Chapter - 4
A Sensitive Spectrophotometric Method for the Determination of Nitrite using Phloroglucinol in Various Environmental Samples.

Summary

A sensitive spectrophotometric method for the determination of nitrite, a very common pollutant at ppm level is described. Proposed method is based on Greiss reaction. Nitrite reacts with p-aminoacetophenone and forms diazonium cation in the acidic medium, which is subsequently coupled with phloroglucinol to form yellow orange dye in alkaline medium having an absorption maximum at 430 nm. The dye is stable and extractable in n-butanol. Beer’s law is obeyed in the range of 0.8 to 8.0 µg per 25 ml of nitrite solution in aqueous medium and 0.1 to 1.0 µg per 100 ml of nitrite solution in extractive system. The molar absorptivity and Sandell’s sensitivity were found to be $1.045 \times 10^5 \text{l mol}^{-1} \text{cm}^{-1}$ and 0.00044 µg cm$^2$ respectively. The method has been successfully applied for the determination of nitrite in polluted water, soil and coke oven effluent.

Published in Chemical Environment Research, 13 (384), 2004, p195-201
Introduction

Nitrite is one of the most important pollutants present in water, soil and preserved food. It is formed in nature as an intermediate compound in nitrogen cycle. It consists of various processes such as nitrification, assimilation, denitrification and fixation of nitrogen etc (1, 2). Nitrification has great importance especially in polluted waters where ammonia, discharges from inadequately treated effluents can constitute toxicity problem. It is also formed by the biodegradation of nitrogenous organic matter and affects biochemical oxygen demand (BOD) of water (3-4).

Nitrite is used in the manufacturing of fertilizers, detergents, dyes as well as in various industries like wood pulping, sugar mill, synthetic fiber making. Effluent from those industries increases the nitrite concentration up to toxic level. It is also used as food preservative for cured meat products and fish, colour enhancing agent and anticorrosive agent (5, 6). It is encountered in the environment by the reduction of less toxic nitrates to more toxic nitrites by micro-organisms. Nitrite containing vegetables like cauliflower, spinach, broccoli, root vegetables and drinking water, cured meat product are the main source of exposure. Nitrite exposure can also occur from certain medications such as antidiarrheal, antimalarial, antidotes for cyanide and hydrogen sulfide poisoning. The abundance of nitrites in water leads into eutrophication in nature. The effluents from domestic water and waste water treatment plants add to its contamination in water (7-9).

Nitrite has been considered as a potentially hazardous compound. It can easily react with secondary amines, tertiary amines and amides to form nitrosamines. Nitrite has suspected as carcinogens and teratogens. (10-12). Use of foodstuffs and drinking water preserved and contaminated with nitrite is objectionable due to its role in causing symptomatic methaemoglobinemia, in which hemoglobin becomes incapable of transporting oxygen due to oxidation of ferrous salts to ferric salts (13-18). Recent studies showed the presence of several N-nitrosamines in normal human urine,
supporting the endogenous reaction of nitrite with amino acids (19-22). The maximum permissible limit specified by the U.S. Public Health Association is 0.06 μg ml\(^{-1}\) of nitrite in potable water (23-24).

Looking to its important role as an atmospheric precursor in the formation of N-nitrosamines and its objectionable presence in water, the determination of nitrite is desirable. Various methods for its determination using different techniques such as flow injection analysis (25), flow injection catalytic spectrophotometry (26), high performance liquid chromatography (27), potentiometry (28), voltametry (29), catalytic photometry (30), capillary electrophoresis (31, 32), fluorimetry (33), polarography (34), etc. are reported in literature.

Many spectrophotometric methods (35-37) have also been reported for the determination of nitrite in various environmental samples. Most of these methods are based on Greiss reaction (38) which involves the formation of diazonium cation by the reaction of aromatic amine with the nitrite in the acidic medium and the subsequent coupling of it with an aromatic compound containing amino or hydroxy substituents. Following above reaction, various methods (39-44) involving reagents like p-rosaniline (45), p-amino benzoic acids (46), mercaptoacetic acid (47), o-nitroaniline - amino naphthalene-2-sulphonic acid (48), p-aminoacetophenone – NEDA (49), PNA – phloroglucinol (50), iodide – LCV (51), neutral red (52), acridine red (53), dapsone-phloroglucinol (54), dapsone- iminodibenzy1 (55) sulfathiazole– NEDA (56), PNA-ethoxyethylene maleicester and ethylcyano acetate (57), PNA- diphenylamine(58) barbituric acid (59) etc. have been reported.

