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RESEARCH ARTICLE

A SIMPLE, HIGHLY SELECTIVE SPECTROPHOTOMETRIC METHOD FOR THE SIMULTANEOUS DETERMINATION OF ARSENIC (III) & ARSENIC (V)) AT NANO-TRACE LEVELS IN SOME GROUNDWATER, REAL, BIOLOGICAL, FOOD AND SOIL SAMPLES USING 2-HYDROXY-1-NAPTHALDEHYDE-ORTHOAMINOPHENOL

[1,*]Jamaluddin Ahmed, M., [1,2]Afrin, Ayesha and [1] Imran Hossain Mollah, M.

[1]Laboratory of Analytical Chemistry, Department of Chemistry, University of Chittagong, Chittagong, Bangladesh
[2]Department of Applied Chemistry and Chemical Engineering, University of Chittagong, Chittagong, Bangladesh

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ABSTRACT

A new spectrophotometric reagent 2-Hydroxy-1-napthaldehyde-orthoaminophenol has been synthesized and characterized. A very simple, ultra-sensitive, highly selective and non-extractive new spectrophotometric method for the simultaneous determination of arsenic (III) and arsenic (V) at nano-trace levels using 2-Hydroxy-1-napthaldehyde-orthoaminophenol (HNA-OAP)has been developed. HNA-OAP has been proposed as a new analytical reagent for the direct non-extractive spectrophotometric determination of arsenic (III&V). This novel spectrophotometric reagent reacts in a slightly acidic (0.00016-0.0004 M H_2SO_4) aqueous solution with arsenic(III) in 20% N,N-dimethylformamide (DMF) to produce highly absorbent red chelate with has an absorption maximum at 505 nm. The absorbance intensity of the metal-chelate reaches a constant value (after heating for 5 min at (45±5) ^{0}C within 15 min and remains stable over 24 h. The average molar absorption coefficient and Sandell's sensitivity were found to be 2.46×10^4 L $mol^{-1}cm^{-1}$ and 5-ng cm^{-2}. Linear calibration graphs were obtained for 0.01 – 60-mgL^{-1} of As, having a detection limit of 1-$ngmL^{-1}$; the quantification limit of the reaction system was found to be 10-$ngmL^{-1}$ and the RSD was 0-3%. The stoichiometric composition of the chelate is 3:2 (As: HNA-OAP). A large excess of over 60 cations, anions and complexion agents (like, chloride, phosphate, azide, tartrate, oxalate, SCN- etc.) do not interfere in the determination. The developed method was successfully used in the determination of total arsenic in several certified reference materials (alloys, steels, ores, human urine, hair, nails, bovine liver and sediments) as well as in some biological fluids (human blood, urine, hair, nail and milk), soil samples, food samples (vegetables, fruits, rice, corn and wheat), solutions containing both arsenic(III) and arsenic(V) speciation and complex synthetic mixtures. The results of the proposed method for assessing groundwater, biological, food and soil samples were comparable with both ICP-OES & AHG-AAS and were found to be in excellent agreement. The method has high precision and accuracy (s = ± 0.01 for 0.5 mg L^{-1}).

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INTRODUCTION

The contamination of groundwater by arsenic in Bangladesh is one of the largest poisoning of a population in the history. The scale of this environmental poisoning disaster is greater than any seen before. It is beyond the accident at Bhopal, India, in 1984, and Chernobyl, Ukraine, in 1986 (Pearce, 2001). Though in 1983, the first arsenic patients seen were from West Bengal, India but it come to consideration and confirmed in 1993 in the Chapai Nawabganj, Bangladesh (Report, 1993).

Corresponding author: **Jamaluddin Ahmed, M.**
Laboratory of Analytical Chemistry, Department of Chemistry, University of Chittagong, Chittagong, Bangladesh.

But at present the contamination has affected 61 out of the 64 districts in Bangladesh where arsenic levels have been found to be above 50-μgL^{-1} (BSTI Standard (http://nora.nerc.ac.uk/id/eprint/11986/). According to the latest statistics that about 165 million inhabitants of Bangladesh between 75 million and 97 million are at risk of drinking contaminated water, being exposed to arsenic contamination (WHO limit is 10 μgL^{-1}) (WHO, 1999). There is no doubt that the arsenic contamination in Bangladesh is a big environmental and social disaster in the human history (Cheng, 2002). However, all the investigations and studies signal arsenic contamination in groundwater of Bangladesh as "Big Disaster!" The determination of arsenic (III) and arsenic (V) in environmental and biological systems is of considerable

current interest because the toxicity of this element to aquatic and terrestrial organism including humans depends on its oxidation state (Cheng, 2002). Arsenic (V) is considered to be essential to mammals for the maintenance of growth, blood cells, normal iron and lipid metabolism, but arsenic (III) is reported to be toxic because of its complexation with coenzymes, coagulation of proteins and uncoupling of phosphorylation and its adverse impact on skin, liver, nose and throad (Bhuiyan, 2015). Strong evidences were provided to indicate that arsenic in drinking water was responsible to cause skin, lung and bladder cancer. Groundwater is the preferred source of drinking water for 99% people in the rural areas Bangladesh. The provisional WHO guideline value of arsenic for drinking water is 10-µg L^{-1}. Therefore, extremely low concentrations of arsenic in groundwater used for potable and domestic purposes should be known accurately. Hence, reliable methods are needed to check the arsenic status of a human and to monitor the occupational exposure to this element by measuring its concentration in bodily fluids.

In the expanding analytical fields such as environmental, biological and material monitoring of trace metals, there is an increasing need to develop simple, sensitive and selective analytical techniques that don't use expensive or complicated test equipment. Many sophisticated techniques, such as NAA, X-ray fluorescence, pulse polarography, ICP-OES, ICP-MS, GF-AAS and AHG-AAS have been used widely to the determination of arsenic. The first four methods are disadvantageous in terms of cost and the instruments used in routine analysis. GF-AAS is often lacking in sensitivity due to sublimation at high temperature, AHG-AAS is sensitive but often affected by matrix conditions of samples such as salinity. There is no direct spectrophotometric method for the determination of arsenic. Only one solvent extractive method is considered as standard method which use Ag-diethyldithiocarbamate (Ag-DDTC) is very less sensitive. ADDC is also insoluble in water but soluble in organic solvents such as pyridine and chloroform which are themselves carcinogenic according to EPA (Chmilenko *et al*., 2001).

Spectrophotometre is essentially an ultra-trace analysis technique and is one of the most powerful and successful tools in chemical analysis. Spectrophotometre is extremely sensitive so much so that sometimes pictogram (10^{-12}g g^{-1}) per gram levels or less can be determined (Talebi, 2005). The goal of the present work was to develop a simpler direct spectrophotometric method for the trace determination of arsenic. In the search for a more sensitive reagent, in this work a new reagent was synthesized according to the method of Sacconi (Sacconi, 1953) and Salam (Salam, 1995) and a complex is formed between arsenic (III) and HNA-OAP. Although HNA-OAP has been reported to be spectrophotometric reagent for selenium (IV) (Deepa, 2014) but has not previously been used for the spectrophotometric determination of arsenic. The method possesses distinct advantages over existing methods (Tom Cherian, 2005; Pereira *et al*., 2008; Ajai, 1999; Zhou Tian-Ze and Wang Ming, 2006; Revanasiddappa, 2007; Gupta and Prabhat, 1986; Nail, 2015; Shahram, 2015; Okeye, 2013; Keisuke Morita and Emiko Kaneko, 2006; Merry, 1980; Palanivelu, 1992; Subrata, 2002; Chand, 2008; Narayana, 2006; Seda Karayunlu, 2010; Lucena-Conde, 2009; Mohamed Noorel Deen Abbas, 2014;)(12-32) with respect to sensitivity, selectivity, range of determination, simplicity, speed, pH/acidity range, thermal stability, accuracy, precision and ease of operation.

The method is based on the color reaction of HNA-OAP in a slightly acidic (0.00016 – 0.0004 M H_2SO_4) solution with As(III) in presence of DMF to produce a colored product, followed by a direct measurement of the absorbance in an aqueous solution at room temperature (25±5)^{o}c. With suitable masking, the reaction can be made to be highly selective and the reagent blank solutions do not show any absorbance.

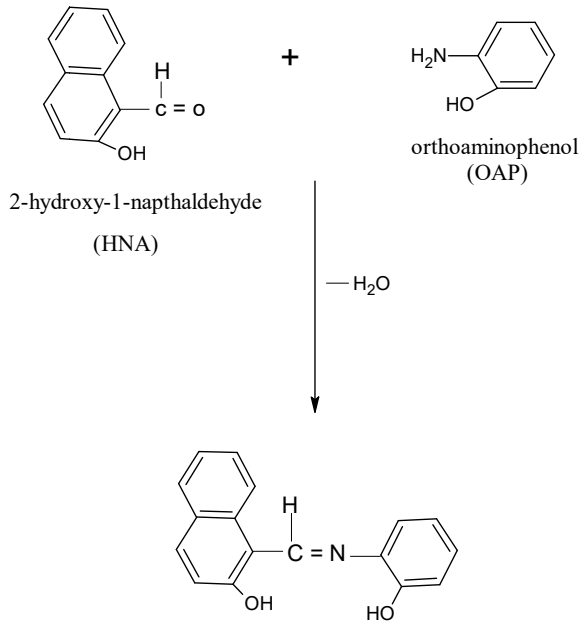
Experimental Section

Apparatus: A Shimadzu (Kyoto, Japan) (Model-1800) double-beam UV/VIS spectrophotometer and a Jenway (England, UK) (Model-3010) pH meter with combination of electrodes were used for measurements of the absorbance and pH, respectively. A Thermo Fisher Scientific (Model-iCE 3000, origin USA) atomic absorption spectrophotometer equipped with a microcomputer – controlled automated hydride – generation coupled to a flow injection analysis system (AGH – AAS) was used to compare of the results at 196.0 nm. Infrared spectrum was recorded with FTIR Spectrophotometer, Shimadzu (Model-IR Prestige 21, Detector-DTGS KBr) in the range 7500-350 cm^{-1} and Model: JEOL500SS, magnetic field strength, solvent used : DMSOD6, standard : TMS, four channel ^{1}HNMR spectrometer with signal-to noise ratio of 5000:1for proton were used for characterization of the ligand.

