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A Spectrophotometric Method for the Determination of Nitrite and Nitrate

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Abstract

A simple, rapid spectrophotometric method for the determination of nitrite and nitrate in water, soil and pharmaceutical preparation samples has been developed. Determination of nitrite is based on the reactions involving sulfanilic acid with methyl anthranilate as the coupling agents and determination of nitrate is based on their reduction to nitrite in the presence of Zn/NaCl. The produced nitrite is subsequently diazotized with sulfanilic acid then coupled with methyl anthranilate to form an azo dye which is measured at 493 nm. The method is optimized for acidity, amount of reagents required and tolerance amount of other ions. The range of linearity for sulfanilic acid-methyl anthranilate couple was found to be 0.2-8.0 µg/mL of nitrite with molar absorptivity be $1.03x10^4$ $Lmol^{-1}cm^{-1}$ and sandell's sensitivity $4.5x10^{-3}$ µg cm^{-2} . The detection limit and quantitation limit of the nitrite determination are found to be 0.93 μgmL^{-1} and 2.82 μgmL^{-1} respectively. This method has been successfully applied to the determination of trace amounts of nitrite and nitrate in water, soil and pharmaceutical preparations.

Keywords:

Spectrophotometry; Diazotization; Nitrite; Nitrate; Methyl anthranilate; Isosorbide

1. Introduction

Nitrite is a versatile chemical agent which has found numerous applications ranging from dye manufacture to food preservation. It produces carcinogenic nitrosamines in the human body through its reaction with amines or amides [1]. Nitrite is one of the pollutants found in the atmosphere and natural water [2] and is an important intermediate in biological nitrogen cycle. Traces of nitrite and nitrate in drinking water may lead to mathemeglobmenia in infants and with long term exposure is a possible cancer risk. Various instrumental methods such as polarography [3], voltammetry [4], fluorimetry [5], biamperometry [6] and flow injection spectrophotometry [7] have been used for nitrite determination. Nitrite is determined spectrophotometrically based on diazo coupling reaction [8, 9] extraction of the azo dye into suitable organic solvent provides a much lower detection limit and improved sensitivity [10].

Nitrate is a well-known contaminant of ground and stream water. It is an important environmental and human health analyte, and thus its detection and quantification are considered to be essential. An excellent review on the detection and determination has been reported by Moorcroft et al [11]. Most of the recent work concerning nitrate determination has embraced the classical reagents. Several reported spectrophotometric methods involve the use of common reactions, such as a reduction reaction followed by diazotization, [12, 13] nitration reactions, [14] or others [15,16]. Other methods involve the use of ion chromatography [17] and specific ion electrodes [18]. The well-known spectrophotometric

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methods for the determination of nitrate are based on the nitration of phenolic compounds [19], chromophoric acids [20], 2,4-xylenol [21], 2,6-xylenol [22], 3,4-xylenol [23], phenoldisulfonic acid [24], brucine[25] and phenol [26] 4-aminoazobenzene [27]. Some sensitive spectrophotometric methods for determine nitrate utilize extractable ion associates of the nitrate ion with basic dyes, like crystal violet [28] and nile blue [29].

In this work, a simple and rapid method has been proposed for the determination of nitrite using methylanthranilate as a coupling agent. Sulfanilic acid was diazotized in acidic medium and coupled with methylanthranilate to give a colored dye having absorption maximum at 493 nm. Determination of nitrate is based on the reduction of nitrate to nitrite in the presence of Zn/NaCl. The produced nitrite is subsequently diazotized with sulfanilic acid and then coupled with methylanthranilate to form an azo dye and was measured at 493 nm. The developed method has been successfully applied to the determination of nitrite and nitrate in different samples. The comparisons of spectrophotometric methods for the determination of nitrite with proposed method are summarized in Table 1.

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

Reagent Remarks	Ref. No	Range $\mu g\ mL^{-1}$	λmax nm	Molar absorptivity ($L\ mol^{-1}\ cm^{-1}$)	Remarks
Neutral red	[30]	0-20	530	2.50×10^4	Extractive and common ions interfere.
Leucocrystal violet	[31]	0.004-0.04	500	1.54×10^4	Less stable and less detection limit
Phenosafranine	[32]	0-12	520	1.03×10^4	Less sensitive.
PNA+ diphenylamine	[33]	0.05-0.80	500	1.45×10^4	Time consuming and less sensitive
PNA+8 quinollinol	[34]	0.01-0.06	550	3.88×10^4	pH dependent and time consuming
MMA+ N,N dimethy- and Aniline	[35]	0.05-2.0	482	2.03×10^4	pH dependent and time consuming low detection limit
SA+EAA	[36]	0.2-3.0	356	1.22×10^4	Less sensitive and less stable.
Sulfanilic acid + Methyl antranilate	[present method]	0.2-8.0	493	1.03×10^4	Simple, rapid, non extractive, high sensitive and stable.