In the present paper a new method based on Greiss reaction has been proposed. Nitrite reacts with p-aminoacetophenone in acidic medium to form a diazonium cation which is subsequently coupled with phloroglucinol to form a yellow orange dye in alkaline medium, having an absorption maximum at 430 nm. The dye is extractable in n-butanol which increases the sensitivity several times. The \(\lambda_{\text{max}}\) of the extracted dye is 440 nm.
Experimental

Apparatus

A Systronics spectrophotometer (Model 109) and Systronics pH meter (Model 331) were used for spectral and pH measurement respectively.

Reagents

All chemicals used were of Analytical Reagents or the best available grade. All solutions were prepared in double distilled water and it was also used throughout the experiment.

Standard Sodium Nitrite Solution (Loba Chemie, Mumbai)

A stock solution (1 mg ml$^{-1}$) containing 0.15 g per 100 ml was prepared in demineralized water. A small amount of chloroform as stabilizer was added and kept in amber coloured flask in cold. Working standard was prepared by appropriate dilution of the stock.

p-Aminoacetophenone (PAAP, Loba Chemie, Mumbai)

1 % (w/v) solution was prepared in 1:4 hydrochloric acid.

Phloroglucinol (Loba Chemie, Mumbai)

1 % (w/v) solution was prepared in 1:4 distilled water.

Sodium Hydroxide (Loba Chemie, Mumbai)

0.2 M aqueous solution was prepared.

EDTA (Merck, Mumbai)

A 10 % (w/v) aqueous solution of disodium salt of EDTA was prepared.
Procedure

For Aqueous System

Aliquots of stock solution containing 0.8 to 8 μg of nitrite was taken in a 25 ml volumetric flask (0.032 to 0.32 μg ml⁻¹). To it 1.0 ml of p-aminoacetophenone was added and kept for two minutes with occasional shaking to ensure complete diazotization, then 1.0 ml of phloroglucinol solution was added and volume was made up to the mark with 0.2 M sodium hydroxide solution. The absorbance of the yellow orange dye was measured at 430 nm against reagent blank.

For Extractive System

Aliquot of stock solution containing 0.1 to 1.0 μg of nitrite was transferred into a 100 ml separatory funnel and 1.0 ml of p-aminoacetophenone was added. Solution was allowed to stand for 2 minutes with occasional shaking. Then 1.0 ml of phloroglucinol was added for coupling and pH was adjusted to 11 to 12 by adding 0.2 M sodium hydroxide. After 5 minutes yellow orange dye formed was extracted with 10 ml and 5 ml aliquots of n-butanol dried over anhydrous sodium sulphate and the absorbance was measured at 440 nm. A reagent blank extracted in n-butanol showed negligible absorbance at this wavelength.

Results and Discussion

Spectral Characteristics

The yellow orange dye showed maximum absorbance at 430 nm in aqueous system and at 440 nm in extractive system.

Effect of Reagent Concentration

1.0 ml of 1% PAAP was required for complete diazotization. Similarly it was observed that 1.0 ml of 1% phloroglucinol was sufficient for complete coupling.
Effect of Time and Temperature

Two minutes time was sufficient for coupling reactions and 5 minutes time was required for full colour development. No significant changes were observed in the absorbance values within the temperature range of $10^0 - 60^0$ C. So all the measurements were carried out at room temperature. Dye is stable for 24 hours in aqueous medium as well as in extractive system.

Effect of pH

Effect of varying pH on the colour reaction has also been studied. It was found that $1.5 - 2.0$ pH was necessary for complete diazotization. Maximum colour development was obtained at pH 11-12 by adding $0.2$ M of sodium hydroxide solution.

