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A New, Fast and Sensitive Polarographic Method for the Determination of Trace Amounts of Nitrate and Application to Raw Potatoes

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ABSTRACT

Nitrate in food stuff can be reduced to nitrite and may form nitrosamines which are known as carcinogenic substances. Thus, its concentration has to be controlled by highly sensitive methods. In this work a new and fast differential pulse polarographic (DPP) method is established for the determination of nitrate. According to the reduction potentials V(II) was found most suitable for the reduction of nitrate. The product of this reaction is V(III) and its polarographic peak is high and sharp, it also displays very good performance in regard to reproducibility. Thus, with this method it is possible to determine low concentrations of nitrate. This reaction has been used first time in this work. The optimum conditions for the quantitative reaction between nitrate and V(II) has been studied. This method is applied to potato samples and nitrate is determined.

***Key words**: Trace Determination, Nitrate, Differential Puls Polarography, Potato.*

1. INTRODUCTION

Nitrate is an important nitrogen source for plants taken from soil as nutrient. It occurs in abundance because of the use of nitrate salts as fertilizer. As a result, nitrate is found in all fruits and vegetables as a natural compound. It can be reduced to nitrite which may interact with amines to form nitrosamines which are known as carcinogenic substances. Thus, even its trace concentration in food-stuff has to be controlled by highly sensitive methods.

The capillary electrophoresis method with direct UV detection is proposed for the determination of nitrite and nitrate in high-salt perchlorate solutions [1]. A new UV-spectrophotometric method for the determination of

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nitrate in vegetables is presented. The method is based on the spectral deconvolution of UV spectra [2].

Spectrophotometric method is proposed for rapid and direct determination of nitrate in small volumes of natural surface waters (maximum 2.0 mL), and optimal experimental conditions, along with other analytical parameters, have been evaluated [3].

A rapid and simple sequential potentiometric method is described for the determination of nitrate and chloride ions in vegetables, by using home-made ion selective electrodes. Nitrate and chloride selective electrodes were constructed, without inner reference solution, by direct applying of the respective PVC and homogeneous crystalline membranes onto a conductive epoxy resin support, as previously described [4].

Amperometric Determination of Nitrate was made at a Copper Electrode Formed ln Situ In a Capillary-fill Sensor Device [5]. A novel approach was developed for nitrate analysis in a FIA configuration with amperometric detection in mineral water and soft drinks .Sensitive and reproducible current measurements were achieved by using a copper electrode activated with a controlled potential protocol [6].

An unsegmented continuous-flow method for the determination of nitrite and nitrate is proposed. Nitrate is photoreduced by UV irradiation with a low-pressure 8 w Hg lamp. The nitrite in the sample and that resulting from the photoreduction of nitrate were used to oxidize iodide ion [7].

Chromatographic determination of Nitrate Esters with Chemiluminescence Detection was developed [8].

Nitrate and nitrite are extracted from food samples, then analyzed by ion chromatography (IC). Commercial samples of ham and salami were analyzed by IC with UV absorbance detection [9].

A polarographic method for the determination of nitrate is based on the reduction of nitrate to nitric oxide which reacts in solution with cobalt (II) and thiocyanate ions forming an electroactive complex that is reduced at the dropping mercury electrode [10]. Determination of nitrate in mineral water and sausage samples was made by using a renewable in situ copper modified electrode [11]. Programmed-Potential Voltammetric Determination of Nitrate was made at a Static Mercury Drop Electrode in Natural Waters and Soil Extracts [12]. Square Wave Voltammetric Determination of Nitrate was made at a Freshly Copper Plated Glassy Carbon Electrode [13].

In one of our studies we developed a new DP polarographic method for the determination of nitrite using sulfite ion as a reductant. In this method while sulfite was oxidized into sulfate, nitrite ion was reduced into NO gas. The polarographic peak of sulfite was used for the determination. Using this method it was possible to determine nitrite ion in sausage sample [14].

