Nitrite/nitrate speciation through arsenomolybdenum blue complex at trace level: Application to Biological and Environmental samples

6.1 Introduction

Nitrate and nitrite are naturally occurring oxyanions of nitrogen and are present everywhere in the environment [1]. Of the two nitrates is the more stable form of oxidized nitrogen but can be reduced to nitrite by microbial action which is a highly reactive form of nitrogen. The chemical and biological processes can further reduce nitrite to various compounds or it can be oxidized to convert it into nitrate [2]. Nitrate has been used extensively in the agricultural sector as in fertilizers, in explosives and as an oxidizing agent in the chemical industries. Nitrate and nitrite are also used as food additives in processed food as preservatives and color fixatives in meat, poultry, fish and cheese [3]. Measurement of nitrite/nitrate levels has become an important parameter in the characterization of water quality throughout the world, especially in industrial effluents. Nitrate is a compound predominantly found in ground and surface waters. The natural sources of nitrate include soil nitrogen, nitrogen-rich geological materials and atmospheric deposition. However, higher concentrations of nitrate are generally associated with human activities and can cause adverse health effects on animals, human beings and plants. The other sources of nitrite/nitrate include radiator coolants, dry land cultivation practices, untreated or poorly treated sewage, livestock wastes, industrial effluents, food processing wastes and decay of organic matter [4]. The nitrogen uptake by the plants is in the form of nitrate which is highly leachable and readily moves with water through the soil. Therefore all sources of nitrogen (including organic nitrogen, ammonia and fertilizers) should be considered as potential sources of nitrite and nitrates [5].

The main concern and focus of almost all the major national and international drinking water quality guidelines produced over the last two decades is to protect human health. According to the standards and guidelines of United States Environmental Protection Agency (USEPA) and World Health Organization (WHO) the threshold limit values of nitrite/nitrate in drinking water are 45 µg/mL\(^{-1}\) and 1 µg/mL\(^{-1}\) respectively [6 - 7].
Nitrate in water is primarily low toxic but microbial action or in vivo reduction is the cause to transform nitrite which then combines with hemoglobin to produce methemoglobinemia that is extremely dangerous especially in infants (blue baby syndrome). Blue baby syndrome is a potentially fatal condition that occurs when the hemoglobin (Fe$^{2+}$) in an infant’s red blood cells is oxidized to methemoglobin (Fe$^{3+}$). Because methemoglobin is unable to transport oxygen, the condition produces symptoms of cyanosis. The affected infants develop an unusual blue - gray or lavender skin color and are often described as irritable or lethargic depending on the severity of their illness. Methemoglobin levels > 50 % can quickly lead to coma and death if the condition is not recognized and treated immediately [8]. In addition to this, the excessive concentration of nitrite/nitrate also decreases the thyroid gland function, leads to low storage of vitamin A and causes production of nitrosamines (which are known as common causes of cancer) [9]. Hence the speciation of nitrite/nitrate in biological and environmental samples has become an important parameter in the diagnosis of the patient in recent years [10]. Natural and anthropogenic effects can cause localised interrelated changes in the cycle. In order to access the impact and extent of the changes, it is essential to develop simple analytical methodology for simultaneous determination of inorganic nitrogen species from a wide variety of biological and environmental samples. Most of these methods are based on the reduction of nitrate to nitrite, which is subsequently determined through colorimetrically through a diazocoupling reaction [11]. Various flow injection procedures have been employed for the simultaneous determination of nitrite/nitrate. Ensafi and Kazemzadeh [12] injected the sample into a carrier stream that was divided into two channels, one containing a reductor column and the other without reductor column. The sum of both the anions was determined in one channel with the column. In the other channel only nitrite was analyzed. Zhang Zhi-Qi, et.al. [13] have introduced a technique of double sample injection, in which one sample zone passed directly to the reaction manifold, there it was merged with the reagents and the nitrite was detected. The other sample zone first passed through the reductor column converting nitrate to nitrite, and then the nitrite plus nitrate was determined. The flow injection based spectrophotometric methods were developed for the individual determination of nitrite or nitrate, and also for the simultaneous determination of nitrite and nitrate in various samples. The simultaneous determination of nitrite/nitrate was based on on-line nitrate reduction in a micro column containing copperised cadmium. A
single chromogenic reagent containing all the necessary reactants was used in both methods [14].

