ELSEVIER

Journal of Chromatography A, 966 (2002) 205–212

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Determination of nitrate in seawater by capillary zone electrophoresis with chloride-induced sample self-stacking

Chuanhong Tu, Hian Kee Lee*

Department of Chemistry, National University of Singapore, 3 Science Drive 3, Singapore 117543, Singapore

Received 14 February 2002; received in revised form 19 April 2002; accepted 3 June 2002

Abstract

A capillary zone electrophoresis (CZE) method was established to determine low concentration nitrate which was online preconcentrated with chloride-induced leading-type sample self-stacking for seawater samples. The sample self-stacking was based on transient isotachophoresis in which chloride served as leading ion, and dihydrogenphosphate in the background electrolyte (0.1 *M* phosphate) as the terminating one. Due to the small mobility difference between nitrate and chloride, the isotachophoresis time was so long that nitrate could not separate from the rear sharp boundary between chloride and the background electrolyte (BGE) when it migrated to the detection window. A zwitterionic surfactant, 3-(*N*,*N*-dimethyldodecylammonio)propane sulfonate was added to the BGE to enlarge the mobility difference for its selective interaction with anions. Thus, a highly conductive sample could be injected in a large volume with about fourfold sensitivity enhancement compared to that of field amplification sample stacking in which nitrate was dissolved in pure water. The relative standard deviations ($n=5$) of migration time, peak area, peak height were 0.1, 3.0, 1.5%, respectively. The limit of detection ($S/N=3$) for nitrate was 35 μg/l in seawater samples with relatively low concentration BGE (0.1 *M* sodium phosphate, pH 6.2). The overall procedure consisting of online preconcentration and separation was as simple as routine CZE except for a slightly longer sample injection time (3–4 min).

Keywords: Sample stacking; Water analysis; Environmental analysis; Nitrate; Inorganic anions

1. Introduction

Many capillary electrophoresis (CE) approaches have been proposed for the determination of inorganic anions in various sample matrices for its high separation efficiency in comparison with ion chromatography (IC) [1–4]. However, for high conductivity samples such as seawater, the determination of minor ionic components is challenging because the mobile salts in the sample will induce electrodispersion, resulting in distortion and broadening of analyte peaks [5]. To overcome these problems, sample dilution has been used [6]. However, sample dilution causes direct sensitivity loss along with the decrease of matrix concentration. Some researchers have used high concentration background electrolytes (BGEs) [7–10]. Although they can alleviate electrodispersion, highly concentrated BGEs lead to high electrical current and excessive Joule heating which are not favorable in CE.

*Corresponding author. Tel.: +65-6874-2995; fax: +65-6779-1691.

E-mail address: chmleehk@nus.edu.sg (H.K. Lee).

0021-9673/02/$ – see front matter
PII: S0021-9673(02)00744-6

Another problem often encountered in inorganic anionic analysis by CE is the low concentration sensitivity due to the short pathlength with online UV detection and the low extinction coefficients of analyte anions [11]. Recently, Breadmore and Haddad reviewed sensitivity enhancement techniques for the determination of inorganic and small organic anions by CE [12]. For a sample in a low conductivity matrix, field amplified sample stacking or field amplified electrokinetic injection can be used to improve the sensitivity [13–16]. Okemgbo et al. determined nitrate and nitrite in a low concentration sample matrix with rapid reverse polarity capillary zone electrophoresis (CZE). The detection limits were 271 n*M* nitrite and 143 n*M* nitrate using on-column sample stacking [17]. Field amplified sample stacking requires a considerable conductivity difference between the BGE and the sample. To preconcentrate trace anions in seawater, the BGE concentration will be unacceptably high.

One possible method for preconcentrating analyte ions in a high concentration ionic matrix is the use of sample self-stacking [5,18,19]. This can be realized by creating a transient isotachophoresis step in the initial state of a CZE separation. The native matrix ion or intentionally added ion can function as a leading or terminating ion and the co-ion of BGE as the other part of isotachophoresis. Gebauer and coworkers have described the criteria for both leading- and terminating-type sample self-stacking [20–22].

