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A Sensitive Spectrofluorimetric Method for the Determination of Nitrite in Agricultural Samples

Masoud Shariati-Rad · Mohsen Irandoust · Farahnaz Niazi

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Abstract A simple, selective, and very sensitive spectrofluorimetric method was developed for the determination of nitrite in vegetables. The method is based on the reaction between nitrite and 4-amino-3-hydroxynaphthalene-1-sulfonic acid (AHNSA) which results in the quenching of the fluorescence of AHNSA. Optimal values of the factors influencing the reaction between nitrite and AHNSA were explored by central composite design (CCD). The factors were pH of the acidic solution and concentration of AHNSA. In optimal conditions, difference between fluorescence intensity of AHNSA and its fluorescence intensity in the presence of nitrite at 442 nm was selected as the analytical signal. The relation between signal and concentration of nitrite was linear in the range of 0.005–0.500 mg L^{-1}. A detection limit of 2.5×10^{-3} mg L^{-1} was obtained for the determination of nitrite by the proposed method. Using the proposed method, it is possible to determine trace amounts of nitrite in vegetable samples with relative errors lower than 6 %. The relative standard deviation (RSD) values of the method for the determination of nitrite in cucumber, cabbage, lettuce, and tomato samples were 0.12, 0.20, 0.86, and 0.13 %, respectively. Moreover, the proposed method was validated by comparison with a standard method.

Keywords Nitrite · Spectrofluorimetric · Central composite design · 4-Amino-3-hydroxynaphthalene-1-sulfonic acid · Vegetable samples

M. Shariati-Rad (✉) · M. Irandoust · F. Niazi
Department of Analytical Chemistry, Faculty of Chemistry, Razi University, Kermanshah, Iran
e-mail: mshariati_rad@yahoo.com

Introduction

Vegetables are an excellent source of vitamins, minerals, and biologically active compounds (Favell 1998; Kidmose et al. 2001). However, vegetables are one of the main sources of entering nitrite to our bodies (Favell 1998; Kidmose et al. 2001). Nitrates are commonly abundant in food primarily because plants take up nitrogen from the soil in this ionic form. Then, nitrates can be reduced to nitrite in foods because of the action of some bacteria (Meah et al. 1994).

Nitrite ion is commonly monitored in agriculture and food control. Nitrite is an important intermediate in the biological nitrogen cycle and is present in soils and surface waters. Nitrite leads to the production of carcinogenic nitrosamines when it reacts with secondary amines in the stomach. In addition, nitrite is known to cause methemoglobinemia (oxygen deficiency) in infants (Seike et al. 2004). Excess concentration of nitrite in drinking water is hazardous to health, especially to pregnant women (Kazemzadeh and Ensafi 2001). Therefore, simple and sensitive methods are required to provide a way for monitoring the legal recommended limits of this ion in agricultural samples and foods.

The most commonly applied methods for the determination of nitrite in addition to spectrophotometry (López Pasquali et al. 2007, 2010; Aydın et al. 2005; Abdul Galil et al. 2007; Afkhami et al. 2005; Borcherding et al. 2000; Ridnour et al. 2000; Chen et al. 2000; Abbas and Mostafa 2000; Miro et al. 2000; Barzcgar et al. 2000; Burakhama et al. 2004; Suvardhana et al. 2005; Yue et al.; 2004) include spectrofluorimetry (Chen et al. 2007; Huang et al. 2000; Wang et al. 2000; Geetha and Balasubramanian 2000; Helaleh and Korenaga 2000a; b; Meininger et al. 2000; Masserini and Fanning 2000; Biswas et al. 2004; Zhang et al. 2002; Argüelles et al.

2004); flow injection analysis (FIA)-kinetic spectrophotometric method (Nouroozi and Mirshafian 2009); electrochemical methods such as differential pulse polarography (Yilmaz and Somer 2008), amperometry (Kalimuthu and John 2009), and potentiometry (Hassan et al. 2003); chemiluminescence (Paolo et al. 2001; Aoki et al. 1997; Wen and Kang 2004; Gao et al. 1990; Markušová and Fedurco 1991; Lu et al. 1992; Butt et al. 2001; Connolly and Paull 2001; He et al. 2011; Akyuz and Ata 2009; Tsikas et al. 1994; Helaleh and Korenaga 2000a; b); electrometric methods (Butt et al. 2001; Connolly and Paull 2001; He et al. 2011; Akyuz and Ata 2009; Tsikas et al. 1994; Helaleh and Korenaga 2000a; b); and chromatography (Butt et al. 2001; Connolly and Paull 2001; He et al. 2011; Akyuz and Ata 2009; Tsikas et al. 1994; Helaleh and Korenaga 2000a; b; Stefanović et al. 2001; Binghui et al. 2006).

