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A SIMPLE SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF ARSENIC IN ENVIRONMENTAL AND MEDICINAL LEAVE SAMPLES USING BENZIL-5-AMINO-1,3, 4- THIADIAZOLE-2-THIOL (BZATDT)

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ABSTRCT

Benzil-5-amino-1,3,4-thiadiazole-2-thiol (BZATDT) 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 4.0, Sodium acetate- acetic acid buffer) to form light yellow colored 1:1 (M:L). The colour reactions are instaneous and the maximum absorbance was obtained at 346 nm and remains constant for over 4 h. The molar absorptivity and Sandell's sensitivity of BZATDT found to be 5.70 x 10^3 L $mol^{-1}cm^{-1}$ and 0.00175 $\mu g cm^{-2}$ of As^{III} respectively. The system obeys Beer's law in the range of 0.126 - 2.64 µg/ml of As^{III}. Since BZATDT method is more sensitive it was applied for determination of Arsenic in some environmental water, soil and medicinal leave samples.

KEYWORDS: Spectrophotometry, Arsenic determination, BZATDT, Environmental and medicinal leave samples.

INTRODUCTION

Arsenic compounds are widely distributed in earth crust and in trace quantities in rocks, soils, water and air.[1,2,3] In recent decades millions of people have suffered from arsenic poisoning as a result of drinking arsenic – contaminated water extracted from shallow tube wells in south and Southeast Asia. Soil arsenic contamination has also occurred in some areas as a result of mining activities. Excessive uptake of arsenic by crop plants may present a food safety problem. It occurs in two forms inorganic arsenic and organic arsenic. Inorganic arsenic species i.e; arsenite and arsenate are present in ground water which cause tremendous epidemic poisoning across the globe. Organic arsenic species (MMA, DMR) are common metabolites found in the human body and are much less toxic. Arsenic also occurs in mineral forms, usually in combination with sulphur and metals, but also as a pure elemental crystal. . 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. sAnimals 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] 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,

Arsenic was also used in various agricultural insecticides and poisons. For example lead hydrogen arsenate was a common insecticide on fruit trees.[10] Arsenic is used as food additive in poultry and swine production and to disease.[11] Arsenic is used in bronzing and pyrotechniques.[12] Arsenic and its compounds, especially the trioxide, are used in the production of pesticides, treated wood products, herbicides, and insecticides. Arsenic is absorbed by all plants but is more concentrated in leafy vegetables, rice, apple, grape, juice and sea food.[13&14] Arsenic (III) is appreciably more toxic than arsenic (V). Usually these species of arsenic in natural water are found at the trace levels.[15] There are only a few analytical techniques available, which have more 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), capillary electrophoresis inductively coupled plasma mass spectrometry (CE-ICP-MS), high performance liquid chromatography–inductively coupled plasma mass spectroscopy[16], electro thermal atomic absorption spectrometry (ETAAS)[17], hydride generation – atomic absorption spectrometry[18], hydride generation – atomic fluorescence spectrometry[19], cathodic stripping Voltammetry[20], anodic stripping voltammetry[21], neutron activation analysis[22], photometric analysis[23], ion selective electrodes[24] and energy dispersive X-ray fluorescence spectrometry[25], 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., Benzil-5-amino-1,3,4- thiadiazole-2-thiol (BZATDT). Since the reagent is more sensitive and it is used for the determination of Arsenic in various water, soil and medicinal plant 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 trace metals in all samples. To determine the trace 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 trace metals were analyzed by Inductively Coupled Plasma-Optical Emission Spectrometry (Varian Vista-MPX CCD Simultaneous Spectrophotometer, Mugrave-Victoria, Australia).[26 & 27] (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 (BZATDT)

Benzil (3g, 0.01426 mol) in 20 ml of N,N- DMF, 5-amino-1,3,4-thiadiazole-2-thiol (1.89gm, 0.01426 mol) dissolved in 20 ml of N,N- DMF were taken in 250 ml round bottom flask. Suitable quantity (1g) of sodium acetate was added to the reaction mixture and refluxed for 8 hours. On cooling the reaction mixture, pale yellow colored product was separated out. It was collected by filtration and washed several times with hot water and N,N- DMF and n-hexane. This compound was recrystallised from N, N- DMF and dried in vacuum. Percent of a Yield is 4.650 gm; m.p.96-98 ^{0}C. The structure of BZATDT is shown in the Fig.1.

