Sequential flow injection spectrophotometric determination of nitrite and nitrate in various samples

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Abstract

A direct spectrophotometric method has been developed for the sequential determination of nitrite and nitrate by flow injection analysis (FIA). The method is based on the reaction of nitrite with safranine O to form a diazonium salt which caused the reddish-orange dye colour of the solution to be changed to blue in acidic media, and which absorbs at 520 nm. The injected sample in the flow injection system is split in two streams. One of the streams is transported through a reductor microcolumn containing copperised cadmium, where nitrate is reduced to nitrite. The two streams are then mixed and treated with the appropriate reagents. The influence of reagent concentrations and manifold parameters was studied. The effect of potentially interfering ion was examined and procedures for elimination of interfering cations are proposed. Nitrite and nitrate can be determined in the range 0.0001–3.00 and 0.005–3.40 μg ml⁻¹, respectively, with a sampling rate of 20 ± 3 h⁻¹. Detection limits (3σ) of 0.5 and 3 ng ml⁻¹ were obtained for nitrite and nitrate, respectively. Nitrite and nitrate were determined in food samples by the proposed method with satisfactory results. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Nitrite; Nitrate; Sequential; Flow injection analysis; Spectrophotometry

1. Introduction

The determination of nitrate is an important factor in the analysis of soils, food and natural waters. Nitrite and nitrate are intimately involved in the overall nitrogen cycle in soil and higher plants [1]. A limit of 45 μg ml⁻¹ nitrate has been proposed for drinking water, since excessive concentrations lead to methaemoglobinemia in infants [2]. Nitrite is formed during the biodegradation of nitrate and ammoniacal nitrogen or nitrogeneous organic matter is an important indicator of fecal pollution of natural water. The determination of nitrite is of general importance because of its harmful impact on human health. The toxicity of nitrite is primarily due to its interaction with blood pigments to produce methaemoglobinemia. The reaction between nitrite and secondary or tertiary cumene leads to the formation of N-nitroso compounds, some of which are known to be carcinogenic, teratogenic and mutagenic [3–5].

Most of the flow injection analysis (FIA) methods for the simultaneous determination of nitrate and nitrate are based on a diazo-coupling reaction [6–9] (Griess method) or liquid–liquid extraction [10]. According to our knowledge, only one paper is based on the diazonium salt system for the simultaneous determination of nitrite and nitrate [11]. Our proposed...
method is based on the reaction of nitrite with safranin O to form a diazonium salt in an acidic solution. Nitrate is reduced to nitrite by using two sequential columns (copper and copper-coated cadmium) [12] and the total nitrite content (initially available nitrite plus produced nitrite) are determined by the proposed method. The method is fast, simple and sensitive compared to all of the present methods for the simultaneous determination of nitrite and nitrate.

2. Experimental

2.1. Reagents

All of the chemicals used in this work were of analytical reagent grade. Distilled water was used throughout, unless otherwise stated.

A nitrite standard solution (1000 μg ml⁻¹) was prepared by dissolving 0.1371 g of dried (for 4 h at 105–110°C) sodium nitrite (Merck) in water and diluting to 100 ml in a standard flask. A pellet of sodium hydroxide was added to prevent the liberation of nitrous acid and 1 ml of chloroform to inhibit bacterial growth. The working standard solutions were freshly prepared daily by diluting the stock solution with water.

A nitrate standard solution (1000 μg ml⁻¹) was prepared by dissolving 0.1500 g of dried (for 1 h at 105–110°C) sodium nitrate (Merck) in water and made up to volume in a 100 ml standard flask. A few drops of chloroform were added to the solution which was kept in a refrigerator for preservation.

A potassium chloride solution (0.05 M) was prepared by dissolving 0.3725 g of anhydrous (dried at 115°C) KCl (Merck) in doubly distilled water and made up to volume in a 100 ml standard flask.

A safranine O solution (4.84 × 10⁻⁵ M) was prepared by dissolving 0.0170 g of the dye (Merck) in water and made up to volume in a 100 ml standard flask.

2.2. Apparatus

A diagram of the flow system employed is shown in Fig. 1. The decrease in absorbance was measured with a Shimadzu model 6AV spectrometer, equipped with a flow-through cell of 20 μl inner volume and 1.0 cm optical path, with its output connected to a Varian strip-chart recorder (Model 9176). A 12-channel peristaltic pump (Desaga, Model PLG) with four silicone rubber tubes (1.0 mm i.d.) was used. PTFE mixing joints and tubing of 1.0 mm i.d. was used for the connections and for the mixing coil. The controlled temperature water bath (Gallenkamp, BLG) was used at a temperature of 30 ± 0.1°C. Sample solutions were injected with a six-port Rheodyne sample injector.

