

Spectrophotometric Determination of Trace Amount of Nitrite in Water with p-Nitroaniline and 1-Naphthol

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ABSTRACT

A simple, selective and sensitive method for the spectrophotometric determination of nitrite in different natural and waste water samples is applied. Nitrite reacts with p-nitroaniline in the presence of hydrochloric acid to form diazonium cation which is subsequently coupled with 1-naphthol to form a stable azo dye. The λ_{\max} for the azo dye is found to be 610 nm. At analytical wavelength 610 nm the Lambert-Beer's law obeys over the concentration range 0.035-0.123 $\mu\text{g/ml}$ of nitrite. The correlation coefficient, molar absorptivity and Sandell's sensitivity of the method were found to be 0.99, $5.24 \times 10^4 \text{ lit. mol}^{-1} \text{ cm}^{-1}$ and $8.77 \times 10^{-4} \mu\text{g cm}^{-2}$ respectively. The percentage recovery was found to range from 93-109% and the interference of foreign ions was also studied.

Keywords: p-Nitroaniline, 1-naphthol, spectrophotometric, molar absorptivity, nitrite

INTRODUCTION

Nitrite has been considered as a potentially hazardous compound for human health. Trace amount of nitrite in drinking water may cause methemoglobinemia, in infants and with long term exposure is possible cancer risk. In potable water occurrence of nitrite is objectionable as it causes methemoglobinemia and anorexia (Calabrese, 1963). The maximum admissible concentration (MAC) is 0.1 mg/l and the U.S Public Health Association specific 0.06 ppm as the maximum permissible limit of nitrite in potable water (SMEWSIW, 1976; Chaurasis & Verma, 1994).

As nitrite shows potential toxicity and harmful effects by forming carcinogenic nitrosoamines, the determination of nitrite is important in environmental protection and public health. Therefore, several methods for nitrite determination have been developed in recent years, such as kinetic method, chromatography, potentiometry, amperometry, polarography, capillary electrophoresis, and spectrophotometry and flow injection analysis (FIA) systems (Butt *et al.* 2001; Siu & Henshall, 1998). Some of these methods have the disadvantage of the employment of large volume of toxic reagents, low sample frequency, application of complicated flow injection systems, poor reproducibility, expensive and time consuming separation procedures and, for certain method, the requirement of high temperature. Researchers developed the methods based on reaction other than Griess reaction has also been developed for nitrite determination. But, in spite of their sensitivity, they suffer from drawback such as tedious synthesis of the reagent and relatively long reaction time.

For the determination of nitrite following diazotization

and coupling reaction is proposed to new reagent system. Nitrite reacts with PNA to form a diazonium cation which is subsequently coupled with 1-naphthol to form a violet dye. Thus the objective of the present study is to apply the developed method for the determination of nitrite in the different natural and waste water samples.

MATERIALS AND METHODS

In this study, a SP meterteke 870 spectrophotometer of Thailand with 1.0 cm matched glass cells were used for absorbance measurements.

All chemicals used were of analytical grade and double distilled water was used for the dilution of reagents and samples. A stock nitrite solution was prepared by dissolving 0.375g of dried sodium nitrite in distilled water and dilute to 250 ml, a pellet of sodium hydroxide and about 1 ml of chloroform were added to prevent the liberation of nitrous acid and to inhibit the bacterial growth respectively. The working solutions were prepared by appropriate dilution of each stock solution with double distilled water. p-nitroaniline (PNA) solution of $1 \times 10^{-3} \text{ M}$ was prepared in 20% aqueous ethanol up to 100 ml. 0.1% of 1-naphthol solution was prepared by dissolving 0.1 gm of the reagents in 100 ml of 1.2M sodium hydroxide up to mark.

RESULTS AND DISCUSSION

Spectral Studies for the Determination of λ_{\max}

Fig. 1 shows the absorption spectrum of the azo dye formed in alkaline medium with 10 $\mu\text{g/ml}$ of nitrite is treated according to the recommended procedure

using 1-naphthol as a coupling reagent. The maximum absorption i.e. λ_{\max} occurs at 610 nm.

