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A Novel Method for the Extraction Spectrophotometric Determination of Nitrate and Nitrite in Water, Waste Water and Effluent

Pranab Kumar Tarafder[*], **S. Roychowdhury**

Department of Atomic Energy, Atomic Minerals Directorate for Exploration and Research, India

[*]**Corresponding author:** Pranab Kumar Tarafder, Department of Atomic Energy, Atomic Minerals Directorate for Exploration and Research, Khasmahal, Jamshedpur-83100, India. Email: pktarafder1954@gmail.com

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Abstract

A novel method for the spectrophotometric determination of nitrite and nitrate in water and effluent samples has been developed. The diazotisable amine, p-aminoacetophenone has been diazotized with nitrite in the presence of hydrochloric acid at room temperature, and the diazotized salt thus produced has been allowed to couple with the reagent, 2,3-dihydroxynaphthalene in alkaline medium to produce a reddish orange azo-dye suitable for the determination of nitrite in ppm level. The λ_{max} of the azo-dye being 475 nm. The very azo-dye is extractable into an organic solvent like ethyl acetate without shifting the λ_{max}. Similarly, nitrate ion forms a strong reddish-yellow nitro product with this reagent in the presence of concentrated sulfuric acid. This nitro product is extractable into ethyl acetate, and its absorbance is measured at 380nm (λ_{max}). The Beer's law for nitrite and nitrate were obeyed in the range, 0.1 to 5.0 ppm and 0.2 to 50 ppm, respectively. The molar absorptivity of nitrite and nitrate are $2.5\text{x}10^4$ and $3.2\text{x}10^3$ $\text{L.mol}^{-1}\text{cm}^{-1}$ respectively. The RSD for both the methods are in the range, 0.5 to 2.5%.

Keywords: 2,3-Dihydroxynaphthalene; Nitrite; Nitrate; Solvent Extraction; Spectrophotometry

Introduction

There are number of methods known for nitrite and nitrate determinations [1]. Mostly nitrite is determined spectrophotometrically by the formation of azo-dyes [2-8]. A number of diazotisable amines are reported for the determination of nitrite [9]. Mention may be made of p-nitro aniline [7], 4-aminobenzoicacid [2,4], 4-aminosalicylic acid [5], 4-nitroaniline [6], 4-aminobenzotrifluoride [10] and 4-aminophenylmercapto acetic acid [11] etc.

Amongst these, 4-aminophenylmercapto acetic acid has been found to be sensitive as well as selective. However, this compound is not commonly available in the market. Most of these methods are based upon amine-amine coupling which always takes place in acidic medium. However, azo-dyes formed of amine-phenol coupling which usually takes place in alkaline medium are also not unprecedented [10]. Similarly; nitrate is generally determined spectrophotometrically based upon nitration of phenols in the presence of concentrated sulfuric acid. The most commonly used spectrophotometric reagents for nitrate determinations are phenol disulfonic acid [12], chromotropic acid [13], brucine [14] etc.

Although in most cases, these determinations are made in aqueous solution but in order to improve the selectivity of determination of these color products, these are often extracted in organic solvents. In water samples, both nitrite and nitrate are important parameters to be determined because of their adverse effects on human beings. While nitrite is a precursor for cancer [15], nitrate when present more than 300 ppm in water samples cause the dreaded disease methamoglobinemia in human beings. Besides, nitrite is an index of organic pollution [16]. Therefore, it is very important to determine nitrite and nitrate in water samples. Usuallyseparate reagents are used for the determination of nitrite and nitrate owing to difficulties in determining them together.

This prompted us to look for a reagent which is capable of determining both nitrite and nitrate. The reagent 2, 3-dihydroxynaphthalene which has been widely used by us in our laboratory for the determination of a host of metal ions like iron [17], manganese [18], uranium [19], thorium [20], molybdenum [21], niobium [22], titanium [23, 24], rare earth elements etc. has been found to couple with diazonium cation of p-aminoacetophenone in alkaline medium to form a stable and intense reddish-orange azo-dye suitable for the spectrophotometric determination of nitrite. At the same time, this very reagent has been found to form a reddish yellow nitro product with nitrate in the presence of conc. sulfuric acid, which is readily extracted into an organic solvent (ethyl acetate), suitable for spectrophotometric determination of nitrate in water samples.

