The chemical generation of NO for the determination of nitrite by high-resolution continuum source molecular absorption spectrometry

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ABSTRACT

In the present work, we propose a method for the determination of nitrite based on the chemical generation of nitric oxide (NO) and its detection by high-resolution continuum source molecular absorption spectrometry. NO is generated by the reduction of nitrite in acidic media with ascorbic acid as the reducing agent and then transferred into a quartz cell by a stream of argon carrier gas. The conditions under which the NO is generated are as follows: $0.4 \, mol \, L^{-1}$ hydrochloric acid, 1.5% (w/v) ascorbic acid, an argon gas pressure of 0.03 MPa and an injection time of the reducing agent of 4 s. All measurements of molecular absorption were performed using the NO line at 215.360 nm, and the signal was measured by peak height. Under these conditions, the method described has limits of detection and quantification of 0.045 and $0.150 \, \mu g \, mL^{-1}$ of nitrite, respectively. The calibration curve is linear for nitrite concentrations in the range 0.15–$15 \, \mu g \, mL^{-1}$. The precision, estimated as the relative standard deviation (RSD), was 3.5% and 4.4% for solutions with nitrite concentrations of 0.5 and $5.0 \, \mu g \, mL^{-1}$, respectively. This method was applied to the analysis of different water samples (well water, drinking water and river water) collected in Cachoeira City, Bahia State, Brazil. The results were in agreement with those obtained by a spectrophotometric method using the Griess reaction. Addition/ recovery tests were also performed to check the validity of the proposed method. Recoveries of 93–106% were achieved.

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1. Introduction

Nitrite is an important nitrogen species with serious implications for both the environment and human health. In natural waters, the presence of nitrite is indicative of pollution, even at low concentrations. In the human body, its toxicity is due to fact that it can react with secondary amines to form nitrosamines, which are known to be carcinogenic agents. Furthermore, when present in high concentrations in the blood, nitrite can react with the iron (III) in hemoglobin to form methemoglobin, which cannot carry oxygen [1–4]. The monitoring of nitrite concentrations in water is therefore relevant, as it represents a potential risk to human health. The World Health Organization (WHO) has established the permissible maximum concentration of nitrite in drinking water at $3 \, mg \, L^{-1}$ [5]. In Brazil, legislation has set the permissible maximum level of nitrite in fresh water at $3.3 \, mg \, L^{-1}$ [6].

Several methods for the quantification of nitrite have been reported in the literature. These include kinetic methods [7,8], ion chromatography [9,10], gas chromatography, mass spectrometry [11], capillary electrophoresis [12], electrochemical methods [13,14] and spectrophotometric methods [4,15–21]. However, the quantification of nitrite has usually been performed by spectrophotometry using the Griess reaction. This colorimetric method is based on the reaction of nitrite with sulfanilamide to form a diazonium salt followed by a coupling reaction with N-(1-naphthyl)-ethylenediamine dihydrochloride to form an azo dye [1]. Although it has a high sensitivity, the Griess reaction involves both potentially carcinogenic compounds and environmental pollutants. Additionally, this reaction suffers from matrix interference due to the turbidity or salinity of the solutions [22].

An interesting alternate method for the determination of nitrite is based on the generation of gaseous species of nitrogen in acidic media, such as nitric oxide (NO) and nitrogen dioxide (NO_2) [3,23]. The gaseous products are separated from the liquid phase by a gas–liquid separator and then transported by a stream of carrier gas to a cell positioned in the light path of the instrument. The analyte detection has been performed by atomic absorption spectrometry [23], UV/vis spectrophotometry [3] or

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chemiluminescence after the reaction of NO with ozone [24]. This strategy requires little to no toxic reagents and minimizes the interference caused by the sample matrix [22].

