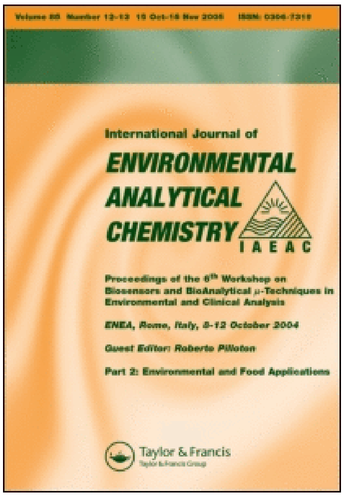

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Determination of Nitrite by Reverse Flow Injection Analysis

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A simple method for the determination of nitrite using reverse flow injection analysis is proposed. Safranine-T is quickly diazotised by nitrite and coupled with an excess of this dye. The reverse FIA mode with a single-channel gives maximum sensitivity. The influence of reaction variables and rFIA manifold, the precision and accuracy of the method and the effects of foreign ions are studied. The procedure is applied to the determination of nitrite in waste waters and cured meats.

KEY WORDS: Nitrite, reverse FIA, spectrophotometry, waste waters, cured meats.

INTRODUCTION

Nitrite determinations are very frequent in environmental analysis. Nitrite does not commonly occur in nature, since it is either oxidized to the more chemically stable nitrate, or reduced by bacteria and other microorganisms to ammonia. However, sodium nitrite is currently used as the conservant for meat products. It has been proved that nitrite is an important precursor of N-nitrosamines, which are potential carcinogens. Thus, methods for the nitrite determination in foods and other biological systems are becoming very important.

Several procedures for determining nitrite ions in solution have been reported. Spectrophotometric methods usually involve the

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diazotisation of an aromatic amine and coupling of the diazocompound with another amine to form a highly coloured azo dye.[1] Formation of a diazo dye using only one reagent was employed by Kamm *et al.*[2] to determine nitrite by the nitrosation of 1-naphthylamine in a large excess of the reactive.

Flow injection analysis (FIA) provides an alternative method of analysis with considerable time saving. Most of the reported procedures for nitrite determination by FIA[3–35] are based on diazotisation-coupling reactions using two different reagents.

In the present study, a simple method, with only one reagent, is described. Safranine-T is quickly diazotised by nitrite and coupled with an excess of this amine to give a stable colour. The reverse FIA mode with a single channel is the most useful for this reaction because it provides an increase in sensitivity, reproducibility and a minimisation in reactive consuming. The procedure has been applied to the determination of nitrite in cured meats and waste waters.

EXPERIMENTAL

Reagents

All chemicals used were of analytical grade and the solutions were prepared with doubly distilled water.

Safranine-T solution, 10^{-3} M. Prepared from the commercial product (Schuchardt. Basic Red 2, C.I. 50240) by dissolving 0.1754 g in 500 ml of water.

Sodium nitrite, 0.01 M. Prepared by dissolving sodium nitrite, dried for 4 h at 110 °C, in doubly distilled water. Working solutions were prepared by dilution just before use.

Apparatus

A Pye Unicam SP8-200 spectrophotometer with a Hellma flow cell (inner volume, 18 μl) was used as a detector. The flow system consisted of a peristaltic pump (Gilson Minipuls HP8) and an injection valve (Omnifit). Flexible teflon tubing (0.5 mm i.d.) was used for the coils.

Flow injection manifold A schematic diagram of the reverse flow injection system is shown in Figure 1. A 135-μl volume of the

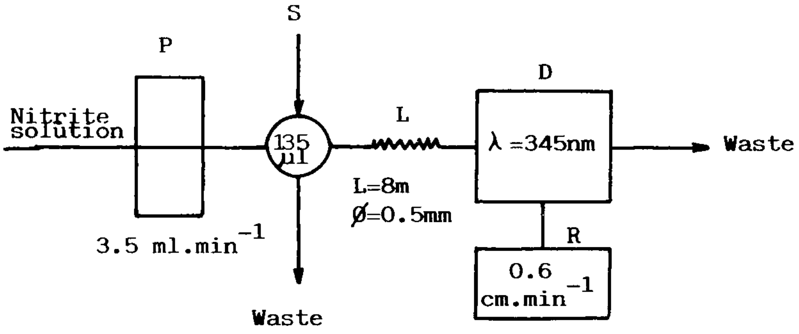

Figure 1 Single-channel manifold for the determination of nitrite by rFIA. Nitrite solution acts as carrier and is propelled at 3.5 ml.min^{-1} by a peristaltic pump, P; S, reagent injector (135 µl loop size); L, reactor (8 m length, 0.5 mm i.d.); D, vis-uv detector at 345 nm; R, recorder (0.6 cm.min^{-1}).