Sensitivity and Precision

The colour system was found to obey Beer's law in the range of $0.8$ to $8.0$ µg of nitrite per 25 ml ($0.032 - 0.32$ µg ml$^{-1}$) in the aqueous and $0.1$ to $1.0$ µg of nitrite per 100 ml ($0.001$ to $0.01$ µg ml$^{-1}$) in the extractive system. The detection limit ($D_L=3.3\sigma/S$) and quantification limit ($Q_L=10\sigma/S$) [where $\sigma$ is the standard deviation of reagent blank ($n=7$) and S is the slope of the calibration curve] of nitrite determination were calculated.

The precision of the method was checked by seven replicate analysis of $4$ µg per 25 ml nitrite solution in aqueous medium. The correlation coefficient of the calibration graph was found to be 1. The various statistical and photometric parameters were evaluated and mentioned in Table 1.

Effect of Foreign Ions.

The effect of various foreign ions was studied by adding known amounts of foreign species to a solution containing $4$ µg of nitrite per 25 ml. It was observed that most of the cations and anions do not interfere. Common interferents $Cu^{2+}$ and $Fe^{3+}$ were found not to interfere up to 1000 µg ml$^{-1}$ when masked with 1 ml solution of 10% EDTA solution. The tolerance limits for the ions observed are given in the Table 2.
Colour Reaction

The proposed colour reaction is based on Greiss reaction. The colour reaction involves two steps which is shown in scheme 1.

1. Nitrite reacts with p-aminoacetophenone in acidic medium and forms a diazonium cation.
2. Diazonium ion couples with phloroglucinol in the alkaline medium and forms yellow orange azo dye.

Applications

It has been reported in literature that small amount of nitrite were found in polluted water, soil, effluent from steel plant. The proposed method has been satisfactorily applied for the determination of nitrite in water samples, soil and in effluent from coke oven plant. The method was valid for samples collected naturally and for those prepared synthetically.

Determination of Nitrite in Water.

In order to check the validity of the proposed method in river water, effluent water and tap water, samples were collected from the different regions. Samples were preserved by treating with 2 ml of mercuric chloride (4 µg per 100 ml) and stored at 0°C. They were filtered through Whatman filter paper No.41 before analysis. Since the samples from tap water were found to be free from nitrite, synthetic samples were prepared by the addition of known amounts of nitrite and analysed by the proposed and reported method (51).

Determination of Nitrite in Soil

Soil samples of manured garden soil, farmland soil and road side soils were taken. 5 g of the soil was dried at 55°C in an oven for 12-16 hours. The dried sample was grind, passed through a 2-mm mesh sieve, sufficient water (containing 1 or 2 drops of concentrated sulfuric acid) was poured on to soak the soil completely. After
a few minutes it was diluted, filtered and leached with water. The filtrate was made upto 100 ml, then aliquots were analysed by the proposed and reported method (51).

Determination of Nitrite in Effluent from Coke Oven

To investigate the applicability of the proposed method various samples of effluent from coke oven of steel plant was collected. Since they gave negative test, synthetic samples were prepared by adding known amounts of nitrite. Then samples were analysed by proposed and reported method (51).

Conclusion

The proposed method has been compared with other spectrophotometric method reported for nitrite and found to be more simple, sensitive and extractive (Table 4). Most of the ions present in the polluted water do not interfere and 95-99 % recovery was obtained. The present method is successfully applied for the determination of nitrite in polluted water, soil and effluent from coke oven.
Scheme 1: Colour Reaction of Nitrite

**Step I - Diazotization**

\[
\text{NO}_2^- + H^+ + \text{p-Aminoacetophenone} \xrightarrow{\text{Acidic Medium}} \text{Diazonium Cation of p-Aminoacetophenone}
\]

**Step II - Coupling**

\[
\text{Diazonium Cation of p-Aminoacetophenone} + \text{Phloroglucinol} \xrightarrow{\text{Alkaline Medium}} \text{Yellow Orange Colour Azo Dye}
\]

\[\lambda_{\text{max}} = 430 \text{ nm}\]
Fig 1: Absorption Spectrum of coloured complex
A. Concentration of nitrite, 8 μg/ 25 ml
B. Reagent blank

Fig 2: Calibration curve for the determination of nitrite
Concentration of nitrite, μg /25 ml
Fig 3: Effect of reagent concentration
Concentration of nitrite, 4 µg/25 ml

Amount of phloroglucinol, ml [B]

