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A simple spectrophotometric method for the determination of arsenic in industrial and environmental samples using vanillin-2-amino nicotinic acid (VANA)

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ABSTRACT

Vanillin-2- amino nicotinic acid (VANA) has been proposed as new analytical reagent for the direct non-extractive spectrophotometric determination of Arsenic. The reagent reacts with arsenic in acidic medium (pH 5.0, Sodium acetate- acetic acid buffer) to form light greenish yellow colored 1:1 (M:L). The colour reactions are instantaneous and the maximum absorbance was obtained at 350 nm and remains constant for over 3 h. The molar absorptivity and Sandell's sensitivity of VANA found to be $6.805 \times 10^3 \text{ L mol}^{-1} \text{ cm}^{-1}$ and $0.00146 \mu\text{gcm}^{-2}$ of As^{III} respectively. The system obeys Beer's law in the range of 0.128 - 2.66 $\mu\text{g/ml}$ of As^{III} . Since VANA method is more sensitive it was applied for determination of Arsenic in some environmental water samples.

Key words: Spectrophotometry, Arsenic determination, VANA, Environmental samples.

INTRODUCTION

Arsenic compounds are widely used and have long been recognized as toxicants [1, 2,3]. Arsenic is widely distributed in the nature. It occurs as inorganic and organic compounds as trivalent. Animals vary in their arsenic accumulation depending upon the type of food they consume (John & Jeanne, 1994) [5, 6]. Acute arsenic exposure can give symptoms with rapid onset of headache, nausea and severe gastrointestinal irritation (Allan et al., 1995) [7]. Increasing industrialization more and more industrial waste get accumulated in various regions and make their passage through soil cause severe environmental pollution and wide life toxicity [4] and also soil into animal body especially in their liver, kidney and lean meat.

Arsenic occurs naturally in the Earth's crust in its inorganic form, trivalent (arsenite) or pentavalent (arsenate). Erosion of arsenic containing surface rocks probably accounts for a significant amount of arsenic in water supplies. It is a ubiquitous element in water, soil and sediments. The occurrence of arsenic in plants and animals generally reflects its accumulation from the environment. The presence of arsenic in drinking water has reached calamitous proportions in many parts of the world. There are numerous reports in the literature based on past and ongoing experience in various countries in Asia and South America concerning the higher risks of skin, bladder, lung, liver, and kidney cancer that result from continued consumption of elevated levels of arsenic in drinking water [8]. Consumption of even low levels of arsenic over a long period can cause a multitude of diseases. The maximum permissible limit for arsenic (III) drinking water is 0.05 mg/L as recommended by WHO [9]. In certain areas in India, Bangladesh,

China, and Mongolia [10], arsenic levels in groundwater exceed 1 ng/ mL. Regarding inorganic arsenic, arsenic (III) is appreciably more toxic than arsenic (V). Usually these species of arsenic in natural water are found at the trace levels [10]. There are only a few analytical techniques available, which have sufficient sensitivity and selectivity to directly determine these species of arsenic, at the trace levels in natural water. Therefore, the development of sensitive and accurate methods for speciation and preconcentration of trace amounts of arsenic (III) and arsenic (V) is necessary. Recently many kinds of conventional analytical techniques such as hydride generatively coupled plasma atomic emission spectrometry(HG-ICP-AES) [5], capillary electrophoresis inductively coupled plasma mass spectrometry (CE-ICP-MS) [4], high performance liquid chromatography-inductively coupled plasma mass spectroscopy [11], electro thermal atomic absorption spectrometry(ETAAS) [12], hydride generation – atomic absorption spectrometry [13], hydride generation – atomic fluorescence spectrometry [14], cathodic stripping Voltammery [15], anodic stripping voltammery [16], neutron activation analysis [17], photometric analysis [18], ion selective electrodes [19] and energy dispersive X-ray fluorescence spectrometry [20], have been used for the determination of low concentrations of arsenic. But all these techniques are costly and require trained staff. Recently most of the spectrophotometric methods have been developed as an alternative for the determination of arsenic instead of conventional techniques.

This paper describes synthesis, characterization and analytical properties of new reagent viz., vanillin-2- amino nicotinic acid (VANA). Since the reagent is more sensitive, it is used for the determination of Arsenic in various water samples.

