New reagent for determination of trace levels of nitrite in environmental samples

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A local industrial product, an intermediate dye, disodium salt of 4,4-diaminostilbene-2,2-disulphonic acid is introduced as a new reagent for a selective and sensitive spectrophotometric determination method of trace levels of nitrite in environmental samples like, water and soil. The method is based on the tetrazo coupling reaction of disodium salt of 4,4-diaminostilbene-2,2-disulphonic acid with a coupling agent of 2-naphthol. The stable water soluble pink azo dye formed under aqueous alkaline condition has an adsorption maximum at 520 nm. The reaction is fast. The dye formed is stable for more than 48 h. Beer’s law is obeyed between 0.04-1.6 ppm of nitrite in a final volume of 25 mL. The molar extinction coefficient of the color system is \(3.0 \times 10^5\) L/mole/cm. Sandal sensitivity is \(1.5335 \times 10^{-3}\) ppm/cm. The relative standard deviation is 2.1 per cent for 10 determinations at 10 ppm level. The method is applied to the determination of nitrite in water and soil samples and the results are compared with the results obtained from the standard method.

Keywords: Nitrite determination, Spectrophotometry, 4,4-diaminostilbene-2,2-disulphonic acid, 2-Naphthol, Pink azo dye

Introduction

The determination of nitrite in environmental samples is of ecological importance, since it is reported that nitrite compounds cause bladder cancer and methenoglobinemia. Nitrite is an active form of nitrogen cycle, resulting from incomplete oxidation of ammonia or from reduction of nitrates. Sometimes proteins are hydrogenated to secondary amines by mycobacteria and these, in turn, promote the formation of carcinogenic N-nitrosamines. Nitrites generally enter surface waters through industrial effluents from food industry, dye industry, petrochemicals, and fertilizers. Owing to these properties analytical methods for nitrites are of interest for application in environmental samples.

It is observed that lot of development in the production of optical brightening agents from the stilbene series has taken place. The principal intermediates in the production of these substances are 4,4-dinitrostilbene-2,2-disulphonic acid (DNSDSA) and 4,4-diaminostilbene-2,2-disulphonic acid (DASDSA). These two intermediates in addition to optical brightening agent are also being manufactured in the Kurnool city. The analytical uses of these intermediates have already been tested. Therefore, it is interesting to introduce 4,4-diaminostilbene-2,2-disulphonic acid (DASDSA) as a new reagent for the spectrophotometric determination of nitrite in the present study.

Many methods have been used for the determination of nitrite. However the most widely used methods are spectrophotometric, most of them based on diazotization. The present paper introduces a new reagent, disodium salt of 4,4-diaminostilbene-2,2-disulphonic acid for the determination of nitrite. The method is based on the tetrazo coupling reaction of disodium salt of 4,4-diaminostilbene-2,2-disulphonic acid with a coupling agent, 2-naphthol. The pink azo dye formed under aqueous alkaline condition has an absorption maximum at 520 nm.

Materials and methods

Apparatus

Chemito UV 2100, UV-Visible spectrophotometer is used for all measurements with 1 cm glass cells. Elico digital pH meter model L1-120 is used for pH adjustments, wherever necessary.

Reagents

All chemicals used are AR grade and distilled water is used for preparing the reagent solutions.
Experimental Procedure

To a series of 25 mL calibrated flasks containing 1 - 40 ppm of nitrite in 1 mL of sample solution, 1 mL of 0.1 M hydrochloric acid solution was added. Mixed well and further added 1.5 mL of 0.1 per cent of disodium salt of 4,4-diaminostilbene-2,2-disulphonic acid solution. Mixed well and it was allowed to stand for 2 min. Added 1 mL of 0.5 per cent 2-naphthol and 0.5 mL of 2 M sodium acetate solution and diluted up to the mark with distilled water. The pink colour developed immediately and was stable for more than 48 h. The absorbance was measured against a reagent blank at 520 nm using 1 cm cell. A straight-line calibration graph passing through the origin was obtained, indicating that Beer’s law was obeyed in the range 0.04 – 1.6 ppm of nitrite in a final volume of 25 mL. The apparent molar absorptivity (referred to nitrite) from the slope of calibration graph was 3.0 X 10^4 L/mol/cm.