Several methods have been reported for the quantitative determination of nitrite/nitrate, including kinetic methods [15], chromatography [16], potentiometry [17], amperometry [18], polarography [19], capillary electrophoresis [20] and flow injection analysis [21] in various biological and environmental samples. However most of these methods use large volumes of toxic reagents, low sample frequency, application of complicated flow injection systems, poor reproducibility, expensive and time consuming procedures. However nitrite and nitrate are often found to co-exist along with other compounds containing nitrogen in nature. Therefore simultaneous measurement of nitrite/nitrate is of great significance in the field of biological and environmental sample analysis.

Among several methods that have been reported, spectrophotometric methods find widespread use due to their simplicity, reproducibility and easy adaptability [22]. It has been established that the concentration of nitrate in biological samples like egg and blood can be determined from the amount of nitrite produced by the reduction of nitrate through copperised cadmium reductor column [23]. Recently phosphomolybdenum blue chemistry has been exploited in the speciation analysis of nitrite and nitrate levels from a variety of sample matrices like meat, vegetables and water samples [24 - 25]. In these methods nitrite oxidizes the phosphomolybdenum blue complex into colorless phosphomolybdic acid. The decrease in the blue color intensity has been correlated to nitrite concentration. However the reaction between nitrite and phosphomolybdneum complex is very slow and a toxic reagent like sodium sulfide has been used as a reducing agent. Herein we report a simple method overcoming the limitations of these methods. In this proposed method, arsenate has been used in place of phosphate to form arsenomolybdenum blue complex and ascorbic acid as a reducing agent. The molar absorptivity of this method has been found to be much higher and the reaction proceeds at room temperature.

6.2 Experimental Section

6.2.1 Apparatus and Reagents

All absorbance measurements were made using the apparatus and reagents as described in the chapter 3 of section 3.2.2
**Sodium arsenate (0.0025M):** Prepared by dissolving 0.08 g of sodium arsenate in distilled water and diluted to 100 mL.

**Ammonium molybdate (0.015M):** Prepared by dissolving 1.854 g of ammonium heptamolybdate tetra hydrate in distilled water and diluted to 100 mL.

**Sulphuric acid (1.25M):** Prepared by diluting 3.5 mL of conc. sulphuric acid in 100 mL distilled water.

**Ascorbic acid (0.01M):** Prepared by dissolving 0.176 g of ascorbic acid in distilled water and diluted to 100 mL.

**Potassium antimony tartarate (0.008M):** Prepared by dissolving 0.267 g of potassium antimony tartarate in distilled water and diluted to 100 mL.

**Zinc sulphate (30 %):** It has been prepared by dissolving 30 g of zinc sulphate in 100 mL using distilled water.

**Trichloroacetic acid (TCA 10 %):** It has been prepared by diluting 10 mL of TCA to 100 mL using distilled water.

**Sodium Carbonate (0.5 %):** It has been prepared by dissolving 0.5 g of sodium carbonate in 100 mL distilled water.

**Sodium hydroxide (1M):** It has been prepared by dissolving 0.4 g of sodium hydroxide in 100 mL distilled water.

**NH₃ - NH₄Cl buffer solution (pH = 10):** It has been prepared by dissolving 0.531 g of NH₄Cl in 80 mL of water, adjusting the pH to 10 with 1:1 ammonia (V/V) and diluted to 100 mL with distilled water.

**Acetate buffer (pH: 3.5):** Dissolve 6.8 g of sodium acetate in 3 mL of acetic acid and adjust the pH to 3.5 with acetic acid and diluting to 100 mL with distilled water.

**6.2.2 Copperized cadmium reductor column:** The procedure for the preparation of copperized cadmium reductor column was explained in the chapter 3 of section 3.2.2

**6.2.3 Preparation and extraction of samples**

**Blood samples**

**Procedure:** The blood samples were collected from different infants of the age group between fifteen days to six months range in sterile plain tube and the samples were centrifuged at 4000 rpm for 20 min. to separate the plasma serum. The resulted serum samples treated with 30 % zinc sulphate (0.05 mLmL⁻¹ of sample) to precipitate the protein.
Precipitated protein was removed by filtration and collected the eluent and washings to 25 mL standard flask and diluted to mark with distilled water.

**Egg samples**

**Procedure:** The egg samples were collected from different breeds of hens at departmental stores in Bangalore. The above samples were weighed and a small hole was created on egg and liquid portion of whole egg was drawn into 100 mL beaker, stirred for few minutes to become homogeneous mass and treated with 10 % trichloroacetic acid (TCA 0.4 mLmL⁻¹ of sample) to precipitate the protein. The precipitated protein was removed by filtration and collected the eluent and washings into 25 mL standard flask and diluted up to the mark with distilled water.