Spectrophotometric methods for the determination of nitrite (AOAC 1997; Kawakami and Igrashi 1996) suffer from poor sensitivity and interference from some anions. However, spectrofluorimetric methods for the determination of nitrite have been developed (Damiani and Burini 1986; Ohta et al. 1986; Jiao et al. 2004), and both sensitivity and selectivity have been improved. In the reported spectrofluorimetric methods, nitrite ion reacts with a reagent or acts as a catalyst for various types of the chemical reactions.

In this work, a simple, selective, and sensitive spectrofluorimetric method is presented for the determination of trace amounts of nitrite in complex vegetable samples. The method is based on the quenching of the fluorescence of 4-amino-3-hydroxynaphthalene-1-sulfonic acid by nitrite in acidic medium.

Experimental

Apparatus and Software

A Jasco spectrofluorimeter (fp 6200) equipped with a xenon discharge lamp and a 1-cm pathlength quartz cell was used for all measurements. A Jenway ion meter model 3345 was employed for pH measurements. Design and analysis of the experiments were carried out by MINITAB (Minitab Inc. Release 16.0) statistical package.

Reagents and Solutions

All chemicals were of analytical reagent grade, and double-distilled water was used for preparation of all solutions. Sodium nitrite, ethanol, and hydrochloric acid were supplied by Merck (Darmstadt, Germany). The reagent 4-amino-3-hydroxynaphthalene-1-sulfonic acid (AHNSA) was supplied by BDH Chemicals Ltd., Poole, England. Stock 100.0 mg L^{-1} nitrite solution was prepared in double-distilled water. Stock 1.0×10^{-3} mol L^{-1} solution of AHNSA was prepared in double-distilled water and ethanol 50:50 % (*v*/*v*). Working solutions were prepared by diluting the stock solutions to an appropriate volume with double-distilled water whenever required. Acidic solution (pH 2.3) was prepared by adding hydrochloric acid and sodium hydroxide to double-distilled water.

Calibration Curve

No significant changes in the fluorescence values were observed within the temperature range of 10–35 °C, and the product was stable for about 2 h in the solution.

Volumes equivalent to 0.5 mL of the stock solution of AHNSA (1.0×10^{-3} mol L^{-1}) were transferred to 5.0-mL volumetric flasks, and after diluting to the volume of 5.0 mL by addition of acidic solution with optimal pH (pH 2.3), 0.25–25 μL of the stock nitrite solution (100.0 mg L^{-1}) was added. It must be mentioned that the maximum fluorescence quenching was reached at 40 min after mixing. After this time, while the solutions have been maintained at room temperature, their fluorescence spectra were recorded. The calibration graph was obtained by plotting the fluorescence changes of the reagent against the concentration of nitrite with excitation at 340 nm and using emission at 442 nm.

Experiments were performed for evaluating the effect of time (30 and 45 min). In most cases, the results showed that the average of the signal at 30 min is about 5.4 % less than the signal at optimum time (40 min). Moreover, for 45 min as the time for obtaining signal, the signal in the average rises only about 0.7 %.

Sample Preparation for the Determination of Nitrite in Vegetable Samples

To analyze dietary products of vegetables and fruits, a volume equivalent to 100 mL of double-distilled water was added to 10 g of the cut, milled, and homogenized sample of each vegetable. The resulted mixture was heated under stirring for approximately 10 min in 75 °C using a water bath. Then, it was allowed to cool and was filtered through a Whatman filter paper. After addition of an excess of EDTA for masking divalent and trivalent interferent cations, it was analyzed within the same conditions for acquiring calibration data. First, pH of the filtrate was adjusted to 2.3. A volume equivalent to 4.5 mL of the resulting sample without dilution was transferred to a 5-mL volumetric flask containing 0.5 mL AHNSA (1.0×10^{-3} mol L^{-1}), and after shaking well and standing for optimal time, its fluorescence spectrum was recorded.

Table 1 Experiments designed based on central composite design with two factors

Experiment number	pH	Concentration of AHNSA (mol L^{-1})	Emission difference
1	2.0	3.4×10^{-4}	34.9
2	1.0	1.0×10^{-4}	11.4
3	2.0	2.0×10^{-4}	32.9
4	2.0	2.0×10^{-4}	39.5
5	2.0	5.8×10^{-5}	38. 2
6	2.0	2.0×10^{-4}	39.7
7	1.0	3.0×10^{-4}	6.5
8	3.0	1.0×10^{-4}	27.2
9	0.6	2.0×10^{-4}	2.6
10	3.4	2.0×10^{-4}	−5.2
11	2.0	2.0×10^{-4}	34.1
12	3.0	3.0×10^{-4}	−16.8

