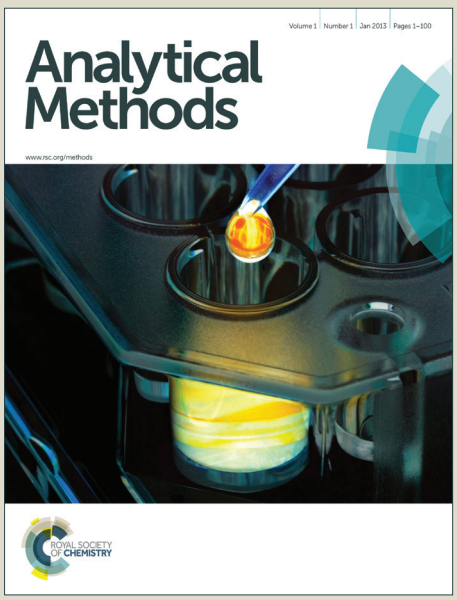
Analytical Methods

Accepted Manuscript



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Highly Selective and Sensitive Spectrophotometric Method for the Determination of Selenium Using 2-Hydroxy- 1-Napthaldehyde-orthoaminophenol

M. Jamaluddin Ahmed*, M. Tazul Islam and Most. Jannatul Nime

A very simple, ultra-sensitive and highly selective non-extractive spectrophotometric method for the determination of trace amounts of selenium using 2-hydroxy-1- napthaldehyde-orthoaminophenol (HNA-OAP) has been developed. HNA-OAP reacts in a slightly acidic (7×10^{-6} - 3.5×10^{-5} M H_2SO_4) aquatic media with selenium (IV) in 40% N, N-dimethylformamide (DMF) to produce highly absorbent an orange red chelate, which has an absorption maximum at 509 nm. The absorbance intensity of the metal-chelate reaches at constant value (after heating of 5 min at 45±5°C) within 15 min and remains stable for over 24 h. The average molar absorption co-efficient and Sandell's sensitivity were found to be 6.30×10^5 L mol^{-1} cm^{-1} and 10 ng cm^{-2} of Se, respectively. Linear calibration graphs were obtained for 0.02 - 7.0 mg L^{-1} of Se, having detection limit of 1.0 μg L^{-1} and quantification limit of the reaction system were found to be 10 μg L^{-1} and RSD 0-2%. The stoichiometric composition of the chelate is 2 : 3 (Se : HNA-OAP). A large excess of over 60 cations, anions and complexting agents do not interfere in the determination. The method was applied successfully in the determination of selenium in several standard reference materials (alloys, steels sediments, tea and bovine liver) as well as in some environmental waters (portable and polluted), biological samples (human blood, urine and hair), soil samples, food samples and pharmaceutical samples, solution containing both selenium(IV) and selenium(VI) and complex synthetic mixtures. The method has high precision and accuracy (s = ± 0.01 for 0.5 mg L^{-1}).

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Introduction

As the toxicological and physiological importance of selenium has become more and more evident[1], during the past forty years, there has been an increasing interest in this essential element. Current interest in the element stems mainly from following observations. First, selenium has been reported to inhibit carcinogenesis by chemical agents such as aminoazo compounds, polycyclic aromatic hydrocarbons, nitrosamines, etc[2]. Selenium compounds may be mutagenic in bacterial systems and there is substantial evidence that they are able to cause DNA damage and chromosomal aberrations in human and animal cells in *vitro*. Secondly, selenium may play an important role in the prevention of cardiovascular disease and myocardial infarction. Selenium is reported to be important in experimentally induced heart diseases in animals[3], inverse epidemiological relationships have been observed among human heart disease and environmental or blood selenium[4]. Deficiency of selenium results in selenium-responsive diseases in various animal species including humans[5]. A final effect of selenium in different organisms is its reported antagonistic action towards various toxic metals[6]. A narrow range of selenium intake that is consistent with health, outside this range there is risk of survival due to either deficiency or toxicity effects for mammals and non-mammals. The range between the essential and toxic concentration levels is 100 fold differences. According to Scott[6a] the nutritional requirement for selenium lies between 0.1 and 0.3 mg kg^{-1}, whereas levels from 2 to 10 mg kg^{-1} may give rise to toxic symptoms. Hence, the reliable methods are needed to cheek the selenium status of man and to monitor the occupational exposure to this element by measuring its concentration in body fluids.

In expanding analytical fields such as environmental, biological and material monitoring of trace metals, there is an increasing need to develop the simple, sensitive and selective analytical techniques that do not use expensive or complicate test equipment. Many sophisticated techniques, such as neutron activation analysis (NAA)[7], X-ray fluorescence[8], pulse polarography[9], inductively coupled plasma optical emission spectrophotometry (ICP-OES)[10], catalytic kinetic spectrophotometric method[11], automated hydride generation atomic absorption spectrophotometry (AHG-AAS)[12], high-performance liquid chromatography (HPLC)[13], graphite furnace atomic absorption spectrophotometry (GF-AAS)[14] and spectrofluorimetry[15] have been widely applied to the determination of selenium. The first four methods[7-10] are disadvantageous in terms of cost and instruments used in routine analysis. GF-AAS[14] is often lacking in

sensitivity due to formation sublime at high temperature. Automated hydride generation-AAS[12] is sensitive but often lacking in sensitivity and affected by matrix conditions of samples such as salinity. Catalytic spectrophotometric[11] and spectrofluorimetric[15] methods are sensitive but are generally lacking simplicity. However, the spectrophotometric method still has the advantages of being simple and without requiring expensive or complicated test equipment. For this reason, a wide variety of spectrophotometric methods[16-32] for determination of selenium has been developed. Several authors have reported on the extractive spectrophotometric determination of selenium(IV) using complexes formed variety of reagents[16-32]. Most of this methods[16-32] is solvent extractive, expensive, toxic and non-recoverable. A composition of few selected methods[16-32], their spectral characteristics and draw backs are summarized for comparison. Most of the organic solvents which were used for those extractions were found to be carcinogenic[6].

The aim of this study was to develop a simpler direct spectrophotometric method for the trace determination of selenium. In the search for a more sensitive reagent, in this work a new Schiff's base reagent 2-hydroxy-1-napthaldehyde-orthoaminophenol (HNA-OAP) was synthesized according to the method of Sacconi[33] and a color reaction of HNA-OAP with Se(IV). HNA-OAP has not previously been used for the spectrophotometric determination of any metal. This paper reports first time on its use in a very sensitive, highly specific spectrophotometric method for the trace determination of selenium. The method possesses distinct advantages over existing methods[16-32] with respect to sensitivity, selectivity, range of determination, simplicity, toxicity, speed, pH / acidity range, thermal stability, accuracy, precision and ease of operation. The method is based on the reaction of nearly non-absorbent HNA-OAP in a slightly acidic solution (7×10^{-6} - 3.5×10^{-5} M H_2SO_4) with Se(IV) in 40% DMF medium to produce a highly absorbent orange red colored 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.

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 hydride - generation coupled to a flow injection analysis system (FIAS - 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}.

Synthesis and characterization of the Reagent

Synthesis of the reagent

The reagent was synthesized in the laboratory according to the method recommended by Sacconi[33] and Salam *et al* [34]. The reagent 2-hydroxy-1-napthaldehyde-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 re-crystallized from ethanol and dried in a desiccator over calcium chloride. Yield of the product was 80%. The structure of the reagent is shown in **Scheme 1.** The reagent 2-hydroxy-1-napthaldehyde-orthoaminophenol (HNA-OAP) is a Schiff-base reagent which is less-toxic than existing spectrophotometric reagents[16-32] for determination of selenium. It has no hazardous effect on living organism including human.

