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A Highly Sensitive Spectrophotometric Method for the Micro level Determination of Nitrite and Nitrate anions by Coupling of Tetrazotized Benzidine and N,N-dimethylaniline

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Abstract

A simple, sensitive and rapid spectrophotometric method for the determination of anions, nitrite and nitrate has been described. Determination of nitrite is by means of reactions involving tetrazotisation of Benzidine, followed by coupling with N,N-dimethylaniline and determination of nitrate is mainly based on reduction of nitrate to nitrite by using granular Zinc in NaCl. The produced nitrite is then availed to tetrazotize the Benzidine, which is then coupled with N,N-dimethylaniline to form pinkish red colored an azo dye in acidic medium. Measured the absorbance of resulted colored dye solution at 538 nm. Amount of reagents required is optimised and the extent of tolerance limit by the diverse ions was investigated. The Beer-Lambert's linearity range for Benzidine- N,N-dimethylaniline couple was found to be 0.05 to 0.3µg/mL of nitrite with molar absorptivity $3.475\text{x}10^4 Lmol^{-1}cm^{-1}$ and sandell's sensitivity $3.3152\text{x}10^{-4}\mu gcm^{-2}$. The evaluated detection limit and quantization limit for the determination of nitrite were $0.00348\mu gmL^{-1}$ and $0.0105\ \mu gmL^{-1}$ respectively. The investigated method is useful for the determination of microgram quantity of anions nitrite and nitrate in water and soil specimens.

Keywords: Benzidine, tetrazotization,N,N-dimethylaniline, nitrite, nitrate, spectrophotometry.

Introduction

The anion Nitrite is a resourceful chemical species which have been utilising in many fields ranging from food preservation to manufacturing process of dyes. Nitrosamines are produced in the organs of human being through the reaction of nitrite with amines or amides[1], Nitrite is produced in water due to the deficiency of oxygen in natural layer of water. Deficiency of oxygen leads to the reduction of nitrates with the formation of nitrites[2]. Nitrite is also produced in water as a result of biodegradation of nitrate, ammonical nitrogen and nitrogenous organic matters[3]. Nitrite is one of the mineral ions present in the atmosphere and natural water[4] cause pollution and is an essential intermediate species in biochemical process of nitrogen cycle. Trace amount of nitrite and nitrate in potable water may lead to oxidation of iron in haemoglobin of the red blood cells to produce methanoglobimenia[5] which inhibits the oxygen transport to the cells is also called blue baby syndrome in infants and such a effect for long term is a risk of causing cancer. Various instrumental methods such as, voltammetry[6], fluorimetry[7], biamperometry[8] .polaraography[9] and flow injection spectrophotometry[10] have reported for the determination of nitrite. Few spectrophotometric methods also reported for determination of nitrite which are mainly based on diazotization and coupling reaction[11,12] extractions process of an azo dye into appropriate organic solvent provides an improved sensitivity and much lower detection limit[13]. Nitrate is a most possible impurity of surface and underground sources of water. Thus its detection and quantitative determination are considered as essential. The wide coverage on the revealing and their quantification has been briefed in review report by Moorcroft et al[14]. Many reported spectrophotometric methods mainly based on the two subsequent chemical reaction, viz a reduction reaction and diazotization[15,16], nitration reactions[17]. Other methods involve the use of specific ion electrodes[18] and ion chromatography[19]. The other known spectrophotometric methods developed for the determination of nitrate involves the nitration reaction of phenolic compounds[20], 2,4-xylenol[21],2,6-xylenol[22], 3,4-xylenol[23], phenoldisulphonicacid[24], brucine[25] and chromophoric acids[26]. Some more sensitive methods being reported by using extraction process of ion associates of the nitrate with dyes such as nile blue[27] and crystal violet[28].

In the present effort, a simple, sensitive and rapid process has been investigated for the determination of nitrite using N,N-dimethylaniline, which couples the tetrazotised benzidine in presence of sodium hydroxide to form a pinkish red colored dye solution in acidic medium showed an absorption maximum 538nm. Determination of nitrate also possible through the similar reaction preceded by the reduction of nitrate to nitrite in the presence of granular Zn in NaCl. The produced nitrite is availed to tetrazotize the benzidine and subsequently coupled with N,N-dimethylaniline to results an azo dye solution and its absorbance was recorded at 538nm. Success of this investigated method has been confirmed by applying the method on different samples containing known amount of nitrite and nitrate. The comparisons of analytical parameters of various reported similar methods for the estimation of nitrite with investigated method are given in table-1.

