HUNGARIAN JOURNAL OF
INDUSTRY AND CHEMISTRY
Vol. 53(2) pp. 73–78 (2025)
hjic.mk.uni-pannon.hu
DOI: 10.33927/hjic-2025-20

NITRITE AND NITRATE ANALYSIS BY CAPILLARY ELECTROPHORESIS WITH DIRECT UV ABSORBANCE DETECTION: A MINI-REVIEW

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The analysis of nitrite and nitrate ions is of great importance in environmental samples, food and various human samples. In contrast to conventional spectrophotometric methods, nitrite and nitrate ions are relatively easy to analyze by capillary electrophoresis as both are negatively charged over a wide pH range as well as absorb over a broad UV range so that they can be analyzed simultaneously by capillary electrophoresis. In this paper, different separation strategies for the analysis of nitrite and nitrate by capillary electrophoresis with direct UV absorbance detection are presented.

Keywords: capillary electrophoresis, nitrite, nitrate, analysis

1. Introduction

The analysis of nitrite (NO_2^-) and nitrate (NO_3^-) ions is of great importance in several aspects, especially in the fields of food safety [1], environmental monitoring [2] and protection [3], drinking water quality management [4] as well as human health [5].

Monitoring nitrate levels in ambient water samples is important for environmental protection as high levels contribute to eutrophication, which reduces the oxygen content of natural bodies of fresh water through algal overgrowth [3]. Nitrate and nitrite are harmful in high concentrations. In the human body, nitrite converts hemoglobin into methemoglobin, which cannot transport oxygen [6], moreover, is also converted in the body into nitrosamines, which are carcinogenic [7]. Nitrite is often used to preserve meat products so monitoring its limit exceedance is a major analytical problem [8]. Nitric oxide (NO), produced in the body, is a vasodilator signal transduction molecule that is not stored, making its direct diagnostic measurement difficult. However, its degradation products (nitrite and nitrate) can be measured, so that the excessive production of nitric oxide can be monitored by determining nitrite and nitrate concentrations in human serum [9]. Since nitrite and nitrate molecules are excreted in urine, their concentrations can also be an indicator of some diseases [10].

The most commonly used methods for nitrate determination can be classified into three types based on their underlying analytical approaches: (1) direct determination (colorimetry, potentiometry, UV absorption and chromatography); (2) reduction of nitrate with ammonium or nitric oxide followed by detection of the concentration of the product (colorimetry, potentiometry, fluorimetry and coulometry); (3) a color-variable complexation reaction of the nitrate [11]. The most widely used method for the determination of nitrite is the Griess test, which is based on the formation of an azo dye in the presence of nitrite in an azo coupling reaction, which allows quantification by spectrophotometric measurement [12].

Nitrite and nitrate have direct absorbance in the UV range 190-250 nm and a single negative charge over a wide pH range, so they can be simultaneously analyzed by the capillary electrophoresis (CE) separation method. In this mini-review paper, capillary electrophoresis separation methods using direct UV absorbance detection for the analysis of nitrite and nitrate ions are presented.

2. Basics of nitrite and nitrate separation by CE

Nitrite and nitrate are relatively small ions, so capillary zone electrophoresis (CZE) is an effective method for

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Received: 18 Aug 2025; Revised: 22 Aug 2025;
Accepted: 22 Aug 2025
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separating these analytes. The electrophoretic mobility of nitrite and nitrate ions is determined by their hydrodynamic volume to charge ratio. Both ions have a negative charge over a wide pH range (~pH > 4), so their separation is due to the difference in their hydrodynamic volume within this pH range. Most capillary electrophoresis methods use this concept to facilitate separation, because the high separation power of CE provides an efficient way to separate such compounds over relatively short analysis times [13]-[25].

