



Spectrophotometric Determination of Trace Amount of Nitrite in Water with 4-Aminophenylacetic Acid and Resorcinol

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Abstract

A simple, sensitive and rapid spectrophotometric method for the determination of nitrite in different natural and wastewater samples is presented. This method is based on diazo coupling reaction where nitrite reacts with 4-aminophenylacetic acid under acidic condition to form diazonium ion which is subsequently coupled with resorcinol to form a stable orange coloured azo-dye. The λ_{max} for the azo dye is found to be 455 nm. The optimum reaction conditions and various analytical parameters have been studied. At analytical wavelength of 455 nm the Lambert-Beer's law obeys over the concentration range 0.1-2.4 $\mu\text{g/ml}$ of nitrite. The correlation coefficient, molar absorptivity and Sandell's sensitivity of the method were found to be 0.9986, $2.27 \times 10^4 \text{ lit.mol}^{-1}\text{cm}^{-1}$ and $2.03 \times 10^{-3} \mu\text{g cm}^{-2}$ respectively. The percentage recovery was found to range from 91.16% -101.4% indicating the suitability of the method for the determination of nitrite in water. The proposed study was compared with others Griess modifications and found to be suitable one. The interference of foreign ions was also examined.

Keywords: 4-aminophenylacetic acid, resorcinol, foreign ions, nitrite, correlation coefficient.

Introduction

Nitrite is one of the important environmental pollutants, it occurs as an intermediate during the biodegradation of nitrogenous wastes by microbes. Nitrite levels, when correlated with other forms of nitrogen in waters, provide indexes of organic pollution in water. Nitrite is a well-known contaminant of ground and stream water. Generally, humans are exposed to nitrite through the ingestion of vegetables, water and cured meats¹⁻³. The occurrence of nitrite in the drinking water and vegetables has been a matter of growing concern that has attracted the attention of analytical chemists. Being a powerful oxidizing agent, it converts iron in the hemoglobin from ferrous to ferric form and due to this hemoglobin loses its property to carry oxygen⁴. It is an important environmental factor and affects our health directly or indirectly, and thus its detection and quantification are considered to be essential. Therefore, many methods for nitrite determination have been developed in recent years, such as kinetic methods, chromatography, potentiometry, capillary electrophoresis, flow injection analysis (FIA) systems and spectrophotometric method^{5, 6}. Recently, some of the related studies have been also reported⁷⁻¹⁰.

There has been no report of using 4-aminophenylacetic acid as diazotisable reagent and resorcinol as coupling reagent for nitrite determination through spectrophotometric technique, though these are extensively used by pairing up with other reagents. These reagents are common and easily available in market. A new reagent system for the nitrite determination is purposed where 4-aminophenylacetic acid is used as diazotisable reagent and resorcinol as coupling reagent. The

nitrite ion reacts with 4-aminophenylacetic acid to form diazonium ion in acidic medium and the diazonium ion couples with resorcinol to form orange colored azo-dye.

Experimental Method

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 a small amount (about 1 ml) of chloroform were added to prevent the liberation of nitrous acid and to inhibit the bacterial growth respectively. The stock solution was titrated with standard solution of potassium permanganate to standardize the stock solution by following the procedure described elsewhere¹¹. Then the nitrite solution of required concentration was prepared by appropriate dilution of stock solution in distilled water daily.

For the calibration curve, 0 ml, 0.5 ml, 1.0 ml, 1.5 ml, 2.0 ml, 2.5 ml, 3.0 ml, 3.5 ml of 10 $\mu\text{g/ml}$ nitrite solutions were transferred to a series of 25 ml volumetric flask and 2.0 ml 4-aminophenylacetic acid solution was added to each flask and shaken well. Each set were left for a 15 minutes to allow the complete diazotization reaction. Then 2 ml of 0.1% resorcinol solution as a coupling reagent was added and each flask had shaken for 1 min. Then the volume was made up to the mark by the addition of water. At last, the absorbance of the resulting colored dye of each flask was measured at 455 nm against the reagent blank by using WPA S- 104 spectrophotometer is shown in figure 1. For all other further works about absorbance was carried out at 455 nm.

