Analytical Methods

Dynamic Article Links

Cite this: *Anal. Methods*, 2011, **3**, 1838

www.rsc.org/methods

PAPER

Nitrite/nitrate speciation through arsenomolybdenum blue complex at trace level: application to biological and environmental samples

M. Pandurangappa* and Y. Venkataramanappa

Received 18th January 2011, Accepted 26th May 2011
DOI: 10.1039/c1ay05025g

A novel and simple method has been proposed for the estimation of nitrite/nitrate based on the reduction of arsenomolybdic acid to arsenomolybdenum blue using ascorbic acid. The obtained arsenomolybdenum blue complex has been oxidized by the addition of nitrite and the decrease in the absorbance of blue color is directly proportional to the nitrite quantity. The absorbance of the arsenomolybdenum blue complex has been measured at 840 nm. All the reaction variables have been optimized and Beer's law has been obeyed in the concentration range 2–10 μg in 25 mL of aqueous phase with a molar absorptivity 4.37×10^4 L mol^{-1} cm^{-1}. The relative standard deviation of the method has been found to be 1.7% for five measurements with a detection limit of 0.003 μg mL^{-1}. The method has been applied to determine nitrite/nitrate levels in biological samples as well as environmental samples.

1. Introduction

Nitrate and nitrite are naturally occurring oxyanions of nitrogen and are present everywhere in the environment.[1] Of the two nitrate 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 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 nitrate/nitrite 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 nitrites 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 nitrate/nitrite 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 nitrate/nitrite 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 changes, it is essential to develop simple analytical methodology for

Department of Studies in Chemistry Bangalore University, Central College Campus, 560 001 Bangalore, India. E-mail: mprangachem@gmail.com; Tel: +91-80-22961352

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 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. Z. Q. Zhang *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 online nitrate reduction in a microcolumn 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 wide spread 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 the 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 the nitrite concentration. However the reaction between nitrite and phosphomolybdenum 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 arsenomolybdneum 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.

2. Experimental

2.1. Apparatus and reagents

Absorbance measurements were made using a Shimadzu UV-VIS-NIR Scanning Spectrophotometer (model UV-3101PC) with 1 cm quartz cuvettes. Control Dynamics (Mumbai) digital pH meter (model APX 175 E/C) was used for all pH measurements. All reagents used were analar grade without further purification. Distilled water was used throughout the experiments.

Nitrite stock solution (1000 $\mu g\ mL^{-1}$): it has been prepared by dissolving 0.15 g of pre-dried sodium nitrite (at 105 ± 5 °C for an hour) in distilled water and diluted to 100 mL. Working standards were prepared from stock solution on the day of use.

NH_3–NH_4Cl buffer solution (pH = 10): it has been prepared by dissolving 0.531 g of NH_4Cl in 80 mL of water, adjusting the pH to 10 with 1 : 1 ammonia (vol/vol) and diluted to 100 mL with water.

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

Formaldehyde (0.5%): it has been prepared by diluting 1.3 mL of formaldehyde (38%) to 100 mL of distilled water.

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

Zinc sulfate (30%): it has been prepared by dissolving 30 g of $ZnSO_4$ in 100 mL of distilled water.

Lead acetate (0.01%): it has been prepared by dissolving 0.01 g of lead acetate in 100 mL of distilled water.

Ascorbic acid (0.01 mol L^{-1}): it has been prepared by dissolving exactly 0.176 g of ascorbic acid in 100 mL of distilled water.

2.2. Copperized cadmium reductor column

Wash 25 g of 20–100 mesh Cd granules with 6 N HCl and rinse with water. Swirl Cd granules with 100 mL of 2% $CuSO_4$ solution for 5 minutes or until blue colour partially fades. Decant and repeat with fresh $CuSO_4$ until a brown colloidal precipitate begins to develop. Gently flush with water to remove all precipitated Cu. Insert a glass wool plug into the bottom of a glass column (30 cm long × 5 mm id) and fill with water. Add sufficient Cu–Cd granules to produce a column of 18.5 cm long. Maintain the water level above Cu–Cd granules to prevent entrapment of air. Wash the column with 200 mL of dilute NH_4Cl–EDTA buffer solution. The column was activated by passing the NH_4Cl–EDTA buffer solution at a flow rate of 7–10 mL min^{-1}. The flow rate was adjusted in such a way that the nitrate solution quantitatively reduces to nitrite after passing through the reductor column. The column was stored using NH_4Cl–EDTA solution. The column should not be allowed to dry. Under these conditions the column can be used for several months. All the column conditions were optimized according to the standard method.[26]

2.3. Calibration procedure

Into a series of 25 mL standard flasks containing 0.7 mL of 1.25 mol L^{-1} H_2SO_4, 1 mL of 0.02 mol L^{-1} ammonium molybdate solution, 0.4 mL of 0.003 mol L^{-1} arsenic(v) solution

followed by 0.5 mL of 0.01 mol L^{-1} ascorbic acid and 0.2 mL of 0.01 mol L^{-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 cuvettes (Fig. 1).

