KINETIC-SPECTROPHOTOMETRIC DETERMINATION OF TRACE AMOUNTS OF NITRITE ION BASED ON ITS REDUCTION REACTION WITH THIONINE IN ACIDIC MEDIA

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ABSTRACT: *A simple, sensitive and rapid method for determination of trace amounts of nitrite ion in real samples is reported. The reaction is initiated by adding known volumes of a nitrite ion solution of identified concentration to acidic solutions of thionine which causes decolorization of reagent with time. The absorbance changes is then monitored spectrophotometrically at* $\lambda_{max} = 602$ *nm at 25 °C. Calibration curve is linear in the range 0.005-1.500* $\mu g\ mL^{-1}$ *of nitrite ion. Many of cations and anions have no interfering effect but iodide, Mo(VI), W(VI) and* $S_2O_3^{2-}$ *ions do interfere. The effect of interfering ions have been eliminated by the use of proper masking agents. This method was used to determine nitrite ion concentration in Karoon river water and foodstuffs such as susages.*

KEY WORDS: *Kinetic-spectrophotometric determination, Thionine, Nitrite ion.*

INTRODUCTION

Nitrite is an active form of the nitrogen cycle, resulting from incomplete oxidation of ammonia or from reduction of nitrates. Under ordinary conditions nitrite levels in waters are low (down to 0.1 $\mu g\ mL^{-1}$), but the increasing use of nitrite as a preservative in the foood industry and as a corrosion inhibitor in the industrial process of water causes these levels occasionally being exceeded as a result of uncontrolled wastes [1,2].

The toxicity of nitrites is due primarily to their interaction with blood pigment to produce methemoglobinemia and their presumptive toxicity relates to their possible reaction, under normally encountered situations, with amines or amides to form toxic nitroso compounds. Nitrites are more toxic than nitrates and restriction of the daily intake for man to 0.4 milligram per kilogram of weight is recommended [3]. Because of these important features of nitrite ion, different analytical methods are applied to determine the concentration of this ion in real samples.

Among different analytical methods like ion-chromatography [4] chemiluminescence [5,6] voltametry [7,8] amperometry [9] potentiometry [9,10] flow-injection [11,12] and spectrophotometry [14-16], the later is the most widely used method. Several reaction-rate methods for the nitrite determination have been published in two recent decades [17-22].

By considering the important role of nitrite ion in

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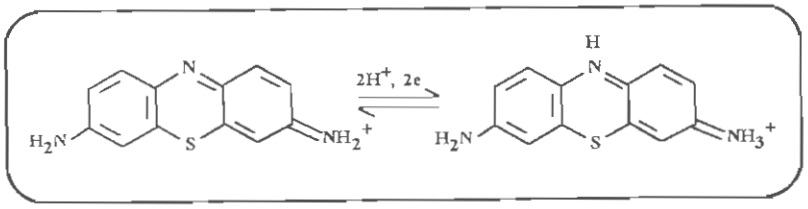

human life, it still seems necessary to introduce new, sensitive, reliable and simple methods for the determination of trace amounts of this anion in water and foodstuffs.

EXPERIMENTAL

All solutions were prepared from distilled demineralized water and analytical grade chemicals *(Merck)*.

Stock nitrite solution (1000 μg mL^{-1}) was prepared by dissolving 1.8478 g of potassium nitrite (*Merck* pro analysis >99.9% pure), dried at 115 °C for 24 hours, in a 1000 mL volumetric flask and diluting to the mark. Working standards were prepared daily.

Stock thionine (3.81×10^{-4} M) was prepared by dissolving 0.05 g of thionine chloride *(Merck)* and diluting to 500 mL in a volumetric flask. The solution was kept in a cold and dark place.

Sulfuric acid (4.0 M) was prepared by introducing 111 mL of concentrated sulfuric acid (95-97% purity and d=1.84 μg mL^{-1}) into a 500 mL volumetric flask and diluting to the mark with distilled demineralized water.

Absorption spectra were recorded on a JASCO Model 7850 UV-Vis spectrophotometer using a 1-cm quartz cell. Absorbance readings at a fixed wavelength (602 nm) were made on a Milton Roy Spectronic 20 D spectrophotometer. A W.C. Heraues Hanau thermostirrer with ±0.1 °C accuracy was used to set the temperature of solutions.

Procedures

Into a 10-mL standard flask, 5 mL of 3.81×10^{-4} M thionine, 2 mL of 4 M sulfuric acid, an aliquot containing 0.1-20 μg mL^{-1} of nitrite ion was introduced and diluted to the volume with demineralized water and the absorbance changes at 602 nm, was rapidly monitored for 2 minutes.

