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Sensitive and selective method for direct determination of nitrite and nitrate by high-performance capillary electrophoresis

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Abstract

In this paper a sensitive and selective method for the direct determination of nitrite and nitrate by high-performance capillary electrophoresis (HPCE) is described. Separation conditions are optimized using a statistic orthogonal design. Optimization results suggest that the effects of several experiment factors on the separation follow the order: cetyltrimethylammonium bromide (CTAB) concentration > buffer pH ≈ buffer concentration > run voltage. The results also suggest that better separation could be achieved at relatively low electric field (10 kV/50 cm). Nitrite, nitrate, and bromide (an internal standard) are well separated in the optimized buffer 20 mmol/l of tetraborate plus 1.1 mmol/l of cetyltrimethylammonium chloride (CTAC). HPCE separation at a CTAC concentration of 1.1 mmol/l is considered to involve an ion-pair association mechanism. The specificity and quantitation–including calibration range, reproducibility, and minimum detection limit are examined. Finally, the method was used to analyze samples such as tap or river water; a large amount of nitrate in the water does not interfere with the determination of a small amount of spiked nitrite.

Keywords: Capillary electrophoresis; Nitrite; Nitrate

1. Introduction

High-performance capillary electrophoresis (HPCE) is a powerful separation technique undergoing rapid development. Recently, capillary ionic electrophoresis [1–8] has been developed for ion analysis. It has several advantages over other ion analysis techniques: high separation efficiency, short run time, vast range of analytes, feasibility in selectivity manipulation, relatively simple instrumental setup, very small sample take-up, and direct sample injection with little pretreatment. Inorganic, organic anions or cations, and ionic surfactants could be separated and determined by this technique. Separation in capillary zone electrophoresis (CZE) is based on the differential electrophoretic mobility of ionic analytes. In principle, HPCE is well suited for direct separation of ions such as inorganic anions. The detection of most inorganic anions is performed by the indirect ultraviolet (UV) method [1–4], while a few anions might be detected

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by UV absorption (or direct UV method). With direct UV detection, absorption spectra of analytes could be acquired, interferences from other anions is decreased, and analytes could be certified with little chance of error.

CZE separation of anions is different from that of cations: the mobility of some inorganic anions can exceed that of the electroosmotic flow. Consequently, some of the anions will never reach the point of detection. In order to detect inorganic anions, two strategies should be used. First, the EOF is reversed by the addition of a special substance (called EOF reverser) to the electrolyte buffer [1–4]. A tetraalkylammonium surfactant could form a molecular bilayer on the capillary inner wall, reverse the direction of the EOF, and might serve as EOF reverser. Second, the HPCE instrument is configured such that samples are injected at the negative terminal of high-voltage power and detected near the positive terminal. With the above two strategies, anions migrate in the same direction as that of the EOF and reach the detector in a shorter time. Consequently, the analysis time of anions is reduced to only a few minutes.

Nitrite is a toxic compound and needs to be detected in several areas such as environmental monitoring and clinical toxicological examinations. Most of the reported analytical methods for nitrite involve azo-derivatization followed by colorimetry or photometry. Recently, simple and direct methods for the determination of nitrite and nitrate by CZE and indirect UV detection have been reported [1,4,8]. In this paper, HPCE separation and direct UV detection of nitrite and nitrate are described. With this method, nitrite is well separated from nitrate, and sample matrices of high ionic strength do not interfere with the determinations because buffers of relatively high concentration are employed in the present study.

