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RESEARCH ARTICLE

A SIMPLE AND SELECTIVE SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF URANIUM AT NANO-TRACE LEVELS IN SOME REAL, ENVIRONMENTAL, BIOLOGICAL FOOD AND SOIL SAMPLES USING 2-HYDROXYNAPHTHALDEHYDEBENZOYLHYDRAZONE

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ABSTRACT

A new spectrophotometric reagent 2-hydroxynaphthaldehydebenzoylhydrazone (HNABH) has been synthesized and characterized. A very simple, ultra-sensitive and highly selective non-extractive new spectrophotometric method for the determination of ultra-trace amount of uranium using 2-hydroxynaphthaldehydebenzoylhydrazone (HNABH) has been developed. HNABH undergoes reaction in a slightly acidic solution (0.00025-0.00075M H_2S0_4) with uranium to give a very light yellow chelate, which has an absorption maximum at 406-nm. The reaction is instantaneous and absorbance remains stable for over 24h. The average molar absorption coefficient and Sandal's sensitivity are found to be 2.87×10^5 L $mol^{-1}cm^{-1}$and 8-ng cm^{-2} of, U respectively. Linear calibration graphs were obtained for 0.01- 60-mg L^{-1} of U having detection limit of 1.0-µg L^{-1} and RSD 0 – 3.0 %. The stoichiometric composition of the chelate is 1:1 (U^{VI}: HNABH). A large excess of over 60 cations, anions and some common complexing agents (such as chloride, azide, tartrate, EDTA, SCN^- etc.) do not interfere in the determination. The method was successfully used in the determination of uranium in several Certified Reference Materials (alloys, steels, ores, standard uranium solution water and marine sediment) as well as in some environmental waters (potable and polluted), biological samples (human blood, urine, milk, hair and nails), soil and oil samples, and complex synthetic mixtures. The results of the proposed method for assessing biological, food, water and vegetables samples were found to be in excellent agreement with those obtained by ICP-OES and AAS, respectively. The method has high precision and accuracy (s =± 0.01 for 0.5 mg L^{-1}).

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INTRODUCTION

Uranium occurs in nature in variety of materials and its abundance in the earth's crust is about 2.7 mg kg^{-1} (IAEA, 2005). Unlike many radioactive elements, its half-life is commensurate with the age of earth because of this, small amount of uranium is found almost everywhere in the soil and water (Yemalyanov *et al.,* 1969). The principal application of uranium is its use as nuclear fuel to generate electric power and to make nuclear weapons (Khan *et al.,* 2008). The mining and milling operations of uranium produce large quantities of low activity wastes in both liquid and solid forms (Leonardo *et al.,* 1999; International Atomic Energy, 1995). The management of these wastes has been a concern in many countries triaging about typical problems like ground water contamination and environmental pollutant (Yemalyanov *et al.,* 1969; International Atomic Energy, 1995). Atomic Spectrophotometric methods have little applications for determination of uranium mainly due to its high spectral background and low sensitivity attainable due to high thermal stability of its oxides (Moor, 1969; Kirkbright *et al.,* 1977).

The determination of uranium by molecular spectrophotometry is important and it requires high accuracy due to its strong association with other elements (Vinogradio, 1963; Aleksandrova *et al.,* 1989). In fact, there is no accepted radiotracer or atomic absorption spectrophotometric technique for its measurement (Khan *et al.,* 1994). Several trace level analytical techniques have been reported for the determination of uranium but most of these involve tedious and painstaking procedures (Slovak *et al.,* 1978; Jaiswal, 1994). However, the uses of organic solvent for spectrophotometric determination of uranium along with other actinides were reported to be simple, selective, and comparatively accurate and have been widely investigated (Sweify *et al.,* 1997). The main advantage of this reagent is stability of its uranium complex which makes possible its analytical utilization in strongly acidic media, where neither hydrolysis nor formation of poly nuclear species occur in the reaction (Khan *et al.,* 2001). Various methods have been reported where organic solvents and mineral acids were used as medium of determination of uranium but most of these were complicated (Venkatesh, 2004; Barakat *et al.,* 2001). Among these, spectrophotometry is essentially a trace analysis technique and is one of the most powerful tools in chemical analysis.

2-hydroxynaphthaldehydebenzoylhydrazone (HNABH) has been reported as a spectrophotometric reagent for aluminum (Hesse, 1972; Starvin *et al.,* 2004; Barakat *et al.,* 2001) and copper (Barakat *et al.,* 2001) but has not previously been used for spectrophotometric determination of uranium. This paper reports its use in a very sensitive, highly specific spectrophotometric method of trace determination of uranium. The method possesses distinct advantages over existing methods (Khan, 2011; Ahmed *et al.,* 2018) with respect to sensitivity, selectivity, range of determination, simplicity, speed, pH /acidity range, thermal stability, accuracy, precision and ease of operation. From above mentioned literature survey (Khan et al – Ahmed et al) it reveals that those methods are lengthy, time-consuming, pH dependent and in most of above mentioned methods, interference was high. It is needless to emphasize further that the direct spectrophotometric method in non-extractive way is more useful if it offers high sensitivity and selectivity. Search should be directed a new in order to develop simpler spectrophotometric method for non-extractive estimation of uranium in very selective and sensitive ways. The method is based on the reaction of non-absorbent HNABH in a slightly acidic (0.00025-0.00075 M H_2SO_4) solution with uranium to produce a highly absorbent very pale yellowish chelate product followed by a direct measurement of the absorbance in an aqueous solution with suitable masking, the reaction can be made highly selective and the reagent blank solutions do not show any absorbance.

MATERIALS AND METHODS

Apparatus: A Shimadzu (Kyoto, Japan) (Model-1800) double beam UV/VIS spectrophotometer and Jenway (England, U.K) (Model-3010) pH meter with a combination of electrodes were used for the measurements of absorbance and pH, respectively. A Shimadzu (Model: AA7000) atomic absorption spectrophotometer (AAS) equipped with microcomputer controlled air-acetylene flame and A Shimadzu (Japan) (Model : 9800) Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES), λ_{max}=418nm, plasma gas flow rate L min^{-1}=15, LOD: 1- 10μ g L^{-1} of U were used for comparison of the results. Infrared spectrum was recorded with FTIR Spectrophotometer, Shimadzu (Model-IR Prestige 21, Detector-DTGS KBr) in the range 7500-350 cm^{-1}. The elemental analyzer Exeter Analytical Inc Model:CE440) equipped with supersensitive thermal conductivity detector for simultaneous determination of CHN was used.

Synthesis and Characterization of the Reagent

Synthesis of the reagent: The reagent was synthesized in our laboratory according to the method of Sacconi (1953) and Salam *et al.,* (1995).