In recent years, the advent of high-resolution continuum source spectrometers [25] has enabled the development of methods for the quantification of non-metal species in air-acetylene flame or graphite furnace [26,27], such as phosphorus [28], sulfur [29] and halogens [30–33]. These methods are based on the absorption spectra of diatomic molecules and represent interesting alternate methods for the determination of the elements previously cited. In this context, Huang et al. developed a method for the determination of nitrate based on the molecular absorption of NO generated by the thermal decomposition of nitrate in graphite furnaces [34].

In this paper, we propose a method for the determination of nitrite by high-resolution continuum source molecular absorption spectrometry based upon the chemical generation of NO. The NO is produced by the reduction of nitrite in acidic media and then transferred by a flow of argon gas to a quartz cell positioned in the light path of a spectrometer.

2. Experimental

2.1. Instrumentation

The molecular absorption of NO was measured with an Analytik Jena AG Model ContrAA 700 high-resolution continuum source spectrometer (Jena, Germany) equipped with a xenon short-arc lamp operating in hot-spot mode as a continuum radiation source. The NO line at 215.360 nm was used for all measurements of molecular absorption and the signal was measured by peak height using three pixels. An HS 50 Hg/hydride system module [35] (Analytik Jena) was used for the chemical generation of NO. The system consisted of a PTFE reaction vessel with a tapered bottom and a bottle for dispensing the reducing agents with a volume of 300 mL. The reducing injection was performed pneumatically in batch mode. A quartz tube cell of 140 mm in length and internal diameter of 8 mm was used with a cell holder placed on the 50 mm burner head. Argon (White Martins, Brazil) of 99.998% purity was used as a purge and transport gas, with flow rates of 12 and 15 L h^{-1}, respectively.

2.2. Reagents and standards

All the reagents used were of analytical grade. All solutions were prepared using ultrapure water obtained from a Milli-Q system (Millipore, Bedford, MA, USA). Hydrochloric acid, ascorbic acid and sodium iodide were of Suprapur^{TM} quality and obtained from Merck (Darmstadt, Germany).

Nitrite stock solution was prepared at a concentration of 1000 µg mL^{-1} by dissolving 150.0 mg of sodium nitrite (Merck) in freshly boiled ultrapure water to a final volume of 100 mL. The stock solution was stored in a freezer at 4 °C. The working solutions were prepared daily by dilution of the stock solution with 2 mL of 2.0 mol L^{-1} HCl, and the volume was filled up to 10 mL with freshly boiled ultrapure water.

### 2.3. Procedure for the determination of nitrite by the proposed method

An 8.0 mL sample of water (well water, drinking water and river water) was transferred to the reaction flask, and 2.0 mL of 2.0 mol L^{-1} hydrochloric acid was added. Solution of 1.5% (w/v) ascorbic acid was pneumatically injected into the reaction flask over 4 s. The nitric oxide (NO) gas generated from the nitrite

reduction reaction was transported to the quartz cell by a stream of argon gas. The measurement of the molecular absorption of NO was performed by the HR-CS MAS over 30 s.

3. Results and discussion

3.1. Study of the experimental conditions for the chemical generation of NO

The chemical generation of NO is based on the reduction reaction of nitrite in acidic media, which is represented by the following equations:

$$NO_{2(aq)}^- + H_{(aq)}^+ \leftrightarrows HNO_{2(aq)} \tag{1}$$

$$HNO_{2(aq)} + H_{(aq)}^+ + e^- \leftrightarrows H_2O_{(l)} + NO_{(g)} \tag{2}$$
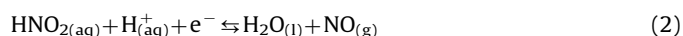
The chemical variables investigated included the type and concentration of the reducing agents as well as the concentration of hydrochloric acid. The HS 50 system variables investigated included the injection time of the reducing agent as well as the pressure of the carrier gas. The study was performed using a univariate methodology for a 10 mL volume of standard solution containing 5.0 µg mL^{-1} of nitrite.