Table-1
Comparison of spectrophotometric methods for the determination of nitrite with proposed method

Reagent used	**Beer-Lambert's range (µgmL^{-1})**	**λ_{max} (nm)**	**Molar absorptivity (L mol^{-1}cm^{-1})**
Neutral red[29]	0-20	530	2.50x10^{4}
4-Amino benzoic acid + 8-hydroxy quinoline[30]	0.1-1.5	499	3.20x10^{4}
PNA+8-quinollinol[31]	0.01-0.06	550	3.88x10^{4}
4-Amiobenzotriflouride[32]	0.03-0.8	510	3.91x10^{4}
Barbituric acid[33]	0.00-3.22	310	1.53x10^{4}
4-Aminophenylaceticacid +1-Naphthol[34]	0.04-1.6	490	2.76x10^{4}
4-Aminosalicylic acid +1-Naphthol[35]	0.1-3.0	520	1.47x10^{4}
Phenolsafranine[36]	0-12	520	1.03x10^{4}
Sulfanilamide+EAA[37]	0.2-3.0	356	1.22X10^{4}
PNA+diphenylamine[38]	0.05-0.80	500	1.45x10^{4}
Leucocrystal violet[39]	0.004- 0.04	500	1.54x10^{4}
PNA+ Ethoxy ethylene maleic ester[40]	0.5-16	439	1.21x10^{4}
MMA+N,N-dimethylaniline[41]	0.05-2.0	482	2.03x10^{4}
Sulfanilic acid+Methylanthranilate[42]	0.2-8.0	493	1.03x10^{4}
PNA + Frusemide[43]	0.4- 2.0	688	3.314x10^{4}
4-Aminophenylacetic acid + Phloroglucinol[44]	0.04-1.8	420	2.40x10^{4}
Benzidine and N,N-dimethylaniline (present method)	0.05- 0.3	538	3.475x10^{4}

MMA:4(1-methyl-1-mesitylcyclobutan-3-yl)-2-aminothiazole; PNA: p-nitroaniline; EAA: ethyl acetoacetate

Material and Methods

Insrtuments: Jasco, model V-630, UV-Visible spectrophotometer with 1cm quartz cell and Digital pH meter model EQ-610 (Equip-tronics) was used.

Reagents and solutions: All chemicals used were with analytical grade purity, and solutions of them are prepared by using distilled water. 0.1500 g of sodium nitrite dissolved in water and diluted to 100 mL to prepare nitrite solution (1000 µgmL^{-1}). Dissolved 0.7220 g of potassium nitrate in water and diluted to 100 mL to prepare nitrate solution (1000 µgmL^{-1}). Operational standard solutions were also prepared by suitable dilution. N,N-dimethylaniline (0.5 mL in 10mL of 3N HCl diluted to 100 mL with water) and benzidine (0.5g in 10 mL of ethyl alcohol, diluted in water) were used. 1M HCl and 0.5 M NaOH were prepared by dissolving exact amounts of those substances.

Determination of Nitrite: Different aliquots of stock solution containing 0.05 to 0.3µgmL^{-1} of nitrite solution were transferred into the series of 25 mL standard flask. To every flask, 1 mL of 0.5% benzidine and 2 mL of 1M hydrochloric acid solutions were added and the solution was shaken occasionally for 3 to 4 minutes to allow the completion of tetrazotization reaction. Then, 1 mL of 0.5% N,N-dimethylaniline and 1 mL of 0.5M sodium hydroxide solutions was added to result an azo dye, it is diluted to 25 mL using water. Recorded the absorbance of the pinkish red colored dye solution at 538nm with reference to the corresponding reagent blank. Then by the calibration plot, results being evaluated which are given in the table-2.

