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Spectrophotometric Determination of Trace Amounts of Arsenic In Sediment, Flyash and Water Samples

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Abstract: A simple, rapid, cost-effective spectrophotometric method is developed to determine arsenic in ppm levels. The present study is based on the reaction of arsenate with acidified molybdate and vanadate to form a molybdovanadoarsenate complex. The heteropoly acid complex formed interacts with cationic dye, Rhodamine B resulting in pink-coloured ion-pair complex whose absorbance is measured at 590 nm. The colour developed is instantaneous and is stabilized with polyvinyl alcohol. Beer's law is obeyed up to 0-0.24 µg $mL^{-1.}$ The molar absorptivity, Sandell's sensitivity, detection limit and limit of quantification (LOQ) are 1.653×10^5 L $mol^{-1}cm^{-1}$, 4.532×10^{-4} µgcm^{-2}, 0.00916 µg mL^{-1} and 0.02785 µg mL^{-1} respectively. The composition of ion-pair complex formed is found to be arsenic: molybdenum: vanadium: Rhodamine B is 1:11:1:4 by mole-ratio method. The proposed method is highly sensitive for arsenic with phosphate alone interferes which is eliminated by reducing arsenate to arsine. The method is useful in determining arsenic at low concentrations in sediment, fly ash and water samples.
Keywords: Arsenic, Spectrophotometer, Heteropoly acid, Rhodamine B, fly ash and sediments.

1. Introduction

Arsenic (As), a ubiquitous semi-metal or metalloid belongs to Group Va (15) of the periodic table exists both in organic and inorganic forms in natural environment. The inorganic forms of As species include As in +3, +5 and in -3 oxidation state while organic form consists of monomethyl arsenic acid, dimethyl arsenic acid, trimethyl arsine etc., Arsenic enters into water ecosystem by natural leaching of arsenopyrite ore, by agricultural run-off due to indiscriminate use of arsenic pesticides like monosodium arsenate, disodium arsenate, lead arsenate and also from poultry washings where roxarsone is used as a nutritional supplement for broilers[1]. Arsenic may accumulate in sediment and soil due to excessive usage of arsenic pesticides, by burning of coal as well as disposal of industrial, municipal and animal waste [2]. Arsenic finds its application in glass industry, pigment, pesticide manufacture, printing etc., It is regarded as a powerful haemolytic poison. The symptoms of acute arsenic poisoning are hyperpigmentation, keratosis, skin cancer, skin ulceration, gastrointenstinal disorder. Long term exposure leads to cardiovascular damage, jaundice and anaemia[3]. Among various forms of arsenic, As(III) is reported to be 25 – 60 times more toxic than As(V) and several hundred times more toxic than organoarsenicals[4].As(III) exhibits its toxicity by interfering with thiol or sulfahydryl groups of enzyme thereby inhibiting their enzyme activity. The maximum permissible limit for arsenic in drinking water as prescribed by WHO and USEPA is 10 ppb. The discharge limit for arsenic in wastewater as recommended by CPCB is 0.2 ppm. High level of arsenic contamination in ground water is seen in Bangladesh followed by West Bengal, India. The arsenic level in groundwater of 111 blocks in 12 districts of West Bengal is in the range of 0.05-3.6 mg/L which is 360 times more than the permissible limit. In these areas, more than 1.5 million people are affected by arsenic poisoning with skin lesions and hyper pigmentation.

In order to determine trace amounts of arsenic in environmental samples numerous analytical methods like atomic absorption spectroscopy (AAS) [5], inductively coupled plasma (ICP) [6], ion chromatography (IC) [7], non-atomic spectrometric methods [8], neutron activation [9] and electro-analytical techniques like voltammetery[10], differential pulse polarography [11] have been reported. Among the methods, HPLC is suggested to be a versatile technique in arsenic speciation with AAS [12] and fluorescence spectrometry [13]. However these techniques, suffer from complicated sample preparation, time consuming for routine analysis of large number of samples and the use of expensive instrument