The reagent 2-hydroxynaphthaldehydebenzoylhydrazone (HNABH) was synthesized by two steps. First, benzoyl-hydrazine (BH) was prepared by refluxing ethyl benzoate (700 mmol) with hydrazine hydrate (700 mmol) at 140°C for 20 hours in a round bottle flux equipped with a condenser and then recrystallized twice from ethanol. An off-white crystalline product was obtained whose melting point was 115°C (Lit. 113-117°C) (Sacconi, 1953). Finally, 2-hydroxynaphthaldehydebenzoylhydrazone was prepared by the condensation of 1:1 molar ratio (30 m mol each) of 2-hydroxynaphthaldehyde (HNP) and benzoyl hydrazine (BH) in ethanol (50-ml). A brown crystalline product was then dried slica gel and calcium chloride in a desiccators. Yield of the product was 80%. The structure of the reagent is shown in Scheme-1.

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 204°C (Lit. 206°C).[56] The result of the elemental analysis (C = 70.10%, O = 10.05 %, N = 9.70 %, H = 5.06 %) of the reagent was in good coincidence with the calculated values (C = 74.45 %, O = 11.03 %, N = 9.65 %, H = 4.83 %). The FTIR spectrum of the prepared reagent (HNABH) is shown in Fig. 1. After analyzing the FTIR spectrum of HNPBH some peaks were found. Among these FTIR peak at 1622.20cm^{-1} (**Fig.1**) was due to the characteristic C=N double bond peak ($v^{C=N}$, 1590-1660 cm^{-1})[58]. Both FTIR spectrum and elemental analysis data indicated the formation of the reagent HNABH. The steadiness of the thermo gravimetric curve indicated that the reagent did not contain any moisture. The elemental analysis and FTIR spectrum were performed by the National Centre of Excellence in Analytical Chemistry, University of Sindh, Jamshoro, Pakistan and FTIR spectra was recorded with FTIR spectrophotometer, a Shimadzu (Model- 1R.prestige21, detector DTGS, KBR) in the range 7500-350 cm^{-1} from our laboratory.

Reagent and solutions: All of the chemicals used were of analytical reagent grade or the highest purity available. Doubly distilled de-ionized water, HPLC-grade ethanol which is non-absorbent under ultraviolet radiation, were used throughout. Glass vessels were cleaned by soaking in acidified solution of $KMnO_4$ or $K_2Cr_2O_7$ followed by washing with concentrated HNO_3 and rinsed several times with de-ionized water. Stock solutions and environmental water samples (1000-mL each) were kept in polypropylene bottles containing 1-mL of concentrated HNO_3. More rigorous contamination control was used when the uranium levels in the specimens were low.

HNABH solution (3.9 ×10^{-3} M): The reagent solution was prepared by dissolving the requisite amount of HNABH in a known volume solution of distilled absolute ethanol. A freshly prepared reagent solution (10^{-3}M) was used as whenever required.

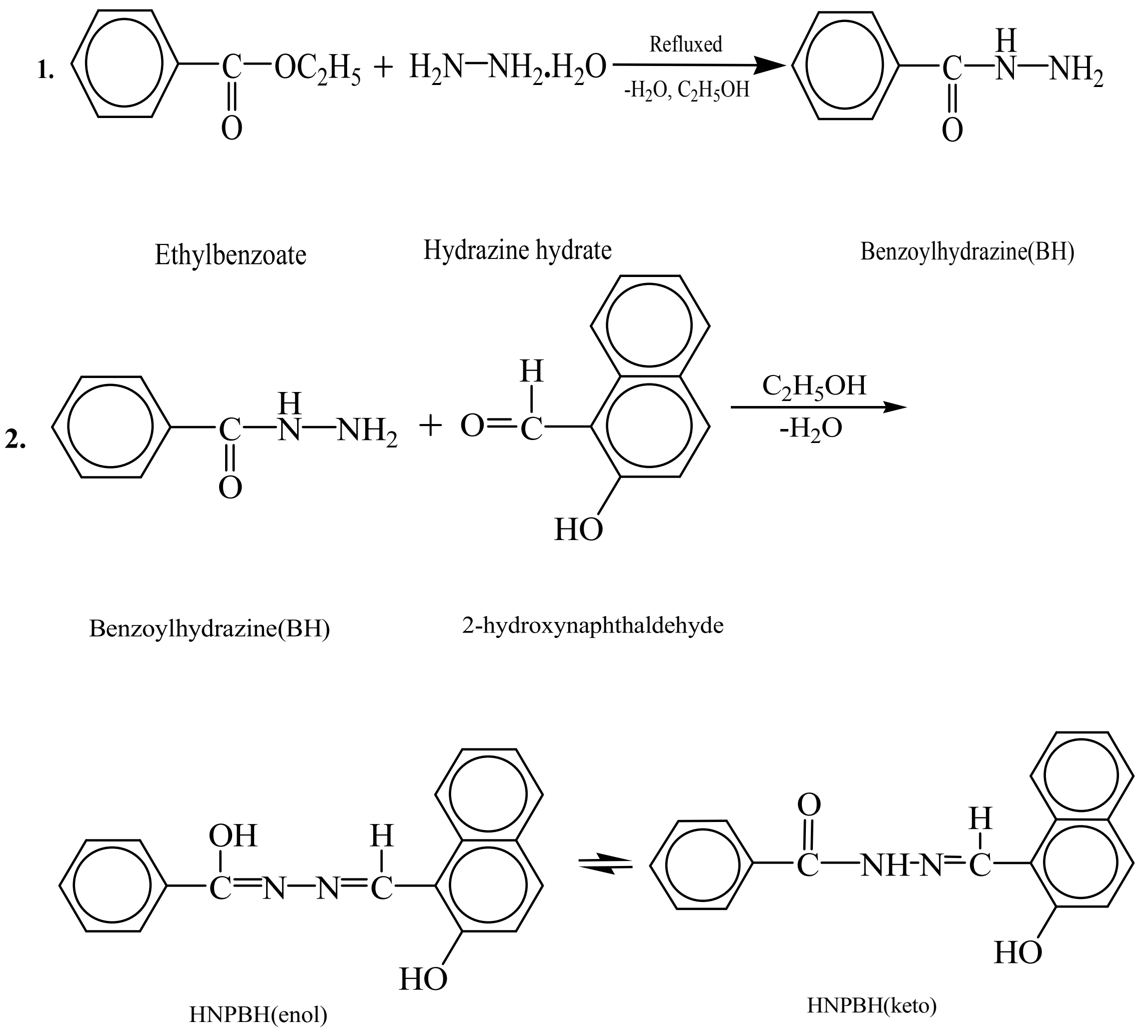
Uranium standard solution (1.53×10^{-2} M): A 100-mL amount of stock solution (1-mg mL^{-1}) of U(VI) was prepared by dissolving 502.13 mg of uranyl nitrate ($UO_2(NO_3).6H_2O$) (Merck pro-analysis grade, 99.7% pure) in doubly distilled de-ionized water. Aliquots of this solution were standardized with EDTA titration using Eriochrome Black T as indicator (Jeffery *et al.,* 1994). Working standard solution was prepared freshly by suitable dilutions of the stock solution as whenever required.