MATERIALS AND METHODS

Apparatus

A Shimadzu (Model-1601) UV-VIS spectrophotometer and ELICO model LI-610 pH meter with combination electrodes were used for measurements of absorbance and pH respectively. ICP-OES (Inductively Coupled Plasma-Optical Emission Spectrometry Model-7000) methods were used for the quantitative analysis of heavy metals in all samples. To determine the heavy metals levels, AOAC methods were used (AOAC 1986, 2003; Jorhem 1993). In this method, the samples were dissolved at 190°C and 400 psi pressure in Mars 5 apparatus (Vessel Type XKP 1500, CEM, Matthews, USA). The heavy metals were analysed by Inductively Coupled Plasma-Optical Emission Spectrometry (Varian Vista-MPX CCD Simultaneous Spectrophotometer, Murgave-Victoria, Australia) [21 & 22]. (Mandal & Suzuki 2002).

Reagent and solutions

All chemicals used were of analytical-reagent grade of the highest purity available procured from Merck. Doubly distilled de-ionized water and glass vessels were cleaned soaking in acidified solutions of $K_2Cr_2O_7$ followed by washing with $Con.HNO_3$, and were rinsed several times with high purity de- ionized water. Stock solutions and environmental water samples were kept in polypropylene bottle containing 1ml of $Conc.HNO_3$.

Preparation of reagent (VANA)

Vanillin (1.5g, 0.0098 mol) in 60 ml of methanol, 2-aminonicotinicacid (1.36 g, 0.0098 mol) dissolved in 60ml of methanol were taken in 250 ml round bottom flask. Suitable quantity (1ml) of 1molar sodium acetate and 2 or 3 drops of $con. H_2SO_4$ were added to the reaction mixture and refluxed for 8 hours. On cooling the reaction mixture, ash colored product was separated out. It was treated with water and ethyl acetate. It was collected by filtration and washed several times with hot water followed by n-hexane. This compound was recrystallised from methanol and dried in vacuum. The ligand is stable for more than 6 months. Percent of Yield is 91; m.p.84-86 °C. The structure of VANA is shown in the Fig.1.

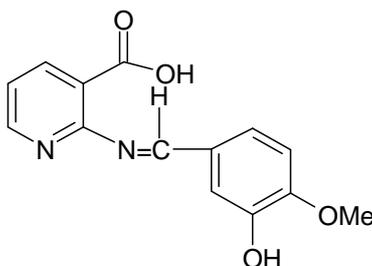


Fig.1. VANA – Structure

Characterization of reagent (VANA)

The reagent has been synthesized and characterized by IR, ¹HNMR and mass spectral data. Infrared spectrum of VANA shows bands at 3375, 3094, 2863, 1714, 1695, 1665, 1662, 1588, 1510, 1464, 1299, 1266, 1067 and 927 respectively corresponding to ν (O-H) symmetric stretch, ν (C-H) stretch (pyridine) (sp^2 -C-H), ν (-OCH₃) stretch, ν (C=O) Aromatic stretch, ν (C=N) Schiffbase, ν (C=C) Aromatic stretch, ν (HC=N) azomethine stretch, ν (C-C) Aromatic stretch, ν (C-C) stretch (pyridine ring), δ (C-H) (Aromatic ring), ν (C=N) stretch (pyridine), ν (C-O) stretch, ν (C-N) stretch and δ (O-H) oop bend (carboxylic acid). ¹H¹NMR spectrum of VANA (CDCl₃+DMSO-d₆) showed signals at 6.75 – 7.43(6H), 9.73 (1H), 4.87(3H), 5.26 (1H) due to benzene and pyridine protons, acid protons, methoxy protons, =C-H. Mass spectrum of VANA shows signal at 273 (M+1) corresponding to its molecular ion peak. The molecular formula of the reagent is C₁₄H₁₂N₂O₄ (M.Wt, 273).

pKa values of reagents

The pKa values were determined by recording the UV-Visible spectra of 1×10^{-3} M solutions of the reagent at various pH values and by taking the arithmetic mean of the values obtained from the measurements at different wave lengths determined spectrophotometrically using Phillips and Merrit method. The values of deprotonation of VANA were 7.0 (pK₁) and 10 (pK₂).

VANA solution

A 1×10^{-2} M solution was prepared by dissolving 0.2722 gm of VANA in 100 ml of methanol. The reagent solution is stable for at least 24 h.

