



P-ISSN2349-8528
E-ISSN 2321-4902
IJCS 2016; 4(3): 101-105
© 2016 JEZS
Received: 15-03-2016
Accepted: 16-04-2016

Nagaraj P
Department of Chemistry,
Dr. M.V. Shetty Institute of
Technology, Moodbidri,
Mangalore-574225, Karnataka,
India.

Gopalakrishna Bhat N
Department of Chemistry,
Srinivas Institute of Technology,
Valachil, Mangalore – 574143,
Karnataka, India.

Chandrashekara KG
Department of Chemistry,
Srinivas Institute of Technology,
Valachil, Mangalore – 574143,
Karnataka, India.

Correspondence
Nagaraj P
Department of Chemistry,
Dr. M.V. Shetty Institute of
Technology, Moodbidri,
Mangalore-574225, Karnataka,
India.

International Journal of Chemical Studies

Spectrophotometric determination of nitrite and nitrate ions by diazo coupling method

Nagaraj P, Gopalakrishna Bhat N and Chandrashekara KG

Abstract

A new spectrophotometric method is investigated for the rapid, sensitive and selective determination of nitrite and nitrate in water and soil samples. It is based on the tetrazotization of benzidine with nitrite, in acid medium to form bisdiazonium ion, which is then coupled with resorcinol. The orange coloured azo dye formed in basic medium, shows absorption maximum at 463.5 nm. The method is optimized for temperature, acidity, amount of reagents required and tolerance amount of anions and cations. The Beer-Lambert's plot shows linearity up to $2.8 \mu\text{g cm}^{-3}$ of nitrite. The molar absorptivity and Sandell's sensitivity are found to be $2.1524 \times 10^4 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$ and $0.0033 \mu\text{g cm}^{-2}$ respectively. The investigated method has detection limit as $0.0049 \mu\text{g cm}^{-3}$ and quantitation limit as $0.0102 \mu\text{g cm}^{-3}$. The method is applied for the determination of trace amount of nitrite and nitrate in water and soil samples. The results are statistically evaluated.

Keywords: Spectrophotometry, nitrite, nitrate, benzidine, tetrazotization, resorcinol

1. Introduction

Excessive accumulation of nitrate and nitrite is a major cause of water pollution, air pollution and various health hazards. Excessive nitrite content results in eutrophication and depletion of dissolved oxygen content in water. Seepage water from animal feedlots, live stock manures, fertilizers, faulty septic systems, erosion of natural deposits etc are some of the important sources of nitrates and nitrites in water and food. For curing and preserving of meat and fish, nitrates and nitrites are extensively used. Drinking water^[1], vegetables^[2, 3], fruits^[4], meats^[5] and other food products^[6, 7] are major sources of dietary nitrates^[8].

Micro flora in the salivary glands and in the stomach reduce the nitrates in to nitrites^[9]. In the human body, nitrites react with amines or amides to generate nitrosamines^[10]. N-Nitroso compounds that are formed endogamously from drinking water and dietary sources, are found to be animal carcinogens^[11]. High intake of N-nitrosodimethylamine is associated with increased risk for gastric cancer^[12]. Nitrite is mutagenic in nature. Under mild acidic conditions phenol reacts with nitrite forming p-diazoquinone. It acted mutagenic in *Salmonella Typhimurium*^[13]. N-nitroso compounds results in DNA alkylation and base modification^[14].

Various methods have been reported for the quantitative determination of nitrite as well as nitrate such as flow injection method^[15], stopped flow analysis^[16], sequential injection analytical technique^[17], and spectrophotometry^[18-23]. Most of the reported methods utilize expensive experimental equipments and time consuming procedures. Since excess nitrite and nitrate concentration is a major threat for environment and flora and fauna, an attempt is made to investigate a rapid, sensitive and selective method for their determination. It is based on the tetrazotization of benzidine in acid medium, followed by coupling with resorcinol in alkaline medium.