This prompted us to investigate the efficacy/potential of this new reagent for the rapid, precise and accurate determination of nitrite and nitrate in water samples. In this paper, the reagent (2, 3-dihydroxynaphthalene) has been introduced by us for the determination of nitrite and nitrate for the first time. For nitrite determination, the reagent p-aminoacetophenone which has already been utilized by earlier workers as a diazotisable amine in the determination of nitrite has beenused by us.

Experimental

Apparatus

Spectrophotometer

Analytik Jena (Germany) make instrument having model No. Specord 250/plus was used for absorbance measurements.

Reagents

Nitrite, 1.0 mg ml^{-1} solution

Weighed 1.4997 g of dried sodium nitrite in 1000 ml volumetric flask, dissolved in water, and the volume was made up to the markwith distilled water. Added a drop of chloroform to preserve it.

Nitrate, 1.0 mg ml^{-1} solution

Weighed 1.371 g of dried sodium nitrate in one-liter volumetric flask, dissolved in water, andthe volume was made up to the mark with distilled water.

2,3-dihydroxynaphthalene, Fluka make (98.5% pure)

The marketed product was used as such without any further purification.

p-A 0.1 % (m/v) aminoacetophenone aqueous solution was used.

Ammonia Solution

Concentrated Sulphuric acid

Procedure

Determination of Nitrite

A 5 ml of sample aliquot containing 1.0-40 µg of nitrite was taken in a 100 ml beaker. To it was added 1 ml of 0.1% p-aminoacetophenone and 2.0 ml of 1:1 HCl and mixed well. To this solution was added 2 ml of 0.1 % 2,3-dihydroxynaphthalene solution dissolved in 5 ml of ammonia (0.1 g of 2,3-dihydroxynapthalene + 5 ml of ammonia, diluted to 100 ml). Transferred this solution into a 125 ml separating funnel and shaken with 10 ml of ethyl acetate. Allowed for phase separation, discarded the aqueous layer and the absorbance of the azo-dye in ethyl acetate was measured at 475 nm against the reagent blank.

Determination of Nitrate

A 0.2 ml of sample aliquot was carefully pipetted out into a 125 ml separating funnel and to it was added 0.05 g solid 2,3-dihydroxynaphthalene followed by 1 ml of conc. sulphuric acid and thoroughly mixed. Immediately added 10 ml of ethyl acetate and shaken the mixture for about 1 min. To it was added 5 ml of water and agitated for a while. Allowed for phase separation, discarded the aqueous layer and the absorbance of reddish-yellow nitro product of2,3-dihydroxynaphthalene in ethyl acetate was measured at 380 nm in a spectrophotometer against the reagent blank.

Results and Discussion

It has been discussed in the prelude ofthe paper, that separate reagents are required for separate determination of nitrite and nitrate in water samples. From the point of view of environmental study, nitrite and nitrate both are required to be determined in the samples under study.However, to the best of our knowledge, a single reagent which could be used forthe determination of both nitrite and nitrate together in the same sample is not known so far.

During the course of our analytical studies with the reagent, 2,3-dihydroxynaphthalene, it has been found that under different reaction conditions, this very reagent can be used for spectrophotometric determination of nitrite as well as nitrate with fairly good sensitivity and selectivity. In what follows are given the details of studies carried out on variables affecting the color development for nitrite and nitrate.

Nitrite

Nitrite is determined by measuring the absorbance of the colored azo-dye formed/synthesized in aqueous solution. The diazotisable amine, 4-aminoacetophenone was diazotized with nitrite in the presence of HCl. The diazonium cation formed was coupled with 2,3-dihydroxynaphthalene in alkaline medium. In order to optimize the reaction conditions, the effect of variables were studied. The under mentioned studies were carried for 25 µg NO_2^-/25ml of aqueous solution.

