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Determination of Arsenic in Environmental and Biological Samples Using Toluidine Blue or Safranine O by Simple Spectrophotometric Method

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Abstract A simple spectrophotometric method has been developed for the determination of arsenic in various environmental and biological samples. The method is based on the reaction of arsenic(III) with potassium iodate in acid medium to liberate iodine. This liberated iodine bleaches the blue color of toluidine blue or pinkish red color of safranine O. The decrease in absorbance at 628 or 532 nm is directly proportional to arsenic(III) concentration and obeys Beer's law in the range of 1.2–10.5 or 0.4–11.5 μg mL^{-1} for arsenic(III). The molar absorptivity, Sandell's sensitivity, detection limit and quantitation limit of the method using toluidine blue or safranine O were found to be 1.076×10^4 or 1.388×10^4 L mol^{-1} cm^{-1}, 9.66×10^{-3} or 7.49×10^{-3} μg cm^{-2}, 0.308 or 0.250 μg mL^{-1}, 0.934 or 0.759 μg mL^{-1} respectively. The relative standard deviation for five replicate analyses of 4 μg mL^{-1} of As(III) using toluidine blue or safranine O were 0.60% or 0.80%. The optimum reaction conditions and other analytical conditions were evaluated. The effect of interfering ions on the determination is described. The proposed method is free from any interference. The method has been used for the determination of arsenic in various environmental and biological samples.

Keywords Arsenic determination · Spectrophotometry · Toluidine blue · Safranine O

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Arsenic is a naturally occurring dissolved element in ground and surface waters throughout the world (kohnhorst et al. 2002). Arsenic exist in a different oxidation states in organic and inorganic forms in many environmental matrices such as natural water and soil. The predominant oxidation states of arsenic are arsenic(III) and arsenic(V) (Smedley and Kinniburgh 2002). Arsenic is a ubiquitous trace element, classed as a semi-metal or metalloid. Due to its toxicity, it needs to be determined in a variety of environmental samples. The toxicity, availability and environmental mobility of arsenic are very much dependent on the chemical forms (Tatken and Lewis 1983; Deveral and Millard 1988). Trace concentrations of arsenic can affect the physical and mechanical properties of metals and metal alloys (Wickstrom et al. 1995). Long term exposure to trace levels of arsenic causes chronic skin and cardiovascular disease. Skin lesions, cancers and cardiovascular diseases are traceable to arsenic poisoning (kohnhorst et al. 2002). In excessive amounts arsenic causes gastrointestinal damage and cardiac damage. The maximum permissible for arsenic in water is 0.05 mg L^{-1} as recommended by WHO. Arsenic and its compounds are reported to be mutagenic, teratogenic and carcinogenic in nature. The determination of arsenic is of critical importance in protecting the population from the health hazards it poses (Morita and Kaneko 2006). Arsenic may accumulate in soils and sediments due to the use of arsenical pesticides, fertilizers in irrigation and oxidation of volatile arsine in air, dust from the burning fossil fuels, as well as disposal of industrial, municipal and animal wastes (Sandbery et al. 1975). Arsenic is used in glass manufacture, pigment production, rodent poisons, insecticides, fungicides, medicines, printing, tanning etc.

Many methods have been reported for the determination of arsenic, such as induced floatation spectrophotometry

(Kunze et al. 1989), coupled plasma mass spectrometry (Samanta et al. 2000), flow injection analysis with hydride generation (Samanta and Chakraborti 1997), neutron activation analysis (Shull and Winefordner 1984) and fluorescence spectroscopy (Madrid et al. 1995). Some of the reported chromogenic reagents used for the spectrophotometric determination of arsenic are diantipyrylmethane (Zaijun et al. 2000), chlorpromazine (Min et al. 1999), alizarine red S (Ahmed and Hassan 1999), methyl orange (Dianwen and Jianping 1996), malachite green (Jie and Wenbin 1985), silver diethyldithiocarbamate (Perez et al. 2002), ammoniumpyrrolidinedithiocarbamate (Yuji et al. 2003), antipyrylazo-4-hydroxybenzenedithiocarboxylic acid (Pastare et al. 1993) and dithiodiantipyrylmethane (Jiayu et al. 1993). However most of these methods suffer from large number of limitations such as low sensitivity (Ahmed and Hassan 1999), need heating or extraction from organic solvents (Zaijun et al. 2000; Yuji et al. 2003) and interference by a large number of ions (Perez et al. 2002). Thus there is a need to develop a new simple, reliable, highly sensitive and inexpensive method which would overcome the existing inadequacies in the determination of trace amounts of arsenic.

