Available online at www.sciencedirect.com
SCIENCE DIRECT®
Talanta 64 (2004) 1290–1298
Talanta
www.elsevier.com/locate/talanta

# Flow analysis–vapor phase generation–Fourier transform infrared (FA–VPG–FTIR) spectrometric determination of nitrite☆

Máximo Gallignani*, Maribel Valero, Carlos Ayala, Maria del Rosario Brunetto, Argenis Sánchez, Jose Luis Burguera, Marcela Burguera

*Faculty of Science, IVAIQUIM (Venezuelan Andean Institute for Chemical Research), University of Los Andes (ULA), Mérida, P.O. Box 440, Mérida 5101-A, Venezuela*

Received 5 March 2004; received in revised form 13 April 2004; accepted 13 April 2004
Available online 20 July 2004

**Abstract**

In this work, the coupling between flow analysis (FA)–vapor phase generation (VPG) and Fourier transform infrared spectrometry (FTIR) has been proposed as a novel and alternative strategy for the determination of nitrite. The analyte was transformed into the gaseous nitric oxide (NO) by on-line reaction with potassium iodide (KI) or ascorbic acid in acidic medium. The gaseous NO generated was transported by means of a $N_2$ gas carrier stream inside the IR gas cell and the corresponding FTIR spectrum was acquired in a continuous mode. The absorbance at 1876 $cm^{-1}$, corrected by a baseline established between 1879 and 1872 $cm^{-1}$ at a nominal resolution of 2 $cm^{-1}$, was selected as a measurement criterion. The effect of different spectroscopic and flow analysis experimental parameters, such as nominal resolution, number of scans, reducing agent and its concentration, acidic medium, reagents and sample flow rates, and the carrier gas flow rate on the analytical signal, and then in the figures of merit were initially evaluated by using a standard short path length (10 cm) IR gas cell. The optimization of the system was carried out by the univariate method. The main aims of this study were: (i) to investigate the on-line generation of gaseous nitric oxide in a continuous flow system, and (ii) the use of Fourier transform infrared spectrometry as an alternative and selective detector for the determination of nitrite. The proposed method was initially tested and applied for the determination of nitrite in samples with very high concentration of nitrite, such as frankfurters.
© 2004 Elsevier B.V. All rights reserved.

*Keywords:* FTIR; Flow analysis; Nitrite; Nitric oxide

## 1. Introduction

Nitrite is an active form of the nitrogen cycle, resulting from incomplete oxidation of ammonia or from reduction of nitrate. Nitrite is also formed during the biodegradation of nitrate and ammoniacal nitrogen or nitrogenous organic matter, and in this case is an important indicator of fecal pollution of natural waters [1]. The occurrence of nitrite salts in the environment, and their use as a preservative in the food industry and a corrosion inhibitor in industrial process water is widespread [2]. On the other hand, nitrite has been reported to cause a harmful impact on human health. The toxicity of nitrite is primarily due to its interaction with blood pigment to produce methemoglobinemia. The reaction between nitrite, and secondary and tertiary amine results in the formation of *N*-nitroso compounds, some of which are known to be carcinogenic, teratogenic and mutagenic [3,4]. Because of these properties, many papers have been published on the determination of nitrite in different samples, but due to different reasons, not all of them are suitable for routine determination. Among these methods, spectrophotometry based on diazotization of an aromatic amine and subsequent coupling to form an azo dye – the Griess–Ilosvay reaction [5] – are the most widely used. These methods are generally sensitive, but often have drawbacks of serious interferences (colored substances, oxidant and reducing agents, copper compounds, etc.), and in some cases, a relatively long reaction time. For these reasons, a series of chromatographic, electrochemical, fluorimetric, chemiluminiscence, etc., methods has been developed, but they suffer from more

☆ Presented at the 12th International Conference on Flow Injection Analysis (ICFIA-2003), held in Mérida, Venezuela, December 7–12, 2003.

* Corresponding author. Fax: +58 274 2714223.
*E-mail addresses:* maximo@ula.ve, aurelio@icnet.com.ve (M. Gallignani).

0039-9140/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2004.04.039

or less time-consuming procedures, the requirement of very specific reagents, or complicated and expensive instrumentation [6]. All these methods have advantages and drawbacks, and most of them have been reviewed by Fox [7] and by Mathew et al. [8]. On the other hand, a number of kinetic spectrophotometric methods, recently reviewed by Gallignani et al. [9], have been reported for nitrite determination. Most of these procedures are based on the catalytic effect of nitrite ion on bromate oxidation of different organic compounds. However, most of them are time consuming, need rigid control over experimental conditions, such as temperature, pH, reaction time, reagents, etc., and generally suffer of serious interferences by oxidant and reducing agents. Additionally, most recently, some procedures based on gas phase molecular absorption spectrometry were also reported for the determination of nitrite [10,11]. Thus, there has been an increasing need in the development of new and selective methods for the determination of nitrite in different natural and artificial samples.

Concerning the chemiluminescence methods (CL), they are simple, elegant, and usually are characterized by interesting figures of merit. The CL methods can be carried out both in liquid [12,13] and gaseous phase [14–19]. However, nowadays the majority of the CL methods used for the determination of nitrite are based on the conversion of nitrite to gaseous nitric oxide (NO(g)), which is transferred to a gas phase, and detected by means of the CL reaction with ozone [12]. Ascorbic acid [14,20], iodide [17,21] and vanadium (III) [16] have been recommended for the quantitative reduction of nitrite to NO. Alternatively, NO is under vacuum released from an acidified solution of nitrite [22]. At this point, it is worthwhile to point out that the gaseous NO is active in the infrared region, showing a well defined band at 1876 $cm^{-1}$ [23–25].