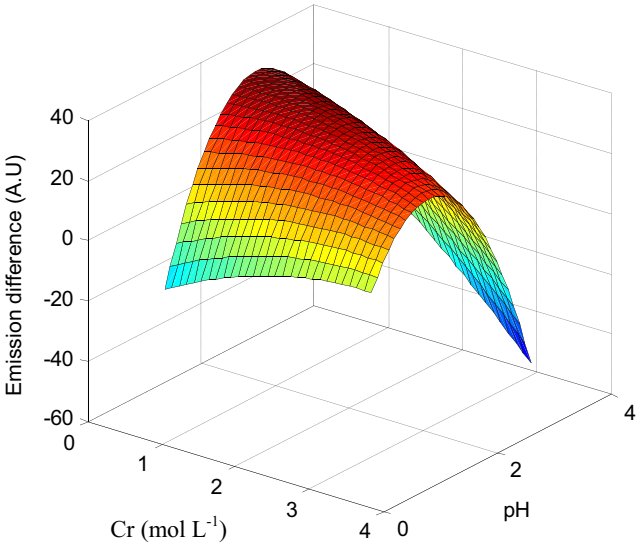


Fig. 1 Variation of the response with concentration of the reagent (Cr) multiplied by 10^4 and pH for the reaction of nitrite with AHNSA

Results and Discussion

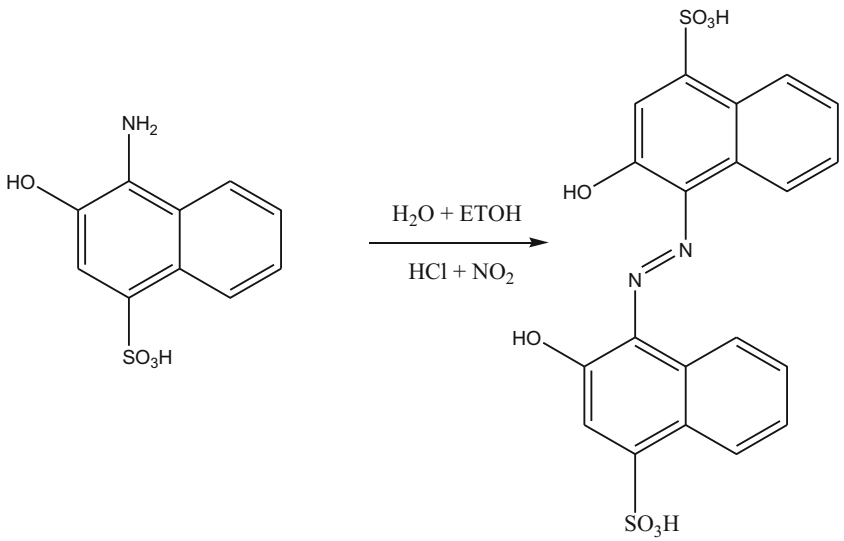
Central Composite Experimental Design and Optimization of the Factors

With design of experiment (DoE), the maximum amount of information of the system is extracted in an economical way (Keeney et al. 1970). Among this information is the interaction between the factors. For this purpose, all factors are changed from one experiment to the next, simultaneously. The reason for performing this type of experiment is that variables can influence each other and the optimal value for one of them may be dependent on the values of the others (Williams 1979).

Central composite design (CCD) is an efficient technique for optimization. CCD provides almost the same information that multilevel full factorial design gives with fewer experiments (West and Ramachandran 1966; Norwitz and Keliher 1978). In CCD, it is assumed that the central point for each factor is 0 and the design is symmetrical around it. Optimal values of the factors for determination of nitrite based on the reaction with AHNSA were obtained by analysis of the results of experiments collected in Table 1.

The possible reaction between nitrite and AHNSA is shown in Scheme 1. Because of the lower rigidity of the product relative to AHNSA, its fluorescence intensity is lower. Moreover, the electron withdrawing group of N=N can be mentioned as another reason for the decrease in the fluorescence intensity of the product.

Scheme 1 The reaction between the AHNSA and nitrite in pH 2.3



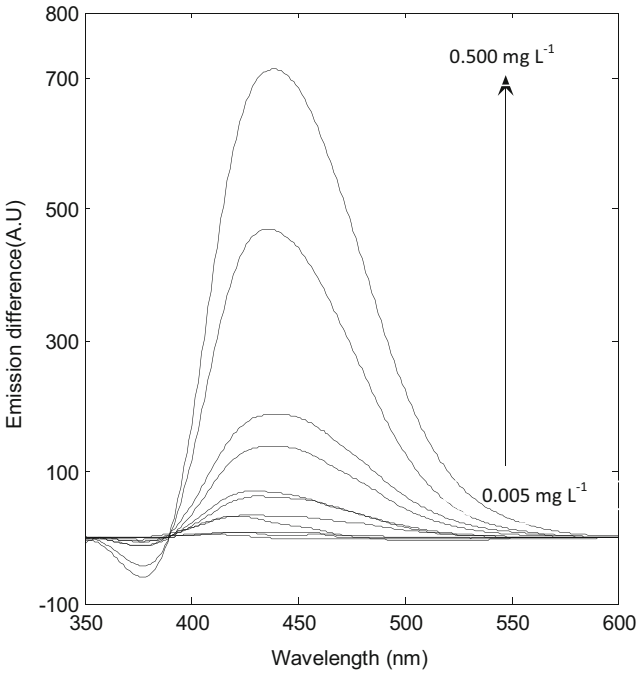


Fig. 2 Fluorescence changes during the reaction between 0.005 and 0.500 mg L^{-1} nitrite solutions and 1.0×10^{-4} mol L^{-1} solution of AHNSA in pH 2.3 after 40 min while the solution has been maintained at room temperature

