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A novel spectrofluorimetric method for the ultra trace analysis of nitrite and nitrate in aqueous medium and its application to air, water, soil and forensic samples

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Abstract

A highly sensitive and virtually specific method has been developed for the trace and ultra trace $5\,\mathrm{ng\,ml^{-1}}$–$1\,\mu\mathrm{g\,ml^{-1}}$ fluorimetric analysis of nitrite. The method is based on the quenching action of nitrite on the native fluorescence of murexide (ammonium purpurate) [$\lambda_{ex} = 349.0\,\mathrm{nm}$, $\lambda_{em} = 444.5\,\mathrm{nm}$] in the acid range of 0.045–0.315 (M) H_2SO_4. The method is very precise and accurate (S.D. $= \pm 0.4877$ and R.S.D. $= 0.4878\%$ for the determination of $0.1\,\mu\mathrm{g\,ml^{-1}}$ of nitrite in 11 replicates). Relatively large excesses of over 35 cations and anions do not interfere. The proposed technique has been successfully applied for the determination of nitrite and nitrate in ground water, surface water and sea water, nitrite in soil and nitrate in forensic samples. The method has also been extended for the analysis of NO_x in air.

Keywords: Murexide; Fluorimetric determination; Nitrite; Nitrate

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1. Introduction

Nitrite and nitrate are wide spread contaminants of ground and surface water worldwide [1,2]. Nitrite is a potential human health threat especially to infants, causing the condition known as methemoglobinemia, also called “blue baby syndrome”. Though not itself harmful, the toxicity of nitrate to humans is mainly attributable to its reduction to nitrite. Chronic consumption of high levels of nitrate may also cause other health problems, like cancers and teratogenic effects [3,4]. Nitrite can react in the human body with dietary components to form toxic, carcinogenic and/or mutagenic nitrosamines [5]. Nitrogen oxides are primary air pollutants and destroy the lining of the respiratory surface, thereby reducing the intake of oxygen for the body leading to respiratory allergies like asthma. Thus, the determination of inorganic nitrogenous species is of great importance in environmental samples.

Nitrates are important constituents of gunpowder and other explosives and fireworks. As such analysis of nitrate is very significant in forensic samples.

Several spectrofluorimetric methods have been developed for the analysis of nitrite [6–17]. These methods are based on the reaction of nitrite with various reagents, such as 2,3-diaminonaphthalene (DAN) [6–8], 2,6-diaminopyridine [9], resorcinol [10], 5-aminofluorescein [11], benzidine [12], pyridoxal-5-phosphate-2-pyridylhydrazone [13], 4-hydroxycoumarin [14], tryptophan [15], 5,6-diamino-1,3-naphthalene disulphonic acid (DANDS) [16] and $4,4',4'',4'''$-tetrasubstituted amino aluminium phthalocyanine [17].

However, there are several disadvantages of using these reagents like, poor reproducibility [11], toxicity of reagent [9,12], involvement of extraction, tedious and time consuming procedure [6–8,16] and narrow detection range [10,13]. In some proposed methods, the reagents have to be synthesised and purified [16,17]. In the present work the authors have described a simple in-expensive, non-extractive and virtually specific fluorimetric method for the determination of nitrite. The technique involves quenching of native fluorescence of murexide in acid medium. The method has also been extended for the analysis of nitrate after its reduction to

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nitrite, thus making the speciation of nitrite and nitrate possible in their mixtures. The method is precise and accurate.

2. Experimental

2.1. Instrumentation

All fluorimetric measurements were performed with a Shimadzu spectrofluorophotometer (RF-5000) equipped with a 150-W xenon lamp, 12″ colour video display, parallel line thermosensitive printer recorder, 1 cm × 1 cm quartz cells with a Shimadzu ASC-5 auto sample changer. Operational performance and sensitivity of the instrument were checked by running the Raman spectrum of distilled water and wavelength error was kept below ± 2.0 nm. A digital pH meter (model pH 5651, Electronic Corporation of India Ltd.) was used for the measurements of the pH. A Hanovia (model 11A) fluorescence UV lamp was used for preliminary qualitative investigations.

