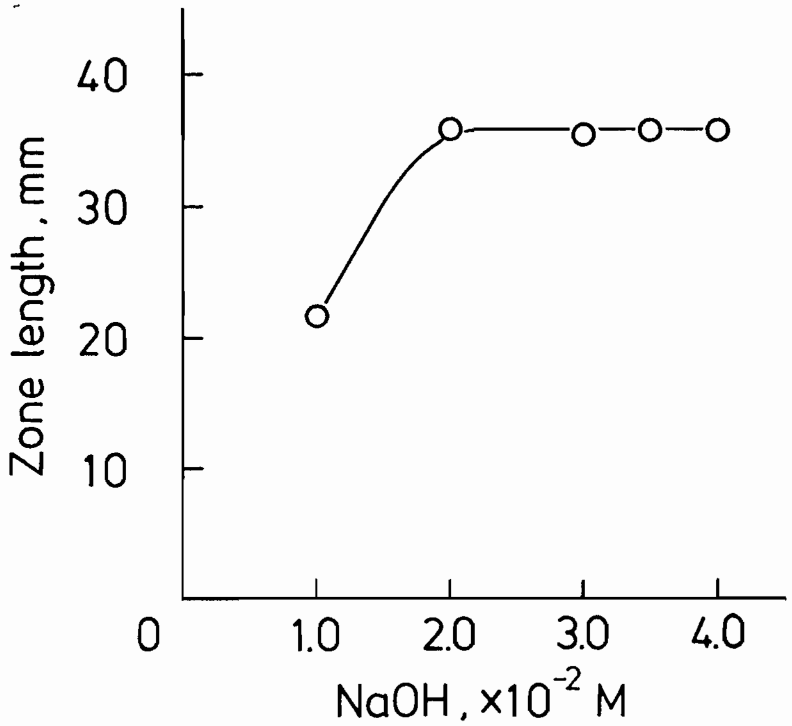

Fig. 7-3 Effect of sodium hydroxide concentration.

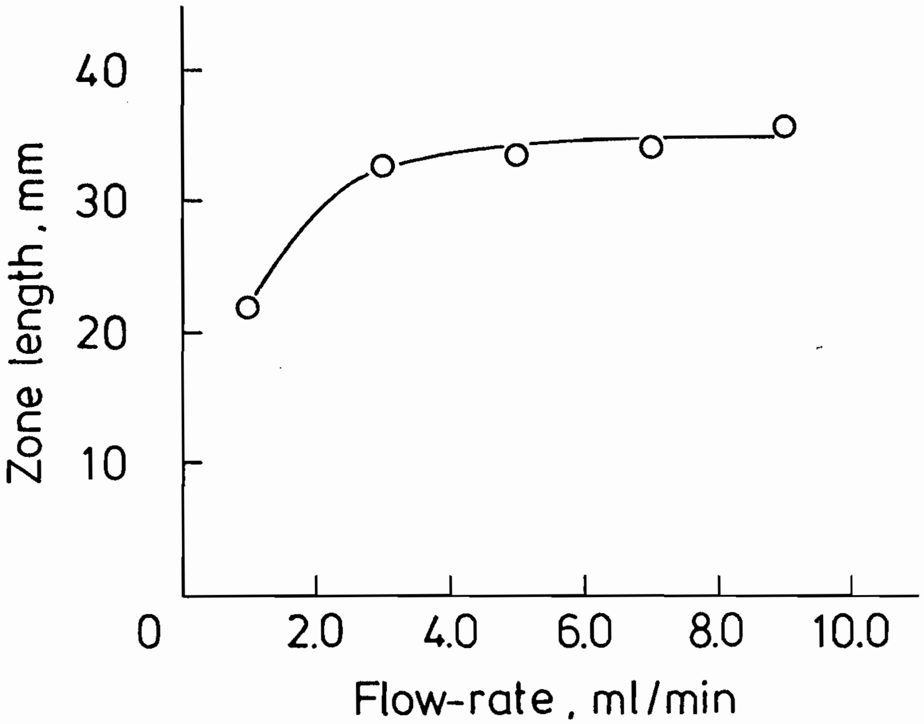

Fig. 7-4 Effect of the flow-rate of sample solution.