**Water samples**

**Procedure:** 10 mL of the water sample was treated with 1 mL of 1 molL⁻¹ sodium hydroxide and centrifuged. The centrifugate has been collected into 50 mL standard flask and the residue was washed with 3×5 mL portions of water and centrifuged again. All the centrifugates were mixed well and diluted to 50 mL in the standard volumetric flask.

**Soil samples**

**Procedure:** The soil samples were collected from agricultural fields, dried and grounded to powder. Finely grounded soil samples were sieved using 20 mesh sieves and a known weight (1 g each) of soil sample was taken into 50 mL beaker and extracted with 5 mL portions of 0.5 % sodium carbonate solution for five times (5×5 mL) and centrifuged repeatedly till to get clear solution. The clear centrifugate solution were collected into 50 mL standard flask and diluted up to the mark with distilled water.

**6.2.4 Recommended procedure**

Into a series of 25 mL standard flasks containing 0.7 mL of 1.25 molL⁻¹ H₂SO₄, 1 mL of 0.02 molL⁻¹ ammonium molybdate solution, 0.4 mL of 0.003 molL⁻¹ arsenic (V) solution followed by 0.5 mL of 0.01 molL⁻¹ ascorbic acid and 0.2 mL of 0.01 molL⁻¹ antimony solution an aliquot of solution containing 0 - 10 µg nitrite was added, the contents were mixed well and allowed to stand for 30 min. to complete the reaction. Then the contents were diluted to the mark with distilled water and absorbance was measured at 840 nm using 1 cm cuvette.
6.3 Results and Discussion

The preliminary studies have been carried out by using various reagents to prepare arsenomolybdenum blue complex as dye in acidic medium by using 5 mL of 0.1 molL\(^{-1}\) ammonium molybdate solution, 5 mL of 0.1 molL\(^{-1}\) arsenic (V) solution followed by 3 mL of 1 molL\(^{-1}\) ascorbic acid and 2 mL of 0.1 molL\(^{-1}\) antimony solution in 25 mL standard flask, then the contents were mixed well and allowed to stand for nearly 30 min. to complete the reaction. Then an aliquot of solution containing 6 µg of nitrite was added and diluted up to the mark with distilled water and the decrease in absorbance was measured at 840 nm using 1 cm path length cuvette. The combination of these reagents gave high blank absorbance and low sample absorbance in acidic medium for the determination of nitrite/nitrate through oxidation reaction.

6.3.1 Optimization study

In order to quantify the nitrite/nitrate at trace level, all the parameters influencing the color development and decrease in the intensity of blue color complex was examined and the optimum values obtained were incorporated in the recommended procedure.

6.3.1.1 Effect of acidity

The effect of acid concentration on arsenomolybdenum blue complex was next investigated. Into a series of 25 mL standard flasks containing 1 mL of 0.02 molL\(^{-1}\) molybdate solution, 0.4 mL of 0.003 molL\(^{-1}\) arsenate solution, 0.5 mL of 0.01 molL\(^{-1}\) ascorbic acid solution and 0.2 mL of 0.01 molL\(^{-1}\) antimony solution, the 1.25 molL\(^{-1}\) H\(_2\)SO\(_4\) solution was varied from 0.1 mL to 2 mL range. It is evident from the graph that the overall acidity in the range 0.6 – 1.2 gave maximum absorbance. Hence 0.7 mL of 1.25 molL\(^{-1}\)H\(_2\)SO\(_4\) was chosen as the optimum concentration to get maximum absorbance (Fig. 6.1).
6.3.1.2 Effect of molybdate concentration