In this regard, spectrophotometers offer a simple, less expensive technique and the accessibility of the instrument in most of the laboratory have resulted in improving and developing a new method for arsenic determination. Most of the spectrophotometric methods reported have used either chromogenic reagent like alizarin red s [14], azure B[15], methyl orange[16], Rhodamine B [17-18], Variamine blue [19], leuco malachite green [20], methyl red [21], Toluidine blue or Safranine O [22], ethyl violet [23], methylene blue [24], or silver diethyldithiocarbamate[25], ammonium pyrrolidinedithiocarbamate[26],acetyl-5-chloro thiophene 5-amino-1,3,4- thiadiazole 2-thiol [27] 2,4-dihydroxy benzophenone-2-amino thiophenol[28]. These methods suffers from limitations such as low sensitivity [14] or extraction into organic solvents like benzene or hexane [17] which are carcinogenic in nature or interferences from large number of ions. Hence a simple, rapid and cost-effective method has to be developed to determine arsenic at these low concentrations without any pretreatment.

The present study focuses on the reaction of arsenic with acidified molybdate and vanadate to form yellow-colouredheteropoly acid. The acid formed interacts with cationic dye, Rhodamine B to form pink-coloured ion-pair complex whose absorbance are measured at 590 nm. The method is advantageous as these reactions occur instantaneously.

2. Materials and methods

2.1 Chemicals

A spectronic-20 spectrophotometer andCarl-Zeiss Spekol UV-Visible spectrophotometer with 10-mm glass cells were used for both absorbance measurements and scanning the spectrum. An Elico-LI-120 digital pH meter equipped with calomel-glass electrode was employed for measuring pH of the solution.All chemicals used for the study were of analytical reagents. Standard As (V) solution (1000 µg/mL) was prepared by dissolving 0.4165g of disodium hydrogenarsenateheptahydrate ($Na_2HAsO_4.7H_2O$) in water with 5mL of 1N H_2SO_4 and diluting to 100 mL. The solution was suitably diluted to obtain 1 ppm of As (V) solution. An acidified ammonium Molybdate solution (1%) was prepared by dissolving 1 g of ammonium heptamolybdate tetrahydrate($(NH_4)_6Mo_7O_{24}.4H_2O$) in minimum amount of water followed by adding 40 mL of 10 N H_2SO_4 and diluted to 100 mL with water. Ammonium vanadate (NH_4VO_3) solution (0.1%) was prepared by dissolving 100 mg of ammonium vanadate in 100 mL of distilled water. The solution was diluted further to 0.01% solution. Rhodamine B (0.02%) was prepared by dissolving 0.10g of Rhodamine B in 500 mL water. Polyvinyl alcohol solution (PVA) (0.1%) was prepared by dissolving 0.1g of PVA in boiling water and diluting to 100 mL. Sediment samples were collected from different places along Adyarriver at Saidapet, canal mix and at Nandambakkam. Fly ash samples were collected from Ennore Thermal power station (ETPS) from boilers and also from stacks. Groundwater samples were collected from Sathyabama University and Saidapet area in Tamilnadu.

2.2 Experimental Procedure

Aliquots of standard solution containing not more than 6µg of As(V) is taken in a series of 25mL standard flask. To this solution added 1mL of 1% acidified molybdate, 1mL of 0.01% ammonium vanadate followed by 2mL each of 0.02% Rhodamine B and 0.1% polyvinyl alcohol. The solution was diluted to the mark with distilled water and the absorbance was measured at 590 nm in 10 mm cells against reagent blank. The concentration of As (V) was determined from the calibration graph.

2.3Arsenic in sediments and fly ash

Sediment samples are dried in sunlight for 48h to remove the moisture content. 0.5g of dried sediment and fly ash is digested with conc. HCl and conc.HNO_3 till white fumes of SO_3 are observed. The digested samples are cooled, filtered through G_4 sintered crucible. The filtrate and washings are made up to 250mL with

distilled water. The filtrate are appropriately diluted and used for analysis by converting arsenic to arsine to eliminate interference due to phosphate.