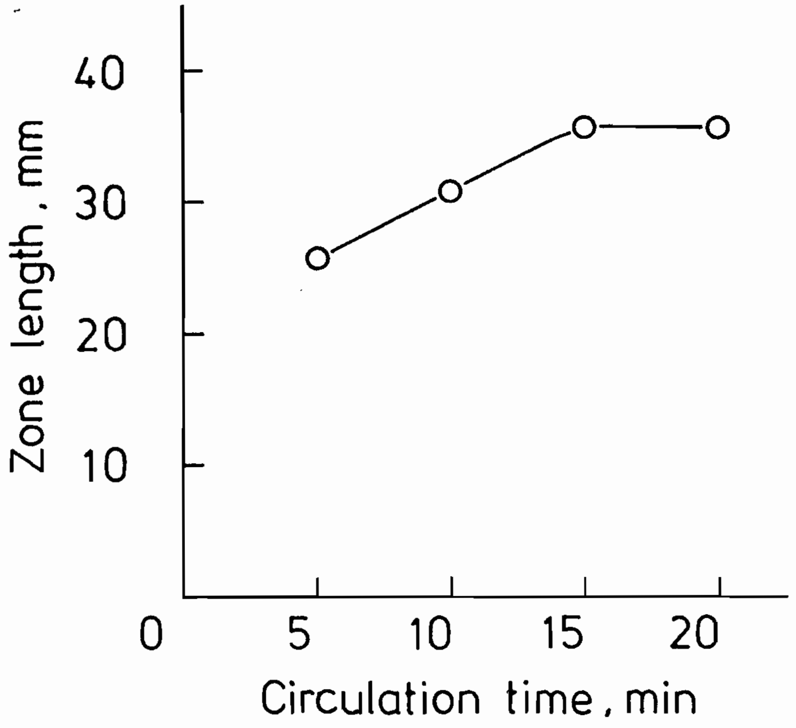

Fig. 7-5 Effect of circulation time.

The temperature of the water-bath was varied in the range 20 ~ 50 ℃. The zone length for hydrogencarbonate ion increased with temperature up to 40℃, but then almost levelled off, as shown in Fig. 7-6. Therefore, 40 ℃ was adopted as the temperature of the water-bath.

7.3.7 Working curve

A linear working curve was obtained for 7 artificial seawater samples containing 10~ 40 mg/l TCO_2. The regression equation was $y=1.28x-0.3$(correlation coefficient 0.999) where x is the concentration of TCO_2 in mg/l and y the zone length in mm when the recording speed is adjusted to 20 mm/min. The relative standard deviation was found to be 0.035 (n=7). The lower determination limit for TCO_2 was 7.8×10^{-2} mg/l, corresponding to a 0.1-mm zone length. The recovery of TCO_2, at 49± 3 %, was less than complete, but this does not matter in practice, because the recovery is almost constant in the range 10~ 40 mg/l TCO_2.

7.3.8 Analysis of seawater samples

The proposed method was applied to the determination of TCO_2 in surface and bottom seawater samples collected around the coastal area of Osaka Bay between Port of Amagasaki and Rokko Island on 15~ 19 December 1986. An isotachopherogram of bottom seawater