The values of emission difference for experiments 10 and 12 in Table 1 are negative. In these experiments, in the presence of nitrite, emission has increased to some extent. As can be seen in Table 1, experiments 10 and 12 have been performed in higher pH values and with relatively high concentrations of AHNSA. In these pH values, (1) in the absence of nitrite, deprotonation of the sulfonic (p*K*a≈2.81) and amine (p*K*a≈3.92) groups of AHNSA increases which results in the increase in the fluorescence intensity of AHNSA, and (2) in the presence of nitrite, formation of HNO_2 with a p*K*a value of 3.40 which is the active agent for producing the product in Scheme 1 is incomplete and nitrite facilitates the two above deprotonations. Therefore, after addition of nitrite, fluorescence intensity of the solution increases.

In Fig. 1, the variation of the emission difference as the response with pH and concentration of the reagent (AHNSA) is shown. As can be seen from Fig. 1, in the lower values of the concentration of the reagent, the emission difference is higher.

Table 2 Analytical data of the constructed calibration curve

Parameters	Results
Linear range (mg L^{-1})	0.005–0.500
Limit of detection (LOD) (mg L^{-1})	2.5×10^{-3}
Limit of quantification (LOQ) (mg L^{-1})	7.6×10^{-3}
Slope (mg^{-1} L) (*b*)	1454.3
Intercept	0.285
Correlation coefficient (*r*)	0.995

Table 3 Tolerance limit of different ions in the determination of nitrite with a concentration of 0.100 mg L^{-1}

Foreign ion	Tolerance limit (mg L^{-1})
SO_4^{2-}	13.5
NO_3^-	14.3
K^+	10.4
Ca^{2+}	6.4
Mg^{2+}	2.4
Fe^{3+}	0.7

Analysis of variance (ANOVA) of the experiments in Table 1 was performed. The resulted model for response (emission difference) can be shown as follows:

Emission difference=−81+103×pH+234,271×(concentration of AHNSA)−22×(pH×pH)−263,510,625×(concentration of AHNSA×concentration of AHNSA)−97,929×(pH×concentration of AHNSA)

$$R^2(\text{adj}) = 78.34\,\%,\ F = 8.96, p = 0.009$$

ANOVA showed that in the studied system, pH with probability value (*p*) of 0.001 is a significant factor at 95 % confidence level ($p<0.05$). Quadratic term of pH (pH×pH) is also a significant term for modeling the response at 95 % confidence level (with $p=0.001$). This is responsible for the curvature of the response surface with variation of pH in Fig. 1. Probability value for the regression model is 0.009 which indicates that the model is robust and can be used for accurate prediction of the response (emission difference).

Optimal values of the factors for the determination of nitrite based on the reaction with AHNSA were obtained by ANOVA. The optimal values for the concentration of AHNSA and pH were 1.0×10^{-4} mol L^{-1} and 2.3, respectively.

Fluorescence Spectra

In the presence of nitrite, the fluorescence intensity of AHNSA decreases. The amount of decrease in the intensity was correlated with the concentration of nitrite. This was the basis of the proposed method for the determination of nitrite.

Analytical Data

Under the optimal experimental conditions, a calibration curve was constructed for nitrite which was linear in the concentration range of 0.005–0.500 mg L^{-1}. The fluorescence changes of the reagent with concentration of nitrite in the optimal conditions are shown in Fig. 2. The statistical data of the calibration curve are reported in Table 2. The limit of