Materials and Methods

A Systronics 2201 UV–VIS Double Beam Spectrophotometer with 1 cm quartz cell was used for the absorbance measurements and a WTW pH 330, pH meter was used. All chemicals used were of analytical grade and distilled water was used for dilution of reagents and samples. Standard arsenic(III) stock solution (1,000 μg mL^{-1}) was prepared by dissolving 0.1734 g of $NaAsO_2$ (Merck Limited, Mumbai) in 100 mL of water. The stock solution was further diluted as needed. Toluidine blue solution (0.01%) or Safranine O solution (0.02%) (S. D. Fine Chem. Limited, Mumbai) was prepared by dissolving 0.01 g of toluidine blue or 0.02 g of safranine O in distilled water and made up to 100 mL each with distilled water. Hydrochloric acid (1 M), potassium iodate (2%) and acetate buffer (pH 4.0) was prepared by dissolving 13.6 g of sodium acetate trihydrate in 80 mL of water. Solution pH was adjusted to 4 with acetic acid, and the mixture was diluted to 100 mL with distilled water. Other reagents were prepared by dissolving appropriate amounts of reagents in distilled water. An aliquot of a sample solution containing 1.2–10.5 or 0.4–11.5 μg mL^{-1} of arsenic(III) was transferred into a series of 10 mL calibrated flasks. Potassium iodate (2%, 1 mL) and hydrochloric acid (1 M, 1 mL) were added and mixture was gently shaken until the appearance of yellow color indicating the liberation of iodine. Toluidine blue (0.01%, 0.5 mL) or safranine O (0.02%, 0.5 mL) was then added and the reaction mixture was shaken for 2 min for maintaining pH 4, 2 mL of acetate buffer was added. The contents were diluted to 10 mL with distilled water and mixed well. The absorbance of the resulting solution was measured at 628 or 532 nm against reagent blank. Water samples from a river receiving effluent of steel plant and fertilizer factory was collected in polyethylene bottles and filtered through Whatman 41 filter paper. A few drops of 10% KI were added to convert any As(V) to As(III). Arsenic content was determined directly according to the recommended procedure. The analytical recovery of arsenic added to ground water, tap water, industrial water and river water ranged between 98% and 100.5%. There was good agreement between the results by the proposed method and the reported method (Pillai et al. 2000). A known weight (about 1 g) of a soil sludge sample was placed in a 50 mL beaker and extracted four times with a 5 mL portion of concentrated hydrochloric acid. The extract was boiled for about half an hour. As(V) if any is reduced to As(III) by the process described above. The solution was cooled and transferred to a 25 mL volumetric flasked with distilled water. Aliquots of the sample were analyzed by the proposed method. A sample of plant material (grass – 5 g) was digested with 10 mL of nitric acid for about 25 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 to volume with water. Suitable aliquot of the sample was analyzed by the proposed and reported method (Pillai et al. 2000).