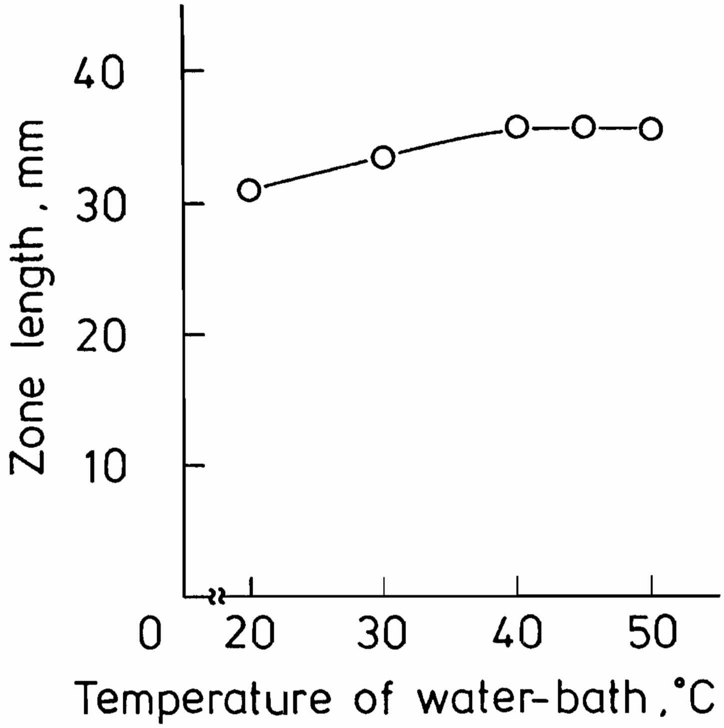

Fig. 7-6 Effect of temperature.

from the mouth of Sumiyoshi river is shown in Fig. 7-7. Then 11 ~ 33 mg/l TCO_2 was added to surface seawater from the Port of Amagasaki and bottom seawater from the mouth of Sumiyoshi river, and analysed. These results are shown in Table 7-1. The mean concentration of TCO_2 for 10 samples agreed with its reported concentration, 28mg/l[28]. The error in the standard addition experiment was less than ± 20.7 %.

Then concentrations of various forms of carbonate compounds were determined with the following calculations. Carbonate compounds exist in seawater as carbon dioxide, carbonic acid, hydrogencarbonate and carbonate ions[1]:

$$CO_2+H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons HCO_3^- \rightleftharpoons CO_3^{2-}$$

H_2CO_3, HCO_3^- and CO_3^{2-} are in the following equilibriums:

$$H_2CO_3 \rightleftharpoons H^+ + HCO_3^-, \quad HCO_3^- \rightleftharpoons H^+ + CO_3^{2-}$$

and

$$\gamma_0[H^+]\,\gamma_2[HCO_3^-]\,/\gamma_1[H_2CO_3] = K_1 \tag{7-1}$$

and

$$\gamma_0[H^+]\,\gamma_3[CO_3^{2-}]\,/\gamma_2[HCO_3^-] = K_2 \tag{7-2}$$

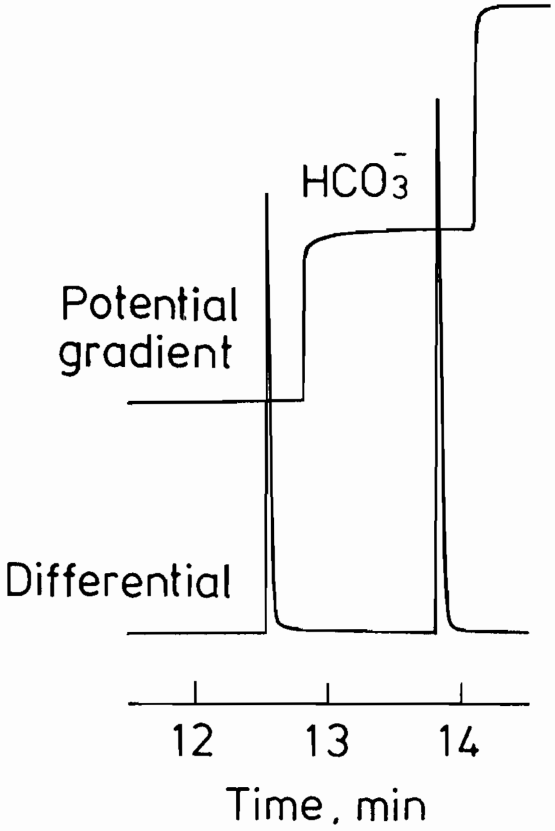

Fig. 7-7 Isotachopherogram of seawater sample treated by the proposed method.