The difference in mobility can be significantly increased if the pH is reduced below 3.5, since the pK_a of nitrite is 3.15. Therefore, the mobility of nitrite is significantly reduced while that of nitrate remains unchanged ($pK_a = -1.3$), resulting in better separation, i.e. higher resolution. Given that its decreased mobility also means that the nitrite and nitrate peaks are shifted in the electropherogram, the migration time of the nitrate peak is shorter as reported in [26]-[30]. These methods are highly sensitive to the pH of the background electrolyte (BGE), moreover, even a small alteration can significantly affect the electrophoretic mobility of nitrite. On the other hand, nitrate is less affected, so the relative migration times of the sample components can vary significantly. Different methods published in the literature based on capillary zone electrophoresis to determine the nitrite and nitrate by absorbance detection are listed in *Table 1*. The sample types, capillary dimensions, separation medium, possible methods for modifying electroosmotic flow (EOF), injection methods, detection wavelength, limit of detection (LOD) values, separation voltage and analysis time are all compared.

3. Electroosmotic flow modification

Electroosmotic flow is often an effective method to improve CE separation. The phenomenon is based on the fact that the silanol groups on the inner wall of the bare fused-silica (BFS) capillary are deprotonated at higher pHs (> 4), so the positively charged buffer components electrostatically attach to the negative surface of the inner capillary wall. The positive layer migrates towards the cathode under the applied electric field, creating a bulk flow across the entire cross-section of the capillary [31].

The negatively charged sample components migrate towards the detector window under reversed polarity, that is, the cathode on the injection side, resulting in upstream EOF (flow towards the inlet) in the BFS capillary. This increases the migration time of the sample components and can also cause peak broadening by increasing electromigration dispersion. To reverse the direction of the EOF in a BFS capillary, it is possible to coat the inner wall of the capillary with temporarily or permanently positively charged materials. In the reviewed publications, cetrimonium chloride (CTAC) [14,18,27]; didodecyldimethylammonium bromide (DDAB) [26]; OFM-OH$^-$ [15,17,19,20,22,23]; tetradecyltrimethylammonium bromide (TTAB) [24],[25]; tetradecyltrimethylammonium hydroxide (TTAOH) [29] and spermine [28] were used as dynamic EOF modifiers. In one case, a polyethyleneimine (PEI) coating as a permanent EOF modifier was used [13]. In another publication, the inner wall of the capillary was protonated in a highly acidic medium, reversing the direction of the EOF [30]. Most publications demonstrate a method that reduces the migration time by reversing the direction of the EOF while still achieving the baseline separation of nitrite and nitrate due to the high efficiency of CE [13-15,17-20,22-30].

Studies have been published where the direction of the EOF remains unchanged so that the upstream EOF is able to remove the large sample matrix from the capillary inlet end, making the degree of conductivity during separation more consistent despite the large volume hydrodynamically injected [16],[21].

4. Sample types

Nitrite and nitrate content were determined on several types of samples such as biological samples (rat brain [20],[27]; human plasma [15,20,21,23-25]; human serum [17]; human urine [17],[19]; human extracellular fluids [25] and food samples [13,22,30]), water samples (drinking water [18]; environmental water [16]) and artificially produced samples [14,26,29].

For liquid samples, with the exception of filtration before separation, sample preparation is by and large unnecessary [16],[18]. Nitrite and nitrate are often extracted from solid samples after homogenization and filtration [13,22,30].

5. Quantitative analysis

One advantage of CZE separation is that given the relatively low concentration of buffer solutions, their viscosity is basically equal to that of water. As a result, they can be efficiently injected hydrodynamically (or hydrostatically) from a sample matrix of similar viscosity, obtaining a high degree of reproducibility. The peak areas of the sample components obtained from the electropherogram are proportional to the volume of the injected sample matrix, so that quantification of the analytes is possible using their areas directly after appropriate calibration curves are recorded.