2. Materials and Method

2.1 Materials

A UV-Visible spectrophotometer (Jasco model V-360 manufactured by Jasco Corporation, Japan) with quartz cell with a path length of 1 cm was used for the measurements of absorbance. The stock solution of sodium nitrite and potassium nitrate were prepared by dissolving 0.1500 g of NaNO_2 and 0.1631 g of KNO_3 in 100 mL distilled water. To each of these solutions, a pellet of sodium hydroxide and 1 mL of chloroform were added. The solutions of, 0.05% benzidine, 5% resorcinol, 2 M sodium hydroxide, 2 M hydrochloric acid, 0.02 M EDTA, 1% sodium carbonate and solutions of various metal ions and anions were

prepared using distilled water. Zinc/NaCl granular mixture was prepared by mixing 1.000 g of finely powered zinc with 200 g of granular sodium chloride in a bottle.

2.2 Procedure

Aliquots of the solution, containing 5 to 70 μg of nitrite were pipetted out in to 25 mL standard flasks, each containing 1 mL of 0.05% benzidine and 1 mL of 2 M hydrochloric acid solutions. The solutions in each standard flask was mixed well and set aside for 3 to 4 minutes to allow the completion of tetrazotization reaction with occasional mixing. Then, 2 mL of 5% resorcinol and 2 mL of 2 M sodium hydroxide solutions were added to each standard flask. Light orange colour was developed. The content of each standard flask was diluted up to the mark using distilled water and mixed well for uniform concentration. The absorbance was measured at 463.5 nm against reagent blank. The nitrite content in the aliquot sample was determined using a concurrently prepared calibration curve.

Nitrate stock solution of volume 10 mL was transferred in to beaker containing 5 mL of 10.2 M hydrochloric acid and 5 g of Zn/NaCl granular mixture. The content was allowed to stand for 15 minutes with occasional shaking. Nitrate was reduced in to nitrite. Then the solution was filtered through Whatman filter paper No. 41 in to a 100 mL standard flask. Then aliquots of the solution, containing 5 to 70 μg of reduced nitrate were pipetted out and determination is carried out as in the case of nitrite.

2.3 Analysis of nitrate and nitrite in water sample

An aliquot (≤ 5 mL) of water sample was treated with 0.5 mL of 1 M NaOH and 5 mL 0.02 M EDTA solution and contents are mixed well. Then it was centrifuged to separate the precipitate. Suitable aliquots of the centrifugate were analysed

for the presence of nitrate and nitrite using proposed procedure. All the tested samples gave negative results. Now to these samples known amounts of nitrite and nitrate were added separately, then analysed for nitrite or nitrate by investigated procedure and also by the reference method [24].

2.4 Analysis of nitrate and nitrite in soil sample

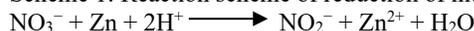
About 1.0 g of soil sample was transferred in to a clean 50 mL beaker. The soil sample was extracted four times with 1% sodium carbonate solution using 5 mL for each time. Then the extract was filtered through Whatman filter paper No. 41 [25]. Suitable aliquots of the filtrate were analysed for the presence of nitrate and nitrite using proposed procedure. All the tested samples gave negative results. Now to these samples known amount of nitrite and nitrate were added separately, then analysed for nitrite or nitrate by investigated procedure and also by the reference method [24].

3. Results and discussion

3.1 Absorption spectrum

In this method benzidine undergoes tetrazotization in the presence of hydrochloric acid, followed by coupling with resorcinol in sodium hydroxide medium to yield orange coloured dye. The azo dye formed by benzidine and resorcinol has maximum absorption at 463.5 nm against the reagent blank. The absorption spectrum of azo dye is shown in Figure 1. The reaction is described in the scheme 1 and 2