2.4 Arsenic in water

Ground water samples were collected from tap waters in polyethylene bottles. The samples were acidified with 1% conc. HCl immediately after collection and filtered through Whatman 41 filter paper. The filtrates are suitably diluted and used for analysis by converting arsenic to arsine.

3. Results and Discussion

3.1 Selection of wavelength

Absorption spectra of Rhodamine B with different concentrations of As(V) in the presence of PVA were scanned from 450 – 700 nm against distilled water is depicted in Fig.1. It is evident from the Fig 1, the reagent blank (curve A) showed a maximum absorbance at 560 nm while in samples, curve B, C and D the absorbance decreases. The curve E represents the spectra of 3µg of arsenic (V) against reagent blank which exhibits a maximum absorbance at 590 nm. Hence, further studies were carried out at 590 nm.

3.2 Effect of experimental variables

Investigation of various parameters involved in the reaction was carried out in order to estimate the reliability of the method developed. The colour system was unstable due to cogulation of colloidal complex and is prevented by adding 2 mL of polyvinyl alcohol [29]. The coloured complex is formed at acidic pH. Optimization experiments for pH were conducted by varying the pH from 0.05- 0.3N. The formation of ion-pair is retarded at both low and high pH. Maximum absorbance was obtained at pH 0.16N with respect to sulphuric acid.

The effect of ammonium molybdate, ammonium vanadate and Rhodamine B in the formation of ternary complex is shown in Fig.2. It is evident from the graphs that the addition of 1ml of 0.01% ammonium vanadate, 1ml of 1% ammonium molybdate and 2ml of 0.02% Rhodamine B was sufficient to give maximum absorbance at 590 nm. At high concentration of either vanadate or molybdate, the absorbance value decreases owing to higher reagent blank values and also due to interaction of excess molybdate or vanadate with Rhodamine B. Low absorbance value is also observed when the concentration of Rhodamine B is decreased below 2mL because of lesser interaction between the heteropoly acid and the cationic dye, Rhodamine B.

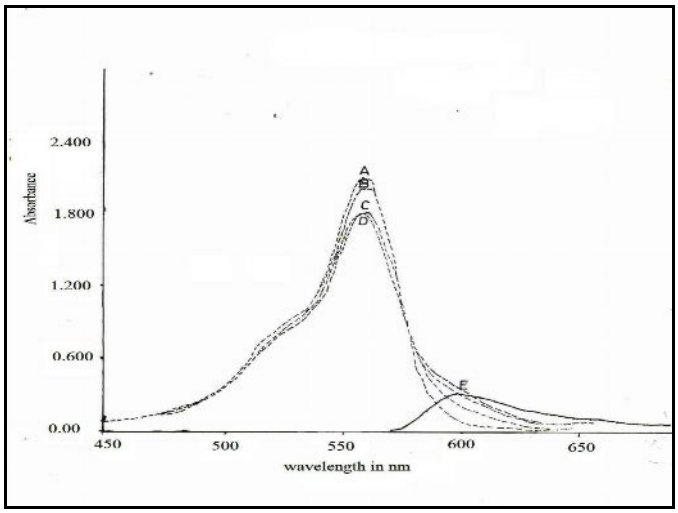

Fig 1. Absorption spectrum of molybdovanadic acid Rhodamine B systems (0.16N H_2SO_4, total volume 25 mL and 10 mm cells), Curve A- 1 ml each of 1% ammonium molybdate and 0.01% ammonium vanadate, 2 ml each of 0.02% Rhodamine B and 0.1% poly vinyl alcohol : reference water. Curve B, C and D –as in A with 1, 2, 3 µg of As(V) respectively: reference water. Curve E- as in D: reference blank

The effect of order of addition of reagents plays an important role in the analysis. The addition of molybdate or vanadate prior to Rhodamine B resulted in high absorbance value. But, the addition of

Rhodamine B before the molybdate or vanadate gave low absorbance value due to difficulty in heteropoly acid formation.