2.2. Reagents and solutions

Ammonium purpurate (murexide) solution: A 10^{-3} (M) solution of ammonium purpurate ($C_8H_4O_6N_5 \cdot NH_4$; molecular weight 284.19) under the trade name of murexide [Loba Chemi, 98% purity (minimum)] was prepared by dissolving the requisite amount (0.0284 g) in de-ionised water followed by acidification with 0.5 ml concentrated sulphuric acid in a 100 ml volumetric flask. It was diluted with water up to the mark. The reagent was stored in a refrigerator. However, a freshly prepared reagent solution was used whenever required. More dilute solutions were prepared from the 10^{-3} (M) stock solution.

Hundred millilitre of nitrite solution [2.1739×10^{-2} (M)] was prepared by dissolving 0.15 g sodium nitrite (E. Merck) in water. More dilute solutions were prepared from the stock solution as required.

Hundred millilitre of nitrate solution [1.6129×10^{-3} (M)] was prepared by dissolving 0.1631 g potassium nitrate (E. Merck) in water. More dilute solutions were prepared from the stock solution as required.

Other solutions: Solutions of inorganic ions were prepared from their water soluble salts (or the oxides and carbonates in acids).

Double distilled demineralised water was used throughout the study. All fluorescence intensity measurements were made at room temperature (25 ± 2 °C).

All glass vessels were cleaned by soaking with acidified solutions of potassium permanganate or potassium dichromate, followed by washing first with concentrated nitric acid and then several times with water.

2.3. General procedure

To a 10 ml volumetric flask containing 0.05–1.0 μg or 1.0–10 μg of standard nitrite solution, 1.5 ml of 10^{-4} (M) or 1.5 ml of 10^{-3} (M) murexide solution and 0.5 ml of 4.5 (M) H_2SO_4 were added. Then the flask was placed in a boiling water bath for 10 min, cooled to room temperature and made up to the mark with water [15]. The fluorescence intensity was measured at 444.5 nm against a corresponding reagent blank, keeping the excitation wavelength at 349.0 nm under the same instrumental settings. From the difference of fluorescence intensities of the solution measured in the absence and in the presence of nitrite; the concentration of nitrite was determined with the help of a standard calibration graph. Calculation expression of fluorescence intensity was as follows

$$\Delta F = F_R - F_{NR}$$

In the equation above, F_{NR} was the fluorescence intensity of test system including nitrite ion; F_R was the fluorescence intensity of reagent blank solution ΔF was the difference of fluorescence intensity. Since the effect was of fluorescence quenching, the value of F_R was greater than the value of F_{NR}.

2.4. Determination of nitrite and nitrate in a mixture

Suitable aliquots of nitrite and nitrate mixture at different mass ratios were taken separately in a 25 ml beaker and reduced the nitrate to nitrite with a pinch of zinc dust in presence of manganous hydroxide as catalyst [18]. Filtered the excess zinc dust, collected the filtrate and the total nitrite and nitrate concentration were determined, following the general procedure. The fluorescence intensity of an equal volume of nitrite and nitrate mixture was measured without reduction of nitrate. This gives the measure of nitrite originally present as nitrite in the mixture. Subtracting this from the total gives the concentration of nitrate present in the mixture.

2.5. Determination of nitrite and nitrate in environmental water samples

The proposed method has been applied to the determination of nitrite and nitrate in ground and surface water samples. The interference of chloride was removed by precipitating the chloride with silver sulphate [19]. Suitable aliquots were taken [16] and analysed for nitrite and nitrate following the general procedure.

2.6. Determination of nitrite in soil samples

One gram of each soil sample (previously air dried, powdered and sieved) was taken in a 100 ml beaker, added 10.0 ml water and filtered. The interference of chloride was removed by precipitating the chloride with silver sulphate. One millilitre of the filtrate was transferred to a 25 ml beaker and determined the nitrite content fluorimetrically according to the general procedure.