Nitrite in Water samples

10 mL water sample was treated with 1 mL 1 M NaOH and 1 mL 0.2 M EDTA. The solution was mixed and centrifuged to remove any precipitate formed. The pH of centrifuge was adjusted to 2 with help of 0.1 M HCl. The pH adjusted centrifuge was transferred to a 25 mL calibrated flask. This mixture was treated with 1.5 mL of 0.1 per cent disodium salt of 4,4-diamino-2,2-disulphonic acid solution, mixed well and allowed to stand for 2 min. 1 mL of 0.5 per cent 2-naphthol solution followed by 0.5 mL of sodium acetate solution were added and diluted to 25 mL with distilled water. Concentration of nitrite was established by reference to the calibration graph prepared.

Nitrite in Soil Samples

A known weight of soil sample (10 g) was placed in a 100 mL beaker and extracted 4-times with 20 mL portions of 1 per cent Na, CO₃. The extract was filtered and made up to 100 mL with distilled water. For nitrite determination, 50 mL of the made up solution was used for colour development by following procedure.

Results and Discussion

In the following experiments, 10 ppm of nitrite was taken and the final volume is made up 25 mL.

Adsorption Spectrum

When nitrite was treated, a pink azo anion was formed. Figure 1 shows the absorption spectra of the azo dye and the reagent blank. The characteristic absorption at 520 nm, shown by the dye under the conditions of the preliminary investigations and of the proposed procedure was adopted for all subsequent experiments.

Choice of Diazonium Component

There appear to be several distinct advantages in using disodium salt of 4,4-diaminostilbene-2,2-disulphonic acid for nitrite determination. It is a new reagent and moreover manufactured locally. The molecule has extended conjugation. After coupling to 2-naphthol, an azo dye with a higher absorbivity was formed.
The effect of disodium salt of 4,4-diaminostilbene-2,2-disulphonic acid concentration on the colour intensity was studied by adding 1.5 mL of 0.01 – 0.5 per cent solutions of the acid in 1 mL of 0.1 M HCl to a series of nitrite solutions. The results have shown that 0.1 per cent of disodium salt of 4,4-diaminostilbene-2,2-disulphonic acid solution is the optimum and hence 0.1 per cent solution of the acid was used in the proposed procedure.

**Hydrochloric Acid Concentration**

Similar experiments were carried out in which the HCl was varied between 0.01 – 0.5 M. 0.1 M HCl gave the maximum colour. Hence, 0.1 M HCl was selected for proposed procedure. Other mineral acid, H₂SO₄, was also tested and found to be unsatisfactory.

**Coupling Agent Concentration**

To a series of nitrite solutions, in 0.1 to 2.5 mL quantity of 0.5 per cent 2-naphthol in methanol were added. 1.0 – 1.5 mL gave the maximum absorbance and therefore 1.0 mL of 0.5 per cent 2-naphthol solution was used.

**Sodium Acetate Concentration**

The effect of the sodium acetate concentration on the absorption was studied. 0.2 to 2.5 mL of 2 M sodium acetate solution was examined. The investigations showed that 0.5 – 2.0 mL gave maximum absorbance, and 0.5 mL was chosen, although the pink colour was formed with 0.1 mL.

**Mechanism of Colour Formation**

The steps involved in the formation of the pink azo dye are shown in Scheme 1.

**Interference Studies**

The interfering effects of various ions at 40 ppm level for determination of 0.4 ppm of nitrite in 25 mL by the proposed method are as follows.