2. Experimental

2.1. Reagents and samples

All solutions were prepared from quartz-redistilled water and domestic analytical or chemical reagents, except when indicated otherwise. Sodium nitrite, sodium nitrate, sodium bromide (internal standard), and sodium or potassium salts of the other inorganic anions used in this study were made up as 1.00 mg/ml solutions. A stock solution of 50 mmol/l sodium tetraborate was prepared by dissolving the desired amount of this substance in water. The pH was adjusted to the desired value with 1 mol/l NaOH or HCl. Cetyltrimethylammonium chloride (CTAC), purchased from Fluka (Switzerland) in the form of 25% (v/v) aqueous solution (equivalent to 0.78 mol/l of CTAC), was diluted with water to make a 5 mmol/l stock solution. A cetyltrimethylammonium bromide (CTAB) solution of 5 mmol/l concentration was obtained by dissolving 0.091 gram of the compound in water. Running electrolyte buffers containing surfactant were prepared by diluting the required aliquots of the stock solutions with water, and the pH was measured after dilution. Nitrite and nitrate stock solutions were diluted with redistilled water for determinations. River or tap water spiked with nitrite was measured directly, without the addition of electrolyte buffer. All the buffer and sample solutions were filtered through a 0.45-μm membrane prior to HPCE measurements.

2.2. Apparatus and electrophoresis

A BioFocus 3000 capillary electrophoresis system (Bio-Rad Laboratories, Hercules, CA, USA) equipped with a high-voltage power supply (30 kV) and control software was used for all separations. A fused-silica capillary with polyimide-coated outer surface (purchased from Yong Nian optic fiber plant, Hebei Province, China), 50 cm in total length (45.5 cm to detector) with 50 μm I.D. and 375 μm O.D., was used. A small section (ca. 0.5 cm) of the capillary coating was burned off with a match to make a window for the on-column UV detector cell. The capillary was washed with 0.1 mol/l NaOH for 5 min, rinsed with water, then equilibrated with the electrolyte for 10 min. Power polarity at the injection end of the capillary was set to negative by computer software, and the other end near the detector was grounded. The electric field was 10 kV/50 cm. Samples were injected with high-pressure

nitrogen, or in the electrophoretic mode. Pressure injection of 48.26 kPa s (7 p.s.i. s) (which is related to sample volume in the range of 7–10 nl for a 50 cm × 50 μm capillary) or electrophoretic injection of 7.5 kV × 5 s were made, except when mentioned otherwise. UV detections were performed at 191, 200 and 214 nm, while peak integrations were done with the data monitored at 200 nm. Electropherograms were recorded and evaluated on a 486 compatible computer with Bio-Rad Spectra and Integrator software. In addition, with this software three-dimensional graphs of absorbance, wavelength, and time can be acquired and plotted. Spectra scanning could be done in the wavelength range from 190 to 800 nm (4 nm per step). Migration-time-calibrated peak area (A/t_m) was used for quantitation in this study.

3. Results and discussion

3.1. Selection of electrolyte buffer and EOF reverser

Nitrite and nitrate have reasonable UV absorptions around 200 nm and could be detected by a UV detector. For sensitive detection of analytes, electrolyte buffers of "high transparency" at the detection wavelengths are required. Tetraborate buffer has this property and was chosen as a candidate for our experiments. Also, an EOF reverser is needed for HPCE separation of anions, and the cationic surfactant CTAB was used in our preliminary experiments. Experimental results showed that tetraborate buffer has little absorption even at the shorter detection wavelength of 191 nm and was suitable for HPCE separation of nitrite and nitrate. However, CTAB was not a good EOF reverser in the present study for two reasons: (i) moderate absorption of 5 mmol/l of CTAB was observed around 200 nm, which might have a negative effect on the sensitive detection of the analytes; (ii) a "ghost peak" between the nitrite and nitrate peaks always appears in a CTAB-containing buffer system, when on-column sample stacking is performed (simply dissolve analytes in pure water or low conductivity buffer). On-column stacking is required to increase injection volumes and sharpen sample peaks for sensitive detection of analytes. However, the ghost peak is undesirable. It is considered to come from bromide in CTAB of the buffer during the on-column stacking process: bromide in CTAB-containing buffer near the rear edge of the sample zone might stack at the boundary between the front edge of sample zone and electrolyte buffer, and exhibit a peak. Based on the above hypothesis, CTAB was replaced by the similar cationic surfactant CTAC, and the ghost peak disappeared. Consequently, tetraborate was chosen as the electrolyte buffer and CTAC as the EOF reverser for the separation of nitrite and nitrate.

