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Capillary zone electrophoresis determination of fluoride in seawater using transient isotachophoresis

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Abstract We developed capillary zone electrophoresis (CZE) with indirect UV detection for determination of fluoride (F^-) in seawater using transient isotachophoresis (tITP) as an on-line concentration procedure. A method of correcting sample salinity effects was also proposed so that F^- concentrations were obtained using a calibration graph. The proposed method is simple: it requires no sample pretreatment aside from dilution. The following optimum conditions were established: background electrolyte (BGE), 5 mM 2,6-pyridinedicarboxylic acid (PDC) adjusted to pH 3.5 containing 0.03% m/v hydroxypropyl methylcellulose (HPMC); detection wavelength, 200 nm; vacuum (50 kPa) injection period of sample, 5 s (254 nL); applied voltage, 23 kV with the sample inlet side as the cathode. The limit of detection (LOD, S/N=3) and limit of quantification (LOQ, S/N=10) for F^- respectively reached 0.024 and 0.070 mg/L. The respective values of the relative standard deviation (RSD) of the peak area, peak height, and migration time for F^- were 2.5, 3.4, and 0.30%. The proposed method was applied to determination of F^- in seawater samples collected from coastal waters of western Japan during August 26–28, 2014. Both results obtained using standard addition method and a calibration graph agreed with those obtained using a conventional spectrophotometric method.

Keywords: Indirect detection · Leading type sample self-stacking · Product of migration time by peak area · Salinity · Working graph

Introduction

Fluoride (F^-) is necessary for the formation of bone and other tissues and for the metabolism of calcium and phosphorus in the human body [1]. When the F^- content in drinking water is inadequate, it can be ingested through F^--added salts [2]. However, excessive intake engenders adverse health effects not only for teeth (dental fluorosis) but also for other body tissues [1]. In general, seawater that contains concentrations of F^- is a main raw material of salts such as edible salts and those used for melting snow on roadway. The regulatory levels of F^- in ocean areas and other areas are restricted respectively to 15 and 8 mg/L by the waste-water standards in Japan [3]. It is therefore important to ascertain the F^- concentration in seawater as well as in salts. Absorptiometry (lanthanum alizarin complexion absorption spectrophotometry or Greenhalgh–Riley method), ion electrode method, and ion chromatography (IC) have been used conventionally for the determination of F^- in seawater [4, 5]. Absorptiometry is an excellent method with small standard deviation (3.8 μg/L) in repeat analysis (n=15) of seawater (F^-, 1.37 mg/L) [5]. However, these methods generally require sample distillation before analysis to avoid interference from coexisting substances or conducting salt error collection. Diffusion, coprecipitation, and solid phase extraction were used instead of distillation to separate F^- from coexisting substances in seawater samples [6]. These pretreatment procedures including distillation are somewhat laborious. Recently, IC methods have been developed for simultaneous determination of anions including F^- in seawater. Fluoride was successfully determined by injecting 17–42-fold diluted samples and switching the detector output range during the analysis [7]. However, F^- could not be determined owing to the severe overlap with chloride (Cl^-) when cycling-column switching system was used [8].

Capillary zone electrophoresis (CZE) is a simple and environmentally friendly method with high resolution. In our earlier studies, we developed CZE methods for the simultaneous determination of nitrite and nitrate [9], iodide and iodate [10], phosphate [11], and bromate [12] in seawater. Artificial seawater (ASW) was used as the background electrolyte (BGE) to eliminate high salt interference (except for phosphate). As an on-line concentration procedure, transient isotachophoresis (tITP) was adopted. There are tITP of roughly two types: terminating type and leading type [13]. In the former, the BGE co-ion has the highest mobility (leading ion). The major sample matrix ion (or a

terminating ion added separately) has the lowest mobility (terminating ion). Analytes will be stacked at the sharp front boundary of the terminating ion. The tITP used in our methods described above corresponds to the terminating type tITP: a high concentration of Cl^- in the BGE acts as the leading ion. Acetate and other materials added to the capillary after the sample was injected act as the terminating ion. In the latter, the major sample matrix ion has the highest mobility; the BGE co-ion has the lowest mobility. Analytes will be stacked at the sharp rear boundary of the sample matrix. The leading type tITP was applied to nitrate determination in seawater [13]. Somewhat different types of tITP have been reported for the determination of strontium and lithium in seawater [14]. In this method, both the leading ion (sodium) and the terminating ion (magnesium and calcium) were contained in seawater samples. We also developed another type of tITP to quantify aniline and pyridine in sewage samples [15]. The BGE co-ion (sodium) in the sample acted as the leading ion and H^+, for which the effective mobility (μ_e) was decreased by deviation of the acid–base balance, acting as a system-induced terminator.

For this study, we developed a leading type tITP–CZE to ascertain F^- in seawater based on our method established for phosphate determination in seawater [11]. Analytical conditions such as applied voltage and sample-injection time were examined as well as the effects of sample salinity on the migration time, peak area, and peak height for F^-. Finally, the proposed method was applied to determination of F^- in seawater samples collected from coastal waters (3 m depth) of western Japan. We also proposed a correction method for sample salinity. Results obtained using the CZE method were compared to those obtained using conventional absorptiometry. No report of the relevant literature describes determination of F^- in seawater using tITP–CZE.