Scheme 1: Reaction scheme of reduction of nitrate to nitrite



Scheme 2: Tetrazotization of benzidine followed by coupling with resorcinol

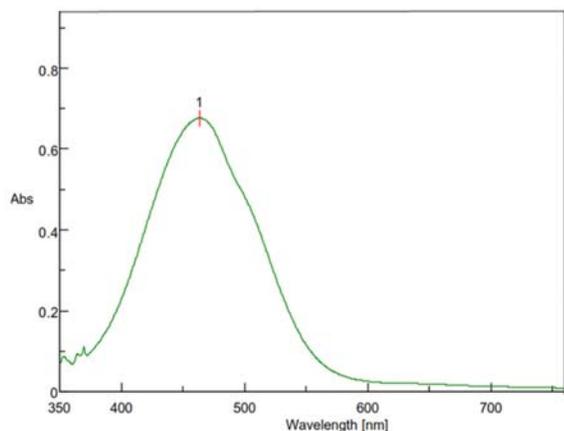
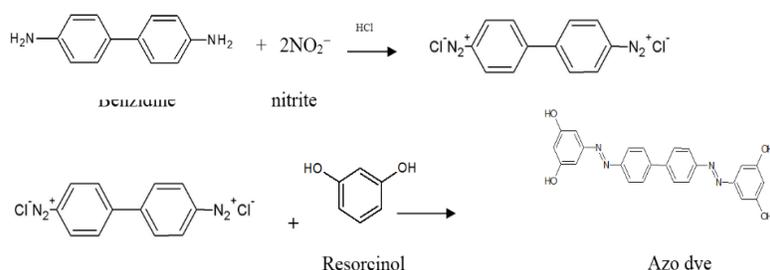


Fig 1: Absorption spectrum of azo dye Vs reagent blank

3.2 Effect of temperature and reagent concentration

Diazotization and coupling reactions are found to be temperature dependent. Diazotization was carried out at 0 $^{\circ}\text{C}$ –

5 $^{\circ}\text{C}$ and coupling reaction was carried out at room temperature. Above 40 $^{\circ}\text{C}$, there is a decrease in intensity of the colour. The reagents, 1 mL of 2.0 M hydrochloric acid, 1 mL of 0.05% benzidine, 2 mL of 5% resorcinol and 2 mL of 2.0 M sodium hydroxide solution per aliquot of the sample (5 to 70 μg of nitrite), result in maximum absorbance.

3.3 Validity of Beer-Lambert's Law

The validity of Beer's law for the system was studied by measuring the absorbance values of a series of solutions having different concentrations of nitrite in the solution. These results are shown in the form of a plot of absorbance verses concentration of nitrite (Figure 2). It is evident from the graph that Lambert-Beer's law is obeyed up to 2.8 $\mu\text{g cm}^{-3}$ of nitrite. For the determination of nitrite, the detection limit [26] was calculated as 0.0049 $\mu\text{g cm}^{-3}$ and quantitation limit [26] was found as 0.0102 $\mu\text{g cm}^{-3}$. The mean value of molar absorptivity is found to be $2.1524 \times 10^4 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$, which indicates that the method is quite sensitive. The Sandell's sensitivity of the method is 0.0033 $\mu\text{g cm}^{-2}$.