Ascorbic acid and sodium iodide in the 0.5–2.5% (w/v) concentration range were initially investigated as reducing agents. This study was performed with an HCl concentration of 0.2 mol L^{-1}, an injection time of the reducing agent of 4 s and an argon carrier gas pressure of 0.03 MPa. The analytical signal was approximately constant for the entire range of concentrations studied for both reducing agents. Due to this finding, ascorbic acid was chosen as the reducing agent for further studies, and a concentration of 1.5% (w/v) was established to ensure the robustness of the method.

The effect of the HCl concentration on the chemical generation of NO was studied in the range of 0.1–0.5 mol L^{-1}. No significant difference was observed in the NO molecular absorption signal in the studied HCl concentration range. However, an acidic media is required for nitrite reduction to NO, as shown in Eqs. (1) and (2). Thus, an HCl concentration of 0.4 mol L^{-1} was chosen for further studies for robustness.

After studying the chemical variables, the carrier gas pressure and the injection time of the reducing agent were investigated. Fig. 1 shows the influence of the argon gas pressure on the analytical signal of NO as the pressure was varied between 0.03 and 0.15 MPa. The analytical signal remained constant up to a pressure of 0.06 MPa, and a nearly linear increase was observed at
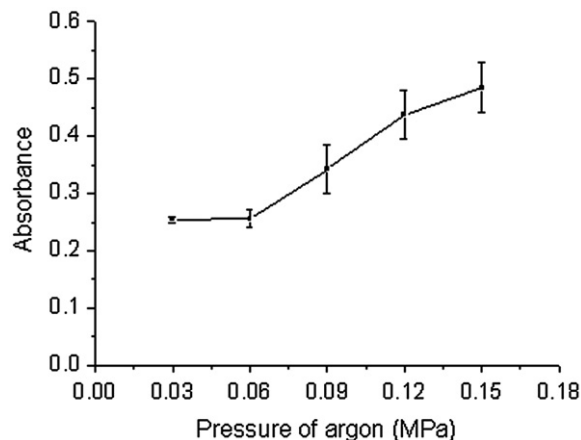

Fig. 1. The effect of the argon gas pressure on the analytical signal of NO.
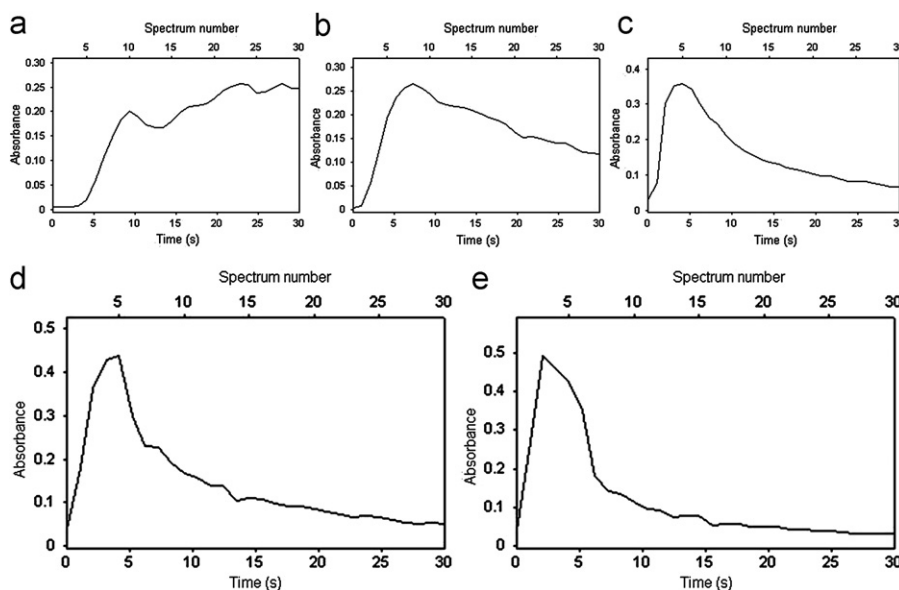
Fig. 2. The influence of the argon gas pressure on the profile of the analytical signal of NO: (a) 0.03, (b) 0.06, (c) 0.09, (d) 0.12 and (e) 0.15 MPa.