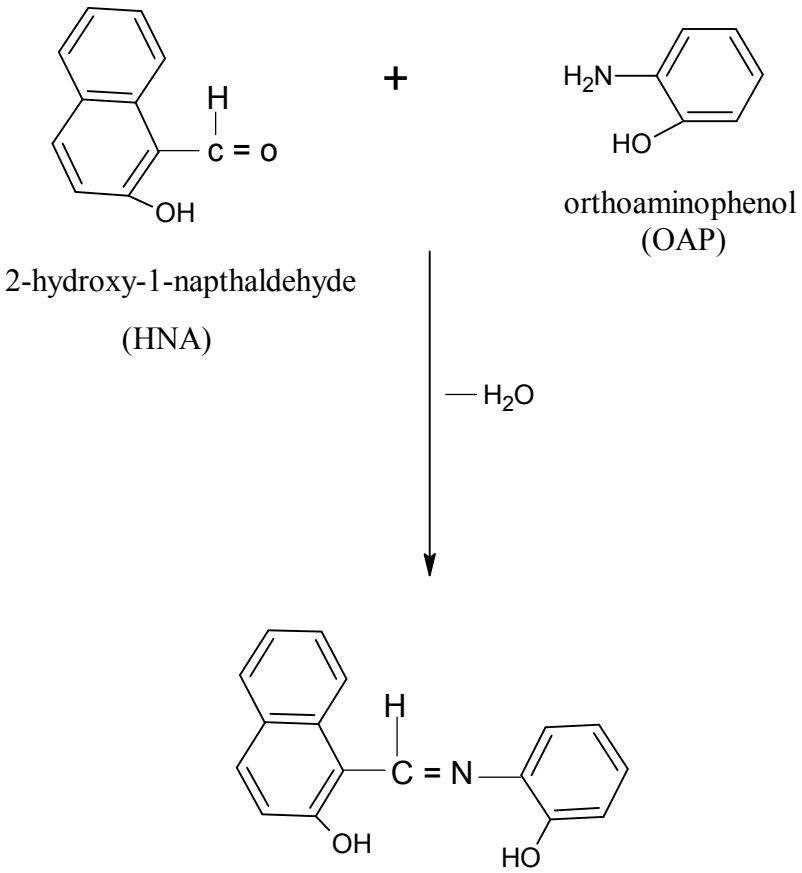
Characterization of the reagent

The reagent was characterized by taking melting point, elemental analysis, and FTIR spectrum and thermogravimetric analysis. The melting point of the reagent was 155-157ºC. (Lit.154 - 156ºC) [34]. The results 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 %) [33]. The FTIR spectrum of prepared reagent (HNA-OAP) is shown in Fig.1. The presence of FTIR peak at 1631.78 cm^{-1} in Fig.1 was due to the characteristic C=N double bond peak ($\nu^{C=N}$, 1583-1618 cm^{-1}) [33] of the Schiff's base reagent indicating the formation of HNA-OAP. The steadiness of the thermogravimetric curve obtained for about 1g of the reagent at 80-90^{o}C indicated that the reagent did not contain any moisture.

The elemental analysis were performed by the National Center of Excellence in Analytical Chemistry, University of Sindh, Pakistan and FTIR spectra was recorded with FTIR spectrophotometer, Shimadzu (Model-IR Prestige 21, Detector-DTGS KBr) in the range 7500-350 cm^{-1} from our laboratory.

Synthesis of the reagent



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

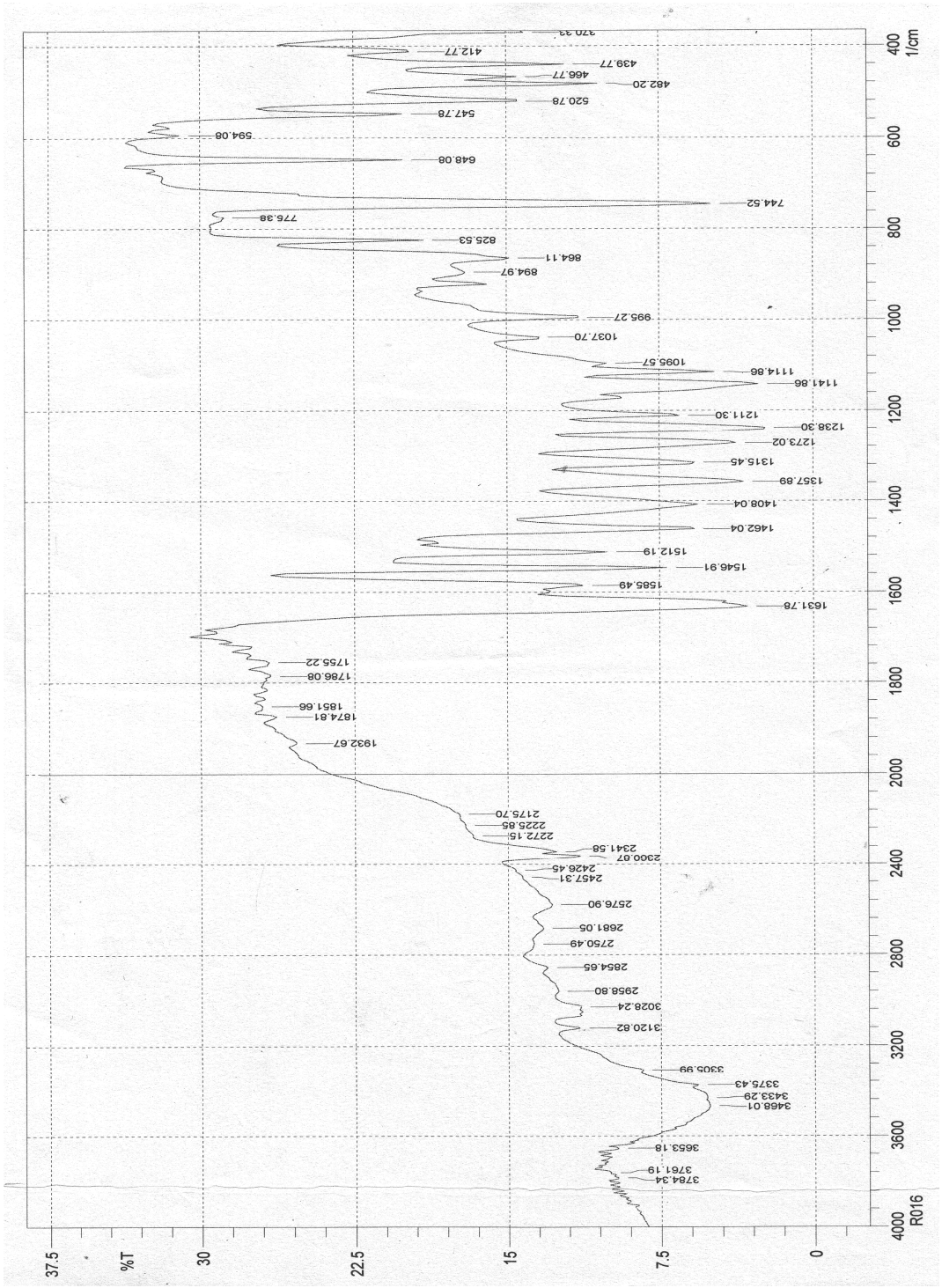


Fig. 1 FTIR spectrum of 2-hydroxy-1-napthaldehyde-orthoaminophenol (HNA-OAP).

Live Subject Statement

We were not aiming to carry out detailed human studies but some samples from individuals were used in our study and as such we abided by all the necessary procedures and regulations and that our University gave consent. University of Chittagong is committed to the protection and safety of human subjects involved in research.