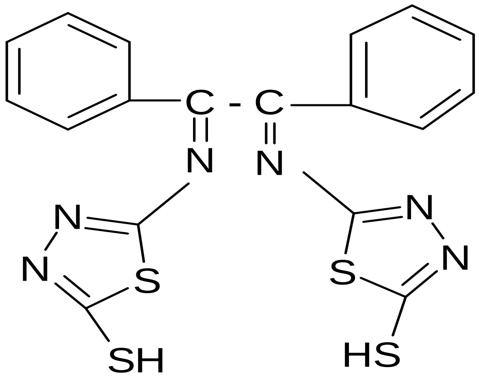

Fig.1. BZATDT – Structure

Characterization of reagent (BZATDT)

The reagent has been synthesized and characterized by IR, ^{1}HNMR and mass spectral data. Infrared spectrum of BZATDT shows bands at 3064, 1676, 1660, 1594, 1510, 1211 and 718 respectively corresponding to ν (C-H) Aromatic stretch (sp^2---C-H), ν (C = N) schiffbase, ν (C =C) Aromatic stretch, ν (C - C) Aromatic stretch, ν (C - N) stretch and ν (C - N) stretch . H^{1}NMR spectrum of BZATDT ($CDCl_3$+DMSO-d_6) showed signals at 7.62 – 7.94 (10H) and 2.51 (1H) due to benzene protons and thiolic protons. Mass spectrum of BZATDT shows signal at 441 (M+1) corresponding to its molecular ion peak. The molecular formula of the reagent is $C_{14} H_{12}N_2O_4$ (M.Wt, 440).

pKa values of reagents

The pKa values were determined by recording the UV-Visible spectra of $1x10^{-3}$ 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 BZATDT were 5.0 (pK_1) and 6.0 (pK_2).

BZATDT solution

It was prepared by taking 0.0110 g of the reagent BZATDT in a 25-ml standard flask. The substance was dissolved in DMF and diluted up to the mark with the same solvent.

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 $Na_2HASO_4.7H_2O$ (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 NH_3 (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) were collected from various places around tirupati and A.P, India. The samples (150 ml) were stored at 5^oC 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 HNO_3 and 5 ml of con H_2SO_4 in a fume cupboard and then cooled to room temperature. The sample was digested in the presence of an excess potassium permanganate solution according to the method recommended by Fifiled et al., The residues were then heated with 10 ml of deionized water in order to dissolved the salts. The solutions were cooled and neutralized with dilute NH_4OH. The digest was transferred into a 25 ml calibrated flask and diluted up to the mark with deionized water.

Soil samples

Preparation of the soil samples were carried out by microwave digestion. Approximately 1 g (dry mass) of soil samples were weighed directly in to the PTFE vessels, to which 10 ml of conc. HNO_3 was added and the vessels were capped immediately. The digestion programme consisted of a ramp time of 10 min tom reach 180^0 C. The power was 800 W. After the completion of program vessels were cooled ,vented and opened and then 2 ml of 30% H_2O_2 as added and filtered the solutions in to 25 ml volumetric flasks and made up with double distilled water. Blanks were prepared by following similar digestion procedure without soil sample.

Preparation of medicinal leaf samples

Sample preparation of the leaf samples were carried out by microwave digestion. Approximately 1 g (dry mass) of leaf sample materials were weighed directly in to the PTFE vessels, to which 10 ml of conc. HNO_3 was added and the vessels were capped immediately. The digestion programme consisted of a ramp time of 10 min tom reach 150^0 C. The power was 800 W. After the completion of program vessels were cooled vented and opened and then 2 ml of 30% H_2O_2 as added and filtered the solutions in to 25 ml volumetric flasks and made up with double distilled water. Blanks were prepared by following similar digestion procedure without plant material.

The reaction of As (III) was tested with (BZATDT) 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 4.0), 1.0 ml of $1x10^{-3}$ M BZATDT, 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 = 4), 1.0 ml of $1x10^{-3}$ M BZATDT and 1ml of As (III) solution made up to the mark with distilled water. Absorbance of the solution was measured at 346 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 BZATDT, 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 BZATDT may be easily prepared. The reagent solutions (0.001M) are found to be stable for 4 hours. The absorption band from 346 nm indicates the formation of As (III)-BZATDT complex in solution. Arsenic (III) reacts with BZATDT in acidic pH (4.0) to give water soluble complexes. The colour reactions are instantaneous at room temperature. The change in the order of addition metal ion, reagent BZATDT 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-4.0 and T= 300 K) and equimolar solutions of Arsenic (III) and BZATDT were used in the calculation of stability constants of the complexes.