Effect of the Concentration of p-aminoacetophenone

In order to maximize the intensity of the colored azo-dye i.e., to optimize the concentration of the diazotizable amine (p-aminoacetophenone), its concentration was varied over a wide range. A 1 ml of 0.1% p-aminoacetophenone in 25 ml of ethyl acetate was found to be optimum for obtaining maximum absorbance of the azo-dye. The dye when extracted in to ethyl acetate remained stable. The λ_{max} (475nm) for the aqueous system did not change on extraction. Similarly, the sensitivity also remainedthe same on extraction.

Effect of HCl Concentration on Diazotization

The concentration of HCl required for diazotization of p-aminoacetophenone was varied over a wide range. A 2ml of 1:1 HCl was found to be optimum for maximum sensitivity and better stability of the azo-dye formed.

Effect of Temperature on Diazotization

The effect of temperature on diazotization was studied by carrying out diazotization over a temperature 0 to 60°C.It was found that a temperature range 0 to 40°C over which the diazotization was complete, as is evident from maximum color intensity of the azo-dye formed, is the most suitable range of temperature for diazotization.

Effect of Coupling Agent

The effect of the concentration of 2,3-dihydroxynaphthalene as a coupling agent was studied over a wide concentration range. A 2 ml of 1.0% aqueous solution of 2,3-dihydroxynaphthalene dissolved in 5 ml of ammonia solution (specific gravity 0.91) was found to be suitable for maximum coupling.

Effect of Alkali/Ammonia Solution

As amine-phenol coupling takes place in alkaline medium, KOH, NaOH and NH_4OH were tried for coupling. Although coupling was found to be more effective in KOH medium, it was avoided because in the process blank too, a purple color is imparted. Hence, ammonia solution (specific gravity 0.91) was successfully used for coupling reaction. A 2ml of 5% (v/v) ammonia solution was found to be optimum.

Effect of Diverse Ions

Effect of foreign ions on the color intensity of the azo-dye formed has been studied in detail. The tolerance limits in ppm are given in parentheses. Cl^-, NH_4^+, HCO_3^-, Br^-, CO_3^{2-}, CH_3COO^-, F^-, I^-, NO_3^-, PO_4^{3-}, SO_4^{2-}(2000), S^{2-}, SO_3^{2-}, CNS^-, $S_2O_3^{2-}$(1500), Cr^{3+}, Ba^{2+}, Ca^{2+}, Mg^{2+}, Hg^{2+}(500), Fe^{3+}, Mn^{2+}, Cu^{2+}(100).

The tolerance of Fe^{3+}, Mn^{2+}, $Cu^{2+}$and Cr^{3+} are low. However, in most natural water samples the content of these elements is never found to exceed 10 ppm. In effluent samples, these elements may exceed 10 ppm. In order to make the method free from interference, solvent extraction of the azo-dye formed in aqueous solution is resorted to. While the azo-dye is easily extracted into ethyl acetate, the elements like Fe^{3+} and Mn^{2+} which form colored anionic complexe with the reagent, 2,3-dihydroxynaphthalene remain in aqueous phase, thereby their interference is eliminated. In this context, it is pertinent to mention here that unlike the proposed method, most of the reported spectrophotometric methods, particularly the ones involving determination of nitrite in aqueous solution, are not free from the serious interference of iron, copper and manganese.

Beer's Law, Molar Absorptivity and Reproducibility of the Method

The Beer's law was obeyed from 0.1 to 5.0 mg/L NO_2^- and the molar absorptivity of the method is $2.5x10^4$ $L.mol^{-1}cm^{-1}$. The relative standard deviation of the method for NO_2^- determination is in the range of 0.5 to 2.5%.