Arsenic (III) Standard solution

A 100 ml amount of stock solution (1.0×10^{-2} M) was prepared by dissolving 0.3120 gm of Sodium arsenate heptahydrate Na₂HASO₄.7H₂O (E. Merck pre analysis) in de-ionized water.

1000 ppm stock solution of arsenic was prepared by dissolving 0.416 gm of Sodium arsenate heptahydrate in 100 ml distilled water.

Buffer Solution

1M Sodium acetate + 0.1M hydrochloric acid (0.5 – 3.0), 0.2M Sodium acetate + 0.2M acetic acid (3.5 – 6.0), 1M Sodium acetate + 0.2M acetic acid (6.5 – 7.5), 2M Ammonia + 2M ammonium chloride (8.0 – 12.0) buffer solutions are prepared in distilled water. Suitable portions of these solutions are mixed to get the desired pH.

Potassium permanganate solution

A 1% potassium permanganate solution was prepared by dissolving in de-ionized water. Aliquots of this solution were standardized with oxalic acid.

Tartrate solution

A 100 ml stock solution of tartrate (0.01% w/v) was prepared by dissolving 10 mg of ACS grade (99%) potassium sodium tartrate tetra hydrate in (100 ml) de-ionized water.

Aqueous ammonia solution

A 100-ml solution of aqueous ammonia was prepared by diluting 10 ml concentrated NH₃ (28–30%, ACS grade) to 100 ml with de-ionized water. The solution was stored in a polypropylene bottle.

Preparation of Sample solutions**Preparation of water samples**

Different water samples (Ground water and Tap water) were collected from various places in around Tirupati, A.P., India. The samples (150 ml) were stored at 5°C in metal free polyethylene bottles. Water samples were filtered through what man filter paper no. 41 and collected into 250 ml beakers. All the filtered environmental water samples were evaporated nearly to dryness with a mixture of 10 ml con HNO₃ and 5ml of con H₂SO₄ in a fume cupboard and then cooled to room temperature. The sample was digested in the presence of an excess potassium permanganate solution according the method recommended by Fified et al.⁵ The residues were then heated with 10 ml of deionized water in order to dissolve the salts. The solutions were cooled and neutralized with dilute NH₄OH. The digest was transferred into a 25- ml calibrated flask and diluted up to the mark with deionized water.

The reaction of As (III) was tested with (VANA) at different pH values. The samples were prepared in 10 ml of the solution containing constant volume of 25 ml of As (III), 2.0 ml of buffer solution (pH 5.0), 1.0 ml of 1×10^{-3} M VANA, 2.0 ml lithium chloride solution. The solution was then shaken with 10.0 ml portions of methanol for two minutes and then allowed to stand each time the combined organic phase of aliquot were taken in 10.0 ml standard flasks and made up to the mark with methanol. The absorbance was measured in 200-800 nm range against reagent blank.

Recommended procedure

A known aliquot of the sample solution was taken in a 25 ml standard flask containing constant volume of 10 ml of buffer solution (pH = 5), 1.0 ml of 1×10^{-3} M VANA and 1ml of As (III) solution made up to the mark with distilled water. Absorbance of the solution was measured at 350 nm against the reagent blank. The absorbance values were referred to the predetermined calibration plot to compute the amount of arsenic.

The author has introduced a new sensitive reagent VANA, for the extractive spectrophotometric determination of trace amounts of As (III).

The proposed method when compared with other spectrophotometric methods is more sensitive and selective. It also offers advantages like reliability and reproducibility in the addition to its simplicity instant color development and less interferences. The results obtained through UV- Visible spectrophotometer have been compared with those obtained through the ICP-OES.

RESULTS AND DISCUSSION

The reagent VANA may be easily prepared. The reagent solutions (0.001M) are found to be stable for 3 hours. The absorption band from 350 nm indicates the formation of As (III)-VANA complex in solution. Arsenic (III) reacts with VANA in acidic pH (5.0) to give water soluble complexes. The colour reactions are instantaneous at room temperature. The change in the order of addition metal ion, reagent VANA and buffer has no effect on the absorbance on the complexes. Analytical characteristics of the complexes are summarized in Table.1. The stoichiometry of the complexes (M:L =1:1) was determined by Job's continuous variation and molar ratio methods. Sodium acetate (0.2M) – acetic acid (0.2M) buffer solution (pH-5.0 and T= 300 K) and equimolar solutions of Arsenic (III) and VANA were used in the calculation of stability constants of the complexes.

