



Rapid Spectrophotometric Determination of Trace Amounts of Nitrate-Nitrogen using Dapsone and α -Naphthol

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Abstract: A simple, rapid and sensitive spectrophotometric method has been developed for the trace determination of nitrite using Dapsone and α -Naphthol. The proposed method is based on the diazotization of Dapsone followed by coupling with α -Naphthol in sodium hydroxide medium to give an intense pink coloured azo dye. The dye shows absorption maximum at 540 nm. The molar absorptivity and Sandell's sensitivity being $5.749 \times 10^4 \text{ l mol}^{-1} \text{ cm}^{-1}$ and 0.24 ng cm^{-3} respectively. Tolerance limit for interfering ions and complexing agents were reported. Other analytical parameters and applications of the method for analysis of tap water, lake water, and industrial effluents have been investigated.

Keywords: Spectrophotometry, nitrite-nitrogen, α -naphthol, industrial effluent

Introduction

Inadequately treated effluents of various industries add the contamination of nitrite in water. The presence of nitrite in water is harmful as it causes methemoglobinemia and forms carcinogenic nitrosamine when present with secondary or tertiary amines^{1, 2}; it also affects the dissolved oxygen content of water. The maximum permissible limit as fixed by U.S. Health Association is $0.06 \mu\text{g cm}^{-3}$ in potable water³. Nitrite concentrations in fresh water are usually very low, 0.001 mg L^{-1} , $\text{NO}_2 - \text{N}$, and rarely higher⁴ than 1.0 mg L^{-1} . In view of the ubiquitous presence of nitrite in the environment, a sensitive method for trace level determination of nitrite is desirable.

Nitrite has been determined by chemiluminescence⁵, fluorometry⁶, polarography⁷, Raman spectroscopy⁸, and gas chromatography-mass spectrometry⁹. Although these methods are quite sensitive, they have several limitations. These methods are difficult, time consuming and requires fairly sophisticated equipments. Recently, many papers have been published on the determination of nitrite based on its catalytic effect on the oxidation of organic compounds by bromate¹⁰⁻¹² or hydrogenperoxide¹³.

These methods are highly sensitive, but suffer from careful control of the temperature and strong interference from sulphide, iodide, thiocyanate, vanadium and iron salts. Although nitrite can be determined by the above mentioned methods, the classical methods are spectrophotometric methods. Most spectrophotometric methods for the determination of nitrite in natural water and waste water were based on the Griess-Ilosvey reaction¹⁴, which was modified several times. Many methods other than diazotization reaction were reported for the determination of nitrite^{15,16}, they are prone to problems of sensitivity and lack of selectivity, mainly due to interference. The spectrophotometric determination of nitrite in various samples by diazotization-coupling techniques¹⁷⁻¹⁹ is a very important and common analysis.

Experimental

Instrument

“SYSTRONICS SPECTROPHOTOMETER 106” model was used for electronic spectral measurements with 10 mm matched quartz cells.

Reagents

All chemicals used were of analytical grade and double distilled water was used throughout the experiments. Standard nitrite stock solution ($1000 \mu\text{g ml}^{-1}$) was prepared by dissolving 150 mg of pre-dried sodium nitrite in de-aerated doubly distilled water and standardized²⁰, a small amount of chloroform was added as a stabiliser and a pellet of sodium hydroxide was added to prevent the liberation of nitrous oxide. The working standard was prepared by appropriate dilution of the stock with de-aerated, doubly distilled water. 0.1%(w/v) dapsone was prepared in 5% ethanol, 0.5% (w/v) α -naphthol in 100 ml of 1.0 mol dm^{-3} sodium hydroxide. 2 mol dm^{-3} hydrochloric acid was prepared in double distilled water. Solutions of foreign ions were prepared according to west²¹.

Sample Preparation

A water sample taken for the determination of nitrite was collected in glass or polythene bottles. A 100 ml of water sample was placed in a beaker and 3.0 ml of 1.0% mercuric chloride solution was added and allowed to stand for 5 min., the solution was filtered to remove any sulphide and iodides present. A known volume of the filtrate was analyzed by the recommended procedure.