Materials and methods

Materials

All reagents were of analytical-reagent grade and were used as received. As a UV-absorbing compound in the BGE, 2,6-pyridinedicarboxylic acid (PDC; Nacalai Tesque, Kyoto, Japan) was

used. Hydroxypropyl methylcellulose (HPMC), used for suppressing electroosmotic flow (EOF), was obtained from Aldrich (Milwaukee, WI, USA). Sodium hydroxide (NaOH) used for flushing the capillary and for adjusting the BGE pH was purchased from Nacalai Tesque. Two-fold concentrated artificial seawater (ASW) without F^- was prepared in the following manner based on the Japanese Standard [16]. First, three groups of mixed aqueous solutions were prepared. They consist of magnesium chloride (22.20 g/L), calcium chloride (2.32 g/L), and strontium chloride (0.08 g/L) in a 200 mL beaker for the first group, potassium chloride (1.38 g/L), sodium hydrogencarbonate (0.40 g/L), potassium bromide (0.20 g/L), and boric acid (0.06 g/L) in a 100 mL beaker for the second group, sodium chloride (49.08 g/L) and sodium sulfate (8.18 g/L) in a 500 mL beaker for the third group [9]. Then the three groups were transferred to a volumetric flask (1 L) and water was filled to the marked line. The salinity of the original ASW (not two-fold concentrated ASW) was 34 (practical salinity, its numerical value ≈ the numerical value for parts per thousand) at 25 °C (pH 7.9). The stock solution of F^- (1000 mg/L) was prepared from sodium fluoride (Wako Pure Chemical Industries, Osaka, Japan) and was diluted serially as required. Standard solutions containing 0–0.30 mg/L F^- in ten-fold diluted ASW were prepared by mixing 2.5 mL of ASW (two-fold concentration), 1.0 mg/L F^- (0–15 mL), and water in a 50 mL volumetric flask. Distilled, demineralized water, obtained from an automatic still (WG220; Yamato Kagaku, Tokyo, Japan) and a high-purity water apparatus (Simpli Lab-UV; Merck Millipore, Tokyo, Japan) was used throughout. All solutions, including seawater samples, were filtered through a 0.45 µm membrane filter (Advantec Tokyo Kaisha, Tokyo, Japan) before use.

Instrumentation

All experiments were conducted using a capillary electrophoresis (CE) instrument equipped with a photo–diode array detector (CAPI-3300; Otsuka Electronics, Osaka, Japan). A polyimide-coated fused-silica capillary (GL Sciences, Tokyo, Japan) with 87.4 cm total length (75 cm effective length) and 75 µm i.d. (375 µm o.d.) was used. The capillary was thermostated at 25 °C. The detection wavelength was set at 200 nm. This experiment used a pH meter (F-22; Horiba, Kyoto, Japan). Seawater samples were collected from 3 m below the surface in coastal waters of western Japan

during August 26–28, 2014 using a pump installed in the training ship at our university. The samples were filtered immediately after bringing back to the laboratory. The filtered samples were stored in the refrigerator at 4 °C until use. Salinity and other parameters were measured using the logger version conductivity and a temperature sensor with a wiper (INFINITY-CTW; JFE Advantech, Tokyo, Japan) set up on the ship. Salinity was obtained from the measured water-temperature, hydraulic pressure, and conductivity automatically.

Procedures

Fluoride contents in seawater samples were quantified using the following CZE procedures. No pretreatment procedures were required except for filtration and dilution. A new capillary was flushed with water for 5 min, then with 1 M NaOH for 40 min, water for 10 min, and BGE (a mixture of 5 mM PDC adjusted to pH 3.5 with 1 M NaOH and 0.03% m/v HPMC) for 10 min (vacuum pressure, 50 kPa). Before the first analysis was conducted each day, the capillary was flushed with water for 5 min and the BGE for 10 min. After the capillary was filled with the BGE for 4 min, the sample solution was vacuum-injected (50 kPa) for 5 s (254 nL, the sample length = 6.6 % of the total capillary-length) into the CE apparatus. The injection period of 1 s corresponds to the sample volume of 50.8 nL (the sample length = 1.3 % of the total capillary-length). Voltage (23 kV) was applied for separation with the sample-inlet side as the cathode. Standard addition method was applied for F^- quantification in seawater samples. In sample vials (700 µL), 60 µL seawater sample, 1.0 mg/L F^- standard solution (0, 30, 60, and 90 µL), and water (540, 510, 480, and 450 µL) were mixed well to produce solutions containing 0–0.15 mg/L F^- in ten-fold diluted seawater samples. In addition, F^- concentrations in seawater samples were obtained using the calibration graph, F^- concentration vs. the product of the migration time by the peak area for F^-. At the end of the day, the capillary was flushed with water for 5 min to fill the capillary with water.

To verify the proposed method, the conventional absorptiometry (lanthanum-alizarin complexone absorptiometry) was conducted according to the procedures shown as below [4]. To prepare lanthanum-alizarin complexone solution, at first 0.192 g alizarin complexone (1,2-dihydroxyanthraquinone-3-yl-methylamine-*N*,*N*-diacetic acid) was dissolved in the mixture of

4 mL aqueous ammonia (1.4 M) and 4 mL ammonium acetate (200 g/L). Then the solution was added into sodium acetate solution (the mixture of 41 g sodium acetate trihydrate / 400 mL water and 24 mL acetic acid). With stirring above solution, 400 mL acetone was added to it gradually and lanthanum solution (0.163 g lanthanum (III) oxide / 10 mL 2 M hydrochloric acid) was added further. After allowing the mixture to cool, the pH was adjusted to ca. 4.7 with acetic acid or aqueous ammonia. The solution was diluted to 1 L with water (lanthanum-alizarin complexone solution). The lanthanum-alizarin complexone solution was added to a seawater sample (20 mL) in a volumetric flask (50 mL). The solution was diluted to the mark with water and mixed well. After being allowed to stand for 1 h, aliquots of the solution were transferred to an absorption cell to measure absorbance at 620 nm. Fluoride concentration in seawater samples was calculated using a calibration graph.

