

Dapsone and Iminodibenzyl as Novel Reagents for the Spectrophotometric Determination of Trace Amounts of Nitrite in Water Samples

Padmarajaiah NAGARAJA,*[†] Mattighatta Shivaswamy HEMANTHA KUMAR,**
and Kanchugarakoppal S. RANGAPPA*

*Department of Chemistry, University of Mysore, Manasagangothri, Mysore-570 006, India

**Department of Environmental Science, University of Mysore, Manasagangothri, Mysore-570 006, India

A rapid, simple, sensitive and selective spectrophotometric determination of nitrite using new diazotizing and coupling reagents is described. The method is based on a diazotization-coupling reaction between dapsone and iminodibenzyl in a hydrochloric acid medium. The molar absorptivity and Sandell's sensitivity were found to be $7.5 \times 10^4 \text{ l mol}^{-1} \text{ cm}^{-1}$ and $0.000613 \mu\text{g ml}^{-1}$, respectively. The interference effects of various cations and anions were also studied and reported. This method has been found to be applicable for the determination of nitrite in various water samples.

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Because nitrites are undesirable in water due to its toxicity,¹ a great deal of interest has been generated concerning their potential health hazards. Nitrites are frequently used as preservatives in food products and their precursors are widely distributed in nature because of nitrogen fertilizers.² They oxidize hemoglobin to methemoglobin, which is unable to transport oxygen,³ and they react with amines and amides to form nitrosamines, which are potent carcinogens.^{4,5} Nitrite concentrations in fresh water are usually very low, 0.001 mg/l $\text{NO}_2\text{-N}$, and rarely higher than 1 mg/l $\text{NO}_2\text{-N}$.⁶ High nitrite concentrations are generally indicative of industrial effluents, and are often associated with an unsatisfactory microbiological quality of water. The maximum permissible limit in potable water is fixed by the U. S. public health service at 0.06 ppm.⁷

In view of the ubiquitous presence of nitrite in the environment, a sensitive and selective method for a trace-level determination of nitrite is desirable. Nitrite has been determined by chemiluminescence,⁸ fluorometry,⁹ polarography¹⁰ and Raman spectroscopy.¹¹ Although these methods are quite sensitive, they are plagued by several limitations. These methods are difficult, time consuming and require fairly sophisticated equipment. Recently, many papers have been published on the determination of nitrite based on its catalytic effect on the oxidation of organic compounds by bromate¹²⁻¹⁴ or hydrogen peroxide.¹⁵ These methods are highly sensitive, but suffer from careful control of the temperature and strong interference from sulfide, iodide, thiocyanate, vanadium and iron salts. Although nitrites can be determined by the above mentioned methods, the classical methods are spectrophotometric procedures. Several authors^{16,17} have proposed flow-injection analysis for determining nitrite through the formation of azo dyes. Most spectrophotometric methods for the determination of nitrite in natural and waste water were based on the Griess-Ilosvey reaction,¹⁸ which was modified

several times. Modifications by Saltzman¹⁹ and Jacobs-Hochheiser²⁰ are widely used and have been adapted as standard procedures.²¹ Although methods based on reactions other than diazocoupling reactions have also been developed for the determination of nitrite,^{22,23} they are prone to problems of sensitivity and a lack of selectivity, mainly due to interference. The spectrophotometric determination of nitrite in various samples by diazotization-coupling techniques²⁴⁻²⁶ is a very important and common analysis. It was the purpose of this work to introduce for the first time dapsone as a new substrate for diazotization coupling reactions and iminodibenzyl (IDB) as a new coupling agent which is economically cheaper than the most widely used coupling reagent, *N*-(1-naphthyl)-ethylenediamine dihydrochloride (NEDA), for the determination of nitrite in various water samples. Diazotized dapsone coupled with IDB in an acid medium gives an intense violet color azo dye. This method is more sensitive and selective than some recently reported methods,²⁷⁻²⁹ except for the porphyrin method.³⁰

Experimental

Reagents and chemicals

All of the chemicals used were of analytical reagent grade, and double-distilled water was used throughout the experiments. A standard sodium nitrite solution (Merck) was also used. A 1 mg/ml stock solution of nitrite was prepared by dissolving 150 mg of pre-dried sodium nitrite in 100 ml of demineralized water. A small amount of chloroform was added as a stabilizer. The working standards were prepared by appropriate dilution of the stock solution.