Fourier transform infrared spectrometry (FTIR) is a fast analytical technique that provides very interesting quantitative information from solid, liquid and gaseous samples. Developments on flow analysis (FA) procedures in the field of infrared spectrometry have helped to solve some of the drawbacks of quantitative determinations using this technique [26]. On the other hand, recent developments in flow analysis–FTIR, based on the on-line generation of vapor phases including volatile hydrides from liquid samples, have improved direct determination by FTIR due to the high transparency of gases, the low background values achieved and the possibilities offered using multiple-pass IR cells to increase the analytical sensitivity [27,28]. Additionally, and in the same way that in HG [29], vapor-phase FTIR allows us an efficient matrix removal, thus reducing spectral interferences [30,31]. However, to the best knowledge of the authors, until now, the determination of nitrite via the on-line generation of gaseous nitric oxide and by using FTIR spectrometry as a detector has not been reported so far.

In this work, a flow analysis–vapor phase generation–Fourier transform infrared (FA–VPG–FTIR) spectrometric method has been developed for the determination of nitrite. The proposed method was initially tested and applied for the determination of nitrite in samples with very high concentration of nitrite, such as frankfurters [32–34]. The main aims of this study were: (i) to investigate the on-line generation of gaseous nitric oxide in a continuous flow system, and (ii) to use the Fourier transform infrared spectrometry as an alternative and selective detector for the determination of nitrite.

## 2. Experimental

### 2.1. Instrumentation

A Perkin Elmer (Norwalk, CT, USA) Spectrum 2000 series FTIR spectrometer equipped with a DGTS detector, a Perkin Elmer MIR–IR source and a KBr beamsplitter were employed to carry out the spectral measurements, accumulating three scans at a nominal resolution of 2 $cm^{-1}$, using a conventional short-pathway IR gas cell Wilmad (New Jersey, USA) with 10 cm of pathway and 60 ml internal volume, equipped with 32 mm × 2 mm circular SeZn windows. The Spectrum 2000 software was used to control the instrument for data acquisition and also to process the results.

Fig. 1 depicts the schematic diagram of the FA–HG–FTIR manifold used in this work. It consists of an assembly of an Ismatec (Glattbrugg, Switzerland) IPC peristaltic pump (P) of four channels, furnished with Tygon tubes, three channels for the transport of samples and reagents ($C_C$, $C_S$, $C_R$), a reaction coil (R), a Varian (Springvale, Australia) gas–liquid separator (GPS), an entry of $N_2$ with an independent flow

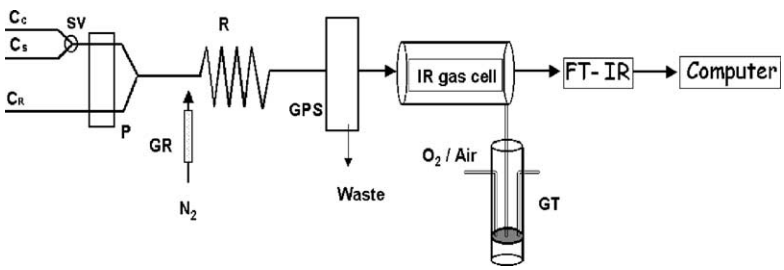


Fig. 1. Schematic diagram of the FA–VPG–FTIR system used in this work. P: peristaltic pumps; SV: selecting valve; $C_C$: carrier (water); $C_S$: sample (standard); $C_R$: reducing agent, GPS: gas phase separator; R: reaction coil; GR: gas flow regulator; GT: gas trapping; detector: FTIR spectrometer.

rate Cole Palmer (Ilinois, USA) regulator (GR), a gas IR cell, a FTIR detector and a home-made gas trap (GT). The experimental design includes a Reodyne (Alltech, Waukegan, USA) manual selecting valve (SV), which permits the selective introduction of the carrier ($C_C$) or the sample flow ($C_S$) in the system. Additionally, a continuous flow of air ($O_2$) is introduced in the GT in order to trap the nitric oxide. Oxygen reacts with the nitrogen monoxide to form nitrogen dioxide, which reacts with water to form nitric acid [35], following the reaction

$$2NO(g) + O_2(g) \leftrightarrows 2NO_2(g)$$

$$2NO_2(g) + H_2O(l) \leftrightarrows H^+(aq) + NO_3^-(aq) + HNO_2(aq)$$

### 2.2. Reagents

All chemicals used were of analytical-reagent grade of the highest purity available (Riedel-de-Haën, Acrõs, Merck and T.J. Baker). Double de-ionized water of 18 MΩ $cm^{-1}$ specific resistivity, obtained in a Milli-Q Plus, Millipore System (Millipore Corporation, Bedford, USA), identified in the text as DI, was used throughout to prepare all the solutions and to rinse the previously cleaned laboratory material.

Nitrite standard solution (1000 mg $l^{-1}$) was prepared by dissolving 0.3787 g of sodium nitrite in DI, and diluting to a final volume of 500 ml. A pellet of sodium hydroxide was added to prevent liberation of nitrous acid and 1 ml of spectroscopic-grade chloroform to inhibit bacterial growth [36]. This standard solution was kept in a refrigerator for preservation, and can be stored for at least 6 months. Working standard solutions were daily prepared by diluting the stock solutions in DI water. The sodium nitrite reagent used was previously dried for 5 h at 105 °C and cooled in a desiccator.