In one work Zr(IV) was used as a catalyst for the destruction nitrite and nitrate and nitrite was determined using DPP (differential pulse polarography) [15]. In another work nitrate and nitrite were reduced into nitric oxide and both ions could be determined by DPP [16]. DPP polarographic determination was used for nitrite, in the presence of o-m- or p- aminobenzoic acids [17].

The amperometric determination of both nitrite and nitrate was made in mineral water and in cured meat using tetraruthenated porphirin- modified electrode [18]. Nitrate and nitrite could simultaneously be determined using Griess reaction . Nitrate was first reduced with V(III) and then the sum was determined spectrophotometrically [19].

In the presence of large amount of nitrite it was not possible to determine nitrate correctly. Nitrite was removed with sulphamic acid. After that nitrate was reduced to nitrite with Vanadium (III) and determined spectrophotometrically using Griess reaction [20]. Liquid chromatography was used for the determination of nitrate and nitrite in atmospheric liquids and lake waters [21].

The classical colorimetric methods, are based on the formation of strongly colored azo dyes. For this purpose an amine is diazotized by means of nitrite and then the intermediate is let to couple with a selected aromatic compound.

Most of the above mentioned methods are laborious, since in many of them diazotization and coupling reactions are used; some of them suffer from matrix interference by oxidizing and reducing agents.

Electrochemical methods are mostly preferred for trace analysis and speciation analysis because of their high selectivity and very low detection limits without necessitating tedious extraction or preconcentration procedures. The results obtained with DPP are very reproducible, since with the use of a dropping mercury electrode, the behavior of the electrode is independent of its past history.

In this work, a new and fast polarographic method was developed where it was possible to determine very low concentrations of nitrate. This method can be applied to many biological materials safely.

2. EXPERIMENTAL

The aim of this work was to determine nitrate ion using the reaction between V (II) and nitrate ion as given below. DP polarography will be used for this purpose

$$2V^{2+} + NO_3^- + 2H^+ \leftrightarrow 2V^{3+} + NO_2^- + H_2O$$

2.1. Apparatus

A polarographic analyzer (PAR 174 A) equipped with a PAR mercury drop timer was used. The drop time of the electrode was in the range 2-3 s (2.35 mg s^{-1}). A Kalousek electrolytic cell with a saturated calomel electrode (SCE), separated by a liquid junction, was used in the three-electrode configuration. The counter electrode was platinum wire. The polarograms were recorded with a Linseis (LY 1600) X- Y recorder under the conditions of a drop life of 1 s, a scan rate of 2-5 mV s^{-1}, and a pulse amplitude of 50 mV.

2.2. Reagents

All chemicals used were of analytical-reagent grade (proanalysis). Triply distilled water was used in the preparation of all solutions. In order to expel the oxygen present in polarographic cell 99.999 % pure nitrogen was passed through the solution. Solutions of 10^{-3} M and more diluted ones were prepared before every use in order to avoid the aging process of solution.

The mercury used in the dropping mercury electrode was obtained from Merck (Darmstadt, Germany). Contaminated mercury was cleaned by passing it successively through dilute HNO_3 (3.0 M) and water columns in the form of fine droplets by using a platinum sieve. This mercury is then washed in the same way until no acidic reaction was observed. The collected mercury was stored in a closed vessel covered with water. It was dried between sheets of filter paper when it was needed. The mercury used is not lost since it was collected quantitatively, without causing any pollution. Thus, no mercury loss is possible and it can be used continuously. Before use, a DPP polarogram of this mercury was recorded in order to confirm the absence of impurities.

2.3. Preparation of reagents

2.3.1. Preparation of 0.03 M V_2O_5

0.2271g V_2O_5 is taken and is added into 30 mL water and 8 mL 12 M HCl, it is stirred for about one hour in a magnetic stirrer using a teflon magnet until it is completely dissolved.