1, 10-Phenanthrolin solution: A 0.1% 1,10-phenanthrolin solution was prepared by dissolving 0.1 gm. amount in 100-mL slightly hot de-ionized water.

1, 5-Diphenylcarbazide solution: A 0.1% 1, 5-diphenylcarbazide solution was prepared by dissolving 0.1 gm amount in 100-mL slightly hot deionized water.

EDTA solution: A 100-mL stock solution of EDTA (0.01%) was prepared by dissolving 10 mg of A.C.S. grade (≥90%) ethylenediaminetetraacetic acid, dissodium salt dehydrate in (100-mL) deionized water.

Synthesis of the reagent



Scheme 1. Synthesis of 2-hydroxynaphthaldehydebenzoylhydrazone (HNABH)

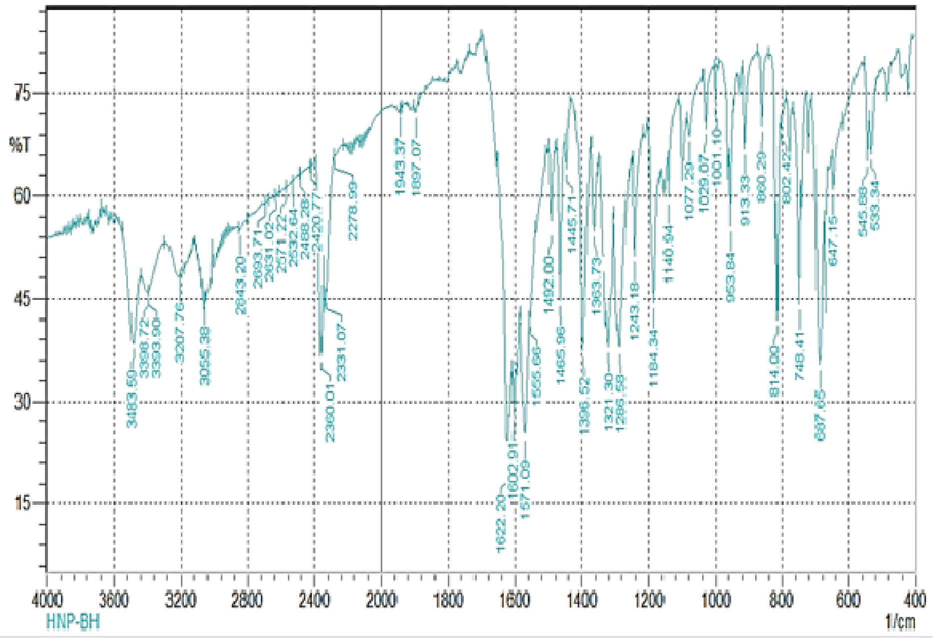


Fig. 1. FTIR Spectrum of 2-hydroxynaphthaldehyde benzoylhydrazone. (HNABH)

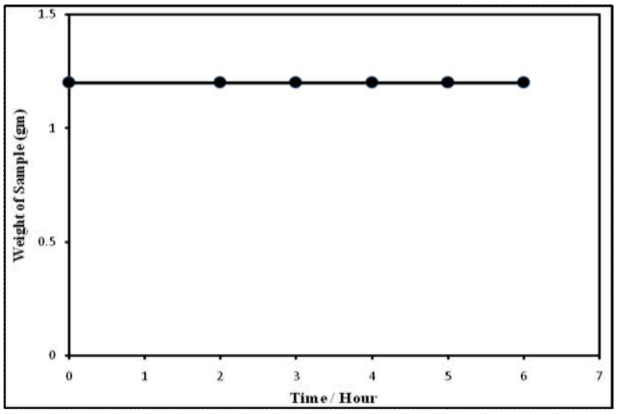


Fig.2. The thermo gravimetric curve of 2-hydroxynaphthaldehydebenzoylhrazone(HNABH) at 85-90º C.

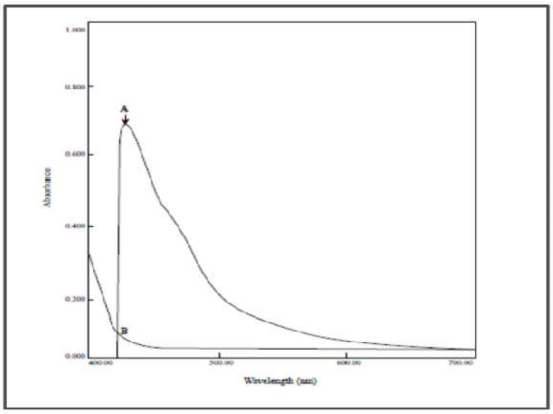


Fig. 3 A and B absorption spectra of the U^{VI} – HNABH system and the reagent blank (λ_{max}=406 nm) in aqueous solutions.