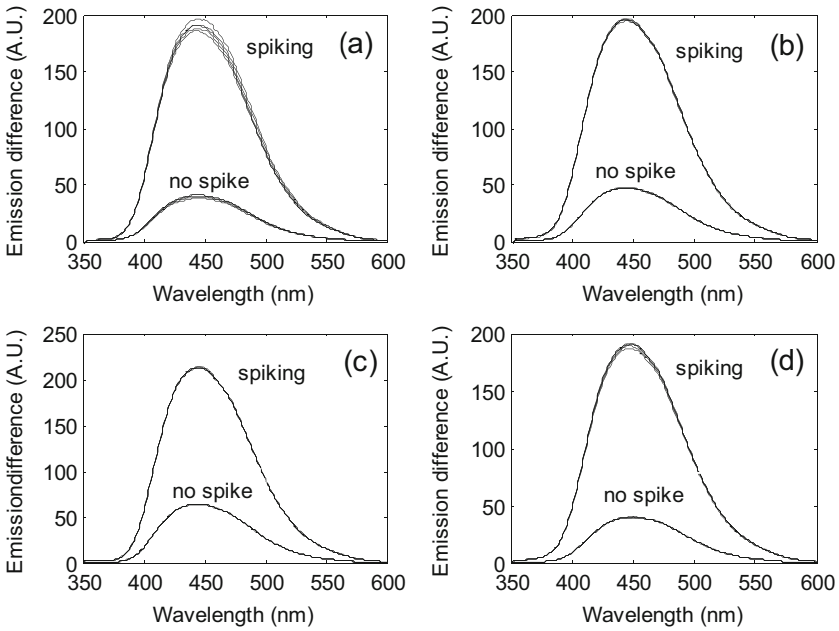


Fig. 3 Difference emission spectra obtained for **a** cucumber, **b** cabbage, **c** lettuce, and **d** tomato in optimal conditions for five replicates

detection (LOD) and quantitation (LOQ) were calculated according to the ICH guidelines using the formulae LOD=3.3×*S*/*b* and LOQ=10×*S*/*b*, respectively (where *S* is the standard deviation of the blank emission signal with optimal excitation and emission wavelengths and *b* is the slope of the calibration plot). The linearity of the calibration curve was validated by the high value of correlation coefficient of the regression equation. The high values of molar absorptivity and low values of LOD indicate that the proposed method is very sensitive.

Effect of Foreign Ions

As the method was developed for the analysis of vegetable samples, the interference from foreign ions commonly present in such samples was studied by adding known amounts of foreign ions to a solution containing nitrite with a concentration of 0.100 mg L^{-1}. The tolerance limit of a potentially interfering ion was taken as its maximum amount causing an error of ≥±5 % during the determination of nitrite in water samples. The tolerance limits for the ions studied are given in Table 3. From Table 3, it is clear that the interference from divalent and trivalent cations may be important. Therefore, in the analysis of the real samples, EDTA was used to mask them.

Application

Under the optimal experimental conditions, validity of the proposed method was checked by determining nitrite in vegetable samples. Samples were also spiked with a known amount of nitrite. The difference emission spectra for different real samples are shown in Fig. 3. As can be seen, the spectra in Fig. 3 are very similar to the spectra shown in Fig. 2. This shows that the species from the analyzed real samples do not interfere in the

Table 4 Determination of nitrite in vegetable samples based on the proposed method

Real sample	Spiked (mg L^{-1})	Found[a] (mg L^{-1})	Reference method (Griess reaction)	*t* statistics[b]	RSD (%)
Cucumber	0.000	0.026 (0.001)	0.025 (0.002)	0.011	1.26
	0.100	0.126 (0.003)	0.123 (0.006)	0.009	0.12
Cabbage	0.000	0.031 (0.001)	0.031 (0.001)	0.001	1.79
	0.100	0.131 (0.001)	0.132 (0.002)	0.006	0.20
Lettuce	0.000	0.042 (0.0003)	0.044 (0.002)	0.017	2.48
	0.100	0.142 (0.001)	0.146 (0.002)	0.030	0.86
Tomato	0.000	0.025 (0.0001)	0.025 (0.002)	0.012	1.30
	0.100	0.125 (0.001)	0.127 (0.002)	0.015	0.13

Values in the parentheses are uncertainty in the predicted concentrations

[a] Mean of five determinations

[b] Critical *t* value for 95 % confidence level with 8 degrees of freedom is 2.31

Table 5 Published results for the spectrofluorimetric determination of nitrite

Matrix	Detection limit (μmol L^{-1})	Linear range (μmol L^{-1})	RSD%	Reaction time (min)	Reference
Water	N/A	0.045–0.072	3.0	60	Lapat et al. (1997)
Water	N/A	0.023–0.072	3.0	60	Lapat et al. (1997)
Water	0.010	0.215–7.170	3	90	Buldt and Karst (1999)
Tap/lake water	0.002	0.017–2.435	4.9	25	Wang et al. (2000)
Water/food	0.054	0.22–13.04	N/A	10	Jie et al. (1999)
Soil/water	N/A	0–8.70	3	5	Geetha and Balasubramanian (2000)
Aqueous	0.059	1.740–28.261	N/A	10	Helaleh and Korenaga (2000a, b)
Water	0.028	1.00–100.00	5.0	1.5	Lee and Field (1984)
Biological samples	0.010	0.013–2.00	N/A		Meininger et al. (2000)
Seawater	0.0046	N/A	N/A	≈3.3	Masserini and Fanning (2000)
Cucumber/cabbage/lettuce/tomato	0.054	0.108–10.870	2.48	40	This method

determination of nitrite by the proposed method. The results of the analysis of the real samples are reported in Table 4. The standard deviations of the results are very low which shows the precision of the proposed method.