Absorption Spectra

The absorption spectrum (NO_2^-, 0.8 ppm) plotted between wavelength vs. absorbance in the spectrophotometer is shown in figure 1. The λ_{max} is found at 475 nm

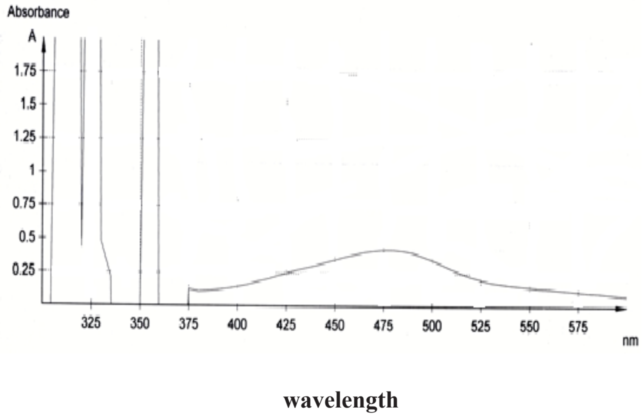
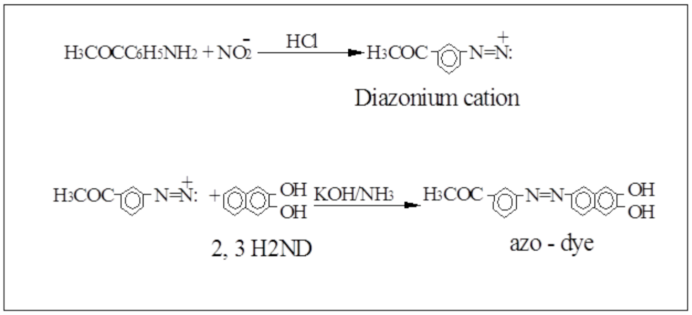


Figure 1: Plot of absorbance vs. wavelength for nitrite (0.8 ppm) determination using 2, 3- dihydroxynaphthalene.

Reaction Scheme

NO_2^- reacts with p-aminoacetophenone in HCl medium to form diazonium cation which is allowed to couple with the reagent , 2,3 dihydroxynaphthalene to produce a reddish- orange a azo dye.



Nitrate

A reddish-yellow nitro-product is formed when nitrate ion (NO_3^-) is allowed to react with 2,3-dihydroxynaphthalene in the presence of conc. H_2SO_4. Sulfuric acid concentration plays a crucial role in the reaction of NO_3^- with 2,3-dihydroxynaphthalene.

In what follows are given the details of studies carried out on variables affecting the reaction of 2,3-dihydroxynaphthalene. Unlike, phenoldisulfonic acid (PDA) which is used for the determination of NO_3^- in aqueous solution, here the determination of NO_3^- is carried out in non-aqueous solvent i.e, ethyl acetate. As the nitration reaction takes place at 10 to 18 mol.L^{-1} concentrated H_2SO_4, determination of NO_3^-in aqueous solution is little difficult because at such high concentration of H_2SO_4, handling of test solution during absorbance measurements is risky. Therefore, solvent extraction of the nitro product formed at minimum volume of aqueous sample solution (0.1 to 0.5ml) and at 10 to 18 mol.L^{-1} H_2SO_4 concentration stabilizes the nitro product formed of NO_3^- and 2,3-dihydroxynaphthalene. This way measurement of absorbance of the extracted nitro product is easily done.

Effect of Volume of Aqueous Aliquot on the Extraction of Nitro-Product

The effect of the volume of the aqueous sample aliquot on the maximum reaction of nitrate with 2,3-dihydroxynaphthalene was studied by varying the volume of the aqueous samplealiquot over the range 0.1 to 10 ml. It was found that at the least volume i.e., 0.1 to 0.5 ml of the aqueous sample aliquot the absorbance of the extracted nitro product remains constant, thereafter it decreases gradually with increase in the volume of aqueous sample aliquot.