2.3.2. Preparation of Jones Reductor

About 50 g of zinc granules were first cleaned in 1 M HCl then they were left in 0.25 M $Hg(NO_3)_2$ about 2-3 minutes so that they were amalgamated with mercury [22]. It was used for the reduction of V(V).

2.3.3. 0.03 M Vanadium (II)

3.0 mL of 0.03 M V (V) solution (yellow) is left for about 12 hours with Zn amalgam granules under nitrogen atmosphere. It was turned into pink-purple color of V (II). Since V (II) may be oxidized into V (III) (green) by air oxygen, during its preparation de-aerated water was used, it was kept with mercury amalgamated zinc granules and the experiments were performed under nitrogen atmosphere**.** Using DC (direct current) polarography the presence of V (III) could be investigated.

2.3.4. 0.03 M Vanadium (III)

V(II) is left under air atmosphere 24 hours so that it is oxidized into V(III) [20]. The color has changed from pink-purple to green during oxidation. The color of V(III) is green. These changes are followed using visible spectrophotometer and also with DC (direct current) polarography.

2.3.5. Preparation of 0.1 M KNO_3

0.253 g KNO_3 is dissolved in 25 mL distilled water.

3. RESULTS AND DISCUSSION

3.1. Polarographic investigation of Vanadium salts

Vanadium V(V) was investigated in 4 M HCl, pH=4 and 5, HAc-NaAc electrolytes. No DP polarographic peak was observed for V(V) in these electrolytes. On the other hand the peaks of V (II) and V (III) were also investigated in 4 M HCl and a peak at about -0.45 V was observed for both. The cathodic or anodic behavior of these peaks had to be studied with DC polarography**.** It was shown that in 4 M HCl, V (II) had an oxidation peak at -0.47 V(anodic) Figure 1 and V (III) had a reduction peak at the same potential (cathodic) Figure 2 and they were reversible.

The reduction potentials of V (II), V (III) and nitrate are as given below

$V^{4+} \leftrightarrow V^{3+} \quad E^0 = 0.337V$

$V^{3+} \leftrightarrow V^{2+} \quad E^0 = -0.255V$

$NO_3^- \leftrightarrow NO_2^- \quad E^0 = +0.94V$

According to these reduction potentials, two below given reactions may take place for the reduction of nitrate.

$$2V^{3+} + NO_3^- + 2H^+ \leftrightarrow 2V^{4+} + NO_2^- + H_2O \qquad \Delta E = +0.6\ V \quad \text{(equation 1)}$$

$$2V^{2+} + NO_3^- + 2H^+ \leftrightarrow 2V^{3+} + NO_2^- + H_2O \qquad \Delta E = +1.2\ V \quad \text{(equation 2)}$$

From their reduction potentials, while Δ E for the first reaction was found as + 0.6 V, for the second one it was found as Δ E = + 1.2 V. According to these results the second reaction will be more quantitative under optimum conditions.

To check the possibilities of these reactions, application for the first equation was made, but as expected the reaction was not quantitative enough. Thus, it was decided to work with the second one, where V (II) was used for the reduction of nitrate. The reaction between V (II) and nitrate may be quantitative only under optimum conditions, as a first step the reaction has been studied under various conditions.

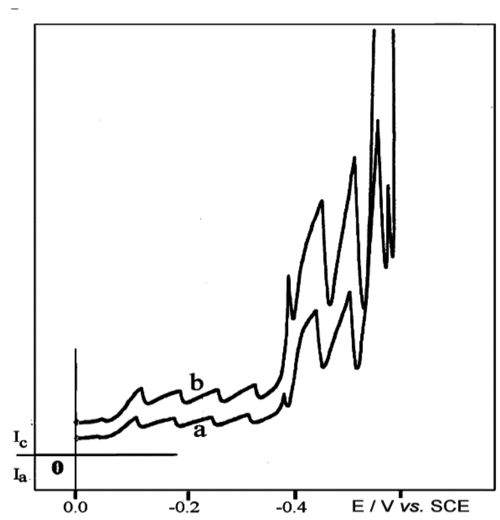

Fig 1. DC (direct current) polarogram of Vanadium (III) (cathodic current)

a) 4 M 10 mL HCl + 0.1 mL 0.05 M V(III)

b) a + 0.1 mL 0.05 M V(III)