2.4. Procedure for the preparation and extraction of biological and environmental samples

2.4.1. 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 tubes and the samples were centrifuged at 4000 rpm for 20 min to separate the plasma serum. The resulted serum samples were treated with 30% zinc sulfate (0.05 mL mL^{-1} of sample) to precipitate the protein. The precipitated protein was removed by filtration and the eluent and washings collected in a 25 mL standard flask and diluted to the mark with distilled water.

2.4.2. 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 the egg and the liquid portion of the whole egg was drawn into 100 mL beaker, stirred for a few minutes to become a homogeneous mass and treated with 10% trichloroacetic acid (TCA 0.4 mL mL^{-1} of sample) to precipitate the protein. The precipitated protein was removed by filtration and the eluent and washings collected into a 25 mL standard flask and diluted up to the mark with distilled water.

2.4.3. Water samples

Procedure. 10 mL of the water sample was treated with 1 mL of 1 mol L^{-1} sodium hydroxide and centrifuged. The centrifugate was collected into a 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.

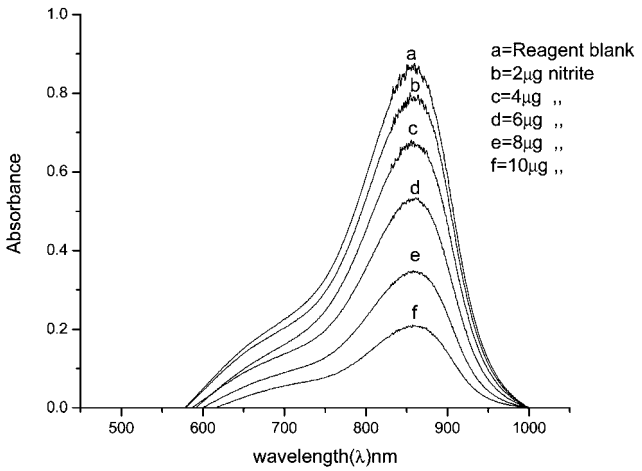


Fig. 1 Absorption spectra.

2.4.4. Soil samples

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

3. Results and discussions

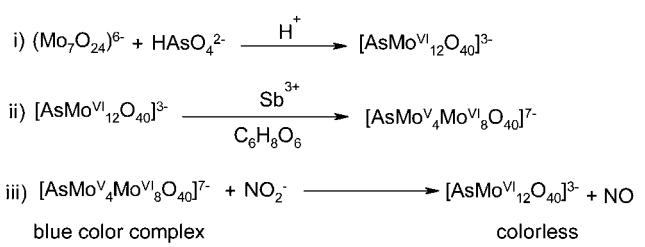
The preliminary studies have been carried out by using various reagents to prepare arsenomolybdenum blue complex as dye in acidic medium by using 5 mL of 0.1 mol L^{-1} ammonium molybdate solution, 5 mL of 0.1 mol L^{-1} arsenic(v) solution followed by 3 mL of 1 mol L^{-1} ascorbic acid and 2 mL of 0.1 mol L^{-1} antimony solution in a 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 cuvettes. 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.

3.1. Species responsible for colour

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 the presence of antimony as catalyst to generate arsenomolybdenum blue colour 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 (Scheme 1).

4. Optimization studies

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



Scheme 1 Species responsible for blue complex formation.

4.1. 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 mol L^{-1} molybdate solution, 0.4 mL of 0.003 mol L^{-1} arsenate solution, 0.5 mL of 0.01 mol L^{-1} ascorbic acid solution and 0.2 mL of 0.01 mol L^{-1} antimony solution, the 1.25 mol L^{-1} H_2SO_4 solution was varied from 0.1 mL to 2 mL range and the results obtained show that the absorbance increases gradually with the increase in acid concentration and attained constant values from 0.6 mL to 1.2 mL and with the further increase in acid concentration the absorbance values decrease. Hence 0.7 mL of 1.25 mol L^{-1} H_2SO_4 was chosen as the optimum concentration to get the maximum absorbance.