The effect of molybdate concentration on arsenomolybdenum blue complex was studied. Into a series of 25 mL standard flasks containing 0.7 mL of 1.25 molL\(^{-1}\) H\(_2\)SO\(_4\), 0.4 mL of 0.003 molL\(^{-1}\)arsenate solution, 0.5 mL of 0.01 molL\(^{-1}\) ascorbic acid solution and 0.2 mL of 0.01 molL\(^{-1}\) antimony solution, the 0.02 molL\(^{-1}\) molybdate solution was varied from 0.1 mL to 1.6 mL range. These studies revealed that 0.9 to 1.6 mL of 0.02 molL\(^{-1}\) molybdate solution is sufficient enough to get maximum absorbance to the sample. Hence 1 mL of 0.02 molL\(^{-1}\) molybdate solutions was used as optimized concentration in all further studies (Fig. 6.2).
The effect of arsenate concentration was studied. Into a series of 25 mL standard flasks containing 0.7 mL of 1.25 molL\(^{-1}\) H\(_2\)SO\(_4\), 1 mL of 0.02 molL\(^{-1}\) molybdate solution, 0.5 mL of 0.01 molL\(^{-1}\) ascorbic acid solution and 0.2 mL of 0.01 molL\(^{-1}\) antimony solution, the 0.003 molL\(^{-1}\) arsenate solution was varied from 0.05 mL to 0.90 mL range and the results obtained shows that the absorbance increases gradually with the increase of arsenate concentration and attained constant values from 0.4 mL to 0.65 mL and with the further increase of arsenate concentration the absorbance values decreases. Hence 0.4 mL of 0.003 molL\(^{-1}\) arsenate solution was chosen as the suitable concentration to get maximum sample absorbance (Fig.6.3).
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6.3.1.4 Effect of ascorbic acid concentration

The effect of ascorbic acid concentration on arsenomolybdenum blue complex was studied. Into a series of 25 mL standard flasks containing 0.7 mL of 1.25 molL\(^{-1}\) H\(_2\)SO\(_4\), 1 mL of 0.02 molL\(^{-1}\) molybdate solution, 0.4 mL of 0.003 molL\(^{-1}\) arsenate solution and 0.2 mL of 0.01 molL\(^{-1}\) antimony solution, the 0.01 molL\(^{-1}\) solution of ascorbic acid was varied from 0.1-1.2 mL range. The maximum absorbance value was obtained in the volume range (0.5 - 0.9 mL). Hence 0.6 mL of 0.01 molL\(^{-1}\) ascorbic acid was chosen as the suitable volume to get maximum absorbance (Fig. 6.4).
6.3.1.5 Effect of antimony concentration

The effect of antimony concentration on arsenomolybdenum blue complex was next investigated. Into a series of 25 mL standard flasks containing 0.7 mL of 1.25 molL\(^{-1}\) H\(_2\)SO\(_4\), 1 mL of 0.02 molL\(^{-1}\) molybdate solution, 0.4 mL of 0.003 molL\(^{-1}\) arsenate solution, 0.5 mL of 0.01 molL\(^{-1}\) ascorbic acid solution, the 0.01 molL\(^{-1}\) antimony solution was varied from 0.05 – 0.5 mL range and the results obtained shows that the absorbance increases gradually with the increase of antimony concentration and attained constant values from 0.2 – 0.45 mL and with the further increase of antimony concentration the absorbance values decreases. Hence 0.2 mL of 0.01 molL\(^{-1}\) antimony solutions was chosen as the suitable volume in all further studies (Fig.6.5).
6.3.1.6 Effect of reaction time on arsenomolybdenum blue complex formation

The effect of time on arsenomolybdenum blue complex formation was next investigated. Into a series of 25 mL standard flasks containing 0.7 mL of 1.25 molL⁻¹ H₂SO₄, 1 mL of 0.02 molL⁻¹ molybdate solution, 0.4 mL of 0.003 molL⁻¹ arsenate solution, 0.5 mL of 0.01 molL⁻¹ ascorbic acid solution and 0.2 mL of 0.01 molL⁻¹ antimony solutions was added. These flasks were allowed to stand for different time intervals and diluted up to the mark with distilled water and the absorbance values were measured at 840 nm. It is evident from the graph that the time required for maximum absorbance is in the range of 25 - 40 min. Hence 30 minutes time period was allowed in all further studies for complete dye formation and to get maximum absorbance (Fig. 6.6).
6.3.1.7 Species responsible for color

The molybdate reacts with sodium arsenate to form colorless arsenomolybdate in acidic medium. The hexavalent molybdenum undergoes reduction to pentavalent Mo by ascorbic acid in presence of antimony as catalyst to generate arsenomolybdenum blue color complex. The absorbance of the arsenomolybdenum blue complex was decreased by the addition of nitrite due to the oxidation of pentavalent Mo to yield colorless arsenomolybdate. The decrease in the absorbance of the complex is directly proportional to the concentration of the nitrite as shown in the scheme 6.1.