In order to approve the accuracy of the proposed method, real samples were also analyzed by the Griess method as a reference method. The results of the application of the reference method are also reported in Table 4. For comparison of the results obtained by the proposed method and the reference method, t statistics was calculated. As can be seen from Table 4, all of the calculated t values are below the critical t value of 2.31 for 95 % confidence level and 8 degrees of freedom. Therefore, the accuracy of the method for the determination of nitrite is confirmed.

Comparison of Reported Methods with the Proposed Method for the Determination of Nitrite

Some of the published spectrofluorimetric methods for the determination of nitrite are collected in Table 5. The methods have been compared based on the linear range, detection limit, time of the analysis, and percent relative standard deviation (RSD%). Based on the RSD% values reported in Table 5, the spectrofluorimetric method introduced here is the most precise method. It must be noted that the reported RSD% value for the proposed method is the highest value obtained for the analyzed real samples.

In the published work by Geetha and Balasubramanian (2000), the reaction step of the analysis requires 5 min; however, the extraction process may take a long time. Masserini and Fanning (2000) introduced a method which possesses a throughput rate of 18 samples per hour (about 3.3 min for each sample). However, their method is based on the flow injection analysis (FIA). In the spectrofluorimetric method proposed by Helaleh and Korenaga (2000a, b), though the overall reaction time is 10 min, it takes place in two steps which may delay more than 10 min. Lee and Field (1984) introduced the reaction of nitrite with Ce(IV). The reaction time was reported to be 1.5 min. They used ion chromatography for the separation and fluorescence for detection. Wang et al. (2000) synthesized a reagent for the spectrofluorimetric determination of nitrite in which its reaction with nitrite is completed in 25 min. However, synthesis of the reagent is time consuming.

As can be seen from the first column of Table 5, most of the published spectrofluorimetric methods for the determination of nitrite have been applied to real samples with simple matrices like water. The dynamic linear range of the present method is wide. Considering the detection limit and lower limit of the linear range, the proposed method is very sensitive. The low LOD obtained by Buldt and Karst has been achieved by HPLC with fluorescence detection (Buldt and Karst 1999). Very low LOD reported by Wang et al. (2000) has been calculated by $LOD=3\times S$. Masserini and Fanning have calculated LOD based on the formula $LOD=2\times S$ (Masserini and Fanning 2000).

Conclusions

A simple spectrofluorimetric method for the sensitive determination of nitrite in vegetable samples was proposed and validated. Excellent precision and recoveries were obtained when the proposed method was applied to complex vegetable samples. The proposed method is very sensitive with low detection limit and lower limit of the linear range. This method offers a simple and cost-effective alternative to existing

methods for nitrite determination. The proposed method requires no control of temperature.

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Compliance with Ethics Requirements This is an original research article that has neither been published previously nor considered presently for publication elsewhere. And, all authors named in the manuscript are entitled to the authorship and have approved the final version of the submitted manuscript.

Conflict of Interest Masoud Shariati-Rad declares that he has no conflict of interest. Mohsen Irandoust declares that he has no conflict of interest. Farahnaz Niazi declares that she has no conflict of interest. This article does not contain any studies with human or animal subjects.

References

Abbas MN, Mostafa GA (2000) Determination of traces of nitrite and nitrate in water by solid phase spectrophotometry. Anal Chim Acta 410:185–192

Abdul Galil MS, Mahadevaiah MS, Yogendra K, Nagendrappa GA (2007) Simple and rapid spectrophotometric method for the determination of nitrite by its decolorizing effect on peroxovanadate complex. Spectrochim Acta A 67:76–82

Afkhami A, Bahram M, Gholami S, Zand Z (2005) Micell-mediated extraction for the spectrophotometric determination of nitrite in water and biological samples based on its reaction with p-nitroaniline in the presence of diphenylamine. Anal Biochem 336: 295–299

Akyuz M, Ata S (2009) Determination of low level nitrite and nitrate in biological, food and environmental samples by gas chromatography–mass spectrometry and liquid chromatography with fluorescence detection. Talanta 79:900–904

AOAC (1995) Official methods of analysis, 16th ed. Association of Analytical Communities, Arlington

Aoki T, Fukuda S, Hosoi Y, Mukai H (1997) Rapid flow injection analysis method for successive determination of ammonia, nitrite, and nitrate in water by gas-phase chemiluminescence. Anal Chim Acta 349:11–16

Argüelles MTF, Cañabate B, Fernández JMC, Pereiro R, Medel AS (2004) Flow injection determination of nitrite by fluorescence quenching. Talanta 62:991–995

Aydın A, Ercan Ö, Tascıoğlu S (2005) A novel method for the spectrophotometric determination of nitrite in water. Talanta 66:1181–1186

Barzcgar M, Mousavi MF, Nemati A (2000) Kinetic spectrophotometric determination of trace amounts of nitrite by its reaction with molybdosilicic acid blue. Microchem J 65:159–163

Binghui Z, Zhixiong Z, Jing Y (2006) Ion chromatographic determination of trace iodate, chlorite, chlorate, bromide, bromate and nitrite in drinking water using suppressed conductivity detection and visible detection. J Chromatogr A 1118:106–110