reagent (5×10^{-4} M safranine-T in 1.5 M HCl) was injected into the nitrite flow (3.5 ml.min^{-1}). Reactor, 8-m long, was coiled on a 6-mm cylinder diameter. The wavelength of the spectrophotometer was fixed at 345 nm. The chart speed for the recorder was 0.6 cm.min^{-1}.

General procedure

Use the single-line manifold showed in Figure 1 and the nitrite solution as carrier (3.5 ml.min^{-1}). Inject 135-µl of the reagent (5×10^{-4} M safranine-T in 1.5 M HCl) and measure the absorbance at 345 nm. The concentration of nitrite is evaluated from the peak heights by using a calibration curve prepared from standards. The curve is linear between 0.05 and 4.0 µg.ml^{-1} of nitrite.

Procedure for the determination of nitrite in the samples

Waste water Filter the sample of waste water and use it as carrier. Then proceed as in General Procedure. Determine the concentration of nitrite from the peak heights by using the calibration curve.

Meat samples Samples of cured meat were purchased from a supermarket and subjected to the following treatment.[35] Blend a 10-g aliquot of a well-homogenized sample for 5 minutes with 70 ml of water and 12 ml of 2% solution of sodium hydroxide. Adjust the pH to about 8 by using more sodium hydroxide solution if necessary. Heat to 50–60°C, add 10 ml of 0.42 M zinc sulphate solution and maintain at 50 °C for 10 minutes with occasional mixing. Cool, filter and dilute to 200 ml.

RESULTS AND DISCUSSION

In acidic medium, safranine-T is diazotised rapidly by nitrite and coupled with excess of the amine to give an azo dye with an absorption maximum at 345 nm. Figure 2 shows the spectral curves of the dye in the absence and in the presence of nitrite.

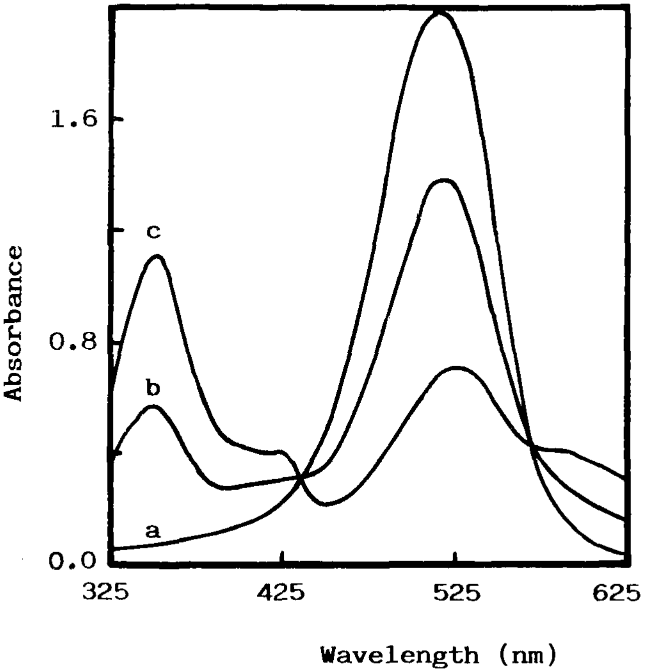

Figure 2 Absorption spectra of safranine-T. 8×10^{-5} M safranine-T; 0.25 M HCl. a, without nitrite; b, 1.0 μg.ml^{-1} nitrite; c, 2.0 μg.ml^{-1}.

Influence of chemical variables

The concentration of the two reagents, safranine-T and hydrochloric acid, are closely related because the absorbance of the dye changes with the acidity of the medium.