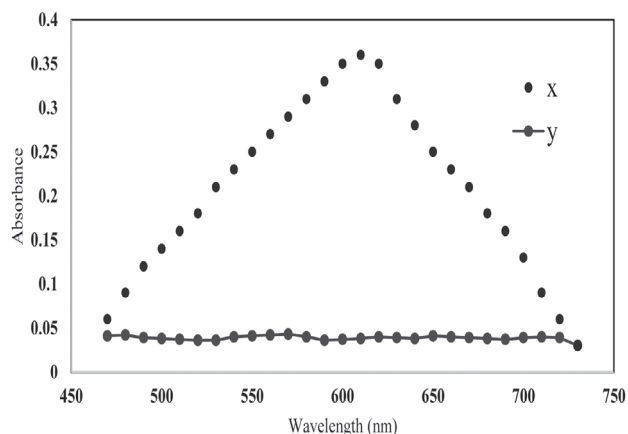


Fig. 1. X- Absorption Spectra of Azo dye Measured against Reagents blank, ($20\mu\text{g}$ of NO_2^- in 25 ml) and Y- Reagents blank against water.

Effect of *p*-Nitroaniline Concentration

To determine the optimum concentration of *p*-nitroaniline on the colour intensity, the concentration of PNA was varied from 0.00028% to 0.00224% in final solution. For this experiment, the 0.014% PNA solution was used. The colour development was then completed according to recommended procedure and plot of absorbance versus concentration of PNA solution for 10 $\mu\text{g}/\text{ml}$ of nitrite is shown in Fig. 2. The curve shows that concentration lower than 0.00112% did not yield satisfactory results and absorption depends upon concentration of PNA. Above 0.00112% of PNA absorption remains constant and independent on the concentration. The optimum concentration was found to be 0.00112% in the final solution i.e. 2.0 ml 0.014 % (w/v) is required in the final volume of 25 ml of the reaction mixture.

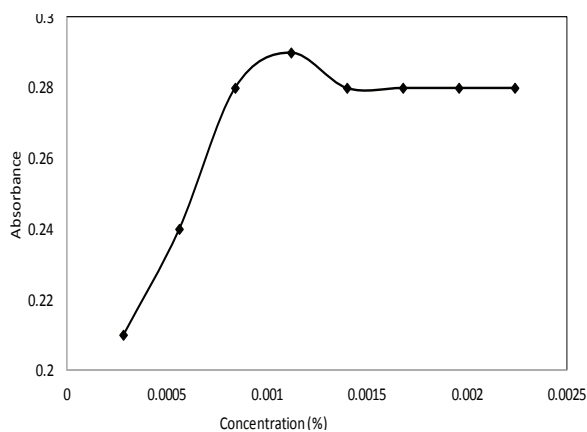


Fig. 2. Effect of PNA Concentration on Diasotization.
Effect of Time Interval between the Addition of

Aromatic Amine and Coupling Agent

The effect of the time interval between the addition of *p*-nitroaniline and 1-naphthol was studied by adding these reagents at different time interval from 5 to 50 minutes. The plot of absorbance versus time for 25 ml nitrite is shown in Fig. 3. It has been shown that absorbance of the azo dye was found to attain a maximum and the absorbance of the azo dye was found to attain a maximum and constant limiting value after 10 minutes and up to 15 minutes. This indicates that the diazotization process takes 10 minutes to be optimum and remains optimum up to 15 minutes. Hence, coupling agents must be added within 10-15 minutes after addition of diazotisable reagent. The coupling was found to be very fast since after addition of 1-naphthol, absorbance of the solutions remains practically constant up to 16 hour. This clearly indicates that the coupling occurs instantaneously and the azo dye formed is stable for at least 16 hour.

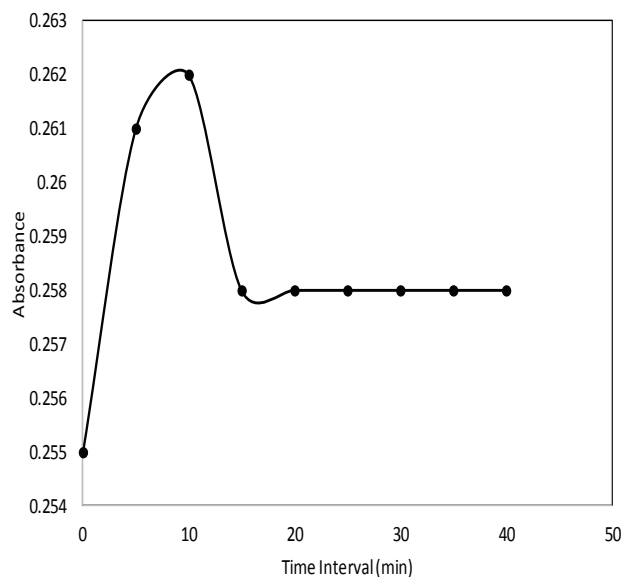


Fig. 3. Effect of Time Interval between the Addition of Aromatic amine and Coupling agent.

