Flow-Injection Catalytic Spectrophotometric Determination of Trace Amounts of Nitrite

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FLOW-INJECTION CATALYTIC SPECTROPHOTOMETRIC DETERMINATION OF TRACE AMOUNTS OF NITRITE

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ABSTRACT

A flow-injection catalytic spectrophotometric method is proposed for the determination of nitrite based on its catalytic effect on potassium bromate oxidation of acridine orange in phosphoric acid medium. The reaction is monitored spectrophotometrically by measuring the decrease in absorbance of acridine orange at 491.5 nm coupling with the stopped-flow technique. The calibration curve was linear between 3.4 and 65 ng/mL, and the detection limit

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was 2.2 ng/mL. Up to 30 samples can be analyzed per hour with a relative precision of ca. 1.9%. The proposed method was successfully applied to the determination of nitrite in waters and food samples with satisfactory results.

INTRODUCTION

There has been an increasing need for a highly sensitive and rapid method for the determination of trace nitrite because of its potential role in producing nitrosoamine, a carcinogenic material[1-2], in the human body. The occurrence of nitrite salts in the environment and their use as food preservation is widespread. The most widely used methods for determination of nitrite are spectrophotometry based on the diazotization of an aromatic amine and subsequent coupling to form an azo dye[3-6]. These methods yield good sensitivity, but often have drawbacks of utilising a toxic reagent[7] and a relatively long coupling time. Other methods such as electrochemical[8], fluorimetry[9], and chromatography[10-12] are also used but suffer from time-consuming procedures and complicated instrumentation.

In view of sensitivity, the catalytic kinetic method is one of the most attractive approaches for the trace determination of nitrite[13-14]. Some batch methods have been reported for the catalytic determination of trace nitrite[15-16], as kinetic methods include time as a variable; in these bath methods care is needed in the mixing of reagents at regular time intervals in order to obtain highly accurate results. Hence, batch methods are sometimes complicated and time consuming, which discourage their use in routine analysis. Such disadvantages in the batch system can be overcome easily in the flow-injection analysis (FIA) system. The reaction time can easily be controlled by fixing the flow rate of the solution and the length of the reaction coil. Besides, the FIA technique is rapid, reproducible and suitable for on-line analysis. In this paper, a sensitive catalytic method based on the catalytic oxidation of acridine orange by potassium bromate has been established with a flow injection system. Although, several catalytic methods
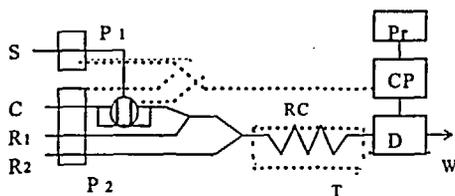
Fig. 1: Schematic diagram of flow injection system used for the determination of nitrite. S, sample; C, carrier; R1, Potassium bromate in phosphoric acid solution; R2, acridine orange solution; P1, P2, peristaltic pumps; V. injection value; RC, reaction coil; T thermostatic water bath; D, detector; CP, computer controlling and data processing system; Pr, printer.

have been described for the determination of nitrite[17-20], so far there has been no report on the use of acridine orange as an indicator for catalytic determination. The proposed procedure is very simple, rapid, sensitive and suitable for on-line determination of the nitrite in natural waters and foods.

EXPERIMENTAL

Apparatus

The FIA system used in this work was a LZ-2000 flow-injection processor (Shengyan · China) with an 18 μL flow cell (light paths, 10 mm), which is equipped with two peristaltic pumps and one rotary valve and controlled by a computer. A schematic diagram of the flow-injection system is shown in Fig. 1. The detector used was a U-3400 spectrophotometer (Hitachi · Japan) which was performed to measure the decrease in absorbance at 491.5 nm (see Fig. 2). A digital pH-3c meter (Shanghai · China) was used for the pH adjustments. A model 501 thermostat was employed to keep the reaction temperature at $25 \pm 0.1^{0}C$ and the manifold for the determination of nitrite .was made of poly(tetrfluoroethylene) (PTFE) tube (0.5 mm I.D.)
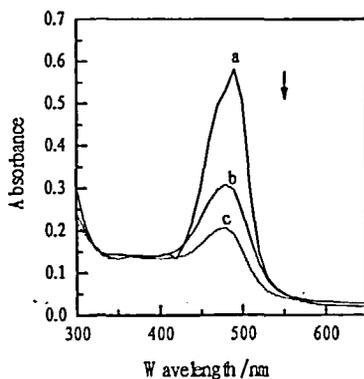
Fig. 2: Absorbance spectra of acridine orange in the presence of 8.0μg/L of nitrite after (a) 20s; (b) 60s; (c) 100s, from initiation of the reaction.

Reagents

All chemicals were of analytical regent grade and doubly-distilled water were used throughout.