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

Absorption spectra of As (III) - BZATDT complex and reagent shows maximum absorbances at 346 nm and 320 nm, respectively (Fig.2). Hence all the spectral measurements of the complex have been carried out at 346 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 3.0 - 5.0, the complex has maximum absorbance in buffer solution of pH (4.0). The analytical studies were therefore, carried out at pH 4.0.

Different molar excess of BZATDT 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 4 hrs. Physicochemical and analytical properties of As (III) complex of BZATDT are summarized in Table – 1.

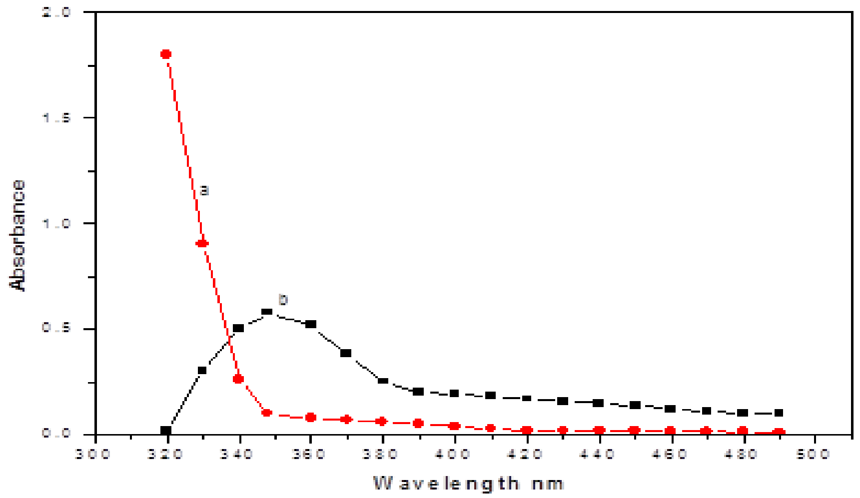

Fig.2 Absorption spectra of (a). As (III) – BZATDT complex (λmax = 346 nm) in aqueous Solution, (b). BZATDT Vs Water blank ($1x10^{-3}$M)

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

S. No.	Characteristics	Results
1	λ_{max} (nm)	346
2	pH range (optimum)	3.0 – 5.0
3	Mean absorbance	0.211 ± 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)	4
6	Beer's law validity range (µg/ml)	0.126 - 2.64
7	Molar absorptivity (L mol^{-1} cm^{-1})	5.70 x 10^3

8	Specific absorptivity (ml g^{-1} cm^{-1})	0.01106
9	Sandell's sensitivity (µg/ml)	0.00175
10	Composition of complex as obtained in Jobs and molar ratio methods (M:L)	1 : 1
11	Stability constant of the complex	1.10×10^{6}
12	Standard deviation in the determination of 2.04 µg/ml of As (III) for ten determinations	0.00117
13	Relative standard deviation (RSD)%	1.857
14	Y-intercept	0.0288
15	Angular coefficient (m)	0.2009
16	Correlation coefficient (v)	0.9972
17	Detection limit (µg ml^{-1})	0.06
18	Determination limit (µg ml^{-1})	0.18

Adherence of the As (III) – BZATDT 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 346 nm. The linear plot between the absorbance and the amount of As (III) is drawn and the straight line obtained the equation A_{346}=0.2009+0.02889 (Fig.3). Further Beer's law is obeyed in the range of 0.126 - 2.64 µg/ml, the molar absorptivity and sandell's sensitivity are found to be 5.705×10^{3} $L.mol^{-1}cm^{-1}$ and 0.00175 µg/cm^{-2} respectively the standard deviation of the method for ten determinations of 1.3932 µg / ml is ± 0.00114.