Results and discussion

Applied voltage

Indirect photometric detection must be adopted for CZE determination of F^- because it has no absorption in the UV-Vis region. According to the procedure established for phosphate determination [17], 5 mM PDC was adopted as the UV absorbing probe. The detection wavelength was also set to 200 nm. To suppress the EOF, 0.01% m/v HPMC was added to the BGE for phosphate determination. Its concentration was increased to 0.03% m/v to enhance the effect of HPMC. By considering of the occurrence of t-ITP, the BGE pH was adjusted to 3.5. At pH 3.5, F^- (μ_e = –36.7 × 10^{-9} $m^2V^{-1}s^{-1}$) will be sandwiched between Cl^- (μ_e = –72.3 × 10^{-9} $m^2V^{-1}s^{-1}$, acts as the leading ion) in seawater samples and the PDC (μ_e = –29.3 × 10^{-9} $m^2V^{-1}s^{-1}$, acts as the terminating ion) in the BGE. The values of μ_e were obtained using simulation software; Peakmaster 5.3 Complex [18, 19]. The standard solution containing 0.14 mg/L F^- in ten-fold diluted ASW was analyzed to investigate the effects of applied voltage (17, 20, 23, 25 kV) on S/N for F^- (sample-injection time, 3 s). To weaken the salt interference with F^- separation, ASW was diluted ten-fold. Because the F^- concentration in open ocean seawater is 1.3–1.4 mg/L [20, 21], its 1/10 concentration was adopted

as the F^- concentration in the standard solution. Analyses were applied twice for each voltage. The S/N, which was obtained using the average peak height, increased linearly with voltage up to 23 kV. Thereafter, it decreased because of the noisy baseline caused by an increase in electric current when the voltage was 25 kV (see Electronic Supplementary Material Fig. S1). Therefore, the optimum voltage adopted for subsequent experiments was 23 kV.

Sample-injection time

The sample-injection time was varied between 3–6 s (152–305 nL) to assess its effects on the migration time, peak area, and peak height for F^-. When the standard solution containing 0.14 mg/L F^- in ten-fold diluted ASW was analyzed, the F^- peak height increased with the sample-injection time up to 5 s (data not shown). Analyses were repeated twice for each sample-injection time. Then the average peak height was calculated. When the injection time was 6 s, the F^- peak was not separated completely from the large mixed peaks of Cl^- and sulfate (SO_4^{2-}) observed in front of the F^- peak. Therefore, the optimum sample-injection time adopted for subsequent experiments was 5 s (254 nL).

Calibration graphs

When ten-fold diluted ASW solutions (salinity, 3.4) containing different concentrations of F^- were analyzed by the proposed procedure, a calibration graph for F^- was linear using the peak area: a regression equation relating the area response to F^- concentration (x, 0–0.30 mg/L) was $y = 69.7\,x + 0.182$ (y: peak area (arbitrary units), correlation coefficient, 0.9989). However, a calibration graph for F^- was not linear when the peak height was used ($y = -60.9\,x^2 + 45.1\,x + 0.185$). Analyses were repeated twice on each concentration. Then the average values were obtained. Table 1 presents the relative standard deviation (RSD), limit of detection (LOD, 0.024 mg/L), and limit of quantification (LOQ, 0.070 mg/L) for F^- using the proposed method. Both the LOD and the LOQ are for 10-fold diluted seawater samples and thus the concentrations of F^- accessible in seawater samples are 10-fold higher. The LOD and the LOQ converted for undiluted seawater samples are 0.24 mg/L and

0.70 mg/L, respectively. The LOQ for the conventional absorptiometry was 0.20 mg/L when the sample volume used for the analysis was 20 mL [4]. If some pre-concentration method is adopted for some analytical method, LOD and LOQ for F^- must be lowered. For example, the LOQ for capillary type isotachophoresis using coprecipitation as the pre-concentration procedure was 0.03 mg/L (the sample volume was 50 mL) [22]. When micro diffusion was used as the pre-concentration method, the LOQ for IC was 0.004 mg/L (the sample volume was 60 mL) [6]. The LOD and the LOQ for solid phase extraction–spectrophotometry were 0.015 mg/L and 0.042 mg/L, respectively (the sample volume was 250 mL) [23]. The LOD (0.24) and the LOQ (0.70 mg/L) for undiluted seawater samples for the proposed procedure was higher than those for above methods. However, they are still sufficiently low to determine F^- in seawater samples (1.3 – 1.4 mg/L for open ocean seawater) precisely. The RSD (2.5%, n=4) for our method was similar to the RSD (3.7%, n=4) for the isotachophoresis method [22] and the RSD (2.0%, 0.8 mg/L, n=10) for solid phase extraction–spectrophotometry [23]. The proposed procedure does not need any complicated pretreatment including distillation. In addition, our method requires much less sample volume (60 μL) than that for above methods.

Effects of salinity in a sample solution

The salinity of coastal seawater samples, especially on the surface, varies because of the effects of river water, insolation, and other influences. In the proposed method, Cl^- in the sample solutions corresponds to the leading ion for tITP. Therefore, it was presumed that the migration time, peak area, and peak height for F^- will vary according to the salinity of the sample solutions. Sample solutions containing 0.14 mg/L F^- in 0–10% (salinity, 0–3.40) ASW were analyzed. Analyses were conducted twice for each sample. Average values were obtained for migration time, peak area, and peak height for F^-. The migration time for F^- increased gradually with the sample salinity up to 3.40 (Fig. 1). This tendency for the analyte migration time in leading type tITP agreed with the tendency for model components obtained using computer simulation [24]. The peak height for F^- increased with the sample salinity, whereas the peak area decreased. A higher Cl^- concentration in the sample solutions results in a longer ITP time leading to higher peak height [13]. Consequently, the peak area