Determination of nitrite-nitrogen (NO_2^-)

An aliquot of a stock solution containing 1.25-20 μg of NO_2^- was transferred into a series of 25 ml calibrated flasks to which 1.0 ml of 0.1% dapsone, 1.0 ml of 2 mol dm^{-3} hydrochloric acid were added and the mixture was allowed to stand for 2 min. for diazotization to be completed. Then 2.5 ml of 0.5% α -naphthol in sodium hydroxide solution was added and diluted to the mark with water, mixed well. The absorbance of the solution was measured at 540 nm against the corresponding reagent blank. The calibration graph was constructed by plotting the absorbance against the amount of nitrite- nitrogen.

Results and Discussion

This method involves the diazotization of two primary amino groups in dapsone in hydrochloric acid medium and each diazonium group would then react with a molecule of α -naphthol in alkali by electrophilic substitution at 4-position to produce an intense pink azo dye. An investigation of the continuous variation method of diazotized dapsone and α -naphthol showed that diazotized dapsone interacts with α -naphthol in the ratio 1:2, similar results have been observed with mole ratio method. The reaction sequence based on the above result is shown in scheme 1. The pink coloured azo dye shows a maximum absorbance at 540 nm. The reagent blank had negligible absorbance at this wavelength. The absorbance spectra of the coloured species are presented in Figure 1.

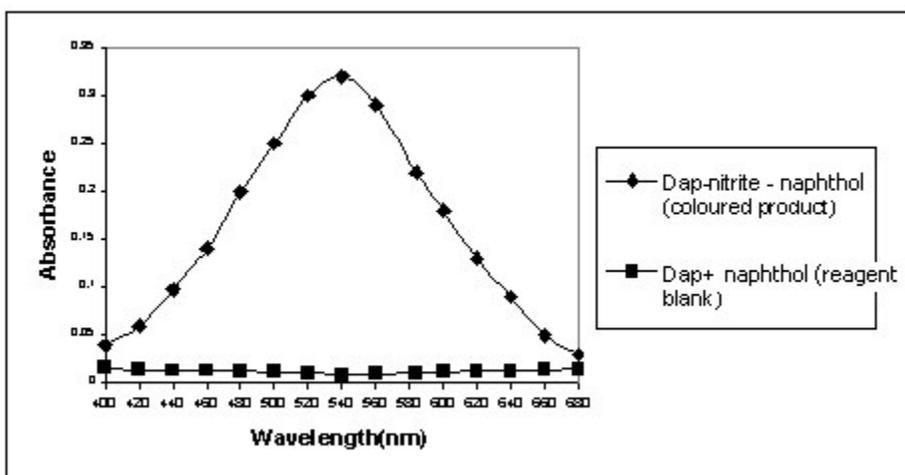


Figure 1 Absorption Spectra of the Reaction Product of Nitrite ($0.4 \mu\text{G MI}^{-1}$) and the Corresponding Reagent Blank

Reactions Conditions

Effect of acidity on diazotization

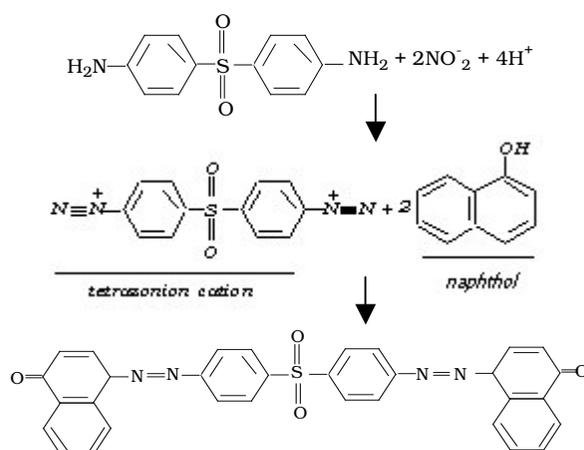
When no acid is present, diazotization does not take place. The effect of acidity on the diazotization was studied. The use of hydrochloric acid as the reaction medium was found to give the better results than use of sulphuric acid for diazotization. The hydrochloric acid concentration of at least 1.0 ml of 2 mol dm^{-3} was found necessary for complete diazotization. A 0.5 – 2.0 ml of 2 mol dm^{-3} hydrochloric acid range gave constant absorbance.

