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

Khadka Deba Bahadur[*] and Duwadi Anjeeta
Central Department of Chemistry, Tribhuvan University, Kirtipur, Kathmandu, NEPAL

Abstract

The developed method is simple, selective and sensitive and has been applied to various water samples for the determination of nitrite. This method is based on diazo coupling reaction, where 4-aminophenylacetic acid is reacted with nitrite in the presence of acid to form diazonium ion. This diazonium ion further reacted with phloroglucinol to form a stable yellow azo-dye. The formed azo-dye has absorption maxima at 420 nm. Various analytical parameters have been studied. The system obeys Lambert-Beer's law in the range of 0.04-1.8 µg /ml. The correlation coefficient, molar absorptivity and Sandell's sensitivity were found to be 0.998, 2.40×10^4 lit.mol^{-1}cm^{-1} and 1.90×10^{-3} µg cm^{-2} respectively. Furthermore, the percentage recovery was found to be in the range of 92.9-103. Some of interference anion and cation was also tested. Although the interference due to foreign ions is negligible except copper and iron, the developed method should be further tested to eliminate such interfering ingredient in future.

Keywords: 4-aminophenylacetic acid, water sample, phloroglucinol, foreign ions, nitrite.

Introduction

Nitrite is one of the important pollutants present in water. Its determination is useful in various fields such as water, food and environmental samples. Nitrite and nitrate ions involve in several important environmental transformations including nitrogen[1]. Nitrite has been considered as a potential hazardous compound for human health. The presence of trace amount of nitrite in drinking water may cause to methemoglobinemia and with long term exposure is a possible cancer risk[2]. It is an important intermediate in the biological nitrogen cycle and is available in surface water and soils. Nitrite is produced in water as pollutants as a result of biodegradation of nitrate and ammonical nitrogen or nitrogenous organic matter[3].

The nitrite is also a versatile chemical agent, which found various applications to food preservation in industry, dye manufacture and corrosion inhibition of industrial process water[4]. It is very useful to determine the nitrite ion in various areas such as biological samples, environmental studies and clinical chemistry[5]. Sodium nitrite or Potassium nitrite find extensive used in the manufacture of azo dyes and explosives. Therefore, effluent from these industries will contain large concentration of nitrites. There is also appearance of nitrites in the water with the deficiency of oxygen in natural layer of water. Deficiency of oxygen leads to the reduction of nitrates with the formation of nitrites[6]. In flowing water where aerobic conditions prevails, only a minutes quantities of nitrites are present because nitrites is unstable in presence of oxygen[7].

A numbers of methods have been developed for the determination of nitrite in water such as electrochemical detection, chromatography, capillary electrophoresis and spectrophotometric method[8,9]. Recently, some of the related studies have been also reported[10-12]. In most of these reported methods have been used expensive experimental set up and chemicals. Considering an importance of nitrite and its determination in water an attempt is made here to develop a sensitive, simple and accurate method which is based on the diazotization of 4-aminophenylacetic acid in presence of acid followed by the coupling reaction with phloroglucinol in presence of alkaline medium.

Material and Methods

250 ml of stock nitrite solution was prepared in distilled water by weighing 0.375g of dried sodium nitrite. About 1 ml of chloroform and a pellet of sodium hydroxide were added to inhibit the bacterial growth and to prevent the liberation of nitrous acid, respectively. The stock solution was titrated with standard solution of potassium permanganate to standardize the stock solution by following the procedure described elsewhere[13]. Then required concentration of nitrite solution was prepared by appropriate dilution of stock solution in distilled water daily.

To prepare calibration curve, 0 ml, 0.5 ml, 1 ml, 1.5ml, 2 ml and 2.5 ml 3 ml, 3.5 ml, 4 ml, 4.5 ml, 5 ml of standard 10 µg /ml nitrite solution were transformed to a series of 25 ml volumetric flask. 2 ml of 0.25 % (w/v) 4-aminophenylacetic acid was added and each set kept aside for 15 minutes to allow the complete diazotization reaction. Then 2 ml of 0.2% (w/v) alkaline solution of phloroglucinol was added as a coupling reagent and each flask shaken for 1 min. Then the volume was made up to

the mark by adding water. Lastly, the absorbance of the resulting colored dye of each flask was measured at 420 nm versus the reagent blank, by using WPA S-104 spectrophotometer.