Although electrokinetic injection often results in a much better LOD (up to nanomolar concentrations [14]), this is not always advantageous in terms of quantification due to conductivity-dependent electrophoretic mobility of analytes. Depending on the conductivity of the sample, different amounts of nitrite and nitrate ions enter the capillary during electrokinetic injection because of the differences in mobility between these ions and also due to the different degree of voltage drop at the boundary between the sample plug and the BGE capillary. Thus, samples containing the same concentration of nitrite and nitrate may have different peak areas, preventing direct concentration determination if electrokinetically injected [32].

Table 1: Separation strategies of nitrate and nitrite by capillary electrophoresis

Sample type	Capillary	Separation media	EOF modifier (reverse EOF)	Sample injection	Detection (nm)	Limit of detection	Sep. volt. (kV)	Sep. time (min)	Ref.
Meat products, vegetables	75 cm (60 cm eff.) 75 µm I.D.	20 mM Tris-HCl; pH 7.5	PEI coating	4 kPa 10 s	210	1.6 µM nitrate; 2.3 µM nitrite	- 28	2.5	[13]
Reagent grade solution	50 cm (45.5 cm eff.) 50 µm I.D.	20 mM tetraborate; pH 8.94	1.1 mM CTAC	(a) 6.9 kPa 7 s; (b) - 7.5 kV 5 s	200	(a) 6.45 µM nitrate; 8.7 µM nitrite; (b) 16.1 nM nitrate; 21.7 nM nitrite	- 10	6	[14]
Human plasma	72 cm; 75 µm I.D.	25 mM sodium phosphate	5% OFM-OH^-	- 6 kV 20 s	214	50 µM nitrate; 50 µM nitrite	- 21.6	N/A	[15]
Environmental water	60 cm (50 cm eff.) 50 µm I.D.	75 mM Tris-HCl; pH 7.25	-	55 kPa 8 s	200	0.21 µM nitrate; 0.43 µM nitrite	- 30	4.5	[16]
Human urine, human serum	60 cm; 100 µm I.D.	15 mM sulfate; pH 8.0,	2.5% OFM-OH^-	10 cm 30 s (hydrostat.)	214	10 mM nitrate	- 15	4	[17]
Drinking water	62 cm (53.5 cm eff.) 75 µm I.D.	50 mM Tris-HCl; pH 7	0.1 mM CTAC	(a) 3 kPa 3 s (b) - 5 kV 15 s	200	(a) 5.3 µM nitrate 14.4 µM nitrite; (b) 160 nM nitrate 65 nM nitrite	- 20	2.2	[18]
Human urine	104 cm eff.; 75 µm I.D.	25 mM sodium sulfate$^-$; pH 4.8	5% OFM-OH^-	3 kPa 30 s - 6 kV 20 s	214	N/A	- 30	5	[19]
Human plasma; rat brain	60 cm; 100 µm I.D.	10 mM sodium sulphate	2.5% OFM-OH^-	10 cm 20 s (hydrostat.)	214	0.4 µM nitrate; 0.54 µM nitrite	- 15	3.1	[20]
Plasma and cerebrospinal fluid (CSF)	36.5 cm (30 cm eff.) 75 µm I.D.	10 mM borate; pH 10	-	3.5 kPa 5 s	214	0.43 µM nitrate; 0.57 µM nitrite	- 15	5.2	[21]

Table 1 (continued): Separation strategies of nitrate and nitrite by capillary electrophoresis

