Reverse Polarity Capillary Zone Electrophoretic Analysis of Nitrate and Nitrite in Natural Water Samples

Asopuru A. Okemgbo,¹ Herbert H. Hill,* and William F. Siems

Department of Chemistry, Washington State University, P.O. Box 644630, Pullman, Washington 99164-4630

Steven G. Metcalf
Numatec Hanford Company, P.O. Box 1300, Richland, Washington 99352

This paper describes the application of reverse polarity capillary zone electrophoresis (CZE) for rapid and accurate determination of nitrate and nitrite in natural water samples. Using hexamethonium bromide (HMB) as an electroosmotic flow modifier in a borate buffer at pH 9.2, the resolution of nitrate and nitrite was accomplished in less than 3 min. CZE was compared with ion chromatographic (IC) and cadmium reduction flow injection analysis (Cd-FIA) methods which are the two most commonly used standard methods for the analysis of natural water samples for nitrate and nitrite. When compared with the IC method for the determination of nitrate and nitrite, CZE reduced analysis time, decreased detection limits by a factor of 10, cut laboratory wastes by more than 2 orders of magnitude, and eliminated interferences commonly associated with IC. When compared with the cadmium reduction method, CZE had the advantage of simultaneous determination of nitrate and nitrite, of being able to be used in the presence of various metallic ions that normally interfere in cadmium reduction, and of decreased detection limits by a factor of 10.

Many methods have been used for the determination of nitrate and nitrite in natural water samples. Most important are spectrophotometric, cadmium reduction, and ion chromatographic (IC) methods. Spectrophotometric methods often involve direct measurement of nitrate and nitrite absorption at wavelengths of 210 nm, 302 nm, and 355 nm.¹ Reduction of nitrate to nitrite, and further conversion into an azo dye coupled with aromatic amines has also been used.² Colorimetric methods, however, are plagued with many interferences such as dissolved organic matter and chromium (VI). Another disadvantage of the azo derivatization of nitrite is that precipitation can occur under test conditions when Sb³⁺, Au³⁺, Bi³⁺, Fe³⁺, Pb²⁺, Hg²⁺, and Ag⁺ ions are present in a sample. The presence of Cu²⁺ ion may catalyze the decomposition of the diazonium salt, and hence, cause low results.

Cadmium reduction flow injection analysis (Cd-FIA) and ion chromatographic methods are the commonly used standard methods for the examination of seawater and wastewater.³–⁶ When operating under optimum conditions, the cadmium reduction method has a detection limit of 10 ppb N for both nitrate and nitrite, with a common analytical operating range of 10 ppb to 1 ppm. Although the cadmium reduction method has been automated and can analyze samples every 2–3 min, the column washing and preconditioning procedures are still tedious and time-consuming. Like the spectrophotometric methods, the cadmium reduction method is matrix sensitive. It may not be used for samples containing high levels of copper, iron, and other metallic ions in solution which lower the efficiency of the reduction process. A primary disadvantage of cadmium reduction is that the volume of hazardous waste generated is high and the cadmium in the solvent waste must be eliminated before waste discharge. For analyses requiring the determination of nitrate as well as nitrite, the sample has to first be analyzed for nitrite omitting the reduction step and then repeated for nitrate via the reduction.

Ion chromatography is the method recommended by the US–EPA for nitrate and nitrite.⁷ The analysis time for suppressed conductivity detection of nitrate and nitrite is less than 5 min, with detection limits of 20 ppb N and 40 ppb N for nitrate and nitrite, respectively. The analytical operating ranges for nitrate and nitrite are 0.42–14.0 ppm N and 0.36–12.0 ppm N, respectively. For samples containing high chloride concentrations, such as seawater, the chloride ion interferences with nitrite.⁸ Moreover, the oxidation of nitrite to nitrate occurs under the acidic conditions of the suppressor column.⁹ As with the cadmium reduction