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

2. Experimental

2.1 Apparatus

A SHIMADZU (Model No: UV-2550) UV-Visible spectrophotometer with 1 cm matching quartz cells were used for the absorbance measurements. A WTW pH 330 pH meter was used.

2.2 Reagents and Solutions

All chemicals used were of analytical reagent grade, and doubly distilled water was used in the preparation of all solutions in the experiments. Nitrite solution (1000 μgmL^{-1}) was prepared by dissolving 0.1500 g sodium nitrite in water and diluting to 100 mL. Nitrate solution (1000 μgmL^{-1}) was prepared by dissolving 0.7220 g potassium nitrate in water and diluting to 100 mL. Working standard solutions were prepared by appropriate dilution. Sulfanilic acid (0.5 g in 100 mL water) and methyl anthranilate (0.5 mL in 100 mL of alcohol) were used. The following reagents were prepared by dissolving appropriate amounts of reagents in water: 2 mol L^{-1} HCl & 2 mol L^{-1} NaOH.

2.3. Procedure

2.3.1. Nitrite determination

Aliquots of stock solution containing 0.2-8.0 μgmL^{-1} of nitrite were transferred in to series of 10 mL calibrated flask. To each flask, 1 mL of 0.5% sulfanilic acid and 1 mL of 2 mol L^{-1} hydrochloric acid solution were added and the solution was shaken thoroughly for 5 min to allow the diazotization reaction to go to completion. Then, 1 mL of 0.5% methyl anthranilate and 2 mL of 2 mol L^{-1} sodium hydroxide solution were added to form an azo dye and the contents were diluted to 10 mL using water. After dilution to 10 mL with water, absorbance of the red colored dye was measured at 493 nm against the corresponding reagent blank and the calibration graph was constructed. The results are summarized in Table 2.

Table 2: Determination of nitrite in water and soil samples using methyl anthranilate as a reagent

Sample	Nitrite added μgmL^{-1}	Proposed method		Reported method [30]		t-test[b]	F-test[c]
		Nitrite found in $\mu gmL^{-1} \pm$ SD[a]	Recovery %	Nitrite found in $\mu gmL^{-1} \pm$ SD[a]	Recovery %		
Tap water	2.0	1.99 ± 0.04	99.50	1.98 ± 0.03	99.00	0.56	1.80
	4.0	4.01 ± 0.04	100.75	4.01 ± 0.05	100.75	0.57	1.71
	6.0	5.98 ± 0.03	99.66	6.01 ± 0.03	100.16	1.49	1.12
River water	2.0	1.99 ± 0.04	99.50	2.01 ± 0.04	100.50	0.56	1.28
	4.0	3.99 ± 0.04	99.75	3.99 ± 0.04	99.75	0.56	1.23
	6.0	5.99 ± 0.03	99.83	5.99 ± 0.05	99.83	0.74	1.97
Ground water	2.0	1.98 ± 0.03	99.00	1.99 ± 0.04	99.50	1.49	1.80
	4.0	4.01 ± 0.05	100.25	4.01 ± 0.04	100.25	0.44	1. 71
	6.0	6.01 ± 0.03	100.16	5.98 ± 0.03	99.66	0.74	1.12
Soil Sample	2.0	2.01 ± 0.04	100.50	1.99 ± 0.04	99.50	0.57	1.28
	4.0	3.99 ± 0.04	99.75	3.99 ± 0.04	99.75	0.56	1.23
	6.0	5.99 ± 0.05	99.83	5.99 ± 0.03	99.83	0.45	1.97

[a] Mean ± Standard deviation (n = 5)
[b] Tabulated t-value for 8 degrees of freedom at P(0.05) is 2.65
[c] Tabulated F- value for (4,4) degrees of freedom at P (0.05) is 5.72

2.3.2. Nitrate determination

Pipetted out 10 mL of nitrate stock solution to a beaker, added 5 mL of Conc. HCl and 2 mL of Zn/NaCl granular mixture0, and was allowed to stand for 30 minutes. with

occasionally stirring to form nitrite, then the solution was filtered to 100 mL standard flask using Whatman No 41 filter paper and diluted up to the mark. Aliquots of stock solution containing 0.26-10.7 μgmL^{-1} of reduced nitrate were transferred in to series of 10 mL standard flask. Added 1 mL of 0.5% sulfanilic acid and 1 mL of 2 mol L^{-1} HCl solution, shaken thoroughly for 5 minutes for the diazotization reaction to go to completion. Then, 1 mL of 0.5% methyl anthranilate and 2 mL of 2 mol L^{-1} sodium hydroxide solution were added to form an azo dye and the contents were diluted to 10 mL with water. After dilution to 10 mL with water, the absorbance of the red colored dye was measured at 493 nm against the corresponding reagent blank. The results are summarized in Table 3.