Sample type	Capillary	Separation media	EOF modifier (reverse EOF)	Sample injection	Detection (nm)	Limit of detection	Sep. volt. (kV)	Sep. time (min)	Ref.
Range of food (cheese, cabbage, puree, fruit juice, water, variety of meat products)	75 cm (50 cm eff.) 75 μm I.D.	1540 ppm NaCl (1000 ppm chloride) electrolyte	0.5 mM OFM-OH$^-$	- 20 kPa•s	210	N/A	- 20	2	[22]
Human plasma	65 cm 75 μm I.D.	750 mM NaCl	5% OFM-OH$^-$	10 cm 90 s (hydrostat.)	214	1.6 μM nitrate	- 20	15.5	[23]
Human plasma	67 cm (60 cm eff.) 50 μm I.D.	200 mM lithium chloride; 5 mM triethylamine	0.7 mM TTAB	3.5 kPa 5 s	200	N/A	- 17	4.5	[24]
Human extracellular fluids	47 cm (40 cm eff.) 50 μm I.D.	5 mM Tris-HCl; 150 mM sodium chloride, pH 7.4	2 mM TTAB	N/A	214	N/A	- 10	3.2	[25]
Reagent grade solution	30 cm (7 cm eff.) 50 μm I.D.	20 mM phosphate; pH 2.5	0.10 mM DDAB	- 5 kV 5 s	214	100 nM nitrate; 1 μM nitrite	- 30	0.17	[26]
Rat brain	40 cm (22 cm eff.); 75 μm I.D.	20 mM phosphate; pH 3.5	2 mM CTAC	- 16 kV 2 s	214	2.86 μM nitrate; 0.96 μM nitrite	- 10	1.5	[27]
Rat plasma	72 cm (22 cm eff.) 75 μm I.D.	50 mM phosphate; pH 3	0.5 mM spermine	17 kPa 1.5 s	214	6.0 μM nitrate; 10 μM nitrite	- 25	3.5	[28]
Rat airway surface liquid (ASL)	72 cm (22 cm eff.) 50 μm I.D.	50 mM phosphate; pH 3	0.5 mM spermine	17 kPa 1.5 s	214	10 μM nitrate; 30 μM nitrite	- 26	6.0	[28]
Reagent grade solution	85 cm (50 cm eff.) 75 μm I.D.	10 mM imidazole-sulfate; 2 mM 18-crown-6; pH 3.8	0.05 mM TTAOH	- 5 kV 5 s	214	N/A	- 30	2.75	[29]
Vegetables (spinach, chard, carrots)	32.5 cm (21.5 cm eff.) 75 μm I.D.	50 mM phosphate; pH 2.5	Reverse EOF due to the acidic buffer	N/A	214	4.8 μM nitrate; 7.4 μM nitrite	- 25	1.9	[30]

In most publications reviewed in this paper, linear calibration equations covering several orders of magnitude with R^2 fit >0.99 assumed for the two sample components [13,14,18-20,23-27,30].

6. Conclusions

The need for the quantification of nitrite and nitrate in the food, environmental protection and diagnostics industries requires the development of sensitive and high-resolution analytical methods. Capillary zone electrophoresis with UV absorbance detection may offer a promising alternative to conventional spectrophotometric methods for determining nitrite and nitrate content which rely on color-change reactions. The great advantages of CZE are extremely short analysis times, few necessary resources for sample preparation, excellent LOD values, the automation capability and the simultaneous determination of the two sample components. In this mini-review, the most popular methods for the CZE-based analysis of nitrite and nitrate by UV detection have been presented.

ABBREVIATIONS

BFS	Bare-fused silica
BGE	Background electrolyte
CE	Capillary electrophoresis
CTAC	Cetrimonium chloride
CZE	Capillary zone electrophoresis
DDAB	Didodecyldimethylammonium bromide
EOF	Electroosmotic flow
I.D.	Inner diameter
LOD	Limit of detection
PEI	Polyethyleneimine
TTAB	Tetradecyltrimethylammonium bromide
TTAOH	Tetradecyltrimethylammonium hydroxide

Acknowledgement

The authors gratefully acknowledge the support of the University of Debrecen Program for Scientific Publications and the TudFin grant #1G3DBK0CTUF247 as well as the ATBG Korea V4 joint project (#2023-1.2.1-ERA_NET-2023-00015), University Research Fellowship Programme (2024-2.1.1-EKÖP), and the ADVANCED_150780 grant from the National Research Development and Innovation Office of Hungary. This is contribution #225 from the Horváth Csaba Memorial Laboratory of Bioseparation Sciences. The research presented in the article was carried out within the framework of the Széchenyi Plan Plus program with the support of the RRF 2.3.1-21-2022-00008 project.

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