The reducing agent solutions: potassium iodide (KI) and acid ascorbic. A 10 % (w/v) potassium iodide was daily prepared by dissolving 25 g in 30 % (v/v) hydrochloric acid, and diluting to 250 ml with the same solution. Ascorbic acid 6% (w/v) was prepared dissolving the adequate amount of $C_6H_8O_6$ in 6% (v/v) $H_2SO_4$. The $N_2$ used in this work (99% purity) was from AGA (Maracaibo, Venezuela), which certifies a purity of 99%; its flow was controlled with a needle valve with a flow meter from Cole Palmer.

### 2.3. Samples

The sausage (frankfurters) samples were initially processed following the method recommended by the AOAC [37]. However, the original procedure – developed for a colorimetric method – was modified in the dilution steps in order to obtain a final solution of 10 g of sample in 100 ml.

*Procedure*: Weigh 5–10 g finely comminuted and thoroly mixed sample into 50 ml beaker. Add ca. 40 ml of $H_2O$ hated to 80 °C. Mix thoroly with glass rod, taking care to break up all lumps, and transfer to 100 ml volumetric flask. Thoroughly wash beaker and rod with successive portions of hot water adding all washing to flask. Add enough hot water to bring volume to ca. 90 ml, transfer flask to steam bath and let stand for 2 h, shaking occasionally. Cool at room temperature, dilute to a final volume and remix. Filter or let settle the solution. At this point, the sample solution is ready for the analysis.

### 2.4. General procedure

Initially, samples and reagents were fed through their respective lines at room temperature as indicated in Fig. 1, under the operating conditions indicated in Table 1. P was on during the analysis to propel continuously the carrier ($C_C$) or the sample ($C_S$), and the reducing agent ($C_R$). The FA–VPG–FTIR system proposed in this work involves three steps: (i) the introduction of the sample in the system and the generation of gaseous nitric oxide, (ii) the stripping of the NO(g) and its transport to the IR gas cell, and (iii) the acquisition of the corresponding FTIR spectrum.

In a preliminary step, the background of the system must be established. For this purpose, the selecting valve was switched to the carrier position. Therefore, P continuously propels the acidic reducing reagent ($C_R$) and the carrier ($C_C$) via R in order to obtain a stable background signal by accumulating three scans in the continuous system. Then, the reference spectrum of the blank was registered, also by accumulating three scans. The initialization of the system – filling, background definition and acquisition of the blank spectrum – took about 2 min, and at this point, the system is ready for the analysis. This step is carried out only at the beginning of the experimental session.

In sequence 1, SV was switched to the sample position in order to introduce selectively the sample or standard ($C_S$) in the system. In this way, the sample solution mixed downstream with the acidic ascorbic acid solution ($C_R$) in R, where the nitrite ion is quantitatively reduced to nitric oxide. The gaseous NO generated in R is completely separated (sequence 2) from the aqueous phase in the gas phase separator (GPS). It is worthwhile to point out that the introduction of a $N_2$ flow in R (see Fig. 1) helps to improve (a) the stripping of the nitric oxide vapor from the solution, and (b) its transport to the gas cell. At this point (sequence 3), three scans were accumulated in the continuous system in order to obtain the FTIR absorption spectrum of the gaseous NO. The previously described sequences (2 and 3) were repeated when the same standard or sample solution was under evaluation. However, prior to the evaluation of a different standard or sample, SV was switched to position 1, and the carrier was introduced – similar that in the preliminary step – for at least 15 s in order to clean the system and then to avoid the carry-over of the previously introduced sample. A complete cycle of the described procedure, including the cleaning step was about 90 s.

Table 1
Operating conditions FA–VPG–FTIR

| Component | Parameter | Value |
|---|---|---|
| FTIR | Radiation source | Perkin Elmer MID–IR |
| | Detector | DGTS |
| | Beamsplitter | KBr |
| | Spectral range | 4500–500 $cm^{-1}$ |
| | Analytical band | Antimony 1876 $cm^{-1}$ |
| | Measurement criterion | Absorbance at 1876 $cm^{-1}$ corrected by means of a baseline established between 1872 and 1879 $cm^{-1}$ |
| | Nominal resolution | 2 $cm^{-1}$ |
| | Background (number of scans accumulated) | 3 $cm^{-1}$ |
| | Number of scans accumulated for each spectrum | 3 $cm^{-1}$ |
| | Gas cell | Conventional short path gas cell – Wilmad<br>Pathway: 10 cm<br>Internal volume: ≅ 60 ml.<br>Windows: ZnSe 32 mm × 2 mm (circular) |
| FA–VPG | Carrier composition ($C_C$) | Water |
| | Carrier flow rate ($C_C$) | 7 ml $min^{-1}$ |
| | Sample (standard) ($C_S$) | Sample (see "Sample preparation").<br>Standard: 0–80 μg $NO_2^-$ $ml^{-1}$ |
| | Sample flow rate ($C_S$) | 7 ml $min^{-1}$ |
| | Reducing agent composition ($C_R$) | Ascorbic acid 6% (w/v) in $H_2SO_4$ 6% (v/v) |
| | Reducing agent flow rate ($C_R$) | 1 ml $min^{-1}$ |
| | Reaction coil (R) | PTFE (70 cm, 0.8 mm i.d.) |
| | $N_2$ flow rate (*Stripping*) | 25 ml $min^{-1}$ |
| | Gas trapping solution | Water |
| | $O_2$ (air) flow rate (GT) | 25 ml $min^{-1}$ |

## 3. Results and discussion

### 3.1. Gaseous FTIR spectrum of the nitric oxide

Fig. 2 shows the absorption of FTIR spectrum of the gaseous nitric oxide (NO(g)) obtained in the proposed system. The spectrum is characterized by a well-defined band between 1879 and 1872 $cm^{-1}$, with maximum absorbance at 1876 $cm^{-1}$. Thus, the absorbance at 1876 $cm^{-1}$ corrected by means of a baseline established between 1879 and 1872 $cm^{-1}$ was selected as the measurement criterion.

