Journal of Environment and Earth Science
ISSN 2224-3216 (Paper) ISSN 2225-0948 (Online)
Vol.3, No.6, 2013- Selected from International Conference on Recent Trends in Applied Sciences with Engineering Applications

Determination of Nitrate in Polluted Water with New Coupling Reagent Hydroxamic Acids : A Rapid and High Potential Reagent for Trace Determination of Nitrate in Polluted Water

Seema Singh*, Jeena Harjit*, H.C. Kataria** and Sulbha Amlathe***
* T.I.E.I.T, Karond Gandhinagar Bhopal (M.P)-462038
** Govt.Geetanjali Girls P.G College Bhopal (M.P)-462038
*** Supervisor, B.U.I.T, Hoshangabad Road Bhopal (M.P)-462026
* Corresponding Author. jeena_harjit@rediffmail.com

Abstract
A rapid, stable and direct visible spectrophotometric method, based on the quantitative nitration of hydroxamic acids in concentrated sulphuric acid, is described for determination of nitrate in water samples from different sites of Bhopal. The nitration product was extracted in n-hexane and yellow complex formed was determined at 462 nm, 458 nm and 450 nm for N-PBHA, N-PClPBHA and N-PClPCHA, respectively. The calibration graph was linear over the range of 0.0001965-0.002751, 0.001965-0.03931 and 0.001965-0.02554 µg/25 ml for N-PBHA, N-PClPBHA and N-PClPCHA, respectively. The molar absorptivities were found to be $3.81x10^6$, $2.65x10^5$ and $4.11x10^5$ L mol^{-1} cm^{-1} for the above three hdroxamic acids. The accuracy of method did not depend on nitrate contents.
Key words: Nitrate, Hydroxamic acid, Water, Spectrophotometry, Potential.

Introduction: The determination of nitrate in water is important, being harmful to human health [1, 2]. Moreover the concentration of nitrate could be used as an important indicator for organic pollution of water [3]. The toxicity of nitrate arises from the capability of human body to reduce it in the lower intenstine to nitrite [4].
It also plays an important role in the nitrogen oxide (NO_2) chemistry in the atmosphere. The primary source for nitrate is the reaction of nitrogen dioxide, NO_2, with ozone. The concentration of NO_3 rises throughout the night. The typical concentration of nitrate range from 10's of parts per trillion (ppt) in remote location to 100's of ppt in more polluted region [5].

Nitrates are one of the most frequently utilized forms of nitrogen from soil. Through root they reach stem and leaf where in photosynthesis process they convert into proteins. Due to exposure to stress situations, and excessive nitrogen fertilization, nitrate accumulation in plant tissues and organs occurs. Nitrates can also accumulate in harmful concentration in soil. Nitrates accumulated in plants have harmful effect on animals which consume plants since they cause various health disorders [6, 7].
Most of the recent work concerning nitrate determination has embraced the classical reagents. Several spectrophotometric [8-10], Sequential flow injection spectrophotometric [11], Polarographic [12] , High Performance Liquid Chromatographic[13], Ion Chromatographic [14], chemiluminescent flow-injection analysis [15] methods were reported for nitrate determination. A potentiometric method has also been suggested [16].
Some of these techniques have the disadvantages of being fairly tedious, while other show non-linear calibration graphs, poor colour stability, low sensitivity and severe interference.
The work described here shows the analysis of water, soil and biological samples. This is simple, reliable and rapid improved spectrophotometric method for measurement of nitrate. Nitration of the reagent (hydroxamic acid) is carried out instantaneously at about 0^oC in 80% sulphuric acid and nitration product is extracted in n-hexane which gives yellow colour, the absorbance of which is measured at 462, 458 and 450 nm with N-PBHA, N-PClPBHA and N-PClPCHA, respectively. Interference from common anions have been investigated.