Water samples were collected from Karoon river and stored in polyethylen bottles. All samples were filtered through a Whatman No. 1 filter paper before analysis. The analysis was performed according to the procedure mentioned above. Another sample taken for analysis was sausage from Demes Co. 5 grams of crushed and grinded sausage was transfered to 100 mL of distilled water and heated up to 80 °C for 30 minutes. The solution was transfered into a 500 mL volumetric flask and heated for 2 hours over a steam bath. 5 mL of saturated $HgCl_2$ solution was added to the sample solution and it was filtered after 10 minutes. The filterate was diluted to the mark in a 500 mL volumetric flask. This solution was used for determination of its nitrite content. The results obtained by the present method and the standard Griess method [25] are given in Table 3.

RESULTS AND DISCUSSION

It was found that nitrite ion can reduce thionine (a redox indicator with the general name thiazines) in sulfuric acid medium. This reaction can be followed spectrophotometrically at λ_{max} of 602 nm.

The absorbance of thionine decreases with time as it is shown in Fig. 1. It should be noted that the reaction was very slow in the abscence of nitrite ion.

The effect of acid concentration on the rate of reaction between thionine and nitrite is shown in Fig. 2. The rate of reaction increased by increasing amount of sulfuric acid until an acid concentration of about 0.8 M is reached. Further addition of H_2SO_4 did not affect the reaction rate considerably. The optimum concentration of sulfuric acid was chosen as 0.8 M in final solution.

The effect of thionine concentration on the rate of reaction at optimum concentration of acid was studied and optimum concentration of thionine in the final solution was chosen to be 1.9×10^{-4} M(Fig. 3).

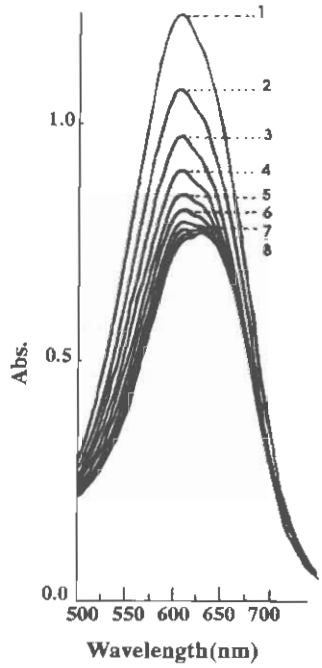

Fig. 1: Absorption spectra of 1.9×10^{-4} M thionine in the presence of 1 $\mu g\ mL^{-1}$ of nitrite ion in 0.8 M sulfuric acid solution. Time intervals 20 s.

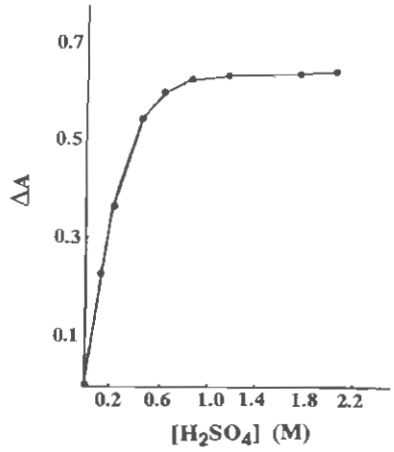

Fig. 2: Effect of sulfuric acid concentration on the reaction between thionine and nitrite. Conditions: nitrite ion, 1 $\mu g\ mL^{-1}$; thionine, 1.9×10^{-4} M.

Concentration ragne of thionine studied was 7.60×10^{-5}–2.67×10^{-4} M. The optimum concentration of thionine showed an absorbance of 1.130 at the begining of the reaction.

The effect of ionic strength on the reaction rate was investigated by using different concentration of sodium chloride and potassium nitrate salts. The ionic strength of solution varied from 0.001 to 0.20 M and no considerable effect on the reaction rate was observed.

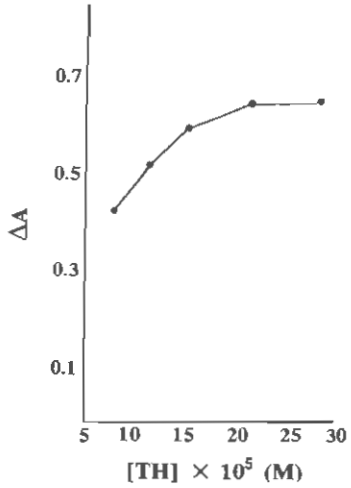

Fig. 3: Effect of thionine concentration on the reaction between thionine and nitrite ion. Conditions: nitrite ion, 1 $\mu g\ mL^{-1}$; sulfuric acid 0.9 M and different concentrations of thionine.