A simple and sensitive method has been developed for the estimation of nitrite/nitrate based on the reduction of arsenomolybdic acid to arsenomolybdenumblue complex using ascorbic acid. The obtained blue complex has been oxidized by the addition of nitrite and the decrease in the absorbance of blue color was correlated to nitrite concentration.
Scheme 6.1 Species responsible for blue color complex formation

\[ (\text{Mo}_7\text{O}_{24})^{6+} + \text{HAsO}_4^{2-} \xrightarrow{\text{H}^+} [\text{AsMo}^{VI}_1\text{O}_{40}]^{3-} \]

\[ [\text{AsMo}^{VI}_1\text{O}_{40}]^{3-} \xrightarrow{\text{Sb}^{3+}} \xrightarrow{\text{C}_6\text{H}_8\text{O}_6} [\text{AsMo}^{V}_{4}\text{Mo}^{VI}_8\text{O}_{40}]^{7-} \]

\[ [\text{AsMo}^{V}_4\text{Mo}^{VI}_8\text{O}_{40}]^{7-} + \text{NO}_2^- \rightarrow [\text{AsMo}^{VI}_1\text{O}_{40}]^{3-} + \text{NO} \]  

**blue color complex**  
**colorless**

**Fig.6.7** Symbolic representation of arsenomolybdneum blue complex (blue color) to arsenomolybdate (colorless) in presence of nitrite
6.3.1.8 Calibration procedure

Into a series of 25 mL standard flasks containing 0.7 mL of 1.25 molL\(^{-1}\) H\(_2\)SO\(_4\), 1 mL of 0.02 molL\(^{-1}\) ammonium molybdate solution, 0.4 mL of 0.003 molL\(^{-1}\) arsenic (V) solution followed by 0.5 mL of 0.01 molL\(^{-1}\) ascorbic acid and 0.2 mL of 0.01 molL\(^{-1}\) antimony solution an aliquot of solution containing 0-10 µg nitrite was added, the contents were mixed well and allowed to stand for 30 min. to complete the reaction. Then the contents were diluted to the mark with distilled water and absorbance values were measured at 840 nm (Fig. 6.7).

![Absorption spectra](Fig. 6.8)
6.3.1.9 Interference study

The effect of interference of the several cations and anions were evaluated to check the suitability of the method for the determination of nitrite and nitrate in water and soil samples. The cations like Hg$^{2+}$, Mg$^{2+}$, Cu$^{2+}$, Co$^{2+}$, Ba$^{2+}$, Na$^+$, Sn$^{2+}$, Fe$^{2+}$, Fe$^{3+}$, Ni$^{2+}$, and Zn$^{2+}$ did not interfere up to 1000 µg level. However Pb$^{2+}$ gave positive interference at 500 µg levels by increasing the absorbance and this was overcome by precipitating as PbS up to 1000 µg level. The anions like CH$_3$COO$^-$, SO$_4^{2-}$, C$_2$O$_4^{2-}$ and PO$_4^{3-}$ did not interfere up to 1000 µg level. But the anions like Br$^-$, I$^-$, Cl$^-$ gave positive interference at 500 µg levels by increasing the absorbance and this was overcome by precipitating as AgX (X= Cl$^-$, Br$^-$ and I$^-$) up to 1000 µg level. However SiO$_3^{2-}$ gave a positive interference at 200 µg level by increasing the absorbance and this was overcome by precipitating as calcium silicate up to 500 µg as shown in the table 6.1.

**Table 6.1 Interference studies**

<table>
<thead>
<tr>
<th>Interferent</th>
<th>Tolerance limit (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH$_3$COO$^-$, SO$_4^{2-}$, PO$_4^{3-}$, C$_2$O$_4^{2-}$</td>
<td>1000</td>
</tr>
<tr>
<td>Br$^-$, Cl$^-$, I$^-$</td>
<td>500</td>
</tr>
<tr>
<td>$^a$Br$^-$, Cl$^-$, I$^-$</td>
<td>1000</td>
</tr>
<tr>
<td>Hg$^{2+}$, Mg$^{2+}$, Cu$^{2+}$, Fe$^{3+}$, Fe$^{2+}$, Na$^+$, Ca$^{2+}$, Co$^{3+}$, Ni$^{2+}$, Zn$^{2+}$</td>
<td>1000</td>
</tr>
<tr>
<td>Pb$^{2+}$</td>
<td>500</td>
</tr>
<tr>
<td>$^b$Pb$^{2+}$</td>
<td>1000</td>
</tr>
<tr>
<td>SiO$_3^{2-}$</td>
<td>200</td>
</tr>
<tr>
<td>$^c$SiO$_3^{2-}$</td>
<td>1000</td>
</tr>
</tbody>
</table>
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*a treated with 0.1 % AgNO₃ solution centrifuged and washed the residue, then the centrifugate and washings were mixed and used to study the decrease in absorbance.