Adherence to Beer-Lambert's Law and Sensitivity

The adherence to the Beer-Lambert's law was tested by reacting aliquots of standard solution containing 1 μg , 2 μg , 3 μg , 4 μg , 5 μg , 10 μg , 15 μg , 20 μg and 25 μg of nitrite in final volume of 25 ml by the recommended procedure. The absorbance of the resulting azo dye is measured at 610 nm. Fig. 4 shows the plot of absorbance against concentration of nitrite. The plot is found to be linear from the origin. From the plot it is clear that the proposed method obeys Beer-Lambert law from 0.035 to 0.123 ppm of nitrite.

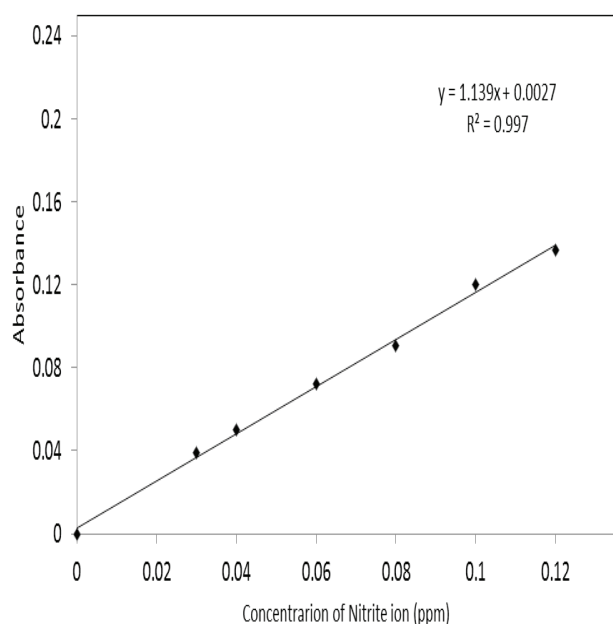


Fig. 4. The Plot of Absorbance vs. Concentration of Nitrite ion in ppm.

Recommended Procedure for the Nitrite Determination in Water

The determination of nitrite ion using p-nitroaniline acid as diazotisable amine and 1-naphthol as a coupling reagent is quite simple, accurate and highly reproducible. The highly sensitivity, simplicity rapidly, elimination of extractive steps and not requiring close adjustment of pH and temperature make the proposed method superior to the most of the Griess modifications. For the better results the following procedure is recommended for the determination of the nitrite in water samples are as follows: i) Take 15-20 ml of water sample in 25 ml volumetric flask. ii) Add 2 ml of p-nitroaniline prepared in 0.05M HCl and shake well then after keep the solution aside for the 15 minutes. iii) Add 1.5 ml EDTA with 1.5 ml 0.1% of 1-naphthol make the solution up to the mark by adding distill water and measure the absorbance at 610 nm. iv) Calculate the amount of nitrite in ppm from the calibration curve.

Accuracy and Precision of the Method

Under the optimum conditions, the proposed method was checked by performing three replicated determination of nitrite by taking and 1, 2, 3, 4, 5, 10, 15, 20, 25 and 30 μg of nitrite in the final volume of 25 ml by the recommended procedure. The percentage recovery was found to be range from 93-109% indicating the suitability of the method for determination of nitrite in water. The results of this analysis are shown in Table 1.

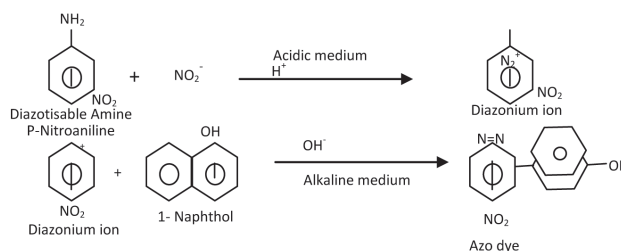
Table1. Accuracy and Precision of the Method

Nitrite Taken(μg)	Nitrite found (μg)#	Mean Recovery (%)	Relative standard deviation (%)
1	00.93 \pm 0.03	093.0	3.2
2	01.98 \pm 0.04	099.0	2.0
3	03.14 \pm 0.10	104.0	3.2
4	04.39 \pm 0.06	109.0	1.4
5	05.48 \pm 0.06	109.0	1.1
10	09.77 \pm 0.73	098.0	7.4
15	14.92 \pm 0.36	099.5	2.4
20	20.06 \pm 0.63	100.0	3.1
25	25.20 \pm 0.36	100.8	1.4
30	30.60 \pm 0.36	102.0	1.2

#Average of three determinations \pm SD

Proposed Reaction Mechanism

The reaction is believed to occur in two steps. In the first step p-nitroaniline reacts with nitrite the presence of acid to form the diazonium cation. In the second step, the diazonium cation subsequently couples with alkaline solution of 1-naphthol to produce the violet colour azo dye. The azo dye has intense color which has absorption maximum in visible region.