4.2. 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 mol L^{-1} H_2SO_4, 0.4 mL of 0.003 mol L^{-1}arsenate solution, 0.5 mL of 0.01 mol L^{-1} ascorbic acid solution and 0.2 mL of 0.01 mol L^{-1} antimony solution, the 0.02 mol L^{-1} molybdate solution was varied from 0.1 mL to 2 mL range and the results obtained show that the absorbance increases gradually with the increase in molybdate concentration and attained constant values from 0.6 mL to 1.2 mL and with the further increase in molybdate concentration the absorbance values decrease. Hence 1 mL of 0.02 mol L^{-1} molybdate solution was used as optimized concentration in all further studies.

4.3. Effect of arsenate concentration

The effect of arsenate concentration was studied. Into a series of 25 mL standard flasks containing 0.7 mL of 1.25 mol L^{-1} H_2SO_4, 1 mL of 0.02 mol L^{-1} molybdate solution, 0.5 mL of 0.01 mol L^{-1} ascorbic acid solution and 0.2 mL of 0.01 mol L^{-1} antimony solution, the 0.003 mol L^{-1} arsenate solution was varied from 0.05 mL to 0.90 mL range and the results obtained show that the absorbance increases gradually with the increase in arsenate concentration and attained constant values from 0.3 mL to 0.5 mL and with the further increase in arsenate concentration the absorbance values decrease. Hence 0.4 mL of 0.003 mol L^{-1} arsenate solution was chosen as the suitable concentration to get the maximum absorbance.

4.4. 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 mol L^{-1} H_2SO_4, 1 mL of 0.02 mol L^{-1} molybdate solution, 0.4 mL of 0.003 mol L^{-1} arsenate solution and 0.2 mL of 0.01 mol L^{-1} antimony solution, the 0.01 mol L^{-1} solution of ascorbic acid was varied from 0.4×10^{-4} to 0.8×10^{-3} mol L^{-1} range and the results obtained show that the absorbance increases gradually with the increase in ascorbic acid concentration and attained constant values from 0.20×10^{-3} to 0.48×10^{-3} mol L^{-1} and with the further increase in ascorbic acid concentration the absorbance values decrease. Hence 0.24×10^{-3} mol L^{-1} solution of ascorbic acid was chosen as the suitable concentration to get the maximum absorbance.

4.5. 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 mol L^{-1} H_2SO_4, 1 mL of 0.02 mol L^{-1} molybdate solution, 0.4 mL of 0.003 mol L^{-1} arsenate solution, 0.5 mL of 0.01 mol L^{-1} ascorbic acid solution, the 0.01 mol L^{-1} antimony solution was varied from 0.1 mL to 2 mL range and the results obtained show that the absorbance increases gradually with the increase in antimony concentration and attained constant values from 0.6 mL to 1.2 mL and with the further increase in antimony concentration the absorbance values decrease. Hence 0.2 mL of 0.01 mol L^{-1} antimony solution was chosen as the suitable concentration to get the maximum absorbance.

4.6. 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 mol L^{-1} H_2SO_4, 1 mL of 0.02 mol L^{-1} molybdate solution, 0.4 mL of 0.003 mol L^{-1} arsenate solution, 0.5 mL of 0.01 mol L^{-1} ascorbic acid solution and 0.2 mL of 0.01 mol L^{-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 the maximum absorbance is in the range of 25–40 min. Hence a 30 minute time period was allowed in all further studies for complete dye formation and to get the maximum absorbance (Fig. 2).

4.7. Interference studies

The effects of interference of the several cations and anions were evaluated to check the suitability of the method for the

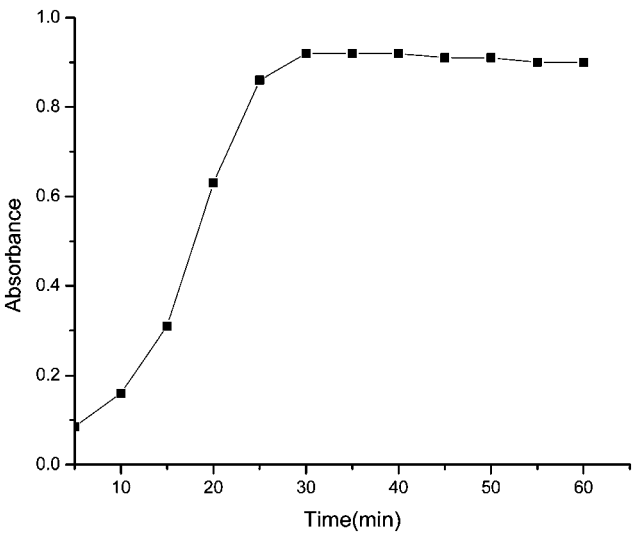


Fig. 2 Effect of reaction time.