Table-2
Determination of nitrite in water and soil samples

Proposed method				**Reported method[29]**			
Sample	**Nitrite Added µgmL^{-1}**	**Nitrite found µgmL^{-1}±SD^{a}**	**Recovery %**	**Nitrite found µgmL^{-1}±SD^{a}**	**Recovery %**	**t-test^{b}**	**F-test^{c}**
Water	0.1	0.098±0.004	98.0	0.096±0.004	96.0	0.66	1.72
	0.2	0.196±0.004	98.0	0.197±0.004	98.5	0.89	1.23
	0.3	0.292±0.03	97.33	0.294±0.005	98.0	1.49	1.02
Soil	5.0	4.90±0.04	98.0	4.92±0.04	98.84	0.66	1.72
	6.0	5.96±0.04	99.33	5.96±0.04	99.33	0.89	1.23
	8.0	7.95±0.05	99.37	7.92±0.05	99.00	0.74	1.26

a Mean± standard deviation (n=5) b Tabulated t-value for 8 degrees of freedom at P(0.95) is 2.65, c Tabulated F-value for(4,4) degrees of freedom at P(0.95) is 5.72

Determination of Nitrate: Pipetted out 10 mL of the nitrate stock solution in to a beaker, added 5 mL of concentrated HCl and 5mL of granular Zn in NaCl mixture, then it was allowed to stand for about 15 minutes with occasional stirring to reduce nitrate in to nitrite, then the solution was filtered through whatman No 41 in to a 100 mL standard flask and developed up to the mark. Series of aliquots of stock solution containing 0.088 to 0.6 μgmL^{-1} of reduced nitrate were transferred into the series of 25 mL standard flask. Added 1 mL of 0.5% benzidine and 1 mL of 1M HCl solution, shaken thoroughly for 5 minutes for the completion of tetrazotization reaction. Then, 1mL of 0.5% N,N-dimethylaniline and 1 mL of 0.5M sodium hydroxide solutions were added to appear an azo dye and the contents were diluted to 25 mL with water. The absorbance of the pinkish red colored dye solution was measured at 538 nm with reference to the corresponding reagent blank.

Determination of nitrite and nitrate in soil sample: A known amounts of nitrite and nitrate were added to about 1.0 to 2.0 g of soil specimen was taken in a glass vessel and repeatedly extracted with small fractions by volume of 0.5% solution of sodium carbonate and filtered by using Whatman no. 41filter paper. The obtained filtrate was made up to the mark in 25mL standard flask. Suitable aliquots of a known volume of the solution was taken into a 25 mL standard flask and estimated as per the proposed method. The results are shown in table-2 and table-3.

Results and Discussion

Absorption spectra: This process is mainly starts with tetrazotization of benzidine in acid medium by using nitrite, it follows the coupling reaction in presence of sodium hydroxide with N,N-dimethylaniline, which gives an azo dye providing pinkish red coloured solution in acidic medium shows absorption maximum at 538 nm. In the case of nitrate, it should be reduced to nitrite by reaction with granulated zinc in NaCl followed by the reaction steps followed in the case of nitrite. Tetrazotization and coupling reactions are favourably carried out at below 10°C as feasibility of these reactions are based on temperature. The absorption spectrum obtained for the colored azo dye solution is showed in figure-1 and the reaction is also described in the scheme -1 and scheme- 2.

Table-3
Determination of nitrate in water and soil samples

	Proposed method			Reported method[29]			
Sample	**Nitrite Added μgmL^{-1}**	**Nitrite found μgmL^{-1}±SD[a]**	**Recovery %**	**Nitrite found μgmL^{-1}±SD[a]**	**Recovery %**	**t-test[b]**	**F-test[c]**
Water	5.0	4.93±0.04	98.6	4.94±0.04	98.8	0.66	1.72
	6.0	5.94±0.04	99.0	5.96±0.04	99.33	0.89	1.23
	8.0	7.95±0.03	97.37	7.95±0. 05	99.37	1.49	1.02
Soil	5.0	4.90±0.04	98.0	4.92±0.04	98.84	0.66	1.72
	6.0	5.96±0.04	99.33	5.96±0.04	99.33	0.89	1.23
	8.0	7.95±0.05	99.37	7.92±0.05	99.00	0.74	1.26

a Mean± standard deviation (n=5) b Tabulated t-value for 8 degrees of freedom at P(0.95) is 2.65, c Tabulated F-value for(4,4) degrees of freedom at P(0.95) is 5.72