Synthesis and characterization of the Reagent

Synthesis of the reagent: The reagent was synthesized in the laboratory according to the method recommended by Sacconi (Talebi, 1953) and Salam (1995)· The reagent 2-hydroxy-1-napthaldehyd-orthoaminophenol (HNA-OAP) was synthesized by following steps. Solutions of 2 - hydroxy - 1 - napthaldehyde (20 mmol) in 30 mL of ethanol and orthoaminophenol (20 mmol) in 30 mL of ethanol were prepared separately by gentle warming. The two solutions mixed together drop wise with continuous stirring. Then it was refluxed at 30 ºC for about 1 hour when a brown crystalline solid appeared on cooling. The product was then filtered off, washed with ethanol and recrystalised from ethanol and dried in a desiccators over calcium chloride. Yield of the product was 80%. The structure of the reagent is shown in Scheme- I.

Characterization of the reagent: The reagent was characterized by taking melting point, elemental analysis, and FTIR spectrum and thermo gravimetric analysis. The melting point of the reagent was 155-157ºC. (Lit. 154 - 156ºC) (9) ·The result of elemental analysis (C = 75.23 %, N = 5.11%, H = 4.74 %) of the reagent was in good coincidence with the calculated values (C = 71.50 %, N = 4.90%, H = 3.98 %) (10). The FTIR spectrum of prepared reagent (HNA-OAP) is shown in Figure 1. The presence of FTIR peak at 1631.78 cm^{-1} in Figure 1 was due to the characteristic C=N double bond peak ($\nu^{C=N}$, 1583-1618 cm^{-1}) (Salam *et al*., 1995) of the Schiff's base reagent indicating the formation of HNA-OAP. The formation of the reagent also tested by ^{1}HNMR spectrum is shown in Figure 2. The presence of ^{1}HNMR peak at^{1}H= δ6.99 (6.8-7.17)^{10}, ^{2}H=δ7.15 (7.2-7.8)^{10} and ^{3}H=δ8.23 (8.2-9.46) (10), respectively of Schiff's base reagent also indicating the formation of HNA-OAP (Salam, 1995). The thermo gravimetric curve of prepared reagent (HNA-OAP) is shown in Figure 3.



Scheme I. 2-hydroxy-1-naphthaldehyde-orthoaminophenol (HNA-OAP)

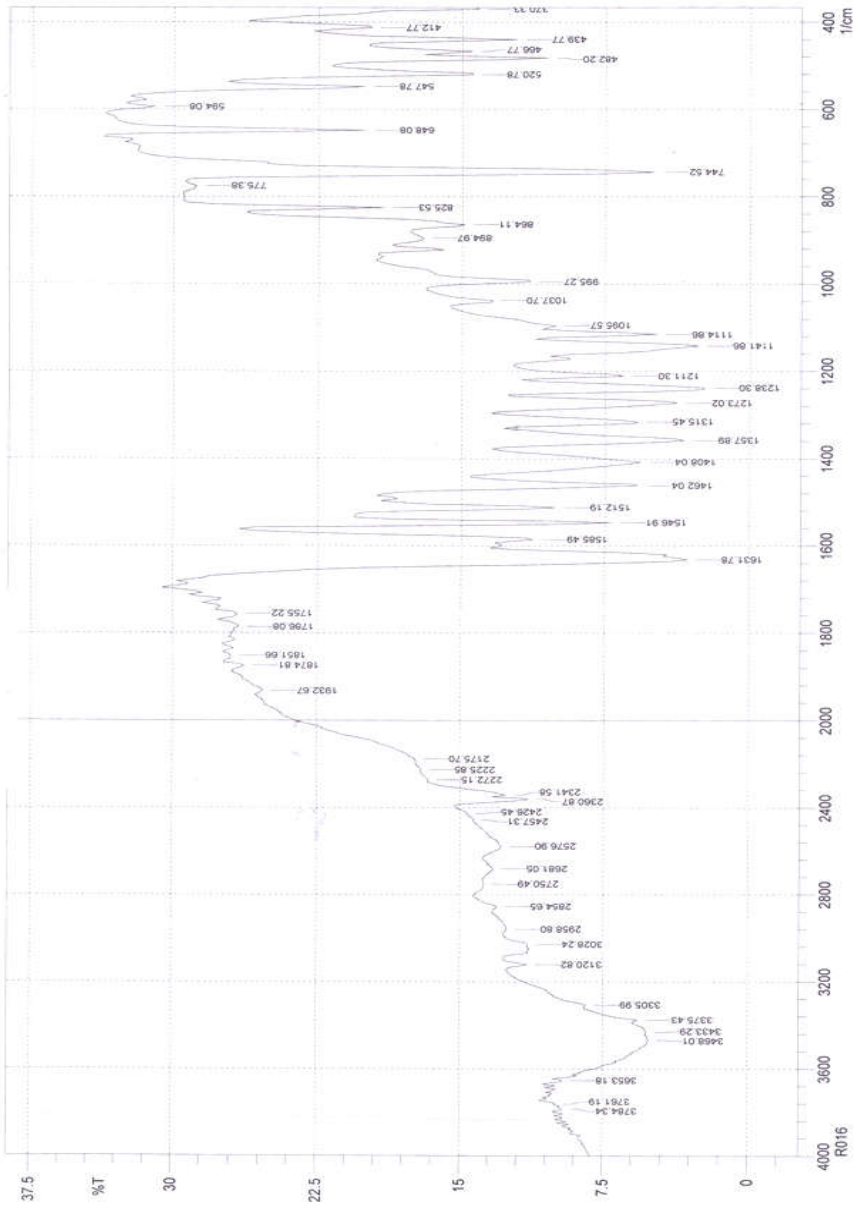


Figure 1. FTIR spectrum of 2-Hydroxy-1- napthaldehyde – aminophenol (HNA – OAP)

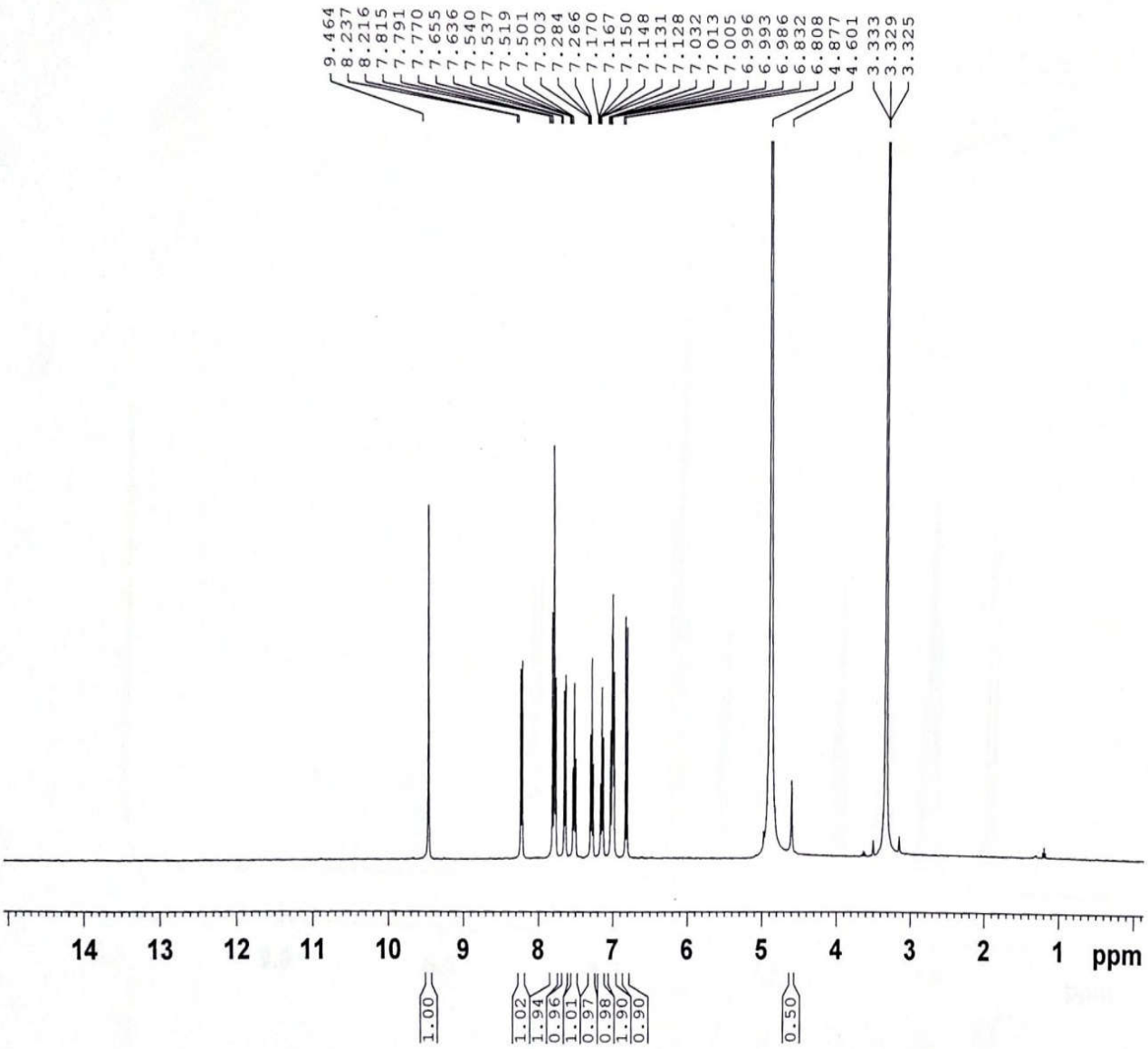


Figure 2. 1HNMR spectrum 2-Hydroxy-1-napthaldehyde aminophenol (HNA – OAP)