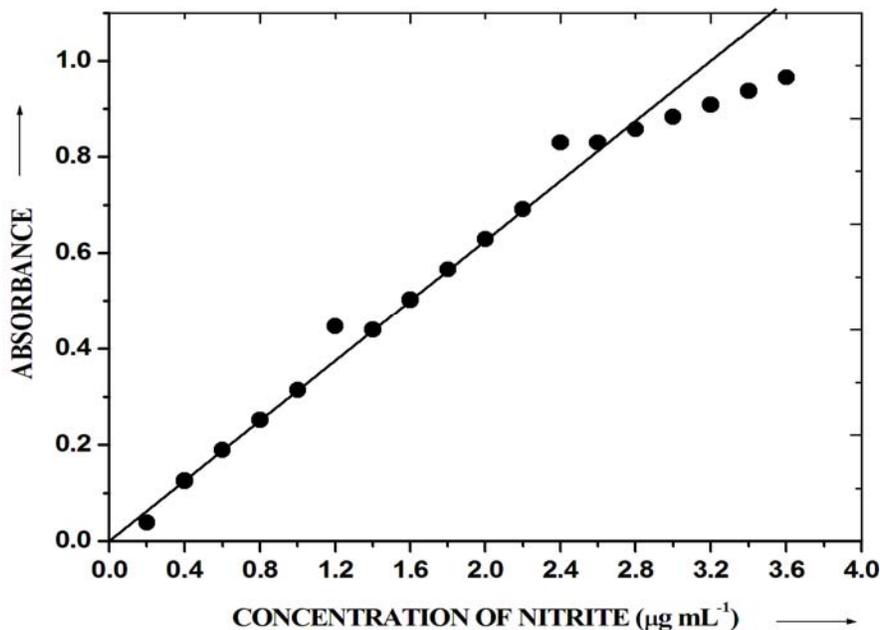


Fig 2: Beer-Lambert's Law plot for azo dye

3.4 Effect of Foreign ions

The absorbance value of azo dye [$2.0 \mu\text{g cm}^{-3}$ nitrite] was studied in the presence of foreign ions. The tolerance limit was fixed as deviation of $\pm 2\%$ in the absorbance value. The results are presented in Table 1. The results indicate that

Sn(II), Co(II), Cu(II), Hg(II), Fe(III) and Ce(IV) interfere severely. Fe(III) and Cu(II) are masked with sodium fluoride and 2-mercaptoethanol respectively. The tolerance level of other ions can be increased by adding 3 mL of 0.02 M EDTA solution.

Table 1: Tolerance limit of diverse ions in the determination of $50 \mu\text{g cm}^{-3}$ nitrite^a

Ion added	Tolerance limit $\mu\text{g cm}^{-3}$
Na(I)	80
K(I)	80
Ba(II)	400
Cd(II)	400
Ca(II)	150
Co(II) ^b	80
Cu(II) ^b	80
Pb(II)	160
Mn(II)	160
Mg(II)	160
Hg(II) ^b	40
Sn(II) ^b	30
Al(III)	400
Fe(III) ^b	70
Ce(IV) ^b	20
Fluoride	1500
Iodide	1500
Chloride	800
Tartarate	800
Citrate	800
Acetate	800
Oxalate	800
Sulphate	1500
Nitrate	800
Phosphate	800

^aAverage of four determinations

^bMasked by masking agents

3.5 Applications

Water and soil samples were explored according to the proposed procedure to affirm the suitability of the investigated method. The results of investigated method has been matched with the results of the reference method^[24]. The Student's t-values at $\alpha=0.05$ and the variance ratio F-values

calculated at $\alpha=0.05$ did not go beyond the theoretical values. The results are presented in Table 2 and Table 3. The accuracy and precision of the results, of investigated method attune with that of the reference method. Hence it can be effectively applied for the analysis of nitrite in soil and water samples with fair degree of accuracy and precision.

Table 2: Determination of nitrite in Water Sample and Soil Sample

Sample	Nitrite added ($\mu\text{g mL}^{-1}$)	Proposed method				Reference method ²⁴				^b F-test	^c t-test
		^a Nitrite found ($\mu\text{g mL}^{-1}$)	Recovery (%)	Relative error (%)	Relative standard deviation (%)	^a Nitrite found ($\mu\text{g mL}^{-1}$)	Recovery (%)	Relative error (%)	Relative standard deviation (%)		
Water Sample	1.0	0.991	99.1	-0.90	0.11	0.992	99.2	-0.80	0.11	1.11	1.49
	1.5	1.499	99.9	-0.07	0.26	1.497	99.8	-0.20	0.31	1.45	0.74
	2.5	2.498	99.9	-0.08	0.38	2.495	99.8	-0.20	0.34	1.27	0.53
Soil Sample	0.8	0.796	99.5	-0.50	1.85	0.801	100.1	+0.13	2.58	1.96	0.44
	1.6	1.603	100.2	+0.19	0.91	1.597	99.8	-0.19	0.67	1.88	0.74
	2.4	2.401	100.1	+0.04	0.35	2.393	99.7	-0.29	0.50	2.02	1.22

^aAverage of five determinations^bTabulated t-value for 8 degree of freedom at P (0.95) is 2.306^cTabulated F-value for (4, 4) degree of freedom at P (0.95) is 6.39