*b treated with 1 mL of 0.1 % hydrogen sulphide solution centrifuged and washed the residue, then the centrifugate and washings were mixed and used to study the decrease in absorbance.

*c treated with 1 mL of 0.1 % calcium oxide centrifuged and washed the residue, then the centrifugate and washings were mixed and used to study the decrease in absorbance.

6.4 Application study

The proposed method has been applied to determine nitrite/nitrate in biological samples like blood and egg, and it has been also extended to environmental samples like water and soil. In order to check the validation of the proposed method, the samples were simultaneously determined by using Griess - Ilosvev reaction as standard method. The results obtained by the proposed method are in good agreement with those obtained by the standard method.

6.4.1 Determination of nitrite/nitrate in blood samples

Nitrite determination: 10 mL of the made up solution was transferred into 25 mL standard flask containing 0.7 mL of 1.25 molL⁻¹ H₂SO₄, 1 mL of 0.02 molL⁻¹ ammonium molybdate solution, 0.4 mL of 0.003 molL⁻¹ arsenic (V) solution followed by 0.5 mL of 0.01 molL⁻¹ ascorbic acid solution and 0.2 mL of 0.01 molL⁻¹ antimony solution. The contents were mixed well and allowed to stand for 30 min. Then the solution was diluted to mark with distilled water and the absorbance was measured at 840 nm.

10 mL of the made up solution was directly used for color development following the procedure described under nitrite in water samples.

Nitrate determination: 10 mL of made up solution was treated with 5 mL of NH₃ - NH₄Cl buffer solution (pH = 10) and passed through copperized cadmium reductor column at a flow rate of 1 mL min⁻¹. The column was washed with 5 × 3 mL portions of water and the eluents were collected into 50 mL standard flask and diluted up to the mark with distilled water, 10 mL of the made up solution was taken and analyzed for total nitrite content. The nitrate content can be calculated by the difference between total nitrite and original nitrite content after reduction (Table 6.2).
6.4.2 Determination of nitrite/nitrate in egg samples

**Nitrite determination**: 10 mL of the made up solution was directly used for color development following the procedure described under nitrite determination in blood samples.

**Nitrate determination**: 10 mL of made up solution was treated with 5 mL of NH₃-NH₄Cl buffer solution (pH = 10) and passed through copperized cadmium reductor column at a flow rate of 1 mL min⁻¹. The column was washed with 5 × 3 mL portions of water and the eluents were collected into 50 mL standard flask and diluted up to the mark with distilled water, 10 mL of the made up solution was taken and analyzed for total nitrite content. The nitrate content can be calculated by the difference between total nitrite and original nitrite content after reduction (Table 6.3).

6.4.3 Determination of nitrite/nitrate in water samples

**Nitrite determination**: 10 mL of the made up solution was transferred into 25 mL standard flask containing 0.7 mL of 1.25 molL⁻¹ H₂SO₄, 1 mL of 0.02 molL⁻¹ ammonium molybdate solution, 0.4 mL of 0.003 molL⁻¹ arsenate solution followed by 0.5 mL of 0.01 molL⁻¹ ascorbic acid solution and 0.2 mL of 0.01 molL⁻¹ antimony solution. The contents were mixed well and allowed to stand for 30 min. Then the solution was diluted to 25 mL with distilled water and the absorbance was measured at 840 nm.

**Nitrate determination**: 10 mL of made up solution was treated with 5 mL of NH₃ - NH₄Cl buffer solution (pH = 10) and passed through copperized cadmium reductor column at a flow rate of 1 mL min⁻¹. The column was washed with 5 × 3 mL portions of water and the eluents were collected into 50 mL standard flask and diluted to the mark with water, 5 mL of the made up solution was taken and analyzed for total nitrite content. The nitrate content can be calculated by the difference between total nitrite and original nitrite content after reduction (Table 6.4).
6.4.4 Determination of nitrite/nitrate in soil samples

**Nitrite determination:** 10 mL of the made up solution was directly used for color development following the procedure described under nitrite in water samples.