and peak height for F^- varied with the sample salinity in the proposed method. Therefore, a calibration graph prepared using standard solutions with constant salinity cannot be used to ascertain the F^- concentrations in seawater samples with different salinities. A standard addition method must be used, but it takes a longer time than a calculation method using a calibration graph. Results demonstrated that the product of the migration time by the peak area of F^- is constant even if the salinity of samples (containing constant F^-) differs (Fig. 2). The reason remains unclear. It can be inferred that the peak area was inversely proportional to the migration time because of the analytical conditions adopted in this procedure. We intend to examine the issue in detail using different analytical conditions (total and effective capillary lengths, capillary inner diameter, sample volume, applied voltage etc.) through a future work. A linear calibration graph was obtained using the product of the migration time by the peak area. A regression equation relating the product (y, arbitrary units) of the migration time by the peak area to concentrations for F^- (x, 0–0.30 mg/L) was $y = 902\,x + 1.27$ (correlation coefficient, 0.9992). Consequently, in spite of differences in the sample salinity, the F^- concentration is obtainable using the proposed calibration graph instead of a standard addition method. This capability is a salient benefit of the proposed method because the procedure for determination with the addition of the standard solutions for F^- to seawater samples is both troublesome and time-consuming.

Analyses of seawater samples

The proposed method was applied for determining F^- in seawater samples collected from coastal waters of western Japan. Each sample was analyzed twice for standard addition method. Then the average values were calculated to obtain the F^- concentration in the samples. Fluoride concentrations were also calculated using the average values (no addition of F^-) and the calibration graph (F^- concentration vs. the product of the migration time by the peak area). For comparison, absorptiometry was also conducted. The influence of sample salinity on the measured values using absorptiometry was corrected as described below. Samples presented in Table 2 were divided into three groups depending on their salinity: group 1 (sample 1, salinity: 3.33), group 2 (samples 2 and 3, average salinity: 25.9), and group 3 (samples 4–15, average salinity: 32.6). The following

equation was used for rough calculations of the Cl^- concentrations added to the standard solutions.

$$S = 1.80655\ Cl$$

Therein, *S* and *Cl* respectively denote the sample salinity and chlorinity [25]. As a result of calculation, the *Cl* values were 1.84, 14.3, and 18.0 for the group 1, the group 2, and the group 3, respectively. These *Cl* values correspond to the addition of 0.37, 2.9, and 3.6 mL of 0.1 g/L Cl^- solution. Three groups of F^- standard solutions with the different salinity were prepared by adding above Cl^- solutions to solutions containing 0–50 μg F^- in 50 mL volumetric flasks. By analyzing the standard solutions using the absorptiometry, calibration graphs of three different levels of salinity were prepared such that salt error can be corrected. Fluoride concentrations in the respective groups were calculated using each calibration graph. Table 2 presents those results. Results for the standard addition method roughly agreed with those obtained using the calibration graph. In addition, both results roughly agreed with those obtained using the absorptiometry. The results presented above were demonstrated statistically using *t*-test (significance level, 5%). Figure 3 depicts an electropherogram of seawater (see Electronic Supplementary Material Fig. S2, the whole electropherogram). A sharp F^- peak was detected within 13 min with baseline separation from high concentrations of Cl^- and SO_4^{2-}. Although F^- should be recorded as the negative peak because it has no UV absorption, the F^- peak was converted to the positive peak by the operating software automatically. The negative peak (in reality positive) just behind F^- peak (at 13 min) was unidentified at present. Fluoride is a conservative element. Its concentration is proportional to the salinity in seawater [26]. The correlation coefficient was 0.7992 between F^- concentrations obtained using the calibration graph and the salinities shown in Table 2. Fairly strong correlation was found. The proposed method using the calibration graph is simple, entailing no extra time or effort. Nevertheless, it provides sufficient sensitivity and accuracy for ascertaining F^- in seawater, requiring only sample dilution before the CZE analysis. The high concentration of Cl^- in sample solutions and PDC in the BGE respectively act as the leading and terminating ions. For the proposed tITP–CZE for F^- determination, it is not necessary to prepare a terminating ion solution.

Conclusions

The proposed procedure is simple, but provides sufficient detection power for determining F^- in seawater. It requires only sample dilution before CZE analysis. As the continuation of the conducted research, the proposed method might be suitable for determination of F^- in salts (0.0425–0.26 mg/g F^- [2]), human urine (0.499–2.210 mg/L F^- [1] and 140 mM Cl^- [27]), human serum (0.033–0.096 mg/L F^- [1] and 100 mM Cl^- [28]), brine, and wastewater samples. To demonstrate the feasibility, it is necessary to examine interference from coexisting substances. In addition, the method sensitivity must be improved to ascertain F^- in serum samples.

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Conflict of interest The authors declare that they have no conflict of interest related to this study.

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Fig. 1 Effects of salinity of sample solutions on the migration time, peak area, and peak height for F^-. (○), migration time; (●), peak area; (△), peak height. Electrophoretic conditions: capillary, 87.4 cm total length (75 cm effective length) and 75 μm i.d. (375 μm o.d.); BGE, 5 mM 2,6-pyridinedicarboxylic acid (PDC) adjusted to pH 3.5 with 1 M NaOH containing 0.03% m/v hydroxypropyl methylcellulose (HPMC); voltage, 23 kV with the sample inlet side as the cathode; wavelength for detection, 200 nm; sample, 0.14 mg/L F^-/ten-fold diluted artificial seawater (ASW); vacuum injection period, 5 s (ca. 254 nL); two determinations for each salinity of samples.

Fig. 2 Relation between the salinity of sample solutions and the product of the migration time by the peak area for F^-. Electrophoretic conditions are identical to those in Fig. 1.

Fig. 3 Electropherogram of the surface seawater sample 11 (ten-fold diluted) in Table 2. Electrophoretic conditions are identical to those in Fig. 1.