Table 3: Determination of nitrate in water and soil samples using methyl anthranilate as a reagent

Sample	Nitrite added μgmL^{-1}	Proposed method		Reported method [30]		t-test[b]	F-test[c]
		Nitrite found in $\mu gmL^{-1} \pm$ SD[a]	Recovery %	Nitrite found in $\mu gmL^{-1} \pm$ SD[a]	Recovery %		
Tap water	4.0	3.90 ± 0.04	97.50	3.93 ± 0.04	98.25	0.66	1.71
	6.0	5.91 ± 0.04	98.50	5.96 ± 0.04	99.33	0.89	1.23
	8.0	7.92 ± 0.03	99.00	7.95 ± 0.05	99.37	1.49	1.02
River water	4.0	3.92 ± 0.04	98.00	3.90 ± 0.04	97.50	0.46	1.28
	6.0	5.93 ± 0.04	98.83	5.91 ± 0.04	98.50	1.06	1.81
	8.0	7.95 ± 0.03	99.37	7.92 ± 0.03	99.00	0.74	1.26
Ground water	4.0	3.96 ± 0.03	99.00	3.92 ± 0.04	98.00	1.49	1.80
	6.0	5.92 ± 0.05	98.66	5.93 ± 0.04	98.83	0.56	1. 71
	8.0	7.92 ± 0.03	99.00	7.95 ± 0.03	99.37	0.89	1.12
Soil Sample	4.0	3.93 ± 0.04	98.25	3.96 ± 0.03	99.00	0.87	1.58
	6.0	5.96 ± 0.04	99.33	5.92 ± 0.05	98.66	1.49	1.81
	8.0	7.95 ± 0.05	99.37	7.92 ± 0.03	99.00	0.56	1.26

[a] Mean ± Standard deviation (n = 5)
[b] Tabulated t-value for 8 degrees of freedom at P(0.05) is 2.65
[c] Tabulated F- value for (4,4) degrees of freedom at P (0.05) is 5.72

2.4. Nitrite and Nitrate Determination in Soil Sample

About 1.0 g of soil sample was taken in a 25 mL beaker and extracted with three mL portions of 0.5% sodium carbonate solution. The extract was filtered through Whatman no. 41 filter paper. The filtrate were collected and diluted to 25 mL. Appropriate aliquots of 1-2 mL of the solution was transferred in to a 10 mL calibrated flask and analyzed according to the general procedure. They tested negative. To these samples known amounts of nitrite and nitrate sample were added and analyzed for nitrite and nitrate following the proposed procedure. The results are summarized in Table 2 and 3.

2.5 Nitrate Determination in Pharmaceutical Samples

Isosorbide dinitrate (0.05 g) sample was taken, dissolved in water and clear solution was made up to 100 mL. Known amount of this solution was taken and analyzed for nitrate content following the procedure described for the analysis of water sample. Isosorbide mononitrate sample was also analyzed by the same procedure. The results are summarized in Table 4.

Table 4: Determination of nitrate in pharmaceutical preparations using sulfanilic acid-Methyl anthranilate as reagents