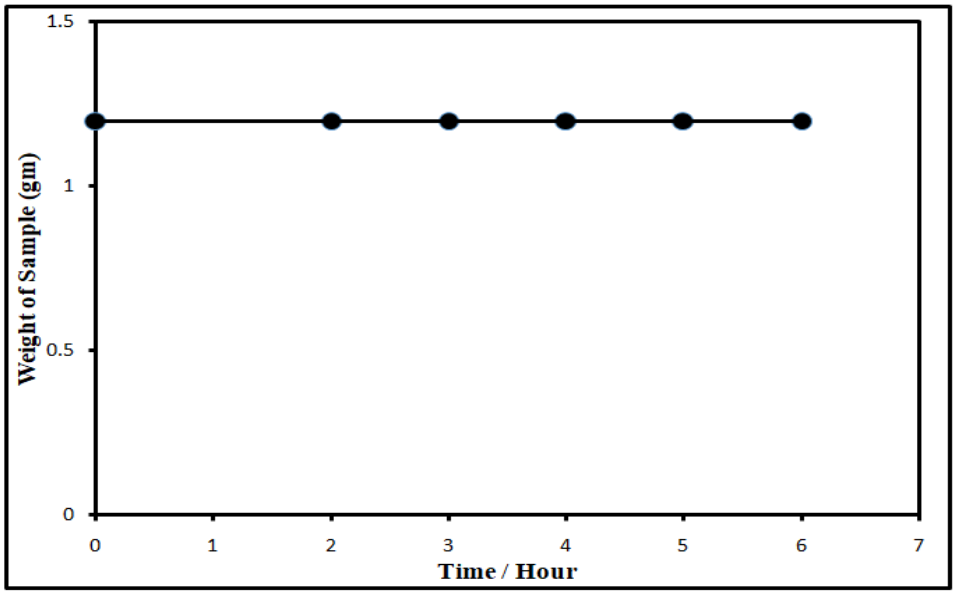


Figure 3. The thermo gravimetric curve of [2-hydroxy-1-napthaldehyde-orthoaminophenol (HNA-OAP)] at 85-90º C

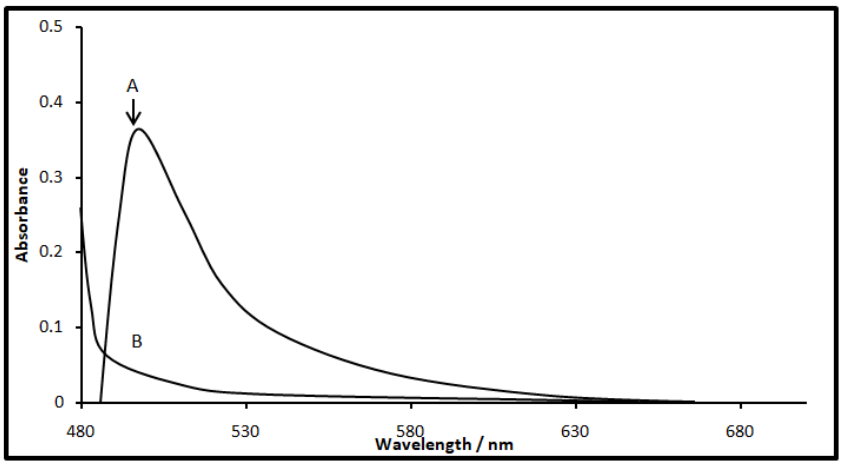


Figure 4 . A & B absorbance spectra of As^{III} - HNA-OAP and the reagent blank in (λ_{max}=495 nm) aqueous solution, respectively.

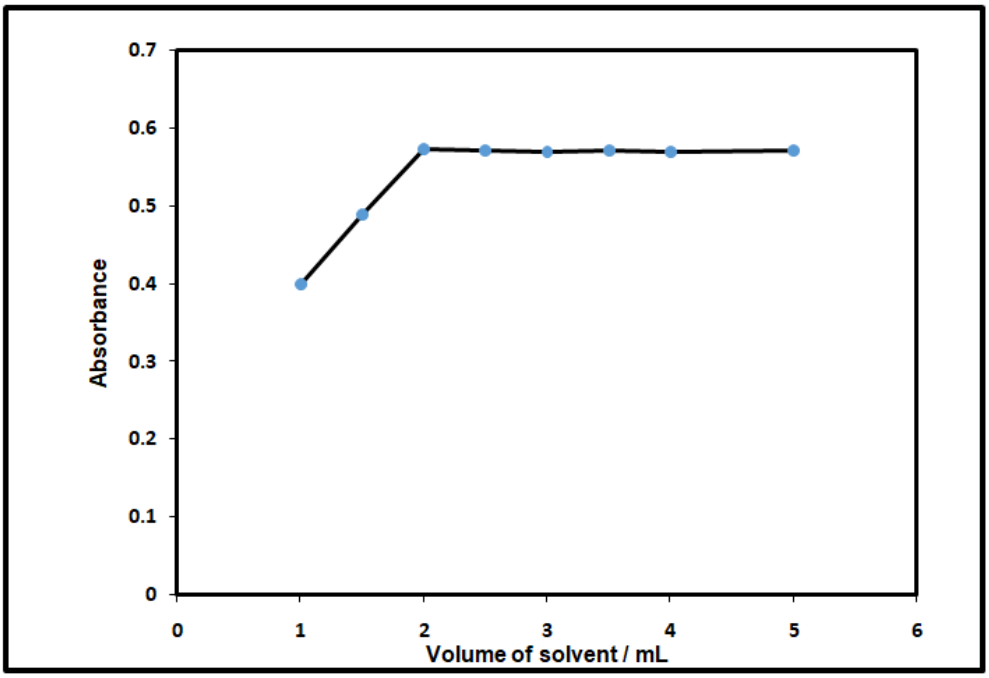


Figure 5. Effect of solvent (DMF) on the absorbance of AS^{III}-HNA-OAP system

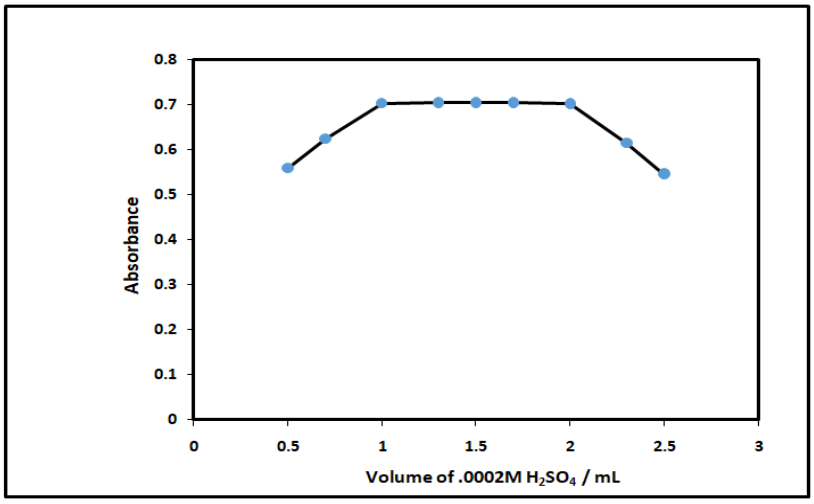


Figure 6. Effect of acidity on the absorbance of As^{III} - HNA-OAP system

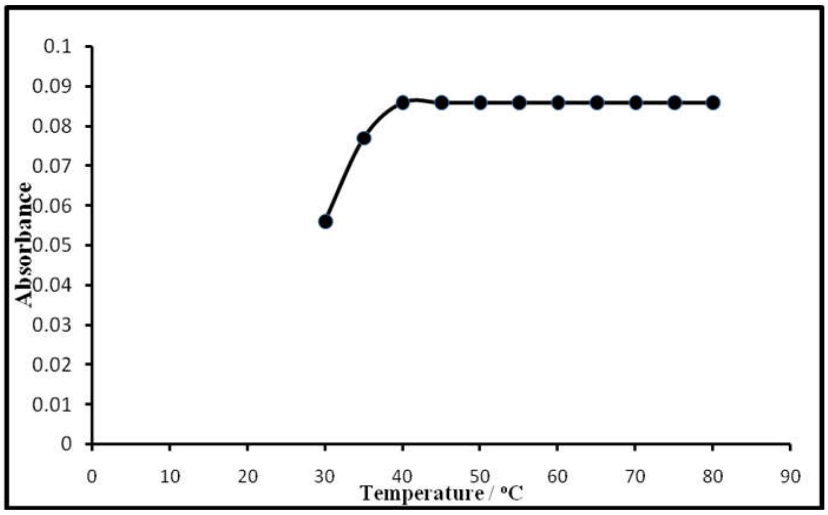


Figure 7. Effect of temperature on the As^{III}- HNA-OAP system

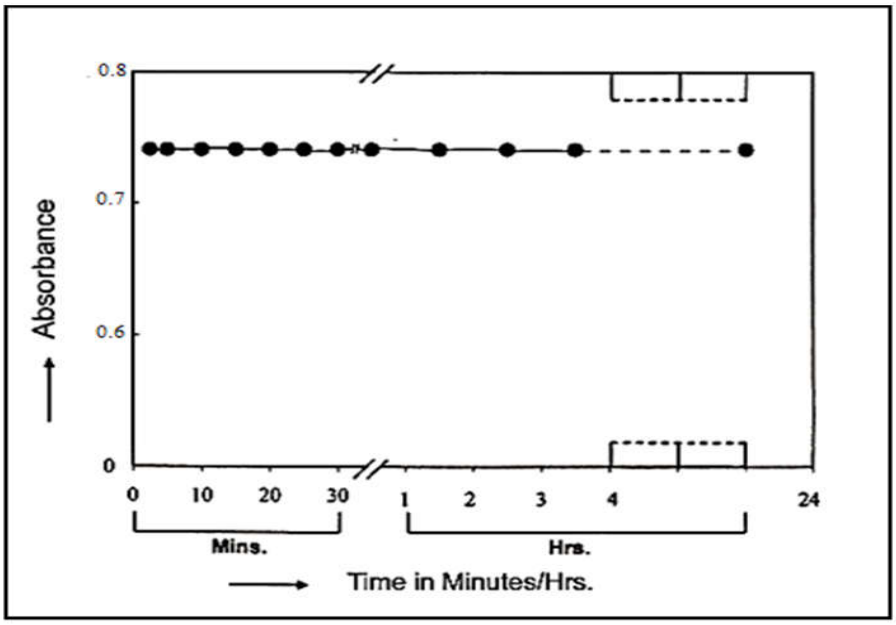


Figure 8. Effect of time on the absorbance of As^{III} - HNA-OAP system

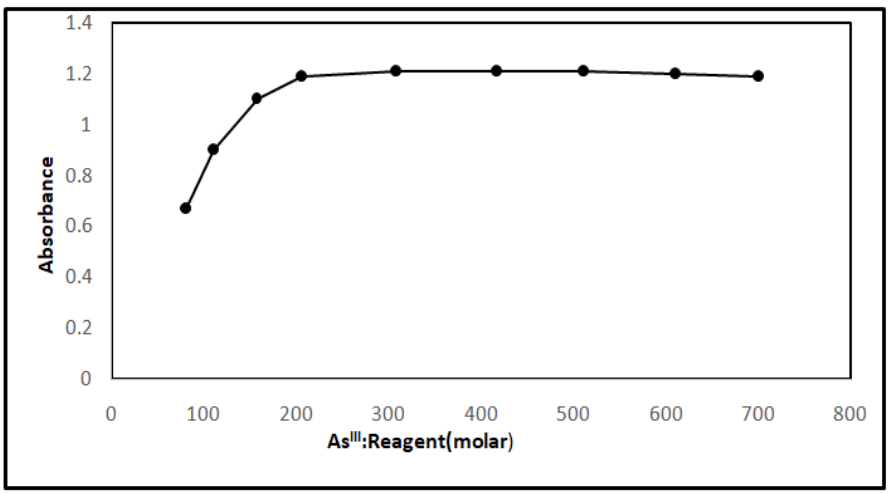


Figure 9. Effect of reagent on the (As^{III}-HNA-OAP molar ratio) of the As^{III}-HNA-OAP system