2.3. Column reactor

The reduction column (R6) was made of a glass tube (10 cm × 3 mm i.d.) filled with copperised cadmium granules (Merck) of particle diameter 0.3–1.50 mm held in position with glass wool plugs. The copper column (R5) before the cadmium was made of a glass tube (20 cm × 3 mm i.d.) filled with copper particles of diameter 0.5–1.50 mm. The columns were coated batchwise with copper by pumping a solution of 0.1 M EDTA at pH 6.80 and 0.1% (w/v) copper sulphate.
3. Real sample analysis

3.1. Preparation of food samples

For the preparation of meat samples, 2.00 g of beef was mixed with sand and homogenised in a mortar. The thoroughly mixed sample was taken in a 100 ml beaker and digested carefully following the method recommended by the AOAC [13].

For the flour samples, 2.00 g of the sample was taken in a 150 ml beaker and mixed with 80 ml of doubly distilled water. The beaker was placed in a water bath at 40°C and the contents digested for 15 min following the method recommended by the AOAC [13].

For cheese samples, any wax coating was removed along with the outer portion of cheese rind and any surface mould. For very hard cheese (Tabriz cheese with <40% moisture), the sample was diced into <6 mm cubes and mixed thoroughly. The sample was ground three times. For soft cheese (Gorgan Cheese with >40% moisture), the sample was made into a paste and mixed well. All cheese samples were stored in glass jars. A cheese–water slurry was prepared. The sample was blended in a high-speed blender at high speed until smooth. An amount of 6 g of the slurry was weighed accurately, placed in a 100 ml beaker and digested following the method recommended by the AOAC [13].

3.2. Procedure

Potassium chloride solution, 0.05 M (Fig. 1, R1), hydrochloric acid, 0.45 M (Fig. 1, R2), safranine O solution, 4.84 × 10⁻⁵ M (Fig. 1, R3) that were previously thermostated at the appropriate temperature (30°C) were pumped at 20.3 ml h⁻¹ for each channel via a peristaltic pump. The standard samples containing (0.0001–2.50 μg ml⁻¹ NO₂⁻, 0.005–3.4 μg ml⁻¹ NO₃⁻) samples were injected into a carrier stream (KCl) at point S (Fig. 1). The sample was then split into two streams. One of the streams was directly treated with other reagents (acid and safranine O and passed to the sample flow cell of the spectrophotometer, when the decrease in absorbance) at 520 nm is due to the initial nitrite concentration (first peak). The other stream was passed through the reduction microcolumns of copper (R4) and copperised cadmium (R5), where nitrate was reduced to nitrite and the delay coil. The sample was treated with the mixed reagent via reaction coil R₆ (250 cm) and the overall mixture was passed to the same cell of the spectrophotometer. The decrease in absorbance due to nitrite plus nitrate was measured (second peak). Thus, the nitrate content was determined by difference. The concentration of nitrite and nitrate were evaluated from the peak height by using the calibration graph prepared from the results obtained for standards.

4. Results and discussion

Safranine O is a dye that can react with nitrite to form a diazonium salt causing the reddish-orange dye colour of the solution to be changed to blue. This is a fast reaction in the presence of nitrite [14,15]. Nitrate can be reduced by passing it through a reducing column such as copperised cadmium [12], in which reaction nitrite can act as a catalyst. By using an automated system such as FIA, one can determine these two species at ultra-trace levels. The reaction is monitored spectrophotometrically by measuring the decrease in absorbance of the dye at 520 nm (see Scheme 1 and Fig. 2).

In order to optimise the flow injection method, the effects of reagent concentration, temperature and manifold variables on the magnitude of the peak height were studied.

4.1. Optimisation of concentrations and temperature

The assembly in Fig. 1 was selected to produce the best compromise between peak height and peak shape. The effect of reagent concentrations and temperature were studied with a fixed flow rate of 20.3 ml h⁻¹ (for each channel), 0.050 μg ml⁻¹ NO₂⁻ and 0.050 μg ml⁻¹ NO₃⁻, a sample volume of 118 μl, and the length of reaction coil of 250 cm at 30°C.