Amount of p-aminoacetophenone, ml [A]

Fig 4: Effect of time on final reaction
Concentration of nitrite 4 µg/25 ml
Fig 5: Effect of pH on final reaction
Concentration of nitrite, 4 μg / 25 ml
Table 1: Photometric and Statistical Parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beer's law range (µg ml(^{-1}))</td>
<td></td>
</tr>
<tr>
<td>1. Aqueous system</td>
<td>0.032-0.32</td>
</tr>
<tr>
<td>2. Extractive system</td>
<td>0.001-0.01</td>
</tr>
<tr>
<td>Molar absorptivity (1 mol(^{-1}) cm(^{-1}))</td>
<td>2.86 x 10(^5)</td>
</tr>
<tr>
<td>Sandell's sensitivity (µg cm(^{-2}))</td>
<td>0.0004</td>
</tr>
<tr>
<td>Detection limit (µg ml(^{-1}))</td>
<td>0.026</td>
</tr>
<tr>
<td>Quantification limit (µg ml(^{-1}))</td>
<td>0.08</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>±0.015</td>
</tr>
<tr>
<td>Relative standard deviation</td>
<td>3.316%</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>1.00</td>
</tr>
<tr>
<td>Regression equation*</td>
<td></td>
</tr>
<tr>
<td>1. Slope (m)</td>
<td>0.1</td>
</tr>
<tr>
<td>2. Intercept (b)</td>
<td>0.0</td>
</tr>
</tbody>
</table>

*\(y = mx + b\), Where \(y\) is the absorbance of the analyte and \(x\) is the concentration of analyte in µg ml\(^{-1}\).

Table 2: Effect of Foreign Ions in the Determination of Nitrite

Concentration of nitrite = 4 µg 25 ml.

<table>
<thead>
<tr>
<th>Foreign Ions</th>
<th>Tolerance Limits* (µg ml(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>SO(_4^{2-}), Bi(^{3+}), Al(^{3+})</td>
<td>1500</td>
</tr>
<tr>
<td>Hg(^{2+}), Sb(^{3+}), Pb(^{2+})</td>
<td>1300</td>
</tr>
<tr>
<td>Se(^{4+}), Zn(^{2+}), PO(_4^{3-})</td>
<td>2000</td>
</tr>
<tr>
<td>Cu(^{2+}), Fe(^{3+})</td>
<td>1000</td>
</tr>
<tr>
<td>V(^{5+}), Sn(^{4+}), F(^-)</td>
<td>400</td>
</tr>
<tr>
<td>CH(_3)C(_2)O(_2^{-})</td>
<td>350</td>
</tr>
</tbody>
</table>

* The tolerance limit is the amount of interferent that causes an error of ± 2% in the absorbance values.
### Table 3: Determination of Nitrite in Various Environmental Samples