Results and Discussion

Effect of Time Interval between the Addition of Aromatic Amine and Coupling Agent: The effect of time interval between the addition of 4-aminophenylacetic acid and resorcinol was studied by adding these reagents at different time interval from 0 to 60 minutes in the interval of every 5 minutes. The plot of absorbance versus time for 25 ml (0.8 µg/ml) nitrite is shown in figure 2. It has been shown that, absorbance of the

azo dye was found to attain a maximum and constant limiting value after 15 minutes. This indicates that, the diazotization process takes 15 minutes to reach optimum condition. Hence coupling agent must be added after 15 minutes of addition of diazotisable reagent. After the addition of resorcinol, coupling was started and it takes another 1 minute for complete colour development. The dye becomes stable for at least 24 hr.

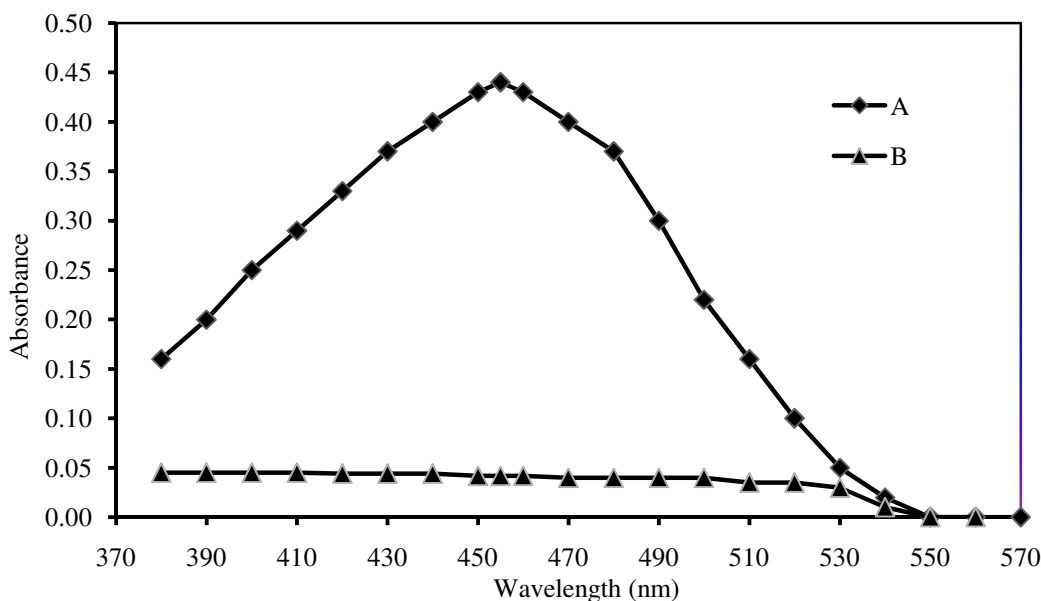


Figure-1

A- Absorption Spectra of Azo dye Measured against Reagent blank, (20 µg of NO₂⁻ in 25 ml) and B- Reagent blank against Distilled water