Effect of Concentration of H_2SO_4

H_2SO_4 concentration plays a crucial role in the formation of nitro-product. In order to see the effect of H_2SO_4concentration on the maximum absorbance of the extracted nitro-product into ethyl acetate, the concentration of $H_2SO_4$was varied over a wide range i.e., 1 to 5 ml of 18 mol.L^{-1} H_2SO_4.

It is found that 1.0 ml concentrated H_2SO_4 (18 mol.L^{-1}) was found to be suitable for obtaining constant absorbance of the extracted nitro-product upto 0.5 ml of aqueous volume before extraction. After extraction of the nitro-product is over, no change in the absorbance was observed with the addition of water up to 10 ml for 10 ml of ethyl acetate used for extraction.

Effect of the Concentration of 2, 3-dihydroxynaphthalene

In order to see the effect, the reagent, 2, 3-dihydroxynaphthalene concentration on the nitration reaction, its concentration was varied in the range 0.01 to 1.0 g, keeping other variables constant. It was found that a 0.05 g of the solid reagent was found to be suitable for obtaining maximum absorbance of the extracted product.

Effect of Time of Extraction and Temperature on the color Formation

For maximum extraction of the nitro-product into the organic solvent (ethyl acetate), extraction for a period of 1 to 2 min was sufficient. Similarly, it was found that the extraction reaction was independent of temperature in the range, 20 to 80°C.

Beer's Law, Molar Absorptivity, Reproducibility and Stability of the Color Extracted

The Beer's law was obeyed in the range, 0.1 to 50 ppm (mg/L) NO_3^-. The molar absorptivity of the method was 3.2x10^3 L.mol^{-1}cm^{-1}. The color formed once, remains stable for 15-20 hours. The Relative Standard Deviation (RSD) of the method varied in the range, 1.0 to 2.5%.

Absorption Spectra

The absorption spectrum of the nitro-product was plotted between absorbance vs. wavelength in the range,300 to 600 nm. The ʎ$_{max}$ was found at 380nm (Figure.2).

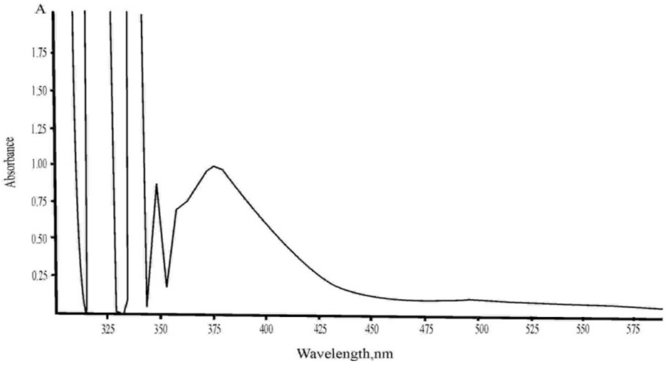


Figure 2: Plot of absorbance vs. wavelength for nitrate (20 ppm) determination using 2, 3- dihydroxynaphthalene.