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_3COO^-, SO_4^{2-}, $C_2O_4^{2-}$ 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 (Table 1).

5. Analytical merits of the method

The analytical parameters like standard deviation and limit of detection were calculated by using the following formulae.

5.1. Standard deviation

The standard deviation was calculated for different concentrations in the range between 2 and 10 μg of nitrite by using the following formula, and all the optimization studies were carried out using a nitrite concentration of 4 μg.

$$\sigma = \sqrt{\frac{\sum d^2}{n-1}}$$

5.2. Limit of detection

The limit of detection was calculated by the following formula:

$$\text{Limit of detection (LOD)} = \frac{\sigma \times 3.3}{\text{Slope}}$$

6. Application study

The proposed method has been applied to determine the 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–Ilosvey reaction as a standard method. The results obtained by the proposed method are in good agreement with those obtained by the standard method.

Table 1 Interference study

Interferent	Tolerance limit/μg
CH_3COO^-, SO_4^{2-}, PO_4^{3-}, $C_2O_4^{2-}$	1000
Br^-, Cl^-, I^-	500
Br^-, Cl^-, I^{-a}	1000
Hg^{2+}, Mg^{2+}, Cu^{2+}, Fe^{3+}, Fe^{2+}, Na^+, Ca^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}	1000
Pb^{2+}	500
Pb^{2+b}	1000
SiO_3^{2-}	200
SiO_3^{2-c}	1000

[a] Treated with 0.1% $AgNO_3$ 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 sulfide 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.

6.1. Determination of nitrite/nitrate in blood samples

6.1.1. Nitrite determination. 10 mL of the made up solution was transferred into a 25 mL standard flask containing 0.7 mL of 1.25 mol L^{-1} H_2SO_4, 1 mL of 0.02 mol L^{-1} ammonium molybdate solution, 0.4 mL of 0.003 mol L^{-1} arsenic(v) solution followed by 0.5 mL of 0.01 mol L^{-1} ascorbic acid solution and 0.2 mL of 0.01 mol L^{-1} antimony solution. The contents were mixed well and allowed to stand for 30 min. Then the solution was diluted to the 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.

6.1.2. Nitrate determination. 10 mL of made up solution was treated with 5 mL of NH_3–NH_4Cl buffer solution (pH = 10) and passed through the 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 a 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 nitrite contents after reduction (Table 2).

6.2. Determination of nitrite/nitrate in egg samples

6.2.1. 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.

6.2.2. Nitrate determination. 10 mL of made up solution was treated with 5 mL of NH_3–NH_4Cl buffer solution (pH = 10) and passed through the 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 a 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 nitrite contents after reduction (Table 3).

6.3. Determination of nitrite/nitrate in water samples

6.3.1. Nitrite determination. 10 mL of the made up solution was transferred into a 25 mL standard flask containing 0.7 mL of 1.25 mol L^{-1} H_2SO_4, 1 mL of 0.02 mol L^{-1} ammonium molybdate solution, 0.4 mL of 0.003 mol L^{-1} arsenate solution followed by 0.5 mL of 0.01 mol L^{-1} ascorbic acid solution and 0.2 mL of 0.01 mol L^{-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.

Table 2 Determination of nitrite/nitrate in blood serum samples[c]

Sample[a]	Nitrite due to nitrate reduction/μg		Added nitrite/μg	Total nitrite[b]/μg		Recovery of added nitrite (%)		RE (%)	RSD (%)	Nitrate found/μg mL^{-1}		RE (%)	RSD (%)
	Proposed	Standard		Proposed	Standard	Proposed	Standard			Proposed	Standard		
A	0.43	0.42	2.0	2.43	2.42	99.6	98.2	±0.42	±1.95	0.32	0.30	±1.6	±2.10
B	0.47	0.45	2.5	2.97	2.95	101.3	99.0	±0.68	±2.02	0.29	0.27	±1.4	±1.81
C	0.49	0.47	3.0	3.49	3.47	99.5	98.9	±0.57	±1.30	0.26	0.23	±1.3	±1.60

[a] Samples have been collected from different infants of age group between 15 days and 6 months. [b] Total nitrite (μg) = nitrite due to nitrate reduction (μg) + added nitrite (μg). Nitrate (μg mL^{-1}) = (nitrite due to nitrate reduction (μg)/volume of blood serum (mL)) × (62/46). [c] RE = Relative Error and RSD = Relative Standard Deviation.