Table 1 Relative standard deviation (RSD), limit of detection (LOD), and limit of quantification (LOQ)

Analyte	RSD (%, n=4)[a]			LOD[b] (S/N=3)	LOQ[b] (S/N=10)
	Time	Area	Height	(mg/L)	(mg/L)
F^-	0.30	2.5	3.4	0.024	0.070

Electrophoretic conditions are identical to those in Fig. 1. Sample, [a]0.14 mg/L and [b]0.05 mg/L F^-/ten-fold diluted ASW

Table 2 Analytical results found for F^- in seawater samples collected from coastal waters (3 m depth) of western Japan during August 26–28, 2014

Sample	F^- (mg/L) in seawater samples			Salinity
	Standard addition method	Calibration graph (time×area) method	Absorptiometry	
1 [a]	0.736	0.636	0.674	3.33
2	0.992	0.856	0.951	23.0
3	0.857	0.853	1.03	28.8
4	1.00	0.930	1.05	31.0
5	1.16	1.15	1.17	32.1
6	1.28	1.19	1.13	31.4
7	1.15	1.19	1.13	33.2
8	1.22	1.29	1.23	33.2
9	1.02	1.10	1.12	32.1
10	1.32	1.25	1.17	34.1
11	1.05	0.979	1.17	34.0
12	1.10	1.11	1.22	34.0
13	1.22	1.14	1.13	32.5
14	1.04	1.06	1.25	31.9
15	1.13	1.15	1.17	31.4

Electrophoretic conditions are identical to those presented in Fig. 1. [a]Collected in the mouth of Yodo river

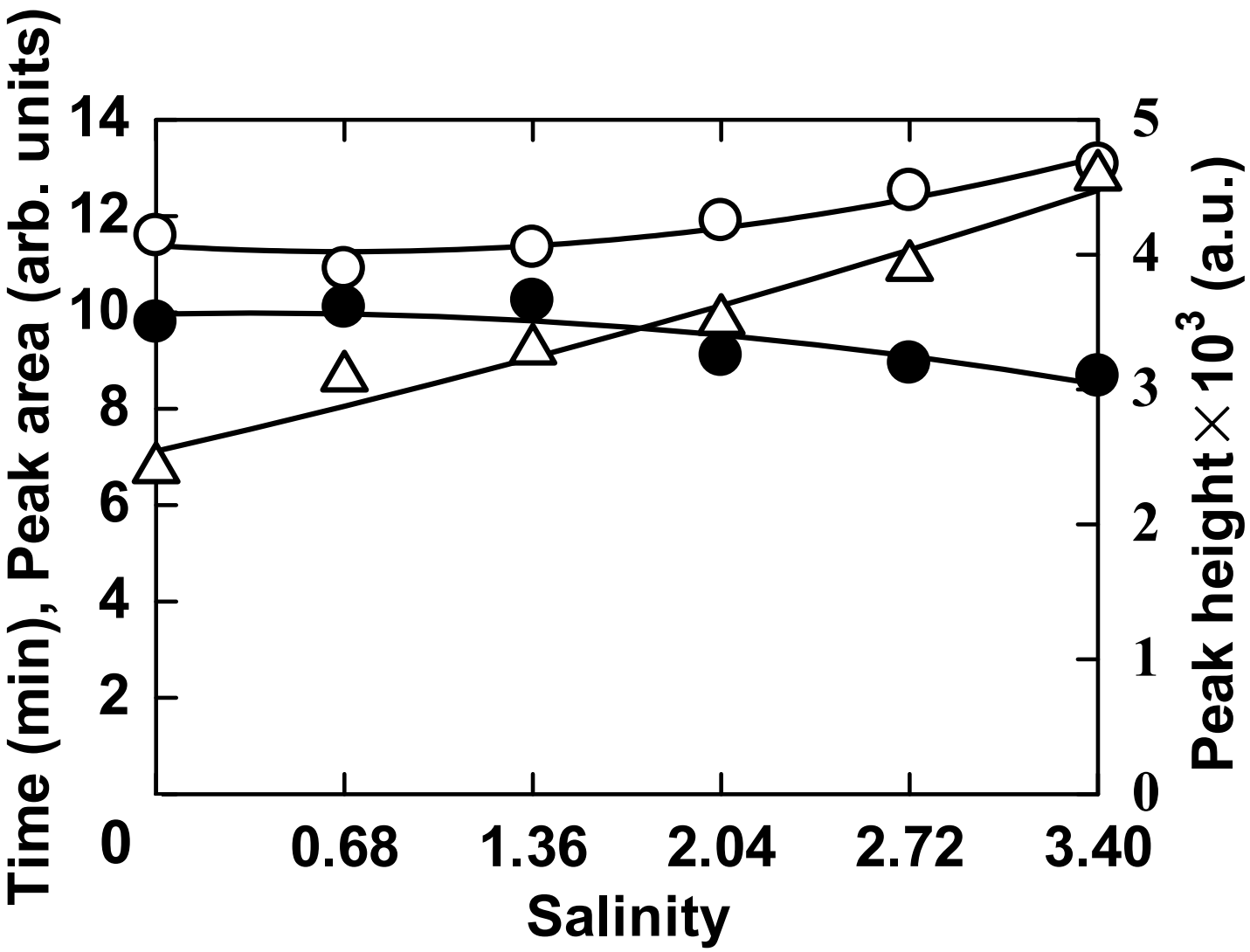
Time (min), Peak area (arb. units)
14
12
10
8
6
4
2
0
0
0.68
1.36
2.04
2.72
3.40
Salinity
5
4
3
2
1
0
Peak height×10^3 (a.u.)