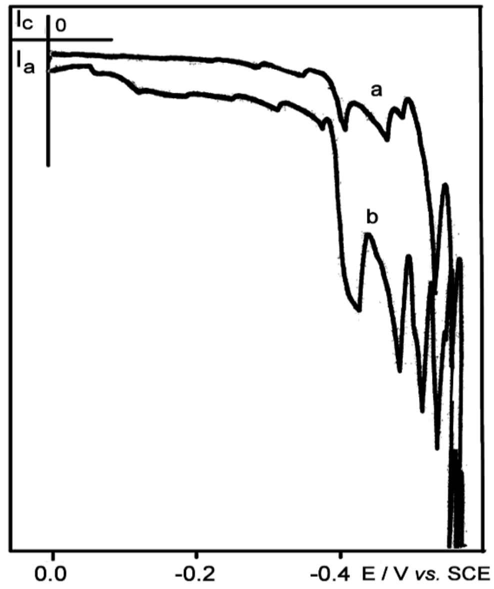

Fig 2. DC polarogram of Vanadium (II) (anodic current)

a) 4 M 10 mL HCl + 0.1 mL 0.05 M V(II)

b)a + 0.1 mL 0.05 M V(II)

Optimum reaction conditions;

For this purpose, nitrate and V (II) solutions were mixed in various ratios and various acidities and polarograms were taken. After the reaction was ended the nitrate concentrations were determined using the polarograms.

In the light of the foregoing findings, in order to have the reaction quantitative, the optimum reaction conditions could be designated as:

1. V (II) concentration has to be at least 4 times larger than nitrate concentration.
2. The acidity has to be 4 M HCl
3. The reaction temperature has to be about 40 ^{o}C and a waiting period about 5 minutes

3.2. Determination of 10^{-5} M nitrate

A synthetic sample is prepared by mixing 0.2 mL 5 x 10^{-3} M V(II) , 0.25 mL 1 x 10^{-3} M nitrate and 10 mL 4 M HCl and it was kept at 40^{o}C for about 5 minutes under nitrogen purging so that the reaction has ended**.**

The DP polarogram is taken at -0.47 V a peak of V(III) reduction was observed which was formed from the reaction with nitrate (eq. 2). First the V(III) formed is determined by standard addition of V(III). The nitrate concentration which was present in sample was calculated according to equation 2 (Figure 3). The same procedure was repeated 4 times. The results are summarized in Table1 and 2.5 x10^{-5} M nitrate was found as (2.4 ± 0.15) x 10^{-5} M. Here we have to mention that before using the standard V(II) solution, it was controlled for the presence of V (III) using DC polarography. As can be seen from Figure 4, no V (III) was present because of the oxidation of V(II) during storing.

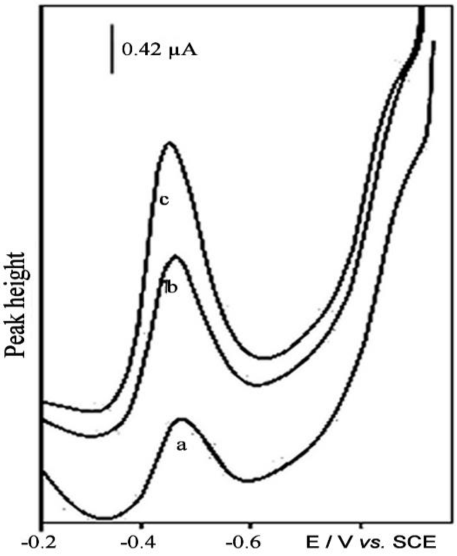

Fig 3. Indirect determination of 2.5 x10 $^{-5}$ M nitrate using V(II) as a reducing agent