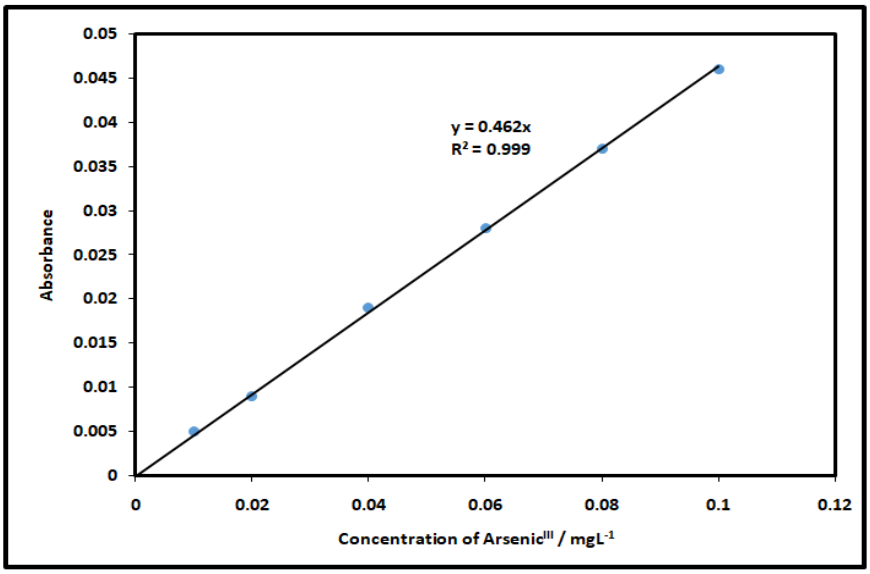


Figure 10. Calibration Graph A: 0.01-0.1-mgL^{-1}of arsenic (III)

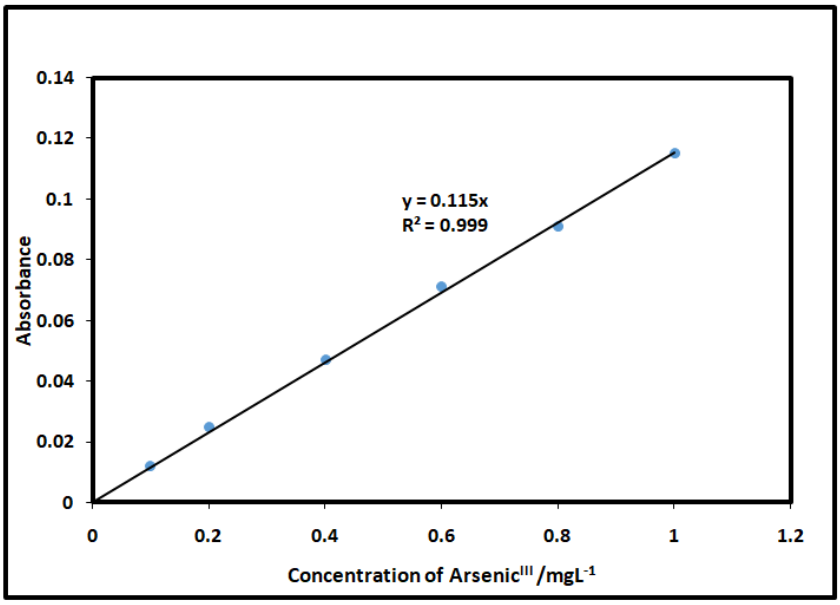


Figure 11. Calibration graph B: 0.1 – 1-mgL^{-1} of arsenic (III)

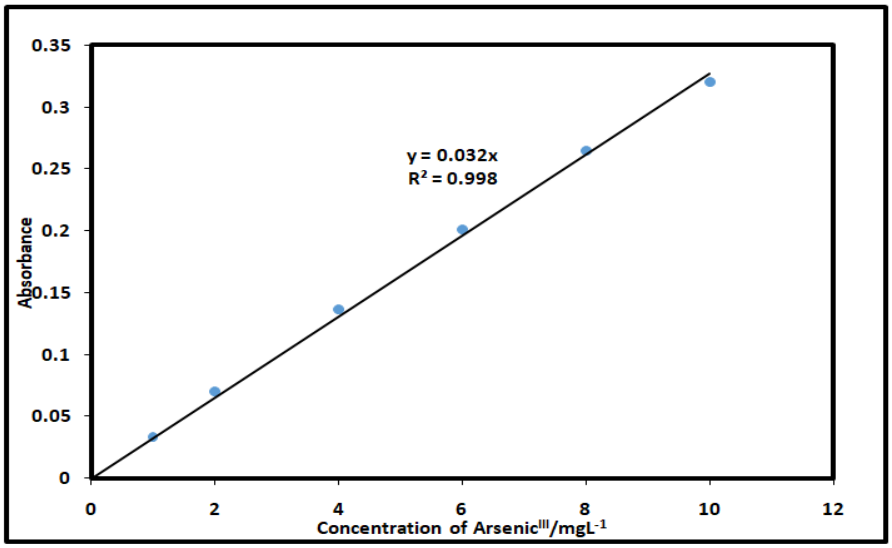


Figure 12. Calibration graph C: 1 – 10-mgL^{-1} of arsenic(III)

Potassium dichromate solution: A 100 mL amount of stock solution (0.1 N) was prepared by dissolving 500 mg of finely powdered $K_2Cr_2O_7$ (Merck) in 100 mL deionized water.

Sodium azide solution: Sodium azide solution (2.5 % w/v) (Fluka purity > 99%) was freshly prepared by dissolving 2.5 gm in 100 mL of deionized water.

Tartrate solution: A 100-mL stock solution of tartrate (0.01 % w/v) was prepared by dissolving 10 mg of A.C.S.-grade (99%) potassium sodium tartrate tetrahydrate in (100-mL) de-ionized water.

Aqueous ammonia solution:A 100-mL solution of an aqueous ammonia solution was prepared by diluting 10 mL concentrated NH_4OH (28-30%, A.C.S.-grade) to 100-mL with de-ionized water. The solution was stored in a polypropylene bottle.

EDTA solution: A 100-mL stock solution of EDTA (0.01 % w/v) was prepared by dissolving 10 mg A.C.S.-grade (>99%) ethylene diamine tetra acetic acid as disodium salt dihydrate in (100-mL) de ionized water.

Other Solutions: Solutions of a large number of inorganic ions and complexing agents were prepared from their AnalaR grade or equivalent grade water-soluble salts (or the oxides and carbonates in hydrochloric acid); those of niobium, tantalum, titanium, zirconium and hafnium were specially prepared from their corresponding oxides (Specpure, Johnson Matthey) according to the recommended procedures of Mukharjee (34). In the case of insoluble substances, special dissolution methods were adopted (Pal, 1984).

Procedure: To 0.1-1.0-mL of a neutral aqueous (pH 6) solution containing 0.1 – 600-µg of arsenic (III) in a 10-mL calibrated flask was mixed with a 1:200 - 1:700 fold molar excess (preferably 1- mL of 3.8×10^{-3} M) of the 2-hydroxy-1-napthaldehyde–orthoaminophenol (HNA-OAP) reagent solution followed by the addition of 0.8 -2.0-mL (preferably 1-mL) of 0.0002 M of sulfuric acid. Then, 2-mL of N, N-dimethylformamide (DMF) was added and the mixture was diluted to the mark with de-ionized water. After heating for 5 min at (45±5) ^oC then cooled at room temperature (25±5)^oC for 15 min, then absorbance was measured at 495 nm against a corresponding reagent blank. The arsenic content in an unknown sample was determined using a concurrently prepared calibration graph.

Sample collection and preservation

Environmental samples: Water and soil samples were collected in polythene bottles from different places of Bangladesh. After collection, HNO_3 (1-mL^{-1}) was added as preservative. *Blood and Urine*: Blood and urine samples were collected in polythene bottles fromeffected persons of Chittagong Medical College Hospital ,Hajiganj and Faridganj Upazila Complex, Chandpur district , Bangladesh. Immediately after collection they were stored in a salt-ice mixture and latter, at the laboratory, were at-20°C. *Soil samples*: Soil samples were collected from different locations of Bangladesh. Samples were dried in air and homogenized with a mortar.

***Food samples*:** Food samples (rice, wheat, tea, fruits and vegetables) were collected from local market of Chittagong, Dhaka, Narsingdi, Comilla and Chandpur districts. After collection the samples (fruits and vegetables) were stored in refrigerator for preservation. Samples (rice, wheat, tea) were used as dry condition and homogenized with a mortar.

RESULTS AND DISCUSSION

Factors Affecting the Absorbance

Absorption spectra: The absorption spectra of the As (III)-HNA-OAP in 0.0002 M sulfuric acid medium was recorded using a spectrophotometer. The absorption spectra of the As(III)-HNA-OAP are a symmetric curve with maximum absorbance at 495 nm; an average molar absorption coefficient of 2.46×10^4 L mol^{-1} cm^{-1} is shown in Figure 4. HNA-OAP did not show any absorbance. In all instances, measurements were made at 495 nm against a reagent blank. The reaction mechanism of the present method is as reported earlier (36).