4. Conclusion

The micro quantities of nitrate and nitrite are determined easily and accurately using UV-Visible spectrophotometer. The azo dye is rapidly formed with excellent reproducibility. The colour development is not dependent on the pH of the solution. The results are compared and statistically analysed with a reference method. It is found that there is no considerable difference in precision and accuracy of the

proposed method and the reference method. The colour of the dye showed excellent stability of more than 24 hours. At the wavelength where dye shows maximum absorbance, reagent blank shows minimum absorbance. The analysis of water and soil samples for nitrate and nitrite using proposed procedure produces excellent results. The method shows high tolerance limit for a number of foreign cations and anions.

Table 3: Determination of nitrate in Water Sample and Soil Sample

Sample	Nitrate added ($\mu\text{g mL}^{-1}$)	Proposed method				Reference method ²⁴				^b F-test	^c t-test
		^a Nitrate found ($\mu\text{g mL}^{-1}$)	Recovery (%)	Relative error (%)	Relative standard deviation (%)	^a Nitrate found ($\mu\text{g mL}^{-1}$)	Recovery (%)	Relative error (%)	Relative standard deviation (%)		
Water Sample	1.2	1.202	100.2	+0.17	0.27	1.199	99.8	-0.08	0.22	1.61	1.58
	1.8	1.795	99.7	-0.28	0.20	1.792	99.6	-0.44	0.22	1.24	0.74
	2.4	2.403	100.1	+0.13	0.19	2.398	99.9	-0.08	0.25	1.53	1.47
Soil Sample	1.5	1.486	99.1	-0.93	0.51	1.491	99.4	-0.60	0.57	1.23	0.98
	2.0	1.988	99.4	-0.60	0.33	1.983	99.2	-0.85	0.29	1.32	1.29
	2.5	2.494	99.6	-0.24	0.21	2.497	99.9	-0.12	0.23	1.12	0.84

^aAverage of five determinations^bTabulated t-value for 8 degree of freedom at P (0.95) is 2.306^cTabulated F-value for (4, 4) degree of freedom at P (0.95) is 6.39

5. Acknowledgement

The authors are grateful to the Srinivas Institute of Technology for providing the laboratory facilities.

6. References

- Thomson BM, Nokes CJ, Cressey P. J Intake and risk assessment of nitrate and nitrite from New Zealand foods and drinking water, *Food Addit. Contam. Part B*. 2007; 24(2):113-121.
- Firdevs Mor, Fatma Sahindokuyucu, Neslihan Erdogan. Nitrate and Nitrite Concentrations of some Vegetables consumed in south province of Turkey, *J Anim Vet Adv*. 2010; 9(15):2013-2016.
- Fatemehsadat Mirmohammed-Makki, Parisa Ziarati. Nitrate and nitrite in Fresh tomato derived products. *Biomed Pharmacol J*. 2015; 8(1):115-122.
- Okafor PN, Ogbona. Nitrate and Nitrite contamination of water sources and fruit juices marketed in South-Eastern Nigeria, *U. I. J food comp Anal*. 2003; 16(2):213-218.
- Ayub Yarmohammadi, Maryam Mirlohi, Zamzam Paknahad, Zahra Gholi. Nitrite assessment in highly used processed meat products in growing age group in Isfahan city, *Int. J Environ. Health Eng*. 2015; 14(3):1-5.
- Mohammad Amer Zamrik. Determination of nitrate and nitrite contents of Syrian White Cheese, *Pharmacol. Pharm*. 2013; 4:171-175.
- Maria Luisa Cortesi, Lucia Vollano, Maria Francesca Peruzzi, Raffaele Marrone, Raffaelina Mercogliano. Determination of nitrate and nitrite levels in infant foods marketed in Southern Italy, *CyTa-J. Food*. 2015; 13(4):629-634.
- Usha Gundimeda, Naidu AN, Kamala Krishnaswamy. Dietary intake of nitrate in India, *Journal of Food Composition and Analysis, J Food Comp and Anal*. 1993; 6(3):242-249.
- Tannenbaum SR. Endogenous formation of N-Nitroso compounds: a current perspective, *Relevance of N-Nitroso Compounds to human cancer: Exposures and Mechanisms*, International agency for research on Cancer, Lyon, France, Publication. 1987, 84.
- Tao Rui. Spectrophotometric determination of nitrite situation Griess-Ilosvay reaction, *Chinese Journal of health laboratory technology Chin. J health lab technol*. 1992; 2:67-71.
- Angela Coss, Kenneth P Cantor, John S Reif, Charles L Lynch, Mary H Ward. Pancreatic Cancer and drinking water and dietary sources of Nitrate and Nitrite, *Am. J of Epidemiol*. 2004; 159(7):693-701.
- Dominique Pobel, Elio Riboli, Jacqueline Cornee, Bertrand Hemon, Monique Guyader, Bertrand Hemon, Monique Guyader. Nitrosamine, nitrate and nitrite in

