

Simple and Sensitive UV-Spectrophotometric Determination for Trace Amounts of Nitrite with Safranin T

WEI-XING MA^{1,2,3,*}, JIAO Ji¹, OU SHA¹ and YING-HONG LIU¹

¹School of Chemical Engineering, Huaihai Institute of Technology, Lianyungang 222005, Jiangsu Province, P.R. China

²Jiangsu-Marine Resources Development Research Institute, Lianyungang 222005, Jiangsu Province, P.R. China

³Key Lab of Applied Chemistry, Huaihai Institute of Technology, Lianyungang 222005, Jiangsu Province, P.R. China

*Corresponding author: Tel: +86 518 85895405, E-mail: weixingma650528@163.com; weixingma650528@126.com

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A new fairly selective, simple and sensitive spectrophotometric method for the determination of trace amounts of nitrite was developed, based on that nitrite reacts with safranin T in HCl medium to form a nitroso-safranin T with the maximum absorption peak at 350 nm. Optimum reaction conditions and other important analytical parameters for the reaction system were established. Beer's law was found to obey for nitrite in the concentration range of 0-1.8 $\mu\text{g mL}^{-1}$ with molar absorptivity of $2.531 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$ and Sandell's sensitivity of $0.0018 \mu\text{g cm}^{-2}$. The effect of interfering ions on the determination is described. The recommended method was applied for the determination of nitrite in different water samples with satisfactory results in agreement with those of the AOAC recommended sulfanilamide-[N-(1-naphthyl)ethylenediamine hydrochloride] (NED) method.

Key Words: Nitrite, Safranin T, Spectrophotometry.

INTRODUCTION

Excess concentration of nitrite as an important water pollutant in drinking water is hazardous to health, especially for infants and pregnant women, because nitrite can react readily with secondary and tertiary amines and amides in human body producing toxic and carcinogenic nitrosamines compounds¹ and also oxidize iron in hemoglobin of the red blood cells to form methemoglobin, which is unable to carry oxygen causing blue baby syndrome².

Ammonia may also be converted into nitrites in distribution system through the activities of microorganisms. Use of nitrites has caused serious pollution problem in food preservation, dye, synthetic fiber, *etc.* So nitrite monitoring has been recognized as the necessity with legislation often levied on permissible levels in drinking water. At present the US EPA³ considered the maximum contaminant level that is allowed for nitrite in drinking water is $1.0 \mu\text{g mL}^{-1}$.

So far, there are some kind of instrumental methods developed for the determination of nitrite in environmental samples, such as spectrofluorometry⁴, chromatography⁵, flow injection analysis⁶, potentiometry⁷, electroanalytical method⁸ and so on. Visible spectrophotometric methods are simple, convenient, sensitive and are relatively inexpensive instrumental method which has been widely applied to the determination

of nitrite⁹⁻¹² in environmental water for faster and cheaper than other instrumental methods. Most of these methods are not suitable for routine determination of nitrite in environmental samples because some of them are costly or need skilled person to handle as well as necessity of use of toxic reagents which may have caused new serious pollution problem. Also, some of them are time consuming or require separation procedures.

Safranin T is a living dye which is used for dying on tissues and so far not applied to the determination of nitrite. In present work, a new simple and sensitive spectrophotometric method for the assay of trace amounts of nitrite was developed, based on the nitroso-reaction of nitrite with safranin T in HCl medium. In the proposed method, there are no complicated sample separation and extraction steps with satisfactory analytical results in agreement with those of the AOAC recommended sulfanilamide-NED [N-(1-naphthyl)ethylenediamine hydrochloride] method¹³.