Results and Discussion

Spectral Studies for the Determination of λ_{max}: For the determination of λ_{max} of dye formed, 2 ml of 20 µg /ml sodium nitrite solution was taken in 25 ml.volumetric flask and 2 ml of 4-aminophenylacetic acid solution is added. This solution is kept aside for 15 minutes to allow the diazotization reaction. Then 2 ml phloroglucinol was mixed and shaken for 1 min and solution was made up to the mark by adding distilled water. The absorbance of the resulting yellow colored dye was measured against a reagent blank (Prepared in the same way, excluding the analyte) at wavelengths from 390 – 600 nm, by using WPA S-104 spectrophotometer is presented in figure 1. The λ_{max} occurs at 420 nm. Hence all other further works about absorbance was carried out at 420 nm.

Effect of Time Interval between the Addition of Aromatic Amine and Coupling Agent: The effect of the time interval between the addition of 4-aminophenylacetic acid and phloroglucinol was studied by adding these reagents at different time interval from 0 to 50 minutes. The plot of absorbance versus time (minutes) for 25ml 0.8 µg /ml nitrite is shown in figure 2. From the curve, it is quite clear that the absorbance of the azo dye is found to attain a constant limiting value after 10 minutes. This indicates that the diazotization process takes some definite time and 15 minutes seems to be optimum. The coupling was found to be very fast and since after addition of

phloroglucinol, absorbance of the solution remains practically constant up to 24 hours. This clearly indicates that the coupling occurs instantaneously.

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 5 µg to 50 µg of nitrite in final volume of 25 ml by the recommended procedure. The absorbance of the resulting azo dye is measured at 420 nm. Figure 3 shows a plot of absorbance versus nitrite concentration. The plot is found to be linear from the origin. From the plot it is clear that the proposed method obeys Beer-Lambert's law from 0.04 to 1.8 ppm. The apparent molar absorptivitiy and Sandell's sensitivity in the region of least photometric error were found to be 2.40×10^4 lit.mol^{-1} cm^{-1} and 1.90×10^{-3} µg cm^{-2} respectively.

Recommended Procedure for the Nitrite Determination in Water: Quantitative determination of nitrite ion using 4-aminophenylacetic acid as diazotisable amine and phloroglucinol as a coupling reagent is quite simple, accurate and highly reproducible. For the better results recommended procedure for the determination of nitrite in water sample are as follows: i. Take 15-20 ml of water sample in 25 ml volumetric flask. ii. Add 2 ml of 0.25% (w/v) 4-aminophenylacetic acid prepared in 0.6M HCl and shake well then after keep the solution aside for 15 minutes. iii. Add 2 ml of 0.2% (w/v) phloroglucinol prepared in 1.2M sodium hydroxide and make the solution up to the mark by adding distil water and measure the absorbance at 420 nm. iv. Calculate the amount of nitrite in ppm from the calibration curve.
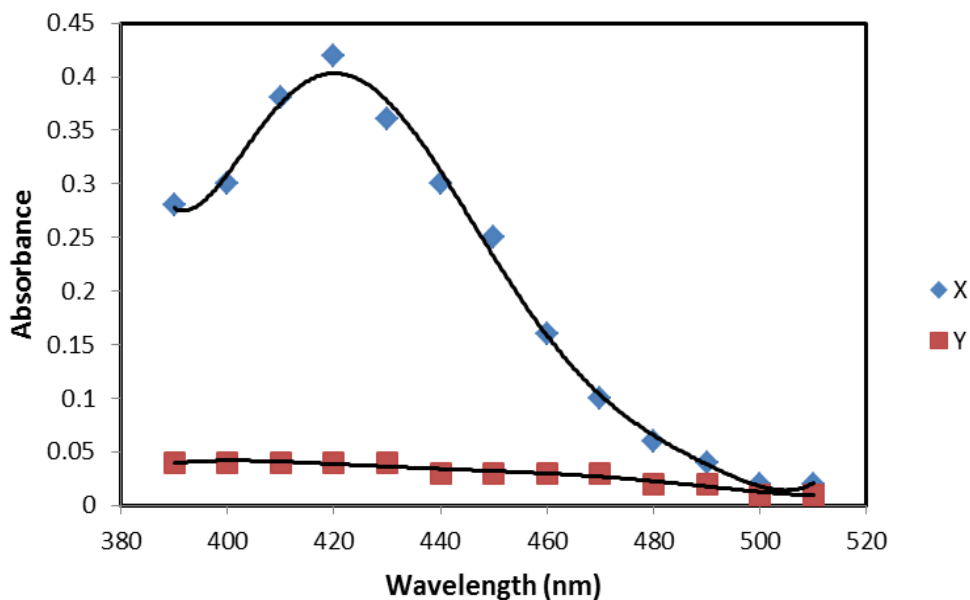