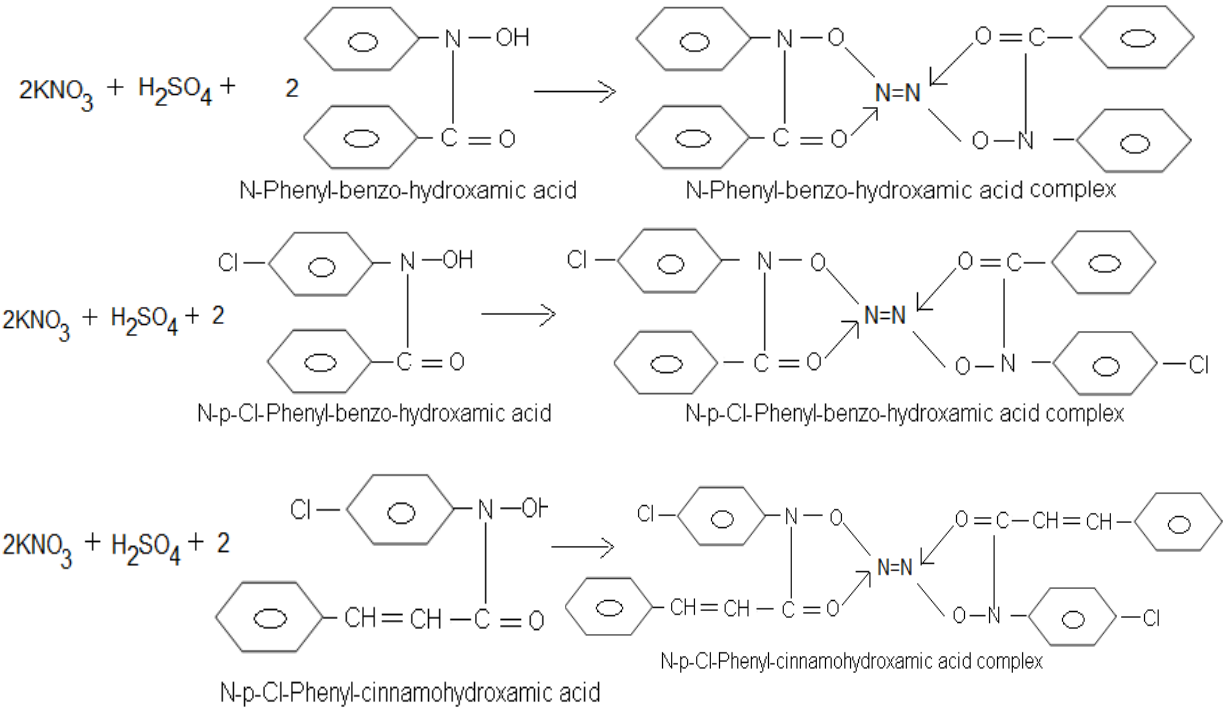
Experimental
"SYSTRONICS SPECTROPHOTOMTER 1700" was used for electronic spectral measurements with 10 mm matched quartz cells. A Hanna 8521 model pH meter was used for pH measurements.

Reagents
All the chemicals used were of AR grade. Double distilled water was used throughout the experiments.

Hydroxamic acids were prepared by the method reported [17].
Sulphuric acid (80%) 100 ml of 98% sulphuric acid was mixed with 22.5 ml of double distilled water. It was allowed to cool at room temperature before use.
Nitrate standard solution: Stock nitrate solution was prepared by dissolving 0.169 KNO_3 in 100 ml of double distilled water; add 1ml chloroform was added to prevent bacterial growth.

Procedure
Prepare calibration graph by transferring with a semi-micro burette 1.0 ml each of standard nitrate solutions, containg 0.0000001965-0.000002751, 0.000001965-0.0000392 and 0.000001965-0.00002553 g/l of nitrate for N-PBHA, N-PClPBHA and N-PClPCHA, respectively into two necked 100 ml round-bottomed flasks. Each solution was nearly evaporated to dryness by heating gently on a hot plate. The flask was then covered and cooled in ice. Then 5ml of 80% sulphuric acid was added followed by 2 ml of 0.0001% hydroxamic acid solution prepared in n-hexane. A reagent blank was taken through the same procedure. The flasks were then shaken for 60s. The contents were carefully transferred into a separating funnel with 80 ml ice cooled distilled water. The two phases were allowed to separate. Aqueous layer was discarded and yellow colour developed in n-hexane hydroxamic acid solution was used for determination of nitrate.

