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Ultra-rapid analysis of nitrate and nitrite by capillary electrophoresis

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Abstract

Rapid analysis of nitrate and nitrite by capillary electrophoresis (CE) has been limited by the ions' very similar electrophoretic mobilities. With a pK_a of 3.15, the mobility of nitrite can be selectively reduced using a low pH buffer in CE. A much shorter capillary can be used and separation voltages can be increased. With this method, nitrate and nitrite are separated in just over 10 s. This is roughly 20 times faster than current separation methods. Direct UV detection at 214 nm was employed and offered sub μM detection limits. Total analysis time (pre-rinse, injection, and separation) was less than 1 min, making this method ideal for high-throughput analysis.

Keywords: Nitrate; Nitrite; Inorganic anions

1. Introduction

Nitrate and nitrite are widespread contaminants present in foods, drinking water, and the environment. Although naturally occurring in the body, abnormally high levels have been linked to diabetes and a number of nervous system disorders. A recent review article cited roughly 400 articles dealing with the analysis and toxicity of nitrate and nitrite [1]. Thus, analytical methods for determining nitrate and nitrite are of considerable importance.

Numerous different techniques have been employed for analyzing nitrate and nitrite. Flow injection analysis (FIA) using spectrophotometric detection has been used extensively [2–4]. These methods are very matrix sensitive and usually involve azo derivatization. The major disadvantage of these methods is their inability to analyze both nitrate and nitrite simultaneously. The sample must be first analyzed for nitrite and then repeated after reduction of nitrate to nitrite with cadmium. High-performance liquid chromatography (HPLC) has been used primarily for analyzing nitrate and nitrite in biological fluids [5–7]. Ion chromatography (IC) has also been used [8], and is the method of choice of the United States Environmental Protection Agency for determining inorganic ions in water samples [9]. Simultaneous analysis of nitrate and nitrite using suppressed conductivity detection can be performed in less than 5 min [9].

Capillary electrophoresis (CE) offers shorter analysis times, simple instrumentation, and is more environmentally friendly. However, analysis times in CE have been limited to 2 to 3 min due to the very similar electrophoretic mobilities of nitrate and nitrite [10–12]. In today's environmental or clinical laboratories, methods capable of high sample throughput are desirable. Cutting an analysis time even by half can greatly reduce costs when dealing with hundreds of samples daily.

Our approach to overcome this problem is to

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selectively alter the mobility of one of the ions. Nitrite has a pK_a of 3.15. Thus use of a low pH buffer can selectively reduce the mobility of nitrite. Extensive studies have investigated the pH range 7–13 for altering the selectivity of anions such as borate, carbonate, and phosphate [13–15]. Surprisingly, very few studies have examined the lower pH range where the selectivity of numerous other anions could be altered. Separations of anionic chloro complexes of gold(III) and platinum group elements at pH 1–2.4 have been studied [16]. The acidic conditions were necessary to minimize hydrolysis of the complexes. As a result, the focus of the studies was on hydrolytic degradation, rather than the effect of pH on mobility. Amran et al. [17] studied the effect of pH from 2 to 8 on the migration behavior bromide, bromate, iodide, iodate, nitrate, nitrite, and selenite. Of these ions, only nitrite and selenite had pK_a in the range studied and differences in their selectivity were observed. Takayanagi et al. also noticed a change in selectivity of nitrate and nitrite at low pH values in a standard anion mixture [18]. Janini et al. demonstrated excellent selectivity between nitrate and nitrite at pH 3.0, resulting in separation of these analytes in just less than 5 min [19].

The greater mobility difference offered by the low pH electrolyte allows the use of a much shorter capillary and increased electric field strengths. Further, use of cationic surfactants such as didodecyldimethylammonium bromide (DDAB) generates a co-electroosmotic flow, which also speeds the separation. This method will illustrate the effectiveness of using pH to alter the mobility of an ion for rapid ion analysis.

2. Experimental

2.1. Apparatus

A P/ACE 2100 (Beckman Instruments, Fullerton, CA, USA) equipped with a UV absorbance detector was used for all experiments. Data acquisition and control was performed using P/ACE Station software (Beckman) for Windows 95 on a 486 personal computer. Untreated silica capillaries (Polymicro Technologies, Phoenix, AZ, USA) with an inner diameter of 50 μm, outer diameter of 365 μm, and a total length of 27 cm (20 cm to the detector) were used unless otherwise noted.

2.2. Reagents

All solutions were prepared in Nanopure ultrapure water (Barnstead). Buffers were prepared from reagent grade orthophosphoric acid and the pH was adjusted using sodium hydroxide (BDH). Nitrate and nitrite sample solutions were prepared in water from their reagent grade potassium and sodium salts (BDH), respectively. The double-chained surfactant didodecyldimethylammonium bromide (DDAB; Aldrich) was used as received.