3.2. Separation of nitrite and nitrate

Nitrite and nitrate were roughly baseline separated by CZE using indirect UV detection because of their similar chemical and electrophoretic properties [1,4]. To obtain better separation, the experimental conditions should be optimized, and a statistic orthogonal design was employed for optimization. The effects of buffer concentration, pH, CTAC concentration and run voltage on the separation were examined. The experiments were arranged according to a $L_9(3^4)$ orthogonal design table, and the resolution between nitrite and nitrate peaks was taken as optimization target function. The experimental results were analyzed using Statistic Analysis System (SAS) software, and are given in Table 1. The statistical results indicate that the influence of the factors on the separation follow the order: CTAC concentration > buffer pH ≈ buffer concentration > run voltage. The optimum conditions are: 0.5 mmol/l CTAC, buffer pH 8.94, 20 mmol/l tetraborate, and 10 kV run voltage.

3.3. Selection of internal standard

Migration time in HPCE might shift from one set of experiments to another because of irreproducibility of the capillary inner wall; peak areas may also vary from one run to another in parallel experiments due to the irreproducibility of injections. These uncertainties in migration time and peak area are always expected to be

Table 1
Optimization of experimental factors using orthogonal design

Buffer pH	Buffer concentration (mmol/l)	CTAC concentration (mmol/l)	Run voltage (kV)	Optimized factor level
A1 (8.943)	B1 (10)	C1 (0.1)	D (7.5)	A1 (8.943)
A2 (9.175)	B2 (20)	C2 (0.3)	D (10)	B2 (20 mmol/l)
A3 (9.383)	B3 (30)	C3 (0.5)	D (12.5)	C3 (0.5 mmol/l)
				D2 (10 kV)

Electropherograms were monitored at 200 nm. The concentrations of nitrite and nitrate are 46.8 and 49.4 μg/ml, respectively. Other conditions are as indicated in the Experimental section.

minimized for precise identification and quantitation. An effective approach is to use internal standard calibration. A good internal standard should be well separated from the tested compounds, have the same or similar chemical or electrophoretic properties, be easily obtained, be stable under the experimental conditions, and not exist in the samples. Among the limited species of UV-absorbing inorganic anions, bromide has all the qualifications, meets these requirements quite well, and so was chosen as internal standard.

Unfortunately, the electrophoretic peak of bromide overlapped with that of nitrite under the above optimized experimental conditions. To separate them, the CTAC concentration, which was the most important experimental parameter, was changed, and the separation results are shown in Table 2. With the increase of the CTAC concentration, the resolution of bromide and nitrite changes. At CTAC concentration of 1.1 mmol/l, the resolution reaches 1.48, and the anions are well separated.

Table 2
Resolution of nitrite and bromide at different CTAC concentrations[a]

	CTAC concentration (mmol/l)				
	0.30	0.50	0.70	0.90	1.1
R_s	0.66	0	0	0.73	1.48

[a] The experimental conditions are the same as in Table 1.

The separation mechanism at 1.1 mmol/l CTAC concentration has been investigated as it might differ from the CZE mode. The migration times of nitrite, bromide, and nitrate at different CTAC concentrations are shown in Fig. 1. In the figure, t_m of the three anions changes little when the CTAC concentration varies in the range from 0.3 to 0.9 mmol/l, while it increases significantly as the CTAC concentration is raised from 0.9 to 1.1 mmol/l. The increase in t_m parallels the increase in resolution shown in Table 2, and it