EXPERIMENTAL

A Shimadze UV-250 pc model UV-visible spectrophotometer (Tokyo, Japan) with 1 cm matched quartz cells was used for the absorbance measurements. Nitrite standard stock solution ($1000 \mu\text{g mL}^{-1}$) was prepared by dissolving 0.1541 g of sodium nitrite in 100 mL volumetric flask and filling it up with water. A small amount of sodium hydroxide was added

to prevent the decomposition of nitrite to nitrous acid. Few drops of chloroform were added to inhibit the bacterial growth and thus make the nitrite solution stable. Working solution was prepared by diluting standard stock solution of sodium nitrite to appropriate volume with distilled water whenever required. 1.0 g L^{-1} safranin T solution was prepared by dissolving 0.1000 g of safranin T and diluting the solution to 100 mL with the distilled water. 6.0 mol/L HCl solution was prepared by diluted 12 mol/L HCl solution. The water used was distilled water and all the reagents were of analytical grade.

A suitable amount of sample solution or nitrite working solution, 2.00 mL of 1.0 g L^{-1} safranin T solution and 2 mL of 6.0 mol L^{-1} HCl solution were transferred into a 50 mL calibrated flasks, diluted to the mark with water. After lying aside for 5 min at room temperature, the absorbance A of the reaction solution against the reagent blank was measured with 1.0 cm cell at 350 nm .

RESULTS AND DISCUSSION

Absorption spectra: In the experimental conditions, the reaction of nitrite with safranin T in HCl medium. The absorption spectra were shown in Fig. 1, it was found that the absorption peak of the reaction solution at 350 nm against the reagent blank, whereas the absorption peak of reagent blank was at 516 nm . Hence, 350 nm was selected for further studies. As we know, the absorption spectrum of the nitrosamino group on nitroso-derivative such as nitroso-diglycolamidic acid¹⁴, was at 350 nm . Therefore the reaction of nitrite with safranin T in HCl medium is a nitrosation reaction and 350 nm is the absorption peak of the nitroso-safranin T.

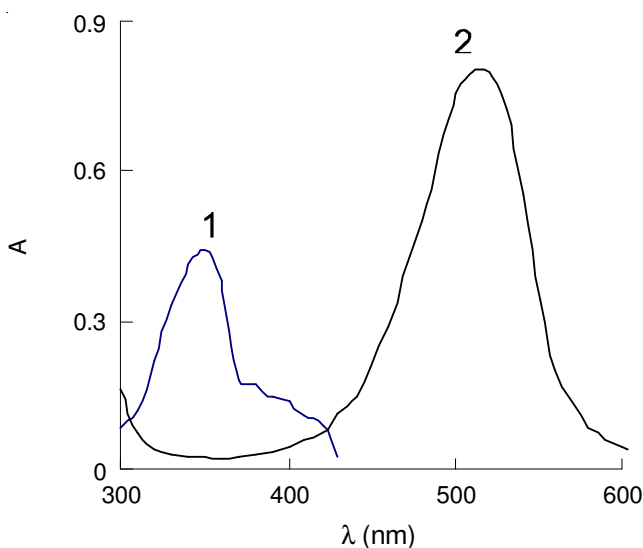


Fig. 1. Absorption spectra: (1) the reagent blank vs. water; (2) the reaction solution vs. the reagent blank. $[\text{nitrite}] = 0.8 \mu\text{g mL}^{-1}$, $[\text{safranin T}] = 0.04 \text{ g L}^{-1}$, $[\text{HCl}] = 0.24 \text{ mol L}^{-1}$

Effect of reaction time: Nitrite reacts fastly with safranin T in HCl medium that the reaction within the time range from 2 min to 150 min at room temperature that gave the maximum absorbance A .

Effect of HCl solution: The effect of HCl solution was investigated on the reaction of nitrite and safranin T and the

results were given in the Fig. 2. It was obvious from Fig. 3 that the volume from 0.50 mL to 6 mL of 6 mol L^{-1} HCl solution was the best medium with the maximum absorbance A . Therefore 2 mL of 6 mol L^{-1} HCl solution was chosen for further studies.