Fig. 1

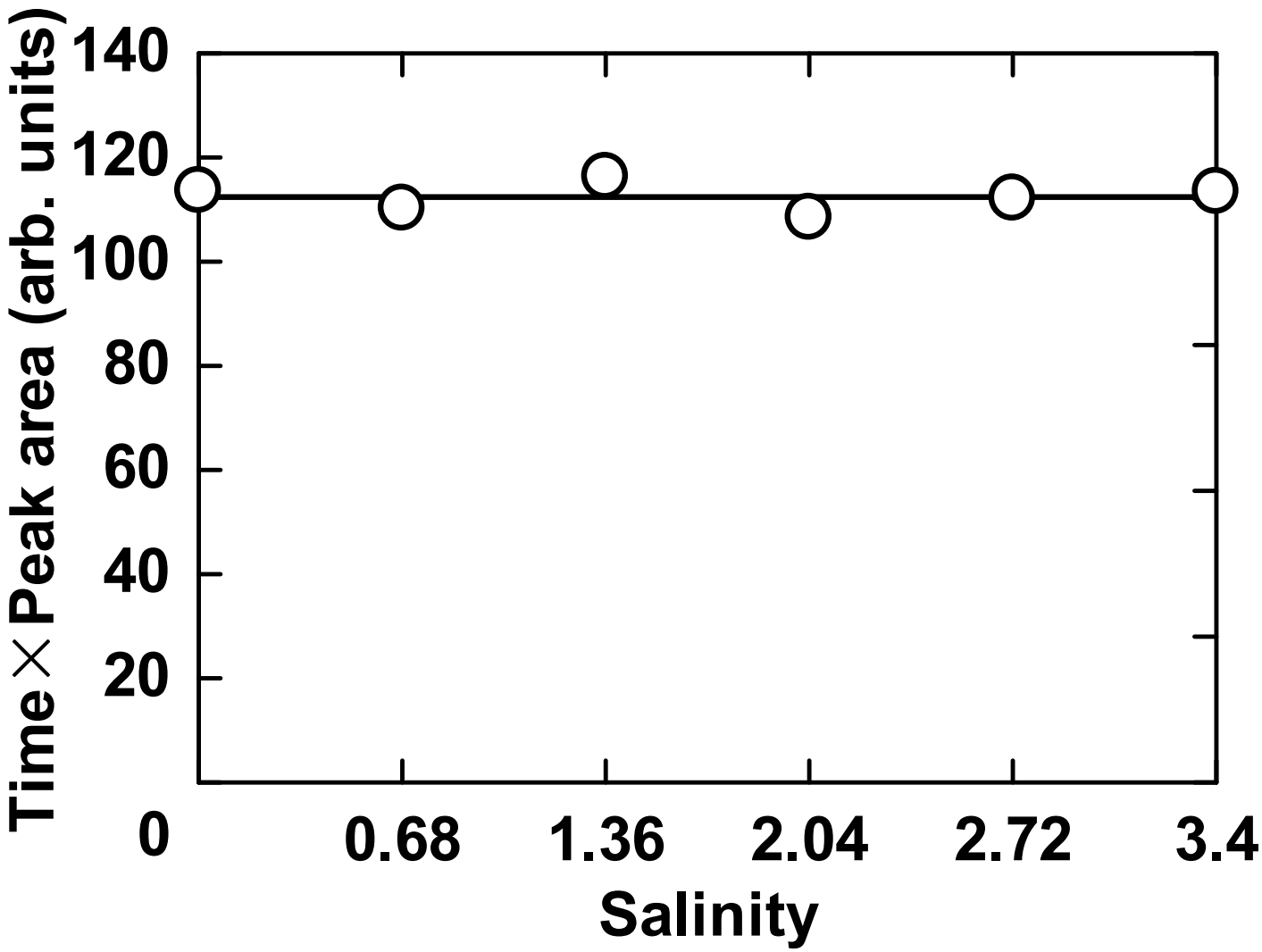
Time×Peak area (arb. units)
140
120
100
80
60
40
20
0
0.68
1.36
2.04
2.72
3.4
Salinity