Effect of Foreign Ions

The effects of foreign ions that often accompany nitrite are examined by carrying out the determination of 20 μg /ml of Nitrite in 25 ml volumetric flask; 2 ml of 20 μg /ml Sodium nitrite solution was taken in 25 ml volumetric flask. Then 2 ml of $1 \times 10^{-3}\text{M}$ PNA solution was added followed by the addition of 3 ml of 0.05M HCl solution. Then the solution was thoroughly shaken and kept aside for 10 minutes to allow the diazotization reaction. Then 1 ml of 10% (w/v) EDTA was added followed by addition of 1.5 ml of 0.1% (w/v) 1-naphthol and shaken for one minute. After that the different foreign ion weight which are reported below are mixed from different compound. The volume was made up to the mark by the addition of water. The effects of foreign ions that often accompany nitrite are examined by carrying out the determination of 20.0 μg of nitrite in 25 ml volumetric flask with the foreign ions following the recommended procedure. The

foreign ions tested are Na⁺, K⁺, NH₄⁺, Ca⁺⁺, Mg⁺⁺, Cu⁺⁺, Cl⁻, NO₃⁻, HCO₃⁻, CO₃⁻, SO₄⁻, PO₄⁻, Fe⁺⁺. The results of this analysis are shown in Table 2. It is apparent that interference due to most of the foreign ions is negligibly small and the method seems to be selective for nitrite. But, however Cu⁺⁺ and Fe⁺⁺ ions interfere seriously while Mg⁺⁺ and PO₄⁻ interfere by causing turbidity to the solution.

Table 2. Effect of Foreign Ions on Absorbance of 10µg/ml of Nitrite Treated by Recommended Procedure

Foreign ions	Amount added(mg)	Ratio of diverse ion/nitrite, wt/wt	Absorbance	Nitrite found (µg)
Sodium	92.00	4600	0.956	21.00
Potassium	39.00	1950	0.952	20.90
Ammonium	18.00	900	0.957	21.01
Calcium	48.00	2000	0.962	21.12
Magnesium	02.40	120	0.911	20.00
Copper	02.54	127	0.820	18.00

Chloride	71.00	3550	0.956	21.00
Nitrate	62.00	3100	1.002	22.00
Bicarbonate	61.00	3050	1.006	22.10
Carbonate	60.00	3000	1.007	22.12
Sulphate	19.20	0960	0.956	21.00
Phosphate	96.00	4800	0.902	19.80
Iron	55.80	2790	0.775	17.00

Analysis of Water Samples

To determine the concentration of nitrite in water samples at first water sample was collected from different source like tap water, ponds, tube wells, wells, rivers and lakes etc. When we collected water from different sources we should be used clean and dry water pots. If we were collected water from river we reach that water which have a motion, and if we cannot test water in 24 hours we can use preservative like as HgCl₂ the proposed method have been applied for the determination of nitrite in water samples by taking the 15 ml of water samples in final volume of 25 ml according to recommended procedure. The concentration of nitrite (ppm) obtained from water samples from various locations are shown in Fig. 5.