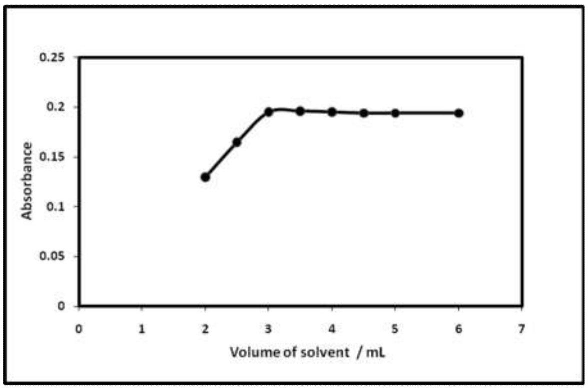


Fig. 4. Effect of solvent (ethanol) on the absorbance of U (VI)-HNABH system

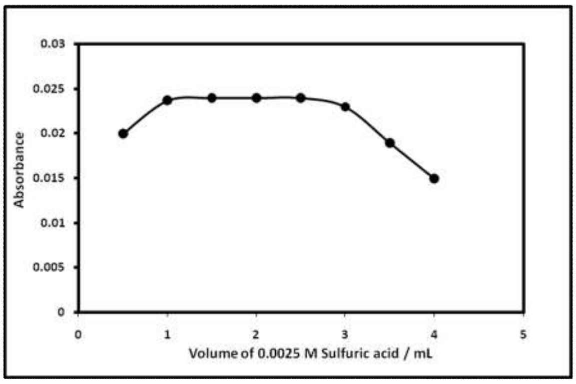


Fig.5 Effect of acidity on the absorbance of U(VI)- HNABH system

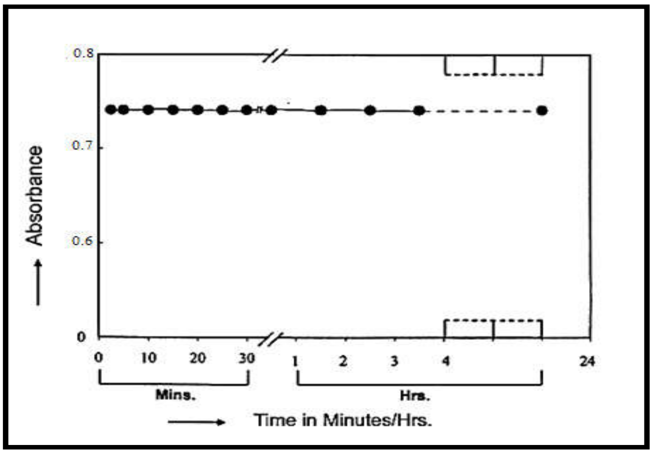


Fig.6. Effect of time on the absorbance of U (IV) - HNABH system

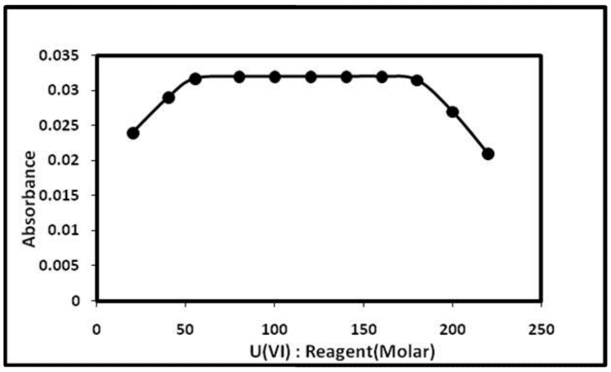


Fig.7. Effect of reagent (U^{VI}-HNABH molar concentration ratio) on the absorbance of U(VI)- HNABH system

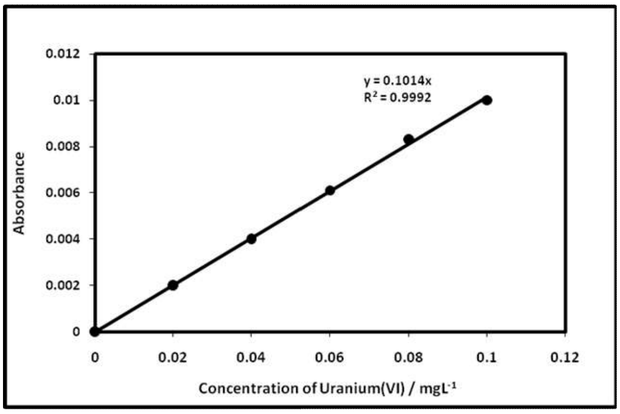


Fig. 8. Calibration graph A:0.01-0.1-mg L^{-1} of uranium(VI)

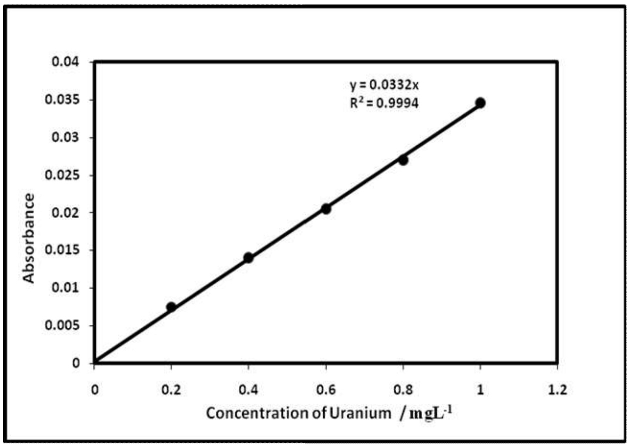


Fig.9 Calibration graph B: 0.1-1-mg L^{-1} of uranium (VI)

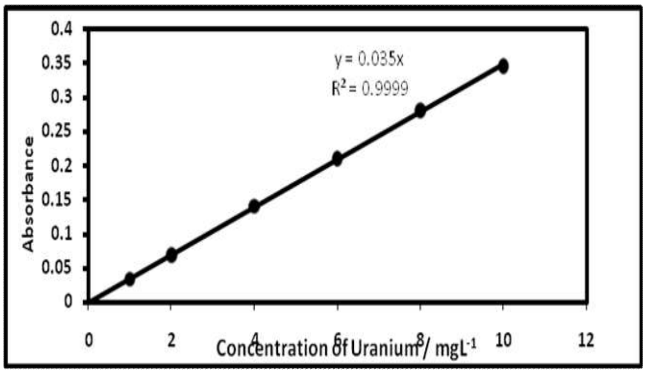


Fig.10 Calibration graph C: 1-10-mg L^{-1} of uranium (VI)

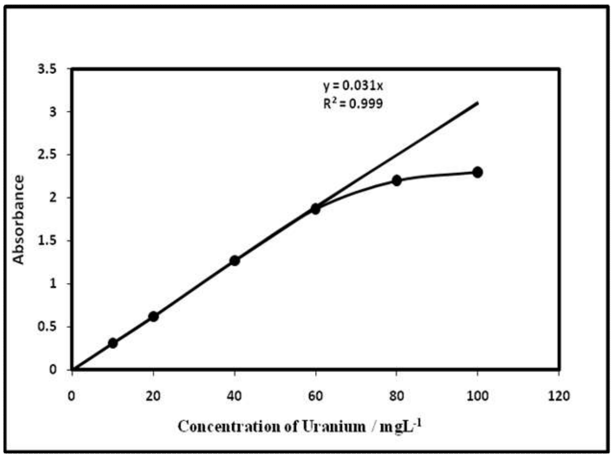


Fig.11 Calibration graph D:10 - 60-mg L^{-1}uranium (VI)

Tartrate solution: A 100-mL stock solution of tartrate (0.01%) was prepared by dissolving 10-mg of A.C.S. grade (99%) potassium sodium tartrate tetra-hydrate in (100-mL) deionized water.