2.3. Separations

New capillaries were used for each set of experiments and were pre-treated with 0.1 *M* NaOH for 10 min before use. A 5 s electrokinetic injection at 5 kV was used for all experiments unless otherwise indicated. Separations were performed under an electric field strength of −926 V/cm unless otherwise noted, with a voltage ramp time of 0.17 min. Direct UV detection at 214 nm was used with a data acquisition rate of 10 Hz and a rise time of 0.1 s.

3. Results and discussion

3.1. Effect of pH on nitrite mobility

Conventionally, anion separations are performed using a chromate buffer at pH 8.0 [13,20]. The similar mobilities of nitrate ($\mu_o = 7.41 \cdot 10^{-4}$ cm^2/V s) and nitrite ($\mu_o = 7.44 \cdot 10^{-4}$ cm^2/V s) result in a resolution of only 1.3 after 3.5 min of migration. However, nitrite has a pK_a of 3.15. Thus, using acidic buffers allows selective retardation of the nitrite peak. Reduction in the mobility of nitrite is first noticeable at pH 4, which is in agreement with Amram et al. [17]. Fig. 1 shows the increased resolution on lowering the pH from 3.5 to 2.5. The enhancement in selectivity is such that even without using an electroosmotic flow (EOF) modifier, baseline separation of nitrate and nitrite can be

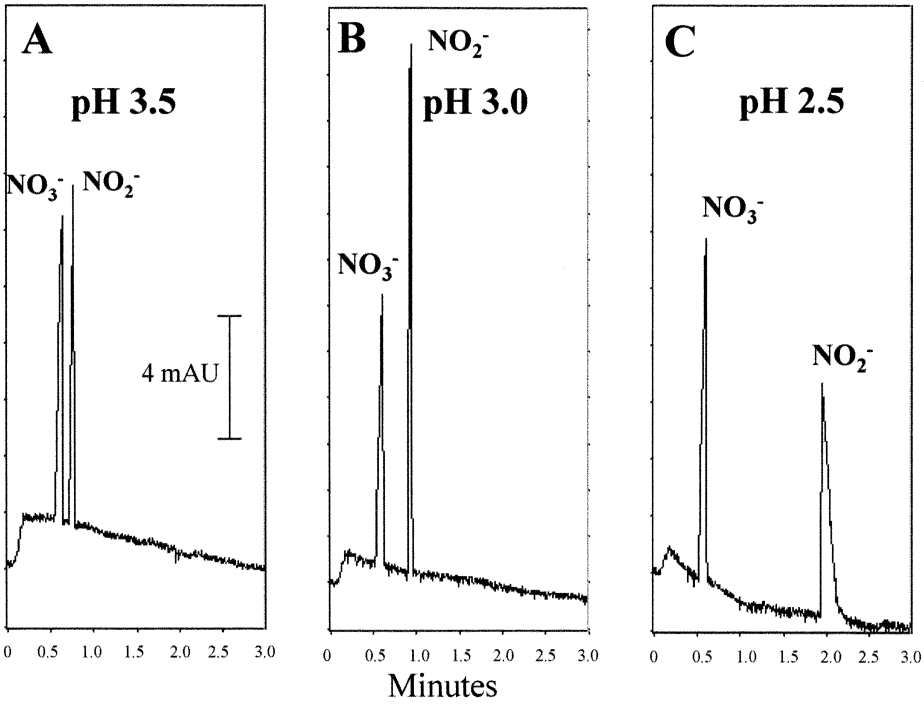

Fig. 1. Effect of pH on resolution. Experimental conditions: 27 cm capillary (20 cm to detector); −926 V/cm; direct UV detection at 214 nm; 20 m*M* phosphate buffer; 1.0 m*M* NO_3^-/NO_2^-.

achieved in 45 s at pH 3.5 (Fig. 1a). However, this separation is performed under counter-EOF conditions. That is, the nitrate and nitrite migrate to the detector in opposition to the EOF. Greater analysis speed would be achieved if the EOF were reversed so as to flow in the same direction as the anion migration.

3.2. Co-electroosmotic flow separation

Typically cationic surfactants such as cetyltrimethylammonium bromide (CTAB) are used to reverse the EOF in capillary electrophoresis [21]. However, CTAB works poorly at low pH. The double-chained cationic surfactant DDAB has been observed to be more effective at reversing the EOF at low pH [22]. Nassar et al. also found the double-chained surfactant DDAOH to be more effective than the single-chained CTAOH in the separation of phosphonic acids at pH 4.0 [23].