The effect of media acidity on the peak height was investigated up to 0.80 M hydrochloric acid, and the results are shown in Fig. 3. It is seen that there is an increase in the peak height when the concentration of hydrochloric acid was varied up to 0.45 M, while
Scheme 1. Reaction between safranine O and nitrite in acidic media.

Fig. 2. Absorption spectra of safranine O in the presence of 2 µg ml⁻¹ of nitrite after (a) 90 s, (b) 180 s, (c) 270 s, (d) 360 s, (e) 450 s, (f) 540 s from initiation of the reaction.
at higher acid concentrations no significant change in the response was observed; 0.45 M hydrochloric acid was therefore used for this study. Fig. 4 shows the effect of safranine O concentration on the peak height. Above $1.3 \times 10^{-4}$ M, the safranine O concentration does not affect the peak heights and $4.84 \times 10^{-5}$ M of the reagents was selected as the optimum concentration.

The effect of potassium chloride concentration on the reduction of nitrate to nitrite was studied in the range 0.010–0.070 M. The result shows that by increasing the concentration up to 0.050 M KCl, the sensitivity increased. Thus, 0.050 M KCl was selected as a carrier solution for the study.

The reaction of safranine O with nitrite in acidic media can occur at a suitable rate at high temperature. The results show that by increasing the temperature to 30 °C, the peak height increases, whereas at higher temperature the peak height decreases. Thus, 30 °C was selected for further study.

4.2. Influence of manifold variables

The optimisation of the proposed flow injection manifold was carried out at 0.050 µg ml$^{-1}$ nitrite and nitrate at 30 °C. The optimum length of the reduction column was established with 0.050 µg ml$^{-1}$ nitrite by using reduction column (R4 and R5) length of 5.0, 10.0, 15.0 and 20.0 cm. For each situation, the percentage of reduced product was determined by comparison of the plateau achieved with that corresponding to 0.050 µg ml$^{-1}$ nitrite processed in the same way. A column length of 10–20 cm was chosen for R4 and R5, because with these lengths, the reduction was complete, reproducibility was good and the back pressure was comparatively low. The lifetime of the reduction column naturally depends on the amount of oxidising material passing through it. In this continuous analysis system, it was found to be >36 h of use. The copperised reduction column can be reactivated on-line by replacing the distilled water with the EDTA/copper sulphate solution.

The peak height depends on the residence time of the sample zone in the system, e.g. on the total flow rate and length of the reduction coil. The effect of the pump flow rate was checked over the range 12–70 ml h$^{-1}$ for each channel. The signal decreased with increasing flow rate. This is due to the fact that at higher flow rate, the residence time was decreased, thus conversion of nitrate to nitrite was decreased. In
addition, the consumption of safranine O is decreased thus causing decreasing peak heights. From the results, 20.3 ml h\(^{-1}\) for each channel was selected for study.

The sample volume injected into the carrier line has a significant effect on the peak height. The signal increases with increasing sample volume up to 118 µl and remains nearly constant for larger volume up to 330 µl. A sample volume of 118 µl was chosen for further experiments.

The influence of reaction coil length on the sensitivity was studied at a constant flow rate. Increase in the length of reduction coil from 100 to 250 cm increased the sensitivity which is related to the longer residence time for the reaction mixture, whereas a longer reaction coil does not affect sensitivity, but only cause peak broadening. Thus, 250 cm for of the reaction coil length (Fig. 1) was selected as the optimum.

4.3. Determination of nitrite and nitrate

The calibration graph for several nitrite and nitrate standards assayed by the proposed flow injection system is shown in Fig. 5, while the performances of the methods for nitrite and nitrate are compared in Table 1, in which the detection limit was calculated as three times the standard deviation of the peak height measured for 10 injections and the precision (R.S.D.%) was obtained from three samples, for each of which 10 replicate determinations were made.

4.4. Effect of interfering ions

The effect of potentially interfering ions for the reaction between safranine O and nitrite was also studied at 0.050 µg ml\(^{-1}\) nitrite. The results in Table 2 show that many cations and anions do not interfere with nitrite and nitrate determination by the proposed method.