Theoretically, the analytes with mobilities ranging between those of the major sample matrix ion and the BGE co-ion can be preconcentrated by this method. However, when the mobility difference between the matrix ion and analyte ions is small, the transient isotachophoresis time is so long that the analytes are very close to the matrix ions at the detection window and cannot be detected as individual peaks [5,20].

In seawater of 35‰ salinity, the chloride concentration is about 0.56 *M* with high mobility [23]. It is easy to find a BGE with slow co-ion to satisfy the transient isotachophoresis conditions for nitrate using chloride as leading ion. However, to our knowledge, no one has reported chloride-induced leading-type sample self-stacking for the determination of nitrate in seawater due to the above-mentioned reason. Recently, Fukushi et al. determined nitrite and nitrate in seawater with artificial seawater as BGE, with high concentration chlorate added into sample to induce terminating-type sample self-stacking. The sensitivity was improved by twofold [10]. However, highly conductive artificial seawater used as BGE resulted in high current and excessive Joule heating.

The aim of this study is to establish a CE method to determine trace nitrate in seawater with a relatively low concentration BGE. Nitrate is preconcentrated online using chloride-induced leading-type sample self-stacking. To overcome the problem of excessive transient isotachophoresis time due to the small mobility difference between chloride and nitrate, a zwitterionic surfactant was added into the BGE for its selective interaction with anions [24]. Thus, high concentration chloride in seawater sample does not interfere with the determination of nitrate, but functions as a leading ion in the transient isotachophoresis for the preconcentration of nitrate.

2. Experimental

2.1. Chemicals

Sodium nitrite, nitrate, chloride and bromide were purchased from Merck (Darmstadt, Germany). All standards and buffers were prepared in 18 MΩ ultrapure water obtained from a Nanopure water purification system (Barnstead, Dubuque, IA, USA). Zwitterionic surfactant 3-(*N*,*N*-dimethyldodecylammonio)propane sulfonate (DDAPS) was a gift from Raschig (Ludwigshafen, Germany).

2.2. Apparatus and procedures

All experiments were performed on a HP-3D CE system (Hewlett-Packard, Waldbronn, Germany) equipped with a diode array detection (DAD) system. The detection wavelength for nitrate was set at 210 nm according to the spectrum obtained with DAD. For monitoring chloride, 195 nm was used. Data were collected and processed with HP Chem Station software.

Untreated 50 μm I.D. fused-silica capillary was supplied by Polymicro Technologies (Phoenix, AZ, USA). Filling the total length (L_T) of a 64.5-cm capillary tubing (56 cm effective length) at 50 mbar

took 1022 s. New capillaries were flushed successively with 1.0 *M* NaOH for 15 min, 0.1 *M* NaOH for 10 min and finally water for 5 min. Between runs, the capillary was flushed with 0.1 *M* NaOH for 2 min, BGE for 5 min. The capillary temperature was maintained at 20 °C. The BGE consisted of 0.1 *M* sodium phosphate, 150 m*M* DDAPS (pH 6.2). All measurements were performed at a constant voltage of −25 kV, with a current of −88 μA.

2.3. Mobility measurements

Dimethyl sulfoxide (DMSO) was used as the neutral electroosmotic flow (EOF) marker. Because the zwitterionic surfactant in BGE can reduce EOF, it took considerable time for the neutral marker to reach the detection window. Since the distance between outlet and detection window was 8.5 cm, much shorter than the effective length of capillary, the DMSO (dissolved in the BGE) was injected at the outlet after sample was injected at the inlet. Once the high voltage of −25 kV was applied, the DMSO was carried to the detection window by the EOF. The anionic analytes migrated to the detection window from the inlet under the influence of the high electrical field. Thus, in one electrophoresis run, the migration times of both EOF and analytes were obtained. The EOF mobility and apparent mobilities of analytes were calculated with the following equations:

$$\mu_{EOF} = -L_T \cdot (L_T - L_{EFF})/tV \quad (1)$$

$$\mu_{APP} = L_T L_{EFF}/tV \quad (2)$$

where L_T and L_{EFF} are the total and effective lengths of the capillary, respectively; t is the migration time of the EOF marker or analytes and V is the applied voltage. The anionic analytes migrate in the reverse direction to the EOF, so the effective mobilities of analytes were calculated using the equation:

$$\mu_{EFF} = \mu_{APP} - \mu_{EOF} \quad (3)$$

2.4. Sample collection and pretreatment

The seawater was collected from the western coast of Singapore, 2 m offshore. Samples were stored under 4 °C prior to analysis. Before analysis, the sample was filtered through a 0.45 μm membrane filter. The standard addition method was used for quantitation, to overcome the influence of the variation of chloride concentration in the sample.

3. Results and discussion

3.1. Chloride-induced leading-type sample self-stacking

Sample self-stacking may occur if the analyte transiently migrates in the stack within the sharp boundary between the major sample matrix ion and the co-ion of the BGE. In leading type sample self-stacking, the major sample matrix ion is the ion with the highest mobility, and the co-ion of the BGE is the least mobile one. The minor analyte ion will be stacked at the sharp rear boundary of the sample matrix. In terminating-type sample self-stacking, the converse situation applies. The front boundary is sharp, and analyte ions will be stacked at the front boundary.

In seawater of 35‰ salinity, chloride concentration is about 0.56 *M* [23]. Its mobility in 0.1 *M* sodium dihydrogenphosphate (pH 6.2) is $79.4 \cdot 10^{-5}$ $cm^2\ V^{-1}\ s^{-1}$. Nitrate occurs in very low concentration of less than 1 mg/l with a mobility of $67.2 \cdot 10^{-5}$ $cm^2\ V^{-1}\ s^{-1}$, so leading-type sample self-stacking will be expected for the preconcentration of nitrate with dihydrogenphosphate as co-ion of the BGE. Leading-type sample self-stacking has at least two advantages over the terminating-type for minor analytes in seawater. Firstly, there is no deliberate need to add additional ions to the sample to induce transient isotachophoresis. Thus, the chance of introducing contaminant will be much reduced or eliminated. Secondly, the co-ion of the BGE functions as terminating ion whose mobility is the lowest; thus, the current for the CZE will be lower, obviating excessive Joule heating.

Due to the small mobility difference between chloride and nitrate, the transient isotachophoresis time for nitrate in the sharp boundary between chloride and the co-ion of BGE is considerably long. According to Boden and Bächmann [5]:

$$t_{ITP} = L_0 \kappa_S (\mu_M - \mu_E)/[i(\mu_M - \mu_A)^2] \quad (4)$$

where t_{ITP} is the time of transient isotachophoresis, κ_S is the conductivity of sample zone, i is the current, μ_A, μ_E and μ_M are the mobilities of the analyte ion, the co-ion of BGE and the matrix ion, respectively. L_0 is the length of sample zone. Fig. 1A shows the electropherograms of 0.4 ppm nitrate in 0.2*M* NaCl solution with 0.1 *M* sodium phosphate (pH 6.2) as BGE. When nitrate migrates to the detection window, it is within the sharp boundary between the chloride and the BGE co-ion, and no separated peak can be detected. EOF for the BGE without DDAPS is threefold higher than in the presence of DDAPS. The migration direction of anionic analyte is against the direction of EOF, the separation time is much longer in the former situation due to the larger EOF, but the nitrate cannot be separated from the sharp rear boundary of chloride. On the other hand, in the presence of 0.15 *M* DDAPS in the BGE, nitrate can be separated from chloride boundary due to the larger mobility difference, as Fig. 1B shows.

