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Spectrophotometric determination of arsenic in environmental and biological samples

B Narayana,* Tom Cherian, Mendalin Mathew & Chand Pasha

Department of Post Graduate Studies and Research in Chemistry, Mangalore University, Mangalagangothri 574 199, India
Email: nbadiadka@yahoo.co.uk

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A simple and sensitive spectrophotometric method has been developed for the determination of arsenic using variamine blue as a chromogenic reagent. The method is based on the reaction of arsenic(III) with potassium iodate in acid medium to liberate iodine, which oxidizes variamine blue to form a violet coloured species having an absorption maximum at 556 nm. Beer's law is obeyed in the range 0.2-14 μg mL^{-1} of As(III). The molar absorptivity, Sandell's sensitivity, detection limit and quantitation limit were found to be 1.43×10^4 L $mol^{-1}cm^{-1}$, 5.26×10^{-2} μg cm^{-2}, 0.022 and 0.072 μg mL^{-1}, respectively. The optimum reaction conditions and other analytical parameters were evaluated. The proposed method has been successfully applied for the determination of arsenic in various environmental and biological samples.

Keywords: Arsenic, Spectrophotometry, Iodate, Variamine blue

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Arsenic compounds are widely used and have long been recognized as toxicants[1-3]. Trace concentrations of arsenic can also affect the physical and mechanical properties of metals and alloys[4]. Arsenic may accumulate in soils and sediments due to the use of arsenical pesticides, fertilizers, irrigation and oxidation of volatile arsine in air, dust from the burning of fossils fuels as well as disposal of industrial, municipal and animal wastes[5]. Arsenic is a constituent of many foods such as meat, fish, poultry, grains and cereals[6]. It is used in medicine, glass manufacture, pigment production, rodent poisons, insecticides, fungicides, printing, tanning, etc. In excessive amounts, arsenic causes gastrointestinal damage and cardiac damage. Chronic doses can cause vascular disorders such as black foot disease[6]. Arsenic and its compounds are reported to be carcinogenic, mutagenic and teratogenic in nature. The toxicity, availability and environmental mobility of arsenic is very much independent on their chemical forms[7].

Arsenic can exist in a variety of oxidation states and in organic and inorganic forms in many environmental matrices such as natural water and soil[8]. The predominant oxidation states of arsenic are As(III) and As(V). Therefore, precise knowledge of the arsenic compounds present in a system is required for an accurate assessment of the environmental and biological impact of arsenic, which have resulted in an increasing need of analytical methods for their determination at micro-trace or ultra-trace levels.

Many methods have been reported for the determination of arsenic such as flow injection analysis with hydride generation[9], atomic absorption spectroscopy[10], gas fluorometry-atomic absorption spectroscopy[11], induced coupled plasma-atomic absorption spectroscopy[12], neutron activation analysis[13], fluorescence spectroscopy[14]. Some of the chromogenic reagent used for the spectrophotometric determination of arsenic are ammonium pyrrolidine-dithiocarbamate[15], silver diethyldithiocarbamate[16], methylene blue[17], diantipyrylmethane[18], alizarine Red S[19], chlorpromazine[20] , methyl orange[21], bismuthiol II[22], dithiodiantipyrylmethane[23], antipyrylazo-4-hydroxybenzenedithiocarboxylic acid[24], malachite green[25] and dithiopyryl-methane[26]. However, most of these methods suffer from certain limitations, such as interference by a large number of ions[15,16], of low sensitivity[19,21] and need of extraction into organic solvents or heating[15,18].

In the proposed method arsenic is reacted with acidified potassium iodate to liberate iodine. The liberated iodine oxidizes variamine blue to form a violet coloured species having an absorption maximum at 556 nm. The proposed method has been successfully applied for the determination of arsenic in various environmental and biological samples.

Experimental Procedure

Apparatus

A Secomam Anthelie NUA 002 UV-VIS spectrophotometer with 1 cm quartz cell was used for the absorbance measurements and a WTW pH 330, pH meter was used for pH measurements.

Reagents

All chemicals used were of analytical grade and distilled water was used for dilution of reagents and samples. Standard arsenic(III) stock solution (1000 μg mL^{-1}) was prepared by dissolving 0.1732 g of $NaAsO_2$ in 100 mL of water. Working standard solution was prepared by dilution of stock solution. Hydrochloric acid, 0.4 M, potassium iodate, 2%, sodium acetate, 2 M were used. A 0.05% solution of variamine blue was prepared by dissolving 0.05 g of variamine blue in 25 mL of ethanol and making up to 100 mL with distilled water. The solution was stored in an amber bottle.

Method

Preparation of calibration curve

An aliquot of a sample solution containing 0.2-14 μg mL^{-1} of arsenic(III) was transferred into a series of 10 mL calibrated flasks. Then, potassium iodate (2%, 1 mL) and hydrochloric acid (0.4 M, 1 mL) were added and mixture was gently shaken. This was followed by addition of variamine blue (0.05%, 1 mL) and 2 mL of 2 M sodium acetate solution. The solution was kept for 5 min and made up to the mark with distilled water. The absorbance of the coloured species was measured at 556 nm against the corresponding reagent blank.