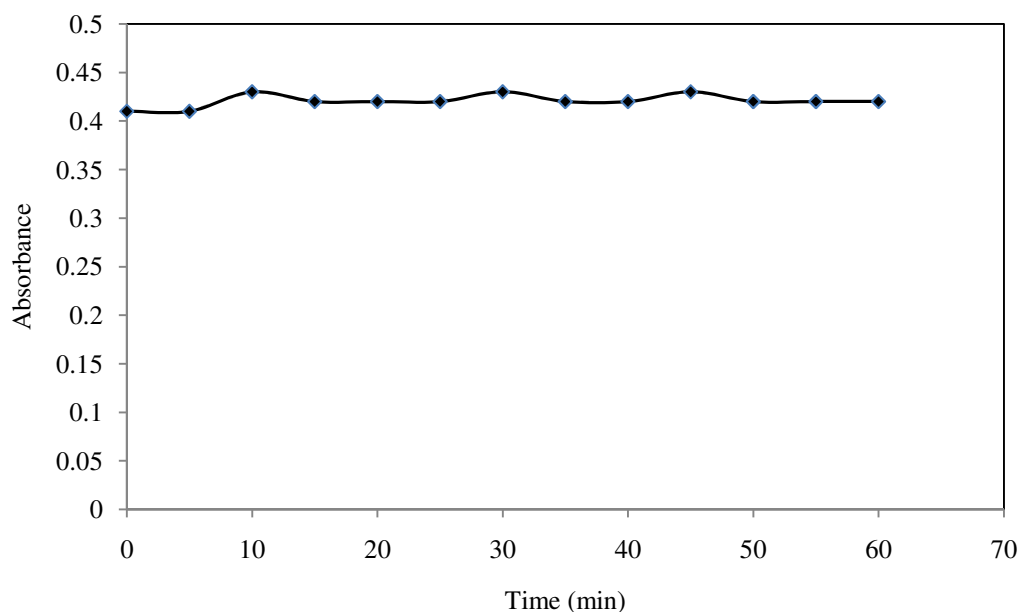


Figure-2

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

Adherence to Beer-Lambert's Law and Sensitivity: The Adherence to Beer-Lambert's law was tested by reacting aliquots of standard solution containing 0.1 to 2.4 ppm of nitrite in final volume of 25 ml and reacted by recommended procedure. The absorbance of the resulting azo dye was measured at 455 nm and plotted against their respective concentration in ppm. The plot was found to be linear is shown in figure 3. From the plot it is clear that the proposed method obeys Beer-Lambert's law from 0.1 to 2.4 ppm of nitrite ion in a final volume of 25 ml. The molar absorptivity and Sandell's sensitivity in the region of least photometric error were found to be $2.27 \times 10^4 \text{ lit. mol}^{-1} \text{ cm}^{-1}$ and $2.03 \times 10^{-3} \mu\text{g cm}^{-2}$ respectively.

Recommended Procedure for the Nitrite Determination in Water: Quantitative determination of nitrite ion by using 4-aminophenylacetic acid as diazotizable amine and resorcinol as coupling reagent is quite simple, sensitive, accurate, and highly reproducible. For the better results, the following procedure is

recommended for the determination of trace amount of nitrite in water sample as follows: i. Take 15 ml of water sample in a 25 ml volumetric flask. ii. Add 2 ml of 0.25% (w/v) 4-aminophenylacetic acid solution prepared in 0.6M HCl and shake well and keep aside for 15 minutes. iii. Add 2 ml of 0.1% resorcinol shake well, keep aside for 1 minute. Then, add distilled water up to the mark, shake well and measure the absorbance at 455 nm. iv. Calculate the amount of nitrite in ppm from the calibration curve.

Accuracy and Precision of the Method: The accuracy and precision of the proposed method was checked by performing five replicate determination of nitrite by taking 1, 2, 5, 10 and 15 μg of nitrite in final volume of 25 ml by the recommended procedure. The results of the analysis are shown in table 1. The percentage recovery was found to range from 91.16% -101.4% indicating the suitability of method for the determination of nitrite in water.