Reagents and solutions

All the chemicals used were of analytical reagent grade of the highest purity available. High-purity DMF (N, N-dimethylformamide) and high-purity de-ionized water, which are non-absorbent under UV radiation, were used throughout. High-purity water was obtained by passing tap water through cellulose absorbent and to mixed-bed ion exchange columns, followed by distillation in a corning AG-11 unit. The Al level in the high-purity water was found to be below the spectrophotometric detection limit (3s of the blank) of 1 $\mu g\ L^{-1}$. Glass vessel were cleaned by soaking in acidified solutions of $KMnO_4$ or $K_2Cr_2O_7$ followed by washing with concentrated HNO_3 and rinsed several times with high purity de-ionized water. Stock solutions and environmental water sample (1000 mL each) were kept in polypropylene bottles containing 1mL concentrated HNO_3. More rigorous contamination control was used when the selenium levels in the specimens were low.

The reagent HNA-OAP stock solution (3.8×10^{-3}M) was prepared by dissolving the requisite amount of HNA-OAP, in a known volume of N, N-dimethylformamide (DMF). More dilute solutions of the reagent were prepared as required. A 100 mL amount of Selenium(IV) stock solution (1.27×10^{-2} M) of tetravalent selenium was prepared by dissolving 219.033 mg of purified-grade (E Merck proanalysis grade) sodium selenite (Na_2SeO_3) (super special Grade J. T. Baker) in doubly distilled de-ionized water and it was subsequently standardized iodometrically with standard sodium thiosulfate[35]. More dilute standard solutions were prepared by appropriate dilution of aliquots from the stock solution with de-ionized water as and when required. A 100-mL amount of Selenium(VI) stock solution (1 $mg\ mL^{-1}$) of hexavalent selenium was prepared by dissolving 239.36 mg of sodium selenate (Na_2SeO_4) (Aldrich A.C.S. grade) in doubly distilled de-ionized water. Aliquots of this solution were standardized with iodometrically[35]. More dilute

standard solutions were prepared by appropriate dilution of aliquots from the stock solution with de-ionized water as and when required. A 100 mL amount of potassium dichromate solution stock solution (0.1 N) was prepared by dissolving 500 mg of finely powdered $K_2Cr_2O_7$ (Merck) in 100 mL de-ionized water. Sodium azide solution (2.5 % w/v) (Fluka purity > 99%) was freshly prepared by dissolving 2.5 gm in 100 mL of de-ionized water. 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. 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. A 100 mL stock solution of EDTA (0.01 % w/v) was prepared by dissolving 10 mg A.C.S.-grade (≥ 99%) ethylenediaminetetraacetic acid as disodium salt dihydrate in (100 mL) de-ionized water. 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[36]. In the case of insoluble substances, special dissolution methods were adopted[37].

Procedure

To 0.1-1.0 mL of a neutral aqueous solution containing 0.2-70 μg of selenium(IV) in a 10 mL calibrated flask was mixed with a 1:100-1:600 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 0.7-3.5 mL (preferably 1 mL) of 1×10^{-5} M of sulfuric acid. Then, 4-mL of N, N-dimethylformamide (DMF) was added and the mixture was diluted to the mark with de-ionized water. After heated for 5 min at 45±5°C then cooled at room temperature (25±5°C) for 15 min and the absorbance was measured at 509 nm against a corresponding reagent blank. The selenium content in an unknown sample was determined using a concurrently prepared calibration graph.

Results and Discussion

Factors Affecting the Absorbance

Absorption spectra. The absorption spectra of the Se(IV)-HNA-OAP in 1×10^{-5} M sulfuric acid medium was recorded using a spectrophotometer. The absorption spectra of the Se(IV)-HNA-OAP is a symmetric curve with maximum absorbance at 509 nm; an average molar absorption coefficient of 6.3×10^5 L mol^{-1} cm^{-1} is shown in Fig. 2. HNA-OAP did not show any absorbance. In all instances, measurements were made at 509 nm against a reagent blank. The reaction mechanism of the present method is as reported earlier[38].

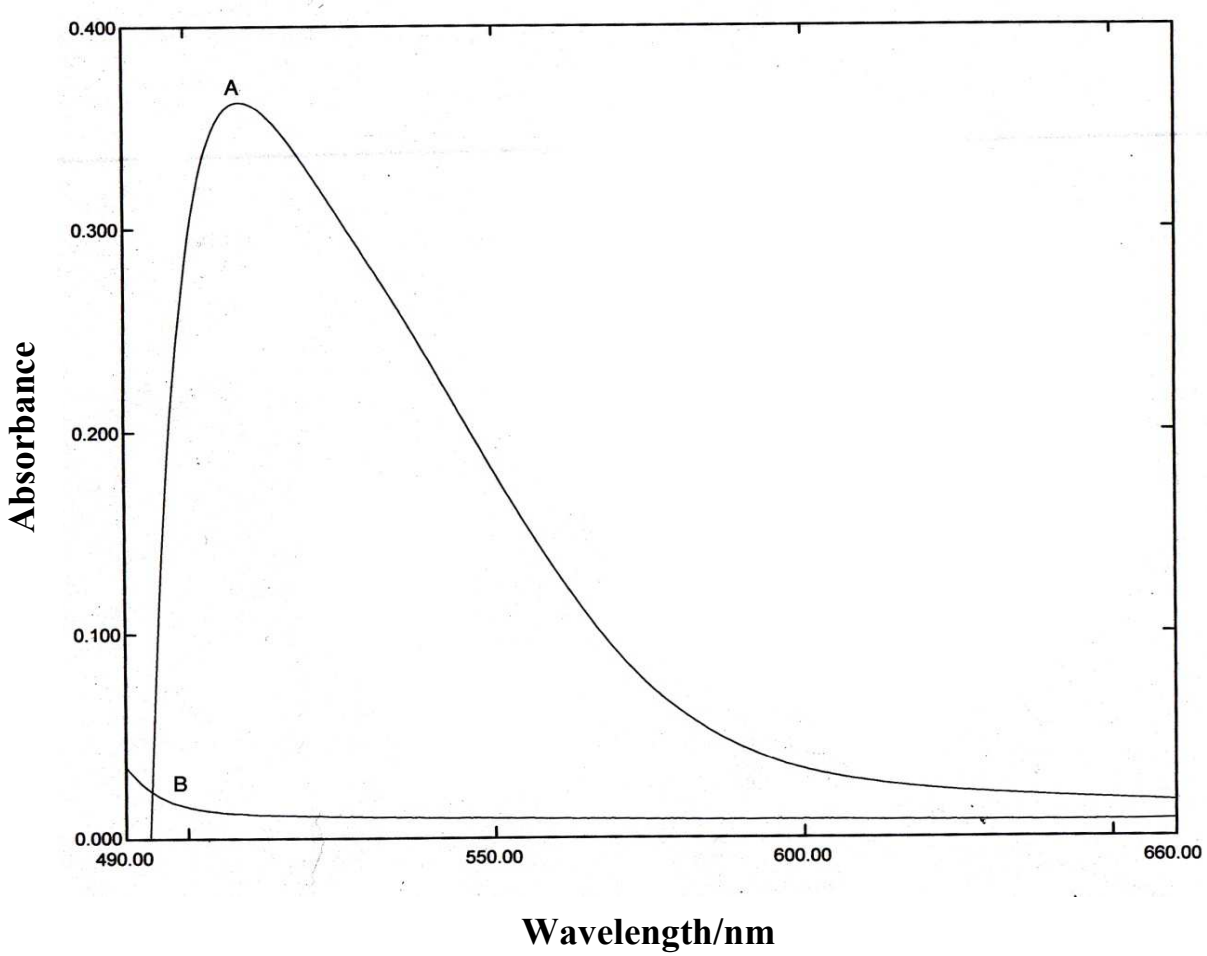


Fig. 2 A and B absorption spectra of Se(IV)-HNA-OAP system and the reagent blank (λ_{max}= 509 nm) in aqueous solutions, respectively.