3.2. Effect of DDAPS concentration on mobility and sample self-stacking

DDAPS is a zwitterionic surfactant with oppositely charged functional groups in close juxtaposition in its hydrophilic part. It can selectively interact with anions like in electrostatic ion chromatography (EIC) due to a few proposed mechanisms [24–28]. As a consequence, this interaction can selectively control the mobilities of anionic analytes [24]. In addition, DDAPS can suppress EOF, which can also be used to improve separation efficiency in CZE [29]. Fig. 2 shows the influence of DDAPS on the mobilities of nitrate, nitrite, chloride and EOF in 0.1 *M* sodium phosphate (pH 6.2). The mobilities of the ions decreased with the increasing of concentration of DDAPS at different rates. When DDAPS concen-

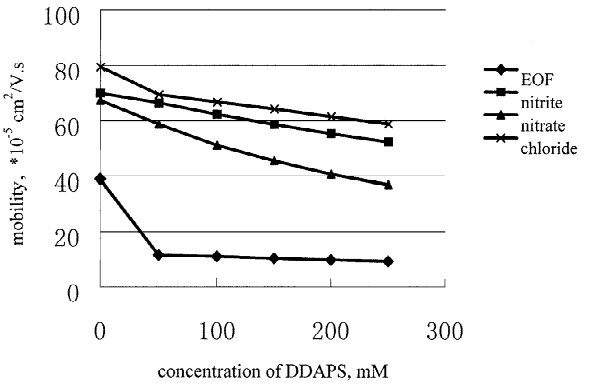

Fig. 2. Effect of DDAPS on mobility of anions and EOF. Experimental conditions: BGE: 0.1 *M* sodium phosphate (pH 6.2) with various concentrations of DDAPS.

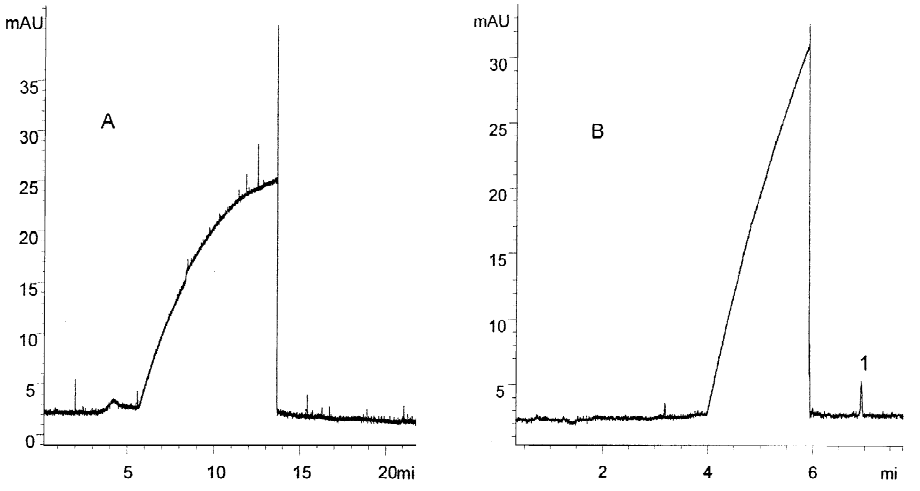

Fig. 1. Chloride-induced leading-type sample self-stacking. Peak 1: nitrate. The broad peak with sharp rear boundary is chloride. Sample: 0.4 mg/l nitrate in 200 m*M* NaCl. BGE: (A) 0.1 *M* sodium phosphate, pH 6.2; (B) 0.1 *M* sodium phosphate, pH 6.2 with 0.15 *M* DDAPS. Detection wavelength 195 nm. Injection 50 mbar, 100 s (124 nl). Separation voltage −25 kV.

tration reached 150 m*M* in BGE, the mobility difference between chloride and nitrate reached $18.7 \cdot 10^{-5}$ cm^2 V^{-1} s^{-1} in comparison with $12.2 \cdot 10^{-5}$ cm^2 V^{-1} s^{-1} in the absence of DDAPS. Transient isotachophoresis time was shortened according to Eq. (4). Thus, nitrate could migrate from the sharp boundary between the chloride and the co-ion (dihydrogenphosphate) of the BGE when it reached the detection window. This is shown in Fig. 1B. At 250 m*M* DDAPS, the mobility of nitrate decreased further to approach the effective mobility of the co-ion (dihydrogenphosphate) in the BGE. The conditions for sample self stacking were therefore violated, resulting in the broadening of the nitrate peak (data not shown).

