

# Application of SPE Technique Using a Newly Obtained Sorbent Based on Silica Gel Covered with Polyaniline to Simultaneous Determination of Nitrate (III) and Nitrate (V) Anions in Water Samples

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## Abstract

Nitrate (III) and nitrate (V) often occur together in many environmental samples; however, high concentrations of other ions, especially chloride, make difficult the correct integration of the peaks. The newly obtained sorbent, based on silica gel modified with polyaniline, was successfully used as a solid phase in SPE technique to remove the undesirable matrix effect.

Commercially available bottled water samples with various contents of mineral components after purification on SPE column were determined using an ion chromatograph DX-500 IC (Dionex USA) with a conductometric detector. As eluent, aqueous solution containing 3.5 mM (0.37 g/L) of  $\text{Na}_2\text{CO}_3$  and 1 mM (0.084 g/L) of  $\text{NaHCO}_3$  was used. Amounts of anions in all investigated samples of water ranged from 1.71 to 22.33 mg/L for nitrate (V) and 0.04-0.10 mg/L for nitrate (III). These values were within Polish standards.

**Keywords:** SPE, ion chromatography (IC), sorbent with polyaniline (PANI), nitrate (III), nitrate (V)

## Introduction

Nitrate (III) and nitrate (V) are commonly occurring forms of nitrogen found in many environmental samples. These anions are formed during decomposition of plants and other organic residues, but their main sources are municipal and industrial wastewater and artificial fertilizers [1]. Nitrates (III) also are used as preservatives in the food industry.

In moderate amounts, nitrogen ions are harmless constituents of food and water. Their high concentrations however, can cause serious problems. Nitrates (III) convert hemoglobin to methemoglobin. This results a reduced oxy-

gen supply to tissues. The main symptoms of methemoglobinemia are bluish color of the skin, headache, dizziness, weakness, and difficulty in breathing. The potential health hazard depends on the individual's reaction. Infants, pregnant women, and adults with reduced stomach acidity are particularly exposed to nitrate (III)-induced methemoglobinemia [2, 3].

An estimated dose of nitrates consumed by humans ranges from 75 to 100 mg daily. The main sources of these ions are food products (80-90%), in particular vegetables, preserved meat, and drinking water (5-10%) [4-6]. The admissible concentration of nitrates (V) and nitrates (III) in drinking water according to Polish regulations is 50 mg/L and 0.5 mg/L, respectively [7].

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The analysis of these ions has been reported in many publications [8-11]. The main methods used for this purpose were electroanalytical techniques, including potentiometric, voltamperometric, amperometric, coulometric, and spectrophotometric (UV/Vis, IR), but ion chromatography (IC) due to many advantages such as short time of analysis, reproducibility, and high sensitivity, is particularly recommended for separation and simultaneous determination of nitrate (V) and nitrate (III) [12-16]. However, environmental samples usually contain chloride ions in concentrations a few times higher than nitrate (III) ions ("matrix effect"), and the proper separation of these ions is the main analytical problem in ion chromatography.

The aim of this study was elaboration of the procedure of sample purification to avoid the matrix effect of chloride ions and determination of the content of nitrate (V) and nitrate (III) in various commercially available drinking waters.

## Experimental Procedures

### Chemicals and Standard Solutions

Reagents: NaHCO<sub>3</sub>, Na<sub>2</sub>CO<sub>3</sub>, AgNO<sub>3</sub>, ammonium persulphate, acetonitrile, chloroform, methanol, and standards of nitrate (V) and nitrate (III) were of analytical grade from Merck (Darmstadt, Germany). Acid Alizarin Violet N (Aldrich-Chemie, Steinheim, Germany) was purified by recrystallization from methanol. Water was redistilled and deionized using the EasyPure RF system (Bearnsted, USA). The efficiency of this process was checked conductometrically; the resistance of water was 18 MΩ/cm. The analyzed samples of mineral water were purchased at a local market. The standard solutions in the concentration range from 0.001 to 5 mg/L and were prepared by dilution of stock solution (1000 mg/L).