Results and Discussion

This method is based on the reaction of arsenic(III) with potassium iodate in acid medium to liberate iodine. This liberated iodine bleaches the blue color of toluidine blue or pinkish red color of safranine O. The decrease in absorbance at 628 or 532 nm is directly proportional to arsenic(III) concentration. The absorption spectra of the colored species of toluidine blue or safranine O is presented in Fig. 1, and the reaction system is represented in Scheme 1. The effect of iodate concentration and acidity on the decolorization was studied with 2 μg mL^{-1} of arsenic solution. The oxidation of iodate to iodine was effective in the pH range 1.0–1.5, which could be maintained by adding 1 mL of 1M HCl in a final volume of 10 mL. The liberation of iodine from KIO_3 in an acid medium was quantitative. The appearance of yellow color indicates the liberation of iodine. Although any excess of iodate in the solution will not interfere. It was found that 1 mL of 2% KI and 1 mL of 1 M HCl were sufficient for

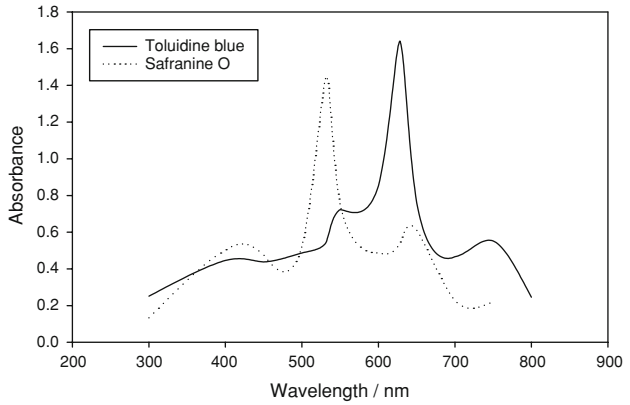

Fig. 1 Absorption spectra of colored species toluidine blue and safranine O

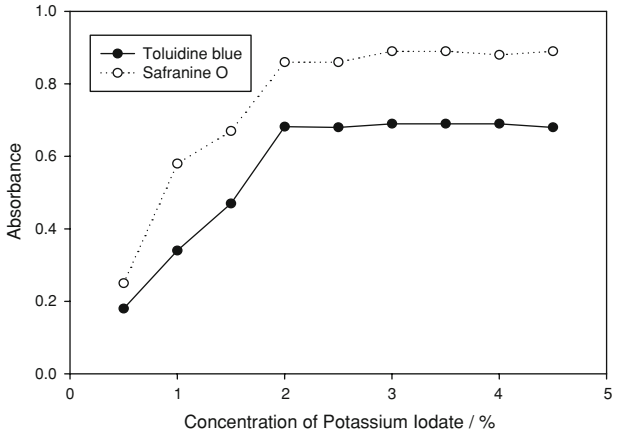

Fig. 2 Effect of concentration of potassium iodate

the liberation of iodine from iodate by arsenic(III) and 0.5 mL of 0.01% toluidine blue or 0.02% safranine O was used for subsequent decolorization. Effect of concentration of iodate in reaction system is presented in Fig. 2. Constant and maximum absorbance values were obtained in the pH = 4 ± 0.2. Hence the pH of the reaction system was maintained at 4 ± 0.2 throughout the study. This could be achieved by the addition of 2 mL of 1 M sodium acetate solution in a total volume of 10 mL. Effect of pH on color stability is presented in Fig. 3.

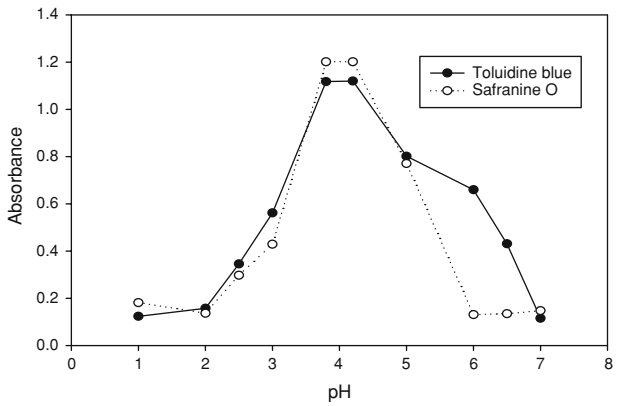

Fig. 3 Effect of pH on color intensity

Proposed Reaction Mechanism

In acid medium arsenic(III) liberate iodine from potassium iodate. This liberated iodine bleaches the blue color of toluidine blue or pinkish red color of safranine O (Scheme 1).

