Capillary electrophoretic speciation of nitrogen in river waters

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A capillary electrophoretic method for simultaneous determination of nitrate, nitrite and ammonium ions has been developed. Direct (NO_3^-, NO_2^-) and indirect (NH_4^+) UV detection at 214 nm in conjunction with electromigration sampling from both ends of the capillary was used. Optimization of the experimental parameters such as electrolyte concentration, pH, nature of counter-ion, was studied. The optimized separations were carried out in 5 mmol/l copper(II) chloride, 10 mmol/l ethylenediamine, 1 mmol/l triethanolamine and 0.05 mmol/l tetradecyltrimethylammonium hydroxide electrolyte (pH 8.2). The method permits excellent separation of three nitrogen species in only 5 min. A 1×10^{-4} mol/l Br^- was used as an internal standard to limit possible electrokinetic injection biases. The proposed system was applied to the speciation of inorganic nitrogen ions in river water samples. The CE results agree with those obtained by spectrophotometric methods.

Key words: capillary electrophoresis, nitrate, ammonium, river waters

INTRODUCTION

The nitrogen cycle is of particular significance in a number of biological and non-biological processes in the environment [1]. Natural and anthropogenic effects can cause localised interrelated changes in the cycle. In order to assess the impact and extent of the changes, it is essential to develop rapid and simple analytical techniques for a simultaneous determination of inorganic nitrogen species in a wide variety of environmental samples.

Many methods have been used for determination of nitrate, nitrite and ammonium in environmental samples. Most important are spectrophotometric and ion chromatographic (IC) methods. Spectrophotometric techniques often involve conversion of nitrite into an azo dye coupled with aromatic amines [2, 3]. Nitrate is previously reduced to nitrite, usually with Cd, and is then determined by difference. In the spectrophotometric determination of ammonium the Nessler [4] and indophenol [5] methods appear to be dominant. All these methods, however, are plagued with many interferences such as dissolved organic matter and common metal ions. Moreover, the use of three separate analyses for each nitrogen species is labour-intensive as well as time-consuming.

The most common analytical technique used for nitrogen species in the last two decades is IC [6]. However, determination of anions and cations by IC requires entirely different conditions (stationary and mobile phases). Mou et al. [7] used a mixed anion–cation-exchange column for simultaneous determination of all three nitrogen ions. Nitrate and nitrite were detected by UV spectrometry and the ammonium ion by the use of a chemically suppressed conductivity detector. The major disadvantage of this system is a relatively low selectivity in comparison with conventional IC columns.

In recent years the use of capillary electrophoresis (CE) for analysis of ionic analytes has grown significantly. Because of its higher resolution, shorter analysis time, lower consumption of reagents, and greater simplicity in operation compared to IC, CE has received a great deal of attention for determination of inorganic ions. Numerous applications of CE have been reported for determination of nitrate and nitrite anions [8–14] or the ammonium cation [15–17] in various aqueous and other samples. However, as the separation by CE is based on the difference in electrophoretic mobilities of the analytes, determination of fast anions and cations in a single analysis under conventional CE conditions is not possible.

In this paper, we report on the CE approach for a simultaneous determination of nitrate, nitrite and ammonium ions based on electrokinetic sample in-

jection from both ends of the capillary and both direct and indirect UV detection. Determination of nitrogen species in river water samples by the CE method was compared to the conventional spectrophotometric methods.

EXPERIMENTAL

Separations were performed on a P/ACE 2100 apparatus (Beckman Instruments Inc., Fullerton, CA, USA) equipped with a UV detector with wavelength filters (200, 214, 230 and 254 nm). Fused silica capillary (Polymicro Technology, Phoenix, AZ, USA) of 75 μm i.d. (375 μm o.d.) and 90 cm total length (50 cm to the detector) was used. Samples were introduced by electromigration injection (5 kV, 5 s) from both ends of the capillary. System Gold software was used for data acquisition. UV detection was employed at 214 nm. All experiments were conducted at 25 °C.