A short rinse with DDAB (0.45 min at 20 p.s.i.; 1 p.s.i.=6894.76 Pa) followed by a rinse with the separation buffer (0.20 min at 20 p.s.i.) before each run yielded a semi-permanent wall coating which provided a strong reversed EOF [22]. The removal of excess DDAB from the buffer lessened ion-pairing effects between the analyte and surfactant and negated any possible loss in sensitivity due to absorption by bromide at 214 nm. Using co-EOF it was necessary to lower the buffer pH to 2.5 to achieve adequate resolution. At pH 3.5 nitrate and nitrite co-migrated resulting in a single peak and at pH 3.0 resolution was limited to 0.8. Under these conditions separation could be achieved in less than 30 s on a 27 cm (20 cm to detector) capillary at pH 2.5, as shown in Fig. 2.

3.3. Ultra-rapid separation

Shortening the capillary to 7 cm to detector allowed nitrate and nitrite to be separated in roughly 12 s, as shown in Fig. 3. The commercial instrument used (Beckman P/ACE) requires a minimum of 27 cm of capillary. This consists of a 20 cm portion

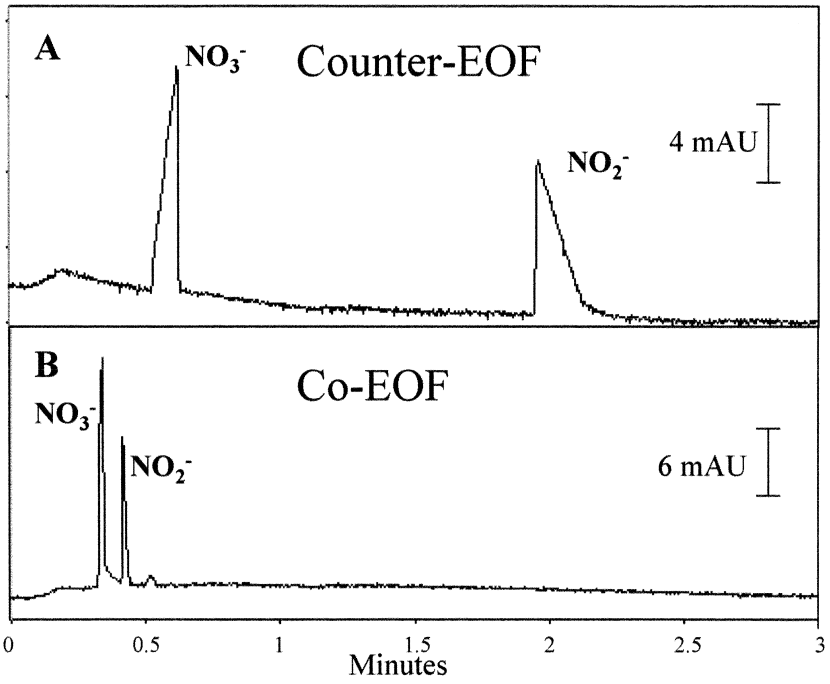

Fig. 2. Counter-EOF versus co-EOF. Experimental conditions: 27 cm capillary (20 cm to detector); −926 V/cm; direct UV detection at 214 nm; 20 m*M* phosphate buffer; pH 2.5; 1.0 m*M* NO_3^-/NO_2^-. (A) No EOF modifier. (B) 1 min pre-rinse with 0.10 m*M* DDABr at high pressure (20 p.s.i.).

from the inlet vial to the detector and a 7 cm portion from the detector to the outlet vial. The 7 cm portion of the capillary was used for the separation by reversing the polarity and electrokinetically injecting at the outlet end of the capillary.

While the 12 s separation is over 10-times faster than previous CE methods, faster separations may have been possible if not for the pre-set voltage ramp time of the instrument. The voltage, as indicated by a plot of current in Fig. 3, required 0.17 min to achieve its set value. This coincides with the migration time of the analytes. Thus, the analytes actually experience an average electric field strength of roughly half the total field strength applied.

3.4. Sample throughput

When dealing with such short separation times, one must consider the overall sample turn-around time (pre-rinse, injection, and separation). Although capillary electrophoresis is considered a relatively fast technique, lengthy pre-conditioning rinses can be required to achieve adequate results. Similarly, certain modes of injection such as gravity injection can be time consuming and add significantly to the sample turn-around time. These aspects of a method are often overlooked and in most cases only the separation time is reported.

This method required a pre-rinse period of only 0.65 min (0.1 m*M* DDAB for 0.45 min at 20 p.s.i.; separation buffer for 0.20 min at 20 p.s.i.) and an injection time of less than 0.1 min, making the sample turn-around time less than 1 min. Table 1 compares sample throughput of this method with other techniques for the analysis of nitrate and nitrite. This sample turn-around time is much faster than conventional chromatographic or CE methods, and is comparable or faster than flow injection methods for nitrate and nitrite [2–12].