Dilute ammonium hydroxide solution: A 100-mL solution of dilute ammonium hydroxide was prepared by diluting 10-mL concentration. NH_4OH (28-30% A.C.S. grade) to 100-mL with deionized water. The solution was stored in a polypropylene bottle.

Other solutions: Solutions of a large number of inorganic ions and complexing agents were prepared from their Analytical 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 (Specupure, Johnson Matthey) according to the recommended procedures of Mukharji (1970) In the case of insoluble substances, special dissolution methods were adopted (Pal *et al.,* 1984).

General Procedure: A volume of 0.1-1.0-mL of neutral aqueous solution containing 0.1-500-µg of uranium in a 10-mL volumetric flask was mixed with a 1:50 to 1:175 fold molar excess (preferably 1-mL of 3.45 $\times 10^{-3}$ M) of 2-hydroxynaphthaldehydebenzoylhydrazone (HNABH) reagent solution followed by the addition of 1.0 – 2.0-mL (preferably 1-mL) of 0.0005M sulfuric acid. The solution was mixed well. After 1 minute 4-mL of ethanol was added. The mixture was diluted up to the mark with deionized water. The absorbance was measured at 406 nm against a corresponding reagent blank. The uranium content in an unknown sample was determined using a concurrently prepared calibration graph.

Sample collection and preservation (Mitra, 2001)

Water: Water samples were collected in polythene bottles from shallow tube-wells, tap-wells, river, sea and drain of different places of Chittagong region, Bangladesh. After collection, HNO_3 (1-mL L^{-1}) was added as preservative.

Blood and Urine: Blood and urine samples were collected in polypropylene bottles from effected persons of Treatment Centre, CSCR Hospital & Chittagong Medical College Hospital, Bangladesh. Immediately after collection they were stored in a salt-ice mixture and later, at the laboratory, were kept at-20^0C.

Soil: Soil (surface) samples were collected from different locations in Chittagong region, Bangladesh. Samples were dried in air and homogenized with a mortar.

RESULTS AND DISCUSSION

Factors Affecting the Absorbance

Absorption spectra: The absorption spectra of a uranium-HNABH system in aqueous medium in presence of 1-mL 0.0005 M sulfuric acid solution, was recorded using the spectrophotometer. The absorption spectrum of the uranium-HNABH is an asymmetric curve with maximum absorbance at 406 nm and an average molar absorptivity of 2.87×10^5 L $mol^{-1}cm^{-1}$ (Fig. 3). The reagent blank exhibited negligible absorbance despite having wavelength at 406 nm. The reaction mechanism of the present method is as reported earlier (Busev, 1981).

Optimization of some Parameters on the Absorbance

Effect of solvent

As HNABH is partially soluble in water, an organic solvent was used for the system, consideration of cost, availability, toxicity and volatility of the solvent etc. Of the various solvents (acetone, benzene, carbon tetrachloride, chloroform, ethanol, 1-butanol, isobutyl methyl ketone, dimethylformamide, methanol and 1,4-dioxane) studied, ethanol was found to be the best solvent for the system. Different volumes (0-6-mL) of ethanol were added to fixed metal ion concentration and the absorbance were measured according to the general procedure. Maximum absorbance was observed in (30 ± 2%) (v/v) ethanol/water medium, hence, a 30% ethanol solution was used in the determination procedure. It was observed that 20-60% (2-6-mL) ethanol produced a constant absorbance of the U (VI)-chelate (**Fig. 4**). For all subsequent measurements, 30% (4-mL) of ethanol was added.

Effect of acidity: Among the various acids (nitric, sulfuric, hydrochloric and phosphoric) studied, sulfuric acid was found to be the best acid for the system. The variation of the absorbance was noted after the addition of 0.05-3.0-mL of 0.0005 M sulfuric acid to every 10-mL of test solution. The maximum and constant absorbance was obtained in the presence of 0.8–2.5-mL of 0.0005 M sulfuric acid at room temperature $(25\pm5)^0$C. Outside this range of acidity, the absorbance decreased (Fig. 5). For all subsequent measurements 1-mL of 0.0005 M sulfuric acid was added.

Effect of time: The reaction is very fast. A constant maximum absorbance was obtained just after dilution within few seconds to volume and remained strictly constant for over 24 h (Fig. 6); a longer period of time was not studied.

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

Parameters	Studied Range	Selected Value
Wavelength/λ_{max}(nm)	200 - 700	406
Solvent /mL	0-8	3.0-6.0 (preferably 4)
Acidity / M H_2SO_4	0.000005-0.005	0.00025-0.00075 (Preferably 0.0005)
pH	6.5 – 2.5	6.0 – 3.0 (Preferably 3.5)
Time / h	0 - 72	1min - 24 h (Preferably 5 min)
Temperature / °C	10 - 90	20 – 80 (Preferably 25 ± 5)
Reagent (fold molar excess, M:R)	1:1 - 1:250	1:50 - 1:175 (Preferably 1:100)
Linear range / mgL^{-1}	0.001 - 100	0.01 – 60
Molar absorptivity	$1.51\times10^5 – 4.23\times10^5$	2.87×10^5
Detection limit / μgL^{-1}	0-100	1.0
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)/U^{VI}(w/w)]

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

Table 3. Determination of uranium in some synthetic mixtures

Sample	Composition of mixtures	Uranium / mgL^{-1}		
	(mgL^{-1})	Added	Found[a] (n = 5)	Recovery ±SD^b(%)
A	U^{VI}	0.5	0.50	100 ± 0.0
		1.00	0.99	99 ± 0.6
B	As in A +Cd + Se(lV) +Zn + Mn(vII)	0.5	0.49	98 ± 0.8
		1.00	1.02	102 ± 0.7
C	As in B + Sn^{2+} + Sb^{3+}+ Mg + Ce^{3+} + Na+EDTA(50)	0.5	0.49	98 ± 1.0
		1.00	0.98	98 ± 0.8
D	As in C+ Cr^{3+} + Se(VI) + $Cr^{(VI)}$ + Ce^{2+} +EDTA(50)	0.5	0.52	104 ± 1.2
		1.00	1.03	103 ± 1.5
E	As in D + Hg^{2+} +Al + V(V)+ Ag+EDTA(50)	0.5	0.53	106 ± 1.5
		1.00	1.05	105 ± 1.3
F	As in E + Fe^{2+}+ Ti (IV) +Ni^{2+} + Cu^{2+}	0.5	0.55	110 ± 1.8
		1.00	1.09	109 ± 1.6

[a] Average of five analyses of each sample,

[b] The measure of precision is the standard deviation (SD).

Effect of temperature: The U^{VI}-HNABH system attained maximum and constant absorbance at room temperature $(25\pm5)^0$C. Outside this range of temperature, the absorbance decreased.