Optimization of some Parameters on the Absorbance

Effect of solvent: Because HNA-OAP is insoluble in water, an organic solvent was used for the system. Of the various solvents (chloroform, benzene, carbon tetrachloride, n-butanol, isobutanol, ethanol, 1, 4-dioxane and N, N-dimethylformamide (DMF)) were tested for the system, DMF was found to be the best solvent for the system. No absorbance was observed in the organic phase with the exception of n-butanol. In 20±2% v/v DMF medium, however maximum absorbance was observed; hence a 20% (2-mL) DMF solution was used in the determination procedure. It was observed that at 1-mgL^{-1} of As-HNA-OAP, 20-70% (2-7-mL) of DMF solution produced a constant absorbance of the As-Chelate (Figure 5).

Effect of acidity: Of the various acids (nitric, sulfuric, hydrochloric and phosphoric) studied, sulfuric acid was found to be the best acid for the system. The absorbance was at a maximum and constant when the 10-mL of solution (1 mg L^{-1}) contained 0.8 - 2.0-mL of 0.0002 M sulfuric acid at temperature (45 ± 5)°C. Outside this range of acidity, the absorbance decreased (Figure 6). For all subsequent measurements 1-mL of 0.0002 M sulfuric acid was added.

Effect of temperature: The As (III) - HNA-OAP system attained maximum and constant absorbance when reaction mixture was heated for 5 minutes at (45±5)°C and then cooled it for 15 minutes (Figure7) at room temperature (25 ±5)°C.

Effect of time: The As^{III}-HNA-OAP complex maximum and constant absorbance was obtained just after the reaction mixture was heated for 5 min (after 45±5) °C and then cooled it for 15 min at room temperature (25 ± 5) °C) and remained strictly unaltered for 24h (Figure 8).

Effect of reagent concentration: Different molar excesses of HNA-OAP were added to a fixed metal ion concentration and absorbances were measured according to the standard procedure. It was observed that at 1-mg L^{-1} As^{III}, the reagent molar ratios of 1:200-1:700 produced a constant absorbance of the As^{III} - chelate (Figure 9).

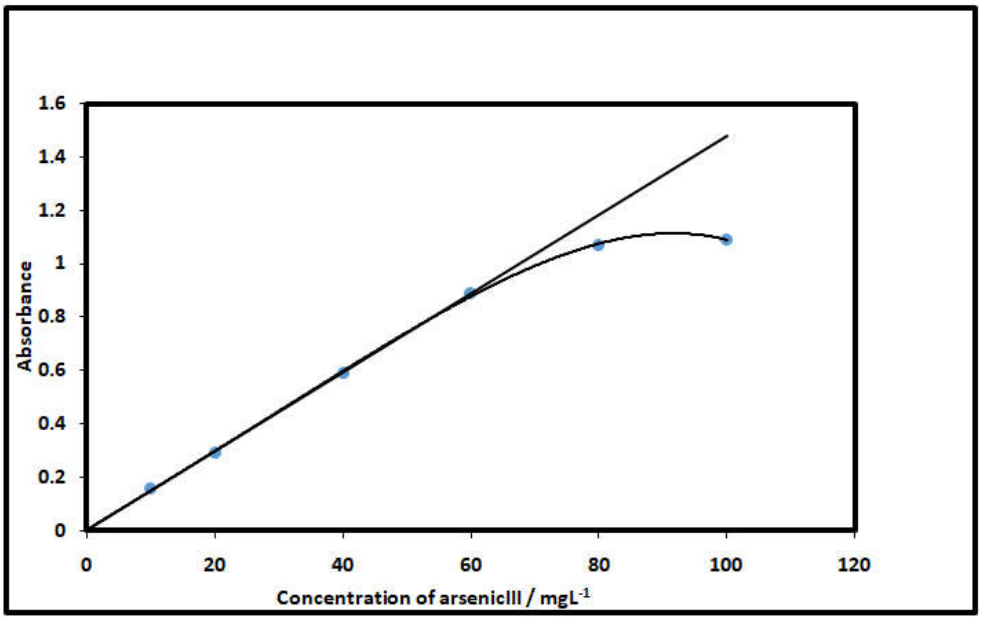


Figure 13. Calibration graph D: 10 – 60-mgL^{-1} of arsenic (III)

Table 1.Summary of selected analytical parameters obtained with optimization experiment

Parameters	Studied range	Selected value
Wavelength / λ_{max}	200- 800	495
Solvent /mL	0-80	2.0- 8.0 (Preferably 2)
Acidity / M H_2SO_4	0.000005-0.005	0.00016-0.0004 (Preferably 0.0002)
pH	6.5 – 2.5	6.0 – 3.0 (Preferably 3.5)
Time / h	0 - 72	1min - 24 h (Preferably 15 min)
Temperature / °C	10 - 80	40 – 80 (Preferably 45 ± 5)
Reagent (fold molar excess, M:R)	1:100 - 1:700	1:200 - 1:700 (Preferably 1:300)
Linear range / mgL^{-1}	0.001 - 100	0.01 - 60
Molar absorptivity	1.51×10^4 – 4.23×10^4	2.46×10^4
Detection limit / µgL^{-1}	0-100	1.0
Sandell's Sensitivity / µgL^{-1}	0.1-50	5.0
Reproducibility (% RSD)	0 - 10	0 - 3
Regression Co-efficient (R^2)	0.9987 –0 .9999	0 .9997

Table 2. Table of tolerance limits of foreign ions*, tolerance ratio [species(X)/As^{III} (w/w)]

Species	Tolerance ratio x/As^{III}(w/w)	Species	Tolerance ratio x/As^{III} (w/w)
Aluminum	50[c]	Lithium	100
Ammonium	100	Lead (II)	100[b]
Antimony	100	Molybdenum (VI)	100
Azide	1000	Manganese (II)	100
Bismuth (III)	100	Manganese (VII)	100
Bromide	100	Nickel	100[d]
Barium	100	Nitrate	100
Cadmium	100	Oxalate	100
Cobalt (II)	100	Phosphate	100
Cobalt (III)	100	Potassium	100
Calcium	100	Selenium (IV)	50[c]
Chloride	100	Selenium (VI)	100
Citrate	100	Strontium	100
Chromium (VI)	50[c]	Sulphate	100
Chromium (III)	50	Sodium	80
Cesium	100	Tartrate	100
Copper (II)	50[b]	Tin (II)	100
Cerium (III)	100	Tin (IV)	100
Cerium (IV)	100	Titanium (IV)	100
EDTA	1000	Tellurium (IV)	50
Fluoride	1000	Thiocyanate	100
Iron (II)	100	Tungsten (VI)	100
Iron (III)	100[b]	Vanadium (V)	100
Iodide	100	Zinc	100

[a]Tolerance limit is defined as the ratio that causes less than ± 5 percent interference.
[b]With10-mgL^{-1}tartrate.
[c]with10-mgL^{-1}EDTA

Table 3.Determination of arsenic in some synthetic mixtures

Sample	Composition of Mixtures (mgL^{-1})	Arsenic /mgL^{-1}		
		Added	Found[a] (n=5)	Recovery ± SD[b] (%)
A	As(III)	0.5	0.49	98± 0.5
		1.0	1.00	100±0.0
B	As in A + Cr^{VI}(50) + V^{VI} + Ti^{IV}(50) + Fe^{3+} (50) +EDTA(50)	0.5	0.502	100.5± 0.6
		1.0	1.02	102.0± 0.7
C	As in B + Pb^{2+} (50)+ Bi^{3+} (50) + Hg^{2+} (50) + Se^{IV}(50)+ Cu^{2+}(50)	0.5	0.49	98±0.5
		1.0	0.99	99±0.3
D	As in C + Sb^{3+}(50) +Ni^{2+}(50) + Ca(50) +Cd(50) + Te^{IV}(50)	0.5	0.51	102±1.0
		1.0	1.02	102±1.2
E	As in D + Mg(50) + Mn^{VII}(50) + W^{VI}(50) + Ba(50) + Ag(50)	0.5	0.53	106±1.5
		1.0	1.06	106±1.6
			0.54	108±1.8
F	As in E +Ce^{III}(50)+ Na(50) + K(50) + Zn(50) + Ce^{IV}(50)	0.5 1.0	1.07	107±1.9

[a]Average of five analyses of each sample.
[b]The measure of precision is the standard deviation (SD).

Table 4. Determination of arsenic in some certified reference materials

Sample No.	Certified Reference Materials (Composition, %)	Arsenic (%)		
		In C.R.M sample	Found (n=5)	RSD[b]
1	GSBH62009-91: Sb=7.95,Bi=0.79,Cu=6.97, As = 1.02 ,Pb=2.0,Fe=0.64.	1.02	0.98	1.5
2	GSBH62005-91: Sb=10.4,Bi=0.103,Cu=3.1,Sn=4.81 As = 1.15 ,Zn=0.68.	1.15	1.12	1.2
3	GBW01637 : Ag = 1 , As= 14, Bi = 0.19 ,Ga = 34 , In = 7.2 , Pb = 3.7 , Sb = 3.3 , Se = 12	14.00	15.05	1.8
4	GBW01622 : Unalloyed steel : Ag = 0.3, As =72 , Bi = 0.5 , Ca = 32 , Cd = 1.9 ,Ga = 28 , In = 0.4 , Mg = 53 , Pb = 2.2,Sb = 7.4 , Se = 43, Sn = 0.164,Te = 0.5,Ti = 8.1 , Zn = 6.8	72.00	70.85	2.0
5	NIES-CRM No. 18 : Human urine[a]	0.134± 0.01	0.132± 0.05	0.06
6	NIST®SRM – 1577c : Bovine liver	19.6 ± 1.4[c]	19.4 ± 1.5	1.5
7	NIST - CRM – Estuarian sediments solution	10.00[d]	9.98	1.0
8	NIST–SRM® – 1643c : Human hair	44.00±1.72[c]	43.52± 2.05	2.0
9	NIST–SRM®– 1643c : Human finger nails	125.00	124.5	2.5
10	SRM-NIST : Groundwater (HS Code : 382200)	1.00[e]	0.99	0.5

[a]The CRMs were obtained from the National Research Council, Govt. Canada.
[b]The measure of precision is the relative standard deviation (RSD).
[c] values in μgg^{-1},
[d]Values in $mgkg^{-1}$,
[e]Values in mgL^{-1}.

