

## Simple Promethazine Method for Direct Spectrophotometry Determination of Nitrite in Water

JUNCUI XU\*<sup>†</sup>, JIAN-HONG BI, XIAOBO WANG and YUBIAO WANG  
*Department of Chemical Engineering, Hefei Teachers College,  
Anhui Province, Hefei 230061, P.R. China  
E-mail: xxjjcz20000214@163.com*

A rapid, simple and most economical spectrophotometric method was proposed for the determination of nitrite in water sample. The method is based on coloured complex formation between nitrite and promethazine in acidic medium. The colourization of the complex by nitrite was exploited to monitor the reaction spectrophotometrically at 249 nm. The method was optimized for effect of concentrations of promethazine, various acids, concentrations of sulphuric acid, order of reagents addition and colour stability. The colour of the complex was found to be stable for *ca.* 2 d. The linearity range of the calibration graph was over 7.00-70.00  $\mu\text{g mL}^{-1}$  of nitrite. The method has been applied for the determination of trace nitrite in lake water with satisfactory results.

**Key Words:** Promethazine, Nitrite determination, Nitrite in water, Spectrophotometry.

### INTRODUCTION

Nitrite is widely applied to dye, food and other aspects as a commonly used chemical and it also has the effect of corrosion resistance in the process of dealing with industrial water<sup>1,2</sup>. Nitrite ion is an important intermediate in the biological nitrogen cycle, which widely exists in soil and water<sup>3</sup>. Nitrite draws public high attention because of its harm to people's health. Its toxin mainly results from the fact that it can form nitrosamine carcinogenic substance<sup>4</sup>, which reacts with diamine and triamine in people's bodies, making people malformed and inducing body mutation<sup>5</sup>. Besides, when the content of nitrite is in the range of 0.4-200 mg/kg. body weight, it will cause poison and if the oral dosage reaches 33-250 mg, it will lead to death<sup>6</sup>. Otherwise, nitrite can react with the ferric iron in haemoglobin, forming ferrihemoglobin and losing the ability of transporting oxygen<sup>7-11</sup>. This disease is called the symptom of high valence hemoglobin<sup>7-11</sup>. Nitrite is a lively intermediate in nitrogen cycle, it forms in the incomplete oxidation of ammonium and the reduction of nitrite, or in the biodegradation of nitrogenous organic matter<sup>12</sup>. Its

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<sup>†</sup>School of Chemical Engineering, Hefei University of Technology, Anhui Province, Hefei 230009, P.R. China.

concentration is one of the important indexes on organic pollution in water<sup>13</sup>. Consequently, it's very important for the determination of nitrite. At present, there are many methods about the determination of nitrite<sup>14-20</sup>, among which spectrophotometry is the most widely used<sup>21</sup>. However, the approach of using promethazine to determine nitrite hasn't been reported yet.

Promethazine has the effect of central diazepam and antihistamine in pharmacology. It is a kind of anti-allergy drug that is universally used and also called phenergan, its hydrochlorate chemical formula is  $C_{17}H_{20}N_2S \cdot HCl$ , chemical name is 10-(2-dimethylamino propyl) dibenzothiazine (Fig. 1).

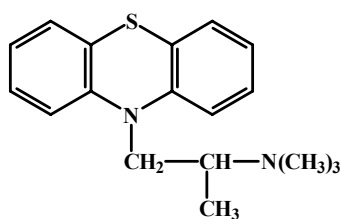


Fig. 1. Structure of promethazine

For the reaction between chlorpromazine, the isotype substance of promethazine and different kinds of ions, we have studied systematically, using spectrophotometry to determine  $V(V)^{22}$ ,  $As(V)^{23}$ ,  $P(V)^{24}$ ; we have studied as well for the reaction between promethazine and ions, applying it to the micro fast determination<sup>25</sup> of  $VO_3^-$  and  $Ce(IV)^{26}$ . In this paper, a novel method is proposed for the determination of nitrite in water. In the sulfuric acid medium, promethazine has a strong absorption peak at 249 nm near the ultraviolet band, after reacting with  $NO_2^-$ , the falling off the absorbance at 249 nm is in direct proportion to the concentration of  $NO_2^-$  in the solution.

## EXPERIMENTAL

Ultraviolet spectrophotometry of type 756 (Shanghai Precise Scientific Instruments Limited Company). Acculab digital balance readable 0.0001 g (Shanghai Ohaus Instrument Company) were used.

All reagents were analytical grade and used without further purification. water used here refers to distilled water.