### 3.2. Optimization of the operating conditions

Preliminary tests were carried out with the aid of different flow assemblies to select the optimal manifold configuration. The assembly shown in Fig. 1 was then selected to produce the best compromise between the quality of the analytical signal and sample throughput.

The FA–VPG–FTIR system proposed in this work involves three steps: (i) the generation of gaseous nitric oxide, (ii) the stripping of the NO(g) and its transport to the IR gas cell, and (iii) the acquisition of the corresponding FTIR

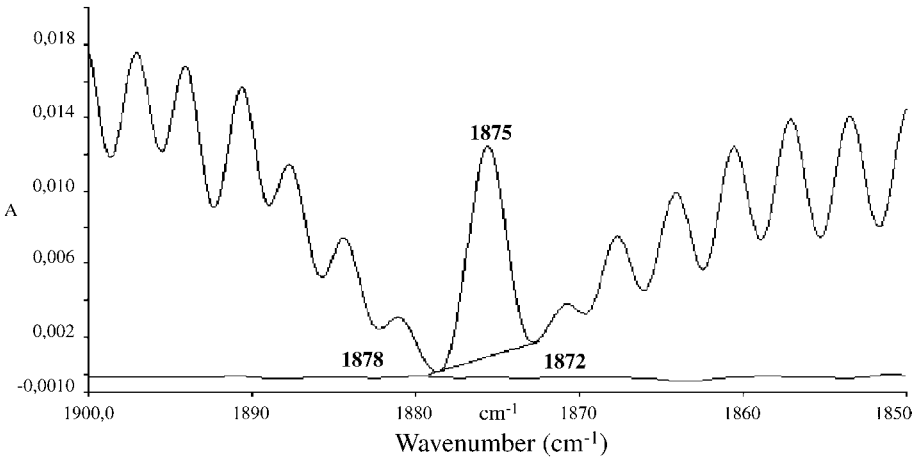


Fig. 2. Gaseous FTIR spectrum of nitric oxide (NO) obtained in the proposed system. Analytical band: 1876 $cm^{-1}$, baseline correction established between 1879 and 1872 $cm^{-1}$, $[NO_2^-] = 50\ \mu g\ ml^{-1}$. Other experimental conditions as indicated in Table 1.

spectrum. Different parameters related to the chemical reaction, the flow analysis system and the FTIR instrumentation were optimized by the univariate optimization method. However, after each optimization, the validity of the previously selected parameters was verified. In this process, a 50 μg $NO_2^-$ ml solution was used for all the experiences.

### 3.3. Effect of chemical and flow analysis parameters

The quantitative reduction of nitrite to nitric oxide has been carried out with different reducing agents, such as KI [17,21], vanadium (III) [16], ascorbic acid, etc. [14,20].The preliminary studies of the proposed system was carried out using potassium iodide as a reducing agent. However, in the optimization process, when the flow rate or the reducing agent concentration was increased, a deposition of a red solid on the tubing walls of the reaction coil and/or in the GPS of the FA system was observed. This fact is probably due to the formation and precipitation of $I_2$ at the high nitrite concentration used in the optimization. Based in these results, the use of KI was discharged, and therefore ascorbic acid in sulfuric acid medium [14,20] was tested as the reducing agent.

| | |
|---|---|
| Redox system I | $2NO_2^- + 4H^+ + 2e^- \leftrightarrows 2NO(g) + 2H_2O$ |
| Nitrite–ascorbic acid | $C_6H_8O_6 \leftrightarrows C_6H_6O_6 + 2H^+ + 2e^-$ |
| | $2NO_2^- 2H^+ + C_6H_8O_6 \leftrightarrows C_6H_6O_6 + 2NO(g) + H_2O$ |
| Redox system II | $2NO_2^- + 4H^+ + 2e^- \leftrightarrows 2NO(g) + 2H_2O$ |
| Nitrite–iodide | $2I^- \leftrightarrows I2 + 2e^-$ |
| | $2NO_2^- + 2I^- + 4H^+ \leftrightarrows 2NO(g) + 2H_2O + I_2(s) \downarrow$ |

The influence of the ascorbic acid concentration and the acidic medium (sulfuric acid concentration) on the NO(g) signal was studied over the range 0–10% (w/v) and 0–10% (v/v), respectively. The analytical response increased with the ascorbic acid concentration up to 4.0 (w/v), as can be shown in Fig. 3(A). Thereafter, a plateau was reached, indicating the completeness of the reduction of $NO_2^-$ to NO(g). On the other hand, the behavior of the analytical response with the sulfuric acid concentration indicates that an acidic medium is required for the developing of the redox system. Similar to that for the reducing agent, the analytical signal grows with increasing the $H_2SO_4$ concentration, reaching a plateau for acid concentrations higher than 3% (v/v) (see Fig. 3B). Based on the described results, an ascorbic acid solution of 6% (p/v) prepared in $H_2SO_4$ 6% (v/v) was selected for further studies. At this point, it is important to point out that the redox reaction between the ascorbic acid and the nitrite ion is carried out in the reaction coil (R) (see Fig. 1). Then, additionally to the chemical parameters described, the effect of the reaction coil length and the total flow rate resulting of the mixture of the sample and reducing agent solutions in R (see Fig. 1), which determine the residence time, had to be studied as well.