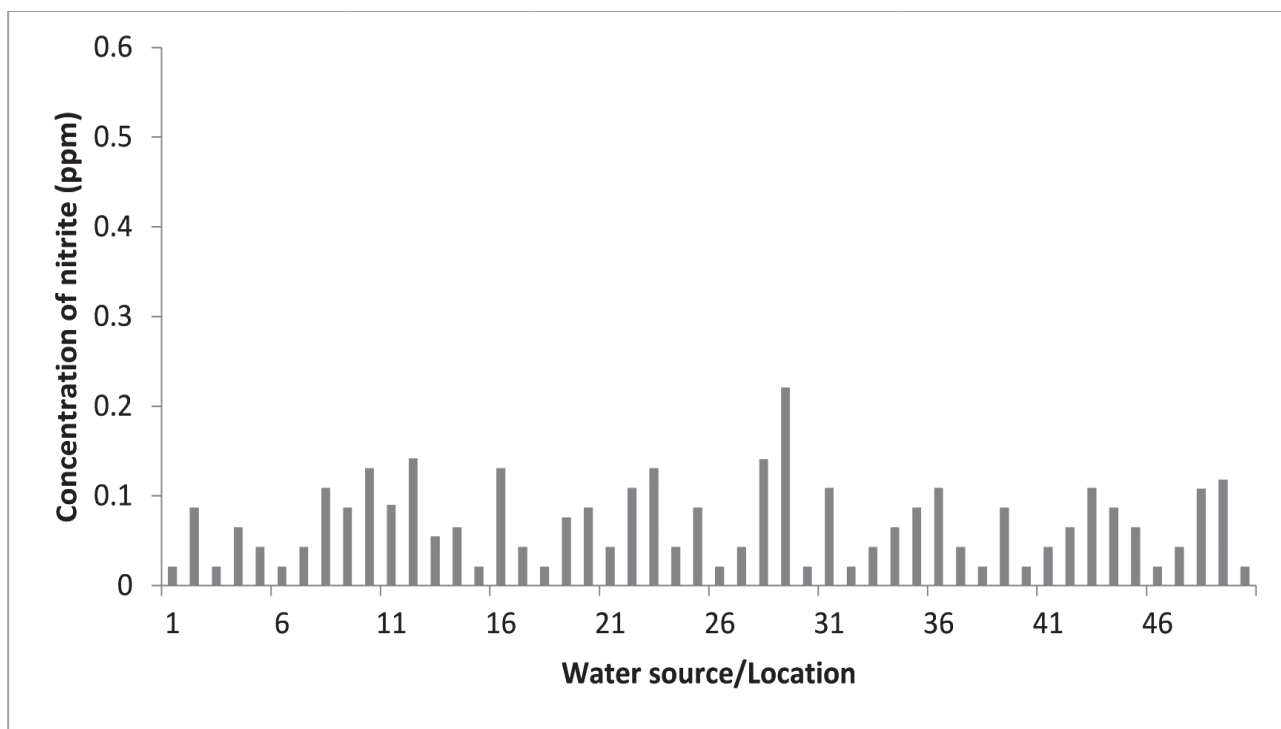


Fig. 5. The Plot of Concentration of Nitrite vs. Water from different sources and locations.

Comparison of the Proposed Method with Some Reported Method

The present method is sensitive, selective and economical in which PNA reagent in the presence of acid reacts with nitrite to form diazonium ion which on coupling

with suitable coupling agent 1- naphthol gives violet colored azo dye. The comparison and the application of the method reflect that the developed is appropriate for the determination of nitrite in water. The result of the comparison is shown in Table 3.

Table 3. Comparison of the Proposed Method with some Reported Methods

Method	Reagent	λ_{\max} (nm)	Molar Absorptivity (litmol ⁻¹ cm ⁻¹)	Determination range (ppm)	Reference
1.	p- Nitroaniline + Frusemide	680	3.314×10^3	0.4-2.0	6
2.	p-Nitroaniline + Ethoxyethylenemaleic ester	439	1.21×10^4	0.5-16.0	7
3.	Barbituric acid	310	1.53×10^4	0.00-3.22	8
4.	4- Aminophenylacetic acid + 1- Naphthol	490	2.70×10^4	0.04-1.6	9
5.	p-Nitroaniline + Diphenylamine	500	1.425×10^4	0.05-0.80	10
6.	4- Aminosalicylic acid + 1-Naphthol	520	1.47×10^4	0.1-3.0	11
7.	Sulfanilic acid + 1-Naphthylamine	520	3.30×10^4	1.4-35	12
8.	p-Aminobenzoic acid + 8-Hydroxyquinoline	499	3.20×10^4	0.1-1.5	13
9.	p-Nitroaniline+ 1- Naphthol [proposed method]	610	5.240×10^4	0.035-0.123	

CONCLUSIONS

The proposed method is simple, sensitive and highly reproducible method for the spectrophotometric determination of trace amount of nitrite present in water sample. The proposed method involves diazotization of the p-nitroaniline followed by coupling with 1-naphthol to give a stable violet coloured azo dye. The optimized amounts of reagents were found to be 2.0 ml 0.014% p-nitroaniline, 2.5 ml HCl, 1.5 ml 10% EDTA and 1.5 ml 0.1% 1-naphthol. This method follows Lambert-Beer's law over range 0.035-0.123 $\mu\text{g/ml}$ of nitrite. The percentage recovery was found to range from 93-109%. The correlation coefficient, molar absorptivity and Sandell's sensitivity of the method were found to be 0.997, $5.24 \times 10^4 \text{ lit. mol}^{-1} \text{ cm}^{-1}$ and $8.77 \times 10^{-4} \mu\text{g cm}^{-2}$ respectively. The validity of the method is also assessed by investigating the effect of common foreign ions. This method should be tested to the soil samples for further study.

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