Expected reaction



Result and discussion

a) Absorption spectrum and calibration curve

After reaction, complex present in organic phase was scanned from 400 nm to 600 nm against reagent blank (figure1). Maximum absorption values were observed at 462nm, 458 nm and 450 nm for nitrate complex with N-PBHA, N-PClPBHA and N-PClPCHA, respectively. Therefore, 462nm, 458 nm and 450 nm were selected with N-PBHA, N-PClPBHA and N-PClPCHA, respectively for the absorbance measurement throughout the experiments.

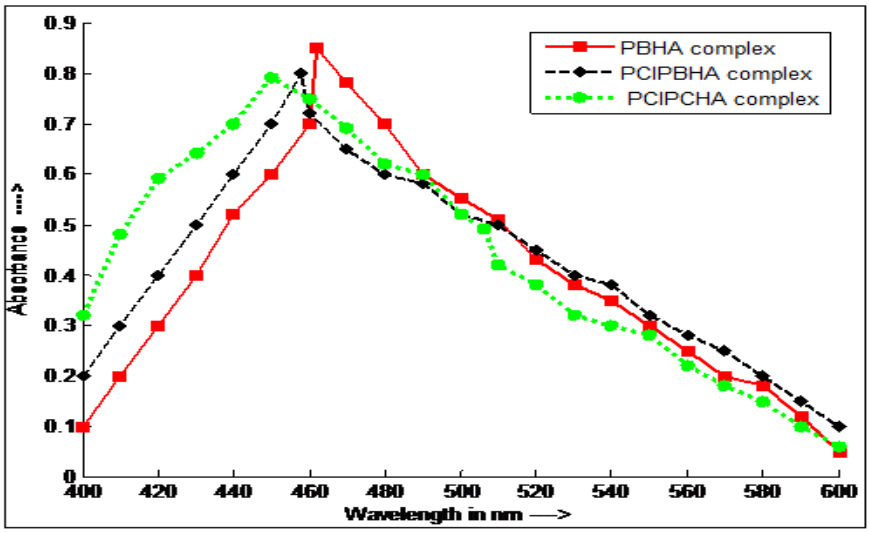


Fig. 1. Absorption spectra of nitrite complexes with hydroxamic acids

A calibration plot of absorbance against concentration of N-PBHA, N-PClPBHA and N-PClPCHA complexes at the absorption maxima gave a linear and reproducible graph in the concentration range of 0.0001965-0.002751, 0.001965-0.03931 and 0.001965-0.02554 µg/25 ml , respectively (fig.2). The beer's law is obeyed in this range.