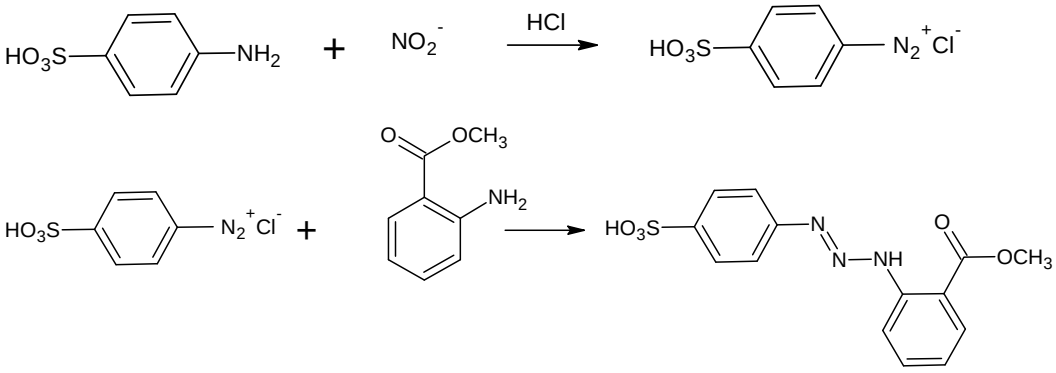
Sample	Nitrate added µgmL^{-1}	Proposed method		Reported method [30]		t-test[b]	F-test[c]
		Nitrate found in µgmL^{-1}± SD[a]	Recovery %	Nitrate found in µgmL^{-1}± SD[a]	Recovery %		
ID[d]	--	1.93 ± 0.05	--	1.94 ± 0.05	--	0.44	1.04
(0.05 g)	5	6.92 ± 0.05	98.00	6.94 ± 0.06	99.50	0.89	1.44
IM[e]	--	1.21 ± 0.04	--	1.19 ± 0.04	--	0.55	1.06
(0.05 g)	5	6.20± 0.03	98.50	6.17 ± 0.04	96.50	0.74	1. 77

[a] Mean±Standard deviation (n = 5). [b] Tabulated t-value for 4 degrees of freedom at P(0.05) is 2.78.[c] Tabulated F44 degrees of freedom at P (0.05) is 5.72. [d] Sample taken 2 µg nitrate (certified). Nichoals Piramal Intra Limited, Himachal pradesh. [e] Sample taken 1.25 µg nitrate (certified). Sun pharmacetical Industries, India.

3. Results and Discussion

3.1. Absorption Spectra

This method is based on the diazotization of sulfanilic acid in acid medium followed by the coupling with methyl anthranilate in alkaline medium, which gives an azo dye with absorption maximum at 493 nm. Diazotization and coupling reactions are found to be temperature dependent. The absorption spectrum of the colored species of azo dye is presented in Fig. 1 and the reaction system is presented in the following scheme.



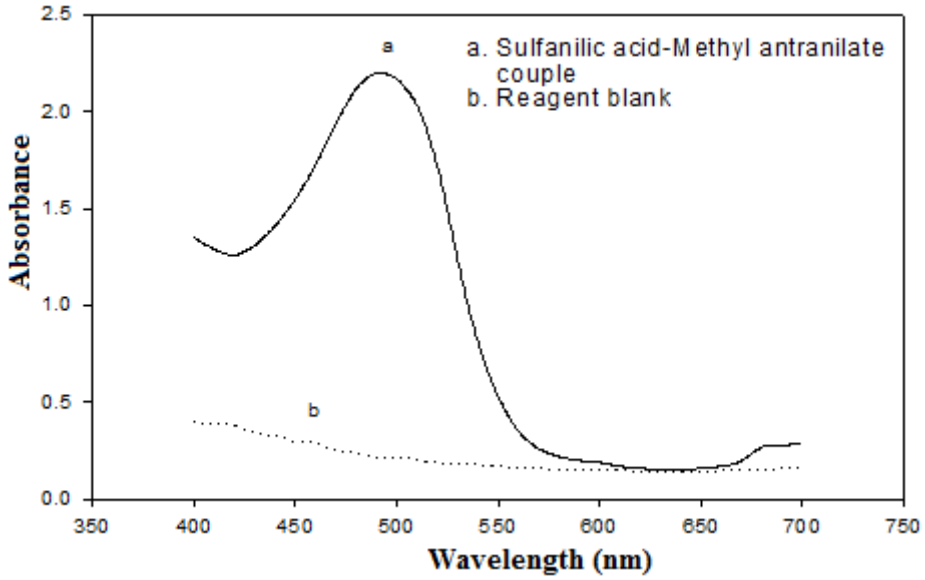


Fig.1. (a) Absorption spectra of azo dye; sulfanilic acid -methyl anthranilate couple and (b) reagent blank vs distilled water

3.2. Effect of Reagent Concentration

A volume of 1 mL of 0.5% sulfanilic acid solution was required for maximum absorbance (Fig.2) and it is found that, addition of 1 mL of methyl anthranilate (0.5%) reagent provides maximum absorbance (Fig.3) The use of larger excess of reagent produced no further increase in the absorbance. Diazotization and coupling reactions are found to be temperature dependent. Diazotization is carried out in cold condition (about 0-5^{0}C) and coupling reaction was carried out at room temperature, above 35^{0}C there was a decrease in intensity of the color (Fig.4). When the acid concentration is increased above 2 mol L^{-1} does not affect the absorbance.