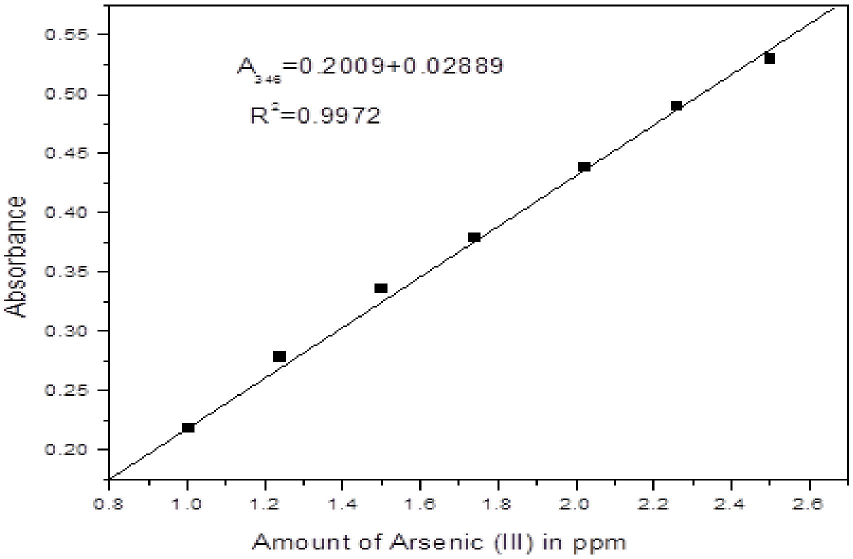

Fig.3. Calibration plot for As (III) determination

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 2.03 µg/ml of Arsenic (III) in solution. The colour reaction is developed as described in the standard procedure. An error of ±2% in the absorbance reading was considered tolerable. The tolerance limit (TL) values in ppm for various anions and cations in the BZATDT 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 (BZATDT) 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 µg/ml	Ion Added	Tolerance limit µg/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) – BZATDT complex

The effect of foreign ions is studied by measuring the absorbance of the reaction mixture containing 2.03 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 ±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 BZATDT forms 1:1 complex with As (III) as shown in the (Fig.4).

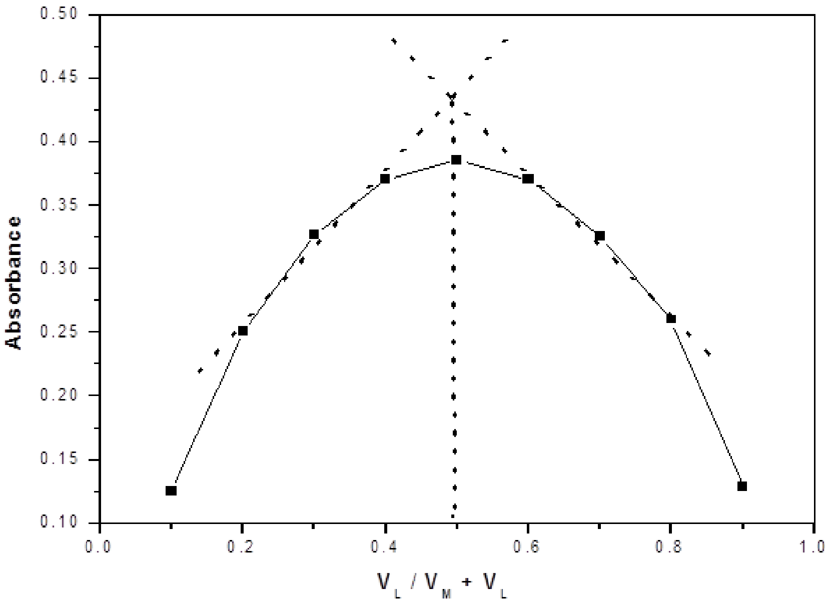

Fig.4. Job's method of continuous variation method As (III) - BZATDT. As (III) and BZATDT 1x10^{-3}M: solvent-Methanol; (pH=4.0)

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]	1.60	1.58
Ground water[c]	1.4	1.37
Industrial waste water[d]	1.05	1.06
Ground water[e]	1.03	1.04

a Average of five determinations
b Collected at Pollur (Chittoor District) A.P, India.
c Collected at Ranipet (Chittor District) A.P, India.
d Collected at Karakambaadi, Tirupati A.P, India.
e Collected at Yaganti, Kurnool (District) 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]	0.0642	0.062
Polluted soil[c]	33.780	31.78

a Average of five determinations
b Collected at kuppam (chittoor), A.P, India.
b Collected at kolar gold field mines ,Karnataka, India

Table: 5. Determination of trace amount of As (III) in Medicinal leaf samples

Name of the sample	Amount of arsenic[a] found µg/g	
	ICP-OES	Proposed Method
Azadirachta indica[b]	2.652	2.641
Dathura [b]	6.542	6.523

a. Average of the five determination
b. Collected at Karakambaadi, 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. In common metal ions such as Fe^{3+}, Pb^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+},Mn^{2+}, Cr^{3+} do not interfere. Urea, bicarbonate, citrate, sulphide, SO_4^{2-}, and PO_4^{3-} 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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