4.5. Determination of nitrite and nitrate in real samples

To check the applicability of the method, the determination of nitrite and nitrate in various samples such as meat products (beef), flour, cheese and environmental samples was carried out. The results are shown in

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Nitrite</th>
<th>Nitrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linear range (µg ml(^{-1}))</td>
<td>0.000–1.00</td>
<td>0.005–3.40</td>
</tr>
<tr>
<td>Detection limit (µg ml(^{-1}))</td>
<td>0.0005</td>
<td>0.003</td>
</tr>
<tr>
<td>Precision (R.S.D. %)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(1) 0.010 µg ml(^{-1}) NO(_2)(^-) and 0.020 µg ml(^{-1}) NO(_3)(^-)</td>
<td>1.80%</td>
<td>1.84%</td>
</tr>
<tr>
<td>(2) 0.050 µg ml(^{-1}) NO(_2)(^-) and 0.100 µg ml(^{-1}) NO(_3)(^-)</td>
<td>1.40%</td>
<td>1.50%</td>
</tr>
<tr>
<td>(3) 0.50 µg ml(^{-1}) NO(_2)(^-) and 0.5 0 µg ml(^{-1}) NO(_3)(^-)</td>
<td>1.25%</td>
<td>1.30%</td>
</tr>
</tbody>
</table>

*For 10 replicate measurements.
Table 2
Effect of interfering ions on the determination of 0.100 mg l\(^{-1}\) nitrite

<table>
<thead>
<tr>
<th>Species Tolerance limit (W_{ion}/W_{NO2})</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>K(^+), Na(^+), OCN(^-), EDTA, CO(_3^{2-}), Al(^3+), Se(VI), Ba(II), Br(^-), I(^-), H(_2)PO(_4^-), HPO(_4^{2-}), C(_2)O(_4^{2-})</td>
<td>1000</td>
</tr>
<tr>
<td>Mn(^2+), Zn(^2+), Pb(^2+), Cu(^2+), Ni(^2+), Mg(^2+), Ba(^2+), Cal(^{6+}), Fe(^3+), Fe(^2+), NO(_3^-), Br(^-), CN(^-), SCN(^-)</td>
<td>500</td>
</tr>
<tr>
<td>Hg(^{2+}), SCN(^-), I(^-)</td>
<td>300</td>
</tr>
<tr>
<td>S(_2)O(_5^{2-}), CH(_3)COO(^-), F(^-)</td>
<td>100</td>
</tr>
<tr>
<td>BrO(_3^-), H(_2)PO(_4^-), HPO(_4^{2-}), C(_2)O(_4^{2-}), IO(_3^-), CH(_3)COO(^-), F(^-)</td>
<td>5</td>
</tr>
</tbody>
</table>

Table 3
Determination of nitrite and nitrate in food samples

<table>
<thead>
<tr>
<th>Samples</th>
<th>Concentration of nitrite found(^a) (mg l(^{-1}))</th>
<th>Concentration of nitrate found(^a) (mg l(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bee meat</td>
<td>0.154 ± 0.02</td>
<td>0.080 ± 0.03</td>
</tr>
<tr>
<td>Calbus (dry)</td>
<td>0.098 ± 0.03</td>
<td>0.062 ± 0.01</td>
</tr>
<tr>
<td>Flour</td>
<td>0.355 ± 0.02</td>
<td>0.062 ± 0.01</td>
</tr>
<tr>
<td>Taibis cheese</td>
<td>0.025 ± 0.04</td>
<td>1.840 ± 0.02</td>
</tr>
<tr>
<td>Gorgan cheese</td>
<td>0.050 ± 0.01</td>
<td>2.22 ± 0.02</td>
</tr>
</tbody>
</table>

\(^a\) Mean of five determinations ± S.D.

Table 4
Determination of nitrite and nitrate in environmental water samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentration of nitrite(^a) (mg l(^{-1}))</th>
<th>Concentration of nitrate(^a) (mg l(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Karaj water Sample 1</td>
<td>0.040 ± 0.002</td>
<td>1.05 ± 0.010</td>
</tr>
<tr>
<td>Sample 2</td>
<td>0.050 ± 0.003</td>
<td>1.62 ± 0.009</td>
</tr>
<tr>
<td>Khazar sea water Sample 1</td>
<td>0.060 ± 0.001</td>
<td>0.60 ± 0.010</td>
</tr>
<tr>
<td>Sample 2</td>
<td>–</td>
<td>0.20 ± 0.020</td>
</tr>
</tbody>
</table>

\(^a\) Mean of five determinations ± S.D.

Tables 3 and 4. They show good reproducibility and accuracy in comparison to the standard method [13].

Acknowledgements

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