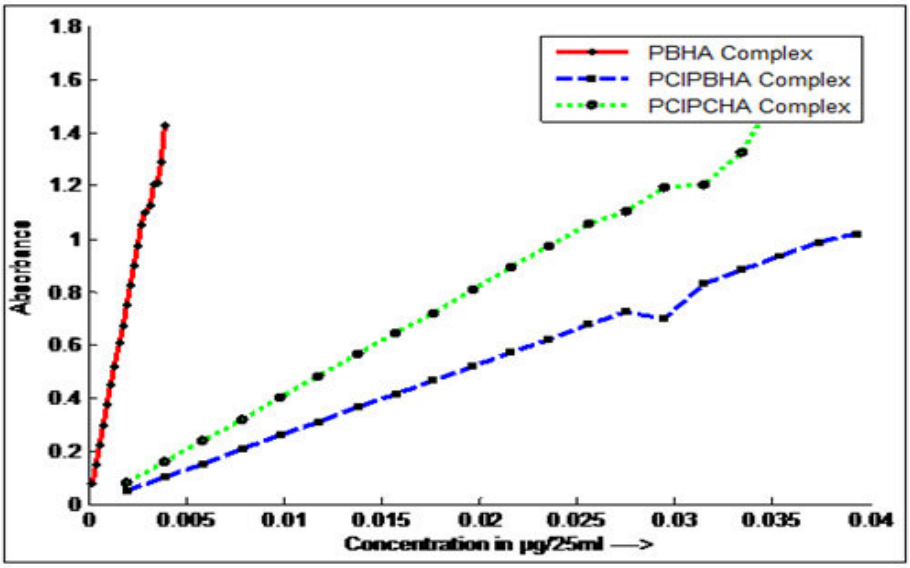


Fig.2: Calibration plot for N-PBHA, N-PClPBHA and N-PClPCHA complexes

Effect of reagent concentration

Optimal concentration of hydroxamic acids were investigated by varying the amount of hydroxamic acids used as reagent.To a series of 1 ml of nitrate standard solution, varying concentration of hydroxamic acids solutions were added and mixed. It was observed that by increasing the concentration of hydroxamic acid absorbance increases and with 2 ml of 0.0001% hydroxamic acid solutions it reaches maximum absorption. Then it decreases rapidly. Therefore 2 ml of 0.0001% concentration of hydroxamic acid solution was selected for all experiments.

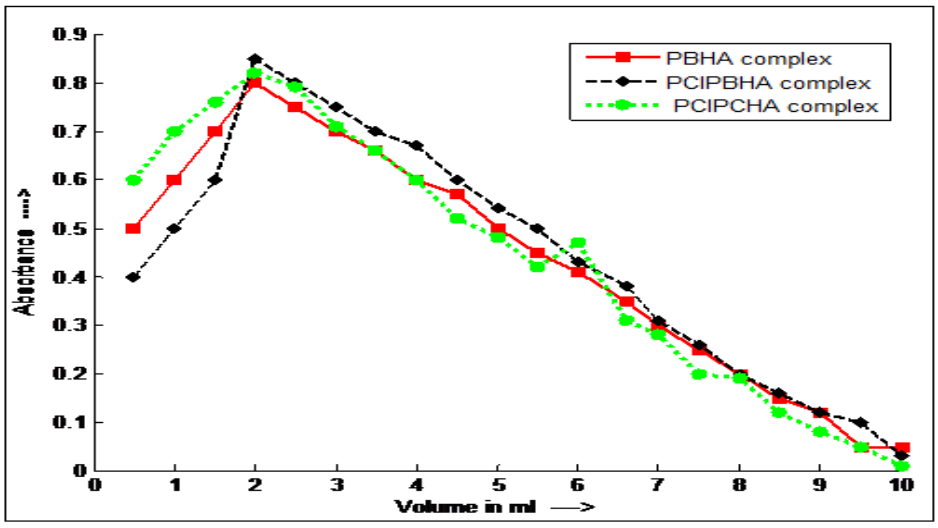


Fig.3: Effect of reagent concentration on absorbance of complexes

Effect of acid concentration

To study the effect of volume and concentration of sulphuric acid on the reaction, varying amounts of different concentration of sulphuric acid were added to standard nitrate solution. It was observed that complex gives the maximum absorbance and better reproducibility, when 5 ml of 80% sulphuric acid was added. Therefore we preferred the use of 5 ml of 80% sulphuric acid over concentrated acid. In figure----, the effect of sulphuric acid volume on absorbance and in figure--- the effect of concentration of sulphuric acid on assorbance is shown. From 2-5 ml sulphuric acid, the absorbance increases sharply, on 5 ml optimum absorbance is obtained and above 5 ml the absorbance slowly decreases.