### SPE Column Preparation

Aniline was distilled under the partial vacuum on the QuickFit apparatus (Great Britain). 30.0 g of the deaerated silica gel LiChrosorb Si 100 particle size 30 μm (Merck, Darmstadt Germany) was placed in a 500 mL flask and mixed with 200 mL of 0.2 M aniline hydrochloride solution and 50 mL of 1 M hydrochloric acid solution.

The flask was immersed in a water bath filled with ice. When the temperature reached 2°C, the slow addition of ammonium persulphate solution was started.

Temperature of the reactionary mixture should not exceed 4°C (the reaction is exothermic); otherwise the low-weight oligomers of aniline were present.

Short-chain polymers of aniline are soluble in acetonitrile, chloroform and methanol; therefore the obtained sorbent was washed several times with small portions of these solvents.

A portion of 1 g of silica gel with polyaniline was placed in a small polypropylene column (55 mm × 5 mm) (J. T. Baker, Phillipsburg, NJ, USA) and was impregnated with 10 mL 0.001 M aqueous solution of Acid Alizarin

Violet N. Then the column was washed with distilled water and aqueous solutions of hydrochloric acid of increasing concentrations to remove excess of the chelating reagent. Next, 20 mL of 0.001 M aqueous solution of AgNO<sub>3</sub> was applied, and the prepared sorbent with chelated silver ions was washed with distilled water and aqueous solutions of hydrochloric acid once again

### Sample Preparation

The samples of commercially available bottled waters were previously degassed using an ultrasonic bath and purified by SPE technique on the chelating sorbent based on silica gel modified with polyaniline to remove bromide and chloride ions. Volume of the sample was 2 mL and flow rate of SPE procedure was 1 mL/min.

### HPLC Condition

The samples were analyzed using an ion chromatograph DX-500 IC (Dionex, USA) with a conductometric detector and suppression of the eluent background in the recirculation mode system. Producer's precolumn AG14A and AS14A column, suppressor ASRS, and sample 25 μL loop were used. As eluent, aqueous solution containing 3.5 mM (0.37 g/L) of Na<sub>2</sub>CO<sub>3</sub> and 1 mM (0.084 g/L) of NaHCO<sub>3</sub> was used. The flow rate was 0.3 mL/min and temperature was 30°C.

## Results

In our investigations SPE technique was successfully used to remove the undesirable matrix effect. The samples of commercially available waters were purified by SPE technique on a sorbent based on silica gel modified with polyaniline to remove bromide and chloride ions (Fig. 1).

Determination of nitrate (III) and nitrate (V) anions was performed via ion chromatography (IC).

The appropriate regression equation to quantification was selected on the basis of the *F* statistic values expressed in formula [17]:

$$F = \frac{R^2}{1 - R^2} \cdot \frac{n - k}{k - 1}$$

...where: *R* – correlation of determination, *n* – number of measurements, *k* – number of explanatory parameters.

The *F* values for different types of regression equations (Table 1) indicate that linear regression is optimal for nitrite, and equation  $y = bx + cx^2$  is better for nitrate.

Table 1. The values of *F* statistics of different regression equations for nitrate (V) and nitrate (III).

Equation	NO <sub>2</sub> <sup>-</sup>	NO <sub>3</sub> <sup>-</sup>
$y = a + bx$	<b>17,308</b>	23,196
$y = a + bx + cx^2$	8,450	134,898
$y = bx$	15,319	24,076
$y = bx + cx^2$	14,657	<b>201,130</b>

Table 2. Linear regression data and validation parameters to quantification of nitrate (V) and nitrate (III).

Parameter	$W_i = \frac{n \cdot s_i^{-2}}{\sum_{i=1}^n s_i^{-2}}$	Standard deviation	$t = W/s$	95% confidence interval		$P > t$	LOD	LOQ
$\text{NO}_2^-$		$y = a + bx$		$R^2 = 0.9987$				
$a$	-0.0049	0.0024	-2.00	-0.0010	0.0002	0.059	0.010	0.04
$b$	0.3745	0.0028	131.56	0.3686	0.3804	0		
$\text{NO}_3^-$		$y = bx + cx^2$		$R^2 = 0.9999$				
$b$	0.3696	0.0050	74.44	0.3594	0.3799	0	0.006	0.02
$c$	0.000964	0.000072	13.32	0.000814	0.001113	0		

The linear regression data and validation parameters such as relative standard deviation, confidence interval, LOD and LOQ are summarized in Table 2. The example of calibration curve obtained for nitrate ions is presented in Fig. 2.