Deionized water was obtained by passing distilled water through a Waters Milli-Q water-purification system (Millipore, Eschborn, Germany). Ethylenediamine (En) and triethanolamine (TrEA) were from Sigma (Sigma, St. Louis, MO, USA). All other reagents, of analytical-reagent grade, were obtained from Merck (Darmstadt, Germany). Stock solutions (0.01 mol/l) of analyte ions and internal standard were prepared from appropriate salts. Tetradecyltrimethylammonium (Merck, Darmstadt, Germany) hydroxide (TTAOH) was prepared from bromide salt by conversion, using an OH^- form anion-exchange material ARA-12P (Reachim, Russia).

The working electrolytes were prepared fresh daily. All electrolyte solutions were filtered through a 0.45 μm membrane filter and degassed by ultrasonication. The capillary was rinsed with 1.0 mol/l sodium hydroxide and water for 5 min, then equilibrated with carrier electrolyte for 30 min at the beginning of each day. Between all electrophoretic separations the capillary was rinsed for 2 min with carrier electrolyte.

RESULTS AND DISCUSSION

In our previous work [18, 19] for the simultaneous CE separation of common inorganic anions and cations based on electromigrative sample introduction from both ends of the capillary and indirect UV detection, we used an electrolyte containing two UV chromophores: cationic imidazole and anionic nitrate or chromate. However, most environmental and biological samples contain large amounts of chloride, sulfate, and hydrogen carbonate anions which interfere in the determination of anionic nitrogen species. Relatively high UV absorbance in the wavelengths range between 200 and 220 nm of both nitrate and nitrite anions enables the use of a direct detection technique for these analytes [14]. This approach has the advantage of eliminating interferences from non-absorbing anions such as chloride, sulfate, etc. Moreover, in this case the carrier electrolyte must contain only one cationic UV chromophore, that significantly improves the baseline stability and detection sensitivity of both anionic and cationic analytes.

Because ammonium has no UV absorbance, for simultaneous UV detection of all three nitrogen species the carrier electrolyte must contain a cationic UV chromophore. However, common cationic UV chromophores used for determination of cations are weak organic bases, which can be used only in acidic medium (pH < 6) due to their deprotonation or low solubility at higher pH values. In our previous work [16] we have introduced copper(II) chelate with ethylenediamine (En) as a cationic UV chromophore. This stable cationic complex exhibits a strong UV absorption over a wide wavelength range below 260 nm. In addition, $Cu(En)_2^{2+}$ does not change the charge and mobilities in the pH range of at least 5–10.

Separation optimization. Preliminary experiments were performed in 7.5 mmol/l $Cu(En)_2^{2+}$ electrolyte. Electrolyte solutions containing $Cu(En)_2^{2+}$ chelate were prepared by neutralization of $CuCl_2$ or $CuSO_4$ standard solutions with an appropriate amount of En. In order to increase the buffering capacity of the electrolyte, 1 mmol/L triethanolamine was also added. An addition of 0.05 mmol/l of TTAOH to the carrier electrolyte additionally reduces the migration times for anions and increases those for cations. Figure 1 demonstrates the separation of a standard mixture of NO_3^-, NO_2^-, NH_4^+ and K^+ ions using copper chloride and sulfate salts for preparation of carrier electrolyte. It is interesting to note that when chloride electrolyte is used, the resolution between nitrate and nitrite is significantly better. It is well known that the highest separation efficiency in CE should be obtained when the effective mobilities of the analytes and of the electrolyte co-ion are similar. Both chloride and sulfate have a similar mobility [20]. Consequently, the peak shapes for nitrogen anions also should be similar in both cases. Moreover, electropherograms in Fig. 1 demonstrate that the nature of the electrolyte anion also influences the peak shapes of the cations studied in the same manner. In order to explain such results, the effective mobility of the $Cu(En)_2^{2+}$ cation was measured in electrolytes containing different anions. The results given in Table 1 demonstrate relatively high variations of the $Cu(En)_2^{2+}$ mobility *vs*. the nature of the counter-ion. It can be concluded that