Figure 3 shows the variation of the peak height with safranine-T concentration at 345 nm. The carrier solution was water (curve *a*) or sodium nitrite solution, $2\,\mu g.ml^{-1}$ (curve *b*). Safranine-T solutions in the range $10^{-5}-8\times10^{-4}$ M in a 1.5 M HCl medium were injected into the carrier solution. A 5×10^{-4} M safranine-T concentration was chosen because higher concentrations did not increase the peak height in the presence of nitrite, but increased the blank values.

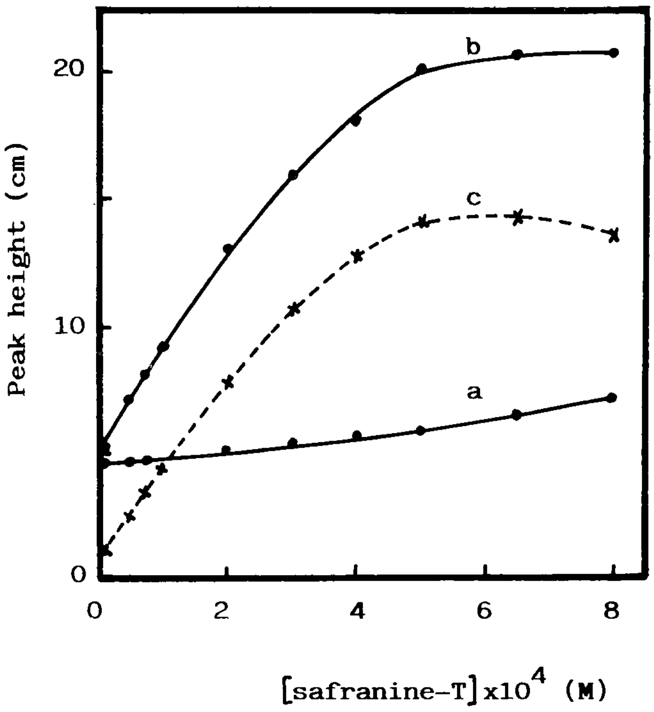

Figure 3 Effect of safranine-T concentration on peak height. 1.5 M HCl; loop size, $135\,\mu l$; reactor length, 8 m; flow rate, $3.5\,ml.min^{-1}$. a, water as carrier; b, $2\,\mu g.ml^{-1}$ nitrite solution as carrier; c, difference between a and b.

The influence of hydrochloric acid concentration on the peak height is represented in Figure 4. In this case, solutions of 5×10^{-4} M safranine-T in different hydrochloric acid media between 0.1–3.0 M were injected in the carrier solutions. A 1.5 M HCl concentration was chosen as a compromise between the higher analytical signal and the lower blank.

Influence of flow injection analysis variables

The reverse FIA method can be particularly advantageous for increasing the sensitivity[37] and the reproducibility.

We have investigated the influence of reaction tube length, flow rate and injected volume of reagent on both the sensitivity of the reaction and the sampling rate.

The influence of the reactor length was studied between 0.5–12 m using a 2 μg.ml^{-1} nitrite solution and water as carriers. As shown in

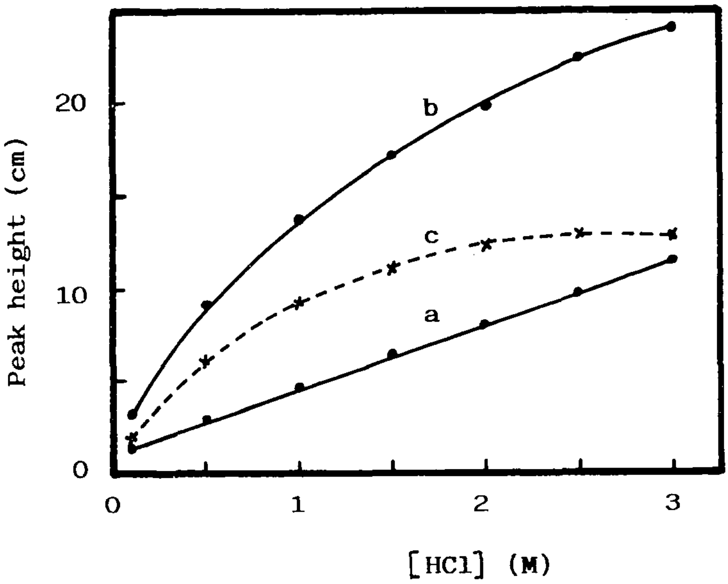

Figure 4 Effect of hydrochloric acid concentration on peak height. Safranine-T, 5×10^{-4} M; loop size, 135-μl; reactor length, 8 m; flow rate, 3.5 ml.min^{-1}. a, water as carrier; b, 2 μg.ml^{-1} nitrite solution as carrier; c, difference between a and b.