2.7. Determination of nitrate in forensic samples

The forensic samples containing nitrate were quantitatively transferred by washing repetitively with minimum amount of water through a filter paper into a 10 ml volumetric flask and volume made up to the mark with water. Suitable aliquots were analysed for nitrate following the general procedure.

2.8. Determination of NO_x in air samples

The air samples were collected by bubbling dust free air through 50.0 ml, 0.1 (M) NaOH at a sampling rate of 200 ml min^{-1} for 24 h [20]. A suitable aliquot was taken from this and analysed for nitrite according to the general procedure.

3. Results and discussions

3.1. Factors affecting the fluorescence intensity

3.1.1. Spectral characteristics

The uncorrected excitation and emission spectra of the murexide in presence and in absence of nitrite, in acidic media were recorded with the spectrofluorimeter. The wavelength maxima of excitation and emission were found to occur at 349.0 and 444.5 nm. (Fig. 1).

3.1.2. Effect of acidity

In the present system sulphuric acid provides a wide range of acidity for the maintenance of constant maximum fluorescence intensity. The constant maximum difference in fluorescence intensity (with respect to corresponding reagent blanks) was observed in the presence of 0.1–0.7 ml of 4.5 (M) sulphuric acid in a total 10.0 ml solution. This corresponds to 0.045–0.315 (M) acidity range in final dilution.

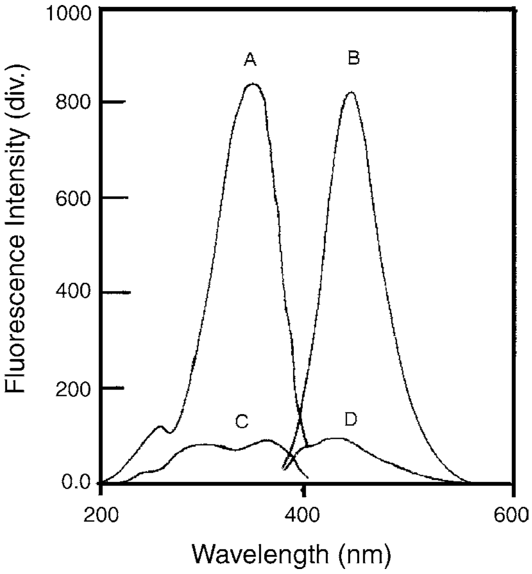

Fig. 1. Fluorescence spectra A and C are the uncorrected excitation spectra of the reagent [murexide] system and [nitrite + murexide] system respectively B and D are the uncorrected emission spectra of the reagent [murexide] system and [nitrite + murexide] system, respectively.

3.1.3. Effect of reagent

For each reagent molecule, two molecules of nitrite reacted as shown in the proposed mechanism. Hence, fluorescence quenching was studied for nitrite: reagent molar ratios in the range 1:0.5–1:90; larger excesses of reagent led to decrease in difference of fluorescence quenching (ΔF) and decrease in sensitivity.

3.1.4. Effect of time

At the very low concentrations where the measurements were carried out, the reaction between murexide and nitrite was found not to be instantaneous. Hence completion of the reaction was attained by placing the volumetric flask in a boiling water bath for 10 min.