Table 7-1 Results for TCO_2 in seawater

Sampling site	Depth (m)	Temp. (°C)	pH	Sal. (‰)	DO (mg/l)	TCO_2		
						Added (mg/l)	Found (mg/l)	Error (%)
Port of Amagasaki	0	12.2	6.65	12.7	4.17	—	26	—
Port of Amagasaki	0					11	37	± 0.0
Port of Amagasaki	0					22	46	-7.7
Port of Amagasaki	0					33	56	-11.5
Port of Amagasaki	5.5	13.3	7.38	27.4	3.90	—	27	—
Nishinomiya harbor	0	12.8	7.93	27.2	7.38	—	26	—
Nishinomiya harbor	3.0	14.0	7.74	30.8	3.82	—	31	—
Pond at KUMM	0	12.9	7.86	27.4	7.89	—	26	—
Pond at KUMM	6.0	12.8	7.98	30.0	6.79	—	26	—
Mouth of Sumiyoshi riv.	0	13.5	7.62	26.1	6.79	—	32	—
Mouth of Sumiyoshi riv.	3.0	13.0	7.91	29.6	6.79	—	29	—
Mouth of Sumiyoshi riv.	3.0					11	39	-3.4
Mouth of Sumiyoshi riv.	3.0					22	51	± 0.0
Mouth of Sumiyoshi riv.	3.0					33	56	-20.7
Rokko Island	0	13.4	7.85	28.3	7.66	—	27	—
Rokko Island	6.0	13.1	8.08	29.8	7.79	—	26	—

Sampling date: 15～19 December 1986.

where $[H_2CO_3]$ =total concentration of free CO_2 and H_2CO_3; K_1, K_2 = first and second apparent dissociation constants of H_2CO_3, respectively; γ_0, γ_1, γ_2, γ_3=activity coefficients of H^+, free H_2CO_3, HCO_3^- and CO_3^{2-}, respectively. Eqn. (7-1) can be represented by

$$[HCO_3^-] / [H_2CO_3] = K_1' / [H^+] \tag{7-3}$$

where $K_1' = K_1\gamma_1/\gamma_0\gamma_2$. Take logarithms of both sides of eqn.(7-3):

$$\log [HCO_3^-] / [H_2CO_3] = pH-pK_1' \tag{7-4}$$

Similarly, eqn. (7-2) can be represented by

$$\log [CO_3^{2-}] / [HCO_3^-] = pH-pK_2' \tag{7-5}$$

where $K_2' = K_2\gamma_2/\gamma_0\gamma_3$.

If pH, pK_1' and pK_2' are known, $[HCO_3^-] / [H_2CO_3]$ and $[CO_3^{2-}] / [HCO_3^-]$ can be obtained from eqns. (7-4) and (7-5), respectively. pK_1' and pK_2' depend on temperature, chlorinity and hydraulic pressure of seawater, and they are listed in tables[29]. Further, the relation between salinity S in ‰ and chlorinity Cl in ‰ is represented by the following equation, as described in

Section 2.3.2.8:

$$S=1.80655\times Cl$$

Thus pK_1' and pK_2' in the conditions of Table 7-1 were calculated by interpolation and extrapolation using the measured values of temperature and salinity. The effects of hydraulic pressure on pK_1' and pK_2' could be neglected in these shallow sea-areas. Then the concentrations of H_2CO_3, HCO_3^- and CO_3^{2-} in the seawater samples were obtained from the concentration of TCO_2 and these mole fractions. These results are shown in Table 7-2.