Absorption Spectra of the Reagent and As (III) - VANA Complex

Absorption spectra of As (III) - VANA complex and reagent shows maximum absorbances at 350 nm and 320 nm, respectively (Fig.2). Hence all the spectral measurements of the complex have been carried out at 350 nm.

The study of the effect of pH on the color intensity of the reaction mixture showed that the constant and maximum color is obtained in the pH range 4.0 - 6.0, the complex has maximum absorbance in buffer solution of pH (5.0). The analytical studies were therefore, carried out at pH 5.0.

Different molar excess of VANA were added to As (III) concentration and the absorbances are measured adopting the standard procedure. It was observed that 10 fold molar excess of reagent with respect to metal ion is necessary to get maximum absorbance. Hence a 10 fold molar excess of reagent was used for further experimental studies.

The absorbance of the solution was measured at different time intervals to ascertain the time stability of the color complex. It was observed that the color development was instantaneous and remained constant for more than 3 hrs. Physicochemical and analytical properties of As (III) complex of VANA are summarized in Table – 1.

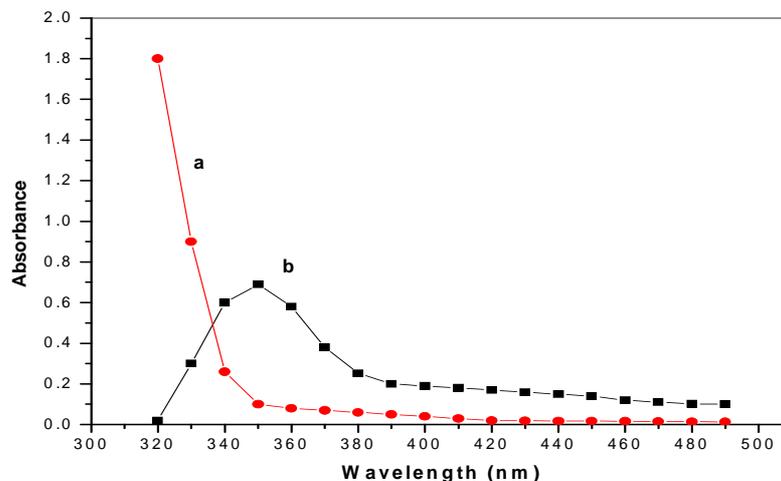


Fig.2. Absorption spectra of (a). As (III) – VANA complex ($\lambda_{\max} = 350$ nm) in aqueous solution, (b). VANA Vs Water blank (1×10^{-3} M)

Table 1. Physico – chemical and analytical characteristics of As (III) – VANA complex

S. No.	Characteristics	Results
1	λ_{\max} (nm)	350
2	pH range (optimum)	3.0 – 6.0
3	Mean absorbance	0.221 ± 0.001
4	Mole of reagent required per mole of metal ion for full color developed	10 Fold
5	Time stability of the complex (in Hrs)	3
6	Beer's law validity range ($\mu\text{g/ml}$)	0.128 - 2.66
7	Molar absorptivity ($\text{L mol}^{-1} \text{cm}^{-1}$)	6.805×10^3
8	Specific absorptivity ($\text{ml g}^{-1} \text{cm}^{-1}$)	0.0196
9	Sandell's sensitivity ($\mu\text{g/ml}$)	0.00146
10	Composition of complex as obtained in Jobs and molar ratio methods (M:L)	1 : 1
11	Stability constant of the complex	1.42×10^6
12	Standard deviation in the determination of $2.04 \mu\text{g/ml}$ of As (III) for ten determinations	0.00114
13	Relative standard deviation (RSD)%	1.8506
14	Y-intercept	-0.0316
15	Angular coefficient (m)	0.0759
16	Correlation coefficient (v)	0.9979
17	Detection limit ($\mu\text{g ml}^{-1}$)	0.06
18	Determination limit ($\mu\text{g ml}^{-1}$)	0.18

Adherence of the As (III) – VANA Complex System to Beer's law

For the possible determination of As (III) at micro level, the absorbance of the solution containing different amounts of the metal ions is measured at 350 nm. The linear plot between the absorbance and the amount of As (III) is drawn and the straight line obtained the equation $A_{350} = 0.0759 - 0.0316$ (Fig.3). Further Beer's law is obeyed in the range of 0.128 - 2.66 $\mu\text{g/ml}$, the molar absorptivity and sandell's sensitivity are found to be $6.805 \times 10^3 \text{ L.mol}^{-1}\text{cm}^{-1}$ and $0.00146 \mu\text{g/cm}^{-2}$ respectively the standard deviation of the method for ten determinations of $2.04 \mu\text{g / ml}$ is ± 0.00114 .