**Nitrate determination:** 10 mL of made up solution was treated with 5 mL of NH₃ - NH₄Cl buffer solution (pH = 10) and passed through copperized cadmium reductor column at a flow rate of 1 mL min⁻¹. The column was washed with 5×3 mL portions of water and the eluents were collected into 50 mL standard flask and diluted to the mark with water, 5 mL of the made up solution was taken and analyzed for total nitrite content. The nitrate content can be calculated by the difference between total nitrite and original nitrite content after reduction (Table 6.5).
Table 6.2 Determination of nitrite/nitrate in blood serum samples

<table>
<thead>
<tr>
<th>sample*</th>
<th>nitrite due to nitrate reduction (µg)</th>
<th>added nitrate (µg)</th>
<th>total nitrite&lt;sup&gt;a&lt;/sup&gt; (µg)</th>
<th>recovery of added nitrite (%)</th>
<th>RE (%)</th>
<th>RSD (%)</th>
<th>nitrate found (µg/mL&lt;sup&gt;−1&lt;/sup&gt;)</th>
<th>RE (%)</th>
<th>RSD (%)</th>
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</thead>
<tbody>
<tr>
<td></td>
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<td>proposed</td>
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<td>proposed</td>
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<td>proposed</td>
</tr>
<tr>
<td>A</td>
<td>0.43</td>
<td>0.42</td>
<td>2.0</td>
<td>2.43</td>
<td>2.42</td>
<td>99.6</td>
<td>98.2</td>
<td>± 0.42</td>
<td>± 1.95</td>
</tr>
<tr>
<td>B</td>
<td>0.47</td>
<td>0.45</td>
<td>2.5</td>
<td>2.97</td>
<td>2.95</td>
<td>101.3</td>
<td>99.0</td>
<td>± 0.68</td>
<td>± 2.02</td>
</tr>
<tr>
<td>C</td>
<td>0.49</td>
<td>0.47</td>
<td>3.0</td>
<td>3.49</td>
<td>3.47</td>
<td>99.5</td>
<td>98.9</td>
<td>± 0.57</td>
<td>± 1.30</td>
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</tbody>
</table>

RE = relative error, RSD = relative standard deviation

*Samples have been collected from different infants of age group between 15 days to 6 months.

<sup>a</sup>Total nitrite (µg) = \{nitrite due to nitrate reduction (µg)\} + \{added nitrate (µg)\}

\[
\text{NO}_3^- (\mu\text{g}\text{mL}^{-1}) = \left(\frac{\text{NO}_2^- \text{ due to NO}_3^- \text{ reduction (µg)}}{\text{volume of blood serum (mL)}}\right) \times \frac{62}{46}
\]
Table 6.3 Determination of nitrite/nitrate in egg sample (scientific name: *Gallus Gallus domesticus*

<table>
<thead>
<tr>
<th>sample*</th>
<th>nitrite due to nitrate reduction (µg)</th>
<th>added nitrite (µg)</th>
<th>total nitrite&lt;sup&gt;a&lt;/sup&gt; (µg)</th>
<th>recovery of added nitrite (%)</th>
<th>RE (%)</th>
<th>RSD (%)</th>
<th>nitrate found (µg·g&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>RE (%)</th>
<th>RSD (%)</th>
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<tr>
<td>A</td>
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<td>96.5</td>
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<td>2.99</td>
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<td>99.0</td>
<td>± 0.3</td>
<td>± 2.0</td>
</tr>
<tr>
<td>C</td>
<td>0.55</td>
<td>0.53</td>
<td>3.0</td>
<td>3.55</td>
<td>3.53</td>
<td>99.5</td>
<td>99.0</td>
<td>± 0.6</td>
<td>± 1.1</td>
</tr>
</tbody>
</table>

RE = relative error, RSD = relative standard deviation

*Egg samples have been collected from different breeds of hens. A - Local egg, B - Farm egg, C - Farm egg

<sup>a</sup>*Total nitrite (µg·g<sup>-1</sup>) = \{nitrite due to nitrate reduction (µg)\} + \{added nitrite (µg)\}*

\[
NO_3^- \ (µg\cdot g^{-1}) = \frac{NO_2^- \ due \ to \ NO_3^- \ reduction \ (µg)}{weight \ of \ whole \ egg \ without \ shell \ (g)} \times \frac{62}{46}
\]
Table 6.4 Determination of nitrite/nitrate in water samples