Table 3 Determination of nitrite/nitrate in egg sample (scientific name: *Gallus Gallus domesticus*)[c]

Sample[a]	Nitrite due to nitrate reduction/μg		Added nitrite/μg	Total nitrite[b]/μg		Recovery of added nitrite (%)		RE (%)	RSD (%)	Nitrate found/μg g^{-1}		RE (%)	RSD (%)
	Proposed	Standard		Proposed	Standard	Proposed	Standard			Proposed	Standard		
A	0.42	0.39	2.0	2.42	2.39	98.5	96.5	±1.3	±1.6	0.30	0.26	±1.3	±1.98
B	0.49	0.48	2.5	2.99	2.98	100.2	99.0	±0.3	±2.0	0.40	0.38	±1.0	±1.30
C	0.55	0.53	3.0	3.55	3.53	99.5	99.0	±0.6	±1.1	0.33	0.31	±2.0	±2.06

[a] Egg samples have been collected from different breeds of hens. A—local egg, B—farm egg, and C—farm egg. [b] Total nitrite (μg g^{-1}) = nitrite due to nitrate reduction (μg) + added nitrite (μg). Nitrate (μg g^{-1}) = (nitrite due to nitrate reduction (μg)/weight of whole egg without shell (g)) × (62/46). [c] RE = Relative Error and RSD = Relative Standard Deviation.

Table 4 Determination of nitrite/nitrate in water samples[c]

Sample[a]	Nitrite originally present/μg		Nitrite due to nitrate reduction/μg		Total nitrite[b]/μg		RE (%)	RSD (%)	Nitrate found/μg L^{-1}		RE (%)	RSD (%)
	Proposed	Standard	Proposed	Standard	Proposed	Standard			Proposed	Standard		
A	163.15	158.20	226.30	194.73	389.45	352.93	±2.3	±1.06	309.62	266.78	±1.8	±1.10
B	142.10	136.02	195.00	181.98	337.10	318.00	±1.6	±2.04	267.15	249.34	±1.7	±1.96
C	173.68	162.10	215.78	198.30	389.46	360.40	±2.4	±1.43	295.92	271.26	±2.1	±1.08

[a] Samples have been collected from Chickkaballapur (Dist), Karnataka. [b] Total nitrite (μg) = nitrite originally present (μg) + nitrite due to nitrate reduction (μg). Nitrate (μg L^{-1}) = (total nitrite (μg) − nitrite originally present (μg)/volume of water sample (L)) × (62/46). [c] RE = Relative Error RSD = Relative Standard Deviation. Samples A and B: borewell water, sample C: lake water.

Table 5 Determination of nitrite/nitrate in soil extract samples[c]

Sample[a]	Nitrite due to nitrate reduction/μg		Added nitrite/μg	Total nitrite[b]/μg		Recovery of added nitrite (%)		RE (%)	RSD (%)	Nitrate found/μg g^{-1}		RE (%)	RSD (%)
	Proposed	Standard		Proposed	Standard	Proposed	Standard			Proposed	Standard		
A	0.18	0.16	2.0	2.18	2.16	98.0	97.5	±0.9	±1.30	0.24	0.22	±2.0	±2.13
B	0.15	0.14	2.5	2.65	2.64	99.5	98.0	±0.4	±2.20	0.20	0.19	±1.2	±1.92
C	0.19	0.17	3.0	3.19	3.17	98.6	97.5	±0.6	±2.0	0.25	0.24	±2.1	±1.68

[a] Soil samples have been collected from regularly irrigated lands of Chickkaballapur (Dist), Karnataka State. [b] Total nitrite (μg) = nitrite due to nitrate reduction (μg) + added nitrite (μg). Nitrate (μg g^{-1}) = (nitrite due to nitrate reduction (μg)/weight of soil sample (g)) × (62/46). [c] RE = Relative Error and RSD = Relative Standard Deviation.

6.3.2. Nitrate determination. 10 mL of made up solution was treated with 5 mL of NH_3–NH_4Cl buffer solution (pH = 10) and passed through the 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 a 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 nitrite contents after reduction (Table 4).