\[
\begin{align*}
\text{NO}_3^-, \text{F}^-, \text{SO}_4^{2-}, \text{CO}_3^{2-}, \text{HCO}_3^-, \text{SO}_3^-, \text{Cl}^-, \text{PO}_4^{3-}, \\
\text{IO}_3^-, \text{Br}^-, \text{Mg}^{2+}, \text{Al}^{3+}, \text{Pb}^{2+}, \text{Mn}^{2+}, \text{Zn}^{2+}, \text{Hg}^{2+}, \text{Sn}^{4+}, \\
\text{Cu}^{2+}, \text{Zn}^{2+}, \text{La}^{3+}, \text{Na}^+, \text{K}^+, \text{titanate, tantalate, silicate, oxalate}, \\
\text{and 2 per cent formaldehyde} \\
\text{SO}_4^{2-}, \text{SiO}_2^4, \text{SiO}_2^5, \text{Fe}^{3+}, \text{Cd}^{2+}, \text{Cu}^{2+}, \text{Ni}^{2+}, \text{AdO}^{2-} \rightarrow \text{Pink colour not developed} \\
\text{Bi}^{3+}, \text{Fe}^{3+} \rightarrow \text{Light pink colour developed} \\
\text{Cr}^{3+}, \text{Co}^{3+}, \text{Ni}^{2+}, \text{Cu}^{2+}, \text{Mo}^{6+} \rightarrow \text{Pink colour developed with interference}
\end{align*}
\]

The interference of SO₄²⁻ and S₂O₃²⁻ was removed by treating with 2 mL of 2 per cent formaldehyde solution. The Cu²⁺ and Cr³⁺ were masked by addition of 1 mL of 0.2 M EDTA. The Ca²⁺, Cd²⁺ and Ni²⁺ metal ions eliminated by precipitation as hydroxide by adding 1 mL of 1 M NaOH. Addition of 1 mL of 1000 ppm fluoride eliminated the interference of Fe³⁺. The interference of Mo⁶⁺ was eliminated by addition of 10 percent potassium thiocyanate. Addition of 2 mL of 5 per cent oxalic acid eliminated the interference due to the presence of 20 ppm of Bi⁵⁺.

**Application of the Method**

To 10 mL water, 1 mL of 1 M NaOH was added and the solution was centrifuged. To the centrifugate, 1 mL 0.2 M EDTA was added. The pH of this solution was adjusted to 2 and analyzed for nitrite by proposed method. These results were compared with that obtained by a standard method⁵ and are given in Table 1.

The proposed method is also used for the determination of nitrite in some soil samples. The nitrite is extracted 4-times with 20 mL aliquots of 1 per cent Na₂CO₃. The extract obtained was made up to 100 mL with distilled water.

To a 50 mL of the made up solution, 10 mL of 1 M NaOH was added and the solution was centrifuged. The
Scheme 1 — Since it is a symmetrical molecule both two above structures are one and the same

Table 1 — Determination of Nitrite in Water and Soil samples

<table>
<thead>
<tr>
<th>SI No.</th>
<th>Name of the sample</th>
<th>Amount of nitrite(ppm)</th>
<th></th>
<th></th>
<th></th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Added</td>
<td>Present method</td>
<td>Standard method</td>
<td>Present in sample</td>
</tr>
<tr>
<td>1</td>
<td>Tap water</td>
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<td>0.10</td>
<td>0.12</td>
<td>0.11</td>
</tr>
<tr>
<td>2</td>
<td>Bisleri water</td>
<td></td>
<td>0.21</td>
<td>0.19</td>
<td>0.20</td>
</tr>
<tr>
<td></td>
<td>(Acqua minerals)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Soils:</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(a) GPREC</td>
<td></td>
<td>0.46</td>
<td>0.48</td>
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<tr>
<td></td>
<td>(b) Code I</td>
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<td>0.65</td>
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</tr>
<tr>
<td></td>
<td>(c) Code II</td>
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<td>0.24</td>
<td>0.24</td>
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<tr>
<td></td>
<td>(d) Tolasipuram</td>
<td></td>
<td>0.18</td>
<td>0.22</td>
<td>0.22</td>
</tr>
<tr>
<td></td>
<td>(e) Rollavanka</td>
<td></td>
<td>0.16</td>
<td>0.16</td>
<td>0.16</td>
</tr>
</tbody>
</table>

Remarks, if any:

- Sample volume is not reduced.
- Sample volume is reduced.

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pH of the centrifugate was adjusted to 7 and the volume of pH adjusted solution was reduced to 10 mL by evaporating on water bath. 1 mL of this solution was taken for nitrite determination by proposed method. These results were compared with that obtained by a standard method\(^5\) and are shown in Table 1.

**Acknowledgement**

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**References**

5. Bacek J, Berka A & Jakubec K. The use of redox reactions in the analysis of dyes and dye production intermediates VI-