A stock nitrite solution (1.0 mg/mL) was prepared by dissolving the required amount of sodium nitrite (dried for 4h at 105~110 ^{o}C) in water, a pellet of sodium hydroxide was added to prevent liberation of nitrite acid and 1.0 mL of spectroscopic grade chloroform to inhibit bacterial growth. The stock solution was kept in a refrigerator for preservation. Working standard nitrite solution were freshly prepared by diluting the stock solution with 0.4 mol/L NH_4Cl.

Acridine orange stock solution (1.0×10^{-4} mol/L) and potassium bromate solution (0.02 mol/L) were prepared by dissolving the required amounts in water, respectively. Phosphoric acid (2.0 mol/L) was prepared from the 85% reagent.

Reagent R1 was obtained by mixing 25.0 mL potassium bromate solution with 25.0 mL phosphoric acid solution. Reagent R2 was obtained by diluting the acridine orange solution.

Preparation of samples

For sausage samples (beef or pork), 2.0 g of the samples was mixed with sand and homogenised in a mortar; the thoroughly mixed samples were then taken in a 100 mL beaker and digested carefully following the method recommended by the AOAC[21].

For flour samples, 2.0 g of the samples was taken in a 150 mL beaker and mixed with 80 mL of doubly-distilled water; the beaker was placed in a water batch at 40 ^{0}C and the contents digested for 15 min following the reference[21].

The milk samples were deproteinized before analysis as follows: to a 250 mL sample was added 50 mL 35% sulfosalicyclic acid. Treated samples were mixed by vortexing every 5 min and allowed to react for 30 min at room temperature, then centrifuged at 1000 rpm for 15 min; 50 mL of the supernatant fluid was taken for analysis.

To all the environmental samples was added 1.0 mol/L NH_4Cl solution with the pH adjusted to pH = 9 in order to prevent the nitrite ion from decomposing at acid conditions[22].

RESULTS AND DISCUSSION

Optimum conditions for FIA

The flow-injection manifold (shown in Fig.1) was investigated in relation to flow variables. The variables studied were the length of reaction coil, sample loop volume, flow rate and reaction temperature. In order to obtain optimum conditions for the system, they were optimized by making all variables constant and varying one at a time, since the individual parameters do not interact during the course of optimization.

In a FIA system, the height of response peak depends on the residence time of the sample zone in the system, i.e., the flow rate and the reaction coil length. Fig. 3 shows the reaction coil length and samples loop volume on the peak height,
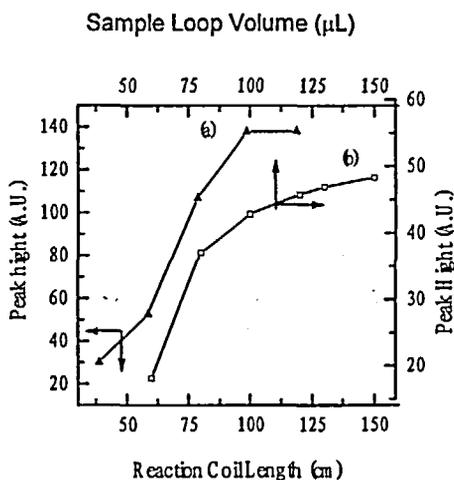
Sample Loop Volume (μL)

Fig. 3: Effect of reaction rate (a), and sample loop volume (b) on the peak height. Conditions: acridine orange, 8.0×10^{-5} mol/L; $KBrO_3$, 0.02mol/L; nitrite 8.0μg/L.

and from curve a, it can be seen that there is a gradual increase in the response peak with increasing length of the reaction coil from 40 to 100 cm. A further increase did not result in any significant change in the sensitivity. A reaction coil length of 100 cm was then selected for the FIA system.

The effect of sample loop volume was also investigated in the range of 60~150 μL. From Fig. 3 curve b, we can see that the analytical signal (Peak Height) increased with increasing sample volume up to 110 μL, above which no significant increase was observed. But at the same time, the sampling frequency decreased with increasing injection volume. 110 μL was chosen as the injection volume, because it provided the best compromise between sensitivity and sampling frequency.

Different flow rates were obtained by changing the pump tube diameters, and the experiments indicated that the lower flow rate gave the higher decrease in
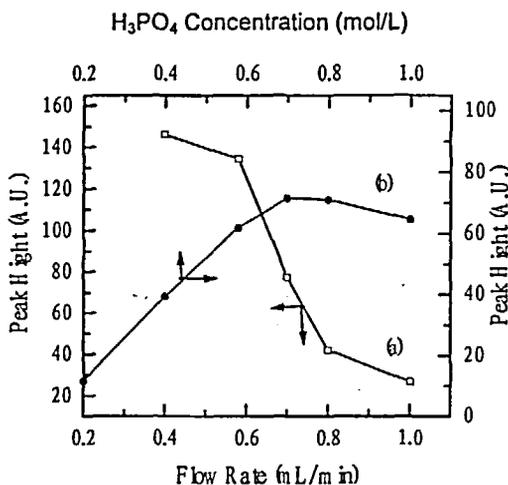
H₃PO₄ Concentration (mol/L)