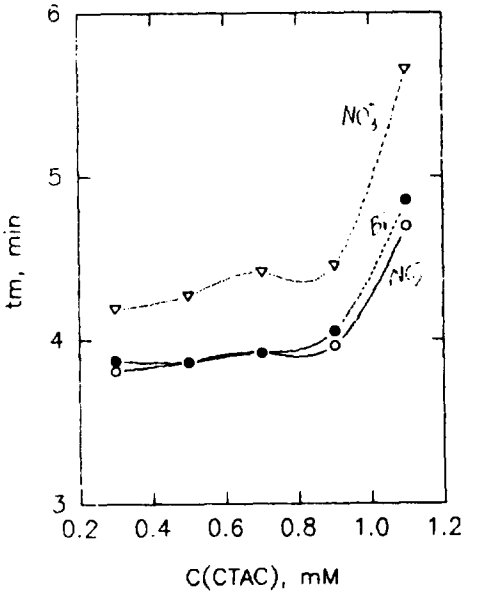

Fig. 1. Migration times of nitrite, bromide, and nitrate at different CTAC concentrations. The experimental conditions are the same as in Table 1.

might be a result of the interaction between anions and the cationic surfactant. The interaction, as a another separation parameter in addition to the electrophoretic mobility, may contribute to the increased resolution. CZE separations of inorganic anions, at a higher concentration of the EOF reverser, might be considered to be partially caused by an ion-pair association mechanism.

3.4. Selection of detection wavelengths

A three-dimensional graph of absorbance, wavelength, and time for nitrite, bromide, and nitrate is shown in Fig. 2. The profile of separation and absorption of the anions is clearly indicated in the figure. From the graph, the UV spectra of these anions could be extracted and are shown in Fig. 3. Nitrite and nitrate have reasonable absorptions over the wavelength range from 220 to 190 nm, while bromide absorbs UV light below 210 nm. Based on the absorption spectra, two commonly used detection wavelengths, 214 and 200 nm, and a shorter wavelength, 191 nm, were initially chosen to monitor the analytes. The electropherogram detected at these wavelengths is shown in Fig. 4. At 191 nm, bromide exhibits a large peak and overlaps with the nitrite peak, and a noisy baseline is observed. Bromide could not be detected at 214 nm. Fortunately, nitrite and bromide are baseline separated when detection is performed at 200 nm. Thus, 200 nm was finally chosen as the detection wavelength.

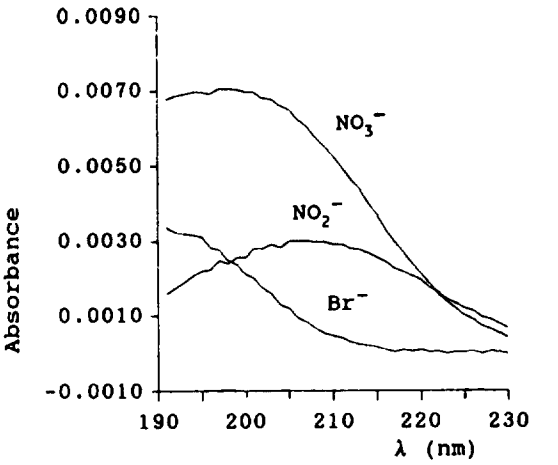

Fig. 3. UV absorption spectra of nitrite, bromide, and nitrate. The experimental conditions are the same as in Fig. 2.

3.5. Identification of the analytes and specificity of the established method

Nitrite and nitrate show absorption at short UV wavelengths, and their absorption spectra, as shown in Fig. 3, could be acquired with the Bio-Focus 3000 capillary electrophoresis system. Identification of the analytes in real samples was done by comparison of their UV spectra and relative migration times (rt_m) with those of the respective pure compounds. Normalized UV