Choice of Compound to be Diazotised and its Concentration Effect on Diazotisation.

Many primary aromatic amines, including o-nitroaniline, p-aminosalicylic acid, p-aminobenzoic acid, and new sulfa compounds like sulfathiazole, sulfacetamide, sulfadiazine and dapsone were tested. Of these only dapsone gave satisfactory results. Moreover it is easily soluble in alcohol, giving colourless solution, is easily diazotized, and gives water soluble azo dye, under the conditions of the determination, thus eliminating the need for an extraction, a time consuming procedure. The effect of dapsone concentration was studied by using a fixed nitrite. It has been found that at least 0.8 ml of 0.1% dapsone

reagent is required to obtain maximum and constant absorbance. There is no appreciable change in the absorbance when the reagent concentration varies in the range of 0.8-1.2 ml of 0.1% dapson. Therefore 1.0 ml of 0.1% dapson was used for further studies.

Choice of coupling agent and its concentration effect with dapson as a diazotising agent, various coupling agents like tiron, resorcinol, 8-hydroxy-quinoline, α -naphthol and β -naphthol were tested for diazotization. α -naphthol gave most useful results and was further studied. Using fixed nitrite and dapson concentration on the colour intensity, 2.0-4.0 ml of 0.5% alkaline α -naphthol in 1.0 mol dm^{-3} was sufficient for complete coupling the diazotized dapson, and hence 2.5 ml of 0.5% an alkaline α -naphthol was used for further studies, a smaller volume decreased the absorbance.



Scheme 1 Proposed Reaction Mechanism of the Formation of Coloured Product.

Colour Stability

Under the optimized conditions, although the pink colour develops instantaneously, 5 min is sufficient to obtain the maximum and constant absorbance. The dye showed no change in absorbance in 48 h. The absorbance remained constant at $10\text{-}70^\circ\text{C}$.

Analytical Characteristics

The colour system was found to obey Beer's law in the range of $1.25\text{-}20 \mu\text{g}$ of nitrite per 25 ml ($0.05\text{-}0.8 \mu\text{g ml}^{-1}$) of dapson – NO_2^- - α -naphthol system. The molar absorptivity and Sandell's sensitivity were found to be $5.749 \times 10^4 \text{ l mol}^{-1} \text{ cm}^{-1}$ and 0.243 ng cm^{-2} respectively with limit of detection 1.12 ng cm^{-3} . The relative standard deviation was found to be 0.0017 for $0.4 \mu\text{g cm}^{-3}$ of NO_2^- .

Effect of Diverse Ions

The interference from foreign ions commonly found in water was studied by adding known amounts of foreign species to a solution containing $0.4 \mu\text{g cm}^{-3}$ of NO_2^- by this method. Sulphide and Fe^{2+} can be masked upto 200 and $400 \mu\text{g cm}^{-3}$ respectively by the additions of 1.0 ml of 1.0% mercuric chloride and 1.0 ml of 5% EDTA solution respectively. Fe^{3+} can be removed as hydroxide with 1.0 ml of 5 mol dm^{-3} and tolerated up to $50 \mu\text{g cm}^{-3}$. The tolerance limits of ions are given in the Table 1.

Table 1. Effect of Diverse Species on the Determination of Nitrite (0.4- $\mu\text{g MI}^{-1}$).

Foreign ions	Tolerance limit ($\mu\text{g ml}^{-1}$)
EDTA	60000
NO_3^- , Al^{3+} , Na^+ , Ca^{2+} , CO_3^{2-} ,	5000
SO_4^{2-} , Ba^{2+} , F^- , Hg^{2+} , Mg^{2+} , Cl^- Tartarate, PO_4^{3-}	4000
Cd^{2+} , K^+	2000
SO_3^{2-} , CH_3COO^- , SCN^- , Pb^{2+}	1500
Cu^{2+} , Fe^{3+} , V^{5+} , Ni^{2+} , Fe^{2+}	1000 ^a
Mn^{2+} , I^- , MoO_4^{2-} , Co^{2+} , Zn^{2+} , Sn^{2+} , CN^-	500
^b S^{2-}	400
Ag^+ , WO_4^{2-}	100

^a Metal ions are removed by precipitation as hydroxide up to 1000 $\mu\text{g ml}^{-1}$

^b Can be masked up to 400 $\mu\text{g ml}^{-1}$ by the addition of 1 ml of 1% HgCl_2

Applications

Analysis of Industrial Effluent.