Fig. 2

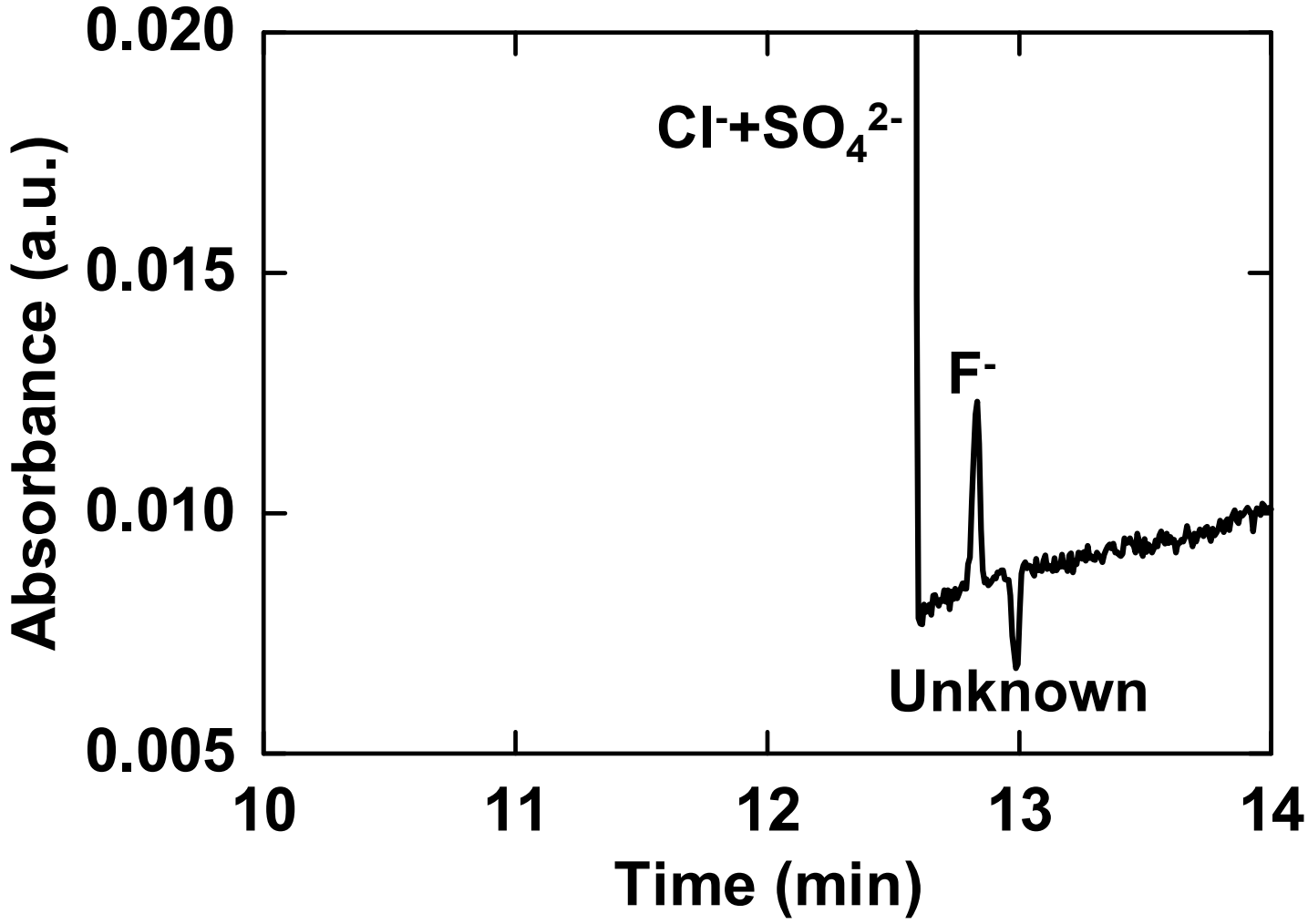
0.020
0.015
0.010
0.005
Absorbance (a.u.)
Cl-+SO4 2-
F-
Unknown
10
11
12
13
14
Time (min)