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 40±2% v/v DMF medium, however maximum absorbance was observed; hence a 40% (4-mL) DMF solution was used in the determination procedure. It was observed that at 1 mg L^{-1} of Se(IV)-HNA-OAP, 40-70% (4-7 mL) of DMF solution produced a constant absorbance of the Se-Chelate (Fig.3).

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. Because sulfuric acid gave maximum absorbance for the system. The short answer is, diprotic acids don't usually dissociate all the way, and for good reason. The absorbance was at a maximum and constant when the 10-mL of solution (1 mg L^{-1}) contained 0.7-3.5 mL of 1×10^{-5} M sulfuric acid(7×10^{-6} - 3.5×10^{-5} M H_2SO_4) after heating at 45±5^{o}C then cooled at room temperature (25±5°C). Outside this range of acidity, the absorbance decreased (Fig.4). For all subsequent measurements 1 mL of 1×10^{-5} M sulfuric acid was added.

Effect of temperature. The Se(IV)-HNA-OAP system attained maximum and constant absorbance when reaction mixture was heated for 5 minutes at 45-80°C and then cooled it for 15 minutes (Fig.5) at room temperature (25 ±5°C). For all subsequent measurements the solution was heated for 5 min at 45 ±5°C and then cooled it for 15 min at room temperature (25 ±5°C).

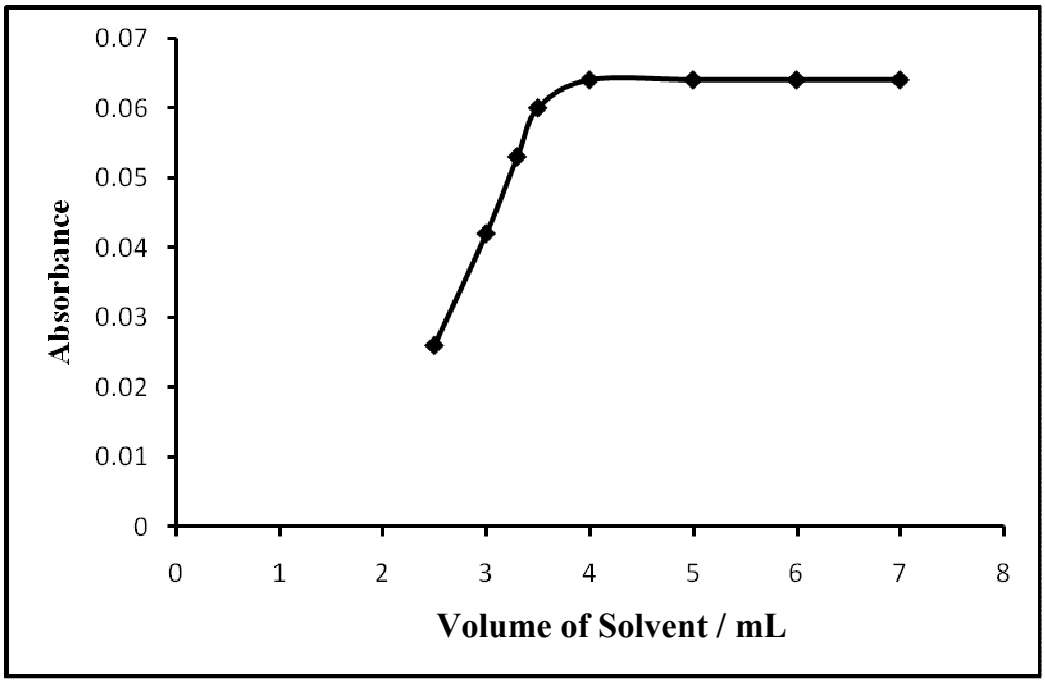


Fig. 3 Effect of solvent (DMF) on the absorbance of Se(IV)-HNA-OAP system.

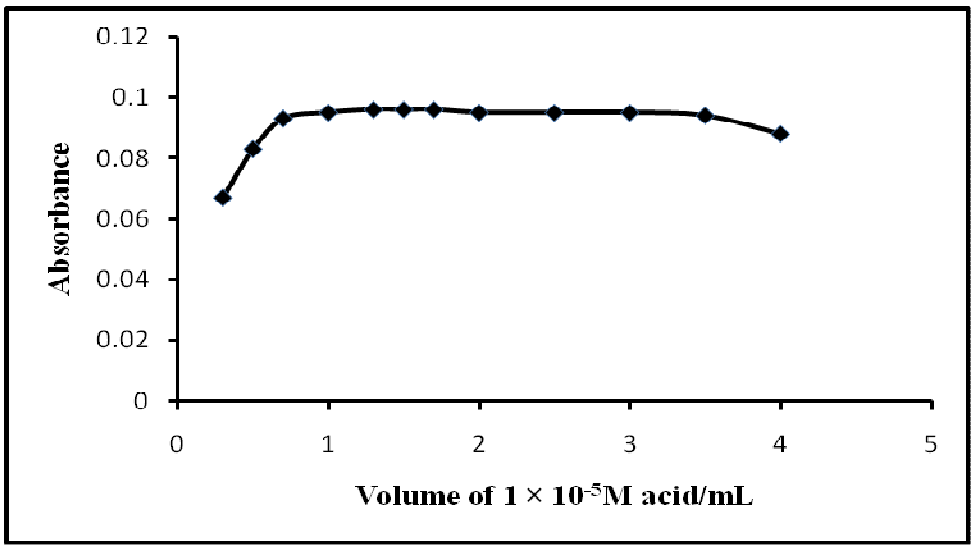


Fig. 4 Effect of acidity on the absorbance of Se(IV)-HNA-OAP system.

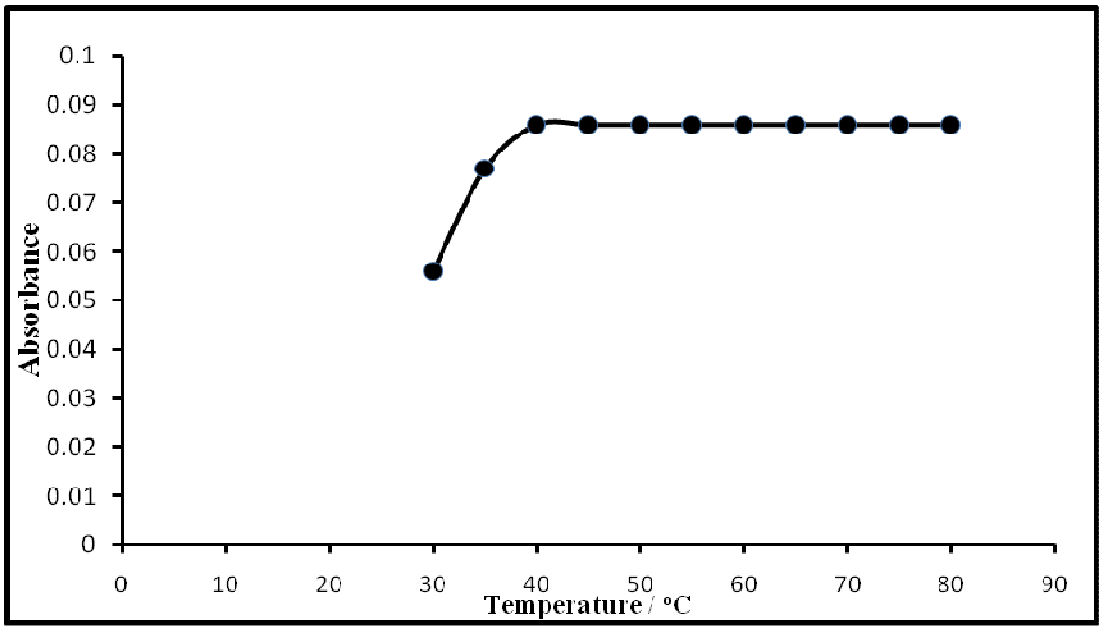


Fig. 5 Effect of temperature on the Se(IV)-HNA-OAP system.