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¹ Current address: Washington State University Tri-Cities, 2710 University Drive, Richland, Washington 99352.
² To whom correspondence should be addressed. (E-mail: hhhill@wsu.edu or FAX: 509-335-8867).
(7) EPA Method 300.0 Determination of Inorganic Anions by Ion Chromatography. United States Environmental Protection Agency, Cincinnati, OH, 1993; p 300.0.
method, the IC method also generates high volumes of wastes. In today's environmental analytical applications, there is a critical need to find and demonstrate technologies that will result in waste minimization and waste avoidance while significantly reducing overall costs. In this study, reverse polarity capillary zone electrophoresis was investigated as an analytical method for nitrate and nitrite that would be suitable for the rapid monitoring of natural waters and aquifers.

Several investigators have reported the use of capillary zone electrophoresis with direct\textsuperscript{15-17} and indirect\textsuperscript{15-21} UV and suppressed conductivity\textsuperscript{21} detection for the analysis of nitrate and nitrite in aqueous and other samples. Amran and co-workers\textsuperscript{12} optimized the analysis of anions by direct detection at 200 nm, in a pH 3 phosphate buffer, separating nitrate ions in about 4 min. They also observed that the presence of chloride interfered with the quantification of the nitrate peak for samples with high chloride content.

Romano et al.\textsuperscript{15} and Jones and Jandik\textsuperscript{16,17} used an indirect detection method at 254 nm, with Nice-Pak OFM Anion BT electroosmotic flow modifier and sodium chromate as electrolyte at pH 8.0, for the separation of anions. The modifier added to the electrolyte caused the electroosmotic flow to move in the same direction as that of the anions, under negative voltage polarity configuration. Poor peak shapes were observed for fluoride and phosphate due to the mismatch of the electrolyte mobility with the mobilities of these ions. The investigators showed that the electroosmotic modifier underwent ion pairing with bromide, nitrate, and sulfate, and hence, lead to a decrease in their mobilities as the osmotic flow modifier (OFM) was increased. Li and Li\textsuperscript{18} also used chromate buffer as indirect UV background for the detection of nitrate and nitrite with cetyltrimethylammonium bromide (CTAB) as electroosmotic flow modifier to reduce and reverse the electroosmotic flow. The major disadvantages of this indirect detection method are the high detection limit and narrow dynamic range of only 2–50 ppm.\textsuperscript{17}

Kelly and Nelson\textsuperscript{19} used 1,2,4,5-benzenetetracarboxylic acid (pyromellitic acid, PMA) as an absorbing background electrolyte for anions at 205 nm. They used diethylenetriamine (DETA) to slow the electroosmotic flow at pH 9.6. Separation of anions was best achieved at 60 °C, with possible comigration of nitrate/sulfate at low temperatures and nitrate/oxalate at 50 °C. Shamsi and Danielson\textsuperscript{20} used naphthalenesulfonate as an electrolyte for indirect photometric detection of anions, organic acids, and surfactants. Borate buffer with DETA as an electroosmotic modifier was also used at pH 8. The detection limit range for the anions was 80–350 ppb. For nitrate and nitrite, detection limits were 110 and 125 ppb, respectively.

Limitations associated with spectrophotometric methods, FIA, IC, and some of the CZE methods for nitrate and nitrite can be summarized as interferences by both cations and anions, high detection limits, narrow dynamic ranges, and poor separation efficiencies. The overall objective of this project, therefore, was to develop a simple and rapid method based on reverse polarity capillary zone electrophoresis which can be used for routine determination of nitrate and nitrite in natural water. In the CZE method, negative voltage polarity was applied at the injection position, and the electroosmotic flow, which ran opposite to the direction of electrophoretic mobility of anions, was reduced by adding an electroosmotic flow modifier, hexamethonium bromide (HMB), to the buffer. The method was, therefore, anion specific. Cations and neutrals were not detected. The specific objectives of this project were to determine the operating conditions that would provide the best combination of low detection limits, a wide dynamic range, reduced waste generation, no cation interferences, and minimal anion interferences for the direct UV detection of the nitrate and nitrite. In addition, the concentrating effects of stacking under prolonged injection times, in both gravity and pressure injection methods, were examined as a means to decrease detection limits. Finally, determination of nitrate in natural water samples for limnological studies of the Lower Snake River\textsuperscript{22} using cadmium reduction and ion chromatography was compared to the novel CZE method. Nitrate level in domestic well water was also measured with the CZE method.