3.3. Effect of BGE concentration

In sample self-stacking, not only must the mobility of the BGE co-ion satisfy the condition of the terminating or leading ion, but its concentration also plays an important role in the preconcentration process. At the initial state of transient electrophoresis, the sample concentration is readjusted to the conditions of BGE according to the Kohlrausch regulating function [20]. In principle, a higher BGE concentration leads to a higher preconcentration factor. On the other hand, from Eq. (4), the isotachophoresis time is inversely proportional to the electrical current which is determined by the concentration of the BGE. A higher BGE concentration produces a larger electrical current and shorter isotachophoresis time, so the analytes will migrate in CZE mode for a longer time before it reaches the detection window, resulting in a more diffuse sample zone. The BGE concentration was increased from 0.05 to 0.1 *M*, the peak height of nitrate increased by twofold. The peak height reached its maximum at 0.1 *M* BGE. Further increasing the BGE concentration caused larger electrical current, shorter isotachophoresis time, greater Joule heating, and loss of sensitivity.

3.4. Influence of chloride concentration

The chloride in the sample functions as a leading ion in the transient isotachophoresis system. The concentration of chloride determines the specific conductivity of the sample zone. According to Eq. (4), a higher chloride concentration in the sample zone results in a longer isotachophoresis time. During transient isotachophoresis, the analyte zone is almost free of diffusion due to electromigrational sharpening effect [20]. When the isotachophoresis condition does not exist any more, the analyte migrates in the BGE under CZE mode, and the analyte zone will broaden due to the diffusion. Longer isotachophoresis time results in a shorter CZE migration time, less diffusion and higher sensitivity. Fig. 3 shows the influence of chloride concentration on the peak height of nitrate. With the same injection volume, the peak height increases with the chloride concentration in the sample due to a longer isotachophoresis time. The sensitivity could be enhanced about fourfold in the presence of 200 m*M* NaCl compared to that when nitrate was dissolved in pure water, in which field amplification sample stacking took place.

It should be noted that the migration time interval between the analytes and the sharp rear boundary of the chloride decreases with chloride concentration. This is especially obvious for nitrite because of its small mobility difference with chloride. In the presence of 10 m*M* NaCl in the sample, nitrite appears close to the rear boundary of chloride (see Fig. 4B). The peak height is about threefold higher than that in pure water. When chloride concentration is increased

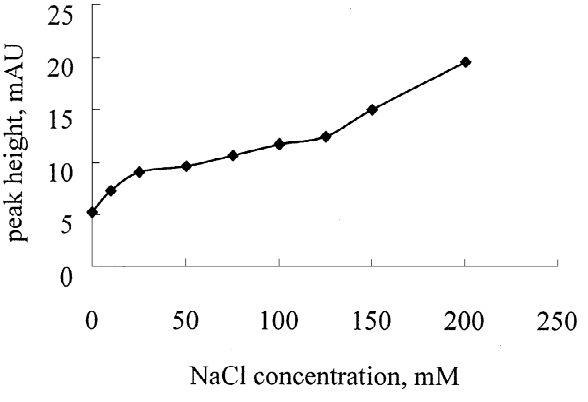

Fig. 3. The effect of NaCl concentration in sample on nitrate peak height. Experimental conditions: BGE: 0.1 *M* sodium phosphate, pH 6.2, 0.15 *M* DDAPS. Sample: 0.4 μg/l nitrate in various concentrations of NaCl. Injection 50 mbar, 200 s (248 nl). Separation voltage −25 kV. Detection wavelength 210 nm.