Effect of reagent concentration: Different molar excesses of HNABH were added to a fixed metal ion concentration and the absorbance was measured according to the general procedure. It was observed that uranium, the reagent molar ratio of 1:50 to 1:175 produced a constant and maximum absorbance of U^{VI}- chelate. Outside this range of acidity, the absorbance decreased (Fig. 7). For different (0.5 and 1-mgL^{-1}) uranium concentrations an identical effect of varying the reagent concentration was noticed. For all subsequent measurements, 1-mL of 3.9×10^{-3} MHNABH reagent was added.

Calibration graph (Beer's law and sensitivity): The well-known equation for a spectrophotometric analysis in a very dilute solution was derived from Beer's law.

Table 4. Determination of uranium in some certified reference materials

Sample No.	Certified Reference Materials (Composition)	Uranium		
		Added/ certified value[a]	Found (n=5)	RSD%
1	GSBH-40101-96,Cr_{12},Mo,V:Dies steel(Cr,Ni,Sn,Cu,Mo,U spike)	1.0[b]	1.05	1.5
2	BAS-10g: high tensile brass(Cu,Pb,Ni,Sn,Al,Zn,MnandU spike)	1.0[b]	1.02	2.0
3	CRM-111: Uranium-233 spike, nitrate solution(99.9%pure)	5.0[c]	4.98	2.5
4	CRM-113-B: Uranium hexafluoride(UF_6,97.5%pure)	4.5[c]	4.49	1.8
5	CRM-125-A: UO_2(Pllet assay and isotropic standard U	5.4[c]	5.48	2.3
6	NBL-CRM- Uranium for water	$^{10.0d}$	9.98	2.5
7	CRM-NRCC-MESS-4: Marine sediment	$^{3.4\pm0.4e}$	3.38±0.5	2.6

[a]These CRMs were from NBL sales@science.doc.gov , [b]Values in mgL^{-1} , [c]values in % , [d]Values in μgL^{-1} , [e]Values in mgKg

Table 6. Determination of Uranium in some human fluids hair and nail samples

Sample No	Sample Source[a]	Sample	Uranium / μgL^{-1}			
			ICP-OES (n = 5)		Proposed Method (n = 5)	
			Found	RSD [b] (%)	Found	RSD [b](%)
1	Normal adult(Male)	Blood	10.0	1.5	10.5	1.5
	(Nonsmoker)	Urine	4.0	0.5	4.8	0.6
	Leukemia patient	Blood	75.8	1.8	76.8	1.8
2	(Male)	Urine	24.5	1.5	25.5	1.6
	Liver cirrhosis patient	Blood	250.0	2.0	252.0	2.5
3	(Female)	Urine	83.5	1.6	84.8	1.8
	Asthma patient	Blood	310.0	2.5	312.5	2.5
4	(male)	Urine	78.5	1.5	79.5	1.6
	Hypertension patient	Blood	370.0	2.5	375.5	2.6
5	(Female)	Urine	93.0	1.6	95.5	1.8
6	Female	Human hair	$^{20.0c}$	1.5	20.8[c]	1.5
7	Female	Human nail	$^{25.0c}$	1.8	25.8	1.8
8	Lacting mother	Human milk	$^{260.0}$	2.0	265.0	2.1

[a]Samples were collected from Chittagong Medical College Hospitals [b]The measure of precision is the relative standard deviation (RSD). [c]Values in $mgkg^{-1}$.

Table7. Determination of uranium in some soil samples

Serial No.	Sample	Uranium/ μgkg^{-1} Found[a]± S (n=5)			
		AAA (n=5)[a]		Proposed Method (n=5)[a]	
		Found[a]	RSD[b] (%)	Found	RSD[b] (%)
1	*Eastern refinery soil (Chattagram)*	16.0	1.6	15.5	1.5
2	Glass Industrial soil(PHP glass)	10.5	1.5	10.0	1.0
3	Steel Industrial soil (Bangladesh Steel Re-rolling Mills Ltd., Chittagong, Bangladesh)	14.0	1.8	12.5	1.6
4	Paint Industry soil (Berger paint)	11.5	1.5	10.8	1.5
5	Ship breaking Industry soil	14.5	2.0	12.8	1.8
6	Industrial soil (Eastern Cables Ltd)	12.5	2.1	11.5	2.0
7	*Agricultural soil (Chittagong University Campus)*	2.7	1.0	2.5	1.2
8	Marine soil (sediments) (Bay of Bengal)	6.0	1.8	5.8	1.5
9	Road side soil (Chittagong to Dhaka)	11.5	2.0	10.8	1.8
10	Karnafully River soil sediment	8.5	1.8	7.5	1.6

[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 et al.

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

Serial No.	Sample	Uranium/ μgkg^{-1} Found[a]± S (n=5)				Sample Source
		ICP-OES (n=5)[a]		Proposed Method (n=5)[a]		
		Found	RSD[b] (%)	Found	RSD[b] (%)	
1	Radish *(Raphanussativas*	258.0	2.0	260.5	2.1	Local Market, Chittagong
2	Rice *(Oryza sativa)*	255.0	2.2	258.0	2.5	Local Market, Chittagong
3	Wheat *(Trictiumaestivum)*	265.0	2.5	263.0	2.8	Local Market, Chittagong
4	Corn *(Zea mays)*	310.0	2.5	312.0	2.6	Local Market , Rajshahi
5	Cabbage *(Brassica oleracca)*	101.5	1.5	102.5	1.6	Local Market, Chittagong
6	Arum*(Arum discorides)*	320.0	1.8	321.5	1.8	Local Market, Chittagong
7	*Carrot (Daucuscarotaativas)*	338.0	2.0	340.0	2.5	Local Market, Chittagong
8	Potato (*Solanumtuberosum)*	239.9	2.2	341.0	2.8	Local Market, Chittagong
9	Spinach *(Spinaciaoleracea)*	497.0	2.5	498.5	3.0	Local Market, Chittagong
10	mango (*Manaiferaindica*)	370.0	2.8	372.0	2.6	Local Market, Chittagong

[a]Average of five replicate 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.