Figure 5, the analytical signal decreased with the length of the reactor up to 4 m. Longer reactors increased the peak heights in the presence of nitrite but did not when water was used as carrier.

Looking for a compromise between the maximum absorbance, i.e., maximum sensitivity, and a good sampling frequency, the length of the reactor chosen was 8 m.

Variation of the flow rate of the nitrite solution over the range 2.0–7.0 ml . min^{-1}, did not produce significant changes in the peak heights. Therefore, a flow rate of 3.5 ml . min^{-1} was used.

As can be seen in Figure 6, the increase in the intensity of the analytical signal as a function of the volume of the reagent injected was almost linear between 60 and 135 μl. Larger volumes of reagent produced smaller increase in the peak heights. A 135 μl loop size was chosen.

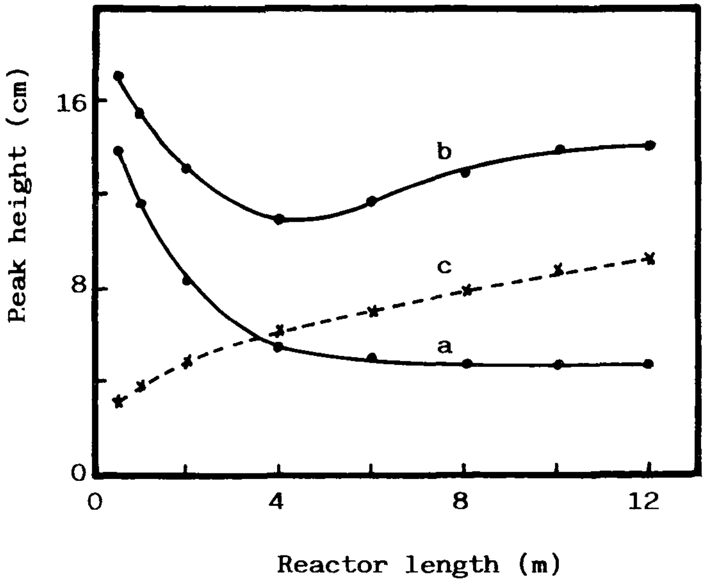

Figure 5 Influence of the reactor length on the peak height. Reagent, 5×10^{-4} M safranine-T in 1.5 M HCl; loop size, 135 μl; flow rate, 3.5 ml . min^{-1}. a, water as carrier; b, 2 μg . ml^{-1} nitrite solution as carrier; c, difference between a and b.

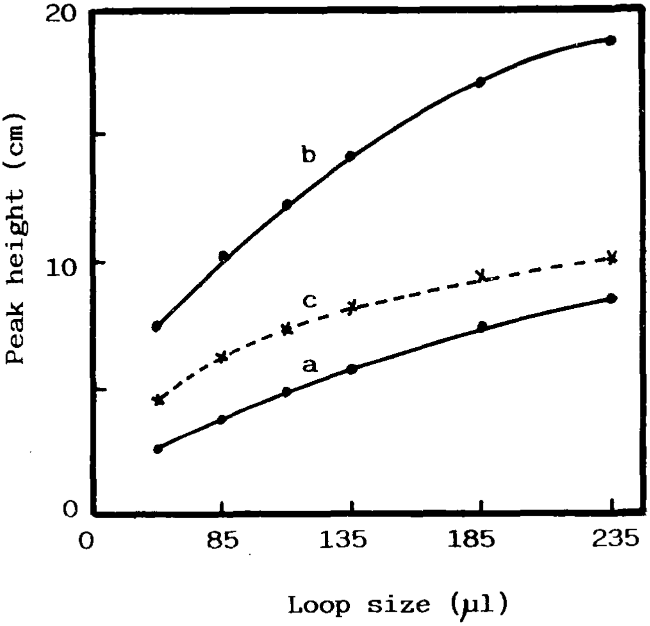

Figure 6 Effect of the loop size on the peak height. Reagent, 5×10^{-4} M safranine-T in 1.5 M HCl; reactor length, 8 m; flow rate, 3.5 ml.min^{-1}. a, water as carrier; b, 2 μg.ml^{-1} nitrite solution as carrier; c, difference between a and b.