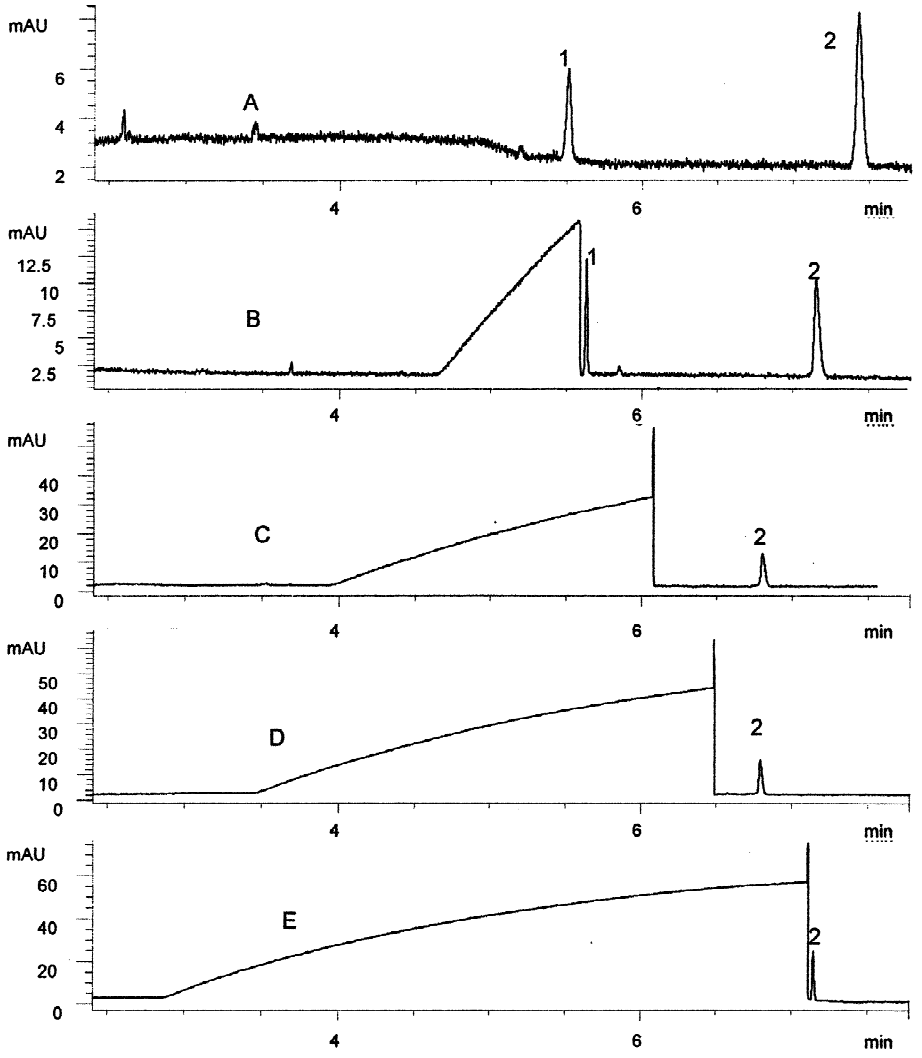

Fig. 4. The effect of chloride concentration on migration time of analytes. Peaks: 1=nitrite; 2=nitrate. The broad peak with sharp rear boundary is chloride. Experimental conditions: sample: 0.4 mg/l nitrite and nitate in: (A) pure water, (B), 10 m*M*, (C) 50 m*M*, (D) 100 m*M*, (E) 200 m*M* NaCl. Detection wavelength 195 nm.

to 25 m*M*, the nitrite cannot be separated from the chloride sharp boundary. Therefore, this method cannot be used to determine nitrite in the presence of a high concentration of chloride.

Another influence of chloride was in the variation of migration time. As shown in Fig. 4, the presence of chloride in sample resulted in shorter nitrate migration time. In their computer simulation, Gebauer et al. showed that the migration times of analyte ions increased with the sample matrix concentration for leading-type sample self stacking [20]. This is different from our results. This difference comes from the injection length and EOF. In Gebauer et al.'s model, the injection volume was 3% of the total capillary volume, and the EOF was assumed to be zero. In our experiment, the injection volume (248 nl) was 20% of the capillary volume, with suppression of the EOF. The effect of sample length was not negligible. The electrical field distribution between the sample zone and the BGE

should be taken into account for the prediction of the migration time. Before the sample matrix zone was pushed out of the capillary by the EOF, the specific conductivity of the sample zone was lower than that of the BGE in case of low concentration of chloride in the sample. The electrical field strength distributed across BGE zone was weaker, resulting in lower migration velocity and longer migration time.