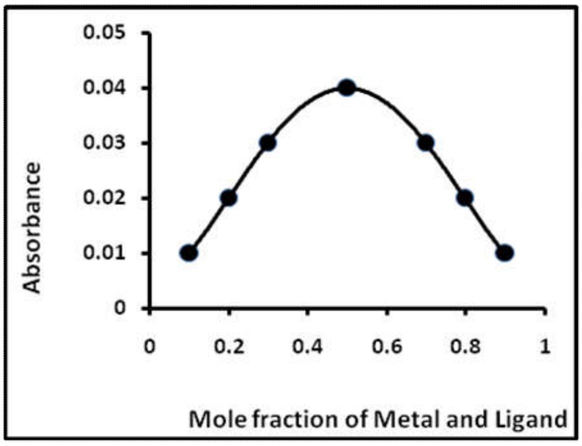
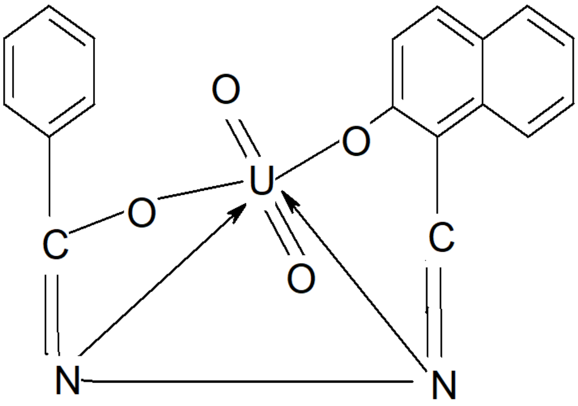


Fig. 12. Job's method for determining the composition of U^{VI}: HNABH (1 : 1) complex



Scheme 2: Probable Structure of [U (HNABH)](1:1) Complex.

The effect of the metal concentration was studied over 0.01-100 mg L^{-1} distributed in four different sets (0.01 -0.1, 0.1-1.0, 1.0-10 and 10.0-100.0-mgL^{-1}) for convenience of the measurement. The absorbance was linear for 0.01-60-mg L^{-1} at 406 nm for representing three graphs (0.01-0.1, 0.1-1.0, 1-10 and 10-100-mg L^{-1}) as shown in Fig. 8, Fig. 9,Fig. 10 and Fig. 11, respectively. Of the four calibration graphs, one showing the limit of the linearity is given in Fig. 11.The next three (Fig. 8, 10, 11) were straight-line graphs passing through the origin (R^2=0.9998). The molar absorption co-efficient and the Sandell's sensitivity (Sandell, 1965) were found to be 2.87×10^5 L mol^{-1} cm^{-1} and 8-ng cm^{-2} of uranium, respectively. The selected analytical parameters obtained with the optimization experiments are summarized in Table 1.

Effect of foreign ions: The effect of over 50 anions, cations and complexing agents on the determination of only 1-mg L^{-1} of uranium was studied. The criterion for an interference (Ojeda *et al.,* 1987) was an absorbance value varying by more than 5% from the expected value for uranium 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 uranium. The interference were from Ni, Al, Fe(III) and Cr(VI) ions. Interference from these ions is probably due to complex formation with HNABH. The greater tolerance limits for these ions can be achieved by using several masking methods. In order to eliminate interference of Al, Fe(II) and Ni; EDTA, tartrate and dimethyl glyoxime used as masking agent, respectively.

During the interference studies, if a precipitate was formed, it was removed by centrifugation. The amount mentioned is not the tolerance limit but the actual amount studied. However, for those ions whose tolerance limit has 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 U^{VI} - HNABH (1:1) complex was indicated by this method. The molar- ratio method (Yoe *et al.,* 1994) was also applied to ascertain the stoichiometric composition of the complex. A U^{VI} – HNABH complex was indicated by both methods and the stoichiometry was also found to be 1:1 (Metal: Ligand). 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 Fig. 12 and the stoichiometry was found to be1:1 (Metal: Ligand).

Precision and accuracy: The precision of the present method was evaluated by determining different concentrations of uranium (each analyzed at least five times). The relative standard deviation (n = 5) was 0-2.0 % for 0.1-500-µg of uranium in10-mL, indicating that this method is highly precise and reproducible. The detection limit (3s of the blank) and Sandell's sensitivity (concentration for 0.001 absorbance unit) for uranium were found to be 1.0-µg L^{-1} and 12-ng cm^{-2}, respectively. The method was also tested by analyzing several synthetic mixtures containing uranium and diverse ions (Table 3).The results for total uranium were in good agreement with certified values (Table 4). The reliability of our U^{VI}-chelate procedure was tested by recovery studies. The average percentage recovery obtained for addition of uranium 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 a ICP-OES (Table 6). Hence, the precision and accuracy of the method were excellent. With suitable masking, the reaction can be made highly selective.

Applications: The proposed method was successfully applied to the determination of uranium 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 uranium in a number of environmental, biological, food, water and soil samples. In view of the unknown composition of environmental water samples, the same equivalent portions of each such sample were analyzed for uranium content; the recoveries in both the "spiked" (added to the samples before the mineralization or dissolution) and the "unspiked" samples are in good agreement (Table 5). The results of biological analyses by spectrophotometric method were found to be in excellent agreement with those obtained by ICP-OES(Table 6). The results of soil samples analyzed by the spectrophotometric method are shown in Table7.The results of water samples by the spectrophotometric method are shown in Table 8.The results of some vegetable and food samples by the spectrophotometric method are shown in Table 9.The results of soil samples by the spectrophotometric method are shown in Table 10. The precision and accuracy of the method were excellent.

Determination of Uranium in some Synthetic Mixtures: Several synthetic mixtures of varying compositions containing uranium and diverse ions of known concentrations were determined by the present method using tartrate or EDTA as masking agent and the results were found to be highly reproducible. The results are shown in **Table 3**. Accurate recoveries were achieved in all solutions.

Determination of Uranium in some Certified Reference Materials

Standard addition to some CRMs and Certified Reference Materials: alloys and some CRMs were analyzed to evaluate the validation of the method. A 0.1-g amount of an alloy or steel or brass containing 1– 5% of uranium was accurately weighed and placed in a 50-mL Erlenmeyer flask following a method recommended by Parker (Sandell, 1965, Parker, 1983.). To it, 10-mL of concentrated HNO_3 and 2-mL of concentrated H_2SO_4 were carefully added. The solution was heated and simmered gently after the addition of another 10-mL of concentrated HNO_3 until all carbides were decomposed. The solution was carefully evaporated in presence of excess oxidizing agent to dense white fumes to drive off the oxides of nitrogen and then cooled to room temperature $(25\pm5)^0$C. After suitable dilution with de ionized water, the contents of the Erlenmeyer flask were warmed to dissolve the soluble salts. The solution was then cooled and neutralized with a dilute NH_4OH solution in the presence of 1-2-mL of 0.01 % (w/v) tartrate solution. The resulting solution filtered, if necessary, through Whatman no. 40 filter paper into a 100-mL calibrated flask. The residue (silica and tungstic 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 uranium content was determined; as described under general procedure using EDTA or tartrate as masking agent. The proposed procedure for spectrophotometric determination of uranium was applied to the analysis of marine sediment (CRM-NRCC-MESS-4), Water (NBL-CRM), standard uranium (CRM-125A) and standard uranium (CRM-113B), CRMs obtained from the NBL sales@science.dog.gov.us of US using tartrate or EDTA as a masking agent, following a method recommended by (Sun *et al.,* 1999). Based on five replicate analyses, the average uranium concentrations determined by spectrophotometric method were found to be in good agreement with the certified values. The results are shown in Table 4.