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

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} Se(IV) metal, the reagent molar ratios of 1:100-1:600 produced a constant absorbance of the Se-chelate. Outside this range of reagent, the absorbance decreased (Fig.6). For all subsequent measurements 1 mL of 3.8×10^{-3} M HNA-OAP reagent was added.

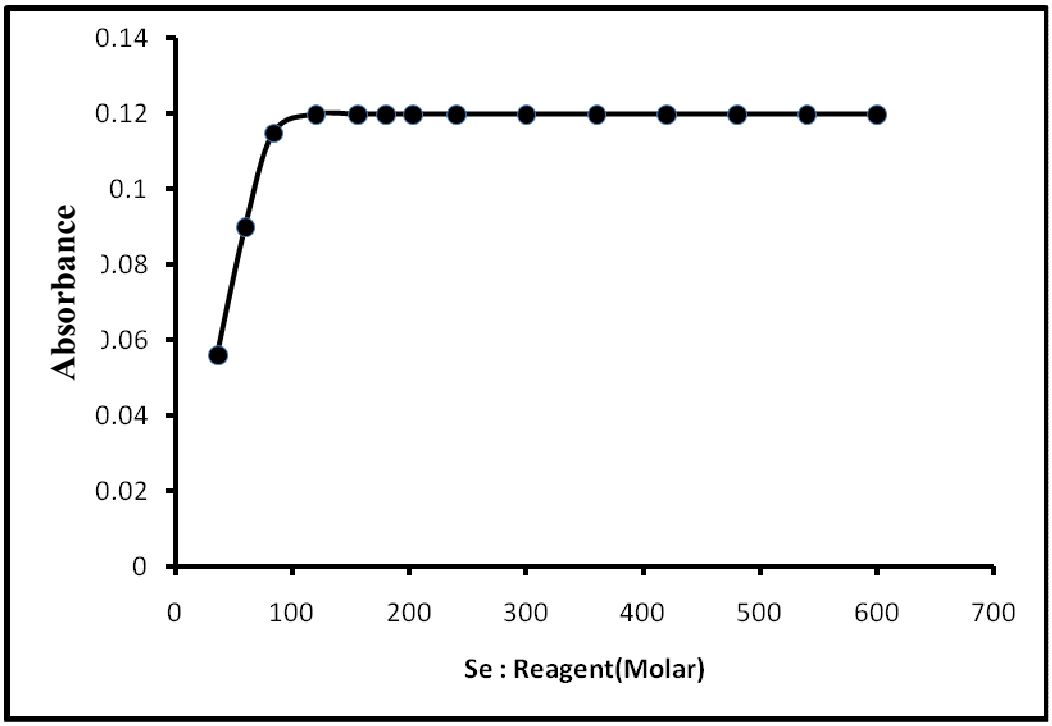


Fig. 6 Effect of reagent on the Se(IV)-HNA-OAP system.

Analytical Parameters

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 0.01-100 mg L^{-1} distributed in four different sets (0.02-0.1, 0.1-1, 1-10 and 10-100 mg L^{-1}) for convenience of measurement. The absorbance was linear for 0.02-7.0 mg L^{-1} of selenium at 509 nm representing three graphs (0.02-0.1, 0.1-1.0 and 1.0-7.0 mg L^{-1}). Of three calibration graphs, the one showing the limit of the linearity range (Fig.7); the next two were straight-line graphs passing through the origin (R^2 = 0.9997). The molar absorptivity and Sandell's sensitivity[39] were found to be 6.3×10^5 L mol^{-1} cm^{-1} and 10 ng cm^{-2} of selenium(IV), respectively. The selected analytical parameters obtained with the optimization experiments are summarized in Table 1.

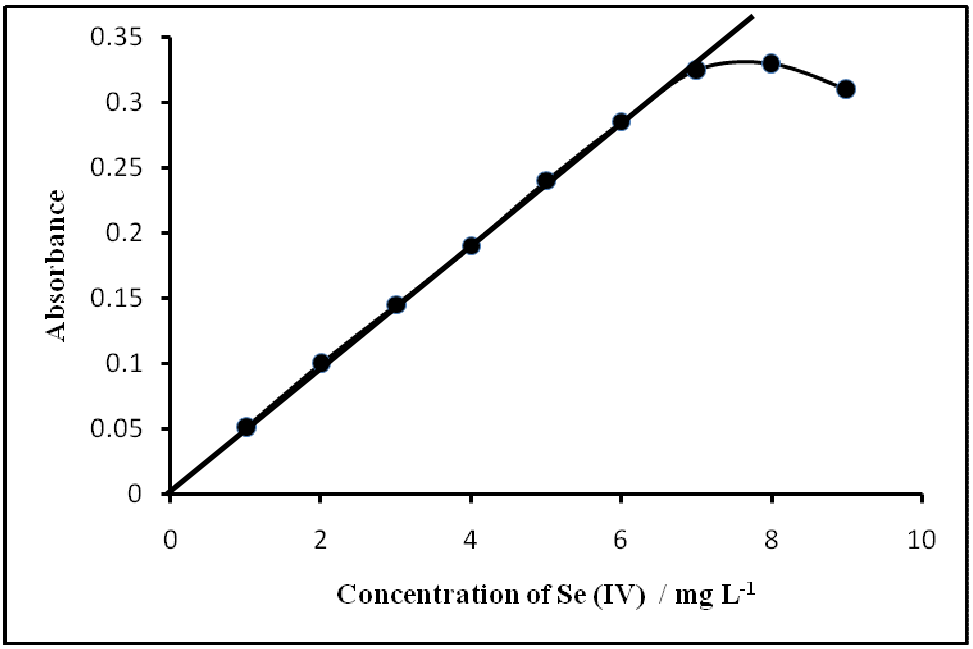


Fig.7 Calibration graph : 1-7 mg L^{-1} of selenium(IV)

Table 1 Selected analytical parameters obtained with the optimization experiments.

Parameters	Studied range	Selected value
Wavelength / λ_{max} (nm)	200 - 800	509
Solvent / % (N, N-dimenthylformamide)	10 - 80	40 - 70 (Preferably 40)
Acidity / M H_2SO_4	1×10^{-6} - 5×10^{-5}	7×10^{-6} - 3.5×10^{-5} (Preferably 1×10^{-5})
pH	6.50 - 4.50	6.01 - 4.90 (Preferably 5.50)
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)	1:1 - 1:600	1:100 - 1:600 (Preferably 1: 120)
Linear range/mg L^{-1} (Beer's Law)	0.01 - 100	0.02 - 7.0
Molar absorption coefficient / L mol^{-1} cm^{-1}	5.6×10^5 - 7.0×10^5	6.3×10^5
Sandell's sensitivity/ ng cm^{-2}	1 - 100	10.0
Detection limit/ µg L^{-1}	0.01 - 10	1.0
Reproducibility (% RSD)	0 - 10	0 - 2 %
Regression coefficient (R^2)	0.9992-0.9999	0.9997

Effect of foreign ions. More than 60 anions, cations and complexing agents were studied individually to investigate their effect on the determination of 1 mg L^{-1} of selenium(IV). The criterion for an interference[40] was an absorbance value varying by more than ± 5% from the expected value for selenium 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 selenium. The most serious interference was from Fe(III) and V(V) 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 Fe(III) and V(V) ions, EDTA and tartrate can be used as masking agents, respectively[41]. A 20-fold excess of Fe(III) and V(V) ions could be masked with EDTA and tartrate, respectively. These two ions were masked because, Fe(III) and V(V) were probably formed complexes 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 selenium(VI) had no reducing effect on selenium(IV). 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.