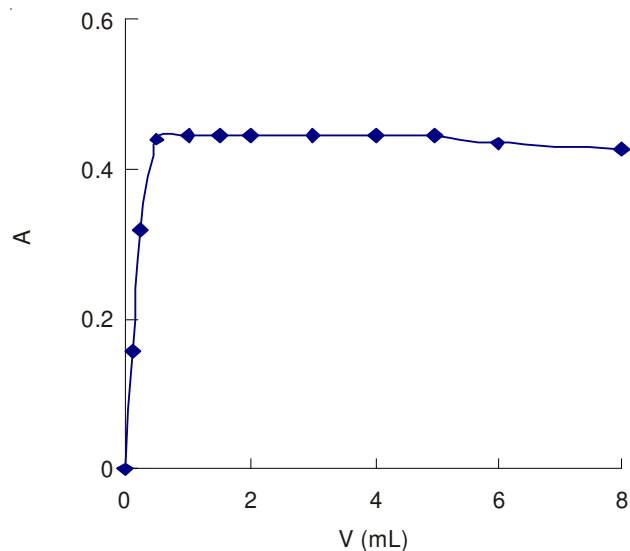


Fig. 2. Effect of HCl solution: $[\text{nitrite}] = 0.8 \mu\text{g mL}^{-1}$, $[\text{safranin T}] = 0.04 \text{ g L}^{-1}$

Effect of safranin T solution: From Fig. 3, $1\text{--}4 \text{ mL}$ safranin T solution could give the maximum absorbance A for the system, so 2 mL 1 g L^{-1} safranin T was chosen in the experiments.

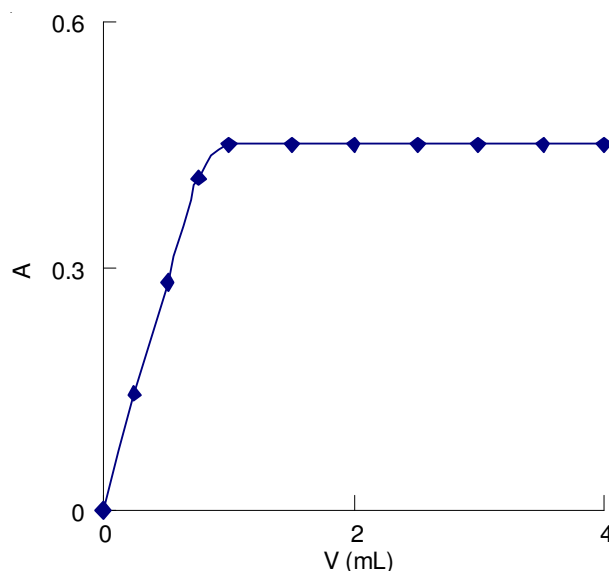


Fig. 3. Effect on volume of safranin T: $[\text{nitrite}] = 0.8 \mu\text{g mL}^{-1}$, $[\text{HCl}] = 0.24 \text{ mol L}^{-1}$

Working curve and detection limit: A series of nitrite working solutions with different concentration were prepared. Under the chosen experimental conditions, the absorbance A of these solutions was measured. The working curve was drawn and shown in Fig. 4. The results showed that Beer's law was obeyed in the concentration range of $0\text{--}1.8 \mu\text{g mL}^{-1}$ for nitrite. The linear regression equation was $A = 0.5503C - 0.0001$ with

the regression coefficient $\gamma = 1.000$ and the molar absorptivity of $2.531 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$ as well as Sandell's sensitivity of $0.0018 \mu\text{g cm}^{-2}$.