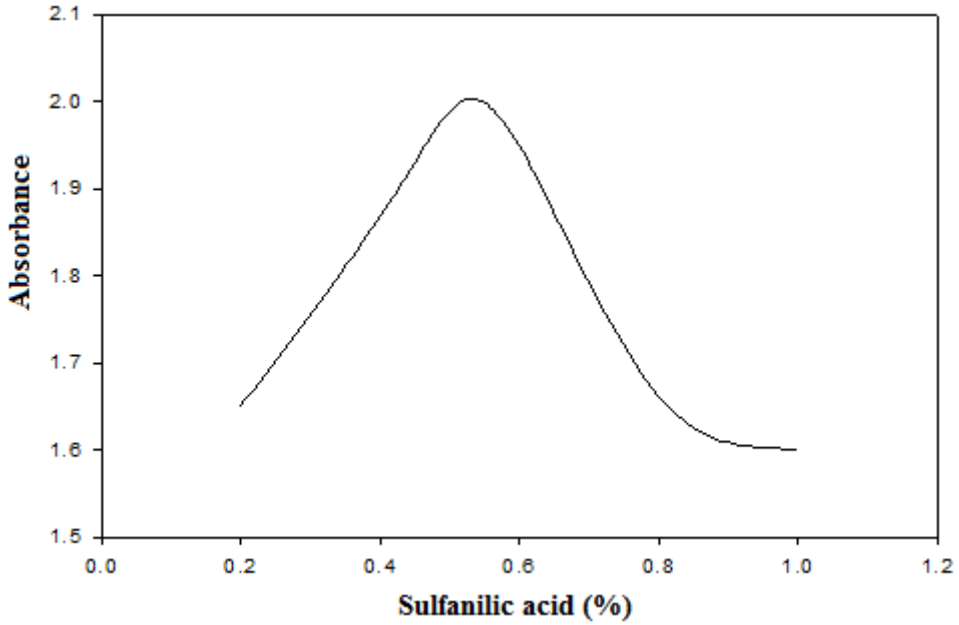


Fig.2: Absorption spectra; Variation of Sulfanilic acid

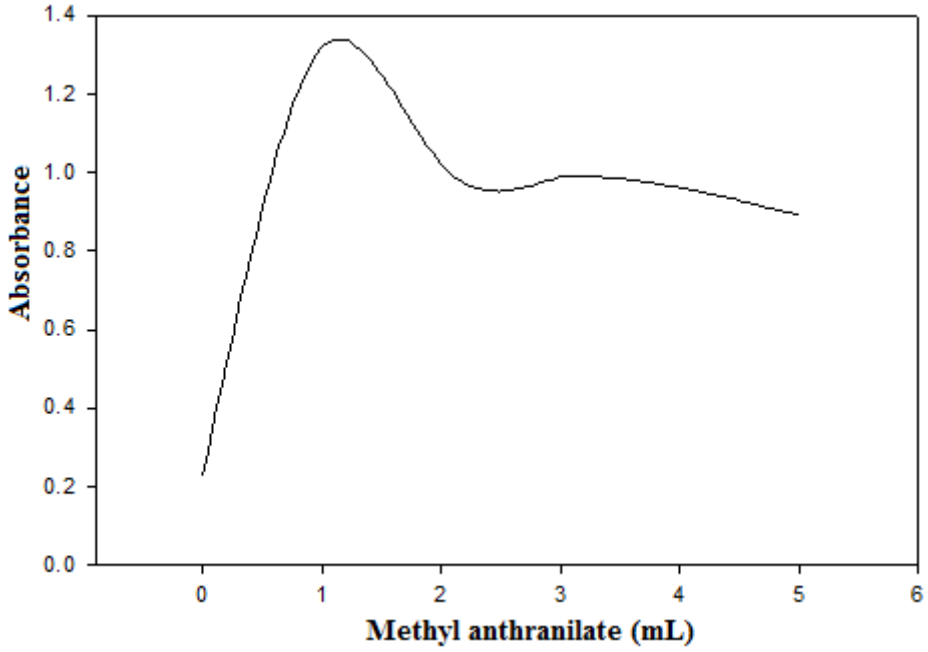


Fig.3. Absorption spectra; Variation of Methyl anthranilate

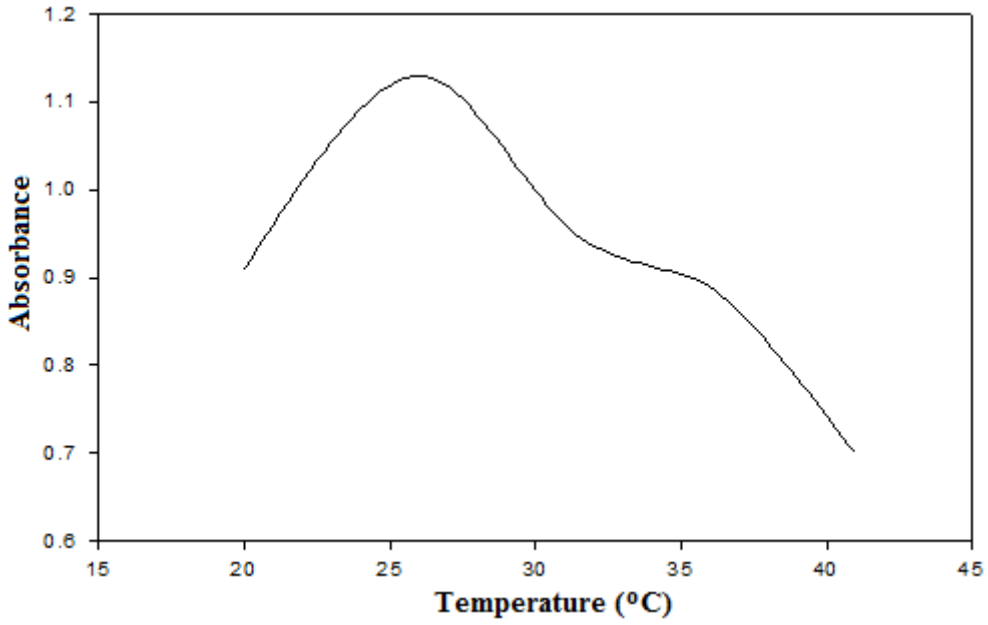


Fig.4. Absorption spectra; Variation of Temperature

3.3. Analytical Data

In this method adherence to Beer's law is studied by measuring the absorbance values of solutions varying nitrite concentration. A straight line graph is obtained by plotting absorbance against concentration of nitrite. Beer's law is obeyed in the concentration range 0.2–8.0 μgmL^{-1} of nitrite. Adherence to Beer's law graph for the