Dapsone solution (Sigma). A 0.1% solution in 5% ethanol was prepared from recrystallized dapsone.

Iminodibenzyl (BDH). A 0.5% solution in ethanol was used.

Hydrochloric acid (BDH). 1 M and 5 M hydrochloric acid was used.

[†] To whom correspondence should be addressed.
E-mail: nagarajap@mailcity.com

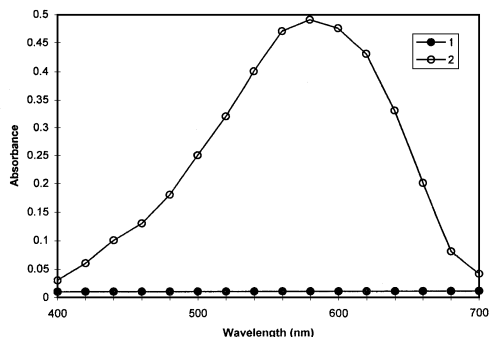


Fig. 1 Absorption spectra of the reaction product of (NO_2^- $0.3 \mu\text{g ml}^{-1}$). 1, Dapsone + IDB (reagent blank); 2, nitrite + dapsone + IDB system.

Apparatus

A JASCO Model UVIDE610 spectrophotometer with a 1.0 cm matched cell was used for electronic spectral measurements.

Procedure

To a solution containing $0.05 - 15 \mu\text{g}$ ($0.002 - 0.6 \mu\text{g ml}^{-1}$) of nitrite was transferred into a series of 25 ml calibrated flask, to which 1.0 ml of dapsone, 1.0 ml of 1 M hydrochloric acid were added; the mixture was allowed 2 min for diazotization. Then, 2 ml of IDB and 7 ml of 10 M hydrochloric acid were added. The maximum violet color developed within 5 min and the absorbance was measured at 570 nm against a reagent blank and a calibration graph was constructed.

Sample preparation

A sample taken for the determination of nitrite should be collected in glass or polyethylene bottles. Place 100 ml of a water sample in a beaker and add 3 ml of a 1% mercuric chloride solution. Let it stand for 5 min, then filter to remove any sulfides and iodide present. Analyze a known volume of the filtrate by the above procedure.

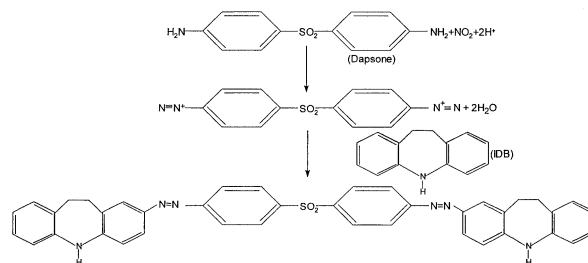
Results and Discussion

Absorption spectra

When IDB was added to the colorless diazotized dapsone in a hydrochloric acid medium, an intense violet azo dye was obtained. The absorption spectrum of the violet dye showed maximum absorption at 570 nm. Under the recommended conditions the dye was stable for up to 50 h, and absorbance varied by not more than 2% over a period of up to seven days. The absorption spectra of this violet azo dye and a colorless reagent blank are shown in Fig. 1.