**EXPERIMENTAL SECTION**

**Apparatus:** A Dionex Capillary Electrophoresis System (CES) I. (Dionex Corporation, Sunnyvale, CA) was used for these studies. This instrument was equipped with positive and negative polarity 30-kV power supplies and an on-column UV–vis absorbance detector. The CE system was interfaced to a Dionex AI-450 Laboratory Automation System with a Dionex Advanced Computer Interface (ACI) and was operated in the remote function. The AI-450 was used for data acquisition, data storage, and data reduction. Ion Chromatography experiments were carried out using a Dionex IC, (Dionex Corporation) equipped with suppressed conductivity and UV detectors. A Technicon AutoAnalyzer II (Technicon Instruments Industrial Systems, Tarrytown, NY), with a 520-nm filter, a 15-mm flow cell, S-10 photo tubes, and a 30 sample/hr cam was used for the cadmium reduction analysis.

**Reagents and Chemicals.** Sodium nitrate (Analytical Reagent, ACS Specifications) was obtained from Mallinckrodt Chemical Works, St. Louis, MO, and sodium nitrite (Baker Analyzed Reagent, ACS Specifications) was from J. T. Baker, Inc., Phillipsburg, NJ. Sodium nitrate and sodium nitrite were dried overnight at 110 °C, cooled and stored in a desiccator. Stock solutions of nitrate and nitrite containing 1000 mg/L (as nitrate nitrogen, and nitrite nitrogen) were made from the dried sample. Sodium borate obtained from Matheson Coleman & Bell, Norwood, OH, and hexamethonium bromide from ICN Biochemicals, Inc., Cleveland, OH, were used to prepare the buffer. Phthalonitrile, used as the...
neutral marker for determining the electroosmotic flow was obtained from Chem Service, West Chester, PA. The reagent water was from an 18 MΩ Nanopure UV ultrapure water system (Barnstead/Thermolyne, Dubuque, IA) and was used for the preparation of all standards and buffer solutions.

**Procedures.** General CZE operating conditions. In the CZE experiments, the electrolytes used were borate buffer solutions with HMB added to control the electroosmotic flow. The borate/HMB were equimolar solutions containing 2.5, 5, 10, and 25 mM borate/HMB dilution from a stock solution of 50 mM borate/50 mM HMB. Hexamethonium bromide is acidic (pH of 100 mM solution in water was 4.62) and partially converts to the hydroxide form on mixing with sodium borate, which maintains the pH of the buffer at 9.3. All solutions were filtered with 0.45 μm membrane filter and degassed prior to use. The reverse polarity mode of the capillary electrophoresis system was operated at −20 kV for the 25 mM borate/HMB buffer to −30 kV for the 2.5 mM borate/HMB buffer. A 50 cm-long × 50 μm i.d. (375 μm o.d.) bare fused silica capillary was used. A small window (about 5 mm in length) for UV detection at 220 nm was created on the capillary by burning off the polyimide coating at 44.9 cm from the injection end. When a capillary was to be used for the first time, it was flushed with 100 mM phosphoric acid for about 3 min in order to remove metal oxides precipitated on the silica walls during manufacture. It was then flushed with 500 mM NaOH for another 16 min in order to reactivate the inner surface of the column. Finally, it was equilibrated with the operating buffer for about 6 min before sample injection. Gravity injection, which has been shown to be more reproducible and exhibit less discrimination before sample injection. Gravity injection, which has been shown to be more reproducible and exhibit less discrimination min before sample injection. Gravity injection, which has been shown to be more reproducible and exhibit less discrimination min before sample injection. Gravity injection, which has been shown to be more reproducible and exhibit less discrimination min before sample injection. Gravity injection, which has been shown to be more reproducible and exhibit less discrimination min before sample injection.