1. 4 M HCl 10 mL + 0.2 mL 5 x 10^{-3} M V(II) + 0.25 mL 1 x 10^{-3} M NO_3^-
2. a + 0.1 mL 5 x 10^{-3} M V(III)
3. b + 0.1 mL 5 x 10^{-3} M V(III)

Table 1. Indirect determination of nitrate in a synthetic sample using V(II)

Nitrate present	$\bar{X}$ found	s	$\bar{X}$ found ± ts/$\sqrt{N}$
2.5 x 10^{-5}M	2.4 x 10^{-5}	0.1 x 10^{-5}	(2.4 ± 0.15) x 10^{-5}

N=4, 95 % CI

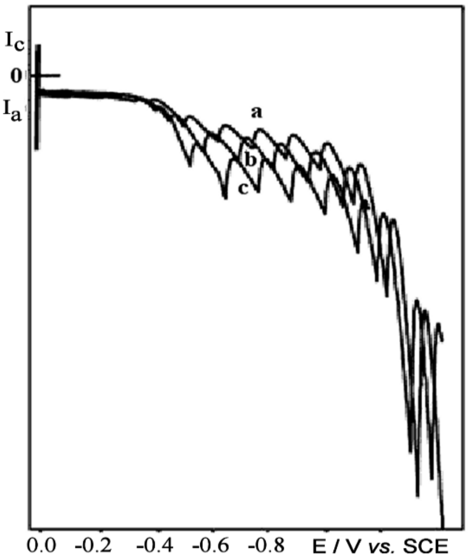

Fig 4. DC (direct current) polarogram of standard Vanadium (II) solution

a) 4 M 10 mL HCl + 0.1 mL 0.005 M V(II)

b) a + 0.1 mL 0.005 M V(II)

c) b + 0.1 mL 0.005 M V(II)

3.3. Interference studies

The most common ions which may be present next to nitrate such as Fe(III), Pb(II), Zn(II), Cu(II) and Cd(II) have been investigated. For this purpose their peak potentials in the same medium (4M HCl) were determined. Cd(II) was at -0.3 V, Pb (II) at -0.25 V , V(II) at -0.47V and Zn(II) at -0.70 V as expected Fe (III) and Cu(II) were both at about 0 V.

As can be seen no interference because of overlapping peaks of Fe(III), Pb(II), Zn(II), Cu(II) ve Cd(II) will be expected. Since sulfate, chloride ions are not electroactive, they will not have a peak overlap either.

To check the possible interferences , a synthetic sample is prepared containing , 9 mL 4 M HCl, 0.25 mL 1x 10^{-3} M nitrate , 0.2 mL 5x 10^{-3} M V (II) and some common cations such as Cd, Pb, Cu and Zn each 0.1 mL 1x10^{-3} M.

It was kept under nitrogen atmosphere by bubbling it for about 5 minutes at 40 °C so that the reaction was quantitative. The DP polarogram is taken, Figure 5, and the peak height of V (III) which was formed as the reaction product is measured, then with standard addition of V (III), its quantity was calculated. The same procedure was repeated 4 times. The results are summarized in Table 2. The nitrate present is found as (2.4 ± 0.4) x 10^{-5} M nitrate.

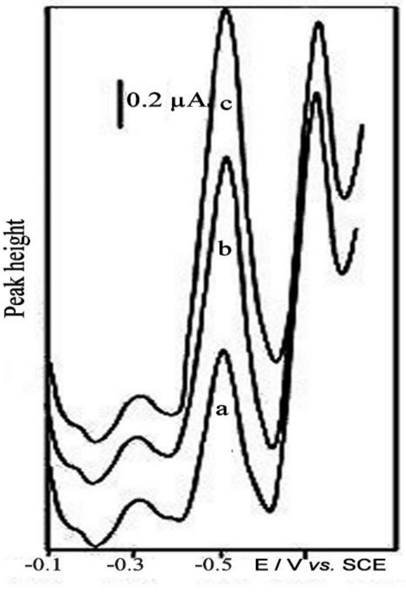

Fig 5. DP polarographic determination of 2.5x 10 $^{-5}$ M nitrate in the presence of Cu, Pb, Cd, Zn, Fe ions