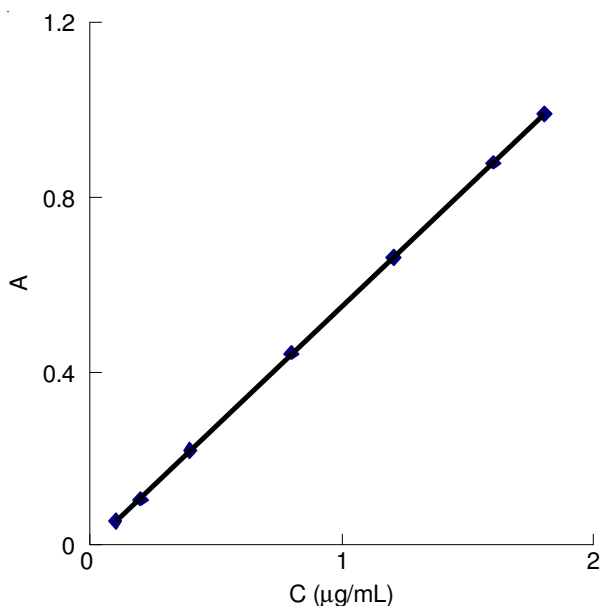


Fig. 4. Working curve: [safranin T] = 0.04 g L^{-1} , [HCl] = 0.24 mol L^{-1}

Interferents: The effect of possible interferents has been studied at a nitrite concentration of $0.5 \mu\text{g mL}^{-1}$. Maximum tolerable concentrations are given in Table-1. Interferents, arising from the suspended solids, can be removed by filtration through a filter with appropriate pore size. The results in Table-1 shows that the proposed method for determination of nitrite is highly selective.

TABLE-1
EFFECT OF FOREIGN IONS ON THE
DETERMINATION OF $0.5 \mu\text{g mL}^{-1}$ NITRITE

Foreign ion	Maximum permissible concentration ($\mu\text{g mL}^{-1}$)	Foreign ion	Maximum permissible concentration ($\mu\text{g mL}^{-1}$)
Ammonium	300	Copper(II)	600
Nitrate	240	Cobalt(II)	500
Chloride	1050	Chromium(III)	800
Bromide	900	Iron(III)	700
Fluoride	950	Lead(II)	750
Sulfate	1200	Manganese(II)	970
Phosphate	800	Magnesium(II)	600
Aluminium(III)	800	Nickel(II)	550
Barium (II)	1000	Potassium(I)	1400
Calcium(II)	1250	Sodium (I)	1350
Cadmium(II)	1300	Zinc(II)	1180

Nitrite analysis in water samples: Water samples were collected and analyzed within 12 h without adding any preservative material. The water samples were filtered through a Whatman No. 1 filter paper before analysis and then an aliquot of the filtrate was taken for the analysis by the proposed method's recommended procedure and the standard method. The satisfactory results were given in Table-2 in agreement with those of the AOAC recommended sulfanilamide-NED [N-(1-naphthyl)ethylenediamine hydrochloride] method¹³.

TABLE-2
NITRITE ANALYSIS IN WATER SAMPLES

Sample	Nitrite added ($\mu\text{g mL}^{-1}$)	Proposed method		AOAC method	
		Nitrite recovered ($\mu\text{g mL}^{-1}$)	Recovery % \pm RSD	Nitrite recovered ($\mu\text{g mL}^{-1}$)	Recovery % \pm RSD
Lake water	0.400	0.393	98.20 \pm 0.53	0.391	97.80 \pm 0.61
	0.800	0.795	99.40 \pm 0.50	0.790	98.70 \pm 0.57
Tap water	0.400	0.396	98.90 \pm 0.47	0.396	98.90 \pm 0.75
	0.800	0.783	97.90 \pm 0.51	0.786	98.30 \pm 0.58
Mineral water	0.400	0.398	99.50 \pm 0.63	0.396	99.10 \pm 0.69
	0.800	0.789	98.60 \pm 0.55	0.791	98.90 \pm 0.65
Well water	0.400	0.395	98.80 \pm 0.52	0.394	98.50 \pm 0.51
	0.800	0.802	100.2 \pm 0.61	0.795	99.40 \pm 0.57
Sea water	0.400	0.402	100.4 \pm 0.48	0.399	99.70 \pm 0.54
	0.800	0.798	99.80 \pm 0.48	0.791	98.90 \pm 0.52

Conclusion

This paper reported that the reaction of nitrite with safranin T could be utilized as a useful method for the spectrophotometric determination of nitrite. The proposed method has the advantages of being simple, cheap, selective, accurate and requires minimum equipments and chemicals. These advantages encourage the application of the proposed method in routine analysis in environmental water.

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