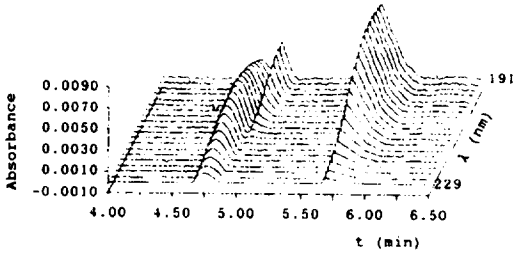

Fig. 2. Absorbance–wavelength–time three-dimensional graph of nitrite, bromide, and nitrate. The experiment was done at 20°C in a buffer of 20 mmol/l tetraborate (pH 8.94) plus 1.1 mmol/l CTAC. Injection volume is −7.5 kV × 5 s and run voltage −10 kV. The concentrations of nitrite, bromide, and nitrate are 78 ng/ml, 0.47 μg/ml and 82 ng/ml, respectively.

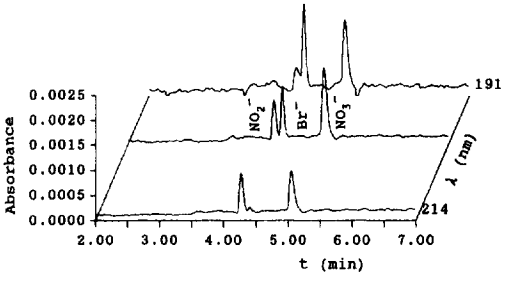

Fig. 4. Electropherogram of nitrite, bromide, and nitrate detected at different wavelengths. The concentrations of nitrite, bromide, and nitrate are 3.12, 4.66 and 3.30 μg/ml, respectively. Sample volumes are injected with 48.26 kPa s (7 p.s.i. s). The other conditions are the same as in Fig. 2.

spectra of samples are compared with those of the standards using Bio-Focus Spectra software. With spectra and rt_m as qualifying parameters, accurate qualitative results could be obtained. For example, nitrate in tap water was recognized by the procedure mentioned above, which will be explained in detail below.

The specificity of the established method was examined, and could be considered excellent. The migration behavior of some common inorganic anions which may have UV absorptions, including I^-, CrO_4^{2-}, $S_2O_3^{2-}$ and SCN^-, was studied. The results indicate that they do not interfere with the determination of nitrite and nitrate. Besides, in principle, cations or non-UV-absorbing anions do not interfere with the direct detection of nitrite and nitrate.

3.6. Quantitation

Several parameters related to quantitation, including linearity, reproducibility, and minimum detectable concentration (MDC), were examined under the above optimized conditions. Using bromide as an internal standard, the peak area (A/t_m) of nitrite or nitrate has a linear relationship with the concentration of nitrite or nitrate, with a correlation coefficient larger than 0.96. The range of the calibration curve, regression equation, and MDC are summarized in Table 3. With the pressure injection mode, the calibration range is from <1 μg/ml to about 10 μg/ml, and the MDC is 0.4 μg/ml, while with the electrophoretic injection mode the calibration range lies in the range 8–80 ng/ml, and the MDC reaches 1 ng/ml.

The within-run and between-run reproducibilities of the established method were also examined. A sample containing 3.12 μg/ml nitrite, 3.30 μg/ml nitrate, and 4.66 μg/ml bromide was successively measured six times in 500 μl electrolyte containing 20 mmol/ml tetraborate (pH 8.94) plus 1.1 mmol/l CTAC. The absolute or relative migration time, the ratio of the calibrated peak areas, and their mean values and relative standard deviation (R.S.D.) are shown in Table 4. The R.S.D. for migration time or relative peak area is moderate, while it is quite small (less than 0.6%) for the relative migration time (rt_m). The between-run reproducibility was examined in the following way: six points of migration time or relative migration time were measured on three days, two points each day. The R.S.D.s of migration time and relative migration time are also shown in Table 4. The between-run R.S.D.s of the relative migration time are also small.