Biswas S, Chowdhury B, Ray BC (2004) A novel spectrofluorimetric method for the ultratrace analysis of nitrite and nitrate in aqueous medium and its application to air, water, soil and forensic samples. Talanta 64:308–312

Borcherding H, Leikefeld S, Frey C, Diekmann S, Steinrücke P (2000) Enzymatic microtiter plate-based nitrate detection in environmental and medical analysis. Anal Biochem 282:1–9

Buldt A, Karst U (1999) Determination of nitrite in waters by microplate fluorescence spectroscopy and HPLC with fluorescence detection. Anal Chem 71:3003–3007

Burakhama R, Oshimab M, Grudpan K, Motomizu S (2004) Simple flow-injection system for the simultaneous determination of nitrite and nitrate in water samples. Talanta 64:1259–1265

Butt SB, Riaz M, Iqbal MZ (2001) Simultaneous determination of nitrite and nitrate by normal phase ion-pair liquid chromatography. Talanta 55:789–797

Chen H, Fang Y, An T, Zhu K, Lu J (2000) Simultaneous spectrophotometric determination of nitrite and nitrate in water samples by flow-injection analysis. Int J Environ Anal Chem 76:89–98

Chen T, Tong A, Zhou Y (2007) 2-Amino-5,7-dimethyl-1,8-naphthyridine as a fluorescent reagent for the determination of nitrite. Spectrochim Acta A 66:586–589

Connolly D, Paull B (2001) Rapid determination of nitrate and nitrite in drinking water samples using ion-interaction liquid chromatography. Anal Chim Acta 441:53–62

Damiani P, Burini G (1986) Fluorometric determination of nitrite. Talanta 33:649–652

Favell DJ (1998) A comparison of the vitamin C content of fresh and frozen vegetables. Food Chem 62:59–64

Gao Z, Wang G, Zhao Z (1990) Determination of trace amounts of nitrite by single-sweep polarography. Anal Chim Acta 230:105–112

Geetha K, Balasubramanian N (2000) An extractive spectrophotometric method for the determination of nitrite using fluorescein amine isomer I. Anal Lett 33:1869–1883

Hassan SSM, Marzouk SAM, Sayour HEM (2003) Selective potentiometric determination of nitrite ion using a novel (4-sulphophenylazo-)1-naphthylamine membrane sensor. Talanta 59: 1237–1244

He LJ, Zhang KG, Wang CJ, Luo XL, Zhang SS (2011) Effective indirect enrichment and determination of nitrite ion in water and biological samples using ionic liquid-dispersive liquid–liquid microextraction combined with high-performance liquid chromatography. J Chromatogr A 1218:3595–3600

Helaleh MIH, Korenaga T (2000a) Fluorometric determination of nitrite with acetaminophen. Microchem J 64:241–246

Helaleh MIH, Korenaga T (2000b) Ion chromatographic method for simultaneous determination of nitrate and nitrite in human saliva. J Chromatogr B 744(2):433–437

Huang Z, Korenaga T, Helaleh MIH (2000) Kinetic spectrofluorimetric determination of nitrite in water samples and nitrogen dioxide in the atmosphere sampled by the liquid droplet method. Mikrochim Acta 134:179–183

Jiao CX, Niu CG, Huana SY, Shen Q, Yang Y, Shen GL, Yu RQ (2004) A reversible chemosensor for nitrite based on the fluorescence quenching of a carbazole derivative. Talanta 64:637–643

Jie NQ, Yang DL, Jiang QB, Zhang Q, Wei L (1999) A fluorescence quenching method for the determination of nitrite with indole. Microchem J 62:371–376

Kalimuthu P, John SA (2009) Highly sensitive and selective amperometric determination of nitrite using electropolymerized film of functionalized thiadiazole modified glassy carbon electrode. Electrochem Commun 11:1065–1068

Kawakami T, Igrashi S (1996) Highly sensitive spectrophotometric determination of nitrite ion using 5,10,15,20-tetrakis(4-aminophenyl)porphine for application to natural waters. Anal Chim Acta 333:175–180

Kazemzadeh A, Ensafi AA (2001) Sequential flow injection spectrophotometric determination of nitrite and nitrate in various samples. Anal Chim Acta 442:319–326

Keeney DR, Byrnes BH, Genson JJ (1970) Determination of nitrate in waters with the nitrate-selective ion electrode. Analyst 95:383–386

Kidmose U, Knuthsen P, Edelenbos M, Justesen U, Hegelund E (2001) Carotenoids and flavonoids in organically grown spinach (*Spinacia*

oleracea L) genotypes after deep frozen storage. J Sci Food Agric 81:918–923

Lapat A, Székelyhidi L, Hornyák I (1997) Spectrofluorimetric determination of 1,3,5-trinitro-1,3,5-triazacyclohexane (hexogen, RDX) as a nitramine type explosive. Biomed Chromatogr 11:102–104