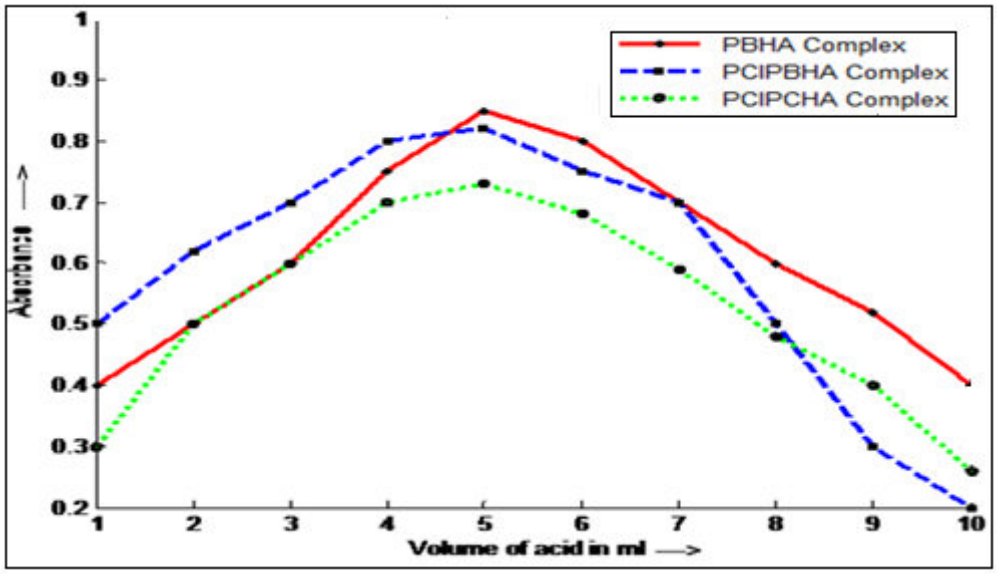


Fig.4: Effect of volume of acid on absorbance of complexes

Effect of temperature

The effect of temperature on colour stability of the complex was studied over the temperature range of 1^0C – 10^0C. It was observed that reaction becomes very fast at the temperature of 1^0C and formed complex gives maximum and stable absorption at the same temperature. Therefore, all experiments performed at the above temperature.

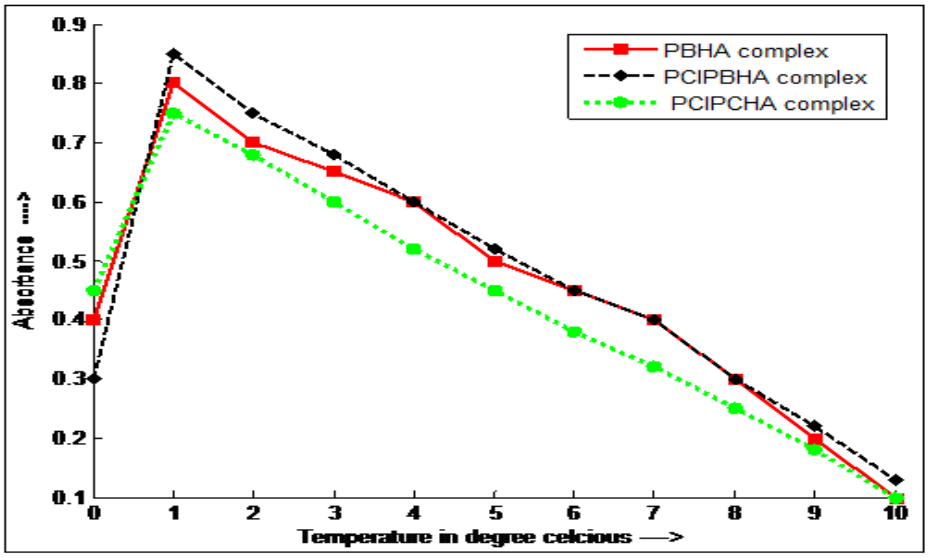


Fig. 5: Effect of temperature on absorbance of complex

Effect of stirring time and rest time

Effect of stirring time and rest time was also studied. For studying the effect of stirring time, the absorbance of formed complex was studied and compared. The stirring time was varied from 1-14 min (Fig.6). As it is clear, after about 2 minutes of stirring and 2 minutes of rest time, the formed complex gives maximum absorption. Therefore above stirring and rest time selected in subsequent studies.