- relation to gastric cancer: A case-study in Marseille, France, *Eur. J Epidemiol.* 1995; 11(1):67-73.
13. Kikugawa K, Kato. Formation of a mutagenic diazoquinone by interaction of phenol with nitrite, *T. Food Chem. Toxicol.* 1988; 26(3):209-214.
 14. Tricker AR, Preussmann. Carcinogenic N-nitrosamines in the diet: occurrence, formation, mechanisms and carcinogenic potential, *R. Mutat. Res.* 1991; 259:277-289.
 15. Siavash Nouroozi, Razieh Mirshafian. Flow injection kinetic spectrophotometric method for the determination of trace amount of nitrite, *Talanta.* 2009; 79(4):1149-1153.
 16. Koupparis MA, Walczak KM, Malmstadt HV. Kinetic determination of nitrite in waters by using a stopped-flow analyser, *Analyst* 1982; 107(1280):1309-1315.
 17. Oliveira SM, Lopes TIMS, Rangel AOSS. Spectrophotometric determination of nitrite and nitrate in cured meat by sequential Injection Analysis, *J food sci.* 2004; 69(9):C690-C695.
 18. Abbas Afkhami, Morteza Bahram, Somayeh Gholami Zahra Zand. Micell-mediated extraction for the spectrophotometric determination of nitrite in water and biological samples based on its reaction with p-nitro aniline in the presence of diphenylamine, *Anal. Biochem.* 2005; 336(2):295-299.
 19. Dayananda BP, Revanasiddappa HD. Determination of nitrites by the formation of diazo dye, *Chem. Pap.* 2007; 61(6):446-451.
 20. Badiadka Narayana, Kenchaiah Sunil. A Spectrophotometric method for the determination of nitrite and nitrate, *Eurasian J Anal Chem.* 2009; 4(2):204-214.
 21. Veena K, Narayana B. Spectrophotometric determination of nitrite using new coupling agents, *Indian J Chem Techn.* 2009; 16:89-92.
 22. Khadka Deba Bahadur, Duwadi Anjeeta. Spectrophotometric determination of trace amount of nitrite in water with 4-Aminophenylacetic acid and Phloroglucinol, *Int. Res. J Environ Sci.* 2014; 3(4):64-69.
 23. Kanchan Nemade, Sanjay Attarde. Development and validation of UV spectrophotometric method for simultaneous estimation of nitrite in water, soil, drug and vegetable samples, *Int. J Adv Chem.* 2014; 2(2):74-79.
 24. Sreekumar NV, Narayana B, Prashant Hegde, Manjunatha BR, Sarojini BK. Determination of nitrite by simple diazotization method, *Microchem. J.* 2003; 74(1):27-32.
 25. Bremner JM. Inorganic Forms of Nitrogen, *Methods of Soil Analysis, Part 2* (Edited by C.A. Black *et al.*) Madison, ASA and SSSA, Agronomy Monograph, 1965, 9.
 26. Alankar Shrivastava, Vipin Gupta B. Methods for the determination of limit of detection and limit of quantitation of the analytical methods, *Chron. Young Sci.* 2011; 2(1):21-25.