In the study of the effects of buffer concentration on sensitivity and resolution, 2.5, 5, 10, and 25 mM borate/HMB buffer concentrations were used. Nitrate and nitrite peaks were monitored by direct UV measurement at 220 nm. Sample concentrations were 130 μM NO₃⁻ and 110 μM NO₂⁻. The separation was carried out in a 50 cm-long × 50 μm i.d. bare fused silica column, with an applied voltage of −20 kV at the injection end and a gravity injection time of 60 s with an injector head height of 100 mm. Dilute samples were prepared in reagent water rather than in dilute buffer to achieve a low buffer-to-sample conductivity ratio. Other CZE conditions described above applied.

Reproducibility and accuracy studies in water samples to which nitrate and nitrite standards had been added were carried out in a 2.5 mM borate/2.5 mM HMB buffer. The water samples used were reagent water, drinking water, and lab tap water. From the stock solutions, mixtures of nitrate and nitrite were prepared at concentrations of 1.0, 1.68, 2.67, and 5.00 ppm. The above levels of nitrate and nitrite were chosen on the basis of EPA method 300.0. Each sample was run for 3 or more replicates. Standard deviation, relative standard deviation (RSD), and amount found were calculated.

In anion interference studies, dilute nitrite and nitrate standards containing up to 5 mM of chloride, sulfate, carbonate, phosphate, fluoride, and oxalate were analyzed under the conditions listed earlier. The ion chromatography experiments were carried out using a 40 mM sodium hydroxide eluent, containing 1.1% acetoniitrite at a flow rate of 1.2 mL/minute. The regenerant solution was 50 mM sulfuric acid at a flow rate of 10 mL/minute. A Dionex Omni Pac PAX-500 column and a Dionex Omni Pac PAX-500 guard column were used for the separations. The sample injection loop was 25 μL. The nitrate and nitrite standards and samples were as described in the CZE section.

The procedures used for the determination of nitrate in natural water was based on Technicon Industrial Method no. 100-70W/B. Nitrate was reduced to nitrite by a copper–cadmium reductor column. The nitrite formed in the reduction was reacted with sulfanilamide under acidic conditions to form a diazo compound, which was then coupled with N-1-naphthylethylenediamine dihydrochloride to form a reddish-purple complex that was measured at 520 nm. QA/QC standards (0.10 mg/L and 0.5 mg/L NO₃⁻/N) were run to check method performance.

A comparison of the CZE method with the IC and cadmium reduction methods was carried out by analyzing real natural water samples. The analysis of natural water samples was carried out in 2.5 mM borate/2.5 mM HMB buffer at pH 9.3, and results were compared with those obtained by IC and cadmium reduction methods. The natural water samples were obtained from the sample sites that are regularly monitored for limnological changes by the Washington State Water Research Center. The River Mile (RM) numbers (see Table 1) were indicative of the location referenced from the confluence of the Snake River and the Columbia River upstream to the Clearwater River. Top samples were those taken at a depth of 1 m; mid samples, those at 17 m; and bottom samples, those at 33 m. A domestic well water sample near Pullman, Washington was also analyzed by the CZE method, and results were compared with those of the Whitman County Department of Health.

In detection limit studies, gravity injection was carried out with injection times from 30–1000 s at an injector head height of 100 mm. The separation was carried out in 2.5 mM borate/2.5 mM HMB buffer. The water samples used were reagent water, drinking water, and lab tap water. From the stock solutions, mixtures of nitrate and nitrite were prepared at concentrations of 1.0, 1.68, 2.67, and 5.00 ppm. The above levels of nitrate and nitrite were chosen on the basis of EPA method 300.0. Each sample was run for 3 or more replicates. Standard deviation, relative standard deviation (RSD), and amount found were calculated.