Reaction conditions

The effect of acidity on the diazotization was studied. The use of hydrochloric acid as the reaction medium was found to give better results than a sulfuric acid medium for diazotization. For diazotization, a 1-ml volume of 1 M hydrochloride acid concentration is sufficient. A hydrochloric acid concentration of at least $5.0 - 10 \text{ ml}$ of 10 M was found to be necessary for the development of violet color; hence, a 7-ml volume of 10 M hydrochloric acid is recommended. With IDB as a new coupling agent, new sulfa compounds, like sulfathiazole, sulfacetamide, sulfadiazine, sulfamethoxazole and dapsone, were tested as a new substrate for diazotization; only dapsone



Scheme 1 Reaction of IDB with diazotized dapsone.

gave a maximum sensitive and a highly selective reaction under the condition for the determination. The effects of IDB and the dapsone concentration on the color intensity was studied by using a fixed nitrite concentration and varying IDB and dapsone concentrations. The results showed that the use of 1 - 4 ml of 0.5% IDB and 1 - 4 ml of 0.1% dapsone gave a constant and maximum absorbance. Therefore, the use of 2 ml of IDB and 1 ml of dapsone is recommended.

Color stability

Under the optimized condition, although the violet color develops instantaneously, 5 min is sufficient to obtain the maximum and constant absorbance. The dye showed no change in absorbance in 50 h. The absorbance remained constant for $10 - 50^\circ\text{C}$.

Reaction sequence and stoichiometry

For the diazotization process, it would be expected that two NH_2 groups in dapsone would be readily diazotized in a hydrochloric acid medium, and that each diazonium group would then react with a molecule of IDB by electrophilic substitution at the 4-position of the coupling agent to produce an intense violet azo dye. An investigation of the continuous molar variation of diazotized dapsone and IDB showed that diazotized dapsone interacts with IDB in the ratio of 1:2. Similar results have been observed with the mole-ratio method. A reaction sequence based on the above results is shown in Scheme 1.

Analytical characteristics

The color system was found to obey Beer's law in the range of $0.05 - 15 \mu\text{g}$ of nitrite per 25 ml ($0.002 - 0.6 \mu\text{g ml}^{-1}$) of the dapsone- NO_2^- -IDB system. The molar absorptivity and Sandell's sensitivity were found to be $7.5 \times 10^4 \text{ l mol}^{-1} \text{ cm}^{-1}$ and $0.000613 \mu\text{g cm}^{-2}$, respectively. The optimum concentration range evaluated by Ringbom's method is found to be between $0.02 - 0.5 \mu\text{g ml}^{-1}$ for $\log I_0/I = 0.001$. The standard deviation and relative standard deviation were found to be ± 0.005 and 1.20% for $0.3 \mu\text{g ml}^{-1}$ of nitrite.

Effects of diverse ions

The interference from foreign ions commonly present in water was studied by adding known amounts of foreign species to a solution containing $0.3 \mu\text{g ml}^{-1}$ of nitrite by this method, which is shown in Table 1. Metal ions, such as iron(II), iron(III), vanadium, copper and nickel, interfere seriously. These metal interferences can be easily overcome by adding 1 ml of a 1 M sodium hydroxide solution to the water sample and centrifuging the solution to remove the precipitated hydroxides prior to analysis. Sulfide interference can be overcome by the addition of 1.0 ml of 1% mercury chloride before a determination by the above procedure.

Table 1 Effect of diverse species on the determination of (0.3 ppm) nitrite

| Foreign ion | Tolerance limit/ $\mu\text{g ml}^{-1}$ |
|---|--|
| EDTA | 6000 |
| NO_3^- , Al^{3+} , Cl^- , Ca^{2+} , CO_3^{2-} , SO_4^{2-} , Na^+ , Ba^{2+} , F^- , Mg^{2+} , Hg^{2+} | 5000 |
| Tartarate, PO_4^{3-} | 4000 |
| Cd^{2+} , K^+ | 2000 |
| SO_3^{2-} , CH_3COO^- , SCN^- , Pb^{2+} | 1500 |
| Fe^{2+} , Fe^{3+} , Cu^{2+} , V^{5+} , Ni^{2+} | 1000 ^b |
| I^- , Mn^{2+} , MoO_4^{2-} , Co^{2+} , Zn^{2+} , CN^- , Sn^{2+} | 500 |
| Ag^+ , WO_4^{2-} | 100 |
| S^{2-} ^a | 20 |

a. S^{2-} can be masked up to $400 \mu\text{g ml}^{-1}$ by the addition of 1 ml of 1% HgCl_2 .