Determination of arsenic in urine and serum

Arsenic is reported to be present in trace amounts in normal urine and serum[27]. If a person is affected by arsenic poisoning the amount of arsenic in urine and serum increases. To check the validity of the method synthetic samples were prepared by adding known amounts of arsenic to serum and urine samples. The samples were deprotonised with trichloroacetic acid and filtered. Aliquots were then analyzed for arsenic by the proposed and reported methods[29].

Determination of arsenic in hair and nails

People drinking arsenic contaminated water have been reported to have high arsenic in their hair and nails. About 0.5 to 1.0 g of hair and nail samples were placed in a tube containing 10 mL of nitric acid. The tube was closed and heated on a hot plate at 100°C for 5 min. After 24 h the lid was opened and 1 mL of concentrated nitric acid was added and evaporated at 100°C until 1 mL of solution remained. Few drops of 10% KI were added to convert As(V) to As(III). The presence of any excess of iodine, indicated by light brown colour was destroyed by adding few drops of ascorbic acid[28]. The sample was cooled, diluted to 5 mL and analyzed by the proposed and also by reported method[29].

Determination of arsenic in plant material

A sample of plant material (5 g) was digested with 10 mL of HNO_3 for about 20 min. After cooling, 1 mL of perchloric acid was added and heating was continued for about another 10 min. As(V) if any, is reduced to As(III) by the process described above. The solution was transferred to a 25 mL volumetric flask and diluted to volume with water. Aliquots of the sample were analysed by the recommended procedure and also by the reported method[29].

Determination of arsenic in soil

A known weight (1 g) of a soil sludge sample was placed in a 50 mL beaker and extracted 4 times with a 5 mL portion of concentrated HCl. The extract was boiled for about 30 min. As(V) if any, is reduced to As(III). The solution was cooled and diluted to 25 mL with distilled water. Aliquots of the sample were analysed by the recommended method and also by the reported method[29].

Determination of arsenic in natural and polluted water

Water samples from a river receiving effluent of steel plant and fertilizer factory were collected in polyethylene bottles and filtered through Whatman 41 filter paper. As(V) if any, is reduced to As(III) by the process described. Arsenic content was determined directly according to the recommended method and also by the reported method[29].

Results and Discussion

This method involves the liberation of iodine by the reaction of arsenic(III) with potassium iodate in an acidic medium. The liberated iodine selectively oxidizes variamine blue in the presence of sodium acetate to form a violet coloured species, which shows a maximum absorbance at 556 nm. The reagent blank had negligible absorbance at this wavelength. The absorption spectrum is plotted in Fig. 1, and the reaction system is given in scheme 1.

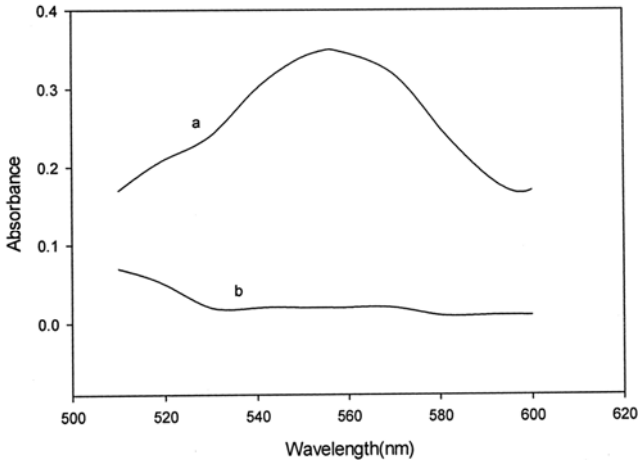
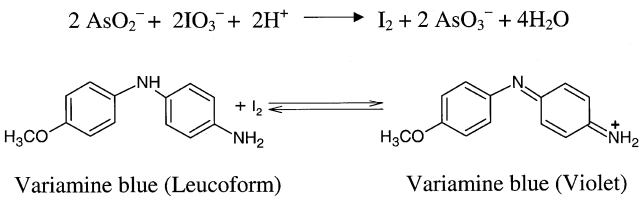


Fig. 1—Absorption spectra of coloured species (As^{3+}, 1 μg mL^{-1}) versus (a) reagent blank and (b) reagent blank versus distilled water



Scheme 1: Oxidation reaction of variamine blue

Analytical data

Beer's law was obeyed in the range of 0.2-14 μg mL^{-1}. The molar absorptivity and Sandell's sensitivity for the coloured system were found to be 1.43×10^4 L $mol^{-1}cm^{-1}$, 5.26×10^{-2} μg cm^{-2} respectively. The detection limit (D_L =3.3σ/S) and quantitation limit (Q_L=10σ/S) [where σ is the standard deviation of the reagent blank (n=5) and S is the slope of the calibration curve] of arsenic determination were found to be 0.022 and 0.072 μg mL^{-1}, respectively.