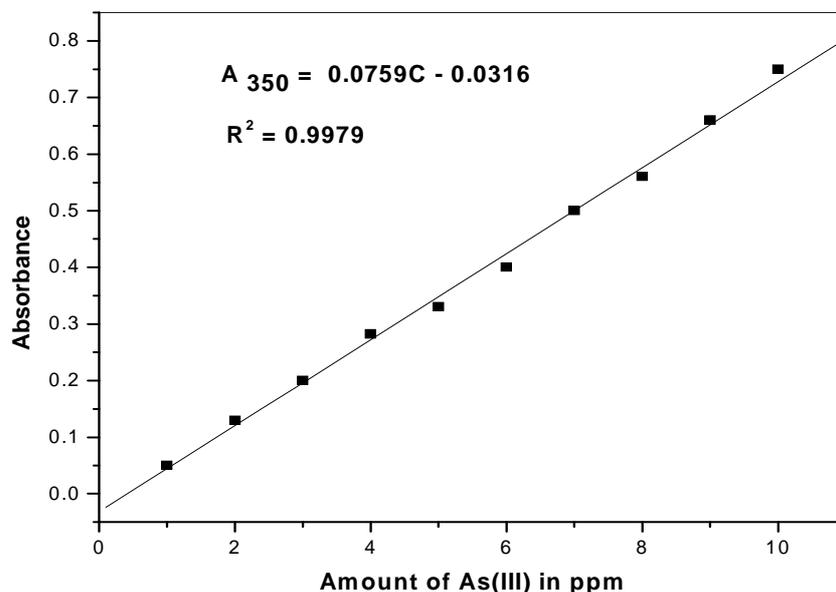


Fig.3. Calibration plot for As (III) determination

Interference of Foreign Ions

The effect of various cations and anions which are generally associated with the metal ion in the determination of As (III) was studied by measuring the absorbance of Arsenic. The complex contains 2.04 $\mu\text{g/ml}$ of Arsenic (III) in solution. The colour reaction is developed as described in the standard procedure. An error of $\pm 2\%$ in the absorbance reading was considered tolerable. The tolerance limit (TL) values in ppm for various anions and cations in the VANA methods respectively are presented in Table.2. Higher amounts of Fe^{3+} do not interfere in the presence of 70 ppm of fluoride. Larger amounts of Hg^{2+} do not interfere in the presence of 600 ppm of iodide.

The present method (VANA) was applied for the determination of Arsenic when present alone and present in water and soil samples. The present ligands containing aromatic ring are found to be potential and cost effective for the determination of Arsenic (III) without the need for extraction using the toxic solvents. Further the reagents are easy to synthesize using commercially available precursors. Moreover the present method is simple, rapid and very sensitive for non-extractive spectrophotometric determination of Arsenic (III) in aqueous medium.

Table 2.Effect of foreign ions

Ion Added	Tolerance limit $\mu\text{g/ml}$	Ion Added	Tolerance limit $\mu\text{g/ml}$
Citrate	384	W(v)	368
Tartarate	296	Mn (II)	22
Urea	288	Pb (II)	0.88
Iodate	254	Cr (VI)	1.2
Bicarbonate	244	Zn (II)	0.13 ^a
Thiocyanate	232	Cd (II)	2.3 ^a
Sulphate	192	Co(II)	0.47
Oxalate	176	Ni (II)	0.47
Thiourea	152	Fe (II)	0.22
Nitrate	124	Au (III)	0.40
Acetate	118	Pt (IV)	0.39
Phosphate	20	Ti (III)	0.42
Bromide	16	Ag (I)	0.22
Chloride	7.1	V (V)	0.20
Fluoride	4.0	Cu (II)	0.14

Effect of foreign ions on the extraction of the As (III) – VANA complex

The effect of foreign ions is studied by measuring the absorbance of the reaction mixture containing 2.04 µg/ml of As (III) in the presence of different amounts of foreign ions. The results are presented in the Table. 2. An error of $\pm 2\%$ in the absorbance value caused by foreign ions is considered as a tolerable limit.