Analytical data A linear calibration graph was obtained for 1.2–10.5 or 0.4–11.5 μg mL^{-1} of arsenic in a final volume of 10 mL. The molar absorptivity and Sandell's sensitivity for the system was found to be 1.076×10^4 or 1.388×10^4 L mol^{-1} cm^{-1}, 9.66×10^{-3} or 7.490×10^{-3} μg cm^{-2} respectively. The detection limit ($D_L = 3.3\sigma/S$) and quantitation limit ($Q_L = 10\sigma/S$) (where σ is the standard deviation of the reagent blank (n = 5) and S is

Scheme 1 Oxidation of toluidine blue or safranine O

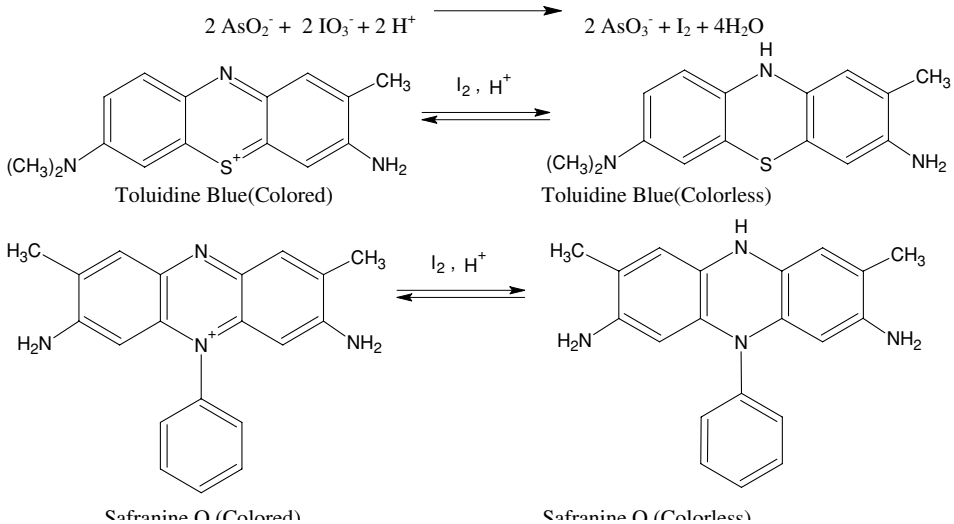

Table 1 Effect of interfering ions on the determinations of 2 μg mL^{-1} Arsenic(III)

Foreign ions	Tolerance limit (μg mL^{-1})		Foreign ions	Tolerance limit (μg L^{-1})	
	Toluidine blue	Safranine O		Toluidine blue	Safranine O
Fe^{3+}	150	100	V^{5+}	125	100
Ni^{2+}	75	100	Zn^{2+}	1,000	1,000
Cu^{2+}	200	200	PO_4^{3-}	1,000	1,000
Cd^{2+}	500	600	Tartarate	1,250	1,000
Ba^{2+}	1,000	750	Oxalate	1,000	1,250
Bi^{3+}	1,000	1,250	Sulfate	750	1,000
Al^{3+}	500	400	Nitrate	750	1,000
Ca^{2+}	400	300	Urea	1,500	1,500
Co^{2+}	175	150	Glucose	1,000	1,200

the slope of the calibration curve) for the arsenic determination using toluidine blue or safranine O were found to be 0.308 or 0.250 μg mL^{-1} and 0.934 or 0.759 μg mL^{-1} respectively. The correlation coefficient and slope of the calibration graph is 0.9996 or 0.9998 and 0.107 or 0.132 respectively. The effect of various foreign ions at microgram levels on the determination of arsenic using toluidine blue or safranine O was studied. The tolerance limits of