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**Evaluations of waste generation for the running of 100 water samples by the CZE, IC, and cadmium reduction methods were carried out.** The comparison was made on calculated values based on all wastes generated through the bulk flow rates, column preparations, and column conditioning.

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Figure 1. Effects of buffer concentration on the response of nitrite and nitrate. Electrolytic buffer: equimolar 25 mM, 50 mM, and 100 mM, and 250 mM borate/hexamethonium bromide, respectively, at pH 9.3; wavelength, 220 nm. Column: 50-cm-long × 50 μm i.d. bare fused silica. Applied voltage: −20 kV, negative polarity. Gravity injection time: 60 s.

RESULTS AND DISCUSSION

Detector Sensitivity. The optimal wavelength in the CZE buffer was found to be 220 nm for both nitrate and nitrite, the same as that reported in the literature. Moreover, the buffer additive, hexamethonium bromide, did not affect the λmax of nitrate and nitrite. Figure 1, however, shows the combined effect of the electrolyte (borate and hexamethonium bromide) concentration on the peak response of nitrate and nitrite at 220 nm. Because peak area gave a better reproducibility than peak height, peak area was used in the evaluation of the response. There was a 25–35% decrease in the peak areas of nitrite and nitrate with a 10 times increase in electrolyte concentration. This decrease in signal was due to the increase in background absorption as the concentration of HM B was increased. Thus, it was advantageous to use a low electrolyte concentration for the analysis of very dilute nitrite and nitrate samples. In addition to increased sensitivity, the use of a low buffer/modified concentration allowed up to 30 kV to be applied across the 50 μm i.d. × 50-cm-long bare silica column, thereby decreasing the analysis time. The high concentration electrolyte produced high electric current when greater than 20 kV was applied. The high power output caused the electrolyte to boil, which, in turn, produced microbubbles in the capillary, causing an arc short within the high-voltage circuit. Moreover, with high voltage and high electrolyte concentrations, increased Joule heating caused increased noise in the analysis.

Table 1. Comparison of Results of Nitrate Analysis of Natural Water by CZE, IC, and Cd Reduction Methods

<table>
<thead>
<tr>
<th>sample</th>
<th>Cd reduction NO3⁻/N(ppm)</th>
<th>CZE NO3⁻/N(ppm)</th>
<th>IC NO3⁻/N(ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RM 118TOP</td>
<td>0.32 ± 0.02</td>
<td>0.33 ± 0.02</td>
<td>0.33 ± 0.04</td>
</tr>
<tr>
<td>RM 118MID</td>
<td>0.29 ± 0.01</td>
<td>0.32 ± 0.03</td>
<td>0.45 ± 0.11</td>
</tr>
<tr>
<td>RM 118BOT</td>
<td>0.29 ± 0.01</td>
<td>0.34 ± 0.09</td>
<td>0.36 ± 0.03</td>
</tr>
<tr>
<td>RM 108TOP</td>
<td>0.40 ± 0.02</td>
<td>0.40 ± 0.04</td>
<td></td>
</tr>
<tr>
<td>RM 108MID</td>
<td>0.37 ± 0.02</td>
<td>0.42 ± 0.07</td>
<td></td>
</tr>
<tr>
<td>RM 129TOP</td>
<td>0.29 ± 0.02</td>
<td>0.39 ± 0.08</td>
<td></td>
</tr>
<tr>
<td>RM 129MID</td>
<td>0.32 ± 0.02</td>
<td>0.42 ± 0.06</td>
<td></td>
</tr>
<tr>
<td>RM 129BOT</td>
<td>0.31 ± 0.02</td>
<td>0.40 ± 0.04</td>
<td></td>
</tr>
<tr>
<td>AB. CONF. TOP</td>
<td>0.43 ± 0.02</td>
<td>0.51 ± 0.04</td>
<td></td>
</tr>
<tr>
<td>AB. CONF. BOT</td>
<td>0.46 ± 0.02</td>
<td>0.51 ± 0.08</td>
<td></td>
</tr>
<tr>
<td>CLEARWATER</td>
<td>0.13 ± 0.01</td>
<td>0.13 ± 0.02</td>
<td></td>
</tr>
</tbody>
</table>