Table 2 Table of tolerance limits of foreign ions[a], tolerance ratio [species (x) / Se (w/w)]

Species x	Tolerance ratio x/Mo (w/w)	Species x	Tolerance ratio x/Mo (w/w)
Ammonium(I), Citrate, Chloride, Cobalt(II), Cadmium, Copper(II), Lead(II), Iron(II), Magnesium, Mercury(II), Nickel(II), Sodium, Strontium, Tin(II & IV), Tungsten(VI), Titanium(IV) and Zinc	50	Arsenic(V), Azide, Ascorbic, Bromide, Barium, Cobalt(III), EDTA, Fluoride, Iodide, Manganese(II), Nitrate, Potassium, Phosphate, Selenium(VI), Sulfate, Tellurium, Thiocyanide and Thiourea	100
Arsenic(III), Aluminium, Bismuth(III), Calcium, Carbonate, Chromium(III & VI) and Thiosulfate	20	Oxalate	200
Iron(III), Lithium	20 [b]	Silver	50[b]
Vanadium(V)	20[c]	Tartrate	300

[a]Tolerance limit was defined as ratio that causes less than 5 percent interference.
[b]with 100 mg L^{-1} tartrate.
[c] with 100 mg L^{-1} EDTA.

Composition of the absorbent complex. Job's method[42] of continuous variation method and the mole ratio method[43] and was applied to ascertain the stoichiometric composition of the complex. A Se(IV)-HNA-OAP(2 :3) complex was indicated by both methods. Experimental data has been shown graphically in Fig.8 and the stoichiometry was found to be 2: 3 (Se : HNA-OAP).

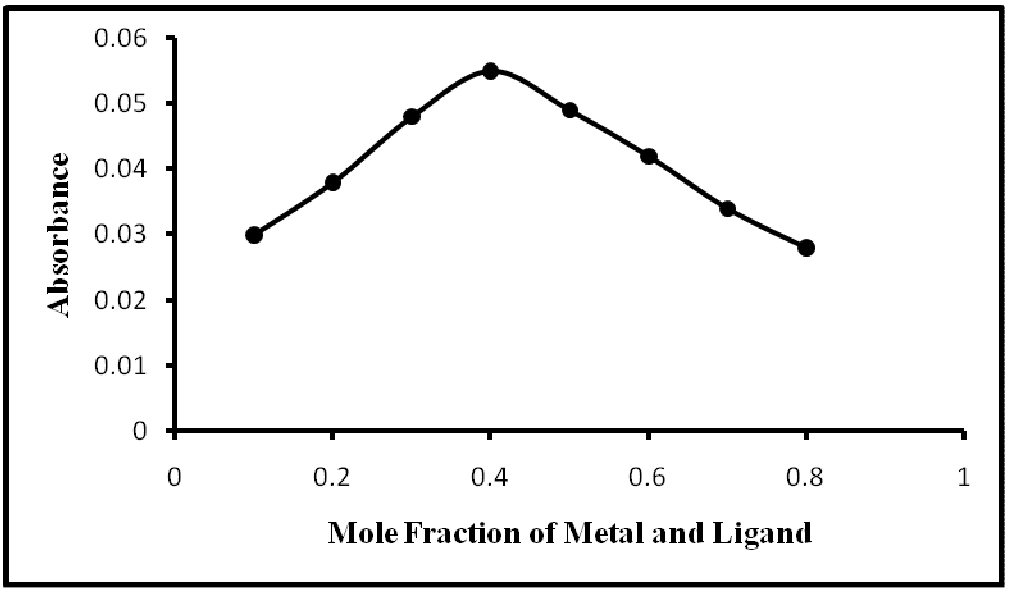


Fig. 8 Job's method for determination the composition of Se(IV): HNA-OAP (2 : 3) Complex.

Precision and accuracy. The precision of the present method was evaluated by determining different concentrations of selenium (each analyzed at least five times). The relative standard deviation (n=5) was 0-2% for 0.2-70 μg of selenium(IV) in 10 mL, indicating that this method is highly precise and reproducible (Table 1). The detection limit (3s of the blank), quantification limit (10 times of detection limit) and Sandell's sensitivity (concentration for 0.001 absorbance unit) for selenium(IV) were found to be 1 μg L^{-1}, 10 μg L^{-1} and 10 ng cm^{-2}, respectively. The method was also tested by analyzing several synthetic mixtures containing selenium(IV) and diverse ions. The results for total selenium were in excellent agreement with certified values (Table 3). The reliability of our selenium-chelate procedure was tested by recovery studies. The average percentage recovery obtained for addition of selenium(IV) spike to some environmental water samples was quantitative, as shown in (Table 4). The results of biological analyses by the spectrophotometric method were in excellent agreement with those obtained by AAS (Table 5). The results of food analyses by spectrophotometric method were also found to be in excellent agreement with those obtained by AAS (Table 8). The results of speciation of selenium(IV) and selenium(VI) in mixtures were highly reproducible. Hence, the precision and accuracy of the method were found to be excellent.

Applications

The present method was successfully applied to the determination of selenium(IV) in a series of synthetic mixtures of various compositions and also in a number of real samples e.g. several certified reference materials (CRM) (Table 3). The method was also extended to the determination of selenium in a number of environmental, biological, soil, food and pharmaceutical samples. In view of the unknown composition of environmental water samples, the same equivalent portions of each such sample were analyzed for selenium 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 4). The results of biological analyses by spectrophotometric method were found to be in excellent agreement with those obtained by AAS (Table 5). The results of soil analyses by the spectrophotometric method are shown in (Table 6). The results of pharmaceutical samples by the spectrophotometric method are shown in (Table 7). The results of food analyses by spectrophotometric method were also found to be in excellent agreement with those obtained by AAS (Table 8). The results of speciation of selenium(IV) and selenium(VI) in mixtures are incorporated in the text.

Determination of selenium in synthetic mixtures. Several synthetic mixtures of varying compositions containing selenium(IV) 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. Accurate recoveries were achieved in all solutions in the range 98±0.8 to 108±1.0%. The reliability of our selenium – chelate procedure was approved by quantitative recovery of selenium(IV) spiked to several synthetic mixture containing selenium(IV) and diverse ions. The method has high precision and accuracy ($s = \pm 0.01$ for 0.5 mg L^{-1}).

Determination of selenium in alloys and steels (certified reference materials).