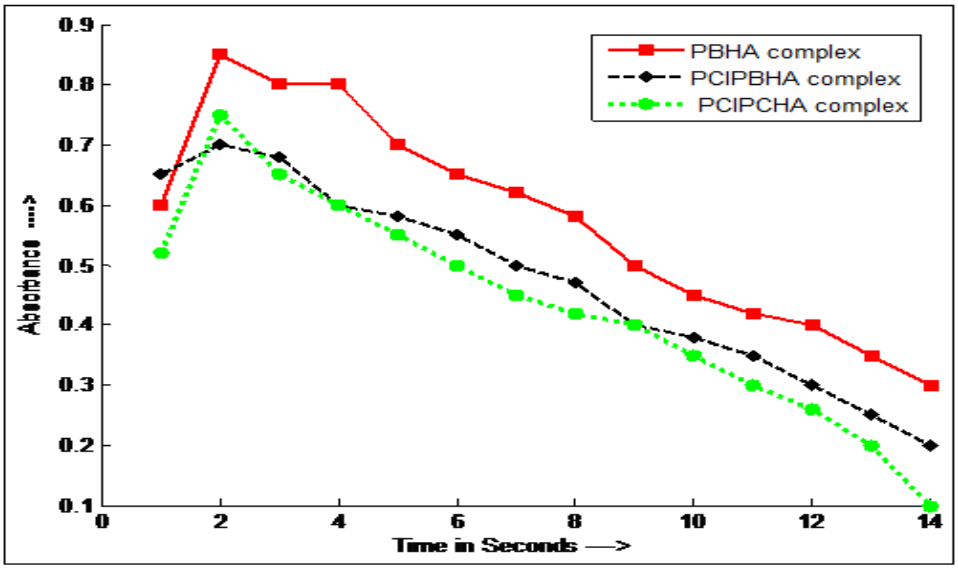


Fig. 6: Effect of stirring time on absorbance of complex

Effect of pH

In order to obtain the optimum condition for the determination of nitrate, absorbance was measured at the pH range 1.0 -10.0 (fig.7). It was observed that the maximum absorbance obtained at 1.5 pH then it started decreasing. Therefore, pH 1.5 was used further for all experiments. The determination was done by using n-hexane as a medium.

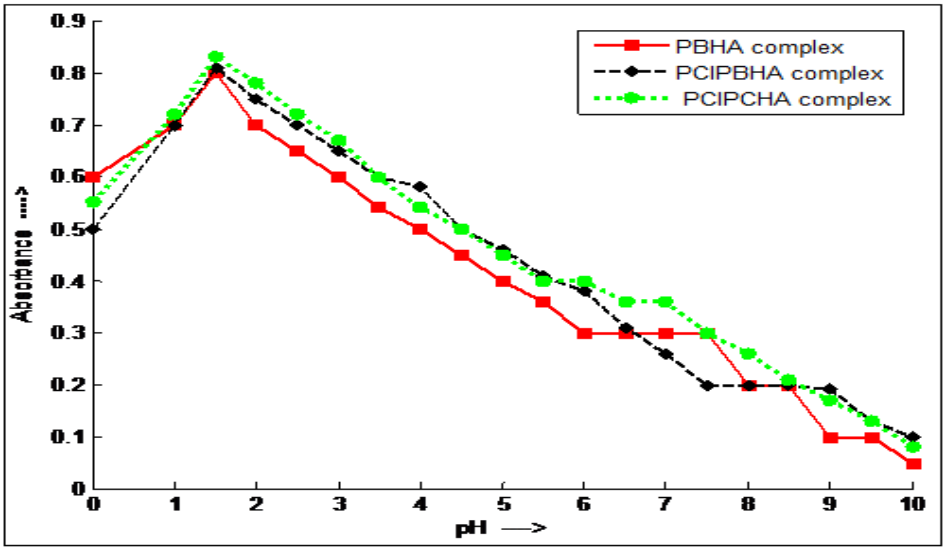


Fig. 7: Effect of pH on absorbance of complex

Effect of different solvent

Different organic solvents like ethanol, n-butanol, diethyl ether, ethyl methyl ketone, ethyl acetate, chloroform, toluene, n- hexane and carbon tetrachloride were used for extraction. n- hexane was found to be most suitable solvent as it gave better and quick phase separation. Therefore, n- hexane was selected for further extraction studies.