In order to study the influence of the total flow rate, two strategies were carried out. In the first one, both the sample and reducing agent were propelled by means of P at the same flow rate. In this approach, the analytical signal grown linearly with the sample flow rate ($Q_S$) in the overall range tested (0.5–10 ml $min^{-1}$), thus indicating a direct dependence between the analytical signal and the rate in which nitrite was introduced in the system (Fig. 3C-a). However, it is worthwhile to point out that under the experimental conditions indicated in Table 1, and by using the commercial Varian GPS, the use of $Q_S$ higher than 8 ml $min^{-1}$ (total flow rate of 16 ml $min^{-1}$) is not recommended. On the other hand, in the second approach, the reducing agent flow rate ($Q_R$) was fixed at 1 ml $min^{-1}$, and $Q_S$ was increased between 0.5 and 12 ml $min^{-1}$. In this case, similar to that in the previous study, the analytical response increased linearly with increasing $Q_S$ in the range 0.5–10 ml $min^{-1}$, as can be seen in Fig. (c)-b. However, for higher values, the slope of the curve decreased, probably because at the fixed experimental conditions used (R = 60 cm, (Ascorbic acid) = 6% w/v in $H_2SO_4$ 6% v/v), the rate in which nitrite is introduced in the system exceeds the rate of generation of the nitric oxide. The selection of the most adequate experimental conditions was obviously a compromise between sensibility, sample and regent consumption, and sample throughput. Based on the obtained results, for this preliminary work, the second strategy was selected, fixing $Q_R$ and $Q_S$ at 1 ml $min^{-1}$ and 7 ml $min^{-1}$, respectively. The first approach will be used in further studies. Concerning the effect of the reaction coil length on the gaseous NO generation, and then on the analytical signal, Fig. 3(D) shows that the analytical response increases with increasing this parameter from 0 to 50 cm, reaching a plateau for higher values, indicating the quantitative conversion of nitrite to gaseous nitric oxide. Thus, a 70 cm reaction coil was selected for further studies.

### 3.4. Effect of the gas carrier flow rate

The functions of the gas carrier in the proposed FA–GPG–FTIR systems are: (i) the stripping of the gaseous NO from the solution, and (ii) its transport to the IR gas cell. It is well documented that the gas carrier flow rate seriously affects the shape of the absorption band in gas phase generation–FTIR systems. The gas carrier drastically influences the speed of the gaseous product introduction into the IR gas cell, but it can also produce its dilution [29–31]. In the configuration used in this work, and by using the commercial Varian GPS, it was not possible to use values lower than 25 ml $min^{-1}$. On the other hand, the use of higher values greatly depresses the analytical sensibility due to a gas–gas dilution effect. It is worthwhile to point out that the additional $N_{2(g)}$ entry, which is generally incorporated in the Varian GPS separator [38,39], was eliminated in order to avoid additional dilution processes. Based in