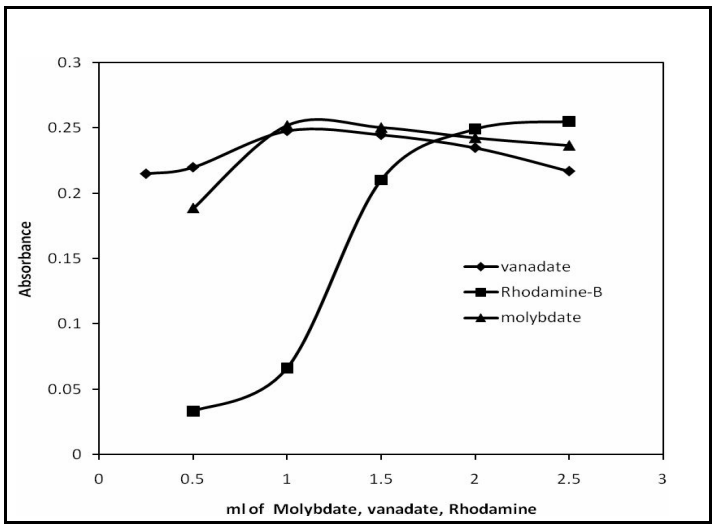

Fig 2. Effect of Molybdate, Vanadate and Rhodamine-B on colour development.

3.3 Calibration graph

At optimum concentration of the reagent, the colour developed by ion-pair complex was instantaneous and remained stable up to 60 minutes. Under these conditions, a calibration graph is depicted for 0-6 µg of As (V)/ 25ml in Fig.3. A straight line passes through the origin indicating the validity of Beer's law. The molar absorptivity and Sandell's sensitivity were calculated from the slope of the line and is found to be 1.629×10^5 L mol^{-1} cm^{-1} and 4.599×10^{-4}µgcm^{-2} respectively for the colour system at 590 nm. The precision for the method developed was established by carrying out 10 separate determinations of standard solution containing 3 µg of As(V)/25ml. The mean absorbance for 10 determinations was found to be 3 µg/25mL with a standard deviation of 0.075 absorbance unit. The relative standard deviation of the method was 2.5%. The detection limit (DL) and limit of quantification (LOQ) were calculated and found to be 0.00916 µg/L and 0.02785µg/L respectively.

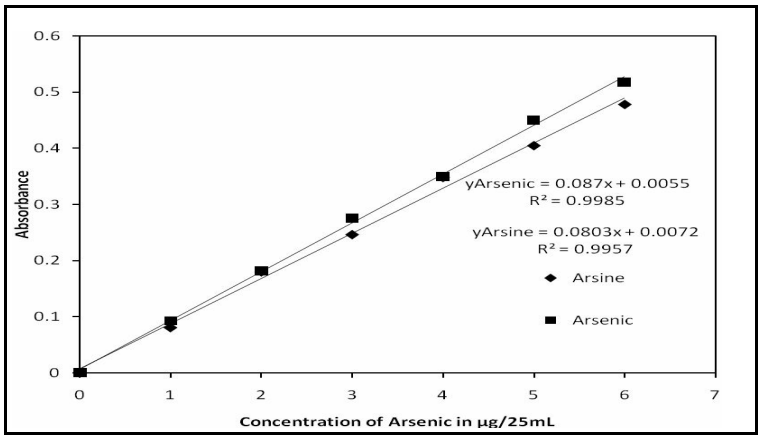

Fig. 3 Calibration graph for As(V) and arsine.

3.4 Composition of ion-pair

The composition of ion-pair was established by mole-ratio method and is shown in Fig 4. The results indicated that the complex forms a molar ratio of arsenic: molybdate as 1:11, arsenic: vanadate as 1: 1 and arsenic: rhodamine as 1: 4. The stoichiometric reactions for the formation of ion-pair complex is given below

$$7AsO_4^{-3} + 11\ Mo_7O_{24}^{-6} + 7VO_3^{-} + 66\ H^{+} \rightarrow 7[AsMo_{11}VO_{40}]^{-4} + 33H_2O$$

$$4[Rh.B]^{+} + [AsMo_{11}VO_{40}]^{-4} \longrightarrow [Rh.B]_4[AsMo_{11}VO_{40}]$$

where Rh represents Rhodamine B.