Figure-1
X- Absorption Spectra of Azo dye Measured against Reagent blank, (20 µg of NO_2^- in 25 ml) and Y- Reagent blank against Distilled water
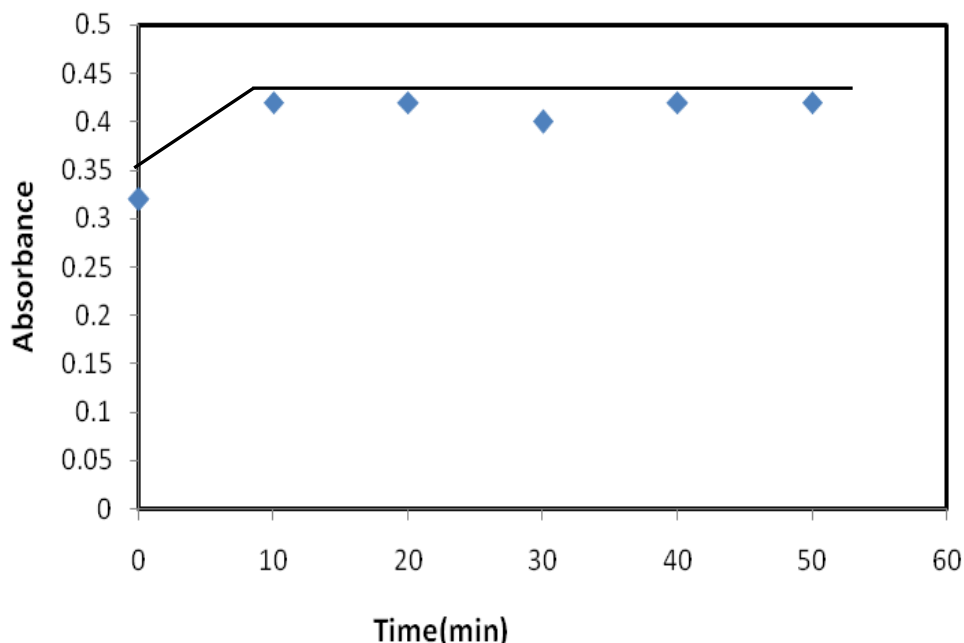
Figure-2
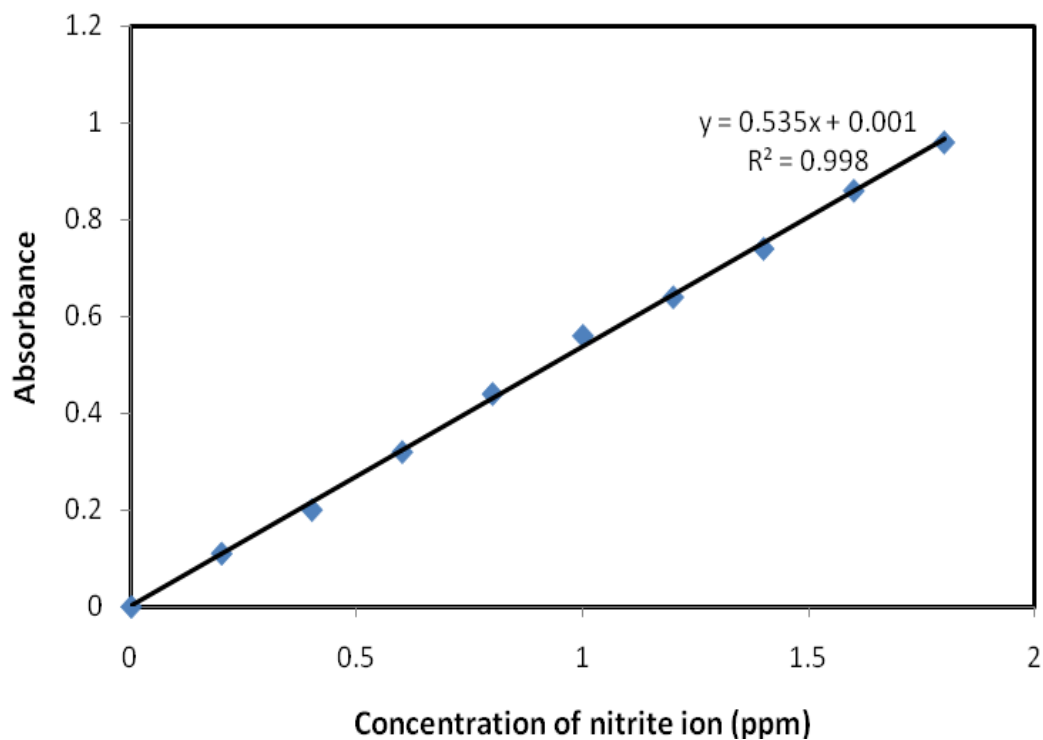**Effect of Time Interval between the Addition of Aromatic amine and Coupling agent**