On the other hand, linear relations were obtained between the concentration of H_2CO_3 and DO in surface and bottom seawaters, as shown in Fig. 7-8. The regression equations of these curves for surface and bottom seawaters were $y=-0.54x+7.7$ and $y=-3.39x+7.8$, respectively. Negative correlations were found between these parameters; the correlation coefficients were -0.978 and -0.818, respectively. In these equations, x is the concentration of H_2CO_3 in mg/l and y the DO in mg/l. The former correlation might be caused by photosynthesis of phytoplankton and the latter by oxidation of organic matter[30,31). Therefore, the degree of activity of phytoplankton in surface seawater and the amount of organic matter in the seabed can be estimated with the proposed isotachophoretic techniques and from the calculated values plotted in Fig. 7-8.

Table 7-2 Calculated concentrations for H_2CO_3, HCO_3^- and CO_3^{2-}

Sampling site	Depth (m)	H_2CO_3 (mg/l)	HCO_3^- (mg/l)	CO_3^{2-} (mg/l)
Port of Amagasaki	0	6.65	19.32	0.03
Port of Amagasaki	5.5	1.33	25.26	0.40
Nishinomiya harbor	0	0.36	24.30	1.33
Nishinomiya harbor	3.0	0.64	29.16	1.19
Pond at KUMM	0	0.43	24.40	1.17
Pond at KUMM	6.0	0.31	24.06	1.63
Mouth of Sumiyoshi river	0	0.93	30.29	0.78
Mouth of Sumiyoshi river	3.0	0.41	27.03	1.55
Rokko Island	0	0.45	25.34	1.21
Rokko Island	6.0	0.24	23.73	2.02

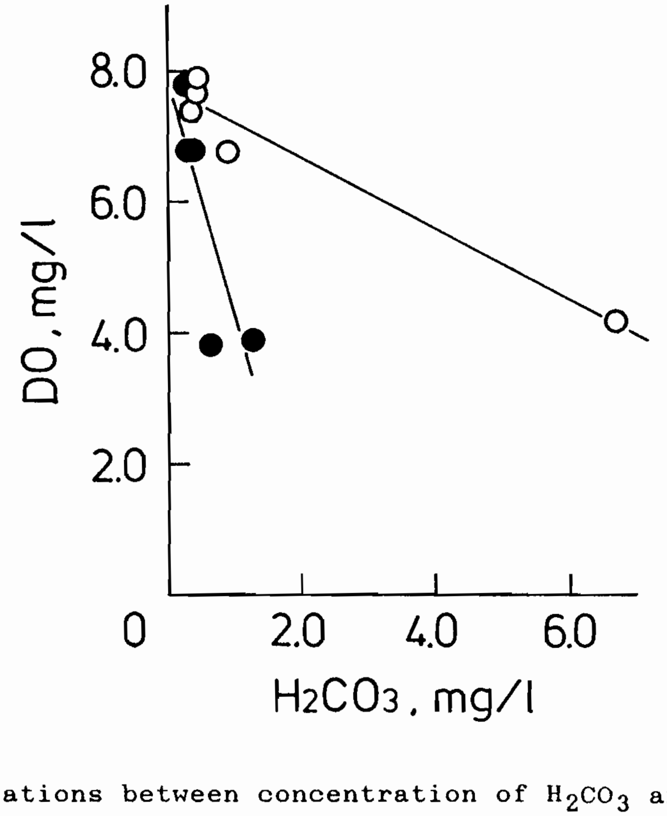

Fig. 7-8 Relations between concentration of H_2CO_3 and DO in surface and bottom seawaters. ○ , Surface seawater; ● , Bottom seawater.

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1. "Simultaneous determination of some sulfur oxoacid ions and measurement of decomposition rate of peroxodisulfate ion by capillary isotachophoresis", K. Fukushi, G. Kondo, K. Hiiro, T.Tanaka, A. Kawahara and S. Wakida, Bunseki Kagaku, 32, 362 (1983).

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