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Journal of Chromatography B, 657 (1994) 419–423

JOURNAL OF CHROMATOGRAPHY B: BIOMEDICAL APPLICATIONS

# Analysis of nitrate and nitrite in water and urine by capillary zone electrophoresis

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**Abstract**

A capillary zone electrophoresis method for the separation and analysis of nitrate and nitrite in water and urine was developed. No interference in the electropherogram from other anions is observed by using a polyacrylamide-coated column with a modified phosphate buffer at pH 3 for the separation, and UV absorption at 214 nm for the detection. The method does not require sample pretreatment or the use of organic solvents. The limit of detection for each analyte ($S/N = 3$), using a 75 $\mu$m I.D. capillary, is 0.5 $\mu$g/ml. Urine samples require 40-fold dilution in order to maintain migration time reproducibility to within 1% relative standard deviation.

## 1. Introduction

Capillary zone electrophoresis offers fast and efficient separation of inorganic ions [1–4]. In general, it compares favorably with ion chromatography [5]. Detection is a problem in both techniques because most of the inorganic ions are non-UV absorbing. Nitrate and nitrite are, however, UV-absorbing species and they can be monitored with a good degree of sensitivity by direct UV at 214 nm. Nitrite, a meat preservative, reacts *in vivo* with amines and amides to form carcinogenic nitrosamines [6]. A limit of 10 mg nitrate per liter is imposed on drinking water to prevent methemoglobin in infants [7]. Furthermore, the recent interest in the physiological importance of nitric oxide have heightened the need for sensitive methods for the determination of nitrite and nitrate in body fluids [8].

The problem with analyzing for nitrate and nitrite in biological samples by high-performance ion chromatography is the presence of high concentration of chloride ions which interfere with the determination of nitrite due to lack of resolution and column saturation [9,10]. The addition of silver reagents or silver-loaded cation-exchange resins will react with the chloride and leads to its elimination as an interfering compound. However, it is reported that the silver chloride precipitation process causes a substantial reduction in the performance of ion-exchange columns used for anions with insoluble silver salts [9,10].

In this communication we report a fast and sensitive capillary zone electrophoresis (CZE) method for the simultaneous determination of nitrate and nitrite in water and urine without the need for sample clean-up, or the use of organic

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*SSDI* 0378-4347(94)00141-U

solvents. The method is overall simple, rugged, easy to perform and requires no special skills.

## 2. Experimental

### 2.1. Reagents

Solutions were prepared using water purified by Barnstead NANOpure ultrapure water system (Dubuque, IA, USA). Sodium nitrate, sodium nitrite, sodium phosphate (mono- and dibasic), acrylamide and ammonium persulfate were purchased from Fisher Scientific (Fair Lawn, NJ, USA). 3-(N,N-Dimethylmyristylammonio)propanesulfonate (DMMAPS) was obtained from Fluka (Buchs, Switzerland) and Brij-35 (30%) was obtained from Sigma (St. Louis, MO, USA). Methacryloxypropyltrimethoxy silane was purchased from Petrarch Systems (Bristol, PA, USA) and N,N,N′,N′-tetramethylethylene diamine (TEMED) was purchased from Bio-Rad Labs. (Richmond, CA, USA).

### 2.2. Apparatus and method

A Beckman CZE (Model P/ACE) System 2000 equipped with a UV detector, an automatic injector, a fluid-cooled column cartridge and a System Gold data station was used in this study. All runs were carried out at 25°C. The 25 m*M* phosphate buffer, used throughout this study, was prepared daily, passed through 0.2-$\mu$m nylon filters and degassed. The capillary inlet and outlet vials were replenished after every ten injections. For each urine sample to be analyzed, 25 $\mu$l were diluted to 1 ml (1:40, v/v) with distilled, deionized water and passed through a 0.2-$\mu$m nylon filter. Injections were made using the pressure mode for 5 s at 0.5 p.s.i. (1 p.s.i. = 6894.76 Pa). Operating conditions are given in the figure captions.

The columns were coated according to a variation of the Hjertén procedure [11]. A 2 m × 75 $\mu$m I.D. fused-silica capillary was rinsed with water, and filled with 20% $HNO_3$. The column was purged with helium, flame-sealed from both ends and placed in an oven at 200°C for 2 h. After cooling to room temperature the seals were broken and the capillary flushed with 1% $HNO_3$ and water, then placed in a gas chromatography oven at 120°C and purged with helium for 2 h. The capillary was cut into three 60-cm pieces and each piece was treated similarly as follows. A detection window was formed by burning off a small section of the polyimide coating. The column was first silylated with methacryloxypropyltrimethoxy silane, then coated with polyacrylamide. The detailed experimental procedure involved first filling the column with 10% silylating reagent in toluene, when attaching both ends with PTFE tubing. After reacting at 60°C for 2 h, the silane solution was withdrawn. After flushing with toluene and purging with helium at 120°C for 1 h, the capillary was filled with de-aerated 10% (w/v) acrylamide solution containing 1 $\mu$l TEMED and 1 mg ammonium persulfate per ml of solution. After about 30 min, excess polyacrylamide was pushed out of the column using a HPLC pump at 4000 p.s.i.