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

Accuracy and Precision of the Method: Under the optimum conditions, the accuracy and precision of the proposed method was checked by performing three replicate determination of nitrite by taking 4, 8, 12, 16 and 20 µg of nitrite in the final volume of 25 ml by the recommended procedure. The percentage recovery was found to be range from 92.9-103. In table 1, the results of this analysis are shown.

Table-1
Accuracy and Precision of the Method

Nitrite taken (µg)	Nitrite found (µg) #	Mean recovery (%)	Relative standard deviation (%)
4	3.89 ± 0.127	97.3	3.7
8	7.74 ± 0.216	96.6	2.8
12	12.14 ± 0.366	101.2	3.0
16	14.86± 0.259	92.9	1.7
20	20.60 ± 0.254	103.0	1.2

mean ± standard deviation

Effect of Foreign ions: 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^+, NH_4^+, Ca^{++}, Mg^{++}, Cu^{++}, Cl^-, NO_3^-, $H_2CO_3^-$, CO_3^{--}, SO_4^{--}, PO_4^{---}, 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 PO_4^{---} interfere by causing turbidity to the solution.

Analysis of Water Samples: Water samples from different pond and wells of Sitapaila, Kirtipur, and Balaju were collected and have been applied for the nitrite determination in different samples. The collected samples of different areas were tested by

taking 20 ml of water sample in final volume of 25 ml according to recommended procedure. In table 3, the results of this analysis are shown.

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)
Sodium	92	4600	20.7
Potassium	39	1950	20.5
Ammonium	18	900	20.8
Calcium	40	2000	20.4
Magnesium	2.4	120	20.1
Copper	2.54	127	17.6
Chloride	71	3550	20.7
Nitrate	62	3100	20.3
Bicarbonate	61	3050	21.1
Carbonate	60	3000	21.3
Sulphate	19.2	960	20.7
Phosphate	96	4800	19.6

Comparison of Some Reported Method with the Proposed Method: The method has been successfully applied to various water samples for the nitrite determination. The result of the comparison is shown in table 4. The application and the comparison of the method reflect that the developed method is appropriate for the nitrite determination in water.

Table-3
Analysis of Water Samples

Sample no.	Volume of water sample (ml)	Source of water	Location	Absorbance	Nitrite found (ppm)
1	20	Pond	Sitapaila	0.14	0.28
2	20	Well	Sitapaila	0.20	0.41
3	20	Well	Sitapaila	0.03	0.06
4	20	Well	Kirtipur	0.16	0.30
5	20	Well	Kirtipur	0.08	0.16
6	20	Well	Balaju	0.08	0.07