Calibration graph and effect of foreign ions

A calibration run obtained for nitrite standards by the proposed reverse FIA system shows that there is a linear relationship between the peak height and the nitrite concentration in the range 0.05–4.0 μg.ml^{-1}.

The sampling rate (using the same nitrite solution as carrier) was about 110 samples per hour.

The coefficient of variation of 10 injections of solutions containing 0.2 and 2.0 μg.ml^{-1} of nitrite were 2.2 and 1.5%, respectively.

In order to apply the method to the determination of nitrite in a variety of matrices, interferences from several common ions that may coexist in the samples were studied. For this purpose, a 1 μg.ml^{-1} nitrite solution containing the foreign ion as the carrier solution was used. No interference from ions such as nitrate, sulphate, phosphate, carbonate, bromide, fluoride, acetate, calcium, magnesium, ammonium and zinc was observed. Silicate was tolerated in a 500:1 ratio to nitrite, and the maximum amount of perchlorate tolerated was 200 $\mu g.ml^{-1}$. The tolerance limit was taken as the concentration which caused an error of no more than $\pm 4\%$ in the nitrite determination. It is clear that the selectivity of the method is satisfactory.

Comparison of reverse FIA and normal FIA

Reverse flow injection analysis (rFIA) was compared with the normal method (nFIA) using the following conditions: for rFIA, sodium nitrite solution, 2 $\mu g.ml^{-1}$ as carrier and 5×10^{-4} M safranine-T in 1.5 M HCl as sample reagent; and for nFIA, 5×10^{-4} M safranine-T in 1.5 M HCl as carrier and sodium nitrite solution, 2 $\mu g.ml^{-1}$ as sample reagent. The ratio of the peak heights obtained with the two techniques was rFIA/nFIA = 10.1/5.1. The sensitivity was two times higher using rFIA.

On the other hand, the values of the coefficient of variation for 10 injections of 2 $\mu g.ml^{-1}$ of nitrite using rFIA and nFIA were 1.5 and 3.6%, respectively. This illustrates the higher reproducibility of rFIA.

Determination of nitrite in waste waters

To investigate the applicability of this method, it has been used to determine nitrite in waste waters. The collected waters were filtered and directly analysed. The analysis of nitrite gave no problems even in the presence of high levels of chloride or nitrate. The results obtained are given in Table 1, and compared with those obtained by using the classical Griess reaction. The concentrations of nitrite obtained by the two methods are in good agreement.

Determination of nitrite in cured meats

Many procedures have been described in the literature for the treatment of food samples. Three steps are generally used in the sample preparation for nitrite determination: (1) extraction of nitrite into solution in a form in which it may be determined, (2) removing substances that interfere in the nitrite determination, and (3)

Table 1 Determination of nitrite in waste waters and cured meats by reverse flow injection analysis

Sample	*Nitrite*,[a] $\mu g.ml^{-1}$	
	rFIA method	*Griess reaction*
Water 1	3.20	3.23
Water 2	1.40	1.45
Water 3	0.95	0.99
Water 4	1.65	1.67
	Nitrite,[a] $\mu g.g^{-1}$	
Cooked ham	27.8	28.3
Bacon	20.9	22.0
Frankfurter	6.7	6.5
Mortadella	33.2	31.6

[a] Mean of triplicate analysis.

removing turbidities that interfere in colorimetric determination.

J. B. Fox[38] summarizes the sample preparation methods in the following way. The alkalization of samples before heating as well as the addition of precipitating reagents, usually zinc, are commonly carried out. The author strongly support the idea that dilution/alcalization/heating/zinc is the preferred method for preparation of samples for nitrite determination.

In the present paper, and because zinc does not interfere in the nitrite determination (see interferences) samples of cured meats were prepared according to this procedure (see Experimental). The extracts obtained were analysed by both rFIA and Griess methods. Table 1 shows that the nitrite contents of each sample determined by the two methods are in good agreement.

Recovery studies were performed on each of the cured meat samples by adding a known amount of nitrite to the sample solution prior to the initial homogenization step. In this way, we proved that during the sample treatment procedure no potential losses occur.

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