Table 5. Determination of arsenic in some groundwater samples

Sample Id	Sample source	Arsenic / mgL^{-1}						
		AHG-AAS (n=5)		Sample Kit	Spectrophotometric Method (n=5)		ICP-OES(n=5)	
		Found	RSD[b] (%)		Found	RSD[b] (%)	Found	RSD[b] (%)
1	Tap Water	0.075	0.6	0.00	0.065	0.75	0.058	0.06
2	Well Water	0.032	0.15	0.00	0.043	0.7	0.035	0.5
3	Shallow Tubewell Water[c]	1.25	1.0	1.00	1.15	1.0	1.12	1.0
4	Shallow Tubewell Water[c]	1.15	0.9	0.5	1.13	1.2	1.15	1.3
5	Shallow Tubewell Water[c]	0.95	0.5	0.5	1.1	1.0	1.98	0.7
6	Shallow Tubewel lWater[c]	1.95	1.0	2.0	1.95	1.5	1.98	1.3
7	Shallow Tubewell Water[c]	1.15	1.0	1.2	1.20	1.0	1.2	1.4
8	Shallow Tubewell Water[c]	0.62	1.0	0.5	0.68	1.3	0.70	1.2
9	Shallow TubewellWater[c]	0.06	1.2	0.05	0.04	0.7	0.65	1.0
10	Shallow Tubewell Water[c]	2.65	1.8	2.5	2.45	1.6	2.72	1.7
11	Shallow Tubewell Water[c]	0.05	0.5	0.00	0.05	0.6	0.05	0.5
12	Shallow Tubewell Water[c]	3.54	1.6	3.0	3.50	1.8	3.60	1.9
13	ShalloTubewell Water[d]	0.04	0.3	0.00	0.00	0.00	0.00	0.00
14	Shallow Tubewell Water[d]	0.92	0.6	0.60	0.95	0.8	0.97	0.8
15	Shallow Tubewell Water[d]	0.05	1.0	0.00	0.00	0.00	0.03	0.6
16	Shallow Tubewell Water[d]	1.55	1.5	1.50	1.58	1.6	1.59	0.8
17	Shallow TubewellWater[d]	1.35	1.0	1.2	1.45	1.2	1.45	1.0
18	Shallow Tubewell Water[d]	0.05	0.1	0.05	0.00	0.00	0.00	0.00
19	Shallow Tubewell Water[d]	0.89	0.6	0.6	0.85	1.0	0.92	0.8
20	Shallow Tubewell Water[d]	0.98	1.0	0.6	0.93	0.8	0.98	1.0
21	Shallow TubewellWater[d]	0.65	0.6	0.5	0.85	1.0	0.61	0.6
22	Shallow TubewellWater[d]	1.21	1.2	1.0	1.35	1.1	1.30	1.2
23	Shallow TubewellWater[d]	0.05	0.5	0.00	0.58	1.3	0.63	1.0
24	Shallow TubewellWater[d]	0.05	0.6	0.00	0.58	1.3	0.63	1.2
25	Shallow TubewellWater[d]	0.85	1.0	0.5	0.95	1.6	0.63	1.2

[a]Average of five replicate determination of each sample.
[b]The measure of precision is the relative standard deviation (RSD).
[c]Shallowtubewell water from Mirsharai Upazila,Chattagram district
[d]Shallowtubewell water from Kachua Upazila, Chandpur district
[e] Shallow tubewell water from Matlab Upazila, Chandpur district
[f]Shallowtubewell water from Faridganj Upazila, Chandpur district.

Table 6. Determination of arsenic in human fluids, hair and nail samples

Sample No	Sample source	sample	Arsenic / mgL^{-1}					
			AHG-AAS (n=5)		Spectrophotometry (n=5)		ICP-OES (n=5)	
			Found	RSD (%)	Found	RSD (%)	Found	RSD (%)
1	Arsenicosis Patients (Skin cancer) (Female)	Blood	5.95	2.0	5.98	1.9	6.00	2.5
		Urine	1.49	1.0	1.51	1.8	1.52	1.6
2	Arsenicosis Patients (Skin cancer) (Male)	Blood	3.55	1.5	3.60	1.6	3.62	1.8
		Urine	0.92	0.8	0.98	0.9	1.00	1.0
3	Liver cirrhosis (Female)	Blood	2.35	1.8	2.41	1.9	2.40	1.5
		Urine	0.65	0.8	0.69	1.0	0.75	1.0
4	Skin disease (Male)	Blood	1.85	1.0	1.95	1.5	1.98	1.6
		Urine	0.49	0.8	0.52	0.9	0.58	0.8
5	Normal adult (Male)	Blood	0.045	0.03	0.052	0.05	0.054	0.05
		Urine	0.002	0.005	0.014	0.003	0.015	0.004
6	Arsenicosis Patients (Female)	Human hair (Female)	10.89[c]	1.5	10.95	1.6	11.00	1.8
7	Arsenicosis Patients (Female)	Human nail (Female)	8.5[c]	1.2	9.02	1.8	9.15	2.0
8	Arsenicosis Patients (Female)	Human milk (Female)	27.5[d]	2.5	27.8	2.0	28.00	2.5

[a]Samples were collected from Chittagong Medical College Hospital, Chittagong district, Hajiganj and Faridganj Upazila Health complex, Chandpur district, Bangladesh.
[b]Values in μgg^{-1}, [c]Values in $mgkg^{-1}$ [d]Values in μgL^{-1}

Table 7. Determination of arsenic in some surface soil samples

Serial No.	Arsenic / $mgkg^{-1}$			Sample Sources[c]
	AHG-AAS (n=5)	Proposed method (n=5)	RSD[b] (%)	
S_1	1.90±0.5	1.95	1.0	Industrial soil (Eastern Refineries), Chittagong
S_2	1.75±0.6	1.80	1.0	Industrial soil (Eastern Cable), Chittagong
S_3	3.95±1.0	3.98	1.5	Industrial soil (BSRM Steel Mill), Chittagong
S_4	2.85±1.0	2.92	1.2	Industrial soil (Madina tannery), Chittagong
S_5	1.68±1.0	1.72	1.4	Road side sample (Dhaka to Chittagong highway}
S_6	0.45±0.1	0.35	0.8	Agricultural soil (Chittagong University Campus)
S_7	0.22	0.25	1.2	Marine soils (sediments) (Bay of Bengal)
S_8	1.95±1.5	1.98	1.8	Pharmaceutical Samples (Glaxo smith kline)
S_9	7.25±1.0	7.51	2.0	Paint industry soil (Berger)
S_{10}	3.58±1.5	3.65	1.8	Paint industry soil(Elite paint)

[a]Average of five analyses of each sample.
[b]The measure of precision is the relative standard deviation (RSD).
[c]Composition of the soil samples: C, N, P, K, Na, Ca, Mg, Ce, Cu, Mo, Fe, Pb, V, Zn,Mn, Co, NO_3, SO_4 etc.

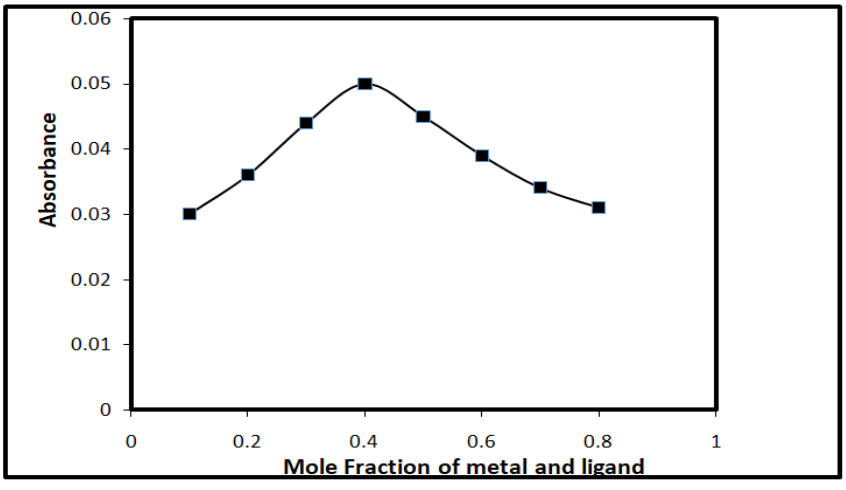


Figure 14. Job'S method for the determination the composition of As^{III}: HNA-OAP(2:3) complex

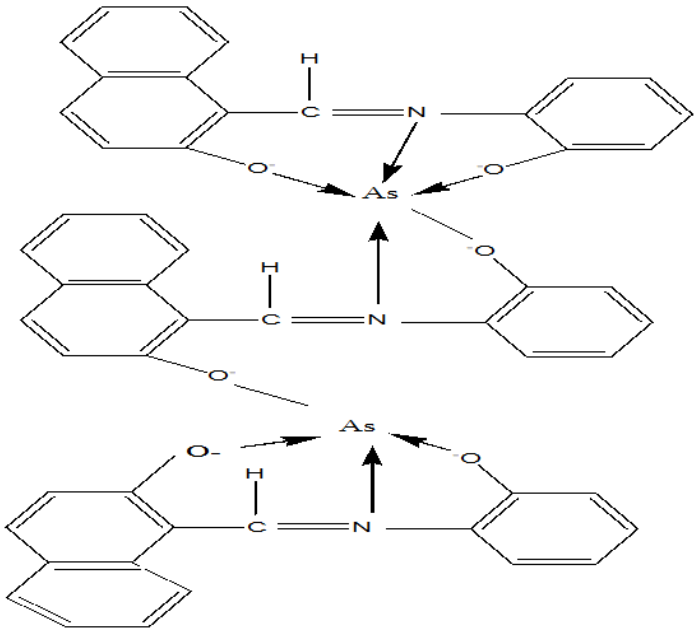
Table 8. Determination of arsenic in some food, fruit and vegetable samples