a) 4 M HCl 9.0 mL + 0.2 mL 5 x 10^{-3} M V (II) + 0.25 mL 1 x 10^{-3} M nitrate + 0.1 mL 1x10^{-3} M Cd, Pb, Cu, Zn

b) a + 0.1 mL 5 x 10^{-3} M V (III)

c) b + 0.1 mL 5 x 10^{-3} M V (III)

Table 2. .Determination of 2.5 x 10^{-5} M nitrate in the presence of interfering ions

Interfering ions	X added nitrate (M)	$\bar{X}$ found nitrate (M)	s	$\bar{X} \pm ts/\sqrt{N}$
1 x 10^{-5}M Zn(II), Cu(II), Cd(II), Pb(II), Fe(III)	2.5 x 10^{-5}	2.4 x 10^{-5}	1.5.x 10^{-6}	(2.4 ± 0.4) x 10^{-5}

95 % CI, N=3

3.4. Application to potato sample

The developed method is applied to potato sample which was grown in Nevşehir, Turkey. For this purpose 100 g of raw, peeled potatoes are whipped in the blender, it is filtered and 21 mL of potato juice is obtained. This juice is diluted into 1000 mL with distilled water in a volumetric flask. A 0.1 mL sample is taken and is added into 10 mL 4 M HCl under nitrogen atmosphere. To this solution which is warmed up to about 40 ^{0}C, 0.2 mL 5x10^{-3} M V (II) is added and it is stirred by purging nitrogen for about 5 minutes so that the reaction was ended. After it is cooled down it was transferred into polarographic cell and the polarogram was taken. The nitrate present is determined by the addition of standard V (III) solution. The result found for nitrate in 100 g of potato was 212 ± 8 mg for N= 4 and 95 % confidence interval. It can be concluded that the nitrate content in Nevşehir city is within normal ranges [24].

4. CONCLUSIONS

A new and fast DP polarographic method for the determination of nitrate has been established. According to the reductions potentials, V (II) was found suitable for the reduction of nitrate. The optimum reaction conditions were studied under various conditions. In order to obtain quantitative reaction between nitrate and V (II), the V (II) concentration had to be at least 4 times higher than nitrate concentration. The acidity had to be 4 M HCl, the reaction temperature had to be about 40^{o}C and a waiting period about 5 minutes was found sufficient.

The limit of detection (LOD) was 5 x 10^{-7} M for (S/N =3). This method is applied for the determination of nitrate in potato sample. It can be concluded that the proposed method can be applied safely for many biological samples.

CONFLICT OF INTEREST

No conflict of interest was declared by the authors.

REFERENCES

[1] Budanova, N., Fourest, B. and Maslennikov, A. “Capillary electrophoresis determination of nitrate and nitrite in high-salt perchlorate solutions for the UC dissolution study”, J. Radioanal. Nucl. Chem, 281: 597–602 (2009).

[2] Nam, P.H., Benitez A. Hommet, F., Bombe, D., Schoefs, O. and Andre, P. “A new quantitative and low-cost determination method of nitrate in vegetables, based on deconvolution of UV spectra” Talanta, 76: 936–940 (2008).

[3] Bulgariu L. and Bulgariu, D. “Direct determination of nitrate in small volumes of natural surface waters using a simple spectrophotometric method” Rev. Anal. Chem., 31: 201-207 (2012).

[4] Perez-Olmos, R., Gabiola X. and Hurtado, J. M. “Sequential Potentiometric Determination of Nitrate and Chloride in Vegetables” Chemistry Journal, 3: 81-85 (2013).