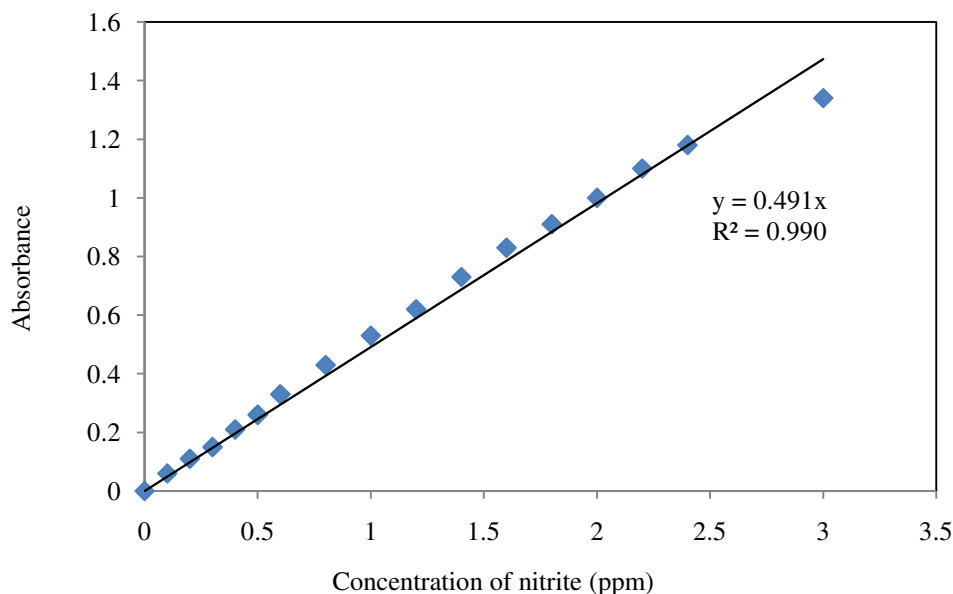


Figure-3
 The Plot of Absorbance vs. Concentration of Nitrite ion in ppm

Table-1
 Accuracy and Precision of the Method

S. No.	Nitrite taken (μg)	Proposed method			T-test**	F-test***
		Nitrite found* (μg)	Mean recovery %	Relative standard deviation (%)		
1	1	0.9116 \pm 0.389	91.16	42.69	-0.508	9.382
2	2	2.028 \pm 0.320	101.40	15.77	0.196	2.195
3	5	4.860 \pm 0.282	97.20	5.80	-1.11	1.709
4	10	10.026 \pm 0.379	100.20	3.78	0.153	2.141
5	15	15.190 \pm 0.660	101.27	4.34	0.644	6.752

*Average of five determination \pm SD, **Tabulated t-value for 5 degree of freedom at 95% probability level is 2.31, ***Tabulated F-value for (4, 4) degree of freedom at 95% probability level is 6.39.

Effect of Foreign ions: The effect of foreign ions that often accompany nitrite, are examined by carrying out the determination of 20µg nitrite ion in 25 ml of volumetric flask along with the foreign ions following the recommended procedure. Variation in absorbance value of $\pm 3\%$ from that obtained for 20 µg of nitrite in final volume of 25 ml in the absence of any interfering ions was taken into consideration of interference. Various foreign ion tested are Na^+ , NH_4^+ , K^+ , Ca^{++} , Mg^{++} , Cu^{++} , Cl^- , NO_3^- , HCO_3^- , CO_3^{--} , SO_4^{--} , PO_4^{--} and Fe^{++} . The result of this analysis is 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^{++} , SO_4^{--} and Fe^{++} ions interfere seriously.

Analysis of Water Samples: The proposed method have been applied for the determination of nitrite in water samples from different sources and locations of Kathmandu valley by taking the 15 ml of water samples in final volume of 25 ml according to recommended procedure. The result of this analysis is shown in figure 4. From the figure it is clear that, the water sample collected from the upper surface of the earth such as ditch, river, well has higher concentration of nitrite while those collected from depth of earth such as tube well, boring has lower concentration of nitrite except some cases.

Comparison of the Proposed Method with Some Reported Method: The present method is sensitive, selective and economical in which 4-aminophenylacetic acid reagent in presence of acid reacts with nitrite to form diazonium ion which on coupling with suitable coupling agent (resorcinol) gives

orange colored azo dye. The comparison and the application of the method reflect that the developed method is appropriate for the determination of nitrite in water. The result of the comparison is shown in table 3.