<table>
<thead>
<tr>
<th>Sample*</th>
<th>nitrite originally present (µg)</th>
<th>nitrite due to nitrate reduction (µg)</th>
<th>total nitrite&lt;sup&gt;a&lt;/sup&gt; (µg)</th>
<th>RE (%)</th>
<th>RSD (%)</th>
<th>nitrate found (µgL&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>RE (%)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>proposed standard</td>
<td>proposed standard</td>
<td>proposed standard</td>
<td></td>
<td></td>
<td>proposed standard</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>163.15 ± 2.3</td>
<td>226.30 ± 194.73</td>
<td>389.45 ± 352.93</td>
<td>± 2.3</td>
<td>± 1.06</td>
<td>309.62 ± 266.78</td>
<td>± 1.8</td>
<td>± 1.10</td>
</tr>
<tr>
<td>B</td>
<td>142.10 ± 1.6</td>
<td>195.00 ± 181.98</td>
<td>337.10 ± 318.00</td>
<td>± 1.6</td>
<td>± 2.04</td>
<td>267.15 ± 249.34</td>
<td>± 1.7</td>
<td>± 1.96</td>
</tr>
<tr>
<td>C</td>
<td>173.68 ± 2.4</td>
<td>215.78 ± 198.30</td>
<td>389.46 ± 360.40</td>
<td>± 2.4</td>
<td>± 1.43</td>
<td>295.92 ± 271.26</td>
<td>± 2.1</td>
<td>± 1.08</td>
</tr>
</tbody>
</table>

RE = relative error, RSD = relative standard deviation, Sample A and B: Borewell water, Sample C: Lake water

*Samples have been collected from Chickkaballapur (Dist), Karnataka.

<sup>a</sup>Total nitrite (µg) = \[ \left\{ \text{nitrite originally present (µg)} \right\} + \left\{ \text{nitrite formed by the reduction of nitrate (µg)} \right\} \]

\[ NO_2^- (\mu g L^{-1}) = \frac{\text{total } NO_2^- (\mu g) - \text{originally present (µg)}}{\text{volume of water sample (L)}} \times \frac{62}{46} \]
Table 6.5 Determination of nitrite/nitrate in soil extract samples

<table>
<thead>
<tr>
<th>sample</th>
<th>nitrite due to nitrate reduction (µg)</th>
<th>added nitrite (µg)</th>
<th>total nitrite (\text{a} ) (µg)</th>
<th>recovery of added nitrite (%)</th>
<th>RE (%)</th>
<th>RSD (%)</th>
<th>nitrate found (µg g(^{-1}))</th>
<th>RE (%)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>proposed</td>
<td>standard</td>
<td>proposed</td>
<td>standard</td>
<td>proposed</td>
<td>standard</td>
<td>proposed</td>
<td>standard</td>
<td>proposed</td>
</tr>
<tr>
<td>A</td>
<td>0.18</td>
<td>0.16</td>
<td>2.0</td>
<td>2.18</td>
<td>2.16</td>
<td>98.0</td>
<td>97.5</td>
<td>± 0.9</td>
<td>± 1.30</td>
</tr>
<tr>
<td>B</td>
<td>0.15</td>
<td>0.14</td>
<td>2.5</td>
<td>2.65</td>
<td>2.64</td>
<td>99.5</td>
<td>98.0</td>
<td>± 0.4</td>
<td>± 2.20</td>
</tr>
<tr>
<td>C</td>
<td>0.19</td>
<td>0.17</td>
<td>3.0</td>
<td>3.19</td>
<td>3.17</td>
<td>98.6</td>
<td>97.5</td>
<td>± 0.6</td>
<td>± 2.0</td>
</tr>
</tbody>
</table>

RE = relative error, RSD = relative standard deviation

*Soil samples have been collected from regularly irrigated lands of Chickkaballapur (Dist), Karnataka State.

\(\text{a} \) Total nitrite (µg) = \{nitrite due to nitrate reduction (µg)\} + \{added nitrite (µg)\}

\[
NO_3^- (\text{µg g}^{-1}) = \left[NO_2^- \text{ due to } NO_3^- \text{ reduction (µg)} \right] \times \frac{62}{46}
\]
6.5 Conclusion

The proposed method is based on the oxidative reaction of nitrite with arsenomolybdenum blue complex to form a colorless arsenomolybdate. The decrease in the absorbance of the blue color is directly proportional to the amount of nitrite added. The reaction conditions have been optimized and the method obeys Beer’s law in the concentration range 0 - 10 µg in 25 mL of aqueous phase. The interference effect of some of the common cations and anions that have been present in water samples has been studied. The proposed method has been applied to determine nitrite/nitrate levels in biological and environmental samples and the results are compared with the standard method [26]. This method can be used for the routine monitoring of nitrite/nitrate levels in environmental samples through flow injection based techniques. It can be used as an alternative method to the existing procedures.

6.6 References