Interference studies

The effect of various non-target species on the determination of nitrate was investigated. The tolerance limit of interfering species were established at those concentrations that do not cause more than ± 2% error in absorbance values of nitrate. 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 1ml of 3% EDTA. The results are given in Table 1.

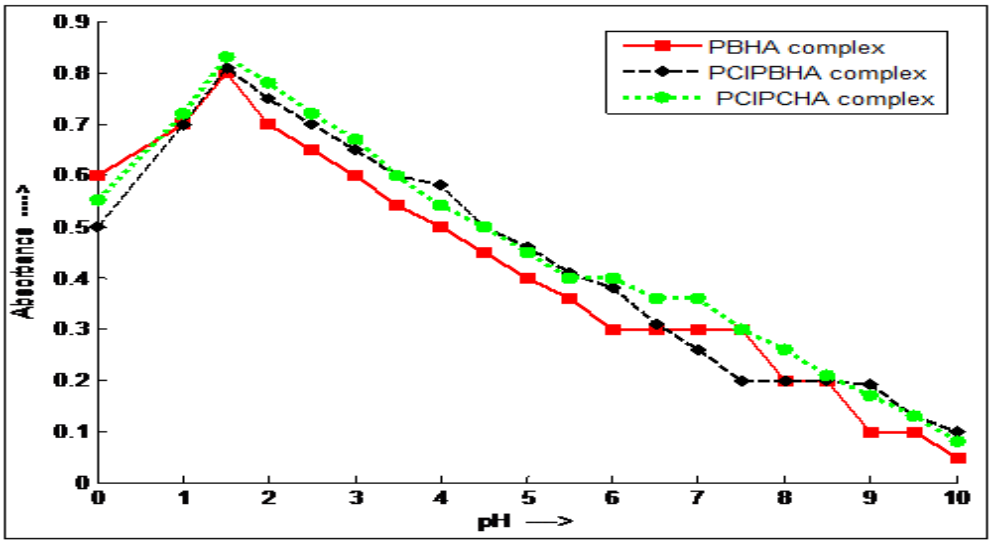
Table 1. Effect of diverse ions on the determination of nitrite using N-PBHA, N-PClPBHA and N-PClPCHA as reagents.

S No.	Diverse ions	Tolerance limit (ppm)		
		N-PBHA	N-PClPBHA	N-PClPCHA
1	Al^{3+}	300	300	285
2	Ba^{2+}	210	200	205
3	Ca^{2+}	500	498	505
4	Cd^{2+}	210	195	200
5	Ce^{4+}*	25	25	20
6	CH_3COO^-	>2025	>2015	>2000
7	citrate	810	800	800
8	Cu^{2+}*	30	30	35
9	Fe^{3+}*	25	25	20
10	NO_2^-	85	70	80
11	Hg^{2+}*	25	20	25
12	K^+	>2010	>2020	>2000
13	Mg^{2+}	510	505	500
14	Mn^{2+}	500	505	502
15	Mo^{6+}*	25	28	25
16	Na^+	>2000	>2020	>2015
17	oxalate	800	795	780
18	Sn^{2+}*	25	25	20
19	Pb^{2+}*	25	25	20
20	tartarate	810	800	785

* Masked by EDTA

Effect of pH

In order to obtain the optimum condition for the determination of nitrate, absorbance was measured at the pH range 1.0 -10.0 (figure 2). It was observed that the absorbance increased upto 4.0 pH then it started decreasing. Therefore, pH 4.0 was used further for all experiments. The determination was done by using n-hexane as a medium.



Applications

In order to evaluate the analytical applicability of the method, the procedure established was used for the determination of nitrate in water samples from different sites of Bhopal district.