Mesityl oxide was injected and separated at 30 kV, using a 25 m*M* phosphate buffer at pH 7, but was not detected after 60 min. The mesityl oxide peak was then pushed through the column by low-pressure separation and was detected as it passed by the detector window. This shows that mesityl oxide was moving towards the negative electrode under electroosmotic forces, but that the flow was negligible.

In our experience, the polyacrylamide-coated columns are stable at neutral and acidic pH and the migration times of small ions are reproducible within 1% relative standard deviation (R.S.D.) for at least 100 injections and to within 2% R.S.D. for several hundred injections over several weeks.

## 3. Results and discussion

Fig. 1 shows the separation of nitrate and nitrite at pH 3. UV-absorption detection at 214 nm was chosen because both analytes have reasonably high extinction coefficients at this wavelength, while other inorganic ions are not

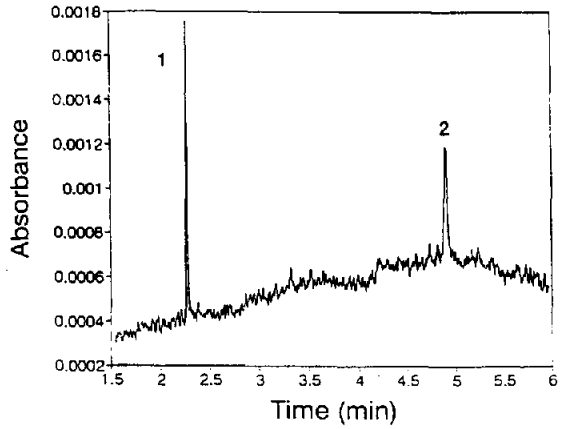


Fig. 1. Electropherogram of the separation of nitrate and nitrite at pH 3. Buffer; 25 m*M* phosphate containing 0.5% DMMAPS and 1.0% Brij-35; Applied voltage: −15 kV; Column: 10% T, polyacrylamide-coated fused-silica [T = (g acrylamide + g N,N′-methylenebisacrylamide)/100 ml solution]; column dimensions: $L_{total}$ = 57 cm, $L_{detection}$ = 50 cm, I.D. = 75 μm; instrument: Beckman Model P/ACE System 2000. Detection: 214 nm. Solutes: 1 = Nitrate (2.5 μg/ml); 2 = Nitrite (2.5 μg/ml).

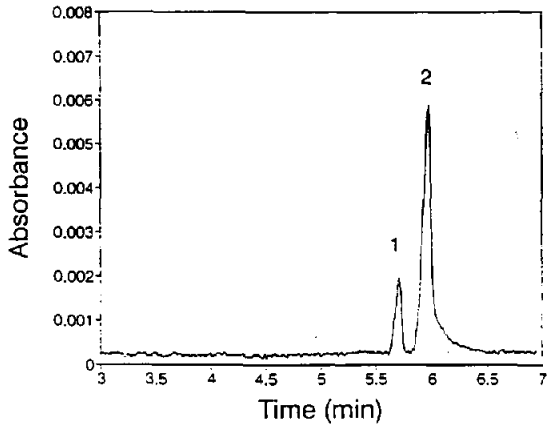


Fig. 2. Electropherogram for the separation of nitrite (1) and nitrate (2) at pH 7. Buffer: 25 m*M* phosphate; other conditions as in Fig. 1.

detected. Organic ions that may be present in urine and absorb at 214 nm, such as oxalate and citrate, do not cause any interference problems in this method presumably because they are protonated at this pH. Positively charged and neutral solutes do not migrate through the column because of the zero electroosmotic flow environment accomplished by the use of polyacrylamide-coated column. Several variations on the experimental procedure, such as changing the applied voltage, pH, buffer type and concentration and the addition of buffer modifiers (sodium dodecyl sulfate, cetyltrimethylammonium bromide, cyclodextrins, zwitterionics) were evaluated.

The use of phosphate buffer at pH 3 rather than acetate buffer at pH 4–5 improved the detection sensitivity because phosphate absorbs less energy at 214 nm. At pH 3, nitrite migrates towards the anode much slower than nitrate (see Fig. 1) because nitrous acid is weaker than nitric acid and nitrite is partially protonated at this pH. As the pH is increased, nitrite is deprotonated and its migration velocity is increased relative to nitrate. At pH 7, nitrite is completely deprotonated and migrates faster than nitrate (Fig. 2), because it has a smaller size and, consequently, a larger charge-to-size ratio.