Fig. 4: Effect of flow rate (a), and H₃PO₄ concentration (b) on the peak height. Conditions: reaction coil length 100cm; acridine orange, 8.0×10^{-5} mol/L: KBrO₃, 0.02mol/L; nitrite 8.0μg/L.

absorbance obviously, but the reproducibility was poor and the peaks were so broad that the sample throughput was very slow. The results are shown in Fig. 4 curve a. A total flow rate of 1.20 mL/min (0.40 mL/min for each channel) were chosen as a compromise between sensitivity and sampling rate. The effect of temperature on the peak height was studied between 25 and 50 ^{0}C. A temperature of 25 ^{0}C was used as the most suitable, because a further increase in temperature did not result in any significant change in the sensitivity.

Optimum conditions of reagents concentration

Experimental results showed that among phosphoric, sulfuric and hydrochloric acids, phosphoric acid gave the best results. It is seen that there is an increase in peak height when the concentration of H₃PO₄ was varied up to 0.7 mol/L (Fig. 4, curve b), while at higher acid concentrations no considerable changes was observed. 0.7 mol/L phosphoric acid in R1 was chosen as optimum.

The effect of acridine orange concentration on sensitivity was evaluated in the range of $0.20 \sim 1.0 \times 10^{-4}$ mol/L; the optimum concentration was in the range $0.60 \sim 0.90 \times 10^{-4}$ mol/L. A working concentration of 8.0×10^{-5} mol/L in R2 was chosen for further studies. It was shown that at a certain concentration of potassium bromate, the higher the concentration the higher the sensitivity. However, if the concentration is too high, the signal will decrease and a steady baseline can not easily be obtained. For this reason, 0.02 mol/L of $KBrO_3$ solution in R1 was adopted in subsequent work.

Calibration curve

Under the optimum conditions described above, in the concentration range of $3.4 \sim 65$ ng/mL of nitrite a quite linear calibration curve was obtained: $H=2.496+579.4C$ ($r=0.9992$), where H and C are the peak height (mm) and nitrite concentration (ng/mL). The detection limit was 2.2 ng/mL, which was calculated by multiplying the standard derivation of 15 blank measurements by three divided by the slope of the linear calibration curve. The relative standard deviation of 20 replicate measurements is 0.87% for 30 ng/mL of nitrite.

Interference of foreign species

The effect of various anions and cations on the determination of 30 ng/mL nitrite were investigated by adding various amounts of these foreign species. The amount at which the species caused an error of no more than 5% was taken as its tolerance limit and the results are shown in Table 1. As can be seen, many anions and cations have no considerable effect on the determination of nitrite. However, the presence of more than five-fold amounts of vanadium(v) and iodide caused a positive error owing to the catalytic effect on the indicator reaction.

Applications

The method described in this work was applied to the determination of nitrite in waters and food samples. The water samples were filtered and analyzed

TABLE 1

Tolerance limit of diverse species in the determination of 30 ng/mL nitrite

Tolerated ratio of foreign ion to nitrite	Foreign ion
1000	Na^+, K^+, Ba^{2+}, Ca^{2+}, Mg^{2+}, Pb^{2+}, NO_3^-, CO_3^{2-}, SO_4^{2-}, $C_2O_4^{2-}$, Cl^-, F^-
500	Al^{3+}, Cd^{2+}, Cu^{2+}, Ag^+, Co^{2+}
130	Zn^{2+}, Ni^{2+}, Fe^{2+}, Cr^{2+}
40	SO_3^{2-}, SCN^-, Hg^{2+}, Br^-
5	I^-, $V(v)$

TABLE 2

Results for the determination of nitrite in real samples: $(\mu g/g)$

Sample	Nitrite added	Proposed Method (n=7)	Standard method [a]	Recovery (%) [b]
River water1	--	0.061±0.001	0.062	--
	0.050	0.110		98
River water2	--	0.073±0.002	0.077	
	0.100	0.170		97
Milk	--	0.105±0.002	0.102	
	0.125	0.225		96
Flour	--	0.322±0.001	0.335	--
Sausage	--	0.647±0.002	0.642	--

[a] Obtained by standard method [21].
[b] Average of seven determinations

directly, and the food samples mentioned above were analyzed using their supernatant fluid. The results are given in Table 2. As can be seen the recovery of added nitrite at different levels in the samples is quantitative, and the results agree well with those obtained by the Griess standard method [21].

CONCLUSIONS

The proposed method is significant with respect to the development of a simple manifold for determining trace levels of nitrite between 3.4 and 65 ng/mL. Its simplicity, excellent reproducibility, higher sensitivity and freedom from pH effect are coupled with the high speed of the FIA technique.

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