Analysis of water samples

For analysis of real sample some pretreatment is necessary. All suspended particles should be removed by suitable procedures. For waste water sample, precentrifugation was used. The samples were transferred in 25 ml calibration flask and 0.5 ml of 0.2 mol^{-L} EDTA solution was added as masking agent and above procedure was applied. The results (table 2) agreed well with those given by the standard method.

Determination of nitrate in water

S No.	Locations in Bhopal	Nitrate found µg ml^{-1}
1.	GI	2.6
2.	MI	4.1
3.	SL	3.6
4.	UL	3.1
5.	ANUC	3.3
6.	GN	1.7

GI-Govindpura industrial area, **MI**-Mandideep Industrial area, **SL**-Shahpura Lake
UL-Upper Lake, **ANUC**-Area Near Union Carbide, **GN**-Gandhi Nagar

Conclusion

An extractive spectrophotometric method was developed for estimation of nitrate. All the hydroxamic acids were successfully used for quantitative determination of nitrite at pH 4.0. Since the reaction time is very less, the method is very quick. The colour developed is very stable. The method offers simple and rapid determination of nitrite in water, soil and biological samples.

As the procedure is free from interference of diverse alkali salt and metal ions, it can be directly applied for analysis of trace nitrite in polluted, natural water, soil and biological samples. The solution of reagent made in organic solvent is very stable and can be used for more than a year.

The results show that with the increase in substitution in hydroxamic acid, the sensitivity of method decreases. Thus, the determination of nitrite with the parent hydroxamic acid, N-PBHA is most sensitive among the hydroxamic acids.

Acknowledgement

We would like to thank Director B.U.I.T Bhopal, Principal Govt.Geetanjali Girls P.G.College Bhopal, Director T.I.E.I.T Bhopal and Director T.I.P Bhopal for providing necessary facilities on time during our research activity.

1. Xu, et al. European Journal of Cancer Prevention, 1,437-443, 1992
2. Beresford, S., International Journal of Epidemiology, 14(1), 57-63,1985
3. Steffii Fried, Brendan Mackie, and Erin Nothwehr, 4, 21-24,2003
4. M. Cornblath, A. F. Hartman, J. Pediat, 32, 421, 1984
5. Finlayson-Pitts , B,J., Pitts, J.N., Academic Press 2000.
6. Z. Nesic, Z. Tomic, V. Krnjaja, D. Tomasevic, Biotechnology in Animal Husbandry, 24 (5-6), 95-104, 2008
7. H. Kroupova1, J. Machova, Z. Svobodova, Vet. Med. – Czech, 50 (11), 461–471,2005
8. Badiadka Narayana1 and Kenchaiah Sunil, Eurasian J. Anal. Chem., 4(2), 204-214, 2009
9. Padmarajaiah Nagaraja, Mattighatta Shivaswamy, Hemant Kumar, Analytical Sciences,18, 355-357,2002
10. E. I. Uwah, J. Abah, N. P. Ndahi, V. O. Ogugbuajaj. J. of Applied Siences in Environmental Sanitation, 4, 3, 233-244, 2009
11. A. Kazemzadeh, Ali A. Ensafi, Analytica Chimica Acta, 442, 319–326, 2001
12. M.I.N. Ximenes, S. Rath, F.G.R. Reyes, Talanta, 51, 49–56, 2000

13. Shin-Shou Chou, Jen-Chien Chung, Deng-Fwu Hwang, Journal of Food and Drug Analysis, 11, 3, 233-238, 2003
14. R. Michalski, I. Kurzyca, Polish Journal of Environmental Studies, 15, 1, 5-18, 2006
15. Pavel Mikuska, Zbynek Vecera, Analytica Chimica Acta, 495, 225–232, 2003
16 Perez-Olmos R, Bezares P, Perez J., Farmaco., 55(2),99-103, 2000
17. Priyadarshini, U.; Tandon, S. G.; J. Chem. Engg. Data, 12, 143, 1967