3.5. Reproducibility and detection limits

The reproducibility of the method was excellent, as shown by the overlay of five consecutive runs in

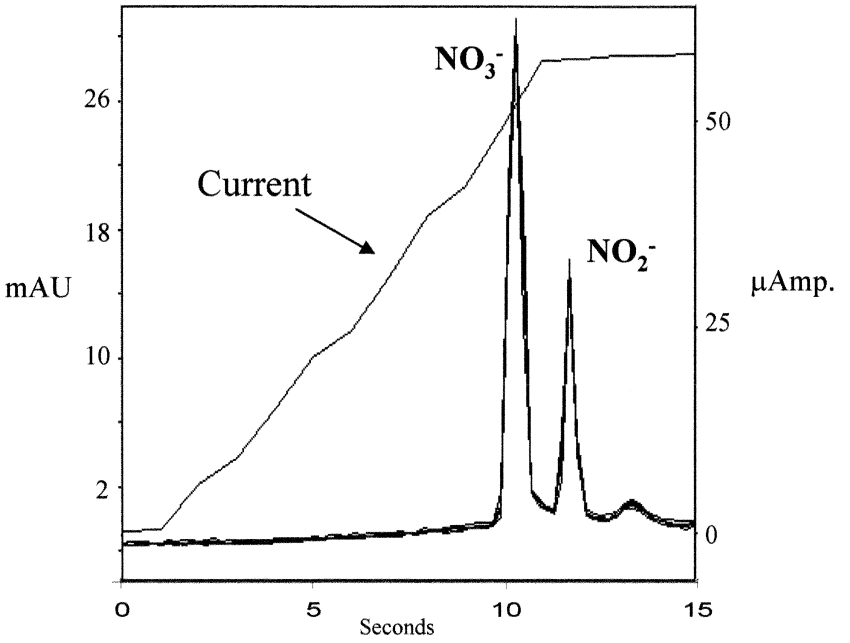

Fig. 3. Optimized separation of nitrate and nitrite. Shown is the overlay of five consecutive runs. Also shown is the voltage ramp (plot of current). Experimental conditions: 7 cm effective length capillary; −1110 V/cm with 0.17 min voltage ramp time; direct UV detection at 214 nm; 20 m*M* phosphate buffer; pH 2.5; 0.45 min pre-rinse with 0.10 m*M* DDABr; 1.0 m*M* NO_3^-/NO_2^-.

Fig. 3. The migration time reproducibility was <0.5% RSD and that for peak area was <0.9% RSD for 1.0 m*M* nitrate and nitrite.

Detection limits were 100 n*M* (6.2 ppb NO_3^-) for nitrate and 1.0 μ*M* (46 ppb NO_2^-) for nitrite using electrokinetic injection (10 kV for 10 s) and 10 μ*M* (0.62 ppm NO_3^- and 0.46 ppm NO_2^-) using hydrodynamic injection (20 p.s.i. for 10 s) in deionized water. Lower detection limits were achieved using electrokinetic injection which is consistent with previous studies [10]. However, electrokinetic injection is limited to low-conductivity samples. Hydrodynamic injection may be required for higher conductivity samples such as seawater.

Table 1
Comparison of sample throughput with other techniques for determining nitrate and nitrite

Technique	Sample throughput (samples/h)	Ref.
HPLC	4	[5]
	12	[7]
FIA	20	[3]
	30	[4]
	50	[2]
IC	10	[8]
	12	[9]
CE	10	[10]
	15	[11,12]
Ultra-rapid CE	60	This work

Linear calibration using electrokinetic injection was achieved with $r=0.99$ and intercept=0 at the 95% confidence interval for both nitrate and nitrite. To achieve linearity over a broader concentration range using electrokinetic injection, 0.3 m*M* KCl was added to the samples to adjust the conductivity of the samples to roughly 1/50 of the conductivity of the buffer. Otherwise, lower concentration samples would experience larger field amplification and undergo more stacking than higher concentration samples [24]. Samples were not prepared in dilute separation buffer (pH 2.5), because the severely reduced mobility of nitrite would result in significant injection bias relative to the higher mobility nitrate [25]. Although adding salt to sample solutions

improved linearity of calibration curves, detection limits suffered due to the reduction in stacking and quantity injected.

4. Conclusions

The ability to alter the mobility of an ion is an important tool for improving resolution and analysis speed in ion analysis. This work demonstrates that rapid separations are possible by simply adjusting the pH to magnify the difference in electrophoretic mobility of ions. Many inorganic anions have pK_a in the pH range possible for capillary electrophoresis, making this type of separation possible for a variety of applications.

This method's suitability for high-throughput analysis makes this type of method ideal for environmental monitoring or clinical analysis. Pushing the limits of a full-scale capillary electrophoresis instrument, this method is well suited for a miniaturized CE device where it could be employed as a portable probe or sensor.

Acknowledgements

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