[5] Fogg, A.G., Scullion S.P. and Edmonds, T.E., “Direct reductive amperometric determination of nitrate at a copper electrode formed *in situ* in a capillary-fill sensor device” Analyst, 116: 573-79 (1991).

[6] Gamboa, C.M.J., Pena, R.C., Paixao T.R.L.C. and Bertotti, M., “A renewable copper electrode as an amperometric flow detector for nitrate determination in mineral water and soft drink samples” Talanta 80:581–585 (2009).

[7] Gil Torro, I., Garcia Mateo J.V. and Martinez Calatayud**,** J., “Flow-injection biamperometric determination of nitrate (by photoreduction) and nitrite with the NO^{-1}_2 /I^{-1} reaction” Anal. Chim. Acta 366:241-249 (1998).

[8] Tsaplev, Yu. B. “High-performance liquid chromatography of nitrate esters with chemiluminescence detection” J. Anal. Chem., 64:299–303 (2009).

[9] Siu, D.C. and Henshall, A. “Ion chromatographic determination of nitrate and nitrite in meat products” J. Chromatogr. A, 804:157–160 (1998).

[10] Ximenes, M.I.N., Rath, S., and Reyes, F.G.R., “Polarographic determination of nitrate in vegetables” Talanta 51:49–56 (2000).

[11] Paixao, T.R.L.C., Cardoso, J. L. and Bertotti, M. “Determination of nitrate in mineral water and sausage samples by using a renewable in situ copper modified electrode” Talanta, 71:186–191 **(**2007).

[12] MarkuSovri, K., Kolzhutova, L. and Dzurov, J. “Programmed-potential voltammetric determination of nitrate at a static mercury drop electrode in natural waters and soil extracts” Electroanalysis 8:582-84 (1996).

[13] Solak A. O. and Çekirdek, P. “Square Wave Voltammetric Determination of Nitrate at a Freshly Copper Plated Glassy Carbon Electrode” Anal. Lett., 38:271-280 (2005).

[14] Yilmaz, U. T. and Somer, G. “Determination of trace nitrite by direct and indirect methods using differential pulse polarography and application” J. Electroanal. Chem. 624: 59-63 (2008).

[15] Sharma, P. and Sharma, R., “Sequential Trace Determination of Nitrate and Nitrite in Natural Waters by Differential Pulse Polarography” Int. J. Environ. Anal. Chem., 82: 7-11 **(**2002).

[16] Walter, H. and Specchio, J. J. “Determination of Nitrite and Nitrate by Differential Pulse Polarography with Simultaneous Nitrogen Purging” Anal. Chem., 64:1313-15 (1992).

[17] Sadallah, T. S. and Banan, A. A. “Differential-Pulse Polarographic Determination of Nitrite” Analyst, 109: 1545-50 (1984).

[18] Caetano da Rocha, J. R., Angnes, L., Bertotti, M., Araki, K. and Toma, H. E., “Amperometric detection of nitrite and nitrate at tetraruthenated porphyrin-modified electrodes in a continuous-flow assembly” Anal. Chim. Acta 452:23-28 (2002).

[19] Miranda, K. M., Espey M. G. and Wink, D. A. “A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite” Nitric Oxide 5:62-71 (2001).

[20] Beda, N. and Nedospasov, A. “A spectrophotometric assay for nitrate in an excess of nitrite” Nitric Oxide 13:93-97 (2005).

[21] Zuo, Y., Wang, C. and Van, T. “Simultaneous determination of nitrite and nitrate in dew, rain, snow and lake water samples by ion-pair high-performance liquid chromatography” Talanta 70:281-85 (2006).

[22] Somer, G. and Kocak, A, “Determination of atmospheric sulfur dioxide by differential-pulse polarography using selenite” Analyst 118:657-59 (1993).

[23] Cieslik, E. and Sikora, E. “Correlation between the levels of nitrates and nitrites and the contents of potassium, calcium and magnesium in potato tubers” Food Chemistry 63(4):525-528 (1998).