higher pressures. The increase in the analytical signal was approximately 90% at a pressure of 0.15 MPa. This increase is due to the increasing transport velocity of the NO molecules from the reaction flask to the quartz cell produced by the increase in argon gas pressure. A larger number of NO molecules reached the quartz cell in a smaller time interval. This fact can also be explained by the change in the profile of the analytical signal, as shown in Fig. 2. The NO signal is nearly continuous at a pressure of 0.03 MPa, and gradually becomes transient with the increasing carrier gas pressure. However, a very high gas pressure promotes a more turbulent flow that can affect the robustness of the method. This effect can be noted by standard deviation obtained to each measuring of pressure in Fig. 1. Based on this information, the argon gas pressure was maintained at 0.03 MPa.

Finally, the effect of the injection time of the reducing agent on the chemical generation of NO was investigated. Injection times in the range of 2–10 s were evaluated, and higher absorbance signals were obtained with injection times above 2 s. Therefore, an injection time of the reducing agent of 4 s was maintained in further experiments.

3.2. Complementary studies

3.2.1. Study of the conversion of NO_2^- to HNO_2

In aqueous media, nitrite exists in both ionic and molecular forms: NO_2^- (nitrite ion) and HNO_2 (nitrous acid). Both species coexist due to the acid–base equilibrium shown in Eq. (1). The conversion of NO_2^- to HNO_2 can be investigated by measuring the analytical signal of NO as a function of the pH of the media. This study was performed under the experimental conditions already established, except for the substitution of hydrochloric acid with a buffer solution at a final concentration of 0.2 mol L^{-1}. As shown in Fig. 3, the analytical signal of NO increased with the decrease in the pH of the media and became constant below a pH of 2.0. The relative signal expected from the distribution of nitrite species as a function of the pH of the media is shown in Fig. 3. The equilibrium constant used to calculate the species distribution was 5.13×10^{-4} [36]. The experimental values obtained from the relative signal of NO agree satisfactorily with those theoretically calculated. Furthermore, these results are in agreement with those obtained in the study of HCl concentration. In this study, the HCl
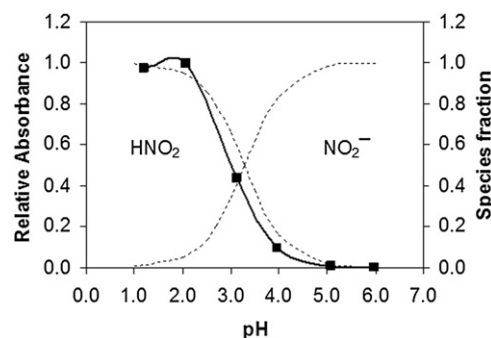

Fig. 3. The distribution of nitrite species as a function of the pH of the media, as measured by the relative signal of the molecular absorption of NO.

concentration was investigated in the range of 0.1–0.5 mol L^{-1}, which corresponds to pH values below 2.0.

3.2.2. Studies of interference

The possible presence of several species that might interfere in the chemical generation of NO was investigated. The species investigated included Ca^{2+}, Mg^{2+}, K^+, Zn^{2+}, Cu^{2+}, Fe^{2+}, Fe^{3+}, F^-, Br^-, I^-, SO_4^{2-}, PO_4^{3-}, CO_3^{2-}, NO_3^-, SO_3^{2-} and humic acid. All species were evaluated at a concentration of 100 $\mu g\ mL^{-1}$ in solutions containing 5.0 $\mu g\ mL^{-1}$ of nitrite. An error of $>10\%$ was adopted as the interference criterion. Under these conditions, only Fe^{2+}, I^- and SO_3^{2-} interfered in the generation of NO (Fig. 4). Obviously, these species are reducing agents and cause the reduction of nitrite prior to analysis. Furthermore, spectral interference was observed for SO_3^{2-} (Fig. 5). A similar phenomenon was observed by Syty and Simmons and also by Haghighi and Tavassoli [3,23]. This spectral interference is due to the molecular absorption of sulfur dioxide formed from sulfite. However, this type of interference can be removed by the equipment software through a least square background correction (LSBC), which is a feature available in high-resolution continuum source spectrometers [37].