determination of nitrite using methyl anthranilate is presented in Fig.5. The molar absorptivity and Sandell's sensitivity of the method is found to be $1.03x10^4$ L mol^{-1} cm^{-1} and $4.5x10^{-3}$ μgcm^{-2}. The correlation coefficient, detection limit (D_L=3.3σ/S) and quantitation limit (Q_L=10σ/S) (where σ is the standard deviation of the regent blank (n=5) and 'S' is the slope of the calibration curve) of the nitrite determination are found to be 0.992, 0.93 μgmL^{-1} and 2.82 μgmL^{-1} respectively.

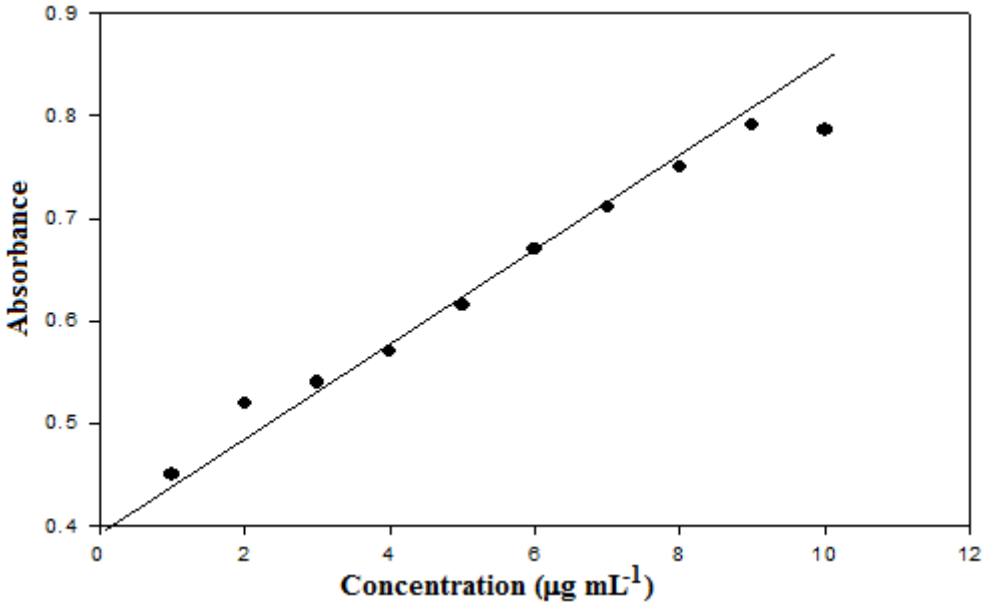


Fig.5. Adherance to beer's law for the determination of nitrite using methyl anthranilate as a reagent