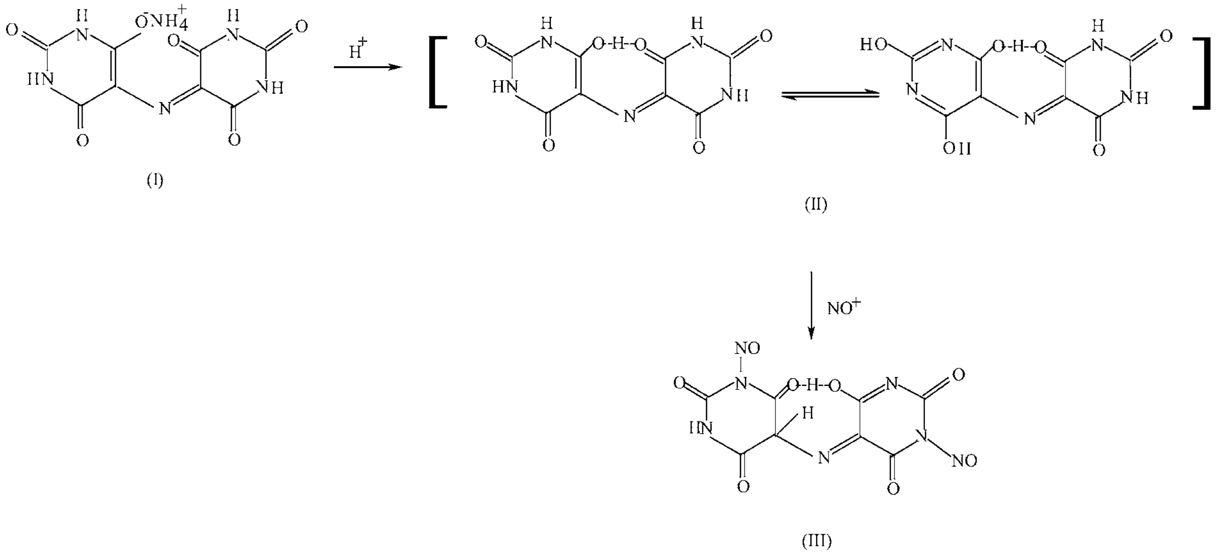

Fig. 2. Proposed mechanism.

Table 1
Speciation of nitrite and nitrate in synthetic mixtures

Serial no.	Nitrite taken (ng ml^{-1})	Nitrate taken (ng ml^{-1})	Nitrite found (ng ml^{-1})[a]	Nitrate found (ng ml^{-1})[a]	Recovery	
					Nitrite (%)	Nitrate (%)
1	500.0	–	499.5	–	99.9	–
2	300.0	200.0	301.5	199.0	100.5	99.5
3	200.0	300.0	200.0	299.0	100.0	99.7
4	–	500.0	–	498.0	–	99.6

[a] Average of five replicate measurements.

Table 2
Analysis of nitrite and nitrate in environmental water samples

Sample source	Nitrite added (ng ml^{-1})	Nitrite found[a] (ng ml^{-1})	Total[a] (nitrite and nitrate) (ng ml^{-1})	Nitrate (by difference) (ng ml^{-1})	Recovery[b]	
					Nitrite (%)	Nitrite (%)
Ground water[c]	0.0	33.0	89.5	56.5	88.0	87.6
	50.0	83.0	140.0	57.0	89.0	89.5
	100.0	132.0	189.0	57.0	88.6	88.3
Surface water[d]	0.0	95.8	221.9	126.1	90.2	87.5
	50.0	146.0	272.0	126.0	89.3	86.2
	100.0	195.2	321.1	125.9	89.4	88.9
Sea water[e]	0.0	90.0	1165.0	1075.0	89.7	92.3
	50.0	139.0	1215.0	1076.0	90.5	91.5
	100.0	190.5	1266.0	1075.5	89.6	91.9

[a] Average of five replicate measurements.
[b] Results were compared to spectrophotometric analysis based on diazotisation of sulphanilamide, followed by coupling with *N*-(1-naphthyl) ethylenediamine dihydrochloride [21].
[c] Collected from Jadavpur University water supply, Jadavpur, Calcutta.
[d] Collected from Ranidighi, Tollygunge, Calcutta.
[e] Collected from Digha, Bay of Bengal.

3.1.5. *Linear range*

The fluorescence intensity exhibited a linear dependence on the nitrite concentration over a wide range (5 ng ml^{-1}–1 $\mu\text{g ml}^{-1}$), distributed over two sets (5–100 ng ml^{-1} and 100 ng ml^{-1}–1 $\mu\text{g ml}^{-1}$) of nitrite concentration for the convenience of fluorescent intensity measurements. The detection limit of the method is 0.6 ng ml^{-1}.