Table-2
Effect of Foreign ions on Absorbance of 20.0 µg of Nitrite Treated by Recommended Procedure

Foreign Ion	Amount added (mg)	Ratio of diverse ion/nitrite, wt/wt	Nitrite found (µg)
Ammonium	18	900	35.95
Bicarbonate	61	3050	22.78
Calcium	40	2000	31.90
Carbonate	60	3000	20.25
Chloride	71	3550	21.77
Copper	2.54	127	41.52
Iron[II]	55.8	2790	96.21
Magnesium	2.4	120	25.31
Nitrate	62	3100	21.77
Potassium	39	1950	21.26
Phosphate	96	4800	28.35
Sodium	92	4600	20.25
Sulphate	19.2	960	60.25

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

Method	Reagent/ Reference	λ_{max} (nm)	Molar Absorptivity ($\text{litmol}^{-1} \text{cm}^{-1}$)	Determination range (ppm)
1.	p-Aminobenzoic acid + 8-Hydroxyquinoline [12]	499	3.20×10^4	0.1-1.5
2.	4-Aminobenzotrifluoride [13]	510	3.91×10^4	0.03-0.8
3.	Barbituric acid [14]	310	1.53×10^4	0.00-3.22
4.	4- Aminosalicylic acid + 1-Naphthol[15]	520	1.47×10^4	0.1-3.0
5.	p- Nitroaniline + Frusemide [16]	680	3.314×10^3	0.4-2.0
6.	4- Aminophenylacetic acid + 1- Naphthol [17]	490	2.70×10^4	0.04-1.6
7.	p-Nitroaniline + Ethoxyethylenemaleic ester[18]	439	1.21×10^4	0.5-16.0
8.	Sulfanilic acid + 1-Naphthylamine [19]	520	3.30×10^4	1.4-35
9.	4-Aminophenylacetic acid + Resorcinol [proposed method]	455	2.27×10^4	0.1-2.4

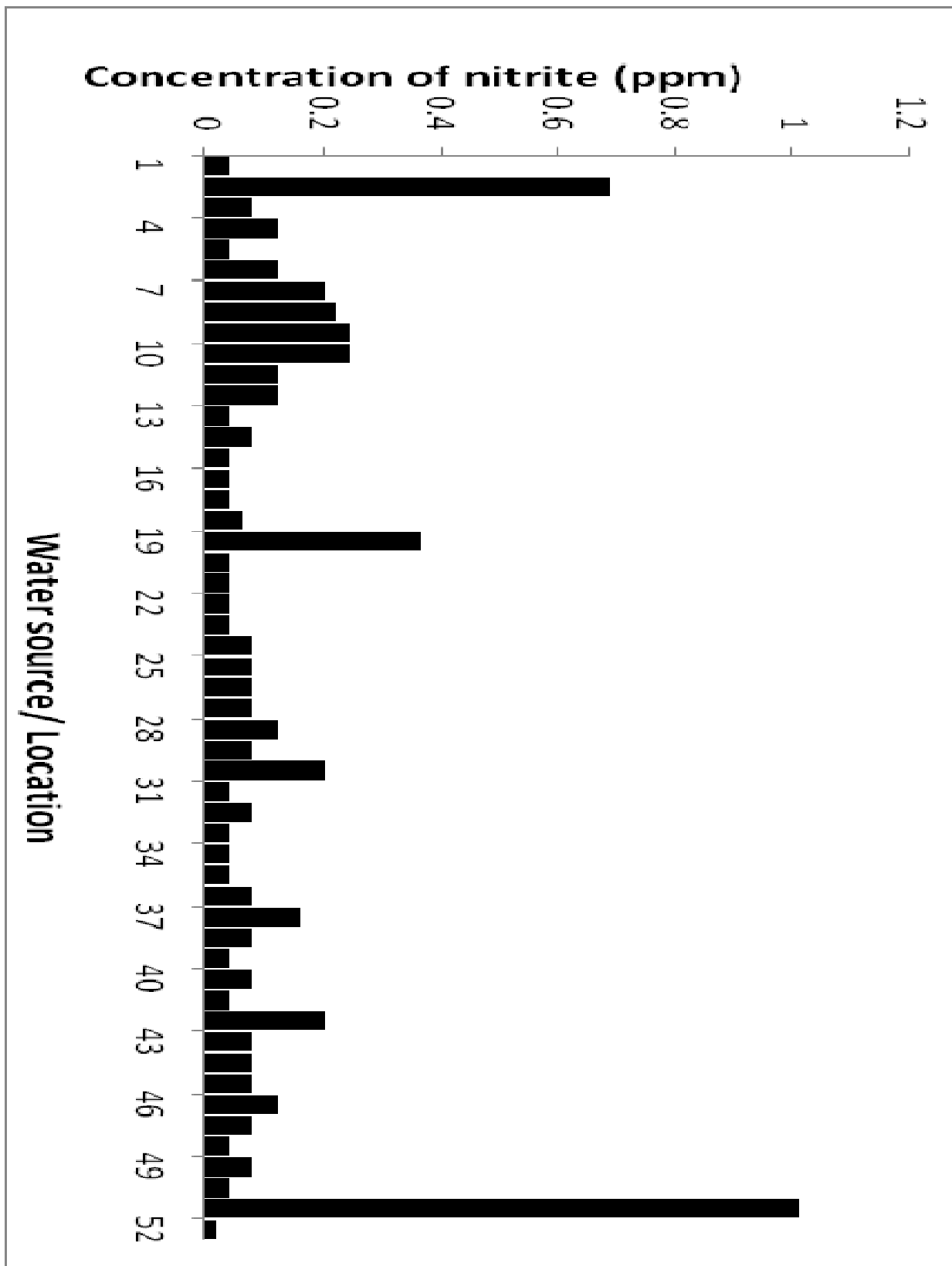
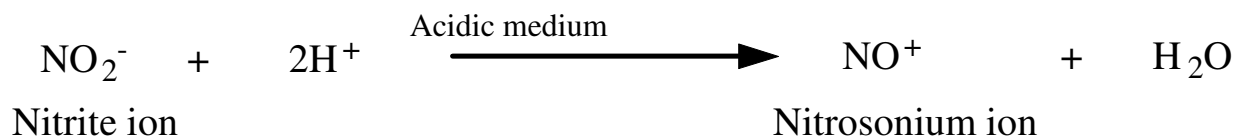