The effect of time on reaction completion is shown in Fig. 4. It is obvious that more than 90% of the reaction is completed 2 minutes after mixing of the reactants.

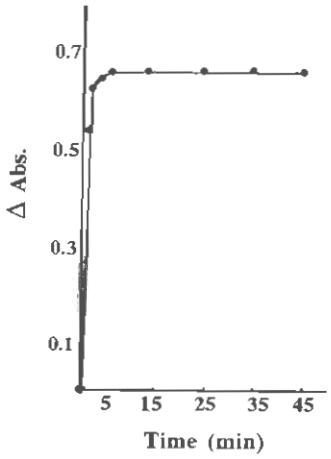

Fig. 4: Effect of time on completion of the reaction. Condtions: thionine, 1.9×10^{-4} M; sulfuric acid, 0.9 M and nitrite ion, 1 $\mu g\ mL^{-1}$

The influence of temperature on the reaction rate was studied and results are summarized in Table 1. It is seen that the rate of reaction increases by increasing temperature. We chose the room temperature for

convenience. The activation energy evaluated from the corresponding Arrhenius plot is E_a=1.32 kcal/mol.

Table 1: The effect of temperature on the rate of reaction

T(K)	ΔA
288	0.600
293	0.615
293	0.640
303	0.665
313	0.715
323	0.765
333	0.815

Calibration graph was obtained by applying the fixed time method under optimum conditions. The calibration curve was linear in the concentration range of 0.005-1.5 $\mu g\ mL^{-1}$ of nitrite ion in the final solution. The following regression equation is obtained: $C_{nitrite}$ ($\mu g\ mL^{-1}$)= 1.720(ΔA)-0.0075 and r=0.998.

The limit of detection obtained from LOD= $3S_{bl}/m$, [24] is 4.4 ng mL^{-1}, where S_{bl} is the standard deviation of blank signals for 12 replicate blank readings (2.5×10^{-3}) and m is the slope of the calibration curve. The LOD of this method shows high sensitivity of this simple, inexpensive and rapid method, compared with the other methods reported for nitrite analysis [17-22]. The relative standard deviation (RSD) of 12 replicate determinations was 0.58% for a 1.0 $\mu g\ mL^{-1}$ of nitrite ion solution.

In order to study the effect of various anions and cations on the determination of nitrite, a fixed amount of nitrite (1 $\mu g\ mL^{-1}$) was taken with defferent amounts of other ions and the recommended procedrue was followed. A relative error of 3% was considered tolerable. The results are summarized in Table 2. As it is seen, a large number of anions and cations used have no considerable effect on the determination of nitrite. However, some anions and cations interfered the nitrite determination. The interfering cations were successfully removed from the solution by passing it from a column containing a strongly acidic cation exchanger of H^+ form (*Merck*, R-SO_3^- type).

Table 2: Tolerance limits of diverse ions on the determination of 1.0 $\mu g\ mL^{-1}$ nitrite

Ion	Tolerated ratio of foreign ion to nitrite	
NO_3^-, Cl^-, F^-, CH_3COO^-, PO_4^{3-}, CO_3^{2-}, HCO_3^-, HPO_4^{2-}, CN^-, IO_3^-, SO_4^{2-}, $S_2O_8^{2-}$, SO_3^{2-}, EDTA, $Cr_2O_7^{2-}$	5000 2000	
SCN^-	0	Interfere
$S_2O_3^{2-}$, WO_4^{2-}, MoO_4^{2-}	1	
$S_2O_5^{2-}$, I^-	1	
Mg(II), Ca(II), Cu(II), Ni(II), Mn(II), Cd(II), Al(III), Hg(II), Pb(II), Hg(I),	2000	
Na(I), K(I)	5000	
Fe(III), Pd(II), Zn(II)	50	

The interference effects of Mo(VI) and W(VI) were eliminated by addition of Hg_2^{2+} [23] and the interference of SCN^-, I^- and $S_2O_3^{2-}$ was removed by introducing Hg^{2+} to the solution [23].

The results show high sensitivity, simplicity, reliability and very low detection limit of this method with respect to the reported methods [17-23].

In order to evaluate the applicability of the method to the real samples it was applied to the determination of nitrite ion in water samples taken from Karoon river and sausage samples from Demes Co. (Table 3).

Table 3: Determination of nitrite in Karoon river and sausage by standard Griess method and the present kinetic method

Sample	$\mu g\ mL^{-1}$ of nitrite Griess method	present method
sausage (1)	10.1	9.9
sausage (2)	10.2	9.9
Karoon water (1)	0.100	0.101
Karoon water (2)	0.099	0.098

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