Figure 2. Effects of buffer concentration on separation efficiency of nitrate and nitrite. Resolution and mobility were reduced at low buffer concentrations. The electropherogram of a 1.3 mM nitrate and 1.1 mM nitrite mixture, using –20 kV in a buffer concentration as follows: (a) 2.5 mM borate/HMB buffer, (b) 25 mM borate/HMB buffer at pH 9.3 and a wavelength of 220 nm. Electrophoretic conditions: 50-cm-long × 50 μm i.d. bare fused silica capillary; applied voltage, –20 kV; negative polarity; and a gravity injection time 60 s.

\[ R_s = \frac{\sqrt{N(\Delta \mu)}}{2(\bar{\mu} + \mu_{eo})} = \frac{1}{4}\sqrt{N\Delta \mu'} \]

where N is the number of theoretical plates; \( \Delta \mu \), \( (\mu_{e1} - \mu_{e2}) \), is the electrophoretic mobility difference between ions 1 and ion 2; \( \bar{\mu} \) is the average mobility of the ions; \( \mu_{eo} \) is the electroosmotic mobility; and \( \Delta \mu' \) is the relative mobility ratio, \( \Delta \mu/(\bar{\mu} + \mu_{eo}) \).

Because a negative potential was applied to the injection end of the column, the electroosmotic flow (EOF) was in the opposite direction of the electrophoretic migration of the ions. The EOF was away from the detector, therefore neutral molecules were not detected. HMB was added to minimize the electroosmotic flow such that the electrophoretic mobilities of nitrite and nitrate were about 4 and 6 times greater than the electroosmotic mobility in the low- and high-concentration electrolytes, respectively.
these anions of interest migrated against the electroosmotic flow to the detector, resulting in longer migration times in the 2.5 mM electrolyte than in the 25 mM electrolyte. Determination of the electroosmotic mobility, $\mu_{eo}$, was carried out with the instrument in normal polarity configuration (20 kV), using 0.6 mM phthalonitrile as a neutral marker in both 2.5 mM borate/2.5 mM HMB and 25 mM borate/25 mM HMB buffers.

Generally, in situations where there is either a decrease in, or a reversal of, direction of the electroosmotic flow, very high resolution can be achieved (see eq 2). The effects of pH, buffer concentration, ionic strength, injected sample volume, applied voltage, and electroosmotic flow velocity, have been investigated by Jorgenson,10 McNair,27 Nielen,13 and Riekkola.28 Because effective electrophoretic mobility and electroosmosis are inversely proportional to the square root of buffer concentration,19,29 low buffer concentration results in higher electrophoretic mobility and electroosmosis.

From eq 2, the enhanced resolution in high concentration (25 mM buffer in this study) was a function of $N$ and $\Delta \mu'$. Both $N$ and $\Delta \mu'$ increased with buffer concentration. Two factors contributed to the increased $N$ with buffer concentration. First, stacking occurred in the high-concentration buffer because the conductivity difference between the buffer and the sample was more pronounced in the 25 mM buffer than in the 2.5 mM buffer.30 Hence, sharper peaks were observed in the 25 mM buffer than in the 2.5 mM buffer (see Figure 2). Second, when band spreading is under diffusion control and minimal dispersion exists as a result of Joule heating, $N$ has been expressed29 as

$$N = \frac{\left(\mu_a + \mu_{eso}\right)V}{D} \quad (3)$$

Here, $D$ is the molecular diffusion coefficient of the ion. Because $\mu_{eso}$ was in the opposite direction of $\mu_a$ for anions and was lower in the 25 mM buffer than in the 2.5 mM buffer, the observed electrophoretic mobility, $\mu_{obs} = \mu_a + \mu_{eso}$, was higher in the 25 mM buffer than in the 2.5 mM buffer. This was so because the 25 mM buffer contained a higher concentration of HMB and, hence, a lower $\mu_{eo}$. This increase in $\mu_{obs}$ at higher buffer concentrations also caused an increase in $\Delta \mu'$. The relative mobility ratio for the 25 mM buffer was about twice that for the 2.5 mM buffer.