The amounts of determined anions in all investigated samples of commercially available bottled water ranged from 1.71 to 22.33 mg/L for nitrate (V) and 0.04-0.10 mg/L for nitrate (III) (Table 3).

### Discussion of Results

Drinking water is a source of nitrogen ions in the human diet. Their content should be monitored, especially nitrates

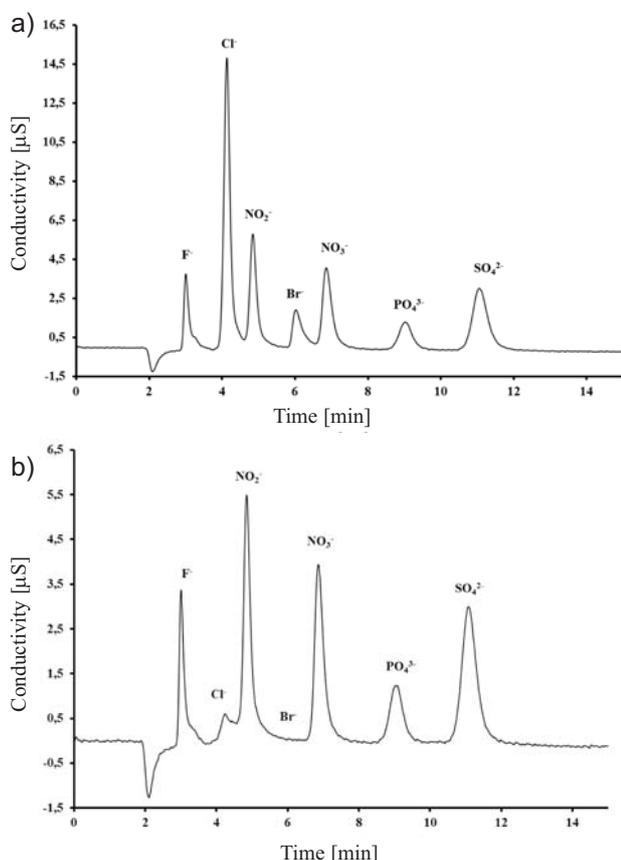


Fig. 1. The example of a chromatogram of investigated water before (a) and after (b) SPE purification.

Table 3. The content of nitrate (V) and nitrate (III) in analyzed bottled waters.

Sample of the water	The content (mg/L)	
	$\text{NO}_2^-$	$\text{NO}_3^-$
	Determined $\pm$ SD (n=5)	Determined $\pm$ SD (n=5)
“Nałęczowianka” carbonated	0.05 $\pm$ 0.01	4.00 $\pm$ 0.48
“Nałęczowianka” noncarbonated	0.08 $\pm$ 0.01	5.52 $\pm$ 0.66
“Cisowianka” carbonated	0.08 $\pm$ 0.01	1.71 $\pm$ 0.20
“Cisowianka” noncarbonated	0.07 $\pm$ 0.01	1.91 $\pm$ 0.23
“Muszynianka”	0.10 $\pm$ 0.02	6.32 $\pm$ 0.76
“Vita” carbonated	0.10 $\pm$ 0.01	10.01 $\pm$ 1.20
“Kropla Beskidu” noncarbonated	0.05 $\pm$ 0.01	7.26 $\pm$ 0.87
“Mazowszanka”	0.09 $\pm$ 0.02	9.40 $\pm$ 1.13
“Arctic” carbonated	0.05 $\pm$ 0.01	6.22 $\pm$ 0.75
“Arctic” noncarbonated	0.04 $\pm$ 0.01	6.03 $\pm$ 0.72
“Primavera” noncarbonated	0.06 $\pm$ 0.01	10.11 $\pm$ 1.21
“Woda Maksymiliana”	0.08 $\pm$ 0.02	18.13 $\pm$ 2.18
“Oaza” carbonated	0.04 $\pm$ 0.01	20.01 $\pm$ 2.41
“Oaza” noncarbonated	0.04 $\pm$ 0.01	22.33 $\pm$ 2.68

(III), because they are toxic in large amounts. According to Polish regulations the maximum permissible limit of nitrate (III) in drinking water is 0.5 mg/L [7]. The presence of nitrate (III) in various amounts was observed in all samples of bottled water and ranged from 0.04 to 0.10 mg/L. These values did not exceed Polish standards. The amounts of nitrate (V) ranged from 1.71 to 22.33 mg/L. The highest total amount of nitrogen ions was determined in “Oaza” water, while the lowest content was in “Cisowianka” water.