Figure – 4
The Plot of Concentration of Nitrite vs. Water from different Sources and Locations

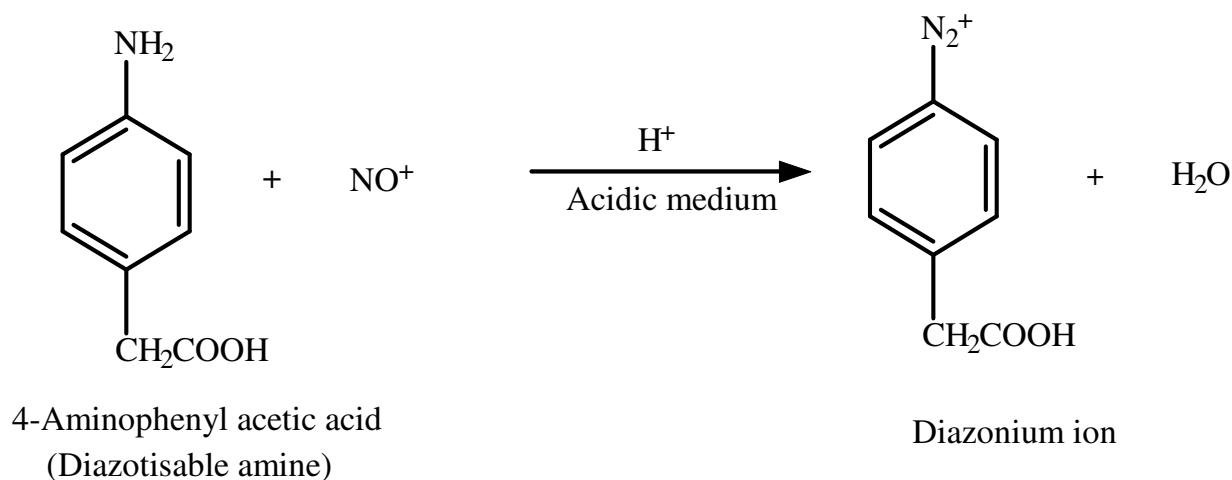
Proposed Reaction Mechanism: The reaction occurs in three steps. In the first step nitrite reacts with acid to form the diazonium cation. In the normal condition the nitrite ion can't attack on the 4-Aminophenylacetic acid due to the presence of negative charge on the reacting site. But when the nitrite ion is treated with the acid it loss one of the oxygen atom and become nitrosyl cation. In the second step the nitrosyl cation attack on