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

Table 2 Determination of nitrite in polluted water and tap water

| Sample | Nitrite added/ μg | Proposed method | | Reported method ²⁶ | |
|-----------------------------|------------------------------|------------------------------|---------------|-------------------------------|---------------|
| | | Nitrite found/ μg | Mean recovery | Nitrite found/ μg | Mean recovery |
| Kukarally lake ^a | — | 0.1 | — | Not found | — |
| Industrial effluent | — | 2.0 | — | 1.98 | — |
| Tap water ^b | 0.4 | 0.39 | 97.5 | Not found | — |
| | 1.0 | 0.99 | 99.0 | 0.98 | 98.0 |
| | 1.4 | 1.39 | 99.28 | 1.38 | 98.57 |

a. Eutrophic lake.

b. Tap water gave no test for nitrite.

Table 3 Comparison of spectrophotometric methods for the determination of nitrite

| | λ_{max} / nm | Range of determination, ppm | Sensitivity/ $1 \text{ mol}^{-1} \text{ cm}^{-1}$ | Remark | Ref. |
|---|--------------------------------|-----------------------------------|--|---|------|
| 5,7-Dihydroxy-4-imino-2-oxochroma | 361 | 0.005 – 0.28 | 2.3×10^4 | Extracted into butanol-ethyl acetate. | 31 |
| Salbutanol sulfate | 410 | 1.84 – 27.6 | 1.8×10^3 | Heating is required. | 32 |
| Rhodamine 6G | 445 | 0.0 – 3.0 | 1.2×10^4 | Less sensitive. | 23 |
| Sulfanilic acid & 1-naphthylamine | 520 | 1.4 – 35.0 | 3.3×10^4 | Not sensitive. | 19 |
| <i>p</i> -Nitroaniline & 2-thenoyltrifluoroacetone | 550 | 0.0 – 5.0 | 3.6×10^4 | Extracted into buton-1-ol. | 34 |
| 4-Aminosalicylic acid and naphth-1-ol | 520 | 0.1 – 3.0 | 1.4×10^4 | Cations strongly interfered with. | 33 |
| <i>p</i> -Aminobenzoic acid & <i>N</i> -(1-naphthyl)-ethylenediamine dihydro chloride | 550 | 0.05 – 1.2 | 2.74×10^4 | Iron(II) and iron(III) strongly interfered with. | 29 |
| Imipramine hydrochloride & 3-methyl-2-benzothiazolinone hydrozone hydrochloride | 640 | 0.02 – 7.0 | 4.9×10^3 | Less sensitive. | 28 |
| 5,10,15,20-Tetrakis(4-aminophenyl)porphine | 434 | 0.0 – 0.018 | 3.0×10^5 | One step reaction. Reacted for 30 min at 30°C . | 30 |
| Dapsone and IDB | 570 | 0.02 – 0.6 | 7.5×10^4 | Present method. | — |

Application

In order to assess the validity of the method, samples were collected from different sources and treated with mercuric chloride to remove any sulfide and as a preservative. Metal ions were removed by precipitation as hydroxides, as described in the earlier procedure. The samples were analyzed by the proposed and reported method (Table 2) and the results are in good agreement. The method was also compared to some reported spectrophotometric methods, and found to be more sensitive (Table 3).

Storage of sample

Never use acid preservation for samples to be analyzed for NO_2^- . Make the determination promptly on fresh samples to prevent bacterial conversion of NO_2^- to NO_3^- or NH_3 . For short-term preservation for 1 – 2 days, freeze at 20°C or store at 4°C .

Conclusion

The results clearly indicate the utility of the proposed method

for the analysis of various water samples. The present method is more sensitive than some of the reported methods. The rapid color development, excellent Beer's law curve and reproducibility as well as freedom from interference by a large group of foreign ions are advantages of this method. This method requires neither extraction nor heating.

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