Table-4
Comparison of some Reported Methods with the Proposed Method

| Method	Reagent/ Reference	λmax (nm)	Molar Absorptivity (litmol^{-1} cm^{-1})	Determination range (ppm)
1.	Sulfanilic acid + 1-Naphthylamine [14]	520	3.30×10^4	1.4-35
2.	p-Nitroaniline + Ethoxyethylenemaleic ester[15]	439	1.21×10^4	0.5-16.0
3.	Barbituric acid [16]	310	1.53×10^4	0.00-3.22
4.	4- Aminophenylacetic acid + 1- Naphthol [17]	490	2.70×10^4	0.04-1.6
5.	p- Nitroaniline + Frusemide [18]	680	3.314×10^3	0.4-2.0
6.	4- Aminosalicylic acid + 1-Naphthol[19]	520	1.47×10^4	0.1-3.0
7.	4-Aminobenzotrifluoride [20]	510	3.91×10^4	0.03-0.8
8.	p-Aminobenzoic acid + 8-Hydroxyquinoline [21]	499	3.20×10^4	0.1-1.5
9.	4-Aminophenylacetic acid + Phloroglucinol [proposed method]	420	2.40×10^4	0.04-1.8

Proposed Reaction Mechanism: The reaction is believed to occur in two steps. In the first step, 4-aminophenylacetic acid reacts with nitrite in presence of acid to form diazonium cation. In the second step the diazonium cation subsequently couples with alkaline solution of phloroglucinol to produce the yellow azo dye. The azo dye has intense color which has absorption maximum in visible region.

Conclusion

This paper reports a simple, rapid, sensitive method for the determination of trace amount of nitrite in water. The proposed method involves diazotization of 4-aminophenylacetic acid followed by the coupling with coupling agent as phloroglucinol to give a stable azodye. The proposed method is a variant of the original Griess method. This indicates that the proposed method is quite simple, sensitive and reproducible. The method obeyed Lambert-Beer's law in the range of 0.04-1.8 µg /ml. The Correlation Coefficient, Molar Absorptivity and Sandell's Sensitivity of the method were found to be 0.998, 2.40×10^4 lit. mol^{-1} cm^{-1} and 1.90×10^{-3} µg cm^{-2} respectively.

This method neither involves extraction nor did demand sophisticate instruments or reagents like others. The required chemical reagents are readily available in the market and are in additional nontoxic and non-carcinogenic. The method is found to be working satisfactorily for the nitrite determination in various water samples. Some of an interference anion and cation was also tested.
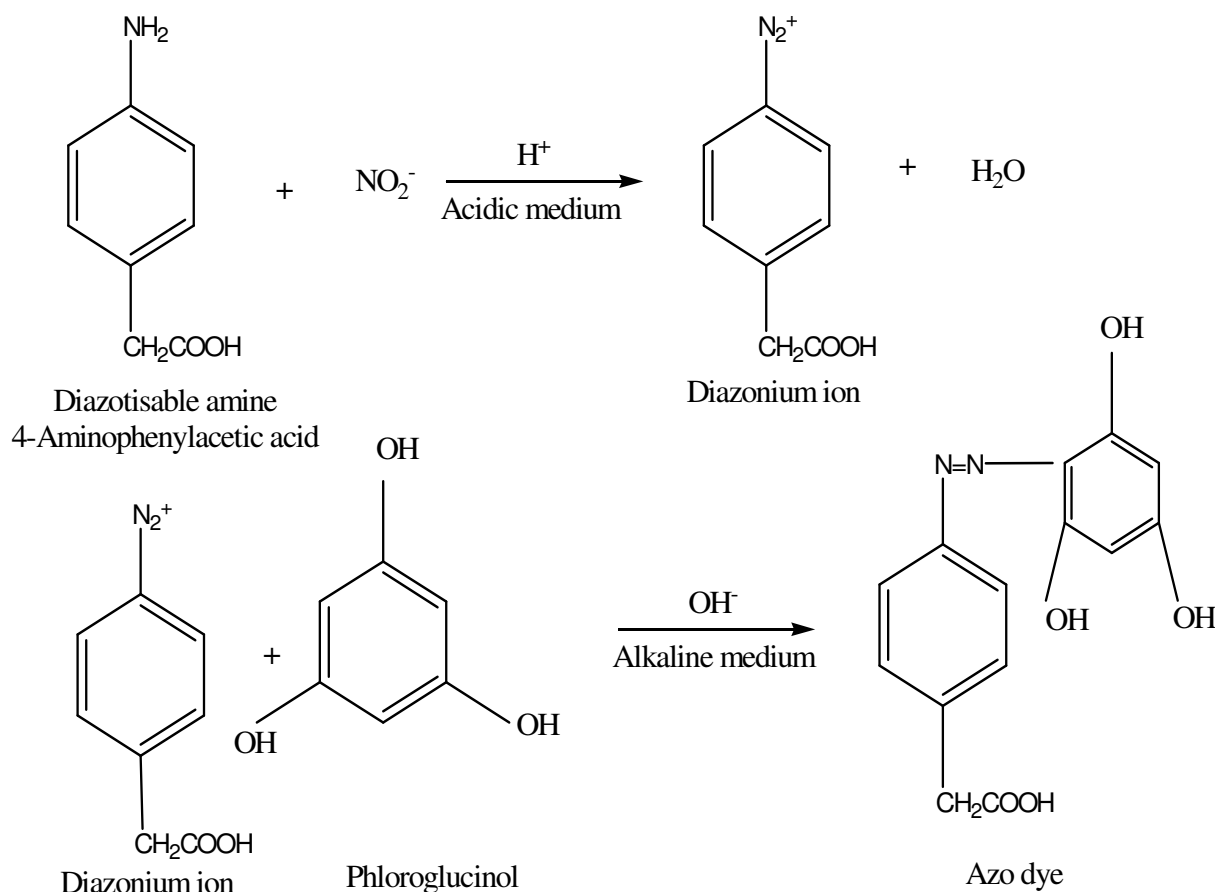
Scheme-1
Proposed Reaction Mechanism