**Standard sodium nitrite solution 700  $\mu g mL^{-1}$ :** Weighed amount, 0.1049 g of sodium nitrite after heated for 4 h at 130 °C was transferred into a 100 mL volumetric flask, it was dissolved and the solution was diluted up to the mark with water. This solution was transferred into a brown bottle and stored it under diffused sunlight. The working solutions as and when required were prepared by appropriate dilution.

**Promethazine solution  $5.0 \times 10^{-4} M$ :** Weighed amount, 0.0401 g of promethazine hydrochloride was transferred into a clean 200 mL beaker, dissolved it with

water, poured the solution into a 250 mL volumetric flask and the solution was diluted to the mark with water. It was saved in refrigeratory and diluted according to need when being used.

**Sulfuric acid solution (1 M):** This solution was prepared by suitable dilution of concentrated sulphuric acid,  $1.98 \text{ g cm}^{-3}$ , 98 %.

**Recommended procedure:** A series of labeled 10 mL volumetric flasks were arranged. To each flask, 2 mL of 1 mol/L sulfuric acid, aliquots of the test solution containing  $7.00\text{-}70.0 \mu\text{g mL}^{-1}$  (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0 mL) of sodium nitrite were added, followed by 0.6 mL of  $5.0 \times 10^{-4}$  mol/L promethazine solution. The solution should be shaken up when adding every kind of reagent. Then, the solution of each flask was diluted to the mark with water. Absorbance of each of the test solution and the blank (the same test solution but not containing sodium nitrite) were measured against water at 249 nm.

**Determination of nitrite in water samples:** Water samples were collected and analyzed within 12 h without adding any preservative material. The water samples were filtered through a filter paper before analysis and then an aliquot of the filtrate was taken for the analysis by the standard method<sup>15</sup> and the proposed method's recommended procedure following standard addition.

## RESULTS AND DISCUSSION

Nitrite reacts with promethazine forming a kind of pink material in 1 M sulphuric acid medium with  $\lambda_{\text{max}}$  249 nm as shown in absorption spectrum. The complex is being colourized by nitrite, which is the basis for the quantitative determination of nitrite in various samples. The reaction conditions as well as the various experimental parameters affecting the development and stability of the coloured complex were carefully investigated and optimized for the quantitative determination of nitrite in various samples analyzed. Every variable was changed many times and was tested each time by constructing the calibration graph to get wide range of nitrite concentrations and higher colour intensity of the complex. The experimental variables such as concentrations of promethazine, types of acids, concentrations of sulphuric acid and order of the reagents addition and also for colour stability of the complex were optimized for the effective determination of nitrite.

**Effect of concentration of promethazine:** The effect of concentration promethazine was studied over the wide range of its concentrations as follows: A series of labeled 10 mL volumetric flasks were arranged. To each flask, 2.5 mL of 1 M sulfuric acid, aliquots of the test solution containing  $7.00 \mu\text{g mL}^{-1}$  (0.1 mL) of sodium nitrite were added, followed by 0.1-1.2 mL (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.2) of  $5.0 \times 10^{-4}$  M promethazine solution. Then, the solution of each flask was diluted to the mark with water. Absorbance of each of the test solutions and the blank (the same test solution but not containing sodium nitrite) were measured at 249 nm against water. The result shows that when the concentration of promethazine is in the range of  $2.0 \times 10^{-5}$  M –  $4.0 \times 10^{-5}$  M (Fig. 2), namely, the absorbance

$\Delta A$  of  $5 \times 10^{-4}$  M promethazine in 2-4 mL reaction system is bigger.  $\Delta A$  will decrease if the range is bigger or smaller. The concentration of  $3.0 \times 10^{-5}$  M promethazine is selected as one of the optimal conditions.

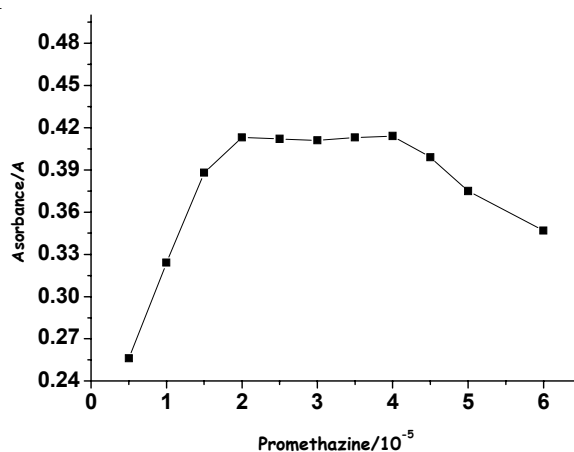


Fig. 2. Absorption spectrum of 2 mL of 1 M sulphuric acid + 0.1 mL of  $700 \mu\text{g mL}^{-1}$  of sodium nitrite + 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.2 mL of  $5 \times 10^{-4}$  M promethazine