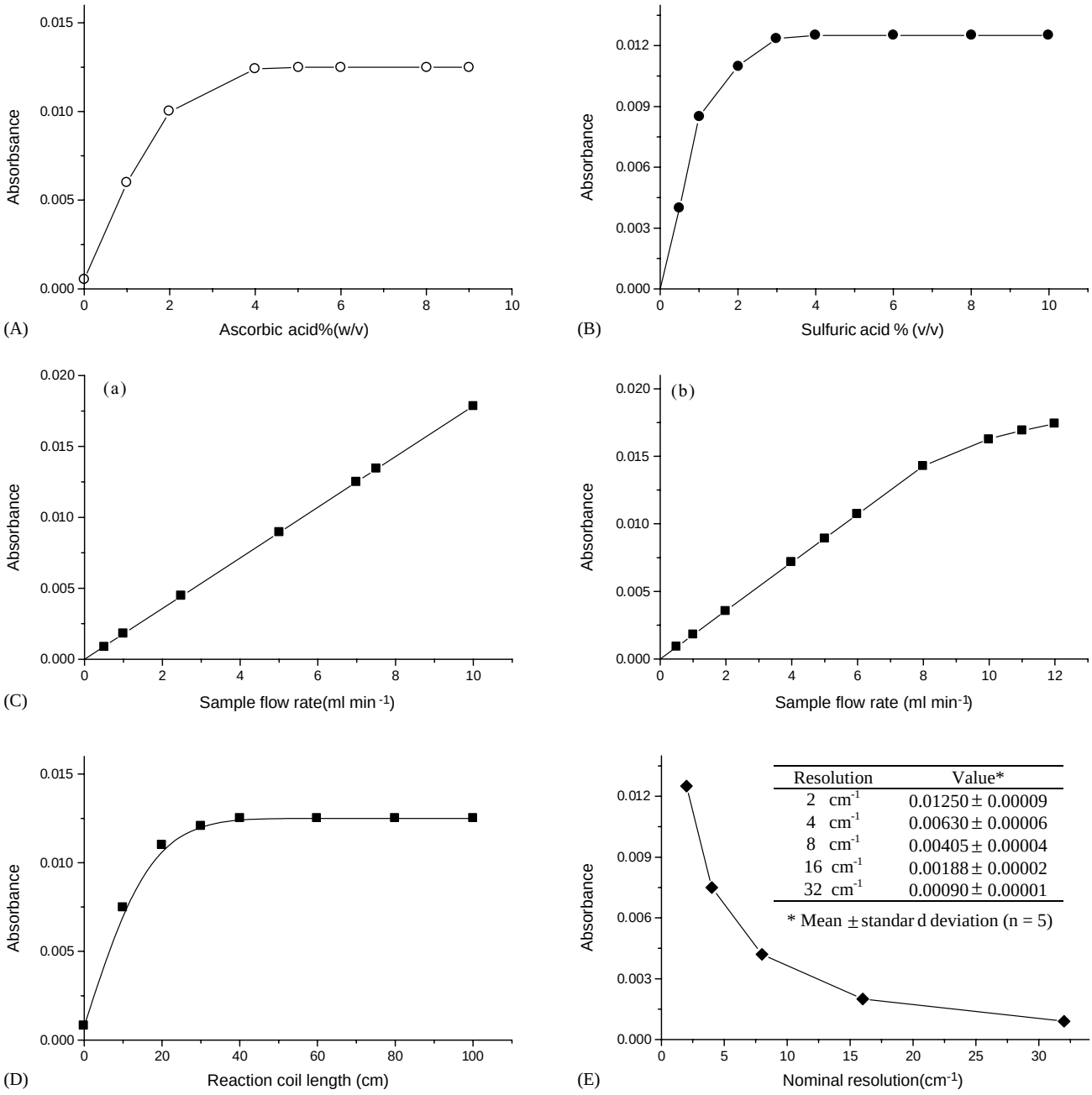


Fig. 3. Effect of chemical, flow analysis and spectroscopic parameters on the NO(g) generation. (A) Influence of the ascorbic acid concentration ([$H_2SO_4$] = 6% v/v). (B) Effect of the sulfuric acid concentration ([ascorbic acid] = 6% w/v). (C) Effect of sample ($Q_S$) and total ($Q_T$) flow rate: (a) the reducing agent flow rate ($Q_R$) was fixed at the same value than the sample flow rate ($Q_T = Q_S + Q_R = 2Q_S$), (b) $Q_R$ was fixed at 1 ml $min^{-1}$ ($Q_T = 1 + Q_S$). (D) Influence of the reaction coil length. (E) Effect of the nominal resolution on the analytical signal. Insert: mean ± standard deviation ($n = 5$) obtained for each nominal resolution from 2 to 32 $cm^{-1}$. In all experiences, a nitrite concentration of 50 μg $ml^{-1}$ was used. Other experimental conditions as indicated in Table 1.

these results, a gas carrier flow of 25 ml $min^{-1}$ was selected for further studies.

### 3.5. Effect of instrumental conditions

The effect of the instrumental parameters, such as the number of scans (the accumulation of scans) and the nominal resolution on the quality of the background and the analytical signal, was studied.

Initially, the influence of the number of scans employed to establish the background and to obtain each spectrum was tested from 1 to 25 scans. Referring to the background definition, three scans are sufficient to establish a stable and clean background. On the other hand, the accumulation of

scans in the proposed system does not have a significant effect on the S/N ratio, probably due to the very clean background obtained. These results are in good agreement with previously obtained results in our laboratory in HG–FTIR systems [30,31]. Thus, three scans were selected for further studies. Higher values do not improve the quality of the analytical signal, and deteriorate the sample throughput. Concerning the nominal resolution, this parameter affects the shape of the absorption band, the sensibility and the acquisition time. Fig. 3(E) shows the behavior of the analytical signal when this parameter increases from 2 to 32 $cm^{-1}$. The insert of the figure presents the information related to the mean ± the standard deviation ($n = 5$), obtained for each experience, as well the time required for the acquisition of each spectrum, accumulating three scans. An increase in the nominal resolution causes the depression and the broadening of the analytical band, and at a same time, drastically reduces the spectrum acquisition time. Then, the selection of this parameter is based on a compromise between analytical sensibility and sample throughput. However, in this case, seeking the best sensibility, a nominal resolution of 2 $cm^{-1}$ was selected for further studies.

### 3.6. Analytical figures of merit

Under the experimental conditions given in Table 1, and by using the standard IR gas cell (10 cm path length, and 60 ml internal volume), the analytical response was linear up to 80 μg $NO_2^-$ $ml^{-1}$, with a theoretical limit of detection – defined as the nitrite concentration which produces an analytical response equivalent to three times the standard deviation of the blank ($3\sigma$) – of 0.3 μg $NO_2^-$ $ml^{-1}$. The equation which describes the simple calibration line was $A = 0.000087 + 0.000243$ [$NO_2^-$], with a regression coefficient ($r$) of 0.9997, where $A$ represents the absorbance at 1876 $cm^{-1}$, corrected by means a baseline established between 1879 and 1872 $cm^{-1}$, and [$NO_2^-$] is the nitrite concentration in μg $ml^{-1}$. A typical signal obtained for a 50 μg $NO_2^-$ $ml^{-1}$ is 0.01250 ± 0.000087. On the other hand, the precision of the procedure was estimated by measuring ten replicates of standard solutions of 50, 20 and 5 mg $ml^{-1}$ nitrite. The relative standard deviations obtained were 0.7, 1.2, and 2.8%, respectively. The sample throughput was about 40 samples per hour.

The accuracy of the proposed method was initially checked by means of recovery experiences. For this purpose, two sample solutions (see samples) were spiked with known amounts of nitrite. In all cases, the quantitative recoveries (97–102.5%) were obtained, as can be seen in Table 2.