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Samples</th>
<th>Nitrite Added (µg)</th>
<th>Proposed Method&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Reported Method&lt;sup&gt;51&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Nitrite Found (µg)</td>
<td>Recovery (%)</td>
</tr>
<tr>
<td>1.</td>
<td>River Water&lt;sup&gt;b&lt;/sup&gt; (5 ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>W&lt;sub&gt;1&lt;/sub&gt;</td>
<td>-</td>
<td>0.9 (± 0.020)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>W&lt;sub&gt;2&lt;/sub&gt;</td>
<td>-</td>
<td>1.0 (± 0.019)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>W&lt;sub&gt;3&lt;/sub&gt;</td>
<td>-</td>
<td>0.8 (± 0.019)</td>
<td>-</td>
</tr>
<tr>
<td>2.</td>
<td>Pond Water (5 ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>W&lt;sub&gt;4&lt;/sub&gt;</td>
<td>-</td>
<td>1.0 (± 0.021)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>W&lt;sub&gt;5&lt;/sub&gt;</td>
<td>-</td>
<td>1.0 (± 0.015)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>W&lt;sub&gt;6&lt;/sub&gt;</td>
<td>-</td>
<td>1.5 (± 0.020)</td>
<td>-</td>
</tr>
<tr>
<td>3.</td>
<td>Tap Water (5 ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>W&lt;sub&gt;7&lt;/sub&gt;</td>
<td>2</td>
<td>1.94 (± 0.017)</td>
<td>97.0</td>
</tr>
<tr>
<td></td>
<td>W&lt;sub&gt;8&lt;/sub&gt;</td>
<td>4</td>
<td>3.90 (± 0.021)</td>
<td>97.5</td>
</tr>
<tr>
<td></td>
<td>W&lt;sub&gt;9&lt;/sub&gt;</td>
<td>6</td>
<td>5.94 (± 0.019)</td>
<td>99.0</td>
</tr>
<tr>
<td>4.</td>
<td>Soil&lt;sup&gt;c&lt;/sup&gt; (5 g)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>S&lt;sub&gt;1&lt;/sub&gt;</td>
<td>-</td>
<td>0.9 (± 0.019)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>S&lt;sub&gt;2&lt;/sub&gt;</td>
<td>-</td>
<td>1.6 (± 0.020)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>S&lt;sub&gt;3&lt;/sub&gt;</td>
<td>-</td>
<td>1.9 (± 0.02)</td>
<td>-</td>
</tr>
<tr>
<td>5.</td>
<td>Coke Oven Effluent&lt;sup&gt;d&lt;/sup&gt; (5 ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>C&lt;sub&gt;1&lt;/sub&gt;</td>
<td>2</td>
<td>1.96 (± 0.021)</td>
<td>98.0</td>
</tr>
<tr>
<td></td>
<td>C&lt;sub&gt;2&lt;/sub&gt;</td>
<td>4</td>
<td>3.96 (± 0.02)</td>
<td>99.0</td>
</tr>
<tr>
<td></td>
<td>C&lt;sub&gt;3&lt;/sub&gt;</td>
<td>6</td>
<td>5.90 (± 0.019)</td>
<td>98.34</td>
</tr>
</tbody>
</table>

<sup>a</sup> Mean of seven replicate analysis.
<sup>b</sup> Samples were collected from different regions from the Shivnath river, which receives effluents from various industries.
<sup>c</sup> Amount of aliquots 5, 10, 15 ml.
<sup>d</sup> Samples were collected from different regions of effluent from coke oven of steel plant.
<table>
<thead>
<tr>
<th>S.No.</th>
<th>Reagents</th>
<th>Range (µg ml(^{-1}))</th>
<th>λ(_{max}) (nm)</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>p-Rosaniline - NEDA (45)</td>
<td>0.08-0.72</td>
<td>565</td>
<td>Fe(^{2+}), Cr(^{6+}), severely interfere</td>
</tr>
<tr>
<td>2</td>
<td>p-Aminophenyl - mercaptoacetic acid (47)</td>
<td>0.1 -1.6</td>
<td>495</td>
<td>S and Sb(^{3+}), interfere</td>
</tr>
<tr>
<td>3</td>
<td>p-Amino benzoic acid (46)</td>
<td>0.1 -1.3</td>
<td>519</td>
<td>Less sensitive</td>
</tr>
<tr>
<td>4</td>
<td>p-Aminoacetophenone - NEDA (49)</td>
<td>0.1 -0.8</td>
<td>545</td>
<td>Less sensitive</td>
</tr>
<tr>
<td>5</td>
<td>o-Nitroaniline, 1-amino naphthalene-2 Sulphonic acid (48)</td>
<td>0.08 -0.68</td>
<td>545</td>
<td>Less sensitive</td>
</tr>
<tr>
<td>6</td>
<td>p-Nitroaniline - phloroglucinol (50)</td>
<td>0.004-0.04</td>
<td>420</td>
<td>Cu(^{2+}) and Fe(^{3+}), interfere</td>
</tr>
<tr>
<td>7</td>
<td>Leucocystal violet (51)</td>
<td>0.004-0.04</td>
<td>590</td>
<td>Reagents costly</td>
</tr>
<tr>
<td>8</td>
<td>p-Aminoacetophenone + phloroglucinol (Proposed method)</td>
<td>0.001-0.01</td>
<td>430</td>
<td>Sensitive, extractive, cost effective reagent</td>
</tr>
</tbody>
</table>
Reference


2. ATSDR, Agency for Toxic Substance and Disease Registry, Department of Health and Human Services, Atlanta, U S, 2001.