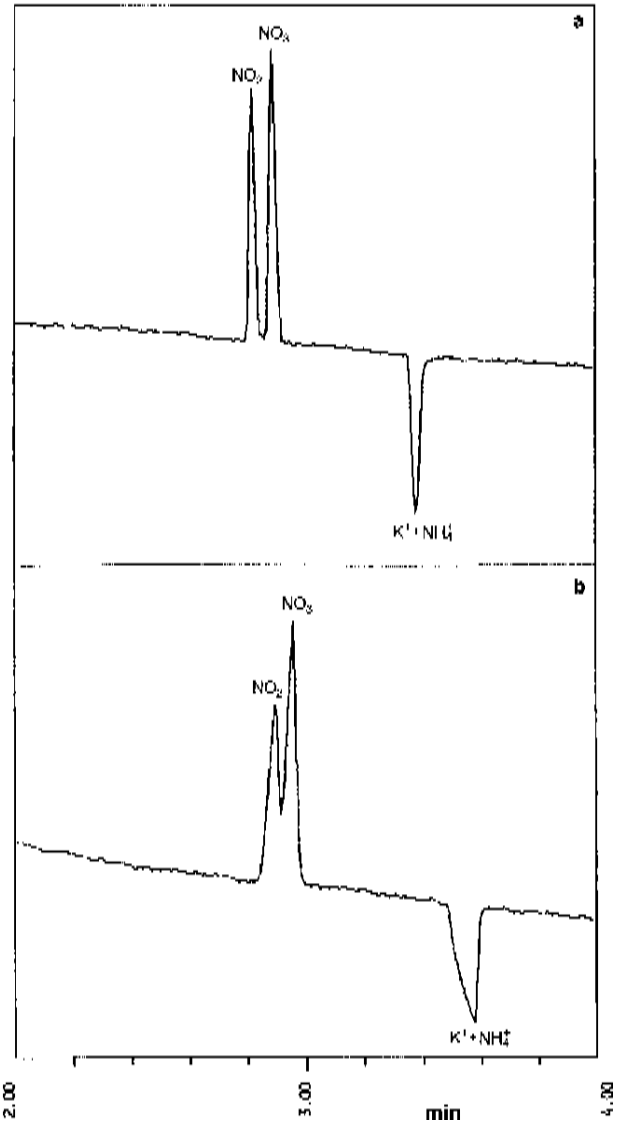

Fig. 1. Electrophoregrams of a standard solution using (*a*) $Cu(En)_2^{2+}$-chloride and (*b*) $Cu(En)_2^{2+}$-sulfate electrolytes. Electrolyte, 7.5 mmol/l copper(II) salt, 15 mmol/l En, 1 mmol/l TrEA, 0.05 mmol/l TTAOH; pH 7.5 (with HCl or with H_2SO_4, respectively); applied voltage, +30 kV

Table 1. **Effect of electrolyte anion on effective mobility (μ_{eff}) of $Cu(En)_2^{2+}$ cation**

Electrolyte (pH 8.0; +25 kV)	$\mu_{eff} \times 10^4$ cm^2 V^{-1} s^{-1}
20 mmol/l CH_3COONa	5.4
20 mmol/l NaCl	5.1
10 mmol/l Na_2SO_4	3.6
10 mmol/l Na_2HPO_4	3.8

hydrophobic chelate tends to form ion-pairs with higher charged anions such as SO_4^{2-} and HPO_4^{2-}. This association process reduces its effective mobility and also the mobility of the counter-ion. Thus, $Cu(En)_2^{2+}$-chloride electrolyte is more suitable for separation of inorganic nitrogen species.

However, the separation of NH_4^+ and K^+ is difficult due to their identical electrophoretic mobilities at a neutral and slightly acidic pH [21]. Increasing the electrolyte pH provides additional possibilities to optimize the separation selectivity. As the pH of the electrolyte is increased, the ammonium ion becomes less protonated (pK^a = 9.25) and its mobility significantly decreases. Results in Fig. 2 demonstrate a good resolution of K^+ and NH_4^+ ions in the pH range above 8.0. In the pH range above 8.5 the peak area of ammonium dramatically decreases. This limits the electrolyte pH to values below 8.5.