Determination of Uranium in Some Environmental Water Samples: Each filtered (with Whatman No. 40) environmental water sample (1000-mL) was evaporated nearly to dryness with a mixture of 2-mL concentrated H_2SO_4 and 10-mL of concentrated HNO_3 in a fume cupboard, following a method recommended by (Greenberg *et al.,* 1999) and was cooled to room temperature. The residue was heated with 10-mL of de ionized water in order to dissolves the salts. The solution was then cooled and neutralized with dilute NH_4OH solution in the presence of a 1–2-mL of 0.01 % (w/v) tartrate or EDTA solution. The resulting solution was then filtered (if necessary) and quantitatively transferred into a 25-mL calibrated flask and made up to the mark with deionized water. An aliquot (1-2-mL) of this pre-concentrated water sample was pipetted into a 10-mL calibrated flask and the zinc content was determined as described under the Procedure, using tartrate or EDTA as a masking agent. The analyses of environmental water samples for uranium from various sources are shown in Table-5. Most spectrophotometric methods for the determination of uranium in natural and sea-water require pre-concentration of uranium (WHO, 1992). The concentration of uranium in natural and sea-water is a few μgL^{-1} in developing countries like India (Khayatian *et al.,* 2013). The mean concentration of uranium found in US drinking waters is 1-mgL^{-1} (WHO, 1992). The biological samples were digested accordingly following a particular method (Ahmed *et al.,* 2010) 20.0-mL of each sample(urine) or 5-mL of blood sample was initially dried in an oven at 120 °C for 24 h. Blood serum samples were further dried in an oven at 200 °C for an additional 24 h. Then, the biological samples were dry-ashed in a muffle furnace at 300 °C for 24 h, then at 450 °C for 4 h. After dry-ashing, samples were wet-ashed with 2-mL concentrated nitric acid and 0.5-mL of 30% hydrogen peroxide. The mixture was heated to just below boiling until complete evaporation. The samples were cooled and wet-ashed three more times in the same manner. At completion, the white residue was dissolved with 10-mL of 1 M HNO_3 by heating and diluted to 20.0-mL for analysis. After neutralizing pH by addition of dilute NH_4OH in the presence of 1-2-mL of a 0.01 % (w/v) tartrate or EDTA solution. The resultant solution was then filtered and transferred quantitatively into a 25-mL calibrated flask and made up to the mark with deionized water. A suitable aliquot (1-2-mL) of the final solution was pipetted into a 10-mL calibrated flask and the uranium content was determined as described under the procedure using tartrate or EDTA 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. The results are shown in Table 6. The abnormally high value for the liver cirrhosis patient is probably due to the involvement of high uranium concentrations with Al and Ni. Occurrence of such high uranium contents are also reported in liver cirrhosis patient from some developed countries (Jaiswal, 1994).

Determination of uranium in some surface soil samples

An air dried homogenized soil sample (100-g) was weighed accurately and placed in a 100-mL micro-Kjeldahl flask. The sample was digested in presence of excess oxidizing agent following the method recommended by Hesse (Hesse, 1972; Jackson, 1965). The solution was then neutralized with dilute NH_4OH in the presence of a 1-2mL of 0.01% (w/v) EDTA or tartrate solution. The resulting solution was then filtrated and quantitatively transferred to a 25-mL calibrated flask and made up to the mark with de ionized water. Suitable aliquots (1-2-mL) were transferred into a 10-mL calibrated flask and a calculated amount of 0.0005 M H_2SO_4 needed to give a final acidity of 0.00025-0.00075 M H_2SO_4 was added followed by 1-mL of 0.01% (w/v) tartrate or EDTA solution as masking agent. The uranium content was then determined by the above procedure and quantified from a calibration graph prepared concurrently. The results of soil analysis by the spectrophotometric method were found to be in excellent agreement with those obtained by AAS. The results are shown in Table 7. The average value of uranium in Chittagong region surface soil was found to be 10.50 mg kg^{-1}.

Determination of uranium in some vegetable and food samples: The vegetable and food samples collected prior to the determination were pretreated in the following way. Edible portion of samples was first washed clean with tap water followed by rewashing with de-ionized water. After removing de-ionized water from the surface of vegetables and fruits, the samples were cut into small pieces and dried at 65° C in oven. An air dried vegetables and fruits samples (10gm) were taken in a 100-mL micro-Kjeldahl flask in presence of oxidizing agent and digested following a method recommended by Stahr (Stahr, 1991). A glass bead and 10-mL of concentrated nitric acid were added and the flask was placed on the digester under gentle heating. When the initial brisk reaction was over, the solution was removed and cooled at room temperature. 1-mL volume of concentrated sulfuric acid was added carefully, followed by the addition of 2-mL of concentrated HF, and heating was continued for at least 0.5 h and then cooled. The solution of flask then neutralized with dilute NH_4OH in the presence of 1-2-mL of a 0.01 % (w/v) tartrate or EDTA solution. The resultant solution was then filtered and transferred quantitatively into a 25-mL calibrated flask and made up to the mark with de-ionized water. A suitable aliquot (1-2-mL) of the final solution was pipetted into a 10-mL calibrated flask and the uranium content was determined as described under the procedure using EDTA 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. The results are shown in Table 8.

Conclusions

In this paper, a new, simple, sensitive, highly selective and inexpensive method with the U^{VI}-HNABH complex was developed for the determination of uranium in some real, environmental, biological, soil, food, vegetable and water samples for continuous monitoring to establish the trace levels of uranium in different sample matrices.

Although many sophisticated techniques such as pulse polarography, HPLC, AAS, ICP-OES and ICP-MS, etc. are available for the determination of uranium at trace levels in numerous complex materials, factors such as the low cost of the uranium instrument, easy handling, lack of requirement for consumables and almost no maintenance have caused spectrophotometry to remain a popular technique, particularly in laboratories of developing countries with limited budget. The sensitivity in terms of molar absorptivity and precision in terms of relative standard deviation of the present method are very reliable for the determination of uranium in real samples down to ng g^{-1} levels in aqueous medium at room temperature (25 ± 5)°C.

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