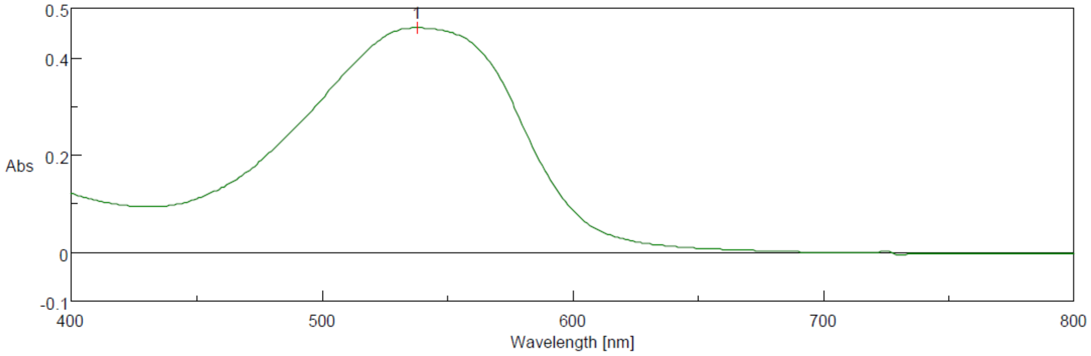
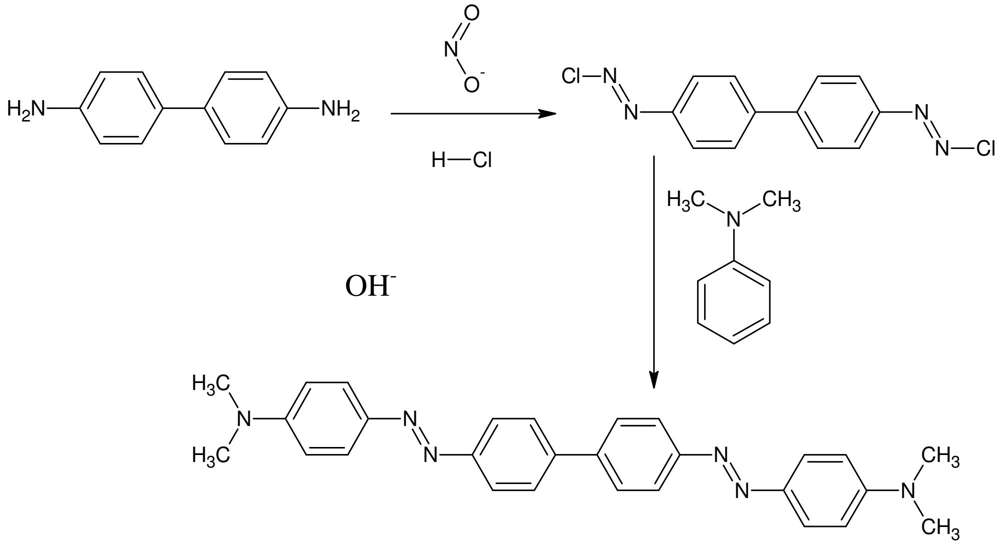


Figure-1
Absorption spectrum of the azo dye Benzidine- N,N-dimethylaniline

$$NO_3^- + Zn + 2H^+ \xrightarrow{\quad} NO_2^- + Zn^{2+} + H_2O$$

Scheme-1
Reaction scheme of reduction of nitrate to nitrite



Scheme-2
Tetrazotization of benzidine followed by coupling with N,N-dimethylaniline

Effect of variation in reagent concentration: A quantity of 1 mL of 0.5% benzidine solution and the addition of 1 mL of N,N-dimethylaniline (0.5%) reagent showed maximum absorbance. The variation in reagent concentration to the larger extent produced no further increasing trend in the absorbance. It is found that tetrazotization and coupling reactions are feasible only at lower temperature. Tetrazotization is carried out in lower temperature (about 0-5^0C) and coupling reaction was carried out at ambient temperature, above 35°C there was a gradual diminish in intensity of the colour. The increase in the concentration of HCl above 1M to 3 M does not vary the absorbance.