**Detection Limit.** On-column preconcentration in CZE, caused by the conductivity mismatch11,13,31–34 between the sample zone and the buffer zone, is known as stacking and can not only increase separation efficiency as discussed in the previous section but also be used to decrease the detection limit of analysis. Figure 3 is a plot of signal-to-noise ratio versus time of gravity injection in a 2.5 mM borate/HMB buffer. There was a steep increase in peak height due to the stacking phenomenon as the injection time increased from 20 to 200 s. No further large gain in signal-to-noise ratio was observed at injection times above 200 s, presumably as a result of the effects of band broadening from the large sample volume. At prolonged injection times, the signal-to-noise per unit time of injection leveled off because as the injected volume increased, peak broadening due to large injection volume became significant and even led to a decrease in peak height. Peak area, however, becomes more reproducible, and hence, a more accurate quantification parameter.

A comparison between pressure and gravity injection methods showed that gravity injection gave better stacking effects than the pressure injection method. The detection limit based on gravity injection under stacking conditions is shown in Figure 4. This figure shows that for a very dilute sample, detection limits ($s/n = 3$) of $3.8 \pm 0.4$ ppb N nitrite and $2.0 \pm 0.2$ ppb N nitrate were obtained for 180-s injection times. For a 180-s injection time, detection limits of 3.8 ppm N nitrite and 2.0 ppm N nitrate standards. Electroosmotic buffer: 2.5 mM borate/2.5 mM hexamethonium bromide; pH, 9.3; wavelength, 220 nm. Column: 50-cm-long $\times$ 50 $\mu$m i.d. bare fused silica. Applied voltage: 220 kV. Gravity injection time: 30-1000 s.

**Interferences.** Anion interference studies showed that because this CZE method was based on direct UV detection, it had

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Anion interferences study. The addition of sodium bromide was adjusted to a concentration of 37.5 mM sodium bromide in the 2.5 mM borate/2.5 mM HMB buffer, for the separation of a sample containing 1 mM each of chloride, sulfate, oxalate, fluoride, and phosphate and 0.29 mM each of nitrite and nitrate. Applied voltage was -22 kV, across a 50-cm-long \times 50 \mu m i.d. bare fused silica column. Gravity injection was with a 100 mm head height for 60 s.

the advantage of reduced interference from nonabsorbing species such as sulfate, chloride, carbonate, fluoride, and phosphate anions. However, because the electroosmotic flow modifier, hexamethonium bromide, used in this method absorbed in the same wavelength region as nitrate and nitrite, nonabsorbing anions were observed as negative peaks in the electropherogram. In Figure 5, the migration times of the potentially interfering anions were found to be different from those of nitrate and nitrite. Thus, these ions do not interfere with the analysis at the concentration level tested. The addition of anions to the sample, however, produced an indirect interference due to increased sample ionic strength, which decreased the resolution of nitrite and nitrate. This problem was solved by adding sodium bromide to increase the ionic strength of the buffer. A more detailed study of the effects of the buffer/sample ionic strength ratio on efficiency and resolution was recently examined. It was found that separation efficiency and resolution in CZE can be approximated to a function of the square root of the buffer/sample ionic strength ratio. It was also suggested that samples with high ionic strength matrix would be best analyzed by using high ionic strength electrolyte. A primary advantage of reverse polarity CZE over standard methods for nitrate and nitrite is that the absorbing neutral compounds and cations migrate away from the detector and do not interfere with the response.