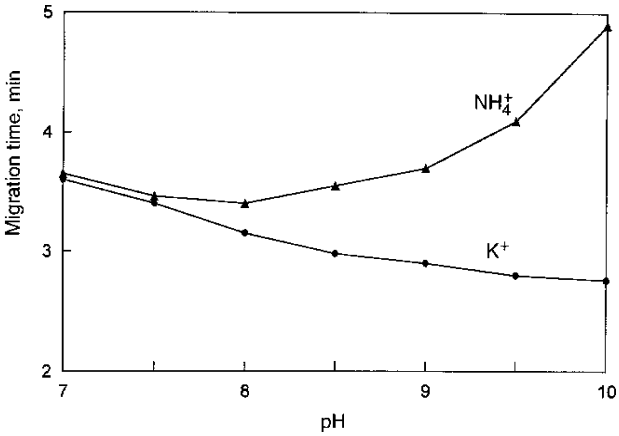

Fig. 2. Effect of electrolyte pH on the migration times of K^+ and NH_4^+ cations. Electrolyte, 7.5 mmol/l $CuCl_2$, 15 mmol/l En, 1 mmol/l TrEA, 0.05 mmol/l TTAOH; applied voltage, +30 kV

The effect of $Cu(En)_2^{2+}$-chloride concentration on the separation efficiency and detection sensitivity was studied in 2.5–10 mmol/l range by maintaining a constant pH value of 8.2. The results showed that with higher electrolyte concentrations a better resolution was observed due to the increasing separation efficiency. The maximum signal-to-noise ratio was in the range of 5.0–7.0 mmol/l $Cu(En)_2^{2+}$-chloride concentrations. Figure 3 shows an example of simultaneous separation of inorganic nitrogen species and potassium ion obtained under optimum conditions. All ions are fully separated in less than 4 min.

Validation of the method. The proposed CE system requires introduction of an electrokinetic (EK) sample. In EK injection the amount of the analyte injected (n_a) can be expressed as follows [22]:

$$n_a = \frac{(\mu_a \pm \mu_{eo}) \cdot r^2 \cdot \pi}{L} \cdot U_{inj} \cdot t_{inj} \cdot c_a,$$

where μ_a is the electrophoretic mobility of the analyte, μ_{eo} is the mobility of the electroosmotic flow (EOF), r is the inner radius of the capillary, L is

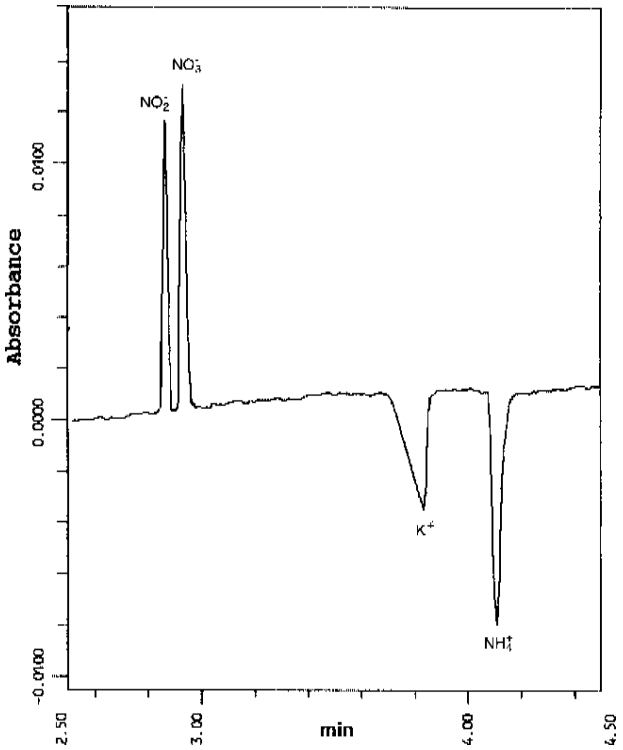

Fig. 3. Electrophoregram of a standard solution under optimum conditions. Electrolyte, 5 mmol/l $CuCl_2$, 10 mmol/l En, 1 mmol/l TrEA, 0.05 mmol/l TTAOH, pH 8.2; applied voltage, +25 kV

the total length of the capillary, U_{inj} is the injection voltage, t_{inj} is the injection time, c_a is the molar concentration of the analyte.