In order to study the possible matrix effect, standard addition graphs were prepared by adding various amounts of nitrite (from 0 to 50 μg $NO_2^-$ $ml^{-1}$) to a 20 ml of sample solution, and diluting to a final volume of 25 ml with DI. The equation obtained for the simple calibration and standard additions for samples 1 and 2 were: $A = 0.000087 + 0.000250$ [$NO_2^-$], $A_1 = 0.00463 + 0.000252$ [$NO_2^-$], and $A_2 = 0.00363 + 0.000248$ [$NO_2^-$], respectively. These curves did not show significant differences in their slope, which denoted the absence of any matrix effect.

Table 2
Recovery studies

| Sample | Endogenous value[a] (μg $ml^{-1}$) | [$NO_2^-$] added (μg $ml^{-1}$) | [$NO_2^-$] found[b] (μg $ml^{-1}$) | Recovery (%) |
|---|---|---|---|---|
| Frankfurter 1[c] | 11.6 ± 0.2 | 0 | 11.5 ± 0.2 | – |
| | | 10 | 21.8 ± 0.3 | 102 |
| | | 25 | 35.9 ± 0.7 | 97.2 |
| | | 50 | 61.9 ± 0.7 | 102.5 |
| Frankfurter 2[c] | 9.7 ± 0.1 | 0 | 9.8 ± 0.3 | – |
| | | 10 | 20.0 ± 0.5 | 103 |
| | | 25 | 35.0 ± 0.8 | 101.2 |
| | | 50 | 58.2 ± 0.9 | 97 |

[a] Correspond to a dilution of the sample solution (20 ml in a final volume of 25 ml).
[b] Mean ± standard deviation of five independent measurements.
[c] Frankfurter 1 and 2: sample solutions (10 g in a final volume of 100 m).

### 3.7. Analysis of real samples

To check the applicability of the proposed method, the determination of nitrite in food samples, such as sausages, was carried out. Samples were also analyzed by two FI-spectrophotometrical procedures. The first one based on the kinetic reaction between thionine and bromate, which is catalyzed by the nitrite ion [40], and the second on in the Shinn reaction [41]. The results obtained by using the simple and standard addition calibration lines, as well those found by the reference procedures agreed well (see Table 3), thus indicating the accuracy of the proposed method, and its viability for the analysis of nitrite in this type of samples.

## 4. Critical evaluation of the developed methodology

In this work, a novel analytical method based on the coupling between flow analysis–vapor phase generation and FTIR spectrometry was developed for the determination of nitrite. The on-line system, which includes a very speedy quantitative homogeneous reduction of nitrite to gaseous nitric oxide with ascorbic acid, reduces sample handling and makes possible the complete automation of the nitrite determination. The sensitivity of the proposed method, by using IR gas cell (path length = 10 cm) is poor (LOD = 0.3 μg $NO_2^-$ $ml^{-1}$). However, it can be easily enhanced by using (i) a multiple path IR gas cell with a path length of various meters, or/and (ii) a pre-concentration step (solid phase extraction). On the other hand, the precision, expressed by means of the relative standard deviation, is comparable or better than those reported in different spectrophotometric procedures [7–9]. In principle, the FA–VPG–FTIR determination of nitrite offers three important advantages, by com-

Table 3
Analysis of real samples

| Sample | FA–VPG–FTIR | | Reference [1][c] $[NO_2^-]$ ($\mu g\,g^{-1}$)[a] | Reference [2][d] $[NO_2^-]$ ($\mu g\,g^{-1}$)[a] |
|---|---|---|---|---|
| | Simple calibration $[NO_2^-]$ ($\mu g\,g^{-1}$)[a] | Standard addition $[NO_2^-]$ ($\mu g\,g^{-1}$)[b] | | |
| Frankfruter 1 | 145 ± 4 | 147 ± 4 | 141 ± 3 | 146 ± 4 |
| Frankfruter 2 | 120 ± 3 | 117 ± 3 | 122 ± 3 | 118 ± 3 |
| Frankfruter 3 | 85 ± 2 | | 83 ± 3 | 82 ± 2 |
| Frankfruter 4 | 78 ± 2 | | 81 ± 2 | 77 ± 3 |
| Frankfruter 5 | 90 ± 2 | | 92 ± 3 | 91 ± 3 |

[a] Mean ± standard deviation of five independent measurements.
[b] Mean ± standard deviation of three measurements.
[c] Reference method 1: FI-kinetic spectrophotometric method [39].
[d] Reference method 2: FI-spectrophotometic method based on the Shinn reaction [40].

parison with other procedures:

(i) The redox reaction between nitrite and ascorbic acid is simple and very speedy, avoiding in this way, complex chemical reactions, which are generally developed in two or three steps, and are time consuming. On the other hand, most of these reactions are subject to several and serious interferences. As an example, the most typical spectrophotometric systems developed for the determination of nitrite are based on the Griees–Ilosvay reaction [7,8], which involves a two-step reaction mechanism, including a coupling reaction. This step is sometimes slow, and is greatly affected by chemical interferences [5].
(ii) The analytical system proposed does not require a special control over experimental parameters, such as temperature, pH, etc. This fact contrasts with the very strict control of these parameters, which is necessary in all the kinetic methods, and in most of the fluorimetric and spectrophotometric procedures described for the determination of nitrite.
(iii) The proposed method allows, in the same way that in hydride generation [29], an efficient matrix removal, thus reducing potential interferences. This fact is very important, and contrasts with the serious interfering effects reported in the traditionally kinetic, fluorimetric, spectrophotomrtic and electrochemical methods.

## Acknowledgements

The authors acknowledge the financial support given by the CDCHT (Consejo de Desarrollo Científico, Humanístico y Tecnológico) of Los Andes University, Mérida, Venezuela (Project C-945-B). Thanks are also due to FONACIT (Fondo Nacional de Ciencia, Tecnología e Innovación) for financial aid through Project S1-2000000815.