A 0.1 g amount of an alloy or steel sample containing 2.04 - 43.0 (μg g^{-1}) of selenium was weighed accurately and placed in a 50 mL Erlenmeyer flask in presence of excess reducing agent to reduce Se(VI) to Se(IV), following a method recommended by Parker[44]. 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 selenium content was determined; as described under general procedure using EDTA or tartrate as masking agent. The proposed procedure for the spectrophotometric determination of selenium was applied to the analysis of estuarine sediment (CEC-CRM-277), Tea (NRC-CRM-C8-05) and Bovine liver (NBS-SRM-1577) 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*.[45] Based on five replicate analyses, average selenium concentration determined by spectrophotometric method was in excellent agreement with the certified values. The results are given in Table 3

Table 3 Determination of selenium(IV) in certified reference materials

Sample	Certified Reference Materials ($\mu g\ g^{-1}$)	Selenium /($\mu g\ g^{-1}$)		
		Certified value	Found (n=5)	RSD.[a] (%)
1	GBW01620*(Ag = 4.6, As = 11, Bi = 0.4, Ca = 21, Cd = 4.6, Ga = 32, In = 2.6, Mg = 16, Pb = 4.1, Sb = 9.5, Se = 16, Sn = 53, Te = 11, Ti = 22, Zn = 32)	16.0	15.89	1.2
2	GBW01622*(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 = 10.4, Te = 0.5, Ti = 8.3, Zn = 20)	43.0	42.95	1.0
3	GBW01637*(As = 14, Bi = 0.19, Ag = 1.0, Ga = 34, In = 7.2, Pb = 3.7, Sb = 3.3, Se = 12, Sn = 8.3, Te = 3.1, Ti = 0.16, Zn = 13)	12.0	11.86	1.5
4	CEC - CRM - 277, Estuarine Sediment	2.04	1.95	0.5
5	NRC- CRM - C85- 05, Tea	0.041	0.043	0.2
6	NBS- SRM -1577, Bovine liver.	1.1	1.17	0.6

* These CRMs obtained from Beijing NCS Analytical Instruments Co. Ltd, China.
[a]The measure of precision is the relative standard deviation (RSD).

Determination of selenium 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 freshly prepared excess sodium azide solution in a fume cupboard to reduce Se(VI) to Se(IV), following a method recommended by Greenberg *et al.*[46] 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 selenium content was determined as described under the general procedure using EDTA or tartrate as masking agent. The results of analyses of environmental water samples from various sources for selenium are shown in Table 4.

Most spectrophotometric methods for determination of selenium in natural and sea-water require preconcentration of selenium[46]. The concentration of selenium in natural and sea water is a few μg L^{-1} in developed countries[47]. The mean concentration of selenium found in U.S. drinking water is greater than 10 μg L^{-1} [47].

Table 4 Determination of selenium(IV) in some environmental water samples

Sample		Selenium /μg L^{-1}		Recovery ± s (%)	s_r [b] (%)
		Added	Found [a]		
Tap water		0	12.0		
		100	112.0	100±0.0	0.00
		500	515.0	100.6±0.5	0.21
Rain Water		0	10.0		
		100	112.0	101.8±0.8	0.29
		500	520.0	102.0±0.5	0.23
Well water		0	15.0		
		100	112.0	97.4±0.5	0.31
		500	525.0	101.9±	0.40
River water	Karnaphully (upper)	0	45.0		
		100	150.0	103.4±0.6	0.25
		500	540.0	99.1±0.4	0.21
	Karnaphully (lower)	0	50.0		
		100	150.0	100±0.0	0.00
		500	560.0	101.8±1.0	0.30
	Halda (upper)	0	30.0		
		100	125.0	96.2±0.5	0.22
		500	535.0	100.9±0.8	0.28
	Halda (lower)	0	35.0		
		100	135.0	100±0.0	0.00
		500	540.0	100.9±0.6	0.24
Sea water	Bay of Bengal (upper)	0	55.0		
		100	155.0	100±0.0	0.00
		500	560.0	100.9±0.7	0.31
	Bay of Bengal (lower)	0	60.0		
		100	155.0	97.0±0.6	0.41
		500	560.0	100±0.0	0.00
Drain water	PHP Glass[c]	0	125.0		
		100	230.0	102±0.8	0.29
		500	635.0	101.6±0.7	0.27
	Elite Paint [d]	0	175.0		
		100	280.0	101.8±0.6	0.28
		500	670.0	99.3±0.5	0.21
	SA Paper[e]	0	85.0		
		100	180.0	97.3±0.8	0.45
		500	570.0	100.9±0.6	0.37
	Eastern cables [f]	0	140.0		
		100	245.0	102.0±1.0	0.48
		500	650.0	101.6±0.8	0.35

[a] average of five replicate determinations of each sample.
[b] The measure precision is the relative standard deviation(s_r).
[c] PHP Glass, Sitakunda, Chittagong.
[d] Elite Paint, Nasirabad,Chittagong.
[e] SA Paper Industry, Bhatiary, Chittagong.
[f] Estern Cables, Patenga, Chittagong.

Determination of selenium 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 reducing agent solution according to the method recommended by Stahr[48]. 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% freshly prepared sodium azide solution to reduce Se(VI) to Se(IV) and heating was continued to dense white fumes, while repeating nitric acid addition, if necessary. Heating was continued for at least 0.5 hr to remove excess azide 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 (1-2mL) of the final solution was pipetted out into a 10 mL calibrated flask and the selenium content was determined as described under general 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 AAS. The results are shown in Table 5.

The abnormally high value for the hair lose and neurological disorder patient is probably due to the involvement of high selenium concentrations with As and Zn. The occurrences of such high selenium contents are also reported in hair lose and neurological disorder patient from some developed countries[49].

Table 5 Determination results of selenium(IV) for human fluids

Serial No.	Sample	Selenium / μg L^{-1}				Sample Source*
		AAS (n=5)		Proposed method (n = 5)		
		Found	RSD[b] (%)	Found	RSD[b] (%)	
1	Blood Urine	200.6 52.8	2.0 1.8	202.5 55.6	1.8 1.3	Hypothyrodism (Female)
2	Blood Urine	413.7 105.5	3.0 2.5	415.8 120.5	2.0 1.5	Hair lose and nail discolor (Female)
3	Blood Urine	314.3 80.5	2.5 2.0	319.5 85.6	2.0 1.5	Neurological Disorder (Male)
4	Blood Urine	273.5 68.0	2.0 1.8	278.8 70.5	2.5 1.5	Pulmonary Edema (Female)
5	Blood Urine	295.8 75.0	1.8 1.4	300.5 78.8	2.2 1.8	Conjunctivities patient (Female)
6	Blood Urine	273.5 70.8	2.0 1.8	275.8 75.0	2.0 1.5	Dental Carries (Male)
7	Blood Urine	100.6 25.8	1.6 1.0	102.5 28.6	1.5 1.0	Normal Adult (Female)
8	Hair	1.55	2.0	1.62	1.8	Normal human hair (Female)
9	Nail	0.75	1.5	0.85	1.0	Normal human nail (Female)

*Samples were from Chittagong Medical College Hospital.
[a]values in μg g^{-1}.
[b]The measure of precision is the relative standard deviation (RSD).

Determination of selenium 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 excess reducing agent (1mL of 2.5% (w/v) fresh sodium azide solution) following a method recommended by Jackson[50]. 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 selenium content was determined as described under general procedure using EDTA or tartrate as masking agent. The selenium content was then determined by the above procedure and quantified from a calibration graph prepared concurrently. The results are shown in Table 6. The average value of selenium in Chittagong region surface soil was found to be 43.29 mg kg^{-1} [51]. The beneficial (0.25mgkg^{-1}) and harmful levels (65.8 mgkg^{-1}) of selenium have been indicated by green mark in the soil samples in Table 6. Food grows in this high selenium containing soil, which will be highly toxic and hazardous for living organisms including human.