3.7. Determination of nitrite in tap and river water

The established method was employed to analyze samples such as tap or river water. The samples were measured directly with no pretreatment other than filtration through a 0.45-μm membrane. Nitrate at the level of several hundred micrograms per milliliter was identified in tap water, as indicated in Fig. 5 by the large peak located at 5.5–5.7 min, by comparing rt_m and UV spectra of the sample with that of the nitrate standard. It is interesting to note that the little peak of nitrite spiked in tap water is well sepa-

Table 3
Calibration range, regression equation, correlation coefficient and minimum detection limit (MDC) for nitrite and nitrate ($n = 5$)

Analyte	Injection mode	Calibration range	Regression equation[a]	r	MDC
Nitrite	High pressure	0.78–7.8 μg/ml	$y = 1.63x + 0.053$	0.99	0.4 μg/ml
	Electrophoretic	7.8 –78 ng/ml	$y = 1.00x + 0.064$	0.97	1 ng/ml
Nitrate	High pressure	0.82–8.2 μg/ml	$y = 2.65x + 0.014$	0.99	0.4 μg/ml
	Electrophoretic	8.2 –82 ng/ml	$y = 1.46x + 2.93$	0.96	1 ng/ml

[a] $y = [(A/t_m)_{NO_3}]/[(A/t_m)_{Br}]$, $x = C_{NO_3}/C_{Br}$. Electrolyte buffer contained 20 mmol/l tetraborate (pH 8.94) and 1.1 mmol/l CTAC.

Table 4
Reproducibility of six measurements[a]

	1	2	3	4	5	6	Mean	R.S.D. (%)
Within-run								
$t_m(NO_2^-)$	4.50	4.42	4.10	4.53	4.27	4.45	4.38	3.7
$t_m(NO_3^-)$	5.35	5.23	4.80	5.39	5.06	5.29	5.19	4.3
$rt_m(NO_2^-)$	0.970	0.971	0.974	0.970	0.970	0.972	0.971	0.17
$rt_m(NO_3^-)$	1.15	1.15	1.14	1.15	1.15	1.16	1.15	0.55
$rA(NO_2^-)$	1.04	0.985	0.982	1.06	1.06	1.06	1.03	3.7
$rA(NO_3^-)$	1.89	1.81	1.84	1.76	2.01	1.80	1.85	4.8
Between-run								
$t_m(NO_2^-)$	4.28	4.38	4.78	4.72	4.50	4.42	4.51	4.4
$t_m(NO_3^-)$	5.05	5.17	5.80	5.72	5.35	5.23	5.39	5.7
$rt_m(NO_2^-)$	0.970	0.969	0.962	0.963	0.970	0.971	0.968	0.41
$rt_m(NO_3^-)$	1.15	1.14	1.17	1.17	1.15	1.15	1.16	1.1

[a] $rt_m = t_m(NO_x)/t_m(Br)$, $rA = (A/t_m)_{NO_x}/(A/t_m)_{Br}$. Conditions are the same as those for Table 3.

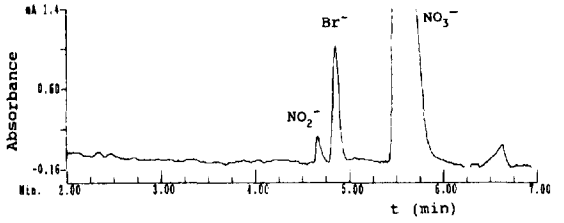

Fig. 5. Electropherogram of tap water spiked with nitrite. Nitrite, 0.78 μg/ml, and bromide, 4.66 μg/ml, are spiked in tap water. Other conditions are the same as in Fig. 2.

rated from the large nitrate peak, as shown in Fig. 5. The good separation suggests a high specificity of the method.

Table 5
Recovery of nitrite at different concentrations from tap water

	Concentration (μg/ml)					Mean	R.S.D. (%)
	0.78	1.56	4.68	6.24	7.80		
Recovery (%)	51	55	50	57	57	54	6.1

The recovery of nitrite spiked in water is measured and given in Table 5. It is moderately low, possibly because nitrite is partly oxidized by oxidants from decontamination agents such as calcium hyperchlorite.

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