Serial No.	Sample	Arsenic / $mgkg^{-1}$ Found[a]± s (n=5)				Sample Source
		ICP-OES (n=5)		Proposed Method (n=5)		
		Found	RSD[b] %	Found	RSD[b] %	
1	Ginger *(Zingiberofficinale)*	2.75	1.5	2.82	1.8	Local Market, Chittagong
2	Carrot *(Daucuscarota)*	4.95	2.0	4.98	2.1	Local Market, Chittagong
3	Garlic (*Allium sativum*)	4.51	2.1	4.59	2.5	Local Market,Dhaka
4	Onion (*Allium cepa)*	2.52	1.6	2.58	1.8	Local Market, Dhaka
5	Tomato (*Lycopersiconesculentum)*	4.15	1.8	4.22	2.0	Local Market,Narsingdi
6	White cabbage (*Brassica -oleraeacupitata)*	2.75	1.9	2.81	2.5	Local Market,Narsingdi
7	Radish *(Raphanussativas)*	3.25	2.0	3.35	2.2	Local Market,comilla
8	Rice (*Oryza sativa*)	1.05	1.8	1.08	2.0	Local Market, Dhaka
9	Arum (Calcasiaantiquorum)	3.99	2.0	4.05	2.1	Local Market, Chandpur
10	Wheat *(Triticumaestivum)*	1.92	1.8	1.98	2.2	Local Market,Dhaka
11	Potatoes(solanumtuberisum)	1.36	1.0	1.41	1.2	Local market,Dhaka
12	Shellfish	100.0	1.5	103.0	1.5	Local Market, Chittagong
13	Milk (Cow milk)	30.0[c]	1.5	31.5[c]	1.6	Local Market, Chittagong
14	Mashrooms (*Agaricusbisporus*)	431.0	1.8	435.0	2.0	Local Market, Chittagong

[a]Average of five replicate analyses of each sample.
[b]The measure of precision is the relative standard deviation (RSD).
[c]Values in μgL^{-1}.

Table 9. Determination of arsenic (III)and arsenic(V) speciation in mixtures

Serial No.	As (III):As (V)	As taken (mgL^{-1})		As found (mgL^{-1})		Error (mgL^{-1})	
		As(III)	As(V)	As(III)	As(V)	As(III)	As(V)
1	1: 1	1.00	1.00	0.99	0.98	0.01	0.02
2	1: 1	1.00	1.00	1.00	1.00	0.00	0.00
3	1: 1	1.00	1.00	0.99	0.98	0.01	0.02
Mean error : As(III) = ±0.0067 ; As(V) = ± 0.013 Standard deviation : As(III) = ±0.005 ; As(V) = ±0.011							
1	1: 5	1.00	5.00	0.99	4.98	0.01	0.02
2	1: 5	1.00	5.00	0.98	4.98	0.02	0.02
3	1: 5	1.00	5.00	0.99	4.99	0.01	0.01
Mean error : As(III) = ±0.013 ; As(V) = ± 0.016 Standard deviation : As(III) = ±0.0058 ; As(V) = ± 0.0006							
1	1:10	1.00	10.00	0.98	9.99	0.02	0.01
2	1:10	1.00	10.00	0.99	9.98	0.01	0.02
3	1:10	1.00	10.00	0.98	9.98	0.02	0.02
Mean error : As(III) = ± 0.016 ; As(V) = ±0.017 Standard deviation (SD) : As(III) = ±0.006 ; As(V) = ±0.0058							



Scheme 2. Probable structure of $[As_2(HNA\text{-}OAP)_3]$ complex (2:3) according to Job's Method

Greater excess concentration of the reagent was not studied. For all subsequent measurements 1-mL of 3.8×10^{-3} M HNA-OAP reagent was added.

Calibration Graph (Beer's Law and Sensitivity): The well-known equation for spectrophotometric analysis in very dilute solutions derived from Beer's law. The effect of metal concentration was studied over 0.01- 60-mg L^{-1} distributed in four different sets (0.01-0.1, 0.1-1, 1-10 and 10-100 mg L^{-1}). For convenience of measurement. The absorbance was linear for 0.01-60-mg L^{-1} of arsenic (III) at 495 nm representing four graphs (0.01-0.1, 0.1-1.0, 1.0-10.0 and 10.0-60.0 mg $L^{-1)}$ as shown in Figure 10, Figure 11, Figure 12 and Figure 13, respectively. Of four calibration graphs, the one showing the limit of the linearity range (Figure13); the next three (Figure 10, Figure 11 and Figure 12) were straight-line graphs passing through the origin (R^2 = 0.9997). The molar absorptive and Sandal's sensitivity (Sandell's, 1965) were found to be 2.46×10^4 L mol^{-1} cm^{-1} and 5-ng cm^{-2} of arsenic (III), respectively. The selected analytical parameters obtained with the optimization experiments are summarized in Table 1.

Effect of Foreign Ions: More than 60 anions, cations and complexing agents were studied individually to investigate their effect on the determination of 1-mgL^{-1} of arsenic (III). The criterion for interference (Ojeda, 1987) was an absorbance value varying by more than ±5% from the expected value for arsenic alone. The results are summarized in Table 2. As can be seen a large number of ions have no significant effect on the determination of arsenic (III). The most serious interference was from selenium (IV) and aluminum (III) ions. Interference from these ions is probably due to complex formation with HNA-OAP. The greater tolerance limits for these ions can be achieved by using several masking agents. In order to eliminate the interference of selenium (IV) and aluminum (III) ions, EDTA and tartrate can be used as masking agents, respectively (Ahmed, 1997). A 50-fold excess of selenium (IV) and aluminum (III) ions could be masked with EDTA and tartrate, respectively. During the interference studies, if a precipitate was formed, it was removed by centrifugation. Strong reducing agents such as tin (II), chloride, iron (II), sulfate, hydroxylamine hydrochloride and sodium azide, which would otherwise reduce arsenic (V) had no reducing effect on arsenic (III). The amount mentioned is not the tolerance limit but the actual amount studied. However, for those ions whose tolerance limits have been studied, their tolerance ratios are mentioned in Table 2.

Composition of the Absorbent complex: Job's method (Job, 1928) of continuous variation method was applied to ascertain the stoichiometric composition of the complex under the optimum conditions (Table 1). A As $^{(III)}$-HNA-OAP (2:3) complex was indicated by this method. The molar- ratio method (Yoe, 1944) was also applied to ascertain the stoichiometric composition of the complex. A As $^{(III)}$-HNA-OAP complex was indicated by both methods and the stoichiometry was also found to be 2:3 (As: HNA-OAP). Job's method of continuous variation was applied to ascertain the stoichiometric composition of the complex according to the general procedure. Experimental data has been shown graphically in Figure14 and the stoichiometry was found to be 2:3 (As: HNA-OAP).

Precision and Accuracy: The precision of the present method was evaluated by determining different concentrations of arsenic (III) (each analyzed at least five times). The relative standard deviation (n=5) was 3-0% for 0.1-600-µg of arsenic (III) in 10-mL, indicating that this method is highly precise and reproducible (Table 1). The detection limit (3s/S of the blank) and Sandell's sensitivity (concentration for 0.001 absorbance unit) for arsenic (III) were found to be 1-µg L^{-1} and 5-ng cm^{-2}, respectively. The method was also tested by analyzing several synthetic mixtures containing arsenic (III) and diverse ions (Table 3). The results for total arsenic were in excellent agreement with certified values (Table 4). The reliability of our arsenic (III)-chelate procedure was tested by recovery studies. The average percentage recovery obtained for addition of arsenic (III) spike to some environmental water samples was quantitative, as shown in Table 5. The results of biological analyses by the spectrophotometric method were in excellent agreement with those obtained by AHG-AAS and ICP-OES (Table 6). The results of food analyses by spectrophotometric method were also found to be in excellent agreement with those obtained by AHG-AAS and ICP-OES (Table 8). The results of speciation of arsenic (III) and arsenic (V) in mixtures were highly reproducible (Table 9). Hence, the precision and accuracy of the method were found to be excellent.

Applications

The present method was successfully applied to the determination of arsenic (III) in a series of synthetic mixtures of various compositions (Table 3) and also in a number of real samples e.g. several certified reference materials (CRMs) (Table 4). The method was also extended to the determination of arsenic (III) in a number of environmental, biological, soil and food samples. In view of the unknown composition of environmental water samples, the same equivalent portions of each such sample were analyzed for arsenic (III) content; the recoveries in both the "spiked" (added to the samples before the mineralization or dissolution) and the "unspiked" samples are in excellent agreement (Table 5).The result of groundwater analyses by spectrophotometric method were found excellent agreement with those obtained by ICP-OES and AHG-AAS. The results of biological analyses by spectrophotometric method were found to be in excellent agreement with those obtained by ICP-OES and AHG-AAS (Table 6). The results of soil analyses by the spectrophotometric method were found excellent agreement with those obtained by AHG-AAS (Table 7). The results of food analyses by spectrophotometric method were also found to be in excellent agreement with those obtained by ICP-OES (Table 9).The results of speciation of arsenic (III) and arsenic (V) in mixtures are shown in Table 10.

Determination of Arsenic in Synthetic Mixtures: The procedure was applied to determine trace amounts of arsenic (III) in some synthetic mixtures with good recovery being achieved. The result indicate the proposed method is suitable and can be successfully applied for determination of As(III).Several synthetic mixtures of varying compositions containing arsenic(III) and diverse ions of known concentrations were determined by the present method using EDTA as masking agent. The results were found to be highly reproducible as shown in Table 3. Accurate recoveries were achieved in all solutions in the range 99.6± 1.5 to 99.9± 0.6. The reliability of our arsenic-HNA-OAP oxidation procedure was approved by quantitative recovery of arsenic (V) spiked in

several synthetic mixtures containing As (III) and diverse ions. This method has high precision and accuracy (s = ± 0.01 for 0.5-μgL^{-1}).

Determination of Arsenic in Alloys and Steels (Certified Reference Materials): A 0.1-g amount of an alloy or steel sample containing 1.02 – 72%of arsenic was weighed accurately and placed in a 50-mL Erlenmeyer flask in presence of excess reducing agent following a method recommended by Parker (Parker, 1983) . To it, 10-mL of 20% sulfuric acid was added while carefully covering with a watch glass until the brisk reaction subsided. The solution was heated and simmered gently after the addition of 10-mL of concentrated HNO_3 until all carbides were decomposed. Then, 2-mL of 1:1 (v/v) H_2SO_4 was added and the solution was carefully evaporated to dense white fumes to drive off the oxides of nitrogen, and then cooled to room temperature (25 ± 5) °C. After suitable dilution with de-ionized water, the contents of the Erlenmeyer flask were warmed so as to dissolve the soluble salts.