The composition of the ion-pair formed is 1:1:11:4 for the proposed system is in accordance with phosphorus system which exhibits 1:1:11:4 for P-V-Mo-Brillant green[30].

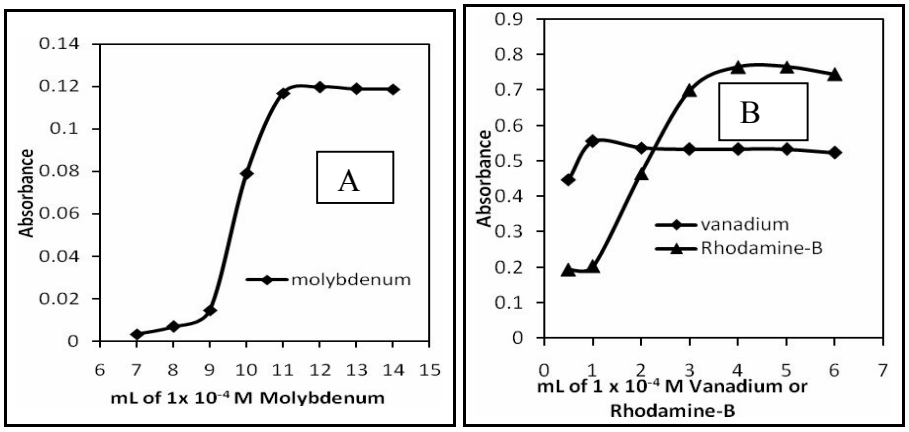

Fig. 4. Mole ratio plot for As: Mo [A] As: V and As: Rhodamine B [B]onion-pair formation.

3.5 Interference study

A systematic study on the interference of 1 mg of several anions and cations on the determination of 2.5 µg of As(V) was carried out and the results are summarized in the Table 1 along with their tolerance limits. The presence of tungstates, phosphates and silicates forms ion-pair complex similar to arsenic thereby enhancing the absorbance value. The ions like Ba^{2+}, Cr^{6+}, F^- decreases the absorbance value as F^- ions decreases the availability of ions and Ba^{2+} ions forms of insoluble precipitates. The negative effect of Cr^{6+} ion on the colour system is due to its oxidizing nature on the dye. It was overcome by converting it to Cr^{3+} by adding 2mL of hydroxyl amine. Phosphate ion alone interferes seriously at all concentrations and necessitates its separation prior to the analysis.

Table 1 Interference study

S.No	Ions	Tolerance limits (mg)
1	K^+, Mg^{2+}, Ca^{2+}, Zn^{2+}, Mn^{2+}, Cd^{2+}, Cr^{3+}, As^{3+}, Cu^{2+}, Ni^{2+}, Hg^{2+}, Fe^{2+}, EDTA, Cl^-, Br^-, NO_3^-, NO_2^-, SO_4^{2-}	<1000
2	SiO_3^{2-} F^- Ba^{2+} WO_4^{2-}	< 250 < 500 < 100 < 50
3	PO_4^{3-} Cr^{6+}	Interfered at all concentrations

3.6 Separation of arsenic as arsine

In order to eliminate the interference due to phosphate and to increase the sensitiveness of the method, arsenic is converted to arsine by using sodium borohydride. To 25mL of solution containing not more than 6 µg of As(V) was taken in the arsine generator. 10mL of concentrated HCl was added to reduce the pH <6.0 and 20 ml of 4% sodium borohydride was added and the solutions were stirred by magnetic stirrer. The arsine generated was allowed to pass through a glass wool impregnated with lead acetate to eliminate H_2S. The evolved arsine is absorbed in a solution containing 3 mL of aqueous KI-I_2 solution and 1mL of $NaHCO_3$ for 10 minutes. The absorbing solution is treated with a drop of metabisulphite in order to decolourise the iodine colour. The solution is used directly for the formation of ion-pair complex using the method developed whose absorbance is measured at 590nm. A calibration graph for arsine is represented in Fig.3 along with As(V). The statistical parameters such as limit of detection (LOD), limit of quantification (LOQ), slope, intercept and co-efficient of regression (R^2) for the proposed method are shown in Table 2. The proposed method is compared with other spectrophotometric heteroploy acid method in Table 3.