Acknowledgements

References

1. Henery J.G. and Heinke G.W., Environmental Science and Engineering, 2nd ed., Prentice Hall, New Jersey **(1996)**

2. Cox R.D. and Frank C.W., Determination of nitrate and nitrite in blood and urine by chemiluminescence, *J. Anal. Toxicol.,* **6(3),** 148-152 **(1982)**

3. Gabbay J., Almog Y., Davidson M. and Donagi A.E., Rapid spectrophotometric micro-determination of nitrites in water, *Analyst,* **102**, 371-376 **(1997)**

4. Manzoori J.L., Sorouraddin M.H. and Haji-Shabani A.M., Spectrophotometric determination of nitrite based on its catalytic effect on the oxidation of carminic acid by bromate, *Talanta,* **46(6),** 1379-1386 **(1998)**

5. Dayananda B.P. and Revanasiddappa H.D., A facile analytical method for the determination of nitrite in water and soil samples, *EJEAF Chem.,* **6(8)**, 2263-2271 **(2007)**

6. Healeh M.I.H. and Korenaga T., Ion chromatographic method for simultaneous determination of nitrate and nitrite in human saliva, *J. Chromatogr.,* **744(2)**, 433-437 **(2000)**

7. Kage S., Kudo K. and Ikeda N., Determination of nitrate in blood by gas chromatography and gas chromatography-mass spectrometry, *J. Chromatogr.,* **742**, 363-368 **(2000)**

8. 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)**

9. Siu D.C. and Henshall A., Ion chromatographic determination of nitrite and nitrate in meat products, *J. Chromatogr.,* **804,** 157-160 **(1998)**

10. 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)**

11. 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)**

12. Chaitanya Lakshmi G., Food coloring: The natural way, *Res. J. Chem. Sci.,* **4(2),** 87-96 **(2014)**

13. Bassett J., Denney R.C., Jeffery G.H. and Mendham J., Vogel's Text Book of Quantitative Analysis, 4th Ed. ELBS, LONGMAN, 447 **(1978)**

14. Saltzman B.E., Colorimetric microdetermination nitrogen dioxide in atmosphere, *Anal. Chem.*, **26(12)**, 1949-1955 **(1954)**

15. 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)**

16. Cotton F.A. and Wilkinson G., Advanced Inorganic Chemistry, 5th ed, John Wiley and Sons Inc. **(1988)**

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. Veena K. and Narayana B., Spectrophotometric determination of nitrite using new coupling agents, *Indian J. Chem. Technol.,* **16**, 89-92 **(2009)**

19. Flamerz S. and Bashir W.A., Spectrophotometric determination of nitrite in waters, *Analyst,* **106**, 243-247 **(1981)**

20. Amin D., Determination of nitrite on using the reaction with 4-aminobenzotrifluoride and 1-naphthol, *Analyst*, **111**, 1335-1337 **(1986)**

21. Bashir W.A. and Flamerz S., Photometric determination of nitrite, *Talanta,* **28(9)**, 697-699 **(1981)**