the lone pair of the nitrogen of 4-aminophenylacetic acid and diazonium cation is formed. In the third step, the diazonium cation subsequently couples with alkaline solution of resorcinol to produce the orange colored azo-dye which has absorption maximum in visible region. The different steps are shown as follows:

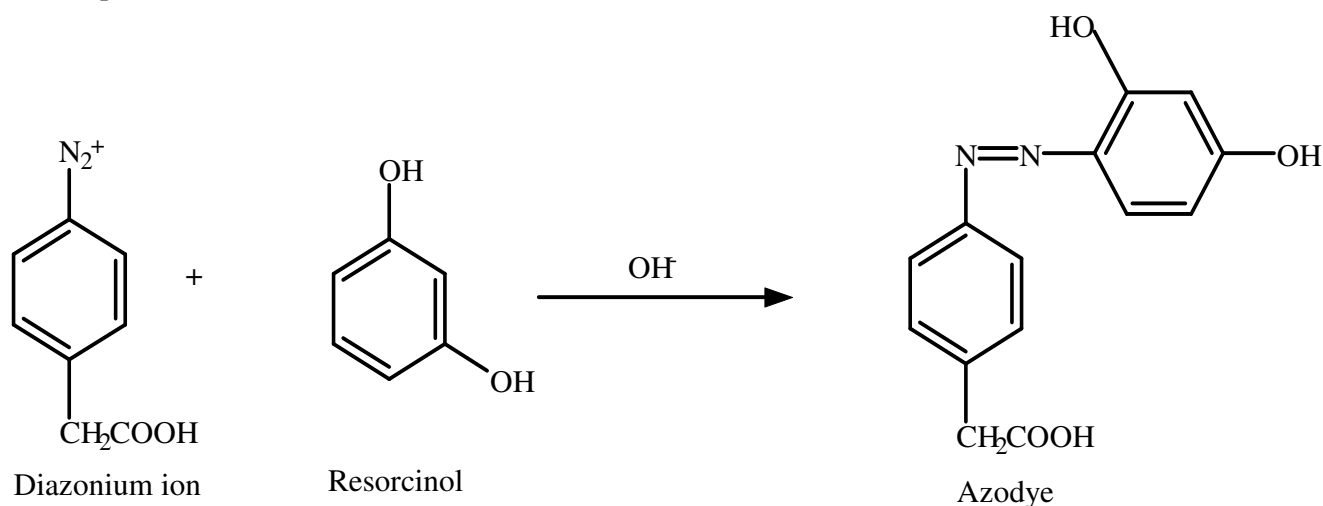
First step:



Second step:



Third step:



Conclusion

A very simple, sensitive and highly reproducible method for the spectrophotometric determination of trace amount of nitrite present in water sample from different sources is proposed and presented. The proposed method is based on the diazotization of the 4-aminophenylacetic acid with nitrite ion followed by coupling with resorcinol to give a stable orange colored azo dye. The optimized amounts of reagents were found to be 2 ml of 0.25% (w/v) 4-aminophenylacetic acid, 2 ml of 0.1% resorcinol solution in final 25 ml solution.

The proposed method compare favorably with the most of other spectrophotometric determination of nitrite in water. This method follows Lambert-Beer's law over the range 0.1-2.4 µg/ml of nitrite. The percentage recovery was found to range from 91.16-101.4% indicating the suitability of the method for the determination of nitrite in water. The correlation coefficient, molar absorptivity and Sandell's sensitivity of the method was found to be 0.9986, 2.27×10^4 lit.mol⁻¹cm⁻¹ and 2.03×10^{-3} µg cm⁻² respectively.