3.4. Effect of Divers Ions

The effect of various non-target species on the determination of nitrite and nitrate were investigated. The tolerance limits of interfering species were established at those concentrations that do not cause more than ± 5% error in absorbance values of nitrite (2 μgmL^{-1}) and nitrate (2.6 μgmL^{-1}) with fixed concentration. The studies revealed that Ce(IV) and Hg(II) showed severe interference. However, the tolerance levels of these ions are increased by the addition of 3 mL of 2% EDTA. The results are given in table 5.

Table 5. Effect of diverse ions on the determination of nitrite (2 μgml^{-1}) and nitrate (2.6 μgml^{-1}) using methyl antranilate as a reagent

Diverse ions	Tolerance limit (μgmL^{-1})
Al^{3+}	300
Ba^{2+}	200
Ca^{2+}	500
Cd^{2+}	200
Ce^{4+}*	25
$CHCOO^{-}$	>2000
citrate	800
Cu^{2+}*	25
Fe^{3+}*	25
Hg^{2+}*	25
K^{+}	>2000
Mg^{2+}	500
Mn^{2+}	500
Mo^{6+}*	25
Na^{+}	>2000
oxalate	800
Sn^{2+}*	25
Pb^{2+}*	25
tartarate	800
W^{6+}*	25

* Masked by EDTA masking agent

4. Applications

The proposed method is applied to the quantitative determinations of nitrite and nitrate in soil and water samples. Statistical analyses of the results by t- and F-tests show that, there is no significant difference in accuracy and precision of the proposed and reported method [30]. The precision of the proposed method is evaluated by replicate analysis of samples containing nitrite and nitrate at five different concentrations (Table 2 and 3).The reagents provide a simple and sensitive method for the spectrophotometric determination of nitrite and nitrate. The proposed method has been successfully applied to the determination of trace amounts of nitrite and nitrate in soil and water samples. This method has (Table 2 and 3) been successfully applied to the determination of trace amounts of nitrate in pharmaceutical preparations.

5. Conclusions

The reagents provide a simple and sensitive method for the spectrophotometric determination of nitrite and nitrate. The reagents have the advantage of high sensitivity and

low absorbance of reagent blank. The developed method does not involve any stringent reaction conditions and offers the advantages of color stability about more than 4 hours. The proposed method has been successfully applied to the determination of trace amounts of nitrite and nitrate in soil and water samples. The proposed method has been successfully applied to the determination of trace amounts of nitrate in pharmaceutical preparations.

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References

1. Ensafi A A, Rezaei B, Nouroozi S (2004) Simultaneous spectrophotometric determination of nitrite and nitrate by flow injection analysis Anal. Sci. 20: 1749.
2. Manzoori J L, Sorouraddin M, Haji-Shabani A M (1998) Spectrophotometric determination of nitrite based on its catalytic effect on the oxidation of carminic acid by bromate. Talanta, 46: 1379.
3. Sabharwal S (1990) Determination of nitrite ion by differential-pulse polarography using *N*-(1-naphthyl) ethylenediamine. Analyst, 115: 1305.
4. Vandenberg C M G, Li H (1988) The determination of nanomolar levels of nitrite in fresh and sea water using cathodic stripping voltammetry. Anal. Chim. Acta, 212: 31.
5. Diallo S, Bastard P, Prognon P, Dauphin C, Hamon M (1996) A new spectrofluorimetric microdetermination of nitrite in water after derivatization with 4-methyl-7-aminocoumarin. Talanta, 43: 359.
6. Jaim L, Maria O R (1993) Determination of nitrite ions in well water by biamperometric standard addition Electroanalysis, 5: 251.
7. Chaurasia A, Verma K K (1994) Flow injection spectrophotometric determination of nitrite. Talanta, 41: 1275.
8. Saltzman B E (1954) Colorimetric microdetermination of nitrogen dioxide in the atmosphere. Anal. Chem., 26: 1949.
9. Kumar B S M, Srikanth T R, Balasubramanian N (1993) Spectrophotometric determination of nitrogen dioxide in air. Fresenius J. Anal. Chem. 345: 592.
10. Shanthi K, Balasubramanian N (1994) Spectrophotometric determination of nitrite and nitrate. J. AOAC Int'l. 77: 1639.
11. Moorcroft M J, Devis J, Compton R G (2001) Detection and determination of nitrate and nitrite: a review Talanta, 54: 785.
12. Horita K, Satake M (1997) Column preconcentration analysis spectrophotometric determination of nitrate and nitrite by a diazotization–coupling reaction. Analyst, 122: 1569.
13. Wang G F, Satake M, Horita K (1998) Spectrophotometric determination of nitrate and nitrite in water and some fruit samples using column preconcentration. Talanta, 46: 671.
14. Hartley A M, Asai R I (1963) Spectrophotometric determination of nitrate with 2,6,-xylenol reagent Anal. Chem. 35: 1207.