Samples are collected in wide-mouthed plastic vessel at different points upstream and downstream from the source of industrial effluents. Samples of potable water were collected from different tanks and different points from lake water. Mercuric chloride (4 mg per 100 ml of sample) was used for preservation and samples were frozen at 0°C within 1 hr of sampling. Samples were filtered through a Whatman No. 41 paper before analysis.

Two sets of experiments were performed to check the validity of the method. In the first set, the volume of test solution was varied and the absorbance was plotted Vs volume taken. For all the samples linear plots were obtained, which could be extrapolated to the same point as that obtained for demineralised water ; this indicates that the determination was quantitative.

In the second set, recovery of NO_2^- was checked by adding various amounts of NO_2^- to a fixed volume of test solution. Recoveries were between 100-100.6%. The results are given in Table 2.

Table 2. Determination of Nitrite in Polluted Water and Tap Water.

Sample	Proposed method			Reported method ³¹	
	Nitrite added (μg)	Nitrite found (μg)	Mean recovery (%)	Nitrite found (μg)	Mean recovery (%)
Kukarally Lake ^a	-	0.1	-	Not found	-
Industrial Effluent	-	3.0	-	2.99	-
Tap Water ^b	0.4	0.4	100	0.4	100
	1.8	1.79	99.4	1.81	100.5
	3.0	3.02	100.6	3.01	100.6

^a Eutrophic Lake, ^b Tap water gave no test for nitrite.

Table 3. Comparison of Spectrophotometric Determination of NO_2^- with Other Methods.

Reagent	λ_{max} (nm)	Beer's law validity range, $\mu\text{g ml}^{-1}$	Molar absorptivity $\text{l mol}^{-1} \text{cm}^{-1}$	Remarks	Ref.
<i>p</i> - Aminoacetophenone + N- (1-Naphthyl) ethylene diamine	545	0.1-0.8	4.6×10^4	Cu^{2+} , Fe^{3+} , S^{2-} , and SO_2 interfered seriously	22
<i>p</i> - Aminobenziocacid + N-(1-Naphthyl) ethylenediamine	550	0.05-1.2	2.74×10^4	Fe^{2+} and Fe^{3+} interfered and less sensitive than the proposed method	23
<i>p</i> - rosaniline + phloroglucinol	420	0.04-0.48	Not reported	Cu^{2+} , Mn^{2+} , Pb^{2+} , Fe^{3+} , Cr^{6+} , V^{5+} , SO_3^{2-} , and S^{2-} interfered	24
<i>p</i> - nitroaniline + 2 - methyl - 8 - quinolinol	585	-	4.72×10^4	Most cations and anions interfered	25
Sulbutamol sulphate	410	1.84-27.6	1.8×10^3	Heating required at 100°C , narrow range of detection	26
5,7-dihydroxy-4-imino-2- oxychroman	361	0.1-5.0	9.2×10^3	Tedious extraction into butanol-ethyl acetate	27
Dapsone + phloroglucinol	425	0.008-1.0	4.28×10^4	-	28
Dapsone + α - naphthol	540	0.05-0.8	5.749×10^4	Simple, rapid and less detection limit	Present method

Conclusion

The proposed method is economical, fairly selective and precise. The method is more sensitive than some of the reported spectrophotometric methods. In this method, the maximum colour developments take place at ambient temperature and there is no need of heating. The azo dye of the reaction product is stable for a sufficient interval of time, which makes the method more practicable. The low values of relative standard deviation and percentage error indicate the high accuracy of the method and the method freedom from interference by a large group of foreign ions are advantages of this method. Therefore the method can be conveniently used for routine analysis of water and industrial effluent analysis.

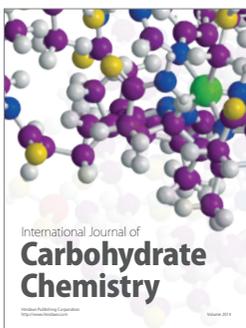
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