**Effect of various acids:** The effect of different types of acids on the reaction system was studied to get the best sensitivity and linearity. The various acids namely sulphuric acid, hydrochloric acid, phosphoric acid and acetic acid were investigated by using 2.5 mL of 1 M acids at a time following the recommended procedure. The results obtained indicate that in case of acetic acid, the reaction of the system does not take place, whereas in case of hydrochloric acid, the linearity range is narrow. Although, phosphoric acid gave high absorbance, but there was no good linearity. Therefore, the sulphuric acid was chosen as an optimal acidic medium for the determination of nitrite.

**Effect of concentration of sulphuric acid:** The effect of sulphuric acid concentrations on the colour intensity and linearity of calibration graph for solutions containing fixed concentration of promethazine was studied. This study was carried out as described above but the change is in the concentration of sulphuric acid (0.5, 0.6, 0.7, 0.8, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0, 6.0 mL). The results obtained are shown in Fig. 3 which indicate that 1-3 mL of 1 M sulphuric acid produce good absorbance values. As a consequence, the 2 mL of 1 M sulphuric acid was chosen to be more convenient for the determination of nitrite.

**Effect of order of addition of the reagents:** Different orders of the reagents addition were studied using optimized amounts of the reagents following the recommended procedure. The results obtained are indicated in Table-1, which show that the orders of reagents addition do not affect absorbance values, but for maintaining the uniformity of the order of reagents addition. Series no. 1 of Table-1 was followed throughout course of the determination of nitrite.

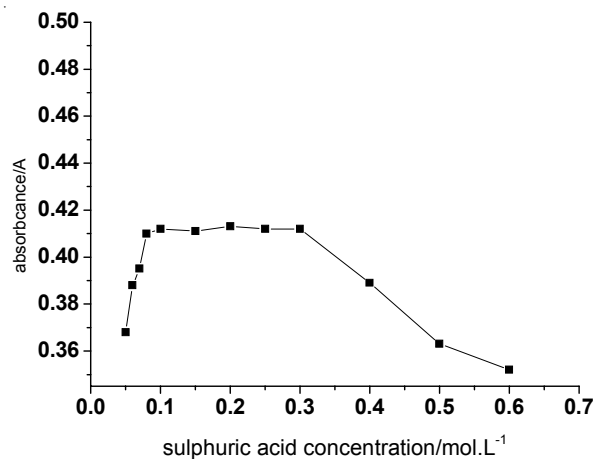


Fig. 3. Absorption spectrum of 0.6 mL of  $5 \times 10^{-4}$  M promethazine + 0.1 mL of  $1000 \mu\text{g mL}^{-1}$  of sodium nitrite + 0.5, 0.6, 0.7, 0.8, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0, 6.0 mL of 1 M sulphuric acid

TABLE-1  
EFFECT OF ORDER OF REAGENTS ADDITION

Number	Order of addition	Absorbance
1	A + B + C	0.414
2	A + C + B	0.413
3	B + A + C	0.413
4	B + C + A	0.412
5	C + A + B	0.413
6	C + B + A	0.415

A = 2 mL of 1 M sulphuric acid, B = 0.1 mL of  $700 \mu\text{g mL}^{-1}$  of sodium nitrite, C = 0.6 mL of  $5 \times 10^{-4}$  M promethazine.

**Colour stability of the complex with time:** The stability of the colour complex was studied according to the recommended procedure using  $20 \mu\text{g mL}^{-1}$  nitrite. The absorbance of the solution was measured at various intervals of time. The results obtained are shown in Table-2, indicate that the reaction of the system is found to be instantaneous at room temperature, since the absorbance values do not change with time. Therefore, colour of the complex is considered to be stable for *ca.* 2 d.

TABLE-2  
STUDY OF COLOUR STABILITY

Time of measurement (h)	Absorbance, $\lambda = 249 \text{ nm}$	Time of measurement (h)	Absorbance, $\lambda = 249 \text{ nm}$
Immediately after make up		3.0	0.547
0.5	0.546	6.0	0.545
1.0	0.545	12.0	0.546
1.5	0.546	24.0	0.547
2.0	0.547	48.0	0.546
2.5	0.546	–	–

**Calibration graph:** Under the optimum conditions, a good linear relationship (Fig. 4) was found between the absorbance of the complex and concentration of nitrite, 7.00-70.00  $\mu\text{g mL}^{-1}$ . The linear regression equation of the calibration graph is found to be (calculated)<sup>27</sup>  $\Delta A = 0.0027C + 0.0297$ , where  $\Delta A$  is absorbance difference value and C is the concentration of nitrite in  $\mu\text{g mL}^{-1}$ , with a correlation coefficient, 0.996.