The validity of the method is also assessed by investigating the effect of common foreign ions and trace amount of nitrite in different water samples and waste water. The comparison of this method with others shows, this method is appropriate for trace level nitrite determination in water. This method should be tested to the soil sample for further study.

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References

1. Hill M.J., Nitrates and Nitrites in Food and Water, 1st ed., Wood Head Publishing Limited, England (1996)
2. Angela Cross, Kenneth P. Cantor, John S. Reif, Charles F. Lynch, and Mary H. Ward, Pancreatic cancer and drinking water and dietary sources of nitrate and nitrite, *Am. J. Epidemiol.*, **159**(7), 693-701 (2004)
3. Rogers M. A. M., Vaughan T. L, Davis S., and David B. Thomas D. B, Consumption of Nitrate, Nitrite, and Nitrosodimethylamine and the Risk of Upper Aerodigestive Tract Cancer, *Cancer Epidemiol. Biomarkers. Prev.*, **4**, 29-36 (1995)
4. Cimpeanu M., Pele M., Compeanu G. H. and Enache M., *Roum. Biotech. Lett.*, **5**, 299-301 (2002)
5. Butt S.B., Riaz M. and Iqbal M.Z., Simultaneous determination of nitrite and nitrate by normal phase ion-pair liquid chromatography, *Talanta*, **55**(4), 789-797 (2001)
6. Siu D.C. and Henshall A., Ion chromatographic determination of nitrite and nitrate in meat products, *J. Chromatogr.*, **804**, 157-160 (1998)
7. Barzegar L., Rezaei Behbehani G., Moosavi M. and Mehreshtiagh M., A calorimetric study on the interaction between Vitamin-B6 and lysozyme, *Res. J. Recent. Sci.*, **1**(ISC-2011), 345-347 (2012)
8. Nwajei G. E., Okwagi P., Nwajei R. I. and Obi-Iyeke G. E, Analytical assessment of trace elements in soils, tomato leaves and fruits in the vicinity of paint industry, Nigeria, *Res. J. Recent. Sci.*, **1**(4), 22-26 (2012)
9. Chaitanya Lakshmi G., Food coloring: The natural way, *Res. J. Chem. Sci.*, **4**(2), 87-96 (2014)
10. Khadka D. B. and Duwadi A., Spectrophotometric determination of trace amount of nitrite in water with 4-aminophenylacetic acid and phloroglucinol, *Int. Res. J. Environment. Sci.*, **3**(4), 64-69 (2014)
11. Bassett J., Denney R. C., Jeffery G. H. and Mendham J., "Vogel's Text Book of Quantitative Analysis" 4th Ed. ELBS, Longman, 447 (1978)
12. Bashir W. A. and Flamerz S., Photometric determination of nitrite, *Talanta*, **28**(9), 697-699 (1981)
13. Amin D., Determination of nitrite on using the reaction with 4-aminobenzotrifluoride and 1-naphthol, *Analyst*, **111**, 1335-1337 (1986)
14. Cotton F.A. and Wilkinson G., Advanced Inorganic Chemistry, 5th ed, John Wiley and Sons Inc. (1988)
15. Flamerz S. and Bashir W. A., Spectrophotometric determination of nitrite in waters, *Analyst*, **106**, 243-247 (1981)
16. Veena K. and Narayana B., Spectrophotometric determination of nitrite using new coupling agents, *Indian J. Chem. Technol.*, **16**, 89-92 (2009)
17. Pradhananga R. R., Khadka D. B. and Sherestha N. K., Spectrophotometric determination of trace amount of nitrite in water with 4-aminophenylacetic Acid and 1-naphthol, *J. Nepal Chem. Soc.*, **11**, 36-43 (1992)
18. Cherian T. and Narayana B., A new system for the spectrophotometric determination of trace amounts of nitrite in environmental samples, *J. Braz. Chem. Soc.*, **17**(3), 577-581 (2006)
19. Saltzman B. E., Colorimetric microdetermination nitrogen dioxide in atmosphere, *Anal. Chem.*, **26**(12), 1949-1955 (1954)