6.4. Determination of nitrite/nitrate in soil samples

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

6.4.2. Nitrate determination. 10 mL of made up solution was treated with 5 mL of NH_3–NH_4Cl buffer solution (pH = 10) and passed through the 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 a 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 nitrite contents after reduction (Table 5).

7. 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 techniques. It can be used as an alternative method to the existing procedures.

Acknowledgements

The authors acknowledge the University Grants Commission (UGC), New Delhi for the financial support.

References

1 Health Canada, *Background Document for Canadian Drinking Water Guidelines: Nitrate/Nitrite*, Ottawa, 1992.
2 L. Pokorny, I. Maturana and W. H. Bortle, *Kirk-Othmer Encyclopedia of Chemical Technology*, John Wiley & Sons, 5th edn, 2006.
3 European Commission, Food Safety Act (Cap. 448) L.N. 54 of 2005 Permitted Food Additives Regulations, 2005, http://www.doi.gov.mt/en/legalnotices/2005/default1.asp.
4 E. A. Graffy, D. R. Helsel and D. K. Mueller, *Nutrients in the Nation's Waters: Identifying Problems and Progress*, US Geological Survey Fact Sheet FS218–96, Geological Survey, Reston, VA, 1996.
5 I. Galaviz-Villa, et al, *J. Agric. Sci.*, 2010, **2**, 17–30.
6 EPA-United States Environmental Protection Agency, *Drinking Water Criteria Document on Nitrate/Nitrite*, Office of Drinking Water, Washington DC, 2006.
7 World Health Organization, *Guidelines for Drinking Water Quality*, 3rd edn, vol. 1, 2008.
8 L. Knobeloch, B. Salna, A. Hogan, J. Postle and H. Anderson, *Environ. Health Perspect.*, 2000, **108**, 675–678.
9 S. K. Gupta, R. C. Gupta, A. B. Gupta, A. K Seth, J. K. Bassin and A. Gupta, *Environ. Health Perspect.*, 2000, **108**, 363–366.
10 W. Stumm and J. Morgan, *Aquatic Chemistry*, John Wiley & Sons, 3rd edn, 1995.
11 M. Watanabe, T. Takamatsu, M. K. Koshikawa, K. Sakamoto and K. Inubushi, *J. Environ. Monit.*, 2006, **8**, 167–173.
12 A. A. Ensafi and A. Kazemzadeh, *Anal. Chim. Acta*, 1999, **382**, 15–21.
13 Z. Q. Zhang, L. J. Gao, H. Y. Zhan and Q. G. Liu, *Anal. Chim. Acta*, 1998, **370**, 59–63.
14 C. E. López Pasquali, A. Gallego-Picó, P. Fernández Hernando, M. Velasco and J. S. Durand Alegría, *Microchem. J.*, 2010, **94**, 79–82.
15 B. Liang, M. Iwatsuki and T. Fukasawa, *Analyst*, 1994, **119**, 2113–2117.
16 S. B. Butt, M. Riaz and M. Z. Iqbal, *Talanta*, 2001, **55**, 789–797.
17 J. Z. Li, X. C. Wu, R. Yuan, H. G. Lin and R. Q. Yu, *Analyst*, 1994, **119**, 1363–1366.
18 M. Bertotti and D. Pletcher, *Anal. Chim. Acta*, 1997, **337**, 49–55.
19 M. I. N. Ximenes, S. Rath and F. G. R. Reyes, *Talanta*, 2000, **51**, 49–56.
20 N. Oztekin, M. S. Nutku and F. B. Erim, *Food Chem.*, 2002, **76**, 103–106.
21 R. Andradea, C. O. Viana, S. G. Guadagnin, F. G. R. Reyesb and R. Rath, *Food Chem.*, 2003, **80**, 597–602.
22 R. Burakhama, M. Oshimab, K. Grudpan and Sh. Motomizu, *Talanta*, 2004, **64**, 1259–1265.
23 X. F. Yue, Zh. Q. Zhang, and H. T. Yan, *Talanta*, 2004, **62**, 97–101.
24 N. A. Zatar, M. A. Abu-Eid and A. F. Eid, *Talanta*, 1999, **50**, 819–826.
25 L. Monser, S. Sadok, G. M. Greenway, I. Shah and R. F. Uglow, *Talanta*, 2002, **57**, 511–518.
26 APHA, *Standard Methods for the Examination of Water and Wastewater Analysis*, 19th edn, Washington DC, 1995, pp. 4-83–4-88.