Determination of selenium in pharmaceutical samples. Finished pharmaceutical samples (each Se containing 1mg tablet or required weight) were quantitatively taken in a beaker and digested in presence of excess reducing agent following a method recommended by Ahmed *et al*[52]. Add 10-mL of concentrated nitric acid and heated to dryness and then added 10 mL of 20% (v/v) of H_2SO_4 and 1-2 drops freshly prepared azide solution. The volume was reduced to 2.5 mL and then cooled to room temperature. The solution was than neutralized with dilute NH_4OH in the presence of a 1-2 mL 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 deionized water.

An aliquot (1-2 mL) of this digested sample was pipetted into a 10 mL calibrated flask and then selenium content was determined as described under the general procedure using EDTA or tartrate as a masking agent. The results of some pharmaceutical analyses are in excellent agreement with the reported values. The analyses of pharmaceutical samples from several pharmaceutical Companies for selenium are given in Table 7.

Table 6 Determination of selenium(IV) in some surface soil

Serial No.	Selenium (mg kg^{-1}) [a]	RSD (%)	Sample Source
S_1 [b]	0.58	1.0	Agriculture soil (Chittagong University Campus)
S_2	15.5	1.5	Marin soil (Bay of Bengal)
S_3	25.8	1.6	Industrial soil (Eastern Cables Chittagong)
S_4	0.25	0.8	Normal soil (Chittagong city)
S_5	45.5	1.8	Glass soil (PHP Glass Industry, Chittagong)
S_6	65.8	2.0	Paint soil (Elite Paint, Chittagong)

[a]Average of five analyses of each sample
[b]Composition of the soil samples: C, N, P, K, Na, Ca, Mg, Cu, Mo, Fe, Pb,V, Se, Zn, Mn, Co, NO_3, SO_4 etc.

Table 7 Determination of selenium(IV) in some pharmaceutical samples

Pharmaceutical Samples	Brand Name	Trade Name	Selenium / $\mu g g^{-1}$		RSD (%)
			Reported (Claimed Value)	Found (n=5)	
Tablet	Square Pharmaceuticals Ltd. (Selenoprotine)	Mulvit plus (Multivitamin-minaral) / 20 mg	20 (Selenium)	21.0	2.0
	Beximco Pharmaceuticals Ltd. (Selenoprotine)	Bextram Gold (A to Z) (Multivitamin-minaral) / 20 mg	20 (Selenium)	19.8	1.8
	Opsonin Pharma (Selenoprotine)	Zovia Gold (A to Z) (Multivitamin-minaral) / 20 mg	20 (Selenium)	19.85	2.5
	Drug International LTD. (Selenoprotine)	Supravit-G (Multivitamin + Minaral) / 20 mg	20 (Selenium)	20.5	1.5
	Holland and Barrett (Selenoprotine)	Selenium 50 µg	50	49.8	2.0

Determination of selenium 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 ashen at 555º C in a muffle furnace in presence of excess reducing agent following the method recommended by Stahr[48], 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 up to 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 selenium content was determined as described under general 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 AAS. The results are shown in Table 8. High value of selenium for *Camellia sinensis* (Green tea) is probably due to the involvement of high selenium concentration in the soil.

Table 8 Determination of selenium(IV) in some food and tea samples

Serial. No.	Sample		Selenium/ µg L^{-1} or µg kg^{-1}			
			AAS(n=5)		Proposed method(n=5)	
			Found	RSD (%)[b]	Found	RSD (%)[b]
1	Milk		0.38	1.0	0.42	1.0
2	Egg (*Gallus domesticus*)		1.41	1.3	1.50	1.2
3	Chicken (*Gallus gallus domesticus*)		1.60	1.6	1.65	1.5
4	Prawn (*Pandulus jordani*)		1.52	1.0	1.61	1.1
5	Loitta (*Harpadon nehereus*)		1.13	1.5	1.15	1.4
6	Garlic *(Allium sativum)*		0.88	1.3	0.95	1.0
7	Oat Meal (*Avena sativa*)		0.48	0.8	0.55	1.0
8	Rice *(Oryza sativa)*		2.88	2.0	2.81	2.0
9	Wheat *(Triticum aestivum)*		1.33	0.8	1.40	1.0
10	Tea *(Camellia sinensis)*	Green Tea	2.5	1.8	2.62	1.9
		Taza Tea	0.55	1.2	0.55	1.4
		Ispahani Tea	0.42	1.0	0.45	1.1

[a]Samples were from local market, Chittagong.
[b]The measure of precision is the relative standard deviation (RSD).

Determination of selenium(IV) and selenium(VI) speciation in mixtures.

Suitable aliquots (1-2 mL) of selenium(IV + VI) 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 hexavalent selenium to tetravalent selenium and heated gently with the further addition of 5 mL of water, if necessary, for 5 min to drive off the excess azide cooled to room temperature. The reaction mixtures was neutralized with dilute NH_4OH and transferred quantitatively into a 10 mL volumetric flask. 1mL of 3.8×10^{-3} M HNA-OAP reagent solution was added followed by the addition of 1 mL of 1×10^{-5} M H_2SO_4 and 4 mL DMF. It was made up to the mark with de-ionized water. The absorbance was measured after heated for at 45±5°C temperature then cooled at room temperature (25±5°C) at 509 nm against a reagent blank. The total selenium content was calculated with the help of a calibration graph prepared concurrently.

An equal aliquot (1-2 mL) of the above selenium (IV + VI) mixture was taken into a 25 mL beaker. The solution was neutralized 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, 1mL of 3.8×10^{-3} M HNA-OAP reagent solution was added, followed by the addition of 1 mL of 1×10^{-5} M H_2SO_4 and 4 mL DMF. It was made up to the mark with de-ionized water. The absorbance was measured following the general procedure at 509 nm against a reagent blank, as before. The selenium concentration was calculated in mg L^{-1} or µg L^{-1} with the aid of a calibration graph. This gives a measure of selenium(IV) originally present in the mixture. This value was subtracted from that of the total selenium to determine the selenium(VI) present in the mixture. The results of a set of determination were tested. The method has high precision and accuracy [s= ± 0.005 Se(IV), s= ± 0.006 Se(VI)] The results of the assessment of speciation of selenium(IV) and selenium(VI) in mixture were highly reproducible. The occurrence of such reproducible results is also reported for different oxidation states of selenium[53]. The present method was also applied to selenium speciation and determination of Se(IV) and Se(VI) in their different binary mixtures in real samples.

Conclusions

A new simple, sensitive and inexpensive method with the selenium(IV)-HNA-OAP complex was developed for the determination of selenium in some real, environmental, biological, soil, food and pharmaceutical samples, for continuous monitoring to establish the trace levels of selenium in difficult samples matrices. Compared with other methods[16-32] in the literatureof, the proposed method has several remarkable analytical characteristics. Firstly : The proposed method is highly sensitive with molar absorptivity of the complex of 6.3×10^5 L mol^{-1} cm^{-1}. Thus, amount of ng g^{-1} of selenium can be determined without preconcentration. Secondly : The proposed method is very simple, rapid and stable. The reaction of selenium(IV) with HNA-OAP is completed rapidly in 5 min at 40°C temperature then after cooling at room temperature (25±5°C) so it does not involve any stringent reaction conditions and offer the advantages of high complex stability (24h). Thirdly : The method has added the advantage of determining individual amounts of Se(IV) and Se(VI). With suitable masking agents, the reaction can be made highly selective. The proposed method using HNA-OAP in presence of aqueous solutions not only is one of the most sensitive methods for the determination of selenium but also is excellent in terms of selectivity and simplicity. Therefore, this method will be successfully applied to the monitoring of trace amounts of selenium in real, environmental, biological, soil, food and pharmaceutical samples.

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