Effect of varying reaction conditions

The effect of various experimental conditions on absorbance was studied. The maximum absorbance was obtained when 1 mL of 2% potassium iodate, 1 mL of 0.4 M HCl and 1 mL of variamine blue were added in the described order. The determination was carried out at room temperature and the time taken for the maximum and constant absorbance was 5 min. The effect of pH (2-7) on absorbance revealed that optimum pH is 4. The colour of the product was stable for more than 8 h.

Effect of interfering ions

The validity of the method was assessed by investigating the effect of foreign species and other com-

Table 1—Estimation of arsenic in biological samples

Samples	Arsenic added μg mL^{-1}	Proposed method		Reference method[29]		*t*- test[b]	*F*-test[c]
		As^{3+} found in μg mL^{-1} ± SD[a]	Recovery %	As^{3+} found in μg mL^{-1} ± SD[a]	Recovery %		
Serum	2.00	1.97± 0.03	98.5	1.95±0.05	98.0	0.79	2.78
	4.00	3.88± 0.02	97.0	3.85± 0.03	96.3	1.89	2.25
	6.00	6.02±0.05	100.3	5.98± 0.08	99.7	0.96	2.56
Urine	2.00	1.98±0.03	99.0	2.01±0.04	100.5	1.35	1.78
	4.00	3.95±0.05	99.0	3.93±0.06	98.3	0.57	1.44
	6.00	5.97±0.02	99.5	5.94±0.03	99.0	1.89	2.25
Hair	2.00	1.98±0.02	99.0	1.96±0.03	98.0	1.24	2.25
	3.00	3.01±0.04	100.3	2.99±0.03	99.7	0.89	1.78
	4.00	3.98±0.03	99.5	3.99±0.06	99.7	0.30	4.00
Nail	2.00	1.98±0.05	99.0	1.97±0.06	98.5	0.57	1.44
	3.00	2.97±0.03	99.0	2.94±0.02	98.0	1.86	2.25
	4.00	3.98±0.05	99.5	4.01±0.03	100.3	1.15	2.78

[a]Mean ± standard deviation (n=5)
[b]Tabulated *t*-value for 8 degrees of freedom at P (0.95) is 2.306
[c]Tabulated *F*-value for (4, 4) degrees of freedom at P (0.95) is 6.39

Table 2—Determination of arsenic in environmental samples

Samples		Proposed method As^{3+} found in μg mL^{-1} ± SD[a]	Reference method[29] As^{3+} found in μg mL^{-1} ± SD[a]	*t*-test[b]	*F*-test[c]
Plant Materials					
(a) Grass		1.40± 0.04	1.34±0.06	1.86	2.25
(b) Mango leaf		1.15±0.03	1.19±0.025	2.29	1.44
Polluted water					
Samples	a.	1.02±0.02	1.01±0.03	0.62	2.25
	b.	2.01±0.05	1.99±0.07	0.52	1.96
	c.	1.18±0.04	1.16±0.05	0.69	1.56
Polluted	a.	1.36±0.02	1.34±0.03	1.24	2.25
Soil samples	b.	1.52±0.05	1.54±0.06	0.57	1.44
	c.	1.40±0.04	1.39±0.03	0.45	1.78

[a]Mean ± standard deviation (n=5)
[b]Tabulated *t*-value for 8 degrees of freedom at P (0.95) is 2.306
[c]Tabulated *F*-value for (4, 4) degrees of freedom at P (0.95) is 6.39

mon ions. Most of the cations like Fe^{3+}, Al^{3+}, Pb^{2+}, Co^{2+}, Na^{+}, K^{+}, Ni^{2+}, Zn^{2+}, Cr^{3+}, Ca^{2+} etc. and anions like SO_4^{2-}, PO_4^{3-} did not interfere. Glucose, urea, uric acid, bicarbonate, citrate, sulphide, aminoacid, phenol etc. also did not interfere.

Application

The proposed method was applied for the quantitative determination of arsenic in various environmental and biological samples. The results of the analysis of the above samples by the proposed and reference methods[29] are given in Table 1 and 2. Statistical analysis of the results by *t*- and *F*-tests showed no significant difference in accuracy and precision of the proposed and reference methods. The precision of the proposed was evaluated by replicate analysis of samples containing arsenic at three different concentrations.

Conclusion

The proposed method is simple, rapid and common metal ions such as Fe^{3+}, Al^{3+}, Pb^{2+}, Co^{2+}, Na^{+}, K^{+}, Ni^{2+}, Zn^{2+}, Cr^{3+}, Ca^{2+} do not interfere. Glucose, urea, uric acid, bicarbonate, citrate, sulphide, aminoacid, SO_4^{2-}, PO_4^{3-} and phenol 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 and biological samples.

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