Lee SH, Field LR (1984) Postcolumn fluorescence detection of nitrite, nitrate, thiosulfate, and iodide anions in high-performance liquid chromatography. Anal Chem 56:2647–2653

López Pasquali E, Fernandez Hernando P, Durand Alegria JS (2007) Spectrophotometric simultaneous determination of nitrite, nitrate and ammonium in soils by flow injection analysis. Anal Chim Acta 600:177–182

López Pasquali CE, Gallego-Picó A, Fernández Hernando P, Velasco M, Durand Alegría JS (2010) Two rapid and sensitive automated methods for the determination of nitrite and nitrate in soil samples. Microchem J 94:79–82

Lu G, He Z, Liu Y (1992) Polarographic determination of atmospheric nitrogen oxides. Talanta 39:123–126

Markušová K, Fedurco M (1991) Vitamin B_{12} as coordinating agent for the voltammetric determination of nitrite in natural waters. Anal Chim Acta 248:109–115

Masserini RT, Fanning KA (2000) A sensor package for the simultaneous determination of nanomolar concentrations of nitrite, nitrate, and ammonia in seawater by fluorescence detection. Mar Chem 68:323–333

Meah MN, Harrison N, Davies A (1994) Nitrate and nitrite in foods and the diet. Food Addit Contam 11:519–532

Meininger H, Li CJ, Wu G (2000) Rapid determination of nitrite by reversed-phase high-performance liquid chromatography with fluorescence detection. J Chromatogr B 746:199–207

Miro M, Cladera A, Estela JM, Cerda V (2000) Sequential injection spectrophotometric analysis of nitrite in natural waters using an on-line solid-phase extraction and preconcentration method. Analyst 125:943–948

Norwitz G, Keliher PN (1978) Inorganic interferences in the 2,4-xylenol spectro-photometric method for nitrate and their elimination. Anal Chem Acta 98:323–333

Nouroozi S, Mirshafian R (2009) Flow injection kinetic spectrophotometric method for the determination of trace amounts of nitrite. Talanta 79:1149–1153

Ohta T, Arai Y, Takitani S (1986) Fluorometric determination of nitrite with 4-hydroxycoumarin. Anal Chem 58:3132–3135

Paolo B, Tiziana Z, Eugenio B, Noemi D, Lino C, Franco Z (2001) Mixed exhaled nitric oxide and plasma nitrites and nitrates in newborn infants. Life Sci 68:2789–2797

Ridnour LA, Sim JE, Hayward MA, Wink DA, Martin SM, Buettner CR, Spitz DR (2000) A spectrophotometric method for the direct detection and quantitation of nitric oxide, nitrite, and nitrate in cell culture media. Anal Biochem 281:223–229

Seike Y, Fukumori R, Senga Y, Oka H, Fujinaga K, Okumura M (2004) A simple and sensitive method for the determination of hydroxylamine in fresh water samples using hypochlorite followed by gas chromatography. Anal Sci 20:139–142

Stefanović SC, Bolanća T, Ćurković L (2001) Simultaneous determination of six inorganic anions in drinking water by non-suppressed ion chromatography. J Chromatogr A 918:325–334

Suvardhana K, Kumara KS, Babua SH, Jayarajb B, Chiranjeevia P (2005) RETRACTED: Simultaneous flow-through determination of nitrites, nitrates and their mixtures in environmental and biological samples using spectrophotometry. Talanta 66:505–512

Tsikas D, Böger RH, B-Böger SM, Gutzki FM, Frölich JC (1994) Quantification of nitrite and nitrate in human urine and plasma as pentafluorobenzyl derivatives by gas chromatography-mass spectrometry using their 15N-labelled analogs. J Chromatogr B 661:185–191

Wang H, Yang W, Liang SC, Zhang ZM, Zhang HS (2000) Spectrofluorimetric determination of nitrite with 5,6-diamino-1,3-naphthalene disulfonic acid. Anal Chim Acta 419:169–173

Wen ZH, Kang TF (2004) Determination of nitrite using sensors based on nickel phthalocyanine polymer modified electrodes. Talanta 62:351–355

West PW, Ramachandran TP (1966) Spectrophotometric determination of nitrate using chromotropic acid. Anal Chem Acta 35:317–324

Williams WJ (1979) Handbook of anion determination. Butterworths, London, p 119

Yilmaz UT, Somer G (2008) Determination of trace nitrite by direct and indirect methods using differential pulse polarography and application. J Electroanal Chem 624:59–63

Yue XF, Zhang ZQ, Yan HT (2004) Flow injection catalytic spectrophotometric simultaneous determination of nitrite and nitrate. Talanta 62:97–101

Zhang X, Wang H, Liang SC, Zhang H-S (2002) Spectrofluorimetric determination of nitric oxide at trace levels with 5,6-diamino-1,3-naphthalene disulfonic acid. Talanta 56:499–504