Fig. 3

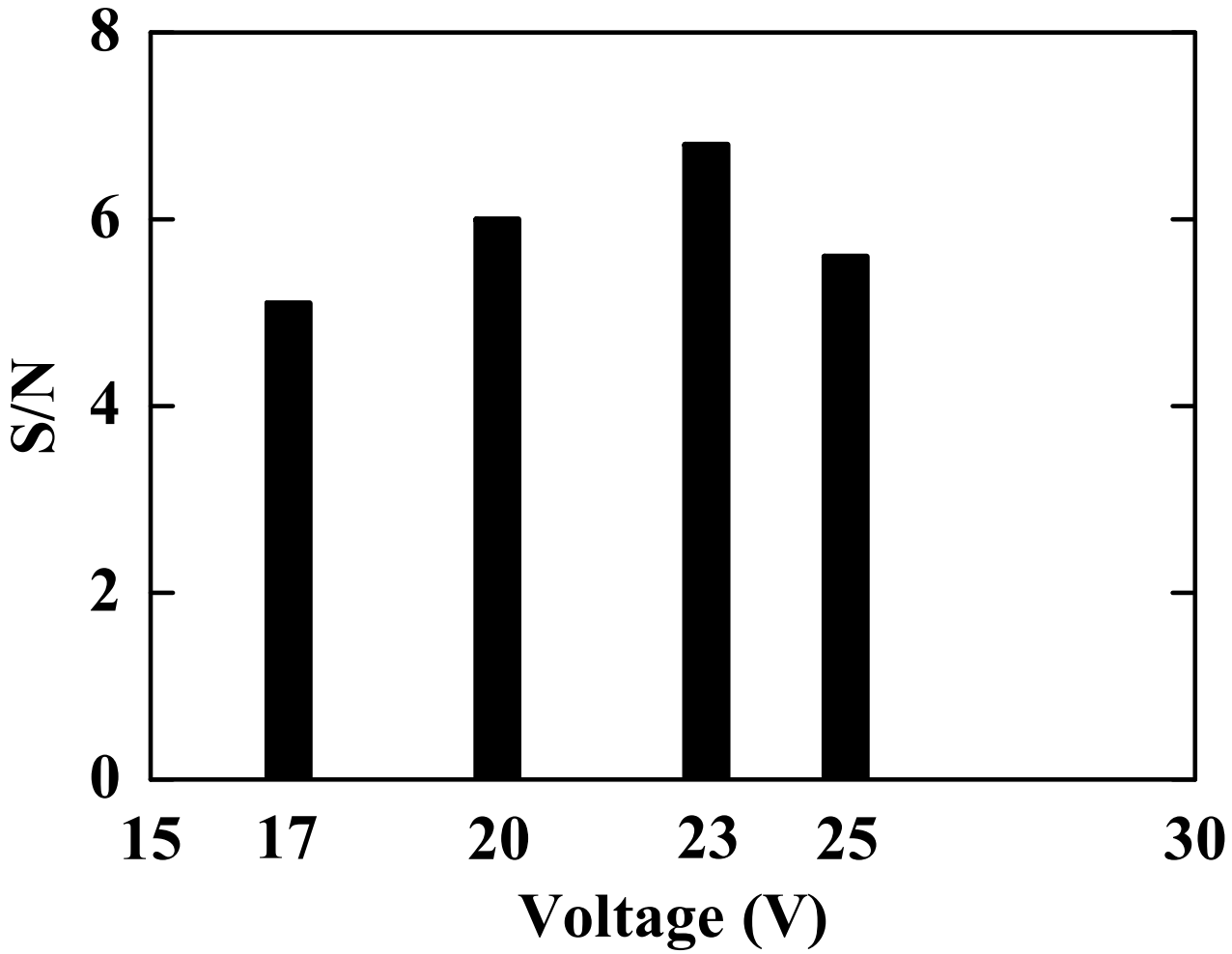

Fig. S1. Effect of applied voltage on S/N for F^-. Electrophoretic conditions: capillary, 87.4 cm total length (75 cm effective length) and 75 μm i.d. (375 μm o.d.); BGE, 5 mM 2,6-pyridinedicarboxylic acid (PDC) adjusted to pH 3.5 with 1 M NaOH containing 0.03% m/v hydroxypropyl methylcellulose (HPMC); voltage, 17–25 kV with the sample inlet side as the cathode; wavelength for detection, 200 nm; sample, 0.14 mg/L F^-/ten-fold diluted artificial seawater (ASW); vacuum injection period, 3 s (ca. 152 nL); two or three determinations for each voltage.

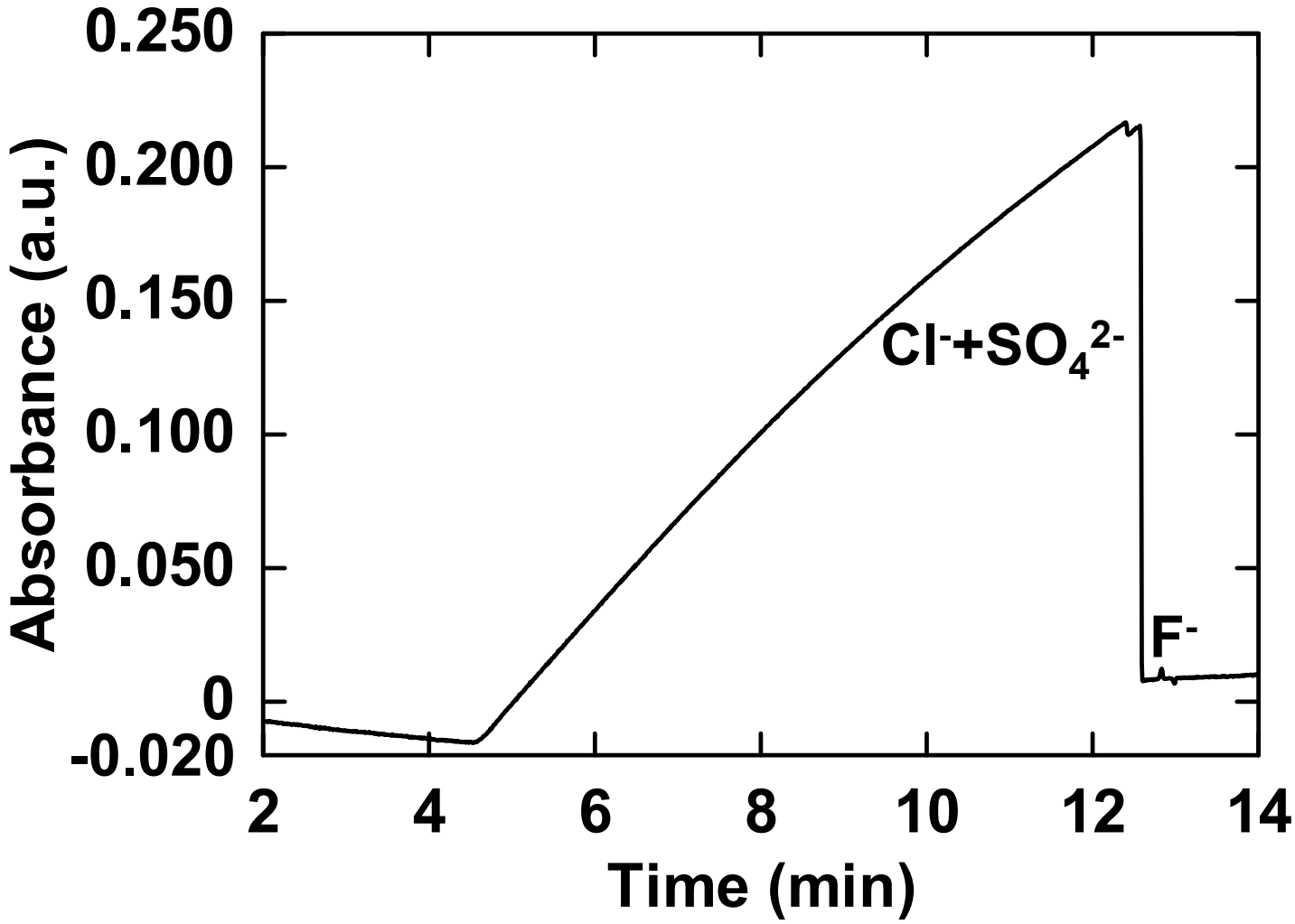

Fig. S2. Whole electropherogram of the surface seawater sample 11 (ten-fold diluted) in Table 2. Electrophoretic conditions: capillary, 87.4 cm total length (75 cm effective length) and 75 µm i.d. (375 µm o.d.); BGE, 5 mM 2,6-pyridinedicarboxylic acid (PDC) adjusted to pH 3.5 with 1 M NaOH containing 0.03% m/v hydroxypropyl methylcellulose (HPMC); voltage, 23 kV with the sample inlet side as the cathode; wavelength for detection, 200 nm; sample, ten-fold diluted seawater 11 (Table 2); vacuum injection period, 5 s (ca. 254 nL).