Effect of Diverse Ions

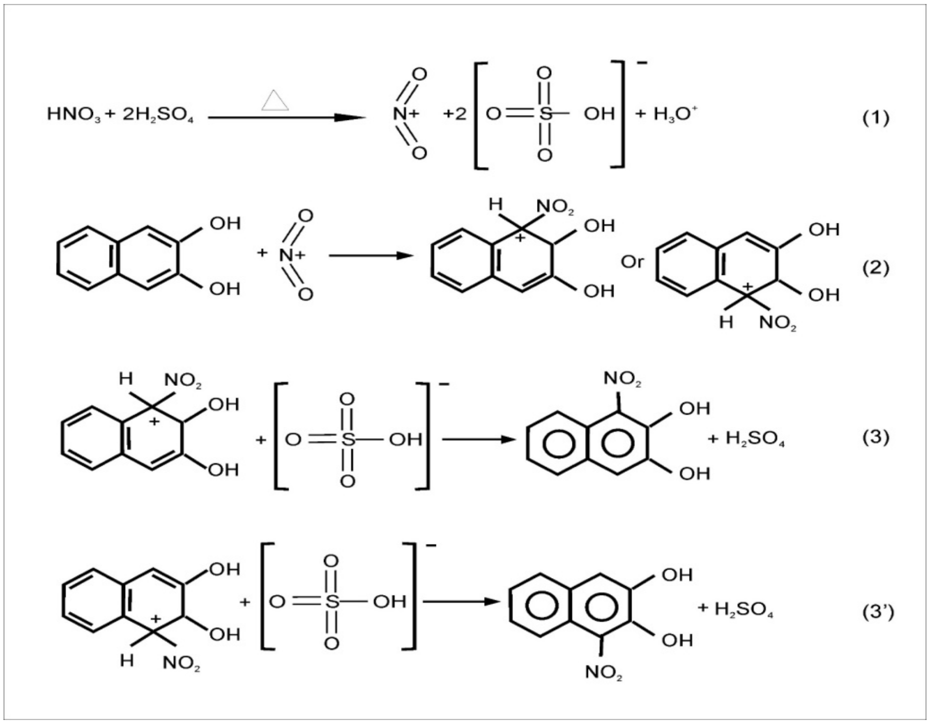
Effect of foreign ions on the color reaction was studied by adding separately increasing concentration of differentions to the 10 mg/L NO_3^- ion. The tolerance limits for the ions

NO_2^-(20), Cl^- (50) , Br^-, CH3COO$^-$, F^-, I^-, PO_4^{3-}, SO_4^{2-} , $C_2O_4^{2-}$(1000),Na^+, K^+, Ca^{2+}, Mg^{2+}, Be^{2+}, Ba^{2+}, Zn^{2+}, Cu^{2+}, Mo^{5+}(500), Fe^{3+}, Mn^{2+}, Cr^{3+}(100).

Like other reported methods for nitrate determination, chloride ion, if present more than 50 mg/L, starts interfering because in the presence of conc. H_2SO_4, chloride starts decomposing NO_3^- into NOCl, a volatile compound. Similarly, NO_2^-, if present, interferes seriously. It is must that before, determining NO_3^- by nitration reaction, prior removal of NO_2^- is done by decomposing the same by adding pinch of sulfamic acid.

Tentative Reaction Scheme

In the presence of conc. H_2SO_4 nitration of the reagent takes with NO_3^- ion as follows.



Application of the Method

The method thus developed has been thoroughly applied to a host of natural water (bore well water, pond, canal, sewage and effluent) samples. The results as shown in table-1 have been found to be favorably comparable with those obtained from standard methods [25, 26].

Sample No.	Nature of sample	NO_2^-, mg/L determined		NO_3^-, mg/L determined	
		Proposed method	Standard method**	Proposed method	Standard method***
W-1	Well water	3.65	3.6	25	24
W-3	Pond water-l	0.21	0.2	4	4.1
W-4	Bore-well water	0.1	0.09	5	4.8
W-5	Sewage water	ND*	ND*	190	187
W-6	Canal water-ll	ND*	ND*	23	22
W-7	Pond water-ll	ND*	ND*	20	20

W-8	Effluent-l	ND*	ND*	76	75
W-9	Effluent-ll	ND*	ND*	12	13
ND*- Not detected, **Sulphanilamide-NED, ***Chromotropic acid					

Table 1: Determination of nitrite and nitrate in water, waste water and effluent samples, n=5

Based on the satisfactory results obtained, the method is recommended for the routine determination of both nitrite and nitrate in the same sample.

Conclusions

It is a unique method for the determination of both nitrate and nitrite together in the same sample using the same reagent (2,3-dihydroxynaphthalene).

The advantages of the method are:

(i) It is rapid and using the same reagent both nitrite (NO_2^-) and nitrate (NO_3^-) can be easily determined in the same sample.

(ii) Being an extraction method, it is mostly free from interference.

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