It is worth noting that the concentration of chloride in the sample is correlated to the maximum injectable volume. According to Eq. (4), both injection volume and chloride concentration influence the transient isotachophoresis time. A longer injection time resulted in more analytes being introduced, a longer isotachophoresis time, and so a greater peak height, but nitrate could not be separated from the boundary between chloride and the co-ion of the BGE at the detection window. For a sample with high concentration of chloride, a satisfactory electropherogram can be obtained with a smaller injection volume. Fig. 5 shows an electropherogram of filtered seawater analyzed without any dilution.

3.5. Determination of nitrate in seawater sample

The major anions in seawater of 35‰ salinity are chloride (ca. 0.56 *M*), sulfate (0.0114 *M*), hydrogencarbonate (0.00143 *M*), and bromide (67 mg/l).

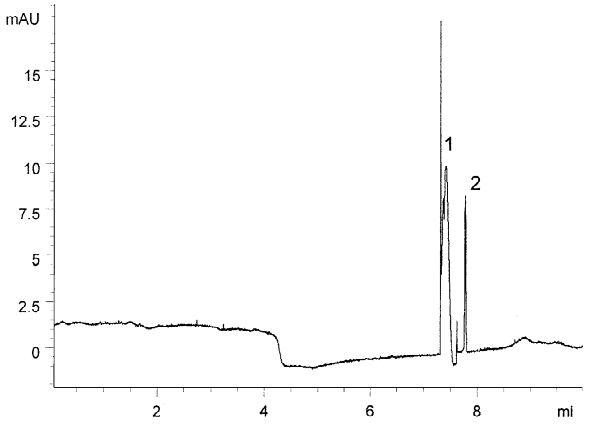

Fig. 5. The electropherogram of undiluted seawater. Peaks: 1 = bromide, 2 = nitrate. Sample: filtered seawater, injection 50 mbar, 60 s (74 nl). BGE: 0.1 *M* sodium phosphate, pH 6.2, 0.15 *M* DDAPS. Other conditions as in Fig. 3.

The minor anions are borate, fluoride and nitrite and nitrate at mg/l or μg/l levels according to the literature [23]. The chloride concentration in our sample was roughly 0.46 *M* measured by titration with silver nitrate. To overcome the peak height changes due to the possible chloride concentration variation in the sample, standard addition was used for nitrate quantitation. The sample was diluted in 2.5-fold with water. 100, 200, 400, 800 μg/l nitrate were spiked into the samples. Both peak areas (*A*) and heights (*H*) were linear with the concentrations (*c*). The calibration equations for areas and heights were $A = 0.0241c + 5.82$ ($r^2 = 0.9983$) and $H = 0.0139c + 3.30$ ($r^2 = 0.9997$), respectively. The standard deviations for the slopes of the calibration curves with areas and heights were $1.13 \cdot 10^{-7}$ and $0.22 \cdot 10^{-7}$, respectively, while for intercepts were $19.2 \cdot 10^{-3}$ and $3.7 \cdot 10^{-3}$, respectively. The determined concentration of nitrate in seawater sample was 0.60 mg/l. The relative standard deviations ($n = 5$) in term of migration time, peak area and height were 0.1, 3.0, and 1.5%, respectively. The limit of detection (LOD) at $S/N = 3$ was 35 μg/l, close to the naturally occurring concentration of nitrate in seawater [23], which is comparable with the results of terminating type sample self-stacking [10], but with a relatively low concentration BGE.

The mobility of bromide was also less than that of chloride in the presence of DDAPS in the BGE (data not shown). Thus, bromide in seawater could also be determined simultaneously with this method (as Fig. 5 shows). The enrichment using sample self stacking was not necessary due to the relatively high concentration of this anion in seawater, although the sample self stacking did occur in the present case.