Calculation of  $F$  statistics was necessary to select the appropriate equation for quantitative analysis. The higher values of  $F$  indicate a better matching of data.  $F$  parameter was calculated for four different types of regression

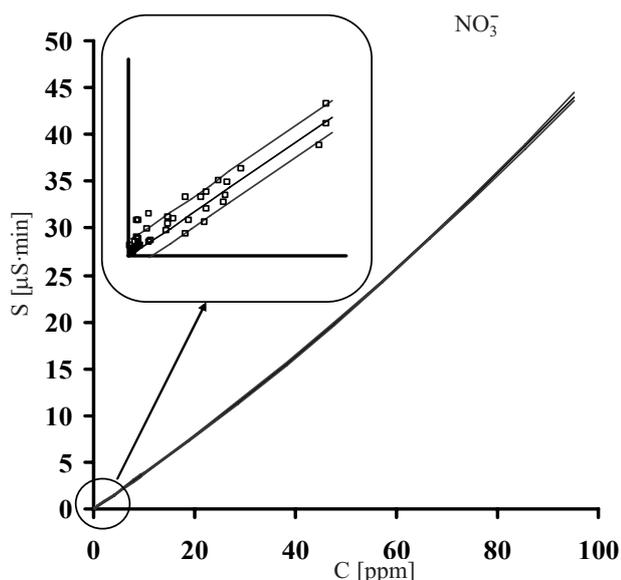


Fig. 2. Calibration curve for nitrate (V) ions.

equations (Table 1). The highest values of  $F$  for nitrates (III) were obtained for the linear regression; however, for nitrate (V), the higher value of  $F$  was for quadratic equation ( $y=bx+cx^2$ ). The established validation parameters such as: linearity, limit of detection, limit of quantification, and precision (Table 1) show that ion chromatography can be used in the routine monitoring of nitrate (V) and nitrate (III).

IC is particularly recommended for speciation analysis of nitrate (V) and nitrate (III) ions, but the high amount of the other ions, especially chloride or bromide (matrix effect), in water samples are the main analytical problem. The high concentration of halogenides ( $\text{Cl}^-$ ,  $\text{Br}^-$ ) makes difficult the correct integration of peaks of nitrate (V) and nitrate (III) ions (Fig. 3). The newly obtained sorbent based on silica gel covered with polyaniline containing chelated silver cations was used to eliminate interfering ions. Chloride and bromide ions were successfully retained during SPE purification of investigated bottled water. Preparing this sorbent is relatively simple and inexpensive and it can be an alternative for commercially available SPE column. The advantage of silica gel coated with a film of

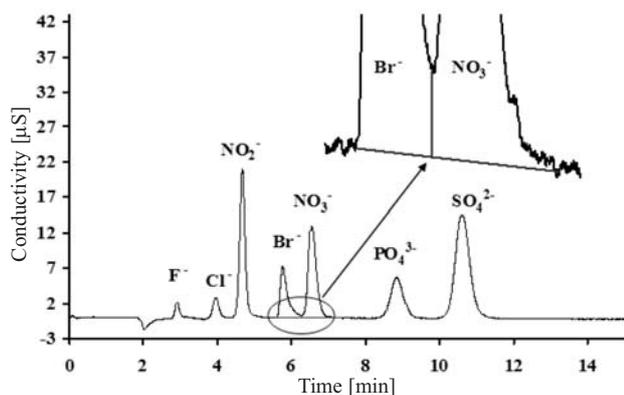


Fig. 3. Example of difficulty with correct integration of the peaks.

polyaniline is its resistance to wide pH range [18], thus it is easy to regenerate and can be used several times in the SPE technique.

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