15. Bastian R, Weberling R, Palilla F (1957) Ultraviolet spectrophotometric determination of nitrate. Application to analysis of alkaline carbonates Anal. Chem. 29: 1795.
16. Borcherding R, Leiukefeld S, Frey C, Diekmann S, Steinrucke P (2000) Determination of low-abundant metabolites in plant extracts by nad(p)h fluorescence with a microtiter plate reader Anal. Biochem. 281: 1.
17. Crowther D, Monaghan J M, Cook K, Gara D (1956) Nitrate and nitrite determination in complex matrices by gradient ion chromatography. Anal. Commum. 33: 51.
18. Keeney D R, Byrnes B H, Genson J J (1970) Determination of nitrate in waters with the nitrate-selective ion electrode Analyst, 95: 383.
19. Williams W. J, *Handbook of Anion Determination* (Butterworths, London,119. 1979)
20. West P W, Ramachandran T P (1966) Spectrophotometricdetermination of nitrate using chromotropic acid. Anal. Chem. Acta. 35: 317.
21. Norwitz G, Keliher P N (1978) Inorganic interferences in the 2,4-xylenol spectrophotometric method for nitrate and their elimination. Anal. Chem. Acta. 98: 323.
22. Andrews D W W (1964) A sensitive method for determining nitrate in water with 2,6-xylenol. Analyst, 89: 730.
23. Osibanjo D, Ajayi S O (1980). Rapid and sensitive spectrophotometric method for the determination of nitrate in rain water using 3, 4-xylenol Analyst, 105: 908.
24. Hora F D, Webber P J (1960) A source of serious error in the determination of nitrates by the phenoldisulphonic acid method and its remedy. Analyst, 85: 567.
25. Jenkins D, Medsker L L (1964) Brucine method for determination of nitrate in ocean, estuarine, and fresh waters. Anal. Chem. 36: 610.
26. Velghe N, Claeys A (1983) Rapid spectrophotometric determination of nitrate with phenol. Analyst, 108: 1018.
27. Dayananda B P, Revanasiddappa H D (2007) Determination of nitrites by the formation of bisazo dye. Chem. Pap., 61: 446
28. Baca P, Freiser H (1977) Determination of trace levels of nitrates by an extraction-photometric method. Anal.Chem. 49: 2249.
29. Pokorny G, Likussar W (1968) Über die verwendung von oxazinen in der analytik Mitteillung. nilblau a zur bestimmung von nitrat. Anal. Chem. Acta. 42: 253.
30. Gayathri N, Balasubramanian N (1999) Spectrophotometric determination of nitrogen dioxide, nitrite and nitrate with Neutral Red. Analusis, 27: 174.
31. Sukumar C, Sunitha M B, Gupta V K (2004) Spectrophotometric determination of nitrite. J. Indian Chem. Soc. 81: 522.
32. Geetha K, Balasubramanian N (2001) A facile analytical method for the determinationof nitrite in water and soil samples. Chem Anal. (Warsaw), 46: 579.
33. Afkhami A, Masahi S, Bahram M (2004) Spectrophotometric determination of nitrite based on its reaction with p-Nitroaniline in the presence of diphenylamine in micellar media. Bull. Kor. Chem. Soc. 25: 1009.
34. Chaube A, Baveja A K, Gupta V K (1982) Extractive spectrophotometric determination of nitrite in polluted waters Anal. Chem. Acta, 143: 273.

35. Ozmen H, Polat F, Cukurovali A (2006) Spectrophotometric determination of nitrite in water samples with 4-(1-methyl-1-mesitylcyclobutane-3-yl)-2-aminothiazole.Anal. Lett. 39: 823.

36. Sreekumar N V, Narayana B, Prashant H, Manjunatha B R, Sarojini B K (2003) Determination of nitrite by simple diazotization method. Microchem. J. 74: 27