Composition and stability constant of the complex

Job's method of continuous variation and molar-ratio methods are applied to ascertain the stoichiometric composition of the complex. It is found that VANA forms 1:1 complex with As (III) as shown in the (Fig.4).

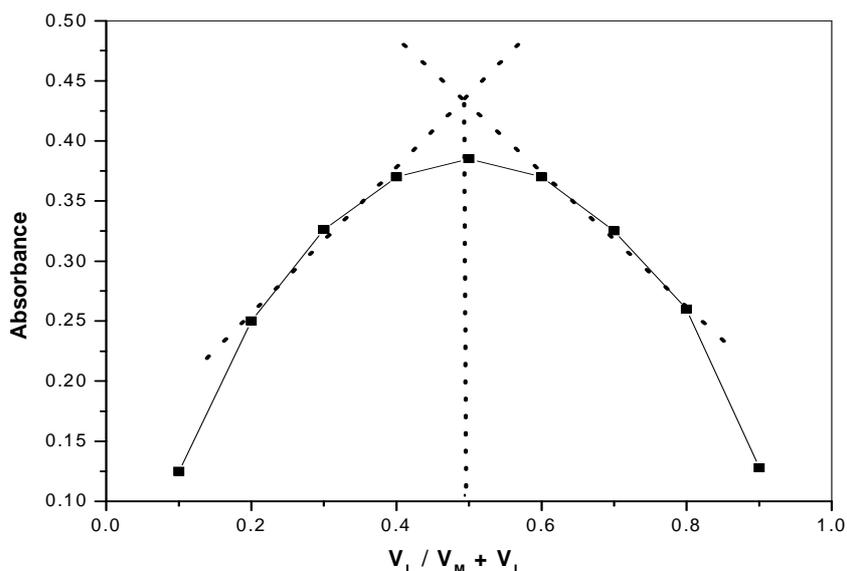


Fig.4. Job's method of continuous variation method As (III)-VANA. As (III) and VANA $1 \times 10^{-3}M$; solvent-Methanol; (pH=5.0)

Application

The proposed extractive spectrophotometric method is applied for the determination of As (III) in water samples. A known aliquot of the sample solutions were taken into a 25 ml separating funnel and the Arsenic content was determined as described in the general procedure. The results are checked with parallel determinations by direct ICP-OES. The data obtained in the analyses of water samples are given in Table 3 and 4.

Table 3. Determination of trace amount of As (III) in water samples

Name of the samples	Amount of arsenic ^a found µg/ml	
	ICP-OES	Proposed Method
Ground water ^b	1.69	1.66
Ground water ^c	1.54	1.36
Industrial waste water ^d	1.051	1.042
Ground water ^e	1.032	1.021
Ground water (upper) ^f	1.02	1.013
Ground water (lower) ^g	0.98	0.965

^a Average of five determinations; ^b Collected at Pollur (Palamaneru-chittoor), A.P, India.; ^c Collected at Ranipet, A.P, India.

^d Collected at Amarraja industry, A.P, India.; ^e Collected at Mahanandi, A.P, India.; ^f Collected at Yaganti (upper), A.P, India.

^g Collected at Yaganti (lower), A.P, India.

Table 4. Determination of trace amount of As (III) in soil sample

Name of the sample	Amount of arsenic ^a found µg/g	
	ICP-OES	Proposed Method
Polluted soil ^b	1.392	1.356

^a Average of five determinations

^b Collected at Pollur (Palamaneru-chittoor), A.P, India.

CONCLUSION

The proposed spectrophotometric method is simple, highly sensitive and selective for the determination of As (III) in water and soil samples. The proposed method is simple, rapid and common metal ions such as Fe³⁺, Pb²⁺, Co²⁺, Ni²⁺, Zn²⁺, Mn²⁺, Cr³⁺ do not interfere. Urea, bicarbonate, citrate, sulphide, SO₄²⁻, and PO₄³⁻ also do not interfere. The proposed method is more sensitive than some reported methods in the literature and has been successfully applied for the determination of arsenic in various environmental samples.

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