## References

[1] A. Kademzadeh, A.A. Ensafi, Anal. Chim. Acta 442 (2001) 319.
[2] R. Montes, J.J. Laserna, Talanta 34 (1987) 1021.
[3] I.A. Wolf, A.E. Wasserman, Science 177 (1972) 15.
[4] R.D. Cox, C.W. Frank, J. Anal. Toxicol. 6 (1982) 148.
[5] J.B. Fox Jr., Anal. Chem. 51 (1979) 1493.
[6] J.L. Manzoori, M.H. Sorouraddin, A.M. Haji-Shabani, Talanta 46 (1998) 1379.
[7] J.B. Fox, CRC Crit. Rev. Anal. Chem. 15 (1985) 283.
[8] J. Matthew, J.D. Moorcroft, R.G. Compton, Talanta 54 (2001) 785.
[9] M. Gallignai, M. Valero, M.R. Brunetto, A. Sanchez, M. Burguera, J.L. Burguera, V. Cerda, Ciencia 11 (2003) 235.
[10] B. Haghighi, A. Tavassoli, Talanta 56 (2002) 137.
[11] B. Haghighi, A. Tavassoli, Frezenius J. Anal. Chem. 371 (2001) 1113.
[12] P. Mikuška, Z. Veèera, Z. Zdráhal, Anal. Chim. Acta 316 (1995) 261.
[13] K. Robards, P.J. Worsfold, Anal. Chim. Acta 266 (1992) 147.
[14] Y. Kanda, M. Taira, Anal. Sci. 19 (2003) 695.
[15] T. Aoki, S. Fukuda, Y. Hosoi, M. Mukai, Anal. Chim. Acta 349 (1997) 11.
[16] R.S. Braman, S.A. Hendrix, Anal. Chem. 61 (1989) 2715.
[17] R.D. Cox, Anal. Chem. 52 (1980) 332.
[18] D. Tsikas, I. Fuchs, F.M. Gutzi, J. Frölich, J. Cromatogr. B. 715 (1998) 441.
[19] F. Yang, E. Troncy, M. Francoeur, B. Vinet, P. Vinay, G. Czaika, G. Blaise, Clin. Chem. 43 (1997) 657.
[20] Y. Kanda, M. Taira, Anal. Chem. 62 (1990) 2084.
[21] T. Aoki, Biomed. Cromatogr. 4 (1990) 128.
[22] A.R. Thornton, J. Pfab, R.C. Massey, Analyst 114 (1989) 747.
[23] J.R. Ferraro, L.J. Basile (Eds.), Fourier Transform Infrared Spectroscopy. Application to Chemical Systems, Academic Press, New York, 1978.
[24] K. Nakamoto, Infrared Raman Spectra of Inorganic and Coordination Compounds, Willey, New York, 1986.
[25] M. Gallignani, M. Valero, A. Sánchez, C. Ayala, M. Brunetto, J.L. Burguera, Flow analysis–gaseous phase generation–Fourier transform infrared spectrometric determination of nitrite. 12th International Conference on Flow Injection Análisis (ICFIA-2003), Poster 1–4, Book of Abstract, December 7–12, 2003, p. 78.
[26] M. Gallignani, S. Garrigues, M. de la Guardia, Analyst 119 (1994) 653.
[27] E. López-Anreus, S. Garrigues, M. de la Guardia, Anal. Chim. Acta 308 (1995) 28.
[28] A. Pérez Ponce, S. Garrigues, M. de la Guardia, Vibr. Spectrosc. 16 (1998) 61.
[29] J. Dedina, D.L Tsalev, Hydride Generation Atomic Absorption Spectrometry, Willey, Chichester, 1995.
[30] M. Gallignani, C. Ayala, M.R. Brunetto, J.L. Burguera, M. Burguera, Analyst 127 (2002) 1705.
[31] M. Gallignani, C. Ayala, M.R. Brunetto, M. Burguera, J.L. Burguera, Talanta 59 (2003) 923.
[32] W. Fiddler, R.C. Doerr, R.A. Gates, J.B. Fox, J. Assoc. Off. Anal. Chem. 67 (1984) 2525.

[33] A.A. Ensafi, G. Bagherian Dehaghei, Frezenius J. Anal. Chem. 363 (1999) 113.
[34] J.H. Dhont, Analyst 85 (1960) 144.
[35] G. Svehla, Vogell's Quantitative Inorganic Chemistry, seventh ed., Longman, 1996.
[36] M. Trojanowicz, W. Matuszewsky, B. Szostek, Anal. Chim. Acta 261 (1992) 391.
[37] K. Helrich (Ed.), Official Methods of Analysis of the Association of Official Analytical Chemist, 15th ed., Association of Official Analytical Chemist, Arlington, VA, 1990.
[38] M. Gallignani, M. Valero, M.R. Brunetto, J.L. Burguera, M. Burguera, J.L. Burguera, Talanta 52 (2000) 1015.
[39] M. Gallignani, H. Bahsas, M.R. Brunetto, J.L. Burguesa, M. Burguera, Y. Petit de Peña, Anal. Chim. Acta 369 (1998) 57.
[40] B. Schweizer, S. Fan, H. Muller, Techniques for Flow Injection Analysis in UV/Vis Spectroscopy, Perkin Elmer Corporation, vol. II, 1993, p. 152.
[41] J.F. Van Staden, Anal. Chim. Acta 138 (1982) 103.