3.1.6. *Effect of foreign ions*

Over 35 cations, anions and some complexing agents were studied individually and found to have negligible interference in the determination of 0.1 $\mu\text{g ml}^{-1}$ of nitrite, when present in relatively large to moderately large mass excess. The study revealed that, 1000-fold excesses of EDTA, tartrate, citrate, oxalate, borate, carbonate, sulphate, phosphate, phosphite, nitrate, alkali and alkaline earth metal ions, 500-fold excesses of Zn^{2+}, Cd^{2+}, Ni^{2+}, Co^{2+}, Al^{3+}, 100-fold excesses of Mn^{2+}, Th^{4+}, Cr^{3+}, Os^{8+}, As^{3+}, Sb^{3+}, Fe^{3+} and Cu^{2+}, 50-fold excesses of Bi^{3+}, Hg^{2+}, HF_2^- have negligible interference. The interference of Cl^- was removed by precipitating with silver sulphate [19]. During the interference studies, any insoluble substances that formed were simply removed by centrifugation and the fluorescence was measured with the centrifugates.

Table 3
Determination of nitrite in soil samples

Serial no.	Sample source	Nitrite added $(\mu\text{g g}^{-1})$	Nitrite found $(\mu\text{g g}^{-1})$[a]	Recovery[b] (%)
1	Soil sample to which fertilisers have not been added; collected from garden of Jadavpur University, Calcutta	0.0	13.5	92.3
		5.0	18.6	91.6
		10.0	23.7	91.8
2	Soil sample to which fertilisers have not been added; collected from garden of B. S. College 24 Parganas (S), West Bengal	0.0	27.3	89.0
		5.0	32.3	90.2
		10.0	37.2	89.7

[a] Average of five replicate measurements.
[b] Results were compared to spectrophotometric analysis [21].

Table 4
Detrmination of nitrate in forensic samples

Serial no.	Sample source	Nitrate found[a]	Recovery[b] (%)
1a	Gun shell residue (1)[c] (μg)	5.92	88.4
1b	Gun shell residue (2)[d](μg)	6.20	89.5
2a	Explosive mixture (1)[e]($\mu g\,g^{-1}$)	143.0	93.5
2b	Explosive mixture (2)[f] (ng)	50.4	87.6

[a] Average of five replicate measurement.
[b] Results were compared to spectrophotometric analysis [21].
[c] Gun shell residue obtained from used cartridges (post firing).
[d] Gun shell residue obtained from used cartridges (post firing).
[e] Explosive mixture (1) obtained from cracker before blasting (weight of explosive mixture ~4 g).
[f] Explosive mixture (2) obtained from cracker after blasting, by washing the residue (~0.092 g) collected from the blasting site.

Table 5
Determination of NO_x in air samples

Serial no.	Sample source	NO_x found[a] ($\mu g\,m^{-3}$)	Recovery[b] (%)
1	Air sample collected from a busy roadside just outside Jadavpur University campus, (Calcutta), on 14th May, 2003.	39.0	89.2
2	Air sample collected from a busy roadside just outside Jadavpur University campus, (Calcutta), on 25th October, 2003.	55.0	88.7

[a] Average of five replicate measurements.
[b] Results were compared to spectrophotometric analysis [21].

3.1.7. *Proposed mechanism*

The reagent murexide (I) in aqueous solution shows a pinkish violet colour. As measurement of fluorescence in presence of colour is not fruitful we have used the protonated form (II). In presence of nitrite ion in acid medium (i.e. NO^+), the fluorescence intensity is quenched probably due to the formation of (III) where extended conjugation and hence $\Pi \rightarrow \Pi^*$ transition is inhibited (Fig. 2). The presence of C–H and N–H bond in (III) is supported by IR peaks at 2932 and 3452 cm^{-1}, respectively.

4. Applications

The present method was applied to determine nitrite and nitrate in synthetic mixtures of the two (Table 1), environmental water samples (Table 2), nitrite in soil samples (Table 3), nitrate in forensic samples (Table 4) and NO_x in air samples (Table 5).

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