In this work, the analysis of nitrate and nitrite was carried out at pH 3 in order to avoid any possible peak overlap, especially if small amounts of one component are to be analyzed in the presence of large excess of the second component. Fig. 2 shows signs of solute adsorption to the capillary wall as manifested in the tailing of the nitrate peak. The addition of 0.5% DMMAPS and 1.0% Brij-35 to the phosphate buffer eliminated this tailing problem as demonstrated in the symmetric peak shapes shown in Fig. 1.

Fig. 3 gives the electropherogram obtained from the direct injection of a well-water sample. The amount of nitrate present in this sample is determined to be 6.9 μg/ml. Because of the low limit of quantitation of this method for water samples (0.5 μg/ml), levels above that imposed on drinking water [7] can be easily monitored.

The analysis of nitrate and nitrite in urine samples by CZE is a delicate problem. If urine is directly injected in a capillary, proteins and other biomolecules in the urine matrix will adsorb to the wall and quickly deteriorate the column performance. Column deterioration is manifested in sample loss, peak asymmetry and shifts in migration times. Also a high chloride ion concentration affects nitrate ion peak position and shape by an isotachophoretic mecha-

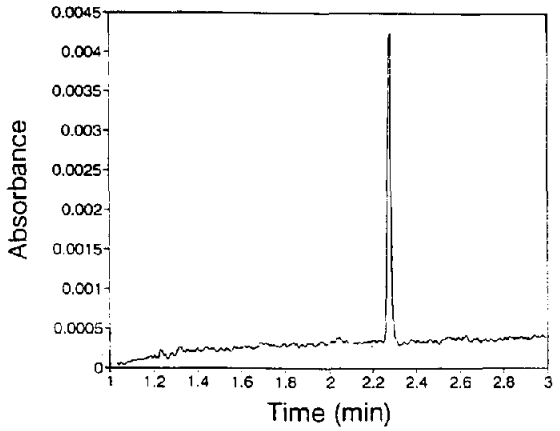


Fig. 3. Electropherogram of a well water sample. Conditions as in Fig. 1.

nism. Urine samples must, therefore, be diluted but not so much as to dilute the analytes of interest beyond their detection limit. As a result of experimentation with different dilution ratios, we observed that a forty-fold dilution of urine samples is needed to keep the column free of adverse matrix effects over extended periods of time. During the course of this work, a single column was used daily (about 30 injections per day) for a period of two weeks without showing any signs of deterioration. The column was rinsed with buffer between injections and rinsed once, at the end of each day, with 0.1 *M* NaOH and water.

Fig. 4 is the electropherogram obtained from the injection of a nitrite-free rat urine sample

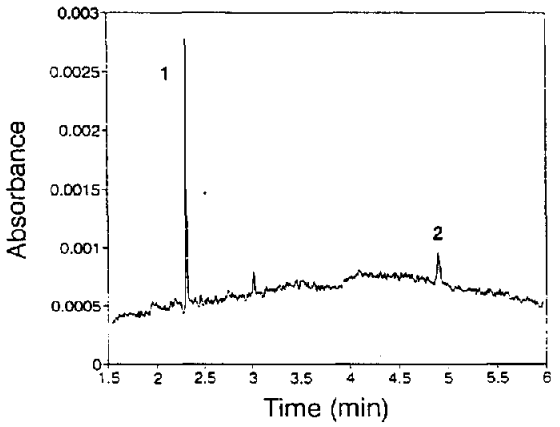


Fig. 4. Electropherogram of a rat urine sample spiked with 50 μg/ml nitrite then diluted 40:1. Conditions as in Fig. 1.

that was spiked with 50 μg/ml nitrite, then diluted forty-fold. The nitrite peak shown in Fig. 4 represents a concentration of 1.25 μg/ml in the injected sample. The concentration of nitrate in the original sample was determined to be 317 ± 6 μg/ml.

The determination of analyte concentrations was done as follows. Standard solutions of nitrate and nitrite in water were prepared at the following concentration: 0.05, 1.0, 2.5, 5.0 and 10.0 μg/ml. Each sample was injected 7 times. The injection time of 5 s at 0.5 p.s.i. was found to be optimal. The migration times for each analyte were reproducible to within 1% R.S.D., while the R.S.D.s for the peak areas were: 1.2% at 10 μg/ml, 3.2% at 5 μg/ml, and 9.5 at 1 μg/ml. The calibration curve was linear over the range 1–10 μg/ml. The R.S.D.s for diluted urine samples were within the same limits presented for water.

## 4. Conclusions

A simple, sensitive and fast CZE method is developed for the analysis of nitrate and nitrite in water and urine samples. The method does not require sample clean-up or the use of organic solvents. The limit of detection for each solutes is 0.5 μg/ml. Urine samples have to be diluted in order to maintain reproducibility of migration times and peak areas. The limit of quantitation of each solute in urine is determined to be 25 μg/ml at 10% R.S.D. This limit of quantitation is comparable to that obtained by ion chromatography using conductivity detection. The method can be easily adapted for the analysis of nitrate and nitrite in serum samples.

## 5. Disclaimer

The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the US Government.

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