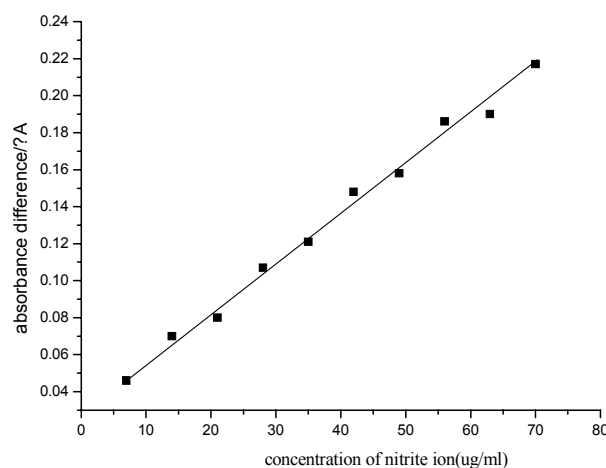


Fig. 4. Calibration graph for the determination of nitrite under optimized conditions

**Analytical application:** In order to investigate the applicability of the proposed method for the analysis of real samples, lake water samples, were analyzed for nitrite. The results obtained for nitrite both by the proposed method and standard method<sup>15</sup> are given in Table-3. The results of the proposed method agree well with those of the standard method.

TABLE-3  
DETERMINATION OF NITRITE IN WATER SAMPLE

Methods	Measured volume ( $\text{NO}_2^-$ )	Standard $\text{NO}_2^-$ added ( $\mu\text{g/m}$ )	$\Sigma\text{NO}_2^-$ found	Recovery (%)	RSD (%)
Present method	0.76	1.00	1.84	108.0	1.7
National standard method	0.78	1.00	1.82	104.0	1.9

**Interference studies:** In order to evaluate the selectivity of the proposed method for the determination of nitrite, the effect of common foreign substances accompanying nitrite was tested in the determination of 20  $\mu\text{g mL}^{-1}$  of nitrite under the optimum conditions given in the recommended procedure. The results obtained are summarized in Table-4. It was found that the presence of common interference of nitrite as indicated in Table-4, do not interfere in the determination of nitrite and the tolerance limit, the maximum concentration of each ion that does not cause more than 4 %

relative error in the determination of nitrite at  $20 \mu\text{g mL}^{-1}$  of its concentration is also shown against the respective ion in Table-4, which account for good selectivity of the method for nitrite.

TABLE-4  
INTERFERENCE OF FOREIGN IONS IN THE  
DETERMINATION OF  $20 \mu\text{g mL}^{-1}$  OF NITRITE

Ion added	Tolerance limit ( $\mu\text{g mL}^{-1}$ )	Ion added	Tolerance limit ( $\mu\text{g mL}^{-1}$ )
$\text{K}^+$	1000	$\text{OAc}^-$	1000
$\text{NH}_4^+$	1000	$\text{Ca}^{2+}$	960
$\text{Cl}^-$	980	$\text{Ba}^{2+}$	980
$\text{F}^-$	1000	$\text{Zn}^{2+}$	900
$\text{I}^-$	980	$\text{Ni}^{2+}$	800
$\text{PO}_4^{3-}$	800	$\text{Cu}^{2+}$	840
$\text{SO}_4^{2-}$	800	$\text{Co}^{2+}$	780
$\text{S}_2\text{O}_3^{2-}$	760	$\text{Cd}^{2+}$	700
$\text{NO}_3^-$	1000	$\text{Fe}^{3+}$	100

### Conclusion

The proposed method for nitrite determination is found to be having excellent selectivity since most of the metal ions (Table-4) could be tolerated in considerable concentration. The absorbance of the complex formed between nitrite and promethazine in 1 M sulphuric acid medium can remain almost unchanged for 2 d. The new proposed method neither involves solvent extraction<sup>28</sup> nor critical control of pH<sup>29</sup> nor temperature<sup>30</sup> nor employs sophisticated instruments<sup>31,32</sup>. Moreover, the proposed new method for nitrite is operationally simple, less time consuming and employs very common and economically cheaper chemicals compared to other methods. The proposed method is as effective as the standard method<sup>15</sup> as indicated by the results (Table-3) but with visible advantages in terms of cheap and without strict control of pH, therefore, it could be either a complementary or a parallel method for the nitrite determination.

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