Table 2 Statistical parameters for the proposed method.

Parameters	**As(V)**	**AsH_3**
Wavelength (λ_{max})	590 nm	590 nm
Beer's law range (µg/mL)	0.02-0.24	0.12-0.24
Molar absorptivity ($Lmol^{-1}cm^{-1}$)	1.629×10^5	1.498×10^5
Regression equation Slope (b) Intercept (a) R^2	Y=0.087b + 0.005a 0.087 0.005 0.998	Y=0.080b+ 0.007a 0.080 0.007 0.995
Sandell sensitivity ($\mu g/cm^2$)	4.599×10^{-4}	5.0×10^{-4}
Limit of Detection (LOD) (µg/mL)	0.00916	0.04089
Limit of quantification (LOQ) (µg/mL)	0.02785	0.1239

Table 3 Comparison of the proposed method with other methods

Method	**Wavelength λ nm**	**Range of determination mg/L**	**Remarks**
Ammonium Molybdate + sodium Vanadate [31]	460	1-30	P, Si interfers, less sensitive
Ammonium Vanadate + crystal violet [32]	545	0.2-0.8	phosphate interfers
Silver diethyldithiocarbamate [33]	525	0.19-0.28	Less sensitive
Ammonium molybdate + SDHA [34]	780	0.02-0.14	P interfers, extraction requitred and time consuming.
As(III) + iodate + Rhodamine B [18]	553	0.04-0.4	High cost of iodate
As(V) + molybdate + vanadate + Rhodamine B (proposed method)	590	0.02-0.24	High colour stability, only phosphate interfers eliminated by arsine formation.
AsH_3 + molybdate + vanadate + Rhodamine B (proposed method)	590	0.12-0.24	High colour stability, no interferences.

3.7 Application to environmental samples

Table 4 represents the analysis of arsenic in sediments, flyash and water samples with and without standard addition of arsenic (V) by the proposed arsine method and its value ranges from 14-38 µg/g, 159-222 µg/g and 0.18-0.20 µg/mL respectively. The recovery of arsenic for the above method using standard addition varies from 92-101% and proves to be satisfactorily in various samples.

Table 4 Application to environmental samples

Sample	**Weight of sample**	**Addition of As(V)**	**Total As obtained**	**Recovery of As %**
Sediment (Adyar River- Saidapet)†	0.5 0.5	- 50	7.2 57.6	 100
Sediment (Adyar River-canal mix)†	0.5 0.5	- 50	12 60.8	 97.6
Sediment	0.5	-	18.8	

(Adyar River Nandambakkam)†	0.5	50	67.2	96.8
Flyash (ETPS-boilers)†	0.5 0.5	- 50	79.7 126.4	 93.4
Flyash (ETPS-Stacks)†	0.5 0.5	- 50	111.0 161.6	 101
Water samples a.Tap water 1* b. Tap water 2*		- 0.100 - 0.100	0.184 0.276 0.204 0.304	 92 100

All the values are the average of 3 determinations
†Concentrations in µg/g *Concentrations in µg/mL

4. Conclusion

The method described is a simple, cost-effective and reliable means of determining trace amount of arsenic by spectrophotometer. It is highly sensitive (ε= 1.5-1.6 × 10^5 $l.mol^{-1}.cm^{-1}$) than standard Molybdenum blue (ε= 2.5× 10^4 $l.mol^{-1}.cm^{-1}$) or silver diethyl dithiocarbamate (ε=1.4 × 10^4 $l.mol^{-1}.cm^{-1}$) and usage of pungent smell of pyridine is avoided. The colour produced is instantaneous and is stable up to 60 minutes. The method can be applied for the determination of trace amounts of As (V) in the range of 0.15 - 6µg /25mL. This method is highly selective and is free from interferences except phosphates which can be removed by converting arsenic to arsine. It is more rapid method and can be used for precise determination of arsenic in natural samples.

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