Data of analytical parameters: In this process validity of the Beer's law is evaluated from the absorbance values of resultant solution by varying the concentration of nitrite. The linear plot of absorbance verses amount of nitrite shows that Beer's law is obeyed in the micro gram range 0.05 to 0.3μgmL^{-1}of amount of nitrite. figure-2 presents the validity of Beer's law plot for the estimation of nitrite using benzidine and N,N-dimethylaniline. The evaluated molar absorptivity and Sandell's sensitivity values of the process are found to be 3.475×10^4 L mol^{-1} cm^{-1} and 3.3152×10^{-4} μgcm^{-2}. From the values 'slope of the calibration curve' (S) and standard deviation of the regent blank (n=5) (σ) The detection limit (DL=3.3σ/S) and quantization limit (QL=10σ/S) were also evaluated for the nitrite estimation, which are 0.00348μgmL^{-1} and 0.0105 μgmL^{-1} respectively.

Study on interference of diverse ions: Investigated the effect of various undesired cations and anions on the estimation of desired anions nitrite and nitrate by means of tolerance ability of interfering ions at those concentrations that will results within ± 5% variation in absorbance values when fixed amount of nitrite (2μgmL^{-1}) and nitrate (2.6 μgmL^{-1}) were taken. The results are given in table-4. The studies showed that interference of most of the diverse ions are negligibly small except Cerium (Ce^{II}) and Mercury (Hg^{II}) caused relentless effect on absorbance, but the tolerance ability by such interfering ions is enhanced by the pre-treatment with 2 or 3 mL of 2 or 1% solution of disodium salt of EDTA.

Table-4
Effect of diverse ion on the determination of nitrite (μgm L^{-1}) by using benzidine and N,N-imethylaniline as coupling agents

Diverse ions	Tolerance limit (μgmL^{-1})	Diverse ions	Tolerance limit (μgmL^{-1})
Al(III)	46	Mg(II)	60
As(V)	34	Mo(VI)	40
Ba(II)	60	Ni(II)	30
Bi(III)	40	Pb(II)	180
Ca(II)	50	Sn(II)	20
Ce(III)	44	Sr(II)	25
Cd(II)	40	Ti(IV)	30
Co(II)	56	Th(IV)	28
Cr(VI)	38	Zr(IV)	40
Cu(II)	32	Acetate	160
Fe(III)	20	Bromide	120
Hg(II)	10	Citrate	130
In(III)	52	Chloride	140
Ir(III)	28	Fluride	160
Mn(II)	30	Nitrate	160
Rh(III)	16	Phosphate	170
Pd(II)	19	Oxalate	130
Tl(III)	110	Sulphate	200

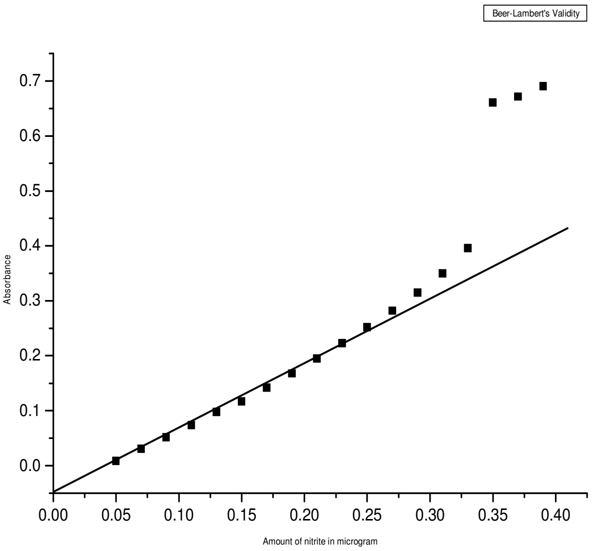


Figure-2
Beer-Lambert's validity plot for nitrite determination

Applications: Quantitative determinations of nitrite and nitrate in water and soil specimens by applying the investigated method were very successful. The success of the method is evident from comparison of the results by statistical approach student's t-test and F-tests, which reveals that, there is no much considerable difference in accuracy and precision among the investigated and reported method[29]. Subjected samples consisting nitrite and nitrate at three dissimilar concentrations by replicate analysis to the investigated method and precision of this method is evaluated (table-2 and 3). The reagents used are suitable for the spectrophotometric method for determination of nitrite and nitrate present in most possible contaminated samples in microgram level.

Conclusion

The proposed organic reagents are readily available and suitable for the estimation of anions, nitrite and nitrate with the advantage of high sensitivity and low absorbance of a reagent blank. The proposed method does not require any tedious chemical reaction conditions and provides the superiority of colour stability for about 14 hours. The investigated method has been successfully adopted for quantification of micro quantity of nitrite and nitrate in water and soil specimens.

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