The solution was then cooled and neutralized with a dilute NH_4OH in the presence of 1-2- mL of 0.01 % (w/v) tartrate solution. The resulting solution was filtered, if necessary, through a Whatman No. 40 filter paper into a 50-mL calibrated flask. The residue (silica and tungstenic acid) was washed with a small volume of hot (1 + 99) H_2SO_4, followed by water; the volume was made up to the mark with de-ionized water. A suitable aliquot (1-2-mL) of the above-mentioned solution was taken into a 10-mL calibrated flask and the arsenic(III) content was determined; as described under Procedure using EDTA or tartrate as masking agent. The proposed procedure for thespectrophotometric determination of arsenic was applied to the analysis of estuarine sediment (CEC – CRM – 277), Tea (NRC – CRM – C85– 05) and Bovine liver (NBS – SRM – 1577) ,the CRM_S obtained from the National Research Council of Canada using tartrate or EDTA as a masking agent, following a method recommended by Sun *et al*. (43) Based on five replicate analyses, average total arsenic concentration determined by spectrophotometric method was in excellent agreement with the certified values. The results are given in Table 4.

Determination of Arsenic in Environmental Water Samples: Each filtered (with Whatman No. 40) environmental water sample (500-mL) was evaporated nearly to dryness with a mixture of 5-mL concentrated H_2SO_4 and 10-mL of concentrated HNO_3 to sulfur trioxide fumes in presence of excess sodium azide solution in a fume cupboard, following a method recommended by Greenberg *et al.* (1992) After cooling addition of 5-mL of concentrated HNO_3 was repeated and heating to a dense fume continued or until the solution become colorless. The residue was then heated with 10-mL of de-ionized water in order to dissolve the salts. The solution was then cooled and neutralized with dilute NH_4OH solution in presence of 1-2 mL of a 0.01% (w/v) tartrate solution. The resulting solution was then filtered and quantitatively transferred into a 25-mL calibrated flask and made up to the mark with de-ionized water. An aliquot (1-2 mL) of this preconcentrated water sample was pipetted into a 10-mL calibrated flask and the arsenic content was determined as described under the general Procedure using EDTA or tartrate as masking agent.The result of groundwater analyses by spectrophotometric method were found excellent agreement with those obtained by ICP-OES ans AHG-AAS. The results of analyses of environmental water samples from various sources for arsenic are shown in Table 5. Most spectrophotometric methods for determination of arsenic in natural water require preconcentration of arsenic (Ramazan, 2015) The concentration of arsenic (III) in natural water is a few μg L^{-1} in developed countries (Kumar, 2004). The mean concentration of arsenic found in U.S. drinking water is less than 5 μg L^{-1} (Kumar, 2004).

- Shallowtubewell water from Kachua Upazila, Chandpur district
- Shallow tubewell water from Matlab Upazila, Chandpur district
- Shallowtubewell water from Faridganj Upazila, Chandpur district.

Determination of Arsenic in Biological Samples: Human blood (2- 4mL) or urine (10-20 mL) or hair (2-5g) sample was taken into a 100-mL micro-Kjeldahl flask. A glass bead and 10-mL of concentrated nitric acid were added, and the flask was placed on the digester under gentle heating. The sample was digested in the presence of an excess sodium azide solution according to the method recommended by Stahr (Stahr, 1991). When the initial brisk reaction was completed, the solution was removed and cooled to room temperature. A 1-mL volume of concentrated sulfuric acid was carefully added, followed by the addition of 1-mL of 2.5% sodium azide solution; and heating was continued to dense white fumes, while repeating nitric acid addition if necessary. Heating was continued for at least 0.5 hr and then cooling was applied. The content of the flask was filtered and neutralized with dilute NH_4OH solution in presence of 1-2-mL of a 0.01% (w/v) tartrate solution. The resultant solution was then filtered and transferred quantitatively into a 10-mL calibrated flask and made up to the mark with de-ionized water. A suitable aliquot (1-2-mL) of the final solution was pipette out into a 10-mL calibrated flask and the arsenic content was determined as described under Procedure using EDTA or tartrate as masking agent. The results of biological analyses by the spectrophotometric method were found to be in excellent agreement with those obtained by ICP-OES and AHG-AAS. The results are shown in Table 6. The abnormally high value for the hair loses and neurological disorder patient is probably due to the involvement of high arsenic concentrations with Se and Zn. The occurrences of such high arsenic contents are also reported in hair lose and neurological disorder patient from some developed countries (Jamaluddin Ahmed, 2019).

Determination of Arsenic in Soil Samples: An air-dried homogenized soil sample (100 g) was accurately weighed and placed in a 100-mL micro-Kjeldahl flask. The sample was digested in the presence of an reducing agent (1-mL of 2.5% (w/v) sodium azide solution) following a method recommended by Jackson (Jackson, 1965). The content of the flask was filtered through a Whatman No.40 filter paper into a 25-mL calibrated flask and neutralized with dilute NH_4OH solution in presence of 1-2-mL of 0.01% (w/v) tartrate solution. The resulting solution was then diluted up to the mark with de-ionized water. A suitable aliquot (1-mL) of the final solution was pipetted out into a 10-mL calibrated flask and the arsenic content was determined as described under Procedure using EDTA or tartrate agent. The arsenic content was then determined by the above procedure and quantified from a

calibration graph prepared concurrently. The results of soil analyses by the spectrophotometric method were found excellent agreement with those obtained by AHG-AAS. The results are shown in Table 7. The average value of arsenic (III) in Chittagong region surface soil was found to be 2.61mg kg^{-1} (Hussam, 2008).

Determination of Arsenic in Food Samples: The food samples used were rice, wheat and tea and these were used as dry condition. Each sample was first ground in a mortar. Tea samples (0.1g) or rice and wheat samples (1.0 g) were weighed accurately and placed in a porcelain crucible and charred in an electric furnace; the sample was a shed at 555º C in a muffle furnace in presence of excess reducing agent following the method recommended by Stahr (47), 2.0-mL of HCl and 10-ml of water were added to the ash. The mixture of each foodstuff was heated below the boiling point for a moment. The solutions were cooled and neutralized with NH_4OH in presence of 1-2-mL of 0.01% (w/v) tartrate or EDTA solution and filtered. The resulting solution was quantitatively transferred into 25-mL calibrated flask and mixed well and made upto the mark with de-ionized water. A suitable aliquot (1-2-mL) of the final solution was pipetted out into a 10-mL calibrated flask and the arsenic **co**ntent was determined as described under Procedure using EDTA or tartrate as masking agent. The results of food analyses by the spectrophotometric method were also found to be in excellent agreement with those obtained by ICP-OES. The results are shown in Table 8. High value of arsenic for *Daucuscarota* (Carrot) is probably due to the involvement of high arsenic concentration in the soil.

Determination of Arsenic (III) and Arsenic (V) Speciation in Mixtures: Suitable aliquots (1-2 mL) of arsenic (III + V) mixtures (preferably 1: 1, 1: 3, 1:5) were taken in a 25-mL conical flask. A few drops (2-3drops) of 4 M H_2SO_4, 3-4 mL of a freshly prepared sodium azide solution (2.5% w/v) was added to reduce the pentavalent arsenic to trivalent arsenic and heated gently with the further addition of 5-mL of water, if necessary, for 5 min to drive off the excess azide then cooled to room temperature. The reaction mixtures was neutralized with dilute NH_4OH and transferred quantitatively into a 10-mL volumetric flask. 1-mL of 3.8 x 10^{-3}M HNA-OAP reagent solution was added followed by the addition of 1-mL of 0.0002 M H_2SO_4 and 2-mL DMF. It was made up to the mark with de-ionized water. The absorbance was measured after 1 min at 495 nm against a reagent blank. The total arsenic content was calculated with the help of a calibration graph prepared concurrently. An equal aliquot (1-2-mL) of the above arsenic (III+V) mixture was taken into a 25-mL beaker. Neutralize the solution with dilute NH_4OH in presence of 1-2-mL of 0.01% (w/v) tartrate solution. After, the content of the beaker was transferred quantitatively into a 10-mL volumetric flask, 1-mL of 3.8 × 10^{-3} M HNA-OAP reagent solution was added, followed by the addition of 1-mL of 0.0002 M H_2SO_4 and 2 mL DMF. It was made up to the mark with de-ionized water. After 1 min the absorbance was measured at 495 nm against a reagent blank, as before. The arsenic concentration was calculated in mg L^{-1} or µg L^{-1} with the aid of a calibration graph. This gives a measure of arsenic (III) originally present in the mixture. This value was subtracted from that of the total arsenic to get the arsenic (III) present in the mixture. The results were found to be highly reproducible.

The occurrence of such reproducible results is also reported for different oxidation states of arsenic (Ahmed, 1997). The results of a set of determination are given in Table 9.

Conclusion

A new simple, sensitive, and inexpensive method with the arsenic (III) -HNA-OAP complex was developed for the determination of arsenic in some real, environmental, biological, soil and food samples, for continuous monitoring to establish the trace levels of arsenic in different samples matrices. Compared with other methods in the literature (12-32), the proposed method has several remarkable analytical characteristics:

- The proposed method is highly sensitive with molar absorptivity of the complex of 2.46×10^4 L mol^{-1} cm^{-1}. Thus, amount of ngg^{-1} levels of arsenic can be determined without preconcentration;
- The proposed method is very simple, rapid and stable. The reaction of arsenic (III) with HNA-OAP is completed rapidly in 5 min at (45±5)°C within 15 min it cooled at temperature(25±5)°C so it does not involve any stringent reaction conditions and offer the advantages of high complex stability (24h).
- The method has added the advantage of determining individual amounts of As(III) and As(V) simultaneously. With suitable masking agents, the reaction can be made highly selective.

The proposed method using HNA-OAP in aqueous solutions not only is one of the most sensitive methods for the determination of arsenic but also is excellent in terms of selectivity and simplicity. Therefore, this method will be successfully applied to the monitoring of nano-trace (ng g^{-1}) amounts of arsenic in real, environmental, biological, soil and food samples.

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Conflicts of Interest

All authors report no conflicts of interest relevant to this article.

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