In the case when the mobility of the analyte is in the opposite direction to that of the EOF only analytes with absolute values of μ_a exceeding the magnitude of the EOF, can be injected. Based on Eq. (1), two different kinds of bias can be observed using the EK injection mode for quantitative analysis [23]. One is brought about by the different mobilities of the species in the sample solution. This effect causes a distortion in the ratio of peak areas for ions having different mobilities. All the three inorganic nitrogen species have similar mobilities (7.40×10^{-4}, 7.46×10^{-4} and 7.60×10^{-4} cm^2/V s for NO_3^-, NO_2^- and NH_4^+, respectively), therefore the mobility bias should be negligible.

The second bias (matrix bias) is related to the ionic strength of the medium in which the analytes are dissolved. Since the mobility of the ions is strongly influenced by the conductivity of the sample, even if the concentration of the analytes injected is held constant, the injected amount of the analytes will vary according to the composition of the sample matrix. This bias means that the peak areas obtained for the same ions in samples of different matrices cannot be compared directly. Matrix bias can be minimized or entirely eliminated by using an internal standard for quantitative analysis [24].

For quantitative analysis we have selected Br^- (as its potassium salt) as an internal standard. The mobility of this anion is close to those of the analytes, therefore mobility bias should be negligible. A 1×10^{-4} mol/l concentration of Br^- was used in all measurements. An injection of this pure solution did not show any presence of the analytes studied.

The linearity of the calibration curves was measured by triplicate injections (5 kV, 5 s) of standards containing 1×10^{-4} mol/l Br^- at seven different concentration levels (5×10^{-6} – 5×10^{-4} mol/l). For evaluation of calibration curves, the peak areas of the analytes were divided by the peak area of the internal standard and plotted *vs*. analyte concentration. Table 2 summarizes the results of the calibration curves for all three analytes. As can be observed, valid calibration is demonstrated at least over two orders of magnitude.

The detection limits (LOD) were determined for EK injection of standard solutions containing 1×10^{-4} mol/L KBr at 5 kV for 10 s based on three times the baseline noise and are also summarized in Table 2. It should be noted that LODs can be reduced at least by a factor of 10 when the concentration of KBr in the standard solutions is of the same order as analytes concentration. The LODs achieved with the proposed method were suitable for natural water samples, and no further optimization was performed.

Table 2. **Calibration data and detection limits for ions studied**

Analyte	Equation of regresion lines	Correlation coefficient (r^2)	Detection limit, mol/l
NO_3^-	$y = -0.0023 + 3.49 \times 10^4 c$	0.999	1×10^{-7}
NO_2^-	$y = -0.0055 + 2.53 \times 10^4 c$	0.998	2×10^{-7}
NH_4^+	$y = 0.0567 + 0.86 \times 10^4 c$	0.996	5×10^{-7}

Analysis of water samples. To evaluate the proposed system for real samples, it was applied to the speciation of inorganic nitrogen ions in river waters. Figure 4 shows a typical electropherogram for a river water sample solution.

Several river water samples were analyzed by the proposed CE method and by spectrophotometric methods [2]. The results are compared in Table 3. No nitrite was detected by both methods in any of the samples in this study. As can be seen, the CE method showed good agreement with data obtained from spectrophotometric techniques. It should be noted that other common cations such as sodium,