Salinity was also investigated as a potential source of interference in the determination of nitrite. Concentrations of NaCl in the range of 1–5% (w/v) were evaluated in solutions containing 5.0 $\mu g\ mL^{-1}$ of nitrite, and the same error criterion as described in previous
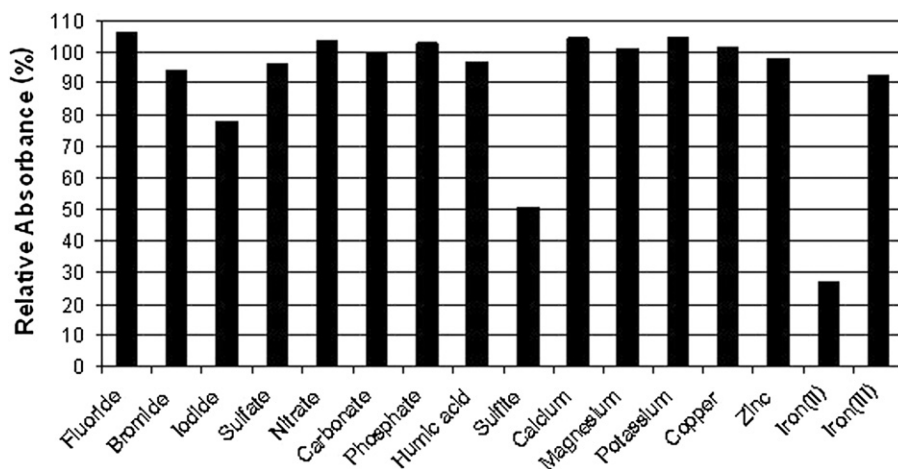
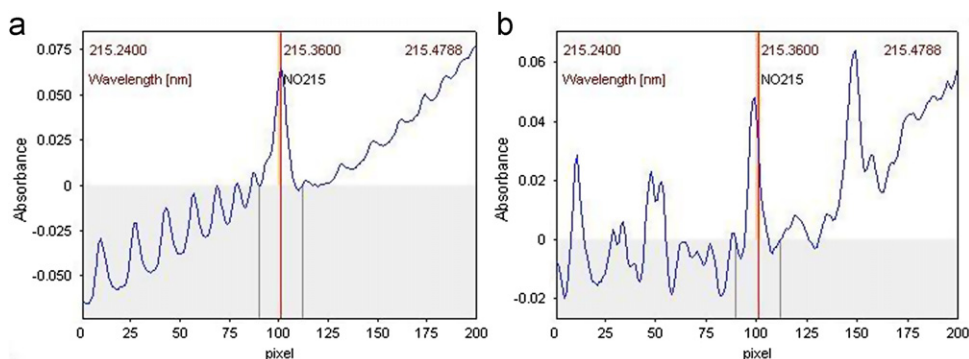**Fig. 4.** The influence of several species on the chemical generation of NO.

Fig. 5. Spectra of molecular absorption: (a) NO spectrum and (b) Spectral interference of sulfur dioxide at NO line.

Table 1
Comparison of the proposed method with chemical generation reported in literature for determination of nitrite.