4. Conclusion

Zwitterionic surfactant DDAPS was used to enlarge the mobility difference between chloride and nitrate, so that leading-type sample self-stacking could be employed to preconcentrate low concentration nitrate in seawater using native chloride in the sample as the leading ion, and the co-ion in the BGE as terminating ion. A detection limit of nitrate of 35 μg/l was achievable for seawater with relatively low concentration BGE. At the concentration of the

LOD, the mole ratio between the matrix and the analyte was around 10^6:1.

Acknowledgements

The authors thank the National University of Singapore for financial support of this work. C.H.T. gratefully acknowledges the award of a research scholarship from the University.

References

[1] G. Bondoux, P. Jandik, W.R. Jones, J. Chromatogr. 602 (1992) 79.
[2] T.G. Huggins, J.D. Henion, Electrophoresis 14 (1993) 531.
[3] P. Kuban, B. Karlberg, Anal. Chem. 70 (1998) 360.
[4] V. Pacakova, K. Stulik, J. Chromatogr. A 789 (1997) 169.
[5] J. Boden, K. Bächmann, J. Chromatogr. A 734 (1996) 319.
[6] K. Fukushi, K. Watanabe, S. Takeda, S.I. Wakida, M. Yamane, K. Higashi, K. Hiiro, J. Chromatogr. A 802 (1998) 211.
[7] A.R. Timerbaev, T. Takayanagi, S. Motomizu, Anal. Commun. 36 (1999) 139.
[8] W.L. Ding, M.J. Thornton, J.S. Fritz, Electrophoresis 19 (1998) 2133.
[9] M.I.T. Carou, P.L. Mahia, S.M. Lorenzo, E.F. Fernandez, D.P. Rodriguez, J. Chromatogr. Sci. 39 (2001) 397.
[10] K. Fukushi, N. Ishio, M. Sumida, S. Takeda, S. Wakida, K. Hiiro, Electrophoresis 21 (2000) 2866.
[11] S.A. Shamsi, N.D. Danielson, Anal. Chem. 66 (1994) 3757.
[12] M.C. Breadmore, P.R. Haddad, Electrophoresis 22 (2001) 2464.
[13] M. Albert, L. Debusschere, C. Demesmay, J.L. Rocca, J. Chromatogr. A 757 (1997) 291.
[14] Y. He, H.K. Lee, Anal. Chem. 71 (1999) 995.
[15] R.L. Chien, D.S. Burgi, Anal. Chem. 64 (1992) 489A.
[16] K. Li, S.F.Y. Li, Analyst 120 (1995) 361.
[17] A.A. Okemgbo, H.H. Hill, W.F. Siems, Anal. Chem. 71 (1999) 2725.
[18] F. Foret, E. Szoko, B.L. Karger, J. Chromatogr. 608 (1992) 3.
[19] F. Foret, E. Szoko, B.L. Karger, Electrophoresis 14 (1993) 417.
[20] P. Gebauer, W. Thormann, P. Bocek, J. Chromatogr. 608 (1992) 47.
[21] P. Gebauer, W. Thormann, P. Bocek, Electrophoresis 16 (1995) 2039.
[22] P. Gebauer, L. Krivankova, P. Pantuckova, P. Bocek, W. Thormann, Electrophoresis 21 (2000) 2797.
[23] M.J. Kennish, in: Practical Handbook of Marine Science, CRC Press, Boca Raton, FL, 2001, p. 60.
[24] M.A. Woodland, C.A. Lucy, Analyst 126 (2001) 28.
[25] W. Hu, H. Tao, H. Haraguchi, Anal. Chem. 66 (1994) 2514.
[26] W. Hu, Langmuir 15 (1999) 7168.
[27] J.M. Patil, T. Okada, Anal. Commun. 36 (1999) 9.
[28] H.A. Cook, W. Hu, J.S. Fritz, P.R. Haddad, Anal. Chem. 73 (2001) 3002.
[29] K.K.C. Yeung, C.A. Lucy, Anal. Chem. 69 (1997) 3435.