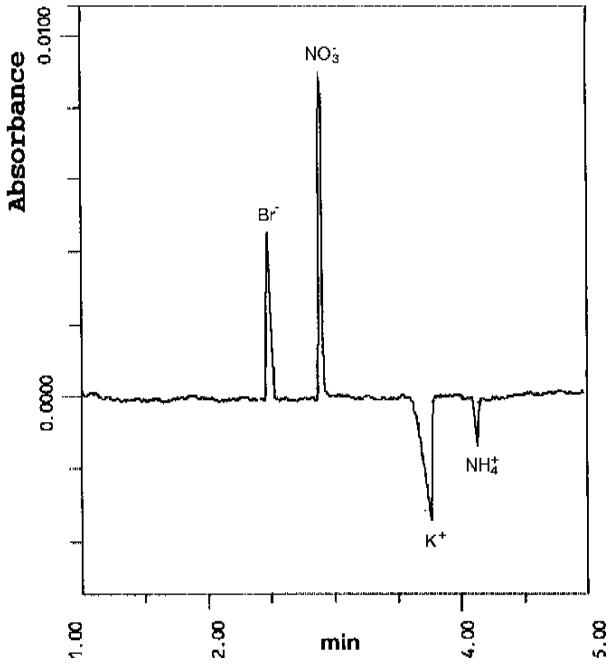

Fig. 4. Example electrophoregram of a river water sample. Conditions as in Figure 3

Table 3. **Data comparison (mg/l) of river water samples (n = 5)**

Sample	Analyte	CE	Spectrophotometry
Neris river water	NO_3^-	18.6 (2.8)[a]	19.8 (2.6)
	NH_4^+	0.21 (5.6)	0.25 (5.1)
Vilnelė river water	NO_3^-	22.5 (2.5)	24.6 (3.1)
	NH_4^+	0.18 (4.8)	0.16 (5.7)
Nevėžis river water	NO_3^-	13.4 (3.0)	12.7 (3.5)
	NH_4^+	0.12 (6.9)	0.15 (6.3)

[a]Values in parentheses are R.S.D. (%).

calcium, magnesium and others migrate slower under conditions used. Consequently, these ions do not interfere with the determination of nitrogen species. The analysis does not require any preliminary treatment of the samples except filtration and addition of the internal standard.

CONCLUSIONS

A new CE system based on the electromigrative sample introduction from both ends of the capillary was developed for single-run determination of nitrate, nitrite and ammonium. Precision of EK injection is improved by using internal standard. The developed CE method is fully suitable for analysis of inorganic nitrogen species in river water samples. The CE method is extremely fast, because it requires one run for all three analytes, provides acceptable precision and can be efficiently used in routine analysis.

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AZOTO FORMŲ NUSTATYMAS UPĖS VANDENYJE KAPILIARINĖS ELEKTROFOREZĖS METODU

S a n t r a u k a

Kapiliarinės elektroforezės metodas optimizuotas azoto formoms atskirti ir nustatyti vieno analizės ciklo metu. Metodas remiasi elektrokinetiniu mėginio įdėjimu į abu kapiliaro galus bei tiesioginiu (NO_3^- ir NO_2^-) ir netiesioginiu (NH_4^+) detektavimu, kai bangos ilgis 214 nm. Ištirta elektrolito pH, koncentracijos bei anijono prigimties įtaka azoto formų atskyrimui. Optimali elektrolito sudėtis: 5 mmol/l $CuCl_2$, 10 mmol/l etilendiaminas, 1 mmol/l trietanolaminas ir 0,05 mmol/l tetradeciltrimetilamonio hidroksidas (pH 8,2). Metodas sėkmingai pritaikytas azoto formoms upės vandenyse greitai nustatyti.

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ОПРЕДЕЛЕНИЕ ФОРМ АЗОТА В РЕЧНОЙ ВОДЕ МЕТОДОМ КАПИЛЛЯРНОГО ЭЛЕКТРОФОРЕЗА

Р е з ю м е

Метод капиллярного электрофореза оптимизирован для одновременного разделения и определения форм азота. Метод основан на электрокинетическом введении пробы в оба конца капилляра, а также на прямом (NO_3^-, NO_2^-) и косвенном (NH_4^+) детектировании при 214 нм. Исследовано влияие pH, концентрации и природы противоиона электролита на разделение форм азота. Оптимальный состав электролита: 5 ммоль/л $CuCl_2$, 10 ммоль/л этилендиамина, 1 ммоль/л триэтаноламина и 0,05 ммоль/л тетрадецилтриметилгидроксид аммония (pH 8,2). Метод успешно применён для быстрого определения форм азота в речной воде.