Technique of detection	LOD ($\mu g\,mL^{-1}$)	Dynamic range ($\mu g\,mL^{-1}$)	Time per replicate (min)	FIA	Sample type	Reference
Spectrophotometry UV	7.5	Up to 1000	2.0	Yes	Spiked water	3
Photometry UV-LED	0.322	1–230	10	Yes	Spiked water	22
AAS	0.23	Up to 65	Less than 2.0	No	Unspecified	23
Chemiluminescence	0.00035	0.003–0.7	1.0	Yes	Water	24
HR-CS MAS	0.045	0.15–15	Less than 1.0	No	Water	This work

paragraph was adopted. No interference was observed in all studied NaCl concentrations.

3.3. Analytical characteristics of the method

The limit of detection (LOD) and limit of quantification (LOQ) of this method were 0.045 and 0.150 $\mu g\,mL^{-1}$ of nitrite, respectively. They were calculated as three and ten times the standard deviation of the blank ($n=10$), divided by the slope of the analytical curve [38]. The method proposed in this work presents a limit of quantification approximately 20 times lower than the permissible maximum levels set by the government agencies [5,6].

The calibration graph was linear in a nitrite concentration range of 0.15–15 $\mu g\,mL^{-1}$, with a correlation coefficient of 0.9989. Precision, as estimated by the relative standard deviation (RSD), was 3.5% and 4.4% for solutions with nitrite concentrations of 0.5 and 5.0 $\mu g\,mL^{-1}$, respectively.

A comparison of the proposed method with those based on the chemical generation is established in Table 1. All these methods are based on the chemical generation of nitrogenous gaseous species for the determination of nitrite. However, different spectrometric techniques are used for the detection. In Table 1 can be seen that this method has lower limit of detection and time per replicate than those methods reported, except by method that employing detection by chemiluminescence which presents higher sensitivity.

Although the proposed method presents a LOD higher than the spectrophotometric method based on the Griess reaction, it offers yet some vantages such as simplicity, lower measuring time and less toxic reagents are required.

3.4. Application of the method

The proposed method was applied to the analysis of different water samples (well water, drinking water and river water) collected in Cachoeira City, Bahia State, Brazil. To check the validity of the

Table 2
Nitrite concentration in water samples determined by both the proposed method as well as spectrophotometrically using the Griess reaction ($n=3$, 95% confidence level).

Sample	Proposed method (HR-CS MAS)			Spectrophotometry
	Added (μg mL^{-1})	Found (μg mL^{-1})	Recovery (%)	Found (μg mL$^{-1)}$)
Well water 1	0.0	0.190 ± 0.015	–	0.203 ± 0.009
	0.300	0.478	96	–
Well water 2	0.0	0.292 ± 0.031	–	0.300 ± 0.004
	0.300	0.596	101	–
River water 1	0.0	< LOQ	–	< LOQ
	0.200	0.193	96	–
River water 2	0.0	< LOQ	–	< LOQ
	0.200	0.185	93	–
Drinking water 1	0.0	< LOQ	–	< LOQ
	0.200	0.201	100	–
Drinking water 2	0.0	< LOQ	–	< LOQ
	0.200	0.211	106	–

proposed method, the samples were also analyzed by spectrophotometry based on the Griess reaction. The results obtained by the proposed method were in agreement with those obtained by the spectrophotometric method. Addition/recovery tests of nitrite were also performed for the purpose of validation. The recoveries achieved were in the range of 93–106%. The concentration of nitrite in the samples and their percent recovery are shown in Table 2. All samples contained nitrite concentrations below the permissible maximum nitrite levels stipulated by both the Brazilian legislature for natural waters (3.3 mg L^{-1}) and by the WHO for drinking water (3 mg L^{-1}).

4. Conclusions

The proposed method was shown to be a simple, rapid and reliable alternative for the determination of nitrite in different water samples. This method requires less toxic reagents and eliminates the timed color development required by the spectrophotometric method based on the Griess reaction. Furthermore, the method was shown to be both precise and accurate, and its quantification limit is suited to the analysis of water. All samples had nitrite concentrations below the permissible maximum levels set by the government agencies. The results demonstrate that this method is suitable for the routine analysis of real samples.

Acknowledgments

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