Analysis. The reproducibility and accuracy studies for spiked samples of nitrate and nitrite were established for this method. The percentage of recovery of nitrite and nitrate in laboratory tap water were comparable to the percentage recovery in reagent water, ranging from 98–100% and 95–101% accuracy for nitrite and nitrate, respectively. The precision for 3 replicate runs was 3.6% relative standard deviation (RSD) for nitrite and 6.5% for nitrate. A slight increase in RSD was observed for lower-concentration standards (<2.0 ppm), but the RSD was always less than 10%. These results are very much comparable with those obtained for IC in this laboratory and with the published results in the IC based Standard Methods6 and the EPA Method 300.0.7

Table 1 is the summary of results obtained for analysis of natural water samples. The CZE results closely resembled those obtained by FIA and IC. Except for a few values that were exactly the same, it was observed that CZE results were consistently higher than those obtained using the cadmium reduction. For this reason, a statistical Student t-test analysis at 95% confidence levels for each sample mean value was performed on the result, between CZE and cadmium reduction. This statistical analysis showed that the two means were not significantly different, except for the AB. CONF. samples, although the reason for the observed small difference between the CZE and the cadmium reduction for these samples was not known. This discrepancy may have been a result of interferences commonly associated with the cadmium reduction method. Note that a 5% standard deviation was applied on all the cadmium reduction data, on the basis of the established precision of a fully optimized method (R. Ramian, personal communication). The standard deviations for CZE and IC were based on 3 replicate measurements.

Figure 6 is the electropherogram of the analysis of another natural water—a domestic well water sample. This well was monitored by the Whitman County Department of Health because of its unusually high level of nitrate. Figure 6 is a 1:5 dilution of the sample and represents 2.06 ± 0.09 ppm NO₃⁻/N. The well water contained 10.1 ppm nitrate. This result compared well to that from the Health Department.

Waste generation was compared for 100 water samples among CZE, Cd-FIA, and IC methods. From calculated values, a 3-min CZE run time generated about 100 mL of waste. For Cd-FIA, in addition to about 120 mL of waste generated during the preparation of the Cd column, the total volume of waste generated during a 3-min analysis time was about 740 mL. For IC, the waste generated from the bulk flow, including the regenerant at about 11.2 mL/min., was about 4.5 L. Therefore, CZE gave analysis waste reduction by greater than 1 order of magnitude when compared with Cd-FIA and by more than 2 orders of magnitude when compared with IC.

CONCLUSIONS

The rapid reverse polarity capillary zone electrophoresis analysis of nitrite and nitrate was used successfully in the presence of anions that would normally interfere in spectrophotometric, cadmium reduction, and ion chromatographic methods. This simple method with high sample throughput was used for the analysis of river and groundwater samples. Resolution of nitrate from nitrite was better in high-buffer concentration than in low-buffer concentration because of both higher separation efficiency and greater relative mobility difference in the higher buffer.
concentration. Increasing the buffer ionic strength (without increasing the buffer concentration) was used to improve the resolution of nitrite and nitrate in samples that have a high sample/buffer ionic strength ratio. This principle will be useful in analyzing samples of high salt content such as seawater. Very low concentration samples can be analyzed using this method because on-column sample preconcentration occurred by stacking. Detection limits of $3.8 \pm 0.4$ ppb N nitrite and $2.0 \pm 0.2$ ppb N nitrate ($271$ and $143$ nM, respectively) were obtained for a gravity injection time of 3 min, and sub ppb limits were measured for prolonged injection times. The method also reduced laboratory waste by a factor of more than 1 order of magnitude when compared with Cd-FIA and greater than 2 orders of magnitude when compared with the IC method.

ACKNOWLEDGMENT

We are indebted to Dr. Steve T. J. Juul of the Water Research Center, Washington State University, Pullman, WA, for providing the natural water samples and for allowing us use their Cd-FIA data. We thank also Mr. Richard Ramian, (Environmental Science/Regional Planning Program, Washington State University, Pullman, WA) who performed the cadmium reduction analyses. We also appreciate the kindness of Drs. John and Ardith Pierce for providing the well water sample. This work was supported in part by a grant from Westinghouse Hanford Company, Richland, WA.

Received for review February 18, 1999. Accepted April 5, 1999.

AC990198+