Table 2 Determination of arsenic in environmental samples using Toluidine blue and Safranine O as reagents

Samples	Toluidine blue				Safranine O		
	As^{3+} added (μg mL^{-1})	As^{3+} found (μg mL^{-1})	Recovery (%)	RSD (%)	As^{3+} found (μg mL^{-1})	Recovery (%)	RSD (%)
Ground water samples[a]	2.00	2.01	100.50	1.99	1.98	99.00	2.02
	4.00	3.96	99.00	3.03	3.98	99.50	1.51
	6.00	5.99	99.83	0.84	5.96	99.33	0.67
	8.00	7.92	99.00	0.25	7.95	99.38	0.88
Tap water samples[a]	2.00	1.94	97.00	1.03	1.96	98.00	3.57
	4.00	3.98	99.50	3.50	3.97	99.25	1.51
	6.00	5.94	99.00	0.50	5.96	99.33	0.16
	8.00	7.96	99.50	0.75	7.98	99.75	1.25
Industrial water samples (collected from the industrial zone of Mangalore city)[a]	2.00	1.99	99.50	1.51	1.96	98.00	0.76
	4.00	3.97	99.25	1.51	3.95	98.75	0.51
	6.00	5.96	99.33	1.34	5.94	99.00	1.34
	8.00	7.95	99.37	1.76	7.97	99.63	0.75
River water samples[b]	2.00	1.96	98.00	1.67	2.00	100.00	2.62
	4.00	3.95	98.75	1.40	3.95	98.75	1.66
	6.00	5.86	98.00	0.72	5.94	99.00	0.57
	8.00	7.89	98.62	0.89	7.96	98.50	0.66
Soil samples[a]	2.00	1.98	99.00	1.20	2.00	100.00	1.81
	4.00	4.01	100.25	1.31	3.96	99.00	0.37
	6.00	5.94	99.00	0.27	5.99	99.83	0.96
	8.00	7.96	99.50	0.32	7.95	99.37	0.97
Plant material (grass samples)[a]	2.00	2.01	100.50	1.32	1.98	99.00	1.00
	4.00	3.96	99.00	0.60	3.96	99.00	0.80
	6.00	6.01	100.17	0.36	5.95	100.17	0.86
	8.00	7.96	99.50	0.55	7.92	99.00	0.89

[a] Arsenic was not detected in ground water, tap water, industrial water, soil and plant material samples

[b] With two fold dilution

interfering species were established at those concentrations that do not cause more than ±2.0% error in absorbance values of arsenic(III) at 2 μg mL^{-1}. The tolerance limits of foreign ions are listed in Table 1. The results indicated, most of common ions did not interfere. The method is also free from the interference of major constituents present in serum. Most of the cations like Fe^{3+}, Ni^{2+}, Cu^{2+}, Cd^{2+}, Ba^{2+}, Bi^{3+}, Al^{3+}, Ca^{2+}, Co^{2+}, V^{5+}, Zn^{2+}, etc., do not interfere. Urea, uric acid, glucose, citrate, tartarate and anions like sulphate and phosphate also do not interfere.

The proposed method was applied to the quantitative determination of arsenic in various environmental and biological samples using toluidine blue or safranine O, the results are presented in Table 2 compare favorably with those with from a reference method (Pillai et al. 2000). The precision of the proposed was evaluated by replicate analysis of samples containing arsenic at three different concentrations. The proposed method for determination of arsenic using toluidine blue or safranine O as a new reagent is facile, rapid, sensitive and highly specific and has a wide analytical range without the need for extraction or heating. Moreover, the procedure is free from any interference and is less time consuming compared with other reported methods, and is also more sensitive than some reported methods in the literature (Ahmed et al 1999; Dianwaen et al. 1996). The developed method does not involve any stringent reaction conditions and offers the advantages of high stability of the bleached reaction system (more than a week). The proposed method has been successfully applied to the determination of trace amounts of arsenic in various environmental and biological samples.

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